The Cumulative Capacity of *Acacia Cyanophylla* Trees for Heavy Metals in Shahat Forest, Libya

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ABSTRACT

This experiment was carried out in the Shahat Forest to evaluate A. Cyanophylla's ability to accumulate heavy metals and absorb them from contaminated soil to potentially clear those heavy metals and rebuild ecosystems. Heavy metals, which include (Zn, Fe, Cd, and Pb) were measured in A. Cyanophylla tissues (aerial parts, roots parts) and soils (0-40 cm) depth beneath. Results showed that concentrations of heavy metals did not exceed the permissible limits of the World Health Organization (WHO), and A. Cyanophylla was efficient in pulling and accumulating heavy metals within its tissues where the Bioaccumulation Factor (BAF) for (Zn, Fe, Cd, and Pb) was (2.2, 6.7, 2.1 and 1.6), respectively, and the Bioconcentration Factor (BCF) for heavy metals in root parts was more than that of aerial parts for all elements, and the Translocation Factor (TF) was also less than 1 for all elements, which means, A. Cyanophylla can be considered as a potential bioaccumulator with phytostabilization strategy. The results of statistical analysis ($p < 0.05$) showed significant differences in concentrations between plant parts.

***Keywords**: Acacia Cyanophylla – Cumulative - Heavy Metals – Shahat.*

القدرة الامتصاصية لأشجار السنط الحقيقي *Acacia Cyanophylla* لبعض المعادن الثقيلة في غابة شحات، ليبيا

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الملخص

تم إجراء هذه التجربة في غابة شحات لتقييم قدرة *A. Cyanophylla* على تراكم المعادن الثقيلة بامتصاصها من التربة الملوثة وإعادة بناء النظم البيئية. تم قياس المعادن الثقيلة والتي تشمل (Zn، Fe، Cd، Pb) في أنسجة *A. Cyanophylla* (الأجزاء الخضرية، أجزاء الجذور) وفي التربة بعمق (0-40 سم). وأظهرت النتائج أن تركيزات المعادن الثقيلة لم تتجاوز الحدود المسموح بها من (منظمة الصحة العالمية). وكان *A. Cyanophylla* فعالاً في امتصاص وتراكم المعادن الثقيلة داخل أنسجته حيث بلغ Bio Accumulation Factor

(BAF) للـ (Zn، Fe، Cd،Pb) (2.2، 6.7، 2.1 و 1.6) على التوالي، وكان Bio Concentration Factor (BCF) في الأجزاء الجذرية اكبر من الأجزاء الخضرية في جميع العناصر، وكان Translocation Factor (TF) أقل من 1 في جميع العناصر أيضًا، وهذا يعني أن *A. Cyanophylla* يمكن اعتباره مراكماً حيوياً متبعاً لاستراتيجية التثبيت النباتي phytostabilization. ايضاً أظهرت نتائج التحليل الإحصائي ($P < 0.05$) وجود فروق معنوية في تركيزات المعادن الثقيلة بين أجزاء النبات.

الكلمات المفتاحية: السنط الحقيقي، Acacia Cyanophylla، القدرة التراكمية، المعادن الثقيلة، شحات.

INTRODUCTION

In nature, heavy metals are substances that can cause cancer and mutagenesis (Gola et al., 2016). Moreover, they are extremely persistent in the atmosphere due to their non-biodegradable nature, which permits deadly quantities to rapidly accumulate (Chandrajith et al., 2005; Naser, et al., 2009; Taghipour & Mosaferi, 2013; Mng'ong'o, et al., 2021; Khan et al., 2023). Plants play an important role in environmental evaluation and cleaning, by eliminating heavy metals (Young et al. 2014). Plants usually use two defense strategies to deal with heavy metal toxicity: avoidance and tolerance, and through these two mechanisms, plants maintain intracellular heavy metal concentrations below toxic threshold levels. Avoidance strategies refer to the ability of plants to limit the uptake of heavy metals and their translocation into plant tissues via root cells, which act as the first line of defense at the extracellular level through a series of mechanisms including root adsorption, precipitation of metal ions, and metal exclusion. Once heavy metal ions enter the cytosol, plants adopt resistance strategies to cope with the toxicity of accumulated metal ions, which is the second line of defense at the subcellular level through various mechanisms such as inactivation, chelation, and compartmentalization of heavy metal ions (Hall, 2002; Dalvi & Bhalerao, 2013; Yu et al., 2019; Kraj et al., 2021; Thakur et al., 2022; Moustakas, 2023).

