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**UNIVERSITY OF
WOLLONGONG**



School of Chemistry

A pre-screening technique for bioavailable metals in sediments

Elvio Diego Amato

**This thesis is presented as part of the requirement for the
Award of the Degree of Doctor of Philosophy
of the
University of Wollongong**

September 2015

ABSTRACT

In this study, a novel application of the diffusive gradients in thin films (DGT) technique as a monitoring tool for bioavailable metals in sediments is presented. DGT was tested using a wide range of marine and fresh water sediments that possessed a variety of chemical and physical properties. These were tested in both the laboratory and in the field. In the laboratory, the use of naturally contaminated sediments allowed the evaluation of the performance of the DGT technique under realistic environmental conditions (i.e. exposure to contaminant mixtures). Strong dose-response relationships were found between DGT-labile metal fluxes and toxicity to the marine amphipod *Melita plumulosa*. The combined flux of metals (Cd, Cu, Ni, Pb and Zn) measured at the sediment water interface (SWI) provided a robust measure of the bioavailable pool of metals present in the sediment and overlying water. A flux normalisation approach was adopted in an attempt to account for different toxicity caused by different metals, and this significantly improved the relationship between DGT and toxicity.

The performance of the DGT technique to predict metal bioavailability was also investigated by comparing DGT-labile metal fluxes from identical metal contaminated sediments deployed in the field and under laboratory conditions with metal bioaccumulation in *Tellina deltoidalis* (marine bivalve) and *Hyridella australis* (freshwater bivalve). The combined laboratory and field results indicated that DGT-labile metal fluxes measured at the SWI provide robust predictions of metal bioaccumulation in *Tellina deltoidalis* (marine bivalve) and *Hyridella australis* (freshwater bivalve), irrespective of the type of exposure (laboratory vs field). There was a mismatch between laboratory and field bioassay results for both species which emphasised the importance of performing *in situ* tests for environmental risk assessments.

The impact of infaunal activities (i.e. borrowing and feeding) to metal bioavailability was investigated in the laboratory by exposing *T. deltoidalis* to sediments with varying degrees of bioturbation (low and high). In the presence of the highly active amphipod *Victoriopisa australiensis*, significantly higher zinc bioaccumulation in *T. deltoidalis* was associated with greater DGT-Zn fluxes measured in the pore and overlying water. This indicated that high infaunal activities may result in

increasing metal exposure and higher risk of adverse effects to sediment dwelling organisms.

Overall, the DGT technique was shown to be a suitable tool for measuring bioavailable metals in sediments irrespective of site-specific parameters (i.e. particles size, acid-extractable metals, organic carbon, sulfides, bioturbation) and environmental conditions (laboratory and field). The combined pool of DGT-labile metals measured in the pore and overlying water and released by weak-binding particulate phases provided predictions of toxicity and metal bioavailability which were consistent with, or stronger than, those obtained using traditional methods based on acid-extractable metal analyses. These results indicate that DGT is a useful tool for monitoring bioavailable metals in sediments and has the potential to improve current sediment quality assessments.

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THESIS CERTIFICATION

I, Elvio Amato Diego, declare this thesis, submitted in fulfilment of the requirements for awarding the degree of Doctor of Philosophy, in the School of Chemistry, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. This document has not been submitted for qualifications at any other academic institution.

Elvio Amato

March, 2015

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3. Amato, E. D.; Simpson, S. L.; Jolley, D. F. (2012).Assessing the performance of the DGT technique for predicting trace metal bioavailability in estuarine sediments. 20th Annual RACI Conference, 11-14th December, Geelong, Australia.
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Chapter 1: Introduction

1.1 Context statement

Australia's current sediment quality guidelines (SQG) offer a risk-based approach in which exceeding a SQG value 'triggers' a tiered assessment framework. The framework for metals proceeds from assessing total concentrations, to bioavailability, to effects (toxicity, ecology). However, the use of inadequate tools for assessing bioavailability frequently results in incorrect conclusions, e.g. 'no impact' when effects are clearly occurring to benthic organisms living within the sediment surface layer.

Existing bioavailability methods have major limitations: porewater measurements are non-time-integrated 'snap-shots'; measurements of acid-volatile sulfides (AVS) over-emphasises the importance of sulfidic sediments; and both of these methods inadequately assess metal bioavailability to benthic organisms living in the surface sediment layer (i.e. the majority of organisms). The diffuse gradients in thin films (DGT) technique is currently the best measure of metal 'release potential'. Field deployments of this simple device can provide time-averaged information on site-specific in-situ metal fluxes from sediments to pore waters under the natural conditions. Through comparison with laboratory and field bioassays, this study will evaluate the DGT technique as a robust *in situ* tool for assessing metal bioavailability.

1.2 Sediments

Concentrations of metals (e.g. Cd, Co, Cr, Cu, Ni, Pb Zn) in aquatic ecosystems have been increasing in the last several decades as a result of urban spread, farming and industrial activities. Once introduced into aquatic environments, metals frequently bind to colloidal and particulate matter and eventually settle to bottom sediments. Increasing metal concentrations in sediments pose a significant risk to the health of sediment biological communities. As these organisms lie at the bottom of the aquatic food chain, adverse effects occurring to these species may significantly impact higher trophic levels and the overall aquatic ecosystem health.

Transport of chemical species across the sediment-water interface occurs due to sedimentation as well as by diffusional migration in pore waters, which is caused by concentration gradients between the two sides of the interface. The chemical composition of pore waters changes due to reactions with solid phases in the sediment and to biogeochemical processes. These processes include aerobic and anaerobic

decomposition of organic matter, oxidation and reduction of iron and manganese phases and production of methane, ammonia and hydrogen sulfide.

The redox conditions in sediments are usually stratified and zones are frequently referred to as oxic, sub-oxic, and anoxic (Jørgensen and Revsbech 1985; Kristensen 2000). In the present study, we refer to oxic sediments as surface sediments penetrated by dissolved oxygen, sub-oxic sediments as those containing mixtures of dissolved Mn(II) and Fe(II) in equilibrium with Fe(III) and Mn(IV) oxyhydroxide phases but containing negligible dissolved oxygen or sulfide, and anoxic sediments as those in which dissolved sulfide and acid volatile sulfides (AVS) are dominant. An understanding of the biogeochemical processes occurring in sediments is essential to predict the risk posed by metal contaminants to benthic organisms and assess the well-being of natural aquatic environments.

1.3 Metal bioavailability in sediments

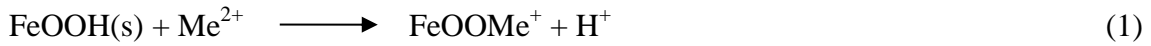
Sediments are complex and heterogeneous systems, where chemicals are present in a wide range of different forms. The toxicological risk associated with a contaminant strongly depends on its bioavailability, namely the various forms in which a contaminant can be available for uptake by organisms. Therefore, assessing the bioavailability of contaminants is critical for predicting toxic effects on benthic organisms and determining sediment ecosystem health.

Metal bioavailability in sediments depends on: 1) metal speciation (e.g. binding with iron and manganese oxyhydroxides, organic carbon and the formation of insoluble sulfides); 2) metal partitioning between water and sediment phases (distribution coefficient K_d dependent on sediment grain size); 3) exposure pathways (e.g. food selectivity and burrow activity); 4) organism physiology (e.g. gut structure, rate of uptake and assimilation efficiency [AE] from ingested particles). In this section, a general description of the various factors influencing bioavailability is provided. Particular emphasis will be placed on the essential role that bioavailability plays in determining metal toxicity in sediments, and its importance as a fundamental part of the sediment quality assessment framework.

1.3.1 Metal speciation in sediments

Chemical speciation, bioavailability, toxicity and mobility of trace metals in sediments are regulated by pH and redox processes mostly associated with biogeochemical cycles

(Borch et al. 2009). Metal contaminants entering aquatic environments are commonly adsorbed to suspended particulate organic matter or on hydrous iron and manganese oxides particles or particle coatings that can be transported to the bottom sediments by flocculation and sedimentation. In the first strata of sediment below the sediment-water interface, the oxygen dissolved in the overlying water penetrates the sediments creating a thin oxic/aerobic layer. Oxic conditions promote the degradation of the organic matter by aerobic bacteria, with the consequent release of the associated metal fraction (Kristensen 2000). The released metals can be subsequently absorbed onto insoluble iron and manganese oxyhydroxides produced by oxidative precipitation (Petersen et al. 1995; Tessier et al. 1996; Van Cappellen et al. 1998) according to the general equation:



where Me^{2+} represents a divalent metal.

The adsorption of metals to hydrous oxides is highly dependent on pH and the metal cation in question (Burdige 1993). Trace metals in marine sediments are frequently associated with iron and manganese oxyhydroxides and changes in the sediment redox chemistry near the sediment water interface can lead to alternating periods of reductive dissolution and oxidation of these phases. As oxygen concentrations become increasingly depleted with depth, reductive dissolution of iron and manganese oxyhydroxides occurs due to anaerobic bacteria using these phases as electron acceptors in organic material mineralization processes (Furrer and Wehrli 1993), and sulfides oxidation processes (Simpson et al. 1998).

Following dissolution of iron and manganese oxyhydroxides, metals associated with these phases are released into the pore water (Hamilton-Taylor et al. 1999; Poulton et al. 2004; Naylor et al. 2006). As anoxic conditions increase, sulfates are used by anaerobic bacteria to degrade organic matter, generating H_2S . Abundant sulfates and organic matter concentrations cause high rates of sulfide production, which can react with reduced forms of iron and manganese generating insoluble monosulfide phases commonly referred as acid-volatile sulfide (AVS), with FeS frequently being the most abundant end product (Canavan et al. 2007) (Equations 2 and 3).





Trace metals released from the reductive dissolution of iron and manganese oxyhydroxide phases can replace iron and manganese to form even more insoluble sulfides, as shown in Equation 4 and 5.



As the solubility of trace metal sulfides is very low, sediments with a molar excess of reactive sulfide to trace metals will exhibit very low dissolved metal concentrations in pore waters (Huerta-Diaz et al. 1998; Di Toro et al. 2005; Burgess et al. 2013). Therefore, with increasing sediment depth, a decrease in porewater metal concentrations is commonly observed.

Another important binding phase regulating metal speciation in sediments is organic matter. Humic substances are common complexing agents in aquatic media, and have a very strong impact on metal speciation in a large variety of natural settings (Christensen et al. 1999; Di Toro et al. 2001; Sánchez-Marín and Beiras 2012; Burgess et al. 2013). Fulvic acids (FA) and the larger humic acids (HA) are highly complex and diverse, with varying amounts of carboxylic and phenolic functional groups. The distribution and abundance of sedimentary organic matter depend on both physical and biological transport processes (e.g. sedimentation, erosion/deposition, lateral transport, resuspension, bioturbation), and on remineralisation and synthesis reactions (Hedges and Keil 1995).

1.3.2 Effects of sediment particles size on metal bioavailability and benthic communities

Sediment particles generally range from sand, silty-sand, to clay. The specific surface area (total surface area per unit of mass) of a sediment varies according to the size of the particles that compose it. As the size of the sediment particles decreases, the specific surface area increases. As a consequence, sediments composed of fine particles will have a larger number of binding sites than sediments made of coarse material, thus a greater ability to lower dissolved metal concentration in the pore water. This directly

affects the partitioning between solid and dissolved phase and the bioavailability of metals (Simpson 2005; Strom et al. 2011; Campana et al. 2013). While sediments composed of fine particles are expected to reduce the exposure to the dissolved phase, they increase the exposure to the particulate phase, as particles having appropriate dimension (e.g. $<63\ \mu\text{m}$) are commonly found in the guts of sediment-ingesting organisms (Tessier et al. 1984).

The sediment particle size and nutrition content also influences whether a sediment is a suitable habitat for benthic fauna. Organisms may prefer a specific sediment due to its particle size, redox status, organic matter and nutrient composition. High porosity sediments, like clays and silts, are usually more compacted (Berner 1980) and harder to penetrate by burrowing organisms than sandy ones. On the other hand, sandy sediments are more susceptible of collapsing. This will affect the depth that a burrowing organism can reach. Given the smaller specific surface area, sandy sediments also offer smaller quantities of organic material that is source of food for many organisms (Mayer et al. 1996).

1.3.3 Exposure pathways

Benthic organisms assimilate contaminants as a result of exposure to dissolved and particulate phases (Lee et al. 2000a; Wiklund and Sundelin 2002; Riba et al. 2003; Di Toro et al. 2005; Simpson 2005). Dissolved metals are assimilated by organisms from the surrounding aquatic medium, i.e. pore water, burrow water and overlying water, whereas the exposure to the particulate phase is due to ingestion of sediment and/or food particles (e.g. algae, plant, phytoplankton or other benthos). The rate at which benthic organisms are exposed to contaminants is therefore dependent on their burrowing and feeding behaviour and the distribution of metals between dissolved and solid phases in the specific compartments they inhabit or are more likely to be exposed to (e.g. deep vs surficial sediment, overlying water).

For burrowing organisms that feed on sediment particles, but use overlying water to irrigate their borrows, the major source of contaminants may be the water column rather than the sediment (Munger and Hare 1997; Hare et al. 2001). Particle ingestion is the main route of metal uptake for many polychaete worms (Wang et al. 1999; Lee et al. 2001; Yan and Wang 2002). Deposit-feeding and/or suspension-feeding species such as amphipods and bivalves are exposed to metals associated with sediment particles as well as the pore water, burrow water and overlying water (Luoma et al.

1992; Fan and Wang 2001; Griscom and Fisher 2002; Wiklund and Sundelin 2002; King et al. 2005)

For polychaete worms, the type of sediments (organic carbon content and sediment grain size) has a little effect on metal assimilation (Wang et al. 1999a), whereas for deposit-feeding clams, the availability of sediment-bound metals is highly dependent on the sediment type (Bryan 1980). Schlekert et al. (1999, 2000) have shown that the estuarine amphipod *Leptocheirus plumulosus* assimilates metals associated with bacterial exopolymer sediment coatings with higher efficiency than those associated with recalcitrant organic carbon, iron oxides or phytoplankton. In mussels, food quality has the major effect on metal assimilation, whereas no substantial effect of food quantity or quality was observed for metal assimilation in marine copepods (Wang and Fisher 1999a). In a study conducted on two clam species, Lee and Luoma (1998) observed an increase of metal concentration (Cd, Zn and Cr) during the spring phytoplankton bloom.

To understand the importance of the dietary exposure as a factor affecting metal bioavailability, the assimilation efficiency (AE) of the contaminants associated with different sources of food must be considered. AE is a physiological parameter that expresses the fraction of ingested elements or compounds that is assimilated into biological tissue. AE can be used to assess the bioavailability of different contaminants associated with different food sources, as long as feeding rates, contaminant concentrations in the food particles and efflux rates of chemicals are known (Wang and Fisher 1999a). It has been shown that, for benthic organisms such as mussels, worms and copepods, the importance of the dietary exposure increases as the AE values increase (Wang and Fisher 1999b). Once they have entered the organism body, the fate of a contaminant is regulated by physiological processes which determine whether a chemical is accumulated or not. Thus, AEs can be used to predict metal bioaccumulation in aquatic invertebrates, particularly in the case where ingested food is the main uptake route (Wang and Fisher 1999a).

A bioenergetics-based kinetic model developed by Landrum et al. (1992) incorporated AE from ingested particles to express metal bioavailability through a bioaccumulation factor defined as the ratio of metal concentration in an organism to the metal total ambient concentration, including both dissolved and particulate phases. Applications of this kinetic model to predict metal body concentrations have shown good accordance with measured concentration in marine bivalves and copepods

collected from the field (Luoma et al. 1992; Wang et al. 1996; Fisher et al. 2000). Further kinetic models have been developed for predicting metal (Cd, Pb, Cu, Zn, Cr, Ni and Hg) bioaccumulation in marine and estuarine amphipods (Clason and Zauke 2000; Clason et al. 2003, 2004).

Although AE is regarded to be a critical parameter for predicting toxicity on a tissue residue base, direct relationships between a contaminant's AE and its toxicity have not been clearly documented. Even though a chemical is assimilated in an organism's tissue, it may be stored in a non-toxic form and hence not represent any risk for the organism (Rainbow 2002, 2007). However, a contaminant cannot exert its toxic effect unless it is accumulated and available to interact with the receptor site where toxic action is initiated, therefore the AE can be related to the potential risk that a contaminant represents as it may be available to interfere with some metabolic process and thus determine toxicity.

Since benthic biota show a great variety of behaviours and dietary habits, careful consideration of the different exposure pathways is necessary to provide a correct evaluation of the bioavailability of contaminants. The relative risk associated with each exposure route has to be considered in relation to the sediment compartment where an organism lives and the distribution of contaminants between dissolved and particulate phase in that given portion of sediment (Simpson and Batley 2007).

1.3.4 Organism physiology

All aquatic invertebrates accumulate metals regardless of whether essential or non-essential. Different organisms living in the same habitat may show different body tissue metal concentrations, sometimes even within individuals belonging to the same taxa (Rainbow 2002). Metals show high affinity for atoms such as sulfur and nitrogen, which are common components of proteins. Consequently, metals bind with a wide range of proteins, and hence are able to interfere with their normal metabolic functions and cause toxicity.

The physiology of organisms plays an essential role in metal accumulation. Some trace metals are essential for metabolism processes so they are required at minimum concentrations. Zinc for example is a fundamental component for many enzymes such as carbonic anhydrase, and respiratory proteins found in some molluscs and crustaceans includes copper in their structure to reversibly bind oxygen molecules.

Conversely, non-essential metals are not required for metabolic purposes and hence are readily excreted or undergo detoxification processes (Rainbow 2002).

The digestive system of benthic organisms strongly affects the bioavailability of sediment-bound contaminants (Mayer et al. 1996; Chen and Mayer 1999). In addition to metabolic and regulation processes, metal assimilation will depend also on other physiological aspects such as gut structure, retention time and acidity. Gut retention time and the importance of intra- and extra-cellular digestion can both affect metal assimilation in bivalves (Decho and Luoma 1996; Wang et al. 1996). Some invertebrates have acidic guts and thus metal desorption from ingested particles may be expected (Wang and Fisher 1999a).

1.3.5 Biological process influencing metal chemistry in sediments

Sediments are inhabited by both macro- and micro-organisms that physically disturb the sediment and pore water. This biologically mediated disturbance is commonly called “bioturbation” and includes activities such as burrow creation and maintenance, sediment ingestion, sediment disposal and burrow irrigation. It has been established that bioturbation in general, and burrowing worms in particular, draw oxygenated water into the sediment and so create an oxic and oxidised zone around their burrows (Aller 1978; Fenchel 1996). These activities affect the vertical redox profile of the sediments, providing oxygen to the sub-oxic and anoxic zones.

As oxygen penetration increases, changes in redox conditions will occur as chemical species undergo oxidation (Williamson et al. 1999). As the form and availability of trace metals is controlled by the chemical species present in the sediment, a change in the redox state of these chemical species will affect trace metals partitioning between sediment and pore water. For example, the oxidation of metal sulfide phases will release trace metals into the pore waters (Peterson et al. 1996; Lagauzère et al. 2009; Simpson et al. 2012a).

Burrows are not permanent, and when abandoned can fill in with surface/sub-surface-derived particles. Abandoned burrows become relic structures around which high CO₂ concentrations and low pH may occur, particularly in poorly buffered freshwater sediments, reflecting locally enhanced sites of microbial metabolic activity (Zhu et al. 2006). Bioturbation is thus expected to have a strong impact on the metal partitioning between solid and dissolved phases and on metal bioavailability in sediments.

1.4 Bioaccumulation

Bioaccumulation is the net retention of a contaminant by an organism over time, hence the influx of the contaminant exceeds its efflux. Aquatic organisms accumulate essential and non-essential metals in their tissue. Essential trace metals, e.g. Cu, Fe, and Zn, are micronutrients essential to life that, if lacking, result in an impairment of organism function that can only be restored by the addition of the metal in question. Non-essential metals, e.g., Cd, Hg, and Pb, are not known to be required in metabolic processes. All metals have the potential to be toxic when present in high concentrations. Contaminated sediments expose benthic organisms to high concentration of trace metals and hence high rates of accumulation in organisms may occur.

Metal bioaccumulation in benthic organisms is strongly related to the bioavailability of the metals in the sediments. The assimilation of metals can be the result of different types of exposure (dissolved and particulate phase and dietary pathway), and the length of retention in their tissue is controlled by their physiology (e.g. gut structure, digestion processes and capacity of detoxification) (Wang and Fisher 1999a; Luoma and Rainbow 2005).

Measurements of body tissue metal concentrations may provide useful information on possible adverse effects only in the case where relationships between bioaccumulation and toxicity are solid and well-documented (Borgmann 2000; Borgmann et al. 2004; Simpson and King 2005). In fact, bioaccumulation tests are not useful when contaminants are regulated by physiological processes or sequestered into non-toxic forms by organisms (Borgmann 2000; Rainbow 2002, 2004).

In a study conducted on the amphipod *Melita plumulosa* and the bivalve *Tellina deltoidalis* in copper-contaminated sediments, Simpson and King (2005) showed that, for these benthic species, calculating lethal body concentrations (LBCs) is not a suitable predictor of toxicity as different LBCs were obtained based on different exposures. Biota-to-sediment accumulation factors (BSAFs), commonly used to estimate the levels of bioaccumulation of non-ionic organic contaminants, showed high variability for metals (McGeer et al. 2003) and hence poor applicability to metal toxicity assessments.

Currently, the application of bioaccumulation tests as a tool for assessing metal contaminant hazards to benthic biota have yet to be fully evaluated. However, a better approach seems to be to express the measured metal concentration in terms of metabolically-available concentration rather than total accumulated concentration

(Rainbow 2002; Vijver et al. 2004; Rainbow 2007). According to the approach described by Rainbow (2002), the accumulated metals in an invertebrate are subdivided into two categories, metabolically-available and detoxified, where the latter represents the fraction of accumulated metals that is no longer available for metabolism purposes. For definition, a metal stored in a detoxified form does not represent any hazard for the organism, thereby there is no theoretical limit to its concentration in the tissue. The fate of a metal will depend on the accumulation pattern adopted by an organism for that metal, for which an exceeding uptake will be regulated by excretion, stored in a non-toxic form or both combined. Hence, toxicity will occur when the accumulation rate of a metabolically-available metal exceeds that of excretion and/or detoxification.

1.5 Bioassay

A healthy ecosystem can be defined as one which satisfactorily supports the development of a biological community (e.g. growth and reproduction) (Maher et al. 1999), although the composition of the community is also important. The introduction of contaminants in ecosystems potentially varies the biological equilibria that occur within it, sometimes causing toxic effects to the biological community. According to the magnitude of the contamination, such effects can lead to changes in biological activities such as growth and reproduction, and, in the worst case, cause mortality within the population. Assessing the effects of contaminants toward a biological community is therefore a straightforward means of determining toxicity in ecosystems. Biological assays are widely used for this purpose and are an essential part of the sediment quality assessment framework (ANZECC/ARMCANZ, 2000).

Whole-sediment toxicity tests are an important tool for assessing the potential effects of contaminated sediments on benthic communities (ANZECC/ARMCANZ, 2000; Chapman and Anderson 2005). Such tests are carried out either for acute or chronic exposure durations. Acute toxicity tests generally measure endpoints such as the survival of organisms in the tested sediments, whereas chronic tests are performed over longer periods and aim to determine sub-lethal endpoints such as growth, development and reproduction. Although the former are more frequently used, longer exposure tests are regarded as better representing the ecological risk associated with episodes of contamination and are more suitable for modelling contaminant effects on population dynamics (Smit et al. 2006; Van den Heuvel-Greve et al. 2007). Nevertheless, chronic tests do not always show higher sensitivity than acute methods (McGeer et al. 2003;

Greenstein et al. 2008) and usually long-term exposure tests are affected by data variability, which results in increasing labour intensiveness and hence higher costs (Kennedy et al. 2009). More recently, rapid sub-lethal chronic tests have been developed (Mann et al. 2009; Perez-Landa and Simpson 2011). Simpson and Spadaro (2011) highlighted the ecological relevance and importance of using the sub-lethal endpoints, and showed that, using 10-day exposure tests on the amphipod *Melita plumulosa* and the copepod *Nitroca spinipes*, the cost required for the whole procedure was approximately 1.5 that of acute tests.

1.6 Approaches and methods for predicting metal toxicity in sediments

The ability to predict metal toxicity is becoming increasingly important for the assessment of contaminated sediments and for the development of sediment quality guidelines (SQGs) (Batley et al. 2005; Wenning 2005). The most common approach has been to interpret metal toxicity only in terms of dissolved metals, using pore water-sediment partitioning models. The equilibrium partitioning (EqP) model based on the ratio of acid-volatile sulfides (AVS) to simultaneously extracted metals (SEM) is widely regarded as being accurate for predicting the lack of toxicity (Cd, Cu, Ni, Pb and Zn) in laboratory metal-spiked sediments and in field-contaminated sediments. Di Toro et al. (2005) extended the AVS/SEM-based approach by coupling it to a biotic ligand model (BLM) with more explicit consideration of particulate organic carbon (POC) as a metal-binding phase (e.g. (SEM-AVS)/fOC). Dissolved organic carbon (DOC) and other competing ligands in pore water are ignored due to their small amounts compared to that of POC. Furthermore, the model assumes that the exposures from dietary sources do not contribute to toxicity.

Biodynamic models or exposure-effect model offer an alternative approach that explicitly consider the effects from both pore water and sediment ingestion exposure (Luoma and Rainbow 2005; Simpson 2005). Similar to the sediment BLM (sBLM), the exposure-effect model uses an EqP approach to predict the exposure to dissolved metals, but also considers metal assimilation by ingested particles (Simpson 2005). Both exposure-effect model and sBLM have been developed on metal-spiked sediment data sets. Inadequate spiking procedures may accentuate the sediment-water partitioning of metals to the dissolved phase and shift the pathway for metal exposure from particulate to pore water (Lee et al. 2000b; Simpson 2005). Given that, in natural environments, dissolved metal concentrations are in the sub- or low- $\mu\text{g/L}$ range, exposure to particle-

bound metals may be the major pathway of metal consumption (Luoma and Rainbow 2005; Simpson 2005). Therefore, toxicity thresholds determined in laboratory tests will not be widely applicable for assessing toxicity in field tests.

Luoma and Rainbow (2005) showed that the dynamic multi-pathway bioaccumulation model could provide a unified explanation of metal bioaccumulation by a wide range of benthic organisms in a range of sediment environments. This approach uses metal bioaccumulation data derived from exposure to dissolved metals combined with a biodynamic model including physiological parameters describing the metal influx and efflux rates for each organisms from each metal and exposure source to predict steady-state metal concentrations in benthic organisms. While the AVS/SEM and sBLM models use data sets derived from metal-spiked sediments which may overly emphasize the contribution of the dissolved exposure, the DYN-BAM approach has been developed on naturally contaminated sediment data sets, and the exposure-effect model may use metal-spiked sediments with low ($\mu\text{g/L}$ range) dissolved metal concentrations. As a consequence, the effects of the porewater metal exposure is not exaggerated to the same extent as the data used for the AVS/SEM and sBLM models, and the important contribution of ingested sediment as an exposure pathway is clear.

While many modelling approaches appear to be suitable for predicting the bioavailability of trace metals in sediments, the major current limitation is the availability of good data sets.

1.7 Sediment quality guidelines

Sediment ecosystem health can be assessed through laboratory biological assays or surrogate chemical measurement which can be linked to adverse effects observed on the living community (Maher et al. 1999). Since the former involves time-consuming and expensive procedures, emphasis has been put by the scientific community on developing more accurate and reliable chemical measurements that can address sediment health. For this purpose, guidelines based upon a biological-effects database have been developed in the USA by Long et al. (1995) and MacDonald et al. (1996), and have subsequently formed the basis of the guidelines adopted by other countries, including Australia and New Zealand (ANZECC/ARMCANZ, 2000).

Sediment quality guidelines (SQG) were included in the Australian and New Zealand Guidelines for Fresh and Marine Water Quality in 2000 (ANZECC/ARMCANZ, 2000). They consist of a large database including contaminant

concentration data combined with biological effects data obtained from metal-spiked sediment toxicity tests and equilibrium partitioning calculations (Long et al. 1995). A distribution of the data yielded concentrations where biological adverse effects on benthic biota were likely, based on the median of effects data and a value below which effects were unlikely based on the 10th percentile of effects data. The latter values were adopted as triggers and further investigation was required to verify any concerns. The procedure adopted for the assessment of the health of sediment is encapsulated in a tiered assessment framework which includes a sequence of steps as described by the decision tree of Figure 1.1. The more a contaminant is regarded to represent an ecological risk for the sediment, on the basis of comparison with trigger values, the more accurate and specific investigations are required. Sediments are an important component of aquatic ecosystems, but only recently the ecological risk that sediment contaminants represent for water ecosystems has been considered by regulatory agencies for the assessment of water health. Nevertheless, the derivation and use of effects data as sediment guideline values has been a much debated question (MacDonald et al. 1996) whilst the understanding of the biological impact of such contaminants was still being developed. Indeed, these values were never intended for use as stand-alone guidelines, but combined with site-specific investigations. For these reasons the guidelines were defined “interim” in recognition that improvements were required.

Metal toxicity in sediments is strongly related to their speciation and hence to the various chemical forms and species in which such contaminants may be available to benthic organisms. The assessment of metal bioavailability as described in the sediment quality guidelines involves the measurement of porewater concentrations, acid-volatile sulfides (AVS) and simultaneously extracted metal (SEM) concentrations and speciation investigations.

A significant portion of the total metal concentration (extracted using concentrated acids) present in the sediment may be associated with strong binding phases and not be bioavailable to organisms (e.g. highly mineralised forms). To determine the fraction of metals that is more likely to be available to organisms, dilute acid-extractable metal (AEM) analyses are frequently used. The most common of these analyses is performed by reacting the wet sediment with cold 1 M hydrochloric acid in a sediment:acid ratio of 1:50 for 1 h. This extraction is analogous to the extraction of metals used in the AVS-SEM (simultaneously extractable metals) analysis.

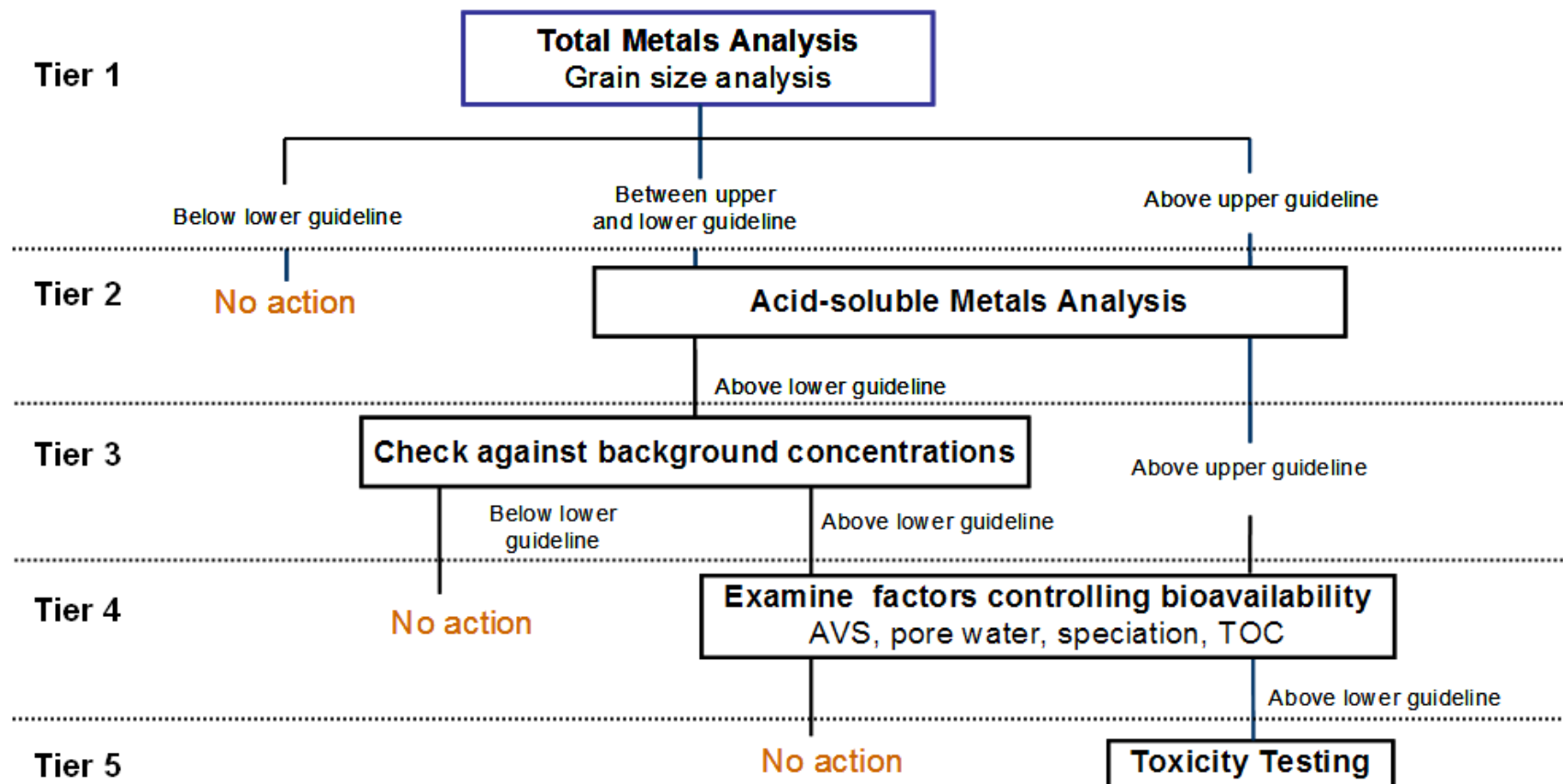


Figure 1.1 Schematic of the tiered-based assessment framework adopted for sediment quality assessment.

Although this extraction is much stronger than can be achieved by organisms, the AEM concentration provides a useful estimate of the ‘potentially bioavailable’ metal concentration, and it is usually suitable to compare this to the guideline value.

The interaction between metal contaminants and AVS and the subsequently formations of metal sulfides is a key process which regulates the bioavailability of several metals in sediments (Di Toro et al. 1990; Ankley et al. 1996; Simpson et al. 1998). The ratio between AVS and SEM has been used to define the fraction of bioavailable metals to the extent that a greater concentration of AVS compared to that of SEM means that metals are likely to be bound with sulfide complexes and hence to be of limited bioavailability. Nevertheless criticism of the AVS/SEM approach has been raised regarding measurement-associated artefacts (Di Toro et al. 1990; Berry et al. 1996) and later the difference between SEM and AVS was recognized to better represent metals bioavailability rather than the ratio approach. However, this comparative method partially predicts the bioavailability of metals since it does not explicitly consider contaminants bound to both particulate and dissolved organic phase (Strom et al. 2011; Besser et al. 2005; Di Toro et al. 2005) as well as the possibility that some benthic organisms may ingest metal sulfide particles (Simpson and King 2005). New approaches and methods resulting from the latest research on predicting metal bioavailability in sediments will be provided further in the text.

In natural water systems, metals are present in a variety of chemical forms and species such as free ions, organic and inorganic complexes, particles and colloids. Studying the speciation of metals is a fundamental process to assess whether a contaminant is present in the sediment in one or more chemical forms which can represent a biological risk for benthic organisms. The guidelines do not provide specific measurement tools for speciation and many of the techniques currently in use are not sufficiently robust for applications in routine laboratories. Metal toxicity to benthos is regarded to be due mainly to inorganic complexes, whereas metal contaminants associated with organic compounds (dissolved and particulate organic matter) are assumed to have low toxicity potential. Hence, according to SQGs, valuable speciation methods should be the ones able to discriminate between metal species that are present in sediment as inorganic or organic complexes (Simpson et al. 2005).

A more recent approach to assessing sediment quality has been recommended as part of the revision of the Australian and New Zealand SQGs (Simpson et al. 2013a) which combines the ANZECC/ARMCANZ framework with multiple lines of evidence

in a weight-of-evidence framework, including investigations such as bioaccumulation/biomagnification tests, multiple species toxicity tests (extended to acute and chronic exposure tests considering the adverse effects on growth and reproduction other than just mortality) and benthic community structure assessments (comparing impacted with reference locations and identifying potential variables that may contribute to such differences) (Simpson et al. 2005; Burton Jr 2002; Wenning et al 2005).

Evaluating metal bioavailability is a well-established component of most SQG assessment frameworks. However, the dynamic equilibria regulating metal speciation are likely to be affected during sample collection, transport, homogenisation and storage required to perform chemical analysis in the laboratory (Simpson and Batley 2003). Laboratory-based bioassays may result in inadequate evaluations of toxicity due to poor representation of field conditions (e.g. solute exchange between pore and overlying waters, tidal cycles, deposition of suspended particulate material, bioturbation) (Peterson et al. 1996; Mann et al. 2010; Belzunce-Segarra et al. 2015) and changes in chemical properties due to sediment homogenisation (Simpson and Batley 2003). Thus, the lack of reliable *in situ* assessments limits the efficacy of current risk assessments. Additional field-based investigations are expected to contribute to stronger and more reliable SQGs.

1.8 Monitoring sediment quality

The most common techniques available for monitoring sediment quality can be subdivided in three main categories: grab sampling and analysis, on-site monitoring and *in situ* monitoring. Grab sampling consists of several steps including collection, preservation, transport, storage, processing and analysis of the sample. This technique has the advantage of being straightforward in its material requirements and interpretation of results, and is ideal for sampling in remote areas as the site has to be visited once (Greenwood et al. 2007; Madrid and Zayas 2007). However, such a long procedure is likely to expose samples to several sources of contamination (including oxygen) and cause unwanted reactions, which may lead to changes in contaminant speciation (Simpson and Batley 2003). As a result, the uncertainty of the analytical data will significantly increase and the accuracy of the results will be compromised (Buffle and Horvai 2000; Vrana et al. 2005). Furthermore, grab sampling provides contaminant concentrations at a single point in time, which makes the technique unsuitable for

measurements in dynamic systems where concentrations of contaminants may vary over time (Greenwood et al. 2007; Madrid et al. 2007).

On-site monitoring techniques alleviate issues of contaminant changes due to sample preservation and transportation. These techniques involve the analysis of the sample in proximity to the collection point (e.g. on the shore of a lake or river, or on board a ship at sea), so that the risk of contaminant modification which may occur between collection and analysis is minimized (Bartram and Ballance 1996). The instruments and equipment required to run the analyses need to be transported to the site, and analytical methods adapted to the field conditions. As a consequence, the reproducibility and detection limits of the methods are considerably lowered and difficulties may arise in matching QA/QC standards (Bartram and Ballance 1996). Additionally, on-site techniques are still affected by changes in contaminant speciation due to sample removal from the environment.

In situ monitoring involves either direct measurements of contaminants on site or direct accumulation of the contaminants in stable forms from the environment, followed by analysis in a laboratory (Buffle and Horvai 2000). This technique clearly avoids all the inconveniences described for the previous two categories, as sample storage, transportation and removal from the environment are no longer required. *In situ* monitoring techniques allow different types of measurement, such as continuous, discrete and time-integrated (Dunn et al. 2007). Contaminant concentrations are sampled directly in their natural matrix, which is the ideal and most desirable procedure for determining contaminant concentrations in natural systems. However, *in situ* methods are not yet available for a considerable number of analytes and usually rely on fragile and expensive equipment (Greenwood et al. 2007; Dunn et al. 2007).

1.9 Diffusive gradients in thin films

The diffusive gradients in thin films (DGT) technique consists of a simple *in situ* device which provides high-resolution time-integrated multi-element measurement of fluxes of dissolved ionic species in aqueous solution. DGT was initially designed for measuring trace metals concentrations in aquatic media (Figure 1.2) (Davison and Zhang 1994; Zhang and Davison 1995), but the development of a sediment device allowed the use of the technique to be extended to sediment deployments (Figure 1.3, Section 1.9.3) (Zhang et al. 1995). The DGT technique is capable of measuring different ionic species (e.g. cationic and anionic metals, sulfides, phosphorous) according to the binding resin

used (e.g. Chelex, ferrihydrite, TiO_2 , AgI, 3-mercaptopropyl-functionalized silica gel) (Naylor et al. 2004; Mason et al. 2010; Panther et al. 2010; Bennett et al. 2011; Price et al. 2013). This technique has been tested in different environmental matrixes, including waters, sediments and soils. For metals, some of the most important applications of the DGT technique include investigation of metal speciation in natural waters (Davison and Zhang 1994; Zhang and Davison 2000), sediment biogeochemical processes (Motelica-Heino and Davison 2003; Tankere-Muller et al. 2007; Stockdale et al. 2010; Wu et al. 2011), and bioavailability assessments in soils (Zhang et al. 2001; Huynh et al. 2010), waters (Camusso and Gasparella 2006; Jordan et al. 2008; Schintu et al. 2008; Schintu et al. 2010) and sediments (Roulier et al. 2008; Costello et al. 2012; Dabrin et al. 2012; Simpson et al. 2012b; Amato et al. 2014). In this section, a detailed description of the principles underpinning the DGT technique is provided. The main focus will be on cationic metal species, but the same principles also apply for other ionic species.

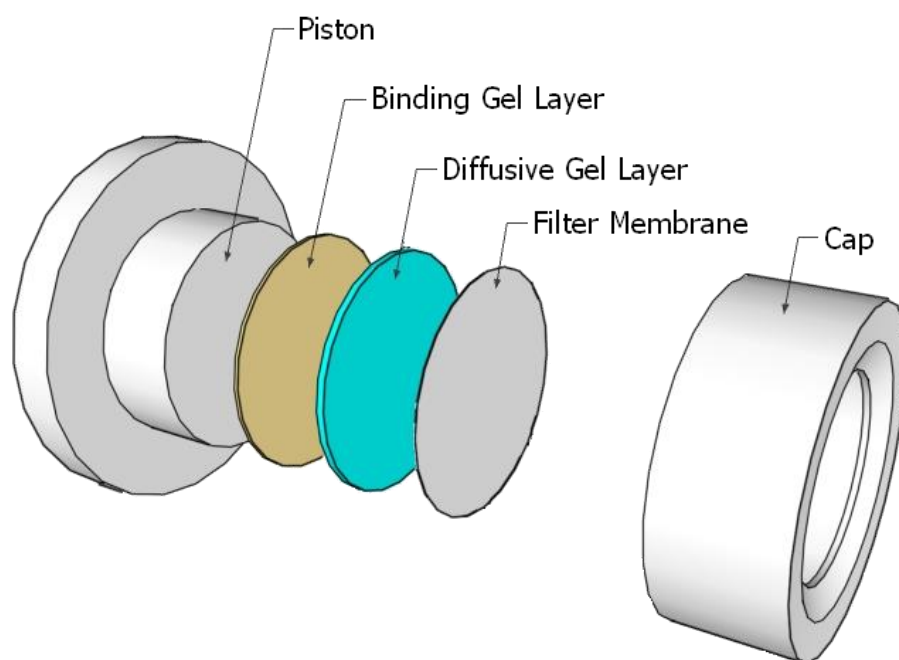


Figure 1.2 Schematic of a DGT piston device (adapted from Bennett et al. 2011).

1.9.1 DGT theoretical principles in waters

The DGT technique is based on the controlled diffusion of metals from a bulk solution through a hydrogel diffusive layer (polyacrylamide) to a cation-exchange resin layer (Chelex) (Figure 1.2). The latter is able to selectively bind and accumulate only the substances of interest according to the type of binding agent used. During natural water deployment, accumulation of particles on the diffusive gel surface exposed to the bulk solution is expected, with the consequent modification of the metal diffusion. A 0.14 mm-thick, 45 μm pore size cellulose nitrate membrane is applied at the interface between the diffusive gel and solution to prevent particles sticking. The transport in the gel layer is restricted to molecular diffusion only, which is governed by kinetics laws, thus diffusion toward the binding-resin is not affected by the bulk solution hydrodynamics (Davison and Zhang 1994). The gel layer and the bulk solution are separated by a diffusive boundary layer (DBL), of thickness δ . In order to reach the binding-resin, metals have to diffuse through the DBL, the filter membrane and the gel-layer of thickness Δg . After a few minutes of deployment, a steady-state concentration gradient is established between the solution and the binding layer. By exploiting this simple steady-state condition, the DGT device can be used for *in situ* concentration measurements. The flux J ($\text{mol cm}^{-2} \text{s}^{-1}$) of an ion from the solution to the chelating resin can be calculated according to Fick's first law of diffusion:

$$J = D (C - C') / (\Delta g - \delta) \quad (6)$$

where D ($\text{cm}^2 \text{s}^{-1}$) is the diffusion coefficient and C and C' are the concentration of the ion in the bulk solution and in the boundary between the binding agent and the diffusive gel, respectively.

If the free metal ions are in a rapid equilibrium with the exchange-resin, i.e. with a large binding constant, the resin/diffusive-gel interface is readily depleted of metals and C' is effectively zero, providing that the resin is not saturated. In well stirred solutions, the DBL thickness is negligibly small compared to the thickness of the diffusive gel (Davison and Zhang 2012). Therefore, assuming that diffusion coefficients in the diffusive gel are the same as in water and the thickness of the DBL is negligible respect to that of the hydrogel, the flux can be calculated as follows:

$$J = DC/\Delta g \quad (7)$$

According to the definition of flux, the mass (M) diffused through an area (A) in a given time (t) is

$$M = J / At \quad (8)$$

Taking the latter into account, the mass of ions that have diffused into the resin layer can be obtained by

$$M = DCtA / \Delta g \quad (9)$$

where t is the deployment time and A the diffusive-gel area exposed to the bulk solution. As the mass of ions accumulated on the resin layer can be analytically determined, the metal concentration in the bulk solution can be quantified as follows:

$$C = M\Delta g / DtA \quad (10)$$

The metal concentration in the exchange resin can be determined by means of techniques capable of analysing solids, such as x-ray fluorescence XRF, proton induced x-ray emission (PIXE) (Davison et al. 1997), laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) (Warnken et al. 2004), or through elution of the bound metals using a known volume of HNO₃ (V_e) solution (Davison and Zhang 1994; Zhang and Davison 1995). The concentration of ions in the acid eluent (C_e) can be then determined by atomic absorption spectrometry(AAS) or inductively coupled plasma atomic emission spectrometry (ICP-MS/ICP-AES). Since the elution is in batch mode, only a fraction of the accumulated metals is able to be removed from the resin. The ratio of the eluted to bound metal is known as the elution factor f_e. Given a known volume of diffusive-gel V_g, the metal concentration into the resin can therefore be calculated by the following equation:

$$M = C_e(V_g + V_e)/f_e \quad (11)$$

When the assumption of negligible diffusive boundary layer (DBL) thickness is not satisfied and the diffusion coefficient in the gel (D_g), filter (D_f) and water (D_w) are different, the accumulated ions mass on the resin is given by

$$\frac{1}{M} = \frac{1}{CA\tau} \left(\frac{\Delta g}{D_g} + \frac{\Delta f}{D_f} + \frac{\delta}{D_w} \right) \quad (12)$$

where Δg and Δf are the thickness of the gel and filter layers, respectively. The thickness of the DBL varies according to the type of natural water system in which the device is deployed (lake, ocean and river). A change of the DBL thickness may affect the mass transfer of the solute to the binding-resin. Provided there is a sufficient thickness of the gel layer and well-stirred solutions, the diffusion of ions within the device is not affected by the thickness of the DBL, which can therefore be considered negligible (Davison and Zhang 2012). However, the DBL thickness can be measured by performing multiple DGT deployments loaded with varying diffusive gel thicknesses (Zhang et al. 1995).

1.9.2 Metal speciation

The polyacrylamide hydrogel features a well-defined pore size structure, where the diffusion of solute through the gel depends on the species dimensions (Zhang and Davison 2000). Large molecule migration is likely to be affected by the hydrogel pore size, whereas metal ions are small enough to diffuse freely through the gel with an effective diffusion coefficient which cannot be distinguished from that of free ions in water. Nevertheless, a considerable fraction of the total amount of metals in water solutions is present as inorganic and organic complexes. The DGT device is able to measure the metal concentration associated with the labile (free and weakly complexed) fraction both kinetically (according to the affinity of the metal with the exchange resin) and by size (on the basis of the molecular dimension). As the accumulation of ions on the resin layer depends on the characteristics of the diffusive layer, by changing the composition of the gel, the DGT device is able to distinguish between inorganic- and organically complexed metal species (Zhang and Davison 2000).

1.9.3 DGT theoretical principles in sediments and soils

Evaluating metal concentration responses provided by DGT deployments in sediments is not as straightforward as for water solution measurements. The successful assessment of DGT metals concentration in aqueous solutions is enabled by the well-mixed conditions in these systems. In contrast, in pore waters the rate of water recycled is considerably lower and depletion of solutes near the DGT device is expected. Furthermore, a wide range of geochemical (e.g. metals cycles and redox profile) and biological processes (mineralization of organic matter and benthic organism behaviour) occur in sediments making the interpretation of DGT deployments even more complex. A good understanding of these processes is thus required to estimate the DGT capabilities to predict metals concentration in sediments. However, after due considerations, the theoretical principles applied to aqueous systems can be taken into account to interpret DGT concentration response into sediments and soils. A better understanding of DGT technique applications in sediments based on different objectives and methodologies is provided by the subdivision in homogeneous and heterogeneous systems. The following section will refer only to sediments, although the results are equally applicable to saturated soils.

1.9.4 Homogeneous systems

In the first DGT studies (Zhang et al. 1995; Harper et al. 1998), homogeneous sediments conditions have been applied in laboratory-spiked sediments in order to simplify the device development and understanding. It has been assumed that the porewater and solid phase concentrations were uniform throughout the sediments. When the DGT device is deployed, metals diffuse through the diffusive-gel layer from the solution to the resin layer (Figure 1.4). At the resin/hydrogel interface, the concentrations of metals decrease rapidly due to their accumulation on the exchange resin, and a steady-state linear concentration gradient throughout the diffusive-gel layer is established. The diffusion of metals from the solution to the exchange resin is thus regulated by the features of the hydrogel, provided that the concentration at the diffusive-gel/solution interface is maintained constant. Due to the low rate of mixing, porewater concentrations near the deployment area become progressively depleted.

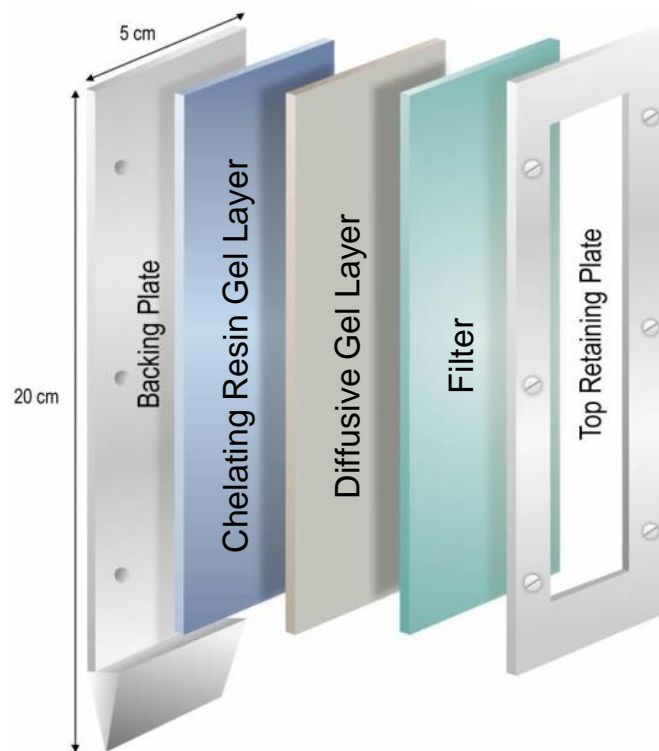


Figure 1.3 Schematic of a DGT probe assembly for sediment deployments.

Schematic cross-section of a DGT planar device deployed in sediments

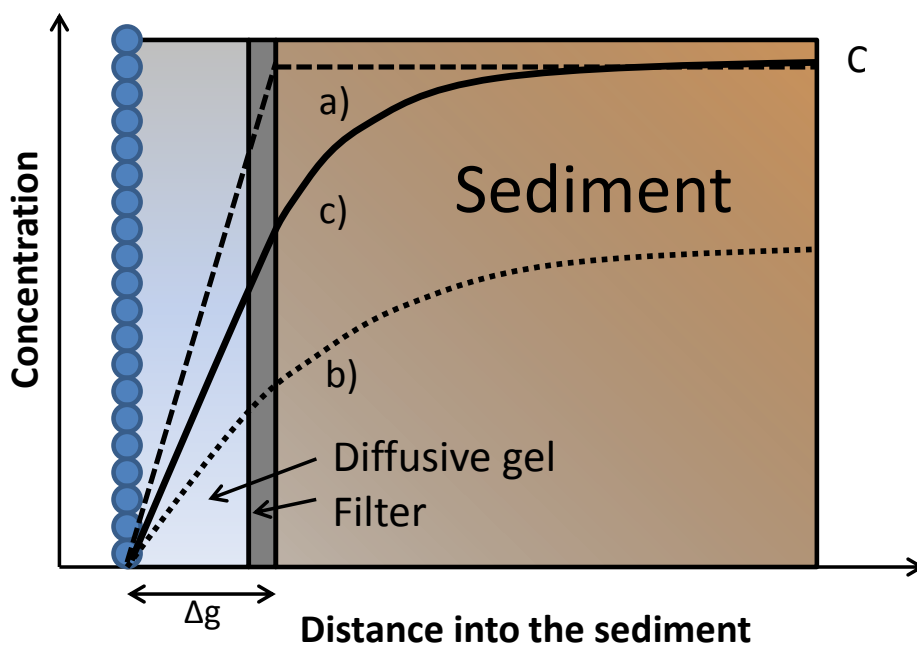


Figure 1.4 Representation of the ionic species in a DGT device and adjacent pore waters during deployment cases where the resupply from the sediment to the pore water is: a) sustained, $R > 0.95$, b) diffusion only, $R = R_{\text{diff}}$, and c) partially sustained, $R_{\text{diff}} < R < 1$.

If there is no resupply mechanism other than diffusion, the flux of solute to the DGT device, which is regulated by the concentration gradient within the diffusive-gel layer, progressively decreases with time. In this case, the metal concentration at the interface between the diffusive-gel layer and sediments (C_a) has been shown to be significantly lower than the concentration in the bulk solution (C).

By exploiting a one dimensional model, Zhang et al. (1995) have shown that, for a 24-h deployment time, the ratio between C_a and C was 0.06, while a two-dimensional model developed by Harper et al. (1998) estimated the ratio to be 0.10. The difference between 1D and 2-D model results becomes negligible when resupply from the solid phase is fast and the depletion of solute occurs to a distance of less than 2 mm from the filter. However, a solute resupply in pore waters adjacent to the DGT device due to metals release from the sediment solid phase is commonly observed (higher values of C_a , as shown by Zhang and Davidson (1995)).

If the remobilization rate of solutes in pore water is sufficiently fast, and hence an adequate resupply to the DGT device is provided, C_a may be considered relatively constant throughout the deployment time (as well as DGT fluxes) and the theory adopted in aqueous systems can be applied to sediment deployments. Considering C_a rather than C , the metal concentration measured by the DGT can be calculated using Equation 10. However, C_a does not remain constant within the whole deployment time due to progressive depletion of metals near the DGT device, and steady-state conditions are not provided. The effective concentration experienced by the DGT device (C_{DGT}) is therefore a time averaged concentration response, which is better defined by the following equation:

$$C_{DGT} = \frac{1}{t} \int_{t_i=0}^1 C_a(t_i) dt \quad 13$$

The flux of solute to the binding resin increases rapidly during the first minute of deployment, until a steady-state condition is established within the diffusive-gel layer. Subsequently, the DGT device gradually depletes the adjacent pore water as the accumulation of ions in the binding resin proceeds. The DGT flux will remain constant according to the sediment capability to resupply metals. However, the initial increase of C_{DGT} has been shown to be negligible after a 1 h deployment time (Zhang et al. 1995).

1.9.5 Solid phase resupply

If independent bulk solution concentration measurements are available, the ratio R between the concentration indicated by the DGT device and the bulk solution concentration can be used to classify sediments according to the capability of solute resupply.

$$R = \frac{C_{DGT}}{C} \quad 14$$

As the concentration measured by the probe is lower or equal to that in the bulk solution, $0 < R < 1$. Ideally the independent solution concentration should be obtained through alternative analytical techniques that measure the same species detected by the DGT device, hence metals associated to the labile fraction of the sediment (e.g. anodic stripping voltammetry). However, Zhang et al. (1995) showed that in many cases the total dissolved concentration can be closely approximated by the DGT measurements.

Taking R into account, characterization of the deployment can be made as one of the following cases: (i) unsustained ($R = R_{diff}$), (ii) sustained ($R > 0.95$), and (iii) partially sustained ($R_{diff} < R < 1$) (Figure 1.4), where R_{diff} is the ratio obtained when metals reach the binding resin only through simple diffusion.

In the first case, the resupply of solute to the DGT device is limited to molecular diffusion through the pore waters, which become progressively depleted as metals continuously diffuse to the resin. The value of R_{diff} depends on the diffusion coefficient of the solute, the time of deployment and the design of the DGT device. For a typical DGT assembly and high porosity sediments, in an unsustained condition, R_{diff} has been estimated at 0.10 (Harper et al. 1998).

In the second case, the rate of solute resupply to the pore water near the DGT device is so rapid that the concentration at the boundary between the diffusive gel and sediment is comparable to that of the bulk pore water. The supply of solutes is sustained throughout the deployment time by both convection and remobilization fluxes from the solid sediment phase. If the resupply rate constant is adequately fast and a large reservoir of metal associated with the solid phase is available, a pseudo steady-state is assumed to be established and C_{DGT} can therefore be interpreted as the effective porewater concentration. In sustained conditions, for the standard case where the gel

thickness $\Delta g = 0.8$ mm, the pseudo steady-state is achieved after a few hours of deployment (Zhang et al. 1995).

The third case describes the circumstance where the solute mixing and the resupply from the solid phase rates are rapid but not sufficiently fast to cope with the solute depletion induced by the DGT device. Although a significant resupply of metals is provided, it is still insufficient to fully sustain the DGT uptake. The exact value of R can be interpreted as the ability of the solid phase to resupply solute to the pore water as a result of the depletion induced by the DGT deployment.

Harper et al. (1998) suggested that for deployment times much lower than 24 h, in many cases there will be insufficient time for the establishment of an invariant response for DGT (pseudo steady-state), whereas for deployment times significantly greater than 24 h, the depletion of metals associated with the solid phase will decrease metal concentrations at the DGT-sediment interface (C_a) with time. More details on kinetics of metal resupply are provided in the following section.

1.9.6 Kinetic of metal exchange and volumetric release

DGT measurements can be used to investigate the kinetics of metal resupply from the sediment solid phase to the dissolved phase. Harper et al. (1998) combined a two-dimensional solid-solution interaction model with simple diffusional transport within a spatial domain to estimate the rate constants for sorption (k_1) and desorption (k_{-1}) between the particulate phase and pore waters. The DGT-induced fluxes in sediments model (DIFS) assumes first-order exchange between the solid phase and solution. The constants k_1 and k_{-1} are related by the equilibrium distribution coefficient K_d which represents the available reservoir of metal sorbed to the solid phase. This relationship is shown by Equation 17, where C_s and C_d are the concentrations of metal sorbed to the particle phase and in the pore water respectively, while P_c is the particle concentration.

$$K_d = \frac{C_s}{C_d} = \frac{1}{P_c} \frac{k_1}{k_{-1}} \quad 15$$

When the DGT device is deployed in sediments, porewater concentrations adjacent to the device become gradually depleted due to the accumulation of metals onto the resin. In response to this perturbation, metals desorb from the solid phase to restore the porewater concentration to its equilibrium value. The time required by the

solid phase to resupply porewater concentrations and hence approach the equilibrium is defined by Equation 16.

$$T_c = \frac{1}{k_1 + k_{-1}} \quad 16$$

The ability of the solid phase to resupply solute to pore water therefore depends on the kinetics of the sorption and desorption processes and the distribution of solute between the solid and dissolved phases (K_d). The two-dimensional model predicts a relationship between R , the available reservoir of metal K_d and the characteristic response time T_c . If R and K_d are known, T_c can be calculated from Equation 17, which is obtained by plotting R against T_c for different values of K_d (where c and d are parameters of the obtained sigmoidal curve).

$$T_c = c \left(\frac{1-R}{R-d} \right)^2 \quad 17$$

DGT measurements of cadmium concentrations in lake sediments (Zhang et al. 1995) showed R values greater than 0.95, implying $K_d > 1.1 \times 10^5 \text{ cm}^3 \text{ g}^{-1}$ and $T_c < 0.8 \text{ s}$. These results suggest that most of the cadmium is sorbed to the sediment solid phase and that the resupply of solute to the pore water is readily sustained by the labile fraction of the sediment. For copper and iron measurements, R values were approximately 0.34 and 0.39, respectively. Such R values are typical of sediments characterized by a partially sustained resupply from the solid phase. In this case, the limited supply of metals to the dissolved phase can be attributed to a small reservoir of available metals (low K_d) or slow rate constant of desorption.

In sediments where P_c and K_d are large, the rate constant of sorption (k_1) will be much greater than that for desorption (k_{-1}) and therefore T_c can be approximated to $1/k_1$. Ernstberger et al. (2005) used the DIFS model to calculate the desorption kinetic constants and the size of the reservoir of available metal of Zn, Cd and Ni in soils, while Zhang et al. (2001) applied the 2-D model to predict the bioavailability of copper to plants in contaminated soils (Lehto et al. 2006; Zhang and Davison 2006). Applications of the model in metal speciation assessments has been shown by Almas et al. (2006) for zinc and cadmium and by Ernstberger et al. (2002) for chromium.

An estimation of the metal release from the solid phase can also be obtained in volumetric terms, as shown by a 1-dimensional model developed by Harper et al. (1998). Provided that some assumptions are taken into account, the flux measured by the DGT device can be related to the mass of solute desorbed from the particulate phase. This model enables the DGT flux to be expressed as a desorptive flux per unit volume of sediment and makes them easier to be compared with concentrations obtained by other analytical techniques.

More powerful and accurate versions of the model designed by Harper et al. (1998) have since been developed. In 2007, Sochaczewski et al. proposed the 2-D DGT-induced fluxes in sediments and soils model (2-D DIFS), an upgraded and modified version of the DIFS model which significantly improved the 2-dimensional description of solute mobilisation processes occurring in sediments and soils. The latest work on DGT modelling was published by the same authors in 2009, where previous results were combined in a three-dimensional model which outlined accurate interpretations of small-scale features of DGT measurements in sediments.

1.9.7 Estimation of porewater concentration by multiple DGT deployments with varying diffusional thickness

DGT measurements obtained by several deployments using devices with different diffusive-gel layer thickness have been used to estimate sediment porewater concentration (Harper et al. 1998). In the sustained case, the metal concentration at the interface between the diffusive-gel layer and sediment, C_a , can be considered equal to that of the pore water, C , since a fast solute resupply from the solid phase occurs. When the pseudo steady-state is reached, the fluxes of solute to the resin gel, J , can be considered to be at their maximum J_{\max} . According to Equation 7 (Section 1.9.1), J is proportional to the reciprocal of the diffusive-gel thickness Δg , providing that C_a remain constant. Therefore, considering multiple DGT deployments, a plot of the measured fluxes against $1/\Delta g$ would be a straight line passing through the origin (given that for $\Delta g \rightarrow \infty$ $1/\Delta g$ and $J \rightarrow 0$) with slope CD .

In the partially sustained case, C_a is lower than C due to the insufficient resupply from the solid phase, and consequently the DGT flux, J , is not at its maximum J_{\max} . The depletion of solute in the pore water adjacent to the DGT device induces a desorptive flux, J_s , from the solid phase which can be considered equal to J when the steady-state is achieved by the system. If Δg is increased, the maximum flux of DGT uptake will

decrease (Equation 7) and thus, for a given thickness of the diffusive gel layer Δg^* , $J = J_s = J_{\max}$. By maintaining $\Delta g > \Delta g^*$ the plot of the measured DGT fluxes obtained with different diffusive layer thicknesses against $1/\Delta g$ will be a straight line passing through the origin with slope CD . The latter can therefore be exploited to calculate the porewater concentration. Alternatively, dissolved concentrations can be estimated by plotting the concentration at the diffusive gel/sediment interface against $1/\Delta g$, where the porewater concentration is given by the point in which the line intercepts the Y-axis. In practice, C_a is usually less than C due to a partial depletion of solute near the DGT device, which occurs although the sustained case is approached. As a result, the concentration provided by DGT measurements is lower than that of the pore water. The linear plot of J against $1/\Delta g$ means that C_a is constant for all the deployments, which is a necessary but not sufficient condition for C_a to be interpreted as C .

