

Heavy Metal Contamination and Risk of Sunflower Germination and Heavy Metal Translocation

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ABSTRACT

Background: Heavy metal contamination in soils adversely affects plant growth, particularly in crops used for phytoremediation, such as sunflower (*Helianthus annuus* L.). Understanding the response of different sunflower varieties to heavy metal stress is crucial for developing effective phytoremediation strategies.

Objective: This study aimed to evaluate the impact of nickel (Ni), cadmium (Cd), and lead (Pb) on the germination, growth attributes, and metal translocation of two sunflower varieties, Hysun-33 and FH-533.

Methods: Sunflower seeds of Hysun-33 and FH-533 were sown in sand-filled pots treated with 0, 50, 100, 150, and 200 mM of Ni, Cd, and Pb. Germination rates, shoot and root lengths, and dry biomass were measured after 20 days. Metal concentrations in roots and shoots were analyzed using atomic absorption spectroscopy, and statistical analyses, including ANOVA and regression, were performed to assess the effects of metal concentrations.

Results: Germination rates for Hysun-33 decreased from 79% at 0 mM to 0% at 200 mM, while FH-533 dropped from 75% to 5%. Shoot lengths decreased by 47% at 150 mM Ni, and root biomass was reduced by 62% under 200 mM Cd for both varieties.

Conclusion: Cadmium posed the greatest threat to sunflower growth, emphasizing the need for enhanced metal tolerance in phytoremediation applications. Reducing heavy metal contamination in crops is vital for improving food safety and public health.

INTRODUCTION

Heavy metal contamination in soils represents a pervasive environmental issue, significantly impacting agricultural productivity and posing risks to human and ecological health. Heavy metals such as nickel (Ni), cadmium (Cd), and lead (Pb) are particularly concerning due to their persistence in the environment and potential to accumulate in plant tissues, disrupting physiological processes and reducing plant viability. These metals, introduced primarily through industrial activities, mining, and the use of contaminated water and fertilizers, adversely affect soil quality and plant growth, impairing key stages like germination and early seedling development, which are critical for the establishment and yield of crops (8). The phytoremediation potential of sunflowers (*Helianthus annuus* L.), which can accumulate significant amounts of heavy metals, offers a dual benefit of remediating contaminated soils while providing biomass that can be utilized for bioenergy production, aligning with sustainable agricultural and environmental management practices (8, 9).

Sunflowers are considered hyperaccumulators of heavy metals, capable of absorbing and translocating metals such as Ni, Cd, and Pb from the soil into their tissues. This ability is influenced by several factors, including the specific sunflower variety, soil conditions, and the type and concentration of metals present. The response of

sunflowers to heavy metal stress varies, with some metals exerting more severe phytotoxic effects than others. Cadmium, for instance, is known for its high toxicity, as it predominantly accumulates in root tissues, restricting its translocation to shoots and thereby impeding overall plant growth and development. This metal interferes with key metabolic pathways, reduces nutrient uptake, and disrupts cellular functions, leading to stunted growth, reduced biomass, and impaired germination rates (9, 10). In contrast, nickel, although essential in trace amounts, becomes toxic at higher concentrations, affecting nutrient homeostasis and enzymatic activity, while lead, which is non-essential and highly toxic, predominantly affects root and shoot growth, often through mechanisms that disrupt photosynthetic and respiratory processes (10).

Recent studies underscore the importance of selecting appropriate sunflower varieties for effective phytoremediation, as different cultivars exhibit varying degrees of tolerance and accumulation capabilities. Hysun-33 and FH-533, the two sunflower varieties examined in this study, show distinct responses to heavy metal stress. Hysun-33 has demonstrated a greater capacity for Ni translocation, which supports better growth and biomass production under lower concentrations of this metal, suggesting its potential suitability for remediating Ni-contaminated soils. However, both varieties are significantly affected by high concentrations of Cd and Pb, which reduce germination rates and inhibit seedling vigor due to the

metals' high retention in root tissues and limited translocation to shoots (10). The ability of these sunflower varieties to accumulate metals in different plant parts—roots, stems, and shoots—plays a crucial role in their effectiveness as phytoremediators. Notably, the accumulation of metals in shoots is desirable for phytoextraction, as it allows for easier harvesting and removal of contaminants from the site.

In addition to phytoremediation, the biomass of sunflowers grown in contaminated soils presents an opportunity for bioenergy production, providing a sustainable approach that integrates environmental remediation with renewable energy generation. The conversion of sunflower biomass into biofuels, such as biodiesel and bioethanol, not only offers an alternative use of contaminated biomass but also contributes to reducing the reliance on fossil fuels, aligning with broader environmental sustainability goals (8). The recent development of enzymatic saccharification techniques has shown promise in processing hyperaccumulated sunflower biomass for biofuel production, although challenges remain due to the inhibitory effects of heavy metals on hydrolytic enzymes (10). Future research is needed to enhance the phytoremediation capacity of sunflowers through genetic modifications or selective breeding, focusing on improving their tolerance and translocation efficiency for various heavy metals. This approach will not only optimize the use of sunflowers in cleaning contaminated soils but also maximize their potential in contributing to sustainable energy solutions.

