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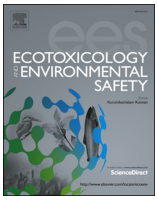

Pb (II) bioavailability to algae (*Chlorella pyrenoidosa*) in relation to its complexation with humic acids of different molecular weight

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ABSTRACT

Humic acid (HA) has a major influence on the environmental fate of metal ions due to its heterogeneity in chemical compositions, structure and functional groups. In this study, we investigated the effect of humic acid (HA) with different molecular weight (Mw) on the bioavailability of Pb for a representative algae-*Chlorella pyrenoidosa*. The results showed that HA with larger Mw had stronger inhibitory effects on the bioavailability of Pb to algae, and the biosorption capacity of Pb decreased with increasing Mw, which is in accordance with the variations of complexation capacities of Pb for HA fraction. In addition, we found that HA with Mw lower than 10 kDa could increase the biosorption capacity of Pb. The considerable differences among the Mw fractions on Pb biosorption were mainly attributed to their properties and corresponding complexation capacities. Phenolic groups were responsible for the variations of binding capacities among different Mw fractions, and it could also better explain the bioaccumulation of Pb to the membranes of algae. By using NICA-Donnan model, we found that over 60% of Pb ions were bound by HAs through specific binding, and the formation of Pb-HAs complex were non-bioavailable to algae, which was proved by the considerably decreasing percentage of internalized Pb. This study provided further insight into the bioavailability of Pb to algae as influenced by the complexation of HA with metal ion such as Pb.

1. Introduction

Heavy metal pollution is one of the main environmental issues aroused long-term attentions. The contaminants that enter the aquatic ecosystem are non-biodegradable and tend to accumulate in living organisms, causing various disorders. Pb has been identified as a hazardous element of environmental concern with high persistence. This metal can induce various morphological, physiological and biochemical dysfunctions in plants such as decrease in seed germination, plant growth, chlorophyll production (Fahr et al., 2013; EI-Banna et al., 2018). Pb could be accumulated in algae in aqueous solution (Atici et al., 2010), and the process of bio-accumulation has been used to control the concentration of Pb in freshwater and wastewater (Wang et al., 2010). Pb in aqueous environment mainly exists as ionic Pb^{2+} and complexes with various ligands such as humic acids. The formation of complex between Pb^{2+} and organic or inorganic ligands could influence its speciation and thus regulating its toxicity to aquatic biota (Shahid et al., 2012; Wang et al., 2018).

Humic acids (HAs), widely distributed in the environment, are an important natural ligands in regulating the speciation, bioavailability, and ultimate fate of trace metal element in the environment (Andrews and Huck, 1994; Prado et al., 2006; Kováčik et al., 2018). For instances, metal toxicity to algae were found to be relieved at the presence of HA via reducing free metal ion concentrations (Worms et al., 2015; Shi et al., 2017; Ondrasek et al., 2018). Several mechanisms were suggested to explain the effects of HA on the bioavailability or toxicity of trace metals to algae, including complexation with metals, alterations to the algae membrane permeability, and formation of ternary Pb-HS complexes on the algae surface (Lamelas et al., 2005; Lamelas and Slaveykova, 2008). Both the complexation of metals by HAs and the effects of HAs types on metal bioavailability/toxicity were intensively addressed, while few studies dealt with the internal relations between the bioavailability of Pb and HA properties such as the fractions of HAs with different molecular weights (Mw). Some studies showed that the toxicity of heavy metals to biota were relevant to the Mw of the HAs: the larger fractions of DOM (Dissolved organic matter) decreased the

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toxicity of copper to lettuce and *Daphnia magna*, while the smaller fraction had the opposite effect (Wang et al., 2010; Al-Reasi and Smith, 2012). However, the criteria of Mw and relevant properties of HA in relation to its roles in determining the toxicity of heavy metals such as Pb are still unclear.

The properties of HA fractions with different Mw, such as chemical compositions, functional groups and aromatic structure, may vary greatly from different sources (Gondar et al., 2006; Fujili et al., 2014; Xiong et al., 2013), among which, functional groups are considered to be the dominant factors influencing the sorption of metal ions on HAs, and the carboxylic and phenolic are recognized as the prevalent binding sites for metal ions (Koukal et al., 2003; Marsac et al., 2017). The relative abundance of these functional groups and the corresponding metal binding capacities varied among different Mw fractions. Generally, the smaller Mw fractions have higher binding capacities than larger Mw fractions due to the differences in content of functional groups. The complexation mechanisms of metals with HAs involve both non-specific (electrostatic) and specific complexation (Gondar et al., 2006; Xu et al., 2016), the relative importance of these two mechanisms varid with the composition of HA. It is believed that the lower Mw and more soluble fractions increase the mobility of heavy metals, while the higher molecular fractions usually inhibit the mobility and bioavailability of heavy metals by forming hydrophobic complexes (Jordan et al., 1997; Chotpantarat et al., 2015; Bahemmat et al., 2016). Thus, the identification of the complexation mechanisms is vital for understanding bioavailability/toxicity of metals to biota. Several complexation models have been developed to identify the complexation mechanisms and calculate the contributions of each mechanism to the apparent complexation. Among them, NICA-Donnan (Non-ideal competitive adsorption-Donnan) model is recognized as one of the reliable models providing good fit to experimental data (Milne et al., 2003; Plaza et al., 2005; Xu et al., 2016), and the evaluation of the metal speciation is essential for the prediction of the bioavailability and potential risk associated with the metal contamination.