1.9.8 Heterogeneous systems

Metal concentrations in sediment pore waters have been shown to be regulated by biogeochemical processes such as mineralization of organic matter, reductive dissolution of iron and manganese oxyhydroxides and insoluble sulfide compounds formation (Section 1.3.1). These diagenetic processes generate a vertical structure of solute concentrations within the sediment pore waters which vary as the distance from the sediment-water interface increases. Small-scale features within sediments have drawn particular attention as the processes occurring at a micro-environmental scale have been hypothesized to contribute significantly to the global sedimentary diagenesis and, as an ultimate consequence, to affect the contaminant behaviour and fate in sediments (Stockdale et al. 2009). Many studies have been focused on the investigation of micro-scale features by assessing solute concentrations in sediments at high resolution. For example, microelectrodes (Revsbech et al. 1980; Stief and Eller 2006) and optodes (Glud et al. 2001; Hulth et al. 2002) have widely been used to measure O_2 , pH and CO_2 concentrations in sediment at a sub-mm scale, revealing frequent solute distribution gradients associated with diagenetic processes occurring in highly localized areas of the sediment.

DGT deployments have been successfully used to investigate vertical profiles and provide a better understanding of the biogeochemical key processes occurring in sediments. By sectioning the ion-exchange resin into slices prior to analysis, it is possible to measure concentrations and metal fluxes at very high resolution. Compared

to conventional techniques such as dialysis, which report concentrations on a centimetre scale, DGT measurements provide vertical profiles at millimetre resolution, typically between 1 and 5 mm. Along with the latter, the volumetric resolution is also considerably high, which can be translated in minimal horizontal averaging. Using a 2.5 x 10 mm resin segment, and taking into account that porewater depletion seldom extends beyond 1 mm from the probe (Harper et al. 1998), the concentration response provided by the DGT device will be representative of a 25 μ L sediment volume, which is 3 orders of magnitudes less than conventional core slicing and squeezing methods (Zhang et al. 2002). Furthermore, ultra-high resolution measurements have been achieved by using a micro-DGT assembly (Davison et al. 1997), which is equipped with a 200- μ m-thick ion-exchange resin layer with 0.2 μ m bead size and features an overall thickness of 1 mm. After deployment, the resin gel is dried onto a cellulose nitrate filter and the masses of metal per unit area of filter are determined using proton induced x-ray emission (PIXE), or, as shown in more recent study, by combining a laser ablation system with an inductively coupled plasma-mass spectrometer (LA-ICP-MS) (Motelica-Heino et al. 2003; Fones et al. 2001; Fones et al. 2004). This particular micro assembly features a vertical resolution of 100 μ m, which allows the investigation of geochemical processes occurring on a sub-millimetre scale.

Applications of the DGT technique in marine sediments have shown pronounced horizontal gradients in remobilization fluxes of iron and manganese and trace metals such as Co, Ni, Cu, Cd, As and Zn (Davison et al. 1997; Zhang et al. 2002; Tankere-Muller et al. 2007) and sulfide (Motelica-Heino et al. 2003). A modelling of the vertical profile of metals in sediment pore waters relies on the assumption that the distribution of solute and solid phase within the sediment is horizontally uniform. However, lateral steep concentration gradients may be formed as a consequence of metal release from decomposition of organic material due to the presence of highly localised sources commonly known as microniches (Aller 1978; Fenchel 1996). The latter are frequently observed in highly populated sediments where the effect of bioturbation due to intense faunal activities is dominant (Aller 1982).

Localised maxima have been attributed by Fones et al. (2004) to metal remobilisation processes driven by the mineralization of organic matter occurring in microniches generated by the transport of material by burrowing organisms. Trace metal releases were consistent with both organic material decomposition and, when associated with iron and manganese release, with oxide reduction processes.

Motelica-Heino et al. (2003) observed simultaneous localized release of metals (Ni, Co, Fe and Mn) and sulfide exploiting a particular DGT assembly which included a layer of AgI overlying the exchange-resin layer. The authors attributed the elevated concentration of metals associated with high production of sulfide to the oxidation of the organic matter occurring in microniches, where metal oxides and sulfate are used as electron acceptors by bacteria.

Horizontal DGT profiles are obtained by slicing the ion-exchange resin gel into squares or rectangles and plotting, on a two-dimensional pattern, the measured concentrations of adjacent cuttings. Conventional slicing methods using a Teflon razor blade on a typical DGT assembly provide DGT measurements capable of distinguishing horizontal concentration gradients on a millimetre scale. Measurements at ultra-high spatial resolution can be performed by deploying a micro-assembly DGT probe. Fones et al. (2004) has shown that horizontal separations up to 300 μm can be obtained analysing the 200- μm -thick resin gel of the micro-assembly with a LA-ICP-MS.

Sochaczewski et al. (2009) outlined a way to interpret the two-dimensional image obtained by DGT measurements in sediments. A mathematical model was formulated in three dimensions to study the interactions between the DGT device and highly localised sources of solutes in sediments. The model was conceived as a DGT deployment into a homogenised sediment where microniches were introduced at a given distance from the device surface. Elevated localised concentrations were shown to be reproduced well by the DGT device.

In the case where $K_d > 10^3$ and a fast solute supply was provided by the solid phase, the DGT peak height was no less than 85% of the peak height obtained if the DGT probe was not present into the sediment. Even for very low K_d values and a slow response time (low T_c), the DGT measurement was no less than 62% of the true concentration. Good accordance between peak width at half height and microniche diameter values was achieved, showing that the size of the local source could be deduced by the DGT measurement (when the microniche was at a distance of 0.2 mm from the device $W_{1/2} = 1.10$ and $\varnothing = 1$ mm). According to the model, DGT measured concentrations declined almost exponentially with increasing distance between microniche and DGT surface, suggesting that sources located more than few millimetre from the device are unlikely to be detected.

The DGT technique was shown to usefully reproduce the distribution of solutes in sediments both vertically and horizontally, yielding accurate concentration

measurements at very high resolution. The affinity of the chelating-resin to a wide range of metals allows the DGT device to simultaneously measure the concentration of different kinds of metals associated with the labile fraction in a well-defined portion of sediment. This feature enables accurate comparisons between metal concentrations and provides significant insights with regard to biogeochemical processes occurring in sediments.

1.9.9 Advantages of DGT as a monitoring tool

The DGT device is a simple and handy tool that has a number of advantages as a monitoring technique. DGT is categorised as an *in situ* technique, therefore measurements are exempted from episodes of contamination due to sample extraction from the environment, storage or transportation (Buffle and Horvai 2000; Vrana et al. 2005). By constantly accumulating metals on the chelating resin, the DGT technique provides time-integrated measurements of cationic metal fluxes that are directly linked to processes occurring in the sediment (Zhang et al 1995). The contribution of each process is averaged in a single measurement which better expresses sediment dynamics than snapshots or discrete measurements, as the former would eventually either overestimate or underestimate episodes of contamination by relying on single point in time measurements, and the latter do not incorporate the variation of contaminant concentrations that may occur over time (Dunn et al. 2007).

Another advantage of accumulating analytes within the chelating resin is that the matrix effect (e.g. Cl^- in sea water) is minimized by the elution process which dilutes interferences to levels that do not interfere with the analysis. The DGT technique is also capable of selectively binding dissolved and labile species in pore and overlying waters, as the binding gel is too weak to accumulate contaminants associated with the inert, strongly bound fraction of the sediment (Davison and Zhang 1994). This characteristic has essential implications for predicting trace metal bioavailability in sediments as concentrations of labile species are regarded as having direct linkages with episodes of trace metal toxicity to sediment-dwelling organisms (Batley and Maher 2001). Additional selectivity can also be achieved by varying the composition of the diffusive gel, e.g. limiting diffusion to small inorganic species only by modifying the gel pore size (Zhang and Davison 2000; Batley et al. 2004).

1.10 Objectives and aim

The aim of this thesis is to evaluate the performance of the DGT technique as a rapid monitoring tool for bioavailable metals in sediments. Based on the literature reviewed here, it is believed that the DGT technique has the potential to provide a significant contribution towards developing more robust and reliable SQGs. The specific objectives of this thesis are:

- To evaluate the performance of the DGT technique in a wide range of different types of sediments. The focus will be on variation in grain size distribution (silty, silty-sand, sandy), TOC and AVS concentrations. Sediment metal concentrations will be quantified as dilute acid-extractable metals (AEM) (labile/weakly-bound) and total recoverable metals TRM (inert/strongly-bound).
- To assess the ability of the DGT technique to predict metal bioavailability in whole sediment bioassays under controlled laboratory conditions. Chronic and acute toxicity tests will be performed using amphipods (*Melita plumulosa* and *Victoriopisa australiensis*) and bivalves (*Tellina deltoidalis* and *Hyridella australis*) and comparisons between predictions of adverse effects based on DGT fluxes and current methods will be provided (TRM, AEM, SEM-AVS, dissolved metal concentrations).
- To evaluate the performance of the DGT technique in predicting metal bioavailability in a field-laboratory bioassay comparison (marine and fresh water sediments). Relationships between DGT-metal fluxes and bioaccumulation in benthic organisms will be investigated *in situ*, and identical bioassays will be simultaneously performed under laboratory conditions. Differences in bioaccumulation rates and DGT-metal fluxes between laboratory and field assays will be evaluated in relation to the use of DGT as a suitable monitoring tool for bioavailable metals in sediments.
- Using the DGT technique to investigate and quantifying the impact of bioturbation on metal bioavailability. Changes in metal bioaccumulation in benthic organisms exposed to contaminated sediments will be investigated in response to varying degrees of bioturbation (zero, low and high) and linked to changes in metal bioavailability predicted by DGT fluxes, AEM and dissolved metal concentrations.

- To provide protocols for the inclusion of the sediment-based DGT technique in the SQG's tiered assessment framework.

Chapter 2: Methods

2.1 General methods and materials

All glass- and plastic-ware used for analyses were new and cleaned by soaking in 10% (v/v) HNO₃ (BDH, AnalaR) for ≥ 24 h followed by rinsing with Milli-Q water (18.2 M Ω ·cm). 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 finally rinsing with Milli-Q water. All chemicals were analytical reagent grade or equivalent analytical purity.

2.2 Test sediments

Sediments were collected using a shovel by removing the first 10-15 cm of surficial sediment during low tide cycles or using a Van Veen grab sampler in deep waters. Control and contaminated sediments were sieved (2-4 mm mesh), homogenized and stored at 4°C in the dark. Test seawater was collected from Cronulla, Sydney, filtered (0.45 μ m, Minisart, Sartorius) and stored in a temperature-controlled room at $21 \pm 1^\circ\text{C}$. Salinity was adjusted to 30 PSU by adding Milli-Q water.

2.3 Test organisms

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 (Spadaro et al. 2008; Mann et al. 2009; Simpson and Spadaro 2011; Strom et al. 2011; Campana et al. 2012). 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. (2008).

Tellina deltoidalis is an estuarine/marine bivalve commonly found in coastal lagoons from southern Queensland to Tasmania and south Western Australia. Although it is believed to be a deposit feeder, it has been demonstrated that *T. deltoidalis* accumulates metals from the solid phase (through ingestion of sediment particles) as well as from the dissolved phase (King et al. 2005; Strom et al. 2011; Campana et al. 2013; Belzunce-Segarra et al. 2015). *T. deltoidalis* (shell lengths of 5-12 mm) were collected from the Lane Cove River (NSW, Australia) and maintained in plastic trays containing sieved (4 mm mesh) sediments collected from the same location filled with filtered (0.45 μ m) sea water and placed in a temperature controlled room ($21 \pm 1^\circ\text{C}$)

until use (Campana et al. 2013; Belzunce-Segarra et al. 2015). During this period, bivalves were fed once a week using Sera Micron fish food (~5 mg/bivalve).

Hyridella australis, a freshwater bivalve commonly found in south-east Australia. *H. australis* (55 ± 5 mm shell length) were collected from the Nepean River, near Menangle, south-west of Sydney, NSW, Australia. After collection, bivalves were placed into a plastic cooler containing sediment and water collected from the same site. Overlaying water was aerated during transportation to the laboratory. Bivalves were maintained in uncontaminated sieved sediments with overlying water in glass aquaria in a temperature-controlled room for acclimation before experimentation. During this period, bivalves were fed once every three days with a commercially available freshwater mussel food (unicellular green algae – *Nannochloropsis* – Nanno 3600, Instant algae, USA.) at 1% (v/w) of total body mass. Half water changes were performed every three days.

The tube-building amphipod *Victoriopisa australiensis* (Melitidae; Chilton, 1923) inhabits estuarine, littoral, mud flats and seagrasses sediments of south-east Australia, from southern Queensland to southern New South Wales (Lowry 2005; Dunn et al. 2009). *V. australiensis* (2-3 cm body length) was collected from Lake Illawarra, a large coastal lagoon located some 50 km south of Sydney, New South Wales. After collection, amphipods were maintained following the procedure previously described for *T. deltoidalis*, except in freshwater sediments.

2.4 Sediment and biota analyses

The fine sediment fraction was determined by wet sieving sediments through a Nylon sieve (<63 μ m mesh) followed by gravimetry. Total recoverable metals (TRM) were analysed following low-pressure aqua-regia (3:1 HNO₃:HCl) microwave digestion of sediments (EM MARS 5). In a pre-weighted centrifuge tube, 6 mL of concentrated HNO₃ and 3 mL of concentrated HCl were added to 0.3 g of finely grained dry sediment (the exact weight was recorded). Samples were microwave heated (800 W) for 50 min at 80°C (ramp of 15 min) and diluted with Milli-Q water to a final volume of 27 mL. Centrifuge tubes were left overnight for the sediment to settle and the exact final weight was recorded. Before analysis, samples were diluted 10-fold using Milli-Q water.

Acid-extractable metals (AEM) determined by a 60 minute 1 M HCl digestion (equivalent to simultaneously extractable metals (SEM)), were determined on wet sediment. Sub-samples were placed in a drying oven 110°C overnight and the dry-

weight to wet-weight ratio was determined by gravimetry. In a 30 mL polycarbonate vial, 25.3 g of 1M HCl was added to 0.5 g of wet sediment (exact weight recorded). Samples were shaken and left to stand for 30 min. This operation was repeated twice. Extracts were filtered (0.45 μ m) and diluted 10-fold before analysis.

Total organic carbon (TOC) was determined by high temperature CO₂ evolution method in a LECO furnace with infrared (IR) detection after removal of inorganic carbonates by acidification.

Acid-volatile sulfides (AVS) were determined in a nitrogen –filled glove box. A small sample of sediment (~0.3 g wet weight) was smeared onto a piece of laboratory film (Parafilm M, American National Can, Chicago, IL, USA), accurately weighed (\pm 3 mg), and transferred to a 50-ml centrifuge tube. Fifty mL of deoxygenated Milli-Q water was added, followed by 5 mL of methylene blue reagent (MBR, 2.8 g of *N,N*-dimethyl-*p*-phenylenediamine hemioxalate salt in 670 mL of H₂SO₄ and 330 mL of Milli-Q water mixed with 200 mL of 0.020 M acidic ferric chloride solution, final solution approximately 22 M (H⁺)) and the centrifuge tube was capped and inverted five times to mix. After 5 min, the sample was centrifuged (2 min, 2,500 rpm) and then allowed to sit for 90 min for the methylene blue colour development. The centrifugation and colour development stages were performed outside the nitrogen–filled glove box with the centrifuge caps tightly sealed. During this period, care was taken not to significantly disturb the sediment (i.e. no further shaking) because the MBR adsorbs to sediment particles. Sulfide standard solutions (50 mL), containing 0 to 0.10 M Na₂S, were prepared and 5 mL of MBR was added. After colour development (90 min), standards and samples were diluted 10-fold with 1 M H₂SO₄ and then analyzed at 670 nm with an ultraviolet–visible light spectrophotometer (LKB Biochrom Ultrospec IIE)).

Filtered aliquots (0.45 μ m) sampled from overlying waters (OLW) were used for dissolved metal analysis. Metals in waters and acid digests were analysed by inductively coupled plasma-atomic emission spectrometry (ICP-AES, Spectro Ciros CCD; ICP-OES, Varian 730-ES). For quality control purposes, acid-digest blanks (10% of samples), replicates (duplicates or triplicates or 20% of samples, according to the level of accuracy required), analyte sample spikes and a certified reference material (CRM) (CC108, European Reference Material) were analysed. Replicates were within 20% and recoveries for spikes and CRM were within 85-115% of expected values.

Biological tissues were digested using microwave-assisted nitric acid extraction (HNO₃ 200°C for 30 min) as previously described in King et al. (2010) and metal

concentrations in digests were analysed by ICP-AES (Varian 730-ES). For quality assurance, acid-digest blanks (10%), replicates (20%) and the CRM DORM-3 (*Mytilus galloprovincialis*, NRCC) were analysed and recovery was within 85-115% of expected values. Alternatively, biological tissues were freeze dried (24 h, Christ Freeze Drier) then digested in a HNO₃/H₂O₂ (2:1, 18 h at 25°C) before microwave heating for 1 h (CEM MARS Microwave Digester; programmed 60°C, 12 mins; 60– 65°C, 10 mins; 65– 70°C; 10 mins and then held at 70°C for 10 mins). Samples were diluted 10-fold with Milli-Q water before metals analysis. Recoveries of DORM-3 were between 75 and 125%.

For the bivalve *H. australis* only, tissues were prepared by Chamani Marasinghe Wadige from University of Canberra. This was performed using microwave-assisted digestion as described by Baldwin et al. (1994). Freeze-dried tissue samples were finely ground to a homogenous powder in an acid-cleaned mortar. Approximately 0.07 g of tissue sample or CRMs (TORT- 2, lobster hepatopancreas tissue and NIST 1566b, oyster tissue) were digested in 1 mL of HNO₃ (Suprapur[®], Merck, Germany) in a microwave oven (CEM MDS-2000, USA) for 2 min at 630 W, 2 min at 0 W and 45 min at 315 W. Metal concentrations measured in the TORT- 2 and NIST 1566b were between 85 and 115% of the certified values.

2.5 Bioassays

All laboratory bioassay for *M. plumulosa*, *T. deltoidalis* and *V. australiensis* were conducted either in an incubator (12:12-h light:dark cycle, light intensity of 3.5 $\mu\text{mol photons/s/m}^2$, Labec Refrigerated Cycling Incubator, Laboratory Equipment) or in a temperature controlled room at $21 \pm 1^\circ\text{C}$ (normally daylight conditions), whereas for *H. australis* experiments were conducted at room temperature (22-25°C). Water quality parameters were measured throughout tests to confirm that major parameters remained within desired ranges (pH 8.2 ± 2 , salinity 31 ± 1 PSU, temperature $21 \pm 1^\circ\text{C}$ and dissolved oxygen >80% saturation).

2.5.1 Amphipod bioassay

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. (2009) according to Simpson and Spadaro (2011). During the 10-day exposure, females underwent 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 (Mann et al. 2009). 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 in 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 (Simpson and Spadaro 2011).

2.5.2 Laboratory and field bioaccumulation bioassay (estuarine sediment)

The laboratory- and field-based bioassays were undertaken for 31 days. The field-deployed bioassays were performed in mesh cages that allowed adequate water circulation and prevented predation (Liber et al. 2007) (Figure A2.4 of Appendix 2). The cages were submerged to a depth of 40 cm in an uncontaminated section of the

Woronora River estuary (Sydney, Australia) (Figure A2.5 of Appendix 2). The test sediments (520 g or 5.1 cm depth) were contained in 1-L LDPE bottles (modified with 3 windows ($4.5 \times 8 \text{ cm}^2$) for field chambers) and had 500 mL overlying water in laboratory bioassays and natural circulation of water in the field. There were two replicates of each sediment sample (sediments 1-8) randomly distributed in both the laboratory and field.

Water quality parameters in the field were measured twice weekly and remained within desired ranges (pH 7.5-8.4, salinity 31-33 PSU, temperature 21-24°C and dissolved oxygen >80% saturation). Each sediment contained seven bivalves that were allowed to bury naturally after water change in the laboratory and before deployment to field cages. Organisms were also fed twice per week with 1 mg/bivalve of Sera Micron (Sera Fishtamins) in the laboratory. At test completion, bivalves were depurated in Milli-Q water for 24 hours, the soft tissue extracted from the shell and stored at -20°C until analysis.

2.5.3 Laboratory and field bioaccumulation bioassay (freshwater sediment)

Bioaccumulation in *H. australis* was assessed by exposing bivalves to control (S1) and contaminated sediments (S2, S3, S4) over a period of 28 days. For laboratory and field bioassays, each treatment was tested in triplicate. For the laboratory bioassay, ~2 kg of sediment was placed in plastic aquariums (12 L) and overlain with 10 L of filtered (0.45 µm) river water and equilibrated for 2 days at room temperature (22-25°C). Overlaying water was aerated using air flow controllers to maintain approximately 100% air-saturation. Bivalves were fed twice a week using unicellular green algae *Nannochloropsis* at 1% (v/w) of total body mass. For the field bioassay, sediments were placed in plastic mesh cages (70×28×12 cm³, 2 cm mesh) which allowed sufficient head-space for bivalve movement, adequate water circulation and prevented predation (Figure A3.1 of Appendix 3). At test commencement, five bivalves were added to each of the three treatment replicates. Bivalves were placed on the sediments and allowed to bury, then cages were locked using plastic cable ties and placed in the same sampling location. Water quality parameters were monitored at beginning, middle and end of test in each of the locations and are available in Table A3.1 of Appendix 3. After 28 days, organisms were retrieved, depurated in clean freshwater for 24 h, and dissected prior to analysis.

2.5.4 Bioaccumulation bioassay for the bioturbation study

The tests were performed in a temperature controlled room (21 ± 3 °C) under normal day-light conditions. Test containers comprised glass beakers (1100 mL capacity) containing 400 mL (or ~550-650 g) of sediment and 600 mL of filtered seawater. The sediments with overlying seawater were equilibrated for 14 days prior to test commencement. For each of the three test sediments (S1, S2, S3), three treatments were prepared corresponding to three different levels of bioturbation activity (zero, low, high), determined by the quantity and type of organisms added to each treatment: zero = no added organisms, low = 5 bivalves (*T. deltoidalis*), high = 5 bivalves + 6 amphipods (*V. australiensis*). Each treatment was prepared in triplicate (producing a total of 27 test vessels). On day 0 of the test, organisms were transferred to designated treatments and tests continued for 28 days.

To ensure that at least a minimal source of nutrition was available to biota, once a week a mixture of Sera Micron fish food and *Tetraselmis* algae (1 mg/organism) was added. The overlying water was removed and replaced with clean seawater 3 times a week (days 0, 2, 4, 7, 9, 11, 14, 16, 18, 21, 22 and 25) to avoid excessive dissolved metal concentrations in the water column. Water quality parameters were periodically monitored throughout the test (temperature = 21 ± 1 °C; NH_3 <0.5 mg/L; dissolved oxygen >85%; pH = 8.0 ± 0.1 ; salinity = 35 ± 1 ‰). On day 28, the sediment mini-cores were collected (as described above) and organisms gently sieved from the sediment, placed in beakers containing clean seawater and depurated for 24 h. The soft tissue of depurated bivalves (euthanized by placing in 4°C water for 2 h) was extracted from the shell using a Teflon-coated blade. Bivalve soft tissues and amphipods (whole body) were freeze-dried (ALPHA 1-2 LDplus, Christ) and stored at -20°C until analysis.

2.6 Diffusive gradients in thin films

Sediment probes casings (24 cm × 4 cm × 0.5 cm, with an open window of 1.8 cm × 15 cm) were purchased from DGT Research (<http://www.dgtresearch.com/>). Gel preparation and probes assembly and handling, before and after deployments, were performed following standard procedures recommended by DGT Research (Lancaster, UK). 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 (Zhang et al. 1995). Probe assembly, handling and gel preparation were

performed following standard procedures recommended by DGT Research. Before deployment, probes were conditioned in NaCl (0.12 M and 0.01 M for marine and fresh water deployments, respectively) and degassed by purging nitrogen gas overnight. A sheet of Chelex gel was added to the conditioning solution to minimize the risk of contamination. All equipment used for DGT gel synthesis (e.g. glass plates, spacers, containers, tweezers, chop board, DGT probes) was detergent-washed, rinsed in Milli-Q water and soaked in 10% HNO₃ for 24 h. The cleaning procedure was completed by an additional acid wash in a second 10% HNO₃ for 24 h. For filter membranes only, 5% HNO₃ and 8 h soaking time were used to avoid corrosion. Gel synthesis, assembling and handling was performed in a laminar flow cabinet (Aurora Vertical SD4). Bio-Rad Chelex[®]-100 resin, (200-400 mesh, sodium form) was used for binding gel synthesis. Ammonium persulfate and tetramethylethylenediamine (TEMED) were purchased from Sigma-Aldrich.

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. Upon retrieval, 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 to the desired resolution using Teflon[®]-coated razor blades. Each slice was weighed and extracted in 500 µL of a 1 M HNO₃ 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). DGT metal fluxes were calculated using Equation 8, whereas metal concentrations were obtained using Equation 10. For all metals, a 0.8 elution factor was used. Blank probes were analysed for laboratory quality assurance. The mass of metals accumulated in blank probes was converted into metal fluxes (or concentrations) and used to estimate the contribution of blanks to DGT measurements. As the slicing procedure and metal concentrations in blank probes varied between different experiments, details are provided in each individual chapter.

Chapter 3: Assessing the performance of the DGT technique to predict metal mixture toxicity to the amphipod *Melita plumulosa*

This Chapter has been published (Appendix 5):

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3.1 Introduction

Evaluating contaminant bioavailability has become a well-established component in many environment quality assessment programs. The ecotoxicological risk associated with a contaminant is dependent 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 (Wang and Fisher 1999b; Simpson and Batley 2007; Rainbow et al. 2009). Many of the procedures for assessing the bioavailability of contaminants in sediments are time-consuming and expensive (Maher et al. 1999), 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 (Long et al. 1995; Macdonald et al. 1996) and ranking these has formed the basis of empirical SQGs for the initial tier of assessments (ANZECC/ARMCANZ, 2000). 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 (Di Toro et al. 2005; Nia et al. 2011; Strom et al. 2011; Campana et al. 2012). 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 (USEPA 2005; Burgess et al. 2013). Like all EqP approaches, the models do not account for contaminant exposure that may occur through ingestion of particles by deposit-feeding organisms (Hare et al. 2001; Simpson and King 2005; Simpson et al. 2012a; Tan et al. 2013).

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 (Zhang et al. 2002; Naylor et al. 2006;

Tankere-Muller et al. 2007). 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 (Zhang et al. 1995). 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 (Roulier et al. 2008; Costello et al. 2012; Dabrin et al. 2012; Simpson et al. 2012b; Teuchies et al. 2012). Roulier et al. (2008) 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. (2012) 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. (2012) 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 (Simpson et al. 2012b). 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.

3.2 Methods

3.2.1 General methods

All glass- and plasticware used for analyses were new and cleaned following the procedures described in Section 2.1. Methods for total recoverable metal (TRM), dilute acid-extractable metal (AEM), acid-volatile sulfide (AVS), total organic carbon (TOC) and particle size analyses are described in Section 2.4.

3.2.2 Sediment sampling and preparation

The control sediment collected from the intertidal estuarine site at Bonnet Bay (BB), Sydney, 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 (Chariton et al. 2010). 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). 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 3.1).

3.2.3 Amphipod bioassay

Detailed procedures for the 10-day amphipod chronic test used to compare DGT metal fluxes with biological responses are provided in Section 2.5.1.

3.2.4 DGT deployment and binding gel slicing

Procedures for DGT gels synthesis, probes assembling and handling, before and after deployment, are provided in Section 2.6. DGT measurements were performed on day 5 of the 10-day toxicity test. One DGT probe was gently inserted into three of the four replicate vessels used for the chronic bioassay. After DGT probes were deployed, amphipods were transferred into test vessels for the second part of the toxicity test (see Section 2.5.1). After 24 h, DGT probes were carefully retrieved, rinsed with Milli-Q water, disassembled and binding gels sliced 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. Gel slices analysis and metal flux calculations were performed according to the procedures provided in Section 2.6. Blank probes were analysed for laboratory quality control and Cd, Cu, Ni and Pb concentrations in elutes contributed for an equivalent flux $< 0.5 \mu\text{g/h/m}^2$. Zinc contamination was consistently detected and typically contributed 15 and 40% of the measured zinc fluxes for contaminated and control sediments, respectively.

3.2.5 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 described (Simpson and Spadaro 2011) using TRM and AEM concentrations of Cd, Cu, Ni, Pb and Zn, and the SQG trigger values (ANZECC/ARMCANZ, 2000). 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: $\text{TU} = \sum(\text{dCd}/5.5 + \text{dCu}/1.3 + \text{dNi}/70 + \text{dPb}/4.4 + \text{dZn}/15)$, 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 (ANZECC/ARMCANZ, 2000), (ii) 50% lethality concentration (LC50) for adult amphipod survival (King et al. 2006), or (iii) sediment quality guidelines (SQG) (ANZECC/ARMCANZ, 2000). 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 3.2). Although there is no agreed equivalent to R^2 in logistic regression, we calculated pseudo- R^2 as an approximate estimate of explained variation.

3.3 Results and discussion

3.3.1 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 3.1 and Table 3.2. Metal concentrations in control sediments were generally below the SQG trigger values (interim SQG-Low (ANZECC/ARMCANZ, 2000)), 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 $\text{AEM} = \text{SEM}$) concentrations indicated a molar excess of SEM over AVS (Table 3.1), and the potential for adverse effects from these metals to the amphipod (USEPA, 2005; Simpson et al. 2012a).

Table 3.1 Physical and chemical properties and toxicity of the control and contaminated test sediments. All concentration are mean (n=2) with variability between measurements of <30%

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	µmol/g	µmol/g	%	%
Control	C1	90 ± 6	100	22	41	100	14	41	88	<0.5	1.9	1.7	50
	C2	90 ± 4	100	38	68*	180	22	70*	150	<0.5	3.2	3.1	90
	C3	90 ± 8	100	35	65*	190	23	59*	160	<0.5	3.1	3.6	100
	C4	85 ± 2	100	26	50	120	13	50	100	<0.5	2.1	2.6	70
	C5	83 ± 5	100	9	21	42	4	17	34	<0.5	0.7	0.7	20
LOW	S1	88 ± 8	41 ± 10	620*	450*	900*	260*	380*	570*	<0.5	15	4.7	100
	S2	88 ± 3	28 ± 11	220*	360*	710*	<1	190*	490*	30.1	-22	4.3	70
	S3	85 ± 2	40 ± 11	120*	170*	340*	20	120*	270*	1.5	4	3.1	30
MEDIUM	S4	77 ± 10	23 ± 17	80*	180*	1600*	3	150*	1600*	7.4	18.5	4	15
	S5	71 ± 5	64 ± 14	230*	310*	620*	3	180*	470*	4.1	4	6.6	30
	S6	63 ± 4	6 ± 2	290*	220*	400*	150*	200*	270*	<0.5	9	1.4	25
HIGH	S7	52 ± 4	22 ± 18	55	110*	1200*	16	120*	1300*	1.9	19.5	1.4	25
	S8	52 ± 11	3 ± 2	1070*	760*	1500*	540*	700*	980*	<0.5	36	5.7	100
	S9	50 ± 6	9 ± 3	510*	360*	680*	240*	340*	480*	<0.5	19	2.9	50
VERY HIGH	S10	25 ± 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. Survival and reproduction are mean ± standard error (*n* = 4). * Concentrations with an asterisk exceed the SQGs (ANZECC/ARMCANZ, 2000).

Table 3.2 Complementary metal concentrations in control and contaminated test 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 (2000), and * concentrations with an asterisk exceed the SQG.

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 ~ 0.8, 1 and 0.7 and AE-Pb/TR-Pb ~ 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 ~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 3.1), 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 3.1). This was attributed to oxidation of the sediments, as was evident by the decrease of AVS concentrations from original to diluted sediments (Table 3.1). 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 Cu₂S, rather than CuS (Simpson et al. 2000)) are poorly soluble in 1 M HCl (Simpson et al. 2012a). 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).

3.3.2 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 3.1). 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 3.2). The increased fluxes of copper, lead and zinc to the DGT probe at this depth observed in most of the contaminated sediments (Figure 3.1) was consistent with previous studies indicating that metal mobility in pore waters is linked to dissolution of iron and manganese oxyhydroxide phases (Fones et al. 2004; Poulton et al. 2004; Naylor et al. 2006). Other processes that may contribute to the formation of DGT maxima in pore waters near the SWI are the degradation of organic matter (Furrer and Wehrli 1993; Petersen et al. 1995; Tankere-Muller et al. 2007) and oxidation of metal sulfides (Lesven et al. 2008; Naylor et al. 2012; Simpson et al. 2012b; Teuchies et al. 2012). 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 (Costello et al. 2012; De Jonge et al. 2012; Simpson et al. 2012a; Simpson et al. 2012b) and was consistent with increasing overlying water copper concentrations measured over the test (Table 3.3). 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 Davison (2000) observed that in a humic-rich freshwater stream more than 50% of the copper was associated with organic substances.

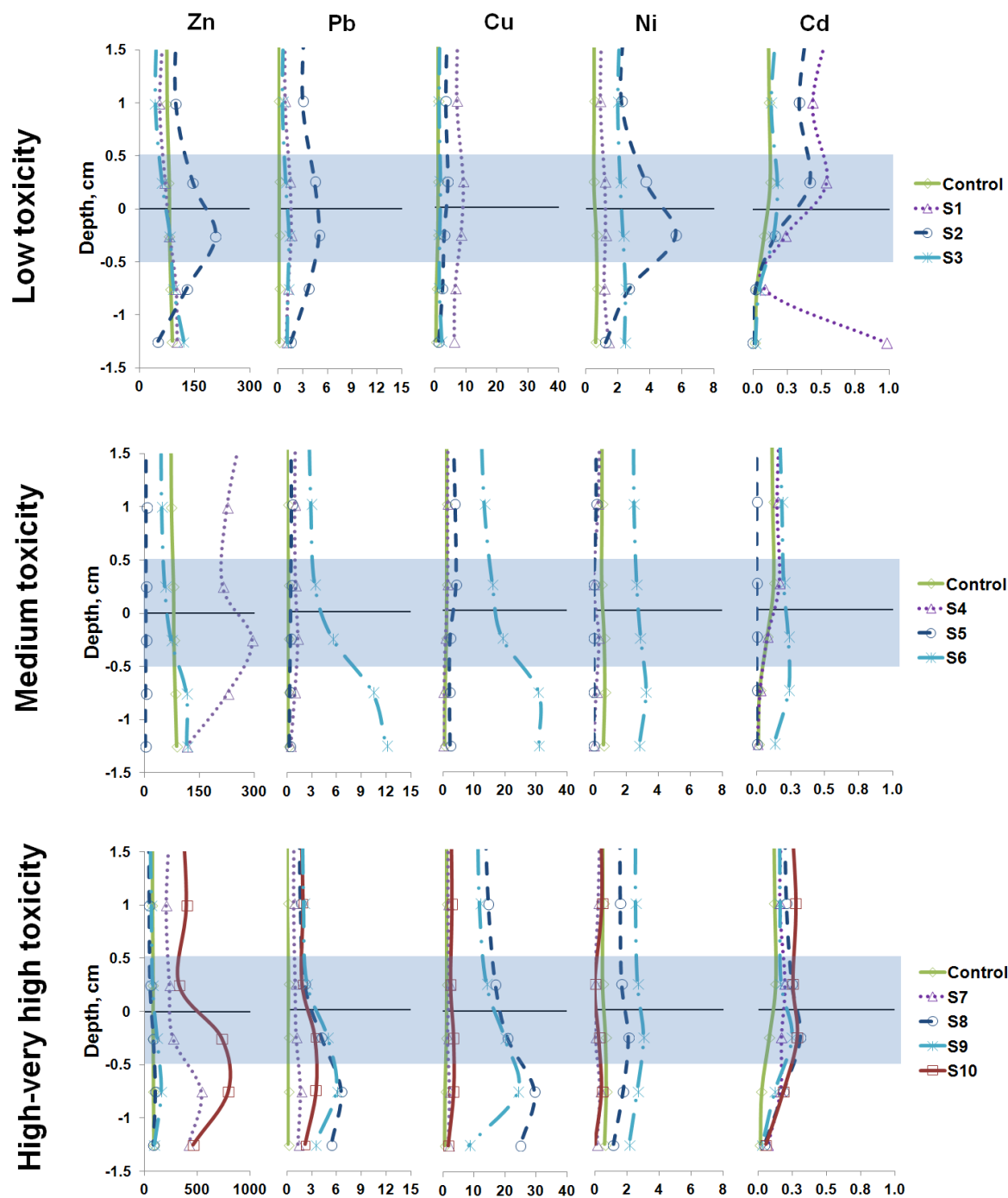


Figure 3.1 DGT profiles of Zn, Pb, Cu, Ni and Cd measured within 1.5 cm of the sediment water interface in test vessels during the amphipod bioassay. Points represent average values of three replicates (standard deviations ranged between 30 and 40% of mean values). Shaded areas indicate the DGT profile from which flux measurements were used when interpreting the toxic exposure.

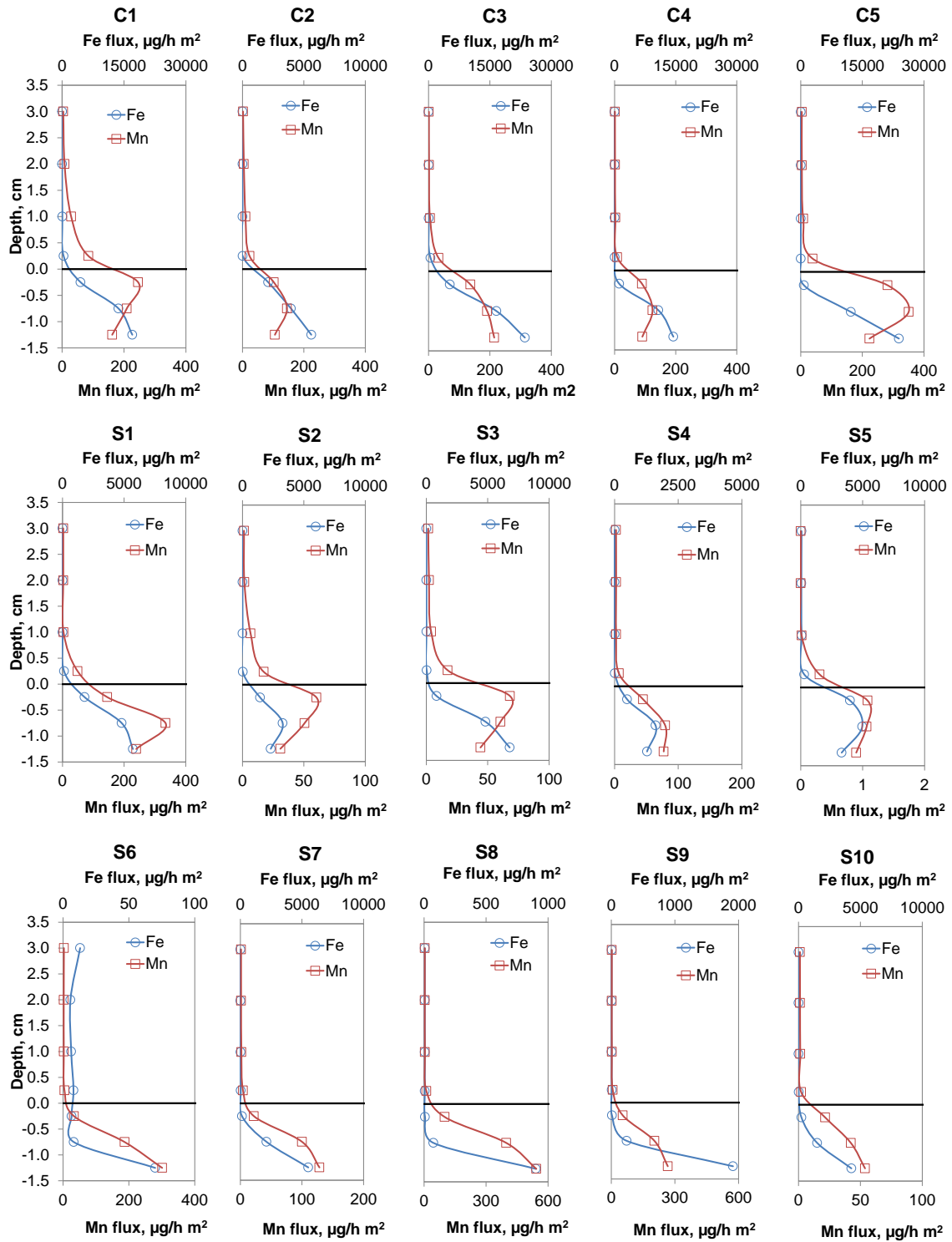


Figure 3.2 DGT-Fe and -Mn vertical profiles in pore waters and overlying waters (average values, $n = 3$, standard deviations ranged between 50 and 60% of mean values).

By separating seawater samples into different fractions, Wells et al. (1998) 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 A1.1 of Appendix 1). Cadmium and nickel fluxes were constantly below $1 \mu\text{g/h/m}^2$, except for nickel in the diluted Kings Bay sediment S2.

Table 3.3 Dissolved Cu, Zn and Pb concentrations in Kings Bay sediment overlying waters

Day	Sediment	Cu, $\mu\text{g/L}$	Zn, $\mu\text{g/L}$	Pb, $\mu\text{g/L}$
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
WQGs		1.3	15	4.4

Values are average concentrations measured throughout the test with standard deviation ($n = 4$). The WQGs are trigger values from ANZECC/ARMCANZ (95% species protection concentrations).

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 3.1), 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 3.1), but a considerable difference in AEM concentrations was observed (Table 3.1). 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 3.1). 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 (Yu et al. 2001). Simpson et al. (2011) 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 A1.1 of Appendix 1), zinc fluxes in Kings Bay sediment S5 were much lower than the other sediments.

3.3.3 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 3.1, Figure 3.3). 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, overlying water concentrations indicated that dissolved zinc (up to 75 ± 14 $\mu\text{g/L}$, Table 3.3) was likely affecting reproduction of *M. plumulosa*, as observed in previous studies (Mann et al. 2011). However, the dissolved zinc concentrations were generally below those causing lethality (10-day LC_{50} , LOEC and NOEC values are 220, 180 and 90 $\mu\text{g Zn/L}$ for juveniles) (Spadaro et al. 2008). 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 (Spadaro et al. 2008; Strom et al. 2011; Campana et al. 2012).

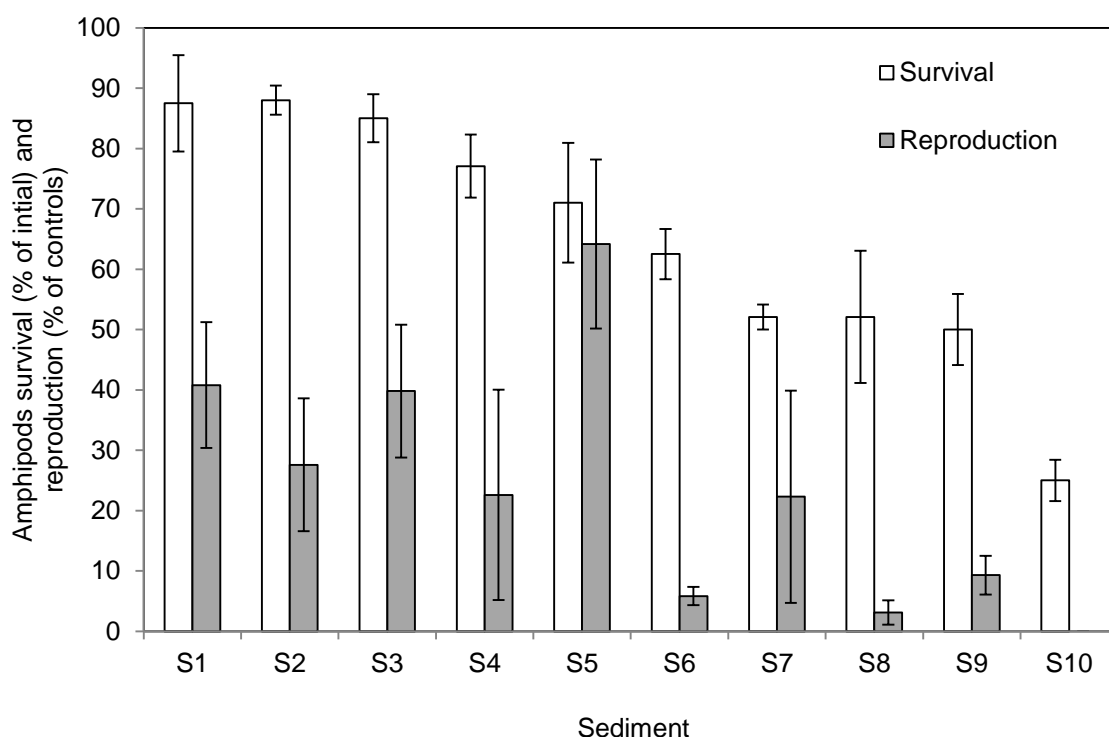


Figure 3.3 Amphipod survival (as a percentage of organisms placed in each test vessel at the beginning of the test) and reproduction (percentage of controls) in contaminated sediments: Kings Bay (S2, S3, S5); Five Dock Bay (S4, S7, S10); Port Kembla (S1, S6, S8, S9). Error bars of average values ($n = 4$) are expressed as standard error.

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 3.4. 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.

3.3.4 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 (Wu et al. 2011). 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 (Strom et al. 2011; Campana et al. 2012), 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. (1998), thus allowing the establishment of a pseudo steady-state and avoiding potential exhaustion of solid phase concentrations (Ciffroy et al. 2011; Nia et al. 2011), as well as metal competition effects on the binding gel (Stockdale et al. 2010). 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’ (Harper et al. 1998).

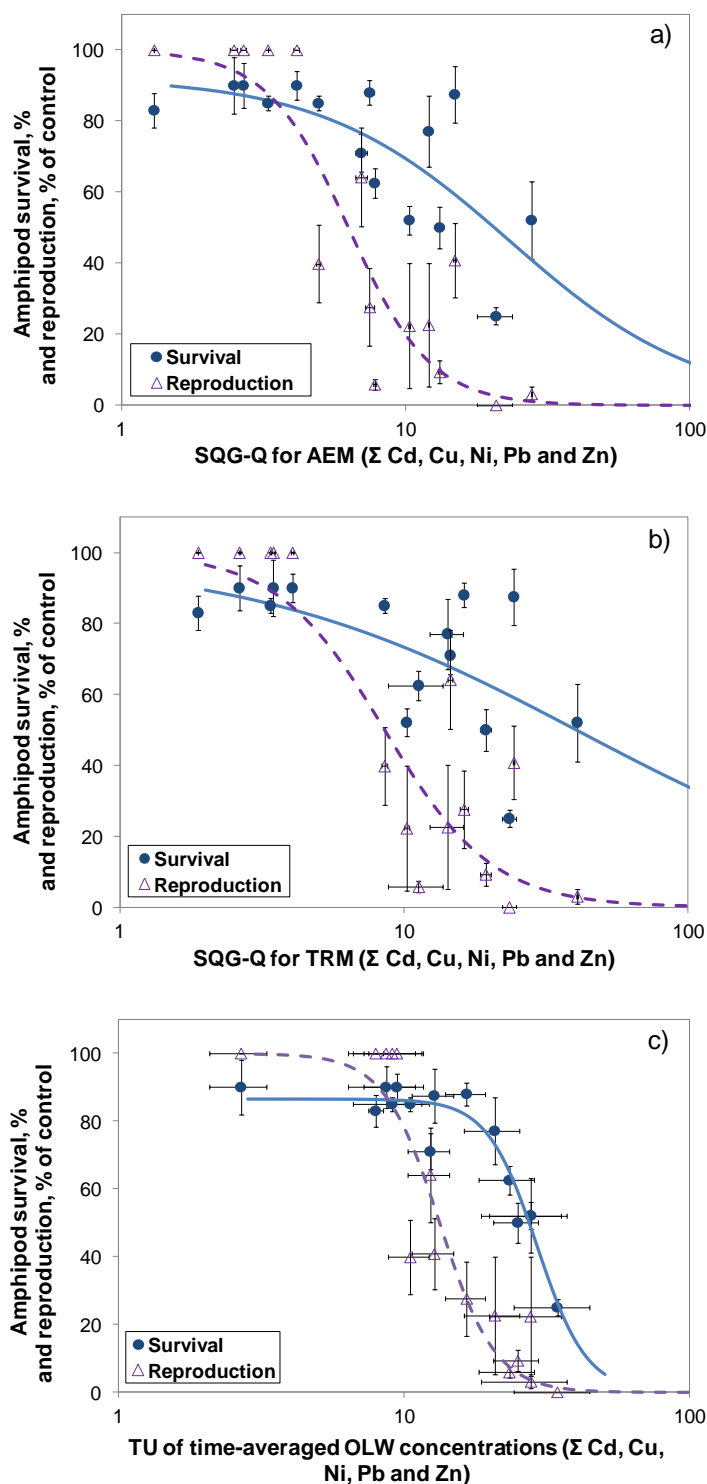


Figure 3.4 Dose-response relationships between amphipod survival and reproduction and different methods: a) AEM concentrations (Σ Cd, Cu, Ni, Pb and Zn) normalized to sediment quality guidelines (SQGs); b) TRM concentrations (Σ Cd, Cu, Ni, Pb and Zn) normalized to SQGs; c) OLW concentrations (Σ Cd, Cu, 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 ($n = 4$).

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 (Zhang et al. 1995). Recent studies (Ciffroy et al. 2011; Nia et al. 2011) 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 (Harper et al. 1998). 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 biologically available after passing through animal guts and yet are potentially not being detected by DGT.

The relationships between the amphipod survival and reproduction and DGT-metal fluxes are shown in Figure 3.5. 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 Section 3.2.5). 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 (ANZECC/ARMCANZ, 2000). The dose-response relationship obtained by normalising DGT fluxes to WQGs provided a better fit than those normalised to the SQGs (Figure 3.5), although all relationships appeared quite similar. For survival, pseudo-R² 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 (Batley et al. 2005). 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* (Strom et al. 2011; Campana et al. 2012). 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 3.5) 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.

3.3.5 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 3.5a). 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) $\mu g_{WQG}/h/m^2$ 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) $\mu g_{WQG}/h/m^2$, respectively. Thus, adverse effects on survival and reproduction were predicted for DGT_{WQG} fluxes exceeding 36 and 17 $\mu g_{WQG}/h/m^2$, respectively.

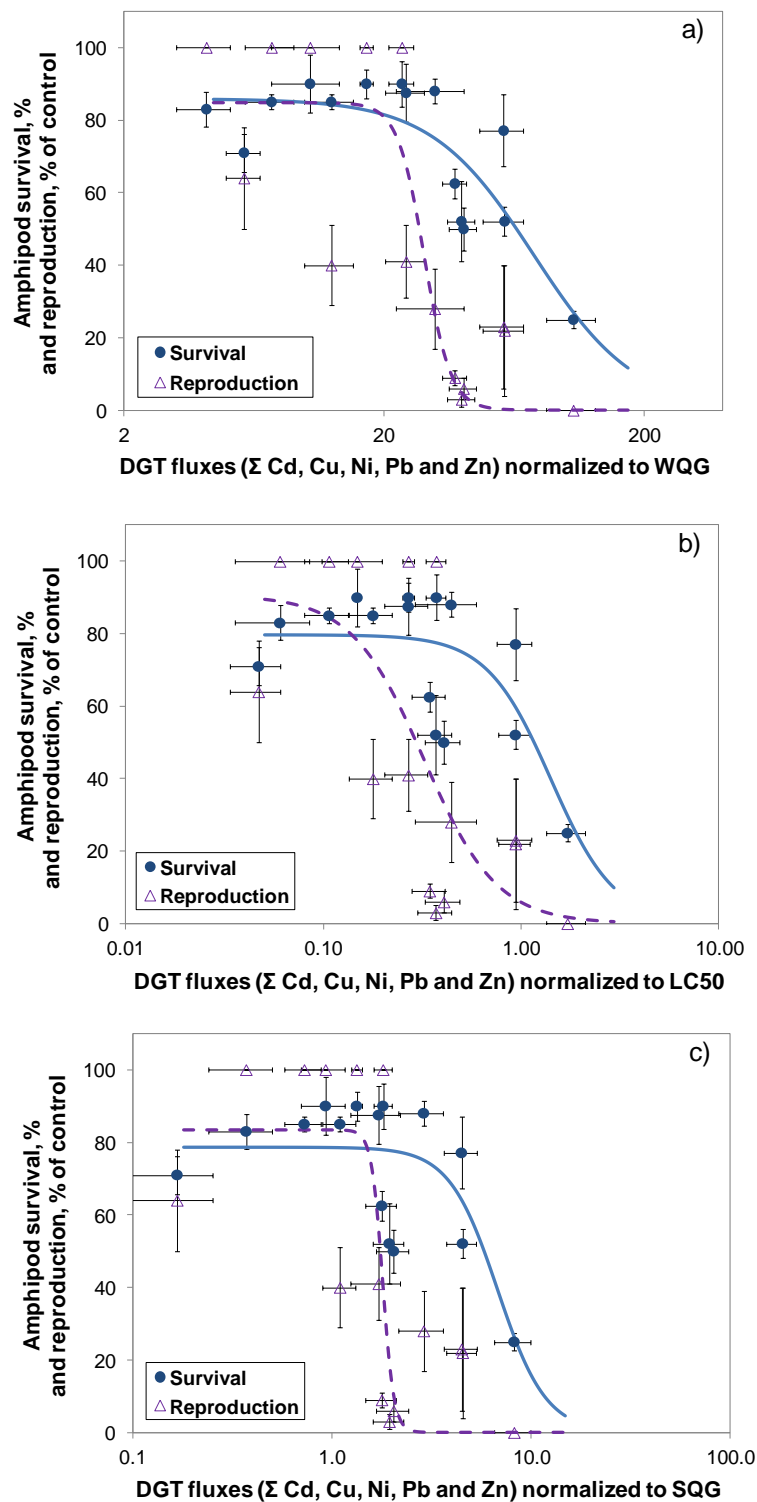


Figure 3.5 Relationships between amphipod survival and reproduction and DGT fluxes normalized to a) WQGs, b) LC₅₀ values and c) SQGs (as described in methods). Mean values of DGT fluxes (Σ Cd, Cu, Ni, Pb and Zn) were calculated for $n = 3$ and reported with standard deviation. Survival and reproduction are mean \pm standard error ($n = 4$).

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 A1.2 of Appendix 1). 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 3.5) were consistent with those created using more traditional measures of metal exposure (Figure 3.4). 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 ‘snap-shot’ in time, or of sediments in

which the metal bioavailability can be highly variable and difficult to characterise using other techniques (Simpson and Batley 2007). 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 (Roulier et al. 2008; Dabrin et al. 2012; Simpson et al. 2012b). Although the technique appears to have limitations to predict toxicity caused by particle ingestion and dietary behaviours (Roulier et al. 2008; Hook et al. 2014), 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.

Chapter 4: Metal fluxes from pore waters and labile sediment phases within surface sediments are useful for predicting metal exposure and bioaccumulation by benthic invertebrates.

The research presented in this Chapter is the result of a collaborative work in which I contributed to the development of the initial concepts and designed the sediment-DGT interactions. I led the DGT preparation, deployments, analyses and interpretation and contributed to field work activities. Dr. Maria Jesus Belzunce Segarra, a research scientist from AZTI-Tecnalia, Marine Research Division, Spain, contributed to the development of the concepts and design of this study and participated to field work activities, biological tissue preparation and analysis, and data analysis. This project resulted in the production of two companion manuscripts, one based on the results presented in this chapter, which focuses on the evaluation of the DGT performance as a monitoring tool for bioavailable metals, and a second manuscript based on the assessment of the mismatch between laboratory and field-based bioassays, where I contributed as a co-author.

This Chapter has been submitted for publication:

Amato, E.D.; Belzunce-Segarra, M.J.; Simpson, S.L.; Jolley, D.F. (submitted 22 Dec 2014). Assessing the performance of the diffusive gradients in thin films technique for predicting trace metal bioavailability in estuarine sediments. *Environmental Science and Technology*. Manuscript ID es-2014-06236h

The accompanying manuscript is under review for publication (Appendix 5):

Belzunce-Segarra, M.J., Simpson, S.L., Amato, E.D., Spadaro, D.A., Hamilton, I., Jarolimek, C., Jolley, D.F. (2015). The mismatch between bioaccumulation in field and laboratory environments: interpreting the differences for metals in benthic bivalves. *Environmental Pollution* (accepted 31 March 2015 ENVPOL-D-14-02079R1)

4.1 Introduction

Increasingly, sediment quality assessment frameworks consider contaminant bioavailability when considering the risk of adverse effects of specific contaminants to benthic organisms (ANZECC/ARMCANZ, 2000; Burgess et al. 2013). For metals, the consideration of metal binding by acid-volatile sulfide (AVS), organic carbon (OC), and iron and manganese oxyhydroxide phases, or non-phase-specific factors such as the fraction of metals present as dilute acid-extractable forms or associated with fine particles, often improves the ability to predict metal bioavailability and risk of toxicity (Besser et al. 2003; Simpson and Batley 2007; Simpson et al. 2013b). However, the choice of which parameters to measure and use in models for predicting metal bioavailability is very challenging as the parameters that are most effective for different metals will vary (Costello et al. 2011; Strom et al. 2011; Campana et al. 2013). Furthermore, the concentration of AVS can be highly variable both spatially and temporally and readily oxidised at the sediment-water interface with overlying water, plant roots and burrowing organisms (Gallon et al. 2008; Simpson and Spadaro 2011).

The fluxes of metals from porewaters and labile sediment phases within surface sediments measured using diffusive gradients in thin films (DGT) have been demonstrated to be useful for predicting metal exposure and lethal and sublethal toxicity to benthic invertebrates (Simpson et al. 2012b; Amato et al. 2014). DGT is an *in situ* technique which provides time-integrated measurements of the combined labile metal fluxes from the sediment porewater and particulate phases (Zhang et al. 1995). An advantage of using metal fluxes in surface sediments for predicting metal bioavailability, and the risk of toxicity to benthic organisms, is that the fluxes from all sediments phases, whether in equilibrium or not, are measured. When the DGT device is deployed in the sediment, metals dissolved in the pore water are rapidly accumulated on the resin, generating a localised zone of depletion in the porewaters and inducing the release of labile, weakly-bound metals absorbed onto sediment particles. While the release rates will differ between sediments and metals, similar processes occur as benthic organisms accumulate metals from porewaters.

When metal concentrations at the interface between the DGT device and sediment are well buffered by metal resupply from the sediment solid phase, Fick's law of diffusion can be used, along with metal diffusion coefficients, deployment time and

diffusive gel thickness, to interpret DGT fluxes as pore water concentrations (Harper et al. 1998). However, in case of partial resupply, or resupply by metal diffusion from more concentrated areas of the sediment, the principles underpinning the DGT technique no longer hold and DGT measurements are the result of dynamic equilibria between the binding strength of the DGT resin and that of the sediment. As the conditions necessary to interpret DGT measurements as pore water concentrations rarely occur, we believe that interpreting DGT measurements as fluxes ($\mu\text{g}/\text{h}/\text{m}^2$) is the most suitable approach for sediment deployments.

The use of DGT-induced metal fluxes to predict the metal toxicity in sediments with varying properties has been limited to laboratory-based toxicity tests: copper and survival of a benthic bivalve (Simpson et al. 2012b); and mixed metals (Cd, Cu, Ni, Pb and Zn) and survival and reproduction of an amphipod (Amato et al. 2014). In this study, we apply the DGT technique to the prediction of metal bioavailability to the bivalve, *Tellina deltoidalis* exposed to an identical series of metal-contaminated marine sediments deployed simultaneously in the field and laboratory over 31 days. As well as evaluating the use of DGT for predicting metal bioavailability, the study was used to better understand the key differences in metal exposure occurring between laboratory and field-based bioassays. In an accompanying study, Belzunce-Segarra et al. (2015) demonstrated that the field and laboratory environments resulted in higher dissolved metal exposures in the laboratory due to lower exchange rates of overlying waters, and that the metal concentrations in the surface sediments changed far more under field conditions due to loss of resuspended sediments and deposition of natural suspended particulate matter from the overlying water.

4.2 Materials and methods

4.2.1 General methods

Plastic-ware used for analyses was new and cleaned following the procedures described in Section 2.1. All chemicals used were analytical reagent grade or equivalent level of purity. Total recoverable metal (TRM), dilute acid-extractable metal (AEM), acid-volatile sulfide (AVS), total organic carbon (TOC) and physical analyses were performed as described in Section 2.4. Biological tissues were analysed as described in Section 2.4. Overlying waters were membrane-filtered (0.45 μm cellulose acetate,

Sartorius Ministart) prior to analyses of dissolved metals (acidified 2% (v/v) with HNO₃ (Tracepur, Merck) immediately after collection) or ammonia (analysed immediately or frozen and analysed within a week). Metal concentrations in overlying waters, acid-digests from sediments, biological tissues and DGT were determined as described in Section 2.4 and Section 2.6.

4.2.2 Test media and organisms

Clean seawater was collected from Port Hacking, Sydney, membrane filtered (0.45 µm) and stored at 4°C. Where necessary, the salinity of the filtered seawater was adjusted to the test salinity of 30 PSU using Milli-Q water. Prior to use in experiments all waters were acclimated in a temperature-controlled room (21±1°C).

Clean and metal-contaminated sediments were collected from a range of estuarine sites near Sydney, Australia. Control sediments were collected from an estuarine location in Bonnet Bay, Sydney, by removing the first 3 cm of oxidised surficial sediment with a plastic spoon. This silty sediment (95% <63 µm) had been previously characterised and found to have relatively low concentrations of metal and organic contaminants (Spadaro et al., 2008), and supported the survival and growth of the bivalve *T. deltoidalis* (King et al. 2010; Campana et al., 2013). The silty control sediment was used either unmodified (Sediment 1) or as a 30:70 mixture with clean sand (98% >180 µm) (Sediment 5). Contaminated sediments were collected from Port Kembla (Sediment 4) and three sites in Sydney Harbour (Sediments 6, 7 and 8). Previous studies found these sediments contained concentrations of Cu, Pb and Zn which greatly exceeded the sediment quality guideline values, and caused toxicity to amphipod reproduction (Amato et al., 2014), but relatively low concentrations of organic contaminants (Chariton et al. 2010; Dafforn et al. 2013). All sediments were sieved (< 1 mm, plastic mesh), homogenized and stored at 4° C in the dark until use. A dilution series was created by mixing Sediment 4 (contaminated) with Sediment 1 (control) (both 95% <63 µm) at ratios of 1:1 (Sediment 3) and 1:3 (Sediment 2). Mixing occurred in a nitrogen atmosphere and the sediments were equilibrated for four weeks before use (Simpson et al., 2004). Test sediments were grouped into two series (silty S1-S4, or sandy S5-S8) according to their physical properties. The deposit feeding benthic bivalve *T. deltoidalis* (shell lengths of 5-12 mm) was collected from Lane Cove River (NSW, Australia) and maintained as described in Section 2.3.

4.2.3 Bioaccumulation bioassays

A detailed description of the procedures followed for the laboratory and field bioassays are provided in Section 2.5.2.

4.2.4 Diffusive gradients in thin films

For DGT gels synthesis and probes preparation and handling refer to Section 2.6. DGT probes were deployed for 24 h in both replicates of the eight test sediments on day 5 and 19 of the bioaccumulation test. In the field, cages were removed from the water, probes carefully inserted in the sediments, then rapidly returned to the same location. Upon retrieval, probes were thoroughly rinsed with Milli-Q water, placed in acid-washed plastic bags and stored at 4°C until analysis. Probes were disassembled and the Chelex-resin sliced at 1-cm intervals below the SWI to a depth of 3 cm, with a 1-cm slice followed by a 2-cm slice obtained above the SWI. Gel slices analysis and metal flux calculations were performed according to the procedures described in Section 2.6. Analyses of blank probes indicated that Cd, Cu, Ni and Pb contributed to DGT measurements for < 0.1 , < 0.1 , < 0.5 and < 0.01 $\mu\text{g}/\text{h}/\text{m}^2$, respectively, based on a 24-h deployment. Zinc contamination was detected in blank probes and estimated to contribute to an equivalent 24-h DGT-Zn flux of 60 ± 14 and 69 ± 17 $\mu\text{g}/\text{h}/\text{m}^2$ for laboratory and field blanks, respectively (mean \pm standard deviation, $n=4$).

