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Metal fluxes from porewaters and labile sediment phases for predicting metal exposure and bioaccumulation in benthic invertebrates

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Amato, Elvio D.; Simpson, Stuart L.; Belzunce-Segarra, María; Jarolimek, Chad V.; and Jolley, Dianne F., "Metal fluxes from porewaters and labile sediment phases for predicting metal exposure and bioaccumulation in benthic invertebrates" (2015). *Faculty of Science, Medicine and Health - Papers: part A*. 3367.

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Abstract

The use of diffusive gradients in thin films (DGT) for predicting metal bioavailability was investigated by exposing the bivalve *Tellina deltoidalis* to an identical series of metal-contaminated sediments deployed simultaneously in the field and laboratory. To understand the differences in metal exposure occurring between laboratory and field-based bioassays, changes in metal fluxes to DGT probes in sediments, and metal concentrations and partitioning to porewaters and overlying waters were investigated. DGT-metal fluxes (Cu, Pb and Zn) were lower in the overlying waters of most field-bioassays compared to the laboratory, causing differences in Pb and Zn bioaccumulation between bivalves exposed to laboratory and field conditions. Overall, DGT-metal fluxes provided predictions of metal bioaccumulation similar to those obtained using dilute-acid extractable metal measurements. This study demonstrates that, irrespective of the physicochemical properties of the sediment and type of exposure (laboratory or field), sediments pose a significant risk of bioaccumulation by *T. deltoidalis* when the Cu, Pb and Zn DGT flux exceeds 3.5, 1.3 and 156 $\mu\text{g}/\text{h}/\text{m}^2$, respectively. The results presented here support the use of the DGT technique for sediment quality assessment and the hypothesis that DGT-metal fluxes may potentially be useful surrogates for the lability of metals for all exposure routes.

Disciplines

Medicine and Health Sciences | Social and Behavioral Sciences

Publication Details

Amato, E. D., Simpson, S. L., Belzunce-Segarra, M. J., Jarolimek, C. V. & Jolley, D. F. (2015). Metal fluxes from porewaters and labile sediment phases for predicting metal exposure and bioaccumulation in benthic invertebrates. *Environmental Science and Technology* (Washington), 49 (24), 14204-14212.

Metal fluxes from porewaters and labile sediment phases for predicting metal exposure and bioaccumulation in benthic invertebrates.

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Rationale: Many sediment quality assessment frameworks consider the bioavailability of contaminants when evaluating the risk posed by contaminants to benthic communities. For metals, analyses of acid-volatile sulfide (AVS), simultaneously extracted metals (SEM) (dilute-acid extractable metals) and organic carbon (OC) are commonly used to assist in the bioavailability assessment. Although these approaches frequently provide useful information, they may result in inadequate predictions of risk of toxicity as they are based on a single measurement ('snapshots' temporally and spatially) that do not adequately reflect how the broader sediment properties (including particle size, iron and manganese oxyhydroxides etc) influence metal bioavailability. Diffusive gradients in thin films (DGT) is an in-situ technique which provides a time-integrated measure of metal fluxes from the pore water and labile sediment phases, allowing the quantitative evaluation of the sediment metal 'release potential'. In this study, we assess the performance of the DGT technique to predict metal bioavailability by comparing DGT metal fluxes with metal bioaccumulation observed in benthic bivalves exposed to identical contaminated sediments under laboratory and field conditions. The suitability of the DGT technique as a monitoring tool for bioavailable metals is evaluated also through the comparison with traditional methods based on particulate sediment analyses.

ABSTRACT

The use of diffusive gradients in thin films (DGT) for predicting metal bioavailability was investigated by exposing the bivalve *Tellina deltoidalis* to an identical series of metal-contaminated sediments deployed simultaneously in the field and laboratory. To understand the differences in metal exposure occurring between laboratory and field based bioassays, changes in metal fluxes to DGT probes in sediments, and metal concentrations and partitioning to porewaters and overlying waters were investigated. DGT-metal fluxes (Cu, Pb and Zn) were lower in the overlying waters of most field-bioassays compared to the laboratory, causing differences in Pb and Zn bioaccumulation between bivalves exposed to laboratory and field conditions. Overall, DGT-metal fluxes provided predictions of metal bioaccumulation similar to those obtained using dilute-acid extractable metal measurements. This study demonstrates that, irrespective of the physicochemical properties of the sediment and type of exposure (laboratory or field), sediments pose a significant risk of bioaccumulation by *T. deltoidalis* when the Cu, Pb and Zn DGT flux exceeds 3.5, 1.3 and 156 $\mu\text{g}/\text{h}/\text{m}^2$, respectively. The results presented here support the use of the DGT technique for sediment quality assessment and the hypothesis that DGT-metal fluxes may potentially be useful surrogates for the lability of metals for all exposure routes.

