



# Ionic analysis of *Prosopis laevigata* response to heavy metals: phytoremediation potential determined by wavelength-dispersive X-ray fluorescence

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Received: 28 January 2023 / Revised: 23 June 2023 / Accepted: 16 October 2023 / Published online: 10 January 2024  
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## Abstract

*Prosopis laevigata* (mesquite) plants can tolerate drought, thermal stress, alkalinity, salinity, and toxicity of heavy metals, making them attractive for phytoremediation. Nonetheless, most of these features have been studied under controlled laboratory conditions. In this work, the bioaccumulation of heavy metals in a free-living population of *P. laevigata* trees growing in a heavily metal-contaminated site (aluminum, chromium, iron, titanium, copper, and zinc) was analyzed. Furthermore, crystal phases of mineral nutrients and trace elements found in *P. laevigata* tissues were determined by X-ray diffraction. *P. laevigata* trees accumulated 705 ( $\pm 17$ ), 47,064 ( $\pm 1459$ ), 14,800 ( $\pm 401$ ) and 30,000 ( $\pm 1719$ ) mg/kg of Cu, Zn, Fe and Al, confirming the potential of these plants to hyper-accumulate metals. The X-ray diffraction analysis showed that *P. laevigata* trees can chelate  $Al^{3+}$  with phosphates to form orthorhombic crystals of aluminum phosphate ( $AlPO_4$ ) in the tissues. This aluminum chelation was probably a mechanism of tolerance used by the plant. The inoculation of seedlings with the endophytic *Bacillus cereus* MH778713 did not prevent Cr-accumulation in the plant but increased metal tolerance and seedling development. These results highlight the use of *P. laevigata* and *B. cereus* MH778713 together as tools for heavy metal bioremediation, particularly on arid and semiarid soils.

**Keywords** Soil X-ray diffraction · WDXRF · *Prosopis* · Hyperaccumulator plants · Microbial inoculation

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Editorial responsibility: Maryam Shabani.

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## Introduction

The contamination of soils, air and water by heavy metals derived from anthropogenic activities, like mining, farming and industrial processes, is an increasing problem that threatens all living organisms. Heavy metal pollution problems arise when the heavy metals are mobilized into the soil and then absorbed by the plants or transported to the groundwater (Wuana and Okieimen 2011). The interaction of heavy metals with cations like calcium, magnesium, and potassium present in soils determines the grade of toxicity of these metals (Palansooriya et al. 2020). The content of organic matter in soils also plays an important role in blocking the toxic action of heavy metals (Stefanowicz et al. 2020). The increasing concentration of toxic elements, in the environment, produces physiological and genetic changes in the fauna and flora of the ecosystem and, in the long-term, human health risks (Singh et al. 2016).

The term phytoremediation refers to a technology based on the use of plants, naturally or genetically modified, or microorganism-plant associations, for the remediation of



polluted environments. Phytoremediation is clean, simple, cost-effective and not environmentally disruptive (Tiodar et al. 2021). On the other hand, bioremediation can be used to remove pollutants such as synthetic organic compounds, xenobiotics, heavy metals and organic hydrocarbons from soils and water (Jaiswal and Shukla 2020).

Semiarid regions are described with an aridity index (ratio of total annual precipitation to potential evapotranspiration) between 0.20 and 0.50, soils of these regions are characterized by a poor structure, low content of organic matter and reduced water retention (Garcia-Franco et al. 2018). *Prosopis* genus contains more than 45 species of spiny trees and shrubs widely distributed in arid and semiarid regions of America, Africa, and Asia, able to produce large amounts of biomass ( $14.5 \text{ T ha}^{-1} \text{ yr}^{-1}$ ) under water deficit conditions (Felker et al. 1983). Plants belonging to the genus *Prosopis* can tolerate alkalinity, high temperatures, salinity, and drought stress, besides fixing nitrogen (associated with nitrogen-fixing bacteria) and adding fertility to soils.

Experiments of *Prosopis* plants under laboratory conditions have shown tolerance and hyper-accumulation of lead (Aldrich et al. 2004; Buendia-Gonzalez et al. 2019), chromium (Aldrich et al. 2003), arsenic (Aldrich et al. 2007; Mokgalaka-Matlala et al. 2008), copper (Zappala et al. 2014) and zinc (Khan et al. 2015). The mechanisms by which *Prosopis* plants can do this remain unclear. Aldrich et al. (2003, 2007) reported that *Prosopis* can actively uptake and reduce Cr (VI) to Cr (III) or As (V) to As (III). Endophyte *Bacillus* of *Prosopis laevigata* isolated from root nodules improved plant tolerance and seed dormancy breakage under chromium-stress experiments (Ramírez et al. 2019, 2020). However, little is known about the capability of native *P. laevigata* trees to accumulate and tolerate heavy metals under wildlife conditions.

The mineral nutrient composition of a living organism is defined as ionome (Salt et al. 2008). Mineral uptake in plants is regulated by both genetic and environmental factors, thus plants can control ionomic homeostasis by regulating the uptake, transport and accumulation (Zhang et al. 2021). The development of high-throughput chemistry analysis has made it possible to evaluate the plant ionome in response to different environmental conditions or plant species (Watanabe et al. 2016, 2021).

Wavelength-dispersive X-ray fluorescence (WDXRF) spectroscopy is a powerful tool used for elemental analysis applications. This technique allows directly analyzing solid samples with multi-element identification, performing qualitative and quantitative determinations. It is highly sensitive to heavy elements, with good resolution for light chemical elements (Marguá et al. 2007). X-ray diffraction (XRD), on the other hand, is a technique used for characterizing crystalline materials; it provides information on

structures, phases, crystal orientations, grain size, deformation, and crystal defects (Bunaciu et al. 2015). Therefore, both chemistry assessments can be useful for plant ionome analysis.

