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Chemical Changes in an Estuarine Sediment During Laboratory Manipulation

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Estuarine sediments serve as natural sinks for aquatic contaminants. The effects of contamination on the associated ecosystem are often assessed by simple chemical analysis, though this has proven unreliable to date (O'Connor and Paul 2000). One alternative is sediment bioassays, where the response of a test organism to contaminated sediment is assessed under controlled laboratory conditions. While such tests have many practical advantages, they are often criticised for a lack of ecological realism (Luoma and Ho 1993; DelValls and Conradi 2000). A particular concern is that laboratory manipulations may significantly alter the complex and dynamic characteristics of natural sediment, and hence alter its toxicity (Swartz 1989; Burgess and McKinney 1997; Chapman et al. 1998). For example, homogenization is recommended as a standard technique (ASTM 1998) to pool multiple field sediment samples, and thus reduce inter-replicate variability. However, Anderson et al. (2001) recently found that homogenization decreased the toxicity of several contaminated sediments to sea urchin embryos exposed at the sediment-water interface.

For heavy metals such as Cd, Cu, Pb, Ni, and Zn, the bioavailability, and hence toxicity, appears largely governed by local redox conditions (Chapman et al. 1998). Redox-active mineral phases such as Fe and Mn oxides (Balistrieri and Murray 1982; Dzombak and Morel 1990), acid volatile sulfides 'AVS' (Di Toro et al. 1992, Simpson et al. 2000) and 'reactive pyrite' (Huerta-Diaz and Morse 1992) readily bind heavy metal ions. On homogenization, anoxic material is brought to the surface, where prolonged oxidising conditions must destroy sulfide minerals and thereby, perhaps briefly, mobilise associated heavy metals (Morse 1994; Di Toro et al. 1996). Also, any free HS^- will coprecipitate freeM^{2+} from other zones. Conversely, as previously oxic sediments are buried, microbiota may switch to alternative oxidants, consuming Fe and Mn oxides and, again, releasing any heavy metal content. More subtly, mixing oxic and anoxic sediments could be expected to remove the endmembers of the redox continuum. Direct reaction of Fe^{III} with HS^- can be rapid (Rickard 1974; Canfield et al. 1992; Taillefert et al. 2000) and even indirect reaction of FeOOH with sulfide minerals can be significantly faster than direct bacterial reduction of FeOOH (Guo et al. 1997). All of these processes are dynamic further complicating toxicity evaluation, for example, AVS concentrations declined throughout the sediment toxicity tests of

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DeWitt et al. (1999). Finally, different sediment depths are typically differentially contaminated (Chapman 1988; Luoma and Ho 1993), and this too will be averaged out by homogenization.

In this experiment we seek to characterise the chemical changes in an estuarine sediment subjected to laboratory manipulations typical of toxicity test protocols. We also examine the response of the same sediment to a hypothetical alternative 'double layer' protocol that aims to avoid mixing the oxic and anoxic material, and hence minimise redox changes in the sediment.

MATERIALS AND METHODS

Sediment samples were collected in September during low tide from Okura River estuary, Auckland, New Zealand (36°40'S, 174°43'E). This estuary is considered to be a relatively uncontaminated area (Nipper et al. 1998). While it is recommended that only the top 2 cm of sediment be collected for toxicity testing (ASTM 1998), depths of as much as 10 cm have been used in recent studies (for example Carr et al. 1996; Nipper et al. 1998). In this study we compromised with a sampling depth of 5 cm. Twenty 5-cm-diameter cores were taken within a *ca.* 10 m^2 area, avoiding shell debris and mud crab burrows. Samples were held in the cores, sealed and ice-cooled, for around 2 h.

