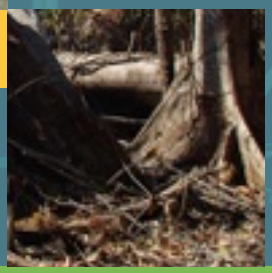


Appendix L

Tailings Ecotoxicology and Geochemistry

DISCLAIMER

This disclaimer applies to and governs the disclosure and use of this Environmental Impact Statement ("EIS"), and by reading, using or relying on any part(s) of the EIS you accept this disclaimer in full.

This Environmental Impact Statement, including the Executive Summary, and all chapters of and attachments and appendices to it and all drawings, plans, models, designs, specifications, reports, photographs, surveys, calculations and other data and information in any format contained and/or referenced in it, is together with this disclaimer referred to as the "**EIS**".

Purpose of EIS

The EIS has been prepared by, for and on behalf of Wafi Mining Limited and Newcrest PNG 2 Limited (together the "**WGJV Participants**"), being the participants in the Wafi-Golpu Joint Venture ("**WGJV**") and the registered holders of exploration licences EL 440 and EL1105, for the sole purpose of an application (the "**Permit Application**") by them for environmental approval under the Environment Act 2000 (the "**Act**") for the proposed construction, operation and (ultimately) closure of an underground copper-gold mine and associated ore processing, concentrate transport and handling, power generation, water and tailings management, and related support facilities and services (the "**Project**") in Morobe Province, Independent State of Papua New Guinea. The EIS was prepared with input from consultants engaged by the WGJV Participants and/or their related bodies corporate ("**Consultants**").

The Permit Application is to be lodged with the Conservation and Environment Protection Authority ("**CEPA**"), Independent State of Papua New Guinea.

Ownership and Copyright

The EIS is the sole property of the WGJV Participants, who reserve and assert all proprietary and copyright ©2018 interests.

Reliance and Use

The EIS is intended and will be made available to CEPA, for review by CEPA and other applicable agencies of the Government of the Independent State of Papua New Guinea ("**Authorised Agencies**"), for the purpose of considering and assessing the Permit Application in accordance with the Act ("**Authorised Purpose**"), and for no other purpose whatsoever.

The EIS shall not be used or relied upon for any purpose other than the Authorised Purpose, unless express written approval is given in advance by the WGJV Participants.

Except for the Authorised Purpose, the EIS, in whole or in part, must not be reproduced, unless express written approval is given in advance by the WGJV Participants.

This disclaimer must accompany every copy of the EIS.

The EIS is meant to be read as a whole, and any part of it should not be read or relied upon out of context.

Limits on investigation and information

The EIS is based in part on information not within the control of either the WGJV Participants or the Consultants. While the WGJV Participants and Consultants believe that the information contained in the EIS should be reliable under the conditions and subject to the limitations set forth in the EIS, they do not guarantee the accuracy of that information.

No Representations or Warranties

While the WGJV Participants, their Related Bodies Corporate and Consultants believe that the information (including any opinions, forecasts or projections) contained in the EIS should be reliable under the conditions and subject to the limitations set out therein, and provide such information in good faith, they make no warranty, guarantee or promise, express or implied, that any of the information will be correct, accurate, complete or up to date, nor that such information will remain unchanged after the date of issue of the EIS to CEPA, nor that any forecasts or projections will be realised. Actual outcomes may vary materially and adversely from projected outcomes.

The use of the EIS shall be at the user's sole risk absolutely and in all respects. Without limitation to the foregoing, and to the maximum extent permitted by applicable law, the WGJV Participants, their Related Bodies Corporate and Consultants:

- do not accept any responsibility, and disclaim all liability whatsoever, for any loss, cost, expense or damage (howsoever arising, including in contract, tort (including negligence) and for breach of statutory duty) that any person or entity may suffer or incur caused by or resulting from any use of or reliance on the EIS or the information contained therein, or any inaccuracies, misstatements, misrepresentations, errors or omissions in its content, or on any other document or information supplied by the WGJV Participants to any Authorised Agency at any time in connection with the Authorised Agency's review of the EIS; and
- expressly disclaim any liability for any consequential, special, contingent or penal damages whatsoever.

The basis of the Consultants' engagement is that the Consultants' liability, whether under the law of contract, tort, statute, equity or otherwise, is limited as set out in the terms of their engagement with the WGJV Participants and/or their related bodies corporate.

Disclosure for Authorised Purpose

The WGJV Participants acknowledge and agree that, for the Authorised Purpose, the EIS may be:

- copied, reproduced and reprinted;
- published or disclosed in whole or in part, including being made available to the general public in accordance with section 55 of the Act. All publications and disclosures are subject to this disclaimer.

Development of Project subject to Approvals, Further Studies and Market and Operating Conditions

Any future development of the Project is subject to further studies, completion of statutory processes, receipt of all necessary or desirable Papua New Guinea Government and WGJV Participant approvals, and market and operating conditions.

Engineering design and other studies are continuing and aspects of the proposed Project design and timetable may change.

NEWCREST MINING LIMITED DISCLAIMER

Newcrest Mining Limited ("**Newcrest**") is the ultimate holding company of Newcrest PNG 2 Limited and any reference below to "Newcrest" or the "Company" includes both Newcrest Mining Limited and Newcrest PNG 2 Limited.

Forward Looking Statements

The EIS includes forward looking statements. Forward looking statements can generally be identified by the use of words such as "may", "will", "expect", "intend", "plan", "estimate", "anticipate", "continue", "outlook" and "guidance", or other similar words and may include, without limitation, statements regarding plans, strategies and objectives of management, anticipated production or construction commencement dates and expected costs or production outputs. The Company continues to distinguish between outlook and guidance. Guidance statements relate to the current financial year. Outlook statements relate to years subsequent to the current financial year.

Forward looking statements inherently involve known and unknown risks, uncertainties and other factors that may cause the Company's actual results, performance and achievements to differ materially from statements in this EIS. Relevant factors may include, but are not limited to, changes in commodity prices, foreign exchange fluctuations and general economic conditions, increased costs and demand for production inputs, the speculative nature of exploration and project development, including the risks of obtaining necessary licences and permits and diminishing quantities or grades of reserves, political and social risks, changes to the regulatory framework within which the Company operates or may in the future operate, environmental conditions including extreme weather conditions, recruitment and retention of personnel, industrial relations issues and litigation.

Forward looking statements are based on the Company's good faith assumptions as to the financial, market, regulatory and other relevant environments that will exist and affect the Company's business and operations in the future.

The Company does not give any assurance that the assumptions will prove to be correct. There may be other factors that could cause actual results or events not to be as anticipated, and many events are beyond the reasonable control of the Company. Readers are cautioned not to place undue reliance on forward looking statements. Forward looking statements in the EIS speak only at the date of issue. Except as required by applicable laws or regulations, the Company does not undertake any obligation to publicly update or revise any of the forward looking statements or to advise of any change in assumptions on which any such statement is based.

Non-IFRS Financial Information

Newcrest results are reported under International Financial Reporting Standards (IFRS) including EBIT and EBITDA. The EIS also includes non-IFRS information including Underlying profit (profit after tax before significant items attributable to owners of the parent company), All-In Sustaining Cost (determined in accordance with the World Gold Council Guidance Note on Non-GAAP Metrics released June 2013), AISC Margin (realised gold price less AISC per ounce sold (where expressed as USD), or realised gold price less AISC per ounce sold divided by realised gold price (where expressed as a %), Interest Coverage Ratio (EBITDA/Interest payable for the relevant period), Free cash flow (cash flow from operating activities less cash flow related to investing activities), EBITDA margin (EBITDA expressed as a percentage of revenue) and EBIT margin (EBIT expressed as a percentage of revenue). These measures are used internally by Management to assess the performance of the business and make decisions on the allocation of resources and are included in the EIS to provide greater understanding of the underlying performance of Newcrest's operations. The non-IFRS information has not been subject to audit or review by Newcrest's external auditor and should be used in addition to IFRS information.

Ore Reserves and Mineral Resources Reporting Requirements

As an Australian Company with securities listed on the Australian Securities Exchange (ASX), Newcrest is subject to Australian disclosure requirements and standards, including the requirements of the Corporations Act 2001 and the ASX. Investors should note that it is a requirement of the ASX listing rules that the reporting of Ore Reserves and Mineral Resources in Australia comply with the 2012 Edition of the Australasian Code for Reporting of Exploration Results, Mineral Resources and Ore Reserves (the JORC Code) and that Newcrest's Ore Reserve and Mineral Resource estimates comply with the JORC Code.

Competent Person's Statement

The information in the EIS that relates to Golpu Ore Reserves is based on information compiled by the Competent Person, Mr Pasqualino Manca, who is a member of The Australasian Institute of Mining and Metallurgy. Mr Pasqualino Manca, is a full-time employee of Newcrest Mining Limited or its relevant subsidiaries, holds options and/or shares in Newcrest Mining Limited and is entitled to participate in Newcrest's executive equity long term incentive plan, details of which are included in Newcrest's 2017 Remuneration Report. Ore Reserve growth is one of the performance measures under recent long term incentive plans. Mr Pasqualino Manca has sufficient experience which is relevant to the styles of mineralisation and type of deposit under consideration and to the activity which he is undertaking to qualify as a Competent Person as defined in the JORC Code 2012. Mr Pasqualino Manca consents to the inclusion of material of the matters based on his information in the form and context in which it appears.

HARMONY GOLD MINING COMPANY LIMITED DISCLAIMER

Harmony Gold Mining Company Limited ("**Harmony**") is the ultimate holding company of Wafi Mining Limited and any reference below to "Harmony" or the "Company" includes both Harmony Gold Mining Company Limited and Wafi Mining Limited.

Forward Looking Statements

These materials contain forward-looking statements within the meaning of the safe harbor provided by Section 21E of the Securities Exchange Act of 1934, as amended, and Section 27A of the Securities Act of 1933, as amended, with respect to our financial condition, results of operations, business strategies, operating efficiencies, competitive positions, growth opportunities for existing services, plans and objectives of management, markets for stock and other matters. These include all statements other than statements of historical fact, including, without limitation, any statements preceded by, followed by, or that include the words "targets", "believes", "expects", "aims", "intends", "will", "may", "anticipates", "would", "should", "could", "estimates", "forecast", "predict", "continue" or similar expressions or the negative thereof.

These forward-looking statements, including, among others, those relating to our future business prospects, revenues and income, wherever they may occur in this EIS and the exhibits to this EIS, are essentially estimates reflecting the best judgment of our senior management and involve a number of risks and uncertainties that could cause actual results to differ materially from those suggested by the forward-looking statements. As a consequence, these forward-looking statements should be considered in light of various important factors, including those set forth in these materials. Important factors that could cause actual results to differ materially from estimates or projections contained in the forward-looking statements include, without limitation: overall economic and business conditions in South Africa, Papua New Guinea, Australia and elsewhere, estimates of future earnings, and the sensitivity of earnings to the gold and other metals prices, estimates of future gold and other metals production and sales, estimates of future cash costs, estimates of future cash flows, and the sensitivity of cash flows to the gold and other metals prices, statements regarding future debt repayments, estimates of future capital expenditures, the success of our business strategy, development activities and other initiatives, estimates of reserves statements regarding future exploration results and the replacement of reserves, the ability to achieve anticipated efficiencies and other cost savings in connection with past and future acquisitions, fluctuations in the market price of gold, the occurrence of hazards associated with underground and surface gold mining, the occurrence of labour disruptions, power cost increases as well as power stoppages, fluctuations and usage constraints, supply chain shortages and increases in the prices of production imports, availability, terms and deployment of capital, changes in government regulation, particularly mining rights and environmental regulation, fluctuations in exchange rates, the adequacy of the Group's insurance coverage and socio-economic or political instability in South Africa and Papua New Guinea and other countries in which we operate.

For a more detailed discussion of such risks and other factors (such as availability of credit or other sources of financing), see the Company's latest Integrated Annual Report and Form 20-F which is on file with the Securities and Exchange Commission, as well as the Company's other Securities and Exchange Commission filings. The Company undertakes no obligation to update publicly or release any revisions to these forward-looking statements to reflect events or circumstances after the date of this EIS or to reflect the occurrence of unanticipated events, except as required by law.

Competent Person's Statement

The Wafi-Golpu Joint Venture is an unincorporated joint venture between a wholly-owned subsidiary of Harmony Gold Mining Company Limited and a wholly-owned subsidiary of Newcrest Mining Limited.

The information in the EIS that relates to Golpu Ore Reserves is based on information compiled by the Competent Person, Mr Pasqualino Manca, who is a member of The Australasian Institute of Mining and Metallurgy. Mr Pasqualino Manca, is a full-time employee of Newcrest Mining Limited or its relevant subsidiaries, holds options and/ or shares in Newcrest Mining Limited and is entitled to participate in Newcrest's executive equity long term incentive plan, details of which are included in Newcrest's 2017 Remuneration Report. Ore Reserve growth is one of the performance measures under recent long term incentive plans. Mr Pasqualino Manca has sufficient experience which is relevant to the styles of mineralisation and type of deposit under consideration and to the activity which he is undertaking to qualify as a Competent Person as defined in the JORC Code 2012. Mr Pasqualino Manca consents to the inclusion of material of the matters based on his information in the form and context in which it appears.

This appendix includes two separate reports prepared by the Commonwealth Scientific and Industrial Research Organisation (CSIRO). The two reports are:

1) Ecotoxicology and Chemistry of Wafi-Golpu Bench-scale Tailings
2) Long-term lab study of Wafi-Golpu tailings: metal geochemistry, release and bioavailability in deposited tailings-sediment mixtures – Stage 1

MINERALS RESOURCES
www.csiro.au

Ecotoxicology and Chemistry of Wafi-Golpu Bench-scale Tailings

Merrin S Adams, David A. Spadaro, Stuart L. Simpson, Monique T. Binet, Joshua J. King, Chad V. Jarolimek, Kitty S. McKnight, Lisa A. Golding and Simon C. Apte

CSIRO Report EP 178086
March 2018

Wafi-Golpu Project

532-1104-FS-REP-0007

Prepared for GDA Consult and IHA Consult

CSIRO Land and Water, CSIRO Mineral Resources

Citation

Adams MS, Spadaro DA, Simpson SL, Binet MT, King JJ, Jarolimek CV, McKnight KS, Golding LA and Apte SC (2018) Ecotoxicology and chemistry of Wafi-Golpu bench-scale tailings. CSIRO Report EP17808, Australia, 79 pp.

Copyright

Contents

Figures

Tables

Glossary

Term	Definition
Acute toxicity	A lethal or adverse sub-lethal effect that occurs after exposure to a chemical for a short period relative to the organism's life span.
Benthic	Referring to organisms living in or on the sediments of aquatic habitats.
Bioaccumulation	A general term describing a process by which chemical substances are accumulated by aquatic organisms from water directly and/or through consumption of food containing the chemicals.
Bioavailable	Able to be taken up by organisms.
Chronic toxicity	A lethal or sub-lethal adverse effect that occurs after exposure to a chemical for a period of time that is a substantial portion of the organism's life span or an adverse effect on a sensitive early life stage.
Contaminants	Biological or chemical substances or entities, not normally present in a system, capable of producing an adverse effect in a biological system, seriously injuring structure or function.
Control	Part of an experimental procedure that is ideally exactly like the treated part except that it is not subject to the test treatment. It is used as a standard of comparison, to check that the outcome of the experiment is a reflection of the test conditions and not of some unknown general factor.
Guideline value	Numerical concentration limit or narrative statement to support and maintain a designated water use. If a GV is exceeded it triggers further investigation or initiates a management response.
Huon Gulf sediment	Sediment collected from the deep ocean environment within the Huon Gulf, PNG
Tailings	A combination of the solid material remaining after the recoverable metals and minerals have been extracted from mined ore, and any remaining process water.
Toxicity	The inherent potential or capacity of a material to cause adverse effects in a living organism.
Toxicity test	The means by which the toxicity of a chemical or other test material is determined. A toxicity test is used to measure the degree of response produced by exposure to a specific level of stimulus (or concentration of chemical) for a specified test period.
Sediment	Unconsolidated mineral and organic particulate material that has settled to the bottom of aquatic environments.

Abbreviations/Acronyms

Abbreviation/ Acronym	Description
AEM	dilute-acid extractable metal(s)
ANZECC	Australian and New Zealand Environment and Conservation Council
ARMCANZ	Agriculture and Resource Management Council of Australia and New Zealand
d	day
DO	Dissolved oxygen
DOC	Dissolved organic carbon
DSTP	Deep sea tailings placement
DTA	Direct toxicity assessment
EC10	The toxicant concentration that is expected to cause one or more specified effects in 10% of a group of organisms under specified conditions
EC50	The toxicant concentration that is expected to cause one or more specified effects in 50% of a group of organisms under specified conditions
GV	Guideline value replaces trigger value (TV) as reported in ANZECC/ARMCANZ (2000) water quality guidelines
h	hour
HC5	Concentration that is hazardous to 5% of species. This term ash the same meaning as the PC95. The PC95 is the preferred terminology in Australia and New Zealand.
IC10	The toxicant concentration that is expected to cause a 10% inhibition in the response of a group of organisms under specified conditions
IC50	The toxicant concentration that is expected to cause a 50% inhibition in the response of a group of organisms under specified conditions
ICP-AES	inductively coupled plasma atomic emission spectrometry
ICP-MS	inductively coupled mass spectrometry
kg	kilogram
L	litre
LOD	Limit of detection
mg	milligram
NOEC	No-observable-effect concentration; the highest tested concentration of a material (toxicant) at which the measured response is statistically indistinguishable from the control response.

PC95(50)	concentration that is protective of 95% of species (with 50% confidence)
PNG	Papua New Guinea
QA/QC	Quality assurance/quality control
SQGV	Sediment quality guideline value (ANZECC/ARMCANZ, 2000)
SSD	Species sensitivity distribution
TDS	Total dissolved solids
TOC	Total organic carbon
TRM	Total recoverable metal(s)
TSS	Total suspended solids
WOE	Weight of evidence
WQGV	Water quality guideline value (ANZECC/ARMCANZ, 2000)

Acknowledgements

The authors would like to acknowledge Intertek for carrying out the fish and tropical sea urchin toxicity tests on the tailings liquor and, Ecotox Services Australasia for carrying out toxicity test with oyster and sea anemone. The authors also thank Geoff Day, Ian Hargreaves, Grant Batterham, David Gwyther, Daniel Moriarty, Stuart Jones, Guy Hamilton, Travis Wood, S. Watson and Andrew Bevan for valuable comments throughout the project.

Executive summary

The Wafi-Golpu Joint Venture (WGJV) is currently undertaking a feasibility study update and an environmental impact assessment (EIA) in order to advance the Wafi-Golpu Project. As part of these studies consideration is being made to determine whether deep sea tailings placement (DSTP) in the Huon Gulf of eastern Papua New Guinea (PNG) is a potentially viable tailings management option for the Wafi-Golpu Project. The bathymetry in the northwestern region of the Huon Gulf comprises steep nearshore seabed slopes along with the occurrence of submarine canyons (depths in excess of 3000 m) in relatively close proximity to the shoreline. The lack of observed oceanic upwelling coupled with a high load of terrestrial sediment from the many rivers draining the Markham River catchments and the Finisterre ranges suggests this physical setting is suitable for consideration of DSTP as a tailings management option. CSIRO was engaged by GDA Consult to provide chemical and ecotoxicological testing as part of the feasibility study update and the environmental impact assessment.

The overall objective of this study was to carry out chemical and ecotoxicology studies to assess the tailings in terms of receiving water quality guideline values (WQGVs) and sediment quality guideline values (SQGVs) (ANZECC/ARMCANZ, 2000) and comparison to the State of PNG *Environment Act 2000* Water Quality Criteria for Aquatic Life Protection.

For this testwork, two tailings were produced from a bench-scale flotation testwork program using samples from drill cores taken from the Golpu resource. These samples comprised of approximately 90% porphyry and 10% metasediments (Tailings 1) and, 25% porphyry and 75% metasediments (Tailings 2).

The objectives of the study were to:

1. Undertake chemical characterisation of the two tailings with comparison to WQGVs and SQGVs, and, *Environmental Act 2000* criteria.
2. Determine the required tailings dilutions (elutriate tests following mixing with seawater) to meet WQGVs and SQGVs, and, *Environmental Act 2000* criteria.
3. Assess the toxicity of a diluted tailings liquor to 8 marine aquatic organisms and derive a ‘safe’ dilution of tailings liquor (using the species sensitivity distribution (SSD) approach).
4. Assess the toxicity of tailings solids to benthic organisms with an emphasis on potential bioavailability using sub-lethal whole-sediment ecotoxicology and bioaccumulation tests.

In considering the results of the study it is important to note the following caveats:

(i) The tailings samples in this study were prepared from aged core samples as used in bench-scale laboratory flotation tests. There was up to 12 months between preparation of the first tailings sample and commencement of test work.

(ii) Preliminary work (not reported herein) suggests that the use of aged core samples results in greater mobility of some metals, particularly zinc, from the solid to dissolved phase, although this has yet to be definitively confirmed.

(iii) As a result, the tailings samples in this study are likely to have had greater reactivity than if fresh core samples had been used. Therefore, the results contained in this report are likely to be conservative (i.e. overestimate impact).

(iv) Additionally, at the time of testing, the scenarios of mixing, dispersion and settling of tailings solids in the laboratory utilised in this study were designed to provide a conservative measure of tailings toxicity to aquatic organisms.

The main conclusions of the study were as follows:

Tailings characterisation, dilution and comparison to water and sediment quality guidelines

1. Both tailings samples were near neutral (pH 7.4 Tailings 1 and pH 7.2 Tailings 2) with dissolved (<0.45 µm) concentrations of Co, Cu and Zn in both tailings exceeding WQGVs. Comparison to PNG water quality criteria for aquatic life protection (Environment Act 2000) indicate that Co, Mn and Cu (Tailings 1 only) exceed the reported criteria concentrations (prior to dilution or any other potential treatment methods).
2. Analyses of total recoverable metal concentrations in tailings solids of Tailings 1 and Tailings 2 showed that Cr, Cu, Ni and Zn exceeded SQGVs. The dilute-acid extractable metal concentrations (a better indicator of potentially bioavailable metals) also exceeded SQGVs for Cu, Ni and Zn. The State of PNG does not provide criteria for sediments (solids).
3. Both tailings solids were shown to contain highly reactive trace metals with elutriate tests (mixing tailings with seawater, 16 h at 30°C) indicating that dissolved (0.45 µm) Cu concentrations continued to exceed the WQGVs (1.3 µg/L) in tailings dilutions of up to, and including, 1 in 10,000. A 1 in 10,000 dilution was sufficient to ensure all other metals did not exceed WQGVs. At a dilution of 1:50,000 copper dissolved metal concentration was also below the guideline value. At a dilution of 1 in 100, Co, Zn and Ni (Tailings 2 only) exceeded WQGVs. A dilution of 1 in 10,000 was sufficient to meet the Environment Act 2000 criteria.
4. Mixing tests examining the effects of time on metals release (1 in 10 dilution over 72 h) indicated a two stage metal release process for Cu, Co, Ni and Zn with an initial rapid release of metals into solution over the first one to five hours followed by a much slower metals release phase. Equilibrium metal concentrations (no further increase in dissolved metal concentrations) were typically achieved after 20 hours of mixing.

Ecotoxicological assessment of tailings liquor

5. The chronic toxicity of tailings to eight aquatic organisms was assessed using a tailings liquor that aimed to simulate the mix/de-aeration tank contents immediately prior to discharge via the DSTP pipeline. Tailings diluted 1 in 4 (m/m) with seawater (equivalent to dilutions of 1 in 4.7 (v/v) for Tailings 1, and 1 in 4.6 (v/v) for Tailings 2) were prepared by mixing for 1 h followed by filtration (0.45 µm). Ultimately, a 1 in 5 dilution (v/v) in the mix-

de-aeration tank will be used. Only the concentrations of Co exceeded the PNG Environment Act 2000 water quality criteria of 0.1 µg/L, by around 40 fold. The concentration of Co, Cu, Zn and Ni (Tailings 2 only) in the tailings liquors exceeded WQGVs by up to a factor of 14 for Cu and 26 for Zn. This was a lot lower than the 1 in 10,000 dilution required in the elutriate tests (point 3 above) in which tailings were mixed with seawater using different dilutions (1 in 10 to 1 in 50,000) and mixing time (16 h). The removal of tailings solids (by filtration) from both tailings liquors also stopped the continuous release of metals from tailings solids over time.

6. Chronic toxicity to microalgae, sea urchins, oysters, sea anemone and fish were of relatively similar sensitivity with EC/IC10 values of 9.4-83% for Tailings 1 and 3.9-69% for Tailings 2. The copepod early life-stage development test was the most sensitive toxicity test to both tailings liquors with EC10 values of 0.36% and 0.19% for Tailings 1 and 2 respectively. The copepod test was also the most sensitive test to individual metals; Cu, Zn, Mn and Ni.
7. The PC95 (or HC5) for tailings liquor mixed with seawater (1 in 4 (m/m)), 1 h followed by filtration (0.45 µm) was 1 in 108 for Tailings 1 and 1 in 263 for Tailings 2 post-discharge dilution (equivalent to 1 in 508 and 1 in 1,210 dilutions of pre-discharge tailings). However, after discharge in the receiving ocean environment, the tailing solids are expected to be rapidly diluted by increasing quantities of entrained seawater and will not be contained within a fixed volume of seawater for one hour as used in the tailing liquor ecotoxicology tests. As a result, the PC95 value derived here is expected to provide a conservative estimate of the PC95.

Ecotoxicological and bioaccumulation assessment of tailings solids

8. Tailings solids that enter the marine environment after discharge from the DSTP pipeline will be mixed (washed) with seawater before being deposited on the sea floor; hence, the tailings solids were washed prior to toxicity testing. Ongoing release of Cu from solids into the dissolved (0.45 µm) phase was observed over 6 days. Dissolved Mn, Ni and Zn were also released from the tailings solid but concentrations in the seawater wash solution started to decrease after about 6 days.
9. The toxicity and bioaccumulation of the tailings solids was assessed by preparing mixtures of tailings and natural deep-sea sediment collected from the Huon Gulf (Huon Gulf sediment); the first time this approach has been utilised. Toxicity of the non-mixed (100%) Huon Gulf sediment was initially assessed and resulted in a lower reproductive output (but not survival) of the amphipod and the copepod compared to a standard sediment control (from shallow waters). The reduced reproduction of the benthic organisms may be due to a lack of natural organic matter and possibly sediment-bound metals. However, it was considered to be acceptable for use in this study because (i) the response was high enough to identify a decrease (toxic) response and, (ii) the reproducibility of the response was reliable.
10. The toxicity of the tailings solids (washed) diluted with the Huon Gulf sediment was carried out using tailings mixed with Huon Gulf sediment. Toxicity of solids to amphipods and copepods required dilution of tailings to 10% for Tailings 1 and <1% for Tailings 2. The

toxicity correlated with dissolved Cu concentrations in overlying water; however modification of the experimental test containers showed that dissolved Cu does not completely explain the observed toxicity. The toxicity was likely to be attributed to Cu (Tailings 1 and 2) and Zn (Tailings 2) partitioned into the liquid phase (e.g. overlying water and pore water), direct contact with solids and dietary (ingestion) exposure of the solid.

11. During the bioaccumulation tests, both tailings samples caused lethality to the bivalve in the lower tailing:sediment dilutions. This prevented bioaccumulation from being assessed reliably in those treatments and hence is the subject of further investigations (to be reported at a later date). In this study, for tailing:sediment dilutions of 30% Tailing 1 and Tailing 2, there was no indication of significant differences in the bioaccumulation of Cu and Zn; the only significant difference detected was for Co. Bivalves exposed to the Huon Gulf sediment (no tailings) showed significant increases in bioaccumulated Cd, Co, Cr, Cu, Mn, Fe, Ni and V when compared to pre-exposed organisms. There were no effects to the survival of the bivalves in the Huon Gulf sediments despite the indication that these natural sediments contained metals that were bioavailable.

1 Introduction

WGJV engaged IHA Consult and GDA Consult to undertake a range of studies including oceanographic, seabed stability, environmental, and tailings characterisation testwork in support of the environmental impact assessment (EIA) for the Wafi-Golpu project. The results of these studies will inform both the ecological impact assessment of DSTP and provide input to the hydrodynamic mixing model which will describe the fate of tailings beyond the DSTP outfall.

CSIRO was engaged by GDA Consult to provide chemical and ecotoxicology testing as part of those studies. This report describes that test program.

1.1 DSTP considerations for the Huon Gulf

The bathymetry in the northwestern region of the Huon Gulf around the port of Lae (including to the south and east of Lae along the coast) comprises steep nearshore seabed slopes along with the occurrence of submarine canyons in relatively close proximity to the shoreline. The deep seabed of the Huon Gulf to the southeast of Lae forms the western slope of the New Britain Trench, which attains maximum depths in excess of 6,000 m. The lack of observed oceanic upwelling coupled with a load of terrestrial sediment from the 12 rivers draining the Markham River catchments and the Finisterre ranges (estimated to be at least 60 Mtpa; WGJV, 2017) suggests that this physical setting is suitable for consideration of DSTP as a tailings management option.

1.2 Previous CSIRO studies on DTSP for the Wafi-Golpu Project

The investigation of DSTP as a Tailings Management Option for the Wafi-Golpu Project commenced in 2011 with an earlier concept study. The objective of the 2011 study was to assess if metals in the tailings are likely to result in potential environmental impacts from DSTP. With regards to the tailings chemistry and potential implications for meeting water quality guideline values (WQGVs), an assessment was completed by CSIRO in August 2012 on the metal concentrations and the potential mobility of metals under discharge conditions using a pilot scale flotation tailings sample.

The conclusions from the 2012 CSIRO study were that dissolved copper (Cu) and zinc (Zn) were determined to be contaminants of potential concern. Dissolved Cu and Zn concentrations in the 1 in 10 tailings:seawater elutriates (i.e. seawater and tailings mixing tests) exceeded their respective WQGV (95% species protection level; ANZECC/ARMCANZ, 2000) by up to a factor of 16. This exceedance was reduced to a maximum of five times for the 1 in 100 elutriates. WQGVs were not expected to be exceeded for any metals for dilutions greater than 1,000.

After discharge, the tailings particulates are predicted to separate from the tailings liquor and deposit on the sea floor. Based on comparison of total recoverable metal (TRM) concentrations within the tailings solids and sediment quality guideline values (SQGVs), the metals of greatest concern to the environment within the deposited solids were chromium (Cr), Cu, nickel (Ni) and

Zn. However, dilute-acid extractable metals (AEM) analyses indicated that less than 20% of the Cr, Cu and Ni were present in potentially bioavailable forms. The majority of the zinc was present in a potentially bioavailable form and represented a significant source of exposure for benthic invertebrates. In order to provide a more comprehensive assessment of the risks posed by the proposed DSTP, the recommendations based on those results were:

1. Aquatic toxicity tests are undertaken to determine 'safe' dilutions for the tailings materials and potential effects due to the mixture of metals originating from the tailings liquid and potential metal release from tailings solids;
2. The bioavailability and potential toxicity of metals in the deposited tailings materials be assessed following mixing with natural sediments to determine the potential effects on benthic invertebrates due to the deposited tailings and metal fluxes from the deposited tailings.

1.3 Current CSIRO testwork objectives

A key objective of the current CSIRO study in 2017 was to carry out chemical and ecotoxicology studies to assess the tailings in terms of receiving WQGVs and SQGVs (ANZECC/ARMCANZ, 2000) and comparison to the State of PNG *Environment Act 2000* Water Quality Criteria for Aquatic Life Protection. The water and sediment quality guidelines for Australian and New Zealand receiving environments provide guidance for a best-practice approach for assessing contaminants in aquatic ecosystems. The *Environment Act 2000* provides legislative criteria for the protection of aquatic life in PNG. Hence, the ANZECC/ARMCANZ (2000) WQGVs and SQGVs provide a more conservative approach for contaminant assessment. Consideration of the high terrestrial sediment loads in to the Huon Gulf along with the influence of townships in the region and activities in the coastal waters off Lea on water quality, ANZECC/ARMCANZ (2000) criteria for a slightly-to-moderately disturbed ecosystem was adopted in this study (i.e. 95% species protection level).

The test work undertaken by CSIRO comprised:

1. Characterising two tailings samples (supplied from the metallurgical testwork) in terms of key chemical and physical variables, including chemistry-based bioavailability analysis and comparison to ANZECC/ARMCANZ (2000) WQGVs, SQGVs and *Environment Act 2000*.
2. Assessing the potential impacts of the tailings discharge on water quality relative to ANZECC/ARMCANZ (2000) WQGVs and *Environmental Act 2000*. This will involve the comparison of metal concentrations from elutriate tests (mixing with seawater) to water quality criteria.
3. Determining the toxicity of the tailings liquor to a suite of sensitive marine test species to derive the concentration that protects 95% of species with 50% confidence (the 'safe' concentration statistically derived from the species sensitivity distribution (SSD) curve).
4. Determining the toxicity of tailings solids and tailings-sediment mixtures to benthic organisms. This includes both whole-sediment ecotoxicology and bioaccumulation tests using a locally collected deep-sea sediment (Huon Gulf sediment).

As new information became available throughout this project, the chemical and ecotoxicological testwork was revised to account for changing conditions regarding the potential DSTP discharge scenario. Additional investigations included:

5. Investigating the cause of toxicity to aquatic biota. Additional single-metal toxicity tests were carried out and published data sourced to identify the toxicity of metals of concern (e.g. Cu, manganese (Mn), Ni and Zn) and compared to the toxicity of the tailings liquor.
6. Additional investigation of Huon Gulf sediment and the potential cause of reduced reproduction to benthic organisms.
7. Additional investigations of metals released from Huon Gulf sediment and the influence on the toxicity of the tailings solids to benthic organisms.
8. Additional chemical testing of sediment trap samples from Huon Gulf (e.g. metal analyses) and additional tailings dilution tests.

1.3.1 Tailings sample selection

This study considers two tailings samples which represent the likely bookend tailings samples expected over the life of mine from the Golpu block cave. The tailings were produced from a laboratory bench-scale flotation testwork program using samples from drill cores taken from the Golpu resource. One tailings sample represented early stage ore feed comprising of approximately 90% porphyry and 10% metasediments. The other tailings sample represented a late-stage ore feed likely to consist of only 25% porphyry and 75% metasediments.

1.3.2 Elutriate (mixing) tests

Following chemical characterisation of the tailings material, the fate of metals in the tailings material were assessed after mixing with natural seawater. The purpose of elutriate (mixing) tests was to provide information on the concentrations of metals released from the solids when the tailings material is mixed with seawater. The methods employed were based on those defined in the National Assessment Guidelines for Dredging (NAGD, 2009). In summary, this involves mixing the tailings material with seawater for a fixed duration (e.g. 1, 12, 24 h) and measurement of the dissolved (<0.45 µm filterable) concentrations of contaminants. The information from elutriate tests was used to determine:

(i) The dilutions required for potential toxicants in the tailings to comply with ambient marine water quality guidelines.

(ii) If there is any significant short-term mobilisation (solubility, precipitation) of potential contaminants when the tailings material is mixed with seawater.

1.3.3 Ecotoxicity testing of tailings liquor to marine biota and liquor ‘safe’ dilutions

The toxicity of the tailings liquor was measured using eight toxicity tests (listed below). Each toxicity test measured chronic toxicity, that is, a biological measurement incorporating a significant part of the organism’s life cycle (e.g. reproduction, early life-stage development). The

chronic toxicity tests included organisms from six general taxonomic groups, seven of which were tropical (or sub-tropical) species. Each bioassay determined the potential biological toxicity of the bioavailable contaminants present in tailings liquor to the individual aquatic biota. The results from these bioassays were combined in SSDs to statistically derive the concentration of tailings liquor likely to protect 95% of species with 50% confidence (PC5(50)) following methods by Batley et al. (2014) and Warne et al. (2015). The use of chronic toxicity data eliminates the need to apply conversion factors on acute toxicity data prior to incorporation into SSDs and hence provides more reliable estimates of the required 'safe' dilutions of tailings liquor.

Test species for this study were selected based on their known sensitivity to contaminants (in particular metals), their availability for use in testing throughout the duration of the project, the availability of standard test protocols, and their known reproducibility as surrogate test species (and test endpoints) for assessing contaminated waters in marine environments. Coral species were not included in this study because coral reefs are absent within 20km of the proposed DSTP site in the Huon Peninsula (WGJV, 2017). The toxicity tests used in this study were:

- Inhibition of growth rate of the tropical microalga *Nitzschia closterium* (tropical strain, cosmopolitan species, 72-h chronic test)
- Inhibition of growth rate of the tropical microalga *Isochrysis galbana* (tropical species, cosmopolitan species, 72-h chronic test)
- Early life-stage development of the tropical copepod *Acartia sinjiensis* (80-h chronic test)
- Larval development of the temperate sea urchin *Heliocidaris tuberculata* (72-h chronic test; the larval development endpoint is more sensitive to metals than the 1-h fertilisation test endpoint)
- Larval development of the tropical sea urchin *Echinometra mathaei* (has been identified in PNG marine waters, 72-h chronic test)
- Larval development of the tropical/sub-tropical oyster, *Saccostrea echinata* (milky oyster) (48-h chronic test)
- Early life-stage development of the tropical/sub-tropical sea anemone *Aiptasia pulchella* (8-d chronic test)
- Embryo development of the tropical/temperate fish *Seriola lalandi* (Yellowtail kingfish, has been identified in PNG marine waters, 7-d chronic test)

Throughout the toxicity tests, Cu, Mn, Ni and Zn were identified as potentially contributing to the toxicity of both tailings liquors. Hence, additional experiments with selected species were carried out using single-metal exposures with these metals to improve the understanding of which components of the liquor were causing toxicity to the marine organisms used in this study.

1.3.4 Ecotoxicity testing and bioaccumulation of tailings solids to benthic biota

After discharge, the tailings is predicted to entrain seawater and become progressively diluted as they transit through the water column on the seafloor (based on physical observations and monitoring from other DSTP systems and modelling of the proposed Wafi-Golpu DSTP system). Results from tests on the tailings material (as generated by the plant) would therefore be overly conservative given that it is likely to contain metals in the liquor and readily soluble metals

associated with the solids that would desorb upon mixing in seawater. Therefore, the approach taken in this study was to simulate the considerable mixing that would occur after discharge from the DSTP outfall by washing the tailings solids with seawater until constant metal concentrations were obtained in wash water. Ultimately, the extent of washing of tailings solids prior to deposition on the sea floor would be dependent on how the tailings are dispersed and move (e.g. as a semi-consolidated mass or fine particles thoroughly washed with seawater).

The washed tailings solid was used for assessing metal bioavailability and potential toxicity. However, exposure of benthic organisms to undiluted deposited tailings solid is unlikely to occur due to the high load of deposited terrestrial sediment into the Huon Gulf. Therefore, toxicity tests were also undertaken on tailings solids diluted with a locally collected deep-sea sediment representing that found at the tailings deposition site.

This is the first study known to incorporate a deep-sea site-specific sediment to dilute tailings solids in ecotoxicity tests. A number of deep-sea sediment samples were collected from the Huon Gulf at a depth of approximately 400–1600 m and suitable sub-samples of sediment (based on observations of particle size and water content) were mixed together to generate a representative sediment. Following investigation of the toxicity of the Huon Gulf sediment to benthic organisms, it was used as the diluent and control (non-contaminated) sediment in all of the toxicity and bioaccumulation tests on the washed tailings solids.

The bioavailability and potential toxicity of metals within the tailings solids following deposition on the ocean floor was evaluated to provide information on the potential for colonisation of deposited tailings solids by benthic biota. The release of metals into overlying water was also investigated to provide further information on the chemical behaviour of metals associated with tailing solids after deposition. The potential toxicity of the deposited tailings-sediment mixtures was assessed using two standard methods that quantify;

- survival and reproduction of the benthic amphipod, *Melita plumulosa*, over 10 d
- survival and reproduction of the benthic harpacticoid copepod, *Nitocra spinipes*, over 10 d.

No standardised whole-sediment toxicity tests exist that utilize deep-sea organisms, so the use of these temperate shallow-water organisms was justified owing to the relatively high sensitivity of the test endpoints to metals (Campana et al., 2012; Simpson et al., 2011; 2013). The amphipod has previously been used for assessing the bioavailability and toxicity of mineral-associated metals in marine sediments (Simpson and Spadaro, 2016).

For the amphipod, the EC50 values for reproduction in whole-sediment tests are in the ranges of 8-20 µg Cu/L and 30-60 µg Zn/L (overlying waters). For the copepod, the EC50s for reproduction in sediments are in the ranges of 23-72 µg Cu/L and 50-400 µg Zn/L (large uncertainty). Data are from Campana et al. (2012) for Cu, and a mixture of published and unpublished studies for Zn (e.g. Simpson et al., 2014; 2016). When expressed based on particulate metal concentrations, effects thresholds are strongly influenced by sediment properties and modified by dietary exposure. The proportion of fine particles (influencing surface area for metal adsorption) and organic carbon (OC) concentrations strongly influence copper bioavailability and toxicity. For *M. plumulosa* and *N. spinipes*, Campana et al. (2012) determined EC10s for reproduction of 5.2 and 4.8 mg <63 µm Cu/g TOC, respectively, thus reflecting the influence of particle size and organic carbon.

The potential bioaccumulation of metals by benthic biota was assessed using the benthic bivalve *Tellina deltoidalis* over 30 d. The bivalve *T. deltoidalis* buries in the top 10–20 cm of sandy or muddy sediments and is a deposit feeder, collecting organic material and particles from surface sediments. The amphipod *M. plumulosa* is a deposit feeder and known to ingest solids while foraging for food. The harpacticoid (benthic) copepod *N. spinipes* is exposed to contaminants present in pore waters, released via fluxes at the sediment-water interface and overlying water and, direct exposure to solids.

Differences in exposure conditions between the laboratory and deep-sea environment include the lower temperature and higher pressure in deep-sea environments. Recent studies of the sensitivity of shallow water organisms to metals have considered these factors (Brown et al. 2017) Comparing effects of temperature and pressure for the shallow-water prawn species *Palaemon varians*, Brown et al. (2017) determined that the sensitivity of the species to Cu and Cd varied by approximately 2–6 fold due to changes in temperature (10 or 20 °C) and less than 2-fold due to changes in pressure (0.1 MPa or 10 MPa). This suggested that shallow-water species may be suitable ecotoxicological proxies for deep-sea species, dependent on their adaptation to habitats with similar environmental variability. For example, shallow water species from polar environments may be a good surrogate for deep-sea organisms due to a similarity in water temperatures (and potential similarity in physiology). In the absence of benthic test species from deep-sea and polar environments, benthic temperate shallow water species were adopted in this study. These tests measure chronic toxicity of a temperate amphipod and copepod species and are 1–3 orders of magnitude more sensitive than the acute toxicity of Cu to prawns reported above. Hence, this study provides a conservative toxicity assessment of tailings solids toxicity to benthic biota.

2 Methods

2.1 Tailings samples

Two tailings samples arrived at CSIRO for chemical and ecotoxicity testing (Table 1). The tailings were produced from a laboratory bench-scale flotation testwork program using samples from drill cores taken from the Golpu resource and are expected to represent the likely bookends of tailings chemistry expected over the life of mine from the Golpu block cave.

The two tailings samples were in two separate drums, each consisting of about 100 kg of each tailings. Upon arrival at CSIRO, the tailings were homogenised and distributed into 20-L containers to obtain smaller sub-samples of the tailings for use in chemical and ecotoxicity tests. Each tailings sample was separated into a wet solids and liquid phase, each phase was homogenised (mixed) thoroughly and re-distributed into 20-L plastic containers to achieve the same solid-to-liquid ratio as the original tailings sample (Tailings 1, 78% solid content, Tailings 2, 72% solid content). The tailings were stored refrigerated until required for testing (up to a period of 12 months).

Throughout this report the terms Tailings 1 and Tailings 2 have been used to describe the two tailings samples.

Table 1. Tailings samples for ecotoxicity testing

Sample	Received	Composition	Also originally known as	S(%) WGJV data	S(%) CSIRO	Total Organic Carbon (%)
Tailings/Drum 1	18 Apr 2016	90% porphyry:10% metasediments	Low S tailings	0.34	0.29	1.7
Tailings/Drum 2	3 Aug 2016	25% porphyry : 75% metasediments	High S tailings	0.5	0.21	1.7

S = sulfur

2.2 Physico-chemical and metal analyses

Water pH, conductivity, dissolved oxygen (DO) and temperature measurements were made using either Thermo Orion (VersaStar Pro-series) or Hanna (HI9819X-series) meters and probes that were calibrated as per manufacturer's instructions.

Sediment, elutriates, pore waters, liquor toxicity test samples (30 mL), undiluted tailings liquor samples and seawater blanks were measured throughout the testing program for total and/or dissolved (0.45 µm filtered) metals. Samples for dissolved metals analysis were filtered through acid-washed 0.45-µm syringe filters (Sartorius, Australia). All samples were acidified with 0.2 % (v/v) concentrated nitric acid (Tracepur, Merck). Moisture and solid content were also measured (CSIRO Method C-202).

Concentrations of metals and ions were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) (CSIRO Method C-229), inductively coupled mass spectrometry (ICP-MS) (CSIRO Method C-209) or aqua regia digestion for total recoverable metals (TRM, CSIRO

Method C-223) and dilute-acid extractable metal (AEM, CSIRO Method C-241). Metal and metalloids analysed included; aluminium (Al), silver (Ag), arsenic (As), calcium (Ca), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), nickel (Ni), lead (Pb), selenium (Se), vanadium (V), zinc (Zn) and mercury (Hg). Metal concentrations in the tissue of bivalves (bioaccumulation test) were also measured by CSIRO (see section 2.4.5). The precision and accuracy of the methods was checked by the analysis of blanks comprising of at least 10% of the sample batch, as well as the analysis of certified reference materials (the exception was for metal analyses that supplemented the toxicity tests). All quality assurance met acceptable criteria (results reported in Appendix A).

2.3 Tailings elutriate (mixing) tests

A series of elutriate tests were undertaken on the tailings material to investigate metal release after mixing with seawater. All tests were carried out in a temperature control room at 30°C, using filtered (0.45 µm) seawater collected from Cronulla, NSW. All plasticware used in tests was acid washed (10% v/v nitric acid) prior to use. All analyses were carried out in triplicate. Filtered seawater blanks were also taken through each process.

2.3.1 Kinetics of metal release

To investigate the kinetics of metal release from the tailings upon mixing with seawater, one part tailings was added to 9 parts seawater in 1-L low density polyethylene bottles (100 mL tailings to 900 mL filtered seawater). Samples were then rolled (60 rpm), with sub-samples taken at time points; 0, 10 min, 1 h, 6 h, 8 h, 24 h, 48 h and 72 h. A portion of each sub-sample was syringe filtered through 0.45 µm filter cartridges (Sartorius), then acidified to 0.2% v/v nitric acid for analysis of dissolved metals by ICP-AES (Varian 730ES) and ICPMS (Agilent 8800). The pH was determined on a separate unfiltered sub-sample (Thermo Orion VersaStar Pro). All instruments were calibrated daily upon use.

2.3.2 Dilution mixing tests

Mixing tests using seawater dilution factors of 10, 100, 1000 and 10,000 fold were carried out by adding the appropriate amount of tailings to filtered seawater in LDPE bottles, then rolling for 16 h (at 60 rpm). A sub-sample of each was syringe filtered through 0.45 µm filter cartridges (Sartorius), then acidified to 0.2% v/v nitric acid for analysis of dissolved metals by ICP-AES (Varian 730ES) and ICP-MS (Agilent 8800). The pH was determined on a separate unfiltered portion (Thermo Orion VersaStar Pro). All instruments were calibrated daily upon use.

2.4 Ecotoxicology assessment of tailings liquor

2.4.1 Preparation of tailings liquors for toxicity testing

Toxicity tests on the tailings liquor were carried out on a filtered tailings liquor sub-sample prepared by simulating the mix/de-aeration tank prior to discharge via the DSTP outfall pipelines. Tailings (liquor and solid phases) were mixed with seawater in a ratio of 1 part tailings and 3 parts

seawater by mass (termed 1 in 4 dilutions (m/m) in this report) which is equivalent to a dilution ratio of 1 in 4.7 (by volume) for Tailings 1, and 1 in 4.6 (by volume) for Tailings 2 (Table 2). This dilution factor was provided to CSIRO at the time of testing; and is slightly lower than the mixing ratio of 1 part tailings and 4 parts seawater (by volume) that is currently being proposed for the mix/de-aeration tank which would result in a five-fold dilution (1 in 5, v/v) of the tailings stream. Hence the results tabled in this study are likely to be more conservative than the expected discharge concentrations if a 1 in 5 v/v dilution ratio is applied.

Upon tailings mixing with seawater, the tailings liquor is expected to neutralise (to seawater pH) and metal concentrations in the liquor are expected to decrease due to dilution and precipitation as the pH of the tailings mixture increases.

For each tailings (Tailings 1 and Tailings 2), a 1 in 4 tailings dilution (m/m) was achieved by combining wet tailings solid, decanted tailings liquid and natural seawater (Table 2). During storage, tailings solids settle to the bottom of the 20-L tailings sub-sample making it very difficult to re-homogenise the tailings to obtain a representative sub-sample for testing. Therefore, tailings liquor was decanted from a 20-L tailings sub-sample and the appropriate weight of wet-solids, decanted liquor (based on pre-determined % solids as wet weight) and seawater were added to 5-L high density polyethylene bottles. The diluted tailings were mixed (rolling) for 1 h at 30°C and the tailings liquor separated by filtration using an acid-washed 0.45 µm cartridge filter (with 0.65 µm pre-filter). The tailings liquor was collected in a 20-L high-density polyethylene container and stored at 4°C in the dark until use. The resulting tailings liquor was defined as 100% tailings liquor (i.e. undiluted tailings liquor)

Table 2. Preparation of 1 in 4 (m/m) diluted tailings liquors for use in liquor toxicity tests (prepared July 2017)

Tailings Sample	% solids (wet weight)	Tailings Solid (wet weight)	Tailings Liquid	Seawater[a]	Final dilution ratio (m/m)	Final dilution ratio (v/v)	Current proposed mixing ratio (v/v)
Tailings 1	47%	470 g	530 g	3000 g	1 in 4	1 in 4.6	1 in 5
Tailings 2	38%	380 g	620 g	3000 g	1 in 4	1 in 4.7	1 in 5

[a] Natural seawater (35‰) filtered to 0.45 µm

Up to eight concentrations of tailings liquor were prepared using filtered (0.45 µm) natural seawater as the diluent and control water. Dissolved (0.45 µm) metal concentrations were measured at the start and end of each toxicity test (Section 2.2).

Each toxicity test included a negative control (seawater) and a positive control (reference toxicant) tested at several concentrations for quality assurance purposes. The reference toxicant for each toxicity test was copper (as copper sulfate). The exception was the copepod test which utilised nickel as the reference toxicant. The pH, salinity, conductivity and dissolved oxygen saturation (excluding the microalgae tests) was measured in all test solutions throughout each toxicity test.

Statistical analyses was carried out using ToxCalc Version 5.0.23 (Tidepool Scientific Software). Following tests for normality and equality of variance, appropriate transformations (e.g. log or arc sine) were used where necessary (e.g. quantal data). Point estimates were then carried out to determine 50% effect or inhibitory concentrations (EC50 or IC50) and 10% effect or inhibitory concentrations (EC10 or IC10). In addition, hypothesis testing was carried out to determine the lowest concentration tested to have a significant effect/inhibition (LOEC) and the highest concentration tested that had no significant effect/inhibition (NOEC).

Microalgae (two species), copepod and sea urchin (temperate) bioassays were carried out by CSIRO Land and Water. Sea urchin (tropical) and fish bioassays were carried out by Intertek and the oyster and sea anemone bioassays were carried out by Ecotox Services Australasia (ESA).

2.4.2 Microalgal toxicity test

The inhibition of growth rate of two marine algae, *N. closterium* and *I. galbana*, exposed to tailings liquor was determined over 72 h. The tests are summarised in Table 3 and based on the OECD Test Guideline 201 (2002) and the protocols of Stauber et al. (1994) and Franklin et al (2005).

The tropical unicellular marine diatom *N. closterium* (Ehrenberg) W. Smith (Strain CS-114, Australian National Algae Culture Collection, CSIRO, Hobart) was originally isolated from the Coral Sea, Queensland. The strain is also known as *Cylindrotheca closterium* and more recently reclassified as *Ceratoneis closterium*. The diatom was cultured in half-strength G medium. The culture was maintained on a 12 h light:12 h dark cycle (Philips TL 40 W fluorescent daylight, 60 µmol photons/s/ m^2) at 27°C without agitation.

Cultures of *I. galbana* (CS-177) were originally obtained from CSIRO, Hobart and originally isolated from Tahiti. This alga has recently been reclassified as *Tisochrysis lutea*. The alga was cultured in a modified half-strength f-medium with the iron and trace element concentrations halved. Cultures were maintained axenically on a 12 h light:12 h dark cycle (Philips TL 40 W cool white light, 40 µmol photons/s/m^2) at 27°C.

Cells in log phase growth were used in the algal bioassay after washing to remove algal culture medium. Test solutions were prepared in triplicate and the cell density in each replicate was determined daily for three days using a FACSCalibur or FACSVerse (BD Bioscience) flow cytometer. A regression line was fitted to a plot of $\log_{10}$ (cell density) versus time (h) for each flask and the cell division rate (µ) determined from the slope. Cell division rates per day were 3.32 x µ x 24.

Table 3. Summary of the test protocol for growth inhibition tests with the tropical microalga *Nitzschia closterium* (strain CS-114) and *Isochrysis galbana* (CS-177)

Parameter	Details
Test type	Static, non-renewal
Temperature	27 ± 2°C
Light quality	Cool white fluorescent lighting
Light intensity	100-150 µmol photons $m^{-2}s^{-1}$
Photoperiod	12 h light : 12 h dark
Test chamber size	250 mL
Test solution volume	50 mL
Renewal of test solutions	None
Age of test organisms	5 days
Initial cell density in test chambers	2-4 x 10^3 cells/mL
No. of replicate chambers/concentration	3
Shaking rate	Twice daily by hand
Dilution water	Natural 0.45 µm filtered seawater
Effluent concentrations	Minimum of 5
Dilution factor	1:2 or 1:3
Test duration	72 h
Endpoint	Growth (cell division rate)
Test acceptability	Cell division rate in controls 1.6 ± 0.4 doublings per day. Variability in the controls <20%. Reference toxicant IC50 within cusum chart limits.

2.4.3 Copepod toxicity tests

The chronic toxicity test with the tropical marine copepod *A. sinjiensis* (Figure 1a) measures early life stage development (hatching and development of larvae from eggs to copepodites) and survival over 80 h. The test protocol is based on methods described in OECD (2005), ISO (2015) and Wollenburger et al. (2002), with modifications for the local tropical isolate of *A. sinjiensis* (Gissi et al., 2013; Binet et al., unpublished) Table 4.

Copepods were originally supplied by the Queensland Department of Primary Industries, Cairns and were cultured in 29-32‰ salinity seawater at 30°C. Cultures of *A. sinjiensis* were fed three times a week with approximately 1-2 x 10^5 cells/mL *Proteomonas sulcata* (previously known as *Cryptomonas* sp.) and 4-8 x 10^4 cells/mL *T. lutea* (previously known as *I. galbana, or* T-ISO) with water changes weekly (Gissi et al., 2013).

A. sinjiensis has 13 stages of development (Figure 1b). The chronic toxicity test encompasses a minimum of 7 life stages: egg hatching; growth and development of six naupliar stages (N1-N6) and; metamorphosis to copepodites (C1-C3). Approximately 40-60 eggs (<24h old) were added to replicate treatment solutions (equilibrated to 30°C) in clean polycarbonate containers. Animals are fed during the test with approximately 3.15 x 10^3 cells/mL *Tetrasemlis chuii* and 4 x 10^4 cells/mL *T. lutea* per day, added on Day 0 (double quantity added to sustain development) and on Day 2. This specialised microalgal diet enables hatched larvae to develop into copepodites within 80h, with around 10-fold less algae than is required in culture to improve toxicant bioavailability (Binet et al., unpublished, Milione and Zeng, 2007) with microalgae grown in culture media with the trace metals removed.

After 48 h, a partial renewal was carried out, whereby 120 mL of freshly prepared test solution was added to the existing 60 mL test solution for each replicate. Physico-chemical parameters were measured throughout the test. The test was terminated when the larval development ratio (LDR) reached >50% in Control treatment (typically 80 h). All solutions were then fixed using Rose Bengal and formalin and refrigerated for a minimum of 24 h to allow the Rose Bengal stain to penetrate. Using microscopy, the number of unhatched eggs, nauplii and copepodites in fixed solutions were enumerated, and three measurements of toxicity were calculated:

Survival, measured as total number of hatched animals: $\sum(Nt+Ct)$

Larval development ratio (LDR): $(LDRt) = \sum Ct/\sum(Nt+Ct)$

Hatching rate (HR): $1-(\sum UEt/\sum UEi)$

Where:
N = number of nauplii
C = number of copepodites
UE = unhatched eggs
t = at the time of test termination
i = at the time of test initiation

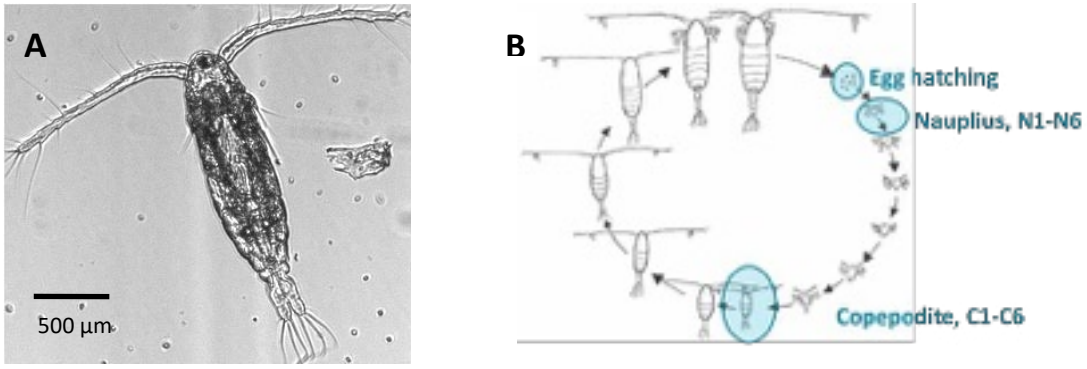


Figure 1. Marine copepod, *Acartia sinjiensis* adult (a) and life cycle (adapted from Mauchline, 1998) (b). The copepod chronic toxicity test measures survival and development of copepods from egg through nauplius (N1-N6) to copepodite life stage (C1-C3).

Table 4. Summary of the test protocol for chronic toxicity tests with the tropical copepod *Acartia sinjiensis*

Parameter	Chronic Toxicity Test
Test type	Static, partial renewal
Test duration	80 h
Temperature	30±1˚C
Salinity	30 - 35‰
Dissolved oxygen	≥ 80% saturation
Light quality	Cool white
Light intensity	8.1 µmols^{-1} m^{-2}
Photoperiod	18 h light; 6 h dark
Test chamber size	250 mL
Test solution volume	60 mL (0-48 h), 180 mL (48-80h)
Renewal of test solutions	Once at 48 h (only partial renewal, additional 120 mL of fresh solution added to existing 60 mL solution)
Age of test organisms	Eggs (< 24 h old)
No. of organisms per test chamber	40-60 (actual number confirmed using egg count controls on Day 0)
No. of replicate chambers per concentration	4
No. of organisms per concentration	160-240
Concentrations	Minimum of 5 and a control
Feeding regime	Day 0: 6.3 x 10^3 cells/mL *T. chuii* and 8 x 10^4 cells/mL *T. lutea* Day 2: 3.15 x 10^3 cells/mL *T. chuii* and 4 x 10^4 cells/mL *T. lutea*
Test chamber aeration	None
Dilution water	0.45 µm filtered seawater
Test endpoint	Larval development ratio; survival; hatching rate
Test acceptability	≥50% LDR in controls; Reference toxicant (copper or nickel) EC50 within acceptable criteria (Cusum chart limits).

2.4.4 Sea urchin toxicity tests

The sea urchin larval development test was carried out with two species; the tropical species *E. mathaei* and the temperate species *H. tuberculate.* The test method is based on methods described by Simon and Laginestra (1997), USEPA (2002) and Byrne et al. (2008) and summarised in Table 5.

Adult *H. tuberculata* were collected from South Maroubra, Sydney, and *E. mathaei* were collected from Abrolhos Island, Geraldton, WA.

Sea urchin gametes were collected following 0.5 M KCl injections to induce sea urchins to spawn (Figure 2). Sperm quality was confirmed by observation of physical characteristics using microscopy. Gamete viability from one male and one female was assessed by presence of vigorously swimming sperm under microscopic examination. Egg quality was confirmed by the ability for healthy sperm to fertilise 90% of the eggs within 10 minutes of combining gametes. Sperm and egg densities were then determined using a haemocytometer and a Sedgwick-Rafter cell, respectively and a final sperm:egg ratio of 100:1 was used to fertilise the egg solution (2000 eggs/mL) and ensure >90% of eggs were fertilised.

Once the eggs were successfully fertilised, approximately 100 or 500 fertilised eggs (depending on the species) were added to each replicate (diluted PFW and controls) at the start of the test. After 72 h, 10% buffered formalin was added to each test tube to preserve the samples and the proportion of normally developed pluteus larvae (first 100 observed under a microscope) were counted and expressed as a percentage of the control.

Figure 2. Injecting KCl into *Heliocidaris tuberculata* to stimulate spawning

Table 5. Summary of the test protocol for larval development tests with the sea urchin *Heliocidaris tuberculata* and *Echimonetra mathaei*

Parameter	*H. tuberculata*	*E. mathaei*
Test type	Static	Static
Temperature	20 ± 1°C	25 ± 1°C
Light quality	Daylight fluorescent lighting	Daylight fluorescent lighting
Light intensity	12-17 $\mu mols^{-1}\ m^{-2}$	Ambient laboratory (<800 Lux)
Test chamber size	9 mL (borosilicate tissue culture tube)	5 mL (glass tubes)
Test solution volume	5 mL	4 mL
Salinity of test solutions	35‰	35‰
Renewal of test solutions	None	None
Source of test organisms	Field collected, Maroubra, NSW	Field collected, Abrolhos Island, Geraldton, WA
Density of fertilised eggs per replicate	500	100
No. of replicates/concentration	4	3
Dilution water	Natural filtered seawater (0.45 µm)	Natural filtered seawater (0.45 µm)
Effluent concentrations	Minimum of 5 and a control	Minimum of 5 and a control
Dilution factor	1:2	1:2
Test duration	72 h	72 h
Endpoint	Larval development to normal pluteas larval stage	Larval development to normal pluteas larval stage
Test acceptability	≥70% normal development in controls, reference toxicant (copper) EC50 within prescribed cusum chart limits	>80% normal development in controls, reference toxicant (copper) EC50 within Cusum chart limits

2.4.5 Oyster toxicity test

This test involves exposing fertilised eggs from field-collected milky oysters, *S. echinata*, to tailings liquor and assessing normal development to the D-veliger larval stage after 48 h. The test protocol is based on APHA (1998) and Krassoi (1995) and is summarised in Table 6.

Oysters were collected from Mackay, Qld. Sperm and eggs were collected from oysters using a gamete stripping procedure and transferred into clean 250-mL beakers containing 200 mL of filtered seawater. The density of each egg and sperm solution were determined using a Sedgwick-Rafter counting chamber and haemocytometer, respectively. Sufficient sperm solution was added to the egg suspension so as to achieve an egg:sperm ratio of 1:100. The egg suspension was then incubated at 25°C for 30 min to allow fertilisation to occur.

Test solutions were prepared in quadruplicate and inoculated with fertilised eggs (final density of 30 ± 5 eggs/mL). Test vessels were covered with cling wrap and incubated at 25°C for 48 h. Tests were terminated after 48 h by adding 10% buffered formalin to each test solution and larvae were examined under 100X magnification using a compound microscope and Sedgwick-Rafter counting chamber. The first 100 larvae were examined and counted as either normal D-veliger larvae or abnormal if the larvae failed to develop from the zygote or trochophore stages, or were misshapen.

Table 6. Summary of test protocol for the larval development test with the tropical oyster *Saccostrea echinata* (milky oyster)

Parameter	Details
Test type	Static non-renewal
Test end-point	Larval development to D-veliger (Prodissochonch I) stage
Test duration	48 h
Test temperature	25 ± 1°C
Test salinity	24-36‰
Test chamber size/volume	5 mL in 9 mL borosilicate glass vial
Source of test organisms	Field collected (Mackay, Qld)
Photoperiod	16:8 light:dark
Light Intensity	600-800 lux
Light quality	Daylight fluorescent lighting
Test concentrations	Minimum of 5 concentrations plus controls
Test acceptability criterion	≥70% normally developed larvae in controls. Reference toxicant, copper, EC50 within chart limits

2.4.6 Sea anemone toxicity test

This test involves exposing lacerates (pre-juvenile developmental stage) produced from laboratory cultured sea anemones (*A. pulchella*) and observing the development of tentacles over 8 d to reach the juvenile developmental stage. The test protocol is based on Howe et al., (2014) and is summarised in Table 7.

Adult sea anemones were originally sourced from the National Marine Science Centre, Charlesworth Bay/ Southern Cross University, Lismore, NSW, Australia and housed at ESA. The adults reproduce asexually by separation of a small piece of pedal disc tissue which is termed a lacerate. Once the lacerate adheres to a substrate and develops at least 8 tentacles, it is termed a juvenile.

New lacerates were harvested from the adult culture tanks and those without tentacles were selected for testing following inspection by stereo microsope. Four lacerates were placed in each of 5 replicate acid-washed test containers for each treatment and control and allowed to acclimate for 3 h to ensure that no test organisms had tentacles at test commencement and to allow adherence of the lacerates to the test containers. Test solutions were renewed every 48 h and physico-chemical parameters were measured. Observations of tentacle development were made at 4, 6, 8, 10 and 14 d using a stereo microscope. A juvenile was recorded if 8 tentacles of any length were visible.

Table 7. Summary of test protocol for the development test with the tropical sea anemone *Aiptasia pulchella*

Parameter	Details
Test type	Static renewal every 48-h
Test end-point	Lacerate development to juvenile stage as evidenced by at least 8 tentacles
Test duration	8 d
Test temperature	25 ± 1°C
Test salinity	24-36‰
Test chamber size/volume	73 mL in 75 mL seawater-aged polyethylene containers
Source of test organisms	Laboratory cultures
Photoperiod	12:12 light:dark
Light Intensity	50-60 µM photons m^2 s^{-1}
Light quality	Daylight fluorescent lighting
Test concentrations	Minimum of 5 concentrations plus controls
Test acceptability criterion	≥ 90% normally developed juveniles in controls. Reference toxicant, copper, EC50 within chart limits

2.4.7 Fish toxicity test

This test involves the exposure of unhatched embryos of the tropical Yellowtail Kingfish *S. lalandi* for 7 d with observations of development and survival recorded daily. The test protocol is based on USEPA (2002a,b) and summarised in Table 8.

Adult fish were maintained for at least 12 months at the Australian Centre for Applied Aquaculture Research (ACAAR) in Fremantle, WA in clean sand-filtered seawater (35‰) at 16-20°C (annual temperature range) and fed a mixed fresh seafood diet. Spawning occurs unassisted on a weekly basis and unhatched eggs <12-h post spawning are used to initiate the test.

The static non-renewal tests were conducted with three replicates per test treatment. Twenty unhatched embryos were randomly selected and introduced into test beakers. Embryos were not fed post-hatch during the test as they have an ample yolk sac which sustains the larvae until day 6. Beakers were covered with cling-wrap film to minimise evaporation and incubated at 22 ± 1°C with 12:12 light:dark cool white fluorescent lighting. Solutions were not aerated but dissolved oxygen was monitored at the beginning and end of the test. Test vessels were checked every 24 hours for abnormal development or mortality of fish and dead fish were removed. By Day 7 the total number of abnormally developed or dead fish was recorded.

Table 8. Summary of the test protocol for the early life-stage development test with *Seriola lalandi*

Parameter	Details
Test type	Static
Test end-point	Early life-stage development (unhatched embryo)
Test duration	7-d
Test temperature	22 ± 1°C
Test salinity	35‰
Test chamber size	600 mL glass beakers
Test solution volume	500 mL
Source of test organisms	Australian Centre for Applied Aquaculture Research
Test concentrations	Minimum of 5 concentrations plus controls
Test acceptability criterion	≥70% normal development/survival in controls, Cu EC50 within chart limits

2.4.8 Species sensitivity distributions and 'safe' dilutions

The risk extrapolation technique of Aldenberg and Slob (1993) has formed the basis of deriving 'safe' concentrations of toxicants from species sensitivity distributions (SSD) and was adopted to derive water quality guideline values for toxicants in the Australian and New Zealand Water Quality Guidelines (ANZECC/ARMCANZ, 2000). The method has been recently modified for the derivation of toxicant guideline values (GVs) in Australia and New Zealand since 2015 (Batley et al., 2014; Warne et al., 2015). Modifications include the preference of EC10 toxicity data over NOECs in SSDs and the recommendation of at least 8 species (previously 5 species) from at least four different taxonomic groups. The toxicity data is then fitted to a Burr Type III distribution and the concentration of toxicant that will protect a defined proportion (e.g. 95%) of species in the receiving environment is derived. An assessment of curve fit and number of species in the SSD also contribute to an assessment of GV reliability (low, moderate, high, very high and the use of only chronic toxicity data providing higher reliability GVs.

The risk extrapolation method has also been used to derive 'safe' dilutions of effluents and waste waters entering aquatic ecosystems. In this study, the derivation of 'safe' dilutions of tailings liquor were derived from SSD with eight EC/IC10 values from only chronic toxicity tests. Sufficient chronic toxicity tests are now available and hence the extrapolation of acute toxicity test data to estimated chronic toxicity values is no longer necessary. A Burr Type III curve was fitted to the data using the Burrlioz 2.0 program (https://research.csiro.au/software/burrlioz/) and the estimated PC95 (concentration of tailings liquor that is protective of 95 of the species in the receiving environment) extrapolated from the curve fit. However, for some data sets the Burrlioz program utilises the inverse Pareto model as the best model curve fit. The equivalent dilutions (1 in X) of tailings liquor was calculated as 100 ÷ PC95 (%). The 95% protection level applies to environments that are slightly-to-moderately disturbed. The PC95 can also be presented as the hazard concentration (HC), that is, the concentration of tailings liquor that would be hazardous to 5% (HC5) of species.

The State of PNG *Environment Act 2000* does not provide methods for deriving 'safe' dilutions.

2.5 Ecotoxicological assessment of tailings solids

2.5.1 Preparation of tailings solids

After discharge via the DSTP outfall pipeline, the tailings is expected to disperse, resulting in the mixing and dilution of tailings liquor (supernatant) with entrained seawater together with additional desorption of metals from tailings solids in contact with seawater. In this study, the tailings solids were mixed with seawater at a ratio of 5 kg solids to 15 L seawater (1 part to 3 parts) for 1 min then allowed to settle for a minimum of 5 h before syphoning the overlying seawater. A minimum settling period was necessary to allow the finer materials to settle out of solution. The seawater rinse liquid was then collected and analysed for dissolved (0.45 µm) metals. This washing procedure was repeated 13 times with 5 to 79 h settling time over a period of 443 h.

2.5.2 Control sediment and tailings treatments

Deep-sea sediments collected from the Huon Gulf in March 2017 (HG) were used as control sediment and as a diluent sediment for mixing with tailings. A sub-sample (25-600 g) of 40 different HG sediments were combined (18 kg in total) within a new 20 L plastic bucket, homogenised thoroughly and stored at 4 °C until time of use. This HG sediment was used to dilute the washed tailings solids from Tailings 1 (Table 9) and Tailings 2 (Table 10), and also to prepare sediment for any test modification/manipulations (Table 13). The diluted tailings (combining washed tailings solid with diluent HG sediment) were homogenised thoroughly and then stored in a zip-lock plastic bag at 4 °C until time of testing. Prior to testing, the tailings/sediment mix was then homogenised, dispensed into the test vessels and allowed to equilibrate with added filtered (0.45 µm) seawater for 48 h at 21°C.

Table 9. Dilution preparation of washed tailings solids from Tailings-1.

Treatment name	Dilutions (%)	Washed tailings solids (g)	Huon Gulf sediment (g)
HG control	0% tailings, 100% HG	-	1250
Tailings-1_1% tailings	1	13	1237
Tailings-1_10% tailings	10	125	1125
Tailings-1_30% tailings	30	375	875
Tailings-1_60% tailings	60	750	500
Tailings-1_90% tailings	90	1125	125

Table 10. Dilution preparation of washed tailings solids from Tailings-2.

Treatment name	Dilutions (%)	Washed tailings solids (g)	Huon Gulf sediment (g)
HG control	0% tailings, 100% HG	-	1250
Tailings-2_1% tailings	1	13	1237
Tailings-2_3% tailings	3	38	1212
Tailings-2_10% tailings	10	125	1125
Tailings-2_30% tailings	30	375	875
Tailings-2_90% tailings	90	1125	125

2.5.3 Water and solids analyses

General methods for physical and chemical analyses of the waters and sediments during the ecotoxicity assessment of tailings solids are provided in Table 11. Clean seawater was collected from The Entrance, Central Coast, New South Wales, Australia, membrane-filtered (1 µm), and acclimated to a room temperature of 21 ± 1°C. The salinity of the filtered seawater was adjusted to the test salinity of 30‰ using Milli-Q deionised water (18 MΩ·cm; Milli-Q® Academic Water System). All plasticware for dissolved metal and ammonia analyses was new, and blanks were used to monitor for possible contamination.

Table 11. General physical and chemical analysis methods for waters.

ANALYTE	METHOD
Water pH, dissolved oxygen (DO) and salinity	Measurements of pH (calibrated against pH 4.0, 7.0 and 10.0 buffers) used a pH meter (HI98191) equipped with a spear-tip FC200B probe (Hanna instruments). DO and temperature measurements were made using a HI5421 DO meter (Hanna) using saturated and zero oxygen solutions. Salinity measurements used a WTW meter (LF 320) with a Tetra-Con 325 probe, and were reported according to the Practical Salinity Scale of 1978 (PSS 78) as dimensionless values.
Dissolved metals by ICP-AES	(APHA 21st ed., 3125; USEPA (2007) SW846 - 6020): The inductively-coupled plasma atomic emission spectrometry (ICP-AES, Varian 730-ES) using in-house methods (C-209 and C-229, respectively). Dissolved metals were those that passed through a 0.45 µm membrane.
Porewater extraction	Porewater was isolated from sediment in an inert atmosphere (nitrogen) by filling a 50 mL centrifuge tube with sediment then centrifuging at 1000 g. The isolated pore water was filtered (<0.45 µm) and acidified to 0.2% HNO_3 for preservation. The concentrations of dissolved metals were determined by ICP-AES.
Dissolved (0.45 µm) total ammonia	Dissolved (0.45 µm) total ammonia (NH_3+NH_4^+) was analysed colorimetrically using an ammonia test kit (API) using a refined method based on the manufacturer's instructions.

2.5.4 Toxicity tests

Standard amphipod survival and reproduction tests

The amphipod reproduction bioassay measures adult survival and reproduction, expressed as the number of embryos and <1-d-old juveniles in the second brood following exposure of *M. plumulosa* to test sediments over a 10 d period. The test was carried out using the standard method described by Spadaro and Simpson (2016a). The test conditions are summarised in Table 12.

In the standard test procedure, 40 g of sediment was placed into 250 mL beakers, filtered seawater (200 mL, 30 ‰) was added and each beaker was incubated at 21°C with aeration for 72 h to allow any resuspended sediments to settle and equilibrate. Four replicates were used per sediment. After the equilibration period, 180 mL of overlying water was siphoned off and replaced with new seawater with care to minimise sediment resuspension.

Amphipods used in the tests were isolated from laboratory cultures and transferred to holding trays 7–10 d before tests commenced. Two days before test commenced males were added to the holding trays for mating. At the start of test (Day 1), six gravid females (gravid for <36 h) from the holding trays and six new males (isolated from laboratory cultures) were randomly assigned to each beaker. Treatments were fed at a rate of 0.5 mg Sera Micron fish food/amphipod twice a week. The sediments were renewed after 5 days by gently sieving away the adults and placing them into the fresh sediments that had been prepared and equilibrated for 72 h as above, thus allowing for the removal of juveniles from the first brood, which are typically unaffected by contaminants in the test sediment because they were already conceived before exposure to test sediments.

On Day 10, the females were carefully removed and the number of embryos per female was counted by microscopy. The sediment was also checked for juvenile amphipods that had escaped the marsupium during the latter stages of the test by sieving the sediment through a 180 µm mesh. The total number of embryos and <1-day-old juveniles were summed and expressed as a percentage of the control. For quality assurance purposes, a minimum of 8 juveniles per female were required in all controls for tests to be considered acceptable. A sediment was considered to be acutely toxic if the survival percent control was <80% and was statistically significantly less ($P<0.05$) than the controls. Chronic toxicity was detected when the reproductive output percent control is <80%, (based on 2 standard deviations of control data n=75) and was statistically significantly less ($P<0.05$) than the controls.

Overlying water concentrations of dissolved metals (<0.45 µm filtered) and ammonia, along with physico-chemical parameters (temperature, pH, salinity and dissolved oxygen) were measured periodically throughout the test. Water was exchanged on Days 3 and 7, sediment renewed on Day 5, ammonia measured on Days 3, 7 and 10, and metals measured on Days 5, 7 and 10. Statistical significance between treatments was calculated using ToxCalc Version 5.0.23 (Tidepool Software).

Table 12. Summary of the standard (original) amphipod survival and reproduction toxicity test conditions

Parameter	Details
Test type	Chronic renewal
Test duration	10 day
Temperature /Salinity	21 ± 1°C / 30 ± 1 ‰
Light intensity	3.5 µmol photons/s/m^2
Photoperiod	12 h light, 12 h dark
Test chamber	250 mL glass beakers
Sediment weight	40 g
Overlying water volume	~220 mL
Total test volume	250 mL
Age/size of test organisms	2-4 month old
No. test organisms/ test chamber	6 females and 6 males
No. replicate beakers / sample	3-4
Feeding regime	0.5 mg Sera micron® fish per amphipod twice a week.
Test chamber aeration	1 outlet with slow bubbling to maintain ≥85% dissolved oxygen throughout test
Control sediment	Uncontaminated sediment with similar physico-chemical parameters (grain size, porewater salinity) to the test sediment. This control was used for quality assurance checks. Huon Gulf sediment used as a diluent control
Overlying water	Fresh uncontaminated seawater (Port Hacking), NSW, 0.45 µm filtered and diluted with deionised water (Milli-Q) to salinity of 30±1‰ Renewal every two days
Endpoint	Adult survival and reproductive output (total embryo/juvenile numbers)
Test acceptability criteria	>80% survival in the controls, >8 embryos/juveniles per female, physico-chemical parameters (dissolved oxygen, pH, salinity and temperature) within acceptable limits throughout the test

Test modification and manipulations to modify exposures

A range of test modifications were applied to provide information on how the tailings exposure conditions may influence the test and assessment outcomes. Summary details of the test modifications are provided in Table 13, and the detailed descriptions provided below.

a. Effects of dissolved metals released from the washed tailings solids

To assess the effects of dissolved metal concentrations in the overlying water on the toxicity observed to amphipod reproduction, test modifications were used to reduce the dissolved metal concentrations in the overlying water compared to the standard test method (Spadaro and Simpson, 2016a). For this purpose, four diluted-tailings treatments were selected for treatment modifications: Tailings-1_10%, Tailings-1_30%, Tailings-2_1% and Tailings-2_10%. For these tailings/concentrations, 400 mL beakers were used containing the same amount of sediments as the standard method and 380 mL of overlying water instead of a 250 mL beaker containing 220 mL of overlying water. The modified treatments are indicated by the suffix 'M1' (Table 13). In addition, the overlying water was exchanged with fresh filtered seawater daily. Additional replicates using the standard method were run in parallel to the modified treatments, the standard treatments were used as controls for comparison of the amphipod reproduction.

b. The effects of layering and mixing of the tailings solids with the diluent sediments

Once the tailings slurry exits the DSTP outfall pipeline and deposit on the ocean floor, the tailings solids could form a layer on top of the existing sediment, and then may mix with the sediment beneath over time (via currents, bioturbation, clean sediment deposition etc.). In the main tailings deposition area (designated footprint) the rate of deposition and depth of tailings may result in the natural sediments being buried below a thick tailings layer. On the edge of the predicted tailings footprint and potentially outside this area small amounts of tailings may deposit as thinner layers. To assess the differences in the risk of toxicity from thin layers of tailings overlying natural sediments, compared to the same proportion mixed within the sediments some additional treatments were prepared. Specifically, the intent was to compare at equivalent %-tailings treatments prepared as a layer of tailings over Huon Gulf sediment with tailings mixed with the Huon Gulf sediment, and test using the standard method.

These 'layer' and 'mixture' treatments were also prepared using a different silty estuarine sediment (the material used for standard controls), instead of the Huon Gulf sediment. The new sediment was from a local estuary known to accommodate high amphipod reproduction (fecundity), and was included because the Huon Gulf sediment (in the absence of tailings) was not resulting in the desired level of reproduction expected for an uncontaminated sediment (effects to reproduction meant that this sediment could not act as a control).

Three concentrations were selected for the layering (suffix 'L', Table 13) and mixed treatments: 0.1%, 1% and 10%. These layered treatments were tested using 0.04, 0.4 and 4 g respectively of undiluted tailings solid from Tailings 1 layered on top of 39.96 g, 39.6 g and 36 g of Huon Gulf sediment in each treatment beaker, respectively, and resulted in tailings layers with depths of approximately 1–1.5 mm for the 10% layered tailings treatment and proportionally thinner layers (but not clearly visible) for the 1% and 0.1% layered tailings treatments. In the 10% layered treatments, the layers were approximately 1 mm thick. For the 1% and 0.1% layered treatments, the tailings solids were spiked into the overlying water and allowed to settle onto the Huon Gulf sediment, to achieve an even, fine layer of tailing solids. No visible layer was observed in these treatments. The 10% layered treatment was tested using both the tailings solids from Tailings 1 and Tailings 2. The treatments using the silty estuarine sediment were indicated by the suffix 'S-L' (Table 13), and used the same amounts of sediments as described for the L-treatments, and also had a matching control (the standard QA control). The remainder of the test protocol was run using the standard methods.

Table 13. Modified treatments

Treatment name	Dilutions (%)	Method
Effects of dissolved metals released from the washed tailings solids		
Standard QA control	0% tailings, 100% S	-
Tailings-1_10% M1	10% tailings, 90% HG	Made in bulk then added to the 400 mL beakers; described in Table 9
Tailings-1_30% M1	30% tailings, 70% HG	
Tailings-2_1% M1	1% tailings, 99% HG	Made in bulk then added to the 400 mL beaker; described in
Tailings-2_10% M1	10% tailings, 90% HG	Table 10
The effects of layering and mixing of the tailings solids with the diluent sediments (HG)		
HG control	0% tailings, 100% HG	-
Tailings-1_0.1% L	0.1% tailings, 99.9% HG	Layered in the beaker; described in Section 2.5.4: Test modification and manipulations to modify exposures, b.'
Tailings-1_1% L	1% tailings, 99% HG	
Tailings-1_10% L	10% tailings, 90% HG	
Tailings-2_10% L	10% tailings, 90% HG	
The effects of layering and mixing of the tailings solids with the diluent sediments (S)		
Standard QA control	0% tailings, 100% S	-
Tailings-1_1% S-L	1% tailings, 99% S	Layered in the beaker; described in Section 2.5.4: Test modification and manipulations to modify exposures, b.'

HG = Huon Gulf sediment; S = standard QA control sediment; M1 = modified overlying water volume; L = tailings layer on surface of diluent sediment (not mixed, homogenised)

Standard copepod reproduction tests

This sub-lethal test measures the reproductive output of the copepod *N. spinipes* following exposure to the test sediments over 10 d. The test was carried out using the standard method described by Spadaro and Simpson (2016b), and is summarised in Table 14. *N. spinipes* was originally isolated from estuarine sediments at Gray's Point on the Woronora River in New South Wales. The copepod is cultured in the laboratory in sand with 30‰ filtered overlying seawater at 21°C (Simpson and Spadaro, 2011). The test conditions are summarised in Table 14.

Sediments were homogenised immediately prior to being added to test vials (0.5 g sediment per 10 mL vial, 4 replicates per sediment). Filtered seawater (30 ‰) was added, and each vial was incubated at 21°C overnight to allow sediments to settle. The following day, overlying water was replaced and five gravid females (3-5 weeks old) were randomly assigned to each vial. Copepods used in the tests were isolated from laboratory cultures. Copepods were fed a diet of 1×10^4 cell/mL of both *I. glabana* (currently *T. lutea*) and *Tetraselmis* sp. as well as 0.3 mg Sera micron® fish food (<63 µm) which was added to each test vial twice a week. After ten days, the number of nauplii (first juvenile lifestage of the copepod) and copepodites (second juvenile lifestage) in each vial was recorded by microscopy. Physio-chemical parameters (temperature, pH, salinity and dissolved oxygen) were monitored throughout the toxicity test.

Reproductive output of the copepods was expressed as the number of juveniles per surviving female. Toxicity was detected when the reproductive output was <75% of the control, (based on 2 standard deviations of control data n=30) and significantly less (P<0.05) than the reproductive

output observed in the control. Tests for significance between treatments and point estimate values (EC50, EC10) were calculated using ToxCalc Version 5.0.23 (Tidepool Software).

Table 14. Summary of the test protocol for sediment toxicity tests with the copepod *Nitocra spinipes*

Parameter	Details
Test type	Static non-renewal
Test duration	10 d
Temperature / Salinity	21 ± 1°C / 30 ± 1 ‰
Photoperiod	12 h light, 12 h dark
Light intensity	3.5 µmol photons/s/m^2
Test chamber	10 ml polycarbonate vial
Sediment weight	0.5 g
Overlying water volume	~9 mL
Total test volume	10 mL
Age / size of test organisms	3-5 week gravid females
No. test organisms / test chamber	5
No. replicate beakers / sample	3
Feeding regime	1×10^4 cell per mL of both alga *I. galbana* and *Tetraselmis* sp. + 0.3 mg Sera micron® fish food (<63 µm) per test vial twice a week.
Test chamber aeration	None
Overlying water	Fresh uncontaminated seawater (Port Hacking), NSW, 0.45 µm filtered and diluted with deionised water (Milli-Q) to salinity of 30±1 ‰
Endpoint	Juvenile production (nauplii + copepodites).
Test acceptability criteria	>20 juveniles per female, physico-chemical parameters within acceptable limits throughout the test

2.5.5 Bivalve survival and bioaccumulation test method

The 30-d bioassay determines whether metals associated with the sediment are bioavailable to the estuarine bivalve, *T. deltoidalis,* by exposing the bivalves to sediment for 30 d and measuring metals that have bioaccumulated in their soft body tissue. This bioassay can also detect toxicity to bivalves by measuring the survival of bivalves after 30 d. The test was carried out using the standard method described by Spadaro and Simpson (2016c), and is summarised in Table 15.

The bivalve/clams, *T. deltoidalis,* were collected at Boronia Park, Lane Cove River at Sydney, NSW, Australia (King et al., 2010). Approximately 500 adult bivalves with shell surface areas from 10 to 60 mm^2 (two dimensional) were collected by gently sieving (2 mm mesh) sediment from a maximum depth of 20 cm.

Bivalves were acclimated for 4 days to the laboratory test conditions (21°C and salinity 30‰) in holding trays with sediment from the bivalve collection site and oxygenated seawater. After acclimation, bivalves were extracted from the sediment, placed in seawater and sorted into groups of 7 individuals with approximately the same size distribution. The bivalves were observed for movement to ensure only live animals were selected for use in the bioaccumulation test.

Approximately 275 mL (1.5 cm depth) of each tailings/sediment treatment was added to 1-L beakers and 900 mL of seawater (30‰) added as overlying water. Each treatment was prepared in triplicate. Overlying water was aerated continuously to maintain dissolved oxygen levels >85% saturation. *T. deltoidalis* were fed twice per week with 1 mg of Sera Micron^{TM} per bivalve. The release of metals from tailings solids to overlying water was monitored by measuring dissolved (0.45 µm filtered) metals in the overlying water before and after water changes.

At the termination of the tests (i.e. after 30 d), surviving bivalves were counted and allowed to depurate for 24 h in clean seawater. Their soft body tissue was dissected from the shell using a Teflon coated razor blade and plastic tweezers. Tissue masses from the same replicate were added to a 30-mL polycarbonate vial and left overnight in a domestic freezer at -20 °C.

Tissues were freeze dried and reweighed to determine the tissue dry weight (DW) and acid digested according to CSIRO Method C-225. Briefly, tissue from each test replicate was digested in duplicate in Teflon digestion tubes by adding 10 mL of Tracepur nitric acid (65%) and a Microwave Accelerated Reactive System (MARS). Digests were made to a final volume of 25 mL with Milli-Q water and metals were measured by ICP-MS (Agilent 7500CE) calibrated with matrix-matched standards. For quality control purposes, one blank (Milli-Q water) and one reference sample (DORM-3, Fish Protein Certified Reference Material, National Research Council Canada) were analysed for every 8 samples.

Table 15. Summary of the test protocol for bioaccumulation tests with the bivalve *Tellina deltoidalis*

Parameter	Details
Test type	Static
Temperature	21 ± 1°C
Light/Photoperiod	Ambient natural light and photoperiod
Test chamber size	1 L glass beaker
Test solution/sediment volume	275 g (equivalent to ~2 cm depth) and made up to 900 mL volume with overlying seawater
Renewal of test solutions	Static renewal (twice per week)
No. of replicate chambers/concentration	3 replicates
Dilution water	Natural seawater (0.22 µm filtered)
Size of organism	10 – 60 mm^2 (two dimensional)
No. of organisms per test chamber	7
Food regime	Sera Micron^{TM} 1mg/bivalve twice per week
Test duration	30 days
Endpoint	Soft tissue metal concentration

3 Results

3.1 Tailings characterisation and mixing (elutriate) tests

3.1.1 Characterisation of tailings samples

Concentrations of metals in the solid and liquor fractions of Tailings 1 and Tailings 2 are presented in Table 16 (solids) and Table 17 (liquor) (Appendix A). Despite the two tailings samples representing two different stages of the mine's operation, concentrations of TRM and AEM in both tailings solids were relatively similar. For the two tailings solids, concentrations of TRM exceeded their respective SQGV and SQGV-high values for Cr, Cu, Ni and Zn. Analyses of TRM on two separate occasions on two separate sub-samples of both tailings solids confirmed the high concentration of these metals in the tailings solids (with As also exceeding the SQGV in Tailings 1 on one occasion). Concentrations of AEM exceeded the SQGV for Cu, Ni and Zn (but not Cr) with Zn also exceeding the SQGV-high. For Cu, AEM concentrations, and hence potentially bioavailable metals, accounted for 15% of the TRM Cu concentration in both tailings samples. AEM concentrations of Ni accounted for 13% and 14% of the TRM Ni concentration for Tailings 1 and Tailings 2 respectively. A higher fraction of Zn was in the AEM phase for Tailings 2 (83%) compared to Tailings 1 (60%).

The pH of the liquor from Tailings 1 and Tailings 2 was near neutral (7.4 and 7.2 respectively), but lower than natural seawater (~8.1-8.2). The conductivity of the two liquors (2,210 and 2,770 µS/cm) was also lower than natural seawater (~5,300 µS/cm).

For both tailings liquors, concentrations of Co, Cu and Zn exceeded their respective WQGVs for 95% species protection level. Australian and New Zealand WQGVs for Mn, Fe and Al in marine waters are currently under review. Based on the submitted GVs for Mn in marine systems, a WQGV of 510 µg/L is proposed for marine waters (when corals are absent, 95% species protection, Golding et al., 2016). Manganese concentrations in both tailings liquors would be expected to exceed these proposed WQGVs. However, Fe concentrations in the tailings would not be expected to exceed the proposed (but not yet accepted) WQGV of 120 µg/L (95% species protection, Golding et al., 2015a).

Comparison of dissolved (0.45 µm) metal concentration in Tailings 1 and Tailings 2 liquors to PNG water quality criteria (*Environment Act 2000*) indicate that Co, Mn and Cu (Tailings 1 only) exceed the reported criteria concentrations. The dissolved Co concentrations in both tailings samples exceed the analytical limit of detection (LOD, 0.1 µg Co/L)[1], while concentrations of Mn and Cu concentrations exceed the criteria concentration by a factor of 1.01 and 1.58 for Mn in Tailings 1 and 2 respectively, and, a factor of 2.3 for Cu in Tailings 1. The State of PNG does not provide criteria for sediments (solids).

[1] The PNG criteria concentration for cobalt (Co) was the analytical limit of detection (LOD), and in this study the limit of detection ranged from 0.01–4.0 µg Co/L. A value of 0.1 µg/L was chosen as a representative LOD for Co

Table 16. Tailings solids composition – metal, metalloid and sulfur concentrations in tailings solids (mg/kg dry weight)

Metals and Metalloids	Tailings 1					Tailings 2					SQVG[a]	
	TRM[b] #1	TRM #2	TRM Average	AEM[c]	% Acid Extractable[d]	TRM #1	TRM #2	TRM Average	AEM	% Acid Extractable	SQGV	SQGV-high
Ag	0.53	0.87	0.70	0.19	27	0.54	0.61	0.58	0.07	13	1	4.0
As	13	**27**	20	3.8	19	14	16	15	3.0	20	20	70
Cd	0.10	0.11	0.10	0.038	37	0.07	0.08	0.07	0.032	43	1.5	10
Co	16	23	19	2.3	12	17	19	18	1.8	9.8	–	–
Cr	**526**	**630**	**578**	58	10	**594**	**650**	**622**	75	12	80	370
Cu	**915**	**1570**	**1240**	**182**	15	**929**	**1050**	**990**	**149**	15	65	270
Fe	55,100	63,700	59,400	5070	8.5	52,200	56,400	54,300	4490	8.3	–	–
Hg	0.02	0.03	0.03	<0.01	<33	0.02	<0.02	0.02	<0.01	<50	0.15	1
Mn	300	366	333	99	30	296	308	302	107	36	–	–
Ni	**234**	**299**	**267**	**33**	13	**274**	**305**	**289**	**40**	14	21	52
Pb	6.1	10	8.0	2.9	37	7.0	7.6	7.3	3.5	48	50	220
Se	3.4	5.9	4.7	0.12	2.6	3.7	3.8	3.8	0.01	0.30	–	–
V	83	104	93	6.7	7.1	79	78	78	7.5	9.6	–	–
Zn	**472**	**840**	**656**	**392**	60	**493**	**552**	**522**	**432**	83	200	410
S	18,400	28,600	23,500	1460	6.2	18,900	20,800	19,850	276	1.4	–	–

[a] sediment quality guideline value (SQGV-high = sediment quality guideline value high), Simpson and Batley, 2016
[b] TRM = total recoverable metals; [c] AEM = dilute-acid extractable metals (1 M HCl) – better indication of bioavailability; [d] % Dilute-acid extractable metal = percentage of TRM (average) present as AEM
Bold values indicate concentration exceeds ANZECC/ARMCANZ (2000) and Simpson and Batley (2016) for sediments

Table 17. Tailings composition – dissolved (<0.45 µm) metal concentrations in tailings liquor

Parameter	Tailings 1	Tailings 2	(WQGV[a]	PNG Water Quality Criteria
			95% species protection	
pH	7.4	7.2	NA	Natural pH
Conductivity (µS/cm)	2210	2770	NA	NR
Alkalinity (mg/L $CaCO_3$)	103	76	NA	NR
Hardness (mg/L, $CaCO_3$)[b]	876	1420	NA	NR
Metals and metalloids (dissolved (<0.45 µm) µg/L)				
Al	NM	NM	24[a]	NR
Ag	0.07	0.14	1.4	NR
As	0.4	0.9	ID	50
Cd	0.1	0.2	5.5	1
Co	**3.4**	**4.4**	1	LoD (0.1)
Cr	1.7	1.0	4.4	10 (as hexavalent)
Cu	**69**	**19**	1.3	30
Fe	2.5	1.4	ID	1000 (in solution)
Mn	2020	3160	ID	2000 (in solution)
Ni	34	62	70	1000
Pb	1.1	1.1	4.4	4
Se	1.5	8.3	ID	10
V	<1	1	100	NR
Zn	**145**	**287**	15	5000
Major ions (dissolved (<0.45 µm, mg/L)				
Ca	265	422	NA	NR
K	54	67	NA	450
Mg	52	88	NA	NR
Na	125	102	NA	NR
S	295	494	NA	NR
Sulphate (SO_4)[c]	801	1341	NA	NR

[a] Water Quality Guideline Value (WQGV) for 95% species protection level, shaded values indicate applicability for slightly-to-moderately disturbed ecosystems, red values indicate concentrations exceed PNG water quality criteria (Environment Act 2000); Aluminium GVs from Golding et al (2015b); [b] Water hardness calculated from concentration of calcium and magnesium; [c] Sulfate concentrations calculated from sulfur concentration (i.e. assuming all S is in the form of SO_4); LoD = Limit of detectability, NM = Not measured; ID = insufficient data; NA = not applicable; unreliable data reported; NR = Not reported; Bold values indicate concentration exceeds ANZECC/ARMCANZ (2000)

3.1.2 Elutriate (mixing) tests with tailings

Upon entering the marine environment, the tailings will mix with seawater and contaminants in tailings will have the potential to disperse and desorb from the particulate material. To assess the mobilisation of metals from the particulates, a series of elutriate tests were undertaken.

The ratios of tailings in seawater (v/v) investigated were 1 in 10, 1 in 100 and 1 in 1000 with dissolved (0.45 µm) metals measured in the elutriates after a 16 h (Table 18, Appendix A) mixing time at 30 ± 1°C. The concentrations of dissolved metals measured in the elutriates were higher than expected; therefore, additional elutriate tests were undertaken using a 1 in 10,000 to establish the dilution required for dissolved metal concentrations to remain below WQGVs.

For Tailings 1 and Tailings 2, dissolved concentrations of Co, Cu and Zn exceeded their respective WQGVs at 1 in 10 and 1 in 100 dilutions with seawater. Dissolved Ni concentrations exceeded the WQGV for Tailings 2, 1 in 10 dilution only. Dissolved Cu concentrations exceeded the WQGV of 1.3 µg/L in all dilutions up to and including 1 in 10,000 dilutions. At 1 in 50,000 dilutions all dissolved metals concentrations were below their respective guideline values. There was a lack of a linear relationship between tailings dilutions and dissolved metal concentrations measured in the elutriates and this was clearly demonstrated for Cu. For example, for Tailings 2, a 1 in 10 dilution with a dissolved Cu concentration of 70 µg/L decreased by a factor of only 23 when compared to the dissolved Cu concentration of 3.1 µg/L in the 1 in 10,000 dilution (i.e. a 100 times more dilute sample decreased Cu concentrations by only a factor of 23).

Upon comparison to PNG *Environment Act 2000* water quality criteria, concentrations of Cu exceeded the criteria concentrations and this was only in the 1 in 10 dilution. Unlike for the ANZECC/ARMCANZ (2000) WQGVs, the PNG criteria concentration for Co is the analytical limit of detection (LOD), and in this study the limit of detection ranged from 0.01–4.0 µg Co/L. A value of 0.1 µg/L was chosen as a representative LOD for Co, to be used as the PNG water quality criterion. Concentrations of Co were greater than 0.1 µg/L in 1 in 10, 1 in 100 and 1 in 1000 tailings dilutions. Hence, for Tailing 1 and Tailings 2, a dilution of 1 in 10,000 was sufficient to meet the *Environment Act 2000* criteria.

3.1.3 Effect of mixing time on metal release

To provide information on whether the dissolved metals released from the tailings to seawater are likely to continue indefinitely or diminish with time, tests were undertaken on a tailings in seawater ratio of 1 in 10 with dissolved (<0.45 µm) metal concentrations measured after 0, 10 min, 1, 6, 8, 24, 48 and 72 h (Table 19, Appendix A). The results (Figure 3 and Table 19) indicated a two stage metal release process for Cu, Co, Ni and Zn with an initial rapid release of metals into solution over the first one to five hours followed by a much slower metals release phase. Equilibrium metal concentrations (no further increase in dissolved metal concentrations) were typically achieved after 20 hours of mixing.

Concentrations of dissolved Co, Cu and Zn exceeded WQGVs for 95% species protection at all time points up to 72 h with dissolved concentrations of each metal increasing over 72 h by more than a factor of 10 (Table 19). Dissolved Co concentrations increased from 1.1 to 14 µg/L (Tailings 1) and 1.5 to 18 µg/L (Tailings 2). Dissolved Cu concentrations increased from 4.7 to 61 µg/L (Tailings 1) and 3.6 to 81 µg/L (Tailings 2) while dissolved Zn concentrations increased from 27 to 262 µg/L

and 45 to 818 µg/L reaching a maximum concentration at the 48 h time point. Dissolved Ni concentrations exceeded the WQGV after 48 h for Tailings 1 and after 6 h for Tailings 2. Dissolved Cd concentrations also increased over time however these remained below the WQGV. Dissolved Mn concentrations also increased over time by about a factor of 2 and remained above the proposed WQGV of 750 µg/L (when corals are not present). The exception was for Tailings 1 with mixing times of 0 and 10 minutes with 694 and 747 µg Mn/L respectively. Trends for Al, As, Cr and V were difficult to interpret because concentrations of these dissolved metals were the same or higher in the seawater blanks compared to the diluted tailings treatments. Dissolved concentrations of Fe and Pb remained consistent with concentrations similar to, or below, the detection limit for each control and tailings treatment.

Comparison to PNG *Environment Act 2000* water quality criteria showed that dissolved concentrations of Cd, Co, Cu and Se were exceeded in the 1 in 10 tailings in seawater dilution at ≥24 h for Co (Tailings 1 and 2), ≥24 h for Cd (Tailings 1 only), ≥6 h for Cu (Tailings 1 and 2) and at 72 h for Se (Tailings 2 only). Note that ANZECC/ARMCANZ (2000) does not provide a WQGV for Se and the *Environment Act 2000* quotes a lower criteria for Cd (1 µg/L) than ANZECC/ARMCANZ (2000) WQGV for receiving waters (5.5 µg/L).

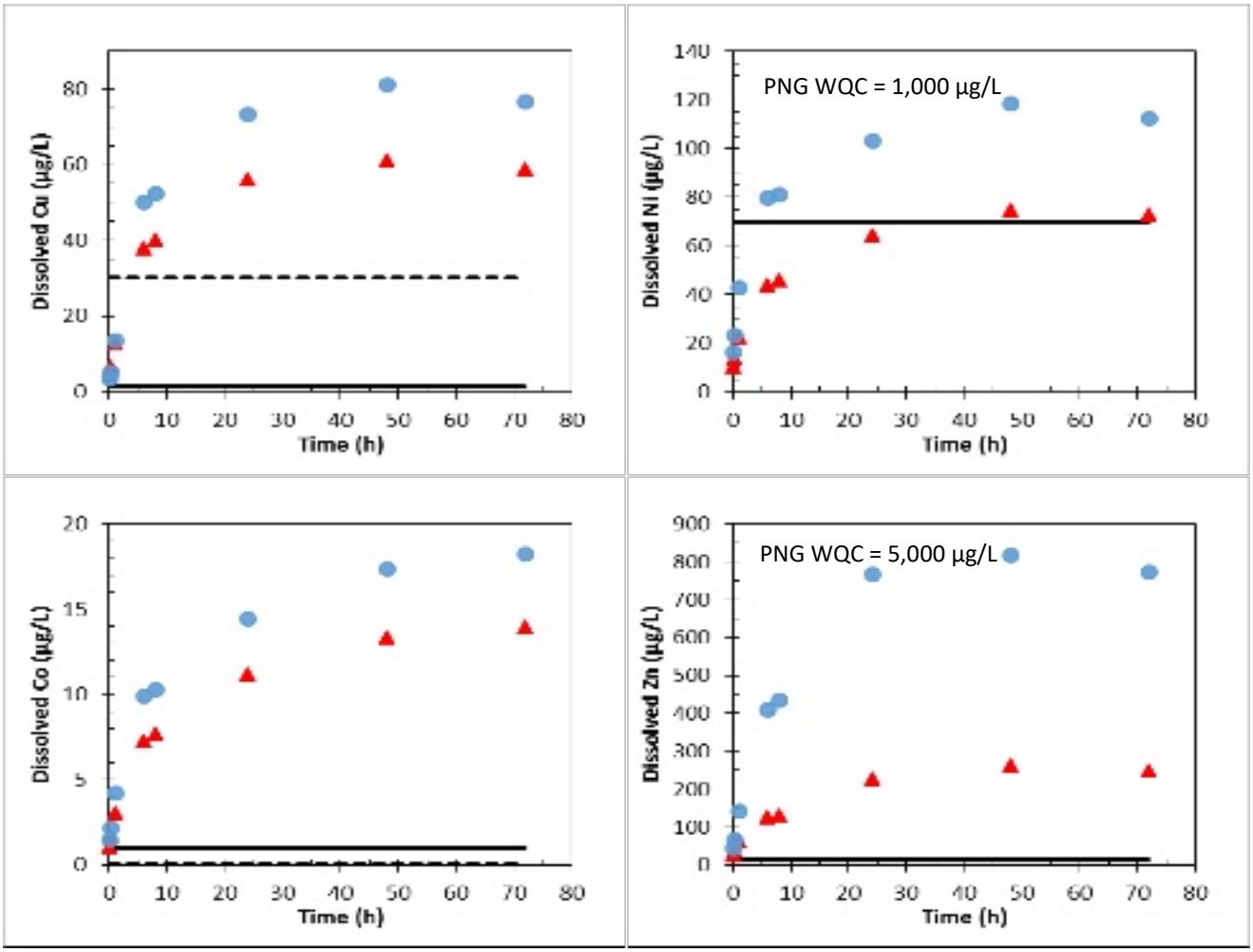


Figure 3. Concentration of dissolved (0.45 µm) copper, cobalt, nickel and zinc in elutriate (mixing) tests. Tailings dilution in elutriate test was 1 part tailings material and 9 parts natural filtered (0.45 µm) (i.e. 1 in 10 dilution). The solid line represents the ANZECC/ARMCANZ (2000) water quality Guideline Value (WQGV). The dash line represents the PNG Water Quality Criteria (State of PNG, *Environment Act 2000*)

Table 18. Chemical analysis of tailings elutriates after a 16 h mixing time (30°C)

Tailings:seawater ratio	Dissolved metal concentration (µg/L)[b]															pH[b]
	Ag	Al	As	Cd	Co	Cr	Cu	Fe	Mn	Mo	Ni	Pb	Se	V	Zn	
Blank 1 (seawater)[a]	1.0	1	3	<1	<4	0.3	<1	0.3	0.3	11	1	4	NM	NM	1	8.07
Blank 2 (seawater)[a]	0.017	3	1.9	0.007	<0.01	0.30	0.26	0.5	0.4	NM	0.18	0.02	0.66	2.3	0.45	8.07
Blank 3 (seawater)[a]	NM	5	2.0	<0.01	<0.01	0.36	0.30	<1	0.8	NM	0.20	0.03	0.20	2.4	0.7	8.07
Blank 4 (seawater)[a]	0.008	2	1.7	<0.01	<0.01	0.21	0.6	<2	0.5	NM	0.18	<0.03	0.33	2.0	<1	8.02
Tailings 1																
1 in 10	1.3	5	2	1	**11**	<0.2	**57**	0.8	1146	35	62	4	NM	NM	**243**	7.34
1 in 100	0.040	10	0.72	0.11	**1.5**	0.30	**19**	0.8	96	NM	8.5	0.01	0.97	0.29	**24**	7.92
1 in 1000	0.022	9	1.5	0.019	0.19	0.21	**11**	0.8	11	NM	1.1	<0.01	0.48	1.3	8.7	8.07
1 in 10,000	NM	6	2.0	<0.01	0.02	0.29	**3.1**	1	1.8	NM	0.30	<0.01	0.15	2.3	1.8	8.03
1 in 50,000	0.005	2	1.7	0.009	<0.01	0.18	0.8	<2	0.6	NM	0.20	<0.03	0.19	2.0	1	8.03
Tailings 2																
1 in 10	1.2	4	3	1	**12**	2.4	**70**	0.5	778	40	**91**	4	NM	NM	**634**	7.32
1 in 100	0.033	9	0.76	0.081	**1.5**	0.25	**20**	0.7	76	NM	11	<0.01	1.1	0.33	**43**	7.98
1 in 1000	0.020	7	1.6	0.013	0.17	0.14	**11**	1.1	8	NM	1.4	0.02	0.49	1.5	12	8.07
1 in 10,000	NM	5	1.9	<0.01	0.02	0.25	**3.1**	<1	1.7	NM	0.36	<0.01	0.14	2.3	2.4	8.04
1 in 50,000	0.007	2	1.7	0.009	<0.01	0.16	0.9	<2	0.6	NM	0.19	<0.03	0.28	1.9	<1	8.04
ANZECC 95% GV[c]	1.4	24[e]	ID	5.5	1	4.4[d]	1.3	ID	ID	ID	70	4.4	ID	100	15	NA
PNG WQC	50	NR	50	1	LOD (0.1)	10	30	1000 (in solution)	2000 (in solution)	NR	1000	4	10	NR	5000	Natural pH

[a] The elutriate tests were carried out over four separate experiments. Blank 1 and 1 in 10 treatment; Blank 2 and 1 in 100, 1 in 1,000 treatments; Blank 3 and 1 in 10,000 treatment, Blank 4 1 in 50,000 treatment.
[b] mean of triplicate treatments
[c] ANZECC/ARMCANZ (2000) Water Quality Guideline Value (WQGV) to protect 95% of species. Shaded GVs are applied to slightly-to-moderately disturbed systems.
[d] WQGV are for Cr(VI), the most toxic form; WQGV for Cr(III) are 27.4 µg/L for 95% species protection
[e] Golding et al., (2015b)
NM = Not measured; ID = insufficient data; NA = not applicable; unreliable data reported; NR = Not reported
Bold values indicate concentrations greater than the 95% species protection value.
Red values indicate concentrations greater than the PNG WQC (*Environment Act 2000*).

Table 19. Effect of mixing time (1 in 10 tailings dilution, 30°C)

Tailings:seawater ratio	Time (h)	Dissolved metal concentration (µg/L)[a]													pH[a]
		Al	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Se	V	Zn	
Blank (seawater)	0	4	2.0	<0.01	<0.01	0.21	0.32	<1	0.75	0.19	0.02	0.18	2.3	0.5	8.04
	10 min	5	2.0	<0.01	<0.01	0.17	0.39	2	0.76	0.19	0.02	0.18	2.3	0.4	8.07
	1	11	1.8	<0.01	<0.01	0.15	0.36	<1	0.76	0.19	0.02	0.17	2.2	0.5	8.12
	6	4	1.8	<0.01	<0.01	0.24	0.23	<1	0.75	0.16	<0.01	0.25	2.2	0.1	8.02
	8	4	1.9	<0.01	<0.01	0.24	0.24	<1	0.69	0.23	<0.01	0.30	2.3	0.2	8.03
	24	4	1.8	0.01	<0.01	0.23	0.22	1	0.74	0.24	<0.01	0.41	2.2	0.2	8.05
	48	4	1.9	<0.01	<0.01	0.20	0.24	<1	0.70	0.19	<0.01	0.47	2.2	0.2	8.07
	72	4	1.8	<0.01	<0.01	0.18	0.22	<1	0.67	1.4	<0.01	1.1	2.4	0.1	8.11
Tailings 1	0	5	0.93	0.18	**1.1**	0.15	**4.7**	<1	694	11	<0.01	0.45	0.54	**27**	7.77
	10 min	6	0.86	0.22	**1.6**	0.13	**6.7**	1	747	14	<0.01	0.61	0.42	**36**	7.77
	1	5	0.80	0.43	**3.0**	0.15	**13**	<1	832	22	<0.01	1.1	0.43	**61**	7.74
	6	6	0.66	0.90	**7.3**	0.30	**38**	1	1020	44	<0.01	1.8	0.24	**127**	7.58
	8	5	0.66	0.93	**7.7**	0.27	**40**	<1	903	46	<0.01	2.1	0.25	**132**	7.54
	24	3	0.58	1.1	**11**	0.09	**56**	<1	997	64	<0.01	2.8	0.20	**226**	7.22
	48	2	0.49	1.2	**13**	0.06	**61**	1	1280	**74**	<0.01	3.4	0.26	**262**	7.24
	72	2	0.50	1.3	**14**	0.05	**59**	<1	1450	**73**	<0.01	5.5	0.44	**249**	7.10
Tailings 2	0	6	1.0	0.02	**1.5**	0.11	**3.6**	<1	764	16	<0.01	0.52	0.62	**45**	7.79
	10 min	6	0.97	0.07	**2.2**	0.13	**5.3**	<1	858	23	<0.01	0.79	0.57	**66**	7.78
	1	5	0.90	0.20	**4.2**	0.26	**14**	<1	901	43	<0.01	1.9	0.63	**143**	7.71
	6	5	0.74	0.54	**9.9**	0.43	**50**	<1	869	**80**	<0.01	2.5	0.29	**412**	7.55
	8	5	0.71	0.58	**10**	0.32	**53**	<1	874	**81**	<0.01	2.8	0.29	**435**	7.48
	24	3	0.52	0.86	**14**	0.06	**73**	<1	979	**103**	<0.01	3.6	0.22	**769**	7.16
	48	2	0.50	0.92	**17**	0.14	**81**	<1	1220	**119**	<0.01	4.0	0.35	**818**	7.14
	72	2	0.51	0.97	**18**	0.03	**77**	<1	1420	**112**	<0.01	12	0.35	**775**	6.93
ANZECC 95% TV[b]		24[d]	ID	5.5	1	4.4[c]	1.3	ID	ID	70	4.4	ID	100	15	NA
PNG WQC		NR	50	1	LOD (0.1)	10	30	1000 (in solution)	2000 (in solution)	1000	4	10	NR	5000	Natural pH

[a] mean of triplicate treatments; [b] ANZECC/ARMCANZ (2000) Water Quality Guideline Value (WQGV) to protect 95% of species. Shaded GVs are applied to slightly-to-moderately disturbed systems.; [c] WQGV is Cr(VI), the most toxic form; WQGV for (III) is 27.4 µg/L for 95% species protection; [d] Golding et al., (2015b)

NM = Not measured; ID = insufficient data; NA = not applicable; unreliable data reported; NR = Not reported

Bold values indicate concentrations greater than the 95% species protection value

Red values indicate concentrations greater than the PNG WQC (*Environment Act 2000*).

3.2 Ecotoxicological assessment of tailings liquid

3.2.1 Preparation of tailings liquid and interpretation of results

The tailings will be mixed with seawater in the mix/de-aeration tank prior to discharge via the DSTP outfall. Therefore, in this study, the toxicity testing on tailings liquors were carried out on diluted tailings to simulate the final tailings liquor composition of water discharged from the mix/de-aeration tank. A tailings dilution of 1 in 4 (that is, 1 part tailings to 3 parts seawater, by mass) was utilised in this study. It is now known that the mix/de-aeration tank will incorporate a 1 in 5 (1 part tailings to 4 parts seawater, by volume) dilution. This is equivalent to a 1 in 4.3 dilution (m/m) for Tailings 1 and 1 in 4.4 dilution (m/m) for Tailings 2. Considering the remobilisation of metals identified in the elutriate (mixing tests) and supporting information on continued metal release from tailings solids (Section 3.1.2 and 3.1.3), there are a few assumptions and limitations that should be considered when interpreting the results presented here that describe the toxicity of the diluted tailings liquors to marine organisms. These assumptions and limitations are;

I. The time frame of pre-discharge dilution mixing. A mixing time of 1 h was adopted in this study; however, results from the kinetic investigation of metals released over 72 h show that metals continue to be released from the tailings particles over time (Section 3.1.3) and on further dilution. Current advice is that the residence time in the mix/deaeration tank will be considerably shorter than 1 h, therefore most of the metal release from tailings will occur in the receiving ocean waters.

II. The 1 in 4 diluted tailings (m/m) material was filtered to 0.45 µm. Removal of the solid particulate phase from the liquor eliminated any ongoing continued release (or re-adsorption) of metals onto the particulate phase (Section 3.2.2).

Hence, the dilution, time frame of mixing and filtration of the tailings liquor prior to testing influences the composition of the tailings liquor, and hence toxicity of the tailings liquor to aquatic organisms. This is further discussed in Section 3.2.6 in relation to liquors of Tailings 1 and Tailings 2 tested in this study.

3.2.2 Metal concentrations in tailings liquor samples used in toxicity tests

Concentrations of dissolved metals in the original undiluted tailings liquors were compared to concentrations of dissolved metals in the diluted (1 in 4, m/m) liquor (Table 20 and Table 21). Concentrations of dissolved metals did not decrease as predicted by serial dilution of tailings with seawater (1 in 4, m/m). This was in agreement to that observed in elutriate (mixing tests) where the dissolved metal concentration was influenced by the dilution factor and release of metals from the tailings particulate phase (Section 3.1.32 and 3.1.3). For both Tailings 1 and Tailings 2, concentrations of dissolved Co, Fe, Ni and Zn were higher in the diluted (1 in 4, m/m) tailings compared to the original undiluted tailings (Table 20 Table 21). Copper concentrations were lower in diluted Tailings 1 (by 81% from 69 µg/L to 13 µg/L) and remained similar in Tailings 2 (18–19 µg/L). Concentrations of Mn were lower in both Tailings 1 and Tailings 2 by 26% and 48% respectively compared to the original undiluted Tailings 1 and Tailings 2.

Comparison of the diluted (1 in 4) tailings liquor to WQGVs shows that concentrations of Co, Cu and Zn exceeded their respective WQGVs for both Tailings 1 and Tailings 2. Concentrations of Ni only exceeded the WQGV for Tailings 2. Concentrations of Mn exceeded the proposed WQGV of 750 µg/L (when corals are absent).

Comparison of the diluted (1 in 4, m/m) tailings liquor to PNG WQC (*Environment Act 2000*) shows that only Co exceeds the WQC in Tailings 1 and Tailings 2.

The 1 in 4 (m/m) diluted and filtered tailings liquor was the highest concentration of liquor tested in the toxicity tests and represented as 100% tailings liquor. Each liquor (Tailings 1 and Tailings 2) was further diluted with seawater (0.45 µm filtered) using a dilution factor of 2 or 3 to obtain a range of tailings liquor concentrations for use in each toxicity test. For both liquors, concentrations of Cu, Mn, Mo, Ni and Zn were generally above the analytical detection limit (1 µg/L). Concentrations of these metals at the start and end of each toxicity test for both Tailings 1 and Tailings 2 followed a linear serial dilution relationship (Appendix B). This was most evident in Tailings 2 for dissolved Mn concentrations (r^2 = 0.9998) followed by Mo (r^2= 0.9964), Ni (r^2 = 0.9978), Cu (r^2 = 0.9853) and Zn (r^2 = 0.9842) (Figure 4). Metal concentrations in test solutions can also decrease throughout the duration of a toxicity test due to adsorption to the test container and adsorption and/or uptake by biota. This was particularly the case for Cu and Zn which are essential nutrients for organisms. The duration of toxicity tests varied from 48 h to 8 d. For Cu and Zn, the relationships improved slightly when only the initial measurements were plotted (Cu r^2 = 0.9925; Zn r^2 = 0.995). Tailings 1 behaved in the same manner as described for Tailings 2 (r^2 values ranging from 0.9996 to 0.9518, Appendix B). Removal of solid particulate matter from the tailings liquor (to less than 0.45 µm) stabilised the metal concentrations in the liquor over time (up to 8 days). This was in contrast to that observed in elutriate test with tailings solids were present over time (Section 3.1.3).

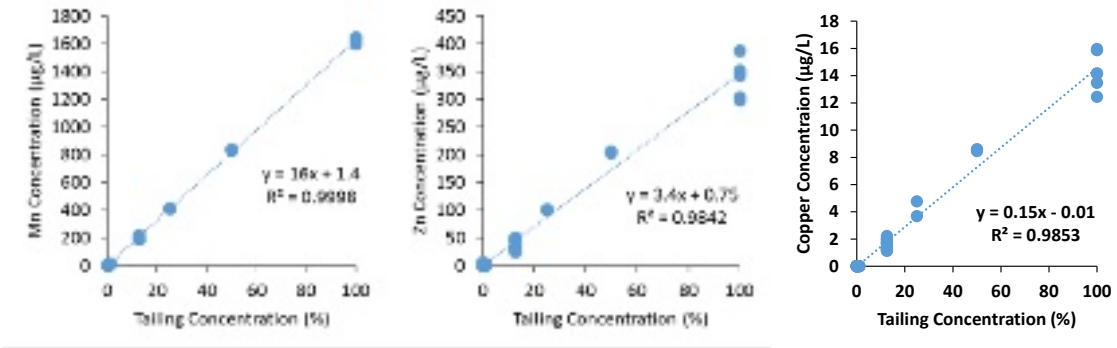


Figure 4. Relationship between Tailings liquor 2 concentration and dissolved (0.45 µm) concentrations of (A) manganese and (B) zinc in all toxicity tests (two tailings concentrations measured per toxicity test). Data points include measured concentrations at the start and end of each toxicity test.

