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Assessment of Native and Endemic Chilean Plants for Removal of Cu, Mo and Pb from Mine Tailings

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Abstract: In Chile, 85% of tailings impoundments are inactive or abandoned and many of them do not have a program of treatment or afforestation. The phytoremediation of tailings with *Oxalis gigantea*, *Cistanthe grandiflora*, *Puya berteroniana* and *Solidago chilensis* have been tested in order to find plants with ornamental value and low water requirements, which enable reductions in molybdenum (Mo), copper (Cu) or lead (Pb) concentrations creating an environmentally friendly surrounding. Ex-situ phytoremediation experiments were carried out for seven months and Mo, Cu and Pb were measured at the beginning and at the end of the growth period. The capacity of these species to phyto-remedy was evaluated using the bioconcentration and translocation factors, along with assessing removal efficiency. *Solidago chilensis* showed the ability to phytoextract Mo while *Puya berteroniana* showed potential for Cu and Mo stabilization. The highest removal efficiencies were obtained for Mo, followed by Cu and Pb. The maximum values of removal efficiency for Mo, Cu and Pb were 28.7% with *Solidago chilensis*, 15.6% with *Puya berteroniana* and 8.8% with *Cistanthe grandiflora*, respectively. Therefore, the most noticeable results were obtained with *Solidago chilensis* for phytoextraction of Mo.

Keywords: phytoremediation; heavy metals; mine tailings; endemic species; native species

1. Introduction

Tailings are a mixture of water and heavy metal-bearing fine-grained minerals [1,2]. In Chile, there exists 757 tailings storage facilities (TSF) of which 173 are abandoned, 111 active, 468 inactive and 5 of them are under construction, according to the last record of mine tailings published on August 10th, 2020 by the National Geology and Mining Agency of Chile [3].

Soil contamination by heavy metals can be particularly hazardous due to the properties of these elements [4]. Central Chile presents climatic conditions that favor the dispersion of particles and the occurrence of metal lixiviation [5].

In Chile, mining from porphyry copper and molybdenum deposits occurs and it is common to find—in the areas surrounding mining activities—high concentrations of As, Cd, Cu, Zn, Pb, and Mo, and thus, soil pollution by potentially toxic elements contained in mining tailings is a latent problem that can cause important environmental damage [6,7].

Lead (Pb) is one of the most toxic metals and it has a significant influence on plant growth and development [8]. Under normal environmental conditions, the mobility of Pb is low but it is increased when more acidic conditions prevail [9]. The toxicity and adverse effects of Pb on plant species have been found to occur at very low concentrations, even at micromolar levels [10]. A consensus exists that the Pb taken up by plants from soils remains in the roots [11,12]. Pb may be translocated from roots to the aerial parts of the plant, however, in the majority of plants (>95%) Pb is accumulated in the roots and only a small portion is translocated to the parts above the ground [9]. The threshold level of Pb for plants is around 2 mg·kg^{-1} [13].

Copper (Cu) is an essential metal for plants; however, it is toxic at high concentrations. Normal values of Cu in plants are between 4 and 15 mg Cu·kg^{-1} dry matter and the critical values in roots are in the range of 100 to 400 mg Cu·kg^{-1} dry matter [14]. Oorts, et al. (2013) indicated the onset of Cu toxicity in shoots and leaves between 5 and 40 mg Cu·kg^{-1} dry matter, while Marschner (2000) specified a concentration higher than 20 or 30 mg Cu·kg^{-1}, depending on plant species [14,15].

In the case of molybdenum (Mo), it can be mobile and bioavailable as $MoO_4{}^{2-}$ [9]. Only small quantities of this element are required by plants, with the normal range for most plant tissues being between 0.3 mg·kg^{-1} and 1.5 mg·kg^{-1}. Moreover, toxicity levels of Mo in plants differ according the species, where values of toxic Mo concentrations have been reported in the range from 100 to 1000 mg·g^{-1} dry matter [16].

