**The bioaccumulative potential of heavy metals in five forest species living in mining environments in the Ecuadorian Amazon region**

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**Abstract**

Pollution caused by heavy metals in soils and ecosystems is an environmental problematic that requires urgent attention due to the ecological problems that it generates. Forest species can be used to mitigate contamination because of their potential to bioaccumulate contaminating metals. Hence, the aim of this research was to identify tree species with good heavy metal bioaccumulating capacities that can contribute to mitigate pollution. The bioconcentration factor for five forest species, such as: Spanish cedar (*Cedrela odorata* L.), cutanga (*Parkia multijuga* Benth.), guaba or ice cream bean (*Inga edulis* Mart.), guarumo (*Cecropia ficifolia* Warb. ex Snethl.) and Amazon tree grape (*Pourouma cecropiifolia* Mart.), commonly found in the Ecuadorian Amazon was analysed, based on the relationship between the leaves and soil concentration of the heavy metals. For heavy metal analysis in leaves and soil samples of each plant species, atomic absorption spectrometry was used. The results showed that *P. cecropiifolia* had the highest bioconcentration factor for lead, *C. odorata* for cadmium and nickel, and *I. edulis* had the highest potential for iron and aluminium absorption. Any kind of correlation between the concentration of each element in soil and leaves was found, which shows that the bioaccumulation capacity of the species studied does not determine the concentration of metals in the soil.

**Keywords**: bioconcentration factor, Ecuadorian Amazon, heavy metals, identification of potential speciesfigure

1. **INTRODUCTION**

Heavy metals are considered toxic elements and one of the world’s largest ecological problems, affecting human health both directly and indirectly (Kulkarni *et al.*, 2018; Latif *et al.*, 2018; Lazo *et al.*, 2019; Palansooriya *et al.*, 2020; Tóth *et al.*, 2016). They are usual nature constituents and it is possible to find them in various environmental components (Garrett, 2000). Among these pollutants, chromium (Cr), copper (Cu), zinc (Zn), nickel (Ni), cadmium (Cd), lead (Pb), and mercury (Hg) are of special concern due to their persistence in the environment, such as its toxicity and possible risk of bioaccumulation (Kołodyńska *et al.*, 2017; Li *et al.*, 2018; Szymanowska *et al.*, 1999). These elements can be together with suspended environmental powder and this way could be carried throw several kilometres by airflow, to settle again as dry or wet deposition (Janhäll, 2015; Lazo *et al.*, 2019).

Recently, heavy metals have been analysed in various natural ecosystems (Tyler, 2005) in both slightly and highly polluted areas, such as mining (Serbula *et al.*, 2013), agricultural (Latif *et al.*, 2018; Ratul *et al.*, 2018) or urban, where heavy metal content originates mainly from various anthropic events (Hu *et al.*, 2014; Ugolini *et al.*, 2013). These human activities could elevate metal content above their normal levels and origin toxic damages in living organisms (Neumann, 2000). Although some heavy metals such as zinc, copper, selenium, are also essential for the normal plant grow (Wuana & Okieimen, 2011); nevertheless, they could be extremely toxic, depending on their specific chemical form or concentration level (Latif *et al.*, 2018; Rehman *et al.*, 2018). They have a tendency to be bioaccumulated in several organisms, rendering them extremely hazardous (Nagajyoti *et al.*, 2010).

Plants are extremely important because they act as an air filter and they release oxygen, regulating that way temperature of the air, thus accumulating some toxic chemical species (Sawidis *et al.*, 2012). Additionally, some authors have been stated that roots and leaves could gather important amounts of heavy metals (Liang *et al.*, 2017; Ugolini *et al.*, 2013), so, they can be environmental pollution markers. Trees can absorb both essential and non-essential metals, and the uptake of heavy metals by both roots and leaves increases when the concentration of heavy metals increases in the external environment (Ugolini *et al.*, 2013), i.e. soil or air. The gathering of metals in plants may lead to conclude that such elements could be present in surrounded soil or air, but in many of then the heavy metals amounts can be several times higher in leaves than in soil (Sawidis *et al.*, 2012). The toxicity of metallic elements in plants hang on several factors, such as: the nature of plant, sort of metal or physical or chemical properties of specific metal chemical specie, concentration level or edaphic characteristics (Nagajyoti *et al.*, 2010; Pajević *et al.*, 2016; Wuana & Okieimen, 2011). Therefore, the leaves of specific tree species have been used as biomonitors of heavy metal pollution in several studies (Çelik *et al.*, 2005; Hu *et al.*, 2014; Monfared *et al.*, 2013; Ŝućur *et al.*, 2010; Suzuki *et al.*, 2009; Tomašević *et al.*, 2004; Tóth *et al.*, 2016). However, metal concentrations in tree organs are not a sufficient indicator of tree contamination, as uptake depends on the plant species and its bioconcentration factors, i.e. the ability of a plant to accumulate heavy metals from soil and air (Alahabadi *et al.*, 2017).

In the Ecuadorian Amazon, given the proximity of volcanoes, soils may have high levels of heavy metals (Mainville *et al.*, 2006). In addition, mining operations are a major cause of environmental contamination, due to inadequate mining waste treatment. Heavy metals are effortlessly released into the environment, posing a potential risk to human health. Small-scale gold mining is one the main causes of metallic elements releasing into the ecosystems, leading to severe pollution (Nakazawa *et al.*, 2016; Xiao *et al.*, 2017). This has been widely reported in developing countries, where inefficient or non-existent environmental regulations exacerbate the problem (Tarras-Wahlber *et al.*, 2000). This fact is particularly worrying in Ecuador as illegal and uncontrolled mining has produced serious environmental pollution, mainly in terms of discharges of potentially toxic elements (Carling *et al.*, 2013; Peña-Carpio & Menéndez-Aguado, 2016). These are not the only causes of heavy metal pollution; the population growth is also to blame in that it has led to the diversification of economic activity (Pimm *et al.*, 2014), the intensive use of toxic agrochemicals (Panday *et al.*, 2015) and so on. In view of the above, this research aimed to identify tree species with good bioaccumulate capacities of heavy metals that can mitigate pollution.