4.2.5 Data analysis

Differences in metal bioaccumulation rates between organisms exposed to laboratory and field conditions, and differences between overlying water concentrations measured in different periods of the tests were investigated using the software Toxcal (Version 5.0.23, TidePool Scientific Software), NCSS (Kaysville, Utah) or Statgraphics Centurion (Warrenton, Virginia). Unless otherwise stated $p = 0.05$ was the level of significance. Data were tested for homogeneity of variance (Levene test) and for normality of residuals distribution (Shapiro-Wilk's test) prior to hypothesis testing. When the data either did not follow a normal distribution or it was not possible to test the data heteroscedasticity, two-way analysis of variance of Kruskal-Wallis test followed by Bennett squares sum partition method were applied to evaluate statistical differences. DGT-based threshold values indicating 'significant' bioaccumulation

corresponded to the lowest DGT flux measured in contaminated sediments above which bioaccumulation was consistently higher than the control mean plus 1 σ .

4.3 Results and discussion

4.3.1 Sediment properties

The chemical and physical properties of the sediments used for the bioaccumulation test are shown in Table 4.1. Concentrations for a greater range of metals and metalloids are provided in Table 4.2 and 4.3. The metal-contaminated sediments (excluding controls S1 and S5) in the silty series had 81-92% <63 μm particles, 4.2-5.7 % total organic carbon (TOC) and <0.1 $\mu\text{mol/g}$ acid-volatile sulfides (AVS), while the sandy series had 10-34% <63 μm particles, 2.1-5.9 % TOC and 4.2-9.7 $\mu\text{mol/g}$ AVS. Copper, lead and zinc were the major contaminants with total recoverable metal (TRM) concentrations ranging from 10 to 1020, 18 to 745, and 55 to 2490 mg/kg, respectively. The dilute acid-extractable metal (AEM) concentrations were 55, 90 and 85% of the TRM for copper, lead and zinc, respectively. The AVS-SEM analyses (where SEM is equivalent to AEM) indicated that all sediments had a molar excess of SEM over AVS, and therefore have potential to cause adverse effects to benthic organisms (Simpson and Batley 2007).

4.3.2 Discrete dissolved metal samples and DGT metal fluxes in the overlying waters

DGT fluxes integrated from 0 to 3 cm above the SWI were interpreted as DGT concentrations in the overlying water. To allow metal concentrations to be calculated ($\mu\text{g/L}$), well-mixed conditions (negligibly small diffusive boundary layer (DBL) thickness), partially provided by the stirring effect of the air purge system, were assumed.²⁰ In laboratory-exposed sediments, DGT concentrations (Cu, Pb and Zn) were overall similar between the two deployments (Figure 4.1), and followed the general trend observed for dissolved metal concentrations measured in discrete water samples (Table 4.1), although the small sample size likely caused an inability to detect significant differences. Increasing DGT metal concentrations (average of first and second deployments) were observed for increasing concentrations of copper in the silty series (0.4 ± 0.1 , 0.9 ± 0.2 , 2.1 ± 0.4 and 4.3 ± 0.8 $\mu\text{g/L}$ for S1, S2, S3 and S4, respectively), and of zinc in the sandy series (<1, 23 ± 25 , 110 ± 50 and 320 ± 10 $\mu\text{g/L}$ for S5, S6, S7 and

S8, respectively), indicating an efflux of these metals from the sediment to the pore water.

Table 4.2 Total recoverable metal concentrations in original sediments in mg/kg, unless specified. All concentration are mean (n=2) with variability between measurements of <30%

Sediment	Al %	Fe %	Mn	Cu	Pb	Zn	As	Cd	Co	Cr	Ni	Sn	V
Silty Series													
S1	1.1	2.5	87	35	65	188	19	1.0	4	29	12	3	41
S2	1.3	3.3	182	303	248	544	30	0.7	8	47	15	60	57
S3	1.4	4.1	275	527	414	828	42	1.5	9	65	18	119	74
S4	1.5	5.5	446	1050	747	1480	64	1.8	16	96	25	234	103
Sandy Series													
S5	0.2	0.4	21	10	18	54	5	0.9	1	5	2	1.0	8
S6	0.7	1.7	107	265	317	705	13	1.3	5	75	15	69	34
S7	0.6	1.2	71	118	198	1550	7	1.1	4	26	11	7	24
S8	0.7	1.7	76	94	218	2640	11	1.4	5	34	13	7	27

Table 4.3. Dilute acid-extractable metal concentrations in original sediments (1 M HCl) in mg/kg, unless specified. All concentration are mean (n=2) with variability between measurements of <30%

Sediment	Al %	Fe %	Mn	Cu	Pb	Zn	As	Cd	Co	Cr	Ni	Sn	V
Silty Series													
S1	0.2	1.1	47	22	63	166	7	0.1	3.0	11	4	1	22
S2	0.3	1.2	85	128	232	387	10	0.4	3.4	19	5	29	29
S3	0.4	1.3	121	252	393	585	16	0.5	3.5	29	6	58	35
S4	0.5	1.6	196	523	721	999	24	1.0	5.4	50	9	117	48
Sandy Series													
S5	0.1	0.3	17	7	17	48	2	0.0	0.7	3	1	0.4	7
S6	0.2	0.6	25	57	242	585	2	1.0	1.5	27	5	26	18
S7	0.2	0.4	17	27	140	1479	2	0.9	1.7	7	2	2	9
S8	0.2	0.5	21	41	199	2405	3	1.2	2.5	15	4	3	12

This was consistent with dissolved metals concentrations in discrete overlying water samples indicating that copper release was higher for the silty series (6 - 20 µg/L) and zinc release was higher for the sandy series (28-450 µg/L) (Table 4.1). DGT-Cu concentrations were <1 µg/L in sandy sediments (S5-S8), whereas DGT-Zn concentrations were <15 µg/L in silty sediments (S1-S4). For all sediments, DGT-Pb concentrations were consistently <2 µg/L. DGT-Cu and -Zn concentrations were generally lower than dissolved copper and zinc concentrations measured in discrete

Table 4.1 Chemical and physical properties of control (S1 and S5) and contaminated sediment and overlying water. All concentration are mean (n=2) with variability between measurements of <30%

Sediment	Total recoverable metals (TRM)					Acid-extractable metals (AEM)					Overlying water concentrations			SEM-AVS μmol/g	TOC %	Particles < 63 μm, %
	Fe %	Mn mg/kg	Cu mg/kg	Pb mg/kg	Zn mg/kg	Fe %	Mn mg/kg	Cu mg/kg	Pb mg/kg	Zn mg/kg	Cu μg/L	Pb μg/L	Zn μg/L			
S1	2.5	87	35	65	188	1.1	47	22	63	166	<2	<2	<2	3	3.6	94
S2	3.3	182	303	248	544	1.2	85	128	232	387	6 ± 3	<2	3 ± 1	9	4.2	92
S3	4.1	275	527	414	828	1.3	121	252	393	585	10 ± 5	<2	6 ± 1	15	4.7	90
S4	5.5	446	1050	747	1480	1.6	196	523	721	1000	20 ± 9	2.2 ± 1.6	18 ± 4	27	5.7	81
S5	0.4	21	10	18	54	0.3	17	5	17	48	3 ± 1	<2	2 ± 1	1	0.9	26
S6	1.7	107	265	317	705	0.6	25	57	242	585	<2	<2	28 ± 9	10	5.9	31
S7	1.2	71	118	198	1550	0.4	17	27	140	1480	<2	<2	120 ± 47	14	4.2	14
S8	1.7	76	94	218	2640	0.5	21	41	199	2400	2 ± 1	<2	450 ± 160	34	2.1	27
Field conc.											(<2)	(<2)	(2.3 ± 1.8)			
SQGs/WQGs			65	50	200			65	50	200	1.3	4.4	15			

AVS = acid-volatile sulfides; AEM-AVS refers to the molar difference, where AEM is equivalent to SEM; TOC = total organic carbon; particles <63 μm is the percentage (by weight) of fine sediment particles. Overlying water concentrations are mean values (with standard deviation) of samples collected during the laboratory experiment (*n*=12) (values in brackets are concentrations measured in the field, *n*=24) SQGs/WQGs = sediment/water quality guidelines trigger values (ANZECC/ARMCANZ, 2000).

overlying water samples (Table 4.1). This may be attributed to the ability of the DGT technique to account for labile, weakly-bound metal species only, whereas the discrete whole water samples provide total dissolved metal concentrations. Although the overlying water in the treatments were mixed by aeration in the laboratory and by tidal currents in the field, it can not be discounted that lower DGT concentrations may also be due to an insufficient water mixing rate, which increases the DBL thickness and affects DGT measurements (Davison and Zhang 2012). On average, DGT-Zn concentrations were about 20% lower than dissolved zinc, whereas DGT-Cu was 75% lower than dissolved copper concentrations. This was consistent with higher affinity of copper for organic ligands, suggesting that a significant amount of copper was likely present as poorly labile dissolved organic complexes in the overlying water (Zhang et al. 2000).

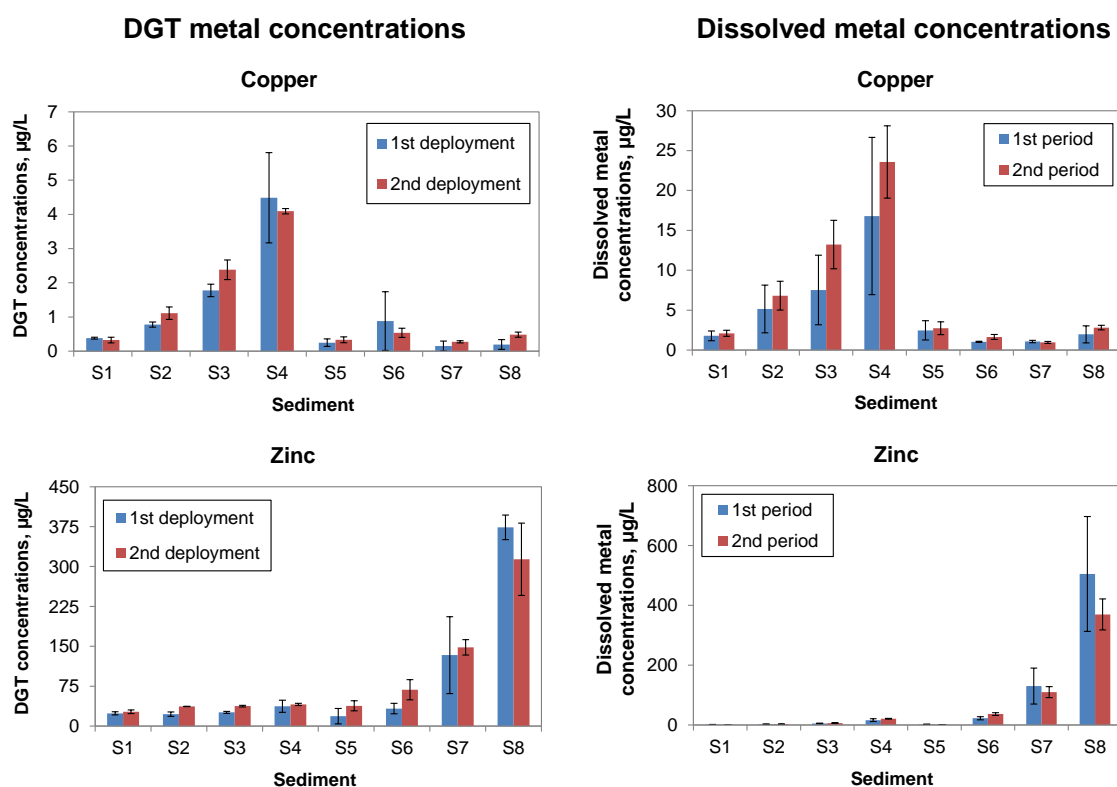


Figure 4.1 Comparison between overlying water DGT and dissolved metal concentrations (Cu and Zn) measured in the first and second DGT deployment under laboratory conditions. DGT probes were deployed on day 5 and 19 for 24 h (from 0 to 3 cm above the SWI) and data point are mean values of two replicates with standard deviation. Dissolved metal concentrations are the average values (with standard deviation) of discrete water samples collected on day 0, 4, 5, 6, 7, 11, 14, for the first period and day 18, 21, 25, 28, 31, for the second period of the test.

As expected, lower metal fluxes (Cu, Pb and Zn) were observed in the overlying water of most field bioassays than for laboratory bioassays (Figure 4.2), and this was attributed to the effect of the water currents rapidly washing away and diluting the metals released across the sediment-water interface to the water column, so that there was inadequate time for metals to bind to the DGT resin. In field deployments, overlying water DGT-metals were similar amongst all treatments ($<1 \mu\text{g/L}$ for Cu and Pb and $32 \pm 10 \mu\text{g/L}$ for Zn, $n=32$). In the field, no significant difference in overlying water metal concentrations were observed between first and second deployments ($n=16$, $\alpha=0.05$), indicating that dissolved metal concentrations varied minimally throughout the *in situ* bioassay. This was expected, as water renewal occurred continuously within the cages and the DGT probes were exposed to the same water column for all treatments.

4.3.3 DGT-metal fluxes from sediments

DGT-Cu, -Pb and -Zn maxima generally occurred approximately 1 cm below the SWI (Figure 4.2) and metal release associated with the reductive dissolution of Fe(III) and Mn(IV) and the degradation of organic matter would have contributed to this (Poulton et al. 2004; Naylor et al. 2006; Amato et al. 2014). It is also likely that minor contributions occurred from metals associated with dissolved organic matter which diffused into the resin. Decreasing DGT-metal fluxes occurred deeper in the sandy sediments (S5, S6, S7 and S8), consistently with increasing reducing conditions that would promote the precipitation of these metals as sulfide phases. This was supported by concurrent studies showing increasing AVS concentrations with increasing sediment depths measured in the sandy sediments (Belzunce-Segarra et al. 2015).

The pore water DGT-Cu, -Pb and -Zn fluxes (below the SWI) were generally lower in field-exposed compared to laboratory-exposed sediments (Figure 4.2). Under field conditions, the diffusion of dissolved metals across the SWI to the overlying water may deplete porewater-metals to a greater degree than in the laboratory. Weakly-bound metals are then released from the solid phase to the pore water as a response to metal depletion. Over time, the ongoing diffusion of metals driven by the concentration gradient may eventually cause the exhaustion of the labile metal-fraction in the sediment and lower the DGT-metal fluxes as a response to the relatively slow metal release from stronger binding phases. Under the laboratory conditions, the lower rate of water renewal results in greater concentrations of metals in the overlying water and

subsequently the dissolved metal gradient becomes weaker and pore water metal concentrations remain higher and closer to equilibrium with the sediments.

Despite the different conditions, similar iron and manganese DGT-profiles were measured in laboratory and field-exposed sediments (Figure 4.3). Increasing Fe(II) and Mn(II) fluxes from 0.5 to 2.5 cm below the SWI were consistent with the reductive dissolution of iron and manganese oxyhydroxide phases used by bacteria for the mineralisation of organic material (Fones et al. 2004; Amato et al. 2014). Differences between iron and manganese reduction boundary depths are usually observed due to the different redox properties of the metals (Teal et al. 2009), but only in sediments S6 and S7 (laboratory and field) such differences were appreciable and consistent with iron being reduced 1 cm deeper than manganese (-1.5 and -0.5 cm, respectively).

The laboratory and field-bioassay were initiated using sediment that had been homogenised and equilibrated in test vessels for one day before test commencement. Such a short equilibration period is a common approach when using whole-sediment bioassays for sediment quality assessment, despite the recognition that this will severely disturb the redox equilibrium which requires considerable time to re-establish (Simpson and Batley 2003; Hutchins et al. 2008). Deposition of solid particulate matter (SPM) was observed in field deployments and affected the physical (grain size) and chemical (e.g., AEM, AVS) properties of the surface sediment (Belzunce-Segarra et al. 2015). The SPM was found to contain relatively low metal concentrations (9 ± 1 , 17 ± 4 , 32 ± 5 , 72 ± 5 , 200 ± 20 mg/kg for As, Cr, Cu, Pb and Zn, respectively) and was mainly composed of fine particles ($90\pm5\%$ $<63\ \mu\text{m}$). Pore water DGT-metal fluxes have been shown to decrease with increasing percentages of $<63\ \mu\text{m}$ particles (Simpson et al. 2012b; Amato et al. 2014), and the deposition of SPM containing lower particulate metal concentrations was expected to lower metal release (and DGT-metal fluxes) near the SWI.

DGT probes were deployed at two different stages, on day 5 and day 19, during the bioassays to provide information on the changes in metal partitioning occurring during the 31-day bioassay. A high degree of variability was observed for the DGT-metal fluxes measured in the pore water, i.e. the difference in fluxes from two replicates sediments under both laboratory and field conditions. The average Cu, Pb and Zn fluxes in the sediments (0 to -3 cm) on day 5 and 19 were generally within a factor of three (Figure 4.4; Figure A2.1 and A2.2 of Appendix 2).

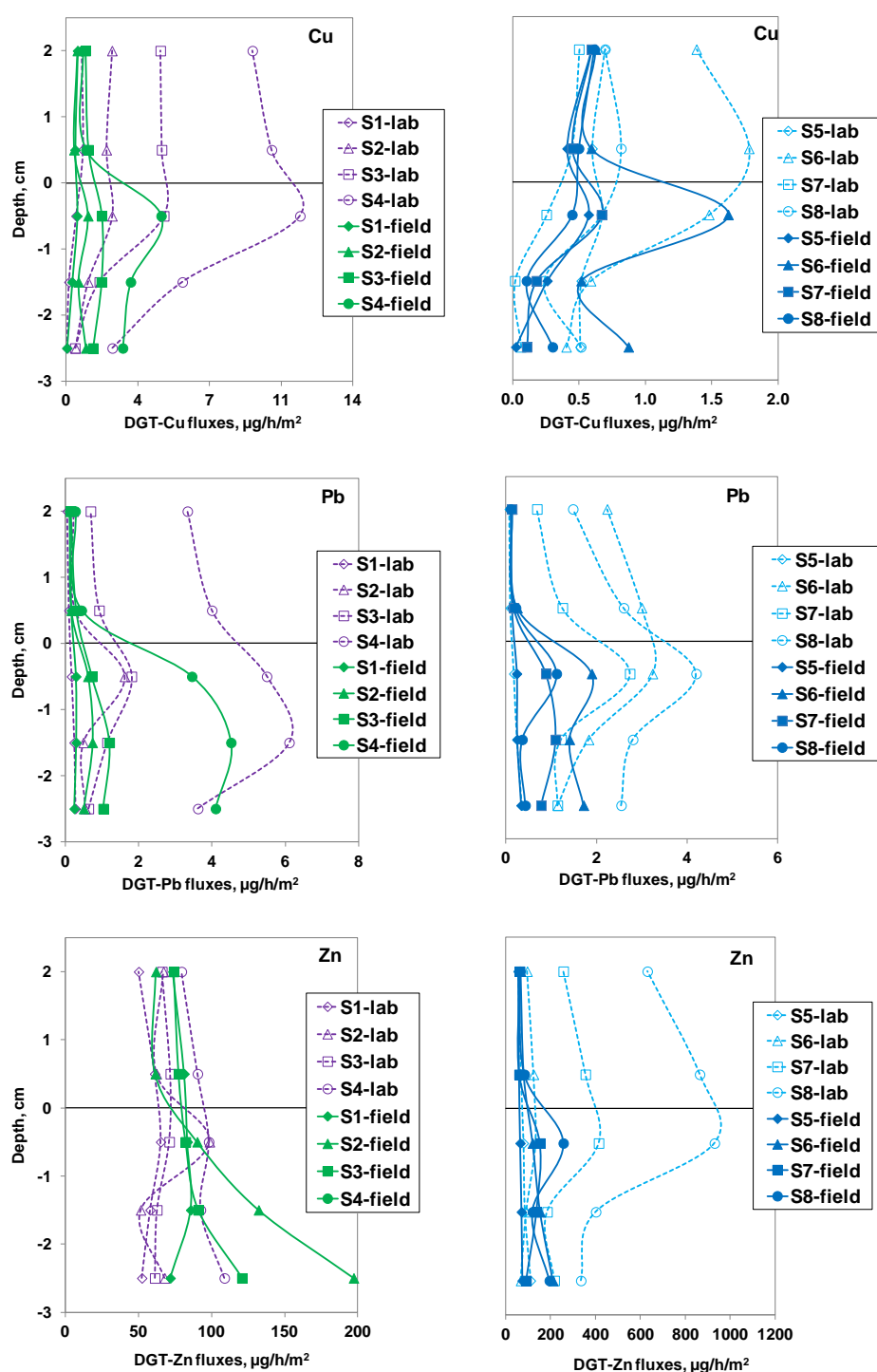


Figure 4.2 DGT-Cu, -Pb and -Zn fluxes measured in silty (left) and sandy (right) sediment series exposed to laboratory (empty symbols) and field (filled symbols) conditions. Data points are mean values of first and second deployment (2 replicates each), with standard deviation averaging 55, 45 and 40% of mean values for Cu, Pb and Zn, respectively.

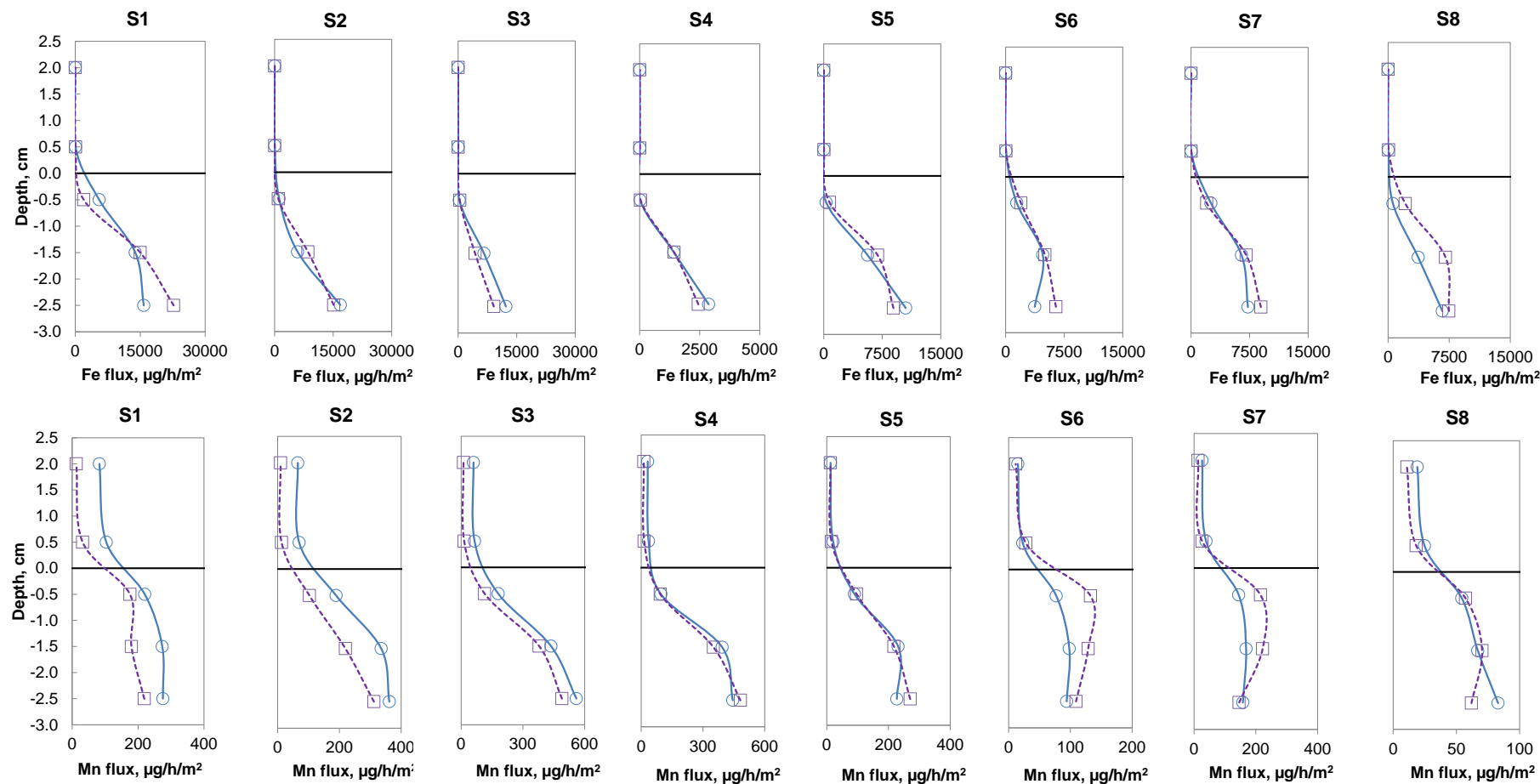


Figure 4.3 DGT-Fe and -Mn fluxes measured in laboratory (circles) and field (squares) deployments (data points represent average values of first and second deployment, standard deviation ranged from 60 to 75% and from 30 to 60% of mean values for iron and manganese, respectively).

4.3.4 Relationships between metal bioaccumulation and DGT fluxes

No mortality to bivalves was observed after the 31-day exposure for any treatments in the laboratory or the field. For many of the exposures, and particularly those with the highest contamination, the bivalve soft-tissue Cu, Pb and Zn concentrations were greater than the baseline concentrations (unexposed bivalves, Figure 4.5). *T. deltoidalis* reside buried in the sediment, but use their siphon to reach the sediment surface to feed on food particles (Campana et al. 2013) and both dietary exposure through ingestion of sediment particles (Simpson and King 2005; Campana et al. 2013) and dissolved exposure (King et al. 2005; Atkinson et al. 2007) have been observed to be significant metal exposure routes. Given the position of *T. deltoidalis* below the SWI and its feeding behaviour at the SWI, four different relationships between DGT metal fluxes (Cu, Pb and Zn, average of first and second deployment) and bioaccumulation were investigated:

- at the SWI (± 1 cm) - exposure from sediments and overlying water;
- in the bulk sediment (from 0 to 3 cm below the SWI) - exposure from the bulk sediments;
- in the deeper sediment (between -2 and -3 cm below the SWI) - exposure from the deeper sediments only; and
- within 1 cm above the SWI - exposure from overlying water only.

Amongst these different approaches, DGT-metal fluxes at the SWI (i.e. ± 1 cm) provided the most consistent relationships with bioaccumulation (Figures 4.6 and 4.7). A comparison between the SWI (i.e. ± 1 cm) relationship and the other three approaches are provided in Figure 4.7 and discussed below. Unless specified otherwise, from here onwards, DGT-fluxes will refer to measurements performed at the SWI only (average of first and second deployment).

Low copper bioaccumulation found in the bivalves in sandy sediments (S5-S8) was consistent with low DGT-Cu fluxes for these sediments, whereas increasing soft tissue concentrations in bivalves exposed to silty sediments (S1-S4) were observed for increasing copper fluxes in those sediments (Figure 4.6). In the silty sediments, similar copper bioaccumulation was observed between laboratory and field-exposed bivalves (except in sediment S2) (Figure 4.5), despite the significantly greater DGT-Cu fluxes in laboratory-exposed sediments (Figures 4.2 and 4.6). This suggests that the use of DGT-Cu fluxes in

laboratory deployments may overestimate the copper exposure and potential for bioaccumulation using these types of sediments (Table 4.1).

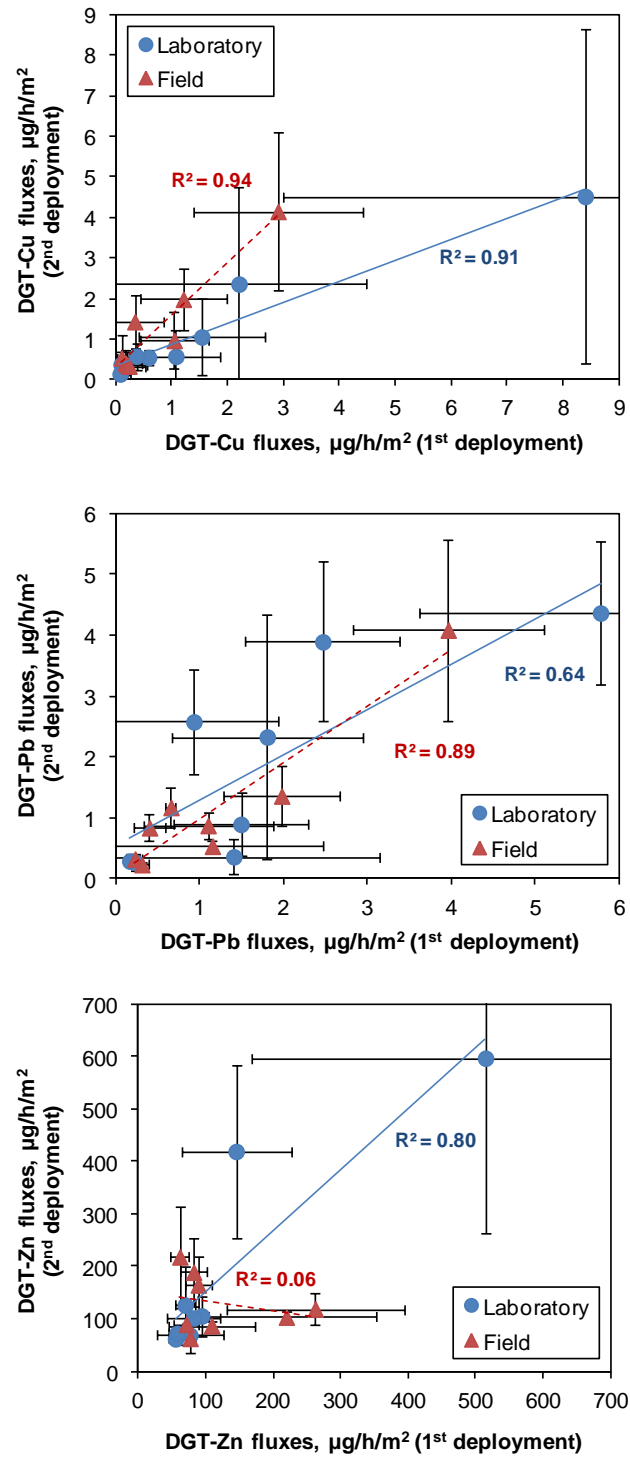


Figure 4.4 Average Cu, Pb and Zn fluxes in the sediments (0 to -3 cm) on day 5 (first deployment) and 19 (second deployment). Data points are average values of two replicates with standard deviation.

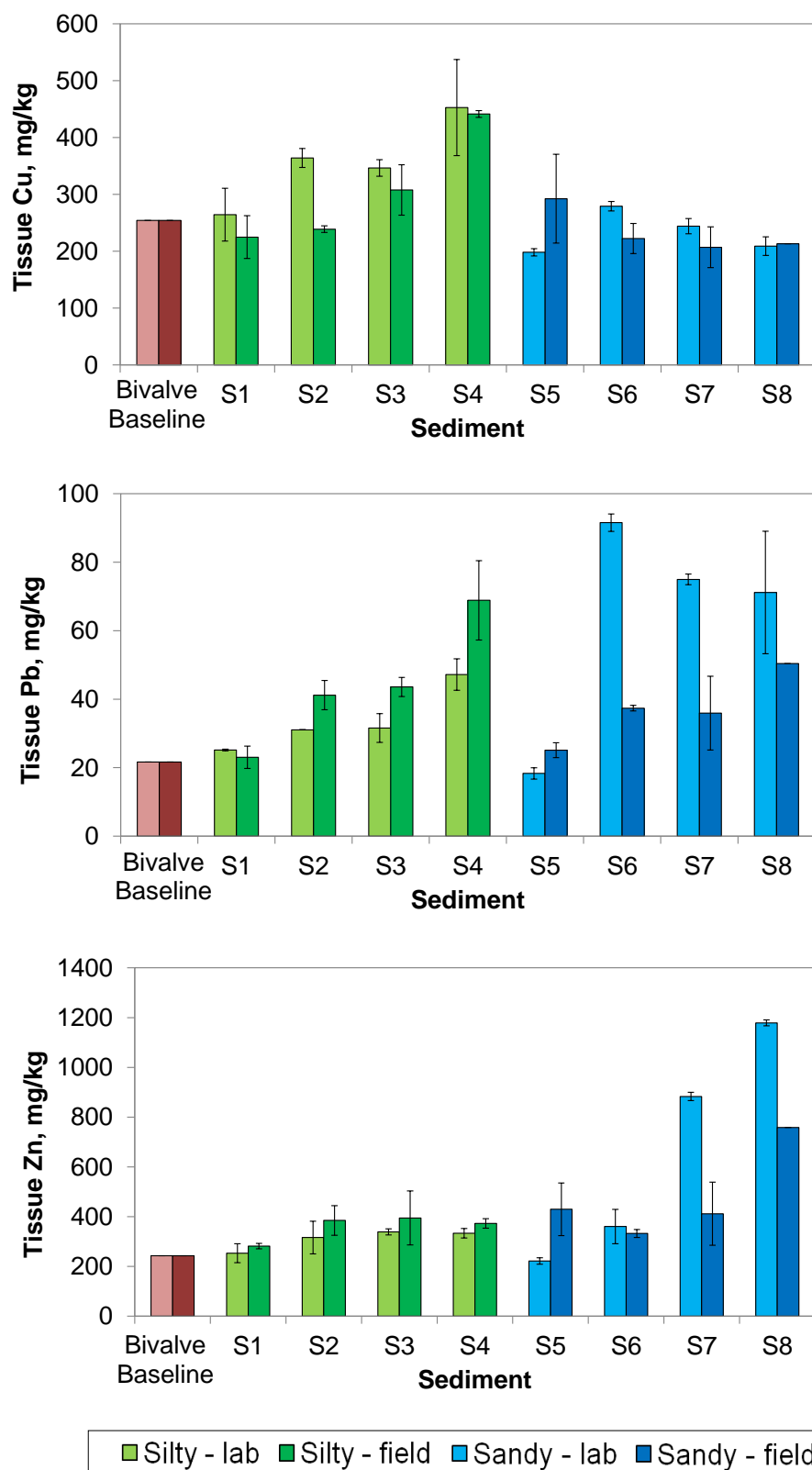


Figure 4.5 Lead, zinc and copper bioaccumulation in laboratory and field-exposed bivalves. Soft tissue concentrations are mean \pm standard errors ($n = 2$). For sediment S8, only one replicate was available for the field experiment. Bivalve baseline is the soft tissue concentrations measured in non-exposed organisms (as described in the methods).

When considering fluxes measured between 0 and -3 cm depth, thus excluding fluxes from the overlying water, the relationship between DGT-Cu fluxes and bioaccumulation improved (R^2 increased from 0.54 to 0.63, Figure 4.7). This suggests that the copper exposure from the overlying water was not contributing to the bioaccumulation of copper as greatly as predicted using DGT-Cu fluxes that incorporate that exposure compartment, which is consistent with the porewater or sediment particle ingestion being the major exposure route for this bivalve (Simpson and King 2005; Campana et al. 2013).

Increasing lead bioaccumulation occurred with increasing DGT-Pb fluxes, although the relationship was quite different for the silty sediments (S1-S4) exposed to laboratory conditions (Figure 4.6). As for copper, this indicated that the use of DGT-Pb fluxes in laboratory deployments may overestimate the lead exposure and potential for bioaccumulation. The results also indicated that dissolved lead in the overlying water was contributing less to the lead bioaccumulation than either the porewater or dietary forms of lead that are easily released from the sediments (and appear proportional to the DGT-labile forms).

The bioaccumulation of zinc increased rapidly when DGT-Zn fluxes exceeded $\sim 420 \mu\text{g}/\text{h}/\text{m}^2$, and a strongly positive relationship existed across all the sediments and exposures (Figure 4.6). This indicated a possible threshold value above which the ability of *T. deltoidalis* to regulate the internal zinc concentrations was inhibited (Rainbow 2002). The strong relationship indicates that the DGT-Zn fluxes at the SWI provide an excellent method for predicting the combined zinc exposure from overlying water, porewater and labile sediment phases that contribute to bioaccumulation irrespective of the sediment properties and exposure conditions. However, similarly strong relationships between bioaccumulation and DGT-Zn fluxes were provided using fluxes measured in the pore water (between 0 and -3 cm depth) and in the overlying water, whereas poor relationships were obtained using fluxes measured in the deeper sediment (>3 cm depth) (Figure 4.7).

4.3.5 Use of DGT-metal fluxes for indicating risk posed by metal contaminated sediments

In the companion paper (Belzunce-Segarra et al. 2015) the average AEM concentrations in the surface sediments before and after exposure were shown to provide the best measurement for predicting bioaccumulation of Cu, Pb and Zn in the bivalve in these same sediments (Figure 4.8).

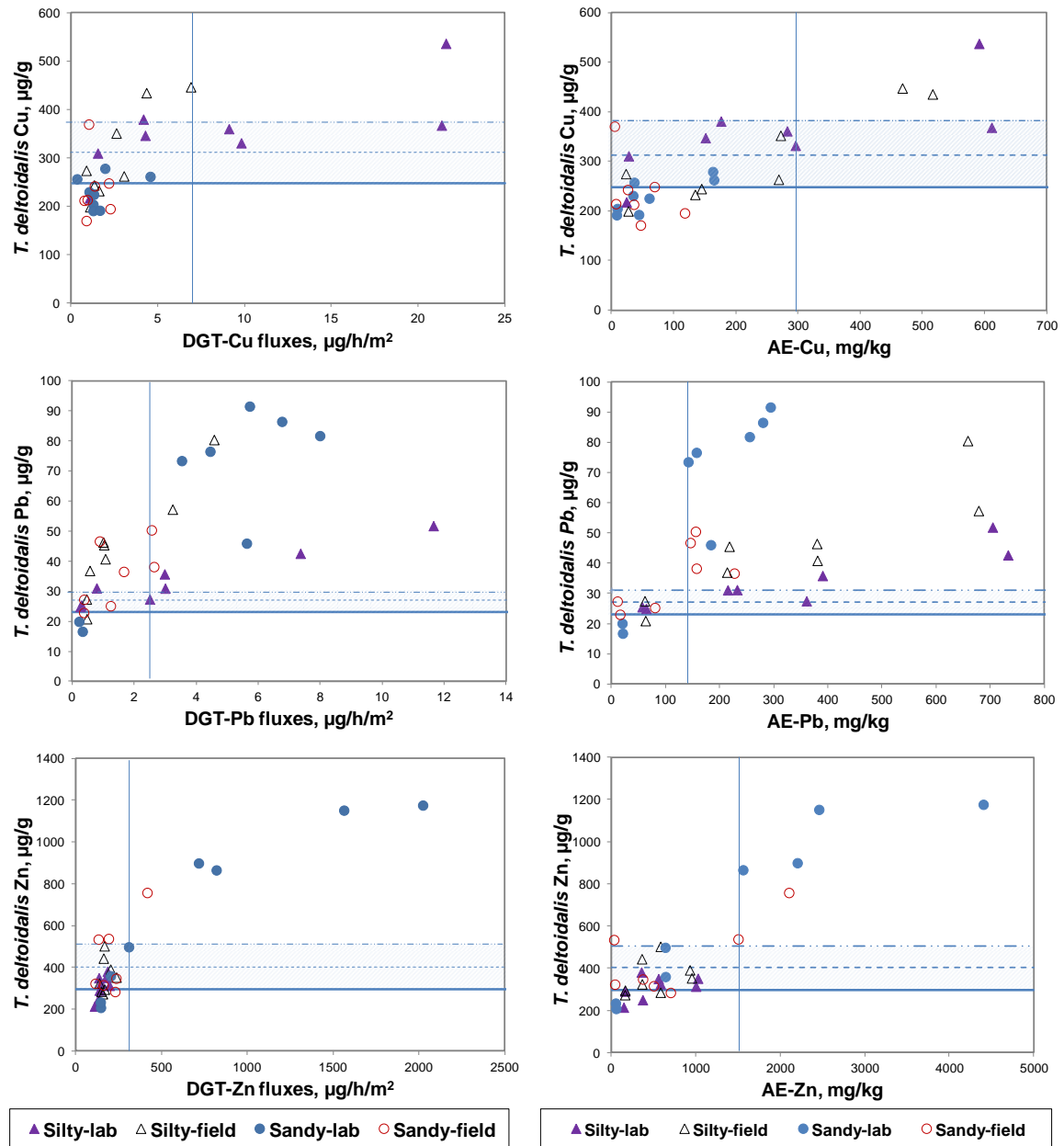


Figure 4.6 Relationships between Cu, Pb and Zn bioaccumulation and DGT fluxes measured at the SWI. The average soft tissue metal concentration found in *T. deltoidalis* exposed to control sediments (blue solid line) is reported with standard deviation (shaded areas, $n=8$). The horizontal continuous line represent the mean of control sediments, whereas dashed lines refer to controls mean value plus 1σ and 2σ . The vertical line corresponds to the DGT flux above which bioaccumulation was consistently higher than controls mean plus 1σ .

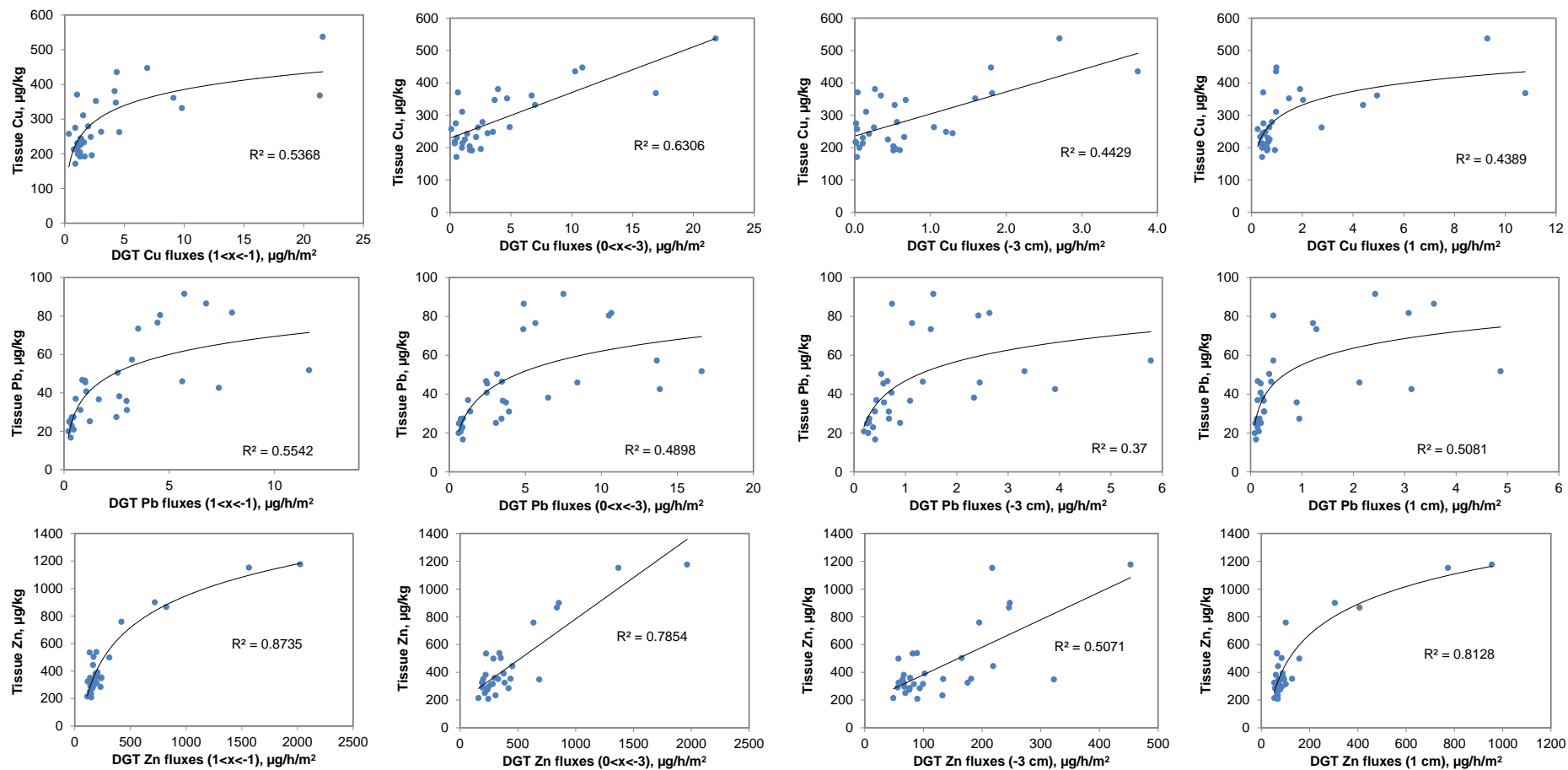


Figure 4.7 Relationships between Cu, Pb and Zn bioaccumulation and DGT fluxes measured (i) at the SWI, (ii) between 0 and -3 cm depth, (iii) at -3 cm depth, and (iv) 1 cm above the SWI (laboratory and field exposure). Lines represent linear or exponential models used to estimate relationships.

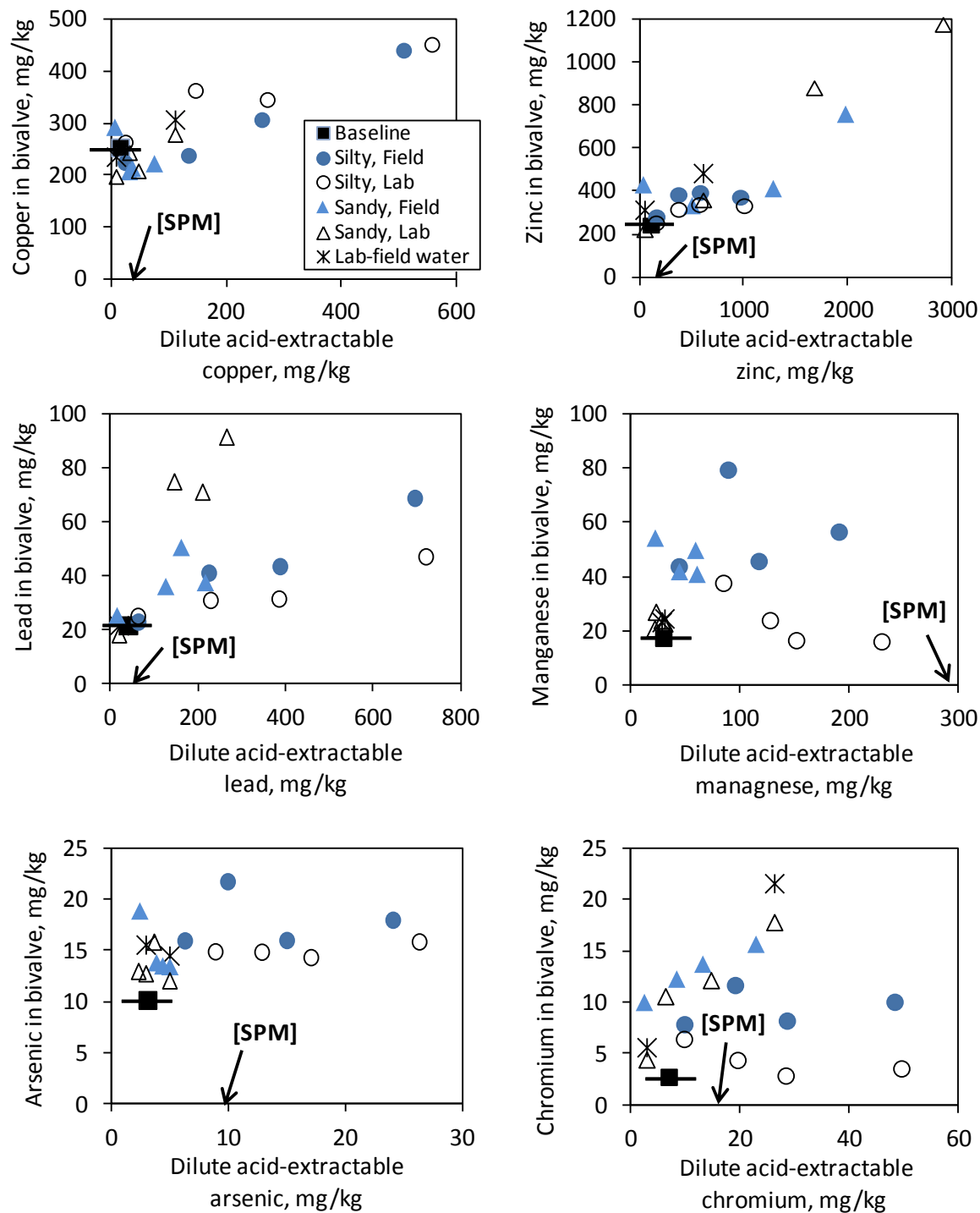


Figure 4.8 The relationship between average (original and final) dilute acid-extractable metal (AEM) concentration in surface sediments and metal bioaccumulation in *T. deltoidealis* after 31 d exposure under laboratory (open symbols) and field (filled symbols) conditions. The solid square with line represents the baseline metal concentrations in the bivalves (unexposed bivalves). The asterisk represents the lab exposures using unfiltered overlying water from the field site. The arrow and [SPM] represents the AEM concentration in the suspended particulate matter at the field site (Belzunche-Segarra et al. 2015).

Strong relationships were observed between DGT-metal fluxes and AEM concentrations (Figure A2.3 of Appendix 2), and with tissue metal concentrations in *T. deltoidalis* (as a measure of potential metal exposure, Figure 4.6). Making multiple measurements on particulate phases through time to predict exposure and risk of effects is not considered practical, and for some assessments the use of DGT may be more effective. For sediments with a wide range of sediment properties, Simpson et al. (2012b) found that DGT-Cu fluxes provided a better predictor of copper bioaccumulation in *T. deltoidalis* than AE-Cu, while for sediments with a wide range of sediment properties, Amato et al. (2014) found that the combined DGT- metal fluxes of Cd, Cu, Ni, Pb and Zn provided a similar predictor of acute and chronic toxicity to the amphipod *Melita plumulosa* in estuarine sediments. In Chapter 3 we showed that the combined DGT- metal fluxes of Cd, Cu, Ni, Pb and Zn provided a similar predictor of acute and chronic toxicity to the amphipod *Melita plumulosa* in estuarine sediments. For freshwater sediments, Roulier et al. (2008) observed useful relationships between DGT-labile copper and lead, but not cadmium, and bioaccumulation of these metals in the freshwater crustacean *Chironomus riparius* (chironomid). Dabrin et al. (2012) observed useful relationships between DGT-Cd and cadmium accumulation in *Potamopyrgus antipodarum* (freshwater mud snail). Costello et al. (2012) found that DGT-Ni measurements were useful for interpreting changes in nickel partitioning in freshwater sediments, but relationships that considered particulate AE-Ni, AVS and OC concentrations were superior to DGT-Ni for predicting freshwater invertebrate responses to sediment nickel.

In order to better utilise DGT-based measurements for predicting the risk posed by metal contaminants to benthic organisms, in Chapter 3 we suggested that the combined DGT-metal fluxes (Cd, Cu, Ni, Pb and Zn) be normalized to water quality guidelines (WQG) trigger values (i.e. as a WQG quotient). The expression of toxicity as a threshold using quotients can be criticised as being inappropriate due to the likely different mechanisms of toxicity of the different metals, however the simplified metric was useful for predicting toxicity to the amphipod *Melita plumulosa*, irrespective of the chemical and physical properties of the sediments. A similar approach can be used to determine a threshold for ‘significant’ bioaccumulation by the bivalve in this study.

For copper, bioaccumulation was significantly greater than that of controls (see Section 4.2.5) for fluxes $>6.9 \mu\text{g}/\text{h}/\text{m}^2$, which when represented as a quotient (i.e. 6.9 divided by the WQG for copper of $1.3 \mu\text{g}/\text{L}$ (95% level of protection)), provides a $\text{DGT}_{\text{WQG-Cu}}$ flux of $5.3 \text{ WQG}/\text{h}/\text{m}^2$. For lead, bioaccumulation was significantly greater than that of controls for fluxes $>2.5 \mu\text{g}/\text{h}/\text{m}^2$, and $\text{DGT}_{\text{WQG-Pb}}$ flux of $0.7 \text{ WQG}/\text{h}/\text{m}^2$ (WQG guideline value for lead = $4.4 \mu\text{g}/\text{L}$, 95% protection level (ANZECC/ARMCANZ, 2000)). For zinc, bioaccumulation was significantly greater than that of controls for fluxes $>310 \mu\text{g}/\text{h}/\text{m}^2$, and $\text{DGT}_{\text{WQG-Zn}}$ flux of $21 \text{ WQG}/\text{h}/\text{m}^2$ (WQG guideline value for zinc = $15 \mu\text{g}/\text{L}$, 95% protection level (ANZECC/ARMCANZ, 2000)). These DGT_{WQG} thresholds are lower for copper and lead, but higher for zinc, than the normalised flux threshold value for sub-lethal effects observed for the amphipod *M. plumulosa* ($17 \text{ WQG}/\text{h}/\text{m}^2$) (see Chapter 3). Similarly, Simpson et al. (2012b) determined DGT_{WQG} threshold for copper of $12 \text{ WQG}/\text{h}/\text{m}^2$ for survival (LC10, 10% effect concentration) in *T. deltoidalis*. Caution is needed in this comparison as both the exposures and the endpoints are very different (i.e.; DGT_{WQG} threshold was derived considering the combined effects of five metals on amphipod reproduction). However, the DGT_{WQG} thresholds for the metal mixture and copper and zinc individually fall within quite a narrow range (5.3-21), and support the use of a value in this range as a potential guideline value.

Based on the combined studies, we suggest that, irrespective of the sediment properties (i.e. whether low or high concentrations of silt, AVS, OC and iron/manganese oxyhydroxides phases), sediments will pose a significant risk of bioaccumulation and potential adverse effects to benthic organisms when the combined flux of metals exceeds a threshold of approximately $20 \mu\text{g}_{\text{WQG}}/\text{h}/\text{m}^2$. Fluxes measured at the SWI appear the most appropriate for making this assessment. The studies indicate that DGT-metal flux measurements more consistently improve the prediction of metal bioavailability than AEM measurements, however further field-based studies are needed to understand the strengths and limitation of the technique. As the DGT technique only measures metals present in dissolved forms, i.e. fluxes from porewaters and labile forms released from sediments within the deployment period, the technique may be expected to be most applicable to assessing effects to organisms exposed predominantly via the dissolved phase. However, dietary exposure (e.g. ingestion of sediment particles) is a major exposure route for both the bivalve, *T. deltoidalis* (King et al. 2005; Simpson and

King 2005; Campana et al. 2013) and amphipod, *M. plumulosa* (Simpson 2005; Strom et al. 2011; Campana et al. 2013). The present study further supports the hypothesis that the DGT-labile metal flux may potentially be a useful surrogate for the lability of metals for all exposure routes.

Chapter 5: Field and laboratory evaluation of the diffusive gradients in thin films (DGT) performance for predicting metal bioaccumulation in the fresh water bivalve *Hyridella australis* exposed to contaminated sediments

The content of this chapter is the result of a collaborative work conducted with Ms. Chamani Marasinghe Wadige, Prof. William Maher and Dr. Anne Taylor from University of Canberra. They developed the concepts and design of the laboratory-field bioassays comparison study. Ms. Wadige identified the locations for the *in situ* bioassay, performed sediment and organisms collection and maintenance, and carried out all biological analysis. I developed the DGT-sediment aspects of the program, prepared, deployed and analysed the DGT probes and performed sediment chemical and physical analyses (TRM, AEM, AVS, sediment particles size) used to interpret metal bioaccumulation in laboratory- and field-exposed organisms. During the 28-day experiment, I assisted Ms. Wadige during field work activities and monitoring. Ms. Wadige, Prof. Maher and Dr. Anne are main authors of a companion paper which is currently in preparation and included in Ms. Wadige's PhD thesis.

This Chapter is being prepared for publication:

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5.1 Introduction

The comparison of total recoverable metals (TRM) concentrations with sediment quality guideline (SQG) values are a common first tier in assessment frameworks (ANZECC/ARMCANZ, 2000; Simpson et al. 2013b). If SQG values are exceeded, then further investigations are generally required, and often the next step involves considering the bioavailability of the contaminants. Analysis of pore waters, diluted-acid extractable metal (AEM), acid volatile sulfide (AVS), and organic carbon (OC) concentrations are frequently used to evaluate and predict the metal bioavailability (Simpson and Batley 2007; Burgess et al. 2013). Where concentrations of bioavailable contaminants are determined to exceed guideline levels, bioassays are usually performed to evaluate toxicity effects resulting from contaminant exposure. Although the chemical analyses used for bioavailability assessment have been shown to be useful for predicting metal toxicity in sediments (Simpson and Spadaro 2011; Burgess et al. 2013), the predictions for more oxidized surface sediments can be quite poor, frequently owing to a broader range of factors influencing metal bioavailability (e.g. sediment particle size, iron and manganese) and also variability in phases that are easily oxidized or reduced (e.g. AVS and Fe(II)) (Strom et al. 2011; Campana et al. 2012; Amato et al. 2014).

Laboratory-based bioassays are powerful tools for assessing the environmental risk associated with contaminants in sediments, but may result in inadequate prediction of biological effects due to difficulties in recreating realistic environmental conditions (; Mann et al. 2010; Belzunche-Segarra et al. 2015). Differences between laboratory and field conditions are likely to occur as a result of alterations to whole sediment properties due to sample homogenisation and storage (Simpson and Batley 2003), site specific factors such as infaunal activities (Forster 1996; Simpson et al. 2002) and difficulties in achieving realistic dissolved metal concentrations in the overlying water, which are often overly high in laboratory bioassays (Belzunche-Segarra et al. 2015). These issues can be overcome using *in situ* bioassays, but they are generally more difficult to perform and not be practical for all environments (Burton et al. 2005; Liber et al. 2007). More effective *in situ* tools for assessing contaminant bioavailability are expected to improve the quality of assessments.

The diffusive gradient in thin films (DGT) technique is increasingly being used as an *in situ* monitoring tool for bioavailable metals in sediments (Costello et al. 2012; Roulier et al. 2008; Dabrin et al. 2012; Simpson et al. 2012b; Amato et al. 2014). The DGT technique has the advantage of providing time-integrated *in situ* measurements of dissolved metal species present in the pore water, and weakly-bound to the solid phase of the sediment (Zhang et al. 1995; Harper et al. 1998; Fones et al. 2004). While these measurements have been shown to be useful for predicting metal bioavailability and toxicity to benthic organisms under laboratory conditions (Roulier et al. 2008; Dabrin et al. 2012; Simpson et al. 2012b; Amato et al. 2014), more research is needed to evaluate the DGT performance in the field.

In this study, the ability of the DGT technique to predict metal bioaccumulation in the bivalve *Hyridella australis* was tested under laboratory and field conditions. The Molonglo River, New South Wales, Australia, was identified as a suitable site for conducting investigations due to its historical metal contamination originated from a mine site located in the upper reaches of the river (Captains Flat) (Norris 1986; Sloane and Norris 2003). Although mining activities ceased several decades ago, metal contaminants from the closed mine site still enter the Molonglo River (Sloane and Norris 2003). Metal bioaccumulation by the bivalve *H. australis* was simultaneously investigated *in situ* and under laboratory conditions using sediments collected in each of the four locations. The performance of the DGT technique for predicting metal bioavailability in sediments was investigated by comparing field and laboratory DGT metal fluxes with bioaccumulation in the bivalve *H. australis* exposed to contaminated sediments over a period of 28 days. Relationships between bioaccumulation rates and DGT fluxes were assessed in relation to the application of DGT as a suitable tool for environmental monitoring.

5.2 Materials and methods

5.2.1 General methods

All glass and plastic-ware used for analyses were new and cleaned by following the procedures described in Section 2.1. For sediments, total recoverable metal (TRM), dilute acid-extractable metal (AEM, 60 min 1-M HCl digestion), total organic carbon (TOC) and acid-volatile sulfide (AVS) analyses were performed according to the

procedures described in Section 2.4. TRM concentrations in biological tissues were analysed following the procedure described by Baldwin et al. (1994) and reported in Section 2.4.

5.2.2 Test media and organisms

Sediments were collected in four sites along the Molonglo River at different distances from a mining complex located at Captains Flat, near Canberra, ACT Australia. Three contaminated and one reference sites were identified along the Molonglo River. The reference sediment was collected at approximately 7.5 km upstream from the mine (sediment S1), and three contaminated sediments at approximately 9 (sediment S2), 28 (sediment S3) and 45 (sediment S4) km downstream from the mine site (Table 5.1). The downstream sites have been previously shown to contain elevated sediment metal concentrations which increased with decreasing distance from the mine site (Sloane and Norris 2003). Sediment sub-samples for chemical analyses (~500 g) were sieved (2 mm), homogenised and stored in the dark at 4° C until use. River water was collected from each location, filtered (0.45 µm) and stored at room temperature. The fresh water bivalve *H. australis* (5.5 ± 0.5 cm) was collected and maintained in freshwater sediment as previously described (Section 2.3).

5.2.3 Bivalve bioassay

Bioaccumulation in *H. australis* was assessed following the procedures described in Section 2.5.3. Dry tissues metal concentrations were determined as described in Section 2.4.

5.2.4 Diffusive gradients in thin films

DGT gels synthesis and probes assembling and handling were performed following the procedures described in Section 2.6.

In the laboratory bioassays, one DGT probe was deployed in each of the three test chamber replicates. In the field, three DGT probes were deployed directly in the sediment near the cages used for the *in situ* bioassay (height limitations prevented probe deployment within cages). DGT probes were deployed in two separate events, in the first and third week of the bioassays (laboratory deployment: day 4 and 16 for sediments S1 and S2 and day 2 and 16 for sediments S3 and S4; field deployment: day 7

and 21 for sediments S1 and S3 and day 2 and 16 for sediments S2 and S4). After 24 h deployment, probes were thoroughly rinsed with Milli-Q water, placed in acid-washed plastic bags and stored at 4°C until analysis.

Probes were disassembled and the Chelex-resin sliced at 1 and 3 cm above the SWI, and -1, -2 and -4 cm below the SWI. In laboratory chambers, the maximum depth achievable for DGT measurements was -3 cm, and when necessary, the slicing procedure was adjusted according to the available depth (-2 and -4 cm for S1 and S4, respectively - first deployment). Metal concentrations in resin slices were used to calculate DGT fluxes as previously described in Section 2.6. For quality assurance, laboratory and field blank probes were analysed and Cu, Mn, Ni and Pb concentrations in blank probes were estimated to contribute for an equivalent flux $<0.2 \mu\text{g/h/m}^2$, whereas cadmium and lead contributed for <0.005 and $<4 \mu\text{g/h/m}^2$, respectively. Significant zinc contamination was found and estimated to contribute for an equivalent flux of $63 \pm 40 \mu\text{g/h/m}^2$.

5.2.5 Data analysis

Statistical analyses were performed using the software R 3.1.2 ($\times 64$) and visually represented using Microsoft Excel 2010. Differences in metal bioaccumulation rates between organisms exposed to different treatments (S1, S2, S3, S4) and exposure conditions (laboratory and field) were investigated using analysis of covariance (ANCOVA) followed by Tukey's test. Data were tested for normality of residuals distribution (Shapiro-Wilk's test) and for homogeneity of variance (Levene test) prior to hypothesis testing. When the data did not follow a normal distribution, Kruskal-Wallis test followed by Wilcoxon–Mann–Whitney test were performed. Unless otherwise stated, $p = 0.05$ was the level of significance.

5.3 Results and discussion

5.3.1 Sediment chemical and physical properties

General physico-chemical characteristics (particle size, TOC, AVS) and total recoverable (TRM) and dilute acid-extractable (AEM) metal concentrations (Cd, Pb and Zn) in field-collected sediment are shown in Table 5.1.

