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Cu and Zn Concentration Gradients Created by Dilution of pH Neutral Metal-Spiked Marine Sediment: A Comparison of Sediment Geochemistry with Direct Methods of Metal Addition

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Received October 23, 2007. Revised manuscript received February 1, 2008. Accepted February 5, 2008.

The geochemistry of artificially metal contaminated sediments prepared using three methods of metal-spiking was compared in this study. Marine sediments with a gradient of Cu and Zn concentrations were prepared by direct-spiking without and with pH-adjustment to pH 7, and also by serial dilution of direct-spiked sediment (4000 $\mu\text{g g}^{-1}$, pH 7 adjusted) with uncontaminated sediment. Porewater concentrations of Cu, Zn, Fe, and Mn in direct-spiked sediments without pH adjustment were orders of magnitude higher than the equivalent sediments adjusted to pH 7 or those prepared by the serial dilution method. Despite pH-adjustment, porewater Cu and Zn concentrations of direct-spiked sediment remained higher than concentrations observed within metal-contaminated natural sediment. The serial dilution of metal-spiked, pH-adjusted sediment substantially decreased Cu and Zn partitioning to the dissolved phase, and minimized the variation of potential competitive ions (H^+ , Fe^{2+} , Mn^{2+}) over the entire gradient of spiked Cu and Zn concentrations. Metal concentration gradients created using serial dilution of Cu- and Zn-spiked, pH-adjusted sediments produced porewater Cu or Zn, Fe, and Mn concentrations that resemble sediment–porewater partitioning (Cu, Zn, Fe, Mn) typical of metal-contaminated natural sediments. This method is recommended for whole-sediment toxicology studies.

Introduction

To assess the effect of metal contamination on benthic ecosystems, and to enable development of appropriate sediment guidelines, an understanding of the metal concentration–response of a range of organisms is required. Concentration–response data are acquired through measuring the biological response of organisms following exposure to sediments that have a graduated range of metal concen-

trations. The use of metal-spiked sediment for this purpose has raised some concerns, however, due to the geochemical changes induced and the inability to replicate field conditions (1). Decreasing pH, due to hydrolysis of aquo–metal complexes, is frequently reported following artificial metal spiking (2–4) with consequent increases in redox potential (5) and porewater metal concentrations (6, 7).

Adjusting sediment pH is a means of minimizing the geochemical changes induced during preparation of metal-spiked sediment (2, 3). A higher pH decreases the quantity of spiked metal in the ionized dissolved form and thus decreases metal fluxes to the porewaters (3), however, dissolved metal concentrations generally far exceed those of in situ metal-contaminated sediment (4). Furthermore, following the equilibration of pH-adjusted sediment, there remains a broad range in pH, Eh, porewater Fe and Mn over the spiked metal concentration gradient (i.e., Cu or Zn 0–4000 $\mu\text{g g}^{-1}$ spiked pH 7 adjusted sediment: pH 6.7–7.3; Eh 200–330 mV; 0.2–13 mg L^{-1} porewater Fe; 0.2–2.0 mg L^{-1} Mn) (3, 7). This variability affects organism exposure and response to metals and obscures the interpretation of concentration–response relationships.

An augmentation of binding phases in metal-spiked sediment, by addition of organic (8, 9) or sulfidic material (10, 11), can decrease the soluble metals available for hydrolysis and minimize pH changes. However, altering the prevalence of these binding phases relative to the original field sediment may be undesirable. Serial dilution of metal-contaminated field sediment with sediment that contains relatively low metal concentrations provides an alternate means of producing concentration gradients, representative of in situ contamination (12). However, the use of contaminated field sediment to obtain concentration–response data for individual metals is usually inappropriate, as these sediments generally contain other toxicants that can exert synergistic or antagonistic effects on organism toxicity (13, 14). To obtain concentration–response data for individual metals, the use of metal-spiked sediments is unavoidable. To date, where sediment dilution has been applied to create concentration gradients in metal-spiked sediment, very little information has been reported on the resulting sediment geochemistry, other than dissolved metals (overlying water) and total spiked-metal concentrations (15, 16).

This study contrasts the different geochemical responses of Cu- or Zn-spiked marine sediments prepared by directly spiking sediments with a graduated range of metal-spike concentrations (i) without and (ii) with pH-adjustment to pH 7, and by (iii) serial dilution of high-concentration direct-spiked sediment with uncontaminated sediment. Changes in sediment pH, redox potential, porewater metals (Cu, Zn, Fe, Mn), particulate metals, acid-volatile sulfide (AVS), and organic carbon were measured.

