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1 **Metal impacts on microbial biomass in the anoxic sediments of a contaminated lake**

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22

23 **Abstract**

24 Little is known about the long-term impacts of metal contamination on the
25 microbiota of anoxic lake sediments. In this study, we examined microbial biomass and
26 metals (arsenic, cadmium, chromium, copper, iron, lead, manganese, and zinc) in the
27 sediments of Lake DePue, a backwater lake located near a former zinc smelter. Sediment
28 core samples were examined using two independent measures for microbial biomass
29 (total microscopic counts and total phospholipid-phosphate concentrations), and for
30 various fractions of each metal (pore water extracts, sequential extractions, and total
31 extracts of all studied metals and zinc speciation by X-ray absorption fine structure
32 (XAFS)). Zinc concentrations were up to 1000 times higher than reported for sediments
33 in the adjacent Illinois River, and ranged from 21,400 mg/kg near the source to 1,680
34 mg/kg near the river. However, solid metal fractions were not well correlated with pore
35 water concentrations, and were not good predictors of biomass concentrations. Instead,
36 biomass, which varied among sites by as much as two-times, was inversely correlated
37 with concentrations of pore water zinc and arsenic as established by multiple linear
38 regression. Monitoring of other parameters known to naturally influence biomass in
39 sediments (*e.g.*, organic carbon concentrations, nitrogen concentrations, pH, sediment
40 texture, and macrophytes) revealed no differences that could explain observed biomass
41 trends. This study provides strong support for control of microbial abundance by pore
42 water metal concentrations in contaminated freshwater sediments.

43 1. Introduction

44 Although the impact of metals on plants and animals is well documented [e.g.,
45 *Posthuma and Vanstraalen*, 1993; *Tyler et al.*, 1989], the relationship between metal
46 contamination and microbial communities is more ambiguous. While metal contamination
47 would generally be anticipated to have a negative impact on a biological system, in aerobic
48 systems such as soils often no biomass changes are observed [e.g., *Bååth et al.*, 1998; *Feris et*
49 *al.*, 2003; *Knight et al.*, 1997]. As a consequence, it has been hypothesized that whole-
50 community biomass recovery will occur during long-term metals exposure [*DiazRavina and*
51 *Bååth*, 1996]. Still, in other studies, metal contamination has been associated with decreased
52 soil microbial biomass [e.g., *Konopka et al.*, 1999; *Pennanen et al.*, 1996]. These apparently
53 contradictory observations reflect, in part, the interference by environmental variables other
54 than metal contamination that also influence microbial populations such as soil texture
55 [*Albrechtsen and Winding*, 1992; *England et al.*, 1993], nutrient availability [*Albrechtsen and*
56 *Winding*, 1992] and area vegetation [*Kandeler et al.*, 2000; *Pennanen et al.*, 1996].
57
58 There is also substantial controversy regarding the toxicity of different metal forms [e.g., *Ankley*
59 *et al.*, 1996; *Worms et al.*, 2006] and there is no consensus regarding appropriate metal fractions
60 for measurement. Free metal ions are often implicated as the most toxic forms [e.g.,
61 *Menkissoglu and Lindow*, 1991; *Poulson et al.*, 1997], as originally suggested by Sunda and
62 *Guillard* [1976]. However, other dissolved-phase metal complexes have been reported to exert
63 greater toxicity [*Errecalde and Campbell*, 2000; *Sarin et al.*, 2000], supporting theories that
64 metal-ion concentration alone may not be able to predict toxicity [e.g., *Hudson*, 1998; *Pinheiro*
65 *and van Leeuwen*, 2001]. Still, many field studies rely on total metal concentrations to asses

66 ecological impacts [e.g., *Gillan et al.*, 2005; *Niklinska et al.*, 2006; *Smit et al.*, 1997], primarily
67 because their higher concentrations make total metals more easily detected.

68

69 The majority of the work examining metals impacts on microbial communities has focused on
70 surface soils, a predominately aerobic system. However, in aquatic systems, sediments are
71 often the final repository for metals, where they precipitate under the often anoxic conditions
72 [*Sprenke et al.*, 2000; *Webster et al.*, 2000]. Thus sediments, which are critical to the health of
73 aquatic systems [*Jones*, 1982], provide a study opportunity for the impacts of metals on
74 anaerobic microbial communities. While laboratory studies have demonstrated metals
75 inhibition to anaerobic microbes [e.g., *Knotek-Smith et al.*, 2003; *Utgikar et al.*, 2001], impacts
76 of metal contaminants to lake sediment communities has not been previously described.

77

78 Our primary interest was to investigate the consequences of long-term metal contamination
79 exposure on lake sediment microbial communities. Specifically, we examined metals speciation
80 and concentrations in association with microbial biomass in a metals-contaminated lake located
81 in Northern Illinois (Lake DePue). Preliminary evaluation revealed that environmental
82 variables other than metal concentration remain relatively consistent in the sediments
83 throughout this small lake. Thus, the lake offers an ideal location to differentiate long-term
84 effects of metal contamination from naturally occurring variation. More detailed analyses
85 revealed a reduction in biomass correlated to a combination of pore water arsenic and zinc
86 concentrations, while total and sequentially extracted metal concentrations were poor predictors.

87

88 **2. Methods**

89 **2.1 Site description**

90 Studies were conducted in Lake DePue, Illinois, USA (latitude 41°19' N, longitude 89°18' W),
91 a shallow, backwater lake connected to the Illinois River by a narrow channel at the west end of
92 the lake. A former zinc smelting facility located north of the lake is currently listed on the
93 United States Environmental Protection agency (USEPA) National Priority List (NPL, aka
94 Superfund, Site ID# ILD062340641). Metal contamination migrated to the lake sediments from
95 this facility over approximately 80 years [*Cahill and Bogner, 2002*]. A man-made creek
96 draining into the northeast corner of the lake is a likely migration path. Characterization of the
97 metals at select sites in Lake DePue was previously described [*Webb et al., 2000*].

98

99 **2.2 Sample collection – preliminary study (June 2000).**

100 Prospective sampling locations were identified in Lake DePue by overlaying a grid pattern on
101 an area topographic map (Figure 1). A global-positioning (GPS) unit was used to direct and
102 document actual sampling locations. Grids with no indicated sample were not accessible by the
103 inflatable sampling boat on the day of sampling either due to winds or shallow water depths.
104 Bulk sediment was collected with an Eckman dredge and split for triplicate analysis. Sediment
105 samples were kept on ice in airtight boxes with N₂ gas headspace for transport to the laboratory.

106

107 **2.3 Sample collection – September 2000**

108 Three replicate cores were collected from sites 1 through site 5 (Figure 2) using a piston coring
109 device with thin-walled PVC sleeves (183-cm long and 6-cm inner diameter) and an O-ring that
110 was held stationary by attached cables to minimize compression and preserve the sediment-
111 water interface. The top two 1-cm intervals were extruded under N₂ gas. Each layer was

112 homogenized by stirring under a stream of N₂ gas. Sub-samples for cell counting, phospholipid
113 phosphate (PLP), and X-ray absorption fine structure (XAFS) analysis were preserved on-site as
114 described below. The remaining sediments were transported on ice in airtight boxes with N₂
115 gas headspace to the laboratory pending further sub-sampling. Pore water was recovered from
116 homogenized bulk sediments by centrifugation (4,000 xg, 15 minutes at 4°C).

117

118 **2.4 Sample collection – July 2004**

119 Duplicate cores from sites 1 through 7 (Figure 2) were collected and sediments from the 0-2cm
120 and 4-6cm intervals were extruded as described for the September 2000 sampling event.

121 Samples were preserved on-site for PLP as described below, and the remainder of the sediment
122 was transported and processed as described for September 2000.

123

124 **2.5 Basic sample characterization**

125 During all sampling events, water column depth, benthic plant growth, sediment color, and
126 sediment texture [ASTM, 2000] were documented on-site. Pore water pH was measured in
127 filtered pore water (0.22 µm cellulose acetate, Millipore) using an Orion® pH meter. Water
128 content was determined by recording the mass lost during overnight drying at 100°C. Bulk
129 sediment densities were calculated by simultaneously recording water displacement (volume)
130 and mass of a sediment aliquot (all samples were saturated). Total organic carbon (TOC, year
131 2004) and dissolved organic carbon (DOC, using 0.45 µm glass-fiber filtered pore water) were
132 measured with a Tekmar-Dohrmann Apollo 9000 TOC Combustion Analyzer that was operated
133 in accordance with USEPA Method 415.1 [1983]. In year 2000, sediments for TOC and total N
134 were dried using a Lyph-Lock 4.5 Liter Benchtop Freeze Dry System Model 77500 (Labconco),

135 inorganic C (carbonate) was volatilized using vapor phase acidification with HCl, and analysis
136 was conducted using a Carlo Erba model 1106 CHN analyzer [*Hedges and Stern, 1984*] .

137

138 **2.6 Metals analysis**

139 **2.6.1 Pore water metal analysis.**

140 Pore waters were filtered (0.22 μm cellulose acetate, Millipore), and preserved using trace metal
141 grade HNO_3 (2% final concentration). Samples were diluted with de-ionized water (Milli-Q
142 RG) to fit the instrumental analytical window, and maintained at the same final pH by addition
143 of HNO_3 . Dissolved pore water metals (As, Cd, Cr, Cu, Mn, Pb and Zn) were analyzed in
144 accordance with EPA Method 200.8 [1991] using a VG elemental inductively coupled plasma-
145 mass spectrometer (ICP-MS) Model PQ ExCell.

146

147 **2.6.2 Total metal analysis**

148 Total acid leachable (“total”) metal concentrations were determined using a concentrated (70%)
149 HNO_3 extraction of oven dried sediments. The supernatant was filtered (0.22 μm cellulose
150 acetate, Millipore) and metals (Cd, Cu, Mn, Pb and Zn) were quantified by flame atomic
151 absorption spectroscopy (FAAS) using a GBC model 920 in year 2000, and using a Thermo
152 Jarrell Ash Autoscan 25 scanning inductively coupled plasma atomic emission spectrometer
153 (ICP-AES) in year 2004.

