



Surface sediment quality – toxicity assessment

Assessment of surface water and sediment quality in the vicinity of power stations and coal ash repositories in Lake Macquarie, NSW

Department of Climate Change,
Energy, the Environment and Water

Acknowledgement of Country

Department of Climate Change, Energy, the Environment and Water acknowledges the Traditional Custodians of the lands where we work and live.

We pay our respects to Elders past, present and emerging.

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Executive summary

This report comprises a toxicity assessment of sediment collected from ten locations within the southern section of Lake Macquarie. This assessment was conducted to determine whether metals present at these locations are at levels likely to be toxic to biota. The method selected for the toxicity assessment was the 10-d whole sediment acute (mortality) and chronic sub-lethal (reproduction) toxicity test using the epibenthic amphipod *Josephosella plumulosa*, formerly *Melita plumulosa* (Zeidler, 1989).

In summary, this study found that:

- The concentrations of dilute acid-extractable metals within surface sediments (top 10 cm) of the ten test sediments were low, and all were below available screening criteria (ANZG, 2018; Buchman, 2008).
- No acute toxicity to *J. plumulosa* was observed in any of the ten test sediments.
- Low-level chronic toxicity to *J. plumulosa* (relative to the control sediment) was observed in two of the ten sediments tested. Both sites were located within the northwest section of the southern lake (Sites 6 and 9).
- No significant differences in the reproductive output of *J. plumulosa* were observed between any of the ten test sediments collected from Lake Macquarie.
- No toxic effects were observed on the test organisms from exposure to the sediments collected closest to the Eraring Power Station and the Vales Point Power Station discharge outflows (Sites 1 and 2).
- No relationships between chronic toxicity and dissolved metal concentrations (in the overlying water of the test chamber) or dilute acid-extractable metal concentrations (estimated bioavailable fraction) of the test sediments were observed.

Overall, the results show that the extent and magnitude of toxicity in surface sediments from the southern section of Lake Macquarie are small, and the metals present pose a low risk of adverse effects to benthic organisms. The assessment of sediment ecosystem health will be further informed by the consideration of additional lines of evidence, such as sediment chemistry (DCCEEW, 2024a; DCCEEW, 2024b) and benthic ecology (Dafforn et al., 2024). The various lines of evidence will then be evaluated within a weight-of-evidence framework to strengthen conclusions regarding whether sediment metals are affecting ecosystem health.

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Glossary of acronyms and common terms

Acronyms

| | |
|--------|---|
| ANOVA | Analysis of variance |
| ANZG | Australian and New Zealand Governments |
| DCCEEW | Department of Climate Change, Energy, the Environment and Water |
| DW | Dry weight |
| EPA | Environment Protection Authority |
| LOR | Limit of reporting |
| NATA | National Association of Testing Authorities |
| NSW | New South Wales |
| PCA | Principal components analysis |
| QA/QC | Quality assurance and quality control |
| SAQP | Sampling, Analysis and Quality Plan |
| SEI | Science Economics and Insights |
| SQGV | Sediment quality guideline value |
| TOC | Total organic carbon |

Units

| | |
|-------|---------------------|
| cm | Centimetre |
| °C | Degree Celsius |
| µm | Micrometre |
| h | Hour |
| mg/kg | Milligrams/kilogram |
| M | Molar |
| pH | Potential Hydrogen |

Definitions*

| | |
|---|---|
| Acute toxicity | An adverse lethal (mortality) or sublethal effect from a toxicant that occurs as a result of an exposure period that is short relative to the organism's lifespan |
| Amphipod | A crustacean of the class Malacostraca and order Amphipoda |
| Benthic organisms | Refers to biota living in or on the sediment at the bottom of a water body |
| Bioavailable/bioavailable fraction | Able to be taken up by an organism / a relative measure of the proportion of a chemical that an organism is exposed to through water, sediment, soil, suspended particles, organic carbon and/or food |
| Concentration | The quantifiable amount of a substance (e.g., in water or sediment) per unit volume or weight |
| Chronic toxicity | Adverse effects over a significant portion of the organism's life span |
| 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 sediment | A sediment possessing similar characteristics to those of the test sediment but without anthropogenic contaminants |
| Dilute acid-extractable metal concentration | Cold extraction using dilute acids (1M HCl for 1 h). Provides a useful estimate of the 'potentially bioavailable' metal concentration |
| Dissolved | Operationally defined as what will pass through a 0.45 µm filter |
| Ecosystem | A community of plants, animals, bacteria and the interrelated chemical and physical environment |
| Epibenthic | Refers to biota that live on or just above the sediment at the bottom of a water body |
| Gravid | Carrying eggs or young |
| Organism | Any living animal or plant; anything capable of carrying on life processes |
| Overlying water | The water above a sediment at a collection site or in a test chamber |
| Pore water | The water that occupies the space between and surrounds individual sediment particles in an aquatic sediment |
| Screening criteria | Measurable quantity (threshold) or condition of an indicator for a specific community value below or, for some stressors, above which we consider to be a low risk of unacceptable effects occurring |
| Sediment | Unconsolidated mineral and organic particulate material that is deposited at the bottom of a water body |
| Sub-lethal | A toxic or deleterious effect below the level of exposure that causes death |
| Total recoverable metal concentration | High temperature extraction (95°C for 2 h) using concentrated acids (HNO ₃ , HCl and H ₂ O ₂) |
| Toxicity | The inherent potential or capacity of a material to cause adverse effects in a living organism |
| Toxicity test | A test used to evaluate the relative potency of a chemical by measuring its effects on a living organism relative to a control or reference |

* Definitions adapted from Simpson and Batley (2016)

1. Introduction

1.1 Project overview

The Science and Insights (S&I) Division of the New South Wales (NSW) Department of Climate Change, Energy, the Environment and Water (DCCEEW) was engaged by the NSW Environment Protection Authority (EPA) to undertake an independent assessment of surface water and sediment quality within the southern section of Lake Macquarie, NSW, Australia. The main purpose of this investigation was to obtain information to facilitate the EPA's contribution to the NSW Government's response to the recommendations in the NSW Parliamentary Inquiry's report into the costs of remediation of coal ash repositories (Parliament of NSW, 2021). The information gathered herein provides a present-day assessment of the contamination status of Lake Macquarie, against which future changes, impacts, and/or mitigation and remediation activities can be assessed.

The scope of works outlined in the Sampling, Analysis, and Quality Plan (SAQP) (DPE, 2023) has been developed to: (i) determine present-day concentrations of metals and metalloids (hereafter referred to as "metals" for simplicity) and nutrients to assess surface water quality; (ii) delineate the extent of metal contamination in sediments; and (iii) assess the potential risks to ecological receptors in the vicinity of the power stations, coal ash repositories, and more broadly within the southern section of Lake Macquarie. The project was divided into a series of stages, as described in the SAQP (DPE, 2023).

The first stage involved a screening assessment of metals in surface sediments collected from 80 locations within Lake Macquarie against adopted screening criteria (ANZG, 2018; Buchman, 2008). The results of this assessment are captured in the companion report, *Surface sediment quality – chemical assessment* (hereafter referred to as the *surface sediment quality report*; DCCEEW, 2024a). Overall, the *surface sediment quality report* found that metals present in the surface sediment (top 5 cm) of the southern section of Lake Macquarie posed a low risk to ecosystem health. The assessment identified areas of the lake where multiple metals were present and would benefit from the investigation of additional lines of evidence to strengthen conclusions about the ecological condition. Additional lines of evidence, including sediment chemistry and a benthic ecological survey, were included in the scope of works, the results of which are captured in the companion reports: *Surface sediment quality – newly depositing sediment assessment* (DCCEEW, 2024b) and *Benthic community composition – Lake Macquarie* (Dafforn et al., 2024). These individual lines of evidence will be considered together within a weight-of-evidence framework to strengthen conclusions about the ecological condition of the southern section of Lake Macquarie.

The current report has focused on providing an ecotoxicological assessment to examine whether contaminants associated with the sediments within key areas of interest within the southern section of Lake Macquarie have the potential to cause toxic effects to biota.

1.2 Aims and objectives

The primary aim of the surface sediment quality investigation was to determine the current concentration of metals in surface sediments within southern Lake Macquarie to assess the distribution of historical inputs and the risk they pose to ecological receptors.

The specific objective to be addressed by this toxicity assessment was to:

- Assess the toxic potential of sediments collected from the southern section of Lake Macquarie to the epibenthic amphipod *Josephosella plumulosa*.

2. Methods

2.1 Site selection

All sites in this study were estuarine sites located within the southern section of Lake Macquarie (Figure 1). For a detailed description of the project location, see Section 1.3 of the *surface sediment quality report*.

A total of 10 sites (Figure 1) were sampled in quadruplicate and processed individually. Site selection was guided by the results presented in the *surface sediment quality report*, and the inclusion of key areas of interest with respect to proximity to the power stations and coal ash repositories. Sites 1 and 2 were selected for their elevated total recoverable copper and selenium concentrations; Site 3 for its elevated total recoverable mercury, copper, lead, and zinc concentrations; and Sites 4 and 5 for their elevated total recoverable copper, lead, and zinc concentrations. Sites 6 to 10 were selected as paired comparison sites to Sites 1 to 5, respectively, as they were located close to Sites 1 to 5 but had lower total recoverable metal concentrations of the main metals of interest for the lake than those measured in Sites 1 to 5. To minimise confounding effects from differences in the physical properties of the sediment, site selection was restrained to locations within the lake with similar total organic carbon (TOC) content (1.5 - 3.0%) and grain size (<0.63 µm fraction > 80%) based on the results reported in the *surface sediment quality report*. This range of TOC content and grain size corresponded with where elevated metal concentrations were occurring within the lake sediments.

2.2 Sediment collection

2.2.1 Test sediment

The sediment samples were collected over two consecutive days on the 31st of January and the 1st of February 2023. All sediments were sampled using a grab sampler (box corer, WILDSCO) operated by a winch from a boat. Before sub-sampling, the overlying water was removed by slow siphoning using a clean tube near one corner of the grab sampler. For the toxicity assessment, the top 10 cm of the sediment grab was scooped out with a clean plastic container and placed into a clean tray. The sediment was homogenised and split into four identical aliquots for analysis (Table 1). The top 10 cm of the sediment was selected for this assessment as this is generally recognised as the depth to which most infauna organisms will occupy (Simpson and Batley, 2016). Four separate sediment samples (replicates) were collected at each site. During field collection, the samples were stored cold (on ice) in an insulated chest.

Profiles of the physicochemical properties of the overlying water column were measured in situ at each site approximately 20 cm above the sediment surface (Simpson and Batley, 2016) using a YSI EXO2 multiparameter water quality sonde. The measured values are provided in Appendix A – Water quality data (field measurement).

