



Multi-scale evaluation of *Alternanthera reineckii* (var. Lilacina) under nickel stress: a molecular, physiological and phytoremediation perspective

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Abstract

Heavy metal contamination in aquatic ecosystems poses a major threat to the environment. While nickel (Ni) plays a vital role in plant growth at low concentrations, excessive concentrations can induce toxicity, leading to genotoxicity and disrupting fundamental biological processes and cellular homeostasis in aquatic plants. This study focuses on evaluating the capacity of *Alternanthera reineckii* Briq (var. Lilacina), a hardy submerged aquatic plant, to remove Ni from freshwater and examines the subsequent physiological and genotoxic effects caused by Ni exposure. Samples were subjected to NiCl₂·6H₂O in a 0.2% Hoagland nutrient solution at concentrations of 0, 10, 25, 50, and 100 µM for 10 days. The results indicated a concentration-dependent increase in Ni accumulation. Although Ni concentrations did not meet the classical hyperaccumulation threshold, the calculated BCFs (ranging from 3 to 9) and TF values consistently > 1 suggest that *A. reineckii* possesses effective internal Ni translocation and moderate accumulation potential. The alterations in essential mineral nutrients, including Ca, Cu, B, and Zn, are indicative of physiological adjustments in response to exposure. ISSR marker analysis revealed noticeable changes in band intensity under various treatments. A high GTS value of 100% and low polymorphic changes suggest that the plant's genetic integrity was largely preserved. The findings indicate that *A. reineckii* exhibits notable accumulation capabilities for Ni and has potential for the remediation of Ni-contaminated freshwater. Moreover, the efficacy of remediation may be further enhanced when used in combination with other plant species.

Keywords Freshwater · Genotoxicity · ISSR · Ni-contaminated water · Ni removal

Introduction

The rapid growth of the global population and industrialization has resulted in an escalation of human activities that pollute natural water resources, particularly in developing countries. The utilization of chemicals in agriculture, irrigation with wastewater, waste incineration, mining,

the burning of fossil fuels, and various industrial processes contribute to heavy metal contamination, thereby posing a serious threat to living organisms (Singh et al. 2024a, b; Mutabaruka and Rana 2025). It is estimated that 25 countries, which collectively represent 25% of the global population, experience 'extremely high' water stress on an annual basis. It is estimated that approximately 4 billion people, representing half of the global population, experience severe water scarcity on an annual basis (United Nations 2025). With the increasing frequency of extreme weather events, such as floods and droughts, it is vital to ensure the protection of water resources from pollution (Kaplan et al. 2023).

Research studies analyzing lake and river water across different continents have shown that approximately 40% of the world's freshwater is polluted by heavy metals, with concentrations increasing notably in Europe and North America (Niu et al. 2021; Kakade et al. 2023). Heavy metals are defined as elements with an atomic number greater than 20 and a density greater than 5 g/cm³. Among these elements,

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cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), and zinc (Zn) are identified as essential, meaning that even small amounts are required for plant growth (Singh et al. 2024a, b; Gjergjizi Nallbani et al. 2025). Of these, Ni is a naturally occurring element in the Earth's crust, with a concentration of almost 3%. It is a highly versatile element, widely used in industrial processes, including the production of stainless steel, alloys, lithium batteries, welding, and electroplating. However, the recycling of Ni-containing waste and products is the largest contributor to environmental pollution. High concentrations of Ni have been shown to exert adverse effects on humans and animals. Ni exposure poses significant health risks including allergies, cardiovascular diseases, lung and kidney diseases in humans and animals. It is also associated with mitochondrial dysfunction and oxidative stress (Rizwan et al. 2024; Sharma et al. 2025).

Ni is essential for the healthy growth and development of plants, contributing to various morphological and physiological processes. It is identified as a beneficial micronutrient, the absence of which cannot be fulfilled by any other nutrient as Ni is a component of urease and a cofactor in many other enzymes including hydrogenases. Furthermore, Ni plays a crucial role in facilitating Fe absorption and seed germination. However, Ni has been observed to become toxic at elevated concentrations, with the ability to disrupt metabolic functions by inhibiting enzyme activity, photosynthetic electron transport, and chlorophyll synthesis (Moreno-Rubio et al. 2022). Ni has been identified as toxic to plant species when concentrations exceed 10 mg kg^{-1} dry weight (DW). However, in tolerant plant species, the toxicity threshold is greater than 50 mg kg^{-1} DW (Amjad et al. 2019). A significant disruption of membrane integrity in plants, resulting in reduced membrane permeability, is one of the primary physiological effects of Ni. This affects various physiological processes, leading to a decrease in photosynthetic pigments, net photosynthesis rate, stomatal conductance, transpiration rate, and water use efficiency (Batoool 2018; Hassan et al. 2019).

Additionally, excessive Ni levels have genotoxic effects on plants, causing DNA damage through interactions with phosphate and carboxyl groups on cell walls and DNA (Ozyigit et al. 2021a; Yilmaz et al. 2023). Consequently, the necessity to eliminate Ni from vital freshwater sources has become a matter of significant scientific and environmental concern, requiring further research and policy attention to mitigate the associated risks.

In recent years, phytoremediation has been identified as a significant eco-friendly technology, utilizing the natural processes of plants to purify soil, air, and water environments. This process involves the extraction, accumulation, and

breakdown of pollutants (including heavy metals, pesticides, organic pollutants, and xenobiotics) through various mechanisms. The uptake of pollutants through phytoremediation encompasses a range of processes, including phytoextraction, phytostabilization, phytovolatilization, and rhizofiltration (Ozyigit et al. 2021b). Although many traditional methods are used in the removal of pollutants, these methods have many disadvantages, such as incomplete pollutant removal, high energy requirements, substantial toxic sludge production, limited applicability to small areas, and high costs when compared with phytoremediation (Sharma et al. 2023, 2025; Phang et al. 2024). In addition, it is an alternative to physical and chemical remediation methods that requires a reduced quantity of equipment, materials, energy, and labour (Jaskulak et al. 2020). Constructed wetlands (CWs) are used widely in wastewater treatment due to their cost-effectiveness. Over the last few decades, these systems have been demonstrated to be capable of eliminating 70–90% of heavy metals and nutrients while maintaining operation at a cost 30–50% lower than that of conventional facilities (Vymazal 2014; Wentzell 2025). Moreover, floating treatment wetlands (FTWs) have also been shown to be effective in the reduction of industrial pollutants, whilst requiring significantly lower installation and maintenance costs in comparison with mechanical filtration systems (Afzal et al. 2019). A further study has indicated that the financial outlay required for phytoremediation is between US\$20 and US\$40 per ton of waste removed, whereas the cost of chemical treatment varies between US\$100 and US\$500 per ton (Kafle et al. 2022). In addition to its economic advantages, it is ecologically significant in terms of removing pollutants from the ecosystem and reducing carbon dioxide emissions through CO_2 sequestration (Phang et al. 2024). These techniques have also been shown to be increasingly effective in reducing the impact of pesticide waste in the aquatic environment. Indeed, it has been reported that the removal of pesticide in freshwater containing plants is 38.9% higher than in water without plants (Cruz et al. 2025).

As demonstrated in the literature, a considerable number of aquatic plants have been shown to bioaccumulate heavy metals and metalloids. Among these, *Alternanthera philoxeroides*, a member of the Amaranthaceae family is of particular interest, as it has been shown to accumulate cadmium (Cd), chromium (Cr), Cu, Fe, lead (Pb), Ni, and Zn (Sudarshan et al. 2020; Sharma et al. 2021; Yan et al. 2023; Zhou et al. 2024). *A. bettzickiana* has been shown to be tolerant of Cd and Pb, and to accumulate them in significant amounts (Tauqeer et al. 2016). *A. reineckii* is a perennial herbaceous plant native to South America, which typically grows in moist areas such as river courses, seepage areas, and in rain pools. This plant can grow



both fully submerged in water and emerged, achieving a height of 15–50 cm and a width of 10–15 cm (USDA 2016). In addition to these features, the variety *A. reineckii* is a particularly popular easy-to-grow aquarium plant, due to the attractive purple-lilac coloration of its leaves.

This study primarily focused on evaluating the potential of *A. reineckii* Briq. (var. Lilacina) for remediating Ni-contaminated freshwater environments. Subsequently, an investigation of the genotoxic effects was conducted of Ni exposure to assess its potential as a hyperaccumulator under elevated heavy metal concentrations. The physiological and molecular assessments conducted in this study were mainly based on the plant's mineral nutrient status, total chlorophyll content, and inter-simple sequence repeat (ISSR) markers to evaluate genomic alterations.

Materials and methods

Experimental design

Aquarium setup and placement of aquatic plants

An aquarium room was set up to cultivate plants under optimal conditions, incorporating shelving and lighting systems. Following the completion of necessary repairs and renovations, a 0.2% Hoagland solution was prepared, in which the plants were cultivated for 2 days prior to being subjected to different concentrations of Ni exposure. To prepare Hoagland's solution, a 1% stock solution was initially prepared using the macro-, and micronutrient, and iron solutions in the specified concentrations shown in Table 1. This 1% solution was then diluted to 0.2% for use and subsequently filled into the tanks, with each tank having a capacity of 21 L. Seedlings of *Alternanthera reineckii* (var.

Lilacina) were obtained from the market and subsequently placed into the tanks. Four pots, each containing six individuals (24 individuals in total), were placed in the aquariums (Fig. 1).

