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

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Abstract

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

1. Introduction

Although the impact of metals on plants and animals is well documented [e.g., *Posthuma and Vanstraalen*, 1993; *Tyler et al.*, 1989], the relationship between metal contamination and microbial communities is more ambiguous. While metal contamination would generally be anticipated to have a negative impact on a biological system, in aerobic systems such as soils often no biomass changes are observed [e.g., *Bååth et al.*, 1998; *Feris et al.*, 2003; *Knight et al.*, 1997]. As a consequence, it has been hypothesized that whole-community biomass recovery will occur during long-term metals exposure [*DiazRavina and Bååth*, 1996]. Still, in other studies, metal contamination has been associated with decreased soil microbial biomass [e.g., *Konopka et al.*, 1999; *Pennanen et al.*, 1996]. These apparently contradictory observations reflect, in part, the interference by environmental variables other than metal contamination that also influence microbial populations such as soil texture [*Albrechtsen and Winding*, 1992; *England et al.*, 1993], nutrient availability [*Albrechtsen and Winding*, 1992] and area vegetation [*Kandeler et al.*, 2000; *Pennanen et al.*, 1996].

There is also substantial controversy regarding the toxicity of different metal forms [e.g., *Ankley et al.*, 1996; *Worms et al.*, 2006] and there is no consensus regarding appropriate metal fractions for measurement. Free metal ions are often implicated as the most toxic forms [e.g., *Menkissoglu and Lindow*, 1991; *Poulson et al.*, 1997], as originally suggested by Sunda and Guillard [1976]. However, other dissolved-phase metal complexes have been reported to exert greater toxicity [*Errecalde and Campbell*, 2000; *Sarin et al.*, 2000], supporting theories that metal-ion concentration alone may not be able to predict toxicity [e.g., *Hudson*, 1998; *Pinheiro and van Leeuwen*, 2001]. Still, many field studies rely on total metal concentrations to assess

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

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

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

2. Methods

2.1 Site description

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

2.2 Sample collection – preliminary study (June 2000).

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

2.3 Sample collection – September 2000

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

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

2.4 Sample collection – July 2004

Duplicate cores from sites 1 through 7 (Figure 2) were collected and sediments from the 0-2cm and 4-6cm intervals were extruded as described for the September 2000 sampling event. Samples were preserved on-site for PLP as described below, and the remainder of the sediment was transported and processed as described for September 2000.

2.5 Basic sample characterization

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

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

2.6 Metals analysis

2.6.1 Pore water metal analysis.

Pore waters were filtered (0.22 µm cellulose acetate, Millipore), and preserved using trace metal grade HNO₃ (2% final concentration). Samples were diluted with de-ionized water (Milli-Q RG) to fit the instrumental analytical window, and maintained at the same final pH by addition of HNO₃. Dissolved pore water metals (As, Cd, Cr, Cu, Mn, Pb and Zn) were analyzed in accordance with EPA Method 200.8 [1991] using a VG elemental inductively coupled plasma-mass spectrometer (ICP-MS) Model PQ ExCell.

2.6.2 Total metal analysis

Total acid leachable (“total”) metal concentrations were determined using a concentrated (70%) HNO₃ extraction of oven dried sediments. The supernatant was filtered (0.22 µm cellulose acetate, Millipore) and metals (Cd, Cu, Mn, Pb and Zn) were quantified by flame atomic absorption spectroscopy (FAAS) using a GBC model 920 in year 2000, and using a Thermo Jarrell Ash Autoscan 25 scanning inductively coupled plasma atomic emission spectrometer (ICP-AES) in year 2004.

