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Prospects for cleaning heavy metal polluted soils using *Helianthus annuus* L. (common sunflower) in a semi-arid area of Tanzania

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Abstract: Natural processes and anthropogenic activities are responsible for the ever-increasing contamination of heavy metals in plants and soils. Elevated levels of heavy metals are reported to threaten physiological processes and functions of plants, also deteriorating microbial activities that, in turn, lower the quality of soils. However, some plant species are capable of tolerating elevated levels of heavy metals by extracting large amounts of the metals without developing any phytotoxic effects. The effectiveness of plants/crops to remove heavy metal pollutants from the soils depends on the nature of the plant and physical, chemical, geological and biological conditions of a particular region. This study aimed to investigate the potential of Helianthus annuus L. (sunflower) to extract lead and cadmium from polluted soils at the University of Dodoma, considering that the soil in that area is reportedly polluted. The levels of lead and cadmium in sunflower roots, leaves and in artificially polluted soils and control (pollutant-free soils) were measured by the atomic absorption spectrophotometer. The results indicate significantly high bioaccumulation factor and translocation factor of sunflower grown in leadpolluted soil and the control soil with cadmium (non-polluted), respectively (p < 0.05). The study concluded that in Dodoma, a semi-arid area, sunflower, which is highly cultivated in that region, is a hyperaccumulator for cadmium and an excluder for lead; thus, suitable for phytoremediation initiatives. Further studies on sunflowers, particularly heavy metal accumulation in seeds, which are borne on the harvestable part of the plant, are recommended.

Keywords: Bioaccumulation factor; cadmium; lead; sunflower; translocation factor

Introduction

Pollution is gradually threatening the safety of the environment in many parts of the world. Heavy metals are among the pollutants predisposed to interact with biotic and abiotic components of ecosystems, consequently altering processes and goods therein. The spread of heavy metals in the environment is linked to industrial production, mining, agrochemicals and utilisation of metal-based compounds (Tchounwou et al., 2012; Sarwar et al., 2017; Fodoué et al., 2022). However, heavy metals such as cobalt, copper, iron, manganese, molybdenum, selenium and zinc play crucial roles in physiological functions in plants and animals, mainly as cofactors that enable enzymes to perform their functions, also governing many cellular functions (Theron et al., 2012; Kumar et al., 2016). All in all, the right amounts of these elements are needed for the proper functioning of the organisms. On the other hand, heavy metals like arsenic, cadmium, lead and mercury are non-essential to organisms and contribute to pronounced problems in soils, plants, animals and air (Duce and Buh, 2010; Johri et al., 2010; Rehman et al., 2017; Islam and Sandhi, 2022). Soils are reservoirs in which plants are mainly harboured. Decomposition of organic matter and cycling of nutrients by microorganisms depend on the health status of soils. In soils, elevated levels of heavy metals decrease the biomass of microorganisms, thereby interfering with enzyme activities that ultimately affect the decomposition of organic matter (Sharma et al., 2007; Hernández, 2008; Hu, 2021). To plants, elevated levels of heavy metals limit reproductive success, cause chlorosis, which tends to inhibit photosynthesis, growth, membrane permeability, nutrient uptake and water absorption due to darkening of roots (Fox, 1995; Parsafar and Marofi, 2013; Nas and Ali, 2018; Nascimento et al., 2021; Musa, 2021).

In heavy metal-contaminated soils, growing plants tend to accumulate the metals in edible and non-edible parts (Gupta et al., 2013). In food crops, such metal accumulation has been linked to bioaccumulation through feeding relationships (Rani and Kaushik, 2023). Despite the negative effects of elevated levels of heavy metals on plants, Baker et al. (1994) and Miranda et al. (2022) recognise two major groups of plants, *viz.*, tolerant and non-tolerant plants. Heavy metal-tolerant plant species are capable of concentrating higher levels of the metals in their shoots without showing any phytotoxic effects. Also, plants

Table 1. The average levels of Pb and Cd in artificially polluted soils and pollutant free soils