This work aimed to investigate the elemental composition of *P. laevigata* plants growing under free-living and laboratory conditions, both subjected to heavy metal stress, using high-throughput elemental analysis technologies (WDXRF spectrometry and X-ray diffraction). Ionomic data showed changes in the metal content of *P. laevigata* tissues in adaptation to heavy metal stress, confirming the potential of mesquite plants for the phytoremediation of metal-contaminated areas.

## Materials and methods

### Site and sample collection

The site of the study was a free-living population of *Prosopis laevigata* trees in the deserted region of Chietla, Puebla, Mexico ( $18^{\circ}31'27.5'' \text{ N}$ ,  $98^{\circ}35'02.0'' \text{ W}$ ), which is a heavy metal-contaminated region due to mining activities. The region of Chietla is located at 1079 m above sea level. Tissues of twenty *P. laevigata* trees were collected, oven-dried at  $60^{\circ} \text{ C}$  for 72 h, ground in a mill, pooled together and divided into two composed samples for further analysis. Leaf samples were taken from the main branches at 2 m height; stem samples were taken at 1.2 m from the soil surface; root samples were taken at 50 cm depth. For each *P. laevigata* tree, a rhizospheric and superficial soil sample was taken at 20 cm deep and surface, for pH analysis. Furthermore, pods from adult trees were taken to the laboratory to obtain seeds for seedlings experiments under hydroponic conditions.

### Soil analysis and determination of pH

Each tree's rhizospheric and superficial soil samples were sieved and pooled together for metal soil composition and pH analysis. Thus, two composite rhizospheric soil samples or superficial soil samples were measured three times to get three pH values for each type of soil. One gram of composite soil sample, previously dried at  $50^{\circ} \text{ C}$  for 48 h, was weighed and transferred into a 100 mL beaker containing 10 mL of deionized water. The suspension was stirred well with a glass rod and allowed to stand for 24 h. The supernatant was used to measure pH in a potentiometer.

Two composite rhizospheric soil samples were used for metal composition analysis using wavelength-dispersive X-ray fluorescence (WDXRF) spectrometry, XRD (X-ray diffraction), and atomic absorption spectrometry.



## WDXRF (wavelength-dispersive X-ray fluorescence) spectrometry

### Soil metal composition

10 g of each composite rhizospheric soil sample was subjected to 500 °C for 48 h; then, samples were finely powdered and then press into a pellet using wax binders for WDXRF analysis (Ramírez et al. 2019). The quantitative analysis of the multi-elements was carried out using a Bruker S8-Tiger WDXRF analyzer equipped with a Rhodium (Rh) tube as an X-ray source, different collimators and filters, a flow proportional counter for light elements (Na to V), a scintillation counter for heavy elements (Cr to Pb), and a high-pressure goniometer for theta and 2 theta angles. Analyses were conducted according to ISO9516-1:2003 using the QUANT-EXPRESS method (fundamental parameters) which includes a unique multipurpose calibration method using certified standards (STG2). The total analysis time for each sample was 20 min with a sensitivity of 20 mg/kg for most elements. Each composite sample was analyzed three times in the WDXRF equipment.

### Tissues from free-living *P. laevigata* trees

Two composite samples of leaf, stem, and root were calcinated at 500 °C for 44 h. Ashes were sieved and combined with wax as a binder to prepare pellets to be used in WDXRF analysis (Ramírez et al. 2019). Each composite sample was analyzed three times in the WDXRF analyzer.

### Tissues from *P. laevigata* seedlings

Composite samples from twelve inoculated and uninoculated seedlings of *P. laevigata* growing over six months with chromium (25 mg/L), iron (2000 mg/L), zinc (3000 mg/L), or without metal supplementation in Jensen's medium were analyzed by WDXRF spectrometry. Composite samples were calcinated, and ashes were finely powdered and then press into a pellet for WDXRF analysis as described before. Each composite sample was analyzed three times in the WDXRF equipment.

### Bioaccumulation factor

Metal concentrations measured by WDXRF were used to calculate the bioaccumulation factor (BF).

$$BF = \frac{[\text{Average of element concentration in root, stem, and leaf}]}{[\text{Element concentration in rhizospheric soil}]}$$

## XRD (X-ray diffraction) assay

The WDXRF is a non-destructive technique used to measure elemental composition. Therefore, the same composite samples used for WDXRF analysis were reused for X-ray diffraction analysis to identify crystalline compounds in plant tissues and rhizospheric soils. The X-ray diffraction (XRD) assay was performed using a Bruker D8 Discover X-ray diffractometer operated at 40 kV and 40 mA applying the CuK $\alpha$ 1 radiation (1.5406 Å). The detector of scintillation is at the angle 2-theta close to zero (~ 4°), in a system connected to a radiation recording measurement as a function of the angle 2 thetas known as  $\lambda$ , the estimated resolution of the interplanar distances was greater than 0.02 Å. The XRD pattern of the sample was identified with the Powder Diffraction File PDF-4 + 2018 from the International Centre of Diffraction Data (ICDD). The input data for the Rietveld refinement (space group, cell parameters, and atomic positions) were taken from the database format PDF-4 + 2018. To model the background a polynomial approach was used, and a pseudo-Voigt function was selected for the profile form. After this, the shape parameters, zero shift, scale factor, unit cell parameters, U, W, and V profile coefficients were refined.