Table 20. Dissolved (<0.45 µm) metal concentrations in Tailings 1 liquor and control (seawater) treatments in toxicity tests

Toxicity Test	Sample	Tailings Treatment (%)	Time Point (day)	Dissolved metal concentration (µg/L)									
				Al	Cd	Co	Cr	Cu	Fe	Mn	Mo	Ni	Zn
Original Tailings 1 liquor		Undiluted	NA	NM	0.1	**3.4**	1.7	**69**	2.5	2020	NM	34	**145**
Diluted Tailings 1 liquor (1 in 4, m/m)		100%	Start of testwork	2.3	<1	**4.0**	<1	**13**	4.0	1485	34	42	**158**
		100%	End of testwork	3.6	<1	<1	<1	**12**	<1	1428	31	42	**150**
I. galbana	Control	0	0	<1	<1	<1	<1	<1	<1	<0.1	2.2	<1	<1
			3 (end)	<1	<1	<1	<1	<1	<1	<0.1	1.1	<1	<1
	Tailings 1	12.5	0	<1	<1	<1	<1	**1.5**	<1	173	4.8	5.0	14
			3 (end)	1.2	<1	<1	<1	**1.6**	<1	174	5.5	5.4	11
		100	0	<1	<1	**3.2**	<1	**13**	1.3	1436	34	48	**139**
			3 (end)	<1	<1	**3.1**	<1	**10**	1.6	1451	35	50	**109**
N. closterium	Control	0	0	<1	<1	<1	<1	<1	<1	<0.1	1.2	<1	<1
			3 (end)	<1	<1	<1	<1	<1	<1	<0.1	<1	<1	<1
	Tailings 1	12.5	0	<1	<1	<1	<1	**1.2**	<1	175	5.5	5.4	10
			3 (end)	<1	<1	<1	<1	**1.1**	<1	176	5.3	4.0	7.4
		100	0	<1	1.0	**3.4**	<1	**12**	1.1	1454	35	43	**138**
			3 (end)	<1	<1	**3.4**	<1	**10**	<1	1450	34	43	**106**
H. tuberculata	Control	0	0	<1	<1	<1	<1	<1	1.6	<0.1	<2	<1	<2
			3 (end)	17	<1	<1	<1	<1	<1	<0.1	<2	<1	6.9
	Tailings 1	12.5	0	<1	<1	<1	<1	**1.4**	<1	175	3.5	4.6	**16**
			3 (end)	14	<1	<1	<1	<1	<1	175	3.7	4.0	**20**
		100	0	<1	<1	**3.9**	<1	**10**	<1	1448	33	42	**139**
			3 (end)	6.0	<1	**3.3**	<1	**9.1**	<1	1455	34	44	**153**
A. sinjiensis	Control	0	0	<1	<1	<1	<1	<1	<1	<0.1	<2	<1	<1
			2 (end)	<1	<1	<1	<1	<1	<1	<0.1	<2	<1	<1
			2 (renewal)	<1	<1	<1	<1	<1	<1	<0.1	<2	1.8	1.3
			3 (end)	<1	<1	<1	<1	<1	<1	<0.1	<2	1.3	<1
	Tailings 1	6.7	0	12	<1	<1	<1	<1	1.0	94	<2	2.8	3.4
			2 (end)	10	<1	<1	<1	<1	<1	92	<2	2.6	1.1
			2 (renewal)	<1	<1	<1	<1	<1	<1	89	<2	2.7	3.7
			3 (end)	3.9	<1	<1	<1	<1	<1	90	<2	2.5	2.8

Toxicity Test	Sample	Tailings Treatment (%)	Time Point (day)	Dissolved metal concentration (µg/L)									
				Al	Cd	Co	Cr	Cu	Fe	Mn	Mo	Ni	Zn
		20	0	<1	<1	<1	1.7	1.1	5.7	287	5.7	9.0	**30**
			2 (end)	<1	<1	<1	<1	<1	<1	287	5.4	8.1	**20**
			2 (renewal)	<1	<1	<1	<1	<1	<1	277	5.1	8.7	**26**
			3 (end)	<1	<1	<1	<1	<1	<1	281	6.0	9.0	**23**
S. lalandi	Control	0	0	<1	<1	<1	<1	<1	<1	9.4	<2	<1	<2
			7 (end)	<1	<1	<1	<1	<1	<1	9.3	<2	<1	2.7
	Tailings 1	12.5	0	<1	<1	<1	<1	1.0	<1	189	4.6	6.6	**34**
			7 (end)	<1	<1	<1	<1	1.0	<1	191	3.6	6.2	**17**
		50	0	1.0	<1	<1	<1	**4.8**	<1	753	16	24	**82**
			7 (end)	<1	<1	<1	<1	**6.0**	<1	756	17	24	**82**
E. mathaei	Control	0	0	<1	<1	**1.2**	<1	<1	<1	6.2	<2	<1	<2
			0	<1	<1	<1	<1	<1	<1	5.9	<2	<1	<2
			3 (end)	<1	<1	<1	<1	<1	<1	5.7	<2	<1	<2
	Tailings 1	12.5	0	<1	<1	<1	<1	**1.9**	<1	189	2.1	6.3	**23**
			3 (end)	<1	<1	<1	<1	1.3	<1	189	3.6	<1	**22**
		50	0	<1	<1	<1	<1	**6.5**	<1	739	15	24	**82**
			3 (end)	<1	<1	**2.3**	<1	**6.2**	<1	760	15	23	**80**
ANZECC 95% TV[a]				24[c]	5.5	1	4.4[b]	1.3	ID	ID	ID	70	15
PNG WQC				NR	1	LOD (0.1)	10	30	1000	2000	NR	1000	5000

[a] ANZECC/ARMCANZ (2000) Water Quality Guideline Value (WQGV) to protect 95% of species. Shaded GVs are applied to slightly-to-moderately disturbed systems.
[b] WQGV is for Cr(VI), the most toxic form; WQGV for Cr(III) is 27.4 µg/L for 95% species protection
[c] Golding et al., (2015b)
NM = Not measured; ID = insufficient data; NA = not applicable; unreliable data reported; NR = Not reported
Bold values indicate concentrations greater than the 95% species protection value.
Red values indicate concentrations greater than the PNG WQC (*Environment Act 2000*)

Table 21. Dissolved (<0.45 µm) metal concentrations in Tailings 2 and control (seawater) treatments in toxicity tests

Toxicity Test	Sample	Tailings Treatment (%)	Time Point (day)	Dissolved metal concentration (µg/L)									
				Al	Cd	Co	Cr	Cu	Fe	Mn	Mo	Ni	Zn
Original Tailings 2 liquor		Undiluted	NA	NM	0.2	**4.4**	1.0	**19**	1.4	3160	NM	62	**287**
Diluted Tailings 2 liquor (1 in 4, m/m)		100%	Start of testwork	2.6	<1	**6.6**	<1	**18**	4.4	1650	44	**90**	**393**
		100%	End of testwork	2.1	11	**11**	<1	**16**	2.0	1621	42	**92**	**382**
I. galbana	Control	0	0	<1	<1	<1	<1	<1	<1	<0.1	2.2	<1	<1
			3 (end)	<1	<1	<1	<1	<1	<1	<0.1	1.1	<1	<1
	Tailings 2	12.5	0	<1	<1	<1	<1	**2.0**	<1	193	5.6	11	**33**
			3 (end)	1.1	<1	<1	<1	**1.4**	<1	195	6.2	11	**27**
		100	0	<1	<1	**5.9**	<1	**16**	<1	1606	43	**90**	**350**
			3 (end)	<1	<1	**5.8**	<1	**14**	2.3	1623	45	**91**	**299**
N. closterium	Control	0	0	<1	<1	<1	<1	<1	<1	<0.1	1.2	<1	<1
			3 (end)	<1	<1	<1	<1	<1	<1	<0.1	<1	<1	<1
	Tailings 2	12.5	0	<1	<1	<1	<1	**2.2**	<1	196	5.7	11	**33**
			3 (end)	<1	<1	<1	<1	**1.7**	<1	197	6.9	10	**26**
		100	0	<1	<1	**5.4**	<1	**16**	2.1	1620	44	**91**	**353**
			3 (end)	<1	<1	**6.0**	<1	**13**	1.3	1629	44	**92**	**304**
H. tuberculata	Control	0	0	<1	<1	<1	<1	<1	1.6	<0.1	<2	<1	<2
			3 (end)	17	<1	<1	<1	<1	<1	<0.1	<2	<1	6.9
	Tailings 2	12.5	0	<1	<1	<1	<1	1.1	<1	193	5.0	12	**34**
			3 (end)	16	<1	<1	<1	1.2	<1	196	3.6	11	**45**
		100	0	3.8	<1	**7.9**	<1	**14**	<1	1632	43	**95**	**344**
			3 (end)	9.1	<1	**6.2**	<1	**12**	<1	1649	42	**95**	**388**
A. sinjiensis	Control	0	0	<1	<1	<1	<1	<1	<1	<0.1	<2	<1	<1
			2 (end)	<1	<1	<1	<1	<1	<1	<0.1	<2	<1	<1
			2 (renewal)	<1	<1	<1	<1	<1	<1	<0.1	<2	1.8	1.3
			3 (end)	<1	<1	<1	<1	<1	<1	<0.1	<2	1.3	<1
	Tailings 2	0.33	0	<1	<1	<1	<1	<1	<1	4.5	<2	<1	<1
			2 (end)	<1	<1	<1	<1	<1	<1	4.2	<2	<1	<1
			2 (renewal)	7.2	<1	<1	<1	<1	<1	4.6	<2	1.3	<1
			3 (end)	<1	<1	<1	<1	<1	<1	4.5	<2	<1	<1

Toxicity Test	Sample	Tailings Treatment (%)	Time Point (day)	Dissolved metal concentration (µg/L)									
				Al	Cd	Co	Cr	Cu	Fe	Mn	Mo	Ni	Zn
		1.0	0	<1	<1	<1	<1	<1	<1	15	<2	1.0	<1
			2 (end)	<1	<1	<1	<1	<1	<1	15	<2	1.9	<1
			2 (renewal)	<1	<1	<1	<1	<1	<1	16	<2	1.0	3.0
			3 (end)	<1	<1	<1	<1	<1	<1	15	<2	1.2	1.5
S. lalandi	Control	0	0	<1	<1	<1	<1	<1	<1	9.4	<2	<1	<2
			7 (end)	<1	<1	<1	<1	<1	<1	9.3	<2	<1	2.7
	Tailings 2	12.5	0	<1	<1	<1	<1	**1.6**	<1	212	4.4	12	**50**
			7 (end)	<1	<1	<1	<1	1.2	<1	213	4.5	13	**51**
		50	0	<1	<1	**2.8**	<1	**8.6**	<1	834	22	52	**206**
			7 (end)	1.8	<1	**2.1**	<1	**8.5**	<1	839	21	51	**205**
E. mathaei	Control	0	0	<1	<1	**1.2**	<1	<1	<1	6.2	<2	<1	<2
			0	<1	<1	<1	<1	<1	<1	5.9	<2	<1	<2
			3 (end)	<1	<1	<1	<1	<1	<1	5.7	<2	<1	<2
	Tailings 2	12.5	0	<1	<1	<1	<1	**2.0**	<1	209	3.1	12	**49**
			3 (end)	<1	<1	<1	<1	**1.8**	<1	215	3.6	13	**50**
		25	0	<1	<1	**2.0**	<1	**4.8**	<1	413	9.6	25	**102**
			3 (end)	<1	<1	<1	<1	**3.7**	<1	415	9.0	25	**101**
ANZECC 95% TV[c]				24[c]	5.5	1	4.4[b]	1.3	ID	ID	ID	70	15
PNG WQC				NR	1	LOD (0.1)	10	30	1000	2000	NR	1000	5000

[a] ANZECC/ARMCANZ (2000) Water Quality Guideline Value (WQGV) to protect 95% of species. Shaded GVs are applied to slightly-to-moderately disturbed systems.
[b] WQGV is for Cr(VI), the most toxic form; WQGV for Cr(III) is 27.4 µg/L for 95% species protection
[c] Golding et al., (2015b)
NM = Not measured; ID = insufficient data; NA = not applicable; unreliable data reported; NR = Not reported
Bold values indicate concentrations greater than the 95% species protection value.
Red values indicate concentrations greater than the PNG WQC (*Environment Act 2000*).

3.2.3 Aquatic toxicity test quality assurance and quality control criteria

All of the toxicity tests meet their respective quality assurance criteria (Table 22) indicating satisfactory performance.

Table 22. Quality assurance criteria[a] for definitive toxicity tests carried out on the tailings liquor

Toxicity Test	Reference Toxicant (positive control)			Control Treatments (negative control)	
	Toxicant	EC50 (or IC50)			
		Acceptability Criteria	Result	Acceptability Criteria	Result
72-h Microalgal growth inhibition (*N. closterium*)	Copper	3.3 ± 0.9 µg/L	3.9 µg/L	2.1 ± 0.3 doublings/day; Control CV<20%	1.9 doublings/day; CV: 2.4%
72-h Microalgal growth inhibition (*I. galbana*)	Copper	4.5 ± 2.6 µg/L	3.7 µg/L	2.3 ± 0.3 doublings/day; Control CV<20%	2.1 doublings/day; CV: 1.4%
80-h Copepod larval development (*A. sinjiensis*)	Nickel	8.6 ± 1.6 µg/L	9.2 µg/L	>50% larval development ratio Control CV<20%	70% larval development ratio CV: 15%
72-h Sea urchin larval development (*E. mathaei*)	Copper	12–32 µg/L	32 µg/L	>70% normal development	99% normal development
72-h Sea urchin larval development (*H. tuberculata*)	Copper	14 ± 9 µg/L	6.6 µg/L	≥70% normal larvae	98% normal larvae
48-h Oyster larval development (*S. echinata*)	Copper	12–17 µg/L	15 µg/L	≥70% normal larvae	77% normal larvae
8-d Anemone development (*A. pulchella*)	Copper	15–54 µg/L	15 µg/L	≥90% normal development (pedal lacerates)	90% normal development
8-d Fish embryo development (*Seriola lalandi*)	Copper	29–75 µg/L	63 µg/L	>70% normal development	94% normal development

[a] all values rounded to 2 significant figures
CV = coefficient of variation

3.2.4 Toxicity of diluted tailings liquor

Chronic toxicity of the diluted tailings liquor to marine organisms was assessed using eight toxicity tests and the results are summarised in Table 23 (Appendix C) with concentration-response curves for each toxicity test presented in Figure 5. The concentrations of diluted tailings liquor tested focused on the concentrations that where low biological effects and were expected to improve the accuracy of calculating EC/IC10 values which are subsequently used in SSDs to derive 'safe' dilutions (Section 3.2.6).

Toxicity tests with microalgae (population growth rate), sea urchins (larval development), oysters (larval development) sea anemone (development) and fish (embryo development) were of relatively similar sensitivity with EC/IC50 values of 28 to >100% and EC/IC10 values of 9.4-83% for Tailings 1 and EC50 values of 14-84% and EC10 values of 3.9-69% for Tailings 2. The copepod early life-stage development test was more sensitive with EC50 and EC10 values of 1.8% and 0.36% respectively for Tailings 1 and 0.38% and 0.19% respectively for Tailings 2.

Table 23. Toxicity of tailings liquor to marine biota. Tailings liquor was prepared by diluting the original tailings material (solids plus liquid) with seawater (1 in 4, m/m) and mixed (1 h) prior to filtration (0.45 µm)

Test	Species	Test Endpoint	Test Date	Tailings 1 Liquor				Tailings 2 Liquor			
				EC/IC50 (%)	EC/IC10 (%)	NOEC (%)	SSD[a] Value	EC50 (%)	EC10 (%)	NOEC (%)	SSD[a] Value
Microalgae	*N. closterium*	72-h growth rate	18/7/2017	28 (24-31)	9.4 (6.4-11)	6.25	9.4	16 (15-18)	3.9 (2.2-5.0)	1	3.9
	I. galbana	72-h growth rate	18/7/2017	98	23 (16-30)	12.5	23	78 (72-82)	30 (20-35)	25	30
Copepod	*A. sinjiensis*	80-h larval development	8/1 2018	1.8 (1.3-2.6)	0.36 (0.12-0.58)	0.25	0.36	0.38 (0.32-0.44)	0.19 (0.11-0.24)	0.11	0.19
Sea urchin	*E. mathaei*	72-h larval development	14/7/2017	34	25	12.5	25	14	12	6.3	12
	H. tuberculata	72-h larval development	9/8/2017	75	54	100	54	37	27	50	27
Oyster	*S. echinata*	48-h larval development	26/7/2017	>100	83 (34-93)	67	83	75 (73-77)	61 (28-72)	33	61
Anemone	*A. pulchella*	8-d development	26/7/2017	>100	83	100	83	84 (83-85)	69 (0-72)	67	69
Fish	*S. lalandi*	8-d embryo development	13/8/2017	41 (33-48)	19 (11-26)	12.5	19	32 (29-35)	16 (13-19)	12.5	16

[a] Species sensitivity distribution; toxicity value used in species sensitivity distribution
Values in parentheses are 95% confidence limits

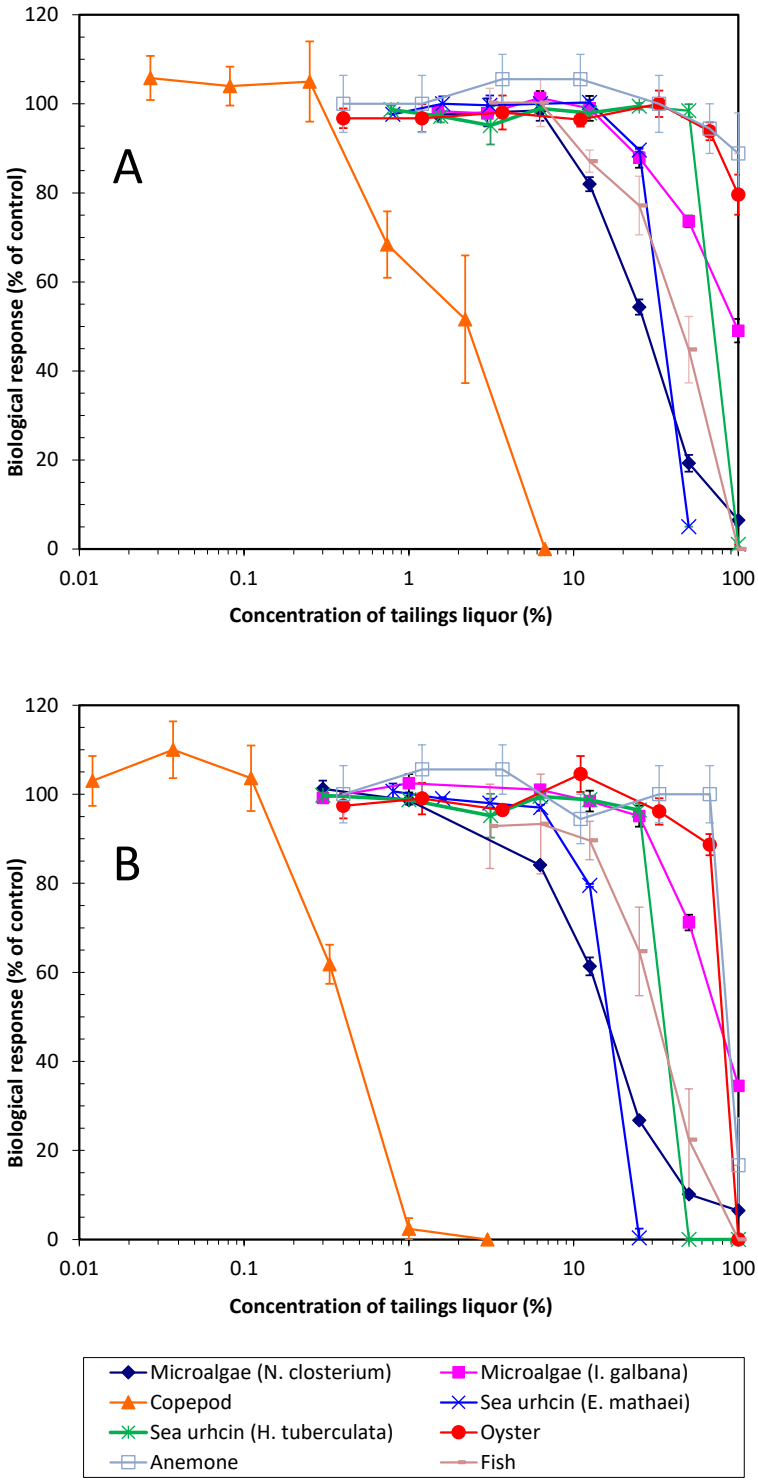


Figure 5. Concentration-response curves for (A) Tailings 1 and (B) Tailings 2 liquor toxicity to marine organisms. Control response is 100% (not shown on the graphs). The 100% tailings liquor concentration represents the 1 in 4 diluted tailings (m/m; 1 h mixing followed by 0.45 µm filtration). Note the logarithmic scales on the x-axes.

3.2.5 Investigating the cause of tailings liquor toxicity to marine biota

The concentration of dissolved (0.45 µm) Co, Cu, Ni and Zn in diluted tailings liquors exceeded WQGVs with concentrations decreasing linearly in a predictable manner as the diluted liquor concentrations decreased from 100% to 6.7% (Tailings 1) and 100% to 0.33% (Tailings 2). This was also true for Mn which exceeded the proposed GV for marine ecosystems. Therefore, the toxicity of these metals to selected species and test endpoints was also investigated in this study by carrying out additional toxicity tests on individual metals and collation of toxicity data published in scientific literature.

Mixtures of contaminants can result in biological effects that can cause a synergistic, additive or antagonistic effect. A synergistic effect is observed when the toxicity of the mixture is more than the sum of the toxic effects of each individual component (contaminant). Additive effects are observed then the mixture has the same toxicity as the sum of the toxicity of the individual components. An antagonistic effect is observed when the mixture toxicity is less than the sum of the toxicity of the individual components. A relatively simple way to indicate if synergistic, additive or antagonistic interactions are observed in the mixture (sample) is to compare the toxic units (TU) of the observed toxicity (OTU) to the predicted toxicity (PTU) by adding the sum of the predicted toxicity of each individual component.

$$OTU = 100 \div EC50_{mixture}$$

The PTU of the mixture is calculated as the sum of the PTU of each individual contaminant (metal).

$$PTU_{metal} = \text{concentration of metal in 100\% liquor} \div EC50_{metal}$$

$$PTU_{mixture} = PTU_{metalA} + PTU_{metalB} + PTU_{metalC} \text{ etc}$$

The observed toxicities of diluted Tailings 1 and Tailings 2 liquors for each toxicity test are presented in Table 24 and Table 25 respectively, along with the PTU for Cu, Mn, Ni and Zn. The toxicity of Co was excluded from the calculations because there was no information on the toxicity of Co to any of the toxicity tests/species utilised in this study. Despite a low WQGV of 1 µg/L, the lowest converted chronic value (NOEC) of 9 µg/L was higher than the concentrations measured in the liquors (ANZECC/ARMCANZ, 2000).

Comparison of the observed and predicted toxicity for diluted Tailings 1 liquor indicates that the OTU of the liquor to microalgae (*N. closterium* and *I. galbana*), the sea urchin *H. tuberculata* and sea anemone (*A. pulchella*) was less than that predicted by the sum of the PTU for Cu, Zn, Mn and Ni, i.e. for these toxicity tests, the contaminants in the liquor are interacting in an antagonistic manner. For the sea urchin (*E. mathaei*), and the fish (*S. lalandi*), OTUs were higher than their respective PTUs. The oyster (*S. echinata*) was of low toxicity (OTU = <1) and by comparison the PTU for Cu was no greater than 1 (PTU = 0.9). However, for these toxicity tests only Cu toxicity data were available and so the effect of other metals on the observed toxicity of the liquor could not be quantified.

The chronic copepod test measuring the early life stage development (from eggs to copepodite) was the most sensitive toxicity test to the tailings liquors and Zn. The toxicity of Zn to copepod development (EC50 of 1.2 µg Zn/L) was at least 10 times more sensitive than for microalgae, sea urchin (*H. tuberculata*) and sea anemone (EC50 of 92–269 µg/L). The OTU for Tailings liquor 1 (OTU = 56) was lower than the PTU based on Zn alone (PTU = 132) and, for the sum of the PTUs for

Cu, Ni, Mn and Zn (PTU = 143) suggesting that the metals in the liquor are acting in an antagonistic manner. While dissolved Zn may be expected to be a major contaminant in the tailings liquor, the copepod development test was also the most sensitive toxicity test to Cu, Mn and Ni. This highlights that microcrustaceans (and in particular copepods) are a relatively sensitive marine taxa and along with their role as a primary consumer in marine ecosystems means that microcrustaceans an important taxonomic group for inclusion in ecotoxicity assessments (Gissi et al., 2016; van Dam et al., 2008).

Comparison of OTUs and PTUs for Tailings 2 liquor followed a similar trend which was not surprising given the similarities in the metal composition of the two liquors; except for Ni and Zn which differed by a factor of 2.1 and 2.5 respectively. The exception was for the toxicity test with the oyster (*S. echinata*) with the observed toxicity slightly higher than the predicted toxicity, but again this was only based on Cu.

Based on this simple TU approach, Cu and Zn were predicted to have a significant contribution to the toxicity of the liquor to aquatic organisms with Ni and Mn also potentially important to copepods. However, these metals have the potential to interact in an antagonistic and synergistic manner and their presence in liquors should be considered in light of other contaminants. For example, Cu toxicity to marine microalgae (*N. closterium*) has been shown to be ameliorated (reduced) when Mn is present (Stauber and Florence, 1985). Other studies on the interactions of metal mixtures on marine organisms are limited.

Table 24. Comparison of diluted Tailings 1 liquor toxicity to the toxicity of copper, zinc, manganese and nickel

Test	Observed Toxicity[a]		Predicted Toxicity[b]												
	EC50 (%)	OTU[a]	Copper			Zinc			Manganese			Nickel			Sum of PTU[c]
			Liquor (µg/L)	EC50 (µg/L)	PTU[b]	Liquor (µg/L)	EC50 (µg/L)	PTU[b]	Liquor (µg/L)	EC50 (µg/L)	PTU[b]	Liquor (µg/L)	EC50 (µg/L)	PTU[b]	
N. closterium	28 (24-31)	**3.6 (3.2-4.2)**	13	3.34[d]	3.9	158	117[d]	1.4	1485	>4790[d]	<0.3	42	6589	0.0	**5.2**
I. galbana	98	**1.0**	13	5.8[d]	2.3	158	92[d]	1.7	1485	>4770[d]	<0.3	42	1933	0.0	**4.0**
A. sinjiensis	1.8 (1.3-2.6)	**56 (38-77)**	13	2.5	5.2	158	1.2[d]	132	1485	1200[d]	1.2	42	8.5	4.9	**143**
E. mathaei	34	**2.9**	13	31[d]	0.4	158	–	–	1485	–	–	42	–	–	**0.4**
H. tuberculata	75	**1.3**	13	6.6[d]	2.0	158	160[e]	1.0	1485	5200[e]	0.3	42	270[e]	0.2	**3.1**
S. echinata	>100	**<1**	13	14.7[d]	0.9	158	–	–	1485	–	–	42	–	–	**0.9**
A. pulchella	>100	**<1**	13	15[d]	0.8	158	269[f]	0.6	1485	–	–	42	>491[f]	<0.1	**1.4**
S. lalandi	41 (33-48)	**2.4 (2.1-3.0)**	13	63[d]	0.2	158	–	–	1485	–	–	42	–	–	**0.2**

[a] Observed toxicity (OTU) = toxicity of diluted tailings liquor expressed as toxic units (100 ÷ EC50)
[b] Predicted toxicity (PTU) of diluted tailings liquor based on the toxicity of an individual metal expressed as toxic units (concentration of individual metal in 100% diluted tailings liquor ÷ EC50 of individual metal)
[c] Sum of PTU = sum of TU_{copper} , TU_{zinc}, $TU_{manganese}$, TU_{nickel}
[d] This study
[e] Doyle et al. (2003)
[f] Howe et al. (2014)
Final toxic units observed (OTU) and predicted (PTU) are in **bold** for ease of comparison.

Table 25. Comparison of diluted Tailings 2 liquor toxicity to the toxicity of copper, zinc, manganese and nickel

Test	Observed Toxicity[a]		Predicted Toxicity[b]												
	EC50 (%)	OTU[a]	Copper			Zinc			Manganese			Nickel			Sum of PTU[c]
			Liquor (µg/L)	EC50 (µg/L)	PTU[b]	Liquor (µg/L)	EC50 (µg/L)	PTU[b]	Liquor (µg/L)	EC50 (µg/L)	PTU[b]	Liquor (µg/L)	EC50 (µg/L)	PTU[b]	
N. closterium	16 (15-18)	**6.3 (5.6-6.7)**	18	3.34[d]	5.4	393	117[d]	3.4	1650	>4790[d]	<0.3	90	6589	0.0	**8.8**
I. galbana	78 (72-82)	**1.3 (1.2-1.4)**	18	5.8_d	3.1	393	92[d]	4.3	1650	>4770[d]	<0.3	90	1933	0.0	**7.4**
A. sinjiensis	0.38 (0.32-0.44)	**263 (227-313)**	18	2.5	7.2	393	1.2[d]	328	1650	1200[d]	1.4	90	8.5	11	**348**
E. mathaei	14	**7.1**	18	31[d]	0.6	393	–	–	1650	–	–	90	–	–	**0.6**
H. tuberculata	37	**2.7**	18	6.6[d]	2.7	393	160[e]	2.5	1650	5200[e]	0.3	90	270[e]	0.3	**5.8**
S. echinata	75 (73-77)	**1.3 (1.3-1.4)**	18	14.7[d]	0.9	393	–	–	1650	–	–	90	–	–	**0.9**
A. pulchella	84 (83-85)	**1.2 (1.2-1.2)**	18	15_d	1.2	393	269[f]	1.5	1650	–	–	90	>491[f]	<0.1	**2.6**
S. lalandi	32 (29-35)	**3.1 (2.9-3.4)**	18	63[d]	0.3	393	–	–	1650	–	–	90	–	–	**0.3**

[a] Observed toxicity (OTU) = toxicity of diluted tailings liquor expressed as toxic units (100 ÷ EC50)
[b] Predicted toxicity (PTU) of diluted tailings liquor based on the toxicity of an individual metal expressed as toxic units (concentration of individual metal in 100% diluted tailings liquor ÷ EC50 of individual metal)
[c] Sum of PTU = sum of TU_{copper} , TU_{zinc}, $TU_{manganese}$, TU_{nickel}
[d] This study
[e] Doyle et al. (2003)
[f] Howe et al. (2014)
Final toxic units observed (OTU) and predicted (PTU) are in **bold** for ease of comparison.

3.2.6 Species sensitivity distributions

Species sensitivity distributions (SSDs) for each tailings sample were generated by plotting histograms of eight chronic EC/IC10 values (Figure 6, Appendix D). There was no need to apply conversion or correction factors to the EC/IC10 values generated in this study because each EC/IC10 value was derived from a chronic toxicity test endpoint (e.g. early life stage development, growth rate) providing greater reliability in the derived 'safe' dilution. The SSDs for both tailings also result in a good curve fit, the exception is the copepod (crustacean) data point at the lower end of the SSD. Considering the higher sensitivity of the copepod test to the tailings and individual metals compared to the other test species, this may be due to the likelihood of the dataset having a bimodal distribution.

The PC95 (or HC5) for tailings liquor that had been prepared by pre-mixing tailings with seawater (1 in 4, m/m) was 1 in 108 (v/v) for Tailings 1 and 1 in 263 (v/v) for Tailings 2 (Table 26) with Tailings 2 requiring 2.4 times more dilutions than Tailings 1. When considering the original tailings (pre-dilution in the mix/de-aeration tank) this would be equivalent to 1 in 508 (v/v) and 1 in 1,210 (v/v) dilutions for Tailings 1 and 2 respectively.

Neither of these dilutions protect the most sensitive species in this study, early life stage development of the tropical copepod *A. sinjiensis*, that is, the copepod IC10 value was lower than the PC95 value. By definition, 5% of the SSD will fall below the PC95 value; hence, it is not always surprising to observe a toxicity data point to fall within the modelled 5% of potentially affected species (Figure 6). For the copepod early life stage development test this is not necessarily unexpected given the relatively high sensitivity to Cu, Zn, Ni and Mn compared to the other test species (and endpoints) used in this study and possible bimodal dataset. Dilutions of tailings required to protect the copepod *A. sinjiensis* were almost double the PC50 value (1 in 278 for Tailings 1 and 1 in 526 for tailings 2).

As mentioned in Section 3.2.1, the 1 in 4 (m/m) dilution of tailings (with 1 h mixing followed by 0.45 µm filtration) used to prepare the liquor for this study was slightly lower than the dilution that will be utilised in the mix/de-aeration tank prior to tailings discharge via the DSTP outfall (1 in 5 (v/v), or 1 in 4.3 Tailings 1 (m/m) and 1 in 4.4 Tailings 2 (m/m)). Elutriate tests showed that dissolved metal concentrations in the tailings decrease with increasing dilution (e.g. Cu, Ni and Zn, Section 3.1.2) within the dilution range of 1 in 10 to 1 in 10,000 (m/m). This would suggest that the dissolved metal concentrations in the tailings liquors tested in this study (using a 1 in 4 dilution, m/m) would be higher than that generated in the mix/de-aeration tank (with a 1 in 5 dilution). If the dissolved metal concentrations in the 1 in 5 tailings dilution (v/v) are in fact lower, it would potentially lead to a lower observed toxicity and lower PC95 value (less required dilutions) for 95% species protection. However, at the time point of mixing with seawater (1 h), the concentration of dissolved metals in elutriates increases rapidly (Figure 3); hence, likely to result in a lack of reliability for estimating accurate concentrations of dissolved metals between treatments that vary slightly in dilution (1 in 4 (m/m), compared to 1 in 4.3 or 1 in 4.4 (m/m) for Tailings 1 and 2, respectively). Regardless, the 1 in 4 tailings dilution (m/m) tested here is likely to be a conservative estimate of the PC95 value and required dilutions of tailings liquors to achieve 95% species protection.

Table 26. Dilutions of tailings liquor required to meet 95% species protection level compared to that required to protect early life stages of copepods[a]

Sample	Species Protection Level	Tailings Liquor (with 1 in 4 dilution, m/m)		Estimated Original Tailings Material
		Concentration (%)	Dilutions (1 in X, v/v)	Dilutions (1 in X, v/v)
Tailings 1	95% (PC95)	0.93	108	508
	Copepod (early life stage development)	0.36	278	1,307
Tailings 2	95% (PC95)	0.38	263	1,210
	Copepod (early life stage development)	0.19	526	2,420

[a] dilutions of tailings liquor have not been rounded off to lower significant figures to allow use of the dilution numbers in further calculations

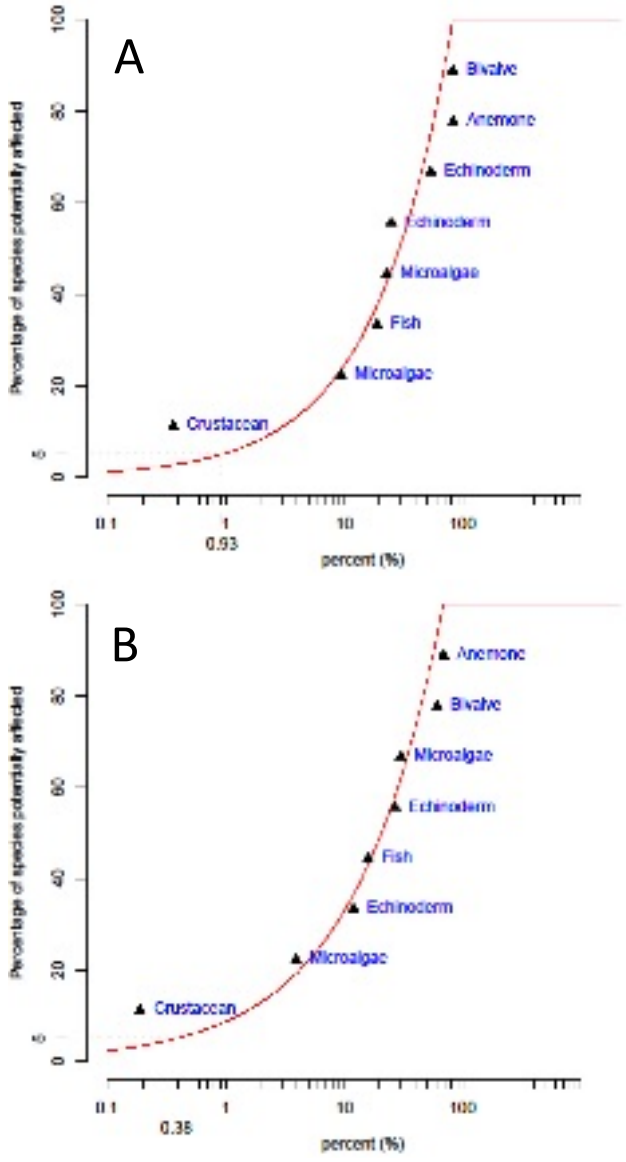


Figure 6. Species sensitivity distribution for 8 species of tailings liquor (1 in 4 dilution with seawater (m/m), mixed for 1 h, filtered to 0.45 µm) for (A) Tailings 1 and (B) Tailings 2. The curve fit is the inverse Pareto model, the dotted line represents the concentration of tailings liquor to achieve 95% species protection level (PC95).

3.3 Ecotoxicological assessment of tailings solids

3.3.1 Washing of tailings prior to toxicity tests

The tailings solids were washed by mixing with seawater (1 in 3 w/w) for 1 min then allowing the tailings solids to settle for 5 to 79 h (settling time was the same for each tailings, and related to logistics) before removing the overlying seawater for analyses. Washing of the tailings occurred 13 times over a period of 443 h and the dissolved (<0.45 µm filtered) concentration of dissolved Cu, Mn, Ni and Zn measured in the overlying waters after each wash are shown in Figure 7. Ongoing release of dissolved Cu was observed to occur that resulted in dissolved Cu concentrations in the range of 10–30 µg/L, while dissolved Zn concentrations decreased (but were variable) to 40–88 µg/L. The release of dissolved Mn, Ni and Zn decreased with consecutive washes, with the greatest decrease observed for dissolved manganese concentrations after 6 days.

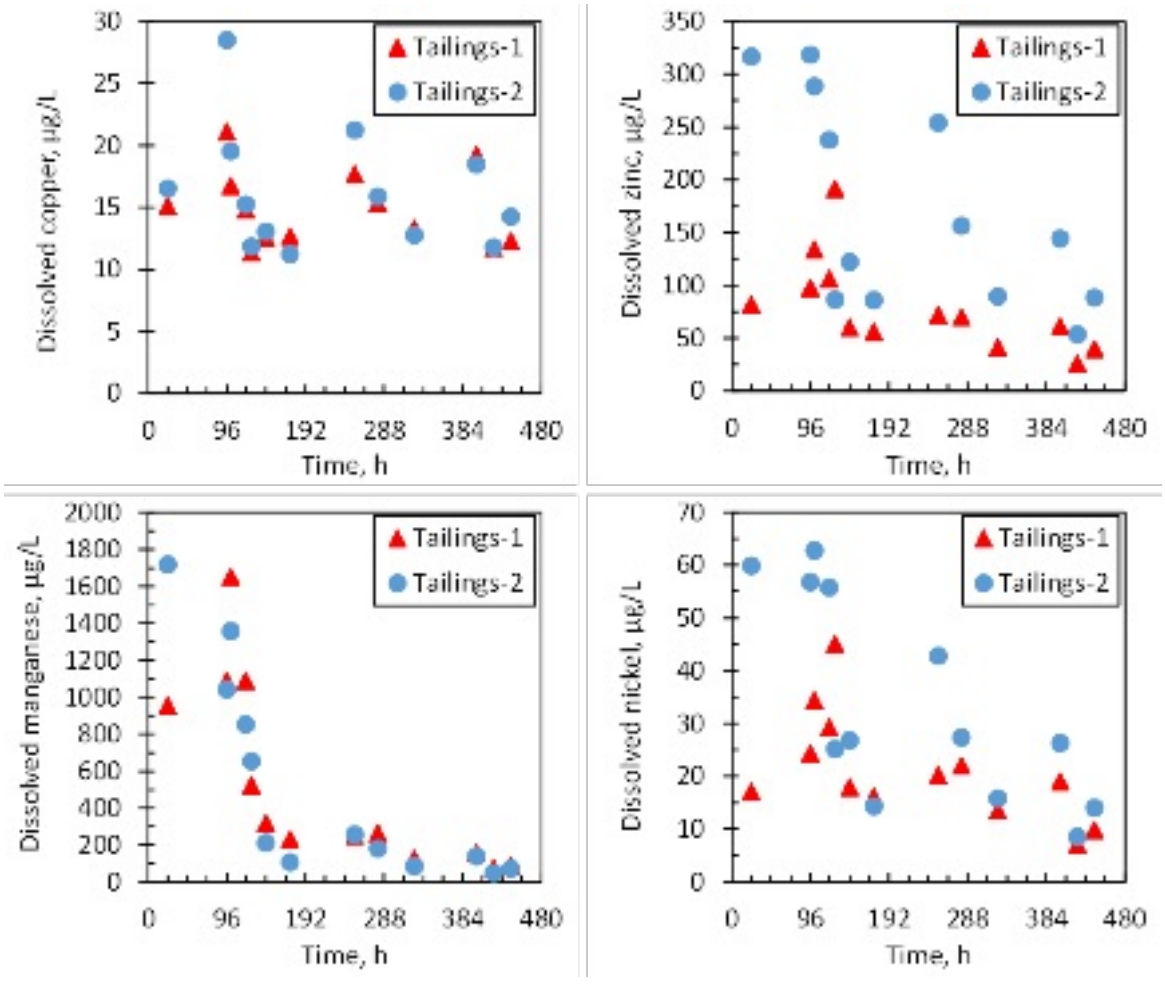


Figure 7. Dissolved metal concentrations in water overlying tailings after each washing.

3.3.2 Sediment toxicity test quality assurance and quality control criteria

All of the toxicity tests meet their respective quality assurance criteria (Table 27). The exception was for the bioaccumulation test. While the survival of bivalves met test acceptability criteria for the standard QA control, bivalve survival in the tailings treatments were low (0–62%). Tailings treatments used in the bioaccumulation test should be low enough to not cause mortality (low survival) to the bivalves, and this was not the case in this study due to the higher than expected toxicity of the tailings to benthic organisms observed in this study.

Table 27. Quality assurance criteria for definitive toxicity and bioaccumulation tests carried out on the tailings solids

Toxicity Test	Control Treatments (negative control)		
	Acceptability Criteria	Result (%)	Criteria Met?
10-d Amphipod survival (*M. plumulosa*)	≥80% survival in standard QA control	90, 96, 92, 92	Yes
10-d Amphipod reproduction (*M. plumulosa*)	≥8 embryos per female in standard QA control	16, 14, 11, 10	Yes
10-d Copepod reproduction (*N. spinipes*)	>20 juveniles per female in standard QA control	22, 22	Yes
30-d Bivalve bioaccumulation (*T. deltoidalis*)	≥80% survival in standard QA control	95	Yes
	≥80% survival in treatments	0 to 65	No (Bioaccumulation assessment not possible for all tailing treatments)

3.3.3 Chemistry and toxicity of the Huon Gulf sediment

The proposed DSTP is intended to result in the tailings solid being deposited in the deep-sea environment. Sediments collected from the proposed deposition site (Huon Gulf sediment) were used initially and throughout the testing to understand the baseline for the response of the two standard tests; toxicity to amphipod and copepod survival and reproduction.

The Huon Gulf sediments were silty (approximately 80-90% <63 µm). A separately collected sediment sample from the Huon Gulf contained generally low concentrations of most metals compared to the tailings with TRM similar for As, Cd, Co, Fe and Pb (Table 17), and lower for Cr (50 mg/kg), Cu (75 mg/kg), Zn (90 mg/kg). The exception was for Mn, which was notably higher in the Huon Gulf sediment (1100 mg Mn/kg) than in the tailings (~300 mg Mn/kg). The AEM concentrations were lower (e.g. AEM: 20-30 mg/kg Cu and Zn, 500 mg/kg Mn and 8400 mg/kg for Fe) than in the tailings. A full characterisation of sediments from the Huon Gulf (including total organic carbon) is the subject of a separate report.

For the Huon Gulf sediment, survival of the amphipod and copepod species were normal (no acute effects); however, the reproductive outputs (chronic effect) of both species were lower when compared to the standard quality assurance (QA) control sediment; silty sediment collected from a coastal estuarine environment (Table 28). Amphipod reproduction in the Huon Gulf sediment was 47-69% (n=7, tested over a seven month period) and copepod reproduction was 62-97% (n=2; Table 28).

During the amphipod toxicity test, measured dissolved concentrations of Mn in the overlying waters of Huon Gulf sediment were in the range of 3000 to 4000 µg/L, and were also initially suspected to cause the lower than expected amphipod reproduction. Several experiments were undertaken to investigate the contribution of dissolved Mn to observed effects. Washing the Huon Gulf sediment (four times using filtered seawater) prior to toxicity testing to lower the release of metals (including Mn), did not improve the reproductive output of the amphipod (Table 28). A separate assessment of the sensitivity of the amphipod to dissolved Mn using Mn-spiked seawater and clean sand as a substrate confirmed that no reproductive effects (no significant difference in reproduction from the control at 3500 µg Mn/L) could be attributed to the dissolved Mn at concentrations measured in the amphipod test (data not shown).

The lower than desired reproductive output of the amphipod in the Huon Gulf sediment compared to the standard coastal estuarine control may also be attributed to a poorer nutritional quality (e.g. reduced total organic carbon amount and quality) of the deep-sea sediment (Huon Gulf sediment <0.05–0.62% compared to 4.0 ± 0.5% for the standard control sediment); but this has not yet been confirmed.

For assessing the effects of the tailings on the reproduction of the two test species, the Huon Gulf sediment was used as the control (results reports are % of Huon Gulf (HG) control); that is, the reduced reproduction of the amphipod and copepod in HG sediment was considered acceptable considering the response was high enough to identify a decreased (toxic) response and the reproducibility of the response was reliable.

Table 28. Toxicity results for Control - Huon Gulf sediment

Treatment	Collected	Test organism	Date tested	Survival (% control)[a]	Reproduction (% control)[a]
Huon Gulf - 1	October 2016	Amphipod	28/11/16	105 ± 7	47 ± 11
Huon Gulf - 2	October 2016	Amphipod	30/1/17	102 ± 2	65 ± 5
Huon Gulf - 3	October 2016	Amphipod	6/2/17	95 ± 5	63 ± 18
Huon Gulf – washed*	October 2016	Amphipod	24/3/17	98 ± 2	65 ± 8
Huon Gulf - 4	March 2017	Amphipod	28/4/17	102 ± 4	51 ± 4
Huon Gulf - 5	March 2017	Amphipod	19/5/17	96 ± 5	69 ± 8
Huon Gulf - 6	March 2017	Amphipod	7/7/17	93 ± 6	56 ± 8
Huon Gulf - 1	March 2017	Copepod	12/5/17	80-100	62 ± 4
Huon Gulf - 2	March 2017	Copepod	30/5/17	80-100	97 ± 9

[a] Compared to silty estuarine control

*Huon Gulf sediment was washed with filtered seawater four times prior to toxicity testing to minimise the release of dissolved metals during the amphipod test

3.3.4 Toxicity of the washed tailings solid to benthic amphipod and copepod

Toxicity of Tailings 1 solid

The Tailings 1 solids and diluted tailings solids were not toxic to amphipod survival, but did cause effects to reproduction of both amphipod and copepod (Table 29, Appendix E). At concentrations of the tailings solids in the range of 1–10%, no toxicity was observed to the reproduction of the two species. Toxic effects to reproduction of both species were observed at tailings solids concentrations of 30% and greater.

The data were adequate for estimating the effects threshold, with EC10 and EC50 values of 14% and 28% tailings for amphipod reproduction, and 5.2% and 27% tailings for copepod reproduction, respectively (Table 30).

The dissolved metals measured in the overlying waters of the amphipod test indicate that dissolved copper was at concentrations previously found to affect the species reproduction for treatments with ≥10% tailings solids (Table 31). The dissolved metals measured in the copepod tests are shown in Table 32, and were generally below concentrations previously found to cause effects to copepod reproduction.

Table 29. Toxicity of Tailings 1 solids to the amphipod and the copepod.

Tailings solid concentration	Amphipod reproduction bioassay				Copepod reproduction bioassay	
	Survival (%)	Survival (% of control)	Juveniles per female	Juveniles per female (% HG control)	Juveniles per female	Juveniles per female (% HG control)
0%	92 ± 3[a]	100 ± 4	8 ± 1	100 ± 9	13 ± 1	100 ± 7
1%	94 ± 6	102 ± 7	11 ± 2	138 ± 18	18 ± 1	137 ± 7
10%	94 ± 4	102 ± 4	10 ± 2	126 ± 19	12 ± 1	93 ± 5
30%	83 ± 6	91 ± 6	4 ± 1	54 ± 8[b]	7 ± 1	53 ± 10[b]
60%	88 ± 2	95 ± 3	1 ± 0	16 ± 5[b]	1 ± 0	4 ± 1[b]
90%	73 ± 11	80 ± 13	1 ± 0	9 ± 4[b]	0 ± 0	3 ± 1[b]

[a] All results are mean ± standard error calculated based on the four replicate tests/sediment.
[b] Statistically less than the control response ($p<0.05$) and below the toxic threshold.

Table 30. Reproduction effects thresholds (percent tailings solid with 95% confidence limits) of the amphipod and the copepod to Tailings 1 solids.

	Percent tailings solid (95% confidence limits)				
	EC10[a]	EC20	EC50	NOEC[b]	LOEC[c]
Copepod	5.2 (2.6-11)	9.4 (4.2-19)	27 (15-43)	10	30
Amphipod	14 (0-15.2)	17 (2.8-20.4)	28 (21.1-39.2)	10	30

[a] Concentration of Tailings 1 solid that results in a 10, 20 or 50% reproduction effect.
[b] Highest concentration that resulted in no observable reproduction effects.
[c] Lowest concentration that resulted in a statistically significant reproduction effect.

Table 31. Dissolved metals concentration in overlying waters of amphipod tests: Tailings 1 solids.

Tailings solid concentration	Dissolved metal, µg/L					
	Cu	Fe	Mn	Ni	Pb	Zn
0%	8.1	2.2	1.4	1.8	1.0	0.7
1%	7.3	3.9	3.7	1.5	2.3	3.1
10%	16	1.9	120	2.6	1.0	1.5
30%	24	2.7	480	4.3	4.3	2.7
60%	34	5.3	780	7.2	1.4	10
90%	51	3.4	520	12	1.4	24
Effect threshold for amphipod reproduction						
EC50	15-30					30-60

Note: Measured concentrations of Al, As, Cd, Cr, Co and V were below the limit of detection (2 µg/L) of the ICP-AES

Table 32. Dissolved metals concentration in overlying waters of copepod tests: Tailings 1 solids.

Tailings solid concentration	Dissolved metal, µg/L					
	Cu	Fe	Mn	Ni	Pb	Zn
0%	3.2	170	2800	1.7	<1	2.9
1%	2.5	210	3200	2.3	<1	0.1
30%	13	190	1700	4.7	<1	6.2
60%	15	220	860	6.4	<1	11
90%	21	600	440	8.3	<1	21
Effect threshold for copepod reproduction						
EC50	23-72					50-400

Note: Measured concentrations of Al, As, Cd, Cr, Co and V were below the limit of detection (2 µg/L) of the ICP-AES.
There was insufficient volume from the 10% concentration to analyse for metals.

Toxicity of Tailings 2 solids

The Tailings 2 solids and diluted tailings solids were not toxic to amphipod survival, but did cause effects to reproduction of both amphipod and copepod (Table 33, Appendix E). Strong relationships were observed between the percent tailings solids and the reproduction of both species, with reproductive output decreasing as the concentration of tailings solids increased. Toxic effects to reproduction of both species were observed in the lowest tailings concentrations tested (1%) and greater.

The data were adequate for estimating effects threshold, with EC10 and EC50 values of 0.37% and 14% tailings for amphipod reproduction, and 0.31% and 1.9% tailings for copepod reproduction, respectively (Table 34). This indicated that the tailings solids from Tailings-2 were more toxic to both species than those from Tailings 1(Table 30 and Table 34).

The dissolved metal concentrations measured in the overlying waters for the amphipod and copepod test are shown in Table 35 and Table 36 respectively. When comparing to previously

determined thresholds for dissolved copper and zinc, the dissolved copper measured in the test may have contributed to the observed reproductive toxicity to the amphipods in all tailings treatments and in tailings treatment of 10% or greater for the copepod. Dissolved zinc may also contribute to the effects in the 90% tailings treatments.

It is important to note here that the laboratory-based toxicity testing may exacerbate the exposure to dissolved metals in the overlying water when compared to what may occur in the field, resulting in more conservative outcomes than may be expected for the same sediments and species in the field.

Table 33. Toxicity of Tailings 2 solids to the amphipod and the copepod.

Tailings solid concentration	Amphipod reproduction bioassay				Copepod reproduction bioassay	
	Survival (%)	Survival (% of control)	Juveniles per female	Juveniles per female (% HG control)	Juveniles per female	Juveniles per female (% HG control)
0%	94 ± 4[a]	100 ± 4	9 ± 1	100 ± 11	21 ± 2	100 ± 10
1%	96 ± 4	102 ± 3	7 ± 1	73 ± 11[b]	15 ± 1	68 ± 4[b]
3%	73 ± 7	78 ± 8[c]	6 ± 1	66 ± 7[b]	6 ± 1	26 ± 3[b]
10%	94 ± 4	100 ± 4	6 ± 1	61 ± 9[b]	1 ± 0	2 ± 1[b]
30%	77 ± 6	82 ± 7	0 ± 0	4 ± 2[b]	0 ± 0	0 ± 0[b]
90%	92 ± 8	98 ± 9	0 ± 0	1 ± 1[b]	0 ± 0	0 ± 0[b]

[a] All results are mean ± standard error calculated based on the four replicate tests/sediment;
[b] Statistically less than the control response ($p<0.05$) and below the toxic threshold.
[c] Not considered to be acutely toxic despite being >80% survival due to the standard error and the relationship between tailings solid concentration and percent survival.

Table 34. Reproduction effects thresholds (percent tailings solid with 95% confidence limits) of the amphipod and the copepod to Tailings 2 solids.

	EC10[a]	EC20	EC50	NOEC[b]	LOEC[c]
Copepod	0.31 (0.15-0.67)	0.62 (0.29-1.3)	1.9 (0.89-2.6)	<1	1
Amphipod	0.37 (0.14-2.4)	0.74 (2.8-5.1)	14 (2.8-19)	<1	1

[a] Concentration of Tailings-2 tailings solid that results in a 10, 20 or 50% reproduction effect.
[b] Highest concentration that resulted in no observable reproduction effects.
[c] Lowest concentration that resulted in a statistically significant reproduction effect.

Table 35. Overlying water metals concentration from the Tailings 2 solid toxicity test (amphipod bioassay)

Tailings solid concentration	Dissolved metal, µg/L				
	Cu	Fe	Mn	Ni	Zn
0%	9	<1	11	<1	<1
1%	14	<1	51	1.4	<1
3%	13	<1	22	1.1	<1
10%	21	3.2	88	2.7	1.7
30%	37	<1	1070	7	8.7
90%	53	7.1	540	26	82
Effect threshold for amphipod reproduction					
EC50	15-30				30-60

Note: Measured concentrations of Al, As, Cd, Cr, Co, Pb and V were below the limit of detection (2 µg/L) of the ICP-AES

Table 36. Overlying water metals concentration from the Tailings 2 solid toxicity test (copepod bioassay)

Tailings solid concentration	Dissolved metal, µg/L				
	Cu	Fe	Mn	Ni	Zn
0%	11	96	1600	1.8	2.9
1%	6.8	35	1500	2.5	<2
3%	7.3	24	1500	2.3	<2
10%	33	140	2500	9.0	24
30%	16	78	2100	7.2	4.4
90%	55	620	620	29	130
Effect threshold for copepod reproduction					
EC50	23-72				50-400

Note: Measured concentrations of Al, As, Cd, Cr, Co and V were below the limit of detection (2 µg/L) of the ICP-AES.

3.3.5 Test modifications and manipulations to modify exposure

Effects of dissolved metals released from the washed tailings solids

The effects to the amphipod reproduction with and without the modifications of the standard methods are summarised in Table 37 (Appendix E) and the changes to the dissolved metal concentrations in the overlying waters are provided in Table 38.

The treatment modifications (M1, see Table 13) resulted in lower dissolved metal exposures during the tests. The dissolved Cu concentrations were at levels expected to cause toxicity to amphipod reproduction in the Tailings 1 60% treatments and potentially also within the Tailings 1 30% treatments. Despite reducing the dissolved Cu concentrations, the M1 treatments for Tailings 1 30% and 60% did not significantly improve the reproductive output of the test organism. The results indicate that sediment bound metals (via an ingesting pathway) may also contribute to the observed toxicity to the amphipods when exposed to the Tailings 1 solids.

The reproductive response from the amphipods in Tailings 2 1% was not significantly different from the standard control. However, the treatment modification significantly improved the reproductive output from the amphipods exposed to tailings from Tailings 2 10%, despite the measured dissolved Cu, Mn, Ni and Zn concentrations in modified and unmodified treatments being below the estimated effect thresholds (both as an average or any single measurement during the test acting as a potential pulse exposure). The estimated EC20 and EC50 values (amphipod reproduction) for dissolved Ni are 125 and 230 µg/L, respectively (unpublished results), so Ni was not responsible for observed differences in reproduction.

When considering all the results together (Tailings 1 and Tailings 2 with and without treatment modifications), there existed a reasonable relationship between increasing dissolved Cu in the overlying water and the decreasing amphipod reproduction (Figure 8). Despite this relationship, dissolved Cu cannot completely explain the toxicity observed to the amphipod reproduction.

Table 37. The effect of dissolved metals released from tailings solids on the reproduction of the amphipod.

Treatment name	Juveniles per female [a]	Juveniles per female (% control)	% M1 treatment control
Standard QA control	11 ± 0	100 ± 2	
Tailings-1_30% M1	3 ± 1	31 ± 6 [b]	100 ± 19
Tailings-1_30%	3 ± 0	29 ± 3 [b]	93 ± 9
Tailings-1_60% M1	1 ± 0	9 ± 2[b]	100 ± 23
Tailings-1_60%	2 ± 0	20 ± 4 [b]	220 ± 40 [c]
Tailings-2 1%_M1	10 ± 1	92 ± 7	100 ± 7
Tailings-2_1%	9 ± 1	82 ± 9	89 ± 10
Tailings-2 10%_M1	11 ± 1	102 ± 9	100 ± 8
Tailings-2_10%	7 ± 1	67 ± 6 [b]	66 ± 6 [d]

[a] All results are mean ± standard error calculated based on the four replicate tests/sediment.
[b] Statistically less than the standard control response ($p<0.05$) and below the toxic threshold.
[c] Note that the very low numbers contribute to large %
[d] Statistically less than the treatment modification control response ($p<0.05$) for the concentration

Table 38. Overlying water metals concentration from method modification amphipod toxicity test

Concentration/treatment	Dissolved metals, µg/L				
	Cu	Fe	Mn	Ni	Zn
Standard QA control	<2	<2	<2	<2	3.1
Tailings-1_30% M1	7.9	15	650	<2	3.8
Tailings-1_30%	13	4.9	1200	4.5	1.8
Tailings-1_60% M1	16	<2	610	3.3	5.5
Tailings-1_60%	45	19	1100	6.8	11
Tailings-2 1%_M1	2.8	<2	34	<2	<2
Tailings-2_1%	5.9	<2	33	<2	6.2
Tailings-2 10%_M1	5.6	<2	200	0.5	4.3
Tailings-2_10%	6.7	<2	480	3.5	8.3

Note: Measured concentrations of Al, As, Cd, Cr, Co and V were below the limit of detection (2 µg/L) of the ICP-AES.

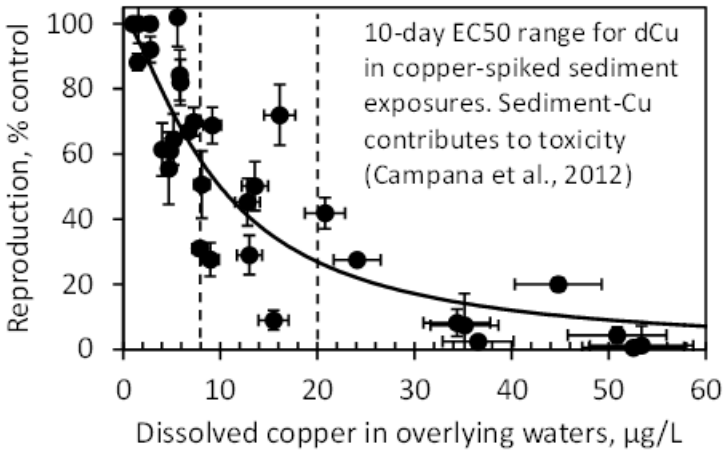


Figure 8. Relationship between dissolved copper in the overlying water and the amphipod reproduction for Bulk Tailings 1 and Tailings 2. Vertical error bars are standard error, horizontal error bars are 10% of the mean. The vertical dashed lines indicate the 10-d EC50 range for dissolved copper observed from past studies using coper spiked sediments, where the observed toxicity is attributed to exposure from both dissolved and particulate copper (Campana et al., 2012).

The effects of layering and mixing of the tailings solids with the diluent sediments

The discharged tailings are expected to mix with other suspended solids within the Huon Gulf water column (inputs from the terrestrial load of suspended solids from the Markham River catchments and Finisterre ranges) resulting in most of the deposition area containing a mixture of tailings and natural loads of sediment. However, for many DSTP operations there is potential for subsurface plumes to split off from the main tailings density current and possibly deposit in areas as a thin layer with minimal mixing with other sediments. For this reason differences in toxicity due to tailings deposited as a mixture and a surface layer (suffix containing 'L') were compared for the same %-tailings within treatments (Table 39 for amphipod and Table 40 for copepod, Appendix E).

The Tailings-1 1% (mixture) and Tailings-1 1%-L (layer) (estimated 0.1–0.2 mm) tailings solids were not toxic when diluted with the standard control (containing the suffix 'S' and 'S-L' respectively) to the reproduction to the amphipods. The Tailings-1 1%-L (layer) (estimated 0.1–0.2 mm) tailings diluted in the Huon Gulf sediment was toxic to the amphipod reproduction, however, no toxicity was observed in the Tailings-1 1% (mixture), Tailings-1 0.1%-L (layer) (estimated 0.1–0.2 mm) and Tailings-1 0.1% (mixture).

The treatments where the tailings solids were layered released more dissolved Cu than the same concentration that was mixed. The dissolved Cu concentration measured in all (mixed or layered) Tailings-1 0.1% and the Tailings-1 1% tailings treatments were below the threshold where reproductive effects are expected.

The 10% layered tailings (1–1.5 mm estimated average layer depth) for Tailings-1 and Tailings-2 had significantly lower reproduction and greater dissolved Cu concentrations in the overlying water than the mixed tailings treatment for both test species.

The high load of terrestrial sediments into the Huon Gulf along with turbidity data suggesting that the formation of regular sub-surface sediment plumes are not an important sediment transport process. This then indicates that tailings are most likely going to be dispersed on the sea floor

mixed with terrestrial sediments, and not as a discreet layer of tailings. Hence, the tailings treatments mixed with Huon Gulf sediment are a more likely scenario, with the tailings tested as a surface layer providing a conservative assessment of tailings toxicity.

Table 39. The effects of layering and mixing of the tailings solids with Huon Gulf sediment on the reproduction of the amphipod.

Treatment name	Juveniles per female	Juveniles per female (% QA control)	Juveniles per female (% Huon Gulf)	Dissolved Cu during exposure (µg/L)
Standard control	10 ± 0[a]	100 ± 3		1.6
Tailings-1_1% S	8 ± 1	88 ± 8		1.5
Tailings-1_1% S-L	8 ± 1	84 ± 11		5.8
Huon Gulf control	5 ± 1	56 ± 8[b]	100 ± 14	4.7
Tailings-1_0.1%	6 ± 0	61 ± 4[b]	113 ± 7	4.0
Tailings-1_0.1% L	6 ± 1	61 ± 6[b]	112 ± 11	4.8
Tailings-1_1%	6 ± 0	64 ± 5[b]	119 ± 10	5.2
Tailings-1_1% L	3 ± 1	28 ± 8[b]	51 ± 15[c]	9.0
Tailings-1_10%	10 ± 2	72 ± 10	126 ± 19	16
Tailings-1_10% L	1 ± 0	7 ± 0	15 ± 1[c]	35
Tailings-2_10%	6 ± 1	42 ± 6	61 ± 9[c]	13
Tailings-2_10% L	0 ± 0	0 ± 0	0 ± 0[c]	21

[a] All results are mean ± standard error calculated based on the four replicate tests/sediment.
[b] Statistically less than the standard control response ($p<0.05$) and below the toxic threshold.
[b] Statistically less than the Huon Gulf (HG) control response ($p<0.05$) and below the toxic threshold.

Table 40. The effects of layering and mixing the tailings solids with standard control sediments on the reproduction of the copepod.

Treatment name	Juveniles per female	Juveniles per female (% HG control)	Dissolved Cu during exposure (µg/L)
Tailings-1_10%	12 ± 1[a]	93 ± 5	NA
Tailings-1_10% L	1 ± 0	9 ± 1[b]	4.2
Tailings-2_10%	1 ± 0	2 ± 1[b]	7.3
Tailings-2_10% L	0 ± 0	0 ± 0[b]	11

[a] All results are mean ± standard error calculated based on the four replicate tests/sediment.
[b] Statistically less than the HG control response ($p<0.05$) and below the toxic threshold.
Note: There was insufficient volume from the 10% concentration to analyse for metals.

3.3.6 Bivalve survival and bioaccumulation results

All tailings treatments caused toxicity to the bivalve survival (Table 41, Appendix E). The dissolved ammonia concentrations in the overlying waters remained below recognised toxicity thresholds (King et al., 2010). The dissolved Cu and Zn concentrations in the overlying waters were high enough to account for the observed toxicity, and could be attributed to release of these metals from the tailings (Table 42). There was a strong relationship between the average dissolved Cu and Zn concentrations measured in the overlying water and the survival of the bivalve (Figure 9).

The concentration of metals within the tissues of the bivalve (dry weight) are summarised in Table 43, and are highly variable as a result of the few bivalves digested for metals analysis due to the low survival numbers. High survival of the bivalves after exposure to the tailings solids mixed with Huon Gulf sediment was not achieved and is a prerequisite for the bioaccumulated soft tissue metal analysis. The poor health of the few surviving bivalves results in the inability to clear the gut during the depuration period resulting in an over-estimation of soft-tissue metal concentration. However, from the bioaccumulation results, it was determined that there was no significant differences in bioaccumulation of Cu or Zn ($p>0.5$). The only significant difference in bioaccumulation of metals in tailings treatments compared to Huon Gulf sediment was for Co ($p=0.46$); but bioaccumulation in the tailings treatment was less (not more) than for the Huon Gulf sediment treatment. The Huon Gulf sediment had significantly greater Cd, Co, Cr, Cu, Mn, Fe, Ni and V than organism tissues pre-test. As a consequence, it was not surprising to observe the bioaccumulation of some metals (Co, Cr, Mn, Fe, Ni and V) that were also significantly greater in the 10% and 20% Tailings treatments. Overall, the bioaccumulation bioassay became more like a survival bioassay and the results indicated that tailings-sediment mixtures comprising 30% of Tailings-1 or 10% of Tailings-2 (wet mass), or greater concentrations, are highly toxic to this bivalve species and requires further investigation.

Table 41. Survival results from the bivalve bioaccumulation bioassay

Concentration/treatment[a]	Survival (% survival)	% of HG Control	Average total ammonia (mg NH_3-N/L)[a]
Huon Gulf sediment	95 ± 5[b]	100 ± 5	0.7
Tailings Solid Tailings-1 30%	10 ± 5	10 ± 5[c]	2.8
Tailings Solid Tailings-1 60%	0 ± 0	0 ± 0[c]	1.5
Tailings Solid Tailings-1 90%	0 ± 0	0 ± 0[c]	4.3
Tailings Solid Tailings-2 10%	62 ± 10	65 ± 10[c]	0.7
Tailings Solid Tailings-2 30%	19 ± 10	20 ± 10[c]	0.9
Tailings Solid Tailings-2 90%	0 ± 0	0 ± 0[c]	5.9

[a] Average ammonia measurements of overlying water in the sediments on day 4, 7, 11, 14, 18, 21 and 25.
[b] All results are mean ± standard error calculated based on the four replicate tests/sediment.
[c] Statistically less than the Huon Gulf sediment response ($p<0.05$).

Table 42. Averaged dissolved metals in the overlying water of the bivalve bioaccumulation bioassay.

Concentration/treatment	Dissolved metals, µg/L				
	Cu	Fe	Mn	Ni	Zn
Huon Gulf sediment	4.9	1.4	410	1.6	1.4
Tailings Solid Tailings-1 30%	24	13	1900	5.0	4.2
Tailings Solid Tailings-1 60%	38	13	1300	7.5	15
Tailings Solid Tailings-1 90%	70	16	570	13	38
Tailings Solid Tailings-2 10%	15	5.0	970	4.1	2.6
Tailings Solid Tailings-2 30%	25	11	1700	6.5	7.0
Tailings Solid Tailings-2 90%	64	99	680	16	56

Note: Measured concentrations of Al, As, Cd, Cr, Co Pb and V were below the limit of detection (2 µg/L) of the ICP-AES.

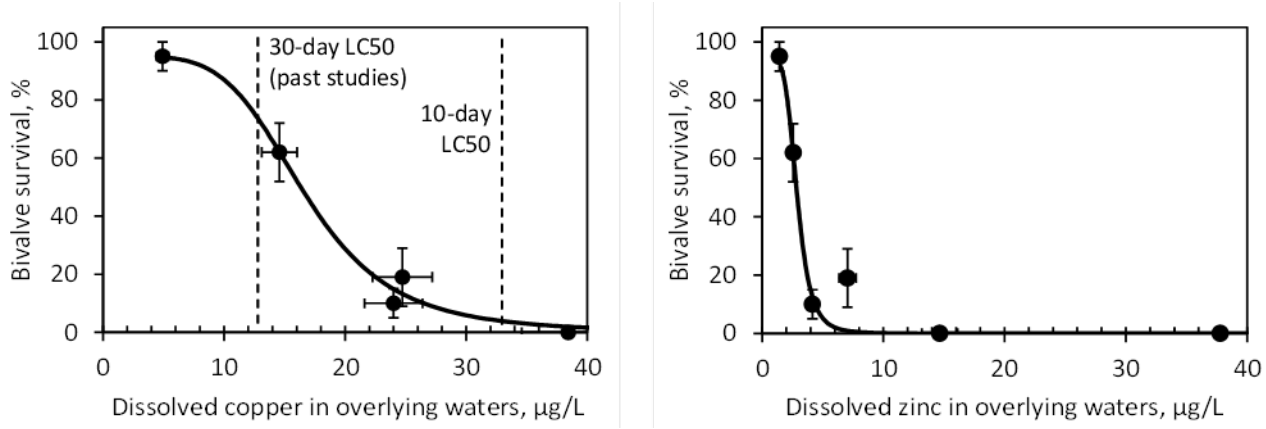


Figure 9. The relationship between the survival of the bivalve after 30 d and the dissolved copper and zinc.

Table 43. Concentrations of metals from the soft tissue of the bivalve following 30 d exposures (dry weight).

Concentration/treatment	Metal concentrations in bivalve tissues, µg/g						
	Ag	As	Cd	Co	Cr	Cu	Hg
Test commencement	5.7 ± 0.63	14 ± 1.9	0.98 ± 0.12	3.1 ± 0.47	4.1 ± 1.4	228 ± 20	0.80 ± 0.12
Huon Gulf	6.3 ± 0.86	14 ± 1.5	1.6 ± 0.08[b]	7.1 ± 0.67[b]	11 ± 1.1[b]	305 ± 32[b]	1.0 ± 0.02
Tailings solid Tailings-1 30%	8.6 ± 6.7	15 ± 8.5	1.4 ± 1.2	4.7 ± 2.8	6.7 ± 7.5	450 ± 360	0.93 ± 0.82
Tailings solid Tailings-2 10%	7.4 ± 4.9	12 ± 2.0	1.2 ± 0.27	5.1 ± 1.0[bc]	8.6 ± 1.3[b]	380 ± 190	0.86 ± 0.22
Tailings solid Tailings-2 30%	5.9 ± 2.4	16 ± 7.9	1.1 ± 0.71	6.0 ± 4.3	26 ± 31	350 ± 150	1.0 ± 0.36
Limit of detection	0.002	0.04	0.01	0.01	0.4	1	0.08
	Mn	**Mo**	**Fe**	**Ni**	**Pb**	**V**	**Zn**
Test commencement	17 ± 2.1	8.9 ± 1.2	2000 ± 310	5.2 ± 1.1	44 ± 7.9	3.5 ± 0.68	390 ± 46
Huon Gulf	150 ± 42 [b]	10 ± 1.2	5800 ± 1400[b]	10 ± 0.56[b]	45 ± 6.5	16 ± 4.8 [b]	460 ± 110
Tailings solid Tailings-1 30%	56 ± 43	14 ± 11	3000 ± 2800	8.1 ± 5.8	64 ± 60	6.7 ± 5.9	320 ± 76
Tailings solid Tailings-2 10%	99 ± 10 [b]	9.5 ± 2.2	4600 ± 850 [b]	11 ± 0.19[b]	43 ± 20	10 ± 1.7 [b]	410 ± 140
Tailings solid Tailings-2 30%	210 ± 220	13 ± .8.4	9700 ± 9300	25 ± 23	44 ± 24	18 ± 20	550 ± 400
Limit of detection	0.5	0.007	10	0.25	0.08	0.2	3

[a] All results are mean ± standard deviation
[b] Statistically significant difference from the test commencement ($p<0.05$).
[c] Statistically significant difference from the HG control ($p<0.05$).

4 Summary and Conclusions

Chemical and ecotoxicological assessments were carried out on two bench scale tailings samples comprising 90% porphyry and 10% metasediments (Tailings 1) and 25% porphyry and 75% metasediments (Tailings 2). A range of chemical and ecotoxicological tests were conducted on the samples. In considering the results of the study it is important to note the following caveats:

(i) The tailings samples in this study were prepared from aged core samples and used in bench-scale laboratory flotation tests. There was up to 12 months between preparation of the first tailings sample and commencement of test work. Hence the results of this study should be interpreted with the results of further studies currently being carried out by CSIRO. In particular, long-term metal mobility from newly prepared tailings material and toxicity and bioaccumulation to benthic organisms.

(ii) Preliminary work (not reported herein) suggests that the use of aged core samples results in greater mobility of some metals, particularly zinc, from the solid to dissolved phase, although this has yet to be definitively confirmed.

(iii) As a result, the tailings samples in this study are likely to have had greater reactivity than if fresh core samples had been used. Therefore, the results contained in this report are likely to be conservative (i.e. overestimate impact).

(iv) Additionally, at the time of testing, the scenarios of mixing, dispersion and settling of tailings solids in the laboratory utilised in this study were designed to provide a conservative measure of tailings toxicity to aquatic organisms. Engineering updates to the project propose to provide a greater level of dilutions at the proposed point of tailings discharge than those modelled in this study.

The main conclusions of the study were as follows:

Tailings characterisation, dilution and comparison to water and sediment quality guidelines

1. Both tailings samples were near neutral (pH 7.4 Tailings 1 and pH 7.2 Tailings 2) with dissolved (<0.45 µm) concentrations of Co, Cu and Zn in both tailings exceeding WQGVs. Comparison to PNG water quality criteria for aquatic life protection (Environment Act 2000) indicate that Co, Mn and Cu (Tailings 1 only) exceed the reported criteria concentrations (prior to dilution or any other potential treatment methods).
2. Analyses of TRM concentrations in tailings solids of Tailings 1 and Tailings 2 showed that Cr, Cu, Ni and Zn exceeded SQGVs. The AEM concentrations (a better indicator of potentially bioavailable metals) also exceeded SQGVs for Cu, Ni and Zn. The State of PNG does not provide criteria for sediments (solids).
3. Both tailings solids were shown to contain reactive trace metals with elutriate tests (mixing tailings with seawater, 16 h at 30°C) indicating that dissolved (0.45 µm) Cu concentrations continued to exceed the WQGVs (1.3 µg/L) in tailings dilutions of up to, and including, 1 in 10,000. A dilution of 1 in 10,000 was sufficient to ensure all other metals did not exceed WQGVs. At a dilution of 1:50,000 copper dissolved metal concentration was also below the

guideline value. At a dilution of 1 in 100, Co, Zn and Ni (Tailings 2 only) exceeded WQGVs. A dilution of 1 in 10,000 was sufficient to meet the Environment Act 2000 criteria.

4. Mixing tests examining the effects of time on metals release (1 in 10 dilution over 72 h) indicated a two stage metal release process for Cu, Co, Ni and Zn with an initial rapid release of metals into solution over the first one to five hours followed by a much slower metals release phase. Equilibrium metal concentrations (no further increase in dissolved metal concentrations) were typically achieved after 20 hours of mixing.

Ecotoxicological assessment of tailings liquor

5. The chronic toxicity of tailings to eight aquatic organisms was assessed using a tailings liquor that aimed to simulate the mix/de-aeration tank contents immediately prior to discharge via the DSTP pipeline. Tailings diluted 1 in 4 (m/m) with seawater (equivalent to dilutions of 1 in 4.7 (v/v) for Tailings 1, and 1 in 4.6 (v/v) for Tailings 2) were prepared by mixing for 1 h followed by filtration (0.45 µm). Ultimately, a 1 in 5 dilution (v/v) in the mix-de-aeration tank will be used. Only the concentrations of Co exceeded the PNG Environment Act 2000 water quality criteria of 0.1 µg/L, by around 40 fold. The concentration of Co, Cu, Zn and Ni (Tailings 2 only) in the tailings liquors exceeded WQGVs by up to a factor of 14 for Cu and 26 for Zn. This was a lot lower than the 1 in 10,000 dilution required in the elutriate tests (point 3 above) in which tailings were mixed with seawater using different dilutions (1 in 10 to 1 in 50,000) and mixing time (16 h). The removal of tailings solids (by filtration) from both tailings liquors also stopped the continuous release of metals from tailings solids over time.

6. Chronic toxicity to microalgae, sea urchins, oysters, sea anemone and fish were of relatively similar sensitivity with EC/IC10 values of 9.4-83% for Tailings 1 and 3.9-69% for Tailings 2. The copepod early life-stage development test was the most sensitive toxicity test to both tailings liquors with EC10 values of 0.36% and 0.19% for Tailings 1 and 2 respectively. The copepod test was also the most sensitive test to individual metals; Cu, Zn, Mn and Ni.

7. The PC95 (or HC5) for tailings liquor mixed with seawater (1 in 4 (m/m)), 1 h followed by filtration (0.45 µm) was 1 in 108 for Tailings 1 and 1 in 263 for Tailings 2 post-discharge dilution (equivalent to 1 in 508 and 1 in 1,210 dilutions of pre-discharge tailings). However, after discharge in the receiving ocean environment, the tailing solids are expected to be rapidly diluted by increasing quantities of entrained seawater and will not be contained within a fixed volume of seawater for one hour as used in the tailing liquor ecotoxicology tests. As a result, the PC95 value derived here is expected to provide a conservative estimate of the PC95.

Ecotoxicological and bioaccumulation assessment of tailings solids

8. Tailings solids that enter the marine environment after discharge from the DSTP pipeline are predicted to be mixed (washed) with seawater before being deposited on the sea floor; hence, the tailings solids were washed prior to toxicity testing. Ongoing release of Cu from solids into the dissolved (0.45 µm) phase was observed over 6 days. Dissolved Mn, Ni and Zn were also released from the tailings solid but concentrations in the seawater wash solution started to decrease after about 6 days.

9. The toxicity and bioaccumulation of the tailings solids was assessed by preparing mixtures of tailings and natural deep-sea sediment collected from the Huon Gulf (Huon Gulf sediment); the first time this approach has been utilised. The toxicity of the non-mixed (100%) Huon Gulf sediment was initially assessed and resulted in a lower reproductive output (but not survival) of the amphipod and the copepod compared to a standard sediment control (from shallow waters). The reduced reproduction of the benthic organisms may be due to a lack of natural organic matter and possibly sediment-bound metals. However, it was considered to be acceptable for use in this study because (i) the response was high enough to identify a decrease (toxic) response and, (ii) the reproducibility of the response was reliable.
10. The toxicity of the tailings solids (washed) diluted with the Huon Gulf sediment was carried out using tailings mixed with Huon Gulf sediment. Toxicity of solids to amphipods and copepods required dilution of tailings to 10% for Tailings 1 and <1% for Tailings 2. The toxicity correlated with dissolved Cu concentrations in overlying water; however modification of the experimental test containers showed that dissolved Cu does not completely explain the observed toxicity. The toxicity was likely to be attributed to Cu (Tailings 1 and 2) and Zn (Tailings 2) partitioned into the liquid phase (e.g. overlying water and pore water), direct contact with solids and dietary (ingestion) exposure of the solid.
11. During the bioaccumulation tests, both tailings samples caused lethality to the bivalve in the lower tailing:sediment dilutions. This prevented bioaccumulation from being assessed in those treatments and hence is the subject of further investigations (to be reported at a later date). In this study, for tailing:sediment dilutions of 30% Tailing 1 and Tailing 2, there was no indication of significant differences in the bioaccumulation of Cu and Zn; the only significant difference detected was for Co. Bivalves exposed to the Huon Gulf sediment (no tailings) showed significant increases in bioaccumulated Cd, Co, Cr, Cu, Mn, Fe, Ni and V when compared to organism before exposure to the tailings or Huon Gulf sediment. There were no effects to the survival of the bivalves in the Huon Gulf sediments despite the indication that these natural sediments contained metals that were bioavailable.

5 References

Aldenberg T and Slob W (1993). Confidence limits for hazardous concentrations based on logistically distributed NOEC toxicity data. Ecotoxicology and Environmental Safety 25(1), 48-63.

ANZECC/ARMCANZ (2000) Australian and New Zealand Guidelines for Fresh and Marine Water Quality, Australia and New Zealand Environment and Conservation Council/Agricultural and Resource Management Council of Australia and New Zealand. Canberra, Australia.

APHA (1998). Standard Methods for the Examination of Water and Wastewater. 20th Edition. American Public Health Association, American Water Works Association, Water Environment Federation, Washington, DC, USA.

APHA (2005). Standard Methods for the Examination of Water and Wastewater. 21st Edition. American Public Health Association, American Water Works Association, Water Environment Federation, Washington, DC, USA.

Batley GE, van Dam RA, Warne MSJ, Chapman JC, Fox DR, Hickey CW and Stauber JL (2014). Technical rationale for changes to the method for deriving Australian and New Zealand water quality guideline values for toxicants. CSIRO Land and Water Report, 37 pp. https://doi.org/10.4225/08/5890d0ac848cc

Brown A, Thatje S and Hauton C (2017). The effects of temperature and hydrostatic pressure on metal toxicity: Insights into toxicity in the deep sea. Environmental Science and Technology 51, 10222-10231.

Byrne M, Oakes DJ, Pollak JK and Laginestra E (2008). Toxicity of landfill leachate to sea urchin development with a focus on ammonia. Cell Biology and Toxicology 24, 503-512.

Campana O, Simpson SL, Spadaro DA and Blasco J (2012). Sub-lethal effects of copper to benthic invertebrates explained by sediment properties and dietary exposure. Environmental Science and Technology 46(12), 6835-6842.

NAGD (2009). National Assessment Guidelines for Dredging, Department of Environment, Water, Heritage and the Arts (DEWHA), Commonwealth of Australia, Canberra, 81 pp.

Doyle CJ, Pablo F, Lim RP and Hyne RV (2003). Assessment of metal toxicity in sediment pore water from Lake Macquarie, Australia. Archives of Environmental Contamination and Toxicology 44, 343-350.

Franklin NM, Stauber JL and Adams MS (2005). Improved methods of conducting microalgal bioassays using flow cytometry. In: Ostrander GK (ed.) Techniques in Aquatic Toxicology. CRC Press, FL, USA, 735 - 756.

Gissi F, Binet MT and Adams MS (2013). Acute toxicity testing with the tropical marine copepod *Acartia sinjiensis*: Optimisation and application. Ecotoxicology and Environmental Safety 97, 86-93.

Gissi F, Stauber JL, Binet MT, Golding LA, Adams MS, Schlekat CE, Garman ER and Jolley DF (2016). A review of nickel toxicity to marine and estuarine tropical biota with particular reference to the South East Asian and Melanesian region. Environmental Pollution 218, 1308-1323.

Golding LA, Adams MS, Batley GE, Binet MT and Stauber JL (2016). Guidelines for the protection of aquatic ecosystems, toxicant trigger values: Manganese - Marine. Australian and New Zealand guidelines for fresh and marine water quality. Draft July 2016. Council of Australian Governments Standing Council on Environment and Water, Canberra, ACT, Australia.

Golding LA, Adams MS, Binet MT, Batley GE and Stauber JL (2015a). Guidelines for the protection of aquatic ecosystems, toxicant trigger values: Iron - Marine. Australian and New Zealand guidelines for fresh and marine water quality. Draft December 2015. Council of Australian Governments Standing Council on Environment and Water, Canberra, ACT, Australia.

Golding LA, Angel BM, Batley GE, Apte SC, Krassoi R and Doyle CJ (2015b). Derivation of a water quality guideline for aluminium in marine waters. Environmental Toxicology and Chemistry 34(1), 141-151.

Guillard RR and Ryther JH (1962). Studies of marine planktonic diatoms. *I. Cyclotella nana* Hustedt, and *Detonula confervacea* (cleve) Gran. Canadian Journal of Microbiology 8, 229-239.

Howe PL, Reichelt-Brushett AJ and Clark MW (2014). Development of a chronic, early life-stage sub-lethal toxicity test and recovery assessment for the tropical zooxanthellate sea anemone *Aiptasia pulchella*. Ecotoxicology and Environmental Safety 100, 138-147.

ISO (2015). Water quality - Calanoid copepod early-life stage test with *Acartia tonsa*. Reference number ISO 16778:2015(E). ISO Geneva, Switzerland.

King CK, Dowse MC and Simpson SL (2010). Toxicity of metals to the bivalve tellina deltoidalis and relationships between metal bioaccumulation and metal partitioning between seawater and marine sediments. Archives of Environmental Contamination and Toxicology 58, 657-665.

Krassoi R (1995). Salinity adjustment of effluents for use with marine bioassays: effects on the larvae of the doughboy scallop *Chlamys asperrimus* and the Sydney rock oyster *Saccostrea commercialis*. . Australasian Journal of Ecotoxicology 1, 143-148.

Milione M and Zeng C (2007). The effects of algal diets on population growth and egg hatching success of the tropical calanoid copepod, *Acartia sinjiensis*. Aquaculture 273, 656-664.

OECD (2002) .Guidelines for Testing of Chemicals No. 201: Freshwater algae and cyanobacteria, growth Inhibition test Organization for Economic Cooperation and Development (OECD) 1-25.

OECD (2005). OECD Draft Guidelines for Testing of Chemicals. Proposal for a New Guideline, Calanoid Copepod Development and Reproduction Test with *Acartia tonsa*. Final Draft Document, Paris.

Simon J and Laginestra E (1997). Bioassay for testing sublethal toxicity in effluents, using gametes of sea urchin *Heliocidaris tuberculata*. National Pulp Mills Research Program Technical Report No. 20. Canberra: CSIRO, 36 pp.

Simpson SL, Campana O and Ho KT (2016). Sediment Toxicity Testing. In: Marine Ecotoxicology: Current Knowledge and Future Issues. Blasco J, Campana O, Chapman P, Hampel M, eds, Elsevier, Amsterdam, Netherlands, pp 199-237.

Simpson SL and Kumar A (2016). Sediment Ecotoxicology. In: Sediment Quality Assessment: A Practical Guide. Simpson SL, Batley GE, eds, CSIRO Publishing, Clayton, Vic, pp 77-122.

Simpson SL and Spadaro DA (2011). Performance and sensitivity of rapid sublethal sediment toxicity tests with the amphipod *Melita plumulosa* and copepod *Nitocra spinipes*. Environmental Toxicology and Chemistry 30, 2326-2334.

Simpson SL and Spadaro DA (2016). Bioavailability and chronic toxicity of metal sulfide minerals to benthic marine invertebrates: implications for deep sea exploration, mining and tailings disposal. Environmental Science and Technology 50, 4061-4070.

Simpson SL, Spadaro DA and O'Brien D (2013). Incorporating bioavailability into management limits for copper in sediments contaminated by antifouling paint used in aquaculture. Chemosphere 93, 2499-2506.

Simpson SL, Spadaro DA and Watters A (2014). Newcastle Port Corporation Port-wide Strategy – future capital dredging assessment. CSIRO Wealth from Oceans Report, Lucas Heights, NSW, 549 pp.

Spadaro DA and Simpson SL (2016a). Appendix E. Protocol for 10-day whole-sediment sub-lethal (reproduction) and acute toxicity tests using the epibenthic amphipod *Melita plumulosa*. In Simpson SL, Batley GE (eds), Sediment Quality Assessment: A Practical Guide. CSIRO Publishing, Clayton, Vic, Australia, pp. 265-275.

Spadaro DA and Simpson SL (2016b). Appendix F. Protocol for whole-sediment sub-lethal (reproduction) toxicity tests using the copepod *Nitocra spinipes* (harpacticoid). In Simpson SL, Batley GE (eds), Sediment Quality Assessment: A Practical Guide. CSIRO Publishing, Clayton, Vic, Australia, pp. 276-284.

Spadaro DA and Simpson SL (2016c). Appendix G. Protocols for 10-day whole-sediment lethality toxicity tests and 30-day bioaccumulation tests using the deposit-feeding benthic bivalve *Tellina deltoidalis*. In Simpson SL, Batley GE (eds), Sediment Quality Assessment: A Practical Guide. CSIRO Publishing, Clayton, Vic, Australia, pp. 285-293.

Stauber J, Tsai J, Vaughan GT, Peterson SM and Brockbank CI (1994). Algae as indicators of toxicity of the effluent from bleached eucalypt kraft pulp mills. National Pulp Mills Research Program Technical Report No. 3 Canberra: CSIRO, 146 pp.

Stauber JL and Florence TM (1985). Interactions of copper and manganese: A mechanism by which manganese alleviates copper toxicity to the marine diatom, *Nitzschia closterium* (Ehrenberg) W. Smith. Aquatic Toxicology 7, 241-254.

USEPA (2002a). Short-term methods for measuring the chronic toxicity of effluents and receiving waters to marine and estuarine organisms. Third Edition. United States Environmental

Protection Agency Report EPA-821-R-02-014, Office of Water, Washington DC, USA, 464 pp.

USEPA (2002b). Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. United States Environmental Protection Report EPA-821-R-02-013, Office of Water (4303T), Washington, DC, USA.

Van Dam R, Harford AJ, Houston MA, Hogan AC and Negri AP (2008). Tropical marine toxicity testing in Australia: A review and recommendations. Australasian Journal of Ecotoxicology 14, 55-88.

Warne MSJ, Batley GE, van Dam RA, Chapman JC, Fox DR, Hickey CW and Stauber JL (2015). Revised Method for Deriving Australian and New Zealand Water Quality Guideline Values for Toxicants. Updated January, 2017. Prepared for the Council of Australian Government's Standing Council on Environment and Water (SCEW). Department of Science, Information Technology and Innovation, Brisbane, Queensland, 48 pp. https://publications.csiro.au/rpr/pub?pid=csiro:EP159161.

WGJV (2017). Wafi-Golpu Joint Venture, Wafi-Golpu Project, Physical, Chemical and Biological Sedimentology of the Huon Gulf, Report No 532-1104-FS-REP-0003.

Appendix A - Chemical analyses reports of metals in tailings material

		Dissolved metals (µg/L)												
Sample ID	**Sample Description**	**Ag**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Se**	**V**	**Zn**
CE414-1F	Golpu Tailings Drum 1 (overlying water)	0.07	0.4	0.1	3.4	1.7	69	2.5	2020	34	1.1	1.5	<1	145
CE414-2F	Golpu Tailings Drum 2 (overlying water)	0.14	0.9	0.2	4.4	1.0	19	1.4	3160	62	1.1	8.3	1	287
LOD (3σ)	**---**	**0.04**	**0.2**	**0.1**	**0.1**	**0.4**	**0.1**	**0.2**	**0.1**	**1**	**0.1**	**0.1**	**1**	**2**
Method Code:	---	**C-209**	**C-209**	**C-209**	**C-209**	**C-209**	**C-209**	**C-229**	**C-229**	**C-209**	**C-209**	**C-209**	**C-209**	**C-209**

Dissolved metals (mg/L)					
Ca	**K**	**Mg**	**Na**	**S**	**Cl**
265	54	52	125	295	pending
422	67	88	102	494	pending
0.03	**0.1**	**0.1**	**0.4**	**2**	
C-229	**C-229**	**C-229**	**C-229**	**C-229**	

Physical Chemistry

Sample ID	Sample Description	pH	Conductivity (µS/cm)	Alkalinity (mg/L $CaCO_3$)
CE414-1F	Golpu Tailings Drum 1 (overlying water)	7.4	2211	103
CE414-2F	Golpu Tailings Drum 2 (overlying water)	7.2	2772	76
Method Code:	---	**C-257**	**C-255**	**C-257**

Quality Control

Spike Recoveries

		Spike Recovery (%)												
Sample ID	**Sample Description**	**Ag**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Se**	**V**	**Zn**
CE414-2F	Golpu Tailings Drum 2 (overlying water)	***93***	***101***	***97***	***97***	***95***	***98***	***91***	---	***98***	***99***	***103***	***97***	***94***

Certified Reference Material

	Dissolved metals (µg/L)												
Sample Description	**Ag**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Se**	**V**	**Zn**
Measured TMDA-54.5	13.6	44.8	160	318	432	416	400	301	333	538	35.9	330	524
Certified value	13.3 ± 1.42	45.2 ± 3.95	157 ± 10	317 ± 23.3	438 ± 30.1	417 ± 37	382 ± 32.5	284 ± 21.9	336 ± 23.8	514 ± 37.9	35.5 ± 3.87	349 ± 22.1	545 ± 46.8
Recovery (%)	***102***	***99***	***102***	***100***	***99***	***100***	***105***	***106***	***99***	***105***	***101***	***94***	***96***

Samples received:

CE414-1 received 18/4/2016; CE414-2 received 3/8/2016

Samples analysed by:

Chad Jarolimek

Josh King

Method codes:

C-209: ICP-MS

C-229: ICP-AES

C-255: Conductivity

C-257: Alkalinity by titration

Miscellaneous information:

A sample of the overlying water was filtered (<0.45 µm) and acidified (0.2% v/v HNO_3) prior to analysis for metals

A sample of the overlying water was filtered (<0.45 µm) prior to analysis for pH, conductivity and alkalinity

			µg/g (dry weight)													
Sample ID	**Sample Description**	**Extract**	**Ag**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Hg**	**Mn**	**Ni**	**Pb**	**Se**	**V**	**Zn**
CE414-1	Golpu Tailings Drum 1	AEM	0.19	3.8	0.038	2.3	58	182	5070	<0.01	99	33	2.9	0.12	6.7	392
		TRM	0.53	13	0.10	16	526	915	55100	0.02	300	234	6.1	3.4	83	472
CE414-2	Golpu Tailings Drum 2	AEM	0.07	3.0	0.032	1.8	75	149	4490	<0.01	107	40	3.5	0.01	7.5	432
		TRM	0.54	14	0.07	17	594	929	52200	0.02	296	274	7.0	3.7	79	493
AEM LOD (3σ)	**---**	**---**	**0.01**	**0.01**	**0.001**	**0.001**	**0.003**	**1**	**0.1**	**0.01**	**0.2**	**0.01**	**0.003**	**0.01**	**0.02**	**0.1**
TRM LOD (3σ)	**---**	**---**	**0.001**	**0.02**	**0.003**	**0.001**	**0.2**	**0.4**	**1**	**0.01**	**0.1**	**1**	**0.01**	**0.01**	**0.3**	**0.1**
Method Code:	**---**	**---**	**C-209**	**C-209**	**C-209**	**C-209**	**C-209/C-229**	**C-229**	**C-229**	**C-229**	**C-229**	**C-209/C-229**	**C-209**	**C-209**	**C-209/C229**	**C-229**

Quality Control

Spike Recoveries

			Spike Recovery (%)													
Sample ID	**Sample Description**	**Extract**	**Ag**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Hg**	**Mn**	**Ni**	**Pb**	**Se**	**V**	**Zn**
CE414-2	Golpu Tailings Drum 2	AEM	***99***	***99***	***98***	***100***	***104***	***101***	---	***106***	***104***	***101***	***97***	***96***	***99***	***101***
		TRM	***97***	***98***	***93***	***95***	***98***	***95***	---	***95***	***95***	***94***	***97***	***94***	***98***	***96***

Certified Reference Materials

		Acid Extractable Metals, µg/g (dry weight)													
Sample ID	**Extract**	**Ag**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Hg**	**Mn**	**Ni**	**Pb**	**Se**	**V**	**Zn**
ERM-CC018 (n=2)	AEM	---	16.6	5.4	2.4	41	60	3100	---	137	12.4	266	---	7.32	239
In-house value		---	17.3	5.59	2.80	45.0	63	3356	---	138	13.9	268	---	7.82	243
Recovery (%)		**---**	***96***	***96***	***84***	***91***	***95***	***92***	**---**	***99***	***89***	***99***	**---**	***94***	***98***

		Total Recoverable Metals, µg/g (dry weight)													
Sample ID	**Extract**	**Ag**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Hg**	**Mn**	**Ni**	**Pb**	**Se**	**V**	**Zn**
ERM-CC018 (n=2)	TRM	---	20.1	5.25	4.49	131	76	---	1.67	---	24.2	288	---	16.1	296
Certified Value		---	22.9 ± 1.3	5.4 ± 0.5	5.9 ± 0.4	129 ± 6	80 ± 4	---	1.38 ± 0.06	---	25.8 ± 1.8	289 ± 10	---	19.4 ± 1.0	313 ± 13
Recovery (%)		**---**	***88***	***97***	***76***	***102***	***95***	**---**	***121***	**---**	***94***	***99***	**---**	***83***	***95***
PACS-3 (n=2)	TRM	0.97	25.2	2.18	8.23	52	297	30836	2.76	247	30	163	0.91	69.7	336
In-house Value		1.1	25.3	2.11	8.12	48	297	30442	2.89	240	28	166	0.97	69.6	335
Recovery (%)		***91***	***100***	***103***	***101***	***109***	***100***	***101***	***96***	***103***	***108***	***98***	***94***	***100***	***100***

Replicates

Acid Extractable Metals

			Acid Extractable Metals, µg/g (dry weight)													
Sample ID	**Sample Description**	**Extract**	**Ag**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Hg**	**Mn**	**Ni**	**Pb**	**Se**	**V**	**Zn**
CE414-1	Golpu Tailings Drum 1	AEM	0.26	3.9	0.035	2.2	57	182	5000	<0.01	96	33	2.9	0.12	6.4	387
CE414-1 dup	Golpu Tailings Drum 1 dup		0.12	3.8	0.041	2.3	59	181	5130	<0.01	102	34	3.0	0.12	6.9	397
CE414-1 avg	**Golpu Tailings Drum 1 avg**		**0.19**	**3.8**	**0.038**	**2.3**	**58**	**182**	**5070**	**<0.01**	**99**	**33**	**2.9**	**0.12**	**6.7**	**392**
CE414-2	Golpu Tailings Drum 2	AEM	0.07	2.9	0.032	1.8	75	145	4450	<0.01	106	40	3.4	0.01	7.5	421
CE414-2 dup	Golpu Tailings Drum 2 dup		0.08	3.2	0.033	1.8	75	154	4540	<0.01	109	40	3.5	0.01	7.5	444
CE414-2 avg	**Golpu Tailings Drum 2 avg**		**0.07**	**3.0**	**0.032**	**1.8**	**75**	**149**	**4490**	**<0.01**	**107**	**40**	**3.5**	**0.01**	**7.5**	**432**

Total Recoverable Metals

			Total Recoverable Metals, µg/g (dry weight)													
Sample ID	**Sample Description**	**Extract**	**Ag**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Hg**	**Mn**	**Ni**	**Pb**	**Se**	**V**	**Zn**
CE414-1	Golpu Tailings Drum 1	TRM	0.53	14	0.09	15	521	912	54600	0.02	299	234	6.1	3.4	83	468
CE414-1 dup	Golpu Tailings Drum 1 dup		0.52	13	0.10	16	531	917	55500	0.02	301	234	6.1	3.3	83	477
CE414-1 avg	**Golpu Tailings Drum 1 avg**		**0.53**	**13**	**0.10**	**16**	**526**	**915**	**55100**	**0.02**	**300**	**234**	**6.1**	**3.4**	**83**	**472**
CE414-2	Golpu Tailings Drum 2	TRM	0.55	15	0.07	17	585	934	50200	0.02	293	275	6.9	4.0	79	494
CE414-2 dup	Golpu Tailings Drum 2 dup		0.54	13	0.07	17	602	924	54200	0.02	300	272	7.1	3.5	79	491
CE414-2 avg	**Golpu Tailings Drum 2 avg**		**0.54**	**14**	**0.07**	**17**	**594**	**929**	**52200**	**0.02**	**296**	**274**	**7.0**	**3.7**	**79**	**493**

Sample ID	**Sample Description**	**Moisture content**	**Solids content**
CE414-1	Golpu Tailings Drum 1	22	78
CE414-2	Golpu Tailings Drum 2	28	72
Method Code:	**---**	**C-202**	**C-202**

Samples received:

CE414-1 received 18/4/2016; CE414-2 received 3/8/2016

Samples analysed by:

Chad Jarolimek

Josh King

Method codes:

C-202: %Moisture % Solids

C-209: ICP-MS

C-229: ICP-AES

C-223: Total recoverable metals

C-241: Dilute acid extractable metals

Miscellaneous information:

TRM analysis performed on dry sample

AEM analysis performed on wet sample, results reported on dry weight basis

			Dissolved metals (µg/L)													
Sample ID	**Sample Description**	**pH**	**Ag**	**Al**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Se**	**V**	**Zn**
SW Blk-1	Cronulla seawater	8.07	0.021	3	1.9	0.005	<0.01	0.33	0.22	0.4	0.4	0.18	0.02	0.65	2.2	0.42
SW Blk-2	Cronulla seawater	8.07	0.015	4	1.9	0.012	<0.01	0.31	0.32	0.6	0.4	0.19	0.01	0.71	2.3	0.47
SW Blk-3	Cronulla seawater	8.07	0.015	3	1.9	0.004	<0.01	0.27	0.25	0.6	0.3	0.17	0.01	0.63	2.4	0.46
Avg Blank	**Cronulla seawater**	**8.07**	**0.017**	**3**	**1.9**	**0.007**	**<0.01**	**0.30**	**0.26**	**0.5**	**0.4**	**0.18**	**0.02**	**0.66**	**2.3**	**0.45**
CE414-1 1,000x R1	Drum-1 1,000x R1	8.08	0.024	9	1.6	0.015	0.19	0.23	11	0.6	11	1.1	<0.01	0.35	1.3	8.5
CE414-1 1,000x R2	Drum-1 1,000x R2	8.08	0.023	8	1.5	0.020	0.20	0.21	11	1.0	11	1.1	<0.01	0.57	1.3	8.7
CE414-1 1,000x R3	Drum-1 1,000x R3	8.06	0.021	8	1.5	0.020	0.19	0.17	11	0.8	10	1.1	0.01	0.52	1.3	8.9
CE414-1 1,000x avg	**Drum-1 1,000x avg**	**8.07**	**0.022**	**9**	**1.5**	**0.019**	**0.19**	**0.21**	**11**	**0.8**	**11**	**1.1**	**<0.01**	**0.48**	**1.3**	**8.7**
CE414-1 100x R1	Drum-1 100x R1	7.94	0.042	10	0.69	0.11	1.6	0.31	20	0.9	96	8.6	0.01	1.0	0.33	24
CE414-1 100x R2	Drum-1 100x R2	7.94	0.041	11	0.73	0.11	1.5	0.31	19	0.7	95	8.4	0.01	0.94	0.27	24
CE414-1 100x R3	Drum-1 100x R3	7.88	0.038	10	0.75	0.11	1.5	0.28	19	0.8	96	8.5	0.01	0.93	0.27	24
CE414-1 100x avg	**Drum-1 100x avg**	**7.92**	**0.040**	**10**	**0.72**	**0.11**	**1.5**	**0.30**	**19**	**0.8**	**96**	**8.5**	**0.01**	**0.97**	**0.29**	**24**
CE414-2 1,000x R1	Drum-2 1,000x R1	8.05	0.021	8	1.6	0.016	0.17	0.16	11	0.6	8.4	1.4	0.02	0.34	1.5	12
CE414-2 1,000x R2	Drum-2 1,000x R2	8.12	0.020	7	1.6	0.012	0.17	0.13	11	0.6	8.3	1.4	0.02	0.54	1.5	12
CE414-2 1,000x R3	Drum-2 1,000x R3	8.05	0.018	6	1.7	0.012	0.17	0.14	11	2.1	8.3	1.4	<0.01	0.58	1.5	12
CE414-2 1,000x avg	**Drum-2 1,000x avg**	**8.07**	**0.020**	**7**	**1.6**	**0.013**	**0.17**	**0.14**	**11**	**1.1**	**8**	**1.4**	**0.02**	**0.49**	**1.5**	**12**
CE414-2 100x R1	Drum-2 100x R1	7.98	0.035	9	0.80	0.084	1.5	0.26	19	0.6	74	10	<0.01	0.99	0.32	41
CE414-2 100x R2	Drum-2 100x R2	7.98	0.033	9	0.68	0.091	1.5	0.24	20	0.6	76	11	<0.01	1.1	0.35	43
CE414-2 100x R3	Drum-2 100x R3	7.97	0.031	9	0.81	0.067	1.5	0.27	20	0.9	76	11	<0.01	1.1	0.32	43
CE414-2 100x avg	**Drum-2 100x avg**	**7.98**	**0.033**	**9**	**0.76**	**0.081**	**1.5**	**0.25**	**20**	**0.7**	**76**	**11**	**<0.01**	**1.1**	**0.33**	**43**
LOD (3σ)	---	---	*0.002*	*1*	*0.01*	*0.001*	*0.01*	*0.02*	*0.003*	*0.3*	*0.1*	*0.02*	*0.01*	*0.04*	*0.004*	*0.03*
Method code	---	C-241	C-209	C-229	C-209	C-209	C-209	C-209	C-209	C-229	C-229	C-209	C-209	C-209	C-209	C-209

Quality Control:

Certified Reference Materials

			Dissolved metals (µg/L)													
Sample ID			**Ag**	**Al**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Se**	**V**	**Zn**
CASS-6	---	---	---	---	---	0.017	0.062	---	0.555	1.68	1.88	0.43	---	---	---	1.68
CASS-6 dup	---	---	---	---	---	0.028	0.061	---	0.564	1.77	1.90	0.40	---	---	---	1.48
CASS-6 avg	---	---	---	---	---	0.022	0.061	---	0.559	1.73	1.89	0.41	---	---	---	1.58
Certified Value	---	---	---	---	---	0.0217 ± 0.0018	0.0672 ± 0.0052	---	0.530 ± 0.032	1.56 ± 0.12	2.22 ± 0.12	0.418 ± 0.040	0.0106 ± 0.0040	---	---	1.27 ± 0.18
Recovery (%)	**---**	**---**	**---**	**---**	**---**	***103***	***91***	**---**	***106***	***111***	***85***	***99***	**---**	**---**	**---**	***124***
Method code	---	C-241	C-209	C-229	C-209	C-209	C-209	C-209	C-209	C-229	C-229	C-209	C-209	C-209	C-209	C-209

			Dissolved metals (µg/L)													
Sample ID			**Ag**	**Al**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Se**	**V**	**Zn**
NASS-6	---	---	---	---	1.61	0.027	---	0.21	0.331	---	---	0.32	---	---	1.76	---
NASS-6 dup	---	---	---	---	1.57	0.033	---	0.18	0.283	---	---	0.32	---	---	1.70	---
NASS-6 avg	---	---	---	---	1.59	0.030	---	0.20	0.307	---	---	0.32	---	---	1.73	---
Certified Value	---	---	---	---	1.43 ± 0.12	0.0311 ± 0.0019	---	0.118 ± 0.008	0.248 ± 0.025	---	---	0.301 ± 0.025	0.006 ± 0.002	---	1.46 ± 0.17	0.257 ± 0.020
Recovery (%)	**---**	**---**	**---**	**---**	***111***	***97***	**---**	***167***	***124***	**---**	**---**	***106***	**---**	**---**	***119***	**---**
Method code	---	C-241	C-209	C-229	C-209	C-209	C-209	C-209	C-209	C-229	C-229	C-209	C-209	C-209	C-209	C-209

Spike Recoveries

			Spike Recovery (%)													
Sample ID	**Sample Description**		**Ag**	**Al**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Se**	**V**	**Zn**
CE414-1 1,000x R3	Drum-1 1,000x R3		88	---	107	87	104	115	93	---	---	99	86	92	121	84
CE414-2 100x R3	Drum-2 100x R3		88	---	108	86	103	112	91	---	---	97	85	92	119	84
Method code	---	C-241	C-209	C-229	C-209	C-209	C-209	C-209	C-209	C-229	C-229	C-209	C-209	C-209	C-209	C-209

Method codes:

C-209: ICP-MS
C-229: ICP-AES
C-241: pH determination

Job number: CE414
Report date: 19/04/2017
Report number: CE414/1
Josh King

			Dissolved metals (µg/L)													
Sample ID	**Sample Description**	**pH**	**Ag**	**Al**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Se**	**V**	**Zn**
SW Blk-1	Cronulla seawater	8.07		5	2.0	<0.01	<0.01	0.36	0.30	1	0.8	0.19	0.04	0.24	2.3	0.7
SW Blk-2	Cronulla seawater	8.07		5	1.9	<0.01	<0.01	0.36	0.30	<1	0.8	0.21	0.03	0.20	2.3	0.6
SW Blk-3	Cronulla seawater	8.07		4	2.1	<0.01	<0.01	0.35	0.30	<1	0.8	0.19	0.03	0.17	2.4	0.6
Avg Blank	**Cronulla seawater**	**8.07**		**5**	**2.0**	**<0.01**	**<0.01**	**0.36**	**0.30**	**<1**	**0.8**	**0.20**	**0.03**	**0.20**	**2.4**	**0.7**
CE414-1 10,000x R1	Drum-1 10,000x R1	8.03		5	2.0	<0.01	0.02	0.32	3.0	1	1.8	0.29	<0.01	0.17	2.3	1.7
CE414-1 10,000x R2	Drum-1 10,000x R2	8.01		9	2.0	<0.01	0.02	0.28	3.2	1	1.8	0.33	<0.01	0.11	2.3	1.9
CE414-1 10,000x R3	Drum-1 10,000x R3	8.04		5	2.0	<0.01	0.02	0.27	3.2	1	1.8	0.29	<0.01	0.16	2.2	1.7
CE414-1 10,000x avg	**Drum-1 10,000x avg**	**8.03**		**6**	**2.0**	**<0.01**	**0.02**	**0.29**	**3.1**	**1**	**1.8**	**0.30**	**<0.01**	**0.15**	**2.3**	**1.8**
CE414-2 10,000x R1	Drum-2 10,000x R1	8.05		5	1.9	<0.01	0.02	0.27	3.1	<1	1.7	0.35	<0.01	0.14	2.3	2.5
CE414-2 10,000x R2	Drum-2 10,000x R2	8.04		5	1.9	<0.01	0.02	0.25	3.2	<1	1.7	0.37	<0.01	0.14	2.3	2.4
CE414-2 10,000x R3	Drum-2 10,000x R3	8.02		5	2.0	<0.01	0.02	0.23	3.2	<1	1.7	0.37	<0.01	0.14	2.3	2.4
CE414-2 10,000x avg	**Drum-2 10,000x avg**	**8.04**		**5**	**1.9**	**<0.01**	**0.02**	**0.25**	**3.1**	**<1**	**1.7**	**0.36**	**<0.01**	**0.14**	**2.3**	**2.4**
LOD (3σ)	---	---		*1*	*0.01*	*0.01*	*0.01*	*0.02*	*0.003*	*1*	*0.03*	*0.01*	*0.01*	*0.01*	*0.001*	*0.2*
Method code	---	C-241	C-209	C-229	C-209	C-209	C-209	C-209	C-209	C-229	C-229	C-209	C-209	C-209	C-209	C-209

Quality Control:

Certified Reference Materials

			Dissolved metals (µg/L)													
Sample ID			**Ag**	**Al**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Se**	**V**	**Zn**
CASS-6	---	---	---	---	---	0.020	0.069	---	0.573	1.48	2.09	0.44	0.016	---	---	1.19
CASS-6 dup	---	---	---	---	---	0.024	0.067	---	0.560	1.44	2.12	0.45	0.010	---	---	1.22
CASS-6 avg	---	---	---	---	---	0.022	0.068	---	0.566	1.46	2.10	0.44	0.013	---	---	1.20
Certified Value	---	---	---	---	---	0.0217 ± 0.0018	0.0672 ± 0.0052	---	0.530 ± 0.032	1.56 ± 0.12	2.22 ± 0.12	0.418 ± 0.040	0.0106 ± 0.0040	---	---	1.27 ± 0.18
Recovery (%)	**---**	**---**	**---**	**---**	**---**	***101***	***101***	**---**	***107***	***94***	***95***	***105***	***122***	**---**	**---**	***95***
Method code	---	---	C-209	C-229	C-209	C-209	C-209	C-209	C-209	C-229	C-229	C-209	C-209	C-209	C-209	C-209

			Dissolved metals (µg/L)													
Sample ID			**Ag**	**Al**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Se**	**V**	**Zn**
NASS-6	---	---	---	---	1.65	0.029	---	---	0.244	---	---	0.33	---	---	1.67	---
NASS-6 dup	---	---	---	---	1.67	0.026	---	---	0.322	---	---	0.34	---	---	1.75	---
NASS-6 avg	---	---	---	---	1.66	0.027	---	---	0.283	---	---	0.34	---	---	1.71	---
Certified Value	---	---	---	---	1.43 ± 0.12	0.0311 ± 0.0019	---	0.118 ± 0.008	0.248 ± 0.025	---	---	0.301 ± 0.025	0.006 ± 0.002	---	1.46 ± 0.17	0.257 ± 0.020
Recovery (%)	**---**	**---**	**---**	**---**	***116***	***88***	**---**	**---**	***114***	**---**	**---**	***111***	**---**	**---**	***117***	**---**
Method code	---	---	C-209	C-229	C-209	C-209	C-209	C-209	C-209	C-229	C-229	C-209	C-209	C-209	C-209	C-209

Spike Recoveries

			Spike Recovery (%)													
Sample ID	**Sample Description**	**---**	**Ag**	**Al**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Se**	**V**	**Zn**
CE414-2 10,000x R3	Drum-2 10,000x R3	---		---	116	88	115	123	101	---	---	107	86	89	131	89
Method code	---	---	C-209	C-229	C-209	C-209	C-209	C-209	C-209	C-229	C-229	C-209	C-209	C-209	C-209	C-209

Method codes:
C-209: ICP-MS
C-229: ICP-AES
C-241: pH determination

Job number: CE414
Report date: 09/05/17
Report number: CE414/2
Josh King

Sample Labels	Final pH	Ag 328.068	Al 167.019	As 188.980	Cd 214.439	Co 228.615	Cr 205.560	Cu 324.754	Fe 238.204	Mn 257.610	Mo 202.032	Ni 231.604	Pb 220.353	Zn 213.857
		Blank Corrected (ug/L)												
SW Blk-1		1.0	1	3	0	1	0.4	0	0.3	0.3	10	1	5	1
SW Blk-2	8.07	1.0	2	4	0	-1	0.3	-1	0.4	0.3	11	2	3	1
SW Blk-3		1.0	1	3	0	2	0.4	0	0.3	0.3	11	1	4	1
Avg SW Blk		**1.0**	**1**	**3**	**0**	**1**	**0.3**	**0**	**0.3**	**0.3**	**11**	**1**	**4**	**1**
LOD (3 σ)		***0.1***	***1***	***2***	***1***	***4***	***0.2***	***1***	***0.3***	***0.01***	***2***	***1***	***3***	***1***
CE414-1 rep 1	7.35	1.3	4	3	2	10	0.2	58	0.8	1193	35	63	3	248
CE414-1 rep 2	7.33	1.3	4	2	1	13	0.0	56	0.8	1125	35	63	5	241
CE414-1 rep 3	7.33	1.4	5	2	1	10	0.1	57	0.9	1118	34	62	4	239
CE414-1 avg	**7.34**	**1.3**	**5**	**2**	**1**	**11**	**0.1**	**57**	**0.8**	**1146**	**35**	**62**	**4**	**243**
CE414-2 rep 1	7.29	1.1	4	3	1	12	2.1	71	0.4	785	41	92	3	683
CE414-2 rep 2	7.33	1.1	4	2	1	12	2.5	68	0.7	758	40	90	3	606
CE414-2 rep 3	7.33	1.4	4	3	1	12	2.6	70	0.5	790	40	91	4	615
CE414-2 avg	**7.32**	**1.2**	**4**	**3**	**1**	**12**	**2.4**	**70**	**0.5**	**778**	**40**	**91**	**4**	**634**
SLEW-3		0.3	1	3	0	0	0	1.8	0.754	1.60	5.5	2	9	0.298
Certified Value								1.55 ± 0.12	0.568 ± 0.059	1.61 ± 0.22	5.1			0.201 ± 0.037
CASS-5		1.0	1	2	0	1	0	-0.790	1.60	2.38	10.2	1	4	1.327
Certified Value									1.44 ± 0.11	2.62 ± 0.20	9.82 ± 0.72			

Sample Labels	Final pH	Ag 328.068	Al 167.019	As 188.980	Cd 214.439	Co 228.615	Cr 205.560	Cu 324.754	Fe 238.204	Mn 257.610	Mo 202.032	Ni 231.604	Pb 220.353	Zn 213.857
		Blank Corrected (ug/L)												
SW Blk-1		1.0	1	3	0	1	0.4	0	0.3	0.3	10	1	5	1
SW Blk-2	8.07	1.0	2	4	0	-1	0.3	-1	0.4	0.3	11	2	3	1
SW Blk-3		1.0	1	3	0	2	0.4	0	0.3	0.3	11	1	4	1
Avg SW Blk		**1.0**	**1**	**3**	**0**	**1**	**0.3**	**0**	**0.3**	**0.3**	**11**	**1**	**4**	**1**
LOD (3 σ)		***0.1***	***1***	***2***	***1***	***4***	***0.2***	***1***	***0.3***	***0.01***	***2***	***1***	***3***	***1***
1:10 dilution														
CE414-1 rep 1	7.35	1.3	4	3	2	10	0.2	58	0.8	1193	35	63	3	248
CE414-1 rep 2	7.33	1.3	4	2	1	13	0.0	56	0.8	1125	35	63	5	241
CE414-1 rep 3	7.33	1.4	5	2	1	10	0.1	57	0.9	1118	34	62	4	239
CE414-1 avg	**7.34**	**1.3**	**5**	**2**	**1**	**11**	**0.1**	**57**	**0.8**	**1146**	**35**	**62**	**4**	**243**
1:10 dilution														
CE414-2 rep 1	7.29	1.1	4	3	1	12	2.1	71	0.4	785	41	92	3	683
CE414-2 rep 2	7.33	1.1	4	2	1	12	2.5	68	0.7	758	40	90	3	606
CE414-2 rep 3	7.33	1.4	4	3	1	12	2.6	70	0.5	790	40	91	4	615
CE414-2 avg	**7.32**	**1.2**	**4**	**3**	**1**	**12**	**2.4**	**70**	**0.5**	**778**	**40**	**91**	**4**	**634**
SLEW-3		0.3	1	3	0	0	0	1.8	0.754	1.60	5.5	2	9	0.298
Certified Value								1.55 ± 0.12	0.568 ± 0.059	1.61 ± 0.22	5.1			0.201 ± 0.037
CASS-5		1.0	1	2	0	1	0	-0.790	1.60	2.38	10.2	1	4	1.327
Certified Value									1.44 ± 0.11	2.62 ± 0.20	9.82 ± 0.72			

					Dissolved metals (µg/L)													
Sample ID	**Sample Description**	**Time Point**	**Hours**	**pH**	**Ag**	**Al**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Se**	**V**	**Zn**
SW Blk-1	Cronulla seawater	0	0	8.05		4	2.0	<0.01	<0.01	0.23	0.33	1	0.72	0.20	0.02	0.16	2.3	0.5
SW Blk-2	Cronulla seawater	0	0	8.03		4	2.0	<0.01	<0.01	0.20	0.31	<1	0.76	0.18	0.02	0.18	2.3	0.4
SW Blk-3	Cronulla seawater	0	0	8.04		4	1.9	<0.01	<0.01	0.19	0.32	<1	0.77	0.19	0.02	0.20	2.3	0.5
Avg Blank	**Cronulla seawater**	**0**	**0**	**8.04**		**4**	**2.0**	**<0.01**	**<0.01**	**0.21**	**0.32**	**<1**	**0.75**	**0.19**	**0.02**	**0.18**	**2.3**	**0.5**
CE414-1 10 R1	Drum-1 10x R1	0	0	7.74		5	0.92	0.46	1.1	0.15	5.0	1	689	15	<0.01	0.42	0.55	27
CE414-1 10x R2	Drum-1 10x R2	0	0	7.79		4	1.0	0.04	1.1	0.16	4.5	<1	695	8.1	<0.01	0.46	0.54	27
CE414-1 10x R3	Drum-1 10x R3	0	0	7.77		5	0.87	0.05	1.1	0.13	4.5	<1	697	8.1	<0.01	0.46	0.52	27
CE414-1 10x avg	**Drum-1 10x avg**	**0**	**0**	**7.77**		**5**	**0.93**	**0.18**	**1.1**	**0.15**	**4.7**	**<1**	**694**	**11**	**<0.01**	**0.45**	**0.54**	**27**
CE414-2 10x R1	Drum-2 10x R1	0	0	7.77		7	1.1	0.02	1.5	0.12	3.6	<1	787	17	<0.01	0.53	0.63	47
CE414-2 10x R2	Drum-2 10x R2	0	0	7.79		5	1.0	0.02	1.5	0.11	3.6	<1	772	16	<0.01	0.49	0.60	45
CE414-2 10x R3	Drum-2 10x R3	0	0	7.80		5	0.98	0.03	1.5	0.11	3.6	<1	734	16	<0.01	0.54	0.64	42
CE414-2 10x avg	**Drum-2 10x avg**	**0**	**0**	**7.79**		**6**	**1.0**	**0.02**	**1.5**	**0.11**	**3.6**	**<1**	**764**	**16**	**<0.01**	**0.52**	**0.62**	**45**
SW Blk	Cronulla seawater	10 min	0.17	8.07		5	2.0	<0.01	<0.01	0.17	0.39	2	0.76	0.19	0.02	0.18	2.3	0.4
CE414-1 10 R1	Drum-1 10x R1	10 min	0.17	7.75		7	0.89	0.46	1.7	0.12	7.2	1	750	19	<0.01	0.60	0.40	38
CE414-1 10x R2	Drum-1 10x R2	10 min	0.17	7.75		5	0.84	0.11	1.6	0.13	6.6	1	758	12	<0.01	0.60	0.41	36
CE414-1 10x R3	Drum-1 10x R3	10 min	0.17	7.82		5	0.85	0.10	1.6	0.13	6.3	<1	735	11	<0.01	0.65	0.46	35
CE414-1 10x avg	**Drum-1 10x avg**	**10 min**	**0.17**	**7.77**		**6**	**0.86**	**0.22**	**1.6**	**0.13**	**6.7**	**1**	**747**	**14**	**<0.01**	**0.61**	**0.42**	**36**
CE414-2 10x R1	Drum-2 10x R1	10 min	0.17	7.76		6	1.0	0.07	2.2	0.14	5.4	<1	878	24	<0.01	0.80	0.56	70
CE414-2 10x R2	Drum-2 10x R2	10 min	0.17	7.79		7	0.93	0.07	2.2	0.14	5.5	<1	837	23	<0.01	0.76	0.58	67
CE414-2 10x R3	Drum-2 10x R3	10 min	0.17	7.80		5	0.99	0.06	2.1	0.11	5.2	<1	860	23	<0.01	0.81	0.59	61
CE414-2 10x avg	**Drum-2 10x avg**	**10 min**	**0.17**	**7.78**		**6**	**0.97**	**0.07**	**2.2**	**0.13**	**5.3**	**<1**	**858**	**23**	**<0.01**	**0.79**	**0.57**	**66**
SW Blk	Cronulla seawater	1 hr	1	8.12		11	1.8	<0.01	<0.01	0.15	0.36	<1	0.76	0.19	0.02	0.17	2.2	0.5
CE414-1 10 R1	Drum-1 10x R1	1 hr	1	7.72		5	0.89	0.64	3.1	0.14	13	<1	827	26	<0.01	1.1	0.41	63
CE414-1 10x R2	Drum-1 10x R2	1 hr	1	7.75		5	0.69	0.32	3.0	0.15	13	<1	718	20	<0.01	1.0	0.43	61
CE414-1 10x R3	Drum-1 10x R3	1 hr	1	7.76		6	0.83	0.32	3.0	0.15	12	<1	950	20	<0.01	1.2	0.45	58
CE414-1 10x avg	**Drum-1 10x avg**	**1 hr**	**1**	**7.74**		**5**	**0.80**	**0.43**	**3.0**	**0.15**	**13**	**<1**	**832**	**22**	**<0.01**	**1.1**	**0.43**	**61**
CE414-2 10x R1	Drum-2 10x R1	1 hr	1	7.69		6	0.93	0.20	4.3	0.25	14	<1	928	44	<0.01	1.7	0.62	151
CE414-2 10x R2	Drum-2 10x R2	1 hr	1	7.66		5	0.98	0.20	4.2	0.26	14	1	920	43	<0.01	1.8	0.62	144
CE414-2 10x R3	Drum-2 10x R3	1 hr	1	7.78		5	0.80	0.20	4.2	0.26	13	<1	856	42	<0.01	2.1	0.66	134
CE414-2 10x avg	**Drum-2 10x avg**	**1 hr**	**1**	**7.71**		**5**	**0.90**	**0.20**	**4.2**	**0.26**	**14**	**<1**	**901**	**43**	**<0.01**	**1.9**	**0.63**	**143**
SW Blk	Cronulla seawater	6 hr	6	8.02		4	1.8	<0.01	<0.01	0.24	0.2	<1	0.75	0.16	<0.01	0.25	2.2	0.1
CE414-1 10 R1	Drum-1 10x R1	6 hr	6	7.60		5	0.66	1.2	7.2	0.31	38	1	1110	47	<0.01	1.8	0.24	128
CE414-1 10x R2	Drum-1 10x R2	6 hr	6	7.53		6	0.71	0.78	7.2	0.29	38	<1	997	42	<0.01	1.8	0.25	127
CE414-1 10x R3	Drum-1 10x R3	6 hr	6	7.60		6	0.62	0.74	7.3	0.30	38	1	969	43	<0.01	1.9	0.24	125
CE414-1 10x avg	**Drum-1 10x avg**	**6 hr**	**6**	**7.58**		**6**	**0.66**	**0.90**	**7.3**	**0.30**	**38**	**1**	**1020**	**44**	**<0.01**	**1.8**	**0.24**	**127**
CE414-2 10x R1	Drum-2 10x R1	6 hr	6	7.47		5	0.73	0.56	9.9	0.49	51	1	914	80	<0.01	2.4	0.29	445
CE414-2 10x R2	Drum-2 10x R2	6 hr	6	7.46		5	0.78	0.53	10	0.39	50	<1	961	80	<0.01	2.5	0.29	437
CE414-2 10x R3	Drum-2 10x R3	6 hr	6	7.71		5	0.72	0.53	9.9	0.41	50	<1	734	79	<0.01	2.6	0.29	353
CE414-2 10x avg	**Drum-2 10x avg**	**6 hr**	**6**	**7.55**		**5**	**0.74**	**0.54**	**9.9**	**0.43**	**50**	**<1**	**869**	**80**	**<0.01**	**2.5**	**0.29**	**412**
SW Blk	Cronulla seawater	8 hr	8	8.03		4	1.9	<0.01	<0.01	0.24	0.2	<1	0.69	0.23	<0.01	0.30	2.3	0.2
CE414-1 10 R1	Drum-1 10x R1	8 hr	8	7.54		5	0.65	1.2	7.8	0.29	41	<1	914	49	<0.01	2.0	0.25	133
CE414-1 10x R2	Drum-1 10x R2	8 hr	8	7.57		5	0.63	0.77	7.7	0.26	40	<1	891	45	<0.01	2.0	0.26	134
CE414-1 10x R3	Drum-1 10x R3	8 hr	8	7.52		5	0.69	0.79	7.6	0.27	40	<1	906	44	<0.01	2.2	0.24	130
CE414-1 10x avg	**Drum-1 10x avg**	**8 hr**	**8**	**7.54**		**5**	**0.66**	**0.93**	**7.7**	**0.27**	**40**	**<1**	**903**	**46**	**<0.01**	**2.1**	**0.25**	**132**
CE414-2 10x R1	Drum-2 10x R1	8 hr	8	7.48		5	0.80	0.57	10	0.29	52	<1	896	81	<0.01	2.7	0.30	446
CE414-2 10x R2	Drum-2 10x R2	8 hr	8	7.47		4	0.67	0.59	10	0.35	52	<1	839	81	<0.01	2.7	0.29	438
CE414-2 10x R3	Drum-2 10x R3	8 hr	8	7.50		5	0.66	0.58	10	0.33	53	<1	888	81	<0.01	2.9	0.29	422
CE414-2 10x avg	**Drum-2 10x avg**	**8 hr**	**8**	**7.48**		**5**	**0.71**	**0.58**	**10**	**0.32**	**53**	**<1**	**874**	**81**	**<0.01**	**2.8**	**0.29**	**435**
SW Blk	Cronulla seawater	24 hr	24	8.05		4	1.8	0.01	<0.01	0.23	0.2	1	0.74	0.24	<0.01	0.41	2.2	0.2

