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Leaching of lead by ammonium salts and EDTA from *Salvinia minima* biomass produced during aquatic phytoremediation

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Abstract

Plant biomass harvested after heavy-metal phytoremediation must be considered as a hazardous waste that should be contained or treated appropriately before disposal or reuse. As a potential method to detoxify the biomass and to convert this material to a suitable fertilizer or mulch, leaching of lead (Pb) from *Salvinia minima* biomass was studied by testing water, several aqueous ammonium salts, and EDTA solution as lead extractants. The research was carried out in two phases: (i) a leaching study to determine the lead-extraction efficiency of the different leachants, and (ii) a thermodynamic analysis to identify the likely reactions and stable Pb(II) species formed in the leaching systems of the most efficient leachants. Experimentally, lead concentrations measured in leached biomass and in leachates were significantly different among the various leachants. It was determined that the extraction strength of the leachants followed the order: EDTA > ammonium oxalate > water $\sim$ ammonium nitrate > ammonium acetate, achieving Pb extraction efficiencies of 99%, 70%, 7.2%, 6.9% and 1.3%, respectively, in single-stage extractions. The thermodynamic study indicated that the dominant species produced by the leaching process should be the soluble species $PbEDTA^{2-}$ for EDTA system, and the insoluble $Pb(COO)_{2S}$ precipitate for the oxalate system.
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1. Introduction

Phytoremediation is an environmental technology that uses plants to degrade, transform, immobilize, or stabilize various organic and inorganic pollutants present in soils, muds or wastewaters [1–5]. This technology is low-cost, simple, sustainable, compatible with the environment and aesthetically more attractive than the conventional technologies. It can be implemented *in situ* to remediate large expanses of contaminated ground [6] or to treat large volumes of dilute wastewater [1]. In Canada, United States and several countries of Europe, phytoremediation has been successfully implemented to treat soils, municipal and industrial wastewater, and groundwater polluted with heavy metals [1,6–9], metalloids (As and Se) [10,11], radionuclides (Cs, Sr, Co, U, and Ra) [12–14], nutrients (phosphates, nitrates, and ammonium), pesticides, herbicides and petroleum derivatives, among other chemicals [3,15].

Many organic contaminants and nutrients are degraded, absorbed and metabolized by plants (through phytodegradation or phytotransformation) in such a way that their basic components are incorporated into the plant tissues [2,3]. Heavy metals, metalloids and radionuclides may be immobilized or taken up by plants (through phytostabilization, phytoextraction, or phytofiltration), but in contrast to organic compounds, these are not metabolized but accumulated in the plant biomass [6,7,16–19]. Hence, the subsequent use of plant biomass harvested after phytoremediation depends on the type of pollutant treated. Biomass generated by phytoremediation of organic contaminants can often be used as fertilizer, forage, mulch or for the production of bio-gas [20]. On the contrary, the use of the biomass produced by phytoremediation of heavy metals is limited by the persistence of these elements.

One of the main drawbacks of heavy-metal phytoremediation is related to handling and disposal of contaminated plant waste [9,21,22]. In the literature it is mentioned that this biomass may be confined in landfills or used as compost [7–10,23]. Nevertheless, these options are questionable because metals could be liberated to the surrounding environment by leaching and other natural processes [21,23] polluting soils, surface water and groundwater and threatening human and animal health. Some authors have mentioned that the biomass can be dried, compacted, and incinerated to recover metals from the ash for recycling if these are valuable, in a similar way as is done for phytomining plants [24,25], or simply for confinement [1,2,6,8,21,26]. Others have proposed that biomass could be used as an energy source [23,22]. However, detailed studies related to the handling and use of the biomass produced by heavy-metal phytoremediation are scarce [21–23,27].

In one of the first articles published concerning the topic in question, co-firing with coal, liquid extraction by leaching, and composting were evaluated to determined the best treatment method to dispose of lead-contaminated plant biomass produced during lead phytoextraction from soil [21]. The authors concluded that leaching was the more effective method to remove lead from the plant biomass because by this process 98% of lead was extracted from the biomass. Co-firing and composting were ruled out because residual ash from the former requires post-treatment before it can be disposed whereas the composting material needs a pre-treatment to avoid lead leaching. Later, Sas-Nowosielska et al. [27] produced a review of phytoextraction-crop disposal methods. They found little information to be available but suggested that incineration could be the preferred disposal method because it is economically feasible and environmentally sound.

Recently, Keller et al. [23] investigated experimentally the thermal behaviour of two different plants used in heavy metals phytoextraction. In that study it was determined that pyrolysis was better than incineration to recover Cd and Zn from plant biomass, but its effectiveness depends on the metal volatility, plant species growth form (i.e., herbs, shrubs or trees), and incineration scheme (i.e., incineration alone or co-incineration with other solid wastes). It is important to note that the plants studied there were *Thlaspi caerulescens*, a Zn hyperaccumulator herb, and *Salix viminalis* (willow), a high biomass tree. To our knowledge, there is no research reported about the handling of aquatic plants produced during heavy-metal phytoremediation, which produces moist biomass with a finer structure and with characteristics different from terrestrial plants.

