Jaisha Batool, 2026, 14:5
ISSN (Online): 2348-4098
ISSN (Print): 2395-4752

International Journal of Science, Engineering and Technology
An Open Access Journal

Comparative Physiological Responses and Burden-Based Root–Stem Partitioning of Five Metals in *Eichhornia Crassipes* Under Citric-Acid-Assisted Hydroponic Exposure

Jaisha Batool[1†*], Eman Waheed[2†*], Eman Fatima[3], Syeda Mahin Ahmed[4]

[1,2]school Of Environment And Resources, Southwest University Of Science And Technology, Mianyang 621010, P.R. China.
[3]department Of Physics, University Of Gujrat, Gujrat, Pakistan
[4]department Of Environmental Sciences, Fatima Jinnah Women University, Rawalpindi, Pakistan
Corresponding Authors *: [1]. Jaisha Batool (Jaishabatool324@Gmail.Com
[2]. Eman Waheed (Eman.Waheed.6991@Gmail.Com
† These Authors Contributed Equally To This Work.

Abstract- Heavy-metal contamination of freshwater ecosystems has increased interest in aquatic plants for metal retention and phytoremediation. This study examined the physiological responses and root–stem partitioning of nickel (Ni), cadmium (Cd), lead (Pb), zinc (Zn), and copper (Cu) in water hyacinth (Eichhornia crassipes (Mart.) Solms) under hydroponic conditions. Plants received dosing solutions containing 100, 250, or 500 µM of each metal salt supplemented with 4.57 mM citric acid over four weeks. Untreated and fertilizer-only plants were included as reference treatments. Vegetative dimensions, tissue fresh and dry masses, dry-matter content, chlorophyll content index, toxicity symptoms, root and stem–petiole metal concentrations, and organ-specific metal burdens were assessed. Responses varied among metals and dosing levels. Lead and cadmium were associated with the clearest reductions in aerial growth and more evident toxicity symptoms at higher dosing levels. Chlorophyll content index declined by 49.0% for Ni and 43.6% for Cd between 100 and 500 µM, whereas Zn and Cu showed non-linear responses. At 500 µM, root Cu and Pb concentrations reached 859.74 and 430.76 mg kg^{-1} dry weight, respectively. Mean root allocation of measured metal burden was 99.7% for Cu, 98.8% for Pb, 92.6% for Zn, 78.0% for Ni, and 69.4% for Cd. Cadmium showed the greatest mean stem–petiole allocation (30.6%), although its burden remained root-dominant. The findings highlight metal-specific physiological patterns and predominantly root-associated metal partitioning, particularly for Cu and Pb, and indicate the relevance of water hyacinth to root-focused metal retention in phytoremediation systems, supporting freshwater environmental management strategies.

Keywords- Eichhornia crassipes; heavy metals; phytoremediation; rhizofiltration; root–stem partitioning

I. INTRODUCTION

The discharge of potentially toxic metals into freshwater environments through industrialization, urbanization, mining, electroplating, battery and fertilizer manufacturing, and untreated wastewater discharge has become a major environmental and public health concern [1]. Metals such as nickel (Ni), cadmium (Cd), lead (Pb), zinc (Zn), and copper (Cu) are persistent, may bioaccumulate, and cannot be degraded into harmless products. They may therefore remain for extended periods in water, sediments, and biological tissues,

creating long-term ecological and human-health risks [1-3]. Conventional treatment technologies, including chemical precipitation, ion exchange, membrane filtration, adsorption, electrochemical treatment, coagulation, and advanced oxidation, can remove metals from wastewater but may be limited by high costs, energy requirements, maintenance demands, sludge generation, and secondary pollution [4]. Phytoremediation has consequently attracted attention

© 2026 Jaisha Batool, This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

as an environmentally friendly and potentially low-cost approach for removing, retaining, or stabilizing metals in aquatic environments [2].

Water hyacinth (*Eichhornia crassipes*) is a fast-growing, free-floating aquatic macrophyte characterized by high biomass production and a dense fibrous root system [5]. Its extensive root surface facilitates the adsorption of metals onto root surfaces and their absorption and retention within plant tissues, although the species is also regarded as a noxious aquatic weed in many regions [5, 6]. A previous review reported removal exceeding 70% for metals including Cu, Pb, Hg, Cd, Al, and As under particular treatment conditions [5], while maximum Zn removal values of 88.3 ± 0.6% and 93.4 ± 0.4% were reported for electroplating wastewater and aqueous solution, respectively [7]. Low translocation factors from roots to leaves have also been observed for Cd, Cu, Pb, and Zn, indicating that water hyacinth may be particularly relevant to rhizofiltration and root-associated metal retention [6, 8].

However, metal-remediation performance is not determined by plant species alone. It is also governed by metal identity, metal availability, dosing level, exposure duration, physiological tolerance, and distribution among plant tissues [2]. Excessive metal availability can impair vegetative development, tissue-mass maintenance, photosynthetic pigments, and cellular redox regulation [9]. Cd and Pb are non-essential metals with recognized phytotoxicity, whereas Ni, Zn, and Cu are required at low availability but may become toxic at excessive levels. Previous investigations of Cd- and Pb-treated water hyacinth have therefore combined tissue-metal measurements with biomass, root development, chlorophyll status, antioxidant responses, and gene expression [10]. Metal behavior may also be modified by chelating compounds. Citric acid can form soluble metal complexes and alter metal mobility and availability, potentially increasing plant–metal interactions while also intensifying phytotoxicity or changing root–stem distribution when mobilization becomes excessive [11].

Despite extensive research on *E. crassipes*, direct comparisons of several metals under a common hydroponic framework remain limited [5, 6]. Previous studies have frequently focused on individual contaminants, specific wastewater sources, or overall removal performance, making it difficult to distinguish how metal identity corresponds with plant physiological responses and internal partitioning [7, 8, 10]. Comparative information is particularly limited when final vegetative measurements, tissue mass and dry-matter content, chlorophyll status, visible toxicity symptoms, tissue-metal concentrations, organ-specific burdens, and root–stem partitioning are evaluated together. This distinction is important because tissue concentration describes metal content per unit dry mass, whereas organ burden incorporates both tissue concentration and organ mass. Evaluating both indices may therefore provide a more complete description of metal distribution within aquatic macrophytes.

The principal contribution of this study is a common-framework comparison of five metals that integrates physiological responses with both concentration-based and burden-based root–stem partitioning, thereby distinguishing tissue enrichment from total organ allocation under citric-acid-assisted conditions. Accordingly, this study examined whether metal identity and dosing-solution level corresponded with distinct physiological and organ-partitioning patterns in water hyacinth. Three biologically grounded expectations guided the analysis. First, the non-essential metals Cd and Pb were expected to show clearer physiological impairment than the essential micronutrients Ni, Zn, and Cu. Second, root–stem metal distribution was expected to differ among metals because of differences in mobility, tissue binding, and internal transport. Third, increasing dosing-solution levels were expected to correspond with reduced plant performance, although non-monotonic responses could occur for essential micronutrients because of their concentration-dependent physiological roles. These expectations were assessed through structured comparisons of final vegetative measurements, tissue dry-matter content, chlorophyll status, visible toxicity symptoms, tissue-metal concentrations, organ-specific burdens, and root–stem partitioning indices.

