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Utilization of ICP/OES for the determination of trace metal binding to different humic fractions

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Abstract

In this study, the use of inductively coupled plasma/optical emission spectrometry (ICP/OES) to determine multi-metal binding to three biomasses, Sphagnum peat moss, humin and humic acids is reported. All the investigations were performed under part per billion (ppb) concentrations. Batch pH profile experiments were performed using multi-metal solutions of Cd(II), Cu(II), Pb(II), Ni(II), Cr(III) and Cr(VI). The results showed that at pH 2 and 3, the metal affinity of the three biomasses exposed to the multi-metal solution that included Cr(III) presented the following order: Cu(II), Pb(II) $>$ Ni(II) $>$ Cr(III) $>$ Cd(II). On the other hand, when Cr(VI) was in the heavy metal mixture, Sphagnum peat moss and humin showed the following affinity: Cu(II), Pb(II) $>$ Ni(II) $>$ Cr(VI) $>$ Cd(II); however, the affinity of the humic acids was: Cu(II) $>$ Pb(II), Cr(VI) $>$ Ni(II) $>$ Cd(II). The results demonstrated that pH values of 4 and 5 were the most favorable for the heavy metal binding process. At pH 5, all the metals, except for Cr(VI), were bound between 90 and 100% to the three biomasses. However, the binding capacity of humic acids decreased at pH 6 in the presence of Cr(VI). The results showed that the ICP/OES permits the determination of heavy metal binding to organic matter at ppb concentration. These results will be very useful in understanding the role of humic substances in the fate and transport of heavy metals, and thus could provide information to develop new methodologies for the removal of low concentrations of toxic heavy metals from contaminated waters.

Keywords: ICP/OES; Humic substances; Trace metal binding; Metal affinity; Batch experiments

1. Introduction

Several human activities contribute to increases in the heavy metal concentrations in localized environments. Once in soil or water, heavy metals enter into the food chain via

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plant uptake and subsequent biomagnifications. Although at trace levels some heavy metals are essential for plants and animals, at higher concentrations they become dangerous for any form of life [1]. Experiments performed in vivo and in vitro have demonstrated that chromium, cadmium, nickel and lead may cause cancer and mutations in living organisms [2–7]. Recently, some heavy metals such as copper have been associated with neurological disorders in humans [8] and behavioral changes in animals [9].

Traditional methods used for the removal of heavy metals from the environment include chemical reduction, precipitation, ion exchange and adsorption by activated carbon. However, these procedures are expensive and potentially risky due to the possibility of by-product generation [10]. Recently, many studies have been focused on the development of new environmentally friendly options for heavy metal removal. Overall, the attention has been directed to the use of living plants for phytoremediation purposes as well as organic materials as biosorbents. These techniques represent low-cost and very effective possibilities for the elimination of heavy metal contamination. For example, immobilized alfalfa biomass (*Medicago sativa*) has been used to remove Cd(II), Cr(III), Cu(II), Pb(II), Ni(II) and Zn(II) from aqueous solution [11,12]; also, the fungi *Mucor meihi* has been efficiently used to remove Cr(III) from a tanning effluent [13]. Furthermore, other materials such as orange peel, bacteria, algae and yeast have also been investigated for heavy metal removal [14,15]. Activated carbon prepared from agricultural by-products provides another adequate option since it is relatively inexpensive and possesses a high capacity to bind heavy metals [16]. Additionally, Sphagnum peat moss has been used to adsorb Ni(II), Pb(II), Cu(II), Cd(II), Zn(II), Cr(III) and Cr(VI) from aqueous solutions [17–20]. Also, a variety of living plants have been used to remove heavy metals from soil and water. Cheng et al. reported the use of *Cyperus alternifolius* and *Villarsia exaltata* for metal removal from polluted waters [21]. *Medicago sativa* [22] and *Helianthus annuus* [23] have been used for the phytoextraction of heavy metals from soil. Most of these studies have been performed using media containing high concentrations of heavy metals (ppm level).

Various methodologies have been developed to determine metal concentrations of different substrata. These methods have evolved from the use of gravimetric, or titrimetric measurements, to the use of instrumental techniques [24]. Currently, spectrometric methods are the most useful tool for metal quantification purposes. This technology is based on the absorption or emission of electromagnetic radiation by atomic or molecular species. The most widely used are flame atomic absorption (FAAS), graphite furnace atomic absorption (GFAAS), and inductively coupled plasma optical emission (ICP/OES), or mass spectrometry (ICP/MS). The first two are essentially single-element detection techniques, while ICP/OES and ICP/MS may be used for multi-metal analysis [25]. Inductively coupled plasma spectrometric excitation sources were first introduced in the 1960s, and since then they have evolved dramatically [26]. ICP/OES may, in principle, be useful to quantify all the elements except argon. For most of the elements, the detection limits are very low and a wide range of concentrations varying from ultra-trace (10 ng/g) to high concentrations (ppm) can be determined [27].

