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Leaching of heavy metal from native plants by chemical leachants

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Abstract

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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. This study provides an evaluation of extractants for the removal of lead from Abutilon Theophrastus biomass. The research was carried out a leaching study to determine the lead-extraction efficiency of the different leachants (water, several aqueous ammonium salts, and ethylenediaminetetraacetic acid solution as lead extractants). The result of this study showed that, lead concentrations measured in leached biomass and in leachates were significantly different among the various leachants. Also the extraction strength of the leachants followed the order ethylenediaminetetraacetic acid>ammonium citrate> water ammonium phosphate > ammonium acetate, to , achieving lead extraction efficiencies of 96%, 67%, 4.2%, 3.9% and 0.3%, respectively, in single-stage extractions. In this study, ethylenediaminetetraacetic acid is the most frequently lead extractants. **(Akbarpour, F et al. Leaching of heavy metal from native plants by chemical leachants. International Journal of Agricultural Science, Research and Technology, 2011; 1(4):149-157).**

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1. Introduction

Phytoremediation is new technology based on the use of higher plants for cleanup process of contaminated environment .Fundamental and applied researches have unequivocally demonstrated that selected plant species have potential to remove, degrade, metabolize, or immobilize a wide range of contaminants. (Dushenkov et al, 1995). 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 or to treat large volumes of dilute wastewater (Dushenkov et al, 1995). In process industries such as: fertilizer breweries, refineries, paper mills, textiles, chemical and petrochemicals, water could be used as coolant, process water, and raw material solvent etc. However, in the process of usage, industrial water becomes polluted and contaminated with various substances it comes in contact with and this gives rise to waste water, which consists of water with variety of potentially harmful substance which is the source of environmental pollution. Sewage sludge is therefore, a by - product of purification of waste water. The resulting sewage sludge has significant organic mater content and contained macro and micro nutrients that are essential for plant growing. These heavy metals which may include cadmium, lead, arsenic etc., are metallic element with relatively high atomic weight that can contaminate ground water, surface water, food etc. and have the potential to be toxic and relatively low concentration metal. All heavy metal exist in waste water in colloidal, particulate and dissolve phases, although in dissolve concentration the colloidal and particulate may be found in hydroxide, oxide, silicate or substance: or absorbed to clay, silica or organic matter. (Lytle et al, 1998), metalloids(As and Se)(Rathinasabapath &Srivastava, 2006) radionuclides (Cs, Sr, Co, U, and Ra) (Dushenkov et al, 2002), nutrients (phosphates, nitrates, and ammonium), pesticides, herbicides and petroleum derivatives, among other chemicals (Macek etal, 2000).Many organic contaminants and nutrients are egraded,absorbed and metabolized by plants (through phytodegradation or phytotransformation) in such a way that their basic components are incorporated into the plant tissues (Reed et al, 1995). 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 (Lytle, 1998).

