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Cumulative Ability Of *Lolium multiflorum* Grasses for Some Heavy Metals in Shahat, Libya

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القدرة التراكمية لنبات الصامة *Lolium multiflorum* لبعض المعادن الثقيلة في شحات، ليبيا

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Abstract

This study was undertaken in the Shahat Forest aimed to evaluate the capacity of *L. multiflorum* to absorb and retain heavy metals in its tissues. heavy metals investigated included (Zn), (Fe), (Cd), & (Pb), with samples obtained from both the aerial and root parts of *L. multiflorum*, as well as from soil at a depth of 0-40 cm beneath *L. multiflorum*. Importantly, the concentrations of these heavy metals were found to be within the permissible limits set by (WHO). The findings indicated that *L. multiflorum* significantly contributes to absorb and accumulate heavy metals, with (BAF) for Zn, Fe, Cd, and Pb recorded at 2.5, 2, 1.7, and 2.2 mg, respectively. Additionally, (BCF) for the roots exceeded 1, while (TF) remained below 1 for all toxic metals examined. Thus, *L. multiflorum* has the potential to function as a bio accumulator utilizing a phytostabilization approach. Statistical evaluations demonstrated significant variations ($p < 0.05$) in heavy metal concentrations across the various parts of the grasses and soils.

Keywords: *Lolium multiflorum*- soil`s heavy metals– Cumulative –ability - shahat .

الملخص

تم إجراء هذه الدراسة في غابة شحات لتقييم قدرة نبات حشائش (الصامة) *L. multiflorum* على امتصاص وتراكم المعادن الثقيلة في أنسجته. حيث تم قياس مستويات المعادن الثقيلة (Zn) و(Fe) و(Cd) و(Pb) في كل من الأجزاء الخضرية والجذرية لنبات *L. multiflorum*، وكذلك في التربة على عمق (0- 40 سم) تحت الحشائش . مبدئياً إن تركيزات المعادن الثقيلة وجدت ضمن الحدود المقبولة لمنظمة الصحة العالمية. ايضاً أظهرت النتائج أن نبات (الصامة) *L. multiflorum* أبدى قدرة كبيرة على امتصاص وتراكم المعادن الثقيلة، حيث كان (BAF) Zn وFe وCd وPb هو (2.5، 2، 1.7، و2.2) على التوالي. بالإضافة إلى ذلك فإن (BCF) الأجزاء الجذرية اكبر من 1 و ايضا (TF) كان اقل من 1 في كل المعادن الثقيلة ، ولذلك يمكن اعتبار *L. multiflorum* مراكماً حيوياً متبعاً لاستراتيجية التثبيت النباتي phytostabilization. ايضا أشار التحليل الإحصائي إلى وجود اختلافات معنوية ($P <0.05$) في تركيزات العناصر الثقيلة بين أجزاء النبات المختلفة.

الكلمات الدالة: حشائش (الصامة). *Lolium multiflorum*. القدرة التراكمية .تلوث بالمعادن الثقيلة .شحات .

Introduction

As important bio monitors, plants are utilized to evaluate the rising levels of trace elements in the ambience (Sarwar et al., 2017; Zhu et al., 2021; Cesur et al., 2021). These plants can absorb heavy metals from their surroundings, often resulting in concentrations that surpass those present in soil. Nonetheless, A capacity for different plant species to uptake these toxic metals varies significantly (Zhao et al., 2014). Additionally, plants are crucial in ecosystems, enabling transfer of elements from non-living to living organisms (Hu et al., 2014; Georgiev et al., 2016; Najafi & Jalali, 2016; Aljerf & Choukaife, 2018). Vegetation in urban areas is especially vital for monitoring and remediation of environmental issues (Young et al., 2014; Bahiru & Yegrem, 2021). Plants can be categorized into three types based on heavy metal accumulation: heavy metal accumulators, heavy metal excluders and indicator plants.

