Advances in heavy metals detoxification, tolerance, accumulation mechanisms, and properties enhancement of *Leersia hexandra* Swartz

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**ABSTRACT**

Heavy metal (HM) pollution is increasingly becoming a serious threat to public and environmental health with more-than-ever rapid industrialization and urbanization activities. Phytoremediation is a sustainable and largely accepted technology because of its low cost, simple operation, environmental safety and recognized as a promising approach for environmental remediation applications. Hyperaccumulator plants are the core of phytoremediation, and the study of their accumulation, detoxification, and HM tolerance mechanisms is fundamental to the progress of phytoremediation. In-depth investigations to understand the physiochemical and dissipation pathways of hyperaccumulators such as *Leersia hexandra* Swartz (*L. hexandra*) which can serve as a useful tool in environmental remediation applications. *L. hexandra* as a chromium hyperaccumulator plant, can also be a remarkable choice to remediate copper and nickel contaminated soils. Therefore, this article summarizes the previous studies on the detoxification strategies/tolerance mechanisms and the enhancement of the properties of *L. hexandra*, which will inspire its future applications in the sustainable environmental cleanup initiatives.

**1. Introduction**

The emergence of rapid industrialization, urbanization, and agriculture, the widespread global plight of soil pollution by heavy metals (HMs) has become a serious environmental concern. Soil remediation, is comparably challenging in comparison to air and water pollution, due to extended biological half-lives, non-biodegradable nature, toxicity, and persistence of residing HMs (Liu et al. 2021a). The bioaccumulation of HMs in food chains via agricultural crops/products poses extensive toxicological implication on the agroecological ecosystems and the subsequent, exposure to living resources in all trophic levels (Ashraf et al. 2019). Therefore, HMs contamination is a widespread challenge with considerable impact on global soil ecosystems (Gavrilescu 2022). The commonly detected HMs with high density include Ni, Pb, Cd, Cr, As, Cu, Zn, Al, and Hg (Pasricha et al. 2021).

Nowadays, the methods generally used to remediate HMs polluted soil can be divided into physicochemical and biological treatments (O’Sullivan et al. 2019). The physicochemical methods include soil replacement, isolation, cleaning, thermal treatment, electrochemical methods, and addition of chemical amendments. However, these techniques have numerous disadvantages such as high cost, irreversible damage to the soil geochemical structure and non-targeted soil biota, as well as might end up in secondary soil pollution (Saeedi et al. 2013; Di Palma et al. 2015; Pasricha et al. 2021). Therefore, these traditional physicochemical methods are not much competent in addressing the HMs pollution. Phytoremediation is a cost-efficient technology with appealing outcomes to recover HMs than other biotechnologies such as microbial and animal remediation, and has a lower disruption in the soil microenvironments (Antoniadis et al. 2017; Yaashikaa et al. 2022). Various studies have demonstrated phytoremediation as a promising green technology to advocate environmental pollution (Gupta et al. 2016). Numerous removal pathways such as absorption, volatilization, root filtration, degradation, and stabilization are the distinctive operatives of phytoremediation technology, which can steadily remove pollutants from soil matrices (Khalid et al. 2017; Khan et al. 2019; Patra et al. 2019; Tiwari et al. 2019). Therefore, this technology could be formidable in achieving the large scale and sustainable environmental remediation projects (Liu et al. 2020; Nedjimi 2021).

Hyperaccumulator plants are capable to accumulate a substantial amount of HMs in their aerial organelles (Pasricha et al. 2021). The concept of ‘hyperaccumulators’ was firstly introduced by Brooks et al. (1977) and these are distinguished from non-hyperaccumulating taxa in many ways (Rascio and Navari-Izzo 2011). For example, their rigorous capacities migrate HMs from rhizosphere to plant tissues to considerably reduce their bioavailability in soil (DalCorso et al. 2019). In general, excessive metal concentrations have minimal impacts on the normal metabolic activities and physiological functions of specifically cultivated hyperaccumulators with no radical growth inhibition symptoms (Yaashikaa et al. 2022). The tolerance and accumulated capacity of plants to heavy metals are mediated through a combination of molecular and physiological mechanisms, different plants exhibit different mechanisms of heavy metal tolerance, and a detailed understanding of these interrelated...
mechanisms is necessary for the development of phytoremediation media (Feng et al. 2021). The mechanisms of metal hyperaccumulation mainly include: stimulation of metal uptake in roots, reduction of metal sequestration in root vesicles, high efficiency of xylem loading and xylem transport, and metal sequestration in leaves. The mechanisms of metal hyperaccumulation mainly include: uptake of heavy metals by roots, chelation and transfer of metals in root vesicles, xylem loading and transport, and chelation and separation of metals in leaves, and enzymatic reactions in the cytoplasm (Verbruggen et al. 2009).

