

Dual-Plant Phytoremediation Strategy for Lead Decontamination Using *Helianthus Annuus* and *Ocimum Sanctum*

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Abstract: Lead (Pb) contamination in soil poses a serious threat to the environmental and human health due to its non-biodegradable and highly toxic nature. Phytoremediation, an eco-friendly and cost-effective technique, utilizes plants to extract, immobilize, or stabilize the heavy metals from contaminated environments. In this work, the phytoremediation ability of *Ocimum sanctum* (tulsi) and *Helianthus annuus* (sunflower) in lead-spiked soils is assessed and contrasted. A controlled pot experiment was conducted over the course of 90 days under consistent climatic conditions, utilising three different lead concentrations (100, 200, and 400 mg/kg) along with a control. Atomic Absorption Spectrophotometry was used to quantify the Pb concentration in root and shoot tissues as well as plant biomass according to standard procedures. The performance of phytoremediation was analysed employing quantitative indicators like the Bioaccumulation Factor, Translocation Factor, and Remediation Efficiency. Because of its increased translocation ability (Translocation Factor (TF) > 1) and shoot Pb accumulation, the study's findings suggested that *Helianthus annuus* (*H. annuus*) offers a significant potential for phytoextraction. However, *Ocimum sanctum* (*O. sanctum*) retained higher Pb in its roots (TF < 1), making it suitable for phytostabilization. Compared to *H. annuus*, *O. sanctum* had a more pronounced decline in biomass as Pb stress increased, suggesting a less effective tolerance. The results suggest that a dual-plant strategy using *Helianthus annuus* for active Pb removal and *O. sanctum* for root-zone root-zone stabilization. This comparative analysis supports species specific phytoremediation technique and provides the promising framework for environmentally sustainable lead decontamination.

Keywords: Phytoremediation, Lead, *Ocimum Sanctum*, *Helianthus Annuus*, Translocation Factor

1.INTRODUCTION

Rapid industrialization and urban expansion have brought significant economic growth but at the cost of increasing environmental pollution[1]. Heavy metals, especially Pb, are the most dangerous toxins that are released into the environment because they stay there for a long time and build up in living things. Lead contamination in soil, largely a result of industrial discharges, lead-based paints, battery manufacturing, smelting as well as waste incineration, remains the pressing environmental challenge globally and specifically in the developing countries[2]. In India, urban and peri-urban areas near electronic as well as chemical industries are often hotspots of such type of contamination. This research emerges from the need to identify and apply sustainable, low-cost as well as effective techniques to remediate lead-polluted soils[3].

Among remediation techniques, phytoremediation has drawn interest as an economical and environmentally beneficial approach. In order to eliminate, limit, or neutralise pollutants from soil and water, green plants are employed. Significant research has been done on well-known hyperaccumulators, but little comparative study has been done on native and culturally important species such as *O. sanctum* and *H. annuus* (sunflower)[4]. The research's inception stems from assessing these species' potential for lead phytoremediation, given that they are

already acclimated to the agroclimatic conditions of India[5]. Additionally, their practical use for urban ecological restoration is made better by their dual function (environmental and ornamental/medical).

1.1. Background of the Research Problem

Lead is a very poisonous element that damages plant metabolism, soil health, and eventually health for humans and animals by contaminating the food chain. Lead and other heavy metals do not biodegrade like organic contaminants[6]. Lead builds up in soils and changes nutrient availability, enzyme activity, and microbial activity[7]. Children in disadvantaged populations close to polluted areas are most affected by prolonged exposure to lead, which causes neurotoxicity, renal failure and haematological consequences in people[8].

Costly, energy-intensive, and potentially more environmentally disruptive than necessary, traditional remedial methods include soil excavation, electrokinetic management & chemical stabilisation. Hence, it is critical to find long-term, easily-accessible solutions for biological remediation in areas with limited resources. Environmentally friendly options include plants that can remove or stabilise heavy metals. Nevertheless, phytoremediation can only be effective if the right species are chosen[9]. Controlled comparison studies across species with varied physical and physiological features are few, in contrast to the abundance of research addressing single-species performance[10].

1.2. Definition of the Research Problem

In this study, researcher aim to answer the following questions: How can the performance of *Helianthus annuus* and *Ocimum sanctum* is measured using standard phytoremediation indices? Also, will these plants show differential potential at phytoremediation for lead-contaminated soil under identical environmental conditions?

