



Cumulative Ability of *Juniperus phoenicea* Trees for Some Heavy Metals in Shahat Forest, Libya.

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Abstract:

This study was carried out in Shahat Forest to evaluate the capacity of *J. phoenicea* to absorb and store heavy metals in its tissues. Concentration of heavy metals, including (Zn), (Fe), (Cd) and (Pb), were measured in both aerial parts and root parts of the plant, as well as in the soil at a depth of 0-40 cm beneath the trees. Importantly, the concentrations of these heavy metals were found to be within the acceptable limits set by (WHO). The findings indicated that *J. phoenicea* exhibited notable efficiency in the uptake and accumulation of heavy metals, with (BAF) for Zn, Fe, Cd, and Pb recorded at 12, 6, 2.9, and 2.2, respectively. Additionally, the (BCF) were greater in the aerial parts than in the root parts, and the (TF) was greater than 1 for all heavy metals. As a result, *J. phoenicea* can be regarded as a promising candidate for bioaccumulation with phytoextraction strategy. Statistical analysis indicated significant differences ($p < 0.05$) in heavy metal concentrations among the different parts of the plant.

Keywords: *Juniperus phoenicea*, Cumulative, Ability, Heavy Metals, Shahat.

القدرة التراكمية لأشجار العرعر الفينيقي *Juniperus phoenicea* لبعض المعادن

الثقيلة في غابة شحات، ليبيا

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الملخص:

أجريت هذه الدراسة في غابة شحات لتقييم قدرة نبات العرعر الفينيقي على امتصاص وتراكم المعادن الثقيلة داخل أنسجته. تم قياس تراكيز المعادن الثقيلة، (Zn)، (Fe)، (Cd)، (Pb)، في كل من الأنسجة الخضرية والجذرية لنبات العرعر الفينيقي وكذلك في التربة على عمق (0 - 40 سم) تحت الأشجار. ومن الجدير بالذكر أن التراكيز المقاسة لهذه المعادن الثقيلة ظلت ضمن الحدود المسموح بها لمنظمة الصحة العالمية. أشارت النتائج إلى أن العرعر الفينيقي أظهر كفاءة ملحوظة في امتصاص وتراكم المعادن الثقيلة، حيث كان معامل التراكم الحيوي (BAF) Bio Accumulation Factor (Zn، Fe، و Cd، و Pb) 12، 6، 2.9، و 2.2 على التوالي. أيضا كانت قيمة معامل التركيز الحيوي (BCF) أعلى في الأجزاء الخضرية مقارنة بالأجزاء الجذرية وكذلك قيمة معامل انتقال العناصر (TF) Translocation Factor كانت أكبر من 1 لكل العناصر. وبناءً على ذلك، يمكن اعتبار ان العرعر الفينيقي مراكماً حيوياً يتبع استراتيجية الاستخلاص الحيوي phytoextraction. كما أظهر التحليل الإحصائي وجود اختلافات معنوية (P < 0.05) في تراكيز العناصر الثقيلة بين أجزاء النبات المختلفة.

الكلمات المفتاحية: العرعر الفينيقي، *Juniperus phoenice*، القدرة التراكمية، التلوث بالمعادن الثقيلة، شحات.

1.Introduction

The concentration of heavy metals in the environment has been increasing each year (Ali *et al.*, 2013). The presence of heavy metals in agricultural soils presents considerable environmental challenges and poses a significant threat to life on Earth. The presence of heavy metals into the food chain is associated with various health risks for humans (Ghosh & Singh, 2005; Sarwar *et al.*, 2017; Bahiru & Yegrem, 2021). In natural ecosystems, heavy metals are recognized for their carcinogenic and mutagenic properties (Gola *et al.*, 2016). Additionally, their non-biodegradable nature contributes to their persistence in the environment, facilitating the rapid accumulation of dangerous levels (Chandrajith *et al.*, 2005; Taghipour & Mosaferi, 2013; Suman *et al.*, 2018; Khan *et al.*, 2023). Plants have been recognized as effective biomonitors for evaluating the rise in heavy metal levels in the atmosphere (Cesur *et al.*, 2021). Plants possess the capability to absorb heavy metals from their surroundings, often accumulating these metals at concentrations that surpass those present in the soil. However, the extent of toxic



