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COPPER SPECIATION IN SOFT, ACID, HUMIC WATERS: EFFECTS ON COPPER
BIOACCUMULATION BY AND TOXICITY TO SIMOCEPHALUS SERRULATUS
(DAPHNIDAE)

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ABSTRACT

The stoichiometry and affinity (conditional stability constants) of Cu chelation by naturally-occurring organic ligands were determined by numerical solutions of Scatchard relationships. These values were then used in the geochemical speciation model, GEOCHEM, to determine the relative importance of each Cu species in dynamic equilibrium with competing metals and ligands. The accumulation of Cu by and toxicity of Cu to the soft water cladoceran Simocephalus serrulatus (Daphnidae) were measured and related to Cu speciation. When $10 \mu\text{g Cu} \cdot \text{L}^{-1}$ were added to pond water in the fall, the distribution of Cu was: Cu^{++} , 36.0%; Cu-organic, 63%; CuOH^- , 0.5% and CuSO_4 , 0.5%. The organics in pond water reduced Cu accumulation and toxicity. However, when the 24 h-LC₅₀ values were reported as Cu^{++} , this species of copper was more toxic in pond water than well water.

INTRODUCTION

Recent studies have demonstrated that copper toxicity to aquatic organisms is related to the concentrations of toxic species rather than total copper concentrations [1]. Toxicity to aquatic organisms has been thought to be related to copper 2+ ion activity. Copper can be associated with particulates or inorganic and organic ligands [2-7]. In hard waters the form of copper is controlled by the pH and alkalinity [8-15]. Copper toxicity is also less in the presence of natural and synthetic organic chelators such as humic acids, sewage effluent, EDTA or NTA [1, 7, 16]. McKnight [17] concluded that humic substances controlled the chemistry of copper added to a reservoir in the northeastern United States. In an attempt to predict the behavior of trace metals in aquatic systems, a number of fractionation techniques have been proposed [18, 19]. Most of these techniques measure operationally-defined species and often produce results, which are specific to a particular aquatic system and have low generality to be

applied in other systems. These techniques often cannot distinguish among several dissolved species, which may or may not be biologically active. The method which would give the most generality would be a complete thermodynamic and kinetic description of all the possible species.

Thermodynamic chemical speciation models have been constructed to predict the relative amount of metal in each possible form under a wide variety of environmental conditions [20]. The stoichiometry and stability constants of soluble complexes and insoluble precipitates are well known for the interactions of metals with inorganic ligands. Natural waters, however, also contain a wide range of dissolved organic constituents, which arise from natural processes in the geosphere [21].

Previous studies have shown that naturally-occurring organics are important in binding copper in soft acid waters that have low inorganic ligand concentrations [2, 5, 22-25]. While metals can be associated with labile organic compounds, such as carbohydrates, amino acids and low molecular weight organic acids, such as acetate and oxylate, most of these organic compounds are rapidly decomposed by microorganisms. In addition to these labile compounds, streams, lakes, ponds and impoundments contain naturally-occurring, colored organic compounds. Neither the stoichiometry (number of metal atoms bound per equivalent of organic matter) nor the affinity of binding (stability constants) are well known for the interaction of trace metals and naturally-occurring organic matter in surface waters. These polymeric compounds are heterogeneous in nature so that they represent a wide spectrum of molecular sizes and potentially many different types of binding sites. Because of the heterogeneous nature of these compounds, Sposito [26] has suggested the use of a quasiparticle model to represent a simplification of the complex situation of metal-organic interactions in surface waters. A quasiparticle model is a mathematical description of an aqueous solution in which the actual assembly of organic compounds is replaced by an assembly of hypothetical identical macromolecules whose mole mass is the number-average mole mass of the actual mixture and whose metal-complexation reactions closely mimic those of the real system [26]. An example of this conceptualization is the determining of overall, conditional stoichiometric and formation constants by Scatchard analyses. Certain classes of metal-organic complexes may have similar stability constants and all of the sites which can be measured on polymeric molecules may not be available for binding metals. For these reasons overall conditional stability constants and maximum binding capacities can be measured for organic ligands by the Scatchard analysis. The technique of Scatchard [27, 28] has been adapted to the measurement of stability constants and number of metal-organic complexes binding sites of heterogeneous mixtures of naturally-occurring organic ligands [29-35].

