RESEARCH ARTICLE

Bioconcentration and Translocation Dynamics of Cadmium and Copper in Wetland Associated Plant Species: Implications for Phytostabilization and Phytoextraction

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Abstract

Heavy metal contamination in soils and aquatic ecosystems poses ecological and health risks, with cadmium (Cd) and copper (Cu) among the most critical pollutants. Conventional remediation is often costly and disruptive, whereas phytoremediation offers a sustainable alternative. Yet, species-specific strategies in metal uptake remain insufficiently understood. This study examined the phytoremediation potential of four wetland-associated plant species viz. *Alternanthera philoxeroides, Juncellus alopecuroides, Physalis minima*, and *Portulaca oleracea* in pot experiments with graded Cd and Cu concentrations (50-400 mg kg^{-1}). Plants were cultivated for 60 days, harvested, and analysed for tissue accumulation using atomic absorption spectrophotometry. Uptake efficiency was assessed using the bioconcentration factor (BCF), the translocation factor (TF), and regression analyses. Results showed a consistent tissue distribution of root > shoot > leaf for both metals, confirming root dominance in sequestration. A. *philoxeroides* and J. *alopecuroides* recorded root BCF > 3 with TF < 1, validating their role as Cd phytostabilizers. In contrast, *J. alopecuroides* displayed strong Cu phytoextraction, with shoot BCF > 2, steep regression slopes, and moderately higher TF values. *A. philoxeroides* showed moderate Cu accumulation, while P. minima and P. oleracea consistently exhibited low uptake, restricting their role to weak stabilization. Two-way ANOVA confirmed significant effects of species and concentration ($p < 0.05$), while regression models ($R^2 > 0.95$) demonstrated predictable dose–response relationships. The study highlights distinct species-specific strategies: Cd phytostabilization and Cu phytoextraction. *J. alopecuroides* is identified as a strong Cu extractor and *A. philoxeroides* as a Cd stabilizer, offering practical applications for sustainable remediation.

Keywords: *Wetland associated plant species*, Bioconcentration factor, Cadmium, Copper, Phytoremediation, Translocation factor.

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Introduction

Heavy metal contamination has emerged as one of the most pressing environmental challenges worldwide due to the persistence, toxicity, and non-biodegradable nature of these elements. Unlike organic pollutants that can degrade over time, heavy metals accumulate in soils, sediments, and water bodies, thereby posing long-term ecological and health risks. Among the wide spectrum of pollutants, cadmium (Cd) and copper (Cu) are of particular concern. Cadmium, a non-essential element, is toxic to plants, animals, and humans even at trace concentrations, disrupting cellular metabolism and causing physiological damage (Briffa, 2020). Copper, while essential as a micronutrient involved in enzymatic and metabolic functions, becomes toxic at elevated levels, impairing plant growth and ecological balance (Ali *et al.*, 2013; Zhao *et al.*, 2015). Recent studies have further highlighted the increasing global burden of heavy metal contamination due to rapid industrialization and urbanization, emphasizing the urgent need for sustainable remediation strategies (Bhat *et al.*, 2022; Pang *et al.*, 2023; Bayuo *et al.*, 2024).

The primary anthropogenic sources of Cd and Cu include mining, smelting, electroplating, fertilizer and pesticide application, as well as industrial and municipal effluents. These pollutants often accumulate in aquatic ecosystems, where they disrupt food webs, diminish biodiversity, and pose risks to human health through bioaccumulation and biomagnification. Addressing such contamination requires reliable, cost-effective, and sustainable remediation strategies (Alengebawy *et al.*, 2021). Conventional remediation technologies, such as soil excavation, washing, chemical immobilization, and electrokinetic extraction, though effective in certain contexts, are constrained by high costs, energy intensity, and potential secondary environmental impacts. These methods can also degrade soil fertility or generate hazardous waste (Sharma *et al.*, 2018). As a result, phytoremediation, the use of plants to extract, stabilize, or detoxify contaminants, has gained prominence as a green, sustainable alternative (Mishra *et al.*, 2008; Yoon *et al.*, 2006; Ekshinge *et al.*, 2022; Kulkarni *et al.*, 2014).

Wetland associated plant species are increasingly recognized for their phytoremediation potential. Their rapid growth, extensive root systems, high biomass productivity, and ecological adaptability enable them to function as natural

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biofilters in wetlands, rivers, and wastewater systems (Liu *et al*., 2018; Lin *et al*., 2024). Importantly, species differ considerably in their remediation strategies. Some act as phytoextractors, translocating metals into aerial biomass that can be harvested and safely removed, whereas others function as phytostabilizers, immobilizing contaminants within root tissues and thereby limiting their environmental mobility.

The effectiveness of these remediation strategies is commonly evaluated using two indices: the bioconcentration factor (BCF) and the translocation factor (TF). BCF quantifies the capacity of plants to accumulate metals relative to soil concentrations, whereas TF measures the efficiency of metal transport from roots to shoots. Plants with BCF > 1 and TF > 1 are considered strong phytoextractors, while those with high BCF in roots but TF < 1 are effective phytostabilizers (Yoon *et al*., 2006).