Standard test sediments were prepared as follows; 10 cores were mixed by hand until visually homogenous, removing any shell fragments and macrofauna. Two 1 L glass jars were filled with ~460 g of this sediment; 100 mL clean seawater (20‰) was added to liquefy. In order to assess the effects of manipulation on a heavy metal spike, $ZnCl_2$ was added (~2.5 µmol Zn/g dry sediment) to the test sediment in two different 1 L glass jars (Figure 1). All four jars were rolled for a total of 4 h. After settling the overlying water was removed, and subsamples (day 0) were taken for analysis. 700 mL of clean seawater was then added to the jars, by placing a polyethylene sheet on top of the sediment during addition to minimise disruption. Sediments were held under artificial lighting at 20°C for 10 d, and subsampled again (day 10). The overlying water was aerated gently, but not renewed (ASTM 1998) (Please note: one of the homogenized unspiked duplicates had to be discarded during the experiment because of an aeration failure).

Zinc was chosen as the spiking element because it is generally by far the most abundant heavy metal contaminant in Auckland sediments (Williamson and Morrisey 2000). Further, Zn may often be the first heavy metal contaminant to become bioavailable; only Ni has a less stable sulfide, and only Cd should fail to out-compete Zn for surface complexation sites on FeOOH, Mn oxides, etc. at marine pH. Finally, Zn forms only metastable ZnS, and does not enter the operationally defined pyrite phase (Huerta-Diaz and Morse 1992).

We considered the natural sediment to be composed of two distinct and approximately homogenous layers, olive-brown 'visually oxic' FeOOH-rich surface sediment and grey-black 'visually anoxic' AVS-rich sediment at depth

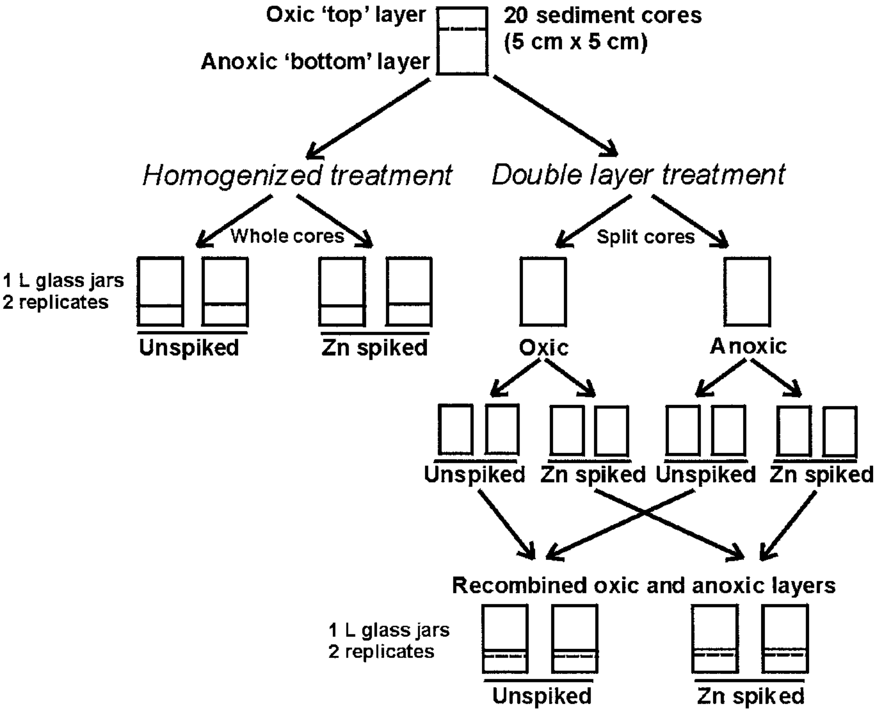

Figure 1. Sediment preparation methodology.

(Williamson et al. 1999). In other sediments from this estuary system, colour quantitatively reflects solid phase iron speciation (Bull and Williamson 2001). Thus, while this model is a simplistic representation of the continuous redox gradient, it does indicate a significant change in the sediment matrix.