Consequently, the high environmental relevance of HA and the lack of knowledge about the relationships between the bioavailability of Pb and the properties of Mw components motivated the present work. The specific objectives for this study were (1) to investigate the complexation mechanism of Pb to individual HA molecular weight fractions. (2) to reveal the correlations between the physicochemical properties of HA and the bioavailability of Pb.

2. Material and method

2.1. Regents and model algae preparation

The stock solutions of Pb (II) were prepared with ultrapure water (Milli-Q system water, 18 MΩ cm^{-1}) from $PbNO_3$ of analytical grade purchased from Sigma-Aldrich Company (USA).

The HA samples were extracted from the surface layer (0–10 cm) of a forest soil in Jinyun mountain, Chongqing, China (N 29°48′, E 106°23′). Before extraction, the soil samples were air-dried and sieved to 2 mm.

Chlorella pyrenoidosa was used as the model algae to investigate the influence of HA on lead (Pb) bioavailability and toxicity. This algae was chosen because of its widespread occurrence and abundance in fresh water. *C. pyrenoidosa* was obtained from the Institute of Hydrobiology, Chinese Academy of Sciences. The algae was cultured in Bristol's solution in an incubator (Hengyi MGC-450HP-2, Shanghai, China) at a room temperature of 25 °C and a regime of 12:12 h light: dark, with shaking twice a day. Algae cells were harvested when algae optical density (OD) was increased from 0.1 to 0.4 by gentle filtration, and re-suspended to an experimental density. Optical density was expressed as an OD value measured at 660 nm on an Aqualog fluorescence spectrophotometer (Varian Inc., USA) in UV–visible absorption mode, cell density was derived with an automated cell counter (AMQAX1000, America). The linear equation between cell number and OD was established base on our pre-experiments in our laboratory, as follows

$$N\text{algae}(\text{cell mL-1}) = 1{\times}10^{7}{\times}OD660 + 916632 \quad (R2 = 0.9996) \tag{1}$$

2.2. Fractionation and characterization of HA

Four molecular weight (Mw) fractions of HA (5–30 kDa) were fractioned by ultrafiltration. The method was described in Supporting information. HAs were characterized to obtain the results of elemental compositions, dissolved organic carbon (DOC); humification index (HIX), fluorescence index (FI); acidic group contents, Please see Supporting information for detail information.

2.3. Complexation measurements

The complexation of Pb with HA fractions (approximately 15 mg L^{-1} of DOC) was measured at two pH values (4.0 and 6.0) and two ionic strength (0.01 mol L^{-1} and 0.1 mol L^{-1} KNO_3). Before complexation, 10 mL HA was added into a beaker. Sorption experiments were performed at room temperature at 25 ± 0.1 °C using a stepwise Pb titration method. The titrants were 0.001 mol L^{-1} $PbNO_3$. After each step the equilibrium Pb^{2+} concentration was measured with a Pb ion-selective electrode (Pb-ISE) combined with an ion densimeter (BPX-931, China). Before starting metal titration, the pH was kept constant at a desirable value for 12 h. Titrations were performed under nitrogen gas atmosphere using an automated titration setup (Metrohm, 836 Titrando). The doses of $PbNO_3$ were gradually increased during the titration. 5 mL of $PbNO_3$ was added stepwise in total without exceeding the limit of precipitation. After each step the equilibrium Pb^{2+} concentration was measured with a Pb ion-selective composite electrode (Pb-ISE) (CS00PB202). The $Pb^{2+}/KNO_3/$was calibrated prior to each titration. The detail process of calibrate was provided in Supporting information. During the titrations, the solutions were stirred for 2 min after each addition of titrant. After metal addition, the pH was readjusted to the desired pH value by adding 0.001 mol L^{-1} NaOH and HNO_3, and then kept constant. The amount of Pb bound to HAs were calculated by subtracting the sum of inorganic lead species from the known total amount of Pb in solution. Three replicates were measured for the titration experiments.

2.4. Complexation data analysis

Conditional distribution coefficient (K_D) is one of the fundamental parameters in a quantitative description of HA binding with Pb^{2+}, which is used to characterize the stability of HA-Pb complex. The K_D was calculated as follows (Buschmann et al., 2006).

$$K_D = \frac{Pb_b}{Pb_f{\times}[HA]} \tag{2}$$

where Pb_b and Pb_f are free and HA-bound concentrations in solutions mmol L^{-1}; [HA] is the concentration of HA in solution in kg L^{-1}

Additionally, in order to investigate the mechanisms involved in the sorption process, Langmuir model and NICA-Donnan model were employed to simulate the experimental data and investigate the distribution of lead speciation.

Langmuir model:

$$Qe = \frac{\mathrm{Qm}kLC\mathrm{e}}{1 + kLC\mathrm{e}} \tag{3}$$

where, C_e (mmol L^{-1}) is the equilibrium Pb^{2+} concentration; Q_e (mmol/g) is the adsorbed amount of Pb^{2+} Q_m (mmol g^{-1}) and k_L is the sorption capacity parameter and sorption affinity constant of the Langmuir model.

NICA-Donnan model: