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Effects of Sediment Characteristics on the Toxicity of Chromium(III) and Chromium(VI) to the Amphipod, *Hyalella azteca*

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We evaluated the influence of sediment characteristics, acid-volatile sulfide (AVS) and organic matter (OM), on the toxicity of chromium (Cr) in freshwater sediments. We conducted chronic (28–42-d) toxicity tests with the amphipod *Hyalella azteca* exposed to Cr(VI) and Cr(III) in water and in spiked sediments. Waterborne Cr(VI) caused reduced survival of amphipods with a median lethal concentration (LC₅₀) of 40 µg/L. Cr(VI) spiked into test sediments with differing levels of AVS resulted in graded decreases in AVS and sediment OM. Only Cr(VI)-spiked sediments with low AVS concentrations (<1 µmol/g) caused significant amphipod mortality. Waterborne Cr(III) concentrations near solubility limits caused decreased survival of amphipods at pH 7 and pH 8 but not at pH 6. Sediments spiked with high levels of Cr(III) did not affect amphipod survival but had minor effects on growth and inconsistent effects on reproduction. Pore waters of some Cr(III)-spiked sediments contained measurable concentrations of Cr(VI), but observed toxic effects did not correspond closely to Cr concentrations in sediment or pore waters. Our results indicate that risks of Cr toxicity are low in freshwater sediments containing substantial concentrations of AVS.

Introduction

Chromium (Cr) occurs in the environment principally as trivalent and hexavalent redox states, Cr(III) and Cr(VI), which differ greatly in their chemistry and toxicology. Cr(III), like other cationic metals, tends to complex with inorganic and organic ligands, sorb to solid surfaces, and precipitate as hydrous oxides (1). Cr(VI) is typically present in water as highly soluble anions that have low affinity for sorption to sediment at circumneutral pH. Because Cr(VI) is more soluble and more toxic than Cr(III) (2–4), concerns about Cr toxicity in surface water and groundwaters are usually related to elevated Cr(VI) concentrations. Cr(VI) tends to be reduced to Cr(III) in sediments by reaction with dissolved and particulate organic matter (OM) and with reduced inorganic species (5, 6), but Cr(III) can also be oxidized to Cr(VI) by reaction with manganese oxides (7–9). The balance between reduction and oxidation processes in sediments is difficult to measure and varies both spatially and temporally (10). However, Masscheleyn et al. (7) noted that wetland soils

sequestered aqueous Cr(IV) effectively until the levels added exceeded the soils' reduction capacity. This finding suggests that risks of Cr(VI) toxicity are low in sediments with substantial pools of labile reduced species.

Management decisions related to sediments contaminated with Cr and other toxic metals are made difficult by the absence of regulatory criteria for protection of benthic organisms. The complexity of Cr speciation in freshwater sediments suggests that empirical sediment quality guidelines based on total Cr concentrations in sediment (11) may not accurately predict risks of Cr toxicity. We evaluated an approach for screening sediments for risks of Cr toxicity based on the ability of acid-volatile sulfide (AVS) to reduce Cr(VI). A similar approach has been recommended for evaluating toxicity risks from other cationic metals that react with AVS to form insoluble metal sulfides (Ag, Cd, Cu, Ni, Pb, and Zn; 12) based on observations that metal toxicity is rarely observed in sediments with AVS concentrations in excess of concentrations of these metals on a molar basis (13). In the case of Cr in sediments, we would expect that sediments containing a substantial pool of AVS have a sufficient reductive capacity to prevent accumulation of toxic levels of Cr(VI) (14). To evaluate this hypothesis, we tested the chronic toxicity of Cr(VI) and Cr(III) in freshwater and in spiked freshwater sediments to the amphipod, *Hyalella azteca*, a versatile test organism that can be used for both water-only and whole-sediment toxicity tests (15, 16). The objectives of these studies were to evaluate the relative toxicity of the two Cr species and to characterize their interactions with AVS and other constituents of freshwater sediments. Similar studies have been conducted to evaluate Cr toxicity to amphipods in marine sediments (14).

Materials and Methods

Toxicity Tests. Chronic water-only and sediment toxicity tests with *H. azteca* were conducted according to published test methods (17, 18). Tests lasted 42 d with end points of survival, growth (carapace length), and reproduction (young per female). In sediment tests, surviving animals were removed from sediment after 28 d and transferred to clean water for 14 d to facilitate reproduction measurements. Test were started with 7-d-old amphipods, stocked in groups of 10 into 300-mL test beakers containing either 200 mL of test solution (and a thin layer of sand) or 100 mL of test sediment and 175 mL of overlying water. Tests started with 16 replicate beakers, with beakers removed at intervals for determinations of survival and growth and for chemical analysis of sediments. Amphipods were fed a preparation of yeast, cereal leaves, and trout chow daily (17). Water was automatically replaced at rates of 6 vol/d (water-only tests) or 2 vol/d (sediment tests). Test water was well water diluted 1:1 with deionized water (pH 8.3, hardness = 1.4 mM, alkalinity = 1.2 mM, conductivity = 330 µS/cm).

For the Cr(VI) water-only test, a proportional diluter delivered control water and five Cr(VI) concentrations in a 50% dilution series, with a high nominal Cr(VI) concentration of 50 µg/L. Because of the low water solubility and low toxicity of Cr(III), the water-only test with Cr(III) was conducted with a single nominal test concentration (100 µg/L) near the expected solubility limit. Control water and the Cr(III) test solutions were delivered at three pH levels (6.0, 7.0, and 8.0). A diluter was modified to deliver test water to 18-L chambers with a 24-h residence time for equilibration of pH-adjusted control water and Cr(III) test solutions. Cr(III) solutions were added to equilibration chambers by automatic dispensers (Hamilton Microlab 500; Reno, NV), and pH was adjusted by

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TABLE 1. Characteristics of Control Sediments Used for Spiked-Sediment Toxicity Tests

parameter	Florissant (FL)	West Bearskin (WB)	Columbia (CW)
sand (%)	3	49	0
silt (%)	31	19	74
clay (%)	66	32	26
moisture (%)	26	84	54
TOC (%)	0.82	9.0	2.2
AVS ($\mu\text{mol/g}$)	<0.01	33	55
SEM metals ($\mu\text{g/g}$)			
Ni	2.9	24	4.6
Cu	3.6	34	<0.14
Zn	7.1	86	17
Ag	0.06	0.13	0.04
Cd	0.09	0.69	0.08
Pb	7.5	14	6.2

automatic pH controllers (Barnant HD PH-P1; Barrington, IL), which added a dilute NaOH solution.

