Pilot-scale evaluation of floating macrophytes for tertiary polishing of dairy wastewater: An integrated assessment ofCd and Pb removal mechanisms

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Title page

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Abstract

The discharge of residual agro-industrial effluents containing potentially toxic elements (PTEs) after conventional biological treatment remains an environmental challenge, since secondary treatment mainly removes organic matter, and not inorganic contaminants. Nature-based polishing technologies utilizing floating aquatic macrophytes are promising alternatives; however, under realistic operating conditions remain limited. This study evaluated the feasibility of using aquatic macrophytes as tertiary polishing systems for dairy effluents previously subjected to secondary treatment, at a pilot scale and under batch-flow conditions. The performance of *Pontederia crassipes*, *Pistia stratiotes*, and *Salvinia auriculata* was compared over 36 days by analyzing water physicochemical parameters, plant growth, nutrient dynamics, and Pb and Cd accumulation. All species improved effluent quality by reducing turbidity, total solids, and chemical oxygen demand, while maintaining dissolved oxygen and pH within environmentally acceptable ranges. *P. crassipes* and *P. stratiotes* produced greater biomass and accumulated higher metal concentrations than *S. auriculata*. The integration of metal data with bioaccumulation and translocation analyses revealed that pH-induced changes in metal speciation influenced bioavailability and uptake, providing mechanistic insights that go beyond conventional removal assessments. Although each treatment utilized a single pilot reactor, repeated temporal monitoring allowed for a robust assessment of treatment dynamics and comparative species performance under consistent operating conditions, substantiating technological viability rather than determining a definitive treatment efficiency. By integrating environmental engineering and plant physiology approaches, our study contributes to the mechanistic understanding of phytoremediation and may inform the optimization and future full-scale implementation of low-cost tertiary polishing systems for agro-industrial effluents.

Keywords

effluent polishing; heavy metals; metal bioaccumulation; physicochemical parameters; plant growth.

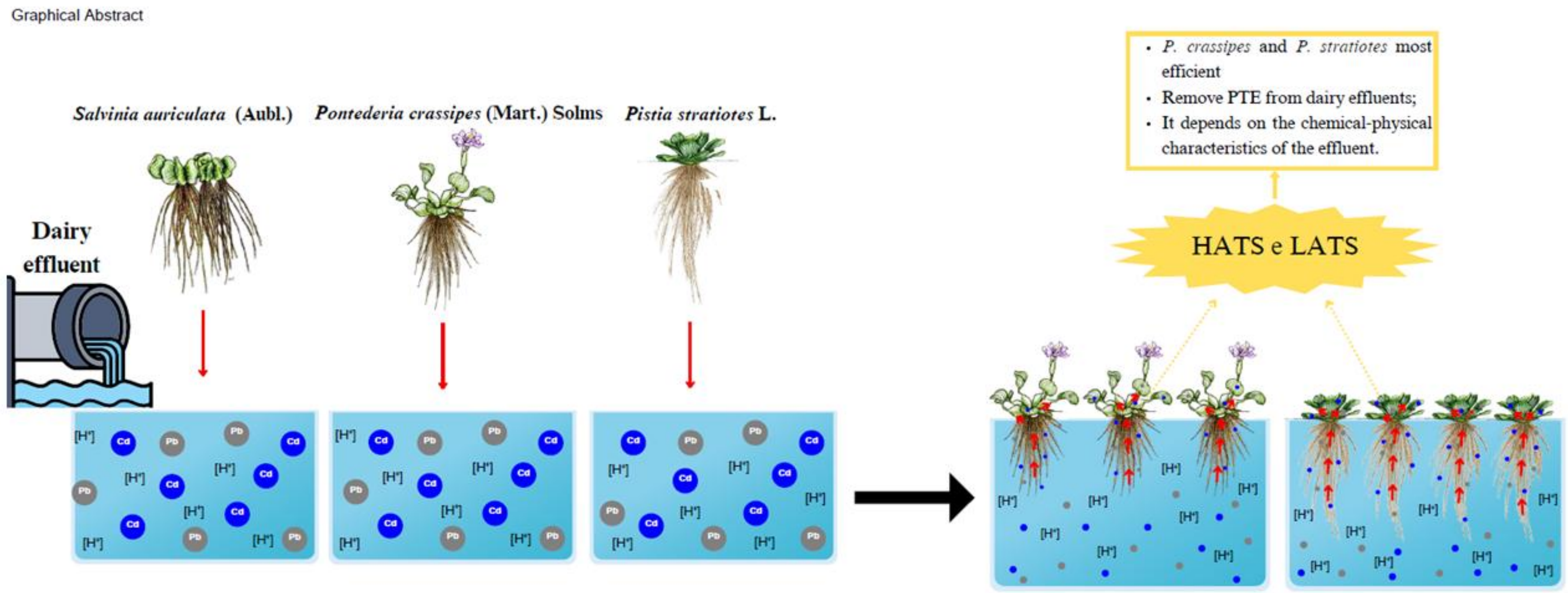
Highlights

Pilot-scale tertiary polishing of dairy wastewater with floating macrophytes.

Integrated assessment of lead/cadmium uptake and translocation.

Pontederia crassipes and Pistia stratiotes showed superior performance.

63 **Graphical abstract**

64 

1. Introduction

The discharge of industrial and agro-industrial effluents containing potentially toxic elements (PTEs) remains a major environmental challenge because conventional wastewater treatment systems are primarily designed to remove organic matter rather than inorganic contaminants. Consequently, treated effluents may still contain residual concentrations of metals capable of accumulating in aquatic ecosystems, posing risks to environmental quality (Shen et al., 2020; Yan et al., 2020). Among these contaminants, lead (Pb) and cadmium (Cd) are non-biodegradability, persistence, high toxicity, and tendency to accumulate in living organisms (Jarup, 2003; Kaur et al., 2017; Collin et al., 2022; Nag and Cummins, 2022; Shi et al., 2023). Although regulatory agencies have established maximum permissible concentrations for these elements in water and industrial effluents (Brasil, 2008; Brasil, 2011; Brasil, 2021; Deviller et al., 2020; Perveen and Amar Ul, 2023; Russ et al., 2022; Zhao et al., 2022), compliance remains challenging when conventional treatment processes are not specifically designed to target metal removal.

The dairy industry constitutes one of the largest agro-industrial sectors globally and is a significant source of wastewater characterized by elevated concentrations of organic matter, nutrients, suspended solids, and, in some cases, residual levels of potentially toxic elements (PTEs). Conventional dairy wastewater treatment systems are primarily engineered to achieve the removal of organic matter, oils and grease, and suspended solids, as reflected by reductions in biochemical oxygen demand (BOD) and chemical oxygen demand (COD). In contrast, the removal of potentially toxic metals is rarely incorporated into treatment system design (Qasim and Mane, 2013; Krishna et al., 2022). Consequently, even after secondary treatment, dairy effluents may still contain contaminants capable of compromising receiving water bodies. Previous studies have reported the occurrence of Cd, Pb, Cu, Zn, Fe, and Ni in dairy products and effluents from different countries (Afolabi et al., 2015; Boudebbouz et al., 2021; Elafify et al., 2023; Năstăsescu et al., 2020; Yan et al., 2022).These shortcomings emphasize the necessity of integrating complementary treatment technologies capable of mitigating inorganic contaminants alongside the removal of conventional wastewater constituents.

Phytoremediation has gained increasing attention as an environmentally sustainable and cost-effective strategy for the polishing of contaminated waters, owing to its ability to remove a wide range of contaminants while requiring low operational inputs and causing minimal environmental disturbance (Cristaldi et al., 2017; Rostami and Azhdarpoor, 2019). In comparison with conventional physicochemical technologies, including activated carbon adsorption, membrane filtration, soil excavation, and reverse osmosis, phytoremediation is associated with lower capital and operational costs and enables the *in situ* remediation of contaminated environments (Wan et al., 2016; Kafle et al., 2022). This approach harnesses natural plant-mediated processes, such as phytoextraction, phytostabilization, rhizofiltration, and phytodegradation, through which contaminants can be absorbed, immobilized, transformed into less toxic forms, or removed from the contaminated matrix (Cristaldi et al., 2017; Kafle et al., 2022).

Among the plant groups investigated for wastewater treatment, free-floating aquatic macrophytes have attracted particular attention because of their rapid growth, high biomass production, extensive root systems, and remarkable tolerance to contaminated environments (Bartmeyer et al., 2019; de Souza et al., 2021; Gomes et al., 2017; Nahar and Hoque, 2021; Ramirez et al., 2021; Zhang et al., 2021). Species such as *Pontederia crassipes*, *Pistia stratiotes*, and *Salvinia* spp. have demonstrated the capacity to remove nutrients, suspended solids, and several metals from polluted waters through rhizofiltration and phytoaccumulation mechanisms (George and Gabriel, 2017; Kodituwakku and Yatawara, 2020). Their application as polishing units after conventional wastewater treatment is particularly attractive because these systems require relatively simple infrastructure and can substantially improve final to improve effluent quality prior to its discharge into receiving water bodies.

In a previous investigation, our research group demonstrated that aquatic macrophytes improve the physicochemical quality of dairy effluents under subtropical conditions (Castro et al., 2017). Based on current knowledge regarding the physiology of floating macrophytes and the influence of aqueous chemistry on metal mobility, our hypothesis investigated the feasibility of using aquatic macrophyte species as tertiary polishing systems effluents by stabilizing PTE for post-treated dairy effluent under pilot-scale discontinuous-flow conditions. Therefore, this study aimed to evaluate the comparative performance of *Pontederia crassipes*, *Pistia stratiotes*, and *Salvinia auriculata* as tertiary polishing systems for secondary-treated dairy wastewater under pilot-scale conditions. Specifically, the objectives were to: (i) assess improvements in physicochemical water quality throughout the treatment period; (ii) evaluate plant growth and biomass production as indicators of treatment sustainability; (iii) quantify Cd and Pb accumulation and translocation within plant tissues; (iv) investigate the influence of theoretical metal speciation on contaminant bioavailability; and (v) integrate these findings to identify the macrophyte species with the greatest potential for application in nature-based tertiary wastewater treatment systems.

Therefore, our innovative proposal goes beyond the isolated evaluation of floating macrophytes as tertiary polishing agents, focusing instead on integrating engineering and plant physiology approaches within a single experimental framework. We emphasize that, by simultaneously evaluating the improvement in effluent quality, plant growth, bioaccumulation of Pb and Cd, and translocation dynamics, our study provides a mechanistic analysis of possible phytoremediation processes. This integrated perspective contributes to a more robust understanding of the factors influencing the performance of nature-based polishing systems and may inform their future optimization for practical applications in wastewater treatment.

2. Materials and methods

2.1. Plant material and experimental implantation

The plant species investigated were *Pontederia crassipes* (Mart.) Solms. (arrow-foot), *Pistia stratiotes* L. (water-lettuce) and *Salvinia auriculata* Aubl. (water fern), which were sampled as soon as they reached the phenological stage of *'young plant'*. Visually, the plants presented a healthy appearance and with completely expanded leaves collected in water bodies without inorganic pollutants. Subsequently, the macrophytes were sanitized with running water and acclimatized in protected cultivation for 20 to 30 days before the start of the experimental implantation. The plants were grouped into sampling units, each comprising 10 plants utilized for chemical and growth analyses. An additional control unit containing wastewater without macrophytes was maintained under identical environmental conditions to distinguish plant-mediated processes from natural physicochemical variations.

