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Keywords

influence, sediment, particle, size, organic, carbon, toxicity, copper, benthic, invertebrates, oxic, suboxic, surface, sediments

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Accounting for the influence of sediment particle size and organic
carbon on toxicity of copper to benthic invertebrates in oxic/sub-oxic
surface sediments

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'The influence of sediment particle size and organic carbon on toxicity of copper to benthic invertebrates in
oxic/suboxic surface sediments.']

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Abstract

The use of sediment quality guidelines (SQGs) to predict the toxicity of metals in sediments is limited by an inadequate understanding of exposure pathways and by poor causal links between exposure and effects. For a 10-day exposure to copper-spiked sediments, toxicity to the amphipod *Melita plumulosa* was demonstrated to occur via a combination of dissolved and dietary copper exposure pathways, but for the bivalves *Spisula trigonella* and *Tellina deltoidalis*, toxicity occurred primarily via exposure to dissolved copper. For relatively oxidised sediments that had moderate amounts of organic carbon (2.6 to 8.3% OC), silt (20 to 100% <63 µm particles) but low acid-volatile sulfide (AVS), acute toxicity thresholds for the three species were derived based on the OC-normalised copper concentration of the <63 µm sediment fraction. For all three species, no effects were observed at concentrations below 10 µg/L of dissolved copper (in pore water and overlying water) or below 12 mg Cu/g OC (for <63 µm sediment). For sediments with silt/OC properties of 20%/0.5%, 50%/1%, or 70%/4%, the particulate-copper based threshold equated to 60, 120, or 480 mg Cu/kg, respectively. For oxic/sub-oxic sediments in which AVS is not limiting metal availability, it is proposed that SQGs of this form will provide both adequate protection against toxicity and improve the prediction of effects for sediments with varying properties.

Keywords: Copper, toxicity, guidelines, organic carbon, particle size, sediment properties

INTRODUCTION

Although sediment quality guidelines (SQGs) are generally useful for predicting when toxic effects due to a specific contaminant will not occur, their major weakness is their poor ability to predict how toxicity thresholds change for different sediment types and different test species [1-3]. The toxicity of metal contaminants in sediments to benthic organisms is influenced by the chemistry and speciation of the metal, the properties of the sediments and the varying exposure pathways of different organisms [4-6]. The exposure and bioavailability of metals from both the dissolved phase (via exposure to pore water, burrow water, or overlying water) and sediment phases (via ingestion of particles), and the sensitivity of the organism to these metal exposures all need to be understood in order to predict effects [7-10].

The prediction of metal bioavailability in sediments is considerably more difficult than in waters due to the greater range of metal-binding phases and processes influencing metal exposure in sediments. For example, solid-phase speciation (metal binding with particulate sulfide, organic carbon, and iron hydroxide phases), sediment-water partitioning characteristics (flux rates), organism behaviour and physiology (burrow irrigation and uptake rates from porewater versus overlying water, feeding selectivity and assimilation efficiencies from particulates) all influence metal bioavailability [3,8,11].

The redox zones of sediments are usually stratified vertically and the distinction between the oxic (oxygen reduction), sub-oxic (nitrate, manganese and iron reduction) and anoxic (sulfate reduction and methanogenesis) layers is important for metal bioavailability [12,13]. It is widely recognised that for anoxic sediments with a molar excess of acid-volatile sulfide (AVS) over simultaneously extractable metals (SEM), the dissolved concentrations of Ag, Cd, Cu, Ni, Pb, and Zn will be negligible and acute or chronic effects should not result from these metals [13].

This knowledge forms the basis of the AVS-SEM equilibrium-partitioning model that is often utilised in SQG frameworks [14]. However, most surface sediments, including the walls of organisms' burrows, are exposed to oxygenated overlying water and their properties can be described as oxic or sub-oxic [8,12,15,16]. While AVS phases that are formed under anoxic conditions may persist in surface sediments, the concentrations are generally low and highly variable. This is when the SEM:AVS equilibrium-partitioning model becomes less useful for predicting metal bioavailability [14,17-19]. In these surface sediments, particulate organic carbon (POC) and iron oxyhydroxide phases are well recognized for their binding of metals, and increased concentrations of POC have been demonstrated to reduce the toxicity of many metals [4,11,13].

Equilibrium partitioning-based models of metal bioavailability neglect the potential exposure to metals that many organisms receive via diet [5,20]. Fine sediments with greater POC concentrations often have elevated metal concentrations due to their affinity for metals. While these properties may reduce porewater metal concentrations, the greater nutritional content and the optimum particle size for ingestion by deposit-feeding organisms may increase the rate of dietary exposure to metals [8,11,21,22]. For benthic organisms, increasing numbers of studies have demonstrated the significance of bioaccumulation and toxicity of metals from dietary exposure [7,21,23-25]. For copper, acute effects from dietary exposure have been demonstrated for the epibenthic amphipod *Melita plumulosa*, a species that ingests significant amounts of sediment while foraging for food [21,26]. For the deposit-feeding polychaete, *Arenicola marina*, metal toxicity occurs when organisms cannot cope with overwhelming influx and the subsequent accumulation rates of metals that occur predominantly through dietary exposure [7,22].

In surface sediments, the exposure of organisms to metals is expected to occur via dietary intake [5], from porewater-derived fluxes at the sediment-water interface [27], and from burrow and overlying waters [28,29]. In these sediments, the bioavailability of copper will be less influenced by AVS, but is expected to be strongly influenced by the concentrations of particulate organic matter, iron hydroxide phases, and the particle size of the sediments [3,4,8]. Costa et al. [30] reported 10-day LC50 values (concentration that resulted in 50% population death) for the amphipod *Gammarus locusta* of 6.8, 57 and >200 mg Cu/kg for sediments with the properties 0.5%FF/0.9%LOI, 25%FF/1.9%LOI, and 75%FF/7.1%LOI, respectively (where FF = fine fraction and LOI = organic matter by loss-on-ignition). Those studies used copper-spiked sediment equilibrated for only 24 h before toxicity tests were undertaken and it is likely that most of the effects were due to dissolved copper that had not reached equilibrium with the sediment binding phases [31].

To determine effects thresholds for copper in sediments, the addition of a soluble copper salt to sediments having known properties is often viewed as the best way to achieve suitable copper exposures. As already noted [3], it is essential that appropriate time be allowed for the added copper to adequately equilibrate with the sediments (e.g. at least 20 days [31,32]. Studies with very short equilibration times often produce effects data of low environmental relevance and would only be appropriate to derive SQGs for application to very recent contamination events. For example, copper-spiked sediments were equilibrated for 2 days before Bat and Raffaelli [33] assessed the survival of the amphipod *Corophium volutator*, and for 9 days before Hagopian-Schlekat et al. [34] assessed survival of the copepod *Amphiascus tenuiremis*. Toxicity to these species occurred only when dissolved copper concentrations in the porewater or overlying water were >100 µg/L, and the toxicity was attributed to dissolved metals. Because of

incomplete equilibration, these dissolved metal concentrations greatly exceeded those observed for similarly contaminated field sediments. LC50 values of 37 and 282 mg Cu/kg for *C. volutator* and *A. tenuiremis*, respectively, determined on the basis of the particulate copper exposures are therefore not appropriate for use in guideline development. It is likely that longer equilibration times would provide much lower porewater copper concentrations for these copper-spiked sediments and higher, but more environmentally realistic, effects thresholds.

This study used copper-spiked oxic/sub-oxic sediments equilibrated for 30 days to achieve the copper concentrations and sediment properties suitable for determining (i) the importance of organism exposure pathways (dissolved versus dietary) and (ii) the influence of sediment particle size and organic carbon on copper exposure toxicity to three benthic organisms, the epibenthic amphipod, *M. plumulosa*, and the benthic bivalves, *Spisula trigonella* and *Tellina deltoidalis*. Before and during tests, detailed measurements were made of sediment properties, the equilibration of the copper-spiked sediments, and the partitioning of copper between different sediment compartments, namely the solid phases and dissolved copper in the pore water and overlying water. Effects thresholds determined for copper in sediments having a range of properties were interpreted in terms of the potential copper exposure pathways, with a view to better predict effects based on these properties.

MATERIALS AND METHODS

General chemistry

Glass- and plastic-ware for analyses were usually new and were cleaned by soaking in 10% (v/v) HNO₃ (BDH Laboratory Supplies, Poole, England) for a minimum of 24 h, followed by thorough rinsing with deionized water (Milli-Q, Millipore, Academic Water System, Sydney, Australia). All chemicals were analytical reagent grade or equivalent analytical purity.