Invasive or fast-growing wetland associated plant species often present ecological paradoxes: although they may outcompete native flora and threaten biodiversity, they also provide ecosystem services such as pollutant removal (Guru *et al*., 2025). For instance, *Alternanthera philoxeroides* (alligator weed) has been reported to tolerate multiple contaminants (Lin *et al*., 2024), while members of the family Cyperaceae, such as *Juncellus alopecuroides*, exhibit strong root-binding capacity and metal sequestration (Liu *et al*., 2018). In contrast, *Physalis minima* and *Portulaca oleracea*, though ecologically adaptable, exhibit variable responses to heavy-metal stress (Ali *et al*., 2013; Sharma & Dubey, 2005). Comparative studies evaluating their simultaneous uptake of Cd and Cu under graded concentrations remain limited.

Recent research emphasizes the importance of integrating physiological tolerance, biomass yield, and ecological adaptability into phytoremediation assessments (Bhat *et al*., 2022; Razzak *et al*., 2022; Yan *et al*., 2020). Such integrative approaches allow for the identification of species that not only accumulate metals effectively but also sustain growth under contaminated conditions.

The present study was therefore designed to evaluate the phytoremediation potential of four wetland associated plant species, *viz*. *Alternanthera philoxeroides, Juncellus alopecuroides, Physalis minima,* and *Portulaca oleracea* under controlled soil experiments with graded concentrations of Cd and Cu. The specific objectives were to: 1) Quantify Cd and Cu accumulation across roots, shoots, and leaves. 2) Determine species-specific remediation strategies using BCF and TF indices. 3) Apply regression and two-way ANOVA to evaluate interspecific variation and dose–response relationships. 4) Compare findings with existing literature to identify potential candidates for phytoextraction or phytostabilization.

This experiment provides new insights into species-specific strategies of wetland associated plant species, with practical implications for integrating them into remediation programs targeting heavy-metal-contaminated soils and aquatic environments.

Materials and Methods

Study Design and Plant Material

A controlled pot experiment was conducted to assess the uptake of cadmium (Cd) and copper (Cu) by four wetland associated plant species: *Alternanthera philoxeroides* (Mart.) Griseb., *Juncellus alopecuroides* (L.) Vahl, *Physalis minima* L., and *Portulaca oleracea* L. These species were selected for their ecological adaptability and contrasting tolerance levels, with *A. philoxeroides* and *J. alopecuroides* known as robust, tolerant macrophytes (Shabani *et al*., 2009b), while *P. minima* and *P. oleracea* are reported to be moderately sensitive in previous phytoremediation studies (Ali *et al*., 2013; Ali *et al*., 2020; Yan *et al*., 2023). Healthy, disease-free propagules of uniform size were collected from natural habitats, thoroughly rinsed to remove surface impurities, and acclimatized in greenhouse conditions for two weeks prior to transplantation. This pre-conditioning step ensured physiological stabilization and minimized transplant shock, following established protocols (Mishra *et al*., 2008; Shabani *et al*., 2009a).

Experimental Setup

The experiment was arranged in a completely randomized design with five graded treatment levels for each heavy metal. This design is widely used in phytoremediation studies to minimize experimental bias and ensure robust statistical comparisons (Yoon *et al*., 2006).

Plastic pots (25 cm diameter × 30 cm height) were filled with a 5:1 soil–cocopeat mixture (w/w), chosen for its favourable aeration and moisture-retention properties, consistent with previous work (Bhat *et al*., 2022; Shabani *et al*., 2009b). The baseline soil was loamy, with a near-neutral pH (6.9–7.2) and negligible background concentrations of Cd and Cu. The physicochemical properties of the soil, including pH, electrical conductivity (EC), organic carbon, and water holding capacity, were analysed prior to experimentation and are presented in Table 1.

Cadmium and copper treatments were introduced separately in the form of cadmium sulphate ($CdSO_4{\cdot}8H_2O$) and copper sulphate ($CuSO_4{\cdot}5H_2O$). Five graded concentrations (50, 100, 200, 300, and 400 mg kg^{-1} soil) were prepared by dissolving metal salts in deionized water and thoroughly mixing them into the soil substrate. This methodology aligns with established phytoremediation protocols (Razzak *et al*., 2022). Control pots without metal addition were maintained for each species. Each treatment was replicated three times. Pots were arranged randomly in a greenhouse under natural light and temperature conditions to avoid positional or environmental bias (Yan *et al*., 2020).

Plant Growth and Maintenance

Following transplantation, plants were grown for 60 days under natural photoperiod and temperature conditions, consistent with standard phytoremediation trials (Ali *et al*., 2013). Soil

Table 1: Physicochemical properties of experimental soil.

