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Estudio de los factores edáficos implicados en la absorción de metales por *Atriplex halimus* en residuos mineros

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Study of the mechanisms involved in the uptake of metals by *Atriplex halimus* in tailings ponds

Abstract

The accumulations of abandoned mining wastes present environmental and public health risks due to the potential transfer of toxic elements through water and wind erosion or leaching. One strategy to reclaim those areas is the use of phytotechnologies. One of the species best adapted to the soils developed over the tailings ponds is *Atriplex halimus*. This species is able to easily colonize the surface of the tailings ponds and develop high biomass, but it can also accumulate metals in their aerial tissues. The main objectives of this study were: to assess the ability of *A. halimus* to change soil physicochemical properties and metals availability when is present, its capability of uptaking metals, and the effect of soil physicochemical on the availability of *A. halimus* to uptake metals, The evolution of different soil properties, different metal fractions and their metals absorption by *A. halimus* was monitored. For this purpose, we sampled rhizospheric soil, non rhizospheric soil and plant tissues from four different tailings ponds. Results showed that *A. halimus*, have an accumulation capacity of metals with a high significant accumulation in leaves, followed by stems. No physicochemical property showed significant differences between soil types (rhizhospheric and non-rhizospheric) indicating that the development of *A. halimus* did not influence these properties in any of the tailings ponds. Therefore, the development and capacity of *A. halimus* are related to the physicochemical properties in each deposit, showing significant differences between the tailings ponds.

Keywords: *metals; tailings; phytoremediation; Atriplex halimus; rhizosphere*

1. Introduction

Mining activity is a factor that greatly influences the development of a country's economy, but at the same time it is a great generator of a strong environmental impact. Mining activities generate large amounts of waste (solids, liquid and gaseous) that can cause different impacts on the environment and its ecosystems, such as: a) impact on water resources (aquatic ecosystems, surface waters, underground and marine), b) variation of the terrain morphology, c) impact on air (atmosphere), d) impact on soils and the flora and fauna associated with them, e) landscape impact, f) environmental pollution by different forms of energy (noise or acoustics, radiations, heat, etc.) (García, 2004).

The impacts of tailings impoundments, waste rock, heap leach, and dump leach facilities on water and soil quality can be severe. These impacts include contamination of groundwater beneath these facilities and surface waters (Environmental Law Alliance Worldwide (ELAW), 2010). Soil pollution occurs when the concentration of a certain substance exceeds its buffering capacity. Soil health is of vital importance to the good functioning of our society, since the soil is involved in a Wide variety of functions such as food and biomass production, and the Storage of nutrients and carbon. In our days, these functions are They are threatened by the degradation caused by the interaction of Bio-physical, socio-economic and political factors.(Xiong, 2016) Therefore, remediation of mining lands is necessary in order to protect the environment from the toxic effects of metal(loid)s from tailings and overburdens, and to conserve the environment for future generations (Glick, 2010).

There are many techniques for the recovery of soils contaminated by heavy metals, but most of these techniques are expensive and invasive for the environment (Simon, 2005). Phytoremediation is an emerging technique that uses the capacity of certain plant species to survive in environments contaminated with heavy metals and organic substances and at the same time extract, accumulate, immobilize or transform these soil contaminants. The plants used in phytoremediation present constitutive mechanisms and adapted to tolerate or accumulate a high content of metals in their rhizosphere and tissues. The success of this treatment is controlled by selecting the right plant species to recover a soil, as well as the careful selection of amendments (organic matter, chelating agents, lime, etc.) that improve soil properties and encourage the survival and growth of plants (Clemente, Walker, & Bernal, 2005). Schwitzguebel (2000) explained different technological subsets of phytoremediation. Phytoextraction (phytoaccumulation) is the removal of pollutants using the plants having the ability to accumulate pollutants from the soil and store them in their shoots so that they can be harvested. Phytostabilization involves the prevention of mobility of pollutants into the soil, and thus reducing its bioavailability. This fact stops pollutants from entering into the food chain (Razzaq, 2017).

Atriplex halimus is a halophytic shrub that is widely distributed in arid and semi-arid regions around the Mediterranean basin. Potential new uses of this versatile plant species include the phytoremediation of soils contaminated by trace elements and the exploitation of its biomass as a source of renewable energy. Such applications, together with its continued use in low-intensity farming systems, should ensure that *A. halimus* remains a vital plant species in low-rainfall regions (Walker et al., 2014). According to Zornoza et al., (2016), *A. halimus* should not be considered for phytostabilization, and has the potential to be used for phytoextraction, since it has fast growth, high biomass, easy harvesting, and can naturally accumulate heavy metals in the aboveground tissues without developing toxicity symptoms, with high bioaccumulation factor.

