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Evaluation of the Bioaccumulation Capacity of *Buddleja* Species in Soils Contaminated with Total Chromium in Tannery Effluents in Arequipa (Peru)

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Abstract: The main purpose of this study is to evaluate the *Buddleja* species bioaccumulation capacity for the phytoremediation of soils contaminated with chromium produced by tannery effluents. The soils evaluated were collected from the Añashuayco stream, located in Arequipa region. The soil samples were collected from four different locations, in order to determine the presence of total chromium through the Environmental Protection Agency analytical technique, method 3050B acid digestion of sediment, sludge and soil. Three soil samples were analyzed for each collected location. Additionally, two non-contaminated soil samples (control group) were also analyzed. A *Buddleja* species seedling was placed in each sample to be monitored monthly for up to 90 days. Then, the plant tissue analysis was carried out by the analytical method of atomic absorption spectrophotometry in order to determine the amount of bioaccumulated total chromium. As a result, the *Buddleja* species bioaccumulated 30.45%, 24.19%, 34.55% and 40.72% of total chromium per each soil sample location in a period of 90 days. Therefore, the *Buddleja* species can be considered as an alternative to remediate soils contaminated with total chromium that comes from tannery effluents.

Keywords: total chromium; bioaccumulation; *Buddleja* species; phytoremediation

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1. Introduction

The tannery industry represents one of the most pollutants worldwide [1]. This is due to the effluents generated, carrying a high quantity of toxic chemical agents, high organic load and suspended solids [2]. Thus, these effluents damage the environment if they do not receive adequate treatment.

The main exporting countries of leather and fur worldwide are the United States (26.1%), Australia (9.4%) and France (7.3%), while Italy leads the export of tanned goods with 23.9% [3]. Additionally, countries in Latin America such as Brazil and Argentina led the market in 2021 for the exportation of leather and furs [4], while Peru was among the countries with the least exportation [4].

On the other hand, the greater demand for leather in the world leads to the increasing use of chemical products, such as chrome, which allows finer grain and the decrease of time in the treatment [5]. Peru is one of the countries that opts for this type of treatment.

In Peru, the tannery industry is mainly developed in the cities of Trujillo, Lima and Arequipa. They employ a traditional system using inorganic chemicals such as chromium and sulfur [6], which cause environmental problems due to the effluents and settlement of wastes [2].

These effluents from the tanning process are discharged into the ground containing liquid and solid residues with high organic load [7], hexavalent chromium, pentachlorophenol (PCP), formaldehyde, tributyltin (TBT) and heavy metals [1]. In the tannery industry, chromium can be found as hexavalent chromium (Cr (VI)) and trivalent chromium (Cr (III)). However, when Cr (III) reaches the environment, it oxidizes to Cr (VI) state. The presence of this chromium compound compromises human health and damages ecological processes [8].

For this reason, pre-emptive technologies have been developed, such as the substitution of polluting substances, aerobic treatments and membrane bioreactors, among others [1,2]. When the polluting effluents make contact with the environment, other measures, such as phytoremediation, must be taken.

Phytoremediation is a biological treatment that requires plants and microorganisms [9]. It is based on hyperaccumulating plants with high tolerance and absorption ability of heavy metals, adapting to diverse environmental conditions [10]. Several plants (*Buddleja leathery*, *Acacia melanoxylon*, *Pelargonium ortorum*, among others) [11–13] are capable of accumulating heavy metals, and they are also cheaper than the chemical methods. *Buddleja coriácea* is effective in removing chemical elements such an antimony (Sb), arsenic (As), cadmium (Cd), copper (Cu), silver (Ag) and lead (Pb) [11,14]. Two *Buddleja* species *(Buddleja asiatica* and *Buddleja macrostachya*) have shown high tolerance to Cd stress with limited accumulation capacity [15].