Accumulator plants possess a concentration ratio of metals that is higher within the plant than in the soil, with a ratio exceeding 1. In contrast, non-accumulating plants have a ratio below 1, while indicator plants maintain a ratio that is roughly 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).). approximately 400 species of hyperaccumulators have been recognized across 22 different families that can hyperaccumulate metals, mainly from families Brassicaceae, Poaceae, Cyperaceae, Asteraceae, Caryophyllaceae, Fabaceae, Lamiaceae, Euphorbiaceae, Violaceae, & Cunoniaceae (Gleba et al., 1999; Prasad & Freitas, 2003; McIntyre, 2003; Ghosh & Singh, 2005; Rascio and Navari-Izzo, 2011; Sladkovska et al., 2022; Khan et al., 2023). Plants typically implement two fundamental strategies to alleviate the toxicity of toxic metals: avoidance and tolerance. Through these strategies, plants can control intracellular concentrations of toxic metals, keeping them at safe levels that do not harm their physiological functions (Raklami et al., 2022; Hall, 2002). In the presence of toxic metals, plants initially attempt to immobilizeing these elements through root adsorption or the alteration of elements ions. Furthermore, various root exudates, including organic acids and amino acids, act as ligands for heavy metals, facilitating the formation of stable complexes within rhizosphere (Yan et al., 2020). This metal exclusion process creates a protective barrier between the root and shoot systems, thereby restricting the absorption of trace elements from soil into roots. Such a mechanism is essential for protecting the above-ground portions of plant from the harmful impacts of heavy metals by regulating their uptake and subsequent movement from the roots to the shoots. Additionally, arbuscular mycorrhizae significantly contribute to reducing entry of toxic metals into the roots through various strategies, including absorp, adsorp, or chelate of these elements in rhizosphere, effectively acting a barrier against elements absorb (Hall, 2002; Anum et al., 2019). When heavy metal ions penetrate cytosol, plants initiate various resistance mechanisms to mitigate toxicity result from accumulation of these ions, representing a secondary defense at subcellular level. These mechanisms include inactivation, chelation, and compartmentalization of toxic metal ions (Dalvi & Bhalerao, 2013). In field of phytoremediation, plants implement a variety of approaches, including phytostabilization, phytoextraction, rhizodegradation, and phytovolatilization, to either passively stabilize or actively remove harmful substances 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 recognized as one of the primary techniques utilized by plants for the removal of toxic metals (Ali et al., 2013; Hao et al., 2014). Phytostabilization is defined as intentional application of metal-tolerant plant species to immobilizeing toxic elements found in soil, thereby reducing their bioavailability and preventing their migration into adjacent ecosystems. This process is vital for minimize risk of toxic metals infiltrating food chain. Several mechanisms contribute to phytostabilization, such as precipitation or reduction of metal valence in rhizosphere, uptake and storage of metals within root tissues, and adsorption of metals onto cell walls of roots (Gerhardt et al., 2017). A significant advantage of phytostabilization is that it negates necessity for removal of hazardous biomass (Wuana & Okueimen, 2011; Sarwar et al., 2017; Ashraf et al., 2019). Careful selection of suitable plant species is critical for successful phytostabilization, as these species must demonstrate tolerance to toxic metals to achieve optimal results. A root systems of these plants play a key role in immobilizing toxic metals, improving soil structure, and mitigating erosion. Therefore, it is essential for these plants to have strong and dense root systems, along with capacity to produce substantial biomass and exhibit 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 are characterized by their rapid and vigorous growth, which makes them important bioindicators for evaluating soil contamination with toxic metals (Ali et al., 2019; Khan et al., 2023). Distribution of heavy metals in both the aboveground and belowground parts of plants is significantly influenced by Poaceae family. Generally, members of this family accumulate fewer chemical elements in their aerial parts than those from other plant families (Chaplaygin, 2018). Poaceae family includes approximately 780 genera and around 12,000 species, showcasing a broad global distribution (Christenhusz & Byng, 2016). Grasses fulfill a variety of functions, such as producing food and fuel, creating lawns and meadows, controlling soil erosion, remediate toxic metals, and restore contaminated ecosystems (Engelhardt and Hawkins, 2016; Rabêlo et al., 2021). Poaceae family has shown significant potential in remediation of contaminated sites, primarily due to its ease of cultivation, rapid growth, and resilience to adverse environmental conditions. These grasses can effectively accumulate large amounts of toxic metals in their rhizosphere while limit their translocation to aboveground parts. Studies have indicated that species within Poaceae family can reduce metal toxicity and enhance uptake of contaminants from polluted environments (Ashraf et al., 2019; Patra et al., 2021).

