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Phytoremediation of Lead Polluted Soils with Native Plant Species

Chávez Rodríguez, Luciana
(Soil Science Deparment, Universidad Nacional Agraria la Molina, Lima, Peru)

Abstract: *Ancient mining activities in Peru have resulted in more than 7000 environmental liabilities that are permanent sources of pollution (heavy metals). Additionally, ongoing mining projects have to deal with soil pollution at the end of their activity. For that reason and taking in account that some of the biggest pollutants (e.g. lead (Pb)) are always attached to mining activities, it is important to find sustainable ways to reduce their impact. Therefore, an experiment was set up, whereby, first, 37 plant species from 12 locations in La Oroya and la Junín Lake, departments of Cerro de Pasco and Junín, Peru, were checked for their Pb content under natural field conditions. Out of them, two plant species of the genus Calamagrostis sp. and Nicotiana sp were selected on the basis of their Pb content. Concentrations of up to 3180 mg kg-1 in roots and 143 mg kg-1 in above-ground biomass for Calamagrostis sp., and 1883 mg kg-1 in flowers and 2136 mg kg-1 in shoots, for Nicotiana sp., were found. With these plant species, a pot experiment (factorial design with four repetitions, level of significance 0.01%, 60 days of growth) was carried out to evaluate the potential for phytoremediation of Pb polluted soils. The plants were grown at three levels of Pb: 700 mg kg-1, 1000 mg kg-1 and 1200 mg kg-1. In addition, Vetiver grass (Vetiveria zizanioides) was also grown to compare its phytoremediation potential with the native species. Nicotiana sp. had a higher amount of above-ground biomass, its high concentration of Pb in the biomass (277 mg kg-1 in roots and 97 mg kg-1 in shoot and leaves), an extraction of Pb (0.3mg of Pb), its natural capacity of translocation of Pb into the above-ground biomass and immobilization in the roots (Translocation factor FT: 0.39).*

Keywords: *Accumulator species, Calamagrostis sp., Nicotiana sp., Vetiveria zizanoides, Translocation factor (FT), bioavailability of heavy metals, speciation, lead.*

I. Introduction

Traditionally, soils polluted with heavy metals are treated with techniques that have serious limitations in terms of alteration and lose of biological, chemical and physical properties of the soil, their high cost [10] and small care of the environment [17]. On the other hand, phytoremediation is becoming a promising technology replacing the traditional ones. It utilizes plants to remove and transform toxic chemicals [36] with the purpose of reducing their threat for humans and ecosystems. It is presented as a cost-effective and environmentally friendly technology that has an additional aesthetical result [10]. It also has a benefit by its contribution to regenerate the natural landscape of the polluted area [9].

Many research projects on phytoremediation have been carried out explaining not only the processes involved but also the plants with the highest potential for specific phytoremediation. Currently, more than 400 species of hyperaccumulation have been documented [35] and current investigation is still continuing to discover new hyperaccumulators. One of the best known plant species used for phytoremediation is Vetiveria zizanioides L due to its fast rate of growth, and its capacity to associate with mycorrhizas. This helps improving the absorption of the pollutant, and the property of repelling herbivores. As such, the entrance of pollutants into the food chain is prevented [1], especially in association with amendments that increase the bioavailability of the metals [6]. Other species commonly used are T. caerulescens, belonging to the brassicaceae family showing a good performance in phytoremediation of Pb, Cd and Zn [3]. Some perennial species such as Salix spp have also been used in controlled plots with successful results in uptake and accumulation of Cd, Zn and Ni in the above ground biomass [18]. In some cases, even edible plants have been used for phytoremediation. Corn (Zea mays), e.g has considerable potential of bioaccumulation of heavy metals due to its high capacity to build up biomass [28]; sunflower (Helianthus annuus) has been used for phytoremediation of Pb, Zc and Cd [20]; mustard and alhova (Trigonella foenum-graecum) were successfully tested in India in association with EDTA to remediate Pb and Cd [32], among many other examples.