The large number of abandoned tailings makes it necessary to find a cost-effective solution and, therefore, to mitigate the negative effects of heavy metals in soils, several methods such as membrane filtration, electrodialysis, and soil washing, among others, have been explored, however, they are expensive and environmentally unfriendly [17]. Among the remediation technologies, several studies have proven the usefulness of phytoremediation as an efficient and environmentally friendly method for removing organic and inorganic contaminants, moreover, it is a cheaper method compared to chemical remediation, biopiles and bioventing, which incorporates the use of plants to remove contaminants from water and soil [18].

Marques et al. (2009) highlighted the three major phytoremediation techniques: phytoextraction, stabilization and volatilization [19]. Additionally, Lam et al. (2018) distinguished two strategies of phytoextraction: the use of plants with a large ability of accumulation in shoots and low biomass, and the use of plant species with high biomass and low ability of extraction [20].

The potential use of certain species for phytoremediation can be evaluated by using the bioconcentration factor (BCF) and translocation factor (TF). BCF is described as the ability of plants for elemental accumulation from the substrate, and the ratio between the concentration of metal present in the plant and the total final metal concentration in soil is considered as an index of bioavailability [21], while TF is used to assess the plant's potential to translocate contaminants [22,23]. BCF values higher than one are indicative of potential success of a certain plant species for phytoremediation, while a TF greater than one indicates the ability to translocate the metal to aerial parts [21]. On the other hand, the consideration of a species as a stabilizer of heavy metals is based on a BCF ≥ 1 and a TF ≤ 1 [24].

Tailings are a poor medium for promoting natural plant growth, they normally have low field capacity, high salinity, high concentration of contaminants such as heavy metals and a lack of organic matter [18]. In order to improve the characteristic of the substrate and to achieve self-sustaining growth of the plants over time, the addition of nutrients, and amendments and/or organic matter are essential for phytoremediation to remediate tailings [25,26].

Prosopis tamarugo, *Schinus molle* and *Artiplex nummularia*, all of them Chilean native species, have been studied for in-situ phytoremediation of tailings in the region of Antofagasta, Chile, with the addition of an organic compost and water for irrigation [27]. All species showed BCF < 1 with different treatments, but *S. molle* has shown features as an accumulator for Cu, Mn, Pb and Zn, and *P. tamarugo* for Mn, Zn and Cd, with TF > 1. *A. nummularia* was the most promising of these species, it showed an accumulator behavior for Mn, Pb and Zn [27]. Lam et al. (2018) evaluated the potential of *Adesmia atacamensis* in the phytoremediation of mine tailings. The results of TF and BCF allowed for the classification of the plant as a Cu hyperaccumulator [20].

Alfonso et al. (2020) obtained auspicious results with the use of indigenous plants for the in-situ phytoremediation of tailings from the Camaquã Mine (Southern Brazil). Eleven different species of spontaneous occurrence in the mine site were assessed. The translocation factor and bioconcentration factor were calculated. Seven of the studied species showed phytoextraction potential for Pb and four species showed some ability for the phytostabilization of Cu [28].

The aim of this study was to determine the potential of Chilean native or endemic plant species, to phyto-remedy mine tailings. Four species from northern Chile: *Oxalis gigantea*, *Cistanthe grandiflora*, *Puya berteroniana* and *Solidago chilensis*, were chosen according to their low water requirements and ornamental value. The potential of these species for phytostabilization or phytoextraction of Mo, Cu and/or Pb in mine tailings was assessed through ex-situ pot experiments.

2. Materials and Methods

2.1. Characterization and Preparation of Mine Tailing

Paste tailing from Compañía Minera Las Cenizas located in Cabildo, Valparaíso Region, Chile was used. The mine company processed copper sulfide and oxide minerals. The sampling location is presented in Figure 1.