Translocation Factor (TF), Remediation Efficiency (RE) and Bioaccumulation Factor (BAF) are three important phytoremediation performance indicators that are used to assess the plants in controlled pot experiments with lead-spiked soil. While *O. sanctum* is historically renowned for its durability and therapeutic qualities, *H. annuus* is noted for its large biomass and shown capability for accumulation[11]. This research aims to find out if these plants can be used together in a two-plant cleanup approach and if they have different botanical roles in the process (phytoextraction and phytostabilization).

1.3. Significance of the Research in the Present Context

This research is significant for various reasons. Firstly, it responds directly to the environmental challenges of the lead pollution, a persistent in industrial as well as semi-urban landscapes. Secondly, it provides scientific evaluation for utilizing native and multifunctional plants, aligning with sustainable development goals (SDGs), specifically SDG 15 (Life on Land) & SDG 3 (Good Health as well as Well-being). The research also provides valuable data for municipalities, NGOs along with environmental agencies looking to implement green solutions in contaminated zones[12].

In addition, the present research encourages a locally environmentally friendly methodology by concentrating on two species that are important to culture. This makes it more likely that

the community will participate and accept the study. The results help with projects to improve cities and restore ecosystems, as well as phytoremediation science. In a larger scientific sense, this work helps us understand how different species handle lead differently and supports a method for comparing assessments that can be used for other metals as well as plant species as well.

2. RELATED WORKS

Many plants class have a traditional history of utilizing phytoremediation to decrease form of rock and roll dirtiness. Heavy metals like lead (Pb), cadmium (Cd), as well as arsenic (As) concede possibility be distant from adulterated soils by using hyperaccumulators like *Brassica juncea*, *Vetiveria zizanioides* & *Populus deltoides*[13]. *Brassica juncea* is a familiar variety in phytoextraction by way of its extreme ore incorporation and fortitude, which have happened widely recognised (Ali and others., 2013). Similar to this, *Vetiveria zizanioides*'s thick root building and volume to readjust to polluted environments have created it a powerful phytostabilizer (Ghosh & Singh, 2005). Even though these classes have shown expected very productive at restoration, not all areas have enlightening agreement or local compliance[14][15].

The majority of the existing research contrasts exotic hyperaccumulators or concentrates on single-species investigations. Comparative evaluations of native, multipurpose plants like *O. sanctum* & *H. annuus* under controlled settings are conspicuously lacking. Additionally, studies often don't use metrics like RE, TF as well as BAF to systematically quantify phytoremediation efficacy. Understanding the mechanistic functions of each species in the absorption, transport, and retention of heavy metals depends on these parameters.

The present study addresses a critical knowledge gap by conducting a controlled experiment comparing two culturally important and geographically adapted species, *H. annuus* & *O. sanctum*. This study offers a practical foundation for choosing plant species matched to site-specific pollution profiles and contributes to developing the phytoremediation paradigm via a rigors pot experiment as well as quantitative performance assessment.

3. METHODOLOGY

3.1. Research Protocol

Consistency in environmental factors such temperature, light exposure as well as moisture was ensured by conducting the research using a controlled pot experiment. Lead (II)nitrate [$\text{Pb}(\text{NO}_3)_2$] was added to soil samples at three different doses (100, 200, and 400 mg/kg) in order to resemble polluted settings. The uncontaminated control group was also added. The comparative capacity of *H. annuus* as well as *Ocimum sanctum* to absorb and withstand lead under the same circumstances was evaluated for each concentration level as shown in Figure 1. To reduce experimental error and ensure statistical robustness, each treatment was duplicated three times. Temperature ($\sim 25^\circ\text{C} \pm 2^\circ\text{C}$), photoperiod (12-hour light/dark cycles) and pH (~ 6.5) were all maintained at consistent ranges. The growth period of ninety

days was chosen because that's enough time for the two plants to become fully grown and show phytoremediation traits that can be seen. This experiment's design made it possible to compare the effectiveness of phytoremediation across species as well as treatment levels. It also confirmed the truth of the differences seen in lead buildup, transfer, and plant reaction.