Measurements of pH of wet sediment used a spear-tipped Hana HI 2031B pH electrode (Hanna Instruments Pty Ltd, Melbourne, Australia). Water salinity, temperature and dissolved oxygen measurements were made with probes from WTW (Wissenschaftlich-Technische Werkstätten, Weilheim, Germany), each calibrated according to manufacturer instructions.

Dissolved metal concentrations in water samples and sediment digests were determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES, Spectroflame EOP, Spectro Analytical Instruments) calibrated with matrix-matched standards (QCD Analysts, Eaglewood, FL, USA). The limit of reporting (LOR) for metals in seawater was 3 to 5 µg/L for Ag, As, Cd, Cu, Cr, Co, Fe, Mn, Ni, Pb and Zn. Dissolved ammonia was analysed colorimetrically using a Merck Spectroquant Kit (14752, Merck). Dissolved organic carbon (DOC) was determined according to standard methods [35].

Methods for measurement of sediment particle size (by wet sieving through 63 µm nylon sieves followed by gravimetry), total organic carbon (OC, Dohrmann DC-190 TOC analyzer, Teledyne Tekmar, Mason, OH), and porewater (PW) extraction (centrifugation at 800 g for 5 min) have been described previously [36]. The PW and overlying water (OW) samples were membrane filtered (0.45 µm cellulose acetate, Sartorius Minisart, Sartorius, Goettingen, Germany) immediately following collection, and acidified with concentrated HNO₃ (2% (v/v) (Tracepur, Merck, Darmstadt, Germany). The concentrations of total recoverable metals (TRM) in sediments were measured following digestion of approximately 0.25 g of sediment in a mixture of 1.0 mL of concentrated HCl and 0.5 ml of concentrated HNO₃ for 24 h, then heated by microwave (2 × 20 min cycles of 100 W), and finally diluted to 25 ml before analysis of dissolved (<0.45 µm) metals. Acid-volatile sulfide and dilute acid extractable metals (AEM)

measurements were made on sub-samples of the same homogenised sediment according to the method described by Simpson [37].

Analyses of filter and digest blanks, replicates for 20% of samples, analyte sample-spikes and the certified reference material (PACS-2, National Research Council Canada, Ottawa, ON, Canada) were undertaken as part of the quality assurance, and recoveries were within 85-110% of expected values. The limits of reporting for the various methods were less than 1/10th of the lowest reported values.

Waters, sediments and copper-spiking

Clean seawater was collected from Port Hacking, Sydney, Australia, membrane filtered (0.45 µm), and acclimated to a room temperature of 21±1°C. Where necessary, the salinity of the filtered seawater was adjusted to the test salinity of 30‰ using Milli-Q water.

Sediments were collected from three locations for use as controls in toxicity tests and for copper-spiked sediment tests: intertidal estuarine sites at Bonnet Bay (silty), Boronia Park (silty-sand) and Grays Point (sandy), from Woronora, Lane Cove, and Port Hacking rivers, respectively (NSW, Australia). The surface layers (upper 2-4 cm) of exposed sediments were collected using clean Teflon spatulas and press-sieved through a 1.1-mm mesh on site to remove coarse sediment particles, unwanted debris and co-occurring biota. The sediment was transferred into clean plastic bags with minimal headspace and stored in a cool room at 4 °C for no longer than one month. The silty sediment that comprised 98% <63 µm-sized sediment was mixed with clean sand (180-500 µm) to create a set of sediments having 100, 50, and 25% silt (<63 µm). The sand was expected to have a low copper-binding affinity and act mostly as a diluent (w/w) for the number of copper-binding sites in the silty sediment. At Bonnet Bay, a deeper silty material was also collected that had very similar characteristics to the surface silt, but one third of the

POC concentration. The <63 μm fractions of the silt, silty-sand and sand surface sediments together with the deeper silt were used to create a sediment series having 2.6, 3.6 and 8.2% TOC (100% <63 μm).

Artificially contaminated copper-spiked sediments were prepared in accordance with modified procedures described by Simpson et al. [31]. All manipulations and pH measurements on the spiked sediments were performed in a nitrogen-filled glove box to minimise oxidation of the sediments. Deoxygenated solutions were prepared by bubbling with high purity nitrogen gas for a minimum of 24 h. The sediments were spiked with varying amounts of copper sulfate (BDH Laboratory Supplies) dissolved in deoxygenated seawater to achieve the desired particulate dry weight concentrations. A concentration series generally consisted of a negative control (i.e. without copper spike) and five treatments with increasing concentrations of particulate copper (up to 2000 mg Cu/kg). A series of 5-6 copper concentrations were used for each series, generally incrementing by 200-300 mg/kg up to 1500-1700 mg/kg. The high density polyethylene plastic bottles (Nalgene) containing the copper-spiked sediments were thoroughly mixed using a Teflon spoon and by shaking for 2 minutes, 3-5 times per week, over a 30-day equilibration period. The sediment pH was measured bi-weekly and if necessary, adjusted to pH 7.5 ± 0.1 (or other specified pH) using 50% (w/v) NaOH solution. Bottles were then purged with nitrogen, shaken and returned to the glove box for storage. Sub-samples of the copper-spiked sediments were taken for analyses of dissolved porewater metals, particulate metals (TRM and AEM), TOC, particle size distribution, AVS, and pH prior to all tests. The porewater copper concentrations were measured at the end of each week of equilibration to confirm that the added copper had adequately equilibrated with the sediments [31].

Test organisms

Juvenile amphipods, *Melita plumulosa*, were obtained from laboratory cultures that were maintained as described previously [36]. Gravid *M. plumulosa* of 5-10 mm body length were identified from cultures by microscopy and placed in small plastic containers (20×15×10 cm) with a thin layer of sediment (3 mm deep) and 3-4 cm of gently aerated overlying water. Juvenile amphipods aged 11±3 d old were used for most tests and isolated from holding trays using a 210 µm sieve immediately prior to test commencement.

Small adult *Spisula trigonella* (4-7 mm shell length) and *Tellina deltoidalis* (5-10 mm shell length) were collected at low tide from the Lane Cove River, Sydney, Australia. The procedures for collection and handling of the bivalves have been described previously [38]. From the time of collection until the tests commenced (2-7 days later), the bivalves were held in trays (16×12 cm, 8 cm depth) with 3 cm depth of sediment and 3 cm overlying seawater (30‰ salinity) (up to 100 *T. deltoidalis* or 200 *S. trigonella* per tray), in a temperature-controlled laboratory at 21±1 °C. Overlying water in the trays was continuously gently aerated and trays were covered with foil to minimise light disturbances to the bivalves. The bivalves were not fed during this acclimation period. Immediately prior to test commencement, bivalves were isolated from the holding sediment by gentle sieving through 1.1 mm sieves.

Toxicity test procedures

All toxicity tests were undertaken at a temperature of 21±1 °C in an environmental chamber (Labec Refrigerated Cycling Incubator) on a 12 h light/12 h dark cycle (light intensity = 3.5 µmol photons/s/m²) for the test duration. Glass beakers and acrylic beaker lids used for toxicity tests were cleaned in a dishwasher (Gallay Scientific Pty Ltd) programmed for a phosphate-free detergent wash (Clean A, Gallay Scientific Pty Ltd), followed by a dilute acid wash (1% HNO₃), and thorough rinsing with Milli-Q water. Test beakers were capped with

plastic lids fitted with an aeration tube to continuously gently bubble the waters with air. For quality control purposes, physico-chemical parameters including dissolved oxygen (>85% saturation), pH, salinity and temperature were monitored at the beginning, periodically throughout, and at the termination of the test in at least one replicate beaker per sample to ensure that they remained within acceptable limits. A salinity of $30 \pm 1\%$ and a pH of 8.0 to 8.2 were maintained in waters throughout the test by the addition of deionised water to beakers in which evaporation had occurred.

Water-only tests had durations of either 4 or 10 days with 3 replicates containing 15-20 organisms per treatment. For *M. plumulosa*, tests were conducted in 250 mL beakers containing 200 mL of test water and a piece of 500 μ m nylon mesh to provide a surface for the amphipods. For *T. deltoidalis* or *S. trigonella*, tests were conducted in 1-L beakers containing 900 mL of test water. Nominal concentrations of copper were achieved by adding the appropriate volume of seawater stock solutions of 100 g/L of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ to test beakers. No food was added to the tests. Beakers were conditioned with the test water for one day prior to test commencement, and then the waters were renewed before adding the test organisms. For *M. plumulosa*, 4-day non-renewal tests and the 10-day renewal tests were undertaken, and water sub-samples were taken (<0.45 μ m filtered) for analyses at the start and finish of all tests, and before and after water renewals (generally on days 3 and 7 of 10-day tests). For *T. deltoidalis* or *S. trigonella* tests, dissolved copper concentrations decreased significantly with time (typically by 10-30% per day) and test solutions were completely renewed on a daily basis, with water samples taken for analyses before and after renewal. Where multiple samples were taken, the reported concentration of dissolved copper in each treatment was the time-averaged concentration [39].