## Atomic absorption spectrometry

Two composite soil and tissue samples were oven-dried at 60 °C for 72 h, finely ground, sieved, weighed, and digested with nitric acid 65% (Suprapur, Merck, Germany). To do it, 0.5 g of soil or tissue composite sample was weighed into a microwave vial. Afterward, 10 ml nitric acid (65%) was added to the vial. Digestion was performed at 175 °C for 5 min in a microwave oven (CEM-MarsX, CEM corporation, Mathews, NC). Digested samples were brought to 60 ml by adding deionized water, then filtered with a Whatman paper (GE Healthcare, UK) for metal composition analysis. Cu, Zn, Fe, Cr, Ca, and K concentrations were measured by an atomic absorption spectrometer (Agilent, Spectra 55B, Australia). Three technical replicates for each composite sample were performed.

## Seedlings experiments: inoculated and uninoculated *P. laevigata* subjected to metal stress

Seeds of *P. laevigata* taken from the site of the study were scarified with concentrated sulfuric acid (98%) for 20 min

in constant agitation and then rinsed several times with deionized sterile water to remove the sulfuric acid excess. Scarified seeds were then placed on agar (1%) for 3 days in the dark for germination and transferred to bottles with Jensen's medium supplemented with 25 mg/L of chromium (VI); 2000 mg/L of iron, or 3000 mg/L of zinc. Controls were not supplemented with any metal. The composition of Jensen's medium was as follows (g/L): KNO<sub>3</sub> 0.5, K<sub>2</sub>HPO<sub>4</sub> 0.2, MgSO<sub>4</sub> 0.2, NaCl 0.2, CaHPO<sub>4</sub> 1.0, FeCl<sub>3</sub> 0.04. Jensen's medium was supplemented with 1 ml/L of trace metal solution, whose composition in g/L was: H<sub>3</sub>BO<sub>3</sub> 2.26, MnSO<sub>4</sub>\*4H<sub>2</sub>O 2.03, ZnSO<sub>4</sub>\*7H<sub>2</sub>O 0.22, CuSO<sub>4</sub>\*5H<sub>2</sub>O 0.08, Na<sub>2</sub>Mo<sub>4</sub>\*2H<sub>2</sub>O 0.09. Twelve plants for each treatment were incubated in a climate chamber at 20 °C with 16 h of light alternating with 8 h of darkness (Ramírez et al. 2019). For the inoculated seedlings assay, the procedure was similar but before transferring *P. laevigata* seedlings into the hydroponic conditions, *Bacillus cereus* MH778713 was inoculated into Jensen's medium at a final concentration of 1 × 10<sup>7</sup> CFU/ml. To do it, *B. cereus* MH778713 was grown overnight in YM liquid medium (yeast extract 1.0 g, mannitol 10 g, dipotassium phosphate 0.5 g, sodium chloride 0.1 g, glutamic acid 0.01 g per liter, pH 7.0), then centrifuged, washed and resuspended in sterile deionized water. The experiment was run for six months when true leaves emerged. Plant growth (fresh and dry weight) was evaluated for each treatment. After taking the measurements, seedlings were dried, cut into 1 cm fragments, crushed, and calcinated for WDXRF and XRD analysis.

## Statistical analysis

The data obtained from the dry and fresh weight of plants exposed to Zn, Fe and Cr were subjected to statistical analysis using one-way analysis of variance (ANOVA), and

significant differences between mean values were determined by the Tukey–Kramer Post Hoc tests or least significant difference using Microsoft 365 Excel for Windows. A significance level of  $p < 0.05$  was used to determine the significant differences in data.

## Results and discussion

The composite rhizospheric samples had 3.38% ( $\pm 0.33$ ) of organic matter, 2.01 ( $\pm 0.10$ ) dS/cm of soil conductivity, 1.52 ( $\pm 0.03$ ) g/ml of apparent density, and 11.81% ( $\pm 0.05$ ) of available nitrogen. Texture analysis of rhizospheric soil resulted in 56% ( $\pm 0.075$ ) sand, 24% ( $\pm 0.078$ ) loam, and 25% ( $\pm 0.09$ ) clay. The wavelength-dispersive X-ray fluorescence (WDXRF) spectrometry was used to determine the abundance of chemical elements in rhizospheric soil and plant organs of a *P. laevigata* population growing in a metal-contaminated zone. The results showed the ability of *P. laevigata* trees to bioaccumulate potassium (K), calcium (Ca), phosphate (P), magnesium (Mg), silicon (Si), sulfur (S), iron (Fe), strontium (Sr), sodium (Na), aluminum (Al), zinc (Zn), and copper (Cu) (Table 1).

Each value is the mean of two composed samples, and the error is presented in parentheses. N. D. means not detected

*P. laevigata* free-living trees of this study were naturally established in a hostile soil containing 53,100 ( $\pm 446$ ) mg Al/kg, 47,450 ( $\pm 199$ ) mg Fe/kg, 402 ( $\pm 96$ ) mg Cu/kg, 322 ( $\pm 46$ ) mg Cr/kg, 5100 ( $\pm 128$ ) mg Ti/kg and 142 ( $\pm 10$ ) mg Zn/kg. High concentrations of metals like aluminum (30,000 mg/kg), iron (14,800 mg/kg), zinc (47,064 mg/kg), and copper (705 mg/kg) were found in tissues of *P. laevigata*; showing the potential of these plants to absorb toxic elements (Table 1). Bioaccumulation factors for Zn,

**Table 1** Ionic analysis of rhizospheric soil and *P. laevigata* tissues by wavelength-dispersive X-ray fluorescence (WDXRF) spectrometry

