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Effect of bioturbation on contaminated sediment deposited over remediated sediment

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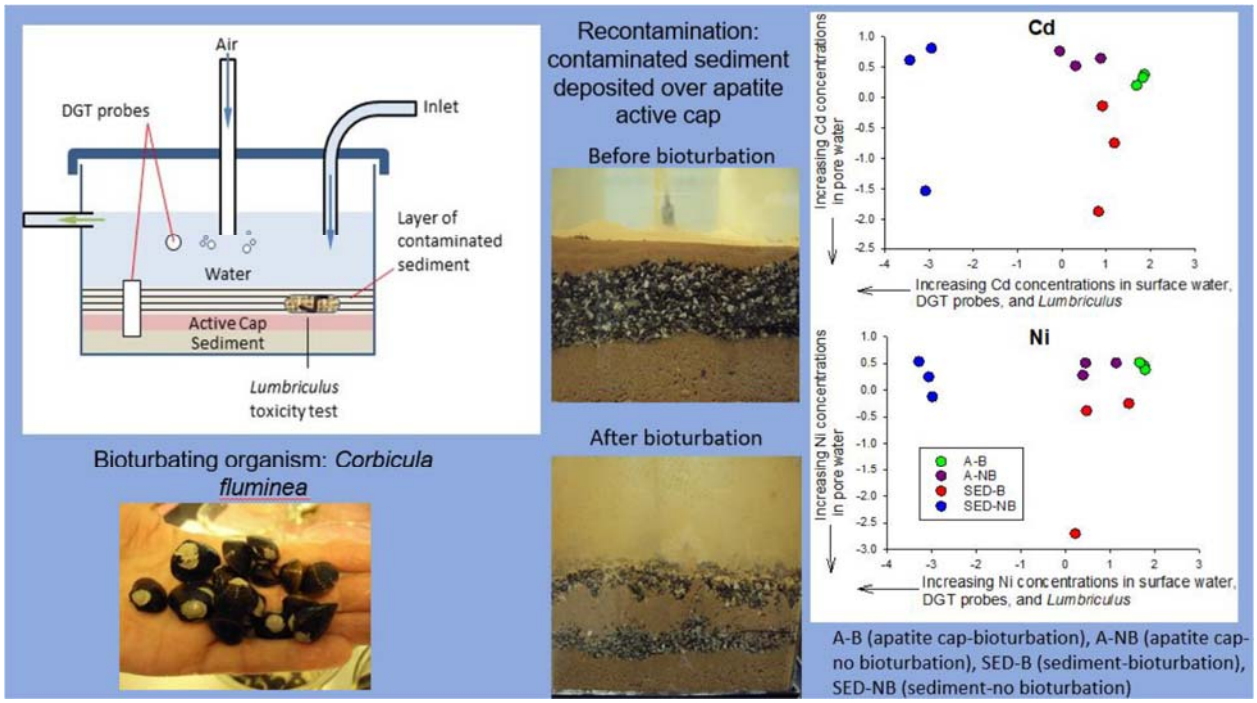
GRAPHICAL ABSTRACT



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HIGHLIGHTS

- Remediated sediment can be re-contaminated by new influxes of contaminated sediment
- Recontamination processes are affected by bioturbation
- Bioturbation did not reduce the ability of apatite caps to control recontamination
- The effects of bioturbation on recontamination of uncapped sediment were metal specific
- Remediation by active caps can mitigate the effects of ongoing sediment contamination

Effect of bioturbation on contaminated sediment deposited over remediated sediment

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ABSTRACT

A challenge to all sediment remediation technologies is the continued influx of contaminants from uncontrolled sources following remediation. However, contaminants deposited on sediments remediated with chemically active sequestering agents may be affected by the sequestering agents resulting in reduced impacts. We deposited sediment contaminated with As, Cd, Cu, Pb, and Zn over clean sediment capped with the sequestering agent, apatite, and clean uncapped sediment in laboratory mesocosms to simulate the recontamination of remediated sediment by influxes of particle-bound contaminants. Cap effectiveness was assessed in the presence and absence of the bioturbating organism *Corbicula fluminea* based on metal fluxes to sediment pore water and surface water, the distribution of dissolved and labile contaminants in sediment and surface water measured by Diffusive Gradients in Thin Films, and contaminant bioaccumulation by *Lumbriculus variegatus*. The metal sequestration capacity of apatite caps was unaffected or improved by bioturbation for all elements except As. Effects with uncapped sediment were metal specific including reductions in the bioavailable pool for Ni, Cd, and to a lesser extent, Pb, increases in the bioavailable pool for As and Cu, and little effect for Zn. It is likely that the reductions observed for some metals in uncapped, clean sediment were the result of burial and dilution of contaminated sediment combined with chemical processes such as sequestration by iron compounds. These results indicate that apatite caps can control recontamination by metals regardless of bioturbation but point to the complexity of sediment recontamination and the need for further study of this problem.

KEYWORDS

Bioturbation; passive caps; active caps; metal contaminants, remediated sediment; re-contamination

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1. Introduction

Bioturbation is the physical disturbance, restructuring, and reworking of sediments by benthic organisms resulting in sediment movement and the alteration of sediment properties. It affects sediment turnover and diffusive and advective processes that transport elements in dissolved and particulate form between the sediments and overlying water. Several studies have demonstrated that bioturbation can influence the transfer of heavy metals between sediment and water (Remaili et al., 2016 and 2017). Tubificid worms renewed particles and adsorption sites for Cd at the interface of sediment and water and increased the Cd content in the upper sediment layer (Ciutat et al., 2005). However, the bivalve *Tellina deltoidalis* had little effect on the release of Zn and Pb from the sediment (Atkinson et al., 2007). Similarly, tubificids had little impact on Pb remobilization from the sediment, and bioturbation by *Monoporeia affinis* was insufficient to release Cu from Cu enriched sediment (Ospina-Alvarez et al., 2014). These findings suggest that the influence of bioturbation on heavy metals fluxes in sediment is complex and may differ among bioturbating organisms, metals, and sediment conditions (He et al., 2017).

The preceding research investigated the effects of bioturbation on metal fluxes in contaminated sediment. However, little is known concerning the effects of bioturbation on metal fluxes in contaminated sediment deposited over remediated sediment. i.e., remediated sediment that has been re-contaminated by continued or new influxes of contaminated sediment over the remediated sediment. Recontamination of remediated sediments by contaminated sediment influxes from permitted discharges, upstream contaminated sites, or stormwater discharge can

90 slow or reverse recovery associated with sediment remediation and is the subject of increasing
91 interest.
92 The most commonly used remediation method is dredging, which involves the
93 removal of contaminated sediment to reduce risks to human and environmental health.
94 However, sediment capping is sometimes preferred because it is often less expensive, and
95 disruptive to the benthic environment. In passive capping, contaminated sediment is
96 covered with a layer (cap) of clean, inert material such as sand, soil, or sediment to
97 physically isolate the contaminants. In active capping chemically reactive amendments are
98 applied to the sediment surface to bind contaminants, thereby reducing pore water
99 contaminant concentrations and bioavailability (Knox et al., 2008; Paller and Knox, 2010;
100 Ghosh et al., 2011; Dixon and Knox, 2012; Knox et al., 2012 and Knox et al., 2014). A
101 variety of amendments are used in active capping including phosphate materials (e.g.,
102 apatite) with metal sequestering abilities (Knox et al., 2012). Regardless of the remediation
103 method, recontamination poses significant challenges that can negate expensive remedial
104 actions, although Knox et al. (2016 and 2019) found that active caps can protect remediated
105 sediment by reducing bioavailable metals in ongoing sources of contamination. However,
106 their research did not address the influence of bioturbation on recontamination.
107 We employed experimental mesocosms to investigate the effects of bioturbation on the
108 recontamination of clean sediment (e.g., sediments remediated by the removal of contaminants
109 through dredging) and sediment that has been capped with a chemically active material that
110 sequesters contaminants (active capping). Bioturbation can influence recontamination processes
111 by mixing newly deposited contaminated sediment with underlying uncontaminated sediment in
112 the case of dredging or sequestering agents in the case of active capping. It may contribute to the

113 release of contaminants from the newly deposited contaminated sediment or to their
114 sequestration or dilution by burial in underlying clean sediments or active capping materials. In
115 the latter case, contaminants may react with the capping materials to reduce their toxicity and
116 bioavailability.

117 The objective of this research was to assess the effects of bioturbation on an incoming
118 particulate contaminant load deposited over underlying clean sediment or active capping material
119 in a freshwater environment. The effects of bioturbation were evaluated based on metal fluxes
120 from contaminated sediment to sediment pore water and overlying surface water, the distribution
121 of dissolved and labile contaminants in the water and sediment measured by Diffusive Gradients
122 in Thin Films (DGT), and contaminant bioaccumulation and toxicity to aquatic organisms. We
123 hypothesized that the bioturbation of contaminated sediment deposited over active caps would
124 increase the contact of the sediment with underlying cap materials, potentially fostering chemical
125 sequestration processes that reduce contaminant bioavailability, toxicity, and release to the water
126 column. Increased contact from bioturbation could result from mixing cap materials with
127 overlying sediment or enhancement of diffusion and advection. We further hypothesized that
128 these effects would be weaker when bioturbation occurs in contaminated sediment deposited
129 over uncontaminated sediment, as would be the case when areas dredged for contaminant
130 removal are subjected to recontamination.

131 **2. Materials and Methods**

132 *2.1. Experimental design*

133 This experiment evaluated the deposition of sediment contaminated with metals (As, Cd,
134 Cu, Ni, Zn, and Pb) over uncapped, clean sediment and clean sediment with an overlying active
135 cap composed of North Carolina apatite in the presence and absence of bioturbating organisms.

136 Apatites ($Ca_5(PO_4)_3(F,Cl,OH)$) are commonly available phosphate minerals usually in the form of
137 carbonate apatite with isomorphic substitution of carbonate for phosphate, F for hydroxyl anion, and
138 minor substitution of Ca^{2+} by Na^{+} and Mg^{2+} atoms. Apatite effectively immobilizes metals in
139 contaminated soils/sediments and reduces their toxicity and bioavailability (Ma et al., 1995;
140 Knox et al., 2003 and 2006).

141 The experiment was conducted in 12 flow-through mesocosms, each consisting of a 20
142 cm wide, 41 cm long, 43 cm high custom-built acrylic aquarium with an acrylic lid. Locally
143 collected, subsurface red clay sediment was added to each mesocosm to produce a layer
144 approximately 5.0 cm thick on the bottom (Table 1). Cu, Zn, As, Cd, and Pb concentrations in
145 this sediment were slightly to moderately elevated, thus simulating conditions often associated
146 with diffuse, nonpoint pollution. Pore water samplers consisting of stainless steel wire mesh
147 screen connected to nylon tubing were buried in the sediment to a depth of about 2.5 cm.
148 Sufficient granular apatite was added to half of the mesocosms to produce a 2.5 cm thick cap
149 over the underlying sediment. Each mesocosms had an airstone with sufficient flow to ensure
150 aeration of the water column without appreciable resuspension of bottom sediments.
151 The mesocosms were divided into four groups of three: apatite caps with bioturbation,
152 apatite caps without bioturbation, uncapped sediment with bioturbation, and uncapped sediment
153 without bioturbation. A single reservoir supplied all mesocosms with 0.5 ml $minute^{-1}$ of
154 uncontaminated, moderately soft, artificial, fresh water prepared according to EPA methods
155 (USEPA 2002). The mesocosms were permitted to equilibrate for six weeks after the initiation of
156 water flow before the experiment was started by adding contaminated sediment to each
157 mesocosm. Our intent was to simulate a remediated benthic ecosystem exposed to an influx of
158 contaminated sediment from an offsite source through an episodic disturbance such as runoff

159 from a storm or release from an upstream construction activity. The contaminated sediment was
160 manually added to each mesocosm to produce a layer about 1.5 cm thick over a bottom layer of
161 uncontaminated sediment (simulating clean [e.g., formerly dredged] sediment) or a bottom layer
162 of uncontaminated sediment with an overlying active cap composed of apatite (i.e., North
163 Carolina rock phosphate). The contaminated sediment was characterized by levels of Cu, As and
164 Cd within the range typical of polluted sediments and levels of Zn, and Pb elevated above those
165 in the uncontaminated layer of bottom sediment but below levels recognized as polluted (Table
166 1).