Whole-sediment tests were conducted over 10 days, with 5 replicate treatments containing 20 organisms per treatment. Tests were conducted in 250 mL beakers with 200 mL seawater overlying the test sediment (20 g for amphipods and 60 g for bivalves). The homogenised sediments were added the day before the test was started and seawater was added slowly to minimise sediment resuspension. On the morning that the tests commenced, the overlying water from each test was removed by gentle siphoning and replaced with new seawater. Test waters were not renewed during the 10-day whole sediment test period for amphipods or bivalves.

Water-only and whole-sediment tests were commenced after organisms were randomly assigned and added to each beaker, then the beakers were again covered and randomly positioned in the environmental chamber. Survival was monitored after water changes and at the completion of tests. For the amphipod, survival was indicated by active movement and confirmed under a dissection microscope. Sediment tests were terminated by gently sieving the sediment tests through a 180 μ m stainless steel sieve, and then the contents were transferred to large amphipod counting trays. Live amphipods were counted and removed and the sieved sediment was then transferred into 120 mL polycarbonate vials with approximately 100 mL of seawater, fixed with 4 mL of 10% v/v neutral phosphate-buffered formalin, and stained with 5 mL of a Rose Bengal solution (0.1 g Rose Bengal salt/ 100 mL Milli-Q water). The sediment was then left for 72 h to enable any surviving amphipods, missed in the initial count, to take up the stain, and then these amphipods were counted. For bivalves, each water and sediment test replicate was rinsed through a 1.1 mm sieve followed by washing into a plastic counting tray and survival was indicated by the presence of a closed shell and observation of unassisted movement.

Tests were considered acceptable when the physico-chemical parameters remained within acceptable limits (salinity $30 \pm 1\text{‰}$, pH 8.1 ± 0.1 , and $>85\%$ DO saturation) throughout the test, and if survival of organisms was on average $\geq 80\%$ in the controls.

Manipulations of dissolved copper in overlying waters of sediment toxicity tests

To help distinguish between toxicity occurring due to copper exposure from the overlying water and sediment compartments, experiments were undertaken that varied the copper concentration in the overlying water while maintaining the same sediment substrate. For the bivalve tests, to reduce concentrations of dissolved copper in the overlying water, a 600 μm mesh sachet containing 3 g of the metal chelating resin, Chelex-100™ (200-400 mesh) was suspended in the water above the test sediment. For the amphipod tests, the dissolved copper in the overlying water was lowered by (i) daily water changes during the 10-day tests, or (ii) variations in the sediment to water ratio. For the second set of experiments, one concentration series of copper-spiked sandy sediments (control, 200, 400, 600 and 800 mg/kg) was prepared and tested for each of three sediment-water ratios of 1:10, 1:5 and 1:1 (w/v) (20 g/200 mL, 20 g/100 mL and 50 g/50 mL of seawater). The other parameters of these sediment toxicity tests and measurements during the tests were the same as those described earlier.

Endpoint data and statistical analysis

Results of the toxicity tests were reported as % survival in each test replicate relative to survival in the control. These proportional data were arc sine transformed and tested for normality of distribution (Shapiro-Wilk's test) and for homogeneity of variance (Bartlett's test) prior to hypothesis testing. Dunnett's Test (parametric) was then used if assumptions of normality and homogeneity of variances were met, and Steel's test (non-parametric) was used when variances were heterogeneous and the distribution unequal. Maximum likelihood

regression using Probit analysis with Abbott's correction or non-linear interpolation with bootstrapping (ICp) if assumptions of the Probit analysis were not met, were also used to determine LC50 and LC15 values and their 95 % confidence limits (CLs). The choice of LC15 values rather than LC10 or LC20 values, was based on the lower variability in responses observed for greater than 30 tests undertaken on control sediments (negligible contamination) that represented a wide range properties (unpublished data). Significance in all statistical tests was set at the $p < 0.05$ level and all statistical analyses were carried out using the software Toxcalc for Microsoft Excel (Version 5.0.23, TidePool Scientific Software, California, U.S.A.).

RESULTS AND DISCUSSION

Effects Thresholds for Dissolved Copper

Without added food, the survival of juvenile *M. plumulosa* in controls was often less than 80% after 10 days, and 10-day water-only effects data were not determined. In a concurrent study, where small amounts of food were added to ensure sustenance did not influence the juvenile survival, Spadaro et al. [36] determined 10-day LC50 and no observed effect concentration (NOEC) values of 76 ± 15 and $36 \mu\text{g/L}$, respectively. Although both bivalve species completely bury themselves when sediments are present, they displayed >85% survival in water-only test controls over 10 days. The 10-day LC50 values for *S. trigonella* and *T. deltoidalis* for dissolved copper were determined to be 15 ± 4 and $5 \pm 2 \mu\text{g/L}$ ($n=2$), respectively. For each species, monitoring of survival over the 10-day tests indicated that survival decreased sharply after approximately 5 days. The 3-, 5- and 7-day LC50 values were 179 (158-214), 59 (48-73) and $25 (18-30) \mu\text{g Cu/L}$ for *S. trigonella*, and 208 (186-246), 102 (90-110), and $9.9 (5.6-15) \mu\text{g Cu/L}$ for *T. deltoidalis*, respectively. The test waters were replaced daily and ammonia concentrations were expected to be well below effects thresholds for all species. King et al. [38]

determined a 10-day LC50 of 67 mg total ammonia/L (pH 8) for *T. deltoidalis* based on porewater concentrations in whole-sediment tests. The results indicated that both species were very sensitive to dissolved copper, but it was not determined whether additional stress due to the absence of a sediment substrate contributed to this sensitivity.

Influence of sediment properties on copper partitioning between sediment and water

The properties of the three field-collected sediments were broadly characterised as silty, silty-sand and sandy substrates (**Table 1**). For the sand-containing sediments (silty-sand and sand), the particulate metal and organic carbon concentrations were greater in the <63 μm fraction than in the bulk sediment. For the silty, silty-sand and sandy sediments, the measured total recoverable copper concentrations (TR-Cu) ranged from 81 to 114% of the nominal concentrations (mean \pm standard deviation = $95 \pm 8\%$). For these sediments, the ratios of copper in the <63 μm fraction to those of the bulk sediment were 1.0 ± 0.0 , 1.9 ± 0.2 and 4.4 ± 0.2 , and the ratios of dilute acid-extractable copper (AE-Cu) to TR-Cu were 0.45 ± 0.07 , 0.34 ± 0.08 and 0.64 ± 0.10 for the silty, silty-sand and sandy sediments, respectively.

The AVS concentrations of the three sediments ranged from 1.6 (silty-sand) to 4.1 (silt) $\mu\text{mol/g}$, respectively, and was likely to comprise of a variety of metal sulfide phases. Sufficient lead and zinc was present in the silty and silty-sand sediments to account for most of the AVS (Table 1), with the remainder likely to comprise of FeS and MnS phases [14]. Copper forms copper sulfides with both copper(I) and copper(II), namely Cu_2S and CuS [40]. These are not usually detected as AVS because they are largely insoluble in dilute acid [17]. The AVS concentrations in each series decreased with increasing added copper (data not shown) and provided evidence for the formation of copper sulfides but these were likely to comprise of less than 250 mg Cu/kg in any treatments. Except for possibly the 200 mg Cu/kg treatments, the

majority of the added copper binding in each series would have been to POC, iron and manganese oxyhydroxide phases, and fine clay particles [3,11].

The porewater copper (PW-Cu) concentrations measured at the start and finish of the experiments were 3 ± 4 $\mu\text{g/L}$ in the silt series. In the silty-sand and sand series, PW-Cu concentrations increased with increasing copper-spike concentration to maximum concentrations of 27 and 66 $\mu\text{g/L}$ for the 1000 mg Cu/kg silty-sand and 1500 mg Cu/kg sand treatments, respectively. For the silty-sand and sand treatments, the PW-Cu concentrations at the end of the 10-day exposures were similar or up to 50% lower than measured at the start of the tests.

