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# Effect of overlying water pH, dissolved oxygen, salinity and sediment disturbances on metal release and sequestration from metal contaminated marine sediments

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# Effect of overlying water pH, dissolved oxygen, salinity and sediment disturbances on metal release and sequestration from metal contaminated marine sediments

## Abstract

Experiments were undertaken to examine the key variables affecting metal release and sequestration processes in marine sediments with metal concentrations in sediments reaching up to 86, 240, 700, and 3000mgkg<sup>-1</sup> (dry weight) for Cd, Cu, Pb and Zn, respectively. The metal release and sequestration rates were affected to a much greater extent by changes in overlying water pH (5.58.0) and sediment disturbance (by physical mixing) than by changes in dissolved oxygen concentration (38mg/l) or salinity (1545 practical salinity units). The physical disturbance of sediments was also found to release metals more rapidly than biological disturbance (bioturbation). The rate of oxidative precipitation of released iron and manganese increased as pH decreased and appeared to greatly influence the sequestration rate of released lead and zinc. Released metals were sequestered less rapidly in waters with lower dissolved oxygen concentrations. Sediments bioturbated by the benthic bivalve *Tellina deltoidalis* caused metal release from the pore waters and higher concentrations of iron and manganese in overlying waters than non-bioturbated sediments. During 21-day sediment exposures? *T. deltoidalis* accumulated significantly higher tissue concentrations of cadmium, lead and zinc from the metal contaminated sediments compared to controls. This study suggests that despite the fact that lead and zinc were most likely bound as sulfide phases in deeper sediments, the metals maintain their bioavailability because of the continued cycling between pore waters and surface sediments due to physical mixing and bioturbation.

## Keywords

Effect, overlying, water, dissolved, oxygen, salinity, sediment, disturbances, metal, release, sequestration, from, metal, contaminated, marine, sediments, CMMB

## Disciplines

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

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1 **Effect of overlying water pH, dissolved oxygen, salinity and sediment disturbances on**  
2 **metal release and sequestration from metal contaminated marine sediments**

3  
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19 **Abstract**

20 Experiments were undertaken to examine the key variables affecting metal release and  
21 sequestration processes in marine sediments with metal concentrations in sediments reaching  
22 up to 86, 240, 700, and 3000 mg kg<sup>-1</sup> (dry weight) for Cd, Cu, Pb and Zn, respectively. The  
23 metal release and sequestration rates were affected to a much greater extent by changes in  
24 overlying water pH (5.5 to 8.0) and sediment disturbance (by physical mixing) than by  
25 changes in dissolved oxygen concentration (3 to 8 mg/L) or salinity (15 to 45 practical  
26 salinity units). The physical disturbance of sediments was also found to release metals more  
27 rapidly than biological disturbance (bioturbation). The rate of oxidative precipitation of  
28 released iron and manganese increased as pH decreased and appeared to greatly influence the  
29 sequestration rate of released lead and zinc. Released metals were sequestered less rapidly in  
30 waters with lower dissolved oxygen concentrations. Sediments bioturbated by the benthic  
31 bivalve *Tellina deltoidalis* caused metal release from the pore waters and higher  
32 concentrations of iron and manganese in overlying waters than non-bioturbated sediments.  
33 During 21-d sediment exposures, *T. deltoidalis* accumulated significantly higher tissue  
34 concentrations of cadmium, lead and zinc from the metal contaminated sediments compared  
35 to controls. This study suggests that despite the fact that lead and zinc were most likely  
36 bound as sulfide phases in deeper sediments, the metals maintain their bioavailability because  
37 of the continued cycling between pore waters and surface sediments due to physical mixing  
38 and bioturbation.

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41 **Key Words** – Metal release and sequestration, contaminated sediments, bioturbation and  
42 physical disturbance, sediment-metal bioavailability , physico-chemical parameters.

43

44

## 45 **Introduction**

46 In most aquatic environments, dissolved metal concentrations in overlying waters are low  
47 due to precipitation as solids or adsorption to suspended particles and the deposition of these  
48 particles as sediments. Once deposited as sediments, biological and chemical  
49 oxidation/reduction and precipitation/dissolution reactions result in the redox stratification of  
50 both dissolved and particulate metals with sediment depth. The oxic fraction of silty  
51 sediments usually extends to depths of 2-5 mm (Kristensen 2000). At greater depths the  
52 sediment becomes sub-oxic, containing mixtures of oxic solid phases (e.g. Fe- and Mn-  
53 (hydr)oxides) in equilibrium with reduced dissolved phases (e.g. Fe(II) and Mn(II)) (Simpson  
54 and Batley 2003). Once the easily reducible Fe- and Mn-(hydr)oxide phases have been  
55 depleted, bacteria reduce sulfate to sulfide, which reacts to form metal sulfide complexes  
56 whose solubility controls the fraction of metals dissolved in solution (Di Toro *et al.* 1992;  
57 Burdige 1993).

58 The fate and toxicity of metals in the sediments is greatly dependent on the partitioning of  
59 metals between the sediment particles and the pore waters (Calmano *et al.* 1993; Simpson  
60 2005). Dissolved metals present in the pore waters are more bioavailable and toxic than  
61 particulate metals (Chapman *et al.* 1998). Physical processes (e.g. water currents,  
62 anthropogenic disturbance) and the activity of benthic organisms (e.g. burrowing, irrigation)  
63 can cause sediment resuspension and mixing of previously redox-stratified sediments with  
64 oxygenated overlying waters. This resuspension and mixing alters metal sediment-water  
65 partitioning and metal speciation in the dissolved phase, i.e., pore waters and overlying waters  
66 (Riedel *et al.* 1997; Simpson *et al.* 2002; Kristensen 2000). These physico-chemical changes  
67 alter the bioavailability of metals in the pore waters (Ciutat and Boudou 2003; Simpson and  
68 Batley 2003) and the release rate (flux) of metals from the sediments (Simpson *et al.* 2002).  
69 The fate (speciation) of metals released from sediments is affected by overlying water  
70 conditions, in particular the pH, salinity, dissolved oxygen concentration and amount of  
71 suspended solids (Simpson *et al.* 2004; Eggleton and Thomas 2004). Understanding the key  
72 factors that influence the release of metals from sediments would allow better prediction of  
73 the changes in metal availability to sediment-dwelling organisms.

74 This research investigated the influence of the overlying water parameters pH, dissolved  
75 oxygen (DO) concentration and salinity on metal release from metal contaminated estuarine  
76 sediments in shallow waters. One at a time, each of the three test parameters was selected and  
77 varied to low, mid and high values of the parameter, whilst maintaining the other parameters  
78 at near constant values. The release of metals across the sediment-water interface into the  
79 overlying water, and subsequent sequestration of these released metals was studied with each  
80 parameter over a 21-d period. These experiments were performed on bioturbated and non-  
81 bioturbated sediments, using the sediment ingesting bivalve *Tellina deltoidalis*. After 21 d  
82 the animals were recovered from the test sediments and analysed for metal accumulation in  
83 comparison to organisms exposed to a control uncontaminated sediment to ascertain the  
84 bioavailability of the sediment metals. Further, in an attempt to understand the effects of the  
85 overlying water parameters in the event of a significant sediment disturbance, the  
86 contaminated sediment that had been observed for 21 d were physically mixed and metal  
87 release to and sequestration from the overlying water were investigated for up to 9 d.

## 88 **Materials and methods**

### 89 *Study site and sampling*

90 Metal contaminated sediments were collected from the Pb-Zn smelter contaminated Cockle  
91 Bay in Lake Macquarie, New South Wales, Australia (151° 30' E, 33° 00' S). Surface (0-5  
92 cm) and depth (10-15 cm) sediment cores were collected from 13 sites (Figure 1) within 3-10  
93 m of the shoreline using a polycarbonate corer (5 cm diameter × 30 cm) to identify the  
94 optimal sediment for this study. Bulk sediment samples for metal release and bioavailability  
95 experiments (30 kg, 0-15 cm depth) were collected, using a shovel from Site 6 (Figure 1), and  
96 sieved on-site through 1 mm mesh to remove large debris. Control bulk sediments, used in  
97 the metal bioavailability study, were collected in the same manner from Boronia Park, an  
98 uncontaminated bay of the estuarine Lane Cove River, Sydney (Table 1). All sediments were  
99 stored in the dark at 4°C and used within one week of collection. The sieved sediments  
100 contained some small benthic organisms (predominantly amphipods at 5 organisms per 100 g  
101 sediment) but were considered non-bioturbated in comparison to the grossly disturbed

102 sediments that were physically mixed or contained bivalves. Clean seawater (salinity of 35-45  
103 PSU) was collected from Fairy Meadow, NSW, Australia.

104 The sediment ingesting bivalves, *Tellina deltoidalis*, were collected from estuarine sand  
105 and mud flats of Lane Cove River, according to the method of King *et al.* (2004).  
106 Approximately 400 bivalves (15-35 mm shell length) were collected for bivalve bioturbation  
107 tests and 50 bivalves ( $16 \pm 1$  mm shell length) for metal bioavailability experiments.

#### 108 *Analytical methods*

109 All chemicals were analytical reagent grade or equivalent analytical purity. High-purity  
110 Milli-Q deionised water ( $18 \text{ M}\Omega \text{ cm}^{-1}$ , Millipore) was used for all solutions. All glass and  
111 plastic-ware was cleaned by soaking in 10 % (v/v)  $\text{HNO}_3$  for >24 h followed by rinsing with  
112 Milli-Q water.

113 Dissolved oxygen (DO) and temperature measurements were made using a YSI 95 meter  
114 (Model 95/25 Ft) and salinity measurements using a WTW meter (LF 320) with a TetraCon  
115 325 probe (both meters were calibrated following manufacturer instructions). Salinity  
116 measurements have been reported according to the Practical Salinity Scale of 1978 (PSS 78)  
117 as dimensionless values. A Sensorex Combination pH electrode (450C) with a Rex pH meter  
118 (Model pH $\beta$ -4) was used for all pH measurements (in the field and in the lab) and was calibrated  
119 against standardised pH 4 and 7 NIST buffers before use. Sediment oxidation-reduction potential  
120 (ORP or redox) was measured using a Metrohm AG 9109 Herisau Combined Pt-wire electrode with  
121 Ag/AgCl reference electrode and Rex pH meter (Model pH $\beta$ -4). The instrument was calibrated using  
122 Orion Application Solution ORP Standard 967961 and all redox readings corrected for  $E_{\text{H}}^{\text{Ref}} = -210 \pm$   
123 3 mV, i.e. reported redox potentials are versus the hydrogen electrode,  $E_{\text{H}}^0 = 0$  mV. Redox  
124 measurements were made when stable potentials were achieved, 1-2 min after insertion of the  
125 probe into the top 5 mm of the test sediment.

126 All samples were filtered using acid-washed (2%  $\text{HNO}_3$ ) and rinsed (Milli-Q water) 0.45  
127  $\mu\text{m}$  cellulose nitrate membrane syringe filters (25 mm, Minisart, Sartorius). In all experiments,  
128 the dissolved metals fraction was defined as the metals passing through a 0.45  $\mu\text{m}$  filter, however, a  
129 portion of the metals measured in the overlying water may have been colloids. Dissolved metal  
130 concentrations were determined by inductively coupled argon plasma-atomic emission

131 spectrometry (ICP-AES) (Spectroflame EOP, Spectro Analytical Instruments) according to  
132 Simpson *et al.* (2002). Detection limits for reporting ICP-AES analyses of metals in seawater  
133 (acidified 0.2 % (v/v) HNO<sub>3</sub>) were 3-10 µg/L for Cd, Cu, Fe, Mn, Pb and Zn.

134 Total organic carbon (TOC) analyses in sediments were made using a high temperature  
135 TOC analyser (Dohrmann DC-190) following the removal of inorganic carbon (carbonates  
136 and bicarbonates) by acidification with 1 M HCl until effervescence was completed.

137 Sediment particle size fractionation was made by wet sieving the sediments through 1000,  
138 250, and 63 µm mesh. Acid-volatile sulfide (AVS) in sediments was determined in bulk  
139 sediments from sediment cores using the methylene blue based rapid screening method  
140 developed by Simpson (2001). Total particulate metals (TPM, *aqua regia* digestion) and  
141 simultaneously-extracted metals (SEM, 1 M HCl) were determined as described in Simpson  
142 *et al.* (2002). Triplicate analyses of AVS and duplicate analyses of SEM and TPM were  
143 performed on sediments.

#### 144 *Experimental set up for metal release assay*

145 Eight purpose-built chambers were constructed for the metal release experiments (Figure  
146 2). Each main chamber was 30 cm × 30 cm × 8 cm deep (surface area = 0.090 m<sup>2</sup>). A 100  
147 mL plastic container containing an aerator and magnetic stirring bar was positioned in the  
148 corner of each chamber, with its upper rim mid-way between the sediment and water surfaces.  
149 The overlying water was stirred and aerated for 0 - 15 min per hour, depending on the DO  
150 concentration required. Homogenised test sediment (4.00 ± 0.05 kg) was placed into the  
151 chamber and 1.5 L clean seawater was added (~1.7 cm water depth).

152 The temperature, pH, salinity and DO of the overlying waters were monitored daily. Over  
153 the 21-day test period, 20 mL samples of overlying waters were taken at the start of the test  
154 and then every second day. Overlying water samples were taken randomly from different  
155 sections of the experimental chamber, immediately filtered (0.45 µm), acidified to 0.2 % (v/v)  
156 HNO<sub>3</sub> and analysed for metals by ICP-AES. Overlying waters in all treatments were  
157 replenished with Milli-Q water following sampling to maintain constant water volume and  
158 required treatment salinity.



159 *Effect of pH, dissolved oxygen, salinity and bioturbation on metal release*

160 The effect of overlying water pH on metal release from sediments was investigated at high  
161 (pH  $8.1 \pm 0.2$ ), mid (pH  $7.2 \pm 0.2$ ) and low (pH  $6 \pm 1$ ) pH values (mean  $\pm$  standard deviation  
162 (SD)). Adjustments to pH were made by drop-wise addition of 1 M HCl to the water into the  
163 aeration chamber to allow rapid mixing and dispersion (15 min stirring by aeration after acid  
164 addition). For the low-pH experiments, continuous pH adjustment and stirring were  
165 necessary to overcome the strong pH-buffering capacity of the sediments. During the pH  
166 experiments the waters were  $19 \pm 1$  °C, salinity  $38 \pm 4$  PSU and  $8 \pm 1$  mg DO L<sup>-1</sup>. The low-pH  
167 experiment was duplicated.

