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Lead complexation behaviour of root exudates of salt marsh plant *Salicornia europaea* L.

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ABSTRACT

Root exudates are considered to have an important role in mobility and bioavailability of heavy metals. High molecular weight (HMW) substances are the main components of root exudates, however, knowledge about their interactions with heavy metals is lacking. In the present study, Pb(II) complexation of the HMW fluorescent fractions in root exudates from *Salicornia europaea* L. was investigated using excitation emission matrix (EEM) fluorescence spectroscopy. Two protein-like fluorescence peaks were identified in the EEM spectrum of root exudates. The fluorescence of both peaks was clearly quenched by Pb(II). The values of conditional stability constants, $\log K_a$, for these two protein-like fluorescence peaks were 4.14 and 3.79. This indicates that the fluorescent substances are strong Pb(II) complexing organic ligands.

Keywords: complexation, fluorescence quenching titration, lead, complexing organic ligands, root exudates, salt marsh plant

INTRODUCTION

Lead (Pb) is one of the most common heavy metal contaminants in soils. It is highly toxic and may exert various harmful effects on human health (Walker *et al*., 1996). Exposure to Pb for a long time can cause high levels of Pb in blood and consequently intellectual impairment, encephalopathy, coma, and even death (Canfield *et al*., 2003). Lower levels of lead in blood may have adverse effects on the central nervous, renal or hematopoietic systems (Meyer *et al*., 2003). Lead poisoning has attracted growing attention in China and many other countries.

Salt marshes are important sinks for anthropogenically derived heavy metals contaminants such as Pb and Cd (Fitzgerald *et al*., 2003). Lead accumulates in salt marshes and its concentration in soil is as high as from several hundreds to several thousands of mg kg^{-1} (Reboreda and Caçador, 2007).

Plants can release soluble organic substances from their roots and it has been estimated that 20–41% of the photosynthetates of a plant are secreted into the soil (Lynch and Whipps, 1990). Root exudates have been demonstrated to affect solubility, mobilization and phytoavailability of heavy metals (Bertin *et al*., 2003; Boularbah *et al*., 1996). Some studies showed that root exudates can improve the solubility of heavy metal ions such as Pb(II), Cu(II) and Cd(II) (Chiang *et al*., 2005; Degryse *et al*., 2008) and thus enhance metal accumulation in plants (Srivastava *et al*., 1999; Luo *et al*., 2008). In contrast, a few studies have reported that root exudates did not enhance metal mobilization and its availability to plants (Zhao *et al*., 2001; Hill and Lion, 2002). In addition, Hill and Lion (2002) showed that phytosiderophore 2′-deoxymugineic acid (DMA) reduced Cd accumulation in *Zea mays* due to the formation of DMA-Cd that might decrease the availability of Cd to *Zea mays*. Despite the major role of root exudates in affecting transport and bioavailability of heavy metals, very limited information is available on quantitative analysis of complexation of root exudates of salt marsh plants with metal ions, which is important for understanding the environmental behaviour of heavy metals in the rhizosphere environment.

The fluorescence excitation emission matrix (EEM) spectrometry is a rapid and sensitive modern technique that provides qualitative and quantitative information on the interaction between fluorescent organic matter and metal ions (Wu *et al*., 2001; Yamashita and Tanoue, 2003; Zhang *et al*., 2010). EEM fluorescence spectroscopy has been extensively used to investigate interaction of dissolved organic matter (DOM) and metals (Wu *et al*., 2001; Yamashita and Tanoue, 2003). Since proteins are also key components of root exudates and they show fluorescent properties, EEM fluorescence spectroscopy should be a powerful technology for the fluorescent components in root exudates and their interaction with metals.