167 Asian clams *Corbicula fluminea* averaging 2-3 cm across the largest dimension of the
168 shell taken locally from the Savannah River near Augusta Georgia were added to half of the
169 sediment and active cap mesocosms 24 hours after the addition of the contaminated sediment.
170 Preliminary experiments showed that Asian clams burrowed to 2 cm or more in depth and
171 significantly affected sediment porosity and vertical zonation. One hundred and twenty clams
172 were added to each mesocosm producing a density of 1,463 individuals/m^2, which simulated
173 natural densities and resulting bioturbation to an extent realistic in rivers with moderate to high
174 densities of Asian clams (Sousa et al., 2008). The clams remained in the mesocosms for 28 days
175 before the experiment was ended. Clams that died during this period were replaced with new
176 clams marked to distinguish them from original clams that remained for the entire 28-day
177 exposure period. Visual observations of the caps and sediment through the transparent mesocosm
178 walls were used to assess the effects of bioturbation on the sediment and cap layers.

179 *2.2. Surface water, pore water, and sediment*

180 Surface water samples for metal analysis were collected eight times at 0, 1, 24, 96, 264,
181 432, 600, and 696 hours. One set of samples for dissolved metals was filtered using a 0.45μm

182 pore diameter membrane filter; a second set of samples for total recoverable metals was not
183 filtered. All samples were acidified with nitric acid to pH<2. Concentrations of As, Cd, Cu, Ni,
184 Pb, and Zn were determined by inductively coupled plasma-mass spectrometry (ICP-MS) using a
185 NexION 300 (Perkin Elmer, Inc.) according to the QA/QC protocols outlined in EPA Method
186 6020B (USEPA, 2014). Surface water in all tanks was monitored for dissolved oxygen,
187 temperature, electrical conductivity, pH, and turbidity with an Accumet XL600 electronic
188 meter. Calcium hardness was measured by ethylenedi-aminetetraacetic acid (EDTA)
189 titration (Hach, 2013).
190 Pore water samples were collected at the end of experiment at time 696 hours (28 days)
191 by extracting water through the pore water samplers with a peristaltic pump. The samples were
192 acidified with nitric acid to pH<2 and analyzed for metal concentrations by ICP-MS. Pore water
193 temperature, electrical conductivity, dissolved oxygen, pH, and oxidation reduction potential
194 were measured in the extracted water.
195 Core samples for measurement of sediment pH were collected from each mesocosm at
196 696 h with a push-tube coring device. The cores from uncapped sediment were divided into a
197 contaminated sediment layer (CL), an A layer (0.0-2.5 cm beneath the contaminated layer), and a
198 B layer (2.5-5.0 cm beneath the contaminated layer). The cores from apatite cap treatments were
199 split into four layers: CL, cap layer (C), A layer (0.0-2.5 cm beneath the cap) and B layer (2.5-
200 5.0 cm beneath the cap). The pH was determined from a 1:1 solid/water equilibrium solution.
201 *2.3. Bioaccumulation and toxicity testing*
202 Eighteen days after the addition of the clams, cylindrical cages composed of fine-mesh,
203 plastic screen containing an average of 5 g of the burrowing annelid California blackworms
204 *Lumbriculus variegates* (obtained from California Blackworm Co.) were embedded within the

top layer of contaminated sediment to assess metal bioavailability. After 10 days, the blackworms were removed from the cages, weighed in aggregate, and transferred to beakers containing uncontaminated water for depuration. Survival was estimated from the change in aggregate wet weight in each cage between the beginning and end of the exposure period. Whole animals were rinsed, freeze-dried to a constant weight, and digested with H_2O_2 and $HN0_3$ at 85 °C. Metals in the extracts were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) using a NexION 300X mass spectrometer and standard QA/QC protocols including internal standards, duplicate samples, blanks, and certified reference materials (TORT-3: Lobster Hepatopancreas Reference Material for Trace Metals) following U.S. EPA Method 6020A (USEPA, 2014). Duplicate samples differed by a maximum of 1.3%. Background samples consisting of worms not exposed in the mesocosms were also analyzed for comparison with worms held in the mesocosms.

The experiment was concluded after removal of the worms (i.e., at the end of the 28-day bioturbation period). At this time, all Asian clams were also removed from the mesocosms, depurated in clean water, and counted to assess survival. Clams present in the mesocosms from the beginning of the bioturbation experiment were dissected to remove soft tissues, which were rinsed and frozen. To economize, tissues from 30 clams were composited to represent average metal concentrations in each mesocosm. Metal analyses were conducted on a 50 mg subsample of freeze-dried tissue from each composite digested and analyzed as described for *Lumbriculus* samples. Background samples consisting of composite samples of 30 clams taken directly from the Savannah River were also analyzed.

2.4. DGT probes

227 Diffusive gradient in thin films (DGT) probes include a collection gel-layer with a
228 medium that selectively binds to the contaminant of interest and a diffusion gel-layer that
229 selectively admits analyte molecules (Davison and Zhang, 1994). DGT tends to exclude
230 non-bioavailable metals strongly bound to organic molecules and other ligands, thus
231 providing a more accurate measure of potentially bioavailable metals than total or dissolved
232 metal measurements. DGT has been successfully used in previous studies to assess metal
233 bioavailability to bioturbating organisms in contaminated sediments (Amato et al. 2016).
234 DGT Chelex 100 water and sediment probes were purchased from DGT Research Ltd
235 (Lancaster, UK). A DGT water probe was suspended in the water with open side facing
236 downward and a DGT sediment probe was vertically inserted into the sediment of each
237 mesocosm for 24 hours one day before retrieving the California blackworms. Water and
238 sediment temperatures were recorded at the times of DGT deployment and retrieval. The
239 sediment probes were deoxygenated before deployment.
240 All probes were rinsed and their Chelex collection gels removed upon retrieval from the
241 mesocosms. The sediment probes were divided into sections corresponding to the surficial
242 contaminated layer (CL), cap (C), underlying sediment layer (A), and deep sediment layer (B), as
243 previously described, to assess the vertical distribution of sediment contaminants. Collection-
244 gels were immersed in 1 M HNO_3 for 24 hours after which the eluent was removed and diluted
245 with deionized water for analysis by ICP-MS. The concentration in the diluted aliquot was
246 adjusted for dilution and the mass of each metal accumulated in the resin gel layer (M) was
247 calculated using equation 1:
248
$$M = \frac{Ce * (V_{NO_3} + V_{gel})}{f_e} \quad (1)$$
249

where

- V_{NO3} = amount of nitrate added (based on the amount of nitric acid required to submerge the resin-gel layer),
- V_{gel} = volume of the resin gel, and
- f_e = elution factor of 0.8 (Zhang and Davison, 1995 and 2001).

The concentration of metal measured by each DGT probe (C_{DGT}) was calculated with equation 2:

$$C_{DGT} = \frac{M * \Delta g}{D * t * A} \quad (2)$$

where:

- Δg = thickness of the diffusive layer and filter layer (0.096 cm) (Zhang and Davison, 1995 and 2001),
- D = diffusion coefficient each metal at the retrieval temperature,
- t = deployment time (24 hr = 86400s), and
- A = exposed area of the DGT unit.

2.5 Statistical analysis

Two-way, factorial analysis-of-variance (ANOVA) was used to assess the significance of differences in surface water metal concentrations, pore water metal concentrations, blackworm whole-body metal concentrations, and DGT surface water concentrations among experimental groups. The main effects in each test were treatment (apatite cap vs uncapped sediment) and bioturbation (presence vs absence). The dependent variable for each metal in the surface water tests was the average concentration in each mesocosm beginning at 264 hours after the introduction of the contaminated sediment and continuing to the end of the experiment, thus eliminating the initial period when metals levels were fluctuating. The dependent variable for each metal in the blackworm tests was the average concentration in the composite samples from

each treatment (N=3 for apatite-bioturbation, N=2 for apatite-no bioturbation [the blackworm cage in one mesocosm was damaged], N=3 for sediment-bioturbation, and N=3 for sediment-no bioturbation). Sample size for each surface water, DGT surface water, and pore water test was three per treatment corresponding to the three replicate mesocosms for each treatment (total of 12 for four treatments).

One-way ANOVA was used to assess the significance of differences in soft tissue metal concentrations among clams held in the bioturbation mesocosms. There were three groups: apatite mesocosms, sediment mesocosms, and clams taken from the field (i.e., background samples). Data points were the composite samples representing each group (N=3 for apatite, N=3 for sediment treatments, and N=6 for background).

Three-way ANOVA was used to analyze DGT sediment metal concentrations with the main effects of treatment (sediment vs. apatite cap), bioturbation (presence/absence), and sediment/cap layers as previously described. The cap layer, which was present only in the apatite cap treatment, was excluded to maintain a balanced design. Average blank concentrations were subtracted when calculating the DGT concentration for each element.

All ANOVA results were considered significant at $\alpha = 0.05$. Most data satisfied tests for normality (Shapiro-Wilk) and equal variance (Brown-Forsythe) making transformations unnecessary. Holm-Sidak pair-wise, multiple comparisons tests (overall $\alpha = 0.05$) were used to assess differences among individual least square means following overall significance in the ANOVAs. Means in statistical tables are least square means (LSMs) derived from linear models, which may differ slightly from observed means (i.e., averages) because of differences in sample size. Separate tests were conducted for As, Cu, Cd, Ni, and Zn. DGT results are not presented

295 for As because Chelex probes are inappropriate for this element, nor for Zn because blank DGT
296 Zn concentrations often exceeded Zn concentrations in the mesocosms.
297 Principal component analysis (PCA) was used to summarize the effects of treatment and
298 bioturbation on metal levels in the mesocosms. PCA is used to analyze interrelationships among
299 several variables and summarize them in terms of a smaller number of variables termed principal
300 components. Correlations between the principal components and the original variables indicate
301 which of the latter contribute most strongly to patterns in the data. We used PCA to summarize
302 the influence of treatment and bioturbation on the average values of the five variables (average
303 surface water, pore water, *Lumbriculus*, DGT water, and DGT sediment concentrations)
304 measured in each mesocosms for Cd, Cu, and Ni and the three variables (average surface water,
305 pore water, and *Lumbriculus* concentrations) measured for As and Zn. PCA results were
306 presented by plotting the scores for each of the 12 mesocosm on principal components 1 and 2
307 together with the Pearson product moment correlations (r) between each component and the
308 three or five variables used to derive the components. Results for only PC1 and PC2 are reported
309 because the other principal components accounted for little additional variance in the data.
310 **3. Results**
311 *3.1. Bioturbation*
312 Bioturbation by the Asian clams strongly affected the integrity and vertical distribution of
313 the layer of contaminated sediment added to the mesocosms. This sediment initially formed a
314 well consolidated, distinct layer after deposition over the caps or uncontaminated sediments in
315 the mesocosms. However, bioturbation by the clams was obvious within 24 hrs. By the end of
316 the 28-day bioturbation period, mixing was extensive, and substantial quantities of apatite and

underlying sediment had been mixed with and translocated above the contaminated sediment (Picture 1). The estimated depth of bioturbation in the mesocosm was about 3 cm.