Special care is currently taken for the use of native flora growing in polluted places. Native species that are already adapted to polluted environments can make the process of phytoremediation easier [9]. As such and due to the high plant diversity in Peru, it is worth to explore the existence of plants as heavy metal hyperaccumulators. This is even more important because of the mining tradition for many years, resulting in about 7000 environmental liabilities (Ministry of Energy and Mines of Peru) that little by little are covered with native vegetation. One of the most noticeable cases of pollution in Peru is the city of La Oroya located in Junín.

It has a mining activity since 1900 [26] until now, and as a consequence, Pb pollution, is one of the major heavy metal polluters of the environment [22].

Nowadays, native vegetation in the surroundings can be seen. This indicates that some plants have the ability to accumulate heavy metals. Within this context, the main objective of this research was to identify, under field conditions, plants that behave as accumulators of Pb, and to test the better ones under controlled conditions (pot experiment). In addition, their capacity was compared with a known hyperaccumulator species in order to verify if native plant species are superior for phytoremediation of their own natural environment.

II. Materials and Methods

2.1 Study sites

To evaluate plants growing in Pb polluted areas, a transect of the areas with known Pb pollution was carried out. Twelve points were selected to have a good representation of the city of La Oroya and the surroundings of Lake Junín (regions of Cerro de Pasco and Junín, in Peru). The majority of the points were located in the region of Junín, between 3400 and 4200 meter above sea level. Predominant vegetation belonged to the gramineae family such as Stipa ichu, as well as cactus, native shrubs, among others. Non- native species such as Pennisetum clandestinum were commonly found close to the towns.

2.2 Soil and plant sampling

At the 12 locations, 1 kg soil was taken from the arable layer without superficial vegetation. Those soil samples were used to characterize the study area in general terms. As the main purpose of the study was to evaluate the potential of plants for phytoremediation, a total of 37 plant samples were taken on the basis of their above-ground biomass production as well as their maturity and physical appearance. In some cases, seeds and living plants were taken for cultivation. The living plants were split up into one part for propagation and the rest was dried at 70°C for 2 days, milled, and digested with a solution of nitric perchloric acid for 1 hour in order to get foliar extracts for analysis of total Pb content by atomic absorption spectroscopy. Finally, two species where selected for the pot experiment. For the pot experiment, 1 kg soil was used. This soil had a pH of 7.4, a relatively high content of $CaCO_3$ (19.6%), a silt-loam texture, and a high Pb content (704.9 mg kg^{-1})

2.3 Plant cultivation

The first species used was Nicotiana sp. This is a 1-meter high growing herbaceous plant belonging to the Solanaceae family. It accumulates Pb mainly in the above-ground biomass under field conditions. The second one was Calamagrostis sp (Calamagrostis vicunarum). It is a small perennial gramineae of about 5-20cm height and normally used as fodder for extensive livestock. This plant accumulates, under field conditions, more metal in the roots than in the above-ground biomass. Additionally, also Vetiveria zizanioides (Vetiver grass) was used. This plant is known as an excellent heavy metal accumulator. The methodology of plant germination was different among the three species used in the study, but all of them were grown in open air conditions. In the case of Nicotiana sp., seeds were germinated in a substrate of sand and organic soil, in a proportion of 3 to 1. The plants were transplanted into the pots when they had a shoot size of about 5cm. In some cases, spontaneous growth of Nicotiana sp. was observed in the substrate used for the pot experiments, so some of those plants were used for treatments T2 and T3. Calamagrostis sp were directly transplanted from the sampled plants collected in the field (slip plants). After one month, new growing was observed. These plants were harvested at 2 cm of stem length for evaluation. The Vetiver grass was transplanted from samples collected from the Agraria University in Lima and put directly in the pots. After two weeks, plants were cut at 4 cm of stem length for evaluation. Plant transplanting and cultivation were carried from December 2013 until March 2014, completing two months of growth for each species. Watering was done generally every two days till the moisture content in the soil reached 50% of water field capacity. Temperature ranged from on average 20°C to 24°C, and air humidity was about 70%. Rainfall was not meaningful during the period of evaluation.

