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Diffusive gradients in thin films technique provide robust prediction of metal bioavailability and toxicity in estuarine sediments


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Abstract

Many sediment quality assessment frameworks incorporate contaminant bioavailability as a critical factor regulating toxicity in aquatic ecosystems. However, current approaches do not always adequately predict metal bioavailability to organisms living in the oxidised sediment surface layers. The deployment of the diffusive gradients in thin films (DGT) probes in sediments allows labile metals present in pore waters and weakly-bound to the particulate phase to be assessed in a time-integrated manner in situ. In this study, relationships between DGT-labile metal fluxes within 5 mm of the sediment-water interface and lethal and sub-lethal effects to the amphipod *Melita plumulosa* were assessed in a range of contaminated estuarine sediments during 10-day laboratory-based bioassays. To account for differing toxicities of metals, DGT fluxes were normalised to water (WQG) or sediment quality guidelines or toxicity thresholds specific for the amphipod. The better dose-response relationships appeared to be the one based on WQG-normalized DGT fluxes, which successfully predicted toxicity despite the wide range of metals and large variations in sediment properties. The study indicated that the labile fraction of metals measured by DGT is useful for predicting metal toxicity to benthic invertebrates, supporting the applicability of this technique as a rapid monitoring tool for sediments quality assessments.

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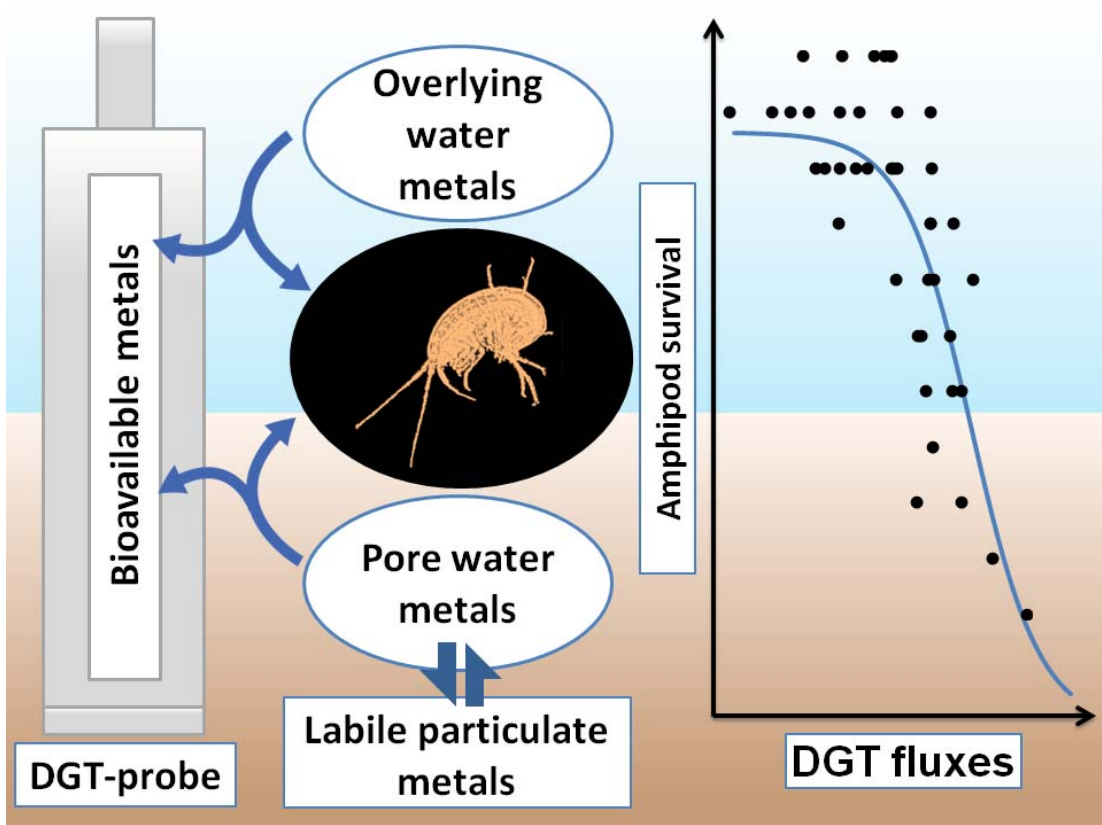
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Rationale: Many regulatory frameworks for sediment quality assessment incorporate procedures for determining contaminant bioavailability, potentially modifying guideline values. However, there are many inadequacies with the methods currently used. The deployment of diffusive gradients in thin films (DGTs) probes in sediments has the potential to provide a time-integrated measure of the pool of potentially bioavailable metals *in situ*. The technique measures fluxes of metal mixtures in pore waters and labile, weakly bound metal forms that are readily released from particles.

In this study, we demonstrate that the combined DGT-labile fluxes of the metals Cd, Cu, Ni, Pb and Zn provide a robust means for predicting toxicity to the amphipod *Melita plumulosa* in estuarine sediments. The DGT technique provided excellent dose-response relationships despite the wide range of metals and concentrations and large variations in sediment properties such as acid-volatile sulfide, organic carbon and particle size. The study supports the applicability of this technique as a rapid monitoring tool for sediments quality assessments.



Diffusive gradients in thin films technique provide robust prediction of metal bioavailability and toxicity in estuarine sediments

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ABSTRACT. Many sediment quality assessment frameworks incorporate contaminant bioavailability as a critical factor regulating toxicity in aquatic ecosystems. However, current approaches do not always adequately predict metal bioavailability to organisms living in the oxidised sediment surface layers. The deployment of the diffusive gradients in thin films (DGT) probes in sediments allows labile metals present in pore waters and weakly-bound to the particulate phase to be assessed in a time-integrated manner *in situ*. In this study, relationships between DGT-labile metal fluxes within 5 mm of the sediment-water interface and lethal and sub-lethal effects to the amphipod *Melita plumulosa* were assessed in a range of contaminated estuarine sediments during 10-day laboratory-based bioassays. To account for differing toxicities of metals, DGT fluxes were normalised to water (WQG) or sediment quality guidelines or toxicity thresholds specific for the amphipod. The better dose-response relationship appeared to be the one based on WQG-normalized DGT fluxes, which successfully predicted toxicity despite the wide range of metals and large variations in sediment properties. The study indicated that the labile fraction of metals measured by DGT is useful for predicting metal toxicity to benthic invertebrates, supporting the applicability of this technique as a rapid monitoring tool for sediments quality assessments.

Keywords: Diffusive gradients in thin films; Metal fluxes; sub-lethal toxicity; amphipod; sediment quality assessment

INTRODUCTION

Evaluating contaminant bioavailability has become a well-established component in many environment quality assessment programs. The ecotoxicological risk associated with a contaminant is dependant upon its bioavailability, which is influenced by the chemistry of the contaminants, the properties of the sediments and the behaviour and physiology of the organism.¹⁻³ Many of the procedures for assessing the bioavailability of contaminants in sediments are time-consuming and expensive,⁴ and may frequently result in equivocal outcomes, thus there is a need to develop more effective methods.

The majority of sediment quality guidelines (SQG) used for assessments are based on empirical relationships between biological effects and contaminant concentrations^{5,6} and ranking these has formed the basis of empirical SQGs for the initial tier of assessments.⁷ While these SQGs are based mostly on total contaminant concentrations, it is well recognised that the bioavailability of contaminants is strongly dependent on the processes that influence the partitioning between the solid and dissolved phase.

For metals, the concentrations of acid-volatile sulfide (AVS), simultaneously extractable metals (SEM), organic carbon (OC), and the oxyhydroxides of iron and manganese are important factors influencing these partitioning processes.⁸⁻¹¹ Several of these factors are employed by equilibrium partitioning (EqP) models to predict metal bioavailability in sediments. The widely used AVS-SEM approach is based on the formation of relatively insoluble metal sulfides from dissolved metals, and thus sediments with an excess of AVS to SEM are predicted to have low dissolved metal concentrations in pore waters and are unlikely to exhibit adverse effects on benthic organisms.^{12,13} Like all EqP approaches, the models do not account for contaminant exposure that may occur through ingestion of particles by deposit-feeding organisms.¹⁴⁻¹⁷

Over the past decade, increasing research has focused on investigating the potential of the diffusive gradients in thin films (DGT) technique as a tool for assessing the lability and dynamics of metals in sediments.¹⁸⁻²⁰ By selectively accumulating divalent metals onto a Chelex[®]-embedded hydrogel layer, the DGT device measures labile metal species present in waters and weakly-bound metals that may be released from the sediment particulate phase.²¹ However, few studies have used the approach for environmental toxicology purposes, where the DGT technique, through the measurement of a combined pool of labile metals, has potential to assist in predicting metal bioavailability, improve the interpretation of exposure-

effects relationships and predicting toxicity.²²⁻²⁶ Roulier et al.²² found a significant correlation between labile copper and lead, but a weak correlation for cadmium, measured by DGT in sediment pore waters and bioaccumulation of these metals in the freshwater crustacean *Chironomus riparius* (chironomid) after a 7-day exposure to contaminated freshwater sediments. Dabrin et al.²³ showed that *C. riparius* could mobilize cadmium from sediment phases and particle ingestion was likely to be a major exposure route, whereas the similar cadmium accumulation rates in *Potamopyrgus antipodarum* (freshwater mud snail) and DGT indicated that pore water was the main exposure route for this species. In a comparison between total concentrations, the AVS-SEM model (normalized to OC) and DGT measurements, Costello et al.²⁴ found that labile nickel measured by DGT (DGT-Ni) was useful for interpreting changes in nickel partitioning from AVS to nickel associated with iron and manganese oxyhydroxide phases, but determined that (SEM-Ni - AVS)/foc relationships were superior to DGT-Ni for predicting freshwater invertebrate responses to sediment nickel. DGT-labile copper fluxes measured at the sediment water interface (SWI) successfully predicted adverse effects on the survival of the deposit-feeding estuarine bivalve *Tellina deltoidalis* in sediments spiked with copper-based antifouling paint particles.²⁵ While these studies are promising, they also indicate that a greater understanding is required before metal lability provided by DGT measurements becomes routinely used for environmental risk assessments.