3.2. Surface water

Surface water turbidity increased from about 3 NTUs to 350-800 NTUs following deposition of the contaminated sediment but decreased to under 10 NTUs within the next 96 hrs as the sediment settled. Turbidity remained about twice as high in mesocosms with bioturbation as mesocosms without bioturbation for the rest of the study (Table 2). Surface water dissolved oxygen concentrations and temperatures were similar among treatments and stable during the study (about 8.0 mg L-1 and 21 ^{o}C, respectively). In contrast, surface water pH, electrical conductivity (EC), and hardness were higher in mesocosms with apatite caps than with uncapped sediment (Table 2).

Metal release to surface waters occurred after the contaminated sediment was added to the mesocosms (Figures 1). Total (i.e., sum of dissolved and particulate) As, Cd, Cu, Ni, Pb, and Zn concentrations peaked at 24 h but decreased to relatively low concentrations by 96 - 264 h (Figures 1). The strongest decreases were observed for Cu, and Pb. The presence of suspended sediment in the water column resulted in relatively high particulate fractions for some metals (especially Cu, As, and Pb) following addition of the contaminated sediment. This fraction decreased rapidly for Cu, As, and Ni and, to a lesser extent, Zn and Cd, as suspended particles settled to the bottom. Pb was almost entirely in particulate form throughout the study (Figure 1).

Two-way ANOVA of surface water concentrations indicated significant ($P \leq 0.05$) interactions between treatment and bioturbation for Cu, As, Ni, and Cd (Figure 2). With Cd and Ni, bioturbation greatly decreased concentrations in mesocosms with uncapped sediment but had less effect in mesocosms with apatite caps, where metal concentrations remained relatively low

regardless of bioturbation (Figure 2). Treatment effects (apatite caps vs uncapped sediment) were smaller for Cu than for Cd and Ni. Bioturbation had little effect on Cu concentrations in mesocosms with active caps but increased Cu concentrations in mesocosms with uncapped sediment. Similar results were observed for Pb, except that differences were not significant because of high inter-replicate variability (Figure 2). Arsenic exhibited a significant interaction in which bioturbation increased concentrations in both apatite cap and uncapped sediment mesocosms but more in the latter. The ANOVA for Zn lacked a significant interaction but indicated significant main effects for treatment and bioturbation: concentrations were lower in mesocosms with active caps and slightly lower in mesocosms with bioturbation. These results showed that bioturbation and capping had interacting effects that differed among metals. Apatite caps maintained relatively low surface water concentrations of all metals except As regardless of bioturbation (Figure 2). In contrast, the ability of uncapped sediment to control surface water metal concentrations was strongly affected by bioturbation, which increased As, Cu, and Pb levels but decreased Cd, Ni, and Zn levels.

3.3. Sediment pore water

The apatite caps increased pore water EC and pH, following the same pattern observed for surface waters, although the differences were greater for pore water (Table 2). The apatite caps also increased sediment pH, as did bioturbation, which was associated with higher sediment pH in both the apatite cap and uncapped sediment mesocosms. Slightly reducing conditions prevailed in the sediments of all mesocosms and were not strongly affected by bioturbation (Table 2).

Two-way ANOVA indicated that pore water concentrations of Ni, Zn, and Cd were significantly lower in mesocosms with apatite caps than mesocosms with uncapped sediment and

363 unaffected by bioturbation. (Figure 3). In contrast, two-way ANOVA of Cu and Pb pore water
364 concentrations indicated a significant interaction between treatment and bioturbation:
365 bioturbation strongly increased porewater concentrations in uncapped sediment mesocosms but
366 not active cap mesocosms. In the latter, porewater Cu and Pb remained low (Figure 3). These
367 results showed that apatite caps kept pore water metal concentrations at relatively low levels,
368 even in the presence of bioturbation that markedly increased concentrations of some metals in
369 the pore water of uncapped sediment.

370 *3.4. DGT surface water*

371 Surface water metal concentrations measured by DGT water probes (Figure 4) were
372 lower than total or dissolved surface water concentrations (Figure 2) because DGT excludes
373 metals bound to particulates and metals strongly bound to large organic molecules and possibly
374 other ligands (Paller et al. 2019). DGT concentrations of Cd and Ni were significantly higher in
375 uncapped sediment without bioturbation than in uncapped sediment with bioturbation and all
376 apatite treatments resulting in statistically significant interactions between treatment and
377 bioturbation (Figure 4). The Cu results were generally similar but did not produce a statistically
378 significant interaction because the Cu data were more variable (Figure 4). These results show
379 that apatite caps reduced DGT surface water concentrations of Cd, Ni, and Cu, and bioturbation
380 reduced DGT surface water concentrations in mesocosms with uncapped sediment. Unlike the
381 preceding metals, DGT Pb concentrations were similar among treatments and unaffected by
382 bioturbation (Figure 4).

383 *3.5. DGT Sediment*

384 Three-way ANOVA of the DGT sediment Cu data indicated significantly lower
385 concentrations in the apatite cap mesocosms than the sediment mesocosms (LSMs of 0.36 and

386 1.17 μg/kg^{-1}, respectively) across sediment layers regardless of bioturbation (Table 3, Figure 5).
387 The Cd sediment data were more complex with significant treatment, bioturbation, and layer
388 effects as well as significant two-way interactions between bioturbation and sediment layers and
389 bioturbation and treatment (Table 4, Figure 5). The multiple comparison results for Cd indicated
390 lower concentrations in mesocosms with apatite caps than mesocosms with uncapped sediment,
391 lower concentrations in mesocosms with bioturbation than in mesocosms without bioturbation,
392 and significant differences among sediment layers in the absence of bioturbation but not in the
393 presence of bioturbation.
394 The three-way ANOVA of the Ni data indicated that treatment effects, bioturbation
395 effects, and all two-way and three-way interactions were significant (Table 5, Figure 5).
396 Multiple comparisons showed that Ni concentrations in all sediment layers of the apatite cap
397 mesocosms were significantly lower than in the uncapped sediment mesocosms when
398 bioturbation was absent. In contrast, Ni concentrations in all sediment layers of the apatite cap
399 mesocosms and sediment mesocosms were similar in the presence of bioturbation. Bioturbation
400 reduced Ni concentrations significantly in all sediment layers within the sediment mesocosms
401 but reduced Ni concentrations only in the contaminated layer (CL) within the apatite mesocosms.
402 The DGT sediment data for Pb indicated significant differences related to treatment and
403 bioturbation and a significant two-way interaction between these two main effects (Table 6,
404 Figure 5). Generally paralleling the results observed with Ni, multiple comparison tests showed
405 that bioturbation significantly reduced DGT Pb levels in the sediment mesocosms but not apatite
406 mesocosms, and apatite caps significantly reduced DGT Pb levels when bioturbation was absent
407 but not when it was present. The Zn data were not analyzed because blank Zn levels were high,
408 often exceeding Zn concentrations in the mesocosms.

3.6. Bioaccumulation and toxicity

Concentrations of the physiologically nonessential elements Cd, Ni, Pb, and to a lesser extent, As in *Lumbriculus* from the mesocosms were higher than background concentrations regardless of treatment or bioturbation (Figure 6). However, two-way ANOVA indicated that concentrations of Cd, Ni, and Pb were significantly lower in *Lumbriculus* from mesocosms with bioturbation than mesocosms without. The opposite pattern was observed for As and Cu. In addition, significant treatment effects indicated that concentrations of Cd, Ni, and Pb were lower in *Lumbriculus* from mesocosms with apatite active caps than mesocosms with uncapped sediment. In contrast, As, Cu, and Zn concentrations were not significantly different between treatments indicating that apatite caps did not reduce the bioaccumulation of these metals. The ability of apatite active caps to significantly reduce bioaccumulation of Ni and Cd compared with uncapped sediment was also indicated by one-way ANOVAs of metals in *Corbicula* tissues, especially Ni, which was near background levels (Figure 7). However, apatite caps did not reduce concentrations of As, Cu, Pb, and Zn in *Corbicula* tissues.

One-way ANOVA indicated that *Lumbriculus* mortality after 10 days of exposure in mesocosms with apatite caps and bioturbation (5.3 %) was significantly lower than mortality in other mesocosms (30.9-36.3%) demonstrating that active caps with bioturbation provided more protection from the influx of metal contaminated sediment than uncapped sediment or capped sediment without bioturbation. Additional toxicity tests conducted with Asian clams after 28 days of exposure in the bioturbation mesocosms showed that average mortality was significantly (Student's t-test, $p = 0.011$) lower in mesocosms with apatite caps (27.5%) than mesocosms with uncapped sediment (45.0 %).

3.7. PCA summary for each metal

The PCAs for Cd and Ni were similar: the first principal component (PC1) accounted for 77-79% of the variance in the five measured variables, and the second (PC2) accounted for 16%-17% (Figure 8). DGT sediment, DGT water, *Lumbriculus*, and surface water concentrations influenced PC1, and pore water concentrations influenced PC2 as indicated by strong correlations (-0.88 – -0.97) between these variables and the PC scores (Figure 8). Mesocosms with apatite caps and mesocosms with uncapped sediment and bioturbation had high scores on PC1 because of their low DGT sediment, DGT surface water, *Lumbriculus*, and surface water concentrations. In contrast, mesocosm with uncapped sediment and no bioturbation had low scores because of high values for these variables. Most mesocosms clustered together on PC2 except for a sediment mesocosm with bioturbation and a sediment mesocosm without bioturbation. In summary, apatite caps kept Ni and Cd concentrations at relatively low levels regardless of bioturbation, and uncapped sediment kept Ni and Cd concentrations at low levels only in the presence of bioturbation.

The PCA for Zn was based on only three variables (Figure 8). PC1 accounted for 49% of the variance and PC2 accounted for 31%. PC1 was weighted by pore and surface water concentrations and PC2 by *Lumbriculus* concentrations. Apatite cap mesocosms with and without bioturbation separated from sediment mesocosms on PC1 because of lower pore and surface water Pb concentrations. PC2 separated one sediment mesocosm without bioturbation from the other mesocosms because of relatively high Zn levels in *Lumbriculus*. Although less pronounced, this pattern resembled Ni and Cd, with apatite caps maintaining pore and surface water metals at relatively low levels regardless of bioturbation.

PC1 and PC2 accounted for 35% and 27%, respectively of the variance in the Pb data, with pore water, *Lumbriculus*, and DGT water concentrations weighting PC1 and DGT sediment

455 and surface water concentrations weighting PC2 (Figure 8). Apatite cap mesocosms with
456 bioturbation clustered near the upper right corner of the PCA plot reflecting lower concentrations
457 of Pb as indicated by all variables except surface water measurements. Pb levels were somewhat
458 higher in apatite cap mesocosms without bioturbation and higher still in sediment mesocosms.
459 PC1 of the Cu PCA accounted for 43% of the variance in the five measured variables,
460 and PC2 accounted for 22% (Figure 8). Pore water, surface water, and DGT sediment
461 concentrations influenced PC1, and DGT water and *Lumbriculus* concentrations influenced PC2.
462 Mesocosms with uncapped sediment and bioturbation had low PC1 scores because of high pore
463 water, surface water, and DGT sediment concentrations. These variables were lower in apatite
464 cap mesocosms and sediment mesocosms without bioturbation resulting in higher scores on PC1.
465 PC2 separated one uncapped sediment mesocosm with relatively high DGT water and low
466 *Lumbriculus* concentrations from the other mesocosms. As observed with Cd and Ni, apatite
467 caps kept Cu concentrations at relatively low levels regardless of bioturbation, but unlike Ni and
468 Cd, uncapped sediment kept Cu concentrations at low levels only in the absence of bioturbation.
469 PC1 of the PCA for As (based on three variables) accounted for 55% of the variance and
470 PC2 for 25% (Figure 8). The relationships among mesocosms differed from the other metals:
471 uncapped sediment mesocosm without bioturbation separated from the other mesocosms on PC1
472 because of lower pore water and surface water concentrations of As, and there was little
473 differentiation among the other mesocosms.

