

Evaluate Cumulative Ability of *Phalaris minor* **Grasses for Some Heavy Metals in area of Shahat, Libya**

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Abstract

This research was carried out in the Shahat Forest to evaluate the ability of *P. minor* to absorb heavy metals in its tissues. The study involved analyzing the concentrations of heavy metals, (Zn), (Fe), (Cd) and (Pb), in both the aerial and root parts of *P. minor*, as well as in the soil at a depth of 0-40 cm beneath the grasses. Importantly, the concentrations of these heavy metals were found to be within the acceptable limits set by the (WHO). The results noted that *P. minor* evidenced a significant ability for the uptake and accumulation of heavy metals, with (BAF) for Zn, Fe, Cd, and Pb recorded at (3.4, 1.9, 1.9, and 2), respectively. Additionally, the (BCF) of the roots parts were higher than (BCF) of the aerial parts, and (TF) remained below (1) for all analyzed elements. As a result, *P. minor* can be considered a potential bioaccumulator with phytostabilization strategy. Statistical analysis indicated significant differences $(p<0.05)$ in heavy metal concentrations among the various parts of the plant.

Keywords: Phalaris minor, Heavy Metals, Cumulative, Shahat, forest.

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تقييم القدرة التراكمية لنبات ابورويس minor Phalaris لبعض المعادن الثقيلة في منطقة شحات، ليبيا

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الملخص

أجري هذا البحث في غابة شحات لتقييم قدرة نبات)ابورويس(minor .P على امتصاص المعادن الثقيلة في أنسجته. حيث تضمنت الدراسة تحديد تركيز العناصر الثقيلة)Zn)و)Fe)و)Cd)و)Pb)في األجزاء الخضرية والجذرية لنبات ابورويس .P minor وكذلك في التربة على عمق (0−40 سم) تحت الأعشاب. وقد كانت تركيزات ً هذه المعادن الثقيلة ضمن الحدود المقبولة التي حددتها منظمة الصحة العالمية. ايضا أشارت النتائج إلى أن نبات ابورويس minor .P أظهر قدرة كبيرة على امتصاص وتراكم المعادن الثقيلة، حيث سجل (BAF) للزنك والحديد والكادميوم والرصاص (3.4، ،9.1 ،9.1 و2(على التوالي. باإلضافة إلى ذلك، كانت)BCF)أعلى في األجزاء الجذرية مقارنة بالأجزاء الخضرية، ايضا (TF) كان أقل من (1) لجميع العناصر التي تم تحليلها. ونتيجة لذلك يمكن اعتبار نبات ابورويس P. minor مراكماً حيوياً متبعاً لاستراتيجية التثبيت النباتي phytostabilization. ايضاً أشارت التحليل الإحصائي إلى وجود اختالفات معنوية)0.05>P)في تر كيز ات العناصر الثقيلة بين أجزاء النبات المختلفة.

الكلمات المفتاحية: minor Phalaris - ابورويس - القدرة التراكمية – المعادن الثقيلة – غابة شحات .

Introduction

Plants have been recognized as effective biomonitors for evaluating the rise in heavy metal levels in the surroundings (Sarwar et al., 2017; Zhu et al., 2021Cesur et al., 2021). Plants have the capability to absorb heavy metals from their surroundings, often accumulating these metals in concentrations that surpass those present in the soil. However, the efficiency of different plant species in taking up toxic metals varies considerably (Zhao et al., 2014). Additionally, plants play a crucial

