World Journal of
Environmental Research
ISSN 2301-2641

Volume 14, Issue 2, (2024) 49-59

www.wjer.eu

Phytoremediation potential of *Helianthus Annuus* L (Sunflower) for the reclamation of lead (Pb) spiked soil

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Suggested Citation:

Ngabra, H., Allamin, I.A., Ismail, H.Y., Ngabra, M., Shettima, H. & Misherima, K.A. (2024). Phytoremediation potential of Helianthus Annuus L (Sunflower) for the reclamation of lead (Pb) spiked soil. *World Journal of Environmental Research*, *14*(2), 49-59. https://doi.org/10.18844/wjer.v14i2.9475

Received from April 11, 2024; revised from July 12, 2024; accepted from December 12, 2024.
Selection and peer review under the responsibility of Prof. Dr. Haluk Soran, Near East University, Cyprus

Abstract

Soil contamination from industrial processes, waste disposal, and natural disasters is a serious issue, endangering human health and ecosystems. Phytoremediation, a low-cost, eco-friendly soil remediation technique, has gained attention. This study evaluated Helianthus annuus L., a local plant, for its potential to remediate lead-contaminated soil under laboratory conditions. Plant phytotolerance was assessed in soil spiked with various lead (Pb) levels. Growth was monitored for lead tolerance, and Pb uptake was measured using atomic absorption spectrophotometry (AAS). Results showed H. annuus exhibited substantial growth at low Pb concentrations compared to controls, with growth declining at higher Pb levels. Biomass analysis over 15 days indicated Pb exposure impacted biomass formation. Pb measurements showed significant root accumulation and translocation within the plant. Findings suggest H. annuus can accumulate lead without compromising biomass, making it a promising candidate for remediating Pb-polluted soils.

Keywords*:* Heavy metal; lead (Pb); phytoremediation; soil; sunflower.

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1. INTRODUCTION

Soil contamination resulting from human activities, such as industrial processes, excessive waste disposal, or natural disasters, has become a significant issue that threatens both human health and ecosystems (Allamin et al., 2021; Yang et al., 2022). The primary sources of contamination stem from human actions, and it is crucial to address these issues to prevent or reduce the transfer of pollutants into terrestrial, atmospheric, or aquatic environments (Iqbal et al., 2024; Sereni et al., 2021; Shimod et al., 2022).

Lead is released from welding activities, led gasoline near busy roads, and lead-acid battery recycling facilities which are detrimental to human life and the ecosystem. Divalent heavy metals such as Pb could cause cardiovascular diseases, mental disorders, retardation, and kidney diseases, it could also damage the renal system if found greater than the permissible limit in the food chain (Bhat et al., 2022; Consentino et al., 2023). Therefore, it is essential to bring Lead within the permissible limits set by various environmental agencies before having any agricultural activities such as vegetable plantation on the soil.

Traditional land reclamation methods are unfriendly to the environment and costly, whereas, Phytoremediation, on the other hand, is both environmentally safe and cost-efficient (Anum et al., 2022). Although cost-effective and environmentally friendly, these technologies have primarily been implemented in field applications within developed countries. They remain largely inaccessible in many developing nations, likely due to limited awareness of their benefits and operational mechanisms. However, as policymakers and the public become more informed about the health risks posed by polluted soils, interest within the scientific community has grown in advancing technologies to remediate contaminated areas (Jabłońska-Czapla et al., 2024).

Affordable and eco-friendly remediation options are essential for restoring polluted lands in densely populated developing countries with limited environmental conservation budgets. Such solutions can help mitigate related risks, make land available for agriculture, enhance food security, and alleviate land tenure challenges (Alves et al., 2022). Given its toxicity and to safeguard our soil and environmental resources, it is essential to remove or to bring lead (Pb) within permissible limits before using the land for agricultural purposes. Therefore, the phytoremediation technique could be efficient in such cases. Several plants were used to remediate contaminated land namely: white willow, Indian mustard, poplar tree, and Indian grass.