2.2. Experimental rationale and study limitations

The pilot units represented physically independent treatment systems operating under realistic environmental conditions; furthermore, logistical and infrastructure constraints limited the construction of replicate reactors for each treatment. Consequently, each macrophyte species was evaluated in a single pilot reactor, monitored continuously throughout the experimental period. To maximize the reliability of the observations, all reactors were subjected to identical hydraulic, operational, and environmental conditions, including the origin of the wastewater, the hydraulic retention time, exposure to climatic conditions, the monitoring protocol, and the analytical procedures. Furthermore, repeated measurements over time allowed for the evaluation of treatment dynamics, rather than just isolated observations of final results, providing a detailed characterization of temporal trends in water quality improvement and plant performance.

The absence of biological replication represents an inherent limitation of this pilot-scale study and should be considered when interpreting the results. Consequently, the statistical analyses were designed to evaluate the temporal consistency and comparative behavior of the treatments, rather than to infer treatment effects at the population level. Therefore, our results should be interpreted as evidence of technological feasibility and the comparative performance of the species, providing a solid foundation for future studies employing experimental designs with replication and full-scale validation.

2.3. Treatments

The effluent was obtained from a polishing pond of a dairy production industry in Toledo, State of Paraná, South of Brazil. We collected 600 L of post-treated effluent directly from the milk and cheese agroindustry. After its complete homogenization, the sampled effluent was distributed in three reactors of 200 L each, in which one species isolated per reactor was cultivated, resulting in three treatments, such as *Salvinia auriculata* Aubl. in polishing of dairy effluent (T1); *Pontederia crassipes* (Mart.) Solms in polishing the dairy effluent (T2); *Pistia stratiotes* in polishing the dairy effluent (T3). The effluent was sampled at the time of implantation (day 0) and on days 4, 8, 12, 16, 20, 24, 28, 32, and 36 after the implantation of the treatments (DAAT).

2.4. Experimental design

Our investigation was conducted as a pilot-scale experiment under controlled discontinuous flow conditions and involved a single experimental cycle. The experimental setup was designed to replicate operating conditions representative of decentralized polishing units, while maintaining strict control over hydraulic load, environmental exposure, and monitoring frequency. Furthermore, each macrophyte species was evaluated in a single reactor, using repeated measurements over time to characterize treatment dynamics. Consequently, the temporal observations represent repeated measurements within the same experimental unit rather than independent biological replicates. We conducted trials involving three unreplicated treatments, with sampling performed at various intervals over a 36-day period—specifically every four days—resulting in a total of ten sampling events and 30 plants (ten plant units per treatment). The experimental design adopted was a randomized complete block design without replication (UCB), in which the blocks were defined as a temporal factor; thus, the treatments were applied to each block, but the blocks themselves were not replicated.

2.5. Experimental evaluations

2.5.1 Analysis of physicochemical parameters in the effluent

The dairy effluent was evaluated between 0 DAAT and 36th DAAT, with sampling and data collection every 4 days. The parameters turbidity, pH, and Dissolved Oxygen (DO) were determined using a multiparametric probe model Hanna HI 982. On the other hand, to determine the chemical oxygen demand (COD), we use the method described by (Baird 2012), as presented in the Standard Methods for the Examination of Water and Wastewater.

2.5.2. Analysis of plant growth

2.5.2.1. Total dry matter mass

The plants were collected, and their organs were separated into the 'aerial part' (leaf limbo, petiole, and stem) and 'root part' (primary and secondary roots). Immediately after collection, they were packed in paper bags (kraft type) and immediately dehydrated in an oven with air circulation [65ºC for 72 hours or until obtaining a mass of constant dry matter (DM)]. At the end of the drying process, we measured the DM on an analytical scale (accuracy of 0.001 g) with its results expressed in g DM^{-1}. Subsequently, the samples were crushed in a knife mill (Willey type) (Araújo et al., 2014) and stored in a dry environment until laboratory analysis.

2.5.3. Analysis of the concentration of nutrients and toxic elements in plant tissue and theoretical diagrams of heavy metal speciation

The total concentrations of the elements K, Ca, Mg, and the HMs Cu, Zn, Fe, Mn, Cd, Pb, Cr, and Al in leaves and root tissue samples were determined by nitro-perchloric digestion (HNO_3 and $HClO_4$)

(Chemists. 2016), followed by Flame Atomic Absorption Spectrometry quantification (FAAS), model GBC 932 AA (Victoria, Australia) with a deuterium lamp for background correction (Welz and Sperling, 1999). Spectroscopy (UV-VIS) determined P foliar levels, while the sulfuric acid digestion method and Kjeldahl with distillation were used for N determination (Tedesco et al., 1995). We investigated the relationship between the theoretical speciation diagrams of HMs, calculated by the Hydra/Medusa software, with the pH values in the effluent and the accumulated levels of metals in plant tissues (Puigdomenech, 2018). Laboratory analysis of nutrients and metals was performed using well-established methods, and the following Limits of Quantification (LOQ, in mg L^{-1}) were achieved: K = 0.01, Ca = 0.005, Mg = 0.005, Cu = 0.005, Fe = 0.01, Mn = 0.01, Zn = 0.005, Cd = 0.005, Pb = 0.01, Cr = 0.01, and Al = 0.01.

2.5.4. Data Analysis

We performed a comparative statistical analysis of the treatments concerning the bioaccumulation of HMs, plant growth, and physicochemical parameters of the treated effluent using the Friedman Test. Initially, the physicochemical data of the effluent, plant growth, and contents of HMs and minerals in plant tissues were explored by descriptive statistical techniques, which we present in profile graphs to describe the behavior of treatments over time. The profiles were compared by the Friedman test for non-replicated designs in blocks, considering the evaluation times as blocks. Subsequently, when the systems showed similar behaviors, the data were modeled from linear and non-linear curves, presenting the prediction margins built with 95% confidence, through which it was possible to establish time intervals that guaranteed the control of the effluent following the national standards. All hypothesis tests were performed considering 5% significance, and all statistical analyses were performed using the statistical software R (Team 2021). The translocation index was calculated using Equation 1 to evaluate the phytoextraction capability of each plant species for the assessed HM.

Eq 1. $$TI = \frac{CAP}{CRP}$$

Where: TI is the translocation index; CAP is the concentration of the element in the aerial part; CRP is the concentration of the element in the radicular part.

3. Results

Unlike laboratory-scale screening studies conducted under highly controlled conditions, our present investigation sought to evaluate the integrated response of biological and physicochemical processes under operationally realistic conditions. Data collection and statistical analysis—employing the Friedman Test as an innovative statistical tool—represent an original contribution to the interpretation of results in phytoremediation studies within environments contaminated by heavy metals (HMs). We evaluated the efficacy of macrophytes in enhancing effluent quality prior to discharge into water bodies. Furthermore, the assessment extended to the phytoremediation potential of these plants, covering aspects such as plant growth and the capacity to accumulate potentially hazardous metals. Given this approach, each treatment was represented by a single pilot-scale reactor, such that repeated observations over time served as the experimental units for temporal comparisons.

Consequently, our statistical analyses focused on identifying consistent temporal differences among treatments, avoiding assumptions incompatible with the experimental design. We selected non-parametric procedures because they are less sensitive to deviations from normality and suitable for repeated observations obtained in pilot-scale environmental systems. Although this analytical approach does not replace true biological replication, it provides a suitable framework for assessing the temporal dynamics of treatments under pilot-scale conditions, while explicitly acknowledging the inferential limitations of the experimental design. Thus, the statistical analyses support a comparative assessment of technological performance rather than definitive estimates of treatment efficiency at the population level. Overall, all macrophyte-based polishing systems reduced turbidity, total solids, and chemical oxygen demand throughout the experimental period, although treatment efficiencies varied among species.

3.1. Physical-chemical parameters

The treatments showed no differences over time regarding pH values (χ^2 = 1.77, df = 2, p = 0.412). All treatments were conducted from a dairy effluent with a pH of 6.24, and after 36 DAAT, the pH rose slightly to the average mark of 7.23 ($\bar{x} \pm S$: 7.03 $\pm$ 0.32). The treatments also showed no differences in the reduction of turbidity over time (χ^2 = 4.17, df = 2, p = 0.124). The fall in turbidity followed a negative exponential curve (Figure 1a). Figure 1a shows the negative exponential adjusted for turbidity data (UNT) over time in the analysis of the efficacy of the species *P. stratiotes, P. crassipes,* and *S. auriculata* during the treatment of tertiary dairy effluent over 36 DAAT, where the equation is as follows $y = A\,exp(-B\,x)$, where $A^* = 116.96$ and $B^* = 0.077$, and x is the chronological time.

For DO data, the Friedman test showed no differences for the three treatments (χ^2 = 0.66, df = 2, p = 0.716). In addition, there was a tendency to increase the DO for the three treatments so that the concentration showed an improvement in its levels, even though it was within the compliance required by CONAMA resolution 430/2011 since day 0 (Figure 1b). The treatments showed small differences among COD over time, according to the Friedman test (χ^2 = 8.35, df = 2, p = 0.015). For the total solids, the Friedman test pointed out that the treatments have no differences (χ^2 = 3.43, df = 2, p = 0.179). In this way, we highlight a decrease in the concentration of total solids for the three treatments (Figure 1c).

As for the total fixed solids (TFS), the Friedman test also showed no differences (χ^2 = 0.21, df = 2, p = 0.900), in which this same pattern was obtained for the data of total volatile solids (χ^2 = 4.76, df = 2, p = 0.092). For the concentrations of phosphorus (P) in the effluent ($mg.L^{-1}$), the Friedman test showed that the treatments showed no differences between each other (χ^2 = 4.66, df = 2, p = 0.096). As for the data on the concentration of nitrogen (N) in the effluent ($mg.L^{-1}$), our results indicate that the treatments differ from each other (χ^2 = 8.66, df = 2, p = 0.131).

3.2. Dry mass, mineral elements, and toxic elements in plant tissues

The plants evaluated in all treatments showed satisfactory growth, even if grown in a contaminated environment. For the average height data (cm), the species of the plants *P. stratiotes, P. crassipes*, and *S. auriculata* presented, respectively, the values of 36.7 ± 9.2; 52.4 ± 8.4; 13.4 ± 1.8) (Figure 2a). For the total dry matter mass (g), the average data of the plant species *P. stratiotes, P. crassipes* and *S. auriculata* were, respectively, 8.28 ± 2.07; 8.50 ± 2.82; 1.40 ± 0.45 (Figure 2b). Concerning the concentration of nitrogen (N) in shoots, the Friedman test pointed out that the treatments presented statistical differences (χ^2 = 9.80, df = 2, p = 0.007). As for the concentration of N in roots, the treatments do not present statistical differences (χ^2 = 0.35, df = 2, p = 0.835). Besides, treatments did not differ concerning P concentration in shoots (χ^2 = 0.80, df = 2, p = 0.670). We report the same pattern for the data of the concentration of P in roots (2 = 1.40, df = 2, p = 0.496). The Friedman test evidence that the treatments have differences in the concentrations over time concerning the K concentration in shoots (χ^2 = 6.20, df = 2, p = 0.045). However, for the K concentration in roots, the treatments showed no differences (χ^2 = 6.20, df = 2, p = 0.045), and there is no difference concerning the concentration of cadmium (Cd) in shoots or roots ($mg.g^{-1}$) (Figure 2c and 2d) (Shoots: χ^2 = 2.64, df = 2, p = 0.266) (Roots: χ^2 = 0.21, df = 2, p = 0.897).The same pattern is observed for Pb foliar and radicular concentrations, with no differences observed (Shoots: χ^2 = 2.00, df = 2, p = 0.367) (Roots: χ^2 = 1.40, df = 2, p = 0.496) (Figure 2e and 2f).

