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Accumulation and microscopic alterations in the leaves of cedar (Juniperus deppeana) induced by heavy metals in mining residues in Guerrero

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Abstract

The concentrations of potentially toxic elements and microscopic changes in the tissues of *Juniperus deppeana* growing in soils contaminated with tailings were evaluated to assess its possible use in the restoration of areas affected by mining residues. Plants, soils, and tailings were collected from the historical mining area of Taxco, Mexico. High concentrations of Zn and Mn were measured in the leaves, whereas Pb, Zn, and Mn exhibited the highest accumulation levels in the roots. Transfer factors for Zn, Mn, and Fe in *J. deppeana* were consistently the highest overall. Optical and scanning electron microscopy (SEM) analyses revealed cellular alterations in the epidermis and palisade parenchyma, along with the presence of phenolic compounds that may be associated with oxidative stress. Mn, Zn, and Cu were observed in the spongy parenchyma, near the vascular bundle, and in some stomata. Pb and Fe were localized in the internal structure of small branches. This study highlights *J. deppeana* as a promising species for the reforestation of areas contaminated with mining residues, due to its tolerance to heavy metals. Besides, establishing this species as a pioneer in regions disturbed by human activities demonstrates its capacity to adapt to such environments.

1. Introduction

In Mexico, mineral exploitation began about twenty-five years after the end of the Spanish conquest, with the first mines established in Taxco, Zumpango, Temascaltepec, Tlalpujahua, Pachuca, and Guanajuato (Coll-Hurtado et al., 2001). The primary minerals extracted include copper, zinc, silver, lead, and gold (Contreras Díaz et al., 2000). Likewise, the mining industry has generated substantial amounts of mine waste or tailings for hundreds of years, leading to environmental contamination by potentially toxic elements (PTEs) such as arsenic, selenium, lead, cadmium, and antimony, among others. These pollutants adversely affect water, air, soil, and vegetation (Dudka and Adriano, 1997).

The physicochemical properties of mining waste vary significantly depending on the mineral source; however, they generally contain high concentrations of PTEs. Additionally, they exhibit low levels of essential nutrients such as phosphorus, nitrogen, and organic matter (Walder and Chavez, 1995; Boulet and Larocque, 1998; Yang et al., 2010; Alloway, 2012). The presence of PTEs leads to severe toxic effects on the environment (Ruiz Huerta and Armienta Hernández, 2012; Montes Rocha, 2016; Soltani et al., 2017; Gavilán-García et al., 2020).

The toxic effects of mine tailings on the environment are most evident in plants that grow in these contaminated areas. In response to the presence of PTEs, plants may develop various adaptive mechanisms (Juárez-Santillán et al., 2010; González-Sánchez et al., 2023). Some species exhibit tolerance to PTEs by employing strategies such as the production of root exudates to limit metal uptake or restricting their translocation from roots to aerial tissues through the formation of stable intracellular compounds. Other plants act as PTEs accumulators, sequestering large quantities of these elements and storing them in their aerial parts. Such plants can mitigate PTEs toxicity through different

mechanisms, including binding metals to the cell wall, compartmentalizing them in vacuoles, or forming stable complexes with organic acids or proteins (Reeves and Baker, 2000).

However, excessive concentrations of PTEs in the environment can lead to phytotoxicity, resulting in chlorosis, stunted plant growth, and reduced nutrient uptake. This toxicity can disrupt plant metabolism and, in the case of leguminous species, impair their ability to fix molecular nitrogen (Broos et al., 2005; Franche et al., 2009). PTEs accumulated in soils are taken up by plants (Siedlecka, 1995), translocated from the roots to various plant tissues (Marschner, 1995; Wang and Hu, 2023), and stored within different cells and compartments (Ernst, 2006; Verkleij and Schat, 2024). Understanding the compartmentalization and speciation of PTEs in phytoextractor trees is essential for optimizing phytoremediation strategies.

Phytoremediation processes are governed by specific criteria: (1) the plant's capacity to accumulate or tolerate PTEs. Plants with a translocation factor (TF) > 1 are considered accumulators, whereas those with a TF < 1 restrict PTE transport to their aerial parts, acting as excluders (Baker and Whiting, 2002). Additionally, successful phytoremediation requires (2) plants with high biomass production, (3) a high translocation factor, and (4) a high bioconcentration factor (Dowdy and McKone, 1997).

One of the defining features of the state of Guerrero is its production and extraction of metallic minerals, particularly in Taxco, where the first silver mining site was established following the arrival of the Spaniards (Servicio Geológico Mexicano, 2020). Taxco is situated in the northern part of the state and is renowned for its polymetallic deposits, characterized by sulfide mineralization, including stockworks and silver veins. In Taxco, Ag, Pb, and Zn deposits (from which these metals are extracted) are associated with hydrothermal activity linked to the magmatism of the Sierra Madre del Sur (Farfán-Panamá et al., 2015). Within the Taxco mining region, two notable study areas are present: the tailings pond “La Concha,” which contains reduced tailings, and “El Fraile,” which holds oxidized tailings.

As a result, a number of studies have been conducted to assess the hazardous nature and environmental impact of mine waste. Besides, few studies have focused on the accumulation of PTEs in plants in Mexico. Understanding which plants can function as accumulators and/or contribute to phytoremediation is crucial. Conifers such as *Juniperus deppeana* Steud. and *Cupressus lindleyi* Klotzsch ex Endl. are among the less-studied species, despite their ability to accumulate PTEs (Morton-Bermea et al., 2014; Osuna-Vallejo et al., 2019; Altonar-Gómez et al., 2021). Additionally, there are reports indicating that *J. deppeana* accumulates arsenic primarily in its stem, a rare characteristic, as plants that accumulate this metalloid typically retain it in the leaves (Puga et al., 2006). In other studies, the bark of *Pinus pinaster* Ait. has been used as a natural adsorbent for PTEs, particularly copper and lead, in contaminated soils (Barreiro, 2017).

Juniperus is one of the most diverse and representative genera of conifers, found from sea level to above the tree line. In the Americas, this genus is distributed across temperate regions, spanning from Canada to Guatemala. It comprises approximately 67 species and 28 varieties. *J. deppeana* var. *deppeana* is a

tree or shrub that ranges from 3 m to 20 m in height, making it one of the most prominent species within this genus (Rangel et al., 2018).

The primary objectives of this study were: i) to determine the concentrations of potentially toxic elements (PTEs) in the substrates and plants of *Juniperus deppeana* growing in soil contaminated with tailings and in the tailings; ii) to perform a physicochemical characterization of the substrates from the mine tailings at “La Concha” and “El Fraile,” comparing them to a control zone in Taxco, Guerrero; and iii) to identify changes or microscopic alterations in tissues caused by PTEs in *J. deppeana*. The ultimate goal is to generate knowledge that contributes to the future application of this species in the restoration of areas impacted by mining residues and other environmental disturbances.