According to the latter approaches, the objectives of this study were i) to assess the ability of *A. halimus* to accumulate metals in its tissues; ii) to evaluate if the growth of *A. halimus* modifies soil physicochemical properties and metal dynamics; and iii) to elucidate if the ability of *A. halimus* to uptake metals is controlled by soil physicochemical properties. We hypothesized that *Atriplex halimus* would be able to uptake high quantities of metals, mainly in the leaves, with different values depending on the tailings ponds where they grow.

2. Materials and method

2.1.Study area and experimental design

The study area was exploited for ~2500 years owing to its large deposits of Pb, Zn and Fe, although mining activities ceased in 1991. It comprises a mountainous range along 25 km of the Mediterranean coastline of the cities of Cartagena and La Unión, with an elevation of 110 m.a.s.l.. Climate is semiarid Mediterranean with mean annual temperature of 18 °C and mean annual precipitation of 275 mm; annual potential evapotranspiration surpasses 900 mm.

Illustration 1 - Location of the study area.

Four tailings pond were selected based on the dominance of *Atriplex halimus* as plant species, neutral-basic conditions and high content of metals. Total metal contents and textural characteristics of the tailings ponds are shown in Table 1. Tailings pond 1 (TP1, 37° 35′ N, 0° 52′ W) was originally neutral (pH = 7.6) but soil characteristics hindered the development of vegetation, mainly high salinity and compactness and low organic matter and nutrient contents (Kabas et al., 2012). As a consequence, a reclamation strategy based on the addition of marble waste and pig slurry was performed in 2010 (see Kabas et al., (2012)). This reclamation led to the spontaneous colonization of the tailings pond by *Zigophyllum fabago, P. miliaceum, Dittrichia viscosa, A. halimus* and *Salsola kali* as most dominant species. Tailings pond 3 (TP3, 37° 35` 28``N , 0° 52` 1`` W) was originally basic (pH = 7.37) and soil characteristics permitted the spontaneous colonization of the surface by native vegetation such as *A. halimus, Piptatherum miliaceum, Phagnalon saxatile, Helychrisum stoechas, Lygeum spartum* and

Tamarix sp., with a mean vegetation cover of 30%. Tailings pond 2 (TP2, 37°35` 7`` N, 0° 52` 4`` W) and Tailings pond 4 (TP4, 37° 35 '38"N, 0º 53' 11"W) were originally acidic (pH = 5.9 and 2.9, respectively). These acidic conditions had hindered the development of vegetation. As a consequence, a reclamation strategy based on aided phytoremediation was performed in both tailings ponds in 2011 (Martínez-Martínez et al., 2018) For this purpose, marble waste, pig slurry and pig manure was applied in 2011, with the planting/sowing of *A. halimus*, *Cistus albidus, H. stoechas, Hyparrhenia hirta, Lavandula dentata, L. spartum, Rosmarinus officinalis, P. saxatile, Cynodon dactylon* and, *Limonium caesium* (see (Martínez-Martínez et al., 2018). All soils were classified as Spolic Technosols (IUSS Working Group, 2015).

Table 1. Soil pH, total concentration of metals and particle size distribution in the four tailings ponds of study. Values are mean ± standard error (n = 6).

Parameters	Tailings pond 1			Tailings pond 2			Tailings pond 3			Tailings pond 4		
PH	7.76	±	0.07	7.52	±	1.17	6.32	±	0.07	7.05	±	0.11
Cd Total (mg/kg)	20.11	±	1.99	9.54	±	2.59	18.16	±	2.34	2.64	±	0.84
Cu Total (mg/kg)	153	±	13	75	±	14	85	±	11	60	±	3
Ni Total (mg/kg)	26.7	±	0.7	18.9	±	3.9	28.6	±	1.8	15.8	±	1.2
Pb Total (mg/kg)	3522	±	386	1969	±	390	2599	±	358	776	±	85
Zn Total (mg/kg)	9984	±	572	4600	±	1374	7939	±	561	3446	±	412
Clay (%)	2.05	±	0.41	2.61	±	0.56	2.31	±	1.81	1.97	±	0.15
Silt (%)	37.03	±	6.88	22.77	±	4.82	22.79	±	13.68	29.29	±	1.57
Sand (%)	60.92	±	7.29	74.62	±	15.30	49.90	±	15.47	68.75	±	1.71
Textural class	Sandy Loam			Sandy Loam			Sandy Loam			Sandy Loam		