Pot no.	Source of artificial	Pb (mg/kg)	Cd (mg/kg)
	pollution		
1	Lead acetate	2134.11	
2	Lead acetate	338.91	
3	Lead acetate	22.11	
4	Lead acetate	102.63	
5	Lead acetate	1221.33	
6	Lead acetate	100.32	
7	Cadmium chloride		4.29
8	Cadmium chloride		299.97
9	Cadmium chloride		45.54
10	Cadmium chloride		198.99
11	Cadmium chloride		91.08
12	Cadmium chloride		4.62
13	None	ND	0.99
14	None	0.33	3.63
15	None	1.98	1.32
16	None	0.99	ND
17	None	32.34	0.99
18	None	0.33	66.99

ND = Not detected.

exhibit heavy metal tolerance through chelate formation between heavy metal ions and plant ligands at the cellular level (Sharma and Dietz, 2006; Manara, 2012). Furthermore, heavy metal tolerance by plants can be achieved by restricting heavy metal ions in the roots through root sorption, metal ion precipitation and exclusion (Dalvi and Bhalerao, 2013). On the other hand, heavy metal non-tolerant plants cannot withstand the negative impacts of heavy metals; instead, they end up being negatively affected.

Phytoremediation is an emerging, cheap technology that uses plants to degrade, extract, contain or immobilise heavy metal pollutants in the environment (Pillon-Smits, 2005; Yan et al., 2020; Zhao et al., 2023). Of the phytoremediation techniques, phytoextraction has recently drawn the attention of environmental ecologists elsewhere. The process makes use of plant species that can take up toxic metals from soils through the root system and accumulate them in the shoots. *Helianthus annuus* L. (sunflower) is among the heavy metal hyperaccumulator and biofuel plants reported in many parts of the world, such as Spain, Britain, Australia and China (Madejón et al., 2003; Pilon-Smits, 2005; Alaboudi et al., 2018). Additionally, the response of crops/plants to elevated levels of heavy metals cannot be generalised due to physical, chemical, biological and geological differences in regions. In Tanzania, there has been limited information regarding the heavy metal hyperaccumulation capacity of the sunflower plant. Sunflower is grown on land which is 6% of the entire productive land of the country (Groot et al., 2019). The leading regions in the production of sunflowers are Dodoma, Singida and Mbeya (Zeng et al., 2017). So far in Dodoma, Pb and Cd pollution have been reported in soils (Mohamed and Haule, 2018; Abu et al., 2021). This called for finding ways of cleaning the soil in that area.

The aim of this study, therefore, was to assess the capacity of *Helianthus annuus* L. (sunflower) to extract Pb and Cd from artificially polluted soil at the University of Dodoma.

Results

The levels of lead and cadmium in artificially polluted soil and pollutant-free soil

The results presented in Table 1 show the levels of lead and cadmium in artificially polluted soils and the control (pollutant-free) soils.

Bioaccumulation factor of Helianthus annuus L. (sunflower) for Pb and Cd

Bioaccumulation factor of sunflower for Pb in artificially Pb polluted soil and in the control (pollutant-free) ranged between 0.70 and 0.88, with mean of 0.79 \pm 0.03, and 0.39 and 0.52, with mean of 0.45 \pm 0.03, respectively (Fig. 1). Furthermore, the bioaccumulation factor for Cd in artificially Cd polluted soil and the control ranged from 0.41 to 0.73, with mean of 0.54 \pm 0.05, and 0.18 to 0.35, with mean of 0.23 \pm 0.03, respectively (Fig. 1). One Way ANOVA revealed a significant difference in bioaccumulation factors of sunflower for lead and cadmium under artificially polluted soils and pollutant-free soils (p < 0.05). The Tukey-Kramer post-test indicated significant differences in bioaccumulation factors by sunflower between all groups, except between Cd-polluted soil and Pb in control soil (pollutant-free soil) (p < 0.05). Bioaccumulation of Pb and Cd from soil to roots by sunflower followed the order: Pb-polluted soil > Cd-polluted soil > pollutant-free soil for Pb > pollutant-free soil for Cd.

Translocation factor for Pb and Cd by Helianthus annuus L. (sunflower)

Translocation factor of Pb by sunflower in artificially Pb-polluted soil and Pb in the control soil (pollutant-free) ranged between 0.13 and 0.41, with a mean of 0.22 \pm 0.04 and 0.63 and 1.58, with a mean of 0.87 \pm 0.18, respectively (Figure 2).