Following this model, 'double layer' test sediments were prepared in duplicate from the remaining cores as follows (Figure 1). Each core was split into visually oxic (the top *ca.* 10 mm) and anoxic layers (promptly transferred to a N_2-filled glove bag), pooled, and mixed by hand. 100 g portions of oxic sediment were weighed out into 500 mL flasks and made up to 300 mL with clean seawater. 400 g portions of anoxic sediment were weighed out into 2 L flasks together with 900 mL N_2-purged clean seawater. Two pairs of oxic and anoxic flasks were spiked with $ZnCl_2$ (~2.5 µmol Zn/g dry sediment). The flasks were then aerated (oxic) or purged with N_2 (anoxic), and agitated vigorously for 2 h. This mixing process is similar to the ammonia removal method of Ferretti et al. (2000), but avoids air oxidation of the anoxic sediment. Dissolved Zn was monitored at intervals by CSWV (DLK-100 electrochemical analyser, AIS Inc, Flemington, NJ, USA) at a Au|Hg microelectrode.

After settling, decantation, a final mix by hand, and subsampling (day 0), 4 cm layers of anoxic sediment were placed into 1 L glass jars, and 1 cm of oxic sediment carefully layered on top (both layers spiked or both unspiked) (Figure 1)

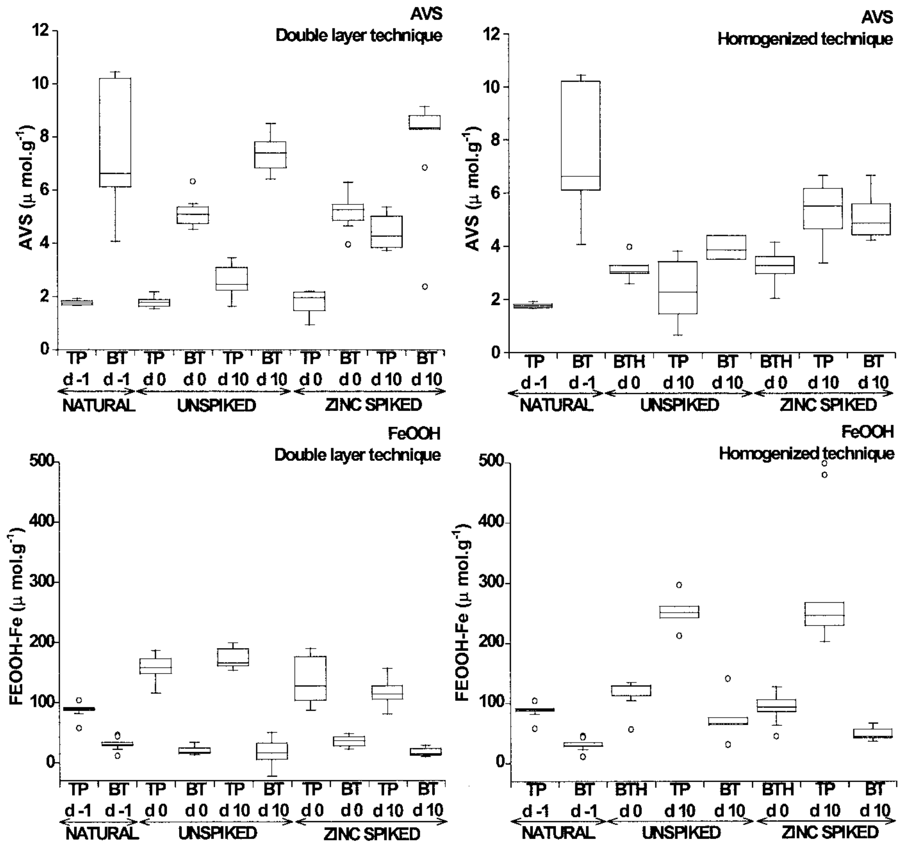

Figure 2. FeOOH and AVS content of unspiked and Zn-spiked sediments: double layer and homogenized techniques. TP, top layer; BT, bottom layer; BTH, both layers.