1.1. Purpose of study

In this research, the researchers use the sunflower (*Helianthus annuus* L.) to remediate the land contaminated with Pb (lead).

2. METHOD AND MATERIALS

2.1. Study area

The experiment was carried out at the University of Maiduguri, with Field work in the Botanical Garden Department of Biological Science and Laboratory work at the Microbiology Laboratory Department of Microbiology. The University of Maiduguri is located at a longitude of 11.50° North and a latitude of 13.15° East, having an elevation of 364m above sea level. The temperature of the metropolis ranges between 35°-42°C in the heating season (March to May) and in cold seasons, it ranges between 19°- 25°C. The amount of rainfall varies between 150-600mm during the short rainy season (June to September) each year.

2.2. Experimental design

2.2.1. Pot preparation

Agriculture soils (pristine) with no history of organic or inorganic contaminants were collected from pristine soil. To each corresponding pot, 3 kg of the soil was transferred which was previously air-dried and sieved using a 4mm sieve to all the corresponding treatments (Asgari et al., 2019).

2.2.2. Treatment of soil with Pb, planting of H. annuus, and sowing of seeds

Lead was weighed into five concentrations (CR5%=150, CR10%=300, CR15%=450, CR20%=600, CR25%=750 mg) homogenously mixed in triplicate. The soil was thoroughly agitated and mixed in a fume hood to ensure even distribution and uniform blending with Pb. The soil sample was taken for physicochemical analysis before treatment (Azeem et al., 2023). After one week, seeds of *H. annuus were* planted accordingly. The viability of the seed of *H. annuus L.* was tested using the floatation method (Ismail et al., 2024). Seed viability was tested by soaking them in distilled water for five minutes. Floating seeds were removed as non-viable, while those that sank were considered viable. The viable seeds then underwent surface sterilization with a 10% hydrogen peroxide solution before planting (Ismail et al., 2024). Seeds were sown at the recommended depth for H. annuus approximately 1.5 to 3 cm in each treatment. For optimal establishment, five seeds were placed in each planting hole. Pots were watered daily, and seedling emergence was monitored closely.

2.2.3. *Collection of soil samples*

Samples were taken at three growth span steps of the plant namely, the early or vegetative (15, to 30 days after seed sowing), pre-flowering (45 days after seed sowing), and maturity (60 days after seed sowing) (Allamin et al., 2021). At each time point, H. annuus plant samples, including roots and above-ground parts, were collected for each treatment and control group. The roots of the uprooted plants were gently shaken to collect the soil adhering to them without damaging the roots or root nodules. This collected soil, termed "rhizosphere soil," was placed into sterile sample bottles for microbiological analysis. For other analyses, however, the soil surrounding the up-rotted plant was sampled using soil auger at a depth of 1 to 5 cm into sterile sampling bottles in triplicates.

2.2.4. *Measurement of plant growth and tolerance*

Physical emergence was observed, and a weekly monitoring and measurement of plant height and shoot number was done for 3 weeks (28 days) where several leaves and plant heights using a measurement ruler were used to measure in all the treatments. In each pot, a plant was harvested in 15-day intervals for 60 days. The plants were washed with distilled water and separated into different parts: shoots (including leaves and stems), and roots, where the wet weight (WW) was recorded, and the dry weight (DW) was also recorded after prior oven drying of the samples at 70^0C for 2 days (Allamin et al., 2020).

2.2.5. *Heavy metals analysis*

Rhizosphere soil, root, shoot, and leaf samples from various treatments were collected and oven-dried at 70 ºC for two days (Allamin et al., 2021). A 1 g portion of each sample was combined with 15 mL of acids (HNO_3, H_2SO_4, and $HClO_4$) in a 5:1:1 ratio at 100 ºC until a clear solution formed. The digested samples were then filtered through the Whatman No. 42 filter paper, and the filtrates were diluted to 50 mL with deionized water before storage for further analysis. Heavy metal concentrations in both dried soil and plant tissue samples were measured using an atomic absorption spectrophotometer (AAS). The bioconcentration factor (BCF) and translocation factor (TF) for heavy metals in H. annuus were calculated based on the specified formula (Allamin et al., 2021).