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role in ecosystems by enabling the transfer of elements from the non-living environment to living organisms (Hu et al., 2014; Georgiev et al., 2016; Najafi & Jalali, 2016; Aljerf & Choukaife, 2018) .Urban vegetation plays a vital role in environmental monitoring and remediation, (Young et al., 2014; Bahiru & Yegrem, 2021). Regarding heavy metal accumulation, plants can be categorized into three distinct groups: heavy-metal accumulators, heavy-metal excluders (or non-accumulators), and indicator plants. Heavy-metal-accumulating plants demonstrate a concentration ratio of the metal within the plant that exceeds that in the soil, with a ratio greater than 1. In contrast, nonaccumulating plants exhibit a ratio of less than 1, while indicator plants maintain a ratio that is approximately equal to 1 (Cunningham & Ow, 1996; Marques et al., 2009; van der Ent et al., 2013; Suman et al., 2018; Yan et al., 2020; Sladkovska et al., 2022; Chitimus et al., 2023; Kord et al., 2024) .To date, around 400 species of hyperaccumulators have been identified across 22 distinct families are capable of hyperaccumulating metals, primarily from families such as Brassicaceae, Poaceae, Cyperaceae, Asteraceae, Caryophyllaceae, Fabaceae, Lamiaceae, Euphorbiaceae, Violaceae, and Cunoniaceae (Gleba et al., 1999; Prasad and Freitas, 2003; McIntyre, 2003; Ghosh & Singh, 2005; Rascio & Navari-Izzo, 2011; Sladkovska et al., 2022; Khan et al., 2023). To counteract heavy metal toxicity, plants generally adopt two main defense mechanisms: avoidance and tolerance. These mechanisms allow plants to maintain intracellular heavy metal concentrations at levels that do not cause harm (Raklami et al., 2022; Hall, 2002). Avoidance strategies enable plants to limit the uptake and movement of heavy metals into their tissues, with root cells acting as the first line of defense at the extracellular level. This is accomplished through various mechanisms, including root adsorption, precipitation of metal ions, and exclusion of metals. Plants facing exposure to heavy metals first engage in strategies to immobilize these elements, primarily through root adsorption or modification of metal ions. Additionally, a range of root exudates, including organic acids and amino acids, serve as ligands for heavy metals, promoting the formation of stable complexes in the rhizosphere (Yan et al., 2020). The mechanism of metal exclusion establishes a protective barrier between the root and shoot systems, thereby limiting the uptake of heavy metals from the soil into the roots. This process is crucial for protect the aerial parts of the plant

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from the detrimental effects of heavy metals by restricting their absorption and subsequent translocation from roots to shoots. Moreover, arbuscular mycorrhizae play a significant role in mitigating the entry of heavy metals into the roots through various mechanisms, such as absorption, adsorption, or chelation of these metals in the rhizosphere, effectively functioning as a barrier against heavy metal uptake (Hall, 2002; Anum et al., 2019). Upon the infiltration of heavy metal ions into the cytosol, plants activate multiple resistance mechanisms to mitigate the toxicity associated with the accumulation of these ions, representing a secondary defense at the subcellular level. The mechanisms involved include inactivation, chelation, and compartmentalization of heavy metal ions (Dalvi & Bhalerao, 2013). In the context of phytoremediation, plants employ a range of strategies such as phytostabilization, phytoextraction, rhizodegradation, and phytovolatilization to either passively stabilize or actively remove contaminants from their environment (Raskin et al., 1994; Tangahu et al., 2011; Patra et al., 2021; Sladkovska et al., 2022; Chitimus et al., 2023; Zia-ul-Haq et al., 2024). Among these approaches, phytostabilization is a key technique utilized for the remediation of heavy metals by plants (Ali et al., 2013; Hao et al., 2014). This method specifically involves the use of metal-tolerant plant species to immobilize heavy metals in the soil, thereby decreasing their bioavailability and preventing their movement into ecosystems, which in turn mitigates the risk of metals entering the food chain. The process can occur through various mechanisms, such as precipitation or reduction of metal valence in the rhizosphere, uptake and sequestration within root tissues, or adsorption onto root cell walls (Gerhardt et al., 2017). A notable benefit of phytostabilization is that it does not necessitate the removal of toxic biomass (Wuana & Okueimen, 2011; Sarwar et al., 2017; Ashraf et al., 2019). The choice of suitable plant species is crucial for effective phytostabilization, as these plants must exhibit tolerance to heavy metals to achieve optimal performance. The root systems of these species are essential for immobilizing heavy metals, maintaining soil structure, and preventing erosion. Consequently, it is essential for these plants to possess robust and dense root systems, along with the ability to generate significant biomass and demonstrate rapid growth (Marques et al., 2009; Ali et al., 2013; Huang et al., 2017; Jacob et al., 2018; YAN et al., 2020; Venegas-Rioseco et al., 2021; Siyar et al., 2022). Grasses, known for their rapid and