Overall, this study aims to evaluate the germination, growth, and heavy metal translocation patterns in sunflower varieties Hysun-33 and FH-533 under varying concentrations of Ni, Cd, and Pb. By assessing these parameters, the research seeks to identify the most suitable sunflower variety for phytoremediation and to enhance understanding of the complex interactions between plants and heavy metals. The findings are expected to inform future strategies for optimizing the use of sunflowers in agricultural and environmental remediation contexts, ultimately contributing to the development of more resilient crop systems and sustainable land management practices.

MATERIAL AND METHODS

The study was conducted to assess the impact of heavy metal contamination on the germination, growth attributes, and metal translocation patterns of two sunflower varieties, Hysun-33 and FH-533, under varying concentrations of nickel (Ni), cadmium (Cd), and lead (Pb). The experiment was carried out in the spring of 2023 at the College of Agriculture, University of Sargodha, Pakistan, using sand-filled pots as the growth medium. Sunflower seeds from both varieties were sown in pots treated with five concentrations of Ni, Cd, and Pb (0, 50, 100, 150, and 200 mM). Each treatment was replicated four times in a fully randomized design, ensuring robust statistical comparisons. A total of 120 pots were used, with each pot containing eight seeds, allowing for sufficient sample sizes to assess germination and growth responses (9).

During the experiment, germination rates were monitored over a week, and the time required for 50% germination (T50) was calculated using established formulas. Key parameters such as germination percentage, germination index, and vigor index were measured to evaluate the effects of heavy metals on seedling vigor. Twenty days after emergence, three plants from each pot were carefully harvested. Roots were washed with distilled water to remove any adhering sand particles, and the plants were divided into root and shoot parts. The lengths and fresh weights of roots and shoots were recorded. Plant samples were then dried in an oven at 70°C for 48 hours to obtain dry weights. Subsequently, dried samples were ground into a fine powder for heavy metal analysis (9).

Heavy metal concentrations in roots and shoots were determined using atomic absorption spectroscopy (AAnalyst-330, Perkin Elmer, Germany). The samples were digested with a mixture of sulfuric acid and hydrogen peroxide (2:1 v/v) at 250°C on a hot plate until they became colorless, indicating complete digestion. The digested samples were filtered, diluted to 50 mL with deionized water, and analyzed against known standards. The translocation factor (TF) of each metal was calculated by dividing the concentration of the metal in the shoots by its concentration in the roots, providing insights into the plant's ability to translocate metals from roots to shoots under different metal stress conditions (10).

Data collection adhered to ethical standards consistent with the Declaration of Helsinki, ensuring responsible conduct and safety in research practices. While the study did not directly involve human subjects, ethical considerations were applied to environmental stewardship and the potential implications for human health, given the broader context of agricultural productivity and food safety. All experimental procedures were reviewed and approved by the relevant institutional review board at the University of Sargodha, ensuring compliance with ethical guidelines for environmental research.

The statistical analysis was conducted using Statistix (Version 8.1, USA), with significance levels set at $p < 0.05$. Analysis of variance (ANOVA) was used to assess the effects of metal concentrations on germination rates, growth parameters, and metal translocation. Post hoc comparisons were performed using the least significant difference (LSD) test to identify specific differences between treatments. Correlations between heavy metal concentrations and growth attributes were also evaluated using Pearson's correlation coefficients, providing a detailed understanding of how each metal influenced plant development and metal accumulation patterns (10).

This methodological approach ensured a comprehensive assessment of the impacts of heavy metals on sunflower varieties, integrating both agronomic and environmental health perspectives. The findings are expected to contribute to the development of phytoremediation strategies that leverage sunflowers for mitigating heavy metal contamination, with potential implications for improving soil quality and safeguarding food systems against metal pollutants. Further studies should explore genetic and

agronomic modifications to enhance the phytoremediation capacity of sunflowers, addressing the challenges of heavy metal stress in contaminated agricultural settings (9, 10).

RESULTS

The study assessed the effects of varying concentrations of nickel (Ni), cadmium (Cd), and lead (Pb) on the germination, growth attributes, and metal translocation of two sunflower varieties, Hysun-33 and FH-533. The detailed analysis of the germination indices and growth attributes revealed differential responses to heavy metal stress, with significant declines observed at higher metal concentrations.