This study was conducted to determine (1) the relative importance of organic carbon in binding copper, (2) the types of binding sites, (3) number of each type of site per unit weight of carbon, (4) conditional stability constants for each type of binding site and (5) relationship between copper speciation and bioaccumulation of copper by and toxicity of copper to the soft water cladoceran, Simocephalus serrulatus (Daphnidae).

METHODS

Skinface Pond is a shallow (2.5 m) two hectare pond located in Aiken County, South Carolina, adjacent to the Department of Energy's Savannah River Plant. Comparative studies were conducted using water from Skinface Pond and from a deep artesian well which is the primary water source for the pond. These waters are of very similar water quality, except for higher concentrations of dissolved organic carbon (DOC) in the pond (Tables 1 and 2). The ionic strength (I) of the pond and well waters is $6 \times 10^{-4} M$, which results in an activity coefficient of 0.90 for copper (calculated from Guntelberg equation). Skinface Pond was constructed by impounding a small stream for recreational purposes in the mid-1940's. Bald cypress (*Taxodium distichum*) are common in the pond basin. The pond is bounded by mature mixed forest of loblolly pine and sweetgum-oak associations. Dense stands of bulrush (*Scirpus* sp.) surround the margins of the pond. The major hydrologic inputs into the pond are from the ephemeral stream, which has the greatest discharge during late fall, winter and early spring, and from the artesian well. There is almost no surface runoff into the pond. The pond receives no direct inputs of either industrial or domestic wastes. The watershed is approximately 14 km^2 of sandy highly

TABLE 1

Water Chemistry of Skinface Pond, April, 1979. Metal concentrations are total.

	Artesian Well Water ^a	Skinface Pond Water ^b
pH (air-equilibrated)	6.1	6.1
pH (ambient)	5.0	5.5
Total alkalinity (air equilibrated)	12.0	12 μ equivalents \cdot L ⁻¹
Redox potential	460	520 mV
Cl ⁻	$9.0 \times 10^{-5} M$	$1.1 \times 10^{-4} M$
SO ₄ ²⁻	$2.4 \times 10^{-5} M$	$2.6 \times 10^{-5} M$
PO ₄ ³⁻	$4.0 \times 10^{-8} M$	$7.6 \times 10^{-7} M$
NO ₃ ²⁻	$1.0 \times 10^{-8} M$	$1.6 \times 10^{-8} M$
CO ₂ ³ ^c	$4.9 \times 10^{-10} M$	$4.9 \times 10^{-10} M$
OH ^{2c}	$1.2 \times 10^{-8} M$	$1.2 \times 10^{-8} M$
Ca	$3.8 \times 10^{-5} M$	$1.3 \times 10^{-4} M$
K	$2.9 \times 10^{-5} M$	$3.9 \times 10^{-5} M$
Na	$7.8 \times 10^{-5} M$	$1.3 \times 10^{-4} M$
Mg	$2.5 \times 10^{-8} M$	$2.9 \times 10^{-8} M$
Ni	$5.1 \times 10^{-9} M$	$8.5 \times 10^{-9} M$
Zn	—	$6.9 \times 10^{-8} M$
Cd	$8.9 \times 10^{-11} M$	$1.8 \times 10^{-10} M$
Fe	$3.3 \times 10^{-7} M$	$7.4 \times 10^{-5} M$
Pb	$5.0 \times 10^{-10} M$	$2.4 \times 10^{-9} M$
Mn	$3.6 \times 10^{-7} M$	$2.0 \times 10^{-6} M$

^aThis study.

^bWiener 1979.

^cCalculated from air equilibrated pH.

TABLE 2

Total copper and dissolved organic carbon, concentrations, stability constants (K_1) and binding capacities (n_1) for copper and ligands in SPW and AWW in April and October, 1979, pH = 6.1

	Pond		Artesian Well
	April	October	
^a K_1	5.1×10^5	3.6×10^6	7.3×10^5
K_2	—	1.9×10^5	—
^b K_o	—	2.5×10^6	—
^c n_1	6.7×10^{-6}	2.1×10^{-6}	9.7×10^{-7}
n_2	—	5.7×10^{-6}	—
$n_1 + n_2$	6.7×10^{-6}	7.8×10^{-6}	9.7×10^{-7}
^d Total Cu	5.0×10^{-8}	6.2×10^{-8}	5.0×10^{-9}
^e Total Organic Carbon	12.4	15.6	0.5

$$^a K = \frac{[Cu\beta]}{[Cu][\beta]} ; \beta = \text{molar concentration of organic ligand}$$

$$^b \text{weighted average stability constant} = K_o \frac{(n_1 \cdot K_1) + (n_2 \cdot K_2)}{(n_1 + n_2)}$$

^c maximum binding capacity of ligand for copper [M]

^d M

^e mg · L⁻¹ TDOC

leached ultisols. The physical and chemical limnology of Skinface Pond and rainwater and surface water inputs are given by Wiener [36].