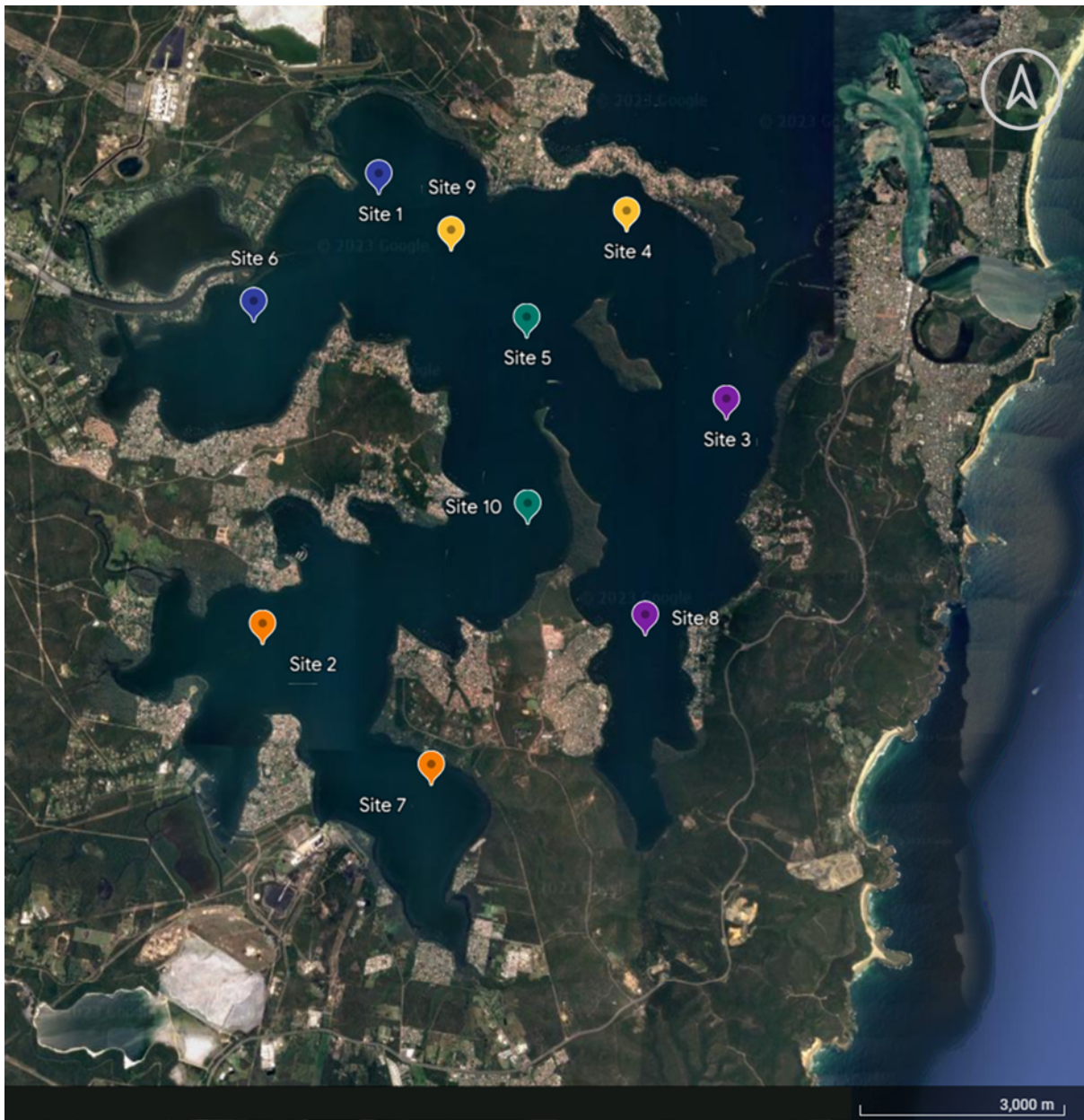


Figure 1. Surface sediment sampling locations (n=10) in Lake Macquarie. Sites 1 to 5 were selected for their elevated total recoverable metal concentrations and Sites 6 to 10 were selected as paired comparison sites (colour indicates site pairings).

Table 1. Sediment sample containers, analyses, and preservation

| Container | Analyses | Preservation |
|---------------------------------------|--|---|
| Plastic container, 250 mL acid-washed | Toxicity testing Particle size determination | Sealed, refrigerated, and stored in darkness |
| Glass jar, 100 mL | Total organic carbon Dilute acid-extractable metals (1 M HCl) | Sealed, refrigerated, and stored in darkness |
| Vial, 50 mL acid-washed | Pore water metals* | Sealed, refrigerated, and stored in darkness. Centrifuged within 36-h of sample collection. |
| Vial, 50 mL | Spare | Frozen upon return to land |

* Pore water chemistry can change rapidly after collection/disturbance (e.g., within 24 hours). Therefore, the concentrations of dissolved metals within pore waters are denoted as 'estimated'.

2.2.2 Control sediment

Assessment of test sediment toxicity was aided by the collection and concurrent testing of control sediments. A control sediment is described as a sediment possessing similar physical characteristics to those of the test sediment but without anthropogenic contaminants. Physical properties of the control sediment, including particle size distribution and percent organic carbon, should bracket those of the test sediment.

The control sediments selected for the current study were collected from Bonnet Bay (34°00'25"S 151°03'24"E) and Grays Point (34°03'51"S 151°05'12"E) near Sydney on the 21st of September 2022 and have a long history of being used for both test-organism culturing and toxicity testing control sediments in our laboratory. Surficial sediment was collected to a depth of 10 cm and gently sieved on site (<2 mm) to remove coarse material (unwanted debris and biota). The control sediments were stored in polyethylene bags and in the dark at 4°C until use.

2.3 Toxicity testing

2.3.1 Rational for toxicity test selection

For this toxicity assessment, the 10-d whole sediment acute (mortality) and chronic sub-lethal (reproduction) toxicity test using the epibenthic amphipod *J. plumulosa*, formerly *Melita plumulosa* (Zeidler, 1989), was used. While toxicity assessments usually utilise multiple test species and/or endpoints, this method was selected as the sole toxicity test for the following reasons:

- *J. plumulosa* is endemic to estuarine and marine sediments of the southeast coast of Australia (Hyne et al., 2005; King et al., 2006).
- *J. plumulosa* is epibenthic and lives in close association with the sediment, at or below the sediment-water interface, which ensures continuous exposure to contaminants released from the sediment into pore water through gills and body surface absorption.
- *J. plumulosa* directly ingest sediment particles and, therefore, have a dietary exposure pathway to sediment-bound contaminants.
- They have broad tolerance to a wide range of salinity, sediment particle sizes and organic matter content, facilitating testing across a range of sediment types.

- They are relatively simple to collect from the field or maintain under laboratory conditions and are amenable to handling.
- *J. plumulosa* has previously been demonstrated to be a sensitive test species for investigating metal-contaminated sediments (King et al., 2006; Mann et al., 2009; Simpson and Spadaro, 2011; Strom et al., 2011).
- Amphipods form an important link in food chains, they are a principal prey item for many birds, fish, and larger invertebrates and hence have the potential for trophic transfer of contaminants from sediments to higher trophic levels.
- The use of a sublethal endpoint provides a chronic measure of toxicity and greater information on potential long-term effects at the individual and population levels (Podlesińska and Dąbrowska, 2019), and the availability of whole-sediment toxicity tests utilising sublethal endpoints is currently limited in Australia (Simpson and Batley, 2016).

2.3.2 Toxicity test method (brief)

For a detailed method description, see Appendix B – Toxicity test method (full). The key details are summarised below and in Table 2. Toxicity tests were conducted on the whole sediment (the sediment and associated pore water). The toxicity testing was split up into two test batches (Test 1: Sites 1, 4, 5, 6, and 9; Test 2: Sites 2, 3, 7, 8, and 10). For the Lake Macquarie test sediments, each site/treatment was tested in quadruplicate, from the four individual grab samples collected at each site. Two control sediments were tested concurrently with each test batch. For the control sediments, each control/treatment was tested in quadruplicate from a single bulk collection of sediment from Bonnet Bay (referred to as Control-BB) and Grays Point (referred to as Control-GP).

The test sediments and control sediments were prepared in duplicate (one beaker for use at test initiation on Day 0, and one beaker for use at the sediment renewal step on Day 5) eight to nine days before test initiation to allow suspended particles to settle out and for the sediment and pore water to equilibrate. The overlying water was periodically replaced (at test initiation and on Days 3, 5, and 7). The physicochemical properties (pH, salinity, specific conductance, dissolved oxygen, and ammonia) of the overlying water were monitored throughout the test (on a composite sample of the four replicates of each treatment) to ensure no adverse conditions were contributing to the test. Samples of the overlying water were collected for dissolved metal analysis on Day 0 (as a composite of the four individual replicates) and Day 3 of the test (in each of the four individual replicates).

The sediments were renewed on Day 5 of the test, by gently sieving the adults through a 500 µm mesh and placing them into fresh sediment. An approximation of the sediment pore water pH was determined on Days 5 and 10. On Day 10, at the termination of the test, the adults and juveniles were recovered from the sediment by wet sieving the contents of each beaker through a 200 µm mesh. The test endpoint, reproduction, was represented as the sum of the number of embryos and juveniles at the end of the test, divided by the original number of females (i.e., six).

A summary of the water quality measurements collected during testing are provided in Appendix C – Water quality data (during toxicity testing).

2.3.3 Data and statistical analyses

Statistical analyses were performed using Minitab® 21 software. The response variable used was the number of offspring produced per female at the end of the test. Data were first tested for normality using the RyanJoiner test. Data were then tested for homogeneity of variance using the F test or Bartlett's test for normally distributed data, or the Levene's test for data not normally distributed.

Data that were normally distributed with equal variances were subsequently compared with control data using a two-sample t-test, while data that did not meet these criteria were compared with control data using the non-parametric Mann-Whitney U test. Analysis of variance (ANOVA; parametric data) was used to compare the means of the ten test sediments from Lake Macquarie to determine if there was a significant difference in test organism response between different locations within the lake. Significance was assessed at the $P < 0.05$ level.

Values below the limit of reporting (LOR) were substituted as half of the LOR for calculating descriptive statistics and performing statistical analyses.

2.3.4 Categorising the level of toxicity

For amphipod acute tests, the test sediment may be classified as toxic when adult mortality is more than 20% higher than the mortality observed in the control sediment, and the difference is statistically significant (Environment Canada, 1998). For amphipod chronic sub-lethal tests, the test sediment may be classified as toxic when the mean reproductive output of the organism was less than the mean reproductive output observed in the control by 15%, and the difference is statistically significant (Simpson and Spadaro, 2011). Sites can be divided into three categories according to their degree of toxicity experienced (Table 3).

Table 2. Summary of test conditions for the 10-d whole sediment chronic sub-lethal (reproduction) and acute (mortality) toxicity test using the epibenthic amphipod *Josephosella plumulosa*. Adapted from Table E.1. of Simpson and Batley (2016).

| Test parameter | Criteria |
|-------------------------------------|--|
| Test type | Ten-day static test with sediment renewal (Day 5) and overlying water renewal (Days 0, 3, 5, and 7) |
| Temperature | 21 ± 1°C |
| pH | 7.2 - 8.2 |
| Ammonia | ≤1 mg/L |
| Test chamber | 250 mL glass beaker |
| Sediment weight | 40 g |
| Overlying water volume | 200 mL |
| No. replicate chambers | 4 |
| No. test organisms per test chamber | 12 (6 males and 6 gravid females at test initiation) |
| Photoperiod | 12-h light:12-h dark |
| Aeration | Slow bubbling to maintain >85% dissolved oxygen saturation |
| Feeding | 0.5 mg of Sera Micron fish food per amphipod (Days 0, 3, 5, and 7) |
| Control sediment | Bonnet Bay (silty) and Grays Point (silty-sand) sediment |
| Overlying water | Uncontaminated seawater was collected from a clean site, filtered to 0.45 µm and diluted to the required salinity with deionised water |
| Standard endpoint | Adult survival and reproduction at test end |
| Test acceptability criteria | ≥80% adult survival and 8-19 juveniles per gravid female in the control. Physicochemical properties of the overlying water within acceptable limits throughout the test. |

Table 3. Toxicity categories for amphipod test

| Toxicity category | Criteria for acute test | Criteria for chronic sub-lethal test |
|--------------------------------------|--|--|
| No acute or chronic toxicity | No difference* in adult mortality was observed between organisms exposed to the test sediment and the control sediment | No difference* in reproductive output was observed between organisms exposed to the test sediment and the control sediment |
| Low-level acute or chronic toxicity | A difference* in adult mortality was observed between test organisms and control organisms between 20 – 50% | A difference* in reproductive output was observed between test organisms and control organisms between 15 – 50% |
| High-level acute or chronic toxicity | A difference* in adult mortality was observed between test organisms and control organisms $\geq 50\%$ | A difference* in reproductive output was observed between test organisms and control organisms $\geq 50\%$ |

* Difference relates to a response greater than 20% (acute) or 15% compared to the control, which is also statistically significant ($P < 0.05$)

2.4 Chemical analyses

The dilute acid-extractable metal concentration was determined by weighing and extracting 5 g of wet sediment with 1 M hydrochloric acid (HCl) for 1 h in a closed digestion tube at room temperature. The extract was analysed by inductively coupled plasma optical emission spectrometry for all metals except mercury. Mercury was analysed by cold vapour atomic absorption spectrometry. Moisture content was determined by heating at $105 \pm 5^\circ\text{C}$ for a minimum of 12-h. The results were moisture-corrected and expressed in milligrams per kilogram and on a dry weight basis (mg/kg DW).

The particle size distribution of the sediment was determined using laser diffraction techniques (Horiba LA 960 particle size distribution analyser). The sediment was added to the circulating high-purity water, ultrasonicated, and the suspended particles passed through a quartz cell. Light from the red and blue lasers refracts different-sized particles at different angles, and the amount of light refracted at each angle is detected by the instrument and used in a mathematical model to determine the particle size present. Particle size was grouped into the three categories of $< 2 \mu\text{m}$ diameter (clay fraction), 2 to $63 \mu\text{m}$ diameter (fine silt fraction), and $63 \mu\text{m}$ to 2 mm diameter (sand fraction). It is noted that fine sediments (e.g., the clay and silt fraction operationally defined as $< 63 \mu\text{m}$) are typically those that are most heavily contaminated due to their high surface area and because their surface chemistry makes them more likely to adsorb organic compounds and metals. This fraction is also considered a suitable representation of the sediment materials that are most readily resuspended and/or potentially ingested by organisms. Therefore, a fourth category, described as the proportion of fine-grained sediments ($< 63 \mu\text{m}$ diameter) was created for data analysis purposes.

TOC was determined on dried and finely ground sediment samples by high-temperature catalytic combustion with a nondispersive infrared sensor.