$$\text{Bioconcentration factor (BCF)} = \frac{\text{Mean heavy metal concentration in the plant.}}{\text{Heavy metals in the soil}}$$

$$\text{Translocation factor (TF)} = \text{Concentration above ground x 1/Concentration in root.}$$

3. RESULTS

Table 1

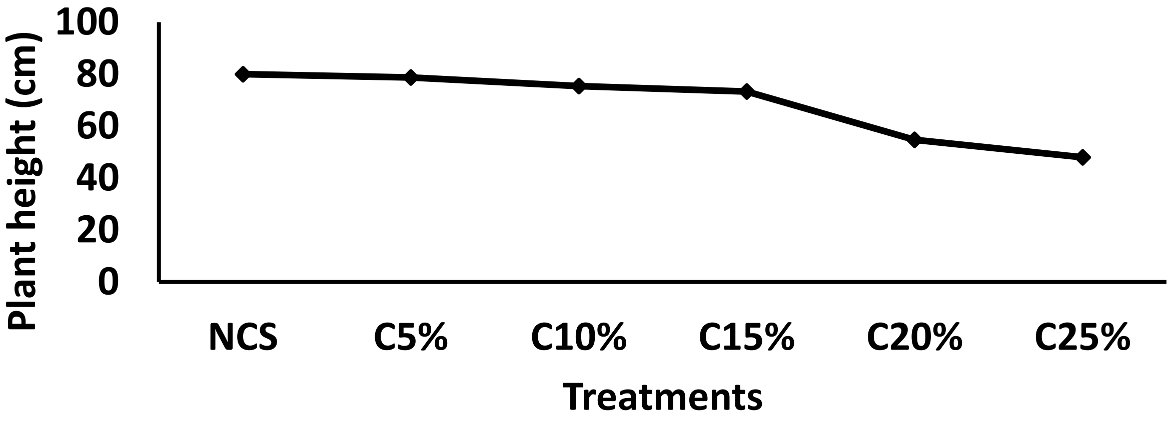
Soil physical and chemical properties before planting

Physico-Chemical Properties	Values
pH	7.10
Temperature	34^0C
Electrical Conductivity	0.15 dsm-1
Total Dissolved Solids	262.00mg/l

Total Nitrogen	3.64g/kg
Organic Carbon	0.23%
Total Organic Matter	0.39%
Soil Texture	Sandy loam
Lead (Pb)	0.00001

Figure 1

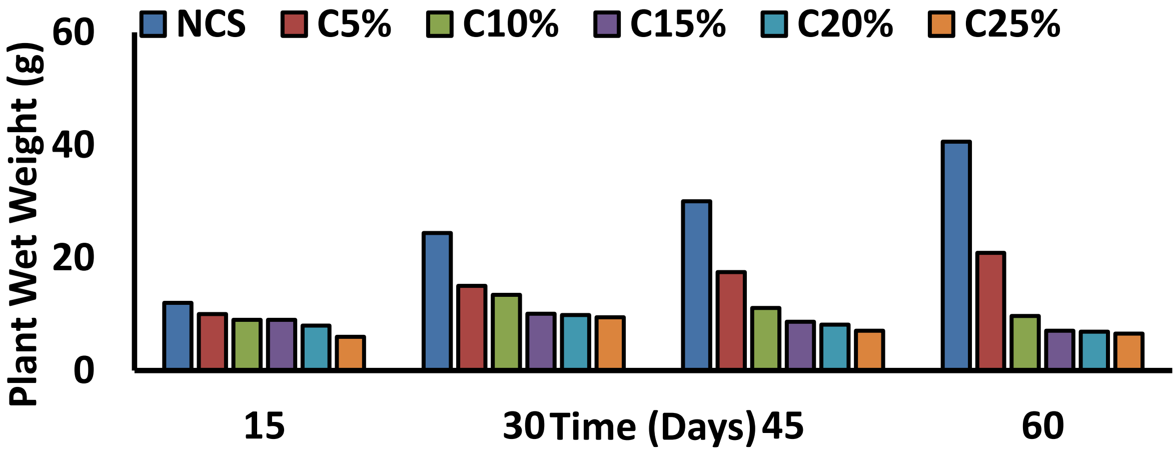
Helianthus annuus L height at 28 days of sowing in soil contaminated with lead (Pb)