2. Materials and methods

2.1. Study site

The study's sampling site is located in Taxco de Alarcón, Guerrero, Mexico (latitude 18.3° N, longitude 99.3° W). This area has an altitude ranging from 1,700 to 2,000 meters above sea level, with a mean annual temperature between 26°C and 30°C. Historical precipitation data indicate an annual average of 1,000 mm (Instituto Nacional de Estadística, Geografía e Informática, 1999). Plants were collected from two locations with PTEs contamination and one control zone, as follows:

1. **Tailing pond “La Concha”**: Located to the north of the San Antonio-“La Concha” mine. Its UTM coordinates are 14Q 432894 E and 2049996 N. The deposit has an irregular shape, with average dimensions of 140 m in length, 50 m in width, and an average thickness of 10 m (Bustamante-García et al., 2004). Two sample points were selected: “La Concha” Tailing (CT) on the plain, and “La Concha” soil contaminated with mine waste (CS) at the base.
2. **Tailing pond “El Fraile”**: Located in the village of the same name, with UTM coordinates of 14Q 433056 E and 2048294 N. The deposit has an irregular shape, with average dimensions of 340 m in length, 227 m in width, and an average thickness of 32 m (Bustamante-García et al., 2004). Three sample points were selected in “El Fraile” tailing (FT) at the base and in “El Fraile” soil contaminated with mine waste (FS) on the plain.
3. **Control Zone (CZ)**: Located in the Taxco Mountain, with UTM coordinates of 14Q 432919 E and 2047966 N, approximately 3 km southeast of the “El Fraile” tailings.

At each of these three locations, a 20 m² plot was defined. Soils were collected from each plot at a depth of 0–25 cm, with three soil samples collected per site (Fig. 1).

2.2. Plant sampling

Three and, if possible, four individual plants were collected from each sampled site. All plants were mature, appeared healthy, and showed no signs of parasitic presence. Species identification was performed at the Metropolitan Herbarium of UAM-I (Universidad Autónoma Metropolitana-Iztapalapa).

The leaves and roots were washed with tap water and rinsed with deionized water (18 MΩ·cm − 1, Milli-Q Millipore). These plant samples were collected from the top layer (0–30 cm), which consisted of a mixture of mining waste and soil. After collection, the samples were thoroughly washed in the laboratory using running tap water, followed by three rinses with deionized water (18 MΩ·cm − 1, Milli-Q Millipore) and a final rinse with tri-distilled water. All plant samples were carefully divided into leaves and roots and subsequently dried at 60°C for 75 hours. The oven-dried plant samples were then ground, sieved (< 325 μm), homogenized, and weighed.

2.3. Physicochemical parameters of soils and tailings

The substrate analyses were conducted according to the Mexican Official Standard NOM-021-SEMARNAT-2000. Soil pH was measured using a pH meter with a 2:1 water/soil ratio. Organic matter (OM) was determined following the Walkley and Black method (Walkley and Black, 1934). Total nitrogen (N_t) was measured using the Kjeldahl method (Bremner, 2018). Phosphorus (P) levels were determined by the Olsen Bray method. Cation exchange capacity (CEC) was evaluated using 1.0 N ammonium acetate at pH 7.0, followed by centrifugation. Carbonates ($CaCO_{3t}$) were analyzed using the Horton-Newson method. Soil texture was determined by the Bouyoucos hydrometer method. The electrical conductivity of the saturated extract (Bower and Wilcox, 2016) was measured after preparing a saturated paste with distilled water. Calcium (Ca) was quantified using atomic absorption spectrophotometry in the paste extract. Sodium (Na) and potassium (K) were assessed by flame emission spectrophotometry in the paste extract. Carbonates (CO_3) and bicarbonates (HCO_3) were measured by sulfuric acid (H_2SO_4) volumetry in the paste extract. Chloride (Cl) was determined using silver nitrate ($AgNO_3$) volumetry in the paste extract, and sulfates (SO_4) were analyzed by barium chloride ($BaCl_2$) turbidimetry in the paste extract.

2.4. Analysis of plants, soil, and tailings

Total PTEs (Pb, Zn, Cu, Fe, and Mn) concentrations in soil and plant samples were directly measured using energy-dispersive X-ray fluorescence spectrometry with a NITON XL3t (Thermo Fisher Scientific), following the EPA 6200 method (Sackett and Martin, 1998). X-ray spectra were analyzed using the Niton Data Transfer software suite. The spectrometer was calibrated for PTEs using certified standards from the National Institute of Standards and Technology (NIST), specifically Montana soil 2586 for soils. Intermediate and high PTEs concentration standards, traceable to NIST, were prepared in-house to create a broad calibration curve. The concentrations of heavy metals in the samples were measured in triplicate. This technique has been approved by the U.S. Environmental Protection Agency for measuring arsenic in dry solid samples (Melamed, 2004). PTEs concentrations were subsequently determined using energy-dispersive X-ray fluorescence. Calibration of the spectrometer for PTEs was conducted with certified standards from NIST, including CRM101 for soils and certified peach leaf standard for plants (SRM1547).

2.5. Translocation factor

PTEs concentration measurements were used to estimate the translocation factor (TF), which is defined as the ratio of PTEs concentration in shoots ([HM] shoots) to the concentration in roots ([HM] roots) (Dinu et al., 2020).

2.6. Microscopical analysis

Microscopic analyses were limited to the leaves to observe potential anatomical effects in the aerial organs. Although analyses of other plant organs would also be valuable, they fall outside the scope of the present study. We focused on the squamiform leaves because they are a part of the plants that are shed after a while and the accumulation in the leaves was investigated. Samples of young *J. deppeana* squamiform leaves, for PTEs translocation analysis, were collected and immediately fixed in a paraformaldehyde/glutaraldehyde solution for at least 4–6 hours (Johansen, 1940). The samples were then washed with a phosphate buffer (6% sodium phosphate monobasic and dibasic). Fixed tissues were dehydrated using a series of ethanol solutions (30%, 50%, 70%, 85%, 96%, and 100%). Dehydrated tissues were cleared with two changes of xylene, infiltrated with two changes of paraffin, and embedded. Paraffin sections, 10 to 12 µm thick, were cut using a rotary microtome. For general observation under a light microscope (LM), safranin-fast green staining was employed using Carl Zeiss 10x to 40x objectives with diascopic light illumination. Micrographs were captured using a digital Moticam 2500 camera, interfaced with Motic Images Plus v.2.0 software.

Elemental mapping was performed on squamiform leaf samples, using dehydrated tissues as described in the previous paragraph. These samples were critical point dried (BAL-TEC CPD030) and coated with carbon (Denton Vacuum LLC Desk II). The material was then examined and photographed using a Jeol JXA-8900 SuperProbe, an electron probe microanalyzer (EPMA) equipped with both a wavelength dispersive X-ray spectrometer (WDS) and an energy dispersive X-ray spectrometer (EDS), operating at an accelerating voltage of 20 kV.

2.7. Statistical analysis

PTEs concentrations in soils and plants were evaluated by a correlation matrix, using data on physicochemical parameters in soils and PTEs concentrations in soils and plants (triplicate). Statistical significance was determined with $P < 0.05$ and $P < 0.01$ using Pearson's r coefficient. Principal component analysis was applied to the physicochemical parameters in soils, as well as to PTEs concentrations in soils (S), roots (Pr), and squamiform leaves (Ph). Statistical analyses were performed using SPSS V.17 software.

3. Results

3.1. Physicochemical characterization

According to the NOM-021-SEMARNAT-2000 (Table 1), samples from the tailing pile and soil contaminated with mining waste at "La Concha" and the control zone exhibited pH values within the moderately alkaline range (7.4–8.5). In the soil contaminated with mining waste at "El Fraile", the pH

ranged from neutral (6.6–7.3) to moderately alkaline. However, in the tailing pile, the pH ranged from strongly acidic (<5) to neutral.

3.2. Potentially toxic elements in the substrates

The concentrations of Pb in the soil contaminated with mining waste from "La Concha" ranged from 14,000 to 20,000 mg kg^{-1}, presenting the highest Pb value among the studied sites. In the tailings of "La Concha", the range was between 12,000 and 18,000 mg kg^{-1} (Table 2). In the soil contaminated with mining waste from "El Fraile", it was 3,700 mg kg^{-1}, and in the tailings, it ranged from 520 to 2,800 mg kg^{-1}. In the control zone, the range was 70-100 mg kg^{-1}. Pb showed a positive association with Zn ($r = 0.887$, $p < 0.01$) and Mn ($r = 0.691$, $p < 0.05$) (Table 3).

