

# Performance of Growth and Remediation Potency of *Jacaranda mimosifolia* in Cadmium and Lead Contaminated Soil

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In a 16-month study addressing global agricultural soil heavy metal contamination, researchers explored plant-centered solutions using *Jacaranda* plants. The impact of different combinations of cadmium nitrate (40, 80, and 120 mg) and lead nitrate (400, 800, and 1200 mg/kg soil) were evaluated relative to *Jacaranda*'s remediation capabilities. Employing a randomized complete block design with 8 applications across 3 repetitions, the study assessed growth traits and chemical characteristics. Untreated plants showed higher growth values, contrasting with reduced values in plants exposed to elevated cadmium (Cd) and lead (Pb) levels. For instance, the treatment with 120 mg Cd/kg soil + 1200 mg Pb/kg soil led to a 28% reduction in plant height, 13% in main stem diameter, 41% in branch number, and 35% in leaf area compared to the control. Despite these challenges, *Jacaranda* plants demonstrated resilience with a 100% survival rate. Plant organs showed increased Cd and Pb contents, with fallen leaves having lower metal content, mitigating pollution hazards. Post-planting, soil characteristics shifted, indicating *Jacaranda*'s potential for Cd phytoextraction (BCF < 1 and, TF > 1) and Pb phytostabilization (BCF and TF < 1). The study establishes *Jacaranda* as a promising candidate for phytoremediation due to its resilience to elevated metal levels.

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## INTRODUCTION

Soil pollution with excessive quantities of heavy metals (HMs) or trace elements is a significant global environmental issue. A significant quantity of toxic HMs is added to the environment by rapid industrialization, advanced agricultural approaches, and other anthropogenic activities. These cause various hazardous impacts on all living organisms, as well as changes in soil characteristics and biological activity (Manoj *et al.* 2020). The release of HMs *via* different utilizations in industry and agriculture increases the harm to

habitat and human health related to exposure to these contaminated locations and dust (Khan *et al.* 2010). HMs are one of the most harmful elements in the soil because of their increasing level of persistence and toxicity to living organisms (Madanan *et al.* 2021). The harm caused by HMs, involving adsorption and distribution through the plant cells, is affected by their bioavailability in soil and different physiological activities in plants (*e.g.*, stage of development or plant physiological age, exposure time, HMs levels in soil, and pH of soil) (Bhargava *et al.* 2012). Benavides *et al.* (2005) and Wang *et al.* (2008) documented that the majority of common visual effects of HMs phytotoxicity are the decline in photosynthesis and reduction in water and nutrient absorption, disturbance in respiration and nitrogen metabolism, leaf chlorosis, and growth inhibition.

Additionally, (HMs) generate oxidative stress by producing a large amount of reactive oxygen species (ROS), which leads to cell death through the peroxidation of membrane lipids, oxidation of proteins, inhibition of enzymes, and damage to nucleic acids. To alleviate the oxidative stress, plants employ the antioxidant system *via* alternation in antioxidative enzyme activities such as superoxide dismutase, ascorbate peroxidase, catalase, glutathione reductase, glutathione S-transferase and guaiacol peroxidase, as well as, the level of low molecular weight antioxidants including ascorbic acid, reduced glutathione, carotenoids, phenolics (Benavides *et al.* 2005; Hossain *et al.* 2012). The equilibrium between reactive oxygen species (ROS) and the antioxidant system is advantageous for plants, as they face challenges in adapting and surviving in unfavourable situations such as heavy metal (HM) contamination (He *et al.* 2011; Sharma *et al.* 2012).

Cadmium (Cd) and lead (Pb) are well-known elements within the category of heavy metals (HMs). The industrial products containing Cd encompass paints and pigments, electroplating, plastics stabilizers, as well as the incineration of Cd-containing materials and phosphate fertilizers. At the same time, the Pb contamination sources include the emission from leaded petrol combustion, herbicides, manufacture of batteries, and pesticides (Saxena *et al.* 2020). A significant threat and/or toxicity can happen to plants, animals, and humans by food chain exposure of Cd and Pb, which can lodge into the ecosystem *via* a natural geological approach or by anthropogenic processes such as municipal and industrial wastes (Aziz *et al.* 2015; Sharma *et al.* 2023). Cadmium has stressful impacts on plants as the retardation of biomass production, soluble protein content, metabolism, enzymatic activities, and increasing ROS production (Ali *et al.* 2014a; Ef *et al.* 2015; Li *et al.* 2015). Also, Pb causes chlorosis, decreases seed germination, decreases plant growth and development of biomass, disturbs photosynthetic rate, reduces element and water absorption and transportation, reduces or alters membrane permeability, and generates excessive anomalous morphology and reactive oxygen species (ROS) in plants, while also inhibiting enzymatic activities in plant cells by interaction with their sulfhydryl groups (Ali *et al.* 2014b; Saxena *et al.* 2020).

Several approaches have been made to avoid the risks of HMs in polluted soil, such as contaminated site excavation and landfilling, soil flushing and washes, efficacy utilizing physiochemical techniques, and electrokinetic applications (Wuana and Okieimen 2011). Previously, Vangronsveld *et al.* (2009) documented that these methods are significantly costly and labor intensive, cause continuous changes in soil traits and decline of indigenous soil microflora.

One phytoremediation strategy is to discover plants that have tolerance against HMs and accumulate the MHs in their aerial organs with large biomass yield (Bhargava *et al.* 2012). In ideal cases, the goal is to accomplish this with no opposing impacts on growth (Ye *et al.* 2017). Great emphasis has been placed on the efficient application of trees in the

remediation of HMs owing to their many good traits, *e.g.*, rapid expansion combined with substantial crop output, a well-developed root system, convenient and cost-effective planting, and their aesthetically acceptable nature (Brunner *et al.* 2008; Luo *et al.* 2016). Phytoremediation has been considered as an inexpensive option that does not require treatment or removal of the soil (Shrestha *et al.* 2019). Furthermore, phytoremediation enhances either physical soil characteristics or biological activities (Wan *et al.* 2016). Phytoremediation can be a cost-effective treatment method relative to ion substitution, solvent extraction, adsorption, oxidation-reduction, and reverse osmosis. Several studies were done on different plant species as promising candidates for the elements cadmium (Cd) and lead (Pb). Phytoremediation has been carried out using poplar species, *Schima superba*, *Salix species*, *Jatropha curcas*, Chinese sweetgum, and Chinese fir (Wu *et al.* 2010; He *et al.* 2011; Bhargava *et al.* 2012; Dai *et al.* 2013; Chang *et al.* 2014; Drzewiecka *et al.* 2017; El-Mahrouk *et al.* 2019, 2020, 2021; Wang *et al.* 2022).

“Several factors affect plant remediation (Wang *et al.* 2020). These include physiochemical soil characteristics, the exudates from plants and microbes, and the bioaccessibility of heavy metals (HMs). The effectiveness of phytoremediation also depends on the capacity of plants to assimilate, adsorb, accumulate, translocate, or sequester various toxicological aspects of metals. (Wang *et al.* 2020). The plants reduce the soil HMs content *via* different ways of phytoextraction. This involves the plants absorbing HMs from the soil and accumulating them in their aboveground portions (Sebastiani *et al.* 2004). Phytostabilization is a process in which certain species stabilize the soil surface by accumulating heavy metals (HMs) in their roots (Marques *et al.* 2009). Additionally, rhizofiltration is HMs from water, or aqueous waste streams are uptook or adsorbed by the plant roots (Erakhrumen and Agbontalor 2007)

According to Sharma *et al.* (2023), it is necessary to find a new species that is used as a phytoremediator of HMs and comprehend the methods by which plant species exhibit resistance to a particular metal. A promising candidate is *Jacaranda mimosifolia* D. Don (Fam. Bignoniaceae). It was widely planted in tropics and subtropics regions (Gentry 1992). It has soft, delicate, fernlike, deciduous foliage, and can grow to be 25 to 40 feet tall with a larger spread (Zaouchi *et al.* 2015; Gilman *et al.* 2019).

Assessing the potential of a plant to contaminate the surrounding environment through leaf shedding in any phytoremediation scenario is advantageous. Plant species, element types, and concentration in the soil affect HM levels in either green or fallen leaves. The study of Rafati *et al.* (2011) found that *Morus alba* fallen leaves contain more Cr and Ni than green leaves, but *Populus alba* exhibited higher concentrations of cadmium (Cd) and chromium (Cr) compared to their green counterparts. El-Mahrouk *et al.* (2021) supported this fact, reporting that fallen leaves from *Jatropha curcas* had Cd and Pb content negligible relative to the green leaves.