Table 5.1 Physical and chemical properties of the reference and contaminated sediments

Sediment	Total recoverable metals (TRM), mg/kg					TOC %	Particles < 63 µm, %
	Fe	Mn	Cd	Pb	Zn		
S1 (reference)	12200±220	230±10	<1	16±1	95±3	1.42	31±2.7
S2	6100±170	250±3	1.21±0.05	33±1	840±20*	4.29	96±0.4
S3	14300±1130	230±1	5.26±0.04*	130±1*	2100±30*	1.66	43±0.4
S4	14100±410	200±10	3.2±0.2*	47.2±0.4	910±4*	1.63	48±0.4
Sediment	Dilute acid-extractable metals (AEM), mg/kg					AVS µmol/g	SEM-AVS µmol/g
	Fe	Mn	Cd	Pb	Zn		
S1 (reference)	6100±200	180±11	<1	8.9±0.4	58±5	<0.5	1
S2	1200±20	200±12	1.0±0.1	23±1	750±60*	<0.5	12
S3	4500±2700	190±12	4.5±0.4*	106±8*	1800±140*	6.6±1.0	22
S4	5100±210	140±3	2.6±0.6*	37±3	790±25*	1.7±0.9	11
SQGs			1.5	50	200		0

Distance from mine: S1 = 8 km (upstream), S2 = 9 km (downstream), S3 = 28 km (downstream), S4 = 45 km (downstream); 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 mean values with standard deviation (n=3). Concentrations for a greater range of metals and metalloids are provided in the Table A3.2 of Appendix 3. *Concentrations with an asterisk exceed the sediment quality guideline values (SQG) (ANZECC/ARMCANZ, 2000).

All sediments collected in sites located downstream from the mine site exhibited significant metal contamination, with sediment quality guideline (SQG) values exceeded by lead in sediment S3, cadmium in sediment S3 and S4, and zinc in sediment S2, S3 and S4 (Table 5.1). In the reference sediment S1, metal concentrations were well below the SQG values (Table A3.2 of Appendix 3). The AEM/TRM ratios were between 0.81-0.85, 0.70-0.80 and 0.87-0.89 in contaminated sediments (S2, S3, S4) for Cd, Pb and Zn, respectively, whereas lower ratios were measured in the reference site S1 (0.74, 0.55 and 0.61 and for Cd, Pb and Zn, respectively). This indicates that in the contaminated sediments, the majority of Cd, Pb and Zn was associated with weaker binding phases and potentially bioavailable. Zinc was the contaminant of major concern, with AE-Zn concentrations exceeding the SQGs by factors between 4 and 9, whereas the highest AE-Cd and AE-Pb concentrations were 3 and 2 times the respective SQGs, respectively.

Total organic carbon (TOC) concentrations were 1.42, 4.29, 1.66 and 1.63 % for site S1, S2, S3 and S4, respectively. Acid-volatile sulfide (AVS) concentrations of 1.7 ± 0.9 and 6.6 ± 1.0 $\mu\text{mol/g}$ were measured in sediment S4 and S3, respectively, whereas in sediments S1 and S2, concentrations were <0.5 $\mu\text{mol/g}$. These more labile sulfide phases strongly influence the metal speciation and bioavailability in sediments as they react with metals forming insoluble compounds. AVS and simultaneously-extractable metal (SEM) concentrations (SEM is equivalent to 1-M HCl AEM) are used by equilibrium partitioning approaches (EqP) to identify increased environmental risk when molar concentrations of SEM exceed AVS concentrations in the sediment, where organic carbon (OC) also modifies the bioavailability of excess SEM (Burgess et al. 2013). Based on calculations of SEM-AVS and (SEM-AVS)/fOC, all contaminated sediments (S2, S3 and S4) indicated potential risk of toxicity to benthic organisms (Table 5.1).

5.3.2 Metal profiles in sediments and overlying waters by DGT

Fe(II) and Mn(II) profiles in sediments and overlying waters are shown in Figure 5.1, with greater details provided in Figures 5.2 and 5.3. Increasing Fe and Mn DGT fluxes observed within 2 cm below the SWI indicated the presence of the suboxic transition zone of insoluble iron and manganese oxyhydroxides phases to soluble reduced forms (Fe(II) and Mn(II)) (Amato et al. 2014).

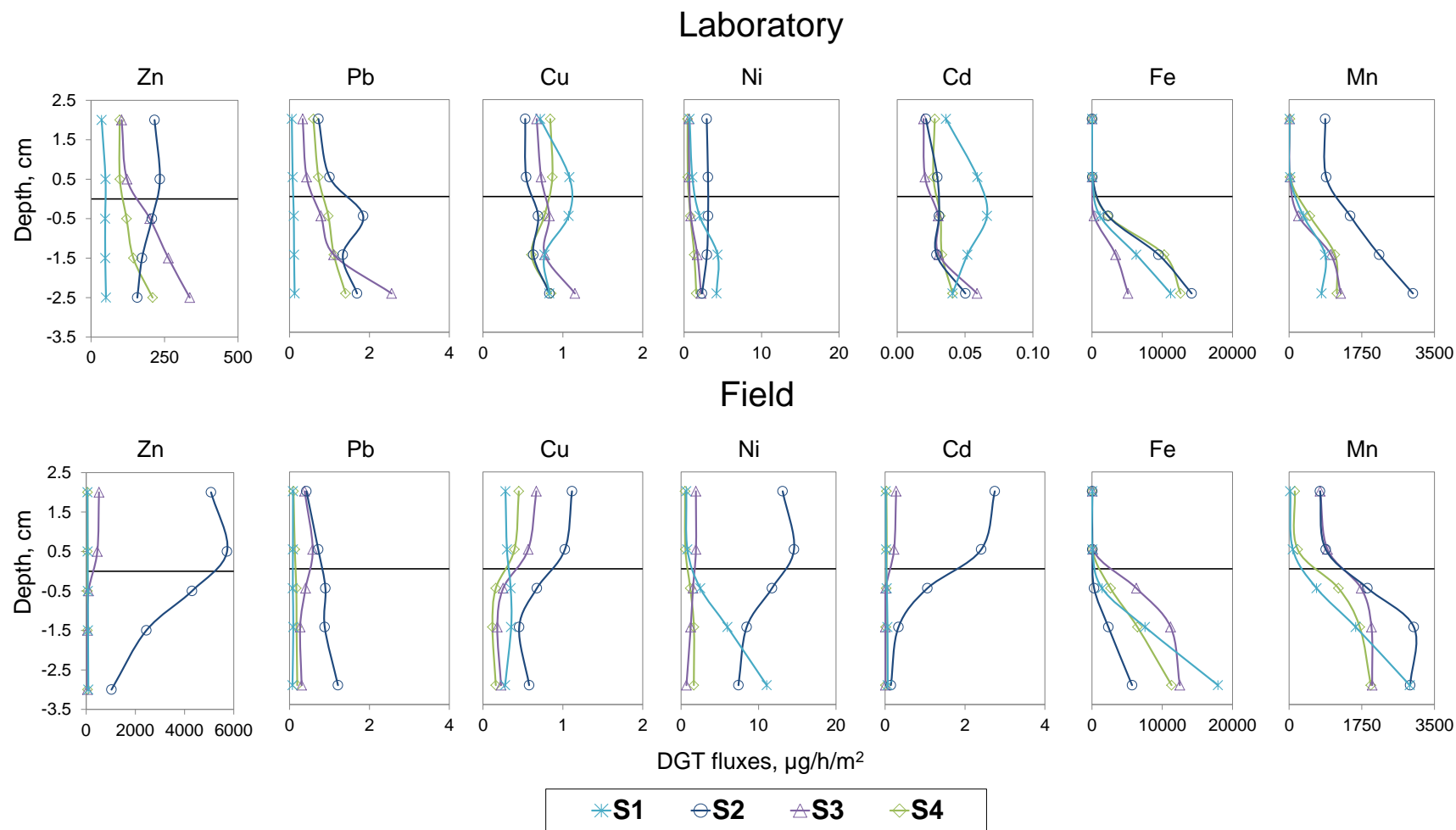


Figure 5.1 DGT profiles of Zn, Pb, Cu, Ni and Cd measured in laboratory and field sediments. Data points are average values of six replicates from the first and second deployments (standard deviations were ~25 and ~30% of mean values for laboratory and field deployments, respectively).

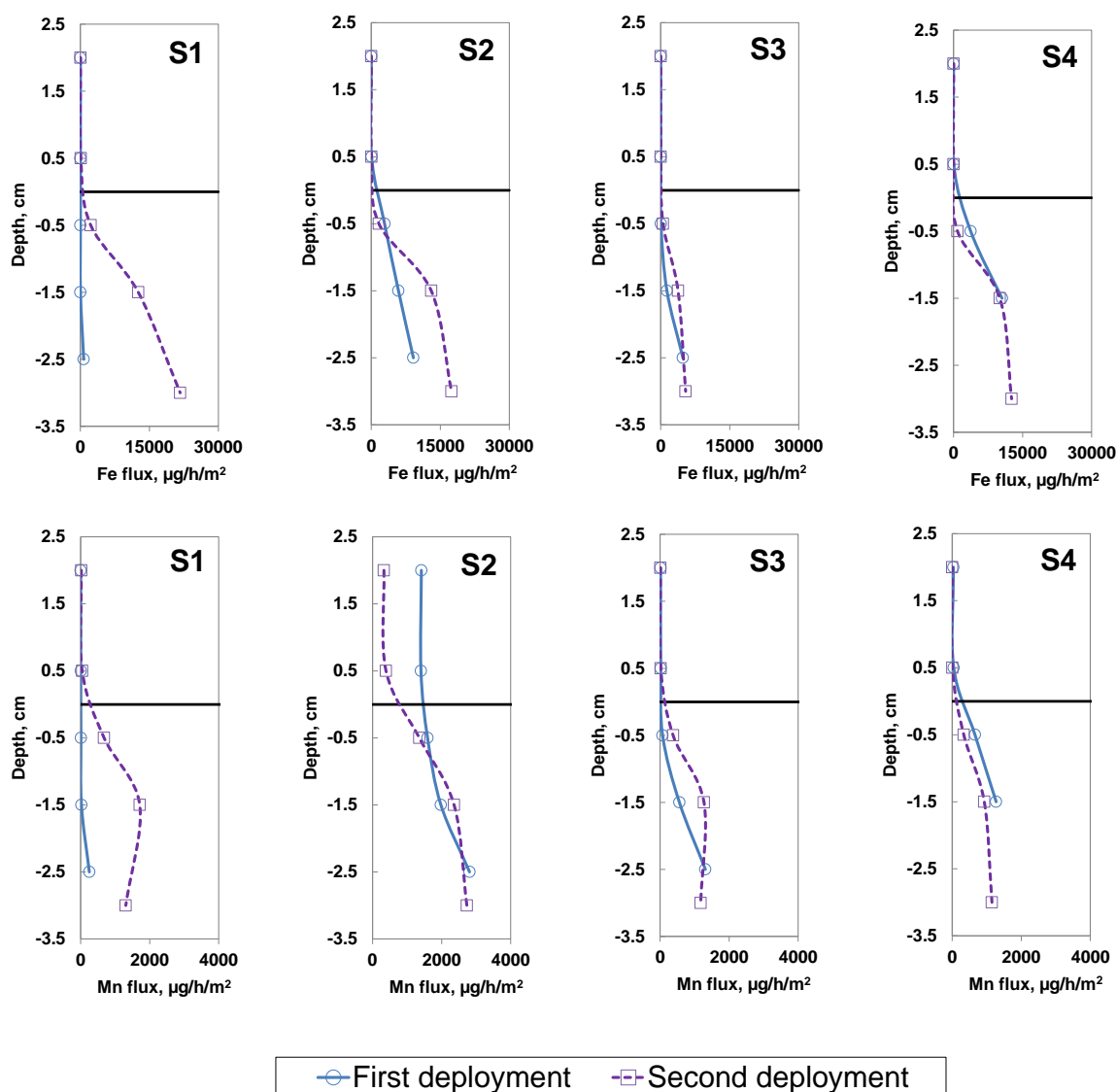


Figure 5.2 Comparison between DGT-Fe and -Mn vertical profiles measured in the first and second deployment in laboratory-exposed. Data points are average values of three replicates (standard deviations were ~35 and ~45% of mean values for Mn and Fe, respectively).

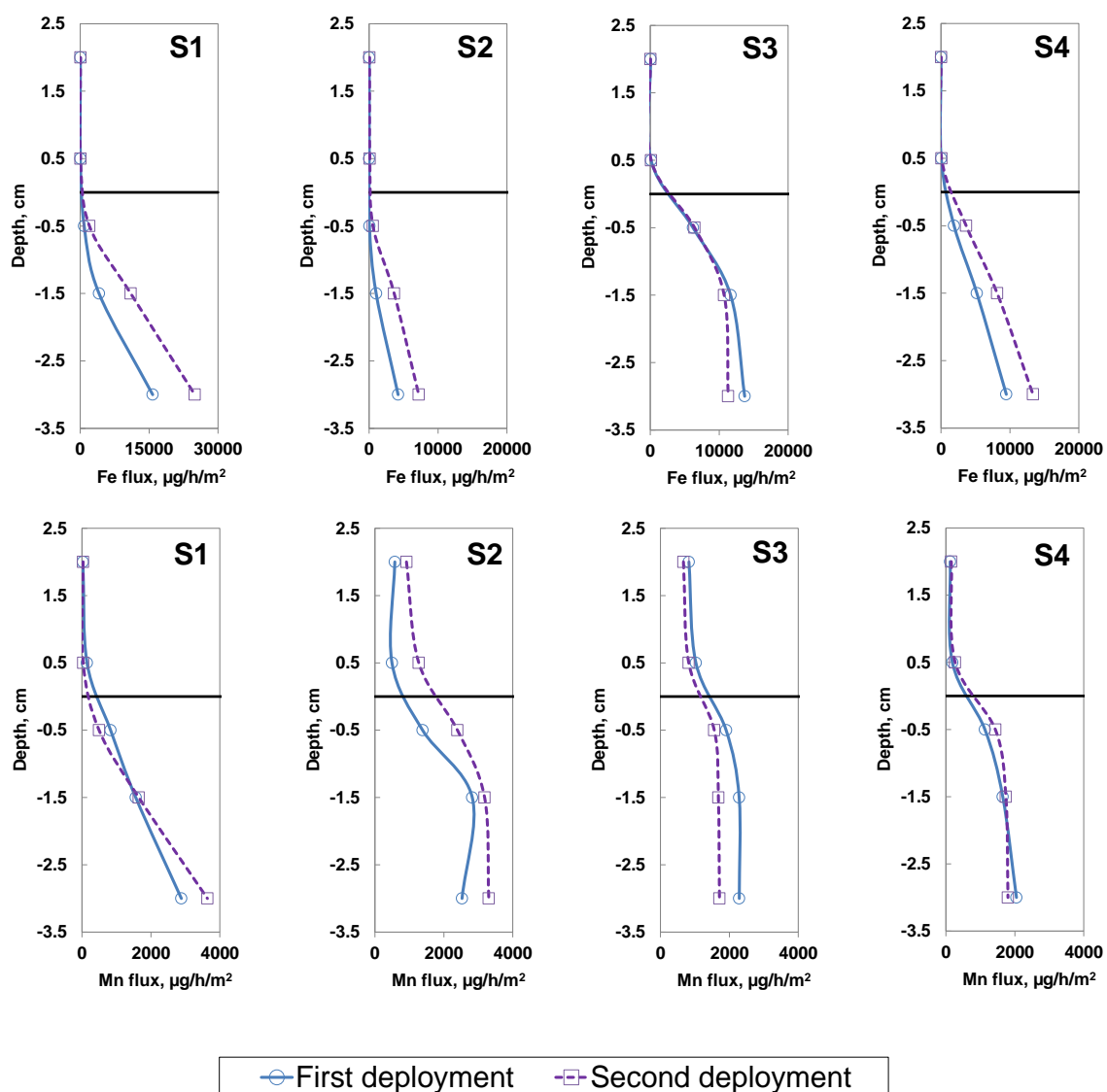


Figure 5.3 Comparison between DGT-Fe and -Mn vertical profiles measured in the first and second field deployments. Data points are average values of three replicates (standard deviations were ~35 and ~45% of mean values for Mn and Fe, respectively).

The mobilization of Cd, Cu, Ni, Pb and Zn in the zone -0.5 and -1.5 cm below the SWI (Figure 5.1) was largely attributed to the reductive dissolution of the iron and manganese solid phases (Naylor et al. 2006; Amato et al. 2014; Chapter 6). Metal release and DGT peaks in the sediment pore waters may also be attributed to degradation of organic matter (Petersen et al. 1995; Tankere-Muller et al. 2007) and oxidation of sulfides phases (Lesven et al. 2008; Naylor et al. 2012).

In laboratory-exposed sediments, similar iron and manganese fluxes were observed during the first and second DGT deployments, except for considerably lower fluxes measured in the first deployment in sediment S1 and moderate differences for the contaminated sediment S2 (Figure 5.2). Test sediments were placed in chambers two days prior to the beginning of the experiment, thus the greater DGT-Fe and -Mn fluxes observed for the second DGT deployment in S1 may be attributed to changes in redox vertical profile occurring as a result of sediment equilibration (Simpson and Batley 2003). In addition, due to the relatively shallow depth of the sediment (~ 5 cm), and the relatively large size of the bivalve (5.5 ± 0.5 cm), increased dissolved oxygen penetration into the pore water may have occurred as a result of bivalves burrowing activities (Aller 1994; Forster 1996; Simpson et al. 2002). In sediment S2, larger DGT-Mn in the overlying water (0 - 3 cm above the SWI) was measured in the first deployment (from 1410 ± 50 to 360 ± 30 $\mu\text{g/h/m}^2$ for 1st and 2nd deployment, respectively).

In field deployments, iron and manganese fluxes were quite similar between first and second deployment for all sediments (Figure 5.3), indicating that changes in sediment redox properties during the 28-day test may have been insignificant.

5.3.3 Differences in DGT-Cd, -Cu, -Ni, -Pb and -Zn fluxes between first and second deployment.

In the laboratory, DGT-Cd, -Cu, -Ni, -Pb and -Zn fluxes in the first deployment were larger than, but generally within a factor of 2, those measured in the second deployment (Figure 5.4). In the first deployment, DGT-Pb fluxes appeared to follow the general trend $S1 < S3 < S4 < S2$, whereas in the second deployment fluxes in contaminated sediments (S2, S3, S4) were lower and within a relatively narrow range. Larger DGT-Zn was measured during the first deployment for sediments S2, but only in the overlying water. DGT-Ni fluxes were similar for sediments S3 and S4, whereas larger fluxes were measured in the overlying water of sediment S2 in the first deployment, and

in the pore water of sediment S1 in the second deployment. DGT-Cd and -Cu were generally very low and similar between first and second deployments, except for slightly larger DGT-Cd and -Cu in the first deployment for sediment S1. However, cadmium and copper fluxes in the reference sediment S1 were within the range measured in control or low toxicity sediments in previous studies (Simpson et al. 2012b; Amato et al. 2014).

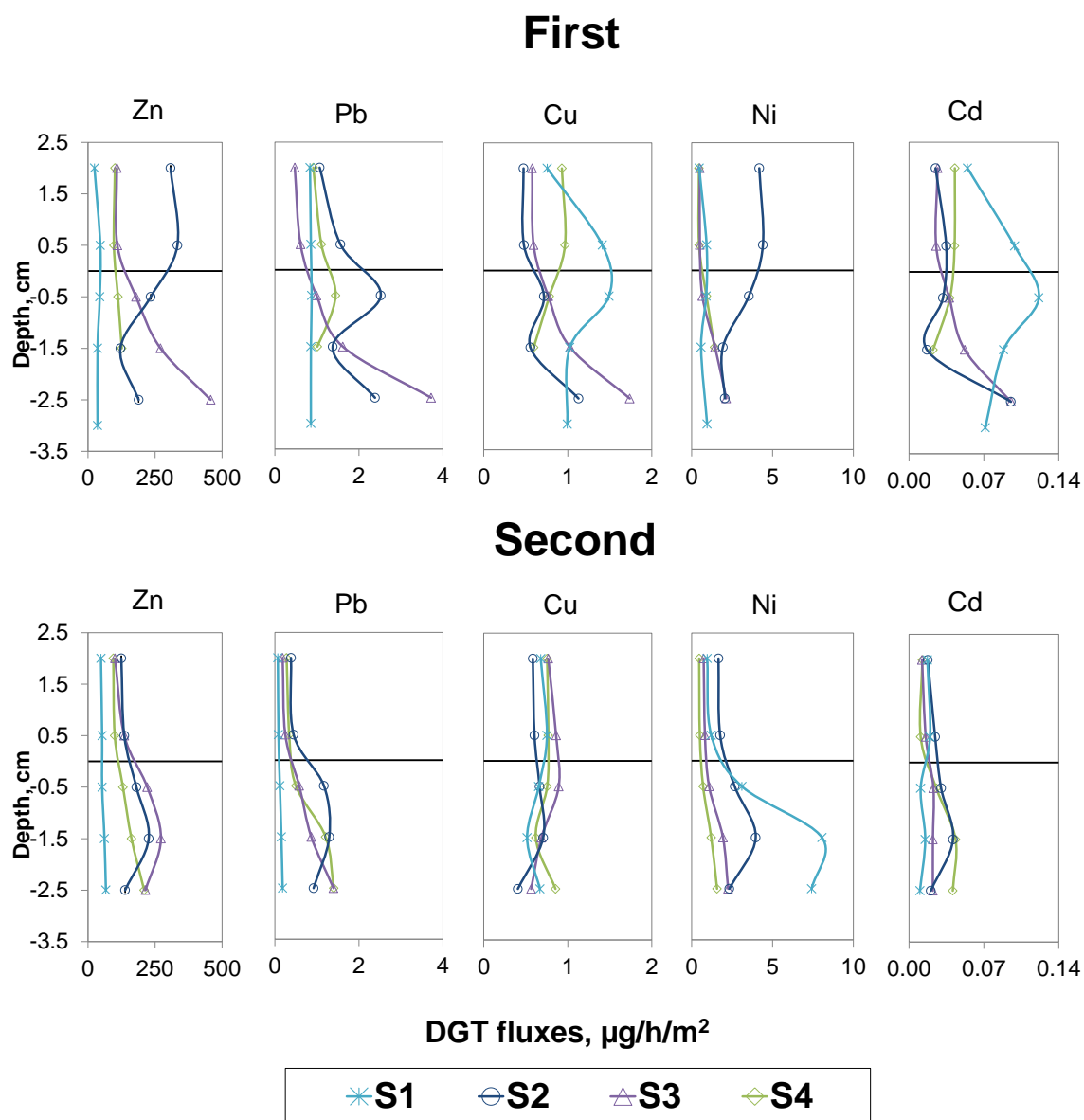


Figure 5.4 Comparison between DGT vertical profiles measured in the first and second deployment in laboratory-exposed sediments. Data points are average values of three replicates (standard deviations were ~25% mean values).

In field deployments, large differences in DGT-metal fluxes were observed between first and second deployment for site S2 and S3, whereas generally similar fluxes were measured for site S1 and S4 (Figure 5.5). Differences in DGT-metal fluxes between different sites also appeared to be more distinct during the second field deployment. In the second deployment, a general increase in DGT-metal fluxes for all metals (Cd, Cu, Ni, Pb, Zn) was observed in the two closest sites located downstream from the mine (S2 and S3). Copper, nickel and lead fluxes in the second deployment of sediment S2 were approximately twice as large as those measured in the first deployment, and cadmium and zinc fluxes were up to ten times larger.

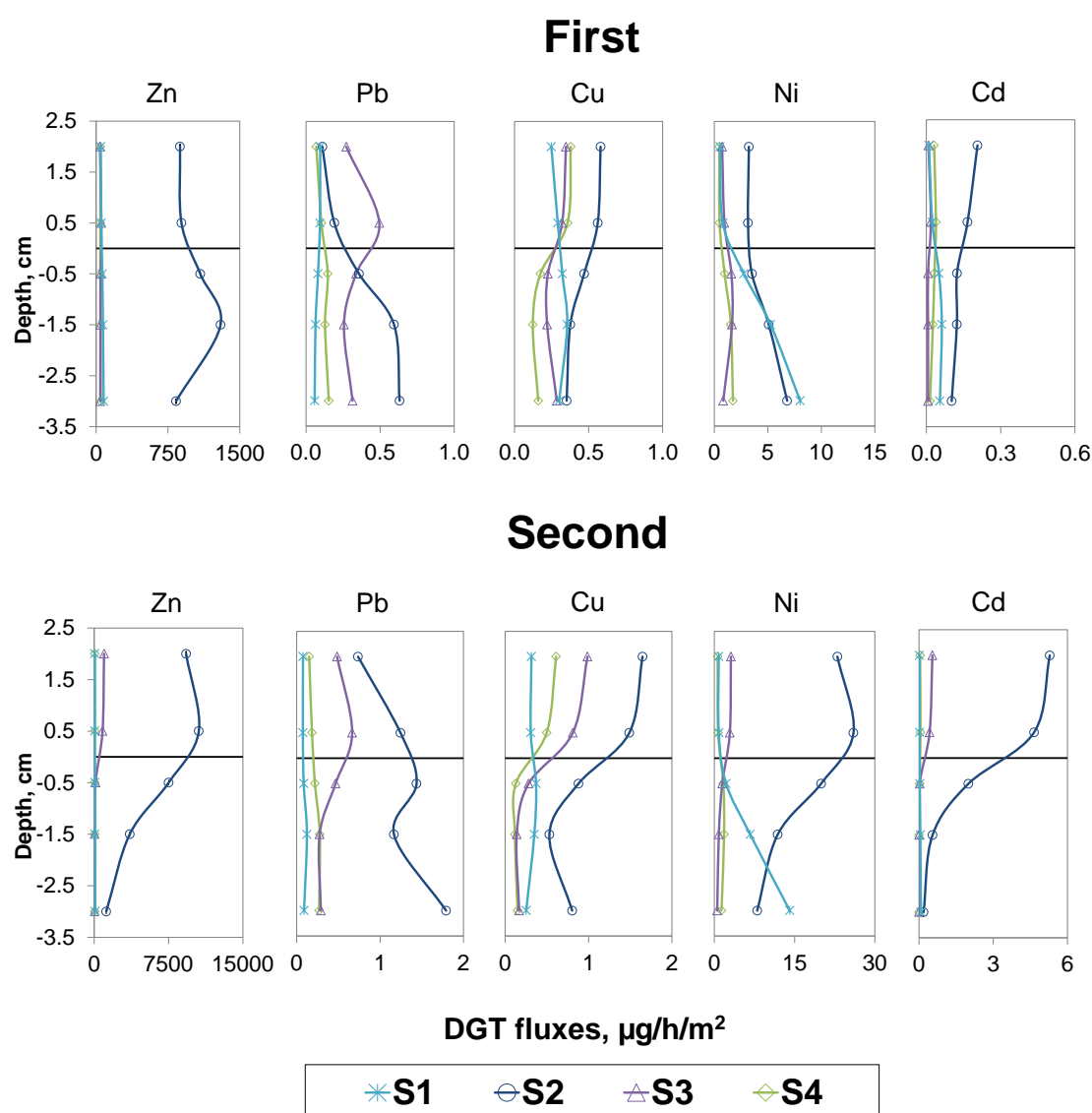


Figure 5.5 Comparison between DGT vertical profiles measured in the first and second field deployments. Data points are average values of three replicates (standard deviations were ~35% mean values).

A similar trend was observed in sediment S3, where DGT-Cu and -Pb fluxes in the second deployment exceeded those of the first deployment by a factor of ~2, and DGT-Cd, -Ni and -Zn by a factor of ~10. These large differences were attributed in part to significant rainfall events which likely caused increasing metal inputs in the river system as indicated by considerably larger DGT fluxes measured in the overlying water and in the pore water (between 0 and 4 cm below the SWI) during the second deployment. This was consistent with decreasing DGT-Cd and -Zn fluxes measured for increasing distances from the mine site, as contaminants concentrations in the river water are expected to be diluted with increasing distance from the source of contamination.

Overall, slightly larger DGT fluxes measured during the first laboratory deployment may indicate that greater metal release from the sediment occurred in the early stages of the test. However, given these differences were relatively small, they also indicate that the metal bioavailability in laboratory-exposed sediments was fairly similar at the time of the first and second DGT-deployment. In field deployments, the observed changes in Cd, Cu, Ni, Pb and Zn fluxes were mainly attributed to significant external inputs (Bierwirth and Pfitzner 2001; Sloane and Norris 2003).

5.3.4 Differences in DGT metal fluxes between laboratory and field deployments

The location within the sediment profiles and magnitude of the DGT-Fe and -Mn fluxes were similar for laboratory and field deployments for sediments S1 and S4, whereas sediment S3 showed larger iron and manganese fluxes in field deployments and sediment S2 larger iron fluxes in laboratory deployments (Figure 5.1 and Figure 5.6). In sediment S3, DGT-Fe fluxes in the pore waters of the field deployments were approximately 5 times larger than those of laboratory, whereas larger DGT-Mn fluxes were measured in both pore water (630 ± 370 and 1900 ± 440 $\mu\text{g}/\text{h}/\text{m}^2$, for laboratory and field deployments, respectively) and overlying water (16 ± 10 and 840 ± 100 $\mu\text{g}/\text{h}/\text{m}^2$, for laboratory and field deployments, respectively). In the laboratory, sediment disturbance by the relatively large bivalves may have facilitated the transport of the more highly oxygenated overlying water into the pore water (Forster 1996; Simpson and Batley 2003). As a consequence, pore water iron and manganese concentrations may have become lower due to precipitation of Fe oxyhydroxides occurring as a result of oxygen penetration in the sediment (see Chapter 6). However, a different trend was observed for

DGT-Fe in sediment S2, where significantly higher DGT-Fe fluxes were measured in the sediment pore waters under laboratory conditions (7600 ± 2600 and 2800 ± 2200 $\mu\text{g/h/m}^2$ for laboratory and field deployments, respectively).

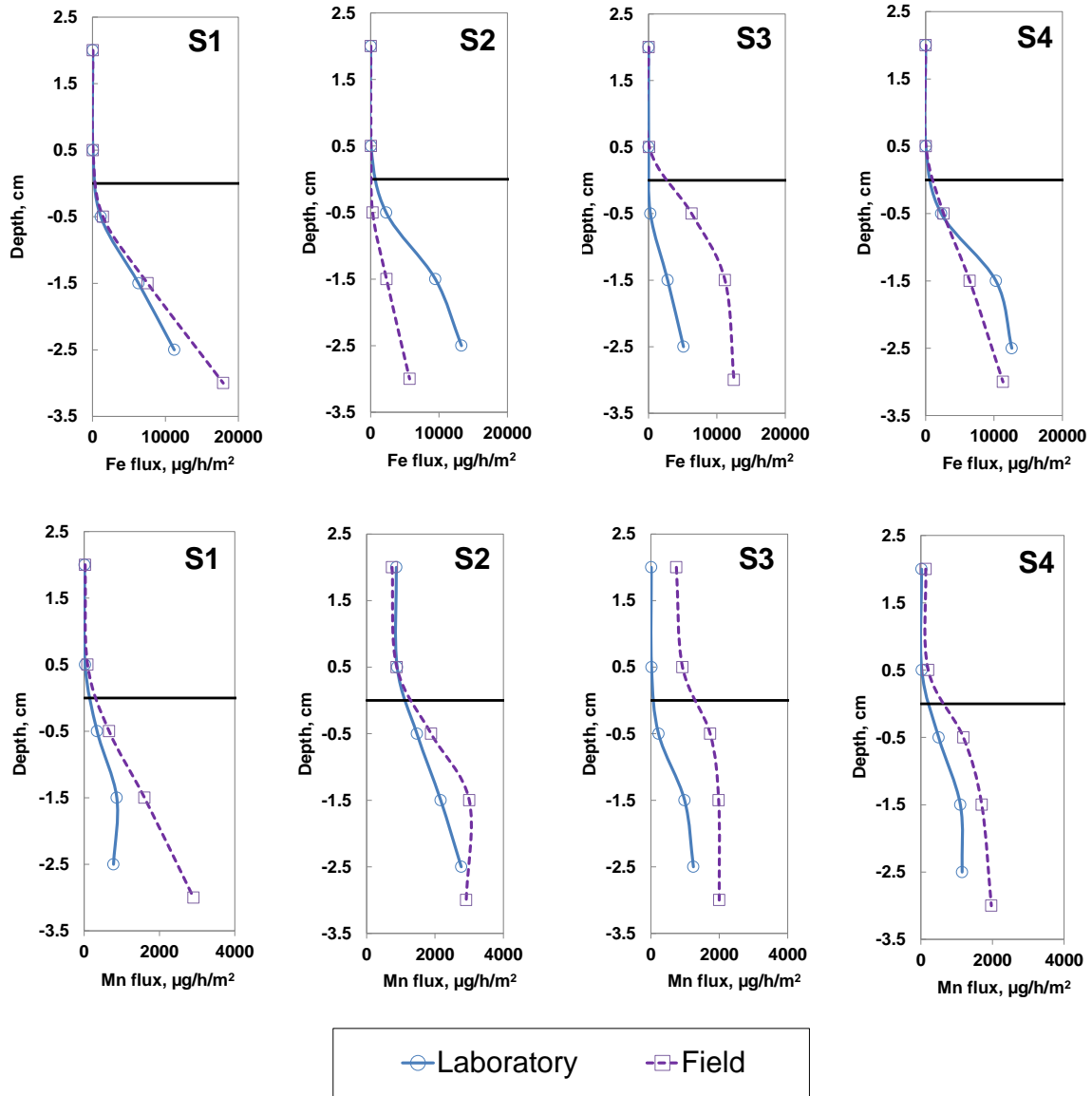


Figure 5.6 Comparison between DGT-Fe and -Mn vertical profiles measured in the laboratory and in the field. Data points are average values of six replicates (on average, standard deviations were ~25 and ~30% of mean values for laboratory and field deployments, respectively).

Lower DGT-Mn fluxes in the overlying water of laboratory deployments may be due to the higher dissolved oxygen concentrations that existed under laboratory conditions ($\approx 100\%$ and 48 ± 23 % saturation for laboratory and field, respectively), which facilitates

the oxidation of dissolved Mn(II) present in the water column and lowered DGT-Mn fluxes. The relatively high DGT-Mn fluxes in the overlying water for the field deployments in sediments S2 and S3 indicate that dissolved manganese persisted in the waters adjacent to the DGTs, i.e. was not diluted and flushed away by overlying water. In contrast, negligible DGT-labile iron was measured, consistently with the expected much faster rate of oxidation of iron than manganese (Stumm and Morgan 1996).

Despite the difference in particulate zinc concentrations (Table 5.1), similar DGT fluxes were measured in laboratory-exposed sediments. Conversely, considerable differences between sites were observed in the field, with a 10-fold increase in Zn fluxes between the first and second deployment for sediment S2 and S3. The DGT-Zn fluxes were larger in laboratory deployments for sediment S4 (48-53 and 97-209 $\mu\text{g}/\text{h}/\text{m}^2$ for laboratory and field deployments, respectively) and in field deployments for sediment S1 (36-51 and 53-87 $\mu\text{g}/\text{h}/\text{m}^2$ for laboratory and field deployments, respectively).

The DGT-Pb fluxes were generally lower in field deployments, except for sediment S2 which exhibited larger copper fluxes under field conditions (Figure 5.1). Little difference was observed in DGT-Cu fluxes between laboratory and field deployments. DGT-Ni fluxes were similar between laboratory and field deployments in sediment S3 and S4, but considerably larger in sediment S2 (and S1 in the pore water) in the field. DGT-Cd fluxes were also considerably larger in field deployments for sediment S2 (2.4 $\mu\text{g}/\text{h}/\text{m}^2$), whereas in the other sediments fluxes were $<0.3 \mu\text{g}/\text{h}/\text{m}^2$.

Overall, little differences were observed between laboratory and field deployments in low contaminated sediments (S1 and S4), whereas considerably larger fluxes were observed for Cd, Ni and Zn in the two sites located the nearest to the mine site (S2 and S3).

5.3.5 Metal bioaccumulation by the bivalve

Differences in metal bioaccumulation between *H. australis* exposed to reference (S1) and contaminated (S2, S3, S4) sediments, under laboratory and field conditions, are shown in Figure 5.7. In the laboratory, cadmium bioaccumulation in bivalves exposed to contaminated sediments (S2, S3, S4) exceeded that of organisms exposed to the reference sediment (S1) ($p < 0.05$). This was consistent with the higher particulate cadmium concentrations found in contaminated sediments (Table 5.1). Under field

conditions, only bivalves exposed to the contaminated site S2 exhibited higher cadmium bioaccumulation than organisms exposed to the reference site (S1) ($p < 0.001$), indicating that differences in cadmium bioaccumulation in the field were not linked to differences in particulate metal concentrations. When comparing cadmium tissue concentrations of bivalves exposed to laboratory and field conditions, bioaccumulation of bivalves deployed in site S2 was considerably greater than those of bivalves exposed to the same sediment under laboratory conditions ($p < 0.001$), whereas lower cadmium bioaccumulation rates were observed in field deployed bivalves for site S3 and S4 ($p < 0.01$). These differences indicate that significant difference in cadmium bioavailability occurred between laboratory and field exposure.

No significant differences ($p > 0.05$) were detected between bivalves exposed to different treatments (S1, S2, S3, S4) and exposures (laboratory and field) for copper and lead. This was consistent with the relatively low particulate copper and lead concentrations found in these sediments (Table 5.1 and Table A3.2 of Appendix 3), and suggested that differences between laboratory and field conditions did not affect metal bioaccumulation in *H. australis*.

Despite the considerably different TR-Zn and AE-Zn concentrations found in reference and contaminated sediments (Table 5.1), no significant differences ($p > 0.05$) in bioaccumulation were observed between bivalves exposed to these sediments under laboratory conditions. AEM/TRM ratios between 0.87-0.89 were measured in contaminated sediments, suggesting that the majority of sediment-bound zinc was associated with phases that may be potentially bioavailable. The similar level of bioaccumulation found in the bivalves suggests that zinc tissue concentrations in *H. australis* did not reflect the TR-Zn and AE-Zn concentrations in the sediments when exposed to laboratory conditions.

When comparing bioaccumulation in bivalves exposed to laboratory and field conditions, significantly higher zinc tissue concentrations were detected for bivalves exposed to S2 and S3 ($p < 0.001$), but not S1 and S4. In addition, higher zinc bioaccumulation was observed in field deployed bivalves exposed to sediment S2 and S3 suggesting that significant differences occurred between laboratory and field exposure. However, this was not the case for bivalves exposed to S1 and S4 suggesting that exposure to laboratory and field conditions did not affect zinc bioaccumulation from these sediments.

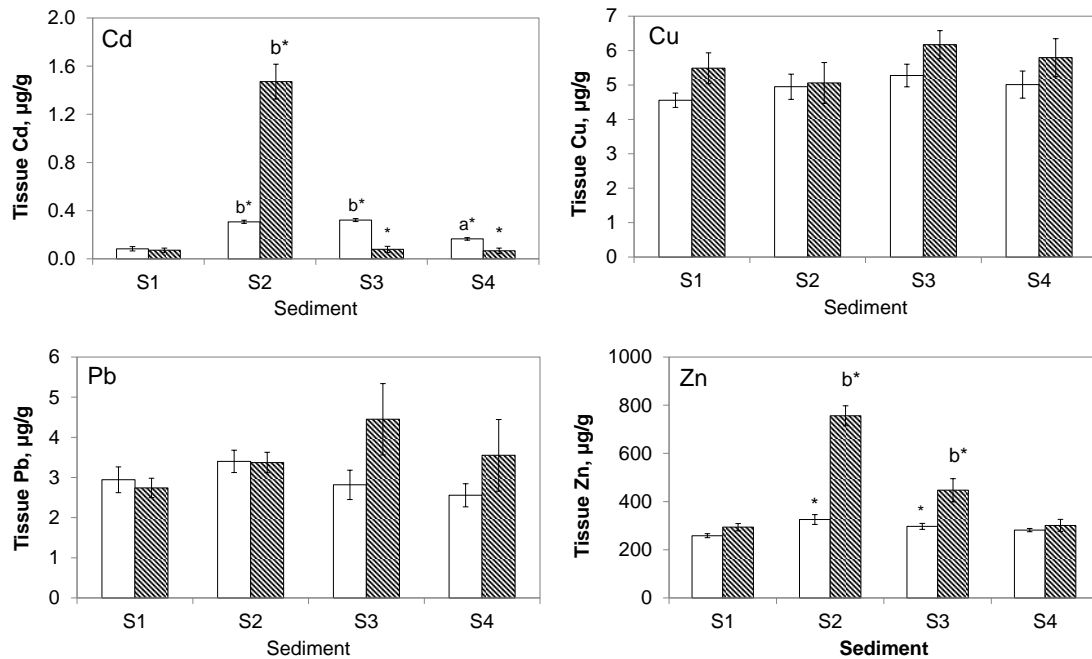


Figure 5.7 Differences in metal bioaccumulation in *H. australis* exposed to laboratory (empty bars) and field conditions (full bars) (mean \pm standard error, $n=5$). Letters a ($p<0.01$) and b ($p<0.001$) indicate significant differences between reference (upstream, S1) and contaminated sediments (downstream, S2, S3, S4), respectively, and asterisks indicate statistical difference between laboratory and field exposure ($p<0.05$).

5.3.6 Comparison between predictions of bioaccumulation based on DGT, TRM and AEM measurements

In general, DGT-metal fluxes (average of first and second deployments) were consistent with bioaccumulation in *H. australis* exposed to different treatments (S1, S2, S3, S4) and test conditions (laboratory and field). In the laboratory, similar DGT-Cu and -Pb fluxes measured in reference (S1) and contaminated (S2, S3, S4) sediments were consistent with similar copper and lead bioaccumulation in bivalves exposed to these sediments (Figure 5.1, 5.7). In the field, the considerably larger DGT-Cd fluxes in sediment S2, and DGT-Zn fluxes in sediment S2 and S3, were consistent with significantly higher cadmium and zinc concentrations in the bivalves (Figure 5.1, 5.7). For sediments S1 and S4, DGT fluxes in laboratory and field deployments were low (<0.1 , <1.5 , <2 and <200 $\mu\text{g}/\text{h}/\text{m}^2$ for Cu, Cd, Pb and Zn, respectively) and within the range of fluxes measured in sediments shown to cause low toxicity to the amphipod *Melita plumulosa* and low bioaccumulation to the bivalve *Tellina deltoidalis* (Figure 5.1) (Amato et al. 2014; Chapter 6).

In order to better evaluate relationships between DGT-metal fluxes and bioaccumulation, considerations on different metal flux exposures should be made. Previous studies have shown that DGT fluxes measured at the SWI were useful for predicting toxicity to the amphipod *M. plumulosa* (Amato et al. 2014) and the bivalve *T. deltoidalis* (Simpson et al. 2012b). *H. australis* is a filter-feeding bivalve which has been shown to accumulate metals in response to exposure to contaminated sediments (Wadige et al. 2014a; Wadige et al. 2014b; Wadige et al. 2014c). Although exposure pathways for metal uptake have not been directly investigated for this species, previous studies indicate that exposure to the dissolved phase may be a major route of uptake for *H. australis* (Wadige et al. 2014a; Wadige et al. 2014b). Given that the bivalve is exposed to metals present in the pore waters and overlying waters, relationships between bioaccumulation and DGT fluxes were investigated using fluxes measured in (i) the water column (between 0 and 3 cm above the SWI), (ii) the pore water (between 0 and -3 or -4 cm below the SWI) and (iii) at the SWI (± 1 cm) (Figure 5.8).

Strong correlations were found between bioaccumulation and DGT-Zn fluxes measured in the overlying water ($R^2=0.936$) and at the SWI ($R^2=0.914$), whereas weaker correlations were obtained using pore water fluxes ($R^2=0.673$) (Figure 5.8). Cadmium bioaccumulation and DGT-Cd fluxes were relatively low for all treatments expect for bivalves exposed to site S2 in the field (Figure 5.1, 5.7). The strong correlation found using fluxes measured in the overlying water suggests that exposure to the dissolved phase was a major contributor for the observed bioaccumulation, consistently with previous studies indicating significantly higher cadmium and zinc bioaccumulation in the gills compared to other tissues (Wadige et al. 2014a; Wadige et al. 2014b). The relationships between zinc and cadmium bioaccumulation in bivalves exposed to laboratory and field conditions and TRM ($R^2<0.23$) and AEM ($R^2<0.30$) concentrations measured in the bulk sediment are shown in Figure 5.9. These comparisons were made under the assumption that TRM and AEM concentrations did not vary between laboratory and field sediments. While TRM concentrations were not expected to change, AEM between the laboratory and field experiment may differ due to alteration of sediment chemical properties caused by sediment collection and homogenization (Simpson and Batley 2003). Poor relationships were found between TR-Zn and bioaccumulation in bivalves exposed to laboratory conditions ($R^2=0.211$) (Figure 5.9).

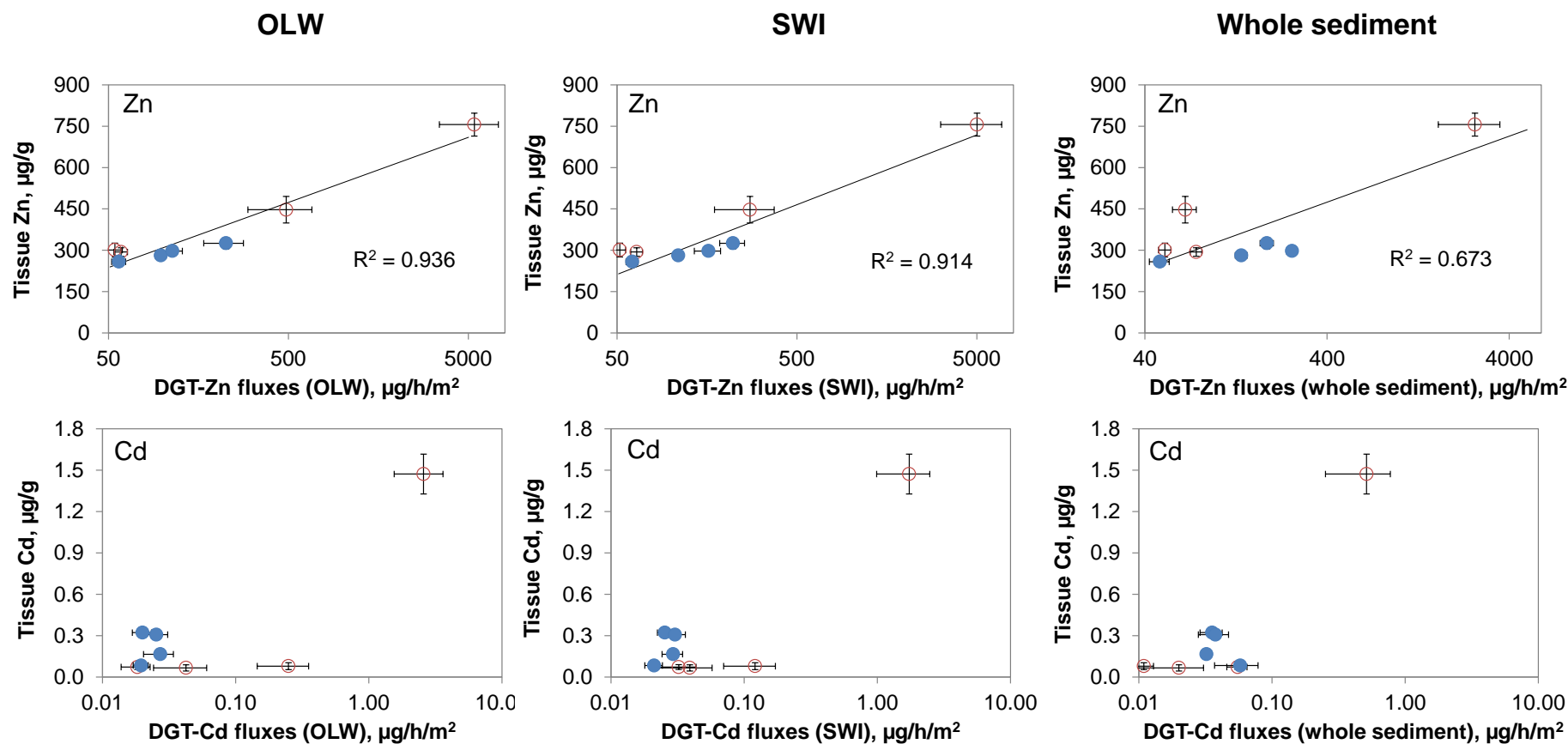


Figure 5.8 Relationships between bioaccumulation and DGT-Cd and -Zn fluxes measured (i) in the overlying water (between 0 and 3 cm above the SWI), (ii) at the SWI (± 1 cm), and (iii) in the pore water (between 0 and -2.5 (laboratory) and between 0 and -3 (field) cm below the SWI). Full and empty circles refer to laboratory and field deployments, respectively. Data points are average values with standard error ($n=15$ and 6 for tissue concentrations and DGT fluxes, respectively).

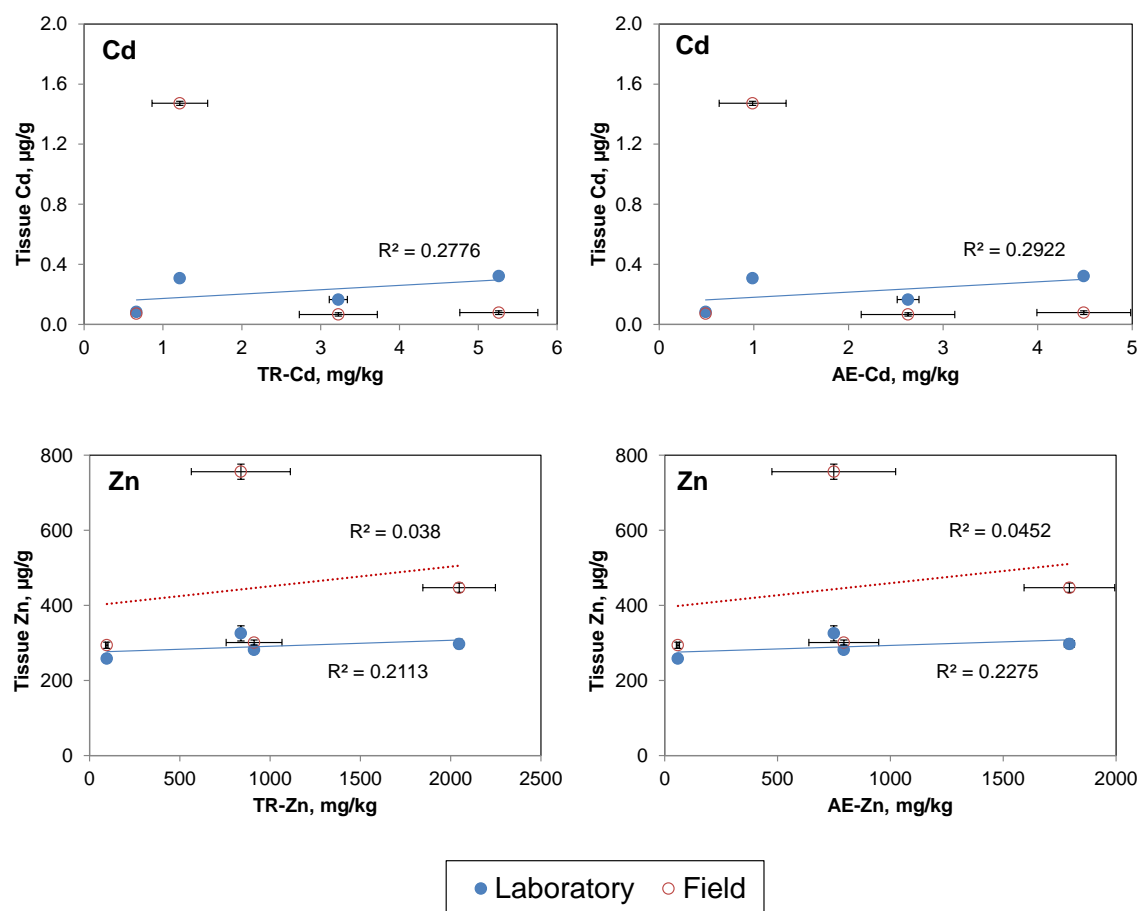


Figure 5.9 Relationships between bioaccumulation in laboratory and field exposed bivalves and particulate metal concentrations (TRM and AEM). Data points are average values with standard error (n=15 and 2 for tissue and particulate metal concentrations, respectively).

AEM concentrations (1 M HCl extractable metals) are often considered better predictors of bioavailability than TRM concentrations as they do not consider the more inert fraction of metals that are only released in concentrated acids. However, poor relationships were obtained when using AE-Zn ($R^2=0.228$). Weak relationships were also observed for cadmium for both TR-Cd and AE-Cd (Figure 5.9), although this may be attributed in part to the relatively low range of cadmium concentrations present in the four sediments. Similarly, poor predictions of cadmium and zinc bioaccumulation were provided for bivalves exposed to field conditions when using TRM and AEM concentrations (Figure 5.9).

When comparing DGT and AEM predictions of zinc bioaccumulation in bivalves exposed to laboratory conditions, the strongest relationships were obtained using zinc fluxes ($R^2=0.927$, 0.993 and 0.531 for DGT fluxes measured in the overlying water, at the SWI and in the pore water below the SWI, respectively, Figure 5.10), but similar weak correlations were found for DGT-Cd (Figure 5.8). TR-Pb and AE-Pb concentrations in sediment S3 were more than two times higher than those measured in sediments S2 and S4 (Table 5.1), but no significantly different lead bioaccumulation was observed between bivalves exposed to these sediments (Figure 5.7).

Overall, this study indicates that differences between laboratory-based and *in situ* (field-based) bioassays may result in significantly different predictions of risk due to varying environmental conditions typical of dynamic systems such as rivers and estuaries. Strong relationships between DGT-Zn fluxes and bioaccumulation were found irrespective of the different chemical and physical properties of the sediments (Table 5.1) and type of exposure (laboratory and field). Based on DGT fluxes measured at the SWI (laboratory and field), the lowest zinc flux causing significantly higher bioaccumulation (compared to that of bivalves exposed to the reference sediments) was $270 \mu\text{g/h/m}^2$, whereas the highest zinc flux measured in treatments causing no significantly different bioaccumulation was $220 \mu\text{g/h/m}^2$. These fluxes may act as possible threshold values above which the risk of zinc bioaccumulation in this species may be expected to increase. For cadmium, significantly higher bioaccumulation was observed only in bivalves exposed to sediment S2 in the field, consistently with the highest DGT-Cd flux measured in this sediment ($1.7 \mu\text{g/h/m}^2$), whereas the highest cadmium flux causing no significantly different bioaccumulation was $0.1 \mu\text{g/h/m}^2$.

In Section 3.3.5, DGT fluxes were normalised using water quality guidelines (WQG) in an attempt to account for the different toxicity expected to be caused by different metals. Although toxicity effects were not assessed in this study, and not applicable when assessing bioaccumulation data, the calculations were made in order to compare between the two studies. For zinc, significant bioaccumulation was observed when normalised fluxes exceeded $18 \mu\text{g}_{\text{WQG}}/\text{h/m}^2$, whereas no significantly different bioaccumulation was observed for normalised fluxes $<15 \mu\text{g}_{\text{WQG}}/\text{h/m}^2$. Similar calculations were not attempted for other metals due to the weak relationships with bioaccumulation data. The value of $18 \mu\text{g}_{\text{WQG}}/\text{h/m}^2$ for zinc bioaccumulation can be

compared to a thresholds for effects to survival and reproduction of the amphipod, *M. plumulosa* of 36 and 17 $\mu\text{g}_{\text{WQG}}/\text{h}/\text{m}^2$, respectively (from multiple metals).

The TRM and AEM concentrations provided poor relationships with cadmium and zinc bioaccumulation, but the performance of these methods could not be assessed in the field. These results suggest that the DGT technique has the potential to be used as a monitoring tool for bioavailable metals in sediments and improve the sediment risk assessment by providing an additional line of evidence based on currently missing *in situ* assessments.

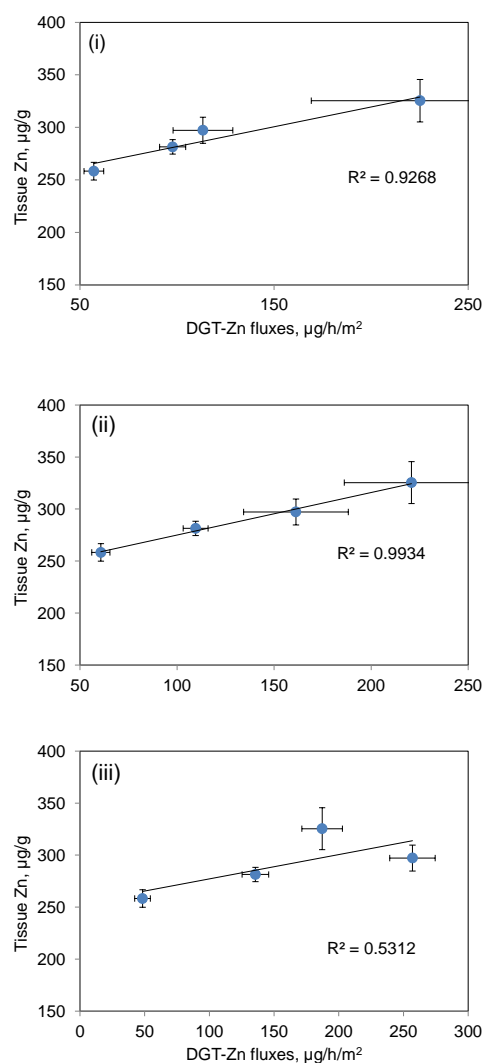


Figure 5.10 Relationships between zinc bioaccumulation in bivalves exposed to laboratory conditions and DGT metal fluxes measured (i) in the overlying water (between 0 and 3 cm above the SWI), (ii) at the SWI (± 1 cm), and (iii) in the pore water (between 0 and -2.5 cm below the SWI). Data points are average values with standard error (n=15 and 6 for tissue concentrations and DGT fluxes, respectively).

Chapter 6: Assessing the effects of bioturbation to metal bioavailability in contaminated sediments by diffusive gradients in thin films (DGT)

The research presented in this Chapter is the result of a collaborative work conducted with Mr. Timothy M. Remaili, a newly commenced PhD student at Wollongong University. Timothy and I jointly contributed to the development of the initial concepts and design of this study. I led the DGT preparation, deployments, analyses and interpretation and contributed to field work activities. Timothy participated to field work activities, performed sediment and water chemical and physical analyses and organisms tissue analyses under my supervision. This project resulted in the production of two companion manuscripts, one based on the results presented in this Chapter, which focuses on the evaluation of the DGT performance as a monitoring tool for bioavailable metals, and a second manuscript based on non-DGT aspects of the greater study, where I contributed as a co-author.

This Chapter has been prepared for publication:

Amato, E. D.; Remaili, T.; Simpson, S. L.; Jolley, D. F. (in preparation 2015) Diffusive gradients in thin films predict bioturbation induced changes in metal bioavailability and bioaccumulation rates in organisms exposed to contaminated sediments. *Environmental Science and Technology*.

The accompanying manuscript (which I co-authored) is being prepared for publication:

Remaili, T.M., Amato, E.D., Spadaro, D.A., Jarolimek, C.V., Jolley, D.F. Simpson, S.L. (in preparation 2015). The impact of single and multiple sources of bioturbation on the assessment of metal exposure and bioaccumulation by benthic organisms. *Environmental Pollution*.

6.1 Introduction

Metal bioavailability is strongly linked to metal speciation, which in sediments is regulated by biogeochemical processes. Burrowing and feeding activities of benthic organisms may alter the speciation of metals in sediments by mixing and transporting fluids and sediment particles (Aller 1994; Fenchel 1996; Teal et al. 2009). The introduction of oxygenated water into sub-oxic to anoxic regions of the sediment may cause oxidation of dissolved Fe(II) and Mn(II) to form iron and manganese oxyhydroxide solid phases, and also oxidation of labile sulfide phases such as acid-volatile sulfide (AVS) (Peterson et al. 1996; Lagauzère et al. 2009; Simpson et al. 2012a). As these processes modify the partitioning of metals between dissolved and particulate phases and the fluxes of metals to the porewaters, they also modify the exposure and bioavailability of metals to the organisms in the sediments and overlying waters (Atkinson et al. 2007; Lopez et al. 2014; Schaller 2014).

As discussed in previous chapters, diffusive gradients in thin films (DGT) is an *in situ* technique which measures fluxes of metals present in the sediment pore water as well as weakly-bound metals that dissociate from solid phases (Zhang et al. 1995). In previous studies we demonstrated that DGT metal fluxes can be used for predicting toxicity to the amphipod *Melita plumulosa* (Amato et al. 2014) and the bivalve *Tellina deltoidalis* (Simpson et al. 2012b) exposed to metal contaminated marine sediments under laboratory conditions. For nickel-spiked freshwater sediments, Costello et al. (2012) found that DGT-nickel concentrations in surface sediments were less useful for predicting effects to macroinvertebrate abundance or diversity than the potentially simpler measurements, for example, dilute acid-extractable nickel (SEM-Ni) or (SEM-AVS)/fOC. Many studies have observed useful relationships between DGT measurements and bioaccumulation: Roulier et al. (2008) for copper and lead in the freshwater chironomid *Chironomus riparius*; van der Geest and Paumen (2008) for copper in the freshwater worm *Tubifex*; Dabrin et al. (2012) cadmium accumulation in the freshwater mud snail *Potamopyrgus antipodarum*; and, Chapter 4 and 5 for Cu, Pb and Zn and *T. deltoidalis* exposed to contaminated marine sediments under laboratory and field conditions.

The majority of sediment toxicity and bioaccumulation studies are undertaken by exposing single species of organisms to contaminants in sediments. However, in

most sediment environments a range of organisms coexist with differing behaviours and sensitivities to contaminants. The exposure and the risk posed by the contaminants to the organisms in natural environments may therefore be significantly influenced by the interactions between species and the behaviours that modify the bioavailability of contaminants in sediments (Simpson and Batley 2003; Atkinson et al. 2007). For sediment quality assessments that rely heavily on laboratory based bioassays to assess potential impacts of contaminants to ecosystem health, the failure to consider inter-organism interactions may result in inaccurate assessment outcomes.

DGT sediment probes that are deployed through the sediment-water interface (SWI) can provide information on metal speciation in different compartments of the sediment (oxic, sub-oxic, anoxic), and potentially provide useful information on changes in metal bioavailability due to biological disturbance. In this study we investigate the use of DGT fluxes for assessing the exposure and predicting the bioaccumulation of metals in contaminated sediments as they are bioturbated by different organisms. Three sediments displaying different levels of contamination and physical properties were left undisturbed (zero bioturbation), or exposed to bioturbation by either the bivalves *T. deltoidalis* alone (low bioturbation) or combined with the amphipod *Victoriopisa australiensis* (high bioturbation). In a companion paper by Remaili et al. (submitted), the bivalve was shown to cause little disturbance to the sediments, whereas the amphipod caused high levels of bioturbation. The DGT-metal fluxes (Cd, Cu, Fe, Mn, Ni, Pb, Zn) and major bioaccumulated metals (Cu, Pb and Zn) were used to assess the degree to which the high bioturbation by the amphipod influenced the metal bioaccumulation by the bivalve, and the benefits gained from using DGT flux measurements as an assessment tool, compared to other more traditional approaches such as total and dilute acid-extractable metal concentrations, and AVS-SEM (Simpson and Batley 2007; Burgess et al. 2013).

6.2 Materials and methods

6.2.1 General methods

All plastic-ware used for analyses was new and cleaned following the procedures described in Section 2.1. All chemicals were analytical reagent grade or equivalent analytical purity. Filtered aliquots (0.45 µm) sampled from overlying waters (OLW)

were combined between replicates and used for dissolved metal analysis. Sediment subsamples were collected before transfer into test vessels. At the end of the experiment, additional sediment mini-cores ($\varnothing=1$ cm, 4 cm deep) were extracted from each test vessel and immediately transferred in a freezer and stored frozen at -20°C until analysis. The extrusion of sediment mini-cores (top and bottom 1.3 ± 0.2 cm) and preparation of sediment for acid-volatile sulfide (AVS) analyses were undertaken in a nitrogen gas-filled glove box. Total recoverable metal (TRM), dilute acid-extractable metal (AEM), AVS and physical analyses were performed as previously described (Section 2.4). Total organic carbon (TOC) was determined by high temperature CO_2 evolution method in a LECO furnace with infra-red (IR) detection after removal of inorganic carbonates by acidification. Metal concentrations in organism tissues were determined following the procedures described in Section 2.4.