Alkalinities, chlorides and dissolved oxygen were determined titrimetrically [37]; pH was measured in the field. Nitrates and total phosphates were determined by Cd reduction and ammonium persulfate methods, respectively [38]. Before sulfate analyses, samples were run through an XAD column to remove organics, which can interfere with the analysis. Samples were subsequently analyzed by the 2-aminoperimidine method [39]. Total organic carbon samples were collected, preserved with two drops of concentrated sulfuric acid, and refrigerated, unless analysis was done on the same day, when samples were not preserved. Total and inorganic carbon concentrations in raw water were measured by a Beckman 915 Total Carbon Analyzer which was equipped with a ceramic combustion chamber. Organic carbon was calculated by difference. Total metal concentrations were determined by atomic absorption spectrophotometry [40]. Organic carbon in water samples was decomposed in a La Jolla Scientific UV photo-oxidizer for six h, with a few drops of hydrogen peroxide to catalyze the reaction. Unfiltered water was used for binding capacity, determination, uptake and toxicity studies.

Binding capacity, defined as the total amount of copper complexed by water, was determined by titration of 50 ml samples with a 7.9×10^{-4} M solution of $Cu(NO_3)_2$. Titrant was added by a Metrohm Dosimat^R E 535 automatic titrimeter. Free copper was measured

with an Orion cupric ion selective electrode, 94-29, and Brinkman model 102 pH meter. Ten molar sodium perchlorate was added as an ionic strength adjuster (0.5 ml, 50 ml sample = 0.1 M). Standard curves were measured before and after each titration, and were linear between 7.9×10^{-7} and 3.0×10^{-5} M. The initial Cu^{++} activity in both SPW and AWW were less than the linear range of the selective ion electrode. The amount of copper added during a titration varied among titrations but was in the range of 1.5×10^{-6} to 3×10^{-5} M Cu. This resulted in a bound to free ratio in the range of 2.8 to 0.14. The binding capacity and stability constants were determined by Scatchard analysis [27, 28]. Data was fit to the model described by equation 1 [41] by the Marquardt nonlinear least squares method [42].

$$\bar{V} = \text{Cu}_f \sum_i \left[\frac{K_i \cdot n_i}{1 + K_i \cdot C_f} \right] \quad (1)$$

where:

\bar{V} = Cu bound, $\mu\text{g atom} \cdot (\text{mg organic carbon})^{-1}$

Cu_f = free copper, $\mu\text{g} \cdot \text{atom} \cdot \text{L}^{-1}$

K_i = conditional stability constants

n_i = maximum number of binding sites of each type

i = number of types of sites present

Components were added to the models sequentially until the residual sums of squares could no longer be reduced. The overall regressions were highly significant ($P < 0.00001$). A typical F-statistic for regression was $F_{4,19} = 63,019$. This analysis was conducted on raw pond water and well water and isolated organics as well as on water that had been photo-oxidized by UV light to remove organics.

Copper speciation was calculated by solving the equations which described the speciation of copper under the conditions existing in Skinface Pond (SPW) and in laboratory experiments. All metals and ligands given in Table 1 are included at the appropriate concentrations for each simulation. Stability constants for all interactions are from the thermodynamic data set GEOTHERM except the Cu and Ca interactions with organics ($\beta 1$ and $\beta 2$) which were measured for organics studied here. The speciation was calculated by the simulation, speciation model GEOCHEM [43]. Several simulations were conducted with conditional stability constants and stoichiometric relationships determined in this study and those given in GEOTHERM [43]. Seasonal changes were followed during the spring and fall in both SPW and artesian well water (AWW).

