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DGT-induced copper flux predicts bioaccumulation and toxicity to bivalves in sediments with varying properties

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Abstract

Many regulatory frameworks for sediment quality assessment include consideration of contaminant bioavailability. However, the “snap-shots” of metal bioavailability provided by analyses of porewaters or acid-volatile sulfidesimultaneously extractable metal (AVS-SEM) relationships do not always contribute sufficient information. The use of inappropriate or inadequate information for assessing metal bioavailability in sediments may result in incorrect assessment decisions. The technique of diffusive gradients in thin films (DGT) enables the in situ measurement of metal concentrations in waters and fluxes from sediment porewaters. We utilized the DGT technique to interpret the bioavailability of copper to the benthic bivalve *Tellina deltoidalis* in sediments of varying properties contaminated with copper-based antifouling paint particles. For a concentration series of copper-paint contaminated sandy, silty-sand, and silty sediment types, DGTprobes were used to measure copper fluxes to the overlying water, at the sedimentwater interface, and in deeper sediments. The overlying water copper concentrations and DGT-Cu fluxes were shown to provide excellent exposure concentration–response relationships in relation to lethal effects occurring to the copper-sensitive benthic bivalve, *T. deltoidalis*. The study demonstrates the strength of the DGT technique, which we expect will become frequently used for assessing metal bioavailability in sediments.

Keywords

induced, copper, flux, predicts, dgt, properties, sediments, varying, bioaccumulation, toxicity, bivalves, CMMB

Disciplines

Life Sciences | Physical Sciences and Mathematics | Social and Behavioral Sciences

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DGT-INDUCED COPPER FLUX PREDICTS BIOACCUMULATION AND TOXICITY TO BIVALVES IN SEDIMENTS WITH VARYING PROPERTIES

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26 **ABSTRACT:** Many regulatory frameworks for sediment quality assessment include
27 consideration of contaminant bioavailability. However, the ‘snap-shots’ of metal
28 bioavailability provided by analyses of pore waters or acid-volatile sulfide - simultaneously
29 extractable metal (AVS-SEM) relationships do not always contribute sufficient information.
30 The use of inappropriate or inadequate information for assessing metal bioavailability in
31 sediments may result in incorrect assessment decisions. The technique of diffusive gradients
32 in thin films (DGT) enables the in situ measurement of metal concentrations in waters and
33 fluxes from sediment pore waters. We utilised the DGT technique to interpret the
34 bioavailability of copper to the benthic bivalve *Tellina deltoidalis* in sediments of varying
35 properties contaminated with copper-based antifouling paint particles. For a concentration
36 series of copper-paint contaminated sandy, silty-sand and silty sediment types, DGT-probes
37 were used to measure copper fluxes to the overlying water, at the sediment-water interface
38 and in deeper sediments. The overlying water copper concentrations and DGT-Cu fluxes
39 were shown to provide excellent exposure concentration-response relationships in relation to
40 lethal effects occurring to the copper-sensitive benthic bivalve, *T. deltoidalis*. The study
41 demonstrates the strength of the DGT technique, which we expect will become frequently
42 used for assessing metal bioavailability in sediments.

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48 Keywords: DGT, copper, antifouling paint, sediment, bivalve, bioaccumulation,
49 ecotoxicology.

50 **Introduction**

51 The technique of diffusive gradients in thin films (DGT) was developed to enable the in situ
52 measurement of metal concentrations in waters¹ and fluxes from sediment pore waters.^{1,2} In a
53 DGT device, dissolved metal species diffuse through a polyacrylamide gel layer and become
54 trapped in a gel impregnated with a metal-exchange resin, which acts as a metal sink.³ The
55 removal of metals from sediment porewaters causes the concentration to decline immediately
56 adjacent to the device. This localised decline could disturb the dynamic equilibrium between
57 the sediment and metal in solution and induced release of metals to solution⁴, the extent of
58 which will depend on the rate of metal resupply from the sediment solid phase to the
59 porewater. If there is rapid resupply, metal concentrations in pore waters may be calculated
60 from the DGT-accumulated metal concentration. However, when resupply from the sediments
61 is limited the DGT-flux provides information on the relative rate of remobilisation of metals
62 from sediments to the pore water.⁵ Hence the DGT directly measures the flux of metal from
63 the sediment during the deployment time, which reflects the concentration in the porewater,
64 its diffusional transport and the supply from the solid phase to solution.⁶ It can be interpreted
65 simply as the average porewater concentration at the interface of the device over the
66 deployment period. The ability of DGT measurements to provide information on the localised
67 remobilisation of metals has been utilised to create high resolution depth profiles of metals
68 within sediments.^{2,7} Such studies have demonstrated the heterogeneity of sediment
69 environments; including the existence of microniches and to characterise zones of metal
70 remobilisation.⁸⁻¹⁰

71 Existing methods for estimating the bioavailability of metals in sediments to benthic
72 organisms have numerous limitations.^{11,12} For example, the usefulness of chemical extractions
73 that provide information on metal lability varies between metals and the degree of
74 contamination and for the organism being studied.^{13,14} Further to this, the equilibrium
75 partitioning relationships between metals and acid-volatile sulfide (AVS) become less
76 appropriate for organisms that create oxic/sub-oxic micro-environments at burrow walls
77 within sulfidic sediments^{15,17}, and pore water analyses provide non time-integrated ‘snap-
78 shots’ of concentrations that change rapidly upon sediment disturbance.^{18,19}

79 The DGT-accumulated metal concentration has been determined to consist of free metal
80 ions, metal ions present as simple inorganic complexes, and labile organic complexes.^{20,21} As
81 these metal fractions represent the most bioavailable metals²⁰⁻²², the time-integrated measure
82 of the bioavailable metal provided by the DGT technique is proving to be a powerful tool for
83 assessing metal bioavailability in waters.²²⁻²⁴ In sediments, the bioavailability of metals to

84 benthic organisms is influenced by the characteristics of the metal, the properties of the
85 sediments and the varying exposure pathways of the organisms.²⁵⁻²⁸ The DGT technique was
86 used by Wegener et al.²⁹ to demonstrate that sediment bioturbation and feeding by the
87 oligochaete *Tubifex* modified porewater metal concentrations within surface sediments. The
88 release of metals from sediment particles to pore water will be more rapid for sediments that
89 contain reactive forms of metals when compared to sediments that contain more inert forms of
90 metals, hence differences in DGT-induced metal fluxes are expected to provide a useful
91 measure of the bioavailability of the metals in sediments. Roulier et al.³⁰ observed total and
92 DGT-accumulated metal concentrations both provided strong relationships with Cu and Pb
93 bioaccumulation from sediments by *Chironomus riparius*, however, only the DGT technique
94 provided an explanation of different organismal uptake for sediments with similar total metal
95 concentrations.

96 In the present study we utilise the DGT technique to interpret the bioavailability of copper
97 to the benthic bivalve *Tellina deltoidalis* in sediments contaminated with copper-based
98 antifouling paint. Copper-based paints are increasingly used by marine industries to reduce
99 biofouling and the abrasion of paint from structures can result in considerable copper
100 concentrations in sediments.³¹ Concentration series of copper-paint contamination were
101 prepared for three sediments, representing sandy, silty-sand and silty environments. DGT-
102 induced copper fluxes were measured in the overlying water, at the sediment-water interface
103 (SWI) and in deeper sediments. The DGT- induced copper flux at the SWI was compared
104 with copper bioaccumulation and toxicity to the bivalve.

