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Fe Removal by *Pistia stratiotes* Under Mixed-Metal Conditions: Evaluating Ionic Competition During Phytoremediation

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Abstract. Heavy metal contamination in industrial wastewater generally occurs as mixtures of several metals, whereas phytoremediation studies have predominantly evaluated metal removal under single-metal conditions. Consequently, the influence of coexisting metals on Fe removal remains insufficiently understood. This study investigated the effect of mixed-metal systems on Fe removal by *Pistia stratiotes* and examined the potential occurrence of ionic interactions during phytoremediation. Batch experiments were conducted for six days using single-metal (Fe) and mixed-metal (Fe–Mn–Co) systems. Fe concentrations were determined by ICP–OES, root morphology and elemental composition were characterized by SEM–EDX, and the data were evaluated using Kruskal–Wallis and Spearman's rank correlation analyses. *P. stratiotes* maintained Fe removal efficiencies exceeding 99% in all treatments. SEM–EDX analysis confirmed the accumulation of Fe, Mn, and Co in the roots, while the porous root morphology provided potential sites for metal adsorption. Statistical analysis indicated that the addition of Mn and Co did not significantly affect Fe removal efficiency ($p = 0.321$), whereas Spearman's rank analysis revealed a weak and non-significant negative correlation between the number of coexisting metals and Fe removal efficiency ($r = -0.293$, $p = 0.482$). These findings suggest that the concentrations of Mn and Co investigated were insufficient to substantially interfere with Fe removal because *P. stratiotes* maintained effective metal homeostasis through regulated uptake and intracellular detoxification. This study contributes to a better understanding of phytoremediation performance under multi-metal conditions and provides relevant information for the application of aquatic plants in industrial wastewater treatment.

Keywords: *Homeostasis, iron, multi-metals, phytoremediation, Pistia stratiotes*

Abstrak. Kontaminasi logam berat dalam air limbah industri umumnya terjadi dalam bentuk campuran beberapa logam, sedangkan penelitian fitoremediasi lebih banyak mengevaluasi penyisihan logam tunggal. Oleh karena itu, pengaruh keberadaan logam secara bersamaan terhadap penyisihan Fe masih belum banyak dipahami. Penelitian ini bertujuan mengkaji pengaruh sistem logam campuran terhadap penyisihan Fe oleh *Pistia stratiotes* serta potensi interaksi ionik selama fitoremediasi. Percobaan batch dilakukan selama enam hari menggunakan sistem logam tunggal (Fe) dan logam campuran (Fe–Mn–Co). Konsentrasi Fe ditentukan menggunakan ICP–OES, sedangkan morfologi akar dan komposisi unsur dikarakterisasi menggunakan SEM–EDX. Data dianalisis menggunakan uji Kruskal–Wallis dan korelasi peringkat Spearman. *P. stratiotes* mempertahankan efisiensi penyisihan Fe lebih dari 99% pada seluruh perlakuan. SEM–EDX mengonfirmasi akumulasi Fe, Mn, dan Co pada akar, sementara morfologi akar yang berpori menyediakan situs potensial untuk adsorpsi logam. Penambahan Mn dan Co tidak memberikan pengaruh signifikan terhadap efisiensi penyisihan Fe ($p = 0,321$). Korelasi Spearman menunjukkan hubungan negatif yang lemah dan tidak signifikan antara jumlah logam yang terdapat secara bersamaan dan efisiensi penyisihan Fe ($r = -0,293$; $p = 0,482$). Hasil ini menunjukkan bahwa *P. stratiotes* mampu mempertahankan penyisihan Fe pada kondisi logam campuran melalui mekanisme homeostasis logam.

Kata kunci: *Homeostasis, besi, multi-logam, fitoremediasi, Pistia stratiotes*

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INTRODUCTION

Heavy metal contamination has become one of the major challenges in aquatic environmental management due to the persistent nature of metals, their resistance to biological degradation, and their ability to accumulate in living organisms through bioaccumulation and biomagnification processes (Briffa et al., 2020). Unlike organic contaminants, which can undergo natural degradation, heavy metals remain in the environment for prolonged periods, thereby posing significant risks to human health and ecosystem stability. In Indonesia, this issue has become increasingly critical as a consequence of rapid industrialization, mining activities, and urban development. According to Statistics Indonesia (Badan Pusat Statistik, 2022), approximately 85.59% of the country's rivers are classified as polluted, with industrial activities representing one of the primary sources of contamination through the discharge of metal-containing wastewater. In addition to industrial effluents, heavy metals may also enter aquatic environments through the corrosion of pipelines, storage tanks, and various metallic infrastructures (Surbakti et al., 2024). Among the metals frequently detected in industrial wastewater, iron (Fe), manganese (Mn), and cobalt (Co) are extensively utilized in manufacturing processes, particularly in the steel industry, where Fe constitutes approximately 97% of the production material (Utomo & Hasan, 2022). Given its predominant role in steel manufacturing and its widespread occurrence in industrial effluents, Fe was selected as the primary focal metal in this study, while Mn and Co were included to represent coexisting metals that may potentially interfere with Fe removal. Although Fe is an essential micronutrient for living organisms, excessive accumulation can stimulate the generation of reactive oxygen species (ROS), induce oxidative stress, and disrupt numerous physiological functions (Samet & Wages, 2018). Moreover, prolonged exposure to elevated concentrations of Fe, Mn, and Co has been associated with hematological disorders, endocrine dysfunction, cardiovascular disease, metabolic disturbances, neurotoxicity, and gastrointestinal disorders (Shinta et al., 2024). Therefore, mitigating heavy metal contamination remains a critical priority in water quality management.

Under real environmental conditions, heavy metals rarely occur as single contaminants. Industrial wastewater typically contains complex mixtures of metals, including Fe, Mn, Co, Ni, Cr, and Cu, originating from the same manufacturing processes. The simultaneous presence of multiple metals may result in competitive, antagonistic, or synergistic interactions that influence their bioavailability and uptake efficiency by plants (Kafle et al., 2022). Such interactions are presumed to arise because several metal ions share common transport pathways across root cell membranes, including members of the ZIP family, such as Iron-Regulated Transporter 1 (IRT1), and NRAMP transporters, allowing the presence of one metal to either inhibit or enhance the uptake of another (Delgado-González et al., 2021). Furthermore, physicochemical characteristics such as ionic radius, hydrated ionic radius, valence state, and transporter affinity play important roles in determining the accumulation behavior of individual metals (Khairuddin et al., 2017). Consequently, metal removal efficiencies obtained from single-metal experiments may

not accurately represent the behavior of phytoremediation systems under realistic multi-metal contamination scenarios. Nevertheless, most phytoremediation studies to date have been conducted using single-metal systems, leaving the influence of metal-metal interactions on remediation performance insufficiently understood.