In all tests, there was a detectable release of copper from the sediments to the overlying water, with the rate of release from the three sediments increasing in the order: silt<silty-sand<sand (**Fig. 1a**). For each concentration series the dissolved copper in the overlying waters increased with the increasing TR-Cu concentration. The dissolved copper concentrations in the overlying waters were significantly higher than the porewater concentrations. The dissolved copper concentrations in the overlying water for the two bivalve tests were similar within 10% (data shown for *S. trigonella*), and were significantly greater than that for the amphipod test. The dissolved copper concentrations were more variable between tests for sand than for silt or silty sand, and, in the sand treatment, there was greater variability for the bivalve than amphipod (Fig. 1a).

The bivalves were much larger organisms than the amphipod, and burrow creation and irrigation, and foraging for food were expected to cause greater disturbance to the sediments in those tests. The copper release to the overlying water is likely to be a combination of diffusive flux from the deeper pore waters and copper release from particulate organic carbon and sulfide phases being oxidised at the sediment-water interface (SWI) [27]. Copper forms strong

complexes with naturally-occurring organic ligands and the bioavailability and toxicity of dissolved copper was expected to decrease with increasing DOC concentrations [4,11]. DOC concentrations in the overlying waters were <1 mg/L in all tests. Porewater DOC was not determined. Although no measurements were made to determine the degree of copper-organic complexation, despite the low DOC concentrations, it is possible that a significant portion of the dissolved copper was present as organic complexes.

In all tests, the observation of greater dissolved copper concentrations in the overlying water compared to the pore water was believed to be partly due to the sampling procedures. The copper concentrations in the centrifuged porewater represent the average in the entire sediment, whereas it is well recognised that depth gradients exist and concentrations might be expected to be greatest near the SWI [27]. It would be expected that the porewater copper concentrations at the SWI would be at least as high as the concentrations in the overlying water. Due to continual mixing of the overlying water caused by the aeration of the waters, no copper gradients were expected in the overlying water column beyond a few millimetres of the SWI. Although it is not possible to determine how well the overlying copper concentrations may represent the actual copper exposure, for at least the epibenthic amphipod, *M. plumulosa*, the dissolved copper exposure (within millimeters of the SWI) may have been significantly greater than that represented by the copper concentrations measured in either the overlying water or porewater. In field environments, the dissolved copper released from the SWI would be diluted to a much greater extent than in the laboratory exposures and overlying water copper (OW-Cu) concentrations would be much lower.

Influence of sediment properties on copper toxicity

For the amphipod and both bivalves, toxicity was lower in the silty sediments than the two sandier sediments (Fig. 1). For each concentration series, LC50s were calculated based on the concentrations of TR-Cu, AE-Cu and copper in the overlying water (OW-Cu) (Table 2). These calculations provide an insight into the effects of sediment properties on the bioavailability and toxicity of copper in the sediments, however, it is not possible to determine which exposure source was responsible for the effects. Although the copper was added to the sediment only a month before these tests, only a fraction of the TR-Cu is likely to be bioavailable. Effects thresholds were not calculated based on PW-Cu concentrations because the concentration-response relationships were weak.

The amphipod, *M. plumulosa*, forages at the sediment-water interface to depths of up to 3 mm, but does not create burrows in the sediment. Given this feeding behaviour, it was hypothesised that the amphipod's exposure to dissolved copper would be predominantly via overlying water rather than pore water. For *M. plumulosa*, the 10-day water-only NOEC is 36 µg/L. In the silty, silty-sand and sandy sediments, greater than 50% lethality to *M. plumulosa* was observed when PW-Cu was <10 µg/L. The maximum PW-Cu concentrations were <30 µg/L except in the 1500 mg Cu/kg sand treatment in which PW-Cu was 41 ± 25 µg/L (mean \pm SD of initial and final PW-Cu) and 100% lethality was observed. These observations indicated that the porewater copper exposure was not the major cause of the observed toxicity.

Dissolved copper concentrations in the overlying water (OW-Cu) were always at least 2-3 times higher than the PW-Cu concentrations and, unlike PW-Cu, the OW-Cu increased fairly uniformly with copper spike concentration (Fig. 1a). However, the LC50 values calculated based on the OW-Cu in the whole-sediment tests with *M. plumulosa* were less than 20 µg/L (Table 2), and indicated that toxic effects were occurring when dissolved copper concentrations were well

below the 10-day NOEC of $\sim 36 \mu\text{g/L}$ [36]. Similarly, during the first 4 days of whole-sediment toxicity tests, significant toxicity was often observed despite OW-Cu concentrations reaching a maximum of $25 \mu\text{g/L}$ compared to a 4-day NOEC for dissolved copper of $81 \pm 17 \mu\text{g/L}$ ($n=5$ replicate tests). Repeated 10-day whole-sediment tests with daily renewal of the overlying water resulted in OW-Cu concentrations of $<10 \mu\text{g/L}$ in the 900 mg Cu/kg silt treatment, but survival was still below 50%, indicating sediment copper exposure influenced toxicity. King et al. [26] used ^{64}Cu -labelled sediment and water to demonstrate that *M. plumulosa* was exposed to considerable amounts of copper through ingestion of fine sediments.

Overall, the results for the copper-spiked sediments were consistent with the exposure to particulate copper being a significant exposure route contributing to the toxicity to *M. plumulosa* [10,21]. However, as discussed earlier, the potential underestimation of the dissolved copper exposure to *M. plumulosa* at the SWI may also cause an over-estimation of role of dietary toxicity compared to water-borne (porewater, interface-water, and overlying water) copper toxicity.

The bivalve *S. trigonella* is primarily a filter feeder which is exposed to copper via the overlying water, however, *T. deltoidalis* is a deposit feeder and will be exposed to dissolved copper in the water and particulate copper via diet [26]. For both species, the exposure to dissolved copper will occur from pore water, burrow water and overlying water [41]. For the silt series, the PW-Cu concentrations were $<5 \mu\text{g/L}$, which was considered too low to account for the toxicity observed for either bivalve species. In the silty-sand and sand sediments in which significant toxicity was observed the PW-Cu concentrations ranged from 7 to $24 \mu\text{g/L}$ for the silty-sand series (mean of PW-Cu at start and finish of test) and 11 to $41 \mu\text{g/L}$ for the sand series. At these PW-Cu concentrations, the porewater exposure was likely to be contributing to the

observed toxicity but the porewater exposure did not account for all of the observed toxicity, e.g. no survival was observed for *S. trigonella* in the 750 mg/kg sand treatment when PW-Cu was just 11 µg/L.

In both bivalve tests, OW-Cu concentrations were between 3 and 10 times higher than the PW-Cu concentrations and increased fairly uniformly with copper spike concentration (Fig. 1a). The LC50 values determined using OW-Cu concentrations during the 10-day whole-sediment tests (Table 2) were greater than the LC50 values of 15 ± 4 and 5 ± 2 µg/L for *S. trigonella* and *T. deltoidalis*, respectively, determined from the 10-day water-only tests. This indicated that the OW-Cu exposure was sufficiently high to account for the toxicity observed for both bivalve species in the sediment tests. To confirm OW-Cu as the cause of toxicity, in *S. trigonella* tests a sachet of the copper-chelating resin Chelex-100 was suspended in the overlying water. In the highest copper-spiked sandy sediment treatment, this reduced the OW-Cu from 150 to <15 µg/L and increased survival of the bivalves from 0% to 100%. Although dissolved ammonia concentrations were observed to increase between water exchanges as a result of nitrogenous waste products excreted by the bivalves, they were not significantly different for the Chelex and non-Chelex treatments. The LC50 values determined based on OW-Cu in the whole-sediment tests (Table 2) were higher than those determined for the 10-day water-only tests (15 µg/L for *S. trigonella* and 5 µg/L for *T. deltoidalis*). While this was attributed mostly to the absence of substrate in the water-only tests causing additional stress to the bivalves, it is also likely that DOC released from the sediments during the tests (<1 mg/L measured in the overlying water) may have formed complexes with the OW-Cu and decreased the bioavailability of the dissolved copper [4,11].

Combined effects of copper from dissolved and dietary exposure

A series of experiments were undertaken to improve our understanding of the relative contributions of water-borne (pore water, SWI, and overlying water) and sediment-particulate copper exposure to the toxicity to *M. plumulosa*. Three test set-ups were prepared using the same copper-spiked silty sediment concentrations series, but with sediment-water ratios of 1:10, 1:5 and 1:1 (**Fig. 2**). By using the same sediment solid phase in each treatment, the exposure of the amphipod to particulate copper, porewater copper within the sediments and the copper flux at the SWI were expected to be very similar. However, the differing volumes of overlying water in the three treatments resulted in lower dissolved copper concentrations at the higher ratios owing to the greater dilution of released copper in overlying water (Fig 2c). In all treatments, the sediment depth was much greater than the typical burrowing depth of *M. plumulosa*, and the difference in the amount of sediment within each treatment should not have influenced the amount of sediment that the amphipods may have ingested.

