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Interactive toxic effects of heavy metals and humic acids on *Vibrio fischeri*

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Abstract

The effect of humic acids (HAs) on the toxicity of copper, zinc, and lead was investigated using the photobacterium *Vibrio fischeri* (Microtox test) as a test organism. The effects of HAs on metal toxicity were evaluated as functions of time and concentration in pure compound solutions. The toxicities of copper and lead were generally comparable, while the toxicity of zinc was lower than those of the other two metals. The toxicity of copper decreased with the addition of HAs, while the toxicity of zinc remained almost constant. On the other hand, the toxicity of lead increased, depending on the concentration of HAs. The interactive effects between copper and zinc and between lead and zinc were synergistic, while the interactive effect between copper and lead on the bioluminescence of *V. fischeri* was additive. The presence of HAs caused relatively high toxicity reduction in the binary mixtures of zinc and copper or zinc and lead, while the toxicity reduction in the case of the binary mixture of copper and lead was negligible.

Keywords: Microtox; Heavy metals; Copper; Zinc; Lead; Interactive effect; Humic acids; Bioavailability; Toxicity

1. Introduction

The presence of heavy metals in solid and liquid wastes is an important issue related to the pollution of the environment. It is generally accepted that the solubility, bioavailability, and toxicity of heavy metals are dependent on various physicochemical parameters such as pH, hardness, interactive effects, and presence of natural organic matter (Janssen et al., 2003; Heijerick et al., 2003; Hadjispyrou et al., 2001; Peijnenburg and Jager, 2003; Manusadzianas et al., 2003). The pH affects the solubility, speciation, and transportation of metals from solid to liquid phase. Additionally, heavy metals are often present in the environment in mixtures, making the assessment of environmental hazards even more difficult due to the antagonistic or synergistic actions that may occur. The investigation of the joint toxic effects of chemicals in a mixture is generally based on comparison of the actual toxic effect of the mixture with the theoretically expected toxic effect deduced by a statistical model, using the toxic effects of the individual chemicals (Aoyama et al., 1987; Kungolos et al., 1999; Mowat and Bundy, 2002)

The bioavailability of metals may be affected by the presence of natural organic matter, such as humic acids (HAs), which are produced by the degradation of dead organic materials. HAs consist of a variety of

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molecular structures such as alcylaromatic, quinoid, and aliphatic structures in the core and amino-acid-like or carbohydrate-like structures and carbonyl, carboxyl, phenyl, and hydroxyl groups in the periphery (Meems et al., 2004). HAs are able to bind a variety of metals at their carboxylic groups, altering the bioavailability and consequently the toxicity of these compounds. Many studies on the effect of complexation on the toxicity of heavy metals have been conducted using various complexing agents, such as ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), and various humic and fulvic acids. Sillanpaa and Oikari (1996) studied the impact of complexation by EDTA and DTPA on the toxicity of various heavy metals using the photobacterium *Vibrio fischeri*. The results indicated that the complexation did not significantly influence the toxicity of copper, cadmium, and mercury and reduced the toxicity of zinc and lead. Sorvari and Sillanpaa (1996) investigated the toxic effects of heavy metals with the presence of EDTA and DTPA on the crustacean *Daphnia magna*, concluding that the toxicity of copper, cadmium, and zinc decreased, while the toxicity of mercury slightly increased. Among the complexing agents, HAs are of major concern for many studies because they consist of natural organic matter ubiquitous in the environment. The assessment of the toxic effects of cadmium and chromium on the crustacean *Daphnia pulex* in the presence of various concentrations of HAs from 0.5 to 50 mg/L showed that the toxicity of cadmium depended on the concentrations of HAs, while the toxicity of chromium was not so influenced (Stackhouse and Benson, 1988). Furthermore, Winner (1984) studied the toxicity and bioaccumulation of cadmium and chromium in the presence of HAs using the crustacean *D. magna*. The results obtained from this study indicated that the presence of HAs decreased the toxicity of copper and increased the toxicity of cadmium, while there was no effect on the bioaccumulation for both metals tested. The effect of HAs on the toxicity of copper has been studied extensively compared with other heavy metals, due to the strong complexation capacity of this metal with HAs (Lubal et al., 1998; Pandey et al., 2000), resulting in most cases in a toxicity reduction (Winner, 1984; Kim et al., 1999; Alberts et al., 2000).

The interpretation of the complexation mechanisms of heavy metals in the presence of HAs and the consequent changes in their toxicity and bioaccumulation are very complicated issues. Therefore, various models have been developed for the identification of complexation mechanisms and of interactions of heavy metals with HAs (Tipping, 1998). Recently, the biotic ligand models (BLMs) providing a quantity framework for the assessment of metal toxicity over a range of metal speciation, pH, hardness, and inorganic and organic complexing agents levels have been developed (Paquin et al., 2000). The BLMs have been successfully applied for the prediction of the toxic effects of heavy metals, such as copper, silver, and zinc, on the crustacean *D. magna*, the microalgae *Selenastrum capricornutum*, and the fathead minnow *Pimephales promelas* (Janssen et al., 2003; Heijerick et al., 2002; De Schamphelaere et al., 2002; Di Toro et al., 2001).

Although many studies on the influence of complexation on the toxicity of heavy metals on various test organisms and on the interactive toxic effects of chemicals have been reported, there is a lack of available data on the effect of HAs on the toxicity of metal mixtures. Furthermore, few studies have dealt with the effect of HAs on the toxicity of metals in *V. fischeri* (Alberts et al., 2000), which does not exhibit high sensitivity to heavy metal toxicity in comparison to other test species, such as *D. magna* or *Lemna minor* (Ince et al., 1999; Kungolos et al., 2004). Still, *V. fischeri* is widely used because of its simplicity and rapid response, while at the same time it is considered a good indicator of cytotoxicity of compounds. The objective of this study was the evaluation of the effects of HAs on the toxicity of copper, zinc, and lead and on their binary mixtures, using *V. fischeri* as a test organism.

2. Materials and methods

2.1. Preparation of metal and HA solutions

The solutions with initial concentrations of 10 mg/L of the tested metals were freshly prepared by dissolving the following chloride salts in deionized water: zinc chloride ($ZnCl_2$) provided by Merck (Germany) and copper chloride dehydrated ($CuCl_2.2H_2O$) and lead chloride ($PbCl_2$) provided by J.T. Baker (The Netherlands). The effect of HA on the toxicity of the tested heavy metals was evaluated using 1, 10, and 20 mg/L HAs that were prepared by a stock HA (Fluka, Germany) solution of 100 mg/L. The stock HA solution was prepared at pH 10, with continuous stirring to achieve a complete dissolution. All chemicals used were of analytical grade and the concentrations of the metals were expressed as mg/L of metal ions. Furthermore, the pH value of all metal solutions was adjusted to 7 ± 0.2, prior to the toxicity tests, by the addition of 0.1 N HCl or 0.1 N NaOH solutions.

2.2. Toxicity assessment of heavy metals

The toxicity of heavy metals was evaluated using the bioluminescence bacteria *V. fischeri* (Microtox test) that were in freeze-dried form (SDI, USA) and activated prior to use by a reconstitution solution. Since *V. fischeri* is a marine organism, an adjustment of the osmotic