The organism used for toxicity testing was Simocephalus serrulatus (Daphnidae). Animals were collected from the littoral zone of Skinface Pond. Individuals used for toxicity and bioaccumulation studies were cultured in the laboratory. Temperature was maintained between 21 and 24°C and the light cycle was 14.5 h light and 9.5 h dark. Animals were fed a suspension of yeast, Cerophyl^R and ground fish food. Bioaccumulation experiments were conducted to

compare the bioavailability of Cu from SPW and AWW. Animals just small enough to swim through a 1 mm² net were isolated and acclimated for at least 24 h to either SPW, or a mixture of SPW and AWW. The animals could not survive in pure AWW, so 10% pond water was added. Animals were collected after 24 h and used in bioassays. Toxicity tests were conducted as described by Giesy et al., 1977. A 10 mg Cu · L⁻¹ stock solution was prepared from Fisher Scientific (1000 mg · L⁻¹ Cu as Cu(NO₃)₂). Dosing solutions were made by adding Cu to either SPW, AWW or a mixture of the two waters. LC₅₀s were determined by the method of Litchfield and Wilcoxon [44].

RESULTS AND DISCUSSION

Inorganic water quality parameters were similar in both (SPW) and (AWW) (Table 1). Ambient pH was lower in AWW, (5.0 well; 5.6 pond) due to dissolved CO₂. Both waters had a similar pH (6.1) and total alkalinity (c.a. 12 μeq · l⁻¹) when air equilibrated. Organic carbon concentrations in SPW were consistently higher than in AWW. Spring runoff increased the total organic carbon concentration of SPW from 3.3 mg TOC · L⁻¹ in February to 14.0 mg TOC · L⁻¹ in March through October. The total copper concentrations in SPW were similar in April and October. Copper concentrations in SPW were approximately 10 times as great as that of AWW (Table 2). Total Cu²⁺ binding capacity of SPW was similar in the spring (6.7 10⁻⁶M) and fall (7.8 10⁻⁶M) and was higher than that of AWW (9.2 10⁻⁷M) (Table 2). The higher binding capacity of the SPW was reduced to values which were essentially the same as well water (1 x 10⁻⁶M), when the organics were removed by photo-oxidation which indicates that the additional binding capacity is due to dissolved organics. Binding capacities were measured on unfiltered samples. Previous multivariate statistical analyses have shown that particulate > 0.15 μm contributed less than 1% to the total binding capacity of SPW. The maximum binding capacities of organics were measured by estimating the asymptotic saturation value (Fig. 1). The binding capacities determined by these two methods were very similar; 11 and 13 x 10⁻⁶M for saturation and Scatchard analyses, respectively. The Scatchard plot for SPW organics is given in Fig. 2. The curvilinear function is indicative of several types of binding sites being present. We fit the data to one-, two- and three-site models. The fit of the two-site model had the best fit (lowest residual variability). The regression was highly significant and observed and predicted values very similar.

The stability constants differed between spring and fall (Table 2). In the spring only one type of binding site was observed. The Scatchard analysis was a straight line and the residual error mean square could not be reduced by the addition of a second binding site to the model. The stability constant for binding of copper was similar for SPW and AWW in April. In October, a two-component model significantly reduced the residual error mean squared, relative to a one-component model, however, a three-component model did not fit the data from SPW significantly better than the two component model. AWW was best described by a one component model. When two binding strengths were observed, an overall conditional stability constant (K_o) was also calculated (Table 2).