Element	Rhizospheric soil	Root	Stem	Leaf
Ca (mg/kg)	58,200 (430)	409,500 (5078)	548,000 (9754)	314,500 (3837)
K (mg/kg)	11,900 (194)	83,450 (1969)	69,450 (1507)	169,650 (4631)
Si (mg/kg)	181,500 (853)	27,850 (1122)	12,100 (342)	31,450 (1195)
Mg (mg/kg)	6550 (147)	25,850 (923)	17,300 (581)	45,650 (1310)
P (mg/kg)	993 (60)	20,850 (847)	10,600 (543)	24,550 (1402)
S (mg/kg)	768 (47)	12,900 (651)	14,250 (445)	18,200 (748)
Fe (mg/kg)	47,450 (199)	7400 (474)	14,800 (401)	4250 (327)
Sr (mg/kg)	2100 (33)	8600 (166)	8100 (240)	3100 (86)
Al (mg/kg)	53,100 (446)	4900 (54)	30,000 (1719)	3700 (49)
Zn (mg/kg)	142 (10)	789 (9)	224 (4)	47,064 (1459)
Na (mg/kg)	6200 (230)	8150 (88)	2700 (28)	1500 (33)
Cu (mg/kg)	402 (96)	414 (10)	705 (17)	N.D
Cr (mg/kg)	322 (46)	N. D	N. D	N. D
Ti (mg/kg)	5100 (128)	N. D	N. D	N. D





**Table 2** Elements concentration in the soil, root, stem, and leaf of *P. laevigata* trees growing in a contaminated zone

Element	Soil	Root	Stem	Leaf
Cu (mg/kg)	0.9 (0.1)	3.3 (0.0)	4.2 (0.8)	8.4 (3.4)
Zn (mg/kg)	2.7 (0.1)	29.2 (0.8)	29.9 (0.4)	33.8 (1.0)
Fe (mg/kg)	1190 (180)	141.6 (41.6)	250 (50)	208 (91)
Cr (mg/kg)	10.9 (9.0)	2.5 (0.8)	5.8 (0.8)	4.2 (0.8)
Ca (mg/kg)	6275 (3765)	14,558 (5775)	19,824 (775)	15,583 (1250)
K (mg/kg)	720 (20)	4083 (250)	4750 (916)	13,750 (1583)
Al (mg/kg)	2700 (100)	N. A	N. A	N. A

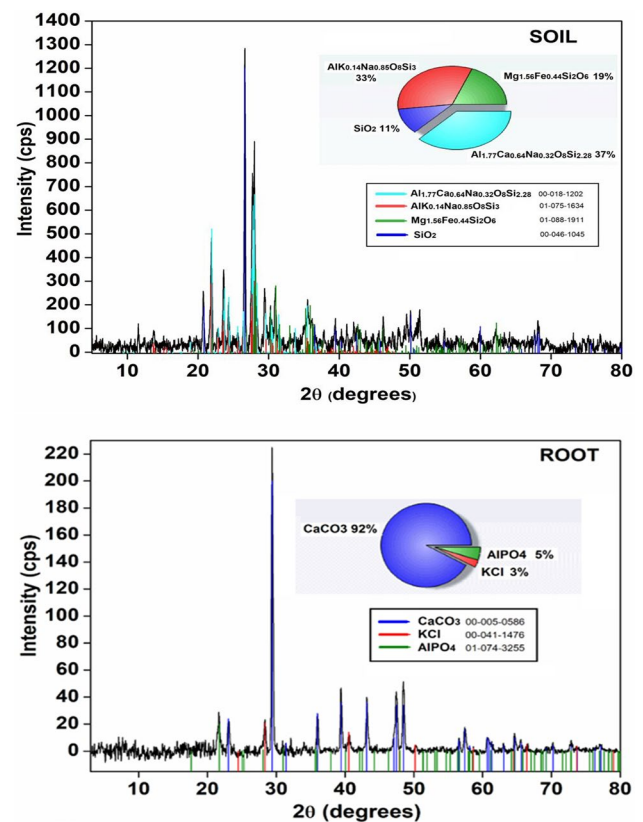
Elements concentration was analyzed by Atomic Absorption Spectrometer

Each value is the mean of two composed samples, the error is presented in parentheses. N. A. means not available

S, P, K and Ca were 113, 20, 18, 9 and 7, determined from measurements in Table 1. However, chromium and titanium were undetected in tissue plants despite their high concentration in soil (Table 1). Knowing that the detection limit of our WDXRF assay was 20 mg/kg, further validation of WDXRF spectrometry data was performed using atomic absorption spectrometry (AAS). The results of tissue accumulation of copper, zinc, iron, chromium, calcium, and potassium determined by AAS are shown in Table 2. The detection limit of AAS was 0.04 mg/kg and provided different element concentrations compared to WDXRF (Tables 1 and 2). There was no linear relationship between values obtained with AAS and WDXRF for Cu and Zn and the  $R^2$  values were lower than 0.39. Conversely, a positive linear relationship between AAS and WDXRF was observed for potassium ( $y = 11.43x + 17,036$ ), calcium ( $y = 35.15x - 161,678$ ) and iron ( $y = 39.17x + 950$ ) with  $R^2$  values bigger than 0.93.

Since mobilization and, hence, the bioavailability of metals in soil depends on the crystalline phases, an X-ray diffraction (XRD) analysis of *P. laevigata* tissues and their rhizospheric soil was performed (Fig. 1 and 2).