154

155 **2.6.3 Sequential metal extraction**

156 Sequential extraction followed a modified Tessier protocol [*Rapins et al. 1986; Tessier et al.*
157 1979] as outlined below. Metals were extracted in a step-wise fashion as follows: step 1)

158 “exchangeable” fraction, 8 ml of 1M MgCl₂ (pH 7.0); step 2) “carbonate-bound” fraction, 8 ml
159 of 1M NaCH₃COO (pH 5.0); step 3) “Fe & Mn-oxide-bound” fraction, 20 ml of 0.04 M
160 hydroxylamine hydrochloride in 25% (v/v) acetic acid; step 4) “organic and sulfide-bound”
161 fraction, 3 ml of 0.02 M HNO₃ and 5 ml of 30% H₂O₂ (pH 2) at 85°C for 2 hours, an additional
162 3 ml of 30% H₂O₂ at 85°C for 3 hours with intermittent shaking, and 5 ml of 3.2 M
163 NH₄CH₃COO in 20% (v/v) HNO₃ and 7 ml deionized water for 30 minutes; and step 5)
164 “residual” fraction, 7.5 ml HCl (37%) and 2.5 ml of HNO₃ (65%) overnight at room
165 temperature and then heated for 2 hours under reflux. Sediment and liquid manipulations for
166 the first three extraction steps were performed in an anaerobic chamber to prevent chemical
167 oxidation of reduced metal species [Wallmann *et al.*, 1993]. Between steps, the sediments were
168 washed with de-ionized water. Following each step the filtrate (0.22 μm multi-cellulose esters,
169 Millipore) was collected and metals (Cd, Cu, Mn, Pb and Zn) were quantified using FAAS. The
170 indicated classifications specify metal fractions typically extracted during each step, though
171 some portion of a metal fraction might be released in earlier steps [Peltier *et al.*, 2005].

172

173 **2.6.4 X-ray absorption fine structure (XAFS)**

174 Immediately after extrusion, sediments were mounted into sample holders with Kapton tape and
175 placed into a portable liquid nitrogen Dewar vessel to minimize chemical transformation prior
176 to analysis. Metal speciation analysis was performed on the bending magnet beam line of the
177 DND-CAT at the Advanced Photon Source, Argonne National Laboratory. Briefly, a Si(111)
178 monochromator was used to vary the X-ray energy from approximately 200 eV below to
179 approximately 1000 eV above the absorption K edge of Zn (9659 eV). The incident and
180 transmitted intensities were measured with IC Spec ionization detectors (Oxford-Danfysik

181 Instruments). Fluorescence was measured with a Canberra 13 element high purity Germanium
182 X-ray detector in step-scanning mode. The contribution of various Zn species to the spectra
183 was determined using quadratic linear programming to fit the sample spectra to a linear
184 combination of standard reference spectra [Gaillard *et al.*, 2001].

185

186 **2.7 Direct cell counts.**

187 Sediment samples for microscopic cell counting were preserved on-site, in duplicate by diluting
188 1:40 (v/v) in filtered (0.22 µm pore size) phosphate-buffered paraformaldehyde fixative solution
189 [Gough and Stahl, 2003], which was made within 24 hours prior to field sampling. Preserved
190 samples were transported, stored, and prepared for analysis as previously described [Gough and
191 Stahl, 2003], using 1:3000 as a final dilution. Samples were arbitrarily grouped (“blocked”) and
192 analyzed in a blocked format to facilitate monitoring of experimental bias [Wardlaw, 1985].

193 Slides were counted within one day of preparation to minimize loss of DAPI (4'-6-diamidino-2-
194 phenylindole) fluorescence using a Zeiss Axioplat microscope (1,000x magnification) equipped
195 with UV fluorescence and an ocular grid. A minimum of either 100 bacteria or 30 grid fields
196 were counted for each slide. Results from duplicate block analyses were evaluated using
197 analysis of variance (ANOVA) to monitor for experimental method variance.

198

199 **2.8 Phospholipid phosphate**

200 Samples for phospholipids-phosphate (PLP) analysis were frozen in triplicate (year 2000) or
201 duplicate (year 2004) in dry ice (on-site) and stored at -80°C until processed. Lipids were
202 extracted from the sediment using solvent extraction and colorimetric methods previously
203 described [Findlay, 1996; Sutton, 2002].

204

205 **2.9 Data analysis**

206 **2.9.1 Descriptive statistics**

207 Deviation of replicates was evaluated by calculating the mean deviation (MD, sometimes called

208 the average deviation) as: $MD = \left(\sum_i^n |x_i - \bar{x}| \right) / n$ where x_i is the discrete value of a single

209 replicate, \bar{x} is the average of the replicates, and n is the number of replicates. Constancy of a

210 parameter among sampling sites was evaluated by calculating the coefficient of variation.

211 Relationships between parameters were evaluated using the Pearson Correlation. Data sets were

212 linearized by logarithmic transformation prior to linear regression analysis when necessary. The

213 95% confidence intervals (CI) on linear regression slopes were evaluated, and correlations

214 whose slope CI did not intersect zero were considered to represent a statistically significant

215 trend. Data points whose absolute normalized residuals were greater than 3 were considered

216 outliers, and were removed from correlation analysis [Zar, 1984]. The influence of individual

217 parameters was considered by comparing correlation analyses both including and excluding

218 extreme values.

219

220 ANOVA with pair-wise comparisons (i.e. Tukey's method) was used to monitor for significant

221 differences among the sampling sites. Data were logarithmically transformed to account for

222 uneven data variance (i.e. heteroscedasticity). Data were compared between the sampling years

223 using a paired t-test.

224

225 **2.9.2 Multiple regression analysis**

226 Multiple regression was conducted in a step-wise elimination process using Microsoft
227 EXCEL™ with the Analyse-It add-on (Analyse-It Software, Ltd., Leeds, England) using all
228 discrete sampling data (i.e., no field replicate averaging). Dependent data (i.e. DAPI cell counts
229 or PLP) were logarithmically transformed. Prior to multiple regression analysis, sets of pore
230 water metal concentrations were evaluated for co-variance using Kendall rank correlation.

231

232 **3. Results**

233 **3.1 Preliminary sediment characterization and site selection.**

234 Sediments were evaluated at 30 locations in the lake in June 2000 to establish an overview of
235 metal distribution patterns in comparison to basic sediment characteristics. Throughout the
236 lake, no benthic vegetation was observed and suspended sediments limited visibility (and hence
237 light penetration) through the water column. Well-graded clays predominated (Table 1), though
238 several areas of the lake were sandy – specifically, the north edge of the lake (S-12, S-15, and S-
239 28) and the southern shoreline at the bend in the lake (S-7).

240

241 Metal concentrations showed high variability throughout the lake (Table 1). Cu, Zn, and Cd
242 showed greater than 140% variability among the sampled sites, and Zn concentrations were
243 detected as high as 100,000 mg/kg (S-28, Table 1), 1000 times higher than background
244 concentrations in the Illinois River [*Cahill and Steele, 1986*]. These metals generally decreased
245 with distance from the creek delta. As an exception to the general distribution pattern, highest
246 Zn, Cd, Pb, and Cu were indicated in the sandy areas along the north shore of the lake (S-15 and
247 S-28), where Fe was also highest.

248

249 Other parameters showed much lower variation within the lake sediments, including Fe and Mn.
250 In general, water content, pore water pH, and TOC were similar throughout the lake with
251 coefficients of variation of less than 20% among the samples (Table 1). DOC showed 61%
252 variation (Table 1); however, this high variation was strongly influenced by S-33, located near
253 the creek delta (Figure 1) where rotting grass may have contributed to higher DOC, particularly
254 as samples collected in the same vicinity (i.e. S-15 and S-41) did not reveal the same high
255 carbon concentrations observed at S-33. Removal of the S-33 data point revealed a variation of
256 23% among the remaining samples.

257

258 Many of the measured parameters monitored during preliminary lake characterization were
259 significantly correlated (Table 2). Strong correlations were indicated between the contaminant
260 metals, Zn, Cd, and Cu, which showed $R^2 > 0.65$. Correlations of these metals with Pb were
261 consistently weaker, suggesting that either the Pb originated from a different contamination
262 source or that migration of Pb through the lake sediments differed than for the other metals.
263 Comparisons of metals with chemical parameters revealed that increased metal contamination
264 correlated with increased TOC and DOC (Table 2), though the relationship with DOC was only
265 statistically significant when the two highly contaminated sandy sites (S-15 and S-33) were not
266 considered (data not shown). pH was also found to have a weak correlation with metal
267 concentrations (Table 2). However, pH did not always accurately predict high metal
268 concentrations. For example, many sites with similar pH had very different total zinc
269 concentrations (for example, compare S-4, S-32, and S-33, Table 1).

270

271 Based on our preliminary study, seven areas in the lake were selected for further study. The
272 selected sites (highlighted by a dagger next to their ID in Table 1) represented a range of metal
273 contamination levels (from 1,600 to 25,000 mg/kg total Zn) and shared the following
274 characteristics: water depths between 1 and 3 m (to ensure boat accessibility), limited light
275 penetration, no indication of benthic plants, similar pH, similar DOC, and clay sediments.

276

277 **3.2 Microbial biomass**

278 Microbial biomass was generally higher in sediments collected further from the metal
279 contamination source (Figure 3). As an exception, in both years 2000 and 2004, biomass
280 concentrations were lower in samples from site 5 than from site 4 (significant difference for
281 DAPI counts, ANOVA with pair-wise difference, $\alpha=0.05$). Biomass concentrations (PLP)
282 were significantly higher at all sites in year 2004 than in year 2000 (paired t-test, $\alpha=0.01$).

283

284 **3.3 Metal concentrations**

285 In confirmation of the preliminary sampling results, metal contamination was generally lower
286 further west of the creek delta (from site 1 toward site 6, Table 3). Total metal concentrations
287 remained relatively unchanged at the sampling sites between sampling events. More easily
288 released metal fractions (“exchangeable”, step 1; “carbonate-bound”, step 2) had greater
289 proportional concentrations at more contaminated sites in year 2000, while the relative
290 proportion of later extraction steps were similar among all the sites (Figure 4). Conversely, for
291 year 2004 samples XAFS revealed that carbonate associated zinc remained proportionally
292 constant among all the tested sites at around 16% and that greater than 60% of the solid-phase
293 zinc was associated with sulfides (Figure 5). As an exception, at the most contaminated site

294 (Site 1) less zinc was associated with sulfides (39%) and complexes with clays, goethites, and
295 phosphates were observed, which were not indicated in samples from the other sites.