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 (Polprascrt, 1996).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 (Ghosh & Singh, 2005). Some reported work on this study showed that biomass may be confined in landfills or used as compost (Rathinasabapath & Srivastava, 2006; Lytle etal, 1998). Nevertheless, these options are questionable because metals could be liberated to the surrounding environment by leaching and other natural processes (Keller et al, 2005) 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 (Brooks et al, 1999), or simply for confinement (Alkorta et al, 2004; Robinson et al, 1999; Dushenkov et al, 1995). Others have proposed that biomass could be used as an energy source (Ghosh & Singh, 2005; Keller et al, 2005). However, detailed studies related to the handling and use of the biomass produced by heavy-metal phytoremediation are scarce (Keller et al, 2005; Sas-Nowosielska et al, 2004).In an earlier study 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(Nowosielska et al .,2004). The authors concluded that leaching was the more effective method to remove lead from the plant biomass because this process leads to the extraction of 98% lead 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 (2004) 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 (2005) investigated experimentally the thermal behavior 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 the best of 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 Abutilon Theophrastus biomass, an aquatic fern with great potential for heavy-metal phytoremediation both in tropical and in subtropical environments (Olgu´ın et al, 2005) which has been identified as a hyperaccumulator of lead (Olgu´ın et al, 2005). Therefore 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 (Gammons & Wood, 2000; Peters, 1999; Quevauviller et al, 1993). Precipitation of lead as a lead citrate during a leaching process could be an additional advantage since the precipitate can be removed from solution by filtration. This characteristic of citrate has been used to recover heavy metals from electrolytic solutions as well as to separate and purify radioactive elements from aqueous nuclear wastes (Gammons & Wood, 2000). Although citrate leaching did not achieve the desired level of residual lead in the biomass, it did achieve 67% extraction in one stage. Thus, considering the advantages that citrate offers as a chelating agent, it may be worth investigating the use of multistage citrate leaching for lead extraction from the biomass. It is worth mentioning that citrate are produced naturally by plants (Mazen et al, 2003), and among other functions they have a fundamental role in metal tolerance and homeostasis of the plants(Franceschi& Nakata, 2005).Two tolerance mechanisms have been identified in which citrate are involved: (a) external exclusion, whereby a plant synthesizes citrate 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 citrate and accumulated in vacuoles or deposited as oxalate crystals in cell walls(Franceschi&Nakata,2005).These processes have been identified in buckwheat (Fagopyrumesculentum) ,rice (Oryza sativa) (Yang et al, 2000)and water hyacinth (Eicchornia crassipes) Mazen &El Maghraby(1997), showing that lead can induce the synthesis of citrate by plants as a tolerance mechanism. Therefore, we might say that a leaching process using citrate is similar to the natural detoxification processes performed by plants. Remarkable differences were observed in the leachates of both leaching system's leaching concentration. As expected, lead concentration in the EDTA leachate was considerably higher than in the citrate 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). Careful assessment and evaluation is required to determine the biodegradation and toxicity of the chelating agents and their metal complexes in soils (Römkens et al., 2002). Although EDTA (ethylenediaminetetraacetic acid) was recognized as the most efficient chelants to increase metal uptake by plants, especially for the uptake of Pb, the low biodegradability of the chemical does not make it a good choice for large-scale field applications (Luo et al., 2005). Most of this kind of research has been carried out in the form of studies comparing the previous EDTA results in metal uptake efficiencies with additional data on the biodegradability of chelants and the metal leaching potential from the application of the chemicals (Luo et al, 2006). The optimization and application of this technology should be based on the full understanding of important processes involved, such as metal solubilization from the application of chelants, the uptake of metals by the roots of plants, and their transport upwards to the shoots of the plants. To prevent the possible movement of metal-chelants into groundwater and to reduce the impact of the remaining chelant on soil microorganisms, the selection of chelants and the amount and process of their application are important, as well as irrigation techniques and the time of the chelant application (Evangelou et al, 2007; Luo et al, 2007). N´u˜nez-L´opez et al (2008) investigated lead concentrations measured in leached biomass were significantly different among the various leachants. It was determined that the extraction strength of the leachants followed the order: EDTA> ammonium oxalate >water.ammonium nitrate > ammonium acetate, Therefore, such leachates would require further treatment before discharge. For comparison, Abutilon biomass was also leached with EDTA because of its strong metal-complexation capacity.

2. Materials and methods

2.1: Material

Lead-contaminated Abutilon biomass A. Theophrastus was cultivated, as an outdoor monoplant culture, under the environmental conditions prevailing from october to November at Babol city in Iran. Culture temperature was maintained between 19 ◦C and 23 ◦C, at pH 6, and the average light intensity was 172 _mol 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, following the methods of (Olgu'in et al, 2005). Later, the dried plants were ground and analyzed at CIDETEQ.A multi-institutional research group on phytoremediation for the sustainable use of water has been established recently in Mexico. The aim for the long term is to consolidate a multi-disciplinary group able to develop phytotechnologies suitable for certain critical problems in the country. The group is formed by researchers from 4 Institutes, located in various regions: INECOL at the east, CIMAV at the north, CICY at the southeast and CIDETEQ at the central region. The multi-institutional group was awarded with a grant from the National Council of Science and Technology for 2 years with certain possibilities of extension. The general aim is to develop phytotechnologies for the removal of Pb (II) from industrial wastewater and As (III) from groundwater (Olgu'in et al, 2005).

