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The Influence of Sediment Constituents and Redox Conditions on the Bioavailability of Copper in Freshwater Sediments

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John Michael Besser

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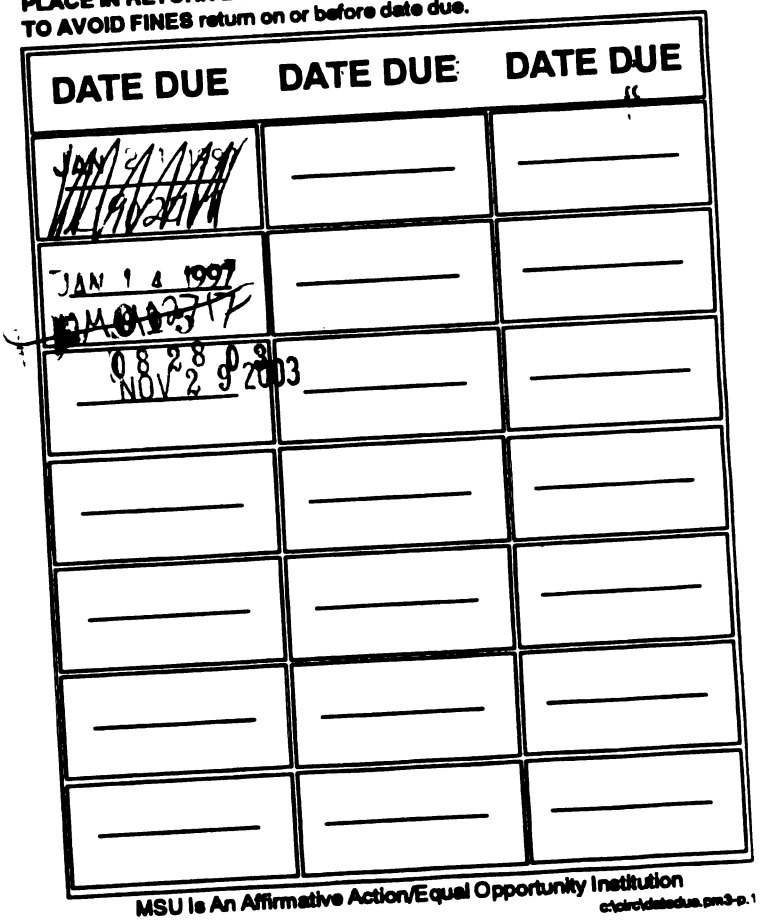

THE INFLUENCE OF SEDIMENT CONSTITUENTS AND REDOX CONDITIONS ON THE BIOAVAILABILITY OF COPPER IN FRESHWATER SEDIMENTS

By

John Michael Besser

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ABSTRACT

THE INFLUENCE OF SEDIMENT CONSTITUENTS AND REDOX CONDITIONS ON THE BIOAVAILABILITY OF COPPER IN FRESHWATER SEDIMENTS

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Sediment bioassays with larvae of the midge, *Chironomus tentans*, were used to assess the bioavailability of copper (Cu) in sediments collected from lakes in Michigan and from the upper Clark Fork River in Montana. The associations of bioassay responses (survival, growth, and metal bioaccumulation) with concentrations of metals in sediment and porewater and with other physicochemical characteristics of sediment and porewater, including acid-volatile sulfide (AVS), were evaluated. Bioaccumulation of Cu and toxic effects on growth and survival of *C. tentans* were negatively correlated with concentrations of sediment organic carbon and AVS and positively correlated with concentrations of Cu in porewater. Bioaccumulation of Cu increased significantly at concentrations of Cu less than those affecting growth and survival. Concentrations of metals in sediment extracts, normalized to concentrations of AVS (SEM:AVS ratios), were weakly correlated with bioassay responses. Although toxicity and bioaccumulation of metals was consistently low in sediments with very low SEM:AVS ratios, the association of Cu bioavailability with SEM:AVS ratios was inconsistent in sediments with ratios close to or greater than 1.0.

Bioassays indicated substantial spatial and temporal variation in metal

bioavailability. Concentrations of AVS, SEM:AVS ratios, and bioassay responses differed significantly between depths in sediment cores from the Montana sites, and bioaccumulation of Cu was significantly greater in deeper core sections, which contained lesser concentrations of AVS. Seasonal changes in AVS concentrations were simulated by incubating sediments under oxic and anoxic conditions in the laboratory. Differences in metal bioavailability between oxic and anoxic treatments varied among sediments from different locations, primarily due to differences in the rates of oxidation of AVS during oxic incubations. Greatest differences in concentrations of AVS between treatments were associated with significant differences in bioaccumulation of Cu and growth of midge larvae in sediments.

Although these results indicate that AVS is a major control on bioavailability of Cu in freshwater sediments, bioaccumulation of Cu and toxicity of Cu-contaminated sediments were more accurately predicted by concentrations of Cu in porewater than by SEM:AVS ratios. Regardless of which measure of metal bioavailability is used, assessments of sediment quality must consider the effects of natural spatial and temporal variation in metal bioavailability.

This dissertation is dedicated to my parents, Theodore and Mary Besser, for encouraging my early interest in nature and for their continuing support, moral and financial, for my extended educational pursuits.

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INTRODUCTION

Problem Statement

The U.S. Environmental Protection Agency and other regulatory agencies have recently begun to develop criteria for protection of aquatic life from toxic effects of sediment-associated contaminants. Field and laboratory methods for detecting the toxicity of sediments to aquatic organisms have been developed in the past decade (Giesy and Hoke 1989, Burton 1991), and these methods have been used to produce site-specific assessments of contaminant impacts in both freshwater and marine environments (Chapman et al. 1987, Reynoldson and Zarull 1989, Becker et al. 1990). However, recent efforts have focused on development of numerical (concentration-based) criteria that will have broader applicability (Chapman 1989). Based on evidence that toxicant concentrations in porewater are good indicators of toxicity to benthic organisms (e.g. Ankley et al. 1991a), efforts to produce sediment quality criteria have focused on prediction of porewater concentrations of toxicant from concentrations in whole sediment. This method, termed "equilibrium partitioning", has been used to produce criteria for non-ionic organic compounds based on concentrations of organic carbon in sediment, partition coefficients for each compound between n-octanol and water (K_{ow}), and the aqueous toxicity of the compound (DiToro et al. 1991a). This method is not easily extended to heavy metals, due to their

complex partitioning behavior. However, there is currently support for the use of acid-volatile sulfide (AVS) concentrations to normalize metal concentrations in sediment. The ratio, [acid-soluble metal concentrations / AVS concentration], has been shown to simplify relationships between metal concentrations and toxicity in metal-contaminated sediments (Carlson et al. 1991, Ankley et al. 1993a).

Proposals to use AVS-normalized metal concentrations to develop of sediment quality criteria for heavy metals raises several important questions about metal associations in freshwater sediments. Although AVS control on metal bioavailability in freshwater sediments has been observed (Carlson et al. 1991), the importance of AVS control over metal bioavailability has not been demonstrated over a wide range of freshwater sediments. The range of AVS concentrations reported from freshwater sediments is similar to that occurring in marine sediments (DiToro et al. 1990), although sulfidic sediments may occur less frequently in freshwater environments (Berner 1981). In sediments containing little or no AVS, metal bioavailability is affected by a variety of sorptive components. The nature of these sorption processes are still poorly understood, especially when multiple competing phases are present (Luoma and Davis 1983, Allard et al. 1987). The instability of sulfides under oxidizing conditions raises the possibility that metal bioavailability could vary seasonally or in response to human activities such as dredging. Changes in metal bioavailability under oxidizing conditions would depend on the kinetics of metal solubilization from the sulfide phase and readsorption on binding components

such as organic matter and iron and manganese hydrous oxides, which are stable under oxidized conditions (Oakley et al. 1980). Although the understanding of these processes needed to produce sediment quality criteria for management of contaminated sediments may be less than that needed to produce accurate mathematical models of metal behavior, our current understanding may fall short of either standard.

LITERATURE REVIEW

Controls on Metal Bioavailability in Freshwater Sediments

The toxicity of dissolved heavy metals entering aquatic ecosystems is modified by a variety of processes. Both chemical and biological activity of metal ions are reduced by formation of complexes with inorganic ligands such as carbonates, sulfate, and chlorides (Mann and Deutscher 1977) and with a variety of dissolved organic compounds (Giesy et al. 1986). Although dissolved metal complexes have a wide range of characteristics, formation of many types of complexes have been shown to produce reduce metal bioaccumulation (Poldoski 1979, Dodge and Theis 1982) and toxicity (Giesy et al. 1983, Borgmann and Ralph 1983) compared to free metal ions. Dissolved metals also become associated with suspended particulates and, particulate-bound metals often make up a large proportion of total metals in surface waters (Gibbs 1973, Hem 1976). Association with particulates reduces metal bioavailability and increases the rate of loss of metals from the water column (Rudd and Turner 1983). Concentrations of metals in the bottom sediments of lakes, rivers, and

harbors have increased as a result of increased metal inputs associated with industrialization (Kemp et al. 1976). Deposition and burial of metals in sediments has sometimes been considered a mechanism for detoxification of overlying waters (Jackson 1978). However, there is evidence that sediment-associated metals can be available to benthic organisms or released to overlying waters as a result of natural changes in sedimentary environments (Lu and Chen 1977, Hunt and Smith 1983) or due to anthropogenic changes such as lake acidification (Schindler et al. 1980).

Metals are associated with a wide variety of components in sediments, and the nature of these associations affects both metal bioavailability and mobility. The primary mechanisms for metal-sediment associations are ion-exchange, adsorption, and coprecipitation, although the nature of these associations are not always distinct, even for individual classes of sediment contaminants (Jenne and Luoma 1977, Allard et al. 1987). Adsorption to iron and manganese hydrous oxides (Davis and Leckie 1978, Lion et al. 1982, Davies-Colley et al., 1984) and adsorption or chelation to organic surfaces (Breteler and Saksa 1985, Swartz et al. 1986) are often the dominant metal associations in aerobic sediments. Under anaerobic conditions, metals can be associated with highly insoluble metal sulfides (Luther et al. 1980, Lee and Kittrick 1984). Mathematical models based on cation-exchange (Hem 1976) and adsorption processes (Oakley et al. 1981) have been used to estimate the relative importance of these metal-sediment associations. The success of such models depends on an understanding of the adsorption characteristics of

heterogeneous sediment surfaces such as Fe/Mn oxides and organic matter (Balistrieri and Murray 1983, Luoma and Davis 1983). Models of copper and cadmium adsorption in oxic estuarine and freshwater sediments have found that concentrations of free metal ions can be predicted based on measurements of organic matter and iron oxides (Davies-Colley et al. 1984, Tessier et al. 1993). However, these models have large uncertainties associated with estimates of partitioning coefficients and binding capacities of sediment components (Luoma and Davis 1983, Honeyman and Santschi 1988).

An alternative to mathematical modeling of metal-binding processes has been the use of chemical extractants to selectively remove sediment metal fractions for direct quantitation. Although many different extractants have been used, most researchers have adopted methods intended to sequentially extract metals in exchangeable (adsorption and cation exchange), carbonate, reducible (Fe/Mn oxides), sulfide plus organic, and residual (crystalline) fractions (Tessier and Campbell 1987). Although often interpreted as if they represent the specific groups suggested by these names, fractions produced by such methods are more accurately interpreted as a broadly overlapping spectrum from very chemically labile to very resistant (Martin et al. 1987). Selective extraction techniques have been used to assess changes in sediment-metal associations during early diagenesis, aeration, and resuspension (McKee et al. 1989a, Khalid et al. 1981, Kersten and Förstner 1986).

Measurement of fractions of chemical extracts and direct measurements of metal binding components have been compared to data from sediment

bioassays to estimate the bioavailability of different sediment phases. Some selective extraction studies with aerobic sediments have found significant positive correlation of metal bioaccumulation with metal concentrations in exchangeable, weak acid, or reducible sediment fractions (Luoma and Bryan 1982, Diks and Allen 1983, Bendell Young and Harvey 1991), but others have found no such associations (Luoma and Bryan, 1978). Organic matter can reduce metal bioavailability in laboratory bioassays (Phelps 1979) and in natural or amended sediments (Breteler and Saksa 1985, Swartz et al. 1986). Recent research has suggested that amorphous metal sulfides, termed acid-volatile sulfide (AVS) because dilute HCl is used to volatilize sulfide for quantitation, can be a major control on metal bioavailability in sediments. The sulfides of heavy metals can be formed by preferential replacement of iron in iron sulfide precipitates, and the solubility of these sulfides is so low that metals can be scavenged very effectively from solution in porewater (DiToro et al. 1990). Several studies have found that metal-contaminated sediments are toxic to benthic invertebrates only if metal concentrations exceed AVS concentrations on a molar basis (DiToro et al. 1990, Ankley et al. 1991b, Carlson et al. 1991). Di Toro et al. (1990) have proposed that the molar ratio of simultaneously-extracted metals to AVS (SEM:AVS) in sediments be used to predict metal toxicity, with no toxicity predicted when SEM:AVS ratios are less than or equal to 1.0.

Sediment components controlling metal bioavailability and mobility are strongly influenced by prevailing geochemical conditions (Salomons et al.

1987). Sediments can be divided into geochemical classifications based on redox status (oxic or anoxic) and dominant mineral phases (sulfidic or nonsulfidic) (Berner 1981). High organic loadings and high sulfate concentrations in estuarine and coastal marine environments favor the development of reduced, sulfidic sediments (Luther et al. 1980). Sulfides are the primary control on metals under these conditions, and several studies have suggested that the release of metals from estuarine and marine sediments is related to oxidation of sulfidic sediments (Lu and Chen 1977, Boulegue et al. 1982, Hunt and Smith 1983, Kersten and Förstner 1986). Nonsulfidic conditions may be more predominant in anoxic freshwater sediments, where development of sulfides is more often limited by sulfate concentrations (Berner 1981). Metal mobility in freshwater sediments is commonly controlled at the interface between oxic surface layers and anoxic, nonsulfidic conditions at depth (Salomons 1985). Solubilization of metals can occur at this oxic-anoxic interface during diagenesis, due to microbial oxidation of organic matter and reduction of manganese oxides (McKee et al. 1989a,b). The partitioning of metals between sediment and dissolved phases differs both qualitatively and quantitatively between sulfidic sediments, where solubility equilibria with sulfides maintain extremely low dissolved metal concentrations regardless of total metal concentrations, and other sediment types, where sorption equilibria produce dissolved metal concentrations proportional to concentrations in the adsorbed phase (Salomons 1985).

Geochemical environments in sediment and associated sediment-metal

equilibria are affected by biological processes in sediments and overlying waters. Organic matter production and deposition in lakes varies both spatially and temporally (Carlton and Klug 1990), and these variations together with seasonal patterns of mixing and stratification can cause changes in sediment environments which affect metal partitioning (Carignan and Lean 1991). Seasonal cycles of biological production and temperature in freshwater lakes produce variation in concentrations of AVS with season and depth (Howard and Evans 1993, Leonard et al. 1993), which may produce corresponding changes in bioavailability of metals. Seasonal changes in redox conditions in surficial sediments can be associated with either increases (Cornett and Ophel 1986, Hesslein 1987) or decreases (Shepard et al. 1980) in metal concentrations in overlying waters. These changes may be more extreme in highly dynamic systems such as rivers or estuaries (Luoma et al. 1985). Burrowing activities of benthic invertebrates (bioturbation) can also increase metal release to overlying waters (Renfro 1973, Graneli 1979). Metal releases caused by bioturbation are apparently related both to excavation and resuspension of sediment particles and alteration of redox gradients (Salomons 1985). These processes can substantially delay the burial of metal-enriched particulates (Robbins 1982).

Metal mobility and bioavailability can be affected by redistribution of metals among sediment components during natural or anthropogenic changes in sedimentary environments. Metals bound to sulfides and organic matter can be shifted to more labile phases during oxidation of estuarine or riverine

sediments (Kersten and Förstner 1986, Khalid et al. 1981). However, the mechanisms controlling metal redistribution among competing sediment phases are poorly understood. Studies with a variety of experimental apparatus have found that affinities for readsorption are specific to different metals, sorptive phases, and sediment conditions. Metals spiked into individual sediment components (iron sulfide, humic acid, clay, sand, and water) were found to equilibrate rapidly among competing components in anoxic model sediments (Oakley et al. 1980). The predominant binding phase for copper was sulfide, but cadmium, lead and zinc were more equally distributed among sulfide, humic acids, and clay. In suspensions of natural sediment (Elliott et al. 1986) and model binding materials (Calmano et al. 1988) under aerobic conditions, cadmium and copper adsorbed preferentially to organic matter compared to inorganic binding sites. Although these redistribution processes remain poorly understood, changing sediment environments and natural gradients at sediment-water and oxic-anoxic interfaces are potentially important influences on the bioavailability and mobility of sediment-associated metals.

RESEARCH GOALS

The three chapters which make up this dissertation address the following research goals:

1. Development of a bioassay of metal bioavailability in freshwater sediments. Sediment bioassays with benthic invertebrates have often relied on the simple respons of lethality, which gives unequivocal evidence of sediment

toxicity, but which may not be sufficiently sensitive to detect subtle differences in the bioavailability of contaminants (Giesy and Hoke 1989). Recently, several methods have been developed for sediment bioassays, which incorporate more sensitive endpoints such as growth and bioaccumulation (USEPA 1994, Environment Canada 1995). A bioassay with larvae of the midge, *Chironomus tentans,* was developed for this research, which used endpoints of growth and bioaccumulation. Growth of larvae of *C. tentans* in sediment bioassays is a sensitive response to toxicants and an indicator of potential effects on benthic communities (Giesy et al. 1988). Bioaccumulation is an appropriate measure of bioavailability of metals, because it is closely associated with concentrations of metals in both water and sediment porewater (Borgmann et al. 1991) and is relatively free of interferences of water quality, sediment characteristics, and effects of other toxic substances. *C. tentans* and other chironomid taxa have also been widely used in studies of bioaccumulation of metals from water and sediment (Krantzberg and Stokes 1989, Hare et al. 1991). The resistance of *C. tentans* to lethal effects of contaminants (Giesy and Hoke 1989) is an advantage for bioaccumulation studies, because larvae can be recovered for chemical analysis from all but the most highly-contaminated sediments.

2. Empirical assessment of associations of metal bioavailability with sediment characteristics. Although proposals for development of sediment quality criteria have focused on associations of contaminants with single sediment phases, such as organic carbon (for non-ionic organic compounds; DiToro et al. 1991a) or acid-volatile sulfide (for metals; DiToro et al. 1991b),

the bioavailability of metals in sediments may be controlled by many different sediment constituents. The associations of metal bioaccumulation and toxicity with metal:AVS ratios in sediments or metal concentrations in porewaters was assessed based on *C. tentans* bioassays with sediments from aquatic systems in Michigan and Montana, which differ in their degree of metal contamination, as well as their water and sediment chemistry, and biological communities. Associations among bioassay responses and various physicochemical influences on metal bioavailability were also assessed with multivariate statistical methods (Johnson et al. 1992, Somerfield et al. 1994).

3. Experimental assessment of the effects of redox conditions on metal bioavailability. The bioavailability of metals in sediments is strongly affected by sediment phases, such as AVS and hydrous oxides, which are not stable during changes between oxic and anoxic conditions. The relative importance of these redox-sensitive sediment phases and more stable phases, such as organic matter and silicate minerals, for controlling the bioavailability of metals in sediments was assessed experimentally by controlling the oxygen content of overlying waters. Bioassays with sediments incubated under oxic and anoxic conditions were used to test predictions of metal bioavailability based on differences in metal:AVS ratios. The effect of these manipulations on concentrations of AVS, metal:AVS ratios, and bioassays of metal bioavailability was used to indicate the likelihood of significant seasonal changes in metal bioavailability in freshwater sediments.

4. Site-specific evaluation of factors affecting metal bioavailability. The research tools developed during this project were applied to a case study of factors affecting metal bioavailability in a complex freshwater system, the upper Clark Fork River of Montana, which is contaminated with metals from mine wastes. This assessment included a survey of metal bioavailability in sediments from a series of sites, which differed in their physicochemical characteristics and degree of metal contamination; an assessment of the spatial variability of metals, AVS, and bioassay responses with depth in core samples; and an evaluation of the potential for seasonal variation in metal bioavailability, using incubation experiments. This assessment was used to evaluate predictions of the bioavailability and toxicity of metal-contaminated sediments based on metal:AVS ratios (DiToro et al. 1991a) or metal concentrations in porewater (Ankley et al. 1991b, 1993), and to assess spatial and temporal variation in AVS which could affect these predictions.

CHAPTER 1

Influences on Copper Bioaccumulation and Growth of the Midge, *Chironomus tentans*, in Metal-Contaminated Sediments

(for submission to *Journal of Aquatic Ecosystem Health*)

Abstract

Sediment bioassays with larvae of the midge, *Chironomus tentans*, were used to evaluate the use of metal bioaccumulation as an indicator of metal bioavailability, and to examine influences on metal bioavailability in sediments with a range of metal concentrations and other physicochemical characteristics. Sediments were collected from lakes in Michigan, USA, and from sites in the Clark Fork River drainage of Montana, USA, which are contaminated with metals from mining activities and from other anthropogenic sources. Bioassays with *C. tentans* larvae were conducted for ten days in a static-renewal test system, with endpoints of survival, growth, and metal bioaccumulation. Bioaccumulation of copper (Cu) by midge larvae was strongly correlated with concentrations of Cu in porewater and was significantly increased, relative to bioaccumulation from reference sediments, at Cu concentrations less than those

affecting growth or survival. Midge survival and growth were weakly correlated with concentrations of Cu and zinc (Zn) in sediment or porewater, and were poorly predicted by ratios of acid-extractable metals to acid volatile sulfide (AVS) in sediments. No toxicity was observed in several sediments with low concentrations of AVS and very high concentrations of copper and zinc in sediment and porewater. Principal components analysis indicated that bioaccumulation of Cu and Cu concentrations in porewater were influenced by AVS, sediment organic carbon, and pH. Toxicity was greatest in sediments with high concentrations of both metals and ammonia and low pH. These results indicate that AVS is a major control on metal bioavailability in freshwater sediments. However, the bioavailability of metals in oxidized sediments, or sediments with low sulfide concentrations, may be controlled primarily by organic matter and metal oxides.

INTRODUCTION

The bioavailability of toxic metals is an important consideration in the development of sediment quality criteria for management of contaminated sediments (Ankley et al. 1994). Investigations of metal toxicity have found that acid-volatile sulfides (AVS), amorphous sulfides of iron and other metals, are the dominant control of metal bioavailability and toxicity in marine sediments (DiToro et al. 1990). These studies have shown that the molar ratio of metals released by dilute acid extraction (simultaneously extracted metals or SEM) to AVS is a good indicator of metal bioavailability. If the molar ratio of

SEM to AVS (SEM:AVS ratio) is less than 1.0, solubility equilibria predict that metals should precipitate as metal sulfides, and porewater in equilibrium with metal sulfides should contain very low concentrations of toxic metals, including cadmium (Cd), Cu, lead (Pb), nickel (Ni) and Zn.

Recent studies with freshwater sediments have suggested that SEM:AVS ratios may also predict metal toxicity in freshwater sediments (Carlson et al. 1991, Ankley et al. 1993, Kemble et al. 1994). However, there are several possible limitations to the use of AVS normalization as a basis for sediment quality criteria. Estimates of metal bioavailability based solely on associations with sulfides may be either over- or under-protective, depending on local conditions. AVS concentrations vary both spatially and temporally in response to differences in redox conditions at the sediment:water interface (Brumbaugh et al. 1994). Seasonal changes in concentrations of AVS, as great as two orders of magnitude, have been reported in freshwater lakes (Howard et al. 1993; Leonard et al. 1993) and oxidation of sulfide-containing sediments can result in increased bioavailability of toxic metals (Calmano et al. 1993; Chapter 3, this volume). Normalization of metal concentrations to AVS does not consider binding of metals to non-sulfide binding phases, such as organic matter or iron and manganese oxides, in oxidized sediments or in sediments with SEM:AVS ratios greater than 1.0 (Fu and Allen 1992, Zhuang et al. 1993).

Sediment bioassays which evaluate the toxicity and bioaccumulation of persistent contaminants in benthic invertebrates can be useful for assessing

influences on the bioavailability of metals in different sediment environments. Many sediment bioassays with aquatic invertebrates have relied on the simple and easily-interpreted toxic response of lethality. However, larvae of the midge, *Chironomus tentans* (Diptera:Chironomidae), have been widely used for sediment bioassays because of the sensitive response of larval growth to sediment-associated toxicants (Giesy et al. 1988). The growth response of *C. tentans* has been incorporated into a standard method for sediment bioassay developed by the U.S. Environmental Protection Agency (USEPA 1994). Larvae of *Chironomus* spp. have also been widely used in studies of bioaccumulation of organic and inorganic contaminants (Besser and Rabeni 1987, Krantzberg and Stokes 1989, Lydy et al. 1992). The insensitivity of the lethality response in *Chironomus* spp. is an advantage in bioaccumulation studies, because larvae can be recovered for chemical analysis from all but the most toxic sediments.

The bioavailability and toxicity of Cu was assessed in sediments from several freshwater study areas in the United States, which differed in their limnologic characteristics and in their degree of contamination with Cu (Figures 1 and 2). Large quantities of Cu have been deposited into lakes in the Keweenaw Peninsula of Michigan, and into the headwaters of the Clark Fork River of Montana, in wastes from historic copper mining and smelting activities. Sediments from both mining areas have produced toxic effects in laboratory bioassays (West et al. 1993, Kemble et al., 1994) and adverse effects on native benthic invertebrate communities (Malueg et al. 1984. Canfield et al. 1994). Lesser quantities of Cu have been deposited into lakes in the lower peninsula

of Michigan, primarily from copper sulfate applications for control of aquatic nuisances.