3.2. Sample Setup

Pb(NO₃)₂ was carefully measured and mixed with deionized water to get the experimental setting ready. Then, it was mixed thoroughly with air-dried as well as sieved yard soil to make sure that the contamination was even. For exact measurements of plant uptake, this process made sure that the lead ions were spread out evenly in the earth. Before being planted, the spiked dirt was left for seven days to settle down and replicate the natural environment. In separate nursery trays in clean soil, researcher planted *Ocimum sanctum* as well as *Helianthus annuus* seeds. After they grew to the right height (approximately 10 to 15 cm), the healthy, uniform seedlings were put into pots with the stimulating soil. Plants were regularly irrigated in distilled water through the growth period to keep them from getting any more dirt on them. To keep the plants healthy without using chemicals from outside, they did pest control by hand and checked on the plants often.

3.3 Gathering Data

After 90 days, plants were carefully rooted and separated from their shoot and root sections. Using distilled water and then tap water, these parts were thoroughly cleaned to get rid of any lead particles which could have adhered to the surface. Researchers recorded each component's fresh and dried biomass. The material had been dried until a constant weight was achieved in a hot-air oven calibrated at 65°C to ensure accurate dry mass measurement. Dried plant tissues were digested using the standard acid digestion method (HNO₃:HClO₄ at a 4:1 ratio) in order to assess lead. In order to quantify the digested samples, they were filtered and diluted. The amount of lead in plant cells was found via Atomic Absorption Spectrophotometry (AAS), an exact and reliable method for analysing trace metals. To examine variations across species, concentrations & plant parts, data were averaged across replicates and statistical methods were employed.

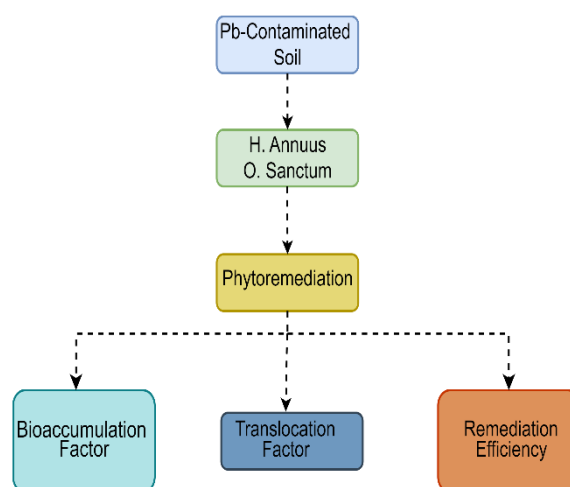


Figure 1 Schematic View of Proposed Framework

3.4. Mathematical Modelling

Helianthus annuus & *Ocimum sanctum*'s phytoremediation capacity was quantitatively assessed using three key indices: Remediation Efficiency (RE), Translocation Factor (TF) & Bioaccumulation Factor (BAF). The BAF, which measures a plant's ability to take up lead in the soil and incorporate it into its tissues, is stated as follows:

$$BAF = \frac{\text{Concentration of Pb in Plant Tissue}}{\text{Concentration of Pb in Soil}}$$

The values for the root & shoot tissues were calculated independently. The plant is efficiently acquiring lead in the soil when the BAF is more than 1, which is ideal for phytoextraction. The plant's preference for translocation or absorption and storage was determined by comparing the BAF levels between the two species as well as between the root and shoot compartments.

The TF assist in evaluating whether the absorbed Pb is effectively transported from the roots to the shoots and is represented as:

$$TF = \frac{\text{Concentration of Pb in Shoot}}{\text{Concentration of Pb in Root}}$$

TF values above 1 mean that lead is easily moved around at the plant system, that is important for plants which will be used for phytoextraction. For example, if the TF is less than 1, it means that the plant performs more effectively at phytostabilization because it keeps the Pb in its roots. The general amount of lead taken from the soil is measured by the third number, called RE. It is found by stating:

$$RE(\%) = \frac{C_0 - C_t}{C_0} \times 100$$

Here C_0 represents the initial Pb concentration in soil while C_t indicates the resultant Pb concentration after the treatment. By combining these indices, a thorough understanding of the phytoremediation dynamics of each plant can be obtained, which helps classify species as either stabilisers or accumulators.

