Hyperaccumulation of Cadmium and Lead in Ruderal Species of the Maria Auxiliadora Campus of the Universidad Politécnica Salesiana Guayaquil, Ecuador

Jaime Naranjo-Moran(✉), Gianna Mawyin-Mora, Tannia Reyes-Vilches, and José Luis Ballesteros-Lara

Grupo de Investigación en Aplicaciones Biotecnológicas, GIAB, Carrera de Biotecnología, Universidad Politécnica Salesiana, UPS, Campus María Auxiliadora, kilómetro 19.5 Vía a La Costa, 090901 Guayaquil, Ecuador
jnaranjo@ups.edu.ec

Abstract. Heavy metal contamination in urban soils is a growing concern. Hyper-accumulating ruderal species offer an alternative for bioremediation of these soils. To characterize Cd and Pb hyper-accumulating ruderal species in the soil adjacent to the María Auxiliadora campus of the Universidad Politécnica Salesiana Guayaquil, Ecuador. Soil samples and more than 50 ruderal species were collected. The three most representative species (*Distimake aegyptius*, *Bidens bipinnata* and *Chenchrus echinatus*) were selected for translocation factor, bioconcentration, germination and mortality analyses. Cd and Pb concentrations were determined by inductively coupled plasma spectroscopy. The soil is contaminated by Cd and Pb, due to a nearby company. Cd concentrations ranged from 1 to 2 ppm. The three selected species showed hyperaccumulation capacity, with *Chenchrus echinatus* standing out as an accumulator and *Distimake aegyptius* as a transporter. The results confirm the presence of ruderal hyper-accumulating species in the study area with potential for bioremediation. The implementation of phytoextraction strategies with *Distimake aegyptius* and phytostabilization with the other species is suggested. The presence of hyperaccumulating ruderal species offers a viable alternative for the bioremediation of soils contaminated by heavy metals.

Keywords: Ruderal species · phytoextraction · heavy metals

1 Introduction

The growing industrialization and urbanization of cities in general have intensified environmental pollution by heavy metals, negatively impacting ecosystems and human health. Among the various pollutants, heavy metals such as cadmium (Cd), lead (Pb), chromium (Cr) and arsenic (As) stand out for their persistent toxicity and bioaccumulation [1].

On the other hand, ruderal plant species are plants that colonize soils disturbed by human activities and have emerged as a promising tool for the bioremediation of soils

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contaminated with heavy metals. Unlike cultivated species, these plants can absorb and accumulate heavy metals in their tissues without suffering adverse effects [2]. This capacity for hyperaccumulation is attributed to various physiological and genetic mechanisms that allow ruderal plants to tolerate and store high concentrations of heavy metals in their various parts, such as leaves, roots and stems [3, 4]. Hyperaccumulation not only facilitates the removal of heavy metals from the soil but can also contribute to the stabilization of contaminants and the reduction of their bioavailability to other organisms [5].

The implementation of bioremediation strategies based on ruderal plant species offers multiple advantages, due to their low cost, effectiveness in degraded environments, and their potential for the ecological restoration of contaminated areas such as roads, landfills or mining tailings [6, 7]. Ecuador is a country with many environmental problems; this nation has exuberant ecosystems such as the Tumbesian dry forests that cover 35% of the coastal region, which have suffered a significant reduction and degradation. Today, only a fraction of this natural treasure remains intact, while most of it has fallen victim to deforestation, urban development, and agriculture [8]. Within this context, herbaceous ruderal species that colonize disturbed land have gained new importance [9]. These species, far from being undesirable intruders, can be valuable tools for environmental assessment and bioremediation [10].

2 Materials and Methods

Study Site. This research was carried out in the province of Guayas, in an area adjacent to the María Auxiliadora campus located on the Vía a "La Costa", kilometer 19.5. The study area was delimited on the ground adjacent to the campus, with a total extension of 500 m. The geographic coordinates of the study area are 2°11′33.4 "S and 80°02′43.7" W. For a better understanding of the location, Fig. 1, with the area of the investigation, is attached.