Considering that biomass harvested at the end of heavy-metal phytoremediation can be quite abundant and that its disposal in landfills represents a potential risk to living beings, we are seeking an alternative method which would allow us on the one hand to detoxify plant biomass in order to use it as bio-fertilizer or mulch and on the other hand to recover lead for confinement or recycling. The main objective of this research was to identify an efficient ammonium-salt solution for leaching Pb from *Salvinia minima* biomass, an aquatic fern with great potential for heavy-metal phytoremediation both in tropical and in subtropical environments [28–32], which has been identified as a hyperaccumulator of lead [28,30]. Our hypothesis is that, besides extracting Pb from plant biomass, ammonium salts can enrich the residual biomass with nitrogen compounds, thus enhancing its nutritional value. We selected ammonium salts because these are organic and biodegradable compounds, which have been used as extractants of heavy metals and other elements presents in soil, muds and radioactive wastes [33–39]. For comparison, *Salvinia* biomass was also leached with EDTA because of its strong metal-complexation capacity.

2. Materials and methods

2.1. Production of lead-contaminated Salvinia biomass

S. minima was cultivated in tap water, as an outdoor mono-plant culture, under the environmental conditions prevailing from October to November at the city of Xalapa, Veracruz, Mexico ($19°32'31''$N and $95°54'35''$W, altitude of 1580 m above sea level). Culture temperature held between $19\,°C$ and $23\,°C$, at pH 6, and the average light intensity was 172 μmol photon $m^{-2}\,s^{-1}$. After 7 days of cultivation plants were exposed for 24 h to various lead concentrations (10–15 mg Pb/L) in the water and then collected. Plants were rinsed with deionised water and dried in an oven at $35\,°C$ to constant weight. For further information see Olguín et al. [28]. Later, the dried plants were ground and analyzed at CIDETEQ using the methods indicated in Section 2.3 of this work.

2.2. Leaching experiments

Leaching experiments were carried out under batch conditions using dried and ground biomass from *Salvinia* plants that had an initial lead concentration of 25 g/kg dry weight. Preliminary screening experiments indicated that extraction performance was better with ground plants than with whole plants [40].

The leachants tested were aqueous ammonium acetate (CH_3COONH_4), ammonium oxalate ($(COONH_4)_2$), ammonium nitrate (NH_4NO_3), EDTA (Na_2-EDTA) and pure water. EDTA was used primarily as a reference leachant because of its strong metal-extraction capacity, and water was considered as a control leachant. The solid-mass-to-solution-volume ratio in our leaching experiments was 1:100; i.e., 2.5 g of plant biomass was contacted with 250 mL of each leachant solution containing the leaching reagent at 0.3 M. This leachant concentration was selected to avoid exceeding the solubility limit of the ammonium oxalate, which in previous experiments, carried out with various ammonium salts at various concentrations, was found to be the most effective agent for extracting Pb from plant biomass [41]. Leaching was performed under continuous stirring for 16 h at room temperature. Experiments were done in triplicate. The pH of each system was measured before and after leaching.

After leaching, the plant biomass was separated from the leachate using a plastic sieve with a mesh size of 18 (1 mm), rinsed with 250 ml of deionised water, drained and dried at $70\,°C$ for 72 h. Leachate samples were filtered to remove fine suspended solids using Whatman 40 filters. Subsequently, both

leachates and rinse-water samples were fixed with 5 ml of HNO_3 and chilled until analysis.

2.3. Analytical methods

Solid and liquid samples were digested with HNO_3 in a MARSX SEM microwave oven. Biomass was digested following the EPA Method 3052, while leachants and rinse water were treated according to EPA Method 3015 [42]. Lead concentration in the resulting liquid samples was determined by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) according to EPA Method 6010B [42] using a Perkin Elmer Optimal 3300DV Emission Spectrophotometer.

2.4. Statistical analysis

In order to test whether the observed differences in lead concentrations determined in both leached biomass and leachates were statistically significant, a one-way analysis of variance (One-way ANOVA) was applied, followed by a means-pairs comparison using the Tukey–Kramer HSD (Honestly Significant Difference) test ($\alpha = 0.05$). The statistical analysis were performed using JMP software version 4.0 (Statistical Analysis Systems Institute, Inc.).

2.5. Solution thermodynamics

A thermodynamic analysis was carried out by applying the Generalized Species and Equilibrium Method (GSEM), which is based on the chemical equilibria among species that can form in a given solution. This method is a combination of Charlot's and Ringbom's methods and was proposed by Rojas Hernández et al. [43–49] to represent the chemical equilibria in aqueous solutions with a simplified and systematic approach. The method makes it possible to construct predominance diagrams that help us to identify the most stable metal species that should form under specified conditions of pH, ligand concentration, etc. Simultaneously, such diagrams provide information concerning the chemical reactions among involved species, species stability, phases formation (i.e. soluble or insoluble species) and phases coexistence.