Phytoremediation has emerged as a promising, environmentally sustainable technology for mitigating heavy metal pollution by exploiting the natural ability of plants to absorb, accumulate, stabilize, and transform contaminants at relatively low operational costs and with minimal ecological impact (Sharma et al., 2023). One of the most extensively investigated hyperaccumulator species is water lettuce (*Pistia stratiotes* L.), which possesses a highly developed fibrous root system with a large surface area that facilitates the uptake of contaminants from aquatic environments. Previous studies have demonstrated the effectiveness of *P. stratiotes* in reducing biochemical oxygen demand (BOD) in domestic wastewater and removing Zn, Ni, Cr, and Cr(VI) from contaminated water (Ardiatma et al., 2023; Fahri Rozanno Putra, 2024; Oktaviani et al., 2020). In addition to its high bioaccumulation capacity, this species has been reported to maintain physiological performance under heavy metal stress through the activation of antioxidant defense mechanisms (Resti Billah et al., 2021). However, these investigations have largely been restricted to single-metal systems and therefore do not adequately explain how the presence of coexisting metals influences the uptake behavior of Fe under multi-metal conditions that more closely resemble actual industrial wastewater.

Based on the current state of knowledge, a substantial research gap remains regarding the influence of mixed-metal contamination on the dynamics of Fe uptake during phytoremediation by *P. stratiotes*. Previous studies have primarily focused on the removal efficiency of individual metals without considering intermetallic interactions that may alter metal transport, bioavailability, and accumulation within plant tissues. A better understanding of these interactions is essential for elucidating phytoremediation mechanisms under environmentally relevant contamination scenarios. Therefore, this study aims to evaluate the effect of mixed-metal systems on the uptake dynamics of Fe by *P. stratiotes*, investigate the relationship between the number of coexisting metals and Fe removal efficiency using correlation analysis, and provide preliminary insights into the occurrence of ionic competition during the phytoremediation process. The findings are expected to advance the current understanding of phytoremediation mechanisms in multi-metal systems and provide a scientific basis for developing wastewater treatment technologies that more accurately reflect real environmental conditions.

MATERIAL AND METHODS

Materials

Water lettuce (*P.stratiotes* L.) was used as the phytoremediation agent in a multi-metal aqueous system. The chemicals employed in this study included $FeSO_4 \cdot 7H_2O$, $MnCl_2 \cdot 4H_2O$, and $CoCl_2 \cdot 6H_2O$ (Merck, Germany) as sources of Fe^{2+}, Mn^{2+}, and Co^{2+} ions, respectively, along with deionized water, a commercial aquatic plant fertilizer, and a 1:1 (v/v) HCl solution for sample preparation prior to elemental analysis.

Instrumentation

Metal concentrations were determined using an Inductively Coupled Plasma-Optical Emission Spectrometer (ICP–OES, Shimadzu ICPE-7510, Japan). The morphology and elemental composition of the plant root tissues after phytoremediation were characterized using a Scanning Electron Microscope equipped with Energy-Dispersive X-ray Spectroscopy (SEM–EDX, JEOL JSM-IT100, Japan). Supporting laboratory equipment included an analytical balance (ME54TE/00, Mettler Toledo), standard laboratory glassware, volumetric pipettes, glass vials, glass funnels, 5 L plastic containers, and Whatman No. 42 filter paper.

Procedure

Plant acclimatization

Prior to the phytoremediation experiments, *P. stratiotes* plants were acclimatized for three days to stabilize their physiological condition before exposure under the experimental conditions. Healthy plants exhibiting green leaves, no visible signs of necrosis, and well-developed root systems were selected following the procedure reported by Ardiatma et al. (2023).

The acclimatization was conducted in 1.5 L of deionized water supplemented with Fe^{2+}, Mn^{2+}, and Co^{2+} at concentrations corresponding to the Indonesian water quality standards established by the Ministry of Environment Regulation No. 22 of 2021, namely 0.3 mg L^{-1} Fe^{2+}, 1.0 mg L^{-1} Mn^{2+}, and 0.2 mg L^{-1} Co^{2+}, respectively. The inclusion of these metal ions during acclimatization was not intended to pre-spike or stress-condition the plants, but to allow gradual physiological adaptation to an ionic environment relevant to the subsequent phytoremediation treatments. After the three-day acclimatization period, the plants were thoroughly rinsed with deionized water to minimize the carry-over of externally adsorbed metal ions before being transferred to the experimental treatment solutions.

Multi-metal phytoremediation experiment

The phytoremediation experiment was conducted based on the method reported by (Fahri Rozanno Putra, 2024) with slight modifications. Twelve *P. stratiotes* plants were placed in individual containers containing 5 L of treatment solution with different combinations of Fe^{2+}, Mn^{2+}, and Co^{2+} ions. The phytoremediation process was performed under static batch conditions for six days.

The treatment solutions consisted of a single-metal Fe system and mixed-metal systems containing Fe in combination with the interfering metals Mn and Co. To maintain adequate nutrient availability throughout the experiment, 12 drops of commercial aquatic plant fertilizer were added to each container. Water samples were collected before and after the phytoremediation process to determine changes in metal concentrations using ICP–OES.

The metal removal efficiency was calculated from the difference between the initial and final metal concentrations according to the following equation:

$$\text{Removal efficiency (\%)} = \frac{C_i - C_e}{C_i} \times 100 \ldots . (1)$$

where (C_i) is the initial metal concentration before phytoremediation (mg L^{-1}), and (C_e) is the final metal concentration after phytoremediation (mg L^{-1}).

ICP-OES analysis

The concentrations of Fe, Mn, and Co were determined using Inductively Coupled Plasma–Optical Emission Spectrometry (ICP-OES). A multi-element standard solution was used to prepare the calibration curves, while the

reagent blank consisted of 10 mL of 1:1 (v/v) HCl diluted to the required volume with deionized water. The analytical calibration was considered acceptable when the coefficient of determination (R^2) was ≥ 0.995.

Following the phytoremediation experiments, the aqueous samples were filtered through Whatman No. 42 filter paper and subjected to a mild acid digestion using 10 mL of 1:1 (v/v) HCl. The samples were heated for 15 min, filtered again, and the resulting filtrates were analyzed by ICP-OES to determine the residual concentrations of Fe, Mn, and Co.

SEM – EDX characterization of plant roots

SEM–EDX analysis was performed to evaluate root surface morphology and the accumulation of metal elements following the phytoremediation process. After the experiment, the roots were thoroughly rinsed with deionized water and oven-dried at 75 °C for 24 h. For SEM morphological observation, intact root segments were used to preserve the root surface architecture. The root segments were mounted on aluminum stubs using conductive carbon tape and coated with a thin layer of platinum (Pt) prior to SEM analysis. For EDX elemental analysis, the dried root material was ground into a fine powder to facilitate bulk elemental characterization.

The prepared samples were examined under high vacuum at an accelerating voltage of 20 kV to obtain SEM micrographs and EDX spectra. EDX analysis was used to identify Fe, Mn, and Co accumulated in the root tissues based on their characteristic X-ray emission energies.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 26. The Fe removal efficiency data were first evaluated for normality using the Shapiro-Wilk test and for homogeneity of variances using Levene's test to determine the suitability of the statistical approach.