Note: NCS=Not Contaminated Sample, C=Contaminated

Figure 2

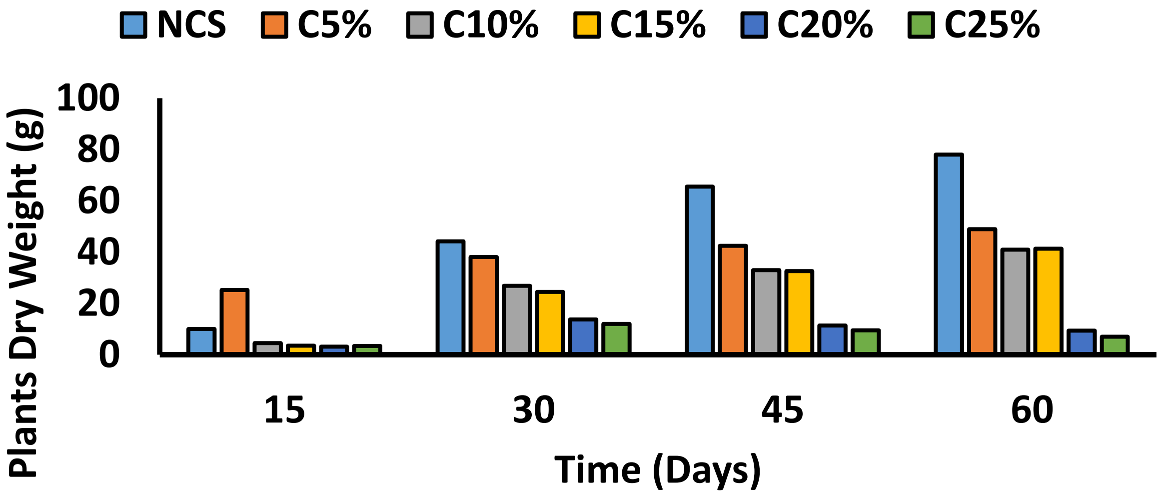
Wet biomass of H. annuus influenced by lead (Pb) uptake from 15 to 60 days of plant growth.



Note: NCS= Not Contaminated Sample, C5%= Contaminated with 5% lead, C10%= Contaminated with 10% lead, C15%= Contaminated with 15% lead, C20%= Contaminated with 20% lead and C25%= Contaminated with 25% lead.