296

297 In contrast to the total metal concentrations, pore water metal concentrations did not always
298 decrease with increased distance from the site, nor did they follow the same rank order as the
299 solid fractions (for examples, consider site 5 for Zn in year 2000, and for As in year 2004, Table
300 3). Additionally, while total metal concentrations remained unchanged, all metals showed
301 statistically lower pore water concentrations in year 2004 (paired t-test, $\alpha=0.01$). The reduction
302 was most pronounced for Cd (on average, 94% lower at each site), Pb (78% lower), Cu (74%
303 lower), and Zn (69% lower).

304

305 **3.4 Sediment characteristics**

306 Environmental characteristics did not vary significantly among the seven sampling sites. All
307 lacked macrophytes and shared comparable features of limited light penetration, dark color, and
308 clay texture. DOC (1.25 to 1.80 mM C), pH (7.5 to 7.9), and total N (3567 to 4822 mg/kg)
309 showed little variation among the sites (Table 4), nor were differences indicated for DOC
310 between the two sampling events (paired t-test, $\alpha=0.05$; pH and total N were not monitored in
311 2004). In both sampling events, water content was highest at sites 3 and 4, and lowest at site 1
312 (ANOVA with pair-wise difference, $\alpha=0.05$, Table 4). A statistically relevant difference was
313 indicated for water content between the two sampling events, representing an increase of
314 between 2% and 14% for year 2004. In year 2000, TOC was highest at sites 1 and 2 (Table 4).
315 TOC was significantly lower in samples collected in year 2004 than year 2000 (paired t-test,
316 $\alpha=0.05$), and variation among the sites was not detected for this later sampling event.

317

318 **3.5 Correlation of biomass and metals.** Biomass concentrations (a combined PLP data set
319 from both year 2000 and 2004) showed statistical correlation with both total Zn and pore water
320 Zn concentrations (metals data was logarithmically normalized), however pore water Zn (Figure
321 6) was a better individual predictor ($R^2=0.381$ and $R^2=0.592$, respectively). Logarithmically
322 normalized pore water As also showed statistically relevant correlations to PLP with $R^2=0.470$.

323

324 Multiple regression analysis was conducted to identify potentially synergistic metals effects.
325 Due to co-variance with pore water Zn, Pb was excluded from the multiple regression analysis
326 of the year 2000 data set, and Cd from the analysis of the year 2004 data set. Both Pb and Cd
327 were excluded from the analysis of the combined data set. Subsequent multiple regression
328 analysis (Table 5) revealed that a combination of pore water As and Zn concentrations best
329 predicted negative correlation to biomass concentrations, both when data sets were considered
330 separately and when combined. In addition, pore water Mn showed a positive influence on
331 correlation to biomass concentrations for samples collected in year 2000. No other modeled
332 pore water metals made significant contributions to the correlation ($p>0.05$). The resulting
333 model showed poorest predictions for samples with highest PLP concentrations, as many were
334 higher than the regression intercept. Further analysis of these points revealed that they all had
335 pore water Zn concentrations less than $0.23 \mu\text{M}$ and pore water As less than $0.10 \mu\text{M}$ (Figure 7).

336

337 **4.0 Discussion**

338 Statistically significant correlations between metal contaminants and microbial biomass have
339 been elusive for many years due to the many confounding variables that naturally impact

340 biomass, and to lack of information regarding the most appropriate metals species for analysis.
341 In particular, aquatic sediments and other anoxic environments have been little studied. In soil
342 systems, it has been demonstrated that sediment texture, prevalence and type of macrophytes,
343 pH, organic carbon concentrations (dissolved and total), nitrogen concentrations, and moisture
344 content can naturally influence microbial biomass concentrations. Previous investigators have
345 found that metal impacts could not easily be distinguished from natural variation caused by one
346 or more of these confounding parameters [e.g., *Kamitani et al.*, 2006; *Kandeler et al.*, 2000;
347 *Pennanen et al.*, 1996]. Expanding on these previous reports, we used a systematic sampling
348 design patterned after experimental approaches established in macro-ecology [*Ruxton and*
349 *Colegrave*, 2003] to select sites that would limit the influence of these confounding variables on
350 interpretation of the experimental results. While all background variability cannot be eliminated
351 in a field-based study, monitoring showed significant differences among the sites for only 2 of
352 the 7 parameters considered: water content and TOC. Still, neither of these two parameters
353 followed a trend that would predict the observed biomass concentrations. For example, in
354 saturated samples, such as lake sediments, water content is only expected to influence biomass
355 at extreme values, and not within the narrow range observed in this study [*Schallenberg and*
356 *Kalff*, 1993]. Further, were TOC causing biomass variation, it would be expected that higher
357 TOC would correlate with higher biomass, which is opposite of the observed trend. Still, the
358 significant correlations of metal concentrations with TOC were of interest, because organic
359 carbons and other substrates might be expected to accumulate if metal stress had inhibited
360 microbial activity. Indeed, many previous studies have reported higher TOC in metal
361 contaminated soils in comparison to uncontaminated control sites [e.g., *Chander and Brookes*,
362 1991; *Kelly and Tate*, 1998; *Valsecchi et al.*, 1995]. Thus, the TOC results in this study support

363 these theories regarding TOC accumulation, and expand these observations to an anoxic and
364 aquatic environment.

365

366 Correlations between biomass and metals concentrations were revealed both by linear
367 regression and multiple regression analysis. In this study, total Zn concentrations were
368 statistically correlated with biomass concentrations, which is in contrast to findings in marine
369 sediments [Gillan *et al.*, 2005]. However the metal contamination levels in Lake DePue were
370 significantly higher than in the Gillan study, where a maximum Zn concentration of 330 mg/kg
371 (HCl-extracted) was recorded. But, regardless, pore water concentrations showed a more
372 predictive correlation in our study than did the total Zn concentration, suggesting that the pore
373 water species exerted greater selective pressure. Still, despite the statistical linear correlation of
374 biomass with individual metals, a mixture of metals may be toxic at lower summed
375 concentration than any one single metal [Dahlin *et al.*, 1997; Fabiano *et al.*, 1994]. Thus,
376 multiple regression analysis was used to provide a more meaningful statistic, and identified pore
377 water zinc and arsenic, in combination, as the most probable controlling variables of biomass in
378 the studied lake system (Table 5). It is important to note that these same variables were
379 identified in two separate sampling years even as the pore water metals concentrations and
380 distribution patterns changed within the lake, suggesting that the higher biomass concentrations
381 observed in 2004 may have been related to decreasing pore water metal contamination. Further
382 review of samples whose biomass concentrations were not as well predicted by the multiple
383 regression model suggested that biomass may not have been impacted in samples with less than
384 0.23 μM pore water Zn and less than 0.10 μM pore water As (Figure 7). As many study sites do
385 not see concentrations above this level, this might explain why some past studies were not able

386 to discern biomass impacts with metal contaminants. However, the microbial populations found
387 in saline or aerobic environments might have different stress responses than those found in
388 anoxic freshwater sediments; therefore, these levels of no observed impacts can not be
389 extrapolated to other environments without further experimental verification.

390
391 In addition to the changes in pore water metals concentrations, another apparent variation
392 between sampling events was the speciation of metals at the sites. XAFS revealed constant
393 relative proportions of carbonate-bound zinc in 2004, in contrast to sequential extractions results
394 for samples collected in year 2000 that indicated far less carbonate-bound zinc further from the
395 source. These weakly bound metal species are often indicative of anthropogenic (rather than
396 natural) metal sources [*Tack and Verloo, 1995*], and followed the expected pattern in year 2000
397 samples by decreasing in relative proportion with distance from the contamination source.
398 Differences were also indicated for sulfide-bound zinc fractions (Figures 4 and 5); however,
399 because the sulfide fraction determined by sequential extraction may be prematurely released
400 with the Fe/Mn-bound fractions in Step 3 [*Peltier et al., 2005*], this observation may be artifact
401 of the method applied.

402

403 **5. Conclusions and Implications**

404 In this study, we have demonstrated a correlation between decreased microbial biomass and
405 increased pore water concentrations of Zn and As in the sediments of a metals contaminated
406 lake. While often environmental impact studies focus on macrophytes, invertebrates or aquatic
407 animals, results of this study further emphasize the potential for microbial impacts. Decreased
408 microbial biomass as a result of metal contamination may cause significant impacts on a lake

409 system by potentially altering the biogeochemical cycles mediated by sediment microorganisms.
410 Indeed, in this study and others [e.g., *Chander and Brookes*, 1991; *Kelly and Tate*, 1998;
411 *Valsecchi et al.*, 1995], organic carbon has been found to be higher in more contaminated
412 sediments, a relationship that was found to be statistically significant in this study. Most
413 importantly, this study demonstrated the effectiveness in field-based studies of controlling
414 confounding variables and of consideration of metals speciation.

415

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424

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438

439

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585 FIGURE CAPTIONS

586 Figure 1. Sampling locations, June 2000, Lake DePue, Illinois, USA (41°19' N, 89°18'
587 W). Grids represent targeted sampling locations. Sample IDs correspond with Table 1.

588 Figure 2. Sampling locations with Zn metal concentrations, September 2000 and July
589 2004, Lake DePue, Illinois USA. Site names correspond with Tables 3 and 4.

