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Competition from Cu(II), Zn(II) and Cd(II) in Pb(II) Binding to Suwannee River Fulvic Acid

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Abstract This is a study of trace metal competition in the complexation of Pb(II) by well-characterized humic substances, namely Suwannee River Fulvic Acid (SRFA) in model solutions. It was found that Cu (II) seems to compete with Pb(II) for strong binding sites of SRFA when present at the same concentration as Pb(II). However, Cd(II) and Zn(II) did not seem to compete with Pb(II) for strong binding sites of SRFA. These two metals did compete with Pb(II) for the weaker binding sites of SRFA. Heterogeneity of SRFA was found to play a crucial role in metal–SRFA interactions. The environmental significance of this research for freshwater is that even at relatively low Pb(II) loadings, the metals associated with lead in minerals, e.g. Cu(II), may successfully compete with Pb(II) for the same binding sites of the naturally occurring organic complexants, with the result that some of the Pb(II) may exist as free Pb^{2+} ions, which has been reported to be one of the toxic forms of Pb in aquatic environment.

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1 Introduction

The complexation of metal ions by naturally occurring humic substances attracts considerable attention because of its importance in regulating metal toxicity, bioavailability, and transport in natural waters (Buffle 1988; Tessier and Turner 1995). Humic substances (HS), present in natural waters are polyfunctional, polyelectrolyte, heterogeneous, supramolecular associations of small and large organic molecules, contain both ‘hard’ (e.g., carboxylic, phenolic) and ‘soft’ (e.g., nitrogen and sulphur containing) complexing sites (Buffle and Filella 1995) for a wide range of metal ions and can be expected to buffer metal ion over a wide range of concentrations. Pb(II) is one of the most extensively investigated heavy metals. Indeed Pb, which has no physiological function and is potentially toxic, has been widely dispersed in the environment since the beginning of metallurgy (Nriagu 1996). One major dispersion pathway for Pb is atmospheric transport because Pb-bearing aerosols are emitted from industrial activities, combustion of coal and leaded gasoline (Nriagu 1979; Shotyk and LeRoux 2005). Its presence in aquatic systems results mostly from anthropogenic activities (Schaule and Paterson 1981).

It is thus of great interest to know and understand the complexation reaction of lead with heterogeneous humic substances present in freshwaters. The significance of humic substances for complexation of Pb in freshwater has been reported in several studies. It has also been reported that Pb is strongly complexed by humic substances in sediments and in soil (Smith and Siccama 1981; Botelho et al. 1994). However, relatively few studies have considered the consequences of ligand heterogeneity and trace metal competitions in Pb^{2+} ion complexation by humic substances.

Still, it is difficult to determine sound physicochemical properties to describe metal complexation by heterogeneous humic substances. One of the few approaches used to describe the stability of metal complexes formed by humic substances that takes the metal loading dependence into account is the Differential Equilibrium Function (DEF). DEF is the cumulative site density as a function of the equilibrium constant. It is based on the differential intensity parameter, K^*, which is a weighted average mean of the equilibrium constants K_i, for all sites present in the humic substance (Gamble 1970; Altmann and Buffle 1988; Buffle and Filella 1995). The DEF is calculated as the first derivative of the product of the weighted average equilibrium function and a weighting factor. The weighting factor depends on the mole fraction of [metal]/[HS], and degree of occupation of each site, *i*. The DEF is not equivalent to a molecular complexation model that describes complexation at each individual site. There are other semianalytical methods presently available to study the heterogeneity of polyfunctional ligands in natural water (Nederlof et al. 1992). However, DEF is relatively simple and useful in making predictions of the complexation behavior of the system as a whole. DEF was used in this work to study the Pb(II) complexation by SRFA. This approach allows us to calculate the heterogeneity parameter (Γ) of humic substances.

The DEF is expressed as:

$$\log \theta = \Gamma \log K^*_\circ - \Gamma \log K*$$

where K^* is the Differential Equilibrium Parameter, and θ is the degree of occupation of sites. $K_0{}^*$ is a constant and has significance that it is the value of K^* when all the sites are fully occupied (i.e. when θ=1). The slope of the DEF, Γ, describes the heterogeneity of a system vis-à-vis complexation. For a simple ligand, Γ=1; as the heterogeneity of the ligand increases, the value of Γ decreases (i.e. slope of DEF decreases). For natural systems, Γ values usually lie between 0.3 and 0.7.