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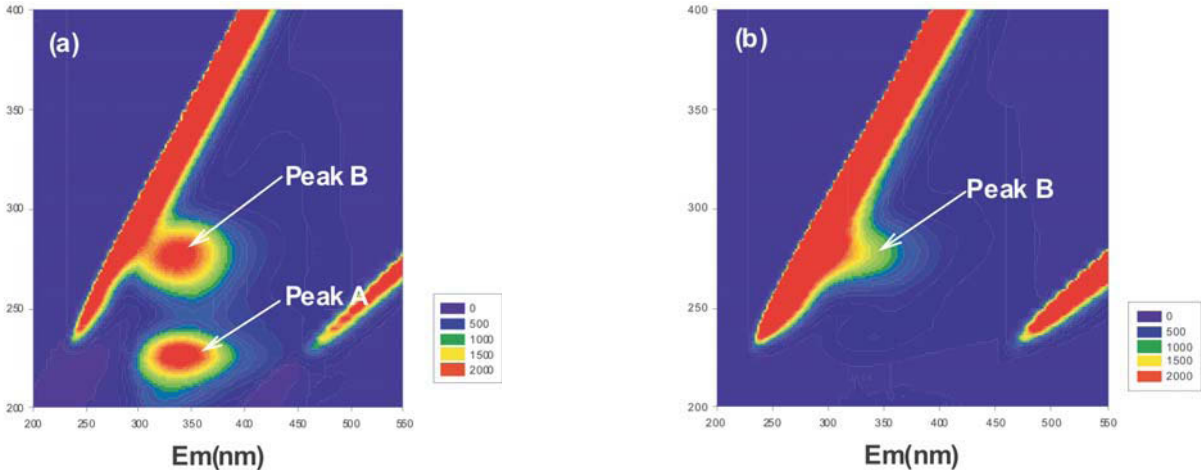

Figure 1 EEM spectra of root exudates in (**a**) absence and (**b**) presence of 0.1 M Pb(II) at 298 K.

The aim of the present study was to investigate complexation between Pb(II) and the root exudates from *Salicornia europaea* using EEM fluorescence spectroscopy. *Salicornia europaea*, was selected because it is a halophyte belonging to the Chenopodiaceae family, which can often be found in coastal and inland salt marshes (Chapman, 1974).

METHODOLOGY

Collection of root exudates

Salicornia europaea L. seedlings were collected from one salt marsh near Urumqi, Xinjiang, China. The soil attached to roots of the seedlings was gently removed with tap water. The seedlings were then hydroponically grown in Hoagland solution (Hoagland and Arnon, 1938) containing 400 mM NaCl. After three weeks, root exudates were collected according to the modified method of Cakmak *et al*. (1996). Briefly, seedling roots were rinsed thoroughly with deionised water and then grown in a black plastic wrapped beaker containing 200 mL of Milli-Q water at 25/20°C (day/night) with a 10 h/14 h light/dark period of illumination (800 µmol m^{-2} s^{-1}). Milli-Q water was used for collection of root exudates instead of $CaCl_2$ solution in order to minimize the effect of Ca^{2+} on the binding capacity of root exudates for lead (Kepert *et al*., 1979). After 3 h, the solution containing root exudates in the beaker was filtered through a 0.22 µm membrane and stored at 4°C (Zhao *et al*., 2001). The collected root exudates were immediately used for fluorescence titration test.

Preparation of Pb(II) solution

Stock Pb(II) solution (0.1 M) was prepared by dissolving $Pb(NO_3)_2$ of analytical grade in Milli-Q water.

Fluorescence quenching titration

The fluorescence spectra of root exudates were recorded with a fluorescence spectrophotometer (F-7000, HITACHI, Japan). A 450-W Xenon lamp was used as the excitation source. EEM were collected every 5 nm over an excitation range of 200–400 nm, with an emission range of 200–550 nm by 2 nm. Both excitation and emission slits were set to 5 nm of band-pass. Scan speed was 1200 nm/min. The response of the fluorometer to a Milli-Q water blank solution was subtracted from the fluorescence spectra recorded for samples containing root exudates and Pb(II) under the same conditions. The EEM data were processed using the software SigmaPlot 10.0 (Systat, US). All the experiments were done in triplicate and the mean values were used.

Root exudate solution was titrated with incremental µL additions of 0.1 M Pb(II) at 25°C, respectively. After each addition of Pb(II), the solution was fully mixed using a magnetic stirrer for 15 min and the fluorescence spectra were recorded. All the titration experiments were conducted in triplicate and the mean values with standard errors were used.

RESULTS AND DISCUSSION

EEM spectra of root exudates

Two fluorescent peaks were identified in the EEM spectrum of root exudates from *Salicornia Europaea* L. (Figure 1a). Peak A was detected at Ex/Em = 225/338–340 nm and peak B at Ex/Em = 275/328–338 nm. Fluorescence of peaks A and B could be assigned to protein-like fluorescence (Coble, 1996; Wu *et al*., 2001; Leenheer and Croué, 2003). The fluorescence intensities of both peaks were significantly reduced after addition of Pb(II) solution and even disappeared in the presence of 0.1 M Pb(II). (Figures 1a and b).