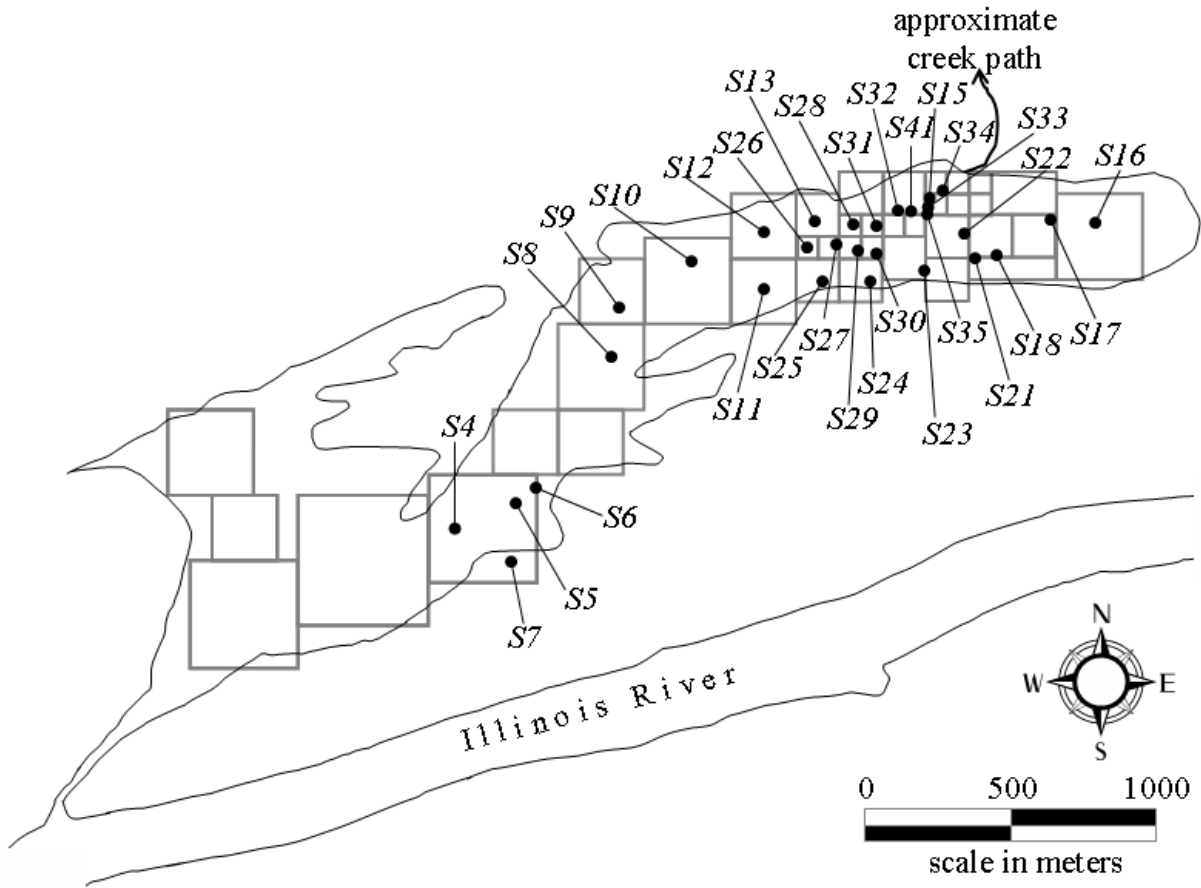
590 Figure 3. Sediment microbial biomass as measured by direct (microscopic) cell counting
591 and phospholipid-phosphate (PLP) analysis. Error bars show mean deviation of two depth
592 intervals in replicate cores (3 cores in September 2000, $n=6$; and 2 cores in July 2004, $n=4$).
593 Values measured by the same method with the same lower case letter were not statistically
594 different based on ANOVA and pair-wise differences ($\alpha=0.05$). Site names correspond with
595 locations in Figure 2.

596 Figure 4. Relative proportions of zinc fractions as determined by sequential extractions
597 (September 2000 samples). Error bars show mean deviation of two depth intervals and three
598 cores ($n=6$). Categories at the right represent chemical species typically released at each
599 extraction step. Site names correspond with locations in Figure 2.

600 Figure 5. Zinc speciation by X-ray absorption fine structure (July 2004 samples). The
601 percentage above each bar shows that species relative proportion in the sample. Error bars for
602 samples from Sites 1 and 2 show the deviation of duplicate samples. Replicates were not
603 processed for Sites 3 or 6. Site names correspond with locations in Figure 2.

604 Figure 6. Sediment microbial biomass (as phospholipid phosphate) versus pore water zinc
605 concentration. Error bars show mean deviation of two depth intervals in replicate cores (3 cores
606 in September 2000, $n=6$; and 2 cores in July 2004, $n=4$).

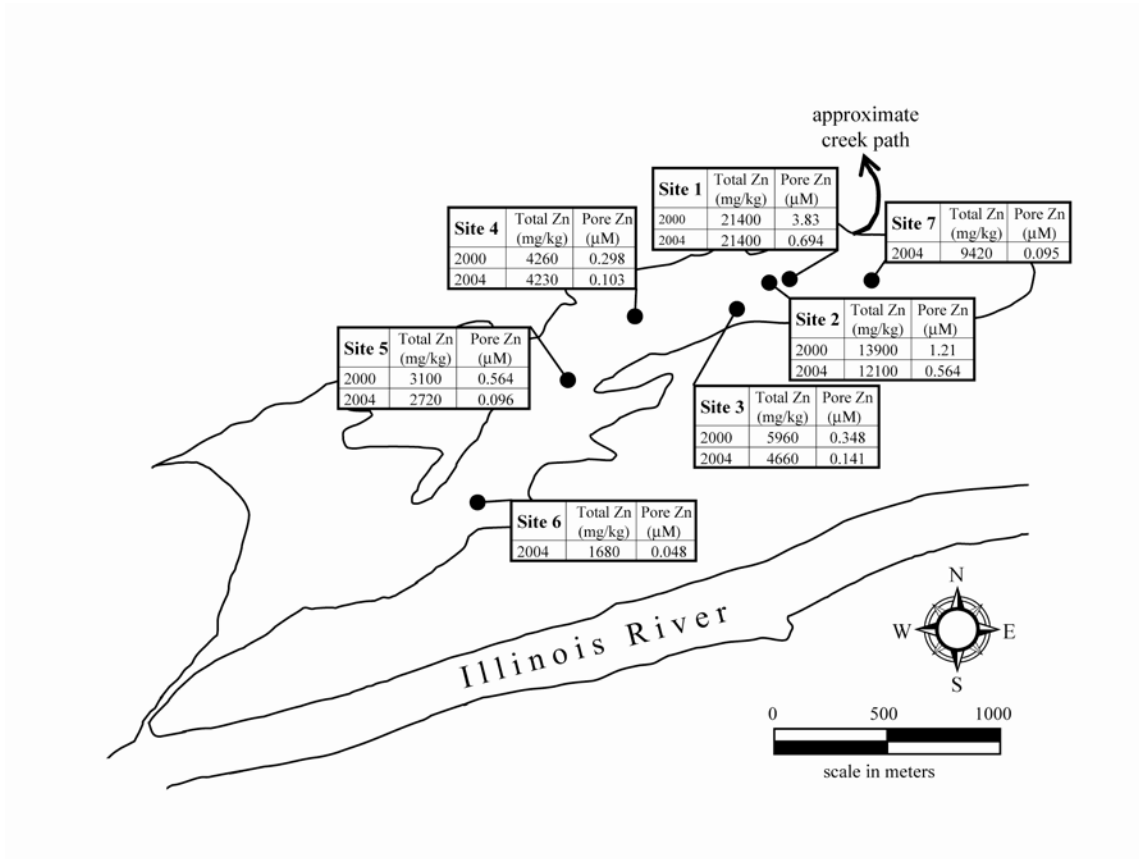
607 Figure 7. Comparison of pore water zinc concentrations verses pore water arsenic
608 concentrations (both negative log normalized) in samples with suspected unimpacted
609 phospholipid phosphate (PLP) and other study samples. “Unimpacted” (solid circles) represent
610 data points whose PLP concentrations were higher than the intercept predicted by multiple
611 regression. “Impacted” (open circles) represent all other data points. The solid line represents
612 the approximate concentrations below which no PLP impact was suggested by the study data.
613



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615 Fig 1

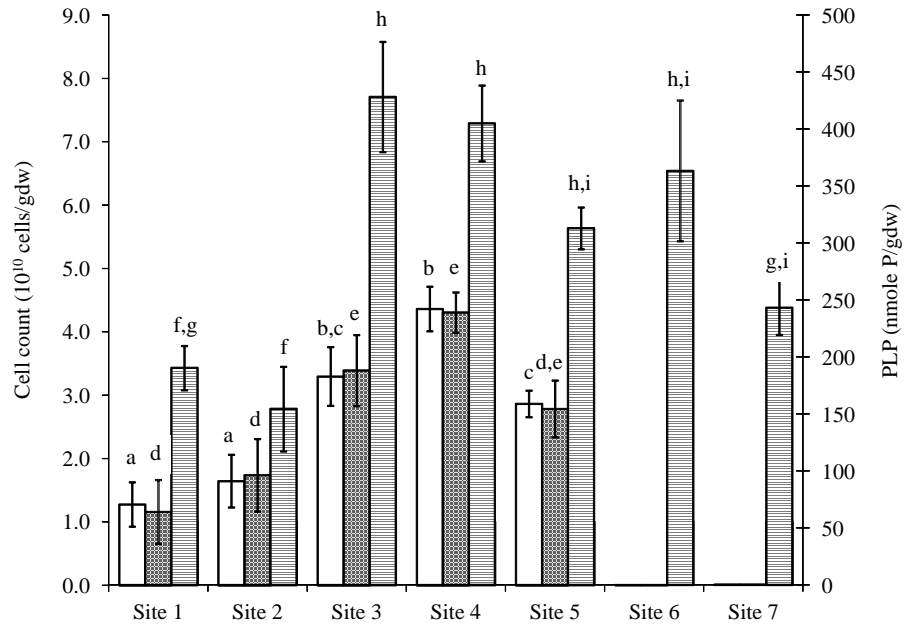
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618 Fig 2

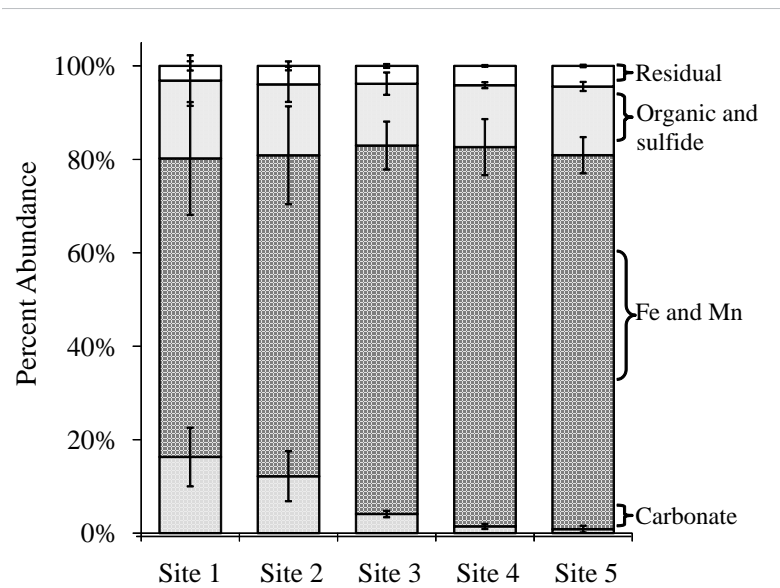
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621 Fig 3

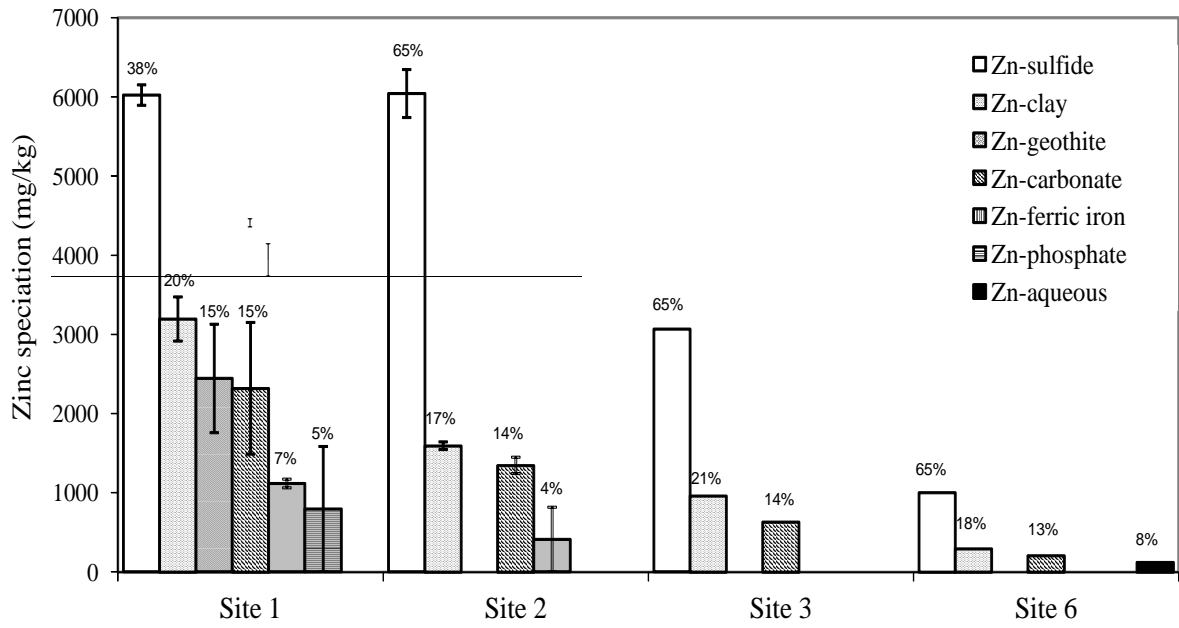
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624 Fig 4

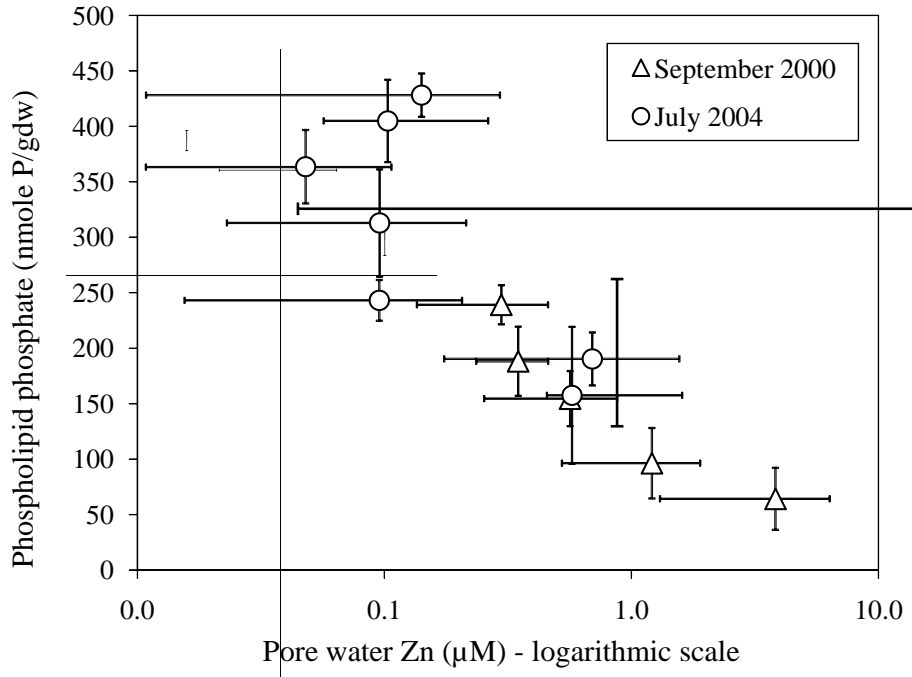
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626 Fig 5
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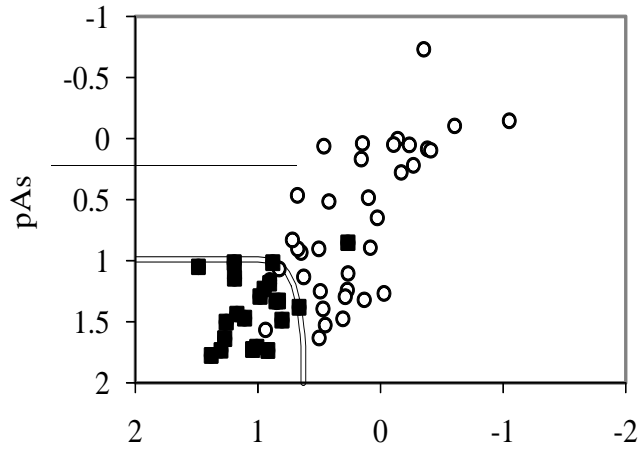
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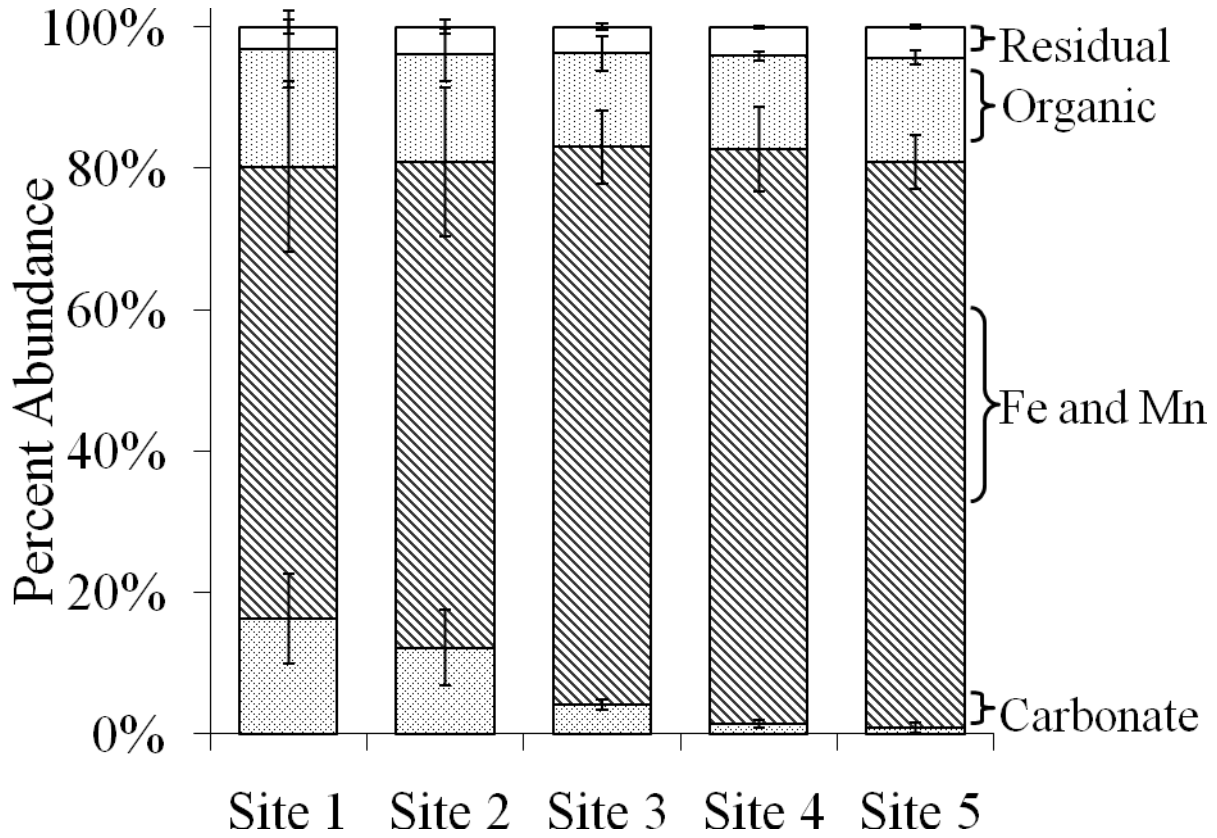
631 Fig 6

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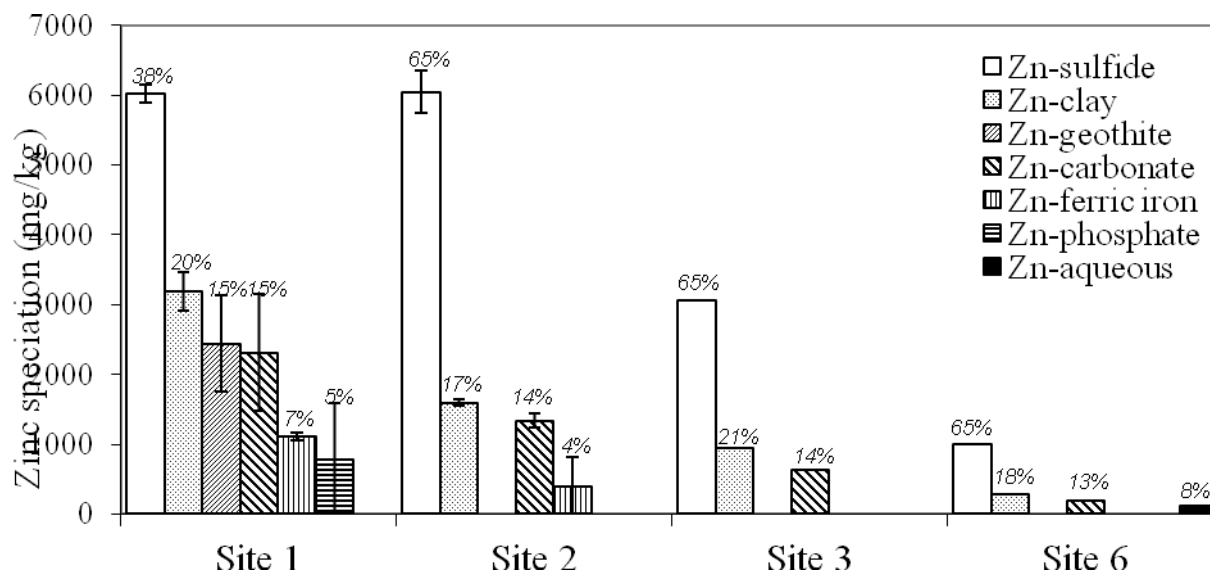
634 Fig 7



