Accumulation, Distribution, and Toxicity of Copper in Sediments of Catfish Ponds Receiving Periodic Copper Sulfate Applications

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ABSTRACT

Copper sulfate ($CuSO_4$) is applied periodically to commercial channel catfish (*Ictalurus punctatus*) ponds as an algicide or parasiticide. Current understanding of the chemistry of copper in soil–water systems suggests that copper may accumulate in pond sediments, although the forms and potential bioavailability of copper in catfish pond sediments are not known. This study investigated the accumulation and distribution of copper in the sediment of catfish ponds receiving periodic additions of $CuSO_4 \cdot 5H_2O$. All ponds were constructed in Sharkey (very-fine, smectitic, thermic Chromic Epiaquert) soil. Nine 0.40-ha ponds received 59 applications of 2.27 kg $CuSO_4 \cdot 5H_2O$ per application per pond over 3 yr; no $CuSO_4 \cdot 5H_2O$ applications were made to nine additional ponds. Total Cu concentration in the sediments of $CuSO_4 \cdot 5H_2O$-amended catfish ponds (172.5 mg kg^{-1}) was four to five times higher than that in the sediments of nonamended ponds (36.1 mg kg^{-1}). Copper accumulated in catfish pond sediments at a rate of 41 μg kg^{-1} dry sediment for each 1 kg ha^{-1} of $CuSO_4 \cdot 5H_2O$ applied to ponds. Copper in the sediments of amended ponds was mainly in the organic matter–bound (30.7%), carbonate-bound (31.8%), and amorphous iron oxide–bound (22.1%) fractions with a considerable fraction (3.4%; 3 to 8 mg kg^{-1}) in soluble and exchangeable fractions. This indicates that Cu accumulates differentially in various fractions, with proportionally greater initial accumulation in potentially bioavailable forms. However, toxicity bioassays with amphipods (*Hyallela azteca*) and common cattail (*Typha latifolia* L.) indicated that the effect of exposure to amended or nonamended pond sediments was not different.

Channel catfish culture in earthen ponds is the most important aquaculture industry in the USA, accounting for more than 50% of the production of all species. In 1999, catfish were cultured in more than 76 600 ha of ponds representing more than 1200 operations (USDA, 1999). About 271 million kg of catfish were processed in 1999, representing total farm-gate sales exceeding \$488 million (USDA, 1999). One of the most serious operational constraints facing channel catfish producers is the potential harvest delay caused by the presence of so-called off-flavors in market-size fish (Tucker, 2000). Some of the odorous compounds that cause off-flavors are produced by certain species of cyanobacteria (blue-green algae) in ponds. Additional costs of production are incurred from the additional feed required to maintain harvest weight and potential revenue is lost from fish mortalities due to diseases that may occur during the holding period until the fish are acceptable for marketing. Estimates of costs of off-flavor to producers range from \$0.04 to \$0.24 kg^{-1} of production (Engle et al., 1995). Off-flavor represents a serious economic impediment to further expansion of this economically important sector.

If the organism responsible for blue-green algae related off-flavor is identified in ponds containing fish near market size, one strategy to minimize the probability of occurrence of off-flavor is to apply an algicide to pond water. Currently, copper sulfate ($CuSO_4$) is the sole USEPA-approved chemical for use in catfish ponds as an algicide. Channel catfish producers apply $CuSO_4$ to ponds as needed throughout typical continuous production periods of 10 to 20 yr prior to pond draining and renovation.

Effective algicidal treatment requires $CuSO_4 \cdot 5H_2O$ application to ponds at 1% of the alkalinity (expressed as mg L^{-1} $CaCO_3$) to obtain free Cu concentrations ranging from 0.25 to 3.0 mg L^{-1} (Gratzek, 1983). However, complete death of the phytoplankton bloom in a fish pond is undesirable as phytoplankton plays a critical role in maintaining suitable water quality for fish production. Regular, low-rate applications of $CuSO_4$ may achieve a more desirable, partial reduction of a phytoplankton bloom (Tucker and van der Ploeg, 1999).

The long-term effects of periodic $CuSO_4 \cdot 5H_2O$ applications to catfish ponds are not known. As a first step, understanding the fractionation of Cu among solid-phase components can provide important fundamental information to assess the potential bioavailability and toxicity of Cu. Results of this study will provide a quantitative assessment of the effects of long-term, low-rate applications of $CuSO_4 \cdot 5H_2O$. The purpose of this study was to investigate the accumulation, distribution, and potential bioavailability of Cu in the sediments of catfish ponds receiving weekly, low-rate applications of $CuSO_4 \cdot 5H_2O$ during the summer growing seasons over 3 yr.