Differences in Fe removal efficiency among the treatment groups were assessed using the Kruskal-Wallis test because the data did not satisfy the assumption of homogeneity of variances. The relationship between the number of coexisting metals in the multi-metal system and the Fe removal efficiency was evaluated using Spearman's rank correlation analysis at a significance level of $p < 0.05$. The correlation coefficient was used to determine the direction and strength of the relationship between metal system complexity and the phytoremediation performance of *Pistia stratiotes*.

RESULT AND DISCUSSION

Acclimatization of *P. stratiotes* L.

The acclimatization stage was conducted to ensure that *P. stratiotes* could adapt to a mixed-metal environment before the phytoremediation experiments. During the three-day acclimatization period in 1.5 L of solution containing Fe (0.3 mg L^{-1}), Mn (1.0 mg L^{-1}), and Co (0.2 mg L^{-1}), corresponding to the Indonesian wastewater quality standards established under Ministry of Environment and Forestry Regulation No. 22 of 2021, the plants maintained stable physiological conditions. After acclimation for 3 days, no visible morphological symptoms of metal toxicity, including chlorosis, necrosis, leaf wilting, or root deterioration, were observed. The leaves remained green, the shoots retained their structural integrity, and the root systems continued to develop normally. These

observations indicate that *P. stratiotes* was able to maintain its physiological performance despite exposure to a mixed-metal solution during the acclimatization period.

The stable physiological condition observed during acclimatization suggests that the concentrations of Fe, Mn, and Co employed in this study remained within the tolerance range of *P. stratiotes*. As a well-recognized aquatic hyperaccumulator, this species possesses multiple adaptive mechanisms that enable the maintenance of cellular homeostasis under heavy metal stress. During the initial exposure stage, Fe^{2+}, Mn^{2+}, and Co^{2+} ions are absorbed through the root tissues via metal transporter proteins. Subsequently, a proportion of the absorbed metal ions is chelated by intracellular ligands, including phytochelatins, metallothioneins, organic acids, and phenolic compounds, thereby reducing their cytotoxicity (Nusyura et al., 2023). These metal-ligand complexes are subsequently sequestered into vacuoles or immobilized within the cell wall, constituting essential detoxification mechanisms that protect cellular metabolism and minimize metal-induced damage (Rizvi et al., 2024).

Successful acclimatization is a critical prerequisite for phytoremediation studies because the initial physiological status of the plants strongly influences their subsequent remediation performance. Plants experiencing stress prior to treatment typically exhibit reduced photosynthetic activity, impaired root development, membrane damage, excessive reactive oxygen species (ROS) production, and consequently diminished metal uptake capacity (Rudy et al., 2024). In contrast, the absence of significant morphological alterations in the present study indicates that *P. stratiotes* successfully initiated physiological tolerance mechanisms without exhibiting visible symptoms of metal toxicity. This favorable physiological condition provided an appropriate baseline for evaluating the influence of mixed-metal systems on Fe uptake during the subsequent phytoremediation experiments.

Phytoremediation Performance of *P. stratiotes* in a Multi-Metal System

Morphological changes in *P.stratiotes* were monitored throughout the phytoremediation experiment to evaluate the physiological responses of the plants to exposure to mixed Fe, Mn, and Co contamination. As presented in Table 1, all plants maintained a healthy physiological condition during the first two days of exposure, with leaves remaining turgid and no visible symptoms of damage. Initial signs of metal-induced stress appeared on the third day, characterized by chlorosis at the leaf margins, which gradually progressed to mild necrosis between days 5 and 6. The most pronounced morphological deterioration was observed in the treatment containing the three-metal mixture (Fe + Mn + Co), where an increased number of chlorotic leaves, partial leaf desiccation, and root detachment at the bottom of the container were evident. Nevertheless, all plants survived until the end of the six-day experiment, indicating that *P. stratiotes* possesses considerable tolerance to mixed-metal exposure despite the development of visible stress symptoms.

The occurrence of chlorosis and necrosis suggests that prolonged exposure to mixed metals induced physiological stress as a consequence of metal accumulation within plant tissues. At relatively low concentrations, essential metals such as Fe, Mn, and Co

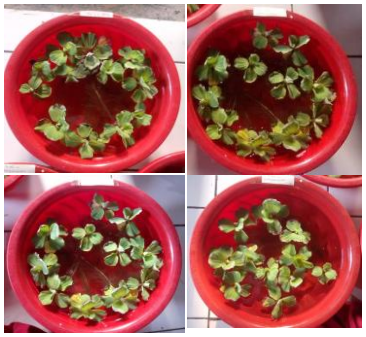
participate in numerous metabolic and enzymatic processes. However, excessive intracellular accumulation disrupts ionic homeostasis and promotes the generation of reactive oxygen species (ROS), leading to oxidative stress (Febriani et al., 2021; Samet & Wages, 2018). Elevated ROS levels can induce lipid peroxidation, protein oxidation, nucleic acid damage, and chlorophyll degradation, ultimately causing leaf chlorosis. Under prolonged stress conditions, oxidative damage compromises membrane integrity, resulting in irreversible cellular injury and tissue necrosis (Li et al, 2022). The morphological responses observed in the present study are consistent with the findings of Oktaviani et al. (2020), who reported that prolonged heavy metal exposure caused leaf discoloration, root deterioration, and reduced plant viability in *P. stratiotes*.

Interestingly, the severity of physiological damage increased with the number of coexisting metals in the treatment system, suggesting that mixed-metal contamination imposes greater physiological stress than single-metal exposure. In multi-metal systems, Fe^{2+}, Mn^{2+}, and Co^{2+} ions may compete for uptake through shared divalent metal transporters, including ZIP, NRAMP, and IRT1 (Li et al., 2022). Beyond transporter-level competition, the simultaneous presence of multiple metal ions may alter the bioavailability of individual metals through changes in metal speciation, binding affinity, and cation–cation interactions in the aqueous phase (Khairuddin et al., 2021) . Consequently, plants are required to allocate greater metabolic resources to maintain ionic homeostasis, regulate metal transport, and activate detoxification pathways, thereby increasing the overall physiological burden under more complex metal mixtures.

Despite the appearance of visible chlorosis and necrosis during the later stages of the experiment, *P. stratiotes* remained viable throughout the phytoremediation period, indicating that the observed physiological damage was insufficient to impair its phytoremediation function. Importantly, these external phenotypic responses were not accompanied by a decline in Fe removal performance. Fe removal efficiency remained above 99% across all treatments, and the differences among treatments were not statistically significant (Kruskal–Wallis, $p = 0.321$). This finding indicates that the visible physiological stress induced by multi-metal exposure did not substantially compromise the plant's capacity to maintain Fe removal under the experimental conditions.