6.2.2 Test media and organisms

Clean seawater was collected from Cronulla (Sydney, Australia), membrane filtered ($0.45\ \mu\text{m}$) and stored in a temperature-controlled room at $21\pm3^{\circ}\text{C}$. In each sediment sampling location, surface sediments were collected (0-15 cm depth) and sieved through a 2 mm plastic mesh in the field to remove coarse material (e.g. detritus and leaves) and minimize the presence of local fauna. The sediments were then homogenised by mixing with a plastic spoon and stored at 4°C in the dark (for a minimum of 2 weeks) before use. One relatively clean sediment (S1) was collected from Lake Illawarra (Wollongong, Australia) and two contaminated sediments (S2 and S3) were collected from Port Kembla (Wollongong) and Kings Bay (Sydney Harbour), respectively. The sediment from Port Kembla was diluted (1:1) using clean sediment collected from Bonnet Bay (Sydney) following the procedure described in Section 4.2.2 to achieve the desired level of contamination (S2). The amphipod *Victoriopisa australiensis* and the bivalve *Tellina deltoidalis* were collected and maintained as previously described (Section 2.3).

6.2.3 Bioaccumulation bioassay

The bioaccumulation bioassay was performed following the procedures described in Section 2.5.4, and metal concentrations in dry tissues were determined as described in Section 2.4.

6.2.4 Diffusive Gradients in Thin Films

Sediment probes were prepared following the procedures described in Section 2.6. For each of the treatments, one DGT probe was deployed in each of the 3 replicate test vessels used for the bioassay on day 8 and day 23 of the 28-day bioaccumulation test. Care was taken to assure that deployments were performed under the same conditions (i.e. 1 day after the previous overlying water renewal). After 24-h deployment, the probes were carefully retrieved from the sediment and the sediment-water interface (SWI) depth recorded. Probes were thoroughly rinsed with Milli-Q water, placed in clean plastic bags and stored at 4°C until analysed. Within 24/48-h of retrieval, DGT probes were disassembled and binding gels sliced using Teflon[®]-coated razor blades. The slicing procedure included two 0.5-cm slices above the SWI and two 0.5-cm slices followed by two 1-cm slices below the SWI (i.e. six slices per probe). Gel slices were analysed as described in Section 2.6. Blank probes were analysed for laboratory quality control and Cd, Cu, Ni and Pb concentrations were found to contribute an equivalent flux of $<0.3 \mu\text{g/h/m}^2$. Zinc contamination was consistently detected and estimated to contribute for $102 \pm 27 \mu\text{g/h/m}^2$, although fluxes as small as $\sim 50 \mu\text{g/h/m}^2$ were consistently measured in some DGT probes deployed in sediment S1 and S2 (Figure A4.5 of Appendix 4).

6.2.5 Data Analysis

Differences in metal fluxes and bioaccumulation rates between different exposures (zero, low and high bioturbation) were investigated using the software R 3.1.2 (×64). Unless otherwise stated, $p=0.05$ was the level of significance. Data were tested for homogeneity of variance (Levene's test) and for normality of residuals distribution (Shapiro-Wilk's test) prior to hypothesis testing and statistical differences between groups were assessed using one-way analysis of variance (ANOVA) followed by Tukey's test. When the data did not follow a normal distribution, Kruskal-Wallis' test was applied to evaluate statistical differences.

6.3 Results and discussion

6.3.1 Sediment chemical and physical properties

The chemical and physical properties of the control (S1) and contaminated (S2, S3) sediments are shown in Table 6.1. Concentrations for a greater range of metals and metalloids are available in the Appendix 4 (Table A4.1). Sediment S1 had total recoverable metal (TRM) concentrations well below the respective sediment quality guideline values (SQGs) (ANZECC/ARMCANZ, 2000; Simpson et al. 2013a) indicating that these metals were unlikely to cause toxicity to benthic organisms (Simpson and Spadaro 2011; Campana et al. 2013; Amato et al. 2014). Sediments S2 and S3 had TRM Cu, Pb and Zn concentrations exceeding SQG values of factors > 10. In sediment S2, AEM/TRM ratios of 0.44, 0.83 and 0.56 for Cu, Pb and Zn, respectively, indicated that a significant fraction of these metals was present in potentially bioavailable forms. Lower AEM/TRM ratios were measured in sediment S3 (<0.01, 0.29 and 0.33 for Cu, Pb and Zn, respectively), suggesting that a smaller fraction of these metals was potentially bioavailable. For copper, the very low AEM/TRM ratio in sediment S3 was expected as this sediment had a much higher AVS concentration than the other sediments (Table 6.1) and copper sulfide phases are poorly soluble in 1 M HCl (Cooper and Morse 1998). Sediments S1 and S3 were relatively sandy with low TOC (~30% <63 μ m, ~1% TOC), while sediment S2 was more silty with higher TOC (76% <63 μ m, 5.7% TOC).

6.3.2 DGT metal fluxes in sediment pore waters and overlying waters

DGT vertical profiles measured in sediments and overlying waters are shown in Figure 6.1 (average of first and second deployment; fluxes measured during the two deployments are shown separately in Figure A4.5, A4.6 and A4.7 of Appendix 4). Peaks in DGT-metal fluxes for Cd, Cu, Ni, Pb and Zn within 1 cm below the SWI were observed in most profiles. These peaks may be linked to increasing Mn and Fe fluxes measured at -0.5 cm and -1 cm depth, respectively, suggesting that metal release may occur as a result of dissolution of Mn and Fe oxyhydroxide phases (Naylor et al. 2004; Naylor et al. 2006; Amato et al. 2014). Other contributions to this metal release may be from degradation of organic matter (Furrer and Wehrli 1993) and oxidation of metal sulfide phases (Naylor et al. 2012; Simpson et al. 2012a).

Table 6.1 Physical and chemical properties and toxicity of the control and contaminated test sediments. All concentration are the mean of two analyses for each sediment, with variability between measurements of <20%

Sediment	Total recoverable metals (TRM), mg/kg							TOC, %	Particle size
	Fe	Mn	Cd	Cu	Ni	Pb	Zn	%	% <63 µm
S1	7600	53	<1	16	2	10	37	1.1	32
S2	42700	289	2*	544*	19	459*	912*	5.7	76
S3	36000	67	8*	1160*	36*	1360*	2830*	1.2	27
Sediment	Dilute acid-extractable metals (AEM = SEM), mg/kg							AVS	SEM-AVS
	Fe	Mn	Cd	Cu	Ni	Pb	Zn	µmol/g	µmol/g
S1	3000	28	<1	8	1	8	28	0.05	0.5
S2	11900	103	1	240*	6	379*	580*	0.2	513
S3	4450	25	2*	3	5	393*	802*	10	4.3
SQGs			1.5	65	21	50	200		0

TRM = total recoverable metals; AEM = dilute acid-extractable metals = SEM; AVS = acid-volatile sulfide; TOC = total organic carbon and % <63 µm refers to the percentage (by weight) of fine sediment particles. * Concentrations with an asterisk exceed the SQGs (ANZECC/ARMCANZ, 2000).

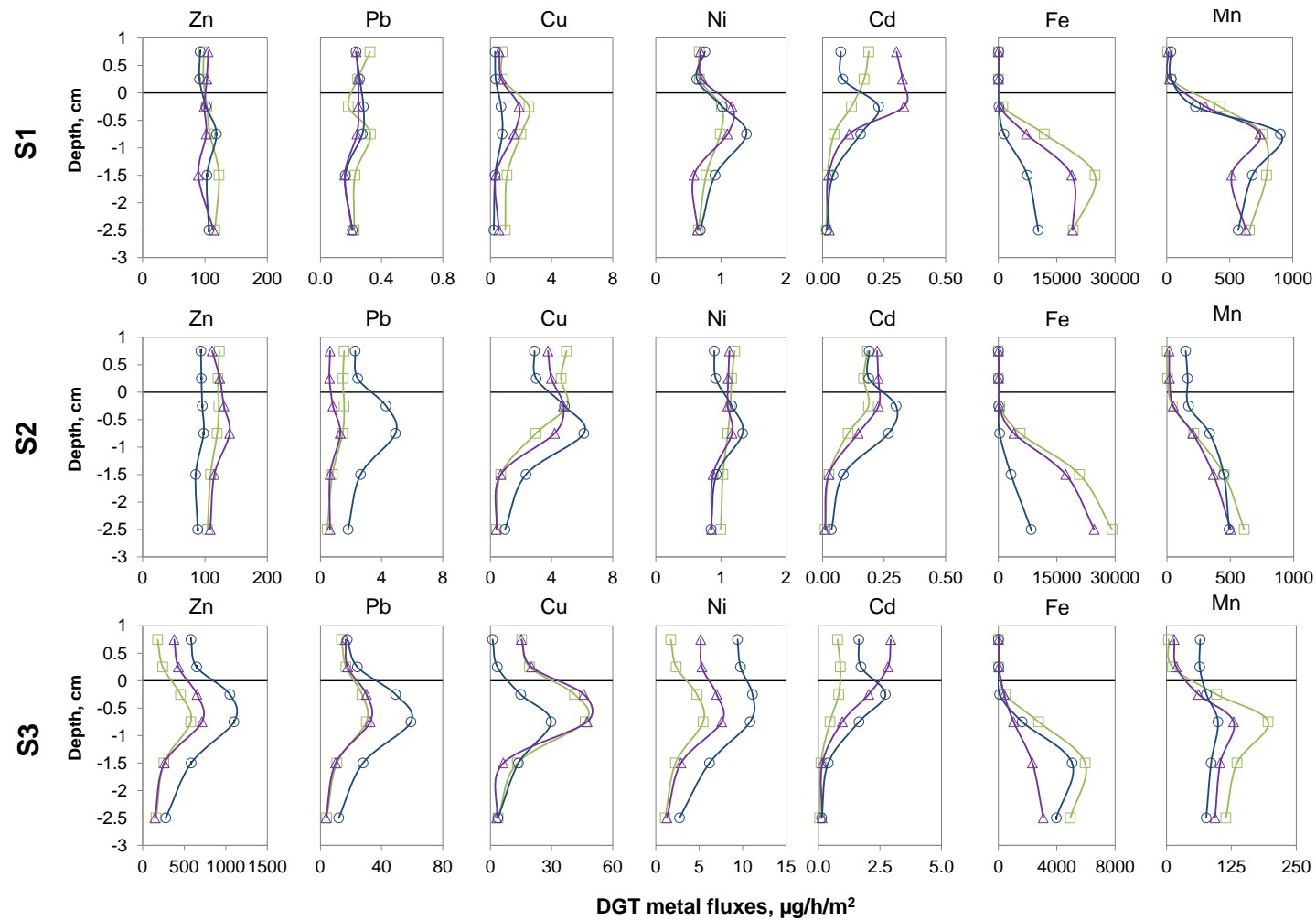


Figure 6.1 DGT vertical profiles measured in pore and overlying waters in sediments exposed to (i) zero (no organisms, squares), (ii) low (bivalves, triangles) and (iii) high (amphipods and bivalves, circles) bioturbation (average of first (day 8) and second (day 23) deployment). Appendix 4 provide profiles for each DGT deployment day.

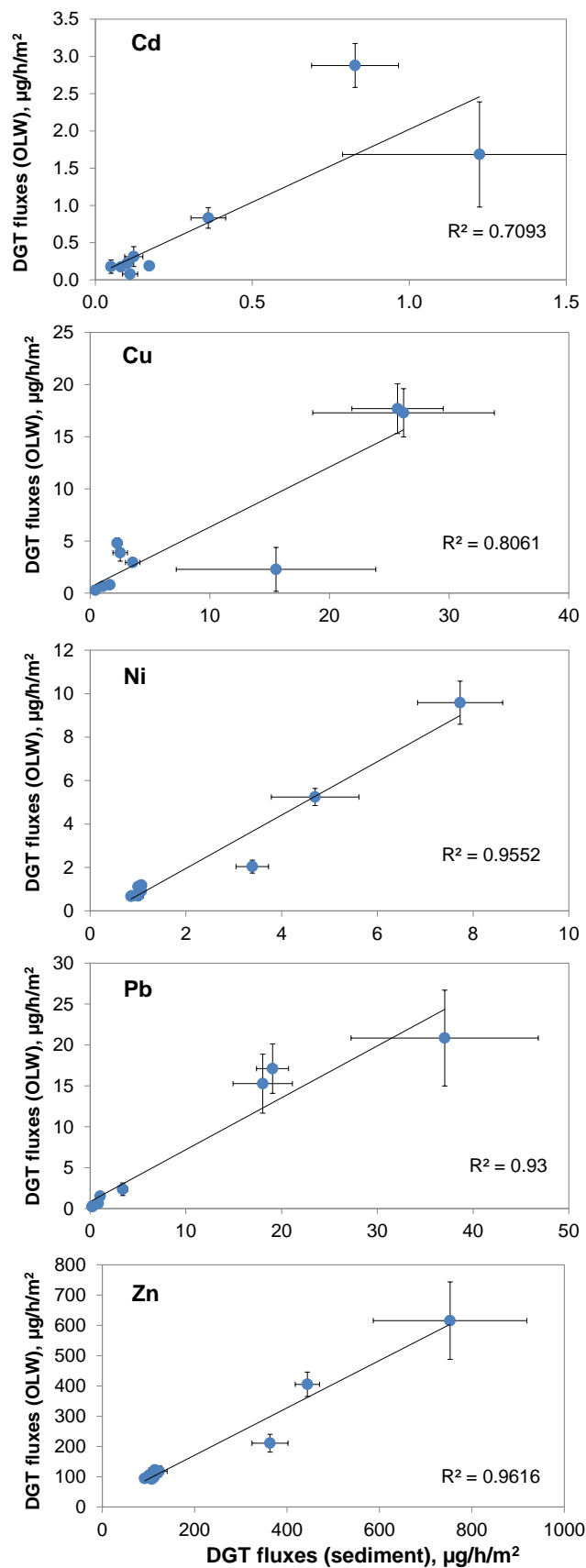


Figure 6.2 Relationships between DGT metal fluxes measured in the sediment (between 0 and - 3 cm depth) and OLW (within 1cm above the SWI) of zero, low and high bioturbation treatments.

In sediment S1, DGT-Ni, -Pb and -Zn fluxes in the low and high bioturbation treatments were similar and not significantly different from those measured in the undisturbed sediment (zero bioturbation) (Figure 6.1, 6.3). Cadmium fluxes were significantly greater ($>100\%$, $p<0.05$) when organisms were present in the sediment, whereas copper fluxes were significantly less in both low ($\sim 30\%$ lower, $p<0.05$) and high ($\sim 70\%$ lower, $p<0.001$) bioturbation treatments (Figure 6.1, 6.3). In sediment S2, no significant differences in DGT fluxes were observed between the zero and low bioturbation treatments, whereas DGT-Cd, -Cu and -Pb fluxes were considerably greater (from 60 to 200% higher, $p<0.001$) in the high bioturbation treatment (Figure 6.1, 6.3). A similar trend was observed in sediment S3, where similar fluxes were measured for the zero and low bioturbation treatments and significantly greater fluxes (Cd, Ni, Pb, Zn) were measured in the high bioturbation treatment (Figure 6.1, 6.3). The increased flux of Cd, Ni, Pb and Zn from sediment S3 was attributed to increasing AVS oxidation occurring as a result of introduction of oxygenated water from the water column. This hypothesis is supported by results shown in the companion paper by Remaili et al. (2015) which indicate that decreasing AVS concentrations occurred for increasing levels of bioturbation in this sediment.

Metal concentrations in the overlying water (OLW) were calculated from DGT measurements by assuming negligible diffusion boundary layer (DBL) (Davison and Zhang 2012). The influence of the three levels of bioturbation on the DGT metal concentrations measured in the overlying waters (DGT_{OLW}) is shown in Figure 6.4. In sediments S1 and S2, similar DGT_{OLW} concentrations (Cd, Cu, Ni, Pb, Zn) were observed for the zero and low bioturbation treatments, whereas significantly lower copper (and nickel for sediment S2) concentrations were measured in treatments exposed to high bioturbation. In sediment S3, DGT_{OLW} -Ni and -Zn concentrations significantly increased for increasing bioturbation levels. The increased nickel and zinc release can be attributed to a number of changes occurring due to the introduction of oxygenated water (dissolved oxygen $> 85\%$) from the overlying water into the sediment resulting from increased bioturbation (e.g. mixing of pore waters with overlying water and oxidation of AVS phases (Remaili et al. 2015). For S3 the DGT_{OLW} -Cd concentrations were significantly higher in the low bioturbation treatment, whereas DGT_{OLW} -Cu concentrations were similar between zero and low bioturbation treatments and considerably lower in the high bioturbation treatment ($p<0.001$).

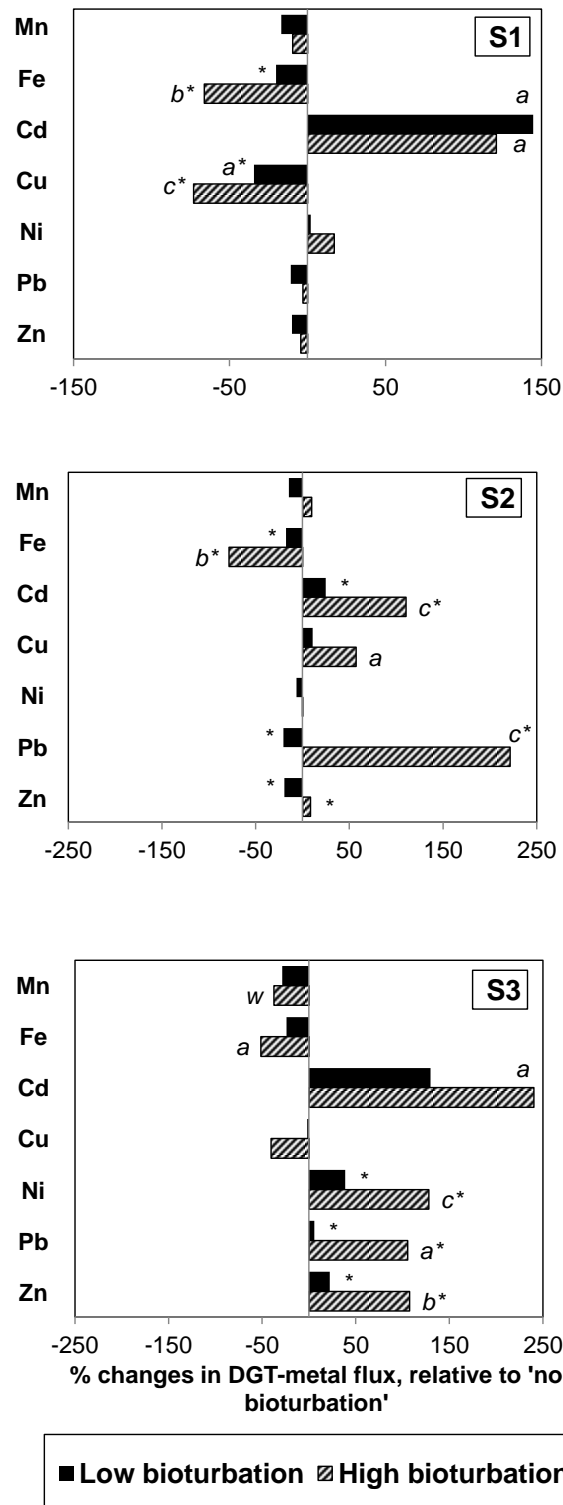


Figure 6.3 Changes in DGT metal fluxes measured in the sediment pore waters (between 0 and -3 cm below the SWI) of sediments exposed to low (bivalves only) and high (bivalves and amphipods) bioturbation expressed as a percentage of fluxes measured in zero bioturbated (undisturbed) sediments. Fluxes are the average of first (day 8) and second (day 23) deployment. Significant differences between treatments exposed to organisms and undisturbed sediments are indicated with the letters *a* ($p < 0.05$), *b* ($p < 0.01$) and *c* ($p < 0.001$), and differences between treatments exposed to organisms are indicated by an asterisk. The letter *w* indicates weak differences ($0.05 < p < 0.06$).

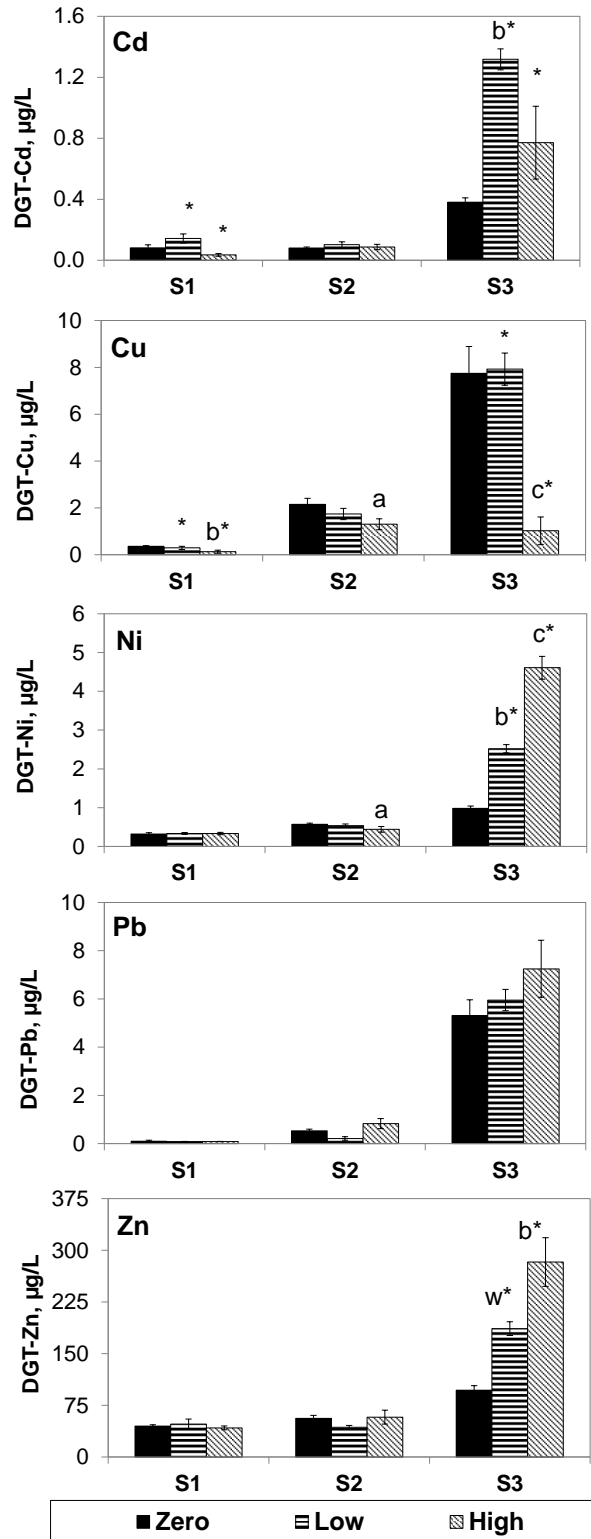


Figure 6.4 DGT metal concentrations measured within 1cm above the SWI in the control (S1) and the contaminated sediments (S2, S3) in presence of (i) no animals (zero bioturbation), (ii) bivalves (low bioturbation) and (iii) amphipods + bivalves (high bioturbation). Mean values are the average of six replicates reported with standard error. Significant differences between treatments exposed to biota and undisturbed sediments are indicated with the letters *a* ($p < 0.05$), *b* ($p < 0.01$) and *c* ($p < 0.001$), and differences between treatments exposed to biota are indicated by an asterisk. The letter *w* indicates weak differences ($0.05 < p < 0.06$).

The differences in OLW metal concentrations between zero, low and high bioturbation were attributed mostly to the physical introduction of oxygenated water in the deeper sediments which promotes greater release of metals to the dissolved phase through disruption of the Fe(II)/iron(III)oxyhydroxides partitioning and oxidation of sulfide phases (Peterson et al. 1996; De Jonge et al. 2009; Simpson et al. 2012a). Metal release through oxidation of AVS appeared to be the dominant process affecting the metal partitioning in the sediment S3. In the contaminated sediment S2, that had much less AVS, the increased release of metals due to higher bioturbation levels may be attributed to other processes such as oxidation of organic matter and equilibrium-kinetics that favour release during the dissolution and formation of manganese and iron oxyhydroxides (Millero 2001).

6.3.4 DGT fluxes and bioaccumulation relationships

In this study, the ability of the DGT technique to predict metal bioavailability in response to varying degrees of bioturbation was investigated by comparing changes in the DGT-labile metal fraction of low and high bioturbated sediments with changes in bioaccumulation rates in bivalves exposed to these sediments. Due to its highly active burrowing behaviour, the amphipod *V. australiensis* was used for the purpose of generating higher degrees of bioturbation in these sediments. The varied degrees of bioaccumulation also provided the ability to further assess the performance of the DGT technique as a metal-bioavailability monitoring tool in sediments.

Previous studies have shown that DGT-metal fluxes measured at the SWI provided useful predictions of toxicity to the amphipod *M. plumulosa* (Amato et al. 2014) and bioaccumulation and survival in the bivalve *T. deltoidalis* (Simpson et al. 2012b; Chapter 4) exposed to metal contaminated sediments with varying properties. In those studies, the relationships between DGT fluxes and biological responses were investigated using fluxes measured in different compartments of the sediment as well as the overlying water (i.e. SWI, top and bottom layer of the sediment, whole sediment) and results indicated that DGT fluxes measured at the SWI (e.g. ± 0.5 cm zone) provided the strongest relationships. This was consistent with the feeding and/or burrowing behaviours of the specific test species (King et al. 2005; Campana et al. 2012).

The behaviour of the amphipod *V. australiensis* is quite different from *M. plumulosa* and *T. deltoidalis*, as it is highly active in creating borrows that it inhabits

and irrigates with overlying water. As multiple exposure sources may contribute to metal bioaccumulation in these species (e.g. food, sediment particles ingestion, pore and overlying waters), relationships between *T. deltoidalis* and *V. australiensis* bioaccumulation of major contaminants (Cu, Pb, Zn, Table 6.1) and the DGT-metal fluxes were investigated using fluxes measured in different compartments of the sediment and the overlying water (i.e. SWI, OLW, whole sediment) (Figure 6.5, and Figure A4.1 and A4.2 of Appendix 4). For *T. deltoidalis*, very similar relationships were obtained using the fluxes derived from these different zones ($R^2=0.903-0.933$, $0.951-0.982$ and $0.581-0.715$ for Zn, Pb and Cu, respectively), whereas the strongest relationships for *V. australiensis* were provided using fluxes measured between ± 1 cm across the SWI ($R^2=0.936$, 0.812 and 0.823 for Zn, Pb and Cu, respectively). For the sake of consistency in the application of the DGT technique, and given that regression models for *T. deltoidalis* provided very similar results (Figure A4.2 of Appendix 4), in this section the relationships between DGT fluxes and bioaccumulation will be discussed using fluxes measured at the SWI (between 0.5 and -0.5 cm depth) (Figure 6.5).

Increasing zinc tissue concentrations in *T. deltoidalis* were observed for increasing DGT-Zn fluxes irrespective of the type of sediment (Table 6.1) and exposure conditions (low or high bioturbation) (Figure 6.5, $R^2=0.907$). In particular, the higher zinc bioaccumulation ($p<0.001$, Figure 6.6) measured in bivalves exposed to sediment S3 under high bioturbation conditions was consistent with greater DGT-Zn fluxes measured in this treatment, suggesting a link between bioturbation, metal release and zinc bioaccumulation (Figure 6.5, Figure 6.6). The increased bioturbation also resulted in increased amounts of suspended solids in the overlying water (Remaili et al. 2015), which may have contributed to bioaccumulation via particle filtration and ingestion.

Increasing DGT-Zn fluxes were also consistent with increasing zinc tissue concentrations in *V. australiensis* ($R^2=0.936$, Figure 6.5). Similarly, lead tissue concentrations in *T. deltoidalis* and *V. australiensis* were significantly correlated with DGT-Pb fluxes, with R^2 values of 0.981 and 0.812 for bivalves and amphipods, respectively.

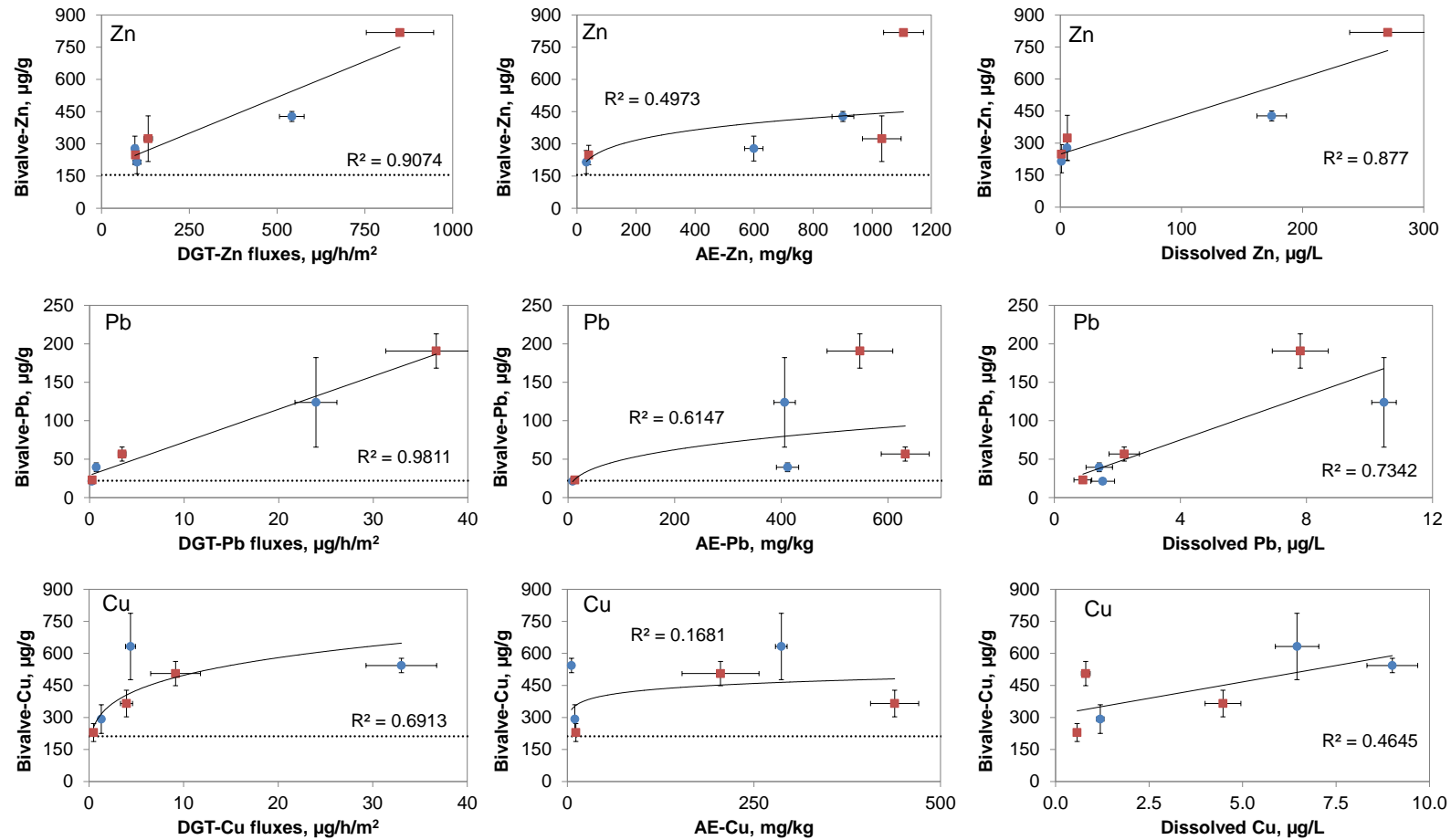


Figure 6.5 Relationships between DGT fluxes measured at the SWI (between 0.5 and -0.5 cm) and AEM concentrations (top 1cm) and metal bioaccumulation in *T. deltoidalis* exposed to low (bivalves only, circles) and high (bivalves and amphipods, squares) bioturbation conditions. DGT fluxes are average values of first (day 8) and second (day 23) deployment. The dotted line indicates average metal concentrations measured in non-exposed bivalves. AEM concentrations are concentrations measured in mini-cores collected at test completion (day 28). Dissolved metal concentrations are average values of overlying water subsamples collected throughout the test. Data points are mean values reported with standard error ($n=3$, 6 and 15 for AEM DGT, and dissolved metal concentrations, respectively).

Weaker relationships were found between copper bioaccumulation in *T. deltoidalis* and DGT-Cu fluxes ($R^2=0.691$). This was due to the lack of consistency between the relatively high copper bioaccumulation found in bivalves exposed to sediment S2 without amphipods (Figure 6.6) and the relatively low DGT-Cu fluxes measured in this treatment (Figure 6.1). This suggests that DGT-labile copper was not the main source of copper for *T. deltoidalis* in this sediment, and copper bioaccumulation likely occurred as a result of particle ingestion (King et al. 2005; Chapter 4). However, the general trend describing the relationship between copper bioaccumulation and DGT fluxes was consistent with that of previous studies (Chapter 4). Positive relationships were also found between DGT-Cu fluxes and bioaccumulation in *V. australiensis* ($R^2=0.823$).

6.3.5 Predicting metal bioavailability in bioturbated sediments and implications for risk assessments

In general, the study indicates that DGT metal fluxes are useful for assessing metal bioavailability in bioturbated sediments and that organisms that cause a high degree of bioturbation can significantly alter, and potentially increase the metal exposure to other organisms within those sediments. While there are many studies that have observed that infaunal activities can affect metal bioavailability in sediments (Atkinson et al. 2007; Lopez et al. 2014; Schaller 2014), the effects of these activities are not frequently considered in risk assessments where outcomes from toxicity tests using single species may be the main driver for assessment decisions (ANZECC/ARMCANZ, 2000). In this study, significant differences in metal bioaccumulation between bivalves exposed to low and high degrees of bioturbation were detected only for zinc ($p<0.001$) in one contaminated sediment (Figure 6.5, Figure 6.6). Thus, in terms of using bioaccumulation data within assessments, the study indicates that the presence of organisms that cause extensive bioturbation may not always modify the assessment outcomes for organisms that bioturbate sediment less. However, the small sample size likely caused an inability to detect significant differences in bioaccumulation for other metals.

Despite only observing changes in bioaccumulation for zinc, significantly greater DGT metal fluxes (Cd, Cu, Ni, Pb, Zn) were measured in high bioturbation

treatments and indicated that the exposure to and risk posed by these metals does increase (Figure 6.3, 6.5).

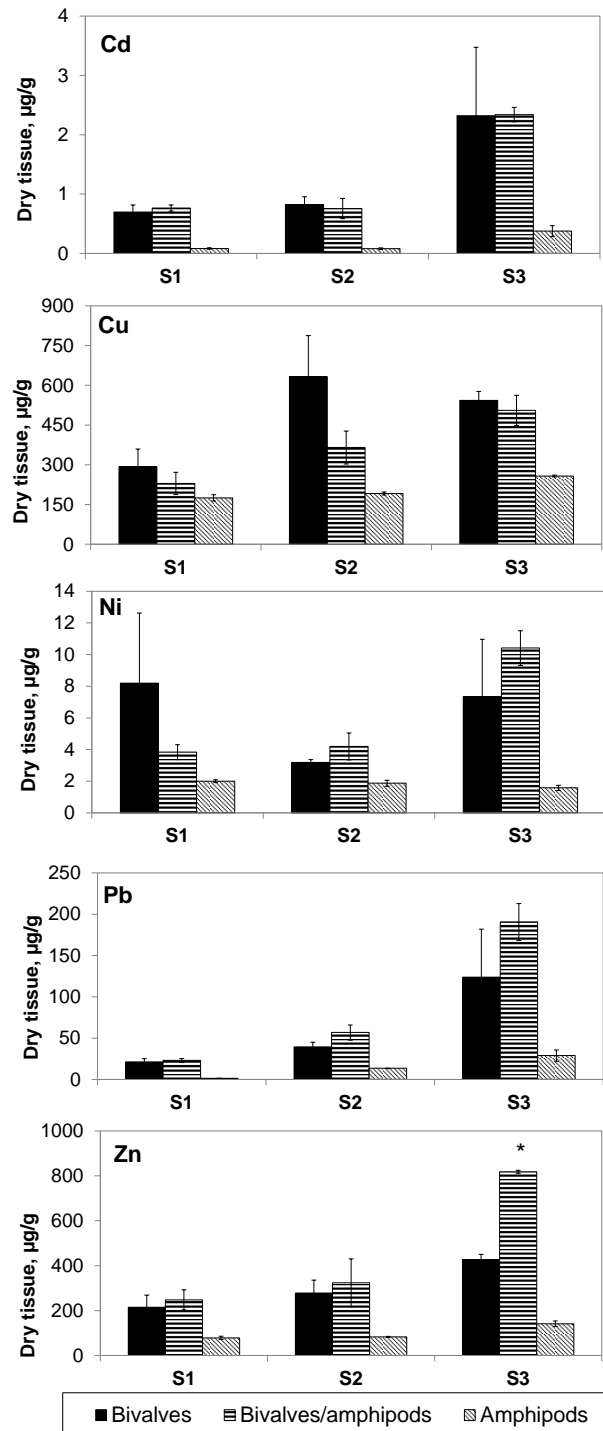


Figure 6.6. Metal concentrations in *T. deltoidalis* and *V. australiensis*. Data are mean values of three replicates with standard error. * The asterisk indicates significant differences ($p < 0.001$) between *T. deltoidalis* exposed to low (dark fill) and high (dark horizontal fill) bioturbation.

Thus, there remains uncertainty as to whether higher degrees of bioturbation may potentially increase the toxicity of sediments, and assessment made using single species may potentially underestimate the risk posed by some metals, when compared to natural environments that contain a mixture of coexisting species. We expect the observations are likely to differ for sediments with differing properties, types and degrees of contamination, as well as for different organisms owing to their varied exposure routes. In previous studies we have proposed that the DGT technique is a suitable tool for assessing the bioavailability and risk posed by the metals such as Cd, Cu, Ni, Pb and Zn in sediments regardless of sediments type. This is based on the premise that sediments exhibiting a higher DGT-induced flux of metals will pose a greater risk of effects to organisms than those exhibiting a lower flux, regardless of the metal-binding phases that are potentially available to modify the metal bioavailability (Simpson et al. 2012b; Amato et al. 2014).

Dilute acid-extractable metal analyses (AEM, 1-M HCl extractions that are equivalent to the simultaneously extractable metal (SEM) fraction in AVS-SEM analyses) are widely used to evaluate the bioavailability of metals in sediments (Burgess et al. 2013; Simpson et al. 2013b). A comparison of relationships between the observed bioaccumulation and the metal exposure predicted by DGT-induced metal flux and AEM concentrations measured at the SWI is provided in Figure 6.5. Strong relationships were found between bioaccumulation and DGT-Pb ($R^2=0.981$) and -Zn ($R^2 = 0.907$) fluxes, whereas weaker relationships were found for copper ($R^2=0.691$). AEM measurements predicted the increased zinc bioaccumulation in bivalves exposed to sediment S3 under high bioturbation conditions (Figure 6.5, Figure 6.6), but appeared to overestimate bioaccumulation in bivalves exposed to sediment S2 (bottom right quadrant, Figure 6.5), causing an overall lower coefficient of determination ($R^2=0.497$). Similarly, also lead bioaccumulation was overestimated in this sediment ($R^2=0.615$), and copper bioaccumulation was poorly predicted using this method ($R^2=0.168$). DGT provided better prediction of bioaccumulation than AEM concentrations also for *V. Australiensis* (Figure A4.1 of Appendix 4).

The exposure pathways and toxicity of metals to the bivalve *T. deltoidalis* has been well studied, and metal uptake occurs from both the dissolved and solid phase exposure, with the dominant exposure route being highly dependent on the partitioning of metals between water and sediment phases (King et al. 2005; Simpson 2005). For the

amphipod *V. Australiensis*, the exposure pathways and sensitivity to metals have not yet been determined. However, several studies indicate that amphipods can be exposed to metals present in the solid and dissolved phases (Besser et al. 2005; Simpson 2005; Simpson and King 2005; King et al. 2006; Campana et al. 2012). The relationships between metal bioaccumulation and dissolved metal concentrations are shown in Figure 6.5, and comparisons between dissolved metal and DGT_{OLW} concentrations are shown in Figure 6.7. For *T. deltoidalis*, increasing bioaccumulation was observed for increasing dissolved lead ($R^2=0.734$) and zinc ($R^2=0.877$) concentrations. A similar trend was also observed for DGT_{OLW} -Pb ($R^2=0.947$) and -Zn ($R^2=0.895$) concentrations. Conversely, relationships with copper bioaccumulation were weak for both dissolved metal ($R^2=0.465$) and DGT_{OLW} ($R^2=0.276$) concentrations.

For *V. Australiensis*, lead and zinc bioaccumulation increased with increasing dissolved metal and DGT_{OLW} concentrations, but both approaches showed no relationships with copper bioaccumulation (Figure A4.3 of Appendix 4).

The relationships between DGT_{OLW} concentrations and dissolved metal concentrations measured in OLW subsamples are shown in Figure 6.8. DGT metal concentrations were generally lower than dissolved metal concentrations for Cd, Cu and Pb, with DGT /dissolved metal concentrations ratios of 1.09-0.51, 0.88-0.22 and 0.93-0.52, respectively. This could be due to the characteristic of the DGT technique to account for bioavailable metal species only. Conversely, DGT_{OLW} -Ni and -Zn concentrations were similar to dissolved zinc concentrations, with DGT /dissolved metal concentrations ratios between 1.10-0.96 and 1.33-1.04, respectively. Overall, positive correlations were found between DGT and dissolved metal concentrations, in particular for nickel and zinc ($R^2 = 0.936, 0.770, 0.984, 0.889$ and 0.994 for Cd, Cu, Ni, Pb and Zn, respectively) (Figure A4.4 of Appendix 4).

Overall, this study demonstrated that higher levels of bioturbation may significantly increase the release of metals in the pore and overlying water for metal-contaminated sediments. Significant increases in zinc body concentrations in *T. deltoidalis* indicated that released zinc was available for uptake by this species, potentially as a result of the combined exposure to pore and overlying waters. The results presented in this study indicate that DGT may be a suitable technique for evaluating metal bioavailability irrespective of the level of bioturbation, whereas

particulate metal concentrations (AEM or TRM) exhibited poor relationships with the observed metal bioaccumulation.

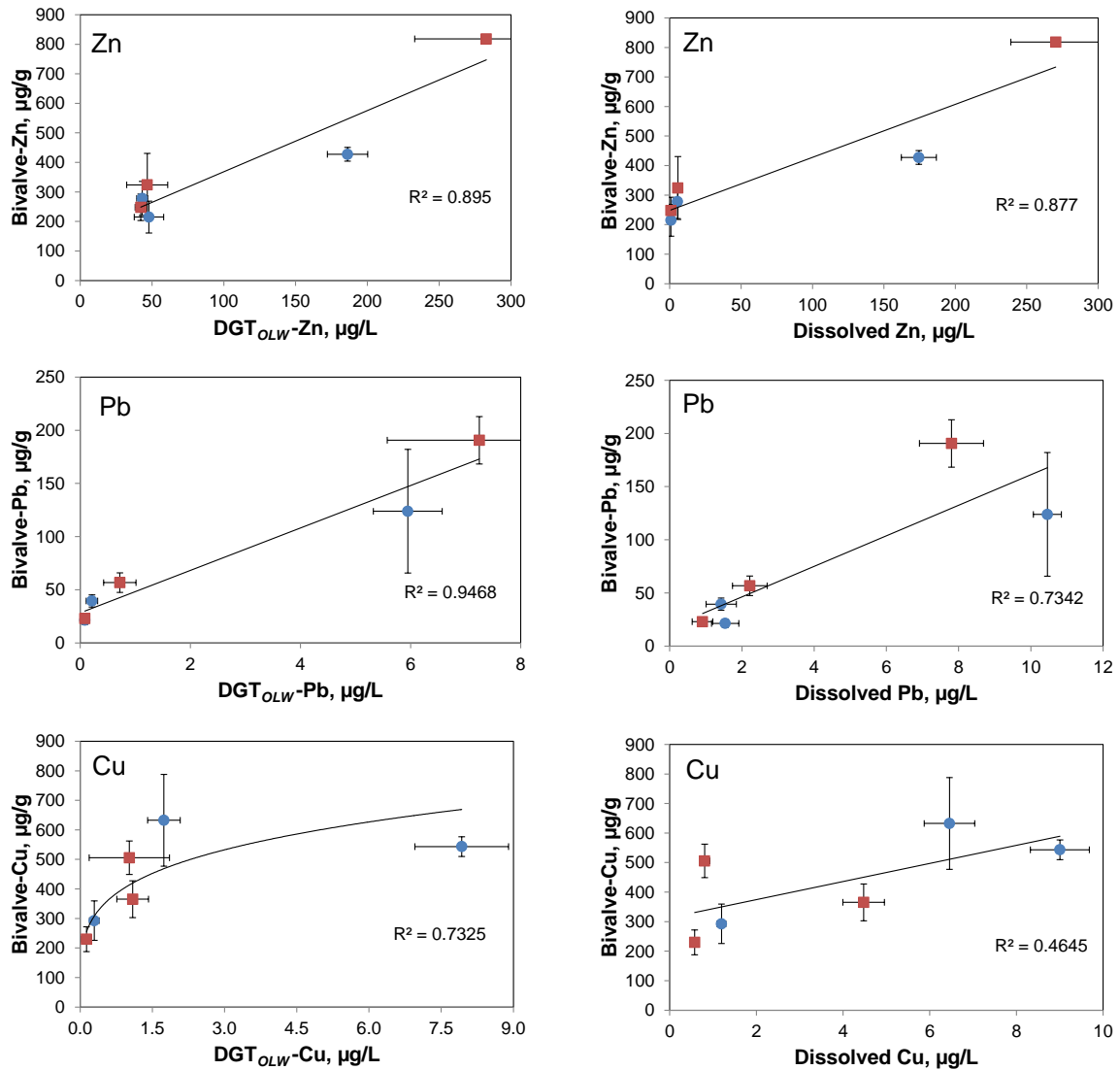


Figure 6.7 Comparison between relationships obtained using DGT and dissolved metal concentrations with bioaccumulation in *T. deltoidalis* exposed to low (bivalves only, circles) and high (bivalves and amphipods, squares) bioturbation conditions. DGT fluxes are average values of first (day 8) and second (day 23) deployment. Dissolved metal concentrations are average values of overlying water subsamples collected throughout the test. Data points are mean values reported with standard error (n=6 and 15 for DGT and dissolved metal concentrations, respectively). The dotted line indicates average metal concentrations measured in non-exposed bivalves. Lines represent linear or exponential models used to estimate relationships.

Based on the relationships with bioaccumulation, DGT appeared to be a more suitable tool for assessing the bioavailable metal pool than particulate metal measurements than AEM concentrations under the experimental conditions investigated in this study. Both dissolved metal and DGT_{OLW} concentrations provided stronger relationships than AEM for lead and zinc bioaccumulation in *T. deltoidalis*, however DGT_{OLW}–bioaccumulation relationships were stronger than the equivalent relationships based on the average exposure from multiple discrete measurements made in the overlying water. Overall DGT-metal flux measurements have the potential to improve the assessment of the risks posed by metals in sediments.

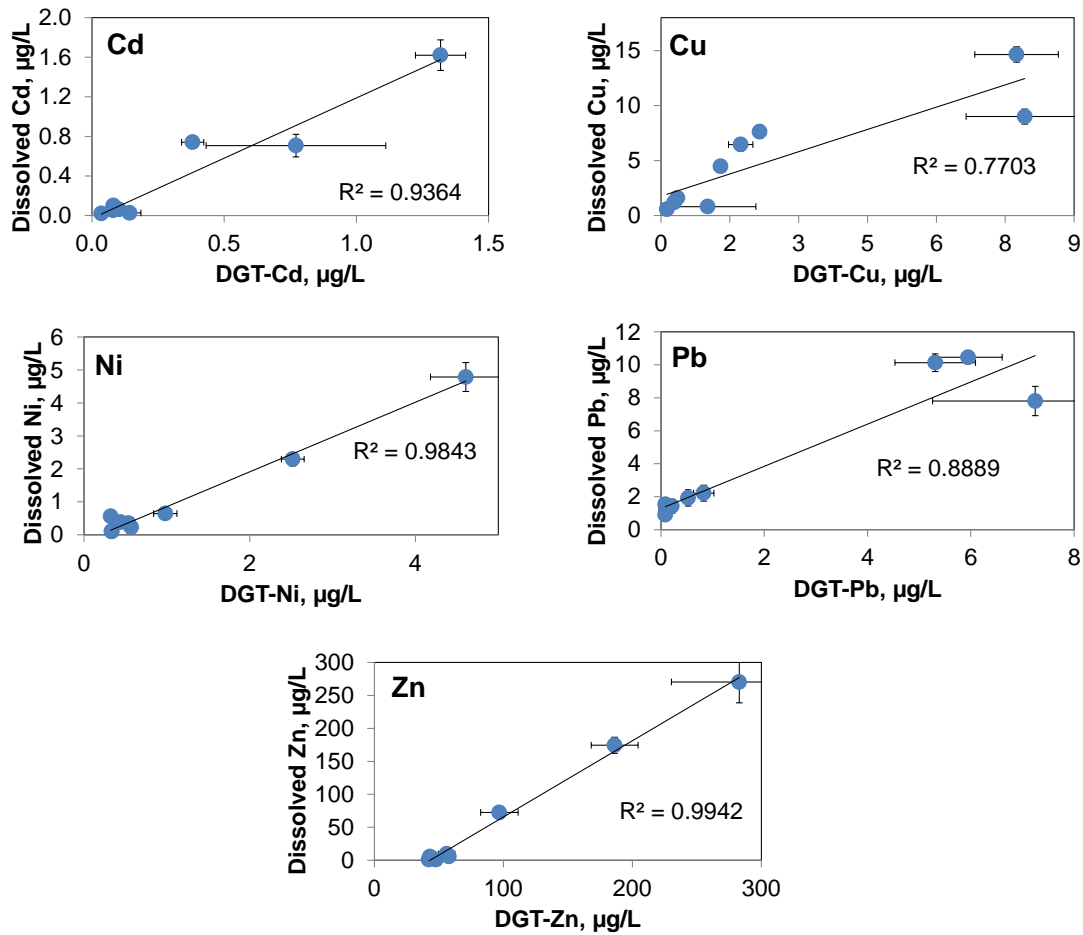


Figure 6.8. Relationships between DGT metal concentrations and dissolved metal concentrations measured in sediments overlying waters. DGT concentrations are mean values with standard error of 3 replicate measurements performed on day 8 and 23 ($n=6$). Dissolved metal concentrations are the average concentration measured in OLW aliquots sampled throughout the test reported with standard error ($n=15$).

Chapter 7: General discussion and conclusions

7.1 General discussion

The aim of this thesis was to assess the performance of the diffusive gradients in thin films (DGT) technique as a rapid monitoring tool for bioavailable metals in sediments. The body of evidence produced in support of this hypothesis was obtained by achieving the following objectives:

- evaluating the performance of the DGT technique in a range of different sediment types;
- assessing the ability of the DGT technique to predict acute and chronic effects to organisms exposed to contaminated sediments under laboratory conditions;
- comparing relationships between DGT measurements and metal bioaccumulation by organisms in field-based assays with those in laboratory-based assays of identical sediments;
- investigating the impact of varying bioturbation activity on metal bioavailability assessments made using the DGT technique; and,
- providing protocols for the use of DGT-based assessments in the SQGs framework.

These main study components were for assessments of metal bioavailability in estuarine-marine sediments, while one additional study was undertaken to evaluate the performance of the DGT technique in assessing metal bioaccumulation by organisms in freshwater sediments. The use of planar sediment DGT probes inserted vertically into sediment allowed the mobilisation of metals of various depths to be investigated, and this also provided the ability to assess whether the overlying water, sediment-water interface, or deeper sediment provided the most useful compartment for assessing the metal exposure.

For all laboratory-based experiments conducted, field collected sediments had varying degrees of metal contamination and physical and chemical properties that influence metal binding and bioavailability (e.g. particle size, acid-volatile sulfide, and organic carbon). A wide range of contamination scenarios were investigated, with Cu, Pb and Zn generally being the major contaminants of concern to benthic organisms, with generally lesser concentrations of Cd and Ni. By using field collected sediments, this study allowed the performance of the DGT technique to be investigated for

sediment deployments in conditions which more closely represented those of real environments.

7.1.1 DGT performance in the laboratory

The ability of the DGT technique to predict metal bioavailability in sediments was initially investigated under laboratory controlled conditions. Robust predictions of acute lethality to the estuarine amphipods *M. plumulosa* exposed to metal contaminated sediments were obtained using DGT-metal fluxes. Dose-response relationships were also investigated using total recoverable metals (TRM), dilute acid-extractable metals (AEM) and overlying water (OLW) dissolved metal concentrations to better represent the potentially bioavailable fraction. The equilibrium partitioning approach combining different sediment binding phases (SEM-AVS)/fOC) did not improve the use of particulate metals data. A comparison between predictions of toxicity obtained using these methods indicated that TRM concentrations were the least useful predictor (pseudo- R^2 for log-logistic concentration-response plot = 0.36), followed by AEM concentrations (pseudo- R^2 =0.54), DGT fluxes (pseudo- R^2 =0.67) and OLW dissolved metal concentrations (average from multiple discrete sampling times) (pseudo- R^2 =0.92). Useful relationships were also obtained for predicting the onset of chronic effects to amphipod reproduction. These results indicated that the performance of the DGT technique was amongst the better of those provided by current methods, but with the advantage of allowing *in situ* applications, and providing time-integrated measurements preferable to “snap-shots” which do not adequately represent dynamic systems such as sediments. While these results give strength to the hypothesis that the DGT technique is a suitable tool for measuring bioavailable metals in sediments, further research is required to evaluate whether the strong relationships observed in laboratory-based experiments are also observed in field situations.

7.1.2 DGT performance in the field

To test the performance of the DGT technique in the field, two *in situ* bioaccumulation bioassays were conducted using estuarine and freshwater sediments, respectively. Each of the experiments was also replicated in the laboratory (using the same sediments) and differences in biological responses between laboratory and field exposures were evaluated. DGT-metal fluxes in the sediments and overlying waters were measured and

compared to organism responses in the laboratory and in the field. In the estuarine experiment, the combined results of laboratory and field experiments indicated that both DGT fluxes ($R^2=0.87$) and AEM concentrations ($R^2=0.79$) were useful for predicting copper and zinc bioaccumulation in the estuarine bivalve *T. deltoidalis*, but weak relationships were obtained for lead. In the freshwater experiment, strong relationships were obtained between zinc bioaccumulation in the freshwater bivalve *H. australis* and DGT fluxes ($R^2=0.91$) irrespective of the type of exposure conditions (laboratory and field), whereas weak relationships were obtained using TRM and AEM concentrations ($R^2<1$). Significant cadmium bioaccumulation was also predicted by DGT in the freshwater study.

Laboratory and field-based experiments performed using identical sediments indicated that significant differences in metal bioaccumulation by the bivalves *T. deltoidalis* and *H. australis* occurred between exposure to laboratory and field conditions. In the estuarine experiment, greater Cu, Pb and Zn fluxes (and dissolved Cu, Pb and Zn concentrations) were measured in the overlying water of laboratory-exposed sediments due to the lower frequency of water exchange compared to continual exchange in the field, whereas in the freshwater experiment, greater fluxes were measured in the field as a result of increased inputs of metal contamination from an upstream mine site caused by rainfall events. This indicates that different environmental conditions occurring between laboratory and field may significantly change the exposure to contaminants and result in inadequate predictions of risk. Thus, the use of reliable *in situ* tools is expected to contribute to more robust sediment risk assessments.

7.1.3 Effects of bioturbation to metal bioavailability and DGT fluxes

There are several factors causing the mismatches between laboratory and field assessments of metal lability: low rates of overlying water exchange may lead to overrepresentation of dissolved metal exposure; space constraints in laboratory-based experiments may result in increased exposure to contaminated sediments due to inability of organisms to avoid exposure; excessive food addition (in the laboratory) may potentially mask effects to organism health that may otherwise be observed in field environments where greater energy expenditure is required to source nutrition; and, bioturbation effects may significantly affect the partitioning of metals between solid and dissolved phases and influence metal bioavailability.

Laboratory-based bioassays are usually performed using one species only, whereas in real sediment environments a range of different organisms typically coexist with different behaviors and sensitivities to contaminants. In a laboratory-based experiment, DGT probes were deployed in sediments exposed to different degrees of bioturbation. The effects of zero (no added organisms), low (bivalve only) and high (bivalve and amphipod) infaunal activities on DGT-labile metal fluxes and the metal bioaccumulation in the bivalve *T. deltoidalis* exposed to these sediments was assessed. DGT indicated significantly greater fluxes (Cd, Pb, Ni, Zn) in presence of the highly active amphipod *V. australiensis*, but significant increases in metal bioaccumulation were observed only for zinc. Overall, a comparison between relationships with metal bioaccumulation in the combined low and high bioturbation treatments obtained using DGT fluxes, AEM and OLW dissolved metal concentrations indicated that the strongest relationships with bioaccumulation were obtained using DGT ($R^2=0.98$, 0.91 and 0.69 for Pb, Zn, and Cu, respectively), followed by dissolved metal concentrations ($R^2=0.73$, 0.88 and 0.47 for Pb, Zn and Cu, respectively) and AEM concentrations ($R^2=0.62$, 0.50 , and 0.17 for Pb, Zn and Cu, respectively).

7.1.4 Influence of sediment particle size on metal bioavailability and risk assessment

One of the key factors affecting metal bioavailability in sediments is the metal partitioning between the solid and dissolved phase. The ability of the solid phase to provide binding sites is influenced by the size of the sediment particles (as well as type), as the total surface available for absorption increases with decreasing particle sizes. Analyses of metal concentrations associated with the percentage of fine sediment particles (e.g. % <63 μm) has often been found to provide a better measure of metal exposure responsible for causing toxicity effects. However, this important factor is not considered in EqP approaches, which consider only the concentration of major metal binding phases (e.g. AVS, TOC for the (SEM-AVS)/fOC approach), but not particle size sediment characteristics. In Chapter 3, we increased the range of sediment types investigated by diluting (1:1 ratio, W/W) one contaminated sediments (100% <63 μm) with (a) a control sediment (100% <63 μm) and (b) a clean sand (0.6-1.5 mm particle size), respectively. The two resulting sediments had a percentage of <63 μm particles of 50 (S9, sandy) and 100% (S1, silty), respectively. Although the diluted sediments had similar TRM and AEM concentrations, amphipod survival was significantly less in the

sandier sediment (88 ± 3 and $50 \pm 6\%$ survival for the silty and sandy sediment, respectively). By following the same procedure, another contaminated sediment was diluted to obtain two new sediments with 30 (S5) and 70% (S2) of particles $< 63 \mu\text{m}$. Similarly, the sandy sediment was the most toxic (71 ± 5 and 88 ± 3 for S5 and S2, respectively), but TRM and AEM were similar (Table 3.1). This indicates that assessments that compare particulate metal concentrations with guideline values that do not consider the influence of particle size differences are likely to result in poor predictions of metal bioavailability and toxicity. Conversely, DGT fluxes measured in the sandy sediment S9 were significantly higher than those measured in the silty sediment S1. This suggests that toxicity was linked to the higher dissolved metal concentrations found in the pore water of the sandy sediment as metals in this sediment are less efficiently retained by the solid phase due to the lower availability of binding sites. However, in the second case, greater DGT fluxes were observed in the least toxic of the two diluted sediments (S2), indicating that in the most toxic sediment exposure to the dissolved phase was not a major contributor to toxicity.

7.1.5 Optimised use DGT metal fluxes for sediment risk assessments

Metals, like many contaminants, are usually present in sediments as mixtures. Adverse effects to benthic organisms may occur as a result of the exposure to one or multiple contaminants. In order to account for the contribution of all metals present in a given sediment, DGT fluxes were expressed as the sum of all metal fluxes measured at the SWI (Chapter 3). However, as different metals have varying potencies and impacts on organisms health, a range of normalisation approaches with the intent of accounting for the different toxicity of different metals were investigated. All of the normalisation approaches trialed improved the relationships between DGT fluxes and toxicity, with a better approach found to be the use of water quality guidelines (WQG)-normalised fluxes (DGT_{WQG} fluxes), i.e. where the flux of each individual metal was divided by its corresponding WQG value before summing all of the ‘WQG-normalised’ fluxes. Based on the dose-response relationships between DGT fluxes (Cd, Cu, Ni, Pb, Zn) and toxicity, three main areas were identified:

- a region which significantly affects survival for DGT_{WQG} fluxes $> 36 \mu\text{g}_{(\text{WQG})}/\text{h}/\text{m}^2$;

- a region which affects reproduction but not survival for fluxes between 17 and $36 \mu\text{g}_{(\text{WQG})}/\text{h}/\text{m}^2$; and
- a no-observed effect (to reproduction or survival) region for fluxes $<17 \mu\text{g}_{(\text{WQG})}/\text{h}/\text{m}^2$.

7.2 Recommendations for the use of the DGT technique for sediment risk assessment

7.2.1 DGT fluxes or concentrations?

The metal release from sediments measured by the DGT technique can be expressed as a flux (Equation 8) or a concentration (Equation 10) (Chapter 1). The assumptions required to express DGT measurements as concentrations in sediments (fast resupply of solute from the solid phase, sufficient pool of labile metals) are rarely satisfied in sediment deployments, and thus the concentrations measured by DGT usually result in underestimation of pore water concentrations. Furthermore, a wide range of geochemical (e.g. metals cycles and redox profile) and biological processes (mineralisation of organic matter and benthic organism behaviour) occur in sediments making the interpretation of DGT data even more complex. By making measurements with DGT devices loaded with different diffusive gel thicknesses, it is possible to evaluate (by plotting the accumulated mass vs. $1/\Delta g$) whether the DGT data can be used as pore water concentrations or maximum flux of metals from the solid phase to the pore water. As metal release in sediments is dependent on redox conditions (oxic, sub-oxic, anoxic), this assessment should be repeated for each given depth investigated, making the overall process time consuming. However, DGT concentrations in sediment deployments can still be used in absence of the conditions required to match the DGT assumptions as long as differences between concentrations ‘experienced’ by the DGT device and real pore water concentrations are understood. While the concept of ‘concentration’ is more familiar to most people than ‘flux’, the calculation of concentrations adds further uncertainty and frequently results in misleading interpretations of DGT data. Thus, expressing DGT measurements as fluxes (e.g. $\mu\text{g}/\text{h}/\text{m}^2$) is suggested here. Thresholds or guidelines can be derived based on the assumption that sediments with greater fluxes of metals pose a greater risk of effects than sediments with lower fluxes of metals.

7.2.2 A multi-compartment approach

One of the most important characteristics of the DGT technique is the ability of simultaneously measure fluxes of metals released in different compartments of the sediment (oxic, sub-oxic, anoxic) and in the overlying water. As the exposure to metals will differ for each biological species according to the organisms borrowing and feeding behaviors, relationships between DGT fluxes and biological responses were investigated using fluxes measured in different compartments representing different exposures (bulk sediment, SWI and overlying water). The most consistent relationships were generally obtained using fluxes measured at the sediment water interface (SWI) (\pm 1 or 2 cm), which represent the combined exposure of metals released in the pore and overlying water. As a large proportion of benthic organisms live in close proximity of the SWI, and are in one way or another affected by the dynamics occurring in this important compartment (e.g. burrowing activities, solute exchange between pore and overlying water, particle deposition) using fluxes measured at the SWI is sound.

7.2.3 DGT deployment duration

An appropriate deployment time is crucial for the establishment of a time invariant response for DGT. Based on two dimensional modelling studies, Harper et al. (1998) suggested that a 24 h deployment time will generally allow the establishment of a pseudo steady-state while avoiding exhaustion of metal concentrations at the DGT-sediment interface, which has been shown to decrease DGT fluxes (Section 1.9.5). The formation of biofilms at the surface of the DGT device, which may act as an additional binding phase, and competition effects causing metal displacement based on the different affinity of metals for the binding resin may occur for deployments longer than 24 h, and affect DGT measurements. Nia et al. (2011) and Ciffroy et al. (2011) proposed a multi-compartmental model accounting for sediment phases with different binding strength to interpret DGT measurements in sediments (DGT-PROFS) and showed that in many cases 24 h deployment time allowed a time invariant response for DGT to be established. Conversely, in sediments where metal release is slow and insufficient to sustain the DGT demand, fluxes will decrease over time. From a risk assessment prospective, this is expected to overestimate potential adverse effects. However, the strong relationships between DGT-metal fluxes and metal accumulation

and toxicity observed in the present study suggest that a 24 h deployment duration is suitable.