2.6.3 Sequential metal extraction

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

“exchangeable” fraction, 8 ml of 1M MgCl_2 (pH 7.0); step 2) “carbonate-bound” fraction, 8 ml of 1M NaCH_3COO (pH 5.0); step 3) “Fe & Mn-oxide-bound” fraction, 20 ml of 0.04 M hydroxylamine hydrochloride in 25% (v/v) acetic acid; step 4) “organic and sulfide-bound” fraction, 3 ml of 0.02 M HNO_3 and 5 ml of 30% H_2O_2 (pH 2) at 85°C for 2 hours, an additional 3 ml of 30% H_2O_2 at 85°C for 3 hours with intermittent shaking, and 5 ml of 3.2 M $\text{NH}_4\text{CH}_3\text{COO}$ in 20% (v/v) HNO_3 and 7 ml deionized water for 30 minutes; and step 5) “residual” fraction, 7.5 ml HCl (37%) and 2.5 ml of HNO_3 (65%) overnight at room temperature and then heated for 2 hours under reflux. Sediment and liquid manipulations for the first three extraction steps were performed in an anaerobic chamber to prevent chemical oxidation of reduced metal species [Wallmann *et al.*, 1993]. Between steps, the sediments were washed with de-ionized water. Following each step the filtrate (0.22 μm multi-cellulose esters, Millipore) was collected and metals (Cd, Cu, Mn, Pb and Zn) were quantified using FAAS. The indicated classifications specify metal fractions typically extracted during each step, though some portion of a metal fraction might be released in earlier steps [Peltier *et al.*, 2005].

2.6.4 X-ray absorption fine structure (XAFS)

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

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

2.7 Direct cell counts.

Sediment samples for microscopic cell counting were preserved on-site, in duplicate by diluting 1:40 (v/v) in filtered (0.22 µm pore size) phosphate-buffered paraformaldehyde fixative solution [Gough and Stahl, 2003], which was made within 24 hours prior to field sampling. Preserved samples were transported, stored, and prepared for analysis as previously described [Gough and Stahl, 2003], using 1:3000 as a final dilution. Samples were arbitrarily grouped (“blocked”) and analyzed in a blocked format to facilitate monitoring of experimental bias [Wardlaw, 1985]. Slides were counted within one day of preparation to minimize loss of DAPI (4'-6-diamidino-2-phenylindole) fluorescence using a Zeiss Axioplat microscope (1,000x magnification) equipped with UV fluorescence and an ocular grid. A minimum of either 100 bacteria or 30 grid fields were counted for each slide. Results from duplicate block analyses were evaluated using analysis of variance (ANOVA) to monitor for experimental method variance.

2.8 Phospholipid phosphate

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

2.9 Data analysis

2.9.1 Descriptive statistics

Deviation of replicates was evaluated by calculating the mean deviation (MD, sometimes called 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 replicate, $\bar{x}$ is the average of the replicates, and n is the number of replicates. Constancy of a parameter among sampling sites was evaluated by calculating the coefficient of variation. Relationships between parameters were evaluated using the Pearson Correlation. Data sets were linearized by logarithmic transformation prior to linear regression analysis when necessary. The 95% confidence intervals (CI) on linear regression slopes were evaluated, and correlations whose slope CI did not intersect zero were considered to represent a statistically significant trend. Data points whose absolute normalized residuals were greater than 3 were considered outliers, and were removed from correlation analysis [Zar, 1984]. The influence of individual parameters was considered by comparing correlation analyses both including and excluding extreme values.

ANOVA with pair-wise comparisons (i.e. Tukey's method) was used to monitor for significant differences among the sampling sites. Data were logarithmically transformed to account for uneven data variance (i.e. heteroscedasticity). Data were compared between the sampling years using a paired t-test.

2.9.2 Multiple regression analysis

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

3. Results

3.1 Preliminary sediment characterization and site selection.

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

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

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

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

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

3.2 Microbial biomass

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

3.3 Metal concentrations

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

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

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

3.4 Sediment characteristics

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

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

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

4.0 Discussion

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

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

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

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

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

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

5. Conclusions and Implications

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

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

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FIGURE CAPTIONS

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

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

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

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

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

Figure 6. Sediment microbial biomass (as phospholipid phosphate) versus pore water zinc concentration. Error bars show mean deviation of two depth intervals in replicate cores (3 cores 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.
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Fig 1

Fig 2

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Fig 4

Fig 5

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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 ± 0.30	3,472 ± 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												18%
												42%
												103%
												214%
												144%
												143%
												61%
												19%
												2%
												12%

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