Figure 3

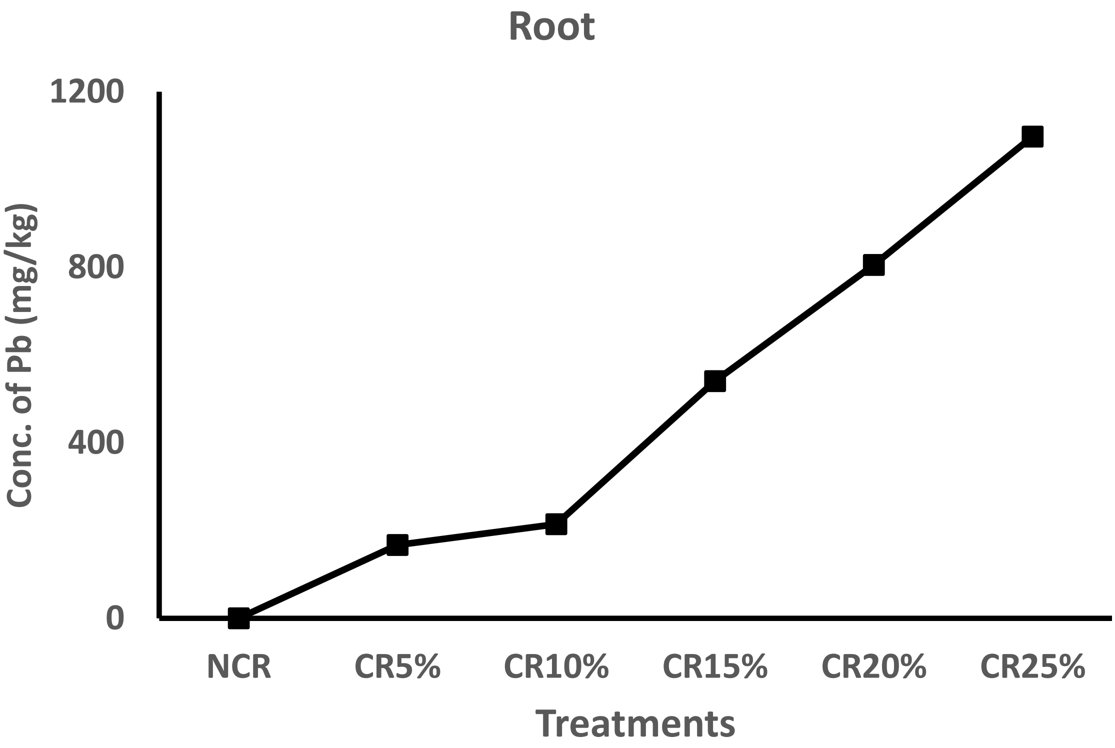
Dry biomass of H. annuus influenced by lead (Pb) uptake from 15 to 60 of plant growth.



Note: NCS= Not Contaminated Sample, C5%= Contaminated with 5% lead, C10%= Contaminated with 10% lead, C15%= Contaminated with 15% lead, C20%= Contaminated with 20% lead and C25%= Contaminated with 25% lead.

Figure 4

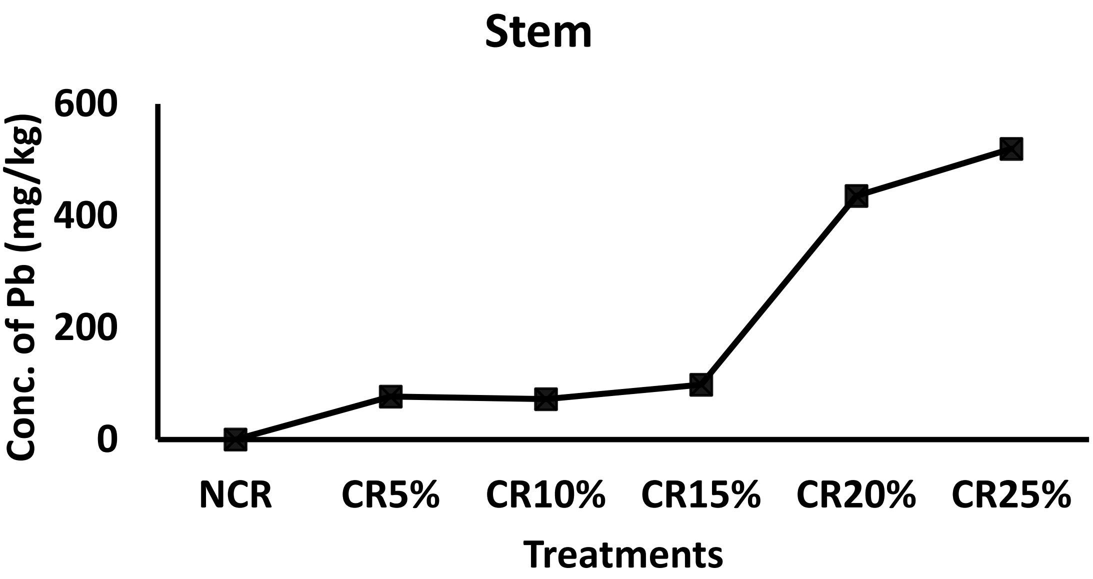
Heavy metals concentrations in roots of H. annuus



Note: NCS= Not Contaminated Sample, C5%= Contaminated with 5% lead, C10%= Contaminated with 10% lead, C15%= Contaminated with 15% lead, C20%= Contaminated with 20% lead and C25%= Contaminated with 25% lead.