There are nearly 721 reported HMs hyperaccumulator plants species, with 72.5% (523 species) exhibiting cardinal potential for nickel dissipation, followed by others Cu, Cd, As, Mn, Pb, Zn, and Cr (Reeves et al. 2018). As majority of present soil pollution is deemed as compound pollution, therefore, plants with inclusive hyperaccumulation endurance are more advantageous. For example, Sedum alfredii Hance has appeared to exhibit remarkable Zn/Cd hyperaccumulator (Xv et al. 2020; Deng et al. 2007), and Arabis paniculata. L. with simultaneous Pb/Zn/Cd hyperaccumulation merits (Tang et al. 2005; Liu et al. 2022a).

The polymetallic-accumulated plant L. hexandra Swartz is a perennial herb with radiant potential to hyperaccumulate Cr, Cu, as well as Ni, and widely distributed near waterfronts and marshes in Southern China (Zhang et al. 2006). L. hexandra was well suited to maintain normal metabolic growth levels in the ambient environment with escalated levels of above-stated HMs (Chen et al. 2008). This suggested that it can be an expedit phytoremediation candidate against Cr, Cu, and Ni. Therefore, this review provides a valuable perspective on exploring the strategies to manifest the uptake, translocation and sequestering potential of L. hexandra against a Cr, Cu, and Ni. Moreover, it also highlights the challenges, opportunities, and the future prospects for improved phytoremediation of HMs.

2. HMs hyperaccumulation in L. hexandra

2.1. Cr accumulation

In the soil environment, Cr exists in two oxidation states, i.e. Cr (VI) and Cr (III), with the latter proven more stable, and at least 100 times more toxic (Pasricha et al. 2021). High concentrations of Cr (III) could inhibit growth in plants, restrain CO₂ uptake, impede chlorophyll synthesis, and chlorosis (Narendrula-Kotha et al. 2019). On the other hand, diminished uptake of essential elements such as iron (Fe), magnesium (Mg), and calcium (Ca) is largely attributed to higher concentrations of Cr (VI) (Ranieri et al. 2020). In most Cr-accumulators, rhizosphere is the primal site of Cr (III) and is less likely to be transferred to above-ground parts (Srivastava et al. 2021). Compared to other HMs, infrequent plant species have been reported with potential Cr-hyperaccumulation capacities. Ranieri et al. (2020) reported two plants including Diocoma nicoloffera Wild and Sutera folina Wild as remarkable Cr-hyperaccumulators, with maximum reported Cr bioaccumulated concentrations of 1500 and 2400 mg/kg, respectively, within dried leaves. Zhang et al. (2007) found that L. hexandra was grown in an average Cr concentration of 114.3 mg/kg sludge, with average Cr concentration 1786.9 mg/kg in the leaves. It is worth considering that the ratio of Cr content in the leaves to the soil samples of the roots was approximately 56.83. However, the ratio of Cr content in the leaves to the roots was around 11.59, and the ratio in the leaves to water was up to 517.86. Liu et al. (2011b) reported that L. hexandra reached the highest Cr content of 1844 mg/kg in the above-ground parts under 330 mg/kg soil Cr treatment (Table 2). Lin et al. (2018) reported that L. hexandra was grown in an average Cr concentration of 60 mg/L nutrient solution (1/2 Hoagland’s nutrient solution), with average Cr concentration 2932 mg/kg DW in the leaves and 33,048 mg/kg DW in the roots (Table 1).

2.2. Cu accumulation

Cu is an essential metal for optimal plant growth and accountable for multitude of vital cellular processes such as photosynthesis, respiration, antioxidant activity, cell wall metabolism, and hormone perception (Vatansever et al. 2017). However, the general Cu requirements for healthy plants are 5–20 mg/g dry weight, and the excess copper can retard plant growth, chlorosis, and invoke cellular oxidative stress (Verret et al. 2004; Mackie et al. 2012; Khan et al. 2021). Cu stress prompts plant growth retardation, chlorosis, and development of oxidative stress. Copper tolerance in metallophytes is considered to arise through adaptation to components of the basic metal homeostasis (Lange et al. 2017). The main strategies are: sequestering Cu ions or binding copper ions to proteins or metabolites (Yruela 2009). Copper is delivered to enzymes via specific proteins, while excess copper can be chelated, such as low molecular weight cysteine-rich metallothioneins (Zheng et al. 2012).

Phytoremediation of Cu using ornamental plants is a delicate subject of current research. Calandula officinalis L. for example, could adapt well in soils with Cu content of 400 mg/kg without exhibiting toxicity, which is generally a highly toxic range for typical plants otherwise (Goswami and Das 2016). Also, Asensio et al. (2018) found two hyperaccumulators species (M. urundeuva and C. langsdorffii) from Brazil could accumulated more than 300 mg/kg Cu in shoots. However, the copper accumulation capacity of these plants is insufficient as accumulators for the remediation of Cu-contaminated soil.

