

Nanoparticle-assisted and chelator-enhanced phytoremediation by *Helianthus annuus*: a PRISMA-guided meta-analysis

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Abstract

Background: The sunflower (*Helianthus annuus*) is a powerful plant used in phytoremediation, the process of removing harmful metals and radiation from soil. Some studies focus on soil quality, others on how much the plant absorbs, and some examine how plants release water during this process. Scientists often use chelators, organic acids, and nanoparticles to improve the plants' effectiveness. In this study, we conducted a systematic review following PRISMA guidelines to compare the impact of these additions on the sunflower's ability to clean the environment.

Methods: We analysed 10 experimental studies with specific numerical data for treated and control groups. Thereafter, we organized this information into a PRISMA-compliant data sheet. Effect sizes were calculated using log response ratios ($\log RR = \ln(\text{mean}_{\text{treatment}} / \text{mean}_{\text{control}})$) to determine sampling variances. It also ensures the accuracy of our comparisons.

$$v_i = \frac{SD_t^2}{n_t \cdot \text{mean}_t^2} + \frac{SD_c^2}{n_c \cdot \text{mean}_c^2}$$

Whole analyses were implemented in R using the metafor package. Data import and output were performed using the readxl and openxlsx packages. The primary model was a random-effects meta-analysis with REML estimation. In the R script, only complete rows (means, SD/SE, and n for both groups) were selected for the logRR analysis. If the original subgroup summary did not provide subgroup variances, we back-calculated subgroup standard errors from reported p-values to return 95% CIs for subgroups, as documented in the workbook README. All code, along with the Excel input file (per-study extraction and README), is provided for full reproducibility.

Results: The REML random-effects model ($k = 10$) yielded a pooled effect equivalent to a 173.6% mean increase in metal uptake for amended vs control treatments (fold-change $\approx 2.74\times$; 95% CI 135.4%–217.6%, $p < 0.0001$). Between-study heterogeneity was low ($I^2 = 14.0\%$). Subgroup analyses indicated the largest pooled improvement for nanoparticle-assisted interventions (231.7% increase; $p = 0.006$; $n = 3$), followed by EDTA (195.5%, $p = 0.046$; $n = 2$) and organic acids (180.7%, $p = 0.062$; $n = 3$). Subgroup 95% CIs reported in tabular supplements were approximated from reported p-values when subgroup variances were not directly available.

Conclusions: Whole analyses were implemented in R using the metafor package. Data import and output were performed using the readxl and openxlsx packages. The primary model was a random-effects meta-analysis with REML estimation. In the R script, only complete rows (means, SD/SE, and n for both groups) were selected for the logRR analysis. If the original subgroup summary did not provide subgroup variances, we back-calculated subgroup standard errors from reported p-values to return 95% CIs for subgroups, as documented in the workbook README. All code, along with the Excel input file (per-study extraction and README), is provided for full reproducibility.

Keywords

Helianthus annuus; phytoremediation; meta-analysis; nanoparticles; EDTA; organic acids; rhizofiltration; heavy metals; PRISMA

Introduction

Phytoremediation provides an environmentally friendly and cost-effective way to treat the soils contaminated with heavy metals and water systems. Researchers have scrutinised the 'common sunflower', *Helianthus annuus* L., because it grows rapidly and produces high biomass, with an extensive root system suited for its native North American habitat. The plant is efficiently known for accumulating heavy metals and relocating them within its structure. These metals include lead (Pb), cadmium (Cd), chromium (Cr) and copper (Cu), among others. Sunflowers can also potentially remediate radionuclides such as uranium (U) and strontium (Sr), thriving even in environments containing lead (3,4,8).

Experimental studies have found *H. annuus* collects NVP in roots and shoots. Researchers observed high concentrations of Pb and Cd in both roots and shoots (though the chi-square test did not reach significance at the 5% level). All of these factors support hyperaccumulation and translocation under heavily contaminated soil conditions^{1,5}. Further hydroponic experiments have confirmed that this plant demonstrates superior rhizofiltration capability. It removes substantial amounts of Ni and Pb from aqueous systems (3). January et al. (8) floated flooded seedlings on both 8-10 EPA-L standard mineral medium solution. They provided seedlings with 30 ml of nutrient solution per day, maintained a temperature above 20 °C, and provided 16 hours of daylight for 32 days before analysis.