Keywords:

bioavailability; diffusive gradients in thin films; dilute-acid extractable metals; acid-volatile sulfide

TOC Art

INTRODUCTION

Increasingly, sediment quality assessment frameworks consider contaminant bioavailability when evaluating the risk of adverse effects of specific contaminants to benthic organisms.^{1, 2} 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.³⁻⁵ 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.⁶⁻⁸ Furthermore, the concentration of AVS near the sediment-water interface (SWI) can be highly variable both spatially and temporally due to accompanied variability in the precursor elements to its formation (e.g. labile C, Fe and S), and sediment disturbance caused by plant roots and organisms feeding and burrowing behaviours.^{9,10}

The fluxes of metals from porewaters and labile sediment phases within surface sediments measured using diffusive gradients in thin films (DGT) deployed within sediments have been demonstrated to be useful for predicting metal exposure and lethal and sublethal toxicity to benthic invertebrates.^{11,12} DGT is an *in situ* technique which provides time-integrated measurements of the combined labile metal fluxes from the sediment porewater and particulate phases.¹³ 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.

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.¹⁴ However, in the 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 equilibriums 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,¹⁵ 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 metal release rates and DGT-metal flux measured will differ depending on the sediment properties and the chemical behaviour of the metals, and the release rate will also influence the accumulation by benthic organisms. This provides the basis for the use of DGT-metal fluxes for assessing metal bioavailability in sediments.

While the majority of studies applying DGT-induced metal fluxes for predicting metal bioavailability and toxicity in sediments have been conducted under laboratory conditions,^{11, 12, 16, 17} considerably less is known about the performance of DGTs for this purpose under field conditions.¹⁸ In this study, we test the performance of the DGT technique for predicting metal bioavailability and bioaccumulation 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 exposures occurring between laboratory and field-based bioassays.

MATERIALS AND METHODS

General methods. Plastic-ware used for analyses was new and washed by soaking in a 10% (v/v) HNO₃ (Suprapur Merck) for ≥ 24 h, followed by thorough rinsing with deionized water (Milli-Q, 18 M Ω •cm). Test vessels used for bioassays were washed with 1% HNO₃ followed by a Milli-Q water rinse. All chemicals used were analytical reagent grade or equivalent level of purity. Sediment chemical analyses were performed on sub-samples of homogenized sediments: particle size < 63 μm (by wet sieving through nylon mesh); organic carbon (OC, by high temperature TOC analyser following removal of inorganic carbon by acidification with 1 M HCl) and total recoverable metals (TRM, by microwave assisted aqua regia digestion);⁷ dilute-acid extractable metals (AEM, 1 M HCl) and acid-volatile sulfide (AVS) according to Simpson.¹⁹ Biological tissues were freeze-dried before microwave-assisted nitric acid extraction (HNO₃ at 200 °C for 30 min).²⁰ Water quality parameters (pH, salinity, temperature and dissolved oxygen) were monitored throughout the test. Overlying waters were membrane-filtered (0.45 μm) and acidified (2% (v/v) with HNO₃) prior to analyses of dissolved metals. Metal concentrations in overlying waters, acid-digests from sediments, biological tissues and DGT extracts were determined by inductively coupled plasma - optical emission spectrometry (ICP-OES, Varian 730-ES) and inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7500ce). Dissolved ammonia was analysed colorimetrically using a Merck Spectroquant Kit (14752, Merck). For quality assurance purposes, acid-digest blanks (10% of samples), replicates (20% of samples), analyte sample-spikes and certified reference materials (CRMs) were analysed. Replicates 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 expected 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, membrane filtered (0.45 μm) and analysed to confirm all metals of interest were < 1 $\mu\text{g/L}$. 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 $\pm$ 1 °C).