4. RESULTS

In a controlled pot-based design, soil samples were intentionally contaminated with graded quantities of lead nitrate (100, 200 & 400 mg/kg) to provide the experimental dataset in this research. To evaluate their capacity for phytoremediation, *Helianthus annuus* & *Ocimum sanctum* were both cultivated for ninety days under similar environmental circumstances. Employing AAS, lead accumulation in shoot and root tissues was measured after harvest. Standard formulae were used to determine important metrics including RE, BAF and TF. The comparison dataset illustrating each plant's capacity to absorb, translocate as well as tolerate lead was produced by this methodical approach.

Here are the results, which show clear differences among the two species in how they take in the pollutants, how they affect their physiology and how well they clean up afterwards.

Helianthus annuus as well as *Ocimum sanctum* biomass production and Pb accumulation behaviour at three different levels of lead contamination (100, 200 & 400 mg/kg) are detailed in Table 1. It contains the dry biomass, the observed Pb concentrations in the tissues of the roots and shoots and derived metrics like the TF and the BAF for the roots and shoots. Having TF values greater than 1, which indicates effective translocation, *H. annuus* exhibited a higher Pb content in shoots than in roots across all treatments. In contrast, *O. sanctum* exhibited TF values under 1, indicating minimal shoot mobility and root retention, and it also collected more Pb in its roots.

Table 1: Pb Accumulation & Biomass in *Helianthus Annuus* and *Ocimum Sanctum*

Pb Level (mg/kg)	Plant	Pb in Root (mg/kg)	Pb in Shoot (mg/kg)	Biomass (g)	BAF (Root)	BAF (Shoot)	TF
100	<i>H. annuus</i>	45	52	5.2	0.45	0.52	1.16
200	<i>H. annuus</i>	82	89	4.7	0.41	0.45	1.08
400	<i>H. annuus</i>	140	150	3.6	0.35	0.38	1.07
100	<i>O. sanctum</i>	38	20	3.9	0.38	0.20	0.53
200	<i>O. sanctum</i>	63	34	3.2	0.32	0.17	0.54
400	<i>O. sanctum</i>	91	56	2.8	0.23	0.14	0.62

The unique accumulation patterns seen in *H. annuus* and *O. sanctum* indicate that these two species are best suited to different aspects of phytoremediation. *H. annuus* is efficient at phytoextraction because it can take up and transfer lead, whereas *O. sanctum* is better at phytostabilization because it can minimise Pb mobility inside the plant. Remarkably, when the Pb content rose, both species showed a slow but steady decrease in biomass, which indicates phytotoxic stress. The premise that these plants vary in their lead handling processes along with remediation capacity is well supported by this table.

Pb deposition of the roots for *Ocimum sanctum* and *Helianthus annuus* at increased soil Pb levels is contrasted in a bar chart in Figure 2. The bars show a definite rising trend of root accumulation with increasing Pb levels in the soil. The root Pb concentration of *H. annuus* was consistently greater than that of *O. sanctum*, especially at both 200 and 400 mg/kg, when

it absorbed between 30 and 50 percent more lead. Under the same circumstances, findings suggests that sunflower roots were more effective in absorbing lead than tulsi roots.

H. annuus is a powerful lead accumulator, as this figure illustrates. *O. sanctum* nonetheless showed detectable Pb absorption in spite of reduced absolute quantities, confirming its ability to stabilise in settings poisoned with lead. The ability distinctions among the two species are further highlighted by this Figure 2, which also makes it easier to understand patterns in root absorption.

