Water management strategies to assessing sunflowers (*Helianthus annuus* L.) for heavy metal phytoremediation

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Abstract
The current study examines the phytoremediation potential of Helianthus annuus (sunflower) and two hybrid species, Teddy Bear L and Little Becka Hybrid, for bioaccumulating and translocating copper (Cu^{2+}) and cadmium (Cd^{2+}) under various water types (well and tap water) and moisture depletion conditions. It was carried out at the Bakrajo Technical Institute farm in Sulaimani, Iraq, during the 2019–2020 growing season. Using a Completely Randomized Design (CRD), statistical analyses including ANOVA revealed significant species and water type effects on the uptake and translocation of Cu^{2+}, but moisture depletion and replicate treatments had no substantial impact on these processes. Despite some minor differences in translocation ratios, both species demonstrated similar capabilities for metal uptake and translocation. While showing that moisture depletion has little effect on metal buildup, our results emphasize the significance of species selection and irrigation water quality in phytoremediation. Further research is recommended to explore the complex interactions between environmental factors, plant physiology, and metal bioavailability in phytoremediation contexts.
Keywords: Phytoremediation, $Cu^{2+}$and Cd^{2+}, *Helianthus annuus* L., Heavy Metal, Environmental Factors.

Introduction
Water contamination by heavy metals represents a major environmental and public health concern worldwide, as these pollutants not only degrade water quality but also accumulate in soils through irrigation, industrial discharge, and agricultural practices, where they persist for long periods due to their non-biodegradable nature. This accumulation leads to soil degradation, reduced microbial activity, and impaired nutrient cycling, while simultaneously increasing the risk of heavy metal uptake by crops and their subsequent transfer into the food chain, posing serious threats to ecosystem stability, food safety, and human health. [1,2]. Industrial activities, agricultural runoff, and urban waste contribute to the accumulation of these toxic elements in soil and water systems, leading to detrimental effects on ecosystems and human health [3]. Phytoremediation, the use of plants to remove, stabilize, or degrade pollutants, has emerged as a promising and sustainable approach to mitigate heavy metal contamination [4]. One plant of particular interest in this field is **Helianthus annuus*

L. Sunflower rapid growth and extensive root system, also, has shown potential in accumulating heavy metals from contaminated soils and waters [5].

Effective water management is crucial for ensuring the sustainable use of water resources, particularly in the face of increasing pollution and environmental challenges [6]. Contaminants such as heavy metals originating from industrial processes, agricultural runoff, and urban waste significantly degrade water quality, posing serious risks to aquatic ecosystems, soil systems, and human health [7]. In this context, soil and water remediation encompasses a wide range of physical, chemical, and biological techniques aimed at removing, immobilizing, or neutralizing pollutants from contaminated environments [8]. Among these approaches, phytoremediation stands out as an eco-friendly, sustainable, and cost-effective method that minimizes environmental disturbance while promoting ecosystem recovery [9]. This approach utilizes plants to absorb, accumulate, and detoxify contaminants from soil and water, thereby restoring water quality, improving soil conditions, and enhancing the overall health and resilience of aquatic and terrestrial ecosystems [10].

One plant with notable potential in this field is *Helianthus annuus* L. (sunflower), which has attracted considerable attention due to its robust growth, high biomass production, and extensive root system that enhances its capacity to absorb and accumulate heavy metals from contaminated soil and water. Moreover, its strong tolerance to various environmental stresses and pollutants allows it to survive in degraded conditions, making it an excellent and cost-effective candidate for phytoremediation strategies aimed at reducing heavy metal concentrations and restoring polluted ecosystems. [11]. Effective water and soil management plays a crucial role in the removal of heavy metals from contaminated environments [12]. Techniques such as phytoremediation, where plants are used to absorb and accumulate heavy metals, can enhance soil health while simultaneously reducing toxicity levels [13]. Additionally, the application of bioremediation methods, which utilize microorganisms to degrade or transform harmful substances, can further mitigate heavy metal contamination in both water and soil [14]. Regular monitoring of soil and water quality, along with implementing sustainable practices like crop rotation and organic amendments, can foster a balanced ecosystem that supports the natural processes of metal removal. By integrating these strategies, can not only improve soil and water quality but also promote environmental sustainability and public health [15].

This study aims to investigate the phytoremediation potential of two sunflower cultivars, *Helianthus annuus* L. (Teddy Bear and Little Becka Hybrid), under different water availability regimes, in order to evaluate their efficiency in removing heavy metals from contaminated soil. The comparison between these two water regimes is essential because water availability directly influences plant growth, root development, and physiological processes that control metal uptake, transport, and accumulation within plant tissues. Adequate water supply can enhance metal mobility in the soil solution, facilitating their absorption by roots, while water stress may limit uptake but increase plant tolerance mechanisms. Therefore, understanding how different irrigation conditions affect the remediation performance of sunflowers provides valuable insight into optimizing phytoremediation strategies for improving water quality and managing polluted soil–water systems in a sustainable and environmentally friendly manner.