Parameter	*Value*
pH	7.0
Electrical conductivity (dS m^{-1})	0.32
Organic Carbon (g kg^{-1})	5.8
Water Holding Capacity (%)	48

moisture was maintained near field capacity by daily irrigation with deionized water. To prevent confounding effects, no fertilizers were added (Sharma & Dubey, 2005). Plant health was regularly monitored, and visible symptoms of stress, including chlorosis, stunted growth, or necrosis, were documented (Lin *et al*., 2024).

Sample collection and processing

At harvest, plants were carefully uprooted and thoroughly washed with tap water followed by distilled water to remove adhering soil particles. Plant tissues were separated into roots, shoots, and leaves, blotted dry, and oven-dried at 70 °C until constant weight was achieved (Mishra *et al*., 2008). Dried samples were ground into fine powder using a stainless-steel mill to avoid contamination.

For metal analysis, 0.5 g of powdered plant tissue was digested using a concentrated nitric acid (HNO_3) and perchloric acid ($HClO_4$) mixture (4:1) until a clear solution was obtained. The digested samples were filtered, diluted to 50 mL with deionized water, and analyzed for Cd and Cu concentrations using Atomic Absorption Spectrophotometry (AAS; Perkin Elmer Analyst 400). This approach follows standard protocols for trace metal estimation (USEPA, 1996). Reagent blanks and certified reference materials were employed for quality control (Zhao *et al*., 2015).

Soil analysis

Soil samples were collected from each pot at the end of the experiment, air-dried, ground, and sieved through a 2 mm mesh. Residual Cd and Cu concentrations were determined following acid digestion (HNO_3: $HClO_4$, 4:1) and quantified using AAS (USEPA, 1996). These values were subsequently used to calculate bioconcentration factors (BCF).

Phytoremediation indices

Bioconcentration factor (BCF) and translocation factor (TF) were calculated to evaluate the phytoremediation potential of the selected macrophytes (Baker, 1981; Yoon *et al*., 2006).

BCF was calculated as

BCF = Croot / Csoil

where Croot is the metal concentration in roots (mg kg^{-1}), and Csoil is the metal concentration in soil (mg kg^{-1}).

TF was calculated as

TF = Cshoot / Croot

where Cshoot is the metal concentration in shoot tissues (mg kg^{-1}).

Values of BCF > 1 indicate efficient uptake, whereas TF > 1 indicates effective translocation of metals to aerial tissues (Yoon *et al*., 2006).

Statistical analyses

Statistical analyses were conducted to evaluate interspecific variation and dose–response relationships of cadmium (Cd) and copper (Cu) uptake among selected macrophytes. A two-way ANOVA (factors: species and concentration) was applied to assess the effects of plant species and metal concentration on metal accumulation in different plant tissues, as well as their interaction effects (Quinn & Keough, 2002; Zar, 2014). Where significant differences were observed, Tukey's Honestly Significant Difference (HSD) test was used for post-hoc comparisons at $\alpha = 0.05$. Dose - response relationships between soil metal concentrations and tissue accumulation were analysed using ordinary least squares (OLS) regression. This approach enabled quantification of uptake efficiency and comparison of regression slopes among species.

$$Y = a + bX + \varepsilon$$

where Y is the tissue concentration (mg kg^{-1}), X is the soil concentration (mg kg^{-1}), a is the intercept, b is the slope, and ε is the residual error. Regression slopes (b) were used to compare uptake efficiency among species, and coefficients of determination (R^2) were used to assess goodness of fit. For each species, 95% confidence intervals (CIs) were calculated for all regression lines to quantify variability.

All analyses were performed using Python 3.11, specifically the libraries *stats models* (Seabold & Perktold, 2010), *pandas* (McKinney, 2010), and *SciPy* (Virtanen *et al*., 2020), ensuring reproducibility and transparency in statistical interpretation.

Results

Tissue partitioning of cadmium and copper

Across all treatments and macrophytes, the partitioning of both Cd and Cu consistently followed the order root > shoot > leaf, indicating that roots acted as the principal sink for metal accumulation (Tables 2a and 2b). This pattern was particularly pronounced at higher exposure levels (300–400 mg kg^{-1}), where roots accumulated disproportionately higher concentrations relative to aerial tissues. Such a distribution reflects the physiological role of roots as the first interface for metal uptake and suggests active sequestration mechanisms at the root–soil boundary.

For cadmium, *Juncellus alopecuroides* showed the maximum root accumulation (174 mg kg^{-1} at 400 mg kg^{-1}), closely followed by *Alternanthera philoxeroides*. Both species demonstrated substantial Cd retention in roots, while translocation to shoots and leaves remained comparatively limited. In *Physalis minima* and *Portulaca oleracea*, Cd accumulation was consistently lower across all tissues, with shoot concentrations particularly suppressed, highlighting their weaker capacity for Cd uptake.

For copper, a similar root-dominant pattern was observed. However, *J. alopecuroides* exhibited an exceptional ability to translocate Cu to shoots, reaching 157 mg kg^{-1} at 400 mg kg^{-1} soil—the highest shoot accumulation recorded among all species. *A. philoxeroides* demonstrated moderate shoot Cu accumulation, while *P. minima* and *P. oleracea* remained poor accumulators across all tissues.