Predominance diagrams are maps that show, in two or three dimensions, the regions of primary stability of each chemical species of analytical interest which can form in a given system. They are constructed by recognizing all chemical reactions involved and their corresponding equilibrium constants [43–50]. The diagrams are drawn on a rectangular graph that usually represents pM' or pL' on the y-axis versus pH on the x-axis, where pM' and pL' represent the negative logarithms of the total concentrations of the metal and ligand, respectively. The prime symbol (') denotes the generalized species concentration of the metal (M) or ligand (L), defined as the sum of all species containing M or L present in a given aqueous system. Lines drawn on the predominance diagrams represent the boundaries between the different stable species potentially formed throughout pH scale. These lines depend on the equilibrium constants and the concentration of each component present in the solution as well

Table 1
Stability constants and solubility products for Pb(II) complexes formed with ligands from the various leachants tested

Ligand	Soluble species	
	Equilibrium	Formation constant
H_2O	$Pb^{2+} + OH^- \Leftrightarrow PbOH^-$	$\log \beta_1 = 6.4$
	$Pb^{2+} + 2OH^- \Leftrightarrow Pb(OH)_2$	$\log \beta_2 = 10.9$
	$Pb^{2+} + 3OH^- \Leftrightarrow Pb(OH)_3{}^-$	$\log \beta_3 = 13.9$
NH_3	$H_2O + NH_3 \Leftrightarrow NH_4{}^+ + OH^-$	$\log K = 9.24$
	$Pb^{2+} + NH_3 \Leftrightarrow PbNH_3{}^{2+}$	$\log K = 1.9$
$NO_3{}^-$	$Pb^{2+} + NO_3{}^- \Leftrightarrow PbNO_3{}^+$	$\log K = 1.17$
CH_3COO^-	$H^+ + CH_3COO^- \Leftrightarrow CH_3COOH$	$\log K = 4.76$
	$Pb^{2+} + CH_3COO^- \Leftrightarrow PbCH_3COO^+$	$\log K = 2.68$
	$Pb^{2+} + 2CH_3COO^- \Leftrightarrow Pb(CH_3COO)_2$	$\log K = 4.08$
$(COO)_2{}^{2-}$	$H^+ + (COO)_2{}^{2-} \Leftrightarrow H(COO)_2{}^-$	$\log K = 3.57$
	$2H^+ + (COO)_2{}^{2-} \Leftrightarrow (COOH)_2$	$\log \beta_2 = 4.57$
	$Pb^{2+} + H(COO)_2{}^- \Leftrightarrow PbH(COO)_2{}^+$	$\log K = 1.42$
	$Pb^{2+} + (COO)_2{}^{2-} \Leftrightarrow Pb(COO)_2$	$\log \beta_1 = 4.16$
	$Pb^{2+} + 2(COO)_2{}^{2-} \Leftrightarrow Pb[(COO)_2]_2{}^{2-}$	$\log \beta_2 = 6.32$
EDTA	$Pb^{2+} + HEDTA^{3-} \Leftrightarrow PbHEDTA^-$	$\log K = 2.8$
	$Pb^{2+} + EDTA^{4-} \Leftrightarrow PbEDTA^{2-}$	$\log K = 18.0$

Ligand	Insoluble species	
	Equilibrium	Solubility product
H_2O	$Pb^{2+} + 2OH^- \Leftrightarrow Pb(OH)_{2S}$	$pK_{sp} = 16.79$
$(COO)_2{}^{2-}$	$Pb^{2+} + (COO)_2{}^{2-} \Leftrightarrow Pb(COO)_{2S}$	$pK_{sp} = 9.02$

Note: $\log K$ values are the logarithms of equilibrium quotients obtained at specific conditions of temperature and ionic strength (IUPAC SC-Data base). In most of the cases we chose the equilibrium constant obtained at 25 °C and at 0 ionic strength, but for oxalates we use the equilibrium constant obtained at 25 °C and at 1 ionic strength in which is considered the oxalate precipitation.

as the pH of the system. Because there are more than two degrees of freedom available in multicomponent systems, all other concentrations and temperature must be specified. In this case the predominance diagrams were constructed considering both the lead concentration leached from the Salvinia biomass at the end of the leaching process, and the leachant concentration used (0.3 M equivalent to pL = 0.52).

Table 1 lists the lead-complexation reactions that are most likely to be significant in the leaching solutions studied here as well as the precipitation reactions for insoluble species. The table also gives the corresponding formation constants and solubility products, which were obtained from the International Union of Pure and Applied Chemistry Stability Constant Database (IUPAC SC-Data base) [85]. In most of the cases we chose the equilibrium constant reported for 25 °C and at 0 ionic strength, but for lead oxalate solubility we used the equilibrium constant obtained at 25 °C and at ionic strength 1.0 M.

For construction of the predominance diagrams we assumed that the anionic counter ion which balances the charge in the solutions is otherwise inert; that the activities of water and solid species are equal to 1.0, and we also took activity coefficients to be unity. The boundaries between regions were calculated to be the loci where the concentrations of equilibrated lead species in solution are equal.
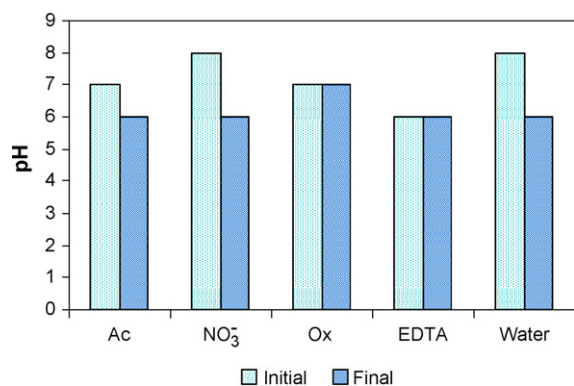
Fig. 1. Initial and final pH levels in the various leaching solutions.