105 **Material and Methods**

106 **General methods.** All glass and plastic-ware for analyses were usually new and were
107 cleaned by soaking in 10% (v/v) HNO₃ (BDH, Analytical Reagent grade) for a minimum of
108 24 h, followed by thorough rinsing with deionized water (Milli-Q, 18 MΩ/cm). All chemicals
109 were analytical reagent grade or equivalent analytical purity. Water pH, salinity, temperature
110 and dissolved oxygen measurements were made with probes calibrated according to
111 manufacturer instructions (WTW). Methods for sediment particle size (by wet sieving
112 through 63 μm nylon sieves followed by gravimetry), total organic carbon (TOC, by high
113 temperature TOC analyzer), porewater (PW) extraction (centrifugation at 800 g for 5 min),
114 and dissolved ammonia analyses are described in Spadaro et al.³². Methods for analyses of
115 total recoverable metals (TRM, by microwave assisted aqua regia), dilute acid extractable
116 metals (AEM, 1 M HCl) and acid-volatile sulfide (AVS) (all determined on sub-samples of
117 the same homogenised sediment) are described previously.³³ Biota tissues were freeze-dried

118 before microwave-assisted (MARS 5, CEM) acid/peroxide extraction (3:1 HNO₃:H₂O₂, 80 °C
119 for 50 min). Dissolved metal concentrations in acid-digests of water, biota and sediment
120 samples were determined by inductively coupled plasma - optical emission spectrometry
121 (ICP-OES, Varian 730-ES) as described in Simpson et al.³⁴ The metal concentrations of the
122 acid-digests of DGT gels were determined by inductively coupled plasma-mass
123 spectrophotometry (ICP-MS, Agilent 7500ce). As part of the quality assurance, analyses of
124 filter and acid-digest blanks, replicates for 20% of samples, analyte sample-spikes and the
125 certified reference materials (CRMs) were performed. Replicates were within 20% and
126 recoveries for spikes and CRMs, PACS-2 for sediment (National Research Council Canada,
127 NRCC) and DORM-3 for biota (fish protein, NRCC), were within 85-115% of expected
128 values. The limits of reporting for the various methods were less than 10% of the lowest
129 measured values.

130 **Test media and organisms.** Clean seawater was collected from Port Hacking, Sydney,
131 Australia, membrane filtered (0.45 µm), and acclimated to a room temperature of 21±1°C.
132 Where necessary, the salinity of the filtered seawater was adjusted to the test salinity of 30
133 PSU using Milli-Q water. Surface sediments (0-3 cm depth) were collected from three
134 intertidal estuarine locations for use as controls and for preparing sediment with a range of
135 particles sizes as described in Strom et al.³⁵ Three sediments were prepared with 10%
136 (sandy), 30% (silty-sand) and 60% (silty) <63 µm particles, respectively. *T. deltoidalis* with
137 shell lengths of 5-12 mm were collected from Lane Cove River (NSW, Australia) and
138 maintained as described previously.^{36,37}

139 **Copper-based paint spiked sediments.** Copper-based antifouling paint (13% copper(I)
140 oxide/4% zinc oxide; Hempanet, Hempel) was dried at 45 °C for 24 h, snap-frozen in liquid
141 nitrogen, crushed using a Teflon beaker and mortar, and the <180 µm particles isolated using
142 a nylon sieve. Before TRM analyses, the paint samples were heated in a porcelain crucible at
143 500°C for 4 h to destroy the organic matrix. Copper concentration series of paint-spiked
144 sediments were prepared by adding the required amounts of paint to the three sediments. The
145 sediments remained at pH 7.7±0.2 without pH-adjustment and were mixed and equilibrated
146 for a period of 30 days.³⁸ Copper concentrations are frequently 100-1000 mg/kg in sediments
147 near marinas and aquaculture leases that use copper-based paints on boats, nets and other
148 marine structures.^{31,39} An initial concentration series of 0, 200 and 1000 mg Cu/kg was
149 prepared and tested. After 8 months (stored refrigerated) the 1000 mg Cu/kg sediment from
150 each particle size composition, was diluted with control sediment of the same composition to
151 create sandy sediments with 0, 50, 100, and 200 mg Cu/kg, and silty-sand and silty sediments

152 with 0, 100, 200, and 400 mg Cu/kg. These dilution series were mixed and equilibrated for a
153 further 30 days to achieve dissolved-particulate copper partitioning similar to that occurring in
154 field-contaminated sediments.⁴⁰ At the completion of the equilibration process, sub-samples
155 of the copper-paint spiked sediments taken for duplicate analyses of pH, dissolved porewater
156 metals, particulate metals (TRM and AEM), particle size distribution, TOC, and AVS.

157 **Diffusive gradients in thin films (DGT).** Plastic DGT assemblies (24×4×0.5 cm, with
158 1.8×15 cm windows) were purchased from DGT Research (<http://www.dgtresearch.com/>).
159 DGT gels were prepared following the procedures recommended by DGT Research (details
160 are provided in Section S1 of the Supporting Information,). The DGT probe cross-section
161 comprised: backing plate, 0.45 µm filter membrane, 0.25 mm Chelex gel, 0.5 mm diffusive
162 gel, 0.45 µm filter membranes, and a front window plate. All components had been carefully
163 prepared to minimise metal contamination. Deoxygenated DGT probes were gently inserted
164 into the sediments to a depth of 4 cm with care to ensure a good contact between the sediment
165 and the DGT membrane. Deployments times of 24 h (DGT equilibration) were selected
166 based on Garmo et al.,⁴¹ as we used 0.5 mm diffusive gel thickness in environmentally
167 relevant PW-Cu concentrations in the presence of humic acids. After 24 h of deployment,
168 DGT probes were gently removed from the beakers and position of the sediment-water
169 interface (SWI) and overlying water depth were recorded. The probes were immediately
170 rinsed with Milli-Q water to remove sediment particles, then held in clean plastic bags at 4°C
171 until disassembly. DGT devices were disassembled (within 10 days of retrieval) and resin gel
172 slices cut using a Teflon coated blade to obtain the desired vertical profile. For the first DGT
173 deployment there was a 1.5 cm slice at the SWI (from -0.75 cm to +0.75 cm), two 2-cm slices
174 in the overlying water above the SWI-slice and 2-cm slice in the sediment above the SWI-
175 slice. For the second DGT deployment there was a 1-cm slice at the SWI (-0.5 cm to 0.5 cm),
176 and 1 cm slices above and below the SWI-slice. The mid-point of the slice was set as the
177 location in data analysis and Figure 1. Slices from below the SWI were washed with Milli-Q
178 water to remove any sediment particles. Each slice was weighed, then eluted with 1 M HNO₃
179 for 16-24 h before analysis by ICP-MS. Undeployed DGT probes were analysed as handling
180 blanks and their copper concentrations were less than 5% of the lowest measured
181 concentration.

182 **Bivalve bioaccumulation bioassays.** The test sediments (400 mL) and overlying water
183 (500 mL) were added to 1 L Pyrex beakers (washed sequentially with phosphate-free
184 detergent, 1% HNO₃ and Milli-Q water) five weeks prior to the start of the tests and kept in a
185 temperature controlled lab (21±3°C, normal day light conditions).³⁷ The beakers were

186 bubbled with air during this period and throughout the tests, and developed stratified redox
187 profiles.⁴² On the day of test commencement the overlying water was removed to a level of 2
188 cm above the sediment surface, 10 bivalves were added to each beaker, and then new
189 seawater was added. Water pH (pH 8.2±2), salinity (31±1‰), temperature (21±1°C,) and
190 dissolved oxygen concentration (>80% saturation), and water levels were monitored
191 throughout the tests. The overlying water was changed 3 times per week after sub-sampling
192 for analyses of dissolved copper and ammonia. The DGT probes were inserted on days 8 and
193 15, and removed 24 h after deployment. The tests were terminated after 30 days and
194 surviving organisms were counted, and allowed to depurate overnight in clean seawater
195 before refrigeration. The bivalve shells were opened using a Teflon coated razor blade and the
196 soft tissue transferred to a pre-weighed 50 mL polycarbonate vial using plastic tweezers and
197 then frozen at -20°C before analyses.