Although several studies on heavy metal binding to different humic fractions have already been published [10,17,28], no references were found on metal and multi-metal binding to humic biomasses at trace or ppb levels. The aim of this work was to determine the Cd(II), Cu(II), Pb(II), Ni(II), Cr(III) and Cr(VI) binding to Sphagnum peat moss and its humin and humic acids at trace level (ppb) using ICP/OES. Because humic fractions are usually present in the all natural surroundings, the information obtained from this investigation will be very valuable since it will also provide data on the fate of heavy metals at low concentrations in the environment.

2. Methodology

2.1. Extraction of humic fractions from Sphagnum peat moss

Gardea-Torresdey et al. [28] previously described the procedure used in this investigation for the extraction of humic fractions from Sphagnum peat moss. A fraction of 100 g of Canadian Sphagnum peat moss was dried at 51 °C during 3 days. The dried peat moss was then ground to a fine powder and sieved through an 80 mesh screen. Afterwards, the powder was washed twice with 500 ml of 0.01 M HCl in order to eliminate debris and possible contamination by metals, and centrifuged for 5 min at 3000 rpm. Subsequently, 500 ml of 0.1 M NaOH was added to the biomass and the pH of the solution was adjusted to 13.5 by the addition of 5 M NaOH. The solution was stirred for 48 h and then centrifuged. The humin fraction and other insoluble material precipitated and the humic acids remained in the supernatant. Subsequently, the supernatant was acidified to pH 0.5 with 6 M HCl and the mixture stirred over a 24 h period. The humic acids precipitated from the acidified supernatant. The humin extracted from the alkaline solution was washed twice with deionized water to eliminate the remaining alkalinity. The humin and the humic acids were freeze dried on a Labconco freeze dryer at $-45\,^{\circ}\mathrm{C}$ and 69×10^{-3} mbar pressure. The dried humin and humic acids were once again ground and sieved through an 80 mesh screen in order to obtain a 180 μm size particle. The fulvic acids fraction, which remained in the solution, was not studied.

2.2. Heavy metals studied

In this study, two multi-metal solutions of 2 μmoles of Cd(II), Cu(II), Pb(II), Ni(II), Cr(III) and Cr(VI) were prepared by successive dilutions from stock solutions of 1000 ppm concentrations. Solution *A* contained Cd(II), Cu(II), Pb(II), Ni(II) and Cr(III), and solution *B* contained Cd(II), Cu(II), Pb(II), Ni(II) and Cr(VI). This procedure was followed because when using ICP/OES it is not possible to quantify Cr(III) or Cr(VI) separately; quantification is made for total chromium. For this purpose, the following salts, reagent grade, were used: $Cd(NO_3)_2{\cdot}4H_2O$, $CuSO_4{\cdot}5H_2O$, $Pb(NO_3)_2$, $Ni(NO_3)_2{\cdot}6H_2O$, $Cr(NO_3)_3{\cdot}9H_2O$ and $K_2Cr_2O_7$. The 2 μM multi-metal solutions *A* and *B* included 225 ppb of Cd(II), 127 ppb of Cu (II), 414 ppb of Pb(II), 118 ppb of Ni(II) and 104 ppb of Cr species.

2.3. The pH profile experiments

A sample of 500 mg of each dry biomass (Sphagnum peat moss, humin and humic acids) was washed twice with 40 ml of 0.01 M HCl in order to eliminate debris and eventual contamination by metals. Subsequently, the respective washed biomass was diluted in 100 ml

Table 1
Selected wavelengths and correlation coefficient for the elements analyzed

Element	Wavelength	Correlation coefficient
Cd	226.502	0.999993
Cu	324.752	0.999995
Pb	220.353	0.999729
Ni	231.604	0.999979
Cr	283.563	0.999997

of 0.01 M HCl in order to achieve a biomass suspension concentration of 5 mg/ml. This procedure was done in sets of five aliquots for each fraction. While stirring, the aliquots were adjusted to pH values of 2, 3, 4, 5 and 6.