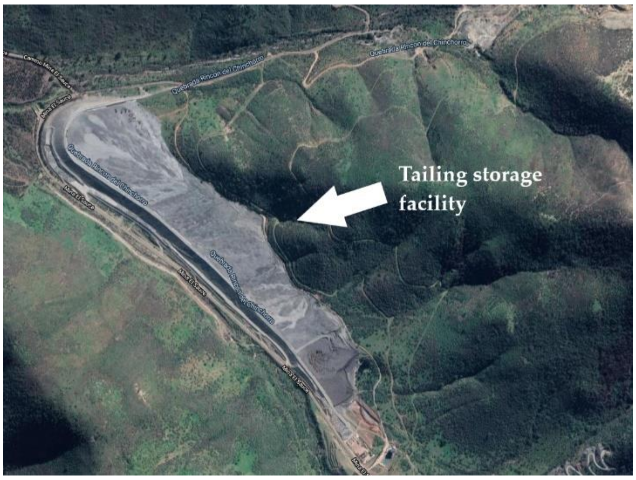

Figure 1. Tailing storage facility (32°28′16.1″ S, 71°05′00.2″ W).

Before the phytoremediation experiments, tailings were dried at 105 °C until achieving constant mass, ground in a ball mill, sieved through an ASTM mesh 19 mm and homogenized [29]. The main properties of the tailings are presented in Table 1. Table 2 shows the initial concentrations of Mo, Cu and Pb measured by ICP-OES.

Table 1. Main geochemical properties of tailing.

Parameter	Value
Specific gravity	2.82
Solid concentration in weight %	83
Granulometry d_{50} micrometers	0.046
Granulometry d_{20} micrometers	0.005
Granulometry d_{80} micrometers	0.240

Table 2. Initial concentration of Mo, Cu and Pb in dry tailing ± confidence interval (IC).

Element	Concentration mg·kg^{-1} Dry Tailing ± IC
Cu	1582.22 ± 78.31
Mo	3.86 ± 0.17
Pb	228.15 ± 2.79
Zn	86.98 ± 3.15
Ni	9.46 ± 0.25
Cd	Under detection limit
Cr	15.46 ± 0.54

2.2. Plants Species

Four different plant species were used for the phytoremediation studies: *Oxalis gigantea*, *Cistanthe grandiflora*, *Puya berteroniana* and *Solidago chilensis*.

Oxalis gigantea Barnéoud (Churqui or Churco) is a very common endemic Chilean plant which belongs to the *Oxalidaceae* family. It grows in northern Chile from the Antofagasta to Coquimbo regions and is hardy to USDA Zone 10 and 11. *Cistanthe grandiflora*, frequently called Doquilla or Pata de guanaco is an endemic Chilean plant of the *Portulacaceae* family, which can be found between the Antofagasta and Ñuble regions. It is hardy to USDA Zone 9. *Puya berteroniana* is an endemic Chilean plant of the *Bromeliaceae* family, commonly called Chagual, Cardón or Magüey and has an excellent ornamental value. This plant grows from the Coquimbo to Maule regions and is hardy to USDA Zone 9. Finally, *Solidago chilensis*, or commonly called Fulel, is a native Chilean plant that can be found between the Arica and Parinacota and Los Lagos regions. This plant belongs to the *Astaraceae* family, *Solidago chilensis* is hardy to USDA Zone 9 and Los Lagos [30,31].

2.3. Potted Experiments

Plants with an initial height of 10 cm were placed into pots with 1440 g of dry tailing. The pots were left outdoors over a seven-month period, under similar environmental conditions to those where the mine tailings impoundment is located.

For each plant species, three specimens were placed in tailing. Potable water and biofertilizer were provided weekly and monthly, respectively. The characterization of foliar organic stimulant is presented in Table 3.

Table 3. Foliar organic stimulant composition (based on marine algae *Ascophyllum nodosum*).

Element	Concentration
Nitrogen	0.1% *w*/*w*
Phosphorous	0.0% *w*/*w*
Potassium	3.0% *w*/*w*
Arsenic	<0.5 mg·kg^{-1}
Cadmium	<0.5 mg·kg^{-1}
Lead	<1 mg·kg^{-1}
Mercury	<0.5 mg·kg^{-1}

2.4. Sample Preparation and ICP-OES Measurements

Upon the expiry of the growth period, leaves and stems (aerial part) and roots were divided with a knife and carefully washed with abundant potable water, distilled water and deionized water to remove tailing particles adhering to them and any other type of dirt. Both parts of the plants were cut to reduce their size and placed into waxed paper envelopes, afterwards they were dried at 45 °C until constant mass was achieved, ground and homogenized.