The copper exposure-toxicity data were used to construct relationships between the observed toxicity and the exposure from particulate copper and overlying water (Fig. 2a,b). For the 1:10, 1:5 and 1:1 ratio series the LC50 values were 560 (520-595), 484 (470-497) and 377 (286-448) mg Cu/kg, respectively (Fig. 2d). As the exposure to particulate, porewater and SWI copper was expected to be the same, the increased toxicity appeared to be consistent with the higher OW-Cu concentrations at lower sediment:water ratios. The greatest variation in amphipod survival occurred in the 400 mg Cu/kg treatment, with the 1:10, 1:5 and 1:1 treatments resulting in OW-Cu exposures of 10, 18 and 33 µg/L and survivals of 100, 75 and 22%, respectively (Fig. 2b). Similar relationships existed when survival was plotted against OW-Cu exposures, with the survival for the 1:10, 1:5 and 1:1 treatments at 400 mg/kg used to demonstrate relative toxicities (Fig. 2c). In combination with the 400 mg/kg sediment copper exposure, an increase in OW-Cu

exposure of 23 µg/L resulting in increased toxicity by up to 78%, despite the dissolved copper concentration being below the water-only NOEC of 36 µg/L.

For the 1:10, 1:5 and 1:1 series, the LC15, LC50 and LC75 concentrations were calculated based on TR-Cu (sediment copper) (Fig. 2a). For each LCx concentration, the OW-Cu concentration that corresponded to these TR-Cu concentrations were determined from Fig. 2c. Regressions were then made of TR-Cu against OW-Cu for each of the three LC15, LC50 and LC75 values (Fig. 2d). These linear regressions were consistent with ‘additive’ contributions to the toxic effects from the combined dissolved (OW-Cu) and particulate copper exposure routes. At higher TR-Cu concentrations, lower OW-Cu concentrations were required to elicit the same toxicity, and vice versa. Based on the LC50 and LC15 relationships, extrapolation to zero copper exposure provided LC50 and LC15 values of 65 ± 6 µg/L and 36 µg/L (OW-Cu), which are very similar to the water-only LC50 and NOEC values of 76 ± 15 µg/L and 36 µg/L determined by Spadaro et al. [36]. Conversely, an extrapolation to zero OW-Cu exposure provided LC50 and LC15 values of 708 and 620 mg/kg (TR-Cu), respectively, which are relevant to sediments in field environments with high dilution rates for copper released to the overlying water. Overall, the results for *M. plumulosa* were consistent with the copper exposure from both the sediments (dietary) and overlying waters (dissolved) contributing additively to the observed toxicity. Furthermore, the close match of the LC50 and LC15 values based on extrapolations to zero TR-Cu for water-only exposures indicated the copper flux at the SWI was unlikely to have been creating a significantly greater dissolved copper exposure than that represented by the OW-Cu measurements.

Normalising for the effect of particle size on the toxicity of copper

For copper-spiked sediments with particle sizes modified to contain 100, 50 and 25% silt (silty sediment diluted with sand, Table 1), the ratios of copper in the <63 μm fraction to that of the bulk sediment were (mean \pm SD) 1.09 ± 0.05 , 1.78 ± 0.05 and 2.92 ± 0.13 , respectively. This was equivalent to 73-100% of the added copper being associated with the <63 μm fraction. However, the ratios of AE-Cu to TR-Cu for 100, 50 and 25% silt were 0.62 ± 0.09 , 0.50 ± 0.09 and 0.49 ± 0.09 , respectively, and indicated that the copper bioavailability may be very similar.

For both *M. plumulosa* and *S. trigonella*, all controls had >95% survival, indicating that differences in food availability were not creating significant differences between the 100, 50 and 25% silt treatments (**Fig. 3**). The LC50 values for the 100, 50 and 25% silt series were 823, 407 and 193 mg Cu/kg for *M. plumulosa*, and 721, 336 and 147 mg Cu/kg for *S. trigonella* (Fig. 3a,c), respectively. As expected, the rate of copper release to the overlying water was greater as the silt content of the sediment decreased (Fig. 3b,d). For *M. plumulosa*, LC50 values calculated based on OW-Cu concentrations were below the water-only NOEC of 36 $\mu\text{g/L}$ (i.e. consistent with the silty, silty-sand, and sandy sediment results), which was consistent with particulate copper contributing to the observed toxicity. For *S. trigonella*, LC50 values based on OW-Cu concentrations were 13-15 $\mu\text{g/L}$, which were lower than those observed for the three sediment types (Table 2). The higher OW-Cu concentrations in the bivalve concentration-response series compared to the amphipod series are likely to be due to both the greater amount of sediment used as substrate and the greater bioturbating activity within the bivalve experiments.

In the 100, 50 and 25% silt experiments, the principal copper-binding phase in each treatment was the silt (4.5% TOC; 2.4% iron; 4.5 $\mu\text{mol AVS/g}$; Table 1), with minimal copper-binding to the sand particles. Analyses of the 100, 50 and 25% silt controls (with no added copper) showed AVS concentrations of 2.1, 1.4 and 0.5 $\mu\text{mol/g}$, respectively. This indicated

that, although the sediments were prepared within a nitrogen atmosphere, the preparation had resulted in oxidation of some of the AVS present in the original silt. This was not unexpected as the sub-oxic silt contained significant concentrations of iron and manganese in both oxidised (e.g. oxyhydroxides) and reduced (e.g. Fe(II)/Mn(II)) states (Table 1). The mixing required to create the altered % silt substrates is likely to have increased contact between the oxidised iron and manganese phases and the reduced FeS, the predominant component of AVS, which would cause oxidation of FeS [42]. Based on the amounts of AVS present, newly formed copper sulfide phases may have comprised up to 130, 90, and 32 mg/kg of the spiked copper in the 100, 50 and 25% silt series, respectively.

For treatments with the same total copper concentration, the less silty sediments had a proportionally higher copper concentration in the <63 μm sediment and also greater copper release to the overlying water (Fig. 3). For the amphipod and bivalve tests, the OW-Cu concentrations correlated better with TR-Cu for the <63 μm fraction than the bulk sediment, indicating this phase was controlling copper bioavailability. The relationships between amphipod and bivalve survival and the <63 μm TR-Cu concentration (**Fig. 4**) indicate that <63 μm TR-Cu is a much better predictor of toxicity than TR-Cu for the bulk sediment (Figs. 2 and 3). However, the ‘normalisation’ to <63 μm TR-Cu concentration did not account for the toxicity observed in the sand series, which caused less toxicity than the other normalised series. This was attributed to differences in the TOC concentration of the <63 μm sediment fraction, which was 10.7% for the sand, but only 4.0 to 4.5% for the silty-sand, silt and the 100, 50 and 25% silt series. Organic matter is recognised as being a major copper-binding phase [3,4,11].

Normalising for the effect of particle size and organic carbon on the toxicity of copper

For copper-spiked sediments with similar particle sizes, but modified to contain 2.6, 3.6 and 8.2% OC, the ratios of copper in the <63 μm fraction to that of the bulk sediment were relatively near 1.0, and the ratios of AE-Cu to TR-Cu were 0.54 ± 0.08 , 0.64 ± 0.11 and 0.47 ± 0.07 , respectively. Analyses of the 2.6, 3.6 and 8.2% OC controls showed AVS concentrations of ≤ 0.1 , ≤ 0.1 and $3.3 \mu\text{mol/g}$, respectively, and up to 200 mg/kg of the spiked copper may have been present as copper sulfide phases in the 8.2% OC series. As in the experiments where particle size was modified, the OW-Cu concentrations were below the dissolved copper effects threshold of 36 $\mu\text{g/L}$ for *M. plumulosa*, but reached concentrations that would cause toxicity for *S. trigonella* (equivalent to an LC50 of 16-21 $\mu\text{g/L}$).

During the toxicity tests, the sediments with less OC had faster rates of copper release to the overlying water, indicating that the copper exposure would likely increase with decreasing TOC concentration. The relationships between amphipod and bivalve survival and the TR-Cu concentration of the bulk sediment and TR-Cu concentration of the <63 μm sediment fraction with and without normalisation to OC concentration are shown in **Fig. 5**. While normalisation of TR-Cu to only OC improved the concentrations-response relationships (Fig 5c,d), the normalisation to both sediment particle size and OC (i.e. <63 μm mg Cu/g OC) further improved the predictions of copper bioavailability and toxicity (Fig 5e,f).