2.4 Lead Treatment

Before transplanting the plants into the pots, a solution of (400 ml, water field capacity) Pb acetate acid ($Pb(CH_3COO)_2 \cdot 3H_2O$) was homogeneously applied to the soil in the pots in order to achieve a specific Pb concentration. Table 1 shows the amount of lead salt used and the concentration of lead for each treatment. Each treatment had 4 repetitions, making a total of 36 pots.

Table 1. Treatments - Lead concentration and quantity of salt used

Treatment	Lead Concentration (mg kg^{-1})/pot	Quantity of salt ($Pb(CH_3COO)_2 \cdot 3H_2O$) in mg/pot
T1	700	0
T2	1000	549.2
T3	1200	915.4

2.5 Statistical Analysis

Results are reported in terms of concentration of Pb in roots and above-ground biomass, Pb uptake as well as the translocation factor. For comparison between the treatments, the Tukey test was used with α of 0.01% under a factorial design having as factor the levels of treatments (700, 1000 and 1200 mgPb Kg^{-1}) and the plant species (Nicotiana sp., Calamagrostis sp., and Vetiver grass). In order to fulfill model conditions, the Box Cox transformation was used. The shoot height and visual appearance were also monitored. The statistical analysis was done using the R project software.

III. Results

3.1 Plants characterization

Of the 37 collected plant species six genus were represented: Agrostis, Desmodium, Calamagrostis sp., Muhlenbergia, Glandularia and Nicotiana sp. (Table 2):

Table 2. Lead concentration of several plant species under field conditions

Genus of species	Lead concentration (mg kg^{-1})		Sample point location
	Above- ground biomass	Roots	
Agrostis	178	505	003
Desmodium	450	406	004
Calamagrostis sp.	143	3180	004
Muhlenbergia	1205	1164	004
Glandularia	394	1045	004
Nicotiana sp.	Flowers: 1882 Stem: 2136		005

From these six species, two belonging to the genus Nicotiana sp. and Calamagrostis sp. were selected for the pot experiment, because they accumulated more than 1000 mg kg^{-1} of Pb in their biomass.

3.2 Growth of the cultivated plant species under controlled conditions

The two native plant species as well as the Vetiver grass were grown for 60 days. During this period, no noticeable effect of Pb was found on the height of the grown plants (Fig. 1). In case of Nicotiana sp., e.g. growth was even higher for the highest polluted treatment (1200 mg kg^{-1} (T3) or 1000 mg kg^{-1} (T2)). For Calamagrostis sp., the behavior was quite similar, but in this case the highest growth was noticed for T2, and a reduction for T3. For Vetiver grass, growth was higher under the lowest pollution (T1). However, those differences were not statistically significant.