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636 Fig 4

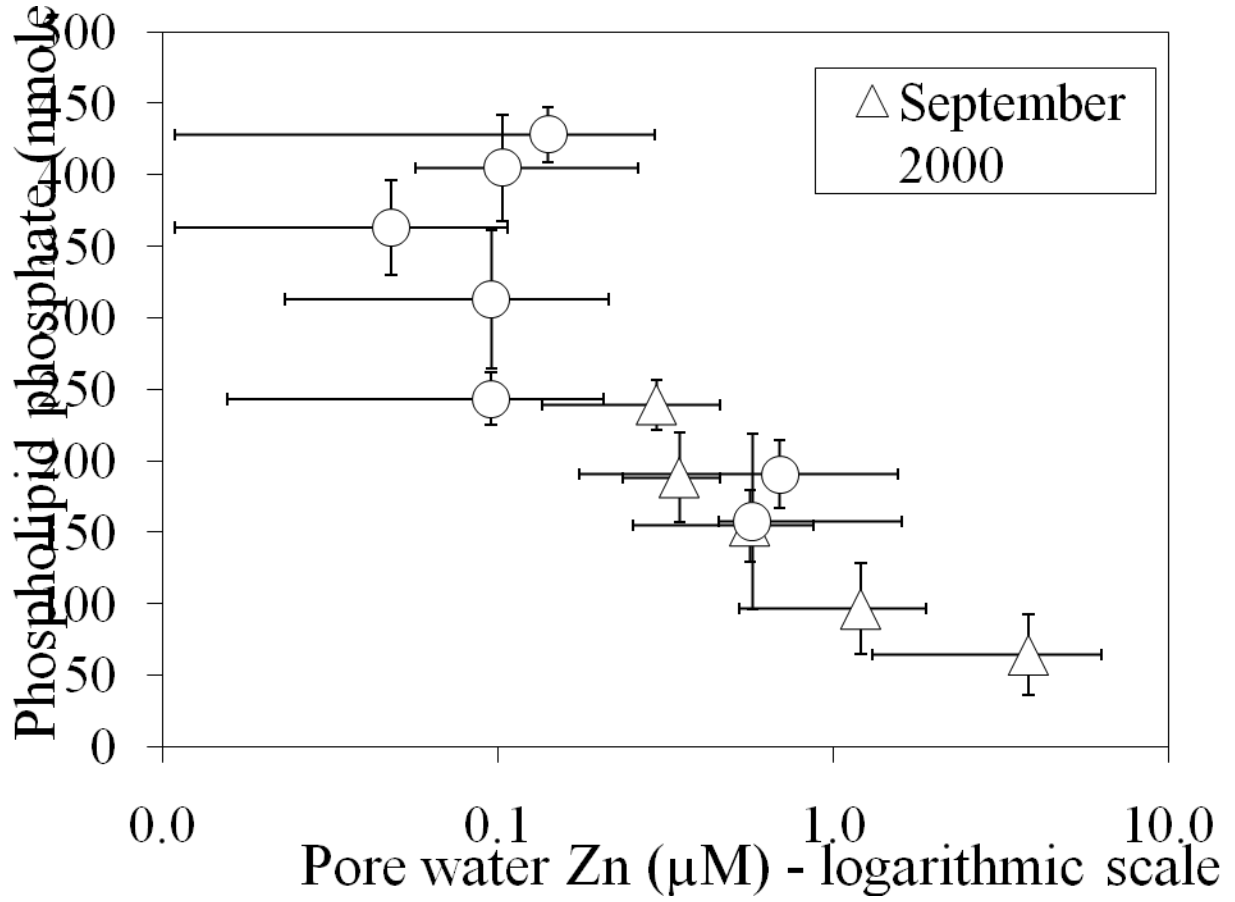
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639 Fig 5

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641

TABLE 1 Analytical results for lake sediment samples, June 2000 samples

Sample ID ^a	Sediment Description ^b	Water depth ^c (m)	Water Content ^d (%)	pH	TOC (mg/kg)	DOC (mM C)	Total Zn (mg/kg)	Total Cd (mg/kg)	Total Cu (mg/kg)	Total Pb (mg/kg)	Total Mn (mg/kg)	Total Fe (mg/kg)
S-4*	-- ^e	2.44	70% ± 1.0%	7.40 ± 0.06	37,126 ± 1025	1.58 ± 0.22	1,627 ± 32	nd	56.5 ± 4.4	nd	690 ± 10	20,384 ± 111
S-5	--	2.13	68% ± 1.0%	7.34 ± 0.06	37,297 ± 1679	1.31 ± 0.29	2,003 ± 27	nd	69.1 ± 2.1	nd	688 ± 19	20,144 ± 697
S-6	Gr SL	1.98	68% ± 1.9%	7.27 ± 0.10	35,226 ± 2064	1.40 ± 0.19	2,400 ± 75	nd	63.4 ± 3.6	nd	757 ± 42	21,563 ± 349
S-7	Br SA	1.37	50% ± 2.8%	7.45 ± 0.16	28,877 ± 2408	--	1,006 ± 17	nd	37.0 ± 3.5	nd	668 ± 58	20,465 ± 866
S-8*	Br Cl	1.83	60% ± 1.9%	7.26 ± 0.07	33,172 ± 1312	0.95 ± 0.29	3,350 ± 79	nd	92.5 ± 3.1	nd	722 ± 32	19,334 ± 326
S-9	Gr Cl	2.74	69% ± 1.8%	7.22 ± 0.03	38,938 ± 1297	1.78 ± 0.20	3,872 ± 35	23.2 ± 0.5	136 ± 5	nd	781 ± 21	21,076 ± 458
S-10*	--	2.90	69% ± 0.7%	7.27 ± 0.01	37,863 ± 1763	1.73 ± 0.20	4,475 ± 28	35.5 ± 4.8	171 ± 12	nd	775 ± 2	21,658 ± 284
S-11	Br Tight Cl	2.90	70% ± 0.5%	7.21 ± 0.02	42,889 ± 1798	2.21 ± 0.15	4,515 ± 102	31.8 ± 2.5	183 ± 8	384 ± 26	755 ± 51	20,739 ± 392
S-12	Br Fine SA	2.44	60% ± 1.3%	7.36 ± 0.05	53,764 ± 2567	2.39 ± 0.19	24,410 ± 525	164 ± 6	236 ± 6	687 ± 26	1,318 ± 48	21,520 ± 319
S-13	--	2.13	64% ± 0.5%	7.41 ± 0.07	42,203 ± 2721	2.45 ± 0.17	16,053 ± 266	254 ± 2	575 ± 33	1,372 ± 181	964 ± 36	19,709 ± 325
S-15	SA w/ organics	0.91	60% ± 0.8%	7.54 ± 0.15	55,870 ± 1802	1.88 ± 0.36	85,338 ± 4495	265 ± 19	7,253 ± 230	nd	1,898 ± 128	24,380 ± 636
S-16	SA	1.37	60% ± 2.4%	7.40 ± 0.17	46,512 ± 1798	2.22 ± 0.19	6,530 ± 164	51.6 ± 5.3	282 ± 13	nd	1,113 ± 23	17,490 ± 279
S-17*	rotting weeds	--	--	--	--	--	--	--	--	--	--	--
S-18	CL w/ SA	1.37	54% ± 1.9%	7.24 ± 0.04	37,934 ± 1520	2.34 ± 0.19	4,452 ± 21	34.3 ± 1.1	191 ± 1	nd	803 ± 47	18,059 ± 81
S-21	SL w/ CL clumps	1.52	61% ± 0.6%	7.36 ± 0.03	36,616 ± 1237	2.30 ± 0.21	7,296 ± 56	48.1 ± 3.6	257 ± 1	nd	958 ± 9	16,975 ± 1142
S-22*	--	1.52	61% ± 1.2%	7.35 ± 0.02	40,683 ± 1873	2.10 ± 0.16	17,538 ± 393	122 ± 4	841 ± 19	378 ± 39	1,057 ± 16	18,160 ± 312
S-23	DrBr Smooth SL	1.68	61% ± 1.8%	7.30 ± 0.04	35,069 ± 1730	2.41 ± 0.17	6,663 ± 74	35.8 ± 0.4	241 ± 9	nd	703 ± 17	16,718 ± 268
S-24	Gr CL	1.98	63% ± 1.0%	7.40 ± 0.01	36,831 ± 2631	2.59 ± 0.17	6,879 ± 259	44.5 ± 0.5	223 ± 20	nd	923 ± 50	17,515 ± 575
S-25*	Br to Gr tight CL	2.74	70% ± 0.8%	7.33 ± 0.03	42,224 ± 1644	1.96 ± 0.14	6,530 ± 14	41.6 ± 0.9	234 ± 4	nd	843 ± 15	18,606 ± 190
S-26	--	2.90	70% ± 0.3%	7.13 ± 0.06	44,489 ± 1894	2.31 ± 0.23	6,249 ± 136	52.3 ± 2.2	267 ± 6	nd	827 ± 25	18,691 ± 269
S-27	Gr CL	2.13	67% ± 0.3%	7.23 ± 0.06	44,114 ± 1494	1.87 ± 0.14	7,798 ± 56	63.7 ± 1.0	342 ± 4	nd	819 ± 19	18,856 ± 447
S-28	Br SA	1.68	39% ± 1.2%	7.69 ± 0.06	49,360 ± 3634	2.89 ± 0.42	99,313 ± 12,787	1,089 ± 62	1,036 ± 70	3,049 ± 62	1,612 ± 51	34,836 ± 1040
S-29	Gr CL	1.98	67% ± 1.9%	7.15 ± 0.04	43,696 ± 1666	2.17 ± 0.15	8,295 ± 60	66.9 ± 2.7	349 ± 5	nd	791 ± 40	18,518 ± 172
S-30*	Dk Br CL w/ SA	1.83	60% ± 1.7%	7.39 ± 0.03	36,340 ± 1515	2.64 ± 0.17	11,602 ± 2360	76.5 ± 11.5	345 ± 25	nd	817 ± 10	17,862 ± 378
S-31	CL w/ SA	1.68	60% ± 1.0%	7.37 ± 0.02	34,019 ± 2740	2.84 ± 0.17	21,129 ± 963	220 ± 21	711 ± 44	593 ± 94	925 ± 29	19,265 ± 1148
S-32*	CL w/ SA and org	1.52	52% ± 0.3%	7.44 ± 0.04	47,923 ± 1654	2.89 ± 0.36	24,960 ± 491	226 ± 10	808 ± 52	467 ± 44	1,356 ± 46	24,762 ± 455
S-33	grass w/ CL and SA	1.07	73% ± 1.0%	7.34 ± 0.01	64,481 ± 5910	9.24 ± 0.65	62,895 ± 1038	318 ± 14	6,788 ± 358	409 ± 11	2,560 ± 288	26,808 ± 1065
S-34*	--	0.91	--	--	--	--	--	--	--	--	--	--
S-35*	rotting grass	1.07	--	--	--	--	--	--	--	--	--	--
S-41	--	0.15	56% ± 0.3%	7.42 ± 0.02	37,390 ± 1217	2.70 ± 0.20	25,670 ± 623	225 ± 20	1,006 ± 73.6	383 ± 28	1,239 ± 20	21,454 ± 244
Coefficient of variation												
12%												
19%												
61%												
143%												
144%												
214%												
103%												
42%												
18%												