The objectives of this study were:

(1) to compare the usefulness of metal bioaccumulation, growth, and survival of midge larvae as indicators of metal bioavailability, and

(2) to assess the influences of sediment and porewater characteristics on metal bioavailability and toxicity in contaminated sediments.

METHODS

Sediment Collection and Sample Preparation

Sediments were collected in 1992 from sixteen lakes and waterways in Michigan, USA (Figure 1), which were collected and tested in two groups. Eight sites from Michigan's Upper Peninsula, including several sites in the Keweenaw mining area (Torch Lake, TL; Keweenaw Waterway, KW and DB; and Lac LaBelle, LL) were sampled in August 1992. Sediments were collected in October 1992 from eight lakes in southern Michigan. Sediments were collected in August 1993 from 12 sites in the Clark Fork River drainage in western Montana, USA (Figure 2), downstream from a copper mining and smelting district, including five sites in the Clark Fork River (CF1-CF5), six sites in Milltown Reservoir, a small impoundment of the Clark Fork which has accumulated extensive deposits of metal-contaminated sediments (MR2 - MR25) and one site in Rock Creek (RC), an uncontaminated tributary of the Clark Fork. Sediments from relatively uncontaminated "reference sites" in each

of the three areas (CL, LO, RC), were treated as controls in statistical comparisons.

Sediment samples were collected with a petite ponar dredge or with a polypropylene scoop (at the Clark Fork River sites) and combined in a polyethylene bucket to produce composite sample of 3 to 8 liters. Sample containers, sampling gear, and laboratory apparatus were washed, rinsed with acid (10% HCl), and rinsed with ultrapure deionized water in the laboratory before each sampling trip. In the field, sampling gear was scrubbed, rinsed with 10% HCl, and rinsed with water from each site before use. Sediment samples from Michigan sites were stored in coolers for the duration of sampling trips (up to five days). Sediment samples from the Montana sites were placed on ice on the day of collection and shipped to the laboratory by the day after collection. All samples were transferred to a walk-in cooler for storage at 4°C before use.

Sediments were homogenized by stirring before samples were withdrawn for bioassays or chemical analyses. Bioassays and chemical analyses of porewater were initiated within 30 days of collection. Porewaters for analysis of metals and water quality parameters were prepared by centrifugation (DuPont Sorvall SS-3): sediments for 40 minutes at 7000 rpm and supernatants (porewater) were centrifuged for 15 additional minutes at 10,000 rpm. Porewater samples for metal analyses were preserved with nitric acid (Baker Instra-Analyzed) to a concentration of 1% (v/v). Porewaters for analysis of organic carbon were filtered through 1.0 μm pore diameter glass-fiber filters (Whatman GF/C), acidified with sulfuric acid to eliminate carbonates,

and refrigerated before analysis.

Bioassays

Sediment bioassays were conducted with larvae of the midge, *Chironomus tentans*, by a procedure similar to that developed by the U.S. Environmental Protection Agency (USEPA 1994). Midge larvae were cultured at 23 °C in dechlorinated tap water (hardness approximately 300 mg/L) with a paper pulp substratum and fed a suspension of blended fish food flakes (Tetra-Min). Cohorts for bioassays were started from eggs deposited on the same date and reared in the test dilution water. Larvae of uniform age (12-14 d after egg deposition) and uniform size were selected for use in bioassays.

Exposure chambers (4 replicates per sediment) were prepared from 300-mL "high-form" borosilicate glass beakers with two 17-mm holes, covered with 100 μm stainless screen, to allow water circulation. Each group of replicates was placed in a 9-liter all-glass aquarium, into which water replacements for were added by gravity from a polyethylene head tank. Dilution water for bioassays with Michigan sediments was well water diluted with ultrapure laboratory water to a hardness of 95 mg/L. Dilution water for bioassays with Montana sediments was moderately-hard reconstituted water, prepared from reagent-grade salts and ultrapure water (hardness 90 mg/L; USEPA 1994). The water replacement rate was one volume per day for bioassays with Michigan sediments and two volumes per day for bioassays with Montana sediments.

Bioassay chambers were filled with 100 mL of test sediment and dilution water and placed into the exposure aquarium (at 23 $\pm$ 1 °C) one day before the start of bioassay. One volume of dilution water was added to each aquarium before the start of the bioassay. Midge larvae were added to the exposure chambers at random to produce a total of ten larvae per chamber and larvae in each chamber were fed 100 μl of fish food suspension (6 mg dry wt.). For tests receiving two water replacements per day (Montana sediments), a second water replacement was started at least eight hours after the start of the first replacement. Feeding and water renewal(s) were continued in this fashion until the bioassay was ended on day 10. Dissolved oxygen (D.O.) was measured in two replicates each morning before the water replacement, to detect decreases in D.O. concentrations which could adversely affect growth and survival of midge larvae. If persistent depressions in D.O. ($<$2.0 mg/L) were measured, feeding rates were temporarily reduced to allow re-oxygenation. Composite samples of overlying water for water quality analysis were collected from bioassay chambers on days 1 and 9.

At the end of the bioassay, exposure chambers were removed from the aquaria and contents of each chamber were sieved through a stainless steel mesh (100 μm pore diameter) and rinsed with dilution water. The number of survivors for each chamber was recorded and surviving larvae from each replicate were transferred to 30-mL plastic cups containing dilution water and a small amount of acid-washed sand. Larvae in each cup were fed the daily ration of food and set aside to allow time for clearance of gut contents. After

twelve hours, larvae from each cup were rinsed with ultrapure water, transferred to labeled aluminum weigh boats, dried for 24 hours at 60 °C, and weighed to the nearest 0.01 mg.

Chemical Analysis

Extraction and analysis of acid-volatile sulfide (AVS) were performed by the method of Allen et al. (1993). Sulfide was quantitated by a colorimetric method (Michigan sediments) or by ion-selective electrode (Montana sediments; Orion Instruments). Sediment extracts containing simultaneously-extracted metals (SEM) were filtered through a 1.0 μm glass-fiber filter (Whatman GF/C). Sediment samples for analysis of total organic carbon (TOC) were dried for 24 hours at 105°C and ground with a glass mortar and pestle. A sample of (1-2 g) was moistened with ultrapure water, treated with HCl to until evolution of CO_2 ceased, dried, and re-weighed. Carbon analyses on these samples were accomplished by a dry combustion technique (Leco Instruments). Water quality characteristics of sediment porewaters and overlying water from bioassays were determined by standard methods (APHA 1985): organic carbon, by persulfate digestion and infrared detection; ammonia, by ion-selective electrode; hardness, by EDTA titration with a colorimetric endpoint; pH, by combination electrode; total alkalinity, by titration with standard acid to pH 4.5; and dissolved oxygen, by electrode.

Midge samples were digested with high-purity reagents (Baker Instra-Analyzed or Ultrex) at sub-boiling temperatures (90-95 °C) in Teflon centrifuge

tubes. Concentrated nitric acid (1.5 ml) was added for the first 24 hours of digestion, the sample was cooled, hydrogen peroxide (1.0 mL of a 30% solution) added, and the digestion was continued for an additional 24 hours. Digested samples were diluted with ultrapure water to 10 mL at a final concentration of 1% nitric acid.

Copper and zinc concentrations in midge larvae and sediment porewater were determined by atomic absorption spectrophotometry with polarized Zeeman background correction (Hitachi Instruments model 180-80). Samples containing concentrations of Cu less than 100 ug/L were analyzed with graphite furnace atomization. Samples with higher concentrations of Cu and all samples for Zn analysis were analyzed with atomization in an air-acetylene flame (USEPA 1986). Metals in one group of SEM extracts were analyzed by inductively-coupled plasma optical emission spectroscopy (Jarrell-Ash Instruments). Quality of metal analyses was assured by analyses of procedural blanks, sample splits, standard reference materials, and matrix spikes (Appendix A).

Statistical Analysis

Statistical analyses were performed with procedures in the SAS statistical package (SAS Institute 1989). Comparisons of bioassay results among sites were made by analysis of variance (ANOVA). Normality was assessed with the Shapiro-Wilks test. Logarithmic transformation was used to improve the normality and homogeneity of metal concentrations in porewater, sediments,

and midges before analysis. Survival data were analyzed by nonparametric ANOVA (Kruskal-Wallis test). Differences between reference and test sediments were assessed with one-tailed Dunnett's tests. Associations among variables affecting metal bioavailability were assessed by Pearson product-moment correlations. Multivariate associations among sediment and porewater characteristics and bioassay responses (including log-transformed metal concentrations) were evaluated with principal components analysis performed on the correlation matrix.

RESULTS AND DISCUSSION

Chemical Characteristics of Sediments and Porewaters

Concentrations of metals and other constituents of sediment and porewater varied widely among the sediments tested (Tables 1 and 2). Concentrations of Cu were high in sediments from several Michigan sites affected by mining activities (DB, KW, LL, TL), but low in other Michigan sites. A gradient of Cu contamination was evident at the Montana sites, with concentrations decreasing from CF1 downstream to CF5 and the Milltown Reservoir sites (except for high Cu concentrations at site MR10). Concentrations of Zn were greater in extracts of Montana sediments than in extracts from Michigan sediments. Sediments from Michigan lakes generally had greater concentrations of organic carbon and AVS, although concentrations of AVS varied substantially among sites at all locations. Porewater characteristics reflected differences in mineralogy and limnological

characteristics among locations, ranging from the softwater lakes of Upper Michigan to the carbonate-derived sediments of many lakes in Lower Michigan. Porewaters from Montana sediments, especially those from the highly-contaminated sediments in the upper Clark Fork River, had high hardness and slightly acidic pH. Concentrations of ammonia and organic carbon were greater in porewaters from the Montana sediments.

Sediment Bioassays

Midge survival, growth, and Cu bioaccumulation responded differently to sediments from the three sutdy areas (Table 3). No significant differences in survival were detected by ANOVA in the two sets of bioassays with Michigan sediments, despite the wide range in metal contamination among sites. However, survival differed significantly among sediments from the Montana study sites. The insensitivity of the survival endpoint is consistent with previous studies with *C. tentans,* which reported LC_{50}s, (concentrations causing mortality of 50% of exposed organisms) of 1,500 to 1,700 μg Cu/L in aqueous exposure (96-hr exposure; Nebeker et al. 1984) and 850 to 2,300 μg Cu/g in sediment (10-d exposure; Cairns et al. 1984). Growth of *C. tentans* was more variable among sites, with significant ANOVAs in all three studies. Growth was less affected in the bioassays with Michigan sediments, only two of which caused significant reductions in growth relative to reference sediments, than in the Montana sediments, all but one of which produced significant reduction in growth. Our results showed less toxicity than that reported in previous *C.*

tentans bioassays with sediments from the Keweenaw mining district (West et al. 1993, Ankley et al. 1993), but greater toxicity than that reported from *C. riparius* bioassays with sediments from the Clark Fork drainage (Kemble et al. 1994). Bioaccumulation of Cu by *C. tentans* differed significantly among sediments from different sites in all three studies (Table 3). Significant differences in Cu bioaccumulation were evident even when little or no toxicity was observed, as in the two Michigan studies. Bioaccumulation of Cu was significantly greater in 22 of 25 test sediments than in reference sediments. Bioaccumulation of Cu from Clark Fork sediments by *C. tentans* was similar to that reported for *Hyalella azteca* in laboratory bioassays and in samples of benthic invertebrates collected from the Clark Fork (Cain et al. 1992, Ingersoll et al. 1994).

Influences on Toxicity and Bioaccumulation

Toxic effects on *C. tentans* larvae were not strongly associated with concentrations of Cu in sediment or porewater (Table 4). Neither survival nor growth of midge larvae had significant negative correlations with Cu concentrations in sediment extracts or porewater, or with midge Cu bioaccumulation. Although several sediments with porewater Cu concentrations greater than 50 ug/L caused significant reductions in survival and/or growth, six sites with similar or greater porewater Cu concentrations produced neither reduced growth nor reduced survival (Figure 3). *C. tentans* tolerated concentrations of Cu in porewater greater than the LC_{50} for Cu in

porewater for *Hyalella azteca* (20 ug/L; Ankley et al 1993). In contrast, midge growth was significantly reduced, relative to growth in the reference sediment, in several sediments from the Montana study which had low concentrations of Cu in porewater. Both survival and growth were significantly negatively correlated with concentrations of Zn in SEM extracts. However, survival and growth did not have significant correlations with SEM:AVS ratios, which were based on the sum of concentrations of Cu, Zn, Cd, Ni and Pb. Similar inconsistent relationships between sediment toxicity and SEM:AVS ratios were reported in a previous study of sediments contaminated with Cu, including sediments from the Keweenaw area (Ankley et al. 1993).

The toxicity observed in sediments which had relatively low concentrations of Cu and other metals may indicate interactions of porewater metals with ammonia or other porewater constituents. Ammonia concentrations in porewater had a significant negative correlation with midge survival, and a weaker negative correlation with growth (Table 4). Ammonia concentrations in porewater were greater in Montana sediments, where effects on both growth and survival were observed, than in the Upper Michigan sediments. Comparable concentrations of ammonia in porewater (17-25 mg N/L) have been linked to toxic effects on fish and microcrustaceans in toxicity identification studies (Ankley et al. 1990). A previous study with Clark Fork sediments also suggested that ammonia contributed to observed toxicity to *Hyalella azteca* (Kemble et al. 1994). Survival and growth of midge larvae were also significantly correlated with hardness, alkalinity, and pH of porewaters.

The positive correlation of survival and growth with pH may reflect the effect of low pH on the toxicity of metals, due to the greater predominance of free metal ions (Dodge and Theis 1982, Borgmann and Ralph 1983). The Montana sediments which had relatively low pH also had increased hardness and, to a lesser extent, greater alkalinity. The lower concentrations of alkalinity, relative to hardness, in these porewaters suggests that acid was being generated in these sediments, probably due to the oxidation of sulfides. Seasonal cycles of sulfate reduction and sulfide oxidation have been documented in metal-contaminated floodplain soils of the Clark Fork drainage (Moore et al. 1991; J. Moore, University of Montana, personal communication) and oxidation of sulfides has been linked to increased metal mobility and bioavailability in sediments (Zhuang et al. 1993; Calmano et al 1993; Chapter 3, this volume). The porewater characteristics measured in this study differ from those previously reported for Clark Fork sediments (Brumbaugh et al. 1994), in which porewater had neutral to slightly alkaline pH and alkalinity approximately equal to hardness.

Bioaccumulation of Cu by midge larvae was strongly associated with Cu concentrations in SEM extracts and in porewater. Bioaccumulation of Cu was significantly correlated with both concentrations of Cu in porewater and with SEM:AVS ratios (Table 4). However, bioaccumulation of Cu did not always correspond to the predictions of the AVS normalization hypothesis (Figure 4). Bioaccumulation of high concentrations of Cu (>100 μg Cu/g) occurred in several sediments which had SEM:AVS ratios less than 1.0 (MT, CF4). This

contrasts with a previous study, which found that benthic invertebrates did not accumulate metals from sediments with SEM:AVS ratios less than 1.0 (Ankley et al 1994b). Conversely, little bioaccumulation occurred (< 10 μg Cu/g) in two sediments with SEM:AVS ratios greater than 1.0 (RL, CL). A previous examination of metal bioaccumulation by native benthic invertebrates from the Clark Fork River, and by *Hyallela azteca* in laboratory bioassays with Clark Fork sediments, reported strong associations of metal bioaccumulation with SEM:AVS ratios (Ingersoll et al. 1994). In the current study, bioaccumulation of Cu was more closely related to concentrations of Cu in porewater than to SEM:AVS ratios (Table 4, Figures 3 and 4).

Principal component analysis (PCA) of the correlation matrix of nine bioassay endpoints and sediment characteristics from the Michigan and Montana studies indicated the interactions of several influences on sediment toxicity and metal bioavailability. The first three principal components incorporated nearly 70% of the variation from the nine original variables (Table 5). The first component, which contained 34% of the total variation, reflected a gradient of metal concentrations, ammonia concentrations, and toxicity. The second and third axes reflect the influence of sites with high concentrations of metals, but relatively low toxicity. The second axis was strongly influenced by sediments from the Keweenaw area of upper Michigan (DB and TL), which had high bioaccumulation of Cu by midge larvae and high concentrations of Cu in porewater. The third axis was strongly influenced by one sediment from the Milltown Reservoir (MR10), which had high concentrations of both Cu and Zn.

A plot of the observations (study sites) on these three axes allowed comparisons of the characteristics of sediments from different locations (Figure 5). This plot illustrates the tendency for high survival and growth of midge larvae, and low porewater Cu concentrations, in sediments with high concentrations of AVS and organic carbon (i.e. positive scores on axis 1). In contrast, sediments which had high concentrations of Cu, Zn, and ammonia (negative scores on axes 1 and 2) caused the greatest reductions in survival and growth of midge larvae. Sediments with high porewater Cu concentrations, but low concentrations of Zn and/or ammonia (positive scores on axes 2 and 3) were not toxic. These sediments had low concentrations of AVS and ammonia, which suggests that they were relatively oxidized. Under oxidizing conditions, metal bioavailability is usually controlled by binding to sediment organic matter and to iron and manganese hydrous oxides (Fu and Allen 1992, Zhuang et al. 1993), rather than by metal sulfides.

Bioaccumulation of Cu by *C. tentans* larvae was more strongly associated with concentrations of Cu in porewater than were toxic effects on growth and survival. The observed toxicity may have resulted from the combined effects of metals and ammonia. The relative toxicity of different sediments was influenced by sediment characteristics which modified metal bioavailability, including AVS, organic carbon, and pH. These results suggest that the concentrations of Cu in porewater is a direct and easily measured indicator of the bioavailability of Cu in sediments. The bioavailability of Cu, as indicated by concentrations of Cu in porewater and bioaccumulation of Cu by *C. tentans*

larvae, was controlled primarily by acid-volatile sulfides in most of the sediments tested. However, AVS normalization may give misleading results for sediments with low AVS concentrations, where even low concentrations of metals can produce SEM:AVS ratios greater than 1.0. In addition, characteristics of porewaters of Clark Fork sediments suggest that oxidation of AVS may affect metal bioavailability in freshwater sediments. Oxidation of AVS releases metals directly, due to dissolution of metal sulfides, and the acidity generated by oxidation of sulfide can further increase metal bioavailability and toxicity. The rate of oxidation of AVS can be rapid in some sediments (Chapter 3, this volume), and oxidation of AVS in surface sediments may increase metal bioavailability even though SEM:AVS ratios of subsurface sediments remain greater than 1.0.

Table 1. Chemical characteristics (expressed on dry wt. basis) of sediment from Michigan lakes and from the upper Clark Fork River drainage of Montana.

Site ID	TOC (%)	Fe + Mn (%)	AVS (μmol/g)	SEM-Cu (μmol/g)	SEM-Zn (μmol/g)	SEM:AVS Ratio
MICHIGAN SITES:						
CL	12	0.5	5.7	1.6	4.9	1.6
DB	11	2.0	0.2	57	3.3	820
DL	10	7.2	65	0.4	3.6	0.1
GL	10	0.8	7.8	2.9	2.9	0.8
KW	3	0.1	1.3	15	2.5	19
LL	8	0.1	0.4	97	2.6	46
RL	6	1.3	0.9	3.0	4.0	8.0
TL	2	1.4	0.1	43	2.0	710
FL	2	1.5	35	1.3	3.8	0.2
HL	19	1.3	43	7.6	3.2	0.2
JL	7	0.5	55	2.1	1.8	0.1
LO	7	2.7	470	4.0	2.6	<0.1
MI	21	3.3	14	8.2	6.8	0.9
MO	10	3.1	340	6.5	6.4	<0.1
MT	30	1.1	40	16	1.3	0.3
WL	21	0.6	23	2.4	2.7	0.2
MONTANA SITES:						
RC	1	0.03	1.9	<0.1	0.2	0.2
CF1	1	0.4	2.9	10	14	8.2
CF2	1	1.9	0.5	6.0	8.8	30
CF3	2	1.0	7.5	0.7	9.4	1.5
CF4	3	0.8	22	0.6	10	0.5
CF5	1	0.4	11	0.8	7.0	0.8
MR19	1	0.5	7.5	0.7	6.1	0.9
MR7	4	0.8	15	1.2	11	0.8
MR2	4	1.9	19	0.6	12	0.7
MR10	6	0.7	0.2	14	66	480
MR11	4	0.2	22	0.6	21	1
MR25	4	0.9	47	0.2	22	0.5

Table 2. Chemical characteristics of porewater from Michigan and Montana sediments.

Site	Conduct. (μmhos)	pH	Alkalinity (mg/L)*	Hardness (mg/L)*	DOC (mg/L)	NH_3 mg-N/L	Cu (μg/L)
MICHIGAN SITES:							
CL	291	7.52	154	173	11	0.3	1
DB	150	7.09	.	66	4	0.8	2400
DL	359	7.26	188	165	9	2.8	5
GL	165	6.75	70	49	5	5.2	18
KW	183	7.17	98	85	11	1.4	580
LL	160	7.02	84	82	10	0.6	150
RL	144	7.11	110	103	8	1.3	36
TL	.	7.10	92	136	22	0.3	890
FL	650	7.56	218	258	14	2.5	2
HL	395	7.00	200	148	6	13.2	13
JL	765	7.73	400	313	25	20.4	9
LO	690	7.91	354	260	12	5.2	2
MI	129	7.44	62	66	4	0.5	26
MO	419	7.82	164	162	10	4.2	3
MT	405	7.56	176	194	12	1.3	63
WL	415	7.55	132	156	6	0.3	2
MONTANA SITES:							
RC	466	7.40	46	230	12	2.3	9
CF1	3620	6.56	970	1460	39	29.7	510
CF2	1055	7.45	243	560	17	12.9	52
CF3	1657	6.99	588	920	46	17.4	47
CF4	1117	7.03	360	540	27	20.2	140
CF5	1351	7.23	444	810	21	4.3	43
MR1	1127	7.04	318	470	21	11.9	33
MR7	1439	6.81	470	462	54	44.4	350
MR2	1320	6.90	382	440	59	20.6	110
MR1	526	6.87	140	220	10	6.0	480
MR1	724	6.98	186	350	10	4.3	20
MR2	818	6.88	236	368	25	15.9	98

* Alkalinity and hardness expressed as equivalent $CaCO_3$.

Table 3. Results of *Chironomus tentans* bioassays with Michigan and Montana sediments. Means, with standard deviation in parentheses (N = 4); and results of ANOVAs (p-values) with Dunnett's test.

LOCATION Site ID	Survival (%)	Growth (mg/larva)	Bioaccumulation (μg Cu/g)
UPPER MICHIGAN			
CL (Ref.)	98 (13)	0.93 (0.16)	7 (1)
DB	100 (0)	1.00 (0.25)	726 (36)
DL	83 (13)	0.79 (0.14)	28 (10)
GL	103 (17)	0.78 (0.11)	19 (11)
KW	95 (10)	1.26 (0.23)	356 (81)
LL	100 (8)	0.98 (0.22)	159 (32)
RL	93 (10)	0.79 (0.14)	8 (1)
TL	98 (05)	1.16 (0.21)	*495 (112)*
ANOVA	p = 0.37	p = 0.01	p < 0.0001
LOWER MICHIGAN			
FL	98 (05)	1.15 (0.05)	*44 (9)*
HL	95 (10)	0.98 (0.11)	*52 (28)*
JL	103 (26)	1.22 (0.24)	*45 (3)*
LO (Ref.)	103 (05)	1.13 (0.08)	19 (5)
MI	98 (05)	*0.78 (0.06)*	*55 (3)*
MO	95 (06)	1.09 (0.11)	*38 (5)*
MT	100 (08)	0.92 (0.11)	*166 (92)*
WL	105 (06)	*0.73 (0.18)*	27 (2)
ANOVA	p = 0.51	p < 0.0001	p < 0.0001

Table 3 (continued).

LOCATION Site ID	Survival (%)	Growth (mg/larva)	Bioaccumulation (μg Cu/g)
MONTANA			
CF1	*18 (05)*	*0.05 (0.01)*	*260 (116)*
CF2	*73 (15)*	*0.23 (0.05)*	*80 (11)*
CF3	*13 (10)*	*0.15 (0.08)*	*278 (216)*
CF4	85 (17)	*1.09 (0.26)*	*145 (59)*
CF5	85 (10)	*0.64 (0.11)*	*89 (16)*
MR2	*55 (06)*	*0.55 (0.11)*	*61 (7)*
MR7	93 (15)	*0.98 (0.03)*	44 (19)
MR10	98 (05)	1.26 (0.18)	*228 (40)*
MR11	95 (10)	*1.09 (0.08)*	*66 (19)*
MR19	78 (19)	*0.87 (0.10)*	*59 (30)*
MR25	*33 (21)*	*0.84 (0.19)*	32 (10)
RC (Ref.)	95 (06)	1.46 (0.12)	19 (4)
ANOVA	$p<0.0001$	$p<0.0001$	$p<0.0001$

* Means in italics are significantly different from reference (Dunnett's test).

Table 4. Correlations among *Chironomus tentans* bioassay endpoints, metal concentrations, and other characteristics of sediment and porewater.