Tailing was carefully cleaned, dried at 105 °C until constant mass was achieved, grounded, sieved through an ASTM mesh N°18 and homogenized.

For ICP-OES measurements, digestion procedure was carried out with 0.200 g of dry sample, which were placed in a Teflon vial for microwave and 8 mL of concentrated HNO_3 and 2.0 mL of concentrated H_2O_2 were added. The vials were covered with parafilm tape and were left to pre-digest for 4 h before the digestion in the microwave. When the samples were at room temperature, they were placed in 25 mL volumetric flasks which were then filled with deionized water to the calibration line. All reagents were of analytical grade.

All samples were prepared in duplicate and digested twice in a microwave Ethos Easy. The temperature program consisted of three segments: the first from 0 to 10 min with an increase in temperature until 180 °C, a second period of 10 min with a constant temperature of 180 °C and the last corresponding to a cool down period of 10 min.

2.5. Heavy Metal Determination

The concentrations of Mo, Cu and Pb were determined in tailing and plants (roots and stems + leaves = aerial part). The metal concentrations in plants and tailings samples were determined by inductively coupled atomic emission spectroscopy (Perkin Elmer), directly from digested solutions at the Institute of Chemistry and Biochemistry, Faculty of Science of Universidad de Valparaíso, Chile.

For the present study, the bioaccumulation factor (BCF) and translocation factor (TF) were calculated with Equations (1)–(3) [4,32–34].

$$\text{TF} = \frac{\text{Metal concentration (Stems + Leaves)}}{\text{Metal concentration in roots}} \tag{1}$$

$$\text{BCF}_{\text{roots}} = \frac{\text{Metal concentration in roots}}{\text{Initial concentration of metal in tailing}} \tag{2}$$

$$\text{BCF}_{\text{aerial}} = \frac{\text{Metal concentration in aerial parts}}{\text{Initial concentration of metal in tailing}} \tag{3}$$

The removal efficiency (RE) was calculated with the Equation (4).

$$\text{RE} = \frac{(C_i - C_f)}{C_i} \times 100\% \tag{4}$$

where C_i and C_f are the initial and final concentration of the element in the tailing.

3. Results

The final concentrations of Mo, Pb and Cu in each plant species, divided into roots and aerial parts, were determined after the growth period. For each plant, three samples of roots and three samples of aerial parts were taken in duplicate, the final mean concentration of each duplicate is presented in Figure 2.

All plant species showed a decreasing trend of Pb and Cu concentrations from tailing to aerial parts (tailings → roots → leaves and stems) but in the case of Mo this decreasing trend is only observed in the case of *Cistanthe grandiflora*.

Oxalis gigantea presented a Mo concentration in aerial parts slightly higher than in roots, while *Puya berteroniana* exhibited a concentration of Mo in roots higher than what was found in the final tailing. *Solidago chilensis* showed the reverse trend with a decreasing concentration of Mo from aerial parts to tailing.

The ability of *Solidago chilensis* and *Puya berteroniana* to accumulate Pb in their roots is notorious, far exceeding the normal threshold levels. Additionally, the same species showed the ability of Cu accumulation in roots.

To evaluate the ability of all species to translocate or stabilize the studied metals, TF, BCF and removal efficiency were calculated; in the case of BCF, this factor was obtained for roots and for aerial parts. The results are presented in Table 4.