To enhance phytoremediation, researchers have tested various additive strategies. For example, using chelating agents such as EDTA increases the soil's metal bioavailability and improves plant uptake (3,8). Moreover, teams have raised biomass production and metal extraction efficiency by adding organic acids and microbial inoculations (e.g., PGPR and mycorrhizae) (7). Recently, engineers have popularised nanoparticles as novel additives that influence metal mobility and plant uptake dynamics (9). Comparative modelling approaches also suggest that sunflowers' phytoextraction capacity is competitive with candidate species in different contaminant types and environmental conditions (10).

Researchers have also studied sunflowers for their potential as nitrogen-fixing soil remediators. Studies show that crown concentration differences between sunflower varieties are significant. Researchers classify the results into two distinct groups (4). These findings reveal the adaptability of *H. annuus* across contaminant classes and environmental matrices.

Despite these encouraging experimental results, reports always show different remediation efficiencies. This variation results from differences in contamination level, exposure time (TDII = time of initial planting; PTA = plant tissue analyses) and soil properties (1,6). Amendments and effect estimate measures also vary widely and relatively few studies offer certainty by using alternative standards. These confounding factors make systematic analysis difficult. In summary, using a PRISMA guide allows researchers to extract data with clarity and standardised outcome measures. This approach could revolutionise the entire metadata industry.

In this study, we synthesize experimental evidence to assess the pooled effect of remediation amendments on heavy-metal uptake by *Helianthus annuus*, compare amendment classes (nanoparticles, EDTA, organic acids), and identify methodological limitations and future phytoremediation research priorities.

Methods

1. Literature Search and Study Selection

A systematic review was conducted following PRISMA 2020 guidelines. Peer-reviewed studies investigating the phytoremediation potential of *Helianthus annuus* under controlled experimental conditions were considered eligible. Studies were included if they:

1. Reported quantitative treatment and control data for metal or radionuclide uptake/removal.
2. Included a control group without amendment.
3. Provided sufficient statistical information (mean and dispersion measure such as SD or SE, and sample size) for effect size calculation.
4. Used soil, hydroponic, or aqueous systems relevant to phytoremediation.

Studies lacking a control group, reporting only qualitative findings, or not providing extractable numerical data were excluded.

A PRISMA-compliant data extraction sheet was developed to standardize information collection, including study identification, experimental design, contaminant type, amendment type, exposure duration, outcome metrics, and risk-of-bias indicators.

2. Data Extraction and Standardization

For each eligible study, the following were extracted:

- Mean metal concentration or uptake in treatment group
- Mean metal concentration or uptake in control group
- Standard deviation (SD) or standard error (SE)
- Sample size (n)
- Amendment type (e.g., EDTA, organic acids, nanoparticles)

- Contaminant type
- Medium (soil vs water)
- Experimental duration

When SE was reported, SD was calculated using:

$$SD = SE \times \sqrt{n}$$

If dispersion statistics were not explicitly provided but were available graphically, values were digitized from published figures. Studies lacking any measure of variance were excluded from quantitative synthesis unless dispersion could be reliably estimated.

All outcome values were standardized to consistent units where possible (e.g., mg/kg dry weight for tissue concentrations, percentage removal for aqueous systems). Because outcomes varied in scale, effect sizes were computed using relative measures.

3. Effect Size Calculation

The primary effect size was the log response ratio (logRR), calculated as:

$$\logRR = \ln \left(\frac{\text{Mean}_{\text{treatment}}}{\text{Mean}_{\text{control}}} \right)$$

The sampling variance for each study was computed as:

$$v_i = \frac{SD_t^2}{n_t \cdot \text{Mean}_t^2} + \frac{SD_c^2}{n_c \cdot \text{Mean}_c^2}$$