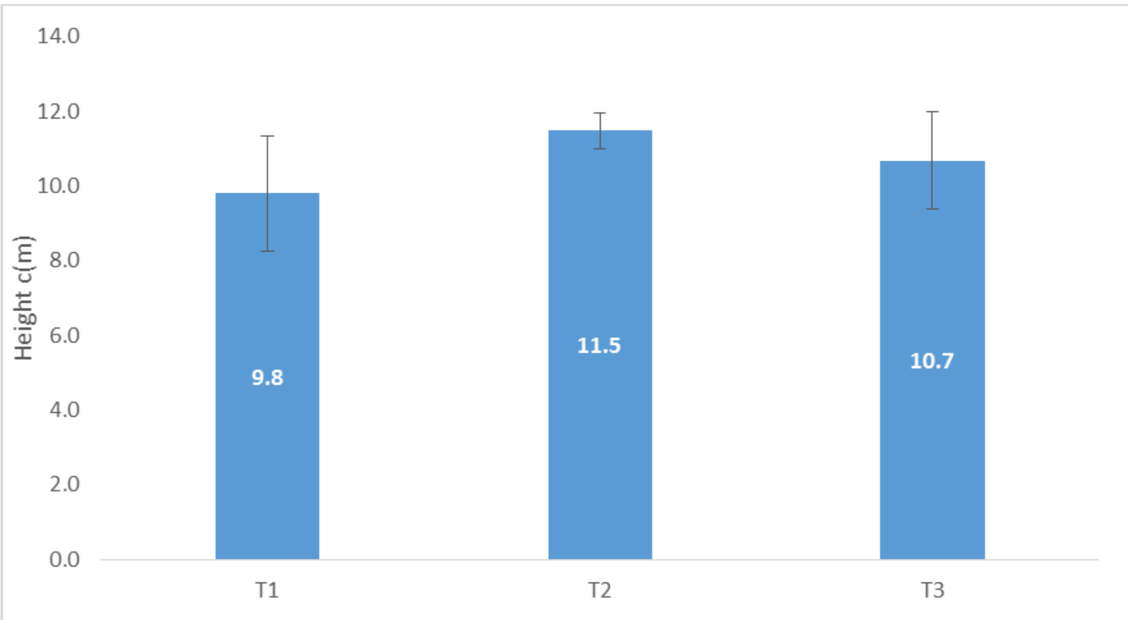

Figure 1a. Mean growth of Calamagrostis sp. under the 3 treatments
Cv: 9.49%

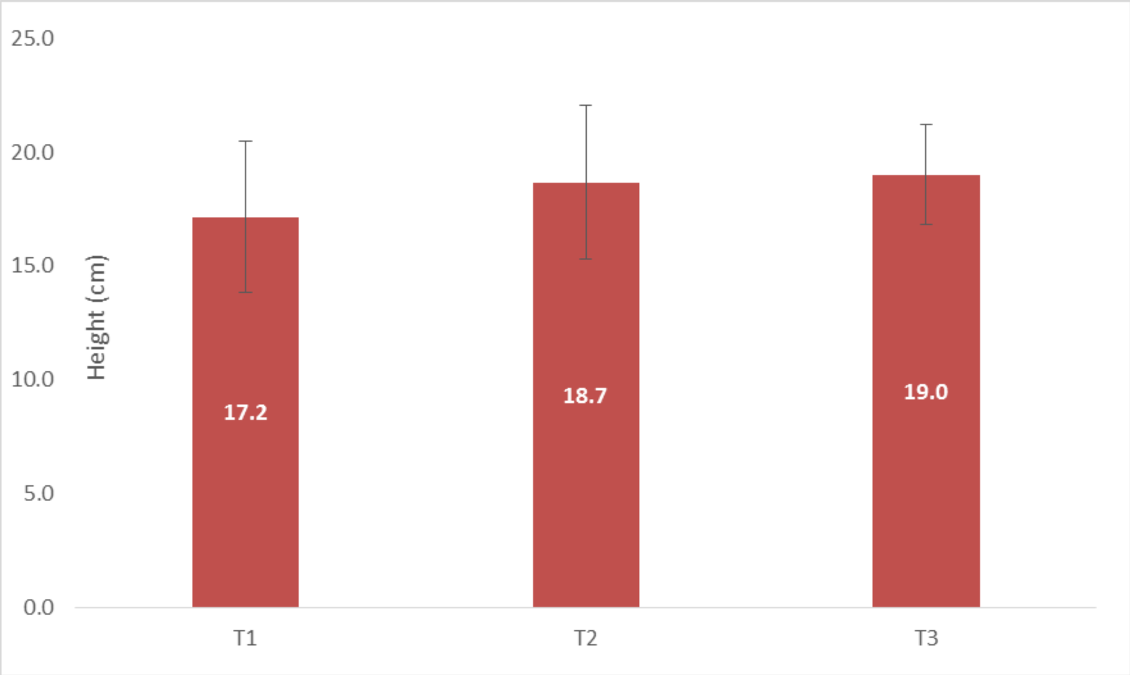

Figure 1b. Mean growth of Nicotiana sp. under the 3 treatments of lead
Cv: 18.88%