	Toxicity		Bioaccum.
	Survival	Growth	Midge Cu (log)
Bioaccumulation			
Midge Cu (log)	-0.29	-0.21	--
Sediment			
SEM-Cu (log)	-0.22	0.06	0.55*
SEM-Zn (log)	-0.42*	-0.37*	0.22
ΣSEM:AVS (log)	-0.09	-0.20	0.60*
AVS	0.17	0.29	-0.31
Organic C	0.39*	0.06	-0.05
Porewater			
Total Cu	-0.32	-0.19	0.78*
Organic C	-0.63*	0.06	-0.05
Ammonia	-0.50*	-0.32	0.08
pH	0.48*	0.39*	-0.35
Hardness	-0.78*	-0.65*	0.24
Alkalinity	-0.71*	-0.58*	0.26

* Asterisk indicates correlations significant at $p < 0.05$ ($N = 2$).

Table 5. Principal components analysis with bioassay responses and characteristics of sediment and porewater. Eigenvalues indicate the proportion of total variation contained in the first five principal components; eigenvectors indicate the contribution of the original variables to each component.

Principal Component	1	2	3	4	5
Eigenvalue	3.03	1.96	1.19	1.02	0.75
% of variation	33.6	21.8	13.2	11.4	8.4
cumulative %	33.6	55.4	68.6	80.0	88.4
Variable	**Eigenvector:**				
Midge Survival	0.45	0.22	0.27	-0.24	-0.20
Midge Growth	0.37	0.16	0.56	0.09	-0.24
Midge Cu (log)	-0.24	0.62	-0.08	0.19	0.07
Porewater Cu (log)	-0.22	0.62	0.01	0.23	0.01
SEM Zn (log)	-0.20	-0.04	0.65	-0.12	0.65
Porewater NH_3	-0.32	-0.34	0.04	0.24	-0.04
Sed. Organic C	0.27	0.16	-0.37	-0.44	0.56
Sediment AVS	0.31	-0.12	-0.02	0.70	0.39
Porewater pH	0.48	0.01	-0.20	0.28	0.08

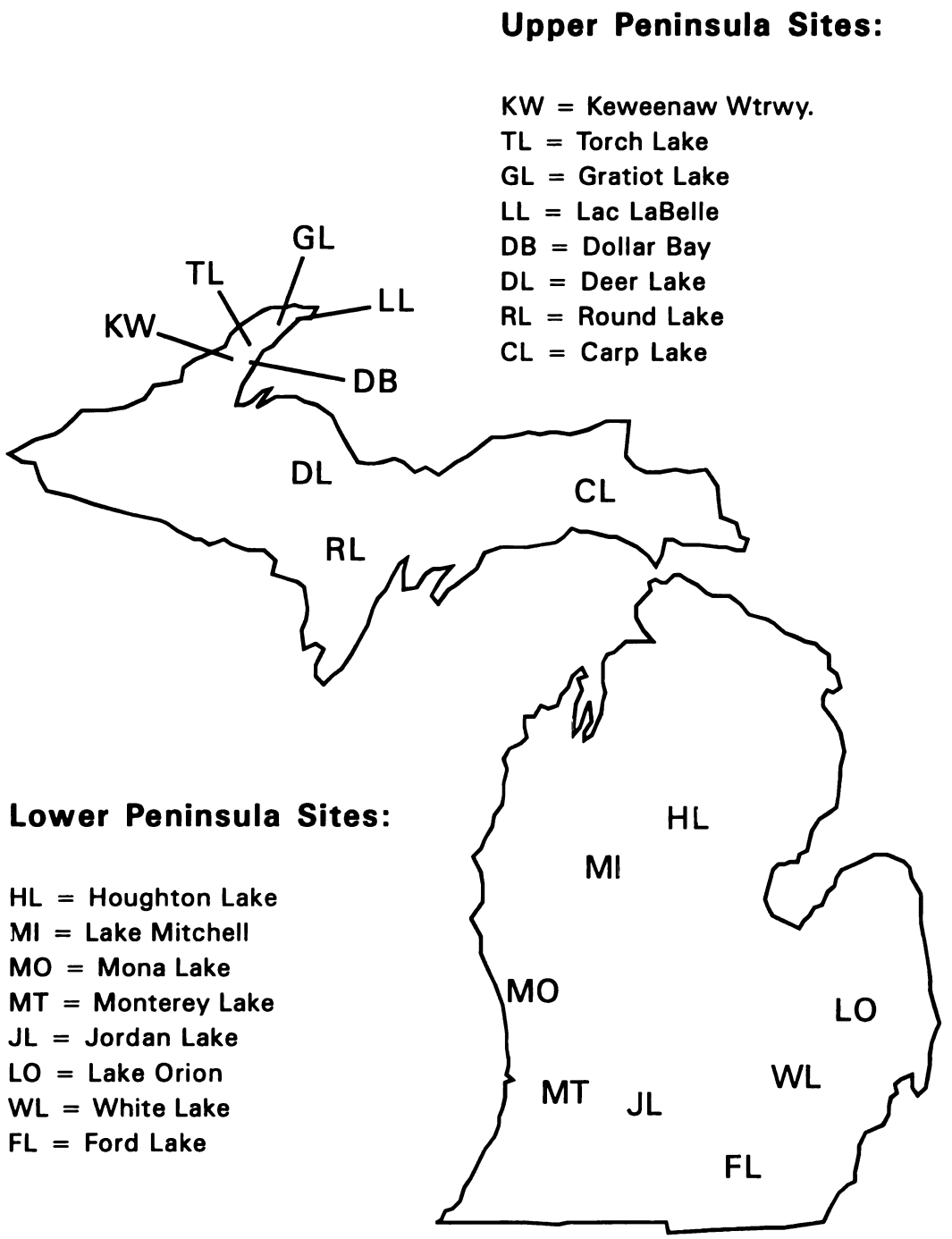


Figure 1. Sampling sites in the Upper and Lower Peninsulas of Michigan, USA.

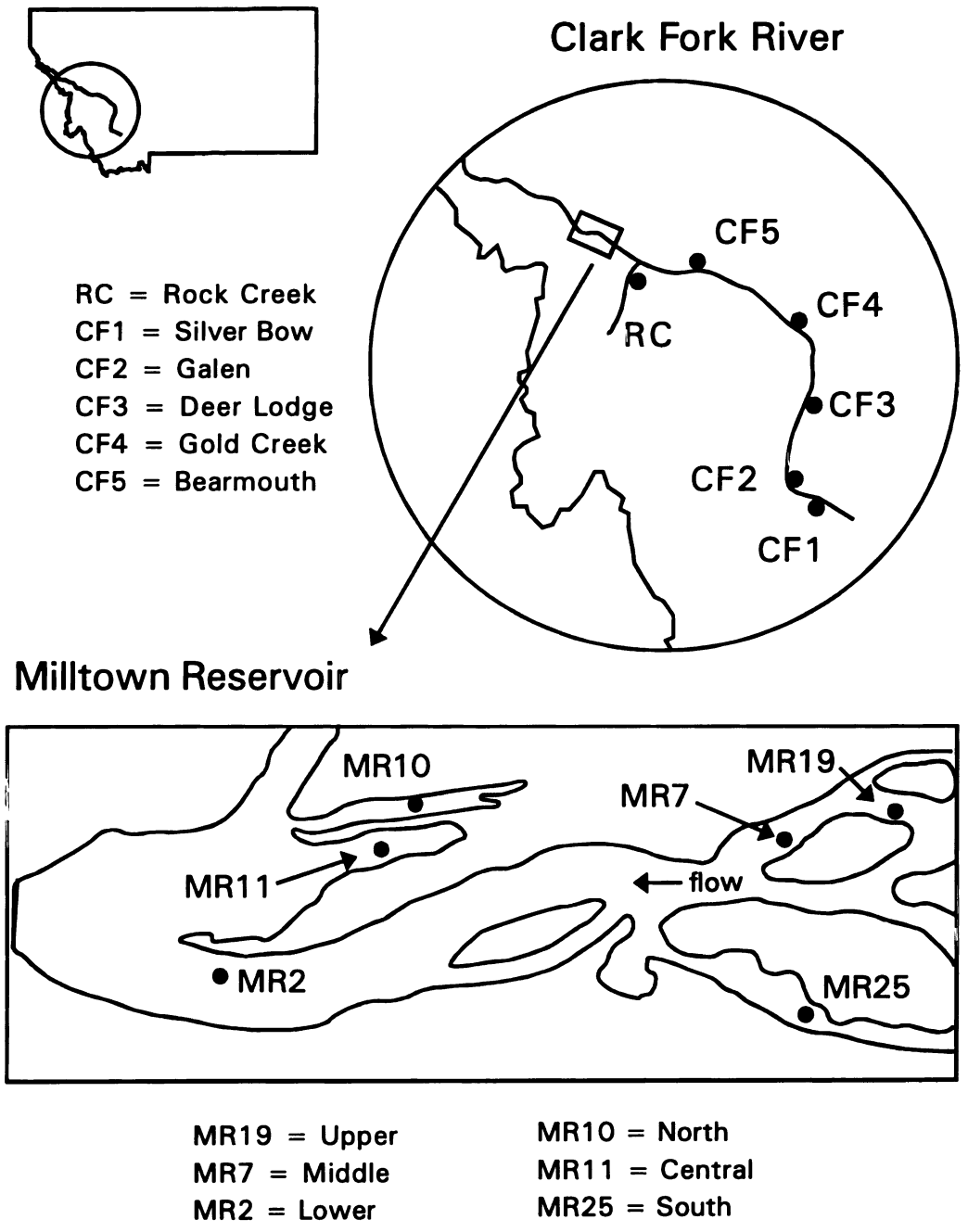


Figure 2. Sampling sites in the upper Clark Fork River drainage of Montana, USA.

Figure 3. Relationships of growth and bioaccumulation of Cu by *C. tentans* larvae to total concentrations of Cu in porewater. (a) growth, with significant reductions from reference sites indicated by hollow symbols ($p < 0.05$, Dunnett's test); (b) bioaccumulation, with linear regression.

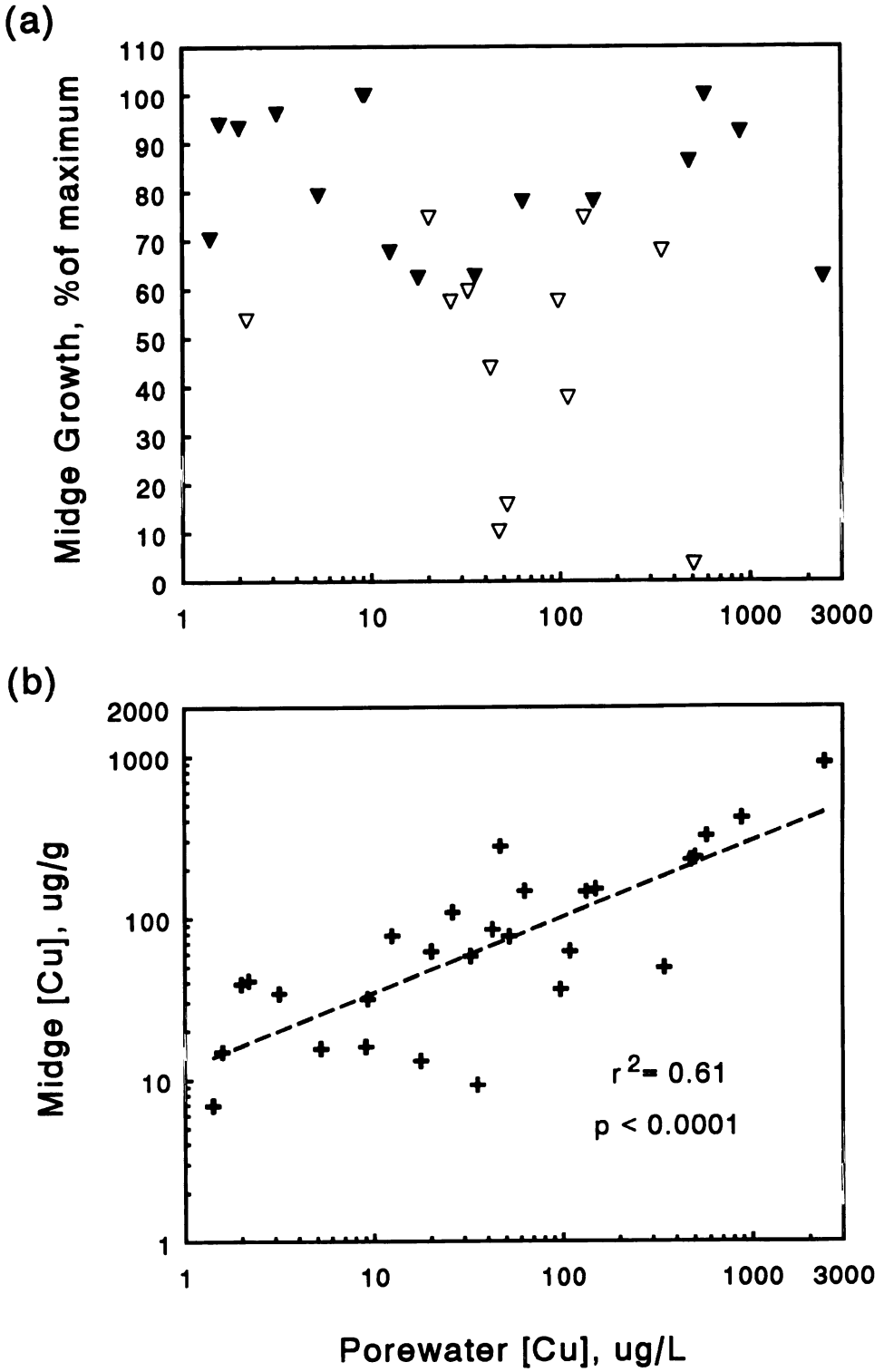
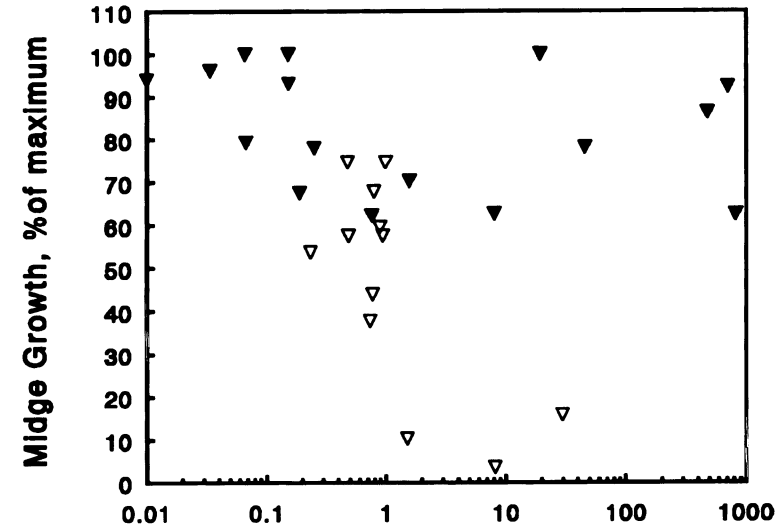
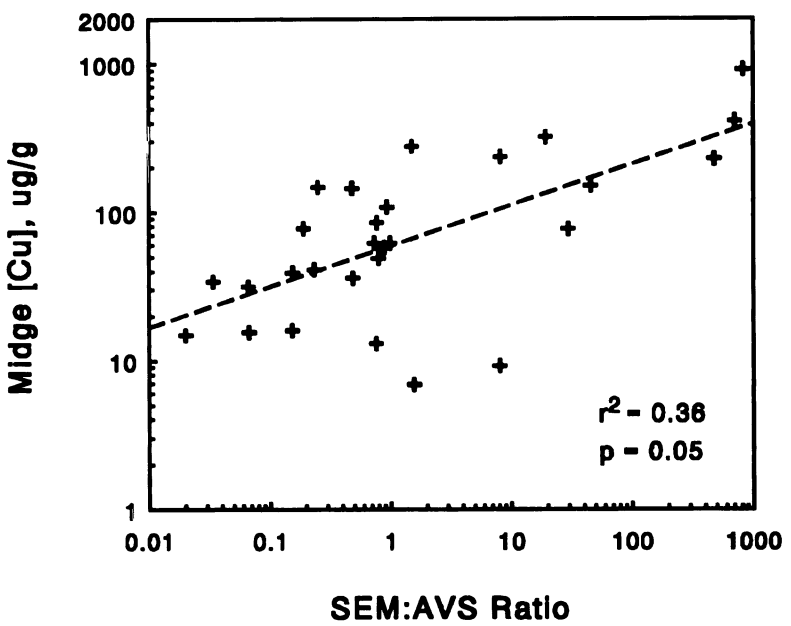

(a)
Midge Growth, %of maximum
110
100
90
80
70
60
50
40
30
20
10
0
1
10
100
1000
3000
(b)
Midge [Cu], ug/g
2000
1000
100
10
1
r^2= 0.61
p < 0.0001
1
10
100
1000
3000
Porewater [Cu], ug/L

Figure 4. Relationships of growth and bioaccumulation of Cu by *C. tentans* larvae to ratios of simultaneously-extracted metals to acid-volatile sulfide (SEM:AVS ratios). (a) growth, with significant reductions from references sediment indicated by hollow symbols (Dunnett's test, $p < 0.05$); (b) bioaccumulation, with linear regression.

(a)


Midge Growth, %of maximum
110
100
90
80
70
60
50
40
30
20
10
0
0.01
0.1
1
10
100
1000

(b)


Midge [Cu], ug/g
2000
1000
100
10
1
0.01
0.1
1
10
100
1000
r2 = 0.36
p = 0.05
SEM:AVS Ratio

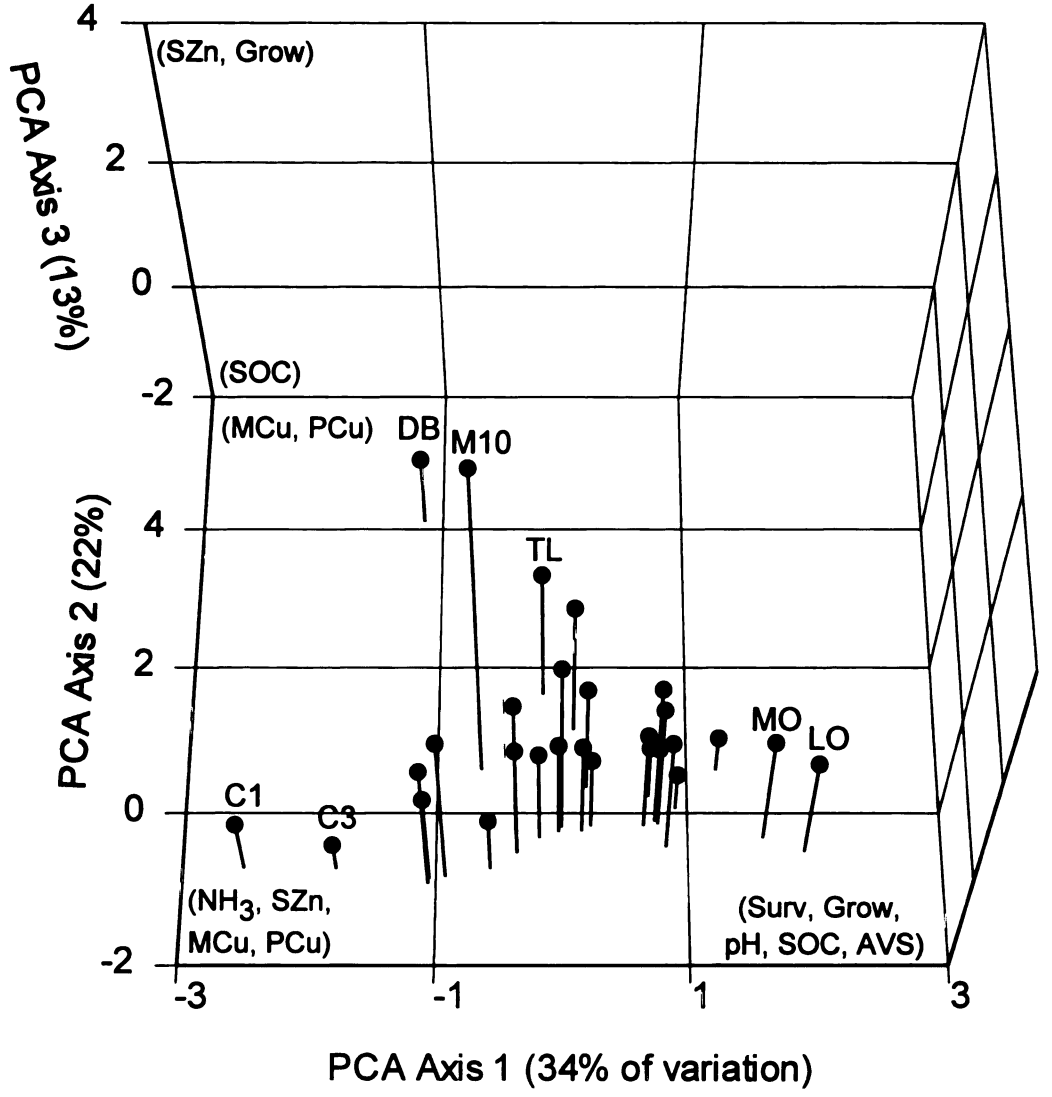


Figure 5. Principal components analysis (PCA) based on bioassay endpoints and sediment characteristics. Points represent scores for sample sites on the first three principal component axes. Influence of original variables are indicated by the location of variable IDs along the PCA axes (Surv = midge survival, Grow = midge growth, MCu = midge Cu bioaccumulation, PCu = porewater Cu, SZn = SEM Zn). Selected sites are identified by site IDs.

CHAPTER 2

Effects of Oxic and Anoxic Conditions on the Bioavailability of Copper in Michigan Lake Sediments to the Midge, *Chironomus tentans*

(for submission to *Archives of Environmental Contamination and Toxicology*)

Abstract

The influence of redox status of overlying waters on the bioavailability of copper (Cu) in lake sediments was examined using bioassays with larvae of the midge, *Chironomus tentans*. Sediments were collected from sixteen lakes and waterways in Michigan which differed in their geochemical characteristics, trophic status, and degree of copper contamination. Subsamples of sediment from each site were incubated for six weeks under air and nitrogen atmospheres. Concentrations of acid-volatile sulfide (AVS) did not decrease in most sediments during incubation with oxic overlying waters, but increased in sediments under anoxic conditions. Responses of bioassay endpoints to incubation treatments varied among sediments from different locations. Midge growth was significantly reduced following incubation with oxic overlying water in sediments which contained greater concentrations of Cu from mining wastes.

Oxic overlying water had lesser effects, primarily increases in bioaccumulation, in sediments which contained lesser concentrations of Cu.

Normalization of sediment metal concentrations to AVS, as ratios of concentrations of acid-extractable metals to AVS (SEM:AVS ratios), did not accurately predict toxicity or metal bioavailability. Toxicity was not observed at SEM:AVS ratios less than 1.0, as predicted, but toxicity was also not observed in some sediments with the greatest SEM:AVS ratios. AVS normalization only predicted bioaccumulation accurately at very small or very large SEM:AVS ratios. Bioaccumulation followed a close dose-response relationship with Cu concentrations in porewater, and growth of midge larvae was linearly related to porewater Cu concentrations at concentrations greater than 10 μg Cu/L. These results suggest that measurement of porewater metal concentrations is a simpler and more accurate predictor of bioaccumulation and toxic effects of metals in freshwater than normalization of metal concentrations to AVS.

INTRODUCTION

Characterization of the bioavailable fraction of metals in sediments is necessary for accurate predictions of toxicity of metal-contaminated sediments. Recent research on controls on metal bioavailability in sediments has focused on the role of acid-volatile sulfides (AVS), the pool of amorphous sulfides of transition metals (Allen et al. 1993). The ability of excess AVS to reduce the toxicity of metals has been well documented (DiToro et al. 1990, Ankley et al.

1991, Carlson et al. 1991, Casas and Crecelius 1994). However, there is also evidence that sorption and complexation of metals with other sediment phases, such as organic matter and hydrous oxides of iron and manganese, are also important controls on metal bioavailability (Lion et al. 1982, Calmano et al. 1988, Fu et al. 1992, Chapter 1, this volume). Although associations of metals with oxides and organic matter are generally weaker than associations with sulfides, these phases may be the dominant controls on metal bioavailability under oxic conditions, where sulfides are not stable; in systems with low sulfur concentrations; or in highly contaminated sediments where inputs of metals exceed the available sulfide pool (Bendell Young and Harvey 1991, Tessier et al. 1993).

The bioavailability of metals in freshwater sediment may undergo substantial seasonal changes. Seasonal changes in temperature and primary productivity in temperate lakes produce corresponding changes in sediments, including concentrations of labile organic matter, rates of benthic metabolism, redox status, and concentrations of redox labile sediment constituents such as AVS and hydrous oxides. Concentrations of AVS undergo substantial seasonal changes in some freshwater lakes (Howard and Evans 1993, Leonard et al. 1993). AVS concentrations in sediments reflect a balance between oxidation of sulfide, which can occur continuously at the sediment surface except during periods of hypolimnetic anoxia, and reduction of sulfate, which is affected by many factors including redox, pH, sulfate concentrations, temperature, and concentrations of organic substrates (Nedwell and Abram 1978, Smith and

Klug 1981, Herlihy and Mills 1985). Oxidation of sulfidic sediments from both freshwater and marine environments can be associated with substantial increases in bioavailability of heavy metals, including Cu, and release of dissolved metals to overlying waters (Lu and Chen 1977, Morfett and Hamilton-Taylor 1988, Kerner and Wallman 1992, Calmano et al. 1993, Zhuang et al. 1994). In contrast, metals associated with oxides of iron (Fe) and manganese (Mn) can be solubilized during periods of anoxia (Balistrieri et al. 1992). The stability of organic matter during redox changes and its strong binding affinity for metals make it an important control on metal bioavailability in some sediments (Fu et al. 1992, Ankley et al. 1993).