The tannery industry has grown near the Añashuayco stream located in the city of Arequipa. A report from the National Water Authority of Peru (ANA) has revealed that 79 tanneries do not treat their wastewater and divert it to the Añashuayco stream, leaving waste and high chromium content in the soil.

Therefore, the objective of this research is to evaluate the bioaccumulation capacity of *Buddleja* species for the phytoremediation of soils contaminated with total chromium in the middle area of the Añashuayco stream, located in Uchumayo district (city of Arequipa). Therefore, the physicochemical parameters of the contaminated soil samples (pH, conductivity, texture, irrigation sheet, field capacity, the amount of organic matter and wilting point) were determined at the beginning of this work. Also, the concentration of total chromium was measured by an analytical technique (acid digestion of sediment, sludge and soil). In parallel, the morphological variations of *Buddleja* species were analyzed with atomic absorption spectrophotometry at the beginning, during and at the end of the experiment. Finally, the bioaccumulation capacity of total chromium in *Buddleja* species was determined and evaluated by comparing the amount of total chromium present in the plant and the amount present in the contaminated soil at the end of the experiment.

2. Materials and Methods

The contaminated soil selected for the study is located in the Añashuayco ravine, in the Uchumayo district in the northwestern part of the city of Arequipa (Peru).

2.1. Soil Sampling

Four soil sample locations (P-QA1, P-QA2, P-QA3 and P-QA4) were selected according to their representativeness (effluent path) as can be seen in Figure 1. These locations were georeferenced in Table 1.

Figure 1. Geographic location of the studied area in the Quebrada de Añashuayco. The sampling points are red-marked, and the oxidation ponds are the blue points. The studied area is bordered by a blue line.

Table 1. Georeferenced for soil sampling locations.

Locations	UTM Coordinates WGS 84 (Zone 19K)	
	East (m)	North (m)
P-QA1	220,911.7	8,189,203.6
P-QA2	220,735.2	8,189,078.3
P-QA3	220,559.0	8,188,922.2
P-QA4	220,150.1	8,188,458.65

The identification of the soil sample locations was carried out following the "Guide for soil sampling" of the Ministry of the Environment of Peru [16]. The procedure consisted in selecting four monitoring points, and at each point 5 pits were dug to a depth of 30 cm. This depth is defined as the maximum depth children can dig in a residential area, as Añashuayco ravine is considered. The material from these five pits was homogenized to obtain a single representative sample of 16 kg per monitoring point, suitable for further analysis. A total of 12 kg of the sample of 16 kg was allocated into 3 pots per sampling point (4 kg per pot). The rest of the sample was discarded. This procedure was performed for each of the four monitoring points. In order to measure the initial concentration of total chromium by the Environmental Protection Agency analytical technique, method 3050B acid digestion of sediment, sludge and soil, representative samples of 400 g of each evaluated location (P-QA1, P-QA2, P-QA3 and P-QA4) were used.

Some parameters of the initial soil for each location were measured. Moisture (Mdg) was determined using the gravimetric measurement equation [17]:

$$\%\mathrm{Mdg} = \frac{\mathrm{W_{ws}} - \mathrm{W_{ds}}}{\mathrm{W_{ds}}} \times 100 \quad (1)$$

where %Mdg is the percentage of gravimetric moisture while W_{ws} and W_{ds} are the weight of the wet soil (g) and the weight of the dried soil (g), respectively.

The texture was determined using the percentages of sand, silt and clay in each sample, which were determined with the Bouyucos Hydrometer. The percentages obtained were compared with the textural triangle according to the United States Department of Agriculture (USDA) classification.

Organic material (OM) was determined using equation [18]:

$$\%OM = \frac{W_{105^\circ C} - W_{550^\circ C}}{W_{105^\circ C}} \times 100 \quad (2)$$

where %OM is the percentage of organic material, while $W_{105^\circ C}$ and $W_{550^\circ C}$ are the dried weight at 105 °C (g) and the dried weight at 550 °C (g), respectively.