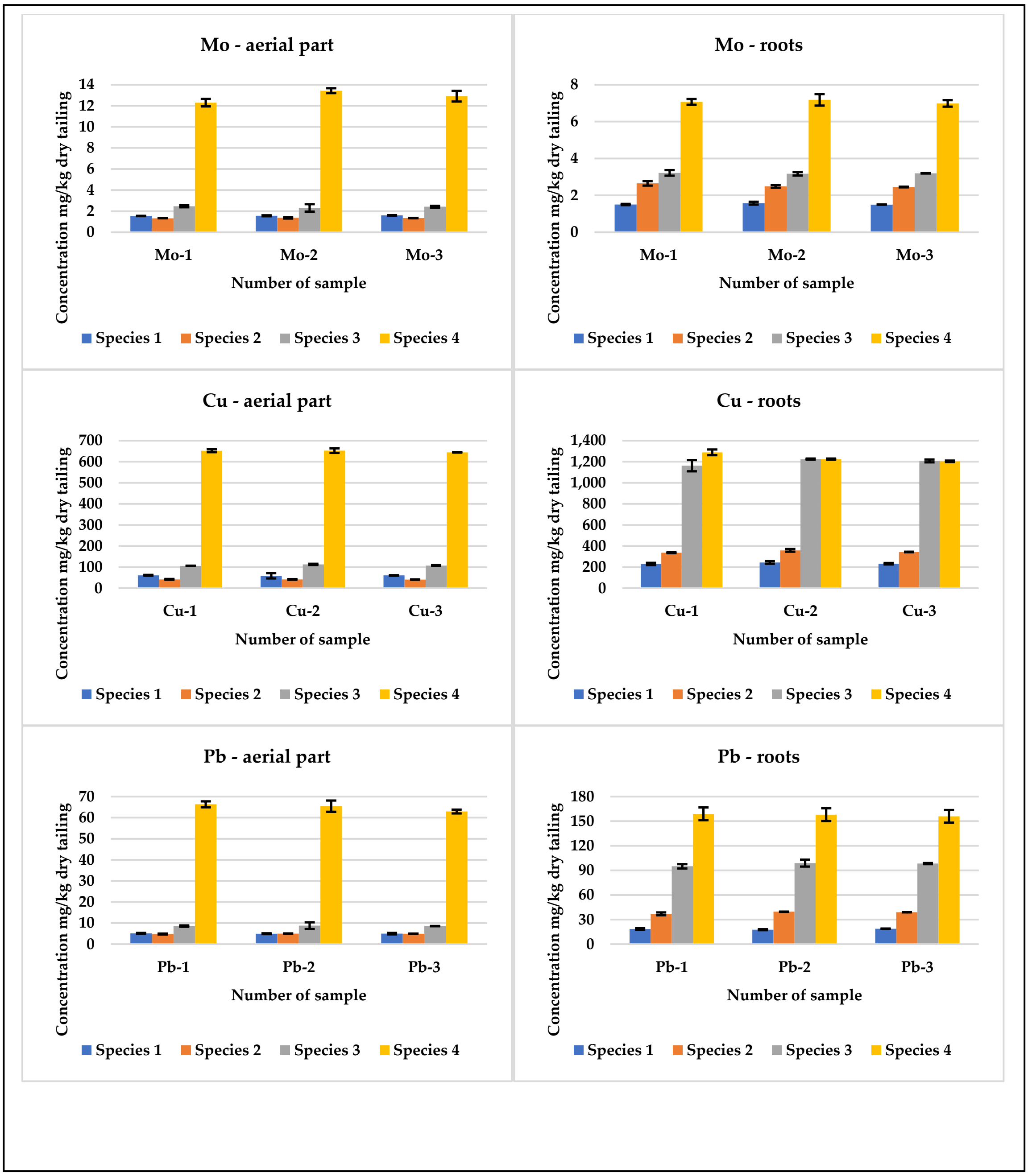

Figure 2. Mean concentration in duplicate samples, aerial part and roots, where species 1: *Oxalis gigantea*, species 2: *Cistanthe grandiflora*, Species 3: *Puya berteroniana*, species 4: *Solidago chilensis*.

Table 4. Translocation factor (TF), bioconcentration factor (BCF) and removal efficiency after the growth period.