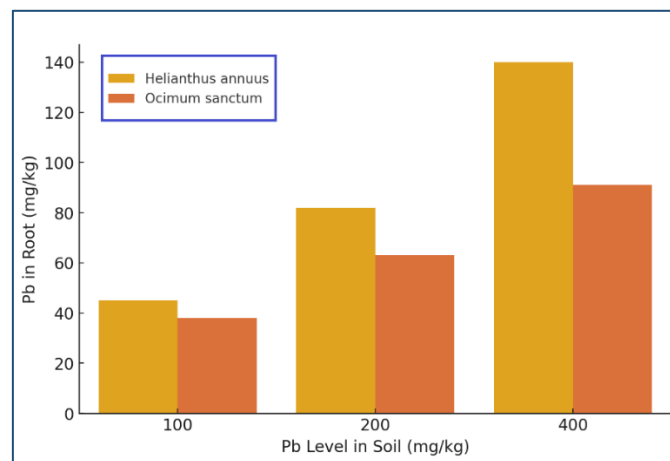


Figure 2 Pb Accumulation in Roots

Figure 3 shows how both plant species' biomass output was affected by increasing Pb concentrations. As the amount of lead in the soil spiked the plant biomass slowly decreased. At all treatment levels, *H. annuus* had more biomass than *O. sanctum*. *O. sanctum* was more sensitive to Pb-induced phytotoxicity, as shown by a steeper biomass decline at 400 mg/kg Pb. Lead exposure has a physiological cost, as this figure 3 illustrates. Superior biomass retention via *H. annuus* points to improved mechanisms for tolerance, which might be achieved by active detoxification pathways, vacuolar compartmentalisation, or sequestration. The larger decrease in *O. sanctum*, on the other hand, suggests that while it is capable of absorbing Pb, it is more negatively impacted by the stress linked to it, which may restrict its use on a field size unless it is cultivated in areas that are only mildly polluted.

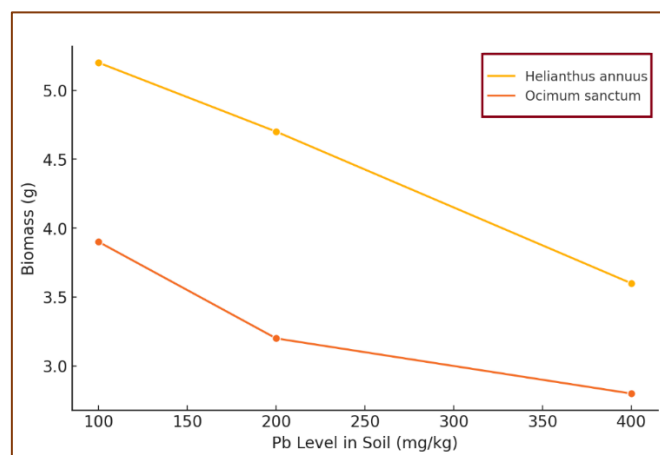


Figure 3 Biomass vs Pb Concentration

Root Pb accumulation at different concentrations is visually compared in Figure 4's heatmap. As a reliable indicator of Pb absorption, the colour gradient shows that *H. annuus* always appears in deeper tones in the roots. At whatever amount of Pb treatment, this visualisation makes it clear that *H. annuus* outperforms *O. sanctum*. Its ability to display patterns related to both plants and concentrations in a single view is what makes this graphic so illuminating. The rate of growth is much higher in *H. annuus*, even though both plants show increased Pb absorption with growing soil Pb. As a result, the heatmap facilitates the selection of remediation options based on the degree of pollution and supports the finding that sunflower is an additional aggressive Pb accumulator.

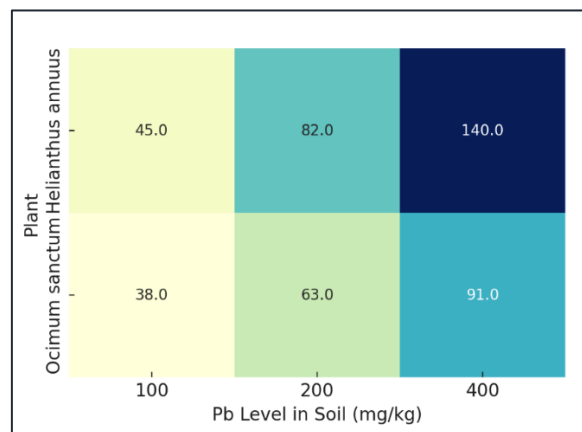


Figure 4 Heatmap of Pb in Root (mg/kg) at Different Pb Levels

The BAF to root as well as shoot and TF for the two species are shown in Figure 5. Each of the three measures for *H. annuus* has a higher median number, but TF always stays above 1. The TF as well as BAF (shoot) numbers for *O. sanctum* are lower, which shows that it can't move as easily. There is greater variation in performance, but on the whole, *H. annuus* has better results since its spread (IQR) is wider. Differentiating each species' involvement in phytoremediation requires the use of this boxplot. By absorbing and transporting Pb into its aerial portions for subsequent harvesting, *H. annuus* proves to be a suitable phytoextraction candidate with higher BAF & TF values. Since root absorption stops Pb leaching or additionally mobility, *O. sanctum*'s lower TF further supports its use in phytostabilization. Through the visualisation of variation & central tendency of the primary performance measures, the boxplot offers statistical depth.