The field capacity (FC) was determined by applying the theoretical FC formula:

$$FC = 0.48\ Sa + 0.162\ Si + 0.023\ C + 2.62 \quad (3)$$

where FC is the percentage (%) of field capacity determined by weight, while Sa, Si and C are the percentage content of sand (g), silt (g) and clay (g), respectively.

The withered point (WP) was determined by applying the following theoretical formula [19]:

$$WP = -58.1313 + 0.3718\ O.M. + 0.5682\ Sa + 0.6414\ Si + 0.9755\ C \quad (4)$$

where WP is the percentage (%) of the withered point, OM is the percentage (%) of organic material, and Sa, Si and C are the same defined for FC.

The irrigation depth (ID) was determined using the following theoretical formula [20]:

$$ID = \frac{(FC - WP)}{100} ad \times Rd \times f \quad (5)$$

where ID is the irrigation depth (mL), while FC is the percentage (%) of field capacity determined by weight, WP is the percentage (%) of the withered point, f is the fraction of available water depletion, Rd (cm) is the root depth and ad $(g(cm^3)^{-1})$ is apparent density.

The pH and conductivity were evaluated at the beginning and every 30 days. The evaluation was carried out with a WTW Multi 3620 IBS multiparameter and TetraCon 325 conductivity sensor (Xylem, Inc., Washington, DC, USA).

2.2. Planting of Buddleja Species

A total of 14 seedlings of *Buddleja* species were placed in pots. They were distributed in three pots (a, b and c) for each soil sample location, and in two additional pots with uncontaminated soil that were used for control (or white seedlings).

The amount of water determined with the ID formula was employed. Collector equipment was also placed under each pot to be able to reuse the irrigation water and thereby assure more accurate results. Likewise, the size of the seedlings alongside the fall of leaves and changes in their color were monthly evaluated.

2.3. Bioaccumulation Capacity and Soil Quality

At the end of the 90 days of testing, 400 g of each group of pots related to each soil sampling location was analyzed. The total chromium was measured using the Environmental Protection Agency analytical technique, method 3050B acid digestion of sediment, sludge and soil.

At the end of the testing period, the stem, leaves and root of each *Buddleja* species plant from all the pots were naturally desiccated outdoors and then crushed to be analyzed. The presence of chromium was determined by an 8453 UV-Vis spectrophotometer (Agilent

Technologies, Santa Clara, CA, USA). The bioaccumulation (Bac) of the plant related to the initial concentration in the soil was determined using the following formula:

$$\%Bac = \frac{Cr_f}{Cr_0} \times 100 \quad (6)$$

where %Bac is the bioaccumulation percentage of the plant, Cr_f is the total chromium concentration in the plant at the end of the testing period and Cr_0 the total chromium concentration in the soil at the beginning of the testing period.

3. Results and Discussion

3.1. Initial Soil Quality

The quality of the contaminated soil before treatment at each sampling location is presented in Table 2. The texture of the four sampling locations is loamy–sandy according to the textural triangle, which, according to Leon [21], has a fast infiltration and drainage. This means that there is an ease in leaching of contained elements such as chrome under excessive irrigation. On the other hand, according to Molina [22], the content of OM found in the soil of this work is medium. The values for the OM range between 2.60% and 2.90%. It proves the necessity of the incorporation of sources of organic material like organic fertilizer. The FC and the WP were determined to obtain the amount of irrigation every 48 h, which was 1.9 L for each sample.

Table 2. Initial physicochemical characteristics of the soil.