Materials and Methods

The current study was conducted in an open field at the Bakrajo Technical Institute Farm, Sulaimani Polytechnic University, in Sulaimani, Kurdistan Region, Iraq, during the 2019-2020 growing season. The region experiences an average annual temperature ranging from 20 to 42 °C and receives annual rainfall between 250 to 500 mm. This climatic context is essential for understanding the agricultural conditions and potential crop performance in the area.

Experiment details

The pot experiment was place at the open field Bakrajo technical Institute, Sulaimani University, Sulaimani city, during the growing season of 2019-2020. The experiment employed plastic pots with a 30-liter capacity. Each container was filled with 30 kg of surface soil (0-20 cm). Sunflower seeds were placed in germination trays on April 4, 2019 to produce seedlings. Two-week-old seedlings were placed into pots. Initially, three seedlings were transferred into each pot, but this was later decreased to one. The plants were harvested after 90 days, on July 14, 2020. The experiment was put up using a randomized full block design with three replications. Sunflower species were the major plots, with water Sources were randomized in subplots, whereas water availability regimes were considered sub-subplots.

Soil preparation

The Bakrajo Technical Institute's agricultural study field provided soil samples, which were then air-dried, gently crushed, and sieved using a 4 mm stainless-steel pot sieve. For physicochemical investigation, a second portion of the soil was sieved through a 2 mm screen.

Water preparation

Sunflowers were irrigated using two distinct water sources. The water used for control was tap water from Bakrajo municipality's pipeline system. The water was gathered from the Bakrajo Technical Institute's well. Well water is collected straight from the raw well water storage facility using a polyethylene tank, with no chemical or biological treatment.

Data Analysis

Data analysis was carried out using Excel, XLSTAT 2019, an XLSTAT 2019 statistical software to evaluate the effects of sunflower types. An analysis of variance (ANOVA) was performed to determine the significance of differences among treatments for all measured variables,Where significant differences were found, Multiple Range Test (Duncan) was applied to identify which treatments differed significantly from one another. This statistical approach ensured a reliable comparison of treatment effects and supported the interpretation of experimental results.

Results and Discussion

The results of the experiment investigating the effects of sunflower species, irrigation water types, and replicate treatments on heavy metal accumulation and plant growth are summarized in Table 1. Statistical analyses were performed to assess significant differences in bioaccumulation factors (BAF), translocation ratios (TL and TR), metal concentrations in plant tissues (roots and shoots), and biomass production (root and shoot weights). The findings revealed that both sunflower species, Teddy Bear L. and Little Becka Hybrid, exhibited distinct responses to the different irrigation water types, indicating variability in their capacity to absorb and translocate heavy metals from contaminated soil. Moreover, irrigation water source (well versus tap water) significantly influenced metal availability and uptake, likely due to differences in water chemistry affecting metal solubility and mobility in the soil. Variations among replicates further reflected the influence of environmental and biological factors on plant performance. Overall, the results demonstrate that sunflower species and water regime play a critical role in determining phytoremediation efficiency, with observable impacts on metal accumulation patterns and plant growth, highlighting the importance of selecting appropriate plant genotypes and irrigation strategies for optimizing remediation of contaminated soil–water systems.

Analysis of variance phytoremediation ratio BAF Cu^{2+} of assessing Sunflowers (*Helianthus annuus* L.) for heavy metal phytoremediation"

The analysis of variance (ANOVA) for the phytoremediation ratio and bioaccumulation factor (BAF) of Cu^{2+} in *Helianthus annuus* (sunflower) revealed significant effects of both species ($p = 0.019$) and irrigation water types ($p = 0.026$), indicating that these factors play a crucial role in influencing the sunflower's capacity to uptake and remove copper from contaminated soil. In contrast, other factors such as moisture depletion ($p = 0.078$), the REP factor ($p = 0.300$), and higher-order interactions ($p > 0.05$) were not statistically significant, suggesting that they do not exert a strong influence on copper accumulation under the conditions of this study. For instance, moisture depletion did not significantly affect copper uptake, likely because the applied soil moisture levels were not sufficiently extreme to induce physiological stress in the plants. Similarly, the non-significant effect of the REP factor may be attributed to the uniformity of experimental conditions and controlled environmental variables [16]. Furthermore, the absence of significant interactions implies that these factors did not act synergistically to alter copper bioaccumulation. Overall, the results highlight that species selection and irrigation water quality are key determinants of phytoremediation efficiency, whereas moisture depletion, replication, and their interactions have a limited impact in this experimental setup, as shown in Table 1.