Aliquots of 4 ml of each biomass suspension (5 mg/ml) were centrifuged at 3000 rpm. The peat moss and humin fractions were centrifuged for 5 min, and the humic acids for 10–15 min. The supernatants were transferred to clean test tubes and the biomass pellets were saved for the binding experiments. Four milliliter of each multi-metal solution previously adjusted to the pH values of 2, 3, 4, 5 and 6 was added to the respective biomass pellet. The supernatants, which were used as control, received the same dose of heavy metal solutions. After that, all treated solutions were equilibrated on a rocker for 1 h, as reported by Gardea-Torresdey et al. [28]. The samples were then centrifuged, the final pH was recorded, and the respective supernatants were analyzed by ICP/OES to quantify the remaining metal. All the experiments were performed in triplicate for quality control.

2.4. *Metal analysis*

The metal analyses were performed using an ICP/OES Optima 4300 DV, with a Perkin-Elmer AS-90 plus auto sampler rack. After performing the background equivalent concentration experiment to test the instrument sensitivity, the following parameters were introduced: nebulizer flow, 0.80 l/min; radio frequency power, 1450 W; sample introduction, 1.45 ml/min; flush time, 10 s; delay time, 60 s; read time, 10 s; wash time, 45 s; replicates, four (each sample read four times). Standards were prepared from 1000 ppm stock solutions and diluted with 0.01 M HCl. The blank and four points were used to obtain the calibration curve. Table 1 shows the wavelengths selected for each metal as well as the respective correlation coefficients. The amount of metal adsorbed to the biomass was calculated by the difference between the concentration found in the control solutions and the concentration of the remaining metals found in the supernatants after the application of treatments.

3. Results and discussion

3.1. *Metal affinity*

The pH profile for the metal affinity to Sphagnum peat moss is shown in Fig. 1a and b. Fig. 1a shows the results found when the peat moss was reacted with solution *A* that contained

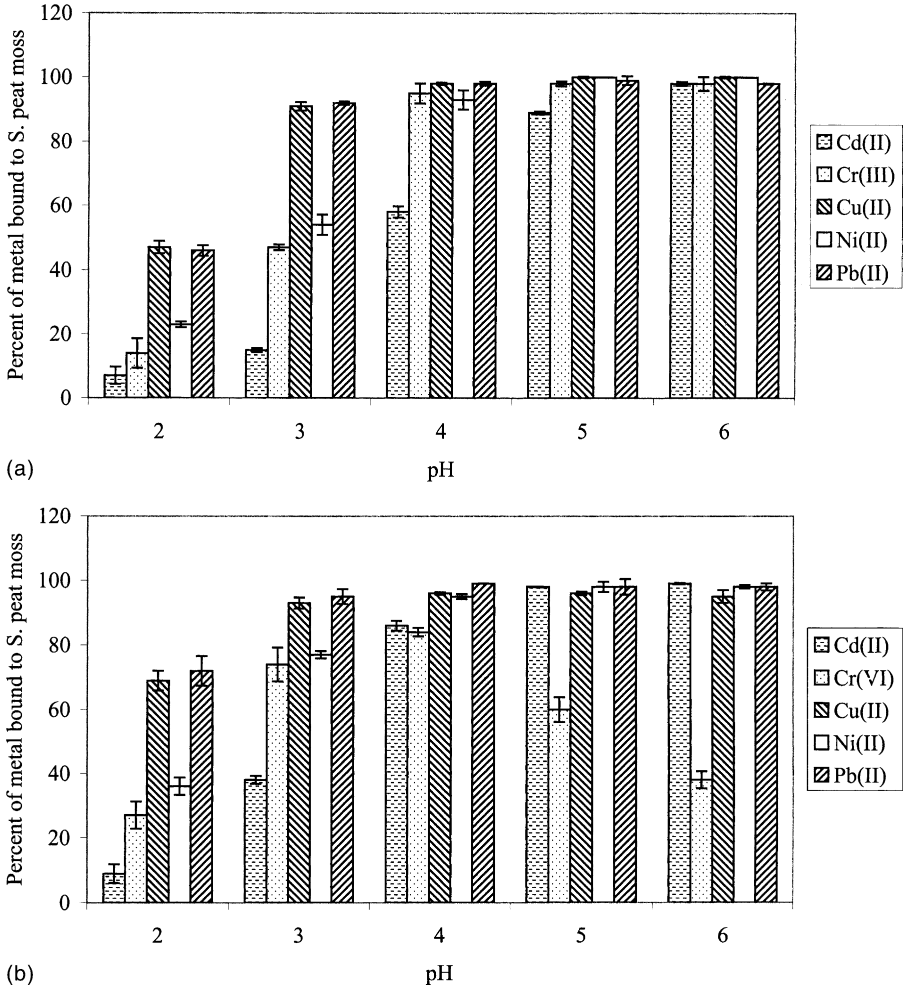

Fig. 1. Percent of metal bound by Sphagnum peat moss after 1 h of equilibration with a multi-metal solution at different pH values. (a) The solution contained 2 μmoles of Cd(II), Cu(II), Pb(II), Ni(II) and Cr(III). (b) The solution contained 2 μmoles of Cd(II), Cu(II), Pb(II), Ni(II) and Cr(VI). Error bars represent 95% confidence interval.