Nickel exposure

In the context of Ni treatment, a solution of $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ was applied at the following concentrations (μM): 0 μM (control, no Ni added), 10 μM (2.37 mg L^{-1}), 25 μM (5.94 mg L^{-1}), 50 μM (11.88 mg L^{-1}), and 100 μM (23.77 mg L^{-1}). The optimal abiotic conditions for plant growth in freshwater ecosystems included a 16:8-h light–dark cycle, a

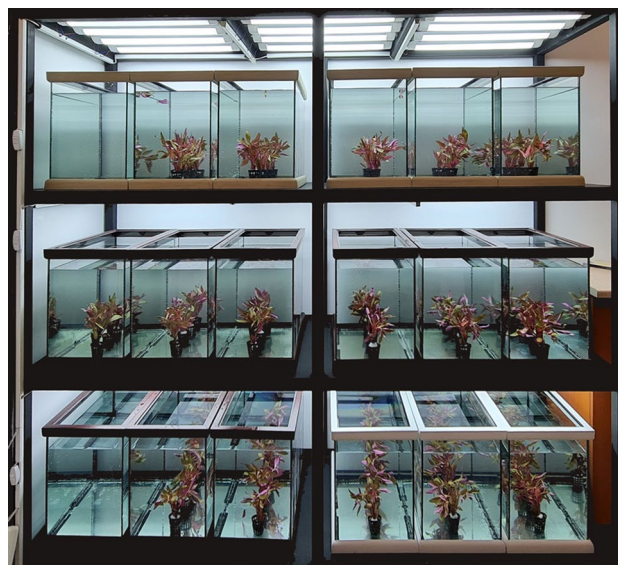


Fig. 1 Placement of *A. reineckii* plants in the aquarium system

Table 1 The composition and preparation of the Hoagland solution used in the study

Component	Chemical name/formula	Amount (g)	Preparation method
Macronutrients	Ammonium dihydrogen phosphate/ $\text{NH}_4\text{H}_2\text{PO}_4$	11.50	Each macronutrient was dissolved separately in 100 mL distilled water to prepare a 1 M stock solution
	Calcium nitrate tetrahydrate/ $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	23.61	
	Magnesium sulfate heptahydrate/ $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	24.64	
	Potassium nitrate/ KNO_3	10.11	
Micronutrients	Copper (II) sulfate pentahydrate/ $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.08	All micronutrients were sequentially dissolved in 900 mL distilled water, then the volume was adjusted to 1 L
	Boric acid/ H_3BO_3	2.86	
	Zinc sulfate heptahydrate/ $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.22	
	Manganese (II) chloride tetrahydrate/ $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	1.81	
	Molybdc acid monohydrate/ $\text{H}_2\text{MoO}_4 \cdot \text{H}_2\text{O}$	0.02	
Iron (Fe-EDTA) Solution	Ethylenediaminetetraacetic acid (EDTA)/ $\text{C}_{10}\text{H}_{16}\text{N}_2\text{O}_8$	26.10	Dissolved in 286 mL ddH ₂ O with 19 g KOH
	Potassium hydroxide/ KOH	19.00	Dissolved together with EDTA
	Iron (II) sulfate heptahydrate/ $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	24.90	Dissolved in 500 mL distilled water, then slowly added to the EDTA solution. pH adjusted to ~7.1



temperature of 23 ± 2 °C, and a light intensity between 4000 and 4200 $\mu\text{mol m}^{-2}\text{s}^{-1}$.

Following a 10-day exposure period to $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ in the aquarium, all plants were harvested. Samples were subsequently divided into two groups: those washed with double-distilled water for molecular and mineral analyses, and those not washed. For the molecular analysis, the leaves (meristematic areas at the tip) were utilized, while the stem and other leaves were used for mineral analysis. Samples designated for mineral analysis were dried in an oven at $+80$ °C for 48 h (Kalra 1997). Samples intended for molecular analysis were kept at -80 °C in a Lexicon II ULT deep freezer (Esco Micro Pte. Ltd., Singapore) until DNA analysis.

Determination of nutrient components

Following the grinding of plant samples in a porcelain mortar and subsequent weighing, 0.2 g of each sample was placed in Teflon vessels, with 8 mL of 65% HNO_3 added to each vessel. The samples were then digested at 150 °C using a Berghof MWS-2 microwave. After cooling, the vessel edges were rinsed with distilled water, and the contents were filtered into 50 mL conical tubes, then adjusted to a final volume of 50 mL. The concentrations of heavy metals and mineral nutrients, including boron (B), calcium (Ca), Cd, Cu, Fe, potassium (K), magnesium (Mg), Mn, sodium (Na), Ni, Pb, and Zn, were measured using the Perkin Elmer Optima 7000DV ICP-OES system. The analysis was performed as previously mentioned by Ozyigit et al. (2021a) and Karahan et al. (2023).

Calibration standards were prepared by diluting a 1000 mg L^{-1} ICP multi-element stock solution (Merck) to obtain six concentrations (0, 50, 100, 250, 500 and 1000 ppm) for calibration curve generation, achieving an R^2 value greater than 0.999. To ensure the accuracy of the results, limits of detection (LOD) and relative standard deviation (RSD) were examined (below 5% for all samples) using standard methods. The LOD was demonstrated to be 1 ng L^{-1} for Ni. (Gjergjizi Nallbani et al. 2025).

Determination of total chlorophyll content

Following a 10-day exposure to Ni (0 μM , 10 μM , 25 μM , 50 μM , and 100 μM) both washed and unwashed fresh leaves of *A. reineckii* were collected, and chlorophyll concentrations were determined using a Chlorophyll Content Meter (CCM)-200plus instrument.

Assessment of water content and phytoaccumulation potential of plants

To evaluate the potential of *A. reineckii* for Ni phytoremediation, several key parameters were analyzed: bioaccumulation index (BAI), bioconcentration factor (BCF), surface adsorption (SA), surface adsorption efficiency (SAE), removal efficiency (RE), translocation factor (TF), and water content (WC). The formulae employed to calculate these parameters are as follows:

Bioaccumulation index

The BAI value was calculated to assess the Ni accumulation per unit DW. This value was calculated using the following formula:

$$\text{BAI} = \frac{C_f(\text{mg kg}^{-1}\text{DW}) - C_i(\text{mg kg}^{-1}\text{DW})}{C_i(\text{mg kg}^{-1}\text{DW})}$$

In this formula, C_i represents the initial concentration of Ni in the plant ($\text{mg kg}^{-1}\text{DW}$), and C_f is the final concentration of Ni in the plant ($\text{mg kg}^{-1}\text{DW}$). The BAI factor was calculated separately for leaf, stem and total plant values.

Bioconcentration factor

The BCF is defined as the capacity of plants to accumulate heavy metals from the environment, which is represented by the ratio of the metal content of the *A. reineckii* (C_p) or its constituent ($\text{mg kg}^{-1}\text{DW}$) in relation to the initial metal concentration in water (C_w) (mg L^{-1}).

$$\text{BCF} = \frac{C_p(\text{mg kg}^{-1}\text{DW})}{C_w(\text{mg L}^{-1})}$$

Surface adsorption

To distinguish between the amount of Ni present on the surface and the amount of biologically accumulated Ni, SA values ($\text{mg kg}^{-1}\text{DW}$) were calculated. These values were obtained simply by calculating the difference between the Ni concentration accumulated in an unwashed sample ($\text{mg kg}^{-1}\text{DW}$) and the Ni concentration accumulated in a washed sample ($\text{mg kg}^{-1}\text{DW}$).

$$\text{SA} = C_{\text{unwashed}}(\text{mg kg}^{-1}\text{DW}) - C_{\text{washed}}(\text{mg kg}^{-1}\text{DW})$$



Surface adsorption efficiency

The SAE value (%) was calculated to evaluate the impact of SA on the removal of Ni from water. The following formula was used in this calculation:

$$SAE(\%) = \frac{SA(\text{mg kg}^{-1} \text{ DW})}{Ci(\text{mg L}^{-1})} \times 100$$

In this formula, SA represents the SA value (mg kg⁻¹ DW) of Ni and Ci represents the initial Ni concentration in water (mg L⁻¹).

Removal efficiency

The Ni RE in water (%), i.e. the proportion of Ni removed from water, is determined by the following formula:

$$RE(\%) = \frac{Ci(\text{mg L}^{-1}) - Cf(\text{mg L}^{-1})}{Ci(\text{mg L}^{-1})} \times 100$$

In this equation, Ci represents the initial Ni concentration in the water (mg L⁻¹), and Cf represents the final Ni concentration in the water (mg L⁻¹).