The concentrations of Zn at the "La Concha" site were as follows: In soil contaminated with mining waste, it ranged from 24,000 to 47,500 mg kg^{-1}, with the highest Zn value among all the studied sites, and in the tailings, it ranged from 25,000 to 30,000 mg kg^{-1}. In the soil contaminated with mining waste from "El Fraile", it was 1,000 mg kg^{-1}, and in the tailings, it ranged from 1,200 to 9,500 mg kg^{-1} (Table 2). In the control zone, the determined range was 290-360 mg kg^{-1}.

For Cu, concentrations in the soil contaminated with mining waste from "La Concha" ranged from 60 to 780 mg kg^{-1}, and in the tailings, they ranged from 575 to 78,000 mg kg^{-1}. At the "El Fraile" site, the soil contaminated with mining waste had a concentration of 102 mg kg^{-1}, and in the tailings, it ranged from 111 to 332 mg kg^{-1} (Table 2). In the control zone, the concentration range was from 50 to 575 mg kg^{-1} (Table 2).

The concentrations of Fe in the tailings of "La Concha" ranged from 380 to 87,500 mg kg^{-1}, and in the soil contaminated with mining waste, they ranged from 75,000 to 97,500 mg kg^{-1}. At the "El Fraile" site, the soil contaminated with mining waste had values of 62,500 mg kg^{-1}, and in the tailings, the range was from 45,000 to 55,000 mg kg^{-1}. In the control zone, the range was from 30,000 to 45,000 mg kg^{-1}.

The Mn content in the substrates (Table 2) ranges from 3,100 to 3,600 mg kg^{-1} for soil contaminated with mining waste from "La Concha" and from 5,000 to 8,250 mg kg^{-1} in the tailings. At the "El Fraile" site, the tailings ranged from 310 to 3,450 mg kg^{-1}, and the soil contaminated with mining waste had 280 mg kg^{-1}. In the control zone, the range was 260 to 340 mg kg^{-1}.

3.3. Analysis of PTEs in the leaves of the selected species through X-ray fluorescence (XRF)

In the case of Zn, the highest concentrations were found in leaves of *J. deppeana* (32-469 mg kg^{-1}) in the soil contaminated with mine waste of “El Fraile”, while in the control zone, a high concentration of Fe in leaves of *J. deppeana* was found to be 341 mg kg^{-1}. Finally, for Mn, the highest concentrations were found in the leaves of *J. deppeana* that grew up in the tailings of "La Concha" (309 mg kg^{-1}), exceeding the phytotoxicity threshold (300 mg kg^{-1} of Mn) proposed by Kabata-Pendias (2000). The lowest Mn values were reported for the leaves of *J. deppeana* that grew up tailings of "El Fraile" (12 mg kg^{-1}), which were lower in the leaves of *J. deppeana* of the control zone (35 mg kg^{-1}) (Table 4).

For Zn, the highest values were found in leaves of *J. deppeana* (469 mg kg^{-1}) in the tailings of "La Concha", surpassing the normal concentration range of 20 to 400 mg kg^{-1}, according to Reeves and Baker (2000). However, for *J. deppeana*, the concentration in the leaves was high in the control zone with 88 mg kg^{-1} compared to 32 mg kg^{-1} of the soil contaminated with mining waste of the tailing pond "El Fraile".

The concentration of Cu in the leaves was within normal ranges (5-25 mg kg^{-1}) for tolerant plants growing in metal-contaminated soils (Reeves and Baker, 2000) (Table 4).

J. deppeana accumulated the highest amounts of Pb in roots, with concentrations of 81 mg kg^{-1}, in the tailing pond "La Concha", while for Zn, Cu, Fe, and Mn (363.18, 30.53, 235.26, and 141.73, respectively) in the soil contaminated with mining waste in the tailing pond "El Fraile" (Table 5). When compared with the normal ranges in plants in Pb (0.1-5 mg kg^{-1}) and Cu (5-25 mg kg^{-1}) were high. Without exceeding the phytotoxic threshold in Mn in all sites (300 mg kg^{-1}).

3.4. Transfer factor

J. deppeana recorded the following transfer factors .06 (control zone), .30 (tailing in “El Fraile”), .19 (tailing in “El Fraile”), .44 (tailing in “La Concha”), and .46 (tailing in “El Fraile”) for Pb, Zn, Cu, Fe, and Mn, respectively (Table 6). In all cases, the transfer factor was less than 1, indicating an exclusion strategy through immobilization of PTEs in the roots.

3.5. Statistical analysis

In the correlation matrix of physicochemical parameters in the substrates, heavy metals in the substrates and plants (leaves and root) at the study sites show a high correlation for PbS with ZnS (.944), CuS (.979), and Cl^- (.953); ZnS with CuS (.985) and Na (.920); CuS with Na (.892) and Cl^- (.895); MnS with $CaCO_3T$ (.991), Cl^- (.957), ZnPh (.917), and MnPh (.932); pH with EC (-.985), Ca (-.967), $SO_{=4}$ (-.989), and CuPr (-.955); OM with Nt (1), P (.996), CEC (.907), and HCO_{-3} (.934); Nt with P (.997), CEC (.913), and HCO_{-3} (.941); CaCO3T with Cl^- (.978) and MnPh (.891); CEC with HCO_{-3} (.898); EC with Ca (.996), $SO_{=4}$ (.986), and CuPr (.983) and finally, Ca with $SO_{=4}$ (.974) and CuPr (.981) (Table 3).

The results of the initial eigenvalues indicate that three principal components were considered and grouped physicochemical parameters in substrates, PTEs content in the substrates, and plants (leaves and roots) into a three-component model accounting for 94.37% of the total variance (Table 7). All elements are consequently well represented by these three principal components.

3.6. Microscopical analysis

In the leaves of *J. deppeana* from the plain of the "La Concha" tailings, in a panoramic view (Figure 3A and B), some areas of the spongy parenchyma were observed without cellular content and with structural alterations. Additionally, regarding zinc accumulation, the following observations were made:

(1) Zn distribution in the leaves overlapped with necrotic spots, (2) Zn accumulation on portions of the abaxial side of the leaves resulted in necrosis and collapse (Figure 3B), along with chemically altered cell walls, and (3) excessive Zn storage within the mesophyll chloroplasts. An irregular cuticle with foreign particles was observed, as evidenced by staining, showing irregularities (Figure 3C). The epidermis and hypodermis are unstratified, with visible cellular content in both (Figure 3C). The palisade parenchyma exhibited an apparently unchanged cellular structure, containing starch grains that were not highly conspicuous, as well as the presence of phenolic compounds in low abundance (Figure 3B and C). The spongy parenchyma appeared loose, with no visible cellular alterations (Figure 3C).

In the leaves of *J. deppeana* growing from the base of the "La Concha" tailings, the spongy parenchyma appeared loose, with a loss of cellular content, which was more pronounced near the vascular bundle (Figure 3D and E). This vascular bundle showed compaction along its edges (Figure 3D), which was characterized by the presence of particles on the cuticle, as well as thickening of the epidermis (Figure 3F). The hypodermis exhibited alterations in the cell wall, resulting in cellular compaction (Figure 3F). The palisade parenchyma contained a few starch grains, which were not present in all cells, and tannins were observed in a generally loose composition (Figure 3F).