Heterogeneity parameter, Γ, is a very important parameter which is directly related to the buffer intensity of the complexing system, which might influence the capacity of natural waters to sustain life after metal pollution events. The same complexing substance might have different heterogeneity for different metal ions (Buffle and Filella 1995; Buffle et al. 1990; Pinheiro et al. 1994) because of the following factors: (1) different complexing sites might be available to different metal ions, and (2) the stabilities of the strongest and weakest sites titrated with the same metal might vary from metal to metal.

The reported Γ values for Cu binding by humic substances are in the range 0.3–0.5 (Town and van Leeuwen 2004; Filella and Town 2000; Vasconcelos and Gomes 1997; Chakraborty et al. 2007), depending on the humic substances. These rather low values of Γ suggest that a relatively wide distribution range of binding sites are available for Cu in humic substances.

The heterogeneity parameter (Γ) reported for Pb(II) binding with humic substances is higher than that for Cu(II), again depending somewhat on the source of the humics, e.g. a value of ~0.60 from various sources (Filella and Town 2000), ~0.70 for SRFA (Filella and Town 2001), ~0.7 for various HA (Botelho et al. 1994). There is some evidence that Γ for a whole freshwater is lower than that for isolated humics (Filella and Town 2000).

It was reported by Ephraim (1992) that the Γ value for Zn(II) was ~0.9. Town and van Leeuwen reported (Town and van Leeuwen 2004) that the extent of heterogeneity of metal binding by several humic substances followed the order Cu(II) > Cd(II).

Xue et al. (1995) found that copper and zinc compete for the strong organic complexants. It was reported by Bizri (1993) that Cu outcompeted Pb(II) and Pb(II) outcompeted Zn for the binding sites of humic substances in freshwater. Kinniburgh et al. (1999) reported that the complexation of Cu^{2+} is highest with humic acid followed by Pb^{2+} and then Cd^{2+} at different pH and free metal ion concentrations.

It was reported in our recent study (Chakraborty et al. 2007) that a comparison of the heterogeneity parameters (Γ) for Zn(II),Cd(II), Pb(II) and Cu(II) complexes in the model solutions of SRFA showed that $\Gamma_{\mathrm{Cd}}>\Gamma_{\mathrm{Zn}}>\Gamma_{\mathrm{Pb}}>\Gamma_{\mathrm{Cu}}$, suggesting that SRFA behaved as a relatively more homogeneous complexant for Zn(II) and Cd(II), whereas it behaved as a relatively more heterogeneous complexant for Pb(II). It was also found (Chakraborty et al. 2007) that the order of stability of M-SRFA complex is typically $K^*_{\mathrm{Cu}}>K^*_{\mathrm{Pb}}>K^*_{\mathrm{Zn}}>K^*_{\mathrm{Cd}}$.

The objective of this paper is to check the hypothesis that the heterogeneity of humic substances and trace metal competition determines the M(II)–DOC interactions in freshwater. To test the hypothesis, heterogeneity of SRFA and its effect on Pb(II) complexation has been studied. This study also investigated the effects of competition of the trace metals Cu(II), Cd(II), and Zn(II) on the Pb(II)–SRFA complexation in model solutions. In this research, Scanned Stripping Voltammetry was employed to study the complexation reaction of Pb(II) with SRFA, using hanging mercury drop electrode (HMDE).

2 Theory

2.1 Scanned Stripping Voltammetry

The voltammetric current–potential curve was used to determine speciation parameters by using the following equation 1, which was developed by Filella et al. (1990).

$$E=E_{\mathrm{o}}+\frac{\mathrm{RT}}{nF}\times\ln\left[\frac{1}{A^{1/\Gamma}C_{\mathrm{L,T}}}\left(\frac{C_{\mathrm{M,T}}}{C_{\mathrm{L,T}}}\right)^{(1-\Gamma)/\Gamma}\left(\frac{D_{\mathrm{R}}}{D_{\mathrm{ML}}}\right)^{1/2}\right]+\frac{\mathrm{RT}}{nF}\ln\left[\frac{\left(1-i/i_{\lim}\right)^{1/\Gamma}}{i/i_{\lim}}\right]\qquad(1)$$

