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# Forest Species with Bioaccumulatory Potential of Heavy Metals in Mining Areas in the Ecuadorian Amazon

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## ABSTRACT

The study aimed to evaluate the potential bioaccumulation of heavy metals in tree and shrub species in the Simón Bolívar zone, El Capricho parish, Carlos Julio Arosemena Tola canton, Napo Province, Ecuador, an area previously affected by illegal gold mining activities. The bioconcentration factor of six species was analyzed based on the ratio of heavy metal concentrations in leaves to soil. Leaf-level results revealed that Iron (Fe) accumulated most in *Acalypha diversifolia* Jacq. and *Cedrela odorata* L., while *Theobroma cacao* L. showed higher Zinc (Zn) accumulation, indicating their potential for phytoremediation in contaminated areas. Regarding soil metal concentrations, *C. odorata* exhibited the highest levels of Fe, Zn, Copper (Cu), and Lead (Pb), whereas *A. diversifolia* had the lowest. Based on the bioconcentration factor (BCF), *A. diversifolia* demonstrated the highest potential for absorbing Fe, Cu, and Pb, significantly differing with elevated values compared to other species. These findings could inform soil remediation strategies using these potential species to mitigate toxic elements in the study area.

**Keywords:** vegetation, soil, contamination, bioaccumulation, phytoremediation

## INTRODUCTION

Currently, the contamination of soils and leaf tissues by heavy metals due to mining activity is on the rise worldwide, causing one of the most serious environmental problems due to anthropic activity (Rodríguez-Eugenio et al., 2019). In Ecuador, mining has been a topic of debate among the economic, social and environmental sectors, as it is considered one of the main strategic sectors and as one of the essential points for the country's economic development (Estupiñan et al., 2021). However, the high loss of biodiversity due to mining expansion mainly the exploitation of gold in the Ecuadorian Amazon (Delgado Fernández et al., 2023), has led to urgent measures for the conservation and remediation of contaminated soils, such as the use of bioaccumulator plants. The bioaccumulatory potential of forest species is a feasible tool to provide solutions to this problem. In addition, many plant species have some ability to bioaccumulate metals and other pollutants, which usually vary depending on the plant species and the nature of the pollutants, which are carried from roots to leaves (Wang et al., 2019).

These plant species are capable of absorbing and storing those metals, which is why they are called hyperaccumulators, which are usually found in soils with a high metal content due to natural geochemical conditions (Delgadillo-López et al., 2011). These plants generally have little biomass as they use more energy in the components needed to get used to the large amounts of metal concentrations within their tissues (Cahuana & Aduvire, 2019). Therefore, accumulator plants play a very important role in decreasing or increasing soil toxicity. The most common hyperaccumulator species of heavy metals are from the families Brassicaceae, Asteraceae, Amaranthaceae, Poaceae, including the Fabaceae family for metals such as Se, Pb and Ni (Bustos, 2021).

The main heavy metals that aid in the metabolism of plant species are magnesium (Mn), nickel (Ni), copper (Cu), zinc (Zn), and molybdenum (Mo), unlike other elements, which are phytotoxic (arsenic (As), cadmium (Cd), mercury (Hg) and lead (Covarrubias & Cabriales, 2017). In addition these metals are considered as heavy elements and can be accumulated in different organs of the plant, there is also a group of heavy metals that are established as higher risk (As, Cr, Cd, Hg Pb), being associated with the Mining Environmental Liabilities (Cahuana & Aduvire, 2019).

For this reason, the mining activities carried out in the Simón Bolívar area, the Capricho of the Carlos Julio Arosemena Tola Canton, Napo Province have highly affected the forests; Deforestation and large-scale agglomeration of tailings of washed gravel have increased soil and water pollution, and for lack of any technical procedure (Napo Prefecture, 2019).