Cr(III). In this figure one can see that at pH 2 the decreasing order of metal affinity was as follows: Cu(II) $>$ Pb(II) $>$ Ni(II) $>$ Cr(III) $>$ Cd(II). At pH values of 3 and 4 the affinity found was as follows: Pb(II) $\approx$ Cu(II) $>$ Ni(II) $>$ Cr(III) $>$ Cd(II). When the pH was raised to 5, the order of affinity was, Cu(II) $\approx$ Ni(II) $\approx$ Pb(II) $>$ Cr(III) $>$ Cd(II). No differences in the order of affinity were observed at pH 6. On the other hand, when Sphagnum peat moss was treated with the multi-metal solution *B*, which included Cr(VI), some changes in the order of affinity were observed. Fig. 1b shows that at pH values of 2 and 3 the affinity was: Pb(II) $>$ Cu(II) $>$ Ni(II) $>$ Cr(VI) $>$ Cd(II). However, at pH 4 the arrangement obtained was Pb(II) > Cu(II) ≈ Ni(II) > Cd(II) > Cr(VI), while at pH 5 the position of Cu(II), Ni(II) and Cd(II) were interchanged in the order affinity, showing the following arrangement: Pb(II) ≈ Ni(II) ≈ Cd(II) > Cu(II) > Cr(VI). When the pH was raised to 6, Cd(II) was slightly preferred to the other metals and the affinity observed was as follows: Cd(II) > Pb(II) ≈ Ni(II) > Cu(II) > Cr(VI). The selectivity observed for Sphagnum peat moss in solution *B* agreed with the results reported by Spinti et al. [29]. These researchers exposed Sphagnum peat moss to acid mine drainage wastewaters at pH between 3 and 4, finding the following order of affinity: Fe > Al > Pb > Cu > Cd, Zn > Ca > Mn > Mg > Na. It was indeed the selectivity observed for Pb(II), Cu(II) and Cd(II) in this investigation. Crist et al. [30] found that ion-exchange constant values for Pb and Cu displacing Ca bound to peat moss are larger that the values for other metals, which confirm that Cu and Pb are strongly bound to peat moss. On the other hand, when Sphagnum peat moss was used in biosorptive flotation experiments that involved solids separation, the selectivity observed was, Pb > Ni > Cu > Cd [20]. The results obtained in this investigation were different as compared to the above mentioned, possibly because the procedures utilized were different.

Fig. 2a shows the heavy metal affinity to humin when Cr(III) is part of the multi-metal solution. The graph illustrates that at pH values of 2 and 3 the order of affinity is: Cu(II) > Pb(II) > Ni(II) ≈ Cr(III) > Cd(II). As the pH increases to 4, 5 and 6, there is a change in the order of affinity. Ni(II) is bound in higher amount when compared to Pb(II) and the order of affinity is: Cu(II) ≈ Ni(II) > Pb(II) > Cr(III) > Cd(II). However, Fig. 2b illustrates that when the multi-metal solution contains Cr(VI), only at pH 2 the humin presents the same affinity as when Cr(III) is in the multi-metal solution. At pH 3, humin adsorbs less Cr(VI) as compared to the adsorption of Cr(III). At this pH level, the order of affinity is: Cu(II) > Pb(II) > Ni(II) > Cr(VI) > Cd(II), but at pH 4, the affinity is Pb(II) > Cu(II) > Ni(II) > Cd(II) > Cr(VI). However, at pH 5 and 6, the concentration of Cd(II) and Ni(II) bound to humin biomass is higher and the affinity is: Ni(II) ≈ Cd(II) > Pb(II) ≈ Cu(II) > Cr(VI). No metal selectivity data for humin was previously found in literature.