2.2. Leaching experiments

Leaching experiments were carried out under batch conditions using dried and ground biomass from Abutilon 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. The leachants tested were aqueous ammonium acetate, ammonium citrate, ammonium phosphate, EDTA and deionised 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 250mL 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 citrate, 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. 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 ◦C70 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 5ml ofHNO3 and chilled until analysis.

2.3. Analytical methods

Solid and liquid samples were digested with HNO_3 in a microwave oven. Biomass was digested following the EPA Method 3052, while leachants and rinse water were treated according to EPA Method 3015(EPA, 1998). Lead concentration in the resulting liquid samples was determined by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) according to EPA Method 6010B (EPA, 1998)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 analyses were performed using SPSS software version 14.0.

3. Results and discussion

3.1. Leachate results

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 citrate and EDTA solutions did not change, but the pH of acetate phosphate and water systems did fall slightly, from a weakly alkaline pH to a weakly acid pH (Fig. 1).

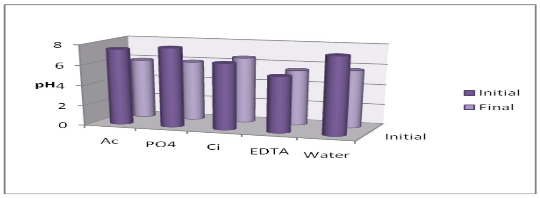

Fig 1. Initial and final pH levels in the various leaching solutions

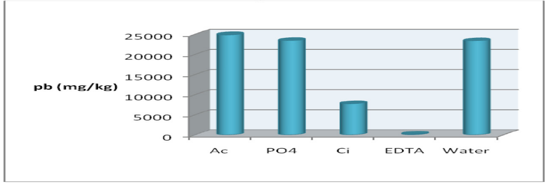

Fig. 2. Lead concentrations in residual biomass after leaching with ammonium acetate (Ac), ammonium phosphate (PO_4), ammonium citrate (Ci), EDTA and water

Table1and Fig 2 show the concentrations of lead determined in the various solid residues. Clearly EDTA was the best leaching agent followed by ammonium citrate, whereas ammonium phosphate, 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 citrate (7.75 g/kg) was much greater than that leached with EDTA but still smaller than that leached with ammonium acetate, phosphate 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 HSD).

According to the Tukey HSD test, the following three groups were significantly different from one another: (i) the biomass leached. With acetates, phosphate and water, (ii) the biomass leached with citrate, and (iii) the biomass leached with EDTA.

Table1. Average lead concentrations measured in samples of leached Abutilon theophrasti 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 balancea [Pb] (mg/kg) Average(STD)
Citrate(Ci)	7747.1(362.1)	5.93(.0.43)	3.31(0.55)	8661.7(389.3)
Acetates(Ac)	24843.1(775.7)	2.67 (0.04)	0.62(0.01)	25172.7(773.4)
Phosphate(po4)	23444.1(547.5)	2.94 (0.42)	1.04 (0.18)	23842.8(543.2)
Water	23369.8 (290.3)	3.29 (1.24)	0.67(0.39)	2376701(188.9)
EDTA	167.6(14.1)	197.4(1.8)	23.34(0.52)	22241.9 (152.8)

Notes: Each value is the average of $n = 3$. Initial lead concentration in *Abutilon biomass*: 25,173 mg/kg dry weight. (STD): Standard deviation a .Total Pb recovered in solid and liquids

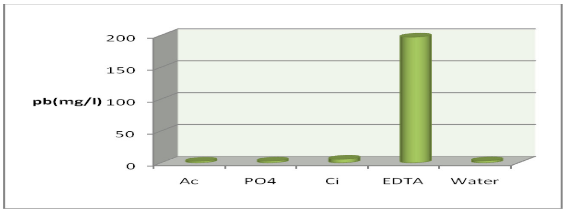

Fig. 3. Lead concentrations in leachates from contacting with ammonium acetate (Ac), ammonium phosphate (PO_4), ammonium citrate (Ci), EDTA, and water,

