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Toxicity assessment of arsenic and cobalt in the presence of aquatic humic substances of different molecular sizes

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ABSTRACT

The release of contaminants in aquatic ecosystems can be influenced by humic acids. In this study, toxicity tests using environmentally relevant concentrations of arsenic and cobalt were conducted both in the presence and absence of aquatic humic substances (AHS) and the fractions of different molecular sizes in the range of (< 5, 5–10;10–30; 30–100 and > 100 kDa) using the microcrustacean *Ceriodaphnia dubia*. AHS together with arsenic reduced the toxicity, and the toxicity decreased in fractions of larger molecular size AHS. Despite the presence of cobalt, the reduction in toxicity was not observed and that depended on the molecular size of AHS. There was a trend of enhanced toxicity for Co in fractions of larger molecular sizes, opposed to that found for arsenic. Thus, the humic substances alter toxicity of trace elements, and this effect varies depending on the size of the humic substances

1. Introduction

The release of contaminants in water surfaces can modify environmental characteristics and, consequently, change the preexistent aquatic life. This environment is considered a complex system composed of metallic ions, dissolved gases and nutrients such as ammonium, phosphate, nitrate and also organic compounds such as amino acids, humic substances and particulate materials. Some metals with low concentrations (micrograms per liter) are degraded neither by chemical nor by biological reactions (Rocha et al., 2004).

Cobalt is an essential metal that is found in the active site of B12 vitamin and represents an important role in biochemical reactions of life. However, it is also considered toxic in high doses (also cumulatively), and its long-term exposure causes adverse health effects (Siegel, 2002; Simonsen et al., 2012). Therefore, the knowledge related to behavior of this metal is an essential requirement for risk assessment, in order to establish the water quality criteria (Lock et al., 2004; De Schamphelaere et al., 2008). Arsenic is specifically mentioned as a metalloid for having both metallic and nonmetallic characteristics. Even for arsenic, other elements such as arsenic and sulphydric groups of proteins could exert toxic effects by binding to nonmetallic constituents of cellular macromolecules (Walker et al., 2012). Jain and Ali (2000) reported the toxicity of different species of arsenic in the following order: Arsenite > Arsenate > monometilarsenate (MMA) > dimetilarsenite (DMA). In terms of toxicity effect, speciation is considered an essential technique for better understanding of mechanisms of toxicity for chemical elements (Craig, 1986). While chemical analysis identifies and quantifies concentrations of substances, toxicity tests assess the effect of these substances on biological systems. In addition to the above mentioned facts, understanding of the physiological behavior of the bodies assists us in understanding their association with related toxic effects (Costa et al., 2008; Dodds, 2002; Wetzel, 2001). Freshwater invertebrates such as *Daphnia magna* and *Ceriodaphnia dubia* are organisms recommended by Environmental Protection Agency (EPA) for toxicity tests in aquatic environments.

The organic matter usually found in aquatic environment influences the reduction of toxicity levels via naturally available elements such as metal ions of organisms present in these environments (Al-Reasi et al., 2011). Formed from the biological and enzymatic decomposition of plant and animal residues, humic substances (HS) are considered the main component of natural organic matter (NOM), globally present in the terrestrial and aquatic environments (Stevenson, 1994). The

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molecular fractionation is a procedure that helps to understand better the complex matrices of the organic matter present in the aquatic environments, which in turn has proven to be one of the most important techniques for studying the behavior of these substances in water (Sargentini Jr. et al., 2001). Limited knowledge about the influence of the fractionally dissolved organic carbon (DOC) in complex solutions, become a factor of concern due to the potential of contamination represented by the pollutants in an aquatic environment (Markich et al., 2002).

The study of humic substances based on molecular size could be considered, in principle, as a simple method to study the nature of complex mixtures of macromolecules by their molecular sizes, thus facilitating the study of the properties of HS (Swift, 1989). This study may be accomplished by using the tangential flow ultra-filtration technique with membrane filters (Burba et al., 1998; Rocha et al., 2000). The methodology focused on the use of fractionation of humic substances using an ultra-filtration system to separate and concentrate the humic substances extracted from both water and soil samples (also useful and suitable to work with samples of high volumes). Ultrafiltration has been established as an efficient and reliable technique for the evaluation of metal–HS stability function (Nifant'eva et al., 1999). In this context, the present study aimed to evaluate the influences of different molecular sizes of Aquatic Humic Substances (AHS) with As e Co, by using *Ceriodaphnia dubia* as part of ecotoxicological tests.

2. Materials and methods

For assessment of arsenic and cobalt interactions with AHS the following procedural steps are depicted in Fig. 1.

2.1. Water collection and AHS extraction

Water used in this experiment was collected from Sorocabinha River which is situated in Iguape city, Brazil (Latitude: 24°41′59″S, Longitude: 47°33′05″W). This region is famous for having high concentration of natural organic matter. Chromatographic method following literature recommendation (International Humic Substances Society, IHSS; Thurman and Malcolm, 1981) using column packed resin Superlite™ DAX-8 (Sigma Aldrich) was employed in extraction procedure. The water collected in Sorocabinha River was acidified to a pH value of 2 and the humic substances were retained in the resin when the water passed through the XAD-8 column in a slow stream. The AHS adsorbed was eluted with Sodium Hydroxide (P.A. Dinâmica) at 0.1 mol L^{-1}. The humic substances extracts were acidified to obtain a pH value of 6.2 using HCl (P.A. Synth) solution at 0.1 mol L^{-1} and stocked. The pH value was adjusted according to *in situ* measurement.