Most studies document the response of plants subjected to the singular stress of a heavy metal despite soil being polluted with multiple metals (He *et al.* 2013a,b; Chen *et al.* 2014). Furthermore, there is currently a lack of evidence regarding the utilization of *Jacaranda mimosifolia* as a phytoremediation agent for soil contaminated with cadmium (Cd) and lead (Pb) in Egypt, as well as the data about it in this aspect, are very limited in the other countries in the global. Furthermore, several regions in Egypt are contaminated with elevated levels of heavy metals (HMs), notably cadmium (Cd) and lead (Pb), stemming from the utilization of sewage effluent, industrial discharge, or drainage water in irrigation practices, as well as the application of sewage sludge as organic fertilizer for soil enrichment. Therefore, a pot experiment was conducted to get available data about the

growth and efficiency of *J. mimosifolia* in the phytoremediation process, specifically addressing the synergistic impacts of cadmium (Cd) and lead (Pb).

## EXPERIMENTAL

An experimental study in pots was carried out within the confines of Antoniadis Garden, Horticulture Research Institute, Alexandria branch, Ministry of Agriculture, Egypt, to investigate the synergistic impact of varying amounts of Cd and Pb on the development and chemical composition of *Jacaranda mimosifolia* during the period of 1<sup>st</sup> April 2020 to 1<sup>st</sup> August 2021. The goal was to study the relationship between the contents of these elements in plant parts and soil levels to estimate the phytoremediation potential and the soil properties after the plantation period.

### Plant Material

A local nursery obtained one-year-old homogenous *Jacaranda mimosifolia* transplants ( $20 \pm 2$  cm in height).

### Pollutant Treatments

Cadmium nitrate Cd (NO<sub>3</sub>)<sub>2</sub> was applied at the rates of 40 [low concentration (L)], 80 [medium concentration (M)], and 120 [high concentration (H)] mg/kg soil, which was equivalent to 14.4, 28.8, and 43.2 mg of elemental Cd/kg soil. Also, lead nitrate Pb (NO<sub>3</sub>)<sub>2</sub> was applied at the rates of 400 [low concentration (L)], 800 [medium concentration (M)], and 1200 [high concentration (H)] mg/kg soil, which was equivalent to 247.7, 495.4, and 743.2 mg of elemental Pb/kg soil. Plastic pots, measuring 40 cm in diameter, were filled with 9 kg of air-dried soil per pot. This soil was blended with metal solutions at the specified concentrations and kept for 60 days before being cultured outdoors under a plastic house (from 29<sup>th</sup> January to 31<sup>st</sup> March)—untreated soil was considered as a control. The treatments of heavy metals were conducted as follows: control, LCd LPb, MCd MPb, H Cd HPb, LCd HPb, HCd LPb, MCd HPb, and HCd MPb.

For context, world-high allowable Cd and Pb levels range from 1 to 5 and 20 to 300 mg/kg soil, respectively according to Kabata-Pendias (2011).

### Transplanting Date

Similar transplants of *Jacaranda mimosifolia* were grown in pots filled with contaminated soil, while other pots contained untreated soil with HMs as a control (one plant/pot) on 1<sup>st</sup> April 2020. The transplants were placed in the open field.

### Experimental Layout

The subject was designed using a randomized complete block design (Snedecor 1989). The experiment contained seven applications in addition to the control (untreated soil) repeated thrice. There were three plants of each replicate, thus nine transplants for each treatment.

### Agricultural Practices

Routine agricultural practices (such as weeding and the controlling of insecticides and pesticides) were done during the experimental period; Tap water (pH 7.20; EC 0.59 ds/m) was used to irrigate the plants when needed.

## Measurements

On 1<sup>st</sup> August 2021, 6 plants were randomly harvested for each utilization (2 plants of each repetition) to measure the following traits.

### Vegetative Growth

Plant height (cm) and stem diameter were measured at a distance of 5 cm from the soil surface, number of branches, area/leaf (cm<sup>2</sup>) using a C1-202 Laser area meter (Cid Bio- Science, USA), software, weights of leaves, stem and roots (fresh and dry) and the longest root length (cm). The selected plants were categorized into their respective parts: roots, stems, and leaves. Subsequently, the specimens underwent two rounds of washing: first with tap water to eliminate any soil residue, and subsequently with deionized water. The plant samples were subjected to oven-drying at a temperature of 80 °C for a duration of 24 hours (Rautio *et al.* 2010). A Minolta SPAD, 502, Japan device was used to measure leaf greenness (SPAD units) (Markwell *et al.* 1995) in the field before harvesting the fifth leaf from the plant top.

### Soil Analysis

Soil analysis was done before and after the plantation. A hydrometer was used to analyze soil physical parameters (the particle size distribution) (Gee and Bauder 1986) before the plantation only. Soil samples were collected from all repetitions for each application and then mixed carefully in one sample (Table 1). The soil air-dried samples were pulverized using a mortar and pestle and then filtered through a stainless-steel test sieve to obtain fractions smaller than 2 mm (Cools and De Vos 2010). In order to assess soil chemical characteristics, a mixture of 20 grams of dried soil and 100 milliliters of distilled water (at a ratio of 1:5) was allowed to sit for 24 h, after which the resulting extract was filtered. The recorded measurements of the soil samples were as follows: EC was measured using an EC-Meter (MI 170, Szeged, Hungary) (Jackson 1973). A volumetric Calimeter was used to determine total carbonate (Nelson and Sommers 1996).

The micro Kjeldahl method was utilized to determine the available nitrogen (N) (Bremner 1982). Available P was assessed according to Olsen *et al.* (1982). Ca, Mg, and Cl were also estimated (Jackson 1973). The method for measuring Na and K used a flame photometer PSP7 (JENEWAY, Staffordshire, UK) (Black 1965). The concentrations of cadmium (Cd) and lead (Pb) were determined using an atomic absorption spectrophotometer ( AAS ) (Page *et al.* 1982). The pH of the soil was determined by measuring the soil suspension (1:2.5, soil: distilled water) with a pH meter after a 30-minute period (JENEWAY3510, Staffordshire, UK) (Jackson 1973). For organic matter (OM) determination, 1 g of soil was blended with 10 mL of 0.1667 M K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> and 20 mL of concentrated H<sub>2</sub>SO<sub>4</sub> containing 1.25% of Ag<sub>2</sub>SO<sub>4</sub>. The mixture was stirred, and after 30 minutes, the green color of chromium sulfate was measured using a spectrophotometer at 660 nm. Sucrose (0.42%) served as a standard, and carbon (C%) was calculated as follows:

$$C\% = \frac{(mg \text{ of observed } C \times 100)}{1000} \quad (1)$$

Finally, organic matter percentage (OM%) was computed as:

$$OM\% = C\% \times 1.724 \text{ (Nelson and Sommers 1996)} \quad (2)$$

## Chemical Composition of Leaves, Stems, and Roots

A metal-free mill (IKa-Werke, M20 Germany) made from stainless steel was used to grind the dry samples of plant organs. 5 mL concentrated sulphuric acid was mixed with 0.2 g a homogenous powder of the plant parts, and the mixture was heated for 10 min. Then 0.5 mL of perchloric acid was added drop by drop, and the heating process was continued until a clear solution was obtained. The solution was then cooled and filtered. After that, distilled water was supplied to bring the total volume of the solution to 50 mL (Evenhuis and Deward 1980). The solution of samples was prepared for estimating N, P, K, and carbohydrate% in leaves (D.W), cadmium, and lead in leaves, stems, and roots (mg/kg D.W.). Also, the fallen leaves were collected during Nov. 2021, and then their Cd and Pb concentrations were determined.

Measurements of N% were obtained by the modified micro-Kjeldahl (Horwitz 1990) method, P% colorimetrically in a spectrophotometer (GT 80 + UK) (Murphy and Riley 1962), K% by flame photometer (Cottenie *et al.* 1982), and total carbohydrate% by (Herbert *et al.* 1971). Cadmium and Pb levels (mg/kg D.W.) were estimated in different plant parts using Perkin, 3300 Atomic Absorption Spectrophotometer (Page *et al.* 1982). The uptake of Cd or Pb was calculated as follows: Cd or Pb concentrations  $\times$  D.W. (leaves, stems, and roots)/ 1000.

$$\text{Total uptake} = \text{uptake of leaves} + \text{stems} + \text{roots} \text{ (mg/plant)} \quad (3)$$

## Indicators to Determine the Efficiency of *Jacaranda mimosifolia* for Phytoextraction of Cd and Pb in Polluted Soil

Bioconcentration (BCF) factor and translocation factor (TF) were calculated as follows,

$$BCF = \frac{PO}{SM} \quad (4)$$

$$TF \% = \frac{MC}{MH} \times 100 \quad (5)$$

where *PO* is the plant organ metal content (mg/kg D.W.), and *SM* is the soil metal content (mg/kg soil), also known as “metal level added + soil metal level before contamination”, *MC* is the shoots metal content (mg/kg D.W.), and *MH* refers to the metal content in the roots (mg/kg D.W.). *TF %* is used to calculate the efficiency of ion transfer from roots to aboveground plant organs (Maiti and Jaiswal 2008), where shoots refer to leaves and stems.