Census of Ruderal Floristic Composition. The circular plant census is a traditional technique to evaluate the composition and structure of plant communities [11], in addition the use of digital tools facilitated the identification for the collection of species remotely, by optimizing the research process and making it more accessible to a wide range of users on the live internet. The cellular applications used for automated plant recognition through images were iNaturalit Ecuador (https://ecuador.inaturalist.org/) and Pl@ntNet (https://identify.plantnet.org/es), these applications have been very useful for researchers in recent years, even where there is not much plant diversity [12]. The circular plant census incorporates a global positioning system (GPS) to determine the exact location of the sampling plots. This will allow the creation of an interactive map that provides the digital repositories with a visualization of the spatial distribution of plant species, and this information will be updated in future surveys. In addition, many apps have tools to record detailed information about each species, such as scientific name, height, stem diameter and conservation status. The counting of plant species, an essential component of the circular census, was simplified by the use of apps. The circular plot consisted of the numbering and geographic location of the PSUs (Permanent Sampling Units) in a flat field. The PSU was 500 × 15 m, where the ruderal species were evaluated. The PSU was

divided into eight sampling subunits with a diameter of 10 m, where the different ruderal species were collected in a 5 m radius. From each central subunit were placed squares with the dimension of 1 m2 × 1 m2, and then three subsamples for each sample were analyzed randomly (Fig. 1). Instead of taking manual notes, users captured images of the plants to identify remotely with the image recognition algorithm. This not only saves time and effort but also reduces the possibility of errors in species identification. A validation with three recognized databases "Herbario Rapid Reference" (https://plantidtools.fieldmuseum.org/es/rrc/5581), "Trópicos" (https://www.tropicos.org/home), and "Bioweb Ecuador" (https://bioweb.bio/) was carried out to contrast the information collected.

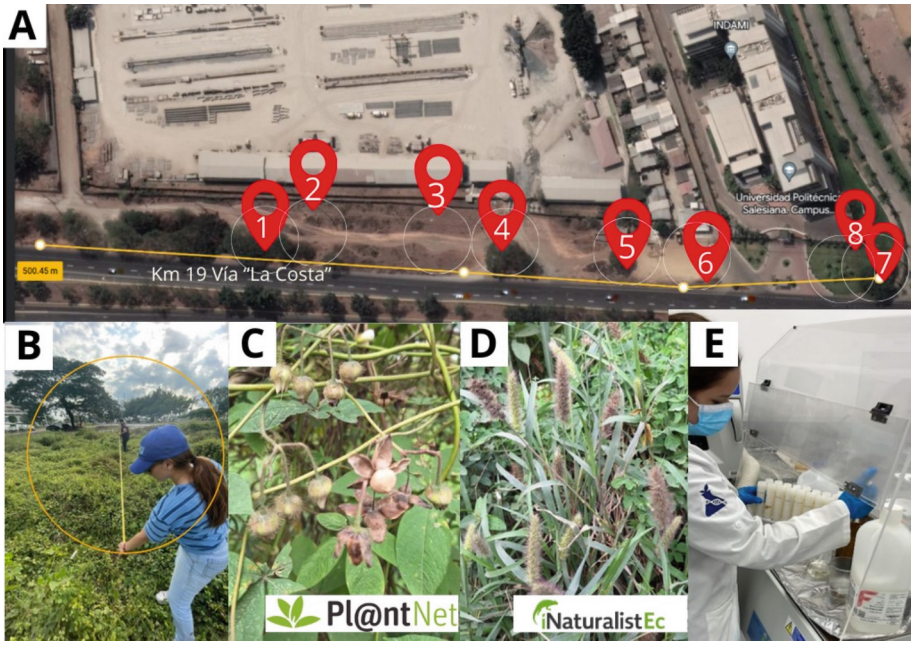


Fig. 1. A) Sampling sites in ruderal zone at km 19 via La Costa; B) Circular sampling installation; C-D) Photographic shots for remote reconnaissance; E) Sample processing in acid digestion.

Elemental Determination Of Metals (Cd and Pb). To determine the concentration of Cd and Pb in soil samples, roots, and leaves, the technique of inductively coupled plasma optical emission spectroscopy (ICP-OES) was used. The samples were prepared by drying, grinding, and sieving according to the EPA-3051A method, developed by the United States Environmental Protection Agency [13]. The leaves were dried, ground, and sieved before acid digestion. A microwave acid digestion was used, 0.5 g of each type of sample (soil, roots, and leaves) were weighed and placed in digestion vessels. Ultrapure nitric acid was added (10 ml for soil and 10 ml for roots and leaves) and different heating-digestion programs were applied according to the type of sample, for sue-lo: 175 °C for 5:30 min. And for roots and leaves: 200 °C for 15:00 min. Once the digestion was finished, the solutions were filtered and diluted to a final volume

of 50 ml with type 1 water. The ICP-OES analysis was performed by calibrating the equipment, where a multi-element solution was prepared with concentrations of 0.2; 0.7; 1.1; 1.1; 1.1; 1.2; and 4.8 ug/g for each element (Cd and Pb) from an individual 1000 mg/L standard solution. For the reading, the diluted samples were introduced into an autosampler and analyzed in a Thermo Scientific ICP-OES equipment model iCAP 7400 Duo. The following wavelengths were used for the reading of each element Cd: 226,502 nm and Pb: 220,353 nm, the validation of the calibration curves for each element showed a coefficient of determination (R2) of 0.9970 for both elements analyzed (Cd and Pb).