300 mL of clean seawater was then added and the sediments stored at 4°C overnight. Again, polyethylene sheets were used to minimise disruption of layers during additions. According to the general theme of creating a more realistic test material, the jars were exposed for 10 days on a wooden platform on the laboratory roof, underneath a transparent plastic cover. Water temperatures in the jars were close to ambient air temperature, ranging between about 5 and 25°C. Overlying water was changed daily, and sampled on days 1, 4, 7, and 10.

Munsell® Soil Colour Charts (Kollmorgen Instruments Corp.) were used to describe the colour characteristics of the top (oxic) and bottom (anoxic) layers, before and after manipulation, and at test termination. Sediment grain size was determined using a Galai time-of-transition particle analyser (Galai CIS-100, Galai Productions, Migdal Haemek, Israel) with sonication. Raw particle diameter data were determined at 0.25φ resolution and processed using the manufacturer's

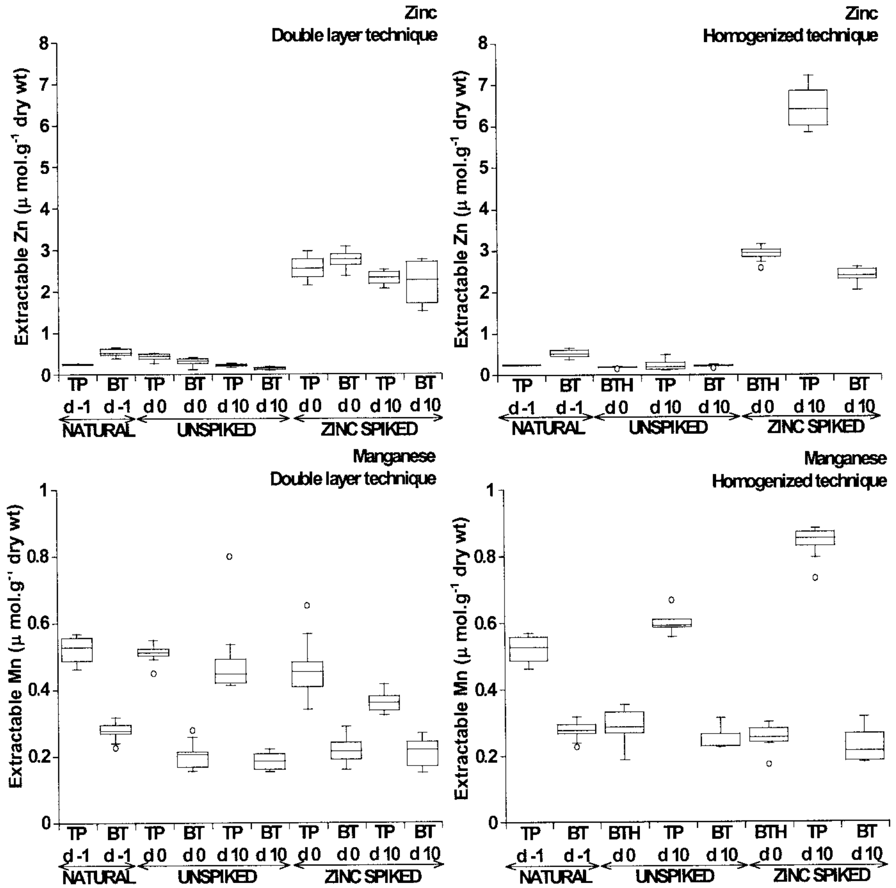

Figure 3. 0.5 M HCl-extractable Mn and Zn content of unspiked and Zn-spiked sediments: double layer and homogenized techniques. TP, top layer; BT, bottom layer; BTH, both layers.

software. Sediment water content was measured as weight loss on drying overnight at 50°C; organic carbon content was estimated from a second overnight drying period at 400°C.