Instead, these responses suggest the activation of adaptive tolerance mechanisms, including metal chelation by phytochelatins and metallothioneins, sequestration of metal ions into vacuoles, and enhancement of antioxidant defense systems to suppress ROS accumulation (Li et al., 2022). The sustained removal performance may reflect the activation of adaptive responses that support ionic homeostasis and metal detoxification Therefore, the morphological alterations observed in this study represent an external phenotypic manifestation of metal-induced stress, whereas the consistently high Fe removal efficiency indicates that the functional phytoremediation capacity of *P. stratiotes* remained largely maintained despite the increased physiological burden imposed by multi-metal exposure.

Table 1. Physiological responses of *P.stratiotes* during the phytoremediation process

Day-	Container	Plant Condition			Documentation
		Healthy	Chlorotic	Dead	
1	1 (Fe)	12	-	-	
	2 (Fe+Co)	12	-	-	
	3(Fe+Mn)	12	-	-	
	4 (Fe+Mn+Co)	12	-	-	
2	1 (Fe)	12	-	-	
	2 (Fe+Co)	12	-	-	
	3(Fe+Mn)	12	-	-	
	4 (Fe+Mn+Co)	12	-	-	
3	1 (Fe)	11	1	-	
	2 (Fe+Co)	11	1	-	
	3(Fe+Mn)	11	1	-	
	4 (Fe+Mn+Co)	11	1	-	
4	1 (Fe)	11	1	-	
	2 (Fe+Co)	10	2	-	
	3(Fe+Mn)	10	2	-	
	4 (Fe+Mn+Co)	9	3	-	
5	1 (Fe)	7	4	1	
	2 (Fe+Co)	3	8	1	
	3(Fe+Mn)	3	8	1	
	4 (Fe+Mn+Co)	3	6	3	

6	1 (Fe)	2	8	2	
	2 (Fe+Co)	2	8	2	
	3(Fe+Mn)	2	8	2	
	4 (Fe+Mn+Co)	1	9	3	

Root Morphology and Characterization of *P. stratiotes* by SEM–EDS

Root morphology was characterized using Scanning Electron Microscopy (SEM) to evaluate structural changes following the phytoremediation process, whereas Energy-Dispersive X-ray Spectroscopy (EDX) was employed to identify the metal elements accumulated within the root tissues. SEM micrographs obtained at magnifications of 500× and 1000× (Figure 1) revealed that the roots of *P.stratiotes* retained their porous architecture and fibrous network after exposure to the mixed-metal system. The root pores exhibited diameters ranging from 47.3 to 50.6 µm, with an average pore width of approximately 14.8 µm. Such a porous structure provides a large effective surface area, thereby enhancing the probability of contact between dissolved metal ions and the root surface. A high surface area is a key structural characteristic that facilitates the initial adsorption of metal ions, promotes their diffusion toward root tissues, and supports subsequent transport into plant cells (Sharma et al., 2023).

Beyond serving as the primary site for surface adsorption, the root surface also represents the initial interface for metal uptake through membrane-bound transporter proteins located in root epidermal cells. During the early stage of exposure, Fe^{2+}, Mn^{2+}, and Co^{2+} ions diffuse toward the root surface and interact with negatively charged functional groups within the cell wall, including hydroxyl, carboxyl, and phosphate groups. These interactions are followed by active transport into the cytoplasm through divalent metal transporters, after which the absorbed ions are distributed to different cellular compartments according to the physiological requirements of the plant (Li et al., 2022). As intracellular metal concentrations increase, a fraction of the absorbed ions is chelated by phytochelatins and metallothioneins, thereby reducing metal toxicity before sequestration into vacuoles as part of the cellular detoxification process (Nusyura et al., 2023). These mechanisms explain why the root tissues largely preserved their structural integrity despite the appearance of physiological stress symptoms during the later stages of phytoremediation.

The EDX spectra clearly showed the presence of Fe, Mn, and Co on the root surface after phytoremediation, with atomic percentages of 1.83%, 0.65%, and 0.19%, respectively (Figure 2). The detection of these elements confirms that all three metals were successfully accumulated within the root tissues of *P. stratiotes*. Among the detected metals, Fe exhibited the highest atomic

percentage, indicating a greater accumulation capacity relative to Mn and Co. This observation is consistent with the ICP–OES results, which demonstrated relatively

high Fe removal efficiency throughout the phytoremediation process, further supporting the role of the root system as the primary site of Fe accumulation prior to its translocation to other plant tissues.

The preferential accumulation of Fe in the roots is likely associated with its physiological role as an essential micronutrient required for chlorophyll biosynthesis, cytochrome formation, electron transport, cellular respiration, and numerous enzymatic reactions in plants (Fahri Rozanno Putra, 2024). Furthermore, Fe uptake is mediated by high-affinity metal transporters, particularly Iron-Regulated Transporter 1 (IRT1), a member of the ZIP transporter family. Although IRT1 is capable of transporting several other divalent metal ions, its affinity for Fe is generally higher, which may explain the predominance of Fe accumulation over Mn and Co under the experimental conditions employed in this study (Rodrigues et al., 2023). Nevertheless, the simultaneous presence of Mn and Co may still induce competition at the transporter level, particularly at elevated metal concentrations or under altered metal ratios. Therefore, the substantial accumulation of Fe observed in this study suggests that Fe uptake by *P. stratiotes* remained effective despite exposure to a mixed-metal environment.

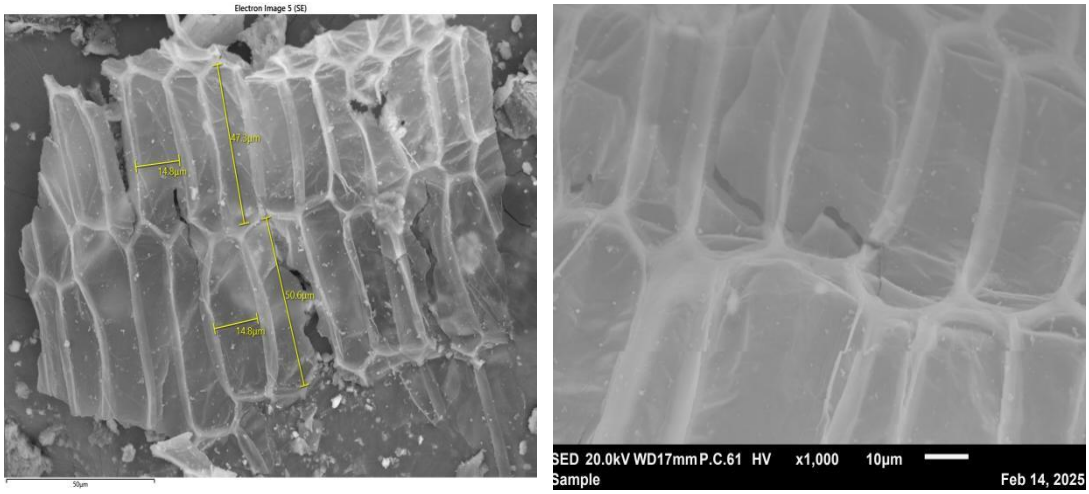


Figure.1. Root morphology of *Pistia stratiotes*: (a) 500× magnification; (b) 1000× magnification