The meaning of each symbols are summarized in the appendix. This equation is based on the following assumptions: (a) free M ion is reversibly reduced into M^0; all complexes are labile at the electrode surface; $[\mathrm{M_b}]>>[\mathrm{M}]$ (i.e. $[\mathrm{M_b}]=\mathrm{C_{M,T}}$) where $[\mathrm{M}]_{\mathrm{b}}$ is the concentration of metal bound to DOC, and [M] is the free metal ion concentration in the sample; the Freundlich adsorption isotherm is followed; and there is no adsorption of complex or complexant at the electrode surface.

In the voltammetric current potential curve, the slope of the rising portion reflects the heterogeneity parameter (Γ) of a polyfunctional molecules. A lower value of Γ means an elongation of the voltammetric current potential curve and vice versa. The meaning of Γ and its constancy make it a very important parameter for describing the properties of heterogeneous complexant such as humic substances, especially for interpretation of voltammetric signals. The advantage of Eq. 1 is that the heterogeneous properties of the complexant (i.e., Γ) is explicitly considered. The parameter Γ can be directly calculated from the polarograms by using Eq. 1.

2.2 Differential Equilibrium Function (DEF)

The DEF is expressed as:

$$\log\theta=\Gamma\log K^*_{\circ}-\Gamma\log K^*\qquad(2)$$

Equation 2 is another form of Freundlich isotherm; metal complexation by many heterogeneous complexants adheres to this relationship fairly closely (Filella et al. 1990). The constant, K_0*, can be determined from the shift of the polarographic wave relative to the Scanned Stripping Voltammogram of the metal aqua complex using Eqs. 3 (Deford and Hume 1951) and 4 (Filella et al. 1990).

$$\ln\alpha=\frac{nF}{\mathrm{RT}}\left(E_{1/2}-E^{L}_{1/2}\right)+\ln\left(\frac{i_{\lim}}{i^{L}_{\lim}}\right)\qquad(3)$$

$$\alpha=c_{\mathrm{L,T}}\frac{K^*\Gamma}{1-\Gamma}\left(\frac{c_{\mathrm{L,T}}}{c_{\mathrm{L,M}}}\right)^{(1-\Gamma)/\Gamma}\qquad(4)$$

2.3 Diffusion Coefficients

Heterogeneous systems give a mean diffusion coefficient, $\overline{D}$ which is a weighted average of D_{M} and D_{ML} (van Leeuwen and Buffle 1990):

$$\overline{D}=\frac{D_{\mathrm{M}}c_{\mathrm{M}}+D_{\mathrm{ML}}c_{\mathrm{ML}}}{c_{\mathrm{M,T}}}\qquad(5)$$

where D_{M} and D_{ML} are the diffusion coefficient of the metal and of the complex (the latter is assumed to be equal to the diffusion coefficient of the ligands), respectively; c_{M}, c_{ML} and $c_{\mathrm{M,T}}$ are the free, complexed and total bulk metal concentrations, respectively.

3 Experimental

3.1 Materials and Methodology

3.1.1 Suwannee River Fulvic Acid (SRFA)

Suwannee River Fulvic Acid (SRFA, (Catalogue. No. 1S101F) was obtained from the International Humic Substances Society (IHSS, USA; Ritchie and Perdue 2003). The concentration of carboxylic and phenolic groups in SRFA has been reported to be 11.44 and 2.91 mmol g^{-1}, respectively (Ritchie and Perdue 2003). The concentration of SRFA considered in this paper is related to the concentration of carboxylic groups (at pH=5.0) instead of the bulk concentration of polydisperse SRFA.

3.1.2 Model Solutions of Suwannee River Fulvic Acid

Three model solutions (to be henceforth called 'test solutions') were prepared in ultrapure water containing various concentrations of SRFA (1×10^{-3} mol L^{-1}, 5×10^{-4} mol L^{-1} and 5×10^{-5} mol L^{-1}) and 5×10^{-7} mol L^{-1}concentrations of Pb(II). The pH of all solutions was adjusted to 5.0 to minimize the polyelectrolytic effect and prevent the hydrolysis of Pb(II) using HAc/NaAc buffer. The test solutions were left to equilibration for 72 h in a dark place before analysis. Metal competition in test solutions was investigated by adding the SRFA to an aqueous solution containing a fixed concentration of the Pb(II) (5×10^{-7} mol L^{-1}) and equimolar concentrations of one of the competing Cu (II), Cd(II) and Zn(II) trace metals. A 15.0-mL aliquot of the test solution was weighed and used as the test solution. The pH of the test solution was checked again immediately before analysis and found to be the same.