Accumulation and Translocation Factors for Cd and Pb. To evaluate the bioaccumulation capacity of plants, the concentration of metal present in their tissues was divided by the amount of metal present in the ruderal soil [14]. This index indicates how much metal the plant has absorbed about the amount available in the soil. As for translocation, the concentration of metal present in the plant biomass (roots and leaves) was divided by the concentration of metal present in the roots. This indicator reflects the efficiency with which the plant transports the metal absorbed from the roots to the aerial parts, i.e. bioaccumulation measures the total amount of metal that the plant has absorbed and retained, while translocation evaluates the capacity of the plant to distribute the metal absorbed by its different parts. Consideration should be given to measuring the quantities of plants and agricultural products in Ecuador [15].

Data Analysis. Once the significant differences were identified by ANOVA, Tukey's test was used to establish which of these differences were statistically relevant. A significance level of $p < 0.05$ was established for all tests.

3 Results

As a result of this research, a total of 56 species belonging to 21 plant families were identified in the eight transects, as shown in Table 1. These species are considered ruderal, due to the high anthropogenic intervention in the area.

As a result of the circular plant census, three representative species were found in several individuals in the eight transects: *Distimake aegyptius*, *Chenchrus echinatus*, and *Bidens bipinnata*, belonging to the families Convolvulaceae, Poaceae, and Asteraceae. From these species, root, leaf, and soil samples were collected for each of the three selected plant species. It is observed that point eight had a higher level of Cd, while point two had a higher level of Pb, as shown in Fig. 2 concerning the other sampling points, which means that the accumulation is well determined by anthropogenic activity.

Cd values in leaves ranged from 0.10 ppm (*D. aegyptius*) to 0.19 ppm (*B. bipinnata*). For Pb, values ranged from 0.10 ppm (*D. aegyptius*) to 0.20 ppm (*B. bipinnata*). No significant differences were found in the accumulation of heavy metals among the leaves of the three species. As for the accumulation in roots, the species *B. bipinnata* and *D. aegyptius* presented values of Cd and Pb uptake in roots lower than 1 ppm. *C. echinatus* showed values greater than 1 ppm, with 1.03 ppm for Cd and 1.40 ppm for Pb. In general, roots of the three species accumulated more heavy metals than leaves. Accumulation in rhizospheric soil showed a higher accumulation of heavy metals for all three plants, with

Table 1. Ruderal species identified at each sampling point at km 19 via La Costa.

Sampling points	Scientific name	Family
First point	*Rhynchosia minima*	Fabaceae
	Argentina adenophora	Asteraceae
	Eclipta prostrata	Asteraceae
	Ludwigia peruviana	Onagraceae
	Rhynchosia minima	Fabaceae
	Ptelea trifoliata	Rutaceae
	Cenchrus echinatus	Poaceae
	Sida rhombifolia	Malvaceae
Second point	*Echinochloa colona*	Poaceae
	Dactyloctenium aegyptium	Poaceae
	Chloris barbata	Poaceae
	Sorghum halepense	Poaceae
	Merremia umbellata	Convolvulaceae
Third point	*Sporobolus pyramidatus*	Poaceae
	Melochia lupulina	Malvaceae
	Achyranthes aspera	Amaranthaceae
	Commelina diffusa	Commelinaceae
	Bidens bipinnata	Asteraceae
	Echinochloa colona	Poaceae
	Cucumis dipsaceus	Cucurbitaceae
	Ageratum conyzoides	Asteraceae
	Distimake aegyptius	Convolvulaceae
	Cyperus rotundus	Cyperaceae
	Mitracarpus hirtus	Rubiaceae
	Sida acuta	Malvaceae
Fourth point	*Tephrosia purpurea*	Fabaceae
	Melochia lupulina	Malvaceae
	Albizia multiflora	Fabaceae
	Ipomoea coccinea	Convolvulaceae
	Rhynchosia minima	Fabaceae
Fifth point	*Portulaca oleracea*	Portulacaceae
	Convolvulus althaeoides	Convolvulaceae