Spiked-sediment tests were conducted with three test sediments with differing physicochemical characteristics (Table 1): a wetted soil from Florissant, MO (FL); sediment from West Bearskin Lake, MN (WB); and sediment from a Columbia, MO, wetlands (CW). Test sediments contained levels of Cr ($<5 \mu\text{g/g}$) and other metals (nickel, zinc, copper, lead, and cadmium) less than sediment quality guidelines (11). Sediments were spiked with one or more levels of Cr(VI) or Cr(III) plus unspiked controls. For the Cr(VI)-spiking study, WB and CW sediments were spiked at three levels of Cr(VI) to evaluate interactions of Cr(VI) with AVS. Spike levels for these sediments were designated as " $n\times$ ", where n equals the molar ratio of Cr to AVS. The WB sediment was spiked at ratios of $1\times$, $3\times$, and $10\times$, and the CW sediment was spiked at $1\times$, $1.5\times$, and $3\times$. The FL sediment was spiked with a single Cr(VI) level (FL-Cr6) equal to the lowest level used for the WB sediment. For the Cr(III)-spiked sediment study, each spike treatment (FL-Cr3, WB-Cr3, CW-Cr3) received a single level of Cr(III) equivalent to the highest Cr(VI) spike level for that sediment. Spike solutions of $\text{K}_2\text{Cr}_2\text{O}_7$ or CrCl_3 were prepared in deionized water, and spiked sediments were prepared by adding 300 mL of spike solution to 3 L of wet sediment in a 4-L jar. Control sediments were prepared by mixing sediments with 300 mL of deionized water. Cr(III) spike solutions were adjusted to pH 7.0 with dilute sodium hydroxide, producing a slurry of Cr(III) precipitate. Jars containing sediment and spiking media were stirred thoroughly and then stored at 4°C for 1 week (with periodic mixing) before the start of the toxicity tests. One day before each toxicity test, sediments were homogenized and added to exposure beakers; beakers were placed in the exposure system; and water replacements were started to allow settling of sediment and flushing of excess spike solutions.

Characterization of Water and Sediment. Pore water samples were isolated by centrifugation at 7000g (IEC model PR-7000; Thermo, Woburn, MA) for 15 min at 4°C . Samples of pore water and overlying water for analysis of filterable Cr (an estimate of dissolved Cr), Cr(VI), and dissolved organic carbon (DOC) were filtered through $0.45\text{-}\mu\text{m}$ polypropylene membrane filters. Samples for analysis of filterable Cr were acidified to 1% (v/v) with HNO_3 . Pore water and overlying water were analyzed for hardness, alkalinity, dissolved oxygen, ammonia, conductivity, pH, and DOC using standard methods. Sediments were analyzed for AVS by ion-selective electrode after room temperature extraction with 1 N HCl (19) and for total organic carbon (TOC) by acidification, combustion, and colorimetric titration with a carbon analyzer (UIC Coulometrics model 5010; Joliet, IL). Total recoverable Cr was extracted from sediment by microwave digestion with concentrated HNO_3 in a sealed Teflon-lined vessel.

Separation of Cr(III) and Cr(VI) in water was accomplished by an adaptation of cation-exchange methods (20, 21). This separation is feasible because Cr(VI) exists as an anion (HCrO_4^- or CrO_4^{2-}) and is soluble over a wide pH range, whereas Cr(III) is primarily soluble only at a pH of about 5 or less, where $\text{Cr}(\text{OH})_2^+$ becomes the dominant species (22). We added a pH 5 buffer to filtered samples, passed them through a strong cation-exchange resin to retain cationic Cr(III) species, and analyzed the eluent for Cr to determine Cr(VI) concentrations (see Supporting Information). This method was designed to minimize artifacts due to Cr(III) passing through the cation-exchange column as anionic Cr(III)-organic complexes (20) or as neutral colloids. We used a resin of fine particle size (100–200 mesh) in the acid form to maximize resin-solute interactions and to maintain the acid pH. We assumed that buffering samples to a pH 5 would lead to rapid conversion of colloidal $\text{Cr}(\text{OH})_3$ to $\text{Cr}(\text{OH})_2^+$, displacement of weakly complexed Cr(III), and protonation of negatively charged moieties on DOC; all of which would increase retention of Cr(III) on the resin. Removal of considerable DOC by the cation-exchange column was indicated by retention of all visible color of pore water samples in the upper few millimeters of the resin bed. Retention of 100 ppb Cr(III) spikes added to pore water samples prior to the ion-exchange separation was consistently greater than 99% (see Supporting Information). The selectivity of our method was also indicated by the absence of quantifiable Cr(VI) levels ($\geq 10 \text{ g/L}$) in pore waters of several sediments containing very high total Cr concentrations (see Results and Discussion).

Chromium was analyzed by ICP-MS, with both chromium masses (52 and 53) monitored to check for potential interferences. Samples of water and pore water were diluted at least 10-fold before analysis. Several additional metals (Ni, Cu, Zn, Ag, Cd, Pb) were analyzed by ICP-MS in sediment extracts from the AVS extraction of control sediments after evaporative digestion with nitric acid to reduce chloride levels. Method detection limits for filtered Cr and Cr(VI) ranged from 0.5 to $2.0 \mu\text{g/L}$, but we used a conservative reporting limit of $10 \mu\text{g/L}$ for Cr(VI) to avoid low-level artifacts of the separation method. Additional QA information is included with the Supporting Information.

Statistical Analysis. Analysis of variance (ANOVA) of toxicity data was conducted using SAS/STAT software (version 8.2; SAS Institute, Cary, NC). All toxicity data were transformed (square root, log, or rank) before ANOVA. Comparisons among multiple treatment means were made by Fisher's LSD procedure, and differences between individual treatments and controls were determined by one-tailed Dunnett's or Wilcoxon tests. Statements of statistical significance refer to a probability of type I error of 5% or less ($p \leq 0.05$). Median lethal concentrations (LC_{50}) were determined by the Trimmed Spearman-Kärber method using TOXSTAT software (version 3.5; Lincoln Software Associates, Bisbee, AZ).