1. Materials and Methods

2. Plant material and experimental conditions

Mature and visibly healthy plants of *Eichhornia crassipes* (Mart.) Solms were collected from the bioremediation ponds of the National Agricultural Research Centre, Islamabad, Pakistan. The plants were thoroughly rinsed with tap water to remove adhering sediment, debris,

insects, and other visible surface materials before being transferred to the greenhouse of Fatima Jinnah Women University, Rawalpindi, Pakistan. The experiment was conducted during May and June 2025 under natural greenhouse light and ventilation. The water temperature during the experimental period was approximately 25–27 °C. The pH values of both the source pond water and the greenhouse tap water were 6.7, as measured using an HI9811-5 multiparameter meter (Hanna Instruments). Before treatment application, the pond water and tap water were screened for background concentrations of Ni, Cd, Pb, Zn and Cu using an AA-7000 flame atomic absorption spectrophotometer (Shimadzu, Kyoto, Japan). Preliminary FAAS screening of the source pond water and greenhouse tap water showed no measurable signals for Ni, Cd, Pb, Zn or Cu under the analytical conditions used.



Figure. 1 Experimental workflow for hydroponic metal treatment of *Eichhornia crassipes*

1. **Experimental design**

Each experimental unit consisted of one pot containing one plant and 3 L of tap water before the first treatment application. The experiment comprised 17 experimental units: 15 metal-treated units representing five metals at three dosing-solution levels, one untreated control, and one fertilizer-only reference. Each treatment was represented by one experimental unit, and pot positions were not changed during the experimental period. Nickel (Ni), cadmium (Cd), lead (Pb), zinc (Zn), and copper (Cu) were tested separately using weekly 1 L dosing solutions containing 100, 250, or 500 µ*M* of the corresponding metal salt. All metal dosing solutions also contained nominally 4.57 m*M* citric acid. The untreated control received no added metal, citric acid, or fertilizer. A fertilizer-only treatment was included as a nutrient-supported, non-metal reference. This treatment received 5 g of commercial diammonium phosphate fertilizer (Sona DAP, Fauji Fertilizer Company, Pakistan) in 1 L of water during each weekly application. Four applications were made over the experimental period, corresponding to a nominal cumulative DAP addition of 20 g per pot. Because no combined fertilizer-plus-metal treatment was included, the fertilizer-only reference was not used to evaluate whether DAP alleviated metal-associated toxicity or altered tissue-metal concentrations.

Final vegetative dimensions, fresh and dry tissue masses, tissue dry-matter content, chlorophyll status, visible toxicity symptoms, root and stem–petiole metal concentrations, organ-specific metal burdens, and root–stem partitioning indices were selected as response variables. Comparable physiological and tissue-metal endpoints have previously been used to characterize the responses of water hyacinth to Cd and Pb stress [10].

II. PREPARATION OF METAL DOSING SOLUTIONS

Metal dosing solutions were prepared separately using anhydrous nickel chloride ($NiCl_2$), cadmium chloride ($CdCl_2$), lead nitrate [$Pb(NO_3)_2$], zinc sulfate ($ZnSO_4$) and copper sulfate ($CuSO_4$). The corresponding molar masses used in the solution-preparation calculations were: 129.6 g mol^{-1} for $NiCl_2$, 183.3 g mol^{-1} for $CdCl_2$, 331.2 g mol^{-1} for $Pb(NO_3)_2$, 161.4 g mol^{-1} for $ZnSO_4$ and 159.6 g mol^{-1} for $CuSO_4$. For each metal, 1 L dosing solutions containing 100, 250 or 500 µ*M* of the corresponding metal salt were prepared in distilled water. The required mass of each salt was calculated using Eq. (1).

m=CVM #1

where *m* is the required mass of the salt (g), *C* is the target molar concentration in mol L^{-1}, *V* is the solution volume in liters, and *M* is the molar mass of the salt in g mol^{-1}.

The masses used to prepare the 100, 250 and 500 µ*M* dosing solutions, respectively, were:

1. 0.01296, 0.03240 and 0.06480 g $NiCl_2$;

2. 0.01833, 0.04583 and 0.09165 g $CdCl_2$;

3. 0.03312, 0.08280 and 0.16560 g $Pb(NO_3)_2$;

4. 0.01614, 0.04035 and 0.08070 g $ZnSO_4$; and

5. 0.01596, 0.03990 and 0.07980 g $CuSO_4$.

Citric acid monohydrate ($C_6H_{10}O_8$) was added to each 1 L metal dosing solution at 0.96 g L^{-1}. This mass corresponded to a nominal concentration of approximately 4.57 m*M*. Citric acid was included as a biodegradable chelating agent capable of modifying metal complexation and availability in phytoremediation systems [11]. Its use as an assisting ligand has also been investigated for Pb phytoremediation by water hyacinth and other floating aquatic macrophytes in industrial effluent [12].

The treatment groups were arranged as follows:

Table 1. Experimental treatments and weekly dosing conditions.

Treatment group	Treatment description
Control group	1 L tap water
Fertilizer	5 g DAP with 1 L water
Ni treatments	1 L containing 100, 250, or 500 µM Ni salt + 4.57 mM citric acid
Cd treatments	1 L containing 100, 250, or 500 µM Cd salt + 4.57 mM citric acid
Pb treatments	1 L containing 100, 250, or 500 µM Pb salt + 4.57 mM citric acid
Zn treatments	1 L containing 100, 250, or 500 µM Zn salt + 4.57 mM citric acid
Cu treatments	1 L containing 100, 250, or 500 µM Cu salt + 4.57 mM citric acid

III. WEEKLY TREATMENT APPLICATION

The first treatment application was made on day 0. One liter of the assigned metal dosing solution was added to the 3 L of tap water initially present in each pot, producing an approximate total hydroponic volume of 4 L. Three additional 1 L applications of the corresponding dosing solution were made on days 7, 14, and 21. Each experimental unit therefore received four treatment applications during the four-week exposure period. The existing hydroponic medium was not removed before subsequent applications. Under the greenhouse conditions, approximately 1 L of water was lost between weekly applications through evaporation and plant transpiration.

This water loss was not replaced separately; instead, each weekly 1 L treatment application restored the hydroponic volume to approximately 4 L. The volume immediately before each application was estimated visually rather than measured volumetrically.

The untreated control received 1 L of tap water at the same weekly intervals as the metal-treated pots. The fertilizer-only reference received 5 g of DAP in 1 L of water during each corresponding application. No existing hydroponic medium was removed from the untreated control, fertilizer-only reference, or metal-treated pots before the weekly additions.

The treatment levels of 100, 250, and 500 µ*M* refer to the concentrations of the weekly 1 L dosing solutions rather than to constant metal concentrations maintained in the complete hydroponic medium.

Following the first addition to the initial 3 L of tap water, the theoretical nominal metal concentrations in the approximately 4 L pot medium were 25, 62.5, and 125 µ*M*, respectively. Assuming that the pot volume returned to approximately 4 L before each application and that all added metal remained within the pot–plant system, the total metal added over four applications was equivalent to nominal cumulative loadings of 100, 250, and 500 µ*M* when expressed relative to a 4 L pot volume.