Pore water was collected by centrifuging an aliquot of the sediment within 36-h of field collection and passing the supernatant through a $0.45 \mu\text{m}$ polyethersulfone filter. The samples were acidified with concentrated nitric acid (HNO_3) to $\text{pH} < 2$, diluted up to 10 times based on the dissolved solids content, estimated from the conductivity, and then analysed by inductively coupled plasma optical emission spectrometry or inductively coupled plasma mass spectrometry. Pore water chemistry can change rapidly after collection/disturbance (e.g., within 24-h); therefore, concentrations of dissolved metals within pore waters are denoted as 'estimated'.

All analyses were performed by National Association of Testing Authorities (NATA)-accredited laboratories using established standard operating procedures. NATA-accredited analyses for compliance with ISO/IEC 17025 – Testing (i.e., results issued in accordance with NATA’s quality assurance and quality control (QA/QC) requirements) included those for TOC and excluded those for particle size and 1M HCl extractable metals. Laboratory QA/QC procedures may include reagent or preparation blanks, spike blanks, certified reference materials and matrix spike duplicates and were compared to the laboratory’s established criteria for reporting.

3. Results and discussion

3.1 Sediment chemistry

The results of the sediment chemical analyses are provided in Appendix D – Sediment chemistry data. While Sites 1 to 5 were selected based on having greater concentrations of key metals (based on total recoverable acid digestion techniques), reported in the *surface sediment quality report*, the concentrations of dilute acid-extractable metal concentrations were low and generally very similar between the paired treatments (often having overlapping confidence intervals) (Figures Figure A1 to Figure A8). All sediments had dilute acid-extractable metal concentrations below their respective screening criteria (ANZG, 2018; Buchman, 2008) (Table A6).

Principal components analysis (PCA) was used to explore the differences in abiotic factors among the ten sediment treatments (Figure 2). The first PCA axis explained 46.2% of the variation across sites, and the main variables the separation was related to were increasing chromium, zinc, nickel, lead, and copper. The second PCA axis explained 18.9% of the variation across sites, and the main variables the separation was related to were increasing arsenic, decreasing particle size, increasing selenium and cadmium, and decreasing TOC. Site 6 was clustering at the top left side of the graph with greater selenium and low proportion of particles <63 µm and TOC. Sites 3, 8, and 5 were clustering on the bottom right with greater lead and zinc. Site 7 was clustering middle right with greater cadmium. Sites 9 and 4 were dispersed, suggesting greater variability of the abiotic factors between the respective replicates.

Pore water results indicated that trace metals, including arsenic, cobalt, iron, manganese, molybdenum, vanadium (all sites), and lead (Site 9), could be released from the sediments upon disturbance (Table A7). Dissolved cadmium, chromium, copper, nickel, selenium, and zinc were not detected above the limit of reporting in pore waters. In the absence of guidelines for pore water, the estimated dissolved metal concentrations of the pore waters were compared to the screening criteria adopted for waters in the current study. Dissolved arsenic (min – max: 0.005 – 0.012 mg/L) and manganese (1.1 – 4.7 mg/L) exceeded their respective screening criteria at all sites and molybdenum (0.014 – 0.024 mg/L) exceeded at Site 7, but concentrations were relatively similar across all sites.

3.2 Toxicity test data

Test acceptability criteria were met for both test batches ($\geq 80\%$ adult survival and ≥ 8 juveniles per gravid female in the control) (Table 4). Physicochemical properties of the overlying water, including pH, salinity, specific conductance, and dissolved oxygen, remained within acceptable ranges (Table A2). Temperature was slightly elevated, fluctuating between 22.5 and 24.5°C throughout the exposure period. However, this range is likely to be slightly elevated compared to that of the test beakers as the temperature logger for the temperature control room was positioned directly under the lights, whereas the test beakers were not positioned under direct light. Ammonia concentrations in the overlying water were ≤ 1 mg/L, which is well below the concentration that may affect the reproduction of the amphipod *J. plumulosa* (Simpson and Spadaro, 2011).

No acute toxicity was observed in any of the ten test sediments (that is, there was $>80\%$ survival of adult organisms across all test sediments) (Table 4).

Chronic toxicity (sub-lethal effects on amphipod reproduction) was observed in test organisms exposed to two out of the ten sediments tested (Sites 6 and 9) when compared to the sediment controls (Table 4, Figure 3). Toxicity in both treatments was categorised as low-level chronic toxicity

based on the criteria in Table 3. However, when comparing reproductive output for Lake Macquarie sites alone, there was no significant difference between sites. The outcomes of the statistical analyses performed are captured in Appendix E – Hypothesis testing.

3.3 Comparison across sites

The sediments identified as having low-level chronic toxicity (Sites 6 and 9) compared to the sediment controls, were both located in the northwest arm of the southern section of the lake and Site 6 was located in close proximity to the entrance to Dora Creek. Interestingly, Sites 6 and 9 were selected as paired reference locations for Sites 1 and 4, respectively. Sites 1 and 4, had higher sediment total recoverable metal concentrations than Sites 6 and 9 identified in the *surface sediment quality report*. Site 1 was located closest to the Eraring Power Station cooling water outlet, and no toxic effects were observed on the test organisms. The next closest samples to Sites 6 and 9 were Sites 4 and 5. Sites 4 and 5 had reduced reproductive output compared to the sediment control, but the response was not statistically significant. Moving further away from Eraring Power Station in a southeast direction, no toxic effects were evident in sediments (Sites 2, 3, 7, 8, and 10). Site 2 is located closest to Vales Point Power Station discharge outflows.

For Sites 6 and 9, detectable concentrations of dissolved arsenic, cadmium, cobalt, copper, manganese, and vanadium were measured in the overlying water, possibly due to their flux from the sediment to pore waters and diffusion to the overlying water during the toxicity tests (concentrations greater than observed in the seawater blank) (Table A3). Site 9 had dissolved arsenic of the overlying water exceed the default guideline value for marine waters of 0.0023 mg/L for As(III) but was below the default guideline value (DGV) of 0.0045 µg/L for As(V) (ANZG, 2018). Dissolved copper in the overlying water exceeded the default guideline value for marine waters of 0.0013 mg/L (ANZG, 2018) at all sites. Similar concentrations of arsenic and copper were observed in the overlying water for all sediment treatments. As such, dissolved arsenic and dissolved copper were not considered to have contributed to the observed toxicity for amphipods in any of the test sediments. No relationship between reproductive output and the dissolved metal concentrations of the overlying water was observed (Figure 4).

The dilute acid-extractable metal concentrations of all test sediments were below their respective screening criteria concentrations (Table A6). No relationship between reproductive output and the dilute acid-extractable metal concentrations of the sediment was observed (Figure 5). There was also no relationship between reproductive output and major physicochemical properties of the sediment (Figure 6).

In summary, the current study targeted five locations with the highest total recoverable metal concentrations as identified in the *surface sediment quality report* and paired them with five site-specific reference sites located nearby with similar sediment physicochemical properties but lower total recoverable metal concentrations. No acute or chronic toxicity was observed to *J. plumulosa* for the five targeted locations (Sites 1 to 5), however, two of the paired reference locations (Sites 6 and 9) exhibited significantly lower reproductive output compared to the sediment controls. Despite this, there was no significant difference in the reproductive output of *J. plumulosa* among the ten Lake Macquarie sites. The marginally lower reproductive output of the test organisms exposed to sediments from Sites 6 and 9 may be due to differences in the biotic or abiotic (physical or chemical) properties between the Lake Macquarie test sediments and the control sediments.

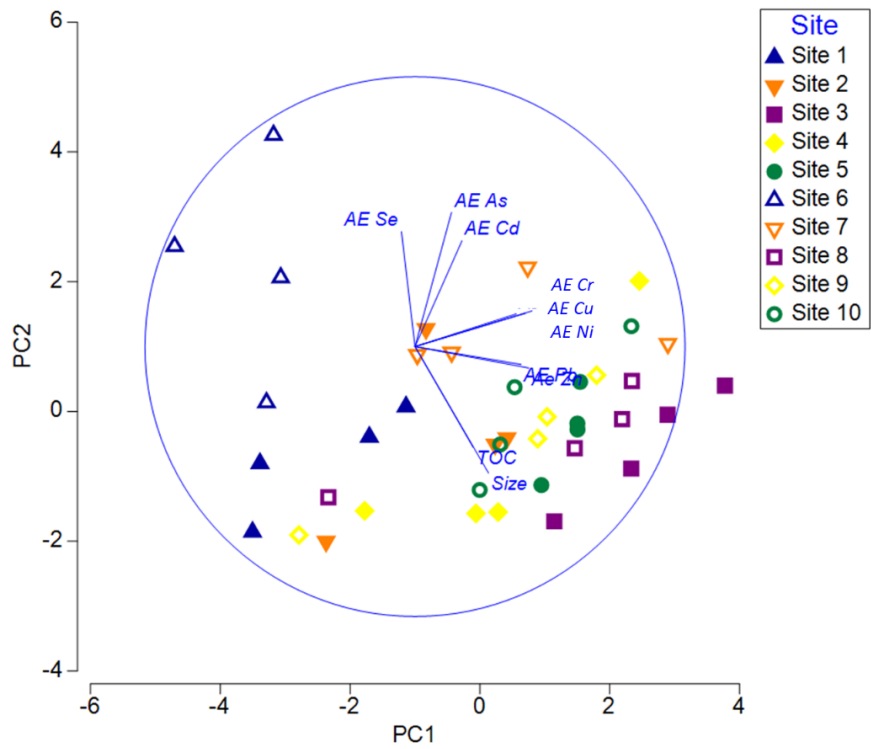


Figure 2. Principal components analysis of ten surface sediments collected from Lake Macquarie. Sites 1 to 5 were selected for their elevated concentrations of total recoverable metals, and sites 6 to 10 were selected as paired comparison sites (colour indicates site pairings).

Table 4. Survival and reproductive output of *Josephosella plumulosa* in the control and test sediments following a 10-day exposure

| Treatment ^a | Acute test | | Chronic sublethal test | | |
|------------------------|-----------------------------------|-------------------|---|--|----------------------------|
| | Control survival (%) ^b | Outcome | Reproductive output (no. offspring per adult female) ^b | Reproductive output (% control) ^c | Outcome |
| Control-BB | 98 ± 5 | Not applicable | 10 ± 1 ^d | 100 ± 8 ^d | Not applicable |
| Control-GP | 94 ± 4 | | | | |
| Control-BB | 98 ± 2 | Not applicable | 10 ± 1 ^d | 100 ± 14 ^d | Not applicable |
| Control-GP | 98 ± 2 | | | | |
| Site 1 | 94 ± 2 | No acute toxicity | 10 ± 1 | 102 ± 10 | No chronic toxicity |
| Site 2 | 94 ± 4 | No acute toxicity | 9 ± 1 | 91 ± 10 | No chronic toxicity |
| Site 3 | 90 ± 2 | No acute toxicity | 8 ± 1 | 86 ± 8 | No chronic toxicity |
| Site 4 | 88 ± 7 | No acute toxicity | 8 ± 2 | 82 ± 15 | No chronic toxicity |
| Site 5 | 86 ± 10 ^d | No acute toxicity | 8 ± 3 ^e | 78 ± 25 ^e | No chronic toxicity |
| Site 6 | 100 ± 0 ^d | No acute toxicity | 5 ± 2 ^e | 52 ± 18 ^e * | Low-level chronic toxicity |
| Site 7 | 92 ± 3 | No acute toxicity | 9 ± 1 | 87 ± 14 | No chronic toxicity |
| Site 8 | 94 ± 3 ^d | No acute toxicity | 8 ± 1 ^e | 84 ± 10 ^e | No chronic toxicity |
| Site 9 | 92 ± 6 | No acute toxicity | 6 ± 1 | 58 ± 10 * | Low-level chronic toxicity |
| Site 10 | 97 ± 3 ^d | No acute toxicity | 9 ± 1 ^e | 94 ± 11 ^e | No chronic toxicity |

* The asterisk indicates the reproductive output in the test sediment was significantly lower compared to the control sediment (two-sample t-test, p<0.05)

^a Treatments (control or test sediments) tested as part of Test one are in black while those tested as part of Test two are in blue

^b Mean ± standard error (n=4) unless otherwise stated

^c The reproductive endpoint was represented as the sum of the number of embryos and juveniles at the test end, divided by the original number of females (i.e. six) and expressed as a percentage of the control treatments (for each test batch the Control-Bonnet Bay (BB) and Control-Grays Point (GP) treatments were combined).