This study examined changes in the bioavailability of metals in sediments from freshwater lakes in Michigan, which have a wide range of physicochemical characteristics, during periods of incubation with oxic and anoxic overlying water. The bioavailability of Cu, which occurs at relatively great concentrations in sediments of these lakes due to a variety of anthropogenic inputs, was assessed with a standard ten-day sediment bioassay with larvae of the midge, *Chironomus tentans* (USEPA 1994). *C. tentans* responds with metal bioaccumulation, growth reductions, and mortality, across a wide range of metal exposure (Chapter 1, this volume).

The objectives of this study were:

(1) to evaluate the effects of changes in oxygen status of overlying water on the bioavailability of metals associated with redox-sensitive sediment phases, including AVS; and

(2) to compare the usefulness of metal:AVS ratios in sediment and metal concentrations in porewater for predicting bioaccumulation and growth of *C. tentans* in sediment bioassays.

METHODS

Sediment Collection and Sample Preparation

Sediments were collected from sixteen lakes and waterways in Michigan, USA (Figure 1). Eight sites from Michigan's Upper Peninsula (U.P.) were sampled in August 1992. U.P. sites included four sites in the Keweenaw Peninsula, which were contaminated with wastes from historic copper mining activities (Lac LaBelle, LL; Torch Lake, TL; Keweenaw Waterway, KW; and Dollar Bay, DB), one site affected by iron mining and ore processing (Deer Lake, DL), and three sites without known anthropogenic metal inputs (Carp Lake, CL; Round Lake, RL; and Gratiot Lake, GL). Sediments were collected in October 1992 from eight lakes in southern Michigan. Lower Peninsula (L.P.) sites included several lakes (Houghton Lake, HL; Lake Mitchell, MI; Monterey Lake, MT; and White Lake, WL), which received applications of copper sulfate for control of aquatic nuisances, and four lakes (Ford Lake, FL; Jordan Lake, JL; and Lake Orion, LO) which have received lesser inputs of metals from point and nonpoint sources.

Sediment samples were collected with a petite ponar dredge and combined in a polyethylene bucket to produce composite samples of 8 to 10 liters. Sample containers, sampling gear, and laboratory apparatus were

washed, rinsed with acid (10% HCl), and rinsed with deionized water in the laboratory before each sampling trip. In the field, sampling gear was scrubbed between sampling stations, rinsed with 10% HCl, and rinsed with site water. Sediment samples were stored in coolers for the duration of sampling trips (up to five days). All samples were transferred to a walk-in cooler for storage at 4°C before use. Sediments were homogenized by stirring before samples were withdrawn for bioassays or chemical analyses. Porewaters for analysis of metals and water quality parameters were prepared by centrifugation (DuPont Sorvall SS-3). Sediments were centrifuged for 40 minutes at 7000 rpm and supernatants for 15 minutes at 10,000 rpm. Samples of porewater for metal analyses were preserved with nitric acid (Baker Instra-Analyzed) to a final concentration of 1% (v/v).

Sediment Incubations

Sediments from each site were incubated for six weeks under oxic and anoxic conditions. Two four-liter portions of each homogenized sample were placed in eight-liter polyethylene containers with 2 liters of test water (described below). These containers were placed inside 5-gallon polyethylene buckets with airtight lids. Two ports were cut into the lids of the outer buckets: one for insertion of plastic tubing leading to an airstone in the overlying water, and another for a bubble trap to allow gas to escape from the container. Gas lines in the air treatment delivered compressed room air continuously and those in the nitrogen treatment delivered nitrogen, which was

passed through an oxygen trap (Baxter), at intervals of 24 to 48 hr, to purge the atmosphere inside the container. Containers were incubated in water baths at 18-20 °C. Containers were opened weekly and the sediments stirred gently to increase exposure to overlying water. Samples were removed after six weeks for chemical analysis and bioassay.

Sediment Bioassays

Sediment bioassays were conducted with larvae of the midge, *Chironomus tentans*, based on the static-renewal method developed by the U.S. Environmental Protection Agency (USEPA 1994). Bioassay chambers were prepared from 300-mL high-form beakers with two 17-mm holes covered with 100 μm stainless screen to allow water circulation. Each group of replicates were placed in a glass aquarium and water replacements were added from separate polyethylene head tanks which contained one replacement volume (9 L). Test water was dechlorinated tap water diluted with ultrapure laboratory water (Barnstead E-Pure) to a hardness of 95 mg/L as $CaCO_3$. Three replicate bioassay chambers per treatment group were filled with 100 mL of sediment and test water, and placed into an exposure aquarium for 24 hr before the start of bioassay. One water replacement was added to each aquarium before the start of the bioassay. *C. tentans* larvae of uniform age (12-14 d after egg deposition) and uniform size were selected for use in bioassays. Ten larvae were added in random fashion to each exposure chambers. A suspension containing 6 mg of flake fish food (Tetra-Min) was added to each chamber

after the daily water renewal during ten-day exposure period. On day ten, the contents of each chamber were rinsed through a stainless steel sieve (100 μm pore diameter). Surviving larvae from each replicate were transferred to 30-mL plastic cups with dilution water and a small amount of acid-washed sand, fed a daily ration of food, and set aside to allow clearance of sediment from gut contents. After 12 hr, larvae from each cup were rinsed with ultrapure water, transferred to aluminum weigh boats, dried at 60 °C, and weighed to the nearest 0.01 mg.

Chemical Analysis

Analysis of acid-volatile sulfide (AVS) in sediments were performed by room-temperature extraction for 30-min with 1N HCl and quantitation of sulfide by the colorimetric method (Allen et al. 1993). Sediment extracts were filtered through a 1.0 μm glass-fiber filter (Whatman GF/C) for analysis of simultaneously-extracted metals (SEM). Water quality characteristics of sediment porewaters were determined by standard methods (APHA 1985): ammonia, by ion-selective electrode; hardness, by EDTA titration with a colorimetric endpoint; pH, by combination electrode; and organic carbon by persulfate digestion and infrared detection.

Midge samples (typically 5-20 mg dry wt.) were digested in teflon tubes with high-purity reagents (Baker Instra-Analyzed or Ultrex) at sub-boiling temperatures (90 to 95 °C). Concentrated nitric acid (1.5 ml) was added before the first 24 hours of digestion, the sample was cooled, 30% hydrogen

peroxide (1.0 ml) was added, and the digestion was continued for an additional 24 hours. Digested samples were diluted to 10 mL with ultrapure water. Copper concentrations in digested midge larvae and sediment porewater were determined by atomic absorption spectrophotometry (AAS) with graphite furnace atomization and polarized Zeeman background correction (Hitachi Model 180-80). Copper and zinc in SEM extracts were analyzed by AAS with atomization in an air-acetylene flame. Quality assurance measures for metal analyses included analyses of procedural blanks, sample splits, standard reference materials, and matrix spikes (Appendix A).

Statistical Analysis

Statistical analyses were performed with the SAS statistical package (SAS Institute 1989). Differences in chemical characteristics and bioassay endpoints among sampling sites and between incubation treatments, and interactions of site and treatment effects were assessed by analysis of variance (ANOVA). If site X treatment interactions were significant, treatment differences within each site were evaluated with t-tests. Associations among variables affecting metal bioavailability were assessed by linear and nonlinear regression. Concentrations of AVS and metals were log-transformed before analysis to improve normality and homogeneity of variance. A critical significance level (probability of type I error) of $p \leq 0.05$ was used for evaluation of results of ANOVA and regression analyses. However, due to the few degrees of freedom of t-tests with three replicates, $p \leq 0.10$ was reported

as "marginally significant."

RESULTS

Characteristics of Sediment and Porewater

The sixteen sediments tested represented a wide range of physicochemical characteristics associated with metal bioavailability (Tables 1 and 2). Sediments from L.P. sites generally contained greater concentrations of AVS and organic carbon and porewaters from L.P. sediments had greater alkalinity and hardness and more alkaline pH than those from Upper Peninsula sediments. Concentrations of Cu in SEM extracts were greatest in the four sediments affected by mining wastes (DB, KW, TL, LL), although several L.P. sediments (HL, MI, MO, MT) contained moderately high concentrations of SEM. Molar ratios of metals in SEM extracts to AVS (SEM:AVS ratios) were substantially greater than 1.0 only in the U.P. sediments affected by mining. Concentrations of Cu in porewater reflected overall variation of Cu concentrations in sediments, although porewaters from two U.P. sediments (GL, RL), which had low concentrations of Cu in SEM extracts, contained relatively high concentrations of Cu in porewater (>10 ug/L).

Six-week incubations with oxic and anoxic overlying waters (air and nitrogen treatments) produced variable effects in different sediments. Although concentrations of AVS averaged 18% less in the air treatment than in the nitrogen treatment in sediments which originally contained greater than 1 μmol/g, there was no significant effect of the incubation treatment on AVS

concentrations (ANOVA; Table 6). The air treatment did not cause net oxidation of AVS. After six weeks, concentrations of AVS in both treatments were generally greater than those measured before incubation. Concentrations of SEM in several U.P. sediments were less in both treatments after incubation than in fresh sediments, whereas SEM concentrations in Lower Peninsula sediments were generally greater after incubation (Table 6). Differences in SEM concentrations between treatments occurred in only a few sediments: SEM concentrations were greater in the air treatment in CL and LO and greater in the nitrogen treatment in HL and WL. SEM:AVS ratios also differed between treatments in several sediments. SEM:AVS ratios in sediments from CL and FL were greater than 1.0 in the air treatment and less than or equal to 1.0 in the nitrogen treatment; and the opposite trend was evident in HL and WL. Concentrations of Cu in porewater did not always correspond to differences, between treatments, in Cu concentrations in SEM extracts or in SEM:AVS ratios. The four sediments which contained greatest concentrations of Cu from mine wastes showed differences in concentrations of Cu in porewater between treatments, despite consistently high SEM:AVS ratios (from 18 to 570) in both treatments. Concentrations of Cu in porewater were greater in the air treatment in two of these sediments (LL and TL) and greater in the nitrogen treatment in the other two (DB and KW). Concentrations of Cu in porewaters in L.P. sediments were generally greater in the air treatment, although the opposite trend was evident in one sediment (MI).

Sediment Bioassays

Bioaccumulation of Cu by *C. tentans* was more strongly affected by the incubation treatments in L.P. sediments than in U.P. sediments (Figure 6). Bioaccumulation of Cu was greatest (>100 μg Cu/g) in the four sediments containing mine wastes and in two L.P. sites (MI and MT). Bioaccumulation differed significantly among sites, but not between incubation treatments (ANOVA). There was a significant interaction between ANOVA effects of site and treatment (site X treatment interaction) for Cu bioaccumulation in Lower Peninsula sediments. This interaction was caused by differences between treatments in sediments from two sites, MI and HL (t-tests). In both of these sediments, differences in Cu bioaccumulation corresponded to Cu concentrations in porewater. The difference in bioaccumulation in the HL sediment also corresponded to a difference in SEM:AVS ratios between treatments.

Growth of *C. tentans* larvae was more strongly affected by the incubation treatments in bioassays with U.P. sediments (Figure 6). Midge growth was significantly reduced in the air treatment in U.P. sediments (ANOVA). The greatest reductions in growth, which occurred in the LL and TL sediments, corresponded to differences in porewater Cu concentrations. There was no significant effect of the incubation treatment on midge growth in bioassays with L.P. sediments. However, there was a significant site X treatment interaction, which corresponded to significantly reduced growth in the air treatment in the FL sediment (t-test). These differences corresponded

to SEM:AVS ratios, but not to porewater Cu concentrations.

DISCUSSION

Effect of Oxic and Anoxic Conditions

The relative rates of oxidation of sulfide and reduction of sulfate in sediments from Michigan lakes were apparently affected by factors other than the redox status of overlying waters. Incubation of the Michigan lake sediments for six weeks under oxic and anoxic conditions had only minor effects on concentrations of AVS on the bioavailability of Cu. The small differences in AVS and bioavailability of Cu in sediments from oxic and anoxic incubations in this study contrast with the rapid rates of oxidation of AVS reported for sediment slurries (Calmano et al. 1993, Zhuang et al. 1994). More rapid rates of AVS oxidation have also been reported in thin layers of sediments from the Clark Fork River of Montana (Chapter 3, this volume). The greater rates of AVS oxidation in the Clark Fork sediments apparently resulted from greater sediment permeability and slower oxygen consumption in subsurface sediments. Greater rates of oxidation of AVS have also been associated with lesser rates of sediment metabolism and greater oxygen penetration in marine sediments (Thode-Anderson and Jorgenson 1989). Seasonal cycles of AVS concentrations in mesotrophic lakes are reportedly associated with changes in water temperatures, with greatest concentrations during summer and minimum concentrations during periods of ice cover (Leonard et al. 1993). In systems which do not experience hypolimnetic anoxia, seasonal variation of AVS is

caused primarily by changes in rates of sulfate reduction, which increase during summer due to greater inputs of oxidizable organic matter and greater activity of sulfate-reducing bacteria, and decrease to very low rates at temperatures of 5 to 10 °C (Nedwell and Floodgate 1972, Herlihy and Mills 1985). The results of the current study suggest that rates of oxidation at sediment surfaces are slower than rates of sulfate reduction in anoxic subsurface layers of most sediments at summer temperatures (15-20 °C).

There were some differences in metal bioavailability between incubation treatments, although the mechanisms for these differences were not always evident. Greater SEM:AVS ratios and/or greater concentrations of Cu in porewater suggested that metal bioavailability was greater in the air treatment in most sediments (Table 6). However, these differences corresponded to significant differences in bioaccumulation or growth between treatments in only a few cases. The significant reduction in growth of midge larvae in the air treatment in U.P. sediments was not consistently associated with greater concentrations of Cu in porewater or greater bioaccumulation of Cu by midge larvae. Oxidation of AVS cannot explain the differences in toxicity of the U.P. sediments, including those containing mine wastes, since these sediments contained very low concentrations of AVS and high SEM:AVS ratios in both air and nitrogen treatments. Of the five L.P. sites which had greater porewater Cu concentrations in the air treatment, the only significant responses in midge bioassays were greater bioaccumulation in the one sediment (HL) and reduced growth in the another (FL). These differences may be related to oxidation of

metal sulfides in these sediments, since SEM:AVS ratios were close to or greater than 1.0 in these sediments after aeration. In contrast, a few sediments showed evidence of greater Cu bioavailability in the nitrogen (anoxic) treatment. The nitrogen treatment was associated with greater porewater Cu concentrations in two sediments from the Keweenaw mining area (KW and DB) and from one L.P. sediment (MI). Bioaccumulation of Cu was greater in the nitrogen treatment in the MI sediment, but not in the two Keweenaw sediments. The greater concentrations of Cu in porewaters of these sediments under anoxic conditions may have resulted from dissolution of Fe and Mn oxides and release of associated Cu into porewater. Two of the three sediments (DB and MI) contained high concentrations of Fe and Mn in SEM extracts (>2%; Table 1). The sediments from the Keweenaw area were apparently oxidized when collected, as indicated by AVS concentrations near the limit of detection. An oxidized surface layer may also have been present in the MI sediments, since this was the most oligotrophic of the L.P. lakes sampled.

Indicators of Cu Bioavailability

SEM:AVS ratios were significantly associated with metal bioavailability in bioassays with incubated sediments from Michigan lakes. There was a significant linear relationship between SEM:AVS ratios and bioaccumulation of Cu by midges (Figure 7). Prediction of bioaccumulation was good at both very low (<0.1) and very high (>10) SEM:AVS ratios, but was less reliable at ratios

close to 1.0, the hypothesized boundary between nontoxic and "potentially toxic" conditions (DiToro et al. 1991b). Bioaccumulation was greater than 100 μg Cu/g in several sediments with SEM:AVS ratios less than 1.0, and less than 40 μg Cu/g in several sediments with SEM:AVS ratios greater than 1.0. This result contrasts with a previous study, which found that oligochaetes did not bioaccumulate Cu or other metals from sediments with SEM:AVS ratios less than 1.0 (Ankley et al. 1994b). The limited bioaccumulation of Cu from some sediments with SEM:AVS ratios greater than 1.0 may reflect the low concentrations of Cu in SEM extracts from some of these sediments. Other researchers have reported low bioavailability of metals in sediments which had SEM:AVS ratios greater than 1.0, but which contained low concentrations of metals (Hare et al. 1994). This preblem in interpretation would be eliminated by normalizing metal concentrations as the difference between concentrations of SEM and AVS, an estimate of the concentrations of "excess" SEM or non-sulfide bound metals, rather than as SEM:AVS ratios. This method would avoid the possibility of over-estimating the toxicity of sediments which do not contain hazardous concentrations of metals. Bioaccumulation of Cu by midge larvae from sediments without excess SEM was not simply an artifact of calculation of SEM:AVS ratios. This unexpected bioaccumulation may indicate a limitation of the practice of measuring SEM and AVS in composites of grab samples, rather than in surface sediment layers. Metals in natural sediment profiles, or in the homogenized grab samples commonly used in sediment bioassays, may not be in equilibrium with the total mass of AVS present. Localized oxidation

of AVS in surface layers in the field or in bioassays could produce a pool of metals available to biota, despite the presence of excess of AVS in the entire sample.

Toxic effects of metals on growth of midge larvae were predicted less accurately by normalization with SEM:AVS ratios (Figure 7). Midge growth was consistently unaffected in sediments with SEM:AVS ratios less than 1.0, but the trend for reduced growth at ratios greater than 1.0 was only weakly linear (r^2 = 0.41), and several of these sediments produced unexpectedly high or low toxicity. The shallow slope of the dose-response curve reflects, in part, the relative insensitivity of *C. tentans* to Cu toxicity (Nebeker et al. 1984, West et al. 1993). However, the ability of *C. tentans* to grow normally in some sediments with high SEM:AVS ratios suggests that metal bioavailability is controlled by constituents other than sulfides in these sediments. Other studies have also reported unexpectedly low toxicity at SEM:AVS ratios greater than 1.0 (Ankley et al. 1993; Chapter 1, this volume).

Concentrations of Cu in porewater were more useful than SEM:AVS ratios for predicting bioaccumulation and toxicity of sediment metals. Concentrations of Cu in porewater were strongly predictive of Cu bioaccumulation by *C. tentans*. Bioaccumulation followed a sigmoidal response to porewater Cu concentrations, with little accumulation at low concentrations, a maximum slope in the range 10 to 100 μg Cu/L, and an apparent maximum bioaccumulation (1000-2000 ug/g) at higher porewater Cu concentrations (Figure 7). Porewater Cu had a weaker linear association with growth in *C.*

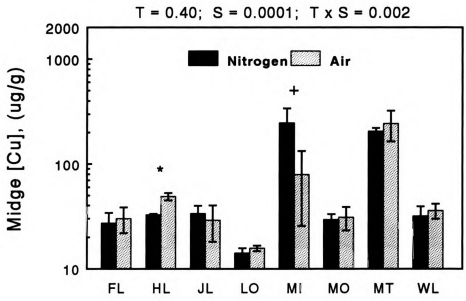
tentans, in part due to variation of midge growth at low porewater Cu concentrations. This association was stronger at porewater Cu concentrations greater than 10 ug/L, in the range where rapid bioaccumulation of Cu was observed. A previous study of the toxicity of Keweenaw sediments, which used sediment bioassays with the amphipod *Hyalella azteca*, also found a strong association of toxic effects with concentrations of Cu in porewater (Ankley et al. 1993a). The strong relationship of bioavailability with Cu concentrations in porewater is consistent with previous studies which have found that the activity of free metal ions is the best predictor of bioavailability and toxicity (Dodge and Theis 1982, Borgmann and Ralph 1983). Accurate measurements of free or dissolved metal concentrations in porewater are not simple, due to precipitation, sorption, or other interactions of dissolved metals with particulates, including filter materials (Carignan et al. 1985). Nevertheless, measurement of concentrations of metals in porewater provide a more direct assessment of the availability of metals to benthic organisms, and the potential for release of metals to overlying water, than do measurements of metals in particulate fractions.

Table 6. Characteristics of sediment and porewater from Michigan lakes after nitrogen (N_2) and air incubations. Means of duplicate analyses for SEM and AVS and single analyses for Cu in porewater.

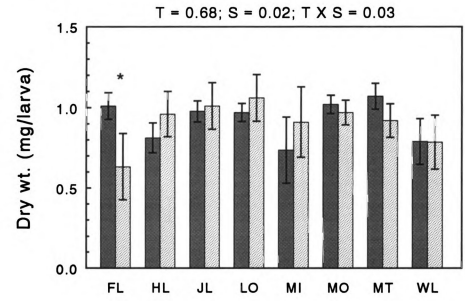
Site	AVS (μmole/g)		SEM Cu + Zn (μmoles/g)		SEM:AVS Ratio		Porewater Cu (ug/L)	
	N_2	Air	N_2	Air	N_2	Air	N_2	Air
Upper Peninsula:								
CL	13	6.4	1.7	8.4	0.2	2.0	0.6	0.3
DB	0.2	0.6	28	74	140	150	1400	610
DL	88	51	1.1	1.6	0.01	0.04	5.6	5.9
GL	28	11	1.5	1.8	0.07	0.21	6.4	11
KW	0.7	<0.1	22	20	31	420	440	130
LL	0.2	0.2	10	8.2	54	42	46	110
RL	8.3	9.3	1.0	--	0.17	--	1.8	1.4
TL	1.1	<0.1	19	16	18	570	360	650
Lower Peninsula:								
FL	52	41	45	63	1.0	1.9	3.0	2.8
HL	55	49	200	27	4.2	0.61	8.4	15
JL	79	61	14	6.4	0.19	0.11	7.7	12
LO	804	840	11	110	0.02	0.17	1.6	4.9
MI	19	17	10	12	0.60	0.81	15	9.2
MO	500	350	19	14	0.04	0.05	1.7	3.2
MT	53	62	14	9.6	0.28	0.16	38	42
WL	26	27	20	10	1.1	0.51	5.0	6.9

Figure 6. Responses in *Chironomus tentans* bioassays with sediments from Michigan lakes, after incubations in nitrogen and air treatments. Means with standard deviation (N = 3); ANOVA main effects (p-values) of site (S) and incubation treatment (T) and site X treatment interactions (S x T); and t-tests for treatment differences by site (+ = $p < 0.10$, * = $p < 0.05$). (a) bioaccumulation in Lower Peninsula sediments; (b) growth in Lower Peninsula sediments; (c) bioaccumulation in Upper Peninsula sediments; (d) growth in Upper Peninsula sediments

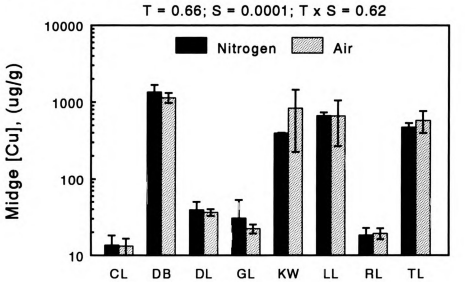
(a) Bioaccumulation -- Lower Peninsula



(b) Growth -- Lower Peninsula



(c) Bioaccumulation -- Upper Peninsula



(d) Growth -- Upper Peninsula

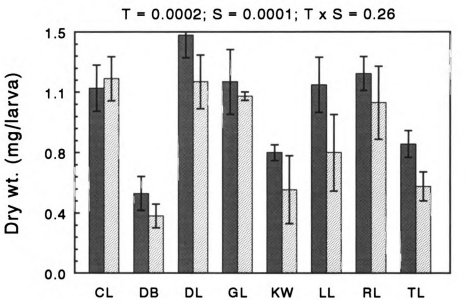
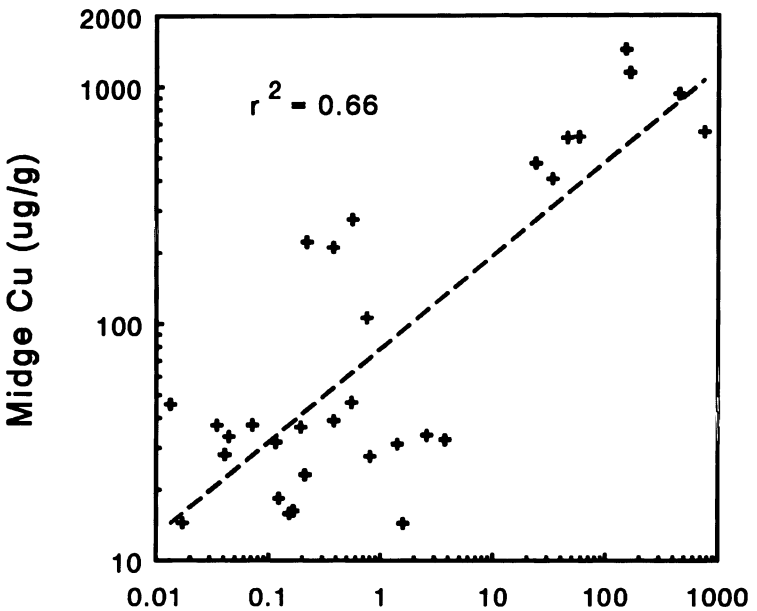


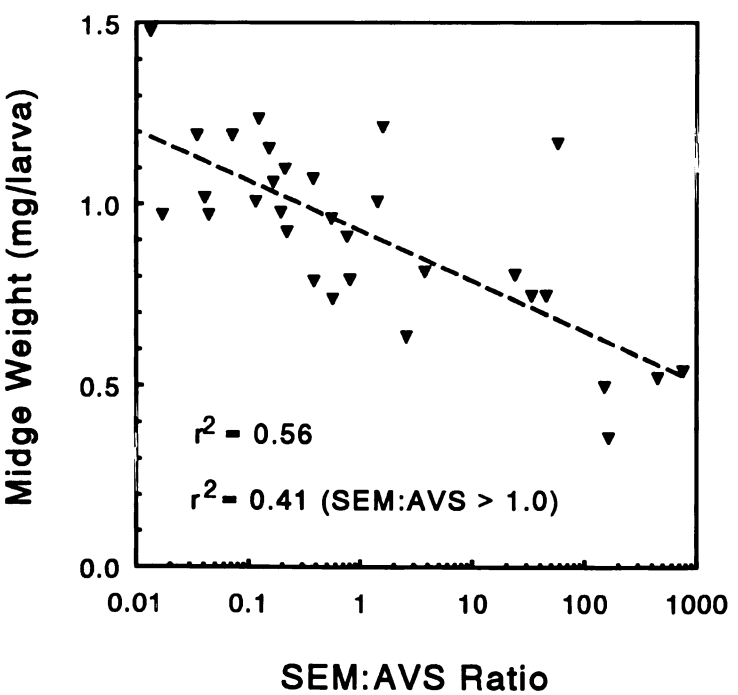
Figure 6 (continued).