Similarly, the nominal citric-acid concentration in the complete pot medium was approximately 1.14 m*M* after the first application, while the cumulative quantity added over four applications was equivalent to 4.57 m*M* relative to an assumed 4 L volume. These values represent theoretical nominal loadings rather than measured dissolved concentrations. Actual dissolved-metal and citric-acid concentrations were not determined during the exposure period and may have been affected by plant uptake, adsorption to roots and container surfaces, precipitation, complexation, and variation in solution volume.

1. Plant Harvesting And Tissue Separation

Plants were harvested after four weeks of exposure and transported to the laboratory for final vegetative measurements, tissue-mass determination, chlorophyll measurement, and tissue-metal analysis. Visible symptoms of toxicity, including chlorosis, leaf curling, discoloration, browning, wilting, and tissue damage, were recorded at harvest. The development of new leaves was also documented. Each plant was separated into root, leaf, and stem–petiole tissues. Roots and stem–petiole tissues were used for metal analysis, whereas leaves were used for chlorophyll measurement. Morphological and physiological indicators, including tissue mass, root development, chlorophyll status, and visible tissue injury, have previously been used to evaluate water-hyacinth responses to metal exposure [10].

2. Growth And Biomass Measurements

At the end of the exposure period, root length, leaf length, and stem–petiole length were measured in centimeters using a ruler. Fresh masses of the separated root, leaf, and stem–petiole tissues were measured using a digital laboratory balance. For dry-mass determination, root and stem–petiole tissues were first dried under sunlight for 6 h and subsequently oven-dried at 70 °C for 10 h. Root and stem–petiole dry masses were then recorded. Tissue dry-matter content was calculated using Eq. (2).

- Dry matter content %= organ dry mass gorgan fresh mass g ×100#2
- The calculated percentages were expressed as tissue dry-matter content.

3. Chlorophyll Measurement

At the end of the four-week exposure period, leaf chlorophyll status was measured non-destructively using a CCM-200 Plus chlorophyll content meter (Opti-Sciences, Hudson, NH, USA). Chlorophyll content has been used as an indicator of photosynthetic and physiological responses of water hyacinth to Cd and Pb exposure [10]. One chlorophyll content index reading was obtained from one leaf of each plant at one position on the selected leaf. Because each treatment contained one plant and one chlorophyll reading, each recorded value represented a single treatment-level observation rather than an average of biological or technical replicates.

4. Plant-Tissue Preparation And Acid Digestion

The dried root and stem–petiole tissues were separately ground into fine powders using a mortar and pestle. A portion of each dried tissue powder was transferred to a digestion vessel, and 10 mL of concentrated nitric acid was added to each sample. The samples were left at room temperature for 20 min to allow the initial acid reaction. The samples were then heated on a hot plate at 100–120 °C for 40 min. After cooling, 5 mL of concentrated perchloric acid was added. The samples were reheated initially at 120 °C, after which the temperature was gradually increased to 180–200 °C. Digestion was continued for approximately 30–60 min or until fumes appeared. After cooling, 30 mL of distilled water was added to each digest. The samples were heated again at 150–180 °C until white fumes appeared. The digests were subsequently cooled, filtered, and diluted to a final volume of 50 mL with distilled water.

5. Determination Of Tissue Metal Concentrations

Following digestion, the samples were filtered and diluted to a final volume of 50 mL with distilled water. Concentrations of Ni, Cd, Pb, Zn and Cu in the digested root and stem–petiole samples were determined using an AA-7000 flame atomic absorption spectrophotometer (Shimadzu, Kyoto, Japan).

Tissue metal concentrations were calculated using Eq. (3).

Ctissue=Cdigest×Vfinal×DFmsample #3

where C_{tissue} is the tissue metal concentration in mg kg^{-1} dry weight, C_{digest} is the metal concentration measured in the digest in mg L^{-1}, V_{final} is the final digest volume in liters, DF is any additional dilution factor and m_{sample} is the dry tissue mass in kilograms. All tissue metal concentrations were expressed as mg kg^{-1} DW.

6. Organ-Specific Metal Burden And Partitioning

Organ-specific metal burden was calculated by multiplying the tissue metal concentration by the corresponding organ dry mass, as shown in Eq. (4).

Aorgan=Ctissue×Morgan#4

where A_{organ} is the metal burden in the organ in µg, C_{tissue} is the metal concentration in µg g^{-1} dry weight, and M_{organ} is the organ dry mass in grams. Because 1 mg kg^{-1} is numerically equivalent to 1 µg g^{-1}, tissue concentrations expressed as mg kg^{-1} DW were multiplied directly by organ dry mass in grams.

Root and stem–petiole allocations were calculated from the corresponding organ burdens:

Root allocation %= ArootAroot+Astem-petiole×100 #5

Stem-petiole allocation %= Astem-petioleAroot+Astem-petiole×100 #6

The stem–petiole/root concentration ratio was calculated as:

Stem-petioleroot concentration ratio=Cstem-petioleCroot #7

Organ-specific metal burdens and tissue-concentration ratios were used as complementary descriptive indices of metal partitioning between root and stem–petiole tissues. Previous studies have evaluated organ-specific accumulation and translocation in water hyacinth, including limited transfer of several metals from roots to leaves and separate quantification of metal concentrations in roots, petioles, stolons, and laminae [13]. Burden-based allocation represented the relative total amount of metal present in the two analyzed tissue fractions and was calculated using tissue concentration and corresponding organ dry mass. In contrast, the stem–petiole/root concentration ratio compared metal concentration per unit dry mass. A ratio below 1 indicated a higher concentration in roots, whereas a ratio above 1 indicated a higher concentration in stem–petiole tissue. A concentration ratio above 1 did not necessarily indicate that the total stem–petiole metal burden exceeded the root burden.

IV. DATA ANALYSIS

Data were summarized descriptively to compare vegetative performance, dry-matter content, chlorophyll status, visible toxicity symptoms, tissue metal concentrations, organ-specific metal burdens, and partitioning indices across metals and dosing levels. The analysis focused on treatment-associated patterns and cross-metal comparisons rather than formal hypothesis testing.

Mean Chlorophyll Across Doses

Mean chlorophyll= C100+C250+C5003 #8

High-Dose Chlorophyll Change

Chlorophyll change %= C500-C100C100 ×100 #9

Chlorophyll Response Range

Response range=Cmax-Cmin #10

where C_{100}, C_{250}, and C_{500} represent chlorophyll values at 100, 250, and 500 µ*M*, respectively. C_{max} and C_{min} represent the maximum and minimum chlorophyll values among the three concentrations for each metal. Derived chlorophyll-response indices were calculated from values extracted from the archived graphical record and were used only for descriptive comparison among metals.

V. RESULTS AND DISCUSSION

1. Final vegetative measurements and visible toxicity symptoms

Final vegetative dimensions and fresh tissue masses were recorded after four weeks of treatment and are presented in Table 2.