Figure 5

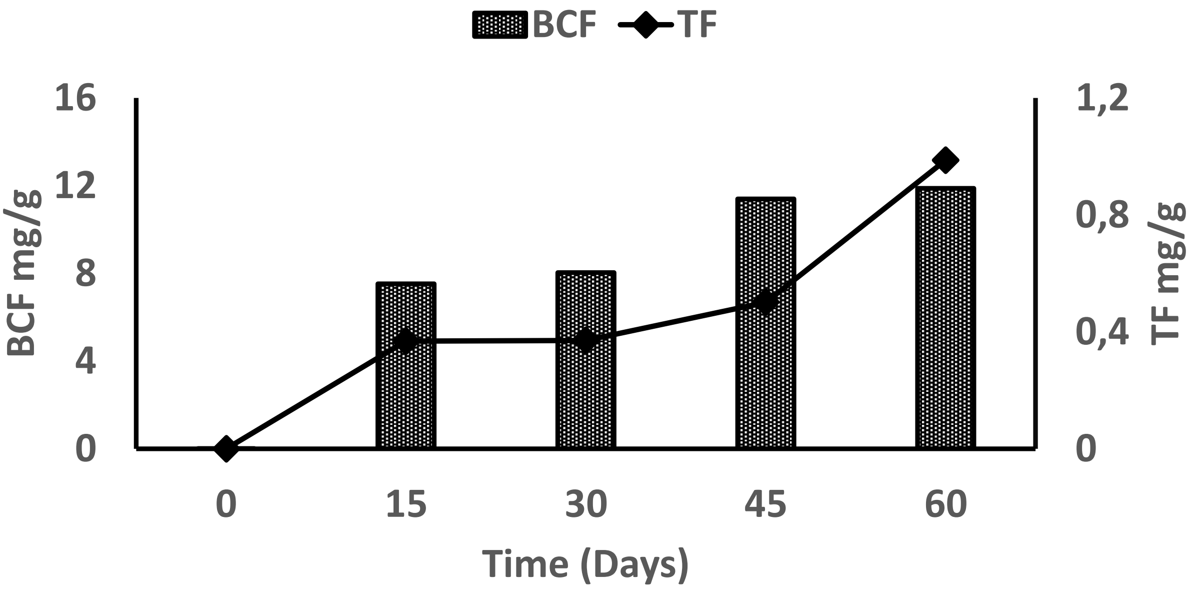
Heavy metals concentrations in the stem of H. annuus



Note:
NCS= Not Contaminated Sample, C5%= Contaminated with 5% lead, C10%= Contaminated with 10% lead, C15%= Contaminated with 15% lead, C20%= Contaminated with 20% lead and C25%= Contaminated with 25% lead.

Figure 6

Bioconcentration Factor (BCF) and Translocation factor (TF) of Lead in the highest Concentration from 0 to 60 days.



4. DISCUSSIONS

In the present study, we screened the phytotolerance of sunflower (*Helianthus annuus L*) towards lead (Pb) spiked soil at different concentration gradients. The physicochemical quality of the pristine soil was determined erstwhile before spiking the soil with various concentrations of Pb (Table 1) the results show that the soil has a neutral pH and is rich with organic carbon and nitrogen respectively. Since both physical and chemical properties influence soil productivity, the assessment of soil physico-chemical attributes in a region is crucial. Soil organic carbon and nitrogen have a significant impact on several soil characteristics, such as color, nutrient holding capacity (cation and anion exchange capacity), nutrient turnover, and stability. These characteristics, in turn, influence water relations, aeration, and workability of the soil (Azeem et al., 2023). The tolerance of (*Helianthus annuus L*) in the early stage of growth after germination was studied phenotypically by measuring the growth of the plant (Figure 1), which shows a significant growth of (*Helianthus annuus L*) in lower concentration compared to the control which was not spiked with lead (Pb) though there is a decrease in the plant growth at high concentration of C25% (Pb). Numerous studies have shown that various heavy metals, such as Cd and Pb, can inhibit plant growth (Farid et al., 2018). Consistent with these findings, this study revealed that lead (Pb) exposure reduced the growth of Helianthus annuus L. Specifically, higher Pb concentrations of 600 and 750 mg per kg of soil decreased plant height by 6.6% and 7.6%, respectively. Elevated heavy metal levels are known to hinder cell division and differentiation, limit cell elongation, and negatively impact overall plant growth and development (Asgari et al., 2019). Helianthus annuus L. exhibited varied growth responses under Pb-induced stress.