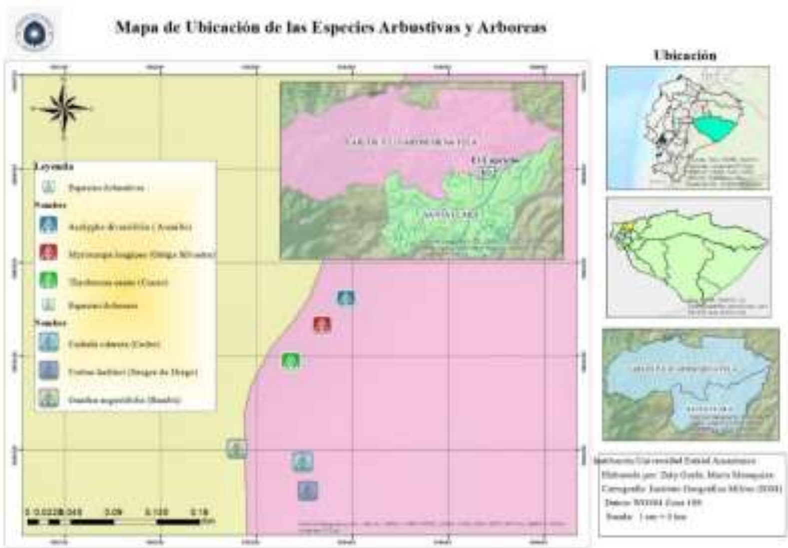
Therefore, the objective of the study was to evaluate the bioaccumulatory potential of heavy metals in six species of forest plants as phytoremedial species in areas of the Ecuadorian Amazon that have been contaminated by mining extraction.

## MATERIALS AND METHODS

### Study Area

This work was carried out 300 m from a mining area where illegal gold extraction activities were carried out approximately four years ago. The place is located in the area of Simón Bolívar, El Capricho parish, in the Carlos Julio Arosemena Tola canton, Napo Province, Ecuador (1°12´03´´S 77°51´51´´W). There, leaf and soil samples were collected from the six forest species located on the banks of the Puno River (Fig. 1). The climate is permanently humid temperate (90%), its temperature ranges between 22°C and 25 °C, with an average annual rainfall between 3800 and

4500 meters above




sea level (Napo Prefecture, 2019).

**Figure 1.** Geographical location of the study area belonging to the parish of El Capricho, Napo. The location of the plant species studied is shown in colored boxes. In original language English

## Plant and soil material sampling

Six forest species were selected on the bank of the Puno River, El Capricho parish near the mining extraction area in January 2023; Three tree species *Cedrela odorata* (Cedar), *Croton lechleri* (Dragon's Blood) and *Guadua angustifolia* Kunth. (Bamboo), three shrub species *Theobroma cacao* L. (Cocoa), *Acalypha diversifolia* (Arenillo) and *Myriocarpa longipes* L. (Wild nettle). The selection criteria for these species were the economic and ecological importance for the region with predominance in the ecosystems of Piedmont evergreen forests, are Fast-growing, easily adaptable species with a wide distribution in the Amazon region (García-Quintana et al., 2024).
The species were identified in the field with the accompaniment of a specialist in the area and the taxonomic identification was carried out through specialized bibliography "Book of Trees of Ecuador: representative species" (Palacios, 2016), Photographic Guide to Trees of the Peruvian Amazon (Flores Bendezú, 2020), Guide for the identification of 24 non-timber species (PROAmazonía, 2020) and virtual resources (WFO, n.d.).

The selection of the plant material was given by collecting adult and young leaves from the trees that were exposed to the sun, leaves from different branches were selected around the entire crown of the plant in a good physical condition, that is, without mechanical damage or the presence of visible pests and diseases. The samples were labeled and transferred to the Environmental Laboratory of the Amazonian State University (UEA) for further analysis.

Soil samples were taken at three different points around the base of the trunk of the selected trees with the use of a drill at a depth of 0-30 cm in a radius of approximately 30 cm from the tree; Next, leaf litter and roots were removed from the sample (Greksa et al., 2019). The samples were labeled and transferred to the UEA soil laboratory for subsequent heavy metal analysis procedure.

**Heavy Metal Analysis in Leaf and Soil Samples**

The samples of the collected plants were subjected to drying by oven at a temperature of 60°C for 72 hours. The dried samples were crushed to a fine substance of particles smaller than 2mm; subsequently, 3 replications were made (R1, R2, R3). For the soil samples, only a replicate was dried at room temperature for four days on filter paper, then they were crushed, passed through a 2 mm mesh sieve and dried in an oven at a temperature of 40 °C for 48 h (Šichorová et al., 2004; Sun et al., 2016). With the previously labeled plant and soil material, wet digestion was carried out with a mixture of nitric acid (HNO3) at 65% and hydrogen peroxide (H2O2) at 37%, in a ratio of 7:1 (Wang et al., 2019). Once the samples were digested, they were placed in a flask of 50ml each, distilled water was added and taken to a volume of 25 ml with 1% HNO3, the mixtures were covered and stirred in order to carry out filtration.