CE414-1 10 R1	Drum-1 10x R1	24 hr	24	7.19		4	0.59	1.4	11	0.12	56	<1	1000	68	<0.01	2.7	0.19	227
CE414-1 10x R2	Drum-1 10x R2	24 hr	24	7.35		3	0.55	0.99	11	0.09	56	<1	966	62	<0.01	2.9	0.21	225
CE414-1 10x R3	Drum-1 10x R3	24 hr	24	7.13		3	0.61	1.0	11	0.08	56	<1	1030	63	<0.01	2.8	0.21	225
CE414-1 10x avg	**Drum-1 10x avg**	**24 hr**	**24**	**7.22**		**3**	**0.58**	**1.1**	**11**	**0.09**	**56**	**<1**	**997**	**64**	**<0.01**	**2.8**	**0.20**	**226**
CE414-2 10x R1	Drum-2 10x R1	24 hr	24	7.13		3	0.52	0.86	14	0.06	74	<1	964	103	<0.01	3.6	0.21	764
CE414-2 10x R2	Drum-2 10x R2	24 hr	24	7.23		3	0.49	0.85	14	0.06	73	<1	1010	103	<0.01	3.5	0.22	788
CE414-2 10x R3	Drum-2 10x R3	24 hr	24	7.12		3	0.56	0.87	15	0.07	73	<1	961	104	<0.01	3.8	0.24	754
CE414-2 10x avg	**Drum-2 10x avg**	**24 hr**	**24**	**7.16**		**3**	**0.52**	**0.86**	**14**	**0.06**	**73**	**<1**	**979**	**103**	**<0.01**	**3.6**	**0.22**	**769**
SW Blk	Cronulla seawater	48 hr	48	8.07		4	1.9	<0.01	<0.01	0.20	0.2	<1	0.70	0.19	<0.01	0.47	2.2	0.2
CE414-1 10 R1	Drum-1 10x R1	48 hr	48	7.24		2	0.55	1.5	14	0.07	62	<1	1250	78	<0.01	3.4	0.25	267
CE414-1 10x R2	Drum-1 10x R2	48 hr	48	7.21		3	0.48	1.0	13	0.06	61	2	1270	72	<0.01	3.3	0.27	261
CE414-1 10x R3	Drum-1 10x R3	48 hr	48	7.27		3	0.45	1.1	13	0.04	60	<1	1320	73	<0.01	3.5	0.28	259
CE414-1 10x avg	**Drum-1 10x avg**	**48 hr**	**48**	**7.24**		**2**	**0.49**	**1.2**	**13**	**0.06**	**61**	**1**	**1280**	**74**	**<0.01**	**3.4**	**0.26**	**262**
CE414-2 10x R1	Drum-2 10x R1	48 hr	48	7.15		2	0.51	0.94	17	0.03	82	<1	1150	118	<0.01	4.0	0.31	783
CE414-2 10x R2	Drum-2 10x R2	48 hr	48	7.14		2	0.52	0.93	17	0.34	81	<1	1250	119	<0.01	4.1	0.39	799
CE414-2 10x R3	Drum-2 10x R3	48 hr	48	7.13		2	0.47	0.90	17	0.04	80	<1	1270	119	<0.01	4.0	0.35	873
CE414-2 10x avg	**Drum-2 10x avg**	**48 hr**	**48**	**7.14**		**2**	**0.50**	**0.92**	**17**	**0.14**	**81**	**<1**	**1220**	**119**	**<0.01**	**4.0**	**0.35**	**818**
SW Blk-1	Cronulla seawater	72 hr	72	8.10		4	1.7	<0.01	0.01	0.18	0.2	<1	0.66	2.2	<0.01	0.79	2.3	0.2
SW Blk-2	Cronulla seawater	72 hr	72	8.12		4	1.8	0.01	<0.01	0.18	0.2	1	0.66	0.79	<0.01	1.2	2.4	0.1
SW Blk-3	Cronulla seawater	72 hr	72	8.12		4	1.9	<0.01	<0.01	0.18	0.2	<1	0.68	1.2	<0.01	1.4	2.3	0.1
Avg Blank	**Cronulla seawater**	**72 hr**	**72**	**8.11**		**4**	**1.8**	**<0.01**	**<0.01**	**0.18**	**0.2**	**<1**	**0.67**	**1.4**	**<0.01**	**1.1**	**2.4**	**0.1**
CE414-1 10 R1	Drum-1 10x R1	72 hr	72	7.08		2	0.51	1.5	14	0.04	59	<1	1470	76	<0.01	4.5	0.45	251
CE414-1 10x R2	Drum-1 10x R2	72 hr	72	7.11		2	0.49	1.2	14	0.05	59	<1	1370	71	<0.01	5.2	0.45	246
CE414-1 10x R3	Drum-1 10x R3	72 hr	72	7.12		2	0.51	1.1	14	0.05	59	<1	1510	71	<0.01	7.0	0.42	250
CE414-1 10x avg	**Drum-1 10x avg**	**72 hr**	**72**	**7.10**		**2**	**0.50**	**1.3**	**14**	**0.05**	**59**	**<1**	**1450**	**73**	**<0.01**	**5.5**	**0.44**	**249**
CE414-2 10x R1	Drum-2 10x R1	72 hr	72	6.93		2	0.50	0.95	18	0.03	78	<1	1340	112	<0.01	9.2	0.39	743
CE414-2 10x R2	Drum-2 10x R2	72 hr	72	6.90		2	0.55	0.96	18	0.03	76	<1	1450	113	<0.01	12	0.34	768
CE414-2 10x R3	Drum-2 10x R3	72 hr	72	6.95		2	0.49	0.99	18	0.04	75	1	1460	112	<0.01	14	0.31	815
CE414-2 10x avg	**Drum-2 10x avg**	**72 hr**	**72**	**6.93**		**2**	**0.51**	**0.97**	**18**	**0.03**	**77**	**<1**	**1420**	**112**	**<0.01**	**12**	**0.35**	**775**
LOD (3σ)	---			---		*1*	*0.01*	*0.01*	*0.01*	*0.02*	*0.003*	*1*	*0.03*	*0.01*	*0.01*	*0.01*	*0.01*	*0.2*
Method code	---			C-241	C-209	C-229	C-209	C-209	C-209	C-209	C-209	C-229	C-229	C-209	C-209	C-209	C-209	C-209/C-229

Quality Control:

Certified Reference Materials

					Dissolved metals (µg/L)													
Sample ID	**---**	**---**	**---**	**---**	**Ag**	**Al**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Se**	**V**	**Zn**
CASS-6	---	---	---	---	---	---	---	0.020	0.069	---	0.573	1.48	2.09	0.436	---	---	---	
CASS-6 dup	---	---	---	---	---	---	---	0.024	0.067	---	0.560	1.44	2.12	0.446	---	---	---	
CASS-6 avg	---	---	---	---	---	---	---	0.022	0.068	---	0.566	1.46	2.10	0.441	---	---	---	
Certified Value	---	---	---	---	---	---	---	0.0217 ± 0.0018	0.0672 ± 0.0052	---	0.530 ± 0.032	1.56 ± 0.12	2.22 ± 0.12	0.418 ± 0.040	0.0106 ± 0.0040	---	---	1.27 ± 0.18
Recovery (%)	**---**	**---**	**---**	**---**	**---**	**---**	**---**	***101***	***101***	**---**	***107***	***94***	***95***	***105***	**---**	**---**	**---**	
Method code	---	---	---	---	C-209	C-229	C-209	C-209	C-209	C-229	C-209	C-229	C-229	C-209	C-209	C-209	C-209	C-209

					Dissolved metals (µg/L)													
Sample ID	**---**	**---**	**---**	**---**	**Ag**	**Al**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Se**	**V**	**Zn**
NASS-6	---	---	---	---	---	---	1.65	0.029	---	---	0.244	---	---	0.33	---	---	1.67	---
NASS-6 dup	---	---	---	---	---	---	1.67	0.026	---	---	0.322	---	---	0.34	---	---	1.75	---
NASS-6 avg	---	---	---	---	---	---	1.66	0.027	---	---	0.283	---	---	0.34	---	---	1.71	---
Certified Value	---	---	---	---	---	---	1.43 ± 0.12	0.0311 ± 0.0019	---	0.118 ± 0.008	0.248 ± 0.025	---	---	0.301 ± 0.025	0.006 ± 0.002	---	1.46 ± 0.17	0.257 ± 0.020

Recovery (%)	---	---	---	---	---	---	***116***	***88***	---	---	***114***	---	---	***111***	---	---	***117***	---
Method code	---	---	---	---	C-209	C-229	C-209	C-209	C-209	C-229	C-209	C-229	C-229	C-209	C-209	C-209	C-209	C-209

Spike Recoveries

					Spike Recovery (%)													
Sample ID	**Sample Description**	**Time Point**	**---**	**---**	**Ag**	**Al**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Se**	**V**	**Zn**
CE414-2 10x R3	Drum-2 10x R3	1 hr	---	---		---	108	88	102	108	91	---	---	95	86	95	115	---
CE414-1 10x R1	Drum-1 10x R1	48 hr	---	---	87	---	102	87	100	110	89	---	---	92	84	92	114	---
CE414-2 10x R3	Drum-2 10x R3	72 hr	---	---	87	---	101	85	96	104	88	---	---	79	86	82	108	---
Method code	---	---	---	---	C-209	C-229	C-209	C-209	C-209	C-229	C-209	C-229	C-229	C-209	C-209	C-209	C-209	C-209/C-229

Method codes:

C-209: ICP-MS

C-229: ICP-AES

C-241: pH determination

Job number: CE414

Report date: 09/05/17

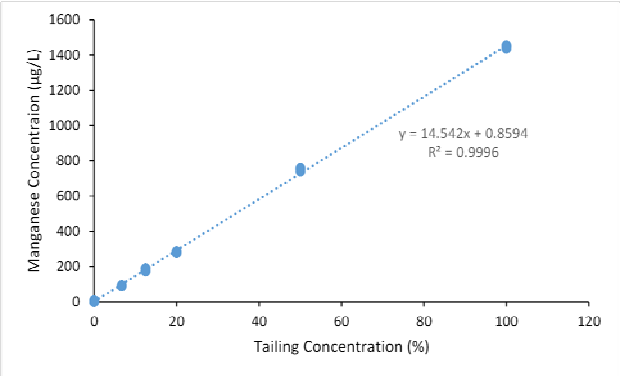
Report number: CE414/2

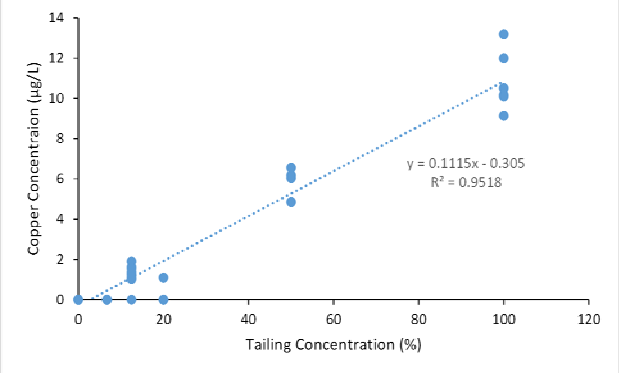
Josh King

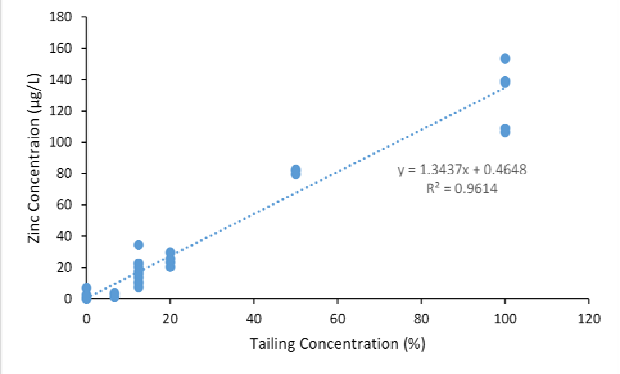
Appendix B - Dissolved metal concentrations in toxicity tests with tailings liquor

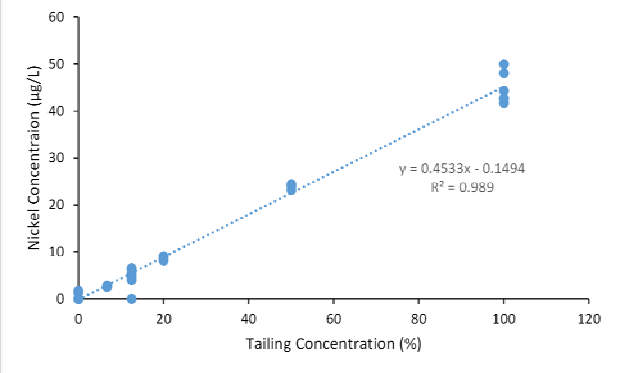
<u>Tailing Liquor 1</u>— Relationship between dissolved metal concentration and tailing liquor concentration (100% tailing was prepared following mixing with seawater (1 in 4) for 1 h and filtration to 0.45 µm)

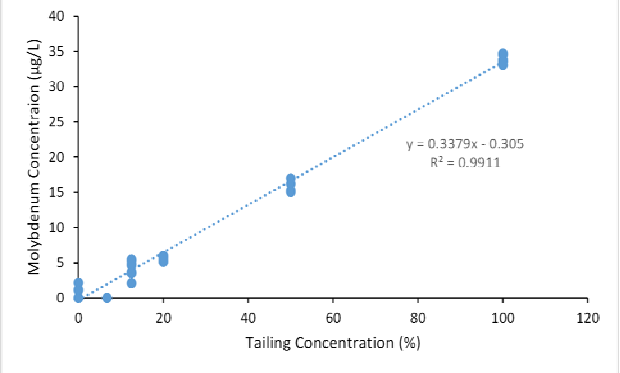
Data includes measurements at the start and end of each toxicity test



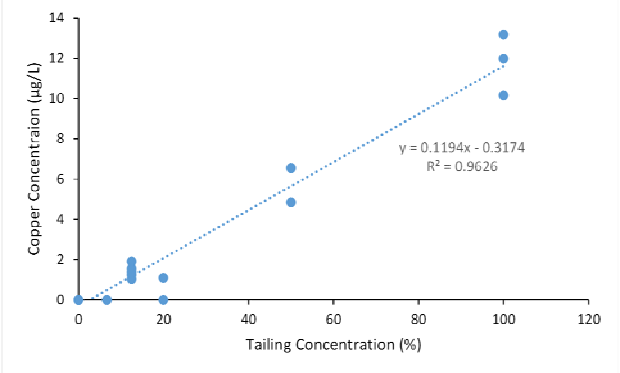


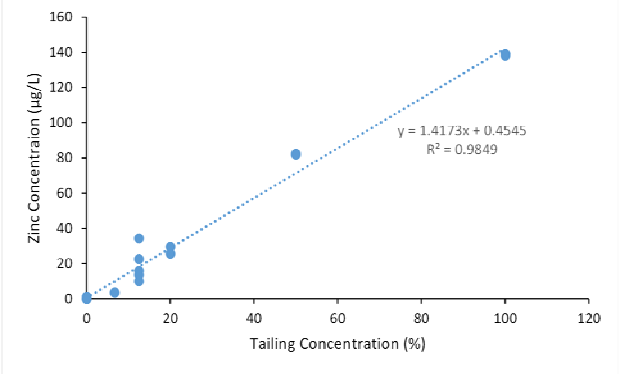






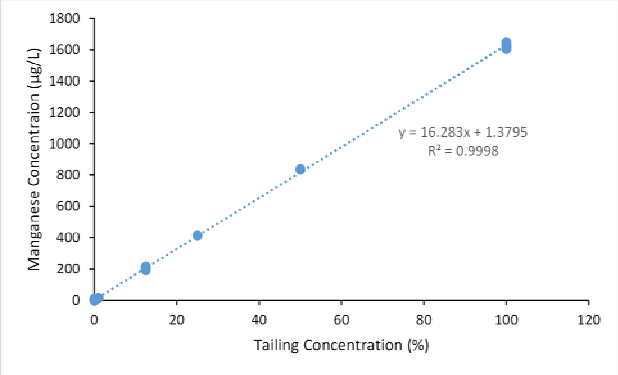
Data includes measurements only at the start of each toxicity test

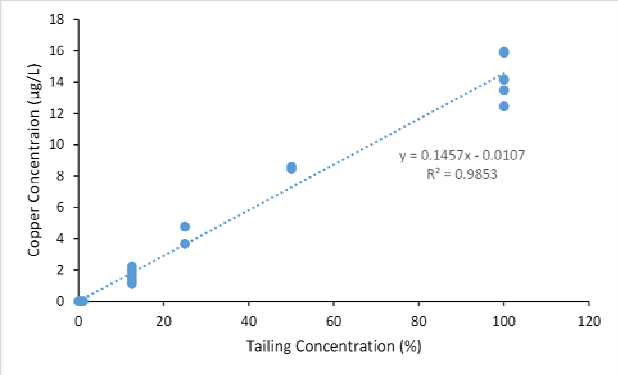


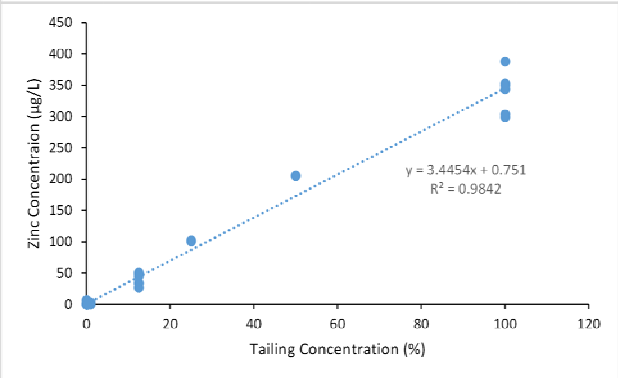


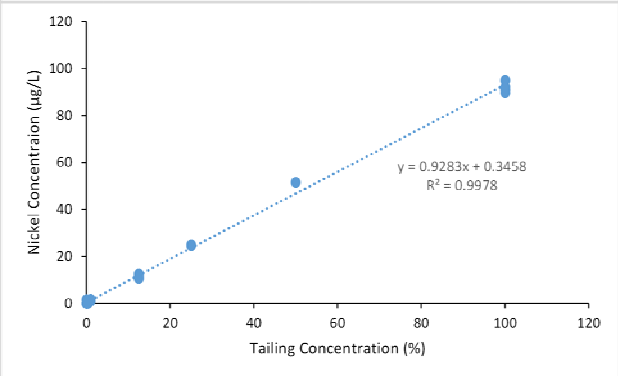
<u>Tailing Liquor 2</u>— Relationship between dissolved metal concentration and tailing liquor concentration (100% tailing was prepared following mixing with seawater (1 in 4) for 1 h and filtration to 0.45 µm)

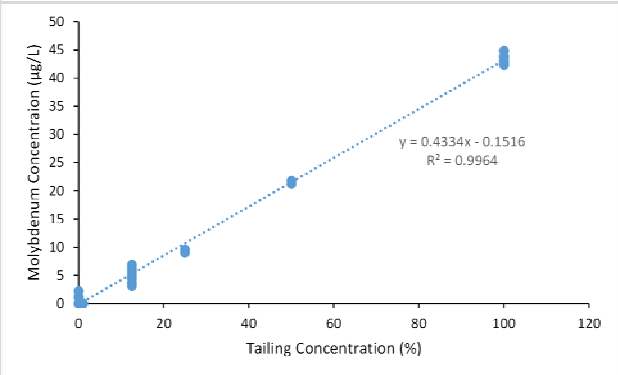
Data includes measurements at the start and end of each toxicity test



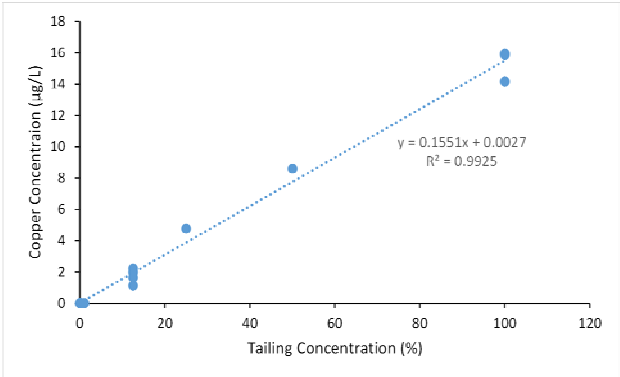


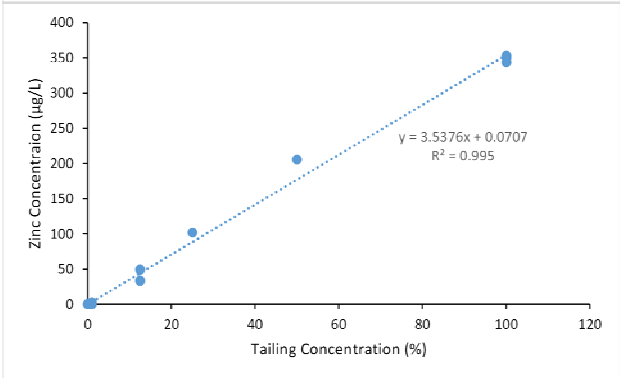






Data includes measurements only at the start of each toxicity test





Appendix C - Test reports for the ecotoxicity of tailings liquor

LAND AND WATER
www.csiro.au

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6831 • **ABN** 41 687 119 230

14 August 2017

Chronic Microalgal Growth Test Report E17021 NCAG

Client: GDA Consult Pty Ltd
Project: Wafi-Golpu Pre-feasibility study of DSTP
Test Performed: 72-h chronic algal growth toxicity test with the tropical marine alga *Nitzschia closterium*

Test Initiated:	18/7/2017	
CSIRO Sample No.	**Sample Name**	**Sample Description**
E17021	Drum 1	Drum 1 tailings
E17022	Drum 2	Drum 2 tailings

Sample Preparation: Prior to toxicity testing, tailings liquor was prepared by simulating pre-discharge mixing with seawater at a dilution of 1 in 4. Natural seawater (3 parts, 3 kg) was added to tailings material (solids and liquor) (1 part, 1 kg) and mixed on a roller for 1 h. The resulting solution was filtered to 0.45 µm using a cartridge filter (with a 0.65 µm pre-filter) and the filtrate (tailings liquor) collected for testing. The tailings liquor was stored at 4°C in the dark prior to toxicity testing. All reference to tailings liquor here on describes the 1 in 4 diluted and filtered tailings-seawater mixture as 100% (or undiluted) tailings liquor.
Physico-Chemistry: The salinity of the tailings liquors from Drum 1 and Drum 2 was 29-30‰ and pH of 7.4 and 7.6 respectively. Each tailings liquor was serially diluted in natural filtered (0.45 µm) seawater prior to testing (Drum 1 1.5–100%, Drum 2 0.3–100% liquor). A salinity/pH control was also prepared by the addition of high purity water (milli-Q) and 1M HCl (drop-wise) to natural seawater to match the salinity and pH of 100% liquor (the highest tailings liquor concentration tested).

Sample	**Physico-Chemistry**				
	pH	**Salinity (‰)**	**Conductivity (mS/cm)**	**DO (% O_2 sat)**	**Comments**
Drum 1 liquor (E17021)	7.40	29.7	46.2	109	-
Drum 2 liquor (E17022)	7.64	29.0	45.4	109	
Seawater (QA control)	8.05	35.8	54.7	109	
Salinity-pH control	7.40	29.2	45.7	109	

Test method: This test measures the decrease (inhibition) in growth rate (cell division) of the tropical marine alga *Nitzschia closterium* (CS-114, also known as *Ceratoneis closterium*) during exposure to the sample for 72 h. A pre-washed suspension of microalgae were added to give an initial starting cell density of 2–4 x 10^3 cells/mL with nutrients (1.5 mg/L nitrate and 0.15 mg/L phosphate) added to each control, reference toxicant and tailings liquor concentration (prepared by serial dilution with seawater) to ensure exponential growth rate could be maintained throughout the test. Test flasks were incubated at 27°C with cell density measured daily using flow cytometry (FACSCalibur or FACSVerse, BD Bioscience). The test protocol is based on Franklin et al., (2005) and the OECD test guideline (1984). The 72-h IC50, IC10, LOEC and NOEC values were calculated using ToxCalc Version 5.0.23 (Tidepool Software). Copper was also tested for quality assurance purposes and the pH of each treatment measured at the beginning and end of the test. Dissolved metals (<0.45 µm) were also measured in selected tailings liquor concentrations (refer to main report for results). A range-finder test (limited concentrations and replicates) was also carried out on

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6831 • **ABN** 41 687 119 230

undiluted liquor (decanted, not filtered) to establish the concentrations of liquor to be tested in the definitive toxicity test. Results of the range-finder test are presented in the appendix.

Sample Results: Tailings liquor from Drum 2 was more toxic than tailings liquor from Drum 1 with an IC10 of 3.9% and 9.4% respectively.
QA Comments: Algal growth rate in the salinity/pH control (1.56 doubling per day) was significantly lower than that in the QA Control (1.88 doublings per day) after 72 h. This suggests that pH and salinity may be a minor contribution to the toxicity at the highest test concentration (100% PFW).
The pH difference (Day 0 to Day 3) in each test concentration throughout the test was ≤0.29 for Drum 1 tailings liquor and ≤0.36 for Drum 2 tailings liquor. The pH difference for the QA Control was 0.27.

Sample	Growth Rate (Doublings/Day)	Growth Rate (% of QA Control)	CV (%)
QA Control (seawater)	1.88	100	2.4
Salinity-pH control	1.56[a]	83[a]	1.6
Drum 1-E17021			
1.5%	1.84	98	0.8
3%	1.85	98	5.6
6.25%	1.86	99	4.1
12.5%	1.54[a]	82[a]	3.4
25%	1.02[a]	54[a]	5.4
50%	0.36[a]	19[a]	17
100%	0.12[a]	6[a]	27
Drum 2-E17022			
0.3%	1.91	101	3.2
1%	1.86	99	1.7
6.25%	1.58[a]	84[a]	1.1
12.5%	1.16[a]	61[a]	5.7
25%	0.50[a]	27[a]	4.0
50%	0.19[a]	10[a]	13
100%	0.12[a]	6[a]	16

Sample	IC50 (%)[b]	IC10 (%)[b]	LOEC (%)[c]	NOEC (%)[d]
Drum 1 – E17021	28 (24-31)	9.4 (6.4-11)	12.5	6.25
Drum 2 – E17022	16 (15-18)	3.9 (2.2-5.0)	6.25	1

[a] Significantly (p≤0.05) less than QA Control
[b] Concentration of the sample to cause a 50% or 10% inhibition in algal growth; values in parentheses are 95% confidence limits
[c] Lowest concentration tested to cause a significant (p≤0.05) inhibition in algal growth compared to the control
[d] Highest concentration tested to have no significant (p≥0.05) inhibition in algal growth compared to the control

Quality Assurance/Quality Control	Criterion	This Test	Criterion Met?
72-h Control growth rate (doublings per day)	2.1 ± 0.3	1.88	Yes
72-h Control growth rate CV (%)	20%	2.4	Yes
Reference toxicant 72-h IC50 (measured copper, µg/L)	3.3 ± 0.9	2.8	Yes

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6831 • **ABN** 41 687 119 230

References:

Franklin, N.M., Stauber, J.L. and Adams, M.S. (2005). Improved methods of conducting microalgal bioassays using flow cytometry. In: Techniques in Aquatic Toxicology (eds) G.K. Ostrander. CRC Press, FL, USA.

OECD (1984). Guideline for testing of chemicals. Algal growth inhibition test. Test Guideline No. 201. Organisation for Economic Cooperation and Development, Paris, France.

Test carried out by:	Kitty McKnight, Monique Binet
Test supervised by:	Monique Binet
Test report prepared by:	Merrin Adams
Test report reviewed by:	Merrin Adams and Lisa Golding

Team Leader | Aquatic Ecotoxicology
Aquatic Contaminants Group
Environmental Contaminant Mitigation and Biotechnologies
CSIRO Land and Water
E merrin.adams@csiro.au **T** +61 2 9710 6831

Date: 7 August 2017

LAND AND WATER
www.csiro.au
New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6831 • **ABN** 41 687 119 230

Statistics-Sample

18/07/2017-21/07/2017 **72-h Chronic Toxicity of tailings liquor, Drum 1 (E17021) and Drum 2 (E17022), to *Tropical Nitzschia closterium***

	Sample	pH		Day 0	Day 1	Day 2	Day 3	Slope	Growth Rate (dblngs		Pearson	% Control	Mean %	CV (%)
Flask No.		Day 0	Day 3	*All cell counts in (cells/mL) by 10^3*						Mean				
1				3.47	8.35	47.38	171.13	0.02430	1.94	1.88	99%	103%	100%	2.4%
2	Control	8.06	8.33	3.47	7.76	38.39	151.68	0.02340	1.86		98%	99%		
3				3.47	8.23	37.00	151.77	0.02323	1.85		99%	98%		
						Mean Control growth rate =		0.02365						
Drum 1 (E17021) (%)														
19				3.47	7.89	37.50	138.83	0.02285	1.82	1.84	99%	97%	98%	0.8%
20	1.5	8.09	8.33	3.47	8.95	37.08	155.04	0.02320	1.85		99%	98%		
21				3.47	8.56	36.06	152.78	0.02315	1.84		99%	98%		
22				3.47	6.70	35.28	129.37	0.02265	1.80	1.85	98%	96%	98%	5.6%
23	3	8.10	8.36	3.47	6.70	29.89	127.15	0.02226	1.77		97%	94%		
24				3.47	7.41	47.48	175.65	0.02467	1.97		98%	104%		
25				3.47	6.76	40.01	169.80	0.02434	1.94	1.86	97%	103%	99%	4.1%
26	6.25	8.10	8.36	3.47	6.92	37.06	139.44	0.02309	1.84		98%	98%		
27				3.47	6.72	31.36	130.16	0.02246	1.79		98%	95%		
28				3.47	7.43	29.91	88.87	0.02013	1.60	1.54	99%	85%	82%	3.4%
29	12.5	8.09	8.32	3.47	7.94	22.13	82.89	0.01908	1.52		99%	81%		
30				3.47	5.70	20.13	74.52	0.01893	1.51		97%	80%		
31				3.47	3.91	9.12	31.85	0.01357	1.08	1.02	90%	57%	54%	5.4%
32	25	8.08	8.29	3.47	5.06	9.67	26.32	0.01217	0.97		96%	51%		
33				3.47	3.79	10.32	26.35	0.01282	1.02		92%	54%		
34				3.47	3.33	4.76	6.06	0.00367	0.29	0.36	86%	16%	19%	17%
35	50	8.04	8.24	3.47	3.60	4.19	8.21	0.00495	0.39		77%	21%		
36				3.47	2.75	4.45	7.49	0.00505	0.40		70%	21%		
37				3.47	1.90	2.88	3.70	0.00110	0.09	0.12	7%	5%	6%	27%
38	100	7.90	8.19	3.47	1.57	2.80	4.08	0.00193	0.15		11%	8%		
39				3.47	2.11	2.32	4.50	0.00158	0.13		10%	7%		
Drum 2 (E17022) (%)														
40				3.47	8.63	37.80	149.54	0.02310	1.84	1.91	99%	98%	101%	3.2%
41	0.3	8.05	8.41	3.47	9.35	51.71	182.29	0.02460	1.96		99%	104%		
42				3.47	8.94	46.88	169.00	0.02409	1.92		99%	102%		
43				3.47	11.22	47.09	146.33	0.02291	1.83	1.86	100%	97%	99%	1.7%
44	1	8.06	8.40	3.47	8.50	50.25	150.36	0.02368	1.89		98%	100%		
45				3.47	9.27	47.09	149.68	0.02338	1.86		99%	99%		
46				3.47	6.13	26.03	87.27	0.02012	1.60	1.58	97%	85%	84%	1.1%
47	6.25	8.07	8.37	3.47	6.48	22.64	86.13	0.01970	1.57		98%	83%		
48				3.47	7.56	24.44	90.16	0.01981	1.58		99%	84%		
49				3.47	3.71	11.37	32.81	0.01422	1.13	1.16	91%	60%	61%	5.7%
50	12.5	8.07	8.23	3.47	4.85	14.31	31.03	0.01385	1.10		%	59%		
51				3.47	5.13	15.66	41.09	0.01544	1.23		97%	65%		
52				3.47	3.19	3.85	10.89	0.00655	0.52	0.50	66%	28%	27%	4.0%
53	25	8.05	8.27	3.47	2.44	4.34	9.25	0.00636	0.51		65%	27%		
54				3.47	3.01	5.09	8.88	0.00605	0.48		80%	26%		
55				3.47	1.92	3.28	4.52	0.00240	0.19	0.19	23%	10%	10%	13%
56	50	8.03	8.23	3.47	2.15	3.35	4.40	0.00209	0.17		25%	9%		
57				3.47	1.97	3.49	4.71	0.00269	0.21		28%	11%		
58				3.47	1.06	2.61	3.54	0.00174	0.14	0.12	5%	7%	6%	16%
59	100	7.96	8.20	3.47	1.15	2.12	3.57	0.00126	0.10		3%	5%		
60				3.47	1.36	2.24	3.93	0.00158	0.13		5%	7%		

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6831 • **ABN** 41 687 119 230

Algal growth-72-h slope

Start Date:	18/07/2017	Test ID:	170718 WG	Sample ID:	E17021
End Date:	21/07/2017	Lab ID:	CSIRO-CSIRO-CECR	Sample Type:	synthetic tailings liquor -Drum 1
Sample Date:		Protocol:	STA-Stauber et al 1994	Test Species:	NC-Nitzschia closterium
Comments:					

Conc-%	1	2	3
Control	0.0243	0.0234	0.0232
1.5	0.0228	0.0232	0.0231
3	0.0226	0.0223	0.0247
6.25	0.0243	0.0231	0.0225
12.5	0.0201	0.0191	0.0189
25	0.0136	0.0122	0.0128
50	0.0037	0.0049	0.0050
100	0.0011	0.0019	0.0016

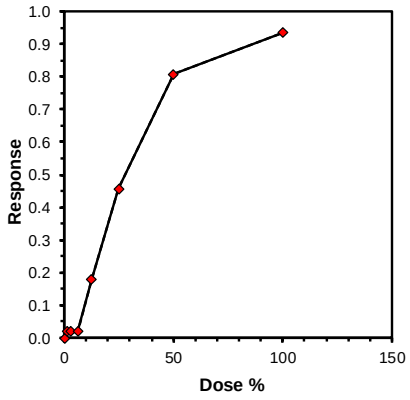
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Control	0.0236	1.0000	0.0236	0.0232	0.0243	2.439	3				0.0236	1.0000
1.5	0.0231	0.9755	0.0231	0.0228	0.0232	0.824	3	0.935	2.560	0.0016	0.0232	0.9805
3	0.0232	0.9808	0.0232	0.0223	0.0247	5.573	3	0.733	2.560	0.0016	0.0232	0.9805
6.25	0.0233	0.9853	0.0233	0.0225	0.0243	4.095	3	0.562	2.560	0.0016	0.0232	0.9805
*12.5	0.0194	0.8196	0.0194	0.0189	0.0201	3.353	3	6.874	2.560	0.0016	0.0194	0.8196
*25	0.0129	0.5436	0.0129	0.0122	0.0136	5.435	3	17.395	2.560	0.0016	0.0129	0.5436
*50	0.0046	0.1927	0.0046	0.0037	0.0050	16.828	3	30.765	2.560	0.0016	0.0046	0.1927
*100	0.0015	0.0650	0.0015	0.0011	0.0019	26.970	3	35.634	2.560	0.0016	0.0015	0.0650

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.963808	0.884	0.518711	-0.27003
Bartlett's Test indicates equal variances (p = 0.56)	5.864472	18.47531		

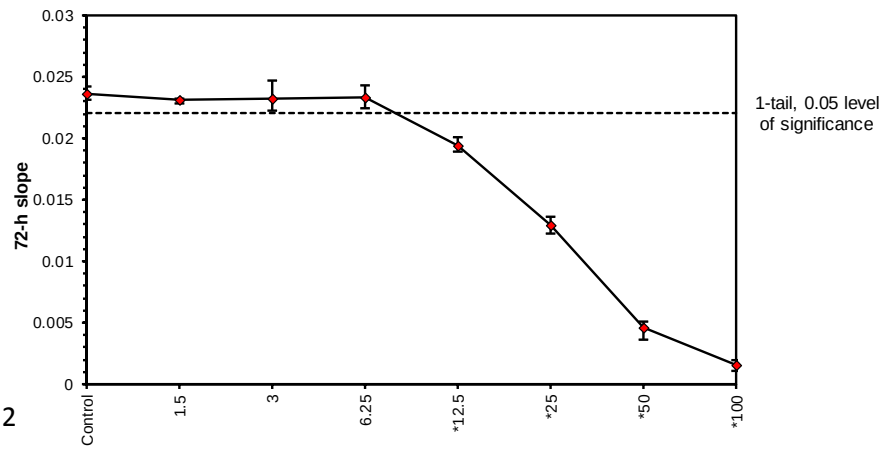
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	6.25	12.5	8.838835	16	0.001588	0.067174	0.000246	5.77E-07	5.8E-17	7, 16

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05	7.435	1.190	0.000	9.053	-3.0022
IC10	9.377	0.569	6.419	11.160	-0.3461
IC15	11.320	0.587	8.704	13.704	0.1130
IC20	13.388	0.658	10.921	15.834	-0.0370
IC25	15.652	0.603	13.227	17.927	-0.2082
IC40	22.444	0.596	20.222	24.707	0.0357
IC50	28.104	0.961	23.988	31.317	-0.1392



Dose-Response Plot



New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6831 • **ABN** 41 687 119 230

Acute Fish Test-72 h growth rate

Start Date:	7/18/2017	Test ID:	Drum 2	Sample ID:	E17022 (Drum 2)
End Date:	7/21/2017	Lab ID:		Sample Type:	Tailings Liquor
Sample Date:		Protocol:	EPAA 91-EPA Acute	Test Species:	PP-Pimephales promelas
Comments:	Drum 2 talings liquor prepared following a 1 in 4 dilution of tailings				

Conc-%	1	2	3
Control	0.0243	0.0234	0.0232
0.3	0.0231	0.0246	0.0241
1	0.0229	0.0237	0.0234
6.25	0.0201	0.0197	0.0198
12.5	0.0142	0.0139	0.0154
25	0.0065	0.0064	0.0061
50	0.0024	0.0021	0.0027
100	0.0017	0.0013	0.0016

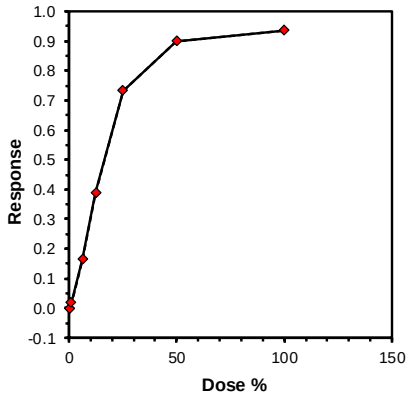
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Control	0.0236	1.0000	0.0236	0.0232	0.0243	2.439	3				0.0238	1.0000
0.3	0.0239	1.0121	0.0239	0.0231	0.0246	3.181	3	-0.701	2.560	0.0010	0.0238	1.0000
1	0.0233	0.9863	0.0233	0.0229	0.0237	1.659	3	0.794	2.560	0.0010	0.0233	0.9803
*6.25	0.0199	0.8406	0.0199	0.0197	0.0201	1.110	3	9.204	2.560	0.0010	0.0199	0.8355
*12.5	0.0145	0.6134	0.0145	0.0139	0.0154	5.719	3	22.327	2.560	0.0010	0.0145	0.6097
*25	0.0063	0.2674	0.0063	0.0061	0.0065	3.977	3	42.309	2.560	0.0010	0.0063	0.2657
*50	0.0024	0.1013	0.0024	0.0021	0.0027	12.562	3	51.897	2.560	0.0010	0.0024	0.1007
*100	0.0015	0.0645	0.0015	0.0013	0.0017	15.937	3	54.022	2.560	0.0010	0.0015	0.0642

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.98	0.884	0.266938	0.110045
Bartlett's Test indicates equal variances (p = 0.48)	6.548854	18.47531		

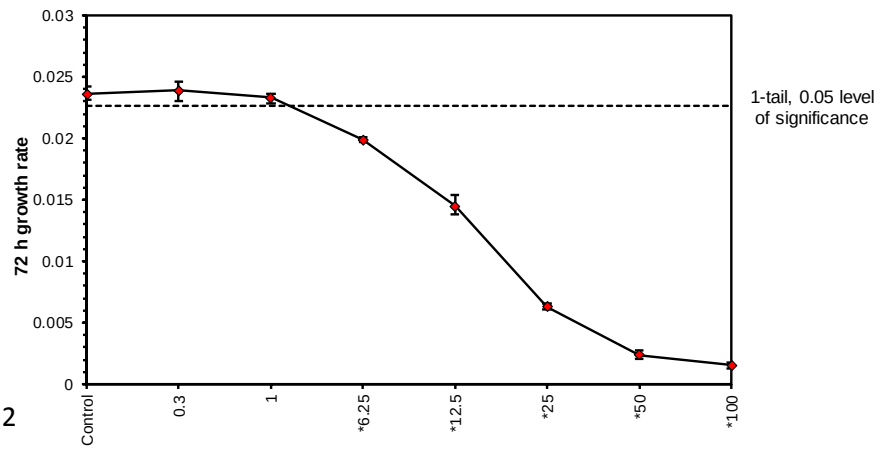
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1	6.25	2.5	100	0.001048	0.044329	0.000283	2.51E-07	2.5E-20	7, 16

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05	2.099	0.377	0.319	3.229	-0.0981
IC10	3.912	0.334	2.228	4.955	-0.0883
IC15	5.726	0.322	4.122	6.816	-0.0729
IC20	7.234	0.250	6.074	8.068	-0.0151
IC25	8.617	0.287	7.442	9.780	0.3077
IC40	12.852	0.579	10.956	15.356	0.4404
IC50	16.486	0.474	14.633	18.361	0.1874



Dose-Response Plot



LAND AND WATER
www.csiro.au
New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6831 • **ABN** 41 687 119 230

Statistics - QA Control

18/07/2017-21/07/2017 **72-h Chronic Toxicity of tailings liquor, Drum 1 (E17021) and Drum 2 (E17022), to Tropical *Nitzschia closterium***

	Sample		pH		Day 0	Day 1	Day 2	Day 3	Slope	Growth Rate (dblngs/d		Pearson	% Contro	Mean %	CV (%)
Flask No.	Nominal	Measured	Day 0	Day 3	*All cell counts in (cells/mL) by 10^3*						Mean				
1					3.47	8.35	47.38	171.13	0.02430	1.94	1.88	99%	103%	100%	2.4%
2	Control		8.06	8.33	3.47	7.76	38.39	151.68	0.02340	1.86		98%	99%		
3					3.47	8.23	37.00	151.77	0.02323	1.85		99%	98%		
							Mean control rate =		***0.02365***						
Salinity/pH Controls*															
4					3.47	7.77	27.51	89.79	0.01995	1.59	1.56	99%	84%	83%	1.6%
5	sal/pH		7.84	8.25	3.47	7.92	29.78	78.50	0.01933	1.54		99%	82%		
6					3.47	7.64	29.10	82.70	0.01963	1.56		99%	83%		
Copper (µg/L) [0.45 µm dissolved measured copper concentrations]															
7					3.47	5.21	10.93	39.14	0.01449	1.15	1.20	94%	61%	64%	4.0%
8	2.5	2.2	8.05	8.23	3.47	6.09	15.02	46.25	0.01569	1.25		98%	66%		
9					3.47	6.06	14.78	40.24	0.01492	1.19		98%	63%		
10					3.47	4.26	6.88	8.81	0.00593	0.47	0.40	98%	25%	21%	17%
11	5	4.2	8.09	8.23	3.47	3.50	6.48	6.93	0.00487	0.39		84%	21%		
12					3.47	3.97	5.44	6.87	0.00428	0.34		98%	18%		
13					3.47	4.28	5.33	5.50	0.00290	0.23	0.19	93%	12%	10%	17%
14	10	8.5	8.10	8.23	3.47	3.65	5.18	4.66	0.00223	0.18		69%	9%		
15					3.47	4.91	5.44	4.99	0.00216	0.17		60%	9%		
16					3.47	2.93	3.09	6.05	0.00311	0.25	0.32	44%	13%	17%	23%
17	20	17.2	8.10	8.23	3.47	3.12	4.92	7.35	0.00490	0.39		81%	21%		
18					3.47	3.33	4.61	6.35	0.00387	0.31		85%	16%		

*** matches sal/pH between top concentrations of Drum 1 and Drum 2**

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6831 • **ABN** 41 687 119 230

Algal growth-72-h slope					
Start Date:	18/07/2017	Test ID:	170718 WG	Sample ID:	REF-Ref Toxicant
End Date:	21/07/2017	Lab ID:	CSIRO-CSIRO-CECR	Sample Type:	CUSO-Copper sulfate
Sample Date:		Protocol:	STA-Stauber et al 1994	Test Species:	NC-Nitzschia closterium
Comments:	Measured concentrations				

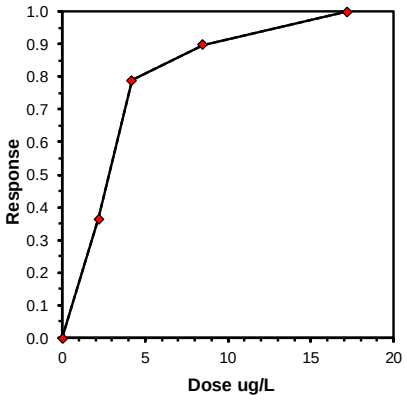
Conc-ug/L	1	2	3
Control	0.0243	0.0234	0.0232
2.2	0.0145	0.0157	0.0149
4.2	0.0059	0.0049	0.0043
8.5	0.0029	0.0022	0.0022
17.2	0.0000	0.0000	0.0000

			Transform: Untransformed					1-Tailed			Isotonic	
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Control	0.0236	1.0000	0.0236	0.0232	0.0243	2.439	3				0.0236	1.0000
*2.2	0.0150	0.6359	0.0150	0.0145	0.0157	4.044	3	16.861	2.420	0.0012	0.0150	0.6359
*4.2	0.0050	0.2125	0.0050	0.0043	0.0059	16.612	3	36.466	2.420	0.0012	0.0050	0.2125
*8.5	0.0024	0.1028	0.0024	0.0022	0.0029	16.744	3	41.547	2.420	0.0012	0.0024	0.1028
17.2	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	3				0.0000	0.0000

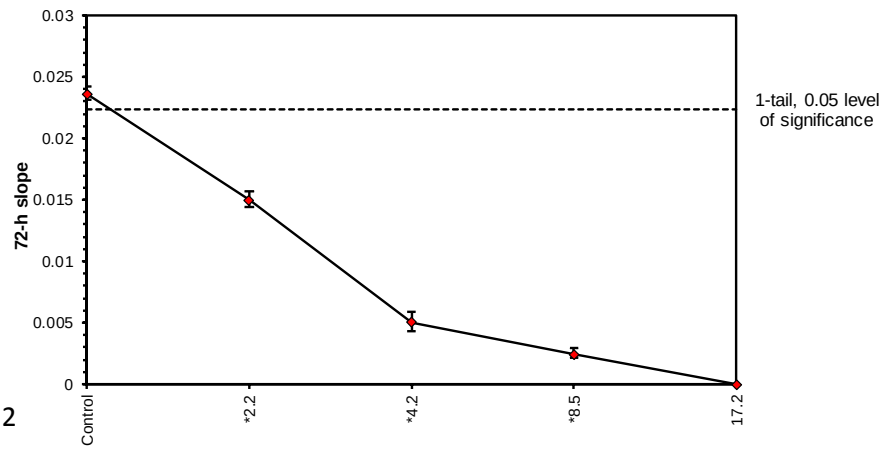
Auxiliary Tests					Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.90509		0.805		0.512334	-1.07317
Bartlett's Test indicates equal variances (p = 0.84)					0.829222		11.34487			
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test	<2.2	2.2			0.001236	0.052262	0.000284	3.91E-07	4.4E-10	3, 8

Linear Interpolation (200 Resamples)					
Point	ug/L	SD	95% CL(Exp)		Skew
IC05*	0.3021	0.0119	0.2596	0.3546	0.2870
IC10*	0.6042	0.0239	0.5192	0.7093	0.2870
IC15*	0.9063	0.0358	0.7789	1.0639	0.2870
IC20*	1.2083	0.0477	1.0385	1.4186	0.2870
IC25*	1.5104	0.0597	1.2981	1.7732	0.2870
IC40	2.3694	0.0636	2.1217	2.6092	-0.0178
IC50	2.8418	0.0582	2.6122	3.0517	0.0316

* indicates IC estimate less than the lowest concentration



Dose-Response Plot



New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6831 • **ABN** 41 687 119 230

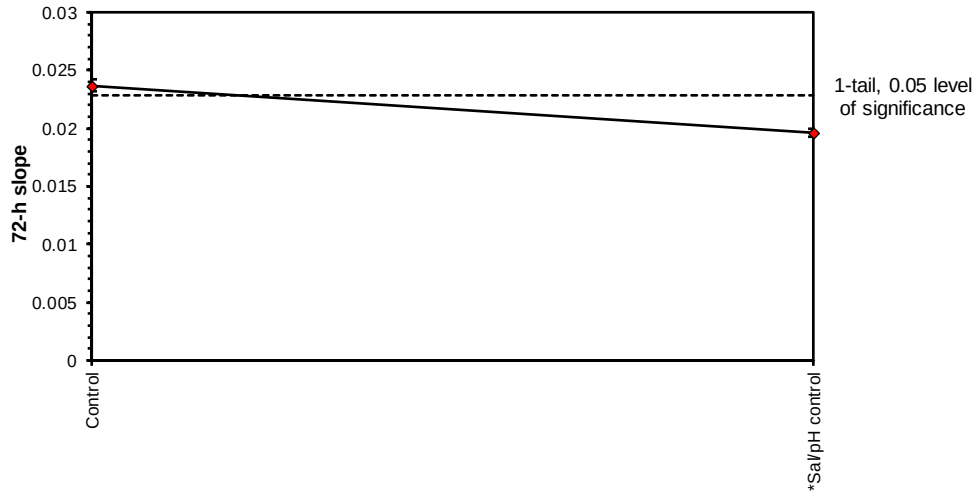
Algal growth-72-h slope					
Start Date:	18/07/2017	Test ID:	170718 WG	Sample ID:	
End Date:	21/07/2017	Lab ID:	CSIRO-CSIRO-CECR	Sample Type:	
Sample Date:		Protocol:	STA-Stauber et al 1994	Test Species:	NC-Nitzschia closterium
Comments:					

Conc-	1	2	3
Control	0.0243	0.0234	0.0232
Sal/pH control	0.0199	0.0193	0.0196

			Transform: Untransformed					1-Tailed		
Conc-	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
Control	0.0236	1.0000	0.0236	0.0232	0.0243	2.439	3			
*Sal/pH control	0.0196	0.8305	0.0196	0.0193	0.0199	1.580	3	10.599	2.132	0.0008

Auxiliary Tests	Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.91372		0.713		0.847928	-0.54666
F-Test indicates equal variances (p = 0.45)	3.454518		199			
Hypothesis Test (1-tail, 0.05)	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Homoscedastic t Test indicates significant differences	0.000806	0.034092	2.41E-05	2.14E-07	4.5E-04	1, 4

Dose-Response Plot



LAND AND WATER
www.csiro.au
New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6831 • **ABN** 41 687 119 230

Statistics-Range-finder test

Date 07/02/2017 - 10/02/2017

Toxicity of Tailings Liquor Drum 1 and Drum 2 to *Tropical N. closterium* - 72-h growth rate inhibition test - Rangefinder

			pH (after algae)		Salinity (psu)		Conductivity (ms)		DO (% sat)		Day 0	Day 1	Day 2	Day 3	Slope	Growth Rate (dblngs/day)		Pearson	% Contro	Mean %	CV (%)
Flask No.	Sample	Measured	Day 0	Day 3	Day 0	Day 3	Day 0	Day 3	Day 0	Day 3	*All cell counts in (cells/mL) by 10^3*						Mean				
1											3.01	10.37	70.30	157.92	0.02496	1.99		98%	99%	100%	1.1%
2	Control	0	8.10	8.28	36.0	36.2	55.0	54.1	110%	93%	3.01	11.21	61.56	186.53	0.02548	2.03	2.01	99%	101%		
3											3.01	10.41	51.61	178.02	0.02505	2.00		100%	99%		
4											3.01	11.02	56.97	190.76	0.02550	2.03		100%	101%		
														mean control =	***0.02525***						
Reference Toxicant - Copper (µg/L)																					
5											3.01	8.47	63.29	182.23	0.02592	2.06		98%	103%		
6	1	Need MS	8.10	8.26	N/S	N/S	N/S	N/S	N/S	N/S	3.01	9.38	41.39	154.09	0.02405	1.92	1.99	100%	95%	99%	5.3%
5											3.01	4.09	12.58	28.61	0.01426	1.14		96%	56%		
6	3	1	8.10	8.21	N/S	N/S	N/S	N/S	N/S	N/S	3.01	4.28	17.74	42.16	0.01690	1.35	1.24	96%	67%	62%	12.0%
7											3.01	3.91	8.71	17.48	0.01100	0.88		96%	44%		
8	6	4	8.10	8.19	N/S	N/S	N/S	N/S	N/S	N/S	3.01	3.80	9.32	15.68	0.01058	0.84	0.86	96%	42%	43%	2.7%
9											3.01	3.21	6.13	5.53	0.00447	0.36		76%	18%		
10	12	8	8.11	8.15	N/S	N/S	N/S	N/S	N/S	N/S	3.01	3.45	5.79	7.44	0.00585	0.47	0.41	96%	23%	20%	18.9%
11											3.01	2.76	4.13	4.10	0.00241	0.19		68%	10%		
12	18	13	8.10	8.16	N/S	N/S	N/S	N/S	N/S	N/S	3.01	2.95	4.22	4.57	0.00291	0.23	0.21	84%	12%	11%	13.5%
Drum 1 (E17021)																					
13											3.01	12.19	74.52	198.77	0.02602	2.07		99%	103%		
14	0.01%	NA	8.09	8.25	36.1	35.9	54.7	54.0	98%	93%	3.01	10.74	61.67	159.96	0.02473	1.97	2.02	99%	98%	101%	3.6%
15											3.01	9.42	52.11	191.55	0.02564	2.04		99%	102%		
16	0.1%	NA	8.10	8.30	36.0	36.1	54.6	54.3	98%	94%	3.01	10.01	61.86	158.38	0.02481	1.98	2.01	99%	98%	100%	2.3%
17											3.01	10.73	47.72	154.36	0.02408	1.92		100%	95%		
18	1%	NA	8.10	8.29	35.7	35.8	54.1	53.8	98%	93%	3.01	10.84	51.74	167.65	0.02465	1.96	1.94	100%	98%	96%	1.7%
19											3.01	6.85	28.76	98.08	0.02151	1.71		99%	85%		
20	10%	NA	8.10	8.28	32.9	32.8	50.3	49.9	100%	93%	3.01	7.31	32.58	124.34	0.02290	1.83	1.77	99%	91%	88%	4.4%
21											3.01	0.97	1.53	3.19	0.00114	0.09		2%	5%		
22	50%	NA	8.11	8.24	18.6	19.1	30.0	30.6	101%	93%	3.01	1.17	1.64	1.58	-0.00289	-0.23	-0.07	27%	-11%	-3%	N/A
Drum 2 (E17022)																					
13											3.01	10.20	49.59	148.92	0.02404	1.92		100%	95%		
14	0.01%	NA	8.09	8.29	36.1	35.9	54.5	54.1	98%	93%	3.01	11.71	47.53	170.18	0.02444	1.95	1.93	100%	97%	96%	1.2%
15											3.01	11.68	51.20	158.40	0.02419	1.93		100%	96%		
16	0.1%	NA	8.09	8.26	36.0	36.4	54.4	54.7	99%	93%	3.01	11.46	52.65	166.74	0.02455	1.96	1.94	100%	97%	97%	1.1%
17											3.01	11.39	55.34	177.25	0.02499	1.99		100%	99%		
18	1%	NA	8.09	8.28	35.8	35.7	54.1	53.9	100%	95%	3.01	11.06	45.40	142.88	0.02351	1.87	1.93	100%	93%	96%	4.3%
19											3.01	3.91	14.78	50.36	0.01770	1.41		94%	70%		
20	10%	NA	8.08	8.22	32.5	32.5	49.7	49.5	100%	95%	3.01	3.91	14.91	42.91	0.01685	1.34	1.38	95%	67%	68%	3.5%
21											3.01	0.83	1.43	1.11	-0.00443	-0.35		33%	-18%		
22	50%	NA	8.05	8.17	18.8	18.6	30.3	30.0	100%	95%	3.01	0.89	0.94	1.17	-0.00503	-0.40	-0.38	40%	-20%	-19%	N/A

S not sampled

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6831 • **ABN** 41 687 119 230

Algal growth-72-h slope

Start Date:	7/02/2017	Test ID:	170207WG	Sample ID:	DRUM1
End Date:	10/02/2017	Lab ID:	CSIRO-CSIRO-CECR	Sample Type:	Tailings Liquor
Sample Date:		Protocol:	STA-Stauber et al 1994	Test Species:	NC-Nitzschia closterium
Comments:					

Conc-%	1	2	3	4
Control	0.0250	0.0255	0.0250	0.0255
0.01	0.0260	0.0247		
0.1	0.0256	0.0248		
1	0.0241	0.0247		
10	0.0215	0.0229		
50	0.0011	0.0000		

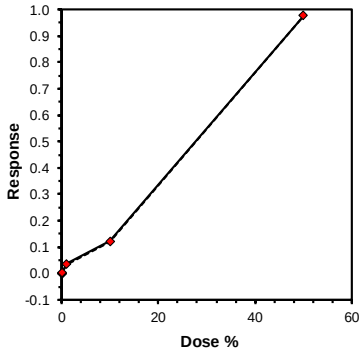
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Control	0.0252	1.0000	0.0252	0.0250	0.0255	1.122	4				0.0253	1.0000
0.01	0.0254	1.0051	0.0254	0.0247	0.0260	3.602	2	-0.237	2.660	0.0015	0.0253	1.0000
0.1	0.0252	0.9992	0.0252	0.0248	0.0256	2.332	2	0.039	2.660	0.0015	0.0252	0.9966
1	0.0244	0.9650	0.0244	0.0241	0.0247	1.673	2	1.611	2.660	0.0015	0.0244	0.9625
*10	0.0222	0.8796	0.0222	0.0215	0.0229	4.445	2	5.540	2.660	0.0015	0.0222	0.8773
*50	0.0006	0.0226	0.0006	0.0000	0.0011	141.421	2	44.969	2.660	0.0015	0.0006	0.0225

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.913639	0.825	-0.00185	-1.58856
Bartlett's Test indicates equal variances (p = 0.73)	2.793181	15.08627		

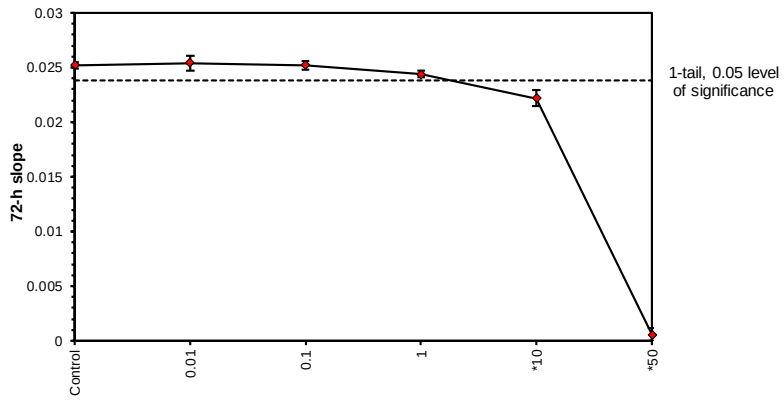
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1	10	3.162278	100	0.00146	0.057816	0.000201	4.02E-07	9.2E-10	5, 8

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05	2.322	1.051	0.000	10.608	0.3484
IC10	7.604	1.729	0.000	18.704	0.1949
IC15	11.279	1.032	2.288	16.865	-0.3698
IC20	13.618	0.911	6.437	18.943	-0.1769
IC25	15.958	0.857	9.192	20.966	-0.1879
IC40	22.977	0.719	16.942	27.507	-0.2220
IC50	27.657	0.652	21.925	32.126	-0.2285



Dose-Response Plot



New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6831 • **ABN** 41 687 119 230

Algal growth-72-h slope				
Start Date:	7/02/2017	Test ID:	170207WG	Sample ID:	DRUM2
End Date:	10/02/2017	Lab ID:	CSIRO-CSIRO-CECR	Sample Type:	Tailings Liquor
Sample Date:		Protocol:	STA-Stauber et al 1994	Test Species:	NC-Nitzschia closterium
Comments:					

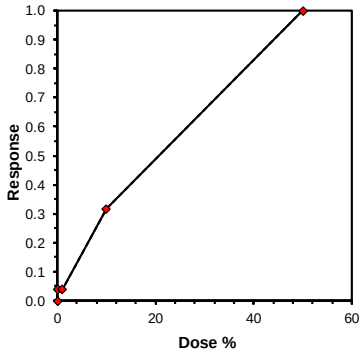
Conc-%	1	2	3	4
Control	0.0250	0.0255	0.0250	0.0255
0.01	0.0240	0.0244		
0.1	0.0242	0.0246		
1	0.0250	0.0235		
10	0.0177	0.0168		
50	0.0000	0.0000		

			Transform: Untransformed					1-Tailed			Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Control	0.0252	1.0000	0.0252	0.0250	0.0255	1.122	4				0.0252	1.0000
0.01	0.0242	0.9601	0.0242	0.0240	0.0244	1.161	2	2.269	2.620	0.0012	0.0243	0.9627
0.1	0.0244	0.9653	0.0244	0.0242	0.0246	1.055	2	1.975	2.620	0.0012	0.0243	0.9627
1	0.0242	0.9604	0.0242	0.0235	0.0250	4.302	2	2.251	2.620	0.0012	0.0242	0.9604
*10	0.0173	0.6842	0.0173	0.0168	0.0177	3.493	2	17.968	2.620	0.0012	0.0173	0.6842
50	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	2				0.0000	0.0000

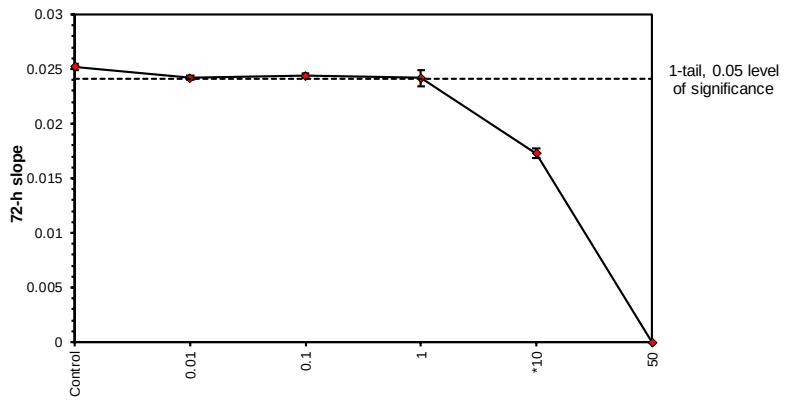
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.968546	0.805	-0.00404	-0.20143
Bartlett's Test indicates equal variances (p = 0.52)	3.252545	13.2767		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1	10	3.162278	100	0.001163	0.046052	2.34E-05	2.63E-07	4.4E-06	4, 7

Linear Interpolation (200 Resamples)					
Point	%	SD	95% CL(Exp)		Skew
IC05	1.340	0.498	0.000	3.748	-0.6734
IC10	2.969	0.449	0.000	5.352	-0.7300
IC15	4.598	0.380	1.282	7.075	-0.6428
IC20	6.227	0.346	3.215	8.757	-0.4796
IC25	7.856	0.357	5.148	10.388	-0.2712
IC40	14.922	0.652	10.641	19.032	-0.2096
IC50	20.768	0.543	17.201	24.193	-0.2096



Dose-Response Plot



LAND AND WATER
www.csiro.au

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6831 • **ABN** 41 687 119 230

14 August 2017

Chronic Microalgal Growth Test Report E17021 IGAG

Client: GDA Consult Pty Ltd
Project: Wafi-Golpu Pre-feasibility study of DSTP
Test Performed: 72-h chronic algal growth toxicity test with the tropical marine alga *Isochrysis galbana*

Test Initiated:	18/7/2017	
CSIRO Sample No.	**Sample Name**	**Sample Description**
E17021	Drum 1	Drum 1 tailings
E17022	Drum 2	Drum 2 tailings

Sample Preparation: Prior to toxicity testing, tailings liquor was prepared by simulating pre-discharge mixing with seawater at a dilution of 1 in 4. Natural seawater (3 parts, 3 kg) was added to tailings material (solids and liquor) (1 part, 1 kg) and mixed on a roller for 1 h. The resulting solution was filtered to 0.45 µm using a cartridge filter (with a 0.65 µm pre-filter) and the filtrate (tailings liquor) collected for testing. The tailings liquor was stored at 4°C in the dark prior to toxicity testing. All reference to tailings liquor here on describes the 1 in 4 diluted and filtered tailings-seawater mixture as 100% (or undiluted) tailings liquor.
Physico-Chemistry: The salinity of the tailings liquors from Drum 1 and Drum 2 was 29-30‰ and pH of 7.4 and 7.6 respectively. Each tailings liquor was serially diluted in natural filtered (0.45 µm) seawater prior to testing (Drum 1 1.5–100%, Drum 2 0.3–100% liquor). A salinity/pH control was also prepared by the addition of high purity water (milli-Q) and 1M HCl (drop-wise) to natural seawater to match the salinity and pH of 100% liquor (the highest tailings liquor concentration tested).

Sample	**Physico-Chemistry**				
	pH	**Salinity (‰)**	**Conductivity (mS/cm)**	**DO (% O_2 sat)**	**Comments**
Drum 1 liquor (E17021)	7.40	29.7	46.2	109	-
Drum 2 liquor (E17022)	7.64	29.0	45.4	109	
Seawater (QA control)	8.05	35.8	54.7	109	
Salinity-pH control	7.40	29.2	45.7	109	

Test method: This test measures the decrease (inhibition) in growth rate (cell division) of the tropical marine alga *Isochrysis galbana* (CS-177) (now *Tisochrysis lutea*) during exposure to the sample for 72 h. A pre-washed suspension of microalgae were added to give an initial starting cell density of 2–4 x 10^3 cells/mL with nutrients (1.5 mg/L nitrate and 0.15 mg/L phosphate) added to each control, reference toxicant and tailings liquor concentration (prepared by serial dilution with seawater) to ensure exponential growth rate could be maintained throughout the test. Test flasks were incubated at 27°C with cell density measured daily using flow cytometry (FACSCalibur or FACSVerse, BD Bioscience). The test protocol is based on Franklin et al., (2005) and the OECD test guideline (1984). The 72-h IC50, IC10, LOEC and NOEC values were calculated using ToxCalc Version 5.0.23 (Tidepool Software). Copper was also tested for quality assurance purposes and the pH of each treatment measured at the beginning and end of the test. Dissolved metals (0.45 µm) were also measured in selected tailings liquor concentrations (refer to main report for results). A range-finder test (limited concentrations and replicates) was also carried out on undiluted liquor (decanted,

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6831 • **ABN** 41 687 119 230

not filtered) to establish the concentrations of liquor to be tested in the definitive toxicity test. Results of the range-finder test are presented in the appendix.

Sample Results: Tailings liquor from Drum 1 and Drum 2 were similarly toxic to algal growth rate with an IC10 of 23% and 30% respectively and overlapping 95% confidence limits (16–30% and 20–35% respectively).
QA Comments: Algal growth rate in the salinity/pH control (2.11 doublings per day) was similar to that in the QA Control (2.10 doublings per day) after 72 h. This suggests that the lower pH and salinity measured in the highest test concentration would not contribute to the toxicity observed at the highest test concentration (100% tailings).
The pH difference (Day 0 to Day 3) in each test concentration throughout the test was ≤0.3 for Drum 1 tailings liquor and ≤0.39 for Drum 2 tailings liquor. The pH difference for the QA Control was 0.08.

Sample	Growth Rate (Doublings/Day)	Growth Rate (% of QA Control)	CV (%)
QA Control (seawater)	2.10	100	1.4
Salinity-pH control	2.11	100	0.4
Drum 1-E17021			
1.5%	2.07	98	0.8
3%	2.06	98	0.8
6.25%	2.13	101	2.7
12.5%	2.08	99	4.9
25%	1.85[a]	88[a]	4.5
50%	1.55[a]	74[a]	3.1
100%	1.03[a]	46[a]	9.3
Drum 2-E17022			
0.3%	2.09	99	1.5
1%	2.16	102	3.5
6.25%	2.13	101	1.9
12.5%	2.07	98	4.1
25%	2.00	95	4.3
50%	1.50[a]	71[a]	4.2
100%	0.73[a]	34[a]	5.2

Sample	IC50 (%)[b]	IC10 (%)[b]	LOEC (%)[c]	NOEC (%)[d]
Drum 1 – E17021	98	23 (16-30)	25	12.5
Drum 2 – E17022	78 (72-82)	30 (20-35)	50	25

[a] Significantly (p≤0.05) less than QA Control
[b] Concentration of the sample to cause a 50% or 10% inhibition in algal growth; values in parentheses are 95% confidence limits
[c] Lowest concentration tested to cause a significant (p≤0.05) inhibition in algal growth compared to the control
[d] Highest concentration tested to have no significant (p≥0.05) inhibition in algal growth compared to the control

Quality Assurance/Quality Control	Criterion	This Test	Criterion Met?
72-h Control growth rate (doublings per day)	2.3 ± 0.3	2.10	Yes
72-h Control growth rate CV (%)	20%	1.4	Yes
Reference toxicant 72-h IC50 (measured copper, µg/L)	4.5 ± 2.6	3.7	Yes

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6831 • **ABN** 41 687 119 230

References:

Franklin, N.M., Stauber, J.L. and Adams, M.S. (2005). Improved methods of conducting microalgal bioassays using flow cytometry. In: Techniques in Aquatic Toxicology (eds) G.K. Ostrander. CRC Press, FL, USA.

OECD (1984). Guideline for testing of chemicals. Algal growth inhibition test. Test Guideline No. 201. Organisation for Economic Cooperation and Development, Paris, France.

Test carried out by:	Kitty McKnight, Monique Binet
Test supervised by:	Monique Binet
Test report prepared by:	Merrin Adams
Test report reviewed by:	Merrin Adams and Lisa Golding

Team Leader | Aquatic Ecotoxicology
Aquatic Contaminants Group
Environmental Contaminant Mitigation and Biotechnologies
CSIRO Land and Water
E merrin.adams@csiro.au **T** +61 2 9710 6831

Date: 14 August 2017

LAND AND WATER
www.csiro.au
New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6831 • **ABN** 41 687 119 230

Statistics-Sample

18/07/2017-21/07/2017 **72-h Chronic Toxicity of tailings liquor, Drum 1 (E17021) and Drum 2 (E17022), to *Isochrysis galbana***

	Sample	pH		Day 0	Day 1	Day 2	Day 3	Slope	Growth Rate		Pearson	% Control	Mean %	CV (%)
Flask No.		Day 0	Day 3	*All cell counts in (cells/mL) by 10^3*					dblngs/day	Mean				
1				3.51	8.97	66.39	223.03	0.02616	2.08	2.10	98%	99%	100%	1.4%
2	Control	8.10	8.18	3.51	8.85	67.99	249.54	0.02684	2.14		98%	102%		
3				3.51	8.61	62.23	228.49	0.02625	2.09		98%	99%		
						Mean Control growth rate =		0.02642						
Drum 1 (E17021) (%)														
19				3.51	8.97	61.46	230.29	0.02619	2.09	2.07	98%	99%	98%	0.8%
20	1.5	8.11	8.31	3.51	9.15	62.39	214.79	0.02581	2.06		99%	98%		
21				3.51	10.87	68.86	225.02	0.02593	2.07		99%	98%		
22				3.51	11.46	65.13	221.56	0.02565	2.04	2.06	99%	97%	98%	0.8%
23	3	8.11	8.33	3.51	12.39	75.06	234.93	0.02608	2.08		99%	99%		
24				3.51	10.87	64.93	224.83	0.02582	2.06		99%	98%		
25				3.51	10.82	61.49	315.84	0.02757	2.20	2.13	99%	104%	101%	2.7%
26	6.25	8.11	8.35	3.51	10.90	62.74	260.36	0.02655	2.12		99%	100%		
27				3.51	11.08	62.33	244.97	0.02617	2.09		99%	99%		
28				3.51	10.06	54.49	204.64	0.02513	2.00	2.08	99%	95%	99%	4.9%
29	12.5	8.10	8.36	3.51	9.65	61.15	217.08	0.02573	2.05		99%	97%		
30				3.51	10.13	61.29	309.64	0.02758	2.20		99%	104%		
31				3.51	8.64	44.40	152.72	0.02344	1.87	1.85	99%	89%	88%	4.5%
32	25	8.08	8.35	3.51	9.54	43.92	179.85	0.02413	1.92		99%	91%		
33				3.51	8.77	40.03	123.68	0.02208	1.76		99%	84%		
34				3.51	5.00	20.26	83.04	0.01971	1.57	1.55	95%	75%	74%	3.1%
35	50	8.05	8.32	3.51	6.43	22.64	89.37	0.01985	1.58		97%	75%		
36				3.51	5.64	20.82	71.89	0.01876	1.49		97%	71%		
37				3.51	4.27	8.48	23.61	0.01159	0.92	1.03	92%	44%	49%	9.3%
38	100	7.97	8.27	3.51	4.32	10.90	33.02	0.01384	1.10		93%	52%		
39				3.51	3.83	10.27	30.04	0.01344	1.07		91%	51%		
Drum 2 (E17022) (%)														
40				3.51	10.52	65.03	248.49	0.02642	2.11	2.09	99%	100%	99%	1.5%
41	0.3	8.06	8.33	3.51	11.75	64.01	258.08	0.02640	2.10		100%	100%		
42				3.51	12.26	59.77	237.48	0.02575	2.05		100%	97%		
43				3.51	10.86	66.88	254.40	0.02654	2.11	2.16	99%	100%	102%	3.5%
44	1	8.07	8.34	3.51	12.50	64.44	363.24	0.02815	2.24		100%	107%		
45			.	3.51	13.20	71.14	263.90	0.02650	2.11		100%	100%		
46				3.51	13.81	78.17	278.66	0.02688	2.14	2.13	100%	102%	101%	1.9%
47	6.25	8.08	8.39	3.51	13.98	79.32	287.35	0.02705	2.16		100%	102%		
48				3.51	12.71	60.30	256.27	0.02611	2.08		100%	99%		
49				3.51	10.38	69.08	281.43	0.02723	2.17	2.07	99%	103%	98%	4.1%
50	12.5	8.08	8.41	3.51	8.55	48.93	207.38	0.02530	2.02		99%	96%		
51				3.51	11.53	55.51	227.69	0.02549	2.03		100%	97%		
52				3.51	7.84	55.44	222.11	0.02606	2.08	2.00	98%	99%	95%	4.3%
53	25	8.06	8.45	3.51	8.38	50.39	206.23	0.02536	2.02		98%	96%		
54				3.51	7.69	40.65	165.65	0.02394	1.91		98%	91%		
55				3.51	6.30	19.51	87.41	0.01950	1.55	1.50	96%	74%	71%	4.2%
56	50	8.04	8.4	3.51	6.13	19.63	78.69	0.01899	1.51		97%	72%		
57				3.51	6.54	18.20	67.87	0.01793	1.43		98%	68%		
58				3.51	3.39	5.27	16.22	0.00911	0.73	0.73	79%	34%	34%	5.2%
59	100	7.98	8.32	3.51	3.75	7.54	16.25	0.00958	0.76		91%	36%		
60				3.51	3.78	6.57	14.34	0.00864	0.69		90%	33%		

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6831 • **ABN** 41 687 119 230

Algal growth-72-h slope

Start Date:	18/07/2017	Test ID:	170818WG	Sample ID:	E17021
End Date:	21/07/2017	Lab ID:	CSIRO-CSIRO-CECR	Sample Type:	Drum 1 - Synthetic tailings liquor
Sample Date:		Protocol:	STA-Stauber et al 1994	Test Species:	ISO-Isochrysis
Comments:					

Conc-%	1	2	3
Control	0.0262	0.0268	0.0262
1.5	0.0262	0.0258	0.0259
3	0.0256	0.0261	0.0258
6.25	0.0276	0.0265	0.0262
12.5	0.0251	0.0257	0.0276
25	0.0234	0.0241	0.0221
50	0.0197	0.0199	0.0188
100	0.0116	0.0138	0.0134

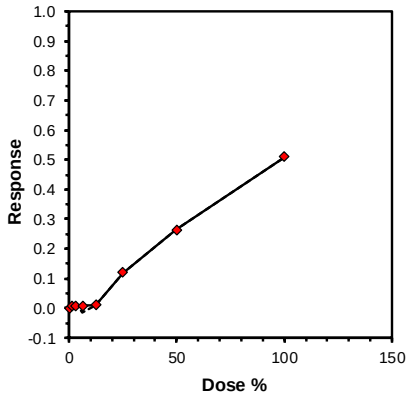
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Control	0.0264	1.0000	0.0264	0.0262	0.0268	1.394	3				0.0264	1.0000
1.5	0.0260	0.9834	0.0260	0.0258	0.0262	0.763	3	0.663	2.560	0.0017	0.0262	0.9917
3	0.0258	0.9785	0.0258	0.0256	0.0261	0.846	3	0.858	2.560	0.0017	0.0262	0.9917
6.25	0.0268	1.0132	0.0268	0.0262	0.0276	2.705	3	-0.525	2.560	0.0017	0.0262	0.9917
12.5	0.0261	0.9898	0.0261	0.0251	0.0276	4.879	3	0.407	2.560	0.0017	0.0261	0.9898
*25	0.0232	0.8791	0.0232	0.0221	0.0241	4.488	3	4.826	2.560	0.0017	0.0232	0.8791
*50	0.0194	0.7358	0.0194	0.0188	0.0199	3.063	3	10.540	2.560	0.0017	0.0194	0.7358
*100	0.0130	0.4905	0.0130	0.0116	0.0138	9.277	3	20.329	2.560	0.0017	0.0130	0.4905

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.983737	0.884	-0.07618	-0.0132
Bartlett's Test indicates equal variances (p = 0.22)	9.5451	18.47531		

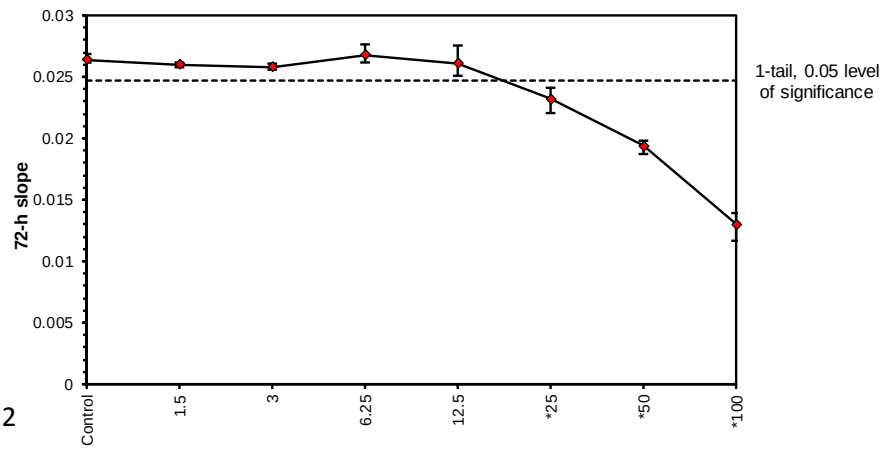
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	12.5	25	17.67767	8	0.001695	0.064159	7.09E-05	6.57E-07	2.9E-12	7, 16

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05	16.992	1.698	8.128	21.414	-0.5947
IC10	22.636	1.877	15.843	30.431	0.1650
IC15	30.072	2.605	18.403	37.699	-0.5354
IC20	38.801	1.991	30.381	45.186	-0.6488
IC25	47.530	1.974	38.785	54.973	-0.2377
IC40	77.687	2.790	67.299	88.461	-0.0140
IC50	98.067				



Dose-Response Plot



New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6831 • **ABN** 41 687 119 230

Algal growth-72-h slope

Start Date:	18/07/2017	Test ID:	170818WG	Sample ID:	E17022
End Date:	21/07/2017	Lab ID:	CSIRO-CSIRO-CECR	Sample Type:	Drum 2 - Synthetic tailings liquor
Sample Date:		Protocol:	STA-Stauber et al 1994	Test Species:	ISO-Isochrysis
Comments:					

Conc-%	1	2	3
Control	0.0262	0.0268	0.0262
0.3	0.0264	0.0264	0.0257
1	0.0265	0.0282	0.0265
6.25	0.0269	0.0271	0.0261
12.5	0.0272	0.0253	0.0255
25	0.0261	0.0254	0.0239
50	0.0195	0.0190	0.0179
100	0.0091	0.0096	0.0086

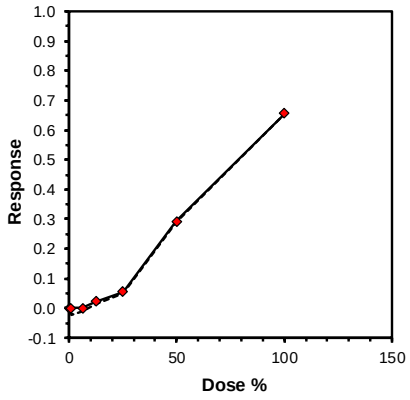
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Control	0.0264	1.0000	0.0264	0.0262	0.0268	1.394	3				0.0266	1.0000
0.3	0.0262	0.9914	0.0262	0.0257	0.0264	1.465	3	0.368	2.560	0.0016	0.0266	1.0000
1	0.0271	1.0246	0.0271	0.0265	0.0282	3.484	3	-1.051	2.560	0.0016	0.0266	1.0000
6.25	0.0267	1.0101	0.0267	0.0261	0.0271	1.888	3	-0.433	2.560	0.0016	0.0266	1.0000
12.5	0.0260	0.9846	0.0260	0.0253	0.0272	4.087	3	0.659	2.560	0.0016	0.0260	0.9782
25	0.0251	0.9508	0.0251	0.0239	0.0261	4.300	3	2.101	2.560	0.0016	0.0251	0.9447
*50	0.0188	0.7119	0.0188	0.0179	0.0195	4.250	3	12.313	2.560	0.0016	0.0188	0.7073
*100	0.0091	0.3449	0.0091	0.0086	0.0096	5.172	3	28.003	2.560	0.0016	0.0091	0.3427

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.970243	0.884	0.193668	-0.59783
Bartlett's Test indicates equal variances (p = 0.73)	4.419228	18.47531		

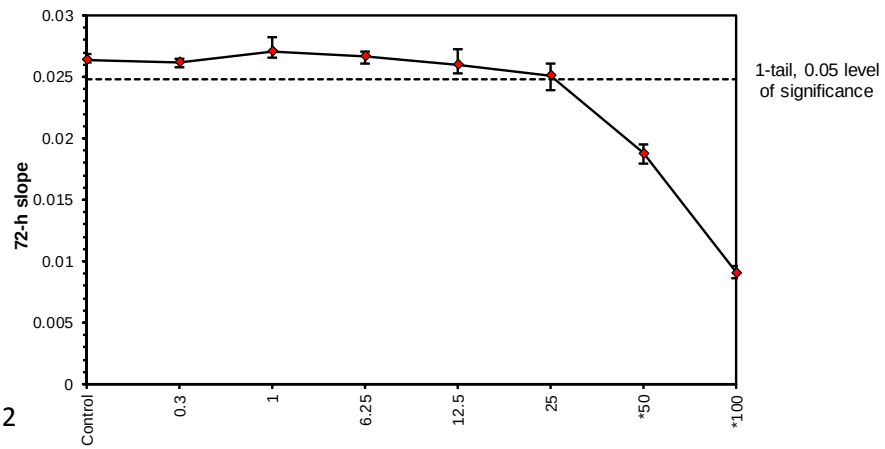
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	25	50	35.35534	4	0.001582	0.059889	0.000118	5.73E-07	1.8E-14	7, 16

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05	23.016	4.312	0.169	31.924	-0.7584
IC10	29.706	1.796	20.004	35.010	-0.7269
IC15	34.972	1.467	28.116	39.581	-0.5982
IC20	40.239	1.309	34.156	44.628	-0.5038
IC25	45.505	1.360	39.470	50.064	-0.3391
IC40	64.716	1.540	57.105	69.455	-0.5854
IC50	78.427	1.204	72.349	82.282	-0.5521



Dose-Response Plot



LAND AND WATER
www.csiro.au
New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6831 • **ABN** 41 687 119 230

Statistics - QA Control

18/07/2017-21/07/2017 **72-h Chronic Toxicity of tailings liquor, Drum 1 (E17021) and Drum 2 (E17022), to *Isochrysis galbana***

	Sample		pH		Day 0	Day 1	Day 2	Day 3	Slope	Growth Rate		Pearson	% Contro	Mean %	CV (%)
Flask No.	Nominal	Measured	Day 0	Day 3	*All cell counts in (cells/mL) by 10^3*					dblngs/day	Mean				
1					3.51	8.97	66.39	223.03	0.02616	2.08	2.10	98%	99%	100%	1.4%
2	Control		8.10	8.18	3.51	8.85	67.99	249.54	0.02684	2.14		98%	102%		
3					3.51	8.61	62.23	228.49	0.02625	2.09		98%	99%		
								Mean control rate =	***0.02642***						
Salinity/pH Controls*															
4					3.51	8.03	61.43	230.54	0.02640	2.10	2.11	98%	100%	100%	0.4%
5	sal/pH		7.92	8.19	3.51	7.95	62.12	229.28	0.02641	2.10		98%	100%		
6					3.51	8.18	61.60	239.22	0.02657	2.12		98%	101%		
Copper (µg/L) [dissolved 0.45 µm measured copper concentrations]															
7					3.51	7.07	25.84	63.56	0.01807	1.44	1.50	99%	68%	71%	9.3%
8	3	2.4	8.10	8.22	3.51	6.95	25.48	57.67	0.01755	1.40		99%	66%		
9					3.51	7.36	30.05	101.66	0.02082	1.66		99%	79%		
10					3.51	5.04	8.23	11.04	0.00711	0.57	0.58	99%	27%	28%	3.7%
11	6	5.0	8.11	8.24	3.51	4.73	8.13	11.92	0.00762	0.61		99%	29%		
12					3.51	4.64	7.53	11.23	0.00719	0.57		99%	27%		
13					3.51	3.63	4.21	4.79	0.00196	0.16	0.13	95%	7%	6%	24%
14	12	10	8.11	8.26	3.51	4.02	4.16	4.64	0.00158	0.13		96%	6%		
15					3.51	3.61	4.10	4.20	0.00120	0.10		92%	5%		
16					3.51	3.62	3.02	2.45	0.00000	0.00	0.00	83%	0%	0%	N/A
17	18	15	8.11	8.27	3.51	3.53	2.91	2.54	0.00000	0.00		89%	0%		
18					3.51	3.41	2.38	2.32	0.00000	0.00		85%	0%		

*** matched initial sal/pH between top concentrations of Drum 1 and Drum 2**

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6831 • **ABN** 41 687 119 230

Algal growth-72-h slope					
Start Date:	18/07/2017	Test ID:	170718 WG	Sample ID:	REF-Ref Toxicant
End Date:	21/07/2017	Lab ID:	CSIRO-CSIRO-CECR	Sample Type:	CUSO-Copper sulfate
Sample Date:		Protocol:	STA-Stauber et al 1994	Test Species:	ISO-Isochrysis
Comments:	Measured concentrations				

Conc-ug/L	1	2	3
Control	0.0262	0.0268	0.0262
2.4	0.0181	0.0175	0.0208
5	0.0071	0.0076	0.0072
10	0.0020	0.0016	0.0012
15.3	0.0000	0.0000	0.0000

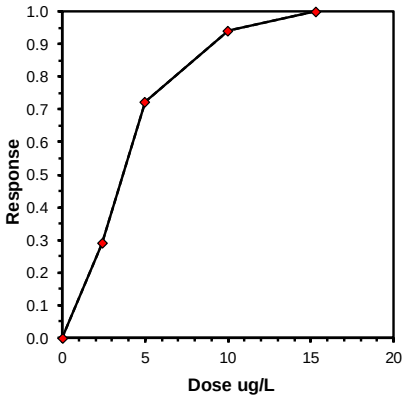
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Control	0.0264	1.0000	0.0264	0.0262	0.0268	1.394	3				0.0264	1.0000
*2.4	0.0188	0.7121	0.0188	0.0175	0.0208	9.345	3	10.040	2.420	0.0018	0.0188	0.7121
*5	0.0073	0.2765	0.0073	0.0071	0.0076	3.744	3	25.231	2.420	0.0018	0.0073	0.2765
*10	0.0016	0.0598	0.0016	0.0012	0.0020	23.793	3	32.791	2.420	0.0018	0.0016	0.0598
15.3	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	3				0.0000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.882422	0.805	1.263135	3.687684
Bartlett's Test indicates equal variances (p = 0.05)	7.978198	11.34487		

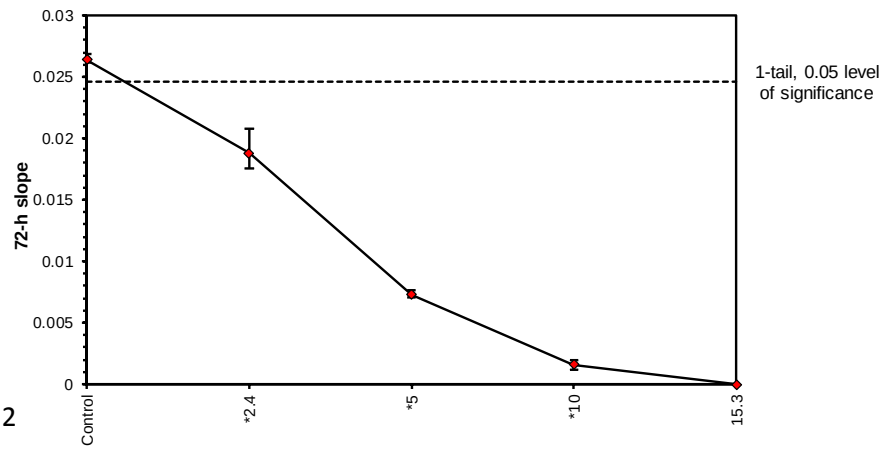
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	<2.4	2.4			0.001833	0.069388	0.000375	8.61E-07	3.3E-09	3, 8

Linear Interpolation (200 Resamples)					
Point	ug/L	SD	95% CL(Exp)		Skew
IC05*	0.4169	0.0499	0.2902	0.6999	1.0471
IC10*	0.8337	0.0998	0.5804	1.3997	1.0471
IC15*	1.2506	0.1497	0.8706	2.0996	1.0471
IC20*	1.6674	0.1996	1.1607	2.7995	1.0471
IC25*	2.0843	0.2209	1.4509	3.0962	0.5714
IC40	3.0693	0.1390	2.5583	3.6251	0.1877
IC50	3.6662	0.0970	3.2935	4.0435	0.1861

* indicates IC estimate less than the lowest concentration



Dose-Response Plot



New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6831 • **ABN** 41 687 119 230

Microalgae Test-72 h growth rate					
Start Date:	7/18/2017	Test ID:	Sal-pH Con	Sample ID:	Sal-pH control
End Date:	7/21/2017	Lab ID:		Sample Type:	diluted seawater
Sample Date:		Protocol:		Test Species:	IG-Isochrysis galbana
Comments:	salinity/pH control for algal toxicity test: 29 ppt, pH 7.9				

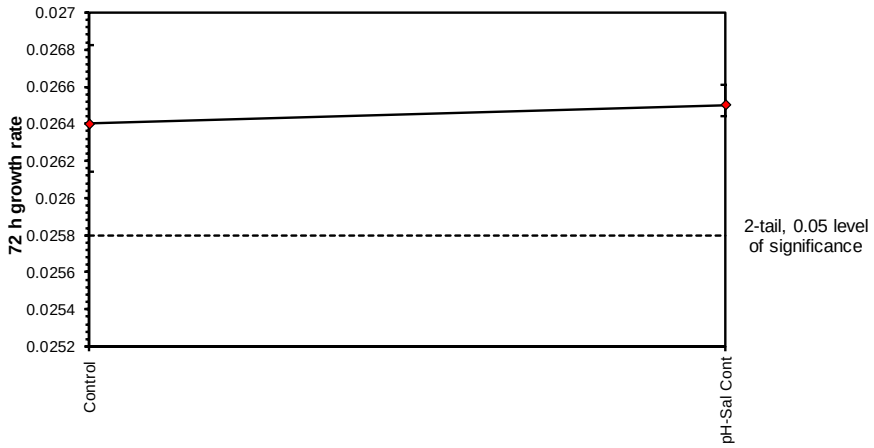
Conc-%	1	2	3
Control	0.0262	0.0268	0.0262
pH-Sal Cont	0.0264	0.0264	0.0266

			Transform: Untransformed					2-Tailed		
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
Control	0.0264	1.0000	0.0264	0.0262	0.0268	1.394	3			
pH-Sal Cont	0.0265	1.0017	0.0265	0.0264	0.0266	0.367	3	0.203	2.776	0.0006

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.913093	0.713	1.18088	1.43745
F-Test indicates equal variances (p = 0.13)	14.40037	199		

Hypothesis Test (2-tail, 0.05)	MSDu	MSDp	MSB	MSE	F-Prob	df
Homoscedastic t Test indicates no significant differences	0.00061	0.023102	2.99E-09	7.25E-08	0.848855	1, 4

Dose-Response Plot



New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6831 • **ABN** 41 687 119 230

Statistics – Range-finder test

Date 07/02/2017 - 10/02/2017

Toxicity of Tailings Liquor Drum 1 and Drum 2 to *I. galbana* - 72-h growth rate inhibition test - Rangefinder

			pH (after algae)		Salinity (psu)		Conductivity (µs)		DO (% sat)		Day 0	Day 1	Day 2	Day 3	Slope	Growth Rate (dblngs/day)		Pearson	% Contro	Mean %	CV (%)
Flask No.	Sample	Measured	Day 0	Day 3			Day 0	Day 3	Day 0	Day 3	*All cell counts in (cells/mL) by 10^3*						Mean				
1											3.23	17.67	148.95	210.99	0.02655	2.12		94%	99%	100%	1.7%
2	Control	0	8.10	8.2	36.0	36.0	55.0	54.1	110%	94%	3.23	14.90	131.55	231.51	0.02713	2.16	2.13	96%	102%		
3											3.23	15.06	127.36	230.22	0.02703	2.15		96%	101%		
4											3.23	20.99	119.78	222.34	0.02612	2.08		96%	98%		
														mean control =	***0.02671***						
Reference Toxicant - Copper (µg/L)																					
5											3.23	14.88	101.58	199.16	0.02585	2.06		97%	97%		
6	1	Need MS	8.10	8.21	N/S	N/S	N/S	N/S	N/S	N/S	3.23	17.04	132.50	220.68	0.02664	2.12	2.09	95%	100%	98%	2.1%
5											3.23	13.77	85.43	151.91	0.02421	1.93		97%	91%		
6	2	1	8.10	8.20	N/S	N/S	N/S	N/S	N/S	N/S	3.23	13.85	68.71	130.05	0.02296	1.83	1.88	97%	86%	88%	3.7%
7											3.23	9.14	24.69	37.73	0.01514	1.21		97%	57%		
8	4	2	8.10	8.20	N/S	N/S	N/S	N/S	N/S	N/S	3.23	9.23	20.57	31.91	0.01388	1.11	1.16	97%	52%	54%	6.1%
9											3.23	4.46	9.05	8.87	0.00676	0.54		88%	25%		
10	8	6	8.11	8.19	N/S	N/S	N/S	N/S	N/S	N/S	3.23	6.16	9.02	9.10	0.00631	0.50	0.52	86%	24%	24%	4.9%
11											3.23	4.11	4.31	4.66	0.00208	0.17		88%	8%		
12	12	9	8.10	8.18	N/S	N/S	N/S	N/S	N/S	N/S	3.23	4.31	5.04	4.93	0.00258	0.21	0.19	81%	10%	9%	15.3%
Drum 1 (E17021)																					
13											3.23	18.38	132.91	236.97	0.02690	2.14		96%	101%		
14	0.01%	NA	8.09	8.21	36.1	36.1	54.7	84.2	98%	94%	3.23	22.50	143.45	236.60	0.02666	2.12	2.13	95%	100%	100%	0.6%
15											3.23	18.40	134.83	252.20	0.02726	2.17		96%	102%		
16	0.1%	NA	8.10	8.20	36.0	36.2	54.6	54.3	98%	94%	3.23	19.90	131.97	257.32	0.02719	2.17	2.17	96%	102%	102%	0.2%
17											3.23	19.45	122.33	249.80	0.02693	2.15		97%	101%		
18	1%	NA	8.10	8.22	35.7	35.0	54.1	53.8	98%	94%	3.23	21.86	128.14	239.43	0.02657	2.12	2.13	96%	100%	100%	0.9%
19											3.23	17.24	108.69	198.58	0.02569	2.05		96%	96%		
20	10%	NA	8.10	8.25	32.9	33.0	50.3	50.0	100%	95%	3.23	22.26	112.47	206.47	0.02550	2.03	2.04	96%	95%	96%	0.5%
21											3.23	6.56	12.21	18.31	0.01054	0.84		99%	39%		
22	50%	NA	8.11	8.27	18.6	19.3	30.0	30.9	101%	94%	3.23	5.50	14.98	17.04	0.01084	0.86	0.85	93%	41%	40%	2.0%
Drum 2 (E17022)																					
13											3.23	16.72	151.38	259.76	0.02780	2.22		96%	104%		
14	0.01%	NA	8.09	8.22	36.1	36.1	54.5	54.2	98%	94%	3.23	21.54	143.55	223.09	0.02642	2.11	2.16	94%	99%	102%	3.6%
15											3.23	20.04	166.52	239.01	0.02720	2.17		94%	102%		
16	0.1%	NA	8.09	8.22	36.0	36.1	54.4	54.2	99%	94%	3.23	17.80	152.28	247.94	0.02745	2.19	2.18	95%	103%	102%	0.7%
17											3.23	18.94	117.44	236.30	0.02661	2.12		97%	100%		
18	1%	NA	8.09	8.22	35.8	35.6	54.1	53.6	100%	94%	3.23	19.38	110.02	218.00	0.02601	2.07	2.10	97%	97%	98%	1.6%
19											3.23	15.43	83.48	154.79	0.02406	1.92		97%	90%		
20	10%	NA	8.08	8.18	32.5	33.0	49.7	50.1	100%	94%	3.23	13.81	85.54	129.61	0.02334	1.86	1.89	95%	87%	89%	2.1%
21											3.23	4.08	23.91	53.91	0.01848	1.47		93%	69%		
22	50%	NA	8.05	8.22	18.8	19.0	30.3	30.4	100%	94%	3.23	5.13	20.13	50.33	0.01738	1.38	1.43	97%	65%	67%	4.3%

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6831 • **ABN** 41 687 119 230

Algal growth-72-h slope						
Start Date:	7/02/2017	Test ID:	170207WG	Sample ID:	DRUM1	
End Date:	10/02/2017	Lab ID:	CSIRO-CSIRO-CECR	Sample Type:	Tailings liquor	
Sample Date:		Protocol:		Test Species:	IG-Isochrysis galbana	
Comments:						

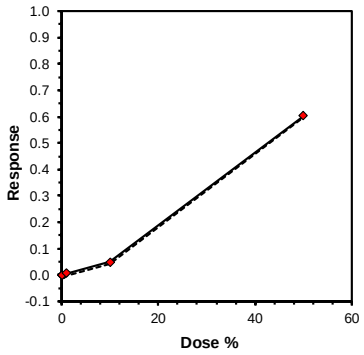
Conc-%	1	2	3	4
Control	0.0265	0.0271	0.0270	0.0261
0.01	0.0269	0.0267		
0.1	0.0273	0.0272		
1	0.0269	0.0266		
10	0.0257	0.0255		
50	0.0105	0.0108		

			Transform: Untransformed					1-Tailed			Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Control	0.0267	1.0000	0.0267	0.0261	0.0271	1.741	4				0.0269	1.0000
0.01	0.0268	1.0027	0.0268	0.0267	0.0269	0.624	2	-0.267	2.660	0.0007	0.0269	1.0000
0.1	0.0272	1.0194	0.0272	0.0272	0.0273	0.186	2	-1.885	2.660	0.0007	0.0269	1.0000
1	0.0268	1.0017	0.0268	0.0266	0.0269	0.945	2	-0.169	2.660	0.0007	0.0268	0.9944
*10	0.0256	0.9584	0.0256	0.0255	0.0257	0.522	2	4.042	2.660	0.0007	0.0256	0.9514
*50	0.0107	0.4003	0.0107	0.0105	0.0108	1.975	2	58.279	2.660	0.0007	0.0107	0.3974

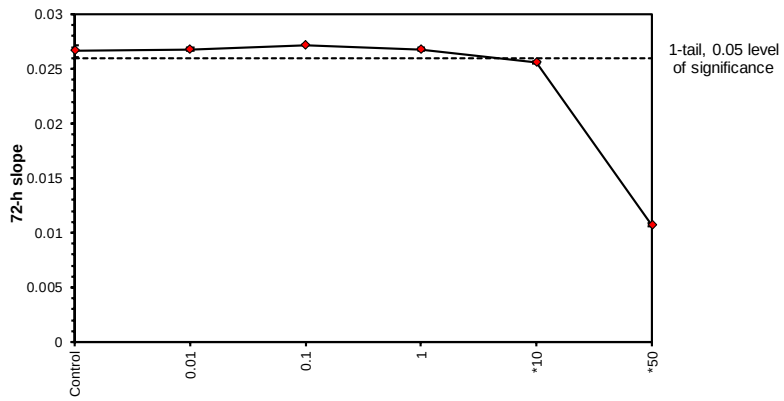
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.954843	0.825	-0.53903	1.352757
Bartlett's Test indicates equal variances (p = 0.52)	4.201273	15.08627		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1	10	3.162278	100	0.000731	0.02737	8.77E-05	1.01E-07	1.0E-10	5, 8

Linear Interpolation (200 Resamples)					
Point	%	SD	95% CL(Exp)		Skew
IC05	10.101	0.456	4.831	11.605	-1.0464
IC10	13.711	0.230	11.711	15.206	-0.0848
IC15	17.321	0.217	15.471	18.787	-0.0424
IC20	20.931	0.208	19.192	22.269	0.0174
IC25	24.542	0.203	22.912	25.912	0.0853
IC40	35.372	0.219	33.867	36.994	0.1980
IC50	42.593	0.250	40.897	44.408	0.1649



Dose-Response Plot



New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6831 • **ABN** 41 687 119 230

Algal growth-72-h slope

Start Date:	7/02/2017	Test ID:	170207WG	Sample ID:	DRUM2
End Date:	10/02/2017	Lab ID:	CSIRO-CSIRO-CECR	Sample Type:	Tailings liquor
Sample Date:		Protocol:		Test Species:	IG-Isochrysis galbana
Comments:					

Conc-%	1	2	3	4
Control	0.0265	0.0271	0.0270	0.0261
0.01	0.0278	0.0264		
0.1	0.0272	0.0274		
1	0.0266	0.0260		
10	0.0241	0.0233		
50	0.0185	0.0174		

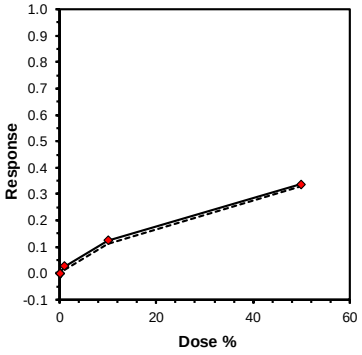
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Control	0.0267	1.0000	0.0267	0.0261	0.0271	1.741	4				0.0270	1.0000
0.01	0.0271	1.0152	0.0271	0.0264	0.0278	3.601	2	-0.812	2.660	0.0013	0.0270	1.0000
0.1	0.0273	1.0230	0.0273	0.0272	0.0274	0.652	2	-1.229	2.660	0.0013	0.0270	1.0000
1	0.0263	0.9850	0.0263	0.0260	0.0266	1.605	2	0.800	2.660	0.0013	0.0263	0.9726
*10	0.0237	0.8875	0.0237	0.0233	0.0241	2.145	2	6.001	2.660	0.0013	0.0237	0.8763
*50	0.0179	0.6714	0.0179	0.0174	0.0185	4.333	2	17.528	2.660	0.0013	0.0179	0.6629

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.940935	0.825	-0.08912	-1.39501
Bartlett's Test indicates equal variances (p = 0.83)	2.158614	15.08627		

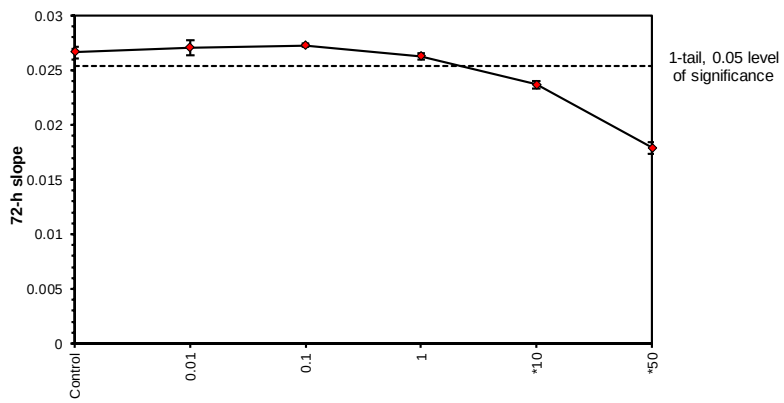
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1	10	3.162278	100	0.001332	0.049867	2.76E-05	3.34E-07	1.2E-06	5, 8

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05	3.112	0.741	0.000	7.608	-0.1475
IC10	7.786	0.853	1.244	14.750	0.2305
IC15	14.933	1.821	0.000	27.136	-0.1204
IC20	24.306	1.667	9.902	36.723	0.0612
IC25	33.679	1.839	21.074	48.115	0.3062
IC40	>50				
IC50	>50				



Dose-Response Plot



CSIRO LAND AND WATER
www.csiro.au

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6812 • **ABN** 41 687 119 230

18 January 2018

Chronic Copepod Larval Development Test Report E17021 CLD

Client:	GDA Consult Pty Ltd
Project:	Wafi-Golpu Pre-feasibility study of DSTP
Test Performed:	80-h larval development test with the marine copepod *Acartia sinjiensis*

Test Initiated:	8/1/2018	
CSIRO Sample No.	**Sample Name:**	**Sample Description:**
E17021	Drum 1	Drum 1 tailings
E17022	Drum 2	Drum 2 tailings

Sample Physico-Chemistry and Preparation: Prior to toxicity testing, tailings liquor was prepared by simulating pre-discharge mixing with seawater at a dilution of 1 in 4. Natural seawater (3 parts, 3 kg) was added to tailings material (solids and liquor) (1 part, 1 kg) and mixed on a roller for 1 h. The resulting solution was filtered to 0.45 µm using a cartridge filter (with a 0.65 µm pre-filter) and the filtrate (tailings liquor) collected for testing. The tailings liquor was stored at 4°C in the dark prior to toxicity testing. All reference to tailings liquor hereon describes the 1 in 4 diluted and filtered tailings-seawater mixture as 100% (or undiluted) tailings liquor. Analyses of dissolved metals confirmed that the sample composition was unchanged since first tested with copepods on 31 July, 2018 (see main report for details).

Sample	**Physico-Chemistry**				
	pH	**Salinity (‰)**	**Conductivity (mS/cm)**	**DO (% O_2 sat)**	**Comments**
Drum 1 liquor, 100% (E17021)	7.8	30	46	99	
Drum 2 liquor, 100% (E17022)	7.7	29	45	99	
Drum 1 liquor, 6.7% (highest test concentration)	8.0	36	54	91	
Drum 2 liquor, 3% (highest test concentration)	8.0	36	55	90	
Seawater (used for controls and diluent)	8.1	36	56	98	

Test method: This test measures the proportion of *A. sinjiensis* eggs that hatch, and develop through 6 naupliar stages to metamorphose to copepodites. This usually takes 80 h, but can be extended if required. The test containers were incubated at 29-30˚C. The test protocol follows an in-house method recently developed for *A. sinjiensis* (Binet et al, unpublished manuscript) and is based on methods described in ISO (2015) and Knuckey et al (2005). The number of eggs, nauplii and copepodites in each test container is counted microscopically after a minimum of 80 h exposure, and used to determine egg hatching rate, the larval development ratio (LDR; calculated as ∑copepodites/∑(copepodites + nauplii), and the total number of hatched animals (proxy for survival). The mean LDR of the QA Controls are compared to those for each treatment to determine the IC50, IC10, LOEC and NOEC values, using ToxCalc Version 5.0.23 (Tidepool Software). Nickel was also tested for quality assurance purposes. Microalgal food is required by copepod larvae to survive, develop and moult. Two strains of microalgae, *Tetraselmsi chuii* and *Tisochrysis lutea* (previously known as *Isochrysis galbana*) were added as food throughout the exposure period. For the QA Control and nickel reference toxicant treatments,

microalgae grown in F2 medium were used. For the tailings treatments and an additional control (Control MOD), microalgae grown in F2 medium without essential trace metal nutrients were used. Dissolved metals (0.45 µm) were also measured in selected tailings liquor concentrations (refer to main report for results). A range-finder test (limited concentrations and replicates) was also carried out on undiluted liquor (decanted, not filtered) to establish the concentrations of liquor to be tested in the definitive toxicity test. Results of the range-finder test are presented in the appendix.

Sample Results: Both tailings liquor samples from Drum 1 (E17021) and Drum 2 (E17022) were toxic to *A. sinjiensis* larval development with IC10 values of 0.36% and 0.19%, and IC50 values of 1.8% and 0.38%, respectively. Egg hatching was not affected by either sample, with similar hatching rates of around 60% in all treatments.

The total number of animals counted in each replicate can be used as a proxy for survival, since only live, or very recently deceased animals stain strongly with Rose Bengal, and only strongly stained animals are counted. Based on this parameter, liquors from Drums 1 and 2 were less toxic to survival than to larval development over the same exposure period, i.e., the LC10 and LC50 values for Drum 1 (0.81% and 2.8%%, respectively) and Drum 2 (0.35% and 0.70%, respectively) were higher than the IC10 and IC50 values determined based on larval development.

QA Comments: The acceptability criteria for this bioassay were achieved; the LDR in the QA control (70%) was >50% with a CV of <20%, and the reference toxicant, nickel IC50 for LDR was 9.2 µg/L, within the cusum chart limit of 8.6 ± 1.7 µg/L. There was no significant difference between the QA Control and the Control MOD in terms of hatch rate, larval development (LDR) or survival.

Sample	**Mean hatch rate (%)**	**Total animals counted (survival)**		**Larval Development Ratio (LDR)**		
		Mean number counted	**Mean % MOD Control**	**Mean LDR (%)**	**Mean % MOD Control**	**CV (%)**
QA Control	66	29	85	70	101	15
MOD Control	65	34	100	69	100	10
Drum 1 (E17021)						
0.027%	57	31	91	73	106	11
0.082%	50	33	98	72	104	9.4
0.25%	55	32	94	73	105	19
0.74%	58	30	89	47[a]	68[a]	24
2.2%	55	22[a]	64[a]	36[a]	52[a]	62
6.7%	52	2[a]	5[a]	0[a]	0[a]	N/A
Drum 2 (E17022)						
0.012%	54	40	116	71	103	11
0.037%	54	35	102	76	110	13
0.11%	60	31	90	72	103	16
0.33%	59	32	94	43[a]	62[a]	16
1.0%	58	8[a]	24[a]	2[a]	2[a]	224
3.0%	55	3[a]	9[a]	0[a]	0[a]	N/A

LDR	**IC50 (%)[b]**	**IC10 (%)[b]**	**LOEC (%)[c]**	**NOEC (%)[d]**
Drum 1 (E17021)	1.8 (1.3-2.6)	0.36 (0.12-0.58)	0.74	0.25
Drum 2 (E17022)	0.38 (0.32-0.44)	0.19 (0.11-0.24)	0.33	0.11
Survival	**LC50 (%)[b]**	**LC10 (%)[b]**	**LOEC (%)[c]**	**NOEC (%)[d]**
Drum 1 (E17021)	2.8 (2.2-3.5)	0.81 (0.38-1.2)	2.2	0.74
Drum 2 (E17022)	0.70 (0.61-0.79)	0.35 (0.25-0.44)	1.0	0.33

[a] Significantly less than Control MOD

[b] Concentration of the sample to cause a 50% or 10% inhibition in the measured endpoint (LDR or survival)

[c] Lowest concentration tested to have a significant (p≤0.05) inhibition in the measured endpoint compared to the control MOD

[d] Highest concentration tested to have no significant (p>0.05) inhibition in the measured endpoint compared to the control MOD

Quality Assurance/Quality Control	Criterion	This Test	Criterion Met?
QA Control larval development ratio (%)	>50%	70%	Yes
QA Control larval development CV (%)	<20%	15%	Yes
Reference toxicant IC50 (measured nickel, µg/L)	8.6 ± 1.7	9.2	Yes

References:

Knuckey, R.M., Semmens, G.L., Mayer, R.J. and Rimmer, M.A. (2005). Development of an optimal microalgal diet for the culture of the calanoid copepod *Acartia sinjiensis*: Effect of algal species and feed concentration on copepod development. Aquaculture 249: 339-351.

ISO (2015). International Standard ISO 16778, Water quality – Calanoid copepod early-life stage test with *Acartia tonsa*, First edition 2015-06-15, Switzerland.