^a Sample ID corresponds to Figure 1; ^b Description codes based on USCS shorthand: Gr, gray; Br, brown; Dk, dark; SL, silt; SA, sand; CL, clay; ^c Water column height above the sediment-water interface was measured to the nearest 0.15 m; ^d All samples were saturated; ^e --, not recorded or analyzed; [†] Sediment could not be recovered for laboratory analysis; nd, not detected; * Sampling location selected for further study.

TABLE 2 Coefficient of determination (R^2) for correlation between sediment parameters, June 2000 samples

	Water Content ^a	pH	Total Zn	Total Cd	Total Cu	Total Pb	Total Mn	Total Fe	TOC
pH	[0.470] ^b								
Total Zn	[0.194]	0.469							
Total Cd	-- ^c	0.212 [*]	0.655 [*]						
Total Cu	--	0.278 ^{+,§}	0.903 [*]	0.708 ^{+,§}					
Total Pb	[0.225]	--	--	0.218	--				
Total Mn	[0.302] [§]	0.476 [§]	0.789	0.619	0.606 ⁺	--			
Total Fe	--	0.351	0.682	0.345 [*]	0.234 ^{+,§}	--	0.505 [*]		
TOC	--	0.063 [§]	0.468	0.179	0.516	0.057	0.731	0.100	
DOC	[0.482] [§]	0.151 [§]	0.475	0.244 [§]	0.469 ^{+,§}	--	0.407 ^{+,§}	--	--

^a column headers represent parameters used as independent variables, and row headers indicate parameters used as dependent variables; ^bbrackets indicate negative correlation; ^c--, correlation was not statistically significant. ⁺data from S-15 was an outlier; ^{*}data from S-28 was an outlier; [§]data from S-33 was an outlier.

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TABLE 3 Analytical results for metal fractions by sequential extractions and pore water analysis

Site	September 2000							July 2004		
	exchangeable (mg/kg)	carbonate bound (mg/kg)	Fe/Min bound (mg/kg)	organic/HIS' bound (mg/kg)	residual (mg/kg)	total (mg/kg)	pore water (μM)	total (mg/kg)	pore water (μM)	
<i>Zinc</i>										
Site 1	7.6 ± 5.5 ^a	4028 ± 1552 ^a	15,793 ± 2987 ^a	4122 ± 1339 ^a	776 ± 243 ^a	21383 ± 4150 ^a	3.83 ± 2.52 ^a	21433 ± 1724 ^a	0.694 ± 0.175 ^a	
Site 2	6.6 ± 3.2 ^a	1695 ± 746 ^b	9,558 ± 1459 ^b	2164 ± 470 ^b	547 ± 113 ^a	13862 ± 2751 ^b	1.21 ± 0.69 ^{ab}	12062 ± 1389 ^b	0.564 ± 0.455 ^{ab}	
Site 3	nd	249 ± 42 ^c	4,797 ± 313 ^c	806 ± 145 ^c	231 ± 24 ^b	5963 ± 136 ^c	0.348 ± 0.112 ^{bc}	4656 ± 328 ^c	0.141 ± 0.011 ^{bc}	
Site 4	nd	67.3 ± 25.4 ^d	3,818 ± 282 ^d	622 ± 29 ^d	194 ± 9 ^c	4255 ± 72 ^d	0.298 ± 0.162 ^c	4230 ± 521 ^c	0.103 ± 0.057 ^{cd}	
Site 5	nd	27.5 ± 23.0 ^e	2,552 ± 124 ^e	469 ± 31 ^e	140 ± 9 ^d	3104 ± 46 ^e	0.564 ± 0.311 ^{bc}	2723 ± 22 ^d	0.096 ± 0.023 ^{cd}	
Site 6								1681 ± 147 ^e	0.048 ± 0.011 ^d	
Site 7								9420 ± 867 ^b	0.095 ± 0.016 ^{cd}	
<i>Cadmium</i>										
Site 1	nd	nd	71.5 ± 11.0 ^a	21.5 ± 3.0 ^a	3.13 ± 0.46 ^a	89.0 ± 7.9 ^a	0.0153 ± 0.0104 ^a	91.9 ± 3.6 ^{ab}	0.00045 ± 0.00008 ^{ab}	
Site 2	nd	nd	66.1 ± 11.6 ^a	17.0 ± 3.7 ^a	2.83 ± 0.38 ^a	90.6 ± 17.2 ^a	0.0088 ± 0.0018 ^a	152 ± 56 ^a	0.00082 ± 0.00036 ^a	
Site 3	nd	nd	31.6 ± 2.3 ^b	5.4 ± 1.2 ^b	nd	37.2 ± 1.7 ^b	0.0075 ± 0.0044 ^a	13.8 ± 0.5 ^c	0.00065 ± 0.00005 ^a	
Site 4	nd	nd	19.7 ± 0.3 ^c	2.9 ± 0.4 ^c	nd	24.5 ± 0.4 ^c	0.0056 ± 0.0020 ^a	17.3 ± 7.1 ^c	0.00022 ± 0.00005 ^{cd}	
Site 5	nd	nd	11.4 ± 0.4 ^d	2.0 ± 0.2 ^d	nd	14.4 ± 0.5 ^d	0.0050 ± 0.0018 ^a	6.62 ± 2.46 ^d	0.00036 ± 0.00007 ^{bc}	
Site 6								5.73 ± 0.46 ^d	0.00013 ± 0.00003 ^d	
Site 7								62.6 ± 5.5 ^b	0.00044 ± 0.00015 ^{ab}	
<i>Lead</i>										
Site 1	nd	nd	42.7 ± 8.1 ^a	147 ± 60 ^a	51.4 ± 28.5 ^a	260 ± 126 ^{ab}	0.0188 ± 0.0092 ^a	480 ± 34 ^a	0.00073 ± 0.00033 ^{ab}	
Site 2	nd	nd	87.3 ± 23.3 ^b	131 ± 25 ^a	30.9 ± 8.5 ^b	288 ± 36 ^{ab}	0.0154 ± 0.0041 ^a	350 ± 100 ^a	0.00149 ± 0.00050 ^{ab}	
Site 3	nd	nd	66.8 ± 9.8 ^b	63.3 ± 8.4 ^b	nd	162 ± 9 ^b	0.0055 ± 0.0019 ^b	112 ± 11 ^b	0.00232 ± 0.00073 ^{bc}	
Site 4	nd	nd	49.1 ± 2.1 ^a	45.9 ± 3.9 ^c	nd	119 ± 2 ^c	0.0045 ± 0.0017 ^b	90.6 ± 7.4 ^b	0.00068 ± 0.00045 ^{bc}	
Site 5	nd	nd	25.0 ± 2.3 ^c	32.6 ± 4.3 ^d	nd	70.5 ± 2 ^d	0.0063 ± 0.0011 ^b	36.9 ± 21.2 ^c	0.00238 ± 0.00037 ^b	
Site 6								32.5 ± 5.6 ^c	0.00031 ± 0.00016 ^a	
Site 7								120 ± 27 ^b	0.00089 ± 0.00057 ^{ab}	
<i>Manganese</i>										
Site 1	193 ± 52 ^a	462 ± 56 ^{ab}	500 ± 182 ^a	138 ± 74 ^a	125 ± 36 ^a	963 ± 174 ^a	24.4 ± 7.8 ^{ab}	1538 ± 182 ^a	17.0 ± 2.1 ^a	
Site 2	127 ± 7 ^b	417 ± 33 ^b	332 ± 51 ^{ab}	71.4 ± 23.7 ^{ab}	106 ± 15 ^{ab}	834 ± 72 ^{ab}	19.8 ± 4.0 ^a	831 ± 108 ^{ab}	16.5 ± 2.0 ^{ab}	
Site 3	179 ± 20 ^{ab}	535 ± 24 ^{ac}	205 ± 49 ^{bc}	26.1 ± 7.4 ^c	95.0 ± 4.0 ^{ab}	777 ± 23 ^{bc}	28.9 ± 5.4 ^{ab}	867 ± 73 ^{ab}	15.7 ± 2.0 ^{ab}	
Site 4	209 ± 7 ^a	767 ± 23 ^d	190 ± 27 ^c	24.4 ± 3.4 ^c	93.5 ± 2.3 ^{ab}	829 ± 12 ^a	33.3 ± 3.6 ^b	988 ± 124 ^{ab}	15.5 ± 1.7 ^{ab}	
Site 5	163 ± 14 ^{ab}	631 ± 46 ^c	190 ± 27 ^c	27.4 ± 6.3 ^{bc}	79.7 ± 2.7 ^b	764 ± 8 ^c	26.9 ± 4.2 ^{ab}	663 ± 264 ^b	16.9 ± 0.9 ^a	
Site 6								798 ± 49 ^{ab}	12.5 ± 0.7 ^b	
Site 7								832 ± 26 ^{ab}	15.4 ± 1.8 ^{ab}	