Based on the sediment particle size and OC normalised effects relationships for the three species, LC50 and LC15 values were 17.3 (15.8-19.0) and 12.3 (10.2-15.6) mg Cu/g OC for *M. plumulosa*, 17.7 (16.8-18.6) and 14.9 (13.1-17.1) mg Cu/g OC for *S. trigonella*, and 25.1 (22.3-28.3) and 19.5 (15.1-25.6) mg Cu/g OC for *T. deltoidalis*, respectively. To put these values in perspective, an effect threshold of 12 mg Cu/g OC would equate to 60 mg Cu/kg for a sediment containing 20% silt (300 mg <63 μm Cu/kg) and 0.5% OC (2.5% <63 μm OC), 120 mg Cu/kg

for a sediment containing 50% silt (240 mg <63 μ m Cu/kg) and 1% OC (2% <63 μ m OC), and 480 mg Cu/kg (686 mg <63 μ m Cu/kg) for a sediment containing 70% silt and 4% OC (~5.7% <63 μ m OC).

These results re-confirmed OC as a major copper-binding phase [3,4,11,43] and that the prediction of copper bioavailability in sediments is significantly improved by normalising particulate copper concentrations to the concentrations of OC. The results were also consistent with the study by Costa et al. [30] which reported 10-day LC50 values for the amphipod *Gammarus locusta* of 6.8, 57 and >200 mg Cu/kg for sediments with increasing amounts of silt and organic matter.

Comparison with (SEM - AVS)/g OC guideline approach

In an extension of the SEM-AVS model, Di Toro et al. [13] proposed that the partitioning of metals to sediment particulate organic carbon could be incorporated by normalizing the molar difference between SEM (Σ Cd,Cu,Ni,Pb,Zn) and AVS to the fraction of organic carbon (foc) in the sediment. In the application of this approach, any sediment in which (SEM-AVS)/foc < 130 μ mol Cu/gOC is predicted to pose a low risk of adverse biological effects [14].

In the present study, the maximum AVS concentration measured in the test sediments (before copper addition) was 4.5 μ mol/g, but most of the sediments contained much lower AVS concentrations. For copper-spiked sediments, a large portion of the copper sulfide phases that form in sediments are very stable in acid [17,44] and will not be measured as AVS. However, the presence of particulate iron(III) phases that dissolve in 1 M HCl may result in the oxidative release of copper from sulfide phases and this will be detected as AE-Cu and SEM-Cu [17]. For the %silt and %OC series, the AVS concentrations measured for the copper spiked sediments were mostly \leq 0.1 μ mol/g, and although the low recovery of copper-sulfide phases was expected

to have only a minor influence on the AE-Cu measurements, the cause-effects relationships were constructed using $<63 \mu\text{m TR-Cu/g OC}$.

Sufficient effects data were generated in this study to calculate effects thresholds based on $(\text{SEM-Cu} - \text{AVS})/\text{foc}$, and LC50 and LC15 (estimated no effects thresholds) values were $163 (123-216)$ and $104 (64-175) \mu\text{mol Cu/g OC}$ for *M. plumulosa* and $123 (106-142)$ and $94 (75-120) \mu\text{mol Cu/g OC}$ for *S. trigonella*, respectively. These acute toxicity results indicate that a value of $\sim 100 \mu\text{mol} (\text{SEM-Cu} - \text{AVS})/\text{g OC}$ may be a more appropriate threshold for predicting a low risk of adverse biological effects when considering sediments in which copper is the major metal contaminant.

The two copper effects thresholds may be compared for different sediment compositions. For a sediment with 50% silt, 1% OC and $0 \mu\text{mol/g AVS}$, a threshold of $100 \mu\text{mol Cu/g OC}$ for the $(\text{SEM-Cu} - \text{AVS})/\text{foc}$ model is equivalent to 64 mg Cu/kg , whereas a threshold of $<63 \mu\text{m} 12 \text{ mg Cu/g OC}$ (from this study) is equivalent to 120 mg Cu/kg . If this same sediment contained 1 or $5 \mu\text{mol/g AVS}$, the $(\text{SEM-Cu} - \text{AVS})/\text{foc}$ threshold would be equivalent to 127 or 381 mg Cu/kg , respectively, but the changes in AVS would not influence the $<63 \mu\text{m} 12 \text{ mg Cu/g OC}$ threshold. While this indicates that the $<63 \mu\text{m} 12 \text{ mg Cu/g OC}$ threshold is overly conservative for sediment containing AVS, for organisms like *M. plumulosa*, that interact predominantly with the oxic surface sediments, or *S. trigonella*, which receives most of its exposure to metals from the overlying water, it is unlikely that AVS concentrations greater than $5 \mu\text{mol/g}$ will exist within the surface sediment layer in which these species reside. The present study indicated that small amounts of AVS within the whole sediment is unlikely to significantly influence copper bioavailability in surface sediments which also contain significant concentrations of other metal-binding phases such as POC.

610 *Copper guidelines for oxic/sub-oxic surface sediments*

611 For the epibenthic amphipod *M. plumulosa*, toxic effects from a 10-day copper exposure
612 to copper-contaminated sediments occur via a combination of dissolved copper and dietary
613 copper exposure pathways. In the copper-spiked sediments, copper toxicity to the benthic
614 bivalves *S. trigonella* and *T. deltoidalis* was primarily via exposure to dissolved copper. For
615 oxic/sub-oxic sediments, in which AVS does not significantly reduce the bioavailability of
616 copper, thresholds that would be protective for acute toxicity for all of these species are 10 µg
617 Cu/L for porewater, burrow water or overlying water, and <63 µm 12 mg Cu/g OC for
618 sediments.

619 While the 30-day equilibration of copper-spiked sediments resulted in partitioning of
620 copper between sediment and water phases that was suitable for study, the copper in these
621 sediments was expected to be more bioavailable than copper in field-contaminated sediments.
622 Furthermore, the use of laboratory-based bioassays that did not have continual renewal of
623 overlying water resulted in greater exposure of organisms to dissolved copper than would be
624 expected to occur for the same sediments at field locations where dilution with overlying water
625 occurs rapidly. Both of these factors will have resulted in the calculated copper effects
626 thresholds being quite conservative with respect to toxic effects occurring in field-contaminated
627 sediments *in situ*.

628 For organisms for which both dissolved and dietary metals exposure are expected to
629 contribute to toxic effects, guideline normalisation approaches may be further improved if the
630 factors that affect metal bioavailability can be incorporated within multi-pathway exposure-effect
631 models [7,8,10]. However, for such models to be appropriately implemented, further research is

also required to determine the usefulness of a combined particle-size and OC normalisation procedure for predicting chronic effects [45].

To accurately predict the onset of chronic effects, a much better understanding of the factors controlling metabolically available metal concentration will be necessary. To improve the use of metal bioavailability considerations in regulatory frameworks for sediment [1], it is equally important that the analytical measurement techniques are both straightforward to apply, but also provide the necessary information on metal bioavailability [3]. As metal toxicity occurs when organisms cannot cope with the overwhelming influx of metals [7,8,46], measurement techniques which assess the likelihood of high metal fluxes will be very useful for this purpose. The technique of diffusive gradients in thin films (DGT) is one technique that may become increasingly useful for providing spatially-resolved information on the bioavailability of metals in sediments, including the SWI and deeper porewaters [26,47].

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Table 1. Physico-chemical properties of the silty, silty-sand and sandy sediments

Sediment	Silty		Silty-sand		Sand	
pH	7.1-7.3		7.3-7.5		7.2-7.4	
Redox potential (mV)	-40±50		-10±20		-30±50	
Particle size: <63, 63-180, >180 µm (%)	98, 2, 0		50, 8, 42		22, 6, 72	
AVS (µmol/g)	4.5±0.5		1.6±0.2		4.1±0.3	
Bulk and <63 µm sediment^a	Bulk	<63 µm	Bulk	<63 µm	Bulk	<63 µm
Organic carbon (TOC), %	4.5	4.5	2.1	4.0	3.3	10.7
TR-Fe (%)	2.4	2.4	1.1	2.0	0.58	2.1
TR-Mn (mg/kg)	70	70	27	44	11	38
TR-Cu (mg/kg)	40	40	34	46	27	75
TR-Pb (mg/kg)	40	40	50	68	14	76
TR-Zn (mg/kg)	210	210	150	254	50	165
Sediment pore waters, mg/L						
Total ammonia	3-5		1-2		<1	
Total sulfide	<0.1		<0.1		<0.1	
Iron	11		6.6		0.43	
Manganese	0.63		0.54		0.10	

^a Bulk concentrations of Cd, Ni and total PAHs were <0.5, <3 and <4 mg/kg, respectively.