198 **Statistical analyses.** At the end of the bioaccumulation bioassays the percent survival in
199 tests relative to survival in the controls was recorded. All statistical analyses were carried out
200 using the software Toxcal (Version 5.0.23, TidePool Scientific Software). Unless otherwise
201 stated $p = 0.05$ was the level of significance. Effect concentration causing $x\%$ of the lethality
202 to the bivalve (LC_x) were estimated from concentration-response data and corresponding
203 copper concentration using linear interpolation as described previously.^{35,37} Correlations
204 (Pearson's product-moment) were performed on the particulate metal concentrations using
205 statistical software (NCSS).

206 **Results and Discussion**

207 **Properties of the paint-spiked sediments.** The TRM copper and zinc concentrations in
208 the paint were 9.3±0.7 mg/kg and 3.0 ±0.2 %, respectively. These compared to
209 concentrations of 13±1 %-Cu and 4.0±1 %-Zn, specified by the manufacturer⁴³ and were
210 similar to the compositions of most copper-based antifouling formulations.⁴⁴

211 The properties of the paint-spiked sediments are shown in Table 1. The pH of the
212 sediments ranged from 7.7-7.9; exhibiting no relationship with spike concentration or
213 sediment type. The sandy, silty-sand and silty sediments had TOC concentrations of 0.7, 1.6,
214 3.1%, respectively, and particle size distributions within 10% of the nominal 10, 30 and 60%
215 <63 µm particles. Less than 4% of each sediment contained 63-180 µm size fraction and the
216 remainder was mostly 180-1100 µm sized sand. The AVS concentrations were <0.5 µmol/g
217 for all the sediments. For total copper concentrations (TR-Cu) were within 25% of the
218 nominal concentrations. Dilute acid-extractable copper concentrations (AE-Cu) comprised

219 48% to 94% (mean \pm SD = 68 \pm 10%) of the TR-Cu, and the copper extractability of the 200
220 mg/kg AE-Cu remained relatively constant over the 8-months between the 1st and 2nd series.
221 This range of copper extractability was significantly greater than we have found for field-
222 collected sediments contaminated with copper-based antifouling paints, where we have
223 measured AE-Cu was 6 \pm 9% of TR-Cu for 67 samples (unpublished results). Porewater copper
224 concentrations (PW-Cu) increased with increasing spiked-copper concentrations, and very
225 high PW-Cu (6 \pm 0.9 mg/L) was measured for the 1000 mg Cu/kg sandy treatment. The silty-
226 sand and sand PW-Cu concentrations were markedly greater in the 2nd series (0, 100, 200,
227 400) than in the 1st series (0, 200, 1000) (Table 1). This is most likely related to the Cu
228 released from the copper-based paints over the 8 months, which would have been removed
229 more effectively from the silty-sediment porewaters than the silty-sand and sand due to the
230 higher concentrations of TOC and finer particles. The time-averaged dissolved copper
231 concentrations in the overlying water (OW-Cu) also increased with increasing spiked-copper
232 concentration. However, the 1st and 2nd silty-sand series did not display anomalously high
233 values, as observed for PW-Cu.

234 **Copper profiles in sediments by diffusive gradients in thin films (DGT).** The principal
235 characteristics of the DGT-Cu flux depth profiles on days 8 and 15 for the three sediment
236 types (Figure 1) were: (i) peaks at the sediment-water interface (SWI), (ii) increased DGT-
237 induced copper flux with increasing spiked-copper concentration, and (iii) lower DGT-
238 induced copper flux in more silty sediments (sand > silty-sand > silt). The peak in the DGT-
239 induced copper flux near to the SWI is consistent with previous studies⁹ and has generally
240 been attributed to its release from organic matter as it is oxidised.^{45,46} Depth profiles of Fe and
241 Mn in the pore water were not obtained for the present study. However subsequent
242 experiments using sediments with similar properties to the silty-sand that had been spiked
243 with a copper mineral phase (unpublished results) indicated that the expected porewater Fe
244 and Mn profiles were established rapidly after sediment disturbance (from collection and
245 spiking) and 1 month equilibration (See Figure S1, Supporting Information). The
246 mobilisation of copper through the reductive dissolution of Fe and Mn oxyhydroxide phases
247 near the SWI is likely to be a major process influencing the copper fluxes.¹⁹ The very slow
248 rate of copper release to the pore water and overlying water in the siltier sediments was
249 consistent with the greater concentrations of TOC and fine particles, which provide copper-
250 binding sites, in the sediments.^{12,35}

251 Several anomalies existed in the DGT-Cu flux profiles for the 2nd deployment (Figure 1f).
252 The 50 mg Cu/kg sand treatment did not exhibit a DGT-Cu flux peak at the SWI, but

253 exhibited unexpectedly high copper mobilisation in the deeper sediments. For the 100 and
254 200 mg Cu/kg sand treatments and 200 and 400 mg Cu/kg silty-sand treatments, the position
255 of the DGT-Cu flux peaks was below the SWI. We believe that burrowing, deposit-feeding
256 activity, and mortality of the bivalves was responsible for several of these observations. The
257 movement of sediments by the bivalves may both increase the flux of copper at the SWI^{34,36}
258 and deplete PW-Cu concentrations in the surface, thereby causing the DGT-Cu flux peak to
259 become located at greater depth within the oxic/sub-oxic zone of the sediments. Bivalve
260 mortalities (% initial) were less than 5% in controls, but 34% and 79% in 100 and 200 mg/kg
261 sandy treatments, respectively, and 30% and 50% in 200 and 400 mg/kg silty-sand treatments,
262 respectively. It is likely that significant mortality occurred within the early stages of the
263 copper exposure⁴⁷, and the decomposition of the organisms will have increased the
264 availability of labile organic matter and stimulated microbial activity near the dead organisms.
265 These processes may have also increased copper mobilisation from particulate organic phases,
266 or decreased copper mobilisation through production of sulfide and formation of copper-
267 sulfide phases.^{10,12} No vertical movement in the DGT-Cu flux peak for the silty treatments
268 was consistent with low toxicity and the low DGT-induced fluxes of PW-Cu in these
269 sediments. As the anomalies were not present during the 1st DGT deployments, the changes
270 within the sediments that caused the new observations may have taken many days to occur.
271 The reason for the highly elevated DGT-Cu flux in the 50 mg Cu/kg sand treatment is not
272 fully understood, however, Sochaczewski et al.⁴ found that DGT maxima could arise from a
273 microniche – a local source with elevated concentration in the porewater, where the intensity
274 of the maxima was related to the distance between the source and the DGT. In this study it is
275 likely that the base of the DGT probe in the 50 mg Cu/kg sand treatment was in close
276 proximity to some copper-based paint particles.