MATERIALS AND METHODS

Ponds and Sampling Protocol

Sediments from eighteen 0.40-ha earthen ponds located at the Thad Cochran National Warmwater Aquaculture Center, Stoneville, Mississippi, were sampled in this study. Ponds were constructed in Sharkey soils. Ponds were stocked with channel catfish at 10 000 ha^{-1} and cultured according to standard, commercial production practices. Nine ponds received 59 applications of 2.27 kg $CuSO_4 \cdot 5H_2O$ per application per pond during the summer growing seasons (May–September) of 3 yr (total = 85.2 kg Cu ha^{-1}); no $CuSO_4 \cdot 5H_2O$ applications were made to nine additional ponds. Crystals of $CuSO_4 \cdot 5H_2O$ were placed in a doubled burlap bag and suspended in the pond about 6 m in front of a paddlewheel aerator. Aerators were operated for

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Abbreviations: AmoFe, amorphous iron oxides; CARB, carbonate; CryFe, crystalline iron oxides; ERO, easily reducible oxides; EXC, exchangeable; OM, organic matter; RES, residual.

approximately 2 h until all crystals were dissolved. Water currents generated by the aerator distributed the dissolved chemical throughout the pond.

Pond sediments were sampled following fish harvest and pond draining. The sampling rate necessary to obtain a representative sample from aquaculture pond sediments was based on recommendations by Ritvo et al. (1998). A composite (37 ha^{-1}) sediment sample was collected using a uniform sampling grid with a 5-cm-diameter core tube from the sediment surface (0 to 5 cm) of each of eight nonamended and eight amended ponds for solid-phase Cu fractionation. In addition, surface (0 to 5 cm) sediment samples were collected using a uniform sampling grid at 74 ha^{-1} from one amended and one nonamended pond to determine spatial distribution of total Cu. Sediments were air-dried, ground with a ceramic mortar and pestle to pass a 2-mm mesh screen, and stored in sealed plastic bags until fractionation and toxicity testing.

Solid-Phase Copper Fractionation

Sediment Cu was separated into six operationally defined solid-phase fractions by selective, sequential dissolution. This method was based on the solubility of individual solid-phase components, and the selectivity and specificity of chemical reagents. The procedure provided a method to assess the gradient of physico–chemical association strength between Cu and various solid particles rather than actual speciation (Martin et al., 1987), thus providing a quantitative indication of the relative availability of Cu. The fractions are more likely to be operationally rather than chemically defined. However, each extractant in the selective, sequential procedure targeted one major solid-phase component. Therefore, the extractant-extractable metal is referred to by the targeted solid-phase component. In no case can an extractant remove all of a targeted solid-phase component without attacking some of the other components. In addition, redistribution and readsorption during sequential dissolution procedures may occur. Despite these shortcomings, common to any chemical extraction procedure, sequential dissolution techniques furnish more useful information on metal binding, mobility, and availability than can be obtained with a single extractant only.

The procedures employed in this study were based on those developed by Tessier et al. (1979) and Shuman (1985). Procedures for measurement of exchangeable, carbonate, residual, and total Cu were modified according to Han and Banin (1996, 1997). The procedure for measurement of the exchangeable fraction was modified by using a neutral nitrate salt (NH_4NO_3) as the extractant to avoid complexation with chloride and exchangeability of Mg that occurs with the original procedure. The procedure for measurement of the carbonate-bound fraction was modified to efficiently remove carbonate-bound metals from soils with varying carbonate content (Han and Banin, 1995). Finally, residual and total Cu were obtained by extraction with 4 M HNO_3.

(i) Ammonium nitrate extractable—Soluble plus exchangeable copper (EXC). Twenty-five milliliters of 1 M NH_4NO_3 (pH adjusted to 7.0 with NH_4OH) were added to 1.1 g of air-dry sediment (equivalent to 1 g of oven-dry sediment) in a 50-mL teflon centrifuge tube and the mixture was shaken for 30 min at 25°C and then centrifuged at 5000 × g for 10 min. The supernatant was decanted and filtered through a 0.45-μm filter. The sediment residue was retained for the next step. The same centrifugation–decantation procedure was used after each of the following extractions.

(ii) Sodium acetate/acetic aid extractable—Copper bound to carbonate (CARB). Twenty-five milliliters of 1 M NaOAc-HOAc at pH 5.0 were added to the residual sediment from step one. The mixture was shaken for 6 h.

(iii) Hydroxylamine hydrochloride extractable—Copper mostly bound to easily reducible oxides (ERO). Twenty-five milliliters of a 0.1 M $NH_2OH \cdot HCl$ + 0.01 M HCl solution (pH 2) were added to the sediment residue from the previous step and shaken for 30 min. Metals extracted in this fraction are mostly from Mn oxides (Shuman, 1982). However, this acid can dissolve some Cu associated with organic matter, resulting in underestimation of organically bound Cu.

(iv) Hydrogen peroxide/ammonium nitrate extractable—Copper bound to organic matter (OM). Three milliliters of a 0.01 M HNO_3 and 5 mL of 30% H_2O_2 were added to the sediment residue from the previous step and the mixture was digested in a water bath at 80°C for 2 h. An additional 2 mL of H_2O_2 were added and the mixture was heated for 1 h. Fifteen milliliters of 1 M NH_4NO_3 were then added and the sample was agitated for 10 min.

(v) Ammonium oxalate in the dark extractable—Copper bound to amorphous iron oxides (AmoFe). Twenty-five milliliters of a 0.2 M oxalate buffer solution (0.2 M $(NH_4)_2C_2O_4$–0.2 M $H_2C_2O_4$ at pH 3.25) were added to the sediment residue from the previous step and the sample was shaken in the dark for 4 h (Shuman, 1982).