168 The effect of overlying water DO on metal release from sediment was investigated at high  
169 ( $8 \pm 1$  mg DO L<sup>-1</sup>), mid ( $6 \pm 1$  mg DO L<sup>-1</sup>) and low ( $2.9 \pm 0.9$  mg DO L<sup>-1</sup>) concentrations.  
170 Variations in DO were obtained by altering the stirring and aeration rate: stirred continuously  
171 and aerated 15 min h<sup>-1</sup> to maintain high-DO, intermittently stirred and aerated (15 min h<sup>-1</sup>) to  
172 maintain mid-DO, and not stirred or aerated but covered with a black plastic lid (limiting light  
173 exposure and photosynthesis) to maintain low-DO conditions, respectively. During the DO  
174 experiments the waters were  $19 \pm 1$  °C, salinity  $35 \pm 3$  PSU and pH  $7.8 \pm 0.2$ . The low-DO  
175 experiment was duplicated.

176 The effect of salinity on metal release from sediments was investigated at high (35 PSU),  
177 mid (25 PSU) and low (15 PSU) salinity. Waters of varying salinity were maintained by  
178 addition of Milli-Q water to the seawater. During one of the first experiments unexpectedly  
179 rapid evaporation produced a very high (45 PSU) super-saline salinity treatment. During the  
180 salinity experiments the waters were  $19 \pm 1$  °C, pH  $8.1 \pm 0.2$  and  $8 \pm 1$  mg DO L<sup>-1</sup>.

181 To investigate the effect of bioturbation on metal release from sediments, 160 benthic  
182 bivalves, *T. deltoidalis*, were added to the sediment chambers. During the bioturbation  
183 experiments the waters were  $19 \pm 2$  °C, pH  $7.7 \pm 0.2$ , salinity  $35 \pm 3$  PSU and  $8 \pm 1$  mg L<sup>-1</sup> DO.  
184 After 21 days, all bivalves were removed from sediment by hand. The bioturbation  
185 experiment was duplicated.

186 *Effect of physical disturbance on metal release*

187 After the initial 21-day release period, sediments and overlying waters for all chambers  
188 were hand mixed for 10 minutes until an homogeneous black slurry was obtained. Water  
189 samples were taken and analysed for dissolved metals every few hours for 48 h, then every  
190 second day for six days. Water samples collected during the first 4 h after mixing were  
191 centrifuged (10 min, 2400 rpm) to remove the large quantity of suspended particles and the  
192 supernatant was filtered (0.45 µm) before metals analysis.

### 193 *Biological availability of the sediment bound metals*

194 In addition to analysis of total particulate and simultaneously-extractable metals, the  
195 biological availability of the sediment bound metals was also investigated. Twenty-five  
196 sediment ingesting *T. deltoidalis* were exposed to metal contaminated sediments, and a  
197 further 25 were exposed to control sediments, for 21 days according to the metal release assay  
198 set up described above, to assess the biological availability of sediment metals by  
199 comparative bioaccumulation. During these experiments the overlying waters were  
200 maintained at  $18.5 \pm 0.5$  °C, pH  $8.0 \pm 0.5$ , salinity  $35 \pm 3$  and  $8.0 \pm 0.5$  mg DO L<sup>-1</sup>.

201 Metal concentrations in soft tissues were determined as described in King *et al.* (2004). In  
202 brief, animals were depurated, tissues dried, digested in nitric acid then hydrogen peroxide,  
203 diluted with Milli-Q water and metal concentrations were measured by ICP-AES. For quality  
204 control purposes, each batch of samples analysed included one blank (Milli-Q water) and two  
205 samples of an oyster tissue standard reference material (SRM 1566b, National Institute of  
206 Standards & Technology).

207 Metal bioavailability data was log<sub>10</sub>-transformed and Analysis of Variance (ANOVA) was  
208 used to test for differences in the concentration of cadmium, copper, lead and zinc within the  
209 whole soft tissue masses between the control and metal-contaminated sediments. All data  
210 figures were prepared using Microsoft® Excel 2000.

## 211 **Results**

### 212 *Sediment characteristics*

213 During sediment collection the overlying waters had temperatures of 22-27°C, pH of 7.8 -  
214 8.2, salinity of 32.2 - 37.2 PSU and DO concentrations of 6.0 - 8.7 mg L<sup>-1</sup>. Surface (0-5 cm)

215 and depth (10-15 cm) sediments had similar pH and redox properties, pH was 6.8-7.8 at the  
216 surface and 6.8-8.1 at depth, and redox potential was -60 to 130 mV at the surface and -60 to  
217 108 mV at depth. TPM (*aqua regia*) and SEM (1 M HCl) concentrations of Cd, Cu, Pb and  
218 Zn in the sediments are shown in Figure 3, along with the Australian sediment quality  
219 guideline (SQG) values for these metals, 1.5, 65, 50, 200 mg kg<sup>-1</sup>, respectively  
220 (ANZECC/ARMCANZ 2000). The maximum measured metal concentrations were 86 mg  
221 kg<sup>-1</sup> Cd, 240 mg kg<sup>-1</sup> Cu, 700 mg kg<sup>-1</sup> Pb, and 3000 mg kg<sup>-1</sup> Zn. Sediment concentrations were  
222 fairly consistent around the study area, with the greatest variability found at sites 7-10, in the  
223 western corner of the bay, which receives direct water flow from the metal-contaminated  
224 creek (Cockle Creek).

225 Acid volatile sulfide (AVS) is considered to be a major metal-binding phase in anoxic  
226 sediments, and pore water metal concentrations are predicted to remain low when there is a  
227 molar excess of AVS over SEM (Di Toro *et al.* 1992; Simpson 1998). Although many of the  
228 sediments appeared to be sub-oxic, most of the sediments had a molar excess of AVS over  
229 SEM (Figure 4). In replicate sediment cores from a single site, greater variations in  
230 concentrations of AVS (factor of 1.5-7.2) were measured than the respective SEM (factor of  
231 1.1-2.5). It was expected that the deeper sediments (10-15 cm depth) would be more anoxic  
232 and contain greater concentrations of AVS than the surface sediments (0-5 cm depth), but this  
233 was observed for only 50% of the sites (Figure 4). This may indicate that in the deeper  
234 sediments contained more pyritic sulfide phases that are not extracted by the AVS method  
235 (Simpson *et al.*, 1998). In addition, greater differences might have been observed if higher  
236 resolution depth profiles were undertaken for AVS analyses. In general, the sediments were  
237 quite heterogenous with respect to AVS, probably due to the burrowing activities of  
238 organisms that introduce oxygen at depth. Particulate iron concentrations (SEM-Fe) were  
239 typically 10-200 μmol g<sup>-1</sup> and AVS analyses indicated that 10-20% of that Fe was present as  
240 iron sulfide, with the remainder expected to be present in iron hydroxide and carbonate phases  
241 (Stumm and Morgan, 1996).

242 Sediments from Site 6 were used to investigate the effects of pH, salinity, DO and  
243 sediment disturbance on metal release rates. This sediment was silty (73% <63 μm, 18% 63-

244 250  $\mu\text{m}$ , 9% 250-1000  $\mu\text{m}$ ) with TOC concentrations of 24-44  $\text{mg kg}^{-1}$  (dry weight) and an  
245 excess of SEM over AVS of 1-7  $\mu\text{mol g}^{-1}$ . SEM concentrations in the bulk Site 6 sediment  
246 were 21  $\text{mg kg}^{-1}$  Cd, 35  $\text{mg kg}^{-1}$  Cu, 250  $\text{mg kg}^{-1}$  Pb, and 950  $\text{mg kg}^{-1}$  Zn. In the <63/63-  
247 250/250-1000  $\mu\text{m}$  particle size fractions the SEM concentrations (in  $\text{mg kg}^{-1}$ ) were 18/22/14  
248 for Cd, 37/19/14 for Cu, 253/119/84 for Pb, and 905/819/587 for Zn. The finest sediment  
249 fraction had the highest metal concentrations of Cu, Pb and Zn and was expected to be the  
250 most easily resuspended by water currents or bioturbation and the most easily ingested by  
251 bivalves. Furthermore, while the highest Cd concentration was found in 63-250  $\mu\text{m}$  sediment,  
252 the greatest amount of Cd is present in the <63  $\mu\text{m}$  fraction as it comprises 73% of the total  
253 sediment volume, compared to 18% for the 63-250  $\mu\text{m}$  fraction.