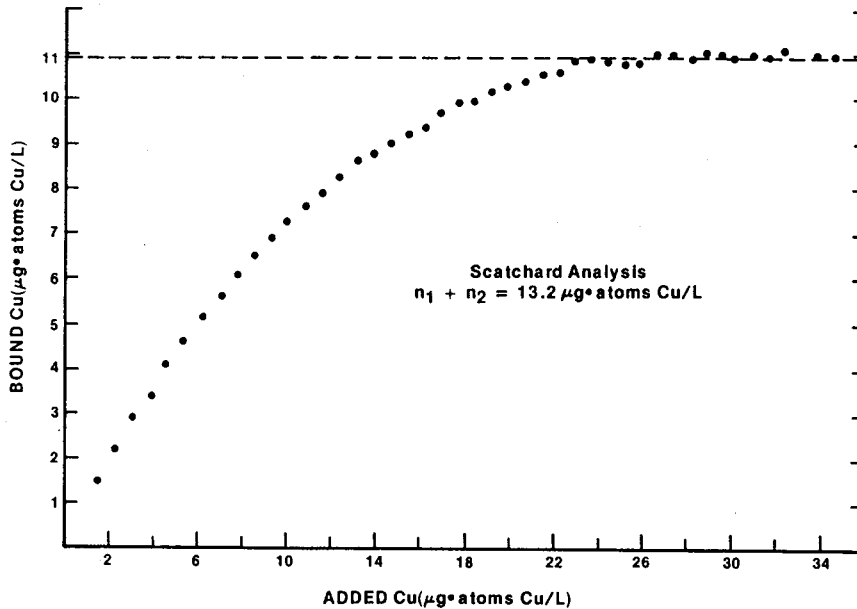


Fig. 1. Titration of Skinface Pond water organics (for April 25, 1980). Bound copper as a function of copper added. The asymptote to the saturation of binding sites, indicated by a dashed line, is approximately $11 \mu\text{g} \cdot \text{atom Cu} \cdot \text{L}^{-1}$.

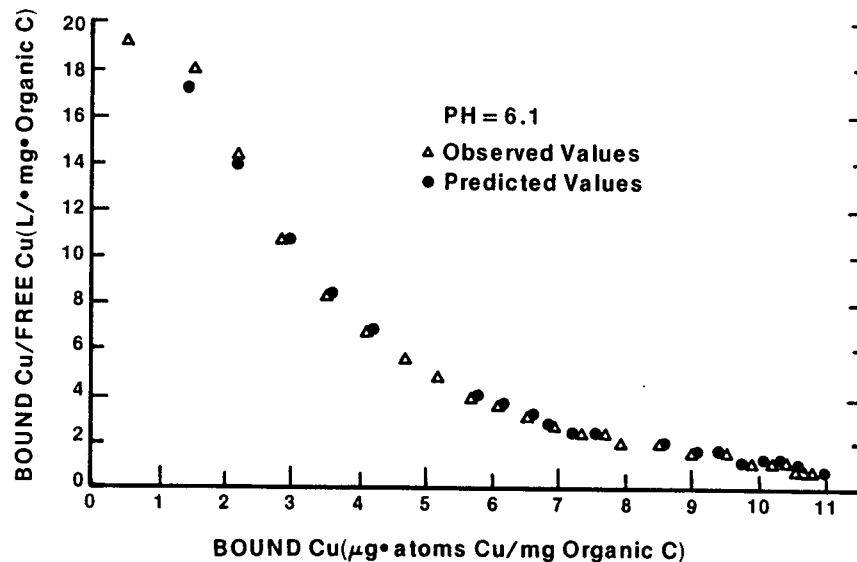


Fig. 2. Scatchard plot of copper binding by Skinface Pond organics for April 25, 1980. The predicted values were generated by a two-site Scatchard model. Least squares non-linear regression for two component model $F_{4,19} = 63,019$ ($pF < 0.00001$).

TABLE 3

Stability constants for copper-complexes with refractory dissolved organic matter. Complexes with known extracellular products are not included.^a

Organic Ligand	pH	Binding Capacity ($g \cdot atom \cdot g^{-1}$)	Stability Constant (K_{stab})	Reference
Kunibetsy River Sediment Extract	6.0	$2 \times 10^{-3}^b$	$1 \times 10^5 - 4.0 \times 10^5$	Matsunaga <i>et al.</i> , 1980 [7]
Lake Water Extract	8.0	—	$3.2 \times 10^8 - 6.3 \times 10^8$	Mantoura and Riley, 1975 [30]
Lake Water Extract	8.0	—	2.6×10^8	Buffle <i>et al.</i> , 1977 [45]
Lake Water Extract	—	—	4.7×10^3	Wilson and Kinney, 1977 [46]
Lake Fulvic	6.5	—	4.7×10^5	Shuman and Cramer, 1979 [47]
Lake Humic	6.5	—	9.2×10^5	Shuman and Cramer, 1979 [47]
Shukunohe River Sediment Extract	6.0	—	$1 \times 10^5 - 4.0 \times 10^5$	Matsunaga <i>et al.</i> , 1979 [48]
Soil Humics	6.0	—	$4.3 \times 10^5 - 4.6 \times 10^7$	Takamatsu and Yoshida, 1978 [49]
Aldrich ^R	6.1	$n_1 = 2.6 \times 10^{-4}$ $n_2 = 4.1 \times 10^{-4}$	$K_1 = 1.3 \times 10^7$ $K_2 = 4.9 \times 10^5$	Giesy (unpublished)
Humic Acid	6.5	$n_1 = 2 \times 10^3$	$K_1 = 1.6 \times 10^6$	Guy and Chakrabarti, 1976 [31]
Tannic Acid	6.8	$n_2 = 5.5 \times 10^{-3}$ $n = 1.1 \times 10^{-2}$	$K_2 = 1.6 \times 10^6$ $K = 2.2 \times 10^5$	Guy and Chakrabarti, 1976 [31]

^aSee Giesy, 1981 for a review of the effects of extracellular products on trace metal speciation [50].

