UNIVERSIDADE FEDERAL DE SÃO CARLOS – UFSCar

CENTRO DE CIÊNCIAS DA NATUREZA – CCN

campus LAGOA DO SINO

CURSO DE GRADUAÇÃO EM ENGENHARIA AGRONÔMICA

JOÃO VITOR CAMARGO

USO DE MACRÓFITAS AQUÁTICAS COMO ESPÉCIES FITORREMEDIADORAS NO TRATAMENTO TERCIÁRIO DE EFLUENTES DE LATICÍNIOS

Buri (SP)

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Trabalho de Conclusão de Curso apresentado ao Curso de Engenharia Agronômica para obtenção do título de Bacharel em Engenharia Agronômica.

Orientação: Prof. Dr. Daniel Baron

Coorientação: Prof. Dr. Iuri Emmanuel de Paula Ferreira

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DEDICATÓRIA

A Deus, por todo apoio e acalento espiritual ao longo dessa jornada.

A minha família, por toda força e confiança que me deram em toda minha vida.

A todos familiares, amigas e amigos.

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"Não é o mais forte das espécies que sobrevive, nem o mais inteligente, mas o que melhor se adapta às mudanças."

(Charles Darwin)

RESUMO

A possível contaminação ambiental por metais pesados (MPs), tais como cádmio (Cd), chumbo (Pb) e cromo (Cr) torna-se recorrente dado o aumento de atividades antropogênicas quando essas não atendem as recomendações técnicas corretas. Dessa maneira, a fim de minimizar os danos causados por atividades irregulares, a fitorremediação surge como uma técnica eficiente e econômica, na qual plantas são utilizadas na descontaminação ambiental. Nesse cenário, destacam-se as macrófitas aquáticas *Pontederia crassipes* (Mart.) Solms (aguapé-de-flecha), *Pistia stratiotes* L. (alface-d'água) e *Salvinia auriculata* Aubl. (orelhinha-de-onça), as quais possuem potencial remediador de poluentes orgânicos inorgânicos presentes em corpos hídricos. A indústria de lácteos está presente em todo território nacional, e quando esse setor industrial não maneja corretamente seus rejeitos antes de o lançarem em corpos hídricos, ocorrem casos graves de contaminação. Portanto, testamos a hipótese se macrófitas aquáticas possuem potencial fitorremediador como ferramenta biotecnológica no tratamento terciário de efluentes de laticínios contaminados com poluentes. Nosso objetivo foi avaliar a eficiência fitorremediadora de macrófitas em corpos hídricos contaminados por metais pesados. Adotamos o delineamento experimental inteiramente casualizado, composto pelas espécies *P. crassipes*, *P.* stratiotes e *S. auriculata* cultivadas em efluentes de laticínios com presença de metais pesados (Cd e Pb). Para isso, realizamos análises dos parâmetros físico-químicos, análise de massa de matéria seca total (MST) indicando boas adaptações das plantas, além de analisar as concentrações de elementos tóxicos nos tecidos vegetais a fim de entender o acúmulo de MPs. Os dados coletados foram submetidos aos testes de Tukey e de Friedman e, após o processamento dos dados, realizamos gráficos de séries temporais para as variáveis analisadas. A literatura reporta a existência da família de transportadores proteicos denominados 'ZIP', a qual está envolvida no transporte de Fe, Zn, Mn e Cd. Outras proteínas e transportadores se destacam e mostram afinidades com íons bivalentes. Especulamos que o transporte de elementos potencialmente tóxicos (EPT) ocorre por sistemas de alta a baixa afinidade (*HATS* e *LATS*, respectivamente). As concentrações de EPT não foram prejudiciais para o crescimento vegetativo das macrófitas e destacamos suas habilidades de 'polimento' frente aos parâmetros físico-químicos do efluente, mostrando a eficiência dessas plantas na 'adequação' do efluente em relação a esses parâmetros antes de seu lançamento em corpos hídricos. Diante disso, aceitamos nossa hipótese inicial de que macrófitas aquáticas possuem potencial fitorremediador como ferramenta biotecnológica em corpos hídricos contaminados com a presença de poluentes

Palavras-chave: Bioacumulação. Efluentes. Fitorremediação. Metais

SUMÁRIO

1. CONSIDERAÇÕES GERAIS PARA ESCOLHA DO TEMA DE ESTUDO

Após diversos debates, a definição de ‘metais pesados’ (MP) refere-se a um metal que tem sua ocorrência de forma natural com o número atômico maior que “20”, além de possuir densidade maior que 5g.cm^{-3} (ALI; KHAN, 2018). Concomitante a isto, tal aspecto teórico ocorrem em meio ao aumento desses poluentes no ambiente, uma vez que, dados da década passada indicam que aproximadamente 245 milhões de hectares de terras agricultáveis estão contaminados por metais pesados (MANI; KUMAR; PATEL, 2015). No Brasil, de acordo com a Companhia Ambiental do Estado de São Paulo, constatou que de 6.434 locais avaliados, 1.273 são áreas contaminadas por metais pesados (CETESB, 2020). O acumulo de MPs no solo aumenta rapidamente devido a processos naturais, além das atividades antropogênicas, bem como o fato dos metais pesados não serem biodegradáveis, mas sim, persistirem no meio ambiente (YAN et al, 2020). Entre os MPs são listado os metais cádmio (Cd), cromo (Cr), chumbo (Pb), zinco (Zn) (DORNE et al., 2011-; SINGH; SINGH; DHAL, 2022) entre outros.

De acordo com Kaur et al, (2017) e Shi et al, colaboradores (2022), afirmam que o Cd é um dos MPs mais tóxicos, considerado mutagênico, neurotóxico, carcinogênico, para os humanos, e fitotóxico, o que acarreta prejuízos às plantas desencadeada pela geração de espécies reativas de oxigênio (EROs). A contaminação por Cd é um tópico de importância para saúde pública, uma vez que este metal ingressa na cadeia alimentar por diferentes vias, tais como inalação e ingestão, acarretando prejuízos aos rins, fígado, pulmões e o coração (JÄRUP, 2003). Outro metal pesado é o Cr, o qual, conforme Taiz et al (2017), possui a capacidade de mimetizar, de forma parcial, alguns outros metais, bem como interagir com o oxigênio (O), de modo a formar EROs. Assim como o Cd e o Cr, o Pb é um MP tóxico, em que seu manuseio causa contaminação ambiental, problemas de saúde além de gerar EROs, acarretando em prejuízos para as plantas, como problema de germinação das sementes, fotossíntese, entre outros. O Pb tetraetila [$Pb.(C_2H_5)_4$], por décadas, utilizado como aditivo na gasolina aumentava o desempenho dos motores. Constatou os prejuízos que o mesmo acarretava, uma vez que liberava partículas de Pb no ar. Essa exposição, a longo prazo, pode acarretar anemia, aumento da pressão arterial, distúrbios sanguíneos, danos graves no cérebro e nos rins, e até morte (WANI; ARA; USMANI, 2015).

A Organização das Nações Unidas (ONU), no ano de 2015, com vistas a reforçar o crescimento econômico sustentável, propôs a ‘Agenda 2030’, a qual é composta por 17 objetivos de desenvolvimento sustentável e 169 metas (NIETO, 2017). Entre esses objetivos destacam-se alguns referentes às áreas da saúde e bem-estar, energia limpa, , acesso à água potável e saneamento, entre outros (MOREIRA et al., 2019). Entre esses objetivos cabe destacar a erradicação da fome, a qual proporciona agricultura sustável, e o acesso à água potável, de modo que tornam necessárias a adoção de técnicas para remediar locais contaminados. Assim, a literatura reporta alguns protocolos para redução dos volumes de resíduos por meio de incineração ou escavação do solo e posterior transferência a aterros sanitários, contudo, esses procedimentos possuem elevado custo. Além dessas, podem ser encontradas práticas de lavagem ácida e fitorremediação para remediar alguns locais contaminados (CRISTALDI et al., 2017; ROSTAMI; AZHDARPOOR, 2019).

De maneira geral, a fitorremediação é reportada como ‘eficiente’, ‘socialmente aceita’, uma vez que não apresenta prejuízos ao ambiente, como também ‘econômica’ (WAN et al., 2016). O uso dessa técnica apresenta superávit econômico em, aproximadamente, 7 anos após sua implantação, além de ser ambientalmente favorável, pois, faz o uso de plantas capazes de remediar ou estabilizar a contaminação de poluentes por meio de mecanismos fisiológicos como fitoestabilização, fitodegradação, entre outros (KAFLE et al., 2022). Algumas espécies possuem potencial de fitoextração e tolerância aos MPs, o que desperta interesse na utilização da fitorremediação como, por exemplo, macrófitas aquáticas que acumulam contaminantes por meio de suas raízes e os transferem para a parte aérea. Entre os mecanismos fisiológicos, a literatura reporta a ‘fitovolatização’, a qual consiste na capacidade das espécies vegetais em absorver metais e, posteriormente, convertê-los em formas menos tóxicas e eliminá-los para a atmosfera por meio da transpiração (KUMARI et al., 2020). Já a ‘fitoestabilização’ realiza a sorção, precipitação, complexação ou alteração da valência do metal na rizosfera, promove redução da mobilidade ou da biodisponibilidade do MP (ALI et al., 2013). A literatura aponta, ainda, o mecanismo ‘fitoextração’ como o mais importante entre as estratégias fitorremediadoras e isso se deve pela capacidade que algumas espécies possuem de absorver, transportar e acumular MPs em suas estruturas aéreas e em órgão extratores (SHEORAN; POONIA, 2016). Dessa forma, as espécies vegetais utilizadas para realizar a fitoextração devem apresentar características como alta taxa de crescimento, produção de biomassa e capacidade de hiperacumular MPs (CRISTALDI et al., 2017).

A espécie vegetal *Pontederia crassipes* (Mart.) Solms, pertencente à família botânica Pontederiaceae, é uma macrófita aquática, conhecida popularmente como aguapé. Essa espécie possui entre 5 a 25 cm de altura, se propaga vegetativamente e/ou via seminífera e pode ser encontrada em seu habitat natural de forma flutuante e/ou enraizada em locais perenemente encharcados (COETZEE et al., 2017). Todavia, essa espécie é também considerada uma planta invasora, a qual se prolifera em diferentes países (JIRAWATTANASOMKUL et al., 2021). Além do mais, trata-se também de espécie de crescimento rápido, alta produção de biomassa e produz sistema radicular extenso, possibilitando uma grande área de contato superficial com poluentes (RAMIREZ et al., 2021; ZHANG et al., 2021). Assim,considera-se que essas plantas sejam indicadas como potencial fitorremediadora em locais contaminados com MPs (BARTMEYER et al., 2019; NAHAR; HOQUE, 2021). Também se encontra a espécie vegetal *Salvinia auriculata* Aubl., pertencente à família botânica Salviniaceae, popularmente conhecida como orelinha-de-onça, encontrada em rios e lagos. A referida espécie também é uma macrófita aquática flutuante de água doce e, ao encontrar condições favoráveis, coloniza uma grande área em um curto período. Tais características, aliadas a sua capacidade em remover e acumular substâncias orgânicas e inorgânicas, permite que essa espécie também seja reportada na literatura como potencial remediadora de metais pesados (GOMES et al., 2017).

As indústrias têxtis, de cosméticos, de alimentos e de laticínios, não raras, são responsáveis pela contaminação ambiental por não tratar seus efluentes, os quais podem ter a presença de MPs. Por exemplo, estudos em diferentes regiões da Romênia reportaram que alguns MPs foram encontrados em laticínios. Entre esses metais são listados o cobre (Cu), Pb, cobalto (Co) e Cd, em quantidades de 3,2 $g.kg^{-1}$ de alimento, de modo a apresentar riscos à saúde humana (NĂSTĂSESCU et al., 2020). Já, Feigl et al (2015), reportam que Zn e o níquel (Ni) em pequenas concentrações são essenciais para que as plantas possam crescer e completar seu ciclo de vida, contudo, MPs não essenciais ao desenvolvimento vegetal (ex.: Cd), assim como altas concentrações dos elementos essenciais, acarretam em toxicidade para o crescimento vegetal.