2.2.Plant and soil sampling

In January 2018, six random plants of *A. halimus* were uprooted from each tailings pond. Each plant was separated into root, stem and mature leaves (leaves developed during past growing seasons). Rhizospheric soil (RS) was also sampled together with each plant. A non-rhizospheric soil (NRS) sample (0-30 cm depth; mean root depth) was collected per each plant, with a minimum distance of 30 cm to the closest plant. Plant samples were carefully washed with tap and deionized water, and then, dried at 55 °C for 72 h. The dried material was ground using a mill (Retsch model ZM 200). Soil samples were air dried for 7 days at room temperature, passed through a 2 mm sieve and stored in plastic bags at room temperature before laboratory analysis. A split of each sample was ground using an agate mortar (RetschRM 100).

2.3. Analytical Methods

Soil pH and electrical conductivity (EC) were measured in distilled water (1:1 and 1:5 w/v, respectively). The texture was measured from a soil/Na-polyphosphate extract and determined by laser diffraction (Mastersizer 2000, Malvern Panalytical). Total organic carbon (TOC), inorganic carbon (IC), total nitrogen (Nt) and total sulfur (St) were determined by an elemental analyzer CNHS-O (EA-1108, Carlo Erba). Total metals (Cd, Cu, Pb and Zn) and macronutrients (Ca, Mg, K and Na) were determined by microwave digestion (Risser & Baker, 1990). Soluble metals and macronutrients were extracted by ionized water in the proportion 1:5 (w:v). The sequential chemical speciation methodology proposed by Tessier et al. (1979) for metals was performed, based on an attempt to reproduce the physicochemical conditions on environmental matrices, considering five phases that define associations of heavy metals to the constituent elements of the soil with the different binding energies. The scheme of Tessier et al. (1979) consists of the application of selective extractants in a sequence, to determine the forms of the decreasing solubility of the physical elements in five fractions: fraction 1: exchangeable (0.5 M $MgCl_2$ (8 mL)), fraction 2: bound to carbonate and specifically adsorbed (1 M NaOAc (8 mL)), fraction 3: reducible or bound to Fe and Mn oxides (0.04 M NH_2OHHCl (20 mL)); fraction 4: oxidizable (0.02 M HNO_3 (3 mL), H_2O_2 30% (5 mL) and 3.2 M NH_4OAc in 20% (v/v) HNO_3 (5 mL)), and e) fraction 5: residual phase (HNO_3 65% (9 mL) and HF 40% (3 mL)). Metal and nutrient concentrations in soils and plants were measured using ICP-MS (Agilent 7500CE). The methodology for total metal(loid)s concentration was referenced using the Certified Reference Material BAM-U110 (Federal Institute for Materials Research and Testing, Germany). Crystallographic structures in soil samples were identified by X-ray diffraction (XRD), with wavelength dispersive X-ray fluorescence spectrometry as complementary assay. Mineral phases were identified using a Bruker D8 Advance instrument in θ-θ mode (Bruker Corporation, Billerica, MA, USA), with CuKα radiation, 40 kV, 30 mA, and a 1-dimensional detector with a window of 3º. Diffraction patterns were evaluated with

DIFFRACplus software and powder diffraction files database PDF2. The Bioaccumulation factor (BF) was also calculated as follows

BF = [metal]plant / [bioavailable metal]soil), for the different plant parts (stem, roof and leaf). Bioavailable metal in soil is the sum of the exchangeable and carbonates fractions.

2.4. Statistical analysis

A two-way ANOVA was developed with soil properties to assess the interaction among the factors: tailings pond (TP1, TP2, TP3 and TP4) and soil type (rhizospheric and non-rhizospheric). For plant properties, a two-way ANOVA was also performed to assess the interaction among the factors: tailings pond (TP1, TP2, TP3 and TP4) and plant part (root, stem and leaf). Statistical analysis was performed with the software IBM SPSS Statistics 20.

3. Results and Discussion

3.1. Physical and Chemical Properties

Most physicochemical properties showed significantly differences among the tailings ponds (Table 2), except for the textural class. The highest levels of pH were found in TP1 (7.76) and the lowest in TP3 (6.32) with an F-value of 22.14 for the factor tailings ponds. TOC and IC showed significantly higher values in TP2. TP2 also showed the significantly highest Nt content, while TP3 the highest content of St. No physicochemical property showed significant differences between soil types (rhizhospheric and non-rhizospheric) indicating that the development of *A. halimus* did not influence these properties in any of the tailings ponds. The mineralogical analysis showed the presence of quartz, muscovite, gypsum, kaolinite, calcite, chlorite-serpentine, dolomite and goethite in all tailings ponds, excepting TP3 and TP4 were also exit the present of albite. But the predominant minerals in all tailings ponds were quartz and muscovite.