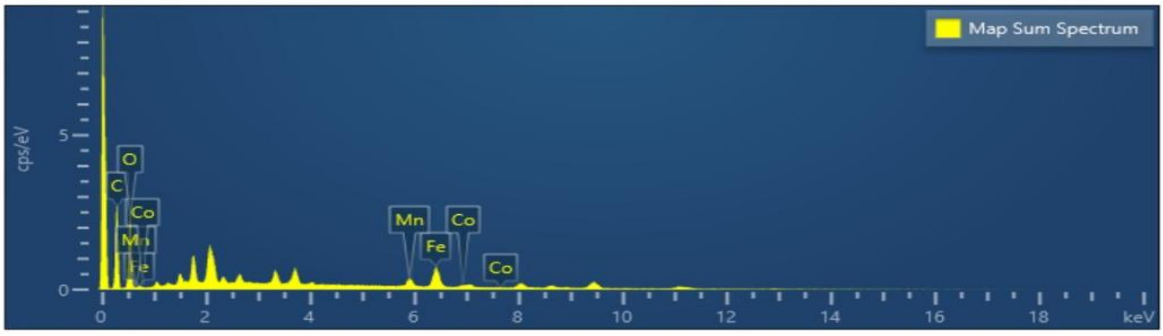


Figure 2. EDX spectrum of *P. stratiotes* roots

Overall, the SEM–EDX characterization provides complementary morphological and elemental evidence supporting the successful phytoremediation of the mixed-metal system.

The preservation of root porosity indicates that the root tissues maintained suitable structural characteristics for metal adsorption and transport, while the detection of Fe, Mn, and Co in the EDX spectra confirms their accumulation within the root tissues. These findings demonstrate that the root system of *P. stratiotes* functions as the principal compartment for metal sequestration and provide mechanistic support for the high Fe removal efficiency quantified by ICP–OES, as discussed in the following section.

Fe Removal Performance in the Multi-Metal System Based on ICP–OES Analysis

The Fe removal performance was evaluated by comparing the metal concentrations before and after phytoremediation using Inductively Coupled Plasma–Optical Emission Spectrometry (ICP–OES). Removal efficiency was calculated from the difference between the initial (C_i) and final (C_e) Fe concentrations and expressed as the percentage reduction in aqueous Fe concentration. As summarized in Table 5, all treatments achieved exceptionally high Fe removal efficiencies, with mean values exceeding 99%. These results demonstrate the remarkable capability of *P. stratiotes* to remove Fe from aqueous media, even in the presence of coexisting metals such as Mn and Co.

The consistently high Fe removal efficiency indicates that the presence of mixed metals did not substantially impair the capacity of *P. stratiotes* to accumulate Fe. This observation is in agreement with the SEM–EDS results, which identified Fe as the predominant element accumulated in the root tissues following phytoremediation. The consistency between the ICP–OES and SEM–EDS analyses suggests that the decrease in aqueous Fe concentration was not solely attributable to surface adsorption but also involved intracellular accumulation within the plant tissues. Therefore, Fe removal in the present study is likely governed by multiple complementary processes, including surface adsorption, membrane transport, intracellular translocation, and sequestration into cellular compartments as part of the plant's metal homeostasis mechanism (Titahena et al., 2023).

Although all treatments exhibited comparable Fe removal efficiencies, the presence of Mn and Co may still influence Fe uptake through ionic competition. Fe^{2+}, Mn^{2+}, and Co^{2+} are divalent metal ions with similar physicochemical characteristics and are known to share common uptake pathways mediated by membrane transporters such as Iron-Regulated Transporter 1 (IRT1), ZIP, and NRAMP proteins (*New Phytologist - 2025 - Rylott - Harnessing Hyperaccumulator Plants to Recover Technology‐critical Metals Where Are We at.Pdf*, n.d.). Theoretically, competition among these ions could reduce Fe uptake when competing metals are present at higher concentrations or exhibit greater affinity for the transport proteins. However, such competitive effects were not evident under the experimental conditions employed in this study, most likely because the metal concentrations were maintained within the Indonesian wastewater quality standards. Under these relatively low exposure levels, the transport capacity and homeostatic regulatory mechanisms of *P. stratiotes* were apparently sufficient to facilitate the simultaneous uptake of Fe, Mn, and Co without substantially compromising Fe accumulation.

In addition to transporter-mediated uptake, the high Fe removal efficiency reflects the ability of *P. stratiotes* to maintain physiological homeostasis during metal accumulation. As an essential micronutrient, Fe is indispensable for chlorophyll biosynthesis, electron transport, cellular respiration, and numerous enzymatic reactions in plants. Consequently, plants possess tightly regulated uptake and storage systems to ensure adequate intracellular Fe availability while preventing metal toxicity. When Fe accumulation exceeds metabolic demand, excess ions are chelated by phytochelatins and metallothioneins and subsequently sequestered into vacuoles, thereby maintaining low concentrations of free Fe in the cytoplasm and minimizing oxidative damage (Rodrigues et al., 2023). This regulatory mechanism enables *P. stratiotes* to sustain efficient Fe uptake while preserving its physiological functions under mixed-metal exposure.

Overall, the ICP–OES results demonstrate that *P. stratiotes* maintained exceptionally high Fe removal efficiency throughout the phytoremediation of the multi-metal system. These findings indicate that the presence of Mn and Co at the investigated concentrations was insufficient to significantly inhibit Fe uptake. Instead, Fe accumulation appears to be primarily driven by the plant's physiological demand for this essential micronutrient, whereas intermetallic competition played only a limited role under the conditions examined. This interpretation is further evaluated in the subsequent statistical analysis, which examines whether the presence of coexisting metals significantly influenced Fe removal efficiency.

Statistical Evaluation of the Effects of Mixed Metals on Fe Removal Efficiency

Prior to hypothesis testing, the Fe removal efficiency data were evaluated to determine the suitability of the statistical methods. The Shapiro–Wilk normality test indicated that the data followed a normal distribution ($p = 0.065$), whereas Levene's test revealed significant heterogeneity of variances ($p < 0.001$). Because the assumption of homogeneity of variances was not satisfied, differences among treatments were evaluated using the non-parametric Kruskal–Wallis test.

The Kruskal–Wallis analysis yielded an Asymp. Sig. value of 0.321 ($p > 0.05$), indicating that the addition of Mn and Co to the treatment system did not significantly affect the Fe removal efficiency of *Pistia stratiotes*. In other words, despite exposure to mixed-metal conditions, the plants maintained a comparable capacity for Fe removal across all treatments. This statistical result corroborates the ICP-OES findings, which demonstrated Fe removal efficiencies exceeding 99% in every treatment, indicating that the concentrations of coexisting metals used in this study were insufficient to significantly reduce the phytoremediation performance of *P. stratiotes*.