2. HIPÓTESE

Testamos a hipótese de que macrófitas aquáticas possuem potencial fitorremediador em corpos hídricos contaminados com a presença de poluentes inorgânicos. Diante disso, segue abaixo o detalhamento da mesma:

- Hipótese de nulidade (‘H0’) = macrófitas aquáticas não serão eficientes como ferramenta biotecnológica no tratamento de efluentes contendo poluentes inorgânicos.

Já na situação em que seja rejeitada a ‘H0’, aceitaremos a hipótese alternativa (‘H1’):

- Hipótese alternativa (‘H1’) = macrófitas aquáticas serão eficientes como ferramenta biotecnológica no tratamento de efluentes contendo poluentes inorgânicos.

3. OBJETIVOS

3.1. Objetivo geral

Avaliar o potencial fitorremediador de macrófitas aquáticas em efluentes de laticínio contaminados com poluentes orgânicos e inorgânicos.

3.2. Objetivos específicos

Analisar a sobrevivência das macrófitas na presença de elementos essenciais e tóxicos, provenientes de rejeitos da indústria de laticínios;

Analisar os parâmetros físicos e químicos do efluente que será tratado;

Mensurar o crescimento vegetativo das macrófitas aquáticas na presença de metais pesados, provenientes de efluentes da indústria de laticínios;

Analisar o acúmulo iônico de elementos essenciais e tóxicos nos tecidos vegetais das macrófitas aquáticas;

Aprimorar habilidades pessoais e profissionais com a realização do projeto;

Redigir um manuscrito científico para posterior submissão a periódico científico com elevado fator de impacto;

Desenvolver biotecnologia que traga benefícios a sociedade de modo geral.

4. REFERÊNCIAS BIBLIOGRÁFICAS

ALI, HAZRAT; KHAN, EZZAT. What are heavy metals? Long-standing controversy over the scientific use of the term ‘heavy metals’–proposal of a comprehensive definition. **Toxicological and Environmental Chemistry**, [*s. l.*], v. 100, n. 1, p. 6–19, 2018.

BARTMEYER, Bruna Carolina; DOS SANTOS OLIVEIRA, Luisa Helena; COELHO, Lucia Helena Gomes. Comparação da retenção de cádmio e chumbo nos tecidos vegetais das macrófitas aquáticas aguapé (*Eichhornia* sp.) e taboa (*Typha* sp.) por biossorção e fitorremediação. **Holos Environment**, v. 19, n. 1, p. 145-159, 2019.

COETZEE, Julie A. et al. Monographs on invasive plants in Europe N° 2: Eichhornia crassipes (Mart.) Solms. **Botany Letters**, v. 164, n. 4, p. 303-326, 2017.

COMPANHIA AMBIENTAL DO ESTADO DE SÃO PAULO, **Relaçao de areas contaminadas**. São Paulo, 2020. Dispoivel em: https://cetesb.sp.gov.br/areascontaminadas/relacao-de-areas-contaminadas/.

CRISTALDI, Antonio *et al.* Phytoremediation of contaminated soils by heavy metals and PAHs. A brief review. **Environmental Technology and Innovation**, v. 8, p. 309–326, 2017.

DORNE, Jean Lou C.M. *et al.* **Human risk assessment of heavy metals: principles and applications,** 2011-. ISSN 15590836.v. 8, 2011.

FEIGL, Gábor *et al.* Comparing the effects of excess copper in the leaves of *Brassica Juncea* (L. Czern) and *Brassica Napus* (L.) seedlings: Growth inhibition, oxidative stress and photosynthetic damage. **Acta**

Biologica Hungarica, v. 66, n. 2, p. 205–221, 2015.

GOMES, Maria Angélica da Conceição *et al*. Salinity effects on photosynthetic pigments, proline, biomass and nitric oxide in *Salvinia auriculata* Aubl. **Acta Limnologica Brasiliensia**, Campos dos Goytacazes, v. 29, 9. ed., p. 1-13, 2017.

JÄRUP, Lars. Hazards of heavy metal contamination. **British Medical Bulletin**, v. 68, p. 167–182, 2003.

JIRAWATTANASOMKUL, Tidarut et al. Use of water hyacinth waste to produce fibre-reinforced polymer composites for concrete confinement: Mechanical performance and environmental assessment. **Journal of Cleaner Production**, v. 292, p. 126041, 2021

KAFLE, Arjun *et al.* Phytoremediation: Mechanisms, plant selection and enhancement by natural and synthetic agents. **Environmental Advances**, v. 8, p. 100203, 2022.

KAUR, Ravdeep *et al.* Co-application of 6-ketone type brassinosteroid and metal chelator alleviates cadmium toxicity in B. juncea L. **Environmental Science and Pollution Research**, v. 24, n. 1, p. 685–700, 2017.

MANI, D.; KUMAR, C.; PATEL, N. K. Hyperaccumulator Oilcake Manure as an Alternative for Chelate-Induced Phytoremediation of Heavy Metals Contaminated Alluvial Soils. **International Journal of Phytoremediation**, v. 17, n. 3, p. 256–263, 2015.

MOREIRA, Marcelo Rasga *et al.* O Brasil rumo a 2030? Percepções de especialistas brasileiros(as) em saúde sobre o potencial de o País cumprir os ODS Brazil heading to 2030. **Saúde em Debate**, v. 43, n. spe7, p. 22–35, 2019.

NAHAR, Kamrun; HOQUE, Sirajul. Phytoremediation to improve eutrophic ecosystem by the floating aquatic macrophyte, water lettuce (*Pistia stratiotes* L.) at lab scale. **The Egyptian Journal of Aquatic Research**, v. 47, n. 2, p. 231-237, 2021.

NĂSTĂSESCU, V. et al. Heavy metal and pesticide levels in dairy products: Evaluation of human health risk. **Food and Chemical Toxicology**, v. 146, 1 dez. 2020.

NIETO, Alejandra Trejo. Economic Growth and Industrialization on the 2030 Agenda: Prospects for Mexico. **Problemas del Desarrollo**, v. 48, n. 188, p. 83–111, 2017.

RAMIREZ, Anyi et al. Utilization of water hyacinth (Eichhornia crassipes) rejects as phosphate-rich fertilizer. **Journal of Environmental Chemical Engineering**, v. 9, n. 1, p. 104776, 2021.

ROSTAMI, Saeid; AZHDARPOOR, Abooalfazl. The application of plant growth regulators to improve phytoremediation of contaminated soils: A review. **Chemosphere**, v. 220, p. 818–827, 2019.

SHI, Jing *et al.* Soil contamination with cadmium and potential risk around various mines in China during 2000–2020. **Journal of Environmental Management**, [*s. l.*], v. 310, n. February, p. 114509, 2022.

SINGH, B. S.Manisha; SINGH, Dipanjali; DHAL, Nabin Kumar. Enhanced phytoremediation strategy for sustainable management of heavy metals and radionuclides. **Case Studies in Chemical and Environmental Engineering**, v. 5, p. 100176, 2022.

TAIZ, Lincoln *et al*. **Fisiologia e Desenvolvimento Vegetal**. Tradução: Alexandra Antunes Matroberti *et al*.: Artmed, 2017. 858 p. Título original: Plant physiology and development. ISBN 978-16-0535-255-8.

WANI, A. L.; ARA, A.; USMANI, J. A. Lead toxicity: A review. **Interdisciplinary Toxicology**, Aligarh, v. 8, n. 2, p. 55-64, jan./abr. 2015.

WAN, X.; LEI, M.; CHEN, T. Cost–benefit calculation of phytoremediation technology for heavy-metal-contaminated soil. **Science of the Total Environment**, v. 563–564, p. 796–802, 2016.

YAN, An *et al.* Phytoremediation: A Promising Approach for Revegetation of Heavy Metal-Polluted Land. **Frontiers in Plant Science**, v. 11, n. April, p. 1–15, 2020.

ZHANG, Liumeng et al. One-pot pyrolysis of a typical invasive plant into nitrogen-doped biochars for efficient sorption of phthalate esters from aqueous solution. **Chemosphere**, v. 280, p. 130712, 2021.

5. ARTIGO DE PESQUISA

Conforme estabelecido pelo Projeto Pedagógico do Curso de Bacharelado em Engenharia Agronômica (PPC, 2016 – link de acesso: https://www.lagoadosino.ufscar.br/cursos/arquivos/ppcs/ppc_engenharia_agronomica_2018_07.pdf), no item '9.4.2. Regulamento do Trabalho de Conclusão de Curso', estabelece que a monografia do trabalho de conclusão do curso se refere a ***"um trabalho acadêmico pode ser monográfico ou de pesquisa o qual poderá ter tema inédito ou advir de pesquisa realizada pelo estudante, no âmbito de sua iniciação científica ou elaborar uma monografia a partir de situações-problema que por ventura vivencie no campo de estágio"***. Assim, ao considerarmos as justificativas apresentadas anteriormente ao longo dessa monografia, redigimos o presente TCC na modalidade '*research paper*'.

AQUATIC MACROPHYTES: A BIOTECHNOLOGICAL TOOL FOR IN SITU TREATMENT OF CONTAMINATED EFFLUENTS

ABSTRACT

Human industrial activities are among the leading causes of environmental heavy metal (HM) accumulation, mainly when improper wastewater management occurs. In this scenario, phytoremediation with aquatic macrophyte species is presented as a suitable technology for agro-industrial effluent treatment systems. Therefore, we tested the hypothesis of whether aquatic macrophytes are efficient in the tertiary treatment of dairy effluents contaminated with pollutants. We collected 600 L of post-treated effluent directly from the milk and cheese agroindustry. After homogenization, we divided into three reactors of 200 L each, applying a macrophyte species, resulting in three treatments for *P. crassipes,* a second with *P. stratiotes,* and another with *S. auriculata.* The effluent was sampled before (day 0) and over 36 days after the implementation of macrophyte treatments to determine the physicochemical parameters. Plant samples were also carried out at the same evaluation intervals to determine plant height, total dry mass, and HMs accumulation (Cd and Pb leaf and root concentrations). Our findings show that plant species can remove and polish effluents with organic and inorganic pollutants, particularly *P. crassipes* and *P. stratiotes*, which, due to their physiological characteristics, were more effective in removal than *S. auriculata*. Also, we offer a pioneering association of transport systems that discuss the Cd^{2+} and Pb^{2+} to elucidate the efficiency of macrophytic species in accumulating these HMs. In this way, we accept our hypothesis that aquatic macrophytes are a biotechnological tool for improving effluent treatment systems, proving to be one of the most promising approaches *in situ* treatment.

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

1. Introduction

Heavy metals (HMs) naturally exist in the environment; however, the unchecked expansion of human activities has significantly heightened the release of hazardous pollutants, both organic and inorganic, into our water bodies and soils. Furthermore, it is worth noting that HMs are non-biodegradable, which means they endure in the environment (Yan et al., 2020). Recent data from this decade reveals that roughly half of agricultural areas exhibit some degree of HM contamination (Khalid et al., 2017). In Brazil, as reported by the Companhia Ambiental do Estado de São Paulo (CETESB) out of 6.434 assessed locations, 1.273 have been identified as contaminated by HMs, including notable elements such as arsenic (As), cadmium (Cd), lead (Pb), copper (Cu), chromium (Cr), iron (Fe), manganese (Mn), mercury (Hg), zinc (Zn), and others (Singh et al., 2021).

Chemical elements can be categorized into essential and non-essential elements based on their essentiality for plant development. Non-essential elements such as arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb) are detrimental to plant cells, leading to oxidative damage (Taiz et al. 2017). Conversely, certain mineral elements are vital for plants and are present in plant tissues, including nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), zinc (Zn), and copper (Cu) (Rengel et al., 2022). Within the category of essential elements, special mention goes to zinc (Zn), copper (Cu), manganese (Mn), and iron (Fe), all of which play critical biological roles in plant development (Bassegio et al., 2020). However, phytotoxicity can occur with these essential elements depending on the plant exposure level. In other words, the toxic effect will be influenced by various factors, including the dose. Even an essential element can harm plant organisms when present in a dose exceeding the plant's tolerance (Gonçalves Jr et al., 2020).