Figure 7. Associations between responses in *Chironomus tentans* bioassays with incubated sediments from Michigan lakes and SEM:AVS ratios or Cu concentrations in porewater. Regression lines and fit (r^2) are reported for linear or logistic regressions. (a) Cu bioaccumulation vs. SEM:AVS ratio; (b) growth vs. SEM:AVS ratio; (c) Cu bioaccumulation vs. porewater Cu concentrations; (d) midge growth vs. porewater Cu concentrations

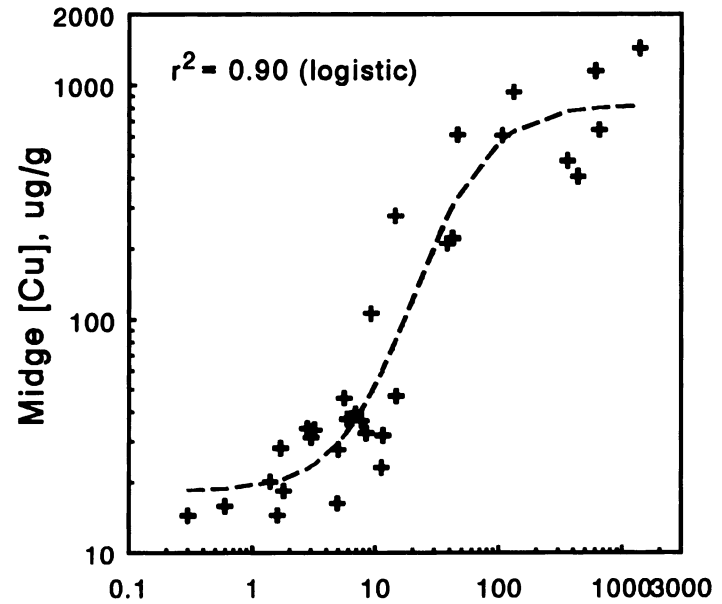
(a)


2000
1000
100
10
Midge Cu (ug/g)
r 2 = 0.66
0.01
0.1
1
10
100
1000

(b)


1.5
1.0
0.5
0.0
Midge Weight (mg/larva)
r2 = 0.56
r2 = 0.41 (SEM:AVS > 1.0)
0.01
0.1
1
10
100
1000
SEM:AVS Ratio

(c)



(d)

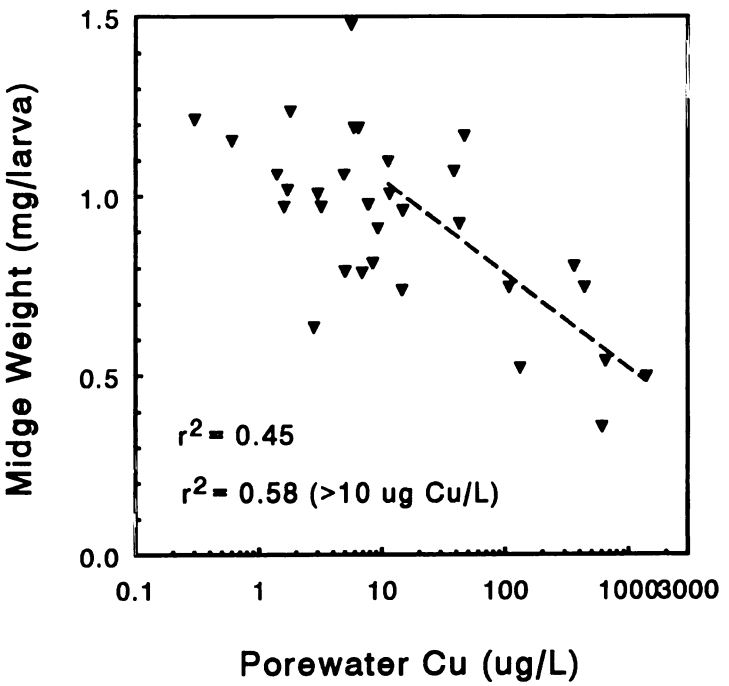


Figure 7 (continued).

CHAPTER 3

Spatial and Temporal Variation in the Bioavailability of Cu and Zn in Sediments from the Clark Fork River, Montana: The Influence of Acid-volatile Sulfide

(for submission to *Environmental Toxicology and Chemistry*)

Abstract

The influence of acid-volatile sulfide (AVS) on spatial and temporal variation of metal bioavailability was assessed by bioassays with sediments from the upper Clark Fork River drainage of Montana, USA. Bioavailability of copper (Cu) and zinc (Zn) was assessed by bioassays of bioaccumulation of metals and inhibition of growth of larvae of the midge, *Chironomus tentans*. Bioaccumulation corresponded to differences in concentrations of AVS between surface and subsurface layers of sediment cores. Bioaccumulation of Cu was significantly less in surface sediments, which contained greater concentrations of AVS, than in subsurface sediments. Bioaccumulation of Cu and growth of midge larvae also differed significantly between sediments incubated in the laboratory with oxic or anoxic overlying water for nine weeks. Rates of

oxidation of AVS during aerobic incubation differed widely among the six sediments tested. Greatest differences in bioavailability of metals between oxic and anoxic treatments occurred in sediments with greatest differences in AVS and SEM:AVS ratios between treatments. These results indicate that AVS is a major control on metal bioavailability in sediments from the upper Clark Fork drainage. Greater bioavailability of metals was indicated by increased bioaccumulation of Cu and Zn and reduced growth by *C. tentans* in sediments with SEM:AVS ratios greater than 1.0. However, little bioaccumulation of Cu or Zn and no inhibition of growth was determined in a some sediments with SEM:AVS ratios much greater than 1.0. The use of AVS normalization to predict metal toxicity in freshwater sediments may be limited by inadequate consideration of other metal-binding phases in sediments, when SEM:AVS ratios are greater than 1.0, and by the difficulty of sampling to adequately address spatial and temporal variation in concentrations of AVS.

INTRODUCTION

Efforts by the U.S. Environmental Protection Agency to develop sediment quality criteria are based on the concept of equilibrium partitioning, which seeks to predict exposure of benthic organisms by estimating concentrations of toxicants in sediment porewater (DiToro et al. 1991a,b). This approach has been used with some success to model the bioavailability of non-ionic organic compounds, by assuming that these compounds partition between water and the organic carbon fraction of sediments (Hoke et al. 1994). However,

predicting the chemical behavior and biological availability of cationic metals is more difficult. A promising approach for predicting the bioavailability and toxicity of cationic metals in sediments is based on the assumption that the primary control on metal bioavailability in sediments is formation of insoluble metal sulfides in subsurface sediments (DiToro et al. 1990, 1991b). Transition metals form sulfide minerals which are highly insoluble, and porewater in equilibrium with these sulfide phases should contain extremely low concentrations of free metals (Stumm and Morgan 1981). The sulfides of most toxic trace metals are less soluble than sulfides of the abundant metal iron (Fe), and should therefore displace Fe from amorphous (noncrystalline) sulfides at equilibrium (DiToro et al. 1991b). Measurements of sulfide and metals released by weak acid extraction, termed acid-volatile sulfides (AVS) and simultaneously-extracted metals (SEM), have been used to estimate the relative abundance of labile sulfides and metals in sediments (Allen et al. 1993). The molar ratio of SEM (including Cd, Cu, Pb, Ni, and Zn) to AVS has been proposed as an index of metal bioavailability in sediments (DiToro et al. 1991b). Several studies with marine sediments have demonstrated that the toxicity of metals such as cadmium and nickel can be predicted based on SEM:AVS ratios, with no toxicity occurring at SEM:AVS ratios less than 1.0 (Ankley et al. 1991, DiToro et al. 1991a, Casas and Crecelius 1994). This AVS normalization method is apparently well suited to assessment of metal bioavailability in nearshore marine sediments, which typically contain high concentrations of sulfides (Berner 1981).

The role of AVS in controlling the bioavailability of metals in freshwater sediments is less well documented. Some studies have reported relatively good prediction of toxicity in freshwater sediments by SEM:AVS ratios (Carlson et al. 1991), although instances of poor prediction of toxicity at SEM:AVS ratios greater than 1.0 have also been reported (Ankley et al. 1993, Hare et al. 1994). The applicability of AVS normalization to freshwater sediments is made less certain by spatial and temporal variability in AVS concentrations in freshwater systems. The formation and stability of sulfides in freshwater sediments are affected by several variables, including concentrations of sulfates in overlying waters, loadings of labile organic matter, redox conditions, and temperature (Herlihy and Mills 1985). Interactions of these biotic and abiotic factors can produce large-and small-scale variation in AVS concentrations. Substantial spatial and temporal variability in AVS has been reported in sediments from freshwater lakes and rivers (Howard and Evans 1993, Leonard et al. 1993, Brumbaugh et al. 1994).

The nature of AVS controls on metal bioavailability was studied in sediments from sites which represent a gradient of metal contamination and riverine, reservoir, and wetland habitat types. The bioavailability of metals in sediments from the upper Clark Fork River drainage of Montana, USA, was assessed with bioassays of growth and bioaccumulation of metals by larvae of the midge, *Chironomus tentans*. The upper Clark Fork and its tributaries have received inputs of metals, predominantly copper (Cu) and zinc (Zn), from mining and smelting operations (Brumbaugh et al. 1994, Kemble et al. 1994).

Although bioaccumulation of metals has been widely used to monitor metal contamination in freshwater systems (Cain et al. 1992, Kiffney and Clements 1993), most previous studies of the influence of AVS on metal bioavailability have relied on determinations of acute lethality. Few studies have examined the response of metal bioaccumulation in sediments with different SEM:AVS ratios (Ankley et al. 1994, Ingersoll et al. 1994).

The objectives of this study were:

(1) to evaluate associations of SEM:AVS ratios with bioaccumulation and toxicity in metal-contaminated freshwater sediments;

(2) to assess differences in concentrations of AVS and bioavailability of metals between surface and subsurface sediment layers; and

(3) to assess the effects of oxic and anoxic conditions on concentrations of AVS and bioavailability of metals ion sediments.

METHODS

Sediment Sampling

Sediments were collected in August 1993 from the upper Clark Fork River drainage of western Montana, USA (Figure 2). Collection sites included five sites in the Clark Fork River, downstream from the historic copper mining and smelting district; six sites in Milltown Reservoir, an impoundment of the Clark Fork which contains extensive deposits of metal-contaminated sediments; and one reference site in Rock Creek (RC), an uncontaminated tributary of the Clark Fork. Sediments were collected in grabs of surficial sediments and in

sediment cores from six primary sites (CF1, CF4, MR2, MR7, MR10, and MR19), which were selected to represent the range of metal contamination and habitat types in the upper Clark Fork drainage.

Sample containers, sampling gear, and laboratory apparatus were cleaned in the laboratory with laboratory detergent, tap water, 10% HCl, and deionized water. Sampling gear was acid-washed in the field between sampling stations and rinsed with site water. Surface grabs were collected with a polypropylene scoop (from sites in the Clark Fork and Rock Creek) or with a petite ponar dredge (from sites in the Milltown Reservoir) and combined to produce a composite sample of 5 to 8 liters from each. Twelve core samples were collected from each of the six primary sites with 5-cm diameter polybutyrate tubes. Cores were obtained from shallow Clark Fork sites by direct insertion of the core tubes into the sediment, and from the deeper Milltown sites with a manual core sampler with a polypropylene nosepiece, which held the core tube inside a stainless steel core barrel (Wildco). Cores were extruded in the field to obtain surface (0-3 cm) and deep (6-9 cm) core sections. Samples from three adjacent cores were combined into each of the four replicate samples from each site and depth. Sediment samples were placed in polyethylene or polycarbonate containers, shipped on ice to the laboratory within 24 hr of collection, and stored in a walk-in cooler at 4°C. Sediments were homogenized by stirring before samples were withdrawn. Bioassays, porewater preparation, and chemical analyses of porewater were conducted within 30 days of collection.

Porewater (interstitial water) was separated from bulk sediments by centrifugation for analysis of metals and water quality parameters. Sediments were centrifuged for 40 minutes at 7000 rpm and the supernatant porewater was centrifuged for 15 minutes at 10,000 rpm (DuPont Sorvall SS-3). Samples of porewater to be analyzed for metals were preserved with nitric acid (Baker Instra-Analyzed) to a final concentration of 1% (v/v). Porewaters for analysis of dissolved organic carbon were filtered through 1.0 μm pore diameter glass-fiber filters (Whatman GF/C), acidified with sulfuric acid to eliminate inorganic carbon, and refrigerated until analysis.

Sediment Incubations

Sediments were incubated in 30 cm X 15 cm glass aquaria equipped with polystyrene under-gravel filter platforms (Penn-Plax) covered with nylon mesh to allow water to recirculate under the sediment layer. Two 2-liter portions of sediment were placed in thin (4 cm) layers and four liters of reconstituted water (described below) were added. Water was lifted from below the sediment layer by gas bubbled up through polystyrene gas-lift tubes in one corner of the platform and passed back through another tube in the opposite corner of the platform. One group of six sediment samples from each primary site (CF1, CF4, MR2, MR7, MR10, and MR19) received compressed room air (Air Treatment) and another group (Nitrogen Treatment) received nitrogen from a gas cylinder, with oxygen removed by an in-line oxygen trap (Baxter). Aquaria were covered with plexiglass lids and sealed with laboratory tape, except for

small holes for the inlet gas tubing and for gas escape. Aquaria were placed in water baths at 18 to 20 °C in continuous darkness. Samples of sediment and overlying water from each aquarium were removed after 21 d and 56 d for analysis of AVS and the incubation was terminated after 63 d. At the end of the incubation period, samples of sediment were removed for sediment bioassays and analysis of metals, AVS, and porewater characteristics.

Bioassays

Sediment bioassays with larvae of the midge, *Chironomus tentans*, were conducted with a static-renewal method based on the procedure developed by the U.S. Environmental Protection Agency (1994). Four replicate exposure chambers per site or treatment group (300-mL glass beakers with two 17-mm windows covered with stainless steel screen) were placed in a 9-liter all-glass aquarium. Each aquarium received two water replacements per day from individual polyethylene head tanks. Replacement of overlying water in the test chambers was accomplished by a drain tube with an intermittent siphon (Benoit et al. 1993). Dilution water was moderately-hard reconstituted water, prepared from reagent-grade salts and ultrapure water (hardness 90 mg/L; USEPA 1994).

Cohorts of midge larvae for bioassays were started from 3 to 5 egg masses, collected on the same date and reared in reconstituted water. Larvae of uniform age (10-12 d after hatching) and uniform size (approx. 5 mm long) were selected for use in bioassays. Midge larvae were added randomly to the

exposure chambers to a total of ten larvae per test chamber. A suspension containing 6 mg dry wt. of fish food (Tetra-Min) was added to each chamber daily during the ten-day exposure period. Composite samples of overlying water from each group of exposure chambers were collected on days 1 and 9 for water quality analysis. After the exposure, groups of exposure chambers were removed in random order and the number of survivors for each chamber was recorded. Surviving larvae from each chamber were transferred to 30-mL plastic cups which contained dilution water and a small amount of acid-washed sand, fed, and set aside to allow clearance of gut contents. After 12 hr, larvae from each cup were rinsed with ultrapure water, dried for 24 hrs at 60 °C, and weighed to the nearest 0.01 mg.

Chemical Analysis

Samples of midge larvae were prepared for metal analysis by digestion in Teflon centrifuge tubes at 90 to 95 °C with high-purity reagents (Baker Instra-Analyzed or Ultrex). Concentrated nitric acid (1.5 ml) was added for the first 24 hrs of digestion, the sample was cooled and a solution of 30% hydrogen peroxide (1.0 ml) added, and the digestion was resumed for an additional 24 hrs. Digested samples were diluted with ultrapure water to a final volume of 10 mL and a final concentration of 10% (v/v) nitric acid.

Sediments were analyzed for acid-volatile sulfide (AVS) and total organic carbon (SOC). AVS was extracted from sediment samples by a 30-min extraction at room temperatures in 1N HCl under an argon atmosphere and

sulfide was quantified by ion-selective electrode (Orion; Allen et al. 1993). Sediment extracts containing simultaneously-extracted metals (SEM) were filtered through a 1.0 μm glass-fiber filter (Whatman GF/C). Samples of sediment for TOC analysis were treated with 1N HCl to volatilize carbonates as CO_2 and remaining, organic carbon was analyzed by a dry combustion-infrared technique (Leco Instruments).

Concentrations of metals in samples of midge larvae, sediment porewater, and SEM extracts were determined by atomic absorption spectrophotometry with polarized Zeeman background correction (AAS) or inductively-coupled plasma optical emission spectroscopy (ICP). Samples of midges and porewater containing low concentrations of Cu (<100 μ/L) were analyzed AAS with graphite furnace atomization (Hitachi Instruments model 180-80). Samples with higher concentrations of Cu and all samples for Zn analysis were analyzed by AAS with atomization in an air-acetylene flame. SEM extracts of composite grab samples from all twelve sites were analyzed by ICP (Jarrell-Ash Instruments). Quality assurance for metal analyses included analysis of procedural blanks, sample splits, standard reference materials for analyses of porewater and midge samples (NIST 1643c and NBS 1577a) and matrix spikes for SEM extracts (Appendix A).

Characteristics of sediment porewaters and overlying water from bioassays were determined by standard methods (APHA 1985): organic carbon, by persulfate digestion and infrared detection; ammonia, by ion-selective electrode; hardness, by EDTA titration with a colorimetric endpoint; pH, by

combination electrode; total alkalinity, by titration to pH 4.5 with standard acid; and dissolved oxygen, by electrode.

Statistical Analysis

Statistical analyses were performed with procedures in the SAS statistical package (SAS Institute 1989). Concentration of metals in porewater, sediment extracts, and midge samples were log-transformed before statistical analysis, to improve the normality and homogeneity of variance. Comparisons of bioassay results among sites were made by analysis of variance (ANOVA), with results assessed at a 5% significance level ($p = 0.05$). Variation among study sites and between treatment groups (core sections or incubation treatments), and interactions of the main effects of site and treatment (site X treatment interactions) were evaluated by two-way ANOVA. If site X treatment interactions were significant, differences between treatment pairs within each site were assessed with t-tests.

RESULTS

Metal Bioavailability in Sediments from Surface Grabs

Characteristics of sediments and porewaters from surface grab samples differed among sampling locations, which included riverine sites in the upper Clark Fork River and deep-water and wetland sites in the Milltown Reservoir. Cu and Zn made up greater than 95% of the total of the five metals (Cd, Cu, Ni, Pb, and Zn) analyzed in SEM extracts (Table 7). Concentrations of Zn and

Cu in SEM extracts showed a decreasing trend with distance downstream in the Clark Fork sites, but high concentrations of these metals also occurred at backwater sites in the Milltown Reservoir (MR10, MR11, and MR25). Concentrations of Zn and Cu in porewater did not follow the same trends as SEM metals, except for high concentrations in the most contaminated sediments (CF1, MR10). AVS concentrations ranged from near zero to nearly 50 μmol/g. Despite the wide range in concentrations of both SEM and AVS, SEM:AVS ratios were quite consistent in most sediments, with ratios from 0.5 to 2.0 in eight of the twelve sites. Three sites (CF1, CF2, and MR10) had greater SEM:AVS ratios, which ranged from 8 to nearly 500. Concentrations of total organic carbon were consistently less in sediments from Clark Fork sites and in the riverine site, MR19, at the Milltown Reservoir. Porewaters were generally hard, with high alkalinity and circumneutral pH. Concentrations of organic carbon and ammonia were greatest in porewaters from the headwater site in the Clark Fork and at the deeper Milltown sites (MR2, MR7).

Sediments from surface grabs from the upper Clark Fork and Milltown Reservoir were toxic and caused increased bioaccumulation of metals in larvae of *C. tentans*. Survival of midge larvae was significantly reduced by sediments from five sites (CF1, CF2, CF3, MR2, and MR25) and growth was significantly reduced by sediments from ten sites (all except MR10), relative to the Rock Creek reference sediment (Chapter 2, this volume). Growth and bioaccumulation of Cu by midge larvae corresponded closely to SEM:AVS ratios (Figure 8). Growth of midge larvae was inhibited and bioaccumulation of Cu

increased in most sediments with SEM:AVS ratios greater than 1.0, relative to sediments with SEM:AVS ratios less than 1.0. There were two exceptions to these trends: growth was not reduced by sediments from MR10, despite a SEM:AVS ratio of nearly 500; and bioaccumulation of Cu and Zn from sediments from CF2 was relatively low (77 μg Cu/g), despite a SEM:AVS ratio of 8.2. Sediments from these two sites had the lowest AVS concentrations measured in this study (<1.0 μmol/g).

Metal Bioavailability in Surface and Subsurface Sediment Layers

Bioavailability of metals differed between sediments from surface (0-3 cm) and subsurface (6-9 cm) sections of core samples. Concentrations of AVS and bioaccumulation and growth of midge larvae differed significantly among sites and between depths (Figure 9), whereas bioaccumulation of Zn differed among sites, but not between depths (Table 9). Concentrations of AVS were greater in surface sediments, although this difference was not consistent among all sites, as indicated by a significant site X depth interaction (Figure 9). Concentrations of AVS were significantly greater in the surface sediments from three sites, but were greater in subsurface sediments at site MR19, although not significantly ($p<0.10$). Trends in Cu bioaccumulation were consistent with differences in AVS, with significantly greater bioaccumulation from deep core sections which had lesser concentrations of AVS. However, trends in midge growth did not always correspond to those in Cu bioaccumulation and AVS. Midge growth was consistently (although not significantly) less in core sections

which had lesser concentrations of AVS in exposures to sediments from riverine sites (CF1, CF4, MR19). The opposite trend was evident in sediments from some sites in the Milltown Reservoir, MR2 and MR7, where midge growth was significantly less in the surface sediments although AVS concentrations were greater in deep sediments.

Effects of Aerobic and Anaerobic Conditions on Metal Bioavailability

The effects of incubation under oxic and anoxic overlying waters on concentrations of AVS and responses in *C. tentans* bioassays varied among sediments from the six primary study sites. Concentrations of AVS remained constant, or increased slightly, in sediments exposed to a nitrogen atmosphere for the nine-week incubation period, but AVS decreased in some sediments under aerobic conditions. AVS concentrations decreased steadily in sediments from CF4 and MR19 during the air treatment, but changed little in sediments from most sites (Figure 10). Rates of AVS oxidation during aeration did not correspond closely to differences in initial AVS concentrations. The sediment with the greatest initial concentration of AVS (CF4) showed the most rapid decrease, whereas several sediments with intermediate or low initial concentrations of AVS showed virtually no change over the nine-week period. Concentrations of Cu and Zn in SEM extracts differed little between treatments (Table 8). Ratios of SEM:AVS differed between air and nitrogen treatments in the Clark Fork sites and in MR19, but not in the other sites in the Milltown Reservoir. The greatest differences occurred in sediments from CF4 and MR19,

which were the only sediments with SEM:AVS ratios less than or equal to 1.0 after the Nitrogen treatment and greater than one after the Air treatment.

Bioaccumulation of Cu and inhibition of growth of *C. tentans* larvae differed significantly between Air and Nitrogen treatments, especially in sediments which had substantial differences in AVS between treatments. Bioaccumulation of Cu, but not Zn was significantly greater in sediments from the air treatment (Figure 11, Table 9). These trends were relatively consistent among sites, as indicated by the absence of significant site X treatment interactions, but were less pronounced for sediments from some of the Milltown sites. Midge growth did not differ significantly between the incubation treatments by ANOVA, but there was a significant site X treatment interaction, and significant differences between sites were evident in several sites by t-test. Midge growth was significantly less in the air treatment from three sites, including the two sites which underwent rapid oxidation of AVS during aeration (t-tests). One site from the Milltown Reservoir (MR7) showed an opposite trend, reduced growth (although not significant) of midge larvae in the nitrogen treatment, in contrast to with significantly reduced bioaccumulation of Cu.