3. Results

3.1. Leaching results

3.1.1. pH variations in the leaching systems

The initial and final pH values for each leaching system are shown in Fig. 1. In general, the initial pH among different systems varied between 6 and 8. After leaching, the pH value of the oxalate and EDTA solutions did not change, but the pH of acetate, nitrate and water systems did fall slightly, from a weakly alkaline pH to a weakly acid pH (Fig. 1).

3.1.2. Lead concentration in Salvinia biomass after leaching

Table 2 and Fig. 2 show the concentrations of lead determined in the various solid residues. Clearly EDTA was the best leaching agent followed by ammonium oxalate, whereas ammonium nitrate, ammonium acetate and water were ineffective to extract lead from the plant biomass. After leaching and washing, the biomass leached with EDTA had the lowest lead concentration, falling from 25 g/kg to 168 mg/kg. Residual lead concentration in the biomass leached with oxalate (7.75 g/kg) was much greater than that leached with EDTA but still smaller than that leached with ammonium acetate, nitrate or water (Fig. 2). The latter samples all contained high residual lead levels. The observed differences in lead content of leached biomass among the various extractants were statistically significant ($P < 0.05$; one-way ANOVA, followed by Tukey–Kramer HSD). According to the Tukey–Kramer HSD test, the following three groups were significantly different from one another: (i) the biomass leached
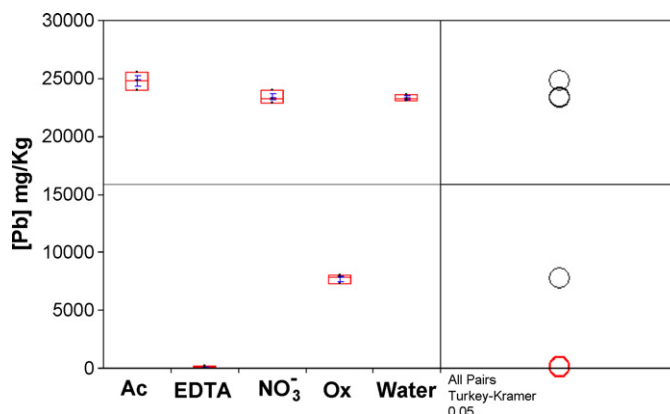

Fig. 2. Lead concentrations in residual biomass after leaching with ammonium acetate (Ac), ammonium nitrate (NO_3^-), ammonium oxalate (Ox), EDTA and water, and identification of statistically significant groups according to the Tukey–Kramer HSD test.

with acetates, nitrates and water, (ii) the biomass leached with oxalate, and (iii) the biomass leached with EDTA. The circles in Fig. 2 represent the groups involved. The centre of each circle is aligned with the mean lead concentration of the group that it represents, and its diameter spans its 95% confidence interval, estimated from the triplicate tests. Groups whose circles do not intersect with another are deemed to be significantly different.

3.1.3. Lead concentration in leachates

Lead concentrations measured in the ammonium acetate, ammonium nitrate, ammonium oxalate, and water leachates were quite low compared to the higher lead concentration in EDTA leachates (Table 2, Fig. 3). Among the former leachates the measured lead concentrations was not statistically different, but compared with EDTA, the difference was highly significant ($P < 0.05$; One-way ANOVA, followed by Tukey–Kramer HSD). The Tukey–Kramer test identified two statistically different groups: one represented by EDTA leachate and the other including the other four solutions (Fig. 3).

3.1.4. Lead mass balance

Table 2 shows also the calculated Pb mass balance for each leaching system, reported as the total mass of lead recovered in the solid and liquid samples after leaching, which may be compared with the original amount, 25,000 mg/kg. The amount of Pb recovered in the case of oxalate is seen to be quite low compared with the amount contained initially in the Salvinia biomass. The

Table 2
Average lead concentrations measured in samples of leached *Salvinia minima* biomass, leachates and rinse water, and the lead mass balance for the various systems

Leachant	Leached biomass [Pb] (mg/kg dry weight) Average (STD)	Leachate [Pb] (mg/L) Average (STD)	Rinse water [Pb] (mg/L) Average (STD)	Lead mass balance[a] [Pb] (mg/kg) Average (STD)
Oxalates (Ox)	7,747.1 (362.1)	5.93 (0.43)	3.31 (0.55)	8,661.7 (389.3)
Acetates (Ac)	24,843.1 (775.7)	2.67 (0.04)	0.62 (0.01)	25,172.7 (773.4)
Nitrates (NO)	23,444.1 (547.5)	2.94 (0.42)	1.04 (0.18)	23,842.8 (543.2)
Water	23,369.8 (290.3)	3.29 (1.24)	0.67 (0.39)	23,767.1 (188.9)
EDTA	167.6 (14.1)	197.4 (1.8)	23.34 (0.52)	22,241.9 (152.8)

Notes: Each value is the average of $n = 3$. Initial lead concentration in *Salvinia* biomass: 25,173 mg/kg dry weight. (STD): Standard deviation.

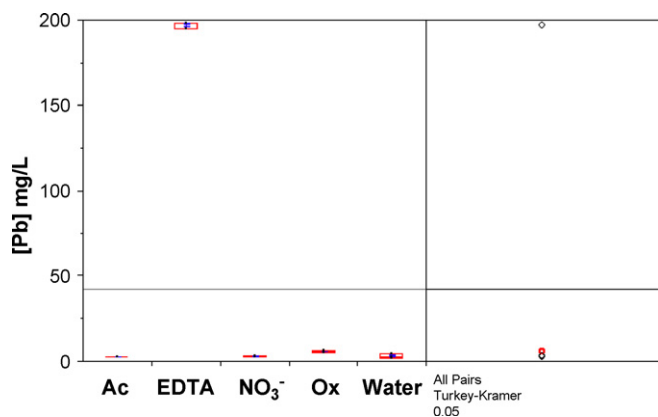[a] Total Pb recovered in solid and liquids.