Both Hysun-33 and FH-533 showed a progressive decrease in germination percentages and indices with increasing concentrations of Ni, Cd, and Pb. In the absence of metals, Hysun-33 exhibited a germination percentage of 79%, which decreased to 0% at 200 mM concentrations. FH-533

followed a similar trend, with an initial germination rate of 75%, dropping to 5% at the highest metal concentration. The germination index showed significant reductions for both varieties, particularly under Cd and Pb stress at higher concentrations, highlighting the pronounced toxicity of these metals (10).

Both sunflower varieties displayed reduced shoot and root lengths and lower dry biomass accumulation as the concentration of heavy metals increased. Hysun-33 maintained higher shoot lengths compared to FH-533 at lower metal concentrations (0 to 100 mM), but growth was severely stunted at 150 mM and completely inhibited at 200 mM. Similarly, root lengths and dry biomass were significantly reduced under metal stress, with Cd showing the most severe inhibitory effects, followed by Pb and Ni. The results clearly demonstrate a concentration-dependent toxicity, with increased metal concentrations leading to progressively worse growth outcomes (10).

Table 1 Germination and Growth Data

| Metal Conc. (mM) | Germ. % H33 | Germ. % FH533 | Shoot L. H33 (cm) |
|------------------|-------------|---------------|-------------------|
| 0 | 79 | 75 | 15 |
| 50 | 75 | 70 | 13 |
| 100 | 70 | 68 | 12 |
| 150 | 40 | 35 | 8 |
| 200 | 0 | 5 | 0 |

Table 2 Biomass Data

| Metal Conc. (mM) | Shoot Dry Bio. H33 (g) | Shoot Dry Bio. FH533 (g) | Root Dry Bio. H33 (g) |
|------------------|------------------------|--------------------------|-----------------------|
| 0 | 0.75 | 0.72 | 0.4 |
| 50 | 0.68 | 0.65 | 0.35 |
| 100 | 0.6 | 0.55 | 0.3 |
| 150 | 0.3 | 0.25 | 0.15 |
| 200 | 0.0 | 0.02 | 0.0 |

Statistical Analysis and Cross-Matching with Worldwide Evidence Advanced statistical analysis, including ANOVA and correlation studies, confirmed significant differences ($p < 0.05$) between treatments. Hysun-33 consistently outperformed FH-533 in terms of germination and growth attributes under Ni and Pb treatments, suggesting a relatively better tolerance to these metals. However, both varieties were highly sensitive to Cd, aligning with global findings where Cd is frequently reported as the most toxic heavy metal to plants, severely impacting root growth and overall plant vigor (10). Cross-referencing these results with worldwide data corroborates the need for selecting appropriate varieties for phytoremediation efforts.

The translocation factor analysis further revealed that Hysun-33 exhibited higher Ni translocation to shoots, correlating with enhanced shoot biomass at lower Ni concentrations, positioning it as a potential candidate for phytoremediation in Ni-contaminated soils. Conversely, Cd and Pb predominantly accumulated in the roots, inhibiting their translocation to above-ground tissues, thus limiting the effectiveness of both sunflower varieties in phytoremediation applications for these metals.

The regression analysis was conducted to examine the impact of heavy metal concentration on the growth attributes of sunflower varieties Hysun-33 and FH-533,

revealing strong linear relationships between metal concentrations and reductions in sunflower growth attributes. Shoot Length: For Hysun-33, R-squared was 0.973 ($p < 0.01$), while FH-533 had an R-squared of 0.961 ($p < 0.01$), indicating significant decreases in shoot length with increasing metal concentrations. Root Length: Hysun-33 had an R-squared of 0.980 ($p < 0.01$), with FH-533 showing an R-squared of 0.972 ($p < 0.01$), demonstrating strong negative correlations with metal concentration. Shoot Dry Biomass: Hysun-33 showed an R-squared of 0.939 ($p < 0.01$), and FH-533 had an R-squared of 0.954 ($p < 0.01$), indicating robust negative effects on shoot biomass under metal stress. Root Dry Biomass: Hysun-33's root dry biomass had an R-squared of 0.951 ($p < 0.01$), while FH-533's was 0.947 ($p < 0.01$), reflecting a similar susceptibility to metal concentration increases.

The high R-squared values underscore a strong linear relationship between metal concentration and reductions in sunflower growth attributes, aligning with global observations that heavy metals like Cd and Pb are particularly detrimental to plant health (10). These findings provide valuable insights for environmental remediation strategies and support the selection of sunflower varieties with enhanced tolerance mechanisms for phytoremediation applications.

The data indicate that heavy metal contamination, especially with Cd, poses a significant threat to sunflower germination and growth. Both Hysun-33 and FH-533 showed substantial declines in performance at elevated metal concentrations, emphasizing the need for targeted breeding and selection of varieties with enhanced metal tolerance and translocation abilities. These findings contribute to the broader understanding of phytoremediation potential in sunflower varieties, offering a scientific basis for optimizing their use in contaminated agricultural landscapes. Future research should focus on genetic enhancements and adaptive strategies to improve the phytoremediation efficiency of sunflowers under severe metal stress conditions.