For metals analysis, five replicates of 0.1-0.3 g wet sediment from each jar were sampled into 15 ml polypropylene tubes with 10 ml 0.5 M HCl, shaken for 1 h, centrifuged at 2000 rpm for 10 min, and the supernatant promptly analysed. Fe, Mn and Zn were determined by FAAS; Fe^{II} spectrophotometrically as the ferrozine complex in HEPES buffer, pH 7.0. For AVS, at least three replicates of 0.1-0.3 g wet sediment, from each jar, were analysed by ion-specific electrode (AgS, Metrohm, CH-9101 Herisau, Switzerland) following by microdiffusion from acid $SnCl_2$ solution into a NaOH / EDTA / ascorbate buffer (Williamson et al. 1999).

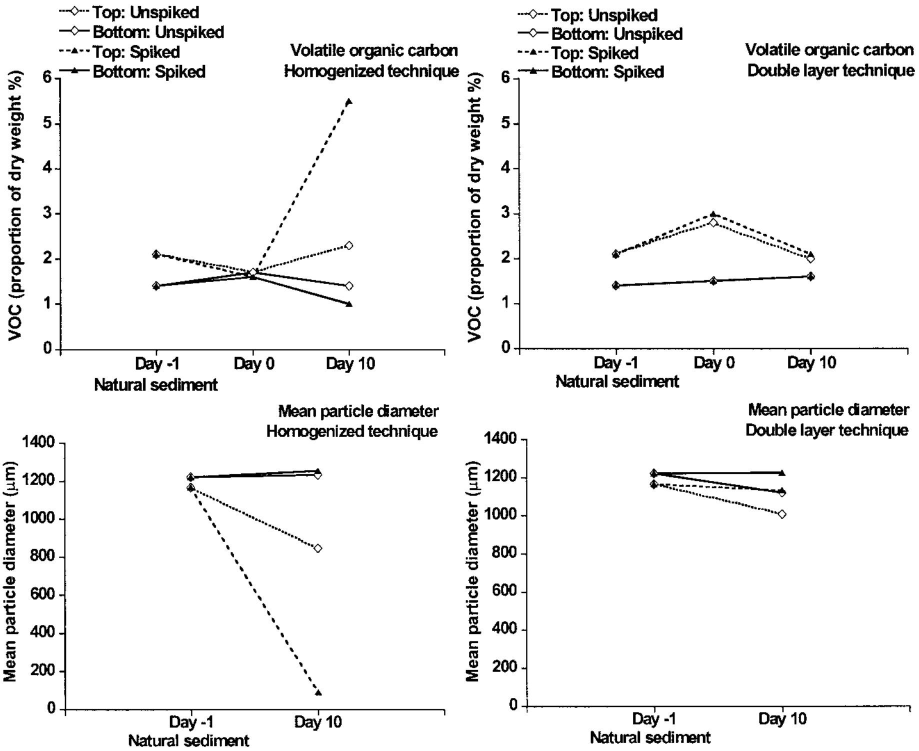

Figure 4. Volatile organic carbon content and r^3-weighted mean particle size of unspiked and Zn-spiked sediments: double layer and homogenized techniques.

Acidity was measured in unfiltered waters using a PHM 92 lab pH meter (Radiometer, Copenhagen) calibrated against standard certified buffers (Fisher). Salinity was measured with a hand-held refractometer (ATAGO). After filtration (Whatman GF/C glass microfiber) subsamples were taken for NH_4-N and metals analysis, preserved respectively by freezing, or addition of HNO_3 to 0.5% v/v.

RESULTS AND DISCUSSION

The natural sediment (day -1) exhibited a fairly consistent and gradual colour change with depth, from Munsell® colour GY 4/2 (olive gray) at the surface to N 2.5/ (black) at 5 cm. This colour can probably be attributed to the iron minerals, as Mn concentrations are low in this estuary (*B. Macaskill, pers. comm.*) and organic matter content is not high.