The DGT technique is capable of measuring labile metals from different compartments of the sediment (overlying water, SWI, deeper sediment), representing different organisms habitats, and to provide *in situ* time-integrated measurements. These unique advantages may considerably improve the assessment of metal bioavailability in sediments. In this study, survival and sub-lethal effects on reproduction of the estuarine-marine amphipod *Melita plumulosa* were assessed in a 10-day whole-sediment toxicity test performed with naturally-contaminated sediments with varying chemical and physical properties. The aim was to compare the dose-response relationships obtained using traditional measurements of metals in sediments and overlying waters with those achieved when DGT-labile metals represented the dose.

MATERIALS AND METHODS

General Methods. All glass and plastic-ware used for analyses were new and cleaned by soaking in 10% (V/V) HNO₃ (BDH, AnalR) for ≥ 24 h. For analytes above trace

concentrations new or recycled acid-washed (10% HNO₃, 24 h) containers were used. Glass beakers used for bioassays were washed in a dishwasher (Gallay Scientific) with detergent followed by acid washing (1% HNO₃) and Milli-Q water rinsing. All chemicals were analytical reagent grade or equivalent analytical purity. The fine sediment fraction was determined by wet sieving sediments with a nylon sieve (<63 µm mesh) followed by gravimetry. Sediment chemical analyses were performed in duplicates on subsamples of homogenized sediment in the week before transfer into test vessels. Filtered aliquots (0.45 µm) sampled from overlying waters (OLW) were used for dissolved metal analysis. Total recoverable metals (TRM) were analysed following low-pressure aqua regia microwave digestion of sediments (3:1 HNO₃:HCl, CEM MARS 5). Metals in waters and acid digests were analysed by inductively coupled plasma-atomic emission spectrometry (ICP-AES, Spectro Ciros CCD). For quality control purposes, all metal analyses were performed in duplicates and recoveries of certified reference material (PACS-2, National Research Council Canada) were between 85 and 120% of the expected value. Methods used for analyses of dilute acid-extractable metals (AEM, 60 minutes 1 M HCl digestion), total organic carbon (TOC, by loss on ignition) and acid-volatile sulfide (AVS) have been previously described.²⁷

Sediment Sampling and Preparation. Control sediments were collected from an intertidal estuarine site at Bonnet Bay (BB) Sydney, Australia, previously demonstrated to be suitable for laboratory culturing and to sustain high rates of reproduction for the amphipod *M. plumulosa*.²⁸ To explore a wider range of less contaminated control sediments, the BB sediment was diluted with clean Sydney sand (SS, 0.6-1.5 mm particle size) to create five controls with 20, 50, 70, 90 and 100% of <63 µm particle fraction (C1 - C5). Five contaminated sediments were sampled from two sites in each of Five Dock Bay (S4 and S10) and Kings Bay (S2 and S5) in Sydney Harbour and one site from Port Kembla (S8), Wollongong, Australia. Sediments from Sydney Harbour have been exposed to many decades of anthropogenic pollution from surrounding areas and the two sites were selected based on past studies showing metals were the major form of contaminants.²⁹ Port Kembla hosts one of the largest Australian industrial complexes comprising a steelworks and a now decommissioned copper smelter, both established in early 1900s. The sediments had varying physical and chemical composition (e.g. levels of contamination, organic carbon (OC) content, AVS concentrations and particle size). Sediments were transported to the laboratory, sieved (4 mm mesh), homogenized and stored at 4°C in the dark. To increase the number and variety of sediments, five additional contaminated sediments were created by diluting the

contaminated sediments with the cleaner BB and SS materials: S7 = S10:BB:SS at ratios of 1:0.25:0.75, S3 = S5:BB:SS at ratios of 1:0.30:0.70, S9 = S8:SS at a ratio of 1:1, S6 = S8:SS at a ratio of 1:3 and S1 = S8:BB at a ratio of 1:1 (Table 1). The collected or prepared sediments were stored for up to 6 months before use. While this storage period would be inappropriate for assessing sediment quality at the specific sites, it was suitable for the purpose of this study which considered the metal bioavailability at the time of testing. Test seawater was collected from Cronulla, Sydney, filtered (0.45 μm) and stored in a temperature-controlled room at $21 \pm 1^\circ\text{C}$. Salinity was adjusted to 30 ‰ by adding Milli-Q water.

Amphipod Bioassay. *Melita plumulosa* is an epi-benthic deposit feeding amphipod found in estuarine and marine sediments in south-east Australia, and is frequently used for assessing acute and chronic effects of sediment contaminants.^{10,11,28,30,31} The species burrows to depths of 5 mm below the sediment water interface, but does not create permanent burrows. *M. plumulosa* was cultured according to Spadaro et al.²⁸ Sediment toxicity was assessed by exposing amphipods to control and contaminated sediments over 10 days. The chronic bioassay assessed both reproduction and survival endpoints, and was adapted from Mann et al.³⁰ according to Simpson and Spadaro.³¹ During the 10-day exposure, females undergo two reproductive cycles producing two separate broods. Adverse effects on amphipod reproduction were assessed by counting the number of embryos and juveniles of the second brood only at test completion, as the first brood is less affected by contamination as conception may have occurred prior to test commencement.³⁰ Homogenized sediments (80 g) and filtered seawater (200 mL) were added to 250 ml glass beakers and incubated (Labec Refrigerated Cycling Incubator, Laboratory Equipment) at $21 \pm 1^\circ\text{C}$ for 14 days prior to the beginning of the test. Oxygen concentrations in overlying waters were kept within 80-110% saturation by using an air purging system, salinity was $30 \pm 1\text{‰}$, pH 8.1 ± 1 and temperature $20 \pm 2^\circ\text{C}$. All sediment bioassays were performed in quadruplicate.

On day 0, amphipods (5 females and 7 males) were randomly assigned to each beaker and placed in the environmental chamber (12:12-h light:dark cycle, light intensity of $3.5 \mu\text{mol photons/s/m}^2$). Animals were fed three times evenly distributed over the test using Sera[®] Micron fish food at a rate of 0.5 mg/amphipod. On day 5, the first brood was discarded by gently sieving the sediment using a 600- μm mesh sieve. Adults trapped in the sieve were transferred to 1 L beakers (more suitable for DGT deployments) containing 500 g of

sediment and 700 mL of filtered sea water. These were prepared and equilibrated at the same time as the previous sediment set up (two weeks before the test commencement). The overlying water was renewed before adults were transferred. Care was taken to maintain the ratio between the sediment:overlying water volume between the two stages of the test. Sediments were placed in a controlled temperature room at $21 \pm 3^\circ\text{C}$ (normal day light conditions). On day 10 sediments were gently sieved and adults separated from juveniles using a 180- μm mesh sieve. The number of juveniles and embryos per female was counted by microscopy and expressed as a percentage of controls. Toxicity was detected when the survival or reproductive output was $<80\%$ of the control, and significantly less ($p < 0.05$) than that observed in the control.³¹

Diffusive Gradients in Thin Films. Plastic planar probes (24 cm \times 4 cm \times 0.5 cm, with open window of 1.8 cm \times 15 cm) were purchased from DGT Research (<http://www.dgtresearch.com/>). The DGT assembly featured a Chelex[®] binding gel and a polyacrylamide diffusive gel of 0.4 mm and 0.8 mm thickness, respectively, topped by a 0.45 μm polysulfone filter membrane.²⁵ Probes assembly, handling and gels preparation were performed following standard procedures recommended by DGT Research (Lancaster, UK). Before amphipods were transferred into new test vessels for sediment renewal (day 5), one DGT probe was gently inserted into three of the four replicate vessels. After a 24-h deployment, the probes were carefully retrieved from the sediment and the SWI depth was recorded by marking both sides of the plastic device. Probes were thoroughly rinsed with Milli-Q water and stored in clean plastic bags at 4°C until analysed. Within three weeks of retrieval, DGT probes were disassembled and binding gels sliced, using Teflon[®]-coated razor blades, to obtain three 0.5-cm slices below the SWI and one 0.5-cm slice followed by three 1-cm slices above the SWI. Each slice was weighed and extracted in 500 μL of a 1 M HNO_3 solution for 24 h. Extracted metals were diluted 10-fold with Milli-Q water and analysed by inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7500ce). Blank probes were analysed for laboratory quality control and Cd, Cu, Ni and Pb concentrations did not contribute for more than an equivalent flux of $0.5 \mu\text{g}/\text{h}/\text{m}^2$ (concentrations were usually below detection limits). Zinc contamination was consistently detected and typically contributed 15% and 40% of the measured zinc fluxes for contaminated and control sediments, respectively.