Fig. 3. Lead concentrations in leachates from contacting with ammonium acetate (Ac), ammonium nitrate (NO_3^-), ammonium oxalate (Ox), EDTA, and water, and identification of statistically significant groups according to the Tukey–Kramer HSD test.

deficit is associated with precipitation of lead oxalate, which was very conspicuous during the processing. This insoluble fraction was retained in the filter during the solid–liquid separation step but was not analytically assessed. The amount of Pb recovered in the solid and liquid samples for the other systems is in fair agreement with the initial Pb concentration in the *Salvinia* biomass, but there are small differences among the nitrates, water, and EDTA. For the two former cases, the discrepancies may be related to a minor amount of Pb precipitation, whereas with EDTA there was probably some small loss of lead during digestion of the leachate samples.

3.1.5. Lead extraction efficiency

Fig. 4 presents the Pb extraction efficiency for the various leachants, based on the amount of lead removed from the *Salvinia* biomass. EDTA exhibited a lead extraction efficiency of 99%, followed by ammonium oxalate which had an efficiency of 70%. Water and ammonium nitrate and ammonium acetate solutions were ineffective leachants; their extraction efficiencies being 7.2%, 6.9%, and 1.3%, respectively.

3.2. Thermodynamic analysis

Lead complexes that may form in the leachates from our five leaching systems are included in Table 1. The only complex pos-
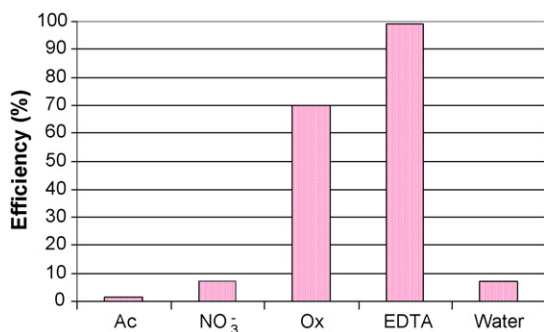

Fig. 4. Single-stage *Salvinia* biomass lead-extraction efficiencies for ammonium acetate (Ac), ammonium nitrate (NO_3^-), ammonium oxalate (Ox), EDTA, and water.

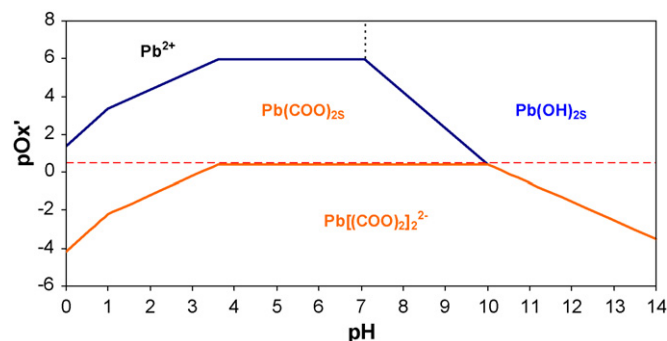Fig. 5. Predominance diagram for the Pb(II)/$C_2O_4^{2-}$/NH_4^+/H_2O system. Specified lead level: $[Pb^{+2}] = 8.41 \times 10^{-4}$ M. The horizontal line represents $[C_2O_4^{2-}] = 0.3$ M.

sible between Pb(II) and ammonia (NH_3) is the lead–ammonia cation ($PbNH_3^{2+}$). In our experiments with the several ammonium solutions, however, this complex does not form because the acidic conditions convert the ammonia to ammonium and because the affinity of Pb(II) for NH_3 is weak compared with that for nitrate (NO_3^-), acetate (CH_3COO^-) and oxalate [$(COO)_2^{2-}$] anions. Increasing the ammonium concentration to 1 M could form the lead–ammonia complex ($PbNH_3^{2+}$), but its region of stability would be limited to a narrow pH interval, i.e. from pH 7.3 to pH 8.2.

The thermodynamics of EDTA and oxalate leachates, the most efficient lead leaching systems, is shown in the predominance diagrams presented in Figs. 5 and 6, which were computed from the parameters given in Table 1.