Results and Discussion

Toxicity of Cr(VI) in Water. Filtered Cr concentrations during the Cr(VI) water-only exposure were close to nominal concentrations (high nominal concentration, $50 \mu\text{g/L}$; Table 2). The relatively low mean value for filterable Cr in the nominal $25 \mu\text{g/L}$ treatment ($18 \mu\text{g/L}$) reflects the influence of a single low measurement ($7.6 \mu\text{g/L}$) on day 0 of the study, probably due to a diluter malfunction, but values measured between days 14 and 42 averaged $21 \mu\text{g/L}$. Speciation of selected samples confirmed that essentially all measured Cr was present as Cr(VI) (see Supporting Information). Water quality characteristics in all Cr(VI) exposure levels remained similar to original characteristics of the test water throughout the test (see Supporting Information).

TABLE 2. Results of a Toxicity Test with the Amphipod, *Hyalella azteca*, Exposed to Cr(VI) in Water^a

filterable Cr ($\mu\text{g/L}$) ($n = 4$)	survival (%)		length (mm)		reproduction (young/female) ($n = 8$)
	day 28 ($n = 12$)	day 42 ($n = 8$)	day 28 ($n = 8$)	day 42 ($n = 8$)	
<2.0 (control)	100 (0)	100 (0)	4.87 (0.11)	8.4 (2.4)	
2.0 (0.3)	90 (7)	90 (7)	4.90 (0.07)	2.3 (1.1)*	
4.7 (1.1)	95 (3)	95 (5)	4.84 (0.09)	2.9 (1.1)	
10 (1.0)	98 (3)	95 (3)	5.27 (0.08)	5.4 (1.5)	
18 (6)	88 (5)*	80 (4)*	5.05 (0.07)	3.3 (1.0)	
48 (2)	38 (5)*	40 (9)*	5.22 (0.17)	1.6 (1.0)*	

^a Means with standard error in parentheses. Asterisks indicate significant difference between treatment and control ($p \leq 0.05$; ANOVA and Dunnett's test with log-transformed data).

Exposure to aqueous Cr(VI) caused decreased survival of *H. azteca*. Survival was significantly decreased at measured Cr(VI) concentrations of 18 $\mu\text{g/L}$ and above on days 28 and 42 (Table 2). Identical LC_{50} values (40 $\mu\text{g/L}$) were determined for the 28- and 42-d exposure periods, and similar results were observed in a 10-d range-finding test (62% mortality at 100 $\mu\text{g/L}$), suggesting that lethal effects of Cr(VI) occurred early in the exposure. Evidence of sublethal effects of Cr(VI) on amphipods was less conclusive. Amphipod growth was not significantly decreased at any Cr(VI) exposure level. Reproduction in all Cr(VI) treatments was at least one-third less than in controls, although statistical analysis indicated that reproduction was significantly less than controls only in the lowest and highest Cr(VI) treatments and not in intermediate treatments. Some of the variation in reproduction rates among treatments may reflect differential mortality of neonate amphipods. Neonates would be exposed to Cr(VI) for up to 7 d between sampling dates (15) and dead neonates would not be visible after only a few days due to rapid decomposition (23). Overall, these results indicate that reduced survival of *H. azteca* is a sensitive response to chronic Cr(VI) exposure. The threshold for Cr(VI) toxicity to *H. azteca*, 14 $\mu\text{g/L}$ (geometric mean of Cr concentrations bracketing the lowest significant effect on survival), is slightly greater than the current U.S. EPA water quality criterion for Cr(VI) (11 $\mu\text{g/L}$; 24). Previous studies have reported chronic values for Cr(VI) between 6 and 40 $\mu\text{g/L}$ for other crustacean taxa and between 264 and 1987 $\mu\text{g/L}$ for fish (3).

Toxicity of Cr(III) in Water. Filterable Cr concentrations in the Cr(III) water-only test were substantially less than the nominal concentration of 100 $\mu\text{g/L}$, indicating that Cr(III) concentrations were limited by solubility (Table 3). Filterable Cr concentrations decreased with increasing pH in the range, pH 6–8. Mean pH values were consistent between Cr(III) treatments and controls, but upward drift of pH in the pH 6 treatment resulted in some overlap in the pH range between pH 6 and pH 7 treatments. Other water quality parameters were similar to unspiked test water, except for the effects of

pH control on alkalinity in the pH 6 and pH 7 treatments (see Supporting Information). Amphipod survival was high ($\geq 90\%$) in controls at all three pH levels (Table 3), but growth and reproduction of control groups were significantly lower at pH 6 and pH 8 than at pH 7. Previous studies in our laboratory suggest that pH 6 may be near the lower limit of pH tolerance for this species, but we have often tested this species successfully at pH 8.

Exposure to Cr(III) decreased survival of amphipods but did not affect growth and reproduction. Survival was significantly less in Cr(III) treatments at pH 7 and pH 8 than in controls (Table 3). Concentrations of aqueous Cr(III) associated with mortality in the pH 7 and pH 8 treatments (29 and 48 $\mu\text{g/L}$, respectively) are less than the current chronic water quality criterion for dissolved Cr(III), 74 $\mu\text{g/L}$ at hardness of 100 mg/L (24). Previous studies with *Daphnia magna* reported chronic toxicity of Cr(III) at 44 $\mu\text{g/L}$ in hard water, but these effects may have been caused by precipitated, rather than dissolved, Cr(III) (3). Toxicity of Cr(III) hydroxy precipitates should not have occurred in our exposure system due to settling of precipitates in the equilibration chambers. Our results suggest that the toxicity of Cr(III) involves more than simply pH-dependent differences in solubility, as no toxicity was observed at pH 6, which had greatest filterable Cr concentrations. However, metastable colloidal species of Cr(III) may have contributed to the observed toxic effects, analogous to effects reported for aluminum hydroxy species (25). Further studies are needed to establish a threshold for chronic toxicity of Cr(III) to *H. azteca* at circumneutral pH.

Chemistry and Toxicity of Cr(VI)-Spiked Sediments.