Once the liquid samples of leaves and soils were filtered, heavy metals were quantified in a Perkin-Elmer 2380 AA atomic absorption spectrophotometer and a standard of 1000 ppm of each metal was used for the calibration curve of the equipment and the corresponding lamp was placed. Subsequently, with the WIBLAB 32 software version 2022, the samples were read to obtain the concentration of the analytical in ppm at wavelengths of 324.8 nm for Cu (copper), 248.3 for Fe (iron), 213.9 nm for Zn (zinc) and 283.3 nm for Pb (lead).

**Bioconcentration Factor (FBC)**

FBC is used to measure the ability of a plant to capture a metal in relation to its concentration in the soil (from the radial and aerial side), as a measure of the efficiency of metal accumulation in biomass, values above 1 indicate that a species is potentially hyperaccumulator, while excluded species have a FBC less than 1, with lower values indicating a greater capacity for exclusion (Alahabadi et al., 2017). Therefore, the FBC (Equation 1) was used to calculate the amount of heavy metals retained and accumulated for each species analyzed, as follows:

$$\boldsymbol{FB} = {}^{\boldsymbol{C_{follaje}}}\!\big/_{\boldsymbol{C_{suelo}}} \qquad [1]$$

Where C foliage and C soil represent the concentration of a specific element in the plant material (foliage) and soil, respectively.

### Data analysis

A simple classification analysis of variance (ANOVA) with a significance level of $P \leq 0.05$ was performed in order to find significant differences between each of the heavy metals (Cu, Fe, Zn, Pb) and the species studied (*C. odorata*, *C. lechleri*, *G. angustifolia*, *T. cacao*, *A. diversifolia* and *M. longipes*), in addition, the Pearson correlation matrix was carried out to establish the similarity between the absorption capacity of heavy metals of the species and to determine the correlations in those concentrations of heavy metals that were analyzed in the plants and the soil.

Cluster analysis (clusters) was also carried out with the aim of classifying the heavy metals in the studied species into groups and then the principal components (PCA) were analyzed in order to find how many components are capable of explaining the total variability of the data and establish the separation of the analyzed plants depending on the concentrations of heavy metals. Statistical analyses were performed using Origin 2022 software.

## RESULTS AND DISCUSSION

### Concentration of heavy metals in leaves and soil

The results obtained in the concentration of heavy metals at the leaf level according to the ANOVA test for Fe, Zn, Cu, Pb in the six species under study (*T. cacao, A. diversifolia, M. longipes, C. odorata, C. lechleri, G. angustifolia*) showed significant differences ($P< 0.05$) (Fig. 2).

The species *A. diversifolia* and *C. odorata* obtained a higher concentration of Fe with a value of 150 mg/kg, while *M. longipes, C. lechleri* and *T. cacao* presented a value lower than 70 mg/kg. The concentration of leaf Zn in the species *T. cacao* had the highest values of 125 mg/kg, and the species *A. diversifolia, M. longipes, G. angustifolia, C. odorata* and *C. lechleri* indicated lower values of 25 mg/kg. Regarding Cu, the species *T. cacao* and *C. lechleri* had a higher value of 11.40 mg/kg and the species *A. diversifolia and C. odorata* presented mean values of 10.35 mg/kg, while the species *M. longipes and G. angustifolia* obtained low values of 8.50 mg/kg, in turn the concentration of Pb in the species *A. diversifolia* and *M. longipes* they indicate a higher value of 4.5 mg/kg and the species *G. angustifolia, C. lechleri* and *C. odorata* presented lower values of 1.5 mg/kg.

These results demonstrated the potential of the species *A. diversifolia* for foliar accumulation of heavy metals such as Fe and Pb and *T. cocoa* for Zn and Cu. Likewise, the species *Solidago canadensis* L. belonging to the Asteraceae family showed that it serves as a bioaccumulator of Zn and Pb by determining that it can be used as a phytostabilizer in soils heavily contaminated with these elements (Bielecka & Królak, 2019).