#### 254 *Effect of pH and physical disturbances on metal release from sediments*

255 Little or no release of Cd, Cu, Pb or Zn was observed for the high- and mid-pH  
256 experiments, but for low-pH experiments Pb and Zn concentrations in the overlying waters  
257 were high (Figure 5). Dissolved copper was <3  $\mu\text{g L}^{-1}$  throughout all experiments. Dissolved  
258 cadmium was <3  $\mu\text{g L}^{-1}$  during the high- and mid-pH experiments, but in the low-pH  
259 experiments was 4-13  $\mu\text{g L}^{-1}$  over the 6-18 day period and 40-50  $\mu\text{g L}^{-1}$  for days 19 and 21.  
260 The release of lead was only significant in the low-pH experiments with lead concentrations  
261 of 100-370  $\mu\text{g L}^{-1}$  measured on days 19 and 20 (Figure 5). Zinc releases were observed in the  
262 mid-pH and low-pH experiments, with average zinc concentrations of 65  $\mu\text{g L}^{-1}$  during the  
263 low-pH experiment and maximum zinc concentrations of 1600  $\mu\text{g L}^{-1}$  in the low-pH  
264 experiments (Figure 5). During the first 21 days of the experiments, release of Mn was  
265 observed only in the low-pH experiments and release of Fe was observed in the low-pH and  
266 mid-pH experiments (Figure 5). The increase in dissolved metal concentrations with time  
267 reflects a steady release rate as the metals in the sediment-water system equilibrated with the  
268 new overlying water condition.

269 At day 21, the sediments were disturbed by physical mixing and then left undisturbed for a  
270 further nine days (Figure 5). Upon sediment disturbance, dissolved iron concentrations  
271 increased in the high-pH experiments and decreased in the mid-pH and low-pH experiments,

272 while dissolved manganese concentrations increased in the mid- and high-pH experiments  
273 and decreased in the low-pH experiments. Following the sediment disturbance the dissolved  
274 Pb and Zn concentrations remained low in the high-pH experiments, but increased in the mid-  
275 pH experiments. For the low-pH experiments, dissolved Pb and Zn concentrations decreased  
276 immediately following sediment disturbance, before increasing again after 24 h, once the  
277 majority of the suspended particles from the sediment disturbance had settled.

278 During the eight days following the sediment disturbance, the metal concentrations in the  
279 overlying waters generally returned to concentrations measured before the disturbance period,  
280 indicating that the release of metals to the overlying waters was only temporary. In the low-  
281 pH experiments, the dissolved concentrations of Fe, Pb and Zn were similar to those  
282 measured before the disturbance period within 72 h. In the mid-pH experiments, dissolved  
283 Mn and Pb concentrations remained elevated compared to concentrations in the overlying  
284 waters before disturbance.

#### 285 *Effect of dissolved oxygen and physical disturbances on metal release from sediments*

286 In all DO experiments the overlying water was pH 8, salinity  $35\pm 3$  PSU and dissolved Cd,  
287 Cu, Pb and Zn were below detection limits until the sediments were disturbed. These results  
288 were consistent with the high pH experiments discussed above. Sediment disturbance resulted  
289 in the release of lead to the overlying water in the low- and mid-DO experiments, and was  
290 greater in low-DO experiments (Figure 6). Following the initial release of lead, immediately  
291 after sediment disturbance the dissolved lead concentrations decreased but remained greater  
292 than pre-disturbance concentrations (below detection). Following sediment disturbance,  
293 dissolved zinc concentrations generally remained  $< 5 \mu\text{g L}^{-1}$  in the high- and mid-DO  
294 experiments, but were  $50\pm 40 \mu\text{g L}^{-1}$  ( $150 \mu\text{g L}^{-1}$  maximum) in the low-DO experiments.  
295 Dissolved copper concentrations remained  $< 3 \mu\text{g L}^{-1}$  in the high-DO experiments, but were 5-  
296  $20 \mu\text{g L}^{-1}$  for the 24-h period following sediment disturbance in the mid- and low-DO  
297 experiments. Dissolved cadmium remained  $< 3 \mu\text{g L}^{-1}$  in all DO experiments.

#### 298 *Effect of salinity on metal release from sediments*

299 Changes in overlying water salinity are most likely to occur due to short-term freshwater  
300 input following rain. In the experiments that started with a salinity of 15 or 25PSU, the  
301 overlying water salinity returned to  $35\pm 3$  within 30 h of the experiments commencing. The  
302 strong salinity-buffering capacity of the sediments and the rapid rate at which overlying  
303 waters returned to the normal water salinity indicated that any overlying water salinity  
304 changes would be only temporary for these sediments. In the natural setting, mixing with  
305 surrounding waters would further increase the dilution rate of freshwater inputs. There was  
306 no significant difference between the rates of metal release in these experiments. In the  
307 evaporated waters experiment with salinity of 45 PSU (maintained throughout the 29-day  
308 experiments), there was no difference in metal release rates compared to experiments where  
309 the salinity was maintained at 35 (both of these experiments also contained bivalves).

#### 310 *Effect of bioturbation on metal release from sediments*

311 There was little change between the release of Pb and Zn from the sediments with or  
312 without the bioturbating bivalve *T. deltoidalis*, however, differences were observed in the  
313 release of Fe and Mn. During the 21-d metal release period, dissolved metal concentrations in  
314 the overlying waters were (mean $\pm$ SD,  $\mu\text{g L}^{-1}$ )  $245\pm 270$  (Fe),  $6\pm 4$  (Mn),  $25\pm 8$  (Pb), and  $33\pm 21$   
315 (Zn) in the original sediments, and  $800\pm 620$  (Fe),  $96\pm 91$  (Mn),  $32\pm 28$  (Pb), and  $55\pm 42$  (Zn) in  
316 the sediments where *T. deltoidalis* was added. Cadmium and copper concentrations under  
317 these natural environmental conditions (pH 8.0, salinity of 35 PSU,  $8.0\text{ mg DO L}^{-1}$ ) were  
318 below detection.

319 Furthermore, the physical disturbance of the sediment that occurred when the bivalves  
320 were removed from the bioturbation treatments on day 21 was very similar to the disturbance  
321 by hand mixing of the original pH, DO and salinity metal release tests. This allowed a direct  
322 comparison between the effect of physical disturbance on the original and the bivalve-  
323 bioturbated sediments (now both in the absence of bivalves). The dissolved metal  
324 concentrations ( $\mu\text{g L}^{-1}$ ) in the overlying waters during the next 48 h were (mean $\pm$ SD)  
325  $840\pm 690$  (Fe) and  $300\pm 300$  (Mn) in the original sediment, and  $640\pm 325$  (Fe) and  $140\pm 110$

326 (Mn) in the bivalve-bioturbated sediment. Concentrations of Pb and Zn did not change  
327 significantly, and Cu and Cd concentrations were below detection.