277 **Copper bioaccumulation by *T. deltoidalis*.** Due to the high level of lethality, tissue-Cu
278 concentrations could not be determined for all 1000 mg Cu/kg treatments, the sandy and silty-
279 sand treatments with 200 mg Cu/kg, and the silty-sand treatments with 400 mg Cu/kg (Table
280 1). For each sediment type, positive relationships existed between tissue-Cu concentrations
281 and the sediment copper concentrations (See Figures S2a&b, Supporting Information). The
282 properties of the sediments influenced the degree of copper bioaccumulation, with tissue-Cu
283 concentrations increasing in the order sandy > silty-sand > silty when compared based on total
284 recoverable Cu or dilute acid-extractable Cu concentrations. A number of studies have
285 demonstrated the importance of sediment properties in influencing the partitioning of metals

286 between the porewaters and sediment phases,^{25,26,28,48} and the influence of these two major
287 exposure routes on the bioaccumulation of metals like copper.^{27,36,49}

288 For each sediment type, linear relationships existed between tissue-Cu and the time-
289 averaged OW-Cu concentration ($r^2=0.96$ for combined data, Figure S2c). Consistent with
290 dissolved copper in the overlying water being the predominant exposure pathway for *T.*
291 *deltoidalis*,^{36,37} these relationships appeared to be largely independent of sediment type.
292 While this bivalve is recognised as being a deposit feeder and accumulated copper from both
293 dissolved and particulate exposure routes⁵⁰, previous studies have found that copper
294 accumulation is mostly attributed to exposure from the overlying water.³⁷ The peak DGT-Cu
295 flux, which occurred near the SWI, also provided strong relationships ($r^2=87$ for combined
296 data) with tissue-Cu concentrations in *T. deltoideal*s. Consistent with the observations by
297 Roulier et al.³⁰, the DGT-Cu concentration provided a better method for predicting copper
298 bioaccumulation than the total copper measurements, i.e. it was less influenced by sediment
299 properties.

300 **Copper lethality to *T. deltoideal*s.** The lethality of the sediments increased with copper
301 concentrations and increased in the order sandy > silty-sand > silty (Figure 2). Dissolved
302 ammonia concentrations were less than 5 mg/L, which is well below the level known to effect
303 *T. deltoideal*s.³⁷ Consistent with copper bioaccumulation results, copper concentration-
304 response models could be created based on the TR-Cu or AE-Cu concentrations for the
305 individual sediments (Figures 2a,b), and also based on OW-Cu for the three sediment
306 collectively (Figure 2c). There also appeared to be a useful relationship between survival and
307 the tissue-Cu concentrations in *T. deltoideal*s (Figure 2d), however, because tissue-Cu
308 concentrations were not determined for organisms that had died, inadequate data exists for the
309 higher exposure concentrations.

310 Five different measures of copper exposure were used to calculate lethal copper
311 concentrations (LC50, LC20, and LC10) for the 30-day exposures of *T. deltoideal*s: TR-Cu
312 and AE-Cu calculations used the separate sand, silty-sand and silt treatments, and OW-Cu,
313 Tissue-Cu and DGT-Cu flux at the SWI used the combined data from all treatments (See
314 Table S1, Supporting Information). As indicated in Figures 2a and b, the LC50 values
315 (concentration causing 50% lethality) increased in the order sand < silty-sand < silt, when
316 calculated based on TR-Cu and AE-Cu concentrations. While neither TR-Cu nor AE-Cu
317 provided a suitable representation of the bioavailable copper concentration, the OW-Cu
318 concentrations were useful for describing the toxicity in all the sediments. LC50 and LC10

319 values of 27 (25-30) and 18 (13-26) $\mu\text{g OW-Cu/L}$ were calculated (Table S1). These results
320 are consistent with the LC50 value of $33\pm 1 \mu\text{g/L}$ determined by Strom et al.³⁵ for copper-
321 spiked sediment with a range of properties.

322 The use of tissue concentrations to express the toxicity of metals is usually more useful
323 for non-essential metals like Cd, Hg, Pb, than essential metals like Cu and Zn.^{12,49,51} However,
324 when the history of the metal exposure is controlled and known, tissue metal concentrations
325 provide both direct evidence of exposure and metal bioavailability to the organisms and also
326 useful expressions for toxicity for essential metals such as copper.⁴⁸ For the 30-day laboratory
327 exposures, owing to the rapid assimilation, the copper accumulation can be treated as
328 metabolically bioavailable,^{27,28} and was used to calculate an LC10 for tissue-Cu of 500 mg/kg
329 (dry weight) (Table S1).

330 A strong concentration-response relationship was also observed when the peak DGT-
331 induced copper flux at the SWI (Figure 1) was used as a measure of the copper exposure
332 concentration to *T. deltoidalis* (Figure 2e). Based on the DGT-Cu flux at the SWI, LC50 and
333 LC10 values of 31 (24-42) and 15 (6-35) $\mu\text{g Cu/m}^2/\text{h}$ were calculated using all the data (Table
334 S1). This relationship has a high degree of environmental significance in terms of the use of
335 DGT for quantifying the risk posed by metal contaminated sediments. Many regulatory
336 frameworks for sediment quality assessment incorporate procedures for determining a
337 contaminants' bioavailability.^{52,53} However, while metal bioavailability is frequently assessed
338 when the total concentration exceeds the sediment quality guideline value, there are many
339 inadequacies with the methods currently utilised.¹² Pore water metal analyses provide non
340 time-integrated 'snap-shots' that are unlikely to reflect the actual exposure for many benthic
341 organisms.^{12,54} It is generally not practical to make multiple field measurements of dissolved
342 metal concentrations, which would allow time-averaged exposures to be accurately assessed.
343 Furthermore, there are numerous artifacts that make porewater analyses problematic in both
344 oxic/sub-oxic and sulfidic sediments.^{19,55,56} The much used relationships between acid-volatile
345 sulfide (AVS) and simultaneously extractable metals (SEM)⁵⁷ can over-emphasise the
346 importance of metal-sulfide binding, as the sediment-microenvironments surrounding many
347 benthic organisms are oxic/sub-oxic either owing to the proximity to the SWI or due to
348 irrigation of burrows with oxygenated waters.^{12,17,42} Consequently, porewater or AVS-SEM
349 measurements may not always provide adequate information on metal bioavailability. While
350 metal concentrations in organism tissues provide a direct measure of exposure, when
351 organisms are collected from the field their exposure history is not usually known and a
352 significant fraction of the accumulated metal is likely to have been converted to forms that are

353 not metabolically available.^{49,58} This limits the use of lethal body concentration approach for
354 assessing metal bioavailability.^{12,59}

355 The DGT-Cu flux – bioaccumulation – toxicity relationships shown in Figure 2
356 demonstrate that DGT-induced metal flux measurements provide a very useful measure of
357 metal bioavailability in sediments with varying properties. We believe the time-integrated
358 metal fluxes derived from laboratory or field deployments of the relatively simple DGT
359 device may provide a more reliable measure of metal bioavailability than porewater and AVS-
360 SEM analyses in many sediments. While, in some respects the accumulation of metals by the
361 DGT probe simulates the bioaccumulation in sedentary benthic organism, the method does
362 not directly assess metal bioavailability arising from dietary exposure (i.e. ingestion of
363 particles). It has yet to be determined whether the DGT-induced metal flux may also provide
364 useful information on the lability of metals on ingested particles. Although the limitations of
365 using the DGT technique for measuring metal fluxes from sediments requires further
366 assessment, we expect the technique will become more frequently used for assessing the
367 bioavailability and potential ecotoxicological effects of metals in sediments.