Soil experiments revealed that L. hexandra leaves grown in the average Cu concentration of 2000 mg/kg soil, the Cu accumulation was 307.89 mg/kg in the leaves (Table 2). The Cu concentration in different tissues of L. hexandra was listed in Table 1. The Cu ratio in L. hexandra leaves to sludge was 0.4–1.75 (Zhang et al. 2008). When L. hexandra grown in the Cu concentration of 40 mg/L nutrient solution (1/2 Hoagland’s nutrient solution), it could be accumulated 2357.26 mg/kg Cu in the leaves (Zhang et al. 2008b) (Table 1). And, Lin et al. (2016) reported that L. hexandra was grown in an average Cu concentration of 38.4 mg/L nutrient

| Metal category | Treatment (mg/kg) | HMs concentration (mg/kg) | Reference |
|----------------|------------------|--------------------------|-----------|
| Cr             | 330              | root: 1844 ± 178         | Lin et al (2011) |
|                |                  | stem: 335 ± 0.34         |           |
|                |                  | leaf: 307 ± 0.02         | Zhang et al (2008a) |
| Cu             | 2000             | root: 323               |           |
|                |                  | stem: 307               |           |
|                |                  | leaf: 0.02              |           |

Results are means ± SD, n = 3.
solutions (1/2 Hoagland’s nutrient solution), with average Cu concentration 1534.76 mg/kg DW in the leaves and 5123.85 mg/kg DW in the roots (Table 1).

2.3. Ni accumulation

Ni$^{2+}$ in soil efficiently enters the root protoplasts through low-affinity transport systems (mainly belong to Zn and Fe transporters) (Deng et al. 2018). In the root cytoplasm, Ni ions are readily chelated by organic compounds, such as histidine, while in the phloem, Ni$^{2+}$ can be chelated by a variety of carboxylic acids, such as malate or citrate (Deng et al. 2016; van der Ent et al. 2017). In general, Ni is compounded in inactive places such as vesicles, plastids, and the other designated primary storage sites (Deng et al. 2018). According to recent research, silicic can transport a large quantity of Ni with subsequent accumulation in young growing tissues (Do Nascimento et al. 2021).

The results of field investigations and nutrient culture experiments showed that L. hexandra was able to migrate Ni from soil and water to leaves and stem. The highest reported Ni concentration in the leaves is approximately 1349 mg/kg (Table 1) and the maximum ratio leaves to water and soil is 415.22 and 18.82, respectively. Under nutrient solution culture conditions, L. hexandra also showed a robust Ni accumulation capacity, with a maximum leaf-based Ni content of 2926 mg/kg as well as a maximum bioaccumulation coefficient of 156.53 (Zhang et al. 2008).

2.4. Cr, Cu, and Ni accumulation

Obviously, single HM contamination rarely occurs in contamination soil, and the co-occurrence of that two or more HM contaminants is too often in contaminated soil environments. The interaction of heavy metals in chemical processes and removal. There are no known enzymes in plants that catalyse the conversion of Cr (VI) to Cr (III) (Cervantes et al. 2001; Howe et al. 2003; Shanker et al. 2009). Importantly, Cr (VI) is approximately many times more harmful than Cr (III) because cellular membranes are often impervious to former. The biotransformation of Cr (VI) to Cr (III) is probed as an essential process to contain Cr (VI) contamination mainly due to the insolubility of Cr (III), which fosters easier precipitation and removal. There are no known enzymes in plants that catalyze the conversion of Cr (VI) to Cr (III) (Cervantes et al. 2001; Howe et al. 2003; Shanker et al. 2009).

Table 1. HMs concentrations in tissue of L. hexandra grown in nutrient solution.

| Metal category | Treatment (mg/L) | HMs concentration (mg/kg DW) | Reference |
|---------------|------------------|-----------------------------|-----------|
| Cr(III)       | 60               | 13.064 ± 1749               | Zhang et al. (2007) |
| Total Cr      | 60               | 33.048 ± 1373               | Lin et al. (2018) |
| Ni            | 50               | 7602 ± 1373                | Zhang et al. (2008b) |
| Cu            | 40               | 7312 ± 810                 | Zhang et al. (2008b) |

Results are means ± SD, n = 3.

A plant that can accumulate for multiple HMs at the same time will have an important impact on phytoremediation. Tao et al. (2020) reported that L. hexandra was able to maintain normal growth in soils containing 8515.69-, 3442.27-, and 2992.55-mg/kg of Cr, Cu, and Ni, respectively. The L. hexandra above-ground Cr, Cu, and Ni concentrations reached 255.23, 191.09, and 185.01 mg/kg, respectively, at 60 days. Bose et al. (2008) reported that Typha angustata L. was grown for 60 days in soil contaminated with Cr, Cu, and Ni concentrations of 400.37, 245.87, and 201.22 mg/kg, respectively. The results suggested that L. hexandra has great potential to treat soils polluted with these HMs. However, it is unconfirmed whether there are synergistic or antagonistic effects among the accumulation of Cr, Cu, and Ni by L. hexandra, and more experiments are needed to confirm this.
mechanism of Cr (VI) removal by this endophytic bacterium was mainly the reduction of Cr (VI) to the less toxic Cr (III).

