EFFECT OF COPPER BINDING BY SUSPENDED PARTICULATE MATTER ON TOXICITY

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Abstract—The kinetics of Cu(II) interactions with Susquehanna River (mid-Atlantic, USA) particle suspensions using the copper ion selective electrode (Cu-ISE) method indicated that the concentration of Cu²⁺ in both the suspension and filtrate was kinetically controlled; the reaction of Cu(II) in the suspension was faster than that in the filtrate. Bioassay tests were performed in continuous flow-through bioassay systems to examine the effect of kinetics of Cu(II) interactions with suspended solids on the toxicity of Cu to Ceriodaphnia dubia. The toxicity curves were displaced to higher total Cu concentration as the reaction time increased, indicating that such interaction of Cu with solids was time dependent. Further, the toxicity curves overlapped for reaction times of 6 and 24 h, indicating that the reaction was relatively rapid and that equilibrium was achieved within 6 h. The survival of organisms was related to the free Cu²⁺ concentration but deviated from the result for bioassays in which dissolved organic matter (DOM) rather than particles reacted with the added Cu(II) to affect the free Cu²⁺ concentration. It may be interpreted that, besides the toxic effect of Cu²⁺, particles exert adverse influences on the organisms.

Keywords—Copper Suspended particles Kinetics Toxicity

INTRODUCTION

The speciation of trace metals in natural waters is important in terms of bioavailability, transport, and fate in the aquatic environment and water quality criteria [1–4]. In addition to complexation with natural organic matter [e.g., 5,6], the significance of sorption by particulate matter in regulating the speciation and concentration of trace metals in the water column is widely recognized. Morel and Hering [7] concluded that the chemistry of trace metals in the water column is dominated by complexation, biological uptake, and sorption to suspended solids. Suspended solids consist of aggregated materials including both biotic (phytoplankton and bacteria) and abiotic (inorganic and detrital organic matter) components that play an important role in removing trace metals (especially Cu and Zn) from the water column [8]. Iron and manganese oxides represent additional scavenger phases with large surface areas, while calcium carbonate is inefficient as a carrier phase [9].

Although there are quite limited studies of the effects of suspended solids on metal toxicity compared with bioassays or toxicity tests of the effects of dissolved organic matter (DOM), evidence does exist that indicates adsorption of metal ions onto suspended solids can decrease their toxicities on the basis of the total concentration of the metal [e.g., 10–12]. Brungs et al. [10] conducted bioassays using a dilution water from a natural stream that received the effluent from a sewage treatment plant. Because the tests were conducted over a nine-month period, water quality varied considerably. For example, hardness ranged from 148 to 340 mg/L expressed as CaCO₃. They reported that toxicity on the basis of total copper concentration varied by a factor of 13, while on the basis of filterable copper, it varied by only a factor of 1.6, suggesting the copper associated with suspended solids was not biologically available. Like bioassays of DOM, almost no studies have addressed the kinetics of copper interaction with suspended particles and its effect on the toxicity to aquatic organisms. Allen et al. [13] indicated that reactions of metal ions with colloidal or particulate matter were rapid, requiring no more than a couple of hours to reach equilibrium. This was substantiated by bioassay tests in the current study in which we investigated the effect of kinetics of copper–particle reaction on the toxicity of copper to Ceriodaphnia dubia.

This investigation is a continuation of our study of the interaction of copper with components of natural waters to further ascertain the relationship between speciation and toxicity. In our previous articles [6,14], we studied the kinetics of copper with natural organic matter and humic substances and the influence of equilibration time on the toxicity of copper. This article concentrates on the reactions of copper with suspended particles in the water column. The effect of such interactions on copper toxicity was examined using the bioassay systems described in the above articles.

MATERIALS AND METHODS

Collection of natural suspended solids

Particulate matter in a suspension is defined as the material that is retained on a 0.4- to 0.5-μm pore-size filter [15]. In this study, a tangential flow filtration (TFF) system was used to concentrate the natural suspended solids collected from river waters. The TFF has received considerable attention as a promising technique for isolating submicron particles and macro-molecules (colloids) in natural waters, especially for large-volume samples [e.g., 16–18].