Accumulation efficiency was calculated based on BCF values and categorized into one of four types. The bioconcentration factor (BCF) is greater than 1 for substances with high concentration, between 1 and 0.1 for substances with medium concentration, between 0.1 and 0.01 for substances with weak concentration, and between 0.01 and 0.001 for substances that do not accumulate (Kabata-Pendias and Pendias 1999). The tolerance index biomass (*TI<sub>b</sub>*) is defined as,

$$TI_b = \frac{TP}{CP} \quad (6)$$

where *TP* is the treated plant D.W. (g/plant), and *CP* is the control plant D.W. (g/plant). Plant D.W. referred to leaves + stems + roots.

The parameter  $TI_b$  was used to evaluate the resistance of *Jacaranda mimosifolia* in soil polluted with Cd and Pb, to calculate ( $TI_b$ ). According to Wilkins (1978), there are 3 values: ( $TI_b$ )  $< 1$  (a net reduction in biomass and a stressed condition of plants),  $TI_b = 1$  (no difference comparing to treatment of control), and  $TI_b > 1$  (a net increase in biomass and correct plant development).

The *Jacaranda mimosifolia* tolerance index of roots ( $TI_r$ ) was also estimated (Wilkins 1978),

$$TI_r = \frac{AR}{LR} \quad (7)$$

where AR is the length of plant roots treated with metal (cm), and LR is the control plant root length (cm).

## Statistical Analysis

The data were analyzed using the SAS program (version 6.12; SAS Institute, Cary, NC). Average separation was performed using Duncan's multiple range test using one-way ANOVA  $\pm$  standard deviation (SD) ( $n = 3$ ). The statistical significance was determined at a level of  $P \leq 0.05$ .

## RESULTS AND DISCUSSION

### Soil Analysis

The used soil for the planting of *Jacaranda mimosifolia* analysis showed that the texture was sandy. The soil had an O.M of 0.53%, a pH of 8.50, and an EC level of 0.37 ds/m (Table 1). After planting, it was found that changes in the values of chemical parameters were to happen relative to prior planting and contamination; the values of pH, EC,  $Na^+$ ,  $Cl^-$ ,  $HCO_3^-$ , and  $SO_4^{2-}$  increased after planting. On the contrary, reductions in O.M%,  $CaCO_3$ ,  $Ca^{++}$ ,  $Mg^{++}$ ,  $K^+$ , and N, P, and K available levels after plantation were noticeable. Indeed, Cd and Pb levels decreased relative to their added quantities to the soil after planting. Also, the most considerable reduction in the available nitrogen, phosphorus, and potassium was to occur in the control treatment, HCd LPb, and LCd HPb, applications, each in turn after the planting. Furthermore, pH rose to 9.10 after applying MCd HPb and HCd MPb treatments. At the same time, EC value increased to 1.29 after using MCd MPb treatment.

Heavy metals application usage affected soil parameters; in this concern, some studies showed that soil characteristics including pH, O.M, and alteration capacity were more related to Cd and Pb retention (Jopony and Young 1994). The addition of metals, causing an increase in cations such as  $Na^+$  and anions such as  $Cl^-$ ,  $HCO_3^-$ , and  $SO_4^{2-}$  may lead to an increase in EC value. Sharma and Raju (2013) reported that high amounts of ions and soluble salts raised EC levels in soil irrigated with industrial effluent. However, the decrease in available N, P, K, soluble cations (regardless of  $Na^+$ ), and concentrations of Cd and Pb at the ending of the trial may be due to the absorption *via* plant roots or the displacement by watering. Cd and Pb soil concentrations have been reduced significantly after applying phytoremediation compared to their initial levels (Durante-Yáñez *et al.* 2022). The soil surface chemistry and metal retention are affected by pH value (Bradl

2004). Also, high pH levels lead to increased metal retention and reduced soil solubility. When the pH decreases, plant Cd uptake rises (Bolan *et al.* 2003). Heavy metals negatively impact microbial activities and the structure of microbial populations (Obbard 2001).

Additionally, Zhao *et al.* (2019) documented that metallic processes may cause alteration in the soil microenvironment, particularly HMs precipitation. The present data were matched with those of El-Mahrouk *et al.* (2019), who found that EC values increased in soil polluted with CdCl<sub>2</sub> at 80 mg/kg soil to 5 ds/m, as well as the available N, P, K. In addition, the Cd, Cu, and Pb levels were decreased after the culture of *Salix mucronata* in comparison to their levels prior to planting. Also, soil characteristics, *e.g.*, pH, CEC, and Ca levels, significantly influence on the Pb soil bioavailability (Zhang *et al.* 2019). Also, El-Mahrouk *et al.* (2020) revealed that EC increased, while the available N, P, K, and Cd, Cu, and Pb levels were reduced with a culture of *Populus nigra* in Cd, Cu, and Pb polluted soil. In contrast, soil pH decreased significantly under Cd stress (Wang *et al.* 2022).

**Table 1.** Soil Physiochemical Parameters before Culture and Soil Chemical Analysis as Impacted by Soil Cd and Pb Levels after Plantation of *Jacaranda*

Parameters	Before Planting	After Plantation							
		Treatments (mg/kg) soil D.W.							
		Control	LCd LPb	MCd MPb	HCd HPb	LCd HPb	HCd LPb	MCd HPb	HCd MPb
pH	8.50	9.02	8.90	8.70	8.92	8.90	9.04	9.10	9.10
EC (ds/m)	0.37	0.62	0.77	1.29	0.70	0.75	0.40	0.49	0.49
CaCO <sub>3</sub> %	2.10	1.80	1.90	1.90	1.46	1.58	1.58	2.00	1.37
O.M%	0.53	0.33	0.36	0.38	0.42	0.40	0.39	0.40	0.40
<b>Soluble cations (meq/L)</b>									
Ca <sup>++</sup>	1.90	0.30	0.70	1.50	0.50	0.10	0.10	0.30	0.35
Mg <sup>++</sup>	1.95	0.20	0.40	0.75	0.30	0.11	0.11	0.19	0.27
Na <sup>+</sup>	1.96	4.17	5.48	8.26	5.65	5.74	2.87	3.30	3.13
K <sup>+</sup>	0.58	0.41	0.51	0.47	0.46	0.46	0.36	0.41	0.41
<b>Soluble anions (meq/L)</b>									
Cl <sup>-</sup>	2.16	4.00	5.00	9.00	5.00	5.00	3.00	2.50	2.50
CO <sub>3</sub> <sup>2-</sup>	—	—	—	—	—	—	—	—	—
HCO <sub>3</sub> <sup>-</sup>	1.10	2.00	2.00	2.00	2.00	3.00	2.00	2.00	1.50
SO <sub>4</sub> <sup>2-</sup>	0.36	1.00	0.90	0.90	1.10	1.10	1.23	1.00	0.80
Available N (mg kg <sup>-1</sup> )	4.50	2.80	3.40	3.60	3.60	3.90	33.00	33.00	38.00
Available P (mg kg <sup>-1</sup> )	5.50	3.90	3.20	4.40	4.80	2.30	1.80	2.80	2.60
Available K (mg kg <sup>-1</sup> )	270	180	160	220	240	120	180	170	160
<b>HMS (mg/kg)</b>									
Cd <sup>++</sup>	0.23	0.10	1.46	3.32	1.41	1.71	0.43	5.61	4.11
Pb <sup>++</sup>	7.40	4.30	39.60	78.90	98.80	122.30	43.00	143.60	56.00
<b>Physical parameters</b>									
Sand (%)	88.90								
Silt (%)	5.00								
Clay (%)	6.10								
Soil texture	Sandy								

## Effect of HMS in Soil on Vegetative Traits

Various combinations of Cd and Pb at different concentrations significantly decreased the majority of vegetative parameters relative to their respective control (Table 2). The highest plant stature measurements (164.68 cm), stem diameter (1.36 cm), branch number (25.33/plant), and area/leaf (205.57 cm<sup>2</sup>) were produced from the negative control group. In addition, the control plants had the maximum fresh and dried weights of leaves (53.44 and 26.27 g/plant), stems (126.01 and 47.57 g/plant), and roots (106.67 and 52.59 g/plant), respectively. The non-treated control reported higher values of the longest root and greenness degrees as 55.67 cm and 36.73 SPSD units in succession. In contrast, HCd HPb treatment had the most negative impact on the traits mentioned above.

The reduction in vegetative traits after applying HCd HPb reached 28% in plant height, 13% in stem diameter, 41% in branch number, 35% in area/ leaf, 34 and 30%, 49 and 41%, and 64 and 55% in leaves, stems, and roots fresh and dry weights, consecutively, 39% in the longest root length, and 26% in SPAD units. It is noticed from the data that some Cd and Pb combinations had a significant impact similar to their respective controls on some vegetative parameters. Also, the effects on vegetative traits of some treatments used were insignificant ( $P \leq 0.05$ ) among themselves, regardless of the control treatments. Despite the negative influence of Cd and Pb combinations, particularly at high concentrations, the *Jacaranda* plants could tolerate and grow with 100% survival in all the used treatments.