Interestingly, Cr is a non-essential element and plants lack any specific mechanism or transporter protein to process its intracellular uptake (Guo et al. 2021). This necessitates the need to complement roots transport of Cr via the carriers of other plant essential elements such as Ca, K, and Fe (Shanker et al. 2005). Non-selective cation channels (NSCCs) are characterized by their lack of ion selectivity, allowing cations of different valence states to pass simultaneously and directly involved in a lot of physiological processes like nutrients uptake, and signal conduction, etc. (Pinto and Ferreira 2015). NSCCs can rapidly transport essential plant nutrients such as K⁺, Ca²⁺, Zn²⁺, etc., and is also a pathway for some toxic ions such as Na⁺, Cr³⁺/Cr⁶⁺, Hg⁺, and Cd²⁺ to enter the cell (Demidchik and Maathuis 2007). Therefore, the inhibition of NSCCs can be used to investigate the pathway of toxic heavy metals into cells (Zhong-Qi and Chang-Bo 2017). The valence of Cr determines its uptake route to be actively operated or passive transfer. For example, Cr (III) is taken up by passive diffusion through cation exchange sites in the plant cell wall. In contrast, Cr (VI) was actively transported in plants via sulfate and phosphate transporters (Zayed and Terry 2003).

2,4-Dinitrophenol (DNP) is an oxidative phosphorylation chemical uncoupling agent that inhibits ATP production by disrupting the proton gradient on both sides of the inner mitochondrial membrane. A recent study also reported that DNP also causes significant hydrolysis of ATP in mitochondria (Tian et al. 2014). Zhang and Liu (2013) reported that in the absence of DNP, elevated Cr uptake by L. hexandra was observed at a Cr concentration of 300 mg/L, the addition of 25 μmol/L DNP caused ~1.4 times lower uptake than former treatments. Whilst it was still ~2 times higher than that with the addition of 50 μmol/L DNP. Additionally, the uptake of Cr was reduced by 34.2% in L. hexandra grown at 2°C compared to that samples at 25°C (room temperature). This suggested that the uptake of Cr by L. hexandra may be an active transport process. Liu et al. (2011a) found that L. hexandra after 4 and 8 h of treatment with 1 mmol/L Ca channel blocker lanthanum chloride (LaCl₃), the Ca content in the roots of L. hexandra was significantly decreased. On the other hand, adding LaCl₃ had no effect on Cr (III) absorption, demonstrating that Ca channels are not receptive to Cr (III) uptake. Similarly, the treatment with K channel blocker tetraethylammonium (TEA), had no inhibitory effect on Cr (III) uptake in L. hexandra roots, demonstrating that the K channels do not implicate in Cr (III) uptake.

Cr (III) and Fe absorption in plants were closely coupled, and both elements may use the similar path of translocation (Singh et al. 2013). Fe hyperaccumulator plants, such as spinach (Spinacia oleracea), have been found to be more effective at transporting Cr (III) to its above-ground tissues than lettuce (Lactuca sativa) and cabbage (Brassica oleracea var. capitata), with no reported Fe accumulation (Srivastava et al. 2021). Liu et al. (2011a) reported that the higher Km values (apparent Michaels constant) for Fe-containing plants and lower Km values for Fe-deficient plants showed that Cr (III) uptake by the root system of L. hexandra was partly mediated by the Fe (III)-plant iron carrier complex transporter protein.

### 3.1.2. Cu uptake

Tabata et al. (1997) firstly reported a P-type ATPase, i.e., PAA1, in Arabidopsis and was involved in Cu transport into the chloroplast. Likewise, Colangelo and Guerinot (2006) observed three P1B-ATPases to be involved in the transport of Cu across the membrane into chloroplasts, and in particular, the P-type ATPase could mediate HM transport across all cellular membranes. This can be further supported by the findings of Pinto et al. (2014) that Cu transporter proteins (COPT) actively participate in its intracellular uptake in the form of Cu (I), and expedites its entry into host cells. Nonetheless, notable inhibition of several P-type-ATPases has observed at small sodium vanadate (Na₃VO₄) dosing (Williams et al. 2000).

Tian et al. (2014) documented that dosing 25 and 50 mol/L ATPase inhibitor (Na₃VO₄) abundantly decreased Cu concentration in the roots of L. hexandra by 26.2% and 31.0%, respectively, compared to the control. This directed that Cu stimulating P-type-ATPase has been present in L. hexandra and evidently regulates Cu uptake. The application of 25 and 50 mol/L DNP dropped Cu concentration by 25.80% and 42.70% in L. hexandra respectively. Moreover, the Cu uptake in the L. hexandra roots is an energy-dependent phenomenon and often stimulated by the ac 2006 tive transport process. The addition of LaCl₃ significantly inhibited Cu uptake by L. hexandra, whereas the addition of TEA did not implicate noticeable inhibition. Therefore, these results showed that Cu uptake by the roots of L. hexandra was most likely attributed to the Ca²⁺ channels with no influence by the K⁺ channels (Qin and Lin 2020).