Test carried out by: Monique Binet, Kitty McKnight, Merrin Adams
Test supervised by: Monique Binet
Test report prepared by: Monique Binet
Test report reviewed by: Merrin Adams and Lisa Golding

Team Leader | Aquatic Ecotoxicology
Aquatic Contaminants Group
Environmental Contaminant Mitigation and Biotechnologies
CSIRO Land and Water
E merrin.adams@csiro.au **T** +61 2 9710 6831

Date: 18 January 2018

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6812 • **ABN** 41 687 119 230

Statistics - Sample

Copepod Larval Development Test - LD43 - Drum 1, modified algal food

Algal food (x 10^4 cells/mL)				
Algae cultured without metal stock, in MODIFIED F2 media				
	Day 0		**Day 2**	
	Target	Actual	*Target*	Actual
T. chuii-MOD	*0.63*	0.61	*0.31*	0.34
I. galbana-MOD	*8.00*	8.04	*4.00*	4.15

Vial	Treatment	Day 0	Day 3										
		Unhatched	Unhatched		Nauplii		Copepodites		Total animals (C+N)		**LDR***		
		eggs (#E)	Eggs (#E)	% hatch	# N	% N	# C	%C	#	% Control	C/(C+N)	% Control	CV
1		57	16	72%	5	9%	20	35%	25	74%	80%	115%	
2		57	15	73%	13	23%	22	39%	35	103%	63%	91%	
3		57	20	65%	12	21%	25	44%	37	109%	68%	97%	
4	Control	57	20	65%	14	25%	22	39%	36	106%	61%	88%	
5		57	26	54%	12	21%	31	55%	43	126%	72%	104%	
6		57	22	61%	7	12%	23	41%	30	88%	77%	110%	
7		57	18	68%	11	19%	21	37%	32	94%	66%	95%	
Mean				65%		19%		41%	**34**	**100%**	**69%**	**100%**	**10%**
Drum 1 (E17021) - with modified algal food													
1		57	27	52%	9	16%	19	34%	28	82%	68%	98%	
2		57	21	63%	13	23%	22	39%	35	103%	63%	91%	
3	0.027%	57	32	43%	8	14%	29	51%	37	109%	78%	113%	
4		57	18	68%	8	14%	25	44%	33	97%	76%	109%	
5		57	24	58%	4	7%	18	32%	22	65%	82%	118%	
Mean				57%		15%		40%	**31**	**91%**	**73%**	**106%**	**11%**
1		57	31	45%	7	12%	27	48%	34	100%	79%	114%	
2		57	24	58%	7	12%	22	39%	29	85%	76%	109%	
3	0.082%	57	21	63%	7	12%	21	37%	28	82%	75%	108%	
4		57	29	49%	10	18%	23	41%	33	97%	70%	100%	
5		57	35	38%	16	28%	26	46%	42	124%	62%	89%	
Mean				50%		17%		42%	**33**	**98%**	**72%**	**104%**	**9.4%**
1		57	39	31%	3	5%	33	58%	36	106%	92%	132%	
2		57	24	58%	9	16%	17	30%	26	76%	65%	94%	
3	0.25%	57	27	52%	6	11%	29	51%	35	103%	83%	119%	
4		57	16	72%	15	27%	20	35%	35	103%	57%	82%	
5		57	21	63%	9	16%	19	34%	28	82%	68%	98%	
Mean				55%		15%		42%	**32**	**94%**	**73%**	**105%**	**19%**
1		57	25	56%	17	30%	11	19%	28	82%	39%	57%	
2		57	27	52%	15	27%	17	30%	32	94%	53%	77%	
3	0.74%	57	24	58%	14	25%	20	35%	34	100%	59%	85%	
4		57	19	66%	12	21%	14	25%	26	76%	54%	78%	
5		57	25	56%	22	39%	10	18%	32	94%	31%	45%	
Mean				58%		28%		25%	**30**	**89%**	**47%**	**68%**	**24%**
1		57	26	54%	9	16%	12	21%	21	62%	57%	82%	
2		57	24	58%	15	27%	2	4%	17	50%	12%	17%	
3	2.2%	57	26	54%	17	30%	3	5%	20	59%	15%	22%	
4		57	22	61%	7	12%	10	18%	17	50%	59%	85%	
5		57	28	50%	21	37%	12	21%	33	97%	36%	52%	
Mean				55%		24%		14%	**22**	**64%**	**36%**	**52%**	**62%**
1		57	44	22%	2	4%	0	0%	2	6%	0%	0%	
2		57	25	56%	1	2%	0	0%	1	3%	0%	0%	
3	6.7%	57	17	70%	2	4%	0	0%	2	6%	0%	0%	
4		57	28	50%	3	5%	0	0%	3	9%	0%	0%	
5		57	21	63%	1	2%	0	0%	1	3%	0%	0%	
Mean				52%		3%		0%	**2**	**5%**	**0%**	**0%**	**N/A**

Copepod Larval Development Test - LD43 - Drum 2, modified algal food

Algal food (x 10^4 cells/mL)				
Algae cultured without metal stock, in MODIFIED F2 media				
	Day 0		Day 2	
	Target	Actual	*Target*	Actual
T. chuii-MOD	*0.63*	0.61	*0.31*	0.34
I. galbana-MOD	*8.00*	8.04	*4.00*	4.15

		Day 0	Day 3										
Vial	Treatment	Unhatched eggs (#E)	Unhatched Eggs (#E)	% hatch	Nauplii # N	Nauplii % N	Copepodites # C	Copepodites %C	Total animals (C+N) #	Total animals (C+N) % Control	**LDR*** C/(C+N)	**LDR*** % Control	CV
1	Control	57	16	72%	5	9%	20	35%	25	74%	80%	115%	
2		57	15	73%	13	23%	22	39%	35	103%	63%	91%	
3		57	20	65%	12	21%	25	44%	37	109%	68%	97%	
4		57	20	65%	14	25%	22	39%	36	106%	61%	88%	
5		57	26	54%	12	21%	31	55%	43	126%	72%	104%	
6		57	22	61%	7	12%	23	41%	30	88%	77%	110%	
7		57	18	68%	11	19%	21	37%	32	94%	66%	95%	
Mean				65%		19%		41%	**34**	**100%**	**69%**	**100%**	**10%**
Drum 2 (E17022) - with modified algal food													
1	0.012%	57	23	59%	11	19%	24	42%	35	103%	69%	99%	
2		57	33	42%	6	11%	28	50%	34	100%	82%	119%	
3		57	26	54%	15	27%	27	48%	42	124%	64%	93%	
4		57	22	61%	14	25%	33	58%	47	138%	70%	101%	
Mean				54%		20%		50%	**40**	**116%**	**71%**	**103%**	**11%**
1	0.037%	57	17	70%	13	23%	20	35%	33	97%	61%	87%	
2		57	31	45%	6	11%	30	53%	36	106%	83%	120%	
3		57	18	68%	6	11%	33	58%	39	115%	85%	122%	
4		57	34	40%	8	14%	22	39%	30	88%	73%	106%	
5		57	29	49%	7	12%	28	50%	35	103%	80%	115%	
Mean				54%		14%		47%	**35**	**102%**	**76%**	**110%**	**13%**
1	0.110%	57	24	58%	3	5%	25	44%	28	82%	89%	129%	
2		57	27	52%	13	23%	18	32%	31	91%	58%	84%	
3		57	23	59%	7	12%	20	35%	27	79%	74%	107%	
4		57	17	70%	9	16%	19	34%	28	82%	68%	98%	
5		57	22	61%	12	21%	27	48%	39	115%	69%	100%	
Mean				60%		16%		39%	**31**	**90%**	**72%**	**103%**	**16%**
1	0.330%	57	27	52%	16	28%	12	21%	28	82%	43%	62%	
2		57	23	59%	16	28%	15	27%	31	91%	48%	70%	
3		57	31	45%	20	35%	13	23%	33	97%	39%	57%	
4		57	20	65%	22	39%	11	19%	33	97%	33%	48%	
5		57	16	72%	17	30%	17	30%	34	100%	50%	72%	
Mean				59%		32%		24%	**32**	**94%**	**43%**	**62%**	**16%**
1	1.00%	57	18	68%	9	16%	0	0%	9	26%	0%	0%	
2		57	21	63%	5	9%	0	0%	5	15%	0%	0%	
3		57	33	42%	11	19%	1	2%	12	35%	8%	12%	
4		57	14	75%	7	12%	0	0%	7	21%	0%	0%	
5		57	32	43%	8	14%	0	0%	8	24%	0%	0%	
Mean				58%		14%		0%	**8**	**24%**	**2%**	**2%**	**224%**
1	3.00%	57	23	59%	1	2%	0	0%	1	3%	0%	0%	
2		57	28	50%	2	4%	0	0%	2	6%	0%	0%	
3		57	28	50%	4	7%	0	0%	4	12%	0%	0%	
4		57	21	63%	3	5%	0	0%	3	9%	0%	0%	
5		57	28	50%	5	9%	0	0%	5	15%	0%	0%	
Mean				55%		5%		0%	**3**	**9%**	**0%**	**0%**	**N/A**

Copepod larval development test-LDR							
Start Date:	8/01/2018	Test ID:	LD43	Sample ID:	E17021-MOD algae		
End Date:	11/01/2018	Lab ID:	CECR-CSIRO	Sample Type:	Drum 1		
Sample Date:		Protocol:	CSIRO-In-house method	Test Species:	AS-Acartia sinjiensis		
Comments:	Algae grown without metals used as food during exposure						

Conc-%	1	2	3	4	5	6	7
Control	0.8000	0.6286	0.6757	0.6111	0.7209	0.7667	0.6563
0.027	0.6786	0.6286	0.7838	0.7576	0.8182		
0.082	0.7941	0.7586	0.7500	0.6970	0.6190		
0.25	0.9167	0.6538	0.8286	0.5714	0.6786		
0.74	0.3929	0.5313	0.5882	0.5385	0.3125		
2.2	0.5714	0.1176	0.1500	0.5882	0.3636		
6.7	0.0000	0.0000	0.0000	0.0000	0.0000		

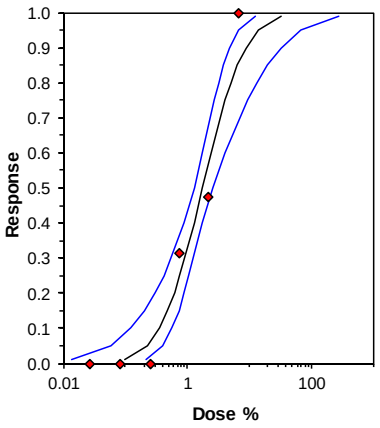
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
Control	0.6942	1.0000	0.9872	0.8974	1.1071	7.952	7				74	238
0.027	0.7333	1.0564	1.0314	0.9154	1.1303	8.530	5	-0.542	2.479	0.2022	42	155
0.082	0.7238	1.0426	1.0195	0.9056	1.0998	7.375	5	-0.397	2.479	0.2022	47	166
0.25	0.7298	1.0513	1.0377	0.8571	1.2780	16.388	5	-0.620	2.479	0.2022	42	160
*0.74	0.4727	0.6809	0.7571	0.5932	0.8741	15.477	5	2.820	2.479	0.2022	80	152
*2.2	0.3582	0.5160	0.6253	0.3501	0.8741	39.472	5	4.436	2.479	0.2022	69	108
6.7	0.0000	0.0000	0.4126	0.2928	0.5236	25.489	5				9	9

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.97623	0.904	-0.0016	-0.1233
Bartlett's Test indicates equal variances (p = 0.08)	9.81502	15.0863		

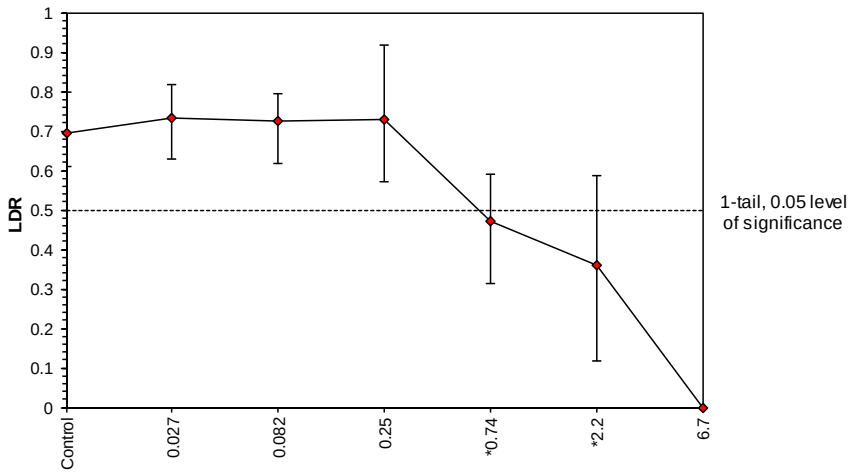
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	0.25	0.74	0.43012	400	0.19679	0.28262	0.15573	0.01942	1.1E-04	5, 26

Maximum Likelihood-Probit											
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	1.83748	0.3829	1.087	2.58795	0.31092	8.31114	9.48773	0.08	0.24921	0.54422	4
Intercept	4.54209	0.13363	4.28018	4.804							
TSCR	0.28269	0.01965	0.24418	0.3212							

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	0.09619	0.01397	0.22159
EC05	3.355	0.22596	0.05813	0.41365
EC10	3.718	0.35625	0.12334	0.58143
EC15	3.964	0.48434	0.20357	0.73639
EC20	4.158	0.61826	0.30111	0.89454
EC25	4.326	0.76231	0.41794	1.06547
EC40	4.747	1.29219	0.89267	1.77065
EC50	5.000	1.77503	1.30044	2.60435
EC60	5.253	2.43828	1.77865	4.08002
EC75	5.674	4.13313	2.7726	9.29039
EC80	5.842	5.09608	3.26554	13.0407
EC85	6.036	6.50516	3.93403	19.4498
EC90	6.282	8.84417	4.95083	32.3064
EC95	6.645	13.9436	6.92273	68.9122
EC99	7.326	32.7538	12.8663	288.007



Dose-Response Plot



Copepod larval development test-LDR							
Start Date:	8/01/2018	Test ID:	LD43	Sample ID:	E17022-MOD algae		
End Date:	11/01/2018	Lab ID:	CECR-CSIRO	Sample Type:	Drum 2		
Sample Date:		Protocol:	CSIRO-In-house method	Test Species:	AS-Acartia sinjiensis		
Comments:	Algae grown without metals used as food during exposure						

Conc-%	1	2	3	4	5	6	7
Control	0.8000	0.6286	0.6757	0.6111	0.7209	0.7667	0.6563
0.012	0.6857	0.8235	0.6429	0.7021			
0.037	0.6061	0.8333	0.8462	0.7333	0.8000		
0.11	0.8929	0.5806	0.7407	0.6786	0.6923		
0.33	0.4286	0.4839	0.3939	0.3333	0.5000		
1	0.0000	0.0000	0.0833	0.0000	0.0000		
3	0.0000	0.0000	0.0000	0.0000	0.0000		

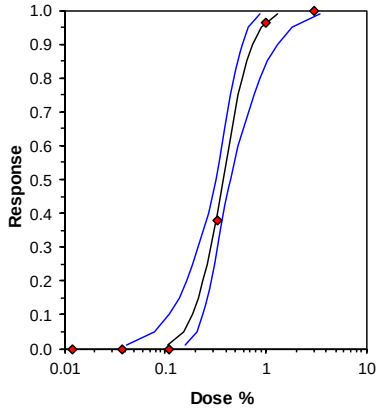
			Transform: Arcsin Square Root					1-Tailed			Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
Control	0.6942	1.0000	0.9872	0.8974	1.1071	7.952	7				74	238
0.012	0.7136	1.0279	1.0092	0.9303	1.1373	8.863	4	-0.377	2.485	0.1449	46	158
0.037	0.7638	1.1003	1.0691	0.8923	1.1677	10.532	5	-1.505	2.485	0.1354	40	173
0.11	0.7170	1.0329	1.0182	0.8664	1.2373	13.463	5	-0.570	2.485	0.1354	44	153
*0.33	0.4279	0.6165	0.7125	0.6155	0.7854	9.700	5	5.043	2.485	0.1354	91	159
*1	0.0167	0.0240	0.2107	0.1674	0.2928	24.138	5	14.255	2.485	0.1354	40	41
3	0.0000	0.0000	0.3312	0.2255	0.5236	35.958	5				15	15

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.97752	0.902	0.3196	0.43135
Bartlett's Test indicates equal variances (p = 0.49)	4.41639	15.0863		

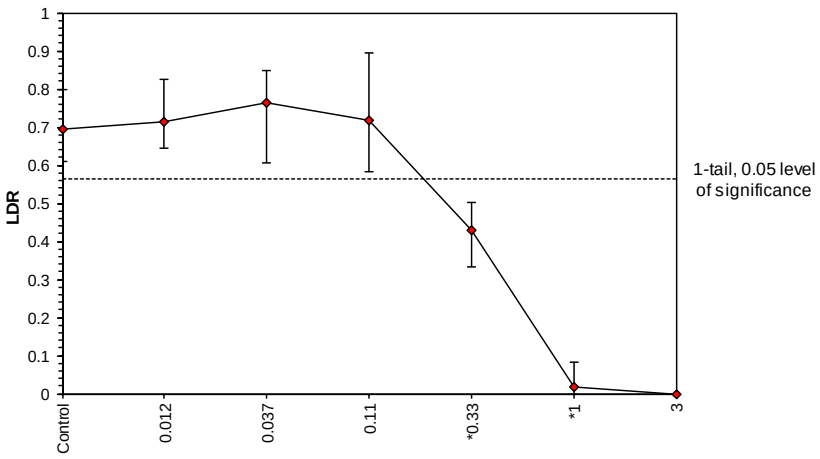
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	0.11	0.33	0.19053	909.091	0.13012	0.18686	0.5497	0.00865	2.1E-13	5, 25

Maximum Likelihood-Probit											
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	4.30242	0.94609	2.44808	6.15676	0.31092	2.19931	9.48773	0.7	-0.4256	0.23243	3
Intercept	6.83097	0.43969	5.96918	7.69276							
TSCR	0.28091	0.01718	0.24724	0.31458							

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	0.10808	0.04185	0.1595
EC05	3.355	0.15564	0.07881	0.20746
EC10	3.718	0.18904	0.11009	0.23943
EC15	3.964	0.21554	0.13759	0.26441
EC20	4.158	0.23923	0.16384	0.28685
EC25	4.326	0.26162	0.18978	0.3085
EC40	4.747	0.32775	0.26836	0.37951
EC50	5.000	0.37535	0.32052	0.44332
EC60	5.253	0.42985	0.37142	0.53376
EC75	5.674	0.53852	0.45368	0.76015
EC80	5.842	0.58891	0.4873	0.8816
EC85	6.036	0.65362	0.52814	1.05083
EC90	6.282	0.74525	0.58283	1.31427
EC95	6.645	0.90519	0.67223	1.83704
EC99	7.326	1.30359	0.87388	3.46141



Dose-Response Plot



Copepod larval survival - (Total hatched animals, C + N)							
Start Date:	8/01/2018	Test ID:	LD43	Sample ID:	E17021		
End Date:	11/01/2018	Lab ID:	CECR-CSIRO	Sample Type:	Drum 1		
Sample Date:		Protocol:	CSIRO-In-house method	Test Species:	AS-Acartia sinjiensis		
Comments:	MODIFIED algae used - no metals stocks in F2 during algal culture						

Conc-%	1	2	3	4	5	6	7
Control	25.000	35.000	37.000	36.000	43.000	30.000	32.000
0.027	28.000	35.000	37.000	33.000	22.000		
0.082	34.000	29.000	28.000	33.000	42.000		
0.25	36.000	26.000	35.000	35.000	28.000		
0.74	28.000	32.000	34.000	26.000	32.000		
2.2	21.000	17.000	20.000	17.000	33.000		
6.7	2.000	1.000	2.000	3.000	1.000		

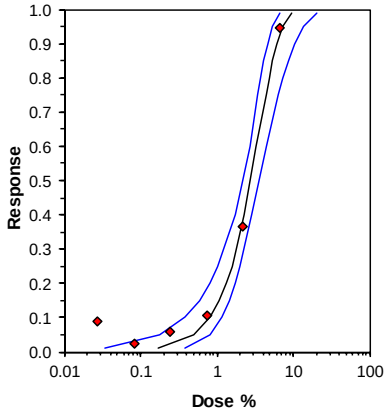
			Transform: Untransformed					2-Tailed				
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Control	34.000	1.0000	34.000	25.000	43.000	16.810	7				34.000	0.0000
0.027	31.000	0.9118	31.000	22.000	37.000	19.489	5	1.010	2.825	8.390	31.000	0.0882
0.082	33.200	0.9765	33.200	28.000	42.000	16.689	5	0.269	2.825	8.390	33.200	0.0235
0.25	32.000	0.9412	32.000	26.000	36.000	14.490	5	0.673	2.825	8.390	32.000	0.0588
0.74	30.400	0.8941	30.400	26.000	34.000	10.810	5	1.212	2.825	8.390	30.400	0.1059
*2.2	21.600	0.6353	21.600	17.000	33.000	30.640	5	4.175	2.825	8.390	21.600	0.3647
*6.7	1.800	0.0529	1.800	1.000	3.000	46.481	5	10.841	2.825	8.390	1.800	0.9471

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.97471	0.914	0.30765	0.23664
Bartlett's Test indicates equal variances (p = 0.06)	11.8699	16.8119		

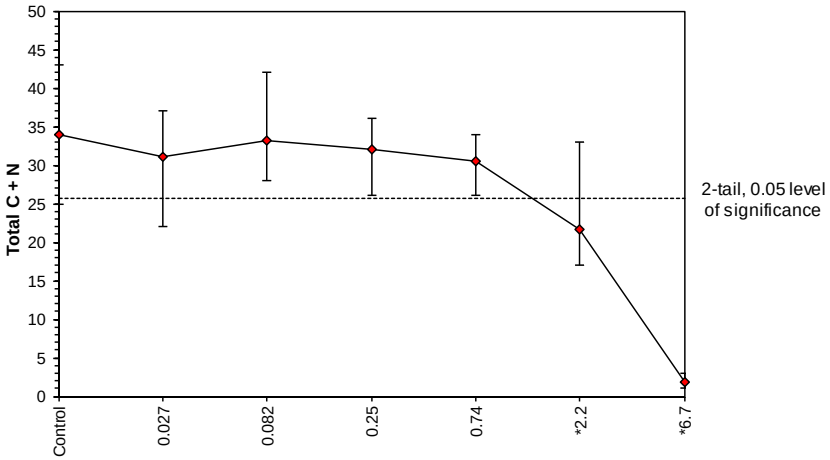
Hypothesis Test (2-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	0.74	2.2	1.27593	135.135	8.39028	0.24677	685.955	25.7333	9.4E-11	6, 30

Maximum Likelihood-Weibull											
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	3.51726	0.65715	2.22924	4.80528	0	2.88576	9.48773	0.58			6
Intercept	-1.9302	0.33713	-2.591	-1.2694							
TSCR											

Point	Weibull	%	95% Fiducial Limits	
EC01	-4.600	0.17414	0.03435	0.37439
EC05	-2.970	0.5062	0.18241	0.82894
EC10	-2.250	0.81091	0.3787	1.18568
EC15	-1.817	1.07695	0.58485	1.47854
EC20	-1.500	1.32535	0.79984	1.74605
EC25	-1.246	1.56515	1.02277	2.00498
EC40	-0.672	2.2793	1.72543	2.83166
EC50	-0.367	2.78341	2.20526	3.51487
EC60	-0.087	3.34138	2.68898	4.39599
EC75	0.327	4.38174	3.4751	6.36182
EC80	0.476	4.83148	3.78332	7.32296
EC85	0.640	5.38067	4.14234	8.57645
EC90	0.834	6.1081	4.59545	10.3615
EC95	1.097	7.25645	5.27228	13.4451
EC99	1.527	9.61559	6.56168	20.6974



Dose-Response Plot



Copepod larval survival - (Total hatched animals, C + N)							
Start Date:	8/01/2018	Test ID:	LD43	Sample ID:	E17022-MOD algae		
End Date:	12/01/2018	Lab ID:	CECR-CSIRO	Sample Type:	Drum 2		
Sample Date:		Protocol:	CSIRO-In-house method	Test Species:	AS-Acartia sinjiensis		
Comments:	Algae grown without metals used as food during exposure						

Conc-%	1	2	3	4	5	6	7
Control	25.000	35.000	37.000	36.000	43.000	30.000	32.000
0.012	35.000	34.000	42.000	47.000			
0.037	33.000	36.000	39.000	30.000	35.000		
0.11	28.000	31.000	27.000	28.000	39.000		
0.33	28.000	31.000	33.000	33.000	34.000		
1	9.000	5.000	12.000	7.000	8.000		
3	1.000	2.000	4.000	3.000	5.000		

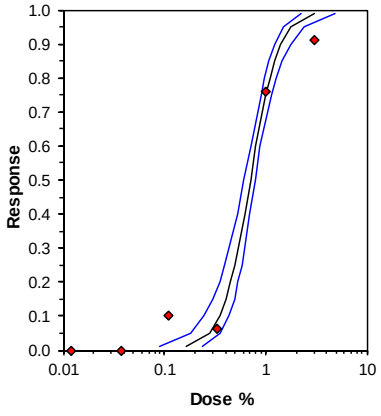
			Transform: Untransformed					2-Tailed				
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Control	34.000	1.0000	34.000	25.000	43.000	16.810	7				34.000	0.0000
0.012	39.500	1.1618	39.500	34.000	47.000	15.538	4	2.090	2.832	7.450	39.500	-0.1618
0.037	34.600	1.0176	34.600	30.000	39.000	9.715	5	0.244	2.832	6.960	34.600	-0.0176
0.11	30.600	0.9000	30.600	27.000	39.000	16.109	5	1.383	2.832	6.960	30.600	0.1000
0.33	31.800	0.9353	31.800	28.000	34.000	7.508	5	0.895	2.832	6.960	31.800	0.0647
*1	8.200	0.2412	8.200	5.000	12.000	31.566	5	10.497	2.832	6.960	8.200	0.7588
*3	3.000	0.0882	3.000	1.000	5.000	52.705	5	12.612	2.832	6.960	3.000	0.9118

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.97227	0.912	0.33682	0.65143
Bartlett's Test indicates equal variances (p = 0.13)	9.77088	16.8119		

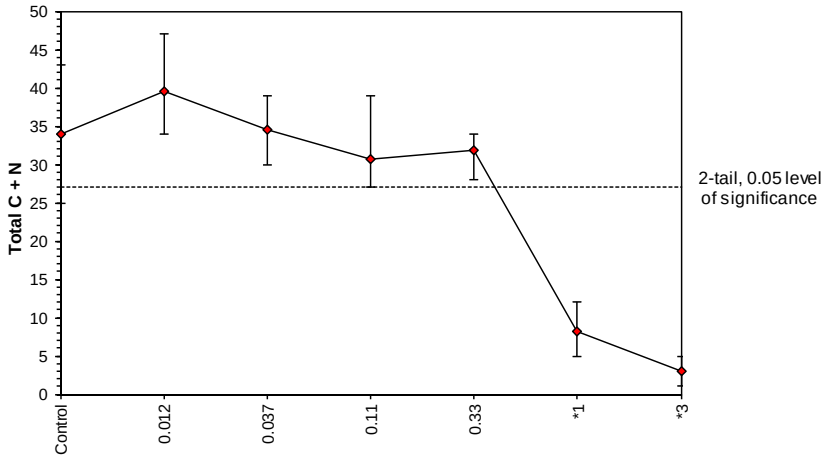
Hypothesis Test (2-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	0.33	1	0.57446	303.03	6.95974	0.2047	1008.33	17.6207	9.3E-15	6, 29

Maximum Likelihood-Logit											
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	7.28	1.00026	5.31949	9.24051	0	7.89942	9.48773	0.1			16
Intercept	1.09295	0.18135	0.7375	1.44841							
TSCR											

Point	Logits	%	95% Fiducial Limits	
EC01	-4.595	0.16545	0.08905	0.23758
EC05	-2.944	0.27888	0.18096	0.36041
EC10	-2.197	0.35323	0.24897	0.43606
EC15	-1.735	0.40888	0.30299	0.49126
EC20	-1.386	0.4565	0.35092	0.5379
EC25	-1.099	0.49999	0.3958	0.58028
EC40	-0.405	0.62255	0.52585	0.70077
EC50	0.000	0.70773	0.61664	0.78796
EC60	0.405	0.80457	0.71669	0.89393
EC75	1.099	1.00179	0.90143	1.1403
EC80	1.386	1.09722	0.98218	1.27343
EC85	1.735	1.22501	1.08429	1.46282
EC90	2.197	1.41803	1.22981	1.7682
EC95	2.944	1.79608	1.49659	2.41877
EC99	4.595	3.02738	2.28129	4.89171



Dose-Response Plot



Statistics – QA Control

Copepod Larval Development Test - LD43 - Wafi definitive - Drum 1 (E17021) and Drum 2 (E17022) with MODIFIED algae

Media: seawater
Eggs = <24h old
250 mL acid washed Polycarbonate containers

Algal food (x 10^4 cells/mL) (grown in F2 media)				
	Day 0		Day 2	
	Target	Actual	*Target*	Actual
T. chuii	*0.63*	0.61	*0.31*	0.31
T. lutea	*8.00*	8.25	*4.00*	3.7

Timing	
Adults Isolated:	7/01/2018, 9am
Eggs Isolated:	8/1/18, 11am
Total number of eggs	11,280
Total eggs after MQ wash:	8,880
Eggs Innoculated:	8/1/18, 12:30-12:50
Day 2 renewal complete:	10/1/18, 12:00-1:15
Day 3 fixation time:	11/1/18, 9:30 pm

Day 0 egg innoculation checks		
1	61	start
2	65	
3	56	
4	44	
		end
Mean	**57**	Target: 60
SD	**3**	

Label	Treatment	TWA	Day 0	Day 3										
		Dissolved (µg/L)	Unhatched eggs (# E)	Unhatched Eggs (# E)	% hatch	Nauplii # N	Nauplii % N	Copepodites # C	Copepodites %C	Total animals (C+ N) #	Total animals (C+ N) % Control	**LDR*** C/(C+N)	**LDR*** % Control	CV
1	Control	<LOD	57	19	66%	8	14%	19	34%	27	92%	70%	101%	
2			57	23	59%	5	9%	22	39%	27	92%	81%	117%	
3			57	26	54%	6	11%	22	39%	28	95%	79%	113%	
4			57	14	75%	16	28%	17	30%	33	112%	52%	74%	
5			57	14	75%	14	25%	19	34%	33	112%	58%	82%	
6			57	19	66%	7	12%	15	27%	22	75%	68%	98%	
7			57	20	65%	9	16%	24	42%	33	112%	73%	104%	
8			57	20	65%	7	12%	25	44%	32	109%	78%	112%	
Mean					66%		16%		36%	**29**	**100%**	**70%**	**100%**	**15%**
1	4 µg/L	4.6	57	20	65%	8	14%	29	51%	37	126%	78%	112%	
2			57	17	70%	3	5%	23	41%	26	89%	88%	127%	
3			57	18	68%	15	27%	11	19%	26	89%	42%	61%	
4			57	23	59%	6	11%	34	60%	40	136%	85%	122%	
5			57	19	66%	10	18%	15	27%	25	85%	60%	86%	
Mean					66%		15%		40%	**31**	**105%**	**71%**	**101%**	**27%**
1	8 µg/L	7.1	57	18	68%	22	39%	12	21%	34	116%	35%	51%	
2			57	19	66%	12	21%	22	39%	34	116%	65%	93%	
3			57	40	29%	18	32%	21	37%	39	133%	54%	77%	
4			57	34	40%	14	25%	17	30%	31	106%	55%	79%	
5			57	25	56%	11	19%	17	30%	28	95%	61%	87%	
Mean					52%		27%		32%	**33**	**113%**	**54%**	**77%**	**21%**
1	12 µg/L	11	57	14	75%	10	18%	17	30%	27	92%	63%	90%	
2			57	18	68%	26	46%	11	19%	37	126%	30%	43%	
3			57	26	54%	22	39%	3	5%	25	85%	12%	17%	
4			57	19	66%	15	27%	9	16%	24	82%	38%	54%	
5			57	25	56%	28	50%	5	9%	33	112%	15%	22%	
Mean					64%		36%		16%	**29**	**99%**	**31%**	**45%**	**65%**
1	16 µg/L	14	57	19	66%	21	37%	0	0%	21	71%	0%	0%	
2			57	35	38%	18	32%	0	0%	18	61%	0%	0%	
3			57	18	68%	24	42%	0	0%	24	82%	0%	0%	
4			57	15	73%	23	41%	0	0%	23	78%	0%	0%	
5			57	20	65%	10	18%	0	0%	10	34%	0%	0%	
Mean					62%		34%		0%	**19**	**65%**	**0%**	**0**	**N/A**

Copepod larval development test-LDR								
Start Date:	8/01/2018	Test ID:	LD43			Sample ID:	REF-Ref Toxicant	
End Date:	11/01/2018	Lab ID:	CECR-CSIRO			Sample Type:	NICL-Nickel chloride	
Sample Date:		Protocol:	CSIRO-In-house method			Test Species:	AS-Acartia sinjiensis	
Comments:	measured - normal F2 algae							

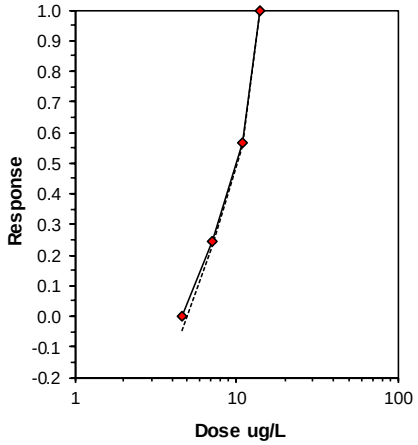
Conc-ug/L	1	2	3	4	5	6	7	8
QA Control	0.7037	0.8148	0.7857	0.5152	0.5758	0.6818	0.7273	0.7813
4.6	0.7838	0.8846	0.4231	0.8500	0.6000			
7.1	0.3529	0.6471	0.5385	0.5484	0.6071			
11	0.6296	0.2973	0.1200	0.3750	0.1515			
14	0.0000	0.0000	0.0000	0.0000	0.0000			

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
QA Control	0.6982	1.0000	0.9937	0.8006	1.1259	11.468	8				72	235
4.6	0.7083	1.0145	1.0157	0.7082	1.2242	21.157	5	-0.231	2.294	0.2187	42	154
7.1	0.5388	0.7717	0.8244	0.6361	0.9347	13.890	5	1.776	2.294	0.2187	77	166
*11	0.3147	0.4507	0.5812	0.3537	0.9165	38.779	5	4.328	2.294	0.2187	101	146
14	0.0000	0.0000	0.1186	0.1022	0.1588	19.635	5				96	96

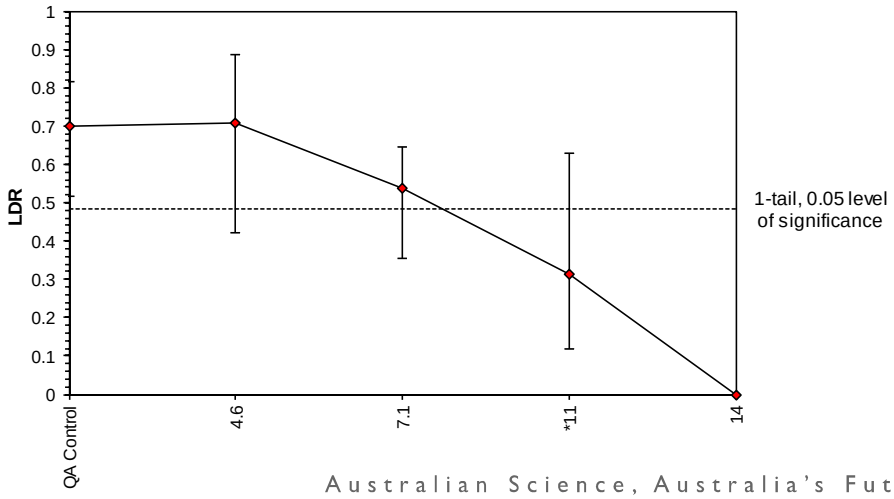
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96893	0.881	-0.1071	-0.2008
Bartlett's Test indicates equal variances (p = 0.30)	3.66083	11.3449		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	7.1	11	8.83742		0.21274	0.30291	0.21868	0.02796	0.00134	3, 19

Trimmed Spearman-Karber			
Trim Level	EC50	95% CL	
0.0%	9.2011	8.8378	9.5794
5.0%	9.3263	8.9232	9.7476
10.0%	9.4493	9.0086	9.9115
20.0%	9.6800	9.1729	10.2152
Auto-0.0%	9.2011	8.8378	9.5794



Dose-Response Plot



Statistics – Range-finder test (microalgal food used was grown in F2 medium with trace metals)

Copepod Larval Development Test - LD36, Wafi Range Finder, 13/2/17

Vial	Treatment	Day 0	Day 3										
		Unhatched	Unhatched		Nauplii		Copepodites		Total animals (C+N)		LDR*		
		eggs (#E)	Eggs (#E)	% hatch	# N	% N	# C	%C	#	% Control	C/(C+N)	% Control	CV
1	Control	60	8	87%	6	10%	32	53%	38	85%	84%	123%	
2		60	12	80%	13	22%	25	42%	38	85%	66%	96%	
3		60	21	65%	13	22%	38	63%	51	114%	75%	109%	
4		60	26	57%	19	32%	31	52%	50	112%	62%	91%	
5		60	15	75%	19	32%	28	47%	47	105%	60%	87%	
8		60	17	72%	16	27%	28	47%	44	99%	64%	93%	
Mean				73%		24%		51%	**45**	**100%**	**68%**	**100%**	**14%**
Drum 1													
1	0.01%	60	13	78%	12	20%	34	57%	46	103%	74%	108%	
2		60	11	82%	12	20%	27	45%	39	87%	69%	101%	
3		60	20	67%	21	35%	22	37%	43	96%	51%	75%	
Mean				76%		25%		46%	**43**	**96%**	**65%**	**95%**	**19%**
1	0.10%	60	11	82%	8	13%	29	48%	37	83%	78%	115%	
2		60	9	85%	22	37%	29	48%	51	114%	57%	83%	
3		60	10	83%	18	30%	21	35%	39	87%	54%	79%	
Mean				83%		27%		44%	**42**	**95%**	**63%**	**92%**	**21%**
1	1.00%	60	13	78%	23	38%	27	45%	50	112%	54%	79%	
2		60	10	83%	24	40%	9	15%	33	74%	27%	40%	
3		60	15	75%	12	20%	9	15%	21	47%	43%	63%	
Mean				79%		33%		25%	**35**	**78%**	**41%**	**61%**	**32%**
1	10%	60	21	65%	13	22%	0	0%	13	29%	0%	0%	
2		60	14	77%	8	13%	7	12%	15	34%	47%	68%	
3		60	19	68%	21	35%	0	0%	21	47%	0%	0%	
Mean				70%		23%		4%	**16**	**37%**	**16%**	**23%**	**173%**
1	50%	60	19	68%	4	7%	0	0%	4	9%	0%	0%	
2		60	27	55%	9	15%	0	0%	9	20%	0%	0%	
3		60	17	72%	2	3%	0	0%	2	4%	0%	0%	
Mean				65%		8%		0%	**5**	**11%**	**0%**	**0%**	**0%**
Drum 2													
1	0.01%	60	14	77%	32	53%	2	3%	34	80%	6%	9%	
2		60	12	80%	17	28%	2	3%	19	52%	11%	15%	
3		60	20	67%	13	22%	0	0%	13	55%	0%	0%	
Mean				74%		34%		2%	**22**	**62%**	**5%**	**8.0%**	**96%**
1	0.10%	60	20	67%	13	22%	0	0%	13	55%	0%	0%	
2		60	15	75%	15	25%	0	0%	15	50%	0%	0%	
3		60	24	60%	11	18%	0	0%	11	58%	0%	0%	
Mean				67%		22%		0%	**13**	**54%**	**0%**	**0%**	**N/A**
1	1.00%	60	28	53%	14	23%	0	0%	14	70%	0%	0%	
2		60	21	65%	20	33%	0	0%	20	68%	0%	0%	
3		60	20	67%	19	32%	0	0%	19	65%	0%	0%	
Mean				62%		29%		0%	**18**	**68%**	**0%**	**0%**	**N/A**
1	10%	60	10	83%	1	2%	0	0%	1	18%	0%	0%	
2		60	14	77%	2	3%	0	0%	2	27%	0%	0%	
3		60	14	77%	0	0%	0	0%	0	23%	0%	0%	
Mean				79%		2%		0%	**1**	**23%**	**0%**	**0%**	**N/A**
1	50%	60	14	77%	0	0%	0	0%	0	23%	0%	0%	
2		60	8	87%	0	0%	0	0%	0	13%	0%	0%	
3		60	4	93%	0	0%	0	0%	0	7%	0%	0%	
Mean				86%		0%		0%	**0**	**14%**	**0%**	**0%**	**N/A**

Note that Tailings liquor from Drum 2 was completely toxic to copepod early life-stage development
A second range-finder test was carried out using higher dilutions of tailings liquor

Copepod larval development test-LDR

Start Date:	13/02/2017	Test ID:	Wafi RF1	Sample ID:	Drum 1
End Date:	16/02/2017	Lab ID:	CECR-CSIRO	Sample Type:	Drum 1
Sample Date:		Protocol:	CSIRO-In-house method	Test Species:	AS-Acartia sinjiensis
Comments:					

Conc-%	1	2	3	4	5	6
Control	0.8421	0.6579	0.7451	0.6200	0.5957	0.6364
0.01	0.7391	0.6923	0.5116			
0.1	0.7838	0.5686	0.5385			
1	0.5400	0.2727	0.4286			
10	0.0000	0.4667	0.0000			
50	0.0000	0.0000	0.0000			

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
Control	0.6829	1.0000	0.9769	0.8817	1.1622	10.862	6				86	268
0.01	0.6477	0.9485	0.9382	0.7970	1.0347	13.321	3	0.301	2.533	0.3260	45	128
0.1	0.6303	0.9230	0.9218	0.8239	1.0872	15.627	3	0.429	2.533	0.3260	48	127
1	0.4138	0.6059	0.6962	0.5495	0.8254	19.939	3	2.181	2.533	0.3260	59	104
*10	0.1556	0.2278	0.3335	0.1093	0.7520	108.779	3	4.999	2.533	0.3260	42	49
50	0.0000	0.0000	0.2605	0.1674	0.3614	37.311	3				15	15

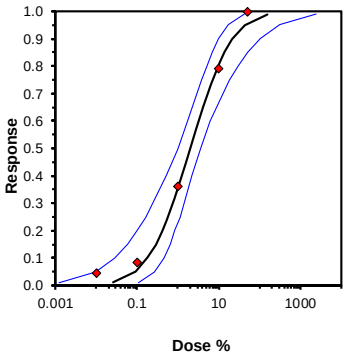
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.939566	0.858	0.990907	1.353358
Bartlett's Test indicates equal variances (p = 0.26)	5.236931	13.2767		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	1	10	3.162278	100	0.319736	0.465489	0.243461	0.033138	0.002538	4, 13

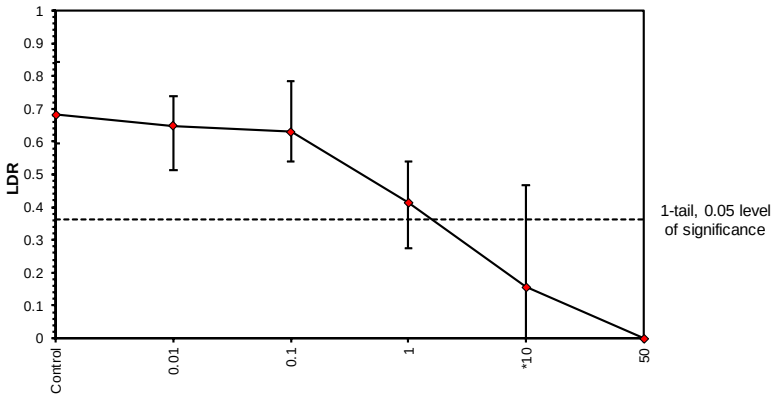
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	1.235524	0.24762	0.750189	1.72086	0.320896	0.747976	7.814728	0.86	0.299782	0.809373	5
Intercept	4.629612	0.179997	4.276817	4.982406							
TSCR	0.332369	0.02308	0.287132	0.377606							

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	0.026116	0.001217	0.104683
EC05	3.355	0.093	0.009539	0.269221
EC10	3.718	0.183033	0.028273	0.45044
EC15	3.964	0.289015	0.058374	0.642646
EC20	4.158	0.415524	0.1031	0.858659
EC25	4.326	0.567373	0.16669	1.109374
EC40	4.747	1.243741	0.530158	2.231996
EC50	5.000	1.994262	0.998179	3.620948
EC60	5.253	3.197677	1.751723	6.302282
EC75	5.674	7.009644	3.839479	18.3993
EC80	5.842	9.571229	5.055543	29.18855
EC85	6.036	13.76079	6.859474	50.76685
EC90	6.282	21.72878	9.911454	103.4939
EC95	6.645	42.76439	16.76858	303.3565
EC99	7.326	152.288	43.55242	2354.444



Dose-Response Plot



Copepod Larval Development Test - LD37, Wafi Range Finder 2, 6/3/17

Vial	Treatment	Day 0	Day 3										
		Unhatched	Unhatched		Nauplii		Copepodites		Total animals (C+N)		LDR*		
		eggs (#E)	Eggs (#E)	% hatch	# N	% N	# C	%C	#	% Control	C/(C+N)	% Control	CV
1	Control	63	19	70%	20	32%	43	69%	63	111%	68%	93%	
2		63	6	90%	27	43%	50	80%	77	136%	65%	89%	
3		63	5	92%	9	14%	34	54%	43	76%	79%	108%	
4		63	10	84%	12	19%	25	40%	37	65%	68%	92%	
5		63	15	76%	10	16%	45	72%	55	97%	82%	112%	
6		63	11	82%	15	24%	52	83%	67	118%	78%	106%	
7		63	10	84%	15	24%	39	62%	54	95%	72%	99%	
Mean				83%		25%		66%	**57**	**100%**	**73%**	**100%**	**8.9%**
Drum 2 (%)													
1	A - D2 0.001%	63	12	81%	6	10%	40	64%	46	81%	87%	119%	
2		63	10	84%	10	16%	52	83%	62	110%	84%	115%	
3		63	11	82%	10	16%	44	70%	54	95%	81%	112%	
Mean				82%		14%		72%	**54**	**95%**	**84%**	**115%**	**3.3%**
1	B - D2 0.01%	63	11	82%	12	19%	3	5%	15	27%	*	*	
2		63	7	89%	10	16%	34	54%	44	78%	77%	106%	
3		63	14	78%	12	19%	42	67%	54	95%	78%	106%	
Mean				83%		18%		42%	**38**	**67%**	**78%**	**106%**	**0.5%**
1	D - D2 0.1%	63	10	84%	8	13%	0	0%	8	14%	0%	0%	
2		63	10	84%	28	45%	0	0%	28	49%	0%	0%	
3		63	8	87%	31	49%	0	0%	31	55%	0%	0%	
Mean				85%		36%		0%	**22**	**39%**	**0%**	**0%**	**N/A**
1	E - D2 1.0%	63	10	84%	14	22%	0	0%	14	25%	0%	0%	
2		63	9	86%	32	51%	1	2%	33	58%	3%	4%	
3		63	6	90%	18	29%	0	0%	18	32%	0%	0%	
Mean				87%		34%		1%	**22**	**38%**	**1%**	**1%**	**173%**
1	F - D2 10%	63	4	94%	2	3%	0	0%	2	4%	0%	0%	
2		63	7	89%	1	2%	0	0%	1	2%	0%	0%	
3		63	8	87%	4	6%	0	0%	4	7%	0%	0%	
Mean				90%		4%		0%	**2**	**4%**	**0%**	**0%**	**N/A**
1	G - D2 50%	63	4	94%	1	2%	0	0%	1	2%	0%	0%	
2		63	3	95%	0	0%	0	0%	0	0%	0%	0%	
3		63	5	92%	1	2%	0	0%	1	2%	0%	0%	
Mean				94%		1%		0%	**1**	**1%**	**0%**	**0%**	**N/A**
Drum 2 (%) new sub-sample from second bucket													
1	T - Drum 2 new 0.1%	63	9	86%	17	27%	3	5%	20	35%	*	*	
2		63	7	89%	30	48%	15	24%	45	80%	33%	46%	
3		63	8	87%	30	48%	11	18%	41	72%	27%	37%	
Mean				87%		41%		15%	**35**	**62%**	**30%**	**41%**	**15%**
1	S - Drum 2 new 1.0%	63	7	89%	4	6%	0	0%	4	7%	*	*	
2		63	6	90%	22	35%	1	2%	23	41%	4%	6%	
3		63	4	94%	27	43%	2	3%	29	51%	7%	9%	
Mean				91%		28%		2%	**19**	**33%**	**6%**	**8%**	**32%**
											LDR		

* LDR calculated after ommitting the first replicate results when obvious effect from measuring phys-chem directly in test solutions. This has not been seen before. However may be due to cross contamination of highly toxic Zn or Drum 2 sample.

Other means and total recovered animals etc still include first rep.

	LDR (%)			LDR (% Control)		
Drum 2 (%)	**Old, LD36**	**Old, LD37**	**New, LD37**	**Old, LD36**	**Old, LD37**	**New, LD37**
0.10%	0%	0%	30%	0%	0%	41%
1.00%	0%	1%	6%	0%	1%	8%

Old = sub-sampled from same bucket as RF1
New = sub-sampled from a new bucket

Copepod larval development test-LDR

Start Date:	6/03/2017	Test ID:	Wafi RF2	Sample ID:	Drum 2 original
End Date:	9/03/2017	Lab ID:	CECR-CSIRO	Sample Type:	Wafi liquor
Sample Date:		Protocol:	CSIRO-In-house method	Test Species:	AS-Acartia sinjiensis
Comments:	Collected from original drum				

Conc-%	1	2	3	4	5	6	7
Control	0.6825	0.6494	0.7907	0.6757	0.8182	0.7761	0.7222
0.001	0.8696	0.8387	0.8148				
0.01	0.7727	0.7778					
0.1	0.0000	0.0000	0.0000				
1	0.0000	0.0303	0.0000				
10	0.0000	0.0000	0.0000				
50	0.0000	0.0000					

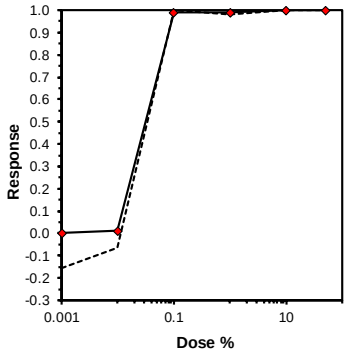
			Transform: Arcsin Square Root					1-Tailed			Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Control	0.7307	1.0000	1.0277	0.9371	1.1303	7.220	7				0.7834	1.0000
0.001	0.8410	1.1510	1.1616	1.1259	1.2013	3.258	3	-2.750	2.673	0.1302	0.7834	1.0000
0.01	0.7753	1.0610	1.0769	1.0739	1.0799	0.397	2	-0.870	2.673	0.1513	0.7755	0.9899
*0.1	0.0000	0.0000	0.1208	0.0899	0.1777	40.893	3	18.622	2.673	0.1302	0.0077	0.0098
*1	0.0101	0.0138	0.1424	0.1181	0.1750	20.598	3	18.178	2.673	0.1302	0.0077	0.0098
*10	0.0000	0.0000	0.3792	0.2527	0.5236	35.953	3	13.315	2.673	0.1302	0.0000	0.0000
*50	0.0000	0.0000	0.5236	0.5236	0.5236	0.000	2	8.908	2.673	0.1513	0.0000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.971978	0.881	0.344939	0.820113
Equality of variance cannot be confirmed				

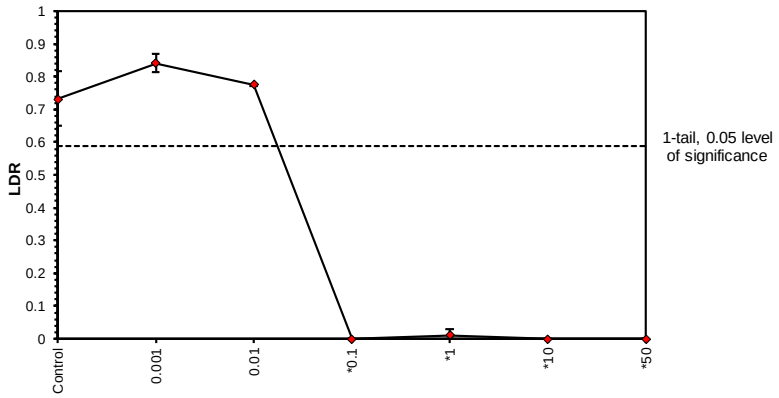
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	0.01	0.1	0.031623	10000	0.142385	0.194275	0.663578	0.004981	9.5E-13	6, 16

Log-Logit Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05	0.0124	0.0008	0.0078	0.0139	-0.2752
IC10	0.0152	0.0012	0.0091	0.0168	-0.0990
IC15	0.0178	0.0016	0.0096	0.0196	-0.2765
IC20	0.0203	0.0020	0.0101	0.0222	-0.3729
IC25	0.0226	0.0025	0.0105	0.0252	-0.4224
IC40	0.0294	0.0038	0.0115	0.0340	-0.4769
IC50	0.0340	0.0047	0.0121	0.0400	-0.4892



Dose-Response Plot



OCEANS AND ATMOSPHERE
www.csiro.au

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6831 • **ABN** 41 687 119 230

16 August 2015

Chronic 72-h Sea Urchin Larval Development Test Report E17021 SULD

Client:	GDA Consult Pty Ltd
Project:	Wafi-Golpu Pre-feasibility study of DSTP
Test Performed:	72-h larval development test with the marine invertebrate *Heliocidaris tuberculata*

Test Initiated:	9 August 2017	
CSIRO Sample No.	**Sample Name:**	**Sample Description:**
E17021	Drum 1	Drum 1 tailings
E17022	Drum 2	Drum 2 tailings

Sample Preparation: Prior to toxicity testing, tailings liquor was prepared by simulating pre-discharge mixing with seawater at a dilution of 1 in 4. Natural seawater (3 parts, 3 kg) was added to tailings material (solids and liquor) (1 part, 1 kg) and mixed on a roller for 1 h. The resulting solution was filtered to 0.45 µm using a cartridge filter (with a 0.65 µm pre-filter) and the filtrate (tailings liquor) collected for testing. The tailings liquor was stored at 4°C in the dark prior to toxicity testing. All reference to tailings liquor here on describes the 1 in 4 diluted and filtered tailings-seawater mixture as 100% (or undiluted) tailings liquor.
Physico-Chemistry: The salinity of the tailings liquors from Drum 1 and Drum 2 was 29‰ and a pH of 7.6 and 7.8 respectively. Each tailings liquor was serially diluted in natural filtered (0.45 µm) seawater prior to testing (Drum 1 0.78–100%, Drum 2 0.3–100% liquor). A salinity/pH control was also prepared by the addition of high purity water (milli-Q) and 1M HCl (drop-wise) to natural seawater to match the salinity and pH of 100% liquor (the highest tailings liquor concentration tested).

Sample Name	**Physico-Chemistry**				
	pH	**Salinity (‰)**	**Conductivity (mS/cm)**	**DO (% O_2 sat)**	**Comments**
Drum 1 liquor (E17021)	7.56	29.3	46.4	105	
Drum 2 liquor (E17022)	7.76	29.1	46.0	106	
Seawater (QA control)	8.10	36.3	55.6	102	
Salinity-pH control	7.50	29.3	45.8	103	

Test method: This test measures the proportion of normally developed larvae from the sea urchin *Heliocidaris turberculata* and is based on the protocol of Simon and Laginestra (1997). Sea urchin sperm and eggs were collected from one male and one female adult animal. Fertilised eggs were exposed to the sample for 72 h at 20°C then fixed with formalin buffer. Normal larval development was assessed microscopically. Toxicity was expressed as the concentration of sample that causes 50% or 10% reduction in normal development (EC50 or EC10 value respectively) and calculated using ToxCalc Version 5.0.23 (Tidepool Software). The lower the EC50, the more toxic the sample. Copper was also tested for quality assurance purposes.

Sample Results:
The tailings liquors were toxic to sea urchin larval development with liquor from Drum 2 more toxic than liquor from Drum 2 (EC10 values of 27% and 54% respectively).

QA Comments:
There were no normally developed larvae in the salinity-pH control matched to the salinity and pH of the undiluted (100%) liquor treatment. Hence, the low salinity (29‰) and pH of the undiluted liquor may be contributing to the observed toxicity of the liquor, especially in the undiluted liquor treatment for both Drum 1 and Drum 2 liquors. It is unclear if low salinity and pH would be contributing to the observed toxicity of the 50% Drum 2 liquor treatment.

Sample	Mean Normal development (%)	CV (%)	Mean Normal development (% of QA Control)	CV (%)
QA Control (seawater)	98	1	100	1
Salinity-pH control	0	NA	0	NA
Drum 1-E17021				
0.78%	97	2	99	2
1.56%	96	3	97	3
3.13%	94	9	95	9
6.25%	97	1	99	1
12.5%	96	2	98	2
25%	98	2	99	2
50%	97	3	98	3
100%	1[a]	78	1[a]	78
Drum 2-E17022				
0.30%	98	2	100	2
1.00%	97	1	99	1
3.13%	94	10	95	10
6.25%	98	1	99	1
12.5%	97	2	99	2
25%	95	3	96	3
50%	0[a]	NA	0[a]	NA
100%	0[a]	NA	0[a]	NA
Summary	**EC50 (%)[b]**	**EC10 (%)[b]**	**LOEC (%)[c]**	**NOEC (%)[d]**
Drum 1 – E17021	75	54	50	100
Drum 2 – E17022	37	27	25	50

NA, Not applicable
[a] Significantly ($p \leq 0.05$) less than the QA control
[b] Concentration of the sample to cause 50% or 10% reduction in sea urchin normal larval development. The 95% confidence limits are not reported because partial toxicity responses were not observed and hence reliable confidence limits could not be calculated.
[c] Lowest concentration tested to have a significant ($p \leq 0.05$) reduction in sea urchin larval development compared to the control
[d] Highest concentration tested to have no significant ($p \geq 0.05$) reduction in sea urchin larval development compared to the control

Quality Assurance/Quality Control	Criterion	This Test	Criterion Met?
Control 72-h normal larval development (%)	≥70	98	Yes
Reference toxicant 72-h EC50 (measured copper, µg/L)	13 ± 8	6.6	Yes

References:
Simon J. and Laginestra E. (1997) Bioassay for testing sublethal toxicity in effluents, using gametes of sea urchin *Heliocidaris tuberculata*. National Pulp Mills Research Program Technical Report No. 20. Canberra: CSIRO, 36 pp.

Test carried out by: Lisa Golding and Monique Binet

Test supervised by:	Lisa Golding
Test report prepared by:	Merrin Adams
Test report reviewed by:	Merrin Adams and Lisa Golding

Team Leader | Aquatic Ecotoxicology
Aquatic Contaminants Group
Environmental Contaminant Mitigation and Technologies
CSIRO Land and Water Flagship
E merrin.adams@csiro.au **T** +61 2 9710 6831

Date: 16 August 2017

Statistics - Sample

Heliocidaris tuberculata 72-h larval development test - E17021 Drum 1

Test date: 9/08/2017

	Sample	pH		Sal (ppt	d (mS/	D.O. (%)		Number of larvae		% Normal			% Control		
Rep		0 h	72 h	0 h	0 h	0 h	72 h	normal	total	72-h	Mean	CV (%)	72-h	Mean	CV (%)
1	sw-35ppt control	8.1	8.1	36	56	104	94	98	100	98%	98%	1%	100%	100%	1%
2								62	62	100%			102%		
3								98	100	98%			100%		
4								97	100	97%			99%		
1	Sal/pH Control (matched to 100% Drum 1 and Drum 2)	7.5	7.9	29	45	101	91	0	5	0%	0%	N/A	0%	0%	N/A
2								0	13	0%			0%		
3								0	48	0%			0%		
4								0	25	0%			0%		

E17021 Drum 1 (%)

1	0.78	8.1	8.1	36	56	103	92	99	100	99%	97%	2%	101%	99%	2%
2								98	100	98%			100%		
3								96	100	96%			98%		
4								95	100	95%			97%		
1	1.56	8.1	8.1	36	56	103	92	96	100	96%	96%	3%	98%	97%	3%
2								99	100	99%			101%		
3								93	100	93%			95%		
4								94	100	94%			96%		
1	3.13	8.1	8.1	36	55	102	92	97	100	97%	94%	9%	99%	95%	9%
2								99	100	99%			101%		
3								97	100	97%			99%		
4								81	100	81%			82%		
1	6.25	8.1	8.1	36	55	103	92	98	100	98%	97%	1%	100%	99%	1%
2								97	100	97%			99%		
3								97	100	97%			99%		
4								97	100	97%			99%		
1	12.5	8.1	8.1	36	55	102	91	94	100	94%	96%	2%	96%	98%	2%
2								98	100	98%			100%		
3								96	100	96%			98%		
4								97	100	97%			99%		
1	25	8.0	8.1	35	53	103	93	97	100	97%	98%	2%	99%	99%	2%
2								89	90	99%			101%		
3								99	100	99%			101%		
4								95	99	96%			98%		
1	50	7.9	8.0	33	51	103	91	99	100	99%	97%	3%	101%	98%	3%
2								93	100	93%			95%		
3								96	100	96%			98%		
4								99	100	99%			101%		
1	100	7.6	7.9	29	46	102	85	1	100	1%	1%	78%	1%	1%	78%
2								1	81	1%			1%		
3								0	100	0%			0%		
4								2	100	2%			2%		

Sea Urchin Larval Development Test-Proportion Normal

Start Date:	9/08/2017	Test ID:	E17021	Sample ID:	E17021-Drum 1	
End Date:	12/08/2017	Lab ID:	LG-Lisa Golding	Sample Type:	EFF2-Industrial	
Sample Date:		Protocol:	USEPA 1995 (modified)	Test Species:	HT-Heliocidaris tuberculata	
Comments:	E17021 Drum 1 diluted with filtered seawater					

Conc-%	1	2	3	4
Control	0.9800	1.0000	0.9800	0.9700
0.78	0.9900	0.9800	0.9600	0.9500
1.56	0.9600	0.9900	0.9300	0.9400
3.13	0.9700	0.9900	0.9700	0.8100
6.25	0.9800	0.9700	0.9700	0.9700
12.5	0.9400	0.9800	0.9600	0.9700
25	0.9700	0.9889	0.9900	0.9596
50	0.9900	0.9300	0.9600	0.9900
100	0.0100	0.0123	0.0000	0.0200

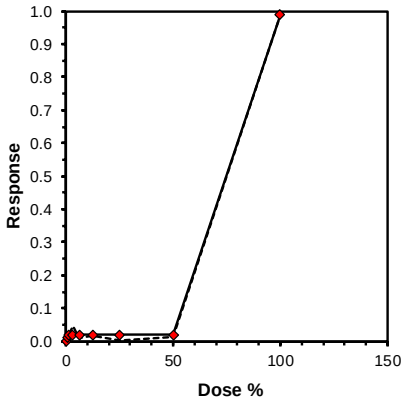
			Transform: Arcsin Square Root					1-Tailed			Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Control	0.9825	1.0000	1.4404	1.3967	1.5073	3.267	4				0.9807	1.0000
0.78	0.9700	0.9873	1.4036	1.3453	1.4706	4.051	4	0.714	2.513	0.1298	0.9700	0.9891
1.56	0.9550	0.9720	1.3666	1.3030	1.4706	5.467	4	1.430	2.513	0.1298	0.9616	0.9805
3.13	0.9350	0.9517	1.3460	1.1198	1.4706	11.498	4	1.829	2.513	0.1298	0.9616	0.9805
6.25	0.9725	0.9898	1.4048	1.3967	1.4289	1.146	4	0.691	2.513	0.1298	0.9616	0.9805
12.5	0.9625	0.9796	1.3796	1.3233	1.4289	3.240	4	1.178	2.513	0.1298	0.9616	0.9805
25	0.9771	0.9945	1.4252	1.3684	1.4706	3.555	4	0.294	2.513	0.1298	0.9616	0.9805
50	0.9675	0.9847	1.4034	1.3030	1.4706	5.856	4	0.717	2.513	0.1298	0.9616	0.9805
*100	0.0106	0.0108	0.1009	0.0500	0.1419	37.881	4	25.936	2.513	0.1298	0.0105	0.0107

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.937364	0.912	-1.01847	3.239448
Bartlett's Test indicates equal variances (p = 0.06)	15.043	20.09023		

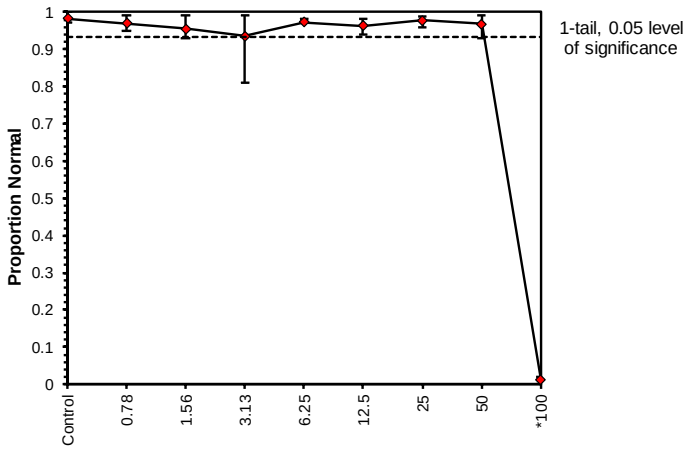
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	50	100	70.71068	2	0.049278	0.050125	0.749066	0.005335	5.8E-20	8, 27

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05	51.574	3.556	49.326	52.542	-12.5606
IC10	54.151	0.517	52.067	55.082	-0.5144
IC15	56.729	0.488	54.774	57.618	-0.5304
IC20	59.307	0.459	57.481	60.144	-0.5474
IC25	61.885	0.431	60.188	62.670	-0.5653
IC40	69.618	0.349	68.246	70.212	-0.6208
IC50	74.774	0.298	73.599	75.298	-0.6496



Dose-Response Plot



Heliocidaris tuberculata 72-h larval development test - E17022 Drum 2

Test date: 9/08/2017

	Sample	pH		Sal (ppt	d (mS/	D.O. (%)		Number of larvae		% Normal			% Control		
Rep		0 h	72 h	0 h	0 h	0 h	72 h	normal	total	72-h	Mean	CV (%)	72-h	Mean	CV (%)
1	sw-35ppt control	8.10	8.09	36	56	104	94	98	100	98%	98%	1%	100%	100%	1%
2								62	62	100%			102%		
3								98	100	98%			100%		
4								97	100	97%			99%		
1	Sal/pH Control (matched to 100% Drum 1 and Drum 2)	7.5	7.9	29	45	101	91	0	5	0%	0%	N/A	0%	0%	N/A
2								0	13	0%			0%		
3								0	48	0%			0%		
4								0	25	0%			0%		

E17022 Drum2 (%)

1	0.30	8.1	8.1	36	56	104	91	98	100	98%	98%	2%	100%	100%	2%
2								92	92	100%			102%		
3								96	100	96%			98%		
4								98	100	98%			100%		
1	1.00	8.1	8.1	36	56	104	91	98	100	98%	97%	1%	100%	99%	1%
2								97	100	97%			99%		
3								96	100	96%			98%		
4								97	100	97%			99%		
1	3.13	8.1	8.1	36	55	104	92	79	100	79%	94%	10%	80%	95%	10%
2								99	100	99%			101%		
3								98	100	98%			100%		
4								98	100	98%			100%		
1	6.25	8.1	8.1	36	55	104	91	98	100	98%	98%	1%	100%	99%	1%
2								98	100	98%			100%		
3								96	100	96%			98%		
4								99	100	99%			101%		
1	12.5	8.1	8.1	35	55	104	92	95	100	95%	97%	2%	97%	99%	2%
2								99	100	99%			101%		
3								97	100	97%			99%		
4								97	100	97%			99%		
1	25	8.0	8.1	35	53	104	92	98	100	98%	95%	3%	100%	96%	3%
2								94	100	94%			96%		
3								96	100	96%			98%		
4								90	99	91%			93%		
1	50	8.0	8.0	33	51	104	89	0	100	0%	0%	N/A	0%	0%	N/A
2								0	100	0%			0%		
3								0	100	0%			0%		
4								0	100	0%			0%		
1	100	7.8	8.0	29	45	108	89	0	100	0%	0%	N/A	0%	0%	N/A
2								0	100	0%			0%		
3								0	100	0%			0%		
4								0	100	0%			0%		

Sea Urchin Larval Development Test-Proportion Normal				
Start Date:	9/08/2017	Test ID:	E17022	Sample ID:	E17022-Drum 2
End Date:	12/08/2017	Lab ID:	LG-Lisa Golding	Sample Type:	EFF2-Industrial
Sample Date:		Protocol:	USEPA 1995 (modified)	Test Species:	HT-Heliocidaris tuberculata
Comments:	E17022 Drum 2 diluted with filtered seawater				

Conc-%	1	2	3	4
Control	0.9800	1.0000	0.9800	0.9700
0.3	0.9800	1.0000	0.9600	0.9800
1	0.9800	0.9700	0.9600	0.9700
3.13	0.7900	0.9900	0.9800	0.9800
6.25	0.9800	0.9800	0.9600	0.9900
12.5	0.9500	0.9900	0.9700	0.9700
25	0.9800	0.9400	0.9600	0.9091
50	0.0000	0.0000	0.0000	0.0000
100	0.0000	0.0000	0.0000	0.0000

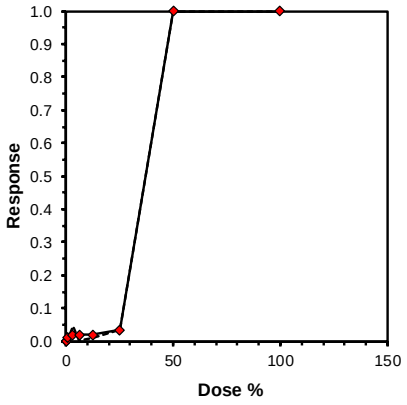
			Transform: Arcsin Square Root					Rank	1-Tailed	Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
Control	0.9825	1.0000	1.4404	1.3967	1.5073	3.267	4			0.9807	1.0000
0.3	0.9800	0.9975	1.4365	1.3694	1.5186	4.284	4	18.00	10.00	0.9796	0.9989
1	0.9700	0.9873	1.3979	1.3694	1.4289	1.739	4	13.00	10.00	0.9700	0.9891
3.13	0.9350	0.9517	1.3558	1.0948	1.4706	12.917	4	17.00	10.00	0.9608	0.9798
6.25	0.9775	0.9949	1.4245	1.3694	1.4706	2.922	4	17.00	10.00	0.9608	0.9798
12.5	0.9700	0.9873	1.4023	1.3453	1.4706	3.678	4	14.00	10.00	0.9608	0.9798
25	0.9473	0.9641	1.3465	1.2645	1.4289	5.176	4	12.00	10.00	0.9474	0.9660
50	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	4			0.0000	0.0000
100	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	4			0.0000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.869486	0.896	-1.60486	5.474216
Bartlett's Test indicates equal variances (p = 0.03)	13.63951	16.81189		

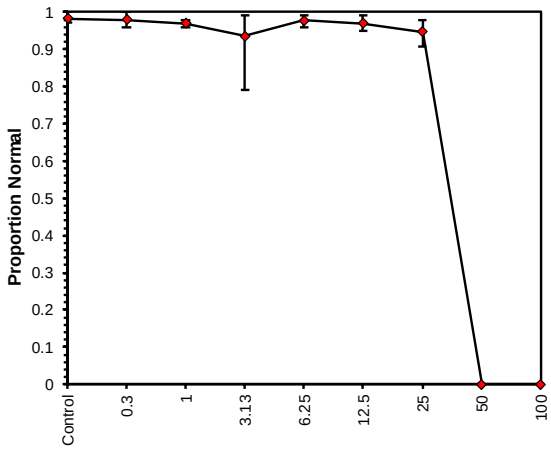
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	25	50	35.35534	4

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05	25.415	4.443	0.000	26.158	-3.8716
IC10	26.709	0.333	25.409	27.413	-0.2019
IC15	28.003	0.315	26.775	28.667	-0.2019
IC20	29.297	0.296	28.141	29.922	-0.2019
IC25	30.591	0.278	29.508	31.177	-0.2019
IC40	34.473	0.222	33.606	34.942	-0.2019
IC50	37.061	0.185	36.338	37.451	-0.2019



Dose-Response Plot



Statistics – Quality Assurance/Quality Control

Heliocidaris tuberculata 72-h larval development test - Quality Assurance

Test date: 9/08/2017

	Copper (µg/L)		pH		Sal (ppt	nd (mS/cr	D.O. (%)		Number of larvae		% Normal			% Control		
Rep	Nominal	Measured dissolved	0 h	72 h	0 h	0 h	0 h	72 h	normal	total	72-h	Mean	CV (%)	72-h	Mean	CV (%)
1	sw-35ppt control	0	8.1	8.1	36	56	104	94	98	100	98%	98%	1%	100%	100%	1%
2									62	62	100%			102%		
3									98	100	98%			100%		
4									97	100	97%			99%		
Reference Toxicant-Copper (µg/L)																
1	4 µg/L	2.7	8.1	8.1	36	55	102	91	96	100	96%	96%	3%	98%	98%	3%
2									87	94	93%			94%		
3									99	100	99%			101%		
4									98	100	98%			100%		
1	8 µg/L	5.4	8.1	8.1	36	56	103	92	60	78	77%	78%	12%	78%	79%	12%
2									66	100	66%			67%		
3									88	100	88%			90%		
4									66	81	81%			83%		
1	12 µg/L	7.5	8.1	8.0	36	56	104	91	29	82	35%	30%	50%	36%	30%	50%
2									40	89	45%			46%		
3									28	100	28%			28%		
4									10	100	10%			10%		
1	16 µg/L	13.0	8.1	8.0	36	56	105	89	0	100	0%	0%	N/A	0%	0%	N/A
2									0	100	0%			0%		
3									0	99	0%			0%		
4									0	100	0%			0%		
1	32 µg/L	25.0	8.1	8.1	36	56	104	87	0	23	0%	0%	N/A	0%	0%	N/A
2									0	39	0%			0%		
3									0	79	0%			0%		
4									0	54	0%			0%		

Sea Urchin Larval Development Test-Proportion Normal						
Start Date:	9/08/2017	Test ID:	AQC053m	Sample ID:	CUSO4-Copper Sulfate	
End Date:	12/08/2017	Lab ID:	LG-Lisa Golding	Sample Type:	CUSO-Copper sulfate	
Sample Date:		Protocol:	SL-Simon Laginestra modifie	Test Species:	HT-Heliocidaris tuberculata	
Comments:	AQC053 Cu in seawater [measured Cu] % normal larval development					

Conc-ug/L	1	2	3	4
Control	0.9800	1.0000	0.9800	0.9700
2.7	0.9600	0.9255	0.9900	0.9800
5.4	0.7692	0.6600	0.8800	0.8148
7.5	0.3537	0.4494	0.2800	0.1000
13	0.0000	0.0000	0.0000	0.0000
25	0.0000	0.0000	0.0000	0.0000

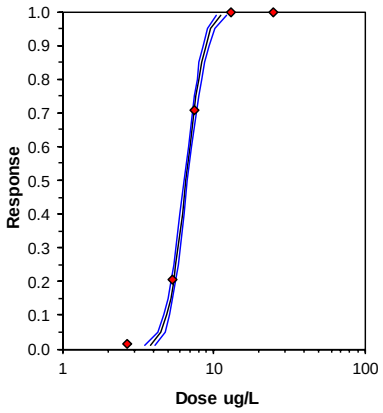
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
Control	0.9825	1.0000	1.4404	1.3967	1.5073	3.267	4				7	362
2.7	0.9639	0.9811	1.3908	1.2944	1.4706	5.503	4	0.617	2.290	0.1842	14	394
*5.4	0.7810	0.7949	1.0902	0.9483	1.2171	10.314	4	4.353	2.290	0.1842	79	359
*7.5	0.2958	0.3010	0.5627	0.3218	0.7347	31.319	4	10.910	2.290	0.1842	264	371
13	0.0000	0.0000	0.0501	0.0500	0.0503	0.252	4				399	399
25	0.0000	0.0000	0.0772	0.0563	0.1044	26.652	4				195	195

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9597	0.844	-0.6809	1.02777
Bartlett's Test indicates equal variances (p = 0.21)	4.4962	11.3449		

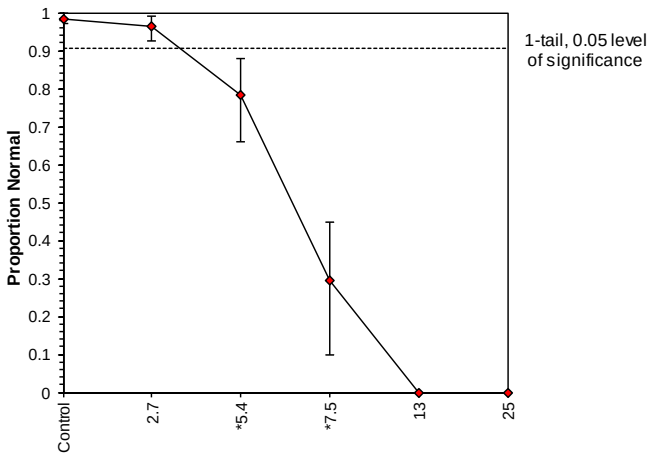
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	2.7	5.4	3.81838		0.07885	0.0802	0.64994	0.01294	4.6E-07	3, 12

Maximum Likelihood-Probit											
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	10.0297	0.66191	8.73233	11.327	0.01934	1.63002	7.81473	0.65	0.81928	0.0997	6
Intercept	-3.2171	0.54521	-4.2857	-2.1485							
TSCR	0.02796	0.006	0.0162	0.03971							

Point	Probits	ug/L	95% Fiducial Limits	
EC01	2.674	3.86664	3.5543	4.1299
EC05	3.355	4.52149	4.24752	4.7508
EC10	3.718	4.91478	4.66825	5.12184
EC15	3.964	5.19928	4.97347	5.39053
EC20	4.158	5.43709	5.22838	5.61606
EC25	4.326	5.64977	5.45551	5.81914
EC40	4.747	6.22329	6.05811	6.37918
EC50	5.000	6.59599	6.43614	6.75859
EC60	5.253	6.99101	6.82179	7.17732
EC75	5.674	7.70069	7.4819	7.9663
EC80	5.842	8.0019	7.75332	8.31143
EC85	6.036	8.3679	8.07847	8.7366
EC90	6.282	8.8523	8.50294	9.30706
EC95	6.645	9.6223	9.16769	10.2282
EC99	7.326	11.2519	10.5467	12.2223



Dose-Response Plot



Sea Urchin Larval Development Test-Proportion Normal				
Start Date:	9/08/2017	Test ID:	E17021-pH	Sample ID:	SAL/PH-Salinity/pH Controls
End Date:	12/08/2017	Lab ID:	LG-Lisa Golding	Sample Type:	FSW-Filtered Seawater
Sample Date:		Protocol:	USEPA 1995 (modified)	Test Species:	HT-Heliocidaris tuberculata
Comments:	salinity/pH control to match 100% E17021 and E17022				

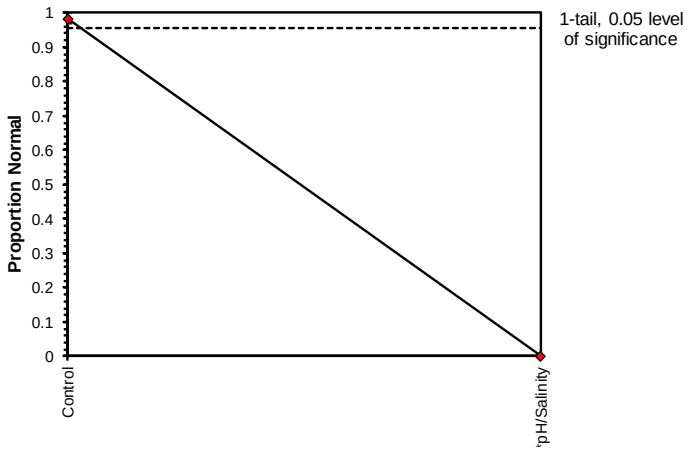
Conc-%	1	2	3	4
Control	0.9800	1.0000	0.9800	0.9700
pH/Salinity	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					1-Tailed		
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
Control	0.9825	1.0000	1.4404	1.3967	1.5073	3.267	4			
*pH/Salinity	0.0000	0.0000	0.1343	0.0722	0.2255	49.706	4	31.992	1.943	0.0793

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.90479	0.749	0.864508	-0.31852
F-Test indicates equal variances (p = 0.58)	2.011408	47.46723		

Hypothesis Test (1-tail, 0.05)	MSDu	MSDp	MSB	MSE	F-Prob	df
Homoscedastic t Test indicates significant differences	0.026433	0.026888	3.412225	0.003334	6.2E-08	1, 6

Dose-Response Plot



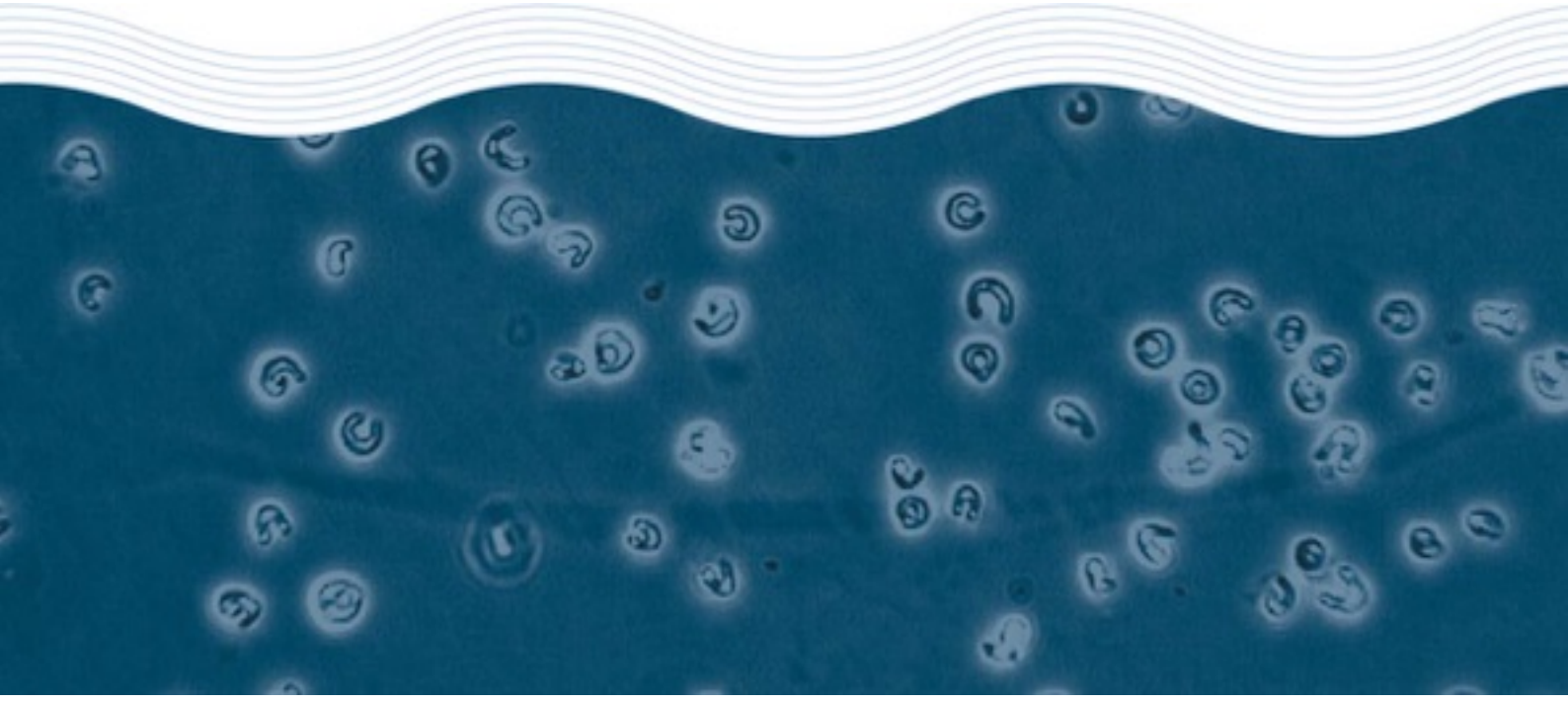
ecotox
SERVICES AUSTRALASIA

Toxicity Assessment of Drum Liquor Samples to Milky Oyster and Anemone Development

CSIRO Land and Water

Test Report

August 2017



Toxicity Test Report: TR1502/1

Accredited for compliance with ISO/IEC 17025

Client:	CSIRO Land and Water	**ESA Job #:**	PR1502
	Locked Bag 2007	**Date Sampled:**	Prepared 11 July 2017
	Kirawee NSW 2232	**Date Received:**	14 June 2017
Attention:	Monique Binet	**Sampled By:**	Client
Client Ref:	Not supplied	**ESA Quote #:**	PL1502_q01

Lab ID No.:	Sample Name:	Sample Description:
8230	Drum 1 Liquor	Aqueous sample, pH 7.6* and salinity 29.6‰*. Sample received at 5ºC* in apparent good condition
8231	Drum 2 Liquor	Aqueous sample, pH 7.7* and salinity 29.2‰*. Sample received at 5ºC* in apparent good condition

*NATA accreditation does not cover the performance of this service

Test Performed:	48-hr larval development test using the milky oyster *Saccostrea echinata*
Test Protocol:	ESA SOP 106 (ESA 2011), based on APHA (1998) and Krassoi (1995)
Test Temperature:	The test was performed at 29±1°C.
Deviations from Protocol:	Nil.
Comments on Solution Preparation:	The sample was not salinity adjusted prior to testing. The sample was serially diluted with filtered sea water (FSW) to achieve the test concentrations. A FSW control was tested concurrently with the sample, including additional FSW controls prepared to match the salinity and pH of the undiluted sample treatment.
Source of Test Organisms:	Field collected from Mackay, QLD.
Test Initiated:	26 July 2017 at 1900h

Controls

Treatment	% Normal larve (Mean ± SD)
FSW Control	77.3 ± 3.5
Salinity Control	72.8 ± 3.5

Sample 8230: *Drum 1 Liquor*

Concentration (%)	% Normal larve (Mean ± SD)
FSW Control	77.3 ± 3.5
0.4	74.8 ± 3.4
1.2	74.8 ± 4.9
3.7	75.8 ± 5.9
11	74.5 ± 2.4
33	77.3 ± 4.6
67	72.5 ± 3.1
100	61.5 ± 7.0*

48-hr EC10 = 83.1 (33.7-93.3)%
48-hr EC50 = >100%
NOEC = 67%
LOEC = 100%

Sample 8231: *Drum 2 Liquor*

Concentration (%)	% Normal larvae (Mean ± SD)
FSW Control	77.3 ± 3.5
0.4	75.3 ± 4.4
1.2	76.5 ± 5.5
3.7	74.5 ± 1.7
11	80.8 ± 6.2
33	74.3 ± 4.6
67	68.5 ±3.7**
100	0.0 ± 0.0

48-hr IC10 = 60.9 (28.1-72.1)%
48-hr EC50 = 74.7 (72.9-76.7)%
NOEC = 33%
LOEC = 67%

* Significant reduction of percent normally developed larvae compared with the FSW Control (Dunnett's test, 1-tail, p=0.05)
**Significant reduction of percent normally developed larvae compared with the FSW Control (Dunnetts t-test, 1-tail, p=0.05)

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean % normal larvae	≥70.0%	77.3%	Yes
Reference Toxicant within cusum chart limits	12.2-17.4 µg Cu/L	14.7µg Cu/L	Yes

Toxicity Test Report: TR1502/1

Test Report Authorised by: Dr Rick Krassoi, Director on 7 August 2017

Results are based on the samples in the condition as received by ESA.

NATA Accredited Laboratory Number: 14709

.

Citations:

APHA (1998) Method 8810 D. Echinoderm Embryo Development Test. In Standard Methods for the Examination of Water and Wastewater, 20th Ed. American Public Health Association, American Water Works Association and the Water Environment Federation, USA.

Doyle, C.J., Pablo, F., Lim, R.P. and Hyne, R.V. (2003) Assessment of metal toxicity in sediment pore water from Lake Macquarie, Australia. *Arch. Environ. Contam. Toxicology*, 44(3): 343-350.

ESA (2014) *ESA SOP 105 - Sea Urchin Larval Development Test*. Issue No. 10. Ecotox Services Australasia, Sydney NSW.

Simon, J. and Laginestra, E.(1997) Bioassay for testing sublethal toxicity in effluents, using gametes of sea urchin *Heliocidaris tuberculata*. National Pulp Mills Research Program Technical Report No. 20. CSIRO, Canberra, ACT.

Toxicity Test Report: TR1502/2

Client:	CSIRO Land and Water Locked Bag 2007 Kirawee NSW 2232	**ESA Job #:** **Date Sampled:** **Date Received:**	PR1502 Prepared 11 July 2017 14 June 2017
Attention:	Monique Binet	**Sampled By:**	Client
Client Ref:	Not supplied	**ESA Quote #:**	PL1502_q01

Lab ID No.:	Sample Name:	Sample Description:
8230	Drum 1 Liquor	Aqueous sample, pH 7.6* and salinity 29.6‰*. Sample received at 5ºC in apparent good condition
8231	Drum 2 Liquor	Aqueous sample, pH 7.7* and salinity 29.2‰*. Sample received at 5ºC in apparent good condition

Test Performed:	8-day Sea anemone pedal lacerate development test using *Aiptasia pulchella*
Test Protocol:	ESA SOP 128 (ESA 2014), based on Howe *et al* (2014)
Test Temperature:	The test was performed at 25±1°C.
Deviations from Protocol:	Nil
Comments on Solution Preparation:	The sample was not salinity adjusted prior to testing. The sample was serially diluted with filtered sea water (FSW) to achieve the test concentrations. A FSW control was tested concurrently with the sample, including additional FSW controls prepared to match the salinity and pH of the undiluted sample treatment.
Source of Test Organisms:	In-house culture, originally sourced from Marine Ecology Research Centre at Southern Cross University, Lismore, NSW
Test Initiated:	26 July 2017 at 2030h

Controls

Treatment	% Normal (Mean ± SD)
FSW Control	90.0 ±11.6
Salinity Control	90.0 ±11.6

Sample 8230: *Drum 1 Liquor*

Concentration (%)	% Normal (Mean ± SD)
FSW Control	90.0 ± 11.6
0.4	90.0 ± 11.6
1.2	90.0 ± 11.6
3.7	90.0 ± 11.6
11	95.0 ± 10.0
33	90.0 ± 11.6
67	85.0 ± 10.0
100	80.0 ± 16.3*

8-d EC10 = 82.5 (CL not reliable)%
8-d EC50 = >100%
NOEC = 100%
LOEC = >100%

Sample 8231: *Drum 2 Liquor*

Concentration (%)	% Normal (Mean ± SD)
FSW Control	90.0 ± 11.6
0.4	90.0 ± 11.6
1.2	95.0 ± 10.0
3.7	95.0 ± 10.0
11	95.0 ± 10.0
33	90.0 ± 11.6
67	90.0 ±11.6
100	15.0 ±19.2*

8-d IC10 = 69.3 (0.0-72.4)%
8-d EC50 = 84.3 (83.3-85.3)%
NOEC = 67%
LOEC = 100%

* Significant reduction of percent normally developed pedal lacerates compared with the FSW Control (Dunnett's test, 1-tail, p=0.05)

QA/QC Parameter	Criterion	This Test	Criterion met?
Control mean % normal pedal lacerates	≥90.0%	90.0%	Yes
Reference Toxicant within cusum chart limits	14.6-53.8 µg Cu/L	15.3µg Cu/L	Yes

Toxicity Test Report: TR1502/2

Test Report Authorised by: Dr Rick Krassoi, Director on 7 August 2017

Results are based on the samples in the condition as received by ESA.

.

Citations:

ESA (2014) SOP 128 – *Sea Anemone Pedal Lacerate Development Toxicity Test*. Issue No 1. Ecotox Services Australasia, Sydney, NSW.

Howe, Pelli L., Reichelt-Brushett, Amanda J. and Clark, Malcolm W (2014) Development of a chronic, early life-stage sub-lethal toxicity test and recovery assessment for the tropical zooxanthellate sea anemone Aiptasia pulchella. Ecotoxicology and Environmental Safety 100: 138-147.

ecotox
SERVICES AUSTRALASIA

Chain-of-Custody Documentation

Centre for Environmental Contaminants Research
CSIRO Land and Water
Post: Locked Bag 2007, Kirrawee , NSW 2232 Australia
Delivery: c/o ANSTO Store, New Illawarra Rd, Building 2, Lucas Heights, NSW 2234
Telephone: (02) 9710 6812 Fax: (02) 9710 6800
Email: monique.binet@csiro.au

CSIRO

CHAIN OF CUSTODY

		Prepared		Water Physico-chemical Parameters						Analyses Required						Additional Information
CSIRO ID (E___)	Sample Description / Sample No	Date	Time	Cond.	Salinity	pH	D.O.	Temp	Other (specify)	Milky oyster larval development	8-d Sea anemone development test					PR1502
(CSIRO Use only)		dd/mm/yy	hh:mm (24h)	mS	‰		mg/L or % sat.	°C								
E17021	Drum 1 liquor	11/07/2017		46	29	7.3	100			X	X					Please refer to email for test concentrations to run and for instructions on sub-sampling during each test. Phys-chem information also TBA in email.
E17022	Drum 2 liquor	11/07/2017		46	29	7.6	99			x	x					
I have also included 30x acid-washed containers, 10x acid washed syringes and 10x 0.45µm filtes for use in sub-sampling.																

				Method of Shipment, Date					
Prepared By:	M. Binet			Toll same-day	Received By:				
Signature					Signature				
For:	CSIRO	Date:	12.7.17	13/7/17.	For:		Date:		

				Method of Shipment, Date					
Collected By:	Kat Melville				Received By:				
Signature:	KMelville				Signature				
For:	Ecotox	Date:	13.7.2017 @ 12:45		For:		Date:		

ecotox
SERVICES AUSTRALASIA

Statistical Printouts for the Milky Oyster Larval Development Tests

Bivalve Larval Development Test-Proportion Normal

Start Date:	26/07/2017 19:30	Test ID:	PR1502/01	Sample ID:	Drum 1 Liquor
End Date:	28/07/2017 19:00	Lab ID:	8230	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 106	Test Species:	SE-Saccostrea echinata
Comments:					

Conc-%	1	2	3	4
FSW Control	0.7600	0.8100	0.7300	0.7900
Salinity Control	0.7500	0.7100	0.7600	0.6900
0.4	0.7200	0.7900	0.7600	0.7200
1.2	0.8100	0.7600	0.7200	0.7000
3.7	0.7600	0.7100	0.7200	0.8400
11	0.7300	0.7600	0.7200	0.7700
33	0.7800	0.7200	0.7600	0.8300
67	0.7200	0.7700	0.7100	0.7000
100	0.6100	0.5300	0.7000	0.6200

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
FSW Control	0.7725	1.0619	1.0744	1.0244	1.1198	3.881	4	*			91	400
Salinity Control	0.7275	1.0000	1.0221	0.9803	1.0588	3.628	4					
0.4	0.7475	1.0275	1.0450	1.0132	1.0948	3.784	4	0.796	2.480	0.0917	101	400
1.2	0.7475	1.0275	1.0457	0.9912	1.1198	5.435	4	0.776	2.480	0.0917	101	400
3.7	0.7575	1.0412	1.0584	1.0021	1.1593	6.767	4	0.435	2.480	0.0917	97	400
11	0.7450	1.0241	1.0418	1.0132	1.0706	2.624	4	0.884	2.480	0.0917	102	400
33	0.7725	1.0619	1.0751	1.0132	1.1458	5.138	4	-0.018	2.480	0.0917	91	400
67	0.7250	0.9966	1.0193	0.9912	1.0706	3.472	4	1.492	2.480	0.0917	110	400
*100	0.6150	0.8454	0.9024	0.8154	0.9912	7.965	4	4.653	2.480	0.0917	154	400

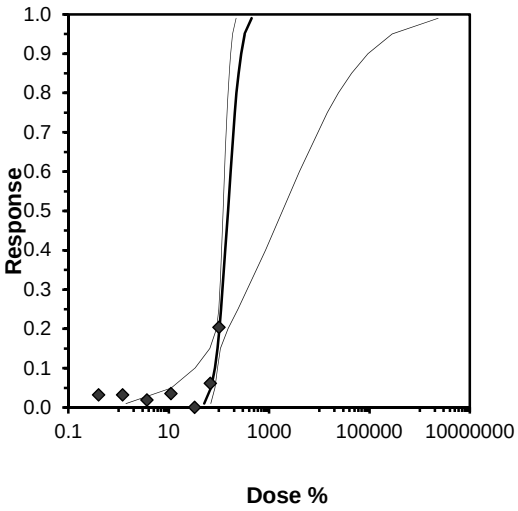
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.969612	0.93	0.447219	-0.29879
Bartlett's Test indicates equal variances (p = 0.76)	4.132527	18.47531		
The control means are not significantly different (p = 0.11)	1.875528	2.446912		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	67	100	81.85353	1.492537	0.080957	0.104703	0.01244	0.002735	0.002374	7, 24
Treatments vs FSW Control										

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	4.918145	2.127189	0.748855	9.087434	0.2275	1.261175	11.0705	0.94	2.180037	0.203329	8
Intercept	-5.72174	4.214671	-13.9825	2.539018							
TSCR	0.242982	0.008831	0.225673	0.260292							

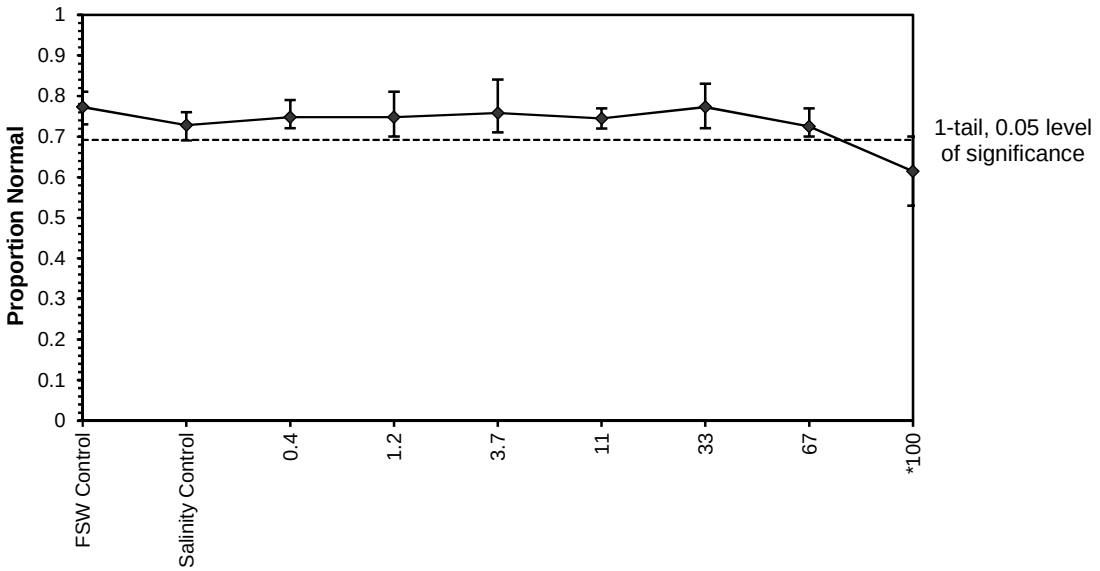
Point	Probits	%	95% Fiducial Limits	
EC01	2.674	50.93585	1.401115	69.19756
EC05	3.355	70.07949	11.26706	83.13716
EC10	3.718	83.07314	33.65854	93.24642
EC15	3.964	93.17517	66.24329	107.123
EC20	4.158	102.0731	89.7695	151.175
EC25	4.326	110.3809	98.70214	239.8039
EC40	4.747	134.4386	113.1975	849.3202
EC50	5.000	151.369	121.3971	1840.311
EC60	5.253	170.4314	129.8816	3997.085
EC75	5.674	207.5774	144.9841	14544.4
EC80	5.842	224.4722	151.3889	24293.84
EC85	6.036	245.9085	159.183	44185.3
EC90	6.282	275.8119	169.527	93806.37
EC95	6.645	326.9511	186.053	286389
EC99	7.326	449.8319	221.3941	2325245



Bivalve Larval Development Test-Proportion Normal

Start Date:	26/07/2017 19:30	Test ID:	PR1502/01	Sample ID:	Drum 1 Liquor
End Date:	28/07/2017 19:00	Lab ID:	8230	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 106	Test Species:	SE-Saccostrea echinata
Comments:					

Dose-Response Plot



Bivalve Larval Development Test-Proportion Normal

Start Date:	26/07/2017 19:30	Test ID:	PR1502/01	Sample ID:	Drum 1 Liquor
End Date:	28/07/2017 19:00	Lab ID:	8230	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 106	Test Species:	SE-Saccostrea echinata
Comments:					

Auxiliary Data Summary

Conc-%	Parameter	Mean	Min	Max	SD	CV%	N
FSW Control	% Normal	77.25	73.00	81.00	3.50	2.42	4
Salinity Control		72.75	69.00	76.00	3.30	2.50	4
0.4		74.75	72.00	79.00	3.40	2.47	4
1.2		74.75	70.00	81.00	4.86	2.95	4
3.7		75.75	71.00	84.00	5.91	3.21	4
11		74.50	72.00	77.00	2.38	2.07	4
33		77.25	72.00	83.00	4.57	2.77	4
67		72.50	70.00	77.00	3.11	2.43	4
100		61.50	53.00	70.00	6.95	4.29	4
FSW Control	pH	8.10	8.10	8.10	0.00	0.00	1
Salinity Control		8.10	8.10	8.10	0.00	0.00	1
0.4		8.10	8.10	8.10	0.00	0.00	1
1.2		8.10	8.10	8.10	0.00	0.00	1
3.7		8.10	8.10	8.10	0.00	0.00	1
11		8.10	8.10	8.10	0.00	0.00	1
33		8.10	8.10	8.10	0.00	0.00	1
67		7.90	7.90	7.90	0.00	0.00	1
100		7.60	7.60	7.60	0.00	0.00	1
FSW Control	Salinity ppt	35.60	35.60	35.60	0.00	0.00	1
Salinity Control		29.30	29.30	29.30	0.00	0.00	1
0.4		35.50	35.50	35.50	0.00	0.00	1
1.2		35.50	35.50	35.50	0.00	0.00	1
3.7		35.10	35.10	35.10	0.00	0.00	1
11		34.70	34.70	34.70	0.00	0.00	1
33		33.90	33.90	33.90	0.00	0.00	1
67		31.90	31.90	31.90	0.00	0.00	1
100		29.60	29.60	29.60	0.00	0.00	1
FSW Control	DO %	105.20	105.20	105.20	0.00	0.00	1
Salinity Control		109.10	109.10	109.10	0.00	0.00	1
0.4		106.30	106.30	106.30	0.00	0.00	1
1.2		107.60	107.60	107.60	0.00	0.00	1
3.7		108.40	108.40	108.40	0.00	0.00	1
11		105.30	105.30	105.30	0.00	0.00	1
33		104.90	104.90	104.90	0.00	0.00	1
67		103.60	103.60	103.60	0.00	0.00	1
100		104.20	104.20	104.20	0.00	0.00	1

-Proportion Normal

Start Date:	26/07/2017 19:30	Test ID:	PR1502/02	Sample ID:	Drum 2 Liquor
End Date:	28/07/2017 19:00	Lab ID:	8231	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 106	Test Species:	SE-Saccostrea echinata
Comments:					

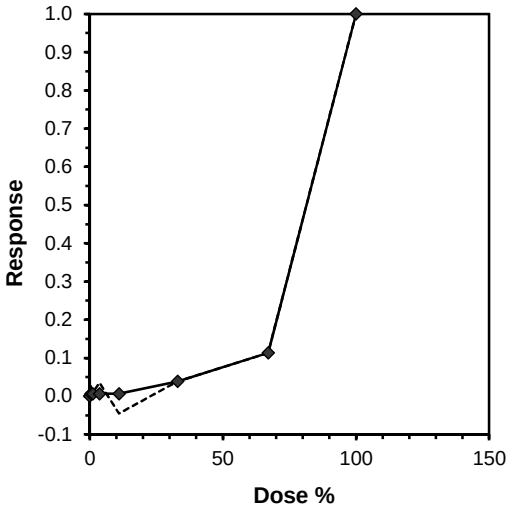
Conc-%	1	2	3	4
FSW Control	0.7600	0.8100	0.7300	0.7900
Salinity Control	0.7500	0.7100	0.7600	0.6900
0.4	0.7600	0.7700	0.7900	0.6900
1.2	0.7300	0.8400	0.7200	0.7700
3.7	0.7600	0.7500	0.7500	0.7200
11	0.7500	0.7700	0.8200	0.8900
33	0.7300	0.7100	0.8100	0.7200
67	0.6400	0.7100	0.7200	0.6700
100	0.0000	0.0000	0.0000	0.0000

			Transform: Arcsin Square Root					Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Mean	N-Mean
FSW Control	0.7725	1.0619	1.0744	1.0244	1.1198	3.881	4	0.7725	1.0000
Salinity Control	0.7275	1.0000	1.0221	0.9803	1.0588	3.628	4		
0.4	0.7525	1.0344	1.0511	0.9803	1.0948	4.712	4	0.7675	0.9935
1.2	0.7650	1.0515	1.0669	1.0132	1.1593	6.226	4	0.7675	0.9935
3.7	0.7450	1.0241	1.0416	1.0132	1.0588	1.893	4	0.7675	0.9935
11	0.8075	1.1100	1.1208	1.0472	1.2327	7.394	4	0.7675	0.9935
33	0.7425	1.0206	1.0399	1.0021	1.1198	5.196	4	0.7425	0.9612
67	0.6850	0.9416	0.9754	0.9273	1.0132	4.071	4	0.6850	0.8867
100	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	4	0.0000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.957904	0.924	0.563047	-0.02913
Bartlett's Test indicates equal variances (p = 0.49)	5.401556	16.81189		
The control means are not significantly different (p = 0.11)	1.875528	2.446912		

Linear Interpolation (200 Resamples)

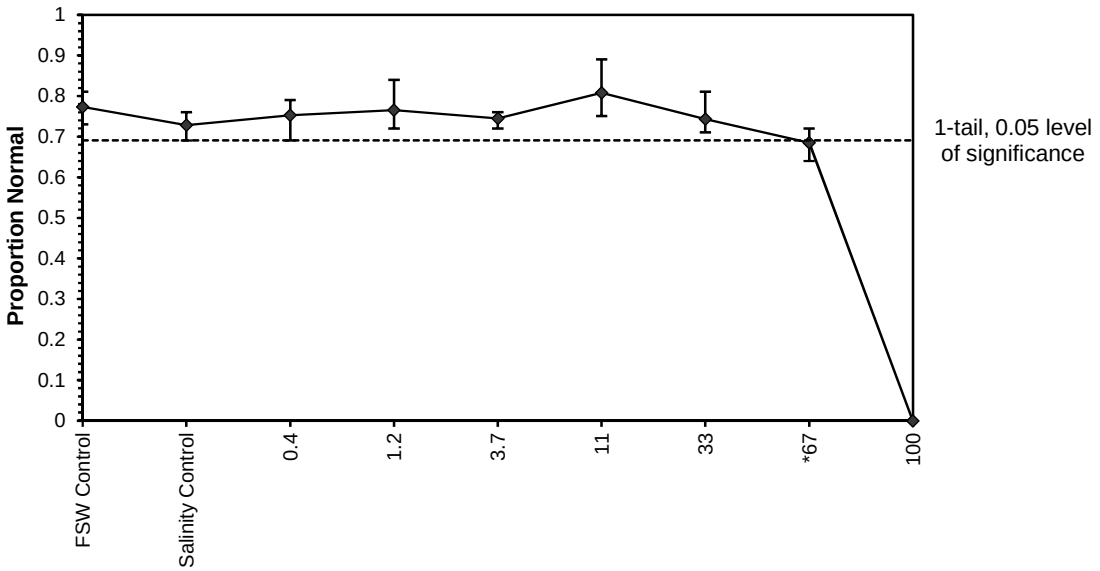
Point	%	SD	95% CL(Exp)		Skew
IC05	38.100	11.964	0.000	58.768	-0.4407
IC10	60.939	8.773	28.073	72.068	-0.6736
IC15	68.367	2.112	55.764	70.465	-3.0701
IC20	70.228	0.846	67.143	72.203	-0.2260
IC25	72.089	0.793	69.197	73.940	-0.2260
IC40	77.671	0.635	75.358	79.152	-0.2260
IC50	81.392	0.529	79.465	82.627	-0.2260



-Proportion Normal

Start Date:	26/07/2017 19:30	Test ID:	PR1502/02	Sample ID:	Drum 2 Liquor
End Date:	28/07/2017 19:00	Lab ID:	8231	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 106	Test Species:	SE-Saccostrea echinata
Comments:					

Dose-Response Plot



-Proportion Normal

Start Date:	26/07/2017 19:30	Test ID:	PR1502/02	Sample ID:	Drum 2 Liquor
End Date:	28/07/2017 19:00	Lab ID:	8231	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 106	Test Species:	SE-Saccostrea echinata
Comments:					

Auxiliary Data Summary

Conc-%	Parameter	Mean	Min	Max	SD	CV%	N
FSW Control	Total Counted	77.25	73.00	81.00	3.50	2.42	4
Salinity Control		72.75	69.00	76.00	3.30	2.50	4
0.4		75.25	69.00	79.00	4.35	2.77	4
1.2		76.50	72.00	84.00	5.45	3.05	4
3.7		74.50	72.00	76.00	1.73	1.77	4
11		80.75	75.00	89.00	6.24	3.09	4
33		74.25	71.00	81.00	4.57	2.88	4
67		68.50	64.00	72.00	3.70	2.81	4
100		0.00	0.00	0.00	0.00		4
FSW Control	Number Normal	8.10	8.10	8.10	0.00	0.00	1
Salinity Control		8.10	8.10	8.10	0.00	0.00	1
0.4		8.10	8.10	8.10	0.00	0.00	1
1.2		8.10	8.10	8.10	0.00	0.00	1
3.7		8.10	8.10	8.10	0.00	0.00	1
11		8.10	8.10	8.10	0.00	0.00	1
33		8.10	8.10	8.10	0.00	0.00	1
67		7.90	7.90	7.90	0.00	0.00	1
100		7.70	7.70	7.70	0.00	0.00	1

-Proportion Normal

Start Date:	26/07/2017 19:30	Test ID:	PR1502/02	Sample ID:	Drum 2 Liquor
End Date:	28/07/2017 19:00	Lab ID:	8231	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 106	Test Species:	SE-Saccostrea echinata
Comments:					

Conc-%	1	2	3	4
FSW Control	0.7600	0.8100	0.7300	0.7900
Salinity Control	0.7500	0.7100	0.7600	0.6900
0.4	0.7600	0.7700	0.7900	0.6900
1.2	0.7300	0.8400	0.7200	0.7700
3.7	0.7600	0.7500	0.7500	0.7200
11	0.7500	0.7700	0.8200	0.8900
33	0.7300	0.7100	0.8100	0.7200
67	0.6400	0.7100	0.7200	0.6700
100	0.0000	0.0000	0.0000	0.0000

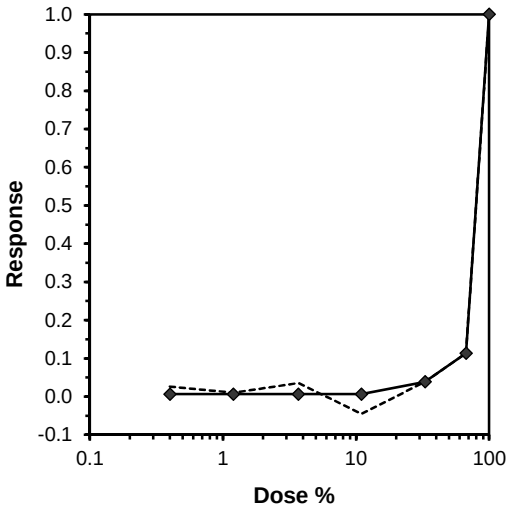
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
FSW Control	0.7725	1.0619	1.0744	1.0244	1.1198	3.881	4	*			91	400
Salinity Control	0.7275	1.0000	1.0221	0.9803	1.0588	3.628	4					
0.4	0.7525	1.0344	1.0511	0.9803	1.0948	4.712	4	0.611	2.451	0.0935	99	400
1.2	0.7650	1.0515	1.0669	1.0132	1.1593	6.226	4	0.198	2.451	0.0935	94	400
3.7	0.7450	1.0241	1.0416	1.0132	1.0588	1.893	4	0.861	2.451	0.0935	102	400
11	0.8075	1.1100	1.1208	1.0472	1.2327	7.394	4	-1.216	2.451	0.0935	77	400
33	0.7425	1.0206	1.0399	1.0021	1.1198	5.196	4	0.906	2.451	0.0935	103	400
*67	0.6850	0.9416	0.9754	0.9273	1.0132	4.071	4	2.598	2.451	0.0935	126	400
100	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	4				400	400

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)	0.957904	0.924	0.563047	-0.02913
Bartlett's Test indicates equal variances (p = 0.49)	5.401556	16.81189		
The control means are not significantly different (p = 0.11)	1.875528	2.446912		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	33	67	47.02127	3.030303	0.082598	0.106825	0.007721	0.002908	0.044707	6, 21
Treatments vs FSW Control										

Trimmed Spearman-Karber

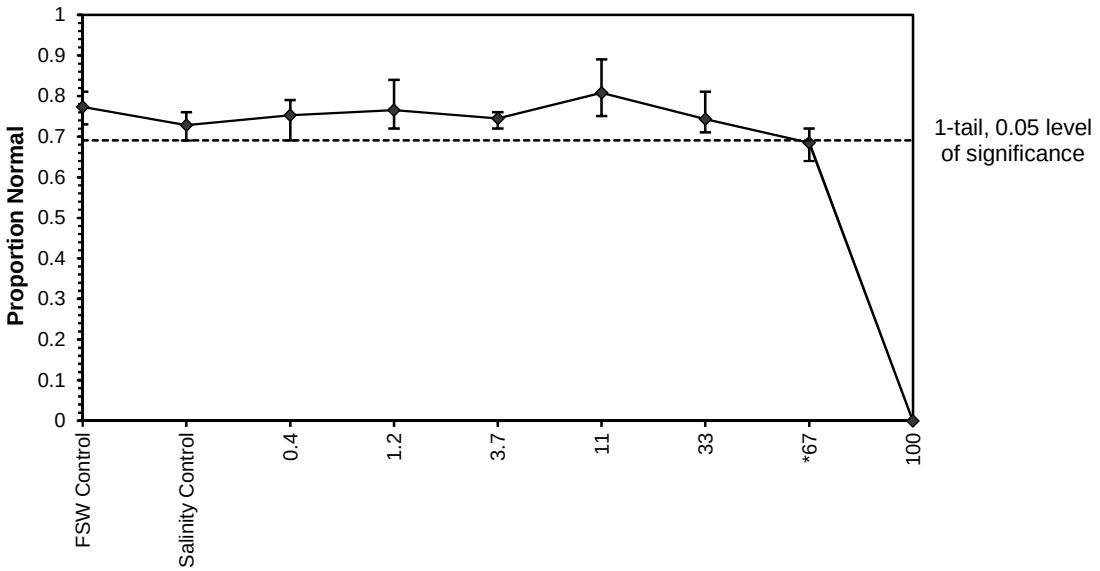
Trim Level	EC50	95% CL	
0.0%			
5.0%	78.195	76.606	79.816
10.0%	79.707	77.248	82.244
20.0%	79.786	79.145	80.433
Auto-0.6%	74.741	72.861	76.670



-Proportion Normal

Start Date:	26/07/2017 19:30	Test ID:	PR1502/02	Sample ID:	Drum 2 Liquor
End Date:	28/07/2017 19:00	Lab ID:	8231	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 106	Test Species:	SE-Saccostrea echinata
Comments:					

Dose-Response Plot



-Proportion Normal

Start Date:	26/07/2017 19:30	Test ID:	PR1502/02	Sample ID:	Drum 2 Liquor
End Date:	28/07/2017 19:00	Lab ID:	8231	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 106	Test Species:	SE-Saccostrea echinata
Comments:					

Auxiliary Data Summary

Conc-%	Parameter	Mean	Min	Max	SD	CV%	N
FSW Control	Total Counted	77.25	73.00	81.00	3.50	2.42	4
Salinity Control		72.75	69.00	76.00	3.30	2.50	4
0.4		75.25	69.00	79.00	4.35	2.77	4
1.2		76.50	72.00	84.00	5.45	3.05	4
3.7		74.50	72.00	76.00	1.73	1.77	4
11		80.75	75.00	89.00	6.24	3.09	4
33		74.25	71.00	81.00	4.57	2.88	4
67		68.50	64.00	72.00	3.70	2.81	4
100		0.00	0.00	0.00	0.00		4
FSW Control	Number Normal	8.10	8.10	8.10	0.00	0.00	1
Salinity Control		8.10	8.10	8.10	0.00	0.00	1
0.4		8.10	8.10	8.10	0.00	0.00	1
1.2		8.10	8.10	8.10	0.00	0.00	1
3.7		8.10	8.10	8.10	0.00	0.00	1
11		8.10	8.10	8.10	0.00	0.00	1
33		8.10	8.10	8.10	0.00	0.00	1
67		7.90	7.90	7.90	0.00	0.00	1
100		7.70	7.70	7.70	0.00	0.00	1

Statistical Printouts for the Sea Anemone Pedal Lacerate Development Test

Anemone Larval Toxicity Test-% Normal

Start Date:	26/07/2017 20:30	Test ID:	PR1502/04	Sample ID:	Drum 1 Liquor
End Date:	3/08/2017 19:30	Lab ID:	8230	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 128	Test Species:	AI-Aiptasia pulchella
Comments:					

Conc-%	1	2	3	4
FSW Control	0.8000	0.8000	1.0000	1.0000
Salinity Control	0.8000	0.8000	1.0000	1.0000
0.4	0.8000	1.0000	0.8000	1.0000
1.2	0.8000	0.8000	1.0000	1.0000
3.7	1.0000	0.8000	1.0000	1.0000
11	1.0000	1.0000	0.8000	1.0000
33	0.8000	0.8000	1.0000	1.0000
67	0.8000	0.8000	0.8000	1.0000
100	0.8000	0.8000	1.0000	0.6000

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
FSW Control	0.9000	1.0000	1.3140	1.1071	1.5208	18.175	4	*			40	400
Salinity Control	0.9000	1.0000	1.3140	1.1071	1.5208	18.175	4					
0.4	0.9000	1.0000	1.3140	1.1071	1.5208	18.175	4	0.000	2.480	0.4050	40	400
1.2	0.9000	1.0000	1.3140	1.1071	1.5208	18.175	4	0.000	2.480	0.4050	40	400
3.7	0.9500	1.0556	1.4174	1.1071	1.5208	14.591	4	-0.633	2.480	0.4050	20	400
11	0.9500	1.0556	1.4174	1.1071	1.5208	14.591	4	-0.633	2.480	0.4050	20	400
33	0.9000	1.0000	1.3140	1.1071	1.5208	18.175	4	0.000	2.480	0.4050	40	400
67	0.8500	0.9444	1.2106	1.1071	1.5208	17.084	4	0.633	2.480	0.4050	60	400
100	0.8000	0.8889	1.1553	0.8861	1.5208	22.939	4	0.972	2.480	0.4050	80	400

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)	0.892625	0.93	0.010463	-1.4491
Bartlett's Test indicates equal variances (p = 1.00)	0.316646	18.47531		
The control means are not significantly different (p = 1.00)	0	2.446912		

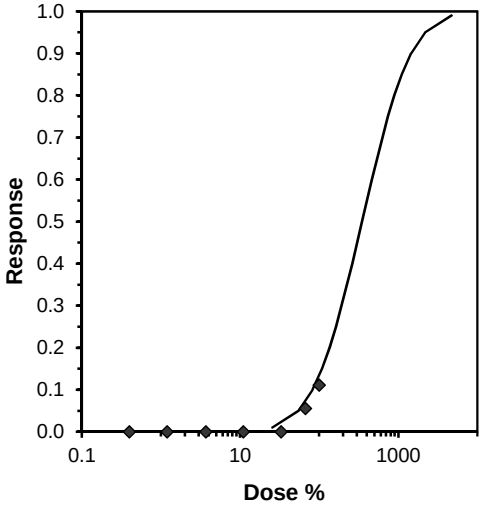
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	100	>100		1	0.313144	0.334744	0.0325	0.053333	0.742502	7, 24
Treatments vs FSW Control										

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	2.052299	1.193426	-1.0155	5.120097	0.1	14.5561	11.0705	1.0E-02	2.540943	0.487258	19
Intercept	-0.21478	2.296293	-6.11759	5.688035							
TSCR	0.080553	0.010569	0.053384	0.107723							

Point	Probits	%	95% Fiducial Limits
EC01	2.674	25.55116	
EC05	3.355	54.88763	
EC10	3.718	82.50808	
EC15	3.964	108.6256	
EC20	4.158	135.1625	
EC25	4.326	163.0394	
EC40	4.747	261.5157	
EC50	5.000	347.4908	
EC60	5.253	461.7308	
EC75	5.674	740.6177	
EC80	5.842	893.3679	
EC85	6.036	1111.615	
EC90	6.282	1463.491	
EC95	6.645	2199.946	
EC99	7.326	4725.808	

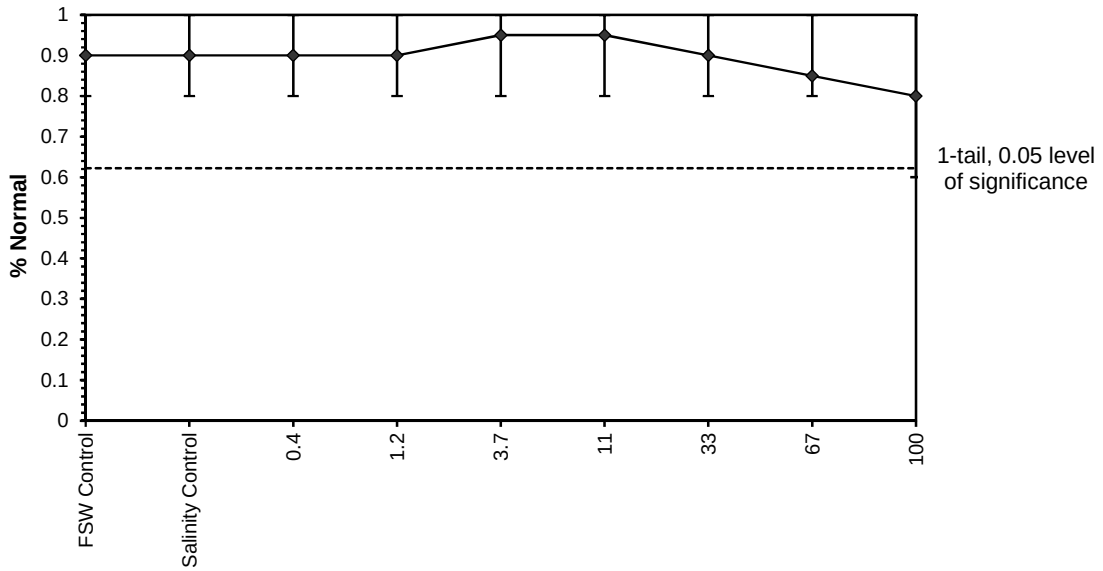
Significant heterogeneity detected (p = 1.00E-02)



Anemone Larval Toxicity Test-% Normal

Start Date:	26/07/2017 20:30	Test ID:	PR1502/04	Sample ID:	Drum 1 Liquor
End Date:	3/08/2017 19:30	Lab ID:	8230	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 128	Test Species:	AI-Aiptasia pulchella
Comments:					

Dose-Response Plot



Anemone Larval Toxicity Test-% Normal

Start Date:	26/07/2017 20:30	Test ID:	PR1502/04	Sample ID:	Drum 1 Liquor
End Date:	3/08/2017 19:30	Lab ID:	8230	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 128	Test Species:	AI-Aiptasia pulchella
Comments:					

Auxiliary Data Summary

Conc-%	Parameter	Mean	Min	Max	SD	CV%	N
FSW Control	% unaffacted	90.00	80.00	100.00	11.55	3.78	4
Salinity Control		90.00	80.00	100.00	11.55	3.78	4
0.4		90.00	80.00	100.00	11.55	3.78	4
1.2		90.00	80.00	100.00	11.55	3.78	4
3.7		95.00	80.00	100.00	10.00	3.33	4
11		95.00	80.00	100.00	10.00	3.33	4
33		90.00	80.00	100.00	11.55	3.78	4
67		85.00	80.00	100.00	10.00	3.72	4
100		80.00	60.00	100.00	16.33	5.05	4
FSW Control	pH	8.10	8.10	8.10	0.00	0.00	1
Salinity Control		8.10	8.10	8.10	0.00	0.00	1
0.4		8.10	8.10	8.10	0.00	0.00	1
1.2		8.10	8.10	8.10	0.00	0.00	1
3.7		8.10	8.10	8.10	0.00	0.00	1
11		8.10	8.10	8.10	0.00	0.00	1
33		8.10	8.10	8.10	0.00	0.00	1
67		7.90	7.90	7.90	0.00	0.00	1
100		7.60	7.60	7.60	0.00	0.00	1
FSW Control	DO, %	35.60	35.60	35.60	0.00	0.00	1
Salinity Control		29.30	29.30	29.30	0.00	0.00	1
0.4		35.50	35.50	35.50	0.00	0.00	1
1.2		35.50	35.50	35.50	0.00	0.00	1
3.7		35.10	35.10	35.10	0.00	0.00	1
11		34.70	34.70	34.70	0.00	0.00	1
33		33.90	33.90	33.90	0.00	0.00	1
67		31.90	31.90	31.90	0.00	0.00	1
100		29.60	29.60	29.60	0.00	0.00	1
FSW Control	Salinity ppt	105.20	105.20	105.20	0.00	0.00	1
Salinity Control		109.10	109.10	109.10	0.00	0.00	1
0.4		106.30	106.30	106.30	0.00	0.00	1
1.2		107.60	107.60	107.60	0.00	0.00	1
3.7		108.40	108.40	108.40	0.00	0.00	1
11		105.30	105.30	105.30	0.00	0.00	1
33		104.90	104.90	104.90	0.00	0.00	1
67		103.60	103.60	103.60	0.00	0.00	1
100		104.20	104.20	104.20	0.00	0.00	1

Anemone Larval Toxicity Test-% Normal

Start Date:	26/07/2017 20:30	Test ID:	PR1502/03	Sample ID:	Drum 2 Liquor
End Date:	3/08/2017 19:30	Lab ID:	8231	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 128	Test Species:	AI-Aiptasia pulchella
Comments:					

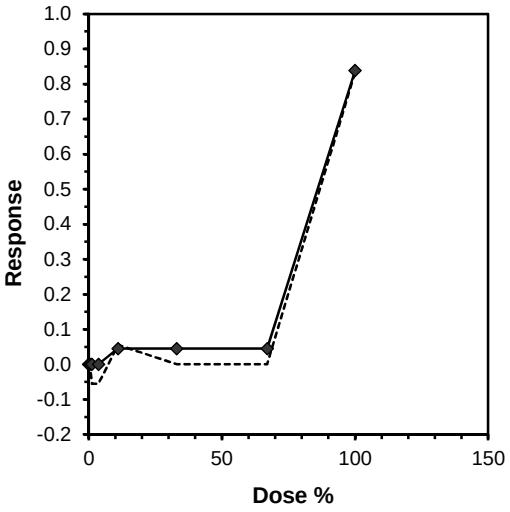
Conc-%	1	2	3	4
FSW Control	0.8000	0.8000	1.0000	1.0000
Salinity Control	0.8000	0.8000	1.0000	1.0000
0.4	1.0000	0.8000	1.0000	0.8000
1.2	0.8000	1.0000	1.0000	1.0000
3.7	0.8000	1.0000	1.0000	1.0000
11	0.8000	0.8000	1.0000	0.8000
33	0.8000	1.0000	0.8000	1.0000
67	1.0000	0.8000	1.0000	0.8000
100	0.4000	0.0000	0.0000	0.2000

			Transform: Arcsin Square Root					Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Mean	N-Mean
FSW Control	0.9000	1.0000	1.3140	1.1071	1.5208	18.175	4	0.9250	1.0000
Salinity Control	0.9000	1.0000	1.3140	1.1071	1.5208	18.175	4		
0.4	0.9000	1.0000	1.3140	1.1071	1.5208	18.175	4	0.9250	1.0000
1.2	0.9500	1.0556	1.4174	1.1071	1.5208	14.591	4	0.9250	1.0000
3.7	0.9500	1.0556	1.4174	1.1071	1.5208	14.591	4	0.9250	1.0000
11	0.8500	0.9444	1.2106	1.1071	1.5208	17.084	4	0.8833	0.9550
33	0.9000	1.0000	1.3140	1.1071	1.5208	18.175	4	0.8833	0.9550
67	0.9000	1.0000	1.3140	1.1071	1.5208	18.175	4	0.8833	0.9550
100	0.1500	0.1667	0.3121	0.0500	0.6847	101.184	4	0.1500	0.1622

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)	0.871884	0.93	-0.02717	-1.55923
Bartlett's Test indicates equal variances (p = 1.00)	0.807681	18.47531		
The control means are not significantly different (p = 1.00)	0	2.446912		

Linear Interpolation (200 Resamples)

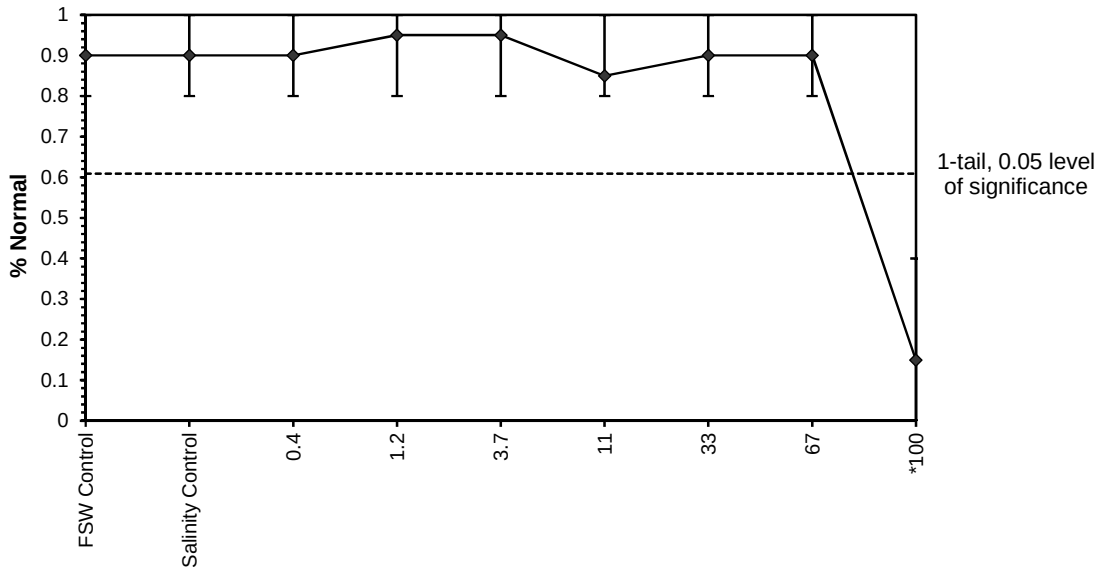
Point	%	SD	95% CL(Exp)		Skew
IC05	67.206	28.641	0.000	70.278	0.3746
IC10	69.288	17.738	0.000	72.432	-2.3622
IC15	71.369	3.018	60.265	74.644	-4.0371
IC20	73.450	1.802	66.368	77.533	-0.3472
IC25	75.531	1.826	68.269	80.055	-0.0456
IC40	81.775	2.140	75.516	88.513	0.6233
IC50	85.938	2.496	79.202	94.156	0.8142



Anemone Larval Toxicity Test-% Normal

Start Date:	26/07/2017 20:30	Test ID:	PR1502/03	Sample ID:	Drum 2 Liquor
End Date:	3/08/2017 19:30	Lab ID:	8231	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 128	Test Species:	AI-Aiptasia pulchella
Comments:					

Dose-Response Plot



Anemone Larval Toxicity Test-% Normal

Start Date:	26/07/2017 20:30	Test ID:	PR1502/03	Sample ID:	Drum 2 Liquor
End Date:	3/08/2017 19:30	Lab ID:	8231	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 128	Test Species:	AI-Aiptasia pulchella
Comments:					

Auxiliary Data Summary

Conc-%	Parameter	Mean	Min	Max	SD	CV%	N
FSW Control	% unaffacted	90.00	80.00	100.00	11.55	3.78	4
Salinity Control		90.00	80.00	100.00	11.55	3.78	4
0.4		90.00	80.00	100.00	11.55	3.78	4
1.2		95.00	80.00	100.00	10.00	3.33	4
3.7		95.00	80.00	100.00	10.00	3.33	4
11		85.00	80.00	100.00	10.00	3.72	4
33		90.00	80.00	100.00	11.55	3.78	4
67		90.00	80.00	100.00	11.55	3.78	4
100		15.00	0.00	40.00	19.15	29.17	4
FSW Control	pH	8.10	8.10	8.10	0.00	0.00	1
Salinity Control		8.10	8.10	8.10	0.00	0.00	1
0.4		8.10	8.10	8.10	0.00	0.00	1
1.2		8.10	8.10	8.10	0.00	0.00	1
3.7		8.10	8.10	8.10	0.00	0.00	1
11		8.10	8.10	8.10	0.00	0.00	1
33		8.10	8.10	8.10	0.00	0.00	1
67		7.90	7.90	7.90	0.00	0.00	1
100		7.70	7.70	7.70	0.00	0.00	1
FSW Control	DO, %	35.60	35.60	35.60	0.00	0.00	1
Salinity Control		29.30	29.30	29.30	0.00	0.00	1
0.4		35.50	35.50	35.50	0.00	0.00	1
1.2		35.50	35.50	35.50	0.00	0.00	1
3.7		35.10	35.10	35.10	0.00	0.00	1
11		34.50	34.50	34.50	0.00	0.00	1
33		33.80	33.80	33.80	0.00	0.00	1
67		31.60	31.60	31.60	0.00	0.00	1
100		29.20	29.20	29.20	0.00	0.00	1
FSW Control	Salinity ppt	105.20	105.20	105.20	0.00	0.00	1
Salinity Control		109.10	109.10	109.10	0.00	0.00	1
0.4		104.10	104.10	104.10	0.00	0.00	1
1.2		106.30	106.30	106.30	0.00	0.00	1
3.7		105.70	105.70	105.70	0.00	0.00	1
11		109.20	109.20	109.20	0.00	0.00	1
33		108.30	108.30	108.30	0.00	0.00	1
67		107.60	107.60	107.60	0.00	0.00	1
100		107.90	107.90	107.90	0.00	0.00	1

Anemone Larval Toxicity Test-% Normal

Start Date:	26/07/2017 20:30	Test ID:	PR1502/03	Sample ID:	Drum 2 Liquor
End Date:	3/08/2017 19:30	Lab ID:	8231	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 128	Test Species:	AI-Aiptasia pulchella
Comments:					

Conc-%	1	2	3	4
FSW Control	0.8000	0.8000	1.0000	1.0000
Salinity Control	0.8000	0.8000	1.0000	1.0000
0.4	1.0000	0.8000	1.0000	0.8000
1.2	0.8000	1.0000	1.0000	1.0000
3.7	0.8000	1.0000	1.0000	1.0000
11	0.8000	0.8000	1.0000	0.8000
33	0.8000	1.0000	0.8000	1.0000
67	1.0000	0.8000	1.0000	0.8000
100	0.4000	0.0000	0.0000	0.2000

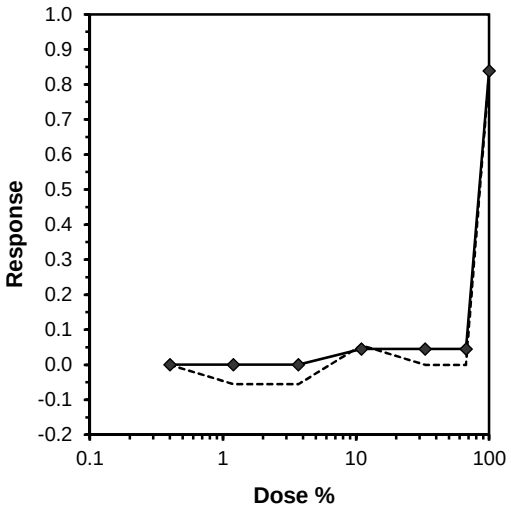
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
FSW Control	0.9000	1.0000	1.3140	1.1071	1.5208	18.175	4	*			40	400
Salinity Control	0.9000	1.0000	1.3140	1.1071	1.5208	18.175	4					
0.4	0.9000	1.0000	1.3140	1.1071	1.5208	18.175	4	0.000	2.480	0.4187	40	400
1.2	0.9500	1.0556	1.4174	1.1071	1.5208	14.591	4	-0.612	2.480	0.4187	20	400
3.7	0.9500	1.0556	1.4174	1.1071	1.5208	14.591	4	-0.612	2.480	0.4187	20	400
11	0.8500	0.9444	1.2106	1.1071	1.5208	17.084	4	0.612	2.480	0.4187	60	400
33	0.9000	1.0000	1.3140	1.1071	1.5208	18.175	4	0.000	2.480	0.4187	40	400
67	0.9000	1.0000	1.3140	1.1071	1.5208	18.175	4	0.000	2.480	0.4187	40	400
*100	0.1500	0.1667	0.3121	0.0500	0.6847	101.184	4	5.933	2.480	0.4187	340	400

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.05)	0.871884	0.93	-0.02717	-1.55923
Bartlett's Test indicates equal variances (p = 1.00)	0.807681	18.47531		
The control means are not significantly different (p = 1.00)	0	2.446912		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	67	100	81.85353	1.492537	0.326536	0.349059	0.534229	0.05702	1.4E-05	7, 24
Treatments vs FSW Control										

Trimmed Spearman-Karber

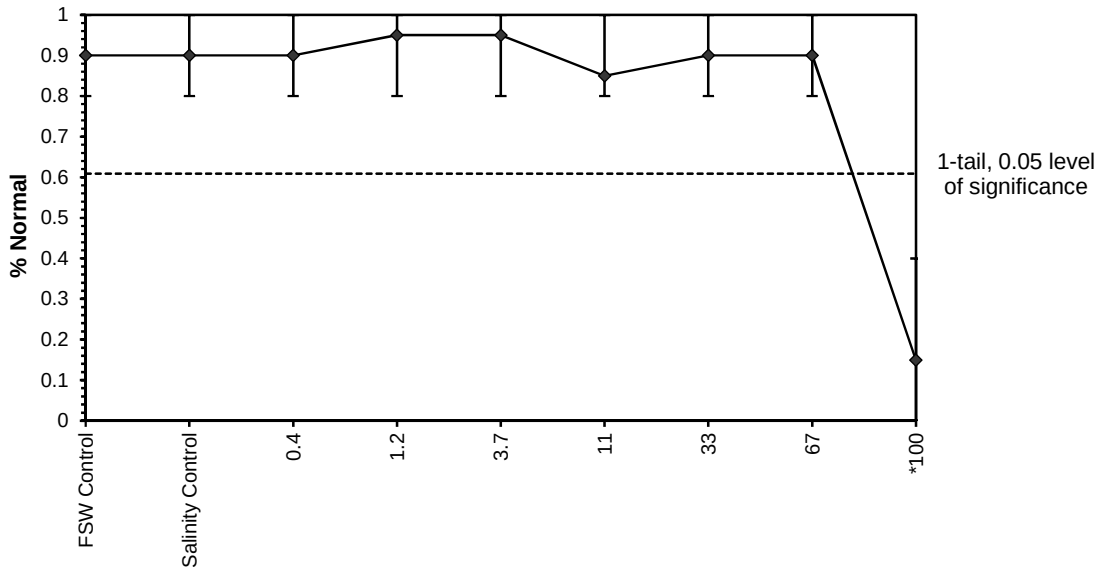
Trim Level	EC50	95% CL	
0.0%			
5.0%			
10.0%			
20.0%	84.311	83.340	85.293
Auto-16.2%	84.311	83.340	85.293



Anemone Larval Toxicity Test-% Normal

Start Date:	26/07/2017 20:30	Test ID:	PR1502/03	Sample ID:	Drum 2 Liquor
End Date:	3/08/2017 19:30	Lab ID:	8231	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 128	Test Species:	AI-Aiptasia pulchella
Comments:					

Dose-Response Plot



Anemone Larval Toxicity Test-% Normal

Start Date:	26/07/2017 20:30	Test ID:	PR1502/03	Sample ID:	Drum 2 Liquor
End Date:	3/08/2017 19:30	Lab ID:	8231	Sample Type:	AQ-Aqueous
Sample Date:		Protocol:	ESA 128	Test Species:	AI-Aiptasia pulchella
Comments:					

Auxiliary Data Summary

Conc-%	Parameter	Mean	Min	Max	SD	CV%	N
FSW Control	% unaffacted	90.00	80.00	100.00	11.55	3.78	4
Salinity Control		90.00	80.00	100.00	11.55	3.78	4
0.4		90.00	80.00	100.00	11.55	3.78	4
1.2		95.00	80.00	100.00	10.00	3.33	4
3.7		95.00	80.00	100.00	10.00	3.33	4
11		85.00	80.00	100.00	10.00	3.72	4
33		90.00	80.00	100.00	11.55	3.78	4
67		90.00	80.00	100.00	11.55	3.78	4
100		15.00	0.00	40.00	19.15	29.17	4
FSW Control	pH	8.10	8.10	8.10	0.00	0.00	1
Salinity Control		8.10	8.10	8.10	0.00	0.00	1
0.4		8.10	8.10	8.10	0.00	0.00	1
1.2		8.10	8.10	8.10	0.00	0.00	1
3.7		8.10	8.10	8.10	0.00	0.00	1
11		8.10	8.10	8.10	0.00	0.00	1
33		8.10	8.10	8.10	0.00	0.00	1
67		7.90	7.90	7.90	0.00	0.00	1
100		7.70	7.70	7.70	0.00	0.00	1
FSW Control	DO, %	35.60	35.60	35.60	0.00	0.00	1
Salinity Control		29.30	29.30	29.30	0.00	0.00	1
0.4		35.50	35.50	35.50	0.00	0.00	1
1.2		35.50	35.50	35.50	0.00	0.00	1
3.7		35.10	35.10	35.10	0.00	0.00	1
11		34.50	34.50	34.50	0.00	0.00	1
33		33.80	33.80	33.80	0.00	0.00	1
67		31.60	31.60	31.60	0.00	0.00	1
100		29.20	29.20	29.20	0.00	0.00	1
FSW Control	Salinity ppt	105.20	105.20	105.20	0.00	0.00	1
Salinity Control		109.10	109.10	109.10	0.00	0.00	1
0.4		104.10	104.10	104.10	0.00	0.00	1
1.2		106.30	106.30	106.30	0.00	0.00	1
3.7		105.70	105.70	105.70	0.00	0.00	1
11		109.20	109.20	109.20	0.00	0.00	1
33		108.30	108.30	108.30	0.00	0.00	1
67		107.60	107.60	107.60	0.00	0.00	1
100		107.90	107.90	107.90	0.00	0.00	1

ECOTOXICOLOGY LABORATORY
TEST REPORT

CLIENT

Monique Binet
Centre for Environmental Contaminants Research (CECR)
CSIRO Land and Water
Locked Bag 2007, Kirrawee NSW 2232, Australia

JOB INFORMATION

JOB REFERENCE	:	ECX17-0713 v2
NO. SAMPLES	:	2
CLIENT ORDER NO.	:	R-09224-01
SAMPLED BY	:	David Spadaro
DATE RECEIVED	:	13/07/17
DATE REPORTED	:	28/8/17

REPORT NOTES

Two mine tailing samples were tested for toxicity testing with the sea urchin larval development bioassay and the fish larval development bioassay.