Clean and metal-contaminated sediments were collected from a range of estuarine sites near Sydney, Australia, as described in Belzunce-Segarra et al.²¹ The sediments had organic contaminants concentrations well below sediment quality guidelines values.¹⁰ All sediments were sieved through a 1 mm plastic mesh, homogenized and stored at 4° C in the dark until use. The sediments were grouped into two series (silty sediments “Silt1-4”, or sandy sediments “Sand1-4”) according to their physical properties, with one relatively uncontaminated sediment in each series. 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 previously.^{8,21}

Bioaccumulation bioassays. The laboratory- and field-based bioassays were undertaken for 31 days as described previously.²¹ The test sediments (Table 1, ~520 g) were contained in 1-L low-density polyethylene (LDPE) bottles (modified with 3 open windows (4.5 cm $\times$ 8 cm) for field chambers). In the laboratory bioassays, sediments had 500 mL of overlying water, whereas in the field, natural circulation of water was allowed to occur through the windows of the modified bottles.²¹ The field-deployed bioassays were

performed within two large mesh cages that each held eight bottles. The cages allowed adequate water circulation and prevented predation.²² The cages were submerged to a depth of 40 cm in an uncontaminated section of the Woronora River estuary (Sydney, Australia). There were two replicates of each test sediment, with all randomly distributed in the laboratory, and one replicate randomly distributed within each of the two field cages. Water quality parameters were measured twice weekly to confirm major parameters 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 before deployment to field cages and after water change in the laboratory. Laboratory organisms were also fed twice per week with 1 mg/bivalve of Sera Micron (Sera Fishtamins). At test completion, bivalves were depurated in clean seawater for 24 h, the soft tissue extracted from the shell and stored at -20°C until analysis.

Diffusive gradients in thin films. DGT sediment probes with overall dimensions of 24 cm × 4 cm × 0.5 cm and open window of 1.8 cm × 15 cm were purchased from DGT Research (<http://www.dgtresearch.com/>). The standard DGT assembly included a Chelex® binding gel and a polyacrylamide diffusive gel of 0.4 mm and 0.8 mm thickness, respectively, and a 0.45 µm pore size, 100 µm-thick polysulfone filter membrane.¹³ Gels and resins were prepared in our laboratory following the procedures recommended by DGT Research (Lancaster, UK), and all DGT manipulations were performed in a laminar flow cabinet. Before deployment, probes were conditioned by soaking overnight in 0.12 M NaCl continuously bubbled with nitrogen gas, then maintained under an inert gas atmosphere until deployment.²³ 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, and then cages were returned to the same location (after 20-30 mins). 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. Resin slices were extracted with 1 M HNO₃, diluted 10-fold with Mill-Q water, analysed by ICP-MS and fluxes calculated based on diffusive coefficients estimated for 21° C.²⁴ 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 in the overlying waters (µg/L), well-mixed conditions (negligibly small diffusive boundary layer (DBL) thickness) were assumed to be provided in both laboratory and field bioassays.¹⁵ Cd, Cu and Pb contributed < 0.1, <0.1 and <0.01 µg/h/m², respectively, to laboratory and field blank probes based on a 24-h deployment. DGT-Zn concentrations were blank corrected as zinc contamination was found in blank probes and estimated to contribute to an equivalent 24-h DGT-Zn fluxes of 60±14 and 69±17 µg/h/m² for laboratory and field blanks, respectively (mean ± standard deviation, *n*=4).

Data analysis. Differences in metal bioaccumulation rates between organisms exposed to laboratory and field conditions were investigated using the software Statgraphics Centurion (Warrenton, Virginia). Unless otherwise stated *p* = 0.05 was the level of significance. Bioaccumulation data were tested for homogeneity of variance (Levene's test) and for normality of residuals distribution (Shapiro-Wilk's test) prior to hypothesis testing. When either data were heteroscedastic or residuals did not follow a normal distribution,

two-way analysis by Kruskal-Wallis test followed by Bennett squares sum partition method were applied to evaluate statistical differences. Statistical differences between DGT concentrations measured in the first and second laboratory deployment were evaluated for individual treatments using paired t-tests followed by Bonferroni correction ($\alpha = 0.05/8$). The normality of the differences was tested using Shapiro-Wilk's test. 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 s.