According to Kaur et al (2017) and Shi et al (2023) , Cd is one of the most toxic HMs to the environment and causes damage to plants because of the generation of reactive oxygen species (EROs). In addition, it damages human health, especially the kidneys, liver, lungs, and heart (Jarup, 2003). Lead stands out among HMs for being highly toxic because it causes various damages to plant and animal organisms. In plants, exposure to Pb is related to a lower biomass accumulation, chlorosis, inhibition of the photosynthetic system, alteration of water balance, and redox homeostasis, among other physiological responses that induce plant death (Collin et al., 2022). In animal organisms such as humans, exposure to Pb can promote damage to the neurological system, the development of carcinomas, and even death, given the ability of Pb to bioaccumulate in cellular tissues (Nag and Cummins, 2022).

The permissible thresholds for HM contaminants in water are inherently tied to the specific intended use of that water in conjunction with prevailing national standards (Perveen and Amar, 2023). This direct relation between water quality and its intended purpose is pivotal in safeguarding public health and preserving environmental integrity (Russ et al., 2022). The standards established for drinking water, for example, are extremely strict, with concentration limits of contaminants set at trace levels to protect the health of people who consume it (Zhao et al., 2022). On the other hand, water used in industrial or agricultural activities may have less restrictive standards since direct human exposure is less likely (Deviller et al., 2020).

For example, the Ministry of Health of Brazil (Brasil, 2021) establishes guidelines related to the procedures for control and surveillance of the water quality intended for human consumption, as well as its portability standards, in which the maximum acceptable limits of total HMs and other inorganic contaminants in drinking water, expressed in milligrams per liter ($mg.L^{-1}$), are 0.01 (As), 0.01 (Pb), 2.0 (Cu), 0.05 (Cr), 0.003 (Cd), 0.07 (Ni) and 0.001 (Hg). In addition, the National Council of the Environment in Brazil addresses groundwater classification and environmental guidelines (Brasil 2008). Groundwater often serves as a direct source for human consumption, sometimes with minimal or no prior treatment. This regulation sets forth maximum allowable concentration limits for HMs in groundwater intended for human consumption, expressed in $\mu g.L^{-1}$, as follows: 10.0 (As), 5.0 (Cd), 10.0 (Pb), 20,000.0 (Cu), 3,000.0 (Fe), 1.0 (Hg), 20.0 (Ni), and 500,000.0 (Zn).

Additionally, concerning to the discharge of treated industrial effluents, the Conselho Nacional de Meio Ambiente (CONAMA) in Brazil to industries are authorized to release their treated effluents only if they adhere to the specified maximum concentrations of total or dissolved HMs in water, expressed in mg/L, which includes 0.5 (As_{total}), 0.2 (Cd_{total}), 0.5 (Pb_{total}), 1.0 ($Cu_{dissolved}$), 0.1 (Cr^{6+}), 1.0 (Cr^{3+}), 15.0 ($Fe_{dissolved}$), 1.0 ($Mn_{dissolved}$), 0.01 (Hg_{total}), 2.0 ($NO_{3\,total}^{-}$), and 5.0 (Zn_{total}) (Brasil, 2011). Dairy industries and other industrial segments (cosmetics, textiles, food, etc.), when they do not sufficiently treat their tailings before releasing them into effluents and water bodies, culminate in severe environmental contamination cases.

According to the Instituto Brasileiro de Geografia e Estatística (IBGE), the Brazilian dairy sector is present throughout the national territory (IBGE, 2021). Among the main challenges related to environmental contamination from industrial and agro-industrial waste, the organic load stands out, evaluated through indicators such as COD (Chemical Oxygen Demand) and BOD (Biochemical Oxygen Demand), which are parameters usually highlighted in the sizing of effluent treatment plants in the food industries (Tabelini et al., 2023).

The literature has previously emphasized the presence of HMs in products stemming from this industry, such as milk, cheese, and other derivatives (Elafify et al., 2023); however, still exists a notable gap in information concerning HM concentrations surpassing the disposal limits observed in effluents already treated across various industrial sectors, with a specific focus on the dairy industry. A few researchers point out that agro-industrial effluent treatment systems are usually sized for the efficient removal of organic load without considering parameters related to HMs of the liquid medium (Qasim and Mane, 2013).

Other studies, such as that of Năstăsescu et al (2020), reported in three different regions of Romania significant concentrations of Pb, Cu and Cd in dairy products, in the value of 3.2 $g.kg^{-1}.day^{-1}$, in such a way that these same authors identified an increased risk of nephrotoxicity, hepatotoxicity, hematotoxicity and cardiotoxicity after the consumption of contaminated products. In the study by Afolabi et al (2015), worrying concentrations ($mg\ L^{-1}$) of Cd (0.09), Fe (1.181), Cu (0.35), Pb (1.095), Zn (0.234) and Ni (0.166) in dairy effluents in Nigeria were found. The authors also describe that conventional effluent treatment systems of this segment employ biological treatments, such as treatment ponds, anaerobic and aerobic reactors, and decanting ponds (Krishna et al., 2022). These treatment systems have relevant efficiency in removing organic matter, solids, oils, and fats but are notably inefficient in removing HMs (Andrade, 2011). Thus, the adoption of innovative and lower-cost techniques that seek to increase treatment efficiency in agro-industrial effluents is necessary.

Several techniques of removal and remediation of environments contaminated by metals (soil and effluents), the literature reports different methodological protocols, such as incineration or excavation of soil and subsequent transfer to landfills, treatments with activated carbon (biochar), membrane treatments (reverse osmosis), among several others that present, for the most part, high installation and maintenance costs. On the other hand, phytoremediation emerges as a sustainable alternative technique with high decontamination potential and low cost, mainly because it enables *in situ* treatment (Cristaldi et al., 2017).

According to Wan and collaborators (Wan et al., 2016), compared to other techniques, phytoremediation is of lower cost, presenting an economic surplus approximately seven years after its implementation. In addition, phytoremediation is environmentally favorable because it employs the use of plants capable of remedying or stabilizing the contamination of pollutants through physiological mechanisms such as phytoextraction, phytostabilization, phytodegradation, among others (Kafle et al., 2022). Plant species that perform phytoextraction must have specific characteristics, such as high growth rate, high biomass production, and hyperaccumulation of HMs (Cristaldi et al., 2017). In this context, aquatic macrophytes from the Pontederiaceae, Salviniaceae, and Araceae families exhibit compelling attributes that make them ideal candidates for phytoremediation. These characteristics include rapid plant growth, prolific biomass generation, and extensive root systems (de Souza et al., 2021). Moreover, these species display significant phytoremediation potential in HM-contaminated areas, owing to their capacity to thrive in environments with elevated HMs (Nahar and Hoque, 2021).

As previously mentioned, the combination of industrial practices and the inadequate performance of wastewater treatment facilities results in unsatisfactory levels of metals in the effluent or sewage sludge. Kodituwakku and Yatawara (2020) investigated the effects of phytoremediation of industrial sewage sludge in constructed wetlands using *P. stratiotes, P. crassipes,* and *S. molesta*. Results show significant reductions in metal concentration, with great reductions in Cd using *P. stratiotes* and Pb using *P. crassipes.* Therefore, through rhizofiltration, macrophytes can absorb, concentrate, and precipitate some HMs (George and Gabriel 2017).

Due to the urgent need to evaluate the innovative treatment and post-treatment techniques for agro-industrial effluents, specifically with the use of the aquatic macrophyte species *P. crassipes* and *S. auriculata* in Brazilian subtropical environments, we previously have investigated the effect of using such plants on the improvement of physical-chemical parameters of the effluent (Castro et al., 2017) and, in the present research, we focus on the absorption and translocation of HMs and the bioaccumulation potential of *P. crassipes*, *P. stratiotes,* and *S. auriculata*.

In this way, we test the hypothesis that aquatic macrophytes are efficient in phytoremediation of potentially toxic elements (PTE) in dairy effluents. From the hypothesis test above, our investigation evaluated the efficiency of aquatic macrophytes [*Pontederia crassipes* (Mart.) Solms*, Pistia stratiotes* L. and *Salvinia auriculata* Aubl.] in a discontinuous flow system to treat effluents generated by the dairy industry to meet national and international standards for releasing effluents into water bodies. We accept our hypothesis that aquatic macrophytes are a biotechnological tool in the treatment of contaminated effluents, proving to be one of the most promising approaches in situ treatment.

Our research paper offers a foundation robust basis for comprehensive the efficiency of the studied macrophytic species in plant growth, metal bioaccumulation, and effluent polishing before discharge. Furthermore, our current study employs a pioneering approach by discussing Cd^{2+} and Pb^{2+} cations to elucidate the effectiveness macrophytic species' in improving the physicochemical parameters of the effluent.

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). 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 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.

2.2. 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, polishing of dairy effluent using: *Salvinia auriculata* Aubl. (T1); *Pontederia crassipes* (Mart.) Solms (T2); *Pistia stratiotes* (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)

2.3. Experimental design.

We conducted trials with three unreplicated treatments with collections at different times over 36 days, every four days, distributed over ten collections, totaling 30 plants (ten plant units per treatment). The experimental design adopted was the unreplicated complete block design (UCB), in which we defined the blocks as a time factor so that the treatments were applied to each block but with no repetitions of the same blocks.

2.4. Experimental evaluations

2.4.1 Analysis of physicochemical parameters in the effluent

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.

2.4.2. Analysis of plant growth

2.4.2.1. Total dry 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). Washed in a 0,08% detergent solution (v-1), rinsed twice in distilled water, and after washing, 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’. Subsequently, the samples were crushed in a knife mill (Willey type) (Araújo et al., 2014) and stored in a dry environment until laboratory analysis.

2.4.3. 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.

2.4.4. 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 using the Friedman test for non-replicated block designs, 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

After collecting and statistically analyzing the data using the Friedman Test as an innovative statistical tool, it represents an original contribution to interpreting results in phytoremediation studies in environments contaminated by heavy metals (HMs). We speculated on the effectiveness of macrophytes in refining and enhancing the quality of effluents before their discharge into water bodies. Besides, our evaluation extended to assessing the phytoremediation potential of these plants, encompassing aspects such as plant growth and their capacity to accumulate potentially hazardous metals.

3.1. Physical-chemical parameters

The effluents showed no differences over time regarding pH values ($\chi^2 = 1.77$, df = 2, p = 0.412). Starting from an initial pH of 6.24 in the dairy effluent, a slight increase to an average of 7.23 ($\bar{x} \pm S$: 7.03 ± 0.32) was observed after 36 DAAT. Similarly, no differences were detected in turbidity reduction over time ($\chi^2 = 4.17$, df = 2, p = 0.124), with turbidity following a negative exponential curve (Figure 1a), modeled as $y = A\, exp(-B\, x)$, where $A^* = 116.96$ and $B^* = 0.077$, and x is the chronological time. For dissolved oxygen (DO), the Friedman test showed no differences among treatments ($\chi^2 = 0.66$, df = 2, p = 0.716), but an increasing trend in DO levels was noted across treatments, remaining within compliance with CONAMA Resolution 430/2011 (Figure 1b). Regarding COD, differences were observed over time ($\chi^2 = 8.35$, df = 2, p = 0.015). For total solids, no differences were found ($\chi^2 = 3.43$, df = 2, p = 0.179), though a decrease in concentration was noted for all treatments (Figure 1c). Both total fixed solids (TFS) and total volatile solids showed no differences over time ($\chi^2 = 0.21$, df = 2, p = 0.900; $\chi^2 = 4.76$, df = 2, p = 0.092, respectively). Phosphorus concentrations in the effluent ($mg \cdot L^{-1}$) also exhibited no significant differences among treatments ($\chi^2 = 4.66$, df = 2, p = 0.096). Finally, while nitrogen concentrations ($mg \cdot L^{-1}$) appeared to differ slightly, this variation was not statistically significant ($\chi^2 = 8.66$, df = 2, p = 0.131).