^d Mean ± standard error (n=8, replicates across the two control sediments were pooled)

^e Replicate excluded (n=3 instead of 4)

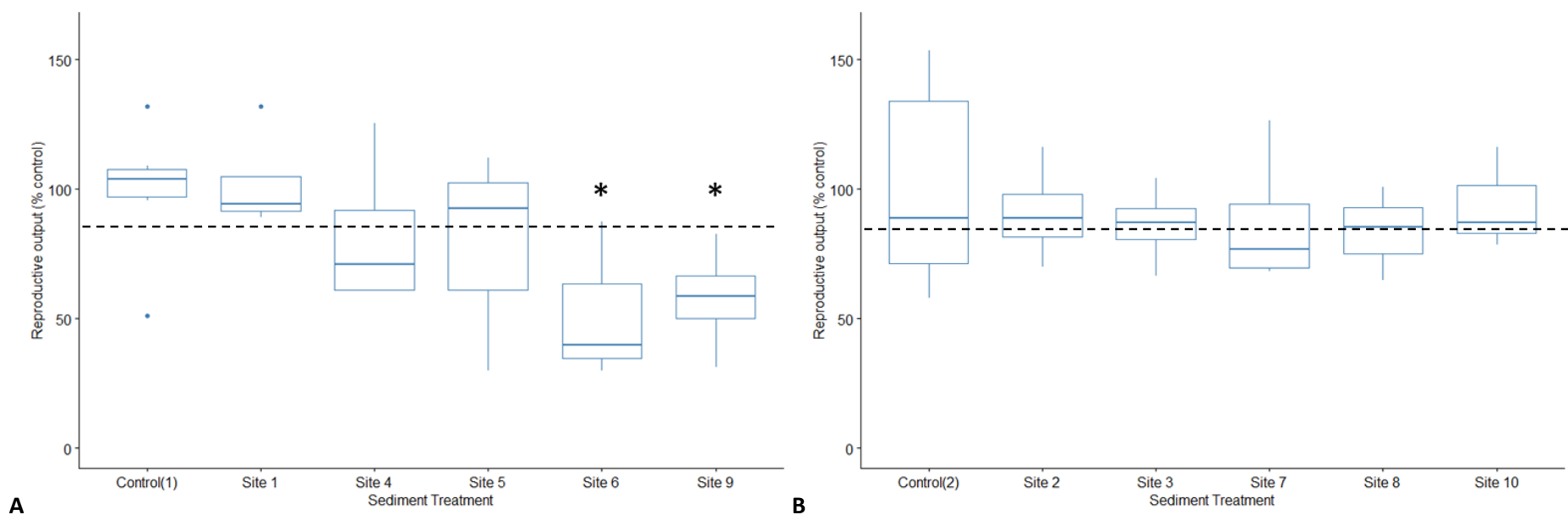


Figure 3. Comparison of the reproductive output of *Josephosella plumulosa* exposed to control and test sediments for 10 d: A) Test 1; and B) Test 2. Test sediments with a reproductive output significantly different to the sediment control are indicated with an asterisk (*). The horizontal black dashed line indicates the no-effect threshold (85% of the control response).

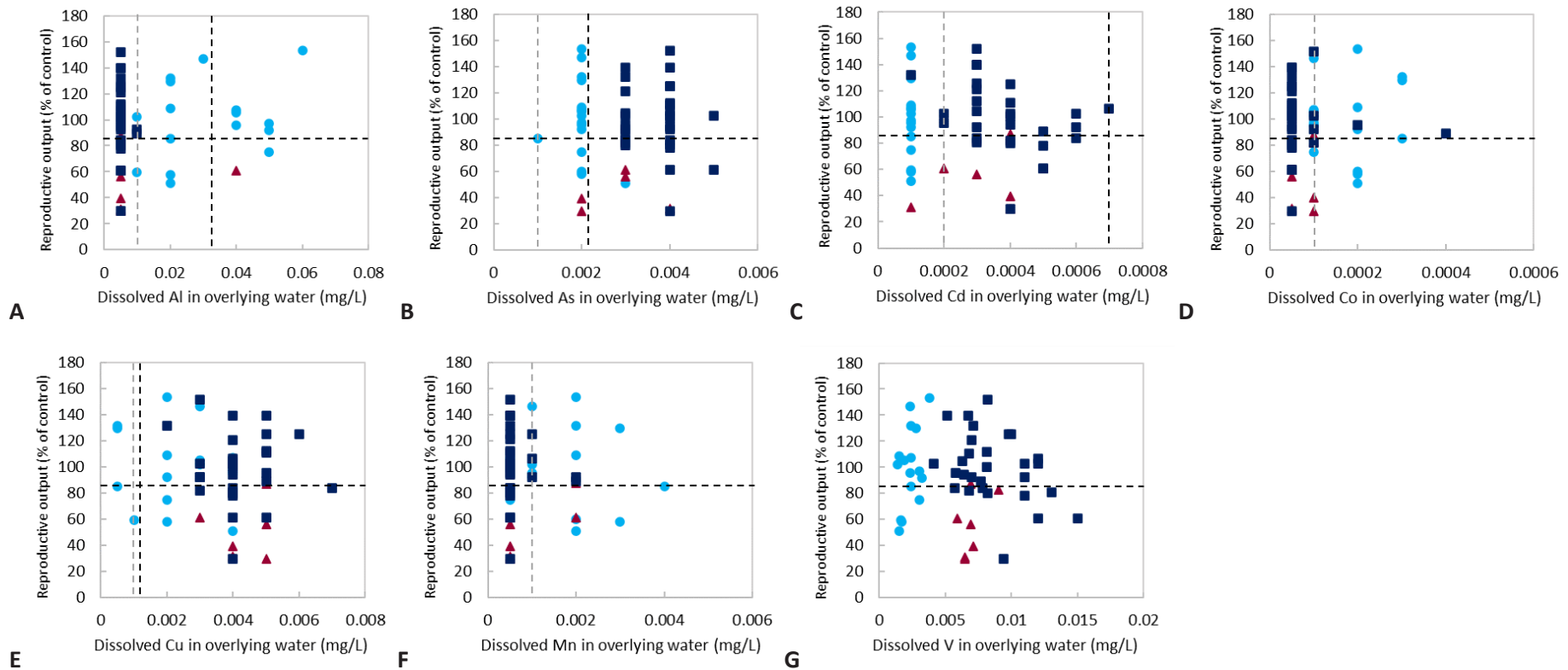


Figure 4. Scatterplots of reproductive output (% of control) and dissolved (<0.45 μm) metal concentrations of the overlying water (mg/L): A) aluminium; B) arsenic; C) cadmium; D) cobalt; E) copper; F) manganese; and G) vanadium. Treatments are indicated by colour and shape: control sediments (*light blue circles*), test sediments that met the criteria for low-level toxicity (Sites 6 and 9) (*red triangles*), and all other test sediments (*dark blue squares*). The horizontal black dashed line indicates the no-effect threshold (85% of the control response). The vertical grey dashed line indicates the limit of reporting (LOR). Values less than the LOR were substituted as half the LOR. All sediments had V concentrations much greater than the LOR. The vertical black dashed line indicates the screening concentration (ANZG, 2018). The screening criteria for Co, Mn and V were greater than the maximum concentration measured in the test sediments.

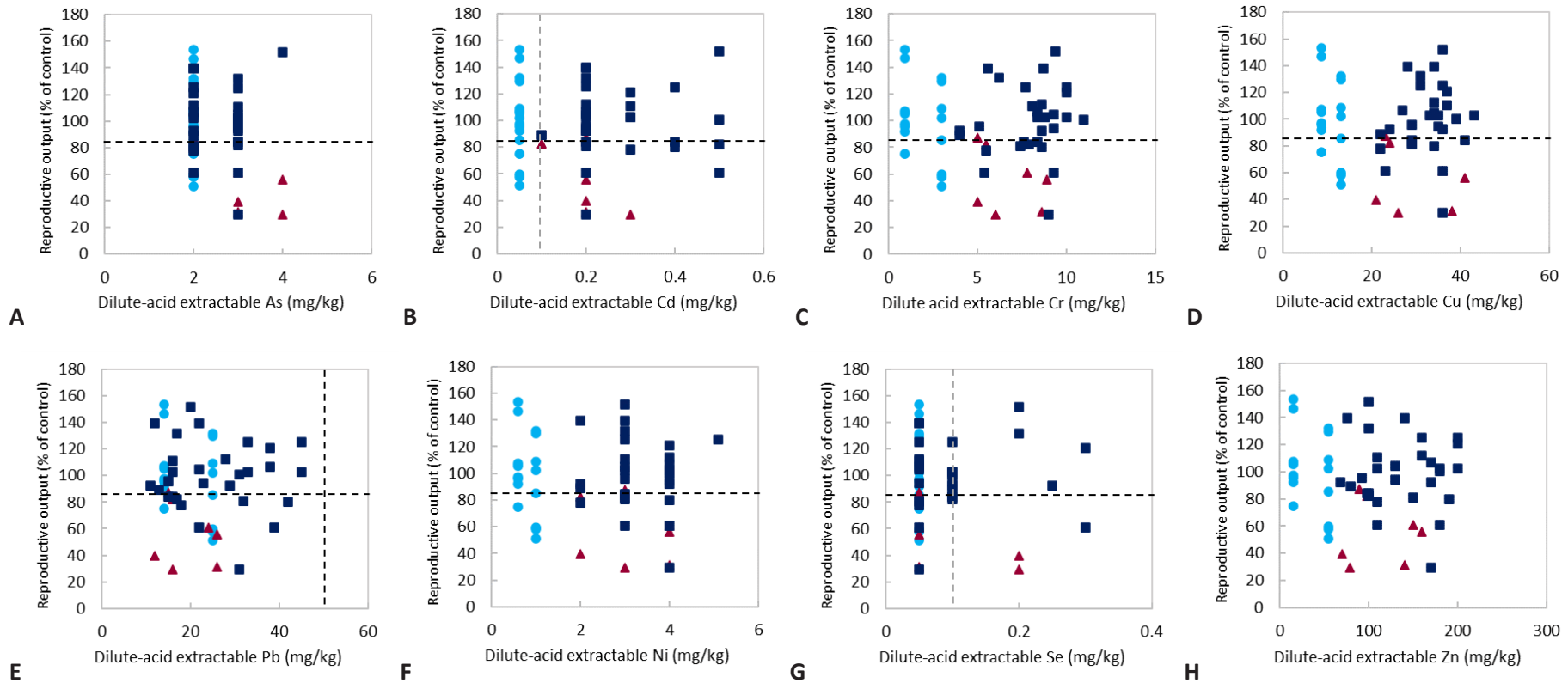


Figure 5. Scatterplots of reproductive output (% of control) and dilute acid-extractable (1M HCl) metal concentrations of the sediment (mg/kg): A) arsenic; B) cadmium; C) chromium; D) copper; E) lead; F) nickel; G) selenium; and H) zinc. Treatments are indicated by colour and shape: control sediments (*light blue circles*), test sediments that met the criteria for low-level toxicity (Sites 6 and 9) (*red triangles*), and all other test sediments (*dark blue squares*). The horizontal black dashed line indicates the no-effect threshold (85% of the control response). The vertical grey dashed line indicates the limit of reporting (LOR). Values less than the LOR were substituted as half the LOR. All sediments had As, Cr, Cu, Pb, Ni, and Zn concentrations much greater than the LOR. The vertical black dashed line indicates the screening concentration (ANZG, 2018). The screening criteria for As, Cd, Cr, Cu, Ni, and Se were greater than the maximum concentration measured in the test sediments.

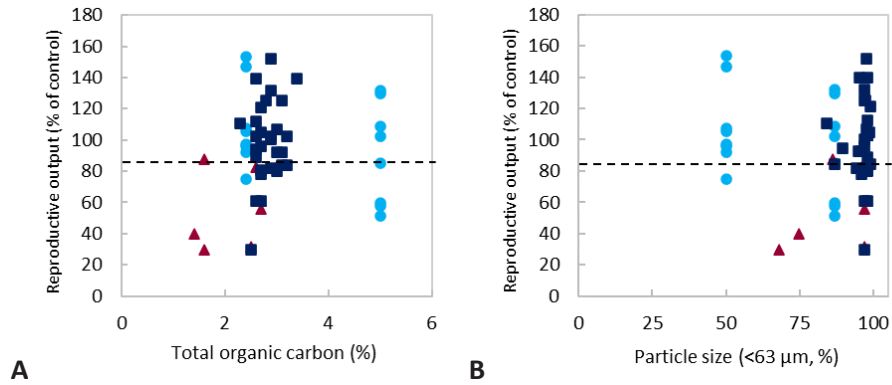


Figure 6. Scatterplots of reproductive output (% of control) and physicochemical properties of the sediment: A) total organic carbon (%); and B) particle size (<63 μm, %). Treatments are indicated by colour and shape: control sediments (*light blue circles*), test sediments with low-level toxicity (i.e., Sites 6 and 9) (*red triangles*), and all other test sediments (*dark blue squares*). The horizontal black dashed line indicates the no-effect threshold (85% of the control response).