TESTED BY

Intertek Ecotoxicology Laboratory
1 Fleet Street (FF19, Block F)
Fremantle, Western Australia 6160
Tel +61 8 9263 0100
Tristan.Stringer@Intertek.com

COMPANY APPROVED SIGNATORY

DR TRISTAN STRINGER
PRINCIPAL ECOTOXICOLOGIST

Sample Information

SAMPLE REFERENCE	INTERTEK REFERENCE
Drum 1 Liquor	ECX17-0713-1

Methodology

The mine liquor sample "Drum 1" was tested with the sea urchin larval development bioassay and the fish larval development bioassay. Water samples were taken (following the instructions provided by CSIRO) at test initiation and termination and returned to CSIRO for chemical analysis.

For quality assurance purposes, a reference toxicant bioassay was tested simultaneously. Statistically calculated effect concentrations (EC_{10}, EC_{50}, NOEC and LOEC) have been reported the samples and reference toxicants.

Bioassay Details

BIOASSAY	PROTOCOL	REFERENCE	TEST SPECIES	TEMPERATURE
Sea Urchin Development *	WIECX-25	ASTM E1563	*E. mathaei*	25°C
Fish Larvae Development*	WIECX-16	USEPA 1004.0	*S. lalandi*	22°C

* NATA Accredited method – Accreditation Number 5646

Physicochemistry

PARAMETER	CONTROL	100%	50%	25%	12.5%	6.3%	3.1%
pH	8.15	7.43	7.91	8.01	8.05	8.07	8.07
Salinity (‰)	34.1	28.3	31.4	32.9	33.6	33.9	34.1
DO (%)	100	109	104	100	100	100	100

PARAMETER	100% PHYS. CHEM. MATCH
pH	7.72
Salinity (‰)	27.8
DO (%)	100

Concentration-Response Data

Concentration (% Sample)	Sea Urchin Development (% Control)	Fish Larvae Development (% Control)
Control	100 ± 1	100 ± 15
3.1	101 ± 3	98 ± 12
6.3	100 ± 1	96 ± 11
12.5	100 ± 2	82 ± 4
25	89 ± 4	73 ± 11
50	5 ± 1	42 ± 12
100	-	0 ± 0
100 Phys Chem	-	94 ± 15

Statistical Effects Data

BIOASSAY	EC_{10} (%)	EC_{50} (%)	NOEC (%)	LOEC (%)
Sea Urchin Development *	25.0	33.9	12.5	25.0
Fish Larvae Development*	19.45	40.5	12.5	25.0

* NATA Accredited method – Accreditation Number 5646

Quality Assurance Limits

BIOASSAY	REFERENCE TOXICANT	OBSERVED EC_{50}	CUSUM CHART LIMITS	COEFFICIENT OF VARIANCE	CONTROL RESPONSE	TEST ACCEPTABILITY
Sea Urchin Development *	Copper	31.3 µg/L	12.4 - 32.0 µg/L	24.0%	>70% Development	YES
Fish Larvae Development*	Copper	62.8 µg/L	38.6 – 74.9 µg/L	33.4%	>70% Development	YES

* NATA Accredited method – Accreditation Number 5646

General Comments

Quality assurance and quality control criteria were within acceptable limits for all bioassays.

Sample Information

SAMPLE REFERENCE	INTERTEK REFERENCE
Drum 2 Liquor	ECX17-0713-1

Methodology

The mine liquor sample "Drum 2" was tested with the sea urchin larval development bioassay and the fish larval development bioassay. Water samples were taken (following the instructions provided by CSIRO) at test initiation and termination and returned to CSIRO for chemical analysis.

For quality assurance purposes, a reference toxicant bioassay was tested simultaneously. Statistically calculated effect concentrations (EC_{10}, EC_{50}, NOEC and LOEC) have been reported the samples and reference toxicants.

Bioassay Details

BIOASSAY	PROTOCOL	REFERENCE	TEST SPECIES	TEMPERATURE
Sea Urchin Development *	WIECX-25	ASTM E1563	*E. mathaei*	25°C
Fish Larvae Development*	WIECX-16	USEPA 1004.0	*S. lalandi*	22°C

* NATA Accredited method – Accreditation Number 5646

Physicochemistry

PARAMETER	CONTROL	100%	50%	25%	12.5%	6.3%	3.1%
pH	8.15	7.69	7.97	8.03	8.07	8.07	8.08
Salinity (‰)	34.1	27.4	32.3	32.7	33.4	33.8	34.0
DO (%)	100	110	100	100	100	99	99

PARAMETER	100% PHYS. CHEM. MATCH
pH	7.72
Salinity (‰)	27.8
DO (%)	100

Concentration-Response Data

Concentration (% Sample)	Sea Urchin Development (% Control)	Fish Larvae Development (% Control)
Control	100 ± 1	100 ± 15
1.6	99 ± 4	-
3.1	98 ± 5	91 ± 11
6.3	97 ± 4	88 ± 15
12.5	79 ± 1	84 ± 7
25	0 ± 0	61 ± 16
50	-	21 ± 17
100	-	0 ± 0
100 Phys Chem	-	94 ± 15

Statistical Effects Data

BIOASSAY	EC_{10} (%)	EC_{50} (%)	NOEC (%)	LOEC (%)
Sea Urchin Development *	11.6	14.4	6.3	12.5
Fish Larvae Development*	16.1	31.9	12.5	25.0

* NATA Accredited method – Accreditation Number 5646

Quality Assurance Limits

BIOASSAY	REFERENCE TOXICANT	OBSERVED EC_{50}	CUSUM CHART LIMITS	COEFFICIENT OF VARIANCE	CONTROL RESPONSE	TEST ACCEPTABILITY
Sea Urchin Development *	Copper	31.3 µg/L	12.4 – 32.0 µg/L	24.0%	>70% Development	YES
Fish Larvae Development*	Copper	62.8 µg/L	38.6 – 74.9 µg/L	33.4%	>70% Development	YES

* NATA Accredited method – Accreditation Number 5646

General Comments

Quality assurance and quality control criteria were within acceptable limits for all bioassays.

APPENDIX - A

Drum 1 (ECX17-0713-1)

Statistical Data

Sea Urchin Larval Development Bioassay

-Proportion Normal

Start Date:	14/7/17	Test ID:	ECX17-0713	Sample ID:	CSIRO
End Date:	17/7/17	Lab ID:	FREO-Geotech Fremantle La	Sample Type:	Drum 1
Sample Date:		Protocol:	WIECX-25	Test Species:	E. MATHAEI
Comments:					

Conc-%	1	2	3
B-Control	0.9900	0.9900	1.0000
0.8	0.9700	0.9400	1.0000
1.6	0.9900	1.0000	0.9900
3.1	1.0000	1.0000	0.9700
6.3	1.0000	1.0000	0.9800
12.5	1.0000	0.9900	1.0000
25	0.9200	0.9000	0.8500
50	0.0600	0.0500	0.0400

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
B-Control	0.9933	1.0000	1.4873	1.4706	1.5208	1.947	3				2	300
0.8	0.9700	0.9765	1.4136	1.3233	1.5208	7.060	3	1.643	2.560	0.1149	9	300
1.6	0.9933	1.0000	1.4873	1.4706	1.5208	1.947	3	0.000	2.560	0.1149	2	300
3.1	0.9900	0.9966	1.4794	1.3967	1.5208	4.842	3	0.177	2.560	0.1149	3	300
6.3	0.9933	1.0000	1.4902	1.4289	1.5208	3.560	3	-0.063	2.560	0.1149	2	300
12.5	0.9967	1.0034	1.5041	1.4706	1.5208	1.925	3	-0.372	2.560	0.1149	1	300
*25	0.8900	0.8960	1.2354	1.1731	1.2840	4.591	3	5.614	2.560	0.1149	33	300
*50	0.0500	0.0503	0.2248	0.2014	0.2475	10.260	3	28.134	2.560	0.1149	285	300

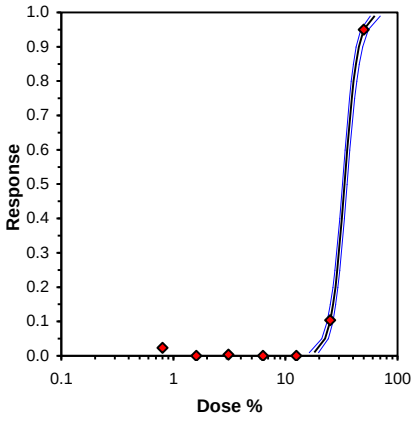
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95833	0.884	-0.1003	0.30286
Bartlett's Test indicates equal variances (p = 0.48)	6.51559	18.4753		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	12.5	25	17.6777	8	0.03188	0.0321	0.57969	0.00302	3.2E-14	7, 16

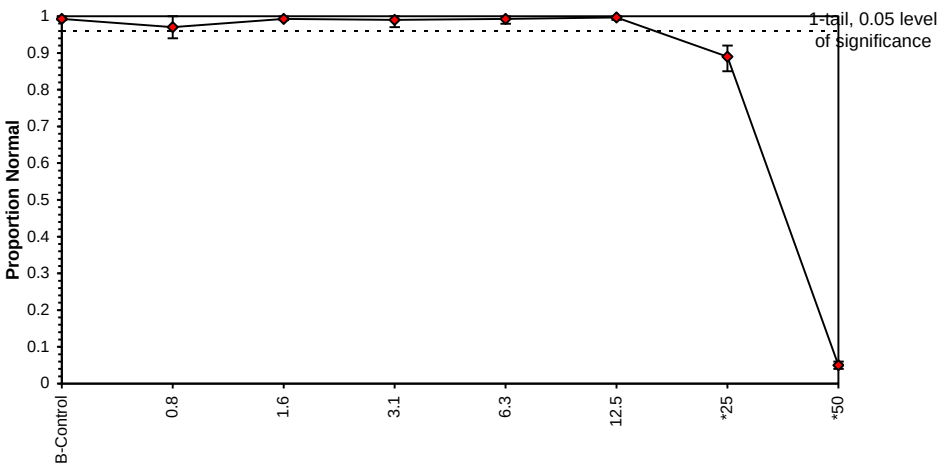
Maximum Likelihood-Logit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	17.0856	0.9127	14.7394	19.4317	0.00667	13.4392	11.0705	0.02			14
Intercept	-26.087	1.38326	-29.642	-22.531							
TSCR	0.01054	0.00397	0.00032	0.02076							

Point	Logits	%	95% Fiducial Limits	
EC01	-4.595	18.1079	16.3072	19.6638
EC05	-2.944	22.6193	20.9804	24.0531
EC10	-2.197	25.0157	23.4711	26.3998
EC15	-1.735	26.625	25.1338	27.9944
EC20	-1.386	27.9046	26.4449	29.2781
EC25	-1.099	29.0077	27.5646	30.3987
EC40	-0.405	31.8481	30.3906	33.3553
EC50	0.000	33.6368	32.1226	35.2751
EC60	0.405	35.526	33.9118	37.3511
EC75	1.099	39.0045	37.1113	41.2901
EC80	1.386	40.5464	38.4968	43.0775
EC85	1.735	42.4951	40.2246	45.3669
EC90	2.197	45.2289	42.6122	48.6285
EC95	2.944	50.0206	46.7177	54.4617
EC99	4.595	62.483	57.0744	70.1569



Dose-Response Plot



Fish Larval Development Bioassay

-Proportion Normal

Start Date:	13/08/2017	Test ID:	ECX17-0713	Sample ID:	CSIRO
End Date:	21/08/2017	Lab ID:	FREO-Geotech Fremantle La	Sample Type:	Drum 1
Sample Date:		Protocol:	WIECX-16	Test Species:	S. lalandi
Comments:					

Conc-%	1	2	3	4
B-Control	0.7719	1.0000	0.9825	1.0000
3.1	0.9123	1.0000	0.9123	
6.3	0.9825	1.0000	0.8421	
12.5	0.8421	0.8421	0.7719	
25	0.7018	0.8421	0.6316	
50	0.4912	0.4912	0.2807	
100	0.0000	0.0000	0.0000	

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
B-Control	0.9386	1.0000	1.3881	1.0729	1.5208	15.397	4				25	400
3.1	0.9415	1.0031	1.3537	1.2701	1.5208	10.691	3	0.288	2.650	0.3164	18	300
6.3	0.9415	1.0031	1.3736	1.1622	1.5208	13.669	3	0.121	2.650	0.3164	18	300
12.5	0.8187	0.8723	1.1324	1.0729	1.1622	4.550	3	2.142	2.650	0.3164	55	300
*25	0.7251	0.7726	1.0246	0.9185	1.1622	12.183	3	3.045	2.650	0.3164	83	300
*50	0.4211	0.4486	0.7039	0.5584	0.7766	17.901	3	5.731	2.650	0.3164	174	300
100	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	3				300	300

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.93021	0.863	-0.7975	0.13029
Bartlett's Test indicates equal variances (p = 0.65)	3.29443	15.0863		

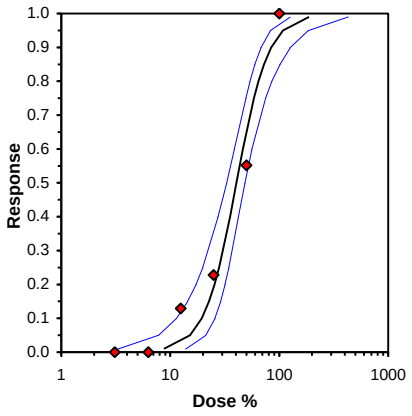
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	12.5	25	17.6777	8	0.1961	0.20279	0.22699	0.02444	6.1E-04	5, 13

Maximum Likelihood-Logit

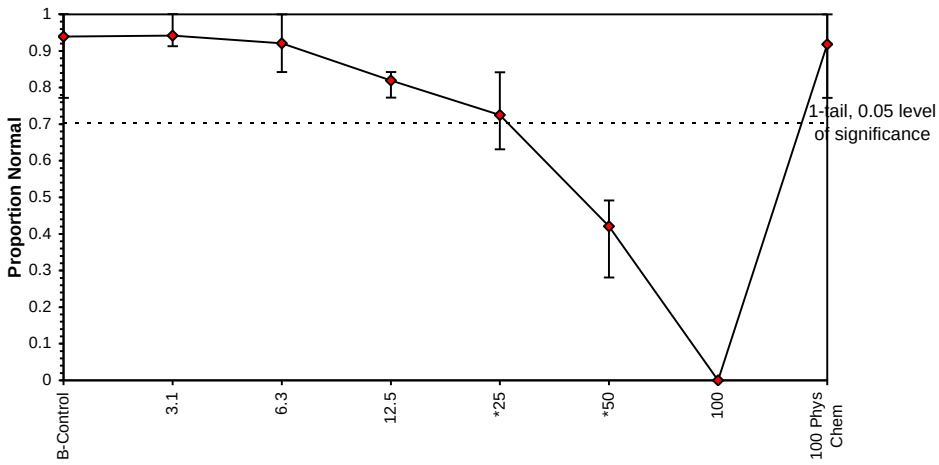
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	6.8963	0.89772	4.40383	9.38878	0.0625	56.1115	9.48773	1.9E-11			20
Intercept	-11.087	1.48736	-15.217	-6.9577							
TSCR	0.07797	0.03074	-0.0074	0.16332							

Point	Logits	%	95% Fiducial Limits	
EC01	-4.595	8.73755	3.32865	13.9174
EC05	-2.944	15.1617	7.80191	21.0994
EC10	-2.197	19.458	11.4252	25.578
EC15	-1.735	22.7081	14.4304	28.892
EC20	-1.386	25.5086	17.1669	31.7361
EC25	-1.099	28.0803	19.7738	34.3654
EC40	-0.405	35.3925	27.4498	42.1595
EC50	0.000	40.5234	32.8062	48.1656
EC60	0.405	46.3982	38.623	55.8604
EC75	1.099	58.4805	49.1601	74.7413
EC80	1.386	64.3763	53.7294	85.2959
EC85	1.735	72.3157	59.4875	100.671
EC90	2.197	84.3947	67.6533	126.289
EC95	2.944	108.309	82.5158	183.814
EC99	4.595	187.942	125.766	428.544

Significant heterogeneity detected (p = 1.90E-11)



Dose-Response Plot



APPENDIX - B

Drum 2 (ECX17-0713-2)

Statistical Data

Sea Urchin Larval Development Bioassay

-Proportion Normal

Start Date:	14/7/17	Test ID:	ECX17-0713	Sample ID:	CSIRO
End Date:	17/7/17	Lab ID:	ITRO-Cedrach Fremantle La	Sample Type:	Drum 2
Sample Date:		Protocol:	WIRCX 25	Test Species:	E. MATHAEI
Comments:					

Conc-%	1	2	3
B-Control	0.9920	0.9936	1.0000
0.8	1.0000	1.0000	0.9985
1.6	1.0000	0.9970	0.9454
3.1	1.0000	0.9853	0.9293
6.3	1.0000	0.9548	0.9401
12.5	0.8018	0.7856	0.7828
25	0.0100	0.0000	0.0000

			Transform: Arcsin Square Root					1-Tailed			Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
B-Control	0.9952	1.0000	1.4975	1.4811	1.5708	1.383	3				2	300
0.8	0.9995	1.0044	1.5250	1.5208	1.5333	0.475	3	-0.467	2.530	0.1499	0	300
1.6	0.9809	0.9856	1.4572	1.3349	1.5208	7.269	3	0.885	2.530	0.1499	5	300
3.1	0.9715	0.9762	1.4239	1.3016	1.5208	7.849	3	1.252	2.530	0.1499	8	300
6.3	0.9649	0.9696	1.4002	1.3236	1.5208	7.547	3	1.653	2.530	0.1499	11	300
*12.5	0.7901	0.7939	1.0649	1.0060	1.1085	1.157	3	6.841	2.530	0.1499	63	300
*25	0.0033	0.0033	0.0067	0.0000	0.1002	43.303	3	24.014	2.530	0.1499	299	300

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro Wilk's Test indicates normal distribution (p > 0.01)	0.9446	0.873	0.2556	0.65562
Bartlett's Test indicates equal variances (p = 0.01)	16.7809	18.0119		

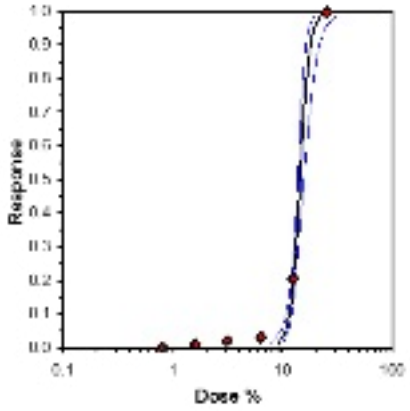
Hypothesis Test (1 tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	6.3	12.5	8.87412	15.873	0.04319	0.04342	0.02259	0.00519	4.8E-12	6, 14

Maximum Likelihood Logit

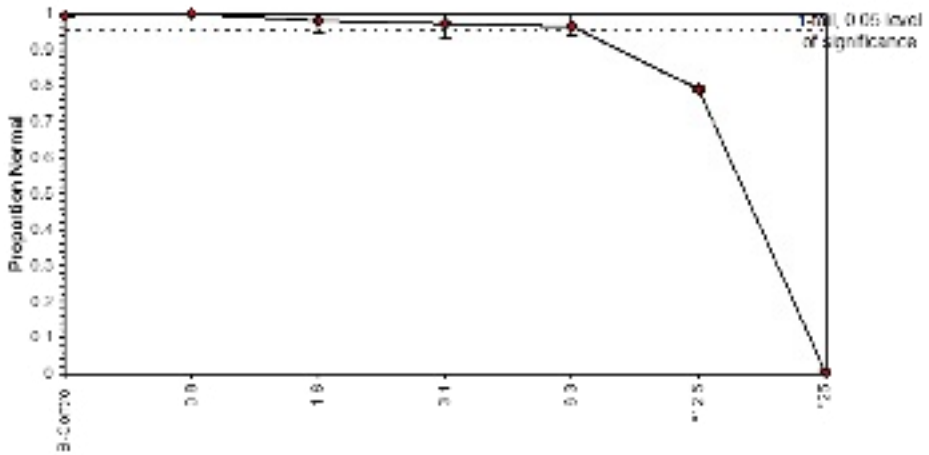
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	23.2099	2.91075	15.1020	31.4769	0.00887	13.2990	9.48773	9.9E-03			19
Intercept	-26.954	3.2599	-36.002	-17.906							
TSCR	0.01723	0.00814	0.00019	0.03427							

Point	Logits	%	95% Fiducial Limits	
EC01	-4.595	9.12036	7.57152	10.0032
EC05	-2.944	10.7371	9.66861	11.3448
EC10	-2.197	11.5603	10.79	12.0574
EC15	-1.735	12.1014	11.4892	12.5693
EC20	-1.386	12.5253	11.9997	13.0184
EC25	-1.099	12.8867	12.3867	13.4466
EC40	-0.405	13.8008	13.2539	14.7058
EC50	0.000	14.3852	13.717	15.5705
EC60	0.405	14.9696	14.1721	16.5141
EC75	1.099	16.0134	14.9662	18.2984
EC80	1.386	16.4751	15.2881	19.1025
EC85	1.735	17.0526	15.6909	20.1275
EC90	2.197	17.8507	16.2482	21.5797
EC95	2.944	19.2193	17.1784	24.1591
EC99	4.595	22.6248	19.4082	31.0309

Significant heterogeneity detected (p = 9.90E-03)



Dose-Response Plot



Fish Larval Development Bioassay

-Proportion Normal

Start Date:	13/08/2017	Test ID:	ECX17-0713	Sample ID:	CSIRO
End Date:	21/08/2017	Lab ID:	FREO-Geotech Fremantle La	Sample Type:	Drum 2
Sample Date:		Protocol:	WIECX-16	Test Species:	S. lalandi
Comments:					

Conc-%	1	2	3	4
B-Control	0.7719	1.0000	0.9825	1.0000
3.1	0.9123	0.7018	1.0000	
6.3	0.7719	0.9825		
12.5	0.9123	0.8421	0.7719	
25	0.4211	0.7018	0.7018	
50	0.0000	0.2807	0.3509	
100	0.0000	0.0000	0.0000	

			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
B-Control	0.9386	1.0000	1.3881	1.0729	1.5208	15.397	4				25	400
3.1	0.8713	0.9283	1.2613	0.9931	1.5208	20.927	3	0.731	2.681	0.4651	39	300
6.3	0.8772	0.9346	1.2554	1.0729	1.4380	20.560	2	0.674	2.681	0.5274	25	200
12.5	0.8421	0.8972	1.1684	1.0729	1.2701	8.452	3	1.266	2.681	0.4651	48	300
*25	0.6082	0.6480	0.8974	0.7061	0.9931	18.461	3	2.828	2.681	0.4651	118	300
*50	0.2105	0.2243	0.4141	0.0500	0.6340	76.687	3	5.614	2.681	0.4651	237	300
100	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	3				300	300

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.91641	0.858	-0.6481	-0.8023
Bartlett's Test indicates equal variances (p = 0.81)	2.27048	15.0863		

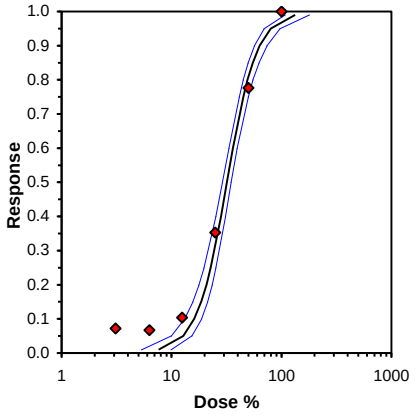
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	12.5	25	17.6777	8	0.33112	0.34243	0.39844	0.05159	0.00185	5, 12

Maximum Likelihood-Logit

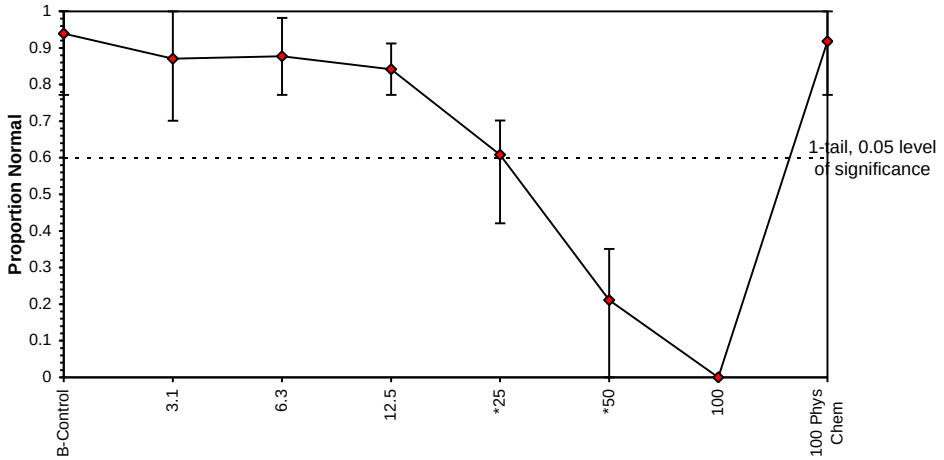
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	7.40541	0.48191	6.06742	8.74339	0.0625	14.0358	9.48773	7.2E-03			15
Intercept	-11.139	0.75344	-13.231	-9.0476							
TSCR	0.1015	0.01831	0.05066	0.15235							

Point	Logits	%	95% Fiducial Limits	
EC01	-4.595	7.65119	5.33485	9.86439
EC05	-2.944	12.7829	9.92532	15.3213
EC10	-2.197	16.1262	13.1206	18.7369
EC15	-1.735	18.621	15.5769	21.2486
EC20	-1.386	20.7509	17.7085	23.3814
EC25	-1.099	22.6926	19.6706	25.3252
EC40	-0.405	28.1504	25.2112	30.8528
EC50	0.000	31.9328	29.0015	34.8071
EC60	0.405	36.2235	33.1801	39.4831
EC75	1.099	44.9356	41.1716	49.6831
EC80	1.386	49.1403	44.8203	54.9097
EC85	1.735	54.7611	49.5417	62.1435
EC90	2.197	63.2329	56.4098	73.4801
EC95	2.944	79.7708	69.2454	96.7711
EC99	4.595	133.274	107.918	179.429

Significant heterogeneity detected (p = 7.18E-03)



Dose-Response Plot



Appendix D - Burrlioz species sensitivity distribution reports

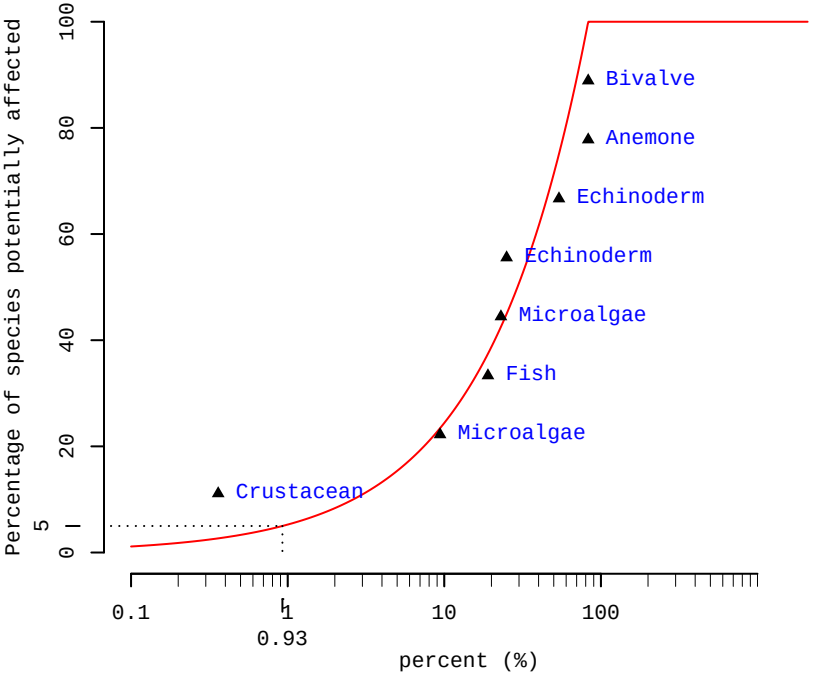
Burrlioz 2.0 report

Toxicant: Tailings 1 Liquor
Input file: C:\Users\ada128\Desktop\New SSDs Wafi\SSD data for Drum 1 tailings_new.csv
Time read: Thu Jan 18 21:24:55 2018
Units: percent (%)
Model: inverse.pareto

Protection level information

Protect. level	Guideline Value	lower 95% CI	upper 95% CI
99%	0.083	0.00031	12
95%	0.93	0.016	17
90%	2.6	0.085	22
80%	7.4	0.5	33

notes:



Data:

Concentration	TaxonomicGroup	CommonName	ScientificName	Duration	Toxicity	Endpoint	Temperature	Type	end
9.4	Microalgae	Microalga	Nitzschia closterium	72 h	Growth rate	IC10	27	Chronic	species
23	Microalgae	Microalga	Isochrysis galbana	72 h	Growth rate	IC10	27	Chronic	species
0.36	Crustacean	Copepod	Acartia sinjiensis	80 h	Development	EC10	30	Chronic	species
83	Anemone	Anemone	Aiptasia pulchella	8 d	Development	EC10	25	Chronic	species
54	Echinoderm	Sea Urchin	Heliocidaris tuberculata	72 h	Larval development	EC10	20	Chronic	species
25	Echinoderm	Sea Urchin	Echinometra mathaei	72 h	Larval development	EC10	25	Chronic	species
83	Bivalve	Oyster	Saccostrea echinata	48 h	Larval development	EC10	29	Chronic	species
19	Fish	Fish	Seriola lalandi	8 d	Imbalance	EC10	22	Chronic	species

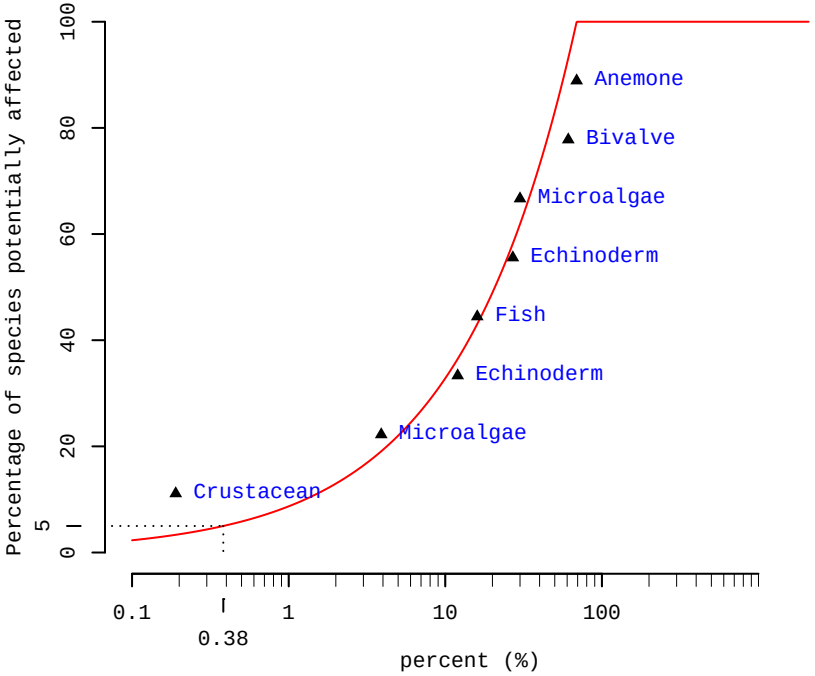
Burrlioz 2.0 report

Toxicant: Tailings 2 Liquor
Input file: C:\Users\ada128\Desktop\New SSDs Wafi\SSD data for Drum 2 tailings_new.csv
Time read: Thu Jan 18 21:27:50 2018
Units: percent (%)
Model: inverse.pareto

Protection level information

Protect. level	Guideline Value	lower 95% CI	upper 95% CI
99%	0.023	0.00012	4.4
95%	0.38	0.0082	9.2
90%	1.3	0.05	14
80%	4.2	0.34	22

notes:



Data:

Concentration	TaxonomicGroup	CommonName	ScientificName	Duration	Toxicity	Endpoint	Temperature	Type	end
3.9	Microalgae	Microalga	Nitzschia closterium	72 h	Growth rate	IC10	27	Chronic	species
30	Microalgae	Microalga	Isochrysis galbana	72 h	Growth rate	IC10	27	Chronic	species
0.19	Crustacean	Copepod	Acartia sinjiensis	80 h	Development	EC10	30	Chronic	species
69	Anemone	Anemone	Aiptasia pulchella	8 d	Development	EC10	25	Chronic	species
27	Echinoderm	Sea Urchin	Heliocidaris tuberculata	72 h	Larval development	EC10	20	Chronic	species
12	Echinoderm	Sea Urchin	Echinometra mathaei	72 h	Larval development	EC10	25	Chronic	species
61	Bivalve	Oyster	Saccostrea echinata	48 h	Larval development	EC10	29	Chronic	species
16	Fish	Fish	Seriola lalandi	8 d	Imbalance	EC10	22	Chronic	species

Appendix E - Test reports for the ecotoxicity and bioaccumulation of tailings solids

CSIRO LAND & WATER
www.csiro.au

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6807 • **ABN** 41 687 119 230

Date: 19/06/2017

Amphipod Reproduction Test Report AR17021

Client: GDA Consult Pty Ltd
Project: Wafi-Goldpu Pre-feasability study of DSTP
Test Performed: 10-day amphipod reproduction toxicity test (sublethal, chronic effects) using the amphipod *Melita plumulosa*

Test Initiated: 28/4/17

CSIRO Sample No.	Sample Name	Sample Description
	QA control	Silty control collected from Bonnet Bay (BB), NSW.
E17024	**Huon Gulf Sediment (HG)**	Deep sea sediment collected from the Huon Gulf
E17021	**Tailing Solid Drum 1 1%**	1% washed Drum 1 tailing solid homogenised with 99% HG
E17021	**Tailing Solid Drum 1 10%**	10% washed Drum 1 tailing solid homogenised with 90% HG
E17021	**Tailing Solid Drum 1 10% Layered**	4 g of 100% washed Drum 1 tailing solid layered on top of 36 g of HG
E17021	**Tailing Solid Drum 1 30%**	30% washed Drum 1 tailing solid homogenised with 70% HG
E17021	**Tailing Solid Drum 1 60%**	60% washed Drum 1 tailing solid homogenised with 40% HG
E17021	**Tailing Solid Drum 1 90%**	90% washed Drum 1 tailing solid homogenised with 10% HG

Test method: The amphipod reproduction bioassay measures adult survival and reproduction, expressed as the number of embryos and <1-d-old juveniles in the second brood following exposure of *Melita plumulosa* to test sediments over a 10-d period. The test was carried out according to the methods described in Simpson and Spadaro (2011). Amphipods used in the tests were isolated from laboratory cultures. Dilutions of the washed tailing solid were made up by diluting the tailing solids with the Huon Gulf (HG) control sediment. These sediments were homogenised immediately prior to being added to test vials (40 g sediment per 250 mL vial, 4 replicates per sediment). A 10% Layered treatment was tested using 4 g of undiluted tailing solid layered on top of 36 g of Huon Gulf control. Filtered seawater (200 mL, 30‰) was added and each beaker was incubated at 21°C with aeration overnight to allow sediments to settle. On the following day, 180 mL of overlying water was siphoned off and replaced with new seawater with care to minimise sediment resuspension. Six gravid females (gravid for <36 h) and six males (isolated from laboratory cultures) were randomly assigned to each beaker. Treatments are fed at a rate of 0.25 mg Sera Micron fish food/amphipod twice a week. The sediments are renewed after 5 d by gently sieving away the adults and placing them into the same fresh sediment that had been equilibrated overnight, thus allowing for the removal of juveniles from the first brood, which is typically unaffected by contaminants in the test sediment because they were already ''conceived'' before exposure to test sediments. On Day 10, the females were carefully removed and the number of embryos per female is counted by microscopy. The sediment was also checked for juvenile amphipods that had escaped the marsupium during the latter stages of the test by sieving the sediment through 180 µm mesh. The total number of embryos and <1-d-old juveniles was summed and expressed as a percentage of the HG control.

For quality assurance (QA) purposes, a minimum of 7 juveniles per female is required in the QA controls for tests to be considered acceptable. A sediment is considered to be acutely toxic if the survival as a

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6807 • **ABN** 41 687 119 230

percentage of the HG control is <80% and is statistically significantly less (P<0.05) than the HG controls. Chronic toxicity is detected when the reproductive output percent control (Huon Gulf) is <85%, (based on 2 standard deviations of control data n=80) and is statistically significant less (P<0.05) than the controls. Physico-chemical parameters (temperature, pH, salinity and dissolved oxygen) were measured throughout the test and subsamples of the overlying water were measured for dissolved metals by inductively coupled plasma atomic emissions spectrometry (ICP-AES). Statistical significance between treatments and effects concentrations were calculated using ToxCalc Version 5.0.23 (Tidepool Software).

Results: The survival of the adults in the test (Table 1) was greater than the minimum acceptability limit of 80% (QA control sediment). The number of embryos per female produced in the QA control sediment was also within the test acceptability limits of ≥8 embryos per female. Dissolved ammonia concentrations remained below levels that may cause effects to the reproduction of the amphipod (Simpson et al., 2013).

Amphipod reproduction in the HG control was observed to have significantly less embryos per females than the amphipods in the QA control and therefore, dilutions of the Drum 1 tailing solid was compared to this control. The 1 and 10% concentrations of the drum 1 tailing solids were observed to have increased the amphipod reproduction when compared to the HG control. Toxic effects to reproduction were observed in concentrations of 30% and greater. The 10% dilution that was layered (reproduction 1 ± 0, dissolved Cu 35 µg/L) was observed to have significantly less embryos per female and more copper in the overlying water than the 10% (reproduction 10 ± 2, dissolved Cu 16 µg/L) that was homogenised prior to testing.

Table 1. Toxicity test results

Sediment	Survival (% survival)	% of Control	Embryos per females	% of Control	Average ammonia (mg NH_3-N/L)[c]
QA control (BB)	90 ± 6[a]	100 ± 7	16 ± 2	100 ± 12	0.7
				% of HG Control	
Huon Gulf (HG) control	92 ± 3	100 ± 4	8 ± 1[b]	100 ± 9	0.3
Tailing Solid Drum 1 1%	94 ± 6	102 ± 7	11 ± 2	138 ± 18	0.3
Tailing Solid Drum 1 10%	94 ± 4	102 ± 4	10 ± 2	126 ± 19	0.6
Tailing Solid Drum 1 10% Layered	92 ± 0	100 ± 0	1 ± 0	15 ± 1[b]	0.4
Tailing Solid Drum 1 30%	83 ± 6	91 ± 6	4 ± 1	54 ± 8[b]	0.8
Tailing Solid Drum 1 60%	88 ± 2	95 ± 3	1 ± 0	16 ± 5[b]	0.8
Tailing Solid Drum 1 90%	73 ± 11	80 ± 13	1 ± 0	9 ± 4[b]	0.6
Drum 1 reproduction effects concentrations	**EC10[d]** 13.6 (0-15.2)	**EC20** 17.2 (2.8-20.4)	**EC50** 28.1 (21.1-39.2)	**NOEC[e]** 30	**LOEC[f]** 60

[a] All results are mean ± standard error calculated based on the four replicate tests/sediment.
[b] Statically less than the control response (p<0.05) and below the toxic threshold.
[c] Average ammonia measurements of overlying water in the sediments on day 3, 5, 7, and 10.
[d] Concentration of drum 1 tailing solid that results in a 10, 20 or 50% reproduction effect.
[e] Highest concentration that resulted in no observable reproduction effects.
[f] Lowest concentration that resulted in a statistically significant reproduction effect.

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6807 • **ABN** 41 687 119 230

Table 2. Time averaged dissolved metals in the overlying water of the amphipod reproduction bioassay.

Sediment	Dissolved metals, µg/L					
	Cu	Fe	Mn	Ni	Pb	Zn
Huon Gulf (HG)	8.1	2.2	1.4	1.8	1.0	0.7
Tailing Solid Drum 1 1%	7.3	3.9	3.7	1.5	2.3	3.1
Tailing Solid Drum 1 10%	16	1.9	120	2.6	1.0	1.5
Tailing Solid Drum 1 10% Layered	35	8.3	95	4.6	0.5	6.4
Tailing Solid Drum 1 30%	24	2.7	480	4.3	4.3	2.7
Tailing Solid Drum 1 60%	34	5.3	780	7.2	1.4	10
Tailing Solid Drum 1 90%	51	3.4	520	12	1.4	24

Note: Measured concentrations of Al, As, Cd, Cr, Co and V were below the limit of detection (2 µg/L) of the ICP-AES

Table 3. Quality assurance/quality control

Quality Assurance/Quality Control Criteria	Range	Criterion Met?
≥80% survival in the QA control (BB)	90 ± 6%	Yes
≥8 embryos per female produced in the QA control	16 ± 2	Yes
pH of overlying water in test beakers	8.0 ± 0.1	Yes
Salinity of overlying water in test beakers	30 ± 0.2‰	Yes
Dissolved oxygen in overlying water in test beakers	>90%	Yes
Temperature of overlying water in test beakers	21 ± 1°C	Yes

Test carried out by:	David Spadaro and Kitty McKnight
Test supervised by:	Stuart Simpson
Test report prepared by:	David Spadaro
Test report reviewed by:	Merrin Adams
Date:	19/6/2017

References

Simpson, S.L., Spadaro, D.A. (2011). Performance and sensitivity of rapid sublethal sediment toxicity tests with the amphipod *Melita plumulosa* and copepod *Nitocra spinipes.* Environmental Toxicology and Chemistry 30, 2326–2334. DOI: 10.1002/etc.633.

Simpson, S.L., Spadaro, D.A., O'Brien, D. (2013). Incorporating bioavailability into management limits for copper and zinc in sediments contaminated by antifouling paint and aquaculture. Chemosphere, 93, 2499–2506.

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6807 • **ABN** 41 687 119 230

Amphipod reproduction - Offspring per female

Start Date:	29/4/17	Test ID:	Drum 1	Sample ID:
End Date:	8/5/2017	Lab ID:		Test Species: Melita plumulosa

Conc-%	1	2	3	4
Huon Gulf	10.167	6.833	8.167	7.667
1	9.500	14.667	8.167	13.000
10	14.000	10.000	11.000	6.333
30	5.333	3.000	5.833	3.667
60	0.833	2.500	0.833	1.167
90	0.167	0.167	1.000	1.500

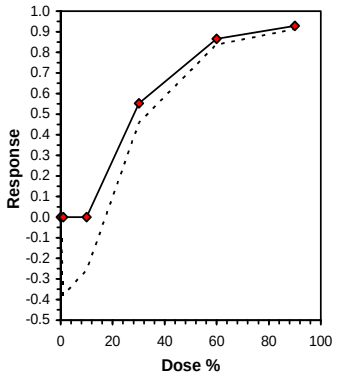
			Transform: Untransformed					2-Tailed			Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Huon Gulf	8.208	1.0000	8.2083	6.8333	10.1667	17.259	4				9.958	1.0000
1	11.333	1.3807	11.3333	8.1667	14.6667	26.606	4	2.211	2.840	4.0135	9.958	1.0000
10	10.333	1.2589	10.3333	6.3333	14.0000	30.603	4	1.504	2.840	4.0135	9.958	1.0000
30	4.458	0.5431	4.4583	3.0000	5.8333	30.120	4	2.654	2.840	4.0135	4.458	0.4477
*60	1.333	0.1624	1.3333	0.8333	2.5000	59.512	4	4.865	2.840	4.0135	1.333	0.1339
*90	0.708	0.0863	0.7083	0.1667	1.5000	92.884	4	5.307	2.840	4.0135	0.708	0.0711

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96997	0.884	-0.0406	0.66772
Bartlett's Test indicates equal variances (p = 0.07)	10.1257	15.0863		

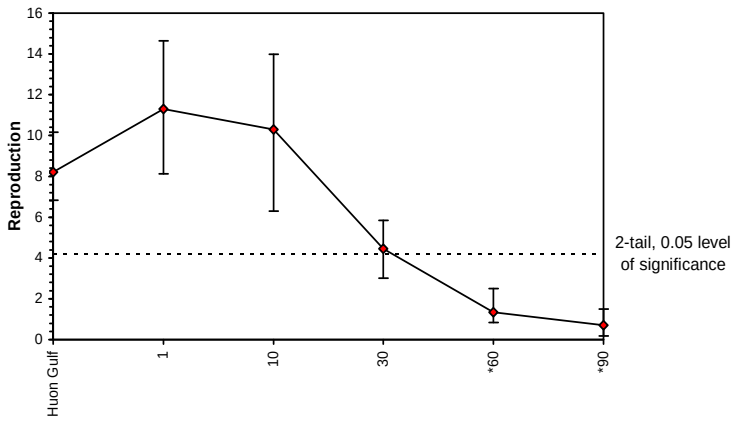
Hypothesis Test (2-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	30	60	42.4264	3.33333	4.01346	0.48895	83.3854	3.99421	6.4E-07	5, 18

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05	11.811	3.156	0.000	12.600	-1.3367
IC10	13.621	2.786	0.000	15.200	-1.6375
IC15	15.432	2.633	0.971	17.800	-1.7343
IC20	17.242	2.589	2.761	20.400	-1.7464
IC25	19.053	2.592	4.551	23.000	-1.6807
IC40	24.485	2.474	15.901	30.994	0.0147
IC50	28.106	2.968	21.080	39.298	1.0035



Dose-Response Plot



CSIRO LAND & WATER
www.csiro.au

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6807 • **ABN** 41 687 119 230

Date: 12/7/2017

Amphipod Reproduction Test Report AR17022

Client: GDA Consult Pty Ltd
Project: Wafi-Golpu Pre-feasability study of DSTP
Test Performed: 10-day amphipod reproduction toxicity test (sublethal, chronic effects) using the amphipod *Melita plumulosa*

Test Initiated: 19/5/17

CSIRO Sample No.	Sample Name	Sample Description
	QA control	Silty control collected from Bonnet Bay (BB), NSW.
E17024	**Huon Gulf Sediment (HG)**	Deep sea sediment collected from the Huon Gulf
E17022	**Tailing Solid Drum 2 1%**	1% washed Drum 1 tailing solid homogenised with 99% HG
E17022	**Tailing Solid Drum 2 3%**	3% washed Drum 1 tailing solid homogenised with 97% HG
E17022	**Tailing Solid Drum 2 10%**	10% washed Drum 2 tailing solid homogenised with 90% HG
E17022	**Tailing Solid Drum 2 10% Layered**	4 g of 100% washed Drum 1 tailing solid layered on top of 36 g of HG
E17022	**Tailing Solid Drum 2 30%**	30% washed Drum 2 tailing solid homogenised with 70% HG
E17022	**Tailing Solid Drum 2 90%**	90% washed Drum 2 tailing solid homogenised with 10% HG

Test method: The amphipod reproduction bioassay measures adult survival and reproduction, expressed as the number of embryos and <1-d-old juveniles in the second brood following exposure of *Melita plumulosa* to test sediments over a 10-d period. The test was carried out according to the methods described in Simpson and Spadaro (2011). Amphipods used in the tests were isolated from laboratory cultures. Dilutions of the washed tailing solid were made up by diluting the tailing solids with the Huon Gulf (HG) control sediment. These sediments were homogenised immediately prior to being added to test vials (40 g sediment per 250 mL vial, 4 replicates per sediment). A 10% Layered treatment was tested using 4 g of undiluted tailing solid layered on top of 36 g of Huon Gulf control. Filtered seawater (200 mL, 30‰) was added and each beaker was incubated at 21°C with aeration overnight to allow sediments to settle. On the following day, 180 mL of overlying water was siphoned off and replaced with new seawater with care to minimise sediment resuspension. Six gravid females (gravid for <36 h) and six males (isolated from laboratory cultures) were randomly assigned to each beaker. Treatments are fed at a rate of 0.25 mg Sera Micron fish food/amphipod twice a week. The sediments are renewed after 5 d by gently sieving away the adults and placing them into the same fresh sediment that had been equilibrated overnight, thus allowing for the removal of juveniles from the first brood, which is typically unaffected by contaminants in the test sediment because they were already ''conceived'' before exposure to test sediments. On Day 10, the females were carefully removed and the number of embryos per female is counted by microscopy. The sediment was also checked for juvenile amphipods that had escaped the marsupium during the latter stages of the test by sieving the sediment through 180 µm mesh. The total number of embryos and <1-d-old juveniles was summed and expressed as a percentage of the HG control.

For quality assurance (QA) purposes, a minimum of 7 juveniles per female is required in the QA controls for tests to be considered acceptable. A sediment is considered to be acutely toxic if the survival as a percentage of the HG control is <80% and is statistically significantly less ($P<0.05$) than the HG controls.

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6807 • **ABN** 41 687 119 230

Chronic toxicity is detected when the reproductive output percent control (Huon Gulf) is <85%, (based on 2 standard deviations of control data n=80) and is statistically significant less (P<0.05) than the controls. Physico-chemical parameters (temperature, pH, salinity and dissolved oxygen) were measured throughout the test and subsamples of the overlying water were measured for dissolved metals by inductively coupled plasma atomic emissions spectrometry (ICP-AES). Statistical significance between treatments and effects concentrations were calculated using ToxCalc Version 5.0.23 (Tidepool Software).

Results: The survival of the adults in the test (Table 1) was within minimum acceptability limit of 80% (QA control sediment) (3% drum 2 tailing was 78% survival however, the standard error was greater than the 80% limit). The number of embryos per female produced in the QA control sediment was also greater than the minimum acceptability limits of 8 embryos per female. Dissolved ammonia concentrations remained below levels that may cause effects to the reproduction of the amphipod (Simpson et al., 2013).

Amphipod reproduction in the HG control was observed to have significantly less embryos per females than the amphipods in the QA control and therefore, dilutions of the Drum 2 tailing solid was compared to this control. Toxic effects to reproduction were observed in the lowest concentrations tested (1%) and greater. A strong relationship was observed between the percent tailing material and the amphipod reproduction; as the concentration of tailing material increased, a decrease in the amount of offspring per female amphipod was observed. The 10% dilution that was layered (reproduction 0 ± 0) was observed to have significantly less embryos per female than the 10% (reproduction 6 ± 1) that was homogenised prior to testing.

Table 1. Toxicity test results

Sediment	Survival (% survival)	% of Control	Embryos per females	% of Control	Average ammonia (mg NH_3-N/L)[c]
QA control	96 ± 2[a]	100 ± 3	14 ± 1	100 ± 6	4.0
				% HG control	
Huon Gulf control	94 ± 4	100 ± 4	9 ± 1[b]	100 ± 11	1.0
Drum 2					
Tailing Solid Drum 2 1%	96 ± 4	102 ± 3	7 ± 1	73 ± 11[b]	2.0
Tailing Solid Drum 2 3%	73 ± 7	78 ± 8	6 ± 1	66 ± 7[b]	1.3
Tailing Solid Drum 2 10%	94 ± 4	100 ± 4	6 ± 1	61 ± 9[b]	1.3
Tailing Solid Drum 2 10% Layered	88 ± 4	93 ± 4	0 ± 0	2 ± 2[b]	1.3
Tailing Solid Drum 2 30%	77 ± 6	82 ± 7	0 ± 0	4 ± 2[b]	2.0
Tailing Solid Drum 2 90%	92 ± 8	98 ± 9	0 ± 0	1 ± 1[b]	1.5
Drum 2 reproduction	**EC10[d]**	**EC20**	**EC50**	**NOEC[e]**	**LOEC[f]**
effects concentrations	0.37 (0.14-2.4)	0.74 (2.8-5.1)	14 (2.8-19)	<1	1

[a] All results are mean ± standard error calculated based on the four replicate tests/sediment.
[b] Statically less than the control response (p<0.05) and below the toxic threshold.
[c] Average ammonia measurements of overlying water in the sediments on day 3, 5, 7, and 10.
[d] Concentration of drum 2 tailing solid that results in a 10, 20 or 50% reproduction effect.
[e] Highest concentration that resulted in no observable reproduction effects.
[f] Lowest concentration that resulted in a statistically significant reproduction effect.

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6807 • **ABN** 41 687 119 230

Table 2. Time averaged dissolved metals in the overlying water of the amphipod reproduction bioassay.

Sediment	Dissolved metals, µg/L				
	Cu	Fe	Mn	Ni	Zn
Huon Gulf control	2.8	2.8	3.0	<1	3.8
Tailing Solid Drum 2 1%	9.2	<1	11	<1	<1
Tailing Solid Drum 2 3%	14	<1	51	1.4	<1
Tailing Solid Drum 2 10%	13	<1	22	1.1	<1
Tailing Solid Drum 2 10% Layered	21	3.2	88	2.7	1.7
Tailing Solid Drum 2 30%	53	1.3	60	3.9	12
Tailing Solid Drum 2 90%	37	<1	1070	7.3	8.7

Note: Measured concentrations of Al, As, Cd, Cr, Co, Pb and V were below the limit of detection (2 µg/L) of the ICP-AES

Table 3. Quality assurance/quality control

Quality Assurance/Quality Control Criteria	Range	Criterion Met?
≥80% survival in the QA control (BB)	96 ± 2%	Yes
≥8 embryos per female produced in the QA control	14 ± 1	Yes
pH of overlying water in test beakers	8.0 ± 0.1	Yes
Salinity of overlying water in test beakers	30 ± 0.2‰	Yes
Dissolved oxygen in overlying water in test beakers	>90%	Yes
Temperature of overlying water in test beakers	21 ± 1°C	Yes

Test carried out by:	David Spadaro
Test supervised by:	Stuart Simpson
Test report prepared by:	David Spadaro
Test report reviewed by:	Merrin Adams
Date:	12/7/2017

References

Simpson, S.L., Spadaro, D.A. (2011). Performance and sensitivity of rapid sublethal sediment toxicity tests with the amphipod *Melita plumulosa* and copepod *Nitocra spinipes.* Environmental Toxicology and Chemistry 30, 2326–2334. DOI: 10.1002/etc.633.

Simpson, S.L., Spadaro, D.A., O'Brien, D. (2013). Incorporating bioavailability into management limits for copper and zinc in sediments contaminated by antifouling paint and aquaculture. Chemosphere, 93, 2499–2506.

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6807 • **ABN** 41 687 119 230

Amphipod Reproduction - Offspring per female

Start Date:	19/5/17	Test ID:	Drum 2	Sample ID:
End Date:	29/5/17	Lab ID:		Test Species: Melita plumulosa

Conc-%	1	2	3	4
Huon Gulf	9.5000	12.0000	9.0000	7.0000
1	4.6667	7.6667	5.8333	9.1667
3	5.1667	8.0000	5.3333	6.0000
10	6.1667	3.3333	6.3333	7.0000
30	0.5000	0.0000	0.0000	0.8333
90	0.0000	0.3333	0.0000	0.0000

			Transform: Untransformed					1-Tailed			Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Huon Gulf	9.3750	1.0000	9.3750	7.0000	12.0000	21.936	4				9.3750	1.0000
*1	6.8333	0.7289	6.8333	4.6667	9.1667	29.064	4	2.471	2.410	2.4790	6.8333	0.7289
*3	6.1250	0.6533	6.1250	5.1667	8.0000	21.238	4	3.160	2.410	2.4790	6.1250	0.6533
*10	5.7083	0.6089	5.7083	3.3333	7.0000	28.445	4	3.565	2.410	2.4790	5.7083	0.6089
*30	0.3333	0.0356	0.3333	0.0000	0.8333	122.474	4	8.790	2.410	2.4790	0.3333	0.0356
*90	0.0833	0.0089	0.0833	0.0000	0.3333	200.000	4	9.033	2.410	2.4790	0.0833	0.0089

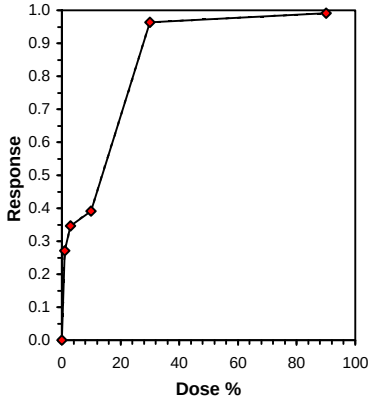
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95199	0.884	0.02523	0.29059
Bartlett's Test indicates equal variances (p = 0.01)	14.7739	15.0863		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	<1	1			2.47898	0.26442	55.8595	2.11613	1.1E-07	5, 18

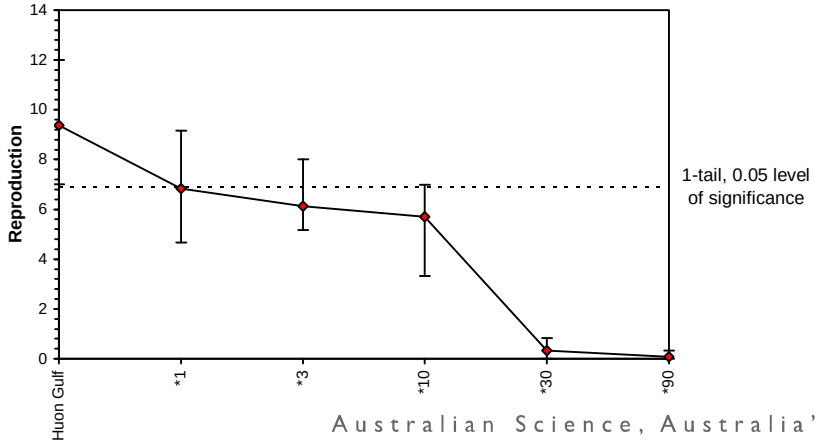
Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05*	0.184	0.248	0.070	1.923	3.4359
IC10*	0.369	0.392	0.141	2.352	3.3254
IC15*	0.553	0.688	0.211	3.354	4.6300
IC20*	0.738	1.167	0.281	5.076	4.7727
IC25*	0.922	2.337	0.352	15.947	2.6176
IC40	10.310	4.705	0.000	17.016	-0.2877
IC50	13.798	2.846	2.808	19.393	-0.8486

* indicates IC estimate less than the lowest concentration



Dose-Response Plot



CSIRO LAND & WATER
www.csiro.au

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6807 • **ABN** 41 687 119 230

Date: 13/7/2017

Amphipod Reproduction Test Report AR17023

Client: GDA Consult Pty Ltd
Project: Wafi-Golpu Pre-feasibility study of DSTP
Test Performed: 10-day amphipod reproduction toxicity test (sublethal, chronic effects) using the amphipod *Melita plumulosa*

Test initiated	**2/6/17**		
CSIRO Sample No.	**Sample Name**	**Sample Description**	**Test Modification**
Control	QA control	Silty control collected from Bonnet Bay (BB), NSW.	None (M0)
E17021	Tailing Solid Drum 1 30%	30% Drum 1 tailing solid; 70% Huon Gulf	Modification 1 (M1)
E17021	Tailing Solid Drum 1 30% M1	30% Drum 1 tailing solid; 70% Huon Gulf	None (M0)
E17021	Tailing Solid Drum 1 60%	60% Drum 1 tailing solid; 40% Huon Gulf	Modification 1 (M1)
E17021	Tailing Solid Drum 1 60% M1	60% Drum 1 tailing solid; 40% Huon Gulf	None (M0)
E17022	Tailing Solid Drum 2 1%	1% Drum 2 tailing solid; 99% Huon Gulf	Modification 1 (M1)
E17022	Tailing Solid Drum 2 1% M1	1% Drum 2 tailing solid; 99% Huon Gulf	None (M0)
E17022	Tailing Solid Drum 2 10%	10% Drum 2 tailing solid; 90% Huon Gulf	Modification 1 (M1)
E17022	Tailing Solid Drum 2 10% M1	10% Drum 2 tailing solid; 90% Huon Gulf	None (M0)

Test method: The amphipod reproduction bioassay measures adult survival and reproduction, expressed as the number of embryos and <1-d-old juveniles in the second brood following exposure of *Melita plumulosa* to test sediments over a 10-d period. The test was carried out according to the methods described in Simpson and Spadaro (2011). Amphipods used in the tests were isolated from laboratory cultures. Dilutions of the washed tailing solid were made up by diluting the tailing solids with the Huon Gulf (HG) control sediment. These sediments were homogenised immediately prior to being added to test vials (40 g sediment per 250 mL vial, 4 replicates per sediment). Filtered seawater (200 mL, 30‰) was added and each beaker was incubated at 21°C with aeration overnight to allow sediments to settle. On the following day, 180 mL of overlying water was siphoned off and replaced with new seawater with care to minimise sediment resuspension. Six gravid females (gravid for <36 h) and six males (isolated from laboratory cultures) were randomly assigned to each beaker. Treatments are fed at a rate of 0.25 mg Sera Micron fish food/amphipod twice a week. The sediments are renewed after 5 d by gently sieving away the adults and placing them into the same fresh sediment that had been equilibrated overnight, thus allowing for the removal of juveniles from the first brood, which is typically unaffected by contaminants in the test sediment because they were already ''conceived'' before exposure to test sediments. On Day 10, the females were carefully removed and the number of embryos per female is counted by microscopy. The sediment was also checked for juvenile amphipods that had escaped the marsupium during the latter stages of the test by sieving the sediment through 180 µm mesh. The total number of embryos and <1-d-old juveniles was summed and expressed as a percentage of the HG control.

For quality assurance (QA) purposes, a minimum of 7 juveniles per female is required in the QA controls for tests to be considered acceptable. A sediment is considered to be acutely toxic if the survival as a percentage of the HG control is <80% and is statistically significantly less ($P<0.05$) than the HG controls. Chronic toxicity is detected when the reproductive output percent control (Huon Gulf) is <85%, (based on 2 standard deviations of control data n=80) and is statistically significant less ($P<0.05$) than the controls. Physico-chemical parameters (temperature, pH, salinity and dissolved oxygen) were measured throughout the test and subsamples of the overlying water were measured for dissolved metals by inductively coupled plasma atomic emissions spectrometry (ICP-AES). Statistical significance between treatments and effects concentrations were calculated using ToxCalc Version 5.0.23 (Tidepool Software).

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6807 • **ABN** 41 687 119 230

Test Method modifications specific to this experiment:

Dissolved metal fluxes in the test beakers can result in the overestimation of toxicity when compared to the tailing disposal site, which has a greater volume of dilution water to reduce the dissolved metal concentration organisms would be exposed to. To assess the effects of dissolved metals fluxing from the tailing material on the reproduction of the amphipods, test modifications where used on the tailing material to reduce the dissolved metal concentration during the test.

Treatment modifications:
M0 – no modification.

Modification 1 (M1) – Two concentrations were selected for treatment modifications from Tailing Solid Drum 1 (10 and 30%) and two concentrations from Tailing Solid Drum 2 (1 and 10%). For these concentrations, 400 mL beakers were used containing 380 mL of overlying water instead of a 250 mL beaker containing 220 mL of overlying water. In addition, the overlying water was exchange with fresh filtered seawater daily. Additional replicates using the standard method were run in parallel to the modified treatments, the TM concentrations were used as controls for comparison of the amphipod reproduction.

Results: The survival of the adults in the test (Table 1) was within minimum acceptability limit of 80% (QA control sediment) (3% drum 2 tailing was 78% survival however, the standard error was greater than the 80% limit). The number of embryos per female produced in the QA control sediment was also greater than the minimum acceptability limits of 8 embryos per female. Dissolved ammonia concentrations remained below levels that may cause effects to the reproduction of the amphipod (Simpson et al., 2013).

The treatment modifications (TM) reduced the amount of dissolved metals in the test beakers during the test. In all the treatments, only the dissolved Cu in Drum 1 60% exceeded the known dissolved metal reproduction effects threshold concentrations (Table 2). However, a toxic response was observed in all Drum 1 treatments (Table 1), suggesting that sediment bound contaminates (via an ingesting pathway) are the main contributor to the observed toxicity to the amphipods. Furthermore, the TM treatments for Drum 1 30% and 60% did not improved the reproductive output of the test organism.

The reproductive response from the amphipods in Drum 2 1% was not significantly different from the QA control. However, the TM treatment significantly improved the reproductive output from the amphipods exposed to tailing from Drum 2 10%, despite the measured dissolved Cu, Mn, Ni and Zn concentrations in those beakers (both as an average or any single measurement during the test acting as a potential pulse exposure) below the known reproductive thresholds.

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6807 • **ABN** 41 687 119 230

Table 1. Toxicity test results

Sediment	Survival (% survival)	% of QA Control	Embryos per females	% of QA Control	% of TM Control	Average ammonia (mg NH_3-N/L)[d]
QA control	92 ± 5[a]	100 ± 5	11 ± 0	100 ± 2		2.2
Drum 1						
Tailing Solid Drum 1 30%	81 ± 7	89 ± 8	3 ± 1	31 ± 6[b]	100 ± 19	0.7
Tailing Solid Drum 1 30% M1	75 ± 3	82 ± 5	3 ± 0	29 ± 3[b]	93 ± 9	1
Tailing Solid Drum 1 60%	67 ± 8	73 ± 8	1 ± 0	9 ± 2[b]	100 ± 23	0.5
Tailing Solid Drum 1 60% M1	73 ± 10	80 ± 11	2 ± 0	20 ± 4[b]	220 ± 40	1
Drum 2						
Tailing Solid Drum 2 1%	83 ± 6	91 ± 6	10 ± 1	92 ± 7	100 ± 7	0.7
Tailing Solid Drum 2 1% M1	88 ± 4	95 ± 5	9 ± 1	82 ± 9	89 ± 10	0.8
Tailing Solid Drum 2 10%	85 ± 7	93 ± 8	11 ± 1	102 ± 9	100 ± 8	0.7
Tailing Solid Drum 2 10% M1	73 ± 24	80 ± 27	7 ± 1	67 ± 6[b]	66 ± 6[c]	0.8

[a] All results are mean ± standard error calculated based on the four replicate tests/sediment.
[b] Statically less than the QA control response ($p<0.05$) and below the toxic threshold.
[c] Statically less than the treatment modification control response ($p<0.05$) for the concentration.
[d] Average ammonia measurements of overlying water in the sediments on day 3, 5, 7, and 10.

Table 2. Time averaged dissolved metals in the overlying water of the amphipod reproduction bioassay.

Sediment	Dissolved metals, µg/L				
	Cu	Fe	Mn	Ni	Zn
QA control	<2	<2	<2	<2	3.1
Drum 1					
Tailing Solid Drum 1 30%	7.9	15	650	<2	3.8
Tailing Solid Drum 1 30% M1	13	4.9	1200	4.5	1.8
Tailing Solid Drum 1 60%	16	<2	610	3.3	5.5
Tailing Solid Drum 1 60% M1	**45**[a]	19	1100	6.8	11
Drum 2					
Tailing Solid Drum 2 1%	2.8	<2	34	<2	<2
Tailing Solid Drum 2 1% M1	5.9	<2	33	<2	6.2
Tailing Solid Drum 2 10%	5.6	<2	200	0.5	4.3
Tailing Solid Drum 2 10% M1	6.7	<2	480	3.5	8.3
Reproductive effects threshold for dissolved metals					
EC10[b]	20 (19-25)	NT	>3500	37 (2.3 -190)	18 (3.0-37)
EC20	22 (20-30)	NT	>3500	72 (5.5-240)	23 (14-45)
EC50	30 (23-47)	NT	>3500	345 (110-830)	49 (NA)

Note: Measured concentrations of Al, As, Cd, Cr, Co Pb and V were below the limit of detection (2 µg/L) of the ICP-AES.
NT: Has not been tested and therefore the reproductive effects thresholds are unknown.
NA: 95% confidence limits are not available as they are larger than the highest concentration tested.
[a] Greater than known reproduction effects thresholds (based on in-house QA database).
[b] Concentration of dissolved metal that results in a 10, 20 or 50% reproduction effect.

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6807 • **ABN** 41 687 119 230

Table 3. Quality assurance/quality control

Quality Assurance/Quality Control Criteria	Range	Criterion Met?
≥80% survival in the QA control (BB)	92 ± 5%	Yes
≥8 embryos per female produced in the QA control	11 ± 0	Yes
pH of overlying water in test beakers	8.0 ± 0.1	Yes
Salinity of overlying water in test beakers	30 ± 0.2‰	Yes
Dissolved oxygen in overlying water in test beakers	>90%	Yes
Temperature of overlying water in test beakers	21 ± 1°C	Yes

Test carried out by:	David Spadaro
Test supervised by:	Stuart Simpson
Test report prepared by:	David Spadaro
Test report reviewed by:	Merrin Adams
Date:	13/7/2017

References

Simpson, S.L., Spadaro, D.A. (2011). Performance and sensitivity of rapid sublethal sediment toxicity tests with the amphipod *Melita plumulosa* and copepod *Nitocra spinipes.* Environmental Toxicology and Chemistry 30, 2326–2334. DOI: 10.1002/etc.633.

Simpson, S.L., Spadaro, D.A., O'Brien, D. (2013). Incorporating bioavailability into management limits for copper and zinc in sediments contaminated by antifouling paint and aquaculture. Chemosphere, 93, 2499–2506.

CSIRO LAND & WATER
www.csiro.au

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6807 • **ABN** 41 687 119 230

Date: 20/7/2017

Amphipod Reproduction Test Report AR17024

Client: GDA Consult Pty Ltd
Project: Wafi-Golpu Pre-feasibility study of DSTP
Test Performed: 10-day amphipod reproduction toxicity test (sublethal, chronic effects) using the amphipod *Melita plumulosa*

Test Initiated: 7/7/17

CSIRO Sample No.	Sample Name	Sample Description
E17021	**QA control (QA)**	Silty control collected from Bonnet Bay (BB), NSW.
E17021	**1% Tail, 99% QA**	1% washed Drum 1 tailing solid homogenised with 99% QA
E17021	**1% Layered QA**	0.4 g of 100% washed Drum 1 tailing solid layered on top of 39.6 g of QA
E17024	**Huon Gulf control (HG)**	Deep sea sediment collected from the Huon Gulf
E17021	**1% Tail, 99% HG**	1% washed Drum 1 tailing solid homogenised with 99% HG
E17021	**1% Layered HG**	0.4 g of 100% washed Drum 1 tailing solid layered on top of 39.6 g of HG
E17021	**0.1% Tail, 99.9% HG**	0.1% washed Drum 1 tailing solid homogenised with 99.9% HG
E17021	**0.1% Layered HG**	0.04 g of 100% washed Drum 1 tailing solid layered on top of 39.96 g of HG

Test method: The amphipod reproduction bioassay measures adult survival and reproduction, expressed as the number of embryos and <1-d-old juveniles in the second brood following exposure of *Melita plumulosa* to test sediments over a 10-d period. The test was carried out according to the methods described in Simpson and Spadaro (2011). Amphipods used in the tests were isolated from laboratory cultures. Dilutions of the washed tailing solid were made up by diluting the tailing solids with the Huon Gulf (HG) control sediment. These sediments were homogenised immediately prior to being added to test vials (40 g sediment per 250 mL vial, 4 replicates per sediment). A 0.1% and 1% Layered treatment was tested using 0.04 and 0.4 g respectively of undiluted tailing solid layered on top of 39.96 g and 39.6 g of Huon Gulf control respectively. Filtered seawater (200 mL, 30‰) was added and each beaker was incubated at 21°C with aeration overnight to allow sediments to settle. On the following day, 180 mL of overlying water was siphoned off and replaced with new seawater with care to minimise sediment resuspension. Six gravid females (gravid for <36 h) and six males (isolated from laboratory cultures) were randomly assigned to each beaker. Treatments are fed at a rate of 0.25 mg Sera Micron fish food/amphipod twice a week. The sediments are renewed after 5 d by gently sieving away the adults and placing them into the same fresh sediment that had been equilibrated overnight, thus allowing for the removal of juveniles from the first brood, which is typically unaffected by contaminants in the test sediment because they were already ''conceived'' before exposure to test sediments. On Day 10, the females were carefully removed and the number of embryos per female is counted by microscopy. The sediment was also checked for juvenile amphipods that had escaped the marsupium during the latter stages of the test by sieving the sediment through 180 µm mesh. The total number of embryos and <1-d-old juveniles was summed and expressed as a percentage of the HG control.

For quality assurance (QA) purposes, a minimum of 7 juveniles per female is required in the QA controls for tests to be considered acceptable. A sediment is considered to be acutely toxic if the survival as a

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6807 • **ABN** 41 687 119 230

percentage of the HG control is <80% and is statistically significantly less (P<0.05) than the HG controls. Chronic toxicity is detected when the reproductive output percent control (Huon Gulf) is <85%, (based on 2 standard deviations of control data n=80) and is statistically significantly less (P<0.05) than the controls. Physico-chemical parameters (temperature, pH, salinity and dissolved oxygen) were measured throughout the test and subsamples of the overlying water were measured for dissolved metals by inductively coupled plasma atomic emissions spectrometry (ICP-AES). Statistical significance between treatments and effects concentrations were calculated using ToxCalc Version 5.0.23 (Tidepool Software).

Results: The survival of the adults in the test (Table 1) was within minimum acceptability limit of 80% (QA control sediment). The number of embryos per female produced in the QA control sediment was also greater than the minimum acceptability limits of 8 embryos per female. Dissolved ammonia concentrations remained below levels that may cause effects to the reproduction of the amphipod (Simpson et al., 2013).

The reproduction in the Huon Gulf control was significantly less than the amphipod reproduction in the QA control. The 1% Homogenised and 1% Layered Drum 1 Tailing Solids were not toxic when diluted with the QA control to the reproduction to the amphipods. The 1% Layered Drum 1 Tailing Solids diluted in the Huon Gulf sediment was toxic to the amphipod reproduction, however, no toxicity was observed in the 1% homogenised, 0.1% layered and 0.1% homogenised.

Table 1. Toxicity test results

Sediment	Survival (% survival)	% of QA Control	Embryos per females	% of QA Control	% of Huon Gulf Control	Average ammonia (mg NH_3-N/L)[d]
QA control (QA)	92 ± 5[a]	100 ± 5	10 ± 0	100 ± 3		0.6
1% Tail, 99% QA (mixed)	92 ± 3	88 ± 8	8 ± 1	88 ± 8		0.3
1% Layered QA	85 ± 6	93 ± 7	8 ± 1	84 ± 11		0.4
Huon Gulf control (HG)	85 ± 5	93 ± 6	5 ± 1	56 ± 8[b]	100 ± 14	0.6
1% Tail, 99% HG (mixed)	94 ± 2	102 ± 2	6 ± 0	64 ± 4[b]	119 ± 10	0.4
1% Layered HG	69 ± 10	75 ± 11	3 ± 1	28 ± 8[b]	51 ± 15[c]	0.6
0.1% Tail, 99.9% HG (mixed)	83 ± 9	91 ± 10	6 ± 0	61 ± 4[b]	108 ± 7	0.5
0.1% Layered HG	85 ± 5	93 ± 6	6 ± 1	61 ± 6[b]	112 ± 11	0.6

[a] All results are mean ± standard error calculated based on the four replicate tests/sediment.
[b] Statically less than the QA control response (p<0.05) and below the toxic threshold.
[c] Statically less than the HG control response (p<0.05) and below the toxic threshold.
[d] Average ammonia measurements of overlying water in the sediments on day 3, 5, 7, and 10.

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6807 • **ABN** 41 687 119 230

Table 2. Time averaged dissolved metals in the overlying water of the amphipod reproduction bioassay.

Sediment	Dissolved metals, µg/L				
	Cu	Fe	Mn	Ni	Zn
QA control (QA)	1.6	<2	<2	<2	8.6
1% Tail, 99% QA	1.5	<2	<2	<2	7.6
1% Layered QA	5.8	2.0	<2	<2	8.7
Huon Gulf control (HG)	4.7	4.7	<2	<2	<2
1% Tail, 99% HG	5.2	5.0	4.5	<2	<2
1% Layered HG	9.0	2.7	5.7	<2	<2
0.1% Tail, 99.9% HG	4.0	3.5	<2	<2	<2
0.1% Layered HG	4.8	3.2	<2	<2	<2

Note: Measured concentrations of Al, As, Cd, Cr, Co Pb and V were below the limit of detection (2 µg/L) of the ICP-AES.

Table 3. Quality assurance/quality control

Quality Assurance/Quality Control Criteria	Range	Criterion Met?
≥80% survival in the QA control (BB)	92 ± 5%	Yes
≥8 embryos per female produced in the QA control	10 ± 0	Yes
pH of overlying water in test beakers	8.0 ± 0.1	Yes
Salinity of overlying water in test beakers	30 ± 0.2‰	Yes
Dissolved oxygen in overlying water in test beakers	>90%	Yes
Temperature of overlying water in test beakers	21 ± 1°C	Yes

Test carried out by:	David Spadaro
Test supervised by:	Stuart Simpson
Test report prepared by:	David Spadaro
Test report reviewed by:	Merrin Adams
Date:	20/7/2017

References

Simpson, S.L., Spadaro, D.A. (2011). Performance and sensitivity of rapid sublethal sediment toxicity tests with the amphipod *Melita plumulosa* and copepod *Nitocra spinipes.* Environmental Toxicology and Chemistry 30, 2326–2334. DOI: 10.1002/etc.633.

Simpson, S.L., Spadaro, D.A., O'Brien, D. (2013). Incorporating bioavailability into management limits for copper and zinc in sediments contaminated by antifouling paint and aquaculture. Chemosphere, 93, 2499–2506.

CSIRO LAND & WATER
www.csiro.au

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6807 • **ABN** 41 687 119 230

Date: 17/7/2017

Copepod Reproduction Test Report CR17021

Client:	GDA Consult Pty Ltd
Project:	Wafi-Golpu Pre-feasibility study of DSTP
Test Performed:	10-day copepod reproduction toxicity test (sublethal, chronic effects) using the copepod *Nitroca spinipes*

Test Initiated: 12/5/17

CSIRO Sample No.	Sample Name	Sample Description
	QA control	Silty control collected from Bonnet Bay (BB), NSW.
E17024	**Huon Gulf Sediment (HG)**	Deep sea sediment collected from the Huon Gulf
E17021	**Tailing Solid Drum 1 1%**	1% washed Drum 1 tailing solid homogenised with 99% HG
E17021	**Tailing Solid Drum 1 10%**	10% washed Drum 1 tailing solid homogenised with 90% HG
E17021	**Tailing Solid Drum 1 10% Layered**	4 g of 100% washed Drum 1 tailing solid layered on top of 36 g of HG
E17021	**Tailing Solid Drum 1 30%**	30% washed Drum 1 tailing solid homogenised with 70% HG
E17021	**Tailing Solid Drum 1 60%**	60% washed Drum 1 tailing solid homogenised with 40% HG
E17021	**Tailing Solid Drum 1 90%**	90% washed Drum 1 tailing solid homogenised with 10% HG

Test Method: This test measures the reproductive output of the copepod *Nitocra spinipes* following exposure to the washed tailing solid (TS) over a 10-d period. TS were homogenised immediately prior to being added to test vials (0.5 g sediment per 10 mL vial, 3 replicates per sediment). A 10% Layered treatment was tested using 50 mg of undiluted tailing solid layered on top of 0.45 g of Huon Gulf control sediment. Filtered seawater (30‰) was added, and each vial was incubated at 21°C overnight to allow sediments to settle. On the following day, overlying water was replaced, five gravid females (3-5 weeks old) were randomly assigned to each vial. Copepods used in the tests were isolated from laboratory cultures. The physico-chemical parameters (temperature, pH, salinity and dissolved oxygen) were monitored throughout the test. Subsamples of the overlying water were collected at the end of the test and measured for dissolved metals by inductively coupled plasma atomic emissions spectrometry (ICP-AES). Treatments were fed a diet of 1×10^4 cell per mL of both *Isochrysis* sp. and *Tetraselmis* sp. as well as 0.3 mg Sera Micron® fish food (<63 µm) per test vial twice a week. After ten days, the combined number of nauplii (first juvenile lifestage of the copepod) and copepodites (second juvenile lifestage) in each vial was recorded by microscopy. Results are expressed as a percentage of the reproductive output in the control sediment. Reproductive toxicity is detected when the reproductive output percent control is <75%, (based on 2 standard deviations of control data n=30) and is statistically significant less ($P<0.05$) than the controls (Simpson and Spadaro, 2011). Statistical significance between treatments and effects concentrations were calculated using ToxCalc Version 5.0.23 (Tidepool Software).

Results: The number of juveniles per female produced in the control sediment was within the test acceptability limits of >20 juveniles per female.

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6807 • **ABN** 41 687 119 230

Copepod reproduction in the Huon Gulf (HG) control was observed to have significantly less embryos per females than the amphipods in the QA control and therefore, dilutions of the Drum 1 tailing solid was compared to this control. The 1% concentration of the Drum 1 tailing solids were observed to have significantly increased the copepod reproduction when compared to the HG control. Toxic effects to reproduction were observed in concentrations of 30% and greater. The 10% dilution that was layered was observed to have significantly less embryos per female than the 10% that was homogenised prior to testing.

Table 1. Toxicity test results

Sediment	Embryos per females	% of Control
QA control	22 ± 2[a]	100 ± 10
Huon Gulf (HG) control	13 ± 1[b]	100 ± 7
Tailing Solid Drum 1 1%	18 ± 1	137 ± 7
Tailing Solid Drum 1 10%	12 ± 1	93 ± 5
Tailing Solid Drum 1 10% Layered	1 ± 0	9 ± 1[c]
Tailing Solid Drum 1 30%	7 ± 1	53 ± 10[c]
Tailing Solid Drum 1 60%	1 ± 0	4 ± 1[c]
Tailing Solid Drum 1 90%	0 ± 0	3 ± 1[c]

Drum 1 reproduction effects concentrations	EC10[d]	EC20	EC50	NOEC[e]	LOEC[f]
	5.2 (2.6-11)	9.4 (4.2-19)	27 (15-43)	10	30

[a] All results are mean ± standard error calculated based on the four replicate tests/sediment.
[b] Statically less than the QA control response ($p<0.05$) and below the toxic threshold.
[c] Statically less than the Huon Gulf control response ($p<0.05$) and below the toxic threshold.
[d] Concentration of Drum 1 tailing solid that results in a 10, 20 or 50% reproduction effect.
[e] Highest concentration that resulted in no observable reproduction effects.
[f] Lowest concentration that resulted in a statistically significant reproduction effect.

Table 2. Dissolved metals in the overlying water of the copepod reproduction bioassay.

Sediment	Dissolved metals, µg/L					
	Cu	Fe	Mn	Ni	Pb	Zn
QA control	<1	190	44	0.7	<1	2.8
Huon Gulf	3.2	170	2800	1.7	<1	2.9
Tailing Solid Drum 1 1%	2.5	210	3200	2.3	<1	0.1
Tailing Solid Drum 1 10% Layered	4.2	150	2500	0.5	<1	1.5
Tailing Solid Drum 1 30%	13	190	1700	4.7	<1	6.2
Tailing Solid Drum 1 60%	15	220	860	6.4	<1	11
Tailing Solid Drum 1 90%	21	600	440	8.3	<1	21

Note: Measured concentrations of Al, As, Cd, Cr, Co and V were below the limit of detection (2 µg/L) of the ICP-AES; There was insufficient volume from the Drum 1 10% concentration to analyse for metals.

Table 3. Quality assurance quality control

Quality Assurance/Quality Control Criteria	Range	Criterion Met?
>20 juveniles per female produced in the QA control	22 ± 2	Yes
pH of overlying water in test beakers	8.0 ± 0.1	Yes

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6807 • **ABN** 41 687 119 230

Salinity of overlying water in test beakers	30 ± 0.2‰	Yes
Dissolved oxygen in overlying water in test beakers	>90%	Yes
Temperature of overlying water in test beakers	21 ± 1°C	Yes

Test carried out by:	David Spadaro
Test supervised by:	Stuart Simpson
Test report prepared by:	David Spadaro
Test report reviewed by:	Stuart Simpson
Date:	17/7/2017

Reference

Simpson, S.L., Spadaro, D.A. (2011). Performance and sensitivity of rapid sublethal sediment toxicity tests with the amphipod *Melita plumulosa* and copepod *Nitocra spinipes.* Environmental Toxicology and Chemistry 30, 2326–2334.

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6807 • **ABN** 41 687 119 230

Copepod Reproduction - Offspring per female

Start Date:	12/5/17	Test ID:	Drum 1	Sample ID:	
End Date:	22/5/17	Lab ID:		Test Species:	Nitocra spinipes

Conc-%	1	2	3
Huon Gulf	15.000	13.000	12.000
1	20.000	17.500	17.250
10	11.250	12.500	13.500
30	7.500	9.000	4.500
60	0.750	0.500	0.500
90	0.250	0.250	0.500

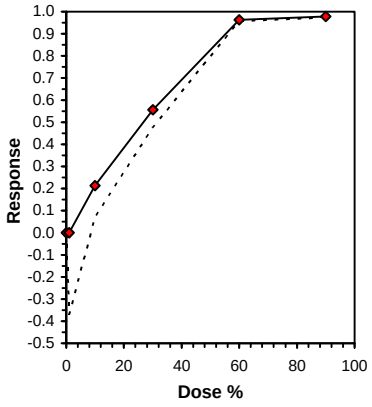
			Transform: Untransformed					2-Tailed			Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Huon Gulf	13.333	1.0000	13.333	12.000	15.000	11.456	3				15.792	1.0000
*1	18.250	1.3688	18.250	17.250	20.000	8.333	3	4.406	3.000	3.348	15.792	1.0000
10	12.417	0.9313	12.417	11.250	13.500	9.079	3	0.821	3.000	3.348	12.417	0.7863
*30	7.000	0.5250	7.000	4.500	9.000	32.733	3	5.675	3.000	3.348	7.000	0.4433
*60	0.583	0.0438	0.583	0.500	0.750	24.744	3	11.425	3.000	3.348	0.583	0.0369
*90	0.333	0.0250	0.333	0.250	0.500	43.301	3	11.649	3.000	3.348	0.333	0.0211

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95826	0.858	-0.0721	0.24132
Bartlett's Test indicates equal variances (p = 0.02)	13.2417	15.0863		

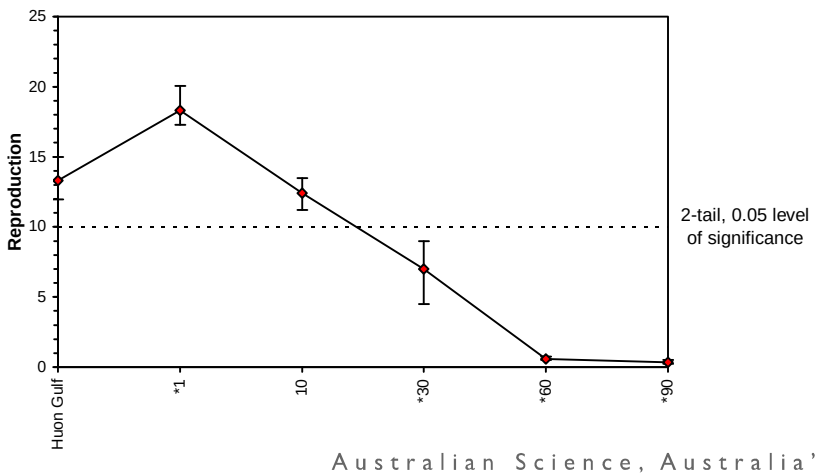
Hypothesis Test (2-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	10	30	17.3205	10	3.34788	0.25109	159.145	1.86806	6.0E-09	5, 12

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05	3.106	0.489	1.816	5.852	1.1468
IC10	5.211	0.978	2.631	10.704	1.1468
IC15	7.317	1.415	3.447	15.069	0.9935
IC20	9.422	1.778	4.262	18.701	0.8194
IC25	12.115	2.243	4.431	21.931	0.4167
IC40	20.862	2.976	11.491	35.742	0.5328
IC50	26.692	3.615	15.398	42.775	0.2644



Dose-Response Plot



CSIRO LAND & WATER
www.csiro.au

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6807 • **ABN** 41 687 119 230

Date: 24/7/2017

Copepod Reproduction Test Report CR17022

Client:	GDA Consult Pty Ltd
Project:	Wafi-Golpu Pre-feasibility study of DSTP
Test Performed:	10-day copepod reproduction toxicity test (sublethal, chronic effects) using the copepod *Nitroca spinipes*

Test Initiated:	30/5/17	
CSIRO Sample No.	**Sample Name**	**Sample Description**
	QA control	Silty control collected from Bonnet Bay (BB), NSW.
E17024	**Huon Gulf Sediment (HG)**	Deep sea sediment collected from the Huon Gulf
E17022	**Tailing Solid Drum 2 1%**	1% washed Drum 1 tailing solid homogenised with 99% HG
E17022	**Tailing Solid Drum 2 3%**	3% washed Drum 1 tailing solid homogenised with 97% HG
E17022	**Tailing Solid Drum 2 10%**	10% washed Drum 2 tailing solid homogenised with 90% HG
E17022	**Tailing Solid Drum 2 10% Layered**	10% washed Drum 1 tailing solid layered on top of 90% HG
E17022	**Tailing Solid Drum 2 30%**	30% washed Drum 2 tailing solid homogenised with 70% HG
E17022	**Tailing Solid Drum 2 90%**	90% washed Drum 2 tailing solid homogenised with 10% HG

Test Method: This test measures the reproductive output of the copepod *Nitocra spinipes* following exposure to the washed tailing solid (TS) over a 10-d period. TS were homogenised immediately prior to being added to test vials (0.5 g sediment per 10 mL vial, 3 replicates per sediment). A 10% Layered treatment was tested using 50 mg of undiluted tailing solid layered on top of 0.45 g of Huon Gulf control sediment. Filtered seawater (30‰) was added, and each vial was incubated at 21°C overnight to allow sediments to settle. On the following day, overlying water was replaced, five gravid females (3-5 weeks old) were randomly assigned to each vial. Copepods used in the tests were isolated from laboratory cultures. The physico-chemical parameters (temperature, pH, salinity and dissolved oxygen) were monitored throughout the test. Subsamples of the overlying water were collected at the end of the test and measured for dissolved metals by inductively coupled plasma atomic emissions spectrometry (ICP-AES). Treatments were fed a diet of 1×10^4 cell per mL of both *Isochrysis* sp. and *Tetraselmis* sp. as well as 0.3 mg Sera Micron® fish food (<63 µm) per test vial twice a week. After ten days, the combined number of nauplii (first juvenile lifestage of the copepod) and copepodites (second juvenile lifestage) in each vial was recorded by microscopy. Results are expressed as a percentage of the reproductive output in the control sediment. Reproductive toxicity is detected when the reproductive output percent control is <75%, (based on 2 standard deviations of control data n=30) and is statistically significant less ($P<0.05$) than the controls (Simpson and Spadaro, 2011). Statistical significance between treatments and effects concentrations were calculated using ToxCalc Version 5.0.23 (Tidepool Software).

Results: The number of juveniles per female produced in the control sediment was within the test acceptability limits of >20 juveniles per female.

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6807 • **ABN** 41 687 119 230

No significant difference was observed between the copepod reproduction in QA control and the Huon Gulf control. All Drum 2 concentrations were considered toxic to the copepod and therefore, no NOEC concentration could be calculated. There was no significant difference between the copepod reproduction between the 10% Layered and the 10% Homogenised concentration, both were highly toxic to the copepods with ≤2% reproduction compared to the controls.

Table 1. Toxicity test results

Sediment	Embryos per females	% of Control
QA control	22 ± 1[a]	100 ± 10
Huon Gulf control	21 ± 2	100 ± 10
Tailing Solid Drum 2 1%	15 ± 1	68 ± 4[b]
Tailing Solid Drum 2 3%	6 ± 1	26 ± 3[b]
Tailing Solid Drum 2 10%	1 ± 0	2 ± 1[b]
Tailing Solid Drum 2 10% Layered	0 ± 0	0 ± 0[b]
Tailing Solid Drum 2 30%	0 ± 0	0 ± 0[b]
Tailing Solid Drum 2 90%	0 ± 0	0 ± 0[b]

Drum 2 reproduction effects concentrations	EC10[c]	EC20	EC50	NOEC[d]	LOEC[e]
	0.31 (0.15-0.67)	0.62 (0.29-1.3)	1.9 (0.89-2.6)	<1	1

[a] All results are mean ± standard error calculated based on the four replicate tests/sediment.
[b] Statically less than the QA and Huon Gulf control response ($p<0.05$) and below the toxic threshold.
[c] Concentration of Drum 2 tailing solid that results in a 10, 20 or 50% reproduction effect.
[d] Highest concentration that resulted in no observable reproduction effects.
[e] Lowest concentration that resulted in a statistically significant reproduction effect.

Table 2. Dissolved metals in the overlying water of the copepod reproduction bioassay.

Sediment	Dissolved metals, µg/L				
	Cu	Fe	Mn	Ni	Zn
QA control	13*	530	70	<2	41
Huon Gulf	11	96	1600	1.8	2.9
Tailing Solid Drum 2 1%	6.8	35	1500	2.5	<2
Tailing Solid Drum 2 3%	7.3	24	1500	2.3	<2
Tailing Solid Drum 2 10%	11	150	1600	1.9	37
Tailing Solid Drum 2 10% Layered	33	140	2500	9.0	24
Tailing Solid Drum 2 30%	16	78	2100	7.2	4.4
Tailing Solid Drum 2 90%	55	620	620	29	130

Note: Measured concentrations of Al, As, Cd, Cr, Co and V were below the limit of detection (2 µg/L) of the ICP-AES.
*Usually high result compared to previous measured copper values under the same condition and therefore could be considered unreliable.

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6807 • **ABN** 41 687 119 230

Table 3. Quality assurance quality control

Quality Assurance/Quality Control Criteria	Range	Criterion Met?
>20 juveniles per female produced in the QA control	22 ± 1	Yes
pH of overlying water in test beakers	8.0 ± 0.1	Yes
Salinity of overlying water in test beakers	30 ± 0.2‰	Yes
Dissolved oxygen in overlying water in test beakers	>90%	Yes
Temperature of overlying water in test beakers	21 ± 1°C	Yes

Test carried out by:	David Spadaro
Test supervised by:	Stuart Simpson
Test report prepared by:	David Spadaro
Test report reviewed by:	Stuart Simpson
Date:	24/7/2017

Reference

Simpson, S.L., Spadaro, D.A. (2011). Performance and sensitivity of rapid sublethal sediment toxicity tests with the amphipod *Melita plumulosa* and copepod *Nitocra spinipes.* Environmental Toxicology and Chemistry 30, 2326–2334.

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6807 • **ABN** 41 687 119 230

Copepod Reproduction - Offspring per female

Start Date:	30/5/17	Test ID:	Drum 2	Sample ID:	
End Date:	8/6/2017	Lab ID:		Test Species:	Nitrocra spinipes

Conc-%	1	2	3
Huon Gulf	20.000	25.500	18.750
1	15.750	12.750	15.000
3	5.250	7.000	4.500
10	0.250	0.500	0.750
30	0.000	0.000	0.000
90	0.000	0.000	0.000

			Transform: Untransformed					1-Tailed			Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Huon Gulf	21.417	1.0000	21.417	18.750	25.500	16.768	3				21.417	1.0000
*1	14.500	0.6770	14.500	12.750	15.750	10.767	3	4.104	2.420	4.078	14.500	0.6770
*3	5.583	0.2607	5.583	4.500	7.000	22.977	3	9.395	2.420	4.078	5.583	0.2607
*10	0.500	0.0233	0.500	0.250	0.750	50.000	3	12.411	2.420	4.078	0.500	0.0233
30	0.000	0.0000	0.000	0.000	0.000	0.000	3				0.000	0.0000
90	0.000	0.0000	0.000	0.000	0.000	0.000	3				0.000	0.0000

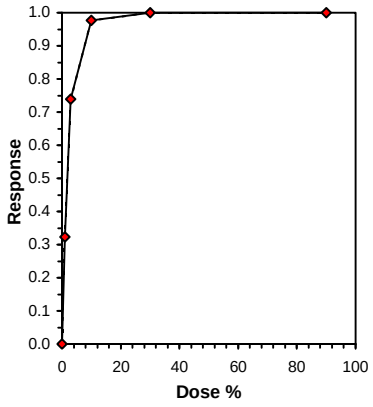
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.94887	0.805	0.8907	1.70983
Bartlett's Test indicates equal variances (p = 0.05)	7.65341	11.3449		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	<1	1			4.07846	0.19043	259.347	4.26042	7.5E-06	3, 8

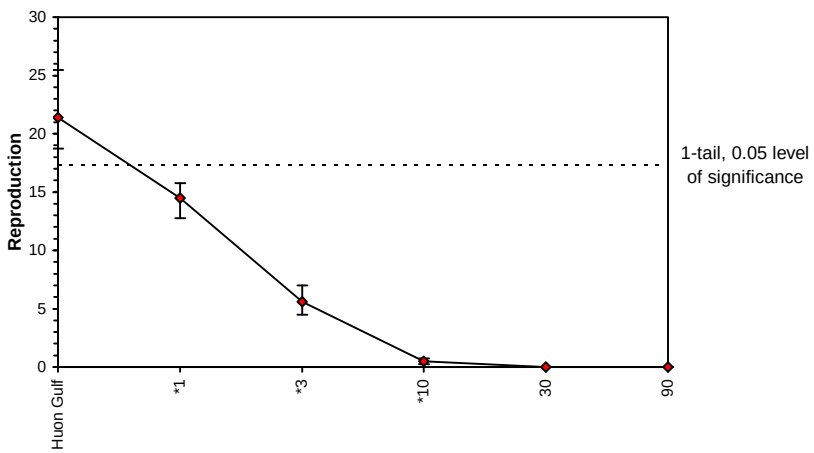
Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05*	0.1548	0.0345	0.0731	0.3341	1.1113
IC10*	0.3096	0.0691	0.1462	0.6682	1.1113
IC15*	0.4645	0.1036	0.2193	1.0023	1.1113
IC20*	0.6193	0.1360	0.2924	1.3360	1.0160
IC25*	0.7741	0.1625	0.3655	1.5944	0.8220
IC40	1.3701	0.2468	0.4402	2.2160	-0.0582
IC50	1.8505	0.2221	0.8888	2.5971	-0.3293

* indicates IC estimate less than the lowest concentration



Dose-Response Plot



CSIRO LAND & WATER
www.csiro.au

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6807 • **ABN** 41 687 119 230

Date: 23/8/2017

Bivalve Bioaccumulation Test Report BB17023

Client:	GDA Consult Pty Ltd
Project:	Wafi-Golpu Pre-feasibility study of DSTP
Test Performed:	30-day bivalve bioaccumulation test using the bivalve *Tellina deltoidalis*

Test Initiated: 27/4/17

CSIRO Sample No.	**Sample Name**	**Sample Description**
E17024	**Huon Gulf (HG)**	Deep sea sediment collected from the Huon Gulf
E17021	**Tailing Solid Drum 1 30%**	30% washed Drum 1 tailing solid homogenised with 70% HG
E17021	**Tailing Solid Drum 1 60%**	60% washed Drum 1 tailing solid homogenised with 40% HG
E17021	**Tailing Solid Drum 1 90%**	90% washed Drum 1 tailing solid homogenised with 10% HG
E17022	**Tailing Solid Drum 2 10%**	10% washed Drum 2 tailing solid homogenised with 90% HG
E17022	**Tailing Solid Drum 2 30%**	30% washed Drum 2 tailing solid homogenised with 70% HG
E17022	**Tailing Solid Drum 2 90%**	90% washed Drum 2 tailing solid homogenised with 10% HG

Test method: The bioassay assesses metal bioaccumulation and survival of the benthic bivalve, *T. deltoidalis,* following exposure to sediments for 30 days (Spadaro and Simpson 2016).

The bivalves were collected at Boronia Park, Lane Cove River estuary (27-32‰), Sydney, New South Wales, Australia. Approximately 300 adult bivalves with shell surface areas from 10 to 60 mm^2 (two dimensional) were collected by gently sieving (2 mm mesh) sediment collected from a maximum depth below the sediment-water interface of 20 cm. Prior to use in tests the bivalves were acclimated for 4 days to the laboratory test conditions (20°C and salinity 30‰) in holding trays with sediment from the bivalve collection site and oxygenated seawater. After acclimation, bivalves were removed from the sediment, placed in seawater and sorted into groups of 7 individuals with approximately the same size distribution. The bivalves were observed over a 1-h period for movement to ensure only live animals were selected for use in the bioaccumulation test.

Approximately 275 mL (1.5 cm depth) of each sediment treatment (including mine tailings) was added to 1-L beakers and 900 mL of seawater (30‰) added as overlying water. Each treatment was prepared in triplicate. Overlying water was aerated continuously to maintain dissolved oxygen (DO) levels >85% saturation. Seven bivalves were added to each test treatment container within 2 h of their removal from the holding trays. During the test, the bivalves were fed twice per week with 4 mg of Sera Micron™ per bivalve. The release of metals from sediments to overlying water was monitored by measuring dissolved (0.45 µm filtered) metals in the overlying water throughout the exposure period, along with DO, pH, temperature and ammonia.

At the termination of the tests (i.e. after 30 days), surviving bivalves were counted and allowed to depurate overnight in clean seawater for 24 h. Following depuration, the soft body tissue of the bivalves was dissected from the shell using a Teflon coated razor blade and plastic tweezers. Tissue masses from

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6807 • **ABN** 41 687 119 230

the same replicate were placed in a 70-mL polycarbonate vial and then stored in a domestic freezer at -20 °C until time of analysis.

For bivalve tissues metal analyses, the tissues were freeze dried and reweighed to determine the tissue dry weight (DW) and acid digested according to CSIRO Method C-225. Briefly, tissue (~0.15 g DW) from each test replicate was digested in duplicate in Teflon digestion tubes by adding 10 mL of Tracepur nitric acid (65%) and a Microwave Accelerated Reactive System (MARS). Digests were made to a final volume of 25 mL with Milli-Q water and metals were measured by inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7500CE) calibrated with matrix-matched standards. For quality control purposes, one blank (Milli-Q water) and one reference sample (DORM-3, Fish Protein Certified Reference Material, National Research Council Canada) were analysed for every 8 samples.

Results: The survival of the bivalves was greater than the minimum acceptability limit of 80% (HG control 95 ± 5%) (Table 1). Quality control data for the bivalve tissue analyses is provided in a separate analytical report, and analytical blanks were less than the limits of reporting and results for certified reference materials were within the expected ranges.

Toxicity to the bivalves, as indicated by reduced survival, was in all treatments that contained mine tailings from Drum 1 and Drum 2 (Table 1). Different levels of metal release to the overlying water were observed for the two tailing solids (Drum 1 and Drum 2) (Table 2). A significant relationship existed between the decreased survival of the bivalve and the dissolved concentration of copper and zinc (Figure 1) in the overlying waters.

Analysis of tissue metal concentrations was only possible where surviving organism provided sufficient tissue for the analysis. This was the case for the treatments Huon Gulf (HG), Tailing solid Drum 1 30%, Tailing solid Drum 2 10%, and Tailing solid Drum 2 30%. Here it is important to note that the low numbers of surviving bivalves in the two 30% tailing treatments results in lower reliability in the analysis results. For these two treatments the surviving organisms may include organisms that were unhealthy and not able to fully depurate their guts before euthanising and analyses, thus potentially resulting in overestimating of accumulated metals.

The tissue metal analyses determined that the concentrations of Cd, Co, Cr, Cu, Mn, Fe, Ni and V were greater in bivalves following the exposure to the HG control treatments than observed for bivalves not exposed to the sediments. The tissue metal concentrations in the bivalves from tailing solids treatments were not significantly different from those observed for the HG control treatment. The variability in the tissue metals data from the bivalves exposed to tailing solids drum 1 30% and tailing solids drum 2 30% is attributed the low survival (10 and 19% respectively) and the inability of the surviving bivalves from these treatments to completely depurate the gut (due to their poor health) during in the 24 h depuration period.

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6807 • **ABN** 41 687 119 230

Table 1. Survival results from the bivalve bioaccumulation bioassay

Sediment	Survival (% survival)	% of HG Control	Average ammonia (mg NH_3-N/L)[c]
Huon Gulf (HG)	95 ± 5[a]	100 ± 5	0.7
Tailing Solid Drum 1 30%	10 ± 5	10 ± 5[b]	2.8
Tailing Solid Drum 1 60%	0 ± 0	0 ± 0[b]	1.5
Tailing Solid Drum 1 90%	0 ± 0	0 ± 0[b]	4.3
Tailing Solid Drum 2 10%	62 ± 10	65 ± 10[b]	0.7
Tailing Solid Drum 2 30%	19 ± 10	20 ± 10[b]	0.9
Tailing Solid Drum 2 90%	0 ± 0	0 ± 0[b]	5.9

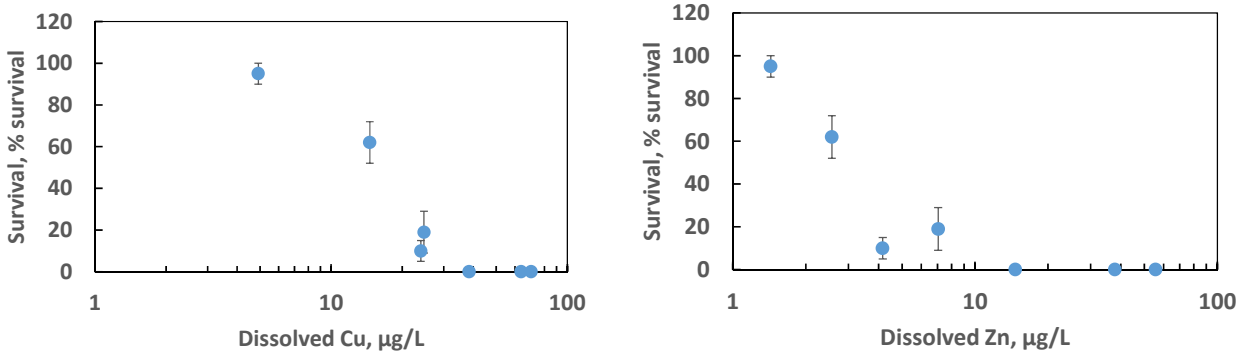
[a] All results are mean ± standard error calculated based on the four replicate tests/sediment.
[b] Statically less than the HG control response ($p<0.05$).
[c] Average ammonia measurements of overlying water in the sediments on day 4, 7, 11, 14, 18, 21 and 25.

Table 2. Averaged dissolved metals in the overlying water of the bivalve bioaccumulation bioassay.

Sediment	Dissolved metals, µg/L				
	Cu	Fe	Mn	Ni	Zn
Huon Gulf (HG)	4.9	1.4	410	1.6	1.4
Tailing Solid Drum 1 30%	24	13	1900	5.0	4.2
Tailing Solid Drum 1 60%	38	13	1300	7.5	15
Tailing Solid Drum 1 90%	70	16	570	13	38
Tailing Solid Drum 2 10%	15	5.0	970	4.1	2.6
Tailing Solid Drum 2 30%	25	11	1700	6.5	7.0
Tailing Solid Drum 2 90%	64	99	680	16	56

Note: Measured concentrations of Al, As, Cd, Cr, Co Pb and V were below the limit of detection (2 µg/L) of the ICP-AES.

Figure 1. The relationship between the survival of the bivalve after 30-d and the dissolved copper and zinc.



New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6807 • **ABN** 41 687 119 230

Table 2. Bioaccumulated metals from the soft tissue of the bivalve.

	Bioaccumulated metals, µg/g						
Sediment	**Ag**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Hg**
Test commencement	5.7 ± 0.63	14 ± 1.9	0.98 ± 0.12	3.1 ± 0.47	4.1 ± 1.4	228 ± 20	0.80 ± 0.12
Huon Gulf	6.3 ± 0.86	14 ± 1.5	1.6 ± 0.08[b]	7.1 ± 0.67[b]	11 ± 1.1[b]	305 ± 32[b]	1.0 ± 0.02
Tailing solid Drum 1 30%	8.6 ± 6.7	15 ± 8.5	1.4 ± 1.2	4.7 ± 2.8	6.7 ± 7.5	450 ± 360	0.93 ± 0.82
Tailing solid Drum 2 10%	7.4 ± 4.9	12 ± 2.0	1.2 ± 0.27	5.1 ± 1.0[bc]	8.6 ± 1.3[b]	380 ± 190	0.86 ± 0.22
Tailing solid Drum 2 30%	5.9 ± 2.4	16 ± 7.9	1.1 ± 0.71	6.0 ± 4.3	26 ± 31	350 ± 150	1.0 ± 0.36
Limit of detection	0.002	0.04	0.01	0.01	0.4	1	0.08

[a] All results are mean ± standard deviation
[b] Statically significant difference from the test commencement ($p<0.05$).
[c] Statically significant difference from the HG control ($p<0.05$).

Table 2 (cont.) Bioaccumulated metals from the soft tissue of the bivalve.

	Bioaccumulated metals, µg/g						
Sediment	**Mn**	**Mo**	**Fe**	**Ni**	**Pb**	**V**	**Zn**
Test commencement	17 ± 2.1	8.9 ± 1.2	2000 ± 310	5.2 ± 1.1	44 ± 7.9	3.5 ± 0.68	390 ± 46
Huon Gulf	150 ± 42 [b]	10 ± 1.2	5800 ± 1400[b]	10 ± 0.56[b]	45 ± 6.5	16 ± 4.8 [b]	460 ± 110
Tailing solid Drum 1 30%	56 ± 43	14 ± 11	3000 ± 2800	8.1 ± 5.8	64 ± 60	6.7 ± 5.9	320 ± 76
Tailing solid Drum 2 10%	99 ± 10 [b]	9.5 ± 2.2	4600 ± 850 [b]	11 ± 0.19[b]	43 ± 20	10 ± 1.7 [b]	410 ± 140
Tailing solid Drum 2 30%	210 ± 220	13 ± .8.4	9700 ± 9300	25 ± 23	44 ± 24	18 ± 20	550 ± 400
Limit of detection	0.5	0.007	10	0.25	0.08	0.2	3

[a] All results are mean ± standard deviation
[b] Statically significant difference from the test commencement ($p<0.05$).
[c] Statically significant difference from the HG control ($p<0.05$).

New Illawarra Rd, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232, Australia
T (02) 9710 6807 • **ABN** 41 687 119 230

Table 3. Quality assurance/quality control

Quality Assurance/Quality Control Criteria	Range	Criterion Met?
≥80% survival in the control (HG)	95 ± 5%	Yes
pH of overlying water in test beakers	8.0 ± 0.1	Yes
Salinity of overlying water in test beakers	30 ± 0.2‰	Yes
Dissolved oxygen in overlying water in test beakers	>90%	Yes
Temperature of overlying water in test beakers	21 ± 1°C	Yes

Test carried out by:	David Spadaro
Test supervised by:	Stuart Simpson
Test report prepared by:	David Spadaro
Test report reviewed by:	Stuart Simpson and Merrin Adams
Date:	23/8/2017

References

Spadaro, D.A., Simpson, S.L. (2016). Appendix G. Protocols for 10-day whole-sediment lethality toxicity tests and 30-day bioaccumulation tests using the deposit-feeding benthic bivalve *Tellina deltoidalis*. In Simpson SL, Batley GE (eds), Sediment Quality Assessment: A Practical Guide. CSIRO Publishing, Victoria, Australia, pp 285-293.

CONTACT US

t 1300 363 400
+61 3 9545 2176
e csiroenquiries@csiro.au
w www.csiro.au

AT CSIRO, WE DO THE EXTRAORDINARY EVERY DAY

We innovate for tomorrow and help improve today – for our customers, all Australians and the world.

Our innovations contribute billions of dollars to the Australian economy every year. As the largest patent holder in the nation, our vast wealth of intellectual property has led to more than 150 spin-off companies.

With more than 5,000 experts and a burning desire to get things done, we are Australia's catalyst for innovation.

CSIRO. WE IMAGINE. WE COLLABORATE. WE INNOVATE.

FOR FURTHER INFORMATION

Land and Water
w www.csiro.au/en/Research/LWF

Merrin Adams
t +61 2 9710 6831
e merrin.adams@csiro.au

Dr Simon Apte
t +61 2 9710 6838
e simon.apte@csiro.au

Dr Stuart Simpson
t +61 2 9710 6807
e stuart.simpson@csiro.au

LAND AND WATER
www.csiro.au

Long-term lab study of Wafi-Golpu tailings: metal geochemistry, release and bioavailability in deposited tailings-sediment mixtures - Stage 1.

Stuart L. Simpson, David A. Spadaro, Joshua J. King, Chad V. Jarolimek, Brett M. Knowles, Leslie L. Huang and Peter R. Teasdale

CSIRO Report EP18834

June 2018

Prepared for Wafi-Golpu Joint Venture (WGJV)

CSIRO Land and Water

Citation

Simpson SL, Spadaro DA, King JJ, Jarolimek CV, Knowles, BM, Huang, LJ, Teasdale, PR (2018) Long-term lab study of Wafi-Golpu tailing: metal geochemistry, release and bioavailability in deposited tailing-sediment mixtures - Stage 1. CSIRO Land and Water Report EP18834, Australia. 131 pp.

Important disclaimer

Contents

Figures

Tables

Abbreviations/Acronyms

Abbreviation / Acronym	Description
µg	Microgram
AEM	Dilute-acid extractable metal
ANZECC	Australian and New Zealand Environment and Conservation Council
ARMCANZ	Agriculture and Resource Management Council of Australia and New Zealand
AVS	Acid volatile sulfide
BICON	Australian Biosecurity Import Conditions
CRM	Certified reference material
d	Day
DET	Diffusive equilibration in thin films
DGT	Diffusive gradients in thin films
DL	diffusive layer
DO	Dissolved oxygen
DOC	Dissolved organic carbon
DSTP	Deep sea tailings placement
EC	Exposure container (mesocosm that contained the TC and surrounding seawater)
Eh	Redox potential
EIA	Environmental impact assessment
GV	Guideline value replaces trigger value (TV) as reported in ANZECC/ARMCANZ (2000) water quality guidelines
h	Hour
kGy	Kilogray (is a radiation absorbed dose measurement unit)
kg	Kilogram
L	Litre
M	mol per litre
mg	Milligram
Milli-Q	High purity deionised water
mL	Millilitre
mm	Millimetre
NOEC	No-observable-effect concentration; the highest tested concentration of a material (toxicant) at which the measured response is statistically indistinguishable from the control response.
PC95(50)	Concentration that is protective of 95% of species (with 50% confidence)
PNG	Papua New Guinea
QA/QC	Quality assurance/quality control
R-value	From DET-DGT measurements Indicates level of metal mobilisation from tailings-sediments to porewaters (R >0.8 indicates very strong short term mobilisation. R <0.2 indicates little to no mobilisation).
Rhizon	A form of porewater suction sampler
SD	Standard deviation
SQGV	Sediment quality guideline value (ANZECC/ARMCANZ, 2000)
SWI	Sediment-water interface
TC	Treatment containers (contained the tailing-sediment treatment)
TOC	Total organic carbon
TRM	Total recoverable metal
TSS	Total suspended solids
WOE	Weight of evidence
WQGV	Water quality guideline value (ANZECC/ARMCANZ, 2000)

Acknowledgments

The authors would like to acknowledge staff at ALS for provision of the tailings. The authors also thank Simon Apte (CSIRO), Geoff Day (GDA) and Grant Batterham (WGJV) for constructive comments during the planning and undertaking of the project. We thank Graeme Batley for review of the draft report.

Executive summary

The Wafi-Golpu Joint Venture (WGJV) has completed an update of the feasibility study (FS) and is currently finalising an Environmental Impact Statement (EIS) to evaluate whether the Markham Canyon in the western Huon Gulf of eastern Papua New Guinea (PNG) is a suitable location for the Wafi-Golpu Project to apply Deep Sea Tailings Placement (DSTP) for the management of its tailings. CSIRO was engaged by WGJV to undertake a six-month study of the geochemistry of tailings-sediment mixtures through time within mesocosms that provided conditions that better simulate the predicted deposition on the ocean floor. Referred to as a 'long term tailings study', the study provided measurements of changes in geochemistry, release of metals, and assessment of metal bioaccumulation and toxicity to benthic organisms. Smaller-scale shorter-term side experiments were used to provide additional information on factors that may influence metal release.

The study used two tailings master composites (BT3 and BT4) to represent the main production 'book ends' over the life of mine (90:10 porphyry:metasediment and 75:25 metasediment:porphyry) and a natural sea floor sediment sample collected from a depth of 3000 metres in the Huon Gulf where both tailings and sediments would likely undergo co-deposition and resuspension as the tailing and natural sediment makes its way towards the likely[1] ultimate deposition location of the New Britain Trench. The tailings used were produced immediately prior to the study. The study included seven treatments (T1 to T7):

- T1 = 100% Huon Gulf sediment (HG)
- T2 = 80:20 BT3:HG
- T3 = 80:20 BT4:HG
- T4 = 20:80 BT3:HG
- T5 = 20:80 BT4:HG
- T6 = T4 (20:80 BT3:HG) covered by 4 cm of HG
- T7 = T5 (20:80 BT4:HG) covered by 4 cm of HG.

The study assessed the geochemistry and release of copper and zinc as chemical toxicants and iron and manganese as indicators of the geochemical status of the deposited sediments using direct measurements of dissolved metals within the overlying waters and sediment pore waters, and through deployment of in-situ passive samplers (diffusive equilibration in thin films (DET) and diffusive gradients in thin films (DGT)). The DET and DGT passive samplers provided high resolution profiles of metals within the sediment porewaters and labile (mobility of) metals released from sediment. The bioaccumulation assessment used a benthic bivalve (mussel) and ecotoxicity assessment an amphipod (small crustacean), both selected owing to their relatively high sensitivity to metals, particularly copper.

[1] Studies are ongoing with regard to sediment transport and deposition along the Markham Canyon and New Britain Trench

This report provides results to the end of week 17 for all 7 treatments, and toxicity and bioaccumulation assessment for tailings-sediment treatments T1, T4, T5, T6 and T7 commenced in week 13. The report also contains results from side-experiments that investigated metal release from 100% tailings and the influence of water temperature, dissolved oxygen and sediment layer thickness on metal release (HG sediment covers).

The total recoverable metal concentrations of two tailings (BT3 and BT4) were 453-525 mg Cu/kg and 34-57 mg Zn/kg. In relation to sediment quality guideline values (SQGVs, (ANZECC/ARMCANZ, 2000)), concentrations (this study) of Cr, Cu and Ni exceeded the SQGVs by factors of 5-14 (SQGV for copper = 65 mg/kg). The metal concentrations were lower than those of the tailing used in the ecotoxicology studies of Adams et al. (2018); those being 915-1570 mg Cu/kg and 472-840 mg Zn/kg, respectively. The differences between the results of this study and Adams et al. (2018) were attributed by the WGJV metallurgist to variability of the ore body for the core samples selected to make up the master composite, which is predominantly based on overall copper and sulfide contents. The dilute-acid extractable metal concentrations of the tailing were 103-113 mg Cu/kg (BT3) and 9-15 mg Zn/kg (BT4), compared to 149-182 mg Cu/kg and 392-432 mg Zn/kg for the corresponding tailings studied by Adams et al. (2018).

The average dissolved copper concentrations in the mesocosms waters exceeded ANZECC/ARMCANZ (2000) water quality guideline values (WQGV (1.3 µg/L)) in the tailings-sediment treatments (T2 to T7), but not in T1 (100% HG). No treatments exceeded the PNG WQGV for dissolved copper of 30 µg/L. No other metals exceeded these WQGVs in any treatments. Dissolved copper concentrations were greater for T2 (7.6 µg/L) and T3 (4.3 µg/L) than the other treatments (generally 1-3 µg/L range). The dissolved copper concentrations were greater for T3 during week 1-6 than thereafter, but T2 concentrations did not decline during the 17 weeks. The copper concentrations in the other treatments were relatively constant.

Porewater metal concentrations showed similar porewater Cu, Fe, and Mn profile patterns in each treatment on week 5 and 11, although concentrations did change according to the composition of the treatment (tailings-sediment). Mobilisation of copper from tailings-sediments occurred 0.5 and 1.5 cm below the sediment-water interface. For T2 and T3 (80% tailings), the porewater peaks of 45-80 µg Cu/L were higher than all other treatments (20-30 µg Cu/L range), while those for T4 and T5 (20% tailings) were not greater than those for T1 (100% HG) nor greater than T6 and T7 that had surface layers of HG. Below 6-8 cm depth the porewater copper concentrations were <10 µg Cu/L for all treatments.

There was no significant bioaccumulation of metals and no acute or chronic toxicity that could be attributed to 20:80 tailings-sediment treatments T4-T7 assessed.

Overall, the benthic fluxes of copper were relatively low and smaller-scale side experiments indicated they may be even lower in the ultimate deep ocean deposition environment. For example, lower dissolved oxygen concentrations and layers of HG sediment as thin as 0.5 cm placed on top of tailings mixtures to 100% tailings and were shown to dramatically lower the dissolved copper release. Therefore the exposure conditions used provided a conservative assessment of the risk of effects to aquatic organisms. Based on comparison of metal concentrations in waters and sediments against guideline values, the risks posed by tailings-sediment mixtures appear low. No adverse effects were determined using direct assessment of toxicity and bioaccumulation using species selected owing to their relatively high sensitivity to copper, the main metal contaminant of concern associated with the tailings. The risk posed by the tailings was demonstrated to be lower for mixtures containing a greater portion of Huon Gulf sediments and for those covered by Huon Gulf sediments. The study provide a high level of confidence for tailings covered by Huon Gulf sediments and for 20:80 tailings:sediment mixtures without an overlying cover of sediment represent a low risk of adverse effects to benthic organism and release negligible copper to overlying waters. The study of the 80:20 tailings:sediment mixtures is ongoing.

1 Introduction

The Wafi-Golpu Joint Venture (WGJV) has completed an updated of the feasibility study (FS) and is currently finalising an Environmental Impact Assessment (EIA) to evaluate whether the Markham Canyon in the western Huon Gulf of eastern Papua New Guinea (PNG) is a suitable location for the Wafi-Golpu Project to apply Deep Sea Tailings Placement (DSTP) for the management of its tailings.

In order to understand the potential impacts of metal based contaminants on the marine environment, the fate (transport and reactions), bioavailability (concentrations and forms available for uptake by aquatic organisms) and potential toxicity (water column and benthic, including dissolved and dietary exposure) need to be understood. In the case of the proposed DSTP, this understanding should extend to tailings in the water column following discharge, during transit to the deep ocean, and following deposition on the ocean floor. To develop the necessary knowledge, a combination of geochemical and ecotoxicological studies, and fate modelling was undertaken. The intent was to develop sufficient knowledge to enable predictions to be made of dissolved and particulate metals concentrations through all of the environments that the tailings may encounter following DSTP-discharge to inform the assessment of potential impacts arising from DTSP in the Huon Gulf.

CSIRO was engaged by Wafi-Golpu Joint Venture (WGJV) to undertake a six-month study (referred to as 'long-term') of tailings-sediment combinations following deposition in seawater, with the intent of enabling measurements of metal geochemistry and release during the study period, and use of bioaccumulation and toxicity bioassays to assess risks of potential adverse effects to benthic organisms towards the end of the study period.

1.1 Conceptual model for DSTP use for the study design

Following discharge from the DSTP pipe at a depth of some 200 m, the tailings are predicted to travel down the steep (15°) canyon walls to the base of the Markham Canyon (700 m). During this process the tailing will entrain and dilute with seawater that contains relatively high concentrations of natural suspended particulate matter (SPM) that originates from multiple rivers (including the Markham and Busu) that discharge into the coastal Huon Gulf environment (estimated at more than 60 Mtpa suspended sediment). The conceptual model (CM) for the tailings depositional environment (TDE) used in developing the study design considered the TDE to be a long gradient (>10 km along the floor of the Markham Canyon) from the near field (500-2500 m water depth) to the far field (2500 to >6000 m). The CM has river-derived SPM (Huon Gulf sediments) as comprising up to 80% (by mass) of the materials co-depositing with the tailings to form sediments along the TDE gradient from the near-field to far-field. The CM has the proposed mixing zone as 2.2 km from end of DSTP pipe through waters from surface to all depths, which includes the zone where plumes may shear off from the density gradient. The CM estimates the time taken for the transit of tailings from the DSTP discharge point to the point of deposition to be some 2 days, but recognised that some materials may deposit early and some later, and that the deposited sediments may continue to move in some areas for months or years after entering the marine environment. Once the DSTP ceases, the CM has the tailings-sediment mixtures becoming covered with natural sediments that will continue to flow into the Huon Gulf.

This is a simplified CM and, to a large extent, relies on the initial results of the hydrodynamic and sediment transport model developed for the Project EIS. Ongoing oceanographic and sedimentology investigations (also undertaken as part of the EIS) strongly indicates a much more complex sediment transport process

where sediments (and tailings) will undergo a continual process of deposition, resuspension, and co mixing associated with fresh natural sediment inputs and the resuspension of previously deposited marine sediments by pervasive bottom attached turbidity currents and mass movement events.

While this CSIRO study was designed to provide knowledge to assist in predicting the risks posed by tailings that deposit on the ocean floor, there are a number of attributes of the predicted depositional environment that were not simulated in the laboratory mesocosms. These included (i) deep-sea conditions relating to low water temperature, higher pressure and low light (complete darkness); (ii) study of a full range of tailings-sediment combinations; and (iii) assessment of risks to benthic organisms by conducting tests on actual deep-sea biota.

The study timeframe was set at 6 months (maximum), with a need to deliver some outcomes within a few months of commencement. A range of measurements were made over the 6-month study period to provide information on the likely 'trajectory' of the metal geochemistry, metal releases and bioavailability.

It was not feasible to conduct a large scale experiment in the laboratory with water temperature or pressure conditions that match those of the deep-sea environment, however, efforts were made to conduct the experiments in the dark (closed containers). A number of shorter-term experiments (weeks) accompanied this long-term study to provide information on how factors such as dissolved oxygen concentrations, temperature and pressure may influence the metal geochemistry.

While a full spectrum of tailings-sediment combinations are possible, only a number of combinations could be considered. This study considered two tailings master composites 'that represent the main production 'book ends' over the life of mine, (i) 90:10 porphyry and metasediment tailings and (ii) 75:25 metasediment and porphry tailings, prepared from a pilot-scale mill-flotation processes. The term master composite means that the sample has been developed from a large number of different drill cores so that it is representative of the ore body. The ratios of tailings:sediment selected for the study were 80:20 and 20:80, to incorporate composition scenarios of majority tailings and majority sediment with recognition that a full spectrum of compositions are likely to exists through the TDE. [Note that a 100% tailings sample was also considered, but during early testing it formed a very compact surface that may have precluded the insertion of testing equipment]. The 20:80 ratio is also reflective of the overall sedimentary inputs to the western Huon Gulf, that being about 16Mtpa of tailings and over 60Mtpa of river inputs. Once the DSTP ceases, these tailings:sediment mixtures will naturally become covered with Huon Gulf sediments, and the study includes treatments that comprise 20:80 tailings:sediment mixtures covered with sediments. A number of shorter bench scale experiments were also conducted looking at different tailings:sediment mixtures and the effect of different sediment cover thicknesses.