(vi) Hot hydroxylamine hydrochloride/acetic acid extractable—Copper bound to crystalline iron oxides (CryFe). Twenty-five milliliters of 0.04 M $NH_2OH \cdot HCl$ in a 25% acetic acid solution were added to the sediment residue from the previous step and the sample was digested in a water bath at 97 to 100°C for 3 h.

(vii) Copper in the residual fraction (RES) and total copper. Twenty-five milliliters of 4 M HNO_3 were added to the residue or sediment and the sample was transferred to a glass digestion tube. Digestion was conducted in a water bath at 80°C for 16 h (Sposito et al., 1982; Han and Banin, 1997). The tube was weighed before and after each step to calculate the remaining solution entrained in the residual sediment, which was subtracted from the measured metal concentration for each step.

In addition, water-soluble Cu was extracted using deionized water at a water to sediment ratio (w/w) of 2. Copper concentration in each extract was determined using atomic absorption spectroscopy. Free Cu ion in the water-soluble fraction was measured using a specific ion electrode.

Sediment Mineralogical Analysis

Samples were subjected to pretreatments to remove salts, carbonates, organic matter, and Fe oxides, and were then separated by sieving and centrifugation into sand-, silt-, and clay-sized fractions (Jackson, 1956; Dixon and White, 1997). Mineralogy of the clay-sized fraction of sediments was determined (Karathanasis and Hajek, 1982; Karathanasis and Harris, 1994). Clays were saturated with Mg and K salts, separately, for X-ray diffraction (XRD) and differential scanning calorimetric (DSC) analyses. A diffractometer (Philips X'Pert-MPD; Philips Electronics, Almelo, the Netherlands) equipped with a ceramic long, fine-focus Cu anode tube was used for XRD analysis. Samples were step-scanned from 2 to 30° 2θ at 1 s per step with a step size of 0.03° 2θ. For DSC analysis (DSC 910S; TA Instruments, New Castle, DE), clay samples (Mg-saturated and dried at 54% relative humidity) were heated in covered aluminum pans from 5 to 625°C at 10°C per minute in an N_2 atmosphere. An empty, covered aluminum pan was used as the reference (Karathanasis and Harris, 1994).

Data Analysis

The following parameters were used to describe the relative binding intensity of Cu in sediments (Banin et al., 1990; Han and Banin, 1997). The partition index (I) and the reduced partition index (I_R) of Cu were calculated as follows:

$$I = \sum_{i=1}^{k}[F_i \times (i)^n] \quad [1]$$

$$I_R = I/(k^n) \quad [2]$$

where i is the extraction step number (1: least aggressive, k: most aggressive) with the values assigned to i in Eq. [1] representing the various steps and components: EXC = 1; CARB = 2; ERO = 3; OM = 4; AmoFe = 5; CryFe = 6; and RES = 7. F_i is the fractional content of Cu in component i out of the total extracted, and n is an integer (usually 1 or 2). In this case, k equals 7 and n equals 2; thus minimal $I_R = 0.02$ and maximal $I_R = 1$. The reduced partition index quantitatively describes the relative binding intensity of metals in soils and enables the comparison of the binding intensity of a given metal among soils, and of different metals in the same soil. The value of i in Eq. [1] increases in accordance with the gradual increase in the aggressiveness of extractants used in the selective sequential dissolution procedure. Thus, a low value of I_R (near the minimum) results from and represents a distribution pattern in which much of the Cu is obtained in the EXC fraction. Conversely, a high value (near 1) results from and represents a situation in which a high proportion of the Cu is bound in the RES fraction. Intermediate values represent various patterns involving metal partitioning among all the solid-phase components.

Comparisons of metal concentrations in the two sediments were made with t-tests. Significance levels of 0.05 and 0.01 were used in the analyses.

Sediment Toxicity Testing

Two model organisms, amphipods and common cattail, have been used previously to evaluate the toxicity of Cu in aquatic sediments (Huggett et al., 1999; Suedel et al., 1996; Deaver and Rodgers, 1996; Muller et al., 2001) and were used for toxicity testing in this study. Amphipods were cultured according to standard methods (USEPA, 1994) and 2- to 3-wk-old amphipods were used for bioassays, which consisted of 10-d static exposures (Nebeker et al., 1984). Cattail inflorescences were collected in the field, placed in plastic bags, transported to the laboratory, and stored at 20°C until testing. Prior to bioassays, viable seeds were separated from nonviable seeds gravimetrically (Moore et al., 1999) by placing part of the inflorescence in a blender with approximately 500 mL of tap water. Inflorescences were homogenized for 10 sec, whereupon floating debris was removed. Viable seeds used for testing were collected from the bottom of the blender. Cattail bioassays consisted of 7-d static exposures (Muller et al., 2001).

A 4:1 mixture (w/w) of water and each pond sediment sample was placed in glass borosilicate beakers. After settling of sediment particles, 10 amphipods and 10 cattail seeds were added to each respective test vessel. The toxicity of each sample was analyzed in three replicate test vessels. Amphipod tests were performed under constant aeration and amphipods were fed every other day with one drop of a solution made from yeast, alfalfa, and digested trout feed. At the end of the exposure period, amphipods were sieved, counted, and placed in 70% ethanol for growth determination. Cattail germination was determined by visual observation and the seedlings were gently placed in a 70% ethanol solution for root and shoot length measurement. Growth of amphipods and cattail roots and shoots after exposure was measured with a Videometric 150 image analyzer with software (American Innovision, San Diego, CA).