DISCUSSION

The responses of growth and bioaccumulation of *C. tentans* in sediment bioassays were consistent with the hypothesis that AVS is a major control on the bioavailability of metals in freshwater sediments. Bioaccumulation of metals and growth of *C. tentans* larvae generally corresponded to predictions of the

AVS normalization method (DiToro et al. 1991b), with inhibition of growth and increased bioaccumulation of Cu occurring at SEM:AVS ratios greater than 1.0 (Figure 8). Additional support for this hypothesis was provided by bioassays with sections of sediment cores, in which differences in AVS between surface and deep sections were associated with opposite trends in Cu bioaccumulation (Figure 9), and by results of sediment incubations, in which oxidation of AVS was associated with reduced growth and increased bioaccumulation of Cu and Zn by midge larvae (Figure 11). However, these studies also indicated two difficulties in the use of AVS normalization to predict metal bioavailability: poor prediction of metal availability in sediments with low concentrations of AVS, and significant spatial, and temporal variation in AVS concentrations.

Bioavailability was poorly predicted by SEM:AVS ratios in sediments containing very low concentrations of AVS. Relatively little bioaccumulation of Cu and Zn and no inhibition of normal growth of *C. tentans* were caused by two sediments (CF2 and MR10), which contained low concentrations of AVS had large SEM:AVS ratios. Similar cases have been reported by other investigators and are generally attributed to the presence of non-sulfide binding phases (Mahony et al. 1991, Ankley et al. 1993, Kemble et al. 1994). The bioavailability of metals which are not bound to sulfides may be reduced by association with other sediment phases such as organic matter or oxides of iron and manganese (Lion et al. 1982, Davies-Colley et al. 1984, Calmano et al. 1988). Extracts of SEM from sediments from the Clark Fork River and Milltown Reservoir sediments contained substantial concentrations of Fe and Mn (up to

1.3%), and oxide precipitates were visible in some sediments when they were exposed to oxygen. Concentrations of organic carbon were low in sediments from Clark Fork sites, but were greater (4-6%) in sediments from sites in Milltown Reservoir. Sediments from MR10, which were not toxic to *C. tentans* despite high concentrations of Cu and Zn in porewater and high SEM:AVS ratios, had the greatest concentrations of Fe, Mn, and organic carbon of the sediments tested. High SEM:AVS ratios may also occur without increased bioavailability of metals, in sediments with very low concentrations of both AVS and metals (Hare et al. 1994, Chapter 1, this volume). Although normalization of metal concentrations to AVS did not accurately predict metal bioavailability in these low-AVS sediments, recent research suggests that measurements of oxide and organic binding sites may allow accurate predictions of metal partitioning and bioavailability in non-sulfidic sediments (Fu et al. 1992, Tessier et al. 1993).

Homogenization of grab samples of sediment for chemical analysis and sediment bioassays may obscure natural variation in AVS and bioavailability with depth in natural sediment profiles. Differences in concentrations of AVS between surface and subsurface sediment layers were associated with differences in metal bioaccumulation and, to a lesser extent, toxicity. AVS concentrations differed between surface and subsurface sediments from all six primary sites, with greater AVS concentrations in surface sediments from five of the six sites. A previous study also reported differences in concentrations of AVS between surface and subsurface sediments from the Clark Fork and

Milltown Reservoir, as well as substantial variation among replicate cores collected within a sampling site (Brumbaugh et al. 1994). AVS depth profiles in lake sediments are typically complex, and often exhibit subsurface maxima (Nriagu 1968, Smith and Klug 1981, Howard and Evans 1993).

Seasonal changes in AVS concentrations also limit the use of AVS normalization to predict metal bioavailability. Oxidation of AVS in Clark Fork and Milltown sediments produced significant increases in metal bioavailability and toxicity. Similar increases in bioavailability have been reported during oxidation of sulfidic estuarine and freshwater sediments (Kerner and Wallman 1992, Calmano et al. 1993, Zhuang et al. 1994). Substantial seasonal changes in AVS concentrations occur in sediments of freshwater lakes (Howard and Evans 1993, Leonard et al. 1993), reflecting the many site-specific influences on AVS formation (Nriagu 1968, Smith and Klug 1981). Since anoxia of overlying waters is unlikely in riverine environments, AVS concentrations in riverine sediments probably reflect a balance between the simultaneous processes of sulfate reduction and AVS oxidation. Rates of sulfate reduction are affected by inputs of organic matter, and by the activity of sulfate-reducing bacteria, which have temperature optima in the range of 15 to 20 °C (Herlihy and Mills 1985). Rates of oxidation of AVS in sediments in contact with oxic overlying water are apparently affected by the permeability of the sediment and the rates of oxygen consumption by decomposition processes (DiToro et al. 1990). In the current study, oxidation of AVS during aerobic incubations was most rapid in sediments from riverine sites, which contained low concentrations

of organic matter and a high content of sand-sized particles, while AVS was oxidized slowly or not at all in the fine-textured, more organic sediments from the Milltown Reservoir. Slow rates of AVS oxidation were also measured in fine-textured, organic sediments from Michigan Lakes during similar incibations with oxic overlying waters (Chapter 2, this volume).

The predominance of SEM:AVS ratios close to 1.0 in most of the sediments tested suggests that concentrations of AVS may determine the concentrations of metals retained in the Clark Fork sediments, which receive continuous inputs of metals from upstream sources. In a few sites with very high metal loadings or low concentrations of AVS, metal bioavailability is apparently controlled by sediment components other than sulfide, such as oxides of Fe and Mn or organic matter. However, it cannot be assumed that concentrations of AVS are always sufficient to provide a sink for metals, even in sediments where AVS was abundant during the sampling period. Concentrations of AVS varied widely both among and within sites, and may be susceptible to rapid change over time. Seasonal cycles of AVS formation and oxidation probably determine whether these sediments are a site of immobilization of metals or a source of metals to porewaters, overlying waters, and biota.

Table 7. Concentrations of metals in sediment extracts (SEM) and unfiltered porewaters from surface grab samples of Montana sediments.

Site	SEM (μmol/g)					Porewater (μg/L)	
	Zn	Cu	Pb	Ni	Cd	Cu	Zn
RC	0.2	0.0	0.003	0.07	0.001	9	13
CF1	8.6	10.3	0.09	0.12	0.04	510	280
CF2	13.5	6.0	0.26	0.06	0.02	52	44
CF3	9.4	0.6	0.28	0.05	0.02	47	27
CF4	10.3	0.5	0.27	0.06	0.02	140	120
CF5	7.0	0.7	0.15	0.10	0.01	43	63
MR2	12.3	0.5	0.24	0.34	0.03	110	190
MR7	10.9	1.2	0.28	0.08	0.03	350	480
MR10	66.3	14.2	0.44	0.16	0.10	480	1400
MR11	21.9	0.5	0.26	0.22	0.05	20	48
MR19	6.1	0.7	0.12	0.03	0.01	33	54
MR25	22.2	0.1	0.22	0.11	0.04	98	280

Table 8. Characteristics of sediment and unfiltered porewater from the Clark Fork River drainage, Montana, after incubation in nitrogen (N_2) and air atmospheres.

Porewater Characteristics:

Site	pH		Alkalinity		Hardness		Ammonia	
	N_2	Air	N_2	Air	N_2	Air	N_2	Air
CF1	5.87	4.93	0	0	709	1031	22	24
CF4	7.63	7.35	344	220	106	985	9	0.2
MR19	7.82	7.81	264	396	847	335	10	0.6
MR7	7.76	7.52	324	222	112	709	13	0.1
MR2	7.49	7.81	142	267	65	106	0.2	0.3
MR10	7.58	7.67	230	452	108	847	1.7	0.5

Sediment Characteristics:

Site	SEM-Cu		SEM-Zn		AVS		SEM:AVS	
	N_2	Air	N_2	Air	N_2	Air	N_2	Air
CF1	7.0	7.9	12	12	5.6	3.5	3.5	5.5
CF4	0.5	5.4	12	11	24	0.9	0.6	18
MR19	1.0	1.9	7.3	7.7	7.5	2.6	1.1	3.7
MR7	1.0	1.6	13	13	13	16	0.5	0.5
MR2	0.8	1.1	15	13	17	15	0.9	1.0
MR10	7.2	8.3	41	43	0.8	0.9	118	91

Table 9. Bioaccumulation of Zn (μg/g dry wt.) by *Chironomus tentans* larvae in bioassays with sediments from the Clark Fork River, Montana. Means with standard deviations in parentheses (N = 4), and results of ANOVA (p-values). (a) exposures with surface (0-3 cm) and deep (6-9) sections of sediment cores; (b) exposures with sediments after incubation with nitrogen and air atmospheres.

Site	(a) Core Sections		(b) Incubated Sediments	
	Surface	Deep	Nitrogen	Air
CF1	507 (337)	408 (121)	432 (203)	722 (238)
CF4	276 (141)	345 (41)	350 (80)	1034 (888)
MR19	310 (79)	340 (130)	293 (99)	1011 (925)
MR7	350 (210)	300 (77)	329 (70)	421 (142)
MR2	316 (540)	383 (122)	366 (71)	462
MR10	630 (169)	956 (164)	894 (280)	694 (443)
RC	281 (226)	157 (32)	249 (89)	243 (159)
ANOVA Effects:				
	Depth	0.28	Depth	0.19
	Site	0.0002	Site	0.009
	Depth X Site	0.76	Depth X Site	0.21

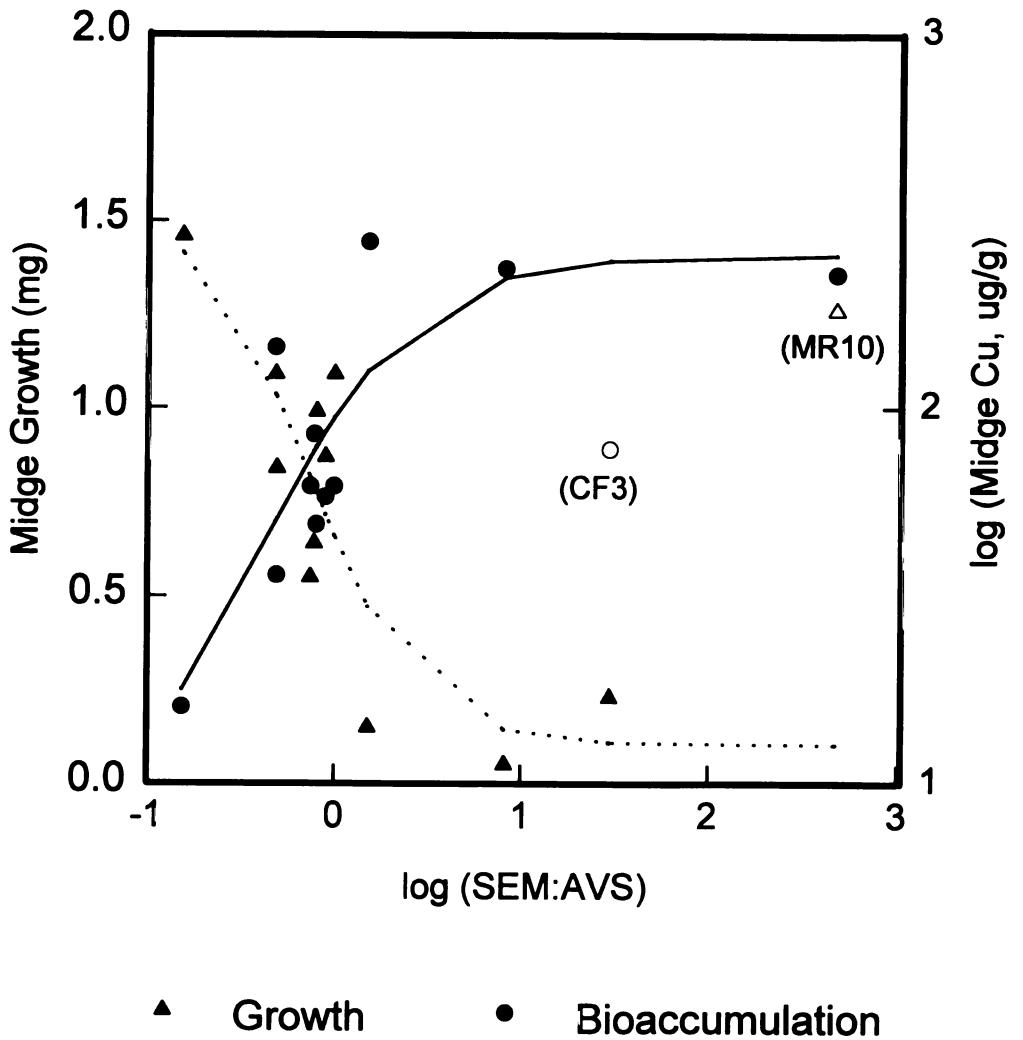
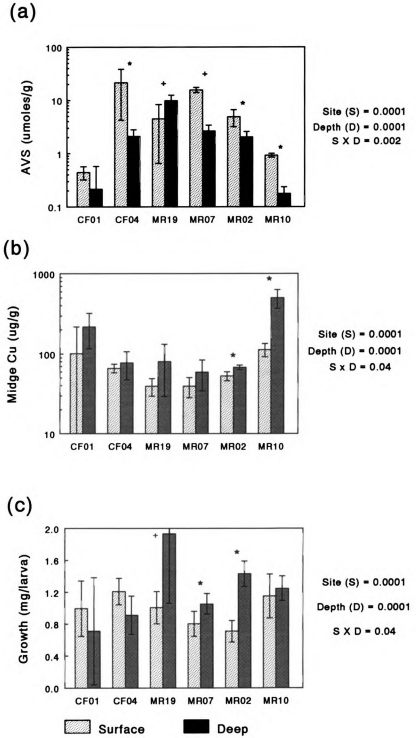


Figure 8. Results of *Chironomus tentans* bioassays with surface grab samples of sediment from the upper Clark Fork drainage. Logistic regressions of midge growth and Cu bioaccumulation vs. SEM:AVS ratios exclude outlying points which are indicated by hollow symbols.

Figure 9. AVS concentrations and responses in *C. tentans* bioassays with surface (0-3 cm) and deep (6-9 cm) sections of sediment cores from the upper Clark Fork River, Montana. Means with standard deviations (N = 4); ANOVA effects of site, depth, and site X depth interaction (p-values); and t-tests for differences between depths within sites (+ = $p < 0.10$, * = $p < 0.05$). (a) AVS; (b) Cu bioaccumulation; and (c) growth.



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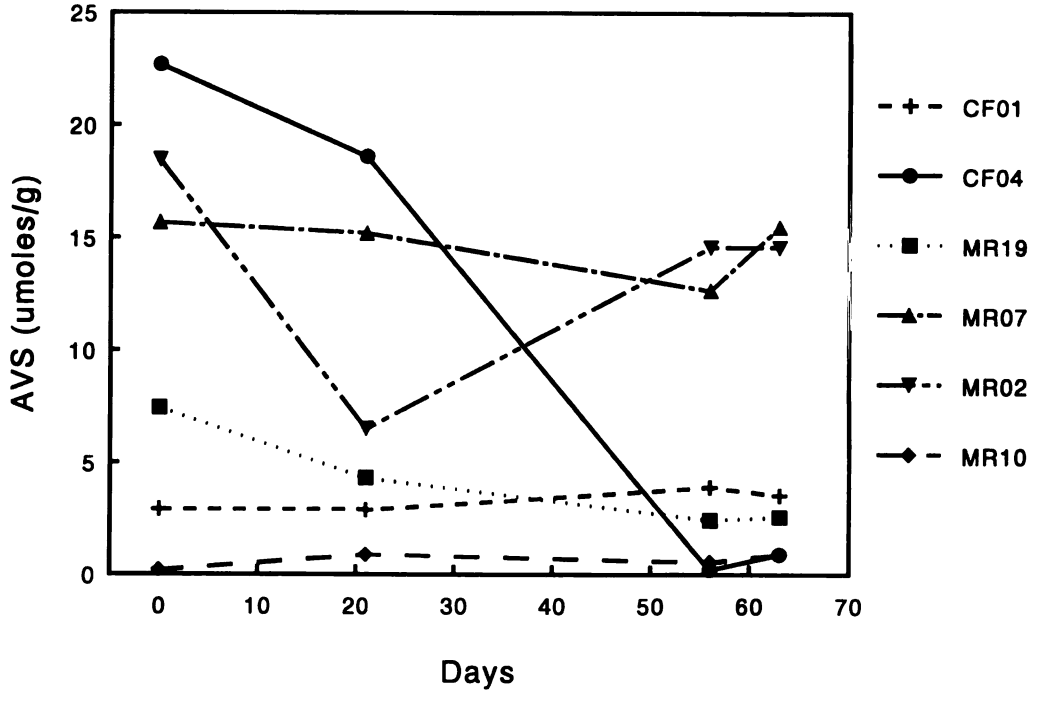
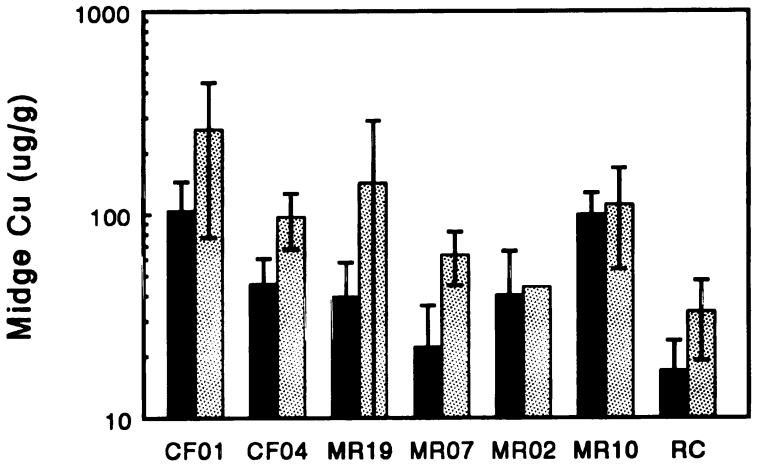


Figure 10. Changes in AVS concentrations in sediments from the Clark Fork River drainage during incubation with aerated overlying waters. Means of duplicate analyses on days 0 and 63; single analyses on other dates.

Figure 11. Results of *Chironomus tentans* bioassays with sediments from the Clark Fork River drainage, after nine-week incubations with air and nitrogen atmospheres. Means and standard deviations (N = 4); ANOVA effects for site, incubation treatment, and site X treatment interactions (p-values); and t-tests for treatment differences within sites (+ = $p < 0.10$, * = $p < 0.05$). (a) Cu bioaccumulation; (b) growth.

(a)

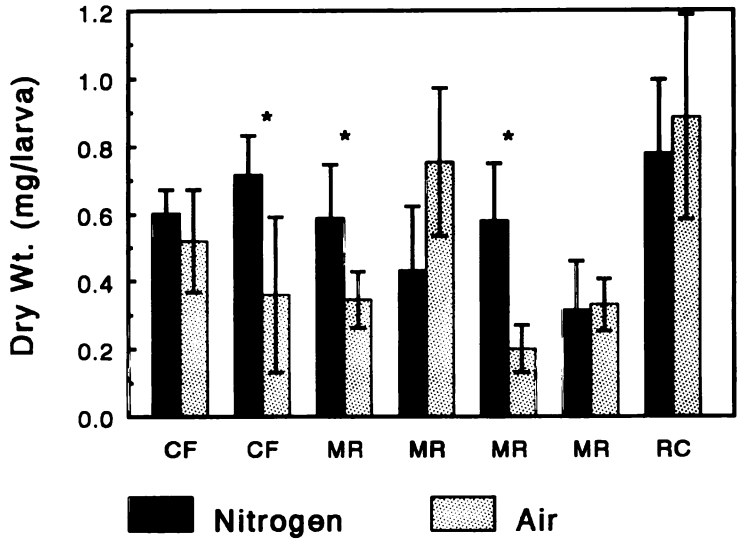


Site (S) = 0.001

Incub. (I) = 0.0002

S X I = 0.48

(b)



Site (S) = 0.0001

Incub. (I) = 0.09

S X T = 0.0004

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site X
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(a) Cu

SUMMARY AND CONCLUSIONS

Quality criteria for protection of aquatic organisms from toxic effects of heavy metals in sediments should be based on understanding of the partitioning of metals between solid components of sediment and dissolved metal species in sediment porewater. The toxicity of metals in sediments and bioaccumulation of metals by sediment-dwelling organisms are closely related to concentrations of dissolved metals in sediment porewater, and metal partitioning between sediment and porewater is controlled by many sediment components, such as organic matter, iron and manganese oxides, and amorphous metal sulfides. Oxide and sulfide phases are sensitive to changes in redox conditions in sediment environments, which can result from natural seasonal cycles of hydrodynamics and biological activity, or by anthropogenic disturbances such as dredging or nutrient enrichment. Redistribution of released metals among competing sediment components during these redox changes may substantially alter metal bioavailability.

Sediment bioassays with larvae of the midge, *Chironomus tentans*, were are a useful method for assessment of the bioavailability of metals, principally copper (Cu), in sediments from lakes in Michigan and from a variety of habitats in the upper Clark Fork River drainage in Montana. The bioassay responses,

survival, growth, and bioaccumulation of metals, were associated with concentrations of metals in sediment and porewater and with concentrations of metal-binding components in sediments, especially acid-volatile sulfide (AVS). Bioaccumulation of Cu was strongly correlated with concentrations of Cu in porewater, and significant increases in bioaccumulation occurred at concentrations of Cu less than those affecting growth and survival. Reductions on growth of midge larvae were less strongly correlated with concentrations of Cu in porewater. Principal components analysis of responses of bioassays and sediment characteristics indicated that bioaccumulation of Cu and toxic effects on growth and survival were negatively associated with concentrations of both AVS and organic carbon in sediment.

Ratios of metal concentrations in sediment extracts (simultaneously-extracted metals or SEM) to concentrations of AVS (SEM:AVS ratios) did not always predict bioavailability of metals. Toxicity and bioaccumulation of metals was consistently low in sediments with very low SEM:AVS ratios, presumably due to formation of insoluble metal sulfides. However, predictions of Cu bioavailability based on SEM:AVS ratios were less reliable in sediments with ratios close to or greater than 1.0. No toxicity and little bioaccumulation were evident in several sediments with SEM:AVS ratios greater than 1.0, in which greater metal bioavailability would be predicted. In some cases, high SEM:AVS ratios simply reflected low concentrations of AVS, without substantial contamination with metals. However, the low bioavailability of metals in several sediments, which had both high SEM:AVS ratios and high

concentrations of toxic metals, was apparently due to binding of metals to sediment components other than sulfides.

Incubation of sediments in the laboratory under oxic and anoxic conditions, to simulate natural seasonal changes in redox status, resulted in significant differences in metal bioavailability. Rates of change of AVS concentrations during aerobic incubation varied widely among sediments from different locations. Little or no decrease in AVS occurred during a six-week aerobic incubation with sediments from Michigan lakes, but substantial decreases in concentrations of AVS occurred in several sediments from the Clark Fork River of Montana during eight-week aerobic incubations. Greatest differences in AVS concentrations and SEM:AVS ratios between aerobic and anaerobic incubations were associated with significant differences in bioaccumulation of Cu and in growth of midge larvae. However, changes in bioavailability also occurred in some sediments without substantial changes in AVS concentrations or SEM:AVS ratios. In these sediments, oxidation of surface sediment layers may have been sufficient to change metal bioavailability to midge larvae without changing bulk sediment characteristics. Concentrations of AVS, SEM:AVS ratios, and bioassay responses also differed significantly between depths in sediment cores from the Montana sites. Bioaccumulation of Cu was significantly greater in deeper core sections, corresponding to lesser concentrations of AVS. These results suggest that laboratory bioassays and chemical measurements based on homogenized sediments samples do not adequately represent conditions in natural sediment profiles.

The statistical associations of AVS and SEM:AVS ratios with bioaccumulation of Cu and toxicity in bioassays with *C. tentans* results support the hypothesis that AVS is a major control on bioavailability of Cu in freshwater sediments. However, neither bioaccumulation of Cu or toxicity of Cu-contaminated sediments were predicted by SEM:AVS ratios with sufficient accuracy for purposes of management of contaminated sediments. Bioaccumulation of Cu and, to a lesser extent, toxic effects in midge larvae were more strongly associated with Cu concentrations in porewater, suggesting that concentrations of metals in sediment porewater may provide a more reliable basis for predicting bioaccumulation and toxicity of metals. However, sediment quality criteria based on either SEM:AVS ratios or metal concentrations in porewater must also consider the natural seasonal changes in concentrations of AVS, and presumably in bioavailability of metals, which may occur in many freshwater sediments.

APPENDIX A -- QUALITY ASSURANCE

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Quality Assurance Characteristics and Measurements

The quality of analytical results was assessed with three measures: detection limits, accuracy, and precision. Instrument detection limits (IDL) for chemical analyses were defined as three times the standard deviation of analyses of standards, at analyte concentrations near zero (s_0; Keith et al. 1994). The value for s_0 was estimated as the Y-intercept of the regression of standard deviation (Y) on analyte concentrations (X). Method detection limits (MDLs) for analyses of midge tissue and sediment extracts (SEM) were adjusted to account for typical sample mass and dilution volume (10 mg and 10 mL for midges, 2.0 g and 320 mL for SEM extracts).