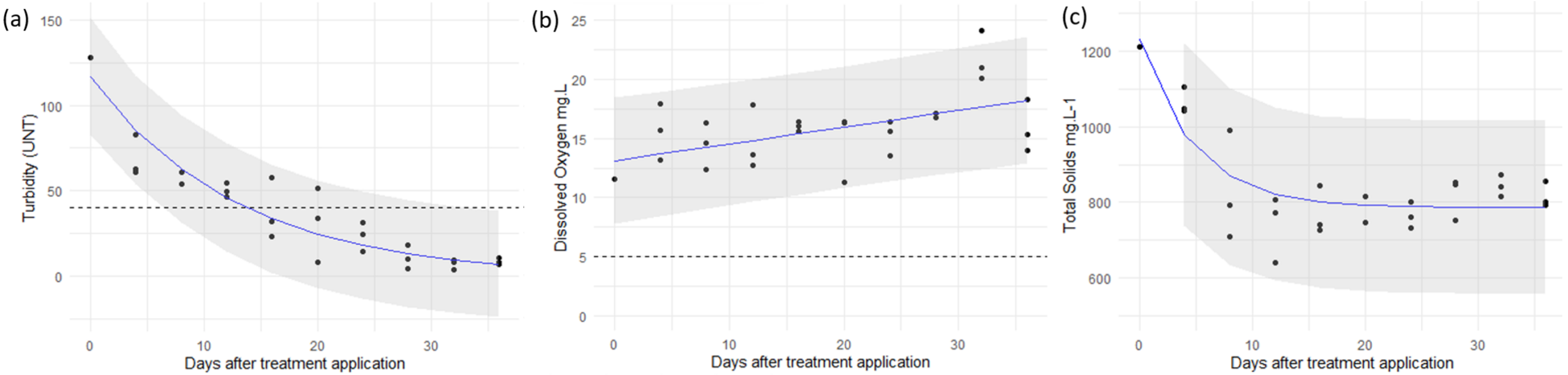


Figure 1. Negative exponential adjustment for turbidity (a), linear adjustment for dissolved oxygen (b), and negative adjustment for total solids (c) of dairy effluents treated with *Pistia stratiotes*, *Pontederia crassipes*, and *Salvinia auriculata* in post-treatment for 36 DAAT. Note: The points 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.

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

The plants in all treatments exhibited satisfactory growth, where we do not observe any damage or setback in the growth of the plants, even in a contaminated environment. For average height (cm), the values observed were 36.7 ± 9.2 for *P. stratiotes*, 52.4 ± 8.4 for *P. crassipes*, and 13.4 ± 1.8 for *S. auriculata* (Figure 2a). Regarding total dry matter (g), the averages were 8.28 ± 2.07 for *P. stratiotes*, 8.50 ± 2.82 for *P. crassipes*, and 1.40 ± 0.45 for *S. auriculata* (Figure 2b). The Friedman test indicated significant differences in nitrogen (N) concentrations in shoots ($\chi^2 = 9.80$, df = 2, p = 0.007), but not in roots ($\chi^2 = 0.35$, df = 2, p = 0.835). For the Phosphorus (P) concentrations showed no differences in either shoots ($\chi^2 = 0.80$, df = 2, p = 0.670) or roots ($\chi^2 = 1.40$, df = 2, p = 0.496). Potassium (K) concentrations in shoots differed significantly over time ($\chi^2 = 6.20$, df = 2, p = 0.045), whereas no differences were observed in roots ($\chi^2 = 6.20$, df = 2, p = 0.045). Similarly, no differences were found in cadmium (Cd) concentrations in shoots or roots (Shoots: $\chi^2 = 2.64$, df = 2, p = 0.266; Roots: $\chi^2 = 0.21$, df = 2, p = 0.897) or in lead (Pb) concentrations in shoots or roots (Shoots: $\chi^2 = 2.00$, df = 2, p = 0.367; Roots: $\chi^2 = 1.40$, df = 2, p = 0.496) (Figures 2c to 2f).

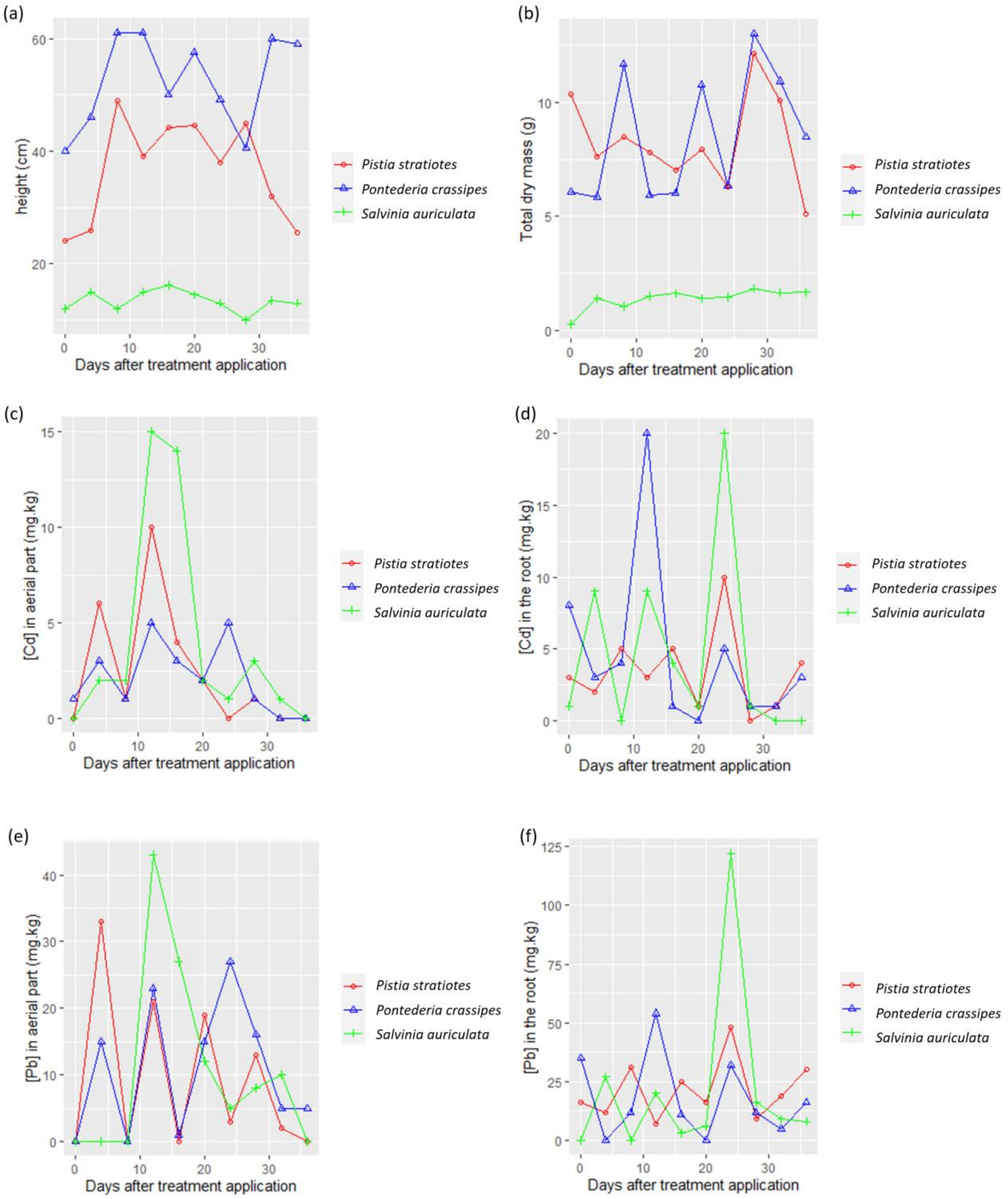


Figure 2. Plant height (a) total dry mass (b), Cd in aerial part (c) and in roots (d), Pb in aerial part (e) and in roots (f) of *Pistia stratiotes*, *Pontederia crassipes*, and *Salvinia auriculata* in post-treatment of dairy effluent for 36 DAAT.

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.

For 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.

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, 0.9 and 0.7, respectively for *S. auriculata*, *P. stratiotes*, and *P. crassipes*; for Pb, with TI of 1.5 and 0.9 for *S. auriculata* and *P. stratiotes*; for Mn, with TI values of 4.9 and 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, $Mn(OH)_2$, $Cd(OH)_2$, $Pb(OH)_2$, $Cr(OH)_2$, $Al(OH)_3$].

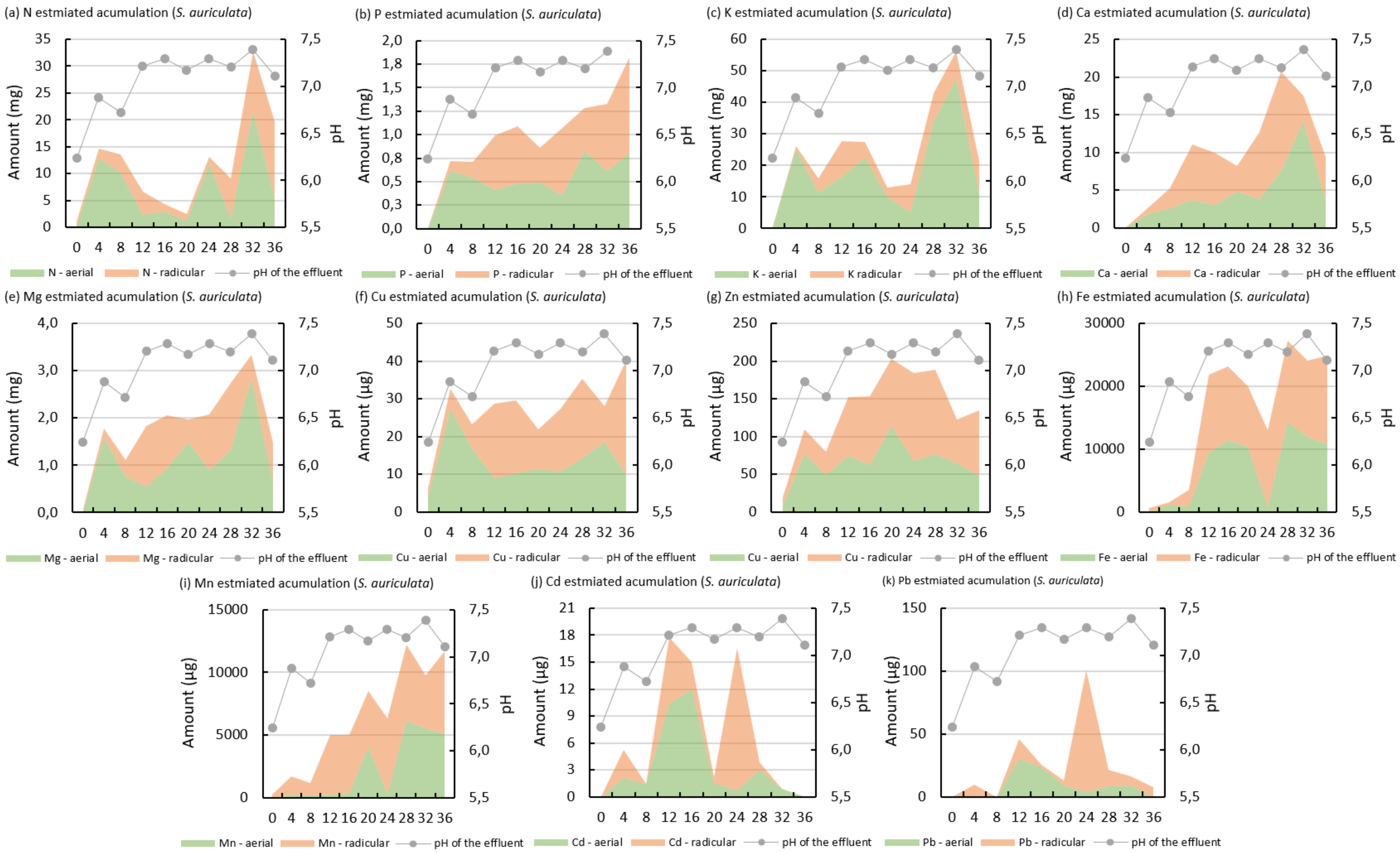


Figure 3. Accumulation of nutrients and metals in the root and leaves of *Salvinia auriculata* plants in post-treatment of dairy effluent for 36 days.