1. **MATERIALS AND METHODS**

**2.1 Study Area**

This study was carried out in a mining area near the banks of the Jatunyacu River in the Yutsupino community, Puerto Napo parish, Napo province, Ecuador (Figure 1), where illegal gold mining activities are carried out.

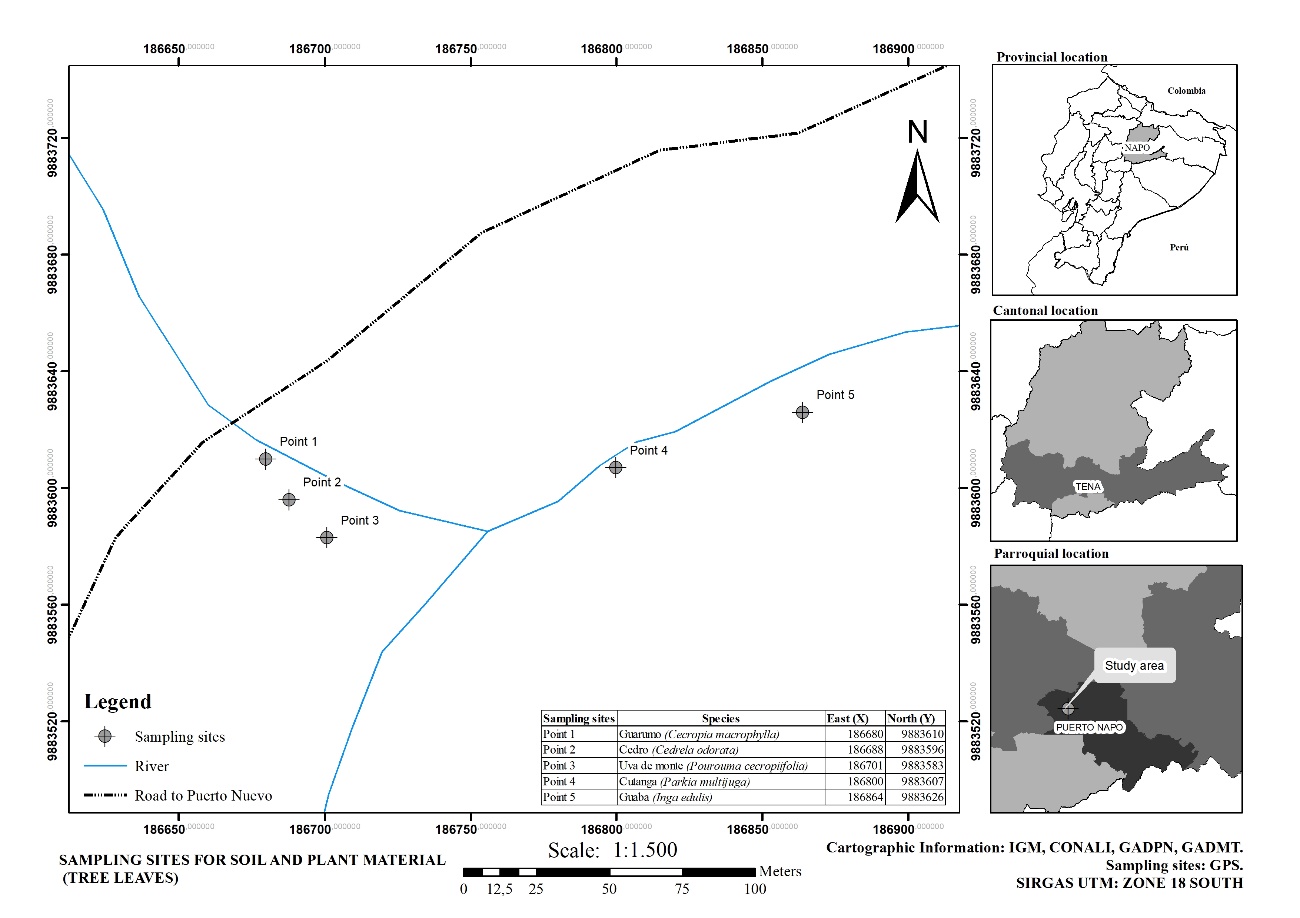


Figure.1 Sampling area for soil and plant material (tree leaves).

**2.2 Sampling of Plant Material and Soil**

For the analysis of heavy metals present in the plant material (leaves), five tree species living in an area close to the mining site were selected: Spanish cedar (*Cedrela odorata* L.), cutanga (*Parkia multijuga* Benth.), guava (*Inga edulis* Mart.), guarumo (*Cecropia ficifolia* and Amazon tree grape (*Pourouma cecropiifolia* Mart.) (http://www.theplantlist.org/). The plant material was collected from different sides of the trees, whereby the branches were cut to avoid the leaves coming into contact with the metal scissors. Leaves without mechanical damage or apparent disease were selected.

Soil samples were taken at three different points at a depth of 0-30cm within 30cm around the selected trees; leaf litter and roots were removed. The samples were labelled and taken to the laboratory for further processing to search for evidence of lead (Pb), cadmium (Cd), nickel (Ni), iron (Fe) and aluminium (Al) presence.

**2.3 Analysis of Soil and Leaf Samples**

The collected soil and plant material samples were air-dried at room temperature for seven days before being taken to the forced circulation oven at a temperature of 40°C for 48 hours. The dried samples were placed in a mortar and pestle to grind them to a completely fine material with particle sizes of less than 2mm (Greksa *et al.*, 2019).

A pre-labelled vial to process the digested soil and plant material samples was used. A mass of 0.5g of each sample was weighed, and then, 7mL of 65% nitric acid (HNO3), was added, followed by 1mL of 30% hydrogen peroxide (H2O2). The vial was closed and placed in a microwave-assisted digestion apparatus (ETHOS ONE) for 50 minutes. Once the digestion process was completed, the sample was allowed to stand at room temperature (Wang *et al.*, 2019).