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vigorous growth, serve as effective bioindicators for evaluating soil contamination by heavy metals (Ali et al., 2019; Khan et al., 2023). Plant family is one of the main factors affecting the heavy metals distribution in the above- and underground parts of plants. Plants from the Poaceae family accumulate less chemical elements in their aboveground parts than the other plants (Chaplaygin, 2018). The Poaceae family, which encompasses grasses, includes around 780 genera and 12,000 species that are widely distributed across the globe (Christenhusz and Byng, 2016). Grasses fulfill various roles, including food and fuel production, the establishment of lawns and meadows, soil erosion control, remediation of toxic metals, and ecological restoration of contaminated environments (Engelhardt and Hawkins, 2016; Rabêlo et al., 2021). The Poaceae family has proven to be highly effective in remediating contaminated environments due to their ease of cultivation, rapid growth rates, and resilience to adverse conditions. These grasses can accumulate significant amounts of heavy metals in their rhizosphere while limiting translocation to above-ground parts. Studies have shown that species within the Poaceae family can alleviate metal toxicity and improve the uptake of contaminants from polluted sites (Ashraf et al., 2019; Patra et al., 2021).

This study was conducted in the Shahat Forest to assess the capacity of *P. minor* to accumulate heavy metals from contaminated soil. and what is the strategy mechanisms will utilizes by *P. minor* in dealing with heavy metals?.

Material and methods

Study aria

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 35.3ºC in summer to 20.1ºC in winter, Minimum temperature ranges from 20.2ºC in summer and 7.5ºC in winter. (Othman & Al-Habbat, 2023).

Sample collection: Ten *P. minor* grasses were selected to conduct this study, fresh samples of *P. minor* grasses 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),

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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 *L. multiflorum* grasses parts and soil parts.

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 until the weight became constant, A known quantity, 2g of each sample (aerial part, root part of *P. minor* grasses 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).

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) at labs of National Institute of Oceanography & Fisheries – Alexandria – Egypt. (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).

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Biological factors:

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:

 $\textit{BCF}=\frac{\textit{C}_\mathrm{plant\,part}}{\textit{C}}$ $c_{\rm soil}$

where *C*_{*plant*} shows the accumulation of heavy metals in plant part (aerial or roots) and *Csoil* denoted the amount 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-**[Translocation factor](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/translocation-factor) (TF)** is an important tool used to assess a plant's potential for [phytoremediation](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/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).

 $TF = \frac{\text{ Metal(aerial parts)}}{\text{Meta(meats next)}}$ Metal(roots parts)

A (TF) value greater than 1 in the metal phytoextactors 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).

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

$$
BAF = \frac{c_{\text{plant}}}{c_{\text{Soil}}}
$$

where *C_{plant}* shows the accumulation of heavy metals in plant (total biomass) and *Csoil* 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

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high values (Usman et al., 2013). If a plant has (BAF) >1 and (TF) $\langle 1$, it can be used as a phytostabilizer; if it has (BAF) $\langle 1 \rangle$ and (TF) >1, it can be used as a phytoextractor (Sopyan et al., 2014; Takarina & Pin, 2017).

Statistical analysis

The obtained data were subjected to the statistical analysis of variance ANOVA one way of the combined analysis in completely randomized design (CRD), the 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).

The results

The data present in Table (1) demonstrates a significant elevation in the concentrations of essential macro elements. Specifically, the concentrations measured in the aerial parts were (0.1.25%, 0.012, 7.74, 2.11 and 0.89 g kg^{-1}) for (N), (P), (K), (Ca), and (Na), respectively. In comparison, the root parts displayed concentrations of $(0.73\%, 0.024, 2.49, 2.38, \text{ and } 0.74 \text{ g kg}^{-1})$ for the same elements. The soil parts revealed concentrations of $(0.55\%, 0.031, 0.765, 3.84$ and $0.16 \text{ g kg}^{-1})$ for N, P, K, Ca, and Na, respectively.

Table (1) The macro elements in T <i>ruttor</i> and son					
parts	N%	$P g kg^{-1}$	K g kg^{-1}	$Ca g kg^{-1}$	Na g kg^{-1}
Aerial	.25	0.012	7.74	2.11	0.89
Roots	37.0	0.024	2.49	2.38	0.74
Soils	0.55	0.031	0.765	3.84	0.16

Table (1) The macro elements in *P. minor* **and soil**

Table (2) presents the concentrations of heavy metals in the aerial parts, recorded as $(0.106, 0.96, 0.044,$ and 0.095 mg kg⁻¹) for (Zn) , (Fe), (Cd), and (Pb), respectively. The root parts exhibited elevated concentrations of heavy metals, with values of (0.426, 1.51, 0.077, and 0.097 mg kg^{-1}) for Zn, Fe, Cd, and Pb, respectively. The soil parts indicated concentrations of (0.153, 1.3, 0.061, and 0.097 mg kg-1) for the same metals. This information suggests that *P. minor* has a tendency to preferentially accumulate heavy metals within its root system rather than in the aerial parts