^bmolecular weight determined to be 515

The stability constants at a pH of 6.1, measured for copper-organic complexes in this study, are similar to those reported by other researchers (Table 3). Mantoura *et al.* [32] also give stability constants for a number of metals and humics, which were determined at a pH of 8.0. This indicates that there is some generality of stability constants and that an average overall value may give some predictability in generalized thermodynamic speciation models. However, there is enough variation among organics in surface waters that some further characterization will be necessary to make accurate predictions of speciation.

The relative concentrations of copper species in AWW and SPW in the spring and fall were calculated by GEOCHEM (Table 4). The pH and ionic strengths were 6.1 and $6.07 \times 10^{-4} M$, respectively, for all four simulations. The total ambient copper concentrations were those

measured by atomic absorption, solids were allowed to precipitate in the simulation. The CO_2 concentration was calculated from the pH; CO_2 equilibrium with the atmosphere was assumed. The speciation simulations predicted that 98% of the Cu present in AWW would exist as Cu^{++} (Table 4). In SPW 88 and 63% of the copper would be bound in copper-organic complexes in spring and fall, respectively. When a weighted average of the two types of binding sites was used in the speciation simulations 90% of the copper was predicted to be bound to organics in the fall (Table 4).

TABLE 4

Relative concentrations (% of total Cu) of major copper species at ambient concentrations calculated by GEOCHEM. All metals and ligands given in Table 1 are included at the appropriate concentrations for each simulation. Stability constants for all interactions are from the thermodynamic data set GEOTHERM^R except the Cu and Ca interactions with organics (β) which were measured for ligands studied here.

	Skinface Pond			Artesian Well
	Spring	Fall Two-Component Model	Weighted ^a Average	One-Component Model
				%
Cu^{++b}	11.7	36.0	10.0	98.2
CuOH^+	0.1	0.4	0.1	0.8
CuSO_4	0.2	0.5	0.1	1.0
$\text{Cu } \beta 1$	88.0	26.1	89.7	— ^b
$\text{Cu } \beta 2$	—	36.9	—	—

^aBased on weighted average stability constant (K_o) = $\frac{(n_1 \cdot K_1) + (n_2 \cdot K_2)}{(n_1 + n_2)}$

^bSpecies which were considered in the model but accounted for less than 0.1% include $\text{Cu}(\text{OH})_2$, CuCO_3 , CuCl^+ and CuCl_2 .

GEOCHEM simulations of the speciation of copper in the uptake study, predicted that in AWW + 10% SPW, 80 and 92% of the copper would exist as Cu^{++} , when the total copper concentration (Cu_T) was 5.0×10^{-9} or 1.7×10^{-7} , respectively. Alternatively, approximately 97% of the copper in SPW would be bound in organic complexes (Table 5).

There were no significant differences among steady state copper concentrations in *S. serrulatus* exposed to $5 \times 10^{-9} \text{M}$ Cu in AWW, $5.0 \times 10^{-8} \text{M}$ Cu in SPW and $1.7 \times 10^{-7} \text{M}$ Cu in SPW (Table 6). Even though the higher copper concentration was 2×10^2 times greater than the lower copper concentration in SPW, the amount of copper assimilated was only twice as great and the difference was not statistically significant (Table 6). The greatest accumulation of copper was from the AWW which contained $1.7 \times 10^{-7} \text{M}$ Cu. The free Cu^{++} concentrations in SPW and in the lower total Cu concentration in AWW were all very similar, approximately 10^{-9}M (Table 7). The predicted Cu^{++} concentration for a total copper concentration of $1.7 \times 10^{-7} \text{M}$ in AWW was $1.6 \times 10^{-7} \text{M}$, which is two orders of magnitude greater than that predicted for SPW containing the same total copper concentration.

TABLE 5

Relative concentrations (% of total Cu) of ambient and elevated total Cu concentrations in the bioaccumulation study calculated by GEOCHEM.