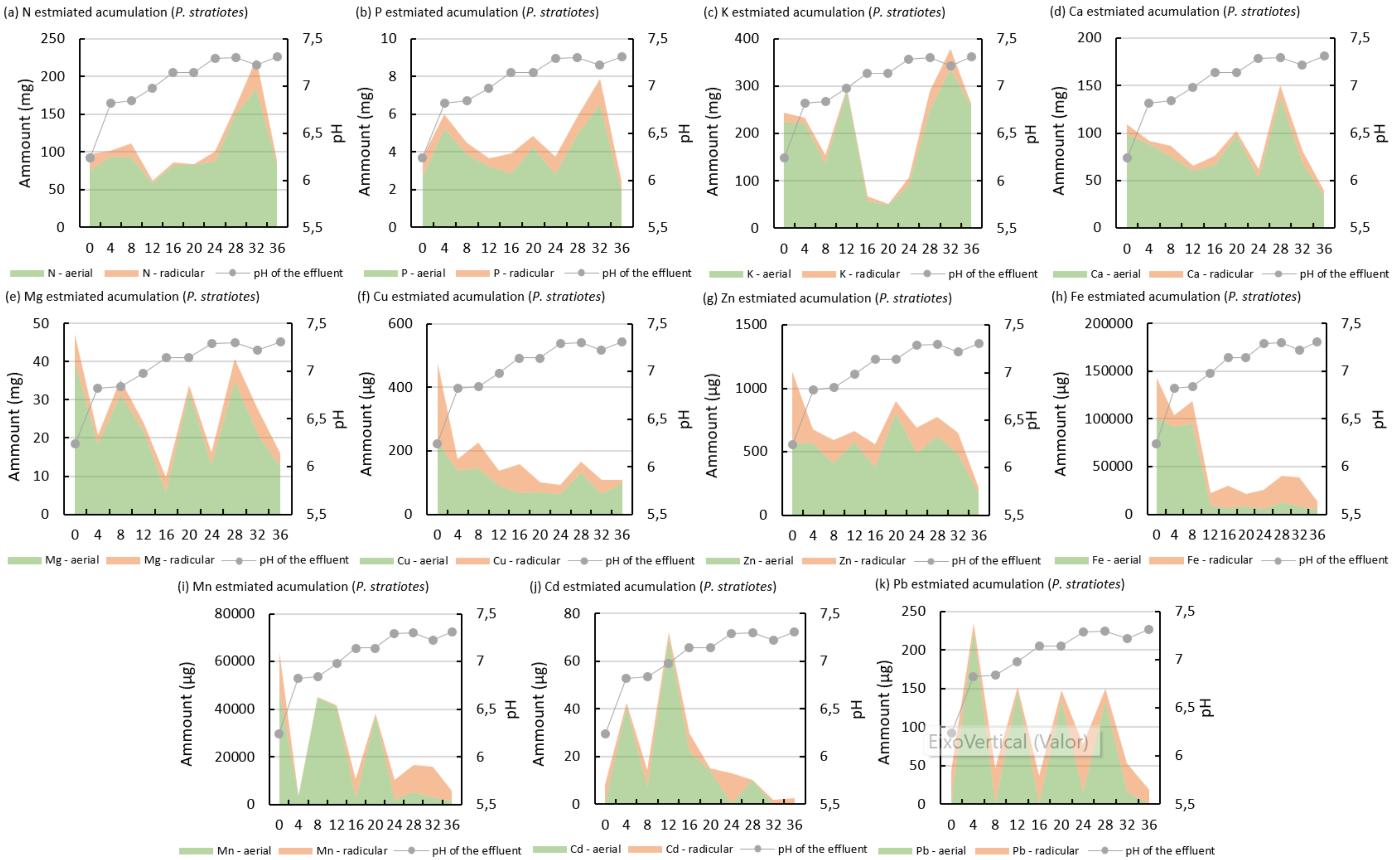


Figure 4. Accumulation of nutrients and metals in the root and leaves of *Pistia stratiotes* plants in post-treatment of dairy effluent for 36 days.

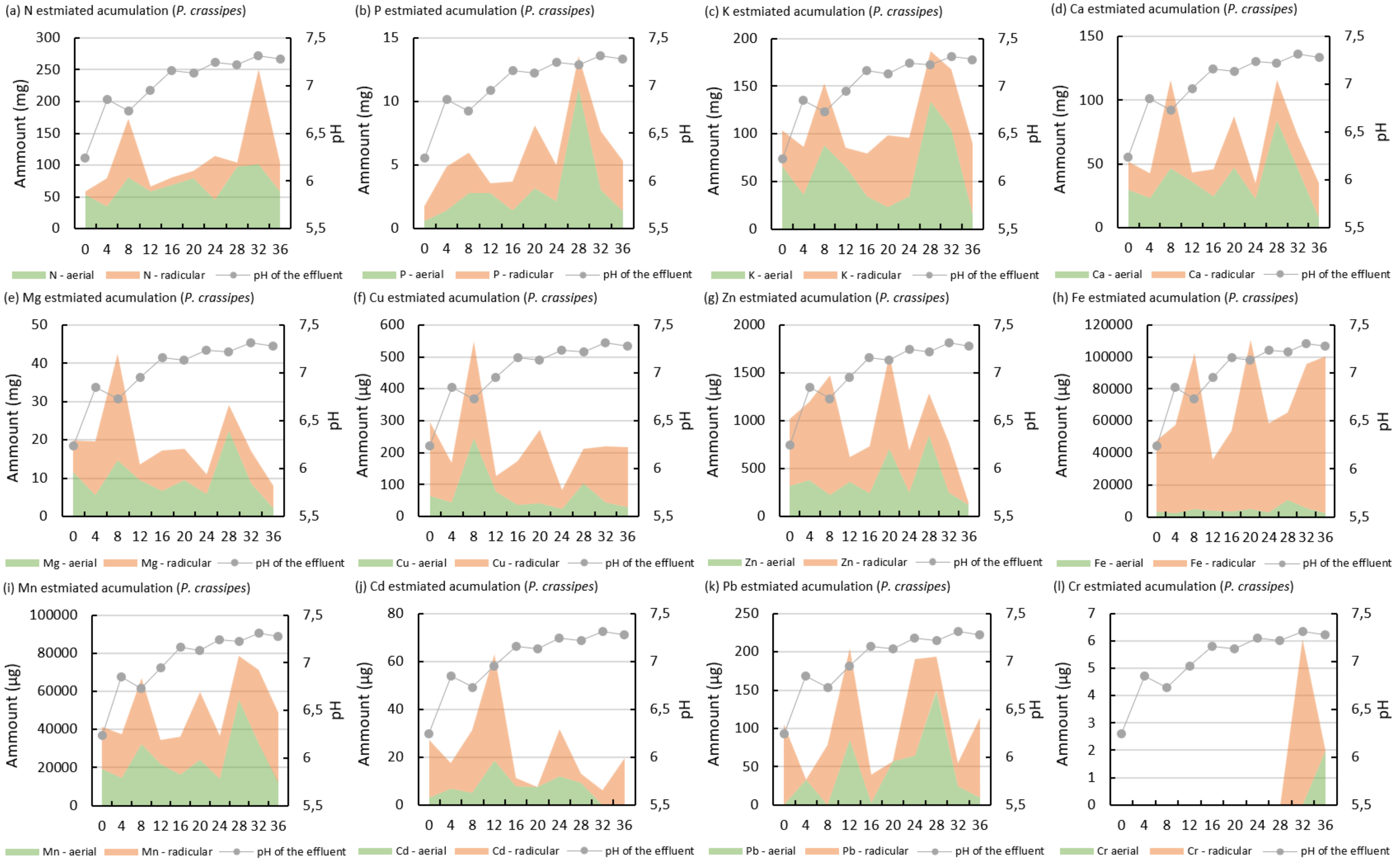
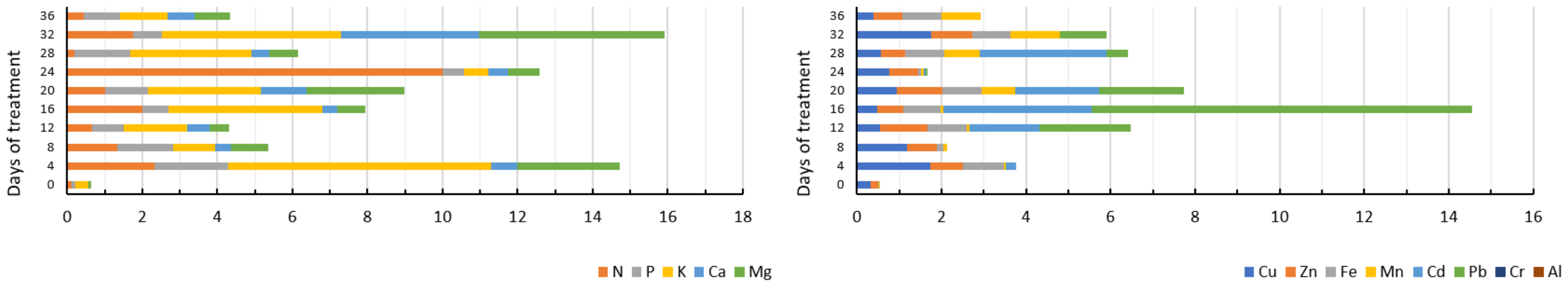
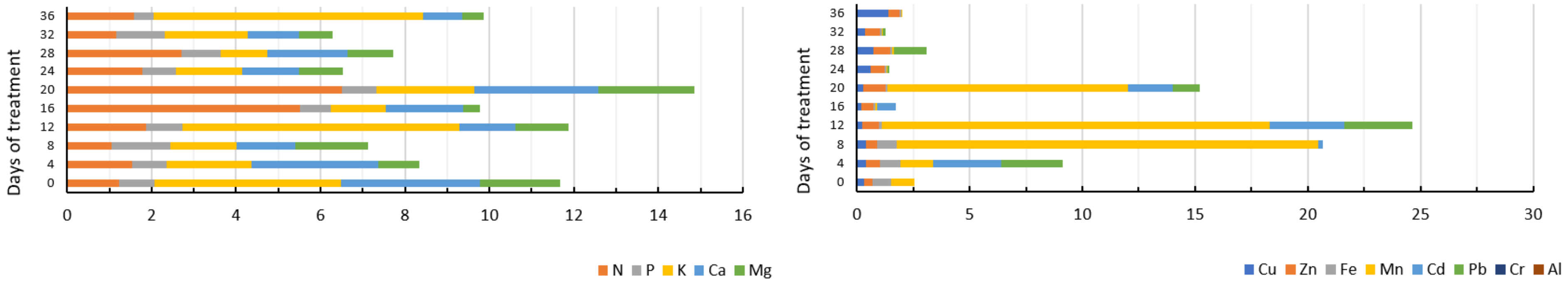


Figure 5. Accumulation of nutrients and metals in the root and leaves of *Pontederia stratiotes* plants in post-treatment of dairy effluent for 36 days.