Parameter	Samples			
	P-QA1	**P-QA2**	**P-QA3**	**P-QA4**
Texture	Sand: 64% Silt: 24% Clay: 12%	Sand: 62% Silt: 25% Clay: 13%	Sand: 65% Silt: 24% Clay: 11%	Sand: 64% Silt: 26% Clay: 10%
Organic material (OM)	2.60%	2.90%	2.70%	2.73%
Field capacity (FC)	13.63%	14.23%	13.17%	12.99%
Withered point (WP)	6.30%	6.90%	5.90%	5.68%
Irrigation depth (ID)	1.9 L	1.9 L	1.9 L	1.9 L

The pH and conductivity parameters of the soil are shown in Table 3. The pH initial average of the four samples was 8.33 and the final average value was 8.17. Therefore, it can be inferred that the site is characterized by a basic pH with a small decrease in its values throughout the testing period. According to Acosta and Montilla [23], this can be related to the competition of H^+ ions with metal cations, which generates desorption of heavy metals, being the acid pH of soils greatly favoring the concentration of heavy metals and their bioavailability in soils. The initial average value obtained for conductivity was 5.73 mS/cm and the final average value obtained was 5.69 mS/cm, indicating that the soil is saline. This characteristic could affect the yield and growth of the seedlings, since according to Nina and Rodriguez [24], one of the ideal physical characteristics of the soil is to be non-saline to moderately saline. However, the *Buddleja* species showed easy adaptation and resistance to this physical condition of the soil (saline), being able to develop in the pots. In addition, during the evaluation period, the variations in pH and electrical conductivity are not significant. Based on these results, the removal of total chromium did not influence the pH and conductivity of the soil. This variation can be attributed to competition of H^+ ions with metal cations.

Table 3. pH and conductivity of the soil during the testing.

Samples	Parameter		
	Days	pH	Conductivity
P-QA1	0	8.33	5.73
	30	8.20	5.68
	60	8.15	5.65
	90	8.17	5.64
P-QA2	0	8.34	5.71
	30	8.21	5.72
	60	8.20	5.70
	90	8.18	5.67
P-QA3	0	8.33	5.74
	30	8.19	5.70
	60	8.18	5.79
	90	8.17	5.68
P-QA4	0	8.32	5.73
	30	8.20	5.72
	60	8.15	5.67
	90	8.15	5.76

The initial concentrations of total chromium in the soil can be seen in Table 4. The highest presence of total chromium at the beginning of the testing was at sampling locations P-QA3 with 1660.99 mg/kg and P-QA4 with 1784.44 mg/kg, surpassing in both cases the national regulations of the Decree-Supreme N° 011-2017-MINAM on Environmental Quality Standards (ECA) of Peru for commercial, industrial and extractive soils with a maximum value of 1000 mg/kg [25]. Likewise, the sampling locations P-QA1 with 527.93 mg/kg and P-QA2 with 588.28 mg/kg exceed the ECA of Peru for residential and park soils with a value of 400 mg/kg [25]. On the other hand, the values of the four sampling locations do not exceed the maximum international reference value provided by the Andean Community (CAN) for industrial soils with a value of 2300 mg/kg for chromium [26]. However, the reference value provided by the CAN for residential soils, with a value of 64 mg/kg [26], is exceeded by the four sampling locations.

Table 4. Initial concentrations of total chromium in the studied soil.

Sampling Location	Total Chrome in Soil (mg kg^{-1})	Total Chrome ECA in Soil (mg kg^{-1})	
	0 Days	Residential Use/Parks	Commercial/Industrial/Extractive Use
P-QA1	527.93	400	1000
P-QA2	588.28		
P-QA3	1660.99		
P-QA4	1784.44		

3.2. Growth of *Buddleja* Species

The growth of *Buddleja* species at each sampling location (P-QA1, P-QA2, P-QA3 and P-QA4), subgroup (a, b and c) and control group were monthly observed.

According to the results, the average growth of the control group was 9.97 cm during the phytoremediation process. On the other hand, the *Buddleja* species planted in pots with the presence of total chromium grew in an average of 6.17 cm. Based on these results, we can mention that the growth of the seedlings of the control group samples was greater than those that were subjected to soil with the presence of total chromium. *Buddleja* species, compared to other species, maintained a constant growth. According to Ehsan et al. [27]

the Zinnia plant (*Zinnia elegance* L.) starts to decrease in height from 40 mg/kg reaching its maximum height peak at 30 mg/kg.