3.1.3 Reagents

Standard solutions (1000 mg L^{-1}) of Cu(II), Zn(II), Cd(II) and Pb(II) were purchased from SCP Science, Montreal, Canada. Ultrapure water of resistivity 18.2 MΩ-cm was obtained direct from a Milli-Q-Plus water purification system (Millipore Corporation). A 2 mol L^{-1} stock solution of sodium acetate was prepared by dissolving an appropriate quantity of sodium acetate trihydrate (ACS grade/BDH) in ultrapure water. A stock solution of 2 mol L^{-1} potassium nitrate (supporting electrolyte) was prepared by dissolving potassium nitrate (Analar, BDH) in ultrapure water. The sodium acetate solution was then purified of metals by electrolysis at −1.5 V vs. E_{SCE} for a minimum of 48 h. immediately prior to its use. While the electrolysis was continued the sodium acetate aqueous solution was drained from the electrolysis cell in order to make sure that the impurity metals removed by electrolysis did not go back into the sodium acetate aqueous solution on the termination of electrolysis. A 2 mol L^{-1} stock solution of acetic acid was prepared by diluting glacial acetic acid (ACS grade/Anachemia) with ultrapure water.

3.2 Apparatus

Voltammetric measurements were made with a computer-controlled Autolab PGSTAT30 potentiostat/galvanostat (Eco Chemie BV, The Netherlands), equipped with a Metrohm 663 VA stand (Metrohm, Switzerland). The working electrode was a static mercury drop electrode (Metrohm, Switzerland). The reference electrode was a Ag/AgCl electrode in a glass tube filled with 3 mol L^{-1} KCl and fitted with a porous Vycor tip (Bioanalytical Systems, Inc., USA). The counter electrode was made of a platinum rod (Metrohm, Switzerland). Analysis of voltammetric peaks was done using the General Purpose Electrochemical Software v4.8 (Eco Chemie BV, The Netherlands).

4 Results and Discussion

Most research on metal complexation by heterogeneous complexants (e.g. SRFA) has used pulse stripping techniques because of the high sensitivity of the method. However, differential pulse anodic stripping voltammetry suffers from induced metal adsorption in the presence of HS (Town and Filella 2000). Town and Filella (2000) reported that in the presence of SRFA, peak height decreases to a much greater extent compared to peak area because of heterogeneity of SRFA and/or insufficient ligand excess. Peak height and peak area were recorded in this work. The ratio of peak area- to-height for Pb(II)–SRFA

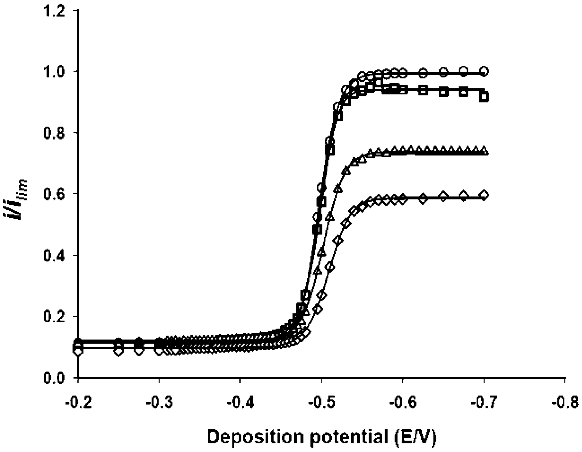

Fig. 1 Typical pseudo-polarograms for Pb(II)–FA complexes in model solutions of SRFA. $c\text{Pb}=5.0\times10^{-7}$ mol L^{-1}; (*empty circles*), Pb(II) + nil FA; (*empty squares*), Pb(II) +CFA (5×10^{-5} mol L^{-1}), (*open triangles*), Pb(II) +CFA (5×10^{-4} mol L^{-1}), (*empty diamonds*), Pb(II) +CFA (1×10^{-3} mol L^{-1}),; HOAc/NaOAc buffer, pH 5.0± 1.0, ionic strength=0.01 mol L^{-1}, temperature= 23± 2°C

was relatively constant across the range of the deposition potentials used in this experiments as was reported in our recent publication (Chakraborty et al. 2007). In this work, peak heights were used in all calculations.