From a physiological perspective, the absence of significant differences among treatments suggests that the metal homeostasis mechanisms of *P. stratiotes* remained effective under simultaneous exposure to multiple divalent metal ions. Although Fe^{2+}, Mn^{2+}, and Co^{2+} are known to share overlapping uptake pathways mediated by transporter families such as ZIP, NRAMP, and Iron-Regulated Transporter 1 (IRT1), plants possess sophisticated regulatory systems that ensure the preferential acquisition

of essential nutrients while maintaining intracellular metal balance (Rodrigues et al., 2023). In addition, metal chelation by phytochelatins and metallothioneins, together with vacuolar sequestration, likely contributed to maintaining low concentrations of free metal ions in the cytoplasm, thereby minimizing metabolic disturbances and preserving efficient Fe uptake (Li et al., 2022). These adaptive mechanisms suggest that the mixed-metal concentrations employed in this study did not exceed the physiological tolerance or detoxification capacity of the plants.

The relationship between the number of coexisting metals and Fe removal efficiency was further examined using Spearman's rank correlation analysis. The analysis produced a correlation coefficient of -0.293 with a significance level of $p = 0.482$, indicating a weak negative correlation that was not statistically significant. Although the addition of more metal species tended to coincide with a slight decrease in Fe removal efficiency, the magnitude of this reduction was too small to establish a meaningful statistical relationship.

Despite its lack of statistical significance, the negative correlation trend remains noteworthy because it may reflect the onset of ionic competition during phytoremediation. Fe^{2+}, Mn^{2+}, and Co^{2+} are divalent cations with similar physicochemical properties and therefore may compete for binding to membrane transporters and intracellular metal-binding ligands. According to the Irving–Williams series, the stability of complexes formed between divalent metal ions and biological ligands generally increases in the order $Mn^{2+} < Fe^{2+} < Co^{2+} < Ni^{2+} < Cu^{2+}$ (Schmidt & Husted, 2019). This series describes the relative thermodynamic stability of divalent metal complexes and therefore provides a chemical basis for considering differences in metal–ligand interactions, rather than direct evidence of in vivo metal transport kinetics. Consequently, Co^{2+} may theoretically have a greater tendency to form stable complexes with available metal-binding ligands than Fe^{2+}, potentially contributing to competition for metal-binding sites. Likewise, Coimbra et al., 2023 (Coimbra & Borges, 2023) reported competitive interactions between Fe and Mn during root uptake when both metals are present at comparable concentrations. Therefore, the possibility of metal–metal competition during plant uptake and binding, although the non-significant correlation does not establish a direct inhibitory effect of Co^{2+} on Fe^{2+} transport.

Nevertheless, the absence of a statistically significant relationship indicates that ionic competition was not the dominant factor controlling Fe removal under the experimental conditions. One plausible explanation is that the metal concentrations were relatively low and remained within the limits of the Indonesian wastewater quality standards, such that the transport capacity and detoxification systems of *P. stratiotes* were not saturated. Furthermore, because Fe is an essential micronutrient required for chlorophyll biosynthesis, cellular respiration, and photosynthetic electron transport, plants generally maintain preferential regulatory mechanisms to secure adequate Fe acquisition even in the presence of competing metal ions. These physiological priorities likely explain why Fe removal efficiency remained consistently high across all treatments, whereas the influence of mixed metals was limited to a weak and statistically insignificant downward trend.

Overall, the statistical analyses demonstrate that the presence of mixed metals did not significantly influence the Fe removal efficiency of *P. stratiotes* under the conditions investigated. However, the observed negative correlation suggests that intermetallic interactions may still occur at the physiological level, although their magnitude was insufficient to impair phytoremediation performance. These findings highlight that phytoremediation efficiency is governed not only by the presence of coexisting metals but also by their concentrations, chemical characteristics, affinity for membrane transporters, and the capacity of plants to maintain intracellular metal homeostasis. Future studies employing a broader range of metal concentrations or more complex multi-metal systems are therefore warranted to further elucidate the mechanisms of ionic competition during phytoremediation.

Mechanisms of Multi-Metal Interactions During Phytoremediation by *Pistia stratiotes*

The combined evidence obtained from morphological observations, SEM–EDX characterization, ICP–OES analysis, and statistical evaluation demonstrates that *P. stratiotes* maintained a high Fe removal efficiency despite exposure to mixed-metal conditions. These findings suggest that phytoremediation performance was governed not only by the intrinsic capacity of the plant to absorb Fe but also by physiological regulatory mechanisms that maintain metal homeostasis under simultaneous exposure to Fe, Mn, and Co. Consequently, metal uptake in a multi-metal system is considerably more complex than in single-metal systems because it involves concurrent processes of ion competition, transporter-mediated uptake, intracellular trafficking, and detoxification. The proposed mechanism of Fe uptake in the mixed-metal system is illustrated in Figure 3.

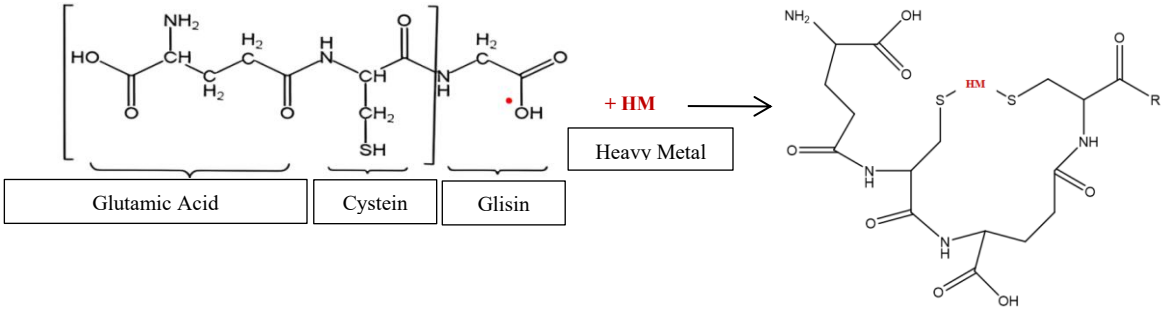


Figure 3. Proposed mechanism of heavy metal interactions with phytochelatin amino acids

Initially, Fe^{2+}, Mn^{2+}, and Co^{2+} ions diffuse from the aqueous solution toward the root surface, where they are adsorbed onto negatively charged functional groups within the cell wall, including hydroxyl, carboxyl, and phosphate groups. These functional groups provide ion-exchange and adsorption sites that facilitate the initial immobilization of metal ions before cellular uptake (Islam et al., 2024). Subsequently, the adsorbed metal ions are transported across the plasma membrane through divalent metal transporters, primarily members of the ZIP and NRAMP families, together with Iron-Regulated Transporter 1 (IRT1), which plays a central role in Fe acquisition by plant roots. Because Fe^{2+}, Mn^{2+},

and Co^{2+} possess the same oxidation state and comparable physicochemical properties, these ions may utilize overlapping transport pathways, creating the potential for competitive uptake.