Table 2. Physicochemical Properties of Soil. Values are mean ± standard deviation (n = 6)

Tailings Pond	Soil Type	PH	Electric Conductivity mS/cm	TOC %	IC %	Nitrogen %	Sulfide %	Clay %	Silt %	Sand %
TP 1	SNR	7.76 ± 0.07	5.73 ± 1.50	0.54 ± 0.09	0.89 ± 0.24	0.04 ± 0.01	0.63 ± 0.16	2.05 ± 0.41	37.03 ± 6.88	60.92 ± 7.29
	SR	7.60 ± 0.03	3.05 ± 0.19	0.54 ± 0.05	0.91 ± 0.23	0.05 ± 0.01	0.38 ± 0.11	1.71 ± 0.30	33.24 ± 1.09	65.05 ± 1.39
TP 2	SNR	7.52 ± 1.17	2.68 ± 0.42	0.75 ± 0.13	1.13 ± 0.36	0.10 ± 0.02	0.05 ± 0.05	2.61 ± 0.56	22.77 ± 4.82	74.62 ± 15.30
	SR	7.51 ± 1.16	2.79 ± 0.43	0.76 ± 0.15	1.16 ± 0.23	0.10 ± 0.02	0.04 ± 0.04	3.87 ± 0.87	32.29 ± 7.22	63.84 ± 13.44
TP 3	SNR	6.32 ± 0.07	4.73 ± 1.96	0.33 ± 0.04	0.15 ± 0.08	0.02 ± 0.00	0.62 ± 0.16	2.31 ± 1.81	22.79 ± 13.68	49.90 ± 15.47
	SR	6.45 ± 0.11	4.09 ± 1.37	0.47 ± 0.08	0.27 ± 0.09	0.03 ± 0.00	0.78 ± 0.11	2.61 ± 2.27	19.10 ± 12.02	53.29 ± 14.28
TP 4	SNR	7.05 ± 0.11	2.54 ± 0.04	0.56 ± 0.05	0.03 ± 0.11	0.05 ± 0.01	0.35 ± 0.16	1.97 ± 0.15	29.29 ± 1.57	68.75 ± 1.71
	SR	6.91 ± 0.11	2.85 ± 0.11	0.74 ± 0.09	0.10 ± 0.11	0.07 ± 0.01	0.27 ± 0.11	2.88 ± 0.25	37.44 ± 3.27	59.68 ± 3.48
F-value										
Tailings Pond (TP)		22,14***	2,99*	5,64**	14,37***	27,91***	13,93***	0,27ns	0,16ns	0,02ns
Soil Type (ST)		0,38 ns	1,12 ns	2,75 ns	0,23 ns	2,27 ns	0,18 ns	0,18 ns	0,03 ns	0,02 ns
TP x ST		1,23 ns	0,92 ns	0,80 ns	0,04 ns	1,04 ns	1,06 ns	0,07 ns	0,10 ns	0,03 ns

Abbreviations: Non-Rhizospheric Soil (SNR), Rhizospheric Soil (SR). Total Organic Carbon (TOC); Inorganic Carbon (IC); No significant (ns); (*) low significant, (**) medium significant, (***) high significant.

3.2.Soil Nutrients

Total and soluble contents of nutrients are shown in Table 3. The tailings pond type significantly influenced all nutrients, except for total Na, indicating different content and availability of nutrients depending on the tailings pond. The development of the plant did not significantly influence any nutrient except for total and soluble K, which tended to be higher in the rhizospheric soil.

Table 3. Soluble and Total Cations in Soil.