In the leaves of *J. deppeana* from the plain of the "El Fraile" tailings in a panoramic view (Figure 3G), some areas of the spongy parenchyma were observed without cellular content and with structural alterations exhibiting granules or aggregations on the cuticle, which were observed in both paradermal and transverse sections (figure not shown). The resin canal exhibited slight compaction, with surrounding cells containing phenolic compounds (Figure 3H). The vascular bundle contained some cells with phenolic content (Figure 3H). The epidermis showed thickening of the cell wall and alterations in cellular content (Figure 3H and I). The hypodermis displayed structural and cellular content alterations, with the presence of lamellar bodies (Figure 3I). The palisade parenchyma appeared loosely arranged, with a low presence of starch grains, and lacked continuity in the transverse sections (Figure 3I).

At the base of the "El Fraile" tailings, the leaves of *J. deppeana* exhibited a uniform cuticle without the presence of foreign particles (Figure 3J). The resin canal showed cellular compaction along its contour, marked by the presence of phenolic compounds (Figure 3K). The palisade parenchyma contained both cellular content and phenolic compounds (Figures 3J, K, and L). The spongy parenchyma appeared loose, with evidence of cellular content in some cells (Figure 3J, K, and L), and in some cases, annular tracheids were observed.

In the leaves of *J. deppeana* from the control zone, the cuticle exhibited some granules (Figure 3M and O). The vascular bundles contained nuclei and annular tracheids (Figure 3N). The palisade parenchyma exhibited clear cellular content and well-defined cell walls, characterized by a low phenolic content. In some cases, large nuclei were observed (Figure 3M and O). The stomata contained nuclei and cellular content (Figure 3O). The spongy parenchyma appeared loosely arranged, with cellular and ergastic content, and showed no signs of cellular alteration (Figure 3M and O). The epidermis displayed cellular content with no evidence of wall thickening (Figure 3M and O).

The analysis of *J. deppeana* leaves from the plain of the "El Fraile" tailings revealed the localization of Mn, Zn, and Cu in the spongy parenchyma and near the vascular bundle, as well as in some stomata (Figure 4B, C, and D). Only Cu was not found outside the internal structure of the leaf. Fe was primarily detected in the epidermis and in particles adhered to the leaf cuticle (Figure 4E). Pb was in the palisade parenchyma and in particles adhered to the leaf surface (Figure 4F).

In the analysis of *J. deppeana* leaves from the control zone, no specific points of PTEs concentration are observed. Figure 5B shows a high concentration of Mn in the yellow-marked areas, primarily located at the periphery of the stomata. Zn, being an essential element for plant development, exhibits a broader distribution than Mn (Figure 5C). Cu does not accumulate significantly in superficial tissues, as shown in Figure 5D. Pb has a wider distribution than the other PTEs, with higher concentrations found in the stomatal cavities (Figure 5E). Fe is more concentrated on the periphery of the stomata, highlighting its importance in plant metabolic pathways (Figure 5F).

4. Discussion

4.1. Physicochemical characterization

The revegetation in the tailing of "El Fraile" could explain the increase in soil pH. Similar results were reported by Pérez-de-Mora et al. (2007) and Yang et al. (2010), who found that vegetation cover increased the soil pH. Plant roots can alter the pH of the local soil through various rhizospheric processes, such as assimilation, production of anions and cations, and the release of organic acids (Nye, 1981; Jones et al., 2004). The optimal pH range for plant development is from 6 to 7.5, although plants can tolerate some alkalinity, up to 8.5. Therefore, these pH conditions may not be a limiting factor for plant establishment in the tailings.

Root exudates can alter the redox state of the rhizosphere, resulting in less oxidized conditions, thereby inhibiting the oxidation of sulfide minerals and resulting in a significant decrease in acid-forming capacity (Adriano et al., 2004; Pérez-de-Mora et al., 2006). However, the residues exposed by the "La Concha" tailings are more susceptible to wind erosion, which can lead to groundwater contamination (Méndez and Armienta, 2003). Changes in pH affect the formation of various chemical species, such as hydroxide and carbonate complexes (Petrović et al., 1999).

The EC values were relatively high in the "El Fraile" tailings, ranging from 2.29 to 2.61 dS/m, which, according to the standard, falls within the moderate saline category. However, in the other areas (Control Zone, "La Concha" tailings, and soil contaminated with mining waste from "El Fraile"), the values ranged from 0.26 to 0.7, which are considered negligible in terms of salinity effects and unlikely to restrict vegetation establishment (Shu et al., 2005). Both pH and EC are regarded as the most critical factors in determining plant colonization in soils contaminated by mining activities (Conesa et al., 2006).

The OM content in the "La Concha" tailings was found to be very low (< 0.5%), while in the base, it ranged from medium (1.6–3.5%) to very high (> 6%). At the "El Fraile" site, OM levels were high in soil

contaminated with mining waste (3.6–6.0%), whereas in the tailings, the levels varied from very low to high (0.6–1.5%). In the control zone, very high OM levels were observed (6.72–17.17%) (Table 1). OM levels in mining residues are typically below 3.4% (Li, 2006; Rotkittikhun et al., 2007). Consequently, OM plays a crucial role in remediation processes, improving conditions in residues for plant colonization. Many heavy metal cations (Cu^{2+}, Cd^{2+}, and Zn^{2+}), are more stable in organic forms, particularly in humic substances (Spark et al., 1997). Another source of OM comes from plant and root residues surrounding the tailings, which can bind heavy metals and alter their availability, fostering environmental health and promoting ecological succession (Pierzynski et al., 2002).

The HCO_3^- content at the "La Concha" site indicates a typical calcareous substrate, whereas values at the "El Fraile" site are low, and those in the control zone are considered moderate according to the standard.

Regarding the Nt percentage (Table 1), the tailing of "La Concha" exhibited a very low level (< 0.05% Nt), while the soil contaminated with mining waste from the same site ranged from low (0.05–0.10%) to very high (> 0.25%). At the "El Fraile" site, the Nt percentages in the soil contaminated with mining waste were high (0.15–0.25%) to very high, while the tailing ranged from very low to low. In the control zone, very high percentages were observed.

The concentrations of P (Table 1) in the tailing of "La Concha" were moderate (5.5–11 mg/kg), while in the soil contaminated with mining waste from the same site, they ranged from low (< 5.5 mg/kg) to moderate. In the soil contaminated with mining waste at "El Fraile", P concentrations ranged from moderate to high (> 11 mg/kg). In the tailing of "El Fraile", the concentrations were low. In the control zone, values varied widely, ranging from low to high.

K levels were low (0.2–0.3 Cmol(+) kg^{-1}) in the tailing of "La Concha", while in the soil contaminated with mining waste from the same site, they ranged from low to high (> 0.6 Cmol(+) kg^{-1}). In the soil contaminated with mining waste at "El Fraile", K was present at high levels, while in the tailing, it varied from very low (< 0.2 Cmol(+) kg^{-1}) to high, a pattern also observed in the control zone (Table 1). For normal plant growth, N, P, and K are essential macronutrients, and these are often deficient in metalliferous soils (Chiu et al., 2006; Yeo and Flowers, 2007). The significant variation in these values may be attributed to the unique mineral composition of the site and the processes involved in extracting precious metals.

The soil texture showed uniformity across the study sites, with sandy loam being predominant, as per NOM-021-SEMARNAT-2000, with sand content exceeding 50%. This is likely since in arid climates, soil tends to be coarser, resulting in lower plasticity. Texture plays a key role in the behavior of PTEs in soils (Estévez et al., 1998). Fine tailings are particularly prone to compaction and cementation (Bradshaw et al., 1978), forming an impenetrable surface with poor aeration that is detrimental to most plants. Similarly, the unfavorable physical structure and anaerobic conditions in the tailings could pose significant limitations for plant establishment. King et al. (2006) found that poor soil aeration greatly

reduces seedling survival in the early stages. However, in the case of this study, the favorable physical structure appears to support the establishment of vegetation.