Table (1): Analysis of variance phytoremediation ratio BAF Cu^{2+} of assessing Sunflowers (*Helianthus annuus* L.) for Heavy Metal Phytoremediation"

Source	Sum of squares	Mean squares	F	Pr > F
Species	1.336	1.336	21.077	0.019
Irrigation Water Types	1.085	1.085	17.105	0.026
Moisture Depletion	0.440	0.440	6.941	0.078
REP	0.234	0.117	1.847	0.300
Species*Irrigation Water Types	0.013	0.013	0.201	0.684
Species*Moisture Depletion	0.078	0.078	1.232	0.348
Species*REP	0.047	0.023	0.369	0.719
Irrigation Water Types*Moisture Depletion	0.051	0.051	0.805	0.436
Irrigation Water Types*REP	0.100	0.050	0.786	0.531
Moisture Depletion*REP	0.019	0.010	0.153	0.864
Species*Irrigation Water Types*Moisture Depletion	0.041	0.041	0.642	0.482
Species*Irrigation Water Types*REP	0.059	0.029	0.465	0.667
Species*Moisture Depletion*REP	0.004	0.002	0.031	0.970
Irrigation Water Types*Moisture Depletion*REP	0.087	0.044	0.687	0.568

Also, the results presented in Table 2 show the analysis of the phytoremediation ratio of $Cu^{2}+$ for two species: Teddy Bear L and Little Becka Hybrid. Both species exhibit similar phytoremediation ratios, with Teddy Bear L showing a mean of 0.4 (±0.1) and Little Becka Hybrid showing a mean of 0.3 (±0.1). The 95% confidence intervals for both species overlap, with the lower bound for both species at 0.2 and 0.1, respectively, and the upper bound for both at 0.6. This overlap suggests that there is no significant statistical difference in the phytoremediation ability between the two species under the conditions tested. The Duncan test results further corroborate this, as both species fall into the same group (A), indicating that their phytoremediation performances are not significantly different. This lack of statistical significance could be due to several factors, such as similar mechanisms of $Cu^{2}+$ uptake and accumulation between the species, or environmental factors that may have had a comparable effect on both species' ability to remediate $Cu^{2}+$. Additionally, the standard error values are relatively large, suggesting some variation in the data, which further supports the conclusion that any differences observed are not large enough to be considered statistically significant. The results agree with study [17,18, 19].

Table (2): Species / Duncan / Analysis of the differences between the species with a confidence interval of 95% phytoremediation ratio BAF Cu^{2+}

Species	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups
Teddy Bear L	0.4	0.1	0.2	0.6	A
Little Becka Hybrid	0.3	0.1	0.1	0.6	A

The results from Table 3 indicate that there is no significant difference in the phytoremediation ratio of Cu^{2+} between tap water and well water. Both tap water (mean = 0.43, SE = 0.07) and well water (mean = 0.29, SE = 0.07) are grouped together under the same letter "A," suggesting that their phytoremediation potential for copper is statistically indistinguishable within the 95% confidence interval (CI). The confidence intervals for both water types overlap substantially, with the lower bound of well water (0.07) and upper bound of tap water (0.66) indicating that the variability in phytoremediation capacity across both water types is not sufficient to establish a significant difference. This non-significance may be attributed to several factors, including the similar mineral composition or pH levels of the water sources, which might not influence the phytoremediation process differently. Additionally, the plant species used in the study could have shown similar uptake and accumulation rates of Cu^{2+}, resulting in comparable remediation efficiencies under the given experimental conditions [20]. Therefore, the lack of significant difference could reflect inherent biological and environmental factors that allow the plants to perform similarly in both water types.

Table (3): Irrigation water types / Duncan / Analysis of the differences between the water types with a confidence interval of 95% phytoremediation ratio BAF Cu^{2+}

Water Types	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups
Tap water	0.43	0.07	0.20	0.66	A
Well water	0.29	0.07	0.07	0.51	A

Table 4 presents the moisture depletion results for phytoremediation of Cu^{2+} with a 95% confidence interval. The species analyzed showed two distinct moisture depletion values: 0.3 (with an LS mean of 0.41) and 0.65 (with an LS mean of 0.31). Both values fall within the same group (A), indicating that the differences in moisture depletion between these two levels are statistically insignificant. The lower and upper bounds of the 95% confidence intervals for the first value (0.18 to 0.64) and the second value (0.09 to 0.53) overlap, further reinforcing that no significant difference exists. The lack of significance suggests that moisture depletion, under the conditions tested, is not strongly influenced by Cu^{2+} concentration in this particular phytoremediation setup. This could be due to factors such as the species' inherent ability to manage moisture loss, the efficiency of Cu^{2+} uptake, or the influence of other environmental variables not accounted for in this specific analysis. Therefore, while differences in moisture depletion were observed, the statistical analysis indicates that these variations are within the margin of error, and do not represent a meaningful difference in moisture depletion due to Cu^{2+} phytoremediation [21].

Table (4): Species / Duncan / Analysis of the differences between the moisture depletions with a confidence interval of 95% phytoremediation ratio BAF Cu^{2+}

Moisture Depletions	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups
0.3	0.41	0.07	0.18	0.64	A
0.65	0.31	0.07	0.09	0.53	A

Analysis of variance translocation ratio TL Cu^{2+} of assessing Sunflowers (*Helianthus annuus* L.) for heavy metal phytoremediation" TL Cu^{2+}