The average data for the species *P. crassipes, P. stratiotes,* and *S. auriculata* concerning the content of Cd in roots, in $mg.kg^{-1}$, were, respectively: 4.60; 3.40; 4.50, and, in shoots, were: 2.10; 2.40; 4.00. Concerning the Pb in roots, in $mg.kg^{-1}$, the following values were found: 17.70; 21.30; 21.10, and in shoots: 10.70; 9.10; 10.50. The estimated accumulation of nutrients and HMs by the shoots of *S. auriculata* during the 36 DAAT (Figures 3, 4, and 5) presented the following means (in mg): 7.1 (N), 0.5 (P), 18.3 (K), 4.5 (Ca), 1.1 (Mg), and in µg: 13.2 (Cu), 64.2 (Zn), 7160.2 (Fe), 2223.2 (Mn), 3.20 (Cd), 8.22 (Pb), and zero (or below LOQ) for Cr and Al. The same plant species presented the following mean accumulation in roots: (in mg): 4.7 (N), 0.5 (P), 6.3 (K), 5.3 (Ca), 0.7 (Mg), and in µg: 14.1 (Cu), 70.5 (Zn), 8834.0 (Fe), 3941.7 (Mn), 3.11 (Cd), 15.69 (Pb), and zero (or below LOQ) for Cr and Al.

The estimated accumulation by the shoots of *P. stratiotes* during the 36 DAAT (Figures 3, 4, and 5) presented the following means (in mg): 99.0 (N), 3.8 (P), 191.7 (K), 78.0 (Ca), 23.1 (Mg), and in µg: 110.2 (Cu), 508.1 (Zn), 34360.1 (Fe), 18873.6 (Mn), 16.41 (Cd), 67.04 (Pb), and zero (or below LOQ) for Cr and Al. The same plant species presented the following mean accumulation in roots: (in mg): 12.6 (N), 0.8 (P), 17.2 (K), 8.6 (Ca), 4.0 (Mg), and in µg: 64.9 (Cu), 179.2 (Zn), 21430.6 (Fe), 6296.8 (Mn), 4.53 (Cd), 29.03 (Pb), and zero (or below LOQ) for Cr and Al.

The estimated accumulation by the shoots of *P. crassipes* during the 36 DAAT (Figures 3, 4, and 5) presented the following means (in mg): 67.9 (N), 3.0 (P), 60.0 (K), 36.9 (Ca), 9.7 (Mg), and in µg: 70.8 (Cu), 364.8 (Zn), 4402.2 (Fe), 24364.6 (Mn), 6.99 (Cd), 42.44 (Pb), 0.20 (Cr), and zero (or below LOQ) for Al. The same plant species presented the following mean accumulation in roots: (in mg): 44.3 (N), 3.0 (P), 54.7 (K), 27.6 (Ca), 9.9 (Mg), and in µg: 160.7 (Cu), 594.0 (Zn), 68458.1 (Fe), 26713.9 (Mn), 15.81 (Cd), 64.65 (Pb), 0.61 (Cr), and zero (or below LOQ) for Al.

The TI of each nutrient and HM is presented in Figure 6, where we highlight values higher than or close to 1, which suggests significant translocation to the aerial part of plants, as observed for Cd, with TI of 1.0, 0.9and 0.7, respectively for *S. auriculata*, *P. stratiotes*, and *P. crassipes*; for Pb, with TI of 1.5and

0.9for *S. auriculata* and *P. stratiotes*; for Mn, with TI values of 4.9and 1.1, observed for *P. stratiotes* and *P. crassipes*; and for Zn, with TI of 2.1observed for *P. crassipes.* Figure 7 shows the mean percentage of nutrients and HMs accumulated by the *S. auriculata*, *P. stratiotes*, and *P. crassipes* during the 36 DAAT.

Finally, in Figures 8 and 9, the theoretical speciation of HMs is presented in function to the pH. The observed forms of HMs may be soluble and readily bioavailable for root absorption (e.g., in acidic pH ranges up to approximately pH 7.0: Cu^{+}, Cu^{2+}, Zn^{2+}, Fe^{2+}, Mn^{2+}, Mn^{2+}, Cd^{2+}, Pb^{2+}, Cr^{2+}, Cr^{2+}, and Al^{2+}) or may be present as insoluble complexes and, therefore, unavailable for root absorption [for example, in pH ranges close to 7.0 to more alkaline ranges: $Cu(OH)_2$, ZnO, Fe_2O_3, $Mn(OH)_2$, $Cd(OH)_2$, $Pb(OH)_2$, $Cr(OH)_2$, $Al(OH)_3$].

4. Discussion

4.1. Efficiency of aquatic macrophytes in tertiary polishing of dairy effluents

Our study demonstrates that floating aquatic macrophytes constitute an effective tertiary polishing strategy for dairy effluents by simultaneously improving the physicochemical quality of the water and promoting the removal of potentially toxic elements (PTEs). Although the three species evaluated showed distinct growth patterns and biomass production, all treatments progressively reduced turbidity, total solids, and chemical oxygen demand, while maintaining dissolved oxygen and pH at levels compatible with environmental standards. These results indicate that polishing performance was not limited to a single species, but reflected the complementary ecological functions commonly performed by floating macrophytes in engineered aquatic systems.

Unlike conventional biological reactors, which are primarily designed to remove biodegradable organic matter, macrophyte-based polishing systems provide additional remediation mechanisms operating simultaneously within the water column and rhizosphere. Besides nutrient assimilation by plants, suspended particles become physically retained within the dense root network, whereas microbial biofilms attached to the roots intensify the degradation of residual organic compounds. Consequently, pollutant removal results from the interaction of biological, physical, and chemical processes rather than from a single removal pathway (Cristaldi et al., 2017; Rostami and Azhdarpoor, 2019; Kafle et al., 2022).

The progressive reduction in turbidity observed throughout the experimental period is consistent with this integrated remediation mechanism. Floating macrophytes develop extensive submerged root systems that reduce water turbulence and increase particle residence time, facilitating the sedimentation of suspended solids while simultaneously promoting the adsorption of fine particulate matter onto root surfaces. Concurrently, rhizosphere microorganisms contribute to the decomposition of dissolved organic compounds, further enhancing water clarity. Similar responses have been reported in constructed wetlands and floating treatment systems utilizing aquatic macrophytes, reinforcing the role of rhizosphere-mediated processes in tertiary wastewater treatment (George and Gabriel, 2017; Kodituwakku and Yatawara, 2020).

The reduction in chemical oxygen demand also suggests that residual biodegradable compounds continued to be mineralized after secondary treatment. Dairy effluents typically contain lactose, lipids, residual proteins, and soluble organic compounds that can persist even after biological treatment (Krishna et al., 2022). The continuous oxygen exchange facilitated by floating vegetation, combined with microbial colonization of root surfaces, creates favorable conditions for the aerobic degradation of these remaining substrates. Therefore, the observed improvements cannot be attributed solely to plant metabolism but rather to the establishment of an integrated plant-microorganism remediation system. It is worth noting that dissolved oxygen levels remained stable or increased slightly during the experimental period, despite the presence of dense floating vegetation. Floating plant systems are often associated with reduced oxygen diffusion due to partial surface coverage; however, root oxygen release, microbial community dynamics, and the relatively moderate plant density used in our study likely prevented oxygen depletion. Maintaining adequate dissolved oxygen levels is particularly important, as oxygen availability regulates microbial metabolism and directly influences the oxidation state and mobility of various metallic elements.

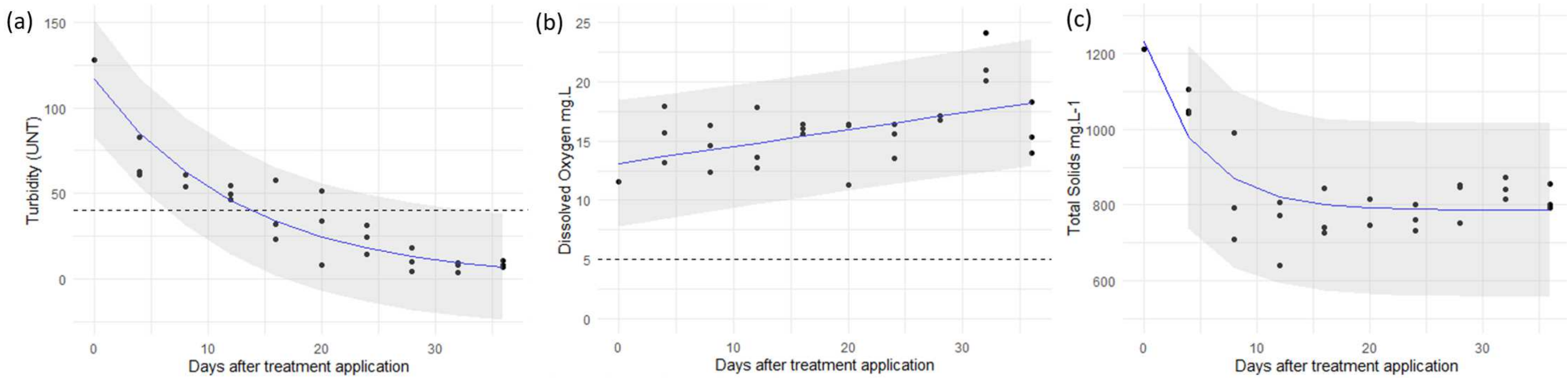


Figure 1. Negative exponential adjustment for turbidity (a), linear adjustment for dissolved oxygen (b), and negative adjustment for total solids (c) of dairy effluents treated with *Pistia stratiotes*, *Pontederia crassipes*, and *Salvinia auriculata* in post-treatment for 36 DAAT. Note: The points are the experimental data; the continuous line in blue represents the adjusted curve; the shaded range is the prediction interval of the response with 95% confidence; and the dotted line is the maximum value of turbidity (40 NTU) and DO (5 $mg.L^{-1}$) for effluent discharge admitted by CONAMA resolution nº 430.

