PHYTON-ANNALES REI BOTANICAE
ISSN: 0079-2047
Volume 66 No. 1 (2026)
https://phyton-annales.com

Growth and physiological response of *Ziziphus jujuba* under Cadmium and lead stress: A case study under greenhouse conditions

Ghulam Muhammad Ehsan[a], Muhammad Ayyoub Tanvir[a], Fahad Rasheed[a*], Muhammad Imran Khan[b], Muhammad Kashif [c]

[a]*Department of Forestry and Range Management, University of Agriculture, Faisalabad, Pakistan*
[b]*Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan*
[c]*Department of Mathematics and Statistics, University of Agriculture, Faisalabad, Pakistan*

[*]*Corresponding authors: Dr. Fahad Rasheed, fahad.rasheed@uaf.edu.pk*

Abstract

Cadmium (Cd) and lead (Pb) contamination in the water, soil, and/or in atmosphere exert drastic negative impacts on the environment. Since phytoremediation is an ecofriendly approach, therefore, growth, biomass production, Cd and Pb accumulation and translocation factor was evidenced in *Ziziphus jujube*, an important multipurpose tree species. Results showed an overall decreasing trend in growth of *Ziziphus jujuba* in all the treatments (Cd, Pb or combination of both). The highest decrease in plant height (67.7%), stem diameter (65.1%) and total biomass (65.4%) was recorded in Cd2 + Pb2 (Cd2, 8mg/kg; Pb2 80mg/kg). Overall, the HM contents were the highest in roots followed by stem and leaves whether Cd and Pb were applied separately or in combination. However, in treatments where Cd and Pb were applied in combination, the absorption of both HM decreased significantly as compared to the treatments where Cd and Pb was applied separately. The translocation factor remined less than one in almost all the treatments for both Cd and Pb, indicating low mobility of both HMs from root to shoot. These findings suggest that although growth was negatively affected but high Cd and Pb accumulation in roots and a TF of less than one, makes *Ziziphus jujube* a suitable candidate for phytoremediating soils contaminated with Cd and Pb.

Key words: Phytoremediation, Growth and biomass production, heavy metals, translocation factor

Introduction

Pakistan is water deficit country with poorly maintained water storage facilities and high volume of wastewater (Nasreen *et al*. 2020). Municipal wastewater usually contains HMs and is commonly used as irrigation water to grow edible crops leading to contaminated food production especially in the developing countries (Othman *et al*. 2021). Globally, phytoremediation technique is used to cope with this issue as an alternate option for the removal toxic pollutants from the soil (Sharma *et al*. 2022). This technique helps retards erosion as well reduces the entry of heavy metals (HMs) to underground water reserves through seepage and percolation (Ali *et al*. 2023).

Among the HMs, cadmium (Cd) and lead. (Pb) are important due to their high mobility, easily accumulation and toxicity to plants. The continuous addition of Cd and Pb in soil can be dangerous for life (Hayat *et al*. 2019). Once Cd and Pb becomes the part of the food chain it can cause cancer, renal failure and mental abnormalities (Fatima *et al*. 2019). In plants, Cd and Pb stress results in decrease in plant growth, which is linked to a decrease in stomatal conductance, transpiration rate and decrease in chlorophyll pigments (Chandra and Kang, 2016). Furthermore, other studies have related the decrease in growth under Cd and Pb stress to over production of oxidants (hydrogen peroxide) and ethylene (Aslam *et al*. 2021). Cd and Pb stress also disturb the absorption of the vital nutrients (potassium, magnesium, calcium and phosphorous) in plants (Huang *et al*. 2017).

Ziziphus jujuba (Beri) is a well-known multipurpose tree (fruit, diseases treatments and wind breaks) (Guo *et al*. 2017). Therefore, owing to the economic importance and suitability of *Ziziphus jujuba* under arid to semi-arid environment, this study was designed to evaluate the growth, phytoremediation potential and translocation of HMs (Pb and Cd) in *Ziziphus jujuba*. Furthermore, translocation factor (TF) of Cd and Pb was also evidenced since TF indicates the mobility of HMs in various plant sections (roots and shoot). Studies have shown that a TF of less than 1 indicates low mobility of HM from root to shoot and vice versa and such species are considered suitable for phytoremediation (Rasheed *et al*. 2019; Li *et al*. 2022). Therefore, this study aims to evidence growth, biomass production, heavy metal accumulation in various plant sections in *Ziziphus jujube* along with TF. The outcome of this research will provide much needed data related to suitability of *Ziziphus jujuba* for phytoremediation of soils contaminated with Cd and Pb.