The analysis of variance (ANOVA) presented in Table 5 for the translocation ratio of Cu^{2+} in sunflowers (*Helianthus annuus* L.) reveals significant effects for species, irrigation water types, and moisture depletion, with p-values of 0.008, 0.004, and 0.016, respectively. These factors are crucial for the phytoremediation potential of sunflowers as they influence the uptake and translocation of heavy metals. The significant interactions between species and irrigation water types ($p = 0.033$) indicate that the type of water used for irrigation modifies the species' efficiency in translocating Cu^{2+}, suggesting that the sunflower's physiological response to different water conditions can vary. However, other factors such as the replication (REP) and most interaction terms, including Species *REP, Irrigation Water Types* Moisture Depletion, and others, did not show significant effects ($p > 0.05$), implying that these variables do not have a strong independent or interactive influence on the Cu^{2+} translocation ratio [22]. The lack of significance in these terms, particularly for REP ($p = 0.245$), suggests that the experimental design and variability due to replication were not large enough to affect the outcomes. Additionally, non-significant interactions such as Species* Moisture Depletion ($p = 0.263$) and others indicate that while these factors may influence plant behavior under certain conditions, their combined effect on Cu^{2+} translocation is minimal in this study's context. This could be due to a variety of reasons, including the range of conditions tested being insufficient to show interaction effects or the experimental scale being too limited to detect subtle changes.

Table (5): Analysis of variance translocation ratio TL Cu^{2+} of assessing Sunflowers (*Helianthus annuus* L.) for heavy metal phytoremediation"

Source	Sum of squares	Mean squares	F	Pr > F
Species	1.26	1.26	39.50	0.008
Irrigation Water Types	1.92	1.92	60.34	0.004
Moisture Depletion	0.79	0.79	24.63	0.016

REP	0.15	0.07	2.33	0.245
Species*Irrigation Water Types	0.45	0.45	14.08	0.033
Species*Moisture Depletion	0.06	0.06	1.89	0.263
Species*REP	0.02	0.01	0.24	0.798
Irrigation Water Types*Moisture Depletion	0.00	0.00	0.00	0.981
Irrigation Water Types*REP	0.06	0.03	0.98	0.471
Moisture Depletion*REP	0.04	0.02	0.65	0.582
Species*Irrigation Water Types*Moisture Depletion	0.03	0.03	1.03	0.384
Species*Irrigation Water Types*REP	0.09	0.04	1.40	0.372
Species*Moisture Depletion*REP	0.24	0.12	3.82	0.150
Irrigation Water Types*Moisture Depletion*REP	0.01	0.01	0.21	0.821

Table 6 presents the results of a Duncan analysis comparing the translocation ratios (TL) of Cu^{2+} across two species, Teddy Bear L and Little Becka Hybrid, with a 95% confidence interval. The LS means for Teddy Bear L and Little Becka Hybrid were 0.46 and 0.32, respectively, with standard errors of 0.05 for both species. The 95% confidence intervals for the translocation ratios were from 0.31 to 0.62 for Teddy Bear L and from 0.16 to 0.49 for Little Becka Hybrid. Both species fall into the same statistical group (A), suggesting no significant difference between them in terms of Cu^{2+} translocation. This lack of significance, despite differences in LS means, could be attributed to the overlapping confidence intervals, which indicate that the true means of the two species could be similar within the bounds of the variability (standard error) [23]. Additionally, the lack of statistical significance may be attributed to limitations in sample size and experimental design, as a relatively small number of replicates or narrow variability among treatments can reduce the statistical power of the analysis, making it difficult to detect real but subtle differences between factors. Furthermore, controlled experimental conditions may have minimized environmental variability, leading to more uniform plant responses and consequently masking potential treatment effects. This highlights the importance of considering statistical power, experimental scale, and treatment intensity when interpreting non-significant results, as true biological effects may exist but remain undetected due to methodological constraints [24].

Table (6): Species / Duncan / analysis of the differences between the species with a confidence interval of 95% translocation ratio TL Cu^{2+}

Species	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups
Teddy Bear L	0.46	0.05	0.31	0.62	A
Little Becka	0.32	0.05	0.16	0.49	A

Hybrid					

The analysis presented in Table 7 shows the translocation ratio of Cu^{2+} (TL Cu^{2+}) across different water types, specifically tap water and well water, with a 95% confidence interval. The LS means indicate that tap water had a significantly higher translocation ratio (0.51 ± 0.05) compared to well water (0.28 ± 0.05). The confidence intervals for the two groups do not overlap, suggesting that tap water promotes a more efficient translocation of Cu^{2+} ions compared to well water. The well water group, with a lower TL Cu^{2+} value, falls within a narrower confidence range, indicating less variation in the results [25]. Additionally, it is possible that the organic content and pH of well water may affect the solubility and mobility of Cu^{2+} ions, leading to reduced translocation compared to tap water. Hence, while tap water appears to support better Cu^{2+} translocation, the underlying mechanisms require further investigation to understand the influence of water chemistry in the bioavailability and plant uptake of heavy metals.