4. Conclusions

The current study assessed the toxicity of ten sediments collected from the southern section of Lake Macquarie to a representative sediment-dwelling organism, the epibenthic amphipod *J. plumulosa*. No acute toxicity to *J. plumulosa* was observed in any of the ten sediments tested. Low-level chronic toxicity to *J. plumulosa* was observed in two of the ten sediments tested when reproductive output was compared to the sediment controls, but these sites were not significantly different to the other test sediments collected within Lake Macquarie. Sites 6 and 9 were both located within the northwest of the southern section of Lake Macquarie; Site 6 was located within Bonnells Bay and in close proximity to Dora Creek, and Site 9 was located north of Pulbah Island and west of Wangi Wangi Point. No toxic effects were observed on the test organisms located closest to the Eraring Power Station and the Vales Point Power Station discharge outflows (Sites 1 and 2).

The dilute acid-extractable metal concentrations in all ten sediments were low, and all were below the screening criteria (ANZG, 2018; Buchman, 2008). No relationship between chronic toxicity and dissolved metals (in the overlying water of the test chamber) or the dilute acid-extractable metal concentrations of the sediment was observed. Overall, the results indicate that the potential for toxicity within surface sediments of the southern section of Lake Macquarie to resident biota is minimal and the trace metals present a low risk of causing adverse effects to benthic organisms. This study is one component of an investigation into sediment quality in the southern section of Lake Macquarie, which includes lines of evidence based on chemistry (DCCEEW 2024a; DCCEEW 2024b) toxicity (the current study) and benthic ecology (Dafforn et al., 2024). This toxicity line of evidence will be further evaluated within a weight-of-evidence framework along with these other lines of evidence to strengthen conclusions regarding whether metals in surface sediments are affecting ecosystem health within the southern section of Lake Macquarie.

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6. Appendices

Appendix A – Water quality data (field measurement)

Table A1. Physicochemical properties of the overlying water within Lake Macquarie. Measurements were collected in situ approximately 20 cm above the sediment surface before sediment collection.

| Site | Latitude (°N) | Longitude (°E) | Depth to sediment (m) | Temperature (°C) | pH | Turbidity (NTU) | Salinity (PSU) | Specific conductance (mS/cm) | Dissolved oxygen | |
|---------|---------------|----------------|-----------------------|------------------|------|-----------------|----------------|------------------------------|------------------|--------|
| | | | | | | | | | (%) | (mg/L) |
| Site 1 | -33.07480 | 151.55444 | 5.4 | 26.5 | 8.55 | 3.81 | 35.0 | 53.1 | 91.8 | 6.06 |
| Site 2 | -33.13450 | 151.53601 | 5.2 | 26.1 | 8.18 | 4.33 | 35.2 | 53.3 | 86.3 | 5.73 |
| Site 3 | -33.10470 | 151.60951 | 10.2 | 25.4 | 8.62 | 1.51 | 35.1 | 53.2 | 79.9 | 5.37 |
| Site 4 | -33.07976 | 151.59370 | 9.2 | 25.2 | 8.51 | 5.62 | 35.1 | 53.2 | 89.7 | 6.05 |
| Site 5 | -33.09385 | 151.57787 | 9.5 | 25.5 | 8.56 | 3.21 | 35.1 | 53.2 | 70.2 | 4.71 |
| Site 6 | -33.09173 | 151.53462 | 4.1 | 27.1 | 8.42 | 3.44 | 35.0 | 53.2 | 99.0 | 6.45 |
| Site 7 | -33.15318 | 151.56274 | 5.0 | 26.3 | 8.46 | 2.67 | 34.7 | 52.7 | 91.3 | 6.07 |
| Site 8 | -33.13333 | 151.59666 | 9.2 | 25.4 | 8.38 | 3.61 | 35.1 | 53.1 | 74.0 | 4.97 |
| Site 9 | -33.08231 | 151.56590 | 7.4 | 25.9 | 8.58 | 3.78 | 35.1 | 53.2 | 74.2 | 4.95 |
| Site 10 | -33.11855 | 151.57803 | 7.0 | 26.3 | 8.74 | 2.34 | 35.0 | 53.1 | 90.7 | 6.01 |

Appendix B – Toxicity test method (full)

The 10-day whole-sediment chronic sub-lethal (reproduction) and acute (mortality) toxicity test using the epibenthic amphipod *J. plumulosa* was undertaken according to the method described in Appendix E of the handbook *Sediment Quality Assessment: A Practical Guide*, edited by Simpson and Batley (2016). The method was developed by Spadaro et al. (2008) and adapted from the work of Mann et al. (2009), which measures the adverse effects of sediment contaminants on amphipod reproduction by counting the number of embryos and juveniles that are produced following exposure.

Toxicity tests were conducted on the whole sediment (the sediment and associated pore water). The toxicity testing was split up into two test batches. Test one commenced on the 10th of February, 2023, and included Sites 1, 4, 5, 6, and 9. Test two commenced on the 17th of February, 2023, and included Sites 2, 3, 7, 8, and 10. For the Lake Macquarie test sediments, each site/treatment was tested in quadruplicate from the four individual grab samples collected at each site. Two control sediments were tested concurrently with each test batch.

The test sediments and control sediments were prepared eight to nine days before test initiation to allow suspended particles to settle out and for the sediment and pore water to equilibrate. For each test replicate, the sample was prepared in duplicate (one beaker for use at test initiation on Day 0, and one beaker for use at the sediment renewal step on Day 5) by placing 40 g (wet weight) of homogenised whole sediment into a 250 mL glass beaker and topping up with filtered (0.45 µm) seawater to give a final approximate volume of 200 mL. The beakers were covered with a Perspex lid, provided with aeration, and placed in a constant environmental chamber at 21±1°C with a 12:12-h light: dark photoperiod for the entire 10-day exposure. Clean seawater was collected from the Sydney region during dry conditions and filtered to 0.45 µm on return to the laboratory. On the day of testing, the overlying water from each of the Day 0 test beakers was removed by gently siphoning with plastic tubing. Fresh overlying water was added gently by pouring down the sides of the beaker.

Amphipods used for the tests were obtained from laboratory cultures maintained by CSIRO Land and Water (Lucas Heights, NSW). Adult female amphipods were collected from the culture trays 9 days before test initiation and placed in a fresh culturing tray. Adult male amphipods were added to the isolated females 2 days before test initiation. On the day of test initiation, the adult amphipods were transferred to trays and 6 males and 6 gravid females (≤2 days into gestation) were picked out and randomly assigned to each Day 0 beaker. Each beaker was then fed at a rate of 0.5 mg of Sera Micron fish food per amphipod, covered, randomised, and returned to the constant environment chamber. On Days 3 and 7 of the test, the overlying water was replaced and fed at the same rate as previously described. The sediments were renewed on Day 5 of the test by gently sieving the adults through a 500 µm mesh and placing them into fresh sediment. The Day 5 beakers were prepared and equilibrated at the same time as the previous beakers used on Day 0. This sediment renewal step facilitates the removal of juveniles from the first brood that are likely to be unaffected from the test sediment because they were already ‘conceived’ before exposure to the test sediment.

On Day 10, at the termination of the test, the adults and juveniles were removed from the sediment by wet sieving the contents of each beaker through a 200 µm mesh. The number of males, females, and juveniles recovered from the sediment was counted. The contents of the sieve and any uncounted juveniles were collected in polycarbonate containers with high-purity water, preserved with formaldehyde, and stained with rose bengal (4,5,6,7-tetrachloro-2',4',5',7-tetraiodofluorescein) solution. These samples were rinsed and counted within 4 days of collection. The number of embryos within the female amphipods recovered at test termination was counted by microscopy.

The test endpoint, reproduction, was represented as the sum of the number of embryos and juveniles at the test end, divided by the original number of females (i.e., six) and expressed as a percentage of the control.

The physicochemical properties (pH, salinity, specific conductance, and dissolved oxygen) of the overlying water were monitored throughout the test (on a composite sample of the four replicates of each treatment) to ensure no adverse conditions were contributing to the test results using a multiparameter meter (multi-3430, WTW GmbH) calibrated as per the manufacturer's instructions. Ammonia was also measured using a rapid test kit (API Aquarium Pharmaceuticals). A composite sample of the four replicates were collected before water renewals on Days 3, 5, 7 and 10. An approximation of the sediment pore water pH was determined on Days 5 and 10 using a gel-filled electrode with spearhead (Sentix 980®, WTW GmbH) calibrated as per the manufacturer's instructions.

Samples of the overlying water were collected for dissolved metal analyses on Day 0 (as a composite of the four individual replicates) and Day 3 of the test (in each of the four individual replicates) and were analysed by inductively coupled plasma optical emission spectrometry or inductively coupled plasma mass spectrometry. Day 3 was used to provide a representative measure of the dissolved metal concentrations during the toxicity test. Temperature was monitored in the constant environmental chamber with a continuous logger (Onset Hobo).

Appendix C – Water quality data (during toxicity testing)

Table A2. Physicochemical properties of the overlying water during toxicity testing. Measured in a composite sample of the replicates for each treatment (n=4) on Days 0, 3, 5, 7 and 10 of the test. For each treatment, all measurements were pooled and presented as a range (minimum –maximum value, n=5)

| Treatment* | pH | Salinity (PSU) | Specific conductance (mS/cm) | Dissolved oxygen (%) | Ammonia (mg/L) |
|------------|-----------|----------------|------------------------------|----------------------|----------------|
| Control-BB | 7.9 – 8.3 | 32.2 – 32.6 | 49.5 – 50.0 | 95 – 103 | 0 – 0.5 |
| Control-BB | 7.9 – 8.0 | 31.8 – 32.5 | 48.9 – 49.8 | 94 – 100 | 0 – 1 |
| Control-GP | 7.9 – 8.4 | 32.4 – 33.1 | 49.9 – 50.6 | 98 – 104 | 0 – 0.5 |
| Control-GP | 7.9 – 8.0 | 32.0 – 33.1 | 49.2 – 50.6 | 90 – 101 | 0 – 1 |
| Site 1 | 8.1 – 8.3 | 32.3 – 32.6 | 49.6 – 50.0 | 95 – 104 | 0 – 0.5 |
| Site 2 | 8.0 – 8.1 | 32.1 – 33.3 | 49.5 – 50.9 | 97 – 102 | 0 – 0.5 |
| Site 3 | 8.0 – 8.1 | 32.2 – 32.9 | 49.6 – 50.4 | 96 – 102 | 0 – 0.5 |
| Site 4 | 8.0 – 8.2 | 32.5 – 32.8 | 49.9 – 50.2 | 98 – 103 | 0 – 0.5 |
| Site 5 | 8.0 – 8.2 | 32.4 – 32.9 | 49.8 – 50.5 | 98 – 105 | 0 – 1 |
| Site 6 | 8.0 – 8.1 | 32.3 – 33.0 | 49.7 – 50.5 | 96 – 104 | 0 – 0.5 |
| Site 7 | 8.0 – 8.1 | 32.2 – 35.2 | 49.5 – 53.5 | 98 – 102 | 0 – 0.5 |
| Site 8 | 8.0 – 8.1 | 32.3 – 33.4 | 49.6 – 51.1 | 94 – 102 | 0 – 0.5 |
| Site 9 | 8.0 – 8.2 | 32.4 – 32.8 | 49.7 – 50.2 | 93 – 104 | 0 – 0.5 |
| Site 10 | 8.0 – 8.1 | 32.4 – 33.9 | 49.7 – 51.7 | 97 – 100 | 0 – 1 |

* Treatments (control or test sediments) tested as part of Test one are in black, while those tested as part of Test two are in blue

Table A3. Dissolved (<0.45 µm filtered) metal concentrations of the overlying water during toxicity testing. Measured in each individual replicate on Day 3 (mean ± standard deviation, n=4). ANZG (2018) marine default guideline values (DGVs) for 95% species protection have been applied as screening criteria unless otherwise indicated (exceedances are indicated by purple font). All values are in mg/L. Table A3 continues on the next page.