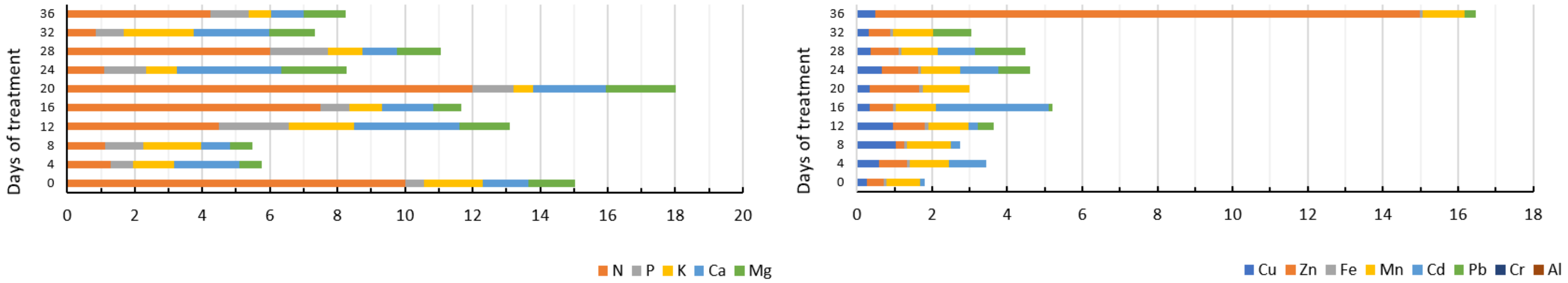
(a) Translocation rate of N, P, K, Ca and Mg - (*S. auriculata*)

(b) Translocation rate of Cu, Zn, Fe, Mn, Cd, Pb, Cr and Al (*S. auriculata*)



(c) Translocation rate of N, P, K, Ca and Mg (*P. stratiotes*)

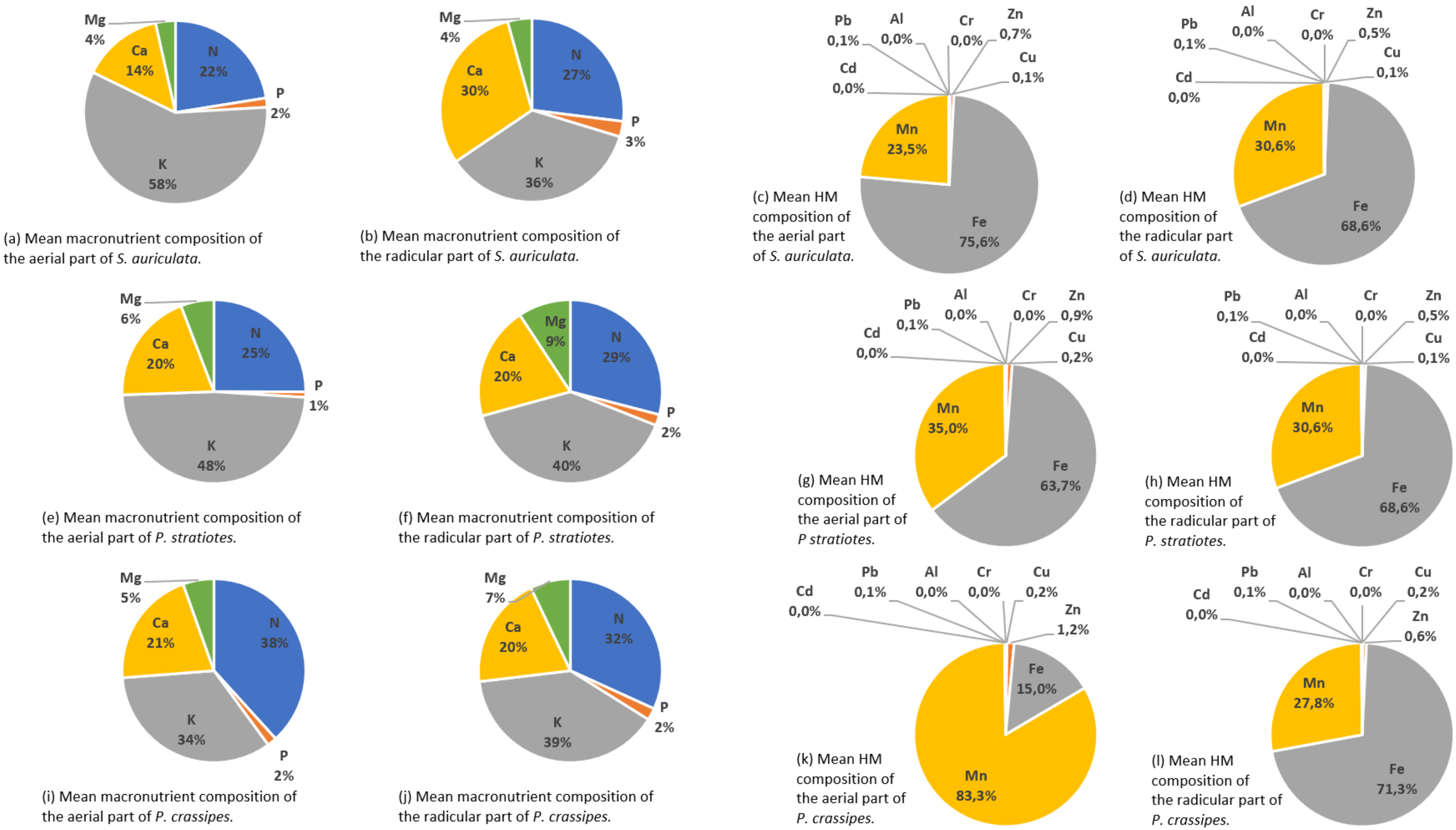
(d) Translocation rate of Cu, Zn, Fe, Mn, Cd, Pb, Cr and Al (*P. stratiotes*)



(e) Translocation rate of N, P, K, Ca and Mg (*P. crassipes*)

(f) Translocation rate of Cu, Zn, Fe, Mn, Cd, Pb, Cr and Al (*P. crassipes*)

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



(a) Mean macronutrient composition of the aerial part of *S. auriculata.*

(b) Mean macronutrient composition of the radicular part of *S. auriculata.*

(c) Mean HM composition of the aerial part of *S. auriculata.*

(d) Mean HM composition of the radicular part of *S. auriculata.*

(e) Mean macronutrient composition of the aerial part of *P. stratiotes.*

(f) Mean macronutrient composition of the radicular part of *P. stratiotes.*

(g) Mean HM composition of the aerial part of *P stratiotes.*

(h) Mean HM composition of the radicular part of *P. stratiotes.*

(i) Mean macronutrient composition of the aerial part of *P. crassipes.*

(j) Mean macronutrient composition of the radicular part of *P. crassipes.*

(k) Mean HM composition of the aerial part of *P. crassipes.*

(l) Mean HM composition of the radicular part of *P. crassipes.*

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.

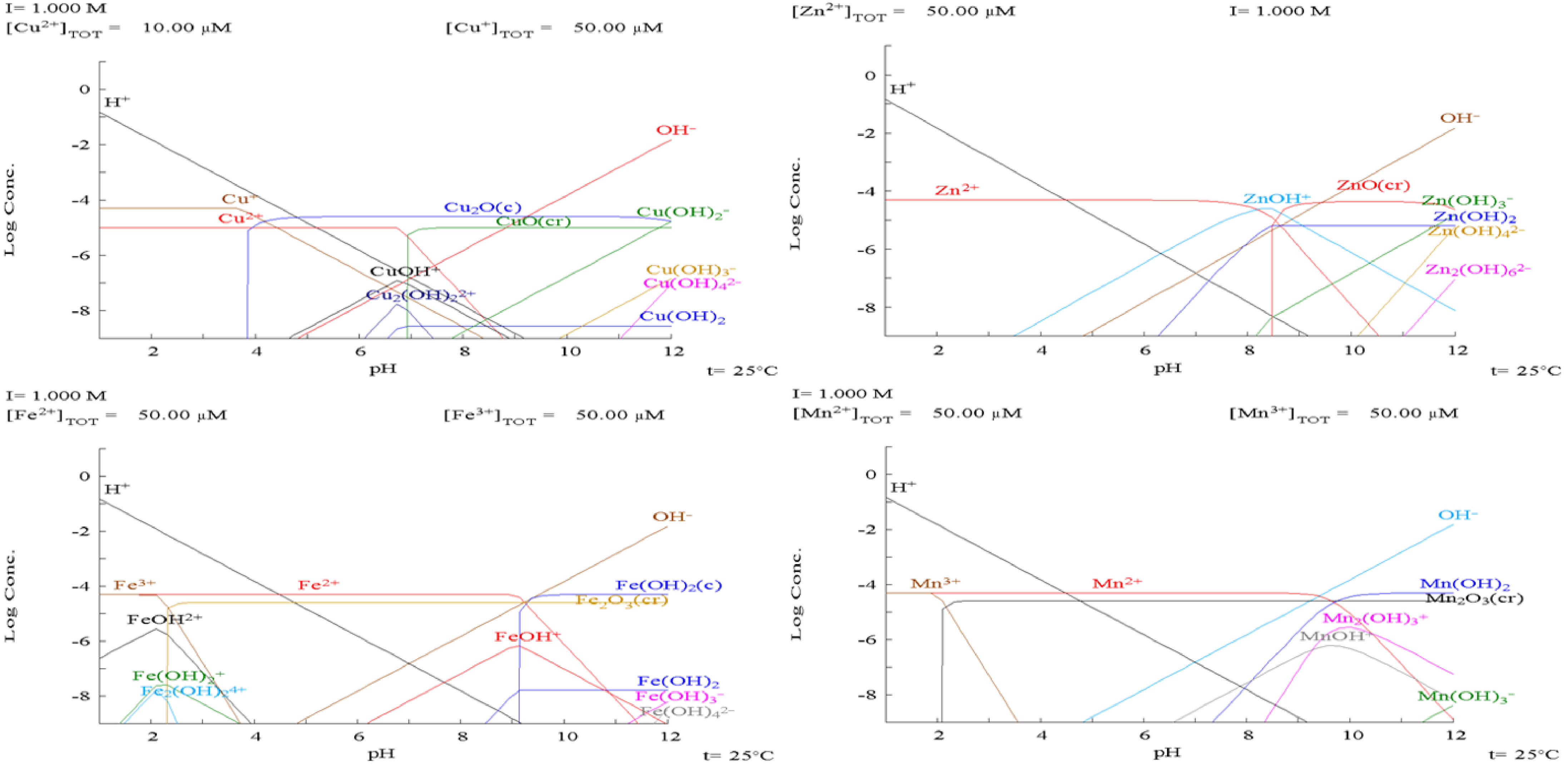


Figure 8. Theoretical diagrams for speciation of Cu, Zn, Fe, and Mn. Source: Hydromedusa Software. Conditions evaluated: pH ranging from 1 to 12; [metals] = 5, 10-5 M; ionic strength = 1 M.

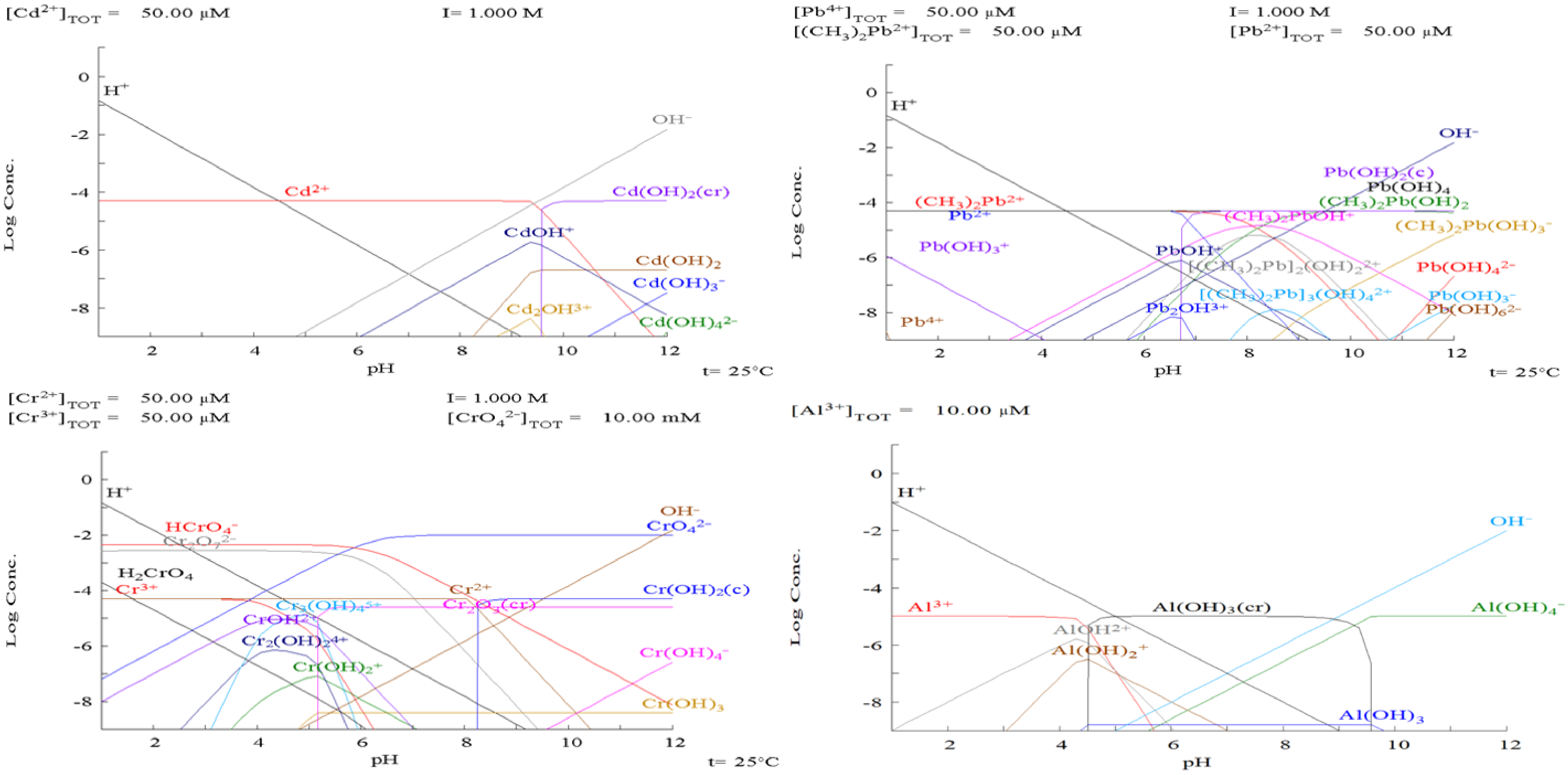


Figure 9. Theoretical diagrams for speciation of Cd, Pb, Cr and Al. Source: Hydromedusa Software. Conditions evaluated: pH ranging from 1 to 12; [metals] = 5, 10-5 M; ionic strength = 1M.