RESULTS AND DISCUSSION

Chemical and Mineralogical Properties of Catfish Pond Sediments

Catfish pond sediments consisted of primarily silt and clay and had a relatively high cation exchange capacity (CEC) (Table 1). Smectite is the predominant clay mineral (43%) in Sharkey soils, but mica (25%), kaolinite (19%), and quartz (10%) are also present (Seifert, 1999). Sediment pH was slightly alkaline. Sediments had intermediate concentrations of carbonate and organic carbon. Carbonate in sediments was mainly calcite (calcium carbonate), indicated by a diffractogram peak at 0.295 nm. The diffractogram peak was not present following the CARB step in the sequential dissolution, indicating that most of the calcite had dissolved. Generally, chemical and mineralogical properties of nonamended and $CuSO_4 \cdot 5H_2O$-amended pond sediments were not different, although $CuSO_4 \cdot 5H_2O$-amended pond sediments contained more sand than nonamended pond sediments (Table 1).

Accumulation of Copper in Copper-Amended Pond Sediments

Copper accumulation in the surface layers of $CuSO_4 \cdot 5H_2O$-amended pond sediments was substantial (Table 2, Fig. 1). Copper concentration in $CuSO_4$-amended pond sediments (172.5 ± 35.2 mg kg^{-1}) was 4.8 times greater than that in nonamended pond sediments (36.1 ± 11.7 mg kg^{-1}). Based on the $CuSO_4 \cdot 5H_2O$ application protocol implemented in this study, the average rate of Cu accumulation in amended sediments over 3 yr was 45.5 mg kg^{-1} yr^{-1}. This is equivalent to a sediment Cu accumulation rate of 41 μg kg^{-1} dry sediment for each 1 kg ha^{-1} of $CuSO_4 \cdot 5H_2O$ applied to ponds.

In a study of 358 freshwater fish ponds, Boyd et al.

Table 1. Chemical properties of catfish pond soils sampled to evaluate copper accumulation (n = 4).

	Nonamended sediment				Amended sediment			
Parameter	$\bar{x}$	**SD**	**min**	**max**	$\bar{x}$	**SD**	**min**	**max**
pH			7.44	7.77			7.16	7.79
Fe_2O_3 (g kg^{-1})	9.2	1.2	7.4	10.0	9.5	0.9	8.5	10.8
$CaCO_3$ (g kg^{-1})	18	9	10	30	14	4	10	21
Organic carbon (g kg^{-1})	14.6	2.1	12.4	16.9	16.5	1.7	14.9	18.3
CEC ($cmol_c$ kg^{-1})†	29	9.5	21	42	21	6.9	25	39
Sand (g kg^{-1})	32	15	13	45	65	46	19	111
Silt (g kg^{-1})	492	71	401	557	553	114	422	626
Clay (g kg^{-1})	477	59	430	555	382	154	277	559

† Cation exchange capacity.

Table 2. Mean (±SD) solid-phase copper fraction concentrations in sediments of catfish ponds receiving periodic applications of $CuSO_4 \cdot 5H_2O$ compared with those in ponds receiving no $CuSO_4 \cdot 5H_2O$ applications (n = 8).

Copper fraction†	Nonamended sediment		Amended sediment		F‡
	mg kg^{-1}	% of total by sum	mg kg^{-1}	% of total by sum	
EXC	0.39 (0.28)	1.2	5.64 (1.50)	3.4	14.5
CARB	5.1 (1.9)	15.8	52.4 (12.2)	31.6	10.3
ERO	0 (0)	0	0.74 (0.38)	0.4	–
OM	6.9 (2.8)	21.4	51.5 (13.5)	31.1	7.5
AmoFe	9.1 (3.5)	28.3	36.6 (12.4)	22.1	8.1
CryFe	6.5 (1.3)	20.2	11.6 (2.9)	7.0	1.8
RES	4.2 (0.7)	13.0	7.1 (1.4)	4.3	1.7
Total (by sum)	32.2		165.6		
Total (by analysis)	36.1 (11.7)		172.5 (35.2)		
H_2O-extractable	0.30 (0.09)		0.88 (0.20)		
I_R§	0.51 (0.04)		0.34 (0.02)		

† EXC = exchangeable, OM = organic matter, ERO = easily reducible oxide, AmoFe = amorphous iron, CryFe = crystalline iron, RES = residual.
‡ F = amended sediment Cu fraction concentration ÷ nonamended sediment Cu fraction concentration.
§ I_R = binding intensity.