Once digested, the samples were transferred to a previously labelled volumetric flask, to which distilled water was added for a final volume of 25mL. The samples were filtered prior to analysis and lastly, heavy metal determinations were performed on an Aurora Intruments LTD atomic absorption spectrometer with a data processer software Trace 1200, under the following conditions: flame type for the elements Pb, Cd, Ni and Fe, air-acetylene and nitrous oxide-acetylene for Al, with an airflow of 1.5Lmin-1 (air pressure 50psi), acetylene flow of 3.5Lmin-1 (acetylene pressure 50psi) and for hollow cathode lamp conditions, 20mA current, slit width 0.2nm and wavelengths of 217nm, 228.8nm, 232nm, 248.3nm and 309.2nm for Pb, Cd, Ni, Fe and Al, respectively.

**2.4 Data Analysis**

An analysis of variance (ANOVA) and Tukey’s test were performed, in order to determine the significance of the differences in heavy metals (Pb, Cd, Zn, Fe and Al) concentrations in the species analysed (*C. odorata, P. multijuga, I. edulis, C. ficifolia* and *P. cecropiifolia*) as well as the concentrations of metals mentioned above in the soil around the place where they grow. A cluster analysis was performed to define the similarity between the heavy metal uptake capacity of the species, and Pearson’s correlation coefficient was used to determine the correlations in the concentrations of the heavy metals analysed in the plants and the soil. A Principal Component Analysis (PCA) to establish the separation of the analysed plants according to the concentrations of heavy metals in soil and trees was done. Origin 2021 software was used for the statistical analyses.

The bioconcentration factor (BCF) was employed to measure each plant’s ability to absorb heavy metals from the soil (Alahabadi *et al.*, 2017). BCF was calculated using Equation (1):

|  |  |
| --- | --- |
|  | (1) |

where and represent the concentration of the element in the plant and in the soil, respectively.

1. **RESULTS**

**3.1 Concentrations of Heavy Metals in Leaves and Soil**

Figure 2a shows the results of the concentration of the heavy metals Pb, Cd, Ni, Fe and Al present in the leaves of *C. odorata*, *P. multijuga, I. edulis, C. ficifolia* and *P. cecropiifolia*, whilst Figure 2b shows the concentrations in the soils where those species grow. Foliar Pb concentrations ranged from 0.25mg kg-1 in *C. odorata* to 7.05mg kg-1 for *P. cecropiifolia*. Foliar Cd concentrations ranged from 0.08mg kg-1 for *P. cecropiifolia* to 0.98mg kg-1 in *C. odorata*. As for Ni, the lowest value was found in *P. multijuga* with a concentration of 1.80mg kg-1 and the highest in *C. ficifolia* with 6.93mg kg-1. Fe values were 69.03mg kg-1 in *C. ficifolia* and 99.02mg kg-1 for *I. edulis*. Al was 109.4mg kg-1 in *C. ficifolia* and 149.6mg kg-1 for *C. odorata*.