Translocation factor

The TF is defined as the ratio of heavy metal concentrations between different plant organs. The factor quantifies the mobility of heavy metals from these anatomical structures. In this study, the final Ni concentration values of stems (*Cstem*) (mg kg⁻¹ DW) and leaves (*Cleaves*) (mg kg⁻¹ DW) were included in the analysis. The equation used is as follows:

$$TF = \frac{Cleaves(\text{mg kg}^{-1} \text{ DW})}{Cstem(\text{mg kg}^{-1} \text{ DW})}$$

Water content

This calculation is key to determining the efficacy of phytoremediation and understanding the WC within the plant (Malar et al. 2014). The WC present in each treatment was estimated by measuring the fresh weights (*FW*) (g) and dry weight (*DW*) (g) of the stems and leaves according to the following formula:

$$WC(\%) = \frac{FW(g) - DW(g)}{FW(g)} \times 100$$

Table 2 ISSR primers used in this study: Sequences and annealing temperatures (Tm)

Primer code	Sequence (5'-3')	Tm (°C)
UBC808	AGA GAG AGA GAG AGA GC	44
UBC810	GAG AGA GAG AGA GAG AT	41
UBC811	GAG AGA GAG AGA GAG AAC	44
UBC812	GAG AGA GAG AGA GAG AA	41
UBC814	CTC TCT CTC TCT CTC TA	41
UBC815	CTC TCT CTC TCT CTC TG	44
UBC822	TCT CTC TCT CTC TCT CA	44
UBC825	ACA CAC ACA CAC ACA CT	44
UBC826	ACA CAC ACA CAC ACA CC	44
UBC827	ACA CAC ACA CAC ACA CG	44
UBC833	GAG AGA GAG AGA GAG AYT	44
UBC834	AGA GAG AGA GAG AGA GYT	44
UBC835	AGA GAG AGA GAG AGA GYC	44
UBC840	CTC TCT CTC TCT CTC TTT	44
UBC841	GAG AGA GAG AGA GAG AYC	44
UBC842	GAG AGA GAG AGA GAG AYG	44
UBC868	GAA GAA GAA GAA GAA GAA	37
UBC872	GAT AGA TAG ATA GAT A	29
UBC876	GAT AGA TAG ACA GAC A	37
UBC881	GGG TGG GGT GGG GTG	48

DNA isolation and ISSR amplification

A quantity of 0.5 g of stem material from each plant was washed with sterile distilled water and stored at -80 °C. Total genomic DNA was then extracted using a modified cetyltrimethylammonium bromide (CTAB) method as described by Doyle and Doyle (1990), with slight modifications.

The subsequent DNA amplification process employed 20 different ISSR primers (Table 2). The PCR mixture with a final volume of 20 µL, included 1 µL of template DNA, 11.1 µL of molecular-grade water, 3 µL 10X Taq Buffer, 3 µL 25 mM MgCl₂, 0.2 µL of 20 mM of each dNTP, 1.5 µL of 10 mM ISSR primer, and 0.2 µL of 5U/µL Taq DNA polymerase (Thermo Scientific). The thermocycler (Aeris Model 96, Esco Micro Pte. Ltd. Singapore) was set to an initial denaturation at 95 °C for 4 min, followed by 36 cycles of 1 min at 94 °C, 40s for annealing, and 1 min at 72 °C for extension, concluding with a final cycle of 7 min at 72 °C. The annealing temperature information, specific to each primer is listed in Table 2.

The amplification products were then subjected to separation on a 1.6% agarose gel in a 1X Tris-borate-EDTA (TBE) buffer (Thermo Fisher Scientific), following ethidium bromide staining and electrophoresis at 90 V for 90 min. Bands were visualized using a UV illuminator (Vilber Lourmat,



France) and their sizes were estimated with a 100-bp ladder (Thermo Fisher Scientific) as the molecular marker (Sorrentino et al. 2017; Filiz et al. 2024).

Determination of polymorphism (%) and assessment of GTS

To ascertain the genetic stability of the experimental groups and to evaluate the effect of environmental stress factors on the genome, the percentage of polymorphism and accordingly genomic template stability (GTS) values were calculated with formulas below:

$$\text{Polymorphism(\%)} = \frac{\text{Number of polymorphic bands in the experimental group}}{\text{Number of polymorphic bands in the control group}} \times 100$$

$$\text{GTS(\%)} = \left[1 - \left(\frac{\text{Number of polymorphic bands in the experimental group}}{\text{Total number of bands in the control group}} \right) \right] \times 100$$

Data analysis

Statistical analyses were performed using multivariate analysis of variance (MANOVA) to evaluate differences within the groups. Tukey's post hoc test was then applied for pairwise comparisons to determine mineral nutrient concentration values. Pearson's correlation coefficient was utilized to analyze the relationship between variables. The significance level was set up at $p < 0.01$ to ensure the robustness of the findings. Band counting and size/intensity assignments for ISSR profiles were performed using GelAnalyzer 2010a software.

Results and discussion

Accumulation of Ni

More than 700 plant species have been documented in the literature as potential Ni accumulators suitable for the remediation of various contaminated regions (van der Ent et al. 2015; Nkrumah et al. 2018). The first documented case of exceptionally high Ni accumulation in plants was reported in the Italian species *Alyssum bertolonii* (Brassicaceae). The term hyperaccumulator was most clearly defined to include plants containing $> 1000 \text{ mg kg}^{-1}$ Ni in dried leaf tissues (Minguzzi and Vegnano 1948; Brooks et al. 1977). Subsequently, Zayed et al. (1998) proposed a revised definition, that a plant may be designated as a good accumulator if it has a $\text{BCF} \geq 1000$. Following that, *Achyranthes aspera*,

Berkheya coddii, *Sebertia acuminata*, *Phidiasia lindavii*, and *Bornmuellera kiyakii* species have been shown to be effective Ni accumulators (Bhargava et al. 2012; Ejaz et al. 2024). While these threshold values are clearly considered the standard for terrestrial plants, there is currently no universally accepted range for the hyperaccumulation thresholds of aquatic plants. *Nasturtium officinale*, the aquatic plant species with the highest Ni accumulation reported to date, accumulated 5758 mg kg^{-1} DW Ni in its roots on the 7th day of 25 mg L^{-1} Ni exposure (Duman and Ozturk 2010). Subsequent studies on *Myriophyllum aquaticum* revealed a 4800 mg kg^{-1} DW Ni accumulation in its leaves

under 7 days of $10 \text{ }\mu\text{M}$ exposure (Harguinteguy et al. 2015). It is therefore evident that a database of hyperaccumulator plants is needed, along with further research to establish hyperaccumulation thresholds in aquatic plants. Table 3 summarizes studies on potential hyperaccumulator plants that may be employed for the purpose of Ni removal within aquatic ecosystems.

However, the application of aquatic plant-based phytoremediation, specifically in the context of heavy metals, remains limited. To address this gap, the present study primarily evaluated Ni and mineral element accumulation in *A. reineckii* under varying $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ concentrations. The results demonstrated a concentration- and time-dependent increase in Ni accumulation in leaf and stem tissues (Fig. 2).

The maximum accumulation value determined in leaf samples was $91.576 \text{ mg kg}^{-1}$ at $100 \text{ }\mu\text{M}$. As demonstrated by González et al. (2015), a similar rising trend was observed in *E. crassipes* aquatic plant samples exposed to Ni, with a maximum value of 290 mg kg^{-1} DW under 4 mg L^{-1} Ni following a 3-day treatment period. Song et al. (2018) reported that the amount of Ni accumulated in the leaves and stems of *Hydrilla verticillata* increased and peaked on the 14th day, with a value of 1600 mg kg^{-1} DW in leaves and 532 mg kg^{-1} DW in stems. In accordance with the results of the present study, it was found that the amount of Ni accumulated in the leaves was higher than that accumulated in the stem. In a similar manner, the maximum Ni value that was reported to accumulate in the leaves of *Phragmites australis* was 38 mg kg^{-1} , while the stem accumulated 2.80 mg kg^{-1} (Hamidian et al. 2024). In contrast to



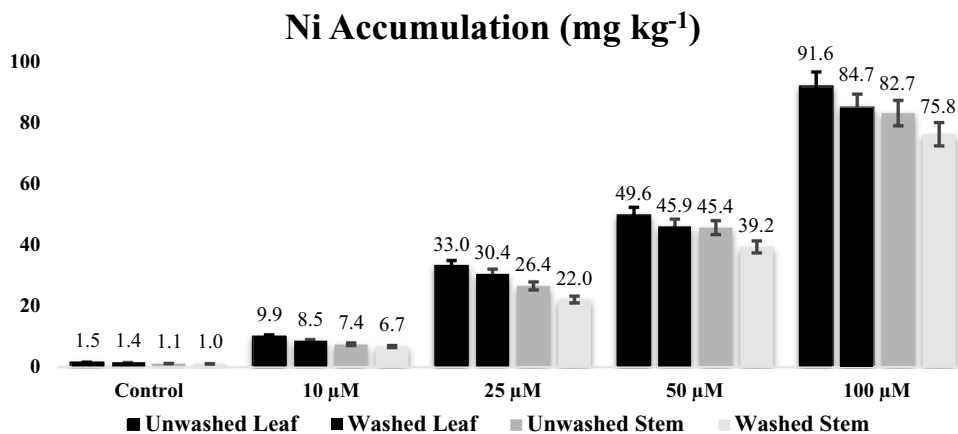
Table 3 Aquatic organisms with potential use in Ni phytoremediation