### 328 *Metal bioavailability in contaminated sediments*

329 No mortality (100% survival) of *T. deltoidalis* occurred over the 21-day exposure to the  
330 contaminated sediments. This was consistent with previous studies that determined lowest  
331 observable effect concentrations (LOEC's) of >100 mg kg<sup>-1</sup> for Cd, Ni and >1000 mg kg<sup>-1</sup> for  
332 Cu, Pb, Zn, for *T. deltoidalis* in 10-d whole-sediment exposures (King *et al.*, unpublished  
333 results).

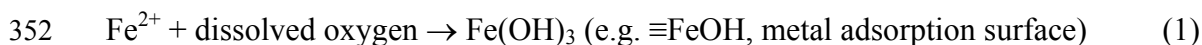
334 Following 21-day sediment exposures, *T. deltoidalis* accumulated significantly higher (P<  
335 0.001, n=20) concentrations of cadmium, lead and zinc in their tissues from metal  
336 contaminated sediments, in comparison to the control sediments (Table 1, Figure 7). No  
337 significant differences were observed for copper bioaccumulation. Measured metal  
338 concentrations in the oyster tissue SRM were in reasonable agreement with certified values  
339 (n=10). Measured (certified) concentrations (mean±SD, µg g<sup>-1</sup> dry weight) of cadmium  
340 2.60±0.01 (2.48±0.08), copper 71.6±1.6 (78.5±0.8), and zinc 1420±46 (1540±8). Lead  
341 recoveries were significantly above the certified values 0.49±0.08 (0.308±0.009) µg g<sup>-1</sup> dry  
342 weight and thus the lead concentrations presented here can only be considered for relative  
343 differences between the treatment and control.

## 344 **Discussion**

### 345 *Effect of pH and physical disturbances on metal release*

346 The metals released from the sediments appeared to be scavenged from the overlying  
347 water by adsorbing to resuspended particles and iron and manganese (hydr)oxide solid phases  
348 (e.g. Fe(OH)<sub>3</sub>, FeOOH, MnO<sub>2</sub>), which were formed through the oxidative precipitation of  
349 dissolved Fe(II) and Mn(II) cations that were simultaneously released from the pore waters  
350 (equations 1 and 2).

351



353



355

356 The influence of pH on these reactions is two-fold: pH influences (i) the rate of oxidation  
357 of Fe(II), and (ii) the binding of metals to organic-, iron- and manganese-based metal-binding  
358 phases. Both of these processes result in faster rates of metal scavenging in waters with high  
359 pH and they concur with the observed results (Figure 5). Santana-Casiano *et al.* (2004) found  
360 that the half life of  $1000 \mu\text{g L}^{-1}$  Fe(II) in seawater (36.7 salinity, 25 °C, air saturated) was ~0.8  
361 min at pH 8, ~20 min at pH 7 and >300 min at pH 6. The rate of oxidation of Mn(II) and  
362 precipitation as MnO<sub>2</sub> is very slow in the pH range 5-9 (Stumm and Morgan 1996).

363 In the absence of sediment disturbance, the reactions depicted in equations 1 and 2 will be  
364 fastest at the sediment-water interface, where oxygen penetration is greatest and the oxidative  
365 precipitation of iron oxyhydroxide phases occurs most rapidly. Following sediment  
366 disturbance, resuspended particulates that contain metal-binding sites in the form of organic-  
367 carbon and iron were expected to be more important for scavenging released metals from the  
368 overlying water. A return to pre-disturbance metal concentrations occurred as the sediment  
369 particulates re-settled, and the sediment redox conditions and sediment-water equilibrium was  
370 re-established.

371 The pH experiments indicate that under high- and mid-pH conditions minimal metal  
372 release from sediments is observed even with considerable physical disturbance. At low pH  
373 (5.5-6) metal release is observable, however, instances of low pH are rare, and natural  
374 dilution and pH buffering would minimise effects to benthic biological organisms.

#### 375 *Effect of dissolved oxygen and physical disturbances on metal release*

376 The results indicated that when DO concentrations were lower, the rate of release of Fe(II)  
377 and Mn(II) from the pore waters was faster and steady-state concentrations in the overlying  
378 waters were higher (Figure 6). This observation is related to the slower rate of oxidative-  
379 precipitation and scavenging of these ions as they diffuse through the sediment-water  
380 interface. Immediately following sediment disturbance, the release of dissolved iron and  
381 manganese was greatest in the high-DO experiments. This was consistent with the sediments  
382 of the high-DO experiments having high concentrations of Fe(II) and Mn(II) in the



383 porewaters (Figure 6), but sediment disturbance (rather than diffusion) was required for its  
384 release to overlying waters. The effect of DO on metals in the overlying water appeared  
385 clearer for Mn than Fe, because of the slower oxidative precipitation of Mn(II) than Fe(II).

#### 386 *Effect of bioturbation metal release*

387 The effect of bioturbating organisms on metal release from sediment was investigated by  
388 the addition of the benthic bivalve *T. deltoidalis* to sediments. The bivalve *T. deltoidalis*  
389 typically resides in the top 3-15 cm of sediments and feeds by sucking up sediment from the  
390 surface with one siphon, and discharging indigestible particles and faeces via the exhalant  
391 siphon to the sediment surface (Beesley *et al.* 1998).

392 While the sediment disturbance caused by bivalve bioturbation was small and continuous  
393 compared to the short but drastic physical disturbances of non-bioturbated sediments, the  
394 pattern of metal release, if not release concentrations, was consistent, with significant Fe and  
395 Mn, and minimal Pb and Zn release observable for both types of tests.

396 Furthermore, bioturbation results have similarities to the DO experiment results.  
397 Bioturbation, like low DO, increased the release of Fe and Mn from the sediment pore waters,  
398 and consequently porewater Fe and Mn concentrations in bioturbated sediments were lower  
399 than those in sediments without bioturbation. When physical disturbance occurred, a greater  
400 release of Fe and Mn occurred from the sediments without bioturbation due to the higher  
401 (trapped) porewater Fe and Mn concentrations.

402

#### 403 *The bioavailability of the sediment-bound-metals*

404 The metal concentrations in bivalves exposed to contaminated sediments varied  
405 considerably, but were of similar magnitude to those typically observed for bioaccumulation  
406 studies (Table 1). Biokinetic studies using Cd and Cu radioisotopes have shown that *T.*  
407 *deltoidalis* would require >40 days exposure to reach steady-state body concentrations of  
408 these metals in sediments (King *et al.* 2005). It is possible that a longer exposure period  
409 would result in a greater metal bioaccumulation in bivalve tissues, and also produce less  
410 variable metal concentrations.

411 The accumulation of cadmium, lead and zinc in the benthic bivalve, *T. deltoidalis*,  
412 demonstrates the bioavailability of the metals in the contaminated sediments used in these  
413 tests. The molar excess of SEM over AVS, and the continued cycling of the metals between  
414 the pore waters and the surface sediments due to the bioturbation process are likely to be a  
415 key process maintaining the bioavailability of the metals in the sediments. The feeding of *T.*  
416 *deltoidalis* on the oxidised surface sediments is likely to be the major route of metal  
417 accumulation.