In terms of metal accumulation, plants can be classified into three categories: heavy-metal accumulators, heavy-metal excluders (or non-accumulators), and indicator plants. Heavy-metal-accumulating plants exhibit a concentration ratio of the metal within the plant to that in the soil greater than 1. In contrast, non-accumulating plants display a significantly lower ratio, while indicator plants maintain a ratio close to 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). In the context of phytoremediation, plants employ various strategies such as phytostabilization, phytoextraction, rhizodegradation, and phytovolatilization to either stabilize (passive phytoremediation) or actively absorb (active phytoremediation) contaminants from their environment (Tangahu et al., 2011; Chitimus et al., 2023). Phytostabilization and phytoextraction are the main methods of heavy metal removal by plants, especially legumes (Hao et al., 2014). Phytostabilization involves using metal-tolerant plant species to immobilize heavy metals below ground and reduce their bioavailability, thus preventing their migration into ecosystems and reducing the chance of metals entering the food chain. Selection of the right plant species is essential for phytostabilization (Gerhardt et al., 2017). To meet the requirements for highly effective phytostabilization, plants must be tolerant to heavy metals. Plant roots play a vital role in immobilizing heavy metals, stabilizing soil structure, and preventing soil erosion. Therefore, plants must have a dense root system and be able to produce a large amount of biomass and grow rapidly (Marques et al., 2009; Tangahu et al., 2011; Yan et al. 2020; Venegas-Rioseco et al., 2021). Due to their symbiotic relationships with nitrogen fixers (rhizobia) and other microorganisms, members of the leguminous family may be hyperaccumulators of heavy metals, Most are associated with mycorrhizal fungi and can absorb more heavy metals from landfill leachate than non-legume species (Yang et al., 2003; Khermandar et al., 2016).

Plant family is one of the main factors affecting the distribution of heavy metals in the above- and underground parts of plants. Plants from the Poaceae family accumulate fewer chemical

elements in their aboveground parts than other plants (Chaplaygin, 2018). The genus Acacia comprises shrubs and trees that are part of the Mimosoideae subfamily within the Fabaceae family. Approximately 960 of the approximately, 1300 species that made up the genus Acacia were native to Australia (Dulama et al., 2012).

Some species of Acacia have potential for phytoremediation because of their rapid growth, large biomass, extensive rooting, and ability to withstand environmental stresses. In addition, these trees form symbiotic nodules with nitrogen-fixing bacteria, enhancing soil fertility (Galiana et al., 1990; Awal, 2024). Acacia, a plant native to the arid zone, showed signs of hypertolerance, accumulated high levels of heavy metals, and had the potential to accumulate more of these elements (Khermandar et al., 2016). According to a greenhouse study, sludge-treated sand tailings provided *Acacia mangium* and *Acacia auriculiformis* with significant amounts of Pb, Cd, As, and Hg accumulation (Aba-Alkhil & Moftah, 2013). More significantly, several studies have documented the Acacia species' growth responses and capacity to accumulate Cd in hazardous, Cd-contaminated environments, in addition to their tolerance to heavy metals (Zhang et al., 2022). However, some plant species limited the transference of heavy metals to the aerial parts by accumulating them in their roots. Among these species was *Acacia albida* Bentha (Anoliefo et al., 2008; Mganga, 2014). Furthermore, the roots of the *Acacia mangium* and *Acacia auriculiformis* seedlings accumulated 1.5–2.0 times more heavy metals than the stems and leaves (Rathna Kumari & Nagaraja, 2023). The zinc concentrations in the root tissues may suggest that *Acacia victoriae* is a suitable choice for phytostabilizing zinc-contaminated soils (Mahdavi & Khermandar, 2015). Approximately 80% of the heavy metals in *Acacia farnesiana* were found in roots with low (TF) and high (BAF) (Maldonado-Magaña et al., 2011).