(*continued*)

Table 1. (*continued*)

Sampling points	Scientific name	Family
	Tetramerium nervosum	Acanthaceae
	Cenchrus echinatus	Poaceae
	Mimosa quadrivalvis	Fabaceae
	Ruellia blechum	Acanthaceae
	Sorghum halepense	Poaceae
	Amaranthus retroflexus	Amaranthaceae
	Distimake aegyptius	Convolvulaceae
	Sida rhombifolia	Malvaceae
	Sida acuta	Malvaceae
Sixth point	*Distimake aegyptius*	Convolvulaceae
	Prestonia mollis	Apocynaceae
	Sida rhombifolia	Malvaceae
	Tecoma castanifolia	Bignoniaceae
	Cenchrus echinatus	Poaceae
	Sorghum vulgare	Poaceae
	Senecio viscosus	Solanaceae
Seventh point	*Cynodon dactylon*	Poaceae
	Solanum nigrum	Solanaceae
	Euphorbia prostrata	Euphorbiaceae
	Alysicarpus vaginalis	Fabaceae
Eighth point	*Murraya paniculata*	Rutaceae
	Synedrella nodiflora	Asteraceae
	Coccoloba uvifera	Polygonaceae
	Cyperus rotundus	Cyperaceae

values above 1 ppm for all metals. Cd values in soil were similar for the three species, ranging from 1.53 ppm (*B. bipinnata*) to 1.60 ppm (*C. echinatus*). For Pb, *C. echinatus* presented the lowest value (1.10 ppm), while *B. bipinnata* (3.70 ppm) and *D. aegyptius* (3.90 ppm) showed the highest values (Fig. 3).

The results for the bioconcentration factor obtained for Cd were as follows: 0.96 for *D. aegyptius,* 0.43 for *B. bipinnata*, and 1.46 for *C. echinatus* (Fig. 4). For Pb they are detailed as follows: 0.62 for *D. aegyptius*, 0.75 for *B. bipinnata*, and 1.20 for *C. echinatus*. The species *C. echinatus* for both metals have the highest bioconcentration factor value, so that, being greater than 1, this species is considered an accumulator of heavy metals. On the other hand, *B. bipinnata* and *D. aegyptius* have values lower than 1, which indicates that they are classified as heavy metal exclusion species. The results

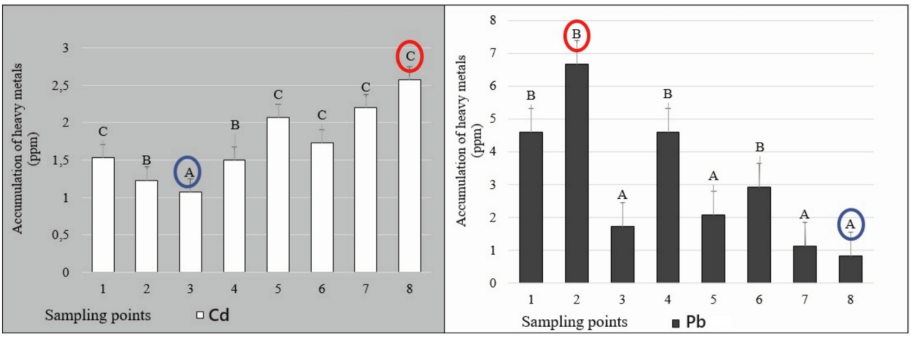


Fig. 2. Quantification of Cd and Pb in the ruderal sampling points of Km 19 Via "La Costa". Different letters in the same row indicate significant statistical differences according to Tukey's test ($p < 0.05$); ± standard error.

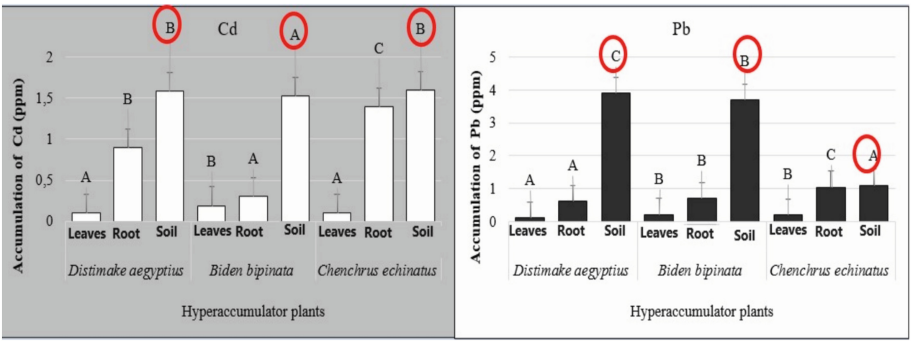


Fig. 3. Cd and Pb accumulation in soil and vegetative structures in the three hyper-accumulating plant species. Different letters in the same row indicate significant statistical differences according to Tukey's test ($p < 0.05$); ± standard error.