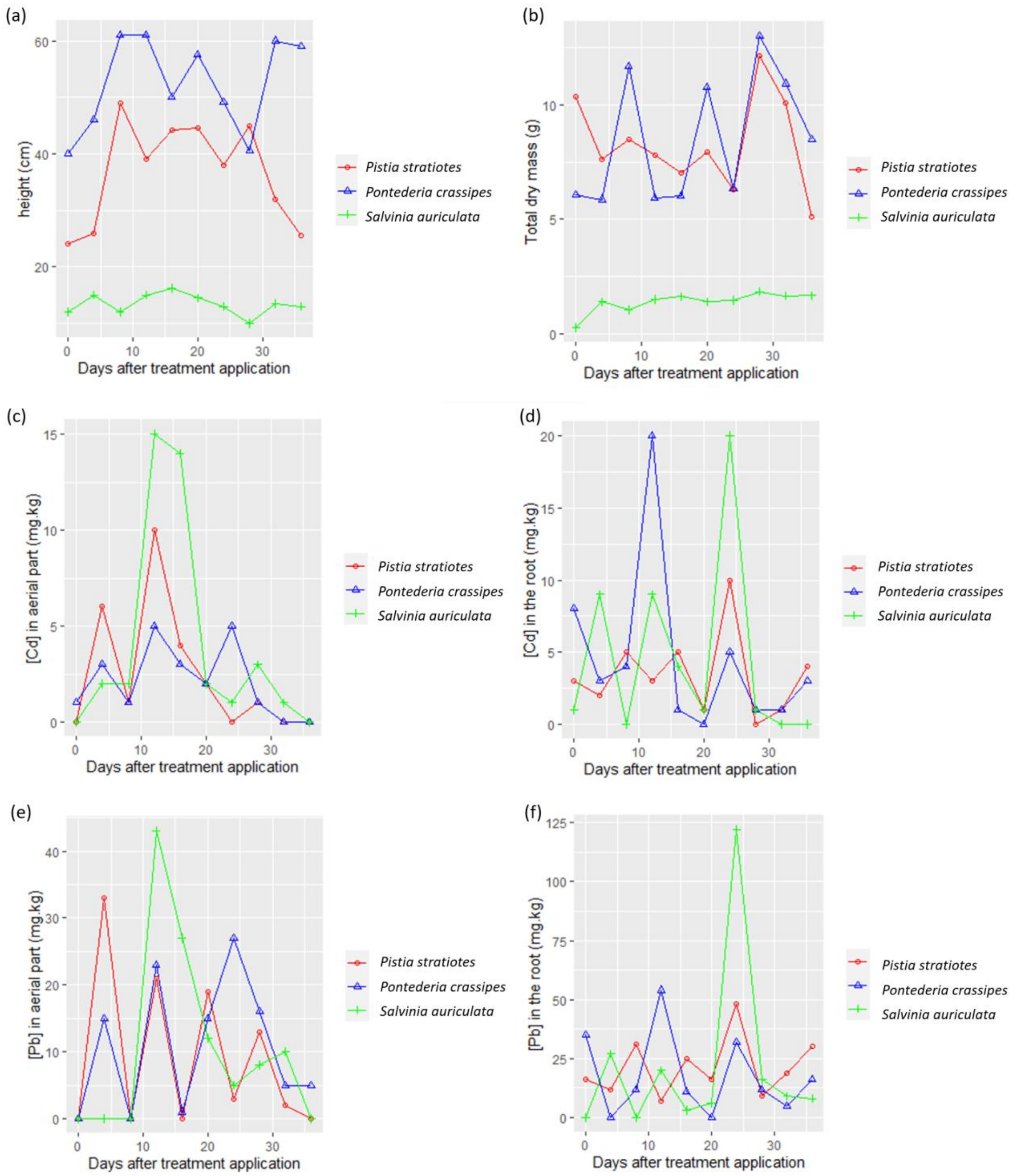


Figure 2. Plant height (a) total dry mass (b), Cd in aerial part (c) and in roots (d), Pb in aerial part (e) and in roots (f) of *Pistia stratiotes*, *Pontederia crassipes*, and *Salvinia auriculata* in post-treatment of dairy effluent for 36 DAAT.

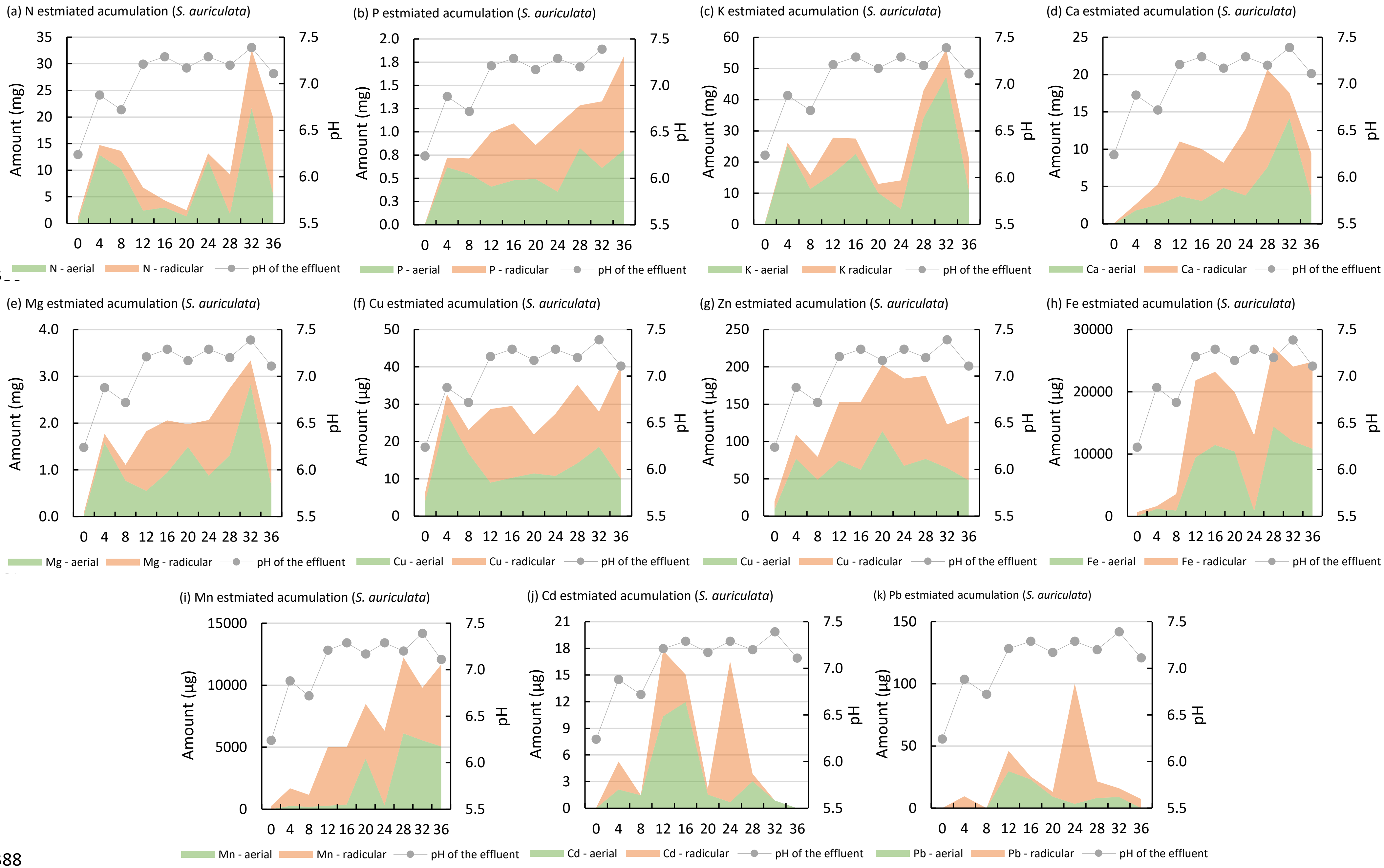


Figure 3. Accumulation of nutrients and metals in the root and leaves of *Salvinia auriculata* plants in post-treatment of dairy effluent for 36 days.

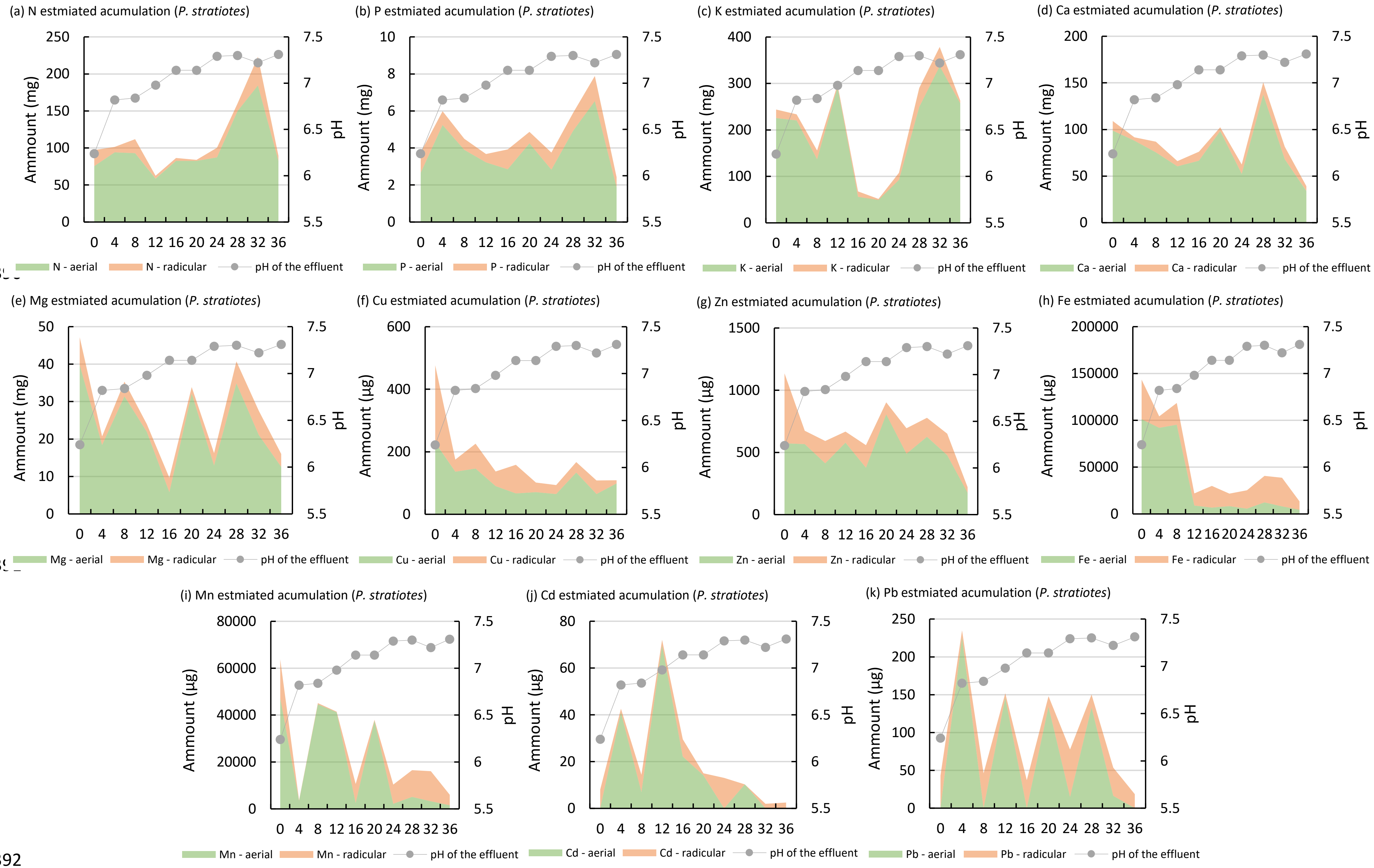


Figure 4. Accumulation of nutrients and metals in the root and leaves of *Pistia stratiotes* plants in post-treatment of dairy effluent for 36 days.