metal accumulation varies significantly among different plant species (Zhao *et al.*, 2014). Additionally, plants are integral to ecosystems, as they facilitate the transfer of elements from the abiotic environment to the biotic one (Hu *et al.*, 2014). Urban vegetation plays a vital role in environmental assessment and remediation by extracting heavy metals from the ecosystem (Young *et al.*, 2014). To counteract heavy metal toxicity, plants generally utilize two primary defense strategies: avoidance and tolerance. These strategies enable them to maintain intracellular heavy metal concentrations at levels that are not harmful (Hall, 2002; Dalvi & Bhalerao, 2013; Yu *et al.*, 2022; Kraj *et al.*, 2021). The existence of a metal exclusion mechanism creates a barrier between the root and shoot systems, thereby restricting the uptake of heavy metals from the soil into the roots. This mechanism is essential for protecting the aerial portions of the plant from the adverse effects of heavy metals by limiting their absorption and subsequent transport from the roots to the shoots (Raskin *et al.*, 1994; Hall, 2002; Dalvi & Bhalerao, 2013). Plants can be categorized based on their capacity for metal accumulation into three distinct groups: heavy-metal accumulators, heavy-metal excluders (or non-accumulators), and indicator species. Heavy-metal-accumulating plants demonstrate a concentration ratio of metals within their tissues that exceeds 1 when compared to the soil concentration. Conversely, non-accumulating plants exhibit a markedly lower ratio, while indicator plants maintain a ratio that approximates 1 (Cunningham & Ow, 1996; Gleba *et al.*, 1999; McIntyre, 2003; Ghosh & Singh, 2005; Rascio & Navari-Izzo, 2011; Yan *et al.*, 2020; Khan *et al.*, 2023; Chitimus *et al.*, 2023; Kord *et al.*, 2024). The efficacy of phytoremediation is closely linked to a plant's ability to absorb metals, transport them from the roots to aerial parts, and sequester them within specific cellular compartments. As a result, a common approach to enhance metal accumulation involves the introduction of genes that code for metal transporters (Venegas-Rioseco *et al.*, 2021). In the realm of phytoremediation, plants utilize a variety of strategies, including (i) phytostabilization—using plants to reduce heavy metal bioavailability in soil, (ii) phytoextraction—using plants to extract and remove heavy metals from soil, (iii) phytovolatilization—using plants to absorb heavy metal from soil and release into the atmosphere as volatile compounds, and (iv) phytofiltration—using hydroponically cultured plants to absorb or adsorb heavy metal ions from groundwater and aqueous waste (Tangahu *et al.*, 2001; Yan *et al.*, 2020; Chitimus *et al.*, 2023). Phytostabilization and



phytoextraction represent the two principal methodologies employed by plants for the remediation of heavy metal contamination (Ali *et al.*, 2013; Hao *et al.*, 2014; Yan *et al.*, 2020). Phytostabilization is characterized by the ability of metal-tolerant plant species to immobilize heavy metals within the soil matrix, thereby reducing their bioavailability and inhibiting their migration into surrounding ecosystems. This process effectively diminishes the potential for heavy metals to enter the food chain. Mechanisms facilitating phytostabilization include precipitation or reduction of metal valence in the rhizosphere, uptake and sequestration within root tissues, and adsorption onto the cell walls of roots (Gerhardt *et al.*, 2017). A notable advantage of phytostabilization, in contrast to phytoextraction, is that it does not require the removal of toxic biomass (Marques *et al.*, 2009; Wuana & Okueimen, 2011; Yan *et al.*, 2020). Conversely, phytoextraction involves the absorption of pollutants from soil or water by plants, which then transport and accumulate these contaminants in their aboveground biomass (Jacob *et al.*, 2018). Recently, phytoextraction has gained prominence as a crucial technique in phytoremediation for the extraction of heavy metals and metalloids from contaminated soils (Sarwar *et al.*, 2017). Unlike phytostabilization, which retains heavy metals within the root zone without their permanent removal, phytoextraction provides a conclusive method for the elimination of heavy metals from polluted environments, thus rendering it suitable for commercial applications (Yan *et al.*, 2020; Siyar *et al.*, 2022).

The Cupressaceae family exhibits a remarkable efficiency in the translocation of nutrients, particularly heavy metals, from the shoot to the plant's aerial parts. Transfer factor (TF) values greater than 1 for this family signify a robust ability for metal accumulation. Within the Cupressaceae, the genus *Juniperus* encompasses around 75 to 80 species, rendering it the second largest genus among conifers. Species of *Juniperus* are commonly found in semiarid regions and are widely employed in landscaping, timber production, and medicinal uses. *Juniperus phoenicea* L. is native to various regions of the Mediterranean basin, the Canary Islands, and North Africa (Abu-Darwish & Ofir, 2014; Angelova, 2022; Asbabou *et al.*, 2024). *Juniperus* acts as a significant bioindicator for heavy metal pollution, as the concentration of heavy metals in its tissues is directly related to the levels of these metals in the surrounding soil (Binxhija & Ylli, 2021). Studies have shown that *Juniperus* species can effectively absorb and



accumulate toxic heavy metals from contaminated sites, thereby positioning them as potential biomonitors or accumulators of environmental pollutants (Achak *et al.*, 2009; Diaconu *et al.*, 2009; Farahat & Linderholm, 2015; Cesur *et al.*, 2021; Siyar *et al.*, 2022; Kord *et al.*, 2024).