### 3.1.3. Ni uptake

To date, no evidence of Ni-specific transporter proteins in plants for Ni uptake has been found (Deng et al. 2018). Non-specific transporter proteins, particularly the ZRT/IRT-like (ZIP) family, appear to accelerate Ni uptake through plant roots (Nishida et al. 2011; Nishida et al. 2015). Hydroponic experiments from Taylor and Macnair (2006) revealed diminished Ni uptake in the Zn/Ni hyperaccumulator plants Noccaea pindicum and Noccaea alpinum var. sylvium at the equimolar Ni and Zn concentrations. Compared to plants treated with Ni alone. However, Ni had no influence on Zn buildup. This was also supported by Assunção et al. (2001) that supplementation of equimolar Ni and Zn concentrations in Zn/Ni hyperaccumulator plant N. caerulescens reduced 80%–90% of Ni uptake but had no effect on Zn absorption. Under Fe-deficient conditions, IRT1 protein worked as a Ni transporter in A. thaliana, indicating that Ni uptake and Fe transport in plants were closely related (García de la Torre et al. 2021). Similarly, Ni uptake Vmax by Alyssum bracteatum was significantly increased by Fe starvation, but this phenomenon was not found in other experimental plans of these genera such as Alyssum inflatum (Mohseni et al. 2018). As a result, these findings suggest that Ni uptake is a plant species-specific mechanism and duly associated with a variety of nonspecific proteins with different transport mechanisms. The transporter proteins for Ni uptake in L. hexandra have not been identified yet, thereby, further experimental work is needed to test the Ni uptake mechanism in L. hexandra.
3.2. Sequestration

Plants have different mechanisms to acclimatize HM ions inside certain intracellular compartments to affirm minimal toxic damage to metabolic pathways and vital cellular organelles after uptake (Sharma et al. 2016). This appropriately prevents HMs ions from entering metabolic organelles. For example, Sinha et al. (2018) observed that plants adsorb Cr ions on cell walls or alternatively store them in vesicles to limit their entry into chloroplasts and mitochondria. Huang et al. (2018) reported the highest assimilation of Cr within the roots of Coptis chinensis Franch followed by the rhizome and lowest inside the leaves. The cultivation of Amaranthus viridis at different concentrations of Cr(VI) in hydroponic experiments revealed the roots as the primary site of Cr accumulation (Liu et al. 2008b). Ultrastructural localization analysis of the plant Raphanus sativus showed that the cell wall of root cortex cells represented high Cr adsorption (Lahouti and Eftehadi 2008). One possible rational includes the adequate production of pectin and extracellular polysaccharides like callus and mucilage in the cell walls of plant root cells, which impede Cu intracellular entry into the cell cytoplasm. Specifically, pectins are polysaccharides with extensive carboxyl groups (–COOH) and are qualified to binding with Cr ions. Similar observations were documented by another study that Cr accumulation on the cell wall of root cells is facilitated by the above-stated mechanism (Miretzky and Cirelli 2010). Liu et al. (2009) found that L. hexandra under the Cr supposition, approximately 43% of the total Cr was detected on the cell wall in roots and 28% was found in the vesicles in leaves. These findings implied that Cr accumulation in the roots of L. hexandra is advantageous to keep minimum disruption in the metabolic activity in the protoplasts, and leaf vesicles are primary sites for Cr accumulation and detoxification due to theirs huge storage capacity which might explain L. hexandra’s exceptional Cr tolerance.

The presence of higher Cu concentrations in the roots limits its translocation from roots to branches due to high Cu concentrations. Cu fixation in the roots is a crucial process to prevent its transfer to branches, thereby, minimizing cellular toxicity (Broadley et al. 2012). From the μXRF elemental mapping and SEM-EDS analysis, Cu was predominately stored in the roots of C. helmsii and subsequently transported to the remaining parts of the plant (Corzo Remijio et al. 2021). This attribute was also observed in Italian ryegrass and Trifolium pretense (red clover), where 60% of the Cu in the root cells was bound to the cell wall and plasma membrane interface (Iwasaki et al. 1990). In the same way, Liang et al. (2016) reported root uptake dominated the overall Cu absorption in L. hexandra, with root cells accounting for 90% of total Cu acclimation. Root retention was found to be the most common pathway of Cu accumulation in L. hexandra. The majority of the Cu contents in the root cells of L. hexandra were found to be in the protein-bound state and the water-insoluble HMs phosphate, accounting for 60%–80% of the total uptake. These findings suggest that Cu was largely fixed in the roots of L. hexandra in a stable, insoluble, or less reactive state. Thus, reducing the generation of reactive oxygen radicals and avoiding cellular damage.

In most Ni hyperaccumulator plants, the branch epidermis is the major site of Ni deposition. However, the vesicles are the main regions of Ni storage in shoot cells at the subcellular level (Küpper et al. 2001). During the seven-day exposure of Thlaspi goingense to Ni, 75% of the Ni was recovered in the vesicles compared to a non-accumulator with 25% recovery capacity. The results demonstrated that the leaf vesicles of hyperaccumulator cells were the major compartment for intracellular Ni fixation (Kramer et al. 2000). Previous studies suggested that IREG/FPN transporter proteins primarily regulate the vesicular uptake of Ni in typical Ni hyperaccumulator plants (Schaaf et al. 2006; Merlot et al. 2014).