3.2.1. Ammonium oxalate ($COONH_4)_2$

Fig. 5 gives the thermodynamic-stability diagram for the lead-ammonium oxalate system. Considering the lead level leached from *Salvinia* biomass (8.41×10^{-4} M equivalent to pPb = 3.07) and the oxalate concentration (0.3 M equivalent to pOx = 0.52) used in the leaching experiments, which is indicated by the horizontal line across Fig. 5, only insoluble species are stable: the precipitate of lead oxalate [$Pb(COO)_{2S}$], from pH 0 to pH 9.9, and the precipitated of lead hydroxide [$Pb(OH)_{2S}$] at pH greater than 9.9. As the pH of the experimental system was pH 7, the favoured species was $Pb(COO)_{2S}$. The lower region of the diagram suggests that a soluble oxalate complex, $Pb[(COO)_2]_2^{2-}$,
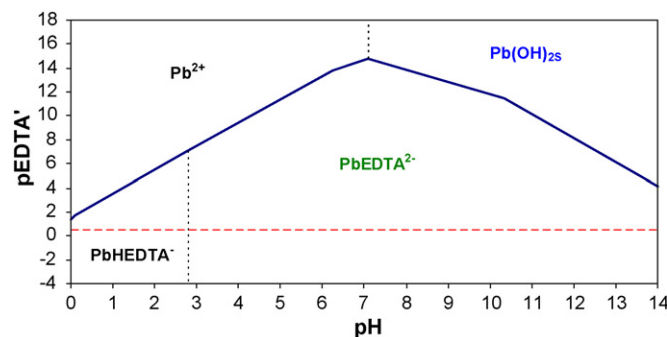

Fig. 6. Predominance diagram for the Pb(II)/H_2O/EDTA system. Specified lead level: $[Pb^{+2}] = 1.2 \times 10^{-3}$ M. The horizontal line represents [EDTA] = 0.3 M.

could form if total oxalate in excess of 0.3 M were provided.

3.2.2. Disodium ethylenediaminetetracetic acid (Na₂-EDTA)

Fig. 6 shows the diagram for EDTA. At the given lead and EDTA levels (pPb = 2.92 and pEDTA = 0.52), only soluble PbEDTA complexes should form over the entire range of pH. It is seen that the protonated species PbHEDTA$^-$ is predominant in acid up to pH 3, whereas above pH 3 the di-anion PbEDTA^{2-} is stable. Considering that the system pH was 6, the predominant species in our leachate was PbEDTA^{2-}.

4. Discussion

Metal-extraction efficiency in a leaching process depends of several factors, such as the matrix characteristics (i.e., substrate structure, chemical composition, texture, grain size, etc.), metal properties, leachant characteristics (i.e., concentration, binding power, solubility, etc.), and, of course, the conditions of the process itself (e.g., pH, temperature, phase ratio, agitation, and extraction time) [36]. Among these factors, pH is one of the more important parameters since it governs speciation, complexation and solubility as well as bioavailability and transport of heavy metals [36,51–55].

Generally, metal extraction from soils and solid wastes is more effective in acid conditions [36,56]. At the same time, acidic pH often modifies or destroys the chemical structure of the treated matrix [36,57]. As we are interested in preserving the biomass for use as a fertilizer or mulch, we did not acidify our leaching systems. Nevertheless, small pH variations were registered during the leaching tests. It was observed that the pH of the EDTA and oxalate systems did not change, but the pH of the acetate, nitrate and water systems did fall, from weakly alkaline or neutral pH to a weakly acid pH. This variation is attributed to proton liberation by both hydrolysis and some breakdown of biomass that occurs during the leaching process. In the case of the water and nitrate systems, the acidity of the solution after the extraction is explained by hydrolysis of free lead ions through the following reaction: $Pb^{2+} + 2H_2O \Leftrightarrow Pb(OH)_{2S} + 2H^+$. Also deprotonation of carboxylic and amino groups from the organic matter during the leaching process could also contribute to H$^+$ liberation [28,36]. It has been found that the deprotonation of different functional groups in plant biomass increases as pH increases [53]. Baig et al. [58] have shown that above pH 4 carboxylic groups in biomass (–COOH) are deprotonated (COO$^-$), thus increasing the availability of cation-binding sites. Considering that the biomass was exposed to a pH between 6 and 8, we suppose that this process together with the cation exchange inherent in the leaching process enriched the *Salvinia* biomass with NH₄$^+$ from the ammonium leachants, but the extent of this effect requires further research.

It is worth mentioning that Gramss et al. [59] recommend the use of NH₄$^+$ as a leaching agent in chelant-enhanced phytoextraction because it serves as an exchanging cation and as a proton donor. These authors explain that NH₄$^+$ reduces soil pH during nitrification process, increasing the solubility of Cd, Cu, Ni and Zn ions and consequently enhancing their bioavailability for plant uptake. Therefore, ammonium-enriched biomass produced during our leaching process could have a added value if it were used as a fertilizer in phytoextraction-plant cultures.

In plants, lead can be adsorbed by carboxyl groups (–COOH) from carbohydrates (galacturonic acid, glucoronic acid and uronic acid) present in the cellular walls of the roots. It can also be bound to different functional groups from internal structures or tissues such as thiol (–SH) from cysteine and glutathione, amino acids of phytochelatins, hydroxyl (–OH) and carboxyl (–COOH) groups from malate, citrate and oxalate, which are accumulated mainly in vacuoles, and carboxyl (–COOH) and amino (–NH₂) groups from amino acids [9,16–19,58]. It has been estimated that in some terrestrial plants the majority of absorbed metals are immobilized in the cellular walls and accumulated in vacuoles, both in roots and leaves [18,19,60], although the proportion appearing in different tissues and cellular structures depends on the type of plant, the metal species and plant phenology. Baranowska-Morek and Wierzbicka [60] have reported that the majority of Pb ions assimilated by plants are retained by polysaccharides synthesized in the cellular walls of the roots. In general it is known that lead in plants is accumulated more in roots than in leaves [8,19]. Such information is important to understand the lead extraction process from plant biomass regardless of the method used.