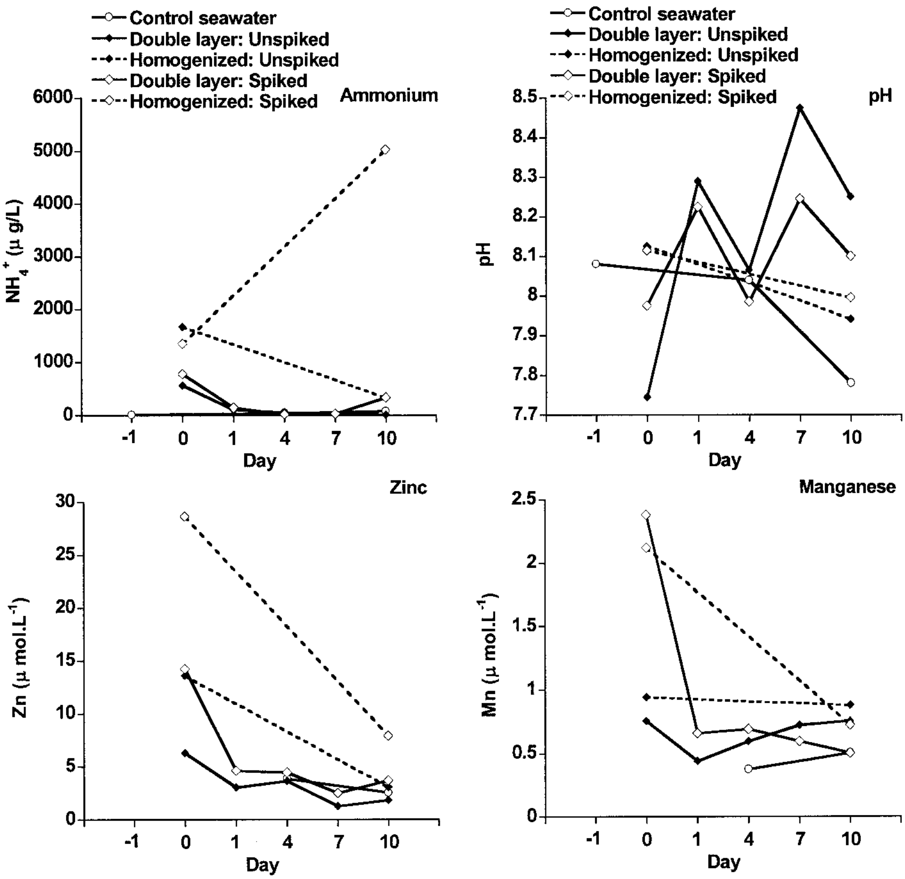

Figure 5. Overlying water concentrations of Zn, Mn, and NH_4^+, and pH.

The homogenized technique initially produced a substantial increase in FeOOH, and some loss of AVS, indicating significant oxidation during the homogenizing procedure (Figure 2). During incubation, the sediment re-stratified, slowly developing quite distinct layers. In all treatments the lower layer darkened to 5Y 3/2 with black mottling N <2.5/ (more prevalent in the unspiked treatment) while the upper 5-8 mm was a lighter shade, 5Y 5/2. The black mottling indicates AVS formation, consistent with the substantial increase observed throughout the sediment. Particle size analysis (Figure 4) indicates differential settling, moving smaller particles to the oxic layer. This effect, probably combined with upward diffusion and oxidation of Mn^{2+} and Fe^{2+}, resulted in substantially elevated extractable metals and organic matter in the oxic layer (Figures 2, 3 and 4). That is, Zn binding phase concentrations changed significantly on homogenization, and increased throughout the sediment during incubation. This implies that Zn bioavailability in the d sediment was different from that in the natural sediment, and probably decreased during incubation. Furthermore, the known toxicant NH_4^+

reached high levels in the overlying water, especially in the spiked treatments (Figure 5), which would have made it difficult to use this sediment as a toxicity test material (Cheung et al. 1997; Ferretti et al. 2000).