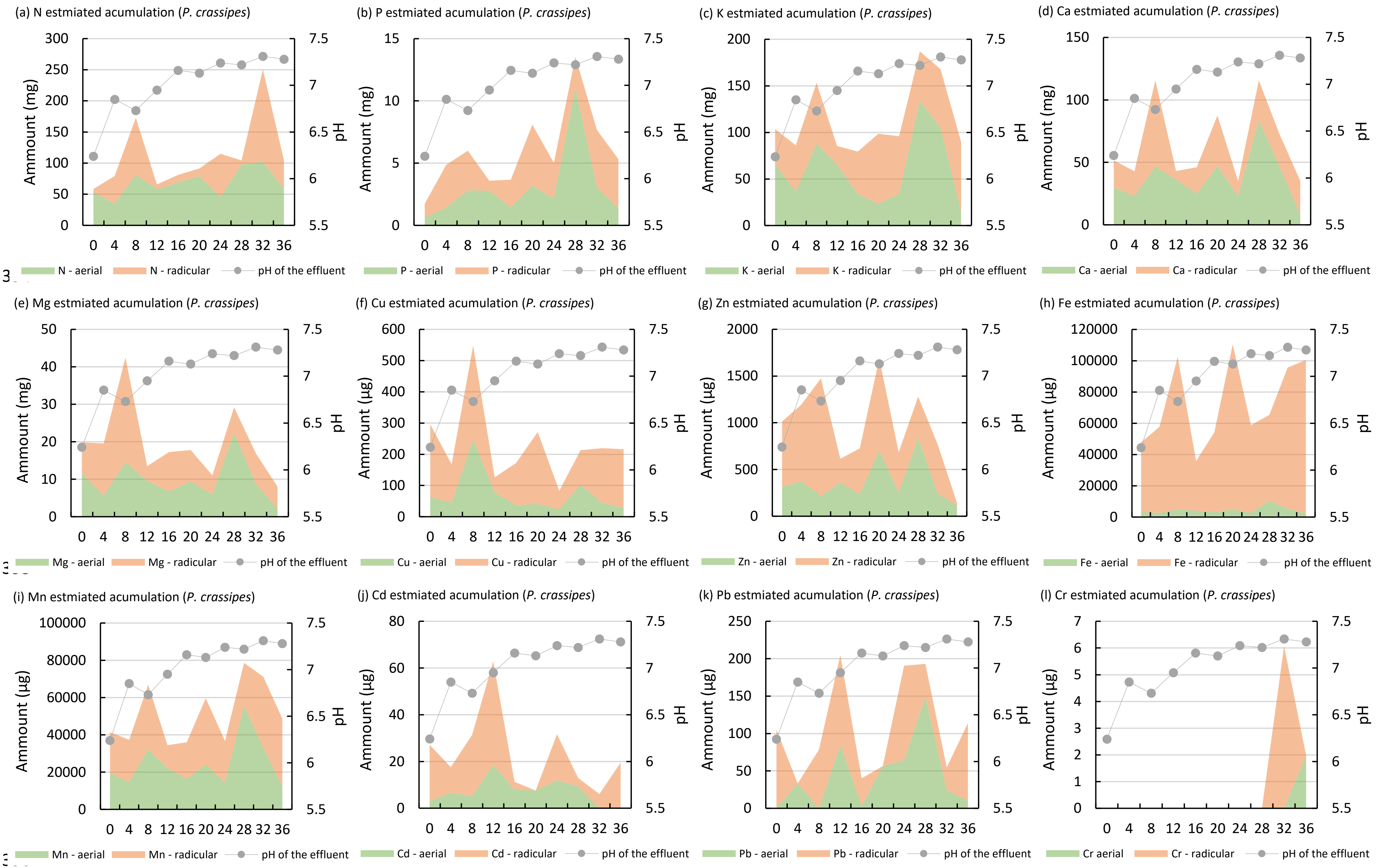


Figure 5. Accumulation of nutrients and metals in the root and leaves of *Pontederia stratiotes* plants in post-treatment of dairy effluent for 36 days.

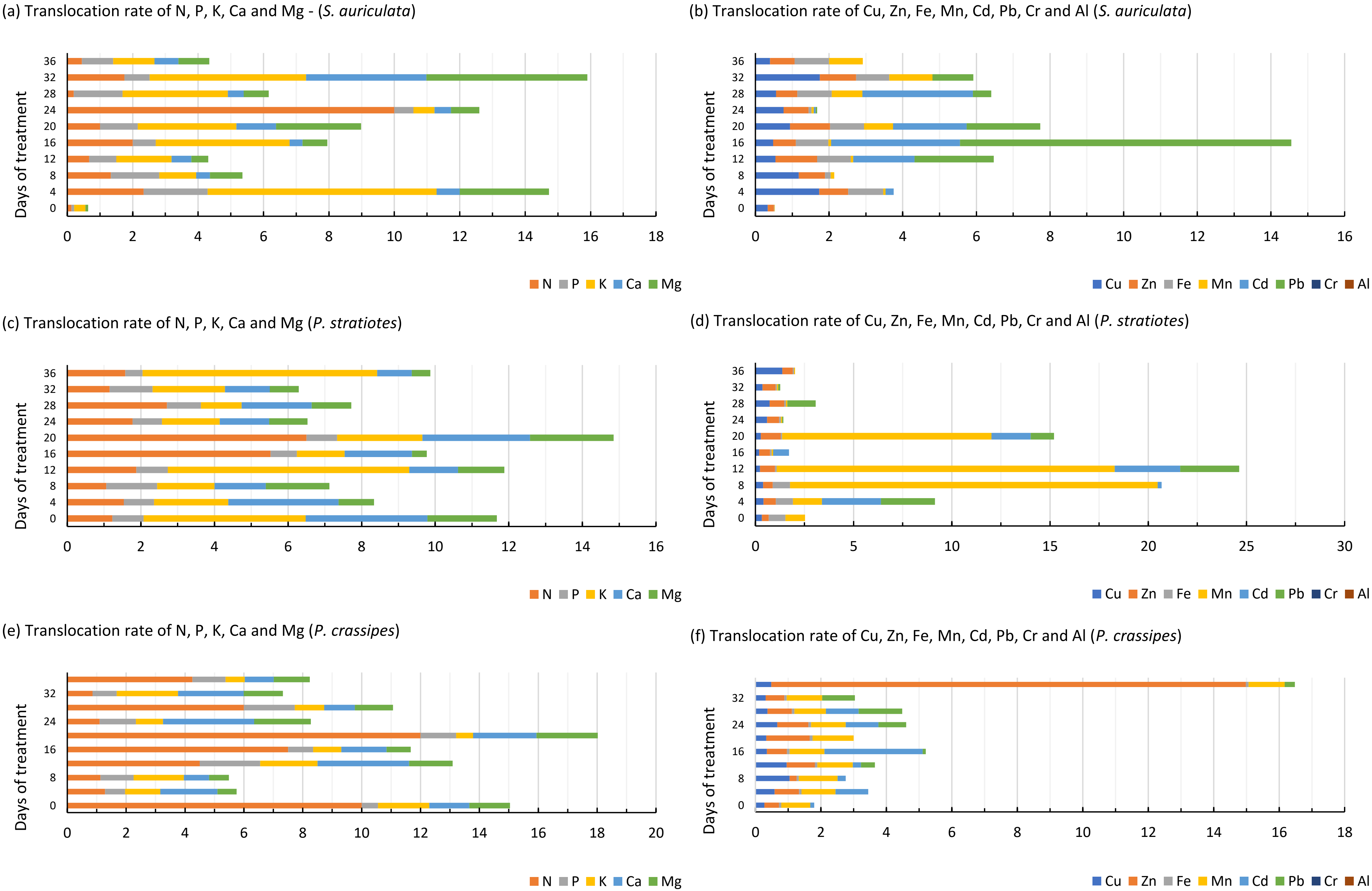


Figure 6. Translocation of nutrients and metals from the roots to leaves of *S. auriculata, P. stratiotes*, and *P. crassipes* during 36 days.

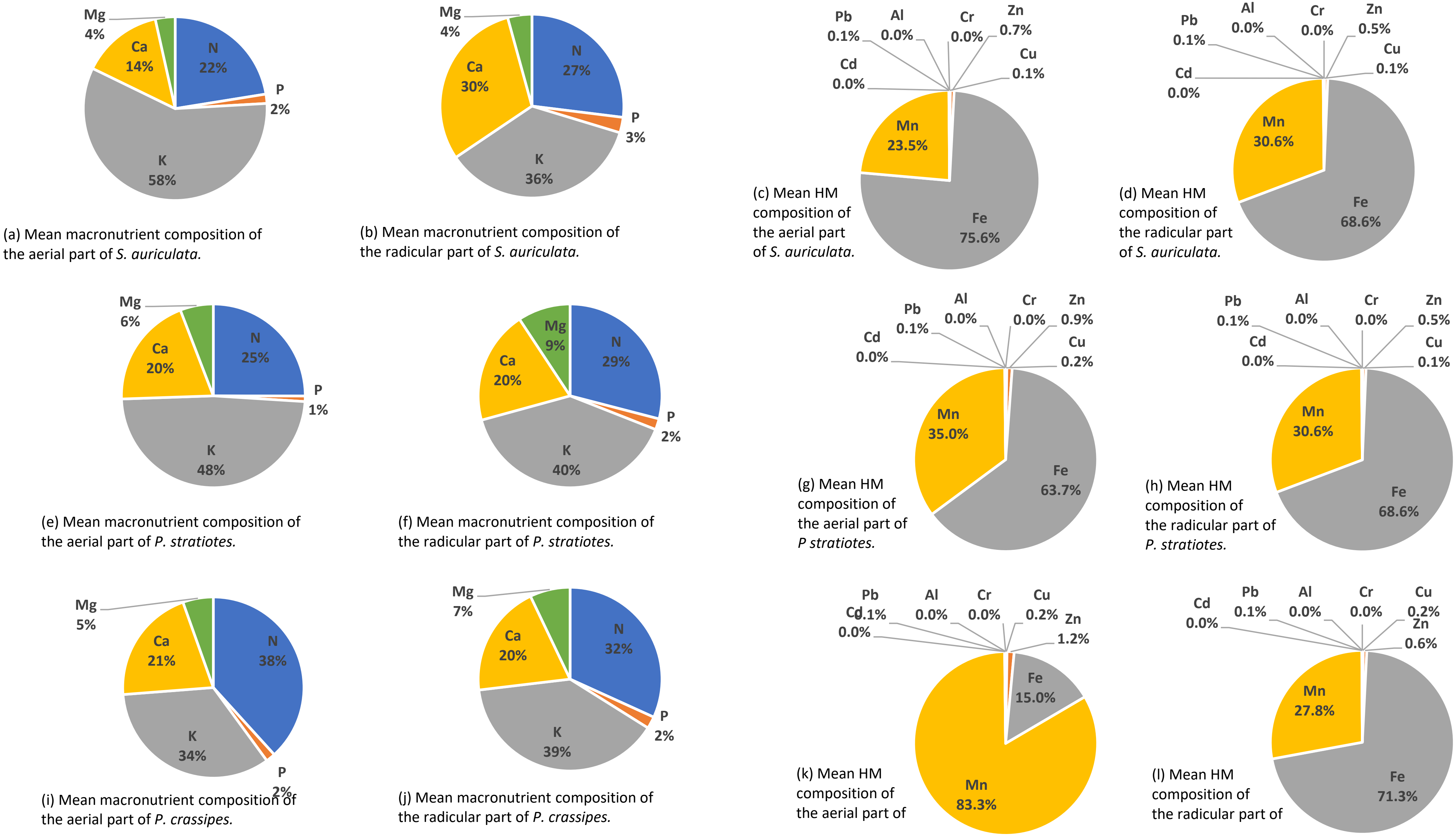


Figure 7. Mean percentual composition of *Salvinia auriculata* (a to d), *Pistia stratiotes* (e to h), and *Pontederia crassipes* (i to l) concerning N, P, K, Ca, Mg, Cu, Zn, Fe, Mn, Cd, Pb, Cr, and Al.