Table 2. LC50 values for *M. plumulosa*, *T. deltoidalis* and *S. trigonella* calculated using concentrations of total recoverable and dilute acid-extractable copper in the sediments, and dissolved copper in the overlying water for whole-sediment tests of three sediments (Table 1)

	<i>M. plumulosa</i>	<i>T. deltoidalis</i>	<i>S. trigonella</i>
Sediment	Sediment: Total recoverable-Cu LC50 (95% CL)^a, mg/kg		
Silt	806 (760-852)	980 (925-1040)	997 (936-1060)
Silty-sand	212 (119-277)	455 (428-483)	708 (642-774)
Sand	198 (123-305)	391 (351-423)	582 (480-611)
	Sediment: Dilute acid extractable-Cu LC50 (95% CL), mg/kg		
Silt	308 (305-322)	386 (336-413)	394 (371-426)
Silty-sand	87 (80-101)	293 (240-328)	480 (446-515)
Sand	59 (31-97)	145 (132-163)	146 (84-166)
	Overlying water-Cu LC50 (95% CL), µg/L		
Silt	19 (18-20)	34 (32-36)	34 (18-43)
Silty-sand	10 (3-10)	32 (20-35)	54 (47-61)
Sand	11 (6-18)	32 (30-35)	55 (27-65)

^a95%CL = 95% confidence limits

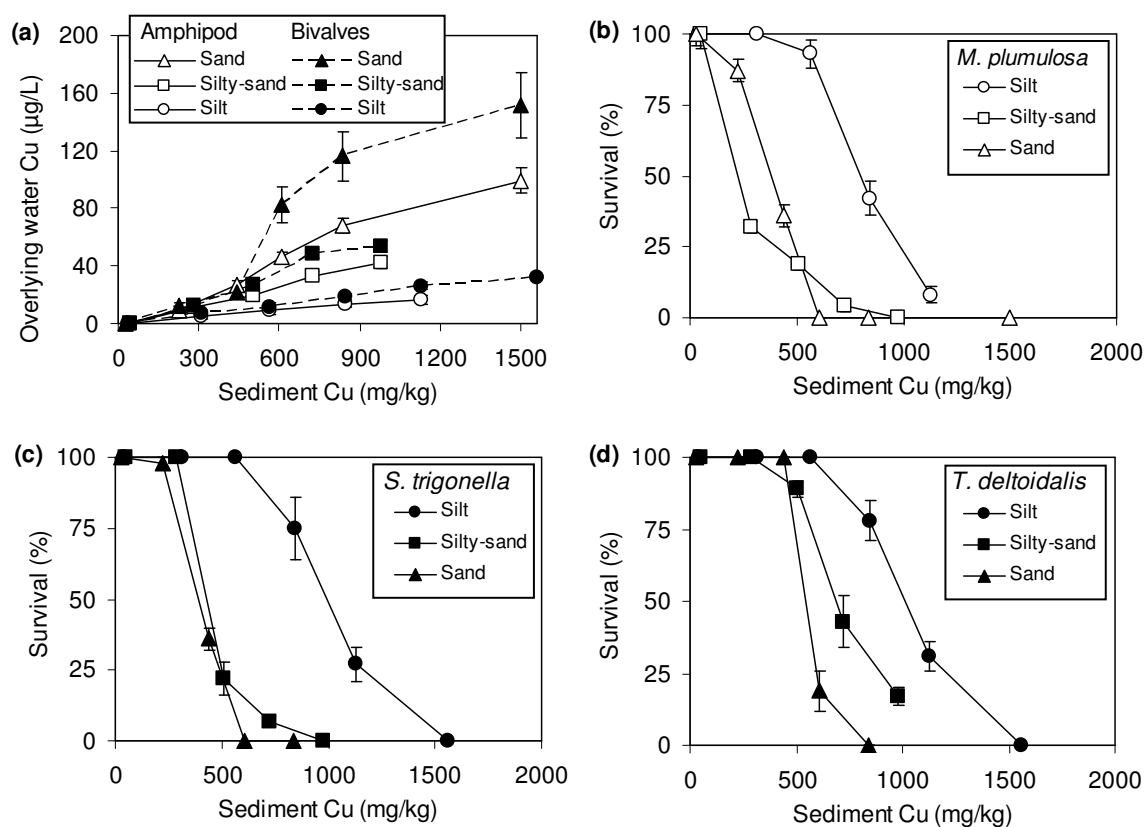


Figure 1. (a) Time-averaged dissolved copper concentrations for amphipod and bivalve tests, and concentration-responses of (b) *M. plumulosa*, (c) *S. trigonella* and (d) *T. deltoidalis* to copper-spiked silt, silty-sand and sand.

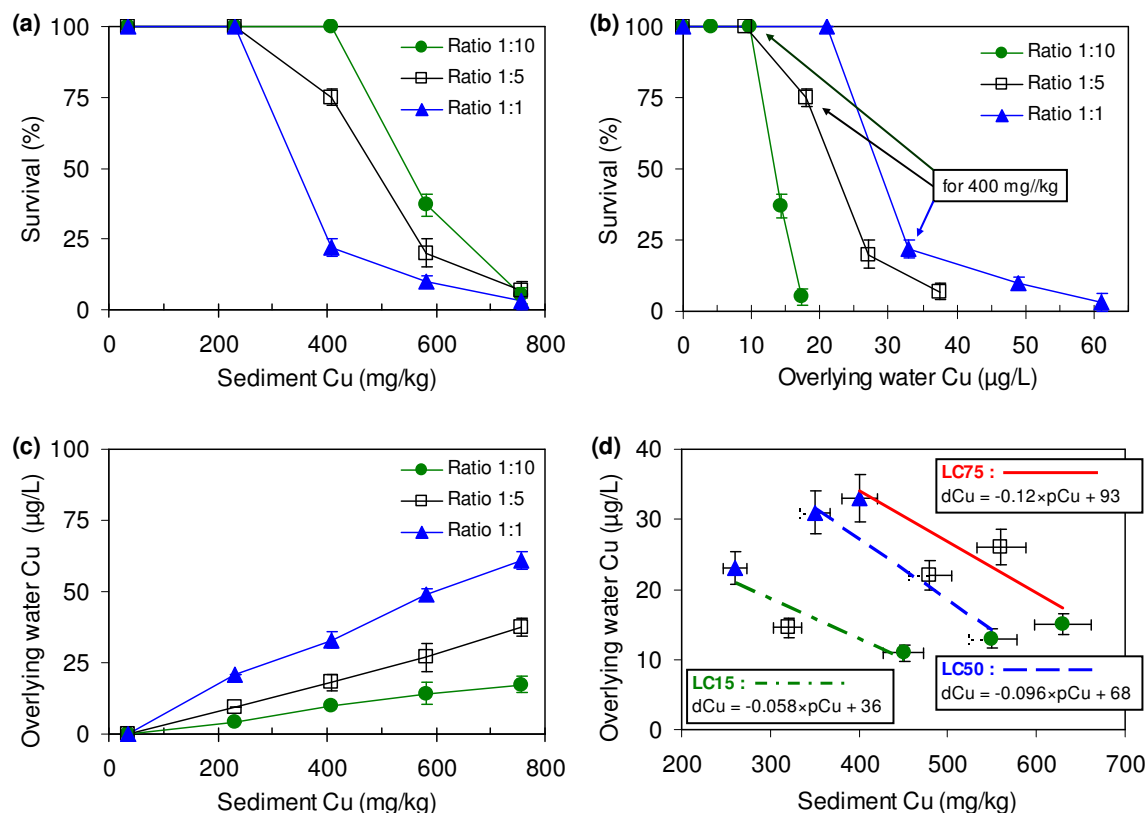


Figure 2. 10-day survival of *M. plumulosa* for the same copper-concentration series undertaken at sediment:overlying water ratios of 1:10, 1:5 and 1:1. (a) survival versus sediment copper, (b) survival versus overlying water copper, (c) sediment copper and the co-occurring dissolved copper concentrations in the overlying water for each treatment, and (d) the relationship between the co-occurring sediment and overlying water copper exposure concentrations resulting in 75, 50 and 15% lethality, respectively.