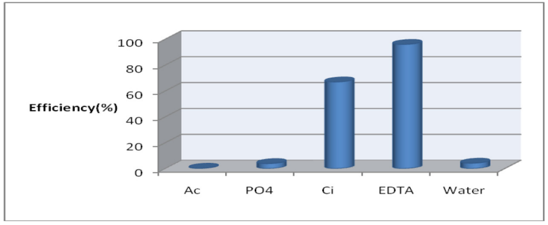

Fig .4. Single –stage Abutilon biomass lead –extraction efficiencies for ammonium acetate (Ac),ammonium phosphate (PO_4) ,ammonium citrate (Ci) , EDTA ,and water

3.2. Lead concentration in leachates

Lead concentrations measured in the ammonium acetate, ammonium nitrate, ammonium citrate, and water leachates were quite low compared to the higher lead concentration in EDTA leachates (Table 1, 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. 3. Lead mass balance

Table 1 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 citrate is seen to be quite low compared with the amount contained initially in the Abutilon biomass. The deficit is associated with precipitation of lead citrate, 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 Abutilon biomass, but there are small differences among the phosphate, water, and EDTA. For the two former cases, the discrepanciesn 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 Fig. 4 presents the Pb extraction efficiency for the various leachants, based on the amount of lead removed from the Abutilon biomass. EDTA exhibited a lead extraction efficiency of 96%, followed by ammonium citrate which had an efficiency of 67%. Water and ammoniumphosphate and ammonium acetate solutions were ineffective leachants; their extraction efficiencies being 4.2%, 3.9%, and0.3%, respectively.

Discussion

Metal-extraction efficiency in a leaching process depends on 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, the conditions of the process itself (e.g., pH, temperature, phase ratio, agitation, and extraction time) (Peters, (1999)). Among these factors, pH is one of the most important parameters since it governs speciation, complexation and solubility as well as bioavailability and transport of heavy metals. Generally, metal extraction from soils and solid wastes is more effective in acid conditions. At the same time, acidic pH often modifies or destroys the chemical structure of the treated matrix (Peters, 1999). Since the objective of the present study was preservation of the biomass for use as a fertilizer or mulch, hence the leaching system was not acidifying.

Nevertheless, small pH variations were registered during the leaching tests. It was observed that the pH of the EDTA and citrate systems did not change, but the pH of the acetate, phosphate 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 phosphate 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 (Olgu´ın etal, 2005; Peters, 1999). It has been found that the deprotonation of different functional groups in plant biomass increases as pH increases. Baig et al (1999) 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 Abutilon biomass with NH4 + from the ammonium leachants, but the extent of this effect requires further research. It is worth mentioning that Gramss et al (2004) 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. We suggested that NH^+_4 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 hytochelatins, 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 (Baig etal.,1999) 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, 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 (2004) 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 (Alkorta etal, 2004). In our leaching study, the extraction power of the tested leachants followed the order EDTA>Cit.>H_2O.Po_4 > Ac. Quantitatively, the differences in extraction power among the leachants were significant, EDTA and citrate being the most effective leachants whereas water ,phosphate,and acetate were practically ineffective Ammonium phosphate and ammonium acetate are considered to be weak extractants(Quevauviller etal.,1993) and are generally used in sequential extraction procedures, at neutral pH, to determine the metal exchangeable fraction in soils (Tandy et al, 2004). This fraction contains highly soluble metals that can be released into the liquid phase by a cation-exchange process. The small amount of lead extracted with acetate, phosphate 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 Abutilon biomass is bound strongly to the plant tissues. Previously, Olgu´ın et al (2005) determined that lead in Abutilon 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 citrate. 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 (Sun etal, 2001;Peters,1999). It has been shown that EDTA is the most effective leachant to extract Pb from polluted soils (Tandy etal, 2004; Kim&Ong, 1999; Peters, 1999).