Spiking of sediments with several levels of Cr(VI) resulted in graded decreases in AVS concentrations and changes in OM concentrations in sediment and pore water. Mean AVS concentrations were decreased by 97% in the WB-10 \times treatment and by 91% in the CW-3 \times treatments on day 0 of the test (Table 4). Sediment TOC also decreased in Cr(VI)-spiked CW and WB sediments. Cr(VI) spikes were associated with increased DOC and decreased hardness and alkalinity in pore waters (Table 5), suggesting that Cr(VI) reacted with particulate organic matter to release dissolved OM and to create additional sites for cation binding. Pore water pH remained near neutral except in the CW-3 \times treatment (pH 8.95) and in the FL-Cr6 treatment (pH 5.85). Water quality of overlying water was unaffected by Cr(VI) spikes (see Supporting Information).

Aqueous Cr(VI) concentrations reflected differences in AVS concentrations among treatments. Initial concentrations of filterable Cr (Table 5) and Cr(VI) in pore water samples were greater than 10 000 $\mu\text{g/L}$ in treatments with lowest AVS concentrations (FL-Cr6, WB-10 \times , and CW-3 \times), but pore water Cr(VI) concentrations remained low (≤ 20 $\mu\text{g/L}$) in treatments with higher AVS concentrations (Figure 1). In all three treatments with quantifiable Cr(VI) concentrations in pore water, these concentrations decreased during the test,

TABLE 3. Results of Toxicity Test with the Amphipod, *Hyalella azteca*, Exposed to Cr(III) in Water at Three pH Levels^a

treatment	filterable Cr ($\mu\text{g/L}$)	pH	survival (%)		length (mm)		reproduction (young/female) ($n = 8$)
			day 28 ($n = 12$)	day 42 ($n = 8$)	day 28 ($n = 4$)	day 42 ($n = 8$)	
control, pH 6	<2	6.44 (6.00–7.12)	94 (1) ab	93 (2)	3.8 (0.1) b	3.3 (0.03) c	0 (0) c
Cr(III), pH 6	76 (63–90)	6.41 (6.00–7.00)	98 (2)	95 (3)	4.3 (0.4)	4.3 (0.4)	1.0 (0.3)
control, pH 7	<2	7.11 (6.90–7.34)	90 (2) b	93 (3)	4.4 (0.1) a	3.9 (0.04) a	1.4 (0.2) a
Cr(III), pH 7	48 (38–54)	7.24 (6.96–7.42)	63 (5)*	60 (7)*	4.1 (0.1)	4.1 (0.1)	1.3 (0.6)
control, pH 8	<2	7.98 (7.79–8.20)	95 (3) a	93 (4)	4.0 (0.2) b	3.6 (0.04) b	0.8 (0.2) b
Cr(III), pH 8	29 (23–35)	7.94 (7.81–8.12)	63 (4)*	53 (5)*	3.9 (0.1)	3.9 (0.1)	2.0 (0.6)

^a Means with range (for pH) or standard error in parentheses. Within a pH level, asterisks indicate significant decreases in test end points in the Cr(III) treatment, relative to the control. For control sediments, means followed by the same letter are not significantly different ($p \leq 0.05$; ANOVA and Fisher's LSD test with log-transformed data).

TABLE 4. Characteristics of Sediments from a Toxicity Test with Cr(VI)-Spiked Sediments^a

treatment	total Cr ($\mu\text{mol/g}$)	TOC (%)	AVS ($\mu\text{mol/g}$)	
			day 0	day 28
FL-control	0.69	0.82	<0.01	<0.01
FL-Cr6	3.3	0.87	<0.01	<0.01
WB-control	1.4	9.0	33	28
WB-1 \times	52	8.8	30	12
WB-3 \times	77	8.9	21	13
WB-10 \times	220	8.5	0.9	2.6
CW-control	1.2	2.2	55	51
CW-1 \times	31	2.2	45	41
CW-1.5 \times	49	2.0	30	35
CW-3 \times	94	1.8	4.9	11

^a Means ($n = 2$), except AVS.

with the greatest decrease occurring in the CW-3 \times treatment. Cr(VI) concentrations in overlying water were much lower than those in pore water but followed similar trends among treatments and over time. Decreases in aqueous Cr(VI) concentrations during the course of the study could have resulted from reaction with AVS and OM and from dilution due to replacement of overlying water. The smallest proportional decrease of Cr(VI) from pore water and overlying water occurred in the FL-Cr6 sediment, which had no AVS and low OM.

These results are consistent with the hypothesis that Cr(VI) concentrations will remain low in sediments containing substantial concentrations of AVS. Berry et al. (14), in studies with Cr(VI)-spiked marine sediments, did not detect Cr(VI) in pore waters of sediments with Cr:AVS ratios of 2.2 or less. In contrast, we measured substantial AVS concentrations in sediments spiked at Cr:AVS ratios of 3:1 (WB-3 \times and CW-3 \times). AVS may have persisted in these treatments due to regeneration of AVS during the test, at least in some treatments. However, our data also suggests that some added Cr(VI) reacted with sediment OM, as has been reported in several previous studies (5, 6, 26, 27). The relationship between Cr(VI) spikes and AVS depletion in the CW sediment was similar to the 2:1 ratio reported by Berry et al. (14) (Figure 2). However, AVS concentrations in the high-OM WB sediment decreased at a ratio of about 1 mol of AVS/8 mol of added Cr(VI). Reaction of Cr(VI) spikes with sediment OM is also suggested by decreases in TOC in the WB and CW sediments (Table 4) and increases in pore water DOC in all three sediments (Table 5). These results suggest that sediment OM also provides some protection against Cr(VI) toxicity in benthic environments, although Cr(VI) toxicity occurred in the spiked WB sediments despite high levels of OM.