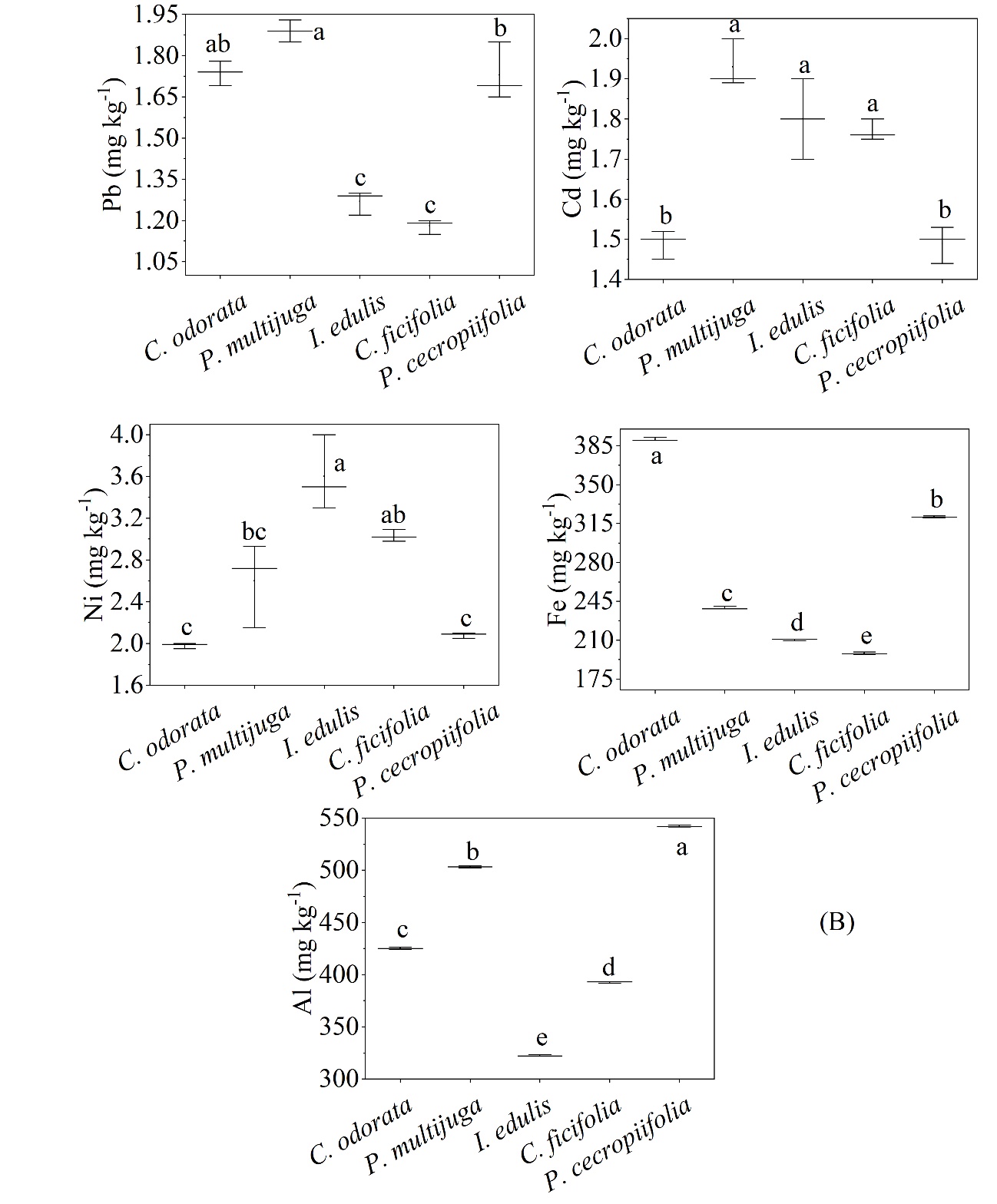
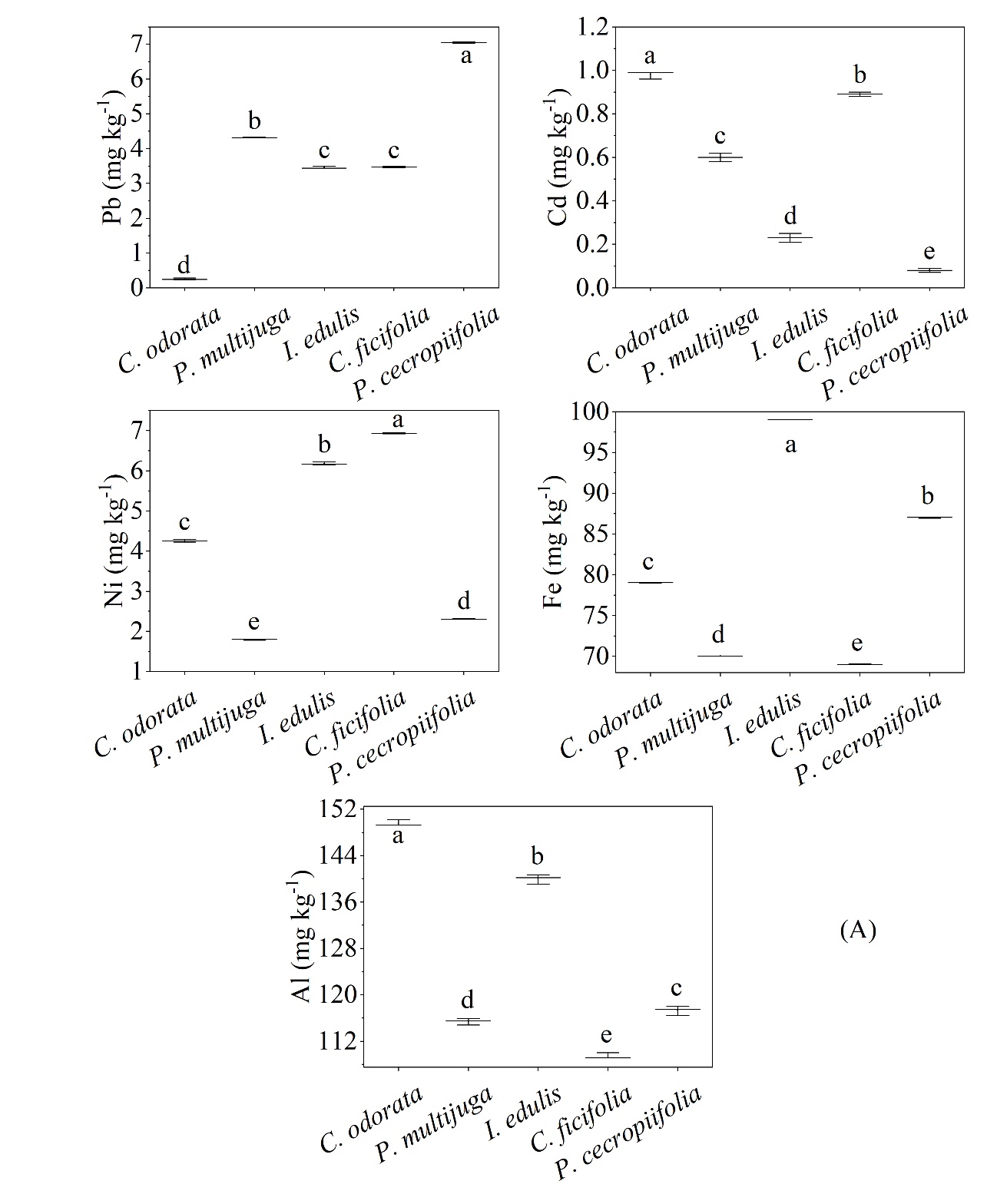


Figure 2. Significance of differences in heavy metal concentrations (Pb, Cd, Ni, Fe and Al) between analysed (A) tree leaves and (B) their concentration in soil (The values within the same heavy metal followed by the same letter do not differ significantly according to Tukey’s test).

Pb concentrations in soil ranged from 1.18mg kg-1 for *C. ficifolia* to 1.89mg kg-1 for *P. multijuga*. Cd values ranged from 1.49mg kg-1 for *C. odorata* and *P. cecropiifolia* to 1.89mg kg-1 in *P. multijuga*. Ni concentrations ranged from 1.98mg kg-1 around the place where *C. odorata* was located, to 3.60mg kg-1 where *I. edulis* was grown. Fe in the soil had a minimum concentration of 198mg kg-1 and a maximum concentration of 390.8mg kg-1, corresponding to *C. ficifolia* and *C. odorata* respectively. Al values ranged from 322.4mg kg-1 in the soil around *I. edulis* to 542.2mg kg-1 for *P. cecropiifolia*.