The Ca content in the tailing of “La Concha” was very low (< 0.2 Cmol (+) Kg^{-1}), while in the soil contaminated with mining waste from the same site, it ranged from low to medium, as in the soil contaminated with mining waste of “El Fraile” and the control zone (Table 1). In contrast, the tailing of “El Fraile” showed a high Ca content (> 10 Cmol (+) Kg^{-1}). The presence of Ca suggests that magnesium is also present, as these two elements are the primary components of the cation exchange complex (Romero et al., 2007), whereas monovalent cations occur in smaller proportions (Monge et al., 1994). Both Ca and Mn support plant growth, although they play a lesser role compared to other elements.

The highest Na values were found at two locations: the first in the soil contaminated with mining waste of “La Concha” (0.96 meq L^{-1}), and the second in the tailing of “El Fraile” (0.85 meq L^{-1}). At the other sampling points, Na concentrations ranged from 0.17 to 0.39 meq L^{-1} (Table 1). High concentrations of salts can exert an osmotic effect on plants, reducing their ability to absorb water (Richards, 1982).

The CEC was low to medium in the tailings of "La Concha" and in the soil contaminated with mining waste from "El Fraile", but it was low in the tailings of "El Fraile". In the control zone, the values ranged from medium to high.

The CO_3^{2-} were only determined at two points: the first, with a value of 0.75 meq L^{-1}, was found in soil contaminated with mining waste from “El Fraile”. This presence can impact the amount of organic matter and humus due to its high percentage. As a result, the exchange of positive and negative ions, as well as the absorption of essential elements for plant development, will be affected. The elevated presence of carbonates blocks the absorption of certain nutrients essential for the plant, such as Fe, Mn, Zn, K, P, and Cu. Furthermore, we can conclude that a high percentage of carbonates will favor the rapid breakdown of organic matter in the tailings, depleting humus (a complex of macromolecules in a colloidal state consisting of proteins, sugars, organic acids, minerals, etc.), as it allows the absorption of H^+ cations and metallic cations like Ca^{2+}, Mg^{2+}, K^+, and Na^+, a function that is diminished in the sample. The second point, the tailings of “La Concha”, had a carbonate value of 0.15 meq L^{-1}, and carbonates were not detected at the other points (Table 1).

Regarding HCO_3^-, the control zone presented the highest values, ranging from 4.38 to 5.49 meq L^{-1}. In the soil contaminated with mining waste from "El Fraile", the range was 2.5 to 2.9 meq L^{-1}, and in the tailings, it ranged from 0.3 to 2.24 meq L^{-1}. In the tailings of "La Concha", the range was between 1.09 and 1.41 meq L^{-1}, and in the soil contaminated with mining waste, it oscillated between 1.53 and 2.13 meq L^{-1} (Table 1).

The Cl^- values ranged from 0.75 to 1.3 meq L^{-1} at almost all sampled points in the zones (Table 1). However, at the tailings of the "El Fraile" site, it was 6 meq L^{-1}. This has an impact on soil composition, as the presence of chlorides contributes to soil salinity.

The highest ranges in SO_4^{2-} measurements correspond to the tailings of "El Fraile", ranging from 12.05 to 18.11 meq L^{-1}, while in the soil contaminated with mining waste, the range was 1.11 to 4.03 meq L^{-1}. In the soil contaminated with mining waste from "La Concha", the range was 0.20 to 2.7 meq L^{-1}, and in the tailings, it ranged from 0.1 to 0.63 meq L^{-1}. In the control zone, the values ranged from 0.05 to 0.76 meq L^{-1} (Table 1). Sulfates may result from sulfide oxidation in the tailings or from the use of sulfate salts in ore processing.

4.2. Potentially toxic elements in the substrates

In mining waste from “La Concha” and “El Fraile” concentrations exceeded the phytotoxic levels proposed by Alloway (2012) for Pb (Table 2). However, they fall within the range of metalliferous soils proposed by Reeves and Baker (2000) (Table 2). In the control zone, the range was 70–100 mg kg^{-1}, while the normal range is 2-300 mg kg^{-1} (Alloway, 2012)

In the soil contaminated with mining waste from “La Concha” and "El Fraile", concentrations exceeded the phytotoxic levels proposed by Alloway (2012) for Zn (Table 2). However, both tailings fall within the range of metalliferous soils proposed by Reeves and Baker (2000) (Table 2). In the control zone, which falls within the normal range but is already considered phytotoxic according to Alloway (2012)

In the control zone, exceeding the phytotoxic range of 60–125 mg kg^{-1} for Cu proposed by Alloway (2012). Phytotoxic concentrations in the soil were exceeded at nearly all sampled sites, and for metalliferous soils, this was the case at only one point, corresponding to the tailings of "La Concha", with a value of 78,000 mg kg^{-1} (Table 2).

In the tailings of the “La Concha”, the concentrations were 1.6 times higher (8,250 mg kg^{-1}) than the range of phytotoxic levels (5,000 mg kg^{-1}) proposed by Alloway (2012) for Mn (Table 2). However, this condition did not limit plant growth.

In general, total concentrations of 5,000 mg kg^{-1} of Mn (Visser, 1993; Alloway, 2012), 60–125 mg kg^{-1} of Cu, and 70–400 mg kg^{-1} of Zn are considered phytotoxic (Kabata-Pendias, 2010). The "La Concha" site was the most contaminated, both in the tailings and in the soil contaminated with mining waste from "La Concha". The highest concentrations of heavy metals were found in the soil contaminated with mining waste from the "La Concha" tailings and in the tailings of "El Fraile". In the control zone, total concentrations of Cu and Zn were within these toxic ranges (Table 2), except for Mn, which was not close to the phytotoxic level. Therefore, native species showed high tolerance to PTEs, thriving in the vicinity of the control zone and mine waste.

Besides total concentrations, the mobility of PTEs is a crucial factor because it determines their bioavailability to plants and microorganisms, which take up elements dissolved in the soil solution (Kabata-Pendias, 2000).

4.3. Analysis of PTEs in the leaves of the selected species through X-ray fluorescence (XRF)

The study focused on the plant selected based on its abundance in the study sites, with *J. deppeana* being the most abundant, as it is present in most of the studied sites. Although the accumulation of PTEs in the leaves was low, their accumulation in the plants was observed (Table 4). In fact, PTEs in the soil-root interface tend to be mobilized by root exudates and microbial activity in the root zone (Remon et al., 2005). As a result, PTEs accumulation reaches a high level, with extractable PTEs grouping around the rhizosphere. A concentration of PTEs in plants above toxic levels implies that internal detoxification mechanisms for tolerance to these metals may exist in *J. deppeana*.

4.4. Transfer factor

Accumulation and exclusion are two basic tolerance strategies involved in plants to cope with high concentrations of PTEs in the environment (Baker, 1987, 1981; Taylor, 1987; Baker and Walker, 1990). PTEs-accumulating plants are characterized by a transfer factor > 1, while plants that exclude the transport of PTEs to the aboveground part are restricted with a transfer factor < 1 (Baker, 1981; Baker and Whiting, 2002). The magnitude of metal translocation to the aboveground part depends not only on plant species but also on the nature of the PTEs.