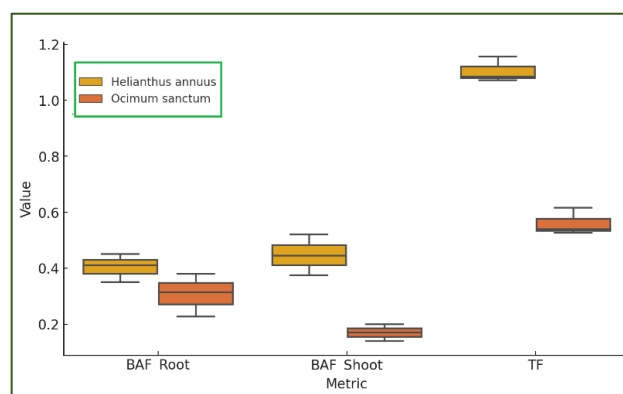


Figure 5 Comparison of BAF and TF

Figure 6 displays a scatter plot that connects the amount of lead in the soil (X-axis) to the amount of living things (Y-axis). The sizes of the bubbles show how much lead the roots take up. A bigger and larger point for *H. annuus* on the plot indicates a higher biomass and a higher Pb absorption rate. The fact that *O. sanctum* is near the bottom of both the directions and the bubble sizes shows that it has a smaller uptake and biomass. In this Figure 6, exposure level, physiological effect, and absorption efficiency are all combined into a one visualisation. The bubble sizes support the greater absorption ability of *H. annuus*, while the diminishing pattern across left to right demonstrates that increasing Pb has a detrimental impact on plant development. This scatter plot supports the previous finding that *O. sanctum* is not as tolerant yet remains helpful in stabilising pollutants close to the root zone, while *H. annuus* is both tolerant and effective at absorbing lead.

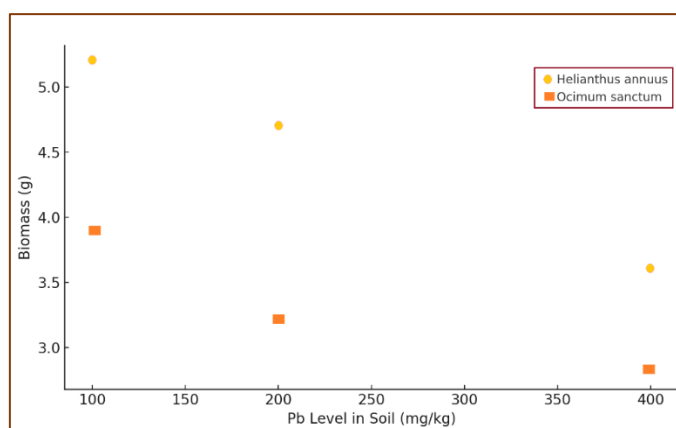


Figure 6 Pb Level vs Biomass with Pb Uptake Size

5. DISCUSSION

The research's findings show that *Helianthus annuus* as well as *Ocimum sanctum* have different ways of cleaning up lead-contaminated grounds and are more effective at it. Overall, *H. annuus* did better at both uptake and transfer, with BAF higher in shoot tissues and TF usually greater than 1. Researchers in Singh et al. (2015) as well as Yoon et al. (2006) found that *H. annuus* was an excellent lead phytoextractor because it grows quickly, has a high biomass, and can move materials around easily. These results support their findings. It is ideal for phytoextraction, which removes contaminants by picking, because *H. annuus* can store Pb in its upper parts.