The dose–response relationships depicted in Figs. 1 and 2 confirm that Cd and Cu uptake increased in proportion to soil concentrations. Importantly, the slopes of the regression lines varied significantly among species, indicating differences in uptake efficiency and tolerance strategies.

Cadmium (Fig. 1)

J. alopecuroides and *A. philoxeroides* exhibited the steepest slopes, reflecting superior uptake and partial transfer to aerial

Table 2a: Cadmium (Cd) uptake (mg kg^{-1}) in roots, shoots, and leaves of selected wetland associated plant species across graded soil concentrations (50–400 mg kg^{-1}).

Species	*Concentration (mg kg^-1)*	*Root (mg kg^{-1})*	*Shoot (mg kg^{-1})*	*Leaves (mg kg^{-1})*	*Soil (mg kg^-1)*
Alternanthera philoxeroides	50	16.5	14.0	4.4	15.1
	100	32.0	13.5	9.0	45.5
	200	71.0	27.3	20.0	81.7
	300	102.0	44.0	30.0	124.0
	400	140.0	60.0	38.5	161.5
Juncellus alopecuroides	50	11.0	5.4	3.3	30.3
	100	43.0	23.0	17.0	17.0
	200	98.0	44.0	28.0	30.0
	300	132.0	61.0	39.0	68.0
	400	174.0	77.0	50.0	99.0
Physalis minima	50	16.2	6.4	3.3	24.1
	100	27.0	11.0	5.3	56.7
	200	59.0	22.0	13.0	106.0
	300	91.0	38.0	19.5	151.5
	400	118.0	54.0	27.0	201.0
Portulaca oleracea	50	10.0	4.2	2.2	33.6
	100	22.0	8.4	4.3	65.3
	200	49.0	16.0	10.0	125.0
	300	75.0	27.0	16.5	181.5
	400	101.0	38.0	22.0	239.0

tissues. In contrast, *P. minima* and *P. oleracea* showed shallow slopes, underscoring their limited Cd uptake. The widening of 95% confidence intervals at higher concentrations indicates greater variability in uptake under elevated Cd stress.

Data points represent observed values, while solid lines represent predicted regression fits for each species, enabling interpretation of slopes and dose-response relationships.

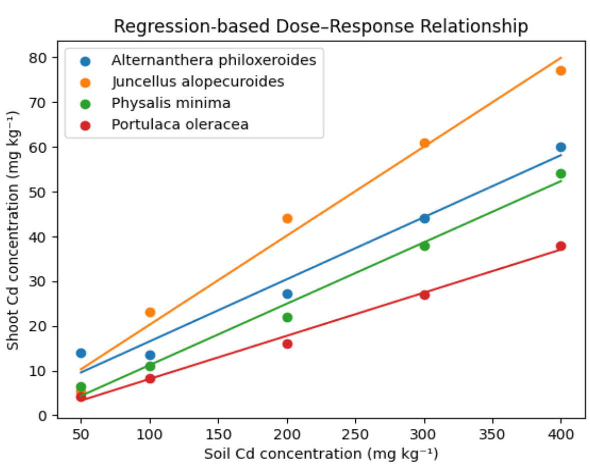

Fig. 1: Relationship between soil Cd concentration and shoot Cd accumulation in selected wetland-associated plant species.

Copper (Fig. 2)

Shoot Cu accumulation displayed strong linearity across all species. *J. alopecuroides* again exhibited the steepest slope, suggesting efficient Cu extraction potential. *A. philoxeroides* displayed moderate but stable uptake, while *P. minima* and *P. oleracea* exhibited flatter slopes, reflecting limited extraction capability. Narrow confidence intervals across most

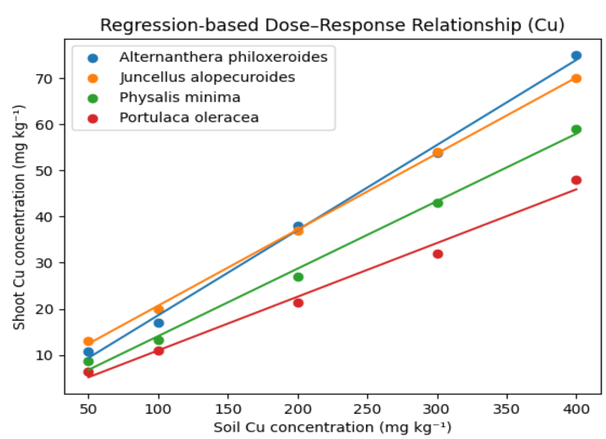

Fig. 2: Relationship between soil Cu concentration and shoot Cu accumulation in selected wetland-associated plant species.