474 **4. Discussion**

475 Bioturbating organisms affect sediments by burrowing, feeding, and respiratory activities
476 that contribute to the bulk movement of buried contaminated sediments to the sediment/surface
477 water interface, create channels for water movement, and agitate sediments (Krantzberg, 1985;

478 Matisoff 1995). Bioturbation usually diminishes the effectiveness of sediment caps by reducing
479 isolation of underlying sediments and promoting contaminant movement into the water column
480 and benthic food chains (Cunningham et al. 1999). However, the vertical transport of dissolved
481 and particulate materials by bioturbation may be beneficial when sediment contaminated with
482 metals is deposited over chemically active caps or clean sediment because bioturbation can mix
483 underlying clean sediment or active cap materials with newly deposited contaminated sediment
484 thereby fostering sequestration and dilution of metals in the latter. We found that the effects of
485 bioturbation were metal specific in this situation, with sequestration enhanced for some metals
486 but unaffected or reduced for others. Bioturbation reduced the release and bioavailability of Ni,
487 Cd, and, to a lesser degree, Pb and Zn, in newly deposited contaminated sediment, particularly in
488 mesocosms with uncapped sediment. However, it increased Cu mobility in mesocosms with
489 uncapped sediment, but not mesocosms with apatite caps, and increased As mobility in all
490 mesocosms.
491 Most variables indicated that concentrations of Cd, Cu, Ni, Pb, and Zn were lower in
492 mesocosms with apatite caps regardless of bioturbation. Apatite sequesters these and other
493 metals in contaminated sediment by various mechanisms (Knox et al. 2003, and 2006). Pb is
494 immobilized through apatite dissolution followed by precipitation of pyromorphite minerals
495 under acidic conditions or precipitation of hydrocerussite [$Pb_3(CO_3)_2(OH)_2$ or $Pb(OH)_2$)] and
496 lead oxide fluoride (Pb_2OF_2) under alkaline conditions (Wright et al. 1995). Cd and Zn are
497 immobilized by the formation of otavite ($CdCO_3$), cadmium hydroxide [$Cd(OH)_2$], and zincite
498 (ZnO) under alkaline conditions. Ma et al., (1995) reported that interactions between apatite and
499 metals in solution is controlled by apatite dissolution resulting in the precipitation of various
500 phosphate phases. In contrast, apatite was not effective for the metalloid, As, as also shown in

501 other studies (Knox et al., 2008 and 2016). The ability of apatite caps to reduce potentially
502 bioavailable metal concentrations in recently deposited contaminated sediments was either
503 unaffected (Cu and Zn) or slightly enhanced (Cd, Ni, and Pb) by bioturbation, probably because
504 of the strong affinity of apatite for metals with or without the mixing of contaminated sediment
505 with apatite facilitated by bioturbation. In general, these results showed that apatite caps
506 effectively treated continued influxes of metals from uncontrolled sources regardless of
507 bioturbation by *Corbicula*.

508 Unlike apatite, the ability of uncapped sediment to reduce bioavailable metals in
509 overlying contaminated sediment was strongly influenced by bioturbation. Sequestration of Cd
510 and Ni in bioturbated, uncapped sediment was much greater than in unbioturbated, uncapped
511 sediment based on all measured variables except pore water concentrations (Figures 2-9). DGT
512 results showed that bioturbation contributed to the uniform vertical distribution of these metals in
513 uncapped sediment compared with steep vertical gradients observed in the absence of
514 bioturbation (Figure 5). These reductions in surficial sediment metal levels undoubtedly
515 contributed to lower surface water concentrations of Cd and Ni in our study. Mixing of the top
516 layer of contaminated sediment with underlying clean sediment by bioturbation could have
517 reduced metal contamination by burial and isolation, dilution with clean sediment, or
518 enhancement of chemical sequestration by iron and other minerals or organic matter in the
519 sediments. Burial of contaminated sediments deeper in the sediment profile where anoxia
520 prevails could have encouraged the formation of insoluble metal sulfides (Simpson et al. 2012).
521 The ability of clean sediments to control metal contamination has also been reported by Simpson
522 et al. (2002), who found that sediment caps were better than sand or zeolite caps at reducing Zn
523 fluxes from contaminated sediments. They attributed this to the high sorption capacity of cleans

524 sediments for most trace metals due to the presence of iron hydroxy, carbonate, and organic
525 materials.

526 Unlike Ni and Cd, bioturbation increased the release of Cu, Pb, and As from uncapped
527 sediments and As from capped and uncapped sediment as indicated by elevated pore and surface
528 water concentrations of these metals compared with unbioturbated sediments. Pb was strongly
529 bound to sediment particles as indicated by the prevalence of the particulate metal fraction in
530 surface water samples (Figures 1 and 2). An increase in suspended particles (as indicated by
531 increased turbidity, Table 2) due to sediment disturbance by bioturbation likely contributed to Pb
532 release in the uncapped sediment mesocosms. However, it is unlikely that this fully accounted
533 for the release of As and Cu, which were largely in the dissolved phase (Figures 1 and 2). DGT
534 sediment and pore water data showed that Cu concentrations in uncapped sediment with
535 bioturbation were elevated compared with uncapped sediment without bioturbation – opposite to
536 the pattern observed for Ni, Cd, and Pb – indicating the potential release of Cu from pore water
537 to surface water (Figures 3 and 5). It is possible that increased oxygenation of the sediment by
538 bioturbation converted strongly bound sulfidized Cu into relatively weakly bound carbonate and
539 exchangeable Cu fractions, as reported by Zoumis et al. (2001). Additionally, release of all three
540 metals may have been facilitated by relatively low pH in the pore water of the uncapped
541 sediments (Table 2).

542 Reductions in bioavailable pore water metal concentrations suggest reduced potential for
543 bioaccumulation and toxicity to benthic organisms as reported by Knox et al. (2016 and 2019).
544 This was supported by Pearson correlations between DGT sediment data (which measured
545 bioaccumulative dissolved and labile metal fractions) and *Lumbriculus* tissue data that were
546 strong for Cd and Ni ($r = 0.92$ and 0,84, respectively, $p<0.001$ for both). Although this

547 correlation was weaker for Pb (r = 0.34), *Lumbriculus* Pb levels were significantly lower in
548 apatite and bioturbation mesocosms corresponding with relatively low Pb levels in the surface
549 and pore waters of these mesocosms (Figures 2-4). There were no DGT sediment data for As,
550 but high As concentrations in *Lumbriculus* from mesocosms with bioturbation corresponded with
551 high As in surface waters and, to a lesser extent, sediment pore waters (Figures 2-4). In contrast,
552 Zn concentrations in *Lumbriculus* differed little among mesocosms despite treatment related
553 differences in sediment pore water, possibly reflecting internal regulation of this physiologically
554 essential metal (Figure 4). Similarly, correlations between DGT sediment concentrations and
555 *Lumbriculus* concentrations were weak (r = -0.03) for Cu, another physiologically essential
556 element.

557 Mortality of *Lumbriculus* and *Corbicula* was observed in all mesocosms, possibly
558 because of suboptimal habitat and crowding as well as metal toxicity. However, mortality rates
559 for both organisms were significantly lower in mesocosms with apatite caps and bioturbation
560 than in other mesocosms suggesting that the reduced ambient concentrations of most metals in
561 these mesocosms was protective (Figure 6). The protective effects of apatite caps were also
562 indicated by reduced uptake of Cd and Ni by *Corbicula* in the bioturbation mesocosms (Figure
563 7). Greater surface water and pore water hardness in the apatite mesocosms (Table 2) may have
564 contributed to these effects since water hardness is inversely correlated with the toxicity of many
565 metals (U.S. EPA 2016). It is likely that metal toxicity was also moderated by the higher pH in
566 the apatite mesocosms because most metals are less bioavailable at higher pHs (Table 2, Kabata-
567 Pendias, 2011). The pH was also affected by bioturbation, which averaged 0.6 higher in the
568 apatite mesocosms and 0.8 higher in the uncapped sediment mesocosms, and likely contributed
569 to reduced bioaccumulation of Pb, Cd, Ni, and Zn by *Lumbriculus* (Table 2, Figure 6). Other

570 researchers have also reported that bioturbation raises sediment pH, perhaps due to the removal
571 of acidic metabolites as a result of greater sediment flushing (Krantzberg 1985).
572 Bioturbation affects physical processes that influence metal transport (diffusion,
573 advection, resuspension) and can change redox potentials, hence biogeochemical reactions, that
574 affect the dissolution, adsorption, and precipitation of metals. The effects of these processes
575 depend upon a metal's affinity for sediment organic matter, strength of adsorption to sediment
576 particles, and behavior under changing redox conditions including tendency to chemically react,
577 adsorb, or co-precipitate with sediment sulfide, iron, and manganese compounds. Various metals
578 respond differently to bioturbation. Xie et al. (2019) reported that bioturbation by bivalves
579 enhanced the release of Mn, Co, Ni, and Zn, but not Cu. In contrast, He et al. (2017) reported
580 that bioturbation and bioirrigation enhanced the transfer of Cu and other metals from pore water
581 to the overlying water. Remaili et al. (2016) reported that bioturbation by the bivalve *Tellina*
582 *deltoidalis* decreased dissolved Cu, increased dissolved Mn, and resulted in greater tissue
583 concentrations for Cr and Zn in some sediments. Amato et al. (2016) reported that bioturbation
584 increased the release of DGT-labile Cd, Ni, Pb, and Zn but not Cu. However, these researchers
585 dealt with preexisting contamination, while our work involved underlying clean sediment that
586 could be mixed with overlying influxes of contaminated sediment by bioturbation resulting in
587 contaminant dilution and enhanced treatment. We found that the effects of bioturbation differed
588 among elements, with the predominant effect with apatite caps being reduction in the
589 bioavailable pool for all elements except As and the predominant effects with clean sediment
590 being reduction in the bioavailable pool for Ni, Cd, and to a lesser extent, Pb, increases in the
591 bioavailable pool for As and Cu, and little effect for Zn. These results indicate the effectiveness

of apatite active caps for the control of sediment recontamination by metals but also point to the complexity of sediment recontamination and the need for further study of this problem.

5. Conclusions

There have been numerous studies of the effects of bioturbation on metal release from un-remediated contaminated sediments and, to a lesser extent, from contaminated sediments remediated by passive capping. However, the effects of bioturbation on the release of metals from remediated sediments that have been re-contaminated have been little studied. We found that the ability of apatite active caps to reduce the bioavailability of most metals in incoming contaminated sediment was unaffected or even enhanced by bioturbation, probably because bioturbation mixed apatite with the incoming sediment, thereby enhancing chemical sequestration of the metals. We also found that bioturbation reduced the bioavailable pool of some metals in contaminated sediment that was deposited over uncapped clean sediment, although it contributed to the release of others. It is likely that the reductions observed for some metals were the result of burial and dilution of the contaminated sediment combined with chemical processes such as sequestration by iron compounds in the clean sediment. These more complex results simulated what may occur when newly dredged, uncapped sediment is challenged by a combination of recontamination and bioturbation. The effects, in this case, will likely be metal specific and influenced by the chemical composition of the sediment. Additional factors unexplored in this study that might be important in determining the effects of bioturbation combined with recontamination include the type of bioturbating organism and depth of bioturbation and cap thickness. However, as observed in previous studies involving recontamination without bioturbation (Knox et al. 2016, 2019), apatite active caps are a promising way to reduce the impacts of ongoing metal contamination from uncontrolled sources.

Conflict of interest

The authors declare no conflict of interest.

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737

Table 1. Metal concentrations (mg kg^{-1}) in sediment layers within the experimental mesocosms*.