From a mechanistic perspective, the simultaneous presence of multiple metal ions is expected to reduce the uptake efficiency of individual metals through competition for transporter proteins and intracellular metal-binding ligands. The extent of this competition is influenced not only by metal concentration but also by physicochemical characteristics such as ionic radius, hydrated ionic radius, transporter affinity, and the thermodynamic stability of metal–ligand complexes (Samet & Wages, 2018). Once inside the plant cells, the absorbed metal ions may interact with a range of intracellular ligands, including phytochelatins, organic acids, proteins, and other metal-binding molecules. In phytochelatins, several functional groups can potentially contribute to metal coordination, including sulfhydryl (–SH), carboxylate ($–COO^-$), and nitrogen-containing groups. Therefore, metal sequestration should not be interpreted as an interaction involving the –SH group alone. The –SH group was emphasized because cysteine residues constitute the characteristic metal-binding sites of phytochelatins, and sulfur atoms can act as electron donors in metal coordination. However, this does not exclude contributions from other functional groups. The carboxylate groups ($–COO^-$), originating primarily from glutamate and cysteine residues, can also provide oxygen donor atoms for coordination with metal ions. Their contribution may be particularly relevant for divalent metals such as Fe^{2+}, Mn^{2+}, and Co^{2+}, for which oxygen-containing ligands can participate in complex formation. Nitrogen-containing groups may also contribute to metal coordination, particularly when available as amine donors. However, nitrogen atoms within peptide amide bonds generally have lower coordinating ability because their lone pairs are delocalized toward the carbonyl groups. Thus, their contribution is expected to be more dependent on the local chemical environment and protonation state.

The relative contribution of these binding groups may differ among metal ions because metal-ligand interactions are governed by the chemical properties of both the metal and the ligand. According to the Irving–Williams series, the stability of complexes formed by divalent transition metals generally follows the order $Mn^{2+} < Fe^{2+} < Co^{2+} < Ni^{2+} < Cu^{2+}$ (Yakovenko et al., 2025). Consequently, Co^{2+} is theoretically expected to compete more effectively than Mn^{2+} for binding sites on transporter proteins and intracellular ligands. Nevertheless, the statistical analyses performed in the present study demonstrated that these competitive interactions were insufficient to significantly reduce Fe removal efficiency, as evidenced by the non-significant Kruskal–Wallis and Spearman correlation results.

The sustained Fe removal efficiency observed throughout the experiment is likely attributable to the highly efficient metal homeostasis mechanisms of *P. stratiotes*. Once transported into the cytoplasm, metal ions are rapidly complexed by phytochelatins, metallothioneins, and other low-molecular-weight organic ligands, thereby minimizing their cytotoxicity and preventing excessive production of reactive oxygen species (ROS) (Li et al., 2022). The resulting metal–ligand complexes are subsequently sequestered into

vacuoles or immobilized within the cell wall, effectively maintaining low concentrations of free metal ions in the cytoplasm while preserving essential metabolic activities. These detoxification mechanisms enable *P. stratiotes* to tolerate mixed-metal exposure without compromising its capacity to continuously remove Fe from the aqueous environment.

The proposed mechanism is further supported by the experimental observations. Although mild chlorosis and necrosis developed during the later stages of phytoremediation, these physiological symptoms were not accompanied by a measurable decline in Fe removal efficiency. This finding indicates that the observed morphological responses primarily reflected adaptive physiological adjustments to increasing metal stress rather than failure of the phytoremediation process itself. In other words, despite the increased physiological burden imposed by mixed-metal exposure, the regulatory networks controlling metal uptake, detoxification, and intracellular homeostasis remained sufficiently effective to sustain efficient Fe accumulation under the experimental conditions.

Overall, the present study demonstrates that metal interactions in phytoremediation systems do not necessarily result in reduced removal efficiency of the target metal. Instead, phytoremediation performance is determined by the dynamic balance among ionic competition, transporter affinity, intracellular detoxification, and the capacity of plants to maintain metal homeostasis. These findings highlight the limitations of evaluating phytoremediation using single-metal systems alone, as industrial wastewater generally contains complex mixtures of metals that interact through multiple physicochemical and biological mechanisms. Therefore, the incorporation of multi-metal experimental systems provides a more realistic framework for understanding phytoremediation processes and for developing treatment technologies that better reflect environmental conditions encountered in practice.

CONCLUSION

This study demonstrated that *P. stratiotes* maintained an exceptionally high Fe removal efficiency (>99%) under both single-metal and mixed-metal (Fe–Mn–Co) conditions. Morphological observations, SEM–EDS characterization, ICP–OES analysis, and statistical evaluation consistently showed that the presence of Mn and Co did not significantly affect Fe removal efficiency, as confirmed by the Kruskal–Wallis test ($p = 0.321$; $p > 0.05$), although prolonged exposure induced mild physiological stress symptoms. These findings indicate that *P. stratiotes* effectively adsorbed, accumulated, and sequestered Fe while maintaining root structural integrity under multi-metal exposure.

Overall, the results suggest that ionic competition among Fe^{2+}, Mn^{2+}, and Co^{2+} was insufficient to impair Fe uptake because the plant maintained efficient metal homeostasis through transporter regulation and intracellular detoxification mechanisms. This study provides new insights into phytoremediation under multi-metal conditions. The findings support the evaluation of mixed-metal systems for improving phytoremediation applications in industrial wastewater treatment.

REFERENCES

Ardiatma, D., Ilyas, N. I., & Sara, N. U. (2023). Efektivitas Metode Fitoremediasi dengan Tanaman Kayu Apu (Pistia stratiotes L .)

terhadap Penurunan Kadar BOD dalam Limbah Domestik di Jakarta peningkatan badan air penerima baik dari aspek kapasitas maupun kualitasnya , Upaya yang harus dilakukan untuk. *Jurnal Sains Dan Terknologi Lingkungan*, *15*, 121–133.

Briffa, J., Sinagra, E., & Blundell, R. (2020). Heavy metal pollution in the environment and their toxicological effects on humans. *Heliyon*, *6*(9), e04691. https://doi.org/10.1016/j.heliyon.2020.e04691

Coimbra, E. C. L., & Borges, A. C. (2023). Removing Mn, Cu and Fe from Real Wastewaters with Macrophytes: Reviewing the Relationship between Environmental Factors and Plants' Uptake Capacity. *Toxics*, *11*(2). https://doi.org/10.3390/toxics11020158

Delgado-González, C. R., Madariaga-Navarrete, A., Fernández-Cortés, J. M., Islas-Pelcastre, M., Oza, G., Iqbal, H. M. N., & Sharma, A. (2021). Advances and applications of water phytoremediation: A potential biotechnological approach for the treatment of heavy metals from contaminated water. *International Journal of Environmental Research and Public Health*, *18*(10). https://doi.org/10.3390/ijerph18105215

Fahri Rozanno Putra. (2024). *No Title*. Sekolah Tinggi Analis Kimia Cilegon.