An approximately 500-L surface water sample was collected from the Susquehanna River (MD, USA) in January 1998 using HNO₃ presoaked 25-L carboys for sample storage and transportation. Suspended particulate matter in the sample...
was immediately concentrated in the laboratory using a Millipore Pellicon® TFF system (Millipore, Bedford, MA, USA) with a 0.45-μm Pellicon filter membrane cartridge. Samples were pumped from a 150-L high-density polyethylene bag-in-bottle reservoir into the membrane cartridge. The permeate flow was diverted to a collection line, and the retentate flow was recycled back to the reservoir. Transmembrane pressure (≈25 psi) was controlled by a clamp on the retentate tube and/or the pump speed. The final volume of the concentrated suspension was about 12 L (i.e., the original water sample was concentrated approximately 40-fold). Both the concentrated suspension and 25 L of permeate solution were stored in the refrigerator at 4°C until use. The TFF system was sequentially cleaned before processing each sample with 1% (v/v) liquid laboratory detergent (Citranox, Alconox, New York, NY, USA), 0.1 N NaOH, and 0.01 N HNO₃ by recycling each cleaning solution through the system under pressure (30 psi) for at least 30 min. After each reagent, the system was flushed with 10 to 20 L distilled water and then 10 to 20 L deionized water. The retentate and permeate solutions of the final flushing with deionized water were monitored for copper content to verify that the TFF system was appropriately cleaned.

Physical and chemical analyses

Inductively coupled plasma atomic emission spectroscopy (Spectro Analytical Instruments, Fitchburg, MA, USA) was used for copper determinations. Total recoverable copper concentrations were determined by the microwave digestion with mixed acids method [19]. The free Cu²⁺ activity was determined by a cupric ion selective electrode (Cu ISE, model 94-29, Orion Research, Boston, MA, USA) and a double junction Ag/AgCl reference electrode (Model 90-02, Orion Research). The calibration of the Cu-ISE and Cu²⁺ activity measurements using the Cu-ISE followed the procedure of Ma et al. [6]. Total suspended solids (TSS) were determined by the standard gravimetric method [20].

Kinetics of copper binding by suspended particles and effect on toxicity

The suspension was filtered through two layers of 43-μm nylon screen. Following filtration, the suspension was diluted as indicated for chemical kinetics measurements or bioassay testing. Chemical kinetics measurements were conducted at pH 6 and bioassays were conducted at pH 8.

Kinetics of copper interactions with the Susquehanna River particle suspension and its filtrate were examined at pH 6.0. The response of the Cu-ISE was not stable and drifted quickly in the presence of solids at pH 6.0. The use of the lower pH minimized the dissolution of organic matter that would complex with the added Cu [21]. The experimental conditions were TSS = 180 mg/L, prepared by dilution of concentrated suspension with the filtrate of river water, adjusted to I = 0.01 M with NaNO₃; pH = 6.0 by adding 0.1 N HNO₃ or NaOH during the experiment; and CuT = 20 μg/L. The kinetics of copper binding by Susquehanna River suspended solids was examined in the titration reactor as described in [6,14]. The potential on the Cu-ISE after the addition of Cu(NO₃)₂ solution to the above suspension was recorded automatically using an in-line computer. Reaction kinetics of copper with colloids and dissolved material in the suspension and with dissolved material in the filtrate were examined.

Medium hardness dilution water, prepared according to the U.S. Environmental Protection Agency procedure [22], was used to adjust the concentrated suspension to 20 mg TSS/L for bioassay tests. The diluted suspension was equilibrated by shaking for at least 2 d prior to the experiment. The pH was 8.0.

The effect of kinetics of copper binding by natural suspended particles on the toxicity of copper to Ceriodaphnia dubia was examined in our bioassay system [6,14]. The system was modified for the particle bioassays to satisfy the requirement of toxicity testing of a suspension by clamping the bioassay chambers into a vertical orientation with fluid entering at the top of the chamber.

The bioassay apparatus, shown in Figure 1, consisted of a reservoir for storage of the suspension or its filtrate, a reservoir for the copper solution, peristaltic pumps, 4 chemical mixing reactors, 12 flow-through bioassay chambers, and 3 peristaltic pumps. The suspension in the sample reservoir (20-L carboy) was stirred continuously at approximately 250 rpm by a StedFast® Stirrer (Model SL2400, Fisher Scientific, Pittsburgh, PA, USA). The stainless steel impeller and its shaft were tightly wrapped with Teflon® film. The suspension was pumped from the sample reservoir with a peristaltic pump, copper was added using another peristaltic pump, and the metal-spiked suspension entered the first of four completely mixed chemical reactors that were connected in series, as has been described in more detail in our earlier publications [6,14]. The chemical reactors and bioassay chambers were constructed of Plexiglas®. A shaded pole gear motor (Dayton Electric, Niles, IL, USA) and a high-density polyethylene stirrer (Nalgene, Rochester, NY, USA) were used to stir the solution in each chemical mixing reactor except for the first mixing reactor (volume = 0.02 L), which used a magnetic stirrer and a stir bar because the water level in it was so low. Two peristaltic pumps delivered the particle suspension and Cu solutions into the first chemical mixing reactor at a constant flow rate of 600 ml/h. The chemical mixing chambers consisted of four com-
of particles in the chambers. The pore size of screened enclosures of bioassay chambers to prevent the escape of the organisms and to minimize the hydrodynamic jet effect on the organisms from the influent stream. The exposure period was 24 h for the toxicity tests.