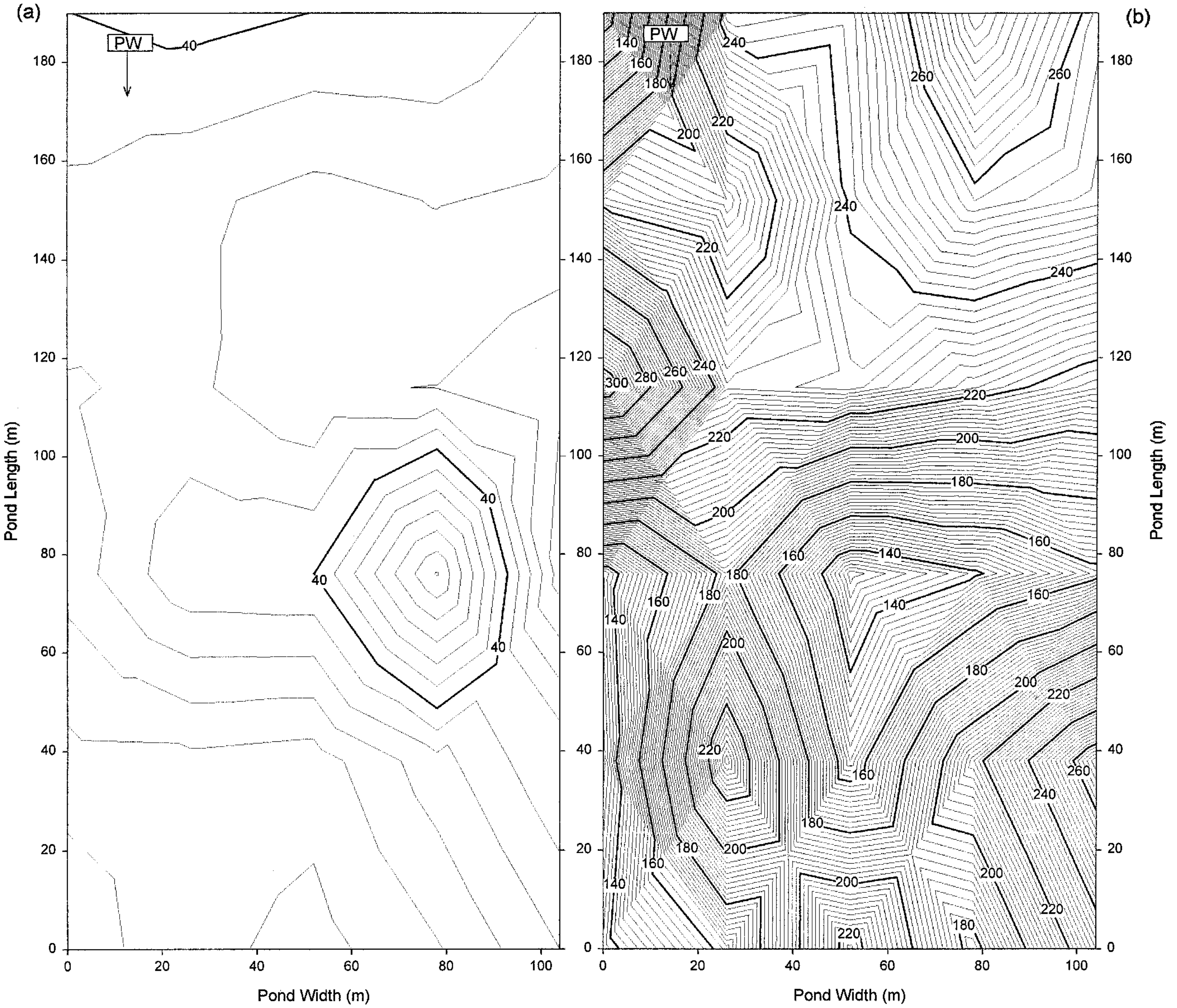

Fig. 1. Total Cu concentration (2 mg kg^{-1} isopleths) in sediments of two 0.40-ha catfish ponds. One pond (*a*) did not receive $CuSO_4 \cdot 5H_2O$ amendments during 3 yr of culture; one pond (*b*) received 59 additions of 2.27 kg $CuSO_4 \cdot 5H_2O$ per addition. PW indicates position of paddlewheel aerator.

(1994) measured an average Cu concentration in sediments of 8.6 mg kg^{-1}. However, nonamended pond sediments in this study had much greater Cu concentrations. The Cu concentration of Sharkey soil between 63.5 and 76.2 cm depth from three forested locations near the ponds sampled in this study ranged from 28.8 to 39.2 mg kg^{-1} (mean = 33.7 mg kg^{-1}) (Pettry and Switzer, 1993). These concentrations are consistent with Cu concentrations measured in nonamended ponds (36.1 mg kg^{-1}).

The spatial distribution of total Cu in nonamended and amended pond sediments was not uniform. Spatial variability of Cu concentrations in amended pond sediments was much greater than that in nonamended sediments. In nonamended ponds, sediment Cu concentrations ranged from 30 to 47 mg kg^{-1} (Fig. 1a). However, Cu concentrations in amended sediments ranged from <100 to >300 mg kg^{-1} (Fig. 1b), indicating that the fate of applied $CuSO_4$ was not uniformly distributed in pond sediments and that composite sampling is essential for representative results. The greatest Cu concentration was measured in the sediment about 65 m down current from the point of $CuSO_4 \cdot 5H_2O$ application (Fig. 1b), suggesting that Cu precipitation from pond water was rapid.