Total [Cu] \underline{M} =	Skinface Pond		Artesian Well + 10% Skinface Pond	
	5.0×10^{-8}	1.7×10^{-7}	5.0×10^{-9}	1.7×10^{-7}
	%			
Cu ⁺⁺	2.1	2.2	80.4	91.6
CuOH ⁺	a	a	0.2	0.3
CuSO ₄	a	a	0.3	0.3
Cu#1	90.9	90.6	17.6	7.4
Cu#2	7.0	7.1	1.5	0.4

^aSpecies which represent less than 0.1% of the total Cu are not reported.

TABLE 6

Mean Cu concentration in *S. serrulatus* (\bar{X} , SD in parentheses) after 6 h exposure (\underline{M} Cu \cdot mg⁻¹, dry weight). Values underlined are not significantly different from one another. One-way ANOVA, $F_{3,8} = 14.36$, $P < 0.005$, $n = 13$; Tukey's HSD critical values; $\alpha = 0.01$, 8.88×10^{-3} , $\alpha = 0.05$, 6.49×10^{-3} .

Artesian Well + 10% Skinface Pond $\underline{Cu_T} = 5.0 \times 10^{-9} \underline{M}$	Skinface Pond $\underline{Cu_T} = 5.0 \times 10^{-8} \underline{M}$	Skinface Pond $\underline{Cu_T} = 1.7 \times 10^{-7} \underline{M}$	Artesian Well + 10% Skinface Pond $\underline{Cu_T} = 1.7 \times 10^{-7} \underline{M}$
3.6×10^{-3} (2.2×10^{-3})	4.9×10^{-3} (1.0×10^{-3})	7.4×10^{-3} (1.5×10^{-3})	1.6×10^{-2} (4.0×10^{-3})

Bioconcentration factors were calculated with total copper concentrations and Cu⁺⁺ concentrations in water (Table 7). When Cu⁺⁺ concentrations were used, there was less variability among BCF than when total copper concentrations were used. This indicates that differences in water chemistry affect the relative amount of copper accumulated.

Further analysis of the effects on copper accumulation by *S. serrulatus* reveals that the amount of Cu⁺⁺ in SPW was 98% less than in AWW when the total copper concentration was $1.7 \times 10^{-7} \underline{M}$. The copper accumulation was 54% less. This result indicates that accumulation of copper is not strictly first order with respect to Cu⁺⁺ concentration.

Acute toxicity bioassays were conducted at different times of the year (Table 8). The amount of organic carbon in SPW changed seasonally as did the values of the 24 h-LC₅₀ (Table 8). The 48 h bioassays did not show any trends with organic carbon content. This was

TABLE 7

Predicted Cu^{++} concentrations and bioconcentration factors (BCF) for copper accumulated by S. serrulatus. $\text{BCF} = (\text{Cu concentration in } \underline{\text{S. serrulatus}}, \text{M Cu} \cdot \text{mg}^{-1}, \text{dry weight}) / (\text{Cu concentration in water, } \underline{\text{M}})$.

	Skinface Pond		Well Water + 10% Pond	
$\text{Cu}_T(\text{M}) =$	5.0×10^{-8}	1.7×10^{-7}	5.0×10^{-9}	1.7×10^{-7}
$\text{Cu}^{++}(\text{M}) =$	1.05×10^{-9}	3.7×10^{-9}	4.02×10^{-9}	1.6×10^{-7}
$\text{BCF}_{\text{Cu}^{++}} =$	4.7×10^0	2.0×10^0	9.0×10^{-1}	1.0×10^{-1}
$\text{BCF}_{\text{Cu}_T} =$	9.6×10^{-2}	4.3×10^{-2}	7.2×10^{-1}	9.4×10^{-2}

TABLE 8

Acute bioassays of copper toxicity to S. serrulatus. Duration of test and total organic carbon concentrations are given as well.

	Test Duration (h)	LC_{50} (M)	Total Organic Carbon ₁ (mg · L ⁻¹)
Pond Water			
February 22, 1981	24	0.12 (7.2) ^a	3
March 19, 1979	48	0.45 (28.5)	11
April 9, 1979	48	0.68 (43.0)	12.4
October 15, 1979	48	0.25 (16.0)	15.6
	24	0.39 (24.5)	15.6
Well Water			
April 2, 1979	24	0.19 (12.0)	0.5

^aValues in parentheses are reported as $\mu\text{g Cu} \cdot \text{L}^{-1}$.

probably due to starvation in the longer duration tests. The LC_{50} values for exposure to copper were positively correlated with total dissolved organic carbon (TDOC) concentration.