Tailings Pond	Soil Type	Na (mg/kg)		Mg (mg/kg)		K (mg/kg)		Ca (mg/kg)	
		Soluble	Total	Soluble	Total	Soluble	Total	Soluble	Total
TP 1	SNR	1288.00 ± 658.20	2190.13 ± 1006.84	552.05 ± 188.78	10646.02 ± 866.01	100.25 ± 29.16	407.60 ± 54.83	2195.58 ± 64.23	43225.83 ± 5156.25
	SR	198.83 ± 60.17	531.88 ± 104.91	125.03 ± 23.65	9019.82 ± 879.33	150.54 ± 28.67	497.28 ± 53.18	2040.12 ± 23.83	43931.30 ± 5082.99
TP 2	SNR	49.65 ± 16.19	444.17 ± 86.38	109.87 ± 20.47	7667.63 ± 1741.95	131.52 ± 59.86	727.23 ± 199.44	2038.50 ± 315.40	40652.58 ± 9152.45
	SR	112.15 ± 17.87	529.14 ± 136.80	139.68 ± 28.47	9258.17 ± 1540.59	177.93 ± 31.77	757.89 ± 139.65	2020.87 ± 312.83	41920.39 ± 8316.54
TP 3	SNR	852.47 ± 616.95	1298.30 ± 761.95	897.27 ± 654.43	12934.69 ± 1143.22	48.52 ± 13.80	399.61 ± 38.99	1635.86 ± 42.75	20293.23 ± 3840.68
	SR	723.00 ± 508.81	1175.10 ± 701.29	638.77 ± 398.19	11530.21 ± 533.88	132.94 ± 32.26	571.85 ± 38.25	1666.93 ± 18.23	16438.97 ± 2019.99
TP 4	SNR	25.77 ± 4.11	292.34 ± 30.16	103.04 ± 24.72	6452.25 ± 683.01	16.30 ± 1.75	497.05 ± 30.91	2032.68 ± 21.71	29764.34 ± 5077.71
	SR	91.88 ± 16.48	491.70 ± 122.65	181.63 ± 66.14	5917.67 ± 530.17	91.44 ± 16.89	830.20 ± 81.80	2043.76 ± 31.10	32526.04 ± 3909.90
F-value									
Tailings Pond (TP)		2,95*	2,34 ns	3,30*	29,82***	3,94*	4,47*	10,15***	8,34***
Soil Type (ST)		1,15ns	1,08ns	0,61ns	0,78ns	10,01*	7,64*	1,63 ns	0,00 ns
TP x ST		1,12 ns	1,40 ns	0,39 ns	1,48 ns	0,32 ns	1,27 ns	2,02 ns	0,201 ns

Abbreviations: Non-Rhizospheric Soil (SNR), Rhizospheric Soil (SR). No significant (ns); (*) low significant, (**) medium significant, (***) high significant.

3.3.Soil Metals

Table 4. Soluble and Total Metals in Soil.

Tailings Pond	Soil Type	Cd mg/kg		Cu mg/kg		Ni mg/kg		Pb mg/kg		Zn mg/kg	
		Soluble	Total	Soluble	Total	Soluble	Total	Soluble	Total	Soluble	Total
TP 1	SNR	0.01 ± 0.00	20.11 ± 1.99	0.04 ± 0.01	152.81 ± 12.88	0.00 ± 0.00	26.68 ± 0.68	0.17 ± 0.03	3522.41 ± 385.61	2.14 ± 0.59	9983.58 ± 572.19
	SR	0.01 ± 0.00	24.27 ± 1.61	0.07 ± 0.00	158.60 ± 9.17	0.00 ± 0.00	27.60 ± 0.69	0.17 ± 0.05	3363.70 ± 352.12	2.84 ± 0.60	10965.54 ± 576.28
TP 2	SNR	0.02 ± 0.01	9.54 ± 2.59	0.06 ± 0.02	75.42 ± 13.79	2.91 ± 1.78	18.89 ± 3.92	0.02 ± 0.01	1969.33 ± 390.24	0.55 ± 0.16	4600.20 ± 1373.96
	SR	0.01 ± 0.00	17.14 ± 6.26	0.09 ± 0.02	90.51 ± 16.09	0.05 ± 0.02	24.80 ± 4.64	0.00 ± 0.00	1976.17 ± 335.04	1.86 ± 0.94	6318.58 ± 1520.86
TP 3	SNR	0.01 ± 0.00	18.16 ± 2.34	0.06 ± 0.01	84.82 ± 10.95	0.01 ± 0.00	28.57 ± 1.76	0.09 ± 0.06	2599.50 ± 358.03	6.09 ± 5.89	7938.68 ± 561.07
	SR	0.02 ± 0.01	21.17 ± 1.84	0.06 ± 0.00	82.89 ± 11.34	0.01 ± 0.00	26.54 ± 1.75	0.13 ± 0.12	2599.75 ± 363.12	4.36 ± 4.07	8566.39 ± 695.56
TP 4	SNR	0.01 ± 0.00	2.64 ± 0.84	0.04 ± 0.01	60.36 ± 3.05	0.00 ± 0.00	15.80 ± 1.23	0.00 ± 0.00	776.32 ± 85.32	0.13 ± 0.03	3445.60 ± 411.64
	SR	0.01 ± 0.00	1.83 ± 0.34	0.04 ± 0.01	59.25 ± 2.88	0.00 ± 0.00	13.72 ± 0.75	0.01 ± 0.00	743.68 ± 76.95	0.07 ± 0.02	3141.78 ± 231.44
F-value Tailings Pond (TP)		1,13 ns	24,76***	3,40*	40,59***	1,03 ns	34,51***	5,84*	35,96***	2,05 ns	37,41***
Soil Type (ST)		0,09 ns	3,47 ns	3,48 ns	0,46 ns	0,97 ns	0,22 ns	0,17 ns	0,05 ns	0,00 ns	1,97 ns
TP x ST		0,94 ns	0,79 ns	1,42 ns	0,38 ns	0,96 ns	2,30 ns	0,15 ns	0,04 ns	0,16 ns	0,04 ns