Effects of Cr(VI)-spiked sediments on amphipods were similar to the effects of Cr(VI) in water. Survival of *H. azteca*

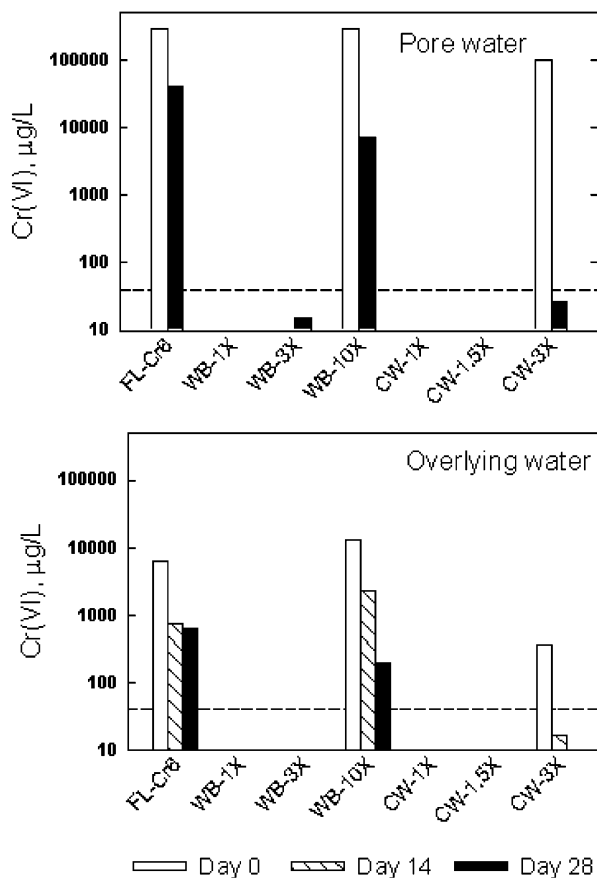


FIGURE 1. Concentrations of Cr(VI) in pore water and overlying water during toxicity test with Cr(VI)-spiked sediment. Dashed line indicates LC₅₀ from water-only toxicity test.

decreased significantly at the highest Cr(VI) spike levels in two of the three sediments tested. The FL-Cr6 and WB-10 \times treatments caused 100% mortality of amphipods by the end of the 28-d sediment exposure (Table 6). Survival of amphipods was also lower in the CW-3 \times treatment (79%) than in controls (95%), but this difference was not statistically significant. Growth and reproduction were not significantly affected in any treatment groups that did not show significant effects on survival. Amphipod mortality did not correspond closely to Cr concentrations in sediments, as significant mortality occurred in treatments with the greatest and lowest total Cr concentrations (220 $\mu\text{mol/g}$ in WB-10 \times and 3.3 $\mu\text{mol/g}$ in FL-Cr6) but not at several intermediate concentrations (Table 4). Survival was significantly decreased in only two of the seven spiked sediments that contained total Cr concentrations greater than a proposed sediment quality

TABLE 5. Characteristics of Pore Waters from a Toxicity Test with Cr(VI)-Spiked Sediments^a

treatment	filterable Cr ($\mu\text{g/L}$)		pH	DOC (mg/L)	alkalinity (mM)	hardness (mM)	ammonia (mg/L)
	day 0	day 28					
FL-control	4	43	6.98	65	0.6	0.6	2.4
FL-Cr6	276 000	39 400	5.85	98	0.7	0.2	1.7
WB-control	2	10	7.46	9.5	4.8	3.8	8.6
WB-1 \times	67	235	8.05	7.0	1.2	1.0	nd
WB-3 \times	1 070	132	7.98	6.8	1.6	0.8	7.9
WB-10 \times	695 000	19 500	8.95	21	1.9	0.2	7.5
CW-control	4	3	6.03	3.0	1.0	2.1	1.6
CW-1 \times	74	358	6.01	2.3	0.4	1.5	1.0
CW-1.5 \times	178	485	7.17	3.0	0.3	0.5	1.1
CW-3 \times	133 000	4 450	7.55	21	2.8	0.4	1.9

^a Means ($n = 2$), except filterable Cr. nd, no data.

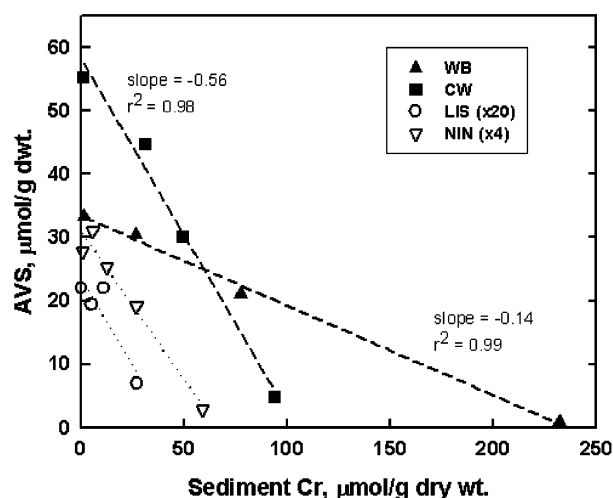


FIGURE 2. Relationship between sediment Cr and AVS in Cr(VI)-spiked sediments. Dashed lines indicate linear regressions. Data from saltwater sediments (LIS and NIN) from Berry et al. (14) are multiplied to facilitate comparisons.

TABLE 6. Results of a Toxicity Test with the Amphipod, *Hyalella azteca*, Exposed to Cr(VI)-Spiked Sediments^a

treatment	survival (%) day 28	length (mm) day 28	reproduction (young/female)
FL-control	96 (3)	4.5 (0.1)	8.7 (1)
FL-Cr6	0*	nd	nd
WB-control	96 (2)	4.5 (0.1)	8.7 (1)
WB-1×	95 (3)	4.6 (0.1)	8.7 (2)
WB-3×	91 (4)	4.7 (0.1)	6.6 (1)
WB-10×	0 *	nd	nd
CW-control	95 (3)	4.5 (0.1)	6.3 (1)
CW-1×	95 (2)	4.7 (0.1)	6.1 (1)
CW-1.5×	91 (4)	4.8 (0.1)	8.2 (4)
CW-3×	79 (7)	4.3 (0.2)	3.8 (8)

^a Means with standard error ($n = 8$ for survival, $n = 4$ for length and reproduction). Asterisk indicates significant decrease in Cr(VI) treatment, relative to appropriate control ($p \leq 0.05$; ANOVA and Dunnett's test with rank-transformed data). nd, no data.

guideline ($2.2 \mu\text{mol/g}$; 11). However, amphipod mortality in Cr(VI)-spiked sediments corresponded closely to concentrations of AVS and Cr(VI). Both toxic sediment treatments, FL-Cr6 and WB-10 \times , had AVS concentrations less than $1 \mu\text{mol/g}$ on day 0 (Table 4) and Cr(VI) concentrations greater than the LC_{50} in pore water and overlying water (Figure 1). Concentrations of Cr(VI) in pore water and overlying water of the CW-3 \times treatment were also high on day 0, but Cr(VI) concentrations in overlying water and pore water were less than the LC_{50} by day 28 (Figure 1), consistent with the lesser effects on survival observed in this treatment.

