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Combined toxicity of copper and phenol derivatives to *Daphnia magna*: Effect of complexation reaction

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Abstract

The complexation of Cu with phenol was investigated in aqueous solution to find the changes in toxicity toward *Daphnia magna* in mixtures of copper and phenol derivatives and determine an appropriate prediction model for the toxicity of these mixtures. In the titration experiment, the results showed that phenol played an important role in the remarkable reduction of the Cu^{2+} concentration, due to its complexation with Cu, with the subsequent reduction in the toxicity of aqueous mixtures containing both Cu and phenol. As a result, it was clearly demonstrated that Cu–phenol formed a non-toxic complex toward *D. magna* as the mortality declined, despite the addition of phenol to a fixed Cu concentration. Meanwhile, prediction of the combined toxicity for binary mixtures of Cu and 11 phenol derivatives more accurately followed an independent action model ($p=0.143$, $df=124$, and $t=-1.475$ in t-test) than an effect summation model ($p\approx 0$, $df=134$, and $t=7.528$ in t-test) due to the dissimilar modes of action and the complexation reactions between Cu and each of the phenolic compounds. Consequently, this study supports the importance of considering complexation reactions in assessing the combined toxicity for the formulation of water quality in mixtures of heavy metals and organic compounds, and in these cases, an independent action model was found to be appropriate.

Keywords: Copper; Phenol derivatives; Complexation; Combined toxicity; *Daphnia magna*

1. Introduction

Toxicity data from tests on single pure chemicals in the laboratory provide fundamental information for the chemical risk assessment or for the formation of water quality criteria. Typically; however, aquatic organisms are not exposed to only a single substance, but rather simultaneously to multiple mixtures of chemicals. Due to the complexity of toxicants, many aquatic organisms are exposed to the mixture of heavy metals and organics in some cases. Most studies on mixture toxicity have been separately performed with heavy metal or organic chemical mixtures (Enserink et al., 1991; Otitoloju, 2002; Altenburger et al., 2000; Faust et al., 2001). However, the speciation due to the interactions between heavy metals and organic chemicals in natural waters is important in the toxicity to aquatic organisms and for water quality criteria (Allen et al., 1980; Allen and Hansen, 1996; Luoma, 1983). Over the last several decades, many studies have demonstrated that the bioavailability or toxicity of trace metals is directly correlated to the concentrations of free metal ions, rather than to the total or complexed metal concentrations (Anderson and Morel, 1978; Brand et al., 1986; Borgmann and Ralph, 1983; Campbell, 1995). Morel and Hering (1993) concluded that the chemistry of trace metals in a water column is dominated by complexation, biological uptake and sorption onto suspended solids.

Copper is able to form complexes with numerous inorganic and organic ligands that are present in water and wastewater. Furthermore, it was well known that the phenolic group of natural materials (i.e., humic substances) is one of the important functional groups in the complexation of copper. The free Cu^{2+} ions that cause toxicity toward aquatic organisms are reduced by their complexation with phenol, while it is not known if the toxicity of the Cu–phenol complexed form is enhanced or reduced. The combined effect of Cu and phenolic compounds can be predicted by two theoretical models, these being effect

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summation (or concentration addition) and independent action. The toxicities of chemical mixtures have been studied by several researchers for the development of new methods; i.e., concentration addition with similar modes of action, and independent action with dissimilar modes of action, for the prediction of mixture toxicities (Backhaus et al., 2000; Faust et al., 2001).

The purpose of this study was to first investigate the reaction of copper ions with phenolic compounds. These reactions were studied by determining the changes in the physical and chemical forms of the copper and by determining the toxicity of the copper to *Daphnia magna* as a function of speciation. In addition, the effects of 11 phenolic compounds following their addition to test organism exposed to Cu was estimated to observe the mixture toxicities when combined with copper and predict the combined effects using the effect summation and independent action methods for verification by a bioassay experiment with *D. magna*.

2. Materials and methods

2.1. Calibration of Cu-ISE and measurement of cupric ions

The free Cu^{2+} concentration was measured using a cupric ion selective electrode (Cu-ISE, model 740, Orion Research, Boston, MA, USA). The electrode was kept in a controlled room and cleaned for accurate measurements. The Cu-ISE was treated with polishing paper, soaked in 0.01 M H_2SO_4 solution, and rinsed with deionized (DI) water to maintain the electrode in good operational condition before each use (Ma et al., 1999). In order to confirm the calibration of free Cu^{2+}, first the Cu-ISE and reference electrode were calibrated using a Cu-ethylenediamine (EN) buffer, over the pCu range 5–12. It has been reported, using a Cu-EN buffer, that the linear response of the Cu-ISE can be obtained for Cu activities of pCu 12 (Benedetti et al., 1995) or even as low as pCu 19 (Avdeef et al., 1983). The calibration curve of the Cu^{2+} activity gave a consistent line in the Cu-EN buffer solution (slope=25.98 mV/pCu, r^2=0.998). The calibration was compared with previous results (Ma et al., 1999), which calibrated the free Cu^{2+} concentration using both Cu-EN buffer ($[Cu^{2+}]=10^{-13}-10^{-6}$ M) and $Cu(NO_3)_2$ (Aldrich Chemical) solution ($[Cu^{2+}]=10^{-7}-10^{-5}$ M) with EN buffer. Unlike in the Cu-EN buffer system, however, the performance of the Cu-ISE in the presence of phenol has been shown to be affected, despite several reports to the contrary with regard to the presence of humic and fulvic acids (Buffle et al., 1977). In this study, titrations were performed to obtain new calibration curves for free Cu^{2+} concentration in the presence of phenols. In order to adjust for the variation of an indirect measurement, a calibration curve was established for different phenol concentrations.