Materials and Methods

General Methods and Reagents. All plasticware and glassware were acid-washed (10% HNO_3 AR grade), rinsed with deionized water (Milli-Q Element, 18 M Ω), dried in a Class-100 laminar flow cabinet, and stored in sealed plastic bags until use. Deoxygenation of water was performed by bubbling high-purity nitrogen through the solution for greater than 12 h. High-purity acids (SupraPur, Merck) were used in metal digestions of sediments and to preserve samples and standards. All other substances used were of analytical reagent grade.

Geochemical Measurements. A pH/redox/temperature meter (TPS LC-80A) was used to measure pH (Orion sure-

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flow, 8165BN) and redox potential (Activon, AEP511) with previously described procedures (4). Sediment moisture content, total organic carbon (TOC) by high-temperature analyzer (Tekmar-Dohrmann DC-190), and particle size were measured. Acid-volatile sulfide (AVS) was extracted (17) and measured (18) as described previously. Acid-extractable metals (AEM) used a 1 M HCl extraction, equivalent to the commonly measured simultaneously extracted metals (SEM) fraction, and porewater metals were obtained as described previously (4). Dissolved metal concentrations were measured using inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500a). The sediment–porewater partition coefficients (K_d , L kg⁻¹) were calculated using the porewater and AEM concentrations. Ten percent of all samples were measured in duplicate. Adequate sample blanks, spike-recoveries, and certified reference materials (95–105% recovery for PACS-2, NRC, Canada) were achieved as part of quality control (4).

Sediment Collection and Metal-Spiking Procedures. Sediment was collected (~30 kg, 0–15 cm depth) from Wellington Point (WP), an intertidal location on Moreton Bay (QLD, Australia) with low background metal concentrations, 2–3 m inside the edge of the mangrove forest. The sediment was sieved (2 mm), the larger fraction was discarded, and the remainder was allowed to consolidate in the dark at 4 °C under 30 cm of seawater for 2 weeks. Clean seawater for all further laboratory manipulations was collected from Main Beach (QLD, Australia).

Metal-spiking, pH-adjustments, and all other sediment manipulations were conducted in a glovebox under a nitrogen atmosphere. Three procedures were used to prepare the concentration gradients of Cu and Zn: direct-spiking without (Direct) and with pH-adjustment (Direct pH 7), and serial dilution of metal-spiked sediment (4000 µg g⁻¹) with pH-adjustment (Dilution pH 7). For Direct (4) and Direct pH 7 (8) concentration gradients, copper (CuCl₂·2H₂O) and zinc (ZnCl₂, anhydrous) metal salt solutions were prepared in deoxygenated water, and mixed with 2 kg of sediment (4:1 sediment/water ratio) and rolled for 2 h to achieve concentrations of 0, 200, 1000, and 4000 µg g⁻¹ Cu or Zn. Metal-spiked sediments undergoing neutralization were equilibrated for 24 h prior to pH adjustment with 1 M NaOH. Preliminary studies with WP sediment determined that adjustment to pH 8 generated a sediment of approximately pH 7 following equilibration.

For sediment dilution experiments (Dilution pH 7), sediment was spiked to a concentration of 4000 µg Cu or Zn g⁻¹, adjusted to pH 8 with 1 M NaOH, and equilibrated for a period of 25 days (final pH ~ 7). This sediment was then serially diluted with unmodified (without added metals) WP sediment (previously sieved and stored at 4 °C) to achieve metal concentrations of 200, 500, 1000, and 2000 µg g⁻¹. The mixing/dilution process resulted in a minor pH decrease, and a small additional pH adjustment at the time of sediment dilution was necessary to obtain pH 7.

Subsamples of Cu- and Zn-spiked sediments prepared by all three methods were transferred to 120-mL vials, capped with a nitrogen gas headspace, and equilibrated for a duration of 1, 3, 5, 10, 20, and 40 days prior to analysis. After each equilibration period, a sample for each of the three metal-spiking procedures at each concentration was analyzed for pH, redox potential, porewater metals, AEM, AVS, and TOC. During the equilibration period a small volume of supernatant developed as sediments settled in their containers. Following pH and redox measurements, this supernatant was removed prior to porewater extraction and sediment analysis.