Furthermore, the accumulation of PTEs in plants may increase the risk of transfer to wild herbivores or livestock (Chaney, 1989; Madejón et al., 2002; Taggart et al., 2005). This contamination can be deposited in vegetation through airborne particles, occurring to a greater extent if the released particle is fine-sized, in areas without vegetation, and therefore physically unstable. The wind carries particles to the surface of plants, providing a significant exposure route for herbivores, such as the direct inhalation of PTEs (Ma and Nomoto, 1996). Khan et al. (2014) show that the uptake and bioaccumulation of these PTEs in plants pose severe health risks to humans, as over 90% of PTEs exposure occurs through the consumption of contaminated vegetables and crops. The remaining 10% stems from direct dermal contact or inhalation of contaminated dust particles.

The pioneering plants in this study are mostly exclusively annual or perennial herbaceous plants. This phenomenon has been previously reported in other plant communities naturally growing in PTE-contaminated sites (Pichtel et al., 2000; Tordoff et al., 2000; Li, 2006; Wang et al., 2008). Herbaceous species have been reported to exhibit a wide ecological spectrum due to their tolerance to PTEs, rapid growth, and high coverage, resulting from long periods of natural selection (Wong, 2003). Additionally, these species support much of the re-vegetation of mine wastes and can restore biogeochemical cycles in the biosphere system.

On the other hand, the diversity of candidate species around mine wastes is of great importance in ecological restoration, as seen in the case of *J. deppeana*. It is well known that plant diversity and niche have a significant influence on ecosystem function. Remediation systems with a greater number of species tend to be more stable and resistant to diseases and pests. Additionally, vegetation cover with a greater number of species facilitates succession, and the annual variation in biomass is lower in such ecosystems (Martínez-Ruiz et al., 2007; Liu et al., 2015; Onésimo et al., 2021).

4.5. Statistical analysis

Principal component analysis (PCA) is a multivariate statistical technique that transforms a large set of correlated variables into a smaller number of new, uncorrelated variables known as principal components (PCs). These components are oriented along the directions of maximum variance in the dataset. PCA helps reduce data dimensionality (Sârbu and Pop, 2005) by identifying the most relevant parameters that describe the overall dataset and summarizing the statistical relationships among variables with minimal loss of the original information (Chandrasekaran et al., 2015; Helena et al., 2000). PCA has been widely used to explain variability in datasets derived from monitoring studies of rivers (Khan et al., 2016, 2017; Singh et al., 2004, 2005), soils (Chandrasekaran et al., 2015), and vegetation (Chudasama et al., 2024).

PCA can reveal relationships between elements in the substrate matrix and plant tissues, providing insights into the sources and translocation pathways of potentially toxic elements (PTEs) in the environment (Bhuiyan et al., 2010). In general, the relationships among metals are consistent with the results obtained from both PCA and the correlation matrix. The correlation matrix is particularly useful for confirming associations between heavy metals in substrates and plant tissues. Positive correlations between heavy metals may indicate that the paired elements in substrate samples share a common source of contamination. In contrast, negative or inverse correlations between heavy metals in substrates and plants suggest that the metal pairs may originate from different sources and are not directly associated in their translocation dynamics.

The PCA loading plots of the physicochemical parameters of the substrates, as well as the PTE contents in substrates and plant tissues (leaves and roots) from Taxco, are presented in Fig. 2, while the PCA results for the variables described above are summarized in Table 3.

4.6. Microscopical analysis

Most studies on the effects of PTEs have focused on the physiological, biochemical, and molecular responses of plants. However, there is limited understanding of the subcellular distribution of metals and the defense mechanisms activated by these PTEs.

The compartmentalization and complex formation of contaminant metals absorbed by plant roots, as well as their translocation through the sap flow to aerial parts, determine plant tolerance to PTEs (Marschner, 1995; Rauser, 1999; Ernst, 2006; Haydon and Cobbett, 2007). This process is regulated by the efficiency of PTEs phytoextraction by plants (Siedlecka, 1995). Therefore, understanding the distribution of these metals in aerial parts is crucial for comprehending the physiological responses that enable plants to thrive in their respective environments. In the leaves of *J. deppeana* from of the “La Concha” the distribution of Zn which may undergo degeneration and destructive processes (Hermle et al., 2007).

5. Conclusions

The physicochemical characteristics of the mine waste sites do not pose a limitation for plant development. However, high sulfate levels were found in "El Fraile," which may pose a risk due to acid drainage. The highest concentrations of Pb, Zn, Cu, Fe, and Mn were detected at the "La Concha" site, primarily in the tailings. A similar pattern was observed in the accumulation of Zn and Mn in the leaves of *J. deppeana* in the tailings of "La Concha." However, at the "El Fraile" site, the highest accumulation occurred in the roots of the plants in the site of tailings for “La Concha” and “El Fraile”, which was confirmed by the transfer factor for Zn, Cu, and Mn. The accumulation of these elements in the leaves was evident through optical microscopy, revealing the presence of PTEs within the tissues. X-ray mapping showed that the metals were mainly located near the vascular bundles, where metabolic activity is minimal. Pb exhibited a different distribution compared to other PTEs, as it was found in the stomatal cavities, suggesting its involvement in plant metabolic pathways. The implementation of *J. deppeana* is a promising alternative for phytoremediation, as it can withstand oxidative stress caused by PTEs without significantly altering its physiological state and demonstrating its capacity to adapt to such environments.

Declarations

Declarations

Data accessibility statement

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

Ethics statement

This work did not require ethical approval from a human subject or animal welfare committee.

Consent for Publication

Not applicable.

Consent to Participate

Not applicable.

Competing interests

The authors declare no competing interests.

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Tables

Tables are available in the Supplementary Files section.