The study performed with humic acids showed that in this biomass Cr species had a strong influence in the selectivity to heavy metal binding. Fig. 3a shows the order of heavy metal binding to humic acids when the multi-metal solution contained Cr(III). In this figure one can see that at pH 2 the order of affinity of the heavy metals toward humic acids was as follows: Cu(II) > Pb(II) > Ni(II) > Cr(III) ≈ Cd(II). At pH 3 Cr(III) was bound in higher amount as compared to Cd(II), but no changes were observed in the order of affinity of the other metals. At pH 4 the order of affinity was Cu(II) ≈ Pb(II) > Cr(III) ≈ Ni(II) > Cd(II), while at pH 6 the affinity changed to the following order: Cu(II) > Pb(II) > Ni(II) > Cr(III) > Cd(II). However, when the multi-metal solution contained Cr(VI) (Fig. 3b) at pH 2, the binding of Cd(II) was minimal, but at pH values of 3 and 4, the behavior was the same as when the Cr(III) was in the multi-metal solution. Important changes in the metal affinity to humic acids were observed when the pH was raised to 5. When the humic acid biomass was reacted with the multi-metal solution that contained Cr(III), the affinity for cadmium was lower as compared to the affinity for the other metals studied; however, in the presence of Cr(VI), the affinity for Cd(II) was the same as that for copper, nickel and lead. No differences in metal affinity were observed when the pH was raised to 6. In experiments performed with crude leonardite humic acids, it was found that in this biomass Cu(II) and

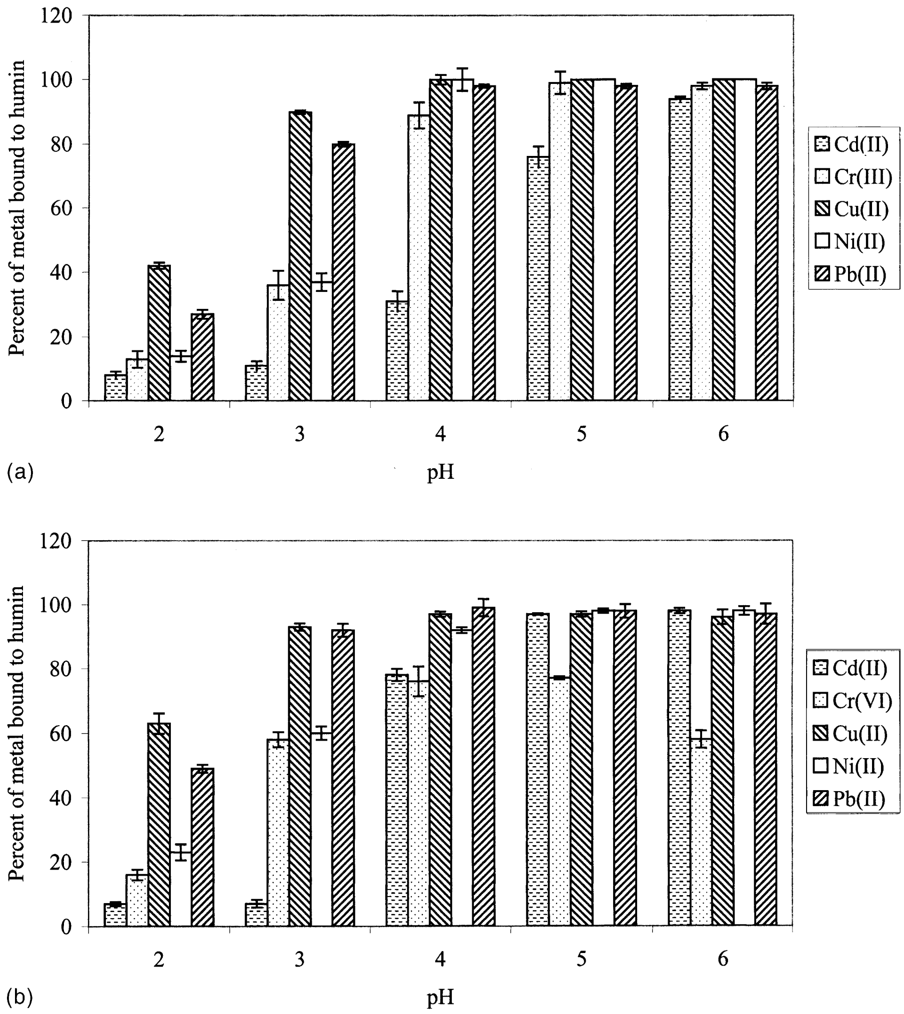

Fig. 2. Percent of metal bound by humin after 1 h of equilibration with a multi-metal solution at different pH values. (a) The solution contained 2 μmoles of Cd(II), Cu(II), Pb(II), Ni(II) and Cr(III). (b) The solution contained 2 μmoles of Cd(II), Cu(II), Pb(II), Ni(II) and Cr(VI). Error bars represent 95% confidence interval.

Pb(II) were selectively bound on a high percentage from multi-metal solutions [31]. Similar results were obtained by Kinniburgh et al. [32], who found that the strength of binding of Cu(II) and Pb(II) to a non purified humic acid is higher than for Cd(II).