This study was conducted in the Shahat Forest to assess the capacity of *J. phoenicea* to accumulate heavy metals from contaminated soil. Additionally, what strategies and mechanisms will *J. phoenicea* employ to manage heavy metal exposure?

2. Material and methods

2.1. Study area: The study was conducted during 2023, in a site exposed to sewage in a forest inside Shahat city, located (32°49'40"N 21°51'44"E) which has a mild climate that tends to be warm and rainy in winter and hot in summer; where maximum temperature in the studied area varies from 35.3°C in summer to 20.1°C in winter. Minimum temperature ranges from 20.2°C in summer and 7.5°C in winter. (Othman & Al-Habbat, 2023).

2.2. Sample collection: Five mature *J. phoenicea* trees were selected to conduct this study, fresh samples of *J. phoenicea* trees from both aerial and root part, soil parts at depth (0 cm - 40 cm) were collected in polyethylene bags and transported to the laboratory for analysis. Five macro elements including total Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca) and Sodium (Na), four heavy metals including zinc (Zn), Iron (Fe), cadmium (Cd) and lead (Pb) were analyzed in the samples of tree and soil parts.

2.3. Sample Preparation: The Collected samples were homogenized and crushed into small particles, decomposed by dry digestion method for the determination of various metals. First of all, the crucibles and the glass wares used in the experiment were washed with distilled water and then dried in oven. Weight of each crucible was made constant by keeping it in muffle furnace at 750 °c for one hour. Then transferred it to desiccator and weighted it. The purpose was to remove all the moisture. This action was repeated till the weight became constant. A known quantity, 2g of each sample (aerial part, root part of trees and soil parts) was introduced into the Porcelain crucible. The crucibles were burned at around 200 °C until the end of organic matter smoke generation, then crucibles kept in muffle furnace at 600 °C for 5 hours, cooled to the room temperature in the

desiccator for 40 minutes. The obtained white ash is moistened with a few drops of de-ionized water, an aliquot of 2.0 mL of concentrated HCl are added, left in contact for 10 minutes and filtered into 100 ml volumetric flasks, the volume was made up to the mark with de-ionized water, all samples were performed in triplicates (Ahmad *et al.*, 2018; Huang *et al.*, 2020).

2.4. Elemental analysis of samples: Determination of element concentrations in all the samples were made directly on each of the final solution by using the appropriate Instrumentation and methods.

1-Total Nitrogen (TN) and total phosphorus (TP) in soil samples were determined using an automatic elemental analyzer (Elemetar Vario Max CN, Germany) and the Olsen method, respectively (Iatrou *et al.*, 2014; Zhao *et al.*, 2022).

2-Total potassium (TK), Sodium (Na) and Calcium (Ca) were determined using an inductively coupled plasma mass spectrometer (ICP-MS) (Agilent 7500ce) (Nogueira *et al.*, 2013; Zhao *et al.*, 2022).

3-Trace elements (Iron, Zinc, Cadmium & lead) were determined by atomic absorption spectrophotometer (AAS) (Oumlouki *et al.*, 2021; Cardoso-Silva *et al.*, 2013).

2.5. biological factors:

2.5.1. Bioconcentration factor (BCF) is described as the ability of plants for elemental accumulation from the substrate. It can be measured for each plant part, such as roots, stems, and leaves using the equation:

$$BCF = C_{\text{plant part}} \backslash C_{\text{soil}}$$

where C_{plant} shows the accumulation of heavy metals in plant part (aerial or roots) and C_{soil} denoted the concentration of heavy metals in soil. BCF values more than 1 demonstrate the potential success of a plant species for phytoremediation (Nouha *et al.*, 2024)

2.5.2. Translocation factor (TF) is an important tool used to assess a plant's potential for phytoremediation purposes. It is calculated from the ratio of the element's presence in the plant's aerial parts compared to that in the plant's roots parts using the equation (Nouha *et al.*, 2024; Wu *et al.*, 2011).

$$TF = \text{Metal (aerial parts)} \backslash \text{Metal (roots parts)}$$



A (TF) value greater than 1 in the metal phytoextractors and less than 1 in the metal phytosabilizer species was observed (Mellem *et al.*, 2012; Pandey *et al.*, 2012; Mishra and Pandey, 2019; Khermandar *et al.*, 2016).