4. Discussion

Dairy effluents present themselves as a worrying environmental contaminant in water bodies near the launch area; thus, it becomes urgent to develop HMs remedial techniques *in situ* for aquatic environments. For example, flooded, dammed, and/or open areas can be places for HM accumulation, harming terrestrial and aquatic biota (Xia et al., 2021). Given this, free-living macrophytes naturally found in nature are potential species for removing inorganic or organic pollutants in water bodies (Kodituwakku and Yatawara, 2020). It is also noteworthy that plants with the potential to remove pollutants may suffer severe injuries if used in media with PTE (Mayonde et al., 2021). On the other hand, in our findings, the accumulation of HMs in plant tissues does not adversely affect plant growth. Wu et al (2021) point out plant physiological mechanisms that mitigate the stress caused by HMs, among which are reported the detection of ions expression of candidate genes in the regulation of intracellular homeostasis of metals, among other mechanisms.

Culturing aquatic macrophytes with inorganic pollutants in aquatic environments shows that high concentrations of pollutants could impact their removal efficiency (Wibowo et al., 2023). Plants grown in the "very short term" (0 to 15 days) are relatively abundant in effluents contaminated with inorganic pollutants. However, in the "short term" (16 to 36 days), investigations are scarce, and such an approach can be explored in developing management strategies with plant species.

In general, the literature indicates that physicochemical characteristics are the main concerns of public authorities regarding environmental monitoring and its correct preservation. Our results indicate that the physicochemical parameters have become 'suitable' to CONAMA (Brasil, 2011) standards in the short term (16 to 36 days). The macrophytes played an essential role in "cleaning" the effluent from the first fortnight of the experiment. We also highlight that the increase in pH is due to the action of phytoplankton since the collections were carried out between 9:00 a.m. and 10:00 a.m. when the light conditions favor liquid photosynthesis (Henares and Camargo, 2014). We also highlight that the slight increase in pH modulates numerous integral membrane proteins to move ions in favor and/or against their concentration gradient (Rengel, 2022).

In this case, a slightly acid-to-neutral pH favors the accumulation of Cd and Pb in the cell interior (Zhang et al., 2023). We speculate that the observed decrease in the concentration of organic matter was achieved through sedimentation at the bottom of the container and fixation by the root system of the plants, as found in the study of Akowanou et al (2023). Our study observed an increase in DO concentrations throughout the experimental period because macrophytes can capture atmospheric O_2 and transport it to the aquatic environment inserted through its structures, such as roots, stems, and leaves. In addition, high DO concentrations promote conditions for the mineralization of the environment and, consequently, help decompose organic matter.

For other parameters, such as turbidity and COD, we observed a significant reduction after the end of the experiment period. Thus, this occurred due to the action of bacteria and microorganisms present in the effluent and rhizosphere of the plant and that they were stimulated by oxygenation in the effluent, degrading organic matter into more straightforward and less toxic products (Selvaraj and Velvizhi, 2021).

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 (Müller, 2023; Shuting et al., 2022), 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 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) (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 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.

Williams and collaborators (Williams et al., 2000) highlighted in their studies that excess micronutrients are as harmful and toxic as much as non-essential metals, such as Cd^{2+}, Hg^{2+}, and Pb^{2+}. In the present research, the PTE observed in the tissues (root and shoot) did not impair plant growth. The non-verification of PTEs harmful effects on plants is also corroborated by previous publications that address the interaction between living organisms (bacteria) and the plant root system to circumvent possible harmful effects on plants (Zhu et al., 2023). In addition, the noteworthy accumulation of metals in plant tissues did not damage the root, which is the leading absorbing site of organic and inorganic pollutants (Kaushal and Mahajan, 2022). The literature reports that plant species can mitigate possible harmful effects through different antioxidant metabolic pathways, whether enzymatic and non-enzymatic, compartmentalization of potentially toxic chemical elements modulated by phytohormones, such as salicylic acid (SA), brassinosteroids (BRs), gibberellic acid (GAs), ethylene, cytokinin (CK), among others (Chen et al., 2021; Wang et al., 2021).

According to Rodrigues and collaborators (Rodrigues et al., 2016), *P. crassipes* cultivated in a stressful environment can modulate part of its biochemical metabolism, allowing its tolerance to this environment. The authors also reported that most of the Cd that the plant accumulates is located in the roots, in which this metal is linked to the negative charges of the cell walls, preventing its migration to the aerial part of the plant and thus preventing possible damage. The study by Wibowo and collaborators (Wibowo et al., 2023) points to the efficiency of *P. stratiotes* in wastewater, highlighting that the bioaccumulation is also higher in the roots than in the stems, so that the levels of translocation of plant materials reached up to 0.9. As for the species *S. auriculata,* we observed in several studies with an approach to species of this same botanical genus that the species can accumulate HMs (Donatus, 2016). Nevertheless, *Salvinia* plants, because they are 'aquatic ferns', have stem tissues without roots and roots filled with root micro-hairs (Biju et al., 2023), i.e., it is an ancestral species with lower efficiency in removing pollutants and, therefore, *P. crassipes and P. stratiotes* (eudicots) have more significant potential in the removal of pollutants.

The present study covers macrophytic plants in experimental conditions in protected cultivation using a discontinuous batch treatment system. However, we must emphasize that our results play a significant role as a preliminary basis for future studies about this technology. Our study could include evaluating the use of macrophytic species in continuous agro-industrial treatment systems, especially when combined with preliminary techniques such as anaerobic, facultative, and aerobic ponds. In addition, our paper offers a solid basis for understanding the efficiency potential of the macrophytic species investigated, both in terms of plant growth and bioaccumulation of metals, as well as in the improvement of the polishing of effluents before their disposal in water bodies. This information can serve as a valuable "starting point" for future research and practical applications in this field of practical study when considering the social, economic, and technological benefits of effluent phytoremediation.

TI close, or higher than 1, is a strong indicator of hyperaccumulation on the aerial part of the element, as observed for *S. auriculata*, *P. stratiotes,* and *P. crassipes* for essential nutrients such as N, P, K, Ca, and Mg. Nevertheless, the same pattern is not often observed for HM, especially toxic HM, with the exception made for plants with phytoextraction pronounced capacities, such as observed by *S. auriculata*, *P. stratiotes*, and *P. crassipes*, respectively, while phytoextracting Cd^{2+} and Pb^{2+}. Also, it is essential to note the higher Zn phytoextraction capacity observed by *P. crassipes*, relatively higher than the TI obtained by Ribeiro and colleagues (Ribeiro et al., 2022), that testing other aquatic macrophytes *Veronica anagallis-aquatica* and *Cakile maritima* in wetlands in the Douro River in Portugal. It is essential to state that although these authors evaluate aquatic macrophytes as bioindicators of HM contamination in an impacted estuarine ecosystem, our results were obtained from the post-treatment dairy wastewater treatment plant. Hence, considering the low levels of HM in both environments, data suggests that the observed behavior of *P. stratiotes* hints at a promising potential for Zn phytoextraction.

Furthermore, it is noteworthy that *P. crassipes* exhibited a notably higher Zn phytoextraction capacity, which is relatively higher than the TI (Ribeiro et al., 2022). In their study, Ribeiro et al. assessed other aquatic macrophytes, namely *Veronica anagallis-aquatica* and *Cakile maritima*, in wetlands along the Douro River in Portugal. In another study, Abdelaal and collaborators (Abdelaal et al., 2021) investigated seven aquatic macrophytes, namely *C. alopecuroides*, *E. stagnina*, *P. australis*, *R. sceleratus*, *T. domingensis*, *E. crassipes*, and *L. stolonifera*, as potential plant species for phytoremediation in the Nile Delta of Egypt. It is important to emphasize that while Ribeiro and colleagues (Ribeiro et al., 2022) and Abdelaal and collaborators (Abdelaal et al., 2021) evaluated aquatic macrophytes in the phytoextraction of HMs in impacted estuarine ecosystems, our results were obtained from the post-treatment of a dairy wastewater treatment plant. Consequently, both environments, estuarine ecosystems and post-treatment reactors, exhibit significantly low levels of HMs. In light of these considerations and maintaining the appropriate context, it can be posited that the observed behavior of *P. crassipes* suggests a promising potential for Zn phytoextraction. However, further investigation should be conducted to characterize phytoextraction capacity of this plant species entirely.

Ultimately, it is imperative to underscore that the elevated values in the deviation about the accumulation of HMs and translocation index (TI) stem (data deviation not shown) from heightened accumulative processes occurring over the 36 DAAT. Specifically, the substantial variation does not manifest imprecision in the measurements; rather, it signifies the progressive (or, in certain instances, regressive) trends in the absorption and translocation of HMs. Additionally, this phenomenon indicates, to a certain extent, the inherent robustness or adaptability of the assessed natural species within an atypical setting, such as an industrial effluent-laden environment.

Furthermore, it is crucial to note that, in a substantial number of observed cases, there is an elevation in the accumulation, or at the very least, the maintenance of the amount of HMs in both the roots and shoots of the investigated aquatic plants during the 36 DAAT. These findings become more attractive when we consider that the pH of the dairy effluent increased; therefore, the aquatic environment was less favorable to dissolved chemical species such as Cu^{2+}, Zn^{2+}, Mn^{2+}, Mn^{3+}, Fe^{2+}, Cd^{2+}, Pb^{2+}, and Cr^{2+}. Consequently, an aquatic environment with increasing pH favors processes such as complexation and precipitation of HMs, making these elements unavailable for plant absorption and accumulation. However, even in these harsh conditions, the studied aquatic plants were able to maintain or even increase the accumulation of several HMs in shoots and/or roots, such as Cu, Zn, Fe, Mn, Cd, and Pb by *S. auriculata*; Cu, Zn, Fe, Mn, Cd, and Pb by *P. stratiotes*; Cu, Zn, Fe, Mn, Cd, and Pb by *P.*

5. Conclusion

Our study evaluated the potential of aquatic macrophytes in polishing effluents from the dairy industry contaminated with organic and inorganic pollutants. In this way, we show that some of the macrophytes used as a study model are biotechnological tools that can potentially remove pollutants with improvements in physicochemical parameters without the metals' uptake in their tissues, causing losses in their plant growth. The species *Pontederia crassipes* and *Pistia stratiotes* stand out as efficient in polishing the contaminated effluent when compared to *Salvinia auriculata.* Thus, we accept our hypothesis that macrophytes are a biotechnological tool in treating contaminated effluents. In summary, our study endorses macrophytes' promising effectiveness in stabilizing and removing PTEs and improving physicochemical parameters in effluents before their release into water bodies.

Forthcoming research must focus on exploring high-affinity and low-affinity transport systems (HATS and LATS) in native plant species to gain a better understand of the mechanisms underlie phytoremediation. Furthermore, we encourage the possibility of applying species in wetlands or ponds of specific treatments according to the specific requirements of each company in the dairy sector.