Figures

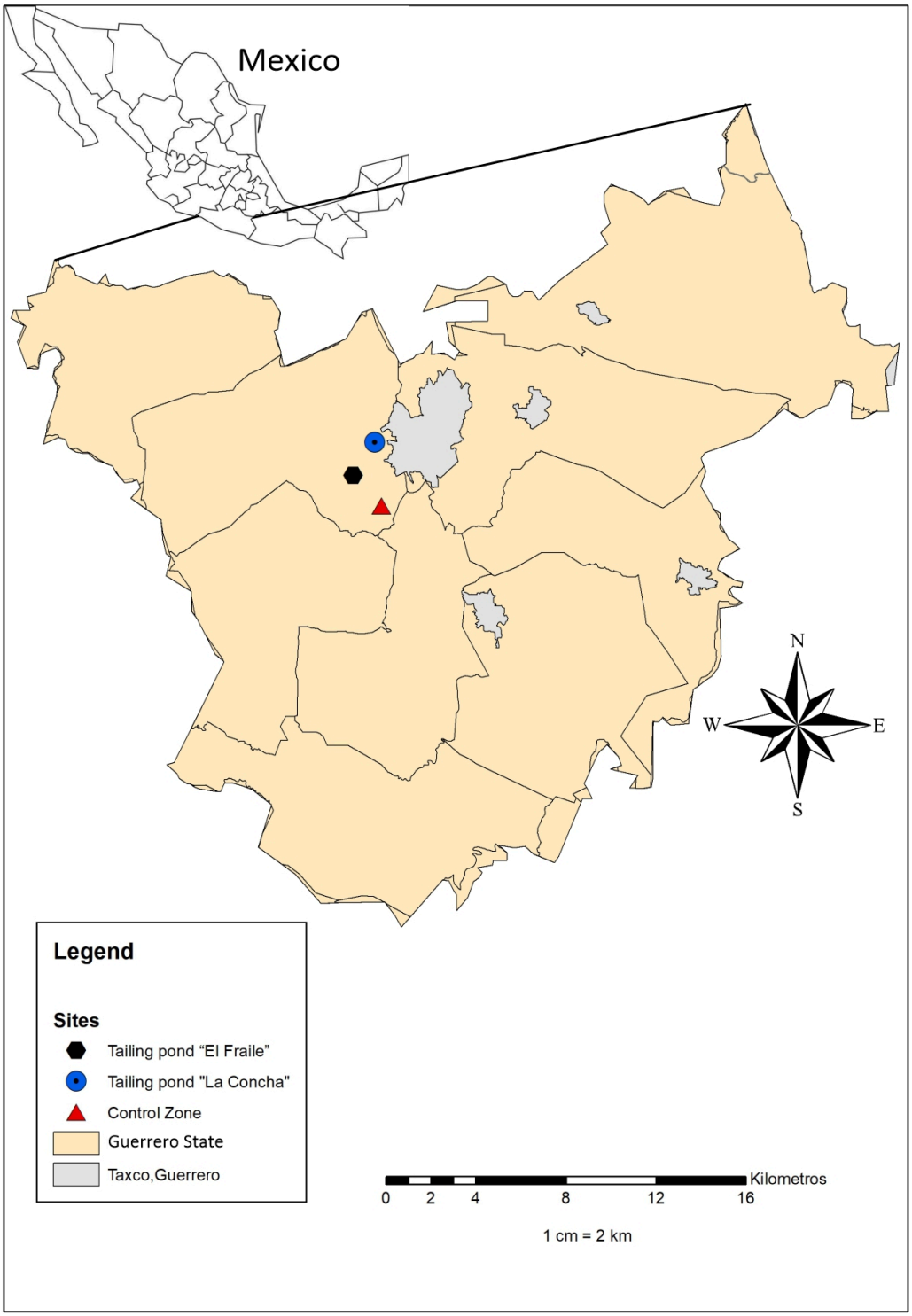


Figure 1

Distribution of sample points at “La Concha” and “El Fraile” tailings in Taxco, Guerrero.

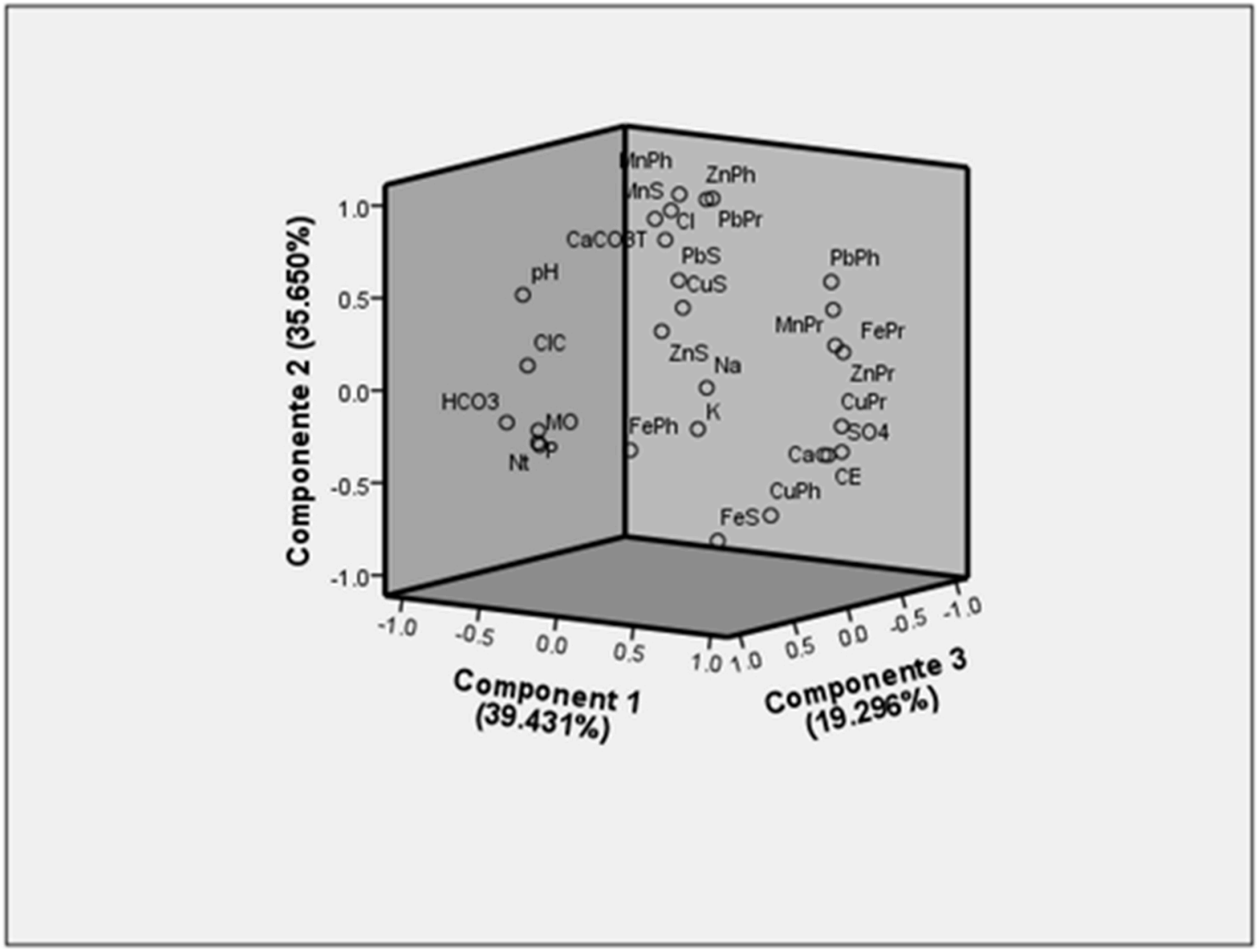


Figure 2

Principal Component Analysis of physicochemical parameters, heavy metals in sediments and in plants of *J. deppeana* in Taxco. Rotation method