Fluorescence quenching titration

The fluorescence intensities of peaks A and B markedly decreased with increasing Pb(II) concentration at all experimental temperatures (Figures 2a and b), indicating that fluorescence of peaks A and B were quenched by Pb(II).

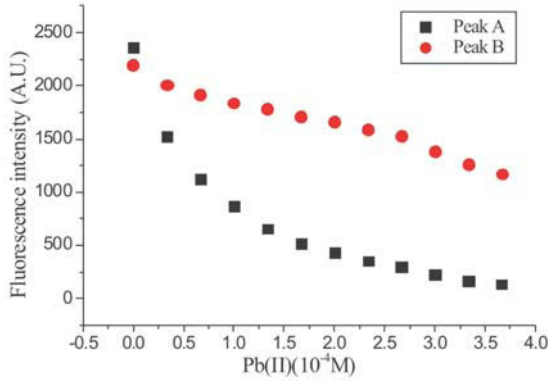

Figure 2 Fluorescence intensities of peaks A and B varies with increasing Pb(II) concentration.

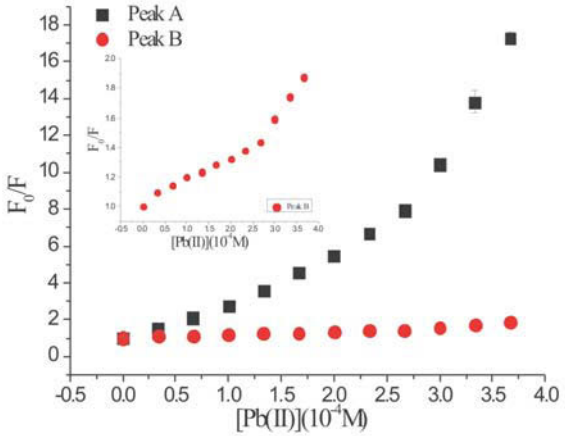

Figure 3 Plots of F_0/F *versus* [Pb(II)] at 298 K; inset is the plot of plots of F_0/F *versus* [Pb(II)] for peak B.

Fluorescence quenching mechanisms

Two types of quenching mechanisms are usually involved in fluorescence quenching: the dynamic and the static quenching. The dynamic quenching is due to collision between the fluorophore and quencher while the static quenching is attributed to the formation of a ground-state complex between the fluorophore and quencher (Lakowicz, 2006).

The fluorescence titration data were fitted with the Stern–Volmer equation (Lakowicz, 2006):

$$\frac{F_0}{F} = 1 + K_{sv}[\mathrm{Q}] = 1 + k_q\tau_0[\mathrm{Q}] \tag{1}$$

where F_0 and F are the steady-state fluorescence intensities in absence and presence of quencher, K_{sv} is the Stern–Volmer quenching constant, and [Q] is the concentration of quencher. k_q is the quenching rate constant of the biological macromolecule and $k_q = K_{sv}/\tau_0$. τ_0 is the average lifetime of the molecule without any quencher and the fluorescence lifetime of the biopolymer is 10^{-8} s (Lakowicz, 2006). And a linear Stern–Volmer plot is generally indicative of a class of fluorophore equally accessible to quencher. For dynamic quenching, the maximum dynamic quenching rate constant (k_q) of various quenchers is 2.0×10^{10} L/(mol·s) (Lakowicz, 2006).

Figure 3 clearly showed that the titration data for peaks A and B could not be fitted with the Stern–Volmer equation. The nonlinearity of Stern–Volmer plot may indicate the simultaneous presence of the dynamic quenching and static fluorescence quenching or the presence of non-linear binding isotherm involving a significant occupation of binding sites and the decrease of free quencher concentration (Borisover, 2006).

The quenching data were further analyzed using the modified Stern–Volmer equation (Lakowicz, 2006):

$$\frac{F_0}{\Delta F} = \frac{F_0}{F_0 - F} = \frac{1}{f_a K_a}\frac{1}{[Pb(II)]} + \frac{1}{f_a}. \tag{2}$$

where K_a is the conditional stability constant (effective quenching constant) for the accessible fluorophores and f_a the fraction of accessible fluorescence.