Using the hydrodynamic modelling results available at the time, together with oceanographic and bathymetry observations, WGJV and its oceanographic experts identified an area at 3000 m depth adjacent to the Markham Canyon (Huon Gulf) where both tailings and sediments would likely co-deposit. At these depths, the current CM considers the natural riverine sediments from multiple sources to be well mixed by their transport down the canyon and thus be generally representative. WGJV recovered three box-core samples from this general area in order for an overall composite Huon Gulf sample to be produced for the experiment. A specific limitation in the use of the Huon Gulf sediments related to the ability to undertake experiments on sediments imported (foreign materials) from Papua New Guinea to Australia (laboratory) and meet Australian Biosecurity Import Conditions (BICON). In this case, any seawater that comes in contact with the Huon Gulf sediments also becomes regulated by BICON. It was considered not feasible to manage the disposal of the proposed high volumes of seawater necessary for the current study (excessive labour and cost), and it was decided that the imported Huon Gulf sediments must be sterilised by means of gamma-irradiation before use in the study. This aspect of the study design is discussed in the report, and

experiments were undertaken to understand how the irradiation of the Huon Gulf sediments may influence the study outcomes.

The seawater used in the experiment was sourced from the south coast of New South Wales, Australia, as very large volumes were required on an ongoing basis. The chemical composition of seawater is generally conservative (similar) and this use is not likely to alter the study outcomes. The seawater was exchanged on a weekly basis to reflect the natural exchange of oceanic seawater within practical experimental limitations.

1.2 Proposed study approach

The primary objective was to undertake a 6-month study of the geochemistry and release of metals from a series of 7 tailing-sediment treatments within mesocosms, and use bioassays towards the end of the study to assess bioaccumulation and toxicity to benthic organisms.

The mesocosms (exposure chambers) were used to create an environment from which measurements and then predictions could be made about the geochemistry of tailings-sediment mixtures (treatments) once they have deposited (stopped moving), which is particularly relevant to benthic animal interactions that rely on a stable sea-bed. Also assessed were closure scenarios whereby mixtures of tailings were covered by natural sediments. A previous study by Adams et al. (2018) indicated that the metals of most concern from a toxicity perspective in the tailings were copper and zinc, but the assessment may also have been influenced by high concentrations of manganese that were being released from the natural Huon Gulf sediments. This study assessed the release of copper and zinc as chemical toxicants and iron and manganese as indicators of the geochemical status of the deposited sediments.

A range of methods was used to provide data on metal geochemistry, metal releases and bioavailability. This included (i) direct measurements of dissolved metals within the overlying waters, (ii) deployment of diffusive equilibration in thin films (DET) and diffusive gradients in thin films (DGT) samplers (probes) to measure porewater concentrations (using DET) and release characteristics (DGT-induced metal fluxes) (Simpson et al., 2012a; Amato et al., 2014; 2015), (iii) Rhizon samplers (Seeberg-Elverfeldt et al., 2005) to extract pore waters in situ from different depths, and also (iv) destructive sampling of pore water (centrifugation techniques) at the end of tests.

It was necessary to use surrogate species for the bioassays (bioaccumulation and toxicity) to assess risk of potential adverse effects on benthic organisms that may live in the deep-ocean environment and in the future colonise the TDE. The species that were used and the test endpoints were selected for their relatively high sensitivity to metals (Campana et al., 2012; 2015; Simpson et al., 2011; 2013). The amphipod, *Melita plumulosa*, has previously been used for assessing the bioavailability and toxicity of mineral-associated metals in marine sediments (Simpson and Spadaro, 2016). The benthic bivalve *Tellina deltoidalis* was used for assessing survival and bioaccumulation, and has been successfully used in previous bioaccumulation studies (King et al., 2010; Campana et al., 2012; 2015). The use of shallow-water species as surrogate organisms for assessing metal bioaccumulation and ecotoxicity relating to deep-sea organisms is discussed further in the appendices.

The reporting is divided into two stages, reflecting the earlier toxicity testing of treatments containing 20% tailings that commenced in week 13, followed by testing of the 80% tailings treatments that started in week 21. The difference in testing times related to the rate of observed decrease in metal concentrations in the overlying waters and sediment porewaters as the study investigated the geochemical changes occurring before the toxicity testing was initiated, which concluded each treatment's study (entailed physical disturbance to the tailings/sediments mixtures).

This report (Stage 1) contains the results to the end of Week 17 and includes results for tailing-sediment geochemistry and release of metals throughout this period for all 7 treatments, and toxicity and

bioaccumulation results for 5 of the 7 tailings-sediment treatments. A second report (Stage 2) will be prepared after 26 weeks of study for the 2 remaining tailings-sediment treatments that had the 80:20 ratio of tailings:sediment.

2 Methods

2.1 Seawater, tailings and Huon Gulf sediment

2.1.1 Preparation of seawater

The seawater for use in the study was sourced from the south coast of New South Wales, Australia. For the purpose of this study, differences in the composition of this seawater and the seawater of the Huon Gulf were considered to be negligible. Two 2-tonne (2000 L) seawater holding tanks were available and were re-supplied on demand with clean seawater (e.g. weekly if required). The seawater quality was checked prior to use and regularly during use, including analyses of dissolved metals. The pumps used to transport seawater from tank to tank and to the chambers had all plastic internal parts and additional checks of water quality made regularly (Appendix A).

2.1.2 Preparation of tailings

Two master composite tailings materials were produced "fresh" for the study (Table 1):

Bulk Tails-3: 90:10 porphyry and metasediment tailings. This was the same mix as Bulk Tails-1 used in the Chemistry-Ecotoxicity study by Adams et al. (2018).
Approximately 60 kg (dry weight equivalent) as a wet tailings solid with overlying tailings liquid, was split across four 20-L containers, delivered to CSIRO on November 14, 2017.

Bulk Tails-4: 75:25 metasediment and porphyry tailings. This was the same mix as Bulk Tails-2 used in the Chemistry-Ecotoxicity study of Adams et al. (2018).
Approximately 50 kg (dry weight equivalent) as a wet tailings solid with overlying tailing liquid, was split across four 20-L containers, delivered CSIRO on November 14, 2017.

Photos of the tailings as received and during preparation are provided in Appendix A.

The main difference in the tailings used in this experiment is that it was freshly prepared and not retained in cold storage for a period (approximately 6 months) prior to use (as in Adams et al. (2018)).

Table 1. Tailings samples for study

Sample	Received	Composition	Also known as
Bulk Tails-3 (BT3)	November 14, 2017	90% porphyry:10% metasediments	P0949 Test 1, P80 = 106 µm
Bulk Tails-4 (BT4)	November 14, 2017	25% porphyry : 75% metasediments	P0949 Test 2, P80 = 106 µm

Upon arrival at CSIRO, and checking of chain of custody forms and labels, the tailings liquid from each container was decanted into a clean 2-L container and the remainder discarded. The tailings solids were highly consolidated (photos in Appendix A), and required considerable force to break apart. Although all four containers (A, B, C, D) of each tailings were expected to have the same composition, sub-samples of each were taken to composite and homogenise (photos in Appendix A). Approximately equal amounts of each of the Bulk Tails were randomly distributed to 88-L clean new plastic containers (photos in Appendix A) (three containers for Tails-3 and 4). Approximately 10 kg of each tailing and several litres of tailings liquid were kept for separate studies.

Based on the conceptual model and predicted initial deposition of the tailings potentially within 2 days of discharge, the tailings were provided several exchanges with clean seawater, simulating the flow down the Markham Canyon as a density-gradient, before being mixed with the Huon Gulf sediments to form the various treatments. It was recognised that some materials will have greater exchanges with seawater, and some will have less depending on where they deposit and how they are transported. During the transport, the tailings would likely mix with some Huon Gulf sediments before depositing on the sea floor, however, both the tailings and Huon Gulf sediments were not available at the same time and their preparation was initially undertaken separately for practical reasons. Similarly, WGJV advised that supporting oceanographic studies also provide evidence of frequent mass movement (landslide) events that help to transport natural sediments down the canyon and will variably mix and co-deposit with tailings, likely over multiple events.

Washing of the tailings with seawater was achieved by adding 50 L of clean seawater to each container of 10-15 kg of tailings and then a nylon spoon was used to mix and resuspend the tailings (for about 5 min) before allowing to settle until the next seawater exchange. The overlying seawater was sub-sampled for dissolved metal analyses before the next exchange, when the overlying seawater was replaced. After the three tailings washing cycles, the overlying water was removed and the separate fractions were combined, homogenised and then stored under refrigeration until use to prepare mixed tailings-sediment treatments. Due to the compact nature of the initial wet tailings, and the time taken for the fine tailings materials to settle out following resuspension in seawater (washing cycle), the tailings were washed initially on a Friday and allowed to settle over 3 days, then washed a second time on the Tuesday and allowed to settle for a further 3 days to enable the added seawater to be exchanged. Finally, to facilitate the preparation of the proportions of tailing required for each of the treatments (tailing:sediment mixtures) the tailing were mixed with a smaller ratio of seawater, and a portion of this seawater was later discharged as the tailing sediment mixtures settled.

2.1.3 Preparing the sediment

The Huon Gulf sediments were collected from approximately 3000 m water depth using a box core, which obtained three separate grab samples during November 25-28 (Geoff Day, personal communication) and were delivered to CSIRO on December 1, 2017. Redox potential measurements (though the entire 42 cm profile), taken immediately after collection were +50-150 mV. To aid in the gamma irradiation treatment on receipt by CSCIRO, the bulk sediments were divided into 48 sediment samples (generally ~6 kg each) and shipped in 16 containers with a total sediment mass of 350 kg (Appendix A). Upon arrival, the contents was checked and then dispatched to ANSTO GATRI facility for gamma irradiation (quarantine treatment of 50 kGy).

The process of gamma irradiation to sterilise the sediments may alter the properties of the sediments as well as killing foreign organisms. Before irradiation and following homogenising, a 1.3-1.5 kg subsample was removed from four of the bags and these sub-samples were then stored separately (BINCON procedures) for the purpose of undertaking experiments to test the influence of irradiation on the properties of the Huon Gulf sediments. Outcomes of these tests are described in Section 4.1.1.

Following gamma-irradiation treatment of the Huon Gulf sediments, a composite of approximately 300 kg of wet sediment was prepared by randomly selecting sterilised bags of sediment from each of the 16 irradiated containers and transferring the contents randomly to one of four clean new 88-L plastic containers. Within these containers the sediments were thoroughly homogenised by gloved hand and then resuspended in clean non-sterile seawater. As the intent of the study was to investigate metal biogeochemistry, the mixed composite (sterile) sediments were inoculated with a small fraction of non-sterile sediment that is used by CSIRO for culturing benthic organisms. This non-sterile sediment had been collected from a local estuary (Bonnet Bay, NSW) and together with the microorganisms present in the seawater was expected to provide a new population of microorganisms that may facilitate biogeochemical cycling of elements such as iron, manganese and sulfur. The amount of Bonnet Bay sediment was 2% by

mass of the total amount of Huon Gulf sediment (dry weight equivalent), and the two were mixed in the presence of the added seawater. This amount was considered sufficient to re-introduce (inoculate) an adequate amount of microorganisms that are present in all sediments. The inoculated Huon Gulf sediment mixture was then allowed to settle before overlying water was removed and then pairs of containers were combined and mixed before separating again. The density and water content of this material was then determined in order to calculate amounts/volumes necessary for the tailings-sediment treatments. The sediments finally were briefly mixed again before use to prepare tailings-sediment mixtures of set up treatments.

2.1.4 Preparation of the washed tailings-sediment mixtures

The treatments that comprise separate tests had the following compositions:

- Treatment-1: 100% Huon Gulf (HG) sediments (composite as described above)
- Treatment-2: Mixture of 80% Bulk Tails-3 (tailings):20% HG composite sediment
- Treatment-3: Mixture of 80% Bulk Tails-4 (tailings):20% HG composite sediment
- Treatment-4: Mixture of 20% Bulk Tails-3 (tailings):80% HG composite sediment
- Treatment-5: Mixture of 20% Bulk Tails-4 (tailings):80% HG composite sediment
- Treatment-6: Treatment-4 (tailings-sediment mixture) overlain by 4 cm of HG composite sediment
- Treatment-7: Treatment-5 (tailings-sediment mixture) overlain by 4 cm of HG composite sediment.

All percentages are on a dry-weight basis.

These treatments incorporate composition scenarios of majority tailings and majority sediments co-deposition, and once the DSTP ceases, the cover of these tailings-sediment mixtures with the continued discharge of large volumes of riverine sediments into the western Huon Gulf (estimated at over 60 Mtpa by GDA Consult Pty Ltd and IHAconsult. 2018). By comparison the anticipated maximum proposed annual discharge of tailings by WGJV is approximately 16.5Mtpa, or ~27% of the total estimated suspended sediment inputs to the Huon Gulf.

Note that a 100% tailings sample was also considered as part of the experimental design, but during early testing it formed a very compacted surface that may have precluded the insertion of testing equipment, so it was decided to proceed with the addition of two treatments with 20% HG composite sediment (Treatments 2 and 3), which is also more representative of longer term co-depositional considerations. Additional side-experiments were undertaken to compare metal release from 100% tailings with the 80:20 and 20:80 tailings: Huon Gulf sediment results (Section 2.4).

The depth of 4 cm was chosen to represent a depth of cover that might readily develop after the DSTP ceases. Additional side-experiments were undertaken to compare metal release from tailings and Huon Gulf sediment with thinner cover depths.

The preparation of the tailings-sediment mixture used wet/dry weight information to calculate the amount of wet tailings and wet Huon Gulf sediment that was to be mixed to achieve the desired %-tailings. The mixtures were repeatedly mixed four times over a week prior to use in the long-term experiments. Excess materials for other tests here then stored refrigerated within heavy duty plastic bags with minimal head space and within 30-L plastic drums.

2.2 Long-term experiments

2.2.1 Design considerations

Mesocosms were used to create an environment more closely representing an open ocean environment; having a greater volume of overlying seawater relative to tailing:sediment mass and surface area, and consequently providing a means for increased dilutions of released metals.

The design of the treatment containers (within the mesocosms) was influenced by the amounts of tailings-sediment materials practically available for the study as well as the study data needs. The tailings-sediment materials were held in 9-L treatment containers (TC, 31 L×22cm width×18 cm height) that were within a larger 110-L chamber (52 L×42 cm width×52 cm height) that contained 75 L of seawater (Exposure chamber (EC) – forming the mesocosms). The EC was able to be interconnected to a second 110-L chamber of seawater (85 L Seawater reservoir (SR) – expanding the mesocosms) to provide a means for increased dilutions where necessary, representing an open ocean environment. The seawater was circulated from the SR (in early stages of the experiments) or from within the EC (in later stages of the experiment when dissolved metal releases where determined to be relatively low) back to the EC at the same depth as the surface of the TC where it was split into a series of water streams that created a water current (flow) across the top to the tailings-sediment mixtures. An additional blank treatment (T0) was prepared and operated that did not have a TC present.

***[Note:** After three weeks of operation, the SR was disconnected as the concentrations of released metals into the EC alone were determined to be achieving relatively low dissolved metals concentrations due to a measured slowing in the rate of release dissolved Cu, Mn and Zn].*

Treatment containers (TC)

Aspects considered for TC design (~9-L; 31 L×22 cm width×18 cm height; ~680 cm^2 surface area):

- Most benthic organisms live within the top 10 cm of sediments. Undisturbed, sediments deeper than 10 cm usually become anoxic as sulfide is produced by microbiologically-mediated processes and accumulates when sufficient labile organic matter is available and oxygen donors before sulfate have been depleted (e.g. dissolved oxygen, nitrate, and labile iron/manganese oxides are low) (Jorgensen and Revsbech, 1985; Canfield et al., 1993; Wang and van Cappellen, 1996). The biogeochemical reactions within these surface sediments have a strong influence on the partitioning of metals between sediment and pore waters and the release (fluxes) of metals to overlying waters and toxicity to organisms (Van Cappellen and Gaillard, 1996; Simpson et al., 2000; Di Toro, 2001; Simpson and Batley, 2003; Simpson et al., 2012b).
- This study sought information on the geochemical profiles of porewater metals that develop in the sediments and influence the metal release (fluxes) to the overlying waters. Information on these geochemical parameters was to be gathered through: (i) direct measurements of dissolved metals within the overlying waters, (ii) deployment of DET and DGT samplers (probes) to measure porewater concentrations (using DET) and release characteristics (DGT-induced metal fluxes) (Zhang et al., 1995; Simpson et al., 2012; Amato et al., 2014; 2015; DGT Research Ltd. http://www.dgtresearch.com/), (iii) Rhizon samplers (Seeberg-Elverfeldt et al., 2005) to extract pore waters in situ from various depths, and also (iv) destructive sampling of pore water (centrifugation techniques) at the end of tests. The treatment and exposure chambers needed to have adequate dimensions to allow for the deployment and sampling using these techniques.
- The DET/DGT probes have a measuring 'window' of approximately 2.5 × 15 cm and were deployed to provide profiles of porewater metals and DGT-induced fluxes within 0-3 cm of overlying water and within the sediments from 0-12 cm deep. A 4-cm depth of 'overlying HG-sediment' in Treatments 6 and 7 was selected to enable a geochemistry redox-profile to develop in this surface material and also enable assessment of changes in the deeper sediments to be examined by the DET and DGT probes.

The 2× DET and 2× DGT (in some cases more) were deployed on 3 or 4 occasions, by inserting into the treatments for 24-48 h.

- The Rhizons were placed within the treatments during set up to enable the extraction of small volumes pore waters at the location (horizontally, 2×40 mm sampling porous cylinder with a mean pore size of 0.1 µm and maximum of 0.2 µm). The uppermost Rhizon samplers were placed 3.5 cm below the initial SWI, recognising that some settling of material may occur so that this becomes 2.5 cm below the SWI. The 2nd and 3rd Rhizon samplers were placed 5 cm and 10 cm further down (i.e. 8.5 and 13.5 cm below the SWI). The height of the TC was 18 cm (~17.7 cm internal) and holes were made for the Rhizon inserts on the side of the container at ~4.5 cm from the end of the container and below the container lip by 3.5, 8.5 and 13.5 cm (the bottom sampler being ~14.2 mm from the bottom). The Rhizons were sampled in the week after the DET and DGT deployments and were positioned at the other end of the TC to where the DET/DGT deployments occurred.
- Destructive sampling of pore water was undertaken at the end of the bioaccumulation test period, with sediment taken in duplicate from three depths to provide data on porewater metals (particularly Cu, Zn, Fe, Mn) in the more oxidized zone (e.g. oxic (0-3 cm), sub-oxic (3-10 cm), and fully anoxic deeper sediment (10 cm-bottom)).

Photographs of the set-up are shown in Appendix A.

Exposure chambers (EC)

Aspects considered for exposure chamber design (~ 110-L chamber; 52 L×42 cm width×52 cm height):

- They were intended to provide a high level of dilution of released metals, in an attempt to mimic ocean conditions, but also not result in such great dilution that measurements of concentrations became too difficult (i.e. analyses of dissolved metals at parts per trillion concentrations (sub-µg/L) is very specialised and expensive).
- They should enable a diffusive flow of seawater to be directed across the surface of the TC, with a flow rate designed to be similar to that at the ocean flow but not resuspend the tailings-sediment materials (not scour the surface) (estimated flow rate 0.05 m/s with relatively low currents, Grant Batterham (WGJV) personal communication). For operations within the EC without the SR, the outlet was at the far end of the TC, and assisted with drawing water across the surface (i.e. flow directed from the front to the back of the TC).
- Flow rate calibration: The design enabled a water current to be directed across the top of the tailings-sediment within the treatment containers (TC), but not through the entire exposure chamber as a cross-section. The flow rate directed to the top cm over the SWI was calibrated by measuring the time taken to pass a known volume of water through the water-splitter (that created 12 jets of water ~2.5 mm diameter each, spanning across a width of 15 cm). Based on multiple measurements over the 16 weeks to date the flow rates achieved varied within ~30% of each other, but were not consistently faster for any one pump or treatment. The mean flow rate from the water splitter was 87 mL/s (77-111 cm^3/s) and 19 cm/s out of the jets. Over the 31 cm length of the TC we assumed dispersion of the water stream occurred to create a current of 0.5 cm depth at the front and 1 cm depth at the end of the TC and spreading to 22 cm width of the container. Based on this the estimated flow near the sediment water interface was 8 cm/s at the front (pushing) and 4 cm/s at the end of the container (drawn towards the outlet).
- To provide the means of increasing the volume of circulating seawater, an interconnecting seawater reservoir (SR) existed that comprised a second 110-L container interconnected with the EC via a port-tube.
- A pump (all plastic internal parts) circulated the seawater taking it from the top of the EC (or SR if in use) and directing the water via a diffuser cross the TC. If the SR was in use, the outlet from the EC would be at the top of the water level and result in unconstrained over-flow back to the SR.

Photographs of the set-up are shown in Appendix A.

2.2.2 Operation

All experiments were undertaken in temperature-controlled (e.g. 19±2ºC) laboratory environment. The environment was regularly cleaned and kept tidy. Acid-washed clean plastic tubing was used for seawater circulation to and from the pump, and, to minimise fouling of tubes internally due to algae-bacterial biofilm growth, the majority of the set-up was covered by thick black plastic to minimise light. Any tubing that appeared to be becoming fouled was replaced with new clean tubing (generally this was done monthly). The pumps circulating the seawater within the tanks were run on a continuous cycle of 1-h_On, 1-h_Off to prevent overheating during the study duration.

The seawater was changed weekly by using additional pumps to draw out approximately 95-98% of the seawater and then replacing this with new seawater. The quality of the incoming seawater was checked weekly at the time of taking samples from the exposure chambers.

A range of measurements were made on the materials in each treatment at the start and completion of the experiment. Measurements included total recoverable metals (TRM), dilute-acid extractable metals (AEM), acid-volatile sulfide (AVS), total organic carbon (TOC), particle size (<63 µm) and pH and redox potential (Eh) at the end of the bioaccumulation test.

Water quality measurements of pH, salinity, dissolved oxygen and temperature were made weekly (Appendix A2) and sampling taken for dissolved metal analyses from all tanks weekly (EC and SR when both in use), usually immediately prior to water changes.

The DET and DGT probes were deployed within the end of the chambers (TC) closest to the seawater flow. Application of DET and DGT samplers occurred on three occasions for T1, T4, T5, T6 and T7 (start of Weeks 5, 11, and 16), and for four occasions for T2 and T3 (Weeks 5, 11, 16 and 24). DET probes provide high resolution concentration profiles of metals in pore waters (e.g. Cu, Zn, Fe, Mn) and enabled flux calculations that provide the geochemical kinetic inputs to a geochemical and hydrodynamic model being developed in parallel by Tetra Tech in collaboration with CSIRO and WGJV. DGT probes provided a direct measure of remobilisation of metals within pore waters and a different insight into the metal fluxes and risks of excessive metal exposure for benthic organisms. Rhizon sampling occurred from the far end of the TC at the start of the week after the DET-DGT deployments (Weeks 6, 12, and 17 and 24).

2.3 Assessment of effects to benthic organisms

During the equilibration period for the long-term study treatments prior to toxicity and bioaccumulation tests (that commenced during week 13), it was expected that the bioavailability of metals within the sediments and release to overlying waters would have decreased significantly (e.g. due to metal release and diagenetic reactions that might result in stronger binding to newly-formed sulfide phases). This did not consider bioturbation (burrowing, burrow-irrigation) activities of benthic macroinvertebrates that might disturb the metal biogeochemistry and alter rates of metal release to pore waters and overlying waters. WGJV have advised that other studies have identified a general absence of macroinvertebrates within the Markham Canyon (proper) due to the highly dynamic natural riverine sediment transport down this conduit, including mass movement (landslide) events. However, bioturbation effects are a consideration for the deep, more stable, oceanic depositional environment.

Bioaccumulation and sub-lethal toxicity tests were used to assess risks that the tailings:sediment mixtures posed to benthic organisms, as per Adams et al. (2018). The bioaccumulation and toxicity (survival) assessment was undertaken over a 30-day period using the bivalve/clam (*Tellina deltoidalis*). The ecotoxicity assessment was undertaken over a 10-day period using an amphipod (*Melita plumulosa*) and assessed the effect on both survival and species reproduction. Both these species offered high sensitivity to metals, multiple exposure routes (dissolved and dietary) and have consistently provided robust outcomes in similar marine sediment quality studies conducted by CSIRO previously.

In-situ testing on undisturbed sediments within the mesocosms was considered for both test methods, but was not practical due to design of the amphipod survival-reproduction test (see Section 2.7). *In-situ* tests

were undertaken for bivalve bioaccumulation, but surface sediments were removed for the amphipod toxicity tests *ex-situ* (standard method). For the *in-situ* tests, a panel was inserted to divide each treatment container (tailings/sediment) into two equal-sized sections (photos in Appendix A). The panel extended to the base of each treatment and to approximately 4 cm above the surface of the treatment into the overlying water. The bivalve bioaccumulation tests were conducted by adding the bivalves to one side of the dividing panel. The amphipod toxicity tests were conducted on surface sediment (0-3 cm) carefully removed from the other side of the panel. In relation to how the tests design may influence the metal exposure to the two species the following points are made: (i) the removal of the sediment surface layer on one side of the divider will have exposed anoxic material to oxygenated water, and potentially facilitated oxidation of metal sulphides uncovered by this manipulation and potentially resulted in greater metal release that would otherwise not have occurred; (ii) the disturbance of the surface sediments during preparation of the amphipod tests may have modified the metal release. For the bivalve bioaccumulation test, the design may result in the outcomes of those tests being more conservative (i.e. greater metal exposure than may have occurred with disturbance). For the amphipod toxicity test, the disturbance of the surface sediment may have increased or decreased metal release, however these tests were conducted in smaller test chambers than the mesocosms and this results in significantly greater concentrations of metals in the overlying waters than occurred in the mesocosms. Consequently the metal exposure in the amphipod tests is considered conservative, i.e. higher than would occur *in-situ* in the mesocoms and higher than what may be expected in a deep open ocean environment.

The bioaccumulation and toxicity test procedures are described in Section 2.7 and Appendix D. Metal release to overlying waters was monitored during the tests to provide information on metal exposures that may contribute to any observed effects.

For treatments T1, T4, T5, T6 and T7, the bioaccumulation and toxicity tests commenced during Week 13 of the study, and DET-DGT deployments were made onto the sediments that contained the bivalves during Week 16 of the study (Week 4 of the bioaccumulation test). Noting that previous DET and DGT deployments were on Weeks 5 and 11 of the study.

The results of the bioaccumulation and toxicity tests on T2 and T3 will be reported in Stage 2. For treatments T2 and T3, the bioaccumulation and toxicity tests will commence during Week 21 of the study (and are not reported in this Stage 1 report). For those treatments, DET and DGT deployments were undertaken for: (i) treatments that did not contain organisms on Week 16, and (ii) treatments that will contain the bivalves during Week 23 of the study (Week 4 of the bioaccumulation test). Noting that previous DET and DGT deployments were also on Weeks 5 and 11 reported in this report (Stage 1). As T1 was sacrificed during the bioaccumulation and toxicity tests of T4 to T7, an additional T1 (T1B) was set up on Week 8 of the study and had seawater exchanges occurring fortnightly throughout the study period in order to be used as a control for the bioaccumulation and toxicity tests of T2 and T3.

2.4 Short-term studies of other factors influencing metal release

2.4.1 The influence of irradiation of the Huon Gulf sediment on metal release

In order to conduct the large-scale long-term experiment within the project timeframe it was necessary to gamma irradiate the sediment as a quarantine treatment to destroy lifeforms. This was undertaken on the Lucas Heights site at ANSTO's GATRI facility to irradiate the sediments being used for the long-term tailings study with 50 kGy. At the time, 4 of the 48 separate bags of the Huon Gulf sediment were separated for an experiment to assess the effect of irradiation on the sediments. The four sediments were homogenised, then split into two amounts of 2-3 kg each and one half of each was irradiated with the other sediments. Following irradiation, both pairs of the 4 sediments were stored refrigerated for later tests on the influence of irradiation on metal release. For one Huon Gulf irradiated/non-irradiated sediment pair, metal concentrations were measured in total and two dilute-acid extractable forms (1 M HCl/60 min and 0.2%

HNO_3/5 min) and also in pore waters, and TOC was analysed before and after irradiation. The tests were set up in 400 mL beakers containing 4 cm depth (~185 g) of each of the Huon Gulf sediments (irradiated and non-irradiated) and 242 mL of overlying water. The beakers were gently aerated and all tests conducted under the standard laboratory conditions (temperature = 19±2ºC, constant darkness). Water changes occurred once a week.

2.4.2 The influence of Huon Gulf sediment layer thickness on metal release

These tests were undertaken to determine whether thin layers of 0.5 or 1 cm of Huon Gulf sediment (HG) were sufficient to cap the tailings and result in a lower dissolved copper and zinc release. The treatments included:

- 100% tailings BT3 (Table 1) with 0, 0.5 and 1 cm depths of HG on top (replicated)
- 80:20 BT3:HG (T2 in mesocosms) and 20:80 BT3:HG (T4 in mesocosms) with 0 and 0.5 cm HG on top
- 100% tailings BT4 and 80:20 BT4:HG (T3 in mesocosms) with 0 cm and 0.5 cm HG on top (replicated).

The tests were set up in 400 mL beakers containing 3 cm depth (~135 g) of each tailings sample, followed by the HG layer and then 242 mL of overlying water. The beakers were gently aerated and all tests conducted under the standard laboratory conditions (19±2°C, at constant darkness) for a duration of 14 days. Water changes occurred once a week. Additional treatments to assess attenuation of metal release from a 2 cm HG layer were undertaken by modification of treatments containing 100% tailings BT3 and BT4 and the 0.5 cm HG layer after 14 days. To each of the original treatments an additional 1.5 cm of HG was added to create a total 2 cm HG layer and the experiment continued for a further 12 days. The new treatments were:

- 100% tailings BT3 with 0 or 2 cm depths of HG on top
- 100% tailings BT4 with 0 or 2 cm depths of HG on top.

Photos of 0.5, 1 and 2 cm layers of Huon Gulf sediment over tailings are shown in Appendix A.

2.4.3 The influence of temperature on metal release

These tests were undertaken to determine whether cooler temperatures more representative of the deep ocean could result in lower dissolved metal release from the tailings. The treatments included 80:20 tailings:HG using BT3 and BT4 (Table 1) at temperatures of:

- 2±1°C (cold room)
- 6±1°C (in refrigerator with thermostat set high; replicated)
- 19±2°C (normal laboratory conditions)
- 29°C (tropical culturing room).

These temperatures spanned the range of Huon Gulf field data provided by WGJV (GDA Consult Pty Ltd and IHAconsult. 2017) that indicates that temperatures decrease from approx. 27°C in the surface waters to 14°C at 300 m depth, 6°C at 700 m depth, then to 1.9°C at 3,200 m depth. The tests were set up in 400 mL beakers containing 3 cm depth of each tailing:HG mixture and then 242 mL of overlying water. The beakers were gently aerated and all tests conducted under the standard laboratory conditions (constant darkness) for 14 days. Water changes occurred once a week. Examples of temperature logs for the test environments are shown in Appendix A.

2.4.4 The influence of dissolved oxygen on metal release

These tests were undertaken to determine whether low dissolved oxygen concentrations resulted in lower dissolved metal release from the tailings. The treatments included 100% BT3 and BT4 (Table 1) and 80:20 BT3:HG with paired treatments having dissolved oxygen:

- 0-7% DO saturation (<0.5 mg/L DO)
- 85-100% DO saturation.

The tests were set up in 400 mL beakers containing 3 cm depth of each tailings or tailings:HG mixture and then 242 mL of overlying water. The beakers were gently aerated and all tests conducted under the standard laboratory conditions (19±2°C, constant darkness) for 14 days. Water changes occurred once a week.

2.5 General test methods

Clean seawater was collected from Port Hacking, Sydney, Australia, membrane-filtered (1 µm), and acclimated to a room temperature of 21 ± 1°C. The salinity of the filtered seawater was adjusted to the test salinity of 30‰ using Milli-Q deionised water (18 MΩ·cm; Milli-Q® Academic Water System).

The analysis of trace metals necessitated the application of rigorous protocols for container preparation, sample collection and analysis, to ensure the accuracy of results. All plasticware used for the processing and analysis of trace metals was new and acid-washed prior to use with a minimum soak of 24 h in 10% v/v nitric acid (Merck, AR grade). Samples for dissolved metals analysis were filtered through acid-washed 0.45-µm syringe filters (Sartorius, Australia). All samples were acidified with 0.2% (v/v) concentrated nitric acid (Tracepur, Merck).

General methods for physical and chemical analyses of the waters and sediments undertaken by CSIRO are provided in Table 2.

2.5.1 Quality control: general procedures and analysis acceptance criteria

For all analyses, at least three analytical blanks were measured per batch of samples for the determination of mean blank metal concentrations and limits of detection (3 × standard deviation (3σ)).

For all analyses, at least 10% of the samples had method duplicates analysed to confirm the precision of analytical procedures.

To assess the potential matrix interferences during metals analyses, at least 10% of the samples included spike recoveries performed (except for weekly monitoring of dissolved metals in seawater).

To confirm the analytical accuracy, aliquots of reference materials from the National Institute of Standards and Technology (NIST), and the Institute for Reference Materials and Measurements (IRMM) were analysed with each batch of samples whenever a suitable reference material was available. Reference standards have certified concentrations of elements for a range of sample matrices such as seawater, sediment and fish, allowing the performance of the analytical procedures to be assessed by a comparison of the results obtained with the certified concentrations. The following reference materials were used: ERM-CC018 (IRMM) for metals in sediments and NIST2976 (NIST) for bivalve tissue metals.

The general acceptance criteria for the analyses included:

- Method duplicates; relative standard deviation is 100% for concentrations ≤5 times limit of detection (LOD), 50% for concentrations between 5 to 10 times the LOD, and 20% for concentrations ≥10 times the LOD;

- Spike recoveries; within 85-115%. Spike recoveries are investigated if outside this range. A common cause of poor spike recoveries is the metal spike is low relative to the concentration in the sample (i.e. spike less than a quarter of the measured concentration). For a batch of samples, if spike recoveries are acceptable for all samples other than ones where the spike is low relative to the measured concentration, the poor spike recoveries are treated as not being representative and are ignored; and,
- Certified reference materials (CRMs); within 85-115% of the certified value specified by certifying authority or within the certified concentration range. CRM material recoveries are investigated if outside this range. Common causes of poor CRM recoveries are the concentrations are low and near the LOD, or are not homogenous for particulate matter.

Table 2. General physical and chemical analysis methods for waters

ANALYTE	METHOD
Water pH, dissolved oxygen (DO) and salinity	Measurements of pH (calibrated against pH 4.0, 7.0 and 10.0 buffers) used a pH meter (HI98191) equipped with a spear-tip FC200B probe (Hanna instruments). DO and temperature measurements were made using a HI5421 DO meter (Hanna) using saturated and zero oxygen solutions. Salinity measurements used a WTW meter (LF 320) with a Tetra-Con 325 probe, and were reported according to the Practical Salinity Scale of 1978 (PSS 78) as dimensionless values.
Dissolved metals by ICP-AES	(APHA 21st ed., 3125; USEPA (2007) SW846 - 6020): The inductively-coupled plasma atomic emission spectrometry (ICP-AES, Varian 730-ES) using in-house methods (C-209 and C-229, respectively). Dissolved metals were those that passed through a 0.45 µm membrane.
Dissolved metals by ICP-MS	(APHA 21st ed., 3125; USEPA SW846 - 6020): The inductively-coupled plasma mass spectrometry (ICP-MS, Agilent 8800) in-house method C-209 utilises a highly efficient argon plasma to ionise selected elements. Ions are then passed into a high-vacuum mass spectrometer, which separates the analytes based on their distinct mass to charge ratios prior to their measurement by a discrete dynode ion detector.
Porewater extraction	Porewater was isolated from sediment by completely filling a 50 mL centrifuge tube with sediment (zero head space) then centrifuging at 1000 g for 3-5 min. Upon opening the overlying porewater was immediately taken using a syringe and filtered (<0.45 µm) within 10 seconds and then acidified to 0.2% HNO_3 for preservation. The concentrations of dissolved metals were determined by ICP-AES.
Dissolved ammonia	Dissolved ammonia was analysed colorimetrically using an ammonia test kit (API) using a refined method based on the manufacturer's instructions.
Total recoverable metals (TRM)	Total recoverable metal (TRM) in tailing-sediment solids were determined following aqua regia digestion [CSIRO Method C-223]. The precision and accuracy of the methods was checked by the analysis of blanks comprising of at least 10% of the sample batch, as well as the analysis of certified reference materials.
Dilute-acid extractable metals (AEM, 1 M HCl)	Dilute-acid extractable metals (AEM) were determined by digesting the sediment in 1 M HCl (~1 g/100 mL) for 60 min, followed by filtration (<0.45 µm) [CSIRO Method C-241]. This metal fraction was designated as the SEM fraction, and the difference between the molar amounts of AVS and SEM (Cd, Cu, Ni, Pb, and Zn) was used according to AVS-SEM theory to predict risk of adverse effects of these metals.
Metals in biota tissues	Metal concentrations in the tissue of bivalves (bioaccumulation test) were determined following a high pressure microwave digestion (200°C) [CSIRO Method C-225]. The precision and accuracy of the methods was checked by the analysis of blanks comprising of at least 10% of the sample batch, as well as the analysis of certified reference materials.
Particle size	The fine sediment fraction (<63 mm) was determined gravimetrically by wet sieving with deionised water through a nylon mesh. Laser particle size analyses were made using a Malvern Mastersizer 3000.
Total organic carbon (TOC)	Total organic carbon (TOC) analysis was conducted using a high temperature CO2 evolution method, in which dried and crushed samples were acid-treated to remove inorganic carbonates followed by combustion (LECO furnace) in the presence of strong oxidants/catalysts and infrared detection of CO2.
Acid volatile sulfide (AVS)	Acid volatile sulfide (AVS) and simultaneously extractable metals (SEM) were analysed according to Simpson (2001). Any samples for AVS analyses were stored frozen in a container with no head space or a nitrogen atmosphere immediately after sampling, and all subsequent handling, including thawing, was undertaken in a nitrogen gas atmosphere (De Lange et al., 2008). In this AVS method, 2.5 ml of methylene blue reagent in 22 M sulfuric acid was reacted directly with 0.1 to 0.4 g of wet sediment in 50 ml of deoxygenated, deionised water (final H^+ concentration, ~1 M). After 1 h, the liberated sulfide was determined colorimetrically at 670 nm using an ultraviolet–visible light spectrophotometer, and the molar AVS concentration was calculated.

2.6 Determination of metal concentrations using DET and DGT

Water DGT measurements (72-h) were made on Weeks 4, 10 and 15, and sediment DET and sediment DGT measurements (24-h) were made on Weeks 5, 11, and 16. The results for only the first two deployments are reported in this first report.

2.6.1 Water DGT

DGT water samplers (n = 2) were deployed in the overlying water for about 3 days (exact times recorded) at <10 cm depth and removed prior to the deployment of sediment samplers. The removed DGT devices were rinsed thoroughly with deionised water. Each binding layer was eluted in 1 mL of 1 M HNO_3 for at least 24 h and diluted 5-fold with 2% HNO_3 before analysis by ICP-MS.

2.6.2 Sediment DET and DGT probe preparation, deployment, removal and analysis

Oxygen was removed from DET and DGT samplers by placing them in narrow-mouth containers in 0.2 M NaCl (with about 10 g of Chelex 20 resin to remove metals), bubbling with N_2 gas for at least 8 h and storing under a N_2 atmosphere.

Photos of the deployed DET-DGTs, their removal and preparation for analysis and shown in Appendix A.

DET sediment samplers (n = 2-4), a DGT sediment sampler with 0.8 mm diffusive layer thickness (n = 1) and a DGT sediment sampler with 0.4 mm diffusive layer thickness (n=1) were deployed in each treatment chamber with sediments (T1-7). All samplers were deployed with 1-2 cm above the sediment-water interface and to at least 10 cm depth. For the Week 11 deployment, an extra step was used for the deployments, with a 0.5 mm thick sheet of acrylic inserted into sediment at the back of the samplers, thereby pushing the sampler forward slightly. The purpose of this was to improve the contact between the sampling window and the sediment. The samplers were removed after they had been deployed for at least 24 h (exact times recorded for DGTs) and were thoroughly rinsed with deionised water to remove every sediment particle.

The gel layer for DET and the binding layer for DGT were removed from the samplers and sliced at resolutions varying from 4 to 20 mm using a Teflon-coated razor blade (changed for each sampler). These sub-samples were eluted in 1 M HNO_3 for at least 24 h and diluted to at least 3 mL with 2% HNO_3 before analysis by ICP-MS. Final volumes for each sub-sample were recorded.

The DET and DGT probes were deployed in the sediment with 1-2.5 cm of the open window above the sediment-water interface and 11.5-13 cm under the sediment-water interface. The deployment time was 24-26 h. After that, the sediment DET and DGT probes were pulled out gently from the sediment and rinsed immediately with Milli-Q water to remove any sediment residues. The rinsed DGT probes were stored in sealed plastic bags with 1-2 mL of Milli-Q water to maintain the moisture, kept cool in the cold room and sliced on the second day.

The rinsed DET probes were taken to the semi-clean room for slicing immediately to avoid any internal diffusion. A Teflon-coated stainless steel scalpel was used to cut though the filter membrane and gel layers from the open window. Plastic tweezers were used to remove the filter membranes and gel layers together from the probes and transfer them onto a plastic cutting sheet. Removing the filter membranes and gel layers together provided protection to the gel layers during transfer as the gel layers were fragile. The filter membrane was then removed. The gel layers were sliced into different widths (5-20 mm) using a cleaned plastic holder with the sharp edge at the bottom. Each piece of gel was transferred into a 5 mL plastic vial which had been acid washed and rinsed with Milli-Q water. The gel pieces of the top 5 mm and the bottom 10 mm were excluded from the study.

For the rinsed DGT probes, the procedure was the same as for the DET probes. The gel layers were cut on a filter membrane. The gel layers were sliced at different depth resolutions (e.g. 4-20 mm) and transferred into 5 mL plastic vials.

A total of 2-4 blanks were used for each sampler type and underwent the same handling and processing as the deployed samplers. In the case of DGT samplers, these blanks were used to determine the method detection limits. All samples were analysed by ICP-MS (Agilent 8800). Notable quality control procedures included several calibration standards <10 µg/L due to the expected low concentrations of copper and zinc, using internal reference standards, regular blank and QC standards (after every 20 samples) and use of a CRM (SRM 1643e from National Institute of Standards and Technology). Repeatability of analysis was determined for samples (one in every 20 samples). Samples were re-analysed if these measures indicated a problem. Recoveries for the CRM were between 93-108% (Appendix A).

2.6.3 Calculations to obtain concentrations and fluxes

DET concentrations were simply obtained from the concentrations of the analysed sample corrected for the dilution factor. DGT concentrations are determined through two steps, which corresponded to the equations below: Equation 1 determining the mass of metals accumulated in the binding layer; and Equation 2 converting this mass into a time-weighted average concentration for the sampled water using an equation derived from Fick's First Law of Diffusion. The symbols in these equations are described in Table 3.

$$M = c_e (V^{bl} + V_e) / f_e \qquad \text{Equation 1}$$

$$c_{DGT} = M \Delta g / D A t \qquad \text{Equation 2}$$

Benthic fluxes are obtained from concentration gradients in DET measurements that cross or are immediately adjacent to the sediment-water interface according to Equation 3 (Sheibley and Paulson, 2014). Fluxes were not determined for zinc because no clear concentration gradient was apparent.

$$J = D_s \varphi \ dc/dz \qquad \text{Equation 3}$$

The diffusion coefficient (D_s) in sediments is obtained from Equation 4 with tortuosity derived from porosity as in equation (v) (Boudreau, 1996).

$$D_s = D_0 / \theta^2 \qquad \text{Equation 4}$$

$$\theta^2 = 1 - \ln(\varphi^2) \qquad \text{Equation 5}$$

R-values are determined according to Equation 4. R-values provide information on the mobilisation of metal analytes from sediment particulates in response to DGT measurements (i.e. a perturbation of the sediment porewater concentrations). The use of DGTs with 0.8 and 0.4 mm diffusion layer thicknesses allows the extent of the perturbation to be varied and therefore the mobilisation to be observed under different conditions.

$$R = c_{DGT} / c_{PW} \qquad \text{Equation 6}$$

Table 3. Description and units of symbols in Equations 1 to 6 with values used to determine concentrations and fluxes.

Symbol	Description	Units	Values used or determined by
M	mass of metal analyte accumulated in DGT binding layer	ng	calculated from equation (i)
c_e	concentration of analyte in eluent (corrected for any dilution)	ng/ml *	analysed by ICP-MS
V^{bl}	volume of binding layer	mL #	varies 0.036-0.144
V_e	eluent volume	mL	water: 1 mL sediment: 1 mL
f_e	elution factor		copper: 0.92. manganese: 0.91 iron: 0.8. zinc: 0.91
c_{DGT}	DGT-labile, time-weighted average concentration of metal analyte in determined solution	ng/mL*	calculated from equation (ii)
Δg	diffusion layer thickness	cm	0.09 for water-DGT. 0.05 and 0.09 for sediment-DGT.
D^{dl}	diffusion coefficient of metal in diffusive layer at 19°C	$cm^2\ s^{-1}$	copper: 4.74×10^{-6}. manganese: 4.46×10^{-6}. iron: 4.65×10^{-6}. zinc: 4.63×10^{-6}.
A	area of DGT sampling interface	cm^2	water: 3.14 sediment: 0.9-3.6
t	deployment time	s	water: ≈259200 sediment: ≈86400
J	diffusive flux	$mg\ m^{-2}\ day^{-1}$	calculated from Equation 3
D_s	diffusion coefficient of metal analyte in treatment sediment at 19°C	$cm^2\ s^{-1}$	calculated from Equation 4
φ	sediment porosity of each treatment sediment (Li and Gregory, 1974)		T1: 0.830 T5: 0.689 T2: 0.522 T6: 0.830 T3: 0.691 T7: 0.830 T4: 0.514
dc/dz	concentration gradient across or adjacent to sediment-water interface	$ng\ cm^{-4}$	Varies; see Appendix D.
D_0	diffusion coefficient of metal analyte in water at 19°C	$cm^2\ s^{-1}$	copper: 6.05×10^{-6} manganese: 5.92×10^{-6} iron: 5.99×10^{-6}
dc/dz	concentration gradient across or adjacent to sediment-water interface	$ng\ cm^{-4}$	Varies; see Appendix D.
θ^2	sediment tortuosity; related to increased diffusional path length within sediments		calculated from Equation 5
R	sediment tortuosity; related to increased diffusional path length within sediments		calculated from Equation 5
θ^2	sediment tortuosity; related to increased diffusional path length within sediments		calculated from Equation 5

* $ng\ mL^{-1}$ used because this value is equivalent to µg/L. # cm^3

2.7 Toxicity and bioaccumulation tests

Standard amphipod survival and reproduction tests

The amphipod reproduction bioassay measures adult survival and reproduction, expressed as the number of embryos and <1-day-old juveniles in the second brood following exposure of *Melita plumulosa* to test sediments over a 10-day period. This species interacts with the surficial sediment porewaters, burrowing to up to 1 cm depth but generally resides within the 0-0.5 cm depth range during the bioassays. The test was carried out using the standard method described by Spadaro and Simpson (2016a). The test conditions are summarised in Table 4.

In the standard test procedure, 40 g of sediment were placed into 250 mL beakers, filtered seawater (200 mL, 30 ‰), and four replicates per test. For this study sediments were taken from the respective treatments after lowering the level of the overlying water to approximately 0.5 cm above the sediment water interface. A plastic spatula was used to carefully collect the top 1-2 cm of the surface sediments. These sediments were laid into the test beakers in a manner that retained the vertical stratification and aimed to cause minimal impact to the sediment profile (40 g sediment per 400 mL vial, 4 replicates per sediment). Filtered seawater (200 mL, 30‰) was added and each beaker was incubated at 21^{o}C with aeration for 72 h to allow sediments to settle. On the commencement of the test, 350 mL of overlying water was siphoned off and replaced with new seawater with care to minimise sediment resuspension.

Amphipods used in the tests were isolated from laboratory cultures and transferred to holding trays 7-10 days before tests commenced. Two days before test commenced males were added to the holding trays for mating. At the start of test (Day 1), six gravid females (gravid for <36 h) and six males (isolated from laboratory cultures) were randomly assigned to each beaker. Treatments are fed at a rate of 0.25 mg Sera Micron fish food/amphipod twice a week. The sediments are renewed after 5 d by gently sieving away the adults and placing them into the same fresh sediment (that had been prepared and equilibrated as described for initial materials), thus allowing for the removal of juveniles from the first brood, which is typically unaffected by contaminants in the test sediment because they were already ‘‘conceived’’ before exposure to test sediments. On Day 10, the females were carefully removed and the number of embryos per female is counted by microscopy. The sediment was also checked for juvenile amphipods that had escaped the marsupium during the latter stages of the test by sieving the sediment through 180 µm mesh. The total number of embryos and <1-d-old juveniles was summed and expressed as a percentage of the control

Overlying water concentrations of dissolved metals (<0.45 µm filtered) and ammonia, along with physico-chemical parameters (temperature, pH, salinity and DO) were measured periodically throughout the test. Water was exchanged on Days 3 and 7, sediment renewed on Day 5, ammonia measured on Days 3, 7 and 10, and metals measured on Days 5, 7 and 10. Statistical significance between treatments was calculated using ToxCalc Version 5.0.23 (Tidepool Software).

Table 4. Summary of the standard (original) amphipod (*Melita plumulosa*) survival and reproduction toxicity test conditions

Parameter	Details
Test type	Chronic renewal
Test duration	10 day
Temperature /Salinity	21 ± 1°C / 30 ± 1 ‰
Light intensity	3.5 µmol photons/s/m^2
Photoperiod	12 h light, 12 h dark
Test chamber	250 mL glass beakers
Sediment weight	40 g
Overlying water volume	~220 mL
Total test volume	250 mL
Age/size of test organisms	2-4 month old
No. test organisms/ test chamber	6 females and 6 males
No. replicate beakers / sample	4
Feeding regime	0.5 mg Sera micron® fish per amphipod twice a week.
Test chamber aeration	1 outlet with slow bubbling to maintain ≥85% dissolved oxygen throughout test
Control sediment	Uncontaminated sediment with similar physico-chemical parameters (grain size, porewater salinity) to the test sediment. This control was used for quality assurance checks. Huon Gulf sediment used as a diluent control
Overlying water	Fresh uncontaminated seawater (Port Hacking), NSW, 0.45 µm filtered and diluted with deionised water (Milli-Q) to salinity of 30±1‰ Renewal every two days
Endpoint	Adult survival and reproductive output (total embryo/juvenile numbers)
Test acceptability criteria	>80% survival in the controls, >8 embryos/juveniles per female, physico-chemical parameters (dissolved oxygen, pH, salinity and temperature) within acceptable limits throughout the test

Bivalve survival and bioaccumulation test method

The 30-day bioassay determines whether metals associated with the tailings:sediments are bioavailable to the deposit-feeding estuarine bivalve, *T. deltoidalis,* by exposing the bivalves to sediment for 30 days and measuring metals that have bioaccumulated in their soft body tissue. This bioassay can also detect toxicity to bivalves by measuring the survival of bivalves after 30 days. This species interacts with the surficial sediment porewaters to a depth of up to 15 cm, but may be constrained to depths of 3-7 cm if sediments are particularly dense and make burrowing difficult.

The test was carried out using the standard method conditions described by Spadaro and Simpson (2016b) and is summarised in Table 5, with modifications for the purpose of undertaking *in-situ* within the mesocosms and a decision that there would be adequate nutrition provided by the substrate and overlying seawater to not provided additional food (Appendix D). Modifications to the standard method included the larger chambers and operating in darkness.

The bivalves were collected at Boronia Park, Lane Cove River estuary (27-32‰), Sydney, New South Wales, Australia (King et al., 2010). Approximately 150 adult bivalves with shell surface areas from 10 to 60 mm^2 (two dimensional) were collected by gently sieving (2 mm mesh) sediment collected from a maximum depth below the sediment-water interface of 20 cm. Prior to use in tests the bivalves were acclimated for 7 days to the laboratory test conditions (20°C and salinity 30‰) in holding trays with sediment from the

bivalve collection site and oxygenated seawater. After acclimation, bivalves were removed from the sediment, placed in seawater and sorted into groups of 10 individuals with approximately the same size distribution. The bivalves were observed over a 1-h period for movement to ensure only live animals were selected for use in the bioaccumulation test.

The bivalves were placed directly into the mesocosm treatments. There was >90% exchange with fresh seawater weekly. Due to the large amount of sediment with natural amounts of algae and bacteria present (from the seawater and inoculated prior to equilibration) and low test organism density, the bivalves were not fed any additional food during the test. Not providing additional food is expected to result in conservative test outcomes, as the bivalve stirs the sediment surface with its siphon while feeding and added food may potentially be preferentially selected over other fine particles. The release of metals from sediments to overlying water was monitored by measuring dissolved (0.45 µm filtered) metals in the overlying water throughout the exposure period, along with DO, pH, temperature and salinity.

At the termination of the tests (i.e. after 30 days), surviving bivalves were counted and allowed to depurate overnight in clean seawater for 24 h. Following depuration, the soft body tissue of the bivalves was dissected from the shell using a Teflon coated razor blade and plastic tweezers. Tissue masses from the same replicate were placed in a 70-mL polycarbonate vial and then stored in a domestic freezer at -20 °C until time of analysis.

Tissues were then freeze dried and reweighed to determine the tissue dry weight (DW) and acid digested according to CSIRO Method C-225. Briefly, tissue from each test replicate was digested in duplicate in Teflon digestion tubes by adding 10 mL of Tracepur nitric acid (65%) and a Microwave Accelerated Reactive System (MARS). Digests were made to a final volume of 25 mL with Milli-Q water and metals were measured by inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7500CE) calibrated with matrix-matched standards. For quality control purposes, one blank (Milli-Q water) and one reference sample (DORM-3, Fish Protein Certified Reference Material, National Research Council Canada) were analysed for every 8 samples.

Table 5. Summary of the test protocol for bioaccumulation tests with the bivalve *Tellina deltoidalis*

Parameter	Details
Test type	Static-renewal – mesocosm
Temperature	21 ± 1°C
Light/Photoperiod	In darkness
Test chamber size	Mesocosms
Test solution/sediment volume	See Section 2.3 and description of mesocosm and Appendix D
Renewal of test solutions	Renewal (once per week)
Dilution water	Natural seawater (0.22 µm filtered)
Size of organism	10 – 60 mm^2 (two dimensional)
No. of organisms per test chamber	10
Food regime	No feeding
Test duration	30 days
Endpoint	≥80% survival providing adequate soft tissue to determine metal concentrations

3 Results

3.1 Physico-chemical properties of tailings, sediments and mixtures

The Huon Gulf sediment was of finer particle size than the tailings materials (BT3 and BT4), having 98% of particles <63 µm, compared to 44% for BT3 and 70% for BT4 (Table 6) and is likely reflective of the depositional environment selected adjacent to the Markham Canyon at 3000m for this test work. Laser particle size analyses were made of these three materials and determined the following distribution percentiles and results: DV10, DV50 and DV90 values of 2.2, 9.7 and 48 µm for Huon Gulf (T1), 16, 83 and 173 µm for BT3, and 6.1, 39, 153 µm for BT4, respectively (sizes below which 10, 50, and 90% of the material is contained, Appendix B). The treatments T2 to T5 (described in section 2.1.4) were tailings-sediment mixtures (80:20 and 20:80) and results are provided for measured fraction <63 µm and the value calculated based on mixing the HG, BT and BT4 combinations (Table 6).

The treatments had negligible concentrations of acid volatile sulphides (AVS; 0.1-0.2 µmol/g). Concentrations of total organic carbon (TOC) were <0.1% in the tailings (BT3 ad BT4), 0.5% in the gamma-irradiated and non- irradiated Huon Gulf sediments. In Week 12, the TOC concentrations of T1, T4, T5, T6 and T7 were 0.6, 0.5, 0.5, 0.6 and 0.6% respectively.

Sediment pH and redox potential (Eh) measurements were made on the surface sediment 1 to 2 cm depth below the sediment water interface at the start of Week 17 (end of the bioaccumulation tests). All treatments had similar pH and Eh, being pH 7.8-8.1 and Eh of -50 to +100 mV.

During the first week of the study all treatments settled and the surface layer dropped 0.5-1 cm below the lip of the treatment container and this was topped up with the respective treatment material at the start of week 2, but at no other stage of the study.

Table 6. Tailings and sediment properties

Treatment	Solid	Particle Size % <63 µm		Organic Carbon TOC, %	AVS µmol/g	pH	Redox Potential Eh, mV
		Measured					
T1	Huon Gulf (HG)	98	**Calculated using base materials mixed**	0.5-0.6	0.2	7.95	-50 to 20
BT3, used in T2, T4, T6	BT3 100%	44		<0.1	<0.1	7.85	-20 to 70
BT4, used in T3, T5, T7	BT4 100%	70		<0.1	<0.1	8.05	-30 to 70
T2	80:20 BT3:HG	63	54	0.3	0.1	7.90	-20 to 90
T3	80:20 BT4:HG	74	75	0.2	0.1	7.92	-50 to 10
T4, T6	20:80 BT3:HG	75	87	0.5-0.6	0.1	7.89	-30 to 40
T5, T7	20:80 BT4:HG	91	92	0.5-0.6	0.1	7.88	-30 to 30

TOC = total organic carbon. AVS = acid-volatile sulfide (sulfide fraction extractable in 1-M HCl).
HG = Huon Gulf (sediment composite used for preparing treatments).
BT3 = Bulk Tails-3 = 90% porphyry:10% metasediments. BT4 = Bulk Tails-4 = 25% porphyry:75% metasediments.
80:20 refers to 80% tailings and 20% sediment mixture. 20:80 refers to 20% tailing and 80% sediment mixture.
pH and redox potential measurements made on week 17 of tests.

3.2 Metal concentrations of tailings, sediments and mixtures

The total recoverable metal (TRM) concentrations are provided in Table 7 (Appendix B) for the two tailings master composite 'book ends' (BT3 and BT4), the Huon Gulf sediment, and the tailings-sediment mixtures at the start of the tests (Week 1) and at Week 12 (start of toxicity bioaccumulation tests for T1, T4, T5, T6 and T7). For the two tailings 'book ends', the concentrations of TR-Cu (453-525 mg/kg) and TR-Zn (34-57 mg/kg) were considerably lower than those in the tailing produced and used in the earlier chemistry and ecotoxicology study by Adams et al. (2018) (TR-Cu was 915-1570 mg/kg and TR-Zn was 472-840 mg/kg, respectively). The concentrations of Cu, Cr and Ni were greater in the tailings than in the Huon Gulf sediments (T1), lower for Mn, V and Zn and similar for the other metals (Table 7). The TR-Cu concentrations in the 80:20 and 20:80 tailing-sediment mixtures were 83-112% of the concentration predicted by mixing the components.

Table 7. Total recoverable metal (TRM) concentrations in the tailings, sediment or tailings-sediment mixture

		Total Recoverable Metals (mg/kg)										
Week	**Sample**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**V**	**Zn**
Two tailing master composite 'book ends'												
Week 1	BT3 initial	1	0.5	12	453	578	48400	437	279	13	55	34
Week 1	BT4 initial	7	0.6	17	525	560	60200	331	299	13	90	57
Huon Gulf sediment												
Week 1	T1 initial	7	0.7	23	47	80	54300	1020	59	11	140	86
Week 12	T1	8	0.8	23	48	82	55400	1170	60	12	141	93
Tailings-sediment mixtures												
Week 1	T2 initial	2	0.6	13	380	528	55400	579	239	15	79	50
Week 1	T3 initial	7	0.7	19	421	449	60300	501	253	15	104	65
Week 1	T4 initial	6	0.5	20	127	197	52800	870	100	16	121	78
Week 12	T4	6	0.6	23	141	216	58200	976	110	14	133	86
Week 1	T5 initial	6	0.6	22	126	159	51500	832	97	14	122	79
Week 12	T5	8	0.8	23	141	175	58300	977	107	13	137	89
Week 12	T6	8	0.7	27	52	89	58800	1100	63	14	150	97
Week 12	T7	9	0.7	23	51	87	58600	1210	64	15	150	98
SQGV [a]		**20**	**1.5**	**NV**	**80**	**65**	**NV**	**NV**	**21**	**50**	**NV**	**200**

[a] The GVs are from ANZECC/ARMCANZ (2000), as per the proposed revision of Simpson et al. (2013). NV= no value listed. QA/QC data is provided in Appendix B. HG = Huon Gulf (sediment composite used for preparing treatments). BT3 = Bulk Tails-3 = 90% porphyry:10% metasediments. BT4 = Bulk Tails-4 = 25% porphyry:75% metasediments. 80:20 refers to 80% tailings and 20% sediment mixture. 20:80 refers to 20% tailings and 80% sediment mixture.

The TRM concentrations of Cr, Cu and Ni in both tailings exceed sediment quality guideline values (SQGVs) (Simpson et al., 2013). A significant portion of the TRM concentration may not be bioavailable to organisms, and for most metal(loid)s, the dilute-acid extractable metal(loid)s (AEM) can be considered the 'maximum bioavailable concentration' (Simpson and Batley, 2016). The concentrations of AEM are shown in Table 8, and the ratio of AEM to TRM in Table 9. The concentrations of AE-Cu (103-113 mg/kg) and AE-Zn (9-15 mg/kg) were considerably lower than those in the tailings produced and used in the earlier chemistry and ecotoxicology study by Adams et al. (2018), in which AE-Cu was 149-182 mg/kg and AE-Zn was 392-432 mg/kg, respectively. The differences between the TRM and AEM results of this study and Adams et al. (2018) were attributed by the WGJV metallurgist to variability of the ore body for the core samples selected to make up the master composite, which is predominantly based on overall Cu and sulphide contents. Note, the tailings used by Adams et al. (2018) had been stored for approximately 6 months before use in those studies and that may have contributed to higher AE-Cu and AE-Zn

concentrations. The fraction of each metal(loid) extractable as AEM (i.e. soluble in 1 M HCl) was lower in the tailings than the Huon Gulf sediments for most metals (similar for zinc).

Table 8. Dilute-acid (1 M HCl) extractable metal concentrations in the tailings, sediment or tailings-sediment mixture

		Dilute-acid Extractable Metals (mg/kg)										
Week	**Sample**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**V**	**Zn**
Two tailings master composite 'book ends'												
Week 1	BT3 initial	<7	<0.5	<2	32	113	3350	116	24	3.5	4	9
Week 1	BT4 initial	<7	<0.5	<2	30	103	4700	95	23	3.3	7	15
Huon Gulf sediment												
Week 1	T1 initial	<7	<0.5	7	4	32	9670	440	8.4	5.2	21	19
Week 12	T1	<7	<0.5	8	6	38	11700	573	11	5.7	24	28
Tailings-sediment mixtures												
Week 1	T2 initial	<7	<0.5	<2	15	191	4210	163	13	8.5	7	13
Week 1	T3 initial	<7	<0.5	2	19	107	5490	156	17	6.5	9	19
Week 1	T4 initial	<7	<0.5	6	6	58	8400	373	8.8	5.4	18	19
Week 12	T4	<7	<0.5	9	11	77	12436	466	15	6.8	24	28
Week 1	T5 initial	<7	<0.5	5	7	53	8470	356	9.8	4.1	17	20
Week 12	T5	<7	<0.5	8	15	66	14040	501	17	14.8 [a]	26	30
Week 12	T6	<7	<0.5	10	9	56	17600	804	17	8.3	35	39
Week 12	T7	<7	<0.5	11	7	43	13430	527	13	6.5	27	31
Control sediment used in bioaccumulation and toxicity tests												
Week 12	Control	<7	<0.5	<2	6	29	8780	52	2	55	24	140
SQGV		**20**	**1.5**	**NV**	**80**	**65**	**NV**	**NV**	**21**	**50**	**NV**	**200**

See footnotes for Table 7. [a] This value appears as erroneous owing to AE-Pb being greater then TR-Pb as indicated by high ratio in Table 9.

Table 9. Ratio of dilute-acid extractable (AEM) to total metals (TRM) in the tailings or tailings-sediment mixture

		AEM/TRM (%)										
Week	**Sample**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**V**	**Zn**
Two tailing master composite 'book ends'												
Week 1	BT3 initial	NV	NV	2	7	20	7	27	9	27	8	27
Week 1	BT4 initial	NV	NV	7	6	18	8	29	8	25	7	27
Huon Gulf sediment												
Week 1	T1 initial	NV	NV	33	9	40	18	43	14	47	15	22
Week 12	T1	NV	NV	37	13	46	21	49	19	45	17	30
Tailings-sediment mixtures												
Week 1	T2 initial	NV	NV	14	4	36	8	28	5	58	9	27
Week 1	T3 initial	NV	NV	13	4	24	9	31	7	43	9	29
Week 1	T4 initial	NV	NV	28	5	29	16	43	9	34	15	25
Week 12	T4	NV	NV	38	8	36	21	48	13	50	18	32
Week 1	T5 initial	NV	NV	24	5	34	16	43	10	30	14	25
Week 12	T5	NV	NV	34	11	38	24	51	16	115 [a]	19	34
Week 12	T6	NV	NV	39	18	63	30	73	28	57	23	40
Week 12	T7	NV	NV	46	14	50	23	44	21	44	18	31

In relation to assessing the risk of bioaccumulation and toxicity of metals, the comparison of measured AEM concentrations with sediment quality guideline values (SQGVs, ANZECC/ARMCANZ, 2000) is considered to be a better predictor than TRM for sediments impacted by mine-derived materials in marine environments (Simpson and Spadaro, 2016). In relation to AEM, copper concentrations exceeded the screening SQGV of 65 mg/kg in the tailings and 80:20 tailings-sediment mixtures, and were close to this value in the 20:80 tailings-sediment mixtures (Table 7). No metals exceeded SQGVs for the HG sediment. The concentrations of metals (Tables 7 and 8) were not significantly different for Weeks 1 and 12 (i.e. 10-20%).

3.3 Overlying waters

3.3.1 Discrete weekly sampling

During the tests water quality was monitored (e.g. DO, temperature, salinity, pH) and samples taken for dissolved metals analyses weekly prior to the waters change. The data provided in Table 10 is the mean±standard deviation to Week 12, as toxicity and bioaccumulation tests started in Week 13. A full summary of data for each week is provided in Appendix B. Where dissolved concentrations were below limits or reporting (LOR), the LOR of 1 µg/L was used for the averaging.

The pH, salinity, dissolved oxygen concentration and temperature of the waters in the treatments were not significantly different and did not vary over the 12-week duration.

No dissolved metals exceeded water quality criteria (WQC) applied in PNG. A potential exception was cobalt, where the WQC is set as the limit of detection (LOD), which in this case was 1 µg/L and was not exceeded. Frequently the WQC for cobalt is assumed to be 0.1 µg/L (i.e. a lower LOD), but there is little support for such a low guideline value (most international guidelines for cobalt are near 1 µg/L).

Dissolved Cu, Zn, and Mn were the metals with concentrations of interest, being either frequently at elevated concentrations or above the ANZECC/ARMCANZ (2000) water quality guideline values (WQGVs) in the case of copper. The main observations for these metals were:

- Dissolved copper concentrations within overlying waters were greater for T2 (7.6±1.5 µg/L) and T3 (4.3±1.5 µg/L) than the other treatments (generally 1-3 µg/L range). At the 12-week stage, the dissolved copper concentrations indicated a small decline for T3: being 4-8 µg/L range for Weeks 1-6 and then 2-4 µg/L range after; but T2 remained relative unchanged (6-8 µg/L range). The other treatments were relatively constant at lower dissolved copper levels.
- In relation to the WQGV, dissolved copper concentrations within overlying waters exceeded the 95% species protection concentrations of 1.3 µg/L (WQGV) in all treatments, and the average dissolved copper concentrations for T2 and T3 exceeded the WQGV by ~6 and 3×, respectively.
- Dissolved zinc concentrations within overlying waters were not significantly different between the seven treatments, although the mean concentrations over the 12-week period appeared marginally greater for T2, T3 and T4 (2.2-2.6 µg/L) than the other treatments (1.2-1.8 µg/L)
- Dissolved manganese concentrations were lower in Treatments T2 and T3 (80% tailings) than in T4 and T5 (20% tailings) and not greater than the treatment with Huon Gulf sediment at the surface (T1, T6, T7). Dissolved manganese concentrations within overlying waters decreased from the first week (100 – 300 µg/L range) to negligible (1-4 µg/L range) by Week 7, after which no concentrations exceeded 3 µg/L (Appendix A2).
- Concentrations of dissolved Cu, Zn and Mn in the 75-L treatment container (TC) and 85-L seawater reservoir (SR) were indistinguishable over the first three weeks of monitoring, demonstrating a well-

mixed experimental design, and were also considered to be relatively low, and consequently the SR was disconnected and not used after Week 3.

The initially high dissolved manganese concentrations is attributed to the manipulation (mixing) of the natural HG sediments. Past studies have observed increases in porewater manganese with homogenised sediments when compared to non-homogenised sediments (Simpson et al., 2003). This is consistent with manganese (hydr)oxide phases in the sediments being reductively dissolved when they are brought into contact with pore-water Fe(II). This reaction ($4Fe^{2+} + 2MnO_{2(s)} + 4H_2O \longrightarrow 2Mn^{2+} + 4FeOOH_{(s)} + 4H^+$) is widely recognized for its importance in the cycling of iron and manganese in sediments (Canfield et al., 1993). This reaction is important because it applies even when sediments are homogenised under anoxic conditions.

Table 10. Water quality and dissolved metals in overlying waters of long-term experiments. Averages to Week 12.

		pH	Salinity	DO	Temp.	Dissolved metals, µg/L		
			‰	%-Sat	ºC	Cu	Zn	Mn
Seawater used to fill mesocosms	Mean	8.2	36.0	88.6	19.2	1.3	1.2	1.1
	SD	0.1	0.2	0.6	0.4	0.2	0.3	0.3
Treatment T0	Mean	8.2	36	90	19	1.0	2.4	1.0
Seawater only	SD	0.1	0.8	2	0.4	0.2	1.3	0.0
Treatment T1	Mean	8.2	36	90	19	1.2	1.2	45
100% Huon Gulf	SD	0.1	0.8	4	0.4	0.4	0.3	85
Treatment T2	Mean	8.2	36	90	19	**7.6**	2.5	34
80% BT3	SD	0.1	0.6	4	0.5	1.5	1.4	44
Treatment T3	Mean	8.2	36	88	19	**4.3**	2.2	39
80% BT4	SD	0.1	0.5	3	0.4	1.5	1.3	51
Treatment T4	Mean	8.2	37	89	19	**2.1**	2.6	83
20% BT3	SD	0.1	0.4	2	0.4	0.9	2.3	96
Treatment T5	Mean	8.2	37	90	19	**2.0**	1.3	97
20% BT4	SD	0.1	0.4	2	0.4	0.8	0.5	111
Treatment T6	Mean	8.2	36	90	19	**1.4**	1.8	92
20% BT3, 4 cm HG	SD	0.1	0.4	2	0.4	0.6	1.0	115
Treatment T7	Mean	8.2	37	90	19	**1.5**	1.4	92
20% BT4, 4 cm HG	SD	0.1	0.3	2	0.3	0.5	0.7	113
WQGV (95% PC)[b]	-	-	-	-	-	1.3	15	NA
WQGV (99% PC)[b]	-	-	-	-	-	0.3	7	NA
PNG WQC	-	-	-	-	-	30	5000	2000 (in solution

[a] mean of triplicate treatments; [b] WQGV to protect 95% or 99% of species ANZECC/ARMCANZ (2000).
Bold values indicate concentrations greater than the 95% species protection value.

3.3.2 Dissolved metals for Weeks 13-17 during bivalve bioaccumulation tests

For Treatments T1, T4, T5, T6 and T7, the top 3-cm of the back half of the treatments was removed for toxicity tests and ten bivalves were added to the front half (separated by a divider) (Photos in Appendix A). For these treatments there was no noticeable influence of this disturbance on the dissolved copper or zinc concentrations measured over the following 4 weeks (Weeks 13-17) (Appendix B), however, dissolved manganese concentrations were in the range of 4-24 µg/L range where they had previously remained <1-2 µg/L range for previous 5-7 weeks. This was consistent with the disturbance of the sediments again resulting in reductive-dissolution of manganese oxide phases.

3.3.3 Water DGT samplers

DGT samplers were deployed within the waters for approximately 72 h over the weekends between Weeks 4 and 5 and between Weeks 10 and 11 (Table 11) and are compared with discrete water sample results for the weeks either side of these (Table 12). Additional information on the Water DGT results is provided in Appendix C. The 72-h DGT samplers provided a measurement of dissolved metals that can be compared to the discrete measurements and also to WQGVs for protection of aquatic organisms.

Table 11. Dissolved metals measured from 74-h deployment of water DGT samplers.

Treatments	Dissolved metal concentrations, µg/L		
	Copper	Zinc	Manganese
Week 4 Water DGT			
Blanks (DGT not deployed)	<0.2	1.4 ± 0.1	<0.1
T0 (mean ±SD)	0.25 ± 0.07	1.9 ± 0.4	<0.1
T1 (mean ±SD)	0.4 ± 0.05	2.0 ± 0.1	30 ± 0.7
T2 (mean ±SD)	5.9 ± 0.9	4.6 ± 0.6	47 ± 6
T3 (mean ±SD)	3.2 ± 0.5	2.4 ± 0.4	56 ± 7
T4 (mean ±SD)	0.70 ± 0.01	2.1 ± 0.1	68 ± 1
T5 (mean ±SD)	0.65 ± 0.07	2.1 ± 0.2	82 ± 1
T6 (mean ±SD)	0.45 ± 0.07	2.1 ± 0.4	47 ± 1
T7 (mean ±SD)	0.4 ± 0.01	2.0 ±0.4	51 ± 2
Week 10 Water DGT			
Blanks (DGT not deployed)	<0.2	2.5	<0.1
T0 (mean ±SD)	0.18 ± 0.03	2.8 ± 1.0	0.06 ± 0.01
T1 (mean ±SD)	0.29 ± 0.01	2.1 ± 0.5	0.09 ± 0.01
T2 (mean ±SD)	4.5 ± 0.03	3.6 ± 0.5	0.30 ± 0.01
T3 (mean ±SD)	1.7 ± 0.07	1.8 ± 0.3	0.23 ± 0.01
T4 (mean ±SD)	0.65 ± 0.01	2.4 ± 0.3	0.09 ± 0.01
T5 (mean ±SD)	0.71 ± 0.02	2.1 ±1.7	0.10 ± 0.01
T6 (mean ±SD)	0.28 ± 0.05	1.7 ± 1.6	0.09 ± 0.02
T7 (mean ±SD)	0.28 ± 0.01	1.2 ±0.1	0.16 ± 0.01
WQGV (95% PC)[a]	1.3	15	NA
WQGV (99% PC)[a]	0.3	7	NA
PNG WQC	30	5000	2000 (in solution

Result are the mean of 2 replicate DGTs, except for T2 which had 4 replicate DGTs for Week 4. Dissolved iron was <2 µg/L.
[a] WQGV to protect 95% or 99% of species (ANZECC/ARMCANZ, 2000).
Bold values indicate concentrations greater than the 95% species protection value

The main observations for these metals were:

- DGT-Cu concentrations decreased in the order T2 > T3 > T4~T5 > T1~T6~T7, indicating that the treatments of the 20:80 tailing-sediment that were capped with 4 cm of Huon Gulf sediments resulting in similar copper release as the 100% Huon Gulf sediment.
- DGT-Zn concentrations were greater for T2 in comparison to all other treatments on Week 4 (all other treatments were not significantly different at around 2 µg/L). DGT-Zn concentrations and lower on Week 12, but greater for T2 than T3.
- DGT-Mn concentrations decreased in the order T5 >T4>T2~T3~T6~T7>T1, and are consistent with the greater disturbance and mixing of the Huon Gulf sediments (i.e. in treatments with greater portion of HG in a mixture) resulting in greater release of manganese due to the process of disturbance causing reductive dissolution of manganese oxide phases (discussed in Section 3.3.1)

- The difference in DGT-metals concentration between T2 and the other treatments was less after 12 weeks than 4 weeks, consistent with decreasing overall Cu, Zn and Mn fluxes.
- DGT-Cu concentrations for T2 and T3 exceeded the WQGV by ~4.5 and 2× on Week 4 and ~3.4 and 1.3× on Week 12, respectively.
- The discrete water samples taken on the weeks either side of the water DGT analyses had moderately higher dissolved copper and manganese concentrations (Table 12). This may indicate the presence of colloids. Overall the magnitudes and relationships were comparable.

Table 12. Discrete monitoring data for Weeks 4,5, 10 and11 for comparison with DGT data

	Week 4	Dissolved metals, µg/L				Week 5	Dissolved metals, µg/L		
Date	Treatment	Cu	Zn	Mn		Treatment	Cu	Zn	Mn
15/1/18	T0	1	1	1	22/1/18	T0	1	1	1
15/1/18	T1	1	1	37	22/1/18	T1	1	1	7
15/1/18	T2	10	4	66	22/1/18	T2	11	3	47
15/1/18	T2 replicate	10	5	66	22/1/18	T2 replicate	10	4	47
15/1/18	T3	5	2	77	22/1/18	T3	6	3	65
15/1/18	T3 replicate	5	2	78	22/1/18	T3 replicate	6	4	66
15/1/18	T4	2	1	95	22/1/18	T4	3	3	42
15/1/18	T5	2	1	107	22/1/18	T5	2	1	30
15/1/18	T6	1	1	61	22/1/18	T6	1	2	15
15/1/18	T7	1	2	65	22/1/18	T7	2	1	25
	Week 10	**Dissolved metals, µg/L**				**Week 11**	**Dissolved metals, µg/L**		
Date	Treatment	Cu	Zn	Mn		Treatment	Cu	Zn	Mn
26/2/18	T0	1	3	1	5/3/18	T0	1	1	1
26/2/18	T1	1	1	1	5/3/18	T1	1	1	1
26/2/18	T2	6	3	1	5/3/18	T2	8	1	1
26/2/18	T2 replicate	6	3	1	5/3/18	T2 replicate	8	1	1
26/2/18	T3	2	2	1	5/3/18	T3	4	1	1
26/2/18	T3 replicate	2	3	1	5/3/18	T3 replicate	4	1	1
26/2/18	T4	1	1	1	5/3/18	T4	2	1	1
26/2/18	T5	1	1	1	5/3/18	T5	2	1	1
26/2/18	T6	1	1	1	5/3/18	T6	1	1	1
26/2/18	T7	1	1	1	5/3/18	T7	1	1	1

3.4 Sediment DET at Weeks 5 and 11

Sediment DET can provide high-resolution concentration profiles of metals in pore waters (e.g. Cu, Fe, Mn) and enable flux calculations across the sediment-water interface that will help to provide geochemical kinetic inputs to a geochemical and hydrodynamic model being developed in parallel by Tetra Tech in collaboration with CSIRO and WGJV. The sediment DET measurements (24-h deployment at start of Weeks 5 and 11) were used to provide profiles of porewater concentrations of dissolved Cu, Mn and Fe from 1-2 cm above the SWI to at least 10 cm below the SWI (the treatment containers were 18 cm deep) (Figure 1, Appendix C for Cu, Fe and Mn profiles). Measurements were made at resolutions varying from 4 to 20 mm.

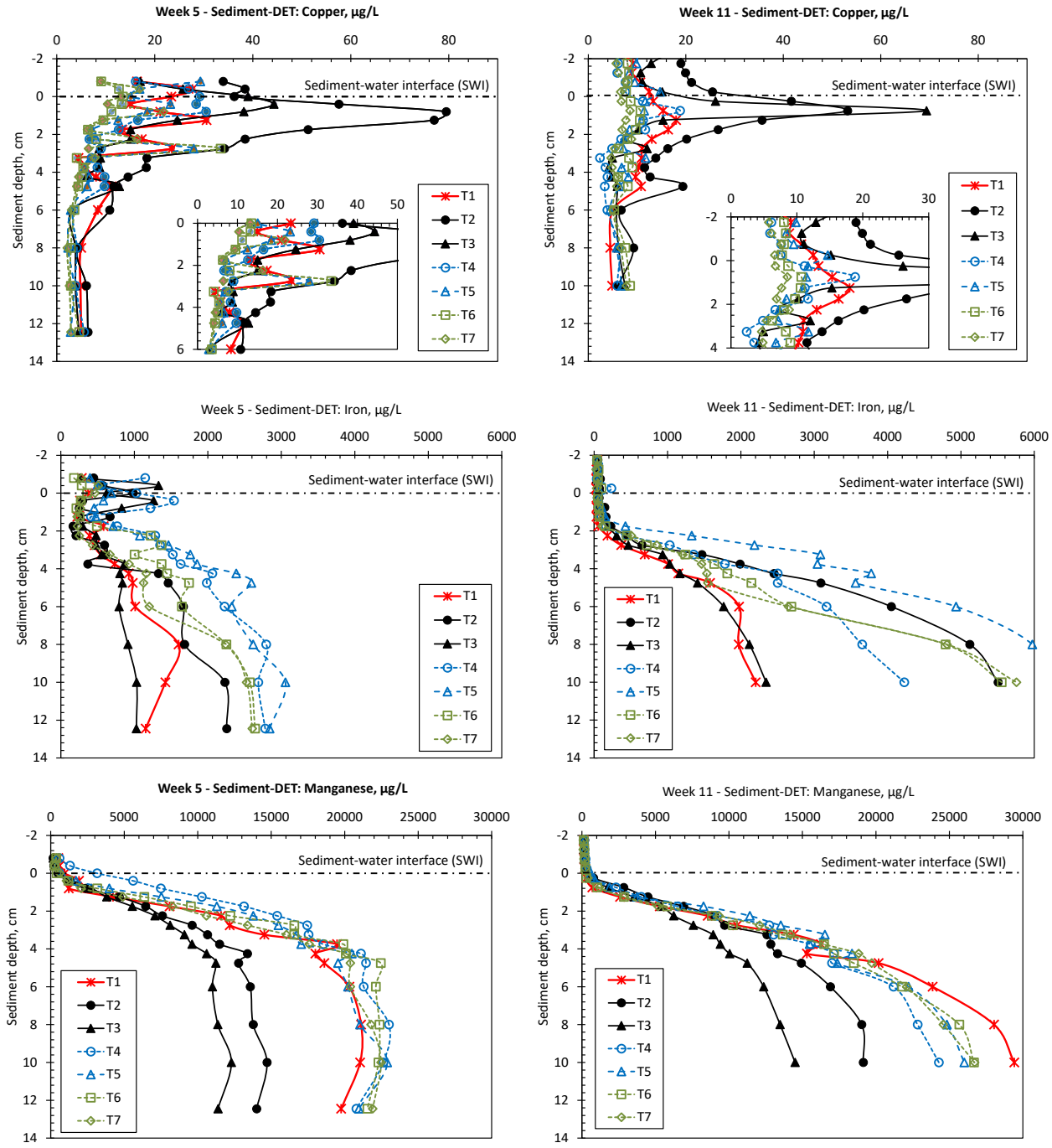


Figure 1. Week 5 and Week 11 comparison of sediment-DET profiles of Cu, Fe and Mn for all treatments. The figures for copper contains data embedded with a larger scale near the mobilisation depth.

3.4.1 Comparison of Cu, Fe and Mn profiles among treatments and weeks

Generally similar profile patterns were observed for each metal regardless of the treatment and week, although concentrations did change according to the composition of the treatment (tailings-sediment) and some features occurred at slightly different depths due to lateral changes within the sediment.

- Diagenetic changes in sediment chemistry were clearly evident between weeks 5 and 11 with the dissolved Mn and Fe geochemical tracers generally increasing in all treatments with depth and over time, indicating a general increase in reducing conditions, but not to the extent that Fe and Mn started

to be precipitated as sulfides (FeS and MnS) at depth. By week 11, sedimentary reducing conditions are clearly established below 2 cm sediment depth on the basis of the porewater Mn and Fe profiles.

- The DET dissolved copper concentrations are generally consistent with the establishment of reducing conditions below 2 cm sediment depth, having a maximum in the surface zone and minimal concentrations below this depth where precipitation and adsorption reactions take place. The exception is in T2, which indicates that lower concentrations were consistently only reflected below a depth of 4-5cm. This treatment had notable bubbles forming during the experimental establishment, which may reflect the presence of residual organics that may complex with copper and lower precipitation/adsorption processes in porewaters (Skrabal et al., 2000; Chapman et al., 2009; Teasdale et al., 2003).
- After 11 weeks, only treatments T2 and T3 have a DET copper concentration that clearly exceeds the Huon Gulf sediment (T1, control) in the surface to 4 cm sediment depth range. Over this same depth range the DET copper concentrations in T6 and T7 were generally lower or similar to the control, T4 and T5, indicating that there had been negligible incursion of copper from the tailing below the HG cap. The equivalent concentrations for both of these two treatments by depth has also decreased between weeks 5 and 11 with the exception of a higher maximum concentration (a single measurement point creating this sharp peak) at 1 cm depth for T3 in week 11. This appears to be an anomaly as the concentrations directly above and below this had also decreased over time and is consistent with the overall observed diagenetic (geochemical) trend. However, microniches of metal mobilization frequently occur within sediments and such phenomena may contribute to this peak. Similarly, the equivalent DGT results for this treatment after 11 weeks (Figure 2, discussed in section 3.5 below) also suggest that the 1 cm depth DET result is an anomaly. These observations will be further verified by the results of the DET testing after 16 and 24 weeks.
- Correlations for copper were investigated for points around the copper peak. These data were split into those points from the peak maxima to lesser depths and from the peak maxima to greater depths (see Table 13) for correlations with manganese and iron. Positive correlations were generally observed for points at lesser depths with manganese, with most being quite strong (> 0.5) and some significant. Negative correlations were observed between copper and manganese for points from the peak maximum to greater depths with many of these being very strong (< -0.75) and significant. These results support the following interpretation of key processes:
    - The manganese and iron profiles indicate the depth at which oxygen penetrates into the sediment, especially for the latter as Fe(II) (the soluble form) is oxidised by O_2 very quickly. The zones of peak Fe(III)- and Mn(IV)-reduction generally occur from 2-3 cm and deeper in the sediment and the decreases in Fe(II) and Mn(II) concentrations at shallower depths therefore indicate the likely presence of O_2, although Fe(II) can also chemically reduce MnO_2. The major copper mobilisation peak occurs at shallower depths and often corresponds to a depth at which iron concentrations are just about at their minimum within the sediment within the surface 0-2 cm sediment depth range, which is clearly evident after diagenetic maturation after 11 weeks.
    - Copper present as sulfide phases (e.g. CuS, $Cu(I)_2S$ or $FeCuS_2$) may be mobilised by: oxidation of these phases (Simpson et al., 2012; Simpson and Spadaro, 2016); metal-metal sulfide exchange reactions; and via mixed metal-sulfide galvanic reactions (Knight, et al., 2018). Copper mobility is generally low with dissolved copper being re-adsorbed or exchanged into other phases quite quickly. As the sediment is dominated by manganese oxide particles, most of the released copper likely adsorbs to this phase. The increasing copper concentrations at the upper side of the mobilisation peak are strongly correlated with manganese (Table 13), especially in T2 and T3, which could indicate that this copper is mobilised by reduction of manganese oxide. However, mobilisation of copper does not continue deeper in the sediment as Mn(IV)-reduction increases, so other processes such as metal sulfide precipitation are likely limiting the availability of copper.
    - The decreasing copper concentrations are strongly negatively correlated with manganese concentrations for all treatments and also iron concentrations (more weakly). This is consistent

with the progression of the major diagenetic processes, where dissolved oxygen does not penetrate below 2 cm sediment depth, having been consumed by ongoing reactions with Mn(II) and Fe(II). It is also consistent with mobilisation of copper occurring only at depths where there is sufficient O_2 to oxidise the sulfide minerals in the first instance.

Table 13. Correlations between copper and manganese and iron and around copper peaks

	Peak maxima to lesser depths				Peak maxima to greater depths			
Treatments	Mn	Sig	Fe	Sig	Mn	Sig	Fe	Sig
T1 (Week 5)	0.50		0.35		-0.80	*	-0.78	*
T1 (Week 11)	0.85	*	0.49		-0.90	**	-0.63	
T2 (Week 5)	0.87	*	-0.42		-0.95	**	-0.31	
T2 (Week 11)	0.91	**	0.77	*	-0.94	**	-0.70	
T3 (Week 5)	0.64		0.46		-0.95	**	-0.60	
T3 (Week 11)	1.0	**	0.10		-0.77	*	-0.46	
T4 (Week 5)	0.72		0.05		-0.95	**	-0.15	
T4 (Week 11)	0.98	**	-0.2		-0.91	**	-0.77	*
T5 (Week 5)	0.89		-0.29		-0.45		-0.29	
T5 (Week 11)	0.25		0.29		-0.68		-0.35	
T6 (Week 5)	0.35		0.90	*	-0.58		-0.12	
T6 (Week 11)	0.96	**	-0.30		-0.62		-0.55	
T7 (Week 5)	0.63		0.14		-0.90	**	-0.76	*
T7 (Week 11)	0.80	*	0.89	**	-0.76	*	-0.79	*

Sig is significance. Correlations significant at p <0.05 (*) and <0.01 (**) are highlighted.
Degrees of freedom vary from 2-6.

3.4.2 Summary of key mechanisms influencing copper mobilisation

The major copper peaks (greater labile forms) only occur in the surface 0-2 cm sediment depth range where the following processes overlap: (i) oxidation of copper sulfide minerals, metal-metal sulphide exchange reactions and mixed metal-sulphide galvanic reactions may occur due to the presence of sufficient O_2 or potential other oxidising agents. The porewater profiles indicate that lower O_2 and establishment of reducing conditions produces less copper release. Importantly, based on this conceptual model, copper adsorbed to manganese and iron oxides should be present in the solid phase at sediment depths above 2-3 cm (mobilisation peak), which should limit concentrations of dissolved copper that may be bioavailable and pose a risk of adverse effects to benthic organisms within these surface sediments. There was no sign of copper mobilisation from the tailing-sediment mixture with a 4 cm HG sediment cover (treatments T6 and T7) as this cover is sufficient to isolate the tailings-sediment below the metal mobility zone. Importantly, this means that any temporary impacts associated with co-deposited tailings is reversible once natural sediment cover the tailings.

3.4.3 Benthic fluxes determined using DET concentration profiles

For Cu, Fe and Mn, the DET-porewater gradients with depth from the sediment-water interface (SWI) were adequate for calculating diffusive flux values (Table 14) using Fick's law of diffusion (see Methods section). The reliability of these data is also further assessed in Appendix C. These data indicate diffusion-controlled

fluxes only and other processes such as the water flow across the SWI may result in an increase of advection of pore waters.

- Copper fluxes were variable between treatments with both positive and negative fluxes observed in Week 5, although in Week 11 all fluxes were positive, but quite low (<0.1 mg m^2/day). The strongest positive fluxes were observed for T2 and T3, the treatments with the highest concentration peaks. Considering these fluxes at a more practical scale, treatments T2 and T3 would result in a benthic flux of 18-29 kg/km^2/year.
- The manganese fluxes were observed to be always positive, quite high (>4 mg/m^2/day[1]) and consistent for Weeks 5 and 11. The fluxes were proportional to the concentrations determined. Due to the shape of the manganese profiles, the confidence in these data is very high.
- The iron fluxes were the most variable, with 5 treatments in Week 5 showing negative fluxes, while all Week 11 fluxes were positive. Week 11 fluxes were generally more reliable due to the improved measurements around the SWI or perhaps due to further consolidation of the sediment. The iron fluxes were moderate to weak and all were <1 mg m^{-2} day^{-1}.

Table 14. Benthic fluxes (mg m^{-2} day^{-1}) for copper, manganese and iron with each treatment

Treatments	Copper	Manganese	Iron
T1 (Week 5)	-0.0089	15	-0.52
T1 (Week 11)	0.011	13	0.0079
T1 (Average)	0.0011 ± 0.014	14 ± 1.4	-0.26 ± 0.37
T2 (Week 5)	0.064	3.6	-0.15
T2 (Week 11)	0.033	3.6	0.045
T2 (Average)	0.049 ± 0.022	3.6 ± 0	-0.052 ± 0.14
T3 (Week 5)	0.048	5.0	-0.97
T3 (Week 11)	0.11	4.8	0.028
T3 (Average)	0.079 ± 0.044	4.7 ± 0.14	-0.47 ± 0.71
T4 (Week 5)	0.0029	4.6	-0.10
T4 (Week 11)	0.013	4.4	0.021
T4 (Average)	0.0080 ± 0.0071	4.5 ± 0.14	-0.040 ± 0.086
T5 (Week 5)	0.0066	9.5	0.020
T5 (Week 11)	0.0034	8.3	0.079
T5 (Average)	0.0050 ± 0.0023	8.7 ± 0.85	0.050 ± 0.042
T6 (Week 5)	-0.0067	16	0.020
T6 (Week 11)	0.0093	13	0.022
T6 (Average)	0.0013 ± 0.011	15 ± 2.1	0.021 ± 0.0014
T7 (Week 5)	-0.026	15	-0.59
T7 (Week 11)	0.0019	14	0.025
T7 (Average)	-0.012 ± 0.020	15 ± 0.71	-0.28 ± 0.43

To convert from mg/m^2/day to kg/km^2/year the data are multiplied by 365.

3.5 Sediment DGT at Weeks 5 and 11

Sediment DGT probes provided an integrated measure of both metals within pore waters and remobilisation of metals from sediment solid phases (resupplied to pore waters after release from solids). DGT provides a different insight to DET in relation to the metal fluxes and risks of excessive metal exposure for benthic organisms because the sampling rates are much higher. Although DGT determines fluxes, these

data can also be presented as concentrations (representing the average porewater concentrations at the interface between the DGT sampler and the sediment over the 24 h deployment) which facilitates comparison with actual porewater measurements (i.e. DET). Where DGT measurements are similar to DET concentrations, this indicates that the metal is mobilised strongly (both quickly and to a large extent). A lower ratio of DGT to DET concentrations signifies weaker mobilisation or even no mobilisation. Using DGT measurements with different diffusive layer thicknesses allows further insight to the degree of mobilisation.

Sediment DGT samplers were deployed (for about 24 h at the start of Weeks 5 and 11) within the sediments on the same dates as the DET samplers and to similar depths, although the measurements were made at lower depth resolution overall. Figure 2, 3, and 4 demonstrate the same general profile patterns as the DET measurements for each metal although the concentrations differ.

3.5.1 Evaluating metal mobilisation from DET and DGT profiles

As noted, DET provides high resolution concentration profiles of metals in pore waters (e.g. Cu, Fe, Mn) (Figure 1), and DGT provides an integrated measure of both metals in pore waters and the remobilisation of metals from sediment solid phases (resupply to pore waters). Comparing the DGT and DET concentrations (Figures 2, 3 and 4) allows the extent of short-term remobilisation (24 h in this instance) to be evaluated. When the ratio of DGT/DET (R) is >0.8, very strong short-term mobilisation from the solid phase is indicated, sufficient to maintain porewater concentrations when metals are being removed from the pore waters (by either a sampler or an organism). An R <0.2 indicates little to no mobilisation. An R of 0.2-0.8 indicates a range of mobilisation (from weak to strong mobilisation with increasing R) from the solid phase. In the case of DGT, probes with two different diffusion layer thicknesses (Δg = 0.4 and 0.8 mm) were deployed to gain greater insights into remobilisation of metals from the tailings. Effectively, the DGT with the 0.4 mm Δg accumulates metals faster than the DGT with the 0.8 mm Δg, which in turn accumulates faster than the DET. When the two DGT measurements are similar it further indicates that the mobilisation occurs quickly. When they are different (i.e. 0.4 mm Δg measures a lower concentration than 0.8 mm) it indicates that the mobilisation is more limited. This is useful because DGT will accumulate metals quicker than most organisms accumulate metals by exposure. Thus, when this information is combined, a stronger insight is gained into the links between geochemistry of metals with sediment pore waters, metal fluxes and the risks of excessive metal exposure for benthic organisms.

Copper mobilisation from tailings-sediments

For copper, DET, DGT (0.8 mm Δg) and DGT (0.4 mm Δg) profiles are compared across treatments in (Figure 2. The copper concentrations are best compared separately around the major peaks and for the background concentrations deeper in the sediment.

- DET provided the equilibrium porewater copper concentrations with depth. For many treatments (but especially T2 and T3) there was a high copper peak at about 0.5-1.5 cm depth.
- The DGT measurements showed similar general profiles with high copper peaks (19-50 µg/L) at about 0.5-1.5 cm depth for several treatments, although the peak width varied somewhat. This was due to slight differences in the profile development laterally within each treatment. For this evaluation, it will be useful to compare the concentrations for the peaks and for the background concentrations.
- In Week 5, the two tailings treatments (T2 and T3) indicated moderate to strong mobilisation, with R-values from 0.4-0.6 for the peaks (Table 15). The T2 treatment gave the highest rates of mobilisation (≈0.6) and notably the R-values were similar for 0.4 and 0.8 mm thicknesses, indicating more rapid mobilisation. The T3 treatment had R-values of 0.54 (0.4 mm) and 0.42 (0.8 mm) indicating slightly lower mobilisation than T2. In Week 11, the results were different, with the T2 DET peak maximum decreasing substantially and the T3 DET peak maximum being narrower and higher (noted as an

anomaly above). The T2 Week 11 R-values (0.78 and 0.91) for the peak indicated very strong mobilisation. For T3 the Week 11 R-values indicating quite weak mobilisation. Treatments T2 and T3 will be monitored twice more using both DET and DGT (to be reported in Stage 2).

- The treatments T4, T5, T6 and T7 indicated weak to very weak copper mobilisation at 0.5-1.5 cm depth (R = 0.25-0.16) in Week 5. In week 11, however, mobilisation was higher in treatments T4 (R = 0.37 and 0.63) and T5 (R = 0.31 and 0.42) and somewhat higher in T7 (R = 0.26 and 0.30). Mobilisation was still generally weak in T6 although an increased R (0.25) value was observed with the 0.4 mm DGT.
- Background concentrations (≈5 cm depth) indicated no mobilisation for any treatments in Weeks 5 and 11.

Overall the results indicated that significant remobilisation of copper from tailings is occurring within the 0-3 cm sediment depth range and maintaining elevated copper concentrations in the surface pore waters.

Manganese mobilisation from tailings

For manganese, DET, DGT (0.8 mm DL) and DGT (0.4 mm DL) profiles were compared across treatments in Figure 3.

- DET provided the equilibrium porewater manganese concentrations with depth, showing a gradual increase in concentration from the sediment-water interface to 4 cm depth below which the concentrations remained steady (≈10-30 mg/L), although as noted earlier the manganese concentrations increased in week 11. The tailings treatments had lower Mn^{2+} concentrations.
- The DGT concentration profiles were the same general shape as the DET measurements with much lower estimated concentrations. The treatments varied from weak to no mobilisation using 0.8 mm DGT samplers and no mobilisation of manganese with 0.4 mm DGT, confirming that mobilisation is not sustained at higher rates of accumulation. The R-values were observed to increase from Week 5 to Week 11, with weak mobilisation observed in all treatments except for T2 (Table 15). This trend will be further evaluated with subsequent samplings.

Overall results indicated high manganese porewater concentrations at depths below 4 cm, but weak to no remobilisation from the solid phase.

Iron mobilisation from tailings

For iron, DET, DGT (0.8 mm Δg) and DGT (0.4 mm Δg) profiles were compared across treatments in Figure 4.

- DET provided the equilibrium porewater iron concentrations with depth. The profiles were not as regular as for the manganese with several treatments indicating small peaks of iron near to the sediment-water interface. The profiles then generally increased in concentration with increasing depth (≈0.8-6 mg/L), with some becoming steady below 4 cm depth. Again concentrations increased from Week 5 to Week 11.
- The DGT concentration profiles were the same general shape as the DET measurements. The 0.8 mm Δg profile had higher maximum concentrations than the 0.4 mm indicating that mobilisation was not sustained at higher rates of accumulation, although the mobilisation with the 0.8 mm Δg ranged from moderate (T1, T3, T4, T5, T6, T7) to strong (T2). Mobilisation varied from weak (T2, T3, T4, T5, T6) to none (T1, T7) with the 0.4 mm probe. The only substantial changes between Weeks 5 and 11 was a decrease in the R-value for T2 to a similar value to those for most other treatments (Table 15). The R-value also decreased for T1 (0.8 mm) and increased for T2, T3, T4 and T7 with the 0.4 mm.

Overall, results indicated moderate iron porewater concentrations at depths of 4 cm and lower but with significant mobilisation from the solid phase under conditions of low accumulation. Mobilisation decreased at higher rates of accumulation although is still present.

Table 15. R-values (C_{DGT}/C_{DET}) using both 0.08 and 0.04 thickness diffusive layers in DGT samplers

	R-values							
	Cu peak*		Cu background#		Mn background#		Fe background#	
Treatments	08 DGT	04 DGT	08 DGT	04 DGT	08 DGT	04 DGT	08 DGT	04 DGT
T1 (Week 5)	0.09	0.10	0.05	0.09	**0.27**	0.14	**0.49**	0.10
T1 (Week 11)	0.20	0.19	0.12	0.38	**0.22**	0.16	**0.30**	0.06
T2 (Week 5)	***0.57***	***0.61***	0.17	0.17	0.20	0.10	***0.85***	**0.22**
T2 (Week 11)	***0.78***	***0.91***	0.11	0.17	0.18	0.12	***0.59***	**0.41**
T3 (Week 5)	***0.54***	**0.42**	0.15	0.15	0.20	0.12	***0.62***	**0.23**
T3 (Week 11)	**0.27**	**0.23**	0.19	0.21	**0.23**	0.13	***0.77***	**0.46**
T4 (Week 5)	**0.25**	0.19	0.14	0.14	0.10	0.11	**0.41**	**0.23**
T4 (Week 11)	***0.63***	**0.37**	0.03	0.11	**0.26**	0.18	***0.54***	**0.34**
T5 (Week 5)	0.16	0.16	0.08	0.08	**0.21**	0.11	**0.47**	**0.28**
T5 (Week 11)	**0.31**	**0.42**	0.07	0.10	**0.27**	0.16	***0.45***	**0.24**
T6 (Week 5)	0.17	0.17	0.10	0.12	0.16	0.10	**0.52**	0.18
T6 (Week 11)	0.13	**0.25**	0.06	0.06	**0.25**	0.15	***0.55***	**0.40**
T7 (Week 5)	0.11	0.11	0.09	0.03	0.10	0.11	***0.59***	0.10
T7 (Week 11)	**0.26**	**0.30**	0.04	0.07	**0.28**	0.13	***0.53***	**0.33**

*concentration maxima between 0.5 and 1.5 cm depth. # background taken from 4-6 cm depth

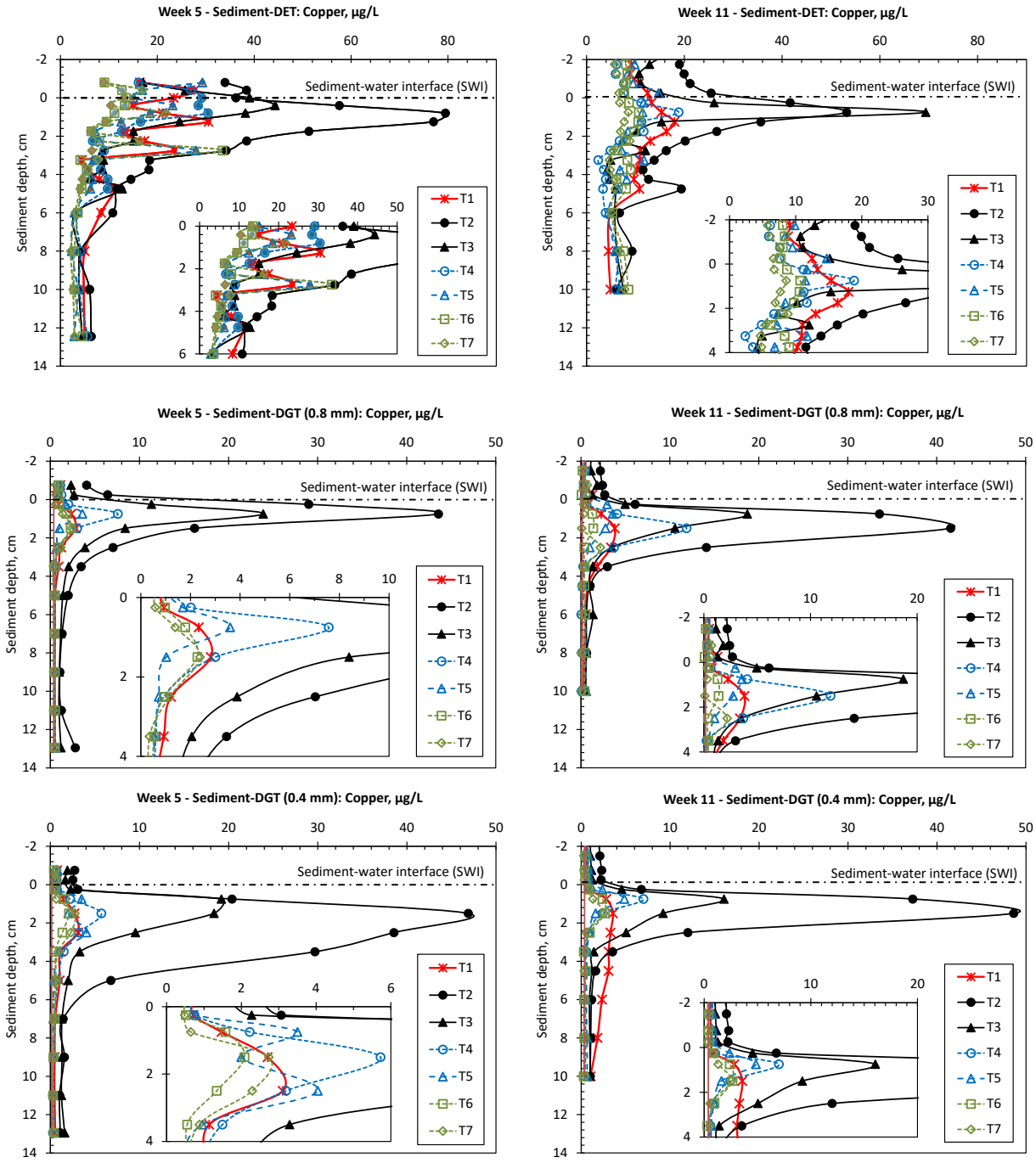


Figure 2. Week 5 and Week 11 comparisons for copper of sediment DET and DGT (0.4 mm Δg) and DGT (0.8 mm Δg) profiles across all treatments. MDL = method detection limit. The figure for sediment DGT Cu contains data embedded with a larger scale near the mobilisation depth.

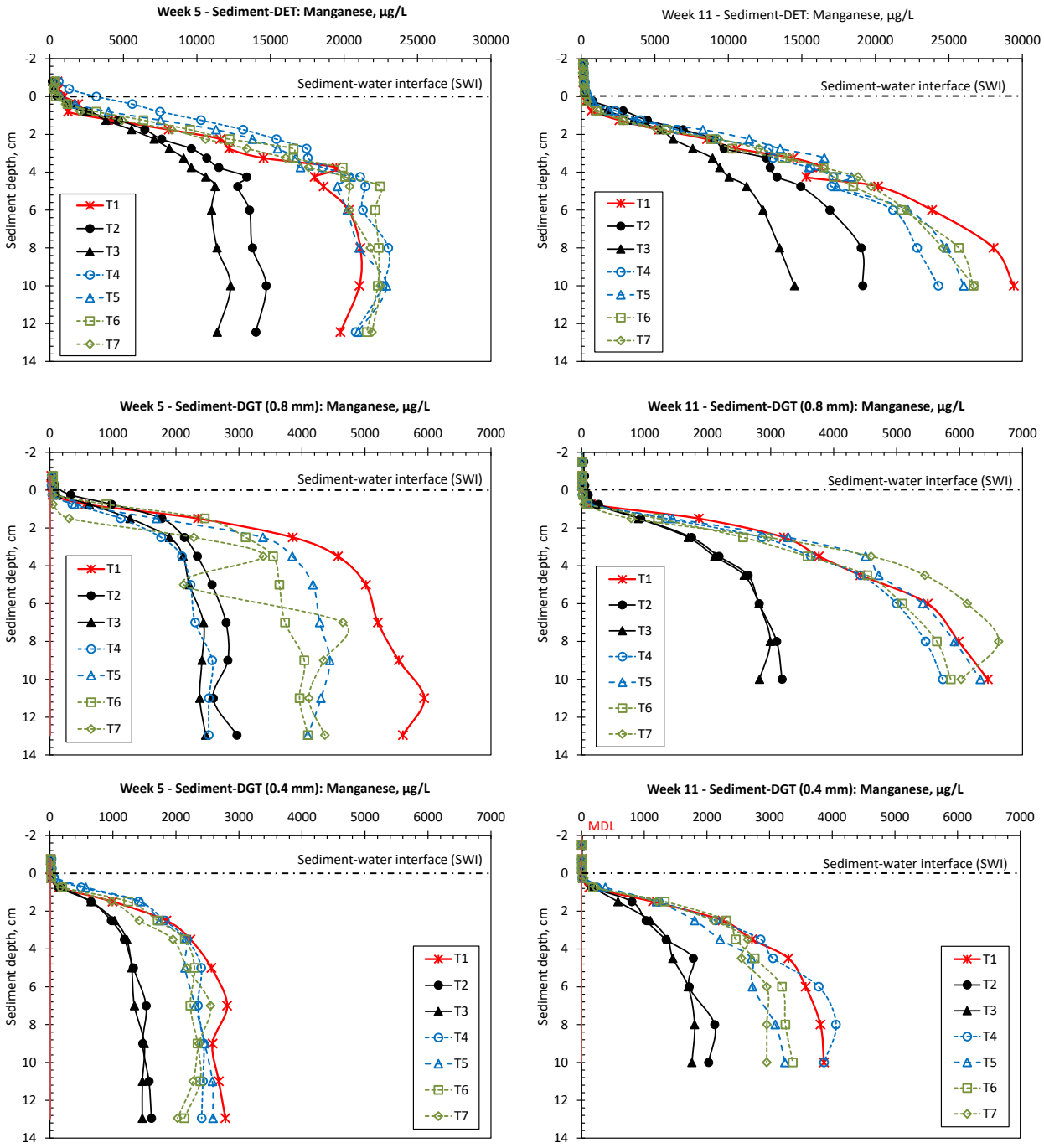


Figure 3. Week 5 and Week 11 comparisons for manganese of sediment DET and DGT (0.4 mm Δg) and DGT (0.8 mm Δg) profiles across all treatments. MDL = method detection limit. The figure for sediment DGT Cu contains data embedded with a larger scale near the mobilisation depth.

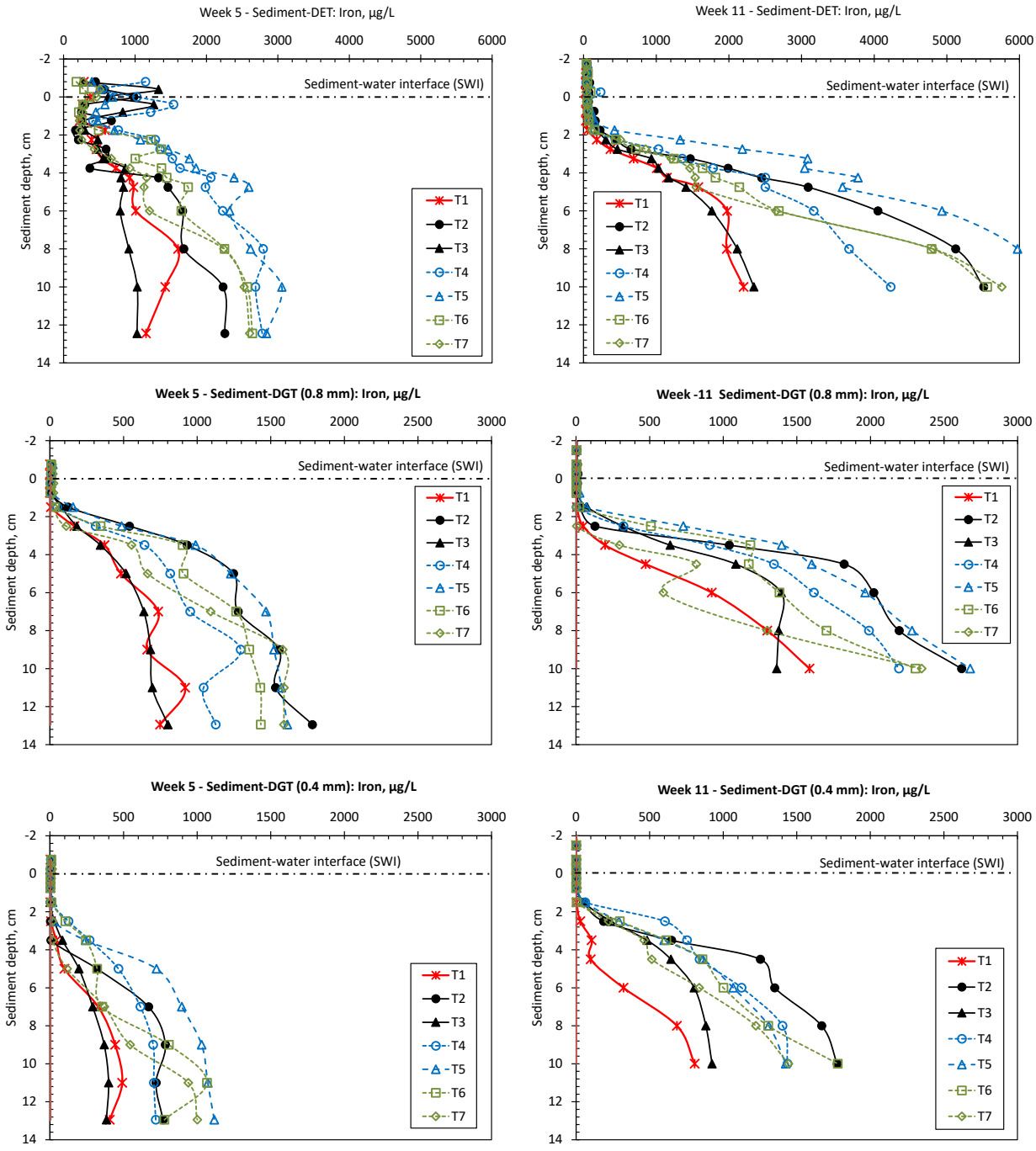


Figure 4. Week 5 and Week 11 comparison for iron of sediment DET and DGT (0.4 mm Δg) and DGT (0.8 mm Δg) profiles across all treatments. MDL = method detection limit. The figure for sediment DGT Cu contains data embedded with a larger scale near the mobilisation depth.

3.6 Metal fluxes to overlying waters

The benthic copper fluxes determined using DET concentration profiles described diffusion controlled fluxes only (Section 3.4.3) and the total net flux may be influenced by the water flow across the sediment-water interface (SWI), which may cause advection of pore waters through the SWI to increase (Zie et al., 2018). In this section, we discuss fluxes calculated using the net dissolved metal release to overlying waters, based on the weekly monitoring data before water changes.

The water flow directed across the SWI was 0.05 m/s during the 17-week period over which data were gathered for this report. The weekly or fortnightly analyses of dissolved metals within the exposure

chambers before water changes enabled net dissolved copper and manganese flux (net advective flux) to be calculated (Figure 5) using the surface area of the tailings-sediment (680 cm^2) and the volume of overlying water (75 L, or 160 L when SR was initially attached (first 3 weeks)). For zinc, the concentrations were not significantly different for any of the treatments and were considered representative of the seawater concentrations influenced by minor amounts of contamination during collection, storage and handling.

Note, there are a range of assumptions that influence the calculated results, including:

(i) The rate of copper release may change over the course of each week up until the sampling before the water change.

(ii) During weeks 2- 12 the tailings-sediment surface level (SWI) gradually dropped 0.5-1.2 cm below the edge of the container, with generally a greater drop closer to the source of the seawater flow indicating minor scouring (e.g. SWI below lip by 0.9-1.2 cm near source and 0.5-0.7 cm at far end). This may create an 'edge effect' in relation to the flow of water. The water current was directed horizontally, but slightly downwards from one end of the container (approximately 1 cm back from the edge). Closer to the container edges, the water currents may have been lower, and therefore potential currents driving an advective flux may have been lower closer to the container edges.

(iii) The flow was directed from one end of the container and was expected to slow as the water current dispersed. Consequently, the potential current 'driving an advective flux may be expected to be lower at the far end of the treatment container.

(iv) There will be a wide range of bottom flow rates in the natural environment. Additional side experiments are to be undertaken to provide information on the potential influence of greater flow rates over the tailings-sediment surface (to be included in a second report).

Additional experiments that investigate these factors will be reported in Stage 2. The variability in the copper release observed for T2 and T3 (Figure 5) may provide some estimation of the magnitude of edge effects as approximately half the drop in the level of the SWI occurred during this period, and the direction of the water splitter that directed the current across the treatments also changed angle by a 5-10º. The variability is about 50%. Preliminary results from additional side experiments that applied (i) a continuous seawater flow in week 18 (not stop-start) and, (ii) a flow rate in week 19 that was continuous and also three times higher than that of weeks 1-17 indicated only small differences in the magnitude of copper release.

Considering these factors collectively in relation to these same treatments (tailing-sediment mixtures) placed in a deep open ocean environment (i.e. unconstrained by the mesocosm design), we may estimate that points (i) to (iv) may result in differences of the flux within a factor of 2-3 (i.e. flux in unconstrained deep-ocean environment may be lower or higher within this range).

The advective fluxes calculated based on the discrete weekly water samples (Figure 5) were considerably higher than those calculated based on diffusion (Fick's law) (Table 14). At Week 5, net advective copper fluxes were 1.5 and 0.78 mg/m^2/day for T2 and T3, compared to diffusion-based fluxes of 0.064 and 0.048 mg/m^2/day, respectively. At Week 11, they were 0.53 and 0.21 mg/m^2/day for T2 and T3, compared to 0.033 and 0.11 mg/m^2/day, respectively. For manganese at Week 5, the net advective fluxes were 7.0 and 9.9 mg/m^2/day for T2 and T3, compared to diffusion-based fluxes of 3.6 and 5.0 mg/m^2/day, respectively. In Week 11 the net advective fluxes of manganese were negligible, but the diffusion-based fluxes remained similar at approximately 5 mg/m^2/day, respectively. A possibly reason for the negligible net advective flux of manganese by Week 6 (in all treatments, Figure 5) is that the surface sediments had become more oxidised over this period, resulting in a faster rate of oxidation of the upward-diffusing iron(II) and manganese(II), and also resulting in higher concentrations of recently precipitated Fe/Mn-oxy(hydro)oxide phases in the surface sediment that can bind metals released by the sub-surface processes.

During the five weeks before the commencement of the bioaccumulation and toxicity tests (weeks 7-12), the average net advective copper fluxes were 0.06 and 0.02 mg/m^2/day for T4 and T5, compared to zero for T1 (0.00 mg/m^2/day), and slightly negative for T6 and T7 (-0.01 mg/m^2/day).

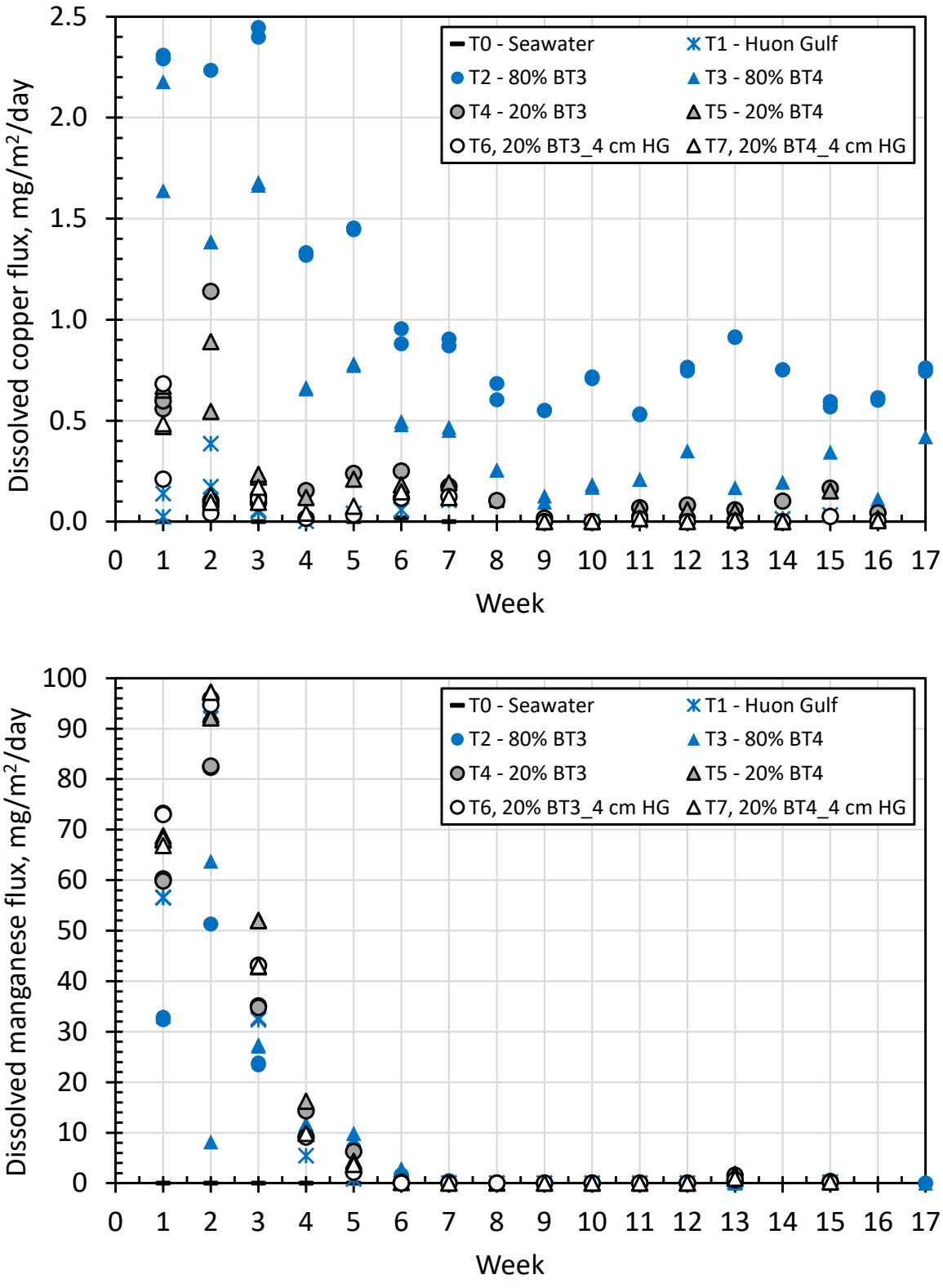


Figure 5. Dissolved copper and manganese fluxes calculated based on the concentrations measured in the overlying waters before water changes (weekly of fortnightly)

Overall, at Week 11 the net advective fluxes of copper were negligible for the T6 and T7 (20:80 tailings:sediment mixture covered by Huon Gulf sediment) and very low for T4 and T5 (20:80 tailings:sediment). For T2 and T3 (80:20 tailings:sediment), the net advective fluxes were16× greater for T2 and 2× for T3 compared to diffusion-based fluxes, and negligible for manganese. Between Weeks 6 and 17, the net advective fluxes of copper appeared to plateau at a lower rate, remaining within the range of 0.5-1 mg/m^2/day for T2 (lower range for T3).

3.7 Porewater analyses

3.7.1 Rhizon samplers

Rhizons are a form of suction sampler, comprising a porous filament (0.1 µm pore size), that were inserted horizontally with each treatment, enabling sampling of 3-15 mL volumes of pore water by sucking from the side port using a syringe. Rhizon samplers were used to extract pore waters in situ from three depths 3, 8 and 13 cm (below the lip of the treatment chamber (TC)). Rhizon sampling occurred at the far end of the TC at the start of the week following the DET-DGT deployments (Weeks 6, 12, and 17 and 24). The results for Weeks 6 and 12 are provided in Tables 16 and 17 respectively. Additional information on the Rhizon results is provided in Appendix C.

Key observations:

- The Rhizon porewater copper concentrations were of a similar magnitude in Weeks 6 and 12, and within the range of 0.5-5 µg/L. The ranges were generally a little greater in Week 12, but there were few if any clear profiles with depth. Overall the results indicate that the tailings were releasing little copper to the porewaters at these depths.
- Porewater zinc concentrations were not consistently greater in the treatments with greater portions of tailing and were generally lower at Week 12 than Week 6. For some treatments on Week 6 the porewater zinc concentrations were higher at greater depths (not T2 or T3).
- Rhizon porewater iron concentrations were generally in the 2-15 mg/L-range and porewater manganese concentrations were generally in the 10-28 mg/L-range. Both frequently greater after 12 weeks than after 6 weeks (T7 the porewater iron and manganese concentrations were lower in Week 12). Porewater aluminium concentrations were low and highly variable and likely to indicate colloidal forms were included in the 0.1-0.2 µm Rhizon filtered fraction.
- Porewater concentrations of molybdenum and nickel were greater in T2 at Weeks 6 and 12 (all depths) than the other treatments, and similar for both sampling periods. T3 also indicated the tailings were a source of these metals to the pore waters, but to a lesser extent.
- Porewater concentrations of arsenic, cobalt and vanadium were lower in T2 and considerably lower in T3 than the other treatments, potentially indicating the Huon Gulf sediment as a greater source of these elements (noting that T7 had lower values on Week 12).

Table 16. Dissolved metals in pore waters extracted using Rhizon samplers within long-term experiments in Week 6.

	Rhizon	Al	Fe	Mn	Dissolved metal concentrations, µg/L (0.1 µm filtered)							
Treatment	Depth, cm		mg/L		Cu	Zn	As	Co	Mo	Ni	Se	V
T1 100% Huon Gulf	3	0.04	1.8	14	1.8	4	14	14	9.1	11	11	3
	8	0.04	4.7	19	1.8	5	25	17	7.9	13	13	4
	13	0.02	3.1	17	2.3	6	16	17	7.6	12	14	4
T2 80% BT3	3	<0.01	6.6	13	1.2	12	11	6	83	47	15	1
	8	<0.01	6.7	13	1.2	9	11	7	82	51	14	1
	13	<0.01	6.9	14	1.1	10	12	7	81	52	8	1
T3 80% BT4	3	0.14	3.3	9.3	2.2	8	8	2	69	28	9	1
	8	<0.01	3.6	11	1.1	6	9	3	70	35	11	<1
	13	0.01	3.4	11	0.9	9	8	3	70	36	12	<1
T4 20% BT3	3	0.02	2.9	17	2.1	4	15	13	20	17	13	3
	8	0.05	4.1	21	2.2	4	17	16	18	22	7	3
	13	<0.01	4.4	20	1.9	9	18	17	18	22	5	3
T5 20% BT4	3	<0.01	3.3	18	1.8	1	15	12	21	19	7	3
	8	<0.01	4.8	21	3.1	7	19	14	21	23	7	3
	13	0.01	4.8	20	2.2	12	18	14	20	22	8	3
T6 20% BT3, 4 cm HG	3	0.05	2.3	19	2.0	6	15	15	13	13	8	3
	8	0.21	4.2	20	3.4	7	18	18	20	24	5	4
	13	0.04	4.2	21	3.1	14	18	17	19	23	6	3
T7 20% BT4, 4 cm HG	3	0.31	2.6	18	2.7	8	17	16	12	14	6	4
	8	0.04	4.5	21	1.8	10	18	14	21	23	6	3
	13	0.27	5.1	22	2.0	20	19	15	21	23	7	4
WQGV (95% PC)					1.3	15	5.5	-	-	70	-	100

Table 17. Dissolved metals in pore waters extracted using Rhizon samplers within long term experiments in Week 12.