3.2. Heavy metal adsorption

Fig. 1 also shows the amount of metals bound to the Sphagnum peat moss biomass related to the presence of Cr species and the changes in pH values. Comparing Fig. 1a and b, it can be seen that at pH values of 2 and 3 the tendency in the adsorption of all the metals was the

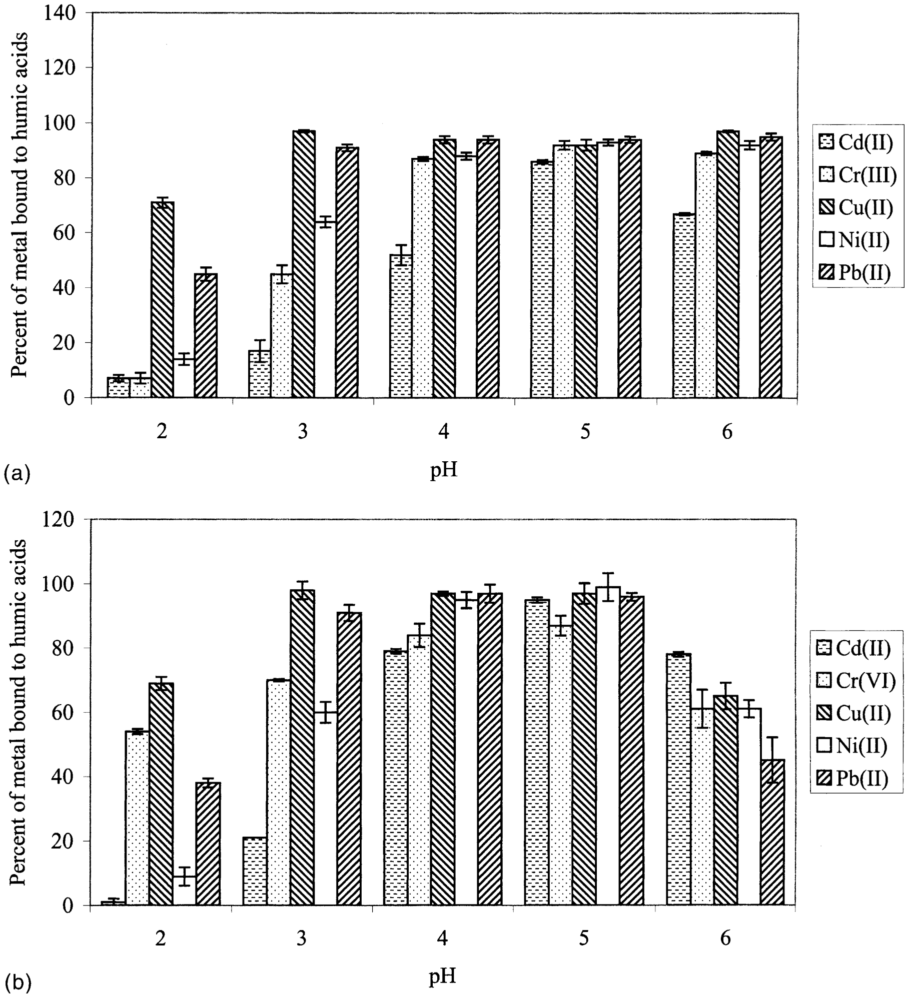

Fig. 3. Percent of metal bound by humic acids after 1 h of equilibration with a multi-metal solution at different pH values. (a) The solution contained 2 μmoles of Cd(II), Cu(II), Pb(II), Ni(II) and Cr(III). (b) The solution contained 2 μmoles of Cd(II), Cu(II), Pb(II), Ni(II) and Cr(VI). Error bars represent 95% confidence interval.