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According to figure (1), (BCF) for the root parts was consistently greater than that of the aerial parts for all heavy metals, with values of (2.7, 1.1, 1.2, and 1.) for Zn, Fe, Cd, and Pb, respectively, in contrast to (0.6, 0.7, 0.7, and 0.9) for the aerial parts. Additionally, (TF) values for all heavy metals remained below (1), with measurements of (0.2, 0.6, 0.5, and 0.9) for Zn, Fe, Cd, and Pb, respectively. Conversely, (BAF) exceed (1) for all heavy metals, with values of (3.4, 1.9, 1.9 and 2) for (Zn, Fe, Cd and Pb) respectively. Figure (1) demonstrates that all assessed parameters, including (BCF) for both aerial and root parts, as well as the (TF) and (BAF), suggest that *P. minor* shows a propensity for heavy metal uptake from the soil and utilizes a phytostabilization mechanism to sequester these metals within its root system.

Figure (1) Comparing biological factors

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Discussion

The observed increases in the concentrations of (N) , (P) , (K) , and (Na) can be linked to the impact of sewage water, as evidenced by the studies conducted by Smith and Giller (1992), Farahat and Linderholm (2015), and Yu et al. (2022). The elevated levels of (K) are further correlated with the presence of various minerals such as feldspar, mica, and illite, which are rich in this element (Ben Mahmoud, 1995). The rise in (Ca) concentration is largely attributed to the parent material of the soil, which contains significant amounts of calcium carbonate (Ben Mahmoud, 1995). Importantly, the concentrations of heavy metals were found to remain within the acceptable thresholds established by the World Health Organization (WHO, 1997). It is significant to note that the root system of P. minor displayed the highest concentrations of heavy metals, aligning with the findings of Prelac et al. (2016), Rabêlo et al. (2021), and Masotla et al. (2023), who reported that certain heavy metals are preferentially absorbed in the root systems compared to the aerial parts of various species within the Poaceae family. The assessment of the transfer factor (TF) indicates values below 1 for all analyzed heavy metals, suggesting that P. minor primarily accumulates these metals in its root systems. Furthermore, the bioaccumulation factor (BAF) values indicate that P. minor is categorized as a bioaccumulator, as it exceeds 1 for all heavy metals, a finding supported by the research of Zhang et al. (2006), Sarathchandra et al. (2022), and Masotla et al. (2023), among others. It has been observed that while the genus Phalaris is not classified as a hyperaccumulator, it possesses the capability to thrive in contaminated environments and effectively uptake heavy metals, as noted in studies by Mugica-Alvarez et al. (2015), Rabêlo et al. (2021), Korzeniowska & Stanislawska-Glubiak (2023), Pinna et al. (2024), and Dradrach et al. (2024). Additionally, researches conducted by Platace et al. (2011), Senze et al. (2022), Ramadan & Balah (2022), Boynukisa et al. (2023), and Khan et al. (2023) suggests that Phalaris may be a viable option for the accumulation of heavy metals. Studies by Kwiatkowska-Malina and Maciejewska (2013), Borowiak et al. (2018), Cakaj et al. (2023), and Ghandali et al. (2024) indicate that several species within the Poaceae family exhibit significant capabilities in the phytoestabilization of heavy metals. These species are characterized by their low water requirements and rapid growth in adverse soil conditions, facilitating the

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establishment of a vegetative cover that reduces the dispersion of contaminated dust while progressively sequestering heavy metals, thereby improving soil quality.

Conclusion

This study was conducted to analyze the capacity of *Phalaris minor* grasses to absorb heavy metals and store them within their tissues, as well as to determine the specific storage parts for these metals within the plant. The results revealed that P. minor grasses effectively absorb heavy metals, with a higher concentration found in the root system compared to the shoot system. Consequently, this plant is recognized as employing a phytoestabilization strategy for the immobilization of heavy metals, therefore can accumulate significant amounts of heavy metals in their rhizosphere while limiting translocation to above-ground parts. The study have shown that P. minor species can alleviate metal toxicity and improve the uptake of contaminants from polluted sites.

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