2.5.3. Bioaccumulation factor (BAF) is used to calculate metals transfer from soil to various plant parts (total biomass) used the following equation:

$$BAF = C_{plant} / C_{soil}$$

where C_{plant} shows the accumulation of heavy metals in plant (total biomass) and C_{soil} denoted the amount of heavy metals in soil. (BAF) values more than 1 demonstrate the potential success of a plant species for bioaccumulation. (Zhuang *et al.*, 2009; Khermandar *et al.*, 2016; He *et al.*, 2021; Hussain *et al.*, 2022;). Plants having both (TF) and (BAF) >1 can be employed as phytoremediators. (BAF) values greater than two are regarded as high values (Usman *et al.*, 2013). If a plant has (BAF) >1 and (TF) <1, it can be used as a phytostabilizer; if it has (BAF) < 1 and (TF) >1, it can be used as a phytoextractor (Sopyan *et al.*, 2014; Takarina & Pin, 2017).

6. Statistical analysis

The obtained data were subjected to the statistical analysis of variance ANOVA of the combined analysis in completely randomized design (CRD) and least significant difference (LSD) at 0.05% was used to compare between the means of treatments using COSTAT software (Pacific Grove, CA, USA) (Ott and Longnecker, 2015).

3. Results:

Table (1) showed an increase in the concentration of macro elements, with the aerial parts exhibiting concentrations of 1.17%, 0.030, 1.01, 3.1, and 0.559 g kg⁻¹ for (N), (P), (K), (Ca), and (Na), respectively. In contrast, the root parts displayed concentrations of 0.74%, 0.012, 0.287, 2.05, and 0.286 g kg⁻¹ for the same elements. The soil revealed concentrations of 0.71%, 0.060, 0.123, 3.1, and 0.140 g kg⁻¹ for N, P, K, Ca, and Na, respectively.

Table (1) The macro elements in *J. phoenicea* and soil

Parts	N%	P g kg ⁻¹	K g kg ⁻¹	Ca g kg ⁻¹	Na g kg ⁻¹
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Aerial	1.17	0.030	1.01	3.1	0.559
Roots	0.74	0.012	0.287	2.05	0.286
Soils	0.71	0.060	0.123	3.1	0.140

*Results resource: field & lab. Study 2023

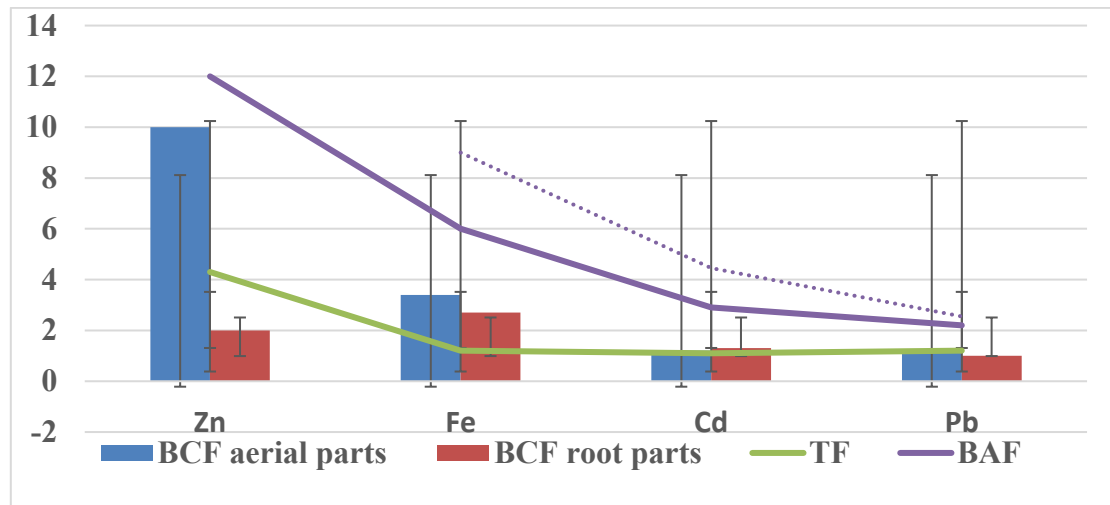
Table (2) We note that there are significant differences at ($p < 0.05$) between plant's parts and soils parts, where were the concentrations of heavy metals in the aerial parts, 1.124, 1.328, 0.069, and 0.116 mg kg⁻¹ for (Zn), (Fe), (Cd), and (Pb), respectively, More than the roots parts which were 0.26, 1.055, 0.061, and 0.099 mg kg⁻¹ for Zn, Fe, Cd, and Pb, respectively, Followed by Soils parts were 0.112, 0.382, 0.50, and 0.097 mg kg⁻¹ for these heavy metals. The data indicate that the aerial parts accumulate a greater quantity of heavy metals compared to the roots, with the accumulation being aerial parts > roots parts > soil. Furthermore, Table (2) suggests that *J. phoenicea* has a tendency to accumulate heavy metals in its aerial parts.