Element	Plant Species	*Oxalis gigantea*	*Cistanthe grandiflora*	*Puya berteroniana*	*Solidago chilensis*
Mo	TF	1.03 ± 0.03	0.53 ± 0.03	0.75 ± 0.02	1.82 ± 0.06
	BCF_{roots}	0.47 ± 0.01	0.78 ± 0.03	1.03 ± 0.01	2.57 ± 0.03
	BCF_{aerial}	0.49 ± 0.00	0.42 ± 0.01	0.73 ± 0.02	4.68 ± 0.17
	% RE	16.38 ± 0.75	15.86 ± 0.34	19.48 ± 0.53	28.70 ± 1.57
Pb	TF	0.27 ± 0.01	0.13 ± 0.01	0.09 ± 0.01	0.41 ± 0.01
	BCF_{roots}	0.09 ± 0.01	0.19 ± 0.01	0.46 ± 0.01	0.72 ± 0.01
	BCF_{aerial}	0.02 ± 0.00	0.02 ± 0.00	0.04 ± 0.00	0.30 ± 0.01
	% RE	7.01 ± 0.87	8.78 ± 0.56	7.62 ± 0.45	4.41 ± 0.22
Cu	TF	0.26 ± 0.01	0.12 ± 0.00	0.09 ± 0.00	0.52 ± 0.01
	BCF_{roots}	0.16 ± 0.01	0.24 ± 0.01	0.90 ± 0.02	0.92 ± 0.03
	BCF_{aerial}	0.04 ± 0.01	0.03 ± 0.00	0.08 ± 0.01	0.48 ± 0.02
	% RE	8.63 ± 0.67	8.72 ± 0.78	15.59 ± 1.03	14.91± 0.98

According to the results shown in Table 4, all studied species presented poor ability to translocate Pb and Cu with a TF < 1. In the case of Mo, *Oxalis gigantea* and *Solidago chilensis* are good candidates for Mo phytoextraction with a TF > 1, where the second appears more promising due to its bioconcentration factor values for roots and aerial parts.

The analysis of the values for BCF highlight the potential use of *Puya berteroniana* for Mo phytostabilization. In the case of Cu, *Puya berteroniana* and *Solidago chilensis* showed a potential for phytostabilization with a BCF close to one. These factors could be improved through the study of the use of nanoparticles and/or chemical solutions, also, the mixture of mine tailings with compost or fertilizers could be considered.

The maximum removal efficiencies were obtained for Mo with all studied species, among which, *Solidago chilensis* showed a value close to 30%, followed by *Puya berteroniana* with a 19.5% removal efficiency. In the case of Pb removal, efficiencies were lower than 9%, *Cistanthe grandiflora* presented the best results with a removal efficiency of 8.8%. For Cu, the maximum values of removal efficiency—close to 15%—were obtained with *Puya berteroniana* and *Solidago chilensis*.

4. Discussion

Figure 3 shows the mean concentration ± IC (confidence interval) for each species and each metal after the experimental period. The Mo accumulated in roots decreases as follows: *Solidago chilensis* > *Puya berteroniana* > *Cistanthe grandiflora* > *Oxalis gigantea*, there is little variation in this trend in the case of aerial parts where the Mo concentration decreases as follows: *Solidago chilensis* > *Puya berteroniana* > *Oxalis gigantea* > *Cistanthe grandiflora*. All species outweighed the normal values for most plant tissues and the accumulation of Mo in aerial parts and roots of *Cistanthe grandiflora* by unit of dry matter is the highest in the group of the studied species.

The concentrations of Cu in the roots of *Oxalis gigantea* and *Cistanthe grandiflora* are in the range of critical values indicated by Oorts et al. (2013), in the case of *Puya berteroniana* and *Solidago chilensis* these values are outweighed. In the case of the aerial parts, all species exceeded the toxic levels indicated by Marschner (2000) and Oorts et al. (2013) [14,15].

In the case of Pb, the threshold level of Pb for plants is clearly surpassed. In terms of mass of Pb by unit of dry matter, the increase in concentration in the roots is as follows: *Oxalis gigantea* < *Cistanthe* *grandiflora* < *Puya berteroniana* < *Solidago chilensis*, with a slight change in the case of aerial parts where the concentration showed by *Oxalis gigantea* is similar to that of *Cistanthe grandiflora*.

It is important to mention that Chile lacks regulations for soil pollutants including heavy metals, and therefore, is not possible to compare with the Chilean norm.