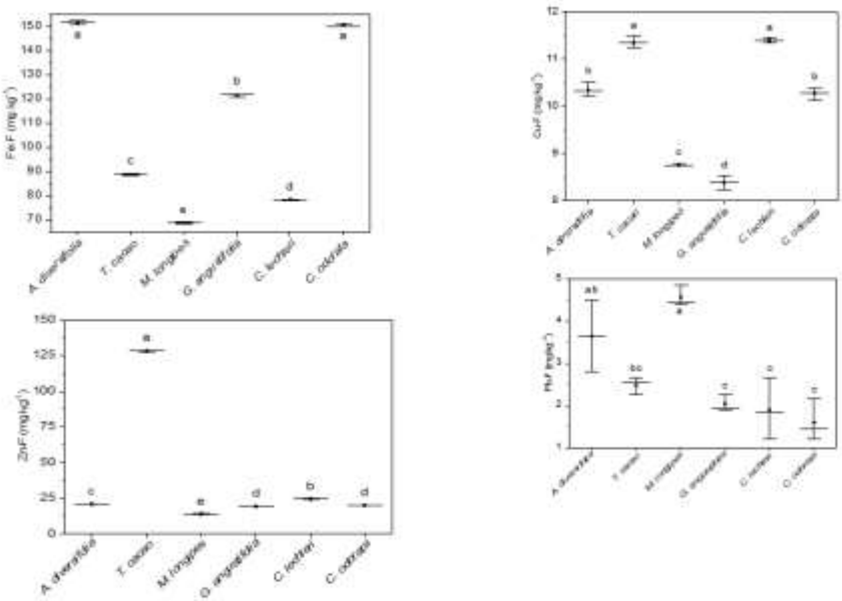


**Figure 2.** ANOVA analysis of variance ($P < 0.05$) of the concentration of heavy metals (a) Fe, (b) Zn, (c) Cu and (d) Pb, present in the leaves of the species *T. cacao*, *A. diversifolia*, *M. longipes, C. odorata*, *C. lechleri*, and *G. angustifolia* (different letters have significant differences). In original language English

The concentrations of heavy metals Fe, Zn, Cu and Pb present in the soil where the species developed *T. cocoa*, *A. diversifolia*, *M. longipes, C. odorata*, *C. lechleri*and *G. angustifolia* showed significant differences (*P< 0.05)* in the concentrations of heavy metals at soil level (Fig. 3), indicating a pattern of variation in the level of species similar to that found in the leaf analysis. Regarding the behavior of Fe, Zn, Cu and Pb, the results showed that the soil where it grows *C. odorata* had high values and *A. diversifolia* the lowest levels, while the Fe concentration values were higher in *T. cocoa* and lower in *A. diversifolia.* The concentration of Pb at ground level ranged from 28 to 30 mg/kg, Cu concentrations ranged from 10 to 44 mg/kg, Zn concentrations ranged from 31 to 89 mg/kg, and Fe concentrations ranged from 1357 to 1552 mg/kg. Therefore, most heavy metals tend to be more accessible to acidic pH soils, which makes it possible to predict whether favorable conditions exist for the accumulation of these metals at the sampled sites (Guerrero Useda and Pineda Acevedo, 2016).