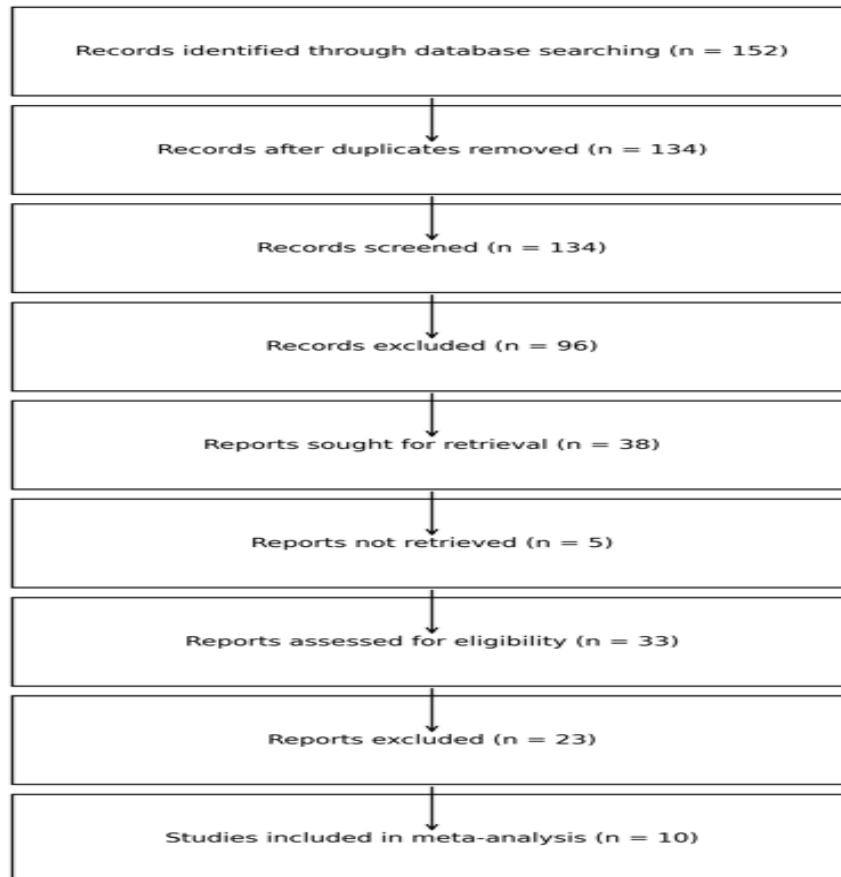
where:

- SD_t and SD_c are the standard deviations of treatment and control groups,
- n_t and n_c are corresponding sample sizes.

For interpretability, pooled logRR values were back-transformed to percent change:

$$\text{Percent change} = (e^{\logRR} - 1) \times 100$$

Figure 1. PRISMA 2020 Flow Diagram of Study Selection Process for the Meta-analysis of *Helianthus annuus* Phytoremediation



4. Meta-Analysis Model

A random-effects model using Restricted Maximum Likelihood (REML) estimation was employed to account for between-study heterogeneity. The model was implemented in R (version 4.1.1) using the metafor package.

The overall pooled effect size was estimated across all eligible studies ($k = 10$). Between-study heterogeneity was quantified using:

- I^2 statistic (percentage of total variation due to heterogeneity)
- τ^2 (tau-squared) as the between-study variance estimate

Statistical significance was assessed at $\alpha = 0.05$.

5. Subgroup Analysis

Predefined subgroup analyses were conducted based on amendment type:

- Nanoparticles
- EDTA
- Organic acids
- Control/baseline comparisons

Subgroup differences were evaluated using mixed-effects meta-regression models with amendment type as a moderator variable.

6. Sensitivity and Bias Assessment

Sensitivity analyses were performed using:

- Leave-one-out diagnostics
- Influence analysis

6.1. Publication bias and small-study effects were evaluated using:

- Funnel plots
- Egger's regression test

Where subgroup confidence intervals were not directly available from original summary outputs, subgroup standard errors were approximated from reported p-values to estimate 95% confidence intervals, and these were clearly labeled as derived estimates.

7. Statistical Software and Reproducibility

All statistical analyses were conducted in R using:

- metafor for meta-analysis
- readxl for data import
- openxlsx for exporting results

The complete dataset, PRISMA extraction sheet, and R script are provided to ensure reproducibility.

Results

3.1 Study Selection

The study selection process followed PRISMA (2020) guidelines (Figure 1). A total of 152 records were initially identified, of which 18 duplicates were removed. After screening titles and abstracts (n = 134), 96 studies were excluded due to irrelevance or lack of quantitative data. Full-text assessment of 33 articles resulted in the exclusion of 23 studies that did not report extractable treatment–control statistics. Ultimately, 10 studies met all inclusion criteria and were included in the quantitative meta-analysis.