The high ability of *Solidago Chilensis* to accumulate Mo, Pb and Cu, in respect to the other species, is also shown in Figure 3.

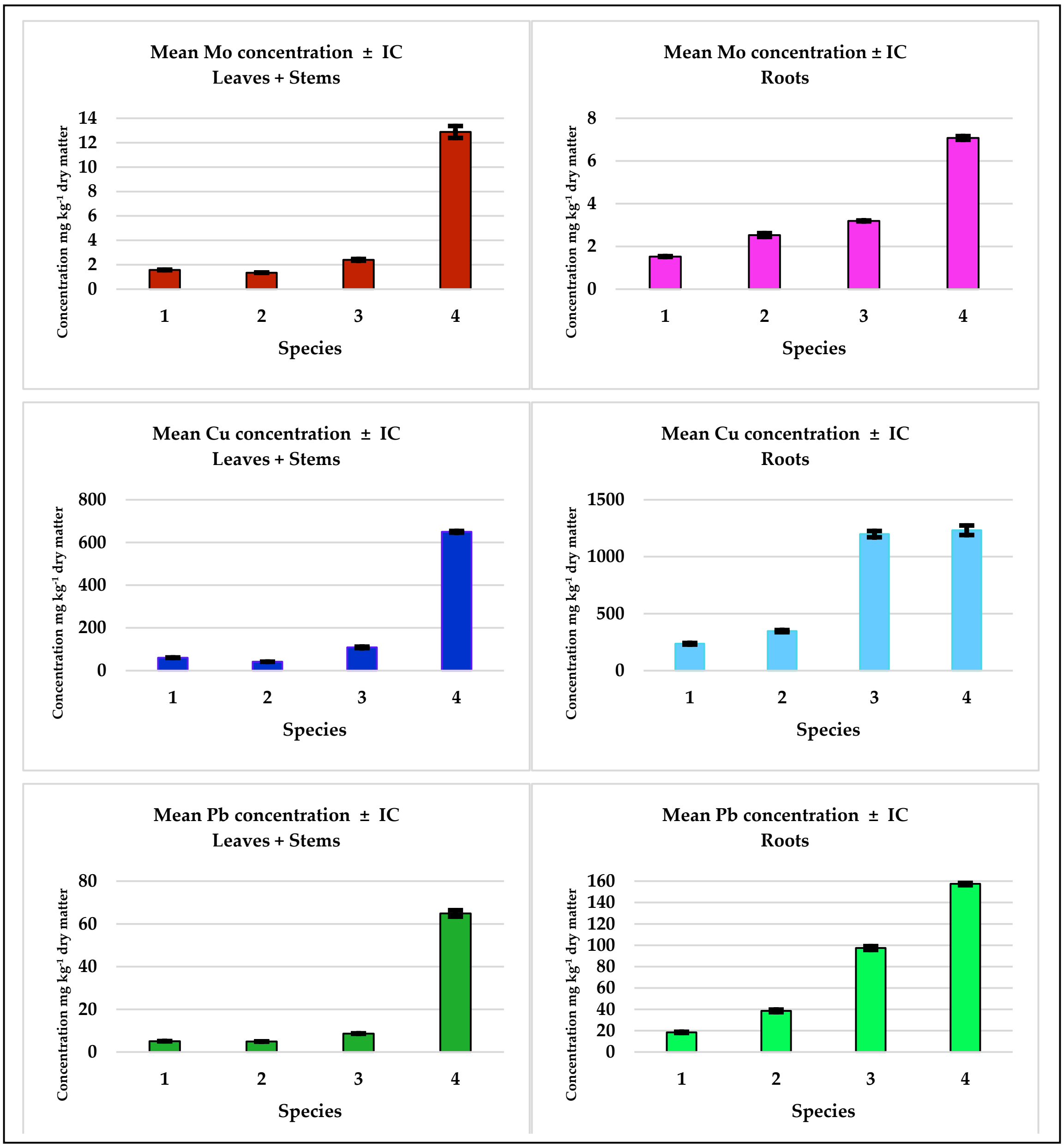

Figure 3. Mean concentration ± IC of Mo, Cu and Pb in roots and aerial parts, where, 1: *Oxalis gigantea*, 2: *Cistanthe grandiflora*, 3: *Puya berteroniana*, 4: *Solidago chilensis*.