4.1 Effect of Varying Concentrations of SRFA on Pb(II) Complexations

Typical scanned stripping voltammograms for the three test solutions of Pb(II) are presented in Fig. 1. The top curve is for a solution of Pb^{2+} ion buffered with HOAc/NaOAc, and represents the Pb(II)–aqua complex. It is a well defined curve with a plateau, indicating a fully reversible electrode process.

The lower curves were for the same concentration of Pb(II), which had been equilibrated for 72 h in three test solutions of SRFA, containing different concentrations of SRFA. A decrease in the limiting current (i) of the Pb(II)–SRFA complexes was observed with the increase of SRFA concentration relative to that for the Pb(II)–aqua complex (i_{lim}).

These experimental observations can be rationalized as follows. The scanned stripping voltammograms of the Pb(II)–SRFA systems present the limiting current that are generated by reduction of Pb^{2+} ions. The observed decrease in the limiting current of the Pb(II)-SRFA system relative to that of the Pb(II)–aqua complex was probably due to the change in the average diffusion coefficient $\left(\overline{D}_{\mathrm{ML}}\right)$ of Pb(II)–SRFA complexes. The $\overline{D}_{\mathrm{ML}}$ for Pb(II)–SRFA complexes was found to be much lower than the diffusion coefficient of free Pb^{2+} ion (D_{M}) in aqueous medium. The $\overline{D}_{\mathrm{ML}}$ of Pb(II)–SRFA complexes decreased as the Pb-to-SRFA mole ratio decreased (Table 1) and this decreased $\overline{D}_{\mathrm{ML}}$ of Pb(II)-SRFA complexes was responsible for the decrease in the limiting current. Complexation parameters calculated from the experimental scanned stripping voltammogram obtained from the test solutions (containing different CPb/CSRFA] mole ratios) systems are summarized in Table 1.

Table 1 shows that the stability of Pb(II) complexation by SRFA (K*) depends on the metal loading. At lower metal loading, stronger binding sites were utilized and thus K* increases with the increase of SRFA concentration. Hence, the decrease in i/i_{lim} value with the increasing concentration of SRFA was the combined effect of the formation of strong Pb(II)–SRFA complexes and the decrease of the weighted average diffusion coefficient of Pb(II)–SRFA complexes. A plot of the log θ versus log K* (differential equilibrium parameter) gave a straight line as shown in Fig. 2. The correlation coefficient for Pb(II)–SRFA system is $R^2=0.99$.

Since the strongest sites of the heterogeneous ligands are occupied first when they bind the metal, as the degree of occupation of all site types increases,

Table 1 Effect of increasing concentration of SRFA on the differential equilibrium functions, mean diffusion coefficients and lability (i/i_{lim}) of Pb(II)–FA complexes in model solutions of Suwannee River fulvic acid

C_{FA}	Γ	log K* [L mol^{-1}]	i/i_{lim}	$\overline{D}_{\mathrm{ML}}$ [m^2 s^{-1}]
0	0.99	–	1	8.28×10^{-10}
5.0×10^{-5}	0.69	6.16± 0.02	0.93± 0.03	7.13×10^{-10}
5.0×10^{-4}	0.70	6.72± 0.01	0.69± 0.01	3.94×10^{-10}
1.0×10^{-3}	0.64	6.93± 0.03	0.54± 0.02	2.41×10^{-10}

$c_{\mathrm{Pb}}=5.0\times10^{-7}$ mol L^{-1}. HOAc/NaOAc buffer, pH 5.0± 1.0, ionic strength=0.01 mol L^{-1}, temperature=23± 2°C.