RESULTS AND DISCUSSION

Sediment properties. The metal-contaminated sediments 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 sulfide (AVS), while the sandy series had 14-31% <63 μm particles, 2.1-5.9 % TOC and 4.2-5.9 $\mu\text{mol/g}$ AVS (Table 1). Copper, lead and zinc were the major contaminants in both sandy and silty sediments. Total recoverable metal (TRM) concentrations in sandy sediments ranged from 10 to 265, 18 to 317, and 54 to 2640 mg/kg for Cu, Pb and Zn, respectively, whereas in silty sediments, they ranged from 35 to 1050, 65 to 747 and 166 to 1000 mg/kg, respectively (Table 1). Concentrations for a greater range of metals and metalloids are provided in Table S1 and S2 of the Supporting Information (SI). The dilute-acid extractable metal (AEM) concentrations were 43 (± 14), 89 (± 10) and 82 (± 11) % of the TRM for Cu, Pb and Zn, 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 (Table 1).⁴

Discrete dissolved metal samples and DGT metal concentrations in the overlying waters.

Dissolved copper and zinc concentrations in discrete overlying water samples measured in the laboratory bioassays generally followed the trend observed for particulate metal concentrations found in the sediments, except for lead concentrations which were consistently <2 $\mu\text{g/L}$ for most treatments (Table 1). Overall, copper and zinc concentrations varied within a small range throughout the laboratory experiment. Moderately (but significant, $p < 0.05$) greater concentrations were observed for copper, in treatments Silt3 (from <1 to 8 $\mu\text{g/L}$), Sand2 (from <1 to 1.4 $\mu\text{g/L}$) and Sand4 (from <1 to 2.4 $\mu\text{g/L}$), and zinc, in treatments Silt4 (from 6 to 20 $\mu\text{g/L}$) and Sand2 (from 22 to 39 $\mu\text{g/L}$) (Figure S1 of the SI).

Overall similar DGT concentrations (Cu, Pb and Zn) were measured between the first and second deployments (except for DGT-Cu in Silt3, $p = 0.001$), although the small sample size likely caused an inability to detect significant differences (Figure S2 of the SI). Increasing DGT metal concentrations in the overlying water (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 Silt1, 2, 3 and 4, respectively), and of zinc in the sandy series (<1, 23 ± 25 , 113 ± 48 and 316 ± 102 $\mu\text{g/L}$ for Sand1, 2, 3 and 4, respectively). DGT-Cu concentrations were <1 $\mu\text{g/L}$ in sandy sediments, whereas DGT-Zn concentrations were <15 $\mu\text{g/L}$ in silty sediments. For all sediments, DGT-Pb concentrations were consistently <2 $\mu\text{g/L}$.

Table 1. Chemical and physical properties of control (Silt1 and Sand1) and contaminated sediment and overlying water.

Sediment	Total recoverable metals (TRM)					Dilute 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			
Silt1	2.5	87	35	65	188	1.1	47	22	63	166	<2	<2	<2	3	3.6	94
Silt2	3.3	182	303	248	544	1.2	85	128	232	387	6 ± 3	<2	3 ± 1	9	4.2	92
Silt3	4.1	275	527	414	828	1.3	121	252	393	585	10 ± 5	<2	6 ± 1	15	4.7	90
Silt4	5.5	446	1050	747	1480	1.6	196	523	721	1000	20 ± 9	2.2 ± 1.6	18 ± 4	27	5.7	81
Sand1	0.4	21	10	18	54	0.3	17	5	17	48	3 ± 1	<2	2 ± 1	1	0.9	26
Sand2	1.7	107	265	317	705	0.6	25	57	242	585	<2	<2	28 ± 9	10	5.9	31
Sand3	1.2	71	118	198	1550	0.4	17	27	140	1480	<2	<2	120 ± 47	14	4.2	14
Sand4	1.7	76	94	218	2640	0.5	21	41	199	2400	2 ± 1	<2	450 ± 160	34	2.1	27
Guideline Values			65	50	200			65	50	200	(<2)	(<2)	(2.3 ± 1.8)			