Additionally, the *Acacia mangium* L's bioaccumulation factor (BAF) value indicated that the heavy metals Hg, As, Cd, and Pb were not accumulated in the leaves of the plant, but rather were present in higher concentrations in the mature plant's roots than in its shoots (Raj, 2019). According to (BAF) and (TF) *Acacia mangium's* primary method of absorbing heavy metals into its plant tissues is phytostabilization (Rosli et al., 2021). Pb is more concentrated in the roots than the aboveground tissues, suggesting that *Acacia victoriae* is a viable choice for phytostabilizing Pb-contaminated soils (Khermandar et al., 2016). The Zn concentration gradients in the various sections of the *Acacia victoria* seedlings are arranged as follows: roots, shoots, and leaves, with high (BCF roots parts) values indicating that the roots have accumulated more metal than the shoots and leaves (Mahdavi & Khermandar, 2015).

This experiment was carried out in the Shahat Forest to evaluate *A. Cyanophylla*'s ability to accumulate heavy metals from contaminated soil. Additionally, what strategy mechanisms are utilized by *A. Cyanophylla* in dealing with heavy metals.

Material and methods

Study area: The study was conducted in 2023, in a site exposed to sewage in a forest inside Shahat city, located (at 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 the maximum temperature in the studied area varies from 35.3ºC in summer to 20.1ºC in winter. The minimum temperature ranges from 20.2ºC in summer and 7.5ºC in winter (Othman & Al-Habbat, 2023).

Sample collection: Five mature *A. Cyanophylla* trees were selected to conduct this study. Fresh samples of *A. Cyanophylla* trees from aerial and root parts and soil parts at the 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 *A. Cyanophylla* tree parts and soil parts.

Sample Preparation: The Collected samples were homogenized and crushed into small particles, decomposed by dry digestion method for determining various metals. First, the crucibles and the glass wares used in the experiment were washed with distilled water and then dried in an oven. The weight of each crucible was made constant by keeping it in a muffle furnace at 750 °c for one hour, then transferred to a desiccator and weighed. The purpose was to remove all the moisture. This action was repeated till the weight became constant. A known quantity, of 2g of each sample (aerial part, root part of *A. Cyanophylla* 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. The crucibles were kept in a muffle furnace at 600 °C for 5 hours and then cooled to room temperature in the desiccator for 40 minutes. The obtained white ash is moistened with a few drops of deionized water. An aliquot of 2.0 mL of concentrated HCl is added, left in contact for 10 minutes, and filtered into 100 ml volumetric flasks. The volume was adjusted to the mark with deionized water. All samples were performed in triplicates (Ahmad et al., 2018; Huang et al., 2020).

Elemental analysis of samples: The determination of element concentrations in all the samples was made directly on each of the final solutions, by using the appropriate Instrumentation and methods.

1-Total Nitrogen (TN %) and total phosphorus (TP g kg^{-1}) in soil samples were determined using an automatic elemental analyzer (Elementar 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) g kg^{-1} 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) mg kg^{-1} were determined by atomic absorption spectrophotometer (AAS) (Oumlouki et al., 2021; Cardoso-Silva et al., 2013).

Biological factors:

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

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

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

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 to that, in the plant's roots parts using the equation (Nouha et al., 2024; Wu et al., 2011).