Surely, the detrimental impacts of Cd and Pb treatments on soil parameters conversely affected vegetative growth. Some harmful influences were noticed on the aged leaves, *e.g.*, leaf edges exhibiting yellow discoloration and desiccation under treatments of high HMs levels. The toxicity symptoms were reduced with increasing plant age. Accordingly, Tu *et al.* (2004) mentioned that tolerance raised by the increment in plant age and some visual harm was reduced in aged *Pteris vittata*. This means that *Jacaranda* could grow in Cd- and Pb-rich environments. The previous studies indicated that growth reduction and leaves abscission in willow tangio (*S. mastudana x S. alba*) resulted from the application of 0.6 to 60.6 µg Cd/g soil (Robinson *et al.* 2000). Also, despite the retardation impacts on the growth traits related to soil Cd and Pb at high concentrations, *Populus maximowiczii x P. nigra* has the ability to thrive in 7.3 and 1368 mg Cd and Pb/kg soil, respectively (Kubátová *et al.* 2016). Conversely, the used HMs levels negatively affected all vegetative traits, and the inhibition impact was correlated with the HM concentration and growth traits. Dong *et al.* (2005) documented that Cd stress causes deleterious effects on the level of photosynthesis and CO<sub>2</sub> level through cells and involves photosynthetic pigments *via* Cd<sup>++</sup> replacing Mg<sup>2+</sup> in chlorophyll structure, resulting in more decrease in fluorescence quantity in comparison to Mg chlorophylls. The two harmful impacts decrease the chlorophyll yield, reducing the photosynthetic rate that leads to aging and cellular demise (Santos *et al.* 2010). In addition, plant physiological and biochemical processes have been deleterious and affected by cadmium (Cd) absorption and retention in plants (Li *et al.* 2023). Similarly, Zacchini *et al.* (2009) found that 38.5 mg/L Cd sulfate negatively affected the willow clone's total leaf area. The study conducted by Tauqueer *et al.* (2016) found that the presence of Cd at a concentration of 0.225 mg/L and Pb at a concentration of 0.414 mg/L resulted in a significant decrease in the fresh and dry weights of *Alternanthera bettzickiana*. Additionally, Pb harm causes photosynthesis reduction, oxidative hazard, DNA harm, and undesirable in mitosis (Küpper 2017). The findings of the present study are consistent with the research conducted by Redovniković *et al.* (2017), which showed that different combinations of Cd (10, 25, and 50 mg/kg soil) and Pb (400,

800, and 1200 mg/kg soil) negatively affected leaves, stem, and root dry weights of *P. nigra* "Italica", and the most negative effect was to be found at the combination of Cd and Pb at high levels. Similarly, El-Mahrouk *et al.* (2021), studying *Jatropha curcas*, found that Cd at 14.4, 28.8, and 43.2 mg/kg soil and Pb at 247.7, 495.4, and 743.2 mg/kg soil in different combinations negatively affected vegetative traits in relative to untreated negative plants. In addition, El-Mahrouk *et al.* (2019, 2020), studying *Salix mucronata* and *Populus nigra*, respectively, concluded that Cd at 3.9, 7.8, 11.9, and 15.6 mg/kg soil, Cu at 14.4, 29.8, 47.7, and 63.6 mg/kg soil, and Pb at 50.0, 91.1, 132.1, and 173.3 mg/kg soil had significantly inhibition influences on the aerial organs and root traits of the two species, and the negative impact was parallel with the HM concentration in the soil. The retardation in root growth and element and water uptake imbalance causes development reduction, structure harmful, reduction in biochemical and physiological processes, which influence on biomass yield negatively (Kumar *et al.* 2017; Wu *et al.* 2018). Also, the root, stem, leaf, and dry biomass of *Clidemia sericea* showed a decrease under Hg, Cd, and Pb stresses (Durante-Yáñez *et al.* 2022). Furthermore, growth inhibition of *Schima superba*, Chinese sweetgum, and Chinese fir was presented at 6, 12, 24, and 36 mg Cd/kg soil (Wang *et al.* 2022). Additionally, Bhat *et al.* (2022) reported that a maximum reduction in the growth rate of *Spirodela polyrhiza* treated with Cu, Pb, and Cd at 0, 0.5, 1, 2, 4, and 8 mg was noticed in treatments of Cd, followed by Pb, then Cu. They added that the highest photosynthetic pigment levels were observed in control plants (untreated). In addition, photosynthetic pigment contents have been differently affected depending on plant species, potentially toxic element levels, and the toxicity degree of metals individually and mixed according to the studies of Fargašová and Molnárová (2010) on *Sinaps alba*, Chinmayee *et al.* (2012) on *Amaranthus spinosus*, Leal-Alvarado *et al.* (2016) on *Salvinia minima* Baker, and Zhang *et al.* (2020) on tobacco. Zhang *et al.* (2020) documented that HMs such as Cr, Ni, Pb, Cd and Zn affected species growth. The reduction in vegetative traits of *Jacaranda* can be attributed to the detrimental impact of Cd and Pb on leaf chemical composition because N, P, and K elements are essential to several compounds, *e.g.*, proteins, carbohydrates, amino acids, phospholipids, nucleic acids, and energy sources. Furthermore, the presence of cadmium (Cd) and lead (Pb) exerts a detrimental impact on the plasma membrane permeability (Sharma *et al.* 2010; Pourrut *et al.* 2011).

**Table 2.** Growth Traits of *Jacaranda* as Impacted by Cd and Pb Levels in the Soil after the Experimental Period

HMS Treatments (mg/kg dry soil)	Plant Height (cm)	Main Stem Diameter (cm)	Branches Number/Plant	Area per Leaf (cm <sup>2</sup> )	Leaf Greenness (SPAD)	Leaves Fresh Weight/Plant (g)
<b>Control</b>	164.7 $\pm$ 2.4a	1.36 $\pm$ 0.02 a	25.33 $\pm$ 1.2 a	205.57 $\pm$ 18.6 a	36.73 $\pm$ 2.5 a	53.44 $\pm$ 4.27 a
<b>LCd LPb</b>	136 $\pm$ 2.1 c	1.31 $\pm$ 0.03abc	17.67 $\pm$ 0.7 c	176.4 $\pm$ 15.3abc	30.2 $\pm$ 1.2 b	40.28 $\pm$ 0.88 cd
<b>MCd MPb</b>	123 $\pm$ 5.1 de	1.30 $\pm$ 0.02abc	16.67 $\pm$ 1.2 cd	139.77 $\pm$ 9.7d	27.77 $\pm$ 2 b	39.2 $\pm$ 3.724 cd
<b>HCd HPb</b>	118 $\pm$ 6.7 e	1.19 $\pm$ 0.04 c	15 $\pm$ 0.0 d	133.3 $\pm$ 3.7 d	27.03 $\pm$ 0.7 b	35.29 $\pm$ 0.48 d
<b>LCd HPb</b>	136 $\pm$ 2.5 c	1.32 $\pm$ 0.02 ab	16.67 $\pm$ 0.3cd	183.73 $\pm$ 9.5abc	28.9 $\pm$ 0.5 b	43.55 $\pm$ 1.02 bc
<b>HCd LPb</b>	130.7 $\pm$ 2.2 cd	1.24 $\pm$ 0.02 bc	17.67 $\pm$ 0.3 c	151.7 $\pm$ 6.0 cd	30.83 $\pm$ 1.1 b	39.2 $\pm$ 0.77 cd
<b>MCd HPb</b>	148.7 $\pm$ 0.9 b	1.36 $\pm$ 0.01a	20 $\pm$ 0.6 b	186.3 $\pm$ 2.1 ab	32.94 $\pm$ 3.4ab	51.99 $\pm$ 0.95 a
<b>HCd MPb</b>	158 $\pm$ 0.6 ab	1.35 $\pm$ 0.04 a	21 $\pm$ 0.6 b	159.5 $\pm$ 3.8 bcd	29.84 $\pm$ 1.9 b	48.28 $\pm$ 1.1ab
HMS Treatments (mg/kg dry soil)	Leaves Dry Weight/Plant (g)	Stems Fresh Weight/Plant (g)	Stems Dry Weight/Plant (g)	Roots Fresh Weight/Plant (g)	Roots Dry Weight/Plant (g)	Length of the Longest Root (cm)
<b>Cont.</b>	26.27 $\pm$ 1.4 a	126.01 $\pm$ 1.7 a	47.57 $\pm$ 4.5 a	106.47 $\pm$ 0.5 a	52.59 $\pm$ 1.6 a	55.67 $\pm$ 1.8 a
<b>LCd LPb</b>	21.91 $\pm$ 0.3 b	77.29 $\pm$ 2.2 d	33.84 $\pm$ 0.5 c	52.46 $\pm$ 0.7d	26.20 $\pm$ 1.8 d	40.50 $\pm$ 3.8 cde
<b>MCd MPb</b>	18.33 $\pm$ 2.4 b	81.92 $\pm$ 0.2 d	36.13 $\pm$ 0.3 bc	63.22 $\pm$ 1.2c	33.82 $\pm$ 0.4 c	36.23 $\pm$ 3.3 de
<b>HCd HPb</b>	18.28 $\pm$ 1.2 b	64.26 $\pm$ 1.03 e	28.17 $\pm$ 0.3 d	38.53 $\pm$ 1.2 e	23.62 $\pm$ 0.8d	34.23 $\pm$ 2.7 e
<b>LCd HPb</b>	21.68 $\pm$ 0.4 b	88.89 $\pm$ 0.8c	39.47 $\pm$ 0.2 b	59.02 $\pm$ 2.1 c	26.62 $\pm$ 1.6d	50.00 $\pm$ 1.7 ab
<b>HCd LPb</b>	19.90 $\pm$ 0.3b	82.01 $\pm$ 2.4 d	36.25 $\pm$ 0.5 bc	70.33 $\pm$ 1.9 b	33.70 $\pm$ 1.4 c	48.00 $\pm$ 1.7 bc
<b>MCd HPb</b>	26.14 $\pm$ 0.4 a	101.71 $\pm$ 2.0 b	44.63 $\pm$ 0.9 a	71.33 $\pm$ 2.1 b	40.02 $\pm$ 1.7 b	43.00 $\pm$ 0.6 bcd
<b>HCd MPb</b>	25.90 $\pm$ 0.9 a	104.69 $\pm$ 0.8 b	46.42 $\pm$ 0.3 a	72.74 $\pm$ 2.9 b	39.84 $\pm$ 1.3 b	41.23 $\pm$ 2.2 cde