TABLE 3 - continued Analytical results for metal fractions by sequential extractions and pore water analysis

Site	September 2000							July 2004		
	exchangeable (mg/kg)	carbonate bound (mg/kg)	Fe/Min bound (mg/kg)	organic/HIS bound (mg/kg)	residual (mg/kg)	total (mg/kg)	pore water (μ M)	total (mg/kg)	pore water (μ M)	
<i>Iron</i>										
Site 1	632 \pm 105 ^a	3363 \pm 216 ^a	2873 \pm 436 ^a	2839 \pm 787 ^a	19,565 \pm 2145 ^a	32,847 \pm 1058 ^a		32,646 \pm 1070 ^a		
Site 2	469 \pm 35 ^b	3252 \pm 209 ^a	2568 \pm 348 ^a	2186 \pm 173 ^{ab}	17,262 \pm 627 ^{ab}	29,687 \pm 1380 ^b		29,848 \pm 1898 ^a		
Site 3	554 \pm 28 ^{ab}	4498 \pm 296 ^b	2743 \pm 804 ^a	1665 \pm 241 ^b	19,307 \pm 857 ^a	26,140 \pm 530 ^c		31,792 \pm 2540 ^a		
Site 4	596 \pm 25 ^{ac}	5273 \pm 114 ^b	3398 \pm 188 ^a	1788 \pm 111 ^b	19,860 \pm 527 ^a	26,560 \pm 647 ^d		34,019 \pm 4039 ^a		
Site 5	497 \pm 41 ^{bc}	4097 \pm 408 ^c	2580 \pm 147 ^a	1865 \pm 240 ^b	16,355 \pm 802 ^b	23,935 \pm 1090 ^c		30,104 \pm 971 ^a		
Site 6								29,738 \pm 1852 ^a		
Site 7								30,876 \pm 4411 ^a		
<i>Copper</i>										
Site 1	nd	nd	5.83 \pm 2.57 ^a	576 \pm 179 ^a	132 \pm 34 ^a	801 \pm 188 ^a	0.203 \pm 0.170 ^a	670 \pm 41 ^a	0.0222 \pm 0.0054 ^a	
Site 2	nd	nd	4.27 \pm 1.23 ^a	291 \pm 46 ^b	69.7 \pm 5.5 ^b	434 \pm 50 ^b	0.0906 \pm 0.0268 ^a	390 \pm 42 ^b	0.0180 \pm 0.0024 ^a	
Site 3	nd	nd	7.82 \pm 1.46 ^a	177 \pm 20 ^c	48.7 \pm 3.0 ^c	237 \pm 9 ^c	0.0468 \pm 0.0399 ^a	197 \pm 19 ^c	0.0239 \pm 0.0064 ^a	
Site 4	nd	nd	6.93 \pm 0.44 ^a	156 \pm 2 ^c	43.8 \pm 1.2 ^c	177 \pm 4 ^d	0.243 \pm 0.310 ^a	181 \pm 21 ^c	0.0103 \pm 0.0008 ^b	
Site 5	nd	nd	4.55 \pm 0.77 ^a	74.8 \pm 5.1 ^d	24.8 \pm 2.2 ^d	101 \pm 4 ^c	0.0535 \pm 0.0386 ^a	116 \pm 3 ^d	0.0228 \pm 0.0028 ^a	
Site 6								84.6 \pm 8.8 ^c	0.00963 \pm 0.00121 ^b	
Site 7								394 \pm 73 ^b	0.0182 \pm 0.0039 ^a	
<i>Arsenic</i>										
Site 1							1.80 \pm 1.20 ^a	29.2 \pm 13.3 ^a	0.526 \pm 0.249 ^a	
Site 2							0.553 \pm 0.335 ^a	28.6 \pm 10.5 ^a	0.331 \pm 0.104 ^a	
Site 3							0.0541 \pm 0.0221 ^b	17.9 \pm 15.9 ^a	0.036 \pm 0.011 ^b	
Site 4							0.0831 \pm 0.0245 ^{bc}	14.6 \pm 7.2 ^a	0.028 \pm 0.009 ^b	
Site 5							0.0924 \pm 0.0338 ^c	5.79 \pm 5.48 ^a	0.049 \pm 0.013 ^b	
Site 6								27.5 \pm 8.0 ^a	0.056 \pm 0.036 ^b	
Site 7								13.4 \pm 13.9 ^a	0.042 \pm 0.019 ^b	
<i>Chromitum</i>										
Site 1							0.168 \pm 0.142 ^a		0.0300 \pm 0.0024 ^a	
Site 2							0.0586 \pm 0.0363 ^a		0.0310 \pm 0.0048 ^a	
Site 3							0.0609 \pm 0.0315 ^a		0.0320 \pm 0.0010 ^a	
Site 4							0.0768 \pm 0.0310 ^a		0.0580 \pm 0.0392 ^a	
Site 5							0.0957 \pm 0.0446 ^a		0.0308 \pm 0.0009 ^a	
Site 6									0.0312 \pm 0.0024 ^a	
Site 7									0.0289 \pm 0.0019 ^a	

Note: Values are the average \pm mean deviation. For September 2000, the average is for 3 cores and 2 depth intervals ($n=6$). For July 2004, the average is for 2 cores and 2 depth intervals ($n=4$). Values within the same metal fraction with the same smaller case letter were not statistically different based on ANOVA and pair-wise differences ($\alpha=0.05$). nd, not detected.

TABLE 4 Environmental characteristics of sampling sites

Site ID	pH	total N (mg/kg)	Water content* (%)				DOC (mM C)		TOC (mg/kg)	
			2000	2000	2004	2004	2000	2004	2000	2004
Site 1	7.8 ± 0.1 ^a	4,313 ± 588 ^a	56% ± 2% ^a	58% ± 3% ^a	1.77 ± 0.06 ^a	1.29 ± 0.03 ^a	53000 ± 7000 ^{ab}	12900 ± 900 ^a		
Site 2	7.9 ± 0.2 ^a	4,822 ± 495 ^a	57% ± 3% ^{ab}	61% ± 4% ^a	1.80 ± 0.19 ^a	1.79 ± 0.46 ^a	57000 ± 3000 ^a	15200 ± 4200 ^a		
Site 3	7.7 ± 0.1 ^{ab}	4,050 ± 487 ^a	64% ± 4% ^{cd}	73% ± 3% ^c	1.65 ± 0.33 ^a	1.57 ± 0.15 ^a	43000 ± 5000 ^{bc}	14100 ± 600 ^a		
Site 4	7.6 ± 0.1 ^b	4,378 ± 391 ^a	70% ± 2% ^c	71% ± 2% ^c	1.25 ± 0.26 ^a	1.50 ± 0.16 ^a	47000 ± 4000 ^{bc}	13400 ± 700 ^a		
Site 5	7.8 ± 0.2 ^{ab}	3,567 ± 792 ^a	62% ± 2% ^{bd}	68% ± 2% ^{bc}	1.36 ± 0.50 ^a	1.40 ± 0.04 ^a	40000 ± 6000 ^c	14000 ± 500 ^a		
Site 6	ns	ns	ns	67% ± 1% ^{bc}	ns	1.25 ± 0.08 ^a	ns	12400 ± 1000 ^a		
Site 7	ns	ns	ns	60% ± 2% ^{ab}	ns	1.36 ± 0.04 ^a	ns	13300 ± 2600 ^a		

Notes: Values are the average ± the average deviation from the mean. For September 2000, the average is for 3 cores and 2 depth intervals (n=6). For July 2004, the average is for 2 cores and 2 depth except values for moisture content at sites 6 and 7 (n=2) due to samples lost in processing. Values with the same smaller case letter in the same column were not statistically different based on ANOVA and pair-wise differences ($\alpha=0.05$); ns, sites 6 and 7 were not sampled in 2000; * All samples were saturated.

TABLE 5 Multiple regression analysis of pore metals with biomass concentrations

	Coeff.	Std error	p-value	95% CI	
<i>DAPI cell count and pore metals</i>					
September 2000, n=30; adjusted R² = 0.84					
Intercept	0.130	0.066	0.0581	-0.0048	0.2651
Zinc	-0.048	0.009	<0.0001	-0.0663	-0.0290
Arsenic	-0.114	0.018	<0.0001	-0.1516	-0.0756
Manganese	0.0136	0.0023	<0.0001	0.0089	0.0183
<i>Total phospholipid and pore metals</i>					
September 2000, n=30; adjusted R² = 0.86					
Intercept	1.81	0.06	<0.0001	1.682	1.944
Zinc	-0.071	0.009	<0.0001	-0.0886	-0.0524
Arsenic	-0.116	0.018	<0.0001	-0.1529	-0.0791
Manganese	0.0164	0.0022	<0.0001	0.0118	0.0210
July 2004, n=28; adjusted R² = 0.57					
Intercept	2.56	0.03	<0.0001	2.495	2.615
Zinc	-0.237	0.089	0.0138	-0.4210	-0.0526
Arsenic	-0.344	0.137	0.0188	-0.6266	-0.0622
Combined 2000 and 2004 data, n=58; adjusted R² = 0.61					
Intercept	2.39	0.27	<0.0001	2.338	2.447
Zinc	-0.101	0.017	<0.0001	-0.1349	-0.0673
Arsenic	-0.140	0.035	0.0002	-0.2110	-0.0698

Note: Multiple regression with stepwise exclusion. Initial metals included were Zn, As, Cr, Cu, Pb, and Mn. Due to Kendall's rank co-variance with Zn, Pb was excluded from the year 2000 data correlations, Cd was excluded from the year 2004 data correlation, and both Pb and Cd were excluded from analysis of the combined data sets.

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