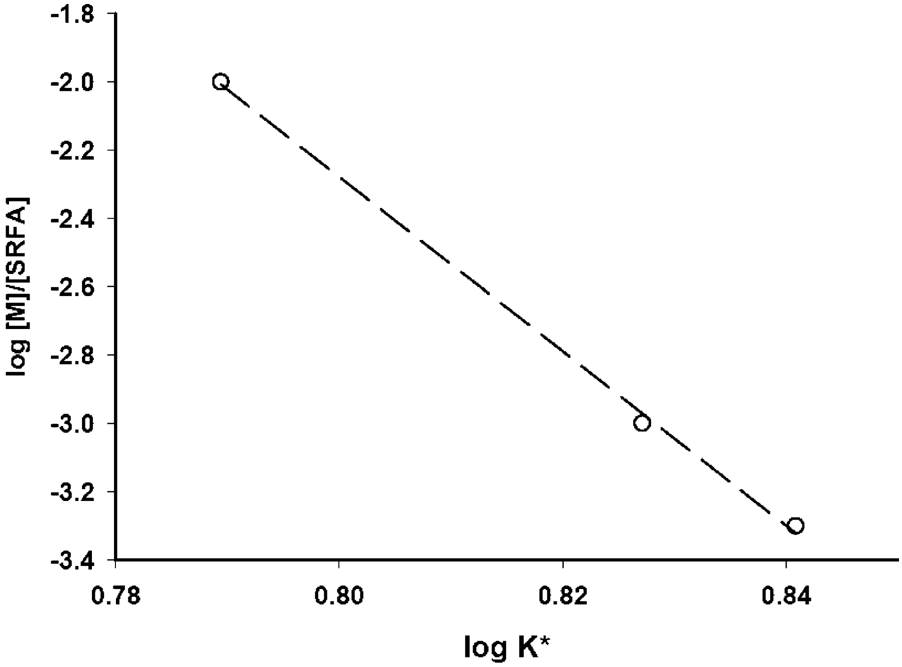

Fig. 2 Differential equilibrium function of Pb(II)–SRFA complexes in model solutions of SRFA. *c*Pb, T=5.0×10^{-7} mol L^{-1}. HOAc/NaOAc buffer, pH 5.0± 0.1, ionic strength =0.01 mol L^{-1}, temperature =23± 2°C. Correlation coefficient for Pb(II)–SRFA system *R*2=0.99

the averaging of the apparent equilibrium between M and the complexing site on L is carried out with an increasing number of weaker sites; hence, *K** decreases.

Table 1 shows that the change in the mole ratio of $C_{\mathrm{Pb,T}}/C_{\mathrm{SRFA}}$ did not appreciably affect the average heterogeneity of the system; Γ remain almost unchanged. It is well known that in the presence of a sufficient excess of complexing ligands, the Γ values are independent of the [*M*]/[*L*] mole ratio. It must be noted that Γ is a constant parameter that defines the Freundlich isotherm, and it should be independent of factors such as the [*M*]/[*L*] ratio. However, different experimental conditions may look at different parts of the same distribution which may be more or less sensitive to Γ (Town and van Leeuwen 2004).

4.2 Effect of Cu(II), Cd(II), and Zn(II) on Pb(II)–SRFA Complexation

The DEFs of the Pb(II)–SRFA complexes ($C_{\mathrm{Pb,T}}$=5.0×10^{-7} mol L^{-1} and C_{SRFA}=1.0×10^{-3} mol L^{-1}) were investigated at constant pH and fixed ionic strength in the presence of either Cu(II), Cd(II), or Zn(II). Figure 3 shows the scanned stripping voltammetric waves for the effect of the above mentioned metals on the Pb(II)–SRFA complexes at pH 5.0 (fixed with HOAc/NaOAc buffer), and at ionic strength 0.01 mol L^{-1}. One concentration of the competing trace metals was used which was the same as of Pb (5.0×10^{-7} mol L^{-1}). At the fixed mole ratio of cPb,T/cSRFA=0.0005, trace metal Cu(II) resulted in higher i/i_{lim} values for Pb(II)–SRFA complexes and