Plant species	Heavy metal	Plant part	Max BAF/BCF	References
<i>Ceratophyllum demersum</i>	Ni	Leaves	4256 -	Borisova et al. 2014
<i>Eichhornia crassipes</i>	Ni	Root	159.2 (BAF)	Eid et al. 2019
	Ni	Total plant	> 1000 (BCF)	Moreno-Rubio et al. 2022
<i>Hydrilla verticillata</i>	Ni	Leaves	1740	Song et al. 2018
		Stem	418	
<i>Lemna gibba</i>	Ni	Total plant	24 -	Bokhari et al. 2019
<i>L. minor</i>	Ni	Total plant	4,471 (BAF)	Polechonska et al. 2021
	Ni	Total plant	> 1000 (BCF)	Moreno-Rubio et al. 2022
<i>L. trisulca</i>	Ni	Total plant	7,123 -	Polechonska et al. 2021
<i>Limnobiium laevigatum</i>	Ni	Leaves	2000	Arán et al. 2017
		Root	6800	
<i>Phalaris arundinacea</i>	Ni	Total plant	7880.50	Senze et al. 2022
<i>Phragmites australis</i>	Ni	Total plant	- 0.86	Bello et al. 2018
<i>Pistia stratiotes</i>	Ni	Total plant	884.7	Şentürk et al. 2023
<i>Potamogeton alpinus</i>	Ni	Leaves	2897	Borisova et al. 2014
<i>Salvinia minima</i>	Ni	Total plant	7429	Fuentes et al. 2014
<i>Sphaerotilus natans</i>	Ni	Total plant	9,684	Das et al. 2021
<i>Typha latifolia</i>	Ni	Total plant	2002	Ben Salem et al. 2017

–: below detection limit

this result, Ni accumulation in *Lessonia trabeculata* (sea-weeds) samples was found to be 33 mg kg⁻¹, and it was found that the accumulation was higher in the stipes, the stem-like structures found in algae, compared to other parts (Sáez et al. 2012). In this study, unwashed leaf samples accumulated 91.576 mg kg⁻¹ Ni at 100 µM, while stems accumulated 82.170 mg kg⁻¹ under the same treatment. These results indicate active transport of Ni to the aerial parts and demonstrate that Ni accumulation varies among aquatic plants species.

In hyperaccumulator plants, transporters such as IREG1-2, IRT1, and NRAMP are responsible for the process of Ni uptake. In leaves, Ni is transported through the xylem and accumulates primarily in epidermal cells. Within the cell, Ni localizes in leaf vacuoles, where it has been observed to form complexes with carboxylic acids, including citrate, malic acid, and malonic acid (Ly et al. 2025). Several factors have been identified as significantly influencing Ni uptake and translocation in plants. These include soil type, environmental conditions, and the plant's capacity to absorb Ni. It has been documented that the process of Ni uptake by the

Fig. 2 Concentration of Ni in washed and unwashed *A. reineckii* leaves and stems

aquatic plant *Halophila stipulacea* occurs via three distinct mechanisms: adsorption to the cell wall, diffusion into the cell, and active accumulation in the protoplasm. Furthermore, it has been determined that a substantial proportion of the Ni content observed in the leaves of the plant is found within the cell wall (Malea et al. 2021).

Results of the ICP-OES analysis revealed a concentration-dependent increase in Ni accumulation in both leaves and stems with increasing $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ concentrations. The greatest Ni accumulation was observed in the unwashed leaf at 100 μM ($91.576 \text{ mg kg}^{-1}$), while the lowest was found in the washed stem at 10 μM (6.706 mg kg^{-1}). Studies indicate that heavy metal accumulation in the stems and leaves of aquatic plants varies considerably. Samples taken from the same aquatic plant in different areas of the same lake have been reported that, in some cases, heavy metal accumulation is higher in the stems, while in others it is higher in the leaves (Bai et al. 2018). This suggests that the accumulation of heavy metals in different plant organs may vary depending on aquatic conditions, the mobility and transport of Ni towards the upper plant parts, and TF-related factors. Moreover, the TF values > 1 observed in *A. reineckii* provide substantial evidence that Ni is effectively transported between the leaves and the stem. As illustrated in Fig. 2, the levels of Ni detected in the leaves and stems of unwashed samples are higher than those detected in washed samples. This finding underscores the significant impact of the washing process on heavy metal accumulation, indicating that washing plays an important role in distinguishing between metals accumulated on the surface and within the tissues (Tables 4 and 5).

Accumulation of other heavy metals and mineral nutrients in relation to Ni

Significant alterations in the concentrations of various mineral elements were detected in the leaves of *A. reineckii* with increasing $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ levels (Tables 4, 6). The concentrations of B, Cu, Fe, K, Mg, Mn, Na and Zn decreased in response to increasing Ni exposure. There was negligible change in Ca concentration which remained high at 100 μM $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ ($12,694.348 \pm 263.596$). Similarly, the stem data revealed declines in the concentrations of B, Cu, Fe, K, Mg, Mn, Na and Zn, compared with the control (Tables 5, 7). It is noteworthy that an increase in Ca levels was observed in the leaves and stems at a concentration of 10 μM . Concurrently, a substantial increase in stem B levels was evident at the 50 μM concentration, while the decline in leaf B levels persisted. A substantial increase in stem Cu levels was also observed at 50 μM .

It has been demonstrated that exposure to Ni results in a dose-dependent reduction in mineral nutrients, with washed tissues reflecting more significant physiological changes and unwashed tissues possibly influenced by surface-associated Ni. It has been determined that leaves exhibit a greater sensitivity to Ni-induced nutrient imbalances in comparison to stems, particularly at elevated concentrations. The maximum mineral concentration was determined to be $13,185.468 \text{ mg kg}^{-1}$ Ca in unwashed leaves at a 10 μM $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ concentration, while the minimum was recorded as 9.889 mg kg^{-1} Cu in washed stem samples.

In the present study, fluctuations in the accumulation of other mineral nutrients under $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ exposure, especially Ca, Cu and B, were noteworthy. An increase in Ca

Table 4 Mineral contents of washed (upper rows) and unwashed (lower rows) leaves under $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ treatments (10–100 μM)

	Control	10 μM	25 μM	50 μM	100 μM
B	21.157 ± 0.425	19.984 ± 0.641	18.094 ± 0.511	16.854 ± 0.451	14.477 ± 0.283
	23.208 ± 0.461	22.091 ± 0.445	20.599 ± 0.474	19.238 ± 0.387	17.196 ± 0.348
Ca	$12,237.953 \pm 254.127$	$12,407.663 \pm 415.590$	$11,829.032 \pm 353.821$	$11,008.433 \pm 304.293$	$10,098.496 \pm 201.346$
	$12,887.028 \pm 267.603$	$13,185.468 \pm 273.790$	$12,770.217 \pm 307.545$	$12,407.060 \pm 257.623$	$12,694.348 \pm 263.596$
Cu	19.019 ± 0.384	17.978 ± 0.586	16.253 ± 0.474	15.98 ± 0.428	14.624 ± 0.278
	19.620 ± 0.385	18.69 ± 0.376	17.512 ± 0.383	17.176 ± 0.341	16.390 ± 0.326
Fe	8696.220 ± 180.568	8027.555 ± 268.852	7127.652 ± 213.185	6708.898 ± 185.440	6007.266 ± 119.765
	8779.384 ± 182.304	8178.584 ± 169.826	7440.159 ± 179.164	7097.578 ± 147.359	6352.234 ± 131.897
K	8296.414 ± 172.271	7836.037 ± 262.446	7078.573 ± 211.693	6660.236 ± 184.088	6042.114 ± 120.469
	8360.053 ± 173.589	7968.431 ± 165.465	7416.976 ± 178.618	7037.466 ± 146.127	6297.239 ± 130.761
Mg	3853.313 ± 80.004	3684.110 ± 123.361	3446.862 ± 103.079	3282.749 ± 90.725	3022.550 ± 60.259
	3974.491 ± 82.523	3834.751 ± 79.618	3613.130 ± 86.998	3569.918 ± 74.114	3310.262 ± 68.722
Mn	296.047 ± 6.133	279.624 ± 9.351	258.483 ± 7.713	234.686 ± 6.470	208.161 ± 4.142
	303.278 ± 6.286	289.101 ± 5.986	269.154 ± 6.445	262.681 ± 5.448	250.449 ± 5.183
Na	4759.399 ± 98.807	4495.278 ± 150.546	4154.861 ± 124.250	3857.415 ± 106.618	3734.503 ± 74.453
	4827.746 ± 100.245	4601.573 ± 95.542	4283.134 ± 103.138	4180.644 ± 86.800	3984.863 ± 82.733
Zn	98.870 ± 2.030	89.756 ± 2.961	79.737 ± 2.339	72.595 ± 1.989	67.550 ± 1.339
	107.086 ± 2.218	98.089 ± 2.024	87.751 ± 2.081	82.289 ± 1.695	75.366 ± 1.548



Table 5 Mineral contents of washed (upper rows) and unwashed (lower rows) stems under $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ treatments (10–100 μM)