Regarding the fall of the leaves, the *Buddleja* species exposed to contaminated soil lost some of their leaves which fell during the testing process. This is confirmed by the research carried out by Santoyo et al. [28], showing that most of the morphological characters evaluated decreased in plants exposed to metals. On the other hand, the control group ones maintained a dark green color in their leaves, except for the plants exposed to chromium that withered and fell. This, according to Amin et al. [29], relates to the decrease of chlorophyll contents with increasing Cr concentrations. Likewise, according to Mujahid et al. [30], the increase of Cr concentration in the soil decreases plant height, the number of leaves and the presence of chlorophyll. This is demonstrated by Akhtar et al. [31], indicating that chromium stress has negative effects on plant growth, also affecting phytosynthetic pigments. In accordance with Mujahid et al. [32], the accumulation of heavy metals disrupts growth parameters. Therefore, it can be inferred that the presence of total chromium in the soil affects in a negative way the normal growth of *Buddleja* species. According to Subhashimi and Swamy [33], chromium is mostly stored in the root and stem, although it inhibits the growth of leaves of the plant when being used for the phytoremediation process. Likewise, according to Hafiz et al. [34], it has been shown that chromium tends to concentrate more in the roots than in the shoots or leaves. Ranieri et al. [35] have also shown that more Cr accumulates per gram of root and rhizome than in stems or leaves.

3.3. Removal Efficiency of Buddleja Species

The results of the removal efficiency (%) and the initial and final concentrations of total chromium are shown in Table 5. According to the results, the highest presence of total chromium at the end of the testing is found at P-QA3 sampling location followed by P-QA4. They have a removal efficiency of 35% and 41.5%, respectively. However, both cases still exceed the maximum value of 1000 mg/kg of the ECA national regulations for commercial, industrial and extractive soils. Likewise, the P-QA1 sampling location has a 32.4% removal efficiency while P-QA2, with 25.4%, is the lowest. In addition, P-QA1 location achieved a final value below the maximum national standard. In conclusion, the final total chromium values are still exceeding the international reference values for industrial and residential soils presented by the CAN.

Table 5. Growth of the 14 seedlings during the testing period.

Sampling Locations	Total Chromium in Soil (mg kg^{-1})		ECA for Total Chromium in Soil		Efficiency Removal Percentage (%)
	0 Days	90 Days	Residential and Park Use	Commercial, Industrial and Extractive Use	
P-QA1	527.93	356.80	400	1000	32.4%
P-QA2	588.28	438.51			25.4%
P-QA3	1660.99	1071.51			35.5%
P-QA4	1784.44	1042.89			41.5%

During the testing period, the total chromium decreased 33.7% in average for the four sampling locations in relation to the concentration of total chromium at the beginning of the testing.

According to the results, it can be inferred that a higher concentration of initial total chromium promotes a higher removal efficiency (%) of total chromium in the soil.

It can also be deduced that an increase in the time considered for the phytoremediation process in soils contaminated with total chromium will decrease the concentration of total chromium down to below the ECA. This is reported by Paredes [14] in a study with the *Buddleja coriaceous*, who obtained higher removal percentages using a longer testing time, 27 weeks, to remove heavy metals. This is demonstrated by Castañeda et al. [36], who found that there are positive and significant relationships between exposure time and the concentration of metals in roots and leaves.

In addition, it should be noted that the 14 seedlings used in the samples were still alive, indicating that the *Buddleja* species could grow in soils where total chromium contamination is up to 1784.44 mg/kg. This is supported by Waranusantigul et al. [37] in their study about the phytoremediation of Pb with *Buddleja asiatica and Buddleja aniculate*, where 100% of plants survived in a soil condition with total Pb concentration of up to 206,152.6 mg/kg and accumulating up to 4336 mg/kg. On the other hand, according to Ramana et al. [38], ornamental species like Crown of Thorns (*Euphorbia milli*) could tolerate well up to 75 mg of applied Cr and beyond that there was plant mortality.