| Treatment | Al | As | Ba | Be | B | Cd | Cr | Co | Cu |
|--------------------|--------------------|---------------------|-----------------|-----------------|-----------|---------------------|-----------------|-----------------|----------------------------|
| Blank ^a | 0.01 ± 0.00 | 0.002 ± 0.000 | 0.0070 ± 0.0002 | <0.0002 | 4.4 ± 0.1 | <0.0002 | 0.0013 ± 0.0003 | <0.0001 | 0.002 ± 0.001 |
| Control-BB | 0.02 ± 0.01 | 0.002 ± 0.001 | 0.0190 ± 0.0008 | <0.0002 | 4.4 ± 0.2 | <0.0002 | <0.0005 | 0.0002 ± 0.0001 | 0.003 ± 0.001 |
| Control-BB | 0.02 ± 0.00 | 0.002 ± 0.001 | 0.0175 ± 0.0006 | <0.0002 | 4.2 ± 0.1 | <0.0002 | <0.0005 | 0.0003 ± 0.0001 | 0.002 ± 0.001 |
| Control-GP | 0.04 ± 0.01 | 0.002 ± 0.000 | 0.0118 ± 0.0005 | <0.0002 | 4.6 ± 0.0 | <0.0002 | 0.0003 ± 0.0001 | 0.0001 ± 0.0000 | 0.004 ± 0.001 |
| Control-GP | 0.05 ± 0.01 | 0.002 ± 0.000 | 0.0099 ± 0.0003 | <0.0002 | 4.3 ± 0.2 | <0.0002 | 0.0009 ± 0.0007 | 0.0002 ± 0.0001 | 0.002 ± 0.001 |
| Site 1 | 0.01 ± 0.00 | 0.003 ± 0.000 | 0.0133 ± 0.0005 | 0.0001 ± 0.0001 | 4.5 ± 0.0 | 0.0004 ± 0.0002 | <0.0005 | 0.0002 ± 0.0002 | 0.004 ± 0.002 |
| Site 2 | <0.01 | 0.003 ± 0.001 | 0.0097 ± 0.0003 | <0.0002 | 4.5 ± 0.1 | 0.0003 ± 0.0001 | 0.0005 ± 0.0001 | <0.0001 | 0.005 ± 0.001 |
| Site 3 | <0.01 | 0.004 ± 0.001 | 0.0102 ± 0.0005 | <0.0002 | 4.4 ± 0.1 | 0.0004 ± 0.0000 | 0.0005 ± 0.0002 | 0.0001 ± 0.0000 | 0.004 ± 0.001 |
| Site 4 | <0.01 | 0.004 ± 0.001 | 0.0115 ± 0.0006 | <0.0002 | 4.4 ± 0.1 | 0.0004 ± 0.0001 | 0.0003 ± 0.0001 | <0.0001 | 0.005 ± 0.001 |
| Site 5 | 0.01 ± 0.01 | 0.004 ± 0.000 | 0.0120 ± 0.0008 | <0.0002 | 4.7 ± 0.1 | 0.0003 ± 0.0001 | 0.0004 ± 0.0001 | <0.0001 | 0.014 ± 0.020 ^e |
| Site 6 | <0.01 | 0.002 ± 0.001 | 0.0114 ± 0.0018 | <0.0002 | 4.5 ± 0.0 | 0.0004 ± 0.0000 | <0.0005 | 0.0001 ± 0.0000 | 0.005 ± 0.001 |
| Site 7 | <0.01 | 0.004 ± 0.001 | 0.0097 ± 0.0002 | <0.0002 | 4.5 ± 0.1 | 0.0004 ± 0.0001 | 0.0003 ± 0.0001 | 0.0001 ± 0.0000 | 0.004 ± 0.001 |
| Site 8 | <0.01 | 0.004 ± 0.001 | 0.0102 ± 0.0006 | <0.0002 | 4.4 ± 0.0 | 0.0005 ± 0.0002 | 0.0007 ± 0.0001 | <0.0001 | 0.004 ± 0.000 |
| Site 9 | 0.01 ± 0.02 | 0.003 ± 0.001 | 0.0123 ± 0.0005 | <0.0002 | 4.4 ± 0.1 | 0.0002 ± 0.0001 | <0.0005 | 0.0001 ± 0.0000 | 0.004 ± 0.001 |
| Site 10 | <0.01 | 0.003 ± 0.001 | 0.0108 ± 0.0010 | <0.0002 | 4.5 ± 0.2 | 0.0003 ± 0.0001 | 0.0004 ± 0.0002 | <0.0001 | 0.004 ± 0.001 |
| DGV | 0.037 ^b | 0.0023 ^c | NA | NA | NA | 0.0007 ^d | 0.0044 | 0.001 | 0.0013 |

^a Treatments (control or test sediments) tested as part of Test one are in black, while those tested as part of Test two are in blue. The blank was clean seawater.

^b Proposed third-party guideline value (G.E. Batley, personal communication)

^c Indicative interim working level

^d To account for the bioaccumulating nature of this toxicant, it is recommended that the 99% species protection level DGV is used for slightly to moderately disturbed systems

^e Dissolved (<0.45 µm filtered) copper, lead, and zinc were elevated due to a single replicate (Replicate 3). Replicate 3 was deemed to be compromised, and the test endpoints (survival and reproduction) for this replicate were excluded from reporting.

Table A3 (continued from the previous page). Dissolved (<0.45 µm filtered) metal concentrations of the overlying water during toxicity testing. Measured in each individual replicate on Day 3 (mean ± standard deviation, n=4). ANZG (2018) marine default guideline values (DGVs) for 95% species protection have been applied as screening criteria unless otherwise indicated (exceedances are indicated in purple font). All values are in mg/L.

| Treatment | Fe | Pb | Mn | Mo | Ni | Se | Ag | Tl | V | Zn |
|------------|-------|------------------------------|--------------------|--------------------|---------------|--------------------|---------|---------|-----------------|----------------------------|
| Blank | <0.02 | <0.0005 | <0.001 | 0.014 ± 0.001 | 0.002 ± 0.001 | <0.002 | <0.0002 | <0.0005 | 0.0018 ± 0.0001 | 0.011 ± 0.004 |
| Control-BB | <0.02 | <0.0005 | 0.002 ± 0.001 | 0.012 ± 0.001 | <0.001 | <0.002 | <0.0002 | <0.0005 | 0.0017 ± 0.0005 | <0.003 |
| Control-BB | <0.02 | <0.0005 | 0.003 ± 0.001 | 0.013 ± 0.001 | <0.001 | <0.002 | <0.0002 | <0.0005 | 0.0021 ± 0.0006 | 0.002 ± 0.002 |
| Control-GP | <0.02 | <0.0005 | 0.001 ± 0.000 | 0.017 ± 0.001 | <0.001 | <0.002 | <0.0002 | <0.0005 | 0.0024 ± 0.0005 | 0.004 ± 0.002 |
| Control-GP | <0.02 | <0.0005 | 0.001 ± 0.001 | 0.017 ± 0.001 | <0.001 | <0.002 | <0.0002 | <0.0005 | 0.0031 ± 0.0006 | <0.003 |
| Site 1 | <0.02 | 0.0004 ± 0.0003 | 0.001 ± 0.001 | 0.016 ± 0.002 | 0.001 ± 0.000 | <0.002 | <0.0002 | <0.0005 | 0.0069 ± 0.0008 | 0.003 ± 0.002 |
| Site 2 | <0.02 | <0.0005 | <0.001 | 0.016 ± 0.001 | 0.001 ± 0.001 | <0.002 | <0.0002 | <0.0005 | 0.0052 ± 0.0008 | <0.003 |
| Site 3 | <0.02 | <0.0005 | <0.001 | 0.016 ± 0.001 | 0.001 ± 0.001 | <0.002 | <0.0002 | <0.0005 | 0.0095 ± 0.0018 | <0.003 |
| Site 4 | <0.02 | <0.0005 | 0.001 ± 0.000 | 0.014 ± 0.000 | 0.001 ± 0.001 | <0.002 | <0.0002 | <0.0005 | 0.0125 ± 0.0021 | <0.003 |
| Site 5 | <0.02 | 0.0005 ± 0.0006 ^e | 0.001 ± 0.001 | 0.017 ± 0.000 | 0.001 ± 0.001 | <0.002 | <0.0002 | <0.0005 | 0.0092 ± 0.0013 | 0.007 ± 0.012 ^e |
| Site 6 | <0.02 | <0.0005 | 0.001 ± 0.001 | 0.015 ± 0.001 | 0.001 ± 0.001 | <0.002 | <0.0002 | <0.0005 | 0.0068 ± 0.0003 | <0.003 |
| Site 7 | <0.02 | <0.0005 | <0.001 | 0.019 ± 0.001 | 0.001 ± 0.001 | <0.002 | <0.0002 | <0.0005 | 0.0074 ± 0.0007 | 0.002 ± 0.001 |
| Site 8 | <0.02 | <0.0005 | 0.001 ± 0.000 | 0.016 ± 0.001 | 0.002 ± 0.001 | <0.002 | <0.0002 | <0.0005 | 0.0103 ± 0.0022 | 0.002 ± 0.001 |
| Site 9 | <0.02 | <0.0005 | 0.001 ± 0.001 | 0.015 ± 0.002 | 0.001 ± 0.000 | <0.002 | <0.0002 | <0.0005 | 0.0071 ± 0.0013 | <0.003 |
| Site 10 | <0.02 | <0.0005 | <0.001 | 0.016 ± 0.001 | 0.001 ± 0.001 | <0.002 | <0.0002 | <0.0005 | 0.0059 ± 0.0012 | <0.003 |
| DGV | NA | 0.0044 | 0.080 ^c | 0.023 ^c | 0.070 | 0.003 ^c | 0.0014 | NA | 0.100 | 0.008 |

^a Treatments (control or test sediments) tested as part of Test one are in black, while those tested as part of Test two are in blue. The blank was clean seawater.

^b Proposed third-party guideline value (G.E. Batley, personal communication)

^c Indicative interim working level

^d To account for the bioaccumulating nature of this toxicant, it is recommended that the 99% species protection level DGV is used for slightly to moderately disturbed systems

^e Dissolved (<0.45 µm filtered) copper, lead, and zinc were elevated due to a single replicate (Replicate 3). Replicate 3 was deemed to be compromised, and the test endpoints (survival and reproduction) for this replicate were excluded from reporting.

Table A4. Physicochemical properties of the sediment pore water during toxicity testing. For each treatment, three measurements were collected (one replicate on Day 5 and two replicates on Day 10), and the results were pooled and presented as a range (minimum – maximum value, n=3).

| Treatment ^a | pH | Redox potential (mV) |
|------------------------|-----------|----------------------|
| Control-BB | 6.2 – 6.7 | 14 – 40 |
| Control-BB | 6.3 – 6.7 | 13 – 37 |
| Control-GP | 7.1 – 7.3 | -20 – -10 |
| Control-GP | 6.9 – 7.1 | -5 – 2 |
| Site 1 | 6.9 – 7.4 | -30 – 2 |
| Site 2 | 7.5 – 7.6 | -35 – -27 |
| Site 3 | 7.4 – 7.7 | -42 – -26 |
| Site 4 | 7.7 – 7.8 | -45 – -39 |
| Site 5 | 7.4 – 7.6 | -33 – -25 |
| Site 6 | 7.6 – 7.7 | -42 – -34 |
| Site 7 | 7.3 – 7.8 | -48 – -18 |
| Site 8 | 7.7 – 7.8 | -46 – -39 |
| Site 9 | 7.4 – 7.7 | -42 – -26 |
| Site 10 | 7.5 – 7.6 | -36 – -30 |

^a Treatments (control or test sediments) tested as part of Test one are in black while those tested as part of Test two are in blue

Appendix D – Sediment chemistry data

Table A5. Summary of the key physicochemical properties of the control sediments and Lake Macquarie test sediments

| Treatment ^a | Total organic carbon ^b (%) | Particle size (%) | | | Particle size ^c (<63 µm, %) | Moisture content (%) |
|------------------------|---------------------------------------|-----------------------|------------------------------|------------------------------|--|----------------------|
| | | <2 µm (clay fraction) | 2-63 µm (fine silt fraction) | 63 µm - 2 mm (sand fraction) | | |
| Control-BB | 5.0 | 4 | 83 | 13 | 87 | 29 |
| Control-GP | 2.4 | 1 | 49 | 51 | 50 | 17 |
| Site 1 | 2.8 ± 0.2 | 12 ± 1 | 85 ± 1 | 3 ± 1 | 97 ± 1 | 54 ± 10 |
| Site 2 | 3.0 ± 0.5 | 9 ± 9 | 85 ± 13 | 6 ± 6 | 94 ± 7 | 66 ± 7 |
| Site 3 | 3.0 ± 0.1 | 11 ± 5 | 87 ± 5 | 2 ± 0 | 98 ± 0 | 74 ± 2 |
| Site 4 | 2.7 ± 0.1 | 17 ± 4 | 81 ± 3 | 2 ± 0 | 98 ± 1 | 67 ± 8 |
| Site 5 | 2.7 ± 0.2 | 20 ± 2 | 78 ± 2 | 2 ± 1 | 98 ± 1 | 76 ± 1 |
| Site 6 | 1.6 ± 0.1 | 12 ± 4 | 67 ± 6 | 22 ± 9 | 78 ± 9 | 57 ± 3 |
| Site 7 | 3.0 ± 0.1 | 9 ± 4 | 85 ± 6 | 6 ± 5 | 94 ± 5 | 71 ± 1 |
| Site 8 | 2.6 ± 0.1 | 18 ± 2 | 80 ± 2 | 2 ± 1 | 98 ± 1 | 75 ± 1 |
| Site 9 | 2.6 ± 0.1 | 16 ± 1 | 81 ± 1 | 3 ± 1 | 97 ± 1 | 69 ± 6 |
| Site 10 | 2.6 ± 0.1 | 8 ± 4 | 89 ± 1 | 4 ± 5 | 96 ± 4 | 75 ± 1 |

^a Control sediment (n=1): BB = Bonnet Bay and GP = Grays Point; Lake Macquarie test sediments (mean ± standard deviation, n=4): Sites 1 to 10.