Abbreviations: Non-Rhizospheric Soil (SNR), Rhizospheric Soil (SR), No significant (ns); (*) low significant, (**) medium significant, (***) high significant.

All metals showed significant differences between tailings ponds with regards to their total concentration. However, for the soluble fraction, Cu and Pb showed significant differences. Cu showed significantly lower concentration in TP4, while Pb showed significantly higher concentration in TP1. There were no significant differences between both types of soil, indicating that the development of the species do not modifies the soluble fraction of the studies metals. Table 4.

Figure 1. Percentage distribution of heavy metals in the five fractions extracted: Abbreviations: No Rhizospheric Soil (NR), Rhizospheric Soil (R)

It has been demonstrated that metals behavior in soils depends of many factors, such as soil properties and characteristics, source of metals, metal distribution in soil constituents, anthropogenic activities, etc. (Gabarrón et al., 2017). The percentage of contribution of each fraction is shown in Figure 1. The exchangeable fraction was very low in all tailings ponds and metals (around 1%), mostly due to the basic pH of all tailings ponds. Cd, as a general pattern in all tailings ponds, showed similar concentration in the carbonates and reducible fractions (35-40 %), follow by residual (15%). Nonetheless, TP1 showed the higher proportion of Cd bound to carbonates, while TP2 and TP4 had the highest percentage of Cd linked to the reducible and residual fractions. These data indicate that the strategy followed in some of the tailings ponds to add calcium carbonate for reclamation is highly effective to immobilize Cd, since a high percentage of this metals is immobilized by carbonates. Cu concentration was mainly associated to the residual phase (70%), followed by organic fraction (20%), reducible (6%) and carbonates (3%). Thus, the addition of organic matter and increase of organic matter by the development of vegetation is a good strategy to immobilize Cu. It is important to highlight that TP4 showed higher percentage of Cu retained in the residual fraction, while TP1 showed the highest proportion of retention in the oxidizable fraction.

Ni concentration was mainly associated to the residual phase (around 70%), followed by reducible (16%), oxidizable (9%) and carbonates (4%). Like for Cu, TP4 showed higher percentage of Cu retained in the residual fraction, but lower in the reducible fraction. Chemical partitioning of Pb was dominated by the residual phase (around 75%), followed by the reducible phase (15%), carbonates (7%) and oxidizable fraction (2%). TP4 showed higher percentage of Pb retained in the residual fraction, while TP1 showed the highest proportion of retention in the reducible and carbonates fractions. Zn concentration was dominated by the residual phase in TP4. However, in the rest of tailings ponds, the most dominant fraction was the reducible.

Thus, according to the analysis of the sequential extraction, TP4 retained the highest metal contents in the residual phase, while TP1 did for the organic, carbonates and reducible fractions. In addition, there are no differences in metal fractionation between rhizospheric and non-rhizospheric soil, suggesting that the development of *A. halimus* did not significantly contribute to changes in metal dynamics.