The main crystalline structures found in soils were anorthite sodium ( $\text{Al}_{1.77}\text{Ca}_{0.64}\text{Na}_{0.32}\text{O}_8\text{Si}_{2.28}$ ), anorthoclase ( $\text{AlK}_{0.14}\text{Na}_{0.85}\text{O}_8\text{Si}_3$ ), enstatite/ferrosilite ( $\text{Mg}_{1.56}\text{Fe}_{0.44}\text{Si}_2\text{O}_6$ ) and moganite ( $\text{SiO}_2$ ). We presume that the elements of these mineralogical phases were scarcely available to the plants because metals bound to silicate structures are scarcely bioavailable. Moreover, when soil pH is above 7, metals tend to form insoluble metal mineral phosphates and carbonates limiting their bioavailability. In fact, rhizospheric and surface soil pH were measured, being  $7.2 \pm 0.9$  and  $8.3 \pm 0.8$ , respectively. The diffractogram obtained from the XRD analysis of root samples (Fig. 1) showed peaks relating to three crystalline phases, calcite ( $\text{CaCO}_3$ ) in the rhombohedral



**Fig. 1** X-ray powder diffraction patterns for soil and root samples

phase, sylvite (KCl) in cubic phase, and aluminum phosphate ( $\text{AlPO}_4$ ) in the orthorhombic phase. The same three elements were found in stem samples, almost in the same proportions (Fig. 2). When leaf samples were analyzed by XRD (Fig. 2), two compounds,  $\text{CaCO}_3$  (in rhombohedral phase) and KCl (in cubic phase), were in common with those compounds found in the root and stem samples. Aluminum phosphate ( $\text{AlPO}_4$ ), namely berlinite, was also identified, but in the hexagonal phase. Two compounds, absent in the root or stem, were identified in leaves, fairchildite ( $\text{K}_2\text{Ca}(\text{CO}_3)_2$ ) in the hexagonal phase and aluminum iron phosphate ( $\text{Al}_{0.67}\text{Fe}_{0.33}\text{PO}_4$ ) in the orthorhombic phase.

*P. laevigata* has been reported as a chromium-hyperaccumulator-plant. These Cr-bioaccumulation traits have been described in seedlings growing in agar-based media or hydroponic conditions; however, in our free-living *P. laevigata* trees, chromium was not detected by WDXRF spectrometry. To clarify this contrasting result, the ability of *P. laevigata* seedlings to bioaccumulate chromium, iron, and zinc, under hydroponic conditions, was assessed by WDXRF spectrometry (Fig. S1). The WDXRF diffractograms of plants exposed to Cr, Fe, or Zn clearly showed the KA and KB radiation emission for the corresponding metal, but not the sample of control plants (Fig. S1 D). An elevated

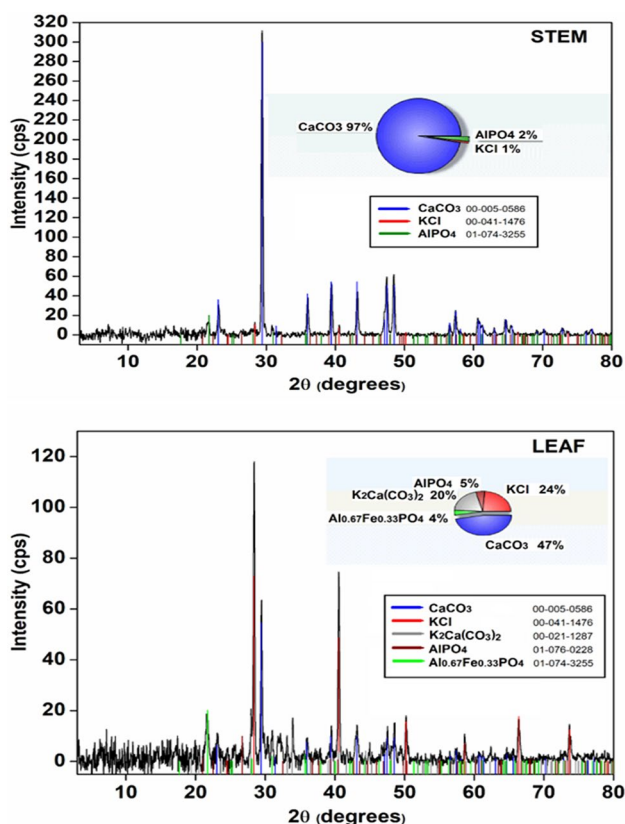
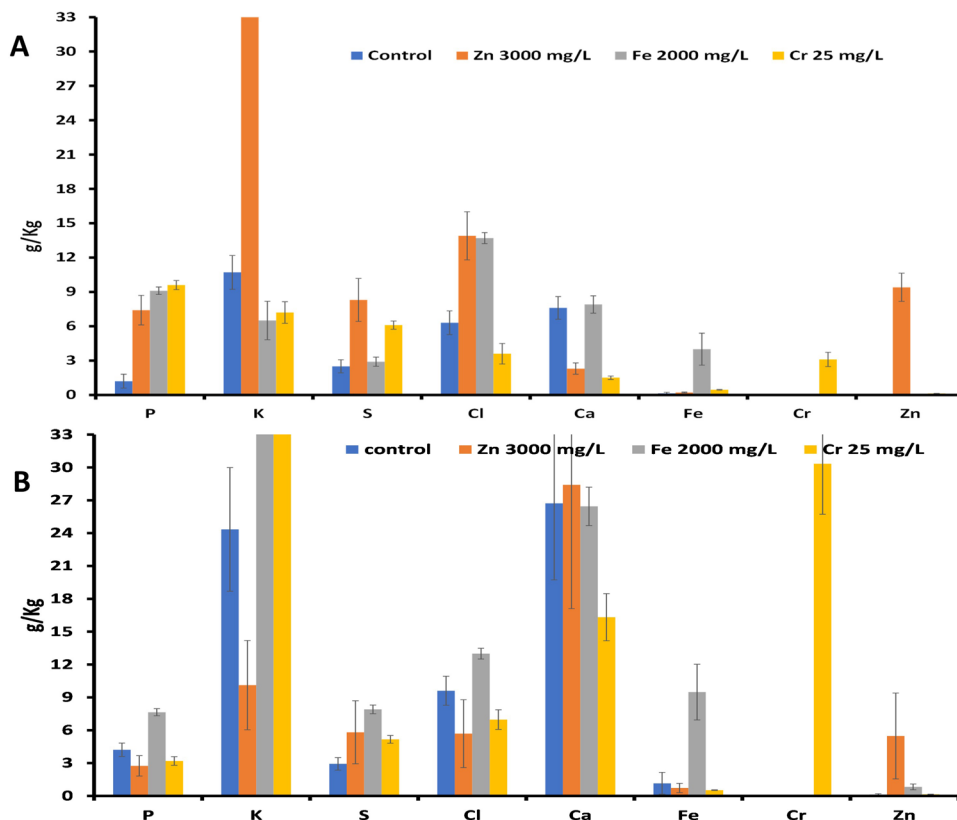