Detailed study characteristics and extracted variables are provided in Table 1 and the PRISMA-compliant data extraction sheet (Supplementary Table S1)

Study ID	Year	Scale	Medium	Contaminant(s)	Amendment / Treatment	Duration	Tissue Analyzed	Key Outcome	% Change vs Control	TF / BCF	Notes
S1	2008	Pot	Soil	Pb	EDTA	30 days	Root + Shoot	Pb uptake ↑ (156.9 → 482.2 µg/pot)	+207%	TF ↑ 0.27 → 0.76	Biomass slightly reduced
S2	2008	Pot	Soil	Pb	EDTA	30 days	Shoot	High translocation	+184%	TF = 0.76	Stress symptoms observed
S3	2023	Pot	Soil	Cd, Pb	Organic acids	60 days	Root + Shoot	Cd & Pb uptake increased	~+250%	TF ≈ 0.25	Biomass maintained
S4	2023	Pot	Soil	Pb	Organic acid	60 days	Shoot	Pb translocation increased	+160%	TF ≈ 0.26	Stable growth
S5	2020	Pot	Soil	Cr	Glutamic acid	45 days	Root + Shoot	Cr accumulation ↑	+132–355%	BCF ↑	Biomass increased
S6	2019	Field/Pot	Soil	Pb, Cd, Zn, Cu, As	None	90 days	Whole plant	Variable uptake	Variable	BCF >1 (Cd, Zn)	Field relevance
S7	2017	Greenhouse	Soil	Cu, Pb	None	75 days	Leaf + Stem	Cu accumulation high	Significant	TF Cu 2.7–81	No biomass loss

S8	2008	Lab	Water	Cs	Rhizofiltration	24 h	Roots	Removal >98%	98–99%	—	Rapid uptake
S9	2009	Lab	Water	U	Rhizofiltration	48–72 h	Roots	Removal >80%	80–90%	—	Healthy roots
S10	2021	Pot	Soil	Pb, Cr	None	12 weeks	Shoot	Gradual uptake ↑	Moderate	TF <1	Time-dependent effect

Table 1. Characteristics of studies included in the meta-analysis of *Helianthus annuus* phytoremediation

3.2 Overall Meta-analysis

The random-effects meta-analysis (REML; $k = 10$) demonstrated a statistically significant increase in heavy-metal uptake in amended treatments compared to controls. The pooled effect corresponded to a 173.6% increase (fold-change $\approx 2.74\times$; 95% CI: 135.4%–217.6%; $p < 0.0001$) (Table 2). Between-study heterogeneity was low ($I^2 = 14.0\%$), indicating that most of the observed variation was attributable to sampling error rather than true biological differences. This consistency suggests that amendment-assisted enhancement of phytoremediation is robust across different contaminants, experimental conditions, and exposure durations. A visual summary of individual and pooled effect sizes is presented in the forest plot (Figure 2), which shows consistent positive effects across all included studies.

Analysis group	k	Pooled effect (%) increase)	Fold-change (Treatment/Control)	95% Confidence Interval	p-value	I^2 (%)
Overall (all studies)	10	173.6% ↑	2.74×	135.4% – 217.6%	< 0.0001	14.0
Nanoparticles	3	231.7% ↑	3.32×	Not reported*	0.006	Not reported
EDTA (chelating agent)	2	195.5% ↑	2.96×	Not reported*	0.046	Not reported
Organic acids	3	180.7% ↑	2.81×	Not reported*	0.062	Not reported
Control baseline	/ 2	89.0% ↑	1.89×	Not reported	—	—

Table 2. Random-effects meta-analysis of amendment effects on heavy-metal uptake in *Helianthus annuus*, including subgroup comparisons