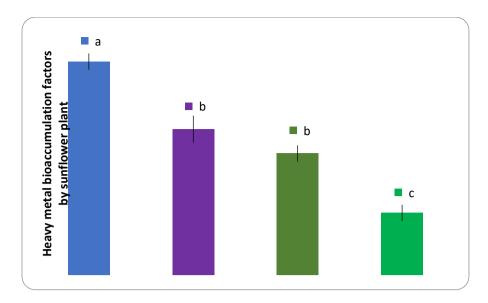


Fig. 1. Heavy metal bioaccumulation (BAF) by sunflower plant in artificially Pb and Cd polluted soils, and in the control.

Furthermore, the translocation factor for Cd by sunflower in artificially Cd-polluted soil and Cd in the control soil ranged from 0.83 to 1.37, with a mean of 1.14 ± 0.09 , and 1.57 to 3.23, with mean of 2.25 ± 0.20 (Figure 2). Kruskal-Wallis test indicated that there is a significant difference in translocation of Pb and Cd in the artificially Pb and Cd-polluted soils and the control soils (KW = 18.9, p < 0.05). Dunn's post hoc test revealed that the translocation factor of sunflower was significantly different only between Pb-polluted soil and Cd in the control soil (pollutant-free soil). The trend of translocation of Pb and Cd from roots to leaves of sunflower followed the order: Cd in pollutant-free soil > Cd-polluted soil > Pb in pollutant-free soil > Pb-polluted soil.

Discussion

This study shows that elevated levels of lead were found in the roots of sunflowers grown in Pb-polluted soil, as depicted by a high bioaccumulation factor. Even for sunflowers grown in non-polluted soils, high levels of lead were found in the roots when compared with cadmium. High levels of Pb in sunflower (*Helianthus annuus* L.) roots and low levels in shoots were also reported in China (Zhou et al., 2020) and Nigeria (Abdullahi et al., 2021). In Germany, higher levels of Pb were found in roots of *Helianthus tuberosus* (Willscher et al., 2017). Likewise, in Iran, the trend of lead storage in sunflower parts was root > stem > leaves (AL-Jobori and Kadhim, 2019). However, our finding contradicts with Crist et al. (2005) regarding the storage of higher levels of lead in the leaves of sunflower. The discrepancy could have resulted from the inducement of lead uptake using a chelating agent, EDTA, which was used in that study.

Furthermore, this study suggests that sunflower was capable of absorbing large amounts of lead from both polluted and non-polluted soils but retaining the metal in the root zone. Ecologically speaking, this is beneficial since the chances of the toxic metal contaminating the harvestable part of the sunflower (the head) will be minimal. In Zambia and Iran, sunflowers were reported as effective plants in removing lead from contaminated soils (Mbuki and Mbewe, 2017; AL-Jobori and Kadhim, 2019). For effective performance, those studies recommended using sunflowers during the early stage of development (Adesodun et al., 2010; Mbuki and Mbewe, 2017). On the contrary, Madejón et al. (2003) did not recommend sunflower for the extraction of lead from polluted soils because of removing only limited levels but leaving much more metals in soils. Based on this background, sunflowers are more suitable for removing lead from lead-contaminated soil. But on the other hand, restriction of lead in the roots of the plant could not be feasible, particularly when considering the least translocation factor of the metal.

If a phytoextraction project aims to completely remove heavy metals from a particular area, then sunflowers could be a candidate plant for eliminating cadmium. This argument is given based on the high translocation factor of sunflowers for cadmium, in that much of the metal is stored in the leaves. This is more appropriate because leaves with elevated levels of cadmium can be harvested and eliminated from a particular area. This finding conforms to Crist et al. (2005), who reported higher levels of cadmium in sunflower leaves in America.

Nevertheless, the detected lead and cadmium levels in the roots and leaves of sunflowers grown in the control suggest heavy metal pollution in the soils of the study area. This supports earlier findings of Mohamed and Haule (2018), who reported on heavy metal pollution in the Dodoma region. The permissible limits for levels of lead and cadmium in soil and plants are 85 mg/kg, 2 mg/kg and 0.8 mg/kg, 0.02 mg/kg, respectively (World Health Organisation (WHO), 1996). In Nigeria, Francis et al. (2017) reported that in sunflowers, the amounts of lead and cadmium were below detection levels because of growing on soil with heavy metals that were below the detection limit.