Accuracy of analyses was estimated from recoveries of analyte from certified standard reference materials (SRMs) and from recoveries of analyte spiked into the sample matrix (matrix spikes). National Institute of Standards and Technology (NIST) SRM 1643c, Trace Elements in Water, and dilutions of a multi-element test solution obtained from the U.S. Environmental Protection Agency (ICAP-19) were used to evaluate water analyses. Bovine Liver (NIST SRM 1577a) was used for assessment of digestion and analysis of midge samples. A commercially-produced certified standard solution (Spike-1; SPEX, Inc.) was used for spiking metals into sediment samples. Recovery was calculated by the formulas:

for <u>matrix spikes</u>:

$$\% \textit{ Recovery} = 100\% \times \left[\frac{(S - U)}{C_{sa}}\right]$$

where

S = measured conc. in spiked sample

U = measured conc. in unspiked sample

C_{sa} = actual conc. of spike added

for <u>reference materials</u>:

$$\% \textit{ Recovery} = 100\% \times \left(\frac{Cm}{Csrm}\right)$$

where Cm = measured conc. in SRM

Csrm = actual concentration in SRM

Precision (reproducibility) of analytical methods was assessed by performing duplicate analyses of selected samples. Analytical precision of metal analyses was assessed by repeated analyses of the same samples. Precision of the method for acid-volatile sulfide analysis was assessed by carrying multiple subsamples of the same sediment through the entire procedure. Precision of duplicate analyses was expressed as relative percent difference (RPD), calculated by the formula:

$$RPD = \frac{(C_1 - C_2) * 100\%}{(C_1 + C_2) / 2}$$

where C1 = first concentration and
C2 = second concentration

Quality Assurance of Metal Analyses

Quality assurance measurements for analysis of Cu and Zn by graphite furnace atomic absorption spectrophotometry (GFAAS) and flame atomic absorption spectrophotometry (FAAS) are summarized in Table 10. Procedures for these analyses were based on the recommendations of the USEPA (1986) manual for analysis of sediment and solid wastes and are summarized in a standard operating procedure (see Appendix B). Detection limits for Cu and Zn are comparable to or better than those reported by USEPA (1986): 1.0 μg/L for Cu and 5.0 μg/L for Zn. The response of acid blanks, solutions of 1% nitric acid (Baker Instra-Analyzed) and deionized water (Barnstead E-pure), indicated that these reagents, used for preservation and dilution of samples, did not add significant amounts of metals to analytical samples. Analyses of Cu and Zn in water had good recovery from SRMs (average recoveries from 90-110%) and good precision (RPD $\leq$ 10%). Procedural blanks from the digestion of midge samples indicated little contamination with Cu, but greater and more variable levels of blank contamination with Zn. The average concentration of Zn in digestion blanks would increase concentrations of Zn in midge samples by 44 μg/g. However, concentrations of Zn in midge samples from sediment bioassays in the Montana study (Chapter 3) generally contained high concentrations of Zn ($<$150 μg/g) and were not corrected for blank contamination. Precision of analyses for both Cu and Zn in midge samples was good (5-10% RPD).

The greatest quality assurance problem was encountered in analyses of metals in SEM extracts. Cu and Zn were measured at detectable concentrations in blanks carried through the SEM extraction, although these concentrations were not great enough to require adjustment of metal concentrations in sediment samples. Analytical precision of duplicate analyses of SEM extracts was good (3-5% RPD). Recoveries of both Cu and Zn from matrix spikes were low,

especially for Zn (19%), and recoveries were highly variable for both metals. Low recoveries should probably be expected from SEM extracts, due to the weak nature of the 1N HCl extraction. Poor recoveries of matrix spikes in the SEM procedure have been reported by other investigators, even when a stronger HCl extractant (3N) was used (Brumbaugh et al. 1994). The variation in recoveries was partially due to poor reproducibility, as indicated by duplicate samples carried through the extraction and analysis step, in which RPD ranged from 1 to 65% (mean = 15.6%). Variable recoveries may be affected by variation of concentrations of carbonate in sediment samples and variation in the mass of sediment in the sample, which could have resulted in variable degree of neutralization of HCl in the extraction solution. The erratic recoveries of Cu and Zn from matrix spikes may also be related to interactions of spiked metals with sulfides or other components of the sediment sample during the extraction, which could have resulted in precipitation or complexation with solid phase components, and subsequent removal during filtration. In light of these problems, future investigations should include: greater replication in SEM extraction and analyses, and measurement of recoveries of metals from a reference sediment which does not contain sulfides (e.g. NIST River Sediment).

Quality Assurance of Analyses of Acid-volatile Sulfide

Results of quality assurance measurements for the two procedures used for analysis of acid-volatile sulfide (AVS) are summarized in Table 11. Both methods are based on the method of Allen et al. (1993) and the final method is summarized in an SOP in Appendix B. The detection limit was lower for the colorimetric method than for the ion-selective electrode method, although both methods were adequate for analyses of sediments containing concentrations of AVS greater than 1.0 μmol/g, the recommended range for use of AVS to predict metal bioavailability (Allen et al. 1993, Ankley et al 1994a). Precision of AVS analyses was better for the ion-selective electrode procedure, probably due to the elimination of sample dilutions necessary in the colorimetric procedure. Accuracy, as measured by recovery of sulfide from spiked blanks and spiked sediments, was similar for both methods (83-88%). Recovery of sulfide spiked into sediment samples was lower (71-73%) and was more variable. The lower recoveries from sediments probably indicates oxidation of sulfide during and after the introduction of sediments into the reaction flask. The greater variability of recoveries from spiked sediments probably indicate interactions of added sulfide with metals or other components in sediment samples, as discussed above for analyses of metals in SEM extracts. Recovery of sulfide from spiked sediments was less variable in 1993 samples, due to improved controls on oxidation of sulfide during the extraction, (longer purge times, control of gas flow rates, and injection of deoxygenated HCl by gas-tight syringe).

Table 10. Quality assurance characteristics for analyses of copper and zinc by atomic absorption spectrophotometry (AAS). GFAAS = graphite furnace AAS, FAAS = flame AAS. Means with standard deviations in parentheses.

QA Sample:	Copper		Zinc
Matrix	GFAAS	FAAS	FAAS
Instrument Detection Limit (µg/L):			
	0.3	13	8
Blank Contamination (ug/L):			
Water	0.4		ND*
Midge	1.1		44
SEM		22	201
Method Detection Limit (µg/g dry wt.):			
Midge	0.3	13	8
SEM		0.8	0.5
Reference Materials or Matrix Spikes (Recovery, %):			
Water	103 (11)		103 (15)
Midge	80 (6)		105 (12)
SEM		70 (64)	19 (36)
Analytical Duplicates (Relative Percent Difference, %):			
Water	11 (13)		2.1 (1.5)
Midge	9.2 (8.7)		5.7 (3.5)
SEM		5.0 (3.7)	3.4 (5.1)

*ND = not detected.

Table 11. Quality assurance characteristics for analyses of acid-volatile sulfide (AVS) in sediments. Means with standard deviation in parentheses.

QA Sample	Colorimetric	Ion-selective Electrode
Matrix	(1992)	(1993)
Method Detection Limit (μmol/g dry wt.):		
Sediment	0.13	0.3
Precision (Relative Percent Difference, %):		
Sediment	28 (27)	12 (14)
Matrix Spike (Recovery, %):		
Blank	83 (10)	88 (5)
Sediment	73 (56)	71 (21)

APPENDIX B -- STANDARD OPERATING PROCEDURES

APPENDIX B -- STANDARD OPERATING PROCEDURES

Aquatic Toxicology Laboratory Standard Operating Procedure
Pesticide Research Center and Department of Fisheries and Wildlife
Michigan State University

Title: Methods for Laboratory Culture and Sediment Bioassays with Larvae of the Midge, *Chironomus tentans*

John M. Besser and Cornell A. Rosiu

Revision 3; October 31, 1994

Description of Method:

This procedure describes methods for laboratory culture of the midge *Chironomus tentans* and for bioassays for determining the toxicity of sediment-bound pollutants. *C. tentans* is maintained in batch cultures with a paper pulp substrate and fed with suspensions of commercially-prepared flake fish food. Cultures are started from egg masses laid on the same day by adults captured from the cultures by aspiration. The static bioassay requires a small volume of test sediment and few test animals, but produces statistically powerful measure of growth inhibition of midge larvae. The test is conducted without replacement of overlying water, a feature which maximizes the likelihood of detecting toxic components in the test sediment. Alternatively, *C. tentans* larvae can be used for sediment bioassays with a static-renewal test system, described in a separate SOP, which minimizes toxicity caused by deterioration of water quality (e.g. ammonia or pH).

Equipment and Supplies:

Water bath or incubator (to control temperature in the range 20-25 C)
Glass aquaria, 18 L (Krislin)
Fiberglass screen covers for aquaria, with loose flap for entry of airline
Polypropyene or glass carboys, 5-gallon
50-mL disposable polypropylene centrifuge tubes (Corning 25330)
Acetal test tube racks (Cole-Parmer), to hold 9 centrifuge tubes
Air pumps or compressed air supply
Air manifolds, with 5-gang valves (Penn-Plax)
Plastic air-line tubing
Plastic pipet tips (200 μL)

Stainless steel syringe needles

Materials:

Food.

These preparations last approximately 1 week without refrigeration. Monitor odor to detect the occurrence of fouling:

1. Culture food formula (100 mg/ml).

   Place 20.0 g Tetra-fin flake food in blender. Fill blender half-way with deionized, reverse-osmosis(RO) water ($\leq$200 mL). Blend jar contents. Pour into a 250 mL ehrlenmeyer flask and bring the fluid level to the 200 mL mark by adding the necessary deionized, RO water. Mix the flask prior to pipetting contents. Feed 400 mg per day to each aquarium. Temporarily reduce the amount fed if cultures develop cloudy water.

2. Bioassay food formula (60 mg/ml)

   Add 12.0 g Tetra-fin flake food to blender. Fill jar half-way with deionized, RO water ($\leq$200 ml). Blend jar contents. Pour into a 250 mL ehrlenmeyer flask and bring the fluid level to the 200 mL mark by adding the necessary deionized, RO water. Swirl mix the flask prior to pipetting contents. Add 6 mg per day to each assay tube.

Paper Pulp substratum

Place shredded strips of ordinary paper towels (brown or white) towel squares in a 1000 mL beaker and cover with deionized water. Boil the water for several minutes, occasionally stirring. Allow contents to cool, then pour off water. Rinse one or more times with clean deionized water. Gradually add portions of the towel mass to approx 500 mL deionized water in a commercial stainless steel blender (Waring) or a laboratory Omni-Mixer and blend until clumps are completely broken up. Continue adding towels until a thick pulp with even consistency pulp is obtained. Store the pulp in a wide-mouth polyethylene bottle and refrigerate for longer storage. The pulp will last for at least a month if refrigerated.

Water

1. Culture water.

   Use well-water that has been chemically characterized and determined safe to culture organisms. Well-water is conditioned at ambient room temperature for at least 24 hours (23 degrees C) by aeration in large, clean polypropylene

carboys before use. Conditioned well-water can then be added by syphon to the culture aquaria.

2. Assay water.

   Use reconstituted HPLC water (10% Perrier/90% HPLC water) in setting up test tube chambers at the start of an assay. Replace water lost during the course of an assay (due to aeration/evaporation) with HPLC water.

Culture Methods:

Collect midge adults daily from 18 liter culture aquaria using a 500 mL ehrlenmeyer flask and 200-250 mL of water in the flask. The flask is equipped with a two-hole rubber stopper equipped with two lengths of flexible plastic tubing. One piece of tubing is used as a mouthpiece for aspiration, and the other is inserted into the culture tank to collect adult midges. It is important to harvest adults daily so that egg masses are not deposited into the established cultures. After collection is complete, cover the flask with foil perforated to allow for ventilation and set it aside overnight. Fill an 18-liter aquarium about one-half full with culture water, stir in about 100 mL of paper pulp and aerate in preparation for addition of egg masses the next day. Next day, carefully pipette 3-6 egg masses from the collection flask and introduce them gently into the newly set-up aquarium, keeping the tip of the pipette underwater and assuring that the eggs come to rest on top of the layer of pulp. Label the aquarium with an ID letter designation, the present date and the number of masses seeded. Enter data on the new aquarium in the Life Cycle Log sheet. Monitor egg incubation over the next several days. After two days, just before the larvae hatch, begin feeding 400 mg of food per day. Cluster of white spots (burrows of newly hatched midges) should be visible near the egg masses. Continue moderate aeration.

Inspect the cultures aquaria daily and collect adults. Feeding rates should be adjusted according to the size and density of larvae in each aquarium. If a foul odor exists or the tank has not been cleaned for more than one week, carefully siphon half of the water into a waste bucket, and refill the tank with conditioned culture water. (Note: Inspect and clean aquaria before you feed). Replace screened covers on all aquaria, and make log entries. Condition more well-water (by aeration) as needed in preparation for use the next day.

Bioassay procedure:

Test chambers consist of individual 50 mL polypropylene centrifuge tubes, with a set of 15 making up one assay unit. Add 7.5 g of wet weight of sediment to each of the 15 tubes. Fill each tube with about 40 mL of assay water such that the level reaches the 50 mL mark.

Repeat for each assay set of 15, including your control. Screw the caps on tightly and invert each set of tubes 5 times, making sure that they mix uniformly. Place the tubes upright in three test tube racks in an 18-L aquarium and allow sediments to settle for 24 hrs. Remove the caps and punch a small hole (0.5 cm) in the center of each with a cork borer to allow addition of food and water. Set up a series of air manifolds (three per test sediment) and attach a short piece of tubing to each. Attach a 200 μL pipet tip to the end of each line with silicone sealant and allow to dry overnight. The following day, clip off the end of the pipet tips to allow slipping a stainless steel syringe needle over each pipet tip. Make a small perforation at the edge of each of the centrifuge tube caps with a separate needle and replace the caps on the tubes. Place the air manifolds along the sides of the aquarium and insert one air line into each tube. Aerate all the tubes for 24 hrs. at a flow rate of 3-4 bubbles per second. Do not aerate so vigorously as to evaporate more than 10 mL of assay water per day, which could cause the pipette tips to become exposed out of the water.

On the following day, if assays are properly synchronized with cultures, one aquarium should have larvae in their 2nd instar stage of development (12 d after egg deposition at 23 C). One second instar larva is placed in the bottom of each tube in the first rack in each aquarium, moving across the full set of assays to mix any influences of selection. The larvae should all be of the same general size, and appearance. After all larvae are in the tubes, feed 6 mg (0.1 ml) of the assay food to each tube and add enough deionized RO water to bring the level back up to the 50 mL mark. Make sure that each tube is bubbling at the correct flow rate (3-4 per second).

Fill out all the information about the test setup on the Bioassay Data Sheet. Prepare the Daily Record sheet to receive data over the next 10 days. Each day of the ten-day bioassay, feed each larva 6 mg of assay food and add deionized RO water to the 50 mL mark on all tubes. Make Daily Record Sheet entries. On the 10th day, label one aluminum weigh boat per test sediment with bioassay and sample identification. Empty the contents of each tube individually onto a benthos screen. Carefully probe the sediment and locate the larva. If after a complete inspection, no larva can be found, enter that individual as DEAD NOT FOUND. If you do find the larva enter it as either DEAD or ALIVE and deposit live individuals in a labeled weighing pan. Make sure that active larvae do not associate too closely with one another, so that after drying they cannot be physically separated from each other. Place the labeled aluminum weighing pans in a convection oven for 24 hours set at 80 C. The individual, dried larva are then weighed on an analytical balance to 0.01 mg weight. Weight data are entered on the blank lines on the Bioassay Data Sheet. Results expressed as percent reduction in weight relative to reference (control) sediment. Depending in study objectives, mortalities may be considered as 100% reduction in growth, although this practice must be clearly stated in any reports.

Aquatic Toxicology Laboratory Standard Operating Procedure
Pesticide Research Center and Department of Fisheries and Wildlife
Michigan State University

Title: Static/Renewal Exposure System for Sediment Bioassays.
Revision 2; December 8, 1994
Prepared by John M. Besser and Jody A. Kubitz

Scope:

This exposure system is suitable for whole-sediment toxicity tests using benthic invertebrates such as midge larvae (*Chironomus tentans* and *Chironomus riparius*), amphipods (*Hyalella azteca*), and oligochaetes (*Lumbriculus variegatus*). This document discusses only the operation of the exposure system. Culture and bioassay procedures for each species are described in separate SOPs.

Summary of Method:

This method is a non-automated adaptation of the water-renewal system described in the draft EPA methods sediment bioassay method (USEPA 1994).

This exposure system allows periodic replacement of the overlying water in bulk sediment toxicity tests. Individual head tanks deliver a volume of replacement water equal to the effective volume of small water baths (aquaria). These aquaria hold replicate exposure chambers containing one of the sediments to be tested. Each water bath is fitted with a siphoning drain mechanism that allows the water level in the bath to fluctuate up and down while water replacement is occurring. As the water level in the bath rises, the fresh overlying water enters the beakers through 250 μm screens, diluting and replacing the resident overlying water in each beaker. After the siphon drain is started, water drains from the bath, removing a portion of the contents of the aquarium, until the siphon is broken and the water level begins to rise again. The system is calibrated to deliver a volume of water equal to the total volume in the water bath over a period of about 4 hours.

Apparatus:

Exposure chambers. 300 mL high-form beakers, drilled with two holes (12-13 mm diameter, centered 8.5 cm above the bottom) Holes are covered with stainless steel screens secured with silicone sealant. Eight replicate exposure chambers are recommended for each sediment to be tested.

Exposure water baths. 10-gallon all-glass aquaria, with 15-20 mm diameter drain hole in one end, centered 8 cm above the bottom of the aquarium. The replacement volume of these exposure aquaria is approximately nine liters. One aquarium is required for each test sediment.

Head tanks. Polyethylene buckets, with 1-cm outlet at bottom of wall. Buckets should be marked at levels corresponding to 1 replacement volume. Outlet is fitted with a rubber stopper with a hole to accept a short piece of glass tubing. Outlet tube is fitted with flexible rubber or plastic hose equipped with an adjustable clamp to control flow rate. One head tank is needed for each exposure water bath.

Siphon drain apparatus. The siphon drain is mounted on a rubber stopper inserted in the drain hole of the exposure water bath. Each drain system is constructed from two pieces of narrow-diameter glass tubing (1/4 in outside diameter), bent into 90 degree elbows, and one piece of wider glass tubing (1/2" o.d.). The ends of the elbow tubes are cut to leave 20 mm lengths on each side of the elbow and the ends are fire-polished. A notch, 1 to 2 mm deep, is cut in one end of one elbow tube, using a fine triangular file or a cutting wheel. This elbow is inserted into the inside end of the stopper, with the notched end facing inward and upward. The other elbow tube is inserted on the outside of the stopper and the elbow is turned downward. The wide-diameter tube is cut to a length of 15 mm, the ends are fire-polished, and one end is plugged with a small cylindrical piece of rubber (e.g. part of a very small rubber stopper or a rubber core extracted from a stopper with a cork borer). Any rubber extending beyond the end of the tube is trimmed off with a razor blade. The wide-diameter tube now forms a "cap" which is placed on top of the inside elbow, and which facilitates the formation of a siphon as water drains out of the water bath. The top of the inner elbow tube should be 10 cm above the bottom of the water bath.

Aeration apparatus. Air pump, flow splitters/manifolds, and tubing with tips for aeration of exposure chambers (if necessary).

Preparation of Apparatus:

The water delivery apparatus should be washed and assembled several days before the test is to begin. Bioassay water of a quality known to be acceptable for the test organisms should be prepared at least 24 hours in advance in a head tank large enough to contain several days' supply. Recommended water types are:

(a) well water (or dechlorinated, charcoal-treated tap water) diluted to desired hardness with ultrapure water (deionized, organic-free water; e.g. from Barnstead E-pure

cartridge system); or

(b) moderately-hard reconstituted water prepared from ultrapure water with high-purity salts (EPA 1994).

Exposure water should be run through the system to check for leaks, malfunctioning siphons, etc. The water reservoirs are calibrated to contain one volume of the exposure water bath. The rate of water delivery should be adjusted to deliver the water volume over a period of approximately four hours.

Bioassay Procedure:

Day -2 (two days before test):

Prepare the apparatus and fill the water bath with bioassay water.

Day -1:

Prepare sediment samples, add test water, and place them into water baths to allow them to equilibrate to the test temperature. Place all beakers containing the same sediment into the same water bath.

Day 0:

(a) Measure the temperature and dissolved oxygen concentration in at least two randomly-selected chambers in each water bath. Remove 25 mL aliquants of the overlying water from each replicate and pool them to make a composite sample. Measure the alkalinity, pH, conductivity, hardness alkalinity and ammonia of the overlying water samples. Run one water replacement cycle.

(b) Place the recommended number of test organisms per test chamber (typically 10) in test water in 30-mL plastic souffle cups (Solo) and float one cup in each chamber. After allowing time for temperature equilibration, gently add the test organisms to each beaker, and feed according to bioassay SOP. Begin the second water replacement 12 hours after the first water replacement began.

Days 1-9:

Measure the temperature and dissolved oxygen concentration of at least two random replicates in each water bath. Start a water replacement cycle, and feed. Begin the second water replacement 12 hours later.

Day 10:

(a) Measure the temperature and dissolved oxygen concentration of at least two random replicates in each water bath. Remove 25 mL aliquants of the overlying water from each replicate and pool them to make a composite sample. Measure the alkalinity, pH, electrical conductivity, hardness, alkalinity, and ammonia of the overlying water

samples. Start a water replacement cycle.

(b) Remove and process the test organisms according to bioassay SOP. Criteria for test acceptability are discussed in the EPA methods (1994) and appropriate bioassay SOPs.

Trouble-shooting:

Siphon malfunction. The siphons may not engage to drain the water bath properly. The siphons must be checked at the start of each water replacement.

Clogging of screens. The beaker screens may become clogged, preventing the exchange of water in and out of the exposure chambers. Periodic cleaning of the screens prevents this problem. Screens should be gently scrubbed from the outside with a test tube brush.

Dissolved oxygen depletion. Dissolved oxygen may decrease below 40% saturation. When this occurs, the frequency of water replacements may be increased (up to four replacements per day), or test chambers can be gently aerated by air lines attached to glass or plastic pipet tips.

Aquatic Toxicology Laboratory Standard Operating Procedure
Pesticide Research Center and Department of Fisheries and Wildlife
Michigan State University

Title: Preparation of Samples of Benthic Invertebrates for Analysis of Metals
Revision 1; February 22, 1994
Prepared by John M. Besser

Summary of Method:

Macroinvertebrate samples from bioassays or field collections are dried at low temperatures, and digested with a sub-boiling nitric acid/hydrogen peroxide procedure. Digestates are diluted to a known volume, and diluted samples are analyzed for metals using atomic absorption spectrophotometry, following guidelines in the appropriate SOP. Results are expressed as micrograms of metal per gram of biomass, on a dry weight basis.

Materials and Equipment:

Polystyrene sample cups, 30 mL (e.g. Solo P-100)
Clean, acid-washed silica sand (sieved to remove fine particles)
Suspension of flake-type fish food (Tetra-Min or Tetra-Fin)
Aluminum weigh boats
Electronic balance capable of weighing to 0.01 mg
Polyethylene microcentrifuge tubes (approx 1.5 mL)
Teflon (PTFE) 15-mL centrifuge tubes, conical
Polyethylene caps for centrifuge tubes, friction-fit
Polypropylene vials, 15 mL with tight-fitting caps
(Corning Snap-Top or equivalent)
Dry-block heating apparatus (Thermolyne) with aluminum digestion blocks
Thermometer reading to >120 C
Adjustable pipettor (0-1000 μL) and disposable pipet tips
Atomic absorption spectrophotometer with flame and graphite furnace atomizers, and Zeeman background correction (Hitachi model 180-80)
Certified Standard Reference Materials for metals in animal tissue (e.g., National Institute for Standards and Technology SRM 1577a, bovine liver)

Reagents:

Clean culture water: diluted well water, diluted mineral water (e.g. 20% Perrier) or reconstituted water

Ultrapure deionized water, Type I reagent grade (Barnstead E-Pure, Nanopure or equivalent)

Wash acid, 10% HCl or 5% HNO3

Ultrapure nitric acid, approx. 70% (Baker Instra-analyzed or equivalent)

Ultrapure hydrogen peroxide, 30% (Baker Instra-analyzed or equivalent)

Procedure:

Cleaning procedure

Care must be made taken to assure that materials and reagents used in this procedure are free of trace metals. Use of glassware should be avoided, as it has exchange sites which may remove metals from solution or serve as a source of metal contamination. Stainless steel implements may be used to manipulate invertebrate samples, but must not be used with acidic solutions. Aluminum weigh boats may be used to dry midge samples, but heat-resistant Teflon or polypropylene materials are preferable. Plasticware should be used whenever possible, with the preference for use being Teflon (best), followed by polymethylpentene (PMP), polypropylene (PP), high-density polyethylene (HDPE), low-density polyethylene (PE) and polystyrene (PS). This ranking is based on metal-binding affinity, durability, and resistance to heat and chemical attack.

Plasticware should be cleaned according to routine laboratory glassware cleaning procedures, followed by an overnight soak in 10% HCl. A separate small acid bath should be prepared which is used only for trace metal analysis, and the acid solution should be replaced regularly to avoid accumulation of metal contaminants. New materials such as sample vials and pipet tips, and autosampler cuvettes can be acid-rinsed just prior to use, if necessary. Acid-washed materials should be rinsed several times with ultrapure water before use. Acid-washed and dried materials should be promptly stored in clean plastic bags to prevent contamination.

Sample preparation

Invertebrate samples can be collected from sediment bioassays or from field collections. It is desirable to hold sediment-dwelling organisms in clean water and substrate to allow clearance of gut contents, which may contain substantial amounts of mineral sediment.

[For *Chironomus tentans*, it has been shown that 12 hours is sufficient to clear an acceptable fraction of gut contents (D. Call, University of Wisconsin-Superior, personal communication). This can be accomplished by transferring living organisms from the

bioassay sediment or from a sieved benthos sample into 30-mL sample cups with culture water and a thin layer of sand. A small amount of food suspension (e.g. 0.5-1.0 mg Tetra-Min for up to 10 *C. tentans* larvae) is added and the larvae are allowed to feed and clear their guts for 12 hours.]

