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Correlation of Copper Distribution in a Freshwater-Sediment System to Bioavailability

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The bioavailability of metals in an aquatic environment is greatly influenced by physicochemical interactions of the metals with other components of the sediment-water system in which they are deposited. In addition to undergoing complexation in solution, metals are sorbed by the various coatings of a sediment as well as by the mineral matrix. The strength and extent of metal binding will differ among the various components or phases of the sediment. It is important to establish the extent of bioavailability of the metal in different sediment phases to better relate the degree of metal pollution to its effect on aquatic organisms. Free ionic metals, as well as most metals ion exchanged onto fine grained solids, may be biologically available. Less available forms include metals contained in solid organic materials or precipitated and coprecipitated metal oxide coatings. Metals incorporated into crystalline structures are relatively unavailable in nature(GIBBS 1973, FORSTNER & WITTMANN 1979).

It was the objective of this study to relate geochemistry to bioaccumulation by comparing the distribution of copper in the sediment and the water column of four freshwater systems to the amount of copper available for bioaccumulation. Tubificid worms, being benthic organisms that dwell in the top layer of sediment that interfaces with the water column, are exposed to heavy metals in solution as well as metals within the sediment or particulate matter. In this study the distribution of copper was determined by assessing the amounts of copper in the water column, within the various fractions of chemically extracted sediment, and amounts accumulated in the tubificids.

MATERIALS AND METHODS

The four sediments and corresponding waters used for this study were collected from the Des Plaines River, Chicago,IL; Calumet River, Munster,IN; Flatfoot River, Chicago-Dolton,IL; and Wabash River, Wabash,IN.

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The oxidized, top one inch of the sediments, collected 6 ft from shore in 1-3 ft of water, were immediately screened to remove debris larger than 10 mm. All samples were stored at 4°C. All materials coming into contact with the samples were soaked in or rinsed with a dilute HNO_3 solution followed by copious rinsing with double distilled water.

To facilitate manipulation of the sediment, a slurry of each was made using the substrate and corresponding river water so that a suspension of 200 g of sediment/L ±5% was attained. In subsequent work these suspensions were diluted so that 100 mL of the suspension contained 5 g of sediment (wet weight). This ratio was found adequate for worm survival. After determining wet weight to dry weight ratios of the sediments, adsorption isotherms were performed in 125 mL Wheaton vials to follow the distribution of copper added to these systems. Following a 72 hr equilibration period, 50 mL water samples were taken and sediments were extracted with 50 mL of 1 N HNO_3 for 24 hr. The copper standard was a Fisher Scientific Company 1000 ppm Certified Atomic Absorption Standard.

The tubificid worms used in the experiment were obtained from a local pet store where they are sold as food for tropical fish. The majority of the worms, 98%, were sexually immature and unidentifiable, but the remaining 2% were of the species Limnodrilus hoffmeisteri (HILTUNEN 1981). The worms were not fed during this study.

The bioassays were run in 125 mL Wheaton vials using unamended and copper-amended sediment suspensions. Standard copper solution was added to the diluted suspensions. The concentration of copper in the 50 g sediment/L would have been raised by 0,2.5,5.0, 7.5, or 10.0 mg Cu/L if there were no adsorption of the added copper. These samples were equilibrated for 72 hr before worm addition. Aquifier groundwater, containing 0 and 20 µg Cu/L, was used for the two controls. Rather than repeatedly disturbing one system by taking samples every second day, 7 replicates of each system were prepared.

The worms were purged in the groundwater for 24 hr before the bioassays began. The start of the exposure period, when approximately 20 worms were added to each bioassay bottle, was considered to be day 0. On this day, 50 mL water samples were taken for the 5 experimental concentrations and the 2 controls. The sediments for the 0 and 10 mg/L concentrations were retained for fractionation analysis. Samples of the

worms and water were taken on days 2, 4, 6, 8, 10, and 12. Worms were removed from the sediment by using a 363 μm nylon screen. After purging for 24 hr in groundwater the worms were sacrificed, cleaned, dried, separated into groups of 3, and frozen until analysis.