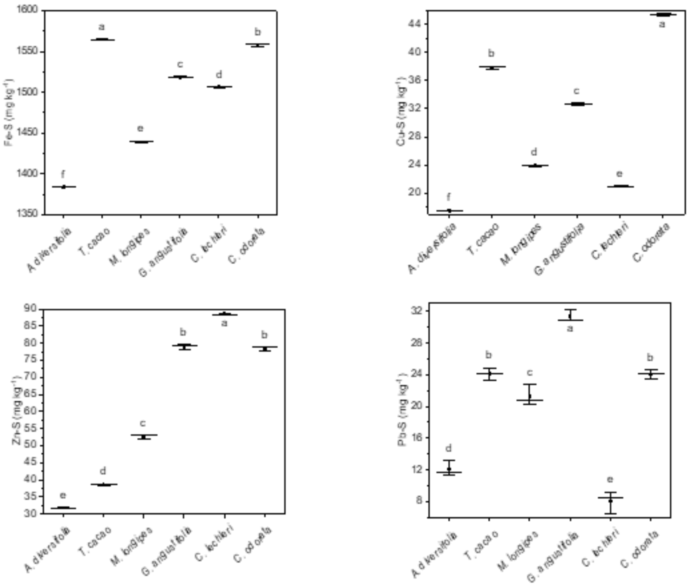



**Figure 3.** Results of the ANOVA analysis of variance ($P < 0.05$) of the concentration of heavy metals (a) Fe, (b) Zn, (c)Cu and (d) Pb, present in the soil where the species *T. cacao*, *A. diversifolia*, *M. longipes, C. odorata*, *C. lechleri*, and *G. angustifolia* (different letters show significant differences) developed. In original language English

## Bioconcentration Factor (FBC)

Subsequently, the results regarding the bioconcentration factor of the species are presented *T. cocoa*, *A. diversifolia*, *M. longipes, C. odorata*, *C. lechleri*and *G. angustifolia* for the heavy metals Fe, Zn, Cu and Pb. The species *T. cocoa* it presented the highest Zn accumulation capacity with a value of 3.35 mg/kg, which also indicates its possible use in areas with high zinc metal content. This species is also a cadmium hyperaccumulator, obtaining a concentration of 0.509 mg.kg-1 in the leaves according to a study in the Peruvian Amazon (Llatance et al., 2018). In the same way, the species *A. diversifolia* presented the highest absorption potential of Fe, Cu and Pb, showing significant differences ($P < 0.05$) with high values compared to other species (Fig. 4), indicating a greater capacity for bioaccumulation of the elements and allowing its use as a phytoremediation species in areas contaminated with Fe, Cu and Pb. According to Guerra Sierra et al., (2021) some species of the family Euphorbiaceae have the bioaccumulatory potential of heavy metals in highly polluted places, as is the case of this species and also because the bioaccumulation of several metals in the same plant turns out to be a species with better phytoremediation potential than those that only accumulate a single metal (Hu et al., 2014).

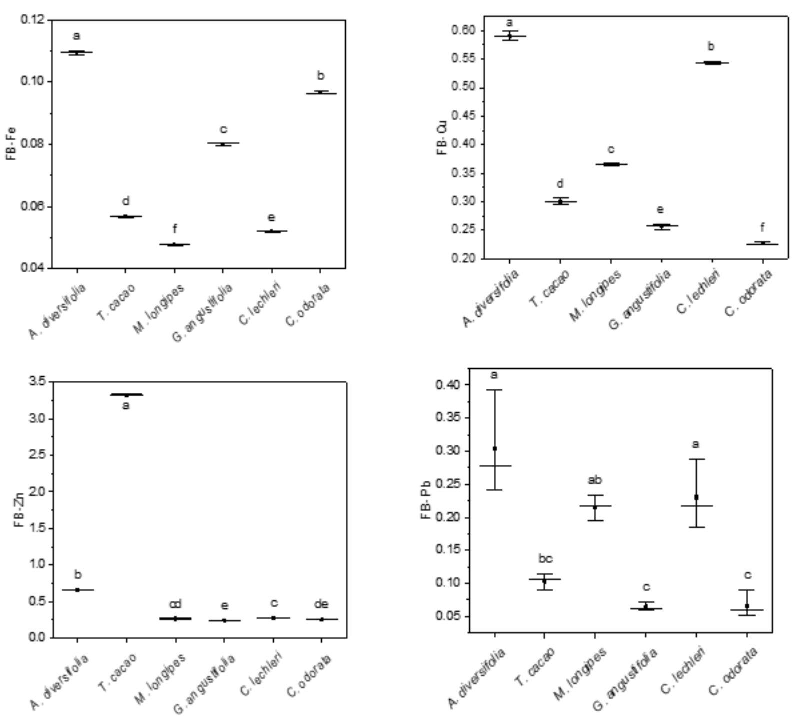



**Figure 4.** Results of the ANOVA analysis of variance ($P < 0.05$) for heavy metal bioconcentration factors (a) Fe, (b) Zn, (c) Cu and (d) Pb, of the species *T. cacao*, *A. diversifolia*, *M. longipes, C. odorata*, *C. lechleri*, and *G. angustifolia* (different letters show significant differences). In original language English

## Heavy metal absorption capacity from plants and soil

The results of Pearson's correlation coefficient (Fig. 5) show a positive and negative correlation, for as the value of one metal increases, the value of another metal decreases near or greater than 50% in the concentration of heavy metals in leaves and soil. The positive correlations of the leaf part between Zn-F and Cu-F (r=0.54) and the positive correlations of the soil elements (Cu-S and Fe-S (r=0.84) and Pb-S and Cu-S (r= 0.7)) are presented. This indicates that the plants studied do not fully absorb soil components, possibly due to the plant's physiology and ability to regulate the uptake of heavy metals under pollution stress (Fernández-Ondoño et al., 2017).