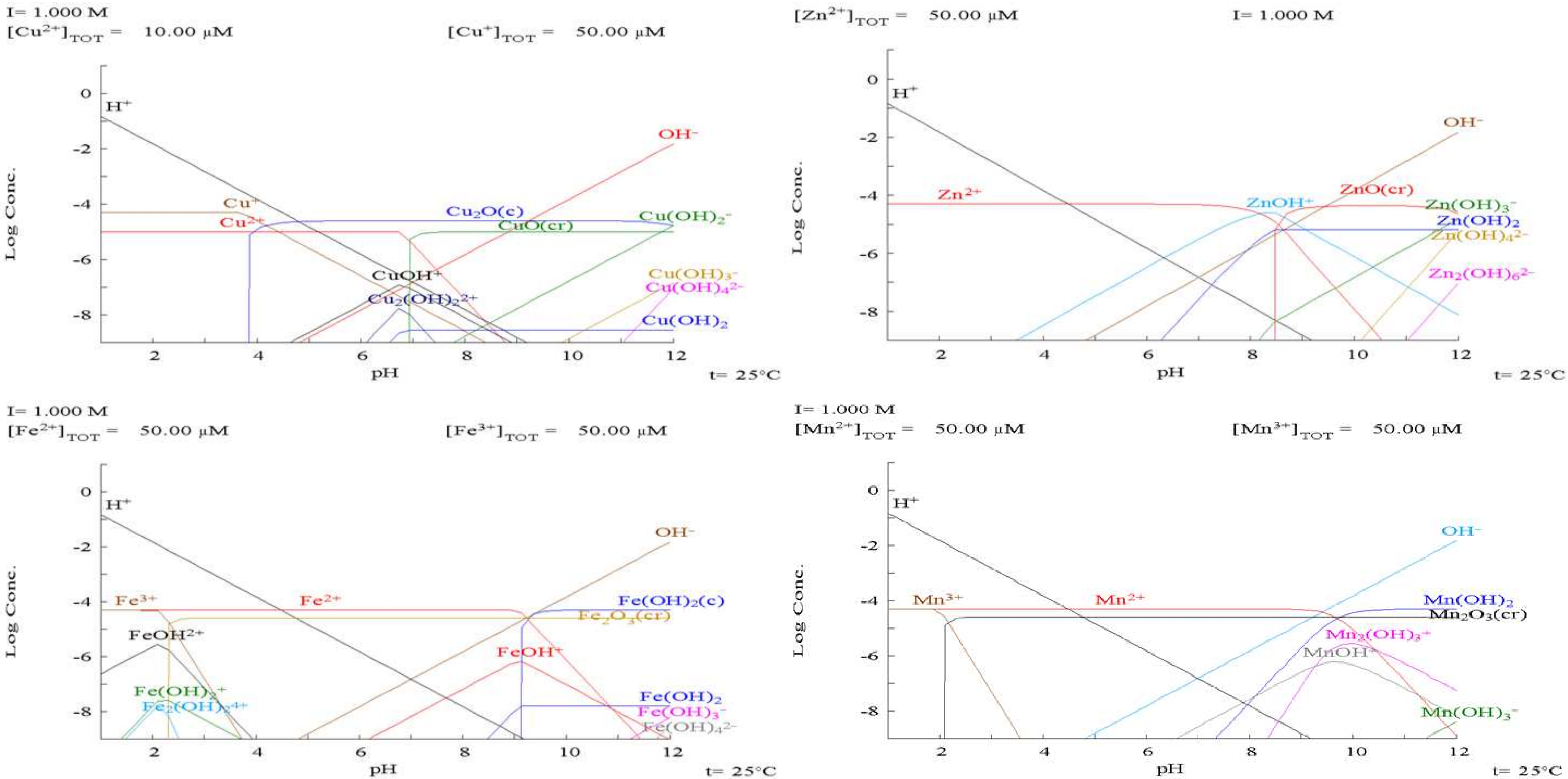


Figure 8. Theoretical diagrams for speciation of Cu, Zn, Fe, and Mn. Source: Hydromedusa Software. Conditions evaluated: pH ranging from 1 to 12; [metals] = 5, 10-5 M; ionic strength = 1 M.

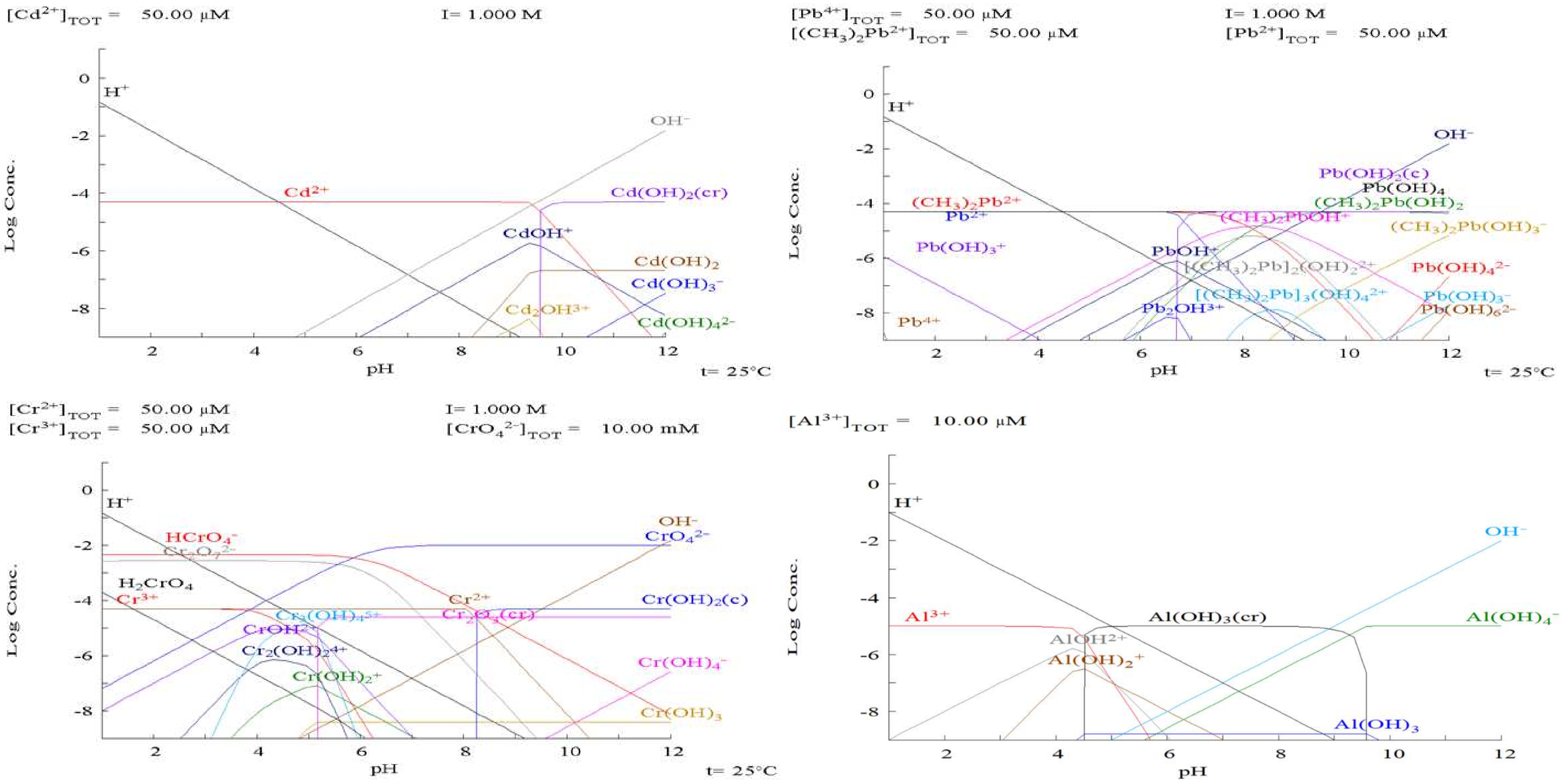


Figure 9. Theoretical diagrams for speciation of Cd, Pb, Cr, and Al. Source: Hydromedusa Software. Conditions evaluated: pH ranging from 1 to 12; [metals] = 5, 10-5 M; ionic strength = 1 M.

The relatively small differences among species regarding physicochemical improvements indicate that the polishing process depends more strongly on the establishment of a functional rhizosphere than on species-specific physiological traits. While differences in biomass production become important for long-term metal accumulation, the short-term improvement of conventional water quality parameters appears to be governed by common ecological processes shared among floating macrophytes. This observation agrees with previous reports indicating that various free-floating species provide comparable reductions in turbidity, suspended solids, and organic load despite differences in morphology (de Souza et al., 2021; Ramirez et al., 2021; Zhang et al., 2021).

From an environmental perspective, these findings reinforce the suitability of macrophytes as treatment units positioned downstream of efficient wastewater treatment plants. Rather than replacing biological reactors, aquatic macrophytes serve as a complementary treatment step capable of removing contaminants that often remain after secondary treatment. This integration is particularly attractive for agro-industrial facilities because it requires relatively simple infrastructure, low energy demand, and minimal operational complexity compared to advanced physicochemical technologies such as membrane filtration, ion exchange, or reverse osmosis (Wan et al., 2016; Cristaldi et al., 2017). Another important aspect concerns the robustness of the polishing process under subtropical environmental conditions. Most phytoremediation studies have been conducted under controlled laboratory conditions or using synthetic wastewater, while information on actual dairy effluents remains scarce. Therefore, the successful performance observed throughout our experimental period indicates that these species tolerate the complex chemical composition of agro-industrial effluents, while maintaining their remediation capacity. Consequently, our findings expand upon the current evidence supporting the use of floating macrophytes under realistic operating conditions and reinforce their potential as nature-based solutions for polishing industrial wastewater.

4.2. Maintenance of plant growth under continuous exposure to potentially toxic elements

An effective phytoremediation system depends on the removal of contaminants as well as the plants' capacity to maintain physiological activity during prolonged exposure to polluted environments. In the present study, all macrophyte species remained feasible throughout the experimental period, exhibiting continuous growth and biomass accumulation despite exposure to residual concentrations of Pb and Cd. This response demonstrates that the investigated species possess a high degree of physiological tolerance to post-treated dairy effluents, an essential prerequisite for sustainable long-term phytoremediation.