In our leaching study, the extraction power of the tested leachants followed the order EDTA ≫ Ox ≫ H₂O ∼ NO₃ > Ac. Quantitatively, the differences in extraction power among the leachants were significant, EDTA and oxalate being the most effective leachants whereas water, nitrate and acetate were practically ineffective.

Ammonium nitrate and ammonium acetate are considered to be weak extractants [33] and are generally used in sequential extraction procedures, at neutral pH, to determine the metal-exchangeable fraction in soils [56,61]. This fraction contains highly soluble metals that can be released into the liquid phase by a cation-exchange process [52]. The small amount of lead extracted with acetate, nitrate and water verifies the poor extraction power of these leachants but also indicates the low lead solubility from the biomass. This result allows us to infer that the major portion of lead in *Salvinia* biomass is bound strongly to the plant tissues. Previously, Olguín et al. [28] determined that lead in *S. minima* is adsorbed extracellularly, presumably by carboxyl groups and hydroxyl groups at the surface of pseudo-roots and fronds, and accumulated intracellularly. Similarly, we may infer that lead in *Salvinia* biomass is distributed between two fractions: (i) weakly adsorbed lead, which is the more soluble fraction, and (ii) lead strongly absorbed or accumulated in internal cell structures. Apparently, most of the lead is in the latter portion and amenable to extraction only by a strong leachant such as EDTA.

EDTA was considerably more effective for extracting Pb from plant biomass than ammonium oxalate. In fact EDTA reduced the lead concentration in *Salvinia* biomass to the permissible levels established in the Mexican environmental law concerning with the use and disposal of sludge and biosolids (NOM-004-SEMARNAT-2002), but oxalate did not.

Generally, EDTA is used widely in *ex situ* soil washing and *in situ* soil flushing to remove Cu, Zn and Pb, among other metals, from contaminated environments [36,62,63]. It has been shown that EDTA is the most effective leachant to extract Pb from polluted soils [36,39,51,56,64]. Furthermore, because of its strong capacity to solubilize metals from soils, making them more available for plant uptake, EDTA is also used widely in chelant-enhanced metal phytoextraction [54,55,65,66]. Nevertheless, EDTA represents a threat of groundwater contamination because most of the metals solubilized from soil are not accumulated by plants but are free to infiltrate the soil profile [65–70]. It has been estimated that only 10% of solubilized metals is taken up by plants; the remaining 90% can be released to groundwater [55]. Moreover, the low biodegradability of EDTA [26,64,66], its high toxicity for plants [55,66,68,71] and soil microorganisms [65], as well as its relatively high cost [51] are other drawbacks that limit the use of EDTA for *in situ* remediation of polluted soils. However, taking into account that: (1) EDTA is able to extract almost all of the lead content from *Salvinia* biomass; (2) the environmental risk may be reduced because biomass leaching could be carried out *ex situ*, under controlled conditions; and (3) the leaching costs could be reduced by recycling EDTA in subsequent leaching cycles [51,63,64,72–75], we suggest that EDTA is a potentially viable leaching agent to extract lead from *Salvinia* and other bioabsorbent plants. Unfortunately, the residual leached biomass could not be used directly as either a bio-fertilizer or as mulch, and its handling would require care.

Ammonium oxalate has also been used to extract metals from soils and other solid wastes. It has been demonstrated that oxalates are very efficient and selective for extracting Cu, Hg, Cd, Ni, Zn and As from soils, but it is less efficient to extract Pb [34–36,39,76]. In soils with high levels of organic matter, however, it has been found that oxalates are more efficient for lead extraction compared to other leachants [34,36]. Sun et al. [62] pointed out that metals bound to organic matter are more labile than those bound to minerals. The explanation is that the hydroxyl and carboxyl groups from organic matter have acid–base characteristics, which contribute to the formation of electrically charged groups, allowing the retention, fixation, exchange, desorption and complexation of metal ions and their ligands [36]. In this context, our results present evidence that ammonium oxalate is indeed an effective extractant for removing lead from predominantly organic matrices.

Unlike EDTA, oxalate is biodegradable, and its cost is lower [35,76]. These are the main advantages that oxalate offers in comparison with EDTA. Precipitation of lead as a lead oxalate during a leaching process could be an additional advantage since the precipitate can be removed from solution by filtration. This characteristic of oxalates has been used to recover heavy metals from electrolytic solutions [38] as well as to separate and purify radioactive elements from aqueous nuclear wastes [37].

Although oxalate leaching did not achieve the desired level of residual lead in the biomass, it did achieve 70% extraction in one stage. Thus, considering the advantages that oxalates offers as a chelating agent, it may be worth investigating the use of multistage oxalate leaching for lead extraction from the biomass.

It is worth mentioning that oxalates are produced naturally by plants [77,78], and among other functions they have a fundamental role in metal tolerance and homeostasis of the plants [79]. Two tolerance mechanisms have been identified in which oxalates are involved: (a) external exclusion, whereby a plant synthesizes oxalate exudates or excretions by its roots to complex and precipitate adsorbed metals in the surrounding environment, thus avoiding assimilation, and (b) internal exclusion, in which assimilated metals are complexed by oxalates and accumulated in vacuoles or deposited as oxalate crystals in cell walls [79,80]. These processes have been identified in buckwheat (*Fagopyrum esculentum*) [81], rice (*Oryza sativa*) [82] and water hyacinth (*Eicchornia crassipes*) [83], showing that lead can induce the synthesis of oxalates by plants as a tolerance mechanism. Therefore, we might say that a leaching process using oxalate is similar to the natural detoxification processes performed by plants.