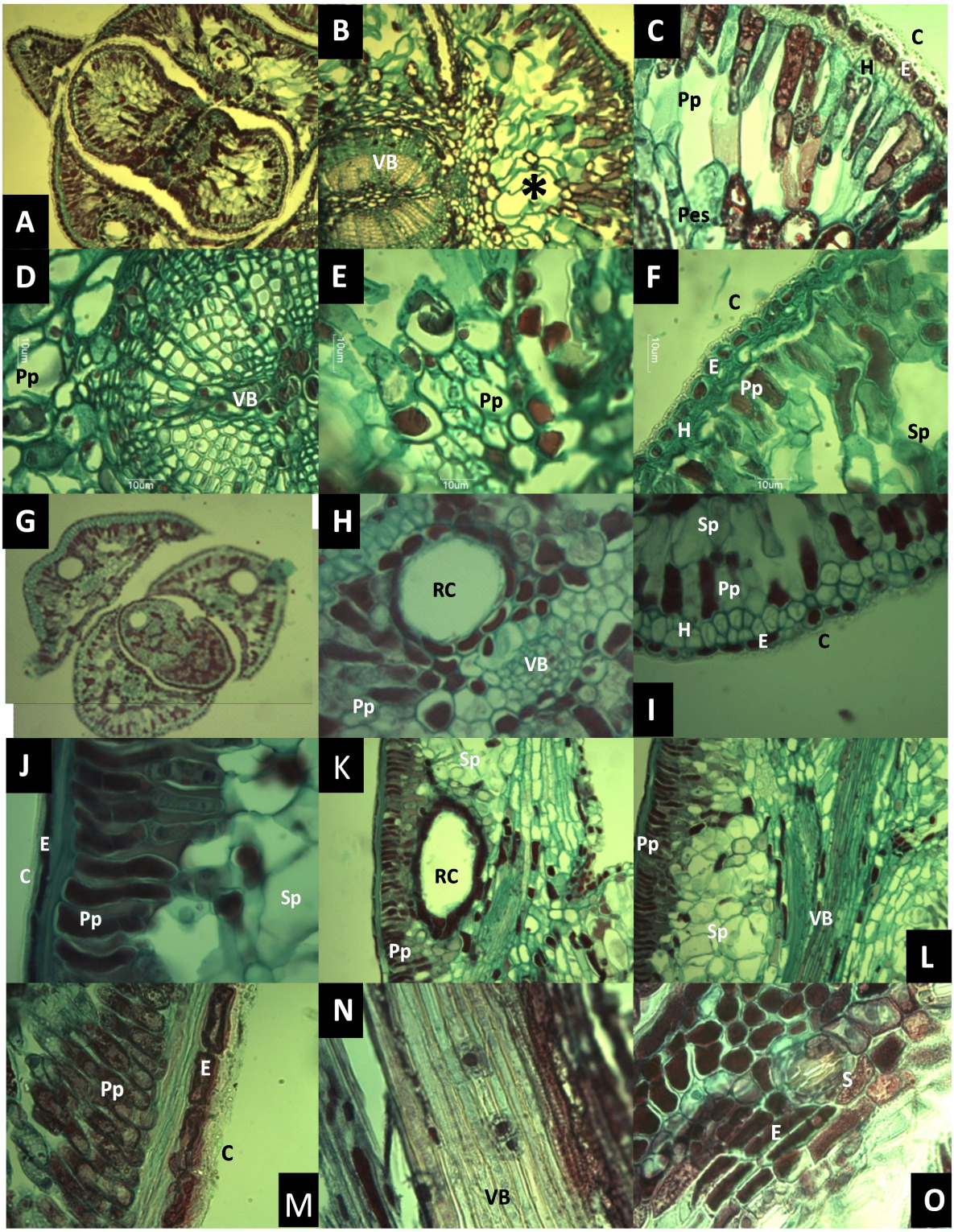


Figure 3

Leaf of *J. deppeana* from the "La Concha" Tailing (CT). (A) Panoramic view. (B) Cellular alterations in the spongy parenchyma and cellular compaction at the periphery of the vascular bundle. (C) Cuticle with a thick layer of cutin and impurities; epidermis and hypodermis without wall thickening. Site "La Concha" – Soil contaminated with mining waste (CS). (D) Some spongy parenchyma cells lacking cellular content. (E) Loss of cellular content in the spongy parenchyma near the leaf cleft. (F) Cuticle with cutin

aggregation, thickening of the epidermis, and cellular alterations in the hypodermis. Site "El Fraile" – Soil contaminated with mining waste (FS). (G) Panoramic cross-sectional view. (H) Vascular bundle surrounded by some cells containing phenolic compounds. (I) Presence of lamellar structures in the hypodermis and phenolic compound-containing cells in the palisade parenchyma. Site "El Fraile" Tailing (FT). (J) Paradermal section showing tannin accumulation and loosely arranged spongy parenchyma. (K) Cellular compaction in cells adjacent to the resin canal. (L) Vascular bundle with visible nuclei and loosely arranged spongy parenchyma. Control Zone. (M) Stoma surrounded by cells with cellular content. (N) Presence of nuclei in the vascular bundle. (O) Paradermal section showing stomata and epidermis with cellular content. Magnifications: A, G – 10X; B, C, D, E, F, H, J, K, L, M, N, O – 40X. Staining: safranin-fast green.

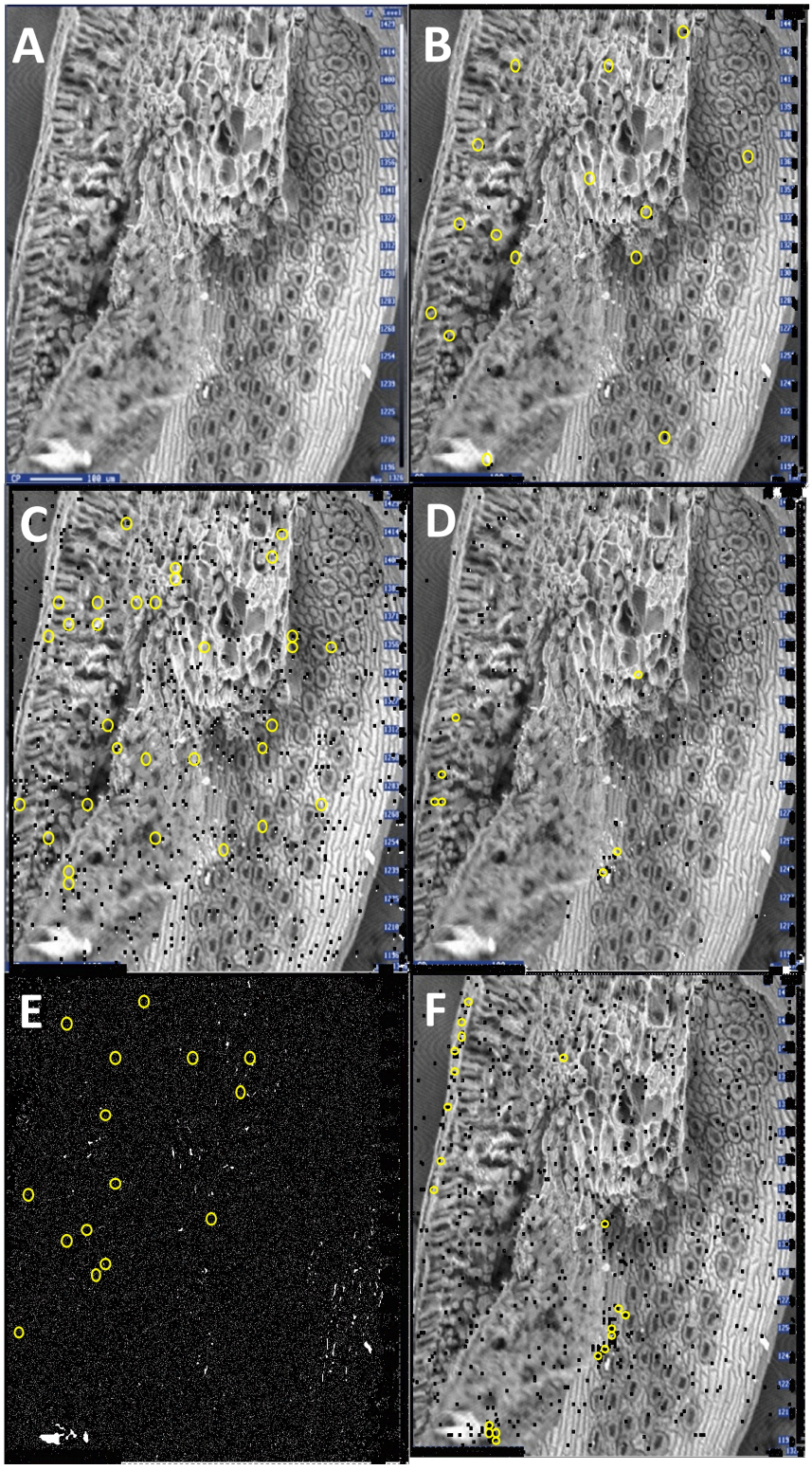


Figure 4

X-ray mapping showing the distribution of elements in the leaf of *J. deppeana* from the plain of “El Fraile”. A) Compositional image, B) Mn mapping, C) Zn mapping, D) Pb mapping, E) Cu mapping, and F) Fe mapping.

Supplementary Files

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