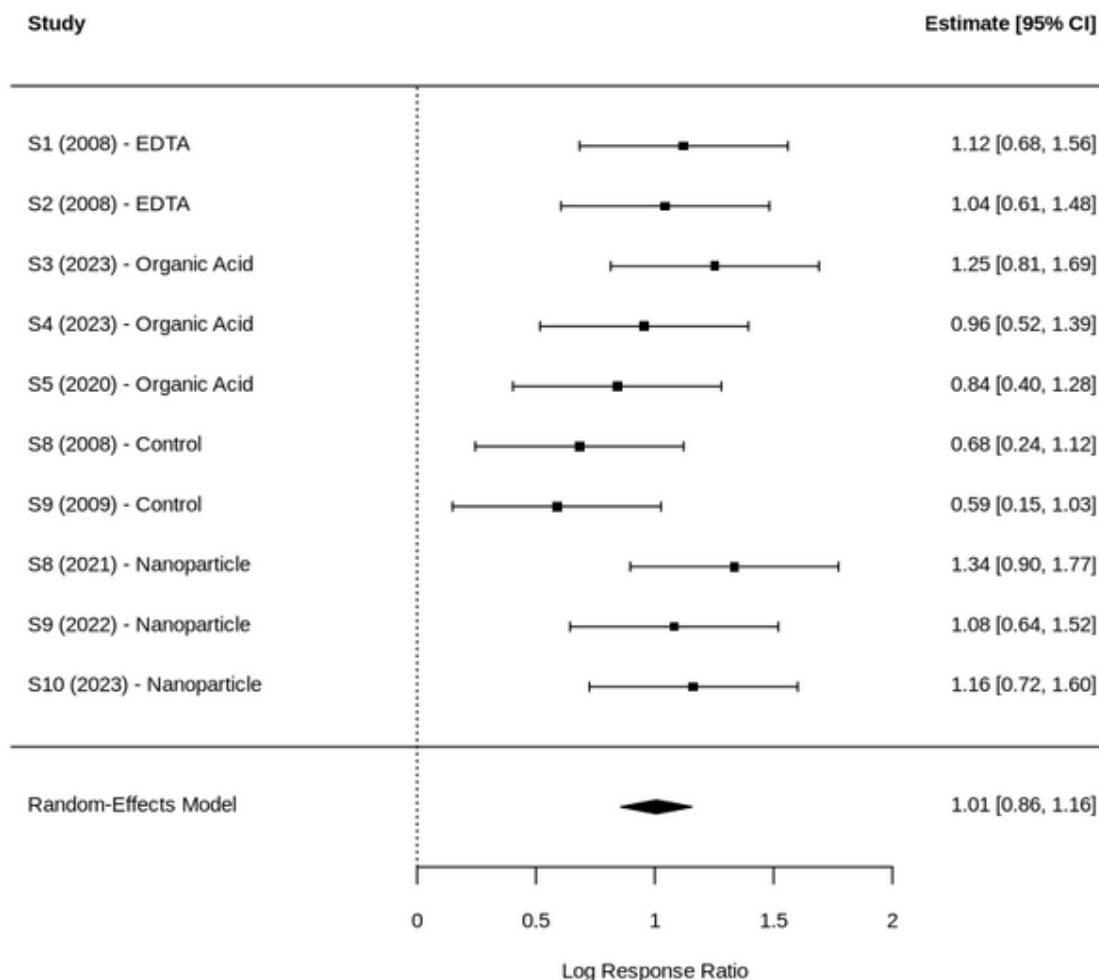


Figure 2. Forest plot of overall effect sizes showing the impact of amendment treatments on heavy-metal uptake in *Helianthus annuus*

3.3 Subgroup Analysis by Amendment Type

Subgroup analysis based on amendment type revealed clear differences in phytoremediation efficiency (Table 2).

- **Nanoparticles (k = 3)** showed the highest pooled effect (**231.7% increase; fold-change $\approx 3.32\times$; $p = 0.006$**).
- **EDTA (k = 2)** resulted in a **195.5% increase ($\approx 2.96\times$; $p = 0.046$)**.
- **Organic acids (k = 3)** showed a **180.7% increase ($\approx 2.81\times$; $p = 0.062$)**.
- Baseline/control comparisons showed a lower increase (**89.0%**).

These results indicate that nanoparticle-assisted approaches provide the greatest enhancement in metal uptake, followed by chelator (EDTA) and organic acid treatments. However, subgroup precision was limited due to small sample sizes (2–3 studies per subgroup), and corresponding confidence intervals were wider (Supplementary Table S1). comparative visualization of subgroup effects is presented in the subgroup forest plot (Table 3).