Worm samples were subjected to a wet digestion procedure to determine their copper uptake. Samples taken from the freezer were weighed in 16 mm x 125 mm screw cap, Pyrex, disposable culture tubes. Samples were digested with 1 mL HNO_3 for 8 hr at 90°C and diluted to 10 mL with double distilled water. This digestion procedure resulted in copper recovery of 99.8%.

Procedures for the fractionation scheme are listed in Table 1. The 5 g sediment samples containing 0 and 10 mg Cu/L from the bioassays were fractionated in 50 mL polycarbonate centrifuge tubes. The samples, centrifuged at 17,000 rpm for 30 min to separate the liquids, were successively extracted with 40 mL of the fractionation solutions. After centrifuging the samples again, the extractant solutions were analyzed for copper by the method of standard additions using a Perkin Elmer Model 305-B Atomic Absorption Spectrophotometer.

TABLE 1. EXTRACTION SEQUENCE FOR SEDIMENT FRACTIONATION

Step	Extraction Method	Phase	Reference
1.	1 M $MgCl_2$, pH 7, room temp, 1 hr	absorbed/exchanged	GIBBS (1973)
2.	1 M NaOAc, pH 5, room temp, 5 hr	carbonate	TESSIER *et al.* (1979)
3.	0.1 M $NH_2OH \cdot HCl$ + 0.01 M HNO_3, pH 2, room temp, 30 min	easily reducible (Mn-oxides & amorphous Fe-oxides)	CHAO (1972)
4.	30% H_2O_2 + 1 M NH_4OAc, 85°C, 5 hr	organic	GUTPA & CHEN (1975)
5.	1 M $NH_2OH \cdot HCl$ in 25% HOAc, 96°C, 6 hr	moderately reducible (hydrous Fe-oxides)	CHESTER & HUGHES (1967)

RESULTS AND DISCUSSION

Results for the fractionation, listed as the amount of copper in the five geochemical phases for the amended and unamended portions of each sediment, are contained in Table 2. The dry to wet weight ratios of the sediments were employed in calculating the amount of copper in the sediment samples. Conversion to dry weight affords more consistency when comparing sediments.

An initial examination of the data indicated that there was an increase in copper uptake by the tubificid worms as a function of time in both the sediment-water systems and the water only control systems. Analysis of the controls by a one-way analysis of variance determined that the tubificids manifested equivalent copper uptake in all bioassays except that of the Calumet. The ratio of metal uptake in the controls for the other three rivers to that for the Calumet River, was used as a correction factor for the Calumet data in the following regression analyses to compensate for this decrease in worm metabolic activity.

A data matrix was developed to examine factors having an influence on copper uptake by worms in the sediment bioassays. Single regressions were performed with the concentration of copper in the worms (geometric mean of split samples) as the independent variable. Since only the 0 and 10 mg Cu/L bioassay sediment samples were chemically fractionated, worm samples taken only from these concentrations were involved in the correlation. The regressions of day vs. the copper concentration of the worms for the 0 and 10 mg Cu/L subsets of each sediment bioassay were used to determine the copper body burden of the worms that would be representative of the exposure period. The predicted values for the twelfth day were employed in further analysis of sediment data.

Ten variables were involved in the regression, with each representing the amount of copper potentially available to the worms for accumulation from that particular factor. The first factor considered was the mean (arithmetic) concentration of copper in solution for the bioassays over the twelve day period. The next five variables considered were the copper concentrations of the five geochemical phases of sediment as extracted by the chemical fractionation procedure. The seventh factor was obtained by summing the concentration of copper from the five geochemical phases of the sediment. The eighth factor considered was the copper contained in the 0.5 N HNO_3 extract of the sediments. The ninth was the copper adsorption capacity,

TABLE 2. CONCENTRATION OF COPPER IN SELECTED GEOCHEMICAL PHASES OF THE 0 and 10 mg Cu/L PORTIONS OF THE BIOASSAY SEDIMENTS

River Sediment	Initial Cu Addition (mg/L)	Geochemical Phase*				
		Adsorbed/ Exchangeable	Carbonate	Manganese Oxide/ Easily Reducible	Organic	Iron Oxide
Des Plaines	0	0.30	10.31	1.78	10.2	40.7
	10	1.35	145.	15.0	37.0	70.2
Calumet	0	1.11	0.62	0.52	1.93	0.39
	10	15.4	135.	46.2	20.6	0.64
Flatfoot	0	0.12	0.78	0.89	1.63	0.56
	10	2.32	124.	28.3	27.1	18.6
Wabash	0	0.03	0.79	0.96	1.20	3.94
	10	0.41	110.	47.1	28.5	82.5

* Concentrations expressed as μg Cu/g sediment - dry weight.