	Rhizon	Al	Fe	Mn	Dissolved metal concentrations, µg/L (0.1 µm filtered)							
Treatment	Depth, cm		mg/L		Cu	Zn	As	Co	Mo	Ni	Se	V
T1 100% Huon Gulf	3	0.05	4.4	19	1.7	Err	16	13	8	10	1	3
	8	0.03	6.0	27	2.5	Err	19	18	7	13	2	3
	13	<0.01	7.1	27	5.0	Err	22	21	7	15	2	3
T2 80% BT3	3	<0.01	12	17	0.7	2	10	7	87	47	2	<1
	8	0.25	15	19	1.1	3	11	8	85	54	2	1
	13	<0.01	15	20	0.9	3	12	9	88	58	3	1
T3 80% BT4	3	<0.01	5.0	10	0.8	3	9	2	67	25	2	<1
	8	<0.01	7.8	16	0.5	3	9	3	67	33	3	<1
	13	<0.01	0.7	0.6	4.2	3	2	<1	15	3	1	1
T4 20% BT3	3	<0.01	5.2	17	1.9	<1	15	12	17	14	3	2
	8	<0.01	7.9	27	2.0	<1	19	19	17	22	4	3
	13	0.45	9.7	28	1.9	<1	20	20	17	23	5	3
T5 20% BT4	3	0.07	6.1	19	4.9	<1	17	10	20	16	5	2
	8	<0.01	9.3	28	2.2	1	20	16	19	23	6	3
	13	<0.01	9.7	28	3.8	3	19	17	19	24	10	3
T6 20% BT3, 4 cm HG	3	<0.01	4.9	20	3.0	5	18	14	12	12	8	3
	8	<0.01	8.8	28	3.1	2	20	19	17	22	9	3
	13	<0.01	9.1	28	3.2	5	23	22	19	26	8	4
T7 20% BT4, 4 cm HG	3	<0.01	0.6	2.9	2.7	<1	3	2	12	2	5	2
	8	<0.01	1.2	6.5	3.7	3	4	5	14	7	2	1
	13	0.05	2.4	11	3.3	2	7	8	15	12	4	2
WQGV (95% PC)					1.3	15	5.5	-	-	70	-	100

Other porewater concentrations: cadmium (Cd), silver (Ag), lead (Pb) <0.1 µg/L, Chromium (Cr) <1 µg/L. WQGV to protect 95% of species ANZECC/ARMCANZ (2000). Err = error in analyses (no additional sample to re-analyse)

3.7.2 Centrifugation of sediments

In Week 16 at the commencement of ecotoxicity work for treatments T1 and T4 to T7, sediments were collected from three depths (0-3 cm, 5-8 cm, and 10-13 cm) for extraction of pore waters using centrifugation. The results (Table 18) show that the concentrations of porewater copper decreased with depth, being consistently greater in the surface sediments (top 0-3 cm). Porewater copper concentrations were not significantly different for T1, T6 and T7, but were greater in the surface sediments of T4 and T5.

The concentrations of porewater iron and manganese increased with depth, with low concentration in surface sediments and relatively similar concentrations in the deeper 5-8 and 10-13 cm depth ranges. Porewater manganese concentrations were also were similar for all treatments. The dissolved iron and manganese concentrations indicate the depths where (i) oxygen penetrates (oxic sediments, (ii) iron and manganese (oxy)hydroxides solid phases form), (ii) reductive dissolution of iron and manganese (oxy)hydroxides phases occurs (sub-oxic) and (iii) sulfide formation occurs (anoxic-sulfidic conditions where lower dissolved iron and manganese that concentrations indicate FeS and MnS formation). The data indicate little sulfide formation was occurring in any of the treatments.

Porewater concentrations of As, Co, Ni were greater below the surface layer, consistent with their mobilisation during the reductive dissolution of iron and manganese oxy(hydr)oxides phases. The presence of cobalt and nickel in the deeper sediments likely indicates minimal sulfide formation was occurring.

Table 18. Sediment porewater metal concentrations

Treatment	Depth, cm	Dissolved metal concentrations, µg/L (0.45 µm filtered)							
		Cu	Fe	Mn	As	Co	Ni	Pb	Zn
T1 100% Huon Gulf	0-3	4.6	10	170	<1	<1	2	<1	47
	5-8	1.2	4000	33000	6.9	17	9	3	66
	10-13	1.1	5200	34000	11	20	12	4	59
T4 20% BT3	0-3	12	30	2030	<1	<1	4	<1	15
	5-8	1.1	6300	30000	7.4	16	17	3	40
	10-13	1.0	9900	34000	11	20	22	4	41
T5 20% BT4	0-3	10	260	2010	1.1	<1	5	<1	53
	5-8	1.4	8000	31000	12	15	20		41
	10-13	0.9	9800	34000	15	18	25	4	47
T6 20% BT3, 4 cm HG	0-3	5.1	10	470	1.0	<1	2	<1	21
	5-8	1.4	6600	31000	10	17	19	2	46
	10-13	1.5	11000	34000	15	20	23	3	40
T7 20% BT4, 4 cm HG	0-3	5.2	20	700	<1	<1	4	<1	42
	5-8	1.3	5100	30000	7.7	14	17	3	70
	10-13	0.7	10000	37000	14	18	24	3	61

Porewater Cd and Cr concentrations were <1 µg/L in all samples

Comparison of porewater data from DET, Rhizon samplers and centrifugation

For the five treatments T1 and T4-T7, a comparison could be made between three techniques providing data on porewater concentrations of Cu, Fe and Mn. In the surface sediments (<3 cm depth) of these five treatments, the DET technique determined porewater copper concentrations of 5-30 µg/L, compared to 1-5 µg/L from the Rhizons and 5-12 µg/L from centrifugation. These differences will be influenced by the different volumes (and depth range) of pore water sampled by each technique. Below 4 cm depth the DET results were 2-11 µg/L range, compared to 1-5 µg/L for Rhizons and 1-2 µg/L from centrifugation.

The DET profiles for iron were erratic above 3 cm depth (in Week 5), but more consistent at greater depths. In the surface sediments, the DET technique determined a porewater iron concentrations range of 0.05-2 mg/L, compared to 0.6-6.1 mg/L from the Rhizons and <0.3 mg/L from centrifugation. The disturbance during sampling and centrifugation of the surface sediments is likely to have resulted in some oxidation of Fe(II) and loss as precipitates within this oxic/sub-oxic zone. Below 4 cm depth, the DET results for iron were 0.8-6 mg/L range, compared to 1.2-9.7 mg/L for Rhizons and 4-11 mg/L from centrifugation.

The DET profiles for manganese were clear and consistent from above the sediment-water interface to 10 cm depth. In the surface sediments, the DET technique determined porewater manganese concentrations of 0.3-16 mg/L (increasing with depth), compared to 9-19 mg/L from the Rhizons and 0.17-2.0 mg/L from centrifugation. Below 4 cm depth, the DET results for manganese were 10-30 mg/L range, compared to 6-28 mg/L for Rhizons and 30-37 mg/L from centrifugation.

The As, Co and Ni concentrations in the surface sample (0-3 cm) determined by centrifugation were lower than the Rhizon results, and indicates there may be a significant gradient to lower concentrations above 3 cm depth (the Rhizon sampler was expected to draw pore water from within 1.5 cm of the sampling depth).

The similar porewater iron and manganese concentrations for all three techniques indicated that any oxidation of pore waters during sampling was not significant in the deeper sediments. The strong concentration profiles obtained using the DETs and the higher resolution enabling location of peak concentrations tended to indicate that the DETs provided a better overall method for assessing porewater metal concentrations.

4 Short-term studies of other factors influencing metal release

A series of smaller-scale and short-term laboratory experiments were used to provide information on a range of factors that may influence metal release from the tailings-sediment treatments. These tests were conducted on 100% tailing, 80:20 and 20:80 tailings:sediment mixtures, and 100% Huon Gulf sediment. The following potential influences on metal release were examined:

(i) irradiation of the Huon Gulf sediment (conducted for import quarantine purposes)

(ii) thickness of Huon Gulf sediment overlying tailings (0.5, 1 and 2cm)

(iii) temperature (2, 6, 19, 29ºC)

(iv) dissolved oxygen (<5% saturation (<2 mg/L DO), 85-100% saturation).

For (i) to (iii) measurements of dissolved metals in the overlying waters were made every 2-3 days over a period of 2-3 weeks, with the seawater being replaced with clean seawater immediately after measurements at the end of each week. Tests assessing the effects of pressure on metal release from the tailing-sediment mixtures will also be undertaken and reported in Stage 2.

4.1.1 The influence of irradiation of the Huon Gulf sediment on metal release

For all four pairs of Huon Gulf sediments, and over the entire 2-week test period, the dissolved concentrations of copper and manganese in the overlying waters were significantly greater for the gamma-irradiated samples than the non-irradiated samples (Table 19). Considering all the test data, the average dissolved copper concentrations in overlying waters were 3.5× greater for the irradiated samples (mean±SD = 7.3±2.3 µg/L) than the non-irradiated samples (2.1±0.4 µg/L), and dissolved manganese concentrations were 5.8× greater for the irradiated samples (mean±SD = 2950±800 µg/L) than the non-irradiated samples (510±400 µg/L). There were no significant differences for dissolved zinc.

Porewater copper concentrations (not shown in a Table) were also greater for the irradiated sediment (14 µg Cu/L) than the non-irradiated sediment (5.7 µg Cu/L).

The total and two dilute-acid extractable metal concentrations measured for the single Huon Gulf irradiated/non-irradiated sediment pair are shown in Table 20. These analyses found a lower 0.2% HNO_3-soluble copper concentration for the irradiated sample than the non-irradiated sediment, but no differences for other metals and no differences for the total or 1-M HCl extractable metal concentrations. TOC was 3.7% for the irradiated sediment and 4.4% for the non-irradiated sediment, which is not a large difference and within the range of variability for TOC analyses of replicate samples.

Disturbances caused by sediment collection and homogenisation require many weeks for reestablishment of equilibrium. Past studies have attributed that increases in porewater manganese following the homogenizing of sediments when compared to the non-homogenized sediments to manganese (hydr)oxide phases in the sediments being reductively dissolved when they are brought into contact with porewater Fe(II) (Simpson and Batley, 2003). This reaction ($4Fe^{2+} + 2MnO_{2(s)} + 4H_2O \longrightarrow 2Mn^{2+} + 4FeOOH_{(s)} + 4H^+$) is widely recognized for its importance in the cycling of iron and manganese in sediments (Canfield et al., 1993). This reaction is important because it applies even when sediments are homogenised under anoxic conditions. The process of gamma irradiation would appear to have a similar influence by increasing the release of manganese. The observation that dissolved copper concentrations are greater for irradiated samples may indicate that a significant portion of the copper within the Huon Gulf sediments is associated

with manganese phases, and the dissolution of those phases results in release of copper. The copper may also be associated with organic matter, and irradiation may be releasing copper from those phases.

Table 19. Effect of gamma irradiation on dissolved Cu, Zn and Mn release for pairs of Huon Gulf sediments

Sediment	Bag	Gamma-irradiation	Day 1	Day 5	Day 7	Day 9	Day 12	Day 14
			Dissolved copper, µg/L					
Huon Gulf	BSS1-E	Yes	3.6	9.4	5.5	8.1	10.8	8.0
Huon Gulf	BSS3-F	Yes	3.7	9.3	6.4	7.8	10.6	7.0
Huon Gulf	BSS4-D	Yes	3.4	8.2	5.4	6.8	9.9	7.0
Huon Gulf	BSS5-J	Yes	3.5	8.7	6.5	7.8	10.4	6.9
Huon Gulf	BSS1-E	No	1.1	2.2	2.2	2.0	1.7	2.1
Huon Gulf	BSS3-F	No	1.6	3.0	2.5	2.3	2.4	2.3
Huon Gulf	BSS4-D	No	1.9	2.9	2.4	2.1	2.3	2.4
Huon Gulf	BSS5-J	No	1.4	2.4	2.2	1.8	2.0	2.2
			Dissolved zinc, µg/L					
			Day 1	Day 5	Day 7	Day 9	Day 12	Day 14
Huon Gulf	BSS1-E	Yes	1.1	1.4	<1	1.0	<1	<1
Huon Gulf	BSS3-F	Yes	2.3	<1	<1	0.5	<1	<1
Huon Gulf	BSS4-D	Yes	1.6	1.5	<1	0.4	<1	<1
Huon Gulf	BSS5-J	Yes	2.1	1.6	<1	0.4	<1	<1
Huon Gulf	BSS1-E	No	1.4	1.6	1.7	0.5	1.3	<1
Huon Gulf	BSS3-F	No	1.2	1.5	1.9	0.6	<1	1.0
Huon Gulf	BSS4-D	No	2.0	1.6	2.8	0.9	1.0	<1
Huon Gulf	BSS5-J	No	2.4	1.1	2.4	0.6	1.5	1.7
			Dissolved manganese, µg/L					
			Day 1	Day 5	Day 7	Day 9	Day 12	Day 14
Huon Gulf	BSS1-E	Yes	1926	<5000	2879	3866	<4000	3530
Huon Gulf	BSS3-F	Yes	1980	<5000	3108	3468	<4000	2820
Huon Gulf	BSS4-D	Yes	1851	<5000	2816	3431	<4000	2826
Huon Gulf	BSS5-J	Yes	1673	4804	3054	3428	<4000	2757
Huon Gulf	BSS1-E	No	421	381	643	43	11	28
Huon Gulf	BSS3-F	No	840	1553	709	644	558	28
Huon Gulf	BSS4-D	No	662	916	303	194	197	212
Huon Gulf	BSS5-J	No	693	1332	96	658	673	403

The monitoring of the dissolved metal release over the 17-week period reported above (Tables 10 and 11) observed that the release of dissolved manganese decreased to <1 µg/L by Week 8. That could be consistent with porewater manganese forming manganese oxides within the surface sediments and consequently not being released to the overlying waters, i.e. equilibrium occurring. Note, the oxidation rate of Mn(II) is quite slow, and much slower than Fe(II) in seawater (Stumm and Morgan, 1996; Millero, 2001; Richard et al., 2013). Over that same period dissolved copper release decreased but did not cease (Table 10).

With respect to the assessment of risks posed by these metals for tailings-sediment mixtures and these mixtures covered with Huon Gulf sediment, the combination of sediment disturbance and irradiation are creating greater dissolved copper and manganese concentrations than may be expected for undisturbed and non-irradiated sediments. Consequently, the comparison of the results with WQGVs and the toxicity

and bioaccumulation test results may provide an overly-conservative assessment of the risk of effects to aquatic organisms.

Table 20. Effect of gamma irradiation on Huon Gulf particulate metal concentrations and forms

Total recoverable metal concentrations, mg/kg										
Irradiated	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Zn**
No	12	0.80	23	53	75	49600	1050	51	12	90
Yes	12	0.80	23	54	76	49300	1040	52	12	91
Ratio	1.00	1.00	1.00	1.02	1.01	0.99	0.99	1.01	1.00	1.01
Dilute-acid (1 M HCl) extractable metal concentrations, mg/kg										
Solid	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Zn**
No	3.8	0.30	9.1	4.1	33	8420	520	7.9	6.7	19
Yes	3.8	0.29	8.4	4.0	33	8260	511	7.8	6.8	18
Ratio	1.00	0.95	0.92	0.98	0.98	0.98	0.98	0.98	1.02	0.98
Dilute-acid (0.2% HNO_3) soluble metal concentrations, mg/kg										
Solid	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Zn**
No	0.77	0.05	1.6	0.10	10	568	188	1.0	0.88	2.6
Yes	0.82	0.06	1.7	0.08	5.7	604	247	0.9	0.83	2.1
Ratio	1.07	1.23	1.05	0.87	0.56	1.06	1.31	0.85	0.95	0.81

4.1.2 Influence of Huon Gulf sediment layer thickness on metal release

These tests were undertaken to examine natural sedimentation and closure scenarios, whereby deposited tailings mixtures are covered by thin layers of 0.5 or 1 cm of Huon Gulf sediment and the sufficiency of the "cap" to lower dissolved copper and zinc release is evaluated.

The dissolved metal concentrations measured in these tests (Tables 21 and 22) are higher than those measured in the long-term experiments (Tables 9 and 10) due to the lower volume of overlying water relative to sediment mass and surface area that was the source of metals. The baseline for metal release may be considered the case of 100% Huon Gulf sediment, which was 7.3±2.3 µg Cu/L, 950±800 µg Mn/L, and 1.3±0.7 µg Zn/L (calculated from Table 19). The results for BT3 show the release of copper generally decreases in the order of treatments: 100% tailing ≥ 80% tailing >20% tailing (mixed with Huon Gulf) (Table 21, C1, C2 and C3). Similarly for BT4 (Table 22, C8, C9). The reverse was observed for dissolved manganese, where greater concentrations were observed with increasing amounts of Huon Gulf sediments, which is clearly the greater source of this metal (as discussed previously).

The 0.5 and 1 cm layers of Huon Gulf sediment over the top of the 100% tailings (BT3 or BT4) resulted in dissolved copper release to the overlying waters being of the same magnitude or lower than that observed for the 20% tailing treatment (e.g. 10-11 µg Cu/L for BT3). For BT3, the copper release from treatments with 0.5 cm layer of Huon Gulf sediment over 20% tailing, and 2 cm layers of Huon Gulf sediment over the top of the 100% tailings, were not greater than the baseline release from 100% Huon Gulf treatments. The reduction for BT4 is also similarly significant but is slightly above the 100% Huon Gulf treatments.

Based on the conceptual model (Section 3.4.2), copper mobilisation requires exposure to dissolved oxygen and these experiments indicated that 0.5 cm may be a sufficient thickness to limit copper mobilisation from sediments comprising 80-100% tailings.

4.1.3 Influence of temperature on metal release

These tests were undertaken to determine whether cooler temperatures present in the deep ocean resulted in lower dissolved metal release from the tailings. The experimental temperatures spanned the range of Huon Gulf field data provided by WGJV (GDA Consult Pty Ltd and IHAconsult. 2017) that indicates that temperatures decrease from approx. 27 °C in the surface waters to 14°C at 300 m depth, 6°C at 700 m depth, then to 1.9°C at 3,200 m depth. The tests were undertaken on the 80% tailings:sediment treatment as this was resulting in the greatest dissolved copper release in the longer-term tests.

For 80% tailings treatments at the temperatures of 27, 19, 6 and 2 ^{o}C, the dissolved copper concentrations were (mean±SD) 25±5, 35±10, 30±10, 32±16 µg/L for BT3 respectively (Table 23), and 19±5, 20±6, 14±4, 12±3 µg/L for BT4 respectively (Table 24). Overall, the results indicate no significant influence of temperature on the copper release from deposited sediments, where some specific differences that are unexplained are considered within the range of experimental variability. Similar conclusions were made for manganese and zinc.

4.1.4 Influence of dissolved oxygen on metal release

These tests were undertaken to determine whether low DO concentrations within the overlying waters resulted in lower dissolved metal release from the tailings and tailing-sediment mixtures. These were undertaken on the 100% tailings and 80% tailings:sediment treatments (T2 and T3) and the results are shown in (Tables 25 and 26). For the 100% tailings treatments, those with <10% DO saturation in overlying waters had dissolved copper concentrations of 5±5 µg/L for BT3 and 8±7 µg/L for BT4. These concentrations can be compared to 41±18 and 39±15 µg/L for 100% BT3 and BT4 tailings in saturated DO conditions (90-100%); noting that the dissolved copper increased with consecutive days of treatment. When comparing 10% DO treatments, dissolved copper release was similar with 100% tailings alone and when covered by a 0.5 cm layer of Huon Gulf sediment (5±4 µg/L for BT3 and 3±4 µg/L for BT4). For the 80% tailings treatments, T2 showed 39±15 and 7±8 µg Cu/L and T3 22±6 and 5±2 µg Cu/L for high and low DO-saturation treatments, respectively. The 10% DO treatments also had lower dissolved zinc release than the 90-100% DO treatments (Table 25). Low and high DO treatments did not have significantly different dissolved manganese concentrations when comparing treatments with similar substrates. This is consistent with Mn(II) being released by the reduction of MnO_2 and Mn(II) having slow oxidation kinetics for precipitation as MnO_2 (Richard et al., 2013).

Overall the results indicated that low DO concentrations in overlying waters resulted in lower fluxes of copper and zinc from the tailings and tailings-sediment mixtures, but may not significantly influence manganese fluxes. The waters within the mesocosms were >80% saturated with DO. The observations are also consistent with the conceptual model proposed based on the DET concentrations (Section 3.4.2), i.e. the copper is initially mobilised from the oxidation of copper sulfide minerals to a labile form in the presence of sufficient dissolved oxygen.

Table 21. Dissolved Cu, Zn and Mn in overlying waters during HG-layer thickness tests on HT3 tailings

				Dissolved copper, µg/L					
Treatment	**Tailings**	**Sediment**	**HG layer**	**Day 1**	**Day 5**	**Day 7**	**Day 9**	**Day 12**	**Day 14**
C1	BT3 100%	Nil	Nil	21	31	31	44	49	63
C1R replicate	BT3 100%	Nil	Nil	21	31	37	54	63	74
C2	BT3 80%	HG 20%	Nil	17	49	40	39	42	33
C3	BT3 20%	HG 80%	Nil	4	12	9	12	17	12
C4	BT3 100%	Nil	0.5 cm	6	12	11	13	14	10
C4R replicate	BT3 100%	Nil	0.5 cm	6	10	9	12	12	9
C5	BT3 100%	Nil	1 cm	8	17	13	16	21	8
C6	BT3 80%	HG 20%	0.5 cm	5	13	10	11	14	11
C7	BT3 20%	HG 80%	0.5 cm	4	Lost	5	6	7	6
#C1	BT3 100%	Nil	Nil	74	80	85	93	85	
#C1R replicate	BT3 100%	Nil	Nil	87	86	86	86	84	
C11	BT3 100%	Nil	2 cm	12	14	6	8	9	
C11R replicate	BT3 100%	Nil	2 cm	14	15	5	7	8	
				Dissolved zinc, µg/L					
Treatment	**Tailings**	**Sediment**	**HG layer**	**Day 1**	**Day 5**	**Day 7**	**Day 9**	**Day 12**	**Day 14**
C1	BT3 100%	Nil	Nil	4	6	4	4	6	7
C1R replicate	BT3 100%	Nil	Nil	5	8	4	6	7	7
C2	BT3 80%	HG 20%	Nil	3	Lost	3	3	3	3
C3	BT3 20%	HG 80%	Nil	2	3	4	2	2	2
C4	BT3 100%	Nil	0.5 cm	1	2	2	1	2	<1
C4R replicate	BT3 100%	Nil	0.5 cm	6	3	1	1	1	<1
C5	BT3 100%	Nil	1 cm	1	4	1	1	2	3
C6	BT3 80%	HG 20%	0.5 cm	1	2	1	1	2	2
C7	BT3 20%	HG 80%	0.5 cm	1	Lost	0	0	1	<1
#C1	BT3 100%	Nil	Nil	6	1	<1	<1	2.7	
#C1R replicate	BT3 100%	Nil	Nil	8	<1	<1	<1	1.9	
C11	BT3 100%	Nil	2 cm	0.6	4.3	2.8	<1	<1	
C11R replicate	BT3 100%	Nil	2 cm	0.3	<1	1.8	<1	<1	
				Dissolved manganese, µg/L					
Treatment	**Tailings**	**Sediment**	**HG layer**	**Day 1**	**Day 5**	**Day 7**	**Day 9**	**Day 12**	**Day 14**
C1	BT3 100%	Nil	Nil	570	1420	840	1120	1820	810
C1R replicate	BT3 100%	Nil	Nil	640	1610	890	1130	1890	950
C2	BT3 80%	HG 20%	Nil	960	2170	1370	1610	2770	1220
C3	BT3 20%	HG 80%	Nil	1780	4250	2410	2930	<4000	2420
C4	BT3 100%	Nil	0.5 cm	2920	4640	2460	2730	<4000	1790
C4R replicate	BT3 100%	Nil	0.5 cm	2860	4890	2710	2890	<4000	2050
C5	BT3 100%	Nil	1 cm	2590	<5000	3740	<5000	<4000	1900
C6	BT3 80%	HG 20%	0.5 cm	1220	2740	1580	1950	3670	1510
C7	BT3 20%	HG 80%	0.5 cm	2670	<5000	2770	3200	<4000	2830
#C1	BT3 100%	Nil	Nil	1000	1340	800	930	1120	
#C1R replicate	BT3 100%	Nil	Nil	1160	1510	790	850	1030	
C11	BT3 100%	Nil	2 cm	<5000	<5000	3140	3840	4470	
C11R replicate	BT3 100%	Nil	2 cm	<5000	<5000	2480	3120	3810	

BT3 = Bulk Tails-3 = 90% porphyry:10% metasediments. 80:20 refers to 80% tailings and 20% sediment mixture. 20:80 refers to 20% tailings and 80% sediment mixture. Treatments #C1 and #C1R were a continuation of C1 and C1R (resulting in Day 1 = Day 16 and Day 14 = Day 28 for these treatments). Treatments C11 and C11R were modifications of C4 and C4R whereby an additional 1.5 cm of HG was added to create a 2 cm layer of HG overlying the tailings, and these are compare against the uncapped #C1 and #C1R treatments.

Table 22. Dissolved Cu, Zn and Mn in overlying waters during HG-layer thickness tests on BT4 tailings

				Dissolved copper, µg/L					
Treatment	Tailings	Sediment	HG layer	Day 1	Day 5	Day 7	Day 9	Day 12	Day 14
C8	BT4 100%	Nil	Nil	20	26	31	44	51	67
C9	BT4 80%	HG 20%	Nil	9	26	18	20	27	21
C10	BT4 100%	Nil	0.5 cm	8	16	12	15	16	15
#C8	BT3 100%	Nil	Nil	97	87	86	96	90	
C12	BT3 100%	Nil	2 cm	13	15	6	7	9	
				Dissolved zinc, µg/L					
Treatment	Tailings	Sediment	HG layer	Day 1	Day 5	Day 7	Day 9	Day 12	Day 14
C8	BT4 100%	Nil	Nil	5	6	4	5	6	5
C9	BT4 80%	HG 20%	Nil	3	6	2	4	4	3
C10	BT4 100%	Nil	0.5 cm	2	2	1	1	2	2
#C8	BT3 100%	Nil	Nil	6.5	<1	<1	<1	1.5	
C12	BT3 100%	Nil	2 cm	0.3	<1	<1	<1	<1	
				Dissolved manganese, µg/L					
Treatment	Tailings	Sediment	HG layer	Day 1	Day 5	Day 7	Day 9	Day 12	Day 14
C8	BT4 100%	Nil	Nil	600	1480	910	1170	1900	890
C9	BT4 80%	HG 20%	Nil	830	2360	1300	1630	3030	1440
C10	BT4 100%	Nil	0.5 cm	2090	3500	1850	2100	3640	1290
#C8	BT3 100%	Nil	Nil	1100	1450	590	710	910	
C12	BT3 100%	Nil	2 cm	<5000	<5000	2090	2520	3120	

Treatment #C8, was a continuation of C8 (resulting in Day 1 = Day 16 and Day 14 = Day 28 for these treatments).
Treatments C12 and C12R were modifications of C10 and C10R, whereby an additional 1.5 cm of HG was added to create a 2 cm layer of HG overlying the tailings, and these are compare against the uncapped #C8 treatment.

Table 23. Dissolved Cu, Zn and Mn in overlying waters for treatments at different temperatures on BT3 tailings

			Temperature	Dissolved copper, µg/L					
Treatment	Tailings	Sediment	ºC	Day 1	Day 5	Day 7	Day 9	Day 12	Day 14
T2	BT3 80%	HG 20%	27	26	35	23	22	25	19
T1	BT3 80%	HG 20%	19	16	44	36	36	44	33
T3	BT3 80%	HG 20%	6	18	25	23	28	40	40
T3R replicate	BT3 80%	HG 20%	6	12	30	27	31	42	40
T4	BT3 80%	HG 20%	2	11	25	23	31	52	48
			Temperature	Dissolved zinc, µg/L					
Treatment	Tailings	Sediment	ºC	Day 1	Day 5	Day 7	Day 9	Day 12	Day 14
T2	BT3 80%	HG 20%	27	2	3	1	3	1	1
T1	BT3 80%	HG 20%	19	5	6	3	3	4	3
T3	BT3 80%	HG 20%	6	4	9	5	6	8	8
T3R replicate	BT3 80%	HG 20%	6	4	8	5	6	8	8
T4	BT3 80%	HG 20%	2	5	7	10	7	9	8
			Temperature	Dissolved manganese, µg/L					
Treatment	Tailings	Sediment	ºC	Day 1	Day 5	Day 7	Day 9	Day 12	Day 14
T2	BT3 80%	HG 20%	27	900	2030	1300	1480	2210	1060
T1	BT3 80%	HG 20%	19	790	2160	1300	1580	2830	1250
T3	BT3 80%	HG 20%	6	640	1300	850	1160	2010	1130
T3R replicate	BT3 80%	HG 20%	6	530	1640	920	1230	2250	1180
T4	BT3 80%	HG 20%	2	820	1630	930	1250	2370	1170

.

Table 24. Dissolved Cu, Zn and Mn in overlying waters for treatments at different temperatures on BT4 tailings

			Temperature	Dissolved copper, µg/L					
Treatment	Tailings	Sediment	ºC	Day 1	Day 5	Day 7	Day 9	Day 12	Day 14
T6	BT4 80%	HG 20%	27	11	25	20	22	22	17
T5	BT4 80%	HG 20%	19	9	22	19	21	28	23
T7	BT4 80%	HG 20%	6	8	14	14	13	14	18
T7R replicate	BT4 80%	HG 20%	6	lost	12	11	16	22	13
T8	BT4 80%	HG 20%	2	7	13	11	12	14	13
			Temperature	Dissolved zinc, µg/L					
Treatment	Tailings	Sediment	ºC	Day 1	Day 5	Day 7	Day 9	Day 12	Day 14
T6	BT4 80%	HG 20%	27	2	4	1	4	2	2
T5	BT4 80%	HG 20%	19	4	9	13 [a]	3	3	3
T7	BT4 80%	HG 20%	6	2	5	6	5	5	5
T7R replicate	BT4 80%	HG 20%	6	lost	5	15 [a]	6	7	5
T8	BT4 80%	HG 20%	2	4	5	3	4	5	5
			Temperature	Dissolved manganese, µg/L					
Treatment	Tailings	Sediment	ºC	Day 1	Day 5	Day 7	Day 9	Day 12	Day 14
T6	BT4 80%	HG 20%	27	810	1970	1450	1840	3050	1290
T5	BT4 80%	HG 20%	19	790	2220	1280	1600	3180	1300
T7	BT4 80%	HG 20%	6	550	1890	1200	1490	2770	1330
T7R replicate	BT4 80%	HG 20%	6	lost	1420	1070	1550	2800	1350
T8	BT4 80%	HG 20%	2	680	1600	880	1240	2480	1060

[a] Potential samples contamination during collection, filtering and analysis.

Table 25. Dissolved Cu, Zn and Mn for treatments with low and high dissolved oxygen: BT3 tailings

			DO	Dissolved copper, µg/L					
Treatment	Tailings	Sediment	% saturation	Day 1	Day 5	Day 7	Day 9	Day 12	Day 14
O1	BT3 100%	Nil	90-100%	22	26	31	44	61	63
O2	BT3 100%	Nil	<10%	6	4	9	2	<1	1
O2R replicate	BT3 100%	Nil	<10%	7	19	1	1	<1	10
O3	BT3 80%	HG 20%	90-100%	14	57	43	41	47	35
O4	BT3 80%	HG 20%	<10%	5	16	13	12	11	10
O5	BT3 100%	5 cm HG layer	<10%	2	2	2	2	14	2
				Dissolved zinc, µg/L					
Treatment	Tailings	Sediment		Day 1	Day 5	Day 7	Day 9	Day 12	Day 14
O1	BT3 100%	Nil	90-100%	3	3	2	4	5	5
O2	BT3 100%	Nil	<10%	1	2	<1	<1	<1	<1
O2R replicate	BT3 100%	Nil	<10%	<1	2	<1	<1	<1	3
O3	BT3 80%	HG 20%	90-100%	3	5	2	2	3	2
O4	BT3 80%	HG 20%	<10%	1	<1	<1	2	<1	1
O5	BT3 100%	5 cm HG layer	<10%	0	1	<1	1	<1	<1
				Dissolved manganese, µg/L					
Treatment	Tailings	Sediment		Day 1	Day 5	Day 7	Day 9	Day 12	Day 14
O1	BT3 100%	Nil	90-100%	790	1700	1490	1630	2740	1310
O2	BT3 100%	Nil	<10%	590	1180	860	970	1360	630
O2R replicate	BT3 100%	Nil	<10%	590	1280	970	950	1300	710
O3	BT3 80%	HG 20%	90-100%	860	2120	2110	2150	3820	1760
O4	BT3 80%	HG 20%	<10%	780	1480	1200	1300	2510	1310
O5	BT3 100%	5 cm HG layer	<10%	3730	4560	3550	3240	<4000	2040

Table 26. Dissolved Cu, Zn and Mn in treatments with low and high dissolved oxygen on BT4 tailings

			DO	Dissolved copper, µg/L					
Treatment	Tailings	Sediment	% saturation	Day 1	Day 5	Day 7	Day 9	Day 12	Day 14
O1	BT4 100%	Nil	90-100%	17	38	32	39	61	50
O2	BT4 100%	Nil	<10%	2	1	1	0	<1	<1
O2R replicate	BT4 100%	Nil	<10%	14	7	7	23	<1	<1
O3	BT4 80%	HG 20%	90-100%	10	27	24	24	28	22
O4	BT4 80%	HG 20%	<10%	5	8	5	4	5	3
O5	BT4 100%	5 cm HG layer	<10%	7	2	2	3	<1	<1
				Dissolved zinc, µg/L					
Treatment	Tailings	Sediment		Day 1	Day 5	Day 7	Day 9	Day 12	Day 14
O1	BT4 100%	Nil	90-100%	2	6	2	3	5	3
O2	BT4 100%	Nil	<10%	<1	2	<1	1	1	<1
O2R replicate	BT4 100%	Nil	<10%	<1	2	<1	1	<1	<1
O3	BT4 80%	HG 20%	90-100%	2	6	2	3	4	4
O4	BT4 80%	HG 20%	<10%	<1	2	<1	<1	<1	<1
O5	BT4 100%	5 cm HG layer	<10%	<1	2	<1	1	<1	<1
				Dissolved manganese, µg/L					
Treatment	Tailings	Sediment		Day 1	Day 5	Day 7	Day 9	Day 12	Day 14
O1	BT4 100%	Nil	90-100%	630	1520	1360	1530	2680	1120
O2	BT4 100%	Nil	<10%	610	1190	980	1050	1720	980
O2R replicate	BT4 100%	Nil	<10%	640	890	660	780	1300	750
O3	BT4 80%	HG 20%	90-100%	920	2180	1810	2000	3590	1610
O4	BT4 80%	HG 20%	<10%	820	1670	1110	1200	1700	860
O5	BT4 100%	5 cm HG layer	<10%	3210	1960	2300	2140	3570	1470

5 Toxicity and bioaccumulation tests

5.1 Toxicity tests

Effects to survival and reproduction of the amphipod *Melita plumulosa* over 10 days of exposure was assessed for treatments T1, T4, T5, T6 and T7, starting on week 13 and results are shown in Table 27. For T2 and T3, the same toxicity tests will be undertaken from week 21 and be reported separately (Stage 2). This amphipod species and the test endpoints were selected owing to its relatively high sensitivity to metals (Campana et al., 2012; Simpson et al., 2011; 2013). The amphipod has previously been used for assessing the bioavailability and toxicity of mineral-associated metals in marine sediments (Simpson and Spadaro, 2016). The use of shallow water species as surrogate organisms for assessing metal bioaccumulation and ecotoxicity relating to deep-sea organisms is discussed further in Appendix D.

The survival and reproduction in the test met the acceptability criteria of (≥80% survival and ≥8 embryos per female in the QA control sediment) (Appendix D). Dissolved ammonia concentrations (0.5-1 mg NH3-N/L) remained below levels that may cause effects to the reproduction of the amphipod (Simpson et al., 2013). During the test the amphipod was observed to burrow to 1 cm (burrowing mostly within the 0.2-0.5 cm range) and therefore interacted with the surficial porewaters just below the sediment-water interface.

No toxicity was observed to the survival of the amphipods (classified as acute toxicity) in any of the test treatments. No acute or chronic toxicity was observed in any of the treatments that contained tailings (i.e. when compared to '% of HG Control').

Table 27. Toxicity tests results

	Amphipod survival		Amphipod reproduction			
Sediment	Survival (% survival)	% of QA Control	Embryos per females	% of QA Control	% of HG Control	Average dissolved copper, µg/L[d]
QA control	88 ± 5[a]	-	10 ± 1	100 ± 12	-	2.2 ± 0.8
T1-HG (Control)	90 ± 5	100 ± 6	6 ± 0	67 ± 3[b]	100 ± 5	5.9 ± 2.9
T4	88 ± 5	98 ± 6	8 ± 1	83 ± 9	125 ± 13	10 ± 5.8[e]
T5	81 ± 5	91 ± 6	7 ± 1	70 ± 9	105 ± 14	9.5 ± 4.8[e]
T6	81 ± 5	91 ± 6	9 ± 1	89 ± 6	134 ± 10[c]	4.8 ± 2.6
T7	92 ± 5	102 ± 5	10 ± 1	106 ± 8	158 ± 12[c]	6.2 ± 3.2

[a] All results are mean ± standard error calculated based on the four replicate tests/sediment.
[b] Statically less than the QA control response ($p<0.05$) and below the toxic threshold.
[c] Statically increase reproduction than the HG control ($p<0.05$).
[d] Average dissolved copper measurements of overlying water in the sediments on days 0, 3, 5, and 7.
[e] Statistically greater dissolved copper concentrations measured in the overlying water (t-test pair-wise comparison of daily copper concentration) compared to T1-HG.

When compared to the QA control, toxicity to amphipod reproduction (classified as chronic toxicity) was assessed to occur in the amphipods exposed to the Huon Gulf sediment (T1-HG (Control) containing no tailings was 67±3% of the QA control). This was the lowest level of reproduction of any of the test treatments, and was consistent with previous studies that found that another Huon Gulf sediment was not an optimal substrate for the species reproduction (Adams et al., 2018). This may be attributed to a lower

nutritional content of the Huon Gulf sediment compared to the sediment used as the QA control, but may also be influenced by the very fine particle size of the Huon Gulf sediment (~95% <63 µm, DV50 ~10 µm).

The reproduction was not significantly different to the QA control for the other tailing-sediment treatments (T4, T5, T6, T7). The particle size of the tailings was greater than the Huon Gulf sediment (e.g. BT3 ~40% <63 µm, DV50 ~80 µm), however, the nutritional value of the tailings would be expected to be lower than the Huon Gulf sediment. The T6 and T7 materials comprised of HG sediment capping to 4 cm depth over the T4 and T5 tailings-sediment mixtures, and after 12 weeks of equilibrating, were expected to have the same physical properties as T1-HG but were potentially impacted by upward diffusion of metals from the underlying tailings. The concentrations of dissolved copper released from the sediments into the overlying water were significantly greater (paired daily concentration, $p<0.05$) in T4 and T5 compared to T1-HG, but were not significant in terms of resultant toxicity (Table 27). There was no significant difference between T1-HG and T6 or T7 indicating that there was minimal or no diffusion of copper through the 4 cm Huon Gulf sediment cap. However, results from the T2 and T3 samples are yet to be determined.

5.2 Bioaccumulation tests

Effects to survival and metal bioaccumulation of the benthic bivalve *T. deltoidalis* were assessed for treatments T1, T4, T5, T6 and T7, starting on Week 13 and ending week 16 after 30 days (Table 28, Appendix D). During the test the bivalves were observed to burrow to 8 cm and therefore interacted with the sediment surficial porewaters.

The survival of the bivalves was 70% (of the survival level in test controls) in T4 treatment and 100% in the other treatments. The bioaccumulated metal concentrations measured in the tissues of the bivalves exposed to the tailing-sediment treatments T4-T7 were not significantly different to those in the T1-HG control for all treatments.

Although the survival of the bivalves in T4 was significantly lower than the control, only two were dead and one had been crushed, possibly during placement of the DET and DGT samplers. As the metal concentrations in the T4 treatment were very similar to those of T1 and T5, the small effects to survival cannot be attributed to metals associated with the tailings (see porewater, overlying water and AEM concentration data above).

Table 28. Benthic bivalve survival and bioaccumulation

Test treatment	Dissolved copper, µg/L	Survival, %	Tissue metal concentration, µg/g (dry weight) Al	As	Cd	Co	Cr	Cu
Tissue concentration at commencement			*1300*	*12*	*0.9*	*<0.9*	*2.3*	*230*
T1-HG	1.2	100	4200±1200[a]	18±1.7	1.4±0.2	5.6±1.2	6.9±0.8	370±160
T4	2.1	70	1900±570	20±0.2	1.6±0.3	4.2±0.1	7.3±1.0	440±42
T5	2.0	100	330±230	20±0.6	1.6±0.1	6.1±0.6	13±2.1	410±110
T6	1.2	100	4900±260	19±1.2	1.4±0.1	4.6±0.3	8.2±1.2	500±52
T7	1.0	100	6500±1100	16±1.4	1.2±0.2	6.3±0.9	8.4±0.7	320±33

	Fe	Mn	Ni	Pb	V	Zn
Tissue concentration at commencement	*1600*	*7.6*	*2.7*	*17*	*2.6*	*150*
T1-HG	5200±1500	110±30	8.5±3.2	55±14	15±5.3	410±150
T4	4100±1300	50±16	8.0±1.1	51±9.5	8.6±1.9	360±88
T5	5600±230	79±6.5	13±0.8	52±0.5	13±1.5	340±87
T6	5900±270	130±15	9.2±0.04	42±7.9	17±1.0	390±21
T7	6600±940	160±41	11±1.1	48±7.2	20±4.2	630±600

[a] All results are mean ± standard deviation

6 Summary

The purpose of the long-term tailings study was to provide a better ability to predict the tailings geochemistry and risks to the environment following deposition of tailings and tailings-sediment mixtures on the deep ocean floor.

The study assessed the risks posed by two new tailings master composites (BT3 and BT4) to represent the main production 'book ends' over the life of mine (90:10 porphyry:metasediment and 75:25 metasediment:porphyry). Using mesocosms (exposure chambers) containing tailings-sediment mixtures in seawater, the assessment of the risk posed by the metals was assessed through three lines of evidence: (i) comparison of concentrations in waters and sediments with guideline values; (ii) direct assessment of metal bioavailability by assessing bioaccumulation with exposed organisms (in this case the bivalve *Tellina deltoidalis*); and, (iii) direct assessment of toxicity using a benthic organism test endpoints that is recognised as being high sensitivity to bioavailable metals (in this case the amphipod *Melita plumulosa*).

While the tailings are moving within the deep ocean (e.g. being transported down canyon-slope or resuspended), the geochemistry will be unstable, and this will influence the partitioning of metals between the dissolved and particulate phases and the risks to the environment. Such risks need to be considered in the context of the progressive mixing and entrainment of seawater (as is the basis for formulating the regulated mixing zone at the tailings outfall), and on the characteristics of the receiving environment, which for Wafi Golpu is described as a dynamic sub-sea canyon that receives significant natural river sediment inputs and experiences regular mass movement events (underwater landslides). Once movement of the sediments has ceased, a range of biogeochemical processes will take place that modify the metal partitioning and bioavailability. Mixing with natural sediments and burial will further modify these processes. This study assessed the geochemistry of tailings and tailings-sediment mixtures (treatments) once they have deposited (stopped moving), and also assessed mixtures covered by natural sediments to evaluate closure scenarios. In an open ocean environment, a large portion of the metals released from the materials will not accumulate indefinitely in the overlying waters, but instead will be flushed by water-mixing. The study subjected the treatments to a circulating current of overlying seawater (0.05 m/s across the sediment-water interface) that was replenished with clean seawater weekly to in part reflect the open ocean environment.

In this report, the final results are described for five tailings-sediment treatments (100% natural Huon Gulf (HG) sediment (the control), 20% tailings and 20% tailings covered by HG sediment) and interim results for two 80% tailings treatments. An earlier study indicated that primarily the toxicity risks associated with the tailings were expected to arise from the copper concentrations that were significantly elevated compared to background (Adams et al. 2018). Consequently, this study chose to focus on copper and zinc as potential chemical toxicants and iron and manganese as indicators of the geochemical status of the deposited sediments.

The total recoverable metal (TRM) concentrations of two tailings (BT3 and BT4) were 453-525 mg Cu/kg and 34-57 mg Zn/kg. In relation to sediment quality guideline values (SQGVs, (ANZECC/ARMCANZ, 2000)), TRM concentrations (this study) of Cr, Cu and Ni exceeded the SQGVs by factors of 5-14 (SQGV for copper = 65 mg/kg). The metal concentrations were lower than those of the tailing used in the ecotoxicology studies of Adams et al. (2018); those being 915-1570 mg Cu/kg and 472-840 mg Zn/kg, respectively.

The differences between the results of this study and Adams et al. (2018) were attributed by the WGJV metallurgist to variability of the ore body for the core samples selected to make up the master composite, which is predominantly based on overall copper and sulfide contents. The dilute-acid extractable metal

(AEM) concentrations of the tailing were 103-113 mg Cu/kg (BT3) and 9-15 mg Zn/kg (BT4), compared to 149-182 mg Cu/kg and 392-432 mg Zn/kg for the corresponding tailings studied by Adams et al. (2018). AEM-Cu was 30-40 mg/kg for T1 (100% HG), 191 mg/kg for T2, 107 mg/kg for T3, and 43-77 mg/kg for T4 to T7. The HG sediment was of finer particle size (98% of particles <63 μm) than BT3 (44%) and BT4 (70%). The HG sediment had greater concentrations of Mn, V and Zn than the tailings, but lower concentrations of Cu, Cr and Ni. No metals exceeded SQGVs for the HG sediment.

During the 12 weeks prior to toxicity and bioaccumulation tests the average dissolved copper concentrations in the mesocosms waters exceeded the ANZECC/ARMCANZ (2000) water quality guideline values (WQGV 1.3 μg/L) in the tailings-sediment treatments (T2 to T7), but not in T1 (100% HG). No treatments exceeded the PNG WQC for dissolved copper of 30 μg/L. No other metals exceeded WQGVs in any treatments. Dissolved copper concentrations were greater for T2 (7.6±1.5 μg/L) and T3 (4.3±1.5 μg/L) than the other treatments (generally 1-3 μg/L range). The dissolved copper concentrations were greater for T3 during week 1-6 (4-8 μg/L range) then after (2-4 μg/L range), but T2 concentrations did not decline during the 17 weeks (generally 6-8 μg/L range). The copper concentrations in the other treatments were relatively constant.

Dissolved manganese concentrations were initially high in all treatments (100-300 μg/L during week 1), but decreased to negligible (1-4 μg/L range) by Week 7, and were lower in Treatments T2 and T3 (80% tailings) than in T4 and T5 (20% tailings) and not greater than the treatment with Huon Gulf sediment at the surface (T1, T6, T7).

The disturbance of the sediments caused by the commencement of bioaccumulation and toxicity tests did not significantly influence the metal concentrations other than an occasional increase in dissolved manganese (4-24 μg/L range). Diffusive gradients in thin films (DGTs) devices deployed in the mesocosm waters for approximately 72 h at the end of Weeks 4 and 10 determined moderately lower dissolved copper and much lower manganese concentrations at week 10. Overall the magnitudes and relationships were comparable.

The dissolved copper concentrations in the long-term tailings mesocosms were lower than those measured in the smaller bench-scale tests of Adams et al. (2018) that had a lower ratio of seawater to tailings-sediment. The higher ratios of seawater to tailings-sediment used in the mesocosms are more comparable to an open-ocean environment. Adams et al. (2018) used the bulk tailings BT1 and BT2, prepared as the same relative geological ore composites as BT3 and BT4 respectively, and the dissolved copper concentrations in the overlying waters ranged from 34 to 51 μg/L for BT1 (60-90% tailing mixture with Huon Gulf sediment) and 15 to 21 μg/L for BT2 (60-90% tailing). Thus, the dissolved copper concentrations in the long-term tailings study were ~4-7× lower in BT3 and ~3-5× lower in BT4, when compared to BT1 and BT2 evaluated in the smaller bench-scale simulations of Adams et al. (2018). These differences may be attributed to both the lower reactive copper concentrations associated with the new tailings used for the long-term tailings study and also the greater dilutions in the larger volume of overlying seawater, which is more representative of the open ocean. However, differences in the diagenetic maturation of the tailing-sediment mixtures in the mesocosms compared to the smaller-scale tests undertaken by Adams et al. (2012) makes a direct comparison between the results of the two studies unwarranted based solely on differences in the ratio of seawater to tailings-sediment. The mesocosm studies provide a more representative assessment of the potential dissolved metal release.

Diffusive Equilibration in thin films (DET) devices were used to measure the dissolved porewater concentration profiles in the tailings/sediment treatments. Measurements were made during Weeks 5 and 11, and provided concentration profiles of porewater Cu, Fe, and Mn at a vertical scale of 4-20 mm and enabled calculations of diffusive fluxes of these metals across the sediment-water interface. Generally similar porewater metal profile patterns were observed in each treatment and week, although

concentrations did change according to the composition of the treatment (tailings-sediment). Diagenetic changes in sediment chemistry were clearly evident between weeks 5 and 11 with the dissolved iron and manganese geochemical tracers generally increasing in all treatments with depth and over time, indicating a general increase in reducing conditions, but not to the extent that iron and manganese started to be precipitated as sulfides (FeS and MnS) at depth. By week 11, sedimentary reducing conditions are clearly established below 2cm on the basis of the porewater iron and manganese profiles.

Sediment porewaters were also sampled directly at three depths using Rhizons on Weeks 6 and 12 and also through separation of porewater by centrifugation for treatments T1, T4-T7 on week 17. For these five treatments, porewater copper concentrations determined by Rhizons (1-5 µg/L) and centrifugation (5-12 µg/L) were lower than those from the DET samplers (5-30 µg/L). Porewater iron and manganese concentrations were more similar for all three techniques.

The mobilisation of copper from tailings-sediments was observed by DET and DGT peaks that occurred 0.5 and 1.5 cm below the sediment-water interface. For T2 and T3 (80% tailings) the DET peaks of 45-80 µg Cu/L were higher than all other treatments (20-30 µg Cu/L range), which were not greater than the control T1 (100% HG). Below 6-8 cm depth the DET concentrations were <10 µg Cu/L. The DGT devices used to examine the mobility of copper in the porewater profiles indicated the rates of mobilisation of copper in T2 and T3 were moderate to high in week 5, and then very high for T2 and low for T3 in week 11. For T4, T5, T6 and T7 the copper mobilisation was low to very low in week 5 and low to moderate in week 11. The results generally indicated that significant remobilisation of copper from tailings is occurring at shallow depths and maintaining elevated copper concentrations in the surface pore waters.

Overall, the porewater profiles indicated that copper mobilisation occurred in the oxic zone (surface) where it is likely first released by the oxidation of sulfide phases (e.g. CuS, $Cu(I)_2S$ or $FeCuS_2$) in the tailings and then rapidly re-adsorbed by iron and manganese (hydr)oxide phases from where it can then be remobilised when those phases are reduced. For Cu, Fe and Mn, DET porewater gradients with depth from the sediment-water interface enabled calculation of diffusive flux values. The copper fluxes for all treatments were quite low (<0.1 mg Cu/m^2/day), with T2 and T3 having the greatest relative positive fluxes with averages for Weeks 5 and 11 equivalent to 18-29 kg Cu/km^2/year. The manganese fluxes were always positive and consistent for Weeks 5 and 11, being higher for treatments T1, T6 and T7 (4700-5900 kg Mn/km^2/year) than those for T2 and T3 that had a greater portion of tailings (e.g. 1300-1800 kg Mn/km^2/year). The iron fluxes were moderate to weak and all were <400 kg Fe/km^2/year.

The advection of pore waters caused by the 0.05 m/s seawater flow across the sediment-water interface was expected to result in total net metal fluxes being higher than the diffusion controlled fluxes calculated from the porewater gradients. For T2 and T3 the net advective fluxes calculated using the dissolved copper release to mesocosm waters were 550 and 280 kg Cu/km^2/year and 190 and 75 kg Cu/km^2/year for T2 and T3 at week 5 and 11, respectively, compared to the average diffusion-based fluxes of <30 kg/km^2/year. For weeks 10-17 the net advective fluxes were <70 kg Cu/km^2/year T3 and T4 and <10 kg Cu/km^2/year for T1, T6 and T7. Between Weeks 6 and 17 the net advective fluxes of copper appeared to plateau at a lower rate and did not decrease.

Factors that may influence metal release from the tailings-sediments treatments include the procedure used for preparing and mixing the materials, the physical-chemical properties of the overlying seawater (dissolved oxygen, temperature, and pressure) and the thickness of the HG sediment that may cover tailing-sediment mixtures. It was necessary to gamma irradiate the HG sediment to meet quarantine import requirements, and four pairs of irradiated/non-irradiated HG sediments were studied to examine the effect that this irradiation may have on the dissolved metal release. The released copper and manganese was 3.4× and 5.8× greater for the irradiated samples than for the non-irradiated samples, respectively. There was also evidence that mixing of the HG sediments increased the release of manganese. In relation to the metal fluxes estimated in the long-term experiment study, the process of irradiating the HG sediment will have

caused greater copper and manganese fluxes than would be expected had the sediments not been irradiated.

HG sediment layers of as little as 0.5 and 1 cm placed over 100% tailings (BT3 and BT4) in side experiments resulted in dissolved copper release of the same magnitude or lower than that observed for T4 and T5 (20% tailings:80% sediment mixture treatment without a HG layer). A 0.5 cm layer of HG over T4 (20% BT3 tailings) and 2 cm HG over 100% BT3 resulted in dissolved copper release no greater than 100% HG (T1 treatment). Based on these studies, copper mobilisation requires exposure to dissolved oxygen and these experiments indicated that 0.5 cm may be a sufficient thickness to limit copper mobilisation from sediments comprising 80-100% tailings.

For T2 and T3 treatments (80% tailings), overlying water temperatures of 27, 19, 6 and 2°C had no significant effect on metal release in side experiments. This indicates that reduced temperatures in the deep ocean of the Huon Gulf (6°C at 700 m depth and 1.9°C at 3,200 m depth - Consult Pty Ltd and IHAconsult. 2017), will not significantly affect metal release from deposited tailings.

The influence of dissolved oxygen (DO) on metal release evaluated scenarios of 90-100% and <10% DO-saturation of overlying waters was also examined in side experiments. This compares to dissolved oxygen measured in the Huon Gulf of approx. 85% at the surface, to a minimum of 34% at between 1,700 and 2,100 m, before increasing again to around 39% at the sea bed (3,200 m; GDA Consult Pty Ltd and IHAconsult. 2017). The test determined that low DO concentrations will result in lower fluxes of copper and zinc from the tailings and tailing-sediment mixtures, but may not significantly influence manganese fluxes. For T2 and T3, the dissolved copper concentrations under low DO-saturation were 18-23% of the high DO-saturation results. The waters within the mesocosms were >80% saturated with DO and are expected to represent a worst-case scenario for copper release. The effects of pressure on metal release will be reported in Stage 2 (experiments not yet undertaken).

Overall, each of these side experiments indicated that the exposure conditions used provided a conservative assessment of the risk of effects to aquatic organisms.

The risks of adverse effects to benthic organisms was assessed using toxicity and bioaccumulation bioassays. Effects to survival and reproduction of the amphipod *M. plumulosa* over 10 days and effects to survival and metal bioaccumulation of the benthic bivalve *T. deltoidalis* were assessed for treatments T1, T4, T5, T6 and T7, starting on Week 13 and ending Week 16 after 30 days. For T2 and T3, the same bioassays will be undertaken from Week 21 and be reported separately (Stage 2). The amphipod and bivalve species and the test endpoints were selected owing to their relatively high sensitivity to copper. These species were noted to interact with the surficial sediment porewaters with the amphipod burrowing to a depth of 0.5-1 cm and the bivalve to 6-8 cm respectively, during the bioassays. No acute or chronic toxicity was observed in any of the treatments that contained tailings, with one exception. The survival of the bivalves was 70% in the T4 treatment and 100% in the other treatments, but the metal concentrations were similar in T4 to those of T1 and T5 and the small effects to survival were not attributed to metals associated with the tailings. The bioaccumulated metal concentrations measured in the tissues of the bivalves exposed for 30 days to the tailings-sediment treatments T4-T7 were not significantly different to those in the Huon Gulf control (T1-HG).

The conclusion drawn from this study after Stage 1 is that the risk of adverse environmental effects in the benthic environment posed by DSTP of two tailings master composites (BT3 and BT4) is low. Stage 2 of this study examines the geochemistry of T2 and T3 for a further 7-8 weeks and then assesses their toxicity and potential to result in elevated levels of metal bioaccumulation to benthic organisms. Stage 2 will therefore provide additional information relating to these conclusions.

7 References

Adams, M.S., Spadaro, D.A., Simpson, S.L., Binet, M.T., King, J.J., Jarolimek, C.V., McKnight, K.S., Golding, L.A. and Apte, S.C. (2018). Ecotoxicology and chemistry of Wafi-Golpu bench-scale tailings. CSIRO Report EP178086, 88 pp.

Amato, E.D., Simpson, S.L., Belzunce-Segarra, M.J., Jarolimek, C.V. and Jolley, D.F. (2015). Metal fluxes from porewaters and labile sediment phases for predicting metal exposure and bioaccumulation in benthic invertebrates. Environ. Sci. Technol., 49, 14204–14212.

Amato, E.D., Simpson, S.L., Jarolimek, C. and Jolley, D.F. (2014). Diffusive gradients in thin films technique provide robust prediction of metal bioavailability and toxicity in estuarine sediments. Environ. Sci. Technol., 48, 4485–4494.

ANZECC/ARMCANZ (2000). Australian and New Zealand Guidelines for Fresh and Marine Water Quality, Australia and New Zealand Environment and Conservation Council/Agricultural and Resource Management Council of Australia and New Zealand. Canberra, Australia.

APHA (2005), Standard Methods for the Examination of Water and Wastewater. 21st Edition. American Public Health Association, American Water Works Association, Water Environment Federation, Washington, DC.

Boudreau, B.P. (1996). The diffusive tortuosity of fine-grained unlithified sediments. Geochim. Cosmochim. Acta., 60, 3139-3142.

Campana, O., Spadaro, D.A., Blasco, J. and Simpson, S.L. (2012). Sublethal effects of copper to benthic invertebrates explained by changes in sediment properties and dietary exposure. Environ. Sci. Technol., 46, 6835−6842.

Campana, O., Taylor, A.M., Blasco, J., Maher, W.A. and Simpson, S.L. (2015). The importance of kinetics of sub-cellular partitioning to the predictability of sub-lethal toxic effects of copper in two deposit feeding organisms. Environ. Sci. Technol., 49, 1806–1814.

King, C.K., Dowse, M.C., and Simpson S.L. (2010). Toxicity of metals to the bivalve Tellina deltoidalis and relationships between metal bioaccumulation and metal partitioning between seawater and marine sediments. Archives of Environmental Contamination and Toxicology, 58, 657–665.

Canfield, D.E., Thamdrup, B., and Hansen, J.W. (1993). The anaerobic degradation of organic matter in Danish coastal sediments: Iron reduction, manganese reduction, and sulfate reduction. Geochim. Cosmochim. Acta, 57, 3867–3883.

Chapman, C.S., Capodaglio, G., Turetta, C., van den Berg, C.M.G. (2009). Marine Benthic fluxes of copper, complexing ligands and thiol compounds in shallow lagoon waters. Environ. Res., 67, 17–24.

De Lange, H.J., Van Griethuysen, C. and Koelmans, A.A. (2008). Sampling method, storage and pretreatment of sediment affect AVS concentrations with consequences for bioassay responses. Environ. Pollut. 151, 243–251.

DGT Research Ltd. Practical Guide for Using DGT for Metals in Waters Website; http://www.dgtresearch.com/.

Di Toro, D.M. (2001). Sediment Flux Modelling. Wiley-Interscience, 624 pages.

GDA Consult Pty Ltd and IHAconsult. (2017). Wafi-Golpu Project. Physical, Chemical and Biological Sedimentology of the Huon Gulf. Draft – November 2017. Report 532-1104-FS-REP-0003.

Jorgensen, B.B. and Revsbech, N.P. (1985). Diffusive boundary layers and the oxygen uptake of sediments and detritus. Limnol. Oceanogr., 30, 111–122.

King, C.K., Dowse, M.C. and Simpson, S.L. (2010). Toxicity of metals to the bivalve *Tellina deltoidalis* and relationships between metal bioaccumulation and metal partitioning between seawater and marine sediments. Archives of Environ. Contam. Toxicol., 58, 657-665.

Knight, R.D., Roberts, S., Cooper, M.J. (2018). Investigating monomineralic and polymineralic reactions during the oxidation of sulphide minerals in seawater: Implications for mining seafloor massive sulphide deposits. Appl. Geochem., 90, 63–74.

Li, Y.-H. and Gregory, S. (1974). Diffusion of ions in seawater and in deep-sea sediments. Geochim. Cosmochim. Acta., 38, 703-714.

Millero, F.J. (2001). Physical Chemistry of Natural Waters. Wiley-Interscience, New York, NY, USA.

Richard, D., Sundby, B., and Mucci, A. (2013) Kinetics of manganese adsorption, desorption, and oxidation in coastal marine sediments. Limnol. Oceanogr., 58, 987–996.

Seeberg-Elverfeldt, J., Schlüter, M., Feseker, T. and Kölling, M. (2005). Rhizon sampling of porewaters near the sediment-water interface of aquatic systems. Limnol. Oceanogr. Methods, 3, 361–371.

Sheibley, R.W. and Pauldon, A.J. (2014). Quantifying Benthic Nitrogen Fluxes in Puget Sound, Washington—A Review of Available Data. USGS Scientific Investigations Report, 2014-5033.

Simpson, S.L. and Batley, G.E. (2003). Disturbances to metal partitioning during toxicity testing of iron(II)-rich porewaters and whole sediments. Environ. Toxicol. Chem., 22, 424–432.

Simpson S.L. and Batley, G.E. (2016). Sediment Quality Assessment: A Practical Guide. CSIRO Publishing, Melbourne, Victoria. 359 pp.
https://publications.csiro.au/rpr/download?pid=csiro:EP165955&dsid=DS1

Simpson, S.L. and Spadaro, D.A. (2011). Performance and sensitivity of rapid sublethal sediment toxicity tests with the amphipod *Melita plumulosa* and copepod *Nitocra spinipes*. Environ. Toxicol. Chem., 30, 2326-2334.

Simpson, S.L. and Spadaro, D.A. (2016). Bioavailability and chronic toxicity of metal sulfide minerals to benthic marine invertebrates: implications for deep sea exploration, mining and tailings disposal. Environ. Sci. Technol., 50, 4061–4070.

Simpson, S.L. (2001). A rapid screening method for acid volatile sulfide in sediments. Environ. Toxicol. Chem., 20, 2657–2661.

Simpson, S.L., Batley, G.E. and Chariton, A.A. (2013). Revision of the ANZECC/ARMCANZ Sediment Quality Guidelines. CSIRO Land and Water Report 8/07. CSIRO, Canberra, Australia, 128 pp.

Simpson, S.L., Rosner, J. and Ellis, J. (2000). Competitive displacement reactions of cadmium, copper, and zinc added to a polluted, sulfidic estuarine sediment. Environ. Toxicol. Chem., 19, 1992–1999.

Simpson, S.L., Spadaro, D.A. and O'Brien, D. (2013). Incorporating bioavailability into management limits for copper in sediments contaminated by antifouling paint used in aquaculture. Chemosphere, 93(10), 2499-2506.

Simpson, S.L., Ward, D., Strom, D. and Jolley, D.F. (2012b). Oxidation of acid-volatile sulfide in surface sediments increases the release and toxicity of copper to the benthic amphipod *Melita plumulosa*. Chemosphere, 88, 953–961.

Simpson, S.L., Yverneau, H., Cremazy, A., Jarolimek, C., Price, H.L. and Jolley, D.F. (2012a). DGT-induced copper flux predicts bioaccumulation and toxicity to bivalves in sediments with varying properties. Environ. Sci. Technol., 46, 9038−9046.

Skrabal, S.A., Donat, J.R. and Burdige, D.J. (2000) Pore water distributions of dissolved copper and copper-complexing ligands in estuarine and coastal marine sediments. Geochim. Cosmochim. Acta, 64, 1843–1857.

Spadaro, D.A. and Simpson, S.L. (2016a). Appendix E. Protocol for 10-day whole-sediment sub-lethal (reproduction) and acute toxicity tests using the epibenthic amphipod *Melita plumulosa*. In Simpson SL, Batley GE (eds), Sediment Quality Assessment: A Practical Guide. CSIRO Publishing, Victoria, Australia, pp 265-275.

Spadaro, D.A. and Simpson, S.L. (2016b). Appendix G. Protocols for 10-day whole-sediment lethality toxicity tests and 30-day bioaccumulation tests using the deposit-feeding benthic bivalve *Tellina deltoidalis*. In Simpson SL, Batley GE (eds), Sediment Quality Assessment: A Practical Guide. CSIRO Publishing, Victoria, Australia, pp 285-293.

Stumm, W. and Morgan, J.J. (1996). Aquatic chemistry: Chemical equilibria and rates in natural waters. John Wiley & Sons.

Teasdale, P.R., Apte, S.C., Ford, P.W., Batley, G.E. and Koehnken, L. (2003). Geochemical cycling and speciation of copper in waters and sediments of Macquarie Harbour, Western Tasmania. Estuar., Coastal Shelf Sci., 57, 475–487.

USEPA (2007). METHOD 6020A Inductively coupled plasma-mass spectrometry. SW846 – 6020. From EPA website: http://www.epa.gov/wastes/hazard/testmethods/sw846/

USEPA (2005). Procedures for the derivation of equilibrium partitioning sediment benchmarks (ESBs) for the protection of benthic organisms: Metal mixtures (cadmium, copper, lead, nickel, silver and zinc). US Environmental Protection Agency, Office of Research and Development Report EPA-600-R-02-011, Washington, DC, USA.

Van Cappellen, P. and Gaillard, J.-F. (1996). Biogeochemical dynamics in aquatic sediments. Rev. Mineral., 34, 335-376.

Wang, Y.F. and van Cappellen, P. (1996). A multicomponent reactive transport model of early diagenesis - application to redox cycling in coastal marine sediments. Geochim. Cosmochim. Acta, 60, 2993-3014.

Xie, M., Wang, N., Gaillard, J.-F. and Packman, A.I. (2018). Interplay between flow and bioturbation enhances metal efflux from low-permeability sediments. J. Hazard. Mat., 341, 304–312.

Zhang, H., Davison, W., Miller, S. and Tych, W. (1995). In situ high resolution measurements of fluxes of Ni, Cu, Fe, and Mn and concentrations of Zn and Cd in porewaters by DGT. Geochim. Cosmochim. Acta, 59, 4181−4192.

Appendix A

Tailings receipt and preparation

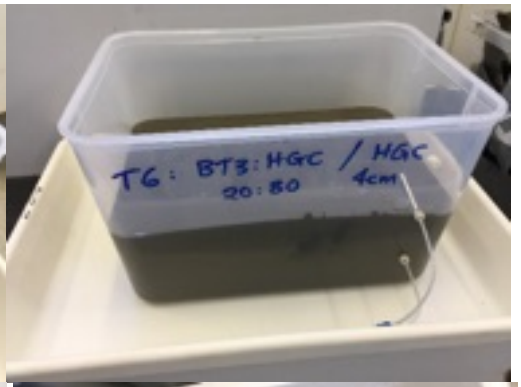
Photos 1 and 2 (top) Bulk Tails-3 (BT3) as received – dense blocks. BT4 was similar, but not quite so dense.

Photos 3 and 4 (middle) Tailings BT3 as received – almost dry state of material after breaking apart the received materials

Photos 5 and 6 (bottom) Tailings BT3 after single wash with seawater and settling for 24 h – reformed very dense material (No visible porewater below 1 cm.

Based on the very dense/consolidated properties of the tailing it was decided that it would not be useful to undertake experiment on 100% tailings. Instead treatments containing 80:20 and 20:80 tailings:sediment mixtures would be prepared and studied.

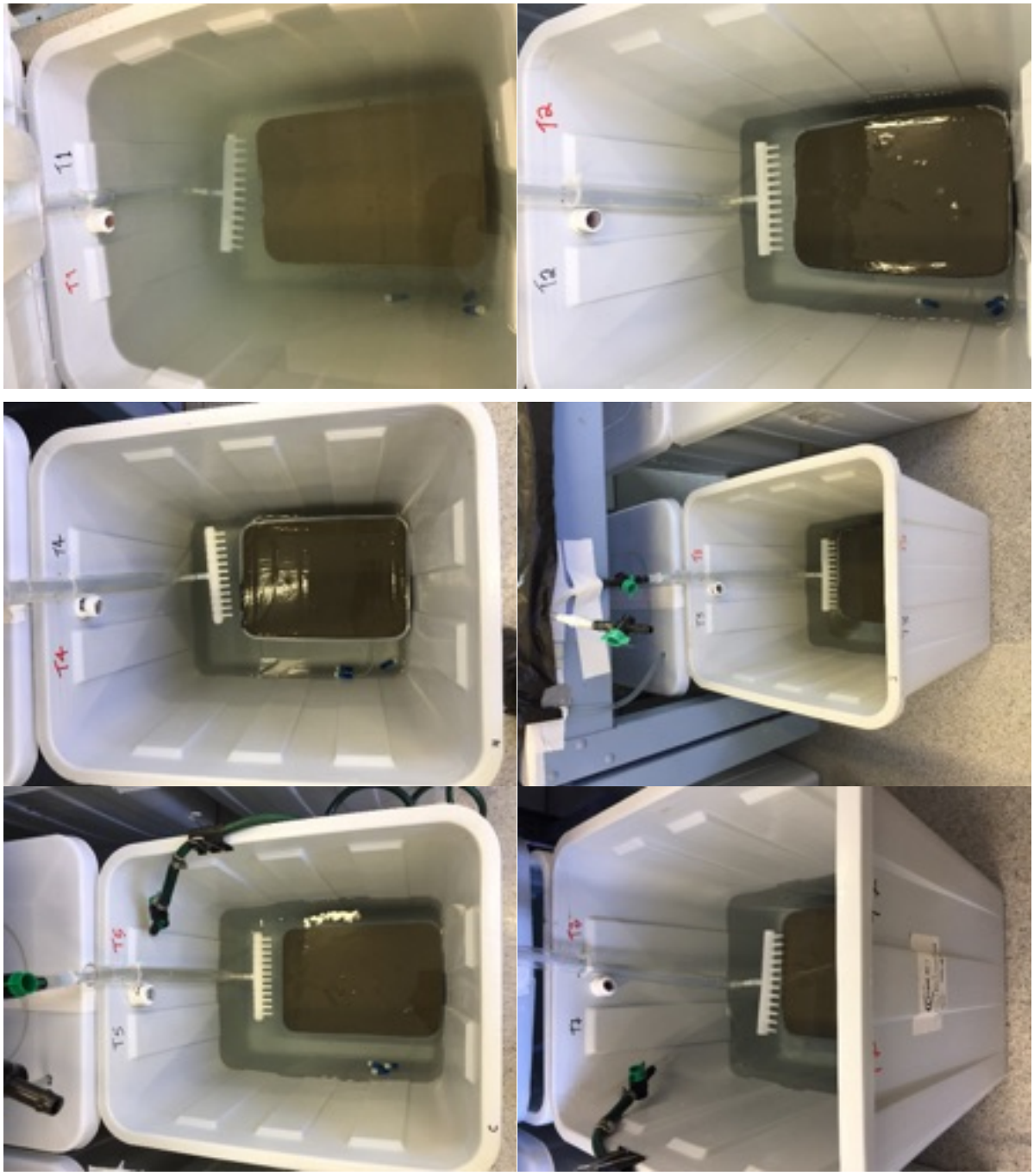
Treatment and exposure chambers



Photos 1 and 2 (top): Treatment containers (TC) showing Rhizon (porewater) sampler ports, an empty TC within the exposure chamber (EC) showing planned position of water splitter that delivered the seawater current of 0.05 m/s

Photos 3-6 (middle, bottom): Example - Treatment (T6) being prepared (tailings:sediment mixture, Rhizons, Huon Gulf layer at top), and then placed in the exposure chamber (EC).

Treatment set up and operating commencing (Day 1)



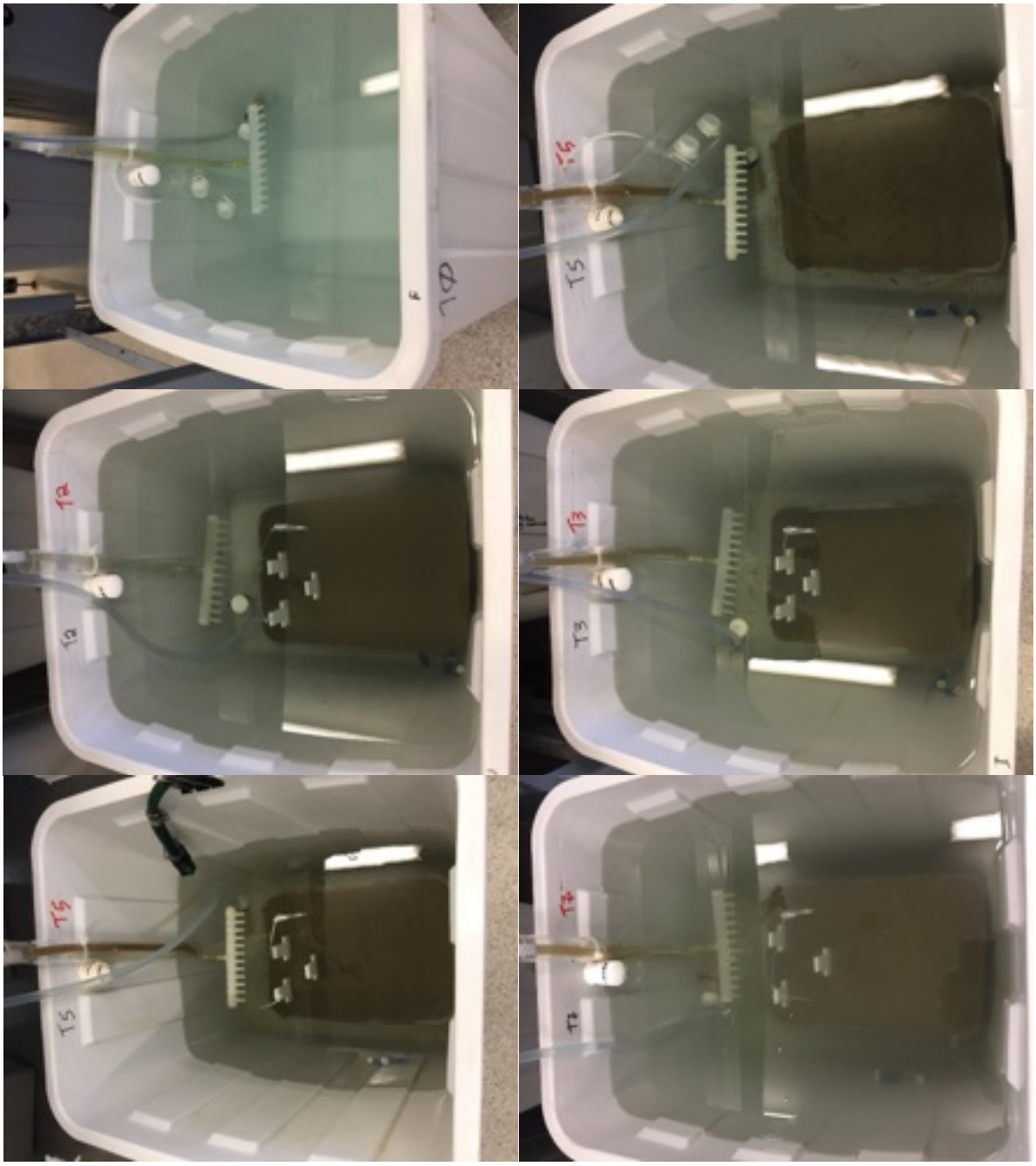
Photos 1-6: Treatment T1, T2, T3, T4, T5, T7 deployed to exposure chambers and seawater being added. Interconnection to seawater reservoir (SR) visible and seawater being added to SR also and interconnected via circulation pumps.

Tailings receipt and preparation

Photos 1-3 (top to middle left): Operations underway – pumps and flow controller on shelves above, thick black plastic keeping light out

Photos 4-6 (middle right to bottom): water changes being made after water quality and dissolved metal sampling after 1 week

DET and DGT deployments in waters and sediments

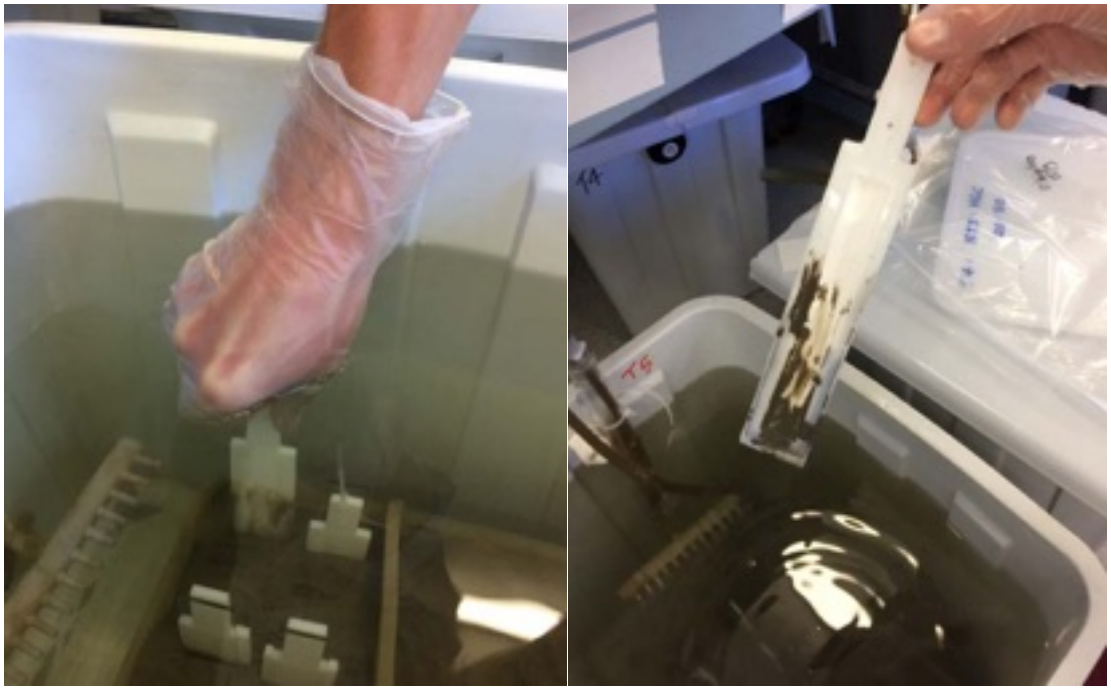


Photos 1 and 2 (top): T0 (no EC) and T5 with water DGTs deployed

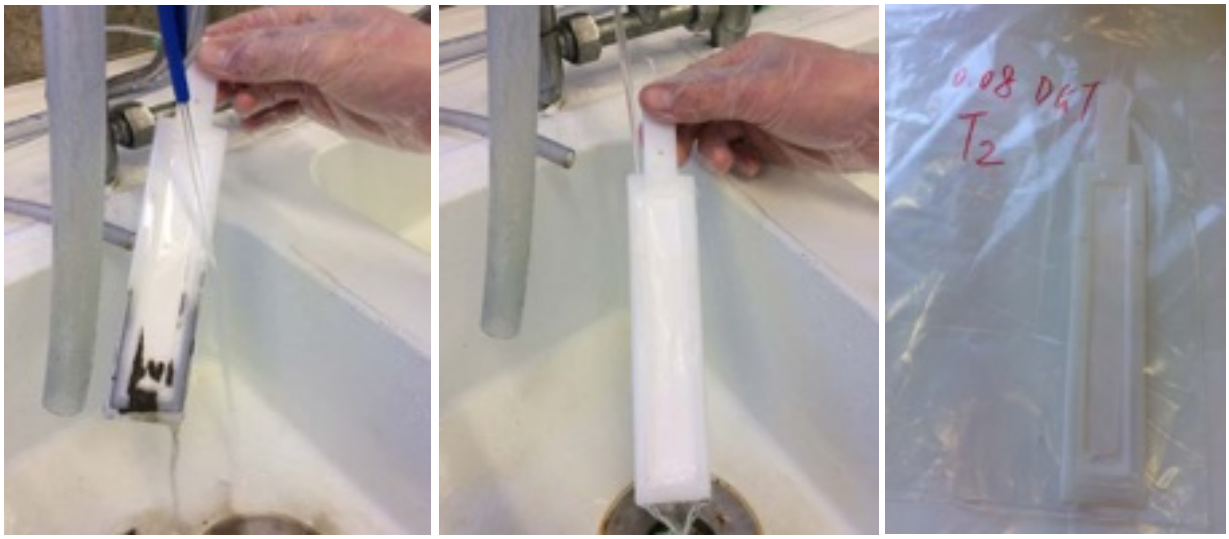
Photos 3 and 4 (middle): T2 and T3 with two sediment DETs and two sediment DGTs deployed

Photos 5 and 6 (bottom): T5 and T7 with two sediment DETs and two sediment DGTs deployed

Processing of DET and DGTs



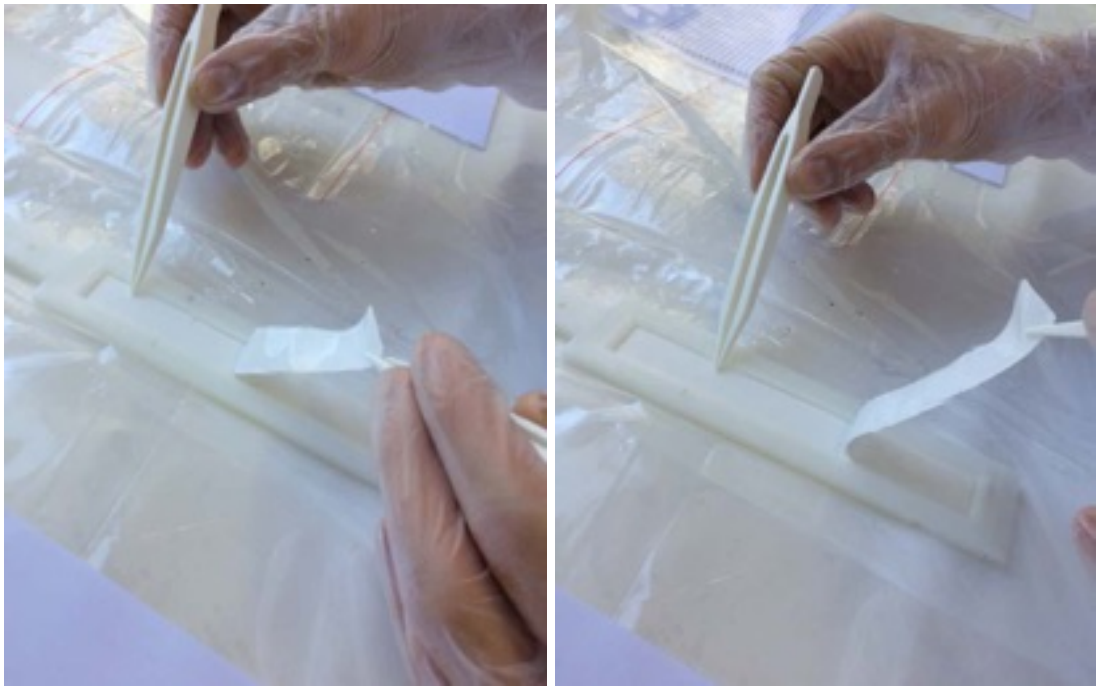
Photos: Collecting sediment DET and DGT probes from the sediment.



Photos: Rinsing the sediment DET and DGT probes until no sediment residues remain.
Rinsed DGT probes in a sealed plastic bag.

Processing of DET and DGT samples

Photos: Using ae scalpel to cut thought the filter membrane and gel layers from the open window.



Photos: Using tweezers to remove the gel layers from the probes.

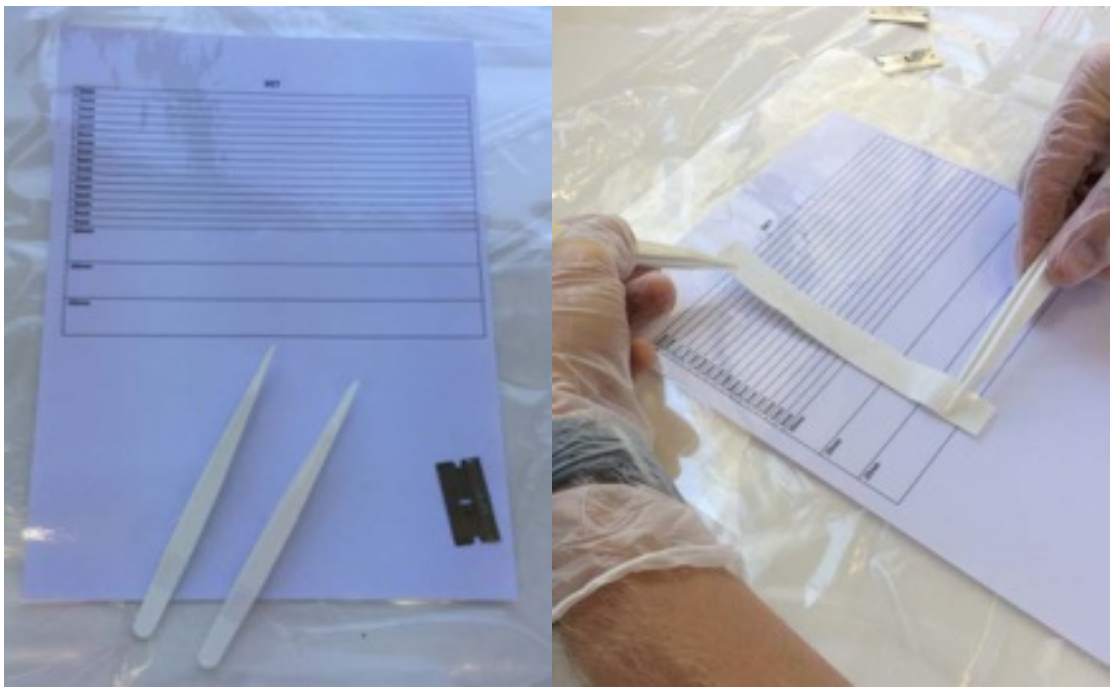


Photo (left): The DET cutting sheet.

Photo (right): Gel layer and filter membrane on the cutting sheet.

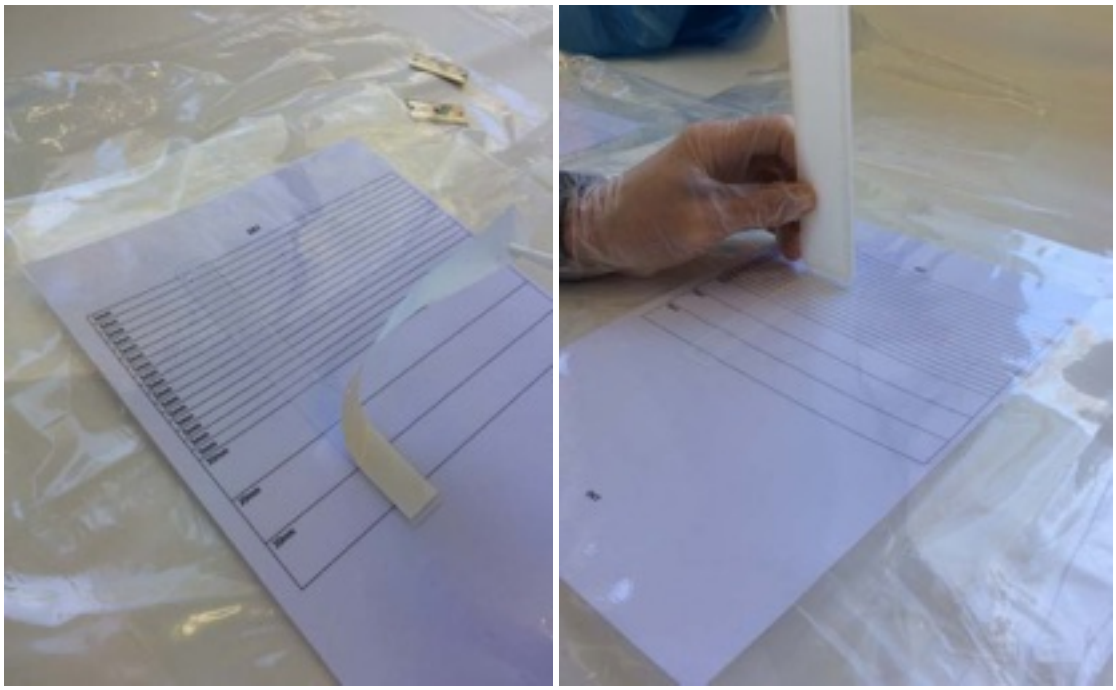


Photo (left): Filter membrane being removed.

Photo (right): Gel layer slicing using a plastic holder with a sharp edge.

Processing of DET of DGT samples

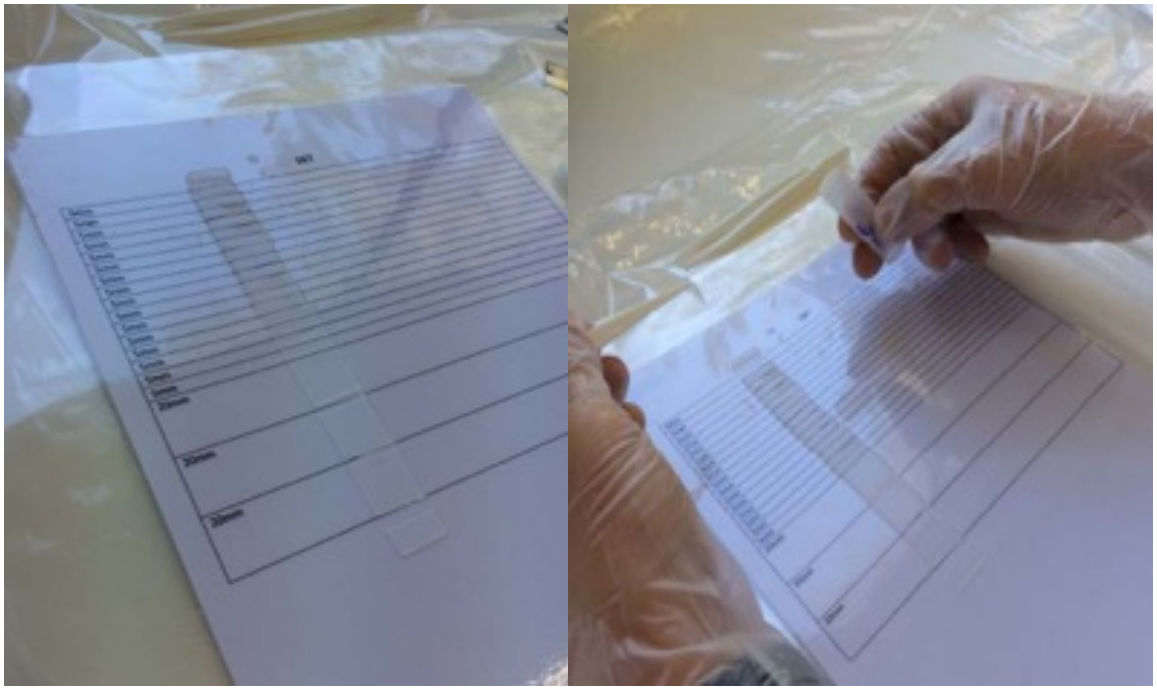


Photo (left): Slicing the gel layer into different millimetre sections.

Photo (right): Transfering a gel piece into a 5 mL vial.

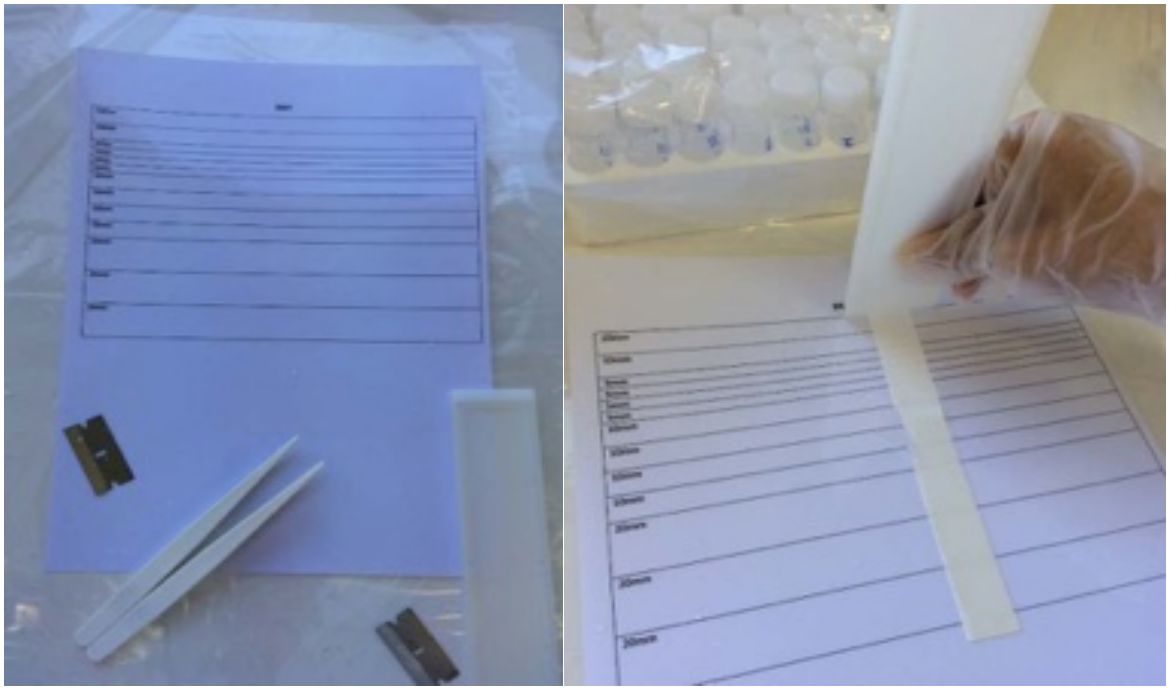


Photo (left): The DGT cutting sheet.

Photo (right): Slicing the DGT gel layers on a filter membrane.

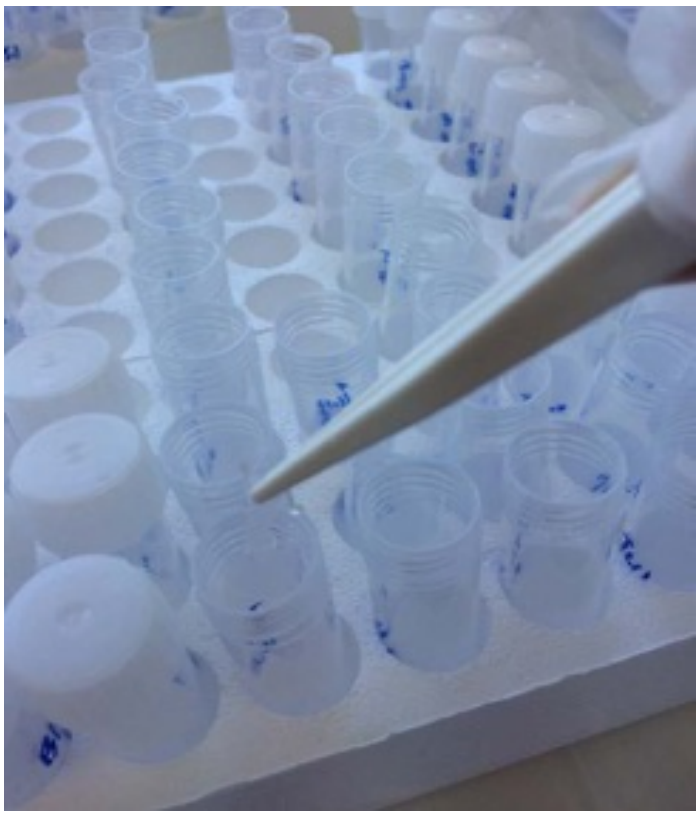
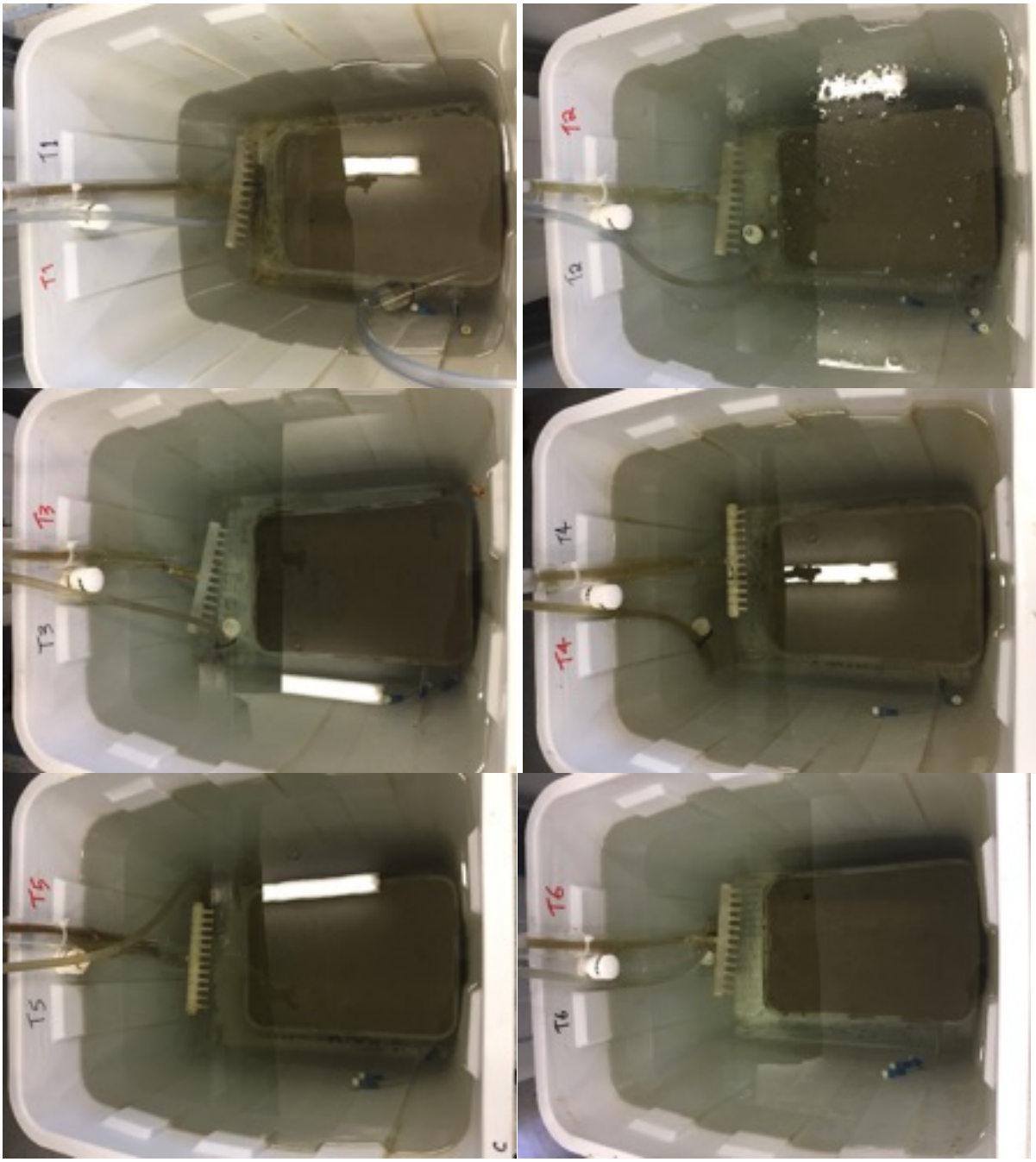


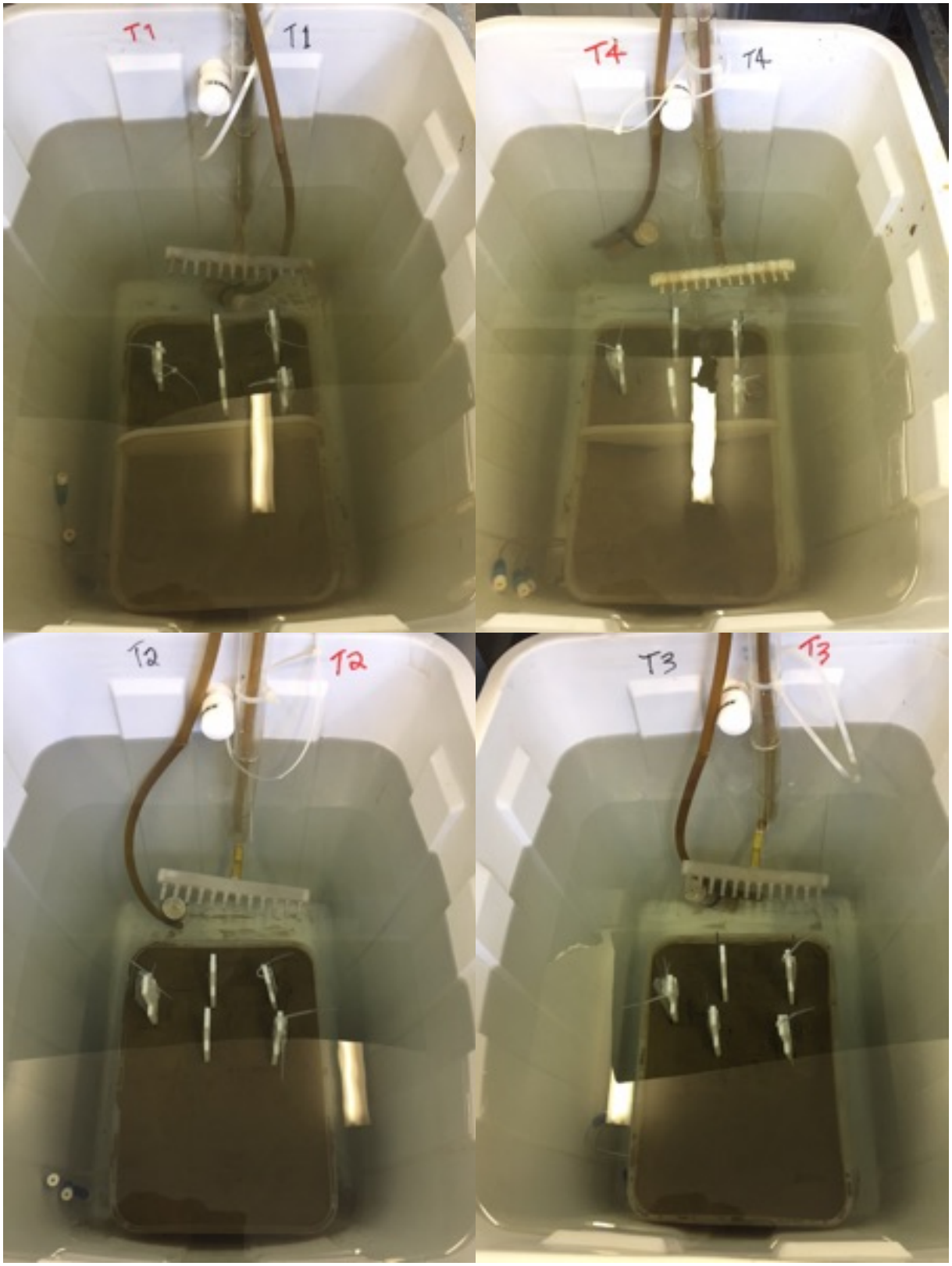
Photo: Transferring each DGT gel layer into a 5 mL plastic vial.

Week 6 of operations



Photos 1-6: Treatments T1 to T6, with now consistent observation that T2 has a lot more bubbles possibly indicating greater organic matter or potentially residual process chemicals. This was evident before water changes from Week 4.

Week 13 of operations (bioaccumulation tests)



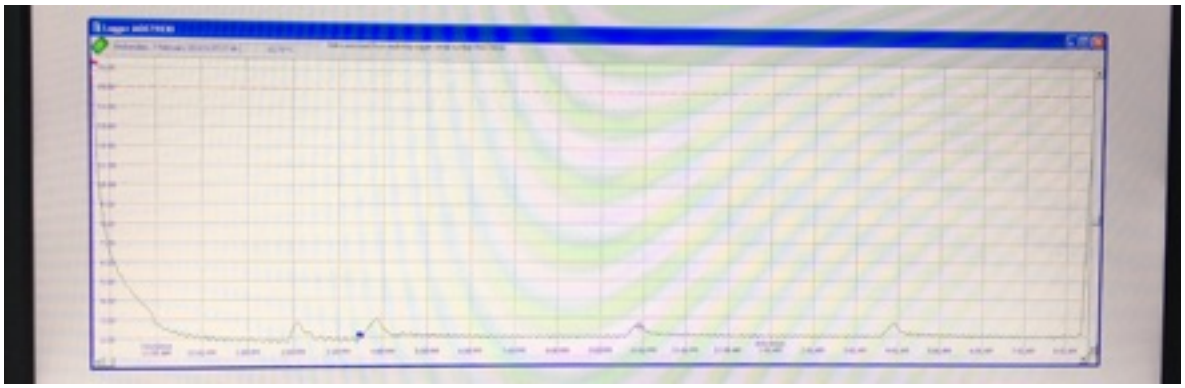
Photos 1-4: Treatments T1 and T4 showing dividing plate where bivalves have been added to the top end and 1-cm surface tailings-sediment has been removed from bottom end (as shown they appear in photos). Treatments T2 and T3 remain as normal, but with DET and DGT deployments underway.

Week 17 of operations (completing bioaccumulation tests)

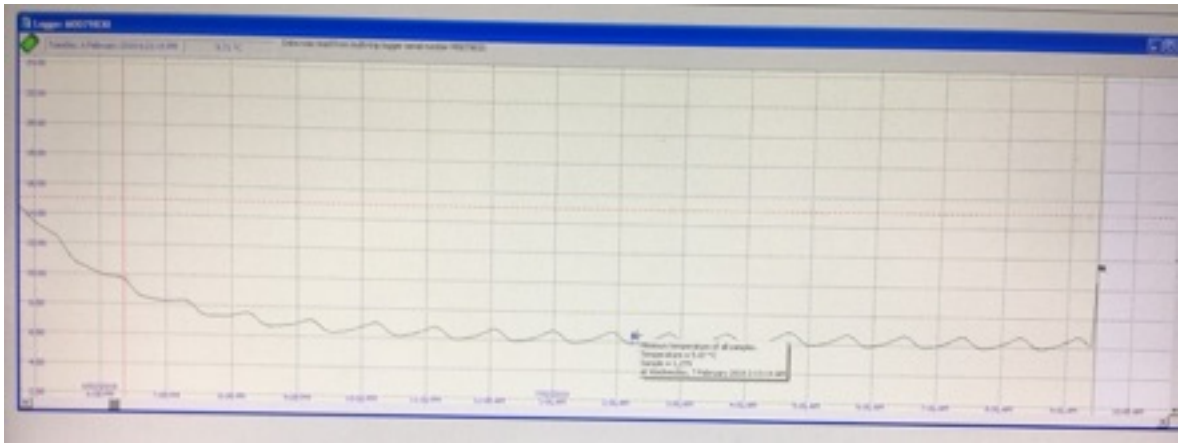
Photos 1-2: Treatment T1 ready for bivalve removal for bioaccumulation assessment.
T4 with water drawn down, Rhizon sampling, prior to bivalve removal for bioaccumulation assessment.

Examples of temperature log for laboratory spaces used in short-term experiments

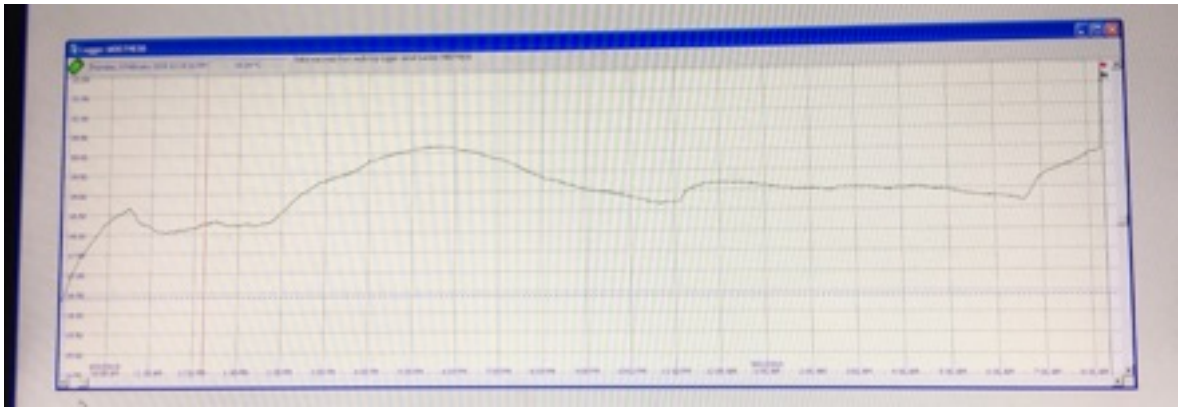
2^{o}C-Temperature logger output from cool-room (refrigerator)



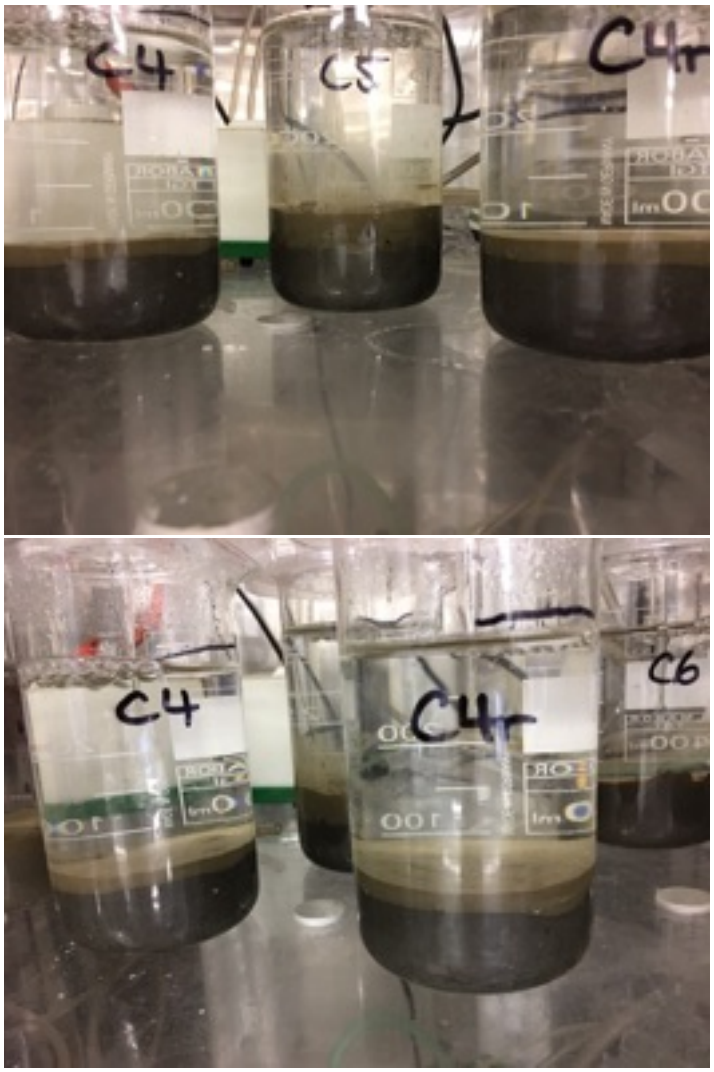
6^{o}C-Temperature logger output from refrigerator in room 116



19C-Temperature logger output from the bench in room 189



Examples of thin layer tests of the short-term experiments



Photos of 0.5, 1 and 2 cm layers of Huon Gulf sediment over tailings

Appendix B – Treatments and operation

Laser particle size analysis of the Huon Gulf sediment and tailings

Huon Gulf sediment (T1)

Measurement Details

Operator Name	willsvdv
Sample Name	Average of 'Initial T1 HG rep 2'
SOP File Name	CSIRO demo.msop

Measurement Details

Analysis Date Time	1/01/2018 12:23:09 PM
Measurement Date Time	1/01/2018 12:23:09 PM
Result Source	Averaged

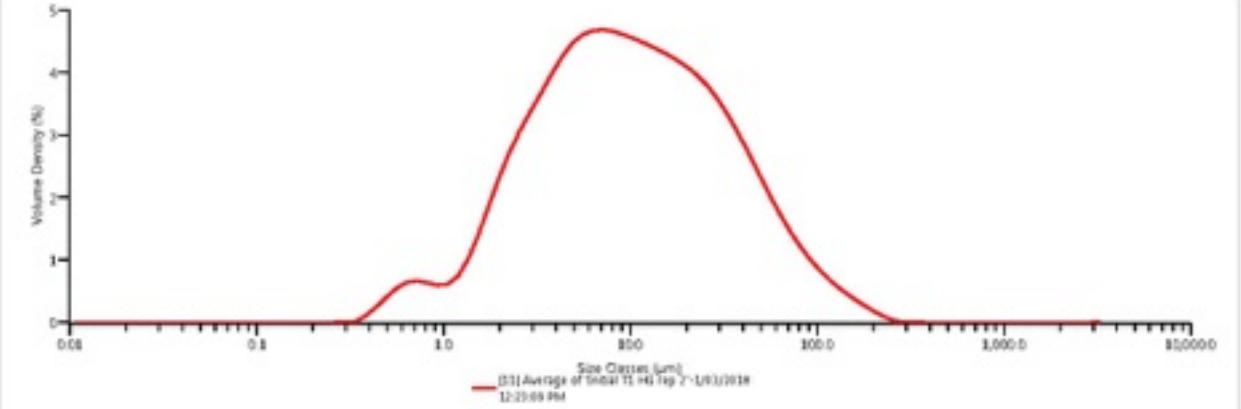
Analysis

Particle Name	China Clay
Particle Refractive Index	1.555
Particle Absorption Index	0.010
Dispersant Name	Water
Dispersant Refractive Index	1.330
Scattering Model	Mie
Analysis Model	General Purpose
Weighted Residual	0.52 %
Laser Obscuration	6.63 %

Result

Concentration	0.0042 %
Span	4.732
Uniformity	1.548
Specific Surface Area	1235 m²/kg
D [3,2]	4.86 µm
D [4,3]	19.5 µm
Dv (10)	2.15 µm
Dv (50)	9.66 µm
Dv (90)	47.7 µm
Volume Below (65) µm	93.77 %

Frequency (compatible)



Result

Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume
0.0000	0.00	0.0463	0.00	0.214	0.00	0.991	0.50	4.58	3.73	21.2	3.31	98.1	0.08	454	0.00	2100	0.0
0.0014	0.00	0.0526	0.00	0.243	0.00	1.13	0.61	5.23	3.85	24.1	3.17	111	0.05	516	0.00	2390	0.0
0.0029	0.00	0.0597	0.00	0.276	0.00	1.28	0.65	5.92	3.93	27.4	2.99	127	0.43	586	0.00	2710	0.0
0.0047	0.00	0.0679	0.00	0.314	0.00	1.45	1.19	6.72	3.91	31.1	1.78	144	0.33	666	0.00	3080	0.0
0.0067	0.00	0.0771	0.00	0.357	0.08	1.65	1.56	7.64	3.88	35.3	2.53	163	0.24	756	0.00	3500	
0.0049	0.00	0.0876	0.00	0.405	0.19	1.88	1.96	8.68	3.84	40.1	2.26	185	0.16	859	0.00		
0.0035	0.00	0.0995	0.00	0.460	0.33	2.13	2.30	9.86	3.78	45.6	1.98	211	0.08	976	0.00		
0.0044	0.00	0.113	0.00	0.523	0.46	2.42	2.57	11.2	3.72	51.8	1.71	240	0.02	1110	0.00		
0.0078	0.00	0.128	0.00	0.594	0.54	2.75	2.82	12.7	3.66	58.9	1.45	272	0.00	1260	0.00		
0.0015	0.00	0.146	0.00	0.675	0.56	3.12	3.07	14.5	3.59	66.9	1.22	310	0.00	1430	0.00		
0.0058	0.00	0.166	0.00	0.767	0.53	3.55	3.32	16.4	3.51	76.0	1.01	352	0.00	1630	0.00		
0.0407	0.00	0.188	0.00	0.871	0.49	4.03	3.55	18.7	3.42	86.4	0.83	400	0.00	1850	0.00		

100% BT3 Tailings

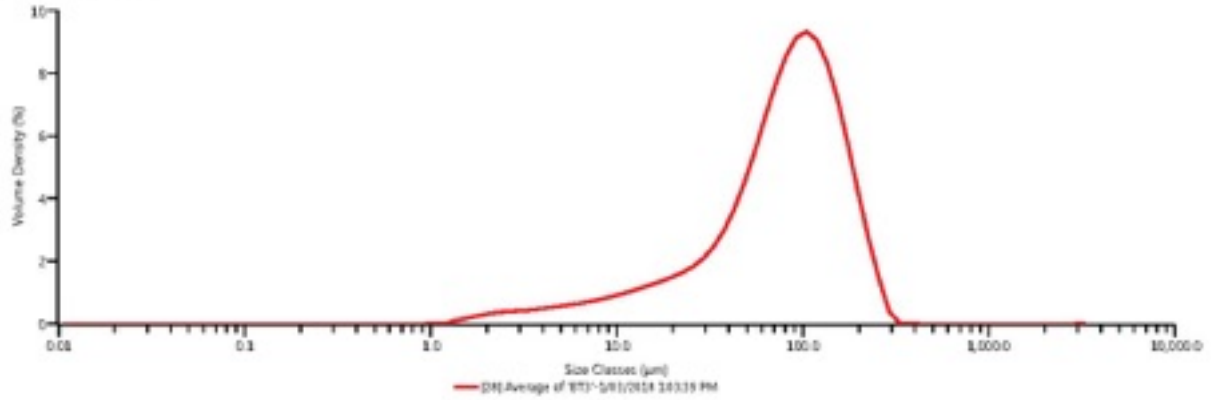
Measurement Details	
Operator Name	willbaxter
Sample Name	Average of 'BT3'
SOP File Name	HydroLV.cfg

Measurement Details	
Analysis Date Time	1/03/2018 1:03:39 PM
Measurement Date Time	1/03/2018 1:03:39 PM
Result Source	Averaged

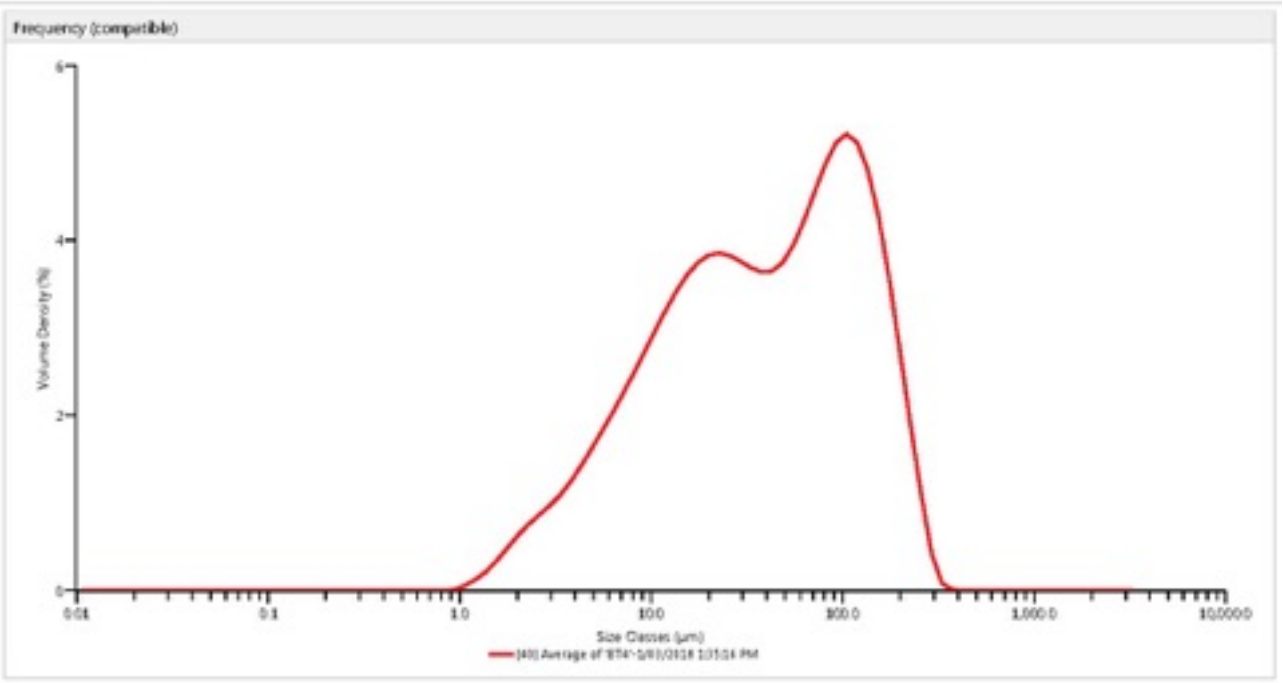
Analysis	
Particle Name	China Clay
Particle Refractive Index	1.555
Particle Absorption Index	0.010
Dispersant Name	Water
Dispersant Refractive Index	1.330
Scattering Model	Mie
Analysis Model	General Purpose
Weighted Residual	0.49 %
Laser Obscuration	6.82 %

Result	
Concentration	0.0252 %
Span	1.874
Uniformity	0.561
Specific Surface Area	191.9 m^2/kg
D [3,2]	31.3 µm
D [4,3]	90.6 µm
Dv (10)	16.4 µm
Dv (50)	83.3 µm
Dv (90)	173 µm
Volume Below (63) µm	35.86 %

Frequency (compatible)



100% BT4 Tailings



Trend

Record Number	Sample Name	Dx (10) (µm)	Dx (50) (µm)	Dx (90) (µm)
40	Average of 'BT4'	6.09	39.3	153

Metal concentrations of tailings, sediments and mixtures

Summary

			Total Recoverable Metals (mg/kg)										
Test week	**Sample ID**		**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**V**	**Zn**
Week 1	BT3 initial	Average	<2	0.5	12	453	578	48400	437	279	13	55	34
Week 1	BT4 initial	Average	7	0.6	17	525	560	60200	331	299	13	90	57
Week 1	T1 initial	Average	7	0.7	23	47	80	54300	1020	59	11	140	86
Week 12	T1	Average	8	0.8	23	48	82	55400	1170	60	12	141	93
Week 1	T2 initial	Average	2	0.6	13	380	528	55400	579	239	15	79	50
Week 1	T3 initial	Average	7	0.7	19	421	449	60300	501	253	15	104	65
Week 1	T4 initial	Average	6	0.5	20	127	197	52800	870	100	16	121	78
Week 12	T4	Average	6	0.6	23	141	216	58200	976	110	14	133	86
Week 1	T5 initial	Average	6	0.6	22	126	159	51500	832	97	14	122	79
Week 12	T5	Average	8	0.8	23	141	175	58300	977	107	13	137	89
Week 12	T6	Average	8	0.7	27	52	89	58800	1100	63	14	150	97
Week 12	T7	Average	9	0.7	23	51	87	58600	1210	64	15	150	98

Quality Control

Replicates

			Total Recoverable Metals (mg/kg)										
	Sample ID	**Replicate**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**V**	**Zn**
Week 1	Limit of reporting		<2	<0.1	<0.3	<0.2	<0.2	<2	<0.2	<0.2	<1	<0.2	<0.2
Week 1	BT3 1	Replicate 1	<2	0.6	13	449	591	49400	444	276	12	56	35
Week 1	BT3 2	Replicate 2	<2	0.5	12	457	564	47400	431	282	14	54	34
Week 1	BT4 1	Replicate 1	8	0.6	17	554	602	61800	352	317	12	92	60
Week 1	BT4 2	Replicate 2	6	0.6	17	496	519	58500	311	280	15	88	53
Week 1	T1-1		7	0.7	23	47	80	54300	1020	59	11	140	86
Week 1	T2-1	Replicate 1	2	0.7	15	389	540	59100	591	244	15	84	51
Week 1	T2-2	Replicate 2	<2	0.5	12	371	516	51700	566	233	15	74	49
Week 1	T3-1		7	0.7	19	421	449	60300	501	253	15	104	65
Week 1	T4-1		6	0.5	20	127	197	52800	870	100	16	121	78
Week 1	T5-1		6	0.6	22	126	159	51500	832	97	14	122	79
Week 12	T1-1	Replicate 1	9	0.8	25	49	83	55800	1180	60	13	143	94
Week 12	T1-2	Replicate 2	8	0.8	20	48	81	55000	1160	60	12	140	93
Week 12	T4-1	Replicate 1	6	0.6	22	142	214	58100	983	111	16	133	87
Week 12	T4-2	Replicate 2	7	0.6	23	140	218	58400	969	110	12	134	86
Week 12	T5-1	Replicate 1	8	0.8	20	143	175	58500	987	108	12	137	89
Week 12	T5-2	Replicate 2	8	0.8	26	139	174	58100	966	106	13	136	89
Week 12	T6-1	Replicate 1	9	0.7	27	54	91	60500	1130	65	16	156	100
Week 12	T6-2	Replicate 2	7	0.8	26	50	86	57000	1080	61	13	144	95
Week 12	T7-1	Replicate 1	8	0.7	25	52	87	58900	1210	64	15	150	98
Week 12	T7-2	Replicate 2	9	0.7	22	51	88	58400	1210	63	14	150	97

Blanks

			Total Recoverable Metals (mg/kg)										
	Sample ID		**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**V**	**Zn**
	Blk 1	Replicate 1	<2	<0.1	<0.5	<0.5	<0.5	4	<0.5	<0.5	<1	<1	<0.5
	Blk 2	Replicate 2	<2	<0.1	<0.5	<0.5	<0.5	2	<0.5	<0.5	<1	<1	<0.5
	Blk 1	Replicate 1	<2	<0.1	<0.5	<0.5	<0.5	<2	<0.5	<0.5	<1	<1	<0.5
	Blk 2	Replicate 2	<2	<0.1	<0.5	<0.5	<0.5	2	<0.5	<0.5	<1	<1	<0.5

Reference Material

			Total Recoverable Metals (mg/kg)										
	Sample ID		**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**V**	**Zn**
	Ref 1	Replicate 1	21.2	5.2	4.6	125	73	9670	184	22	280	15	287
	Ref 2	Replicate 2	22.0	5.5	4.9	128	72	9650	188	23	287	15	297
	ERM-CC018	Certified Value	22.9 ± 1.3	5.4 ± 0.5	5.9 ± 0.4	129 ± 6	80 ± 4	---	---	25.8 ± 1.8	289 ± 10	19.4 ± 1.0	313 ± 13

Summary

			Dilute-acid Extractable Metals (mg/kg)										
	Sample ID		**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**V**	**Zn**
Week 12	BB initial	Average	<7	<0.5	<2	6	29	8780	52	2	55	24	139
Week 1	BT3 initial	Average	<7	<0.5	<2	32	113	3350	116	24	3.5	4	9
Week 1	BT4 initial	Average	<7	<0.5	<2	30	103	4700	95	23	3.3	7	15
Week 1	T1 initial	Average	<7	<0.5	7	4	32	9670	440	8.4	5.2	21	19
Week 12	T1	Average	<7	<0.5	8	6	38	11700	573	11	5.7	24	28
Week 1	T2 initial	Average	<7	<0.5	<2	15	191	4210	163	13	8.5	7	13
Week 1	T3 initial	Average	<7	<0.5	2	19	107	5490	156	17	6.5	9	19
Week 1	T4 initial	Average	<7	<0.5	6	6	58	8400	373	8.8	5.4	18	19
Week 12	T4	Average	<7	<0.5	9	11	77	12436	466	15	6.8	24	28
Week 1	T5 initial	Average	<7	<0.5	5	7	53	8470	356	9.8	4.1	17	20
Week 12	T5	Average	<7	<0.5	8	15	66	14040	501	17	14.8	26	30
Week 12	T6	Average	<7	<0.5	10	9	56	17600	804	17	8.3	35	39
Week 12	T7	Average	<7	<0.5	11	7	43	13430	527	13	6.5	27	31

Quality Control

Replicates

			Dilute-acid Extractable Metals (mg/kg)										
Test week	**Sample ID**	**Replicate**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**V**	**Zn**
Week 1	Limit of reporting		<7	<1	<2	<2	<2	<10	<2	<2	<5	<2	<2
Week 1	BT3 1	Replicate 1	<7	<1	<2	34	111	3590	119	25	<5	5	10
Week 1	BT3 2	Replicate 2	<7	<1	<2	31	115	3120	113	23	<5	4	9
Week 1	BT4 1	Replicate 1	<7	<1	<2	33	107	5110	100	25	<5	7	16
Week 1	BT4 2	Replicate 2	<7	<1	<2	27	98	4290	90	21	<5	6	15
Week 1	T1-1	Replicate 1	<7	<1	7	5	32	9850	445	9	5	21	20
Week 1	T1-2	Replicate 2	<7	<1	8	4	32	9490	436	8	5	21	19
Week 1	T2-1	Replicate 1	<7	<1	2	15	187	4230	161	13	6	7	12
Week 1	T2-2	Replicate 2	<7	<1	2	15	196	4200	164	13	11	7	15
Week 1	T3-1	Replicate 1	<7	<1	3	18	111	5480	155	16	5	9	16
Week 1	T3-2	Replicate 2	<7	<1	<2	19	103	5510	156	17	8	10	21
Week 1	T4-1	Replicate 1	<7	<1	6	6	59	8510	375	9	5	18	20
Week 1	T4-2	Replicate 2	<7	<1	5	6	57	8300	372	8	6	18	19
Week 1	T5-1	Replicate 1	<7	<1	5	7	53	8400	357	10	5	18	20
Week 1	T5-2	Replicate 2	<7	<1	6	7	54	8540	355	10	4	17	20
Week 12	T1-1	Replicate 1	<7	<1	10	7	42	13000	637	13	6	26	31
Week 12	T1-2	Replicate 2	<7	<1	6	6	33	10500	509	10	5	21	25
Week 12	T4-1	Replicate 1	<7	<1	8	9	75	12200	459	14	7	24	27
Week 12	T4-2	Replicate 2	<7	<1	9	12	78	12700	473	16	6	25	29
Week 12	T5-1	Replicate 1	<7	<1	7	16	67	14100	514	18	24	27	30
Week 12	T5-2	Replicate 2	<7	<1	9	15	66	13900	488	17	5	26	29
Week 12	T6-1	Replicate 1	<7	<1	9	8	49	15500	702	15	8	30	34
Week 12	T6-2	Replicate 2	<7	<1	12	10	62	19700	905	20	8	40	45
Week 12	T7-1	Replicate 1	<7	<1	11	8	45	13800	531	14	6	27	32
Week 12	T7-2	Replicate 2	<7	<1	11	7	42	13000	523	12	7	27	29
Week 1	BB1	Replicate 1	<7	<1	<2	6	26	8790	55	2	56	24	139
Week 1	BB2	Replicate 2	7	<1	<2	6	31	8760	50	3	55	24	138

Blanks

			Dilute-acid Extractable Metals (mg/kg)										
	Sample ID		**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**V**	**Zn**
	Blk 1	Replicate 1	<5	<0.5	<2	<2	<1	<10	<2	<2	<5	<2	<2
	Blk 2	Replicate 2	<5	<0.5	<2	<2	<1	<10	<2	<2	<5	<2	<2

			Dilute-acid Extractable Metals (mg/kg)										
	Sample ID		**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**V**	**Zn**
	Ref 1	Replicate 1	16	5.3	1.8	39	60	2830	125	13	257	7	232
	Ref 2	Replicate 2	17	5.3	2.3	40	59	2890	126	13	259	7	234
	In-house Value	ERM-CC018	17	5.6	2.8	45	63	3350	138	14	268	8	243

Overlying waters from mesocosms

Weekly discrete sampling of overlying water monitoring data to Week 12

Wafi long-term laboratory tailings-sediment study - Monitoring data to Week 12

			Water quality parameters				Dissolved Cu, Zn, Mn, µg/L		
Date	Treatment	Week	pH	Salinity, ‰	DO, %-Sat	Temp., ºC	Cu	Zn	Mn
Seawater supply used for all experiments									
28/12/17	Seawater-1	1					2	1	1
2/1/18	Seawater-1	2					2	1	1
8/1/18	Seawater-1	3					1	1	1
15/1/18	Seawater-1	4					1	2	1
22/1/18	Seawater-1	5					1	1	1
29/1/18	Seawater-1	6					1	1	1
5/2/18	Seawater-1	7					1	1	1
12/2/18	Seawater-1	8					2	1	1
19/2/18	Seawater-1	9							
26/2/18	Seawater-1	10					1	1	3
5/3/18	Seawater-1	11							
12/3/18	Seawater-1	12							
Mean	Weeks 1-12						1.3	1.2	1.2
SD							0.4	0.3	0.7

Wafi long-term laboratory tailings-sediment study - Monitoring data to Week 12

			Water quality parameters				Dissolved Cu, Zn, Mn, µg/L		
Date	Treatment	Week	pH	Salinity, ‰	DO, %-Sat	Temp., ºC	Cu	Zn	Mn
Seawater supply used for all experiments									
28/12/17	Seawater-2	1					1	2	1
2/1/18	Seawater-2	2					2	1	1
8/1/18	Seawater-2	3					1	1	1
15/1/18	Seawater-2	4					1	1	1
22/1/18	Seawater-2	5					1	2	1
29/1/18	Seawater-2	6					1	1	1
5/2/18	Seawater-2	7					1	1	1
12/2/18	Seawater-2	8					1	1	1
19/2/18	Seawater-2	9					1	1	1
26/2/18	Seawater-2	10					1	1	1
5/3/18	Seawater-2	11					1	1	1
12/3/18	Seawater-2	12					1	1	1
Mean	Weeks 1-12						1.3	1.1	1.0
SD							0.3	0.2	0.0

Wafi long-term laboratory tailings-sediment study - Monitoring data to Week 12

			Water quality parameters				Dissolved Cu, Zn, Mn, µg/L		
Date	Treatment	Week	pH	Salinity, ‰	DO, %-Sat	Temp., ºC	Cu	Zn	Mn
Seawater supply used for all experiments									
28/12/17	Seawater-3	1					1	2	1
2/1/18	Seawater-3	2					2	1	1
8/1/18	Seawater-3	3					1	1	1
15/1/18	Seawater-3	4					1	2	1
22/1/18	Seawater-3	5					1	1	1
29/1/18	Seawater-3	6					2	1	1
5/2/18	Seawater-3	7					2	1	1
12/2/18	Seawater-3	8					2	1	1
19/2/18	Seawater-3	9					2	1	1
26/2/18	Seawater-3	10					2	1	1
5/3/18	Seawater-3	11					2	1	1
12/3/18	Seawater-3	12					2	1	1
Mean	Weeks 1-12						1.5	1.2	1.0
SD							0.3	0.4	0.0

Wafi long-term laboratory tailings-sediment study - Monitoring data to Week 12

			Water quality parameters				Dissolved Cu, Zn, Mn, µg/L		
Date	Treatment	Week	pH	Salinity, ‰	DO, %-Sat	Temp., ºC	Cu	Zn	Mn
Treatment T0 – has no tailing-sediment treatment, but same water circulation and exchanges									
2/1/18	T0	1					1	2	1
2/1/18	T0 back	1					3	1	1
28/12/17	T0	2	8.17	36.2	89	19.0	1	4	1
28/12/17	T0 back	2					1	4	1
8/1/18	T0	3	8.18	36.3	89	19.7	1	2	1
8/1/18	T0 back	3					1	3	1
15/1/18	T0	4					1	1	1
22/1/18	T0	5					1	1	1
29/1/18	T0	6	8.18	36.2	91	19.2	1	5	1
5/2/18	T0	7					1	1	1
12/2/18	T0	8	8.18	36.7	90	19.8	1	3	1
19/2/18	T0	9	8.28	36.6	88	19.4	2	3	1
26/2/18	T0	10	8.23	36.2	89	19.9	1	3	1
5/3/18	T0	11					1	1	1
12/3/18	T0	12					1	1	1
Mean	Weeks 1-12		8.2	36	90	19	1.0	2.4	1.0
SD			0.1	0.8	2	0.4	0.2	1.3	0.0
19/3/18	T0	13					1	6	<1
26/3/18	T0	14					<1		<1
2/4/18	T0	15					0.7	8	<1
9/3/18	T0	16					<1		<1

Note: the Seawater reservoir (SR) tank the provided additional dilution was removed after week 3.
This was the data indicated as 'back', i.e. back tank = SR.