Materials and Methods

A field trial was setup in Forestry Research Area, Department of Forestry and Range Management (DFRM), University of Agriculture Faisalabad (UAF), Pakistan. A total of 27 pots were arranged by following CRD with five replicates and nine treatments (Table 1). Cadmium chloride ($CdCl_2$) and lead acetate Pb $(CH_3COO)_2$ were used to spike soil with Cd and Pb. The duration of the experiment was six months.

Growth and biomass production in various plant sections

At the end of the experiment, shoot length and collar diameter of *Ziziphus jujuba* were measured by a measuring tape. Thereafter, plants were excised and fresh and/or dried biomass of shoot (leaf + stem) and root weas measured by using a potable weight balance. The plant samples were dried in a heat oven at 70°C for 24 hours.

Heavy metal concentration in plant sections

Plant sample of 0.5g weight was taken, and digestion was done using di-acid (HNO_3 and $HC1O_4$) in a ratio of 2:1 followed by filtration. Subsequent supernatant was used to measure the concentration of Pb and Cd using Atomic Absorption Spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Japan). The limits of detection for Cd and Pb were 0.014 mg kg^{-1} and 0.011 mg kg^{-1} respectively.

Translocation factor

The translocation factor (TF) of Cd and Pb from roots to shoot was calculated using the following equation (Rasheed *et al*. 2020).

$$TF = \frac{HMs\ (\text{mg}\ kg^{-1})\ )}{HM_R(\text{mg}\ kg^{-1})}$$

where HMs and HM_R are heavy metal concentration in the shoot (leaves + stem) and root, respectively.

Statistical analysis

Normality of the data was tested using Q-Q plots and all traits were analyzed using one-way ANOVA for treatment effect. The Tukey's honestly significance difference (HSD) test was used to compare the treatment means which are expressed with their standard error, at 5% level of confidence. All the analysis were done in STATISTICA 12.5 (Maison Alford, France)

Results and Discussion

Effect of Pb and Cd on growth attributes

Results showed a significant reduction in the growth attributes (plant height, diameter, shoot, root, and total biomass) of *Ziziphus jujuba* (Table 2). The highest decrease in plant height (67.7%), stem diameter (65.1%), shoot dry weight (62.7%), root dry weight (74.2%), and total dry weight (65.3%) was evidenced in Cd2+Pb2 (Table 2). These results are in lines earlier reports highlighting the synergistic phytotoxic effects of heavy metals on plant growth and biomass production (khan *et al*., 2022). In previous studies, reductions in plant growth are attributable to disruptions in cell division, inhibition of enzymatic activity, and hormonal imbalances (Sharma *et al*. 2022). Since Cd and Pb can disrupt cell division, alter hormonal regulation, and interfere with nutrient uptake, leading to impaired development, growth inhibition under heavy metal exposure is also attributed to interference of heavy metal with essential physiological processes (Shankar, 2020). However, relatively less decline in root dry weight observed in this study which suggests that *Zizyphus jujuba* prioritizes root development under duress, enhancing survival of *Ziziphus jujuba* in Cd and Pb contaminated environments.

Concentration of Pb and Cd in different plant sections

Results showed that the highest increase in the concentration of cadmium was found in roots followed by stem and leaves (Figure 1). The concentration of Cd was the highest in roots and stem under Cd2 (4.31 and 2.76 folds; Figure 1B,C). In leaves, the highest concentration of HM (Cd and Pb) was found in Cd2+Pb1 as compared to control (Figure 1A). These results suggest that although the absorption of Cd was the highest in roots when applied alone, but the absorption of Cd and Pb decreased when applied in combination. Similar was the pattern for Pb where the increase in the concentration of Pb was the highest in roots under Pb2 (36.43 folds) followed by stem and leaves under Pb2 (9.7 folds, 24.3 folds, respectively) as compared to control (Figure 1(F). Among the treatments where Pb was applied in various combinations with Cd, the highest increase in the concentration of Cd and Pb was found in roots of plants under Cd1+Pb2 (31.4 folds) by followed by in stem and leaves (19.4 and 7.3 folds, Figure 3). These results suggest that although the absorption of Cd and Pb was the highest in roots when applied alone, but the absorption of Cd and Pb decreased significantly when applied in combination. Overall, the high concentration of HMs was found in roots as compared to leaves, suggest a root accumulation strategy in *Ziziphus jujuba*. This strategy that reduces heavy metal mobility from roots to leaves has also been observed in other species (Günthardt-Goerg *et al*., 2023). High accumulation of Cd and Pb in roots rather shoot (leaves and stem) also indicate that *Ziziphus jujuba* minimize metal toxicity in photosynthetic tissues like leaves. These mechanisms in plant species immobilize heavy metals in root tissues, reducing their bioavailability and preventing entry into the food chain is preferred while selection species for phytoremediation (Wang *et al*. 2023).