Table 2b: Copper (Cu) uptake (mg kg^{-1}) in roots, shoots, and leaves of selected wetland associated plant species across graded soil concentrations (50–400 mg kg^{-1})

Species	*Concentration (mg kg^-1)*	*Root (mg kg^{-1})*	*Shoot (mg kg^{-1})*	*Leaves (mg kg^{-1})*	*Soil (mg kg^-1)*
Alternanthera philoxeroides	50	21.9	10.7	8.0	3.4
	100	38.0	17.0	11.2	33.8
	200	81.0	38.0	22.0	59.0
	300	118.0	53.7	30.8	97.5
	400	150.1	75.0	43.1	131.8
Juncellus alopecuroides	50	27.0	13.0	8.5	1.5
	100	39.0	20.0	13.4	27.6
	200	81.0	37.0	20.0	62.0
	300	116.0	54.0	33.0	97.0
	400	157.0	70.0	43.9	372.1
Physalis minima	50	20.6	8.6	5.2	15.6
	100	31.0	13.2	9.0	46.8
	200	68.0	27.0	16.4	88.6
	300	107.0	43.0	22.0	128.0
	400	143.0	59.0	32.0	166.0
Portulaca oleracea	50	15.0	6.5	3.2	25.3
	100	26.0	11.0	5.4	57.6
	200	57.0	21.4	13.0	108.6
	300	85.0	32.0	19.3	163.7
	400	172.0	48.0	27.0	153.0

concentration ranges underscored the reliability of these uptake responses.

Data points represent observed values, and solid lines represent predicted regression fits for each species, enabling clear interpretation of slope and dose–response relationships.

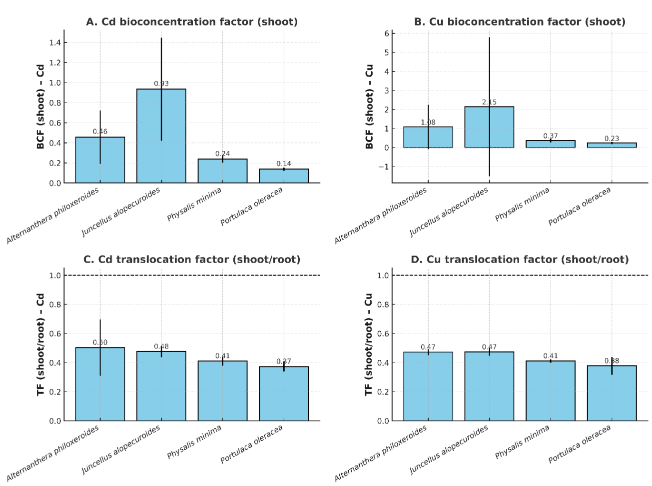

Fig. 3: Bioconcentration factor (BCF; shoot) and translocation factor (TF; shoot/root) for cadmium (Cd) and copper (Cu) in four wetland associated plant species. (A) BCF – Cd; (B) BCF – Cu; (C) TF – Cd; (D) TF – Cu. Error bars represent mean ± standard deviation (SD).

Bioconcentration Factors (BCF)

BCF values provided a quantitative measure of uptake efficiency (Table 3). For Cd, root BCF values frequently exceeded 3 in *A. philoxeroides* and *J. alopecuroides*, indicating their strong ability to accumulate Cd relative to soil concentrations. However, shoot BCF values across all species remained ≤ 1, highlighting the limited potential of these macrophytes as Cd phytoextractors (Fig. 3A). This suggests that while Cd is readily absorbed, its upward translocation is biologically restricted, reflecting a root sequestration–dominated stabilization mechanism.

In contrast, Cu exhibited greater mobility within plant tissues. *J. alopecuroides* achieved the highest shoot BCF values (≈2.15), surpassing the phytoextraction threshold and confirming its potential as a Cu phytoextractor. The relatively higher standard deviation observed in *J. alopecuroides*, particularly for Cu BCF values, indicates greater variability in metal uptake under increasing concentration levels. This variability may be attributed to species-specific physiological responses, differential root absorption efficiency, and possible saturation effects at higher metal concentrations. Such variability suggests that while *J. alopecuroides* exhibits strong phytoextraction potential, its uptake response may be more sensitive to environmental and experimental conditions compared to other species. *A. philoxeroides* also demonstrated moderate shoot BCF values (~1.5), suggesting versatility in Cu uptake. Conversely, *P. minima* and *P. oleracea* consistently exhibited shoot BCF < 1, reflecting

Table 3: Bioconcentration factor (BCF) and translocation factor (TF) (mean ± SD) for cadmium (Cd) and copper (Cu) in selected wetland-associated plant species.