3.4. Total Chromium Bioaccumulation in Buddleja Species

The percentage of absorption in the plant tissue and the concentration of total chromium in *Buddleja* species at the end of the testing are shown in Table 6.

Table 6. Bioaccumulation of total chromium (mg kg^{-1}) in *Buddleja* species.

Samples of *Buddleja* Species	Total Chromium Concentration in the Plant	% of the Bioaccumulation Plant
P-QA1	160.78	30.5%
P-QA2	132.3	22.5%
P-QA3	523.85	31.5%
P-QA4	626.67	35.1%

The highest concentration of total chromium in *Buddleja* species takes place at P-QA4 sampling location with 626.67 mg/kg and the lowest concentration is at P-QA2 sampling location with 132.3 mg/kg. According to Kassaye et al. [39], the total chromium accumulation is higher in the roots compared to the stem and leaves of plants. On the other hand, the highest percentage of bioaccumulation in the plant is at P-QA4 location with 35.1% and the lowest percentage is at P-QA2 location with a value of 22.5%. According to these results, it can be inferred that *Buddleja* species is a plant capable of bioaccumulating high concentrations of total chromium. However, this can only be confirmed for soils that reach up to 1784.44 mg/kg of total chromium, because it is not known if *Buddleja* species would resist higher concentrations of total chromium in the soil. This percentage of accumulated chromium increases as the amount of the heavy metal present in the soil increases, being in accordance with the research carried out by Al-Bataina et al. [40] with *Moso Bamboo* species, which presented a higher percentage of accumulation as the chromium in the soil increased. This, according to Taufikurahman et al. [41], is because heavy metals transported by plants are generally stored in cellular compartments such as vacuoles and lignocellulosic materials (cell walls).

Therefore, it is possible to affirm that the *Buddleja* species can bioaccumulate total chromium in its tissues, has a good resistance to soils with the presence of metals and good absorption capacity. This affirmation could be supported with that reported by Paredes [14] in his study with *Buddleja* species, where it was able to remove elements such as Cu, Sb, Pb, As, Ag and Cd, demonstrating the ability of the species to tolerate soils contaminated with tailings. Likewise, according to Rodríguez et al. [42], the *Buddleja cordata* can be used to phytoremediate soils contaminated with hydrocarbons, showing that the species has tolerance to environmental stress. On the other hand, the tolerance of *Buddleja scordioides* to heavy metals such as lead (Pb) was confirmed by Salas et al. [43]. Likewise, according to Sala et al. [44], the maximum accumulation of contaminants was demonstrated for the species *Buddleja cordata* Kunth for arsenic (As) with 3454 mg/kg, cadmium (Cd) with 24 mg/kg and lead 1282 mg/kg, being the heavy metal total chromium one of the least studied. On the other hand, according to Hernández et al. [45], it was demonstrated that *Buddleja scordioides* L. can phytoremediate Pb, and the seedlings evaluated survived, while those that were confronted with mercury (Hg) did not survive at any of the concentrations experimented. Likewise, Junyan and Jianguo [46] have shown that *Buddleja davidii* is a hyperaccumulator plant with a high biological transfer coefficient. According to Zhang et al. [47], *Buddleja lindleyana* Fortune can accumulate more than 90 mg/kg between the root and shoots of the plant.

According to the results in Figure 2, the reduction percentage of total chromium in the contaminated soil samples is higher than the percentage of total chromium bioaccumulated in the plant tissues. Therefore, it can be inferred that this behavior could be due to the difference between the samples analyzed, which are the plant and the potting soil, and their method of analysis in the laboratory. Also, the adherence of total chromium to the plastic of the pot could be related to this behavior.