AVS = acid-volatile sulfides; SEM-AVS refers to the molar difference, where SEM is equivalent to AEM; TOC = total organic carbon; particles <63 μm is the percentage (by weight) of fine sediment particles. Metal concentrations in sediments (dry weight) are mean values of 2 replicate analyses, and relative standard deviations were generally within 20% of the calculated values. Dissolved metal 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, $n=24$). Guideline Values = sediment and water quality guideline values.¹

DGT-Cu and -Zn concentrations were generally lower than dissolved copper and zinc concentrations measured in discrete overlying water samples (Figure S3 of the SI). 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.¹⁵ On average, DGT-Zn concentrations were about 20% lower than dissolved zinc, whereas DGT-Cu was 75% lower than dissolved copper concentrations. This could be due to higher affinity of copper for organic ligands, which has been previously reported to reduce copper lability to DGT,²⁵ and also the presence of colloidal metal forms.

As expected, lower metal concentrations (i.e. lower metal fluxes) (Cu, Pb and Zn) were observed in the overlying water of most field bioassays than for laboratory bioassays (Figure 1). 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. As a consequence, DGT-metal concentrations in the overlying water for field bioassays were similar amongst all treatments and between the first and second deployments ($<1 \mu\text{g/L}$ for Cu and Pb and $32 \pm 10 \mu\text{g/L}$ for Zn, $n=64$).

DGT-metal fluxes from sediments. DGT-Cu, -Pb and -Zn maxima generally occurred approximately 1 cm below the SWI (Figure 1). In this region of the sediment, a number of processes would have contributed to the observed maxima, including metal release associated with the reductive dissolution of Fe(III) and Mn(IV),^{12,26,27} degradation of organic matter²⁸⁻³⁰ and/or oxidation of AVS phases.^{31,32} Due to the low slicing resolution (1 cm), it was not possible to distinguish the different processes. It is also likely that minor contributions occurred from metals associated with dissolved organic matter which diffused into the resin. Lower DGT-metal fluxes occurred deeper in the sandy sediments, 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 same sandy sediments.²¹

The pore water DGT-Cu, -Pb and -Zn fluxes (below the SWI) were generally lower in field-exposed compared to laboratory-exposed sediments (Figure 1). 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 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.

Figure 1. 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 and 2 replicates ($n=4$), with standard errors averaging $18 (\pm 11)$, $20 (\pm 12)$ and $28 (\pm 20)$ % of mean values for Zn, Pb and Cu, respectively (values in brackets are standard deviations, $n=80$). The lines connecting the measurements for each individual DGT profile are for visual aid only.

Despite the different conditions, similar iron and manganese DGT-profiles were measured in laboratory and field-exposed sediments (Figure S4 of the SI). The lower Fe(II) and Mn(II) fluxes closer to the SWI were consistent with oxygen penetration in surface sediments causing the formation of insoluble Fe(III) and Mn(IV) oxyhydroxides, and the production of Fe(II) and Mn(II) in the deeper sediments by bacterially-assisted reductive dissolution of these oxyhydroxides phases. The oxidation rate of Mn(II) is slower than Fe(II), and the profiles also indicate a flux of Mn(II) from the sediments to the overlying water, but not of Fe(II). Differences between iron and manganese reduction boundary depths are usually observed due to the different redox properties of the metals,³³ but only in sediments Sand2 and Sand3 (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 was added to test vessels on the morning the tests commenced. This was for logistical reasons, and did not provide time for complete redox stratification within the sediments to re-establish.^{34, 35} 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.²¹ The SPM contained 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±5% <63 µm). Pore water DGT-metal fluxes have been shown to decrease with increasing percentages of <63 µm particles,^{11,12} 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 times during the bioassays (on day 5 and 19) 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, i.e. the difference in fluxes from two replicates sediments under both laboratory and field conditions. A comparison between pore water fluxes (average of measurements at -0.5, -1.5, -2.5 cm) obtained in the first and second deployment is provided in Figure 2. Plots indicated linear relationships between DGT fluxes measured in the first and second deployment under both laboratory and field conditions ($R^2=0.64-0.94$), except for DGT-Zn in the field ($R^2=0.06$). Slopes differed from that of a 1:1 line by a factor of <28%, except for copper under laboratory conditions (~50%). This suggests that overall there were relatively little changes in metal partitioning during the tests.