Macrophyte species	*Cd BCF (Shoot) (mean ± SD)*	*Cd TF (Shoot/Root) (mean ± SD)*	*Cu BCF (Shoot) (mean ± SD)*	*Cu TF (Shoot/ Root) (mean ± SD)*
Alternanthera philoxeroides	0.46 ± 0.26	0.50 ± 0.19	1.08 ± 1.16	0.47 ± 0.02
Juncellus alopecuroides	0.93 ± 0.51	0.48 ± 0.04	2.15 ± 3.65	0.47 ± 0.03
Physalis minima	0.24 ± 0.03	0.41 ± 0.03	0.37 ± 0.11	0.41 ± 0.01
Portulaca oleracea	0.14 ± 0.02	0.37 ± 0.03	0.23 ± 0.05	0.38 ± 0.06

Table 4: Regression slopes and R^2 (tissue vs. soil concentration)

Species	*Metal*	*Tissue*	*Slope (mg kg^{-1} per mg kg^-1 soil)*	*Intercept*	*R^2*
Alternanthera philoxeroides	Cd	Root	0.8557	-0.917	0.992
Juncellus alopecuroides			1.7035	8.369	0.77
Physalis minima			0.5992	-2.391	0.993
Portulaca oleracea			0.4469	-6.196	0.999
Alternanthera philoxeroides	Cd	Shoot	0.3337	3.206	0.953
Juncellus alopecuroides			0.7323	6.299	0.749
Physalis minima			0.2744	-3.321	0.981
Portulaca oleracea			0.1644	-2.471	0.991
Alternanthera philoxeroides	Cu	Leaves	0.2804	4.766	0.978
Juncellus alopecuroides			0.0875	13.958	0.812
Physalis minima			0.1747	1.371	0.984
Portulaca oleracea			0.1533	-2.001	0.867
Alternanthera philoxeroides	Cu	Root	1.0463	13.687	0.984
Juncellus alopecuroides			0.3205	48.089	0.792
Physalis minima			0.8439	-1.187	0.983
Portulaca oleracea			0.8539	-15.794	0.662
Alternanthera philoxeroides	Cu	Shoot	0.5152	5.339	0.979
Juncellus alopecuroides			0.1384	23.295	0.772
Physalis minima			0.3435	-0.408	0.98
Portulaca oleracea			0.253	-1.937	0.816
Alternanthera philoxeroides	Cd	Leaves	0.2407	-0.218	0.994
Juncellus alopecuroides			0.4619	4.894	0.735
Physalis minima			0.1382	-1.287	0.991
Portulaca oleracea			0.0988	-1.731	0.996

their poor efficiency in Cu translocation and confirming their limited remediation role (Fig. 3B).

Translocation Factors (TF)

Translocation factors (TF), representing shoot-to-root ratios, were consistently < 1 for both metals across all species and concentrations (Table 3). This confirms that metals were largely retained in root tissues, with limited upward movement into shoots and leaves.

Cadmium TF values ranged from 0.37 to 0.50, underscoring strong root sequestration and minimal shoot allocation (Fig. 3C). This pattern highlights the phytostabilization potential of the tested species, particularly *A. philoxeroides* and *J. alopecuroides*.

Copper TF values also remained below unity but were comparatively higher in *J. alopecuroides* and moderately elevated in *A. philoxeroides*, reflecting a greater physiological mobility of Cu (Fig. 3D). This suggests that Cu, being an essential micronutrient, is translocated more effectively within plant tissues than Cd.

Taken together, these results emphasize distinct species-specific strategies: *J. alopecuroides* combines high BCF and moderate TF values for Cu, making it a strong phytoextractor,

Table 5: Comparative bioconcentration factor (BCF), translocation factor (TF), and remediation roles of four wetland associated plant species.

Species	*Cd BCF (Root)*	*Cd TF*	*Cd Role*	*Cu BCF (Shoot)*	*Cu TF*	*Cu Role*
Alternanthera philoxeroides	>3	<1 (0.37-0.50)	Strong Phytostabilizer	~1.5	<1 (Moderate)	Moderate Extractor
Juncellus alopecuroides	>3	<1 (0.37-0.50)	Strong Phytostabilizer	>2	<1 (Higher)	Strong Extractor
Physalis minima	<2	<1	Weak Stabilizer	<1	<1 (Low)	Limited Role
Portulaca oleracea	<2	<1	Weak Stabilizer	<1	<1 (Low)	Limited Role

while *A. philoxeroides* acts primarily as a stabilizer for Cd but demonstrates moderate Cu extraction ability. *P. minima* and *P. oleracea* remain poor performers, functioning more as stabilizers than extractors.

Dose–response relationships

Linear regression models showed strong dose–response relationships between soil metal concentrations and tissue accumulation (Table 4). Coefficients of determination (R^2) ranged between 0.95 and 0.99, indicating highly predictable uptake dynamics across the tested gradient.

For Cd, the BCF (shoot) values (Fig. 3A) were modest across all species, with *J. alopecuroides* and *A. philoxeroides* displayed steeper slopes in shoots, confirming their higher Cd accumulation efficiency. In contrast, *P. minima* and *P. oleracea* recorded shallow slopes, indicative of weaker uptake ability.