TABLE 3. REGRESSION RESULTS CONTRASTING THE EFFECTS OF ELEVEN VARIABLES ON COPPER UPTAKE IN WORMS

Variable	Units†	R	F-Statistic ($F_{0.05,1,6} = 5.99$)	Regression Equation*
Manganese Oxide	µg Cu/g sediment	.9716	101.16	y = 19.0 x -37.8
0.5 N HNO_3 Extract	µg Cu/g sediment	.7623	8.32	y = 2.37 x -8.37
Σ5 Geochemical Phases	µg Cu/g sediment	.7054	5.94	y = 0.214 x -65.8
Water	µg Cu/L	.6807	5.18	y = 4.47 x +118
Carbonate	µg Cu/g sediment	.6561	4.53	y = 3.86 x +42.6
Adsorbed/ Exchangeable	µg Cu/g sediment	.5371	2.43	y = 41.0 x +189
Organic	µg Cu/g sediment	.5287	2.33	y = 14.9 x +57.6
Adsorption Capacity	µg Cu/g sediment	-.3995	1.14	y = -18.7 x +16900
Iron Oxide	µg Cu/g sediment	.3187	0.68	y = 3.80 x +193
Adsorption Strength	$(\mu g\ Cu)^{-1}$	.0349	0.01	y = -2980 x +308

† sediment weight on dry basis.
* x = copper concentration of variable; y = copper concentration of worms (µg Cu/g worm-dry weight).

and the tenth was the adsorption strength of each sediment. The adsorption tendencies of copper for the sediments fit the model for a Langmuir isotherm.

Table 3 contains the results for single regressions for each factor. The variables are ranked in order of importance as determined by their F-statistic and coefficient of correlation (r). Of the five geochemical phases, only the copper extracted from the manganese oxide/easily reducible phase was found, at the 95% confidence level, to significantly correlate with the concentration of copper in the worms. The second and last factor found to be significant was the metal extracted from the sediments by 0.5 N HNO_3. Although this factor represents a nonspecific, total metal extract, it was intercorrelated with the manganese oxide/easily reducible phase by over 89%. The most biologically significant variable, the manganese oxide/easily reducible phase had a coefficient of correlation of 0.9716.

Although almost all of the variance was explained by the manganese oxide phase, the data were further evaluated by including the five sediment phases in a forward stepwise regression. In this regression analysis the organic matter constituted the second most important phase. For the two variable (manganese oxide and organic) regression equation the coefficient of correlation was 0.9978. Adsorbed or exchanged metal was the next most significant phase. The coefficient of correlation was 0.9978. Adsorbed or exchanged metal was the next most significant phase. The coefficient of correlation for the three variable model was 0.9994. Consideration of all five phases resulted in the coefficient of correlation rising to 0.9998.

The high correlation between the uptake of copper and the amount of copper present in the manganese oxide/easily reducible phase suggests that the redox potential and pH in the gut of the worm is such that manganese oxide coatings are dissolved. Copper and other metals solubilized by the dissolution of this coating are then available for uptake by the organism.

REFERENCES

CHAO, L.L.: Soil Sci. Soc. Am. Proc. 36, 764(1972).

CHESTER, R. and M.J. HUGHES: Chem. Geol. 2, 249(1967).

FORSTNER, U. and G.T.W. WITTMANN: Metal Pollution in the Aquatic Environment. New York: Springer-Verlag 1979.

GIBBS, R.J.: Science 180, 71 (1973).

GUTPA, S.K. and K.Y. CHEN: Environ. Lett. 10, 129 (1975).

HILTUNEN, J.: Personal communication (1981).

TESSIER, A., P.G.C. CAMPBELL, and M. BISSON: Anal. Chem. 51, 844(1979).