The total concentrations of Cu and EN, and the measured pH, were input into the MINEQL+ program (Schecher, 2001) to calculate the activity of free Cu^{2+} at each of the points in the calibration. In order to determine the binding ability of phenolic solution, copper titrations with phenol were performed under given conditions. Freshly prepared 5×10^{-6} M total Cu solution was used as the titrant, which was added to the phenol solution every 10–20 min, as this was the time required to attain a stable potential reading.

2.2. Bioassay experiment for mixture toxicity

The 11 phenol derivatives and the copper, as $CuSO_4{\cdot}5H_2O$, were purchased from Aldrich (Milwaukee, WI, USA). The test organism, *D. magna*, was obtained from the Korean Institute of Toxicology (Daejon, South Korea). The food, *Selenastrum capricornutum*, and yeast, trout chow and Cerophyll® (YTC) mixture were purchased from Aquatic Biosystem Inc. (Fort Collins, CO). The organisms were cultured and handled according to the procedures outlined in the EPA manual (U.S. Environmental Protection Agency, 1993). Culture water for *D. magna* was reconstituted hard water ($CaSO^4{\cdot}H_2O$ 120 mg/L, $NaHCO_3$ 192 mg/L, $MgSO_4$ 120 mg/L, KCl 8 mg/L) with a hardness of 150±10 mg/L as $CaCO_3$, alkalinity of 121±10 mg/L as $CaCO_3$, and pH of 8.0±0.2.

In order to determine the changes in the toxicity of the 11 binary mixtures of the copper and phenol derivatives, 11 identical experiments were set up. Acute 48-h toxicity tests of copper and the phenolic compounds were examined under static non-renewal conditions, using *D. magna*, in a temperature-controlled room, at 25±1 °C, maintained with a 16-h light and 8-h dark photocycle. At a minimum of 2 h prior to the test, *D. magna* were fed on YTC mixture and the green alga, *S. capricornutum*. Four replicates, holding five neonates of less than 24 h old, were set up for each concentration of the copper and 11 phenol derivatives binary mixtures, in 25 ml of test water. Each test set was comprised of different mixture concentrations and a control. The mortality was defined as the number of dead organisms remaining after 48 h of exposure. The target toxic units (TUs) of the 11 binary mixtures were prepared by equitoxic combination, where the amount of each chemical exposed to *D. magna* was calculated based on the concentration showing an equal individual 48 h LC50 value. For observing the changing toxicity with the variation in cupric ions, the phenol concentration in the first experiment was added to fixed copper at 0.5 TU (e.g., 1 TU=17 μg/L for Cu). The phenol concentrations ranged from 0 to 2.0 TU, in increments of 0.1 TU (e.g., 1 TU=11.6 mg/L for phenol). In the second experiment, the Cu concentrations spiked ranged from 0 to 1.6 TU, in increments of 0.1 TU, to a fixed phenol concentration of 0.43 TU. In this set, cupric ions (Cu^{2+}) were also measured using an ion selective electrode (ISE) throughout the entire bioassay experiments. Meanwhile, in order to determine the combined toxicity of the binary mixtures of Cu and the 11 phenolic compounds, bioassays were conducted within various TU ranges, from Σ0.1 TU to Σ3.0 TU, with a control, and the synergistic, additive or antagonistic effects of combined mixtures evaluated.

2.3. Prediction model of mixture

The combined effect of Cu and the phenolic compounds can be predicted using two theoretical models, these being effect summation (or concentration addition) and independent action (Backhaus et al., 2000; Faust et al., 2001). For expressing every concentration in relation to the EC_{50}, the concept of the toxic unit (TU) is introduced.

$$TU = \frac{c}{EC50}; \quad TU_{mixture} = TU_1 + TU_2 + \ldots + TU_n. \tag{1}$$

Here, the EC50 corresponds to 1 TU. After the acute toxicity test using the target chemicals, the effect summation can be calculated using the toxic unit obtained from mathematical and graphical methods. In a similar action for a

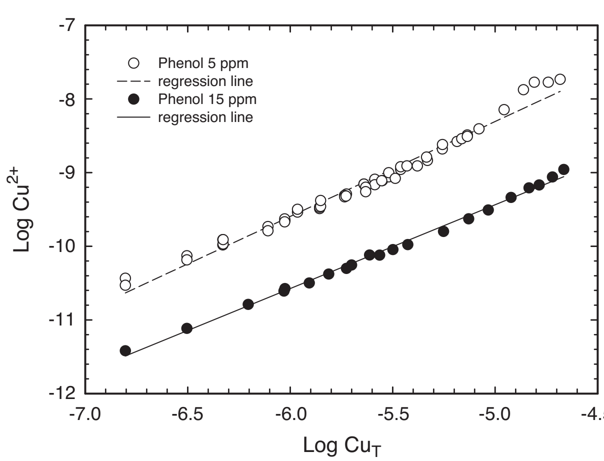

Fig. 1. Relationship between the free copper (Cu^{2+}) and total copper (Cu_T) concentrations at different phenol concentrations; 5 and 15 mg/L. Free copper concentrations were measured using an ion-selective electrode (ISE).