Table (7): Irrigation water types / Duncan / Analysis of the differences between the water types with a confidence interval of 95% translocation ratio TL Cu^{2+}

Water Types	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups	
Tap water	0.51	0.05	0.34	0.67	A	
Well water	0.28	0.05	0.12	0.44		B

The results in Table 8, which present the moisture depletion data under a Duncan test with a 95% confidence interval for the translocation ratio (TL) of Cu^{2+}, reveal non-significant differences between the groups. Specifically, the moisture depletions at two different levels (0.3 and 0.65) show LS means of 0.49 and 0.30, respectively, both of which fall within the overlapping confidence intervals (0.32–0.65 and 0.15–0.46), suggesting no significant difference in moisture depletion across the groups. The standard errors for both levels are 0.05, indicating a small variability in the measurements, further reinforcing the lack of significant difference. The absence of significance can be attributed to the similarity in the observed moisture depletion between the two groups, potentially reflecting a threshold in the Cu^{2+} translocation process where further increases in moisture depletion do not lead to significant changes in the results under the experimental conditions. Similar findings have been reported in studies of ion transport and moisture interactions, where incremental changes in moisture levels do not always translate into statistically significant differences [26]. These results may indicate that moisture depletion is more sensitive to other environmental factors or that Cu^{2+} translocation does not substantially affect moisture dynamics within the range studied.

Table (8): Moisture Depletion / Duncan / Analysis of the differences between the moisture depletions with a confidence interval of 95% translocation ratio TL Cu^{2+}

Moisture depletions	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups
0.3	0.49	0.05	0.32	0.65	A
0.65	0.30	0.05	0.15	0.46	A

Analysis of variance phytoremediation" ratio BAF Cd^{2+} of assessing Sunflowers (*Helianthus annuus* L.) for heavy metal phytoremediation" BAF Cd^{2+}

In the analysis of variance (ANOVA) conducted to assess the phytoremediation" ratio (BAF) of Cd^{2+} in sunflowers (*Helianthus annuus* L.) for phytoremediation, several factors were evaluated for their influence on heavy metal uptake. Notably, the species factor approached significance with a p-value of 0.056, suggesting a potential difference in the BAF between sunflower varieties, although this did not reach the standard threshold of 0.05 for statistical significance. Other interactions between species, irrigation types, moisture depletion, and replications (REP) were non-significant ($p > 0.05$), suggesting that while these factors may have some effect, their combined influence was insufficient to alter the Cd^{2+} uptake significantly in this experimental setup [27]. The lack of significance in many interactions could be attributed to the complex, multifactorial nature of phytoremediation, where plant responses to pollutants depend on numerous interconnected environmental and physiological factors, often requiring more refined experimental designs to capture subtle effects.

Table (9): Analysis of variance phytoremediation" ratio BAF Cd^{2+} of assessing Sunflowers (*Helianthus annuus* L.) for heavy metal phytoremediation" BAF Cd^{2+}

Source	Sum of squares	Mean squares	F	P. > F
Species	0.55	0.55	9.22	0.056
Irrigation Water Types	1.12	1.12	18.76	0.023
Moisture Depletion	0.42	0.42	7.00	0.077
REP	0.26	0.13	2.22	0.257
Species*Irrigation Water Types	0.20	0.20	3.30	0.167
Species*Moisture Depletion	0.03	0.03	0.56	0.510
Species*REP	0.09	0.05	0.79	0.531
Irrigation Water Types*Moisture Depletion	0.02	0.02	0.37	0.588
Irrigation Water Types*REP	0.01	0.01	0.10	0.908
Moisture Depletion*REP	0.06	0.03	0.46	0.668
Species*Irrigation Water Types*Moisture Depletion	0.01	0.01	0.24	0.659
Species*Irrigation Water Types*REP	0.02	0.01	0.18	0.845
Species*Moisture Depletion*REP	0.03	0.01	0.25	0.793

Irrigation Water Types*Moisture Depletion*REP	0.02	0.01	0.14	0.872

The results from Table 10 show the Bioaccumulation Factor (BAF) for Cd^{2+} in two species: *Teddy Bear L* and *Little Becka Hybrid*, with both species sharing the same statistical grouping (Group A). The *Teddy Bear L* species had a BAF value of 0.39 (±0.07), with a 95% confidence interval ranging from 0.18 to 0.61, while *Little Becka Hybrid* had a lower BAF of 0.21 (±0.07), with a 95% confidence interval from 0.01 to 0.44. Despite these numerical differences, the lack of statistical significance between these species suggests that both species have similar capacities for the uptake of Cd^{2+} from the environment. This non-significance may be due to the inherent biological variability in the uptake mechanisms or the effect of environmental factors such as soil composition, availability of Cd^{2+}, or other unaccounted-for variables influencing the plants' uptake efficiency [28]. Additionally, differences in the rooting systems, biomass production, or the physiological tolerance of the species could result in overlapping confidence intervals, reflecting the complex and often non-linear interactions between plant species and heavy metals in phytoremediation contexts [28]. These findings align with similar studies where minor differences in BAF values do not always result in significant outcomes due to such underlying complexities.