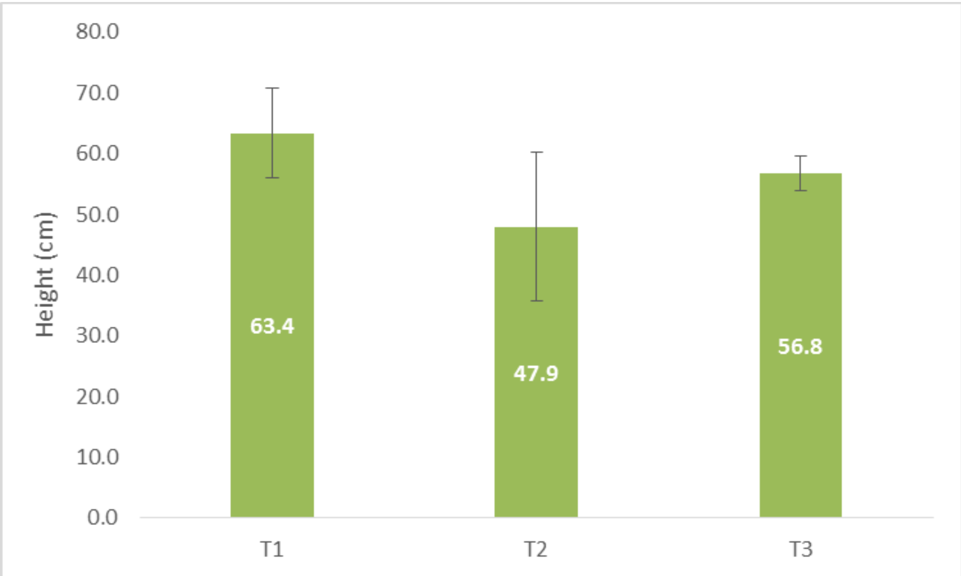

Figure 1c. Mean growth of Vetiver grass under the 3 treatments of lead
Cv: 14.03%

3.3 Lead uptake by plant species under controlled conditions

The mean Pb concentration, under the conditions of the pot experiment, was statistically significantly higher in the native species than in the Vetiver grass (Fig. 2). In the case of Nicotiana sp., the Pb concentration in the above ground biomass (96.5 mg kg-1) was statistically higher than in the Vetiver grass (15.6 mg kg^{-1}) and Calamagrostis sp. (48.4 mg kg^{-1}). However, the Pb concentration in the roots was higher in both native species (276.7 mg kg^{-1} and 299.8 mg kg^{-1} for Nicotiana sp. and Calamagrostis sp., respectively) than in the Vetiver grass (93.5 mg kg^{-1}).

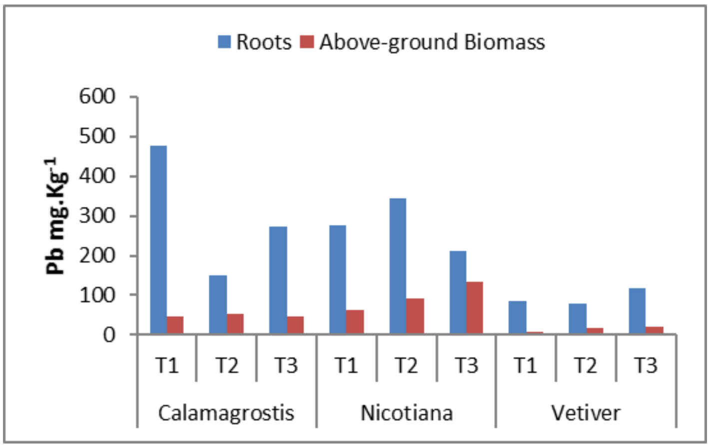

Figure 2. Pb concentration in plant species under the three Pb treatments
CV (above-ground biomass): 21.7%; CV (roots): 0.79%