Our results also indicate that reaction with AVS and OM can reduce Cr(VI) concentrations below toxic levels in freshwater sediments. Spiked sediments with substantial concentrations of AVS ($\geq 5 \mu\text{mol/g}$) had concentrations of Cr(VI) near detection limits in pore water and caused no toxicity to *H. azteca*. In several instances, our data suggest that elevated Cr(VI) concentrations in pore water coexisted with substantial concentrations of AVS. These results could reflect either kinetic limitations on the reaction between Cr(VI) spikes and AVS or spatial variation in concentrations of Cr(VI) and AVS. Decreases in aqueous Cr(VI) concentrations in the CW-3 \times sediment during the course of the test may reflect a slow reaction of Cr(VI) spikes with AVS and OM. Although complete reaction of Cr(VI) with ferrous iron occurs rapidly (in minutes to hours; 28), studies with wetted soils and wetland sediments indicate that Cr(VI) reduction may

be incomplete 10 d or more after spiking (7, 29). Our measurements of low Cr(VI) levels in pore water in the presence of AVS in the CW-3 \times treatment may have been an artifact of the speciation method. However, AVS concentrations increased during the course of the study in the WB-10 \times treatment (Table 4) despite the presence of high concentrations of Cr(VI) in pore water (Figure 1). This apparent coexistence of AVS and Cr(VI) suggests that spiked sediments were not adequately mixed or that redox gradients developed during the course of the study. In field-collected sediments, AVS concentrations may differ substantially between surficial sediments inhabited by benthic fauna and deeper sediment layers (10). Therefore, characterization of sediment samples that include deeper sediments could either over- or underestimate the ability of ambient AVS to protect against toxicity of Cr(VI), depending on the nature of vertical gradients in AVS concentrations.

Chemistry and Toxicity of Cr(III)-Spiked Sediments.

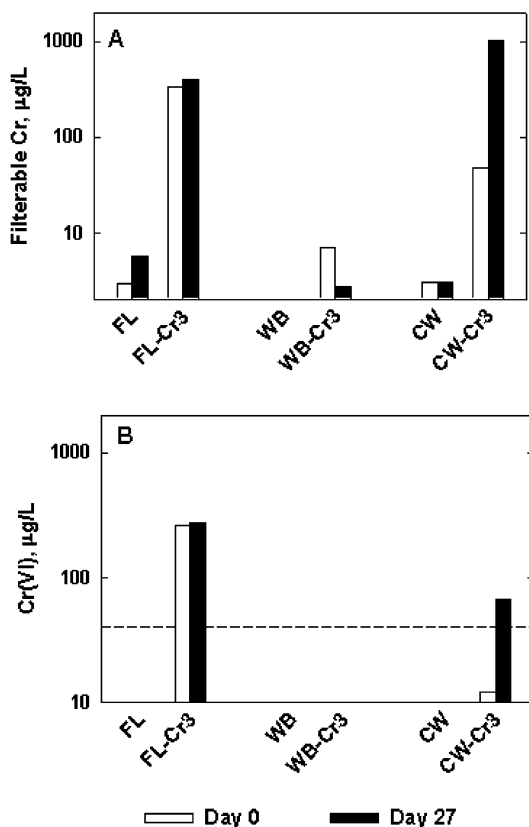
Spiking sediments with Cr(III) produced high levels of sediment Cr without substantially altering other characteristics of sediment or pore water. Cr(III)-spiked sediments had total Cr concentrations (Table 7) equal to or greater than sediments spiked with comparable Cr(VI) spike solutions (Table 4). AVS concentrations were similar to those measured in the earlier Cr(VI) spiking study, except the WB sediment had only about one-third the AVS measured during the previous study. AVS concentrations in the WB-control and WB-Cr3 sediments decreased further during the course of the study. Cr(III) spikes resulted in increases in pH (0.3–0.5 units), hardness, and ammonia in pore waters of all three Cr(III)-spiked sediments (Table 7). Water quality of overlying water showed few differences among sediment types or Cr(III) spike treatments (see Supporting Information).

Concentrations of filterable Cr and Cr(VI) were greater than expected in pore water and overlying water of some Cr(III)-spiked sediments. Pore waters of the FL-Cr3 and CW-Cr3 treatments had filterable Cr concentrations (Figure 3) comparable to those in several treatments from the Cr(VI) spiking study, but much lower than treatments that received Cr(VI) spikes with comparable Cr levels (Table 5). In the FL-Cr3 treatment, concentrations of filterable Cr and Cr(VI) were similar in pore water and were stable over the course of the study. Only the FL-Cr3 treatment had measurable concentrations of filterable Cr ($9.4 \mu\text{g/L}$) and Cr(VI) ($12 \mu\text{g/L}$) in overlying waters (see Supporting Information). Cr(VI) concentrations in pore waters of the FL-Cr3 treatment ($266 \mu\text{g/L}$) were greater than the LC_{50} , but remained an order of magnitude lower than concentrations associated with toxicity in the Cr(VI) spiking study. Pore waters of the CW-Cr3 treatment also had high levels of filterable Cr, but Cr(VI) concentrations were substantially lower than those in the FL-Cr3 treatment. Concentrations of both filterable Cr and Cr(VI) increased in pore waters of CW-Cr3 treatment during the course of the study, but neither filterable Cr nor Cr(VI) was measurable in overlying waters. The high levels of filterable Cr measured in the CW-Cr3 treatment, combined with relatively low measured concentrations of Cr(VI), suggest that the solubility of Cr(III) in these pore waters was enhanced by formation of organic complexes. This also suggests that the Cr(VI) concentrations measured in these samples may have been influenced by passage of Cr(III)–organic complexes through the cation-exchange resin. However, the proportion of filterable Cr that was measured as Cr(VI) ($\sim 10\%$) was much greater than the proportion of any Cr(III) spikes recovered in the Cr(VI) fraction ($< 1\%$).