Wafi long-term laboratory tailings-sediment study - Monitoring data to Week 12

			Water quality parameters				Dissolved Cu, Zn, Mn, µg/L		
Date	Treatment	Week	pH	Salinity, ‰	DO, %-Sat	Temp., ºC	Cu	Zn	Mn
Treatment T1 – 100% HG sediment									
2/1/18	T1	1	8.33	36.5	91	19.3	1	1	174
2/1/18	T1 back	1					1	1	174
28/12/17	T1	2	8.10	36.3	90	18.4	2	3	283
28/12/17	T1 back	2					2	2	286
8/1/18	T1	3	8.14	36.3	89	19.9	1	2	101
8/1/18	T1 back	3					1	1	100
15/1/18	T1	4	8.25	34.6	88	19.1	1	1	37
22/1/18	T1	5	8.30	36.7		19.0	1	1	7
29/1/18	T1	6	8.20	36.5		18.9	1	1	1
5/2/18	T1	7	8.27	36.5	86	19.3	2	1	1
12/2/18	T1	8	8.21	36.3	92	19.3	1	1	1
19/2/18	T1	9	8.16	36.7	91	19.8	1	2	1
26/2/18	T1	10	8.21	36.7	90	19.8	1	1	1
5/3/18	T1	11	8.22	37.4	91	18.9	1	1	1
12/3/18	T1	12					1	1	1
Mean	Weeks 1-12		8.2	36	90	19	1.2	1.2	44.8
SD			0.1	0.8	4	0.4	0.4	0.3	85.1
19/3/18	T1	13					1	2	5
26/3/18	T1	14					1		3
2/4/18	T1	15					1	2	2
9/3/18	T1	16					1		5

Note: the Seawater reservoir (SR) tank the provided additional dilution was removed after week 3.
This was the data indicated as 'back', i.e. back tank = SR.

Wafi long-term laboratory tailings-sediment study - Monitoring data to Week 12

			Water quality parameters				Dissolved Cu, Zn, Mn, µg/L		
Date	Treatment	Week	pH	Salinity, ‰	DO, %-Sat	Temp., ºC	Cu	Zn	Mn
Treatment T2 – 80% Bulk-Tails-3 mix:20% HG sediment (BT3 = 90% porphyry:10% metasediments)									
2/1/18	T2	1	8.27	36.4	89	19.2	8	1	100
2/1/18	T2 back	1					8	1	102
28/12/17	T2	2	8.14	36.1	87	18.4	8	3	158
28/12/17	T2 back	2							
8/1/18	T2	3	8.13	36.2	89	19.7	8	2	74
8/1/18	T2 back	3					8	2	73
15/1/18	T2	4	8.28	35.1	87	19.0	10	4	66
15/1/18	T2 replicate	4					10	5	66
22/1/18	T2	5	8.24	36.6		19.0	11	3	47
22/1/18	T2 replicate	5					10	4	47
29/1/18	T2	6	8.20	36.4		18.9	7	1	12
29/1/18	T2 replicate	6					7	1	12
5/2/18	T2	7	8.22	36.8	87	19.1	7	1	4
5/2/18	T2 replicate	7					7	2	4
12/2/18	T2	8	8.19	36.9	98	19.2	6	4	1
12/2/18	T2 replicate	8					5	4	1
19/2/18	T2	9	8.17	36.9	90	19.8	8	5	1
19/2/18	T2 replicate	9					8	5	1
26/2/18	T2	10	8.24	36.4	91	19.9	6	3	1
26/2/18	T2 replicate	10					6	3	1
5/3/18	T2	11	8.17	37.3	92	18.7	8	1	1
5/3/18	T2 replicate	11					8	1	1
12/3/18	T2	12					7	1	1
12/3/18	T2 replicate	12					7	2	1
Mean	Weeks 1-12		8.2	36	90	19	7.6	2.5	33.7
SD			0.1	0.6	4	0.5	1.5	1.4	44.4
19/3/18	T2	13					6	4	<1
26/3/18	T2 replicate	13					6	4	<1
2/4/18	T2	14					7		<1
26/3/18	T2 replicate	14					7		<1
2/4/18	T2	15					6	5	<1
26/3/18	T2 replicate	15					6	6	<1
2/4/18	T2	16					4		1
9/3/18	T2 replicate	16					4		2

Note: the Seawater reservoir (SR) tank the provided additional dilution was removed after week 3.
This was the data indicated as 'back', i.e. back tank = SR.

Wafi long-term laboratory tailings-sediment study - Monitoring data to Week 12

Date	Treatment	Week	Water quality parameters pH	Salinity, ‰	DO, %-Sat	Temp., ºC	Dissolved Cu, Zn, Mn, µg/L Cu	Zn	Mn
Treatment T3 – 80% Bulk-Tails-4 mix:20% HG sediment (BT4 = 25% porphyry : 75% metasediments)									
2/1/18	T3	1	8.35	36.1	87	19.1	6	2	102
2/1/18	T3 back	1					8	2	102
28/12/17	T3	2	8.11	36.0	81	18.5	5	5	196
28/12/17	T3 back	2					5	2	26
8/1/18	T3	3	8.14	36.2	90	19.7	6		85
8/1/18	T3 back	3					6	2	84
15/1/18	T3	4	8.31	35.5	89	19.1	5	2	77
15/1/18	T3 replicate	4					5	2	78
22/1/18	T3	5	8.23	36.5		18.8	6	3	65
22/1/18	T3 replicate	5					6	4	66
29/1/18	T3	6	8.18	36.5		18.9	4	2	20
29/1/18	T3 replicate	6					4	1	19
5/2/18	T3	7	8.25	36.8	85	19.1	4	1	3
5/2/18	T3 replicate	7					4	5	3
12/2/18	T3	8	8.2	35.9	89	19.2	3	3	1
12/2/18	T3 replicate	8					3	2	1
19/2/18	T3	9	8.17	36.8	92	19.8	2	3	1
19/2/18	T3 replicate	9					3	2	1
26/2/18	T3	10	8.22	36.5	90	19.9	2	2	1
26/2/18	T3 replicate	10					2	3	1
5/3/18	T3	11	8.16	37.2	91	18.8	4	1	1
5/3/18	T3 replicate	11					4	1	1
12/3/18	T3	12					3	1	1
12/3/18	T3 replicate	12					3	1	1
Mean	Weeks 1-12		8.2	36	88	19	4	2	39
SD			0.1	0.5	3	0.4	2	1	51
19/3/18	T3	13					3	3	<1
26/3/18	T3 replicate	13					3	3	<1
2/4/18	T3	14					4		<1
26/3/18	T3 replicate	14					4		<1
2/4/18	T3	15					3	5	<1
26/3/18	T3 replicate	15					3	4	<1
2/4/18	T3	16					3		1
9/3/18	T3 replicate	16					2		1

Note: the Seawater reservoir (SR) tank the provided additional dilution was removed after week 3.
This was the data indicated as 'back', i.e. back tank = SR.

Wafi long-term laboratory tailings-sediment study - Monitoring data to Week 12

			Water quality parameters				Dissolved Cu, Zn, Mn, µg/L		
Date	Treatment	Week	pH	Salinity, ‰	DO, %-Sat	Temp., ºC	Cu	Zn	Mn
Treatment T4 – 80% Bulk-Tails-3 mix:80% HG sediment									
2/1/18	T4	1	8.32	36.2	91	19.2	3	1	186
2/1/18	T4 back	1					3	2	184
28/12/17	T4	2	8.14	36.1	89	18.7	4	8	253
28/12/17	T4 back	2					1	7	254
8/1/18	T4	3	8.14	36.2	90	19.8	1	1	108
8/1/18	T4 back	3					1	1	108
15/1/18	T4	4	8.36	36.6	90	19.1	2	1	95
22/1/18	T4	5	8.22	36.4		19.0	3	3	42
29/1/18	T4	6	8.16	37.1		18.7	3	1	2
5/2/18	T4	7	8.27	36.5	84	19.2	2	1	1
12/2/18	T4	8	8.18	36.7	88	19.3	2	3	1
19/2/18	T4	9	8.16	36.4	89	19.8	1	5	1
26/2/18	T4	10	8.23	36.5	88	19.8	1	1	1
5/3/18	T4	11	8.18	37.3	93	18.9	2	1	1
12/3/18	T4	12					2	1	1
Mean	Weeks 1-12		8.2	37	89	19	2	3	83
SD			0.1	0.4	2	0.4	1	2	96
19/3/18	T4	13					3	3	<1
26/3/18	T4	14					3	3	<1
2/4/18	T4	15					4		<1
9/3/18	T4	16					4		<1

Note: the Seawater reservoir (SR) tank the provided additional dilution was removed after week 3.
This was the data indicated as 'back', i.e. back tank = SR.

Wafi long- term laboratory tailings-sediment study - Monitoring data to Week 12

Date	Treatment	Week	Water quality parameters pH	Salinity, ‰	DO, %-Sat	Temp., ºC	Dissolved Cu, Zn, Mn, µg/L Cu	Zn	Mn
Treatment T5 – 80% Bulk-Tails-4 mix:20% HG sediment									
2/1/18	T5	1	8.20	36.1	90	19.3	3	1	212
2/1/18	T5 back	1					3	1	209
28/12/17	T5	2	8.15	36.1	88	18.8	3	2	285
28/12/17	T5 back	2					4	3	283
8/1/18	T5	3	8.20	36.2	91	19.9	2	1	160
8/1/18	T5 back	3					2	1	160
15/1/18	T5	4	8.36	36.5	91	19.2	2	1	107
22/1/18	T5	5	8.25	36.7		19.0	2	1	30
29/1/18	T5	6	8.14	36.5		19.3	2	1	2
5/2/18	T5	7	8.26	36.4	86	19.5	2	1	1
12/2/18	T5	8	8.18	36.5	94	19.2	1	2	1
19/2/18	T5	9	8.14	36.7	89	19.8	1	2	1
26/2/18	T5	10	8.2	36.6	89	19.9	1	1	1
5/3/18	T5	11	8.2	37.5	89	18.8	2	1	1
12/3/18	T5	12					1	1	1
Mean			8.2	37	90	19	2	1	97
SD			0.1	0.4	2	0.4	1	1	111
19/3/18	T5	13					2	3	24
26/3/18	T5	14					<1		6
2/4/18	T5	15					2	3	4
9/3/18	T5	16					1		1

Note: the Seawater reservoir (SR) tank the provided additional dilution was removed after week 3.
This was the data indicated as 'back', i.e. back tank = SR.

Wafi long-term laboratory tailings-sediment study - Monitoring data to Week 12

			Water quality parameters				Dissolved Cu, Zn, Mn, µg/L		
Date	Treatment	Week	pH	Salinity, ‰	DO, %-Sat	Temp., ºC	Cu	Zn	Mn
Treatment T6 – 80% Bulk-Tails-3 mix:80% HG sediment, overlaid with 4 cm of 100% HG sediment									
2/1/18	T6	1					3	1	225
2/1/18	T6 back	1	8.14	36.0	90	18.6	1	4	295
28/12/17	T6	2					1	3	291
28/12/17	T6 back	2	8.21	36.3	90	19.6	1	1	133
8/1/18	T6	3					1	1	133
8/1/18	T6 back	3	8.35	36.4	87	19.2	1	1	61
15/1/18	T6	4	8.22	36.4		19.1	1	2	15
22/1/18	T6	5	8.18	36.5		19.4	2	1	1
29/1/18	T6	6	8.25	36.4	87	19.5	2	2	1
5/2/18	T6	7	8.17	36.2	93	19.4	1	3	1
12/2/18	T6	8	8.11	36.6	91	19.8	1	3	1
19/2/18	T6	9	8.2	36.4	91	20.0	1	1	1
26/2/18	T6	10	8.21	37.5	90	19.2	1	1	1
5/3/18	T6	11					1	1	1
12/3/18	T6	12					1	1	1
Mean	Weeks 1-12		8.2	36	90	19	1	2	92
SD			0.1	0.4	2	0.4	1	1	115
19/3/18	T6	13					1	3	10
26/3/18	T6	14					<1	22	<1
2/4/18	T6	15					1	2	5
9/3/18	T6	16					1	20	<1

Note: the Seawater reservoir (SR) tank the provided additional dilution was removed after week 3.
This was the data indicated as 'back', i.e. back tank = SR.

Wafi long-term laboratory tailings-sediment study - Monitoring data to Week 12

			Water quality parameters				Dissolved Cu, Zn, Mn, µg/L		
Date	Treatment	Week	pH	Salinity, ‰	DO, %-Sat	Temp., ºC	Cu	Zn	Mn
Treatment T7 – 80% Bulk-Tails-4 mix:20% HG sediment, overlaid with 4 cm of 100% HG sediment									
2/1/18	T7	1	8.30	36.5	91	19.3	2	1	210
2/1/18	T7 back	1					2	1	206
28/12/17	T7	2	8.14	36.0	87	18.8	1	3	299
28/12/17	T7 back	2					1	3	299
8/1/18	T7	3	8.11	36.2	89	19.8	1	1	132
8/1/18	T7 back	3					2	1	133
15/1/18	T7	4	8.38	36.3	91	19.2	1	2	65
22/1/18	T7	5	8.23	36.5		19.2	2	1	25
29/1/18	T7	6	8.18	36.4		19.1	2	1	1
5/2/18	T7	7	8.28	36.7	86	19.4	2	1	1
12/2/18	T7	8	8.23	36.5	92	19.2	1	2	0
19/2/18	T7	9	8.17	36.8	92	19.7	1	2	1
26/2/18	T7	10	8.19	36.5	92	19.8	1	1	1
5/3/18	T7	11	8.21	37.2	91	19.1	1	1	1
12/3/18	T7	12					1	1	3
Mean	Weeks 1-12		8.2	37	90	19	1	1	92
SD			0.1	0.3	2	0.3	1	1	113
19/3/18	T7	13					1	2	14
26/3/18	T7	14					<1	31	<1
2/4/18	T7	15					1	3	2
9/3/18	T7	16					1	30	<1

Note: the Seawater reservoir (SR) tank the provided additional dilution was removed after week 3.
This was the data indicated as 'back', i.e. back tank = SR.

Appendix C – Water DGT, sediment DET, DGT and Rhizon data

Water DGT data for week 5 and 12

Week 10 DGT - Dissolved metals in water, as measured from 74- deployment of water DGTs

Treatments	Dissolved metal concentrations, µg/L		
	Copper	Zinc	Manganese
Blanks (DGT not deployed)	<0.2	1.4	<0.1
	<0.2	1.5	<0.1
T0	0.3	2.1	<0.1
T0 duplicate	0.2	1.6	<0.1
T1	0.4	1.9	30
T1 duplicate	0.4	2.0	29
T2	5.4	4.1	44
T2 duplicate-2	5.3	4.7	43
T2 duplicate-3	5.8	4.2	45
T2 duplicate-4	7.2	5.5	56
T3	3.5	2.6	61
T3 duplicate	2.8	2.1	51
T4	0.7	2.2	68
T4 duplicate	0.7	2.0	68
T5	0.7	2.2	82
T5 duplicate	0.6	1.9	82
T6	0.4	1.8	47
T6 duplicate	0.5	2.3	46
T7	0.4	2.3	49
T7 duplicate	0.4	1.7	52

Dissolved iron was <2 µg/L

Week 5 and Week 5 discrete monitoring data for comparison

	Week 4	Dissolved metals, µg/L				Week 5	Dissolved metals, µg/L		
Date	Treatment	Cu	Zn	Mn		Treatment	Cu	Zn	Mn
15/1/18	T0	1	1	1	22/1/18	T0	1	1	1
15/1/18	T1	1	1	37	22/1/18	T1	1	1	7
15/1/18	T2	10	4	66	22/1/18	T2	11	3	47
15/1/18	T2 replicate	10	5	66	22/1/18	T2 replicate	10	4	47
15/1/18	T3	5	2	77	22/1/18	T3	6	3	65
15/1/18	T3 replicate	5	2	78	22/1/18	T3 replicate	6	4	66
15/1/18	T4	2	1	95	22/1/18	T4	3	3	42
15/1/18	T5	2	1	107	22/1/18	T5	2	1	30
15/1/18	T6	1	1	61	22/1/18	T6	1	2	15
15/1/18	T7	1	2	65	22/1/18	T7	2	1	25

Water DGT data for week 10

Week 10 DGT - Dissolved metals in water, as measured from 74-h deployment of water DGTs

Treatments	Dissolved metal concentrations, µg/L		
	Copper	Zinc	Manganese
Blanks (DGT not deployed)	<0.2	2.5	<0.1
T0 (mean ±SD)	0.18 ± 0.03	2.8 ± 1.0	0.06 ± 0.01
T1 (mean ±SD)	0.29 ± 0.01	2.1 ± 0.5	0.09 ± 0.01
T2 (mean ±SD)	4.45 ± 0.03	3.6 ± 0.5	0.30 ± 0.01
T3 (mean ±SD)	1.68 ± 0.07	1.8 ± 0.3	0.23 ± 0.00
T4 (mean ±SD)	0.65 ± 0.01	2.4 ± 0.3	0.09 ± 0.01
T5 (mean ±SD)	0.71 ± 0.02	2.1 ±1.7	0.10 ± 0.01
T6 (mean ±SD)	0.28 ± 0.05	1.7 ± 1.6	0.09 ± 0.02
T7 (mean ±SD)	0.28 ± 0.01	1.2 ±0.1	0.16 ± 0.01

Dissolved iron was <2 µg/L

Week 10 and Week 11 discrete monitoring data for comparison

	Week 10	Dissolved metals, µg/L				Week 11	Dissolved metals, µg/L		
Date	Treatment	Cu	Zn	Mn		Treatment	Cu	Zn	Mn
26/2/18	T0	1	3	1	5/3/18	T0	1	1	1
26/2/18	T1	1	1	1	5/3/18	T1	1	1	1
26/2/18	T2	6	3	1	5/3/18	T2	8	1	1
26/2/18	T2 replicate	6	3	1	5/3/18	T2 replicate	8	1	1
26/2/18	T3	2	2	1	5/3/18	T3	4	1	1
26/2/18	T3 replicate	2	3	1	5/3/18	T3 replicate	4	1	1
26/2/18	T4	1	1	1	5/3/18	T4	2	1	1
26/2/18	T5	1	1	1	5/3/18	T5	2	1	1
26/2/18	T6	1	1	1	5/3/18	T6	1	1	1
26/2/18	T7	1	1	1	5/3/18	T7	1	1	1

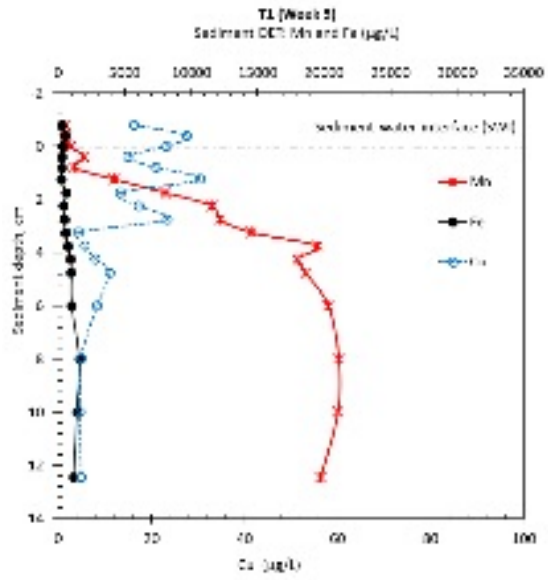
Week 5 quality control for DET andDGT sample analysis

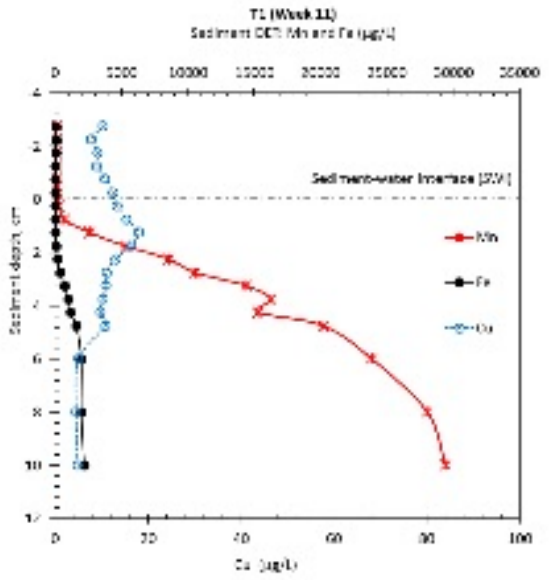
ICP-MS	Dissolved metal concentrations, µg/L			
	Copper	Zinc	Manganese	Iron
Blanks (n = 10)	0.03 ± 0.02	0.2 ± 0.06	0.07 ± 0.02	0.12 ± 0.3
Quality control (%)	81-103	84-101	84-100	83-93
Repetitiveness (%)	97-107	97-106	98-107	99-109
CRM recovery (%)	98-102	97-101	95-100	97-105

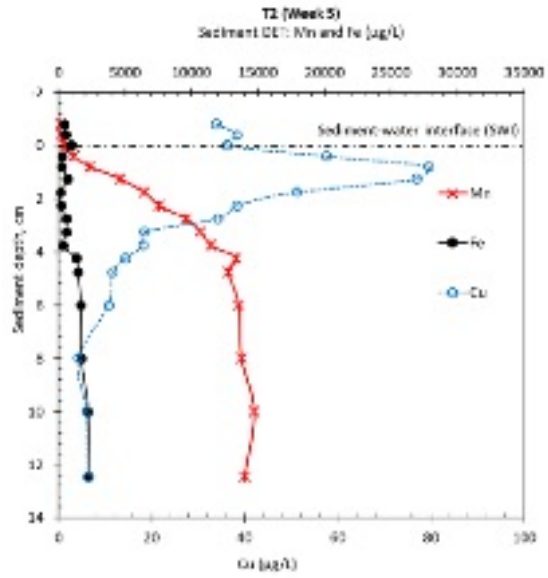
Week 11 quality control for DET and DGT sample analysis

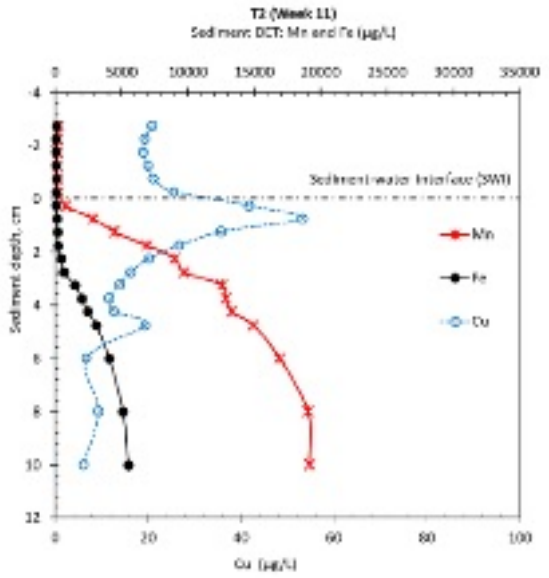
ICP-MS	Dissolved metal concentrations, µg/L			
	Copper	Zinc	Manganese	Iron
Blanks (n = 10)	0.06 ± 0.07	0.1 ± 0.09	0.05 ± 0.1	0.16 ± 0.08
Quality control (%)	100-108	100-107	100-107	95-102
Repetitiveness (%)	90-110	97-106	97-103	96-104
CRM recovery (%)	103-104	107-108	93-105	102-104

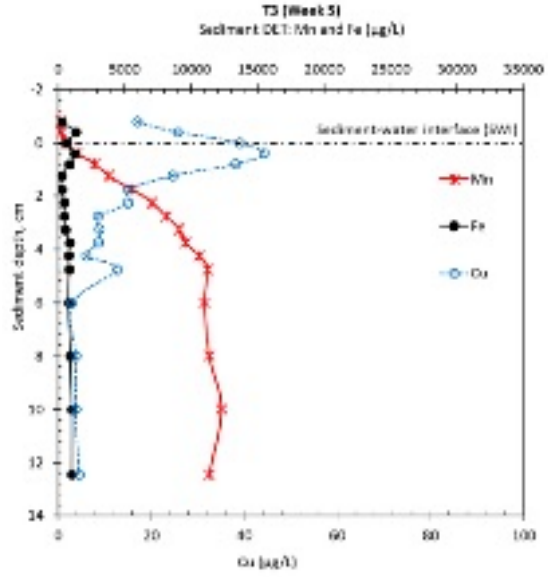
Sediment DET Figures

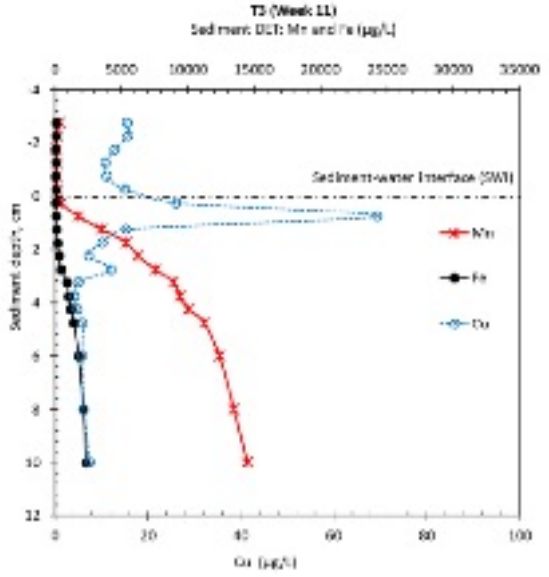


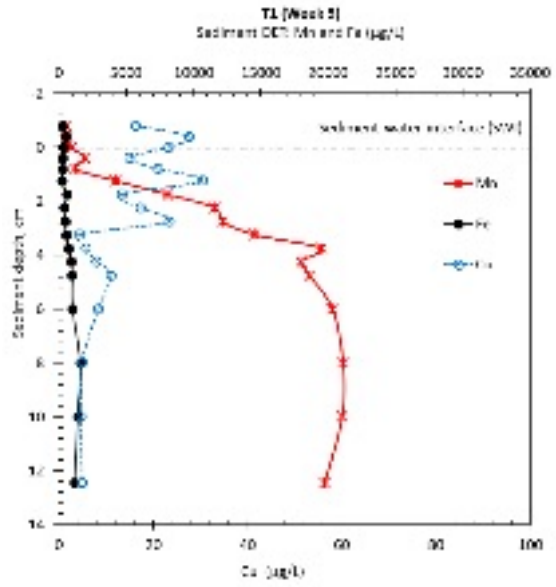


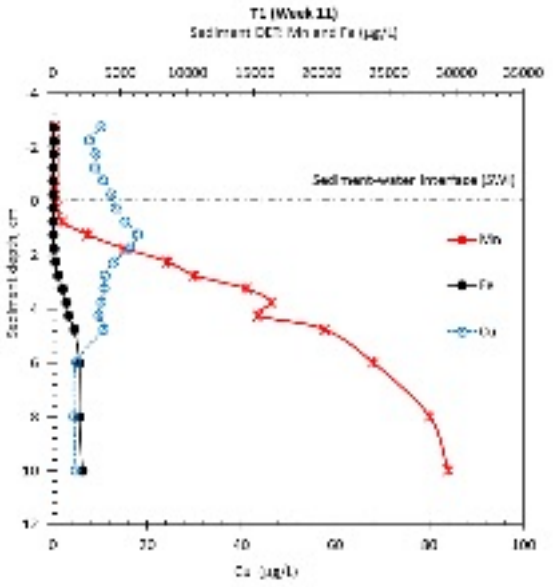


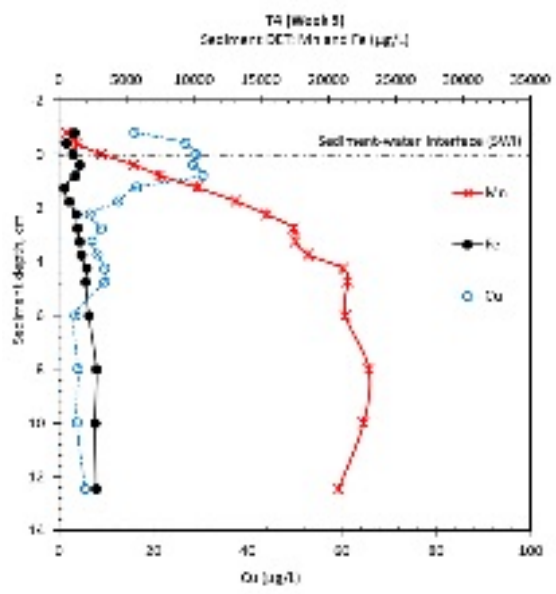


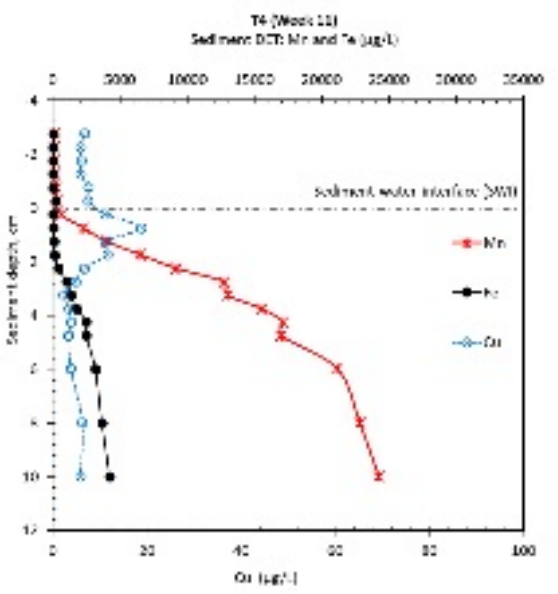


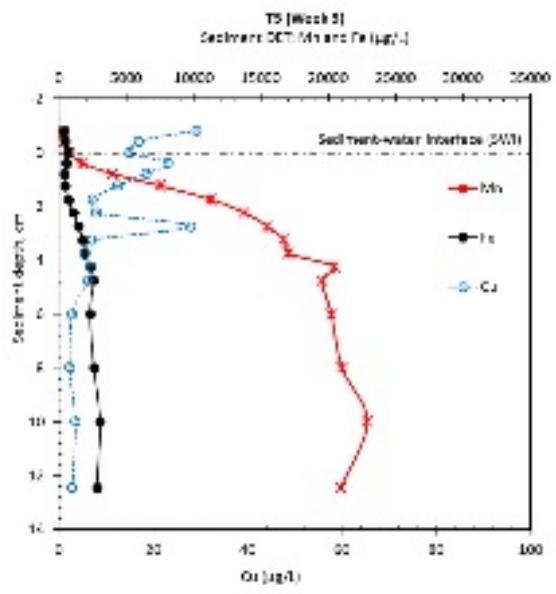


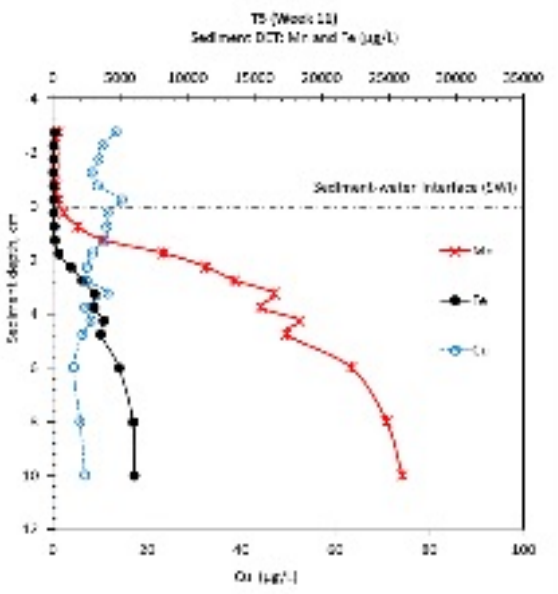

T1 (Week 5)
Sediment-water interface (SWI)
Mn
Fe
Cu

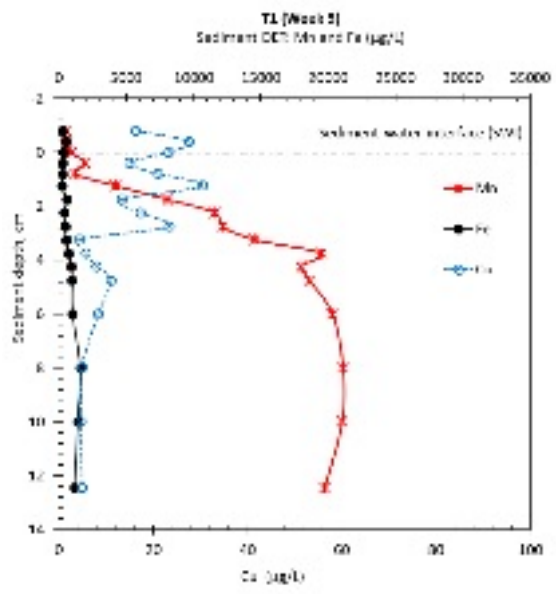

T1 (Week 11)
Sediment-water interface (SWI)
Mn
Fe
Cu

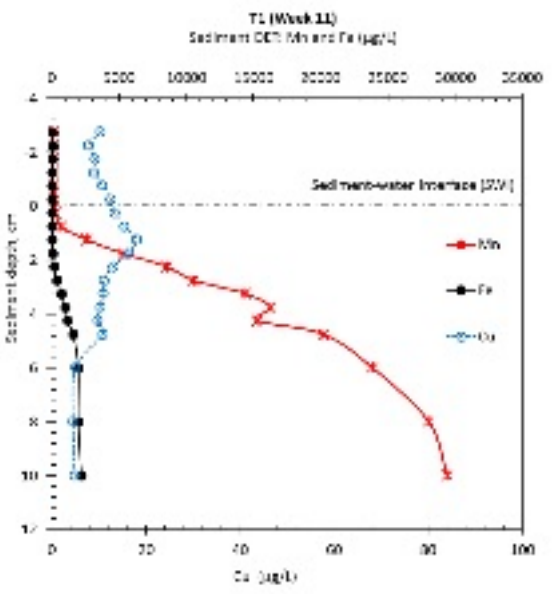

T4 (Week 5)
Sediment-water interface (SWI)
Mn
Fe
Cu

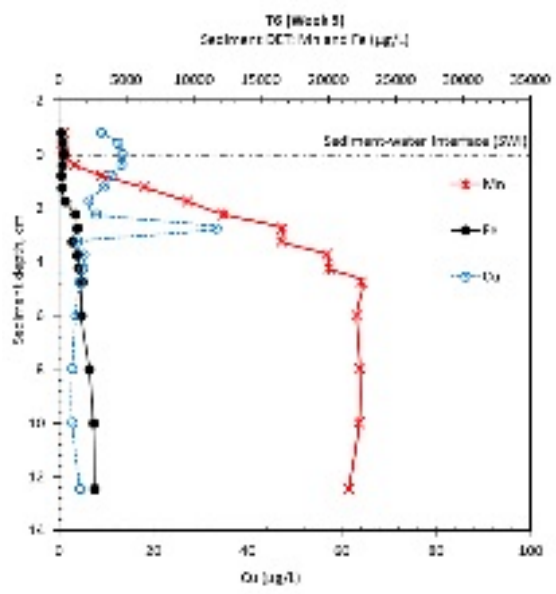

T4 (Week 11)
Sediment-water interface (SWI)
Mn
Fe
Cu

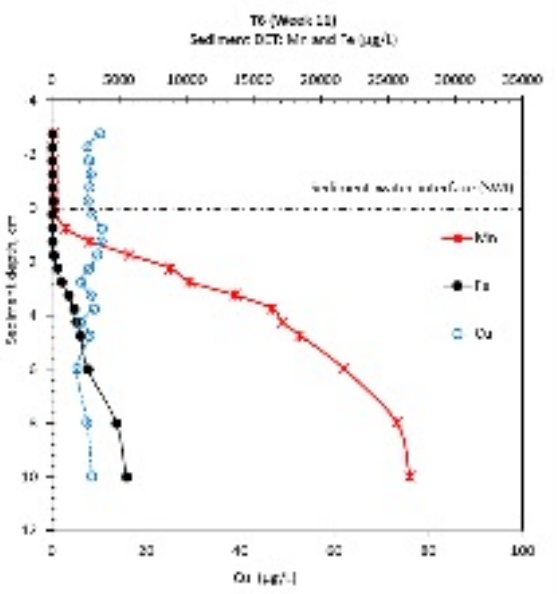

T5 (Week 5)
Sediment-water interface (SWI)
Mn
Fe
Cu

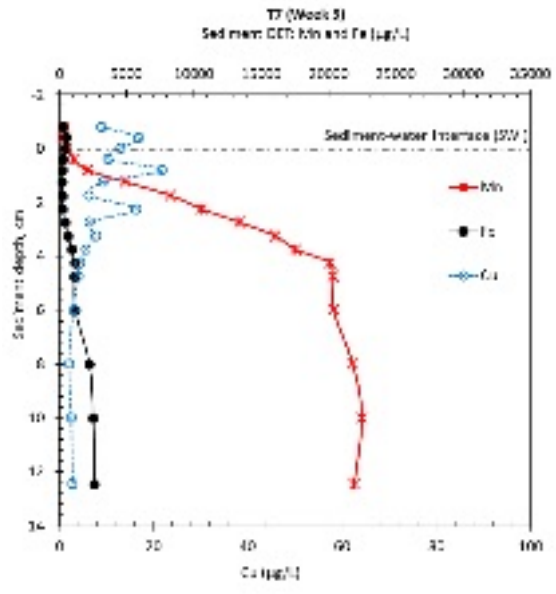

T5 (Week 11)
Sediment-water interface (SWI)
Mn
Fe
Cu

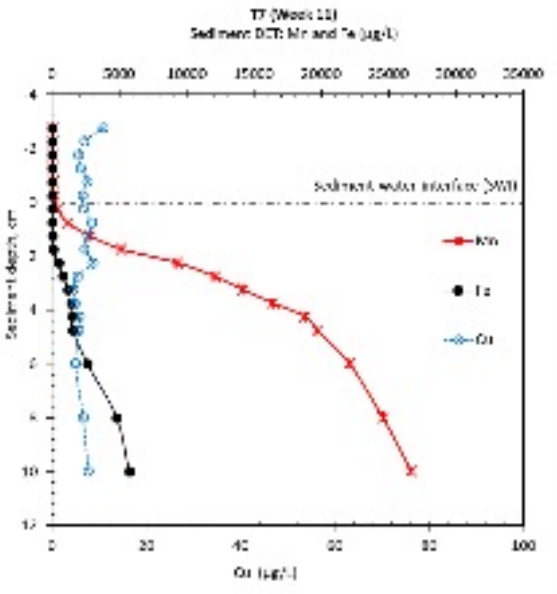

T1 (Week 5)
Sediment DGT: Mn and Fe (µg/L)
Sediment-water interface (SWI)
Mn
Fe
Cu
Sediment depth, cm
Cu (µg/L)


T1 (Week 11)
Sediment DGT: Mn and Fe (µg/L)
Sediment-water interface (SWI)
Mn
Fe
Cu
Sediment depth, cm
Cu (µg/L)


T6 (Week 5)
Sediment DGT: Mn and Fe (µg/L)
Sediment-water interface (SWI)
Mn
Fe
Cu
Sediment depth, cm
Cu (µg/L)


T6 (Week 11)
Sediment DGT: Mn and Fe (µg/L)
Sediment-water interface (SWI)
Mn
Fe
Cu
Sediment depth, cm
Cu (µg/L)


T7 (Week 5)
Sediment DGT: Mn and Fe (µg/L)
Sediment-water interface (SWI)
Mn
Fe
Cu
Sediment depth, cm
Cu (µg/L)


T7 (Week 11)
Sediment DGT: Mn and Fe (µg/L)
Sediment-water interface (SWI)
Mn
Fe
Cu
Sediment depth, cm
Cu (µg/L)

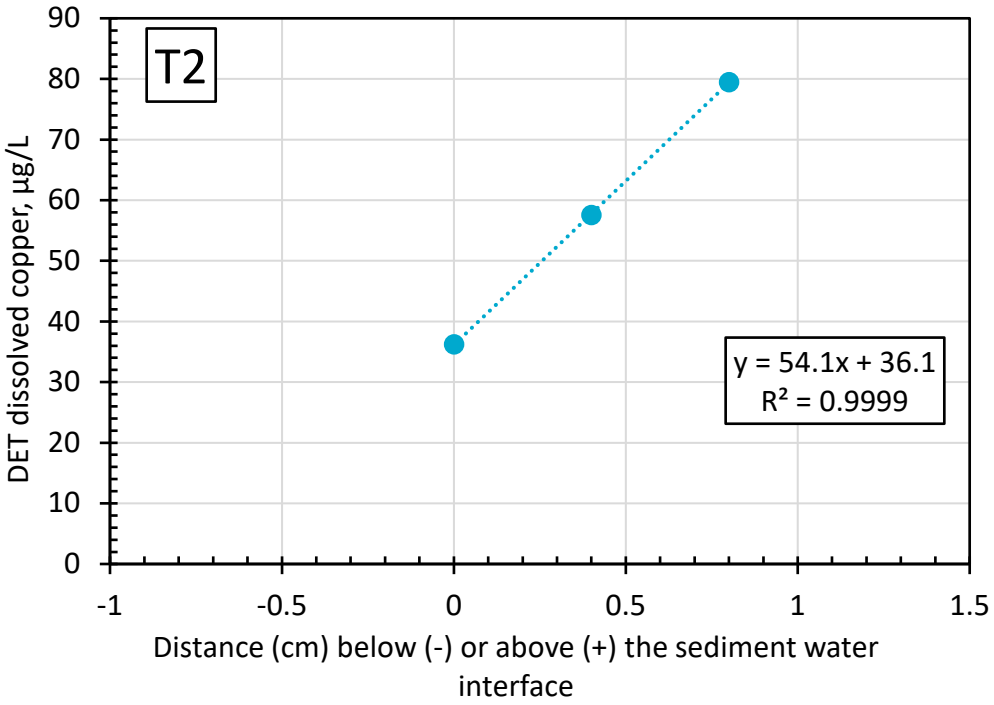
DET metal diffusive flux calculations

Copper flux calculation metadata

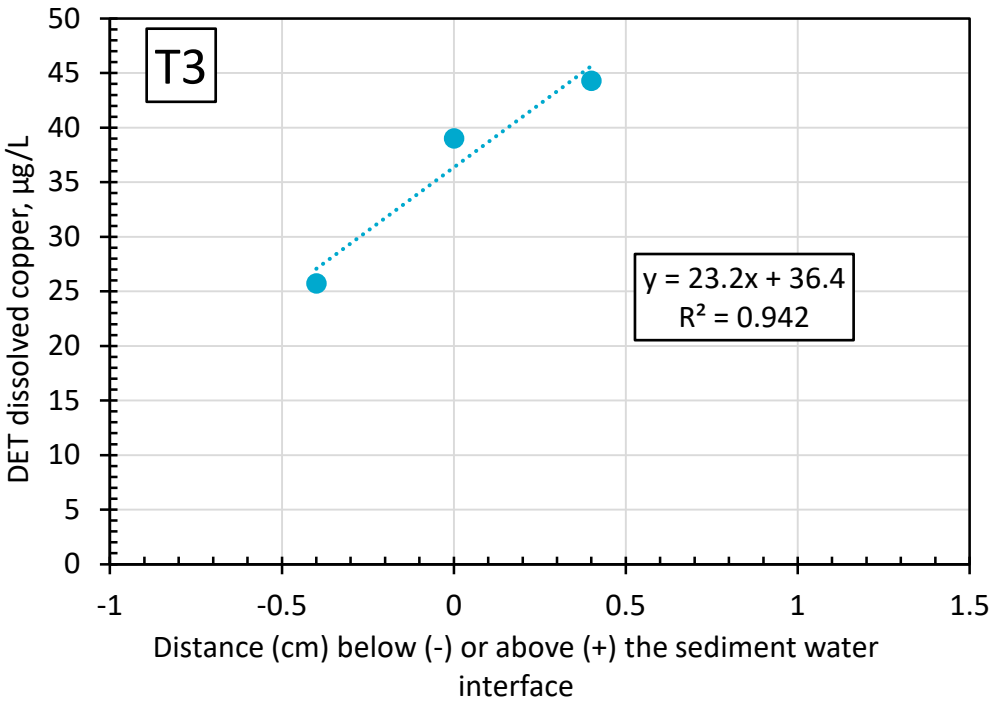
Treatments	Slope (ng cm^{-4})	R^2	n	Confidence*
T1 (week 5)	-2.8	0.0679	3	very low
T1 (week 11)	3.5	0.9812	6	very high
T2 (week 5)	54	0.9999	3	very high
T2 (week 11)	28	0.9908	3	very high
T3 (week 5)	23	0.9420	3	high
T3 (week 11)	54	0.8941	3	high
T4 (week 5)	2.5	0.7535	4	high
T4 (week 11)	11	1.000	3	very high
T5 (week 5)	3.2	0.2292	4	low
T5 (week 11)	1.7	0.9192	4	high
T6 (week 5)	-2.1	0.6847	5	moderate
T6 (week 11)	2.9	0.9561	3	very high
T7 (week 5)	-8.2	0.9932	3	very high
T7 (week 11)	0.60	0.4083	6	moderate

*A qualitative measure of confidence in the flux data based on R^2 and n values.

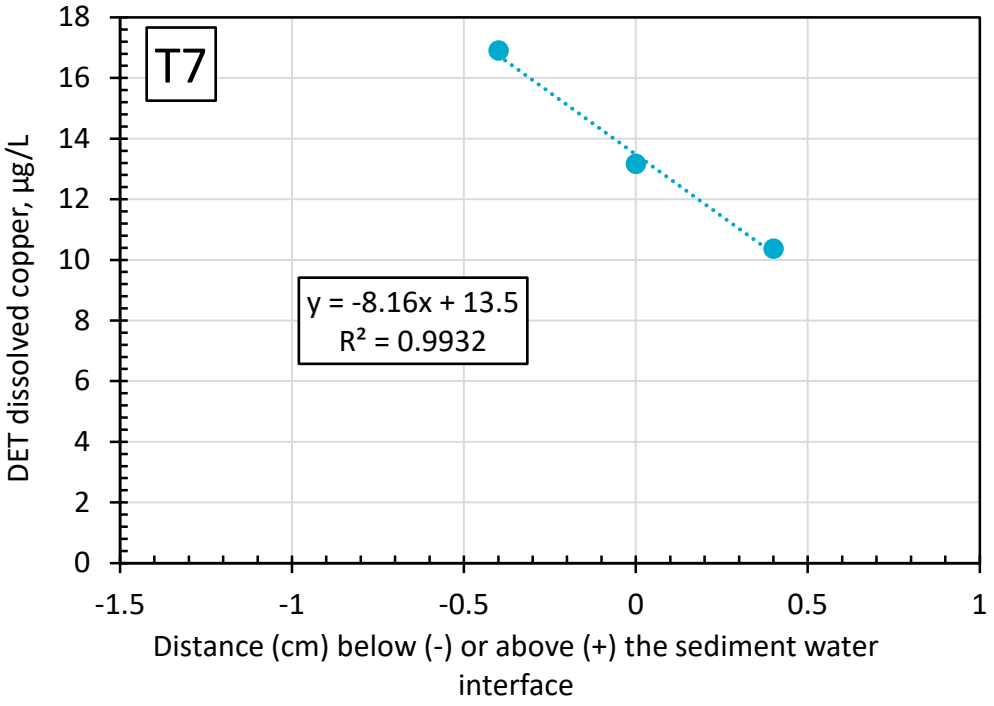
Examples below of data analysis



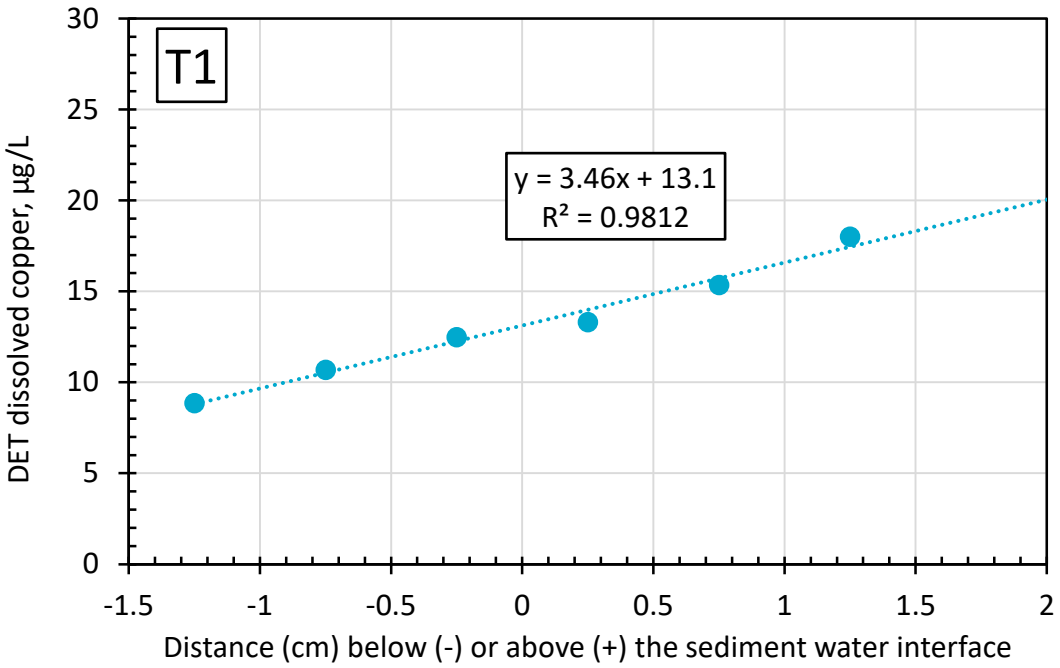
Copper porewater concentration gradient (dC/dz) across sediment-water interface used to calculate benthic fluxes for Treatment 2 after 5 weeks



Copper porewater concentration gradient (dC/dz) across sediment-water interface used to calculate benthic fluxes for Treatment 3 after 5 weeks



Copper porewater concentration gradient (dC/dz) across sediment-water interface used to calculate benthic fluxes for Treatment 7 after 5 weeks



Copper porewater concentration gradient (dC/dz) across sediment-water interface used to calculate benthic fluxes for Treatment 1 after 11 weeks

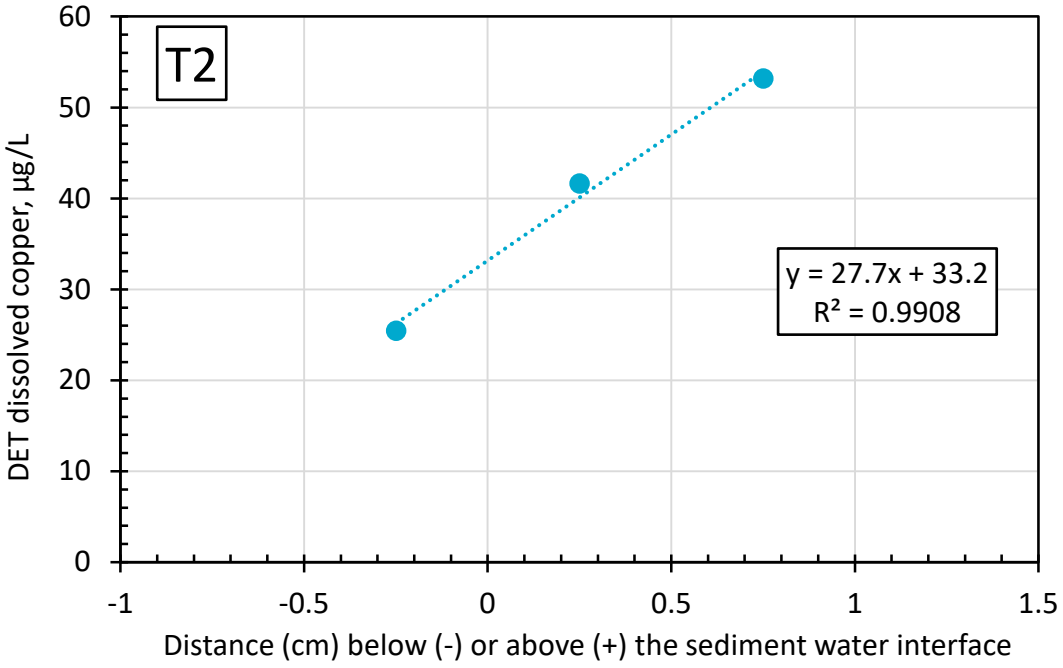


Figure E. Copper porewater concentration gradient (dC/dz) across sediment-water interface used to calculate benthic fluxes for Treatment 2 after 11 weeks

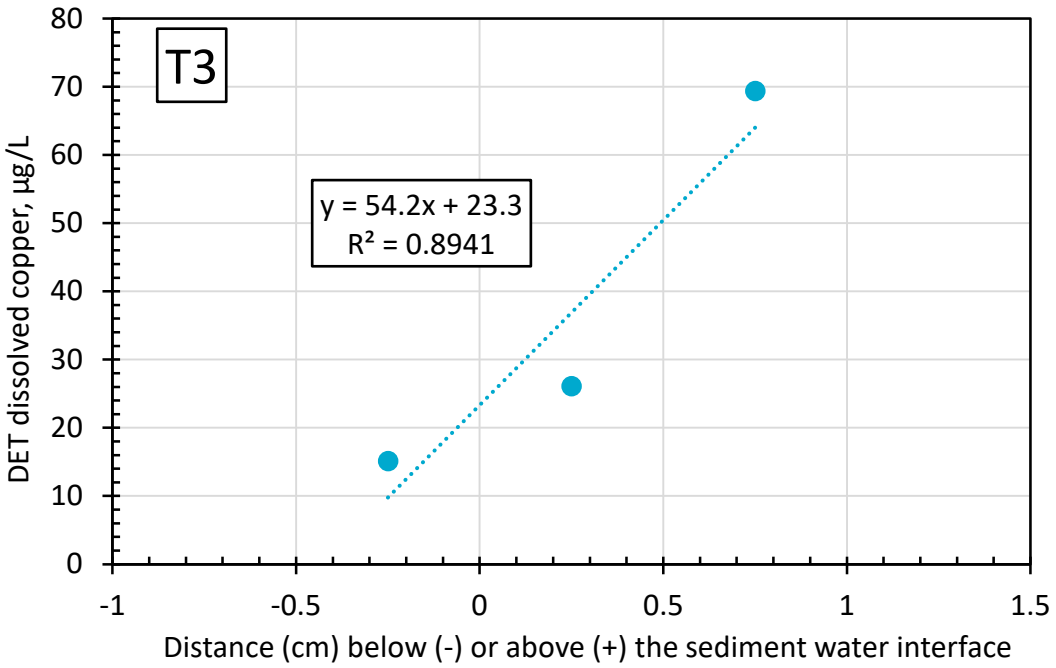
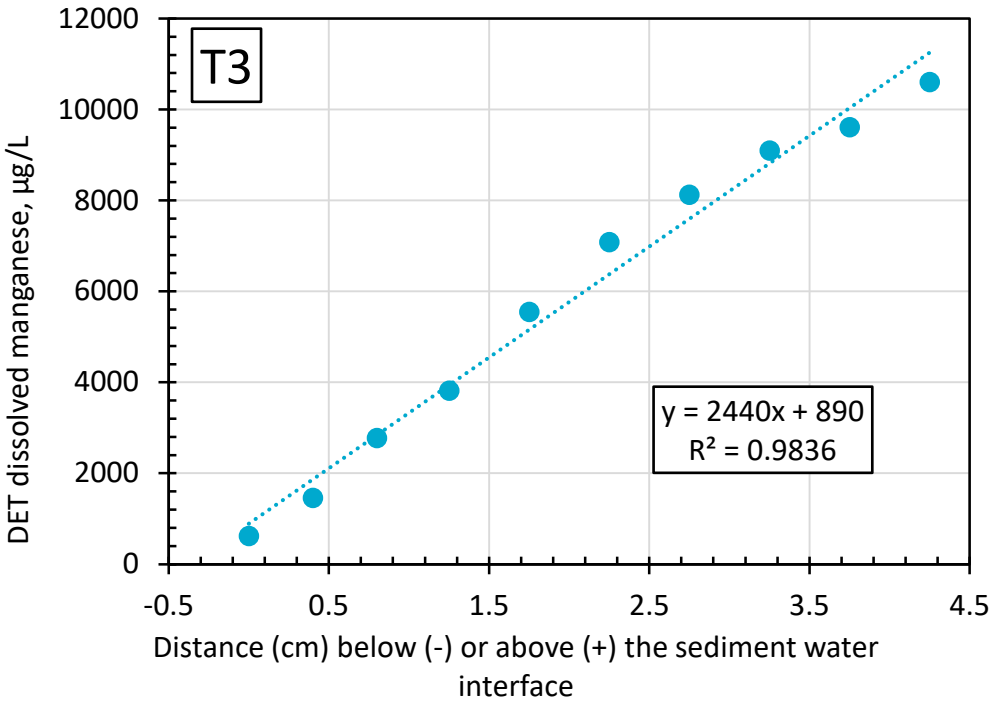


Figure F. Copper porewater concentration gradient (dC/dz) across sediment-water interface used to calculate benthic fluxes for Treatment 3 after 11 weeks

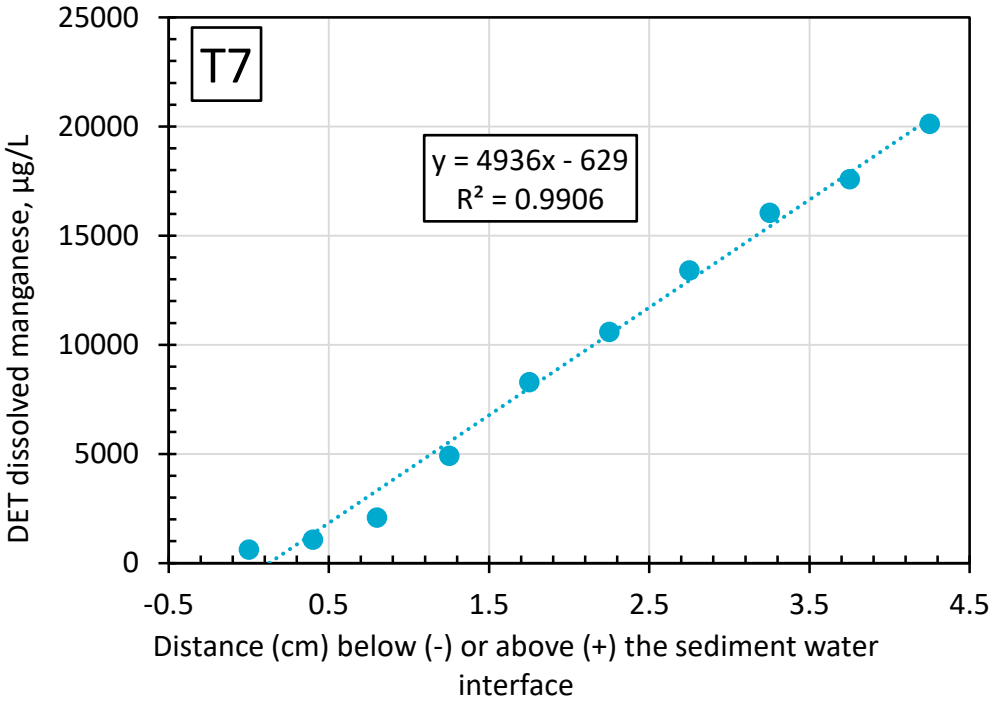
Manganese flux calculation metadata

Treatments	Slope (ng cm^{-4})	R^2	n	Confidence*
T1 (week 5)	4700	0.9589	10	very high
T1 (week 11)	4300	0.9618	11	very high
T2 (week 5)	3100	0.9895	10	very high
T2 (week 11)	3100	0.9767	11	very high
T3 (week 5)	2400	0.9836	10	very high
T3 (week 11)	2400	0.9823	11	very high
T4 (week 5)	4100	0.9613	10	very high
T4 (week 11)	3900	0.9753	11	very high
T5 (week 5)	4700	0.9631	10	very high
T5 (week 11)	4100	0.9425	11	very high
T6 (week 5)	5200	0.9769	10	very high
T6 (week 11)	4200	0.9743	11	very high
T7 (week 5)	4900	0.9906	10	very high
T7 (week 11)	4500	0.9732	11	very high

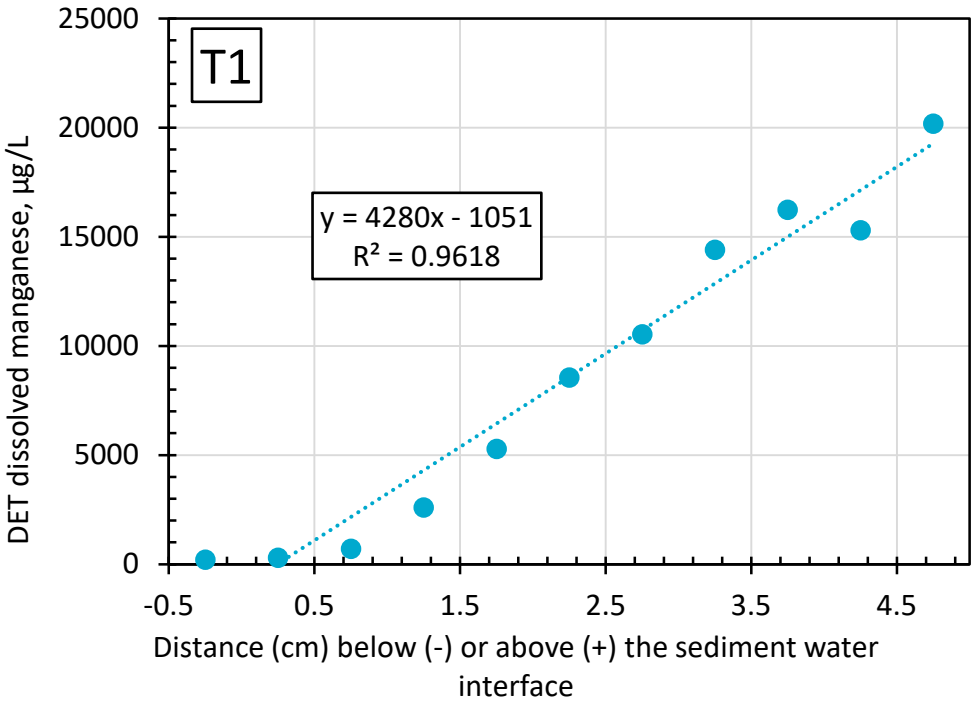
*A qualitative measure of confidence in the flux data based on R^2 and n values.



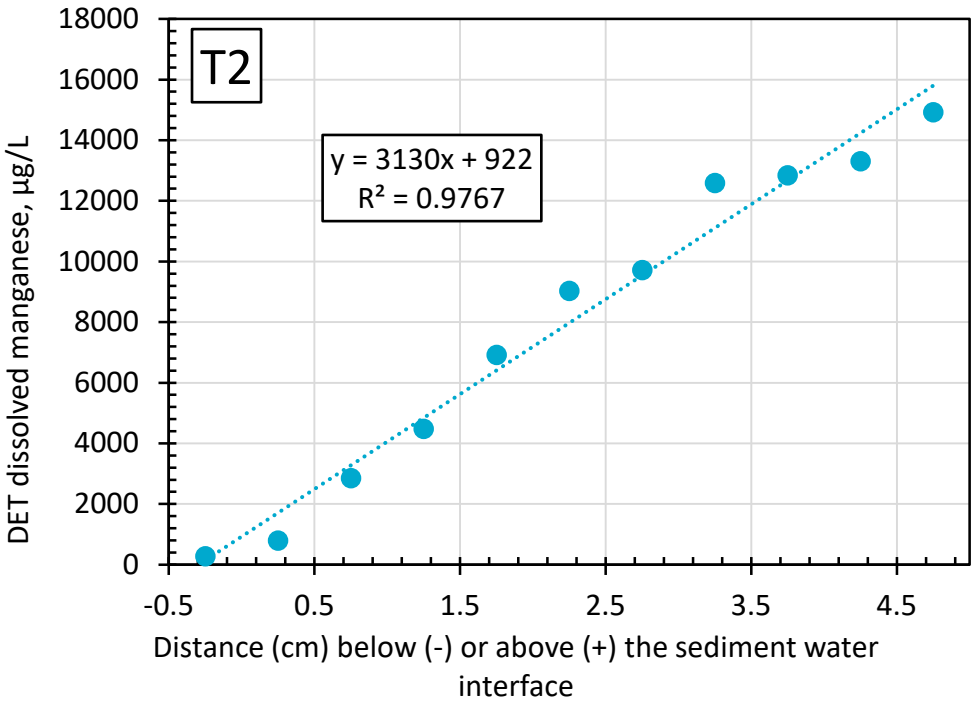
Manganese porewater concentration gradient (dC/dz) across sediment-water interface used to calculate benthic fluxes for Treatment 3 after 5 weeks



Manganese porewater concentration gradient (dC/dz) across sediment-water interface used to calculate benthic fluxes for Treatment 7 after 5 weeks



Manganese porewater concentration gradient (dC/dz) across sediment-water interface used to calculate benthic fluxes for Treatment 1 after 11 weeks

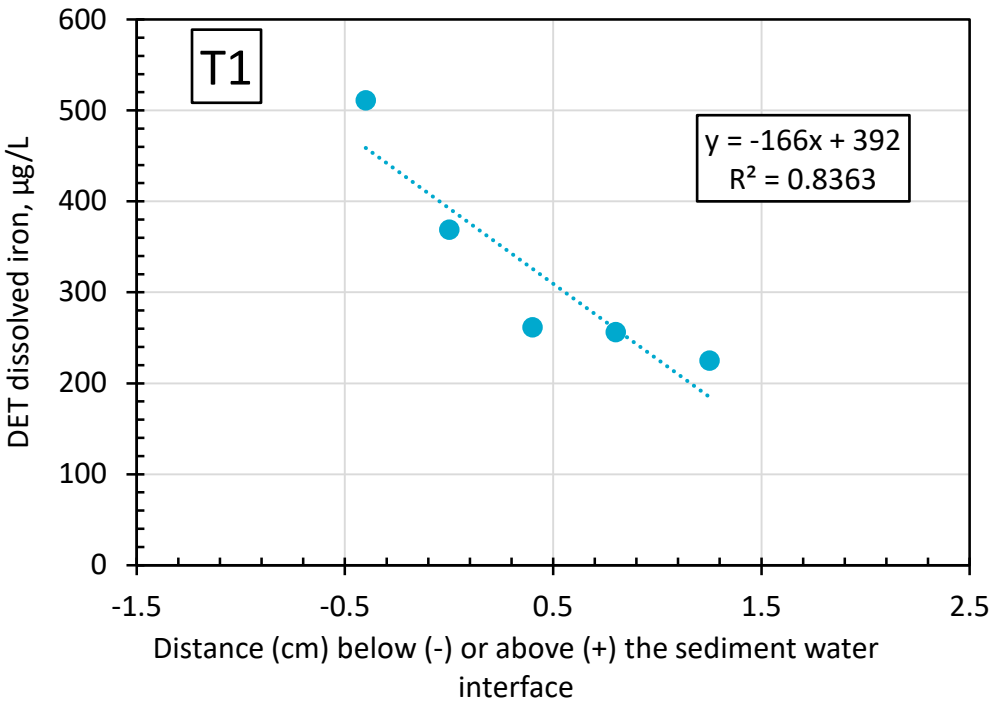


Manganese porewater concentration gradient (dC/dz) across sediment-water interface used to calculate benthic fluxes for Treatment 2 after 11 weeks

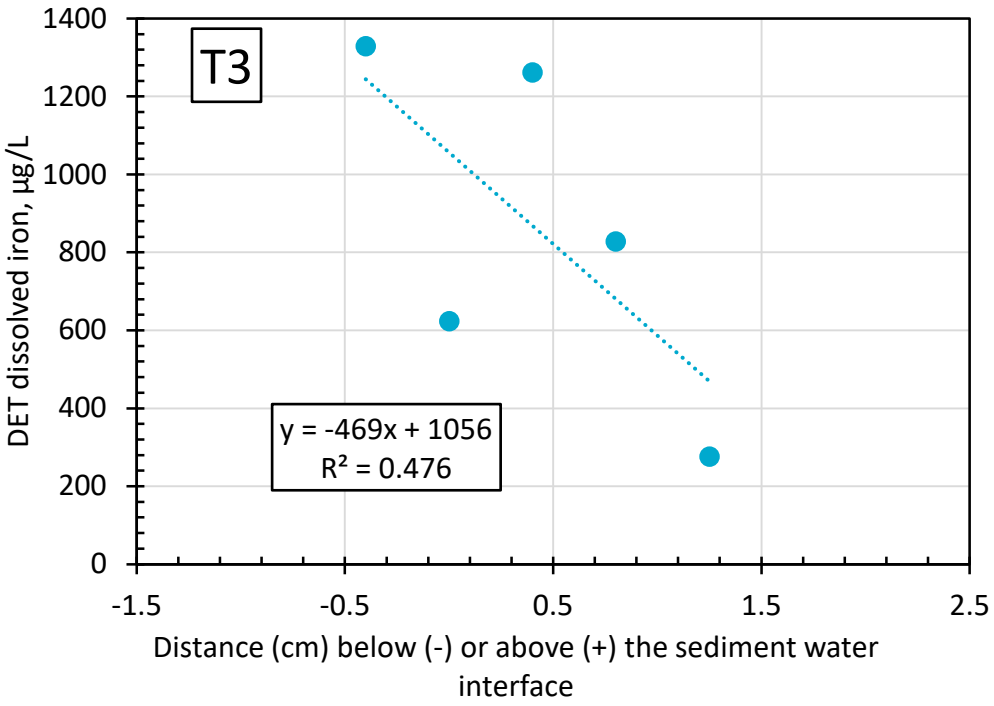
Iron flux calculation metadata

Treatments	Slope (ng cm^{-4})	R^2	n	Confidence*
T1 (week 5)	-170	0.8363	5	high
T1 (week 11)	4300	0.9618	11	very high
T2 (week 5)	-120	0.073	5	very low
T2 (week 11)	3100	0.9767	11	very high
T3 (week 5)	-470	0.476	5	moderate
T3 (week 11)	2400	0.9823	11	very high
T4 (week 5)	-90	0.0255	6	very low
T4 (week 11)	3900	0.9753	11	very high
T5 (week 5)	9.8	0.0052	6	very low
T5 (week 11)	4100	0.9425	11	very high
T6 (week 5)	5.0	0.0038	6	very low
T6 (week 11)	4200	0.9743	11	very high
T7 (week 5)	-190	0.8693	5	high
T7 (week 11)	4500	0.9732	11	very high

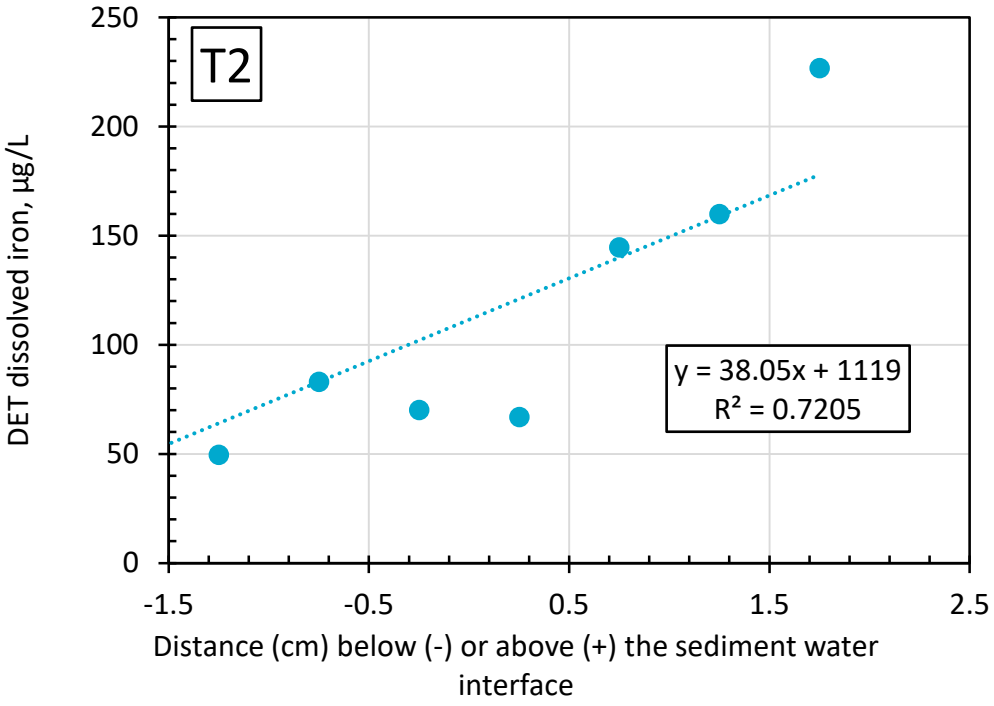
*A qualitative measure of confidence in the flux data based on R^2 and n values.



Iron porewater concentration gradient (dC/dz) across sediment-water interface used to calculate benthic fluxes for Treatment 1 after 5 weeks

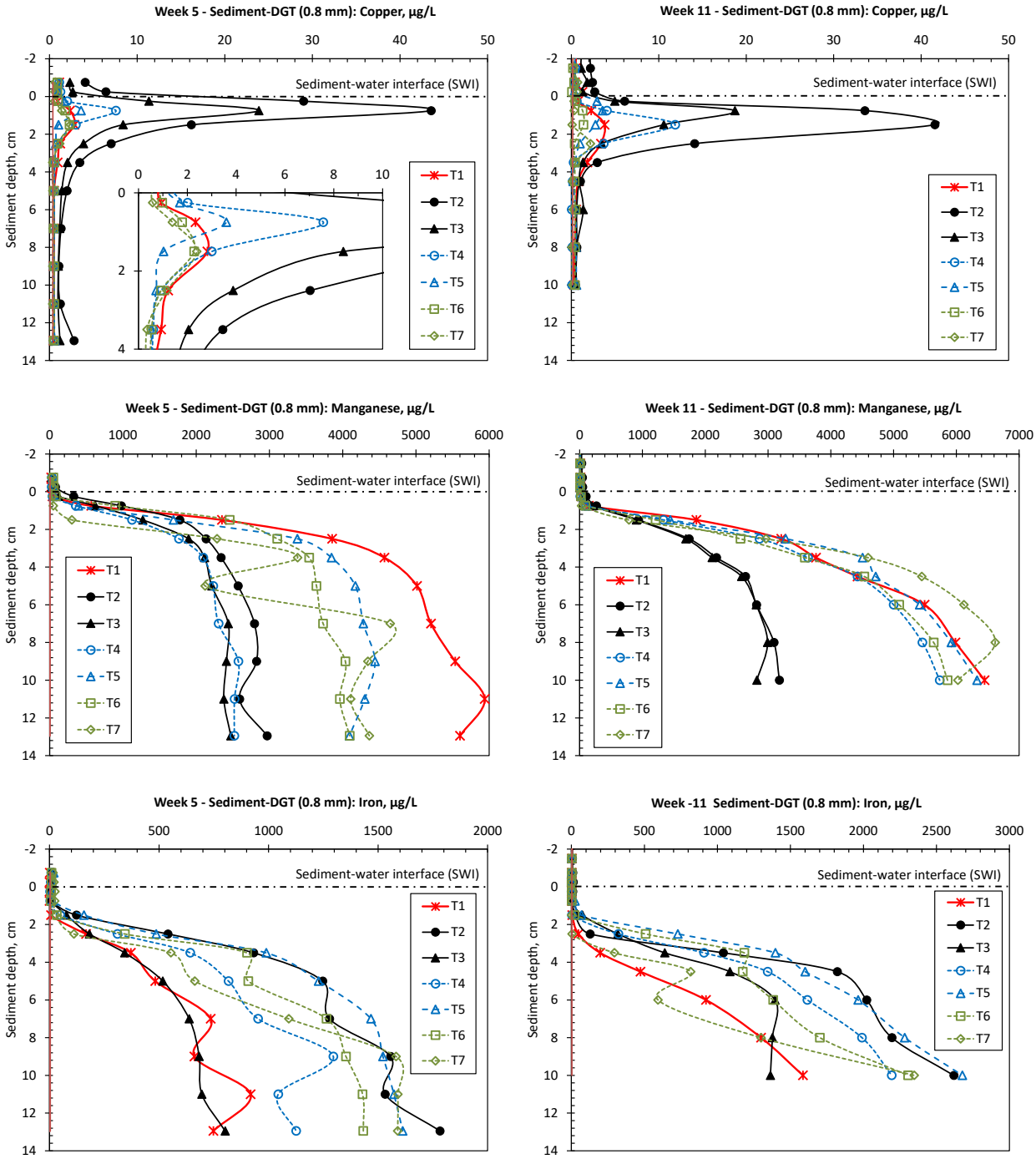


Iron porewater concentration gradient (dC/dz) across sediment-water interface used to calculate benthic fluxes for Treatment 3 after 5 weeks

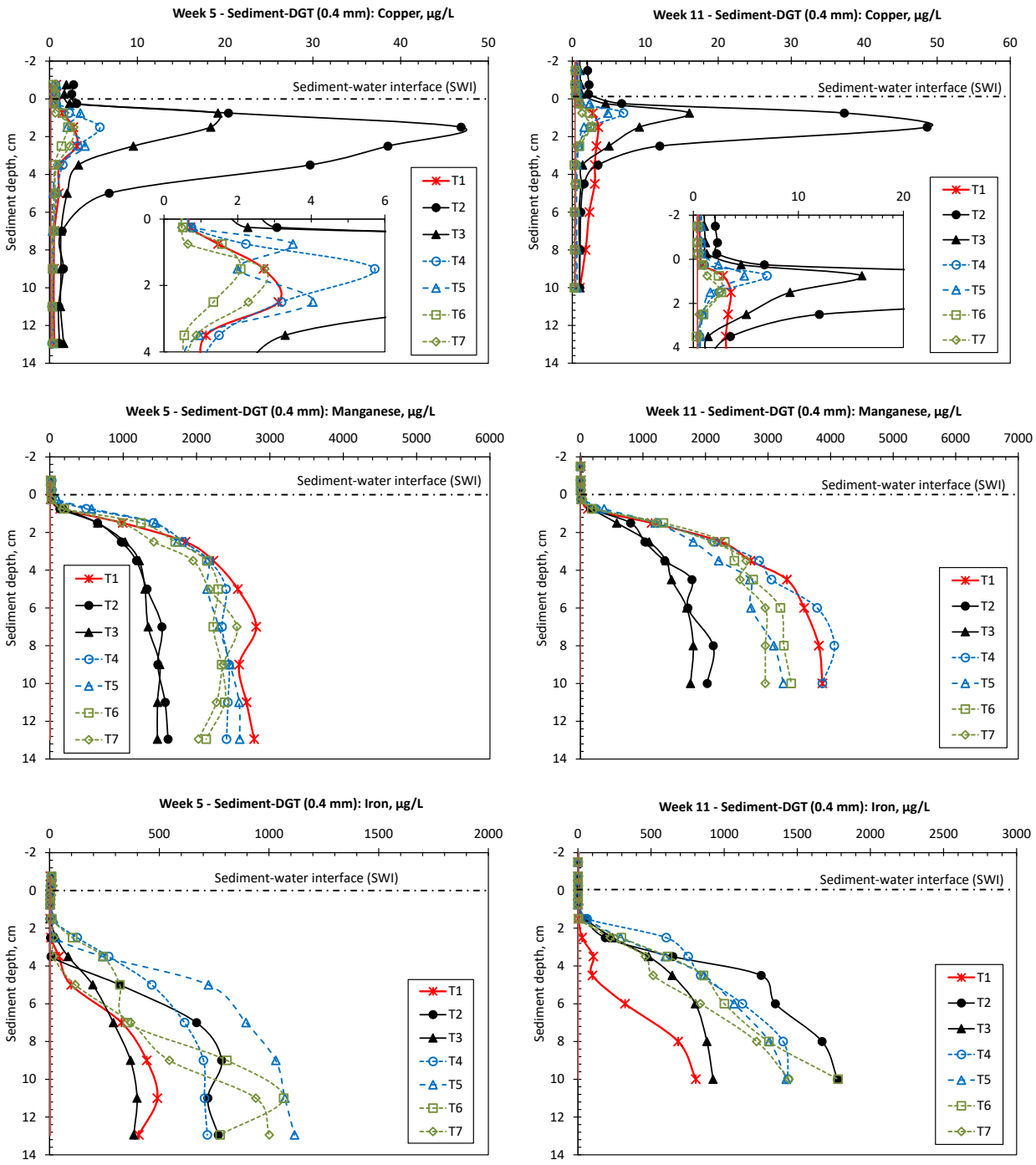


Iron porewater concentration gradient (dC/dz) across sediment-water interface used to calculate benthic fluxes for Treatment 2 after 11 weeks

DGT metal profiles



Comparison of sediment DGT (0.8 mm) profiles of Cu, Fe and Mn for all treatments. The method detection limit is indicated buy the red line when visible. The figure for sediment DGT Cu contains data embedded with a larger scale near the mobilisation depth.



Comparison of sediment DGT (0.4 mm) profiles of Cu, Fe and Mn for all treatments. The method detection limit is indicated buy the red line when visible. The figure for sediment DGT Cu contains data embedded with a larger scale near the mobilisation depth.

Rhizon (porewater) data

Treatment	Rhizon	Dissolved metal concentrations, µg/L (0.1 µm filtered)														
	Depth, cm	Cu	Zn	Mn	Fe	Ag	Al	As	Cd	Co	Cr	Mo	Ni	Pb	Se	V
T1	3	1.8	4			0.043	44	14	0.019	14	0.52	9.1	11	<0.1	11	3.1
T1	8	1.8	5			0.042	43	25	0.020	17	0.54	7.9	13	0.1	13	4.0
T1	13	2.3	6			0.040	17	16	0.025	17	0.52	7.6	12	<0.1	14	3.5
T2	3	1.2	12			0.036	8	11	0.033	6.4	0.76	83	47	<0.1	15	1.1
T2	8	1.2	9			0.031	9	11	0.037	7.1	0.71	82	51	<0.1	14	1.1
T2	13	1.1	10			0.034	13	12	0.033	7.2	0.78	81	52	<0.1	8.4	1.2
T3	3	2.2	8			0.071	142	7.8	0.040	2.2	1.8	69	28	0.1	9.3	1.1
T3	8	1.1	6			0.042	9	9.2	0.028	2.8	0.46	70	35	<0.1	11	0.50
T3	13	0.9	9			0.039	13	8.2	0.030	2.9	0.46	70	36	<0.1	12	0.52
T4	3	2.1	4			0.040	19	15	0.021	13	0.49	20	17	<0.1	13	2.8
T4	8	2.2	4			0.037	105	17	0.028	16	0.73	18	22	<0.1	7.0	3.4
T4	13	1.9	9			0.039	7	18	0.033	17	0.65	18	22	<0.1	5.1	3.1
T5	3	1.8	1			0.041	6	15	0.017	12	0.63	21	19	<0.1	7.0	2.5
T5	8	3.1	7			0.062	10	19	0.021	14	0.67	21	23	<0.1	6.9	3.0
T5	13	2.2	12			0.051	14	18	0.028	14	0.63	20	22	<0.1	7.6	2.7
T6	3	2.0	6			0.039	49	15	0.021	15	0.49	13	13	<0.1	7.7	2.9
T6	8	3.4	7			0.040	213	18	0.033	18	0.78	20	24	0.1	4.8	3.9
T6	13	3.1	14			0.044	38	18	0.031	17	0.57	19	23	0.1	5.6	3.2
T7	3	2.7	8			0.039	312	17	0.020	16	0.95	12	14	0.1	5.6	4.3
T7	8	1.8	10			0.045	37	18	0.020	14	0.61	21	23	<0.1	6.2	2.9
T7	13	2.0	20			0.058	274	19	0.027	15	0.89	21	23	0.1	6.9	3.5

Data for Fe and Mn were not available at the time of this interim report

Appendix D – Sediment Toxicity and Bioaccumulation Tests

Use of surrogate species to assess risk of adverse effects of benthic organisms in the deep sea

For many benthic organisms, the toxicity is frequently well predicted by the dissolved concentrations in overlying waters measured during exposures. For the amphipod, the EC50 values for reproduction in whole-sediment tests are in the ranges of 8-20 µg Cu/L and 30-60 µg Zn/L (overlying waters). For the copepod, the EC50 values for reproduction in sediments are in the ranges of 23-72 µg Cu/L and 50-400 µg Zn/L (large uncertainty). Data are from Campana et al. (2012) for copper, and a mixture of published and unpublished studies for zinc (e.g. Simpson et al., 2014; 2016). When expressed based on particulate metal concentrations, effects thresholds are strongly influenced by sediment properties and modified by dietary exposure. The proportion of fine particles (influencing surface area for metal adsorption) and organic carbon (OC) concentrations strongly influence copper bioavailability and toxicity. For *M. plumulosa* and *N. spinipes*, Campana et al. (2012) determined EC10s for reproduction of 5.2 and 4.8 mg <63 µm Cu/g TOC, respectively, thus reflecting the influence of particle size and organic carbon.

The potential bioaccumulation of metals by benthic biota was assessed using the benthic bivalve *Tellina deltoidalis* over 30 days. The bivalve *T. deltoidalis* buries in the top 10–20 cm of sandy or muddy sediments and is a deposit feeder, collecting organic material and particles from surface sediments. The amphipod *M. plumulosa* is a deposit feeder and known to ingest solids while foraging for food. No standardized whole-sediment toxicity tests exist that utilize deep-sea organisms, so the use of these surrogate organisms was justified owing to the relatively high sensitivity of the test end points to metals (Campana et al., 2012; Simpson and Spadaro., 2011; Simpson et al., 2013). The amphipod has previously been used for assessing the bioavailability and toxicity of mineral-associated metals in marine sediments (Simpson and Spadaro, 2016).

It is noted that differences in exposure conditions between the laboratory and deep-sea environment will include lower temperature and higher pressure. Recent studies of the sensitivity of deep-sea organisms to metals have considered these factors (MIDAS, 2015; Brown et al. 2017) and indicate that shallow-water species may be suitable ecotoxicological proxies for deep-sea species, dependent on adaptation to habitats with similar environmental variability. Of deep-sea species and proposed shallow-water proxies that have been studied to date (MIDAS, 2015), the sensitivity to metals has been 1-2 orders of magnitude less than that of the amphipod and copepod being used to provide conservative outcomes for this assessment. Comparing effects of temperature and pressure for the prawn species, Brown et al. (2017) determined that the LC50s for copper and cadmium were respectively 13.7 mg/L and 9.8 mg/L at 20°C and 0.1 MPa using standard method, 26.9 mg/L and 61.4 mg/L at 10°C and 0.1 MPa using standard method (lower temperature), and 24.3 mg/L and 58.4 mg/L at 10°C and 0.1 MPa using hyperbaric method, and 15.9 mg/L and 55.6 mg/L at 10°C and 10.0 MPa using hyperbaric method (higher pressure). The point here being the very low sensitivity of the species studied and potentially a 2-fold difference in sensitivity owing to change in temperature, which is within the confidence limited of most effects threshold used for deriving WQGVs.

It is important to note here that the laboratory-based toxicity testing may exacerbate the exposure to dissolved metals in the overlying water when compared to what may occur in the field, resulting in a more

conservative outcomes than may be expected for the same sediments and species in the field (Mann et al., 2010; Belzunce-Segarra et al., 2015).

References

Belzunce-Segarra, M.J., Simpson, S.L., Amato, E.D., Spadaro, D.A., Hamilton, I.L., Jarolimek, C.V. and Jolley, D.F. (2015). The mismatch between bioaccumulation in field and laboratory environments: Interpreting the differences for metals in benthic bivalves. Environ. Pollut., 204, 48-57.

Brown, A., Thatje, S. and Hauton, C. (2017). The effects of temperature and hydrostatic pressure on metal toxicity: Insights into toxicity in the deep sea. Environ. Sci. Technol.. 51, 10222-10231.

Canfield DE, Thamdrup B, and Hansen JW. (1993). The anaerobic degradation of organic matter in Danish coastal sediments: Iron reduction, manganese reduction, and sulfate reduction. Geochim. Cosmochim. Acta 57:3867–3883.

Campana, O., Spadaro, D.A., Blasco, J. and Simpson, S.L. (2012). Sublethal effects of copper to benthic invertebrates explained by changes in sediment properties and dietary exposure. Environ. Sci. Technol., 46, 6835−6842.

Mann, R.M., Hyne, R.V., Simandjuntak, D.L. and Simpson, S.L. (2010). A rapid amphipod reproduction test for sediment quality assessment: In situ bioassays do not replicate laboratory bioassays. Environ. Toxicol. Chem., 29, 2566-2574.

MIDAS (2015). Managing impacts of deep sea resource exploitation. European Union Seventh Framework Programme (FP7/2007-2013), grant agreement nº 603418.

Remaili, T., Simpson, S.L., Amato, E.D., Spadaro, D.A., Jarolimek, C.V. and Jolley, D.F. (2016). The impact of sediment bioturbation by secondary organisms on metal bioavailability, bioaccumulation and toxicity to target organisms in benthic bioassays: implications for sediment quality assessment. Environ. Pollut., 208, 590–599.

Simpson, S.L. and Spadaro, D.A. (2011). Performance and sensitivity of rapid sublethal sediment toxicity tests with the amphipod *Melita plumulosa* and copepod *Nitocra spinipes*. Environ. Toxicol. Chem., 30, 2326-2334.

Simpson, S.L. and Spadaro, D.A. (2016). Bioavailability and chronic toxicity of metal sulfide minerals to benthic marine invertebrates: implications for deep sea exploration, mining and tailings disposal. Environ. Sci. Technol., 50, 4061–4070.

Simpson, S.L., Spadaro, D.A. and O'Brien, D. (2013). Incorporating bioavailability into management limits for copper in sediments contaminated by antifouling paint used in aquaculture. Chemosphere, 93, 2499-2506.

Simpson, S.L., Spadaro, D.A. and Batley, G.E. (2016). Development of remediation targets for Kendall Bay sediments. CSIRO Land and Water Report EP162025. 81 pages.

Simpson, S.L., Spadaro, D.A. and Watters, A. (2014). Newcastle Port Corporation Port-wide Strategy – future capital and - maintenance dredging assessment. CSIRO Wealth from Oceans Reports EP14246 (196 pages.) and EP143733 (549 pages).

Amphipod Reproduction Test Report AR17079

Project:	Wafi-Golpu long-term study of DSTP
Test Performed:	10-day amphipod reproduction toxicity test (sublethal, chronic effects) using the amphipod *Melita plumulosa*

Test Initiated:	16/3/18	
CSIRO Sample No.	**Sample Name**	**Sample Description**
	QA	Silty sediment collected from Bonnet Bay, NSW
E17079	**T1-HG**[a]	Huon Gulf (HG) sediment (previously gamma irradiated)
E17077	**T4**	20% BT3 tailings, 80% HG
E17078	**T5**	20% BT4, tailings, 80% HG
E17077	**T6**	**T4** with a 4 cm layer of HG on the surface
E17078	**T7**	**T5** with a 4 cm layer of HG on the surface

Test method: The amphipod reproduction bioassay measures adult survival and reproduction, expressed as the number of embryos and <1-d-old juveniles in the second brood following exposure of *Melita plumulosa* to test sediments over a 10-d period. The test was carried out according to the methods described in Simpson and Spadaro (2011). Amphipods used in the tests were isolated from laboratory cultures. The sediment T1-HG and tailings-sediment samples T4 to T7 were taken from mesocosm treatments of the long-term lab study of Wafi-Golpu tailings (described in this report – Simpson et al. (2018)). They had been equilibrating for 12 weeks in 110 L tanks of seawater (with >90% exchange with fresh seawater weekly). Sediment were taken from the respective treatments after lowering the level of the overlying water to the level of the SWI. A plastic spatula was used to carefully collect the top 1-2 cm of the surface sediments. These sediments were laid into the test beakers in a manner that retained the vertical stratification and aimed to cause minimal impact to the sediment profile (40 g sediment per 400 mL vial, 4 replicates per sediment). Filtered seawater (200 mL, 30‰) was added and each beaker was incubated at 21^{o}C with aeration for 72 h to allow sediments to settle. On the commencement of the test, 350 mL of overlying water was siphoned off and replaced with new seawater with care to minimise sediment resuspension. Six gravid females (gravid for <36 h) and six males (isolated from laboratory cultures) were randomly assigned to each beaker. Treatments are fed at a rate of 0.25 mg Sera Micron fish food/amphipod twice a week. The sediments are renewed after 5 d by gently sieving away the adults and placing them into the same fresh sediment that had been equilibrated, thus allowing for the removal of juveniles from the first brood, which is typically unaffected by contaminants in the test sediment because they were already ‘‘conceived’’ before exposure to test sediments. On Day 10, the females were carefully removed and the number of embryos per female is counted by microscopy. The sediment was also checked for juvenile amphipods that had escaped the marsupium during the latter stages of the test by sieving the sediment through 180 µm mesh. The total number of embryos and <1-d-old juveniles was summed and expressed as a percentage of the control.

For QA purposes, a minimum of 7 juveniles per female is required in the QA controls for tests to be considered acceptable. For this test, effects to adult survival are classified as acute toxicity and effects to reproduction are classified as chronic toxicity. A sediment is considered to be acutely toxic if the survival as a percentage of the HG control is <80% and is statistically significantly less ($P<0.05$) than the HG controls. Chronic toxicity is detected when the reproductive output percent control (Huon Gulf) is <85%, (based on 2 standard deviations of control data n=80) and is statistically significant less ($P<0.05$) than the controls. Physico-chemical parameters (temperature, pH, salinity and dissolved oxygen) were measured throughout the test and sub-samples of the overlying water were measured for dissolved metals by inductively coupled plasma atomic emission spectrometry (ICP-AES). Statistical significance between treatments and effects concentrations were calculated using ToxCalc Version 5.0.23 (Tidepool Software).

Results: The survival of the adults in the test (Table 1) was within minimum acceptability limit of 80% (QA control sediment). The number of embryos per female produced in the QA control sediment was also greater than the minimum acceptability limits of 8 embryos per female (Table 2). Dissolved ammonia concentrations (0.5-1 mg NH_3-N/L) remained below levels that may cause effects to the reproduction of the amphipod (Simpson et al., 2013).

No acute or chronic toxicity was observed in any of the treatments that contained tailings.

Table 1. Toxicity test results

	Amphipod survival		Amphipod reproduction			
Sediment	**Survival (% survival)**	**% of QA Control**	**Embryos per females**	**% of QA Control**	**% of HG Control**	**Average dissolved copper, µg/L[d]**
QA control	88 ± 5[a]	-	10 ± 1	100 ± 12	-	2.2 ± 0.8
T1-HG	90 ± 5	100 ± 6	6 ± 0	67 ± 3[b]	100 ± 5	5.9 ± 2.9
T4	88 ± 5	98 ± 6	8 ± 1	83 ± 9	125 ± 13	10 ± 5.8[e]
T5	81 ± 5	91 ± 6	7 ± 1	70 ± 9	105 ± 14	9.5 ± 4.8[e]
T6	81 ± 5	91 ± 6	9 ± 1	89 ± 6	134 ± 10[c]	4.8 ± 2.6
T7	92 ± 5	102 ± 5	10 ± 1	106 ± 8	158 ± 12[c]	6.2 ± 3.2

[a] All results are mean ± standard error calculated based on the four replicate tests/sediment.
[b] Statically less than the QA control response ($p<0.05$) and below the toxic threshold.
[c] Statically increase reproduction than the HG control ($p<0.05$).
[d] Average dissolved copper measurements of overlying water in the sediments on day 0, 3, 5, and 7.
[e] Statistically greater dissolved copper concentrations measured in the overlying water (t-test pair-wise comparison of daily copper concentration) compared to T1-HG.

Acute toxicity - No toxicity was observed to the survival of the amphipods in any of the test treatments.

Chronic toxicity - When compared to the QA control, toxicity to amphipod reproduction was assessed to occur in the amphipods exposed to the Huon Gulf sediment (67±3% of QA control). This was the lowest level of reproduction of any of the test treatments, and was consistent with previous studies that found that the Huon Gulf sediment was not an optimal substrate for the species reproduction (Adams et al., 2018). This may be attributed to a lower nutritional content of the Huon Gulf sediment compared to the sediment used as the QA control, but may also be influenced by the very fine particle size of the Huon Gulf sediment (~95% <63 µm, DV50 ~10 µm).

The reproduction was not significantly different to the QA control for the other tailings-sediment treatments (T4, T5, T6, T7). The particle size of the tailings was greater than the Huon Gulf sediment (e.g. BT3 ~40% <63 µm, DV50 ~80 µm), however the nutritional value of the tailings would be expected to be lower than the Huon Gulf sediment. The T6 and T7 materials comprised of HG sediment capping the T4 and T5 tailings-sediment mixtures, and after 12 weeks of equilibrating were expect to have the same physical properties as T1-HG but were potentially impacted by upward diffusion of metals from the underlying tailings. The concentrations of dissolved copper released from the sediments into the overlying water were significantly greater (paired daily concentration, $p<0.05$) in T4 and T5 compared to T1-HG, however, no significant difference between T1-HG and T6 or T7 (Table 1).

Table 2. Quality assurance/quality control

Quality Assurance/Quality Control Criteria	Range	Criterion Met?
≥80% survival in the QA control (BB)	88 ± 5%	Yes
≥8 embryos per female produced in the QA control	10 ± 1	Yes
pH of overlying water in test beakers	8.0 ± 0.1	Yes
Salinity of overlying water in test beakers	30 ± 0.2‰	Yes
Dissolved oxygen in overlying water in test beakers	>90%	Yes
Temperature of overlying water in test beakers	21 ± 1°C	Yes

References

Adams, M.S., Spadaro, D.A., Simpson, S.L., Binet, M.T., King, J.J., Jarolimek, C.V., McKnight, K.S., Golding, L.A. and Apte, S.C. (2018). Ecotoxicology and chemistry of Wafi-Golpu bench-scale tailings. CSIRO Report EP178086, 88 pp.

Simpson, S.L. and Spadaro, D.A. (2011). Performance and sensitivity of rapid sublethal sediment toxicity tests with the amphipod *Melita plumulosa* and copepod *Nitocra spinipes.* Environ. Toxicol. Chem., 30, 2326–2334. DOI: 10.1002/etc.633.

Simpson, S.L., Spadaro, D.A. and O'Brien, D. (2013). Incorporating bioavailability into management limits for copper and zinc in sediments contaminated by antifouling paint and aquaculture. Chemosphere, 93, 2499–2506.

Bivalve Bioaccumulation Test Report BB17079

Project:	Wafi-Golpu long-term study of DSTP
Test Performed:	30-day bivalve survival and bioaccumulation test using the benthic bivalve *Tellina deltoidalis*

Test Initiated:	16/3/18	
CSIRO Sample No.	**Sample Name**	**Sample Description**
E17079	**T1-HG**	Gamma irradiated Huon Gulf sediment
E17077	**T4**	20% BT3, 80% HG
E17078	**T5**	20% BT4, 80% HG
E17077	**T6**	**T4** with a 4 cm layer of HG on the surface
E17078	**T7**	**T5** with a 4 cm layer of HG on the surface

Test method: The bioassay assesses metal bioaccumulation and survival of the benthic bivalve, *T. deltoidalis,* following exposure to sediments for 30 days (Spadaro and Simpson, 2016).

The bivalves were collected at Boronia Park, Lane Cove River estuary (27-32‰), Sydney, New South Wales, Australia. Approximately 150 adult bivalves with shell surface areas from 10 to 60 mm^2 (two dimensional) were collected by gently sieving (2 mm mesh) sediment collected from a maximum depth below the sediment-water interface of 20 cm. Prior to use in tests the bivalves were acclimated for 7 days to the laboratory test conditions (20°C and salinity 30‰) in holding trays with sediment from the bivalve collection site and oxygenated seawater. After acclimation, bivalves were removed from the sediment, placed in seawater and sorted into groups of 10 individuals with approximately the same size distribution. The bivalves were observed over a 1-h period for movement to ensure only live animals were selected for use in the bioaccumulation test.

The bivalves were placed directly into the mesocosm treatments (T1-HG and tailing-sediment samples T4 to T7) of the long-term lab study of Wafi-Golpu tailings (described in this report – Simpson et al. (2018)). The mesocosm treatments had been equilibrating for 12 weeks in 110 L tanks of seawater (with >90% exchange with fresh seawater weekly). Due to the large amount of sediment with natural amount of algae and bacteria present (from the seawater and inoculated prior to equilibration) and low test organism density, the bivalves were not fed any additional food during the test. The release of metals from sediments to overlying water was monitored by measuring dissolved (0.45 µm filtered) metals in the overlying water throughout the exposure period, along with DO, pH, temperature and salinity.

At the termination of the tests (i.e. after 30 days), surviving bivalves were counted and allowed to depurate overnight in clean seawater for 24 h. Following depuration, the soft body tissue of the bivalves was dissected from the shell using a Teflon coated razor blade and plastic tweezers. Tissue masses from the same replicate were placed in a 70-mL polycarbonate vial and then stored in a domestic freezer at -20 °C until time of analysis.

For bivalve tissues metal analyses, the tissues were freeze dried and reweighed to determine the tissue dry weight (DW) and acid digested according to CSIRO Method C-225. Briefly, tissue (~0.15 g DW) from each test replicate was digested in duplicate in Teflon digestion tubes by adding 10 mL of Tracepur nitric acid (65%) and a Microwave Accelerated Reactive System (MARS). Digests were made to a final volume of 25 mL with Milli-Q water and metals were measured by inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7500CE) calibrated with matrix-matched standards. For quality control purposes, one blank (Milli-Q

water) and one reference sample (DORM-3, Fish Protein Certified Reference Material, National Research Council Canada) were analysed for every 8 samples.

Results: The water quality criteria were met for all treatments and survival of the bivalves in the Huon Gulf control sediment treatment was greater than the minimum acceptability limit of 80% (Table 1). The QA/QC criteria for the bivalve tissue metal analysis was within acceptable limits and is outlined in the accompanying analysis report.

The survival of the bivalves was 70% in T4 treatment and 100% in the other treatments (Table 2). In treatment T4, two bivalves were found dead (full opened shells recovered) with a third dead bivalve recovered with only cracked pieces of shell after the soft tissue had decomposed, which was suspected damage from the DET-DGT deployments. The reduced survival in T4 could not be attributed to the dissolved or tissue metal concentrations measured. In all treatments the numbers of surviving organisms were sufficient for tissue metal bioaccumulation analysis. The bioaccumulated metal concentrations measured in the tissues of the bivalves exposed to the tailing-sediment treatments T4-T7 were not significantly different to those in the T1-HG control (Table 3).

Table 1. Quality assurance/quality control

Quality Assurance/Quality Control Criteria	Range	Criterion Met?
≥80% survival in the control (HG)	100%	Yes
pH of overlying water in mesocosms	8.0 ± 0.1	Yes
Salinity of overlying water in mesocosms	33 ± 2‰	Yes
Dissolved oxygen in overlying water in mesocosms	>90%	Yes
Temperature of overlying water in mesocosms	19± 1^{o}C	Yes

Table 2. Bivalve survival and dissolved metal concentration during the experiment.

Sediment	Survival, %	Dissolved metals, µg/L				
		Cu	Fe	Mn	Ni	Zn
T1-HG	100	1.2	5.8	1.6	1.5	1.7
T4	70	2.1	1.3	2.7	1.4	2.6
T5	100	2.0	2.9	3.9	<1	2.0
T6	100	1.2	1.0	5.4	2.8	2.4
T7	100	1.0	3.4	2.2	2.5	2.7

Note: Measured concentrations of Al, As, Cd, Cr, Co Pb and V were below the limit of detection (1 µg/L) of the ICP-AES.

Table 3. Metal concentrations in the soft tissue of the bivalves at time of test commencement and after 30 days exposure to the test sediments (treatment)

	Tissue metal concentration, µg/g (dry weight)					
	Al	**As**	**Cd**	**Co**	**Cr**	**Cu**
Test commencement	1300	12	0.9	<0.9	2.3	230
Test treatment						
T1-HG	4200 ± 1200[a]	18 ± 1.7	1.4 ± 0.2	5.6 ± 1.2	6.9 ± 0.8	370 ± 160
T4	1900 ± 570	20 ± 0.2	1.6 ± 0.3	4.2 ± 0.1	7.3 ± 1.0	440 ± 42
T5	330 ± 230	20 ± 0.6	1.6 ± 0.1	6.1 ± 0.6	13 ± 2.1	410 ± 110
T6	4900 ± 260	19 ± 1.2	1.4 ± 0.1	4.6 ± 0.3	8.2 ± 1.2	500 ± 52
T7	6500 ± 1100	16 ± 1.4	1.2 ± 0.2	6.3 ± 0.9	8.4 ± 0.7	320 ± 33
Limit of detection	20	1	0.1	0.9	0.4	1
	Fe	**Mn**	**Ni**	**Pb**	**V**	**Zn**
Test commencement	1600	7.6	2.7	17	2.6	150
Test treatment						
T1-HG	5200 ± 1500	110 ± 30	8.5 ± 3.2	55 ± 14	15 ± 5.3	410 ± 150
T4	4100 ± 1300	50 ± 16	8.0 ± 1.1	51 ± 9.5	8.6 ± 1.9	360 ± 88
T5	5600 ± 230	79 ± 6.5	13 ± 0.8	52 ± 0.5	13 ± 1.5	340 ± 87
T6	5900 ± 270	130 ± 15	9.2 ± 0.04	42 ± 7.9	17 ± 1.0	390 ± 21
T7	6600 ± 940	160 ± 41	11 ± 1.1	48 ± 7.2	20 ± 4.2	630 ± 600
Limit of detection	1	0.1	0.8	2	0.2	1

[a] All results are mean ± standard deviation

References

Spadaro, D.A. and Simpson, S.L. (2016). Appendix G. Protocols for 10-day whole-sediment lethality toxicity tests and 30-day bioaccumulation tests using the deposit-feeding benthic bivalve *Tellina deltoidalis*. In Simpson SL, Batley GE (eds), Sediment Quality Assessment: A Practical Guide. CSIRO Publishing, Victoria, Australia, pp 285-293.

Metal bioaccumulation analysis report

Centre for Environmental Contaminants Research
CSIRO Land and Water
New Illawarra Road, Lucas Heights NSW 2234
Locked Bag 2007, Kirrawee NSW 2232 Australia
Telephone: +61 2 9710 6777 Facsimile: +61 2 9710 6800
www.csiro.au
ABN 41 687 119 230

Final analysis report - total recoverable metals

Client information:

Name: Dr Stuart Simpson
Company: CSIRO Land and Water
Address: Building 2, New Illawarra Rd, Lucas Heights NSW 2234
Email: stuart.simpson@csiro.au

Summary of submitted samples:

Laboratory I.D.	Client Identification:
CE475-1	Wafi Tellina Day 0 Background
CE475-2	B81
CE475-3	B82
CE475-4	T1-1
CE475-5	T4-1
CE475-6	T5-1
CE475-7	T6-1
CE475-8	T7-1
CE475-9	T1-2
CE475-10	T4-2
CE475-11	T5-2
CE475-12	T6-2
CE475-13	T7-2

Accredited for compliance with ISO/IEC 17025.
Accreditation number: 11624

Miscellaneous information:
Samples received: 20/04/18
Samples submitted by: Dr Stuart Simpson and David Spadaro
Samples tested as received

Method codes:
C-225 Total Recoverable Metals in Biota by High Pressure Microwave Digestion (200°C)
C-229 Inductively Coupled Plasma - Atomic Emission Spectrometry (ICP-AES)
C-209 Inductively Coupled Plasma - Mass Spectrometry (ICP-MS)
Samples were freeze dried and ground in an agate mortal and pestle

Quality control:
Analysis of certified reference material
Recoveries:
2 post digestion spike recoveries

Samples prepared and analysed by:
Chad Jarolimek and Josh King

Batch information:
Report date: 24/04/18
Batch number: CE475
Report number: CE475/1

Report prepared by:

Chad Jarolimek
Research Projects Officer

Accredited for compliance with ISO/IEC 17025.
Accreditation number: 11624

Total recoverable metals data summary

		µg/g dry-weight				
Laboratory I.D. No.:	Client Identification:	Ag	Al	As	Cd	Co
CE475-1	Wafi Tailing Day 0	4.8	1280	11	0.34	1.5
CE475-2	BB1	7.7	2800	23	0.75	3.2
CE475-3	BB2	9.4	4400	25	0.93	4.5
CE475-4	T1-1	5.4	3400	17	0.88	4.6
CE475-5	T4-1	8.3	1500	20	1.3	5.2
CE475-6	T5-1	9.2	3000	20	1.2	6.4
CE475-7	T6-1	11	4700	19	1.1	5.6
CE475-8	T7-1	5.8	5800	16	1.1	6.0
CE475-9	T1-2	10	5100	20	1.4	6.9
CE475-10	T4-2	9.0	2300	20	0.98	5.2
CE475-11	T5-2	8.2	3400	21	1.4	7.0
CE475-12	T6-2	9.5	5100	20	0.98	5.9
CE475-13	T7-2	8.9	7300	19	1.4	7.7
LOD [3σ]	---	0.003	30	0.01	0.01	0.004
Method code:	---	C-209	C-229	C-209	C-209	C-209

		µg/g dry-weight				
Laboratory I.D. No.:	Client Identification:	Cr	Cu	Fe	Hg	Mn
CE475-1	Wafi Tailing Day 0	2.4	230	1700	0.64	7.6
CE475-2	BB1	5.2	371	5600	0.80	20
CE475-3	BB2	6.3	404	5400	0.84	15
CE475-4	T1-1	4.8	256	4200	1.2	93
CE475-5	T4-1	4.8	413	3200	1.4	39
CE475-6	T5-1	8.9	291	5900	1.3	71
CE475-7	T6-1	6.4	532	5900	0.99	116
CE475-8	T7-1	7.0	301	6100	1.0	127
CE475-9	T1-2	7.6	478	6200	1.4	135
CE475-10	T4-2	6.0	472	5100	1.0	62
CE475-11	T5-2	13	475	5900	1.4	82
CE475-12	T6-2	7.4	459	6900	0.98	137
CE475-13	T7-2	9.1	348	7400	1.1	185
LOD [3σ]	---	0.01	1	1	0.02	0.1
Method code:	---	C-209	C-229	C-229	C-209	C-229

Accredited for compliance with ISO/IEC 17025.
Accreditation number: 11624

Total recoverable metals data summary continued

		µg/g dry-weight				
Laboratory I.D. No.:	Client identification:	Mo	Ni	Pb	Se	Zn
CE475-1	Wafi Tailing Day 0	5.2	2.7	19	4.0	149
CE475-2	BB1	7.8	5.5	40	5.4	194
CE475-3	BB2	9.6	6.2	57	6.0	403
CE475-4	T1-1	9.0	6.2	48	5.4	304
CE475-5	T4-1	13	7.3	61	7.1	296
CE475-6	T5-1	12	12	53	6.6	241
CE475-7	T6-1	12	9.2	48	5.8	408
CE475-8	T7-1	11	9.9	45	5.9	202
CE475-9	T1-2	12	11	68	7.6	511
CE475-10	T4-2	11	8.7	44	6.2	420
CE475-11	T5-2	13	13	53	6.4	391
CE475-12	T6-2	12	9.2	37	6.5	378
CE475-13	T7-2	15	11	55	6.6	1060
LOD (3σ)	---	0.01	1	0.01	0.01	1
Method code:	---	C-209	C-229	C-206	C-209	C-229

NATA
ACCREDITATION

Accredited for compliance with ISO/IEC17025.
Accreditation number: 11624

Quality control replicates

		µg/g dry-weight				
Laboratory I.D. No.:	Client Identification:	Ag	Al	As	Cd	Co
CE475-2	BB1	7.68	2746	23	0.75	3.23
CE475-2 duplicate	BB1 duplicate	7.63	2775	23	0.74	3.26
CE475-2 average	BB1 average	7.65	2800	23	0.75	3.24
CE475-11 duplicate	T5-2 duplicate	8.15	3437	21	1.44	6.98
CE475-11 average	T5-2 average	8.15	3436.83	21	1.44	6.98
LOD (3σ)	---	0.008	30	0.01	0.01	0.004
Method code:	---	C-209	C-229	C-209	C-209	C-209

		µg/g dry-weight				
Laboratory I.D. No.:	Client Identification:	Cr	Cu	Fe	Hg	Mn
CE475-2	BB1	5.24	372	5506	0.81	20
CE475-2	BB1	5.25	371	5628	0.80	20
CE475-2 average	BB1 average	5.24	371	5600	0.80	20
CE475-11	T5-2	12.8	473	5903	1.46	83
CE475-11 duplicate	T5-2 duplicate	13.0	473	5885	1.36	82
CE475-11 average	T5-2 average	12.9	473	5894	1.41	82
LOD (3σ)	---	0.01	1	1	0.02	0.1
Method code:	---	C-209	C-229	C-225	C-209	C-229

		µg/g dry-weight				
Laboratory I.D. No.:	Client Identification:	Mo	Ni	Pb	Se	Zn
CE475-2	BB1	7.8	5.2	40	5.35	194
CE475-2	BB1	7.9	5.4	40	5.38	194
CE475-2 average	BB1 average	7.8	5.3	40	5.37	194
CE475-11	T5-2	13	13.7	53	6.34	391
CE475-11 duplicate	T5-2 duplicate	13	13.2	52	6.41	392
CE475-11 average	T5-2 average	13	13.4	53	6.37	391
LOD (3σ)	---	0.01	1	0.01	0.01	1
Method code:	---	C-209	C-229	C-209	C-209	C-229

(Dilution duplicates were performed as there was insufficient sample to allow digestion duplicates)

Accredited for compliance with ISO/IEC 17025.
Accreditation number: 11624

Quality control - certified reference material

		µg/g dry-weight				
Laboratory I.D	Sample Identification:	As	Cd	Cu	Fe	Pb
---	NIST2976 replicate-1	12.2	0.74	3.91	148	1.08
---	NIST2976 replicate-2	12.1	0.77	3.16	151	1.04
---	NIST2976 average n=2	12.1	0.76	3.53	149	1.05
---	Certified value	14.3±1.8	0.82±0.16	4.02±0.33	171±4.9	1.19±0.18
---	Recovery (%)	91	92	88	87	88

		µg/g dry-weight		
Laboratory I.D.	Sample Identification:	Se	Hg	Zn
---	NIST2976-1	1.7	0.039	115
---	NIST2976-2	1.5	0.066	116
---	NIST2976 average n=2	1.6	0.052	115
---	Certified value	1.80±0.15	0.061±0.0035	137±13
---	Recovery (%)	89	86	84

NIST2976 - Mussels (Mytilus galloprainciallis)
National Institute of Standards and Technology, Standard Reference Material

Accredited for compliance with ISO/IEC17025.
Accreditation number 11624

Quality control - spike recoveries

		% Recovery				
Laboratory I.D.	Client Identification:	Ag	Al	As	Cd	Co
CE475-1	Wafi Tailing Day 0 Background	93	102	97	98	98
CE475-10	T4-2	90	---	97	96	96
LOD [3σ]	---	0.009	30	0.01	0.01	0.004
Method code:	---	C-209	C-229	C-209	C-209	C-209

		% Recovery				
Laboratory I.D.	Client Identification:	Cr	Cu	Fe	Hg	Mn
CE475-1	Wafi Tailing Day 0 Background	97	96	95	101	95
CE475-2	BB1	95	---	---	100	90
LOD [3σ]	---	0.01	1	1	0.02	0.1
Method code:	---	C-209	C-229	C-229	C-209	C-229

		% Recovery				
Laboratory I.D.	Client Identification:	Mo	Ni	Pb	Se	Zn
CE475-1	Wafi Tailing Day 0 Background	99	96	96	98	92
CE475-2	BB1	95	97	95	97	109
LOD [3σ]	---	0.01	1	0.01	0.01	1
Method code:	---	C-209	C-229	C-209	C-209	C-229

Accredited for compliance with ISO/IEC17025.
Accreditation number: 11624

CONTACT US

t 1300 363 400
+61 3 9545 2176
e csiroenquiries@csiro.au
w www.csiro.au

AT CSIRO, WE DO THE EXTRAORDINARY EVERY DAY

We innovate for tomorrow and help improve today – for our customers, all Australians and the world.

Our innovations contribute billions of dollars to the Australian economy every year. As the largest patent holder in the nation, our vast wealth of intellectual property has led to more than 150 spin-off companies.

With more than 5,000 experts and a burning desire to get things done, we are Australia's catalyst for innovation.

CSIRO. WE IMAGINE. WE COLLABORATE. WE INNOVATE.

FOR FURTHER INFORMATION

Land and Water
w www.csiro.au/en/Research/LWF

Dr Stuart Simpson
t +61 2 9710 6807
e stuart.simpson@csiro.au