### 418 **Conclusions and summary**

419 The release of metals from sediments (and pore waters) occurred during sediment  
420 disturbance, with significant releases during severe mixing and less significant releases from  
421 biological mixing. This study demonstrated that metal release from metal-contaminated  
422 sediments was influenced by iron and manganese redox chemistry. Larger releases into  
423 overlying waters occurred at lower pH (5.5) and DO (3 mg/L) as the sediment bound iron and  
424 manganese were reduced, with pH effecting release rates up to 10 fold greater than those for  
425 DO. Upon mixing the released and resuspended metals became oxidised, sequestered to  
426 particulate phases in the water column, and precipitated with the oxidised iron and  
427 manganese. At higher pH and DO (pH 8.0 and DO 8.0 mg L<sup>-1</sup>) there were faster oxidation  
428 rates of Fe and Mn (and hence faster rates of metal precipitation), which generated lower  
429 dissolved metal concentrations in the overlying water. Hence in natural environments, the pH  
430 and dissolved oxygen of the overlying water will govern the rate at which released metals will  
431 be scavenged from the water column and returned to the sediment. During this study, for mid  
432 to high pH and DO conditions, the overlying water metal concentrations generally returned to  
433 pre-disturbance conditions within eight days, the metal release was only temporary. The  
434 accumulation of metals in the *T. deltoidalis* demonstrates the bioavailability of the metals in  
435 contaminated sediments, where the bioavailability is likely to be a result of the molar excess  
436 of SEM over AVS, and the continued cycling of the metals between the pore waters and the  
437 surface sediments due to the bioturbation process.

438

### 439 **Acknowledgments**

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442 reviewers for constructive comments on this manuscript.

443

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505 List of Figure and Table captions.

506

507

508 Figure 1. The sediment collection sites in Lake Macquarie, New South Wales, Australia. The  
509 bulk sediments for metal release experiments were collected from site 6 (GPS co-  
510 ordinates 0370303, 6351070 WGS84).

511

512 Figure 2. Schematic representation of metal release experimental setup. Each container was  
513  $30 \times 30 \times 8$  cm, contained 4 kg of sediment and 1.5 L overlying water.

514

515 Figure 3. TPM ( $\circ\bullet$ ) and SEM ( $\blacktriangle\triangle$ ) concentrations for 0-5 cm surface ( $\circ\triangle$ ) and 10-15  
516 cm depth ( $\blacktriangle\bullet$ ) sediments from contaminated sediments at Cockle Bay. The dashed line  
517 represents the Australian sediment quality guideline value (ISQG-low value,  
518 ANZECC/ARMCANZ, 2000). The standard error (bars shown) between replicate  
519 analyses was generally 10-30%.

520

521 Figure 4. Concentrations of AVS ( $\square\triangle$ ) and the molar AVS-SEM difference ( $\blacksquare\blacktriangle$ ) in the 0-  
522 5 cm surface ( $\triangle\blacktriangle$ ) and 10-15 cm depth ( $\square\blacksquare$ ) sediments from contaminated sediments at  
523 Cockle Bay. Replicates shown at sites 4, 5, 6, 7 and 13. The standard error between  
524 duplicate analyses was generally 10-30%.

525

526 Figure 5. Concentrations of dissolved Fe, Mn, Pb, and Zn in the overlying waters over the  
527 29-d metal-release experiments at low-pH ( $6\pm 1$ ) ( $\circ$ ), mid-pH ( $7.2\pm 0.2$ ) ( $\blacksquare$ ) and high-pH  
528 ( $8.1\pm 0.2$ ) ( $\triangle$ ). Error bars represent standard errors for duplicate experiments. On day 21  
529 the sediment was disturbed. Data below the detection limit, represented by the dashed  
530 line, are not shown.

531

532 Figure 6. Concentration of dissolved Fe, Mn, and Pb in the overlying waters (pH 8) over the  
533 29-d metal-release experiments at low-DO ( $3 \text{ mg L}^{-1}$ ) ( $\circ$ ), mid-DO ( $6 \text{ mg L}^{-1}$ ) ( $\blacksquare$ ) and  
534 high-DO ( $8 \text{ mg L}^{-1}$ ) ( $\triangle$ ) concentrations. Error bars represent standard errors for duplicate  
535 experiments. On day 21 the sediment was disturbed. Data below the detection limit,  
536 represented by the dashed line, are not shown.

537

538 Figure 7. Concentration of cadmium (mg/kg, dry mass) in the soft tissue mass of *Tellina*  
539 *deltoidalis* following 21-day exposure to control (■) and contaminated (△) bulk  
540 sediments. Overlying waters were maintained at  $18.5 \pm 0.5^\circ\text{C}$ , pH  $8.0 \pm 0.5$ , salinity  $35 \pm 3\text{‰}$   
541 and  $8.0 \pm 0.5 \text{ mg DO L}^{-1}$ .

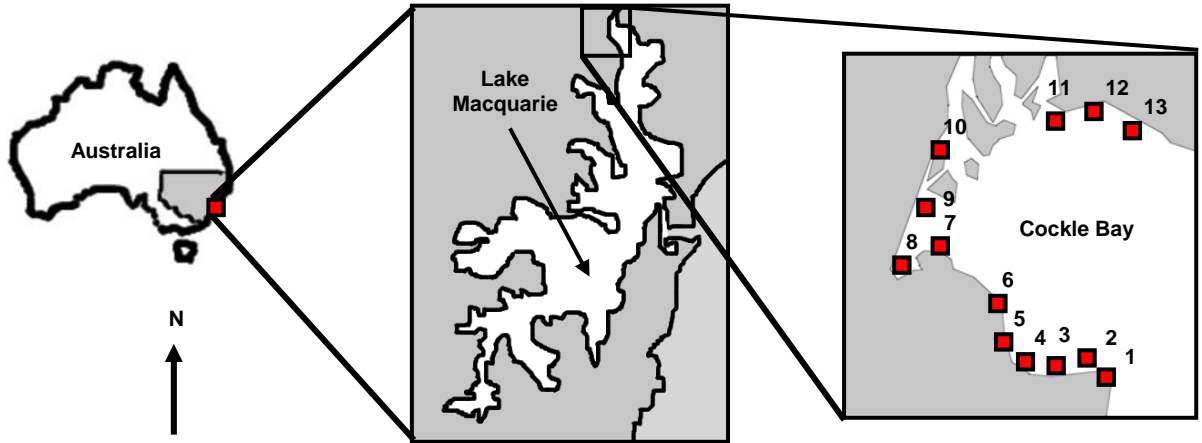
542

543 Table 1. Metal concentrations in test sediments and *T. deltoidalis* following 21-day exposure.  
544 Control and contaminated sediments, mean  $\pm$  std error ( $\text{mg kg}^{-1}$ , dry mass).