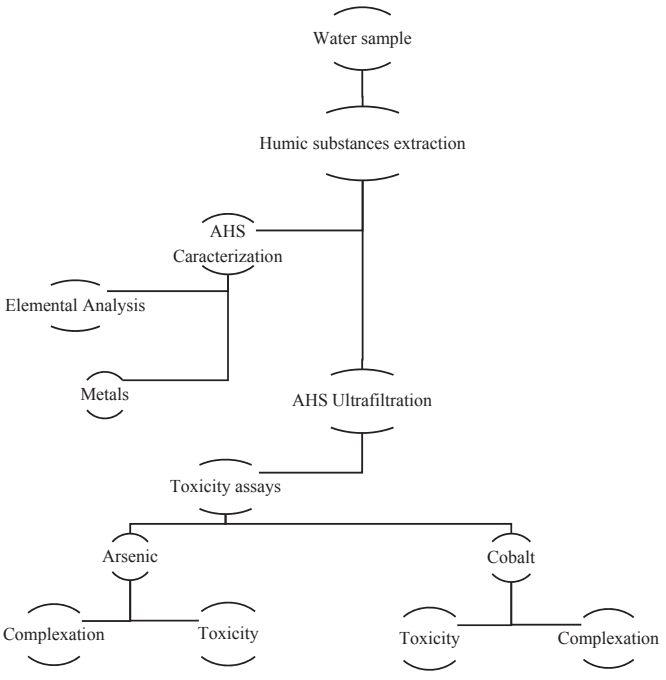

Fig. 1. Diagram of the steps for ecotoxicology assesment of arsenic and cobalt using *C. dubia* at this work.

2.2. Molecular fractionation using ultra-filtration process

With use of a sequential UF through a series of appropriate membranes, it was possible to characterize the distribution of HS and its metal species as a function of molecular size (Burba et al., 1998). The extracted AHS based on procedures described in item 2.1 was subjected to a sequence of molecular fractionation, comprising an ultra-filtration system (UF System) with specific sizes of regenerated cellulose membrane (Millipore of 76 mm diameter) and UF cell, adapted from the experimental description (Burba et al., 1998; Rocha et al., 2000). The system was connected to Tygon® brand tubes with flow controllers using a peristaltic pump. Initially, the system was cleansed with ultra-pure water, HCl solution at 0.001 mol L^{-1} and NaOH solution at 0.1 mol L^{-1}. Towards the end of the procedure as described before, the residue was withdrawn for further cleansing to achieve an earlier ultra-filtration level before initializing the fractionation. Then, 250 mL of AHS extract, 1.6 g L^{-1} TOC was primarily subjected to ultra-filtration process using a membrane of 100 kDa porosity. After complete ultra-filtration (about 12 h), the UF cell was carefully opened, the retained material was collected using ultra-pure water and measured by means of a volumetric flask of 25 mL, for subsequent quantification of the carbon content present in the fraction. The same procedure was sequentially repeated using different membranes with porosity values of 30, 10 and 5 kDa respectively to enable filtering the contents of the material under consideration in the descending order, related to the size of the aforementioned pore membranes. At the end of the 4th sequential step of ultrafiltration, 5 fractions of different molecular sizes were obtained: > 100 kDa, between 30 and 100 kDa, between 10 and 30 kDa, between 5 and 10 kDa, and lower than 5 kDa: F1, F2, F3, F4 and F5, respectively. The dissolved organic carbon (DOC) was determined at high temperature (850 °C) oxidation using carbon analyzer (AnalytikJena multi N/C 3100). Potassium hydrogen phthalate (P.A., Synth) was used to determine organic carbon while sodium carbonate (P.A., Qhemis) and sodium hydrogen carbonate (P.A. Synth) were used to determine inorganic carbon.

2.3. AHS characterization

For elemental analysis, 50 mL of AHS was dried using lyophilization process, a low heat method that obtains solid sample, as described in Aiken (1985 apud Rocha and Rosa, 2003). Part of dried sample was conducted by an elemental analyzer (CE Instruments), thus obtaining percentual of carbon (C), hydrogen (H), nitrogen (N) and sulfur (S). An acid digestion was performed on AHS in order to determine the concentration of metals. Therefore, based on the method 3050b by US Environmental Protection Agency (US EPA, 1996), AHS was dried at a temperature of 35 °C and 0.1 g of humic material was weighed in triplicate. Using a sub-boiling system, the acids used in digestion process were previously purified by distillation process. Concentrated hydrochloric and nitric acids and concentrated hydrogen peroxide were added at temperature of up to 100 °C using the heating support plate. Subsequently after completing digestion, each sample was measured using a volumetric flask and quantified using ICP-OES, 720 series Model, Agilent equipment. The instrument parameters used in this experimental work were 1.1 kW (power), 15.0 L min^{-1} (plasma flow), 1.5 L min^{-1} (auxiliary flow), 0.75 L min^{-1} (nebulizer flow), 1.0 s