Results and Discussion

Wellington Point (WP) sediment was suboxic (redox potential of 35 to -15 mV) with a pH of 6.9–7.1 following sieving and

storage for 2 weeks at 4 °C. The particle size distribution of WP sediment (34.4% <38 µm, 6.5% 38–63 µm, 21.6% 63–180 µm, 30.6% 180–600 µm, and 6.8% >600 µm) and the content of AEM-Fe (7900 µg g⁻¹), TOC (1.9%), and AVS (<0.05 µmol/g) suggested a relatively low metal-binding capacity. Sediment metal AEM concentrations (in µg g⁻¹) taken before metal addition were 0.2 (Cd), 4.5 (Cu), 6.9 (Mn), 39 (Ni), 6.0 (Pb), and 10.8 (Zn). The porewater metal concentrations (in µg L⁻¹) were <1.8 (Cd), 7.6 (Cu), 500–1500 (Fe), 30–120 (Mn), 7.2 (Ni), <5.4 (Pb), and <25 (Zn). Sediment sieving and storage was likely to have disturbed sediment redox conditions and sediment–porewater metal partitioning from that of the in situ sediment. Throughout all experiments, measured concentrations of TOC, AVS, and AEM-Fe and Mn did not change significantly (±10%). Similarly, AEM-Cu and Zn did not deviate significantly (±10%) from the nominal spiked concentrations.

pH and Redox Potential. For Direct and Dilution spiking methods, the sediment pH stabilized (±2–7%) 3–10 days following metal addition (Figure S1). pH decreased substantially for the Direct method of metal addition for both Cu- and Zn-spiked sediments (to 6.6 and 5.8 for 200 and 1000 µg Cu g⁻¹ and to 6.7 and 6.1 for 200 and 1000 µg Zn g⁻¹ at day 10 (Figures 1a and 2a). pH-adjustment of metal-spiked sediment in both Direct pH 7 and Dilution pH 7 methods effectively neutralized sediments across the metal concentration gradient. Over the Cu concentration series, the Dilution (pH 7) method produced an equivalent or slightly smaller range in pH (0.46 pH units) than the Direct pH 7 method (0.54 pH units). A similar range of pH was observed for Zn Direct pH 7 and Dilution methods (Figure 2a).

The pH decrease observed over the concentration gradient (0–2000 µg g⁻¹) in whole sediment toxicity tests using Direct (non pH-adjusted) metal-spiked sediments (1.21 and 0.85 pH units for Cu- and Zn-spiked sediments, respectively) is likely to affect metal exposure, uptake, and organism response (19). The decreased pH augments metal availability (dissolved metals), however, it may also simultaneously increase H⁺ ion competition for metal binding sites on biotic ligands (organism binding sites) (20). Conversely, at higher pH, despite lower porewater metal concentration, decreased H⁺ competition can produce an increase in the biota bound metals and thus an enhanced metal uptake and toxic response (21).

Redox potential for direct and dilution methods of Cu and Zn addition were between 200 and 400 mV (data not shown). For all experiments, the redox potential after 40 days was greater than that of the natural WP sediment (-15 to 35 mV), indicating a degree of sediment oxidation during sediment manipulation despite nitrogen headspace. Despite sediment sieving (2 mm) and homogenization, microenvironments of oxidized and reduced zones develop within the sediment due to differences in bacterial activity and the availability of organic matter (22). Redox microenvironments may be more likely using the Dilution method as the longer duration of sediment storage prior to dilution (i.e., additional 25 days) allows greater microbial degradation (3) and oxidation of organic material, reducing a series of electron acceptors in the sediment.

Porewater Cu and Zn. Porewater metal concentrations (Cu and Zn) generally stabilized (±5–10%) within 5–10 days of metal additions (Figure S2). As porewater Cu and Zn varied minimally after this time, porewater metal concentrations of the three metal-spiking methods will be principally compared after 10 days equilibration. The primary exception to this was in Direct pH 7 1000 µg Zn g⁻¹ sediments, in which dissolved Zn decreased 70% (4.6 to 1.4 mg L⁻¹) between 5 and 40 days. Borgmann and Norwood (15) observed a similar stabilization of dissolved Pb concentrations within a week in artificial sediments created using dilution of a Pb-spiked sediment, with