**3.2 Statistical Analysis**

The Analysis of variance (ANOVA) and Tukey’s test showed statistical differences in the concentration of the metallic elements Pb, Cd, Ni, Fe and Al in the leaves of the species studied, as well as in the soil where the species were grown (Figures 2A and 2B). For instance, *C. odorata* leaves had a significantly lower Pb concentration than *P. cecropiifolia* leaves. However, *C. odorata* had the highest Cd concentration, while *P. cecropiifolia* had the lowest Cd content. As for Ni, *P. multijuga* had the lowest concentration with significant differences to *C. ficifolia,* which was the species with the highest value. Yet *C. ficifolia* showed the lowest Fe and Al concentrations, whilst *I. edulis* and *C. odorata* had the highest Fe and Al values respectively (Fig. 2A).

In general, the soil where the species grow showed significant differences in the concentration of heavy metals (Fig. 2B). The lowest Pb concentration was in the areas of *I. edulis* and *C. ficifolia*, whereas the highest concentration was in the soil around *P. multijuga* and *C. odorata*. Cd concentration was similar in the locations of *C. odorata* and *P. cecropiifolia*. Indeed, the lowest and highest values of Cd were similar for the rest of the soil where the other species were found. Moreover, the presence of Ni in the soil had significant differences with lower values in soil surrounding *P. cecropiifolia* than in soil around *I. edulis*. As for the presence of Fe, where *C. odorata* is present, it had a higher content with significant differences to *C. ficifolia*. Al was higher in the soil around *P. cecropiifolia* and lower in *I. edulis* areas.

According to Pearson’s correlation coefficient (Fig. 3), when analysing the concentration of metals in the leaves of the species studied, only a positive correlation (higher than 50%) was found between Al and Fe (r = 0.54). Meanwhile, other elements showed negative correlations: Cd and Pb (r = -0.79), Fe and Cd (r = -0.71) and Al and Pb (r = -0.69). Positive correlations higher than 50% between soil element concentrations were between Ni and Cd (r = 0.70), Fe and Pb (r = 0.62) and Al and Pb (r = 0.80), whereas Ni presented negative correlations with negative Pb, Al and Fe elements (r = -0.76, r = -0.77, r = -0.87), respectively.

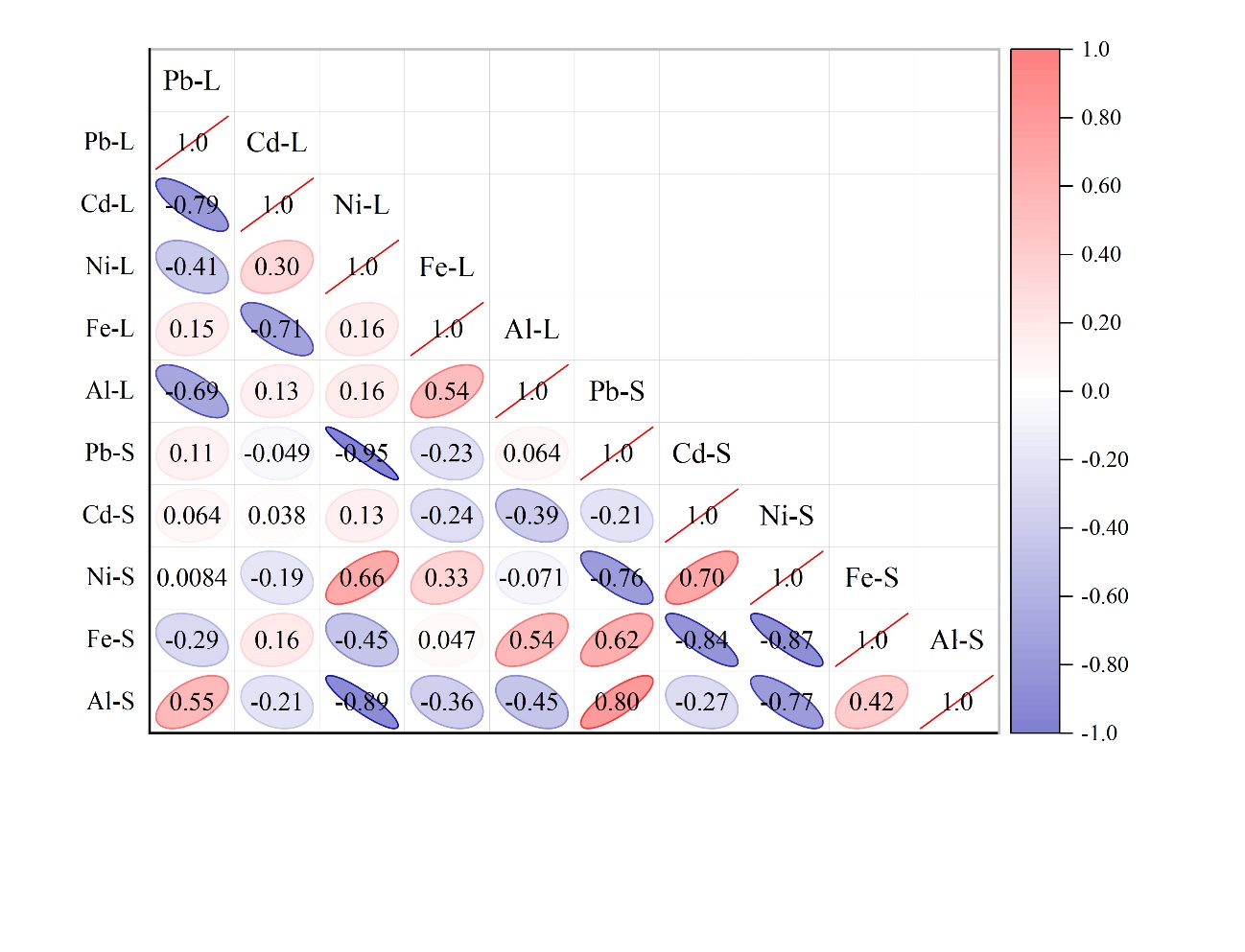


Figure 3. Correlation between the concentration of heavy metals in the leaves of the analysed species and the soil (The numerical value corresponds to Pearson’s correlation coefficient).