Means  $\pm$  standard deviation (SD) (n = 3) with the same letters in a column are non-significant differences (P  $\leq$  0.05) according to Duncan's multiple range test.

## Leaf Chemical Analysis

It is necessary to determine leaf N, P, and K nutrient levels because the soil pH in which *Jacaranda* was grown increased after contamination of Cd and Pb metals from pH 8.5 to 9.1. This pH implied that the absorption of the vital elements *via* plant roots, particularly N, P, K, Mg, and Zn, is not enough. Our results indicated that *Jacaranda* grown in HMS polluted soil had significantly lower amounts of N, P, K, and total carbohydrates percentages than respective controls (Table 3), with some exceptions. Meanwhile, MCd MPb, LCd HPb, and MCd HPb treatments resulted in a non-significant lower P% compared to the control. Additionally, the applications of LCd LPb, MCd MPb, and HCd MPb recorded the same significant level of K% of control. Distinctly, leaf N, P, K, and total carbohydrate status depend on the concentration of HM in the application. This may be due to the synergistic effect of each HM used. Furthermore, lower significant levels of N, P, K, and total carbohydrates % were exhibited in HCd HPb treated plants. Such a treatment decreased N, P, K, and total carbohydrates by 25.35, 64.30, 32.26, and 19.89%, respectively, less than the control.

Foliage nutrient levels depend on soil or soil chemistry levels and the consanguinity of *Salix* species or clones (Mosseler and Major 2017). Heavy metals lead to hazards in plants *via* (i) an obstacle for uptake at root surface as a result to similarities with nutrient cations, like the competition between AS and P and Cd against Zn, (ii) a function collapse of necessary nutrients because of vital cations exclusion from their binding sites (Sharma and Dietz 2009; DalCorso *et al.* 2013). Also, reducing absorptions and transference of nitrate and nitrate reductase by Cd affect N metabolism (Lea and Miflin 2003).

Moreover, Küpper *et al.* (1996) documented that magnesium in the antenna and efficacy centers of chlorophyll can be substituted by Cu, which affects the composition and function of chlorophyll, causing a decrease in build-up carbohydrate. Moreover, the ADP or ATP phosphate group displaced by vital ions were reacted by Pb accumulation in the plant tissue, thus weakening essential nutrient uptake like Mg and Fe, and inducing CO<sub>2</sub> retardation because of stomatal closure (Pourrut *et al.* 2011). Previously, Pietrini *et al.* (2009) cleared various nutrients (*e.g.*, Fe, Zn, and Mg); their uptake, transport, and use in poplar clones were negatively affected by Cd SO<sub>4</sub> at 50 µM. Also, Cd impairs the Calvin cycle enzymes, photosynthetic rate, and metabolism of carbohydrates (Tang *et al.* 2017) and changes the metabolism of antioxidants (Khan *et al.* 2009). Cadmium also is an obstacle to the absorption of Ca, K, P, Mg, and water and retards nitrate transportation and uptake by affecting nitrate reductase. Likewise, El-Mahrouk *et al.* (2019) on *Salix mucronata* and El-Mahrouk *et al.* (2020) on *Populus nigra* reported that leaf N, P, K, and total carbohydrates of two species significantly reduced Cd CL2 at 20, 40, 60, and 80, respectively, and Pb acetate at 250, 450, 600, and 850 mg/kg soil, respectively, relative to the control.

**Table 3.** Effect of Soil Cd and Pb Levels on the Chemical Composition of *Jacaranda* Leaves Following the Experiment Period

HMS Treatments (mg/kg dry soil)	N (%)	P (%)	K (%)	Total Carbohydrates (%)
<b>Control</b>	1.42 $\pm$ 0.01 a	0.14 $\pm$ 0.01 a	1.55 $\pm$ 0.07 a	10.71 $\pm$ 0.1 a
<b>LCd LPb</b>	1.30 $\pm$ 0.03 bc	0.09 $\pm$ 0.002 bcd	1.30 $\pm$ 0.12 ab	8.78 $\pm$ 0.3 bc
<b>MCd MPb</b>	1.31 $\pm$ 0.05 b	0.10 $\pm$ 0.008 abc	1.30 $\pm$ 0.17 ab	8.64 $\pm$ 0.2 c
<b>HCd HPb</b>	1.06 $\pm$ 0.02 e	0.05 $\pm$ 0.001 d	1.05 $\pm$ 0.03b	8.58 $\pm$ 0.1 c
<b>LCd H Pb</b>	1.21 $\pm$ 0.02 cd	0.12 $\pm$ 0.002 ab	1.15 $\pm$ 0.09 b	8.99 $\pm$ 0.1 bc
<b>HCd LPb</b>	1.26 $\pm$ 0.01 bcd	0.07 $\pm$ 0.002 cd	1.10 $\pm$ 0.06 b	9.17 $\pm$ 0.1 b
<b>MCd HPb</b>	1.18 $\pm$ 0.01 d	0.10 $\pm$ 0.008 abc	1.25 $\pm$ 0.03 b	9.03 $\pm$ 0.04 bc
<b>HCd MPb</b>	1.21 $\pm$ 0.04 cd	0.07 $\pm$ 0.001 cd	1.3 $\pm$ 0.06 ab	8.84 $\pm$ 0.2 bc

Means  $\pm$  (SD) (n = 3) with the same letters in a column are non-significant differences (P  $\leq$  0.05) according to Duncan's multiple range test.

### Relationship between Cd and Pb Levels and Uptake in Plant Organs and their Soil Concentrations

The results in Table 4 show that the used Cd and Pb combinations were significantly elevated (P  $\leq$  0.05) in leaves (green leaf and fallen leaf), stems, and roots Cd and Pb contents and uptake, as well as in plant total absorption relative to control untreated plants. Also, the Cd and Pb levels of different plant parts strongly depend on their soil levels. Further, combinations containing high Cd levels (HCd HPb, HCd LPb, and HCd MPb) recorded higher leaf, stem, and root Cd concentrations than the other treatments. This was found in Pb content in the plant parts, where HCd HPb, LCd HPb, and MCd HPb combinations resulted in higher Pb content. Data indicated that contents of Cd and Pb were in the order of roots > stems >, and leaves under the most treatments.

Moreover, Cd or Pb transfer in various vegetative parts and total plant absorption were found to be dependent on their soil contents and plant organs. Higher significant Cd uptake in leaves stems, and roots resulted from applying HCd MPb treatment, besides HCd LPb treatment in the case of root uptake. Higher significant leaf, stem, and root Pb uptake was recorded for MCd and HPb treatment besides HCd and MPb in the case of root uptake. At the same time, total Cd and Pb plant uptake was significantly the highest at HCd MPb treatment (0.44 mg Cd/ plant) and MCd HPb (7.50 mg Pb/ plant), respectively. Generally, Cd and Pb uptake was in an order of roots > stem > leaves. The results indicated that the Cd and Pb contents of fallen leaves were negligible relative to their contents in the green leaves under the all-tested Cd and Pb combinations. The maximum significant content of Cd and Pb in the fallen leaves reached 0.03 (for the treatment content of high Cd level) and 0.08 (for the treatment of LCd HPb) mg/kg D.W. against 3.30 (HCd HPb) and 45.70 (MCd HPb) mg/kg D.W. in the green leaves, respectively.