368 **Bioavailability of Cu(I)oxide-based paints in sediments.** The source of bioavailable
369 copper in the tested sediments was copper-based antifouling paint particles and was chosen
370 because of the increasing use by marine industries and concerns about the effects to biota
371 within sediments contaminated with these materials.³¹ The release of copper from the paint
372 particles to the pore water and overlying seawater, along with the bioaccumulation by the
373 bivalves confirmed the presence of bioavailable copper in sediments. In the present study, the
374 measurements of dilute acid-extractable copper provided no obvious advantage of total copper
375 measurements for predicting the bioavailability of copper in the three sediments. This
376 observation may be due, in part, to the use of sediments that were all artificially copper-
377 contaminated and equilibrated for the same duration before testing, i.e. the partitioning
378 between TR-Cu and AE-Cu forms was the similar for all sediments. For naturally
379 contaminated sediments, which form over longer duration, dilute acid-extractable metal
380 measurements and analyses of metal concentrations of the fine sediment fraction are
381 frequently demonstrated to provide a better measure of metal bioavailability than analyses of
382 total metal concentrations.^{12,60} Both the TR-Cu and AE-Cu measurements indicted that the
383 bioavailability of the copper was significantly lower in the more silty sediments.

384 The toxicity to *T. deltoidalis* in the laboratory tests indicates copper-based paint
385 contamination may cause significant effects to benthic organisms (Figure 2). However, as the

386 majority of the copper exposure to *T. deltoidalis* was from the overlying water compartment,
387 the same paint-contaminated sediments may not cause this degree of toxicity if assessed in
388 situ, i.e. when situated with a deep water column.⁶¹ The copper extractability from the paint-
389 spiked sediments was also significantly greater than we have found for field-collected
390 sediments contaminated with copper-based antifouling paints, and this may also contribute to
391 an overestimation of the bioavailability compared to paint-contaminated sediments in the
392 field.

393 For *T. deltoidalis* a large portion of the copper exposure was from the overlying water
394 compartment³⁷, which was controlled by the DGT-Cu flux from the sediments in these
395 laboratory tests. In a field location the in situ copper exposure and toxicity of the same paint-
396 contaminated sediments would be lower due to the greater dilution of the released copper
397 within the larger water column.⁶¹ For organisms that receive a greater exposure from the
398 sediment pore waters, or may ingest fine paint particles within the sediments⁶², the in situ
399 exposure may be more important.

400 **Acknowledgements**

401 Ian Hamilton and David Spadaro are thanked for assisting with collection and handling of
402 bivalves and undertaking the bioassays. Robert Jung is thanked for assisting with paint and
403 bivalve sample analyses.

404 **Supporting Information**

405 Supporting Information includes further information on the preparation of the DGT probes,
406 profiles of porewater Mn and Fe concentrations, and relationships between copper
407 bioaccumulation and a table lethal copper concentrations for the bivalve is provided for each
408 of the different copper exposures. This information is available free of charge via the internet
409 at <http://pubs.acs.org/>

410 **References**

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571 Tables and Figures

572

573 Table 1. Copper concentrations in sediments and waters and copper bioaccumulation by *T. deltoidalis* in
 574 the Cu(I)oxide-paint spiked sediment types

	Nominal mg/kg	TR-Cu mg/kg		AE-Cu mg/kg		PW-Cu µg/L		OW-Cu µg/L		Tissue-Cu µg/g		
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Sandy (10% <63 µm)	Series 1	0 ^a	5	2	3	1	4.4	11	2.6	3.1	86	28
		200 ^a	200	15	134	5	5.8	5	45	16	ND	ND
		1000 ^a	1000	75	690	130	6000	900	1450	300	ND	ND
	Series 2	0	5	2	3	1	3.7	2	1.6	1.2	114	82
		50	38	7	29	3	12.7	3	7.5	1.8	275	ND
		100	109	8	60	9	17.2	5	14	3.3	314	ND
	200	176	13	107	5	115	47	34	5.1	1370	ND	
Silty-sand (30% <63 µm)	Series 1	0 ^a	10	4	7	2	0.2	3	1.1	1.5	86	89
		200 ^a	200	15	134	15	2.4	2	19	6.5	677	58
		1000 ^a	1000	75	670	150	4.6	2	76	28	ND	ND
	Series 2	0	12	1	11	7	0.5	0	1.7	2.5	154	21
		100	86	8	81	1	18.5	4	12	1.4	431	ND
		200	159	8	128	16	35.4	6	23	5.2	ND	ND
	400	315	17	212	73	62.1	21	26	5.4	ND	ND	
Silty (60% <63 µm)	Series 1	0 ^a	30	10	20	4	0.3	1	0.9	0.8	85	11
		200 ^a	200	15	134	15	7.2	3	15	6	499	40
		1000 ^a	950	75	640	150	6.4	6	49	12	ND	ND
	Series 2	0	27	3	13	7	0.5	0	0.5	0.6	134	24
		100	143	39	96	11	5.3	6	1.7	1.1	194	24
		200	234	11	113	20	1.1	1	2	1	276	22
	400	426	91	250	77	3.9	3	7.7	2.2	414	72	

589 TR-Cu = total recoverable copper (n=2). AE-Cu = dilute acid-extractable copper (n=2).

590 ND = not determined due to inadequate tissue mass due to poor survival.

591 PW-Cu (n=2, at start and finish) and OW-Cu (n=10, time average of samples approximately every 3 days) are dissolved
 592 copper in porewater and overlying water, respectively.

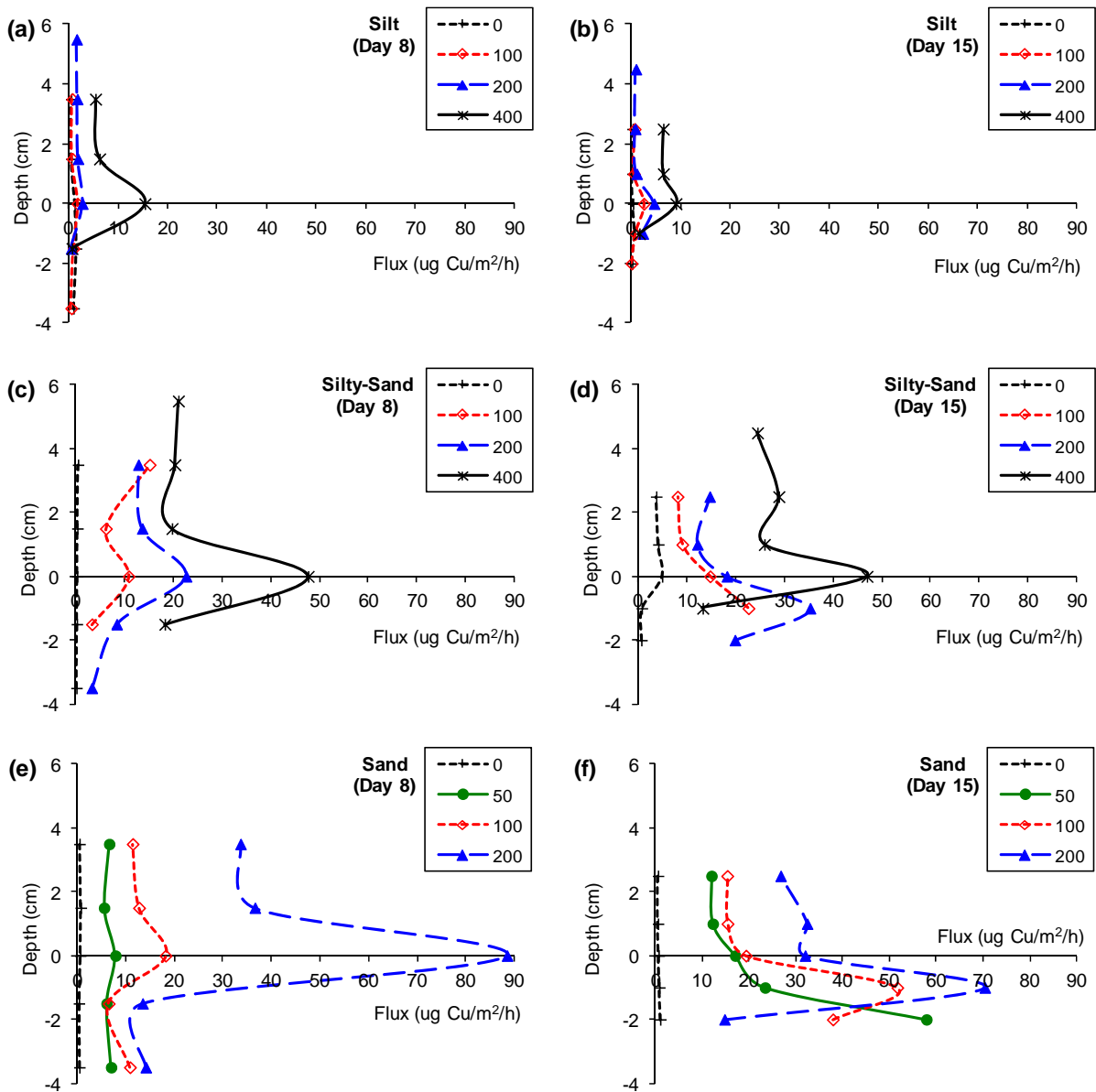
593 ^a First concentration series.