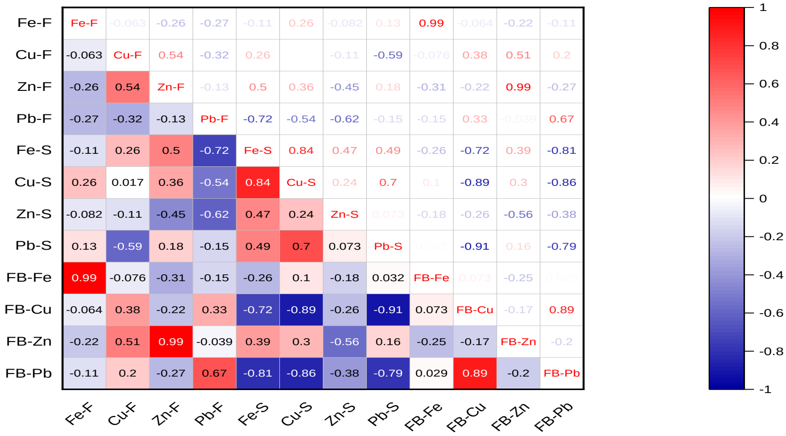



**Figure 5.** Correlation between the concentration of heavy metals in the leaves of the species analyzed and the soil (the numerical value corresponds to Pearson's correlation coefficient and the intense blue and red colors correspond to values close to +1 and -1 with high correlation and light colors low correlation). The nomenclature on the left means the coded variables (Fe-F (Foliar Iron), Cu-F (Foliar Copper), Zn-F (Foliar Zinc), Pb-F (Foliar Lead), (Fe-S (Soil Iron), Cu-S (Soil Copper), Zn-S (Soil Zinc), Pb-S (Soil Lead)).

## Cluster Analysis

The cluster analysis grouped all the species studied into three groups according to the concentration of heavy metals (Fe, Zn, Cu and Pb) in the leaves (Fig. 6). The concentration of Fe and Zn in sheets of *A. diversifolia, C. odorata, T. cacao* and *G. angustifolia* formed a group, while the concentrations of Fe in the species of *T. cacao, M. longipes, C. lechleri* formed the second group and finally the third group was made up of the concentrations of Cu, Pb, Zn in all the species analyzed. These groupings allowed the species to be classified according to their ability to accumulate different heavy metals, facilitating their selection as phytoremedial species in lands contaminated by mining. How can be observed Fe has similar concentrations in the plants studied, perhaps because this element is common in acidic soils with pH values < 5, and generally the Amazon region has pH values below 6 (Díaz, 2018).