As for Cd and Pb content and uptake in the plant organs, Samuilov *et al.* (2016) mentioned that Pb concentrations in *Populus tremula* x *P. alba* vegetative parts are conjunctive with their content in the soil. Previously, Zhivotovsky *et al.* (2011) documented that Cd and Pb accumulated in roots of several tree species more than in stems and leaves. Redovniković *et al.* (2017) reported that Cd and Pb accumulated in roots of *P. nigra* cv. 'Italica' rather than in leaves and stem when grown in Cd at 10, 25, and 50 mg/kg soil and Pb at 400, 800, and 1200 mg/kg soil. Likewise, Krajcarová *et al.* (2016) showed

that when treated with Cd, *Salix polaris* plant parts have different Cd concentrations. In studies of Tang *et al.* (2017) on *Salix matsudana*, Pb in all vegetative organs was in an order of roots > cuttings > twigs > leaves. Additionally, roots of *S. mucronata* and *P. nigra* have Cd and Pb content and uptake more than in stems or leaves (El-Mahrouk *et al.* 2019, 2020) of *Salix* and poplar, respectively. In addition, Durante-Yáñez *et al.* (2022) documented that the levels of Hg, Pb, and Cd in roots, stem, and leaves of *Clidemia sericea* indicated significant differences among the used treatments, and the roots had higher concentrations than leaves or stems. According to Tatian *et al.* (2023), Cd and Pb uptake by *Festuca* species was mainly in the roots rather than aerial parts. Also, Bhat *et al.* (2022) treated *Spirodela polyrhiza* with Cu, Pb, and Cd at 0, 1, 2, 4, and 8 mg/L and they reported that elements levels in the plant was influenced by its behavior. The plant's ability to accumulate these metals was directly related to the concentration of the metals in the soil, with higher concentrations leading to increased uptake by the plant. Furthermore, accumulation of HMs in the plant organs related to HM kind and plant variety. Accordingly, Rahman *et al.* (2022) revealed that the seasonal HMs contents in either leaves or bark of some tree species decreased in the order of Zn > Pb > Cu > Cd in the industrial and residential areas. They added that the tree species markedly showed significantly various capacities for HM accumulation. Cadmium and lead contents in fallen leaves were negligible. That may be due to Cd and Pb transported from leaves to stem and root at the senescence stage. Also, the root of *Jacaranda* had more accumulation of Cd and Pb than the leaves or stems. Hence, the potential environmental hazard posed from falling leaves appears to be minimal, as proposed by Baker (1981), pointing out that a number of species of deciduous plants move the stored HMs to their aerial parts prior to senescence. In addition, willow stand (*Salix viminalis* L. 'Orm') reduces the pollution hazardous to the wider habitat within leaf fall when they do not accumulate HMs in their leaves (Vervaeke *et al.* 2003).

Also, the green leaves of *Morus alba* grown in 40 mg Cd/kg soil had higher content of Cd than fallen leaves; in contrast, fallen leaves had more Cr in a level of 60 mg/kg soil and Ni in a level of 1200 mg/kg soil than the green leaves (Rafati *et al.* 2011). They added that green and fallen leaves of *P. alba* Cd uptake levels did not significantly differ when treated with 40, 80, and 120 mg Cd/kg soil because green leaves occupied their Cd uptake until the fall season. This means that built-up Cd did not undergo translocation into the stem and root structures. Additionally, *Jatropha curcas* grown under 40, 80, and 120 mg Cd nitrate/kg soil and Pb nitrate at 400, 800, and 1200 mg/kg soil in various combinations, the green leaves had a considerable amount of Cd and Pb more than fallen leaves in all used combinations ( El-Mahrouk *et al.* 2021).

**Table 4.** Effect of the Different Cd and Pb Combinations on their Concentrations and Absorptions in Various Plant organs, and Plant Total Uptake

HMS Treatments (mg/kg soil)	Leaves		Fallen leaves	Stems		Roots		Total plant uptake (mg/ plant)
	Content (mg <sup>-1</sup> kg D.W.)	Uptake (mg)	Content (mg <sup>-1</sup> kg D.W.)	Content (mg <sup>-1</sup> kg D.W.)	Uptake (mg)	Content (mg <sup>-1</sup> kg D.W.)	Uptake (mg)	
<b>Cd</b>								
<b>Control</b>	0.09 ±0.15 g	0.002±0.01d	0.01±0.01c	0.07 ±0.017e	0.003±0.01e	0.06 ±0.012d	0.003±0.001e	0.007±0.01e
<b>LCd LPb</b>	2.20 ±0.15 de	0.05 ±0.01 c	0.02±0.01b	1.70 ±0.20 d	0.06 ±0.01d	2.20 ±0.15 c	0.06±0.000cd	0.17±0.001d
<b>MCd MPb</b>	2.50 ±0.20 cd	0.05±0.01bc	0.02±0.01b	3.10 ±0.20 b	0.11 ±0.01 c	2.40 ±0.12 c	0.08±0.001cd	0.24 ±0.02 c
<b>HCd HPb</b>	3.30 ±0.15 a	0.06 ±0.01 b	0.03±0.01a	3.90 ±0.26 a	0.11 ±0.01 c	4.9 ±0.47 b	0.12±0.002bc	0.28±0.02bc
<b>LCd HPb</b>	1.60 ±0.17 f	0.03 ±0.01 d	0.02±0.01b	1.40 ±0.12 d	0.06±0.01de	2.00 ±0.06 c	0.05±0.001cd	0.014±0.01d
<b>HCd LPb</b>	2.70±0.12 bc	0.05±0.01bc	0.03±0.00a	3.90 ±0.15 a	0.14 ±0.01 b	5.80 ±0.43 a	0.20 ±0.002 a	0.39 ±0.03 a
<b>MCd HPb</b>	2.00 ±0.23 ef	0.05±0.00bc	0.02±0.00b	2.40 ±0.12 c	0.11±0.001c	1.70 ±0.20 c	0.07±0.001cd	0.23 ±0.02 c
<b>HCd MPb</b>	3.10 ±0.12 ab	0.08 ±0.00 a	0.03±0.00a	3.80 ±0.20 a	0.18±0.001a	4.50 ±0.17 b	0.18 ±0.002 a	0.44 ±0.03 a
<b>Pb</b>								
<b>control</b>	2.26 ±0.15 e	0.06±0.01d	0.03±0.01e	1.88 ±0.02d	0.09 ±0.01 e	4.50 ±0.28 e	0.24±0.02d	0.39±0.04f
<b>LCd LPb</b>	34.40±1.87bc	0.75±0.02bc	0.04±0.01d	21.65 ±0.32c	0.73 ±0.01 d	99.15 ±3.39 d	2.60 ±0.5 c	4.08±0.34e
<b>MCd MPb</b>	24.30 ±2.32 d	0.45 ±0.01d	0.05±0.01c	22.60 ±1.21c	0.82 ±0.01 d	105.3±4.14cd	3.56 ±0.2 b	4.82±0.43d
<b>HCd HPb</b>	40.40±2.63ab	0.74±0.03bc	0.08±0.01a	42.20 ±3.57a	1.19±0.02bc	125.7±4.02a	2.97 ±0.3 c	4.00±0.54d
<b>LCd HPb</b>	41.00±2.57ab	0.88 ±0.02 b	0.08±0.01a	41.90 ±2.14a	1.65±0.01ab	114.3±4.70b	3.04±0.6bc	5.58±0.51cd
<b>HCd LPb</b>	25.80±1.57cd	0.51±0.01cd	0.04±0.01d	28.45 ±1.27 b	1.03±0.01c	106.2±3.08d	3.58±0.03b	5.12 ±0.55 d
<b>MCd HPb</b>	45.70 ±2.56a	1.20 ±0.01 a	0.05±0.01c	40.70 ±2.57a	1.82±0.03a	109.4±3.30bc	4.38±0.03a	7.50 ±0.79 a
<b>HCd MPb</b>	29.00±1.39cd	0.75±0.02bc	0.06±0.01b	23.25±1.31bc	1.07±0.01c	108.4±3.87bc	4.32±0.06a	6.14 ±0.68 b

Means± (SD) (n = 3) with the same letters in a column are non-significant differences (P≤ 0.05). By Duncan's multiple rang test.

## BCF, TF, and TI

To estimate *Jacaranda* phytoremediation potential in Cd and Pb aggregation, BCF and TF were determined (Table 5). BCF was done to measure the transfer of ions from soil to different parts of the plant. According to the data, regardless of the control, Cd and Pb BCF shoots (BCFs) values under the all-tested HMS combinations had the same significant level. Moreover, the control plants recorded higher significant Cd BCF shoots (0.69) and Pb BCF shoots (0.56). In general, Cd and Pb BCFs values were decreased by raising the Cd and Pb concentrations in the used combinations. Regardless of the control treatment, higher Cd BCFs (0.27) was in LCd LPb treatment, and lower Cd BCFS (0.15) was in HCd LPb and MCd HPb. Also, higher Pb BCFs (0.22) was in LCd LPb, and lower Pb BCFs (0.09) was in MCd MPb. Also, BCF roots (BCFr) of Cd and Pb took a similar trend to their BCFs, with some exceptions. The control plants recorded the highest significant Cd and Pb BCFr of 0.26 and 0.61, respectively. Furthermore, regardless of the control, Cd BCFr under the used combinations of HMS have the same significant level ( $P \leq 0.05$ ), and maximum and minimum Cd BCFr (0.15 and 0.06) were in LCd LPb and MCd HPb, consecutively. Regarding Pb BCFr, also relative to control, all used combinations of HMs significantly reduced Pb BCFr. In addition, the treatments containing L Pb levels had higher significant Pb BCFr of 0.39 and 0.42 for LCd LPb and HCd LPb, respectively, with the same significant level. Meanwhile, under HCd HPb, LCd HPb, and MCd HPb, the movement of Pb from soil to roots was poor compared to the other treatments, and Pb BCFr was 0.17, 0.15, and 0.15, respectively, with the same significant level ( $P \leq 0.05$ ).