^b Combustion method

^c The <63 µm sediment particle size fraction (clay and silt) is considered a suitable representation of the sediment materials that are most readily resuspended or potentially ingested by organisms

Table A6. Summary of the dilute acid-extractable (1M HCl) metal concentrations of the control sediments and Lake Macquarie test sediments. ANZG (2018) sediment quality guideline values (SQGVs) have been applied as screening criteria unless otherwise indicated. All values are in mg/kg.

| Treatment ^a | As | Cd | Cr | Cu | Pb | Hg | Ni | Se | Zn |
|------------------------|-----------|-------------|-----------|--------|--------|-------|-----------|----------------|----------|
| Control-BB | 2 | <0.1 | 3 | 13 | 25 | <0.05 | 1 | <0.1 | 55 |
| Control-GP | 2 | <0.1 | 0.9 | 8.6 | 14 | <0.05 | 0.6 | <0.1 | 15 |
| Site 1 | 2.5 ± 0.6 | 0.18 ± 0.05 | 4.8 ± 1.1 | 27 ± 4 | 14 ± 3 | <0.05 | 2.5 ± 0.6 | 0.16 ± 0.08 | 85 ± 14 |
| Site 2 | 2.8 ± 0.5 | 0.23 ± 0.05 | 7.6 ± 1.4 | 37 ± 7 | 15 ± 2 | <0.05 | 2.8 ± 0.5 | 0.08 ± 0.03 | 99 ± 16 |
| Site 3 | 2.5 ± 0.6 | 0.33 ± 0.10 | 9.3 ± 0.9 | 33 ± 4 | 43 ± 3 | <0.05 | 4.3 ± 0.5 | 0.08 ± 0.03 | 190 ± 14 |
| Site 4 | 2.3 ± 0.5 | 0.28 ± 0.15 | 7.5 ± 1.6 | 30 ± 5 | 32 ± 7 | <0.05 | 3.3 ± 0.5 | 0.11 ± 0.13 | 150 ± 29 |
| Site 5 | 2.8 ± 0.5 | 0.20 ± 0.00 | 8.9 ± 0.4 | 36 ± 1 | 29 ± 1 | <0.05 | 4.0 ± 0.0 | 0.10 ± 0.07 | 168 ± 5 |
| Site 6 | 2.8 ± 1.0 | 0.25 ± 0.06 | 5.3 ± 0.5 | 24 ± 3 | 14 ± 2 | <0.05 | 2.8 ± 0.5 | 0.19 ± 0.10 | 81 ± 9 |
| Site 7 | 3.3 ± 0.5 | 0.48 ± 0.05 | 9.0 ± 1.6 | 33 ± 5 | 21 ± 7 | <0.05 | 3.3 ± 0.5 | 0.13 ± 0.05 | 120 ± 40 |
| Site 8 | 2.3 ± 0.5 | 0.28 ± 0.05 | 8.6 ± 2.1 | 32 ± 7 | 32 ± 9 | <0.05 | 3.5 ± 1.0 | 0.14 ± 0.11 | 173 ± 43 |
| Site 9 | 3.0 ± 0.8 | 0.18 ± 0.05 | 7.7 ± 1.5 | 35 ± 7 | 23 ± 5 | <0.05 | 3.5 ± 1.0 | 0.05 ± 0.00 | 137 ± 27 |
| Site 10 | 3.0 ± 0.8 | 0.23 ± 0.05 | 9.6 ± 1.0 | 36 ± 3 | 23 ± 2 | <0.05 | 3.5 ± 0.6 | 0.06 ± 0.03 | 138 ± 10 |
| SQGV | 20 | 1.5 | 80 | 65 | 50 | 0.15 | 21 | 1 ^b | 200 |
| SQGV-high | 70 | 10 | 370 | 270 | 220 | 1.0 | 52 | | 410 |

^a Control sediment (n=1): BB = Bonnet Bay and GP = Grays Point; Lake Macquarie test sediments (mean ± standard deviation, n=4): Sites 1 to 10.

^b Buchman, 2008

Table A7. Summary of the estimated dissolved (<0.45 µm filtered) metal concentrations within pore waters of the sediments collected from Lake Macquarie (mean ± standard deviation, n=4). ANZG (2018) marine default guideline values (DGVs) for 95% species protection have been applied as screening criteria unless otherwise indicated (exceedances are indicated in purple font). All values are in mg/L. Table A7 continues on the next page.

| Treatment | Al | As | Ba | Be | B (mg/L) | Cd | Cr | Co | Cu | Fe |
|-----------|--------------------|---------------------|---------------|---------|-----------|---------------------|---------|-----------------|--------|-------------|
| Site 1 | <0.01 | 0.010 ± 0.002 | 0.021 ± 0.003 | <0.0002 | 3.7 ± 0.1 | <0.0002 | <0.0005 | 0.0003 ± 0.0001 | <0.001 | 0.23 ± 0.04 |
| Site 2 | <0.01 | 0.010 ± 0.003 | 0.016 ± 0.001 | <0.0002 | 3.5 ± 0.1 | <0.0002 | <0.0005 | 0.0003 ± 0.0001 | <0.001 | 0.30 ± 0.07 |
| Site 3 | <0.01 | 0.012 ± 0.001 | 0.015 ± 0.001 | <0.0002 | 4.0 ± 0.2 | <0.0002 | <0.0005 | 0.0002 ± 0.0000 | <0.001 | 0.13 ± 0.12 |
| Site 4 | <0.01 | 0.009 ± 0.002 | 0.016 ± 0.001 | <0.0002 | 4.0 ± 0.1 | <0.0002 | <0.0005 | 0.0002 ± 0.0000 | <0.001 | 0.19 ± 0.04 |
| Site 5 | <0.01 | 0.008 ± 0.001 | 0.017 ± 0.003 | <0.0002 | 3.6 ± 0.0 | <0.0002 | <0.0005 | 0.0002 ± 0.0001 | <0.001 | 0.15 ± 0.02 |
| Site 6 | <0.01 | 0.005 ± 0.001 | 0.019 ± 0.005 | <0.0002 | 3.5 ± 0.1 | <0.0002 | <0.0005 | 0.0002 ± 0.0001 | <0.001 | 0.48 ± 0.20 |
| Site 7 | <0.01 | 0.010 ± 0.002 | 0.015 ± 0.001 | <0.0002 | 3.4 ± 0.1 | <0.0002 | <0.0005 | 0.0002 ± 0.0000 | <0.001 | 0.23 ± 0.02 |
| Site 8 | 0.009 ± 0.008 | 0.010 ± 0.002 | 0.016 ± 0.001 | <0.0002 | 4.0 ± 0.3 | <0.0002 | <0.0005 | 0.0002 ± 0.0000 | <0.001 | 0.12 ± 0.01 |
| Site 9 | <0.01 | 0.009 ± 0.001 | 0.019 ± 0.001 | <0.0002 | 3.9 ± 0.1 | <0.0002 | <0.0005 | 0.0003 ± 0.0001 | <0.001 | 0.26 ± 0.03 |
| Site 10 | <0.01 | 0.008 ± 0.001 | 0.017 ± 0.002 | <0.0002 | 3.8 ± 0.1 | <0.0002 | <0.0005 | 0.0003 ± 0.0001 | <0.001 | 0.27 ± 0.05 |
| DGV | 0.037 ^a | 0.0023 ^b | - | - | - | 0.0007 ^c | 0.0044 | 0.001 | 0.0013 | - |

^a Proposed third-party guideline value (G.E. Batley, personal communication)

^b Indicative interim working level

^c To account for the bioaccumulating nature of this toxicant, it is recommended that the 99% species protection level DGV is used for slightly to moderately disturbed systems

Table A7 (continued from the previous page). Summary of the estimated dissolved (<0.45 µm filtered) metal concentrations within pore waters of the sediments collected from Lake Macquarie (mean ± standard deviation, n=4). ANZG (2018) marine default guideline values (DGVs) for 95% species protection have been applied as screening criteria unless otherwise indicated (exceedances are indicated in purple font). All values are in mg/L.

| | Pb | Hg | Mn (mg/L) | Mo | Ni | Se | Ag | Tl | V | Zn |
|---------|-----------------|---------------------|--------------------|--------------------|--------|--------------------|---------|---------|-----------------|--------|
| Site 1 | <0.0005 | <0.0004 | 2.2 ± 0.5 | 0.016 ± 0.002 | <0.001 | <0.002 | <0.0002 | <0.0005 | 0.0009 ± 0.0001 | <0.003 |
| Site 2 | <0.0005 | <0.0004 | 4.7 ± 0.9 | 0.017 ± 0.002 | <0.001 | <0.002 | <0.0002 | <0.0005 | 0.0017 ± 0.0006 | <0.003 |
| Site 3 | <0.0005 | <0.0004 | 3.4 ± 0.8 | 0.014 ± 0.001 | <0.001 | <0.002 | <0.0002 | <0.0005 | 0.0029 ± 0.0012 | <0.003 |
| Site 4 | <0.0005 | <0.0004 | 3.7 ± 0.3 | 0.015 ± 0.001 | <0.001 | <0.002 | <0.0002 | <0.0005 | 0.0025 ± 0.0005 | <0.003 |
| Site 5 | <0.0005 | <0.0004 | 4.0 ± 1.0 | 0.018 ± 0.002 | <0.001 | <0.002 | <0.0002 | <0.0005 | 0.0028 ± 0.0003 | <0.003 |
| Site 6 | <0.0005 | <0.0004 | 1.1 ± 0.3 | 0.017 ± 0.001 | <0.001 | <0.002 | <0.0002 | <0.0005 | 0.0005 ± 0.0004 | <0.003 |
| Site 7 | <0.0005 | <0.0004 | 2.9 ± 0.6 | 0.024 ± 0.003 | <0.001 | <0.002 | <0.0002 | <0.0005 | 0.0013 ± 0.0003 | <0.003 |
| Site 8 | <0.0005 | <0.0004 | 3.5 ± 0.8 | 0.016 ± 0.001 | <0.001 | <0.002 | <0.0002 | <0.0005 | 0.0029 ± 0.0006 | <0.003 |
| Site 9 | 0.0003 ± 0.0002 | <0.0004 | 2.9 ± 0.6 | 0.017 ± 0.001 | <0.001 | <0.002 | <0.0002 | <0.0005 | 0.0009 ± 0.0002 | <0.003 |
| Site 10 | <0.0005 | <0.0004 | 3.3 ± 0.6 | 0.019 ± 0.001 | <0.001 | <0.002 | <0.0002 | <0.0005 | 0.0011 ± 0.0003 | <0.003 |
| DGV | 0.0044 | 0.0001 ^c | 0.080 ^b | 0.023 ^b | 0.070 | 0.003 ^b | 0.0014 | NA | 0.100 | 0.008 |

^a Proposed third-party guideline value (G.E. Batley, personal communication)

^b Indicative interim working level

^c To account for the bioaccumulating nature of this toxicant, it is recommended that the 99% species protection level DGV is used for slightly to moderately disturbed systems

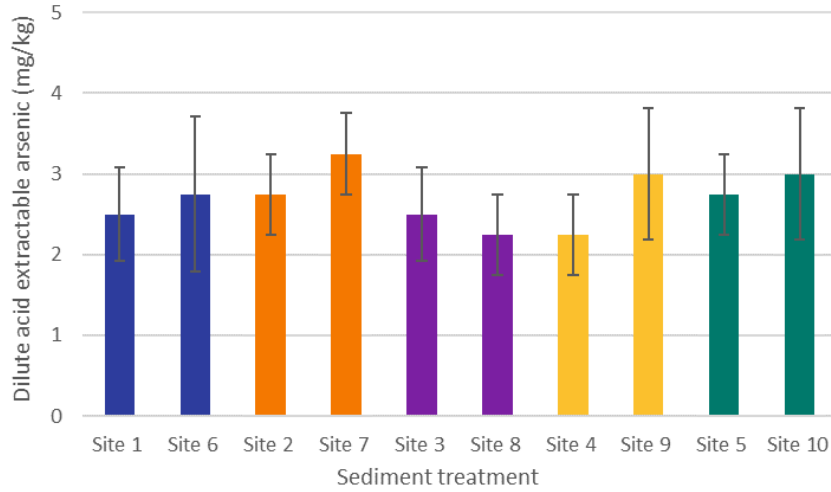


Figure A1. Dilute acid-extractable arsenic concentrations (mg/kg) in surface sediments collected from ten locations within southern Lake Macquarie, Jan-Feb 2023