Sediment description	Mn	Fe	Ni	Cu	Zn	As	Cd	Pb
Clean sediment - bottom layer (average; n=13)	169.8	5845.8	7.8	3.7	7.3	1.0	0.3	5.4
Contaminated sediment - top layer (average; n=7)	117.5	8564.3	9.6	19.4	23.1	11.0	5.4	14.8
Moderately polluted (U.S. EPA, 1977)	300-500		20-50	25-50	90-200	3-8		40-60
Heavily polluted (U.S. EPA, 1977)	>500		>50	>50	>200	>8	>6	>60
Moderate Contamination (New York DEC, 1994)				16-110			0.6-10	
Substantially higher element concentrations in the top layer				YES		YES	YES	
Slightly higher element concentrations in the top layer		YES			YES			YES

* Sediments were digested with H_2O_2 and $HN0_3$, and metals in the extracts were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) using a NexION 300X mass spectrometer (Perkin Elmer, Inc.) according to standard QA/QC protocols outlined in EPA Method 6020A (USEPA, 2014).

Table 2. Surface water, pore water, and sediment properties in four types of experimental mesocosms: apatite cap with bioturbation (A-B), apatite cap without bioturbation (A-NB), uncapped sediment with bioturbation (SED-B), and uncapped sediment without bioturbation (SED-B). Pore water and sediment properties are average values for samples collected at the end of the experiment (696 hours), and surface water properties are average values for eight samples collected from 264-696 hours after initiation of the experiment.

Variable	A-B	A-NB	SED-B	SED-NB
	Surface water			
Dissolved oxygen (mg L^{-1})	7.9	7.8	7.9	7.7
Electrical conductivity (µS cm^{-1})	554.4	410.4	261.9	173.1
Hardness (mg L^{-1} $CaCO_3$)	317.1	248.3	88.2	52.1
pH	8.1	7.8	7.7	7.3
Turbidity (NTU)	7.4	3.3	5.9	3.1
Temperature (ºC)	20.3	20.3	20.2	20.3
	Pore water			
Electrical conductivity (µS cm^{-1})	916.4	1056.8	230.1	162.0
Oxidation reduction potential (mV)	-29.0	-26.3	-32.0	-12.7
pH	7.8	7.7	6.2	5.3
Temperature (ºC)	20.6	20.6	20.6	20.6
	Sediment			
pH	8.1	7.5	6.2	5.4

Table 3. Three-way analysis of variance (ANOVA) of DGT-Cu sediment data. Also shown are the results of multiple comparisons tests among means conducted with the Holm-Sidak all pairwise comparison procedure (overall significance level = 0.05). Multiple comparison results are shown for significant main effects.

Source of Variation	Degrees of freedom	Sum of squares	Mean squares	F ratio	P
Treatment (apatite cap vs. uncapped)	1	11.11	11.11	7.55	0.008
Bioturbation (presence vs. absence)	1	0.93	0.93	0.64	0.429
Layers (CL, A, B)	2	1.04	0.52	0.35	0.704
Treatment x bioturbation	1	0.14	0.14	0.09	0.762
Treatment x layers	2	2.04	1.02	0.69	0.504
Bioturbation x layers	2	0.09	0.04	0.03	0.971
Treatment x bioturbation x layers	2	0.57	0.29	0.19	0.824
Residual	56	82.45	1.47		
Total	67	98.35	1.47		

Multiple comparison results for treatment. Units for least square means (LSMs) are $\mu g\ kg^{-1}$.

Comparison	LSM #1	LSM #2	Difference	t	P
Effect of treatment	1.17 SED*	0.36 A	0.82	2.75	0.008

*SED = uncapped sediment, A = apatite cap

Table 4. Three-way analysis of variance (ANOVA) of DGT-Cd sediment data. Also shown are the results of multiple comparisons tests among means conducted with the Holm-Sidak all pairwise comparison procedure (overall significance level = 0.05). Multiple comparison results are shown for significant interactions.

Source of Variation	Degrees of freedom	Sum of squares	Mean squares	F ratio	P
Treatment (apatite cap vs. uncapped)	1	234.95	234.95	114.53	<0.001
Bioturbation (presence vs. absence)	1	381.43	381.43	185.94	<0.001
Layers (CL, A, B)	2	142.84	71.42	34.82	<0.001
Treatment x bioturbation	1	60.34	60.34	29.41	<0.001
Treatment x layers	2	5.34	2.67	1.30	0.280
Bioturbation x layers	2	88.99	44.49	21.69	<0.001
Treatment x bioturbation x layers	2	3.38	1.69	0.82	0.444
Residual	60	123.08	2.05		
Total	71	1040.35	14.65		

Multiple comparison results for treatment x bioturbation interaction. Units for least square means (LSMs) are $\mu g\ kg^{-1}$.

Comparisons	LSM #1	LSM #2	Difference	t	P
Effect of bioturbation					
Within uncapped sediment mesocosms	9.12 NB*	2.69 B	6.43	13.48	<0.001
Within apatite cap mesocosms	3.68 NB	0.90 B	2.77	5.81	<0.001
Effect of treatment					
Without bioturbation	9.12 SED	3.68 A	5.44	11.40	<0.001
With bioturbation	2.69 SED	0.90 A	1.78	3.73	<0.001

Multiple comparison results for bioturbation x layers interaction. Units for least square means (LSMs) are $\mu g\ kg^{-1}$.

Differences among layers without bioturbation	LSM #1	LSM #2	Difference	t	P
CL vs BL	9.84 Cl	3.88 BL	5.96	10.19	<0.001
CL vs AL	9.84 CL	5.47 AL	4.37	7.47	<0.001
Al vs BL	5.47 AL	3.88 BL	1.59	2.72	0.009
Differences among layers with bioturbation					
CL vs BL	2.22 CL	1.61 BL	0.67	1.14	0.592
CL vs AL	2.22 CL	1.55 AL	0.61	1.04	0.513
Al vs BL	1.61 AL	1.55 BL	0.06	0.10	0.920
Effect of bioturbation on layers					
On CL	9.84 NB	2.22 B	7.62	13.03	<0.001
On AL	5.47 NB	1.61 B	3.86	6.60	<0.001
On BL	3.88 NB	1.55 B	2.33	3.98	<0.001

*SED = uncapped sediment; A = apatite cap, B = bioturbation, NB = no bioturbation, CL = contaminated layer, BL = B layer, AL = A layer.

Table 5. Three-way analysis of variance (ANOVA) of DGT-Ni sediment data. Also shown are the results of multiple comparisons tests among means conducted with the Holm-Sidak all pairwise comparison procedure (overall significance level = 0.05). Multiple comparison results are shown for the 3-way interaction.

Source of Variation	Degrees of freedom	Sum of squares	Mean squares	F ratio	P
Treatment (apatite cap vs. uncapped)	1	36.38	36.38	189.45	<0.001
Bioturbation (presence vs. absence)	1	50.07	50.07	260.77	<0.001
Layers (CL, A, B)	2	10.80	5.40	28.11	<0.001
Treatment x bioturbation	1	25.18	25.18	131.13	<0.001
Treatment x layers	2	3.41	1.71	8.88	<0.001
Bioturbation x layers	2	8.57	4.29	22.32	<0.001
Treatment x bioturbation x layers	2	2.14	1.07	5.58	0.006
Residual	60	11.52	0.19		
Total	71	148.06	2.09		

Multiple comparison results for treatment x bioturbation x layer interaction. Units for least square means (LSMs) are $\mu g\ kg^{-1}$.

Comparison	LSM #1	LSM #2	Difference	t	P
Effect of treatment without bioturbation					
Within contaminated layer	5.01 SED*	1.39 A	3.62	14.31	<0.001
Within A layer	3.30 SED	0.85 A	2.46	9.70	<0.001
Within B layer	2.31 SED	0.58 A	1.74	6.87	<0.001
Effect of treatment with bioturbation					
Within contaminated layer	0.87 SED	0.47 A	0.40	1.59	0.117
Within A layer	0.50 SED	0.39 A	0.11	0.42	0.678
Within B layer	0.71 SED	0.50 A	0.27	1.08	0.412
Effect of bioturbation within uncapped sediment mesocosms					
Within contaminated layer	5.01 NB	0.87 B	4.14	16.37	<0.001
Within A layer	3.30 NB	0.50 B	2.80	11.08	<0.001
Within B layer	2.31 NB	0.71 B	1.61	6.35	<0.001
Effects of bioturbation within apatite cap mesocosms					
Within contaminated layer	1.39 NB	0.47 B	0.92	3.65	<0.001
Within A layer	0.85 NB	0.39 B	0.46	1.80	0.077
Within B layer	0.58 NB	0.50 B	0.08	0.31	0.759

*SED = uncapped sediment, A = apatite cap, B = bioturbation, NB = no bioturbation.

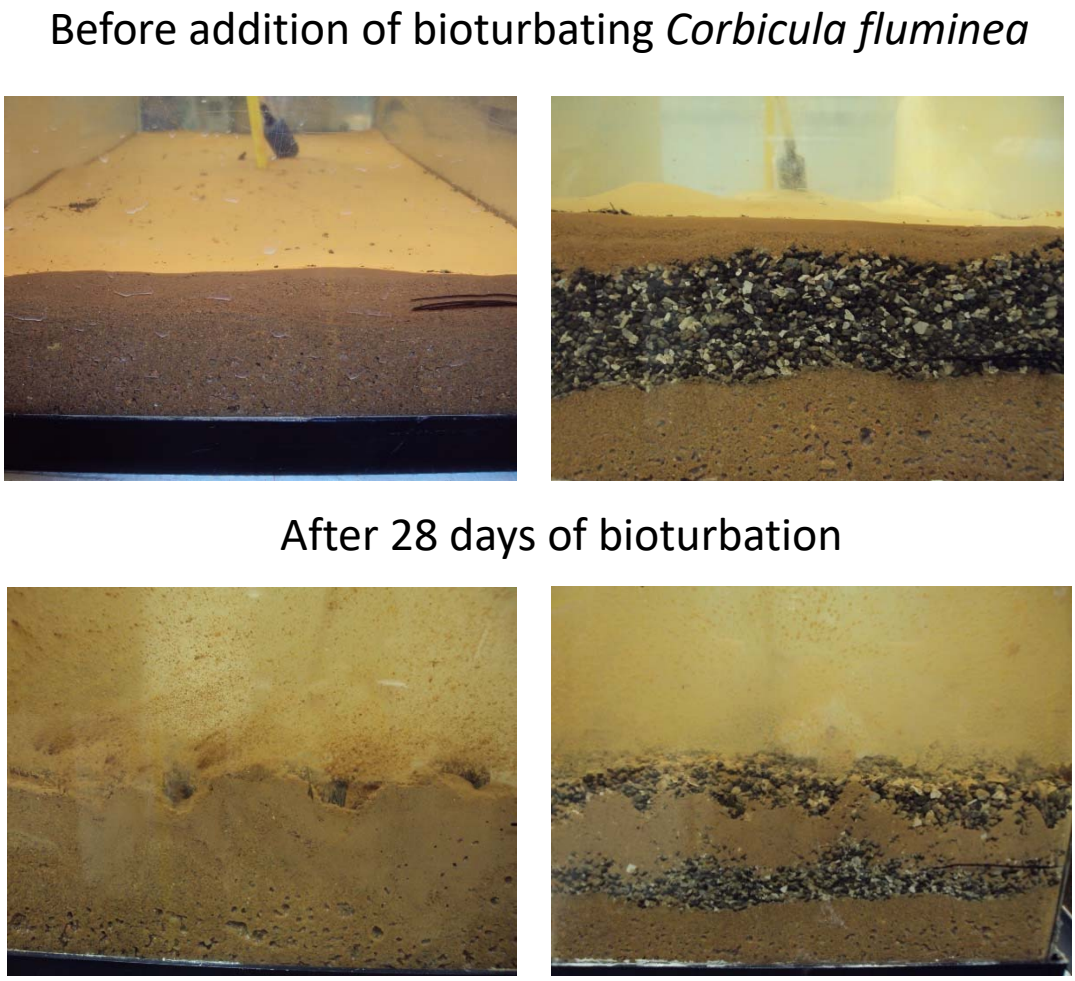
Table 6. Three-way analysis of variance (ANOVA) of DGT-Pb sediment data. Also shown are the results of multiple comparisons tests among means conducted with the Holm-Sidak all pairwise comparison procedure (overall significance level = 0.05). Multiple comparison results are shown for significant interactions.