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

A (TF) value greater than 1 in the metal phytoextractors and less than 1 in the metal phytostabilizer 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) using the following equation:

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

Where C_{plant} shows the accumulation of heavy metals in the plant (total biomass) and C_{soil}

denotes the amount of heavy metals in the soil. (BAF) values more than 1 demonstrate the potential success of a plant species for bioaccumulation. (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 (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

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

The result

From Table (1), an increase in the concentration of N, P, K, Ca, and Na was noticed, whereas in aerial parts were (1.63%, 0.096, 1.4, 3.5, and 0.377 g kg^{-1}) for (N, P, K, Ca, and Na) respectively, in roots parts were (1.05%, 0.030, 0.998, 2.5 and 0.308 g kg^{-1}) for (N, P, K, Ca, and Na) respectively and in soil parts were (0.56%, 0.080, 0.103, 3.1, and 0.102 g kg^{-1}) for (N, P, K, Ca, and Na), respectively.

Table (1) The macro elements in A. *Cyanophylla* and soils

Parts	N%	P g kg^{-1}	K g kg^{-1}	Ca g kg^{-1}	Na g kg^{-1}
Aerial	1.63	0.096	1.4	3.5	0.377
Roots	1.05	0.030	0.998	2.5	0.308
Soils	0.56	0.080	0.103	3.1	0.102

From Table (2) We note that there are significant differences at ($p < 0.05$) between the plant`s parts and soil's contents, where the root parts contained the highest concentration of heavy metals, followed by the soil parts, and finally the aerial parts, whereas concentrations of heavy metals of aerial parts were (0.751, 1.01, 0.059, and 0.084 mg kg^{-1}) for (Zn, Fe, Cd, and Pb), respectively. Heavy metals of roots parts were 1.103, 6.51, 0.098, and 0.166 mg kg^{-1}) for (Zn, Fe, Cd, and Pb), respectively, and heavy metals of soil parts were (0.823, 1.12, 0.0725, and 0.155 mg kg^{-1}), respectively. Table (2) shows that the root parts contain the highest quantity of heavy metals, and the accumulation was in roots > soil parts > Arial part. On the other hand, Table (2) also shows that *A. Cyanophylla* tends to accumulate heavy metals in its root parts and limits them to transfer to aerial parts.

Table (2) Concentration of heavy metals in A. *Cyanophylla* and soils

parts	Zn mg kg^{-1}	Fe mg kg^{-1}	Cd mg kg^{-1}	Pb mg kg^{-1}
Aerial	0.751^{C}	1.01^{B}	$0{,}059^{C}$	0.084^{B}
Roots	1.103^{A}	6.51^{A}	0.098^{A}	$0{,}166^{A}$
Soils	0.823^{B}	1.12^{B}	0.073^{AB}	0.155^{A}

From figure (1) we find that the (BCF) of the aerial parts did not exceed (1) for all heavy metals whereas were (0.9, 0.9, 0.8, and 0.5) for (Zn, Fe, Cd, and Pb) respectively, while the (BCF) of the root was greater than (1) for all heavy metals whereas were (1.3, 5.8, 1.3 and 1) for (Zn, Fe, Cd, and Pb) respectively. Also, looking at the (TF) value, we find that it did not exceed (1) in all heavy metals whereas were (0.6, 0.2, 0.6, and 0.5) for (Zn, Fe, Cd, and Pb) respectively.

On the contrary, the (BAF) value exceeded (1) for all heavy metals whereas were (2.2, 6.7, 2.1, and 1.6) for (Zn, Fe, Cd, and Pb) respectively, On the other hand, figure (1) showed that roots parts contain the highest quantity of heavy metals and all indicators (BCF aerial parts, BCF root parts, TF, and BAF) indices that *A. Cyanophylla* tend to absorb heavy metals from the soil to the root parts but restrict it by phytosablization strategy from transfer to aerial parts.