7.3 Conclusions and future directions

The body of evidence presented in this thesis indicates that the diffusive gradients in thin films (DGT) technique has a strong potential to be used for assessing risks posed by metals in contaminated sediments. The fundamental assumption that increasing DGT fluxes may lead to increasing risks of adverse effects to benthic organisms has been extensively documented in this thesis. An overview of the DGT performance in relation to the different experimental conditions (e.g. type of sediment, exposures, bioassay) and organisms used is provided in Table 7.1. Despite the difficulty in predicting adverse effects in sediments containing mixtures of metals and displaying different chemical and physical properties, the suggested normalisation approach allowed to provide robust prediction of toxicity by using the DGT technique. In laboratory and field-based experiments, DGT fluxes provided generally strong correlations with bioaccumulation and toxicity. In comparison to total (TRM) and dilute acid-extractable (AEM) metal concentrations, DGT-metal fluxes provided similar or stronger predictions of toxicity and bioaccumulation. TRM concentrations were consistently the weakest predictor of adverse effects, whereas dissolved metal concentrations measured in the overlying water were generally in accordance with DGT measurements. In the studies, AVS and DOC analyses were made in order to evaluate the (SEM-AVS) and (SEM-AVS)/FOC equilibrium partitioning (EqP) models, that is frequently used for evaluating the risk posed by the metals Cd, Cu, Ni, Pb and Zn. Poor relationships between these models and the observed metal bioaccumulation or toxicity were found (not shown as Figures).

The DGT device is a simple and handy tool which presents a number of advantages as a monitoring technique. DGT belongs to the category of *in-situ* technique, therefore measurements are exempted from episodes of contamination due to sample extraction from the environment, storage or transportation. By accumulating metals through time on the chelating resin, the DGT technique provides time-integrated measurements of cationic metal fluxes which are directly linked to processes occurring through time in sediment. The contribution of metals from each sediment process is averaged in a single measurement which we believe better expresses sediment dynamics

Table 7.1 DGT performance in relation to different experimental conditions and organism used and comparison with AEM method

Endpoint	Species	Type of sediment*	Exposure	Dose	Metal	Prediction model (DGT)**	Prediction ability	
							DGT	AEM
Survival	<i>Melita plumulosa</i> (Amphipod)	Sandy, Silty, Sandy-Silty	Laboratory	Combined WQG-normalised DGT flux (Cd, Cu, Ni, Pb, Zn)	Mixture (Cd, Cu, Ni, Pb, Zn)	Logistic regression, $p < 0.05$, pseudo- $R^2 = 0.67$	Strong	Strong
Reproduction	<i>Melita plumulosa</i> (Amphipod)	Sandy, Silty, Sandy-Silty	Laboratory	Combined WQG-normalised DGT flux (Cd, Cu, Ni, Pb, Zn)	Mixture (Cd, Cu, Ni, Pb, Zn)	Logistic regression, $p < 0.05$, pseudo- $R^2 = 0.82$	Strong	Strong
Bioaccumulation	<i>Victoriopisa Australiensis</i> (Amphipod)	Sandy and Silty	Laboratory	DGT flux ($\mu\text{g}/\text{h}/\text{m}^2$)	Cu	Linear regression, $p < 0.05$, $R^2 = 0.82$	Strong	Weak
					Pb	Linear regression, $p < 0.05$, $R^2 = 0.81$	Strong	Weak
					Zn	Linear regression, $p < 0.05$, $R^2 = 0.94$	Strong	Weak
Bioaccumulation	<i>Tellina deltoidalis</i> (Bivalve)	Sandy and Silty	Laboratory (combined low-high bioturbation)	DGT flux ($\mu\text{g}/\text{h}/\text{m}^2$)	Cu	Exponential, $p < 0.05$, $R^2 = 0.69$	Moderate	Weak
					Pb	Linear regression, $p < 0.05$, $R^2 = 0.98$	Strong	Weak
					Zn	Linear regression, $p < 0.05$, $R^2 = 0.91$	Strong	Weak
Bioaccumulation	<i>Tellina deltoidalis</i> (Bivalve)	Sandy, Silty, Sandy-Silty	Combined Laboratory/Field	DGT flux ($\mu\text{g}/\text{h}/\text{m}^2$)	Cu	Quadrant analysis	Moderate	Moderate
					Pb	Quadrant analysis	Moderate	Moderate
					Zn	Quadrant analysis	Strong	Strong
Bioaccumulation	<i>Hyridella australis</i> (Bivalve)	Sandy, Silty, Sandy-Silty	Combined Laboratory/Field	DGT flux ($\mu\text{g}/\text{h}/\text{m}^2$)	Zn	Linear regression, $p < 0.05$, $R^2 = 0.91$	Strong	Weak

*Details on chemical and physical properties of the sediments are provided in Tables 3.1, 3.2, 4.1, 4.2, 4.3, 5.1, 6.1.

**Data refer to DGT measurements performed at the SWI (± 0.5 or 1 cm) over a 24 h deployment time.

that may be experienced by an organism rather than snapshots or discrete measurements.

Another advantage of accumulating analytes (within the chelating resin) is that it allows for lower detection limits but also reduces the matrix effect during analysis (e.g. Cl^- in sea water). The DGT technique is also capable of selectively binding the more bioavailable dissolved and labile species in pore and overlying waters, while excluding the less bioavailable metal forms generally associated with organic carbon (dissolved and particulate), carbonates or other strong binding phase of the sediment. This characteristic has important implications for assessments, as concentrations of these labile and more bioavailable species have been shown to better predict metal toxicity to sediment-dwelling organisms. Additional selectivity can be also achieved by varying the composition of the diffusive gel, e.g. limiting diffusion to small inorganic species only by modifying the pore size distribution. By measuring fluxes at the SWI, the DGT technique integrates the fluxes of metals present in the pore and overlying water within this zone, thus representing multiple sources of metals which often contribute to the overall exposure.

Concern still remains as to whether DGT fluxes may be representative of exposure to the particulate phases (e.g. dietary exposure). While strong dose-response relationships between WQG-normalized DGT fluxes and toxicity suggest that DGT fluxes may be representative of the combined dissolved and dietary exposure of the deposit feeding amphipod *M. plumulosa*, inconsistency in some cases between DGT fluxes and bioaccumulation of copper and lead in the deposit feeding bivalve *T. deltoidalis* indicated possible limitations for the use of the DGT technique in risk assessments. These limitations were attributed to inadequacy of the DGT technique to represent exposure occurring as a result of particles ingestion, and/or overemphasis of the exposure to metals present in the overlying water of laboratory exposed sediments. Although in Chapter 4 it was shown that DGT-Cu fluxes may overemphasis the contribution of dissolved copper to the observed bioaccumulation, previous studies have shown that lethal effects of copper contaminated sediments to *T. deltoidalis* were successfully predicted by the DGT technique. Copper is an essential metal for many benthic organisms, thus internal copper concentrations can be more efficiently regulated by these organisms in response to increasing concentrations in the surrounding environment. This was consistent with our results showing that only four sediments

caused significant copper bioaccumulation despite the high level of copper contamination in the sediments. However, when the exposure to copper reaches thresholds capable of causing lethal effects, previous studies indicated that the DGT technique is a suitable tool for predicting effects to survival for this bivalve. Lead is not an essential metal, and the elevated number of sediments causing significant lead bioaccumulation in *T. deltoidalis* confirmed this. DGT-Pb fluxes provided overall strong relationships with lead bioaccumulation, except for bivalves exposed to silty sediments under laboratory conditions. However, under field conditions, predictions of lead bioaccumulation were strong for all sediments types. Further investigations assessing links between DGT fluxes and adverse effects caused by exposure to particles ingestion will better evaluate the performance of the DGT technique as a monitoring tool.

Although *in situ* techniques allow the direct measurements of contaminants in the environment, thus reducing the risk of causing changes in sample properties due to extraction, transport and storage of the sample, the *in situ* sampling is often viewed as difficult to perform. In this study, *in situ* deployments were performed in shallow waters (<1 m), therefore no specific equipment was required. However, for DGT deployments in deeper waters, specifically designed benthic landers have been previously used to perform DGT measurements up to a depth of 77 m. This allows the use of DGT probes without the additional cost of hiring professional divers.

Overall, these limitations indicate that further research is required to optimise the use of the DGT technique for sediment monitoring. However, the strong evidence provided in this study indicates that the DGT technique has the potential to improve the sediment risk assessment framework by providing robust *in situ* measurements which have been shown to incorporate the contribution of the main factors affecting metal bioavailability in sediments (AVS, TOC, pore and overlying water concentrations, particles size). The evidence presented here suggests that DGT will provide industries with an effective *in situ* monitoring tool which provides an improved and highly viable alternative to the many combined analyses (e.g. TRM, AEM, AVS, TOC, dissolved metal concentration) that are frequently used to inform SQG frameworks of the bioavailability and risk posed by metals in sediments.

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Appendix 1

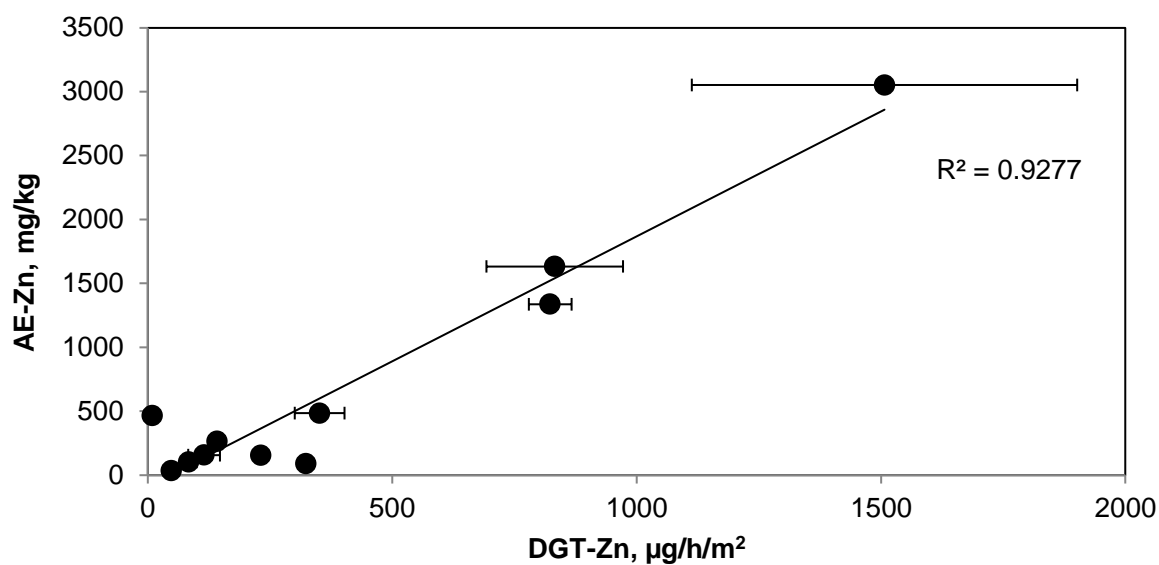


Figure A1.1. Relationships between DGT-Zn fluxes and AE-Zn concentrations in control (C1-C5) and contaminated sandy sediments (S2, S3, S4, S5, S7, S10). DGT data are reported as mean values ($n=3$) with standard deviation. Variability in metal concentration between treatments (of the same homogenised sediments) was less than 30%.

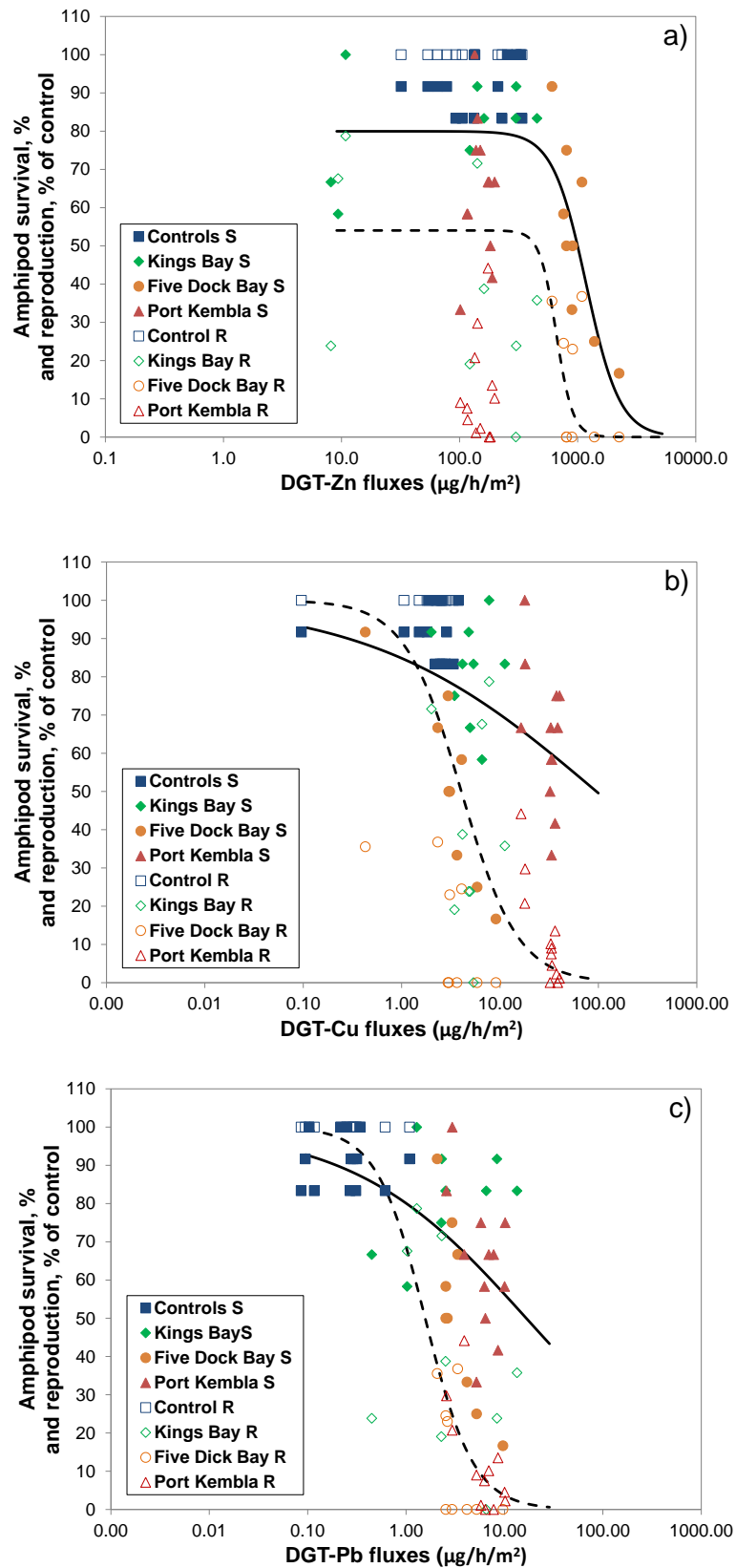


Figure A1.2. Dose-response relationships between amphipod survival and reproduction and DGT-Zn, -Cu and -Pb fluxes. Solid and dashed lines represent survival and reproduction fitting curves, respectively.

Appendix 2

Laboratory

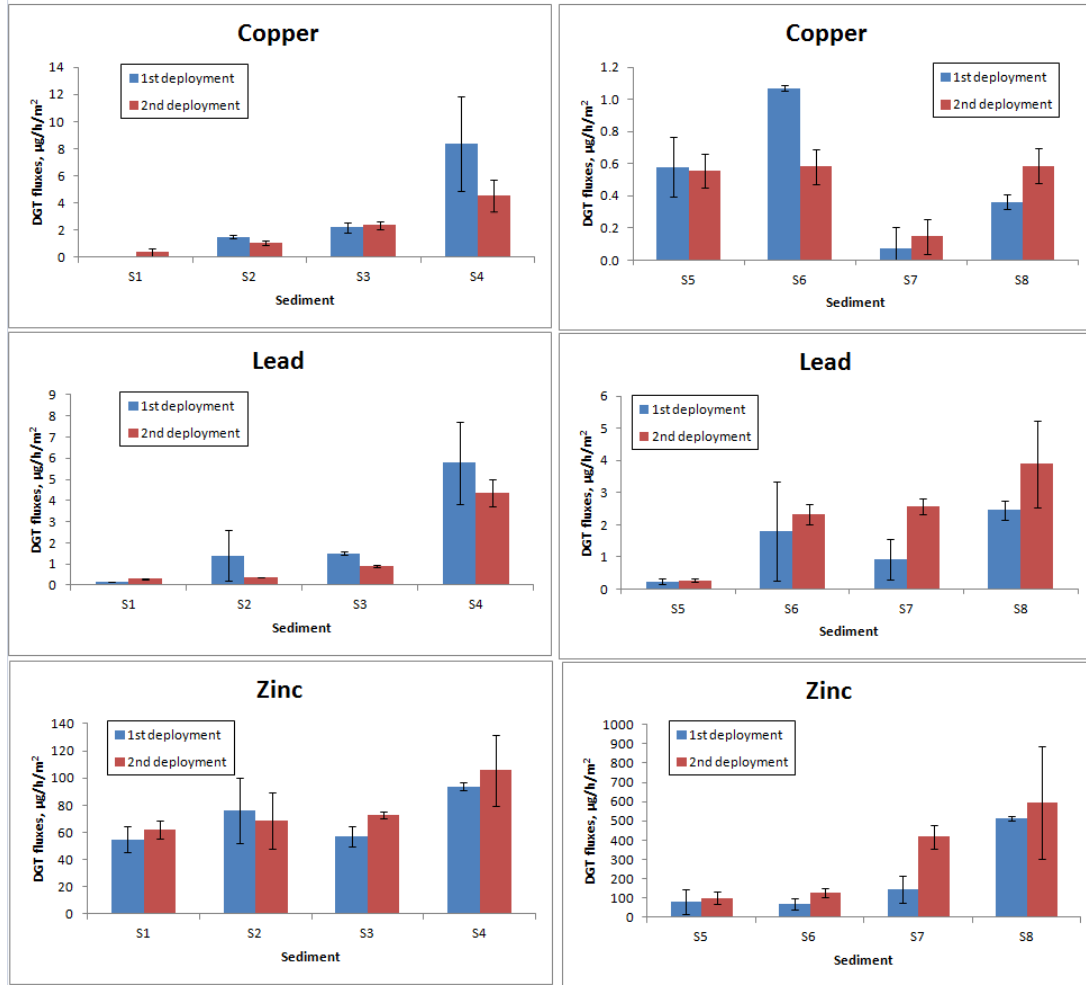


Figure A2.1 Comparison between DGT metal fluxes (Cu, Pb and Zn) measured in the first (day 5) and second (day 19) deployment in laboratory-deployed sediment (from 0 to -3 cm below the SWI). Data are mean values of two replicates reported with standard deviation.

Field

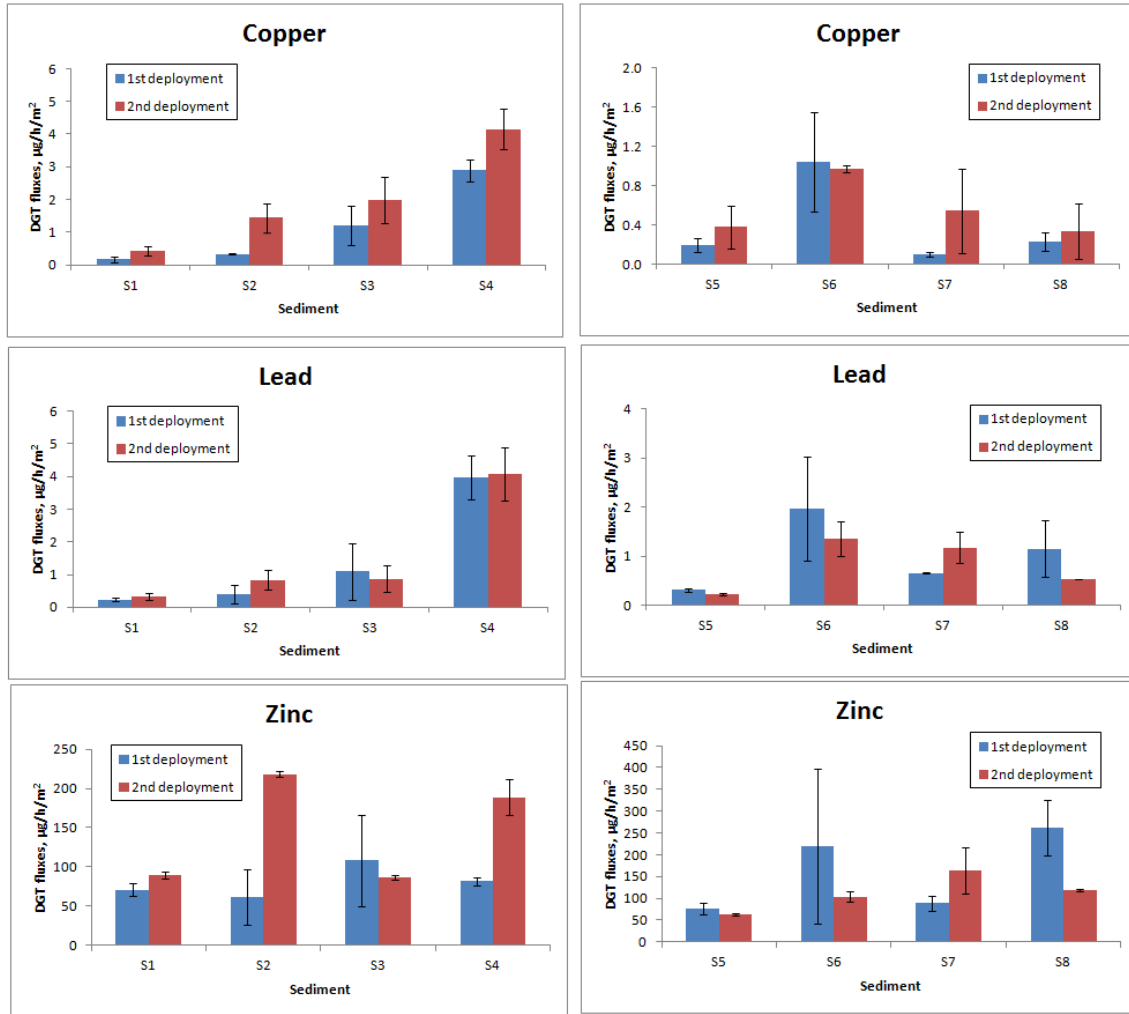


Figure A2.2 Comparison between DGT metal fluxes (Cu, Pb and Zn) measured in the first (day 5) and second (day 19) deployment in field-deployed sediment (from 0 to -3 cm below the SWI). Data are mean values of two replicates reported with standard deviation.

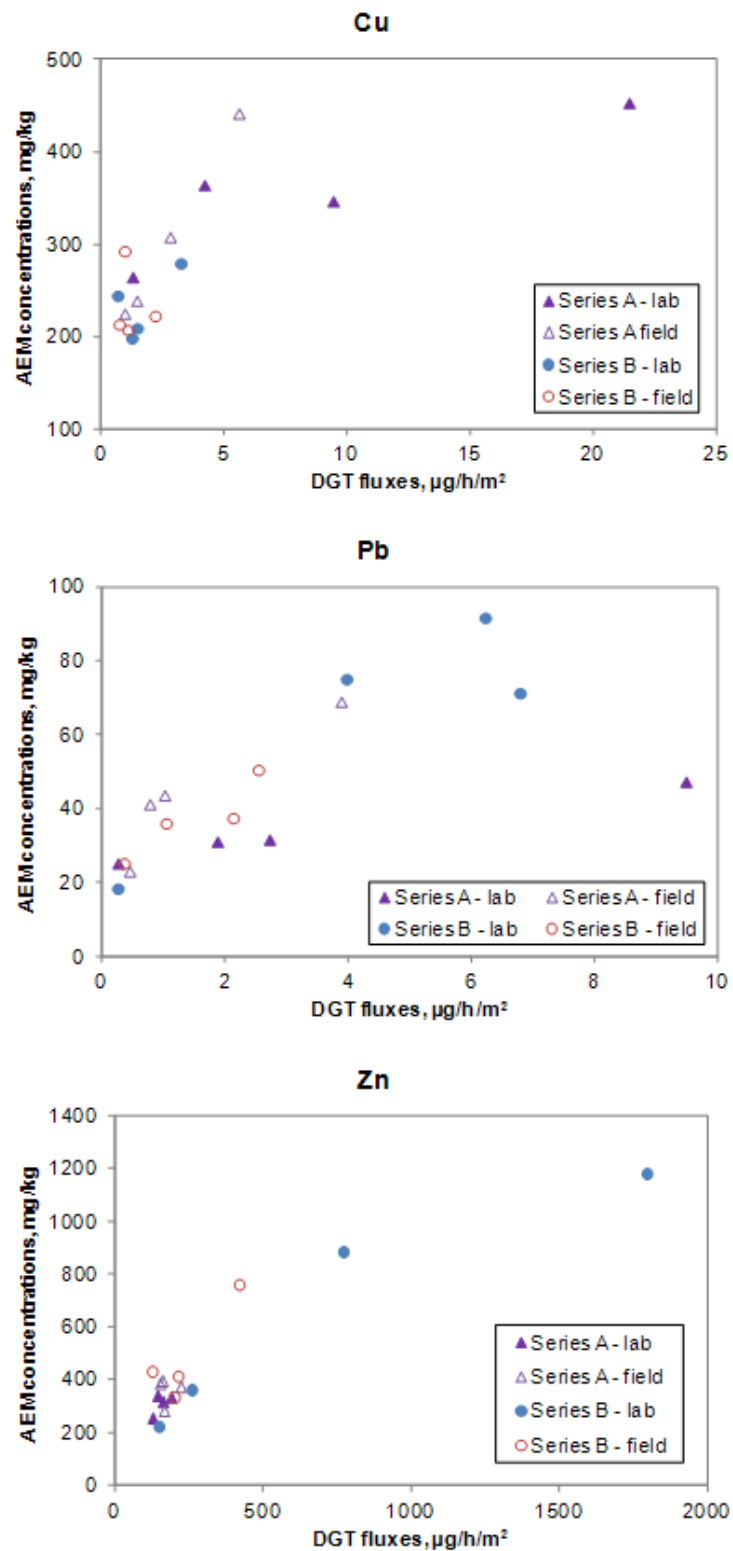


Figure A2.3 Relationships between DGT fluxes measured at the SWI (± 1 cm) and AEM concentrations (average of initial and final surface concentrations). Series A = silty sediments and Series B = sandy sediments.



Figure A2.4 Stainless cage used for the *in situ* bioassay.



Figure A2.5 Deployment of the stainless cages used for the *in situ* bioassay.

Appendix 3



Figure A3.1 Plastic cages (2 cm mesh) used to for the *in situ* bioassay.

Table A3.1 Water quality parameters measured during the field bioassay.

Site	Temperature, ° C	pH	DO, mg/L	Conductivity, ms/cm	Turbidity (NTU)
S1	14.8 ± 0.3	7.3 ± 0.2	7.4 ± 1.5	0.059 ± 0.001	38.3 ± 37.4
S2	18.7 ± 1.3	6.9 ± 0.6	6.5 ± 1.3	0.482 ± 0.288	3.3 ± 3.3
S3	20.1 ± 1.0	7.1 ± 0.1	4.4 ± 2.0	0.385 ± 0.022	73.1 ± 44.2
S4	21 ± 1.1	7.8 ± 0.2	5.9 ± 1.1	0.456 ± 0.051	25.7 ± 14.4

Table A3.2 Metal concentrations in the test sediments*TRM Concentration (mg/Kg)*

Sample	As	Cd	Co	Cr	Cu	Ni	Pb	Zn
S1	7±1	<1	13±1	7.3±0.3	10.0±0.4	12±1	16±1	95±3
S2	4.3±0.3	1.20±0.05	4±1	19.0±0.3	26±1	14.0±0.4	33±1	840±17
S3	6±1	5.30±0.04	8±1	12±1	55±1	9.5±0.2	130±1	2100±31
S4	3±1	3.2±0.2	6.8±0.5	14±1	27±2	11.1±0.1	47.0±0.4	910±4
SQGs	20	1.5	n.a.	80	65	21	50	200

AEM Concentration (mg/Kg)

Sample	As	Cd	Co	Cr	Cu	Ni	Pb	Zn
S1	1.9±0.2	<1	11±1	1.0±0.1	5.9±0.2	6.4±0.3	8.9±0.4	58±5
S2	1.5±0.3	1.0±0.1	1.9±0.2	<1	9.0±0.4	6.4±0.3	23±1	750±58
S3	<1	4.5±0.4	3.6±0.2	1.10±0.04	5±1	3.1±0.2	110±8	1800±140
S4	<1	2.6±0.6	3.3±0.3	1.3±0.1	5±1	3.4±0.1	37±3	790±25
SQGs	20	1.5	n.a.	80	65	21	50	200

Distance from mine: S1 = 8 km (upstream), S2 = 9 km (downstream), S3 = 28 km (downstream), S4 = 45 km (downstream); TRM = total recoverable metals; AEM = dilute acid-extractable metals. Concentrations are mean values with standard deviation (n=3).

Appendix 4

Table A4.1 Total recoverable metal and metalloid concentrations in sediments.

Sediment	Total Recoverable Metals (TRM), mg/kg									
	Al	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
S1	2860	2	0	5	16	7600	53	2	10	37
S2	907	39	2	69	544	42700	289	19	459	912
S3	10500	50	8	392	1160	36000	67	36	1360	2830

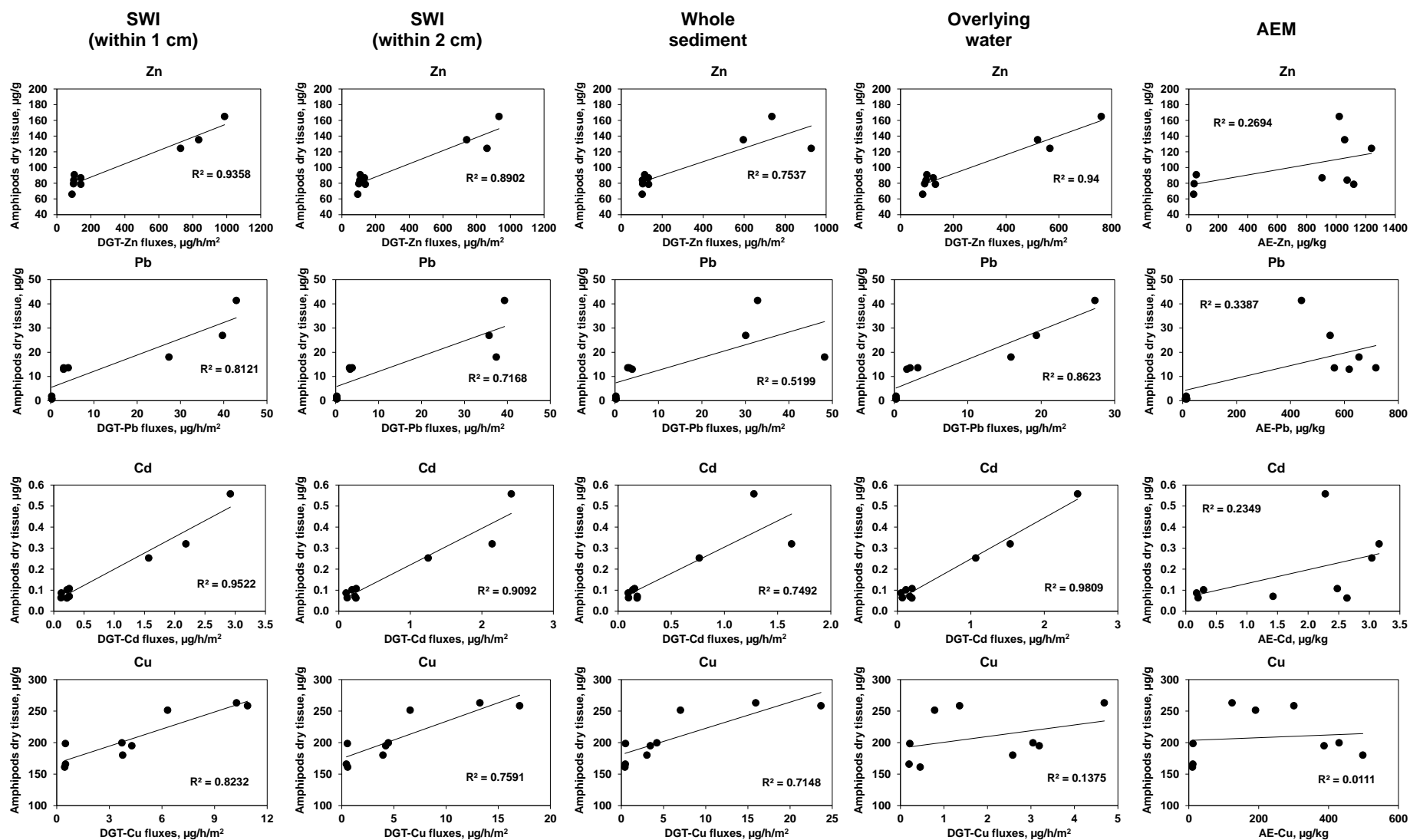


Figure A4.1 Relationships between *V. australiensis* bioaccumulation and DGT metal fluxes (measured in different compartments of the sediment and overlying water) and AEM concentrations (measured in the top 1cm of the sediment).

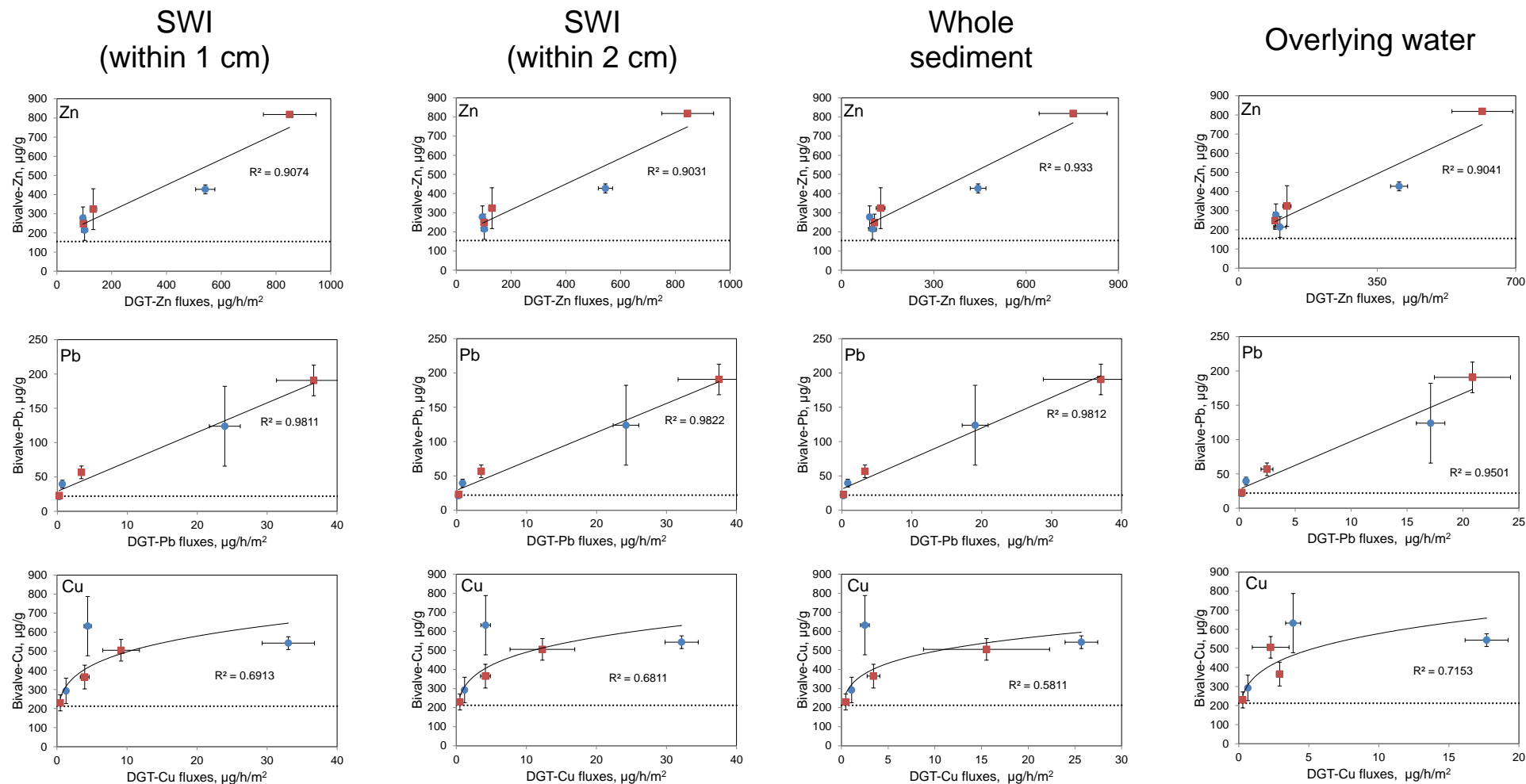


Figure A4.2 Relationships between metal bioaccumulation in *T. deltoidalis* exposed to low (bivalves only, circles) and high (bivalves and amphipods, squares) bioturbation conditions and DGT metal fluxes measured in different compartments of the sediment and overlying water. DGT fluxes are average values of first (day 8) and second (day 23) deployment. Data points are mean values with standard error (n=3 and 6 for tissue concentrations and DGT fluxes, respectively). The dotted line indicates average metal concentrations measured in non-exposed bivalves.

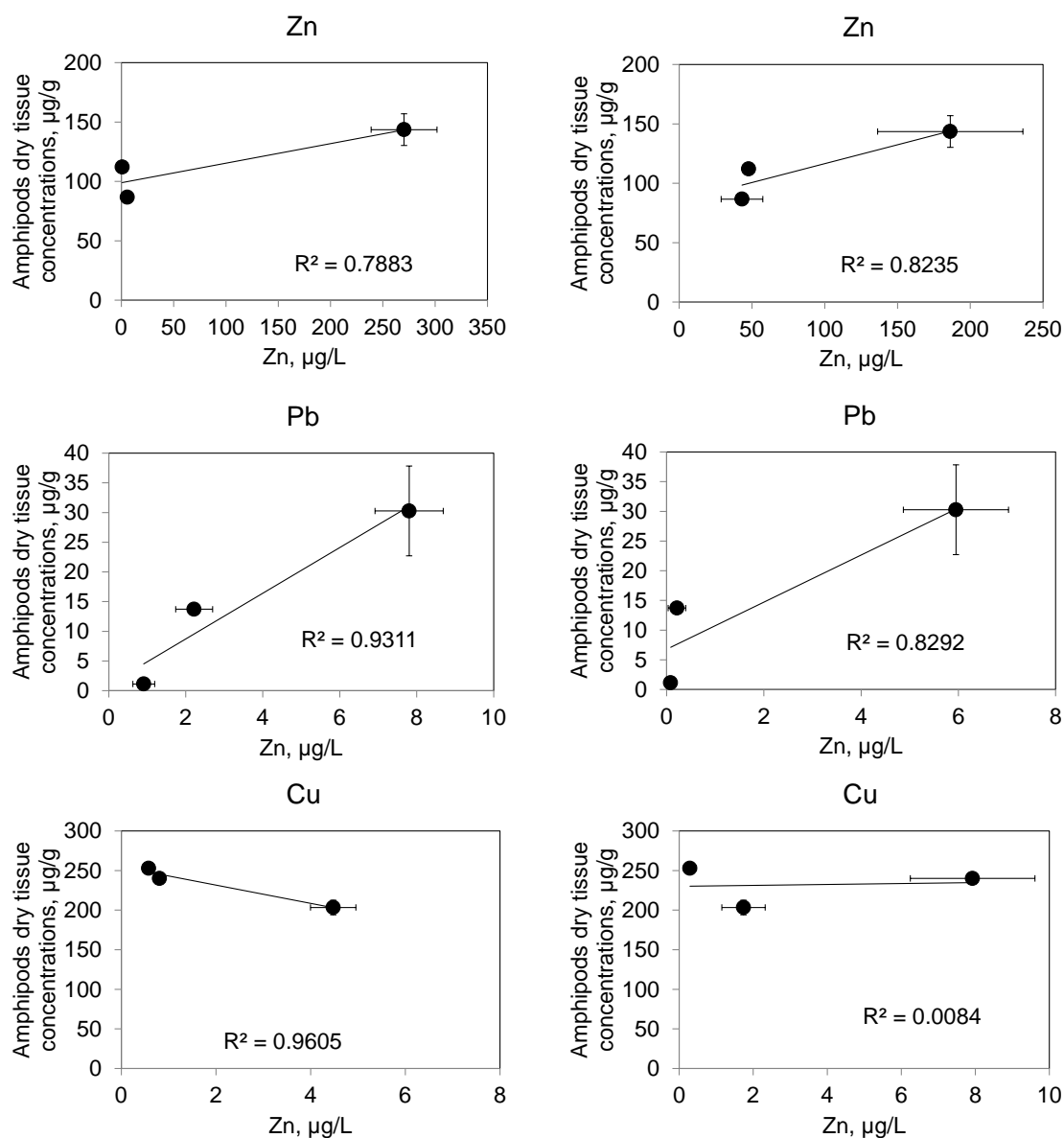


Figure A4.3 Comparison between relationships obtained using DGT and dissolved metal concentrations with bioaccumulation in *V. australiensis*. DGT fluxes are average values of first (day 8) and second (day 23) deployment. Dissolved metal concentrations are average values of overlying water subsamples collected throughout the test. Data points are mean values reported with standard error (n=6 and 15 for DGT and dissolved metal concentrations, respectively).

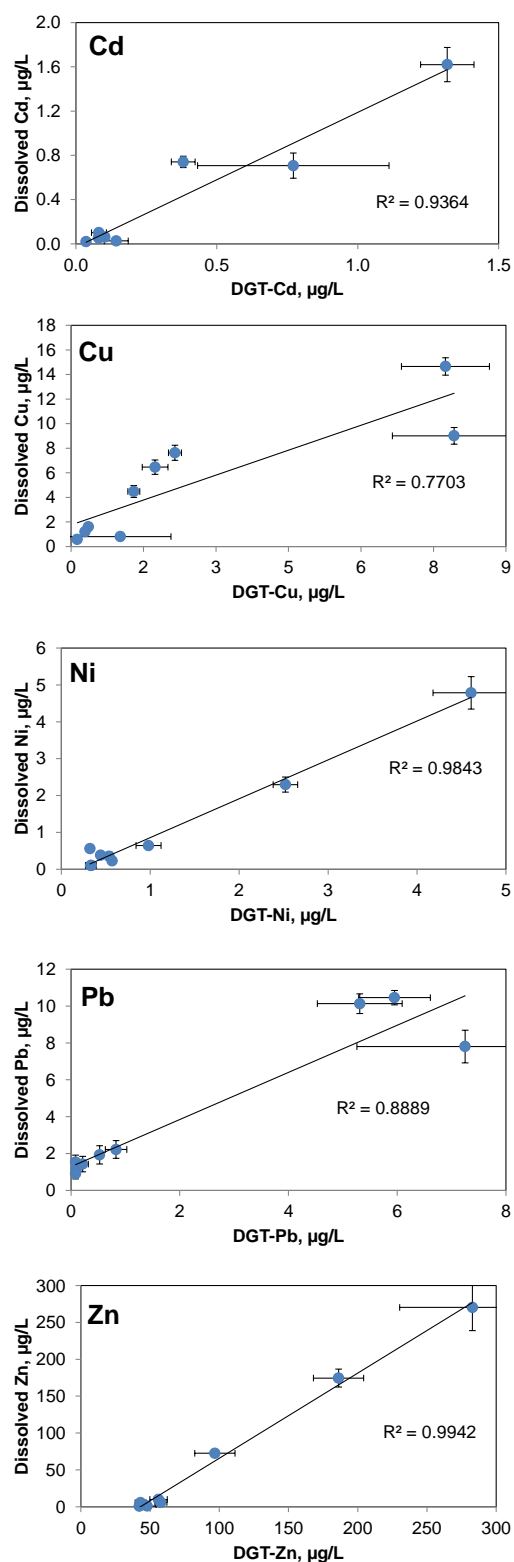


Figure A4.4 Relationships between DGT metal concentrations and dissolved metal concentrations measured in sediments overlying waters. DGT concentrations are mean values with standard error of 3 replicate measurements performed on day 8 and 23 (n=6). Dissolved metal concentrations are the average concentration measured in OLW aliquots sampled throughout the test reported with standard error (n=15).

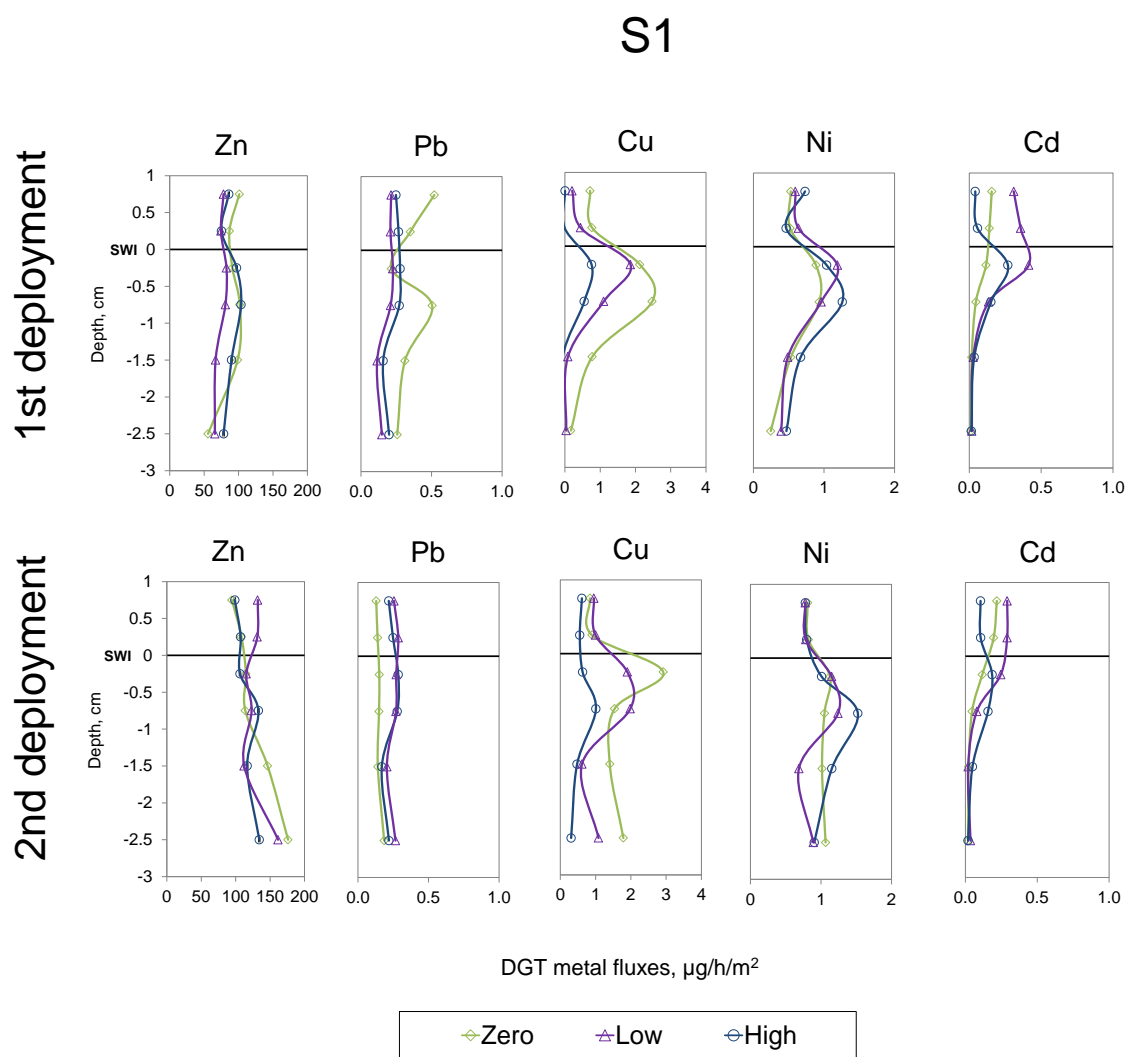


Figure A4.5 DGT vertical profiles measured in pore and overlying waters in sediment S1 exposed to i) zero (no organisms), ii) low (bivalves) and iii) high (amphipods and bivalves) bioturbation (average of first (day 8) and second (day 23) deployment).

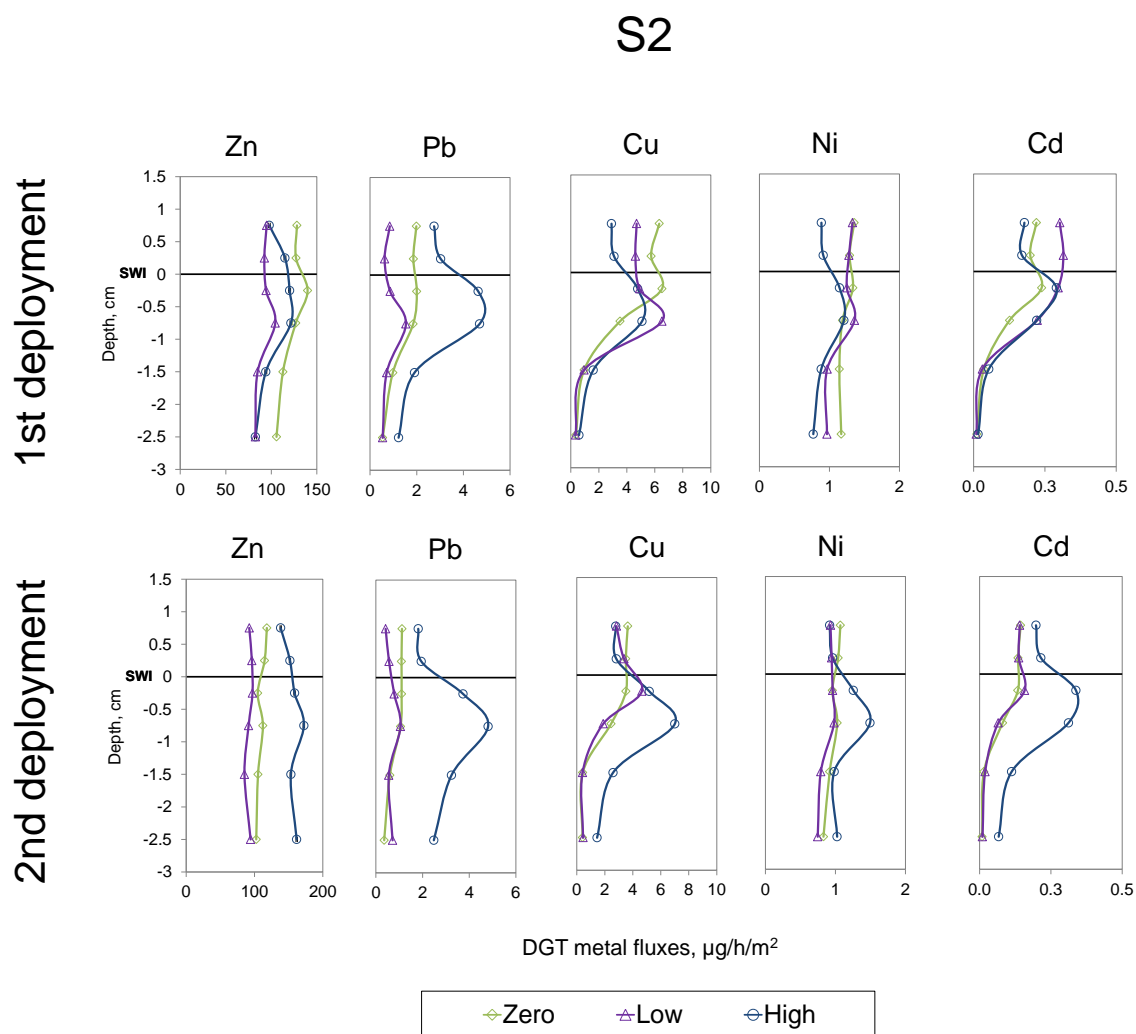


Figure A4.6 DGT vertical profiles measured in pore and overlying waters in sediment S2 exposed to i) zero (no organisms), ii) low (bivalves) and iii) high (amphipods and bivalves) bioturbation (average of first (day 8) and second (day 23) deployment).

S3

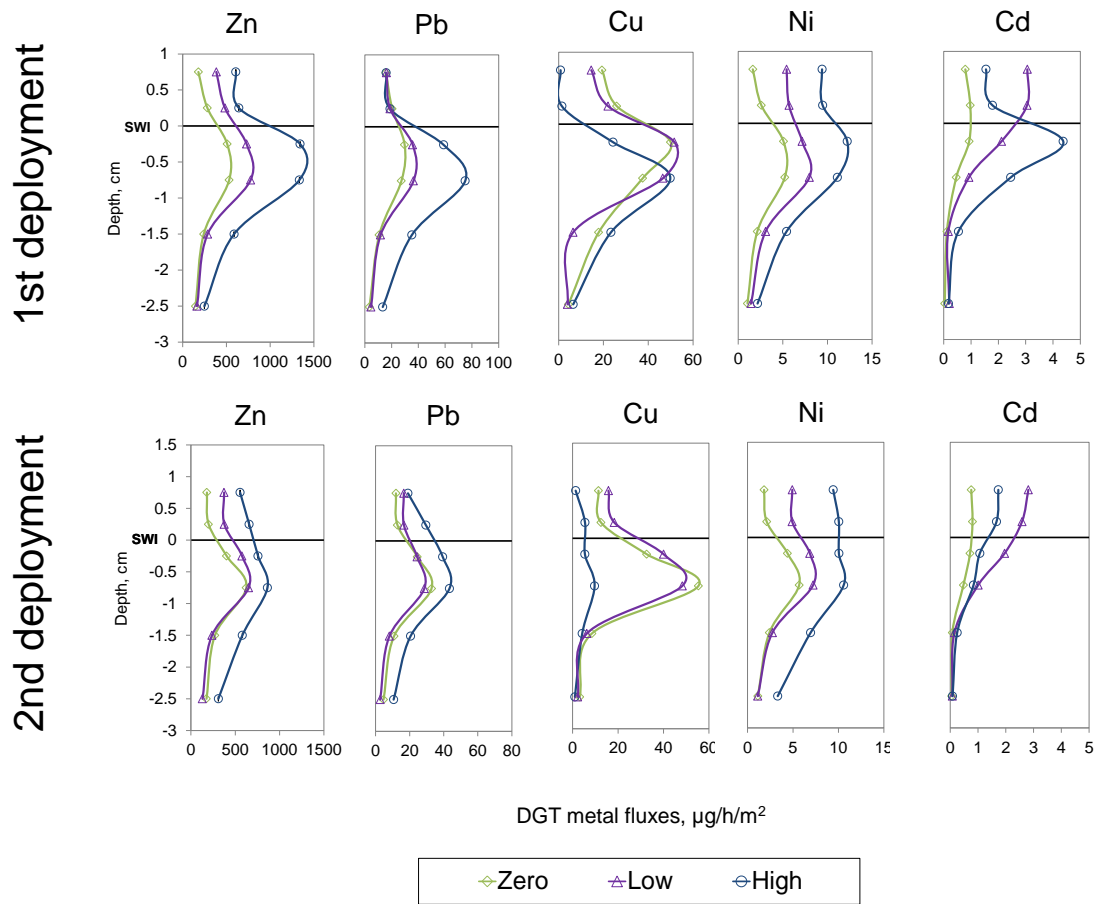


Figure A4.7 DGT vertical profiles measured in pore and overlying waters in sediment S3 exposed to i) zero (no organisms), ii) low (bivalves) and iii) high (amphipods and bivalves) bioturbation (average of first (day 8) and second (day 23) deployment).

Appendix 5

1 **Running title:** Diffusive gradients in thin films predict metal-mixture toxicity in sediments

2
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6
7 **Rationale:** Many regulatory frameworks for sediment quality assessment incorporate procedures for
8 determining contaminant bioavailability and potentially modifying guideline values. However, there
9 are many inadequacies with the methods currently used. The deployment of diffusive gradients in thin
10 films (DGTs) probes in sediments has the potential to provide a time-integrated measure of the pool
11 of potentially bioavailable metals *in situ*. The technique measures fluxes of metal mixtures in pore
12 waters and labile, weakly bound metal forms that are readily released from particles.

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

Article Type: Research article

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

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

47
48
49 Keywords: Diffusive gradients in thin films; Metal fluxes; sub-lethal toxicity; amphipod;
50 sediment quality assessment

51

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 Roullet 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 sediment and overlying water 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 ($24\text{ cm} \times 4\text{ cm} \times 0.5\text{ cm}$, with open window of $1.8\text{ cm} \times 15\text{ cm}$) 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 $0.5\text{ }\mu\text{g/h/m}^2$ (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 described²⁹ 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 (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 3.1%) 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 ($\Sigma \text{Cd, Cu, Ni, Pb}$ and Zn , where $\text{AEM} = \text{SEM}$) 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 ($\text{AE-Zn/TR-Zn} \sim 0.8, 1$ and 0.7 and $\text{AE-Pb/TR-Pb} \sim 0.6, 0.9$ 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 ~ 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, , 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 $1 \mu\text{g/h/m}^2$, 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. From the logistic regressions for survival, r^2 values were 0.536, 0.364, 0.920 for AEM_{SQGQ}, TRM_{SQGQ} and OLW_{TU}, respectively. Due to the large explained variance 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} correlations 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). The purpose of the normalisation was to account for, as best as possible, the differing toxicity of the different metals. 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 normalized to the SQGs (Figure 3), although all relationships appeared quite similar. From the logistic regressions for survival, pseudo- r^2 of 0.669, 0.496, 0.503 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) $\mu g_{WQG}/h/m^2$ 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) $\mu g_{WQG}/h/m^2$, respectively. Thus, adverse effects on survival and reproduction were predicted for DGT_{WQG} fluxes exceeding 36 and 17 $\mu g_{WQG}/h/m^2$, 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 ($p = 0.0464$), 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 ($p=0.00177$). For the Kings Bay and Five Dock Bay sediments, sub-lethal effects to reproduction could not be attributed to any one metal or combination (statistical analyses were not performed due to the large variance of the data), 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 $31 \mu\text{g}_{(\text{WQG})}/\text{h}/\text{m}^2$) and DGT-Pb fluxes (up to $2.3 \mu\text{g}_{(\text{WQG})}/\text{h}/\text{m}^2$, 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 ($36 \mu\text{g}_{(\text{WQG})}/\text{h}/\text{m}^2$) 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 $>36 \mu\text{g}_{(\text{WQG})}/\text{h}/\text{m}^2$; (ii) a region which affects reproduction but not survival for fluxes between 17 and $36 \mu\text{g}_{(\text{WQG})}/\text{h}/\text{m}^2$; and (iii) a no-observed effect (to reproduction or survival) region for fluxes $<17 \mu\text{g}_{(\text{WQG})}/\text{h}/\text{m}^2$.

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.²²⁻²⁴ Although the technique appears to have limitations to predict toxicity caused by particle ingestion and dietary behaviours,²² 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.

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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).

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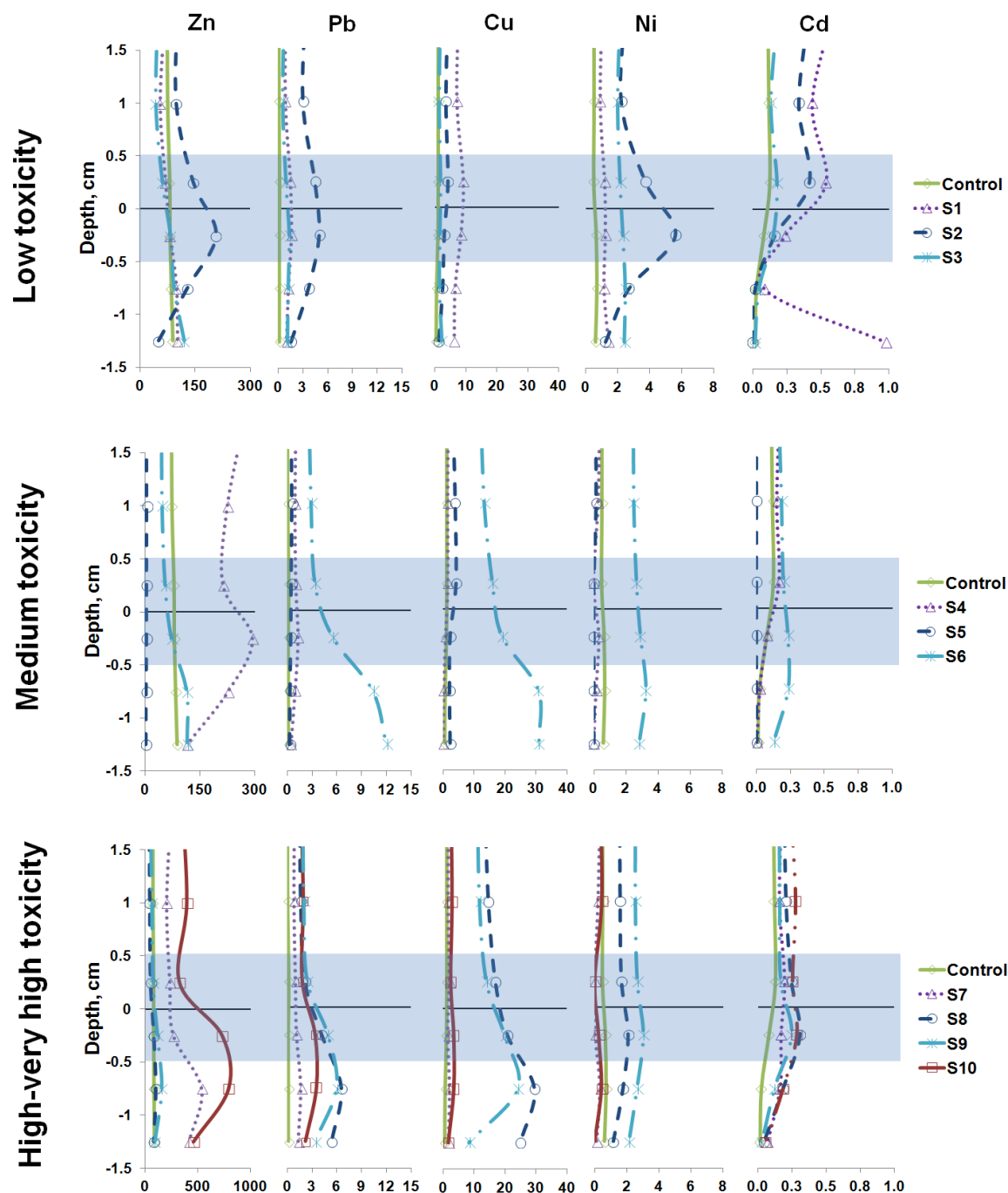


Figure 1. DGT profiles of Zn, Pb, Cu, Ni and Cd measured within 1.5 cm of the sediment water interface in test vessels during the amphipod bioassay. Points represent average values of three replicates (standard deviations ranged between 30 and 40% of mean values). Shaded areas indicate the DGT profile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

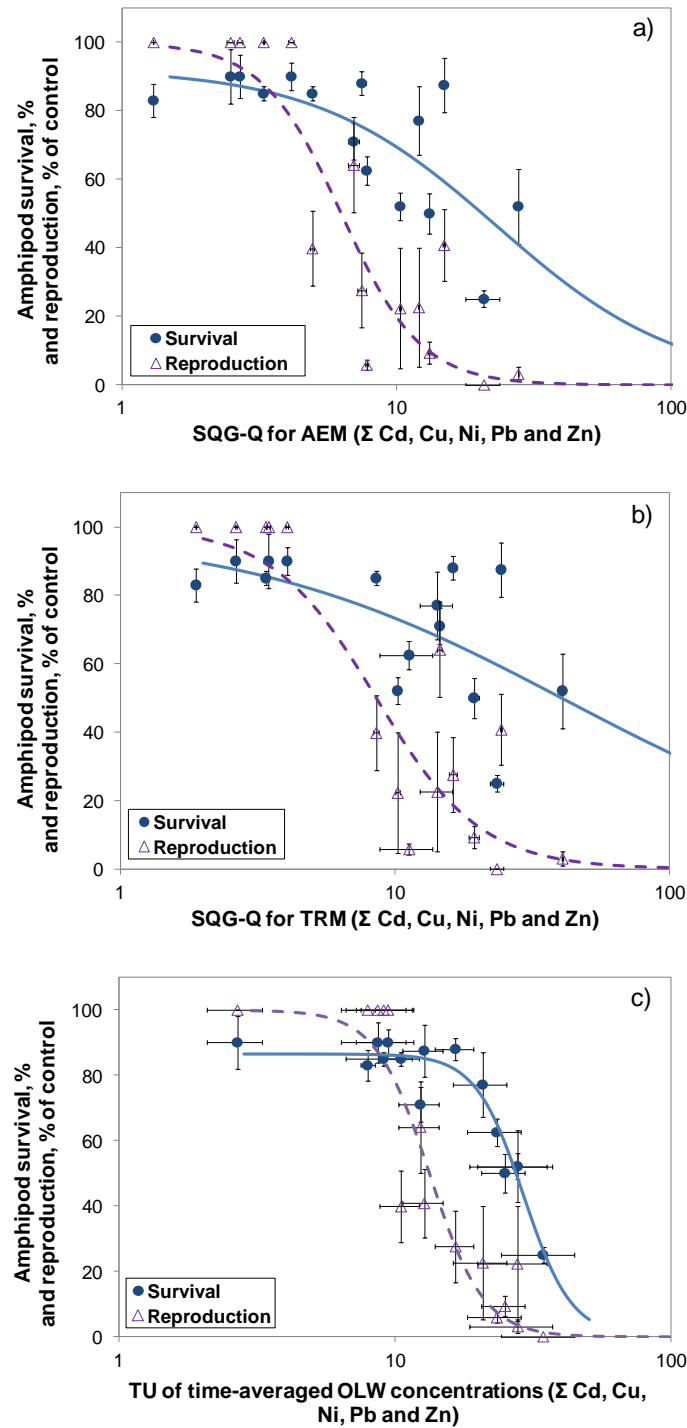


Figure 2. Dose-response relationships between amphipod survival and reproduction and different methods: a) AEM concentrations (Σ Cd, Cu, Ni, Pb and Zn) normalized to sediment quality guidelines (SQGs); b) TRM concentrations (Σ Cd, Cu, Ni, Pb and Zn) normalized to SQGs; c) OLW concentrations (Σ Cd, Cu, 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 ($n = 4$).