The cluster analysis grouped all studied species into three groups based on the concentrations of heavy metals (Pb, Cd, Ni, Fe and Al) in leaves (Figure 4). Al concentrations in leaves of *C. odorata* *and I. edulis* formed one group; Fe concentrations in *P. cecropiifolia, I. edulis, C. ficifolia, P. multijuga* and *C. odorata,* and Al concentrations in *P. cecropiifolia, C. ficifolia* and *P. multijuga* formed the second group; while the third group included Cd, Ni and Pb concentrations.

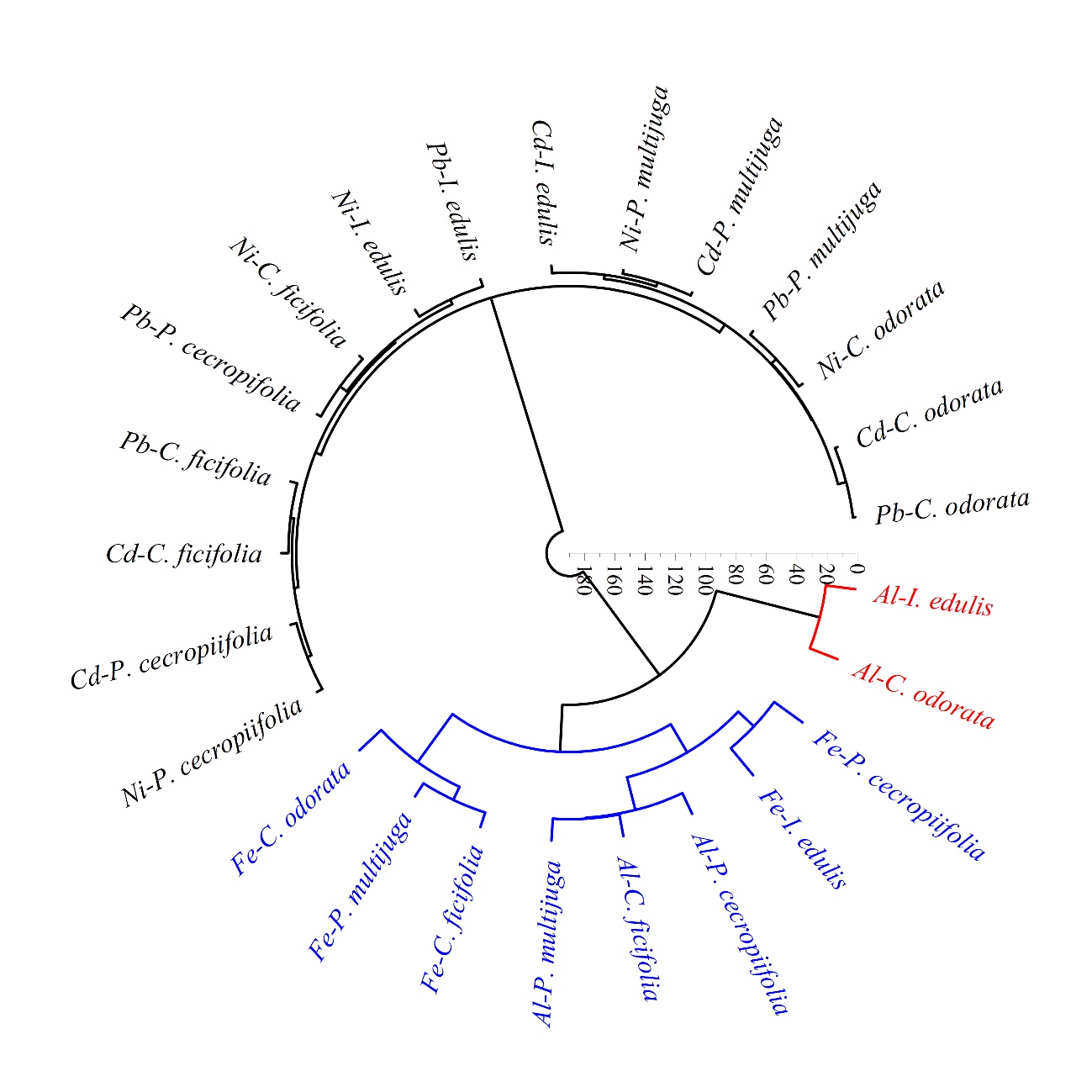


Figure 4. Cluster analysis based on the concentrations of heavy metals (Pb, Cd, Ni, Fe and Al) in leaves of the studied species.

The bioconcentration factor was used to estimate the plants’ ability to absorb certain heavy metals from the soil. The BFC values can be seen in Figure 5. The species analysed differ significantly in their capacity to absorb the heavy metals studied, with *P. cecropiifolia* having the greatest capacity to retain Pb, *C. odorata* accumulating the greatest amount of Cd, and *C. ficifolia* and *C. odorata* absorbing the most Ni. As for Fe and Al, *I. edulis* had the highest bioaccumulation potential for both elements.