Vegetative measurements varied among the metal treatments and dosing-solution levels (Table 2), indicating metal-specific patterns in the response of *E. crassipes*. Root length ranged from 33 to 45 cm across the metal treatments and did not show a consistent decline with increasing dosing level. In contrast, stem–petiole and leaf dimensions showed clearer changes, particularly under Cd and Pb treatment. Under Pb exposure, leaf length decreased from 7.0 cm at 100 µ*M* to 3.5 cm at 500 µ*M*, while stem–petiole length decreased from 12.5 to 9.5 cm. Under Cd exposure, leaf length decreased from 7.5 to 5.0 cm, although stem–petiole length did not show a consistent concentration-related decline. Zn- and Cu-treated plants retained comparatively greater root lengths, whereas the fertilizer-only reference showed the greatest root length of 46 cm. In short, final leaf and stem–petiole dimensions showed clearer high-level reductions than root length, particularly under Cd and Pb exposure. Visible symptoms, including chlorosis, leaf curling, browning, wilting, tissue damage, and reduced

formation of new leaves, became more evident at higher dosing-solution levels, especially under Cd and Pb treatment. Similar changes in biomass, root development, chlorophyll status,

Table 2. Final vegetative dimensions and fresh tissue masses of *Eichhornia crassipes* after four weeks of metal treatment.

Heavy Metal	Treatments	Root length (cm)	Root fresh mass (g)	Stem–petiole length (cm)	Stem–petiole fresh mass (g)	Leaf length (cm)	Leaf fresh mass (g)
Nickel	100 µM	40	6.1	17	13.9	8.5	0.97
	250 µM	40	43.7	12.5	10.195	5.5	0.84
	500 µM	39	32.9	13	12.286	8	0.74
Cadmium	100 µM	42	4.3	14	14.236	7.5	0.78
	250 µM	35	27.1	16	8.415	5.5	0.71
	500 µM	40	30.6	14	11.103	5	0.68
Lead	100 µM	34	17.6	12.5	8.55	7	0.96
	250 µM	40	31.3	14	10.291	6	0.74
	500 µM	33	23.6	9.5	8.617	3.5	0.68
Zinc	100 µM	40	10.8	20	9.012	9	0.93
	250 µM	39	11.3	15	9.336	8	0.82
	500 µM	39	25.4	17	9.545	5.5	0.75
Copper	100 µM	45	7.4	16	7.74	8.5	0.72
	250 µM	39	12.5	11	10.536	7	0.79
	500 µM	43	32.7	14.5	7.794	6	0.91
	Control group	34	6.3	13	14.119	5.5	0.93
	Fertilizer	46	23.04	15	2.043	6.5	0.95

antioxidant responses, and metal accumulation have been reported for water hyacinth exposed to Cd and Pb [10]. Heavy-metal stress can disrupt photosynthesis, nutrient uptake, redox balance, cellular metabolism, and detoxification processes, thereby affecting plant development and physiological condition [14].

Previous research on the aquatic macrophyte *Elodea canadensis* showed dose-dependent Ni and Co accumulation and reduced leaf-cell viability under combined metal and elevated-temperature stress [15]. The clearest reductions at the 500 µ*M* dosing level occurred under Cd and Pb treatment, whereas Zn and Cu showed comparatively variable responses at the lower and intermediate levels. This pattern is consistent with the recognized phytotoxicity of the non-essential metals Cd and Pb and with previous reports describing their adverse effects on vegetative development and photosynthetic processes in aquatic plants [16, 17]. Nevertheless, the responses were not uniformly concentration-dependent across all measurements, indicating that plant response was influenced by both metal identity and dosing-solution level.

1. **Tissue dry-matter response under different metal treatments**

Root and stem–petiole dry-matter contents varied among metal types and dosing-solution levels, as presented in Table 3.

Table 3. Root and stem–petiole dry-matter content of *Eichhornia crassipes* across the metal dosing-solution levels.

Treatment	Root dry-matter content (%)	Root dry-matter content (%)	Stem–petiole dry-matter content (%)
100 µM Ni	16.42	16.42	9.02
250 µM Ni	9.23	9.23	8.39
500 µM Ni	8.92	8.92	2.78
100 µM Cd	100 µM Cd	52.33	5.77
250 µM Cd	250 µM Cd	8.31	7.64
500 µM Cd	500 µM Cd	5.83	3.44
100 µM Pb	100 µM Pb	11.02	12.52
250 µM Pb	250 µM Pb	13.11	7.09
500 µM Pb	500 µM Pb	5.87	3.20
100 µM Zn	100 µM Zn	21.40	8.24
250 µM Zn	250 µM Zn	32.91	7.94
500 µM Zn	500 µM Zn	7.79	4.62
100 µM Cu	100 µM Cu	31.24	8.97
250 µM Cu	250 µM Cu	21.69	6.10
500 µM Cu	500 µM Cu	7.43	3.48

In Ni-treated plants, root and stem–petiole dry-matter content decreased progressively with increasing dosing-solution level (Table 3). Root dry-matter content declined from 16.42% at 100 µ*M* to 8.92% at 500 µ*M*, while stem–petiole dry-matter content decreased from 9.02% to 2.78%. Cd also produced lower dry-matter content at the highest dosing level. Cd root dry-matter content decreased from 52.33% at 100 µ*M* to 8.31% at 250 µ*M* and 5.83% at 500 µ*M*. However, stem–petiole dry-matter content showed a non-monotonic response, increasing from 5.77% at 100 µ*M* to 7.64% at 250 µ*M* before declining to 3.44% at 500 µ*M*. Under Pb treatment, root dry-matter content increased slightly from 11.02% at 100 µ*M* to 13.11% at 250 µ*M* and then decreased to 5.87% at 500 µ*M*, whereas stem–petiole dry-matter content declined from 12.52% to 3.20%. Zn showed a different pattern. Root dry-matter content increased from 21.40% at 100 µ*M* to 32.91% at 250 µ*M* before declining sharply to 7.79% at 500 µ*M*. This intermediate-level response may reflect the physiological role of Zn as an essential micronutrient, although excessive Zn availability can become toxic to plants [18]. Cu showed a progressive decline in dry-matter content: root dry-matter content decreased from 31.24% at 100 µ*M* to 7.43% at 500 µ*M*, while stem–petiole dry-matter content decreased from 8.97% to 3.48%. In short, dry-matter content was generally lower at the 500 µ*M* dosing level, although the responses were not uniformly concentration-dependent across all metals. Because dry-matter content represents the proportion of dry material in fresh tissue, it should be interpreted together with the actual fresh and dry tissue masses presented in Figure 2. These tissue-mass measurements are relevant to phytoremediation because total organ metal burden depends on both tissue-metal concentration and organ dry mass [2].