Pretests on the particle concentration pumped from the sample reservoir were performed to ensure that it was constant with time. The particle concentrations from the four completely mixed chemical reactors in series. The volume of each reactor was selected to provide the target hydraulic residence times (HRT) of 2 min and 1, 5, and 23 h for reaction of Cu with the suspended particulate matter. A portion of the reactor content from each mixing reactor was continuously delivered to each of three 50-ml flow-through bioassay chambers containing Ceriodaphnia dubia neonates that were less than 24 h old. The liquid exchange rate in the bioassay chamber was 24 per h; the HRT in the bioassay chamber was 1 h. Thus, the overall HRT, or chemical reaction time, was equal to the sum of the HRT in the mixing reactor plus that in the bioassay chamber, or 62 min and 2, 6, and 24 h. The test organisms were confined in Nytex (Tetko, Briarcliff Manor, NY, USA) screened enclosures of bioassay chambers to prevent the escape of the organisms and to determine that the reaction rate constants that are reported are net and are valid only for the conditions of measurement. The change of $[\text{Cu}^{2+}]$ as a function of time may be expressed as

$$[\text{Cu}^{2+}]_i = [\text{Cu}^{2+}]_0 \sum_{i=1}^5 [p_i \exp(-k_i t)]$$  \hspace{1cm} (1)$$

where $[\text{Cu}^{2+}]_i$ is the $\text{Cu}^{2+}$ concentration remaining in the solution after time $t$, $[\text{Cu}^{2+}]_0$ is the initial $\text{Cu}^{2+}$ concentration, $p_i$ is the proportion of the initial $\text{Cu}^{2+}$ concentration that reacts with the $i$th site, $k_i$ is the rate constant for the reaction of $\text{Cu}^{2+}$ with the $i$th site, and $t$ is time. Although in many cases the reaction kinetics can be described equally well by different models and the model fitting alone does not allow one to reach any conclusions regarding reaction mechanisms, this model allowed comparison of the results for suspended solids with those for filtrates.

The fitted results indicated that the reaction of copper with the suspension ($k_1 = 2.24 \text{ h}^{-1}$, $k_2 = 0.00599 \text{ h}^{-1}$) was somewhat faster than the reaction of copper with the filtrate ($k_1 = 1.04/\text{h}$, $k_2 = 0.005/\text{h}$). This is consistent with published results. For example, Allen et al. [13] reported that reactions of Pb and Cd with colloidal or particulate matter from Lake Michigan were rapid, requiring no more than a couple of hours to reach equilibrium, while complexes of Pb and Cd with soluble organic matter had not reached equilibrium after 24 h. Yin [23] demonstrated that the time required to reach equilibrium for Hg sorption on soils depended on soil properties. The higher the soil organic matter content, the longer the time needed for the reaction to reach equilibrium. Our results also showed that the sorption reaction of Cu at pH 6.0 was even faster than the complexation reaction of Cu with DOM at pH 8.0 (high pH was favored for the fast reaction for both adsorption and complexation; data not shown). The bioassays were then applied to investigate the kinetics of copper interaction with the suspension (in dilution water) at pH 8.0 and its effect on copper toxicity to Ceriodaphnia dubia.

More precautions were taken in bioassays for suspensions, as mentioned in the Materials and Methods section, because the bioassay systems were originally designed for solution toxicity tests. One of the most important factors taken into account was the particle size of the suspended solids. A laminar sedimentation equation could be used to calculate the settling velocity of the particles if the Reynolds number was less than one. For example, if the maximum diameter (assuming the particle is a spherical shape) of the particles is 177 μm (the pore size of screens in bioassay chambers), assuming the density of particles is 1.05 g/cm^3 and the viscosity of the river water is 0.01 g/cm-s, then the Reynolds number can be calculated by

$$R_e = \frac{d_p V_s \rho_i}{\mu}$$  \hspace{1cm} (2)$$

where $R_e$ is the Reynolds number, $d_p$ is the diameter of the particle, $\rho_i$ is the density of liquid (we assumed the density of the river water was 1.0 g/cm^3), $\mu$ is the viscosity of the liquid, and $V_s$ is the settling velocity of the particle, which was computed as

$$V_s = \frac{d_p^2 (\rho_i - \rho_p) g}{18 \mu}$$  \hspace{1cm} (3)$$

Since the experimental conditions were not favored for the reverse reaction (desorption), the rate constant for desorption was not considered. Nevertheless, the rates that were observed and consequently the rate constants that are reported are net and are valid only for the conditions of measurement.
Effect of suspended particulate matter on copper toxicity

Environ. Toxicol. Chem. 21, 2002 713

where \( p_0 \) is the density of the particles and \( g = 980 \text{ cm/s}^2 \).