Relationships between metal bioaccumulation and DGT fluxes. No mortality to bivalves was observed after the 31-day exposure for any treatments. 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 S5 of the SI). *T. deltoidalis* reside buried in the sediment, but use their siphon to reach the sediment surface to feed on food particles⁸ and both dietary exposure through ingestion of sediment particles^{8,36} and dissolved exposure^{37,38} 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 1st and 2nd deployment) and bioaccumulation were investigated (Figure S6 of Supporting Information):

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

(iv) within 1 cm above the SWI - exposure from overlying water only.

Overall, similar results were obtained using the different approaches. Due to the maxima in the DGT flux observed near the SWI (Figure 1), and the feeding behaviour of the bivalve, DGT-metal fluxes at the SWI (i.e. ± 1 cm) were used for the interpretation of the relationships with bioaccumulation. Further discussion of the choice of the DGT slice-range to use in interpreting the metal bioaccumulation by the bivalve is provided in this section. 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 was consistent with low DGT-Cu fluxes for these sediments, whereas increasing soft tissue concentrations in bivalves exposed to silty sediments were observed for increasing copper fluxes in those sediments (Figure 3). In the silty sediments, similar copper bioaccumulation was observed between laboratory and field-exposed bivalves (except in sediment Silt2) (Figure S5 of the SI), despite the significantly greater DGT-Cu fluxes in laboratory-exposed sediments (Figures 1 and 3). 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 1). 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 did not largely improve (R^2 increased from 0.54 to 0.63, Figure S6 of the SI). However, these results together suggest that the copper exposure from the overlying water was unlikely to have contributed to the bioaccumulation of copper as greatly as predicted using DGT-Cu fluxes, consistent with the porewater or sediment particle ingestion being the major exposure route for this bivalve.^{8,36}

Increasing lead bioaccumulation occurred with increasing DGT-Pb fluxes, although the relationship was quite different for the silty sediments exposed to laboratory conditions (Figure 3). As for copper, this indicated that the use of DGT-Pb fluxes in laboratory deployments may overestimate the lead exposure and potential for bioaccumulation. Lead bioaccumulation in bivalves deployed in silty sediments was higher under field conditions, whereas the opposite trend was observed for bivalves deployed in sandy sediments (Figure S5). However, DGT-Pb fluxes were generally greater under laboratory conditions (Figure 3) for both sandy and silty sediments. Given that dissolved and DGT lead concentrations measured in laboratory and field overlying waters were consistently $<2 \mu\text{g/L}$ for all treatments, dissolved lead in the overlying water likely contributed less to the lead bioaccumulation than either the porewater or particulate (dietary exposure) forms of lead in the sediments.

The bioaccumulation of zinc increased rapidly when DGT-Zn fluxes exceeded $\sim 160 \mu\text{g/h/m}^2$, and a strongly positive relationship existed across all the sediments and exposures (Figure 3). This indicated a possible threshold value above which the ability of *T. deltoidalis* to regulate the internal zinc concentrations was inhibited.^{39,40} 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 (between -2 and -3 cm depth) (Figure S6). This was consistent with the low zinc bioavailability indicated by DGT in the deeper region of the sediment, as opposed to the considerable release of DGT-labile zinc observed in the proximity of the SWI.

Figure 2. DGT-Cu, -Pb and -Zn fluxes measured in the pore water (average of measurements at -0.5, -1.5, -2.5 cm depth) on day 5 (first deployment) and 19 (second deployment). Data points are means with standard error (n=6).

Figure 3. Relationships between Cu, Pb and Zn bioaccumulation and DGT fluxes measured at the SWI and AEM concentrations. 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 and vertical continuous lines are used to separate the data into four quadrants: the continuous horizontal line represents the mean concentration found in bivalves exposed to control sediments, whereas dashed lines refer to controls mean value plus 1s and 2s. The continuous vertical line corresponds to the DGT flux or AEM concentration above which bioaccumulation was consistently higher than controls mean plus 1s.