The binding capacity of SPW and AWW + 10% SPW was positively correlated with total organic carbon concentration. The 24 h- LC_{50} value for SPW was twice as great as for AWW (Table 8), while the concentration of Cu^{++} ion was predicted to be two orders of magnitude less (Table 9). This result indicates that the relationship between free copper and toxicity may not be directly proportional in soft waters, such as those studied here. If the Cu^{++} is the toxic species and there is no synergism, antagonism or additivity due to other chemical species, the LC_{50} , calculated from the Cu^{++} concentration rather than the total Cu concentration, in waters with different binding capacities should be the same. It follows that LC_{50} values based on the total Cu concentration should be positively correlated with the binding capacity for Cu,

TABLE 9

Relative concentrations of Cu species at a total Cu concentration of $10 \mu\text{g} \cdot \text{L}^{-1}$ ($1.6 \times 10^{-7} \text{M}$) in toxicity studies calculated by GEOCHEM.

	Skinface Pond ^b (%)	Artesian Well ^c (%)
Cu ⁺⁺	1.1	91.4
CuOH ⁺	1.1	8.4
CuSO ₄	a	0.1
Cu β 1	97.7	a
Cu β 2	a	a

^aSpecies which represent less than 0.1% of the total Cu are not reported.

^bOctober 15, 1979, 24 hr-LC₅₀ bioassay

^cApril 2, 1979, 24 h-LC₅₀ bioassay

as was observed in this study. However, when LC₅₀ values were calculated from Cu⁺⁺ we found that the values were much lower in SPW than they were in AWW with 10% SPW. When calculated in this way, the 24 h-LC₅₀ in AWW was $1.7 \times 10^{-7} \text{M}$ and the 24 h-LC₅₀ in SPW in February and October were 1.3×10^{-9} and $4.3 \times 10^{-9} \text{M}$, respectively. The conclusion from this finding is that Cu⁺⁺ is much more toxic in the presence of some constituent of SPW. The SPW alone cannot be the source of the toxicity, since the *S. serrulatus* occur in this water naturally and no mortality was observed in controls.

Guy and Kean [15] found that in addition to Cu⁺⁺ the citrate complex (Cu CIT OH⁻²) was a toxic species to the green alga, *Selenastrum capricornutum*. Guglielmucci et al. [51] found that, in addition to the ionic copper, the copper bound to low molecular weight organic compounds are available and reactive toward test organisms. Giesy et al. [52] observed increased toxicity of Cd due to an organic carbon with a nominal ultrafiltration pore diameter of less than 0.009 μm , which had been extracted from Skinface Pond, even though organic carbon in this fraction exhibited binding capacity for Cd. These results suggest that toxicity may not be readily predicted from calculation of metal speciation in all cases if Cu⁺⁺ is assumed to be the most available and the most toxic form.

CONCLUSIONS

We measured conditional stability constants for Cu interaction with naturally-occurring organic compounds in the soft-acid waters of the southeastern coastal plain. We concluded that these organic materials are important in determining the form of Cu.

The presence of organic matter decreased the accumulation of Cu by the softwater cladoceran *Simocephalus serrulatus*. When the bioconcentration factors (BCF) were calculated, using Cu⁺⁺ instead of total Cu concentration, the BCF were similar for all water types, while the BCF varied greatly due to varying amounts of organic matter. From this we concluded that

most of the copper accumulated by *S. serrulatus* was Cu^{++} or the labile aquatic forms and that a decrease in Cu^{++} due to binding of Cu by organic matter reduces accumulation of Cu.

Organic matter reduced the toxicity of Cu to *S. serrulatus*. However, the increase in 24 hr-LC₅₀ was not proportional to the decrease in Cu^{++} predicted by the simulation model GEOCHEM. From this we conclude that organic matter reduces the amount of Cu available for accumulation and the overall toxicity of Cu. However, the toxicity, reported as the 24 hr-LC₅₀ for Cu^{++} , was enhanced by the presence of organic matter. This has possibly far-reaching implications. One implication is that Cu^{++} is not the only toxic species and that Cu toxicity may be facilitated by the presence of organic matter. Therefore, toxicity may not be predictable directly from geochemical speciation. This also indicates that toxicity of Cu may not be directly proportional to Cu accumulation by microcrustaceans.

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