Fig. 2 X-ray powder diffraction patterns for stem and leaf samples

**Fig. 3** Ionic analysis of *P. laevigata* seedling determined by wavelength-dispersive X-ray fluorescence (WDXRF) spectrometry. **A** Uninoculated seedlings growing for six months in Jensen's medium supplemented with 3000 mg/L of Zn, 25 mg/L of Cr, and 2000 mg/L of Fe. **B** Seedling inoculated with *B. cereus* MH778713 growing for six months in Jensen's medium supplemented with 3000 mg/L of Zn, 25 mg/L of Cr, and 2000 mg/L of Fe



concentration of essential elements like K, P, Ca and S were detected in all conditions (Fig. S1).

*P. laevigata* seedlings were able to bioaccumulate up to 3100 ( $\pm$  618) mg Cr/kg, 9400 ( $\pm$  1230) mg Zn/kg, and 4000 ( $\pm$  1398) mg Fe/kg (Fig. 3A), representing a bioaccumulation factor of 123, 3, and 2, calculated as the ratio of element concentration in plant and element concentration in the medium knowing that culture medium contained 25.2 mg Cr/kg, 3017.5 mg Zn/kg and 2019.4 mg Fe/kg. The presence of chromium, iron, or zinc in Jensen's medium increased the plant content of phosphorous (P) by 6- to eightfold (Fig. 3A). Our results showed that *P. laevigata* could uptake Cr from culture medium under hydroponic conditions but no from rhizospheric soil, even when Cr concentration of rhizospheric soil was eightfold higher than hydroponic conditions.

The pH of rhizospheric soil ( $7.2 \pm 0.9$ ) could not be the factor preventing Cr uptake on wild *P. laevigata* trees because the pH of the hydroponic medium containing 25 mg Cr/L ( $\text{pH} = 7.28 \pm 0.15$ ) was similar. However, physical and chemical characteristics of rhizospheric soil (ionic force, organic matter, clay minerals, cation exchange capacity) can be the factors determining the solubility and therefore availability of heavy metals to the plants.

The root nodules of wild *P. laevigata* trees were colonized by *Bacillus* species with important chromium-accumulation characteristics (Ramírez et al. 2019). To rule out that the Cr-hyperaccumulator MH778713 strain, isolated

from *P. laevigata* nodules, could be impairing the Cr uptake of plants, an absorption assay inoculating seedlings of *P. laevigata* with *Bacillus cereus* MH778713 was performed (Fig. 3B). *P. laevigata* seedlings inoculated with *B. cereus* MH778713 bioaccumulated up to 30,334 ( $\pm 4618$ ) mg Cr/kg, representing a bioaccumulation factor of 1213. Cr-accumulation in inoculated seedlings was tenfold higher than in uninoculated *P. laevigata* seedlings (Fig. 3A, B). Inoculated *P. laevigata* seedlings accumulated 2.4-fold more iron and 1.7-fold less zinc than uninoculated seedlings (Fig. 3A, B). In all conditions, the Ca-accumulation of inoculated *P. laevigata* seedlings was 3.3-fold to tenfold higher than uninoculated seedlings. In contrast to inoculated seedlings, the presence of chromium and zinc increased the content of phosphorous and sulfur in uninoculated seedlings (Fig. 3A), probably to cope with metal stress. Increased P and S assimilation can be required for plants to synthesize thio-rich compounds, such as glutathione, phytochelatins, and metallothioneins, which play important roles in metal

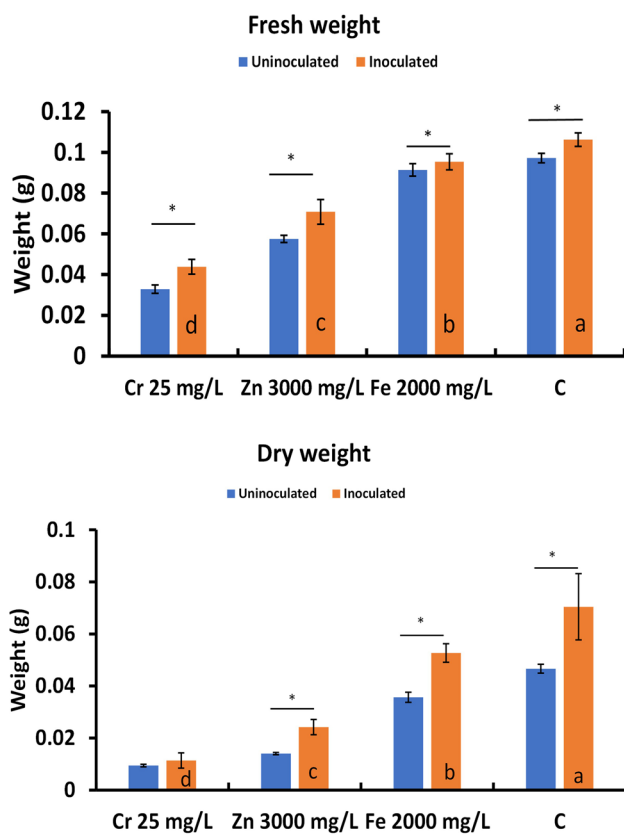
immobilization, sequestration, and detoxification (Li et al. 2023).