The wet and dry biomass of *Helianthus annuus L* in a timeframe of 15 days was analyzed (Figure 2 and 3 respectively) which shows the significant effect of the heavy metal in the biomass formation of *Helianthus annuus L*. The mild effects on physiological parameters may influence plant growth responses and dry matter production. Notably, the application of 200 mg Pb per kg of soil led to an increase in root, shoot, and total plant dry weight compared to other Pb concentrations and the control, suggesting minimal toxicity within this concentration range. However, Pb concentrations exceeding 600 mg resulted in reductions in root, shoot, and total plant dry matter. Analysis of dissected plant organs showed that shoot dry weight was significantly greater than root dry weight. Similar findings have been reported by other researchers, who observed reductions in dry matter production across various plant species with increasing Pb doses in both soil and nutrient solution experiments (Li et al., 2019; Jehan et al., 2022; Khan et al., 2021; Khan et al., 2023).

The absorption of lead (Pb) by Helianthus annuus L was assessed using Atomic Absorption Spectroscopy (AAS), revealing significant Pb accumulation in the plant's roots, with the highest concentrations occurring at elevated Pb levels (Figure 4). Furthermore, the study identified the potential transfer of Pb from roots to stems at higher Pb concentrations (Figure 5). These results confirm that Helianthus annuus L is capable of absorbing heavy metals into its tissues, consistent with findings by Thangavel and Subbhuraam (2004). The data also showed that most of the Pb taken up by the plant is stored in the roots rather than in the stems or leaves. This occurs because heavy metals are absorbed by the roots from the soil and later transported to the leaves via xylem vessels, where they are stored in vacuoles (Omara et al., 2022). Root accumulation of Pb is considered a key factor in enhancing the plant's tolerance to Pb toxicity by limiting metal movement to the leaves (Nkoh et al., 2022).

Additionally, as soil Pb concentration increased, so did the amount of Pb absorbed by the plant. Figure 4 indicates that Pb levels in the entire plant were slightly lower than those in the soil post-harvest, emphasizing the significant role of roots in Pb storage. The high Pb concentration found mainly in the roots suggests that Helianthus annuus L minimizes toxicity in the plant's more active parts by reducing Pb translocation to the above-ground parts and by re-translocating the toxic metal from the shoots back to the roots (Madanan et al., 2021). Farid et al. (2018) confirmed the potential of Helianthus annuus L for remediating metals from contaminated environments. Pb concentration in the roots increased in a linear fashion ($p \geq 0.05$) as Pb doses in the soil were elevated. Heavy metal contamination can disrupt soil microbial communities and degrade soil quality (Xie et al., 2016), with Alaboudi et al. (2018) also noting a rise in Pb levels in soil treated with 200 mg Pb/kg.

Bioconcentration (BCF) and Translocation Factor (TF) are key indicators for assessing a plant's ability for phytoextraction and phytostabilization. As seen in Figure 6, BCF and TF values significantly increased in the roots, stems, and leaves at higher Pb concentrations. The highest BCF of Pb was found in the roots, followed by the stems and leaves of Helianthus annuus L. Plants with a root BCF >1 and TF <1 is suitable for phytostabilization, as they effectively limit the transfer of Pb to the above-ground parts, a crucial feature for phytostabilization species. All Pb concentrations tested resulted in TF values of less than 1, confirming the plant's limited ability to translocate Pb from the roots to the shoots. This limitation is common in the phytoremediation of toxic heavy metals but can be addressed by removing the plants to extract accumulated metals from the roots (Asgari et al., 2019).