The plant species used in the present research have not been studied before for phytoremediation. Some native and endemic species of plants have been previously used but not with the consideration of water requirements.

Lam et al. (2017) studied native Chilean species for the phytoremediation of tailings, among which *Schinus molle* showed the ability to translocate Cu and Pb with TF = 2.78 and 1.33, respectively, and a

BCF < 1 in both cases in tailings without amendments [27]. In the same study, *Atriplex nummularia* presented a TF = 1.33 and a BCF < 1 under the same conditions.

In a later study, Lam et al., (2018) established the potential of *Adesmia atacamensis* (TF = 2.47 and BCF = 0.05) to accumulate Pb in aerial part in tailings without treatment [20].

For comparison, the Pb concentrations of *Adesmia atacamensis* reported by Lam et al. (2018) were 4.7 mg·kg^{-1} in roots and 11.6 mg·kg^{-1} in aerial parts. In the case of this study, *Oxalis Gigantea* and *Cistanthe grandiflora* showed concentrations of 5.05 ± 0.07 mg·kg^{-1} and 4.95 ± 0.08 mg·kg^{-1} in the aerial parts and 18.39 ± 0.53 mg·kg^{-1} and 38.56 ± 1.15 mg·kg^{-1} in the roots, respectively. Although *Cistanthe grandiflora* is capable of accumulating higher concentrations of Pb in roots than *Adesmia atcamensis*, it lack the ability to translocate it.

The same behavior for Pb is observed in the case of *Puya berteroniana* and *Solidago chilensis* (8.66 ± 0.10 mg·kg^{-1} and 64.89 ±1.51 mg·kg^{-1} in the aerial parts and 97.39 ± 1.80 mg·kg^{-1} and 157.58 ± 1.34 mg·kg^{-1} in the roots), where both species showed higher concentrations than *Adesmia atacamensis*.

Ortiz-Calderón et al. (2008) analyzed the concentration of Cu in leaves and roots of several species, among them two Chilean native species: *Schinus polygamous* and *Atriplex deserticota* presented a Cu concentration in leaves of 1.213 and 1.358 mg·kg^{-1} dry mass, respectively, while in roots the concentration was 260 and 2160 mg·kg^{-1} dry mass, respectively [35]. Among them *Schinus polygamous* showed a clear ability to translocate Cu and therefore, to extract Cu. In the case of the present study *Puya berteroniana* and *Solidago chilensis* presented a TF close to one, which should be improved in order to increase the ability of these species to translocate Cu.

While this research was carried out ex-situ, it provides substantive information about the potential ability of the studied species to phyto-remedy Cu, Mo and Pb in mine tailings. Future work must be undertaken in order to improve this ability, for example, using joint implementation with another technology. Additionally, experiments in-situ must be performed accompanied by a sequential extraction procedure of the mine tailings for each studied element.

5. Conclusions

This study covers the potential ability of three endemic Chilean plant species and one native plant species, all of them from northern Chile, for the phytoremediation of Mo, Cu and Pb in mine tailings.

The ability of *Solidago chilensis* for the phytoextraction of Mo is highlighted, as is—to a lesser extent—the ability of *Oxalis* gigantea. In the case of Cu, *Puya berteroniana* and *Solidago chilensis* showed potential for phytostabilization which could be increased with the addition of chemicals or via joint implementation of another technique of remediation, which will be the subject of future studies.

It is important to mention that all these species have ornamental value, therefore, the phytoremediation with them, not only serves to decrease the concentration of the studied elements, but also provides a pleasant environment to the community. In addition to the above, the low water requirements of these species allow for their growth and development in water shortage scenarios.

Finally, the most noticeable results were obtained in the case of Mo, where *Solidago chilensis* should be the chosen species for Mo phytoextraction, while with *Puya berteroniana*, high removal efficiencies for Cu and Pb were obtained.

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