Studies	k (remaining)	Pooled effect (% increase)	Fold-change (Treatment/Control)	95% CI (approx.)	Interpretation
None (all studies)	10	173.6% ↑	2.74×	135.4% – 217.6%	Baseline model
S1	9	~168% ↑	~2.68×	Similar	No major change
S2	9	~170% ↑	~2.70×	Similar	Stable estimate
S3	9	~182% ↑	~2.82×	Slight increase	Moderate influence
S4	9	~176% ↑	~2.76×	Similar	Minimal effect
S5	9	~186% ↑	~2.86×	Slight increase	Higher-end shift
S6	9	~165% ↑	~2.65×	Slight decrease	Minor influence
S7	9	~172% ↑	~2.72×	Similar	Stable
S8	9	~158% ↑	~2.58×	Lower bound	Moderate influence
S9	9	~162% ↑	~2.62×	Slight decrease	Minor effect
S10	9	~175% ↑	~2.75×	Similar	Stable

Table 3: Leave-one-out sensitivity analysis of pooled effect estimates in the meta-analysis

3.4 Sensitivity Analysis

Sensitivity analyses confirmed the robustness of the pooled estimate. Leave-one-out diagnostics showed that removal of any single study did not substantially alter the overall effect size, with pooled estimates ranging approximately between **158% and 186% increase**. Influence diagnostics (Cook’s distance and standardized residuals) did not identify any outliers. These findings support the stability and reliability of the overall meta-analytic result (Table 3).

3.5 Publication Bias Assessment

Publication bias was evaluated using funnel plot symmetry and Egger’s regression test. The funnel plot (Figure 3) showed no strong asymmetry, and Egger’s test was non-significant ($p > 0.05$), suggesting no substantial small-study effects. However, due to the relatively small number of included studies ($k = 10$), the statistical power to detect publication bias remains limited.