Furthermore, the effect of chromium, iron, and zinc stress on plant development was analyzed in inoculated and uninoculated *P. laevigata* seedlings (Fig. 4).

Compared with their controls, uninoculated and inoculated seedlings treated with chromium (25 mg/L) or zinc (3000 mg/L) displayed a significant reduction of dry and fresh weight (Fig. 4). At any condition evaluated, inoculated seedlings had higher plant weight compared to uninoculated seedlings (Fig. 4).

## Discussion

The ionic profile of wild *P. laevigata* trees growing in a heavy metal-contaminated site displayed the bioaccumulation of toxic elements. The accumulation of potentially toxic metals in plants depends on various factors including plant species, organs, mineral concentration and mineralogical phases present in the environment. Thus, depending on *Prosopis* species and the heavy metal concentration in soil, free-living trees of *P. laevigata* have been shown to accumulate up to 14, 19, 24, 104, 427, and 1331 mg/kg of Pb, Cd, Cu, Fe, Cr, and Zn (Beramendi-Orosco et al. 2013; Heredia et al. 2022; Khan et al. 2015; Muro-González et al. 2020; Senthilkumar et al. 2005). Our results showed that *P. laevigata* trees accumulated 705, 47,064, 14,800 and 30,000 mg/kg dry weight of Cu, Zn, Fe and Al, confirming the potential of these plants to hyper-accumulate metals. Aluminum is one of the most abundant metals in soils, which is normally bounded to silicates (e.g., anorthoclase, anorthite) and other nonphytotoxic mineral complexes. Under acid soil conditions or acidification by the rhizospheric microorganism, aluminosilicates can release toxic  $Al^{3+}$  cations into the soil, causing toxicity to the plants (Bojorquez-Quintal et al. 2017). Plants have developed two main mechanisms of tolerance,  $Al^{3+}$  exclusion from root cells and internal  $Al^{3+}$  ion tolerance (Brunner and Sperisen 2013). Our results suggest that *P. laevigata* trees used an internal tolerance mechanism by chelating  $Al^{3+}$  cations with phosphates to form aluminum phosphate ( $AlPO_4$ ) in the orthorhombic phase in both roots and stem (Fig. 1 and 2). Nonetheless, leaves accumulated aluminum phosphate ( $AlPO_4$ ) in the hexagonal phase. The crystalline phase change of  $AlPO_4$  could be due to the different mineral composition of each plant tissue; leaves, for example, contained 47% of calcite ( $CaCO_3$ ) in rhombohedral form while roots and stems contained 92% and 97% of  $CaCO_3$  in the same mineralogical phase. The high content of calcium carbonate (calcite) found in roots and stems could be helping plants to cope with heavy metal toxicity, because calcite increases pH, enhancing the precipitation of metal carbonates (Ali et al. 2020).  $Al^{3+}$  toxicity can be



**Fig. 4** Effect of chromium, iron, and zinc in *P. laevigata* seedlings development. Blue bars represent uninoculated seedlings growing in Jensen's medium for six months. Orange bars represent seedlings inoculated with *B. cereus* MH778713 growing in Jensen's medium for six months. Different letters indicate significant differences between treatments according to the least significant difference at  $p=0.05$ . \*means significant difference  $p<0.05$



the most deleterious limiting factor in acid soils ( $\text{pH} < 5.0$ ), since *P. laevisgata* trees accumulated 30,000 mg/kg of Al, it will be interesting to test the potential of *P. laevisgata* for Al-phytoextraction in acid soils.

Conversely, *P. laevisgata* trees did not accumulate chromium (Table 1) or scarcely (Table 2), albeit the Cr concentration of rhizospheric soil was 322 ( $\pm 46$ ) mg/kg (Table 1). When *P. juliflora* trees were grown in a tannery effluent contaminated soil containing 2542 mg Cr/kg, trees were able to accumulate up to 427 mg Cr/kg in roots (Khan et al. 2015). Under laboratory conditions, where culture medium or substrate is supplied with  $\text{K}_2\text{CrO}_4$ , seedlings of *P. laevisgata* or *P. juliflora* can accumulate up to 20 to 10,983 mg Cr/kg (Aldrich et al. 2003; Buendia-Gonzalez et al. 2012, 2019; Rai et al. 2004). In line with these studies, our *P. laevisgata* seedlings subjected to 25 mg Cr/L in hydroponic systems accumulated up to 3100 mg Cr/kg of plant ashes (Fig. 3A). The factors inhibiting Cr uptake in our *P. laevisgata* trees are not clear, but probably the high content of calcium-aluminum-silicate mineral forms in soils (Fig. 1 and 2), the low Cr concentration in soil (322 mg/kg) and the high soil pH were the culprits of blocking Cr uptake. Cation exchange in soil and hydroponic conditions is completely different. The hydrous aluminosilicates (clay minerals) of soils have strong physical and chemical interactions with dissolved species, especially with heavy metals (Uddin 2017). Thus, the high adsorbing capacity of clay minerals of rhizospheric soils can be one factor blocking Cr uptake in wild *P. laevisgata* trees. Under hydroponic cultures (Fig. 3), conversely, chromium amorphous structures were freely available to plants for bioaccumulation (Grigatti et al. 2015).