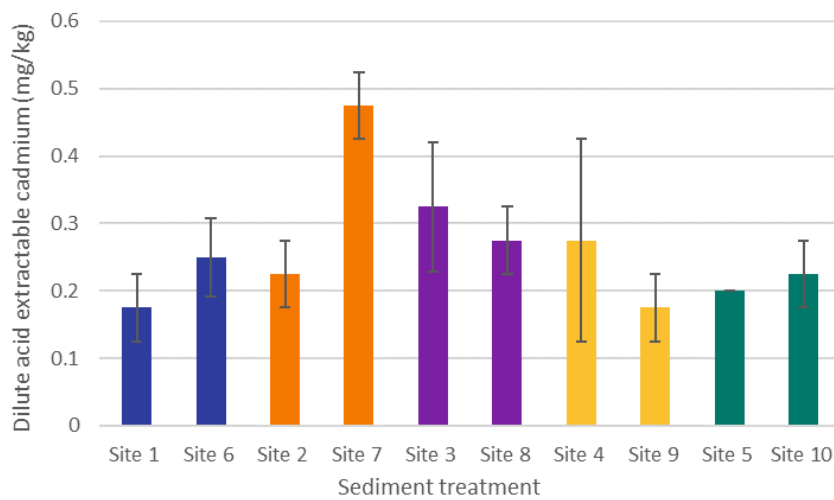


Figure A2. Dilute acid-extractable cadmium concentrations (mg/kg) in surface sediments collected from ten locations within southern Lake Macquarie, Jan-Feb 2023

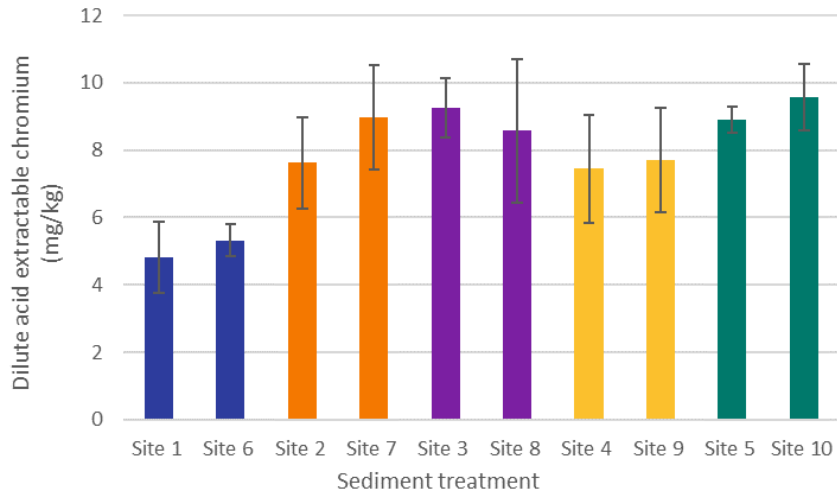


Figure A3. Dilute acid-extractable chromium concentrations (mg/kg) in surface sediments collected from ten locations within southern Lake Macquarie, Jan-Feb 2023

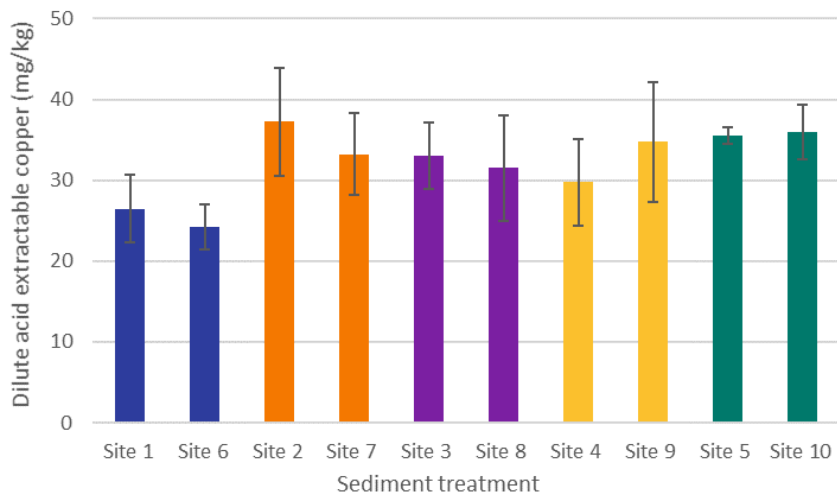


Figure A4. Dilute acid-extractable copper concentrations (mg/kg) in surface sediments collected from ten locations within southern Lake Macquarie, Jan-Feb 2023

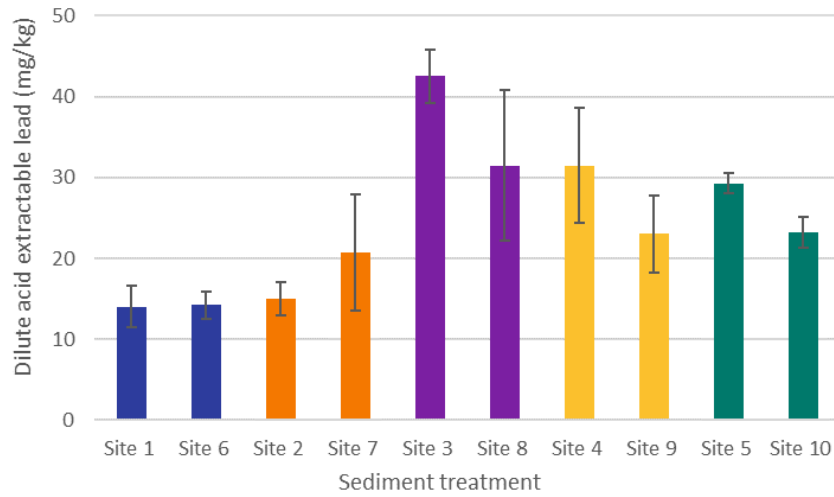


Figure A5. Dilute acid-extractable lead concentrations (mg/kg) in surface sediments collected from ten locations within southern Lake Macquarie, Jan-Feb 2023

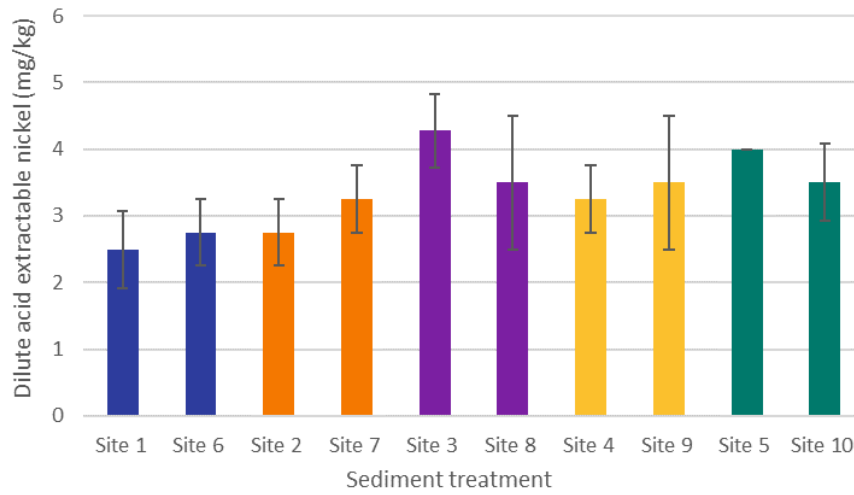


Figure A6. Dilute acid-extractable nickel concentrations (mg/kg) in surface sediments collected from ten locations within southern Lake Macquarie, Jan-Feb 2023

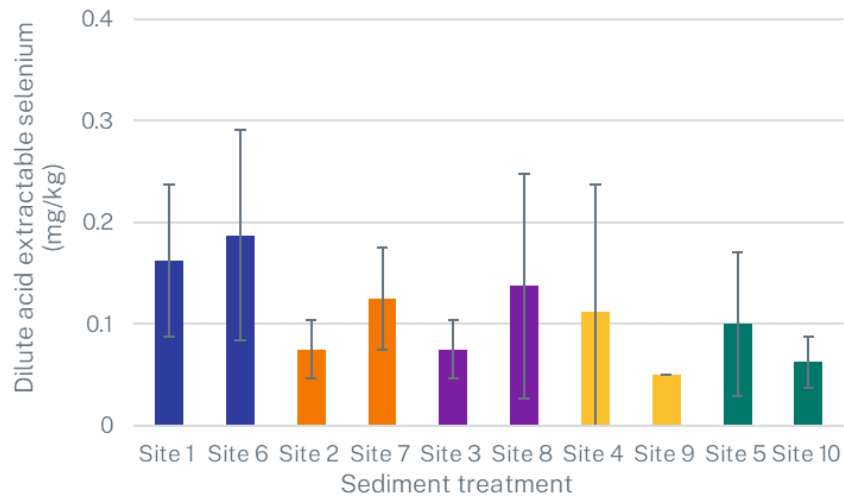


Figure A7. Dilute acid-extractable selenium concentrations (mg/kg) in surface sediments collected from ten locations within southern Lake Macquarie, Jan-Feb 2023

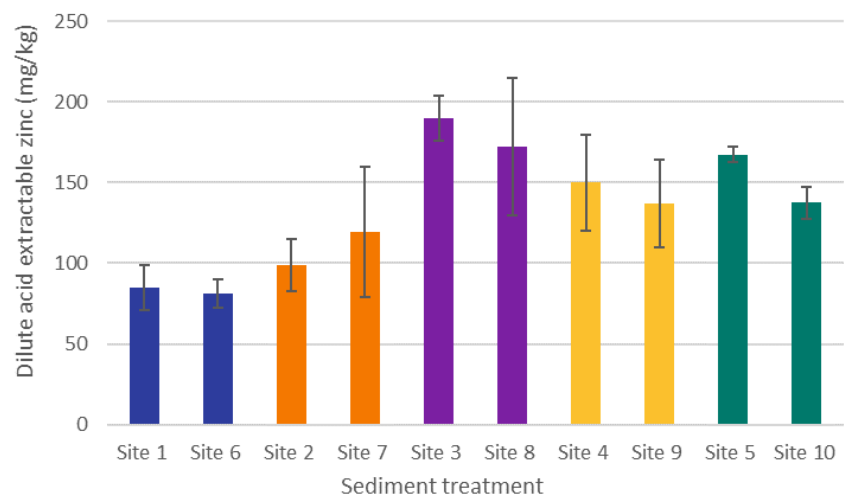


Figure A8. Dilute acid-extractable zinc concentrations (mg/kg) in surface sediments collected from ten locations within southern Lake Macquarie, Jan-Feb 2023

Appendix E – Hypothesis testing

Table A8. A summary of the hypothesis testing outcomes performed in Minitab. Treatments underwent the 10-day whole sediment chronic sub-lethal (reproduction) toxicity test using the epibenthic amphipod *Josephosella plumulosa*. Significance was assessed at the $P < 0.05$ level.

| Comparison | Data type ^a | Analysis performed | P-value | Outcome |
|--|------------------------|---------------------|---------|--|
| Control-BB (Test 1) vs Control-GP (Test 1) | Non-parametric | Mann-Whitney U test | 1.000 | No significant difference between treatments |
| Control-BB (Test 2) vs Control-GP (Test 2) | Non-parametric | Mann-Whitney U test | 1.000 | No significant difference between treatments |
| Combined control (Test 1) vs Site 4 | Parametric | Two-sample T-test | 0.273 | No significant difference between treatments |
| Combined control (Test 1) vs Site 5 | Non-parametric | Mann-Whitney U test | 0.292 | No significant difference between treatments |
| Combined control (Test 1) vs Site 6 | Parametric | Two-sample T-test | 0.021 | Significant difference between treatments |
| Combined control (Test 1) vs Site 9 | Parametric | Two-sample T-test | 0.009 | Significant difference between treatments |
| Combined control (Test 2) vs Site 8 | Parametric | Two-sample T-test | | No significant difference between treatments |
| Combined controls (Test 1) vs combined controls (Test 2) | Parametric | Two-sample T-test | 0.811 | No significant difference between treatments |
| Test sediments from Sites 1 to 10 | Parametric | One-way ANOVA | 0.507 | No significant difference between treatments |

^a Data were first tested for normality using the Ryan Joiner test. Data were then tested for homogeneity of variance using the F test or Bartlett's test for normally distributed data, or the Levene's test for data not normally distributed.

7. Acknowledgements

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