Plant tolerance to contaminated environments is determined by the balance between metal uptake and detoxification. Although Pb and Cd are recognized for inducing oxidative stress, membrane damage, inhibition of photosynthesis, and disruption of nutrient homeostasis (Dias et al., 2019; Collin et al., 2022; Shi et al., 2023), these adverse effects generally occur when metal uptake exceeds the capacity of cellular defense mechanisms. Under the experimental conditions adopted here, the absence of severe growth inhibition suggests that the concentrations remaining after conventional wastewater treatment were sufficiently low to avoid acute phytotoxicity while still allowing measurable accumulation within plant tissues. Consequently, the evaluated macrophytes functioned as effective biological filters without experiencing physiological collapse. The maintenance of plant biomass also indicates that contaminant uptake occurred without substantially compromising primary metabolism. Floating macrophytes are characterized by high relative growth rates, rapid leaf turnover, and efficient nutrient acquisition, traits that support continuous biomass production even under moderate environmental stress (Bartmeyer et al., 2019; Gomes et al., 2017; Nahar and Hoque, 2021). These characteristics are particularly advantageous for wastewater polishing because contaminant removal is directly associated with biomass production. As plants continue to grow, they continuously generate new tissues capable of incorporating nutrients and metals from the surrounding water, thereby extending the remediation capacity of the system.

An additional factor contributing to sustained growth is the preferential retention of metals within belowground tissues. Root sequestration is widely recognized as a major tolerance strategy in aquatic macrophytes because cell walls, vacuoles, and extracellular mucilage provide effective compartments for immobilizing toxic ions before they interfere with essential metabolic pathways (George and Gabriel, 2017; Kafle et al., 2022). This compartmentalization minimizes cellular damage while allowing continued nutrient assimilation and carbon fixation. Macronutrients such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) continued to accumulate in plant tissues, indicating that nutrient uptake mechanisms remained active despite simultaneous exposure to toxic elements. Since several essential nutrients and heavy metals share common membrane transporters, excessive metal toxicity frequently results in nutritional imbalance through competitive inhibition of nutrient absorption (Marschner, 2012; Bassegio et al., 2020). The absence of severe reductions in nutrient acquisition therefore suggests that the residual concentrations of Cd and Pb did not substantially impair transporter function or overall nutrient homeostasis.

However, differences in biomass production among the species provide important insights into their remediation strategies. *P. crassipes* and *P. stratiotes* produced considerably more biomass than *S. auriculata*, indicating larger plant architecture and more extensive root systems. Higher biomass represents a significant advantage, as the total removal of contaminants depends not only on tissue concentration but also on the quantity of plant material produced over time. Consequently, species exhibiting rapid biomass accumulation can remove larger amounts of contaminants, even when tissue concentrations are comparable to those observed in smaller plants. The extensive fibrous root systems of *P.*

crassipes and *P. stratiotes* likely contributed to both their superior growth and their remediation performance. Thus, larger root surface areas increase contact with the surrounding wastewater, facilitating nutrient interception, microbial colonization, and the adsorption of dissolved contaminants. Root-associated microbial communities further stimulate nutrient cycling and organic matter degradation, creating a positive feedback loop that boosts plant productivity while simultaneously improving water quality (de Souza et al., 2021; Ramirez et al., 2021).

Another important aspect concerns the resilience of floating macrophytes when faced with the variable conditions of wastewater. Industrial effluents often exhibit temporal variations in organic load, nutrient availability, pH, and contaminant concentrations. Plant species capable of maintaining stable growth despite these fluctuations are more likely to exhibit consistent performance under operating conditions than highly specialized plants that require restricted environmental ranges. The continuous growth observed throughout the 36-day experimental period thus highlights the ecological plasticity of the evaluated species and supports their application in full-scale wastewater polishing systems. From a technological perspective, maintaining vigorous plant growth offers additional operational benefits beyond contaminant removal. Continuous biomass production increases the capacity for successive harvests by allowing the permanent removal of contaminants accumulated in the treatment system and avoiding the recycling of metals resulting from tissue senescence. Furthermore, high biomass productivity facilitates system recovery after harvesting, thereby sustaining long-term remediation efficiency through relatively simple management practices.

4.3. Cd and Pb bioaccumulation: mechanisms underlying phytoremediation efficiency

The accumulation of Cd and Pb observed throughout the experimental period is consistent with the well-established capacity of floating macrophytes to remove dissolved metals through rhizofiltration. In this process, contaminants are initially adsorbed onto negatively charged functional groups present in root cell walls and extracellular polysaccharides before being transported into root tissues (George and Gabriel, 2017; Kafle et al., 2022). Because the root system remains permanently submerged, it constitutes the primary interface between plants and contaminated water, maximizing contact with dissolved ions and suspended particles. Consequently, rhizofiltration represents the first and often the most important barrier preventing the dispersion of metals into receiving aquatic ecosystems. In addition to surface adsorption, metal removal also involves active physiological transport across root membranes. Lead (Pb) and cadmium (Cd) lack specific transport systems; instead, they are taken up by plant cells via transporters primarily responsible for the uptake of essential divalent cations, such as Ca^{2+}, Fe^{2+}, Mn^{2+}, and Zn^{2+} (Marschner, 2012; Bassegio et al., 2020). This low selectivity explains why aquatic macrophytes can efficiently accumulate toxic metals, even when these elements are present at relatively low concentrations in treated effluents. However, once inside the root cells, the ultimate fate of each metal depends on species-specific detoxification mechanisms that regulate intracellular compartmentalization and long-distance transport.

The literature indicates that for satisfactory plant growth and development, the absorption of essential metals by the plasma membrane, such as Fe, Cu, Mn, and Zn, should not be excessive to avoid plant poisoning. According to the studies of Müller, Shuting and collaborators and Zhang and collaborators (Müller, 2023; Shuting et al., 2022; Zhang et al., 2017), there is the existence of the family of protein transporters called ZIP (Proteins such as IRT and ZRT), both involved in the transport of Fe, Zn, Mn, and Cd. In addition, the Nramps carrier family was identified as a mediator of the absorption of Mn^{+2}, Cu^{+2}, Co^{+2}, Cd^{+2}, and Fe^{+2} (Bozzi and Gaudet, 2021; Ma et al., 2023). Another transporter with the primary role in absorbing Ca^{+2} and Cd^{+2} is entitled LCT1 (low-affinity cation transporter) (Pasricha et al., 2021). Besides, it is reported the existence of the IRT transporter (Fe^{+2} regulation transporter), which also shows an affinity for other bivalent ions such as in addition to Fe^{+2}, Zn^{+2}, Mn^{+2}, and Cd^{+2} (Huang and Dai, 2015), has been reported in tomatoes and its function is predominantly expressed in the roots (Ahmad et al., 2023).

The studies of Karley and White; Puig and Penarrubia (Karley and White, 2009; Puig and Peñarrubia, 2009) show that protein transporters in native plants have distinct characteristics. When nutrient concentrations, whether in the soil or in the aquatic environment in which the plant is grown, are low, the whole proteins that constitute the high-affinity transport system (HATS) are responsible for the cations uptake. On the other hand, the whole membrane proteins that constitute the low-affinity transport system (LATS) are expressed when plant species are grown with high nutrient concentrations. In addition, P-type HM ATPases play a vital role in transporting essential and potentially toxic metals through the membrane in various organisms, including plant species (Palmgren and Axelsen, 1998). Several studies on the dynamics of ionic absorption of mineral elements (enzymatic kinetics) have been the subject of interest to researchers over the years. In these studies, the primary approach generally refers to the multiplicity of transport systems that capture and distribute nitrogen (N). The plant-model *Arabidopsis thaliana* L. (arabidopsis) uptake N predominantly through the roots, where LATS and HATS are expressed. The families of nitrate transport genes encode these (NRT1/NPF and NRT2) (O'Brien et al., 2016; Rashid et al., 2018; Wang et al., 2021).

LATS allows the transport of nitrates in high external concentrations (>0.5 mM), while HATS external concentrations are relatively low (<0.5 mM). It is worth noting that each type of transport system (HATS and LATS) has constitutive and inducible forms, and its expression depends on external concentrations of nitrate perceived for efficient uptake (Miller et al., 2007). Given the capacity of the LATS, we speculate that for our 'short-term' results (16 to 36 DAAT), the expression of this transport system has predominated in the plant species investigated. Although the high-affinity system was expressed quickly, we speculate that saturation may have occurred, leading the plant to express the low-affinity system. The literature uses cations and anions to discuss the central role of HATS and LATS, such as ammonium (cation) and/or nitrate (anion), respectively, because these models explain the metabolic dynamics of the transport systems in question. Thus, in our present study, we offer an elegant and pioneering association of transport systems that discuss the Cd^{2+} and Pb^{2+} cations to elucidate the efficiency of macrophytic species in the improvement of the physicochemical parameters of the effluent. However, we express the need for more studies to establish this association.

An interesting finding of our study is that the evaluated species maintained considerable metal accumulation without exhibiting marked reductions in biomass production. This result suggests that contaminant uptake remained compatible with the activation of detoxification pathways capable of minimizing cellular toxicity. We speculate that one of the primary mechanisms responsible for this tolerance is metal sequestration in root vacuoles or immobilization through binding to cell wall components, phytochelatins, and metallothioneins. These compounds reduce the concentration of free metal ions in the cytoplasm, thereby limiting oxidative damage and preserving metabolic activity (Collin et al., 2022; Shi et al., 2023). Such compartmentalization allows the plants to function simultaneously as contaminant accumulators and biologically active treatment units. Although Pb and Cd showed similar general accumulation trends, important physiological differences should be considered. While Pb tends to remain preferentially retained in underground tissues—due to its higher affinity for negatively charged cell wall components and lower mobility within the xylem—Cd generally exhibits greater mobility within the plant, as it enters the transpiration stream more readily following root uptake (Jarup, 2003; Nag and Cummins, 2022). Although Cd and Pb concentrations in the tissues were generally comparable across species, the greater biomass produced by these macrophytes resulted in higher total metal removal from the wastewater. This distinction highlights an important limitation frequently found in phytoremediation studies, in which remediation efficiency is inferred exclusively from tissue concentrations. Since contaminant removal is determined by the product of concentration and biomass, high-productivity species can remove substantially larger contaminant loads, even when exhibiting elemental concentrations similar to those of smaller plants. Therefore, biomass productivity should be considered a fundamental selection criterion when designing full-scale phytoremediation systems.

The persistence of contaminant accumulation over the 36-day experimental period also indicates that metal uptake was not restricted to an initial adsorption phase but represented a continuous physiological process. Such sustained accumulation suggests that newly produced tissues remained capable of incorporating contaminants throughout plant development, thereby extending the operational lifespan of the polishing system. This characteristic is particularly advantageous in industrial applications because it reduces the frequency of biomass replacement while maintaining stable treatment performance over extended operational periods. An equally important implication concerns the environmental safety of contaminant removal. Metals accumulated in plant biomass are effectively transferred from the aqueous phase to the solid biological compartment, substantially reducing their immediate bioavailability in the treated effluent. However, this process should be interpreted as a transfer of contaminants, not their destruction. Unlike organic pollutants, Cd and Pb cannot be degraded through biological metabolism. Consequently, the long-term success of phytoremediation depends on the proper management of contaminated biomass after harvest. Therefore, biomass harvesting should be considered an integral component of the phytoremediation process, rather than merely a maintenance operation.