Increased toxicity was observed with higher metal concentrations, with more severe effects on the leaves than on the roots and stems. Since Helianthus annuus L stores most of the Pb in its roots, it is concluded that the plant serves a phytostabilizing function for Pb. Li et al. (2019) noted that effective hyperaccumulators can accumulate 1-3% Pb levels in their leaves and stems. This study supports the suitability of Helianthus annuus L for phytoremediation due to its ability to accumulate and tolerate substantial Pb concentrations. Except for hyperaccumulators, low Pb translocation and high root accumulation are typical in many plant species (Li et al., 2019; Younas et al., 2022). Current findings show that Pb is retained predominantly in the roots with minimal translocation to the upper parts of the plant. However, it should be noted that these results are based on pot experiments, and further validation in field conditions is necessary.

Phytoremediation is a promising technique for reclaiming heavy metal-contaminated soils, offering public acceptance and several advantages over traditional methods. The use of hyperaccumulator plants for phytoremediation is an effective strategy, with numerous such plants identified. However, this process is slow, requiring long periods to clean contaminated sites, particularly those with moderate to high contamination levels, partly due to the slow growth rates and low biomass production of hyperaccumulators. Enhancing plant performance is thus essential for improving phytoremediation efficiency. Additionally, increasing the bioavailability of heavy metals in soil is crucial for successful phytoextraction. Many heavy metals, including Pb, are not readily available in the soil for absorption by plants, as only a small portion exists in soluble forms (Ismail et al., 2014; Khan et al., 2019). Metals like Zn and Cd are more mobile and bioavailable to plants than Pb (Kumari et al., 2022). Heavy metals can be categorized by their bioavailability: readily bioavailable metals (Cd, Ni, Zn, As, Se, Cu), moderately bioavailable metals (for instance, Co, Mn, Fe), and those with low bioavailability (for instance, Pb, Cr) (Mishra et al., 2009; Khan et al., 2021; Ozyyigit et al., 2022; Salbitani et al., 2023).

The low bioavailability of Pb limits its uptake, reducing the effectiveness of phytoextraction. Soil properties, such as pH, microbial activity, and the presence of chelating agents, can affect the solubility and bioavailability of heavy metals (Nivetha et al., 2023). Plants can enhance metal bioavailability through root exudates, which acidify the rhizosphere, releasing metals from soil complexes (Thangavel and Subbhuraam, 2004). Moreover, genetic engineering is emerging as a powerful tool to develop plants with enhanced traits, such as rapid growth, high biomass, and increased tolerance and accumulation of heavy metals.

5. CONCLUSION

A comprehensive understanding of the mechanisms involved in the uptake, translocation, and detoxification of heavy metals in plants, along with the identification and characterization of relevant molecules and signaling pathways, is crucial for developing ideal plant species for phytoremediation through genetic engineering. By manipulating genes related to heavy metal uptake, translocation, sequestration, and tolerance, it is possible to enhance a plant's ability to accumulate or tolerate heavy metals.

Phytoremediation is an effective strategy for addressing soil contamination by heavy metals. In this study, the potential of *Helianthus annuus L.* (sunflower) for phytoremediation of lead (Pb) from contaminated soil was evaluated. The results showed that *Helianthus annuus L.* effectively absorbed lead without showing significant stress throughout its growth period. Therefore, sunflower (*Helianthus annuus L.*) proves to be a suitable plant species for the phytoremediation of Pb-contaminated soils, offering an environmentally friendly solution for Pb removal and the restoration of biogeochemical cycles in polluted soils.

Acknowledgment: The authors would like to thank Northeast Center for the Biotechnology University of Maiduguri for supporting the research.

Conflict of Interest: The authors declare no conflict of interest.

Ethical Approval: The study adheres to the ethical guidelines for conducting research.

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