same either if Cr(III) or Cr(VI) were present on the solution. However, at pH 2 Sphagnum peat moss adsorbed more than twice Cr(VI) (27%) as compared to Cr(III) (14%). Also, the adsorption of Ni(II), Pb(II) and Cu(II) was 65, 57 and 48% higher in the presence of Cr(VI) (Fig. 1b) than the adsorption observed in the presence of Cr(III) (Fig. 1a), respectively. No differences were observed in the adsorption of Cd(II). As the pH increased, the amount of metal adsorbed to Sphagnum peat moss also increased. At pH 3, Sphagnum peat moss adsorbed 74% of Cr(VI) and 47% of Cr(III), which indicated that the adsorption of Cr(VI) was 57% higher than that of Cr(III). Furthermore, Cr species altered the adsorption of the other metals. When the multi-metal solution contained Cr(VI), Sphagnum peat moss adsorbed 153 and 45% more Cd(II) and Ni(II), respectively, as compared to the adsorption found in the presence of Cr(III). At pH 3 Cu(II) and Pb(II) were adsorbed in more than 90%. As the pH was raised to 4, the adsorption of Cr(III) surpassed that of Cr(VI) by 13%, but the adsorption of Cd(II) was still 48% higher in presence of Cr(VI). The other metals were adsorbed by more than 90% at this pH. Significant changes were observed in the adsorption of Cr species at pH 6. At this pH, the adsorption of Cr(III) to Sphagnum peat moss was 155% higher as compared to the adsorption of Cr(VI). However, the adsorption of the other metals was almost at 100%. Ho and McKay [19], found that Pb(II) and Cu(II) were most strongly adsorbed by Sphagnum peat moss at pH 5, results that were similar to those found in this study. However, the results found in *Sargassum* seaweed by Kratochvil et al. [33], were slightly different. In seaweed, the maximal adsorption of metals was with Cr(III) at pH 4.

As it can be seen in Fig. 1, at low pH values and in the presence of Cr(VI) the binding capacity of Sphagnum peat moss for Cd(II), Cu(II), Pb(II) and Ni(II) showed a small increase as compared to the adsorption observed in the presence of Cr(III). This observation supports the idea that Cr(VI) oxidizes part of the organic matter present on peat moss. It could be possible that functional groups involved in this oxidation reaction might be alcohols, giving as products carboxylic acids. A reduction of Cr(VI) binding should be expected with an increase of pH since Cr(VI) is a negatively charged species, mainly present as chromate ($CrO_4{}^{-2}$) or dichromate ($Cr_2O_7{}^{-2}$) ion [10]. Nevertheless, in this study the adsorption of Cr(VI) increased when the pH was raised from 2 to 4, and decreased at higher pH values. Sharma and Forster [17], suggested that at low pH, Cr(VI) is reduced to Cr(III) through an oxidation reaction caused by the peat moss organic matter. This reaction is represented below

$$[C] + \mathrm{HCrO_4}^- + 3\mathrm{H}^+ \rightarrow \mathrm{Cr}^{3+} + 2\mathrm{H_2O} + \mathrm{CO_2}$$

where $[C]$ denotes organic matter present on peat moss.

Because the concentrations used in this study were at ppb level, it might be possible that part of the Cr(VI) was reduced to Cr(III), which at pH 2 is less probable to be adsorbed. However, as the pH increases from 3 to 4, Cr(III) produced in the reduction reaction was better bound to the biomass. At pH 5 and 6, less reduction of Cr(VI) occurs, since hydrogen ions needed in this reaction are less abundant. So if the binding groups are negatively charged, Cr(VI) does not bind and stays on the solution, which could explain the results observed for Cr binding in this study.

The heavy metal adsorption to humin is presented in Fig. 2. Fig. 2a shows the bound percentages when Cr(III) is present in the heavy metal solution, and Fig. 2b shows the bound percentages when Cr(VI) is part of the heavy metal mixture. It is important to note in this figure that at pH 2, there were no differences in the percent of Cd and Cr bound to the humin biomass in the multi-metal solutions containing Cr(III) and Cr(VI). However, Pb(II), Ni(II) and Cu(II), were bound 81, 64 and 50%, respectively higher when Cr(VI) was present in the multi-metal solution. Significant changes were observed in the binding percentage of the heavy metals to humin biomass at pH 3. At this pH the adsorption of Cr(VI) was 61% higher than that of Cr(III). Additionally, Cr(VI) favored the adsorption of Ni(II) and Pb(II). At pH 3 humin adsorbed 62 and 15% more Ni(II) and Pb(II), respectively, as compared with the adsorption obtained in the presence of Cr(III). Also, an important change in the adsorption of Cd(II) was noted at pH 3. If Cr(VI) was present in the multi-metal solution, no changes in the adsorption of Cd(II) to humin were observed when the pH was changed from 2 to 3; however, in the presence of Cr(III), humin adsorbed 38% more Cd(II) at pH 3 than at 2 (Fig. 2a and b). A significant increase in Cd(II) binding to humin biomass was observed at pH 4. At this pH, the amount of Cd(II) adsorbed to humin was 152% higher in presence of Cr(VI) and the adsorption of Ni(II) was 19% higher in these conditions (Fig. 2a and b). At pH 4, the adsorption of Cr(III) to humin was 17% higher than the adsorption of Cr(VI). When the pH was raised to 5 and 6, in the presence of Cr(VI) the adsorption of Cd(II) was increased to 97 and 98%, respectively; however, in the presence of Cr(III) the percent of Cd(II) bound to humin changed from 76% at pH 5 to 94% at pH 6, which was an important increase. It is important to note that the adsorption of Cr(III) was 98% at pH 6, but that of Cr(VI) decreased up to 58% at the same pH.