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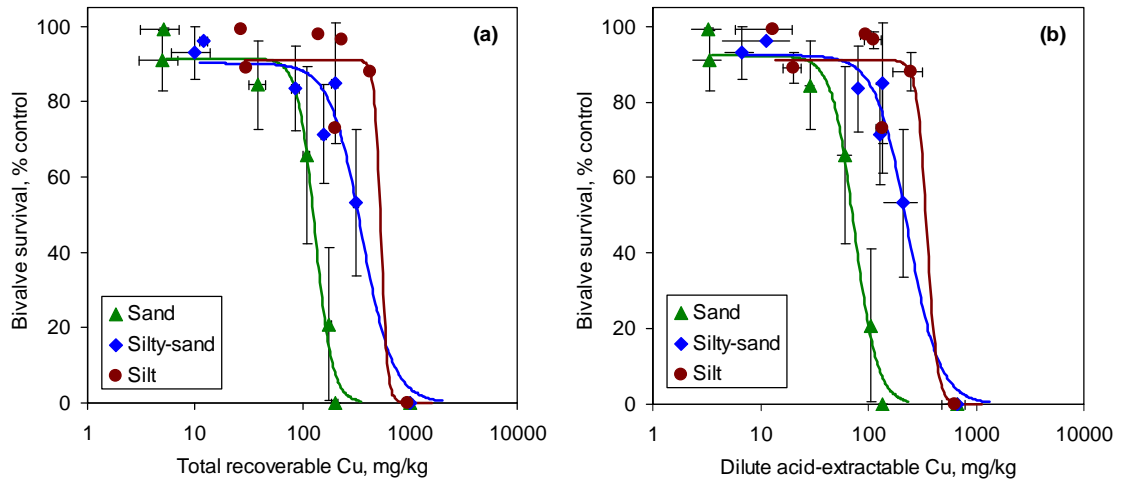
600 Figure 1. Depth profiles of the 24-h DGT copper flux on days 8 and 15 for the three copper
 601 concentration series: 0, 50, 100, 200 mg Cu/kg for the sandy, and 0, 100, 200, 400 mg Cu/kg for the
 602 silty-sand and silty series. Error bars have been omitted for clarity (Table S1 of Supplementary
 603 Information).

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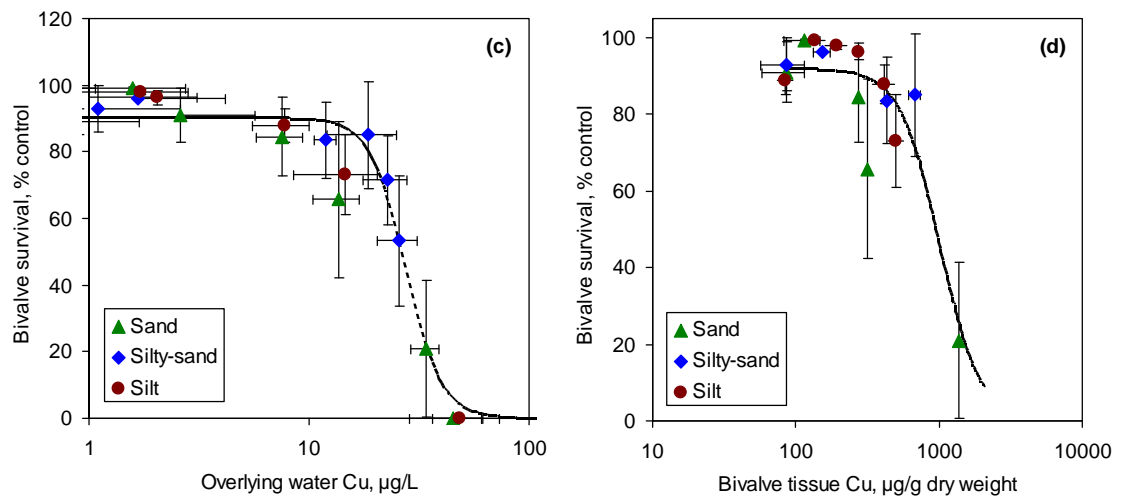
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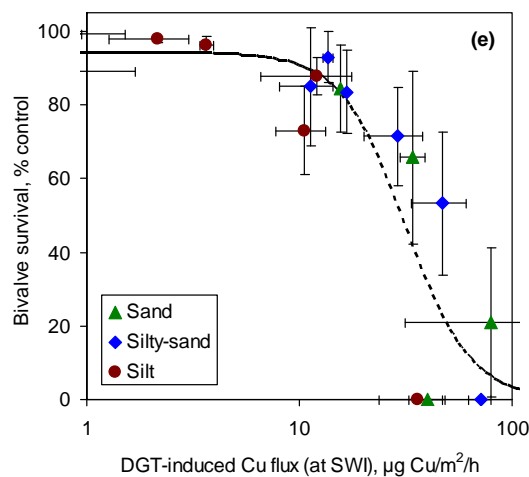
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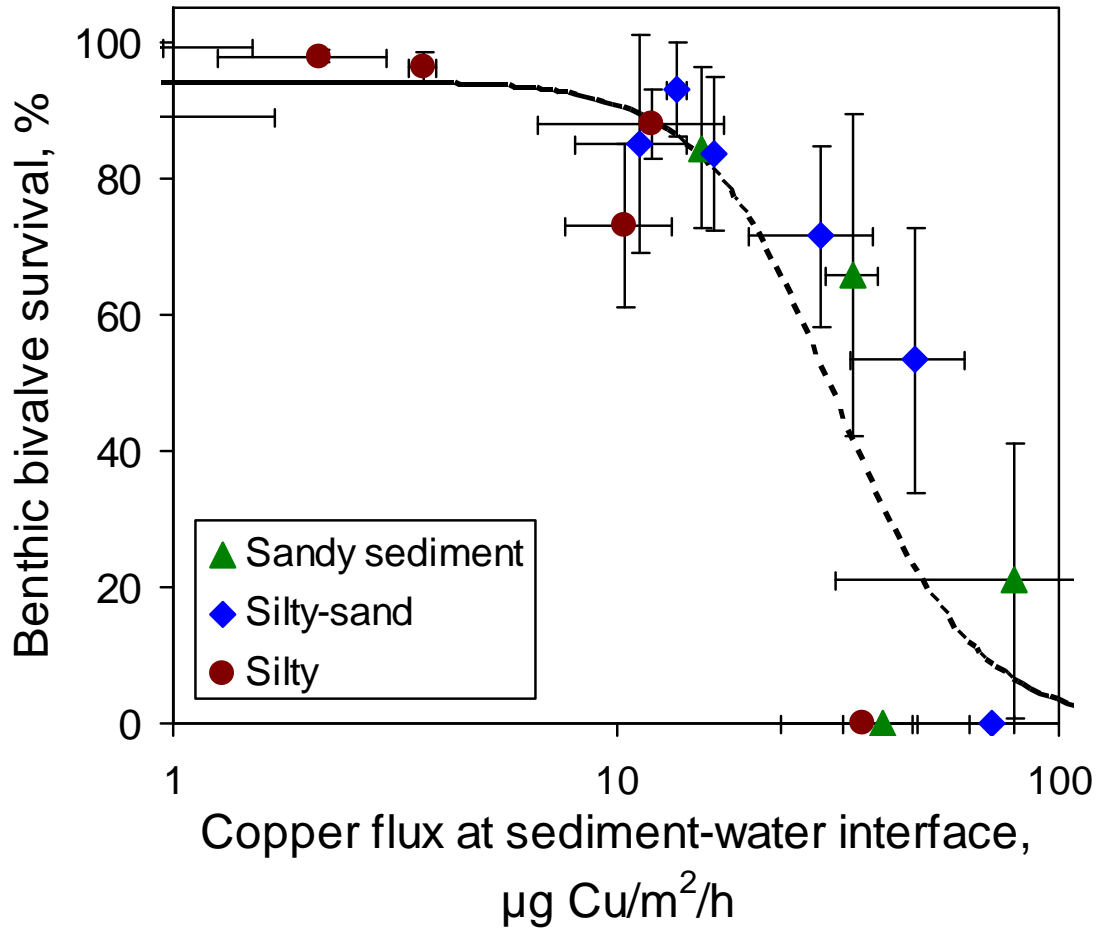