Collectively, these findings demonstrate that the evaluated macrophytes remove Cd and Pb through an integrated sequence of rhizofiltration, physiological uptake, intracellular sequestration, and controlled translocation while maintaining sufficient biomass production to sustain long-term remediation. The observed differences among species primarily reflect variations in biomass productivity and metal partitioning rather than fundamental differences in uptake capacity. Consequently, successful phytoremediation should be interpreted as the outcome of coordinated physiological and ecological processes that maximize contaminant retention while preserving plant performance. Understanding these mechanisms is essential for optimizing species selection and treatment design and provides the basis for interpreting the influence of metal chemical speciation on remediation efficiency, discussed in the following section.

4.4. Influence of metal speciation on phytoremediation efficiency

In the present study, we report the theoretical speciation diagrams provide mechanistic support for the observed patterns of Cd and Pb accumulation, demonstrating that changes in pH directly influence the availability of metal ions for root uptake. Under acidic to near-neutral conditions, Cd and Pb predominantly occur as free divalent cations (Cd^{2+} and Pb^{2+}), which represent the most mobile and bioavailable forms for plant absorption. These ionic species readily diffuse toward the rhizosphere and are transported across root membranes through carriers primarily involved in the acquisition of essential nutrients such as Ca^{2+}, Fe^{2+}, Mn^{2+}, and Zn^{2+} (Marschner, 2012; Bassegio et al., 2020). Therefore, the relatively high accumulation of Cd and Pb observed during the experimental period is consistent with the predominance of these soluble forms within the pH range recorded in the treated dairy effluent.

The role of the rhizosphere becomes particularly important when considering adsorption processes. Even when metals become less soluble because of hydroxide formation, precipitation onto root surfaces or association with extracellular biofilms may continue to remove contaminants from the water column. Thus, decreases in dissolved metal concentrations are not exclusively attributable to intracellular accumulation but also result from physicochemical retention within the root microenvironment. This combined action of biological uptake and surface immobilization explains why efficient contaminant removal can occur even under conditions in which metal bioavailability gradually declines. Microbial communities associated with the root system likely intensified these processes. Rhizosphere microorganisms influence nutrient cycling, organic matter decomposition, and the transformation of metallic species through oxidation, reduction, complexation, and biosorption reactions (George and Gabriel, 2017; Kafle et al., 2022). Although microbial populations were not directly characterized in the present study, their contribution is strongly supported by the simultaneous improvement of physicochemical parameters and sustained contaminant removal. The treatment system therefore functioned as an integrated plant–microbe remediation unit rather than as an exclusively plant-mediated process.

The chemical behavior of other metallic elements measured in plant tissues further reinforces this interpretation. Essential micronutrients such as Fe, Mn, Zn, and Cu exhibit marked changes in solubility across relatively narrow pH ranges, directly affecting their availability for plant uptake. The accumulation patterns observed for these elements suggest that nutrient acquisition remained active throughout the experimental period despite the concurrent presence of Cd and Pb. This finding indicates that the modest pH increase achieved during treatment was sufficient to reduce the mobility of potentially toxic elements without substantially impairing the availability of essential nutrients required for plant growth. Such selective regulation contributes to maintaining physiological performance while simultaneously enhancing contaminant stabilization.

From a practical standpoint, pH should be considered a critical operational variable in phytoremediation systems. Excessively acidic conditions increase the solubility and mobility of toxic metals, which can intensify absorption, but also increase phytotoxicity and the risk of contaminant dispersion. On the other hand, excessively alkaline conditions favor the precipitation of metals, reducing bioavailability and potentially limiting the efficiency of phytoextraction. The near-neutral pH, maintained throughout the experimental period, appears to represent a favorable balance, allowing sufficient bioavailability for the continuous absorption of contaminants while minimizing acute toxicity to the plants. This operational range is particularly advantageous for industrial wastewater treatment, as it can often be maintained without the need for intensive chemical adjustment, thereby reducing operating costs.

4.5. Technological implications for wastewater treatment systems

Our findings demonstrate that floating aquatic macrophytes hold considerable potential as nature-based technologies for tertiary wastewater treatment, particularly for polishing agro-industrial effluents containing residual concentrations of potentially toxic elements (PTEs). However, instead of replacing conventional treatment units, phytoremediation should be seen as a complementary process capable of addressing limitations that often persist after biological treatment, especially the persistence of inorganic contaminants and residual nutrients. This integrated approach aligns with the current transition toward more sustainable wastewater management strategies, in which ecological processes are incorporated to enhance treatment efficiency while simultaneously minimizing operational costs and environmental impacts. Furthermore, one of the main technological advantages observed is the simultaneous improvement of multiple water quality parameters in a single treatment unit. Conventional advanced technologies for metal removal—including chemical precipitation, ion exchange, membrane filtration, electrocoagulation, and activated carbon adsorption—are generally designed to target specific contaminants and often require high energy consumption, continuous chemical dosing, or sophisticated operational control (Wan et al., 2016; Cristaldi et al., 2017). In contrast, floating macrophytes simultaneously reduced residual organic matter, suspended solids, turbidity, and metal concentrations through the combined action of plant metabolism, rhizosphere microorganisms, and physicochemical retention processes. This multifunctionality represents a significant operational advantage, as various treatment objectives can be achieved without increasing system complexity

When approaching the subject from an environmental perspective, the integration of macrophyte-based polishing units into existing wastewater treatment plants is particularly attractive, as it requires only minor modifications to conventional treatment lines. Floating treatment cells can be installed downstream of secondary clarifiers or biological reactors, where residual pollutant concentrations are low enough to avoid severe phytotoxicity, while still allowing for effective contaminant removal. Under these conditions, macrophytes act as biological polishing units that improve the quality of the final effluent before it is released into the environment or the water is reused. Such modular integration facilitates the retrofitting of existing facilities without requiring the replacement of established infrastructure, thereby reducing implementation costs. Furthermore, the sustained growth observed in all evaluated macrophyte species has direct operational implications. Continuous biomass production allows for periodic harvesting, which permanently removes accumulated contaminants from the treatment system while simultaneously renewing the population of metabolically active tissues responsible for ongoing contaminant uptake. Harvesting should therefore be considered an essential operational component in future studies, rather than merely a maintenance procedure. Appropriate harvesting intervals maximize remediation efficiency by preventing plant senescence, minimizing nutrient recycling, and maintaining high photosynthetic activity throughout the treatment cycle.

The comparative performance of the evaluated species further highlights the importance of selecting plants based on environmental engineering objectives rather than solely on contaminant accumulation. Although all three species contributed effectively to wastewater polishing, *P. crassipes* and *P. stratiotes* produced a greater amount of biomass and developed more extensive root systems, characteristics that enhance total contaminant removal and improve hydraulic interaction between the wastewater and the rhizosphere. Conversely, smaller species, such as *S. auriculata*, may be advantageous when rapid surface coverage or simplified biomass handling is prioritized. Thus, species selection should consider multiple operational criteria, including biomass productivity, harvesting frequency, climate adaptation, hydraulic behavior, and long-term maintenance requirements. An additional technological implication concerns process robustness. Industrial effluents are inherently dynamic, with temporal fluctuations in hydraulic flow, organic loading, nutrient concentrations, and contaminant composition. Treatment technologies intended for industrial application must therefore tolerate operational variability without significant reductions in performance. The ability of the evaluated macrophytes to maintain growth and remediation capacity throughout the experimental period suggests a high degree of ecological resilience, indicating that biological polishing systems may buffer moderate fluctuations in wastewater characteristics. Nevertheless, future studies should investigate system performance under variable hydraulic loading and seasonal environmental conditions to better simulate industrial operation.

4.6. Industrial applicability, limitations, and future perspectives

Integrating biological polishing systems downstream of conventional treatment processes represents a practical strategy for addressing contaminants that often persist after secondary treatment, thereby contributing to more sustainable wastewater management. From an industrial perspective, this approach is particularly attractive because it combines low operating costs, lower energy demand, and relatively simple infrastructure with the ability to simultaneously improve multiple physicochemical parameters. Plant species capable of rapid propagation, continuous biomass renewal, and tolerance to moderate fluctuations in effluent composition are more likely to maintain stable remediation performance over extended operational periods. In this context, the superior biomass production observed for *P. crassipes* and *P. stratiotes* suggests that these species may offer greater operational advantages for long-term industrial applications, although final selection must also take into account local environmental conditions, regulatory constraints, and maintenance requirements.

Although we have reported promising results, several limitations must be acknowledged before extrapolating these findings to full-scale treatment systems. First, our experiment was conducted under controlled conditions using pre-treated dairy effluent with relatively low residual concentrations of Pb and Cd. Industrial wastewater treatment plants may exhibit significant temporal variations in hydraulic flow, contaminant loads, nutrient availability, and physicochemical characteristics—factors that can affect contaminant removal efficiency—as well as seasonal variations in temperature, solar radiation, and precipitation affecting the plant species. Therefore, further long-term field studies are needed to assess the system's resilience under realistic operational conditions. A second limitation concerns the limited number of contaminants evaluated. Although Pb and Cd represent potentially toxic elements of environmental relevance, industrial effluents often contain complex mixtures of metals, nutrients, pharmaceuticals, detergents, and emerging contaminants, capable of interacting through synergistic or antagonistic mechanisms. Likewise, incorporating molecular analyses of rhizosphere microbial communities would enhance the understanding of the biological mechanisms contributing to contaminant removal and system stability.

Therefore, future research should focus on bridging the gap between laboratory-scale experiments and industrial implementation. We speculate that future studies should devote special attention to pilot-scale validation under continuous flow conditions, as well as to assessments of scenarios with mixed contaminants, seasonal performance, the development of automated biomass harvesting strategies, and life cycle assessments of integrated treatment systems. Technical and economic analyses comparing phytoremediation with competing polishing technologies will also be essential to define the operational contexts in which macrophyte-based systems offer the greatest environmental and economic benefits.

5. Conclusion

We accept our hypothesis which demonstrates that floating macrophytes represent promising components of sustainable tertiary wastewater treatment systems and provides a mechanistic and engineering-oriented framework for future technological development. The integration of treatment performance, bioaccumulation, translocation behavior, and chemical speciation constitutes the principal scientific contribution of this work and establishes a foundation for the design, optimization, and scale-up of nature-based polishing technologies for wastewater containing residual potentially toxic elements. Overall, our results could support the application of aquatic macrophytes as a sustainable nature-based solution for tertiary wastewater treatment prior to discharge into receiving water bodies. Future research should investigate the role of high- and low-affinity transport systems (HATS and LATS) in native species to improve the mechanistic understanding of phytoremediation processes and optimize species selection for constructed wetlands and other engineered treatment systems tailored to dairy wastewater management.

However, our results should be interpreted according to the characteristics highlighted in the scope of the experimental design, since we conducted the experiment on a pilot scale using one reactor per treatment. Thus, the findings primarily demonstrate the technological feasibility and comparative performance of the species, rather than definitive estimates of treatment efficiency applicable to all operating conditions. Repeated temporal monitoring throughout the experimental period provided robust information on the dynamics of the treatment; nevertheless, future investigations employing replicated pilot systems, longer operational periods, seasonal assessments, and full-scale validation will be essential to strengthen statistical inference and confirm the long-term reliability of the process.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The authors further declare that no commercial or financial organization had any role in the design of the study, collection, analysis, or interpretation of data, writing of the manuscript, or the decision to submit the manuscript for publication.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this manuscript, the author(s) used ChatGPT for exclusively to improve the English language, enhance readability, and assist in the organization and restructuring of the manuscript. The author(s) reviewed and edited the output as needed and take full responsibility for the content of the published article.

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