Source of Variation	Degrees of freedom	Sum of squares	Mean squares	F ratio	P
Treatment (apatite cap vs. uncapped)	1	0.05	0.05	9.61	0.003
Bioturbation (presence vs. absence)	1	0.07	0.07	13.49	<0.001
Layers (CL, A, B)	2	0.03	0.02	2.81	0.069
Treatment x bioturbation	1	0.03	0.03	6.23	0.015
Treatment x layers	2	0.01	<0.01	0.54	0.587
Bioturbation x layers	2	0.01	<0.01	0.53	0.594
Treatment x bioturbation x layers	2	<0.01	<0.01	0.39	0.679
Residual	58	0.31	0.01		
Total	69	0.50	0.01		

Multiple comparison results for treatment x bioturbation interaction. Units for least square means (LSMs) are $\mu g\ kg^{-1}$.

Comparison	LSM #1	LSM #2	Difference	t	P
Effects of bioturbation					
Within uncapped sediment mesocosms	0.16 NB*	0.05 B	0.11	4.29	<0.001
Within apatite cap mesocosms	0.06 NB	0.04 B	0.02	0.85	0.401
Effects of treatment					
With no bioturbation	0.16 SED	0.06 A	0.10	3.96	<0.001
With bioturbation	0.05 SED	0.04 A	0.01	0.43	0.671

*SED =



Picture 1. Bioturbation in the experimental mesocosms: uncapped sediment (left column) and apatite cap (right column).

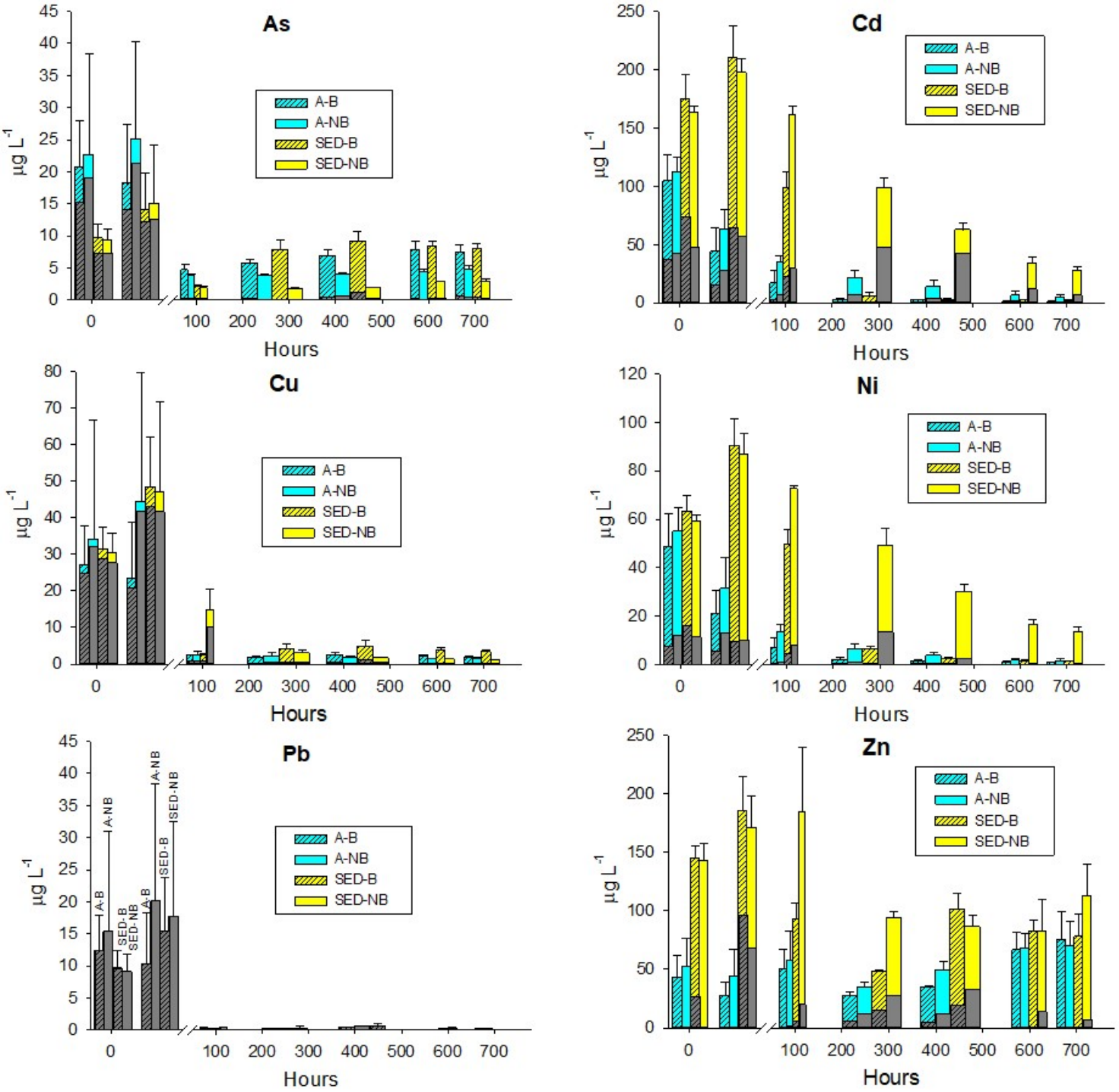


Figure 1. Surface water concentrations of metals in four types of experimental mesocosms (n=3 for each) with influxes of metal-contaminated sediment: apatite cap with bioturbation (A-B), apatite cap without bioturbation (A-NB), uncapped sediment with bioturbation (SED-B), and uncapped sediment without bioturbation (SED-NB). Gray shading indicates particulate metals, other colors dissolved metals.

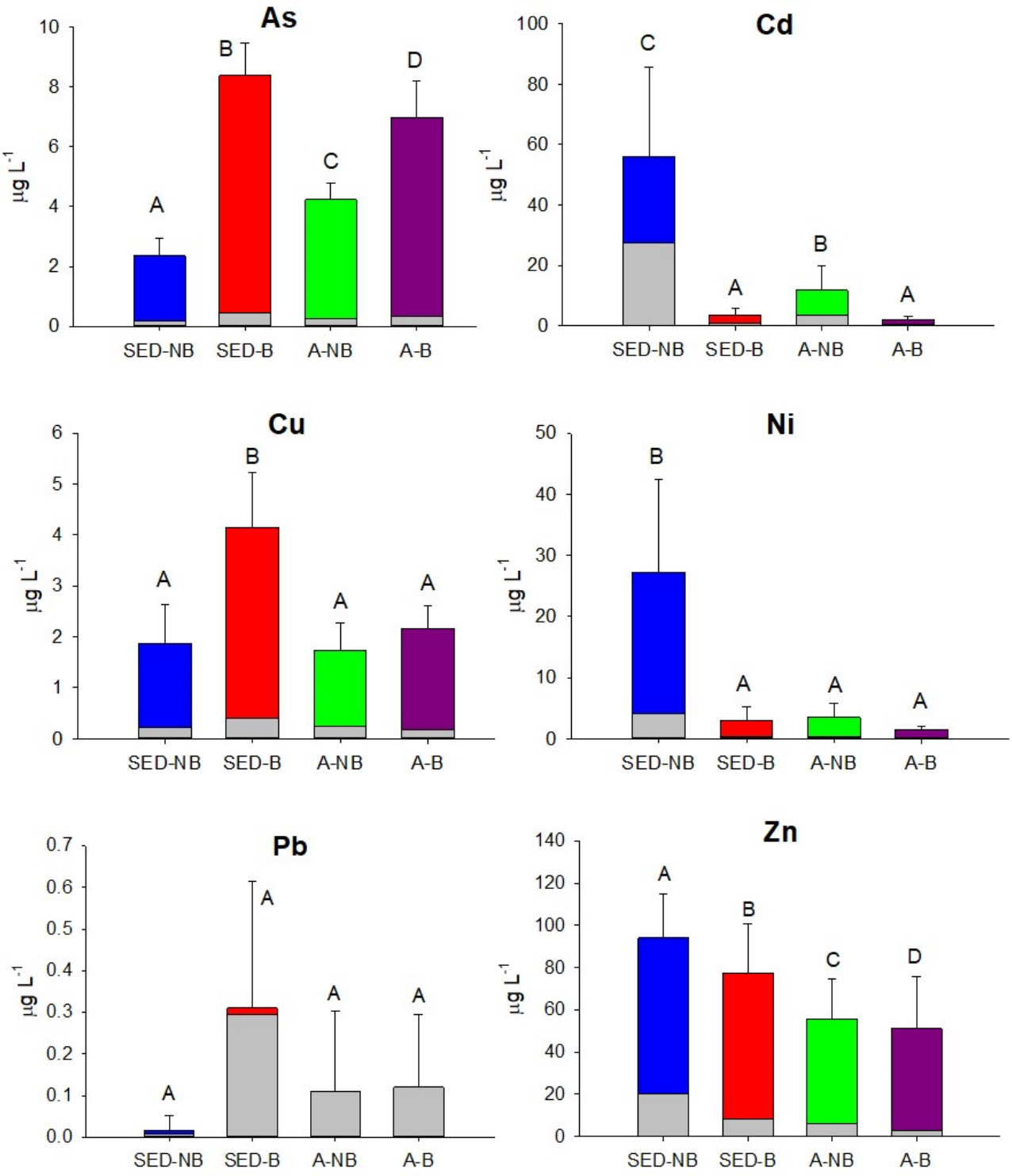


Figure 2. Mean surface water metal concentrations (from 264-296 hrs) for mesocosms with two main effects: treatment (apatite caps [A] vs uncapped sediment [SED]) and bioturbation (presence [B] or absence [NB]). Means represented by different letters are significantly ($p<0.05$). Gray shading indicates particulate fraction. Error bars are standard deviations.