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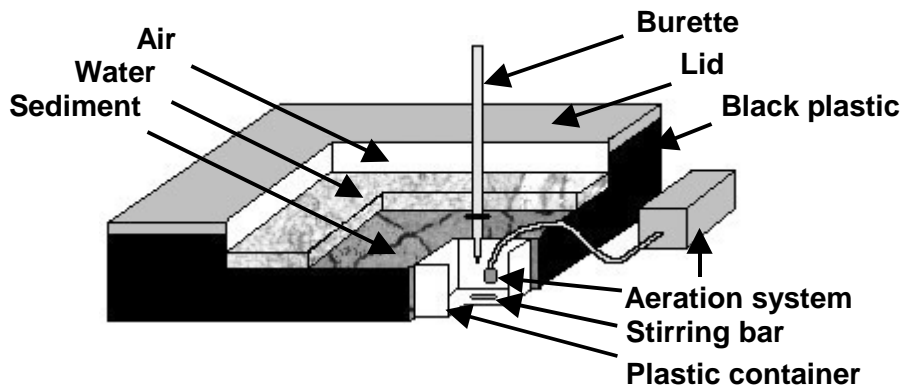
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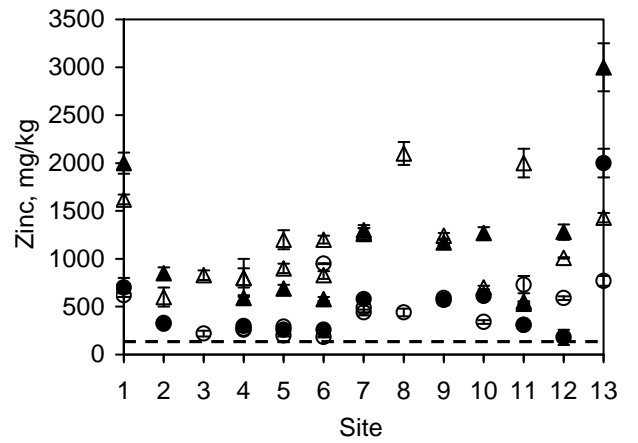
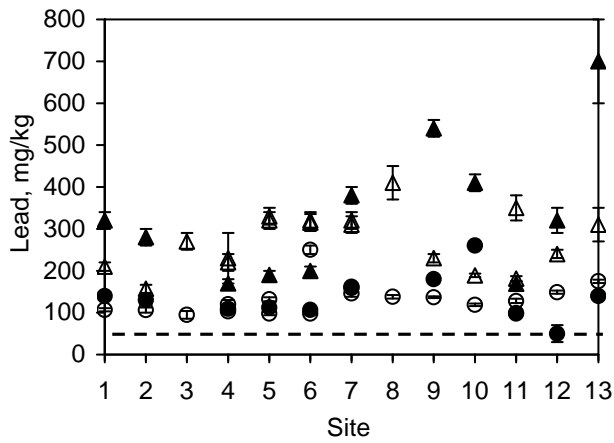
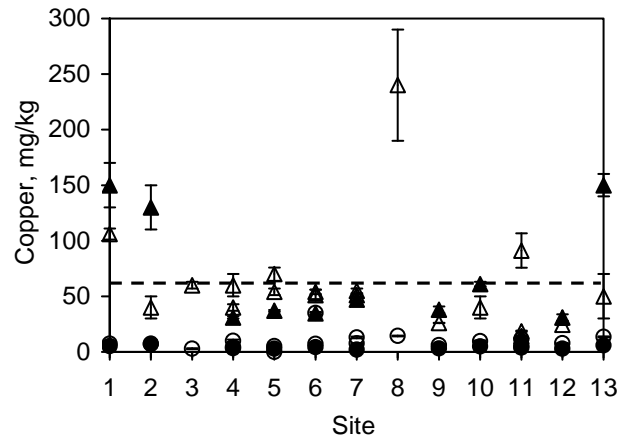
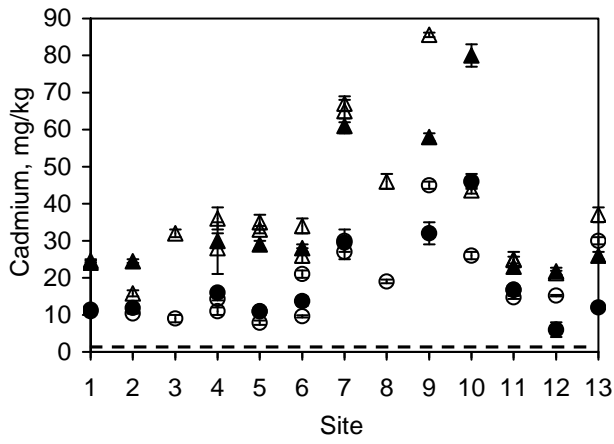
Figure 1.



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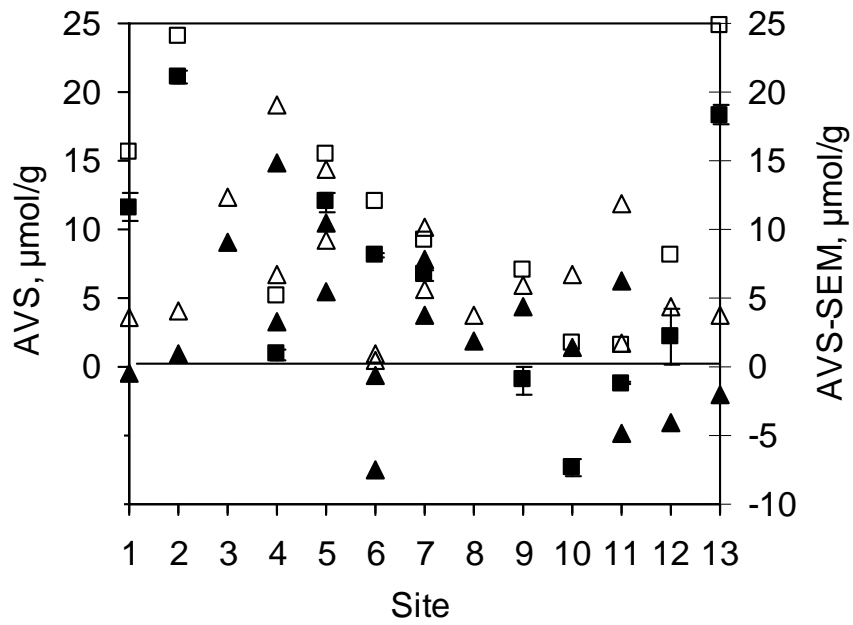
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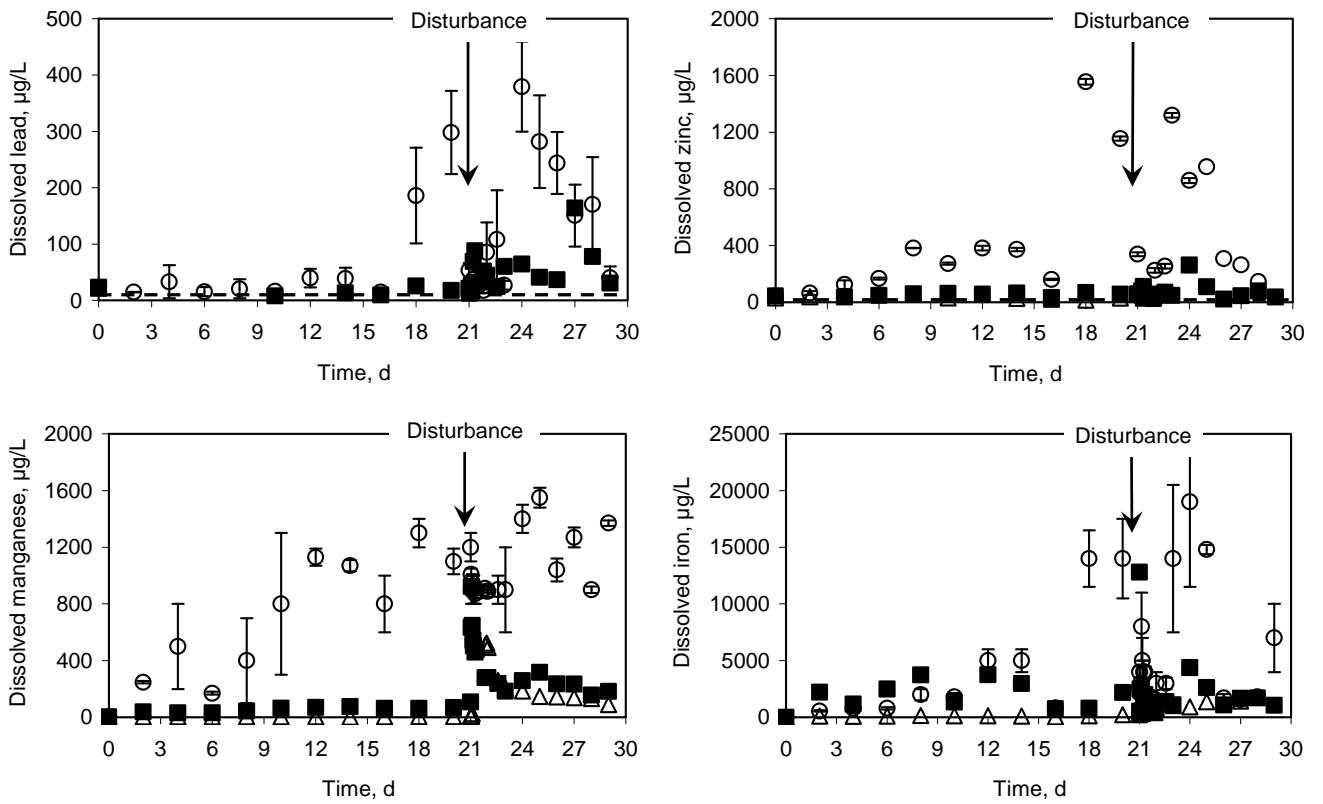
Figure 3.