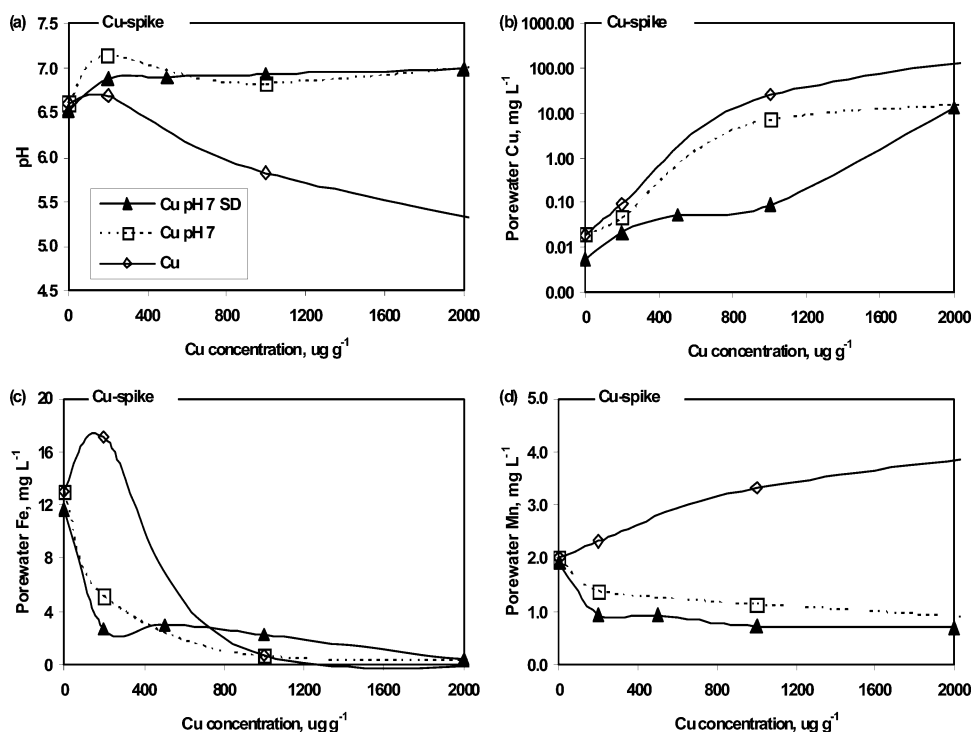


FIGURE 1. Copper-spiked sediments prepared using Direct (\diamond , no pH adjustment), Direct (\square) pH 7, and Dilution (\triangle , pH 7) methods. Sediment pH (a) and porewater concentrations of Cu (b), Fe (c), and Mn (d) after 10 days equilibration for additions of 0–2000 $\mu\text{g g}^{-1}$. Standard deviations for duplicate experiments were 0.04 pH units, <14.4%, <14.9%, and <7.2% for pH, Cu, Fe, and Mn, respectively.

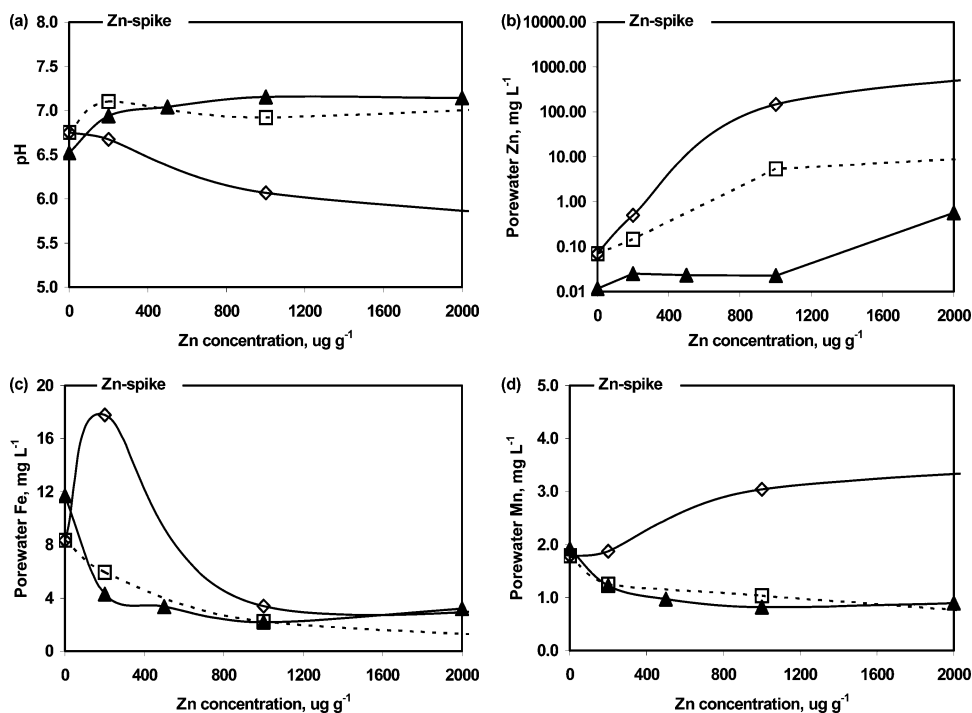


FIGURE 2. Zinc-spiked sediments prepared using Direct (\diamond , no pH adjustment), Direct (\square) pH 7 and Dilution (\triangle , pH 7) methods. Sediment pH (a) and porewater concentrations of Cu (b), Fe (c), and Mn (d) after 10 days equilibration for additions of 0–2000 $\mu\text{g g}^{-1}$. Standard deviations for duplicate experiments were 0.05 pH units, <15.0%, <12.2%, and <14.0% for pH, Cu, Fe, and Mn, respectively.

dissolved Pb concentrations declining after 2 weeks. They suggested that the late decrease in the dissolved Pb concentration may be a result of degradation of DOC limiting its capacity and extent of metal binding. However, despite the continued decrease in porewater Zn in Direct pH 7 1000 $\mu\text{g Zn g}^{-1}$ experiments, dissolved Zn concentrations at day 40 remained

more than 10-fold greater than in Dilution experiments at day 10 (i.e., 35 days after metal addition) (Figure S2).