The location of Ni accumulation in L. hexandra under Ni stress and the role of regulatory transporter proteins were not clear. Based on the studies of other Ni-hyperaccumulator plants, it can be speculated that Ni is largely stored in leaf cell vesicles of L. hexandra. Since most Ni transport proteins rely on the IREG/FPN family, therefore, it is also possible that the mechanism of Ni transport in L. hexandra relies on the IREG/FPN family. However, as Ni tolerance varies between plants, more research is needed to explore the validity of such speculations.

3.3. Plant ligands

In general, only a small fraction of unbound HM ions was reported in plants, which suggested that their major proportion is bound to low molecular weight ligands or proteins. Plants produced many ligands, including organic acids, amino acids, and metallothioneins, and cell wall proteins/pectins/polyphenols. Hence, chelation is a noteworthy factor to cut off the concentration of free HMs ions to relatively low levels (Ehsan et al. 2014; Liu et al. 2015; Osmolovskaya et al. 2018). This could be another important mechanism by which plants prompt HM fixation (Hall 2002; Sharma and Dietz 2006; Gupta et al. 2013).

Elevated levels of several carboxylic acids including citric acid, isocitric acid, oxalic acid, tartaric acid, malic acid, malonic acid, and aconitic acid have been observed in the cytosol vesicles of photosynthetic tissues. Many studies have revealed that these acids have been largely linked to hyperaccumulation (Kramer et al. 2000; Sarret et al. 2002b; Terry and Banuelos 2020). For example, acetic acid and citric acid (C₃H₄O₇) bind to Cd in Solanum nigrum leaves (Sun et al. 2008). Malic acid was involved in the chelation of Zn in Arabidopsis thaliana (Sarret et al., 2002a). Schaumloffel et al. (2003) reported that most of Ni ions were bonded to citrate in the latex of S. acuminate. In addition, citrate is also deemed to combine with Ni in the buds of T. goingense (Krämer et al. 2000). Cu was found to bind with oxalate in Cu-tolerant species of the Cd/Zn hyperaccumulator plant Thlaspi caerulescens (Mijovilovich et al. 2009). Zhang et al. (2009) reported a high proportion of oxalic-Cr complex in the leaves of L. hexandra. Wang et al. (2012) reported that oxalate supply alleviated Cr-induced biomass reduction and root growth inhibition. These results suggested that as an important phytochelatin in plants, oxalic acid (C₃H₂O₄) could be involved in detoxifying Cr impacts in L. hexandra. These findings recommend that C₃H₂O₄ is among the major chelator in L. hexandra for Cr accumulation. Therefore, finding the ligands which ideally combined with Cr in the root cells of L. hexandra is a critical step to thoroughly exploring the opportunities for efficient phytoremediation.
Liu et al. (2008b) reported that the addition of organic acids augmented Cu accumulation in the above-ground parts of *L. hexandra* compared to the control group. The addition of EDTA and C₆H₅O₇ increased Cu accumulation in the aerial parts of *L. hexandra* and particularly the EDTA, which increased by 282.9%–418.9% compared to the control group without EDTA addition. It is possible that Ni accumulation in *L. hexandra* is chained to C₆H₅O₇ because most of the results of Ni hyperaccumulator plants suggest that Ni-C₆H₅O₇ complexes are the cardinal to Ni tolerance.

Histidine (His) is considered to be the most important free amino acid involved in metal hyperaccumulation. It can act as a tridentate ligand via its carboxyloxy, amine, and imadazole functional group (Callahan et al. 2006). Persans et al. (2001) reported that histidine was detected at relatively higher concentrations in the roots of metal hyperaccumulator plants. Supplementation of His in the roots of *Noccaea caerulescens* was observed to enhance Ni accumulation in roots and stem, but prevented its translocation to leaf vesicles (Kozhevnikova et al. 2020). In Ni accumulation studies on plants such as *Pisum sativum* L, *Noccaea caerulescens* F.K. Mey., and *A. lesbiacum*, His aids in the endurance of plants against Ni and reinforces its transport from roots to branches (Callahan et al. 2006; Kozhevnikova et al. 2021). However, no increase in Ni tolerance was observed in the non-accumulating *Alyssum montanum* L (Kerkeb and Kramer 2003). Overall, these results suggested that His is involved in the hyperaccumulation of Ni in the majority of the experimented plant species with a few probable exemptions. This encourages to conduct more studies to unveil the Ni accumulation process in *L. hexandra* that is largely undetermined at present.