3.4. Plants Nutrients and Metals

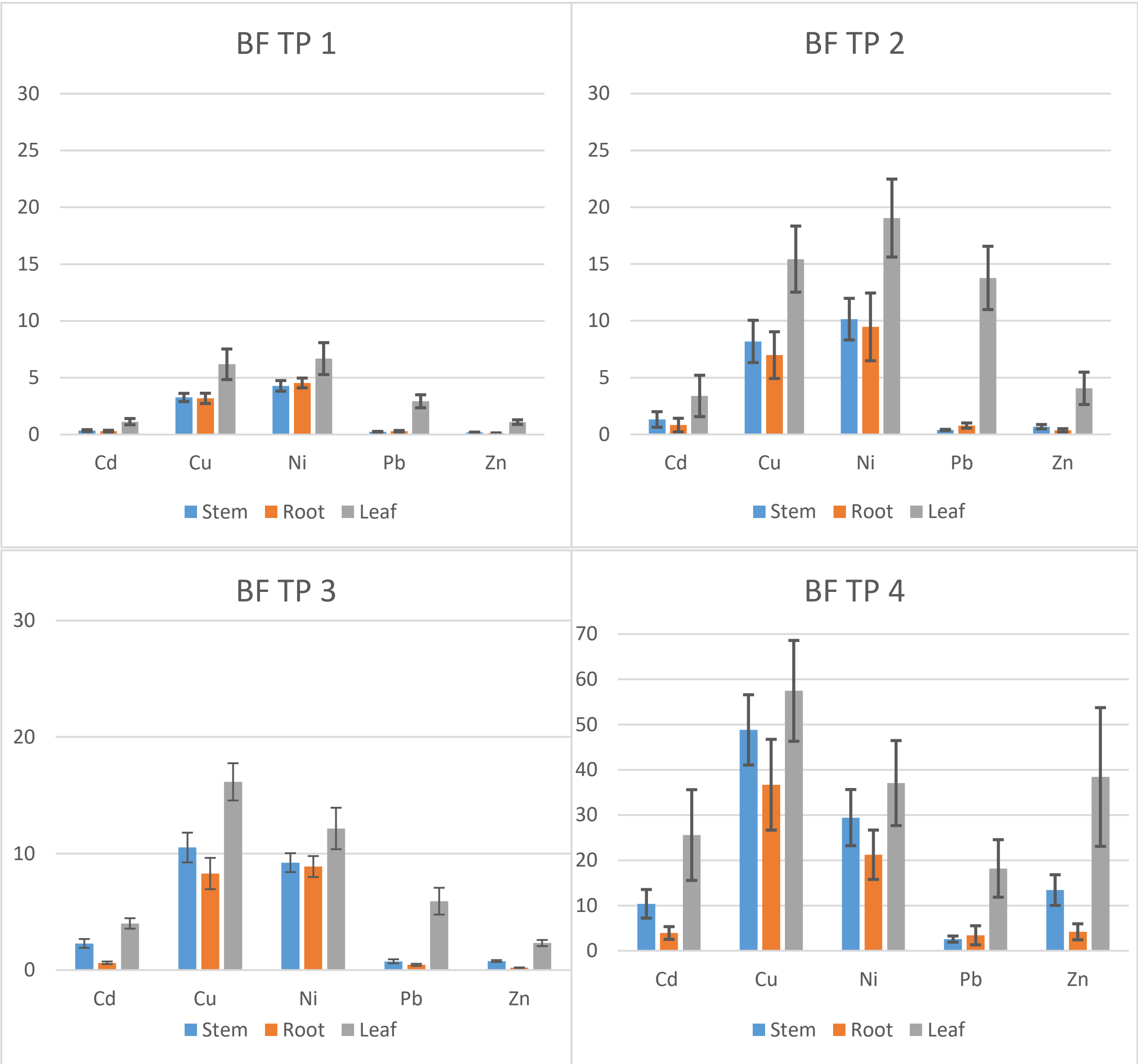

Figure 2. Bioaccumulation factor of the four tailings ponds. Error bars denote standard error

Figure 2 shows the results of the bioaccumulation factor in the four tailings ponds analyzed, evaluating the different accumulation in the stem, root and leaf by metal. We can appreciate that in all tailings ponds, the highest accumulation of metals takes part in the leaf, followed by the stem and then the root. As a general pattern, TP1 has the lowest capacity of accumulation with a maximum of 7 in the leaf for Ni and Cu. TP2 has the highest accumulation in the leaf for Ni, Cu and Pb with values around 15. TP3 showed a BF of 12 in the leaf for Cu and Ni. TP4 is the one with the highest BF values, with 58 in the leaf for Cu, Ni and Zn.

Table 5. Nutrient concentrations in the plant tissues. Values are mean ± standard error