	Control	10 μM	25 μM	50 μM	100 μM
B	14.630 $\pm$ 0.296	13.860 $\pm$ 0.434	12.538 $\pm$ 0.346	11.705 $\pm$ 0.292	10.017 $\pm$ 0.191
	16.067 $\pm$ 0.327	15.344 $\pm$ 0.314	14.365 $\pm$ 0.303	19.234 $\pm$ 0.377	12.870 $\pm$ 0.255
Ca	9070.336 $\pm$ 188.333	9196.087 $\pm$ 308.020	8767.259 $\pm$ 262.207	8158.996 $\pm$ 225.535	7484.616 $\pm$ 149.236
	9146.971 $\pm$ 189.932	9358.849 $\pm$ 194.333	9935.423 $\pm$ 239.283	8993.859 $\pm$ 186.760	9202.165 $\pm$ 191.078
Cu	12.834 $\pm$ 0.254	12.139 $\pm$ 0.379	10.980 $\pm$ 0.307	10.750 $\pm$ 0.271	9.889 $\pm$ 0.186
	14.103 $\pm$ 0.282	13.475 $\pm$ 0.264	12.535 $\pm$ 0.276	13.207 $\pm$ 0.256	12.593 $\pm$ 0.250
Fe	6560.720 $\pm$ 136.229	6056.209 $\pm$ 202.845	5377.322 $\pm$ 160.826	5061.387 $\pm$ 139.894	4532.078 $\pm$ 90.355
	6652.711 $\pm$ 138.130	6197.438 $\pm$ 128.683	5637.827 $\pm$ 135.776	5378.297 $\pm$ 111.660	4986.936 $\pm$ 103.543
K	6385.568 $\pm$ 132.587	6031.163 $\pm$ 201.993	5448.184 $\pm$ 162.937	5126.239 $\pm$ 141.683	4650.454 $\pm$ 92.721
	6442.240 $\pm$ 133.767	6140.470 $\pm$ 127.508	5715.515 $\pm$ 137.623	6356.796 $\pm$ 131.984	5050.618 $\pm$ 104.868
Mg	2999.393 $\pm$ 62.265	2867.682 $\pm$ 96.032	2683.024 $\pm$ 80.223	2555.288 $\pm$ 70.615	2352.734 $\pm$ 46.907
	3079.862 $\pm$ 63.940	2971.613 $\pm$ 61.695	2799.901 $\pm$ 67.421	2766.423 $\pm$ 57.433	2469.910 $\pm$ 51.279
Mn	234.855 $\pm$ 4.859	221.854 $\pm$ 7.403	205.054 $\pm$ 6.108	186.191 $\pm$ 5.123	165.158 $\pm$ 3.279
	240.070 $\pm$ 4.978	228.831 $\pm$ 4.746	213.009 $\pm$ 5.103	207.958 $\pm$ 4.293	198.243 $\pm$ 4.103
Na	3822.108 $\pm$ 79.358	3609.962 $\pm$ 120.901	3336.643 $\pm$ 99.775	3097.795 $\pm$ 85.608	2999.062 $\pm$ 59.797
	3952.970 $\pm$ 82.076	3767.843 $\pm$ 78.223	3507.123 $\pm$ 84.427	3423.184 $\pm$ 71.068	3262.827 $\pm$ 67.746
Zn	75.519 $\pm$ 1.552	68.554 $\pm$ 2.271	60.894 $\pm$ 1.782	55.443 $\pm$ 1.517	51.641 $\pm$ 1.013
	77.832 $\pm$ 1.596	71.291 $\pm$ 1.473	63.742 $\pm$ 1.516	59.815 $\pm$ 1.225	54.838 $\pm$ 1.110

concentrations in both leaves and stems was observed at 10 μM , whereas a substantial increase in stem B levels was evident at the 50 μM , while the decline of B in leaf tissues persisted. Ca plays a pivotal role in a variety of physiological processes, including cell division, plant growth, and photosynthesis. The interaction between Ni and Ca has been demonstrated to enhance nutrient balance and gas exchange

in tomatoes. Certain levels of Ni (0.5–1.0 mg/dm^3) have the capacity to induce enzymatic responses that result in the reduction of blossom-end rot, a physiological disorder that affects fruit formation (Macedo et al. 2022). Furthermore, it has been documented that Ca protects plants against Ni toxicity by specifically enhancing the antioxidant defense mechanisms (Aqeel et al. 2022). These results suggest that

Table 6 Percentage changes in washed (upper rows) and unwashed (lower rows) leaf mineral nutrient levels under $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ treatments (10–100 μM) relative to control

Treatment	B	Ca	Cu	Fe	K	Mg	Mn	Na	Zn
10 μM	−5.543	1.387	−5.474	−7.689	−5.549	−4.391	−5.548	−5.549	−9.218
	−4.816	2.316	−4.334	−6.843	−4.684	−3.516	−4.674	−4.685	−8.402
25 μM	−14.477	−3.341	−14.546	−18.037	−14.679	−10.548	−12.689	−12.702	−19.352
	−11.244	−0.906	−10.741	−15.254	−11.281	−9.092	−11.252	−11.281	−18.055
50 μM	−20.339	−10.047	−16.413	−22.853	−19.722	−14.807	−20.727	−18.952	−26.576
	−17.108	−3.724	−12.455	−19.156	−15.820	−10.179	−13.386	−13.404	−23.156
100 μM	−31.571	−17.482	−23.111	−30.921	−27.172	−21.560	−29.687	−21.534	−31.678
	−25.906	−1.495	−16.460	−27.646	−24.675	−16.712	−17.419	−17.459	−29.621

Table 7 Percentage changes in washed (upper rows) and unwashed (lower rows) stem mineral nutrient levels under $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ treatments (10–100 μM) relative to control

Treatment	B	Ca	Cu	Fe	K	Mg	Mn	Na	Zn
10 μM	−5.259	1.386	−5.414	−7.690	−5.550	−4.391	−5.536	−5.551	−9.223
	−4.501	2.316	−4.456	−6.843	−4.684	−3.515	−4.682	−4.683	−8.404
25 μM	−14.297	−3.341	−14.449	−18.038	−14.680	−10.548	−12.689	−12.701	−19.366
	−10.592	8.620	−11.118	−15.255	−11.281	−9.090	−11.272	−11.279	−18.103
50 μM	−19.989	−10.047	−16.238	−22.853	−19.721	−14.806	−20.721	−18.951	−26.584
	19.711	−1.674	−6.353	−19.156	−1.326	−10.177	−13.376	−13.402	−23.148
100 μM	−31.532	−17.482	−22.947	−30.921	−27.172	−21.560	−29.677	−21.534	−31.619
	−19.897	0.603	−10.708	−25.039	−21.602	−19.805	−17.423	−17.459	−29.542



A. reineckii samples may employ a protection mechanism through enhanced Ca accumulation. In addition, Cu has been demonstrated to play a key role in several significant stages, including its participation in the structure of enzymes, cell wall metabolism, photosynthesis, respiration and antioxidant systems in plants (Chen et al. 2022). The increase in Cu level at 50 μM exposure suggests that the plant develops defense responses against Ni-induced damage. B is an essential element for growth, development and reproduction, and is responsible for the elasticity of the cell wall by taking part in cell wall biosynthesis (Vera-Maldonado et al. 2024). The findings of a previous study revealed a significant decrease in B concentration in the medium was observed for *L. minor* (39%) in Ni treatments, while no change was observed for the other two species, *S. polyrhiza* and *L. punctata* (99–101%) (Oláh et al. 2023). Moreover, the same study revealed a significant decrease in Mn levels ranging from 45 to 55% in all plants following Ni exposure. Conversely, a decline in Mn value was detected in the present study, accompanied by variations in B concentration. Fluctuations in the B level may indicate that cell wall and growth processes are also affected by the Ni exposure. Furthermore, a substantial decline in essential elements such as Fe, K, and Zn suggests that Ni has a detrimental effect on the plant's nutrient uptake and metabolic processes. It has been proposed that Ni uptake in Ni hyperaccumulators is due to the sustained high expression of a structurally altered Zn transporter that shows high affinity for Ni and low affinity for Zn (Mohtadi and Schat, 2024). In this study, it was clearly demonstrated that Zn accumulation decreased in response to increasing Ni accumulation. Overall, the mineral profile of both leaves and stems demonstrates that exposure to heavy metals profoundly alters the nutritional balance of *A. reineckii*.

The MANOVA analysis revealed that $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ exposure significantly affected multiple mineral nutrient variables simultaneously ($p < 0.01$). However, no distinct clustering patterns among the treatment groups were observed. A

Pearson's correlation analysis was conducted to ascertain the relationships between the elements present in the plant following the Ni exposure. The correlation matrix of elements quantified in the tissues of *A. reineckii* reveals a consistent, moderate negative relationship between Ni and all other elements. Particularly, the correlations between Ni-Fe (-0.542), Ni-Zn (-0.534), Ni-K (-0.515), Ni-Mn (-0.497), and Ni-Na (-0.466) are moderately negative and statistically significant. (Table 8). The findings indicate that increasing Ni accumulation significantly suppresses the uptake of these elements. On the other hand, strong positive correlations are observed between Fe-Zn (0.990) and Mn-Zn (0.980). Such high correlation coefficients imply a coordinated behavior among Fe, Mn and Zn, potentially reflecting shared physiological pathways or common regulatory mechanisms governing their uptake and distribution. Within this framework, it is essential to thoroughly investigate the interactions between mineral nutrients in plants under Ni exposure in terms of stress response, ion transport dynamics and the regulation of metabolic pathways.