Direct metal-spiked sediments without pH adjustment developed high concentrations of porewater metals and poorly resembled those typically observed for in situ metal-contaminated sediment (Figures 1b and 2b). Dissolved Cu

and Zn for 200 $\mu\text{g g}^{-1}$ (Cu or Zn) treatments were between 0.092 and 0.158 mg L^{-1} and 0.50 and 2.20 mg L^{-1} , respectively, and for 1000 $\mu\text{g g}^{-1}$ experiments were 26–59 mg L^{-1} and 100–260 mg L^{-1} , respectively. Dissolved Cu concentrations of between <0.001 and 0.070 mg L^{-1} with surface maxima of 0.25 mg L^{-1} and 0.52 mg L^{-1} (23) have been reported in highly contaminated (500–1500 $\mu\text{g Cu g}^{-1}$) field sediments with similar organic carbon and AVS content (<0.5 $\mu\text{mol g}^{-1}$ AVS and 1–2% organic carbon) as WP, yet greater silt and Fe content (70–75% <63 μm particle size and <100 mg g^{-1} total particulate Fe content). Such porewater Cu concentrations, which are rarely reported above these values for field-contaminated sediments (24–26), are 100-fold less than those observed using the Direct method without pH adjustment.

Sediment adjustment to pH 7 substantially decreased the flux of spiked metals to the porewater for Cu- and Zn-spiked sediment (0.046 and 0.15 mg L^{-1} for 200 $\mu\text{g g}^{-1}$ Cu- and Zn-spiked sediment, respectively, for the Direct pH 7 method). However, at higher spike concentrations (1000 $\mu\text{g g}^{-1}$) porewater metal concentrations remained between 1 and 10 mg L^{-1} (7.4 and 4.6 mg L^{-1} for Cu and Zn, respectively). Metal-spiked sediment prepared using the Dilution pH 7 method had markedly lower porewater metal concentrations for Cu- and Zn-spiked sediments (0.021 and 0.088 mg L^{-1} for Cu 200 and 1000 $\mu\text{g g}^{-1}$, respectively, and 0.024 and 0.023 mg L^{-1} for Zn 200 and 1000 $\mu\text{g g}^{-1}$, respectively). The additional 25 days of equilibration prior to sediment dilution in metal-spiked sediment prepared using the Dilution method does not account for the lower porewater metal concentrations observed using the Dilution method. Dissolved metal concentrations measured after 40 days in the Direct (0.093 and 34 mg L^{-1} for Cu 200 and 1000 $\mu\text{g g}^{-1}$, respectively, and 0.73 and 100 mg L^{-1} for Zn 200 and 1000 $\mu\text{g g}^{-1}$, respectively) and the Direct pH 7 (0.048 and 6.44 mg L^{-1} for Cu 200 and 1000 $\mu\text{g g}^{-1}$, respectively, and 0.38 and 1.4 mg L^{-1} for Zn 200 and 1000 $\mu\text{g g}^{-1}$, respectively) methods remained substantially higher than the equivalent treatments for the Dilution method.

The lower porewater metal concentrations in diluted sediment are reflected in sediment–porewater partition coefficients ($K_d = \text{AEM/PW}$) (Table S1). Sediment K_d values representative of naturally metal-contaminated marine environments (in situ), are typically between 1×10^3 to $1 \times 10^5 \text{ L kg}^{-1}$ (27, 28). For Direct metal-spiked sediments, K_d values (day 10) were in the range 1×10^1 to $3 \times 10^3 \text{ L kg}^{-1}$ (3×10^3 and 4×10^1 for Cu 200 and 1000 $\mu\text{g g}^{-1}$, respectively, and 4×10^2 and 1×10^1 for Zn 200 and 1000 $\mu\text{g g}^{-1}$, respectively). pH adjustment increased the sediment-bound metals in Direct pH 7 experiments (5×10^3 and 2×10^2 for Cu 200 and 1000 $\mu\text{g g}^{-1}$, respectively and 1×10^4 and 2×10^2 for Zn 200 and 1000 $\mu\text{g g}^{-1}$, respectively), yet K_d remained well below $1 \times 10^4 \text{ L kg}^{-1}$ (Table S1). In previous studies employing metal-spiked sediments to concentrations of $\sim 500 \mu\text{g g}^{-1}$, K_d values of the sediments have rarely been greater than $1 \times 10^4 \text{ L kg}^{-1}$ (9, 10) despite large ranges in AVS (<0.5–30 $\mu\text{mol g}^{-1}$) and TOC (0.8–12%) content. A higher degree of metal partitioning to the sediments is often observed following the amendment of sediment with organic matter (30) or sulfide (10, 31) metal-binding phases, or in the case of sediments with naturally high AVS concentrations (1, 25, 27). For the Dilution experiments the metal partitioning between porewater and sediments was similar to that observed for naturally contaminated systems (1×10^4 for Cu 200 and 1000 $\mu\text{g g}^{-1}$, respectively and 0.9×10^4 and 4×10^4 for Zn 200 and 1000 $\mu\text{g g}^{-1}$, respectively), without additional organic or sulfide amendments beyond that of the original sediment characteristics.