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 (Nowack etal, 2006). 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 (Kos etal, 2003). It has been estimated that only 10% of solubilized metals is taken up by plants; the remaining 90% can be released to groundwater Nowack et al (2006). Moreover, the low biodegradability of EDTA(Robinson etal.,2000), its high toxicity for plants (Nowack et al, 2006) and soil microorganisms as well as its relatively high cost Kim,Ong (1999) are the 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 Abutilon 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 (Zeng et al, 2005), we suggest that EDTA is a potentially viable leaching agent to extract lead from Abutilon 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 citrate 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 (Elliot & Herzig, 1999; Peters, 1999). In soils with high levels of organic matter, however, it has been found that citrate are more efficient for lead extraction compared to other leachants (Peters, 1999) Sun et al (2001) 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 (Peters,1999).In this context, our results present evidence that ammonium citrate is indeed an effective extractant for removing lead from predominantly organic matrices. Unlike EDTA, citrate is biodegradable, and its cost is lower (Elliot&Herzig, 1999). These are the main advantages that citrate offers in comparison with EDTA .N´u˜nez-L´opez etal. (2008) presented that the extraction strength of the leachants followed the order: EDTA> ammonium oxalate >water.ammonium nitrate > ammonium acetate, achieving Pb extraction efficiencies of 99%, 70%, 7.2%, 6.9% and 1.3%.

The metal can be absorbed by plant roots and transferred as a Pb-EDTA complex. The complexes of Pb-EDTA cannot be split through the reduction or oxidation of Pb. It is also unlikely that Pb-EDTA or EDTA can diffuse across the plasma membrane at any significant rate, as they are too large and polar to move the plasma lemma lipid bilayer. It can be concluded that the uptake of Pb-EDTA by plants can take place in the location where suberization of the root cell walls has not yet occurred and at breaks in the root endodermis and the Casparian strip. Therefore, some damage to the root may be helpful for the indiscriminate uptake of Pb-EDTA by plant roots. The damage could be caused by the toxicity of metals, chelants. The soluble lead contained in EDTA leachates could be recovered by precipitation or by electro deposition. The same applies to the lead complexes from oxalate leachates. Since the soluble lead oxalate (Pb ((COO) 2)2 2−) is an electro active species, it can be recovered by electrochemical means, while the lead oxalate precipitates, which forms as fine dense crystals, can be separated from the leached biomass by screening and filtering or other mechanical separation processes. 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. However, this criterion is limited because it considers only the soluble species, neglecting the possible formation of insoluble species during the extraction process. Although sufficient information is not available to characterize the thermodynamic properties of lead bound to the solid biomass phases, the existence of solution-phase species is much more stable than Pb+2, which indicates a large driving force for extraction. The important issues concerning the selection of chelants and the development of leaching solutions are summarized as follows:

- Extraction strength. The chelant should be able to form strong, stable complexes with toxic metals over a wide pH range.
- Extraction selectivity towards target toxic metals.
- The potential for recovering the spent chelant. If the chelant is to be recycled and reused in the process several times, it should have low biodegradability in soil.
- The metal-chelant complexes should have low adsorption affinity towards solid soil surfaces.
- The chelant should have low toxicity and a low potential to harm the environment.
- The chelant should be cost-effective.

In this study, EDTA is the most frequently chelating agent for extracting potentially toxic trace metals from soils, because of its efficiency, availability and relatively low cost.

4. Conclusion and Recommendations

Among the ammonium salts studied the most effective leaching agent to extract lead from Abutilon biomass was found to be ammonium citrate .Its lead extraction efficiency was considerably higher (67%) than the efficiencies of ammonium phosphate (3.9%) or ammonium acetate (0.3%).The lead concentration in biomass leached by citrate was reduced significantly but not enough to satisfy the limit set by Mexican legislation related to biosolids disposal .Sequential leaching with citrate 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 citrate precipitates as lead citrate ,and only a small fraction remains in a soluble form(Pb (C2O4)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 (96%) than ammonium citrate (67%) in extracting lead from Abutilon 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 citrate are efficient leachants for extraction of lead from Abutilon biomass and may also useful for detoxifying ex situ, under controlled condition ,the biomass of other plants produced during heavy –metal phytoremediation.

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