The uptake and aggregation of Cd and Pb in various *Jacaranda* parts are crucial factors limiting its phytoremediation. It is noted that Cd and Pb BCFs and BCFr values of the control treatment were significantly higher than the others, which may be attributed to the control soil having the lowest Cd and Pb concentrations. Whereas, the BCFs or BCFr represents the ratio of HM levels in plant organs to soil HM levels (Saraswat and Rai 2009; Zacchini *et al.* 2009). The results are similar to Redovniković *et al.* (2017) on poplar, who found that Cd and Pb BCF values were lower than the unit under Cd at 10, 25, and 50 mg/kg soil and Pb at 400, 800, and 1200 mg/kg soil. Also with an increase in soil Cd levels, the Cd bioconcentration factor (Cd BCF) was found to decrease. They concluded that the Pb BCF was lower (0.23) at the highest application levels of Pb, as previously clarified by Mette *et al.* (2014).

The present data showed that Cd and Pb BCF of shoots or roots were medium (BCF = 1- 0.1) with three exceptions. These exceptions were, 1.) Pb BCFs under MCd MPb (0.09); 2.) Cd BCFr under MCd MPb; and 3.) MCd HPb (0.08 and 0.06, respectively), consistent with the findings of Kabata-Pendias and Pendias (1999). Cadmium BCFr of *P. alba* and Cd BCFs or Cd BCFr of *Morus alba* in 40, 80, and 160 mg Cd/kg soil was lower than 1, and the values were reduced by increasing Cd level in soil (Rafati *et al.* 2011). The bioavailability of Pb in soil has been significantly ( $P \leq 0.05$ ) affected by some soil characteristics such as pH, CEC, and Ca levels (Zhang *et al.* 2019). In addition, in Pb, co-accumulation may have decreased Cd accumulation. Some studies are in accordance with the present results in which BCF < 1, such as El-Mahrouk *et al.* (2019) on *Salix mucronata* and (El-Mahrouk *et al.* 2020) on *P. nigra*. The latter authors found that Cd, Cu, and Pb BCF of leaves, stems, and roots were lower than 1 under 20, 40, 60, and 80 mg CdCl<sub>2</sub>/kg soil, 50, 100, 150, and 200 CuCl<sub>2</sub>/kg soil, and 250, 450, 650, and 850 mg Pb acetate/kg soil. In addition, Bhat *et al.* (2022) documented that BCF of Cu, Pb, and Cd decreased by

increasing their concentrations in *Spirodela polyrhiza* medium. Also, Rahman *et al.* (2022) found that metal accumulation index  $> 1$  and bioconcentration index  $< 1$  of Cd and Pb for aerial parts of *Azadirachta indica*, *Cassia fistula*, *Eucalyptus camaldulensis*, *Morus alba* and *Populus deltoides* grown in industrial and residential areas. The present data were in contrast to the finding of Domínguez *et al.* (2008), who found that Cd BCF leaves of *P. alba* were nearly 2 and (Zacchini *et al.* 2009) (BCF of aerial parts of *P. alba* L. clones 6K3 and 14P11 were 2.5 and 4.0). Migeon *et al.* (2009) reported leaf Cd BCF of about 1.39, 2.26, and 1.98 for *P. deltoides* x *P. nigra*, *P. tremula* x *P. tremuloides*, and *P. trichocarpa* x *P. deltoides*, in succession. Furthermore, Jakovljević *et al.* (2014) on poplar and Mette *et al.* (2014) on willow and poplar indicated that Cd BCF was higher than 1 and did not depend on soil Cd content. Moreover, Bhat *et al.* (2022) found that Cd and Pb BCF for *Spirodela polyrhiza* were 600 and 405, consecutively at 0.5 mg concentration. Additionally, Durante-Yáñez *et al.* (2022) on *Clidemia sericea* documented that higher BCF values were calculated for Cd ( $> 1$ ), because it is higher mobility than Pb and Hg, resulting in bioconcentrates more easily in plant roots (Huang *et al.* 2019).

The TF value was determined as a means to calculate ion transportation from roots to vegetative parts (shoots = leaves + stems) (Maiti and Jaiswal 2008). Where TF% is the ion level in shoot/root, data showed that Cd and Pb TF% under the used treatments exhibited different values among all combinations of Cd and Pb with significant levels ( $P \leq 0.05$ ) among some treatments. The Cd TF was higher than 1 ( $> 100$ ), while Pb TF was lesser than the unit ( $< 100$ ) under all treatments. The highest significant Cd TF% reached 266.7, 233.3, and 258.8 % for control, MCd MPb, and MCd HPb, respectively, with the same significant level. However, lower significant Cd TF% was 113.8 % for HCd LPb with the same significant level with LCd LPb, HCd HPb, and HCd MPb utilizations. The untreated negative plants and MCd HPb treated ones have recorded higher significant Pb migration from roots to shoots as registered Pb TF% of 92.0 and 79.0%, respectively. Conversely, lower significant Pb TF% was 44.5 and 48.2% for the treated plants with MCd MPb and HCd MPb, respectively.

Generally, the treatments containing high Pb levels caused more Pb movement toward shoots, in contrast to Cd translocation through plant organs. TF  $> 1$  for Cd and higher Cd TF values were obtained from low and medium Cd levels, and TF  $< 1$  for Pb and its higher values resulted from a high level of Pb, regardless of the control. This can be due to the least soil Cd and Pb concentrations, which are almost the main factors accounting for the substantial quantities of accumulation in the roots, which helps metals transportation during the plant detoxification mechanisms development by ions sequestration in the vacuole *via* union with ligands (proteins, organic acids, and peptides), causing more transference amounts (Nworie and Lin 2021; Sharma *et al.* 2021). Previously, Niu *et al.* (2007), Fulekar *et al.* (2009), and Pourrut *et al.* (2011) mentioned that Cd and Pb cellular displacement can affect the free levels, and therefore, metal transfer through the plant can be affected. The TF Pb values at different concentrations in the soil were not higher ( $< 1$  or  $< 100\%$ ); this may be due to its ability to sequester in the vacuole as a result of a Pb-phytoextraction complex (Lux *et al.* 2011). TF Cd  $> 1$  in the present results agrees with Zacchini *et al.* (2009), which made it clear that TF Cd of *P. alba* was nearly ten. Also, TF Cd in *P. alba* and *M. alba* was higher than 1 at 40, 80, and 160 mg Cd/kg soil (Rafati *et al.* 2011). Also, in the study of Redovniković *et al.* (2017), who reported that TF Cd  $> 1$  and TF Pb  $< 1$  in *P. nigra* cv. 'Italica' grown under 25 and 50 mg Cd/kg soil and 800 and 1200 mg Pb/kg soil. This is in accordance with the present results.

In contrast, El-Mahrouk *et al.* (2019, 2020) on *S. mucronata* and *P. nigra* found that TF Cd < 1 and TF Pb > 1 in the two species grown under 20, 40, 60, and 80 mg Cd/kg soil and 450, 650, and 850 mg Pb/kg soil. This was supported by Durante-Yáñez *et al.* (2022) on *Clidemia sericea*. BCF Cd or Pb of shoots or roots were medium, and TF Pb < 1, which indicates that the metal movement is limiting in plant tissue under higher levels of potentially toxic elements (Khan *et al.* 2009). Also, TF Pb < 1 agrees with the resistance mechanism, resulting in Pb mainly still in the root and a slow transfer to aerial organs (Fahr *et al.* 2013). This phenomenon may be attributed to a plant process that involves altering the permeability of the membrane and modifying the capacity of the cell walls or secretion of many chelating compounds to keep the physiological levels of vital elements and reduce the exposure to non-necessary HMS, which then alleviates harmful impacts to aerial parts (Kalaivanan and Ganeshamurthy 2016). Also, the majority of plant species record limitations in the movement of Pb and Cd from roots to shoots, so in several plants, the TF is lesser than 0.07 according to the findings of Małkowski *et al.* (2019). Thus, BCFs and TF for HMS are fundamental values necessary to assess a plant's efficiency for phytoextraction and phytostabilization. The *Jacaranda* plant, which shows Cd BCFs or CFr < 1 and TF Cd > 1, can be employed as a phytoextractor for Cd under the tested concentrations. At the same time, *Jacaranda* exhibited Pb bioconcentration factors (BCFs) or bioconcentration ratios (BCFr) less than 1, as well as transfer factors (TF) for Pb also less than 1, indicating its potential for Pb phytostabilization at the examined levels.