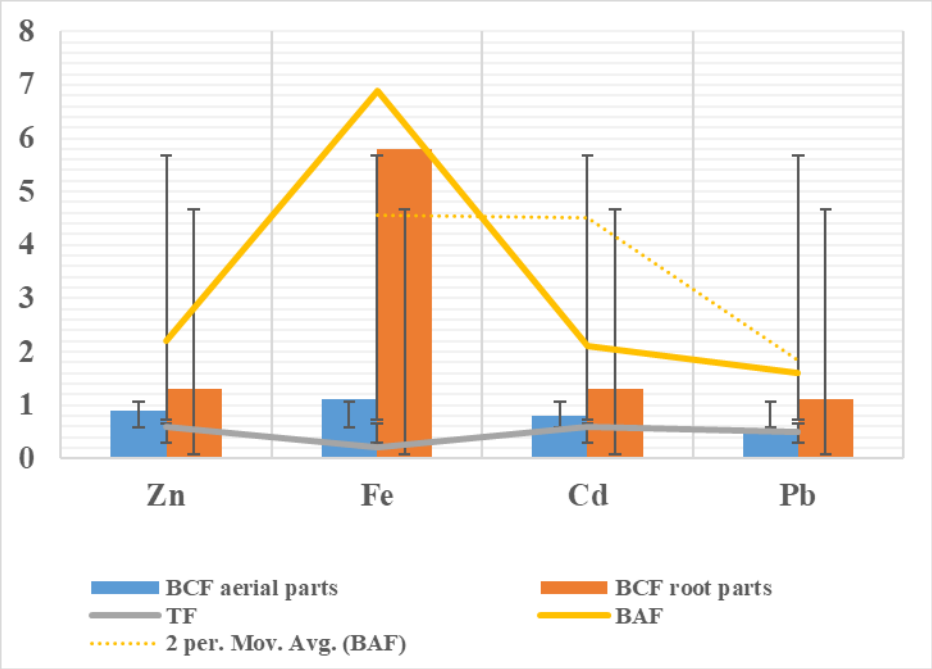


Figure (1) Comparing biological factors

The discussion

An increase in the concentration of N, P K, and Na due to the content of sewage water (Smith and Giller, 1992; Yu et al., 2022). Also, the high concentration of K is due to the presence of several minerals that contain this element, such as feldspar, mica, and illite (Ben Mahmoud, 1995). Regarding the Ca concentration, its increase is due to the soil's original material, which is characterized by a high level of calcium carbonate (Ben Mahmoud, 1995). The concentrations of heavy metals did not exceed the permissible limits of the World Health Organization Standards (WHO, 1996). The root parts contain the highest quantity of heavy metals. The accumulation in roots was greater than in the aerial parts. This was mentioned by (Masvodza et al., 2013; Gerhardt et al., 2017; Lago-Vila et al., 2019), who reported that some heavy metals' absorption in root parts was greater than aerial parts in some legume plants. Also, looking at the (TF) value, we find that it did not exceed 1, and this leads us to the fact that *A. Cyanophylla* accumulated heavy metals in the root system. Similar results were also observed by (Smith & Giller, 1992; Majid et al., 2011; Masvodza et al., 2013; Mganga, 2014; Khermandar et al., 2016). Also, the (BAF) indicates *that A. Cyanophylla* can be considered a bioaccumulator, as the (BAF) value exceeded 1 for all heavy metals, according to the report of (Majid et al., 2011; Khermandar et al., 2016). On the other hand, figure (1) shows that Acacia tends to accumulate heavy metals in its root parts, limiting its transfer to aerial parts, according to (Anoliefo et al., 2008; Mganga. 2014; Raj, 2019). According to (Anoliefo et al., 2008; Maldonado-Magaña et al., 2011; Mganga, 2014; Sopyan et al., 2014; Khermandar et al., 2016; Takarina & Pin, 2017; Suman et al., 2018; Rosli et al., 2021; Rathna Kumari & Nagaraja, 2023) Acacia employs a phytostabilization strategy to accumulate heavy metals below ground, but it is not considered important in the phytoremediation processes because it accumulates the heavy metals in the root system area, while the process of phytoremediation requires accumulating heavy metals in the

aerial parts (not root parts) in order to facilitate the process of harvesting it, which is reported by (Usman et al., 2013; Mishra and Pandey, 2019; Venegas-Rioseco et al., 2021; Nouha et al., 2024).

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