Translocation factor

Translocation factor (TF) for Cd and Pb was found less than 1 in almost all the treatments (Table 2), again indicating a restricted movement of Cd and Pb from roots to aerial parts (stem and leaves). Low TF values are characteristic of phytostabilizing species that accumulate heavy metals in their root tissues (Li *et al*. 2022). This strategy protects aboveground tissues from toxic damage and prevents metals from entering the food chain through leaves or fruits. Similar low translocation factor values (less than 1) have been reported in *Morus alba* and *Conocarpus lacifolius* under heavy metal exposure, emphasizing that restricted translocation is a common adaptation in woody plants (Rasheed *et al*. 2019; El-Keblawy *et al*. 2024). Therefore, a TF of <1 found for *Zizzphus* jujuba makes this species a suitable candidate for phytoremediation of soil contaminated with Cd and Pb.

Conclusion

Ziziphus jujuba exhibited substantial decrease in growth and biomass production under Cd and Pb stress, with the most detrimental effects observed under combined exposure. Highest concentration of Cd and Pb was found in roots followed by stem and leaves, which showed a limiting translocation of Cd and Pb from roots to leaves. Although *Ziziphus jujuba* showed negative impact on growth but survival, high concentration of HMs in roots and a TF of less than 1 underscore its suitability for phytoremediation potential in soils contaminated with Cd and Pb.

Acknowledgement

The authors are highly thankful to the Higher Education Commission, Islamabad for providing funding (PIN NO. 518-77947-2AV5-041-50042807) to Mr. Muhammad Ghulam Ehsan, Ph.D. scholar for this research.

References

Aslam M, Aslam A, Sheraz M, Ali B, Ulhassan Z, Najeeb U, Zhou W, Gill RA 2021. Lead toxicity in cereals: mechanistic insight into toxicity, mode of action, and management. Frontiers in plant science. 11:587785.

Ali, M.H. M.I. Khan, M. Naveed and M.A. Tanvir 2023. Microbe-Assisted Rhizoremediation of Hydrocarbons and Growth Promotion of Chickpea Plants in Petroleum Hydrocarbons-Contaminated Soil. Sustainability. 15:6081.

Chandra R, Kang H 2016. Mixed heavy metal stress on photosynthesis, transpiration rate, and chlorophyll content in poplar hybrids. Forest Science and Technology. 12(2):55-61.

El-Keblawy A, Almehdi AM, Elsheikh EA, Abouleish MY, Sheteiwy MS, Galal TM 2024 . Native desert plants have the potential for phytoremediation of phytotoxic metals in urban cities: implications for cities sustainability in arid environments. Scientific reports. 14(1):13761.

Fatima G, Raza AM, Hadi N, Nigam N, Mahdi AA 2019. Cadmium in human diseases: It's more than just a mere metal. Indian Journal of Clinical Biochemistry. 34(4):371-8.

Günthardt-Goerg MS, Vollenweider P, Schulin R 2022. Metal accumulation and biomass production in young afforestations established on soil contaminated by heavy metals. Plants. 11(4): 523.

Guo, S., J.A. Duan, Y. Li, R. Wang, H. Yan and D. Qian. 2017. Comparison of the bioactive components in two seeds of Ziziphus species by different analytical approaches combined with chemometrics. Front. Pharmacol. 8:609.

Huang D, Gong X, Liu Y, Zeng G, Lai C, Bashir H, Zhou L, Wang D, Xu P, Cheng M, Wan J 2017. Effects of calcium at toxic concentrations of cadmium in plants. 245(5):863-73.

Hayat MT, Nauman M, Nazir N, Ali S, Bangash N 2019. Environmental hazards of cadmium: past, present, and future. In Cadmium toxicity and tolerance in plants. Academic Press. 163-183.

Khan N, Bolan N, Clark I, Meier S, Lewis D, Sánchez-Monedero MA 2023. Synergistic effect of multiple metals present at slightly lower concentration than the Australian investigation level Can induce phytotoxicity. Land. 16;12(3): 698.

Li X, Xiao J, Salam MM, Chen G 2022. Evaluation of dendroremediation potential of ten Quercus spp. for heavy metals contaminated soil: A three-year field trial. Sci.Total Environ. 851:158232.

Nasreen S, Ashraf MA 2020. Inadequate supply of water in agriculture sector of Pakistan due to depleting water reservoirs and redundant irrigation system. Water conservation & management. 5(1):13-9.