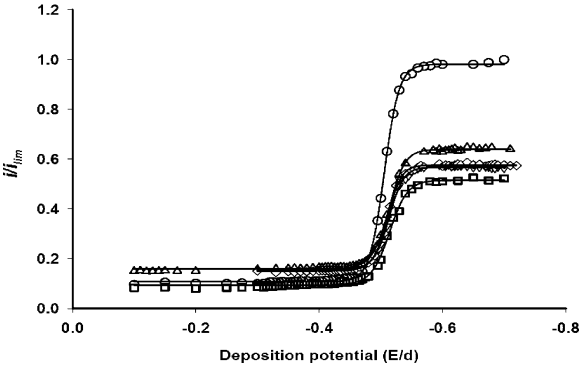

Fig. 3 Effect of competition by trace metals on the pseudo-polarographic waves of Pb(II)–SRFA complexes in model solutions of SRFA, using DPASV at a SMDE. *c*Pb,T=5.0×10^{-7} mol L^{-1}, *c*SRFA=1.0×10^{-3} mol L^{-1}. HOAc/NaOAc buffer, pH 5.0± 0.1, ionic strength=0.01 mol L^{-1}, temperature=23± 2 °C. Concentration of Pb(II) is the same for all curves. (*empty circles*) Pb(II) + nil SRFA, (*empty squares*) Pb(II)–SRFA, (*empty triangles*) Pb(II)–SRFA + Cu(II) (5.0×10^{-7} mol L^{-1}), (*filled triangles*) Pb(II)–SRFA + Zn(II) (5.0×10^{-7} mol L^{-1}), (*empty diamonds*) Pb(II)–SRFA + Cd(II) (5.0×10^{-7} mol L^{-1})

Table 2 Differential equilibrium functions, mean diffusion coefficients, and normalized peak current (i/i_{lim}) of Pb(II)–SRFA complexes in model solutions of SRFA in the presence of various competing metals Cu(II), Zn(II), and Cd(II)

Competing metal, M(II)	Γ	logK^* (L mol^{-1})	i/i_{lim}	$\overline{D}_{\mathrm{ML}}$ (m^{2}s^{-1})
None	0.68	6.93±0.03	0.49±0.03	1.95×10^{-10}
Cu(II)	0.69	6.30±0.05	0.61±0.01	2.60×10^{-10}
Zn(II)	0.66	6.40±0.02	0.48±0.02	1.90×10^{-10}
Cd(II)	0.69	6.50±0.01	0.49±0.01	1.97×10^{-10}

The properties of the solutions were $c_{\mathrm{Pb,T}}=5.0\times10^{-7}$ mol L^{-1}, $c_{\mathrm{SRFA}}=1.0\times10^{-3}$ mol L^{-1}, CM$_{\mathrm{(II)}}=5.0\times10^{-7}$ mol L^{-1}, HOAc/NaOAc buffer, pH 5.0± 1.0, ionic strength=0.01 mol L^{-1}, temperature=23± 2°C

shifted the half-wave potential towards more a positive direction, indicating an increase in the average diffusion coefficient of Pb(II)–SRFA complexes, and a decrease in the overall stability of the Pb(II)–SRFA complexes (lower K*).

The log K^* values of the Pb(II)–SRFA complexes decreased from 6.9 to 6.3 (Table 2) in the presence of increasing concentrations of Cu(II) from 0 to 5.0×10^{-7} mol L^{-1}. The results can be rationalized by hypothesizing progressive displacement of Pb(II) by Cu(II) from the binding sites of SRFA, resulting in the formation of more free Pb^{2+} ions, which enhanced the limiting currents, (i) and mean diffusion coefficient $\left(\overline{D}_{\mathrm{ML}}\right)$ of Pb(II)–SRFA complexes (Table 2). If this hypothesis is true, then Cu(II) and Pb(II) compete for the same binding sites on the SRFA and Cu outcompetes Pb. This effect was not observed when same concentrations of Zn(II) or Cd(II) added to a fixed concentration of Pb(II) (5.0×10^{-7} mol L^{-1}) as shown in Table 2. At a fixed mole ratio of cPb,T/cSRFA= 0.0005, addition of equimolar concentration of Cd(II) or Zn(II) did not change i/$i$lim, contrary to what has been observed for Cu(II) (Table 2).