Table (10): Species / Duncan / Analysis of the differences between the species with a confidence interval of 95% for heavy metal phytoremediation” BAF Cd^{2+}

Species	LS means	Standard error	Lower bound (95%)	Upper bound (95%)	Groups
Teddy Bear L	0.39	0.07	0.18	0.61	A
Little Becka Hybrid	0.21	0.07	0.01	0.44	A

Analysis of variance shoots concentration contain in mg L^{-1} Cu^{2+} of assessing Sunflowers (*Helianthus annuus* L.) for heavy metal concentration” Cu^{2+} in shoots (mg L^{-1})

The results from the analysis of variance (ANOVA) for the copper (Cu^{2+}) concentration in sunflower (*Helianthus annuus* L.) shoots reveal significant effects for species, irrigation water types, and moisture depletion (table 11) on the Cu^{2+} concentration. Specifically, species had a highly significant effect ($p = 0.005$), with $F = 57.2983$, indicating that different sunflower species vary in their ability to accumulate copper. Similarly, the type of irrigation water significantly affected Cu^{2+} concentration ($p = 0.001$, $F = 145.2671$), suggesting that different water types may influence the uptake or bioavailability of copper in the plant. Moisture depletion also showed a significant effect ($p = 0.007$, $F = 43.1240$), which could be due to changes in plant physiological processes such as transpiration and water uptake, influencing metal absorption. However, the interaction terms involving REP (replication), such as Species *REP (p = 0.164), and other two- and three-way interactions, including Species* Moisture Depletion ($p = 0.226$), Irrigation Water Types*Moisture Depletion (p = 0.564), and the three-way interactions, all yielded non-significant results. These non-significant outcomes suggest that while individual factors (species, irrigation, moisture) strongly influence Cu^{2+} concentration, the complexity of their interactions does not consistently alter the plant's copper uptake across replications or under varying moisture levels. The lack of significance in these interactions may be attributed to limited variability or small effects within the experimental conditions, or possibly due to the dominance of the main effects over the interactions. Such findings are consistent with prior studies, which report that main factors like species and water quality often exert a stronger influence on metal uptake than their interactions [29]. Thus, these results highlight the importance of considering each factor independently rather than their combinations when assessing heavy metal uptake in plants.

Table (11): Analysis of variance shoots concentration contain in mg L^{-1} Cu^{2+} of assessing Sunflowers (*Helianthus annuus* L.) for heavy metal concentration" Cu^{2+} in shoots (mg L^{-1})

Source	Sum of squares	Mean squares	F	Pr > F
Species	0.0313	0.0313	57.2983	0.005
Irrigation Water Types	0.0793	0.0793	145.2671	0.001
Moisture Depletion	0.0235	0.0235	43.1240	0.007
REP	0.0047	0.0024	4.3128	0.131
Species*Irrigation Water Types	0.0163	0.0163	29.9423	0.012
Species*Moisture Depletion	0.0013	0.0013	2.3044	0.226
Species*REP	0.0038	0.0019	3.5050	0.164
Irrigation Water Types*Moisture Depletion	0.0002	0.0002	0.4188	0.564
Irrigation Water Types*REP	0.0008	0.0004	0.7420	0.547
Moisture Depletion*REP	0.0007	0.0004	0.6797	0.571
Species*Irrigation Water Types*Moisture Depletion	0.0024	0.0024	4.3596	0.128
Species*Irrigation Water Types*REP	0.0048	0.0024	4.4120	0.128
Species*Moisture Depletion*REP	0.0039	0.0020	3.6120	0.159
Irrigation Water Types*Moisture Depletion*REP	0.0011	0.0006	1.0368	0.455

Analysis of variance roots concentration contain in mg L^{-1} Cu^{2+} in roots of assessing Sunflowers (*Helianthus annuus* L.) for heavy metal concentration" Cu^{2+} in roots (mg L^{-1})

The results at the table 12 the analysis of variance (ANOVA) for root concentrations of Cu^{2+} in *Helianthus annuus* (sunflower) demonstrate significant effects for species (p = 0.010) and irrigation water types (p = 0.016), indicating that both factors independently influence the concentration of Cu^{2+} in sunflower roots. In contrast, other factors, such as moisture depletion ($p = 0.078$) and replication ($p = 0.106$), showed trends towards significance but did not meet the standard threshold for significance ($p < 0.05$). The interactions between species and moisture depletion ($p = 0.441$), species and irrigation water types ($p = 0.141$), and species, irrigation water types, and moisture depletion ($p = 0.3$) were also not significant, suggesting that these combined factors do not significantly alter the Cu^{2+} concentration in sunflower roots under the experimental conditions. These non-significant results could be due to the high variability within the treatments, particularly with the moisture depletion and replication factors, which might not have had enough impact to show a clear statistical effect on heavy metal uptake. Such variability might arise from factors such as the heterogeneity in soil properties, plant genetic differences, or the inherent adaptability of sunflowers to varying environmental stressors, which could dampen the overall interaction effects. This finding is consistent with studies that suggest that single factors, like species and water types, may play more direct roles in metal uptake than complex interactions [28,29].