This research was conducted in the Shahat Forest to assess ability of L. multiflorum (genus) to absorb toxic metals from contaminated soil. Additionally, what strategies and mechanisms will L. multiflorum employ to cope with heavy metals?.

Material and methods

Study aria: In 2023, a study was performed in a forested area of Shahat city, which is affected by sewage. Geographical coordinates of this site are 32°49′40″N 21°51′44″E. Climate generally mild, winters that are warm and rainy, and summers that are notably hot. Maximum temperatures in the study area range from 35.3ºC in summer to 20.1ºC in winter, while minimum temperatures vary from 20.2ºC in summer to 7.5ºC in winter (Othman & Al-Habbat, 2023).

Sample collection: Ten *L. multiflorum* grasses were selected to conduct this study, fresh samples of *L. multiflorum* grasses from both aerial, root part & soil parts at depth (0 cm - 40 cm) were gathered in polyethylene bags and subsequently transported to laboratory for further analysis. Analysis focused on five macroelements: total Nitrogen (N), total Phosphorus (P), total Potassium (K), total Calcium (Ca), and total Sodium (Na), as well as four heavy metals: zinc (Zn), Iron (Fe), cadmium (Cd), and lead (Pb), in both the L. multiflorum grass parts and the soil parts.

Sample Preparation: Collected samples homogenized and crushed into small particles, decomposed by dry digestion process for determinate various metals. At first all crucibles and glass wares washed with distilled water and dried in oven. Weight of each crucible made constant by keep it in muffle furnace at 750 °c for one hour. Then transferred to desiccator and weighted it. Purpose was to remove all moisture. This action was repeated till weight became constant., 2g of each sample (aerial part, root part of *L. multiflorum* grasses and soil parts) was introduced into crucible. crucibles were burned at around 200 °C until end of organic matter smoke generation, then crucibles kept in muffle furnace at 600 °C for 5 hours, cooled to room temperature in desiccator for 40 minutes. Obtained white ash is moistened with a 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, Volume was made up to the mark with deonized water, All samples performed in triplicates (Ahmad et al., 2018; Huang et al., 2020).

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

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

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

3-Trace elements (Iron, Zinc, Cadmium & lead) determined by (AAS) Atomic Absorption Spectrophotometer (Oumlouki et al., 2021; Cardoso-Silva et al., 2013).

biological factors:

1- Bioconcentration factor (BCF) is ability of plants for elemental accumulate from environment. It can be measured for each plant part, such as roots, stems, and leaves using equation:

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

where C_{plant} shows accumulation of toxic metals in plant parts (aerial or roots) and C_{soil} amount of toxic metals in soil. BCF more than 1 is potential success of a plant species for phytoremediation (Nouha et al., 2024).

2-Translocation factor (TF) used to assess a plant's potential for phytoremediation purposes. It calculated from ratio of metlas's presence in plant's aerial parts compared to that in plant's roots parts using equation (Nouha et al., 2024; Wu et al., 2011).

$$TF = Metal(aerial\ parts) \backslash Metal(roots\ parts)$$

A (TF) greater than 1 in metal phytoextactors and less than 1 in 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) used to calculate elements transfer from soil to various plant parts (total biomass) used the following equation :

$$BAF= C_{plant} \backslash C_{soil}$$

where C_{plant} shows accumulation of toxic metals in plant (total biomass) and C_{soil} amount of heavy metals in soil. (BAF) values more than 1 demonstrate 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).