Regarding to the lead concentration in the leachates of both leaching systems, remarkable differences were observed. As expected, lead concentration in the EDTA leachate was considerably higher than in the oxalate system. In both cases, however, lead concentration exceeded the permissible limit set on Mexican environmental legislation governing wastewater discharge to municipal sewer systems (NOM-002-Ecol-1996). Therefore, such leachates would require further treatment before discharge.

According to the thermodynamic analysis, under our leaching conditions, the favored complex in EDTA system was the soluble species PbEDTA^{2-}, whereas for the oxalate system the favored species was the precipitate of lead oxalate Pb(COO)$_{2S}$ although a small fraction of lead oxalate can remain in solution as Pb[(COO)$_2$]$_2^{2-}$. The predominance diagrams calculated in this study also show that for both the Pb(II)–oxalate and Pb(II)–EDTA systems neither pH nor leachant concentration should affect the complex-formation to any great extent. In the former system, for example, only the insoluble species [Pb(COO)$_{2S}$ and Pb(OH)$_{2S}$] will be favoured throughout the pH range, i.e., no adjustment of pH will produce a significant amount of Pb[(COO)$_2$]$_2^{2-}$ in solution. Seemingly, the soluble species could, in principle, be favoured by increasing the oxalate concentration to at least 0.62 M. Reaching this condition may be difficult, however, because at the working temperature the ammonium oxalate solubility is only at 0.358 M [84]. Redissolving the lead oxalate with excess oxalate would presumably require using several stages and adjusting both phase ratios and pH, or perhaps temperature.

In this way, the thermodynamic study allow us to know which species are favoured under our leaching conditions and what is the pH or leachant concentration required to favour the formation of a particular species. Such insight can guide the selection of suitable conditions for achieving efficient metal recovery, e.g., one can choice to produce a soluble and electroactive species for electrochemical recovery or to induce the formation of a precipitate that could be recovered by filtration.

The soluble lead contained in EDTA leachates could be recovered by precipitation [51,63,72,73] or by electrodeposition [74,75]. The same applies for the lead complexes from oxalate leachates. Since the soluble lead oxalate (Pb[(COO)$_2$]$_2^{2-}$) is an electroactive species it can be recovered by electrochemi-

cal means, while the lead oxalate precipitate, which forms as fine dense crystals, can be separated from the leached biomass by screening and filtration or other mechanical separation processes. We are currently working with the leachates from *Salvinia* biomass in order to recover the soluble lead by electrochemical means.

Traditionally, thermodynamic considerations in studies of metal extraction from soils have been limited to the comparison of formation constants, defined by IUPAC as an equilibrium constant that express the propensity of a substance to form from its component parts. Several researchers have pointed out that ligands with higher formation constants should produce more stable complexes and therefore be more effective for metal extraction than the ligands with lower formation constants [34,35,51,62,63]. However, this criterion is limited because it considers only the soluble species, neglecting the possible formation of insoluble species during the extraction process. Such effect was experimentally evident in our various leaching systems in which lead complexes extracted from *Salvinia* biomass occurred in both soluble and insoluble forms. Although sufficient information is not available to characterize the thermodynamic properties of lead bound to the solid biomass phases, the predominance diagrams showed here can indicate the relative strengths of various leaching solutions. In particular, the existence of solution-phase species much more stable than Pb^{+2} indicates a large driving force for extraction.

5. Conclusions and recommendations

Among the ammonium salts studied, the most effective leaching agent to extract lead from *Salvinia* biomass was found to be ammonium oxalate. Its lead extraction efficiency was considerably higher (70%) than the efficiencies of ammonium nitrate (6.9%) or ammonium acetate (1.3%). The lead concentration in biomass leached by oxalates was reduced significantly but not enough to satisfy the limit set by Mexican legislation related to biosolids disposal. Sequential leaching with oxalates could be an option to reduce the lead level in the leached biomass to the permissible limits.

Most of the lead extracted from plant biomass by oxalate precipitates as lead oxalate $[Pb(COO)_{2S}]$, and only a small fraction remains in a soluble form $[Pb(C_2O_4)_2{}^{2-}]$. The insoluble compound may be removed from the leachate by a proper filtration process, whereas soluble lead might be removed by precipitation or electrodeposition in order to meet the standards for wastewater discharges.

EDTA was much more efficient (99%) than ammonium oxalate (70%) in extracting lead from *Salvinia* biomass. EDTA reduced the lead content in the plant biomass to permissible levels for biosolids waste in a single step. Further treatment of leachates should be done to recover lead and perhaps to recycle EDTA.

In general, both EDTA and oxalates are efficient leachants for extraction of lead from *Salvinia* biomass and may also be useful for detoxifying *ex situ*, under controlled conditions, the biomass of other plants produced during heavy-metal phytoremediation.

In addition to studying the optimization of the leaching process using oxalates and lead recovery methods, it is suggested that further research be done considering both synthetic and natural chelating agents with structures similar to EDTA and oxalate that are biodegradable, such as [S,S]-ethylenediamine disuccinic acid (EDDS), ammonium citrate and other organic acids that have been shown to be efficient extractants for lead and other metals.

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