The results of this study imply that sunflower (*Helianthus annuus* L.) is a good absorber of heavy metals, though their sequestration to the aerial parts may be governed by the type and characteristics of the metallic ion. This study, therefore, identifies sunflower as a hyperaccumulator for cadmium and an excluder for lead. A plant is recognised as a

hyperaccumulator for heavy metals if its translocation factor exceeds 1; or an excluder if it retains high levels of the metals in the roots but with a translocation factor below 1 (Ginocchio and Baker, 2004; Yang et al., 2005; Boularbah et al., 2006; Rotkittikhun et al., 2006). The deep root system of sunflowers is reported as a suitable architecture for facilitation of heavy metal uptake from soils (Tomé et al., 2008). The possible tolerance of sunflower for elevated levels of cadmium and lead polluted soils is in agreement with Niu et al. (2007), who reported on the capacity of sunflower for Pb and Cd uptake in China.

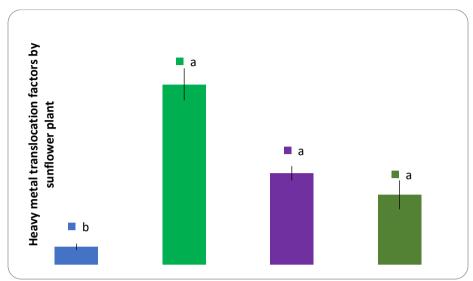


Fig. 2. Heavy metal translocation factor (TF) by sunflower plant in artificially Pb and Cd polluted soils, and the control. Groups with the same letters do not differ statistically, while those with different letters show significant differences.

Materials and Methods

The study area

This study was carried out at the University of Dodoma, which is located in Dodoma District, in the central part of Tanzania. The University of Dodoma is located along latitude 6°10′23"S and longitude 35°44′31"E. Dodoma is a semi-arid region receiving a modest amount of rainfall, which ranges between 550 and 600 mm per year; the rainy season usually starts from December to April (Gayo, 2021). In that area, the mean annual temperature is 29°C, with the lowest and highest peaks of 13°C and 30°C experienced in July and November, respectively (Kayombo et al., 2020). The soils of Dodoma are categorised based on hydrological characteristics, these include phaeozems and leptosols with poor groundwater retention, as well as vertisols and acrisols with moderately suitable groundwater permeability and retention properties respectively (Mseli et al., 2021). In Dodoma, the soil textural classes consist of coarse sand, loamy and clays underlaid with hard sub-soils (Msanya et al., 2018). Dodoma Region has a population of 3,085,625 people (United Republic of Tanzania (URT), 2022). The main economic activity in that area is agriculture, involving farming and livestock keeping. Sunflowers are among the crops cultivated. In Tanzania, Dodoma is among the best three regions in the production of sunflowers. Yet heavy metal pollution from Pb and Cd has been reported in soils of that region (Mohamed and Haule, 2018; Abu et al., 2021).

Experimental set-up

The experiment was done in the greenhouse at the Department of Biology (University of Dodoma) using 18 pots of 30 cm height and diameter of 40 cm with at least three holes at the bottom. The height of 30 cm was chosen based on the deeprooting nature of the sunflower plant (Ennos, 2000). The pot with a diameter of 40 cm was used to give seedling spacing of 30 cm as recommended by Wright (2022). Thirty-six kg of fresh soil collected from the above ground of canopy trees was used. Two kilograms of fresh soil were independently put in each of the eighteen pots.

Artificial pollution by heavy metal salts

Initially, 2000 mg lead acetate ($Pb(C_2H_3O_2)_2$) was added to each of the first six pots, while 200 mg cadmium chloride ($CdCl_2$) was separately put into the other six pots to artificially polluting the soils as recommended by Wang et al. (2013) and Ugwu et al. (2019). The resulting mixtures were well-mixed. In the last six pots, no heavy metal salt was added as they served as controls (Chunilall et al., 2005). The samples in all pots were left for one month to give time for thorough mixing particularly for the artificially polluted pots. The soils were dampened twice a week.