Phytoremediation related factors: BCF, TF, BAI, SA, SAE, RE, WC

As one of the principal phytoremediation indicators, BCF values varied among leaves, stems, and total biomass under different Ni concentrations. In this study, BCF values were calculated separately for leaves, stems and the total plant. The BCF values were determined as 4, 5, 4, 4 in leaves; 3, 4, 3, 3 in stems; and 6, 9, 7, 7 in the total plant under 10, 25, 50 and 100 μM Ni treatments, respectively. These results indicate that the BCF values of leaves, stems, and the total plant reached their maximum levels at 25 μM Ni treatment (Table 9). A noticeable decline in BCF with increasing Ni concentrations, particularly after 25 μM exposure, suggests that metal uptake may be limited by concentration-dependent physiological constraints and/or a potential saturation effect in Ni accumulation (Wang et al. 2022). Previous

Table 8 Pearson correlation matrix of elemental concentrations in plant tissues

	B	Ca	Cu	Fe	K	Mg	Mn	Na	Zn
Ni	-,376**	-,135	-,252*	-,542**	-,515**	-,402**	-,497**	-,466**	-,534**
B		,873**	,937**	,894**	,954**	,924**	,921**	,911**	,896**
Ca			,954**	,857**	,861**	,933**	,904**	,892**	,856**
Cu				,937**	,945**	,974**	,949**	,960**	,929**
Fe					,980**	,977**	,981**	,990**	,990**
K						,973**	,971**	,977**	,967**
Mg							,979**	,985**	,971**
Mn								,988**	,980**
Na									,987**

** . Correlation is significant at the 0.01 level (2-tailed)

* . Correlation is significant at the 0.05 level (2-tailed)



Table 9 Phytoremediation parameters

Treatment	Bioaccumulation index (l/s/t)	Bioconcentration factor (l/s/t)	%Removal efficiency (w)	Surface adsorption (l/s/t)	Surface adsorption efficiency (l/s/t)	Translocation factor (l/s)	Water content (l/s/t) (%)
10 μ M	5.1 /5.5 /5.2	4 /3 /6	8	1 /1 /2	62 /28/90	1.26	93.3 /92.6 /92.7
25 μ M	20.8 /20.1 /20.5	5 /4 /9	9	3 /4 /7	44 /74 /118	1.38	92.5 /92.6 /92.2
50 μ M	31.9 /36.7 /33.9	4 /3 /7	9	4 /6/10	31 /53 /84	1.17	93.9 /93.1 /93.2
100 μ M	59.7 /72.0 /64.9	4 /3 /7	10	7 /7 /14	29 /29 /59	1.12	92.6 /92.2 /92.2

The 'l/s/t/w' format indicates the values for leaves, stem, total plant and water, respectively

research on the related species, *Alternanthera philoxeroides*, reported high heavy metal accumulation with BCF values exceeding 5, consistent with the present findings (Nan et al. 2013). However, reported BCFs for Ni demonstrate considerable variation in the literature. For instance, several studies have documented that *Typha latifolia* L., a native aquatic macrophyte, displays BCF values ranging from moderate (< 1) to exceptionally high (> 2000) (Ben Salem et al. 2017; Haghazadeh et al. 2021). A recent investigation of *T. angustifolia* samples collected from the Chenab River reported a BCF value of 76.12 for Ni, while the same species obtained from the Ravi River exhibited a slightly lower BCF value of 66.58 (Afzaal et al. 2024). This wide variation indicates that Ni accumulation capacity is strongly influenced by species-species traits, exposure concentration, environmental conditions, and sampling site characteristics. Furthermore, it has been documented that *Pistia stratiotes* has been reported to reach a maximum BCF of $884.7 \text{ L kg}^{-1} \text{ DW}$ at a Ni concentration of 0.75 mg L^{-1} ; this result is considerably higher than the values determined in the present study (Senturk et al. 2023).

BAI values were calculated as 5.1, 20.8, 31.9, and 59.7 in leaves; 5.5, 20.1, 36.7, and 72.0 in stems; and 5.2, 20.5, 33.9, and 64.9 in the total plant in the 10, 25, 50, and 100 μM $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ treatment groups, respectively (Table 9). The BAI values ranging from 5.1 to 64.9 demonstrated a pronounced increase in response to elevated Ni exposure. The 100 μM treatment resulted in the highest BAI value, suggesting that the plant accumulated

The 10 μM $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ treatment group exhibited the lowest RE at 8%, while the highest RE was observed under the 100 μM treatment (10%). The RE values for both the 25 μM and 50 μM treatments were identical, recorded as 9% (Table 9). These findings suggest that an increase in Ni concentration does not necessarily enhance removal from water, indicating a possible saturation effect or limited uptake efficiency, which aligns with Monero-Rubio et al. (2022), who reported RE values for Ni ranging from 50 and 68% for *L. minor* and *E. crassipes*. In contrast, *Potamogeton malaianus*

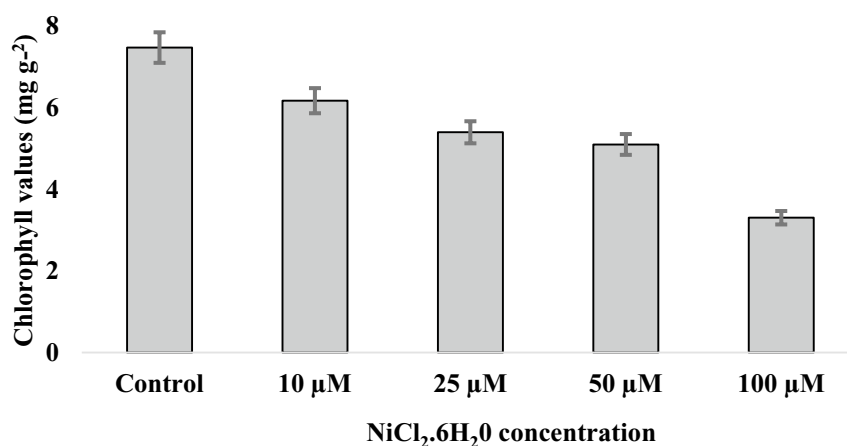
demonstrated a substantially higher RE exceeding 94%, indicating that the capacity for Ni uptake varies among different aquatic plant species (Lyu et al. 2021).

With increasing Ni concentration, the SA values increased, reaching 1, 3, 4, and 7 in leaves; 1, 4, 6, and 7 in stems; and 2, 7, 10, and 14 in the total plant. On the other hand, the SAE was calculated as 62, 44, 31, and 29 in leaves; 28, 74, 53, and 29 in stems; and 90, 118, 84, and 59 in the total plant for the 10, 25, 50, and 100 μM treatments (Table 9). Both SA and SAE varied across treatments, with the highest SA (14) detected in the total plant at 100 μM , while the highest SAE (118) was observed in the total plant at 25 μM .

The WC values remained relatively stable across treatments, ranging from 92.2% to 93.9%, indicating that Ni exposure did not exert a substantial influence on the plant's water retention capacity. Among the TF values calculated to obtain information about Ni translocation between plant parts, the highest value was observed in the 25 μM $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ treatment group with a value of 1.38, indicating moderate translocation from stems to leaves. (Table 9). In other groups, the results were 1.26, 1.17, and 1.12, for the treatments with 10 μM , 50 μM , and 100 μM $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, respectively. The TF values greater than 1 are generally interpreted as an indication of effective aboveground translocation and phytoextraction potential, rather than being sufficient alone to classify a plant as a hyperaccumulator (Ghafoori et al. 2022). The findings of this study demonstrate that *A. reineckii* samples exhibited TF values greater than 1 across all tested concentrations, thereby indicating the ability of the plant to translocate Ni efficiently from stems to leaves. This attribute suggests that *A. reineckii* may have potential for phytoextraction-based phytoremediation of Ni-contaminated aquatic environments. In contrast, *P. stratiotes* displayed TF values below 1, emphasizing its efficacy in phytostabilization (Galal et al. 2018). Complementing these findings, *Suaeda maritima* was investigated for its phytoremediation potential in the Khorkhoran International Wetland (Baharvand et al. 2023). The results indicated that higher



Fig. 3 Total chlorophyll levels in samples under different $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ treatments



concentrations of Ni (3.34 mg kg^{-1}) were present in the leaves compared to roots, demonstrating active translocation. In addition, $\text{TF} > 1$, further confirmed its potential for Ni phytoextraction.

Total chlorophyll content

Chlorophyll plays a crucial role in photosynthesis; therefore, quantifying total chlorophyll content is a key parameter for understanding the physiological state of plants following heavy metal exposure (Sitarska et al. 2023). The results of the total chlorophyll analyses are presented in Fig. 3. The data show a progressive decline in chlorophyll content with increasing $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ concentrations, decreasing from 7.47 mg m^{-2} in the $10 \mu\text{M}$, 5.4 mg m^{-2} in the $25 \mu\text{M}$, 5.09 mg m^{-2} in the $50 \mu\text{M}$, and 3.3 mg m^{-2} in the $100 \mu\text{M}$ group. These findings clearly show that chlorophyll levels decrease under increasing $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ exposure, demonstrating the negative impact of Ni stress on plant growth and development. This decline may be attributed to several mechanisms, including reduced Fe content, diminished efficiency of enzymes involved in chlorophyll biosynthesis, and the substitution of central Mg^{2+} ions within chlorophylls by heavy metals (Uruc Parlak 2016).

Total chlorophyll content in plants has been shown to be a reliable indicator of heavy metal tolerance and overall photosynthetic activity. Ni exposure has been reported to impair photosynthetic processes by reducing CO_2 assimilation and Fe uptake. An increase in Ni concentration has been shown to result in a decrease in chlorophyll content by inhibiting the activities of enzymes involved in chlorophyll biosynthesis and indirectly affecting chlorophyll production (Zaid et al. 2019; Heidari et al. 2020). Furthermore, Ni toxicity has been shown to reduce the net photosynthetic rate through alterations in photosynthetic pigment concentration, stomatal conductance, transpiration rate and water-use efficiency.