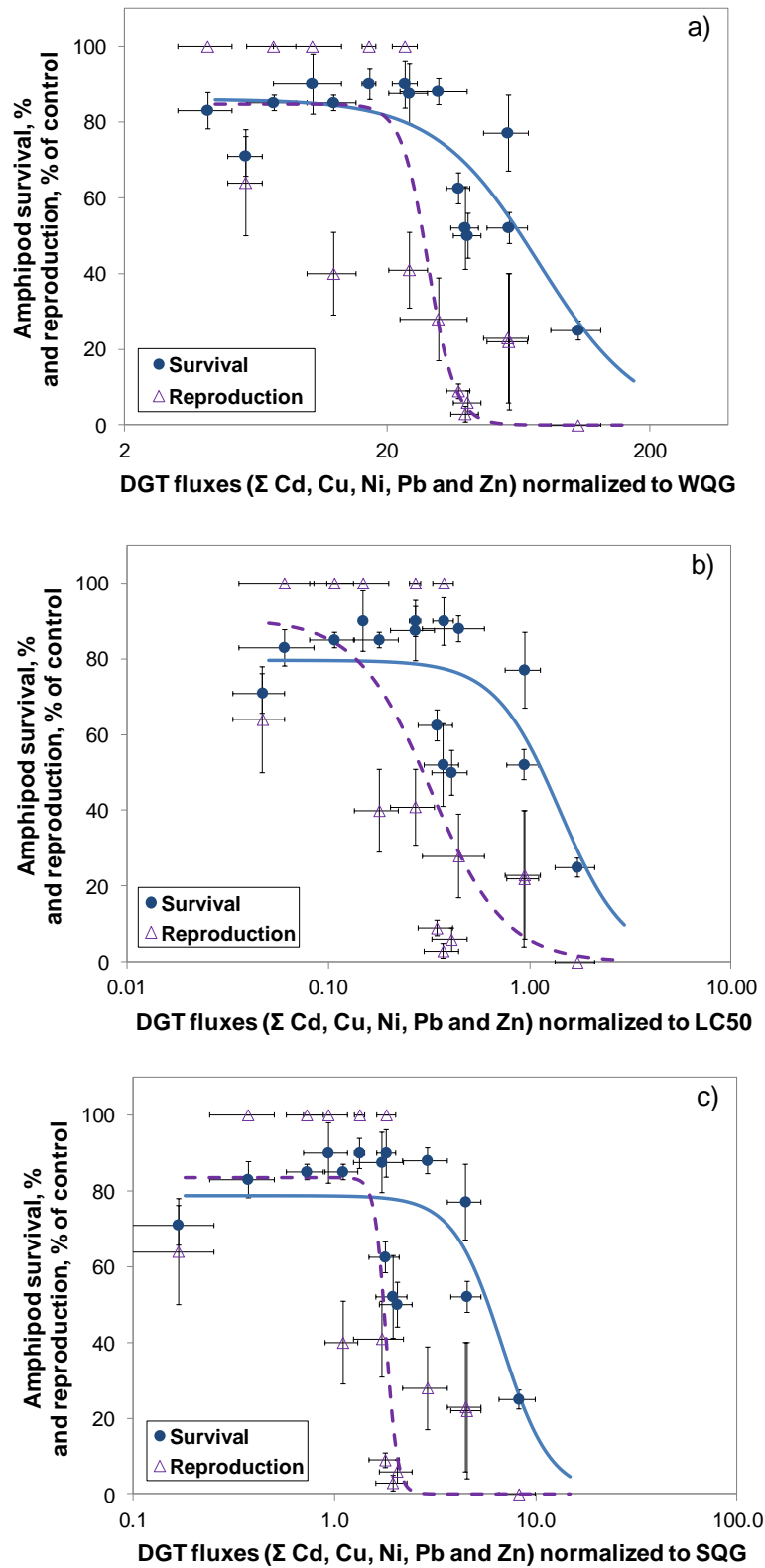


Figure 3. Relationships between amphipod survival and reproduction and DGT fluxes normalized to a) WQGs, b) LC50 values and c) SQGs (as described in methods). Mean values of DGT fluxes (Σ Cd, Cu, Ni, Pb and Zn) were calculated for $n = 3$ and reported with standard deviation. Survival and reproduction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

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	µmol/g	µmol/g	%	%
Control	C1	90 ± 6	100	22	41	100	14	41	88	<0.5	1.9	1.7	50
	C2	90 ± 4	100	38	68*	180	22	70*	150	<0.5	3.2	3.1	90
	C3	90 ± 8	100	35	65*	190	23	59*	160	<0.5	3.1	3.6	100
	C4	85 ± 2	100	26	50	120	13	50	100	<0.5	2.1	2.6	70
	C5	83 ± 5	100	9	21	42	4	17	34	<0.5	0.7	0.7	20
LOW	S1	88 ± 8	41 ± 10	620*	450*	900*	260*	380*	570*	<0.5	15	4.7	100
	S2	88 ± 3	28 ± 11	220*	360*	710*	<1	190*	490*	30.1	-22	4.3	70
	S3	85 ± 2	40 ± 11	120*	170*	340*	20	120*	270*	1.5	4	3.1	30
MEDIUM	S4	77 ± 10	23 ± 17	80*	180*	1600*	3	150*	1600*	7.4	18.5	4	15
	S5	71 ± 5	64 ± 14	230*	310*	620*	3	180*	470*	4.1	4	6.6	30
	S6	63 ± 4	6 ± 2	290*	220*	400*	150*	200*	270*	<0.5	9	1.4	25
HIGH	S7	52 ± 4	22 ± 18	55	110*	1200*	16	120*	1300*	1.9	19.5	1.4	25
	S8	52 ± 11	3 ± 2	1070*	760*	1500*	540*	700*	980*	<0.5	36	5.7	100
	S9	50 ± 6	9 ± 3	510*	360*	680*	240*	340*	480*	<0.5	19	2.9	50
VERY HIGH	S10	25 ± 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 ± 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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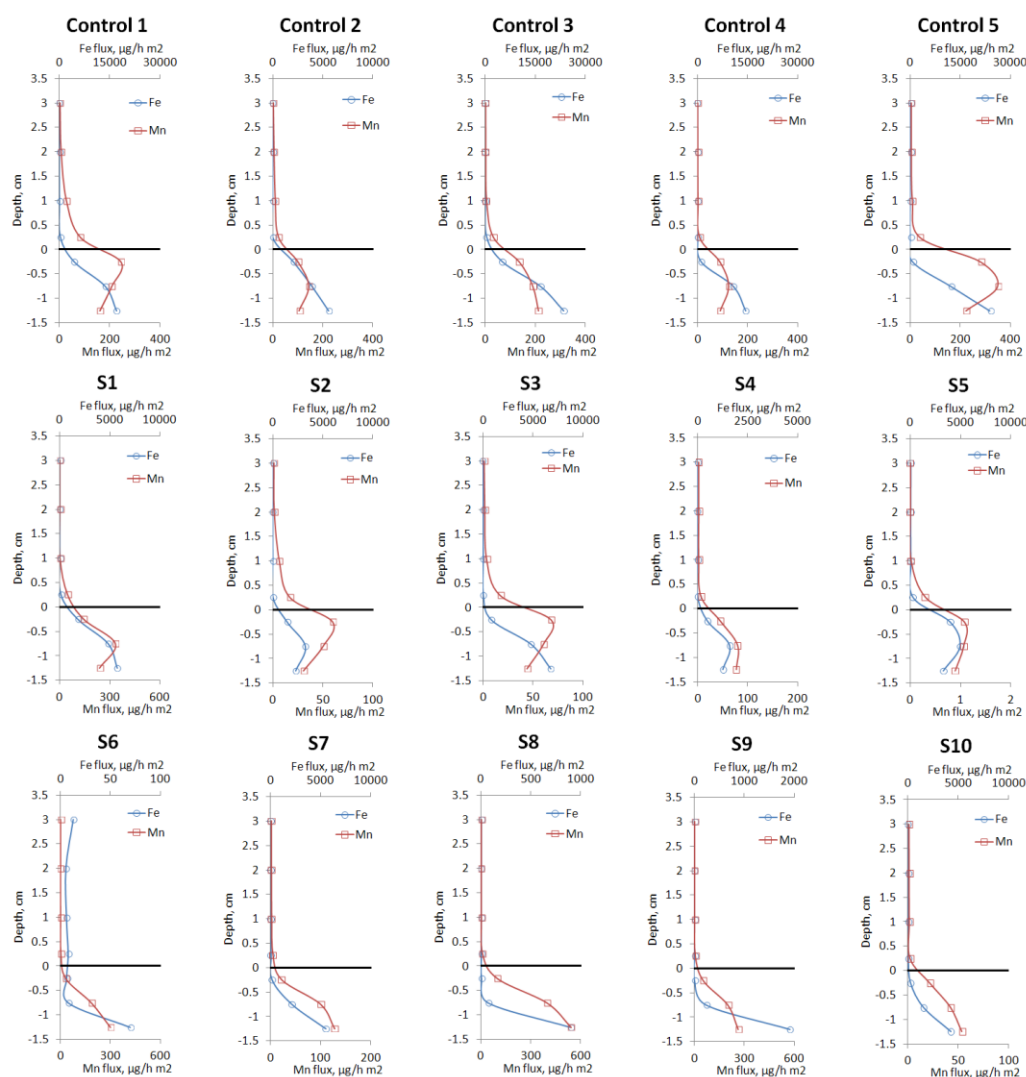


Figure S1. DGT-Fe and -Mn vertical profiles in pore waters and overlying waters (average values, $n = 3$, standard deviations ranged between 50 and 60% of mean values).

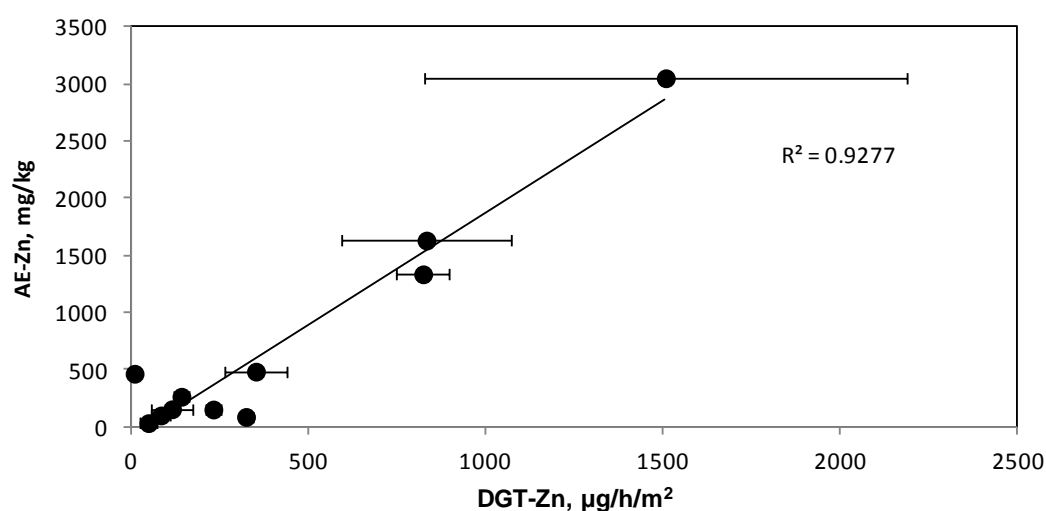


Figure S2. Relationships between DGT-Zn fluxes and AE-Zn concentrations in control (C1-C5) and contaminated sandy sediments (S2, S3, S4, S5, S7, S10). DGT data are reported as mean values ($n=3$) with standard deviation. Variability in metal concentration between treatments (of the same homogenised sediments)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

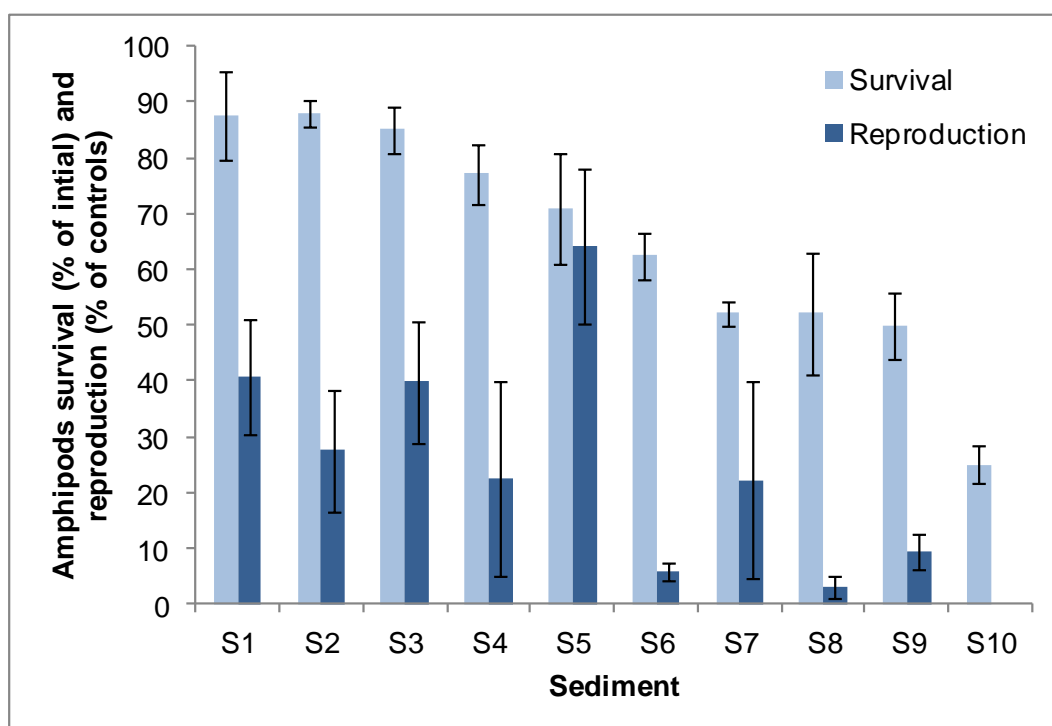


Figure S3. Amphipod survival (percentage of the number of animals placed in each test vessel at the beginning of the test) and reproduction (percentage of controls) in contaminated sediments: Kings Bay (S2, S3, S5); Five Dock Bay (S4, S7, S10); Port Kembla (S1, S6, S8, S9). Error bars of average values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

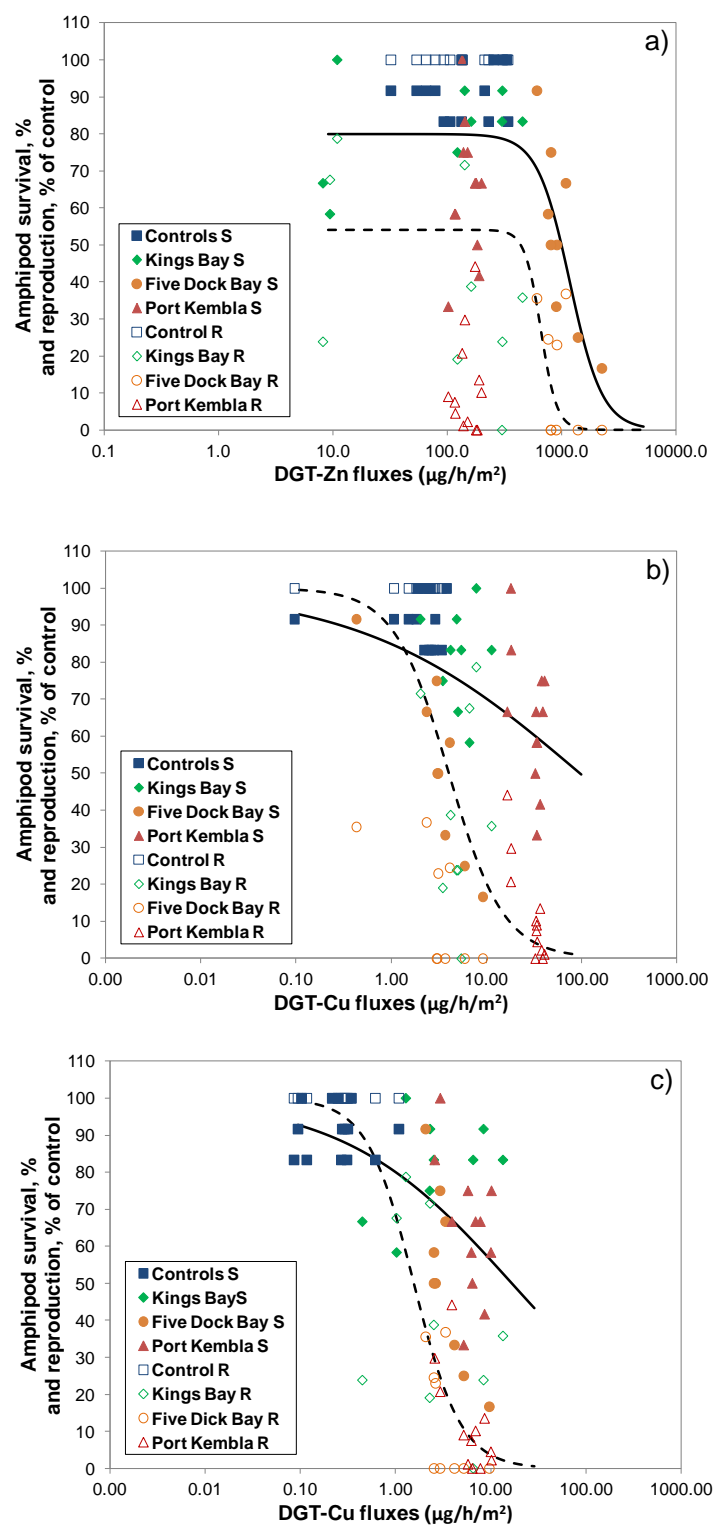


Figure S4. Dose-response relationships between amphipod survival and reproduction and DGT-Zn, -Cu and -Pb fluxes. Solid and dashed lines represent survival and reproduction fitting curves, respectively.

Table S1. Metal concentrations in the control and contaminated sediments.

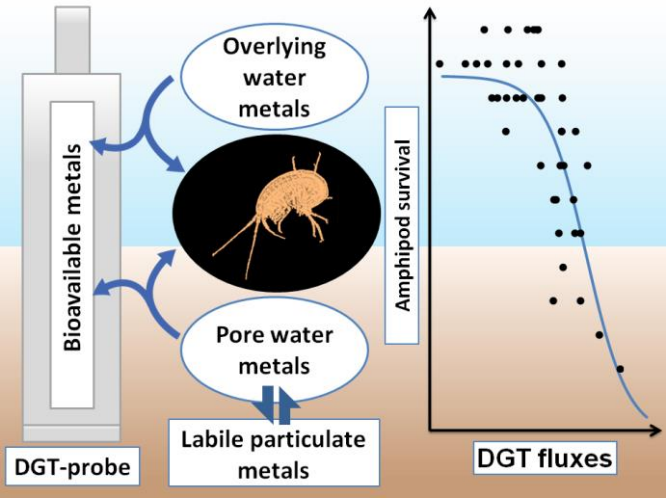
Sediment	TRM, mg/kg				AEM, mg/kg			
	Cd	Ni	Fe	Mn	Cd	Ni	Fe	Mn
C1	<1	13	17800	66	<1	11	5100	38
C2	<1	7	6350	28	<1	5	1800	22
C3	<1	9	15200	56	<1	7	5400	37
C4	<1	13	24500	87	<1	9	9400	58
C5	<1	12	25800	100	<1	3	10900	71
S1	<3	19	44800	320	<1	5	12400	130
S2	<1	22*	17200	76	<1	5	4300	23
S3	<1	14	12500	57	<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

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**The mismatch between bioaccumulation in field and laboratory environments:
interpreting the differences for metals in benthic bivalves.**

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routes, condition-index, benthic bivalve.

ABSTRACT

Laboratory-based bioaccumulation and toxicity bioassays are frequently used to predict the ecological risk of contaminated sediments in the field. This study investigates the bioassay conditions most relevant to achieving environmentally relevant field exposures. An identical series of metal-contaminated marine sediments were deployed in the field and laboratory over 31 days. Changes in metal concentrations and partitioning in both sediments and waters were used to interpret differences in metal exposure and bioaccumulation to the benthic bivalve *Tellina deltoidalis*. Loss of resuspended sediments and deposition of suspended particulate matter from the overlying water resulted in the concentrations of Cu, Pb and Zn (major contaminants) becoming lower in the 1-cm surface layer of field-deployed sediments. Lower exchange rates of overlying waters in the laboratory resulted in higher dissolved metal exposures. The prediction of metal bioaccumulation by the bivalves in field and laboratory was improved by considering the metal partitioning within the surface sediments.

Capsule

To improve the value of field- and laboratory-based sediment bioassays in ecological risk assessments, it is necessary to create exposure conditions that resemble those in the field.

Introduction

Laboratory-based bioassays are routinely used during the assessment of risk posed by contaminated sediments (ASTM, 2010; Casado-Martinez et al., 2006; Kennedy et al., 2009; Simpson and Spadaro, 2011). However, the conditions by which organisms are exposed to sediments in the laboratory can be quite different from those that occur from the same sediments in the field (Burton et al., 2005; Liber et al., 2007). Static laboratory-based bioassays frequently result in much higher dissolved contaminant concentrations in overlying waters than occurs for the same sediment in the field. This is due to the higher sediment to overlying water ratios that occur in laboratory vessels in comparison to field sites, and continuous renewal of overlying water at field locations, further diluting contaminant fluxes from the sediments to the overlying water (Mann et al., 2010; Rosen et al., 2012). Organisms within field-based bioassays have to contend with greater variation in conditions than laboratory assays, including dissolved oxygen, light, temperature, turbidity and resuspension of sediments, tidal flows and competition from other organisms, including predation (Ringwood and Keppler, 2002; Anderson et al., 2004). Uncaged organisms in field locations may also reduce their exposure to contaminants by avoidance behaviours (Ward et al., 2013a,b). As such, the relationship between laboratory and field exposures has been the topic of ongoing debate.

Organisms behaviours is influenced by the surrounding environment and, in particular, food availability and quality. Aquatic organisms obtain nutrition from both the dissolved and particulate phases of the environment, utilising both passive uptake and active ingestion (Rainbow, 2007). For benthic organisms, ingestion processes, establishing/maintaining habitats (e.g., burrowing) and irrigation of such habitats often cause sediment bioturbation, a process which alters sediment substrata by perturbing redox stratification, porewater equilibria and increases oxidation of metal-binding phases such as acid-volatile sulfide (AVS) (Riedel et al., 1997; Simpson and Batley, 2003; Atkinson et al., 2007; Simpson et al., 2012). These activities influence contaminant bioavailability from the perspective of both chemical (contaminant partitioning and speciation) and biological exposures (organisms burrowing and feeding behaviour) (Ciutat and Boudou, 2003; Atkinson et al., 2007; Simpson et al., 2012).

Here we compare the changes in the dissolved and particulate exposure and bioaccumulation of metals to the estuarine benthic deposit-feeding bivalve *Tellina deltoidalis* for the same series of metal-contaminated sediments in the laboratory and field over 31 days. The deployed sediments allowed the influence of contaminant concentrations and properties on the metal exposure and bioaccumulation to be assessed. Changes in metal concentrations

and sediment properties influencing metal bioavailability (e.g. particle size, AVS, TOC) in the surface and deeper sediments, metal concentrations in overlying waters, and metal bioaccumulation and bivalve health using a Condition Index were used in the interpretation of the results.

Material and Methods

General methods. All glass and plastic-ware for analyses were new and cleaned by soaking in 10% (v/v) HNO₃ (BDH, Analytical Reagent grade) for ≥ 24 h, followed by thorough rinsing with deionized water (Milli-Q, 18 M Ω ·cm). Test vessels used for bioassays were washed in a dishwasher (Gallay Scientific) sequentially with phosphate-free detergent, 1% HNO₃ and Milli-Q water. All chemicals were analytical reagent grade or equivalent analytical purity. Water pH, salinity, temperature and dissolved oxygen measurements were made with probes from WTW (Wissenschaftlich-Technische Werkstätten) calibrated according to manufacturer instructions. Dissolved ammonia was analysed colorimetrically using a Merck Spectroquant Kit (14752, Merck). The fraction of fine sediment (<63 μ m) was determined by wet sieving through 63 μ m nylon mesh followed by gravimetry, and total organic carbon (TOC) determined by high temperature TOC analyser (OC) after the removal of inorganic carbon with 1 M HCl until effervescence was complete.

Overlying water samples were membrane filtered (0.45 μ m cellulose acetate, Sartorius Ministart) immediately after collection and acidified with concentrated HNO₃ (2% (v/v), Tracepur, Merck). Methods for analyses of total recoverable metals (TRM, by microwave assisted aqua regia) were performed as per Strom et al. (2011), and dilute acid-extractable metals (AEM, 1 M HCl) and acid-volatile sulfide (AVS) (all determined on sub-samples of the same homogenised sediment) as per Simpson (2001). Biota tissues (pool of seven individuals per sample) were freeze-dried before microwave-assisted (MARS Express) nitric acid extraction (HNO₃ at 200 °C for 30 min) as described in King et al. (2010). Dissolved metal concentrations in acid-digests of waters, sediment, and biological tissue samples were determined by a combination of inductively coupled plasma - optical emission spectrometry (ICP-OES, Varian 730-ES) and inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7500ce).

For quality assurance, filter and acid-digest blanks, duplicate analyses for 20% of all samples, sample spike recovery and certified reference material (CRMs) analyses were performed. Duplicates were within 20% and recoveries for spikes and CRMs, PACS-2 for sediment (National Research Council Canada, NRCC, Ottawa, ON, Canada) and DORM-3

for biota (*Mytilus galloprovincialis*, NRCC), were within 85-115% of certified values. The limits of reporting for the various methods were less than 10% of the lowest measured values.

Test media and organisms. Clean seawater was collected from Port Hacking, Sydney (New South Wales, Australia), membrane filtered (0.45 µm) and stored in the dark at 4°C. Where necessary, the salinity of the filtered seawater was adjusted to the test salinity of 30 PSU using deionised water. Prior to use in experiments all waters were acclimated in a temperature-controlled room (21±1°C).

Control sediments were collected from an estuarine location in Bonnet Bay, Sydney, following the procedure previously described by Strom et al. (2011). This silty sediment (95% <63 µm) had been previously characterised and found to have relatively low concentrations of metal and organic contaminants (Spadaro et al., 2008), and supported the survival and growth of the bivalve *T. deltoidalis* (King et al., 2010; Campana et al., 2013). The silty control sediment was used either unmodified (Sediment 1) or as a 30:70 mixture with clean sand (98% >180 µm) (Sediment 5). Contaminated sediments were collected from Port Kembla (Sediment 4) and three sites in Sydney Harbour (Sediments 6, 7 and 8). Previous studies found these sediments contained concentrations of Cu, Pb and Zn which greatly exceeded the sediment quality guideline values, and caused toxicity to amphipod reproduction (Amato et al., 2014), but relatively low concentrations of organic contaminants (Chariton et al., 2010; Dafforn et al., 2012). All sediments were sieved (< 1 mm, plastic mesh), homogenized and stored at 4° C in the dark until use. A dilution series was created by mixing Sediment 4 (contaminated) with Sediment 1 (control) (both 95% <63 µm) at ratios of 1:1 (Sediment 3) and 1:3 (Sediment 2). Mixing occurred in a nitrogen atmosphere and the sediments were equilibrated for four weeks before use (Simpson et al., 2004). Test sediments were grouped into two series (silty S1-S4, or sandy S5-S8) according to their physical properties.

The *T. deltoidalis* (shell lengths of 5-12 mm) were collected from Lane Cove River (NSW, Australia) and maintained as described previously (Atkinson et al., 2007; King et al., 2010; Campana et al., 2013). Bivalves were analysed for both metal content (soft tissues after 24-h depuration) and Condition Index. Condition Index calculations were made on seven bivalves per sediment sample according to Freeman (1974), in which Condition Index = (dry soft body mass/dry shell mass) × 100. Further details about the sediments and bivalve are provided in the Supporting Information.

Field bioaccumulation bioassays. The field-deployed bioassays were performed in chambers (1-L Nalgene bottles with three 4.5 cm × 8 cm openings cut in the sides to allow water circulation) held within cages deployed in an uncontaminated section of the Woronora

River estuary (Sydney, NSW, Australia) for 31 days. Dimensions and photographs of the chambers and cages are provided in the Supporting Information. The cages were suspended from a floating boat pontoon that allowed cages to maintain a submerged depth of 40 cm, irrespective of tidal cycles. There were two replicates of each sediment sample (sediments 1-8) and these were randomly distributed in the cages.

Water quality parameters were measured twice weekly in the field for pH (7.5-8), salinity (31-33 PSU), temperature (21-24°C) and dissolved oxygen concentration (>80% saturation). Suspended particulate matter (SPM) was collected using sediment traps (100 mL polycarbonate vials, 4 cm diameter × 9 cm high) secured within the inner section of each cage on day 1 and changed on days 5, 10 and 20 (16 vials in total). Dissolved metals were monitored using diffusive gradients in thin films (DGT). Piston covers were obtained from DGT Research Ltd (Lancaster, UK) and DGTs were prepared and assembled following standard procedures (Zhang and Davison, 1995; <http://www.dgtresearch.com/>) with Chelex binding resin. DGTs were deployed between the cages (40 cm below surface) for 5 days in triplicate on days 0, 10, 17, 21 and 26 of the deployment period (15 DGTs in total). Upon retrieval the pistons were thoroughly rinsed with deionised water, placed individually in acid-washed plastic bags in a cooler box and transported to the laboratory. Pistons were disassembled within 24 to 48 h of retrieval, the Chelex resins were eluted in 1 M HNO₃ (Merck, Suprapur) and analysed by ICP-MS (Agilent-7500). Approximately 10% of all DGT pistons prepared were used for blank measurements. The limit of reporting (3 × detection limit determined from the blanks) was 0.1 µg/L for DGT-Cu, -Pb, -Mn and -Zn. Further details of the DGT preparation and analyses are provided in Supporting Information.

On day 1 the seven bivalves were added to test sediments (520 g or 5.1 cm depth) contained in the modified 1-L LDPE bottles (see Supporting Information) and allowed to bury into sediments before deployment from the pontoon. With care not to disturb the sediments, cages were lowered into the water at the field site allowing field seawater to circulate naturally. The tests were terminated on day 31 when the cages were removed from the water, returned to the laboratory, and disassembled within 2 h. Samples of sediments were taken from three depths (surface layer (1 cm), 2-3 cm below the sediment surface, and >3 cm depth) using a spatula as these layers were removed to locate the bivalves. Surviving organisms were counted, depurated without feeding for 24 h in clean seawater under the same laboratory conditions, and shells opened using a Teflon coated razor blade. The soft tissue was stored at -20°C until analysis.

Laboratory bioaccumulation bioassays. The laboratory-deployed bioassays were conducted in a temperature controlled room (21±3°C) under ambient austral spring light

conditions (1000 lux) from dawn (8 am) to dusk (5 pm) for 31 days. One day prior to the start of the bioassay, two replicates of test sediments (520 g wet weight or 5.1 cm depth) and overlying water (500 mL) were added to 1-L LDPE bottles with aeration to provide circulation (King et al., 2010). At the beginning of the test, the overlying water was renewed and seven bivalves were added to each test bottle. Overlying waters were continuously aerated and renewed twice a week after sub-sampling for dissolved metals. Organisms were also fed twice per week with 1 mg/bivalve of Sera Micron (Sera Fishtamins) which contained 3, <0.2, 2.8, 16, 1300, 54, 2.8, 1, and 34 mg/kg of As, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn, respectively (total metals). The overlying water was monitored every second day to confirm major parameters remained within desired ranges (pH 8.2±2, salinity 31±1 PSU, temperature 21±1°C and dissolved oxygen >80% saturation). The tests were terminated on day 31 in the same manner as the field-deployed sediments.

To investigate the influence of the seawater quality on metal bioaccumulation and bivalve CI, additional replicates of Sediments 5 and 6 (named 5FW (sandy control) and 6FW (sandy contaminated), respectively) were tested using unfiltered seawater collected from the field deployment site. No food was supplied to these additional replicates.

Statistical analyses. At test completion the percent survival in tests relative to controls was recorded. Statistical analyses were carried out using the software Toxcalc (Version 5.0.23, TidePool Scientific Software), NCSS (Kaysville, Utah) or Statgraphics Centurion (Warrenton, Virginia). Unless otherwise stated $p = 0.05$ was the level of significance. Correlations (Pearson's product-moment) were performed on the particulate metal concentrations. Condition Index data and concentration of metals bioaccumulated in *Tellina deltoidalis* were tested for significant statistical differences. Prior to the hypothesis testing, data were tested for homogeneity of variance (Levene test) and for normality of residuals distribution (Shapiro-Wilk's test). Since the data either did not follow a normal distribution or it was not possible to test the data heteroscedasticity, two-way analysis of variance of Kruskal-Wallis test followed by Bennett squares sum partition method were applied to evaluate statistical differences. Logistics relating to time and resources limited the sediment replicates to n=2 containers. Although low replicates can lead to an inability to detect differences (type II error), the two replicates were found to be suitable for detecting differences between the laboratory and field data in this study.

Results and Discussion

Properties of the sediments

The chemical and physical properties of control and metal contaminated sediments are shown in Table 1. Total recoverable (TRM) and dilute acid-extractable (AEM) metal concentrations for a greater range of metals and metalloids (Al, Fe, Mn, As, Cd, Co, Cr, Cu, Ni, Pb, Sn, V and Zn) are provided in the Tables S1 and S2 of the Supporting Information. Metal concentrations in diluted silty sediments were within 20% of the expected values, and the AEM concentrations provided a contamination gradient for Cu, Pb and Zn from 22 to 525, 63 to 721 and 166 to 1000 mg/kg, respectively. Excluding the controls (Sediments 1 and 5), the %-silt (<63 µm), AVS and TOC were 81-94%, <0.1 µmol/g and 3.6-5.7% in the silty sediments, and 14-31%, 4.2-9.7 µmol/g and 2.1-5.9% in the sandy sediments, respectively. For sandy sediments the AEM concentrations were much higher for zinc (48-2410 mg/kg) than copper (5-57 mg/kg) and lead (17-242 mg/kg). When the control sediments were excluded, the silty sediments had the highest copper, generally higher lead and lower zinc concentrations than sandy sediments.

The average AEM/TRM ratios in the silty (S2-S4) and sandy (S6-S8) sediments were 0.95 and 0.80 for Pb, 0.70 and 0.90 for Zn, and 0.47 to 0.29 for Cu, respectively (Table S3). The copper sulfide phases that form in sediments (CuS, Cu₂S) are mostly insoluble in 1 M HCl (Simpson et al., 1998; 2000), and the low AE-Cu/TR-Cu ratio of 0.29 reflects this. AEM concentrations of arsenic (up to 24 mg/kg), chromium (up to 50 mg/kg), and tin (up to 117 mg/kg) were higher in the silty sediments, with the likely source of tin being paint flakes from shipping operations (tributyl tin) in Port Kembla (Table S2 of the Supporting Information).

Sediment changes during the field and laboratory exposures

Significant changes occurred in the sediments during the 31-d exposure period, particularly between the surface sediments of the field and laboratory exposures. The major physico-chemical properties of the top 1-cm of field and laboratory deployed sediments after the exposure are shown in Table 2. The particle size of the silty sediments did not change following deployment, however the proportion of fine sediment (<63 µm) in the surface layer of field-exposed sandy sediments was 11±5.5% greater than the original material. This was attributed to the deposition of fine suspended particulate matter (SPM) from the water column, of which 90±5% was <63 µm.

TOC concentrations of the surface sediments were not greatly affected by deployment (Table 2), with TOC concentrations of deposited SPM being similar to silty sediments, and only marginally higher than the sandy sediments. Sediment 6 (sandy series) had 7.9% TOC at the completion of the laboratory exposures, compared to 5.9% in the original sediment,

which may be due to localised heterogeneity. It is unlikely that TOC from the added food contributed to this as similar trends were not observed in the other sediments.

In the 0-1 cm surface sediment layers, AVS concentrations remained $<0.1 \mu\text{mol/g}$ in the silty sediments (i.e. same at start and end of the deployment), while AVS concentrations were lower in all the sandy sediments at the end of the deployments (Tables 1 and 2). Only the sandy laboratory deployed S7 and S8 sediments retained considerable AVS (2.6 and $1.8 \mu\text{mol/g}$, respectively), indicating that the bioturbation activities of the bivalves were not resulting in complete oxidation of the surface sediments in the laboratory. In general, sediments exposed under laboratory conditions maintained higher AVS concentrations than those in the field. However, AVS concentrations measured from the bottom layer of the sediments (not shown) generally remained similar to the original sediment (Table 1).

After the 31-d exposure, changes in AEM concentrations in the surface layer of the silty sediments (S1-S4) were generally greater for the laboratory- than for field-deployed, but the reverse occurred in sandy sediments (S6-S8) (Table 2, Figure 1). The changes in TRM were generally less pronounced (Figure S1 of the Supporting Information), and the AEM measurement was expected to provide a better explanation of metal bioaccumulation and effects than the total metal concentration (Chapman et al., 1998; Simpson and Spadaro, 2011). The changes in metal concentrations were much greater for the surface sediments than the deeper sediments (Figure 2, Figure S2 of the Supporting Information).

The final AEM concentrations of As, Mn, Fe and Cu in the surface layer for most sediments were greater than the original concentrations, particularly for sandy sediments (i.e. AEM exposed/original >1 in Figure 1). Similar observations were made for TRM concentrations of As, Mn and Fe in the sandy sediments, but differences between the original and final concentrations were generally less than was observed for AEM (Figure S1 of the Supporting Information). However, total recoverable copper (TR-Cu) concentrations indicated a loss of copper from the surface layer (TR-Cu exposed/original < 1). The final AEM concentrations of Pb and Zn were lower than the original concentrations in the surface layer of the silty (both field and laboratory) and field-deployed sandy sediments, but greater than the original concentrations in the surface layer of the laboratory-deployed sandy sediments (Figure 1). Changes in TR-Pb and TR-Zn were similar to those observed for AE-Pb and AE-Zn.

The increases in AEM concentrations of As, Mn and Fe in the surface of the laboratory-deployed sediments (Figure 1) are best explained by geochemical migration processes, where greater dissolved metal concentrations exist under more reduced conditions. These ions migrate to the oxic surface layer, Mn(II) and Fe(II) oxidise to form oxyhydroxides, which

have a high binding affinity for the dissolved metals. For the field-deployed silty sediments, few changes occurred in AEM (<20%), and these differences were attributed to the permanent loss of these metals (compared to laboratory deployments) when released from the sediments combined with deposition of SPM from the overlying water. The SPM had much higher manganese, arsenic and iron concentrations than the original sandy sediments (lower for silty sediments) (Table 2), which contributed to the increase in these elements in the surface layer for the field sandy series deployments (Figures 1 and 2).

It is likely that the loss of fine sediment particles due to water currents and dilution with SPM were the main factors contributing to the decreased concentration of TR-Cu, Pb and Zn and AE-Pb and AE-Zn in the surface of field-deployed sediments. The surface sediments were >50% lower in TRM Cu, Pb and Zn in sandy sediments after field-exposure (Figures S1 and S2 of the Supporting Information). The trends for AE-Cu are complicated by the potential formation of copper sulfide phases that are not extracted as AE-Cu (Simpson et al., 1998). For TR-Cu in the sandy sediments, the concentrations decreased ~40% in the surface sediments, but not the deeper sediments (Figure S2 of the Supporting Information). However, AE-Cu concentrations decreased considerably in the deeper sediments, consistent with the formation of insoluble copper sulfide phases (Figure 2).

Bivalve bioaccumulation and health

Bioaccumulation of As, Cr, Cu, Mn, Pb and Zn occurred in the bivalve tissues and the patterns were quite different in the field- and laboratory-deployed by *T. deltoidalis* for the same sediments (Figure 3; Figure S3). This bivalve feeds on fine materials from the sediment by using its extruded siphon to stir the nearby sediment (Campana et al., 2013). Metal bioaccumulation is also presented in relation to the concentrations of AEM and TRM in the original sediments (Figures S4 and S5) and depth profiles of metal concentrations (Figures S6 and S7 of the Supporting Information).

Differences in metal bioaccumulation were generally consistent with metal contamination in sediments (Figures 3 and S3), however, the field-deployed bivalves in the silty sediments had significantly higher accumulation of As, Cr and Mn than laboratory-deployed bivalves ($p < 0.05$, $DF = 1$, $\chi^2_{As} = 4.64$, $\chi^2_{Cr} = 5.24$, $\chi^2_{Mn} = 18.91$). In contrast, bivalves in the sandy sediments displayed higher accumulation of Pb and Zn in the laboratory than in the field exposures. Copper accumulation was higher in all laboratory exposures, (except S5 and S8), especially in silty sediments. For the laboratory exposures that used field-collected overlying water (asterisk in Figure 3), the bioaccumulation was higher for Cu, Pb and Zn than observed for the same laboratory-deployed sediments with clean filtered seawater. While there appeared to be significant relationships between sediment Cu, Pb and Zn concentrations

and bioaccumulation ($p < 0.05$, $DF = 1$, $r^2\text{Cu} = 0.82$, $r^2\text{Zn} = 0.91$ in laboratory exposure and $p < 0.05$, $DF = 1$, $r^2\text{Cu} = 0.76$, $r^2\text{Pb} = 0.66$, $r^2\text{Zn} = 0.47$ in field exposure), similar relationships were not observed for the other metals or metalloids, $p > 0.05$ (Figure 3).

Bivalve health (Condition Index) was calculated for each sediment treatment following the laboratory and field exposures (Figure 4, and discussed in detail in the Supplementary Information). Irrespective of sediment type (silty or sandy), the sediments with the greatest metal contamination (S4 and S8) had the lowest Condition Index in both laboratory and field exposures. The Condition Indexes showed significantly higher biomass of bivalves after the laboratory-deployments compared to field-deployments ($p < 0.05$, $DF = 1$, $\chi^2 = 7.57$), which may be related to differences in nutrition sources, physical stress in different environments, transplantation, and possible gametogenesis processes during the exposure period (Jolley et al., 2004). Thus, for the same sediments, the laboratory exposures appear to cause less stress to the bivalves. The temperature range and variability was similar in the field (21 to 24.5°C) than the laboratory (21±3°C), and was therefore not expected to have contributed to these differences.

Dissolved and particulate metal exposure contribution to bioaccumulation

Past studies have shown that *T. deltoidalis* accumulates metals from both dissolved and particulate sources (King et al., 2005; Simpson and King, 2005; King et al., 2010; Campana et al., 2013). The bioturbating activity vertically mixed the sediments, and in field deployments, this lead to the incorporation of SPM and the loss of fine sediments to overlying waters. This altered the physico-chemical properties of the surface sediments as shown in Table 2. During the 31-day exposure period, dissolved metal concentrations were also measured (by DGT in the field and discrete samples in the laboratory) but most dissolved metals were below the limit of reporting, however time-averaged (DGT) concentrations were determined for Cu, Mn, Pb and Zn (Figure 5).

For copper and zinc, bioaccumulation increased markedly with increasing sediment-metals in both the field and laboratory (Figure 3), with similar relationships observed when considering AEM and TRM concentrations from the surface, middle or bottom layers (Figures S6 and S7 of the Supporting Information). The dissolved copper concentrations were generally $< 3 \mu\text{g/L}$, except for the silty sediment laboratory exposures for which greater copper bioaccumulation occurred in exposures with greater dissolved copper concentrations (Figure 5). The range of dissolved zinc concentrations was much greater (except for S7), and there was a significant relationship with zinc bioaccumulation, $p < 0.05$, $DF = 1$, $r^2\text{Zn} = 0.83$ in laboratory samples (Figure 5). Dissolved copper and zinc concentrations were markedly higher in the laboratory than field exposures (Figure 5), and these differences were attributed

to the continuous and higher rate of overlying water exchange in the field. In assessing the risk posed by the contaminated sediments, the laboratory exposures may considerably overestimate bioaccumulation or toxicity of sediments compared to field exposures. Similar observations have been made previously (Mann et al., 2010). Due to the similarities in bioaccumulation of copper and zinc for dissolved and particulate exposure concentrations, it was difficult to determine whether dissolved or particulate sources represented the major exposure route for bioaccumulation. However it is likely that the dissolved route contributes much more to bioaccumulation in the laboratory than in the field exposures for both copper and zinc.

Lead has no known metabolic requirements for bivalves (Fukunaga and Anderson 2011) and substantial lead bioaccumulation was expected to occur during the 31-d period for the sediment with the higher lead contamination. However, bioaccumulation differed for the silty and sandy sediments and for field and laboratory exposures (Figure 3). Lead bioaccumulation was relatively linear with increasing sediment lead concentration, but was considerably greater for the sandy than silty sediments. For the silty sediments, greater lead bioaccumulation occurred in the field than the laboratory (Figures 3 and S3), whereas, the opposite trend was observed for sandy sediments. The dissolved lead in the field and laboratory overlying waters only ranged from 1 to 2.2 $\mu\text{g/L}$ and displayed poor relationships with lead bioaccumulation (Figure 5). This suggests that particle ingestion (dietary exposure) was likely to contribute to lead bioaccumulation in all sediments. Figures 1 and 3 show that exposure to lead during the 31-d period was considerably different between the field- and laboratory-deployments (particularly evident for sandy treatments), most likely due to dilution of lead-contaminated particles with clean sediments (deposition of suspended particulate matter) and loss of fine sediments through resuspension.

For the field-deployments, a large portion of the change in sediment Cu, Pb and Zn concentrations was attributed to dilution with SPM depositing from the overlying water. However, despite the major changes to the surface sediments during the exposure period, the interpretation of the metal accumulation and Condition Index of the bivalves was not improved appreciably when the changes in the metal concentrations within the sediment profile were considered. The accumulation occurring as a result of metal exposure were considered in relation to (i) the average (original and final) concentrations in the surface sediments (Figure 3), (ii) the original AEM concentrations in the surface sediments (Figure S4), (iii) the AEM concentrations of the different sediment depths (surface, middle, bottom) (Figure S6), and (iv) the TRM concentrations of the different sediment depths (Figure S7).

No significant relationships were observed between manganese bioaccumulation and concentrations in the sediments, $p>0.05$, $DF=1$, $r^2<0.15$ in both laboratory and field deployments (Figure 3) and water, $p>0.05$ (Figure 5). However, bioaccumulated manganese concentrations were generally significantly greater ($p<0.05$, $DF=1$, $\chi^2Mn=18.91$) for the field- than laboratory-deployments, despite considerably greater dissolved manganese exposure in most of the laboratory exposures. For both laboratory and field sediment, the concentrations of Mn (and As) were considerably greater in the top 0-1 cm surface layers at the end of the 31-d exposures. This was attributed to the higher concentrations in the SPM (field only) as well as migration of these ions due to diagenic processes (laboratory and field). Despite analyses of manganese in the surface or bulk sediments, we were not able to effectively account for greater manganese bioaccumulation (Figures 3 and 5). It is possible that finer-scale analyses may have been needed both spatially and temporally within the sediments and overlying waters, or that other organism-specific factors were influencing this.

In laboratory exposures, the bivalves were observed to regularly extend their siphons to feed directly on the upper most surface layer resulting in a greater exposure to the overlying water and metal forms within this upper most surface layer. When sectioning the sediments at the end of the exposures, a greater portion (not accurately quantified) of the bivalves in the field exposure were at the very bottom of the chambers. We consider it likely that, due to the more dynamic conditions within the sediment surface of the field exposures, the bivalves avoided the sediment surface and were potentially feeding (ingesting) a greater portion of the sediment from the deeper (bottom and middle) layers in the field.

Conclusions

While it is difficult to generalise, these results indicate that the particulate metals sources are the dominant metal exposure route in most of the laboratory and field exposures, with a dominant dissolved metal exposure clearly evident only for zinc, and partly evident for copper, in laboratory tests.

The study highlighted many of the challenges in trying to create laboratory bioassays that provide for exposure conditions reflecting those in the field. There are also ongoing challenges for determining the measurements that are most useful for the interpretation of bioaccumulation and toxicity assessments results. A cost-benefit analysis is often a consideration for many assessments, and the present study indicates that many of the additional simple measurements may not significantly improve study outcomes. Recent studies show that diffusive gradients in thin film techniques (DGT) provide a robust prediction of metal bioavailability and are being increasingly applied for bioavailability

measurements (Amato et al., 2014a,b). The present study highlights the following major differences between laboratory and field exposures:

- Overlying water conditions typically maintained in laboratory bioassays will often poorly represent the conditions in the field. Major differences will include the over-representation of the dissolved metal exposure in the laboratory due to lower rates of water exchange and an inability to replicate the SPM conditions, which may act as an additional source of metal exposure, a metal adsorption phase for dissolved metal releases from sediments, and also as a potentially additional form of nutrition.
- Sediment resuspension and losses of fine sediments to the external environment, and deposition of fine SPM from the overlying water occur in the field, but not in laboratory. For long-duration exposures, monitoring of changes in the composition of the fine sediments may be important for many field studies.
- For most benthic organisms it is necessary to consider both dissolved and particulate exposure pathways to adequately interpret metal bioaccumulation and toxic effects. Differences in food quality and quantity were likely to have been major factors confounding the interpretation of the metal exposure routes in the present study.
- By constraining contaminated sediments within laboratory-exposure chambers, the exposure of test organisms to contaminants is generally higher owing to the increased dissolved exposure and inability of organisms to avoid the exposure (Ward et al., 2013a,b). However, excessive food addition may potentially mask effects to organism health that may otherwise be observed in field environments where greater energy expenditure is required to source nutrition.
- In general, under similar laboratory and field deployments conditions, laboratory bioassays will result in higher bioaccumulation of major metals (Cu, Pb, Zn): this can lead to misleading sediment quality evaluation of bioaccumulation data if used without considering differences in exposure.

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Supporting Information

Supporting Information includes further information on the sediment preparation, field cages deployment, preparation of the DGT pistons, DGTs processing and analyses, Condition Index results and discussion. This information is available free of charge via the internet at <http://pubs.acs.org/>

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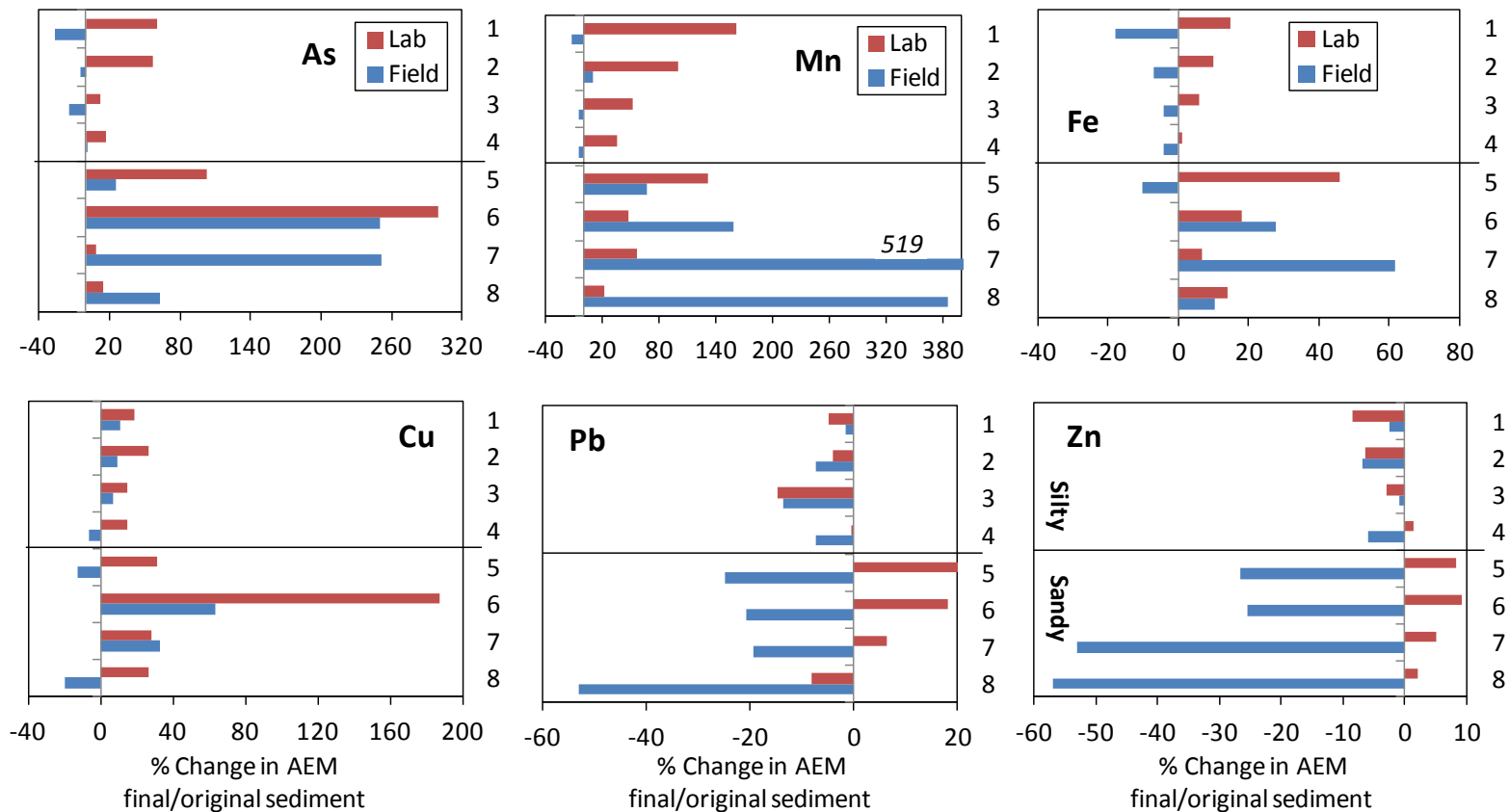
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623 Figure 1. Comparison between laboratory (red) and field (blue) changes in dilute acid-extractable metal concentration (AEM, as % change) in the
624 surface 1 cm of sediments original (pre-deployment) and final (post-deployment). Sediments (numbered down the right side) are silty (1 (control), 2, 3,
625 4) and sandy (5 (control), 6, 7, 8).

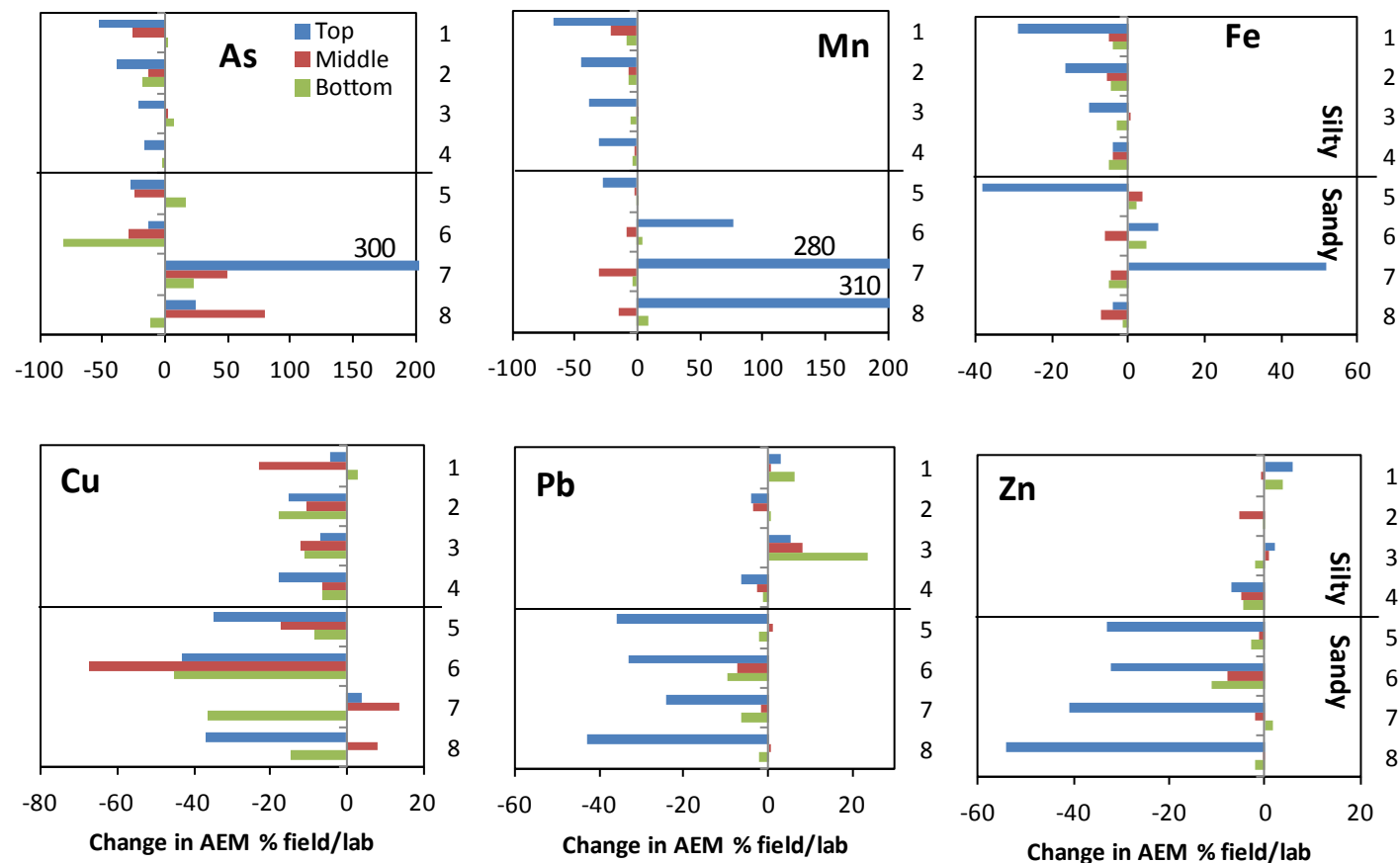


Figure 2. The percent change in dilute acid-extractable metal (AEM) concentration at different depths in sediments (as described in Figure 1) deployed for 31 d in field compared to sediments exposed under laboratory conditions. Top (blue) = 0-1 cm surface layer; Middle (red) = 2-3 cm depth; Bottom (green) = 3-4 cm depth.

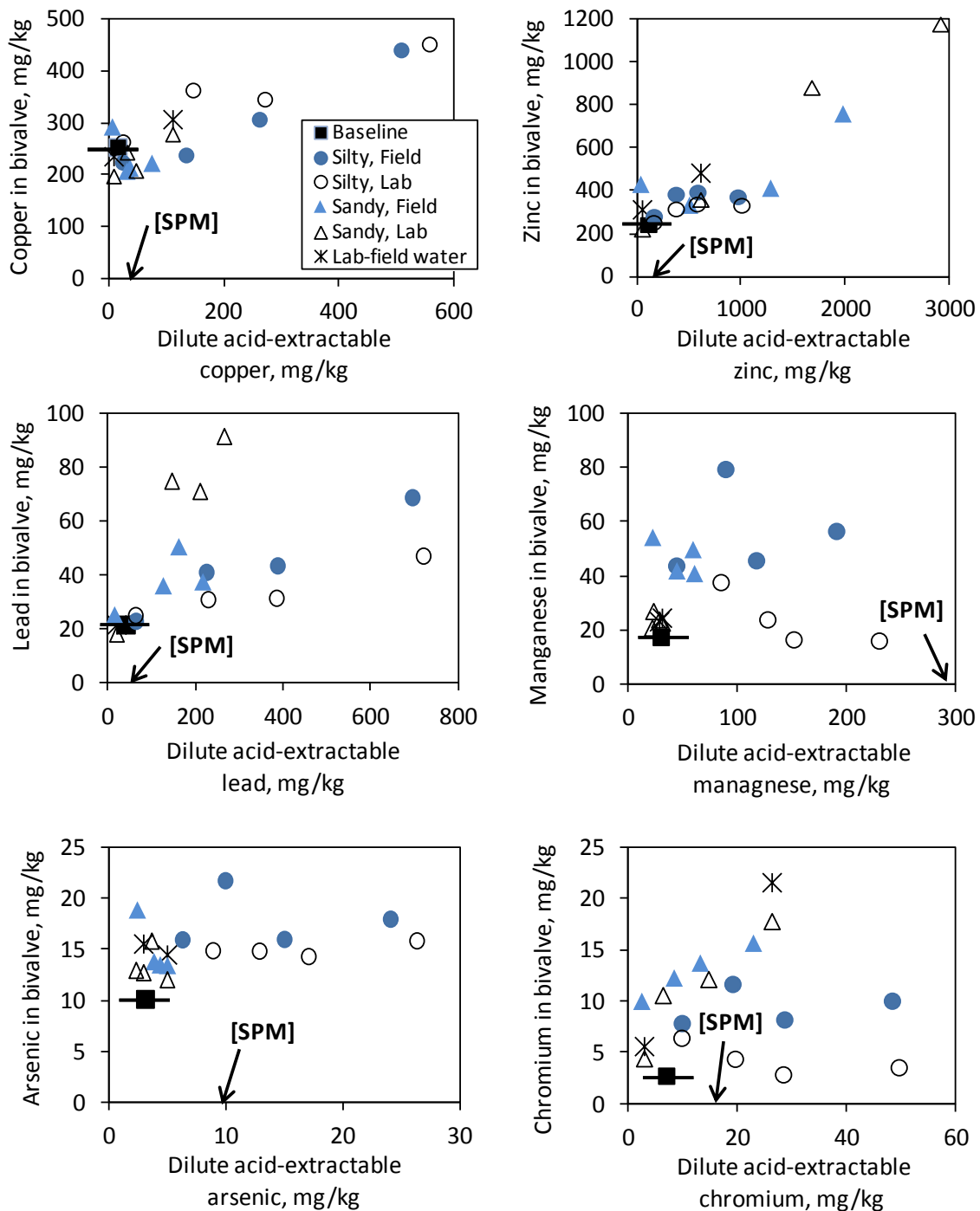
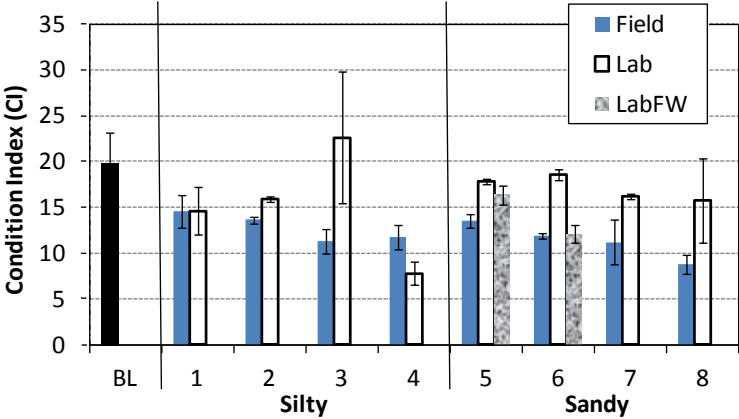


Figure 3. The relationship between average (original and final) dilute acid-extractable metal (AEM) concentration in surface sediments and metal bioaccumulation in *T. deltoidalis* (mean, n=2) after 31 d exposure under laboratory (open symbols) and field (filled symbols) conditions. The solid square with line represents the baseline metal concentrations in the bivalves (unexposed bivalves). The asterisk represents the lab exposures using unfiltered overlying water from the field site. The arrow and [SPM] represents the AEM concentration in the suspended particulate matter at the field site (mean, n=4).