Data Analysis. To assist in the analysis of effects from the mixtures of the metals (Cd, Cu, Ni, Pb, Zn), a range of normalisation approaches were investigated to account for the known differences in the toxicity of the different metals. To provide comparison with effects-relationships based on particulate metal concentrations, mean sediment quality guideline quotients (SQGQ) were also calculated as previously described³¹ using TRM and AEM concentrations of Cd, Cu, Ni, Pb and Zn, and the SQG trigger values.⁷ Similarly, using the time-averaged overlying water concentrations, a toxic unit (TU) approach was applied to provide a conservative estimate of joint toxicity of the metals by summing the potential contributions: $TU = \sum(dCd/5.5 + dCu/1.3 + dNi/70 + dPb/4.4 + dZn/15)$, where the numerators are the dissolved metal concentrations and the denominators of 5.5, 1.3, 70, 4.4 and 15 $\mu\text{g/L}$ are the corresponding WQG threshold values. Three approaches were investigated using the measured dissolved metal flux (DGT-M) divided by either the corresponding (i) water quality guideline (WQG) values designed to protect 95% of species,⁷ (ii) 50% lethality concentration (LC50) for adult amphipod survival,³² or (iii) the corresponding SQG.⁷ These 'normalised' fluxes for metal mixtures are referred to as DGT_{WQG} , DGT_{LC50} , and DGT_{SQG} , respectively. For the DGT flux – toxicity relationships, log–logistic concentration response curves were calculated. Individual and combined effects of DGT-Cu, -Pb and -Zn fluxes to amphipod survival were investigated using a logistic regression model (using R), assuming binomial response (survival vs death) and considering interactions between up to three metal fluxes at a time (where possible). Cadmium and nickel fluxes were not considered in the regression as concentrations in the sediments were generally below SQGs threshold values and were not found to have significant effect on survival (Table S1 of the Supporting Information). Although there is no agreed equivalent to R^2 in logistic regression, we calculated pseudo- R^2 as an approximate estimate of explained variation.

RESULTS AND DISCUSSION

Sediment Chemical and Physical Properties. Concentrations of Cd, Cu, Ni, Pb and Zn in the five control and ten contaminated sediments as total recoverable (TRM) and dilute acid-extractable (AEM) metals are shown in Table 1 and Table S1 of the Supporting Information. Metal concentrations in control sediments were generally below the SQG trigger values (interim SQG-Low⁷), whereas the concentrations of Cu, Pb and Zn greatly exceeded the SQGs in most of the contaminated sediments. The TOC concentrations were marginally lower in the control sediments (from 0.7 to 3.2%) than the contaminated sediments (from 1.4

to 6.6%). The fine sediment fraction (<63 μm) ranged from 20 to 100% and from 15 to 100% in control and contaminated sediments, respectively. The AVS concentrations in contaminated sediments ranged from <0.5 to 7.4 $\mu\text{mol/g}$, except for sediment S2 which was considerably higher (30 $\mu\text{mol/g}$). The difference between AVS and SEM (Σ Cd, Cu, Ni, Pb and Zn, where AEM = SEM) concentrations indicated a molar excess of SEM over AVS (Table 1), and the potential for adverse effects from these metals to the amphipod.^{12,16}

The AEM measurements (1 M HCl) provide information on the portion of metals associated with the potentially more labile and biologically available sediment phases. When AEM and TRM concentrations are similar, it indicates that the labile fraction of metals may represent a large portion of the total sediment metals. In contaminated sediments, TRM and AEM concentrations of zinc and lead were very similar, suggesting that a large portion of these metals was present in potentially bioavailable forms (AE-Zn/TR-Zn \sim 0.8, 1 and 0.7 and AE-Pb/TR-Pb \sim 0.6, 0.9 and 0.9 for the Kings Bay (S2, S3, S5), Five Dock Bay (S4, S7, S10) and Port Kembla (S1, S6, S8, S9) sediments, respectively). In the Port Kembla sediments the AE-Cu/TR-Cu ratio was \sim 0.5, whereas in Kings Bay and Five Dock Bay sediments the AE-Cu/TR-Cu ratio was <0.2, indicating stronger binding of copper for those sediments.

For the diluted contaminated sediments (S1, S3, S6, S7, S9; Table 1), the TR-Cu, Pb and Zn concentrations were within 10% of the concentrations expected based on the undiluted materials. The AE-Pb and AE-Zn concentrations of the diluted sediment were within 25% of that expected based on dilution, while AE-Cu was considerably greater in the diluted sediments S3 and S7 compared to the original sediments (S5 and S10, respectively) (Table 1). This was attributed to oxidation of the sediments, as was evident by the decrease of AVS concentrations from original to diluted sediments (Table 1). While PbS and ZnS phases are readily extracted in 1 M HCl (as AE-Pb and AE-Zn), copper sulfide phases (expected to be predominantly Cu_2S , rather than CuS^{33}) are poorly soluble in 1 M HCl.¹⁶ The oxidation of copper sulfide phases will result in increased amounts of copper phases measured as AE-Cu (e.g. copper associated with organic matter and iron oxyhydroxide phases).

DGT Profiles in Sediments and Overlying Waters. DGT-labile copper, lead and zinc showed similar magnitude of fluxes and vertical profiles for all sediments (Figure 1). Fluxes of Fe(II) and Mn(II) indicated regions of reductive dissolution between 0 and 1.5 cm below the SWI, with the reduction zone of oxyhydroxide phases of iron 0.5-1.5 cm below the SWI and manganese typically 0.5 cm above zone for iron (Figure S1 of the Supporting

Information). The increased fluxes of copper, lead and zinc to the DGT probe at this depth observed in most of the contaminated sediments (Figure 1) was consistent with previous studies indicating that metal mobility in pore waters is linked to dissolution of iron and manganese oxyhydroxide phases.^{19,34,35} Other processes that may contribute to the formation of DGT maxima in pore waters near the SWI are the degradation of organic matter^{20,36,37} and oxidation of metal sulfides.^{25,26, 38,39} In the undiluted Kings Bay sediments S2 and S5, copper fluxes were greater in the overlying waters, indicating a considerable release of copper from the sediment to the water column. Such metal release is usually observed due to oxidation of organic matter and AVS in surficial sediments^{16,24,25,40} and was consistent with increasing overlying water copper concentrations measured over the test (Table S2 of the Supporting Information). Differences in DGT metal fluxes measured in the overlying water may be related to the different affinity of metals for organic ligands that were likely to have been released to the water column, as well as the partitioning of the dissolved organic matter between colloidal and soluble phases. Zhang and Davison⁴¹ observed that in a humic-rich freshwater stream more than 50% of the copper was associated with organic substances. By separating seawater samples into different fractions, Wells et al.⁴² found that copper was largely associated with colloidal organic ligands (>1 KDa), while the majority of zinc and cadmium were bound to smaller organic compounds (<1 KDa). They also showed that the weaker copper-binding fraction was predominantly colloidal, while the <1 KDa fraction showed a higher binding strength. As a consequence, greater DGT-Cu fluxes measured in the overlying waters of the Kings Bay sediments S2 and S5 may be related to the presence of weak colloidal copper-binding organic ligands resuspended from the sediment which rapidly released labile copper to the dissolved phase.