It has also been reported that chlorophyll a decreased by up to 73%, chlorophyll b by 58% and carotenoids by 49% in plants under Ni exposure (Ahmad et al. 2023; Kovacik and Vydra 2024). The findings of the present study align with the existing literature, showing a decrease in total chlorophyll content from 7.47 in the control group to 3.3 at $100 \mu\text{M}$ Ni treatment.

During the experiments, it was observed that as Ni concentration increased, older leaves exhibited chlorosis due to chlorophyll loss and subsequently fell off. This response suggests that, under stress, the plant limits the uptake of minerals and nutrients from the external environment and instead remobilizes minerals from older leaves to younger parts (Maillard et al 2015; Hu et al 2019). This interpretation is consistent with the ICP-OES results, which indicate a protective physiological adjustment by the plant.

ISSR data analysis

The genotoxic effects of heavy metals on genetic material can be assessed by examining alterations in DNA banding profiles. This approach employs PCR-based molecular markers (e.g. RAPD, AFLP and ISSR) to evaluate genotoxic effects in plants by observing the appearance of new bands and the loss or gain of bands or increases/decreases in band densities in DNA profiles. This approach enables the estimation of genomic template stability (Yazicioglu et al. 2024; Sorrentino et al. 2017). DNA damage and changes in the efficiency of DNA repair and replication processes can affect genomic stability. In this study, genomic DNA samples were amplified with 20 different ISSR primers, since ISSRs are more reliable than RAPD due to their longer primers and higher stringency. This reduced the risk of misinterpretation caused by nonspecific amplification or DNA contamination (Ozyigit et al. 2021a).



Table 10 Molecular sizes and intensities of obtained bands using ISSR primers under different $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ exposure

Primers	Molecular size of bands	Control	10 μM	25 μM	50 μM	100 μM
UBC812	869	8.74	13.22	8.62	8.51	8.33
	646	22.64	36.66	23.76	37.72	31.53
	497	11.22	10.25	11.57	10.57	10.53
UBC814	1340	13.33	13.82	13.25	14.40	14.53
	1050	13.18	13.14	13.92	13.21	13.42
	895	26.37	24.43	23.75	32.46	37.24
	765	17.48	16.97	18.06	19.53	20.05
	630	35.91	29.45	28.21	60.63	89.19
	505	13.72	13.47	13.36	13.10	13.09
	365	13.30	13.30	13.88	13.60	13.80
UBC825	1054	11.32	21.02	11.88	13.45	10.40
	861	43.51	76.05	35.12	56.92	35.63
	733	39.89	32.80	21.16	31.58	24.25
	646	24.86	21.55	18.15	26.07	20.49
	573	17.05	14.80	14.63	17.02	12.13
UBC827	457	10.92	9.82	10.44	10.85	10.87
	767	38.35	38.88	46.61	43.04	48.80
	615	39.18	33.05	32.57	27.63	39.73
	443	45.13	39.10	39.24	38.52	45.36
UBC834	944	14.75	22.12	8.43	13.72	14.20
	701	12.64	16.28	8.60	14.49	18.83
	588	65.92	66.19	34.61	61.95	57.03
	482	35.15	36.37	33.66	36.71	18.06
	391	24.86	23.32	14.96	22.77	23.05

Of the 20 ISSR primers selected for molecular analysis, UBC 812, UBC 814, UBC 825, UBC 827, and UBC 834 generated the most optimal results, producing clear and distinguishable band patterns. The band profiles obtained with these primers and the change percentages in these profiles are presented in Tables 10 and 11. Increasing concentrations of $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ led to noticeable variations in band density. All five primers exhibited increases in band intensity. Primer UBC 814 showed the most pronounced increase at 630 bp under the 100 μM treatment and the least pronounced increase at 365 bp under the 10 μM treatment.

The primer UBC 812 generated clear and distinguishable band profiles, with fragment sizes ranging from 497 to 869 bp (Fig. 4a). Although no new bands or band losses were observed, variations in band intensity were detected across all treatment groups. The highest increase in band intensity was observed at 50 μM Ni concentration at 646 bp, whereas the lowest was recorded at 497 bp under 25 μM treatment. Additionally, an increase in band intensity was observed at 646 bp across all Ni concentrations. Conversely, an increase was detected only at 869 bp and 497 bp under 10 μM and 25 μM , respectively. Moreover, the decline in band intensity

was observed at 497 bp under the 10, 50, and 100 μM , and at 869 bp under 25, 50, and 100 μM Ni treatments.

The UBC 814 primer produced well-defined and clearly distinguishable band profiles, with fragment sizes ranging from 465 to 1340 bp (Fig. 4b). Although no new bands or band losses were detected; notable variations in band intensity were observed in all experimental groups. The strongest increase in band intensity was observed at 630 bp under the 100 μM treatment, while the smallest increase was recorded at 365 bp under the 10 μM treatment. A consistent increase in band intensity was detected only at 365 bp, while a consistent decrease was observed at 505 bp. At 630 and 895 bp, decreases in band density were detected at 10 and 25 μM , followed by increases at 50 and 100 μM . At 1340 bp, a decrease was detected only at 25 μM , while at 1050 bp and 765 bp, it was detected only at 10 μM .

The UBC 825 primer produced clear and distinguishable band profiles, with fragment sizes ranging from 457 to 1054 bp (Fig. 4c). No new bands or band losses were observed. The highest increase in band intensity was observed at 1054 bp under 10 μM Ni treatment, while the lowest was recorded at 50 μM at 646 bp. Consistent



Table 11 Changes in band intensity (%) using ISSR primers under different Ni exposure levels

Primers	Molecular size of bands	Band intensity changes (%) in 10 μ M	Band intensity changes (%) in 25 μ M	Band intensity changes (%) in 50 μ M	Band intensity changes (%) in 100 μ M
UBC812	869	51,26(+)	1,42(−)	2,59(−)	4,74(−)
	646	61,94(+)	4,98(+)	66,62(+)	39,31(+)
	497	8,62(−)	3,10(+)	5,80(−)	6,15(−)
UBC814	1340	3,71(+)	0,60(−)	8,04(+)	9,04(+)
	1050	0,29(−)	5,55(+)	0,23(+)	1,81(+)
	895	7,37(−)	9,93(−)	23,07(+)	41,22(+)
	765	2,94(−)	3,27(+)	11,69(+)	14,66(+)
	630	17,99(−)	21,43(−)	68,86(+)	148,38(+)
	505	1,86(−)	2,63(−)	4,51(−)	4,60(−)
	365	0,02(+)	4,32(+)	2,21(+)	3,74(+)
	1054	85,74(+)	4,95(+)	18,83(+)	8,15(−)
UBC825	861	74,80(+)	19,27(−)	30,83(+)	18,10(−)
	733	17,76(−)	46,96(−)	20,82(−)	39,21(−)
	646	13,31(−)	27,02(−)	4,85(+)	17,59(−)
	573	13,18(−)	14,20(−)	0,16(−)	28,85(−)
	457	10,10(−)	4,41(−)	0,61(−)	0,42(−)
	767	1,36(+)	21,54(+)	12,21(+)	27,25(+)
UBC827	615	15,67(−)	16,88(−)	29,48(−)	1,38(+)
	443	13,37(−)	13,04(−)	14,63(−)	0,51(+)
	944	49,89(+)	42,88(−)	7,05(−)	3,75(−)
UBC834	701	28,83(+)	31,95(−)	14,71(+)	49,04(+)
	588	0,41(+)	47,49(−)	6,01(−)	13,48(−)
	482	3,45(+)	4,25(−)	4,43(+)	48,63(−)
	391	6,18(−)	39,83(−)	8,39(−)	7,26(−)

Plus (+) and minus (−) indicate percentage increase and decrease, respectively, in comparison with the control group

reductions in intensity were noted at 457, 573, and 733 bp across all Ni concentrations. Increases at 646 bp were observed only at 50 μ M, at 861 bp at 10 and 50 μ M, and at 1054 bp at 10, 25, and 50 μ M.

The UBC 827 primer produced well-defined band profiles, with fragment sizes ranging from 443 to 767 bp (Fig. 4d). No new bands or band losses were observed. The highest increase in band intensity was observed at 767 bp under 100 μ M treatment, while the lowest was recorded at 100 μ M at 443 bp. The band intensity at 767 bp exhibited an increase at all concentrations, while the band intensities at 615 and 443 bp demonstrated an increase only at 100 μ M.

The UBC 834 primer generated clear and distinguishable band profiles, with fragment sizes ranging from 391 to 944 bp (Fig. 4e). No new bands or band losses were observed. The highest increase in band intensity was observed at 944 bp under 10 μ M Ni treatment, while the lowest was recorded at 588 bp under 10 μ M treatment. As Ni concentration increased from 10 to 25 μ M, a general decrease in band intensity was observed across all base

pairs. At 10 μ M, declines were detected at all fragments except 391 bp, which remained unchanged. In contrast, at 944 bp and 588 bp, the band intensities were reduced at 50 and 100 μ M, whereas the 701 bp band exhibited increased intensity at the same concentrations. Additionally, the 482 bp fragment demonstrated increased intensity at 50 μ M but decreased again at 100 μ M.

Overall, the five primers examined (UBC 812, UBC 814, UBC 825, UBC 827 and UBC 834) produced clear and distinct banding patterns. Observable variations in band intensity indicated potential Ni-induced genomic alterations. Among the primers, UBC 814 exhibited the most significant increase in band intensity, particularly at elevated Ni concentrations. Conversely, UBC 825 and UBC 834 demonstrated a general decline in band intensity, which may be attributable to their heightened sensitivity to Ni exposure.