Statistical analysis

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

The results

Data presented in Table (1) indicates a notable elevation in the concentrations of macro elements. The values recorded for the aerial parts were 0.76%, 0.012, 9.8, 0.875, & 1.68 g kg-1 corresponding to N, P, K, Ca & Na respectively. In contrast, the concentrations observed in root parts were 1.61%, 0.012, 2.5, 1.58 & 0.743 g kg-1 for same macro elements. soil parts exhibited concentrations of 1.1%, 0.050, 0.55, 3.38, & 0.436 g kg-1 for N, P, K, Ca, & Na, respectively.

Table (1) The macro elements in L. multiflorum and soil

parts	**N%**	**P** g kg^{-1}	**K** g kg^{-1}	**Ca** g kg^{-1}	**Na** g kg^{-1}
Aerial	0.76	0.012	9.8	0.875	1.68
Roots	1.61	0.012	2.5	1.58	0.743
Soils	1.1	0.050	0.55	3.38	0.436

Table (2) illustrates the concentrations of heavy metals found in the aerial parts of L. multiflorum, which were measured at 0.182, 0.55, 0.041 & 0.097 mg/L for Zn, Fe, Cd & Pb, respectively. In contrast, root parts displayed significantly higher concentrations, with values of 0.93, 1.68, 0.094 0.121 mg/L for Zn, Fe, Cd & Pb, respectively. Additionally, soil parts revealed concentrations of 0.449, 1.07 0.077 & 0.097 mg/L for Zn, Fe, Cd & Pb, respectively. These results suggest that L. multiflorum has a greater ability to accumulate toxic metals within root parts than in aerial parts.

Table (2) concentration of heavy metals in L. multiflorum and soil

parts	**Zn** mg kg^{-1}	**Fe** mg kg^{-1}	**Cd** mg kg^{-1}	**Pb** mg kg^{-1}
Aerial	0.182^{C}	0.55^{C}	0,041^{C}	0.097^{B}
Roots	0.93^{A}	1.68^{A}	0.094^{A}	0,121^{A}
Soils	0.449^{B}	1.07^{B}	0.077^{B}	0.097^{B}
LSD	**0.174**	**0.130**	**0.015**	**0.005**

Data presented in figure (1) showed that (BCF) for root parts consistently surpassed that of aerial parts across all examined heavy metals. Specifically, (BCF) values for aerial parts were recorded at 0.4, 0.5, 0.5, and 1 for Zn, Fe, Cd & (Pb), respectively. In contrast, root parts exhibited (BCF) values of 2, 1.5, 1.2, & 1.2 for same metals. Furthermore, (TF) remained below 1 for all heavy metals, with values of 0.2, 0.3, 0.4, & 0.8 for Zn, Fe, Cd, & Pb, respectively. Conversely, (BAF) exceeded 1 for all heavy metals, with values of 2.5, 2, 1.7, & 2.2 for Zn, Fe, Cd, & Pb, respectively. Additionally, figure (1) indicates that all measured parameters, including the (BCF) for both aerial and root parts, TF, and BAF, suggest that L. multiflorum demonstrates a propensity for heavy metal absorption from soil. This species appears to utilize a phytostabilization mechanism to primarily sequester these metals within its root system.