The double layer technique had more success in retaining the large-scale chemical characteristics of the natural sediment. The solid phase analytes (Figures 2 and 3) exhibit relatively little change during preparation, with the exception of oxic layer FeOOH, which increased by more than 50%. This probably indicates oxidation of iron (II) and could perhaps be reduced by omitting aeration. A small amount (1-6%) of total extractable Mn and Zn moved into the overlying water during homogenization (Figure 5). A considerable amount of ammonia was also removed from the anoxic sediments; and a trace of sulfide, equivalent to 0.6% of the AVS, from the anoxic unspiked sediment (Figure 5). Presumably ZnS formation prevented any such loss from the spiked sediment. Further sulfide might have been lost to the N_2 stream in the form of H_2S, but the characteristic odour was not observed. No other species, such as Fe^{2+}, were observed at the microelectrode.

During incubation, AVS increased substantially (Figure 2). The layers remained distinct; and the final colour of the oxic layer was 5Y 5/3, i.e. a higher colour saturation, consistent with increased FeOOH content (Bull and Williamson 2001). The anoxic layer finished at 10Y 3/1, except for a few very fine U-shaped burrows extending down from the oxic layer; there was none of the black mottling seen in the homogenized treatment. Mn and NH_4^+ concentrations in the overlying water were low; dissolved Zn was fairly constant throughout, and consistently higher in the spiked treatments (Figure 5).

In this experiment the manipulated material was a poor analogue of the natural sediment. Gradual restratification and differential settling caused further changes during incubation. We must conclude that the end (consistency across replicates) did not justify the mean (homogenization)! The 'double layer' protocol reduced these problems somewhat by separating visually oxic and anoxic zones during manipulation, and by keeping anoxic sediments deoxygenated. As a side benefit, NH_4^+ is initially flushed from the sediment.

Since both metal-releasing and metal-sequestering reactions occur in sediments, it is not obvious whether homogenization would generally increase or decrease heavy metal bioavailability, let alone to what extent. The chemical changes observed here will not be precisely replicated in other sediments. Particular caution should be taken when comparing Zn-spiked treatments with natural sediments. Though the great majority of the Zn was effectively bound onto the sediment during spiking, 'aged' contaminants in natural systems are in thermodynamically and kinetically more stable states (Swartz 1989; Simpson et al. 2000). Nonetheless, the 'averaging out' of the redox gradient on homogenization, and consequent dynamic changes during incubation, seem likely to have significant effects on dissolved Zn concentrations, and hence on the bioavailability of sedimentary Zn. The same should apply to other heavy metal contaminants.

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REFERENCES

Anderson BS, Hunt JW, Phillips BM, Fairey R, Puckett HM, Stephenson M, Taberski K, Newman J, Tjeerdema RS (2001) Influence of sample manipulation on contamination and toxicity at the sediment-water interface. Mar Environ Res 51:191-211

American Society for Testing and Materials (1998) Standard guide for conducting 10-day static sediment toxicity tests with marine and estuarine amphipods. E 1367-92. In: Annual Book of ASTM Standards, vol 11. West Conshohocken, Pa, pp 732-757

Balistrieri LS, Murray JW (1982) The adsorption of Cu, Pb, Zn, and Cd on goethite from major ion seawater. Geochim Cosmochim Acta 46:1253-1265

Bull DC, Williamson RB (2001) Prediction of principal metal-binding solid phases in estuarine sediments from colour image analysis. Environ Sci Technol 35:1658-1662

Burgess RM, McKinney RA (1997) Effects of sediment homogenisation in interstitial water PCB geochemistry. Arch Environ Contam Toxicol 33:125-129

Canfield DE, Raiswell R, Bottrell S. 1992. The reactivity of sedimentary iron minerals toward sulfide. American J Sci 292:659-683

Carr RS, Chapman DC, Howard CL, Biedenbach JM (1996) Sediment quality triad assessment survey of the Galveston Bay, Texas system. Ecotoxicology 5:341-364.