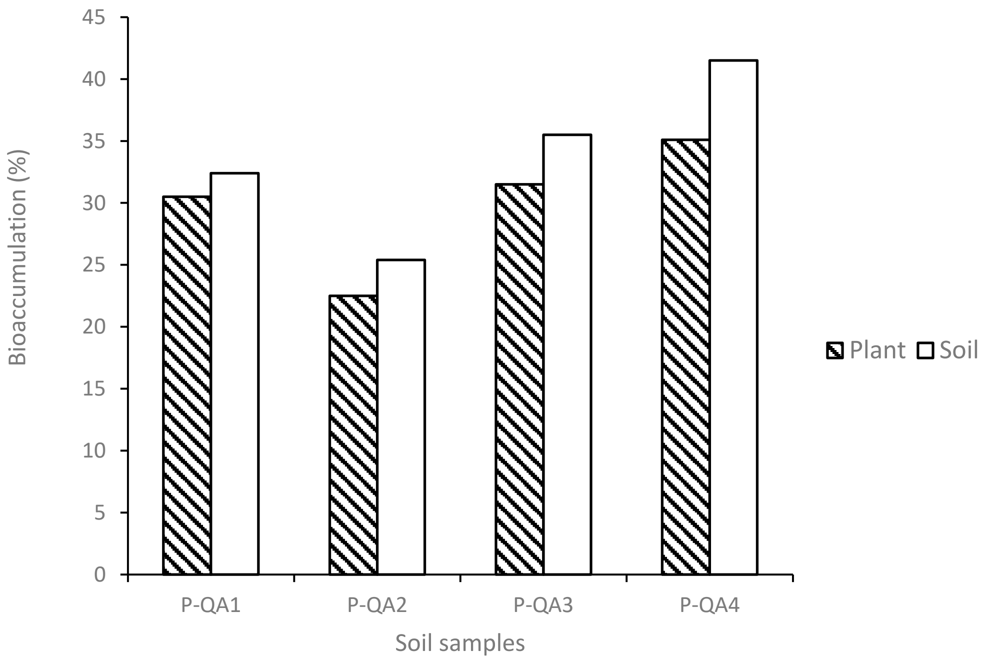

Figure 2. Percentage of total chromium reduction (%) in the *plant* and in soil samples at the end of the testing.

The Peruvian national standard was used to calculate the phytoremediation efficiency, as the study was evaluated on Peru (Figure 1 and Table 1). The values obtained were compared with the National Quality Standards (ECA). Although when compared with other international values such as the Canadian Soil Quality Guidelines and Environmental Protection Agency (EPA) Soil Quality Guidelines, they also exceed the values for residential soil of 64 (mg/kg) and 230 (mg/kg), respectively, and for industrial soil of 87 (mg/kg) and 510 (mg/kg), respectively [48]. In both cases, both the initial values exceed these values, while in the final values only the residential values exceed them; additionally, the industrial values for the Environmental Protection Agency (EPA) Soil Quality Guidelines both in point P-QA1 and P-QA2 do not exceed them, remaining below them.

4. Conclusions

According to the study, it can be drawn that the removal of total chromium does not influence the pH and conductivity of the soil. Also, the higher the concentration of total chromium, the higher the removal efficiency percentage (%) in the soil. For that reason, the average value for the removal efficiency percentage (%) of the four sampling locations was 33.7%. In addition, a longer period of time for the phytoremediation process will lead to a more efficient removal of chromium in the soils; therefore, the chromium concentration in soils would be under the maximum permissible in the Peruvian regulations.

In conclusion, the *Buddleja* species was able to reduce total chromium from soils contaminated by tanning activity due to its ability to bioaccumulate total chromium in its tissues and its resistance to soils contaminated with metals. However, this can only be confirmed for soils that reach 1784.44 mg kg^{-1} of total chromium concentration approximately. It is recommended to conduct future studies with higher concentrations of chromium and a longer time of phytoremediation process in order to evaluate the resistance of *Buddleja* species.

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