The results suggest that Cd(II) and Zn(II) did not compete with Pb(II) for the strong SRFA binding sites under the experimental conditions used in this study. However, the log K^* values of the Pb(II)–SRFA complexes decreased from 6.9 to 6.5 and to 6.4 (Table 2) in the presence of increasing concentrations of Cd(II) and Zn(II), respectively, from 0 to 5.0×10^{-7} mol L^{-1}. These contradictory results are probably due to progressive displacement of Pb(II) either by Cd(II) or Zn (II) from the weaker binding sites of SRFA, resulting in the formation of free Pb^{2+} ions. However, weak Pb (II)–SRFA complexes dissociate and generate Pb^{2+} ions on electrode surface and contribute to the total current measured at different electrode potentials during the experiment. As a result, the limiting currents, remain unchanged even in the presence of Cd (II) and Zn(II).

$$K^* = \frac{\sum_{i=1}^{n} K_i\theta_i(1-\theta_i)\Delta X_i}{\sum_{i=1}^{n} \theta_i(1-\theta_i)\Delta X_i} \tag{6}$$

This explanation can be further supported by the Equation 6 (van Leeuwen and Buffle 1990). The displacement of Pb^{2+} from the weaker site of SRFA, either by Cd(II) or Zn(II) would decrease the total concentration of Pb(II)–SRFA weaker complexes.

As a result $\sum_{i=1}^{n} K_i\theta_i(1-\theta_i)\Delta X_i$ decreases. Hence, the average equilibrium function (log K^*), and so the DEF would decrease.

Table 2 shows that the increasing order of effects of trace metal competition on the K^* values, of the Pb (II)–SRFA complexes are: Cu(II) > Zn(II) > Cd(II). The average Γ values of Pb(II)–SRFA complexes in the presence of competing metals was ~0.7, which is similar to that reported in the literature for Pb binding by humics. This result suggests that the average heterogeneity of Pb(II)–SRFA complexes was constant in the presence of competing metals. The similar observation was reported by Town and van Leeuwen for Cu. They measured a significant displacement of Cu by Pb from Cu(II)–HS complexes but no change in the Γ value for Cu. These results are expected for humic substances. Since the ligand (SRFA) is heterogeneous, the competing metal affects a range of sites (to different extents depending on θ), but does not specifically “block” a group of sites—so the conditional binding by metal appears slightly weaker, but its Γ is not measurably affected.

It has been found that the extent of competition of different metals for binding with humic substances depends on the relative concentrations of the metals (and ligands) and on their conditional stability constants of M(II)–Humate complex under the given conditions. In addition to these factors, Γ (heterogeneity parameter) also has relevant significance in metal competition, since it determines an effective range of sites of humic substances at which binding occurs.

5 Environmental Significance

This study has shown that trace metal Cu(II) competes with trace metal Pb(II) for binding by SRFA even when the Cu(II) are in the same concentrations as Pb (II). The environmental significance of this research for freshwater is that even at relatively low lead loadings, the metals associated with lead in minerals, e.g. copper, may successfully compete with Pb(II) for the same binding sites of the naturally occurring organic complexants (HS), with the result that some of the Pb(II) may exist as free Pb^{2+} ions, which has been reported to be one of the toxic forms of Pb in aquatic environment (Kungolos et al. 2006).

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Appendix

List of symbols. In the following list the most important or most frequently used symbols have been collected.

M	Metal
ML	Metal–ligand complex
E_0	standard potential (V) of the redox couple
R	Universal Gas Constant
T	absolute temperature (K)
n	number of electrons involved in the balanced reaction
F	Faraday constant
D_R	the diffusion coefficient of reduced form of M in mercury
$\overline{D}_{\mathrm{ML}}$	the diffusion coefficient of ML in the solution
D_{M}	the diffusion coefficient of M (aqua) in the solution
i_{lim}	the diffusion-limited limiting current in absence of ligand
i	the measured current
A	Freundlich Constant
$c_{\mathrm{M,T}}$	the total concentrations of M in the bulk solution
$c_{\mathrm{L,T}}$	the total concentrations of L in the bulk solution.
Γ	the degree of heterogeneity of the system
K^*	the Differential Equilibrium Parameter
Θ	the degree of occupation of sites
K_0^*	a constant
$E_{1/2}$	half-wave potential in the absence of ligand
$EL_{1/2}$	half-wave potential in the presence of ligand
$i_{\mathrm{lim}}^{\mathrm{L}}$	limiting current in the presence of ligand
α	degree of complexation of the ligand
K_{i}	Equilibrium constant of a metal at a specific site "*i*" in HA
ΔX	Total binding sites of humic acid with different affinities for M

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