Copper Fractionation in Sediments

Total Cu extracted by 4 *M* HNO_3 was strongly correlated with the sum of the fractions by sequential dissolution. The proportion of total Cu extracted by 4 *M* HNO_3 represented by the sum of the fractions obtained by sequential dissolution was 92.4% for nonamended sediment and 96.9% for Cu-amended sediment. These results indicate that the sequential dissolution technique resulted in excellent recovery of Cu from catfish pond sediments.

The fractionation of Cu was different between amended and nonamended pond sediments. In nonamended ponds, sediment Cu was mostly present in the AmoFe (28.3%), OM (21.4%), and CryFe (20.2%) fractions (Table 2). This indicates that Fe oxides or oxyhydroxides and organic matter play very important roles in binding Cu in nonamended sediments. Copper readily coprecipitates and forms solid solutions with Fe oxides and oxyhydroxides (Lindsay, 1979). The solubility of Cu oxide (tenorite) and hydroxide minerals is low and that of Cu carbonate is relatively high (Stumm and Morgan, 1981). However, Cu^{2+} may be chemisorbed or occluded in iron oxide coatings rather than as a separate phase through the formation of Cu^{2+}–O–Fe^{3+} or Cu–O–Al bonds (McBride, 1981). McIntosh et al. (1978) reported that 50% of heavy metals were in the occluded phase of Fe and Mn oxides, and the concentration and distribution of Cu as well as Cd, Pb, Ni, and Zn were positively correlated to the amount of hydrous iron and manganese oxides in the sediments. These observations support the observation that Cu in nonamended sediments was distributed primarily in fractions associated with Fe (i.e., AmoFe and CryFe fractions).

In contrast to nonamended pond sediments, sediment Cu in $CuSO_4$–amended ponds was mainly present in the CARB (31.6%) and OM (31.1%) fractions. There was also a large proportion of Cu in the AmoFe (22.1%) fraction. Water used to fill the ponds sampled in this study had moderately high alkalinity (100 to 200 mg L^{-1} as $CaCO_3$) that would promote precipitation of $CuCO_3$ despite the relatively high solubility of this compound. The association of excess soluble Cu added to soils with the carbonate phase tends to be temporary. $CuCO_3$ is not very stable in aqueous alkaline environments due to larger ionic size differences between Cu (0.72×10^{-10} m) and Ca (0.99×10^{-10} m) (McBride, 1981) and the relatively high solubility of $CuCO_3$ ($K_{sp} = 1.4 \times 10^{-10}$) (Dean, 1973). Thus, following initial precipitation, Cu in $CuSO_4$–amended pond sediments may redistribute with time from the CARB fraction into the AmoFe-bound and OM fractions. Han and Banin (1997, 1999) reported that added soluble Cu was redistributed from the EXC and CARB fraction into the ERO and OM fractions in arid-zone soils. However, Cu^{2+} may substitute freely for Mg in magnesium carbonate or Fe in iron carbonate (McBride, 1981).

The large proportion of Cu in the OM fraction can be related to the uptake by and subsequent sedimentation of dead phytoplankton. Additionally, Cu can be adsorbed to organic matter, particularly humic substances. Copper can form the core of a very stable inner sphere complex with humic substances (McBride, 1981).

Copper extracted from many river and lake sediments is primarily in the residual fraction, followed by the organic matter–bound fraction. In two Quebec river sediments, Cu was mostly in the residual (38%), organic matter–bound (25%), Fe and Mn oxide–bound (20%), and carbonate-bound (15%) fractions (Tessier et al., 1979). Copper was mainly in the residual (53%) and organic matter–bound (30%) fractions in sediments of the Gulf Intracoastal Waterway near Galveston, Texas (Lindau and Hossner, 1982). These results indicate that Cu will ultimately redistribute to fractions of low bioavailability. Copper in $CuSO_4$–amended catfish pond sediments was present in more labile fractions than in many river and lake sediments, reflecting a history of recent $CuSO_4$ additions. Additional research is required to assess the kinetics of Cu redistribution in aquatic sediments.

The relative binding intensity (I_R) of Cu in nonamended sediments was 1.5 times greater than that of Cu in $CuSO_4$–amended sediments, indicating that accumulation of Cu in amended sediments over the time frame (3 yr) of $CuSO_4 \cdot 5H_2O$ applications occurred in fractions with potentially greater bioavailability. The greatest proportional increases of Cu in amended pond sediments occurred in the EXC and CARB fractions (Table 2). As discussed previously, I_R can range from 0.02 to 1. The value of I_R was strongly related to the total inputs of Cu, sediment properties, and time scale (Table 2). Han and Banin (1997, 1999) reported that the I_R of Cu was inversely related to Cu loading in arid-zone soils, and I_R in a loessal soil was higher than that in a sandy soil. Binding intensity (as indicated by I_R) can be expected to increase with time as a result of redistribution of Cu from more labile into more stable fractions. McLaren and Ritchie (1993) reported that a high proportion of the applied Cu as copper sulfate in a lateritic sandy soil in western Australia was initially associated with the soil organic matter. During the course of the trial (20 yr), a substantial proportion of Cu redistributed to the residual fraction.

Solution Copper and Availability of Copper in Sediments

Copper concentrations in the soluble and EXC fractions in amended sediments were much higher than those in nonamended sediments (Table 2). Water-soluble and EXC Cu in the amended sediments were 3 and 11 times higher than those in nonamended sediments, respectively. This indicates that the potential bioavailability of Cu in amended pond sediments was greater than that in nonamended sediments.