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Figure 4. Comparison of Condition Index (CI) for *Tellina deltoidalis* exposed to test sediments for 31 days in both the field and laboratory (mean \pm standard errors, n=2). The filled black bar (BL) represents the baseline CI (unexposed bivalves). The shaded bars show the laboratory experiments using field water.

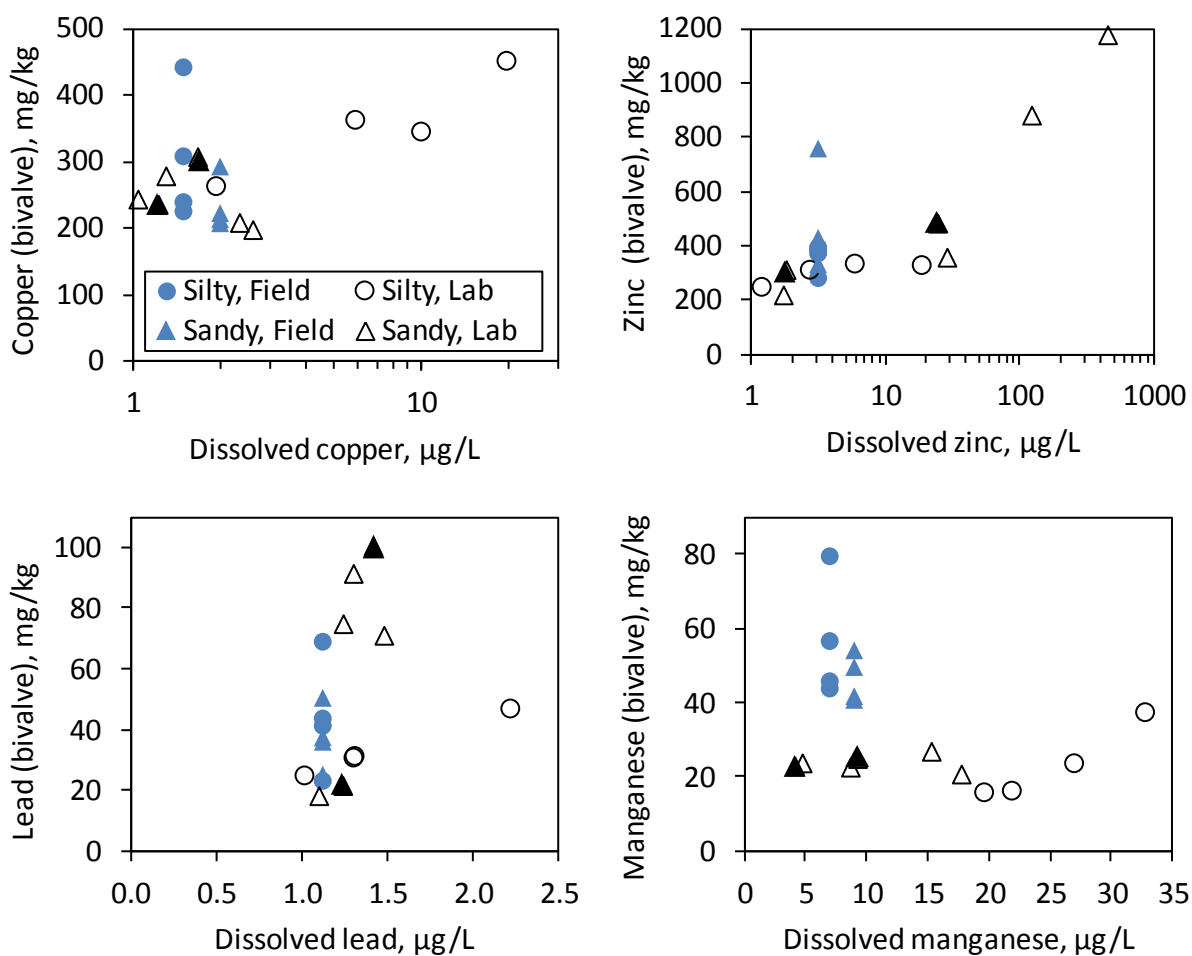


Figure 5. The relationship between dissolved Cu, Pb, Mn and Zn concentrations in the overlying water (mean, $n=10$ in Lab samples; $n=14$ in Field samples) and metal accumulation in *T. deltoidalis* (mean, $n=2$) after 31 d exposure in sediments under laboratory (open symbols) and field (filled symbols) conditions. The filled black triangles represent the laboratory exposures with field water. Standard errors are recorded in Table S5 of SI.

668 Table 1. Sediment chemical and physical properties of the control (S1 and S5) and contaminated test sediments prior to deployment (original
669 sediments). Concentrations are in mg/kg, dry mass, unless specified. Where n>1, mean±SD.

	Dilute acid-extractable metals (AEM)								Fine fraction	TOC	AVS	SEM-AVS
	Al	Fe	Mn	Cu	Pb	Zn	Cr	As	% <63 µm	%	µmol/g	µmol/g
Silty Sediments	%	%	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg				
S1 (control)	0.2	1.1	47	22	63	166	11	7	94	3.6±0.21	<0.1	3
S2	0.3	1.2	85	128	232	387	19	10	92	4.2±0.11	<0.1	9
S3	0.4	1.3	121	252	393	585	29	16	90	4.7±0.09	<0.1	15
S4	0.5	1.6	196	523	721	1000	50	24	81	5.7±0.30	<0.1	27
Sandy Sediment												
S5 (control)	0.1	0.3	17	5	17	48	3	2	26	0.9±0.06	<0.1	1
S6	0.2	0.6	25	57	242	585	27	2	31	5.9±0.49	5.8±1.2	10
S7	0.2	0.4	17	27	140	1480	7	2	14	4.2±0.25	9.7±2.0	14
S8	0.2	0.5	21	41	199	2400	15	3	27	2.1±0.04	4.2±1.5	34

670 For the metal analyses, replicate analyses were made on 20% of samples. For those results the mean values are provided and the standard deviations (n=2) were
671 generally similar in magnitude as those shown for metal analyses in Table 2 (where analyses were made on all replicate samples).

672 %<63 µm refers to the percentage (by mass) of fine sediment particles; TOC = total organic carbon (dry mass, mean n=2);

673 AVS = acid-volatile sulphide (mean n=4), SEM-AVS = the molar difference, where SEM (simultaneously extractable metals) is equivalent to AEM.

674 Table 2. Fine sediment fraction (<63 µm) and chemical parameters of test sediments (top 1 cm) in field and laboratory scenarios, after 31 days
675 exposure (final sediments). Data for new deposited sediments in field (trapped sediments) are also included. Where n>1, mean±SD.

		Size, % <63 µm	TOC %	AVS µmol/g	Dilute acid-extractable metals (AEM) mg/kg								
Sediment					Al %	Fe %	Mn	As	Cr	Cu	Pb	Zn	
Trapped sediments (SPM) - Silty		90±5	3.7±0.3	<0.1	0.3±0.01	1.1±0.1	300±20	10±0.1	17±4	29±3	67±4	190±20	
Silty Sediment	Field	S1	93	3.9	<0.1	0.2±0.01	0.9±0.01	41±6	5±0.3	9±1	25±3	62±1	160±5
		S2	92	3.7	<0.1	0.3±0.01	1.1±0.01	93±4	10±0.3	19±1	140±10	220±10	360±10
		S3	87	4.6	<0.1	0.4±0.01	1.3±0.01	110±10	14±1	28±1	270±20	380±10	580±10
		S4	75	5.3	<0.1	0.5±0.01	1.6±0.01	190±20	24±1	47±3	490±40	670±20	940±16
	Lab	S1	94	3.7	<0.1	0.2±0.01	1.2±0.1	120±20	11±0.6	9±0.5	26±3	60±5	150±10
		S2	92	4.4	<0.1	0.3±0.01	1.3±0.01	170±14	16±0.2	20±0.5	160±20	220±20	360±10
		S3	90	4.7	<0.1	0.4±0.01	1.4±0.01	180±10	18±0.8	28±0.5	290±10	380±20	570±20
		S4	81	5.6	<0.1	0.5±0.01	1.7±0.1	260±20	29±0.1	49±2	600±20	720±20	1010±20
Trapped sediments (SPM) - Sandy		3.9±0.1	3.9±0.1	<0.1	0.3±0.01	1.1±0.1	300±20	9±1	17±4	32±5	72±5	200±20	
Sandy Sediment	Field	S5	17	0.6	<0.1	0.1±0.01	0.2±0.1	29±9	3±0.8	2±0	6±1	13±3	35±8
		S6	43	5.3	<0.1	0.2±0.01	0.8±0.1	65±25	7±0.6	19±3	93±30	190±50	440±10
		S7	32	3.0	0.1±0.05	0.2±0.04	0.9±0.4	100±40	8±3	10±2	36±15	110±50	700±10
		S8	32	1.7	0.2±0.19	0.2±0.02	0.6±0.2	100±90	5±2	12±2	33±4	94±43	1030±40
	Lab	S5	26	0.9	<0.1	0.1±0.01	0.4±0.01	40±0	4±0.7	3±0.5	9±1	20±1	52±4
		S6	31	7.9	0.3±0.08	0.2±0.01	0.7±0.01	37±6	8±1	26±2	160±10	290±10	640±10
		S7	14	4.0	2.6±0.17	0.2±0.01	0.4±0.01	27±4	2±0.5	6±0.5	35±1	150±10	1560±50
		S8	27	1.6	1.8±0.76	0.2±0.06	0.6±0.1	25±9	4±2	14±1	52±12	180±50	2460±100

676 % <63 µm refers to the percentage (by mass) of fine sediment particles; TOC = total organic carbon (dry mass); AVS = acid-volatile sulphide (mean, n= 2),
677 AEM = acid extractable metals (mean, n= 2)
678 SPM = Suspended particulate matter (In the SPM: AVS <0.1 µmol/g (n=6) and <63 µm = 90 ±5% (n=6), metals (n = 4))

Supporting Information

The mismatch between bioaccumulation in field and laboratory environments: interpreting the differences for metals in benthic bivalves

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Supporting Information: The mismatch between bioaccumulation in field and laboratory environments: interpreting the differences for metals in benthic bivalves.

Material and Methods

Test Sediments. The contaminated sediments were collected from: the outer harbour of Port Kembla (Sediment 4), Australia, an industrial site hosting a steelworks and a decommissioned copper smelter; and from inner Sydney Harbour at the estuarine sites of Kings Bay (Sediment 6) and Five Dock Bay (Sediments 7 and 8). These sediments had been previously found to contain concentrations of copper, lead and zinc which greatly exceeded the sediment quality guideline values, and caused toxicity to amphipod reproduction (Amato et al., 2014a). Past studies had shown that concentrations of organic contaminants are relatively low in these sediments (Chariton et al., 2010; Dafforn et al., 2012), and were not tested in the present study. All sediments were sieved in the field (4 mm plastic mesh) and then in the laboratory (1 mm plastic mesh), homogenized and stored at 4° C in the dark until use.

Test sediments were grouped into two series (silty sediments S1-S4, or sandy sediments S5-S8) according to their physical properties. Silty sediment series (>81% of <63 µm particles) included Sediment 1 (silty control), Sediment 4 (metal contaminated silty sediment), and two extra sediments obtained by mixing Sediments 1 and 4 (at ratios 1:1 for Sediment 3, and 3:1 for Sediment 2); Sandy sediment series (<31% of <63 µm particle size) included Sediment 5 (sandy control), and three metal contaminated sandy sediments (Sediments 6, 7 and 8).

Acid volatile sulfide (AVS) is an important metal binding phase and the relationship between AVS and the simultaneously extractable metals (SEM) forms the basis the commonly used equilibrium partitioning (EqP) model for predicting porewater-metal lability and the associated risk of adverse effects (USEPA 2005; Burgess et al. 2013). The AVS concentrations were negligible in the silty sediments, but considerable in the sandy sediments (Table 1). The AEM measurements are equivalent to SEM (i.e. same acid concentration and extraction duration) and all sediments had a molar excess of SEM over AVS, indicating that AVS concentrations were insufficient to immobilize all the available metals as sulfide phases.

Test Organism. The benthic bivalve *T. deltoidalis* (family Tellinidae) is endemic to Australia and ranges from southern Queensland to Tasmania and south Western Australia. It lives in estuarine and coastal lagoons and grows to approximately 25 mm in length. The biology of this species has not been extensively studied, but like other tellinids, *T. deltoidalis* is a deposit feeder, visibly collecting organic material and particles from surface sediments (Campana et al., 2013). *T. deltoidalis* with shell lengths of 5-12 mm were collected from Lane Cove River (NSW, Australia) and maintained as described previously (Atkinson et al., 2007; King et al., 2010; Campana et al., 2013).

Preparation of diffusive gradients in thin films (DGT) pistons. DGT piston covers were obtained from DGT Research Ltd (Lancaster, UK) and assembled following standard procedures recommended by DGT Research (<http://www.dgtresearch.com/>). All glass and plasticware for DGT piston preparation were cleaned by soaking in detergent (commercial detergent diluted in tap water) for 24 h, then in 10% (v/v) HNO₃ (70%, AR grade, Ajax Finechem Pty Ltd) for 24 h and rinsed thoroughly with MQ water. All glass and plasticware for DGT probes analysis were cleaned by soaking in 10% (v/v) HNO₃ for 24 h and rinsed thoroughly with MQ water.

DGT gels were prepared from a stock solution comprising of 15% (w/v) acrylamide (40% acrylamide solution, Electrophoresis Purity Reagent, Bio-Rad Laboratories) and 0.3% (w/v) DGT cross-linker (2% aqueous solution DGT Cross Linker, DGT Research, Lancaster, U.K.). The gel chemical polymerization for diffusive gels (0.50 mm-thick) was initiated by adding 75 µL of 10% (w/v) freshly made APS (98+% ammonium persulfate, for analysis ACS, Acros Organics) to 10 mL of stock solution and catalysed by adding 25 µL of TEMED (99% N,N,N,N-tetramethylethylenediamine, Molecular Biology tested, Sigma). The solution was stirred for 3½ minutes, then immediately cast between a pair of glass plates separated by a 0.5 mm plastic spacer. The gel solution was left to polymerize for 1 h at 45°C.

Wet Chelex resin was prepared by mixing 2 g of dry Chelex resin with 10 mL of MQ water, then allowing the resin to settle and withdrawing the overlying water with a pipette. Chelex gels (0.25 mm-thick) were prepared by adding 5 mL of stock solution to 2 g of wet chelating resin, then 25 µL of initiator (10% (w/v) freshly made APS) and 7.5 µL of catalyst (TEMED). The solution was mixed for 3 minutes, then immediately cast between a pair of glass plates separated by a 0.25 mm plastic spacer. The solution was then left to polymerize for 1 h at 45°C.

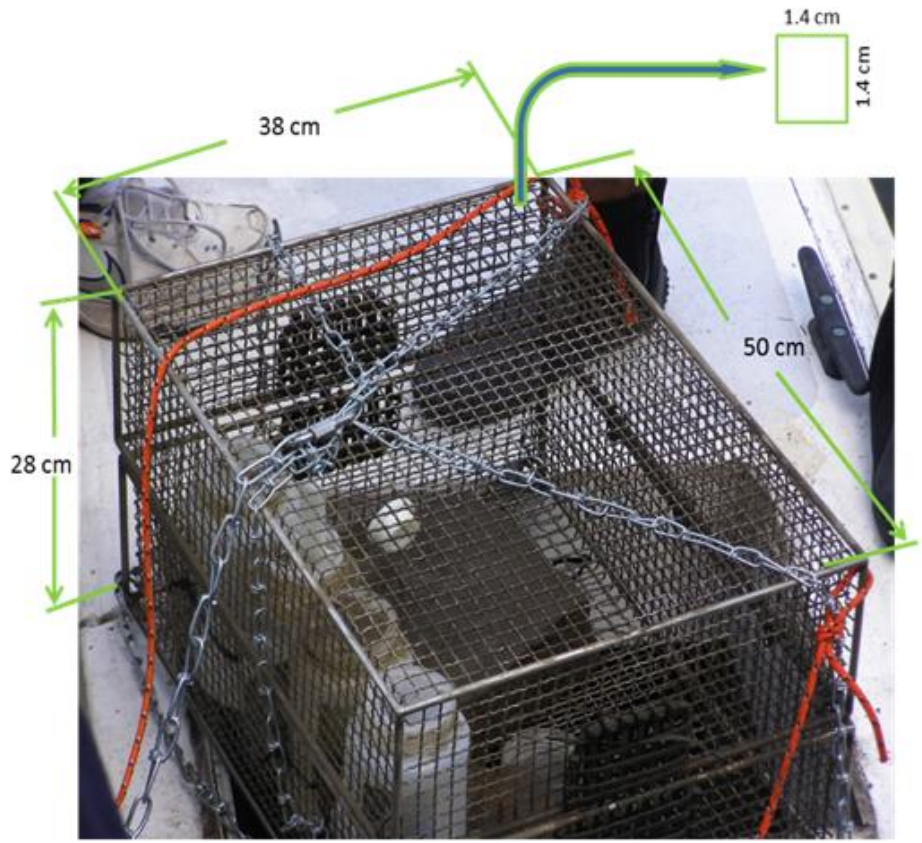
The resulting diffusive and Chelex gels were removed from the glass plates and hydrated in Milli-Q water for 24 h, replenishing the water three times to remove all unreacted chemicals. Diffusive gels were stored in 0.01 M NaNO₃ (AR grade, Chem Supply) at room temperature and Chelex gels were stored in Milli-Q water in a refrigerator at 4°C until use for probe construction. Gels were cut with a Teflon coated razor blade using a plastic rectangular strip of the desired dimensions in order to fit in the DGT device. Acid-cleaned 0.45 µm filter membranes were cut and stored in MQ water.

Pistons were assembled carefully to ensure no air bubbles were trapped within the layers. The DGT units were kept in sealed clean plastic bags containing few drops of MQ water to avoid gel drying and stored in a refrigerator until deployment.

To prevent the introduction of oxygen into the sediments during the deployment, DGT pistons were deoxygenated for 24 h prior to deployment by immersing them in a 0.05 M NaCl (>95.5%, Sigma) solution saturated with nitrogen gas (continually bubbling to remove the dissolved oxygen). DGT pistons were deployed between the cages (40 cm below surface) for 5 days in triplicate on days 0, 10, 17, 21 and 26 of the deployment period (i.e. 15 DGTs). Upon retrieval the pistons were thoroughly rinsed with deionised water, placed individually in acid-washed plastic bags in a cooler box during transport to the laboratory and kept in a cool room (4°C) until disassembly. DGT devices were disassembled and resin discs were weighed and eluted with 4.5 mL of 1 M HNO₃ (Merck, Suprapur) for 48 h and analysed by ICP-MS (Agilent-7500). Undeployed DGT probes were analysed as handling blanks.

The elution factor used for all metals was 0.8, and the DGT-labile metals concentrations were calculated using the DGT equation: $CDGT = \Delta gM/DA t$, where Δg = diffusive layer thickness (0.08 cm), M = mean mass of metal accumulated, D = diffusion coefficient at 23±2°C (www.dgtresearch.com; Zhang and Davison, 1995), A = surface area interface (3.14 cm²) and t = deployment time (seconds).

Field deployment test vessels. The field-deployed bioassays were performed in cages deployed in an uncontaminated section of the Woronora River estuary (Sydney, Australia) for 31 days. The cages were suspended from a floating boat pontoon that allowed cages to maintain a submerged depth of 40 cm, irrespective of tidal cycles. Cages comprised of two sections hinged together with cable ties to create a fully enclosed cage of dimensions 50 × 37 × 26 cm³ (L × W × H) stainless steel with an approximate mesh size of 1-cm² which allowed free estuarine water flow through the cage. The exposure chambers comprised 520 g of sediment (approximately 5.1 cm depth, 44 cm² surface area at SWI) and 7 bivalves within 1 L low-density polyethylene bottle (LDPE, Nalgene) that had three 4.5×8 cm² windows cut in the sides to allow water flow. There were two replicates of each treatment (sediments 1-8) and these were randomly distributed in the cages as shown in photographs 1-5.



Photographs 1 and 2. Stainless steel cage used for field deployment



Photographs 3 and 4. Field-deployment site: boat pontoon in Woronora estuary, Sydney, Australia



Photograph 5. Hanging cage with exposure chambers deployed under water

Supporting Information: The mismatch between bioaccumulation in field and laboratory environments: interpreting the differences for metals in benthic bivalves.

Results and Discussion

Bivalve health

Differences in metal bioaccumulation were generally consistent with metal contamination in sediments (Figure 3), however, the field-deployed bivalves in the silty sediments had significantly higher accumulation of As, Cr and Mn than laboratory-deployed bivalves ($p < 0.05$, $DF = 1$, $\chi^2_{As} = 4.64$, $\chi^2_{Cr} = 5.24$, $\chi^2_{Mn} = 18.91$). In contrast, bivalves in the sandy sediments displayed higher accumulation of Pb and Zn in the laboratory than in the field exposures. Copper accumulation was higher in all laboratory exposures, (except S5 and S8), especially in silty sediments. For the laboratory exposures that used field-collected overlying water (asterisk in Figure 3), the bioaccumulation was higher for Cu, Pb and Zn than observed for the same laboratory-deployed sediments with clean filtered seawater. While there appeared to be significant relationships between sediment Cu, Pb and Zn concentrations and bioaccumulation ($p < 0.05$, $DF = 1$, $r^2_{Cu} = 0.82$, $r^2_{Zn} = 0.91$ in laboratory exposure and $p < 0.05$, $DF = 1$, $r^2_{Cu} = 0.76$, $r^2_{Pb} = 0.66$, $r^2_{Zn} = 0.47$ in field exposure), similar relationships were not observed for the other metals or metalloids, $p > 0.05$ (Figures 3).

Bivalve health (Condition Index, CI) was calculated for each sediment treatment following the laboratory and field exposures (Figure 4). CI was lower than baseline (calculated from non-exposed bivalves) following both laboratory (except S3) and field exposures, and lower in the contaminated sediments (S2-S4, S6-S8) than controls (S1 and S5). The lowest CI was recorded for sediment S4, which is consistent with the high level of contamination. There were no significant differences in CI between bivalves exposed to the silty or sandy sediments ($p > 0.05$, $DF = 1$, $\chi^2 = 7.88$), but CI in silty series were lower than in sandy series for laboratory deployments (Figure 4). Copper is more toxic to *T. deltoidalis* than lead and zinc (King et al., 2004, 2010), and the higher copper concentrations in the silty sediments may have contributed to the lower CI.

The CI of laboratory-deployed bivalves in which the overlying water was from the field site (S5-FW and S6-FW) were not different to the equivalent field and lab-deployed bivalves, but were lower than the CI for Sediments 5 and 6 in laboratory-deployments (Figure 4).

847 The CIs showed significantly higher biomass of bivalves after the laboratory-deployments
848 compared to field-deployments ($p < 0.05$, $DF = 1$, $\chi^2 = 7.57$). Differences in nutrition may have been a
849 major contributor to this, as laboratory-deployed bivalves were provided with additional food (easily
850 resuspended and preferentially consumed), while the field-deployed bivalves sourced additional
851 nutrition from the SPM that deposited during the exposure period. Besides, in field deployments
852 bivalves suffer physical stress as need to deal with sediment remobilization processes. Other factors
853 affecting CI include stress caused by the transplantation (individuals liberate gametes and lose
854 weight), and possible gametogenesis processes during the exposure period (demands energy and
855 implies biomass loss) (Jolley et al., 2004). Thus, for the same sediments, the laboratory exposures
856 appear to cause less stress to the bivalves.

857 Irrespective of sediment type (silty or sandy), the sediments with the greatest metal
858 contamination (S4 and S8) had the lowest CI in both laboratory and field exposures. The major metal
859 contaminants were Cu, Pb and Zn (Table 1), and the CI of the deployed bivalves follows an inverse
860 relationship with sediment zinc concentration (Figure S8). Laboratory deployed sediments with the
861 highest bioaccumulation of Zn (sandy) and Cu (silty) corresponded with lower CI values (Figures 4 and
862 S8), indicating that the bioaccumulated copper and zinc may be a useful indicator of bivalve health in
863 the laboratory. No clear relationships existed for the other bioaccumulated metals/metalloids or
864 between CI and sediment contaminant concentrations for laboratory exposures.

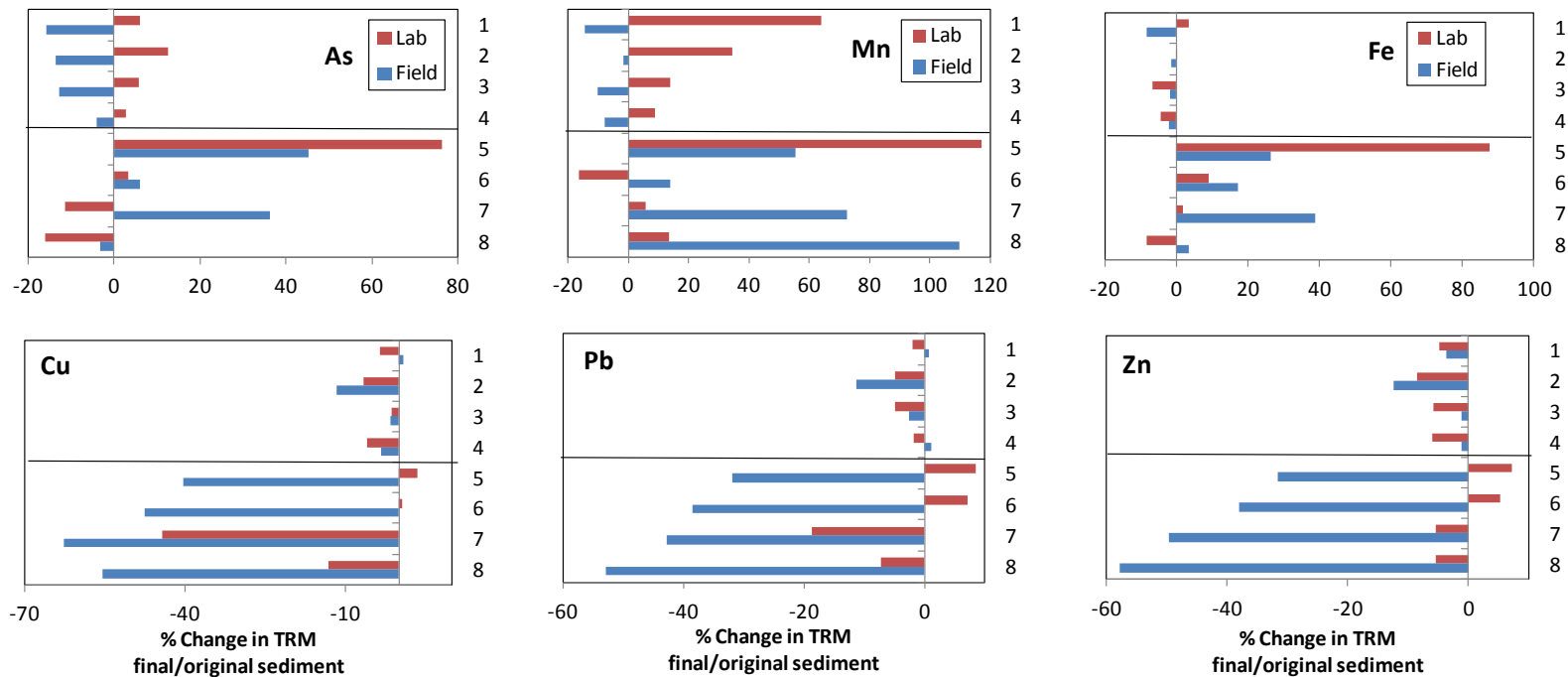
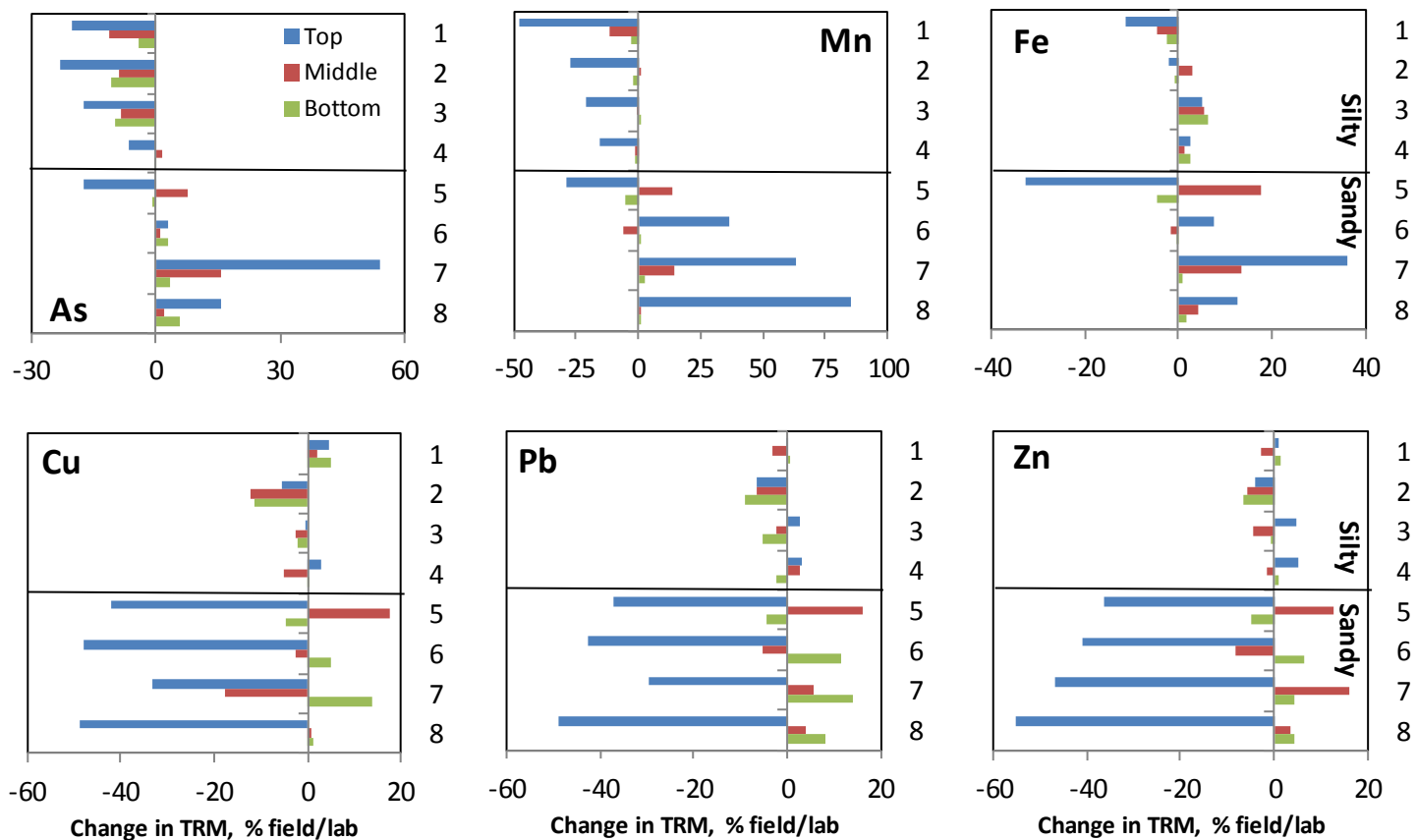


Figure S1. The percent change in total recoverable metal (TRM) concentrations in the surface 1 cm of sediments final (post-deployment) compared to original (pre-deployment) under laboratory (red) and field (blue) conditions Sediments (numbered down the right side) are silty (1 (control), 2-4) and sandy (5 (control), 6-8).



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Figure S2. The percent change in total recoverable metal (TRM) concentrations in sediments deployed for 31 d in field compared to sediments exposed under laboratory conditions. Sediments (numbered down the right side) are silty (1 (control), 2-4) and sandy (5 (control), 6-8), and were collected from the top (surface 1 cm layer), the middle (2-3cm depth layer) and bottom (> 3 cm layer).

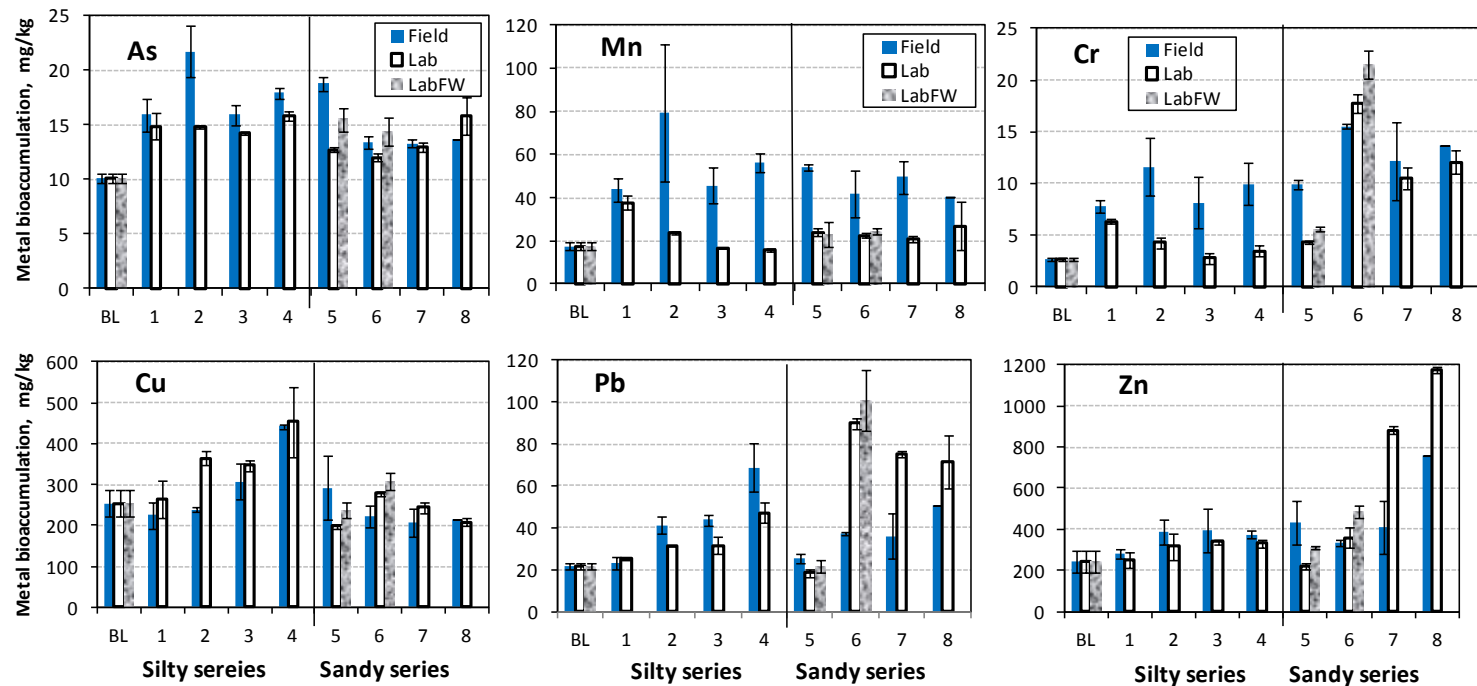


Figure S3. The metal accumulation in *T. deltoidalis* after 31 d exposure in sediments under laboratory and field conditions. Sediments are silty (1 (control), 2-4) and sandy (5 (control), 6-8), mean \pm SE, n=2. LabFW = samples deployed in laboratory with overlaying water supplied from the field. BL = baseline sample.

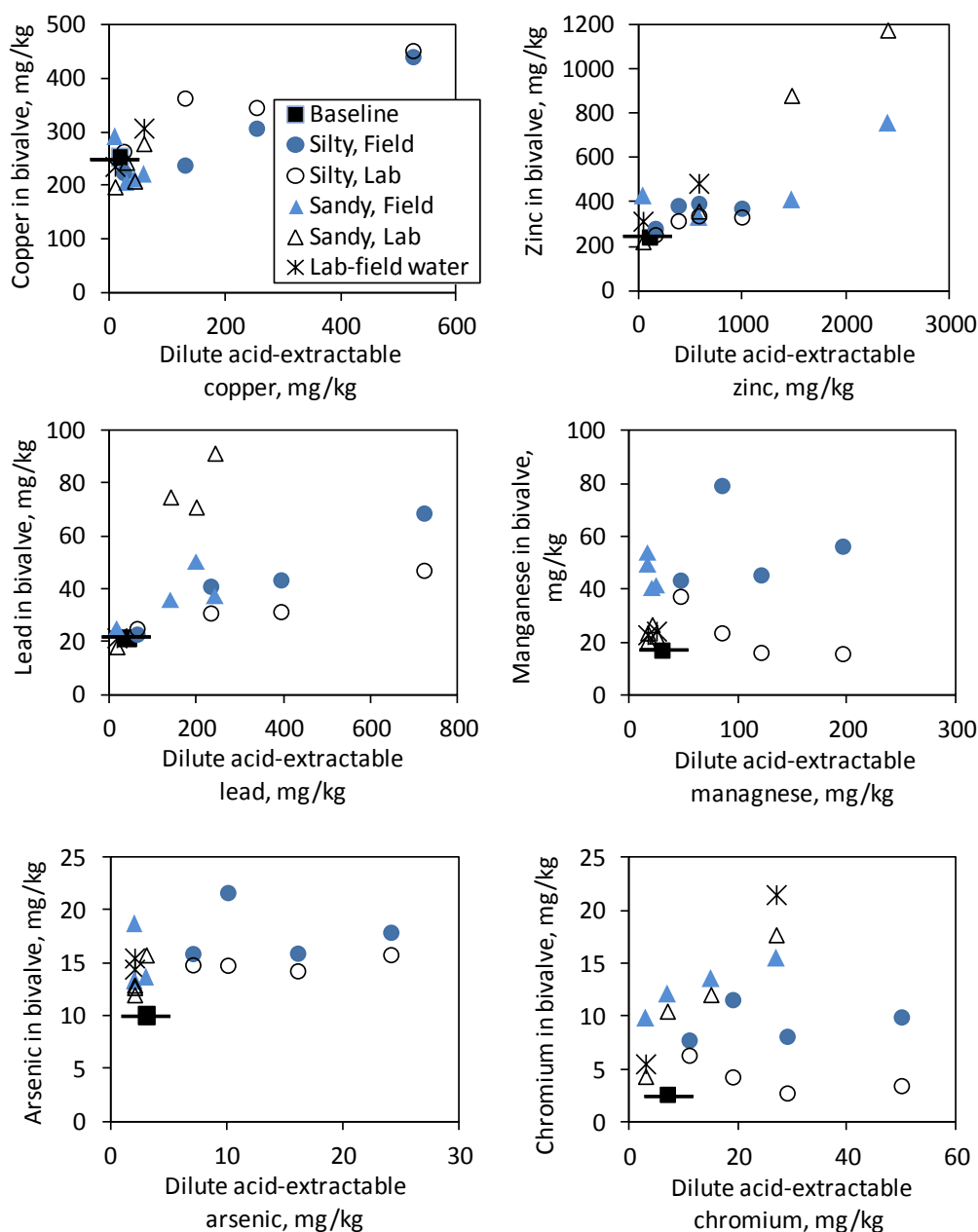


Figure S4. The relationship between original dilute acid-extractable metal (AEM) concentration in surface sediments and metal bioaccumulation in *T. deltoidealis* after 31 d exposure (mean, n=2) under laboratory (open symbols) and field (filled symbols) conditions. The solid square with line represents the baseline metal concentrations in the bivalves (unexposed bivalves). The asterisk represents the lab exposures using unfiltered overlying water from the field site. The arrow and [SPM] represents the AEM concentration in the suspended particulate matter at the field site (mean, n=4). For the metal analyses in sediments, duplicate analyses were made on 20% of all samples. For those results the mean values are plotted.

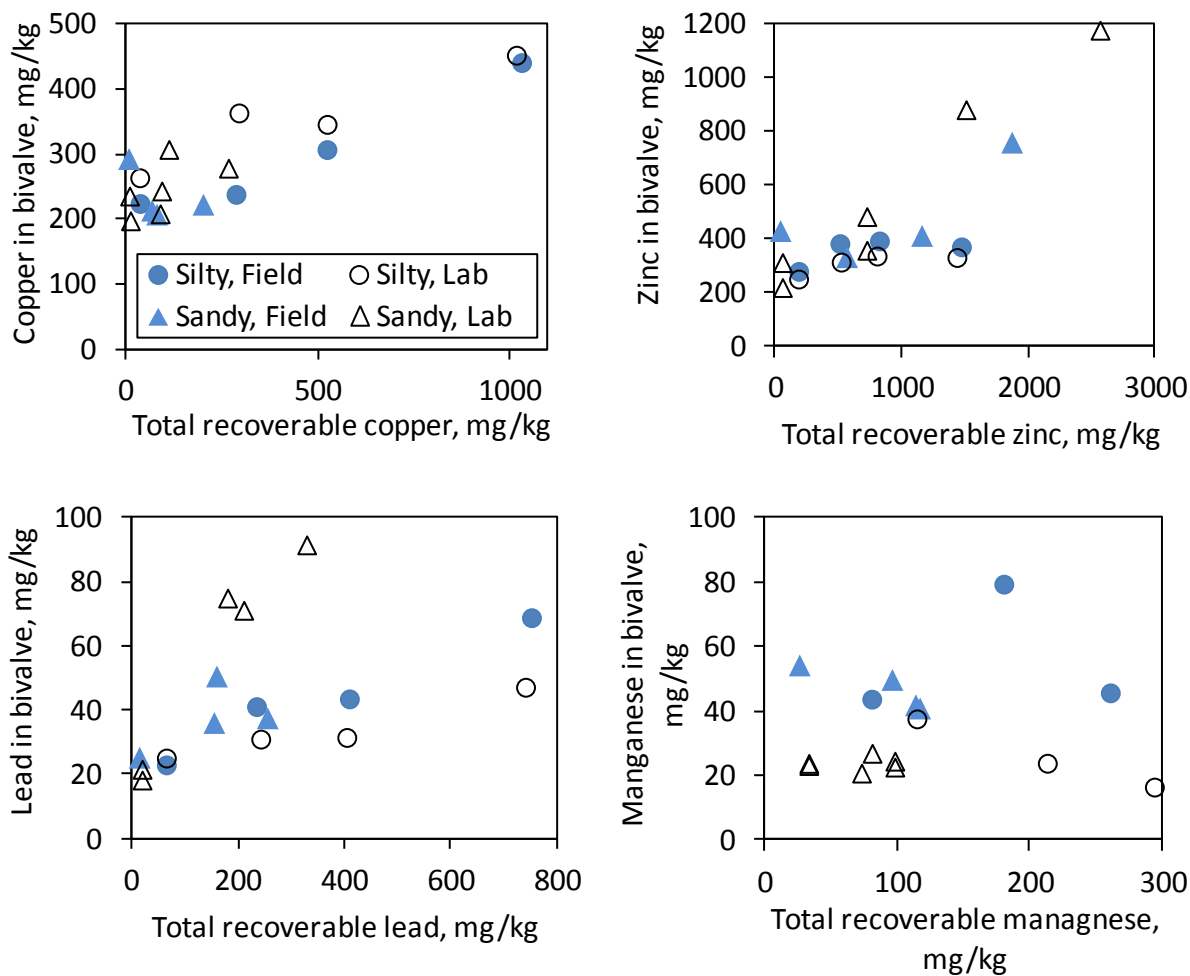


Figure S5. The relationship between original total recoverable metal (TRM) concentration in surface sediments and metal bioaccumulation in *T. deltoidalis* (mean, n=2) after 31 d exposure under laboratory (open symbols) and field (filled symbols) conditions. For the metal analyses in sediments, duplicate analyses were made on 20% of all samples. For those results the mean values are plotted.

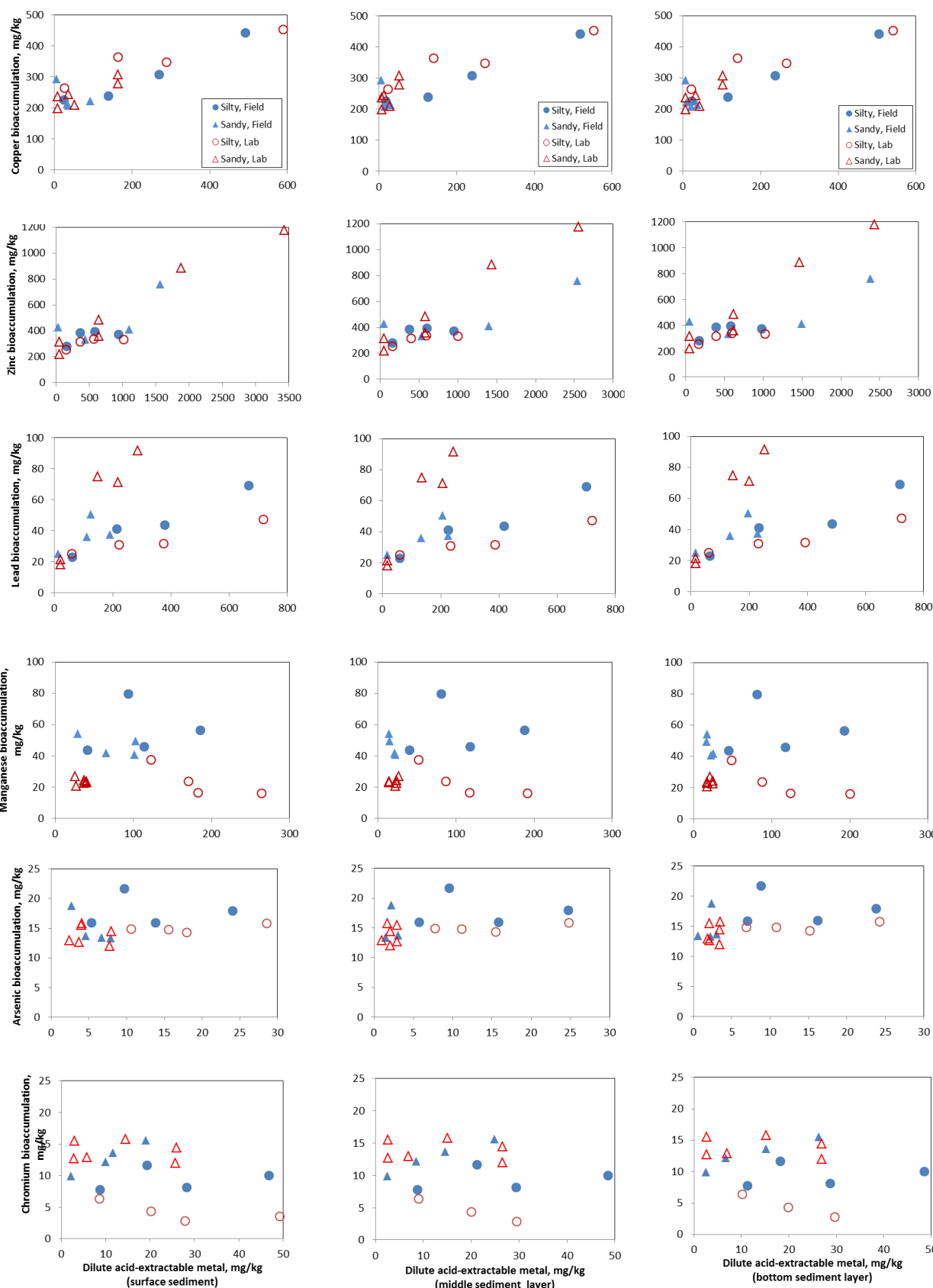


Figure S6. The relationship between the metal accumulated in bivalves, mean, n=2, (mg/kg, dry mass) and the dilute acid-extractable metal (AEM) concentrations of the different sediment depths after 31 days deployment under laboratory (red open symbols) and field (blue filled symbols) conditions: surface 1 cm (left column of figures), middle 2-3 cm (middle column) and bottom >3cm (right column). For the metal analyses in sediments, duplicate analyses were made on 20% of all samples. For those results the mean values are plotted.

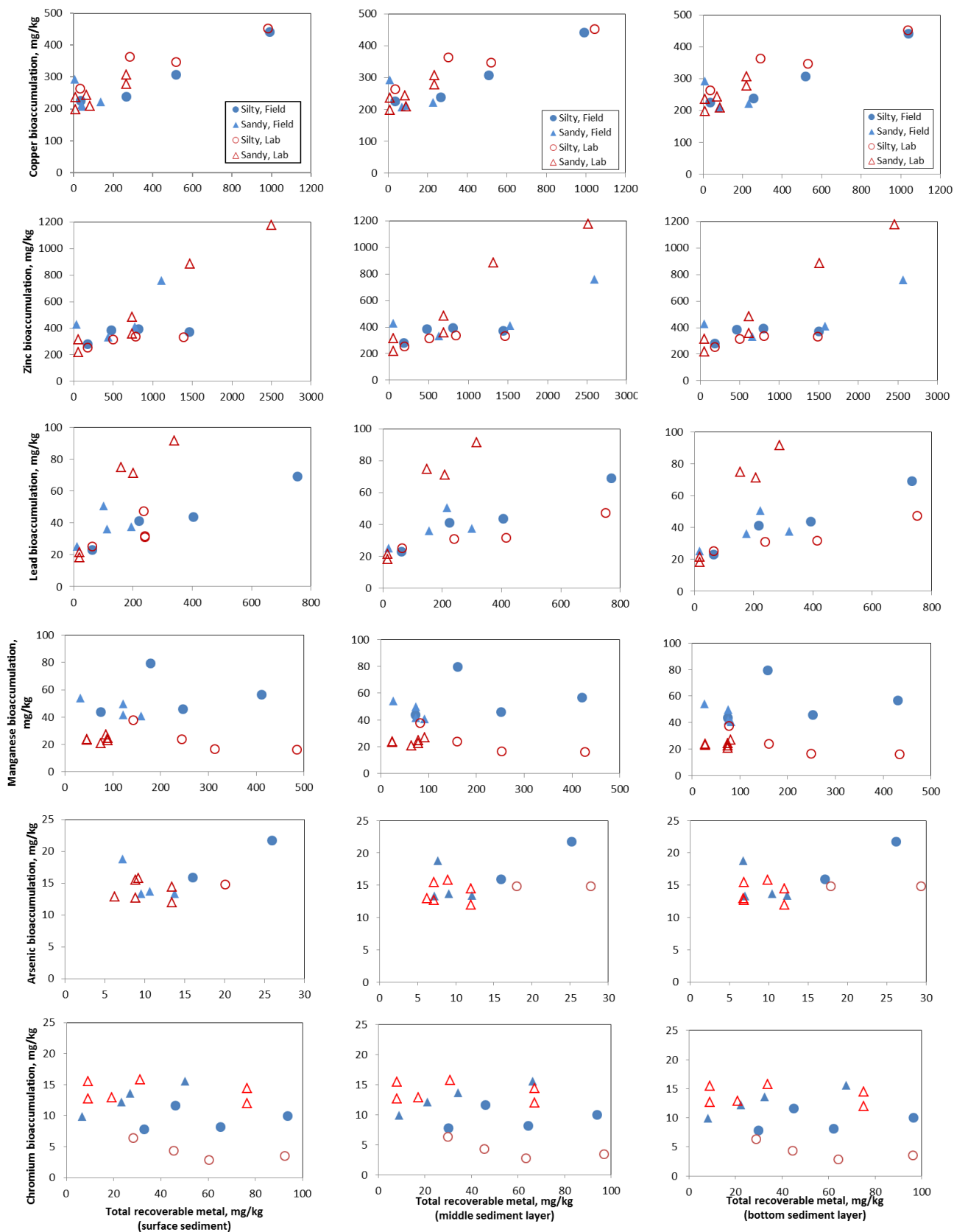


Figure S7. The relationship between the metal accumulated in bivalves, mean, $n=2$, (mg/kg, dry mass) and the total recoverable metals (TRM) concentrations of the different sediment depths after 31 days deployment under laboratory (red open symbols) and field (blue filled symbols) conditions: surface 1 cm (left column of figures), middle 2-3 cm (middle column) and bottom >3cm (right column). For the metal analyses in sediments, duplicate analyses were made on 20% of all samples. For those results the mean values are plotted.

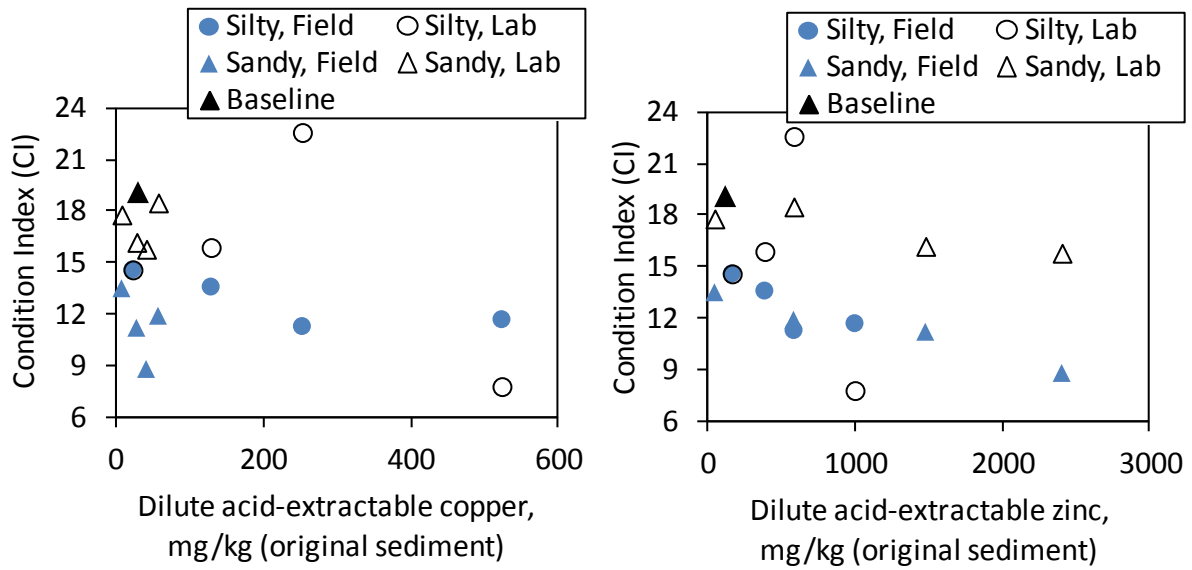


Figure S8. Comparison of Condition Index (CI) for *Tellina deltoidalis* exposed to test sediments for 31 days in both the field (filled symbols) and laboratory (open symbols) (mean, n=2) and dilute acid-extractable copper and zinc concentrations measured in the original sediments. The filled black triangles represent the baseline CI (unexposed bivalves). For metal analyses duplicate analyses were made on 20% of all samples. For those results the mean values are plotted.

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Table S1. Total recoverable metal concentrations in original sediments (prior to deployment) in mg/kg, dry mass, unless specified. Control sediments S1 and S5.

Sediment	Al %	Fe %	Mn	Cu	Pb	Zn	As	Cd	Co	Cr	Ni	Sn	V
Silty Series													
S1	1.1	2.5	87	35	65	188	19	1.0	4	29	12	3	41
S2	1.3	3.3	182	303	248	544	30	0.7	8	47	15	60	57
S3	1.4	4.1	275	527	414	828	42	1.5	9	65	18	119	74
S4	1.5	5.5	446	1050	747	1480	64	1.8	16	96	25	234	103
Sandy Series													
S5	0.2	0.4	21	10	18	54	5	0.9	1	5	2	1.0	8
S6	0.7	1.7	107	265	317	705	13	1.3	5	75	15	69	34
S7	0.6	1.2	71	118	198	1550	7	1.1	4	26	11	7	24
S8	0.7	1.7	76	94	218	2640	11	1.4	5	34	13	7	27

931

Table S2. Dilute acid-extractable metal concentrations in original sediments (1 M HCl) in mg/kg, dry mass, unless specified. Control sediments S1 and S5.

Sediment	Al %	Fe %	Mn	Cu	Pb	Zn	As	Cd	Co	Cr	Ni	Sn	V
Silty Series													
S1	0.2	1.1	47	22	63	166	7	0.1	3.0	11	4	1	22
S2	0.3	1.2	85	128	232	387	10	0.4	3.4	19	5	29	29
S3	0.4	1.3	121	252	393	585	16	0.5	3.5	29	6	58	35
S4	0.5	1.6	196	523	721	999	24	1.0	5.4	50	9	117	48
Sandy Series													
S5	0.1	0.3	17	7	17	48	2	0.0	0.7	3	1	0.4	7
S6	0.2	0.6	25	57	242	585	2	1.0	1.5	27	5	26	18
S7	0.2	0.4	17	27	140	1479	2	0.9	1.7	7	2	2	9
S8	0.2	0.5	21	41	199	2405	3	1.2	2.5	15	4	3	12

932

Table S3. Dilute acid-extractable metal concentration, as a percent of the total recoverable metal concentration (AEM/TRM ×100%) in original sediments, unless specified. Control sediments S1 and S5.

Sediment	Al	Fe	Mn	Cu	Pb	Zn	As	Cd	Co	Cr	Ni	Sn	V
Silty Series													
S1	22	42	54	63	97	88	37	13	75	37	31	34	55
S2	25	36	47	42	94	71	33	54	43	41	32	48	50
S3	29	32	44	48	95	71	37	34	39	45	35	49	48
S4	37	30	44	50	97	68	38	58	34	52	35	50	46
Average (S2,S3,S4)	30	33	45	47	95	70	36	49	38	46	34	49	48
Sandy Series													
S5	36	58	83	72	97	90	42	5	70	55	62	40	84
S6	30	38	23	21	76	83	16	76	30	36	31	38	52
S7	35	33	24	23	71	95	28	80	43	26	22	26	37
S8	29	31	28	43	91	91	31	81	50	45	28	41	44
Average (S6,S7,S8)	31	34	25	29	80	90	25	79	41	36	27	35	44

For the metal analyses, replicate analyses were made on 20% of samples. For those results the mean values are provided and the standard deviations (n=2) were generally similar in magnitude as those shown for metal analyses in Table 2 (where analyses were made on all replicate samples).

937 **Table S4.** Fine sediment fraction (<63 µm) and chemical parameters of test sediments (top layer) in field and laboratory scenarios after 31 days
938 exposure. Concentrations are in mg/kg, dry mass, unless specified. Control sediments S1 and S5.
939

		Size, %	TOC	AVS	Total recoverable metals (TRM) mg/kg								Dilute acid-extractable metals (AEM) mg/kg								
		<63 μm	%	μmol/g	Al %	Fe %	Mn	As	Cr	Cu	Pb	Zn	Al %	Fe %	Mn	As	Cr	Cu	Pb	Zn	
Sediment																					
Silty Series	Field	S1	93	3.9	<0.1	0.9	2.3	75	16	33	35	63	181	0.2	0.9	41	5	9	25	62	161
		S2	92	3.7	<0.1	1.3	3.2	179	26	46	268	220	477	0.3	1.1	93	10	19	139	215	361
		S3	87	4.6	<0.1	1.4	4.1	247	37	65	519	403	818	0.4	1.3	114	14	28	270	380	580
		S4	75	5.3	<0.1	1.4	5.3	412	61	94	1010	755	1460	0.5	1.6	186	24	47	492	668	939
	Lab	S1	94	3.7	<0.1	1.1	2.6	143	20	28	34	64	179	0.2	1.2	123	11	9	26	60	152
		S2	92	4.4	<0.1	1.1	3.3	245	34	45	283	236	498	0.3	1.3	170	16	20	163	223	362
		S3	90	4.7	<0.1	1.1	3.9	313	44	60	519	393	781	0.4	1.4	183	18	28	289	375	568
		S4	81	5.6	<0.1	1.3	5.2	486	66	92	984	733	1390	0.5	1.7	264	29	49	601	718	1010
Sandy Series	Field	S5	17	0.6	<0.1	0.2	0.6	33	7	7	6	12	37	0.1	0.2	29	3	2	6	13	35
		S6	43	5.3	<0.1	0.7	1.9	122	14	50	139	195	438	0.2	0.8	65	7	19	93	192	436
		S7	32	3.0	0.1	0.6	1.6	123	10	23	44	113	780	0.2	0.9	103	8	10	36	113	700
		S8	32	1.7	0.2	0.7	1.8	159	11	27	42	103	1120	0.2	0.6	102	5	12	33	94	1030
	Lab	S5	26	0.9	<0.1	0.3	0.8	46	9	9	10	20	58	0.1	0.4	40	4	3	9	20	52
		S6	31	7.9	0.3	0.7	1.8	89	13	77	266	340	742	0.2	0.7	37	8	26	164	286	639
		S7	14	4.0	2.6	0.5	1.2	75	6	19	66	161	1470	0.2	0.4	27	2	6	35	149	1560
		S8	27	1.6	1.8	0.7	1.6	86	9	31	82	202	2500	0.2	0.6	25	4	14	52	183	2460

940

941 TRM = total recoverable metals; %<63 µm refers to the percentage (by mass) of fine sediment particles; TOC = total organic carbon (dry mass); AVS = acid-
942 volatile sulfide, (mean, n=2), AEM = dilute acid extractable metals (average values for 2 replicates).
943 Standard deviations are recorded in Table 2.

Table S5. Concentration of metals in the overlaying waters of tested sediments in laboratory and in field deployments ($\mu\text{g/L}$), mean values \pm standard errors).

	Laboratory exposures				Field exposures			
	Cu	Mn	Pb	Zn	Cu	Mn	Pb	Zn
	$\mu\text{g/L}$ (n=10)				$\mu\text{g/L}$ (n=14)			
S1	2 \pm 0.2	33 \pm 11	1.0 \pm 0.1	1 \pm 0.1	1.5 \pm 0.1	7 \pm 5.8	1.1 \pm 0.1	2.7 \pm 0.8
S2	6 \pm 0.8	27 \pm 10	1.3 \pm 0.2	3 \pm 0.3	1.5 \pm 0.1	7 \pm 5.8	1.1 \pm 0.1	2.7 \pm 0.8
S3	10 \pm 1.4	22 \pm 8	1.3 \pm 0.2	6 \pm 0.4	1.5 \pm 0.1	7 \pm 5.8	1.1 \pm 0.1	2.7 \pm 0.8
S4	20 \pm 2.5	20 \pm 7	2.2 \pm 0.5	18 \pm 1.3	1.5 \pm 0.1	7 \pm 5.8	1.1 \pm 0.1	2.7 \pm 0.8
S5	3 \pm 0.3	5 \pm 2	1.1 \pm 0.1	2 \pm 0.3	1.7 \pm 0.6	9 \pm 3.2	1.1 \pm 0.1	3.5 \pm 1.4
S6	1 \pm 0.1	9 \pm 2	1.3 \pm 0.2	28 \pm 2.7	1.7 \pm 0.6	9 \pm 3.2	1.1 \pm 0.1	3.5 \pm 1.4
S7	1 \pm 0.1	18 \pm 3	1.2 \pm 0.1	122 \pm 15	1.7 \pm 0.6	9 \pm 3.2	1.1 \pm 0.1	3.5 \pm 1.4
S8	2 \pm 0.3	15 \pm 4	1.5 \pm 0.3	448 \pm 51	1.7 \pm 0.6	9 \pm 3.2	1.1 \pm 0.1	3.5 \pm 1.4
S5 FW	1 \pm 0.1	4 \pm 1	1.2 \pm 0.2	2 \pm 0.3				
S6 FW	2 \pm 0.2	9 \pm 1	1.4 \pm 0.3	24 \pm 3				

S5FW and S6FW: sediment samples deployed in laboratory with overlaying water supplied from the field.

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