When *P. laevisgata* seedlings were inoculated with *B. cereus* MH778713 and grown under hydroponic conditions without any metal stress, a plant growth promotion was observed (Fig. 4). This was likely the result of higher P, K, Ca, and Fe uptake (Fig. 3B), since inoculated seedlings accumulated 3.5-, 2.1-, 3.5- and 9.3-fold more minerals than the uninoculated seedlings. Zn- or Cr-stress induced higher phosphorus and sulfur assimilation in plants (Fig. 3). Under heavy metal stress, thio-rich compounds, such as glutathione, phytochelatins, and metallothioneins, are induced to keep the metal ion homeostasis by binding with heavy metal ions (Li et al. 2023). In fact, there is a differential metallothionein induction in response to cadmium, copper or zinc suggesting that each metallothionein has a specific role in *P. juliflora* (Usha et al. 2009).

Under all hydroponic conditions tested, chromium was the most toxic metal (Fig. 4). High concentrations of hexavalent chromium disrupt plant tissues integrity (Scoccianti et al. 2008; Sharma et al. 2003), disturbing the biosynthesis of chlorophyll (Shanker et al. 2005) and germination process (Ramírez et al. 2020). Metabolic alterations caused by hexavalent chromium promote the expression of enzymes and

metabolites responsible for the detoxification and elimination of ROS (reactive oxygen species) (Shanker et al. 2005). The phytotoxic effects of Cr in uninoculated seedlings were associated with a reduction of K and Ca uptake (Fig. 3A) affecting probably potassium physiological processes that influence plant growth and metabolism, calcium sensors, plant cell walls and membranes. Potassium plays a crucial role in photosynthetic activity, osmoregulation, stomatal movements, energy transfer, phloem transport, and cation–anion balance. It also plays a mitigating role in various abiotic stresses such as drought, salinity, and metal toxicity (Wang et al. 2013). Phytotoxic effects of Cr in *P. laevisgata* seedlings were also associated with an eightfold increase in phosphorus accumulation (Fig. 3A), and P reduces the heavy metal toxicity diluting the metal or forming a metal complex avoiding the transit of the toxic metal (Sarwar et al. 2010).

The presence of zinc in the environment also affects mesquite plant development (Fig. 4). High levels of zinc cause morphological, biochemical, and physiological disorders (Balafrej et al. 2020), starting with the disruption of the nuclear membrane and the loss of the integrity of cell organelles (Dong et al. 2006). Wavelength-dispersive X-ray fluorescence analysis (Table 1, Fig. 3A) showed the accumulation of zinc in mesquite plants up to 47,064 mg/kg under free-living conditions or 9400 mg/Kg under hydroponic conditions. Zinc hyperaccumulating plants, unlike chromium, can accumulate high concentrations of zinc in aerial tissues (Reeves and Baker 2000).

There was some variation in the elemental content of samples determined by atomic absorption spectroscopy and WDXRF (Tables 1 and 2), with WDXRF's tendency to show higher element concentrations. The differences can be attributed to the physical interaction between the elements during the analysis. In the dry chemical analysis (WDXRF), all elements of the samples are in strong contact with each other, generating interferences of the fluorescence emission between elements, which is called the matrix effect. Wet chemistry analysis (atomic absorption spectroscopy), on the other hand, requires sample digestion with acid, reducing or eliminating element interactions.

## Conclusion

*P. laevisgata* plants growing under free-living and/or laboratory-controlled conditions can accumulate high concentrations of Zn, Fe, Al, and Cr, confirming the potential of this plant to hyper-accumulate heavy metals. The X-ray diffraction analysis showed that *P. laevisgata* trees can chelate  $\text{Al}^{3+}$  cations with phosphates to form orthorhombic phases of aluminum phosphate ( $\text{AlPO}_4$ ), probably as an internal aluminum tolerance mechanism. The inoculation of seedlings with an endophytic *Bacillus* did not prevent Cr-accumulation





in the plant but increased metal tolerance and seedling development. Our results highlight the use of *P. laevigata* with *Bacillus cereus* MH778713 as valuable tools for heavy metal bioremediation of arid lands.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s13762-023-05297-7>.

**Acknowledgements** Financial support was received from Vicerrectoría de Investigación y Estudios de Posgrado (VIEP-BUAP), Instituto de Ciencias (ICUAP), Posgrado en Ciencias (Microbiología) and CONACyT research grant number 252847. Verónica Ramírez and Dolores López are grateful for grant numbers 273291 and 994841 from CONACyT.

**Author contributions** VR, AB, and JAM conceived, designed, and directed the project, contributed to the interpretation of the results, and designed the figures. VR, DL, VQ, PL, GJ and JM participated in the acquisition of the data and developed the methodologies. AB took the lead in the writing of the manuscript. All authors provided critical feedback, helped to shape the research, discussed the results, and contributed to the final version of the manuscript.

**Funding** This work was supported by Vicerrectoría de Investigación y Estudios de Posgrado (VIEP-BUAP), Instituto de Ciencias (ICUAP), Posgrado en Ciencias (Microbiología) and CONACyT research grant number 252847. Verónica Ramírez and Dolores López are grateful for grant numbers 273291 and 994841 from CONACyT.

## Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

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