In the water-soluble fraction, no free Cu ion was detected by specific ion electrode (detection limit = 10^{-8} *M*; Orion Research, 1996) in extracts from both nonamended and amended sediments (data not shown), implying that Cu in the water-soluble fraction was in complexes formed with inorganic and organic ligands. Thus, the availability of Cu in the amended pond sediment systems may be relatively low.

Exchangeable Cu is the key fraction bridging solid-phase and solution components. There was a strong correlation between water-soluble and EXC Cu in sediment from both treatments (Table 3). Bioavailability, as indicated by water-soluble and EXC Cu concentrations, was strongly controlled by solid-phase components in nonamended sediments. Copper concentrations in water-soluble and EXC extracts of nonamended pond sediments were strongly correlated with sediment solid-phase Cu concentrations in CARB, AmoFe, CryFe, or OM fractions (Table 3). In contrast, Cu concentrations in water-soluble and EXC extracts of amended pond sediments were not correlated with any solid-phase Cu fraction concentrations (Table 3). These results suggest that the bioavailability of Cu is directly related to solid-phase Cu concentrations at low solid-phase Cu concentrations, but increases in bioavailability are not directly related to solid-phase Cu concentrations above certain threshold solid-phase Cu concentrations at relatively high solid-phase Cu concentrations.

Table 3. Pearson correlation coefficients between water-soluble or exchangeable (EXC) Cu and solid-phase fractions and soil pH.

	Nonamended sediment		Amended sediment	
Fraction†	**Water-soluble**	**EXC**	**Water-soluble**	**EXC**
EXC	**0.766***		**0.764***	
CARB	**0.884****	**0.815***	**0.262**	**0.602**
OM	**0.626**	**0.752***	**−0.349**	**−0.173**
AmoFe	**0.877****	**0.884****	**0.149**	**0.267**
CryFe	**0.798***	**0.921****	**0.022**	**0.169**
RES	**−0.070**	**0.150**	**0.075**	**0.012**
Total	**0.861****	**0.896****	**0.129**	**0.327**
pH	**−0.430**	**−0.573**	**0.453**	**0.229**

*** The regression coefficient is significant at the 95% level.**
**** The regression coefficient is significant at the 99% level.**
† CARB = carbonate, OM = organic matter, AmoFe = amorphous iron, CryFe = crystalline iron, RES = residual.

Sediment Toxicity Testing

Amphipod survival and growth and cattail germination and root and shoot growth were not different when exposed to rewetted nonamended and amended sediments (Table 4). Although results of the binding intensity calculation indicate the greater potential bioavailability of Cu in $CuSO_4$–amended pond sediments, apparently these concentrations were insufficient to affect the response of the toxicity test organisms to sediment exposure. In bioassays with amphipods, Huggett et al. (1999) reported a no observed effects concentration (NOEC) of 2010 mg kg^{-1} Cu. Suedel et al. (1996) observed an amphipod 10-d LC_{50} of 262 mg kg^{-1} Cu. The 7-d NOEC for cattail was 19 mg kg^{-1} Cu in sandy (75%) Cu-amended sediments (Muller et al., 2001), but 1245 mg kg^{-1} Cu in Cu-amended stream sediments (Huggett et al., 2001). The higher NOEC in the amended stream sediment was related to a large proportion of sediment in the clay fraction and a high sediment organic C concentration (3.4%). Organic C can account for an additional 10 to 100 μmol g^{-1} of Cu binding relative to low organic C soils (Mahoney et al., 1996).

The results of the toxicity testing should be interpreted with caution as the bioavailability of Cu in sediments is strongly correlated with interstitial free Cu ion concentration (Ankley et al., 1993; Chapman et al., 1998). Ankley et al. (1993) suggested that interstitial water is the primary media through which benthic organisms are exposed to sediment contaminants. To assess the potential effect of exposure to interstitial water, an interstitial water toxicity unit (IWTU) can be calculated by dividing the interstitial free Cu ion concentration by the 10-d aqueous LC_{50} of the test organism. If the IWTU is greater than 1.0, then effects are predicted to occur, otherwise effects are predicted to not occur (Ankley et al., 1996). The 10-d LC_{50} to amphipods determined in our laboratory was 63 μg Cu L^{-1} (Huggett et al., 2001), implying that an interstitial water concentration ≥63 μg Cu L^{-1} may cause effects to amphipods.

Nonetheless, IWTU may underestimate Cu toxicity because interstitial water is not the only route of exposure. Amphipods feed on detrital particles at the sedi-

Table 4. The effect of exposure to control and $CuSO_4 \cdot 5H_2O$-amended catfish pond sediments on amphipod and cattail mean (SD) survival and growth.