Labile zinc fluxes were up to two orders of magnitude greater than other metals and correlated ($R^2 = 0.93$) with AE-Zn concentrations in control (C1-C5) and contaminated sandy sediments (S2, S3, S4, S5, S7, S10) (Figure S2 of the Supporting Information). Cadmium and nickel fluxes were constantly below $1 \mu\text{g}/\text{h}/\text{m}^2$, except for nickel in the diluted Kings Bay sediment S2. The DGT-Cu fluxes were low and also consistent with the low AE-Cu concentrations, although the increase of AE-Cu observed in the diluted sediments S3 and S7 compared to the original sediments S5 and S10, respectively (Table 1), did not result in a greater DGT-Cu flux. We attributed the increase in AE-Cu to a shift in copper binding from sulfide to more oxidised phases such as organic matter and iron oxyhydroxides. It is likely that copper in the porewater was also being complexed by dissolved organic matter and these

complexes were sufficiently non-labile to not be measured by the DGT. Similar magnitudes of fluxes were measured for copper and lead (Figure 1), but a considerable difference in AEM concentrations was observed (Table 1). In general, the results indicate that the AEM measurements provide an overestimation of the potentially labile lead and an underestimation of the potentially labile copper. The difference between the two techniques emphasises the complexity of evaluating the potentially bioavailable fraction of metals in sediments and a potential deficiency of using a 1 M HCl-extractable metal concentrations as the only measurement method. The DGT fluxes of Cu, Pb and Zn in control sediments were consistently lower than those measured in the medium, high and very high toxicity sediments, except DGT-Zn in the sediments S5 and S6 (Figure 1). Sediment S5 showed unexpectedly very low fluxes of Cd, Ni and Zn, which could be related to the high TOC concentration providing an additional strong metal-binding phase.⁴³ Simpson et al.⁴⁴ showed that copper bioavailability to a range of benthic organisms (including amphipod) in sediments with varying properties decreased with increasing OC concentrations. However, it was unexpected that, despite the very high zinc concentrations measured and the strong relationships between DGT-Zn and AE-Zn concentrations (Figure S2 of the Supporting Information), zinc fluxes in Kings Bay sediment S5 were much lower than the other sediments.

Survival and Reproduction of the Amphipod *M. plumulosa*. Amphipod survival and reproduction in control sediments was within acceptable levels, while adverse effects (<80% and significantly different $p < 0.05$ to controls) on survival or reproduction occurred in contaminated sediments (Table 1, Figure S3 of the Supporting Information). Decreased survival was observed in the Five Dock Bay sediments S7 and S10 and the Port Kembla sediments S6, S8 and S9, with survival ranging from 25 to 77% and from 50 to 63%, respectively, whereas in the Kings Bay sediments (S2, S3, S5) the only treatment to affect survival was S5 (71%). Significant effects to reproduction were observed for all the contaminated sediments compared to controls (C1-C5). For sediments S2 and S3 the reproduction rates decreased to 28 and 40% of that in the control sediments, respectively. In these sediments, dissolved zinc concentrations in overlying waters (up to 75 ± 14 $\mu\text{g/L}$, Table S2 of the Supporting Information) indicated that dissolved zinc was likely affecting reproduction of *M. plumulosa*, as observed in previous studies.⁴⁵ However, the dissolved zinc concentrations were generally below those causing lethality (10-day LC₅₀, LOEC and NOEC values are 220, 180 and 90 $\mu\text{g Zn/L}$ for juveniles).²⁸ For this amphipod, dietary exposure to metals by ingestion of particles is an important exposure pathway, so it is likely that metal

uptake from both overlying water and sediment was contributing to reproductive toxicity.^{10,11,28}

Relationships between amphipod survival and reproduction and particulate metal concentrations (TRM and AEM) and time-averaged dissolved concentrations in the overlying waters (OLW) are shown in Figure 2. In these relationships the combined effects of the five metals (Cd, Cu, Ni, Pb and Zn) was evaluated using SQGQ (TRM_{SQGQ}, AEM_{SQGQ}) or toxic WQG-based unit approaches (OLW_{TU}). Increasing toxicity was observed with increasing AEM_{SQGQ}, TRM_{SQGQ} and OLW_{TU} concentrations. OLW_{TU} provided the best prediction of toxicity to survival, while little difference was observed between predictions of adverse effects to reproduction. For survival, pseudo-R² values were 0.54, 0.36 and 0.92 for AEM_{SQGQ}, TRM_{SQGQ} and OLW_{TU}, respectively. Due to the large residual variation, no values were calculated for reproduction.

Relationships between DGT-metal Fluxes and Toxicity. The DGT technique has the advantage of being able to measure labile metal fluxes in different regions of the sediment profile, ranging from the overlying water up to several centimeters depth in the sediment.⁴⁶ Benthic organisms can inhabit different regions of the sediment strata, but most species reside in the top 0-15 cm region of the sediments. As *M. plumulosa* resides in the top 5 mm of sediments, but may sometimes be observed swimming a few mm above the SWI,^{10,11} relationships between DGT fluxes and biological responses were investigated considering metal fluxes measured between 5 mm above and 5 mm below the SWI only.

The rate at which the sediment responds to the localized perturbation generated by DGT device influences the time required to establish a steady-state relationship between the rates of metal uptake by DGT and porewater metal resupply by the sediments. In this study, DGT probes were deployed for 24 hours according to Harper et al.,⁴⁷ thus allowing the establishment of a pseudo steady-state and avoiding potential exhaustion of solid phase concentrations,^{9,48} as well as metal competition effects on the binding gel.⁴⁹ When the kinetics of metal desorption from the solid phase are fast enough to counteract pore water concentration depletions, pseudo steady-state conditions are rapidly approached (few hours) and a time invariant response for DGT is established. In this case, the metal resupply to the DGT device has been described as ‘sustained’ or ‘partially sustained’.⁴⁷ Conversely, in case of slow resupply or ‘diffusion only’, significant depletion of metal concentrations near the device will occur, and porewater concentrations will be resupplied by diffusion of metals in

adjacent pore water along a concentration gradient toward the DGT device. As a consequence, longer deployment times (or different probe assembly) are required.²¹ Recent studies^{9,48} have shown that, for some metals and sediment types, 24 hours is a sufficient time to establish pseudo steady-state conditions (using standard 0.8 mm diffusive gel thickness). If such conditions are not approached within the deployment time, metal fluxes will be overestimated resulting in overly protective estimations of toxicity, which is still a preferred scenario to underestimating potential risks. Slow or 'diffusion only' rates of resupply are due to either a small pool of metals or slow kinetics of metal desorption from the solid phase to the pore water.⁴⁷ It is reasonable to assume that, in these cases, metal exposure to benthic organisms will be limited. However, a portion of relatively strongly-bound metals may become available after passing through animal guts and potentially not being detected by DGT.

The relationships between the amphipod survival and reproduction and DGT-metal fluxes are shown in Figure 3. In an attempt to account for the varying degree of toxicity caused by different metals, the time-integrated DGT-metal fluxes of each metal, which represented the dose in the dose-response relationships, were normalised based on WQGs, LC50s, or SQGs (as described in methods). Overall, all three approaches resulted in similar dose-response relationships between normalized DGT fluxes and amphipod survival. This was despite the wide range of metal concentrations and large variations in sediment properties such as AVS, TOC and particle size. Normalisation of DGT fluxes based on the LC50s did not improve the correlation between DGT fluxes and biological responses compared to WQGs, even though the LC50 values are specific to *M. plumulosa*, whereas the WQGs were derived by exposing a wide range of organisms to individual contaminants.⁷ The dose-response relationship obtained by normalising DGT fluxes to WQGs provided a better fit than those normalised to the SQGs (Figure 3), although all relationships appeared quite similar. For survival, pseudo- R^2 of 0.67, 0.50 and 0.50 were calculated for fluxes normalized to WQGs, SQGs and LC50s, respectively. The SQGs are based on effects databases obtained by combining biological and chemical data from laboratory or field toxicity tests where animals were exposed to sediments containing mixtures of contaminants. Toxicity effects were thus equally ascribed to all metals present in the sediment although some contaminants might have not been present in concentrations sufficient to cause the observed adverse effects.⁵⁰ As a result, SQGs derived from this empirical approach may be considerably lower than necessary to provide protection for some metals.

As WQGs are intended to be protective of effects of dissolved metals to all aquatic species, and the DGT-fluxes can be most closely related to this exposure, we consider the normalisation to WQGs to be the most appropriate of these approaches when considering metal mixtures (e.g. DGT_{WQG} fluxes). Like EqP-approaches, this approach does not explicitly consider the potential effects of dietary metal exposure, which is particularly important for *M. plumulosa*.^{10,11} Also not considered are the possible interactive effects of metal mixtures. In relation to dietary metal exposure, at least for the sediments studied, the strong relationships between the DGT-metal fluxes and toxicity (Figure 3) indicates that dietary metal exposure has a minor contribution to the observed effects or is proportional to the DGT_{WQG} fluxes. If the latter is true, the labile fraction of metals represented by the DGT flux may potentially be a useful surrogate for the lability of metals for all exposure routes.

Applying DGT-metal Fluxes in Assessments. The DGT_{WQG} fluxes provided a strong dose-response relationship and a robust predictor of the combined toxicity of the metals Cd, Cu, Ni, Pb and Zn to the survival of *M. plumulosa* (Figure 3a). While this is the first such application of the DGT technique for this purpose, the DGT_{WQG} fluxes allow the calculation of LC10, LC20 and LC50 values (95% confidence limits) of 24 (15-51), 36 (26-56) and 72 (56-93) $\mu\text{g}_{WQG}/\text{h}/\text{m}^2$ for this species and these may be suitable as a preliminary acute effects thresholds for protection of benthic invertebrates in these sediments. The thresholds for sub-lethal effects to reproduction based on calculated effects concentrations (EC) of EC10, EC20 and EC50 will be 14 (8-27), 17 (11-28) and 25 (20-32) $\mu\text{g}_{WQG}/\text{h}/\text{m}^2$, respectively. Thus, adverse effects on survival and reproduction were predicted for DGT_{WQG} fluxes exceeding 36 and 17 $\mu\text{g}_{WQG}/\text{h}/\text{m}^2$, respectively.