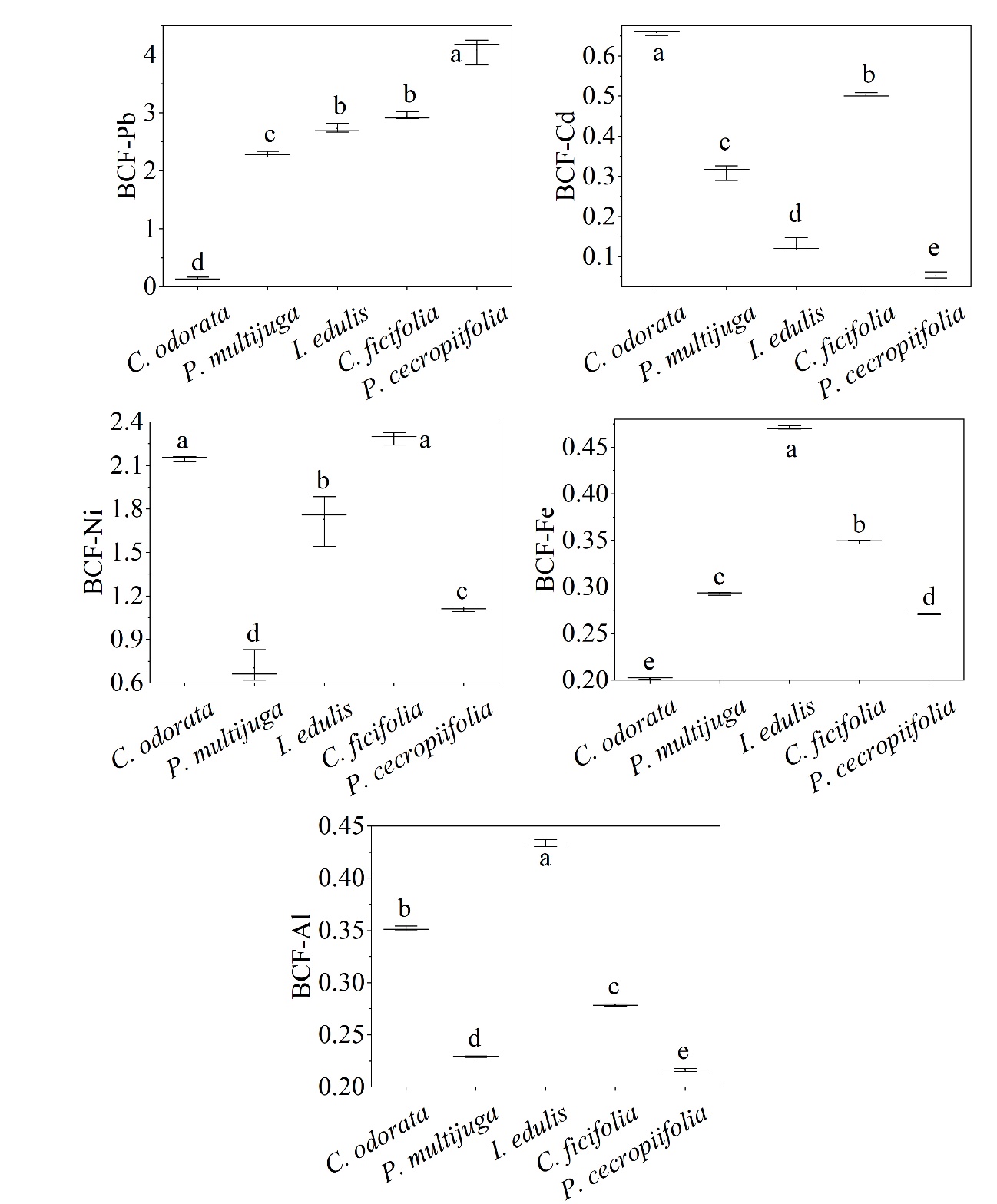


Figure 5. BCF results for the analysed tree species (The values within the same heavy metal followed by the same letter do not differ significantly according to Tukey’s test).

From the eigenvalues of the correlation matrix of the heavy metals studied and using the PCA method, the initial data set was reduced to two principal components that explained 70.6% of the variability (Figure 6). Based on the PCA analysis, Ni and Cd concentrations in the soil were lower than those of Fe, Pb and Al (PC 1).