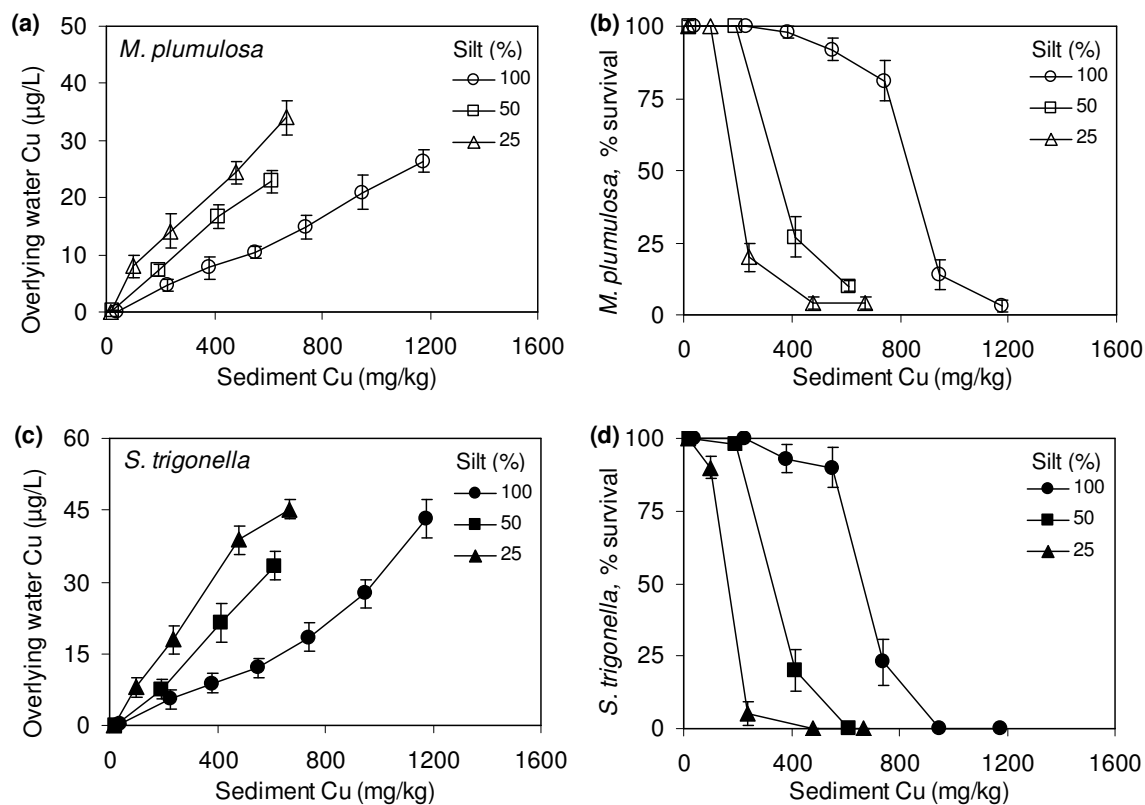


Figure 3. Relationships between copper in the overlying water and sediment and concentration-responses of *M. plumulosa* and *S. trigonella* for 100, 50 and 25% silt treatments

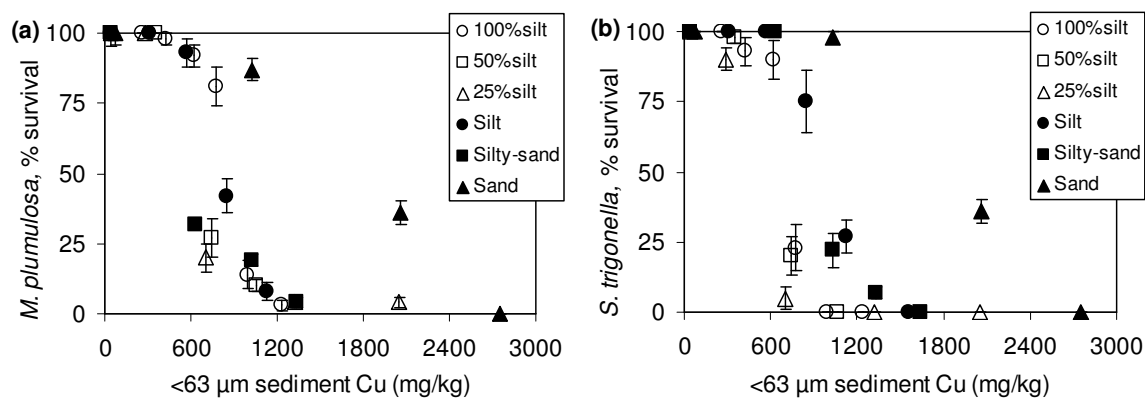


Figure 4. Relationships between survival of (a) *M. plumulosa* and (b) *S. trigonella* and the <63 µm sediment copper concentration for 100, 50 and 25% silt treatments

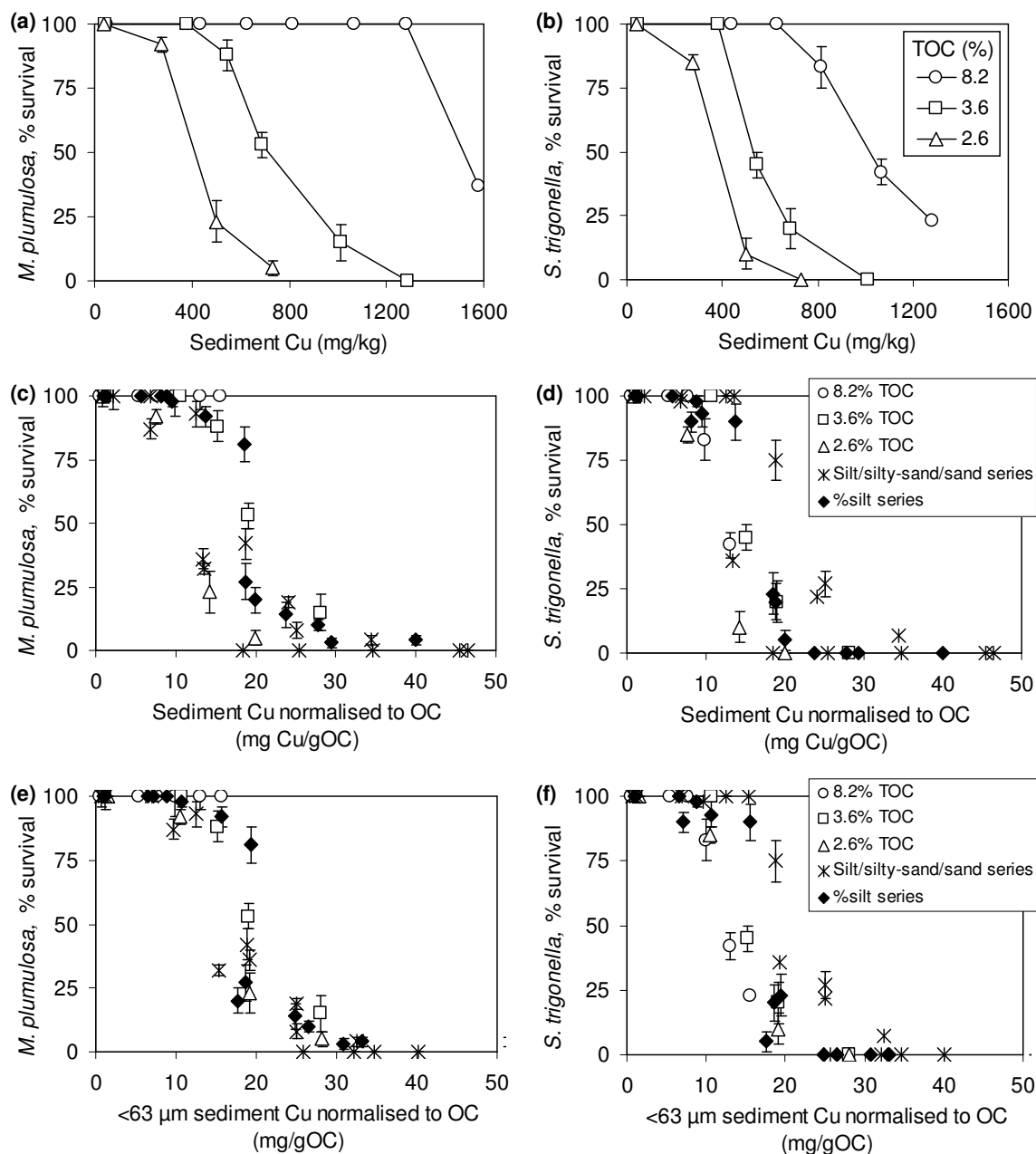


Figure 5. Relationships between survival (of *M. plumulosa* and *S. trigonella*) and (a, b) sediment copper concentration for the 8.2, 3.6 and 2.6 %OC series (100% <63 µm), (c, d) sediment copper concentration normalised to OC concentration of the whole sediment for all data sets, and (e, f) the sediment copper concentration normalised to OC concentration of the <63 µm sediment fraction for all data sets.