Processing of soils and sunflowers for heavy metal analysis

After one month of mixing, all soils were analysed for levels of Cd and Pb before sowing of *Helianthus annuus* (sunflower) seeds. After heavy metal analysis in soils, four sunflower seeds (commercial hybrid AGUARA 4) that were purchased from a vendor in Dodoma City were sown 30 cm apart in each of the 18 pots. Watering was done early in the morning or late evening as needed until the vegetative stage was attained (Schneiter and Miller, 1981). After one month of growing the

sunflowers, about $10\,\mathrm{g}$ of roots and leaves were separately collected from each pot for heavy metal analysis. Before analysis, the roots and leaves were thoroughly washed with distilled water to remove all adhering particles. The roots and leaves were oven-dried at 105° C to constant weights. The dried roots and leaves were cooled and ground to powder using a wearing blender as recommended by Allen (1989). Five grams from each sample were used for analysis of Pb and Cd after digestion with 5 ml of 5:1 concentrated Perchloric (HClO₄) and Nitric (HNO₃) acid mixture (Allen, 1989). The resulting mixtures were then heated in a Kjeldahl Thermo at 200° C to complete digestion. After digestion, samples were left to cool, and then distilled water was added up to the 50-ml mark. The digested samples were filtered to get clear solutions for heavy metal analysis.

Data collection

The levels of Pb and Cd in soils were analysed by the atomic absorption spectrophotometer 4210 MP-AES, Model G8007A, at the College of Earth Sciences and Engineering of the University of Dodoma. Likewise, the levels of heavy metals Pb and Cd in roots and leaves were analysed by the atomic absorption spectrophotometer 4210 MP-AES, Model G8007A. Standard Reference Material of National Institute of Standards and Technology (NIST) 2709 San Joaquin soil and tomato leaf (SRM 1573a) were used, with percentage recoveries of 83% Pb and 85% Cd.

Data analysis

In this study bioaccumulation factor (BAF) of Pb and Cd from the soil to *Helianthus annuus* L. was determined by calculating the ratio of metal levels in the root to that of the soil as:

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BAF = \frac{\text{Metal level in the roots (mg/kg)}}{\text{Metal level in the soil (mg/kg)}}
(Equation 1)
(Fitz and Wenzel, 2002)
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Pb and Cd translocation from root to shoot of *Helianthus annuus* L. was measured by translocation factor (TF) which is given by:

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TF = \frac{\text{Metal level in the leaves (mg/kg)}}{\text{Metal level in the roots (mg/kg)}}
(Equation 2)
(Baker and Brooks, 1989; Al Salman and Abdul Aziz, 2002)
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Bioaccumulation factor values of sunflowers grown in Pb-polluted soil, Cd-polluted soil and control (pollutant-free) were assessed using One Way ANOVA due to their parametric nature. On the other hand, data on translocation factors of sunflowers for Pb and Cd in polluted and control (pollutant-free) soils were assessed using Kruskal-Wallis because they were nonparametric.

Conclusions

In this study, it was concluded that sunflower (*Helianthus annuus* L.) has potential as a candidate plant for cleaning lead and cadmium-polluted soils. This is evidenced by the high bioaccumulation factors for lead and translocation factors for cadmium. The fate of extracted lead from lead polluted soil is retaining the metal in the roots. On the other hand, the amounts of cadmium extracted by sunflower from cadmium polluted soil are transported and subsequently stored in leaves. Therefore, sunflower is a hyperaccumulator for cadmium and an excluder for lead in heavy metal-polluted soils. In many regions, heavy metals mostly found in contaminated soils are lead and cadmium (Musa, 2021). Thus, related studies using native crops/plants are recommended, considering regional variations in physical, chemical, geological and biological conditions. Also, a thorough investigation of heavy metal contents in sunflower seeds is recommended considering that these are the common harvested and consumed plant parts that can result into bioaccumulation of toxic metals in herbivores and/or omnivores in food chains/webs.

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Authors' contributions

Both authors formulated the conception and design of this study. MM participated in data collection, and then the data were analysed by NM. NM drafted the manuscript, which was then revised by both authors. NM and MM approved the manuscript for submission.

Conflict of interests

There are no competing interests to declare.

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