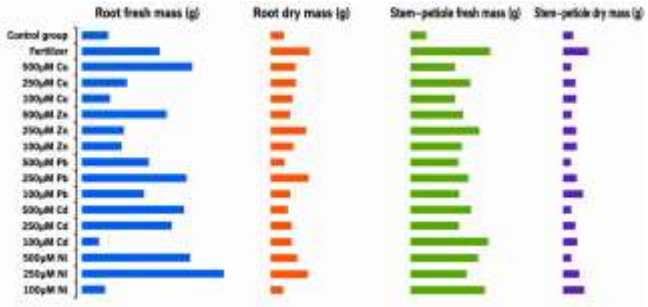


Figure. 2 Fresh and dry masses of root and stem–petiole tissues of *Eichhornia crassipes* after different metal treatments

Root and stem–petiole fresh and dry masses varied among metals and dosing-solution levels (Figure 2), indicating metal-specific differences in tissue production. Several treatments showed lower dry masses at the 500 µ*M* dosing level; however, the

responses were not uniformly concentration-dependent because fresh and dry masses also increased at some intermediate or high dosing levels. The lower tissue masses observed in some treatments may reflect metal-induced disruption of root activity, nutrient uptake, photosynthesis, and cellular detoxification processes. Plant tissue mass is relevant to phytoremediation performance because total organ metal burden depends on both tissue-metal concentration and organ dry mass [2]. Previous studies of *E. crassipes* have reported predominantly root-associated metal accumulation and limited transfer to above-ground tissues, emphasizing the importance of maintaining root tissue mass for root-associated metal retention [6, 13]. Consequently, reductions in root or stem–petiole dry mass at some 500 µ*M* dosing levels may limit the total quantity of metal retained in the analyzed tissues, even when tissue-metal concentrations remain comparatively high.

1. Chlorophyll Response Under Metal Treatments

Leaf chlorophyll content index varied among metals and dosing-solution levels (Figure 3). The chlorophyll content in the plants varied with the type and concentration of metals (as shown in Figure 3), suggesting that these different metal ions influenced the chlorophyll status of the plants in different ways. For Ni treatment, the lowest chlorophyll content index was recorded at the 500 µ*M* Ni dosing level, indicating that high exposure to Ni had a negative effect on the status of chlorophyll in photosynthetic pigments [19]. Cd stress affected leaf physiological performance, as chlorophyll content decreased with increasing Cd dosing level [10]. The chlorophyll content was also affected by Pb treatment, but not necessarily in a dose-dependent manner [10, 16].

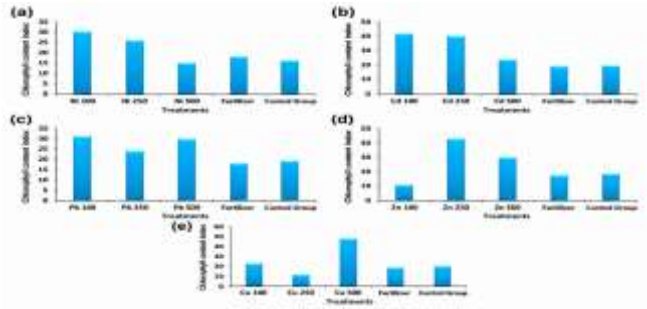


Figure. 3 Chlorophyll content index of *Eichhornia crassipes* at the 100, 250 and 500 µ*M* metal dosing-solution levels and in the fertilizer-only and untreated reference treatments: (a) Ni, (b) Cd, (c) Pb, (d) Zn and (e) Cu. Each bar represents one treatment-level observation

However, Zn and Cu showed non-linear chlorophyll responses, which may reflect their roles as essential micronutrients at low concentrations and their toxicity at higher concentrations [18, 20]. Similar Cu-Zn-related effects on chlorophyll synthesis and photosystem function have also been reported in plants [20]. Derived chlorophyll-response indices were also calculated for the various metals according to the formulas presented in Section 2.11 to further compare chlorophyll responses among metals.

Table 4. Derived chlorophyll-response indices of *Eichhornia crassipes* across the metal dosing-solution levels.

Metal	Mean chlorophyll across doses	Chlorophyll change from 100 to 500 µM (%)	Response range	Interpretation
Ni	26.1	-49.0	16.3	High-dose chlorophyll suppression
Cd	37.1	-43.6	19.3	Clear dose-related chlorophyll decline
Pb	28.6	-3.2	7.0	Relatively variable but less high-dose decline
Zn	28.5	+151.8	34.1	Strong non-linear response
Cu	29.5	+110.7	38.3	Strong non-linear response

The derived chlorophyll-response indices further supported that chlorophyll response was metal-specific, as seen in Table 4. Both Ni and Cd exhibited a distinct high-dose chlorophyll suppression, with chlorophyll decreasing by ~49.0% and 43.6%, respectively, from 100 µ*M* to 500 µ*M* [19, 21]. Cd also exhibited a relatively broad response range, which was also important in terms of its effect on photosynthetic status [21]. By comparison, the change was small for

Pb, but the response was different for each concentration. The positive high-dose changes and larger response range of Zn and Cu suggested that there were non-linear relationships for chlorophyll, rather than dose-dependent inhibition [18, 20]. These patterns suggest that the chlorophyll response in *E. crassipes* was affected by both the type and concentration of the metals, with Ni and Cd demonstrating a greater suppression of pigment, whereas Zn and Cu demonstrated greater variability in physiological responses.

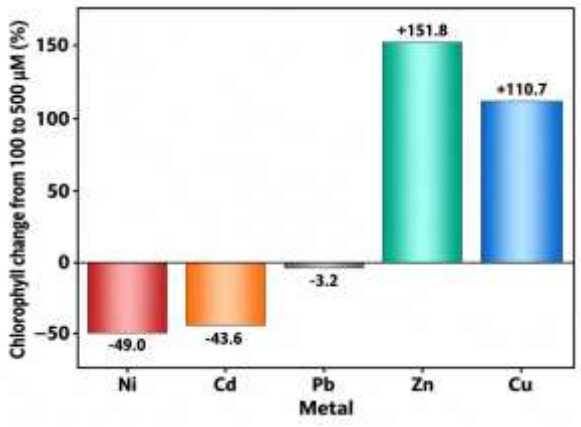


Figure. 4 Change in the chlorophyll content index of *Eichhornia crassipes* between the 100 and 500 µ*M* metal dosing-solution levels

Figure 4 further depicts the chlorophyll changes occurring at high doses of each metal. The chlorophyll change was negative for Ni (around −49.0%) and Cd (around −43.6%), respectively, suggesting higher pigment suppression at the highest concentration. A relatively weak high-dose chlorophyll response was observed for Pb, with just a slight decrease of −3.2%. However, both Zn and Cu exhibited positive increases of +151.8% and +110.7%, respectively, indicating that their chlorophyll responses were not merely dose-dependent. These patterns further illustrate the dependence of the chlorophyll response of *E. crassipes* on the type of metal and its concentration. Changes in chlorophyll content were consistent with visible chlorosis and damage to the leaves seen under heavy-metal stress. The decrease in chlorophyll may result from inhibition of chlorophyll biosynthesis, damage to the chloroplast, oxidative stress, and disruption of photosynthetic metabolism [14]. Chlorophyll and photosynthesis-related responses have been reported for water hyacinth under Cd/Pb stress [10]. A recent study reported that Cd stress reduced photosynthesis and affected root structure and respiration in water hyacinth [21]. Similarly, previous work on Cd and Pb toxicity indicates that these elements can impair photosynthetic pathways, reduce chlorophyll synthesis, and disturb plant physiological performance [16, 22].