For the overall trend, logistic regression models showed significant relationships between survival and DGT-Zn and DGT-Cu fluxes ($p < 0.001$). Contributions from combined effects of metal fluxes were not significant ($\alpha = 0.05$) and therefore excluded by the model. In Five Dock Bay sediments (S4, S7, S10), significant effects to survival were only observed for DGT-Cu ($p = 0.046$), although a trend between DGT-Zn and survival clearly occurred (Figure S4 of the Supporting Information). This was likely due to interactions between DGT-Cu and DGT-Zn variables causing an underestimation of any DGT-Zn influence on toxicity. When DGT-Cu and -Pb were excluded by the model, the relationship between DGT-Zn and survival was significant ($p = 0.002$). For the Kings Bay and Five Dock Bay sediments, sub-lethal effects to reproduction could not be attributed to any one metal or combination, but based on the higher

DGT fluxes, Cu, Pb and Zn were considered to be the major contributors to the toxicity. For the Port Kembla sediments (S1, S6, S8, S9), the relatively high DGT-Cu fluxes (up to $31 \mu\text{g}_{(WQG)}/\text{h}/\text{m}^2$) and DGT-Pb fluxes (up to $2.3 \mu\text{g}_{(WQG)}/\text{h}/\text{m}^2$, ten-fold higher than the highest measured in controls) indicated that copper and lead may be the major contributors to the toxicity (other metal fluxes were similar or slightly higher than controls), although no significant relationships were observed ($\alpha=0.05$).

The frequent observation of sub-lethal effects at metal fluxes below the LC20 for survival ($36 \mu\text{g}_{(WQG)}/\text{h}/\text{m}^2$) highlights the importance of evaluating sub-lethal endpoints when assessing sediment quality. The dose-response relationships between DGT_{WQG} fluxes and amphipod survival and reproduction identifies three main areas which describe the relationships between DGT and toxicity: (i) a region which significantly affects survival for DGT_{WQG} fluxes $>36 \mu\text{g}_{(WQG)}/\text{h}/\text{m}^2$; (ii) a region which affects reproduction but not survival for fluxes between 17 and $36 \mu\text{g}_{(WQG)}/\text{h}/\text{m}^2$; and (iii) a no-observed effect (to reproduction or survival) region for fluxes $<17 \mu\text{g}_{(WQG)}/\text{h}/\text{m}^2$.

Evaluating contaminants bioavailability in the environment is a very complex task and there is the need of rapid and effective tools to overcome otherwise laborious and time consuming practices. The dose-response relationships based on the DGT-metal fluxes (Figure 3) were consistent with those created using more traditional measures of metal exposure (Figure 2). The considerable advantages of providing time-integrated *in-situ* measurements makes DGT a more powerful technique compared to grab samples of water which provide a single 'snapshot' in time, or of sediments in which the metal bioavailability can be highly variable and difficult to characterise using other techniques.² The present study adds further elements to the increasing body of evidence sustaining the suitability of the DGT technique as a tool for predicting metal toxicity in sediments.^{22,23,25} Although the technique appears to have limitations to predict toxicity caused by particle ingestion and dietary behaviours,^{22,51} in this study adverse effects to survival and reproduction of the deposit-feeder amphipod *M. plumulosa* were well predicted by DGT. This supports the hypothesis that the DGT-labile metal flux may potentially be a useful surrogate for the lability of metals for all exposure routes. However, further research is required specially to evaluate whether relationships observed in laboratory-based experiments apply to real environmental scenarios. DGT applicability in the field should be further investigated and difference between laboratory and field adequately evaluated.

■ ACKNOWLEDGMENTS

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Supporting Information

Supporting Figures: S1 (DGT-Fe and DGT-Mn vertical profiles in pore waters and overlying waters), S2 (Relationships between DGT-Zn fluxes and AE-Zn concentrations in control and contaminated sandy sediments), S3 (Amphipod survival and reproduction in contaminated sediments), S4 (Dose-response relationships between amphipod survival and reproduction and DGT-Zn, -Cu and -Pb fluxes).

Supporting Tables: S1 (Metal concentrations in the control and contaminated sediments), S2 (Dissolved Cu, Zn and Pb concentrations in Kings Bay contaminated sediment overlying waters measured throughout the test and respective Water Quality Guidelines).

This information is available free of charge via the Internet at <http://pubs.acs.org/>.

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List of Figures

Figure 1. DGT profiles of Zn, Pb, Cu, Ni and Cd measured within 1.5 cm of the sediment water interface in test vessels during the amphipod bioassay. Points represent average values of three replicates (standard deviations ranged between 30 and 40% of mean values). Shaded areas indicate the DGT profile from which flux measurements were used when interpreting the toxic exposure.

Figure 2. Dose-response relationships between amphipod survival and reproduction and different methods: a) AEM concentrations (Σ Cd, Cu, Ni, Pb and Zn) normalized to sediment quality guidelines (SQGs); b) TRM concentrations (Σ Cd, Cu, Ni, Pb and Zn) normalized to SQGs; c) OLW concentrations (Σ Cd, Cu, Ni, Pb and Zn) normalized to water quality guidelines (WQGs). Mean values of AEM and TRM were calculated for $n = 2$ and reported with standard deviation, while OLW concentration means are calculated for $n = 20$ and $2 < n < 6$ for contaminated and control sediments, respectively, reported with standard deviation. Survival and reproduction were mean \pm standard error ($n = 4$).

Figure 3. Relationships between amphipod survival and reproduction and DGT fluxes normalized to a) WQGs, b) LC50 values and c) SQGs (as described in methods). Mean values of DGT fluxes (Σ Cd, Cu, Ni, Pb and Zn) were calculated for $n = 3$ and reported with standard deviation. Survival and reproduction are mean \pm standard error ($n = 4$).

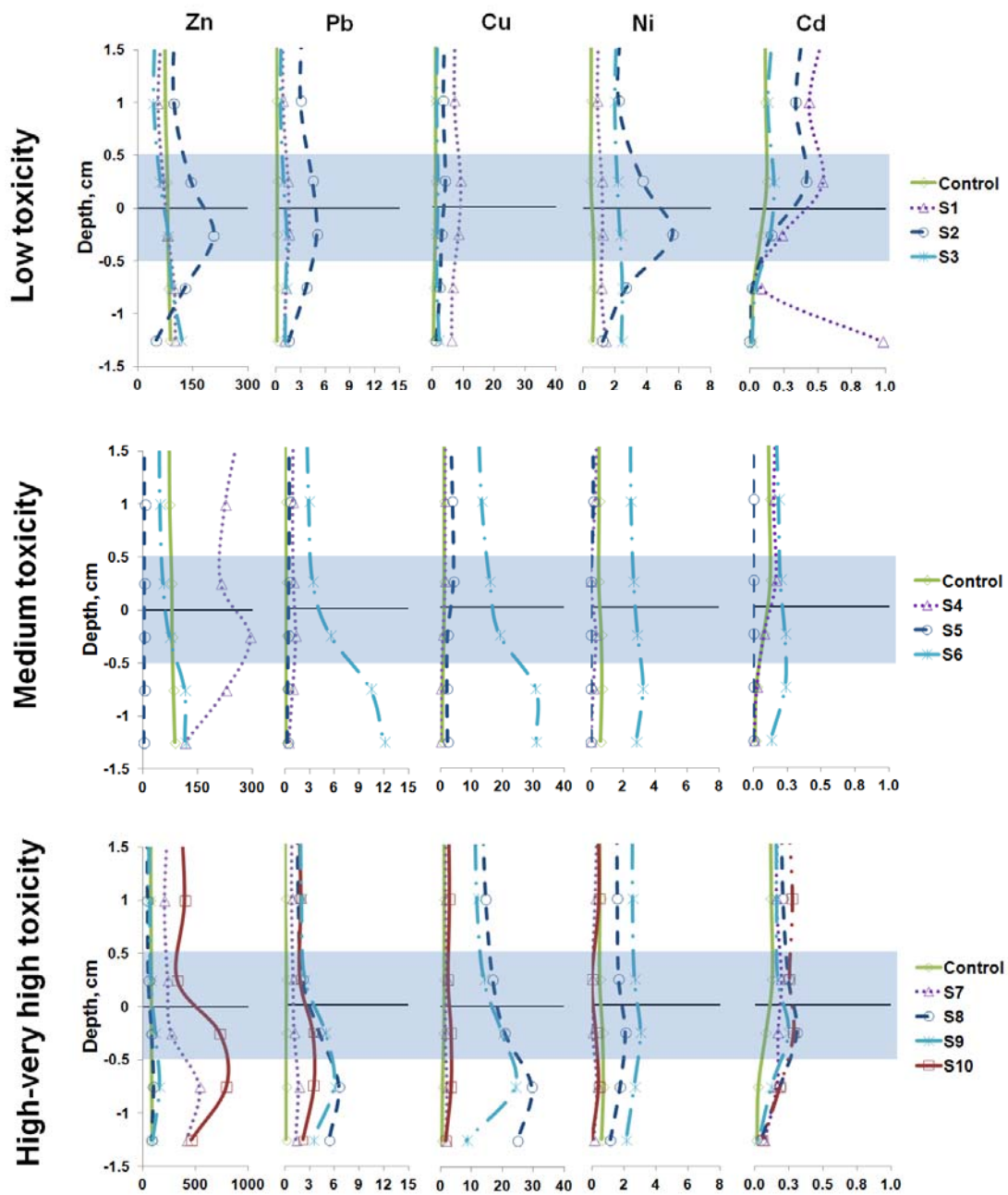


Figure 1.