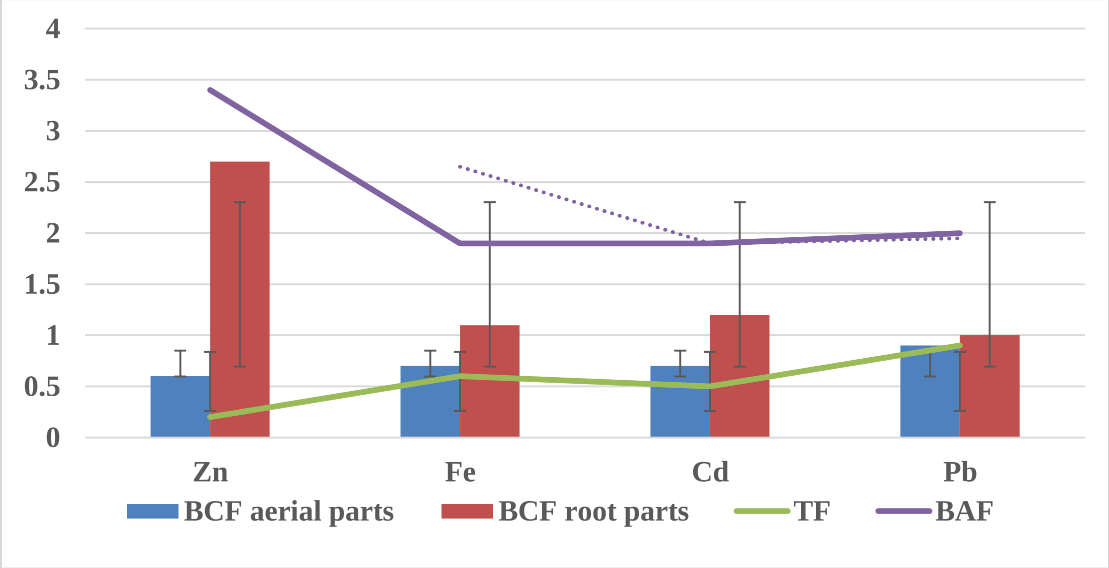

Figure (1) Comparing biological factors

Discussion

The elevated levels of N, P, K, & Na can be linked to the release of sewage effluent, as noted by Smith and Giller (1992), Farahat and Linderholm (2015), and Yu et al. (2022). The significant concentrations of (K) are attributed to the presence of minerals such as feldspar, mica, and illite, which are known to contain this nutrient (Ben Mahmoud, 1995). In terms of (Ca) levels, the increase is largely associated with the soil's parent material, which is rich in calcium carbonate (Ben Mahmoud, 1995). Importantly, the levels of heavy metals remained within the acceptable limits set by the World Health Organization (WHO, 1997). Research indicates that the root parts of the genus Lolium show highest concentrations of trace elements, a finding corroborated by Prelac et al. (2016), Rabêlo et al. (2021), & Masotla et al. (2023), who observed certain species within Poaceae family showed a greater capacity for heavy metal uptake in their roots parts compared to their aerial parts. Furthermore, (TF) for all heavy metals did not surpass 1, suggesting that the genus Lolium primarily retains heavy metals in its root systems. Additionally, (BAF) indicates that L. multiflorum acts as a bioaccumulator, as its (BAF) values for all trace elements were greater than 1. This claim is supported by the findings of Zhang et al. (2006), Sarathchandra et al. (2022), Masotla et al. (2023), Prelac et al. (2016), Kumar et al. (2017), Wen et al. (2018), Sladkovska et al. (2022), Suman et al. (2018), Mishra & Pandey (2019), and Venegas-Rioseco et al. (2021). Furthermore, Mugica-Alvarez et al. (2015), Rabêlo et al. (2021), Korzeniowska & Stanislawska-Glubiak (2023), Pinna et al. (2024), & Dradrach et al. (2024) indicate that genus Lolium, commonly known as ryegrass, is not classified as a hyperaccumulator; nevertheless, it exhibits a notable capacity to thrive in contaminated soils & effectively absorb heavy metals. Additionally, Kwiatkowska-Malina and Maciejewska (2013), along with Borowiak et al. (2018), Cakaj et al. (2023), and Ghandali et al. (2024), highlight that Italian ryegrass (genus Lolium) is particularly advantageous for phytostabilization. This species requires minimal irrigation and shows rapid growth even under challenging soil conditions, thereby establishing a vegetative cover that mitigates the spread of contaminated dust while gradually absorbing heavy metals, ultimately enhancing soil quality.

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