The ability of *O. sanctum* to retain lead mostly in roots, on the other hand, was shown by TF values that were continuously below 1. This is consistent with the results of Sharma et al. (2018), who observed that *O. sanctum* exhibited little translocation but significant heavy metal absorption in roots. This behaviour points to its efficacy as a phytostabilizer, which is essential for reducing Pb mobility & environmental dispersion. Both species showed a decrease in biomass at greater Pb concentrations, indicating phytotoxic stress; nevertheless, *H. annuus* kept a substantially higher biomass, indicating improved tolerance, maybe due to detoxifying processes including cell wall binding or vacuolar sequestration. Raskin et al. (1997) agreed and said that both uptake as well as tolerance traits are important when choosing phytoremediation species[19]. This work reinforces the significance of plant-

specific techniques in remediation efforts by merging experimental results with previous findings and confirming the appropriateness of *O. sanctum* for confinement roles and *H. annuus* in active decontamination.

State-of-the-Art (SOTA) Comparison Table

Plant Species	Phytoremediation Strategy	TF	BAF (Shoot)	Biomass Tolerance	Suitable For
<i>Helianthus annuus</i>	Phytoextraction	>1	High	High	Taking out Pb through harvestable shoots
<i>Ocimum sanctum</i>	Phytostabilization	<1	Low	Moderate to Low	Stabilising the root zone in soils with mild pollution
<i>Brassica juncea</i> ¹	Phytoextraction	>1	Medium to High	Medium	Pb/Cd uptake of industrial soils
<i>Vetiveria zizanioides</i> ²	Phytostabilization	<1	Low	High	Stabilisation in places with mine waste or a lot of weathering
<i>Populus deltoides</i> ³	Phytoextraction (Deep-rooted)	>1	Medium	High	Long-term decontamination in agroforestry zones

The experimental results are analysed and placed within the larger framework of the existing phytoremediation literature in this discussion. It further supports the concept that both *H. annuus* and *O. sanctum* are potential phytoremediation options for lead, although they serve different purposes. For effective site-adapted environmental management, it is recommended to use both species together.

6. CONCLUSION AND FUTURE WORK

This research successfully showed that *Helianthus annuus* as well as *Ocimum sanctum* have different levels of ability to clean up lead-contaminated grounds when they are exposed to them in controlled circumstances. The research methodically evaluated both plant species' levels of tolerance, translocation, and bioaccumulation using a standardised experimental technique. The various remediation tactics shown by each plant were specified using

quantitative indicators including RE, BAF and TF. The concept that different kinds of plants have diverse coping strategies for handling heavy metal stress thus may be successfully used for various remediation purposes is supported by these data.

Helianthus annuus showed promising phytoextraction results, including increased lead deposition in the shoots, TF values that remained constantly above 1, and reasonably stable biomass even when exposed to high levels of lead. For field-based cleaning methods, the fact that it can move lead in roots to plants that can be harvested is useful. *Ocimum sanctum*, on the other hand, had more Pb staying in the root tissues, less movement ($TF < 1$), as well as a larger decrease in biomass when exposed to lead stress, which makes it more of an antioxidant for plants. When these plants are used strategically, based on the type and amount of soil pollution, their different behaviours make it possible.

The findings from this research also match what other research has said about phytoremediation. For example, *H. annuus* has been described as a hyperaccumulator, as well as *O. sanctum* has been looked into for its ability to handle metals and potential medical use. Besides supporting these roles, the data also gives comparison views under the same testing settings, which is something that other similar works frequently lack. These graphs and models show that species-specific placement is beneficial. They also suggest that accumulator-stabilizer plant pairs could be used in a dual-strategy approach to cleanup, which would remove more pollutants while causing less damage to the environment.

The research has limitations despite these encouraging findings. The experiment was limited to pot-based settings using Lead(II) nitrate $[Pb(NO_3)_2]$ for simulated contamination, which may not accurately represent the intricacy of metal distribution & speciation at the field level. Furthermore, this controlled setting did not account for plant-to-plant competition, seasonal fluctuations, or interactions with the soil microbiota. Future research should take these factors into consideration as they may have a substantial impact on remediation results.

Going ahead, it is suggested that the research be broadened to incorporate tests in the field with different types of dirt and levels of pollution. Adding plant growth-promoting rhizobacteria (PGPR) or microbial communities could assist plants take in more metals and handle stress better. Advanced molecular research can also help find genetic traits that give substances hyperaccumulative qualities and create them. Lastly, it is important to look into how phytoremediation can be used to restore the environment in the real world, including whether it is cost-effective and how the biomass can be used after the process (for example, for biofuel or safe burning).

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