2. Root–Stem Metal Concentrations, Burdens, And Partitioning

Root–stem metal partitioning was evaluated to determine whether the metals showed predominantly root-associated or stem–petiole-associated distribution.

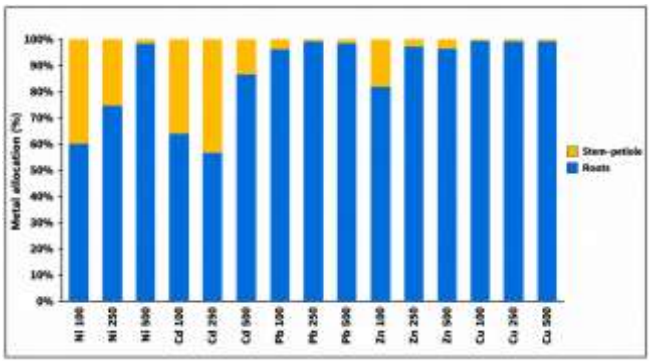


Figure. 5 Burden-based metal allocation between root and stem–petiole tissues of *Eichhornia crassipes* at the 100, 250, and 500 µ*M* dosing-solution levels. Allocation percentages were calculated from tissue metal concentration and the corresponding organ dry mass. Each stacked bar represents one treatment-level observation

Burden-based metal allocation differed descriptively among metals and dosing-solution levels. Root allocation exceeded stem–petiole allocation for all five metals at each dosing level (Figure 5). Cu and Pb showed the strongest root-dominant distributions, with root allocations exceeding 97% at all three dosing levels. Zn also showed predominantly root-associated burdens, particularly at the 250 and 500 µ*M* dosing-solution levels. Ni showed greater variability, with root allocation increasing from 60.3% at the 100 µ*M* dosing level to 99.0% at the 500 µ*M* dosing level. Cd showed the greatest relative allocation to stem–petiole tissue, particularly at the 250 µ*M* dosing level, where 43.0% of the measured burden occurred in the stem–petiole

fraction. Nevertheless, the total Cd burden remained greater in roots than in stem–petiole tissue at all three dosing levels. These burden-based findings are consistent with recent concentration-based studies reporting preferential metal accumulation in water-hyacinth roots and limited transfer to above-ground tissues [23, 24].

Table 5. Mean burden-based allocation of Ni, Cd, Pb, Zn and Cu between root and stem–petiole tissues of *Eichhornia crassipes* across the three dosing-solution levels.

Metal	Mean root allocation (%)	Mean stem–petiole allocation (%)	Interpretation
Ni	78.0	22.0	Variable but predominantly root-associated
Cd	69.4	30.6	Greatest relative stem–petiole allocation
Pb	98.8	1.2	Strongly root-dominant allocation
Zn	92.6	7.4	Predominantly root-dominant allocation
Cu	99.7	0.3	Strongest root-dominant allocation

Descriptive mean root allocation across the three dosing-solution levels followed the sequence Cu (99.7%) > Pb (98.8%) > Zn (92.6%) > Ni (78.0%) > Cd (69.4%) (Table 5). Cu and Pb therefore showed the strongest root-dominant allocation, whereas Cd showed the greatest relative allocation to stem–petiole tissue, with a mean value of 30.6%. Nevertheless, the total Cd burden remained predominantly root-associated.

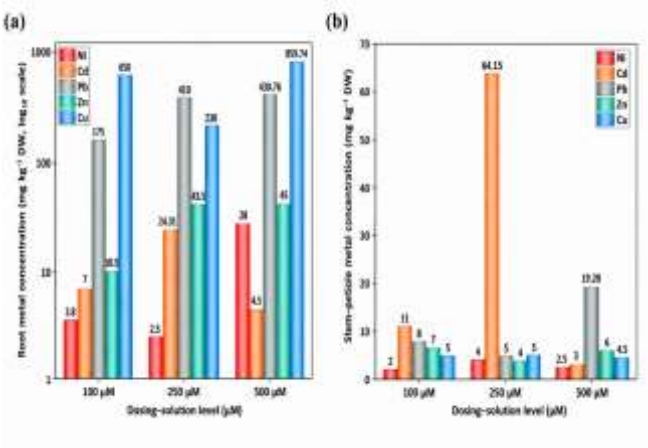


Figure. 6 Root and stem–petiole concentrations of Ni, Cd, Pb, Zn and Cu in *Eichhornia crassipes* at the 100, 250 and 500 µ*M* dosing-solution levels

These results demonstrate that tissue-concentration ratios and burden-based allocation describe different aspects of metal distribution because total organ burden is influenced by both tissue concentration and corresponding organ dry mass. Metal-specific organ partitioning has also been reported in other aquatic macrophytes, with Cd, Zn, and Cu occurring mainly in above-ground eelgrass tissues, whereas Pb was preferentially retained in below-ground tissues [25].

(a) Root metal concentrations are presented on a $\log_{10}$ scale because of the large differences among metals. (b) Stem–petiole metal concentrations are presented on a linear scale. Concentrations are expressed as mg kg^{-1} DW, and each bar represents one treatment-level observation Root and stem–petiole metal concentrations differed descriptively among metals and dosing-solution levels (Figure 6). Cu showed the highest measured root concentration, reaching 859.74 mg kg^{-1} DW at the 500 µ*M* dosing level, while Pb reached 430.76 mg kg^{-1} DW at the same level (Figure 6a). These results indicate high root-associated concentrations of Cu and Pb under the present experimental conditions. By comparison, Ni, Cd and Zn generally showed lower root concentrations. Stem–petiole concentrations displayed a different pattern (Figure 6b). Cd reached its highest stem–petiole concentration of 64.15 mg kg^{-1} DW at the 250 µ*M* dosing level, whereas Cu concentrations in stem–petiole tissue remained between 4.5 and 5.0 mg kg^{-1} DW (Figure 6b). Thus, Cu and Pb showed strongly root-dominant concentrations relative to the analyzed stem–petiole fraction, while Cd showed comparatively

greater stem–petiole concentration at the intermediate dosing level.

Table 6. Stem–petiole/root concentration ratios of Ni, Cd, Pb, Zn, and Cu in *Eichhornia crassipes* at the three dosing-solution levels

Metal	Treatment	Root concentration (mg kg-1 DW)	Stem–petiole concentration (mg kg-1 DW)	Stem–petiole/root concentration ratio	Stem–petiole/root concentration ratio
Ni	100 µM	3.8	2.0	0.53	
	250 µM	2.5	4.0	1.60	
	500 µM	28.0	2.5	0.09	
Cd	100 µM	7.0	11.0	1.57	
	250 µM	24.31	64.15	2.64	
	500 µM	4.5	3.0	0.67	
Pb	100 µM	175.0	8.0	0.05	
	250 µM	410.0	5.0	0.01	
	500 µM	430.76	19.28	0.04	
Zn	100 µM	10.5	7.0	0.67	
	250 µM	43.5	4.0	0.09	
	500 µM	45.0	6.0	0.13	
Cu	100 µM	650.0	5.0	0.01	
	250 µM	230.0	5.0	0.02	
	500 µM	859.74	4.5	0.01	

The stem–petiole/root concentration ratio differed descriptively among metals and dosing levels (Table 6). Ratios below 1 were observed for Pb, Zn and Cu at all three dosing levels, indicating higher concentrations in roots than in stem–petiole tissue. Cd showed ratios above 1 at the 100 and 250 µ*M* dosing-solution levels, indicating that its concentration per unit dry mass was higher in stem–petiole tissue under these conditions. Ni also showed a ratio above 1 at the 250 µ*M* dosing level. However, the larger root dry masses resulted in greater total root burdens even when the stem–petiole/root concentration ratio exceeded 1. Consequently, concentration ratios and burden-based allocation describe different aspects of metal partitioning and should not be interpreted interchangeably.