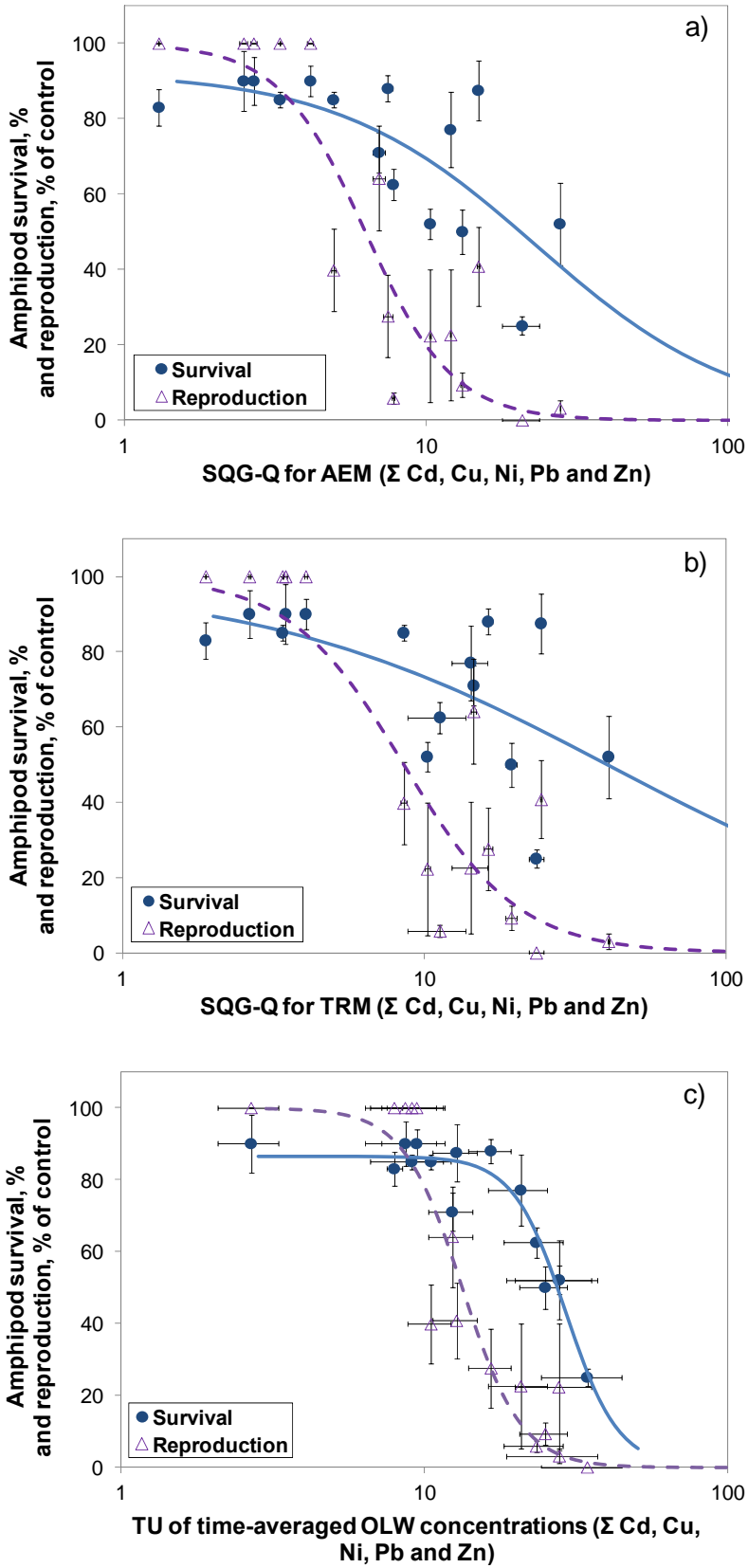


Figure 2.

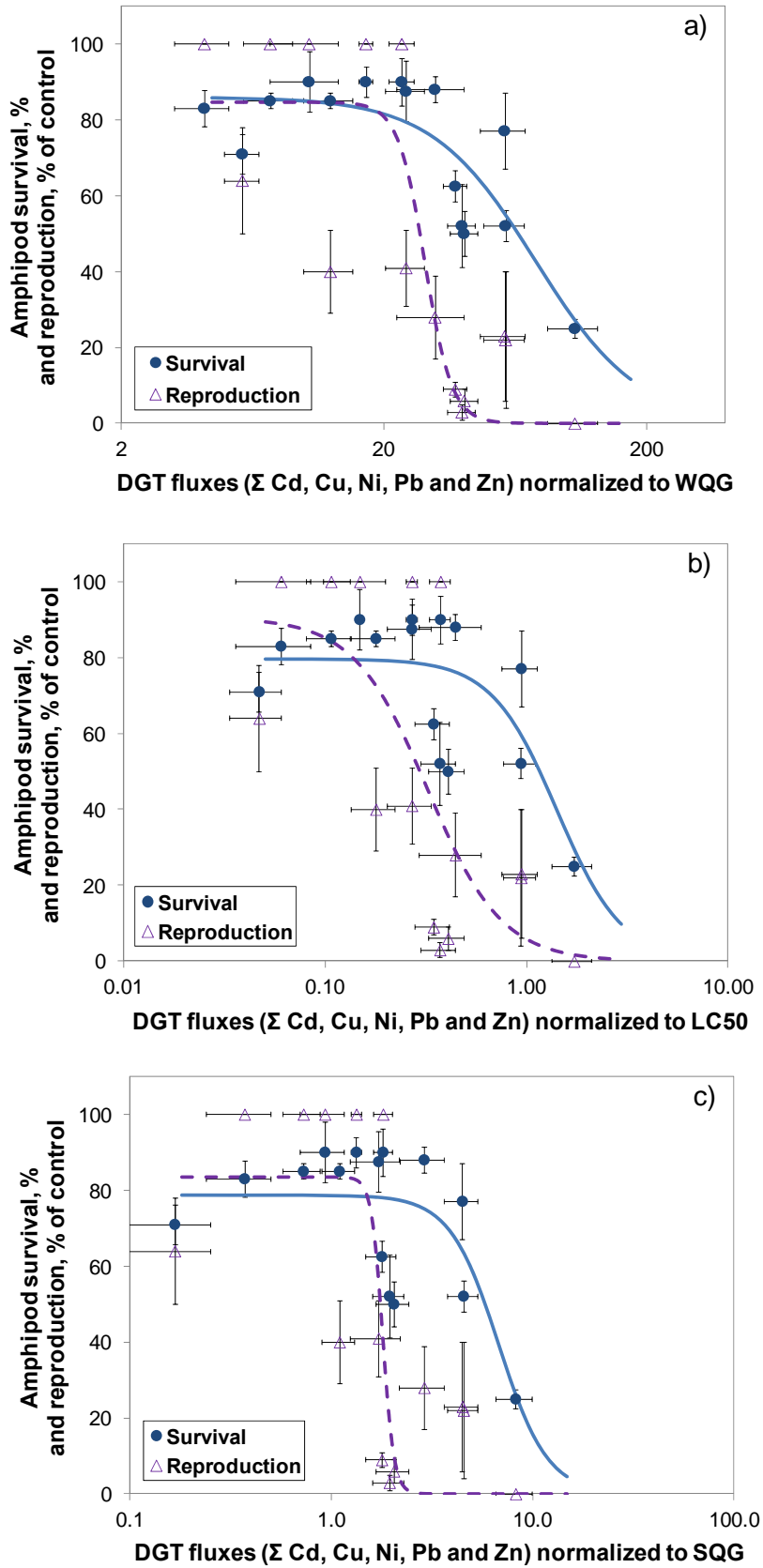


Figure 3.

Table 1. Physical and chemical properties and toxicity of the control and contaminated test sediments.