obtained for the translocation factor for Cd were as follows: 1.40 in *D. aegyptius*, 0.65 in *B. bipinnata,* and 0.08 in *C. echinatus.* On the other hand, for Pb, 0.18 was obtained in *D. aegyptius*, 0.29 in *B. bipinnata,* and 0.19 in *C. echinatus.* The species *D. aegyptius* was the only one that reported a value higher than 1 for the translocation factor, specifically for Cd. This means that the plant has a greater capacity to accumulate and transport metals from the soil. It could be used for phytoextraction purposes in soils contaminated by pe-rated metals. However, the analyzed species present Pb values lower than 1.

4 Discussion

Research on heavy metal contamination in soil adjacent to an industrial zone has shown that Cd and Pb levels range from 0.13–.88 ppm for Cd and 4.64–18.21 ppm for Pb [16]. Although these values do not exceed the national average for uncontaminated soils [17], like the results of this investigation, they represent a warning sign. The results

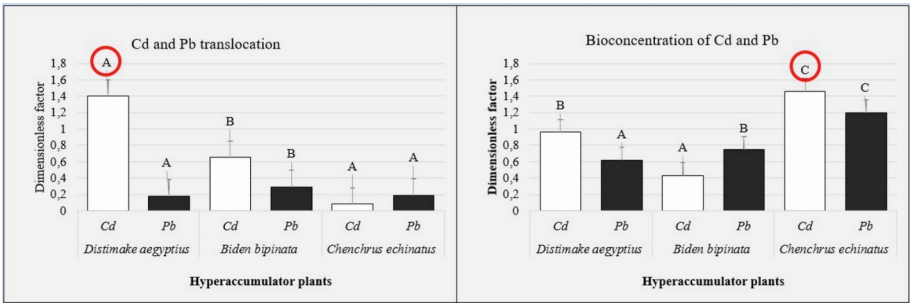


Fig. 4. Cd and Pb accumulation and translocation factors for three hyperaccumulating plants. Different letters in the same row indicate significant statistical differences according to Tukey's test ($p < 0.05$); ± standard error.

suggest the need to implement remediation and prevention strategies for soil contamination at km 19 via La Costa. The accumulation of heavy metals in the leaves of the three species studied (*Bidens bipinnata*, *Distimake aegyptius* and *Chenchrus echinatus*) showed a low accumulation of heavy metals. Other research carried out on the species *Ambrosia ambrosioides* (Asteraceae), found accumulation values of 249.6 ppm for Pb and 2227.8 ppm for Cd in roots, and 420.1 ppm for Cd and 710.7 ppm for Pb in leaves [18]. The root presented the highest accumulation of Cd. Researchers indicate that in the species of the genus *Brachiaria spp.* (Poaceae), values of 0.01 to 0.024 ppm for Cd and 1.01 to 1.32 ppm for Pb in leaves, and 0.02 to 0.034 ppm for Cd and 1.72 to 1.92 ppm for Pb in roots were recorded. Values in soil ranged from 0.1 to 1.11 ppm for Cd and 12.90 to 23.43 ppm for Pb [19]. It is commented that in *Ipomoea batatas* (Convolvulaceae), values from 4.08 to 77.1 ppm for Pb in roots and 0.99 to 43.43 ppm for Pb in leaves were found. These values exceed the maximum limits established for human consumption [20]. For these reasons, it is of vital importance to monitor the ruderal plant community and, finally, to promote bioprospecting of ruderal plant species in bioremediation processes.

5 Conclusions

The study shows that the soil adjacent to the Salesian Polytechnic University is contaminated with heavy metals, which negatively affects the germination as well as survival of the ruderal plant species present in the area. *Chenchrus echinatus* and *Distimake aegyptius* species, due to their ability to accumulate and tolerate heavy metals, could be used for phytoextraction and phytostabilization strategies, respectively. Further research is needed to determine the optimal conditions for germination and growth of these species in contaminated soils and to evaluate their potential for the remediation of contaminated sites.

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