A higher degree of metal-partitioning to the particulate phase was observed for 0–1000 $\mu\text{g g}^{-1}$ Dilution treatments than 2000 $\mu\text{g g}^{-1}$. The dilution of directly spiked sediment with metal-free sediment provides additional metal binding sites, with little change in the circumneutral pH. This sudden

influx of new metal-binding phases induces the rapid adsorption of free metal ions in the porewater. High porewater Cu and Zn concentrations occurred in sediments prepared by Dilution method at Cu and Zn concentrations greater than 1000 $\mu\text{g g}^{-1}$ (Figures 1b and 2b), and were due to the exhaustion of newly available metal-binding phases in added WP sediment. Similar saturation of binding phases has been observed in previous studies using sediment dilution of metal-spiked sediment, with threshold sediment metal concentrations of $\sim 1000 \mu\text{g g}^{-1}$ (15, 16). Moreover, when the 4000 $\mu\text{g g}^{-1}$ directly spiked sediment was diluted to 2000 $\mu\text{g g}^{-1}$, porewater metals did not reach a stable state, even after 40 days, indicating a similar saturation of metal-binding sites. Disturbance of weakly bound metal phases (particularly CuOH) during sediment dilution, which are likely to be saturated at high spike concentrations (1), and the gradual re-equilibration of these phases may contribute to the changing porewater Cu or Zn following sediment dilution.

Numerous studies and models (i.e., the free ion activity model) highlight the importance of dissolved metals in determining metal bioavailability, accumulation, and toxic response of test organisms (32, 33). While it is now widely recognized that the extent to which free ions bind to receptor sites on the biotic ligand and competition at the receptor further regulate metal accumulation and toxic response, the dissolved metal concentration remains a crucial determinant of potential metal uptake (34). Thus, the large variations in porewater metal partitioning observed using the three methods of metal-spiking sediment is likely to substantially dictate the resultant toxicities observed in toxicity tests using these sediments.

Porewater Mn. Porewater Mn concentrations in metal-spiked sediments had generally stabilized after 5–10 days equilibration. The concentrations were predominantly dictated by changes in the sediment pH (porewater Mn = $-1.52 \text{ pH} + 11.80$, $n = 144$, $r = -0.927$). In Cu- and Zn-spiked sediments (Direct method), dissolved Mn concentrations increased over the metal concentration gradients (2.0 to 3.0 and 1.8 to 3.3 mg L^{-1} for 0 to 2000 $\mu\text{g g}^{-1}$ Cu and Zn, respectively) as a consequence of decreasing sediment pH (Figures 1d and 2d). Dissolved Mn, as a potential competitor of the spiked metal for binding sites, may affect test organism metal accumulation. Adjustment to pH 7 in the Direct and Dilution experiments lowered the variation in porewater Mn concentration over the spiked metal concentration gradients (0.7–1.4 mg L^{-1} porewater Mn, except 0 $\mu\text{g g}^{-1}$ sediments). By decreasing the range of porewater Mn concentrations over the spiked metal gradient (200–2000 $\mu\text{g g}^{-1}$), Direct pH 7 and Dilution methods reduce the possibility that changing porewater Mn concentrations could affect metal–biotic ligand interaction and metal accumulation in whole sediment toxicity tests.