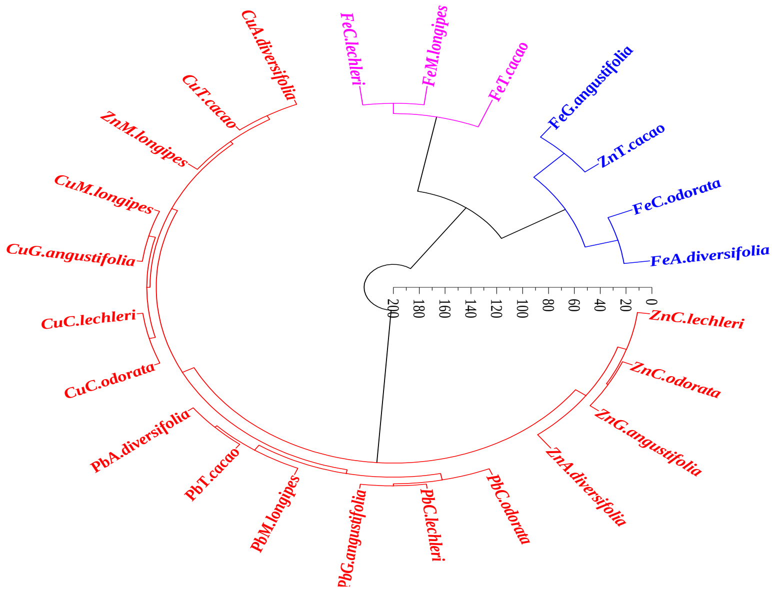



**Figure 6.** Cluster analysis based on the concentration (mg/kg) of heavy metals (Fe, Zn, Cu, Pb) in leaves of the species *T. cacao*, *A. diversifolia*, *M. longipes, C. odorata*, *C. lechleri*, and *G. angustifolia*. In original language English

**Principal Component Analysis (PCA)**

According to the eigenvalues of the heavy metal correlation matrix investigated and using the principal component analysis (PCA) method (Fig. 7), the initial set of data was simplified to two principal components that explain 62.97 % of the total variability of the data. According to the PCA analysis, it reported the concentrations of Zn, Cu and Pb in foliars lower than the Fe of PC1.

The coordinate system defined by the principal components allows us to determine that the species *A. diversifolia,* which is located on the positive side of the axis, is separated on the PC2 axis, with lower values of Zn and Cu concentration in the soil and with a higher concentration of Zn in the plant, while the species *T. cacao* it was found on the negative side of the axis, and was characterized by higher concentrations of Fe in the soil, however, the species *G. angustifolia* presented a higher ratio of Pb in the soil on the positive axis. Therefore, there is a variability in the absorption of metals depending on the species, in this case we can deduce that the species *A. diversifolia, T. cacao* and *G. angustifolia* absorb Zn, Fe and Pb better respectively. However, Akintola and Bodede (2019) found that *C. odorata* is an effective species for accumulating and distributing heavy metals such as Cu, Pb, Zn, Cd and Co. in its tissues.

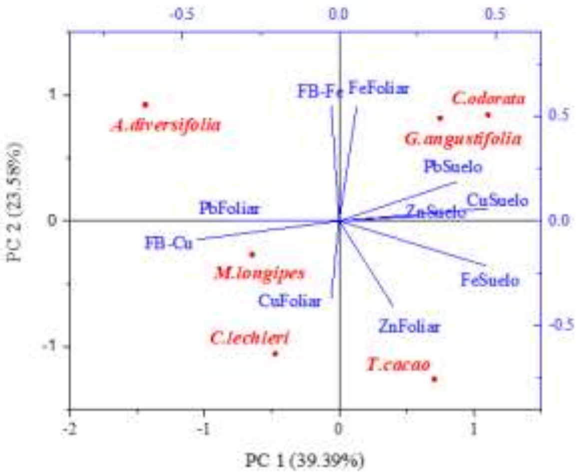



**Figure 7.** Results of the PCA analysis of heavy metal concentration (Fe, Zn, Cu, Pb) in leaf (foliar) and soil samples of the species *T. cacao*, *A. diversifolia*, *M. longipes, C. odorata*, *C. lechleri*, and *G. angustifolia*. In original language English

## CONCLUSIONS

The analysis of leaf material from six forest species that inhabit mining areas in the Amazon region of Ecuador, as well as soil samples where each species grows, allowed us to identify that plants such as grit (*A. diversifolia*), cocoa (*T. cacao*) and cedar (*C. odorata*) have bioaccumulatory potential mainly of Fe, Zn and Cu. which indicates that they have a high capacity for bioaccumulation at the leaf level and can be used as phytoremedial species in areas contaminated with heavy metals.

The species *A. diversifolia* and *T. cacao* exhibited a greater capacity for absorption of heavy metals such as Fe and Zn respectively, with significant differences between them and the other species studied. However, *C. odorata* obtained the highest values in terms of the concentration of Fe, Zn, Cu and Pb in the soil where this species lives, possibly due to the physiology of the plant, the absorption capacity of these elements under pollution stress and the acidic pH of the soil.

There is no strong correlation between the elements of the soil and the leaves of the species analyzed, which indicates that the bioconcentration capacity of the plant species determines the amount of absorption. These findings serve as a first attempt to identify potentially bioaccumulator species based on the pollutant in mining areas.

## THANKS

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