The calculated settling velocity for 177 \( \mu \text{m} \)-diameter particles is about \( 8.53 \times 10^{-2} \text{ cm/s} \) and \( R_e = 0.151 < 1 \). Because \( R_e \) value is less than one, we can use the laminar sedimentation equation to calculate the settling velocity of particles.

For particles of the same composition and shape, the larger the particle, the faster it settles. If the bioassay chamber is placed in a horizontal orientation, two velocities affect the flow of the suspension through the chamber. The maximum settling velocity for which the particles will flow through the chamber without being deposited in the bottom of the chamber can be obtained. Under ideal conditions, particles with the settling velocity of \( 5.55 \times 10^{-3} \text{ cm/s} \) or greater will accumulate in the chamber if no eddy flow occurs. This critical settling \( \left( V_s^c \right) \) was estimated from a mean retention time of 1.5 h obtained from previously reported measurements for these bioassay chambers [6]. From Equations 2 and 3, we calculated that all particles larger than 14.3 \( \mu \text{m} \) would be retained in the chamber. Even for particles having a settling velocity less than \( V_s^c \), they would be removed in the proportion \( V_s^c/V_s \). For instance, about 50% of 10-\( \mu \text{m} \) particles would accumulate in the chamber. This accumulation of particles in the chambers would shift the distribution of particle-bound copper and soluble copper with time, resulting in a decreased concentration of bioavailable copper. In order to avoid the problem associated with the accumulation of particles in the bioassay chambers, we modified the arrangement of bioassay chambers by placing them in a vertical orientation, as shown in Figure 1.

The results of bioassay tests for the suspensions and filtrates plotted as survival (%) of organisms versus \( Cu_T \) concentration (\( \mu \text{M} \)) are shown in Figure 3. The toxicity curves were displaced to higher copper concentration as the reaction time of copper with the suspensions and filtrates increased, indicating that such reactions were kinetically controlled. In addition, the toxicity curves of suspensions for reaction times of 6 and 24 h (i.e., HRT = 6 h and HRT = 24 h) in Figure 3 overlapped, illustrating similar toxicity at these reaction times. This implied that the reaction of Cu with the suspension was rapid and the equilibrium was achieved within 6 h after the initiation of the reaction. During bioassay tests, the suspension samples from the effluent of each chemical mixing reactor and bioassay chamber were collected for two purposes. One aliquot was used to determine the free Cu\(^{2+}\) concentration in the suspension using the Cu-ISE method; the other one was used to determine the total solids concentration by the standard method. Although there was slight accumulation of particles in bioassay chambers (especially in low HRT such as 1- and 2-h chambers) near the entrance and exit ports, the particle concentrations determined for all samples were between 18.0 and 25.2 mg/L (Table 1). Furthermore, the particle concentration from the sample reservoir was monitored over a 48-h period and it was constant, around 20 mg/L.

Like the analyses of bioassay results for DOM in Ma et al. [6], we normalized the toxicity results in Figure 3 by using the Cu\(^{2+}\) concentration determined for each HRT instead of total copper concentration. The results plotted as survival (%) of organisms versus measured Cu\(^{2+}\) concentration are shown in Figure 4. There was a linear relationship between the percentage survival of Ceriodaphnia dubia and the measured Cu\(^{2+}\) concentration. The regression of all data, except for 0 and 100% survival, gave an equation of survival (%) = 127.25 \( - \frac{5.556 \times 10^{10}[\text{Cu}^{2+}]}{M} \), with a correlation coefficient of 0.9572. It was observed that this relationship deviated from
the regression line for DOM bioassay tests shown as the dashed line in Figure 4. This should not be interpreted as an example not conforming to the free-ion activity model [24] since the biological response in this system was still related to the Cu$^{2+}$ concentration rather than to the total copper concentration. This deviation suggests that, besides the toxic effect of free Cu$^{2+}$, particles may exert additional adverse effects on the organisms. In addition to the physical stress resulting from the presence of particles, this deviation may also be attributed to the exchange of free Cu$^{2+}$ from the low binding affinity sites on the particle surfaces to the high binding affinity site on the biotic surfaces. Furthermore, ingestion of Cu-laden particles may also contribute to exposure.

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REFERENCES