With regard to the uptake of Pb in the above-ground biomass only a small amount was found in Calamagrostis sp. because of the time of growing (sixty days). Nicotiana sp. and Vetiver grass showed the same performance in terms of Pb uptake. However, there was more potential in the Nicotiana sp. This species can accumulate more heavy metal during its first stage of growth (Table 3).

Table 3. Biomass and Pb Extraction per plant species under the three treatments

Plant Species	Level of Treatment	Mean of Above-Ground Biomass	Standard Deviation	Mean of Pb Extraction	Standard Deviation
Calamagrostis sp	T1	0.27	0.12	0.02	0.01
	T2	0.42	0.32	0.03	0.01
	T3	0.25	0.05	0.03	0.01
Mean Value		**0.31**	**0.20**	**0.03**	**0.01**
Nicotiana sp	T1	1.69	0.88	0.19	0.06
	T2	3.03	0.18	0.32	0.04
	T3	3.10	0.46	0.39	0.10
Mean Value		**2.60**	**0.86**	**0.30**	**0.10**
Vetiver grass	T1	5.19	1.98	0.24	0.13
	T2	5.30	1.72	0.26	0.11
	T3	5.05	1.73	0.30	0.14
Mean Value		**5.18**	**1.65**	**0.27**	**0.12**

In the present study, the translocation factor (TF), defined as the relation between Pb concentration in the above ground biomass and Pb concentration in the roots [28], was lower than 1 (Table. 4). In terms of mean values, Vetiver grass had the lowest TF value (0.14), while no statistical differences were found between the TFs of Nicotiana sp. and Calamagrostis sp. Additionally, it was found that the highest value was registered with Nicotiana sp. under the highest Pb treatment (T3) with a mean value of 0.69.

Table 4. Translocation factor of the plant species under the three treatments

Plant Species	Level of treatment	Translocation Factor F per level of treatment	Standard Deviation
Calamagrostis sp	T1	0.29	0.13
	T2	0.41	0.21
	T3	0.22	0.08
Mean value		**0.31**	
Nicotiana sp	T1	0.24	0.09
	T2	0.22	0.03
	T3	0.69	0.06
Mean value		**0.38**	
Vetiver grass	T1	0.17	0.09
	T2	0.17	0.11
	T3	0.08	0.07
Mean value		**0.14**	

IV. Discussion

In the study area, the main source of Pb pollution is atmospheric deposition by the activity of the La Oroya foundry. This kind of pollution is also seen in other areas such as Spain where Pb, Cd and Zn pollution is highly observed in fields and grasslands due to the influence of an old Zn and Pb mine [29]. Another main source of Pb pollution is deposition from vehicular emissions like in Taiwan due the heavy metal content of diesel [41], or in Ibadan (Nigeria) where a high Pb concentration (205 mg kg^{-1}) is found in areas close to the Ibadan-Ilorin and Ibadan-Iwo motorways [21].

Under field conditions, a high diversity of plants can be found in highly Pb polluted areas due to favorable weather conditions that promote plant growing, as it was explained by Kim and Owens [12]. Of the six plant species shown in Table 2, Calamagrostis sp. and Nicotiana sp. were chosen, because they accumulated more than 1000 mg $kg^{-1}$of Pb in their biomass. This pollution level is used to define a plant species as hyperaccumulator of a particular metal in other studies [30], and it is a key factor for phytoremediation processes [5]. Other members of the gramineae family such as Cynodon dactylon and Lolium perenne have also shown to have a good potential for phytoremediation [34]. The same has been observed with Nicotiana glauca [2].