Level of toxicity	Sediment	Toxicity			TRM, mg/kg			AEM, mg/kg			AVS	SEM-AVS	TOC	<63 μm
		Survival %	Reproduction%		Cu	Pb	Zn	Cu	Pb	Zn	$\mu\text{mol/g}$	$\mu\text{mol/g}$	%	%
Control	C1	90 \pm 6	100		22	41	100	14	41	88	<0.5	1.9	1.7	50
	C2	90 \pm 4	100		38	68*	180	22	70*	150	<0.5	3.2	3.1	90
	C3	90 \pm 8	100		35	65*	190	23	59*	160	<0.5	3.1	3.6	100
	C4	85 \pm 2	100		26	50	120	13	50	100	<0.5	2.1	2.6	70
	C5	83 \pm 5	100		9	21	42	4	17	34	<0.5	0.7	0.7	20
LOW	S1	88 \pm 8	41	\pm 10	620*	450*	900*	260*	380*	570*	<0.5	15	4.7	100
	S2	88 \pm 3	28	\pm 11	220*	360*	710*	<1	190*	490*	30.1	-22	4.3	70
	S3	85 \pm 2	40	\pm 11	120*	170*	340*	20	120*	270*	1.5	4	3.1	30
MEDIUM	S4	77 \pm 10	23	\pm 17	80*	180*	1600*	3	150*	1600*	7.4	18.5	4	15
	S5	71 \pm 5	64	\pm 14	230*	310*	620*	3	180*	470*	4.1	4	6.6	30
	S6	63 \pm 4	6	\pm 2	290*	220*	400*	150*	200*	270*	<0.5	9	1.4	25
HIGH	S7	52 \pm 4	22	\pm 18	55	110*	1200*	16	120*	1300*	1.9	19.5	1.4	25
	S8	52 \pm 11	3	\pm 2	1070*	760*	1500*	540*	700*	980*	<0.5	36	5.7	100
	S9	50 \pm 6	9	\pm 3	510*	360*	680*	240*	340*	480*	<0.5	19	2.9	50
VERY HIGH	S10	25 \pm 2	0		110*	260*	2900*	6	220*	3000*	5.1	42.8	1.8	25
	SQGs				65	50	200	65	50	200				

TRM = total recoverable metals; AEM = dilute acid-extractable metals; AVS = acid-volatile sulfide; SEM-AVS = the molar difference, where SEM is equivalent to AEM. TOC = total organic carbon and % <63 μm refers to the percentage (by weight) of fine sediment particles. All concentration are the mean of two analyses for each sediment, with variability between measurements of <30%. Survival and reproduction are mean \pm standard error ($n = 4$). Concentrations of cadmium and nickel were generally below the SQGs for TRM and always for AEM and are provided, along with iron and manganese, in the Table S1 of the Supporting Information. Concentrations with an asterisk exceed the SQGs⁷

Supporting information:

Diffusive gradients in thin films technique provide robust prediction of metal bioavailability and toxicity in estuarine sediments

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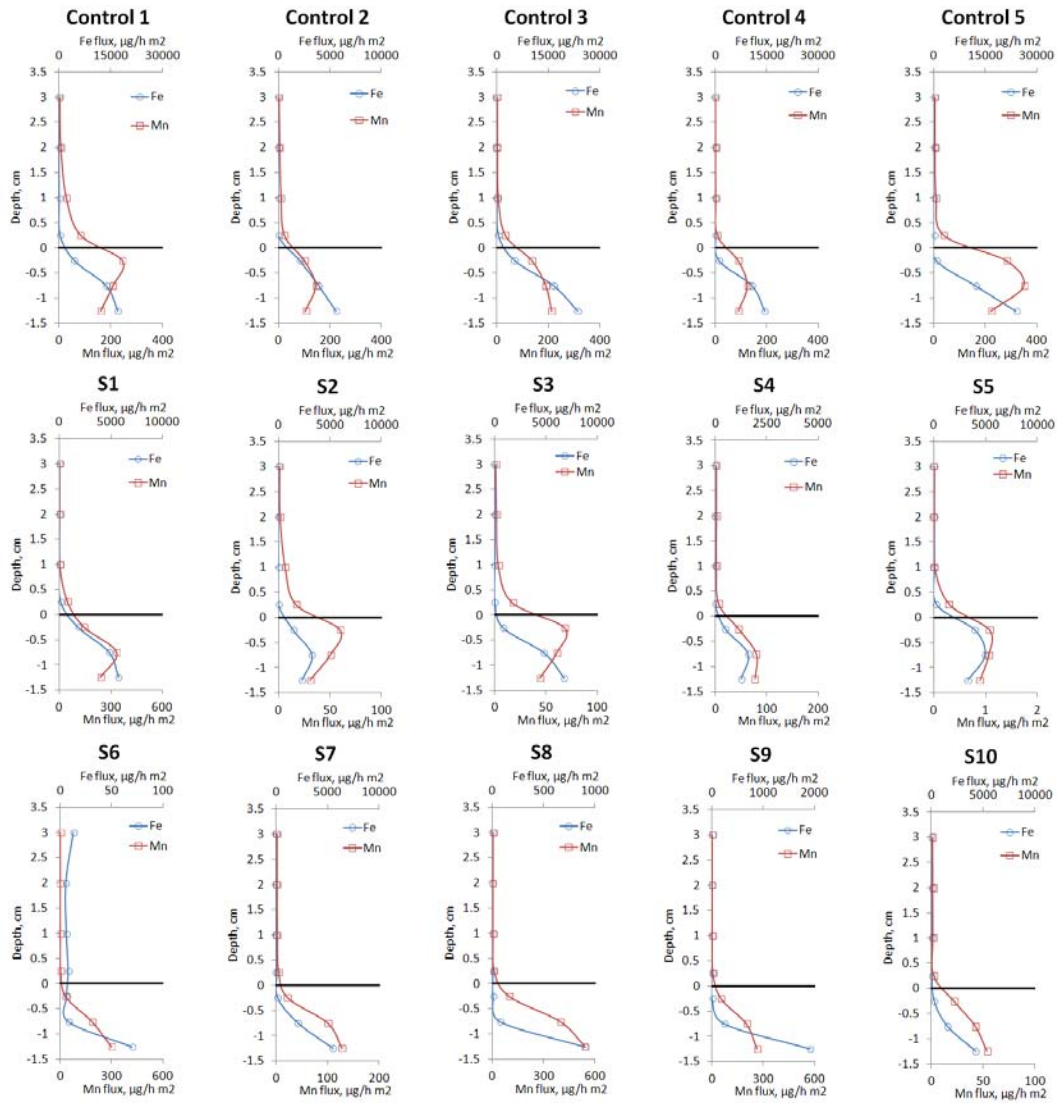


Figure S1. DGT-Fe and -Mn vertical profiles in pore waters and overlying waters (average values, $n = 3$, standard deviations ranged between 50 and 60% of mean values).

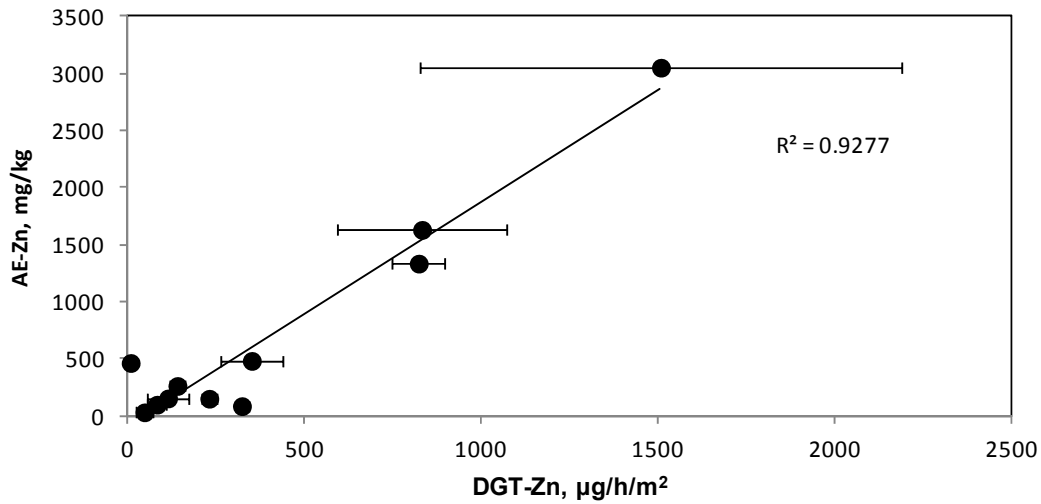


Figure S2. Relationships between DGT-Zn fluxes and AE-Zn concentrations in control (C1-C5) and contaminated sandy sediments (S2, S3, S4, S5, S7, S10). DGT data are reported as mean values ($n=3$) with standard deviation. Variability in metal concentration between treatments (of the same homogenised sediments) was less than 30%.