Fig. 3 illustrates the pH profile for the heavy metal adsorption to humic acids treated with the multi-metal solutions that contained either Cr(III) or Cr(VI). The heavy metal adsorption to humic acids presented significant differences as compared to Sphagnum peat moss and humin. Furthermore, the Cr species and the solution pH determined the amount of metal adsorbed to humic acids. Comparing Fig. 3a and b, one can see that at pH 2 the adsorption of Cr(VI) to humic acids (54%) was more than 70 times higher than the adsorption of Cr(III) (0.7%). Also, at pH 2 the adsorption of Cd(II) to humic acids was 1% in the presence of Cr(VI), but 7% in the presence of Cr(III). In addition, the adsorption of Ni(II) at pH 2 was also affected by the Cr species. If the multi-metal solution contained Cr(VI) the adsorption of nickel to humic acids was 9%, but if the multi-metal solution contained Cr(III), the adsorption of nickel was 14%, which represents a difference of 55% higher. No differences were observed in the percent of Cu(II) and Pb(II) bound to humic acids at pH 2. When the pH was raised to 3, the adsorption of the heavy metals studied to humic acids was not affected by the Cr speciation; however, at this pH the adsorption of Cr(VI) was 55% higher than that of Cr(III). At pH 4, only the cadmium adsorption to humic acids was affected by the presence of chromium. At this pH the adsorption of Cd(II) was 52% higher in the presence of Cr(VI) as compared to the adsorption of this metal in the presence of Cr(III). At pH 5 all the metals were bound at the same level in solutions *A* and *B*. Finally, it was observed that at pH 6 the percentage of all the metals bound decreased dramatically for every metal, in the presence of Cr(VI).

Gardea-Torresdey et al. [28], demonstrated that carboxylic acids present in Sphagnum peat moss, humin and humic acids, are the main, though not the only, groups responsible for copper binding at ppm level. Previous studies conducted with alfalfa biomass also showed that carboxyl groups are the sites where most of metal binding occurs [11]. Additionally, recent experiments performed with alfalfa biomass and heavy metals at ppm concentration suggested that the functional groups responsible for metal binding at low and high concentrations, might be the same [34–36]. This may also imply that the sites responsible for Cu(II) and Ni(II) binding at trace and high level in these materials are the same.

Relevant information has been reported for stability constants obtained from humic and fulvic acids extracted from soil and water [37–43]. Even though polyacrylic acids have been used as models in order to study the interactions of organic substances with heavy metals, there is no complete agreement in this matter [44]. Previous results indicated that is difficult to perform a reliable model to predict the behavior of humic and fulvic acids due to complexity of these substances [45]. However, most of the information found in the literature suggest that the order of stability constant follows the Irving–Williams series [46], which is Pd $>$ Cu $>$ Ni $>$ Co $>$ Zn $>$ Cd $>$ Fe $>$ Mn $>$ Mg [46]. Indeed it was the order found for Cu, Ni and Cd in this study.

This study showed that ICP/OES is a reliable technique in determining heavy metal binding to Sphagnum peat moss, humin, and humic acids at ppb level. These results demonstrated that the heavy metal binding increases as pH increases. In general, at low pH values the percentage of metal bound to the three biomasses was strongly affected by the presence of the Cr species. Also, the highest percent of heavy metal binding was found at pH values of 4 and 5 for all the metals, with the exception of Cr(VI). However, the binding capacity of humic acids decreased at pH 6 in the presence of Cr(VI). The three biomasses showed a great affinity and selectivity for copper and lead. The order of affinity found in this study agreed with previous results reported in the literature. At low pH values of 2 and 3, the metal affinity for the three biomasses for a multi-metal solution containing Cr(III) was: Cu(II), Pb(II) $>$ Ni(II) $>$ Cr(III) $>$ Cd(II); if the metal mixture contained Cr(VI), Sphagnum peat moss and humin showed the following arrangement: Cu(II), Pb(II) $>$ Ni(II) $>$ Cr(VI) $>$ Cd(II), and humic acids showed the following arrangement: Cu(II) $>$ Pb(II), Cr(VI) $>$ Ni(II) $>$ Cd(II). This information will be very useful in understanding the fate and transport of heavy metals in aquatic environments at low concentrations.

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