In this modified equation, both the quenchable fluorophores and the quencher inaccessible fluorophores were considered. Following Lakowicz (2006), the total fluorescence (F_0) fluorophores in the absence of quencher, F_0, equals to F_{0a} and F_{0b}.

$$F_0 = F_{0a} + F_{0b} \tag{3}$$

where F_{0a} is the fluorescence of the fluorophore moieties that can complex with quencher and F_{0b} is the fluorescence of the inaccessible fluorophore moieties.

In the presence of quencher, only the F_{0a} will change and the observed fluorescence intensity will be provided to form the modified Stern–Volmer equation to evaluate the complexation parameters, *i.e.* conditional stability constants and binding capacities.

$$f = F_{0a}/(F_{0a} + F_{0b}) \tag{4}$$

Good linear relationship between $F_0/(F_0 - F)$ and $[\mathrm{Pb(II)}]^{-1}$ for peaks A ($R^2 = 0.999$) and B ($R^2 = 0.953$) was observed (Figure 4). The values of conditional stability constant ($\log K_a$) for peaks A and B were 4.14 and 3.79, respectively. The larger value of $\log K_a$ for peaks A than that for peak B implies that the fluorophores represented by peak A has greater binding capacity for Pb(II) than Peak B. Values of f_a for peaks A and B were 1.11 and 0.48, respectively. This suggest that most of fluorescence of peak A was accessible to Pb(II) while only about half of the fluorescence of peak B was accessible to Pb(II).

The values of $\log K_a$ for root exudates-Pb(II) system in the present study were close to overall stability constants ($\log K$) for root exudates-Pb(II) and other heavy metals systems determined by other methods (Mench *et al.*, 1987). Morel *et al.* (1986) reported that the overall stability constant ($\log K$) values for complexation of root mucilage from maize (*Zea mays* L.) with Pb(II), Cu(II) and Cd(II) were 4.17–5.6, 4.14–5.4 and 4.17–5.3, respectively. Furthermore, they found that the root mucilage was mainly composed of HMW polysaccharides, proteins and uronic acids. The overall stability constants were deter-

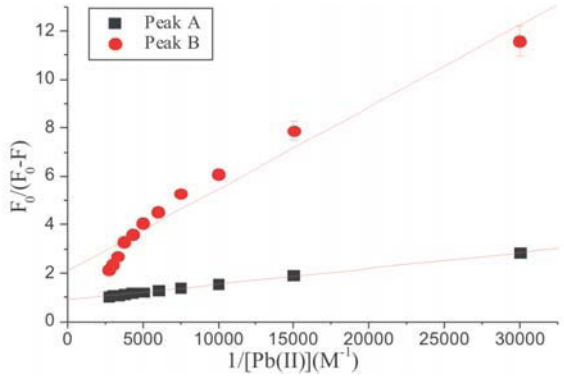

Figure 4 Modified Stern–Volmer plots for the quenching of root exudates by Pb(II).

mined from the data of equilibrium dialysis by the linear Langmuir Equation. Mench *et al*. (1987), using dialysis and ion-selective electrode titrations, estimated the values of overall stability constants (log *K*) for metals (Pb, Cu, Cd and Zn) and high molecular weight soluble exudates from maize (*Zea mays* L.) to be 3.65–3.15. The overall stability constant for Pb(II) and root exudates was 3.65. Although the values of stability constants for complexation of root exudates and heavy metals seem to be close in these studies and the present study, caution should be taken when they are compared with each other because of the different methods used. Despite its advantages, including rapidness, high sensitivity and simplicity, EEM fluorescence titration was only applicable to the fluorescent components in root exudates. This means that binding capacity of other important fractions that do not emit fluorescence can not be determined by this method and the conditional stability constant estimated by this method may be underestimated.

CONCLUSIONS

Two protein-like fluorescence peaks were identified in the EEM spectrum of root exudates from salt marsh plant *Salicornia europaea* L. and fluorescence of both peaks could be quenched by Pb(II). The values of conditional stability constant (log K_a) indicate that the protein-like substances in root exudates were strong complexing organic ligands for Pb(II), which implies that the fluorescent substances in root exudates may affect transport and fate of lead in soils. We conclude that more attention should be paid to the effect of fluorescent substances in root exudates on the chemical forms and mobility of heavy metals in soils.

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