The across-dose mean stem–petiole/root concentration ratio varied among the tested metals (Figure 7). Cd showed the highest mean ratio of 1.63, followed by Ni (0.74), Zn (0.30), Pb (0.03) and Cu (0.01). Cd was the only metal with an across-dose mean ratio above 1, indicating a higher average concentration per unit dry mass in stem–petiole tissue than in roots. In contrast, the very low ratios of Pb and Cu indicated

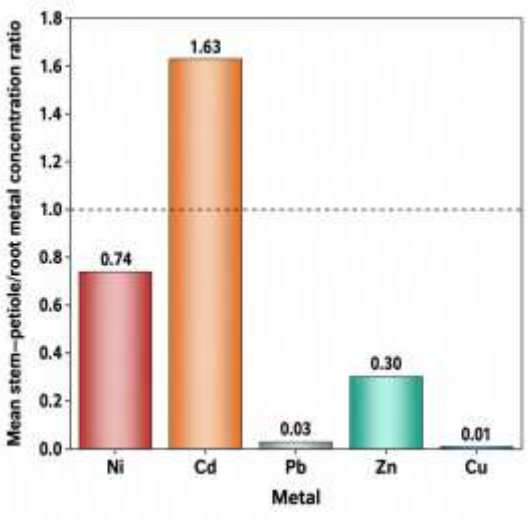


Figure. 7 Across-dose mean stem–petiole/root concentration ratio of Ni, Cd, Pb, Zn and Cu in *Eichhornia crassipes*. Values were calculated as arithmetic means across the 100, 250 and 500 µ*M* dosing-solution levels

strongly root-dominant tissue concentrations. These concentration-based patterns complemented the burden-based allocation results but did not represent total organ metal amounts. Therefore, the concentration ratios were interpreted together with corresponding organ dry masses rather than as direct measures of total translocation.

VIII. METAL RESPONSES UNDER CITRIC-ACID-ASSISTED CONDITIONS

Citric acid may have influenced the uptake and translocation of metals in the present study. The metal treatments were supplemented with citric acid as a biodegradable chelating agent to enhance metal solubility and availability for plant uptake [26]. Because all metal treatments contained citric acid, the present study evaluates metal accumulation under citric-acid-assisted conditions, but it does not independently quantify the contribution of citric acid. Chelator-assisted phytoremediation can improve metal availability, metal uptake, and partial translocation, but also can result in phytotoxicity where excessive metal mobilization leads to reduced plant development and tissue mass [11]. Biodegradable chelators have been suggested as greener chelators in chelate-assisted phytoremediation due to their ability to enhance metal mobility and uptake, with the added benefit of being less persistent in the environment than synthetic chelators [11]. Under the citric-acid-assisted conditions, Cd showed comparatively greater distribution to stem–petiole tissue, whereas Pb and Cu remained predominantly root-associated. However, because treatments without citric acid were not included, the independent effect of citric acid cannot be separated from the effect of metal exposure alone. Thus, the importance of citric acid needs to be interpreted with some caution, and further experiments are needed to confirm its specific contribution by using treatments with and without chelator additions.

1. Plant Performance Under Fertilizer Application

The fertilizer-treated plant showed comparatively favorable final vegetative condition and no clear toxicity symptoms under nutrient-supported, non-metal conditions. This observation indicates that nutrient availability supported plant development under the hydroponic conditions used. However, the fertilizer treatment was not combined with metal exposure and therefore does not provide evidence that fertilizer reduced metal uptake or alleviated metal-associated toxicity. Nutrient availability may nevertheless influence plant responses to metal stress by affecting membrane stability, ionic balance, antioxidant defense, and competition between essential nutrients and metal ions [27].

2. Implications For Rhizofiltration And Root-Associated Metal Retention

Because residual metal concentrations in the hydroponic solution were not measured after treatment, the present study does not directly quantify metal-removal efficiency from water. Therefore, the results should be interpreted mainly in terms of tissue-metal concentrations, root-associated burdens, and root–stem partitioning. The findings indicate that *E. crassipes* may be particularly relevant to rhizofiltration and root-associated metal retention rather than shoot-based phytoextraction. This interpretation is supported by the higher root concentrations and root-dominant burdens observed particularly for Cu and Pb. The dense fibrous root system of *E. crassipes* provides a large surface area for metal adsorption, absorption, and sequestration, which may favor root-associated retention and limited distribution to above-ground tissues. Similar root-dominant metal distribution and limited transfer to above-ground tissues have been reported in previous studies of *E. crassipes* [28].

The high root-associated concentrations and burdens of Cu and Pb suggest that *E. crassipes* has potential for retaining these metals in controlled hydroponic systems. However, metal-enriched root biomass must be harvested and managed appropriately to prevent secondary contamination [29]. The potential use of *E. crassipes* and other aquatic macrophytes for removing metals from polluted aquatic environments has also been emphasized, although such systems require

further validation under real wastewater conditions and appropriate management of contaminated biomass [12, 23]. Field-scale hybrid constructed wetlands have likewise demonstrated the removal of Cu, Zn, Cr, and Pb through the combined contributions of sedimentation, plant uptake, and root–microorganism–substrate interactions [30]. Microbe-assisted phytoremediation may also be considered in future studies because beneficial microbial communities can improve metal tolerance, promote metal transformation, and enhance overall remediation performance under wastewater conditions [31, 32].

IX. CONCLUSION

This study demonstrated that the physiological response and organ-level metal partitioning of *Eichhornia crassipes* varied according to metal identity and dosing-solution level under citric-acid-assisted hydroponic conditions. Pb and Cd produced the clearest reductions in final aerial dimensions and the most evident toxicity symptoms at higher dosing levels, while tissue dry-matter content was generally lower at 500 µ*M* across metals. Ni and Cd showed the clearest high-dose chlorophyll declines, whereas Zn and Cu displayed variable, non-linear chlorophyll responses. Metal distribution was predominantly root-associated.

Mean burden-based root allocation followed the sequence Cu (99.7%) > Pb (98.8%) > Zn (92.6%) > Ni (78.0%) > Cd (69.4%). Cu and Pb showed the strongest root-dominant allocation, whereas Cd exhibited the greatest relative allocation to stem–petiole tissue (30.6%), although its total burden remained higher in roots at all dosing levels. Stem–petiole/root concentration ratios provided complementary information on tissue concentrations but differed from burden-based allocation because organ burden also depended on tissue dry mass.