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611 Figure 2. Relationships between survival of the bivalve, *Tellina deltoidalis*, and different
 612 copper exposures: (a) total recoverable copper, (b) dilute acid-extractable copper, (c)
 613 dissolved copper in overlying water, (d) bivalve tissue-Cu concentrations, and (e) peak DGT-
 614 induced Cu flux at the sediment water interface (DGT-Cu flux). Data presented for three
 615 sediment types for the different Cu-spike concentrations (mean \pm SD, n=2). The lines
 616 represent log-logistic concentration response curves calculated for the three different sediment
 617 types for (a) and (b) and for the combined data for (c), (d) and (e). The LC50, LC20 and LC10
 618 values for each relationship are provided in Table S1 of the Supporting Information.

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626 **SUPPORTING INFORMATION:**

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628 **DGT-INDUCED COPPER FLUX PREDICTS BIOACCUMULATION AND TOXICITY TO**
629 **BIVALVES IN SEDIMENTS WITH VARYING PROPERTIES**

630

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641

642 **S1 Methodology:**

643 **S1.1 Preparation of diffusive gradients in thin films (DGT) probes**

644 Plastic DGT assemblies with open windows of 1.8 × 15 cm and overall dimensions of 24 × 4 × 0.5 cm were
645 purchased from DGT Research (Lancaster, UK). All glass and plasticware for DGT probes preparation were
646 cleaned by soaking in detergent (commercial detergent diluted in tap water) for 24 h, then in 10% (v/v) HNO₃
647 (70%, AR grade, Ajax Finechem Pty Ltd) for 24 h and rinsed thoroughly with MQ water. All glass and
648 plasticware for DGT probes analysis were cleaned by soaking in 10% (v/v) HNO₃ for 24 h and rinsed thoroughly
649 with MQ water.

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

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

664 The resulting diffusive and Chelex gels were removed from the glass plates and hydrated in Milli-Q water
665 for 24 h, replenishing the water three times to remove all unreacted chemicals. Diffusive gels were stored in

666 0.01 M NaNO₃ (AR grade, Chem Supply) at room temperature and Chelex gels were stored in Milli-Q water in a
667 refrigerator at 4°C until use for probe construction. Gels were cut with a Teflon coated razor blade using a
668 plastic rectangular strip of the desired dimensions in order to fit in the DGT device. Acid-cleaned 0.45 µm filter
669 membranes were cut and stored in MQ water. Probes were assembled by laying a wet filter membrane on the
670 base, overlaying a Chelex gel layer, then a diffusive gel layer and finally another wet filter membrane on the
671 DGT backing plates and closing the devices with the front window plates. Care was taken to ensure no air
672 bubbles were trapped within the layers. The DGT units were kept in sealed clean plastic bags containing few
673 drops of MQ water to avoid gel drying and stored in a refrigerator until deployment.

674 ***S1.1 DGT probes deployment, retrieval and analysis***

675 To prevent the introduction of oxygen into the sediments during the deployment, DGT probes were
676 deoxygenated for 24 h prior to deployment by immersing them in a 0.05 M NaCl (>95.5%, Sigma) solution
677 saturated with nitrogen gas (continually bubbling to remove the dissolved oxygen). DGT devices were
678 immediately gently inserted into the test beakers to a depth of 4 cm with care to ensure a good contact between
679 the sediment and the DGT membrane. After 24 h of deployment, DGT probes were gently removed from the
680 beakers and both sediment and overlying water levels were noted. Devices were immediately rinsed with MQ
681 water to remove all remaining sediment particles. Probes were put in clean plastic bags and kept in a cool room
682 (4°C) until disassembly.

683 DGT devices were disassembled (within 10 days of retrieval) and resin gel slices cut using a Teflon coated
684 blade to obtain the desired vertical profile: two 2-cm slices in the overlying water, a one 1-cm slice at the
685 sediment-water interface (SWI) and one 2-cm slice in the sediment. Some of the slices from below the SWI had
686 sediment particles adhered to them which were removed by washing with MQ water. Each slice was weighed
687 and put into a 5-mL vial, then eluted with 1 mL of 1 M HNO₃ for 16-24 h before analysis by ICP-MS.
688 Undeployed DGT probes were analysed as handling blanks and their copper concentrations were less than 5% of
689 the lowest measured concentration.

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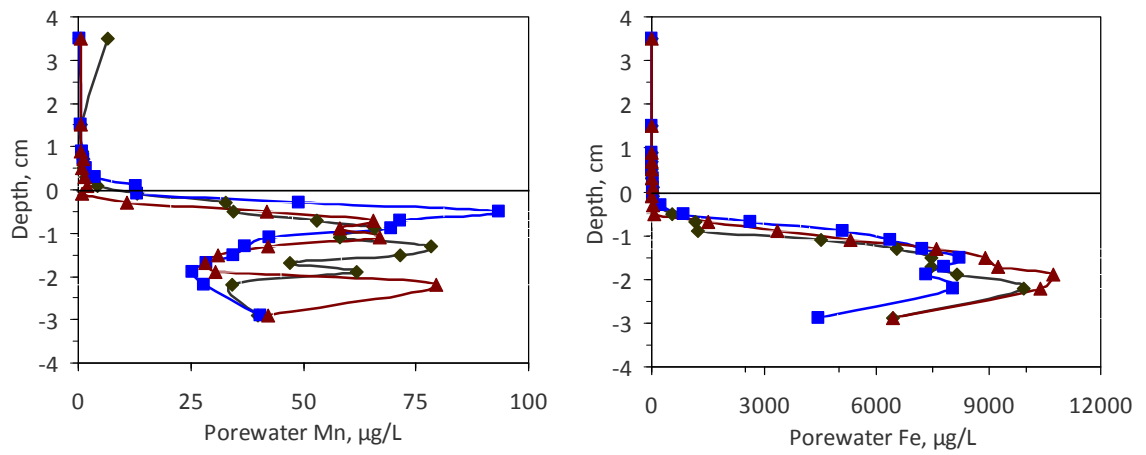
691