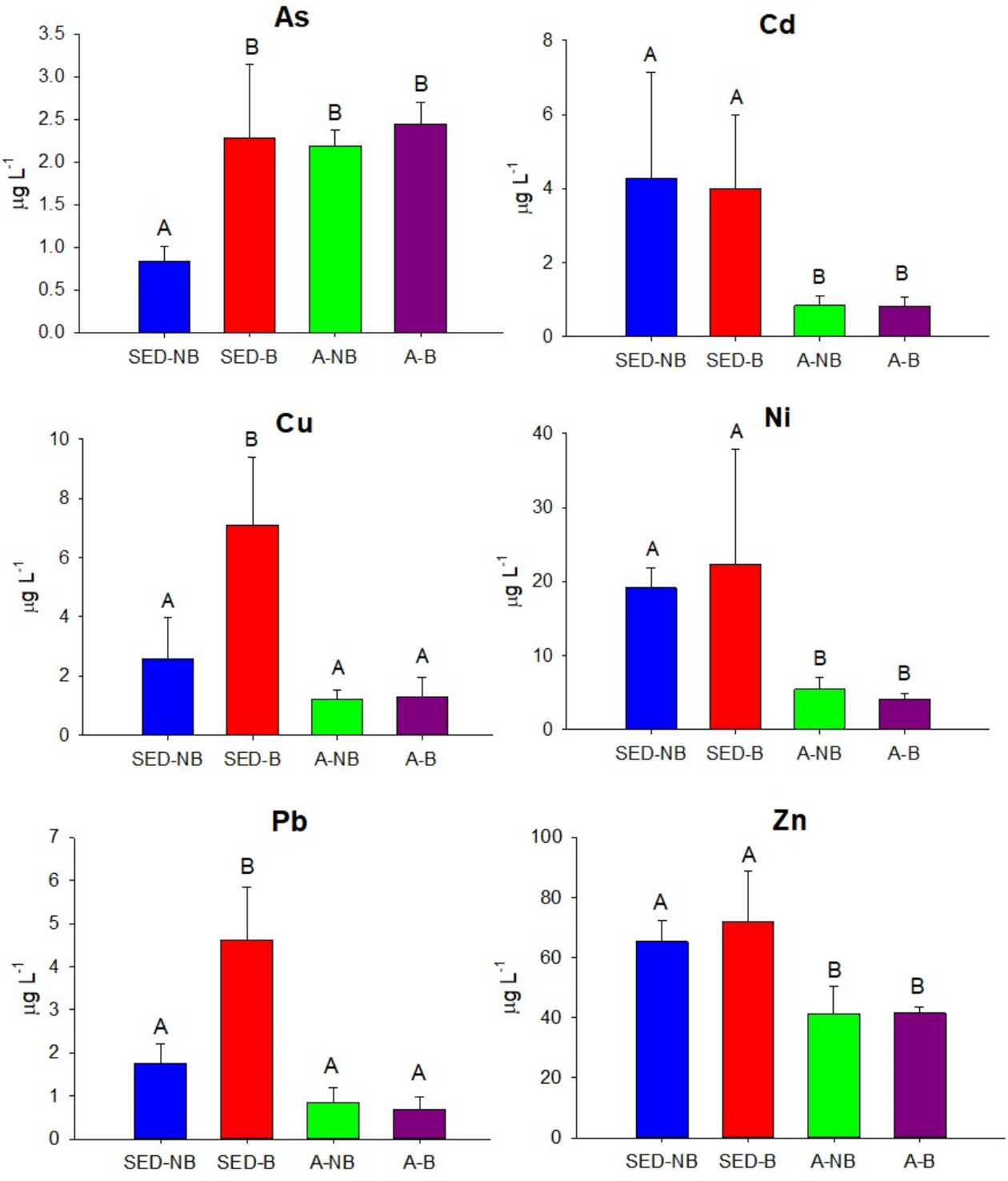


Figure 3. Mean pore water metal concentrations for mesocosms with two main effects: treatment (apatite caps [A] vs uncapped sediment [SED]) and bioturbation (presence [B] or absence [NB]). Means represented by different letters are significantly ($p<0.05$) different. Error bars are standard deviations.

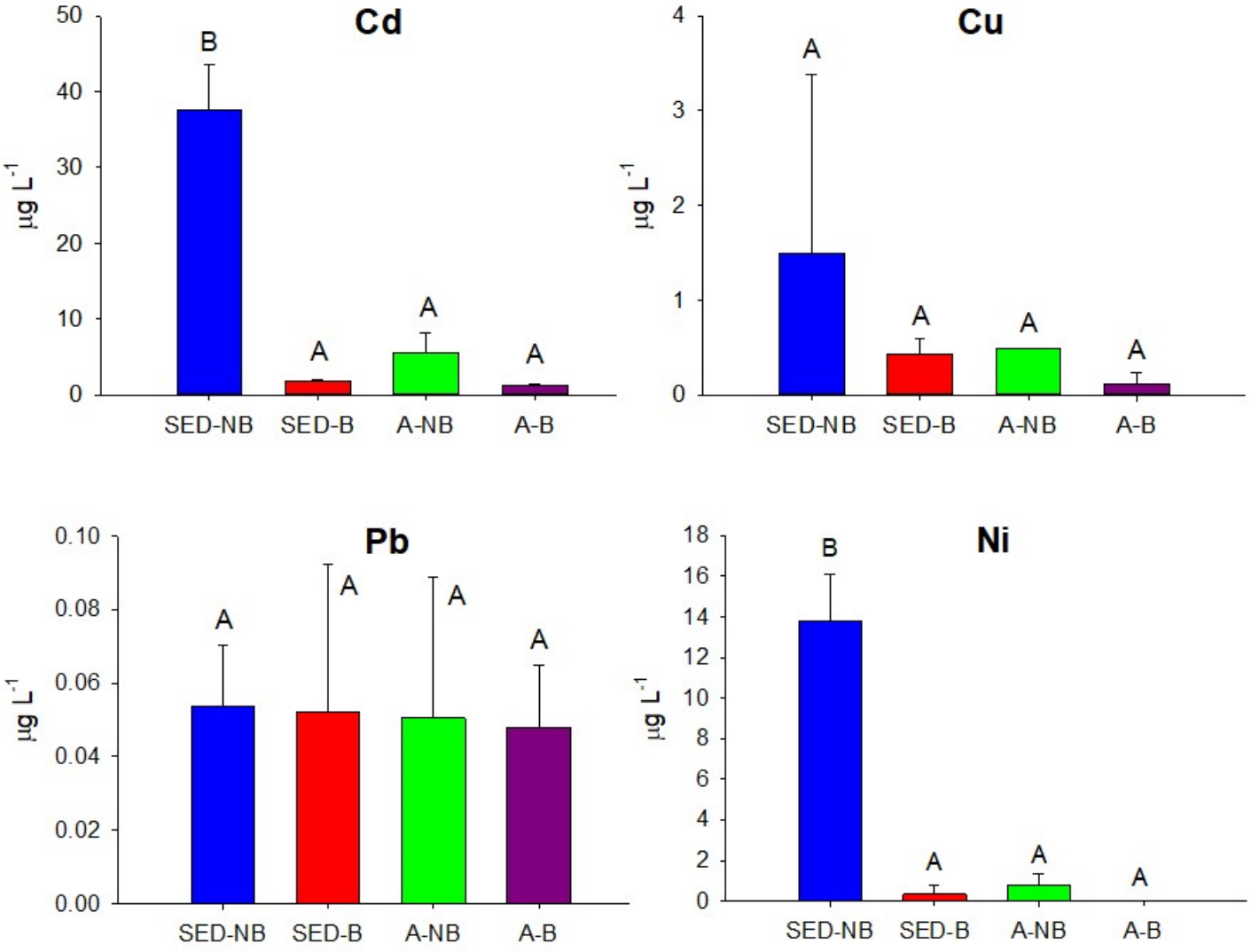


Figure 4. Mean DGT surface water metal concentrations for mesocosms with two main effects: treatment (apatite caps [A] vs uncapped sediment [SED]) and bioturbation (presence [B] or absence [NB]). Means represented by different letters are significantly ($p<0.05$) different. Error bars are standard deviations.

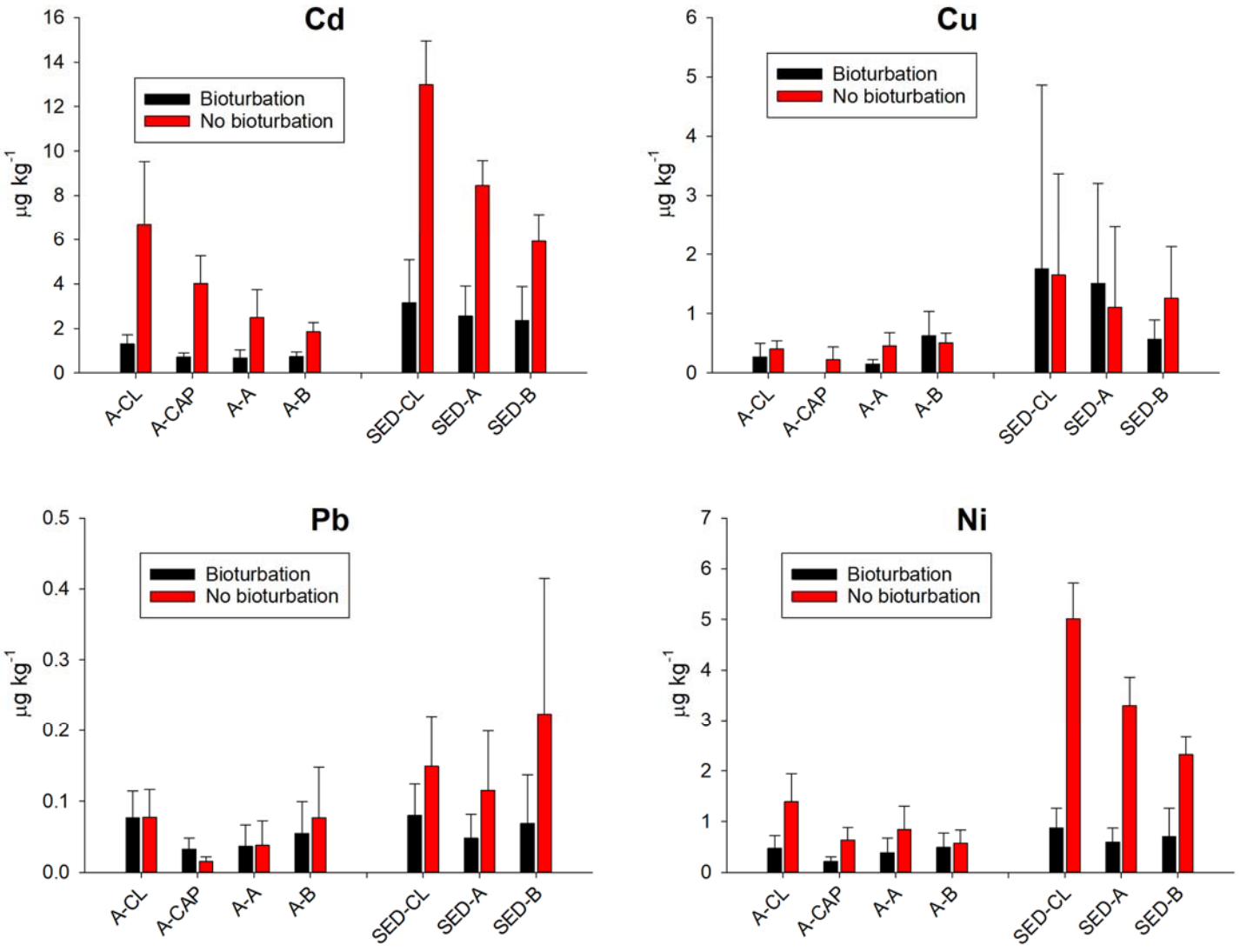


Figure 5. Metal concentrations measured by DGT in the sediment of experimental mesocosms with untreated sediment (SED) and with sediment treated with apatite caps (A). Three sediment layers are represented: surficial contaminated layer (CL), cap layer (for mesocosms with caps, CAP), sediment later beneath the cap or surficial contaminated later (A), and deep sediment layer (B).