These results suggest that the oxidation of Cr(III) was affected by characteristics of the test sediments. The occurrence of quantifiable concentrations of Cr(VI) in pore waters of two of the three Cr(III)-spiked sediments indicated that a small fraction of added Cr(III) ($< 0.1\%$) was oxidized

TABLE 7. Characteristics of Sediment and Pore Water from a Toxicity Test with Cr(III)-Spiked Sediments^a

treatment	total Cr ($\mu\text{mol/g}$)	AVS ($\mu\text{mol/g}$)		pH	alkalinity (mM)	hardness (mM)	ammonia (mg/L)
		day 0	day 28				
FL-control	0.6	<0.01	<0.01	6.69	0.9	1.1	0.97
FL-Cr3	5.6	<0.01	<0.01	7.02	2.0	3.4	2.0
WB-control	1.4	13	6.9	6.59	0.6	2.5	2.2
WB-Cr3	340	13	9.4	6.86	0.5	6.7	11
CW-control	1.1	86	87	7.16	4.1	3.1	12
CW-Cr3	350	79	83	7.59	1.3	7.8	14

^a Means ($n = 2$), except AVS.FIGURE 3. Pore water Cr concentrations during toxicity tests with Cr(III)-spiked sediments: (A) filterable Cr and (B) Cr(VI). Dashed line indicates LC_{50} for Cr(VI).

to Cr(VI). Previous studies have documented oxidation of Cr(III) by manganese oxides and reported that rates of Cr(III) oxidation are inversely related to sediment OM (7, 9). This mechanism is consistent with our observation that pore water Cr(VI) concentrations were greatest in the low-TOC, oxidized FL soil, despite much greater Cr(III) spikes in the CW-Cr3 and WB-Cr3 sediments. There was no evidence of net oxidation of Cr(III) in the highly organic WB-Cr3 sediment. Filterable Cr and Cr(VI) accumulated over time in the CW-Cr3 sediment despite the presence of AVS, suggesting that oxidation of Cr(III) occurred in oxic surface layers. The lower rates of net Cr(III) oxidation in the WB-Cr3 and CW-Cr3 sediments can be explained by several mechanisms, including sorption of Cr(III) to OM, interaction of OM with potentially reactive sites on manganese oxides (9), and/or reduction of Cr(VI) by AVS.

Exposure to Cr(III)-spiked sediments was associated with sublethal toxic effects on *H. azteca*. Amphipod survival did not differ significantly between control and Cr(III) treatments for any of the sediments, and mean survival ranged from 90% to 99% in five of six treatment groups on day 28 (Table

TABLE 8. Results of a Toxicity Test with the Amphipod, *Hyaella azteca*, Exposed to Cr(III)-Spiked Sediments^a

treatment	survival (%)	length (mm)		reproduction (young/female)
	day 28	day 28	day 42	
FL-control	91 (5)	3.6 (0.04)	4.3 (0.10)	4.0 (2.0)
FL-Cr3	80 (11)	3.3 (0.12) ^b *	4.1 (0.15)	4.5 (2.2) ^b
WB-control	99 (2)	4.1 (0.09)	4.2 (0.04)	2.8 (0.54)
WB-Cr3	96 (3)	3.6 (0.15)*	4.2 (0.11)	0.93 (0.37)*
CW-control	96 (2)	4.8 (0.11)	5.0 (0.12)	6.6 (1.7)
CW-Cr3	90 (4)	4.4 (0.06)*	4.4 (0.09)*	7.0 (0.91)

^a Means with standard error ($n = 4$, except $n = 8$ for 28-d survival, or as noted). Asterisks indicate significant decreases in Cr(VI) treatment, relative to control ($p \leq 0.05$; ANOVA and Dunnett's test on rank-transformed data). ^b $n = 3$.

8). The lower mean survival (80%) in the FL-CR3 treatment is attributable to one replicate out of eight that had no animals recovered, perhaps reflecting a stocking error. Small but significant reductions in growth (8–13% less than controls) occurred in all three Cr(III)-spiked sediments by day 28. A significant reduction in growth was still evident in the CW-Cr3 treatment on day 42, after amphipods were held in clean water for 14 d. Reproduction was substantially decreased only in the WB-Cr3 treatment. These toxic effects were not consistent with results of our studies of the toxicity of Cr(III) or Cr(VI) in water or sediment and did not correspond in a concentration-dependent fashion with constituents of sediment or pore water. Effects of Cr(VI) on growth and reproduction seem unlikely because our studies of Cr(VI) toxicity in water and Cr(VI)-spiked sediments showed no significant sublethal effects at Cr(VI) concentrations equal to or greater than those measured in the Cr(III) spiking study. Also, Cr(VI) concentrations were low in pore waters of the WB-Cr3 treatment, which had significant effects on both growth and reproduction. Our water-only test with Cr(III) also showed little evidence of sublethal effects, as discussed above. Concentrations of Cr(III) in pore waters of FL-Cr3 and CW-Cr3 sediments were greater than concentrations associated with decreased survival in the water-only study but were low in the WB-Cr3 sediment, which showed equal or greater sublethal effects (Table 8). None of the other pore water characteristics associated with the Cr(III) spikes, such as increased pH or hardness (Table 7), are plausible causes for the observed effects on growth and reproduction. Total Cr levels in our Cr(III)-spiked sediments approached (FL-Cr3) or greatly exceeded (WB-Cr3 and CW-Cr3) the 99th percentile of sediment Cr concentrations reported in a national sediment quality database ($6.5 \mu\text{mol/g}$; 30). At such high levels of spiked Cr(III), solid-phase constituents, such as precipitated $\text{Cr}(\text{OH})_3$, may have contributed to the observed sublethal effects by acting as an irritant or by reducing the quality or availability of food. Thus, the sublethal effects observed in our Cr(III)-spiked sediments may not be representative of risks of sediment toxicity in Cr-contaminated aquatic environments.