Table (2) concentration of heavy metals in *J. phoenicea* and soil

Parts	Zn mg kg⁻¹	Fe mg kg⁻¹	Cd mg kg⁻¹	Pb mg kg⁻¹
Aerial	1.124 ^A	1.328 ^A	0,069 ^A	0.116 ^A
Roots	0.26 ^B	1.055 ^B	0.061 ^B	0,099 ^A
Soils	0.112 ^C	0.382 ^C	0.050 ^C	0.097 ^A
LSD	0,035	0.058	0.005	0.022

*Results resource: field & lab. Study 2023

According to figure (1) (BCF) for the aerial parts was greater than that for the root parts across all heavy metals, with values of 10, 3.4, 1.5, and 1.2 for Zn, Fe, Cd, and Pb, respectively. In comparison, (BCF) for the roots parts was 2, 2.7, 1.3, and 1 for the same metals. (TF) values exceeded (1) for all heavy metals, where was 4.3, 1.2, 1.1, and 1.2 for Zn, Fe, Cd, and Pb, respectively. Conversely, (BAF) exceeded (1) for all heavy metals where (12, 6, 2.9 and 2.2) for (Zn, Fe, Cd and Pb) respectively, on other hand figure (1) showed that all indicators (BCF aerial parts, BCF root parts, TF, and BAF) indices that *J. phoenicea* trend to absorb heavy metals from the soil parts to the biomass parts, but transfer it by Phytoextraction strategy from roots parts to aerial parts.



*Results resource: field & lab. Study 2023

Figure (1) Comparing biological factors

4. Discussion:

The elevated concentrations of (N), (P), (K), and s (Na) can be attributed to the influx of sewage water, as noted by Smith and Giller (1992), Farahat and Linderholm (2015), and Yu *et al.* (2022). The significant levels of (K) are linked to the presence of various minerals, including feldspar, mica, and illite, which are known to contain this element (Ben Mahmoud, 1995). The increase (Ca) concentration is primarily due to the soil's parent material, which is characterized by a high content of calcium carbonate (Ben Mahmoud, 1995).

Importantly, the concentrations of heavy metals remained within the permissible limits established by the World Health Organization WHO, 1997. The aerial parts of the plants exhibited the highest accumulation of heavy metals, with a greater concentration observed in the aerial parts compared to the root systems. This finding accord with the observations of Zhao *et al.* (2014), Farahat and Linderholm (2015), Cesur *et al.* (2021), Siyar *et al.* (2022), and Kord *et al.* (2024), who reported a higher absorption of heavy metals in the aerial parts of certain Cupressaceae species than in their roots . Furthermore, (TF) values exceeded (1) for all heavy metals, indicating that *Juniperus phoenicea* effectively accumulates these metals in its aerial parts. Similar findings were reported by Farahat and Linderholm (2015) and Kord *et al.* (2024). (BAF) also suggests that *Juniperus*



phoenicea can be classified as a bioaccumulator, as the (BAF) of heavy metals exceed 1, its according to Achak *et al.* (2009) and Diaconu *et al.* (2009). Additionally, figure (1) explain that Juniperus phoenicea tends to transfer heavy metals from its roots to its aerial parts, corroborating the findings of Farahat and Linderholm (2015) and Siyar *et al.* (2022). According to the findings of Usman *et al.* (2013), Mishra and Pandey (2019), Venegas-Rioseco *et al.* (2021), Siyar *et al.* (2022), Kord *et al.* (2024), and Nouha *et al.* (2024), *Juniperus phoenicea* demonstrates a significant capacity for phytoextraction, enabling it to accumulate heavy metals in its above-ground structures. This species plays a crucial role in phytoremediation efforts, as it effectively sequesters heavy metals in the aerial portions of the plant, which is essential for the successful harvesting of these contaminants, as the process necessitates accumulation in the above-ground parts rather than the root system.

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