Febriani, H., Puspitasari, D. J., & Sosidi, H. (2021). Adsorbsi Ion Logam Cu (II) Menggunakan Biomassa Daun Genjer (Limnocharis flava) [Adsorption of Cu (II) Ions by Biomass of Genjer Leaves (Limnocharis flava)]. *Jurnal Riset Kimia*, *7*(2), 131–136.

Islam, M. M., Saxena, N., & Sharma, D. (2024). Phytoremediation as a green and sustainable prospective method for heavy metal contamination: a review. *RSC Sustainability*, *2*(5), 1269–1288. https://doi.org/10.1039/d3su00440f

Kafle, A., Timilsina, A., Gautam, A., Adhikari, K., Bhattarai, A., & Aryal, N. (2022). Phytoremediation: Mechanisms, plant selection and enhancement by natural and synthetic agents. *Environmental Advances*, *8*(February), 100203. https://doi.org/10.1016/j.envadv.2022.100203

Khairuddin, Sikanna, R., & Sabaruddin. (2017). Assessment Ability Plants of Kale Land Roots (Ipomoea reptans Poir) in Absorbing Metallic of Mercury (Hg) on Polluted Soil. *KOVALEN: Jurnal Riset Kimia*, *3*(3), 303–312.

Khairuddin, Wengkau, W., Puspitasari, D. J., Sosidi, H., & Inda, N. I. (2021). Adsorpsi Logam Merkuri (Hg) dari Limbah Tanah Tercemar Menggunakan Tanaman Sawi (Brassica juncea L) pada Berbagai Waktu Tanam. *KOVALEN: Jurnal Riset Kimia*, *7*(1), 65–71. https://doi.org/10.22487/kovalen.2021.v7.i1.13666

Li, Y., Xin, J., & Tian, R. (2022). Physiological defense and metabolic strategy of *Pistia stratiotes* in response to zinc–cadmium co-pollution. *Plant Physiology and Biochemistry, 178*, 1–11.

Li, Y., Xin, J., Ge, W., & Tian, R. (2022). Tolerance mechanism and phytoremediation potential of *Pistia stratiotes* to zinc and cadmium co-contamination. *International Journal of Phytoremediation, 24*(12).

New Phytologist - 2025 - Rylott - Harnessing hyperaccumulator plants to recover technology‐critical metals where are we at.pdf. (n.d.).

Nusyura, R., Azizah, E., Ningsih, D. P., & Dewi, N. (2023). Analisis Kadar Logam Kadmium, Mangan dan Seng dalam Air Limbah secara Inductively Coupled Plasma-Optical Emission Spectrometry. *Jurnal Serambi Engineering*, *VIII*(1), 4529–4537.

Oktaviani, L., Nilandita, W., & Suprayogi, D. (2020). Fitoremediasi Tanaman Apu-Apu (Pistia Stratiotes) terhadap Kadar Logam Zn Berdasarkan Variasi Jumlah Tanaman. *Al-Ard: Jurnal Teknik Lingkungan*, *6*(1), 44–52. https://doi.org/10.29080/alard.v6i1.981

Resti Billah, A., Dewi M, A., & Trirahayu N, P. (2021). Phythoremediasi Chromium Total (Cr-T) menggunakan kayu apu (Pistia stratiotes L.) pada limbah cair batik. *Jurnal Biologi Udayana*, *24*(1), 47–54.

Rizvi, Z. F., Jamal, M., Parveen, H., Sarfraz, W., Nasreen, S., Khalid, N., & Muzammil, K. (2024). Phytoremediation potential of Pistia stratiotes, Eichhornia crassipes, and Typha latifolia for chromium with stimulation of secondary metabolites. *Heliyon*, *10*(7), e29078. https://doi.org/10.1016/j.heliyon.2024.e29078

Rodrigues, W. F. C., Lisboa, A. B. P., Lima, J. E., Ricachenevsky, F. K., & Del-Bem, L. E. (2023). Ferrous iron uptake via IRT1/ZIP evolved at least twice in green plants. *New Phytologist*, *237*(6), 1951–1961. https://doi.org/10.1111/nph.18661

Rudy, S. U., Harso, W., Ramadanil, Iqbal, M., & Prismawiryanti. (2024). Fitoremediasi Air Tercemar Nikel (Ni) dan Merkuri (Hg)

Menggunakan Tanaman Azolla filiculoides Lam. *KOVALEN: Jurnal Riset Kimia*, *10*(2), 185–192. https://doi.org/10.22487/kovalen.2024.v10.i2.17343

Samet, J. M., & Wages, P. A. (2018). Oxidative stress from environmental exposures. *Current Opinion in Toxicology*, *7*(2017), 60–66. https://doi.org/10.1016/j.cotox.2017.10.008

Schmidt, S. B., & Husted, S. (2019). The biochemical properties of manganese in plants. *Plants*, *8*(10). https://doi.org/10.3390/plants8100381

Sharma, J. K., Kumar, N., Singh, N. P., & Santal, A. R. (2023). Phytoremediation technologies and their mechanism for removal of heavy metal from contaminated soil: An approach for a sustainable environment. *Frontiers in Plant Science*, *14*(January), 1–13. https://doi.org/10.3389/fpls.2023.1076876

Shinta, D. Y., Juliandi, M. D., & Primal, D. (2024). Dampak Paparan Logam Berat Besi (Fe) Terhadap Hemoglobin (Hb) Pada Darah Tikus Wistar. *Jurnal Zona*, *8*(1), 57–63. https://doi.org/10.52364/zona.v8i1.111

Surbakti, N. A. B., Febriani, H., & Syukriah, S. (2024). Kandungan logam berat besi (Fe) pada air dan daging ikan lemeduk (Barbonymus schwanenfeldii) di Sungai Belumai Deli Serdang. *Jurnal Akuakultur Sungai Dan Danau*, *9*(1), 69. https://doi.org/10.33087/akuakultur.v9i1.203

Titahena, M. F., Mariwy, A., & Sunarti, S. (2023). STUDI REMEDIASI TANAH TERCEMAR LOGAM MERKURI (Hg) MENGGUNAKAN TUMBUHAN TEKI (Cyperus rotundus). *Jurnal Reka Lingkungan*, *11*(2), 95–104. https://doi.org/10.26760/rekalingkungan.v11i2.95-104

Utomo, W. B., & Hasan, B. (2022). Penentuan Laju Korosi Baja Konstruksi Menggunakan Metode TafelAnalisis. *Prosiding 6th Seminar Nasional Penelitian & Pengabdian Kepada Masyarakat*, *1*(6), 19–24.

Yakovenko, M., Khokha, Y., & Pavlyuk, M. (2025). Vertical Distribution of Mobile Trace Elements in Peat Deposits of the Lviv Region and Their Relationships with Physico-Chemical Properties. *Visnyk of V. N. Karazin Kharkiv National University, Series “Geology. Geography. Ecology,” 2025*(63), 113–127. https://doi.org/10.26565/2410-7360-2025-63-09