Nicotinamide (NA) is mainly involved in the transport of micronutrients in plants and has been reported to be involved in the (Fe) and Cu metabolism (Von Wiren et al. 1999; Liao et al. 2000). Trampczynska et al. (2010) also reported that NA combined with Zn and Ni in vivo to form Zn-NA and Ni-NA complexes in xylem, which reduced the bioavailability of both HMs in *tobacco* and *A. thaliana* plants with improved tolerance. Irtelli et al. (2009) reported that NA is an impactful Cu chelator in *Brassica carinata*. Douchkov et al. (2005) observed that NA’s gene of *Arabidopsis thaliana* was ectopically expressed in transgenic tobacco plants. Cultivation of these plants in Ni comprising medium, with NA co-supplementation resulted in an increased Ni tolerance of the plants, with non-negligible influence on the uptake rate. In addition, Cr uptake by *L. hexandra* was related to Fe metabolism. Therefore, it can be inferred that NA may be involved in the process of Cr, Cu, and Ni accumulation in *L. hexandra* (Verbruggen et al. 2009). However, since the mechanism of hyperaccumulation may be vary between plants to plants and also depends on the different accumulate HMs, further experiments are desirable to support these speculations.

### 3.4. Antioxidant response

Excess HM ions in the cytoplasm disrupt cellular redox homeostasis and trigger oxidative stress by generating reactive oxygen species (ROS), active nitrogen species (RNS), and reactive carbonyl species (RCS) (Hoque et al. 2021). Overproduction of ROS, RNS, and RCS inhibit cellular metabolic activities, damage protein as well as DNA structures and also destroy the structure of chloroplasts and cause cell death (Liu et al. 2017; Parshica et al. 2021). And, Liu et al. (2022b) reported that HMs accumulated in plants impair plant tolerance to other abiotic stresses by negatively affecting ROS and ion homeostasis. To combat the overproduction of ROS, plants activate their powerful antioxidant defense mechanisms. The deleterious HM impacts in plants are protected by scavenging excess ROS through stimulating the activity of their antioxidant enzymes, which inhibit the oxidative processes by interrupting free radical chain reactions (Yan et al. 2020). These enzymes include: superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and glutathione reductase (GR), as well as non-enzymatic antioxidant compounds including glutathione, flavonoids, carotenoids, ascorbates, and tocopherols (Sankar Ganesh et al. 2008; Gill and Tuteja 2010; Sinha et al. 2018; DalCorso et al. 2019). Shanker et al. (2004) studied the toxicant impacts of Cr (VI) treatment on *Zea. mays*, *Solanum lycopersicum*, and *Brassica oleracea*. These results disclosed that high Cr levels accelerated glutathione (GSH) content in the roots and leaves of experimented plants. Similarly, Freeman et al. (2004) reported that Ni accumulation had strong relation with GSH in the genus *Noccaea*.

According to Huang et al. (2012), Cr treatment decreased SOD and POD activity; however, increased CAT content in *L. hexandra*. In addition, this study also discovered that several vital cellular compounds such as chlorophyll, soluble sugars, MDA, flavonoid compounds, proline, and soluble protein in leaves could adapt to and regulate Cr stress. Man et al. (2019) found an upsurge in the activity of MDA, SOD, CAT, POD along with up and downs of APX content in *L. hexandra* under the high Cu concentration (Figure 1).

### 4. Enhancement of HMs enrichment capacity

#### 4.1. NPK fertilizer

The addition of chemical amendments such as chelating agents, lime, soil acidifiers, and fertilizers to the soil is a common strategy to enhance the phytoremediation process, and of all the commonly used soil amendments, fertilizers are the most widely used in the world (Xu et al. 2018). Chevalier and Rossignol (2011) found N, P, and K are involved in the enhancement of HMs enrichment capacity, and fertilizers to the soil is a common strategy to enhance the phytoremediation process, and of all the commonly used soil amendments, fertilizers are the most widely used in the world (Xu et al. 2018). Chevalier and Rossignol (2011) found N, P, and K are involved in the response of plants to important mechanisms such as photosynthesis, energy metabolism, and stress-related metabolism. They are also important components of plant amino acids, proteins, genetic material, and enzymes (Iltisham et al. 2020). Different fertilization methods have significant effects on improving plant growth, soil physicochemical properties, and heavy metal extraction capacity of plants (Rao et al. 2018; Wang et al. 2020). The supply of NPK will increase plant biomass, enzyme activity, protein content, etc., thus enhancing the effect of phytoremediation (Deng et al. 2014).

Several studies showed that applying nitrogen, phosphates, potassium (NPK) fertilizer can significantly increase HM uptake in plants. Appropriate fertilization could increase the plant biomass content and may enhance their ability to uptake and tolerate HMs (Wei et al. 2010; Titah et al. 2013). However, the accumulation, detoxification, and
tolerance mechanisms of different HMs are a species’ specific phenomenon, and corresponding studies have been done for *L. hexandra*. Liu et al. (2011a) reported that the application of 100 ml NPK (0.75 mmol/K₂SO₄, 0.25 mmol/L KH₂PO₄, 0.1 mmol/L KCl, and 2.0 mmol/L Ca(NO₃)₂) stimulated 45% shoot biomass and 26% Cr uptake compared with the non-fertilized cultures. The results of Lin et al. (2018) showed that the addition of NPK fertilizers greatly increased the biomass of *L. hexandra* with 52.3% higher Cu extraction efficiency.