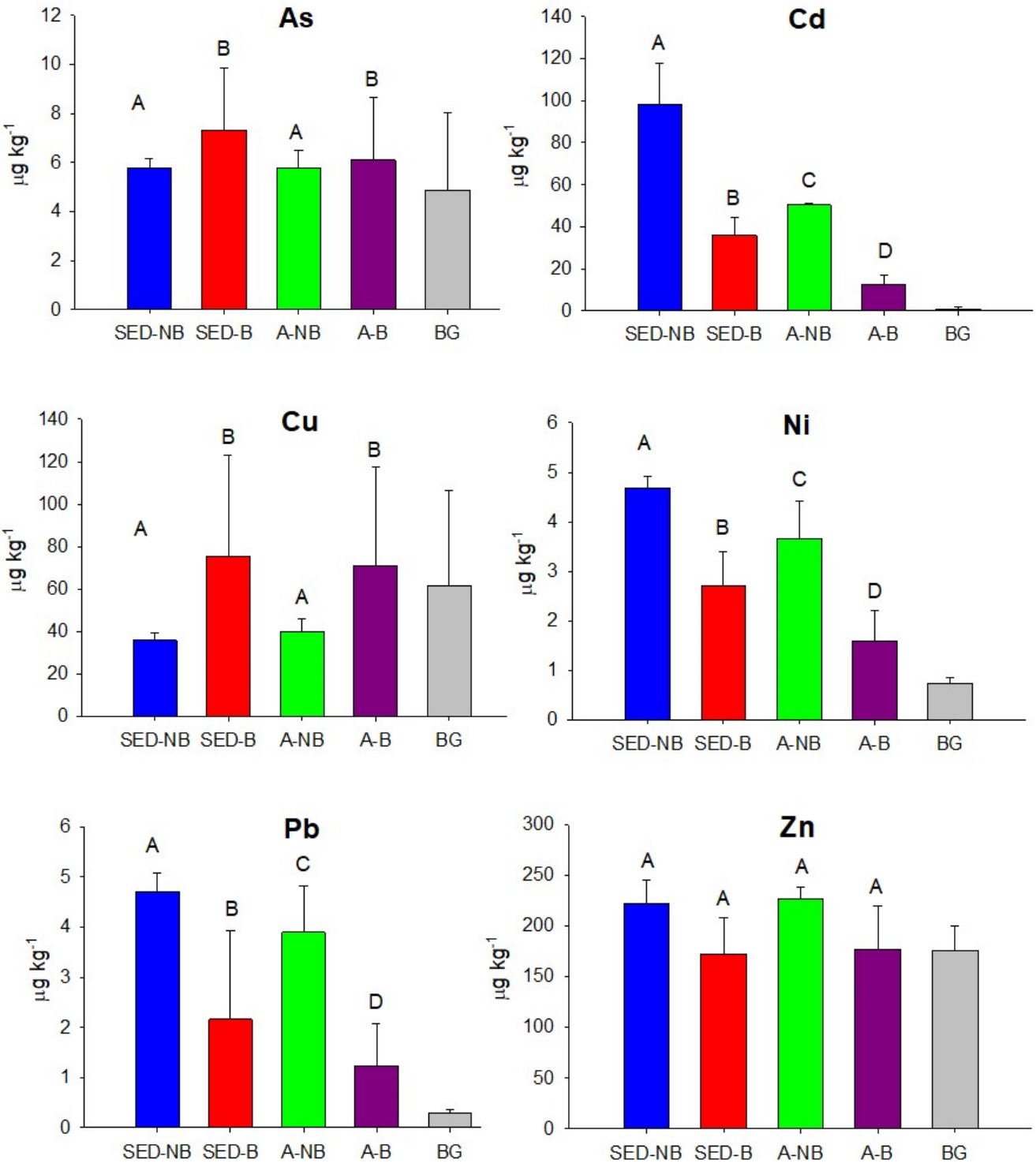


Figure 6. Mean element concentrations (whole body) in *Lumbriculus variegatus* from mesocosms with two main effects: treatment (apatite caps [A] vs uncapped sediment [SED]) and bioturbation (presence [B] or absence [NB]). Means represented by different letters are significantly ($p<0.05$) different. Metal concentrations in worms not exposed in the experimental mesocosms (BG) are shown for comparison but are not included in the statistical tests. Error bars are standard deviations.

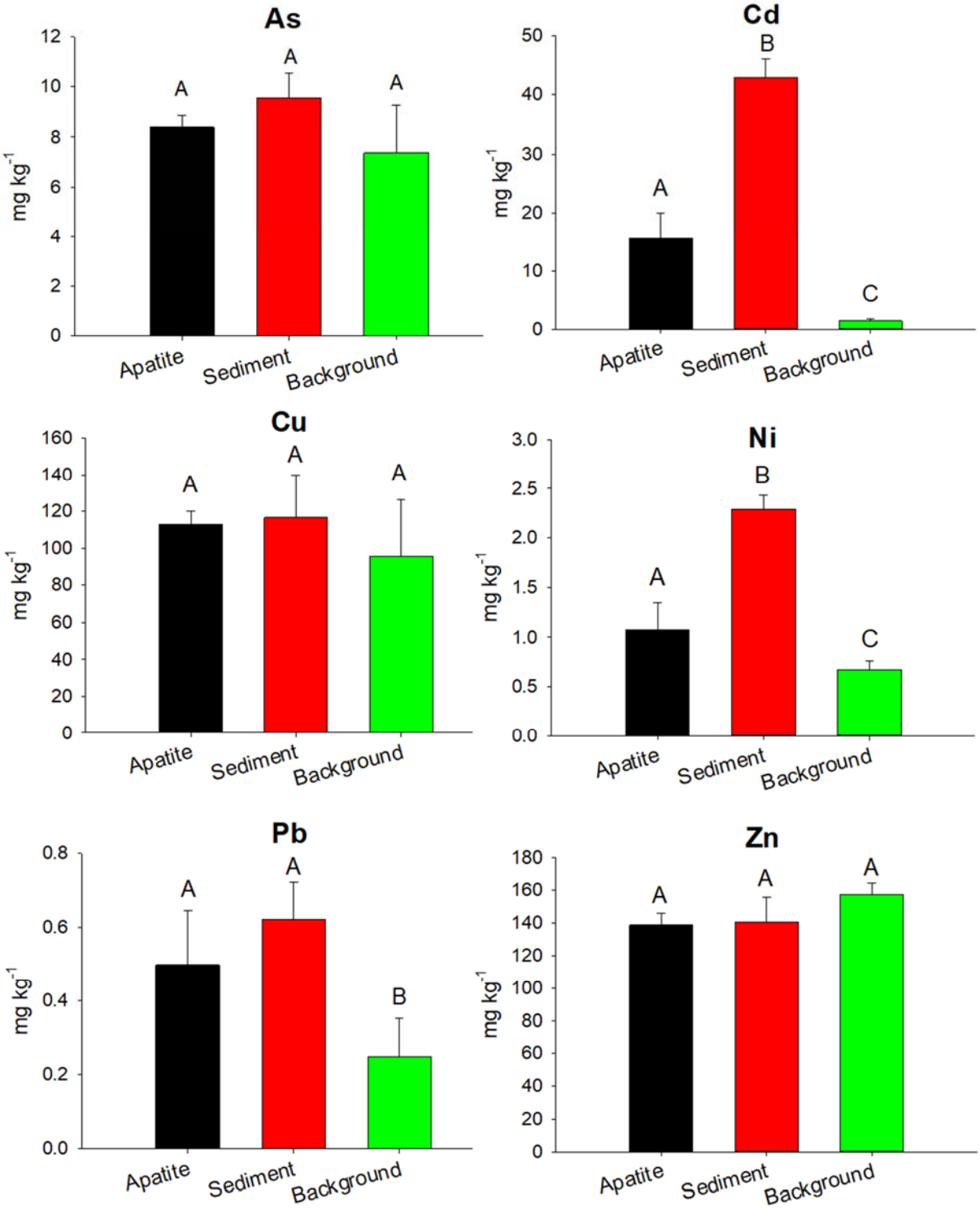


Figure 7. Mean element concentrations (whole body, 10-day exposure) in *Corbicula fluminea* from mesocosms with apatite caps and mesocosms with uncapped sediment. Background concentrations in *Corbicula* are included for comparison. Means represented by different letters are significantly ($p<0.05$) different.

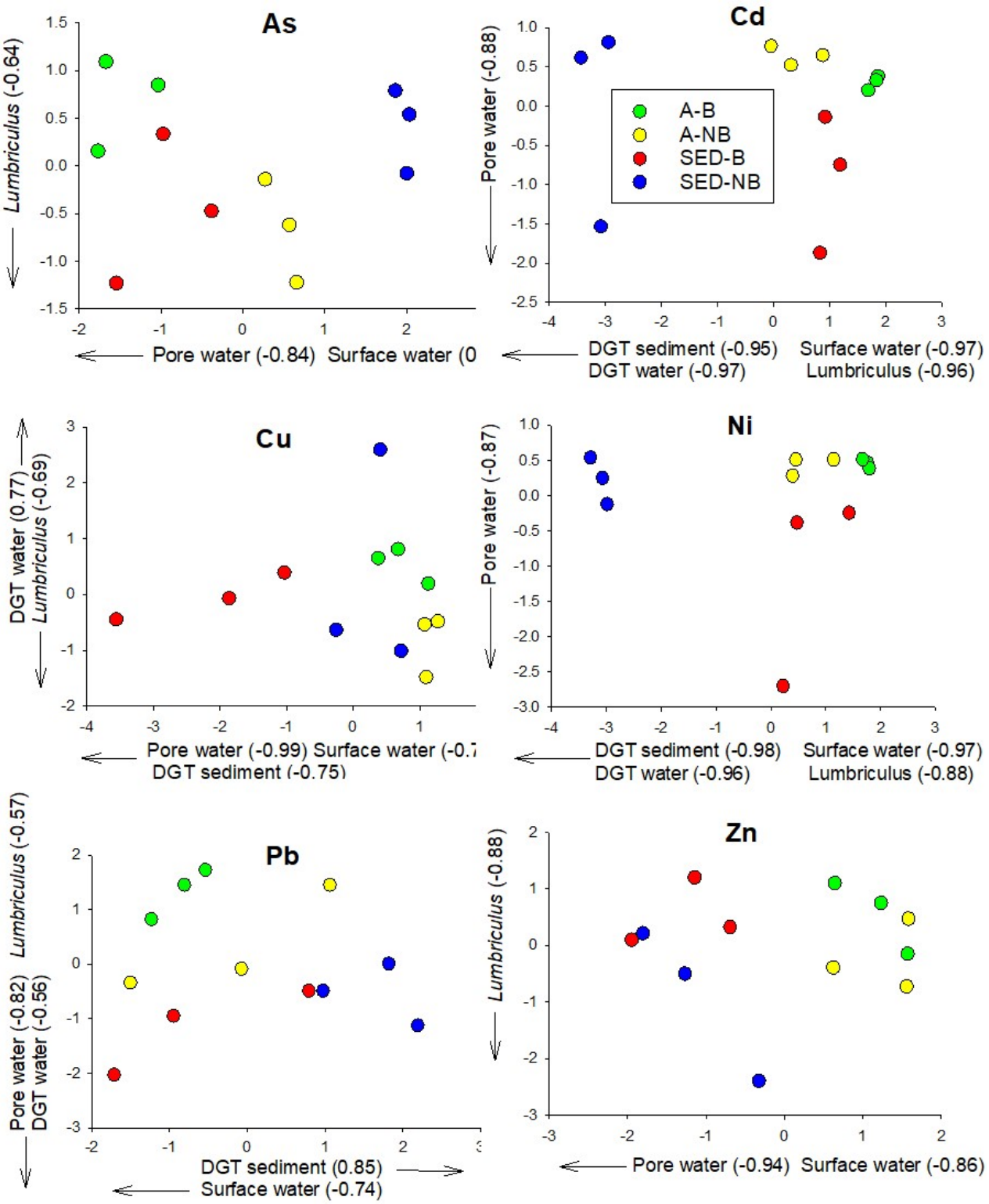


Figure 8. Principal component analysis of metal concentrations in four types of experimental mesocosms (apatite caps with bioturbation [A-B], apatite caps without bioturbation [A-NB], uncapped sediment with bioturbation [SED-B], and uncapped sediment without bioturbation SED-NB]). PC1 (principal component 1) is represented by the abscissa, and PC2 (principal component 2) is represented by the ordinate.