Othman YA, Al-Assaf A, Tadros MJ, Albalawneh A 2021. Heavy metals and microbes accumulation in soil and food crops irrigated with wastewater and the potential human health risk: a metadata analysis. Water. 13(23):3405.

Rasheed F, Zafar Z, Waseem ZA, Rafay M, Abdullah M, Salam MM, Mohsin M, Khan WR 2019. Phytoaccumulation of Zn, Pb, and Cd in Conocarpus lancifolius irrigated with wastewater: does physiological response influence heavy metal uptake?. International journal of phytoremediation. 22(3): 287-94.

Shankar SR 2020. Effect of heavy metals on growth and development of cultivated plants with reference to cadmium, chromium and lead–a review. Journal of Stress Physiology & Biochemistry. 16(3):84-102.

Sharma A, Kapoor D, Gautam S, Landi M, Kandhol N, Araniti F, Ramakrishnan M, Satish L, Singh VP, Sharma P, Bhardwaj R 2022. Heavy metal induced regulation of plant biology: Recent insights. Physiol. Plant. 174(3): 13688.

Wang Y, Wang X, Deng Q, Mao M, Xia H, Wang J, Lv X, Wang Y, Zhang H, Zhang XA, Luo X 2023. Living plants and straws of four hyperaccumulator plants increase the cadmium uptake of Ziziphus acidojujuba seedlings. International Journal of Environmental Analytical Chemistry. 103(8):1804-16.

PHYTON-ANNALES REI BOTANICAE
ISSN: 0079-2047
Volume 66 No. 1 (2026)
https://phyton-annales.com

Tables

Table 1. Physical and chemical attributes of soil before spiking.

Properties (Units)	Values
Soil texture %	Sandy loam (sand 50, silt 27, clay 23)
Ph	6.80 ± 0.01
Available P (mg kg^{-1})	5.20 ± 0.35
Available N (mg kg^{-1})	132 ± 4.50
Extractable K (mg kg^{-1})	92.2 ± 2.10
Cd (mg kg^{-1})	0.35±0.01
Pb (mg kg^{-1})	2.19±0.14

PHYTON-ANNALES REI BOTANICAE
ISSN: 0079-2047
Volume 66 No. 1 (2026)
https://phyton-annales.com

Table 2. Stem, root, total biomass and translocation factor of *Zizyphus jujuba* grown on contaminated soil. All means are presented with their standard errors, and small letters represent the significant differences among various means. Treatment effect (T-effect) was taken significant at P<0.05.

Treatments	**Plant height (cm)**	**S. diameter (cm)**	**Shoot biomass (g)**	**Root biomass (g)**	**Total biomass (g)**	**TF (Cd)**	**TF (Pb)**
C	210±4.36a	72.33±2.40 a	28.16±0.92a	8.53±0.41a	36.7±0.90 a	0.95±0.07	1±0.01
Cd1	154±3.63b	59±2.08b	23.2±0.52b	6.9±0.20b	30.1±0.68 b	0.91±0.08	1±0.01
Cd2	106±2.20de	43.66±2.40 c	17.4±1.07c	5.6±0.26bc	23±1.22 c	0.89±0.02	1.29±0.05
Pb1	165±4.16b	60.33±1.45 b	22.6±0.72b	6.16±0.14bc	28.7±0.57 b	1.05±0.05	0.92±0.06
Pb2	125±1.54c	38±1.52cd	16.36±1.13cd	5.1±0.17cd	21.4±0.95 c	1.06±0.06	0.82±0.06
Cd1+Pb1	100±0.88de	43.33±1.85 c	17.13±0.93c	6.03±0.29bc	23.2±1.10 c	0.96±0.04	1.13±0.06
Cd1+Pb2	88.9±3.50ef	31±1.15de	13.63±0.42e	3.9±0.28d	17.5±0.46 d	0.98±0.05	0.89±0.08
Cd2+Pb1	78.4±3.53fg	33.66±1.45 de	14.8±0.79de	5.06±0.29cd	19.8±0.96 d	0.93±0.03	1.10±0.04
Cd2+Pb2	65.7±4.31g	23.33±2.33 e	10.5±0.73e	2.2±0.20e	12.7±0.55 e	1.02±0.02	0.89±0.05
T-effect	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P = 0.001	P = 0.001

PHYTON-ANNALES REI BOTANICAE
ISSN: 0079-2047
Volume 66 No. 1 (2026)
https://phyton-annales.com

Figure 1: Accumulation of Cd and Pb in leaves **(A),** stem **(B)** and roots **(C)** after 180 days of experiment in *Zizyphus jujuba* saplings. Each bar represents the means values and standard errors under each treatment and small letters indicate the pairwise differences between means. Tests were taken significant $P \leq 0.05$.