Table (12): Analysis of variance roots concentration contain in mg L^{-1} Cu^{2+} of assessing Sunflowers (*Helianthus annuus* L.) for heavy metal concentration" Cu^{2+} in roots (mg L^{-1})

Source	Sum of squares	Mean squares	F	Pr > F
Species	0.289	0.289	33.10	0.010
Irrigation Water Types	0.216	0.216	24.72	0.016
Moisture Depletion	0.060	0.060	6.914	0.078
REP	0.090	0.045	5.183	0.106
Species*Irrigation Water Types	0.034	0.034	3.942	0.141
Species*Moisture Depletion	0.007	0.007	0.783	0.441
Species*REP	0.019	0.009	1.075	0.445
Irrigation Water Types*Moisture Depletion	0.028	0.028	3.262	0.169
Irrigation Water Types*REP	0.050	0.025	2.888	0.200
Moisture Depletion*REP	0.005	0.002	0.270	0.780
Species*Irrigation Water Types*Moisture Depletion	0.014	0.014	1.561	0.300
Species*Irrigation Water Types*REP	0.035	0.018	2.012	0.279
Species*Moisture Depletion*REP	0.001	0.00001	0.032	0.969
Irrigation Water Types*Moisture Depletion*REP	0.019	0.009	1.082	0.443

Analysis of variance soil concentration contain in mg L^{-1} Cu^{2+} of assessing Sunflowers (*Helianthus annuus* L.) for heavy metal concentration" Cu^{2+} in soil (mg L^{-1})

The results presented in Table 13 show an analysis of variance (ANOVA) assessing the concentration of Cu^{2+} in soil (mg L^{-1}) in relation to the growth of sunflowers (*Helianthus annuus L.*), focusing on the effects of species, irrigation water types, moisture depletion, and their interactions. The factor *species* (F = 42.222, p = 0.007) exhibited a significant effect on Cu^{2+} concentration, indicating that different sunflower species respond differently to Cu^{2+} accumulation. This is consistent with the known ability of plant species to vary in metal uptake, influenced by root morphology and metal transport mechanisms (Krämer, 2020). However, the factor *irrigation water types* (F = 10.005, p = 0.051) approached significance but did not reach the conventional threshold of $p < 0.05$. This marginal result suggests that while irrigation water quality may influence heavy metal uptake, its impact might not be as pronounced as expected, potentially due to the buffering capacity of the soil or the plants' inherent ability to regulate metal uptake under varying water conditions [29]. Similarly, *moisture depletion* (F = 4.004, p = 0.139) did not significantly affect Cu^{2+} concentration, indicating that water stress, within the range tested, may not significantly alter Cu^{2+} uptake, likely because other factors such as root architecture and soil characteristics override the direct effects of moisture levels on metal absorption. Interaction terms such as *species × irrigation water types* and *species × moisture depletion* also showed no significant effects, which further suggests that while species-specific responses to Cu^{2+} exist, their interaction with these factors does not significantly influence metal accumulation, possibly due to the low variability in the treatments or the buffering effects within the experimental setup. The non-significant results may reflect inherent plant resilience or the complexity of environmental variables in metal uptake processes, emphasizing the need for further studies to better understand the environmental interactions that govern heavy metal bioavailability and accumulation in crops.

Table (13): Analysis of variance soil concentration contain in mg L^{-1} Cu^{2+} of assessing Sunflowers (*Helianthus annuus* L.) for heavy metal concentration" Cu^{2+} in soil (mg L^{-1})

Source	Sum of squares	Mean squares	F	Pr > F
Species	2.867	2.867	42.222	0.007
Irrigation Water Types	0.679	0.679	10.005	0.051
Moisture Depletion	0.272	0.272	4.004	0.139
REP	0.755	0.377	5.55	0.09

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Species*Irrigation Water Types	0.502	0.502	7.400	0.073
Species*Moisture Depletion	0.048	0.048	0.706	0.463
Species*REP	0.099	0.049	0.726	0.553
Irrigation Water Types*Moisture Depletion	0.112	0.112	1.648	0.289
Irrigation Water Types*REP	0.157	0.078	1.156	0.425
Moisture Depletion*REP	0.029	0.014	0.213	0.820
Species*Irrigation Water Types*Moisture Depletion	0.047	0.047	0.687	0.468
Species*Irrigation Water Types*REP	0.092	0.046	0.677	0.572
Species*Moisture Depletion*REP	0.026	0.013	0.192	0.835
Irrigation Water Types*Moisture Depletion*REP	0.012	0.006	0.086	0.920

Species / Duncan / Analysis of variance concentration contain in mg L^{-1} Cd^{2+} of assessing Sunflowers (*Helianthus annuus* L.) for heavy metal concentration" Cd^{2+} in shoots, roots and soil (mg L^{-1})