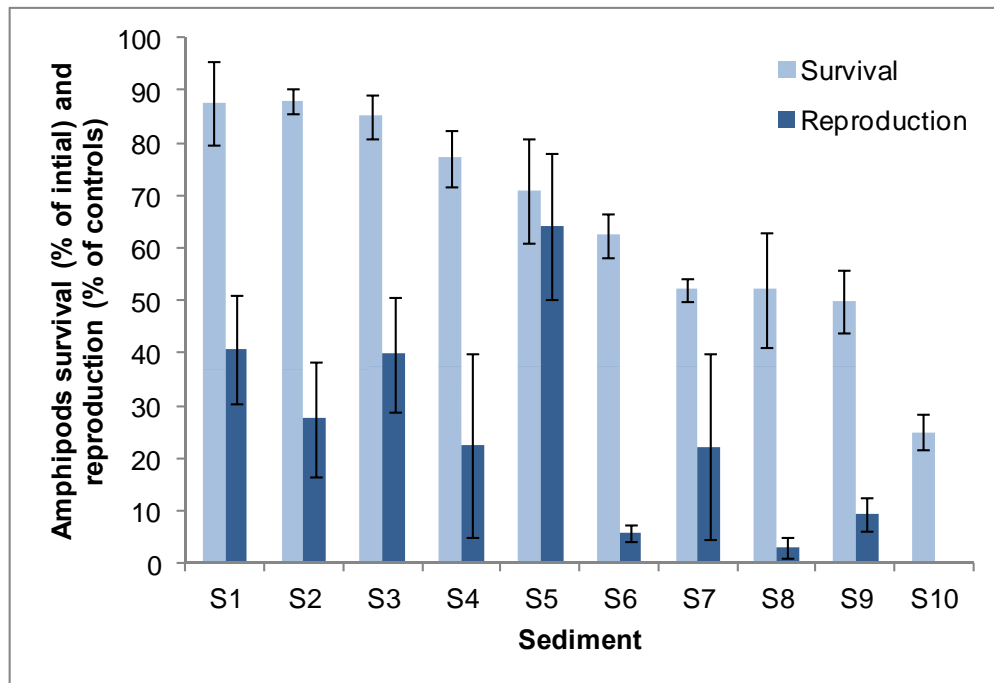


Figure S3. Amphipod survival (percentage of the number of animals placed in each test vessel at the beginning of the test) and reproduction (percentage of controls) in contaminated sediments: Kings Bay (S2, S3, S5); Five Dock Bay (S4, S7, S10); Port Kembla (S1, S6, S8, S9). Error bars of average values ($n = 4$) are expressed as standard error.

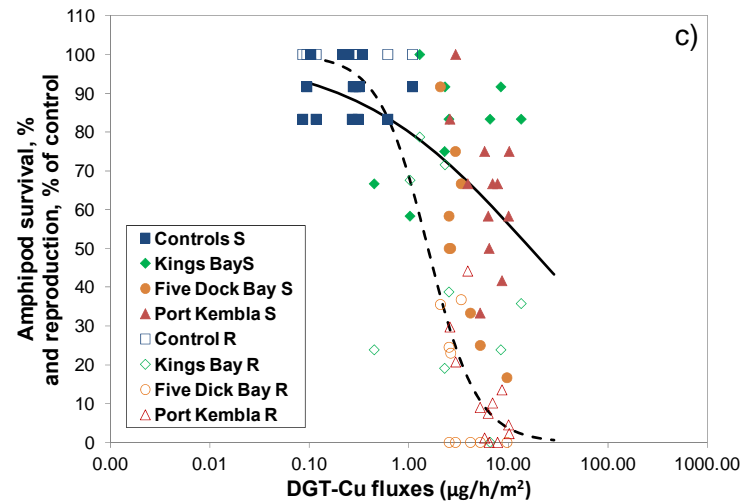
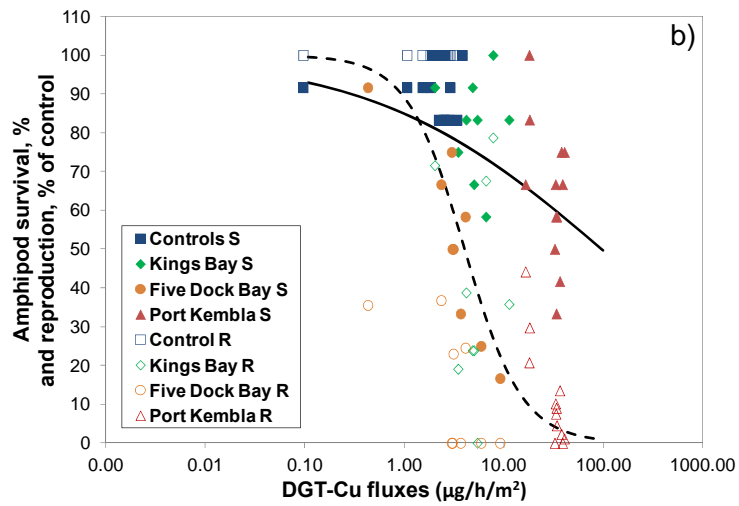
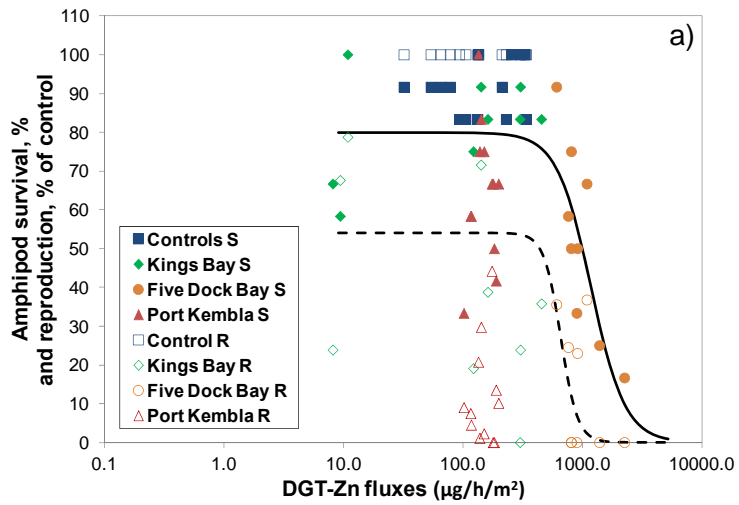


Figure S4. Dose-response relationships between amphipod survival and reproduction and DGT-Zn, -Cu and -Pb fluxes. Solid and dashed lines represent survival and reproduction fitting curves, respectively.

Table S1. Metal concentrations in the control and contaminated sediments

Sediment	TRM, mg/kg				AEM, mg/kg			
	Cd	Ni	Fe	Mn	Cd	Ni	Fe	Mn
C1	<1	13	17800	66	<1	11	5100	38
C2	<1	7	6350	28	<1	5	1800	22
C3	<1	9	15200	56	<1	7	5400	37
C4	<1	13	24500	87	<1	9	9400	58
C5	<1	12	25800	100	<1	3	10900	71
S1	<3	19	44800	320	<1	5	12400	130
S2	<1	22*	17200	76	<1	5	4300	23
S3	<1	14	12500	57	<1	5	4000	21
S4	<1	13	12200	81	<1	5	4100	16
S5	<1	18	15900	74	<1	7	5200	19
S6	<3	7	15800	140	<1	8	5000	19
S7	<1	11	12300	61	<1	8	15300	200
S8	<3	23*	56900	500	<1	4	4100	32
S9	<3	13	27900	230	<1	2	4100	57
S10	<1	17	17700	100	<1	6	6000	20
SQGs	5	21			5	21		

TRM = Total recoverable metals; AEM = dilute acid-extractable metals. The SQGs are the trigger values from ANZECC/ARMCANZ,⁷ and concentrations with an asterisk exceed the SQG.

Table S2. Dissolved Cu, Zn and Pb concentrations (average values with standard deviation, $n = 4$) in Kings Bay contaminated sediment overlying waters measured throughout the test and respective Water Quality Guidelines (95% species protection concentrations, ANZECC/ARMCANZ⁷).

	Sediment	Cu, µg/L	Zn, µg/L	Pb, µg/L
Day 3	S2	4.5 ± 1.0	58.7 ± 15.7	12.6 ± 5.7
	S3	3.5 ± 0.3	6.3 ± 1.7	10.2 ± 6.9
	S5	1.6 ± 0.7	30.3 ± 8.8	10.3 ± 8.7
Day 5	S2	6.1 ± 1.0	70.8 ± 22.8	16.5 ± 4.6
	S3	4.2 ± 0.7	6.1 ± 2.2	10.3 ± 3.6
	S5	3.4 ± 0.8	33.5 ± 8.0	3.5 ± 9.4
OLW water and sediment renewed				
Day 6	S2	3.7 ± 0.3	74.8 ± 13.5	36.6 ± 9.5
	S3	1.5 ± 0.6	28.9 ± 3.8	28.5 ± 4.7
	S5	2.5 ± 0.4	48.6 ± 6.9	33.8 ± 10.8
Day 7	S2	9.9 ± 1.3	59.8 ± 12.2	34.5 ± 5.3
	S3	4.2 ± 1.7	21.5 ± 3.1	31.8 ± 1.9
	S5	5.9 ± 0.9	38.6 ± 5.0	37.6 ± 7.9
Day 10	S2	10.9 ± 0.8	50.8 ± 5.5	17.6 ± 6.5
	S3	7.0 ± 0.8	23.6 ± 1.7	23.1 ± 5.2
	S5	8.0 ± 0.9	33.5 ± 4.1	26.3 ± 4.4
	WQG	1.3	15	4.4