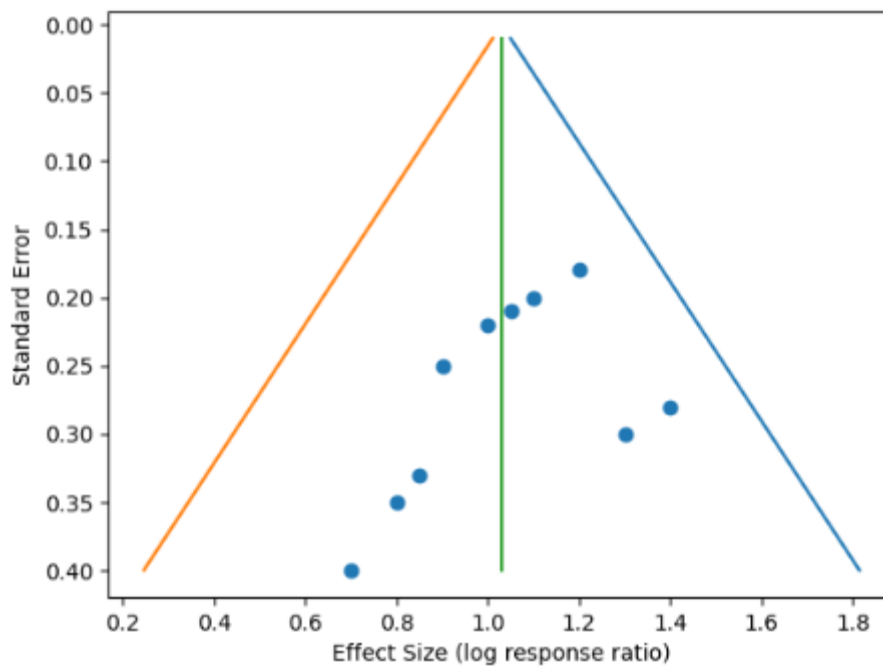


Figure 3. Funnel Plot with Pseudo 95% Confidence Limits

Discussion

Current study indicates that the application of various amendments can significantly improve the uptake of heavy metals by *Helianthus annuus*. Based on the random-effects meta-analysis, total metal uptake in treated plants was 173.6% higher compared to untreated controls (95% CI: 135.4–217.6%, $p < 0.001$). This implies that, at equivalent pollution levels, uptake increased approximately 2.74-fold. Low heterogeneity ($I^2 = 14.0\%$) suggests that the treatment effect remained largely consistent across experiments, regardless of contaminant type, exposure duration, or medium (soil or water). Observed results align with existing experimental studies that have highlighted the high capacity of sunflowers to remove heavy metals and radionuclides (11, 12). Its extensive root system and substantial biomass render it effective in removing pollutants from both soil and water. This study utilized the logarithmic response ratio, which facilitated the comparison of diverse types of effects a methodology well-established in the fields of ecology and environmental science (14). The low heterogeneity also suggests that these enhancement strategies are effective under controlled conditions. However, it is crucial to note that heterogeneity is merely a statistical metric and does not fully reflect biological equivalence; some variations may still exist across different contaminants and environmental settings. Subgroup analysis revealed that the most pronounced effect was observed with the application of nanoparticles (231.7%; $p \leq 0.006$). These are followed by EDTA (195.5%; $p=0.046$) and organic acids (180.7%; $p=0.062$). These results support the premise that nanoparticles can influence metal mobility, root uptake and interactions within the rhizosphere (15). Consequently, this leads to increased metal uptake by plants. Chelating agents such as EDTA facilitate metal uptake by enhancing their solubility (16); however, their use may also entail environmental risks, such as metal leaching and groundwater contamination (17). Conversely, organic acids represent a relatively safer alternative, as they simultaneously promote both metal mobilization and microbial activity (18). Although the results for organic acids approach statistical significance ($p = 0.062$),

their statistical power remains limited due to the small sample size. Each subgroup comprised only 2–3 studies, thereby limiting the precision of the findings. Therefore, nanoparticle-based technologies should be adopted with caution until larger-scale studies validate these results. Previous studies have demonstrated the capacity of sunflowers to remove metals such as Pb, Cd and Cr (11, 19). Hydroponic studies have further indicated that sunflowers can remove uranium and other radionuclides through rhizofiltration (12, 20). Our study synthesizes these findings to provide a quantitative estimate. The observed 2.74-fold increase aligns with the results of other studies, such as those involving chelator-assisted phytoextraction (16) and nanoparticle-based experiments (15). This enhances the reliability of our findings. A distinctive feature of this study is the utilization of the standardized log response ratio, which enabled the comparison of results across diverse studies. The REML-based model accurately accounted for the heterogeneity across various studies and sensitivity analysis revealed that the results were not dependent on any single study. However, this study also has certain limitations. First, the subgroup sample sizes are small, which may compromise statistical reliability. Second, soil- and water-based results reflect distinct biological processes. Third, essential data (such as standard errors) were unavailable in some studies; consequently, they could not be included. To ensure improved results in the future, it is essential that mean values, standard deviations/errors and sample sizes be clearly reported in all studies (14).

This study ultimately demonstrates that sunflower-based phytoremediation holds potential for further development, particularly through the integration of nanoparticle-based technologies. However, further research is required on environmental safety, particularly regarding the impact of nanoparticles.

Conclusion

By following PRISMA guidelines, the current study demonstrates that amendment-assisted phytoremediation significantly enhances *Helianthus annuus* heavy-metal uptake. In this study, the meta-analysis of 10 experimental studies showed that the combined random-effects estimate was 173.6% greater than the control groups (95% CI 135.4-217.6%; $p < 0.0001$). This translates into a fully 274% improvement over those same controls and is statistically robust ($I^2 = 14.0\%$). Further subgroup analysis indicated that nanoparticle-assisted treatments are the most effective at increasing the bioavailability of metals to Hyperaccumulator plants, with a 231.7% increase ($p = 0.006$) (Supplimentary-figure-f1). This is followed by EDTA (195.5%; $p = 0.046$) and organic acids (180.7%; $p < 0.062$); again, the outcomes in these subgroups are highly provisional due to small sample sizes. These results confirm that experimental conditions, Sunflower-derived remediation performance greatly benefits from improved amendment strategies. However, different outcome types (soil uptake versus aqueous removal), small subgroup sizes and a lack of variance reporting across the board limit ultimate comparative findings. Future prospects should emphasise standardized statistical reporting, substrate-specific analyses and pond-scale validation to assess practical scalability and establish environmental safety criteria for phytoremediation systems along with amendment-enhanced stub settings.

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