TI<sub>b</sub> and TI<sub>r</sub> were calculated to evaluate the *Jacaranda* tolerance against Cd and Pb and to estimate the growth development in the suggested HMS combinations (Table 5). A notable decrease in TI<sub>b</sub> and TI<sub>r</sub> values was observed across all tested Cd and Pb levels in the soil in comparison to the untreated plants, except TI<sub>r</sub> in LCd HPb and HCd LPb values, which take place at the same significant level of the control. Likewise, TI<sub>b</sub> and TI<sub>r</sub> values were lower than the unity (< 1) at all used Cd and Pb combinations, and the least value of either TI<sub>b</sub> (0.55) or TI<sub>r</sub> (0.62) was found in the HCd HPb treatment. This means that the *Jacaranda* plants were under stress, with a net reduction in biomass and stressed plant condition (Wilkins 1978), and the root length was limited.

The results indicated that TI<sub>b</sub> or TI<sub>r</sub> values differed under the various tested HMS combinations. Utmaian *et al.* (2007) reported that metal tolerances differed among 20 different willow and poplar clones treated by Cd and Zn. Despite the reduction in TI<sub>b</sub> or TI<sub>r</sub>, the *Jacaranda* plant has shown better tolerance under some tested HMS treatments, whereas TI<sub>b</sub> reached 0.88 and 0.89 in MCd HPb and HCd MPb and TI<sub>r</sub> got 0.90 and 0.86 in LCd HPb and HCd LPb. This implies that TI<sub>b</sub> or TI<sub>r</sub> is related to the type of metal and its level in the soil. Additionally, Wu *et al.* (2010) declared that at 4.35 g Cd/kg purple soil, there was a significant reduction in TI of *P. deltoides* x *P. nigra*. Dickinson *et al.* (1994) reported that metal resistance or absorption depended on species and clones of willow. The *S. viminalis* biomass or root tolerance index was < 1 at three  $\mu$ M Cu (NO<sub>3</sub>)<sub>2</sub> and at a ratio of Ca/Mg (20: 1 and 1: 10) (Mleczek *et al.* 2013). The TI<sub>b</sub> and TI<sub>r</sub> of each *P. nigra* or *S. mucronata* were less than the unity at the following CdCl<sub>2</sub> levels (20, 40, 60, and 80 mg/kg soil) and Pb acetate levels (250, 450, 650, and 850 mg/kg soil) (El-Mahrouk *et al.* 2019, 2020). El-Mahrouk *et al.* (2021) documented that tolerance index biomass or roots of *Jatropha curcas* was < 1 under different Cd and Pb combinations, except that Pb TI<sub>r</sub> was > 1 at 40 mg Cd nitrate + 400 mg Pb nitrate. Generally, BCF, TF and TI<sub>b</sub> or TI<sub>r</sub> are keys that can be estimated to define Cd and Pb hyperaccumulator. The plausible species for

phytoremediation could have a big accumulation quantity in its tissues (particularly in aerial organs) and greater metal tolerances (Shi *et al.* 2009). Therefore, *Jacaranda mimosifolia* emerges as a promising candidate for phytoremediation applications in soil contaminated with Cd and Pb at the examined levels.

## CONCLUSIONS

1. The study was done to estimate the behavior of *Jacaranda* growth and its efficiency in phytoremediation strategies under Cd and Pb stresses in various combinations.
2. Despite Cd and Pb treatments having impaired impacts on the growth and essential chemical parameters, *Jacaranda* plant could grow without exhibiting toxicity symptoms with increasing age, and the survival was 100% until the end of the experiment.
3. Cd and Pb contents and uptake in the plant parts were related to the metal concentration in the tested treatments. *Jacaranda* exhibited good tolerance against Cd and Pb, particularly at low and medium levels.
4. The Cd and Pb contents of fallen leaves were negligible relative to their contents in the green leaves; thus, the contamination damage worldwide from the leaf fall is expected to be relatively small.
5. Cd BCFr was  $< 1$ , and Cd TF was  $> 1$ ; therefore, the tested *Jacaranda* can be a suitable candidate for Cd phytoextraction. Pb BCFr and TF values were  $< 1$ ; thus, *Jacaranda* can be suitable only as a phytostabilizer for Pb remediation.
6. The experiment period was 16 months (1<sup>st</sup> April 2020 to 1<sup>st</sup> August 2021). It is beneficial to carry out studies for prolonged exposure in open fields and include environmental conditions for the long-life cycle of woody species to identify more realistic changes in growth traits and HMS accumulation and contents in the plant tissues.
7. *Jacaranda*-mediated phytoremediation offers economic feasibility and scalability advantages compared to conventional remediation methods. Its lower initial costs, adaptability to various environments, and long-term sustainability make it an attractive option for addressing soil contamination.
8. Future studies on *Jacaranda* will estimate the enzymatic activity, reactive oxygenates species (ROS), electrolyte leakage, total antioxidant activity, amino-acids, and pigments (Carotenoids, chlorophylls) because these measurements are strongly related to resistance mechanism against heavy metal stress.
9. The discovered species as hyperaccumulators for heavy metals (HMs) can acclimate them to grow under different environmental conditions.
10. Harvested organs of either long-life plants (trees and shrubs) or short-life plants (annual and genial herbs), which are intermediate to phytoremediation can be used in biogas production rather than being composted or burned on site.

**Table 5.** BCF Shoots, BCF Roots, TF%, TI<sub>b</sub>, and TI<sub>r</sub> of *Jacaranda* were Affected by Cd and Pb Levels in the Soil after the Experimental Period

HMS Treatments (mg/kg dry soil)	BCF Shoots		BCF Roots		TF (%)		TI Biomass	TI Roots
	Cd	Pb	Cd	Pb	Cd	Pb		
<b>Cont.</b>	0.69 $\pm$ 0.109a	0.56 $\pm$ 0.023a	0.26 $\pm$ 0.052a	0.61 $\pm$ 0.038a	266.7 $\pm$ 36.2a	92.0 $\pm$ 3.7a	1.00 $\pm$ 0.01 a	1.00 $\pm$ 0.01 a
<b>LCd LPb</b>	0.27 $\pm$ 0.002b	0.22 $\pm$ 0.005b	0.15 $\pm$ 0.001b	0.39 $\pm$ 0.002b	177.3 $\pm$ 22.5bc	46.4 $\pm$ 6.5de	0.64 $\pm$ 0.04de	0.73 $\pm$ 0.08cde
<b>MCd MPb</b>	0.19 $\pm$ 0.002b	0.09 $\pm$ 0.000b	0.08 $\pm$ 0.000b	0.21 $\pm$ 0.001c	233.3 $\pm$ 5.6ab	44.5 $\pm$ 0.2 e	0.70 $\pm$ 0.04d	0.65 $\pm$ 0.06de
<b>HCd HPb</b>	0.17 $\pm$ 0.001b	0.11 $\pm$ 0.002b	0.11 $\pm$ 0.001b	0.17 $\pm$ 0.001c	146.9 $\pm$ 10.5 c	65.7 $\pm$ 4.6cd	0.55 $\pm$ 0.02 e	0.62 $\pm$ 0.05 c
<b>LCd HPb</b>	0.21 $\pm$ 0.001b	0.11 $\pm$ 0.001b	0.14 $\pm$ 0.001b	0.15 $\pm$ 0.001c	150.0 $\pm$ 11.8 c	72.5 $\pm$ 0.3bc	0.70 $\pm$ 0.04cd	0.90 $\pm$ 0.05ab
<b>HCd LPb</b>	0.15 $\pm$ 0.001b	0.21 $\pm$ 0.004b	0.13 $\pm$ 0.001b	0.42 $\pm$ 0.003b	113.8 $\pm$ 5.1c	51.1 $\pm$ 4.4de	0.72 $\pm$ 0.05c	0.86 $\pm$ 0.03abc
<b>MCd HPb</b>	0.15 $\pm$ 0.001b	0.12 $\pm$ 0.001b	0.06 $\pm$ 0.001b	0.15 $\pm$ 0.001c	258.8 $\pm$ 50.9a	79.0 $\pm$ 3.4ab	0.88 $\pm$ 0.04b	0.77 $\pm$ 0.03bcd
<b>HCd MPb</b>	0.16 $\pm$ 0.001b	0.10 $\pm$ 0.002b	0.10 $\pm$ 0.001b	0.22 $\pm$ 0.002c	153.3 $\pm$ 6.5c	48.2 $\pm$ 5.2 e	0.89 $\pm$ 0.05b	0.74 $\pm$ 0.04cde

Means  $\pm$  (SD) (n = 3) have similar letters in a column with non-significant differences (P $\leq$ 0.05) by Duncan's multiple range test.

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