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Figure 4.

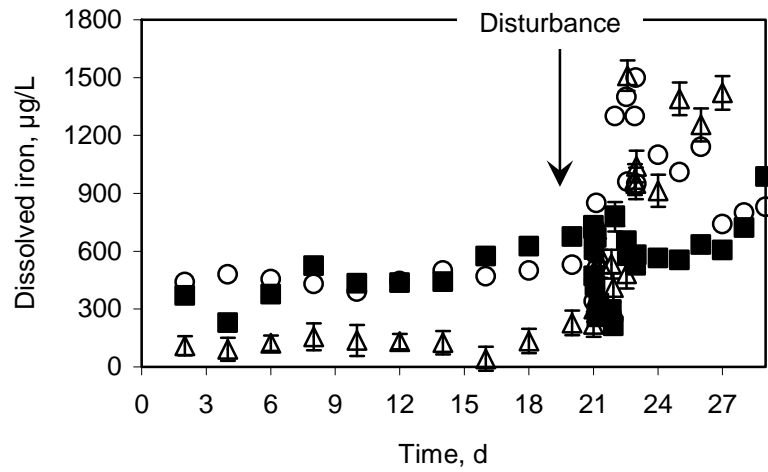
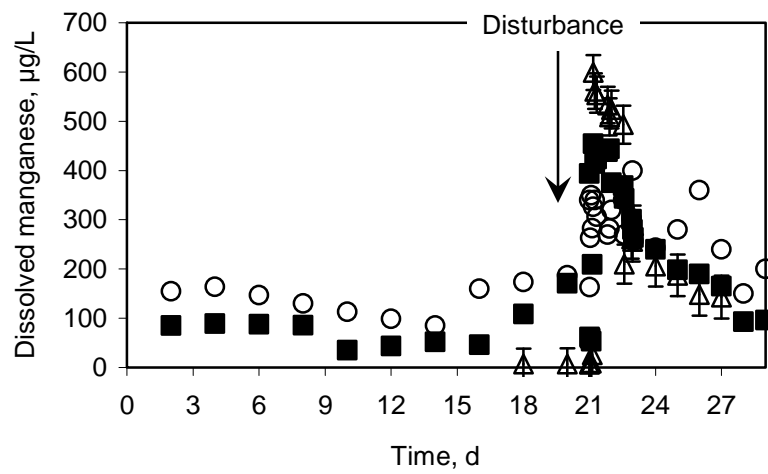
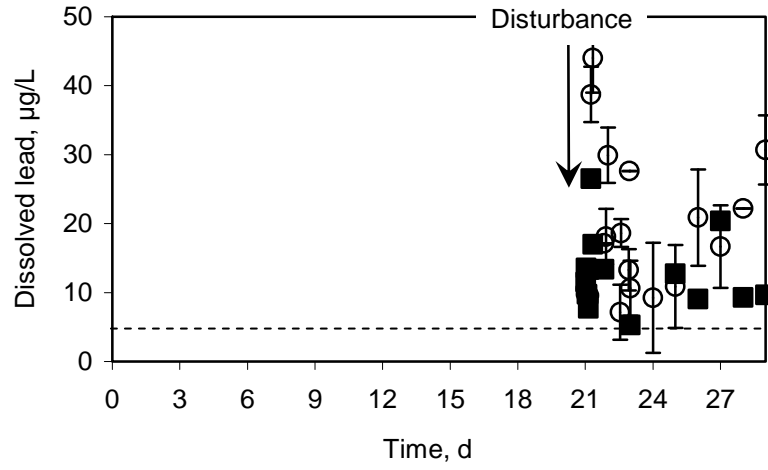
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Figure 5.

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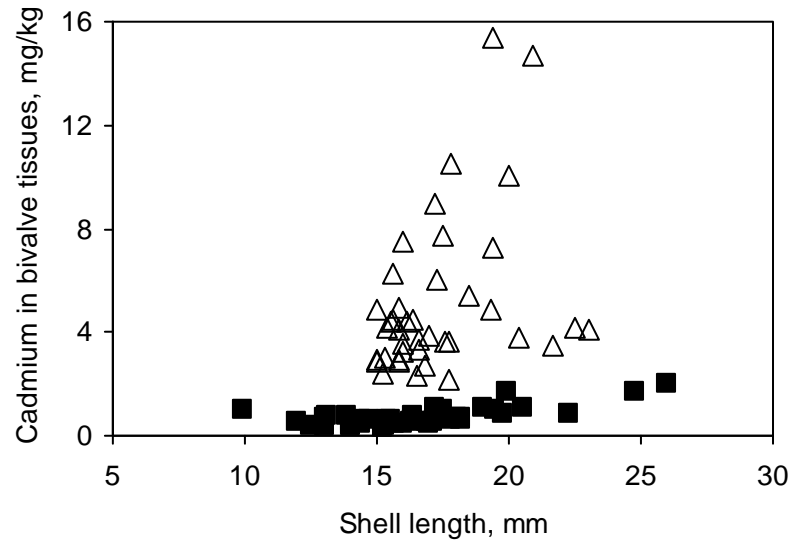
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**Figure 6.**



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**Figure 7.**

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**Table 1.**

Metal	Total metals in sediment		Bivalve tissue concentrations		*Significance
	Boronia Park (n=5)	Cockle Bay (n=6)	Boronia Park (n=20)	Cockle Bay (n=20)	Differences in bivalve metals
Cadmium	0.6 ± 0.1	25.5 ± 0.5	0.73 ± 0.08	5.1 ± 0.7	P < 0.001
Copper	25 ± 10	51 ± 3	220 ± 30	290 ± 45	P = 0.161
Lead	35 ± 15	314 ± 4	26 ± 3	55 ± 8	P < 0.001
Zinc	90 ± 20	1200 ± 60	230 ± 20	370 ± 50	P < 0.001

597 \* Significant differences between log-transformed data from Boronia Park and Cockle Bay bivalve  
598 tissues.

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600