Risks of Toxicity of Chromium-Contaminated Sediments. Results of our studies are consistent with the hypothesis that the presence of substantial concentrations of AVS in freshwater sediments greatly reduces risks of Cr toxicity to benthic organisms. Concentrations of AVS and, to a lesser extent, OM decreased in sediments spiked with Cr(VI). Cr(VI) spikes produced 100% mortality of amphipods in sediments without AVS and in sediments where nearly all AVS was eliminated by reaction with excess Cr(VI), but no significant toxic effects were observed in Cr(VI)-spiked sediments that contained AVS concentrations greater than 1 $\mu\text{mol/g}$ after spiking. Our toxicity tests did not produce clear evidence of Cr(III) toxicity in sediment. Amphipod survival was not significantly reduced by exposure to Cr(III)-spiked sediments containing extremely high total Cr concentrations (near 2% by weight). Growth and (in one case) reproduction were reduced in amphipods exposed to Cr(III)-spiked sediments, but these responses did not correspond to levels of total Cr in sediments or to levels of filterable Cr or Cr(VI) in pore water. We observed significant oxidation of Cr(III) to Cr(VI) only in oxic sediments without AVS. Overall, our results indicate that toxic effects of Cr are unlikely in freshwater sediments containing substantial concentrations of AVS and/or OM.

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Supporting Information Available

Detailed descriptions of methods for Cr(VI) speciation method, quality assurance for Cr analyses, and toxicity testing along with tables of additional data on water quality during toxicity tests. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Literature Cited

- (1) Luther, G. W. In *Metal Contaminated Aquatic Sediments*; Allen, H. E., Ed.; Ann Arbor Press: Chelsea, MI, 1995; pp 65–80.
- (2) Pawlicz, A. V.; Kent, R. A.; Schnieder, U. A.; Jefferson, C. *Environ. Toxicol. Water Qual.* **1997**, *12*, 185.
- (3) *Quality Criteria for Water—1986*; U.S. Environmental Protection Agency, Office of Water: Washington, DC, 1986; EPA 440/5-86-001.
- (4) *1995 Updates: Water Quality Criteria Documents for the Protection of Aquatic Life in Ambient Water*; U.S. Environmental Protection Agency, Office of Water: Washington, DC, 1996; EPA 820-B-96-001.
- (5) Wittbrodt, P. R.; Palmer, C. D. *Environ. Sci. Technol.* **1995**, *29*, 255.
- (6) Elovitz, M. S.; Fish, W. *Environ. Sci. Technol.* **1995**, *29*, 1933.
- (7) Masscheleyn, P. H.; Pardue, J. H.; DeLaune, R. D.; Patrick, W. H., Jr. *Environ. Sci. Technol.* **1992**, *26*, 1217.
- (8) Eary, L. E.; Rai, D. *Environ. Sci. Technol.* **1988**, *22*, 972.
- (9) Hassan, S. M.; Garrison, A. W. *Chem. Speciation Bioavailability* **1996**, *8*, 85.
- (10) Besser, J. M.; Ingersoll, C. G.; Giesy, J. P. *Environ. Toxicol. Chem.* **1996**, *15*, 286.
- (11) MacDonald, D. D.; Ingersoll, C. G.; Berger, T. A., *Arch. Environ. Contam. Toxicol.* **2000**, *39*, 20.
- (12) *Procedures for Deriving Equilibrium Partitioning Sediment Guidelines (ESGs) for the Protection of Benthic Organisms: Metal Mixtures (Cadmium, Copper, Lead, Nickel, Silver, and Zinc)*; U.S. Environmental Protection Agency, Office of Science and Technology/Office of Research and Development: Washington, DC, 2000; EPA-822-R-00-005.
- (13) Ankley, G. T.; DiToro, D. M.; Hansen, D. J.; Berry, W. J. *Environ. Toxicol. Chem.* **1996**, *15*, 2056.
- (14) Berry, W. J.; Boothman, W. S.; Serbst, J.; Edwards, P. A. *Environ. Toxicol. Chem.* **2004**, *23* (12), in press.
- (15) Ingersoll, C. G.; Brunson, E. L.; Dwyer, F. J.; Hardesty, D. K.; Kemble, N. E. *Environ. Toxicol. Chem.* **1998**, *17*, 1508.
- (16) Besser, J. M.; Brumbaugh, W. G.; May, T. W.; Ingersoll, C. G. *Environ. Toxicol. Chem.* **2003**, *22*, 805.
- (17) *Methods for Measuring the Toxicity and Bioaccumulation of Sediment-Associated Contaminants with Freshwater Invertebrates*; U.S. Environmental Protection Agency, Office of Water: Washington, DC, 2000; EPA/600/R-99/064.
- (18) Standard test methods for measuring the toxicity of sediment-associated contaminants with freshwater invertebrates, In *Annual Book of Standards*, Vol. 11.05; ASTM International: West Conshohocken, PA, 2003; ASTM E1706-00.
- (19) Brumbaugh, W. G.; Ingersoll, C. G.; Kemble, N. E.; May, T. W.; Zajicek, J. L. *Environ. Toxicol. Chem.* **1994**, *12*, 1971.
- (20) Subramanian, K. S.; Méranger, J. C.; Wan, C. C.; Corsini, A. *Int. J. Environ. Anal. Chem.* **1985**, *19*, 261.
- (21) Icopini, G. A.; Long, D. T. *Environ. Sci. Technol.* **2002**, *36*, 2994.
- (22) Richard, F. C.; Bourg, A. C. M. *Water Res.* **1991**, *25*, 807.
- (23) Tomasovic, M.; Dwyer, F. J.; Greer, I. E.; Ingersoll, C. G. *Environ. Toxicol. Chem.* **1995**, *14*, 1177.
- (24) *National Recommended Water Quality Criteria: 2002*; U.S. Environmental Protection Agency, Office of Water: Washington, DC, 2002; EPA 822-R-02-047.
- (25) Poléo, A. B. S. *Aquat. Toxicol.* **1995**, *31*, 347.
- (26) Makos, J. D.; Hrnecir, D. C. *Environ. Sci. Technol.* **1995**, *29*, 2414.
- (27) Wang, W.-X.; Griscom, S. B.; Fisher, N. S. *Environ. Sci. Technol.* **1997**, *31*, 603.
- (28) Fendorf, S. E.; Li, G. *Environ. Sci. Technol.* **1996**, *30*, 1614.
- (29) Kozuh, N.; Stupar, J.; Gorenc, B. *Environ. Sci. Technol.* **2000**, *34*, 112.
- (30) *National Sediment Quality Survey Database—1980 to 1999*; U.S. Environmental Protection Agency, Office of Science and Technology: Washington, DC, 2001; EPA-823-C-01-001.

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