DISCUSSION

The findings of this study highlighted the substantial impact of heavy metal contamination on the germination, growth, and metal translocation abilities of sunflower varieties Hysun-33 and FH-533, with cadmium identified as the most phytotoxic element. Both varieties exhibited a marked decline in germination rates and growth attributes as the concentrations of nickel, cadmium, and lead increased, demonstrating a clear concentration-dependent toxicity. This aligns with previous studies which have consistently reported cadmium as highly detrimental to plant growth, particularly affecting root elongation and biomass accumulation due to its strong affinity for root tissues and its disruptive impact on essential metabolic pathways (Ahmad et al., 2007; Gopal and Rizvi, 2008). The observed severe effects of cadmium on root growth and its limited translocation to shoots corroborate earlier findings that suggest cadmium's toxicity largely arises from its high retention in root tissues, restricting the overall nutrient and water uptake essential for healthy plant development (Singh et al., 2004).

The study also revealed that both nickel and lead, while less toxic than cadmium, still significantly inhibited growth at higher concentrations. Nickel, although required in trace amounts for plant enzymatic functions, exhibited toxic effects at elevated levels, disrupting cellular homeostasis and nutrient balance, which is consistent with global literature that indicates nickel's dual role as both an essential micronutrient and a potential toxin (Chen et al., 2009; Ghori et al., 2019). Lead, being a non-essential and highly toxic metal, showed considerable accumulation in root and shoot tissues, adversely affecting plant growth by impairing photosynthetic efficiency and inducing oxidative stress, which aligns with findings from previous studies highlighting lead's propensity to inhibit root and shoot development across various plant species (Kopittke et al., 2007; Sharma and Dubey, 2005).

The differential responses between the two sunflower varieties suggest that Hysun-33 possesses a relatively higher tolerance to nickel and lead compared to FH-533, particularly at lower concentrations. Hysun-33's superior translocation factor for nickel, leading to enhanced shoot biomass, underscores its potential utility in phytoremediation applications in nickel-contaminated

environments. However, the limited ability of both varieties to translocate cadmium and lead to above-ground parts presents a significant challenge for their use in remediating soils heavily contaminated with these metals. This pattern of metal accumulation in roots with limited translocation to shoots has been widely observed in hyperaccumulator species and points to the need for targeted breeding or genetic modification strategies aimed at enhancing the translocation efficiency for these metals (Page and Feller, 2015; Uzu et al., 2011).

A major strength of this study lies in its comprehensive analysis of both germination and detailed growth attributes under varying metal concentrations, providing a robust dataset that elucidates the concentration-dependent effects of each metal. Moreover, the inclusion of multiple heavy metals allows for a comparative assessment of their relative toxicity, which is valuable for identifying specific phytoremediation applications. However, the study is limited by its scope to only two sunflower varieties and the focus on early growth stages; further research is needed to explore long-term effects and the potential for recovery in mature plants under sustained metal stress. Additionally, while the study provides insights into the phytoremediation potential of sunflowers, it does not account for possible interactions with other soil contaminants or environmental factors that could influence metal uptake and translocation. Future research should aim to expand the genetic and varietal screening of sunflowers, incorporating advanced breeding techniques and biotechnological interventions to enhance metal tolerance and translocation capabilities. Exploring the underlying genetic and molecular mechanisms that govern metal uptake and transport in sunflowers could lead to the development of more resilient varieties tailored for specific phytoremediation needs. Additionally, integrating field trials that consider a broader range of environmental variables would provide a more holistic understanding of sunflower performance in real-world contaminated sites. The findings of this study underscore the potential of sunflower varieties in phytoremediation but also highlight the need for ongoing innovation and refinement to fully harness their capabilities in mitigating soil heavy metal contamination (Salt et al., 1995; Pilon-Smits and Pilon, 2002).

CONCLUSION

The study demonstrated that heavy metal contamination, particularly with cadmium, significantly impairs the germination and growth of sunflower varieties Hysun-33 and FH-533, with both showing substantial declines in performance at elevated metal concentrations. The results emphasize the need for targeted breeding and genetic enhancements to improve metal tolerance and translocation abilities in sunflowers, making them more effective for phytoremediation. In terms of human healthcare implications, the ability of sunflowers to mitigate soil contamination could play a critical role in reducing heavy metal exposure from agricultural produce, thereby enhancing food safety and public health. By optimizing sunflower varieties for environmental remediation, it is

possible to contribute to cleaner agricultural ecosystems, ultimately protecting human health from the adverse effects of heavy metal accumulation in the food chain.

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