Tailings Pond	Plant Part	Na mg/kg	Mg mg/kg	K mg/kg	Ca mg/kg
TP 1	Stem	15288 ± 961	6994 ± 428	22372.6 ± 715.5	6781.3 ± 294.5
	Root	14536 ± 9322	11801 ± 7314	29768.1 ± 19792.9	8051.7 ± 5359.1
	Leaf	20815 ± 3328	20877 ± 2151	15044.9 ± 1857.9	34086.5 ± 2157.5
TP 2	Stem	23202 ± 1801	11169 ± 1837	33031.7 ± 2585.5	6978.3 ± 618.7
	root	2317 ± 333	3642 ± 257	13826.6 ± 144.3	2670.8 ± 271.0
	Root	2317 ± 333	3642 ± 257	13826.6 ± 144.3	2670.8 ± 271.0
	Leaf	9340 ± 1338	16141 ± 1834	9237.1 ± 1820.4	30790.9 ± 920.2
TP 3	Stem	22873 ± 3058	10041 ± 1044	24233.1 ± 2711.1	6137.0 ± 517.0
	Root	5768 ± 898	4715 ± 428	11339.8 ± 1136.7	2370.9 ± 430.4
	Leaf	29783 ± 4505	21772 ± 1656	24189.4 ± 3330.2	15708.7 ± 1077.8
TP 4	Stem	16894 ± 1632	13345 ± 792	27706.4 ± 5518.6	11470.5 ± 1502.3
	Root	2253 ± 336	4715 ± 434	10882.8 ± 1032.6	3104.4 ± 306.2
	Leaf	16200 ± 3303	24365 ± 2645	22902.0 ± 6486.8	29939.6 ± 4320.3
F-value					
Tailings Pond (TP)		3,33*	1,10 ns	0,15 ns	7,67***
Plant Part (PP)		16,41***	32,53***	2,86 ns	123,96***
TP x PP		2,91*	1,77 ns	1,59 ns	4,00*

Abbreviations: No significant (ns); (*) low significant, (**) medium significant, (***) high significant.

Tailings pond type had only significant effect on the accumulation of Na and Ca in the plant tissues. TP4 caused the lowest accumulation of Na in all tissues, while TP3 caused the lowest accumulation of Ca in the tissues, just the tailings pond with lowest carbonates content since no external addition was applied. Plant part had a significant effect on all nutrients except for K, with significant highest accumulation in leaves, followed by stems. Table 5.

Table 6. Total metals in plant tissues.

Tailings Pond	Plant Part	Cd mg/kg	Cu mg/kg	Ni mg/kg	Pb mg/kg	Zn mg/kg
TP 1	stem	4.1 ± 0.9	25.9 ± 0.6	6.4 ± 0.2	52 ± 3	364 ± 29
	root	3.3 ± 0.8	24.8 ± 1.0	6.9 ± 0.3	69 ± 12	237 ± 35
	leaf	12.6 ± 1.7	48.1 ± 3.7	9.9 ± 0.6	707 ± 73	2083 ± 131
TP 2	stem	3.9 ± 1.4	23.7 ± 0.9	6.6 ± 0.2	22 ± 4	397 ± 79
	root	2.8 ± 1.3	22.9 ± 0.7	7.3 ± 0.4	46 ± 7	244 ± 42
	leaf	11.2 ± 3.2	47.3 ± 6.4	12.8 ± 1.0	754 ± 189	2498 ± 562
TP 3	stem	19.0 ± 3.6	28.0 ± 1.9	8.8 ± 0.8	78 ± 15	871 ± 100
	root	4.9 ± 0.9	21.2 ± 1.1	8.3 ± 0.3	48 ± 7	225 ± 21
	leaf	33.0 ± 5.9	44.3 ± 4.6	11.1 ± 0.6	652 ± 159	2614 ± 323
TP 4	stem	3.7 ± 0.6	23.7 ± 1.1	7.3 ± 0.4	19 ± 2	647 ± 265
	root	2.1 ± 0.4	22.3 ± 0.8	7.7 ± 0.2	27 ± 9	292 ± 148
	leaf	8.7 ± 2.2	27.3 ± 2.8	8.9 ± 0.5	149 ± 40	1243 ± 336
F-value						
Tailings Pond (TP)		20,87***	5.04*	6.44*	5.06*	2.43 ns
Plant Part (PP)		27,66***	51.51***	48.53***	60.23***	68.25***
TP x PP		4,17**	3.59*	4.21*	3.98*	2.62*

Abbreviations: No significant (ns); (*) low significant, (**) medium significant, (***) high significant.

Table 6 shows that the tailings pond type have significantly accumulation in all metals, except for Zn. Plant part type had a high significant effect on the accumulation in all metals, with significant highest accumulation in leaves, followed by stems.

4. Conclusion

The study confirms the accumulation capacity of metals in *A. halimus*, with a high significant accumulation in leaves, followed by stems. No physicochemical property showed significant differences between soil types (rhizospheric and non-rhizospheric), which indicates that the development of *A. halimus* did not influence these properties in any of the tailings ponds. Therefore, the development and capacity of *A. halimus* are related to the physicochemical properties in each deposit which showed significant differences between the tailings ponds.

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