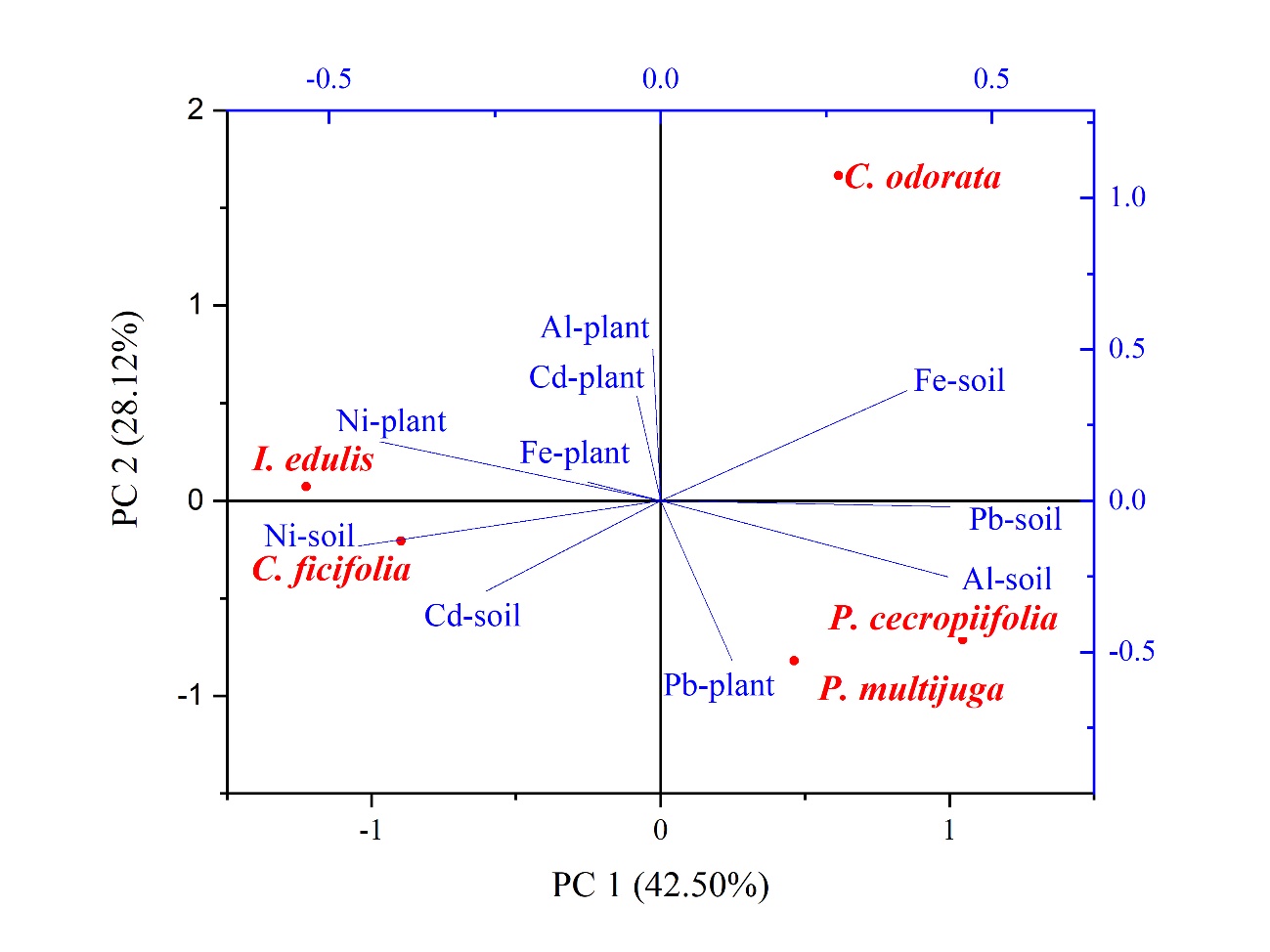


Figure 6. PCA analysis of concentrations of heavy metals (Pb, Cd, Ni, Fe and Al) in the leaves and soil of *C. odorata, P. multijuga, I. edulis, P. cecropiifolia* and *C. ficifolia*.

When looking at the plants in the coordinate system, determined by the principal components, it can be noted that the PC 1 axis separates the species *C. odorata, P. cecropiifolia* and *P. multijuga*, which are on the positive side of the axis and had lower values of Cd and Ni in the soil, from *I. edulis* and *C. ficifolia*, which are on the negative side of the axis and are characterised by higher concentrations of Cd and Ni in the soil. The second axis, PC 2, separates the species *C. odorata*, on the positive side of the axis and with a lower Pb concentration, from the rest of the species.

1. **DISCUSSION**

The leaf material of five forest species, *C. odorata, P. multijuga, I. edulis, C. ficifolia* and *P. cecropiifolia*, which commonly inhabit mining areas in the Ecuadorian Amazon region, along with samples of soil found close to each species under study were tested. The objective was to identify which species had the potential to bioaccumulate Pb, Cd, Ni, Fe and Al in order to mitigate possible contamination by any of these elements in Amazonian ecosystems or in soils of different uses, whether agricultural or forestry, in any region of the country where the species grow. A soil analysis between 0 and 30cm was used as an indicator of contamination, because according to some authors, the average maximum concentration is found in the soil layer within this depth range (Šichorová *et al.*, 2004; Wu *et al.*, 2013). Concentrations of heavy metals might be higher at a depth above 30cm, but their chemical forms differ with depth and therefore may not be readily available to plants (Wu *et al.*, 2013). According to Ecuadorian regulations (MAE-TULSMA, 2015), it was found that Cd concentration exceeded the permissible values (0.5mg kg-1), Pb and Ni were below the established limits, while Fe and Al are not regulated. It is important to highlight the bioaccumulate potential of the species, regardless of whether the soil can be considered contaminated or not. If the plants are able to absorb any of the elements considered as contaminants, then this could be a viable option for the bioremediation of contaminated soils.

Previous studies have documented the heavy metal biosorption potential of *C. odorata*. For example, Akintola and Bodede, (2019) studied seedlings grown in landfill soils and deduced that heavy metal concentrations in contaminated soils indicated metal enrichment in plant tissues. Enrichment coefficients and distribution factors showed the potential of *C. odorata* as a bioaccumulator species. Thus, they concluded that seedlings of this species can be used to clean up or rehabilitate soils that are contaminated with the heavy metals studied (Cu, Pb, Zn and Co).

The Hg bioaccumulator potential of *I. edulis* has also been reported, as it has been used for phytoremediation in a mining site contaminated with this heavy metal (Marrugo-Negrete *et al.*, 2016).

For the species *P. multijuga, C. ficifolia* and *P. cecropiifolia*, no studies have been reported on the absorption capacity of heavy metals, so this research reports for the first time the bioaccumulate potential of these species.

1. **CONCLUSION**

The bioconcentration factor made it possible to identify the studied species’ (*C. odorata, P. multijuga, I. edulis, P. cecropiifolia* and *C. ficifolia*) capacity to bioaccumulate the heavy metals Pb, Cd, Ni, Fe and Al, making it possible to propose reforestation in areas contaminated with these elements to mitigate the negative impacts they may cause on the ecosystem. For Pb-contaminated soils, *P. cecropiifolia* is recommended as it showed the highest CBF-Pb value. Furthermore, the species *C. odorata* can be used in Cd and Ni contaminated sites, while *I. edulis* showed the highest potential for Fe and Al uptake.

As no correlation was found between the concentration of the element in the soil and the leaves, it can be asserted that absorption depends on the bioconcentration capacity of the species. It is therefore suggested that more forest species inhabiting the Ecuadorian Amazon region could be studied as well as other heavy metals in order to broaden the identification of potential species, depending on the pollutant.

**COMPETING INTERESTS AND FUNDING**

**Author Contribution**

* Yudel Gracía-Quintana,Luis Ramón Bravo-Sánchez, Yasiel Arteaga-Crespo: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.
* Dixon Andi Grefa, Jorge de Jesús Guerrón-Torres, Jenny Elizabeth Rosero-Sanchez: Performed the experiments; Contributed reagents, materials, analysis tools or data.

All the authors read and approved the inal manuscript.

**FUNDING**

This work was supported by Universidad Estatal Amazónica. Ecuador.

**CONLICTS OF INTEREST**

The authors declare no competing interests.

**DATA AVAILABILITY STATEMENTS**

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

**ACKNOWLEDGEMENTS**

Thanks to the Universidad Estatal Amazónica for their financial support.

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