After gut clearance, invertebrates are transferred to weighing boats and dried in a gravity convection oven at 60 C for 24 hours. Dried samples are placed in a desiccator to cool, then weighed at 0.01 mg resolution. It is important that the balances be located on a stable platform in a location free of drafts and vibrations which may affect accuracy. Dried and weighed samples can be stored in acid-washed microcentrifuge tubes. Samples should be labeled with adhesive label tape with the following information: study ID, sample ID, replicate number, and date collected. This information should also be recorded on a data sheet, along with taxonomic information, number of organisms in each sample, and sample dry weight.

Sample digestion

The heating block is turned on and set to a temperature of 90 C $\pm$ 5 C. The temperature setting should be determined beforehand to prevent boiling of samples. A digestion tube, which is partially filled with water and has a thermometer inserted though the cap, is used to monitor temperatures in the heating block. The heating block should be located in a well-ventilated area, but not in a fume hood, due to the cooling effects of continuous air flow.

Dried invertebrate samples are transferred to clean Teflon digestion tubes, 1.5 mL nitric acid (70%) is added and tubes are capped loosely. Sample labels are transferred from the storage vials to the digestion tubes and the tubes are placed in the wells of the digestion block. Once the proper temperature range is achieved, the samples are allowed to reflux (sub-boiling temperatures) for 24 hours. After 24 hours, the samples are cooled to room temperature, by turning off the heating block or removing samples to cool more rapidly in a test tube rack). Once cooled, the caps are removed carefully, to avoid losing any digestate condensed on the inside the caps, and 1.0 mL of hydrogen peroxide solution is added. The samples are heated **slowly** to 90 $\pm$ 5 C for an additional 24 hours.

[Caution must be taken to avoid adding hydrogen peroxide to a hot sample or to a samples containing a large amount of organic matter, to avoid loss of sample due to vigorous evolution of gas . It is best to raise the temperature to a lower temperature, 60-70 C, for an hour or two before it is raised to the reflux temperature.]

Extract preparation

Digestion tubes are placed in a test tube rack. The cap of the sample to be diluted is removed carefully and the inside of the cap is rinsed with about 1 mL of ultrapure water from a squirt bottle and the rinse is added to the tube contents. The contents of the digestion tube are mixed several times with the 1000-μL pipet and the sample is pipetted into the sample tube, drawing from the bottom of the tube to assure transfer of any remaining particulates. Two or more rinses of 1-2 mL water are added and transferred to the sample vial, and the sample is brought up to the final volume of 10 mL, using graduations on the tube. A new pipet tip should be used for each sample. Alternately, the volume of the sample can be determined gravimetrically in a tared sample tube. The net weight of the diluted sample should 10.6 g (which is adjusted for the density of 10% HNO3).

Metal analysis

Invertebrate samples are analyzed by atomic absorption spectrophotometry (AAS), following the SOP, "Analysis of Metals by Atomic Absorption Spectrophotometry." The small sample biomass typical of invertebrate samples usually requires the use of graphite furnace atomization for elements such as Cu, which is much more sensitive than the flame technique. Analysis of certain elements such as mercury, selenium, and arsenic typically requires specialized procedures such as cold-vapor trapping (Hg), hydride generation (Se) or arsine generation (As). The Zeeman background correction should eliminate the need for time-consuming procedures such as standard additions or use of matrix modifiers.

Analysis by inductively-coupled plasma emission spectroscopy (ICPES) may allow rapid analysis of multiple elements in invertebrate samples. This type of analysis is available on campus at a reasonable cost (at the Toxicology Analytical Laboratory in the MSU Veterinary Medical Research Center), but the relatively high detection limits of this technique generally makes it inappropriate for dilute samples.

Quality Assurance:

Procedural blanks

One or more blanks must be carried through the digestion procedure with each batch of samples. Blank digestion tubes are randomly inserted into the batch of samples and receive the same digestion reagents and rinses as other samples. Substantial contamination of extraction blanks should be subtracted from metal concentrations in the corresponding batch of samples. A control chart which plots metal concentrations in blanks over time can be used to identify problems with laboratory contamination (e.g., associated with change in technician, reagents, or materials).

Reference materials

Certified standard reference materials (SRMs) are used to verify the quantitiative recovery of metals from biological tissues. One SRM sample is normally analyzed with each batch of samples. Appropriate SRMs available from the National Institute for Standards and Technology (NIST) for this procedure include bovine liver (1577a), oyster tissue, and lobster tissue. The choice of SRM should be made based on their concentrations of the element(s) of interest, which should be similar to the expected range in the samples to be analyzed.

Aquatic Toxicology Laboratory Standard Operating Procedure
Pesticide Research Center and Department of Fisheries and Wildlife
Michigan State University

Title: Determination of Acid-volatile Sulfide and Simultaneously-extracted Metals in Sediment

Revision 1; December 8, 1994

Prepared by John M. Besser

Summary of Method:

Acid-volatile sulfides (AVS) and simultaneously-extracted metals (SEM) are determined in sediments by digestion of sample in 2N HCl under an oxygen-free atmosphere. Under acid conditions, AVS is solubilized as H2S, which is purged in a stream of nitrogen into an alkaline trapping solution. After AVS is removed, the acid sediment suspension is filtered to produce an extract containing SEM. Sulfide in the trapping solution is analyzed by ion-selective electrode. Metals in SEM extract are analyzed by atomic absorption spectrophotometry (AAS) or inductively-coupled plasma emission spectroscopy (ICPES).

Equipment and Materials:

Fume hood
250-mL glass extraction flasks with septum inlet (Ace Glass 6934)
Rubber septa
Vacuum take-off adapter (Ace 5193)
Nitrogen gas cylinder and regulator with output of 10 psi
Oxygen traps with 1/4" fittings (large-capacity and indicating)
Gas flow meter(s) adjustable within range of 0-100 mL/min
Polypropylene disposable syringe, 20-50 mL, with luer connector
Stainless steel syringe needles (22 ga., 1")
1/4" pipe thread to 1/4" hose barb connectors
7-mm o.d. glass tubing
1/4" i.d. Tygon and Teflon tubing
Teflon stopcock, three-way
7-mm diameter coarse fritted gas dispersion tubes
200-mL glass hydrometer cylinders (or graduated cylinders) marked at 100 mL
Two-hole rubber stoppers
Magnetic stir plate(s) and stir bars
Silver/sulfide specific ion electrode (Orion)

Double-junction reference electrode (Orion) and filling solutions

pH/ion-selective electrode meter (Orion 811/901 or equivalent)

Ring stands or laboratory support rack and clamps

Titration buret and stand

Filtration apparatus

Reagents:

Deionized water (DW), from cartridge purification system (Barnstead E-pure).

Deoxygenated deionized water (DDW), purged with nitrogen for 1 hour or more.

Sulfide antioxidant buffer (SAOB), a 2N solution of NaOH (80 g/L), disodium EDTA dihydrate (74.4g/L), and ascorbic acid (35.2 g/L). Dissolve NaOH first and chill under cold tap water before addition of other reagents and filling to volume.

2N HCl, high-purity for metal analysis (Baker Instra-Analyzed), deoxygenated.

Sulfide stock solution, ~0.1M. Dissolve 2.4g $Na_2S.9H_2O$ in 100 mL DDW and store in airtight amber or foil-covered bottle. Prepare weekly and standardize daily.

$Cd(NO_3)_2$. 0.100M.

Apparatus Setup:

Assembled digestion/trapping apparatus is shown in Allen et al. (1992). Tubing between oxygen traps and reaction flasks is tygon. Tubing connection at inlet of reaction flask is made with an insert of Teflon tubing to allow easy disassembly. Tubing between reaction and trapping flask is glass with tygon outer sleeve to allow disassembly. Dispersion tubes in reaction and trapping flasks are connected to glass tubing with outer sleeves of Teflon tubing.

Procedure:

Standardization of sulfide stock (daily)

Connect sulfide electrode reference electrode to meter and set meter to REL MV mode. Place electrodes in sample containing 50 mL SAOB, 40 mL DDW, and 10.0 mL sulfide stock. Titrate with 0.100M Cd(NO3)2 with constant magnetic stirring, recording changes in voltage during titration. (Place thin sheet of styrofoam between sample and stir plate to avoid heat transfer which may affect electrode response.) Determine inflection point of titration curve graphically and calculate concentration of sulfide stock. After titration, clean sulfide deposits from surface of sulfide electrode by soaking in 1% HNO3 and polishing according to manufacturer's instructions.

Calibration of meter

Prepare low sulfide standard by adding 0.10 mL sulfide stock to 100 mL of 1 + 1 SAOB (50 mL SAOB + 50 mL DDW). Set meter on CONCN mode and adjust STD VALUE thumbwheels to indicate total amount of sulfide in the standard: e.g. 10.0 μmoles if stock concentration is 0.10M. Set slope thumbwheels to -29.00. Place electrodes in standard with gentle stirring, push CLEAR/READ MV button, wait 10-20 minutes for a stable reading (approx. -750 to -800 mV), and push SET CONCN button. Prepare a high standard (approx. 100 μmole) by adding 1.0 mL of sulfide stock to 50 mL SAOB and 49 mL DDW, rinse the electrodes and place them in the standard, and wait for the reading to stabilize (5-10 minutes). Adjust the slope thumbwheels to produce the correct reading in μmoles. A slope of -24 to -30 indicates improper calibration or aberrant electrode behavior. Refer to troubleshooting suggestions in electrode manual.

Sample preparation

Samples of sediment should be refrigerated at 4 C before analysis. Analysis within two weeks of sample collection is recommended To minimize errors due to AVS oxidation during prolonged storage, store samples in gas-impermeable containers, use containers with a minimum of headspace above sample (or purge headspace with nitrogen before sealing), and do not remove subsamples until just before analysis.

Collect replicate subsamples of homogenized sediment for AVS analysis and moisture content determination. Place one or more samples of sediments for moisture determination (approx. 5 g) on preweighed foil weigh boats and record wet weight. Determine dry weight and moisture content after drying for 24 hours at 100 C. Samples for AVS analysis are weighed on tared 30-mL polystyrene sample cups, covered with Parafilm and refrigerated until analysis. Samples for AVS analysis should weigh between 2 g and 10 g wet weight: AVS oxidation can become significant on smaller samples and larger samples (especially those with high carbonate content) may affect on the acidity of the sediment suspension.

Sample digestion and sulfide analysis

Purge reagents (HCL and DDW) and apparatus for 1 hour before starting first digestion. To prepare for sample addition, add 90 mL DDW to reaction flask, add 80 mL 1 + 1 SAOB to trapping flask, and purge the system with nitrogen at 100 mL/min for 10 minutes. During this period, open the stopcock to purge oxygen from the septum port, then insert the septum into the port.

Rinse the sediment sample into the reaction flask with a minimum of DDW. Insert a plastic powder funnel into the neck of the flask to prevent sediment from interfering with the

ground glass seal. After adding the sample, reseal the flask and continue purging the system for 10 more minutes.

Add deoxygenated HCl to the reaction flask with a polypropylene and rubber syringe. Withdraw about 25 mL of deoxygenated HCl into the syringe through a short length of Teflon tubing, expel any gas bubbles from the syringe, and replace the tubing with a stainless steel syringe needle. Reduce the gas flow rate to approx 25 mL/minute. After expelling any gas bubbles and a small volume of acid through the needle, insert the syringe through the septum and inject 20 mL of acid into the sediment suspension. After the acid has been added, close the stopcock, increase gas flow to 100 mL/min, and start magnetic stirring fast enough to keep the sediment in suspension. Continue the digestion for 30 minutes.

After digestion, disconnect the glass tubing from the outlet of the digestion flask and use a squirt bottle of DW to rinse the inside of the tubing into the trapping flask. Bring the volume in the trapping flask to 100 mL. If the sample is not being analyzed immediately, transfer it from the trapping flask to a polypropylene sample bottle. Transfer the sample to a small beaker for analysis, place the beaker on a stir plate with continuous slow stirring, and insert the sulfide selective electrode. After 5 minutes, read the sulfide content of the sample directly from the meter.

SEM extract preparation and analysis

Rinse the sediment suspension from the digestion flask into a graduated cylinder and record the total volume. Mix the extract thoroughly, allow large particulates to settle, and decant at least 50 mL for filtration. Filter this portion through a glass-fiber filter (1.0 μm, Whatman GF/C) and store in a polyethylene or polypropylene bottle. This sample can be analyzed by ICPES by AAS.

Cleaning of Glassware

Apparatus downstream from the inlet to the reaction should be disassembled and cleaned after each sample. Glassware and tubing should be rinsed with dilute HCl (5-10%) and thoroughly rinsed with DW. Tubing and dispersion tubes should be cleared with air from an empty squirt bottle before reassembly

Quality Assurance:

Calibration

Calibration of the meter should be checked every 2 hours during analyses. Calibration can be checked using the original standard, although preparation of a new standards is recommended after four hours.

Blanks

Procedural blanks (full procedure without sample addition) are run to detect contamination with sulfide, metals, or both, from apparatus or reagents. Blanks should be analyzed to detect possible contamination from any new batches of reagents, and enough blanks should be included with every batch of samples to quantify the level of contamination in the laboratory environment.

Spikes

Spikes are used to determine recoveries of known amounts of sulfide and metals added to the digestion flask. *Spiked blanks*, additions of sulfide to the digestion flask without sediment present, are used to indicate losses of sulfide due to oxidation, leakage, or retention on the apparatus. Spiked blanks covering an appropriate range of sulfide concentrations should be run before each study to ensure that the apparatus and experimental conditions are working correctly. Recoveries of spiked blanks should average greater than 90% over the working range of the samples to be analyzed.

Spiked samples measure the recovery of sulfide and/or metals added to the digestion flask with sediment present. Sulfide-spiked samples indicate reduced recovery attributable to interactions with sediment components as well as the same types of losses affecting spiked blanks. Similarly, metal-spiked sediments indicate reduced recovery of added the metals due to physical and chemical interactions with dissolved and suspended components in the digestion flask and losses to digestion or filtration apparatus. A sufficient number of sulfide- and metal-spiked samples should be run during each study to document the effectiveness of the AVS/SEM digestion across the range of sample characteristics that are encountered (e.g. particle-size distribution, organic content, AVS concentrations, metal concentrations).

Aquatic Toxicology Laboratory Standard Operating Procedure
Pesticide Research Center and Department of Fisheries and Wildlife
Michigan State University

Title: Analysis of Metals by Atomic Absorption Spectrophotometry

John M. Besser and Richard A. Othoudt

Revision 1; December 8, 1994

Scope:

This SOP covers analysis of heavy metals in aqueous samples by atomic absorption spectrophotometry (AAS). It includes general methods for AAS with atomization by air-acetylene flame or graphite furnace. Methods for preparation of extracts of solid samples, such as blood, tissues of fish or invertebrates, or sediment are described in other SOPS. Specialized sample preparation techniques such as cold-vapor or hydride-generation are not covered.

Summary of Method:

This procedure describes methods for the quantification of metals in standard solutions, reference materials, and unknown samples. Samples are atomized in an air-acetylene flame or in an electrothermal graphite furnace, absorbance of a specific wavelength of light by metal atoms is measured and concentrations of metals are determined by linear regression of absorbance vs. standard concentrations, performed with spreadsheet software on a microcomputer. Methods are described for sample preparation, preparation of the Hitachi 180-80 AAS, calibration and analysis, and quality assurance.

Interferences:

The use of the Zeeman background correction removes most interferences from background absorption by non-target elements in sample matrices. Evidence of background

Apparatus:

Hitachi model 180-80 atomic absorption spectrophotometer, with air-acetylene burner, electrothermal graphite furnace, automatic temperature controller, autosampler, and data processing unit.

Hollow cathode lamp for elements of interest

Cooling water supply and drain

Ventilation hood

Air compressor and air filter and desiccator cartridges

Acetylene tank and regulator

Argon tank and regulator

Reagents:

Certified standard for atomic absorption spectroscopy, 1000 ppm

Certified Standard Reference Solution (e.g. NIST Standard Reference Materials 1643b or 1643c, Trace Elements in Water)

Standard Reference Material for tissue or sediment (e.g. NIST 1577a, Bovine Liver, or NIST 1645 River Sediment), as appropriate

Type II deionized water (Barnstead E-Pure or Nano-Pure or equivalent)

Ultrapure HNO_3 HCl (Baker Instra-analyzed)

Materials and Replacement Parts:

Hitachi pyrolytically-coated graphite cuvette (part #180-7444).

Replacement rings for graphite furnace (part #180-7401)

Ink ribbons and paper rolls for printed output

Replacement nozzle assembly for autosampler (part #170-6322)

Cleaning wires for flame aspirator

Sample cuvettes, 0.5 mL volume (Baxter or Fisher)

Adjustable pipettes, 10-100 μL, 100-1000 μL, and 500-5000 μL and tips

Disposaple polypropylene pipette tips

PMP Volumetric flasks

Latex or vinyl gloves

Kimwipes

Personal computer and spreadsheet software

Hazards and Precautions:

Care should be taken to avoid the ingestion of inhalation of toxic metals. Be sure that the vent hood above the A.A. is on when analyzing samples or standards. Gloves, safety glasses, and lab coat should be worn whenever working with acid samples or reagents.

Sample Preparation:

Plasticware should be washed according to normal laboratory procedures, soaked overnight in an acid bath of 10% HCl or 5% HNO_3 (or rinsed several times with wash acid), and rinsed thoroughly with type II water. Pipette tips should be rinsed with wash acid and type II water before use. Autosampler cups and pipette tips used to dispense samples for analysis

should be rinsed with wash acid and type II water. Autosampler cups should also be rinsed once with 0.5 mL sample before final addition of sample.

Standard solutions are prepared in PMP volumetric flasks by serial dilution from certified standards. Dilutions of volumes less than 0.5 mL should be avoided. Samples should be stored in acid-washed polypropylene or high-density polyethylene containers. All samples and standards must contain nitric acid. 1% HNO_3 on a volume basis is recommended: e.g, 1 mL concentrated HNO_3 per 100 mL. It is convenient to add HNO_3 **after** dilution of standards to the desired volume; this allows addition of 1% HNO_3 to samples prior to analysis without requiring adjustment of analytical results.

Preparation of Apparatus:

Consult a reference (e.g. EPA 1986) before planning your analyses and read through the operating manual for the Hitachi 180-80 and familiarize yourself with the instrument before you start working on the A.A. Default instrument parameters for determination of the metals of interest are stored in the CMOS memory of the 180-80 data station. Select the element of interest and the method of atomization. The 'Analysis' display screen will give you necessary information on such parameters as lamp current and wavelength, sample volume and gas flow rates. When the appropriate hollow cathode lamp is installed, turn on the instrument and select the correct lamp current. The lamp should be warmed up for 30 minutes before attempting any analyses. Select the 'Measurement' display to monitor the lamp output. Select the lamp wavelength by adjusting the coarse and fine wavelength adjustment knobs to obtain peak lamp output and adjust the lamp output with the gain dial to a reading of 100. The lamp output will vary somewhat until the lamp is warmed up. Select the appropriate flow path for cooling water, depending on whether flame or furnace atomization will be used, and turn on the cooling water flow. An audible click indicates that flow is adequate; do not turn the flow up much beyond this flow rate. Turn on the exhaust hood.

<u>AAS with Flame Atomization</u>

Clean the burner slot according to the instructions (moistened KimWipes work well). If the burner is encrusted with oxides and carbonates, a dilute solution of wash acid helps dissolve them; if encrustation in severe, a razor blade can be used to scrape them off, although the magnets make this difficult (by the way, this is a good time to take off your watch). Turn on the air compressor and adjust the air flow to the burner with the oxidant regulator dial. Make sure the gas flow switch and the gas flow cock are turned to off and the gas regulator dial is completely closed (counterclockwise), then open the main valve on the acetylene tank (adjust the main regulator to the required pressure if necessary). Turn on the gas flow switch (not the

flow cock) and adjust the gas regulator on the 180-80 to the specified pressure. When the gas and oxidant settings are correct, turn on the flame sensor switch, open the gas flow cock, and push the ignite button to light the burner. Hold this button down for several seconds, then release it and check to see if the flame remains lit; it may take several tries. If the flame will not stay lit after several tries, check the gas flow rate. It may be necessary to turn the gas flow up **slightly** to get the flame to light, then turn it down slowly to the specified setting. Aspirate a dilute (1-5%) solution of clean HNO_3 in type II water into the flame for 5-10 minutes to clean the burner. Adjust the lamp output to 100 again after the flame is lit.

Graphite Furnace Atomization

The furnace should be cleaned at regular intervals; daily cleaning is ideal. Make sure that the water and power are off before you start cleaning. Remove the cuvette, the light sensor; the fiber optics tubing on the right hand side of the furnace and the electrodes. Use ethanol and a KimWipe to clean the graphite rings, discard the KimWipe, and continue cleaning the remainder of the furnace and magnets. Check to make sure that the quartz windows are also clean. If the windows need to be cleaned take care not to scratch them. Reassemble the furnace and put in cuvette.

When the furnace is ready, open the main valve on the argon cylinder, then turn on the power supply for the furnace. An annoying beep at this point probably means that flow of cooling water or carrier gas are inadequate. Select the 'GA' screen, then select 'dry' and push the check button to start the dry cycle, and adjust the argon flow if necessary. When the dry cycle is finished (30 sec), push 'boost' to heat the cuvette to its peak temperature. The cuvette should be burned several times to atomize any dust or contamination; a new cuvette will need more burns to stabilize the cuvette surface. If the cuvette has been changed or moved the auto sampler tip must be readjusted. This takes practice to get it right and it is very important that it be adjusted properly or the coefficient of variance will be above 10%, which is out of the accepted range. The tip also needs to be cut every 300-400 injections. The instruction manual gives a detailed procedure for doing this. Before each session the auto sampler tip should be rinsed with type II water, and wash acid to remove any contamination. Turn on the autosampler and wait for it to go through its rinse cycle.

Procedure:

Flame AAS

Select the desired measurement conditions. Readings of absorbance with '5-second integration' and duplicate measurements per sample are recommended for flame AAS. [If small sample volume is a problem, rapid 'Direct' readout of absorbance can be used combined with

more replicate measurements per sample (e.g. 4 or 5) .] Although the instrument will calculate sample concentrations directly based on a series of three standards and an blank, it is generally preferable to select output of absorbance readings, for both standards and samples, and calculate sample concentrations based on linear regressions of standard curves on a PC with spreadsheet software such as Lotus or Excel.

When you are ready to make readings, aspirate an acid blank (1% HNO_3) for several seconds, then push the 'zero' button on the data station. Repeat the analysis of the blank several times to assure a stable baseline, then proceed with your standards and samples. Analyze samples by aspirating the sample into the flame and pushing the 'start/stop' button on the data station. The absorbance of the sample will be displayed and printed after each reading. After you have made the selected number of readings (usually 2), the mean and standard deviation of the absorbance will be printed out Follow the instruction the manual for extinguishing the flame and shutting down the instrument.

Furnace AAS:

Select the desired measurement conditions: redout of absorbance is recommended, with two replicates per sample. Make sure that the same number of replicates are selected in the data station display and on the autosampler control box. Set up several cuvettes with acid blanks, then push 'start/stop' on the autosampler to start the sample queue. The autosampler performs the selected number of injections for each sample; a typical graphite furnace cycle will take between one and two minutes per injection. Continue running blanks until stable, low absorbance readings are produced (<0.0050 is as good target), then set up a queue of standards and samples. The 'start/stop' button allows you to start or stop the autosampler as needed. Shut down the instrument by turning off the power supply, autosampler, gas and water flow, turn down the lamp current and torn off main power switch.

Quality Assurance:

A calibration check (standard or reference solution) should be run after about every five samples to assure the stability of the calibration curve. If the check sample differs by more than 10% from its nominal or previously-determined concentration range, it should be re-analyzed along with a second check sample; if both of these samples are not within 10% of their nominal value, a new calibration curve should be established and all samples analyzed since the previous check sample should be re-analyzed. Periodic changes in calibration are normal during furnace AAS, due to the continuously changing environment in the cuvette.

The precision of replicate measurements, reported as coefficient of variation (CV, %) is reported for each sample and should be monitored continuously to assure the reliability of

each analysis. A good objective for precision of the analytical replicates is a CV of less than 10%. Samples which do not meet this objective should be re-analyzed. Poor precision of duplicates may indicate deterioration of the graphite cuvette. The performance of the cuvette changes rapidly after about 150 burns, resulting in poor precision, and the cuvette should be replaced at this time.

Trouble-shooting:

The instrument manual has a table of common problems and their likely causes and solutions. Document all problems, as well as routine information on calibration and lamp usage, in the instrument logbook for future reference. The following are common problems encountered in our laboratory:

Fogging of the quartz windows on the graphite furnace. Results in erratic and unpredictable absorbance readings and poor calibration. This has occurred during humid summer months, due to excessive cooling of the furnace assembly (usually due to cooling water flow through the furnace assembly during analyses by flame AAS). The cooling water flow should be directed to either the burner or the furnace, not both.

Inadequate flow of cooling water through the burner head. Results in erratic absorbance due to variable flame temperature. This is less likely now that cooling lines are separated. Monitor flow at the drain and check for clogging and/or leaks in the tubing around the burner.

Clogging of the aspirator. Causes decreases in absorbance during flame AAS, which may be mistaken for changes in lamp output or flame temperature. Samples which contain particulates or high concentration of minerals may clog the aspirator feeding sample to the flame. Clean the aspirator regularly with the special cleaning wires, available from Hitachi.

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