Generally, the growth, in terms of height and turgidity, of Calamagrostis sp., Nicotiana sp. and Vetiver grass was normal and no influence of Pb could be seen. The negative influence of Pb on growing hormones such as gibberellins, auxins and cytokinins [8] is well documented, but in our present research, Pb had a positive influence especially in the case of Nicotiana sp. However, more studies on biological and physiological aspects of Pb pollution should be done. Hormesis [33], defined as the doses of a toxic substance able to stimulate growth, should be considered for further research, as well as the presence of entophytic bacteria associated to the roots of plants that can promote plant growth. The same effect was found with Oryza sativa when grown in Cd enriched substrates in association with some bacteria of the genus Methylobacterium oryzae and Burkholderia Sp. They helped this plant to deal with Cd and to stimulate its growth [25].

In terms of biomass development, it was lowest with Calamagrostis sp. This should be attributed to the short time of growing (2 months), which was not enough to let this species reach the normal height it shows under natural growing conditions [27]. Obviously, under field conditions, the period of growth is much longer and, as a consequence, the level of accumulation higher [4]. Moreover, the growing conditions during the pot experiment differed importantly from the conditions in which native plant locally grow. Despite this, the level of Pb concentration found in the pot experiment for the native plant species was relatively high, especially in comparison with Vetiver grass. The reason of this behavior was not one the objectives of the present experiment. However, it could be somehow explained by the activity of the rhizosphere of the native plants, already adapted to polluted conditions and their exudates that can either increase bacterial activity, and as such, Pb speciation to forms which can be better assimilated by plants [14]. An additional possibility is the increased dissolution and bioavailability [13]. It is worth to mention that the growing period of 60 days of evaluation was selected to standardize the evaluation time for all species, and also because of the growth period of Vetiver grass that reached in some cases even 1m high in that period of time.

With regard to the uptake of Pb, only a small amount was found in the Calamagrostis sp. because of the low height and biomass obtained after 60 days of growing. Nicotiana sp. and Vetiver grass showed the same performance in terms of Pb uptake. However, there was more potential with Nicotiana Sp. because this species accumulates more Pb in its first stage of growth. It is important to mention that in Nicotiana sp., even if the difference is not significant, Pb uptake increased when the Pb concentration in the substrate increased. This result is similar to observations with Siam weed Chromolaena odorata (L.) and Cd, Pb and Zn enriched substrates [38].

In the present study, the translocation factor (TF), defined as the relation between Pb concentration in the above-ground biomass and Pb concentration in the roots [28], was lower than 1. Low values mean that the processes mostly occurring in plants are phytostabilization [43] and immobilization of Pb in the roots [11]. The observation is useful especially for species such as Calamagrostis sp. which are mainly used as fodder [27]. It avoids problems of bioaccumulation and biomagnification within the food chain [39]. It is the same with Nicotiana sp. There are no reports on its use whereby Pb can be transferred into the food chain. For this species, a high TF value was found in the highest polluted Pb treatment (T3). This result is similar with the one reported for rice, cultivar Japonica [15], showing that this species is more suitable for growing in polluted substrates.

V. Conclusion

It was found that Nicotiana sp. had the best potential for phytoremediation because of its high amount of above-ground biomass, its high concentration of Pb in the biomass (277 mg kg^{-1} in roots and 97 mg kg^{-1} in shoot and leaves), its extraction of Pb (0.3mg of Pb), its natural capacity of translocation of Pb into the above-ground biomass and immobilization in the roots (Translocation factor FT: 0.39). With this experiment, it was confirmed that native species are most appropriate for phytoremediation in natural areas. However, it is known the limitations of plastic pot based experiments, in terms of overestimation of the real capacity of phytoremediation of the species, so it is important to do further research in such conditions that let Pb equilibrates in the soil environment. Despite this, it is important to carry out this kind of experiments to have much more control in the experiment, understand the processes behind phytoremediation, and try to identify the species, tasks quite difficult to do under field conditions.

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