In contrast, Cu displayed a distinct behaviour. *J. alopecuroides* exhibited the steepest slope in shoots, aligning with its high BCF values and confirming its superior Cu extraction capacity. *A. philoxeroides* maintained moderate slopes, while *P. minima* and *P. oleracea* again demonstrated flatter slopes, reflecting limited extraction efficiency and higher sensitivity to Cu stress.

These results not only underscore interspecific differences but also highlight the reliability of regression models in predicting metal uptake potential under increasing contamination loads.

Statistical analysis

Two-way ANOVA (Species × Concentration, additive model) indicated statistically significant main effects of both species and concentration ($p < 0.05$) on Cd and Cu accumulation across roots, shoots, and leaves. Metal concentration exerted the strongest influence, confirming dose-dependent uptake trends across all species.

Species effects were also highly significant. *J. alopecuroides* consistently outperformed all other macrophytes in Cu uptake, particularly in shoots, while *A. philoxeroides* excelled in Cd sequestration within roots. Post-hoc Tukey's HSD comparisons reinforced these differences, grouping *J. alopecuroides* and *A. philoxeroides* as high-performing species distinct from the lower-performing *P. minima* and *P. oleracea*.

Integrated interpretation of results

Overall, the results reveal clear species-specific remediation strategies. Cd uptake was predominantly restricted to roots, with *A. philoxeroides* and *J. alopecuroides* functioning as effective phytostabilizers. In contrast, Cu demonstrated greater physiological mobility, with *J. alopecuroides* emerging as a promising phytoextractor due to its elevated shoot BCF values (>2), steeper regression slopes, and moderately higher TF values compared to other species. *A. philoxeroides* displayed intermediate performance for Cu, while *P. minima* and *P. oleracea* exhibited consistently weak accumulation across both metals.

The Table 5 consolidates the BCF, TF, and species-specific remediation roles, reinforcing the uptake and partitioning trends presented in Tables 2–5 and Figs. 1–3. Both *Alternanthera philoxeroides* and *Juncellus alopecuroides* consistently exhibited high root BCF (>3) for Cd with TF values < 1, validating the root-dominant accumulation patterns illustrated in Figs. 1 and 3c. This demonstrates their role as effective Cd phytostabilizers, in agreement with regression slopes that showed strong but root-limited uptake responses. In contrast, *J. alopecuroides* showed markedly different behaviour for Cu, with shoot BCF > 2 and comparatively higher TF values (Fig. 3D), aligning with the steep regression slopes observed in Fig. 2. This confirms its dual strategy: stabilizing Cd while efficiently extracting Cu into harvestable shoots. *A. philoxeroides* exhibited intermediate Cu accumulation (shoot BCF ~1.5), which corroborates its role as a moderate extractor, as suggested by its linear but less steep regression trends. In contrast, *Physalis minima* and *Portulaca oleracea* consistently recorded low BCF (<2) and TF < 1, in agreement with their flatter regression slopes and limited accumulation across tissues. Their contribution is therefore restricted to weak stabilization, with minimal practical remediation potential. Collectively, these results and the synthesized table emphasize a functional divergence among species: robust Cd stabilization by *A. philoxeroides* and *J. alopecuroides*, strong Cu extraction by *J. alopecuroides*, and only marginal contributions from *P. minima* and *P. oleracea*.

These findings underscore the importance of remediation strategies to specific plant traits and metal types, with *J. alopecuroides* serving as a candidate for Cu phytoextraction and *A. philoxeroides* for Cd phytostabilization.

Discussion

The consistent partitioning of both Cd and Cu in the order root > shoot > leaf across all four macrophytes highlights the critical role of roots as the primary site of metal sequestration. This pattern aligns with widely reported trends in aquatic and wetland species, where rhizodermal and cortical tissues act as the first barrier to heavy metal uptake and translocation (Mishra *et al*., 2008; Zhao *et al*., 2015). The exceptionally high root BCF values (>3) observed in *Alternanthera philoxeroides* and *Juncellus alopecuroides* confirm their ability to immobilize Cd efficiently within the rhizosphere, thereby limiting its bioavailability to aboveground parts. Phytostabilization provides dual benefits by reducing the risk of metal leaching into surrounding water systems and limiting trophic transfer through confinement of metals within belowground biomass. The consistently low translocation factor (TF < 1) observed for Cd further supports this stabilization mechanism, indicating minimal upward movement. At the physiological level, Cd retention is typically achieved through cell wall binding, precipitation as insoluble complexes, or vacuolar sequestration mediated by phytochelatins and metallothioneins (Sharma & Dubey, 2005; Khan *et al*., 2024; Munyai & Dalu, 2023). These mechanisms prevent Cd from interfering with photosynthetic tissues and reduce oxidative stress in shoots, thereby validating the potential of *A. philoxeroides* and *J. alopecuroides* as effective phytostabilizers in Cd-contaminated environments.