692 Table S1. Lethal copper concentrations for the bivalve, *Tellina deltoidalis* (Figure 2)

	TR-Cu, mg/kg (Sand)	TR-Cu, mg/kg (Silty-sand)	TR-Cu, mg/kg (Silt)
LC50 (95% CL)	130 (110-156)	355 (244-518)	530 (---)
LC20 (95% CL)	102 (78-136)	223 (120-415)	490 (---)
LC10 (95% CL)	88 (63-132)	170 (67-414)	460 (---)
	AE-Cu, mg/kg (Sand)	AE-Cu, mg/kg (Silty-sand)	AE-Cu, mg/kg (Silt)
LC50 (95% CL)	75 (61-91)	178 (93-339)	350 (---)
LC20 (95% CL)	54 (39-77)	87 (35-224)	300 (---)
LC10 (95% CL)	44 (29-74)	57 (16-217)	280 (---)
	OW-Cu, µg/L (All treatments)	Tissue-Cu, mg/kg (All treatments)	DGT-Cu flux, ug Cu/m ² /h (All treatments)
LC50 (95% CL)	27 (25-30)	1000 (770-1300)	31 (24-42)
LC20 (95% CL)	21 (17-27)	650 (380-1100)	19 (11-36)
LC10 (95% CL)	18 (13-26)	500 (230-1100)	15 (6-35)

- 693 LC50 (95% CL) = concentration causing 50% lethality (measured after 30 days).
694 LC20 and LC10 represent 20% and 10% effect concentrations, respectively.
695 95% CL = 95% confidence limit (--- = not possible to calculate 95% CL).
696 TR-Cu = total recoverable copper concentration (aqua regia, mg/kg).
697 AE-Cu = dilute acid-extractable copper concentration (1-M HCl, mg/kg)
698 OW-Cu = overlying water copper concentrations (30-day time averaged concentration, µg/L)
699 Tissue-Cu = copper concentrations after 30 days in surviving bivalves (mg/kg, dry weight)
700 DGT-Cu flux = Peak DGT-induced Cu flux at the sediment-water interface (ug Cu/m²/h)
701 Effects thresholds for TR-Cu and AE-Cu were calculated separately for sand, silty-sand and silt treatments
702 Effects thresholds for OW-Cu, Tissue-Cu and DGT-Cu flux were calculated using the combined data from treatments
703

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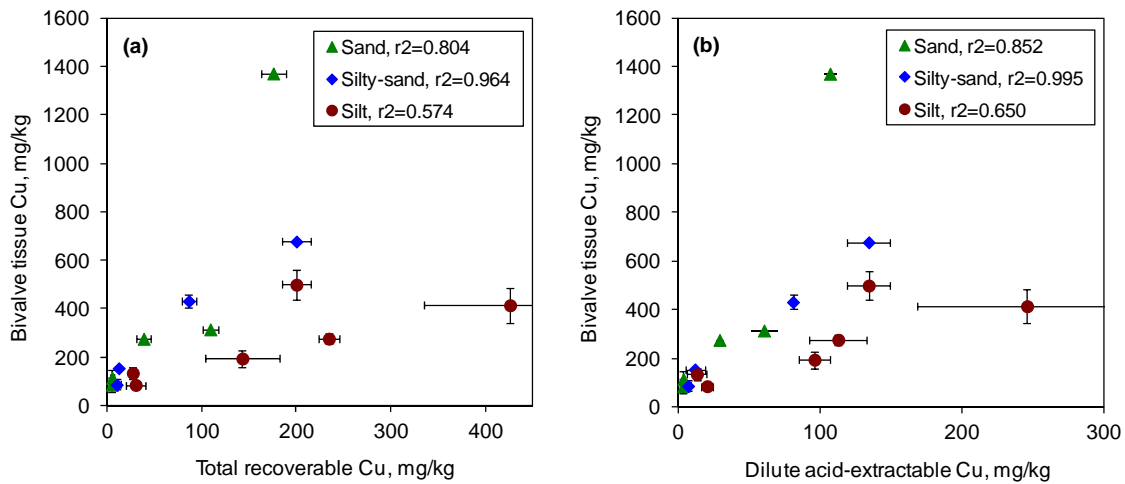


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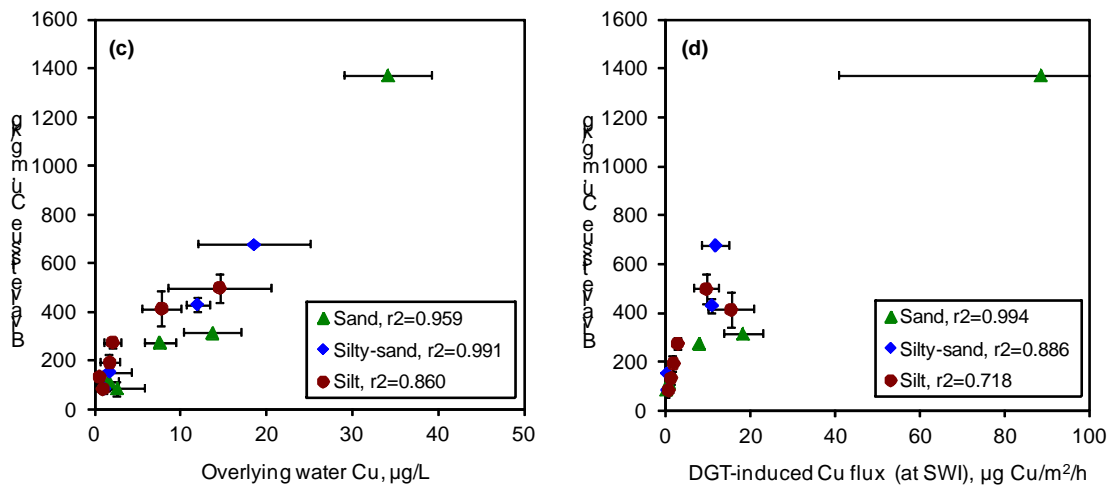
706

707 Figure S1. Porewater Mn and Fe concentrations within silty-sand that had been spiked with a copper
708 mineral phase (unpublished results), confirming that the expected porewater Fe and Mn profiles
709 develop following sediment disturbance and one month equilibration following spiking. The three
710 porewater metal profiles (●, ■, ▲) are from three DGTs from separate experiments.
711

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715 Figure S2. Relationships between copper bioaccumulation by bivalve, *Tellina deltoidalis*, and
 716 different copper exposures: (a) total recoverable copper (TR-Cu), (b) dilute acid-extractable
 717 copper (AE-Cu), (c) dissolved copper in overlying water (OW-Cu, time averaged) and (d) peak
 718 DGT-induced Cu flux at the sediment-water interface (DGT-Cu flux). Data presented for three
 719 sediment types for the different Cu-spike concentrations (mean \pm SD, n=2).

720 Correlations (r^2) between copper bioaccumulation by bivalve and copper concentrations for
 721 each of the sediment types

Sediment type	(a) TR-Cu	(b) AE-Cu	(c) OW-Cu	(d) DGT flux
Sand	0.804	0.852	0.959	0.994
Silty-sand	0.964	0.995	0.991	0.886
Silt	0.574	0.650	0.860	0.718
Combined data	NA	NA	0.962	0.865

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