In short, *E. crassipes* exhibited predominantly root-associated metal partitioning, particularly for Cu and Pb, highlighting its potential for rhizofiltration and root-associated metal retention under controlled hydroponic conditions. These findings identify *E. crassipes* as a promising aquatic macrophyte for use in managed phytoremediation systems, provided that metal-enriched biomass is regularly harvested and safely managed.

1. Author Contributions

Jaisha Batool: Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Eman Waheed:** Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Eman Fatima:** Investigation, Resources. **Syeda Mahin Ahmed:** Investigation, Resources.

2. Declaration Of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

3. Data Availability

The data supporting the findings of this study are included within the article and are available from the corresponding author upon reasonable request.

4. Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

REFERENCES

1. Jomova, K., et al., Heavy metals: toxicity and human health effects. Archives of toxicology, 2025. 99(1): p. 153-209.
2. Bayuo, J., et al., Recent and sustainable advances in phytoremediation of heavy metals from wastewater using aquatic plant species: Green approach. J Environ Manage, 2024. 370: p. 122523.
3. Dasari, M.R. and V.K. Mishra, Harnessing phytoremediation of heavy metals for sustainable water cleanup: a comprehensive review and future directions. J Environ Manage, 2026. 404: p. 129271.
4. Fei, Y. and Y.H. Hu, Recent progress in removal of heavy metals from wastewater: A comprehensive review. Chemosphere, 2023. 335: p. 139077.
5. Monroy-Licht, A., et al., Unlocking the potential of Eichhornia crassipes for wastewater treatment:

phytoremediation of aquatic pollutants, a strategy for advancing Sustainable Development Goal-06 clean water. Environmental Science and Pollution Research, 2024. 31(31): p. 43561-43582.

6. Fan, Y., et al., The Potential of Pontederia crassipes to Remediate Heavy Metals in Water. Plants, 2025. 14(23): p. 3604.
7. Durairaj, S., Sorption capacity of Eichhornia crassipes (Mart.) Solms for zinc removal from electroplating industry wastewater. Environ Sci Pollut Res Int, 2024. 31(21): p. 30849-30866.
8. Du, Y., et al., Accumulation and translocation of heavy metals in water hyacinth: Maximising the use of green resources to remediate sites impacted by e-waste recycling activities. Ecological Indicators, 2020. 115: p. 106384.
9. Ghuge, S.A., et al., Comprehensive mechanisms of heavy metal toxicity in plants, detoxification, and remediation. J Hazard Mater, 2023. 450: p. 131039.
10. Chen, Q., et al., Effects of functional carbon nanodots on water hyacinth response to Cd/Pb stress: Implication for phytoremediation. Journal of environmental management, 2021. 299: p. 113624.
11. Yin, F., et al., Biodegradable chelating agents for enhancing phytoremediation: mechanisms, market feasibility, and future studies. Ecotoxicology and environmental safety, 2024. 272: p. 116113.
12. Kaushal, J., et al., Phytoremediation of paper mill effluent contaminated with lead using EDTA and citric acid supported aquatic plant species: Eichhornia crassipes, Lemna minor, and Salvinia molesta. Bioresource Technology Reports, 2026: p. 102733.
13. Eid, E.M., et al., Uptake prediction of nine heavy metals by Eichhornia crassipes grown in irrigation canals: A biomonitoring approach. Science of The Total Environment, 2021. 782: p. 146887.
14. Mohamed, H.I., et al., Heavy metals toxicity in plants: understanding mechanisms and developing coping strategies for remediation: a review. Bioresour Bioprocess, 2025. 12(1): p. 95.
15. Polechońska, L., A. Klink, and K. Sokołowska, Rising temperature impacts the trace metal uptake and toxicity in aquatic plants - A case study of Ni and Co in Elodea canadensis Michx. Sci Total Environ, 2024. 955: p. 177307.
16. Gupta, M., et al., Lead toxicity in plants: mechanistic insights into toxicity, physiological responses of plants and mitigation strategies. Plant Signaling & Behavior, 2024. 19(1): p. 2365576.
17. Hu, Y., et al., Cadmium toxicity in plants: from transport to tolerance mechanisms. Plant Signal Behav, 2025. 20(1): p. 2544316.
18. Kaur, H. and N. Garg, Zinc toxicity in plants: a review. Planta, 2021. 253(6): p. 129.
19. Kováčik, J. and M. Vydra, The impact of nickel on plant growth and oxidative balance. Physiologia plantarum, 2024. 176(6): p. e14595.
20. Wang, S., S. Yang, and J. Wang, The combined effects of copper and zinc on Arabidopsis involve differential regulation of chlorophyll synthesis and photosystem function. Plant Physiology and Biochemistry, 2024. 216: p. 109160.
21. Liu, L., et al., Long-term adaptation of water hyacinth to low cadmium involves antioxidant enzyme and metallothionein transcriptional regulation. Chemosphere, 2024. 365: p. 143346.
22. Song, J., et al., Exploring the deleterious effects of heavy metal cadmium on antioxidant defense and photosynthetic pathways in higher plants. Plant Stress, 2025. 15: p. 100716.
23. Bramhankar, D., et al., A Comparative Study of Phytoremediation Potential of Water Lettuce (Pistia stratiotes L.) and Water Hyacinth (Eichhornia crassipes [Mart.] Solms) for Mitigating Contaminants in the Nag River, Maharashtra, India. Cleaner Water, 2026: p. 100252.
24. Ramadan, S., M.M. Elshamy, and E.M. Nafea, Evaluation of Pontederia crassipes as bioindicator of heavy metals in Lake Manzala, Egypt. Scientific Reports, 2026. 16(1): p. 14643.
25. Xu, S., et al., Comparison of metals in eelgrass (Zostera marina L.) and the environment across the North Pacific Ocean: Environmental processes drive source delivery. Environmental Pollution, 2024. 343: p. 123096.
26. Mobin, F., J.M. Deloya, and L. Guo, The impact of citric acid on metal accumulation in Lemna minor. Water, 2025. 17(6): p. 830.
27. WU, X.-Z., Suppression effect of fertilizers on heavy metal stress in aquatic plants studied by the probe beam deflection/fluorescence quenching method.

Environmental Monitoring and Contaminants Research, 2024. 4: p. 111-116.

28. Guayjarernpanishk, W. and P. Sampanpanish, Efficiency of sodium phytate in the remediation of As, Mn, and Cu contamination in acid mine drainage using water hyacinth. Heliyon, 2024. 10(4).
29. Li, G., et al., Insight into the pyrolytic characteristic and heavy metal immobilization during the co-pyrolysis of phytoremediation biomass with duckweed. Journal of the Energy Institute, 2026: p. 102528.
30. Bao, J.a., et al., Removal characteristics of heavy metals from polluted river water purified by hybrid constructed wetlands. Science of The Total Environment, 2025. 959: p. 178162.
31. Ali, M.H., et al., Microbes-assisted phytoremediation of lead and petroleum hydrocarbons contaminated water by water hyacinth. International Journal of Phytoremediation, 2024. 26(3): p. 405-415.
32. Heisi, H.D., R. Nkuna, and T. Matambo, Rhizosphere microbial community structure and PICRUSt2 predicted metagenomes function in heavy metal contaminated sites: A case study of the Blesbokspruit wetland. Science of the Total Environment, 2025. 959: p. 178147.