Chapman PM (1988) Marine sediment toxicity tests. In: Lichtenberg JJ, Winter FA, Weber CI, and Fradkin L (eds) Chemical and Biological Characterisation of Sludges, Sediments, Dredge Spoils, and Drilling Muds. ASTM STP 976. Philadelphia, pp 391-402

Chapman PM, Wang F, Janssen C, Persoone G, Allen HE (1998) Ecotoxicology of metals in aquatic sediments; binding and release, bioavailability, risk assessment, and remediation. Canadian J Fish Aquat Sci 55:2221-2243

Cheung YH, Neller A, Chu KH, Tam NFY, Wong CK, Wong YS, Wong MH (1997) Assessment of sediment toxicity using different trophic organisms. Arch Environ Contam Toxicol 32:260-267

DelValls TA, Conradi M (2000) Advances in marine ecotoxicology: Laboratory tests versus field assessment data on sediment quality studies. Ciencias Marinas 26:39-64

DeWitt TH, Hickey CW, Morrisey DJ, Nipper MG, Roper DS, Williamson RB, Van Dam L, Williams EK (1999) Do amphipods have the same concentration-response to contaminated sediment in situ as in vitro? Environ Toxicol Chem 18:1026-1037

Di Toro DM, Mahony JD, Hansen DJ, Scott KJ, Carlson AR, Ankley GT (1992) Acid volatile sulfide predicts the acute toxicity of cadmium and nickel in sediments. Environ Sci Technol 26:96-101

Di Toro DM, Mahony JD, Hansen DJ, Berry WJ (1996) A model of the oxidation of iron and cadmium sulfide in sediments. Environ Toxicol Chem 15:2168-2186

Dzombak DA, Morel FMM (1990) Surface complexation modelling: hydrous ferric oxide. John Wiley and Sons, New York.

Ferretti JA, Calesso DF, Hermon TA (2000) Evaluation of methods to remove ammonia interference in marine sediment toxicity tests. Environ Toxicol Chem 19:1935-1941

Guo T, DeLaune RD, Patrick WH Jr (1997) The influence of sediment redox chemistry on chemically active forms of arsenic, cadmium, chromium, and zinc in estuarine sediment. Environ Int 23:305-316

Huerta-Diaz MA, Morse JW (1992) Pyritisation of trace metals in anoxic marine sediments. Geochim Cosmochim Acta 56:2681-2702

Luoma SN, Ho HT (1993) Appropriate uses of marine and estuarine sediment bioassays. In: Calow P (ed) Handbook of Ecotoxicology, vol 1. Blackwell Scientific Publications, pp 193-226

Morse JW (1994) Interactions of trace metals with authigenic sulfide minerals: implications for their bioavailability. Mar Chem 46:1-6

Nipper MG, Roper DS, Williams EK, Martin ML, Van Dam LF, Mills GN (1998) Sediment toxicity and benthic communities in mildly contaminated mudflats. Environ Toxicol Chem 17:502-510

O'Connor T, Paul JF (2000) Misfit between sediment toxicity and chemistry. Mar Pollut Bull 40:59-64

Rickard DT (1974) Kinetics and mechanism of the sulfidation of goethite. American J Sci 274:941-952

Simpson SL, Rosner J, Ellis J (2000) Competitive displacement reactions of cadmium, copper, and zinc added to a polluted, sulfidic estuarine sediment. Environ Toxicol Chem 19:1992-1999

Swartz RC (1989) Marine sediment toxicity tests. In: Contaminated Marine Sediments-Assessment and Remediation, National Academy Press, Washington, DC, pp 115-129

Taillefert M, Bono AB, Luther GW III (2000) Reactivity of freshly formed Fe(III) in synthetic solutions and (pore)waters: voltammetric evidence of an aging process. Environ Sci Technol 34:2169-2177

Williamson RB, Wilcock RJ, Wise BE, Pickmere SE (1999) Effect of burrowing by the crab *Helice crassa* on chemistry of intertidal muddy sediments. Environ Toxicol Chem 18:2078-2086

Williamson RB, Morrisey DJ (2000) Stormwater contamination of urban estuaries 1. Predicting the buildup of heavy metals in sediments. Estuaries 23:56-66