The analysis from the (table 14) explain the effect of Cd^{2+} (cadmium) concentrations on sunflowers (*Helianthus annuus* L.) in terms of shoots, roots, and soil showed no significant differences between the species for all measured parameters, as indicated by the p-values (Pr > F) of 0.573 for shoots, 0.352 for roots, and 0.151 for soil. These p-values are well above the conventional significance threshold of 0.05, suggesting that the variations in Cd^{2+} concentration in shoots, roots, and soil across different sunflower species are not statistically significant. This result implies that neither of the two species, Little Becka Hybrid and Teddy Bear L, demonstrated a notable difference in cadmium uptake or accumulation across the examined tissue types (shoots and roots) or in soil concentration. Such findings may suggest that both species exhibit similar physiological responses to cadmium exposure, and factors such as metal tolerance or metal sequestration mechanisms might not vary significantly between these species under the specific experimental conditions. These results are consistent with other studies that show sunflower species often display similar cadmium uptake characteristics, with overall cadmium concentrations in the plant tissues remaining below levels that would lead to significant species-specific responses [14,30]. The non-significant differences across these parameters also point to the need for further studies with more variable environmental conditions or different metal concentrations to assess the potential ecological impact of cadmium, which is known to be a persistent environmental pollutant. Given that Cd^{2+} is a toxic heavy metal, it remains a significant concern for soil and water contamination, potentially affecting plant growth, soil fertility, and overall ecosystem health. The results underline the necessity for continued research on the bioaccumulation of cadmium in crops and its implications for food safety and environmental sustainability [14]

Table (14): Species / Duncan / Analysis of variance concentration contain in mg L^{-1} Cd^{2+} of assessing Sunflowers (*Helianthus annuus* L.) for heavy metal concentration" Cd^{2+} in shoots, roots and soil (mg L^{-1})

Species	Cd^{2+} Shoot	Cd^{2+} Root	Cd^{2+} Soil
Little Becka Hybrid	0.069 [a]	0.155 [a]	0.723 [a]
Teddy Bear L	0.047 [a]	0.152 [a]	0.388 [a]
Pr > F(Model)	0.573	0.352	0.151
Significant	No	No	No

Water types / Duncan / Analysis of variance concentration contain in mg L^{-1} Cd^{2+} of assessing Sunflowers (*Helianthus annuus* L.) for heavy metal concentration" Cd^{2+} in shoots, roots and soil (mg L^{-1})

In this study, the effect of different water types (tap water and well water) show in the table 15 for the concentration of cadmium (Cd^{2+}) in the shoots, roots, and soil of *Helianthus annuus L.* (sunflowers) was assessed. The cadmium concentrations in the shoots, roots, and soil were reported in mg L^{-1}, with tap water showing values of 0.062 mg L^{-1}, 0.159 mg L^{-1}, and 0.508 mg L^{-1}, respectively, while well water exhibited 0.054 mg L^{-1}, 0.147 mg L^{-1}, and 0.602 mg L^{-1}, respectively. Analysis of variance (ANOVA) results revealed that the differences in cadmium concentrations between the two water types were not statistically significant (Pr > F values for shoots, roots, and soil were 0.573, 0.352, and 0.151, respectively). This indicates that the type of water (tap vs. well) did not have a significant impact on the accumulation of Cd^{2+} in sunflower tissues or the soil. The lack of statistical significance in all three measured categories suggests that factors other than water type, such as soil properties, sunflower variety, or environmental conditions, may be more influential in determining the uptake of Cd^{2+}. This finding is consistent with the broader scientific understanding that heavy metal uptake in plants can be influenced by a complex interaction of soil pH, organic matter, and plant physiology, rather than just the water source. Although the concentrations observed were low, the presence of cadmium in the soil and plant tissues still raises concerns about potential environmental pollution and bioaccumulation, especially in agricultural areas exposed to contaminated water sources [14,29]. The implications of these findings stress the need for further research into the mobility of cadmium in different environmental settings, and the potential risks to plant health and food safety in contaminated areas.

Table (15): Water types / Duncan / Analysis of variance concentration contain in mg L^{-1} Cd^{2+} of assessing Sunflowers (*Helianthus annuus* L.) for heavy metal concentration" Cd^{2+} in shoots, roots and soil (mg L^{-1})

Water types	Cd SHOOT	Cd ROOT	Cd soil
Tap water	0.062 [a]	0.159 [a]	0.508 [a]
Well water	0.054 [a]	0.147 [a]	0.602 [a]
Pr > F(Model)	0.573	0.352	0.151
Significant	No	No	No

The study's findings provide insight into what influences the bioaccumulation of copper (Cu^{2+}) and cadmium (Cd^{2+}) in sunflowers (*Helianthus annuus* L.) for phytoremediation. The analysis indicated that species selection, particularly between Teddy Bear L and Little Becka Hybrid, significantly influenced metal uptake, with both species showing similar capacities for copper and cadmium remediation. However, irrigation water types, moisture depletion, and their interactions did not significantly affect metal bioaccumulation, suggesting that these factors were less important in the experimental setup. The lack of significant effects from water type and moisture levels suggests that factors like soil conditions or plant genetics may have a more substantial impact on metal uptake than initially expected. While the study highlighted the importance of species choice in optimizing phytoremediation strategies, it also revealed the complexity of interpreting environmental factors. Despite some differences in moisture depletion and metal concentrations, these variations did not lead to statistically significant changes in the phytoremediation process. The findings suggest that water quality, including the type of irrigation water used, plays a more limited role in metal translocation under controlled conditions. Further illustrating the complexity of phytoremediation processes, the lack of significant interactions between moisture depletion, water type, and species in terms of metal uptake suggests that more varied environmental conditions or alternative plant species may need to be tested in order to better understand the findings' wider applicability.

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