Porewater Fe. Homogenization of sediment alone, during the metal addition process, initiates a release of iron to the porewater phase caused by a desorption of Fe from dissolved organic matter and solid phases, or an enhanced reduction of Fe(III) by microbes due to greater substrate availability (35). In uncontaminated laboratory-manipulated and natural sediment, this is accompanied by a subsequent decrease in porewater Fe and bacterial activity after approximately 15 days, attributed to substrate limitation and continued oxidation of Fe(II) and precipitation of Fe(III) (35). For Cu- and Zn-spiked sediments, a similar initial release of Fe to the porewater was generally observed, peaking day 5–20 and decreasing thereafter. This occurred for both the Direct and Dilution methods (Figure 3a and b), with the exception of Cu 1000 $\mu\text{g g}^{-1}$ Direct non-pH adjusted experiments. In Cu or Zn 200 $\mu\text{g g}^{-1}$ Direct pH 7 experiments, the peak porewater Fe concentrations of 5.4 mg L^{-1} (day 5) and 6.0 mg L^{-1} (day 10) for Cu and Zn, respectively, were substantially less than

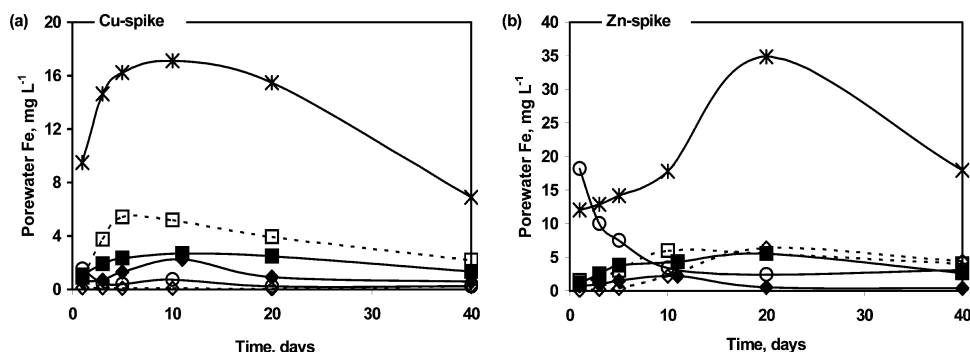


FIGURE 3. Porewater Fe for (a) Cu or (b) Zn spiking methods are shown: Direct non-pH adjusted (*○), Direct pH 7 (□◇), and Dilution (■◆). Metal concentrations = 200 $\mu\text{g g}^{-1}$ (*□) and 1000 $\mu\text{g g}^{-1}$ (○◇). Standard deviations for duplicate experiments were <14.9% and <12.2% for Cu and Zn experiments, respectively.

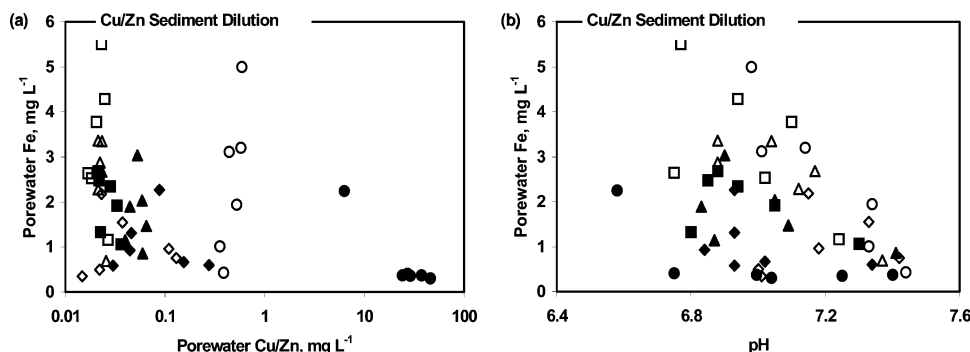


FIGURE 4. Pore-water Fe relation with porewater Cu or Zn (a), and pH (b) for Cu-spiked (closed symbol) and Zn-spiked (open symbol) sediment dilution experiments at spike concentrations of 200(□■), 500(△▲), 1000(◇◆), and 2000(○●) (days 1–40).

those in non pH-neutralized sediments (max 17.1 and 34.8 mg L^{-1} for 200 $\mu\text{g g}^{-1}$ Cu- and Zn-spiked sediment, respectively). For the Direct 1000 $\mu\text{g g}^{-1}$ Cu g^{-1} experiments, the porewater Fe concentration in both pH 7 and non pH-neutralized sediments were both relatively low (0.6–0.7 mg L^{-1}). The comparatively low concentration of Fe in the dissolved phase is likely due to an inhibition of the bacterial reduction of Fe(III) by toxic concentrations of dissolved Cu, as has been reported previously (4, 36). For the Direct 1000 $\mu\text{g Zn g}^{-1}$ experiments, at day 10 the non pH-neutralized experiment had a porewater Fe concentration of 3.4 mg L^{-1} compared to 2.2 mg L^{-1} for the Direct pH 7 experiments. With regard to Direct Zn-spiked pH 7 experiments, the porewater-Fe versus time profiles were similar for the 200 and 1000 $\mu\text{g Zn g}^{-1}$ experiments, however, the peaks in porewater Fe of 6.0 and 6.4 mg L^{-1} occurred on days 10 and 20 for 200 and 1000 $\mu\text{g g}^{-1}$ Zn pH 7 experiments, respectively. Toxicity of porewater metals to Fe(III)-reducing bacteria has been previously shown to slow the rate of bacterially mediated Fe(III) reduction, but has no significant effect on the total amount of Fe reduced over longer time periods (36).