Use of DGT-metal fluxes for predicting risk of bioaccumulation in metal contaminated

sediments. In the companion paper²¹ the average AEM concentrations in the surface sediments before and after exposure were shown to provide a better measurement than TRM and OLV concentrations for predicting bioaccumulation of Cu, Pb and Zn in the bivalve in these same sediments. Strong relationships were observed between DGT-metal fluxes and AEM concentrations (Figure S7 of the SI), and with tissue metal concentrations in *T. deltoidalis* (as a measure of potential metal exposure, Figure 3). To assist with the comparison between predictions of bioaccumulation obtained by DGT and AEM a quadrant approach was adopted (Figure 3). Overall, DGT-metal fluxes and AEM concentrations provided similar predictions of bioaccumulation of Cu, Pb and Zn. DGT and AEM predicted that 4 and 6 of the sediments posed potential risk of copper bioaccumulation, respectively. Both methods identified the same silty sediments under laboratory exposure conditions to exhibit potential risk, although AEM indicated a greater risk of copper bioaccumulation for two additional silty sediments deployed in the field. For lead, AEM concentrations appeared to provide a clearer break-out of points between the upper-right and bottom-left quadrant (indicating a greater ability to separate 'risk of significant and frequent bioaccumulation' from 'no risk' situations - no significant bioaccumulation or infrequent observation of significant bioaccumulation), whereas in the DGT plot some data points laid on the upper-left quadrant. For zinc, similar predictions of bioaccumulation were obtained using the two methods, i.e. the same sediments were indicated to pose risk of bioaccumulation by AEM and DGT. Significant bioaccumulation was occasionally observed in the upper-left quadrant for both AEM and DGT, which indicated that this approach may result in the overestimation of risk thresholds. The overall similar predictions of bioaccumulation obtained by the two techniques is likely due to the ability of both techniques to provide information on the less strongly-bound (and thus more bioavailable) metals which are more likely to be released in the porewater and/or within the organisms following particles ingestion. However, the observations were influenced by the choice of sediment used in this study, and a greater range of sediment metal concentrations and properties needs to be studied to determine the advantages of each approach.

Bioaccumulation in *T. deltoidalis* exposed to contaminated sediments was significantly greater than that of bivalves exposed to control sediments for fluxes $>3.5 \mu\text{g/h/m}^2$ for copper, $>1.3 \mu\text{g/h/m}^2$ for lead, and $>156 \mu\text{g/h/m}^2$ for zinc. These values can be used as potential guideline values to identify risk of bioaccumulation in benthic bivalves. The ability of the DGT technique to provide *in situ* measurements reduces the risk of sample alteration associated with sample grabbing procedures and storage, and DGT time-integrated measurements allow us to incorporate changes in metal lability that may occur over time as opposed to 'snapshot' measurements typical of particulate metal analyses. This study indicates that predictions of bioaccumulation provided by DGT-metal flux measurements were overall similar to those obtained using dilute-acid extractable metal 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*^{8,36,37} and amphipod, *M. plumulosa*.^{7,8,41} The present study further supports the hypothesis of Amato et al.¹² that the DGT-labile metal flux may potentially be a useful surrogate for the lability of metals for all exposure routes.

■ ACKNOWLEDGMENTS

David Spadaro and Ian Hamilton are thanked for assisting with culturing and handling of amphipod and advice on tests. Katelyn Edge is thanked for collecting the sediments from Port Kembla, NSW. Robert Jung and Josh King are thanked for assisting with bivalve sample analyses. Iñigo Muxika is thanked for data analysis support. The authors acknowledge the financial support of the NSW Environmental Trust (Research Project APP2010-RD-0177), the University of Wollongong scholarship support for E Amato, the CSIRO Wealth from Oceans Flagship, and the Basque Government for financial support for M. Belzunce-Segarra.

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Supporting information

Metal fluxes from porewaters and labile sediment phases for predicting metal exposure and bioaccumulation in benthic invertebrates.

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Figure S7: Relationships between DGT fluxes measured at the SWI (± 1 cm) and AEM concentrations (average of initial and final surface concentrations).

Table S1. Total recoverable metal concentrations in original sediments in mg/kg, unless specified.