In contrast, Cu exhibited comparatively higher mobility within plant tissues, particularly in *J. alopecuroides*, which achieved the highest shoot BCF values (≈2.15), surpassing the phytoextraction threshold and confirming its potential as a Cu phytoextractor. The relatively higher standard deviation observed in *J. alopecuroides*, particularly for Cu BCF values, indicates greater variability in metal uptake under increasing concentration levels. This variability may be attributed to species-specific physiological responses, differential root absorption efficiency, and possible saturation effects at higher metal concentrations. Such variability suggests that while *J. alopecuroides* exhibits strong phytoextraction potential, its uptake response may be more sensitive to environmental and experimental conditions compared to other species. *Alternanthera philoxeroides* also demonstrated moderate shoot BCF values (~1.5), reflecting its versatility and adaptability across contaminants. Conversely, *Physalis minima* and *Portulaca oleracea* consistently exhibited shoot BCF < 1, indicating limited translocation efficiency and confirming their reduced suitability for active phytoextraction.

One of the most significant outcomes of this study is the strong linear relationship observed between soil metal concentrations and plant tissue accumulation, with coefficients of determination (R^2) exceeding 0.95 across most species–metal combinations. Such predictable dose - response relationships are critical for scaling phytoremediation strategies from laboratory to field conditions, as they allow estimation of metal removal rates and remediation timelines under varying contamination levels (Yoon *et al*., 2006). Notably, *J. alopecuroides* displayed the steepest regression slopes for Cu, indicating superior uptake efficiency under higher contamination levels. In contrast, the relatively shallow slopes observed in *Physalis minima* and *Portulaca oleracea* further reinforce their limited role in remediation under high contamination scenarios. The widening confidence intervals at elevated concentrations suggest increased variability and physiological stress under extreme exposure conditions, highlighting the need for caution when extrapolating laboratory findings to field applications.

The present findings are consistent with established literature, which generally identifies Cd as more suitable for phytostabilization and Cu as more amenable to phytoextraction processes (Yan *et al*., 2020; Bhat *et al*., 2022; Pang *et al*., 2023). The strong Cd retention observed in *A. philoxeroides* and *J. alopecuroides* corroborates earlier studies demonstrating root-dominant Cd sequestration in wetland associated plant species (Mishra *et al*., 2008; Zhao *et al*., 2015). Similarly, the high Cu accumulation in *J. alopecuroides* supports emerging evidence that members of the Cyperaceae family are effective yet underutilized phytoremediators (Emenike *et al*., 2018). However, the ecological implications of using invasive species such as *A. philoxeroides* must be carefully considered, as their uncontrolled proliferation may adversely affect native biodiversity (Shen *et al*., 2022; Razzak *et al*., 2022)

From an applied perspective, the results highlight distinct remediation pathways for contaminated environments. In Cd-contaminated systems, the strong root sequestration capacity of *A. philoxeroides* and *J. alopecuroides* supports their use in phytostabilization strategies, particularly in wetlands and riparian buffer zones where immobilization reduces metal mobility and leaching (Ali *et al*., 2013). In Cu-contaminated sites, the superior phytoextraction efficiency of *J. alopecuroides* makes it a suitable candidate for biomass-based removal systems, where periodic harvesting can effectively reduce metal loads (Yoon *et al*., 2006). *Alternanthera philoxeroides* may serve as a complementary species in multi-species remediation systems. The consistently low TF values observed across species also reduce the risk of metal transfer into aboveground biomass consumed by herbivores, thereby minimizing food-chain contamination. However, harvested biomass must be managed carefully, preferably through controlled disposal methods such as incineration, biochar production, or bioenergy recovery, to prevent secondary pollution (Bayuo *et al*., 2024).

Despite the clear outcomes observed under controlled conditions, certain limitations must be acknowledged. Pot experiments do not fully replicate field conditions, where factors such as soil heterogeneity, microbial activity, pH fluctuations, and redox dynamics may influence metal bioavailability and plant uptake. Additionally, the long-term ecological risks associated with the use of invasive species must be carefully evaluated. These limitations highlight the need for cautious interpretation and application of the findings under real-world conditions.

Conclusion

The present study demonstrated clear species-specific phytoremediation responses among selected wetland associated plant species under cadmium (Cd) and copper (Cu) contamination. A consistent accumulation pattern (root > shoot > leaf) across all species highlighted the dominant role of roots in metal sequestration. *Alternanthera philoxeroides* and *Juncellus alopecuroides* exhibited strong Cd phytostabilization potential, characterized by high root accumulation and limited translocation, thereby reducing the risk of metal mobility and trophic transfer. In contrast, *J. alopecuroides* showed superior performance for Cu phytoextraction, with higher leaf accumulation and efficient uptake across increasing concentrations, while *A. philoxeroides* demonstrated moderate extraction ability.

Overall, the findings emphasize the importance of selecting appropriate macrophyte species based on the target metal and remediation objective. *J. alopecuroides* emerges as a promising candidate for integrated phytoremediation strategies, offering potential for both stabilization and extraction under varying contamination conditions. The application of such species in wetlands and contaminated sites can contribute to sustainable and eco-friendly management of heavy metal pollution.

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