In this study, DNA banding patterns from control and Ni-treated plants were compared to evaluate polymorphism and GTS in *A. reineckii*. Analyses of primer profiles showed 0% polymorphism and a GTS value of 100% (Table 12). The results indicate that *A. reineckii* responds to Ni exposure



Fig. 4 ISSR primer band profiles under different Ni concentrations (a = Intensity increase b = Intensity decrease). (4a: UBC812, 4b: UBC814, 4c: UBC825, 4d: UBC827, 4e: UBC834)

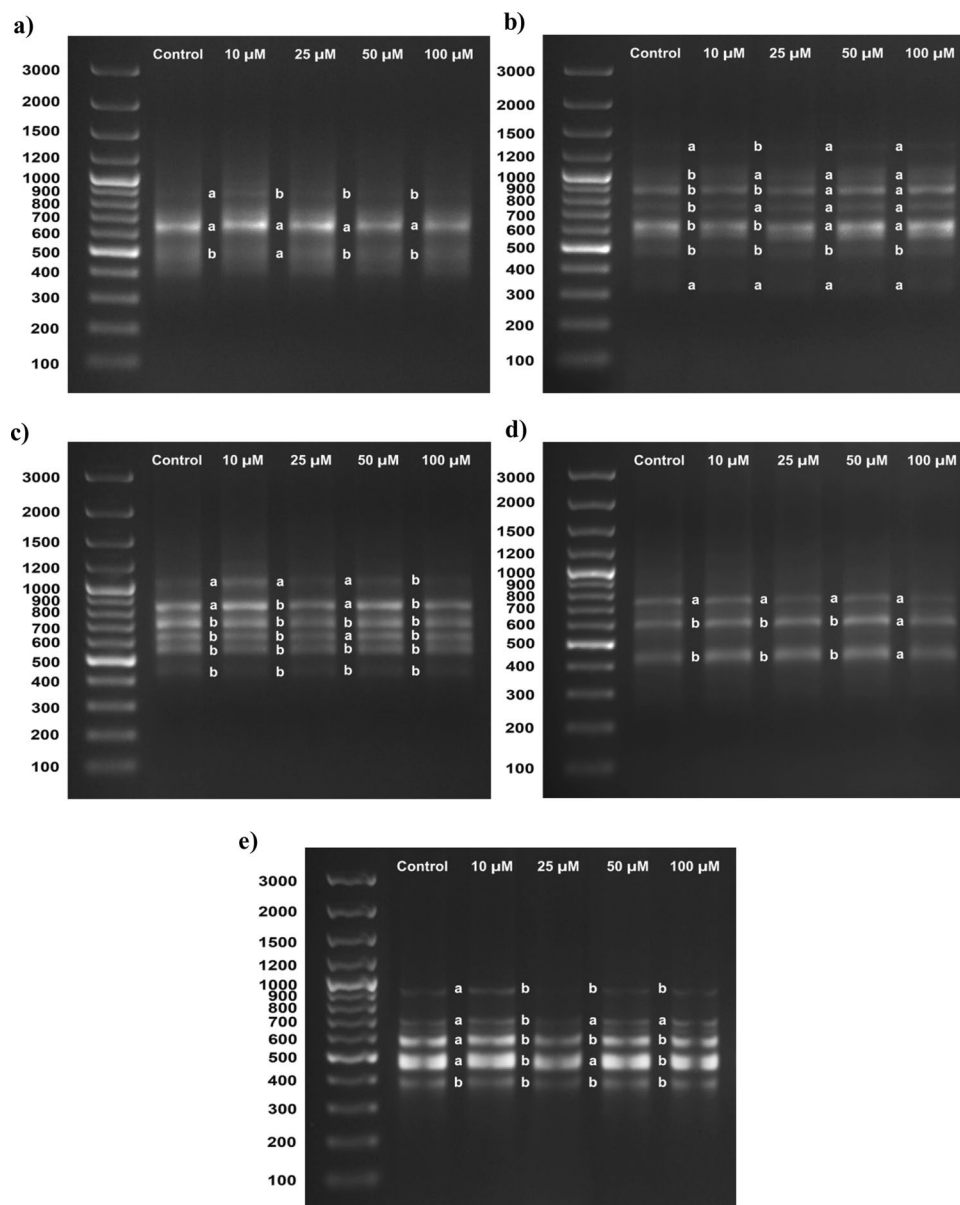


Table 12 Polymorphism and GTS in *A. reineckii* exposed to $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, based on individual ISSR primers and combined primer analysis (TB = Total bands, PB = Polymorphic bands)

Primer	0 μM	10 μM		25 μM		50 μM		100 μM		Polymorphism (%)	GTS (%)
	TB	TB	PB	TB	PB	TB	PB	TB	PB		
UBC812	3	3	0	3	0	3	0	3	0	0	100
UBC814	7	7	0	7	0	7	0	7	0	0	100
UBC825	6	6	0	6	0	6	0	6	0	0	100
UBC827	3	3	0	3	0	3	0	3	0	0	100
UBC834	5	5	0	5	0	5	0	5	0	0	100
Total	24	24	0	24	0	24	0	24	0	0	100



in a relatively limited manner at the genomic level, maintaining its genomic stability with minimal polymorphism. These findings align with previous studies on the aquatic plant *L. minor*, which exhibited high GTS and low polymorphism percentages, as well as significant intensity changes in response to Cd and Ni co-exposure (Ozyigit et al. 2021a). Similarly, a study on *Azolla pinnata* and *L. minor*, two different aquatic macrophytes, reported increases in band intensity under Mn exposure, (Md Nasir et al. 2024) although *L. minor* exhibited 19% polymorphism. This finding suggests that *L. minor* exhibits a higher degree of genomic responder under Mn exposure.

In addition to the use of plants individually in the removal of heavy metals, it has been demonstrated that plant combinations can also enhance RE. For instance, the combination of *Phragmites australis* and *T. latifolia* was found to enhance Ni removal rates by up to 61% (Kumari and Tripathi 2015). Furthermore, studies have emphasized the hyperaccumulation potential of *Salvinia minima* and *Potamogeton alpinus*, respectively, with high BCF values exceeding 1000 (Borisova et al. 2014; Fuentes et al. 2014). In the present study, 10% Ni removal was calculated for *A. reineckii* in freshwater systems on day 10, with a considerably higher BCF value of 6.82 in the total plant. The results of this study suggest that while the tested species demonstrates promising phytoremediation capabilities, its efficiency may be further improved by optimizing strategies such as combined plant cultures or modifying environmental conditions.

Conclusion

This study highlights *Alternanthera reineckii* Briq. (var Lilacina) as a species with a considerable capacity for Ni accumulation and internal translocation, along with notable genomic stability (100%) under Ni stress, as shown through ISSR marker analysis. Despite fluctuations in band intensity, no new bands or band losses were detected, indicating genomic stability and limited genotoxic effects at the tested concentrations. This stability implies that *A. reineckii* maintains its DNA integrity even under heavy metal exposure, which is a critical factor for its survival and adaptation in polluted habitats.

Shifts in mineral nutrient levels, particularly Ca, Cu, and B, may reflect adaptive physiological responses that could contribute to mitigating Ni toxicity. The observed decreases in essential elements such as Fe, K, and Zn underscore the disruptive impact of Ni on nutrient uptake and metabolic functions. Additionally, reductions in total chlorophyll content and photosynthetic activity further illustrate the physiological stress imposed by Ni.

The measured BCFs and TFs confirm that *A. reineckii* has promising potential for phytoremediation, as it effectively accumulates and mobilizes Ni within its tissues. Indicators such as BAI and RE data also highlight the plant's capacity to tolerate and remove Ni from contaminated environments, though saturation effects at higher concentrations suggest a limit to uptake capacity.

Although *A. reineckii* does not exceed the classical hyperaccumulator threshold for Ni ($>1000 \text{ mg kg}^{-1}$ in shoot tissues), its BCF values are higher than those reported for some species considered effective in phytoremediation studies. This finding suggests that, despite not meeting hyperaccumulator criteria, *A. reineckii* demonstrates efficient Ni uptake and translocation capabilities that are relevant to practical phytoremediation applications.

Overall, the findings indicate that *A. reineckii* displays moderate Ni accumulation and limited RE, which vary depending on exposure concentrations. The observed BCF and TF values further confirm that this species possesses considerable potential for Ni phytoremediation in contaminated freshwater ecosystems. To further enhance the efficacy of Ni removal, future research should focus on optimizing growth conditions and investigating the potential synergistic effects of mixed plant cultures. Moreover, given the paucity of studies on Ni-induced genotoxicity in aquatic plants using ISSR markers, the results of the present study may contribute to future research and the existing literature in this field.

In conclusion, these results provide valuable insights into the physiological and genetic responses of *A. reineckii* to Ni exposure, reinforcing its potential use as a sustainable and effective agent for phytoremediation. Further investigations are encouraged to elucidate the molecular basis of Ni tolerance and to evaluate field-scale applications to maximize ecological benefits.

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Author contributions B.N.E. conducted the experiments, performed the data analysis, prepared the figures, and wrote the main text, A.H.O., Z.M., H.Y. and I.E.Y. assisted in conducting the experiments and performed the data analysis, I.I.O. conceptualized the study, wrote, edited, and revised the manuscript. All authors made contributions in revisions and critical discussions for the manuscript.

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Data availability The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.



Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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