Sediment	Al %	Fe %	Mn	Cu	Pb	Zn	As	Cd	Co	Cr	Ni	Sn	V
Silt1	1.1	2.5	87	35	65	188	19	1.0	4	29	12	3	41
Silt2	1.3	3.3	182	303	248	544	30	0.7	8	47	15	60	57
Silt3	1.4	4.1	275	527	414	828	42	1.5	9	65	18	119	74
Silt4	1.5	5.5	446	1050	747	1480	64	1.8	16	96	25	234	103
Sand1	0.2	0.4	21	10	18	54	5	0.9	1	5	2	1.0	8
Sand2	0.7	1.7	107	265	317	705	13	1.3	5	75	15	69	34
Sand3	0.6	1.2	71	118	198	1550	7	1.1	4	26	11	7	24
Sand4	0.7	1.7	76	94	218	2640	11	1.4	5	34	13	7	27

Table S2. Dilute acid-extractable metal concentrations in original sediments (1 M HCl) in mg/kg, unless specified.

Sediment	Al %	Fe %	Mn	Cu	Pb	Zn	As	Cd	Co	Cr	Ni	Sn	V
Silt1	0.2	1.1	47	22	63	166	7	0.1	3.0	11	4	1	22
Silt2	0.3	1.2	85	128	232	387	10	0.4	3.4	19	5	29	29
Silt3	0.4	1.3	121	252	393	585	16	0.5	3.5	29	6	58	35
Silt4	0.5	1.6	196	523	721	999	24	1.0	5.4	50	9	117	48
Sand1	0.1	0.3	17	7	17	48	2	0.0	0.7	3	1	0.4	7
Sand2	0.2	0.6	25	57	242	585	2	1.0	1.5	27	5	26	18
Sand3	0.2	0.4	17	27	140	1479	2	0.9	1.7	7	2	2	9
Sand4	0.2	0.5	21	41	199	2405	3	1.2	2.5	15	4	3	12

Figure S1. Changes in dissolved copper (a and b) and zinc (c and d) concentrations measured in discrete overlying water samples collected throughout the test. Plots 'a' and 'c' show the full data set for Cu and Zn, respectively, whereas plots 'b' and 'd' show concentrations lower than 5 and 50 $\mu\text{g/L}$ for Cu and Zn, respectively. Curves are linear regressions reported for $p < 0.05$.

Figure S2. Comparison between DGT metal concentrations (Cu and Zn) measured in the first (day 5) and second (day 19) deployment under laboratory conditions (from 0 to 3 cm above the SWI). Data points are means of four values with standard deviation. * The asterisk indicates significant differences between deployments (Bonferroni correction: $\alpha=0.05/8$), whereas the letter w indicates weak differences ($0.006 < p < 0.01$). DGT-Pb concentrations were consistently $< 2 \mu\text{g/L}$.

Figure S3. Relationships between DGT and dissolved metal concentrations. Data points are means with standard deviations (n=8 and 12 for DGT and dissolved metal concentrations, respectively).

Figure S4. DGT-Fe and -Mn fluxes measured in laboratory and field deployments (data points represent average values of first and second deployment and 2 replicates ($n=4$), standard errors were on average $26 (\pm 13)$ and $29 (\pm 16)$ % of mean values for iron and manganese, respectively). Continuous and dashed lines represent field and laboratory deployments, respectively. The lines connecting the measurements for each individual DGT profile are for visual aid only.

Figure S5. Lead, zinc and copper bioaccumulation in laboratory and field-exposed bivalves. Soft tissue concentrations are mean $\pm$ standard errors ($n = 2$). For sediment Sand4, only one replicate was available for the field experiment. *T. deltoidalis* baseline is the metal concentrations measured in non-exposed biota (as described in the methods). Metal concentrations (dry weight) in organisms exposed to different sediment types (Sed. type = sandy and silty) and exposure conditions (Sed. exp = laboratory and field) were tested for statistical differences. Interaction effects (Inter.) between sediment types and exposures were also tested for statistical significance.

Figure S6. Relationships between Cu, Pb and Zn bioaccumulation and DGT fluxes measured (i) at the SWI (± 1 cm), (ii) between 0 and -3 cm depth, (iii) at -3 cm depth, and (iv) 1 cm above the SWI (laboratory and field exposure).

Figure S7. Relationships between DGT fluxes measured at the SWI (± 1 cm) and AEM concentrations (average of initial and final surface concentrations).