### 4.2. Nitrogen fertilizer

Nitrogen plays an important role in plant growth because it is a component of all plant structures such as proteins, enzymes, and chlorophyll (Maqsood et al. 2016). Proper nitrogen supply can increase leaf area, photosynthesis, and also promote plant growth (Ahmad et al. 2009). Zhang et al. (2011) reported that the application of nitrogen fertilizer (NH₄)₂SO₄ in 400 mg/kg Cr treatment soils elicited 1.5–4 times higher Cr accumulation in *L. hexandra* compared to the control group (none N fertilizer supply) (Figure 2). Moreover, the N fertilizer-treated *L. hexandra* had higher protein and chlorophyll content. The results indicated that N fertilizer supply can catalyze the growth rate, total carbohydrate levels of *L. hexandra*, and can improve its capacity to accumulate Cr. Zhang et al. (2017) reported that under 500 mg/kg Cu treatment soils, the N fertilizer (Ca(NO₃)₂) supply 2.5–4 times improved Cu accumulation in *L. hexandra* than the control group (Figure 3). Moreover, an up-rise in the biochemical parameters such as plant biomass, chlorophyll, soluble proteins, and metallothionein was also observed compared to the control group. Many studies showed that the application of nitrogen fertilizer can effectively increase the cellular biomass and the bioaccumulation of both Cr and Cu in *L. hexandra*.

### 4.3. Phosphate fertilizers

Phosphorus is an essential plant nutrient with an abundant role in metabolic functions, and a major component of
4.4. Microorganism

Soil is an environment colonized by many different microorganisms that play a key role in nutrient cycling and soil formation (Ahemad and Khan 2013). Many studies have identified that the relationship between plants and soil microorganisms can facilitate phytoextraction and phytostabilization processes to enhance the phytoremediation of heavy metals, as well as promote plant growth and increase plant tolerance to adversity (Khan et al. 2009; Ma et al. 2015; Zloch et al. 2017). The interrelationship between plants and microorganisms plays a key role in phytoremediation (Hryniewicz et al. 2018). Secretions from plant roots are a source of nutrients for microorganisms and also increase the solubility of trace elements, affecting the activity of microorganisms associated with the plant root system (Iqbal and Ahemad 2015). Microorganisms can reduce the toxicity of harmful pollutants, improve soil structure, regulate plant defense responses to stress factors, and promote plant growth to indirectly enhance plant uptake of heavy metals (Elsgaard et al. 2001).

Lin et al. (2020) reported that the Cr reduction by the synergistic effect of the root exudates and Bacillus was more than 99%, and was about 40% and 70% higher than the groups treated with Bacillus and root exudate alone, respectively. Multiple allelopathic substances in root exudates of L. hexandra were decomposed on the expense of growth metabolism after Bacillus inoculation. Notably, a phenolic allelopathic substance (2,4-Di-tert-butylphenol) was detected in the root exudates, which was seemingly a potential indicator of response mechanism of L. hexandra for Cr (VI) stress.

5. Conclusion and discussion

The uptake of Cr(III) and Cu(II) by L. hexandra may be an active transport process. The carrier of Cr(III) may be the same as the carrier of Fe(III) and Cu uptake by the roots of L. hexandra may be attributed to the Ca\(^{2+}\) channels. Approximately 74% of total Cr(III) and 90% of total Cu were acclimated in the L. hexandra roots. In L. hexandra root cell, the cell wall was the major site for Cr(III) accumulation and the leaf vesicles were primary sites for Cr(III) accumulation and detoxification. That were the strategies of L. hexandra to minimal toxic damage to metabolic pathways after uptake. L. hexandra could produce many ligands to cut off the concentration of free HMs ions, oxalic acid could be the primary organic acid to detoxify Cr(III). Under the Cr treatment, L. hexandra increased CAT content; however, deceased SOD and POD activity to protected cellular metabolic activities and DNA structures from the surplus ROS. An upsurge in the activity of MDA, SOD, CAT, POD along with up and downs of APX content in L. hexandra could be the primary organic acid to detoxify Cr(III).

The mechanisms of accumulation and detoxification of Cr or Cu in L. hexandra were widely discussed, and many studies have investigated that changing fertilization regimes considerably improve its acclimation capacities. As an excellent Cr hyperaccumulator, L. hexandra also emerges to absorb large quantities of both Cu and Ni. In case of Ni stress, the accumulation and detoxification mechanisms are yet largely undetermined, therefore, understanding the insights of the dissipation mechanisms in L. hexandra is essential to the actual pollution treatment.

As majority of the hyperaccumulator plants are contaminate-specific and slow-growing, thus, exploring mutual biotechnologies are recommended to investigate in the future research. Moreover, using molecular biology and transgenic
technology. His research interests include water treatment technology, heavy metal pollution phytoremediation, and environmental impact assessment.

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