		Amphipod		Cattail		
Treatment	***n***	**Survival**	**Growth**	**Germination**	**Root growth**	**Shoot growth**
		%	**mm d^{-1}**	**%**	**mm d^{-1}**	
Nonamended	**3**	**84.3 (1.8)**	**3.8 (0.1)**	**87.7 (4.1)**	**7.2 (0.7)**	**10.3 (0.6)**
Amended	**8**	**87.9 (5.3)**	**3.7 (0.2)**	**93.8 (4.5)**	**7.0 (0.7)**	**10.6 (0.5)**

ment–water interface (Chapman et al., 1998). Dietary exposure to Cu may be enhanced by the consumption of settled phytoplankton killed through Cu uptake. In addition, organisms may be exposed to Cu by the diffusion of free Cu from sediments into the overlying water. Suedel et al. (1996) observed a relationship between the overlying water Cu concentrations and toxicity to amphipods in Cu-amended sediment bioassays.

In this study, sediments were air-dried following collection for the Cu fractionation study and then rewetted for toxicity testing. The bioavailability of Cu may have been altered (reduced) by precipitation of Cu oxides and carbonates upon drying. Further studies are required to evaluate the toxicity of freshly collected sediment, which should include measurement of porewater Cu concentrations. Additional research that relates sediment Cu concentration determined from air-dried samples to interstitial free Cu ion concentration is also required. Finally, the effect of sediment Cu concentration on microbial activity in catfish ponds is not known. Inhibition of microbial activity could have profound effects on catfish pond oxygen dynamics and the regeneration of nutrients from organic matter mineralization.

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Elevated Lead and Zinc Contents in Remote Alpine Soils of the Swiss National Park

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ABSTRACT

Weathering of bedrock and pedogenic processes can result in elevated heavy metal concentrations in the soil. Small-scale variations in bedrock composition can therefore cause local variations in the metal content of the soil. Such a case was found in the remote alpine area of the Swiss National Park. Soil profiles were sampled at an altitude of about 2400 m, representing soils developed above different bedrocks. The concentration of lead in the profiles was found to be strongly dependent on the metal content in the bedrock underlying the soil and was strongly enriched in the top 10 cm. The dolomitic bedrock in the study area contains elevated lead concentrations compared with other dolomites. Dissolution of dolomite and accumulation of weathering residues during soil formation resulted in high lead concentrations throughout the soil profile. The enrichment of lead in the topsoil, however, is largely attributed to atmospheric input. The isotopic signature of the lead clearly indicates that it is mainly of natural origin and that atmospheric deposition of anthropogenic lead contributed to about 20 to 40% to the lead concentration in the topsoil on the bedrock with elevated lead concentrations. In the soils on bedrock with normal lead concentrations, the anthropogenic contribution is estimated to be about 75%. Also, zinc was very strongly enriched in the topsoil. This enrichment was closely correlated with the organic matter distribution in the profiles, suggesting that recycling through plant uptake and litter deposition was a dominant process in the long-term retention of this metal in the soil.

The major external source of heavy metals in soils is usually pollution caused by anthropogenic activities, such as metal mining, smelting, and processing. Additionally, diffuse pollution by wet and dry deposition has resulted in the long-term accumulation of heavy metals all over the world, for example in Greenland ice (Rosman et al., 1993), Scandinavian soils (Bindler et al., 1999; Rosman et al., 1998), or peat bogs (Weiss et al., 1999). While this is true for most contaminated soils, some high metal concentrations in soils are of natural origin, resulting from weathering of the underlying bedrock. Soils in the Mendip region in Great Britain, for example, are highly enriched in lead, zinc, and cadmium due to the high concentrations of these metals in the bedrock and the presence of mineralized veins (Fuge et al., 1991). This area has a long history of mining and smelting. Soils developed on serpentinite are highly enriched in nickel and chromium and sustain a specialized plant community composed of Ni-tolerant species (Proctor and Baker, 1994). These soils are restricted to small areas and are easily recognized by their special plant community.

Weathering of bedrock with only slightly elevated metal concentrations may result in an enrichment of metals by pedogenic processes (Fleige and Hindel, 1987; Blaser et al., 2000). Therefore, soils in areas that are not known to have elevated metal concentrations in the bedrock may also show naturally elevated metal concentrations.

In the course of the implementation of the Swiss National Soil Monitoring Network, unexpectedly high HNO_3–extractable lead concentrations of up to 140 mg kg^{-1} were found in the topsoil of a remote site (Munt la Schera) in the Swiss National Park at an altitude of 2400 m above sea level, far away from industry and major traffic routes (Federal Office of Environment, Forests and Landscape, 1993). These high contents fall far outside the normal ranges given in the literature for lead in soils not exposed to direct air pollution, for example 10 to 30 mg kg^{-1} (Alloway, 1990) or 2 to 60 mg kg^{-1} (Scheffer and Schachtschabel, 1992). They clearly exceed the guide value of 50 mg kg^{-1} for lead given by the Swiss Ordinance Relating on Burdens in Soil (Schweizerischer Bundesrat, 1998). In addition, the zinc concentrations at the Munt la Schera site, averaging around 100 mg kg^{-1} in the topsoil, are beyond the normal range for unpolluted soils.

Given the remote location of the Swiss National Park, which had been kept free from direct human impacts, except for hiking on a few trails since the beginning of the century, speculations were raised that the high metal contents may have resulted from the deposition of aerosols originating from the industrial centers of Northern Italy (Scheurer et al., 1993). Investigations of Steinnes et al. (1989) in southern Norway showed that atmospheric long-distance transport may indeed lead to heavy metal pollution of areas located far away from large industrial

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