The Dilution method similarly resulted in the release of porewater Fe concentrations, which decreased after 20 days to that of Direct pH 7 experiments, however, exhibited a smaller range of porewater Fe concentrations over the spiked metal concentration gradient (Figures 1c and 2c). This was a product of decreased variation in pH and porewater spiked metal concentrations over the concentration gradient (with the exception of Zn 200 and Cu 2000 $\mu\text{g g}^{-1}$ spike concentrations), two factors known to influence porewater Fe concentrations. For the 2000 $\mu\text{g Cu g}^{-1}$ Dilution experiment, the porewater Cu concentration was generally > 10 mg L^{-1} and porewater Fe concentration was less than 0.40 mg L^{-1} (Figure 4a), suggesting inhibition of bacterially mediated Fe(III) reduction. The one exception in which Fe concentration was 2.2 mg L^{-1} at a dissolved Cu concentration of 6.3 mg L^{-1} (Dilution 2000 $\mu\text{g Cu g}^{-1}$ day 40) was associated with a decrease in pH

to 6.6. While, Zn has similarly been shown to exhibit toxic effects on bacteria (37), this was not apparent in Zn Dilution experiments (Figure 4a). pH appears to have had a greater influence on porewater Fe concentrations (porewater Fe = 4.84 pH + 36.7, $r = 0.652$, $n = 24$, Figure 4b, open symbols) than the concentration of dissolved Zn. Inhibition of bacterially mediated Fe(III) reduction in Dilution experiments due to dissolved Zn is less probable due to requirement at greater concentrations for bacterial metabolism.

Unlike pH and porewater spiked metals, porewater Fe concentrations did not stabilize (Direct, Direct pH 7, Dilution pH 7) even after a 40-day equilibration period. Nonetheless, Figures 1c and 2c provide a comparison of porewater Fe concentrations across Cu and Zn concentration gradients after 10 days equilibration, by which time the pH and porewater spiked metal concentrations had generally stabilized. For Direct Cu- and Zn-spiked (non-neutralized) sediment, in which pH and porewater spiked metal concentrations varied greatly over the concentration gradient, so too did porewater Fe (from 0.7 to 17.1 and 3.4 to 17.8 mg L^{-1} for Cu and Zn, respectively). Changing porewater Fe concentrations are likely to affect the toxic response of test organisms both indirectly and directly. Greater concentrations of dissolved Fe affect porewater metal concentrations via competition for binding sites on nonbiotic ligands (i.e., OC, DOC, particulate matter) (38). Simultaneously, Fe competition for binding at the receptor site is likely to decrease metal toxicity. However, elevated porewater Fe concentrations may increase internalized free Fe, known to promote the production of deleterious hydroxyl free radicals via the Fenton reaction (39).

Variation in porewater Fe over the spiked metal concentration gradient, and thus the potential competitive interference that this may have on organism metal accumulation and subtoxic/toxic response, was progressively minimized using the Direct pH 7 method and the Dilution method. Porewater Fe concentration profiles that developed in

sediment using the Dilution method were particularly stable between 200 and 1000 $\mu\text{g g}^{-1}$ for Cu and Zn which is within observed in situ metal contamination ranges (23).

The Dilution method of metal addition, therefore, decreased both the range of porewater Fe, Mn, and H^+ over the spiked Cu or Zn concentration gradient and the partitioning of spiked metals to the dissolved phase relative to Direct spike methods. It is thus our recommendation as the preferential means of metal spiking sediment. To what extent the means of sediment metal addition affects metal accumulation and organism response, however, remains speculative and is the focus of ongoing research.

Acknowledgments

Greatly appreciated technical assistance was provided by K. O'Halloran (ICP-MS). An Australian Postgraduate Awards grant provided the basis of funding for this work.

Supporting Information Available

pH and porewater spiked-metal profiles (40 days) for Direct, Direct pH 7, and Dilution pH 7 methods are provided in Figures S1 and S2, and partition coefficients (K_d) for Dilution pH 7 experiments are provided in Table S1. This information is available free of charge via the Internet at <http://pubs.acs.org>.

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