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References

Abdelaal, M., I.A. Mashaly, D.S. Srour, M.A. Dakhil, M.A. El-Liethy, A. El-Keblawy, R.F. El-Barougy, M.W.A. Halmy and G.A. El-Sherbeny. 2021. Phytoremediation Perspectives of Seven Aquatic Macrophytes for Removal of Heavy Metals from Polluted Drains in the Nile Delta of Egypt. *In* Biology.

Afolabi, T., A. Alade, M. Jimoh and I. Fashola. 2015. Heavy metal ions adsorption from dairy industrial wastewater using activated carbon from milk bush kernel shell. Desalination and Water Treatment. 57:1-13.

Ahmad, I., A. Rawoof, Priyanka, K. Islam, J. Momo, T. Anju, A. Kumar and N. Ramchiary. 2023. Diversity and expression analysis of ZIP transporters and associated metabolites under zinc and iron stress in Capsicum. Plant Physiology and Biochemistry. 196:415-430.

Akowanou, A.V.O., H.E.J. Deguenon, K.C. Balogoun, M.M.A. Daouda and M.P. Aina. 2023. The combined effect of three floating macrophytes in domestic wastewater treatment. Scientific African. 20:e01630.

Andrade, L. 2011. Tratamento de efluente de indústria de laticínios por duas configurações de biorreator com membranas e nanofiltração visando o reúso. Thesis.

Araújo, L., E. Alves, C. Rodrigues and A. Rodrigues. 2014. Emergence and early growth of seedlings of Eugenia jambolana Lam. After removal of the pulp. Ciência Rural. 45:14-18.

Baird, R.B.*e.a.* 2012. Standard methods for the examination of water and wastewater. American public health association, Washington, DC.

Bassegio, C., M.A. Campagnolo, D. Schwantes, A.C. Gonçalves Junior, J. Manfrin, A.d.P. Schiller and D. Bassegio. 2020. Growth and accumulation of Pb by roots and shoots of Brassica juncea L. International Journal of Phytoremediation. 22:134-139.

Biju, L.M., V.G. K, P. Senthil Kumar, R. Kavitha, R. Rajagopal and G. Rangasamy. 2023. Application of Salvinia sps. in remediation of reactive mixed azo dyes and Cr (VI) - Its pathway elucidation. Environmental Research. 216:114635.

Bozzi, A.T. and R. Gaudet. 2021. Molecular Mechanism of Nramp-Family Transition Metal Transport. Journal of Molecular Biology. 433:166991.

Brasil, C.C.N.d.M.A.d. 2008. Resolução nº 396 do Conselho Nacional do Meio Ambiente do Brasil.

Brasil, C.C.N.d.M.A.d. 2011. Resolução do Conama 430 de 2011(Resolução CONAMA Nº 430 DE 13/05/2011).

Castro, G., D. Schwantes, A. Gonçalves Jr, A. Richart, T. Veiga and A. Rosenberger. 2017. Treatment of dairy effluents in wetlands systems with floating Aquatic Macrophytes. Revista de Ciências Ambientais. 11:25.

Chemists., A.A.o.O.A. 2016. Official methods of analysis of AOAC International. Association of Official Analytical Chemists, 20th Edn. AOAC International, Rockville, Maryland.

Chen, L., H. Yang, Y. Fang, W. Guo, H. Chen, X. Zhang, W. Dai, S. Chen, Q. Hao and S. Yuan. 2021. Overexpression of GmMYB14 improves high-density yield and drought tolerance of soybean through regulating plant architecture mediated by the brassinosteroid pathway. Plant biotechnology journal. 19:702-716.

Collin, S., A. Baskar, D.M. Geevarghese, MNVS Ali, P. Bahubali, R. Choudhary, V. Lvov, G.I. Tovar, F. Senatov, S. Koppala and S. Swamiappan. 2022. Bioaccumulation of lead (Pb) and its effects in plants: A review. Journal of Hazardous Materials Letters. 3:100064.

Cristaldi, A., G.O. Conti, E.H. Jho, P. Zuccarello, A. Grasso, C. Copat and M. Ferrante. 2017. Phytoremediation of contaminated soils by heavy metals and PAHs. A brief review. Environmental Technology & Innovation. 8:309-326.

Deviller, G., L. Lundy and D. Fatta-Kassinos. 2020. Recommendations to derive quality standards for chemical pollutants in reclaimed water intended for reuse in agricultural irrigation. Chemosphere. 240:124911.

Donatus, M. 2016. Removal of heavy metals from industrial effluent using Salvinia molesta. Int. J. Chemtech. Res. 9:608-613.

Elafify, M., M. El-Toukhy, K.I. Sallam, NM. Sadoma, S. Mohammed Abd-Elghany, A. Abdelkhalek and A.H. El-Baz. 2023. Heavy metal residues in milk and some dairy products with insight into their health risk assessment and the role of Lactobacillus rhamnosus in reducing the lead and cadmium load in cheese. Food Chemistry Advances. 2:100261.

George, G.T. and J.J. Gabriel. 2017. Phytoremediation of heavy metals from municipal waste water by Salvinia molesta Mitchell. The Saudi Journal of Life Sciences. 2:8.

Gonçalves Jr, A., D. Schwantes, R. Sousa, T. Silva, V. Guimarães, M. Campagnolo, E. Vasconcelos and J. Zimmermann. 2020. Phytoremediation capacity, growth and physiological responses of Crambe abyssinica Hochst on soil contaminated with Cd and Pb. Journal of Environmental Management. 262:110342.

Wibowo, Y., A. Tyaz Nugraha and A. Rohman. 2023. Phytoremediation of several wastewater sources using Pistia stratiotes and Eichhornia crassipes in Indonesia. Environmental Nanotechnology, Monitoring & Management. 20:100781.

Henares, M.N.P. and A.F.M. Camargo. 2014. Estimating nitrogen and phosphorus saturation point for Eichhornia crassipes (Mart.) Solms and Salvinia molesta Mitchell in mesocosms used to treating aquaculture effluent. Acta Limnologica Brasiliensia. 26

Huang, D. and W. Dai. 2015. Two iron-regulated transporter (IRT) genes showed differential expression in poplar trees under iron or zinc deficiency. Journal of Plant Physiology. 186-187:59-67.

I.B.G.E. 2021. Indicadores IBGE: Estatística da Produção Pecuária, Rio de Janeiro.

Jarup, L. 2003. Hazards of heavy metal contamination. British Medical Bulletin. 68:167-182.

Kafle, A., A. Timilsina, A. Gautam, K. Adhikari, A. Bhattarai and N. Aryal. 2022. Phytoremediation: Mechanisms, plant selection and enhancement by natural and synthetic agents. Environmental Advances. 8:100203.

Kaur, R., P. Yadav, A. Thukral, A. Walia and R. Bhardwaj. 2017. Co-application of 6-ketone type brassinosteroid and metal chelator alleviates cadmium toxicity in B. juncea L. Environmental Science and Pollution Research. 24

Kaushal, J. and P. Mahajan. 2022. Kinetic Evaluation for Removal of an Anionic Diazo Direct Red 28 by Using Phytoremediation Potential of Salvinia molesta Mitchell. Bulletin of Environmental Contamination and Toxicology. 108:437-442.

Khalid, S., M. Shahid, N.K. Niazi, B. Murtaza, I. Bibi and C. Dumat. 2017. A comparison of technologies for remediation of heavy metal contaminated soils. Journal of Geochemical Exploration. 182:247-268.

Kodituwakku, K.A.R.K. and M. Yatawara. 2020. Phytoremediation of Industrial Sewage Sludge with Eichhornia crassipes, Salvinia molesta and Pistia stratiotes in Batch Fed Free Water Flow Constructed Wetlands. Bulletin of Environmental Contamination and Toxicology. 104:627-633.

Krishna, B.R., S. Bhuvaneshwari, F. Majeed, M. M, E. Jose and A. Mohan. 2022. Different treatment methodologies and reactors employed for dairy effluent treatment - A review. Journal of Water Process Engineering. 46:102622.

Ma, X., H. Yang, Y. Bu, Y. Zhang, N. Sun, X. Wu and Y. Jing. 2023. Genome-wide identification of the NRAMP gene family in Populus trichocarpa and their function as heavy metal transporters. Ecotoxicology and Environmental Safety. 261:115110.

Mayonde, S., G.V. Cron, K.L. Glennon and M.J. Byrne. 2021. Effects of cadmium toxicity on the physiology and growth of a halophytic plant, Tamarix usneoides (E. Mey. ex Bunge). International Journal of Phytoremediation. 23:130-138.

Miller, A.J., X. Fan, M. Orsel, S.J. Smith and D.M. Wells. 2007. Nitrate transport and signalling. Journal of Experimental Botany. 58:2297-2306.

Müller, B. 2023. Iron transport mechanisms and their evolution focusing on chloroplasts. Journal of Plant Physiology. 288:154059.

Nag, R. and E. Cummins. 2022. Human health risk assessment of lead (Pb) through the environmental-food pathway. Science of The Total Environment. 810:151168.

Nahar, K. and S. Hoque. 2021. Phytoremediation to improve eutrophic ecosystem by the floating aquatic macrophyte, water lettuce (Pistia stratiotes L.) at lab scale. The Egyptian Journal of Aquatic Research. 47

Năstăsescu, V., M. Magdalena, M. Goumenou, D. Anca Oana, E. Renieri, D. Udeanu, E. Oprea, A. Arsene, C. Dinu-Pîrvu and M. Ghica. 2020. Heavy metal and pesticide levels in dairy products: Evaluation of human health risk. Food and Chemical Toxicology. 146:111844.

Palmgren, M.G. and K.B. Axelsen. 1998. Evolution of P-type ATPases. Biochimica et Biophysica Acta (BBA) - Bioenergetics. 1365:37-45.

Pasricha, S., V. Mathur, A. Garg, S. Lenka, K. Verma and S. Agarwal. 2021. Molecular mechanisms underlying heavy metal uptake, translocation and tolerance in hyperaccumulators-an analysis: Heavy metal tolerance in hyperaccumulators. Environmental Challenges. 4:100197.

Perveen, S. and H. Amar Ul. 2023. Drinking water quality monitoring, assessment and management in Pakistan: A review. Heliyon. 9

Puig, S. and L. Peñarrubia. 2009. Placing metal micronutrients in context: transport and distribution in plants. Current Opinion in Plant Biology. 12:299-306.

Puigdomenech, I. 2018. HYDRA (Hydrochemical Equilibrium- Constant Database) and MEDUSA (Make Equilibrium Diagrams Using Sophisticated Algorithms) Programs. Royal Institute of Technology, Sweden.

Qasim, W. and A.V. Mane. 2013. Characterization and treatment of selected food industrial effluents by coagulation and adsorption techniques. Water Resources and Industry. 4:1-12.

Rengel, Z., Cakmak, I., White, P.J. 2022. Marschner's Mineral Nutrition of Plants. London: Academic Press.

Ribeiro, C., A. Almeida and C. Couto. 2022. The Aquatic macrophytes as bioindicators of heavy metals contamination in estuarine ecosystems. Scientific Letters. 1:5.

Rodrigues, A., A. Santos, F. Santos, A. Pereira and N. Sobrinho. 2016. Response Mechanisms of Plants to Heavy Metal Pollution: Possibility of Using Macrophytes for Remediation of Contaminated Aquatic Environments. Revista Virtual de Química. 8

Russ, J., E. Zaveri, S. Desbureaux, R. Damania and A.-S. Rodella. 2022. The impact of water quality on GDP growth: Evidence from around the world. Water Security. 17:100130.

Saúde, B.M.d. 2021. Portaria Nº 888, de 4 de maio de 2021, Brasília, Brasil.

Selvaraj, D. and G. Velvizhi. 2021. Sustainable ecological engineering systems for the treatment of domestic wastewater using emerging, floating and submerged macrophytes. Journal of Environmental Management. 286:112253.

Shi, J., X. Wang and E. Wang. 2023. Mycorrhizal symbiosis in plant growth and stress adaptation: From genes to ecosystems. Annual Review of Plant Biology. 74:569-607.

Shuting, Z., D. Hongwei, M. Qing, H. Rui, T. Huarong and Y. Lianyu. 2022. Identification and expression analysis of the ZRT, IRT-like protein (ZIP) gene family in Camellia sinensis (L.) O. Kuntze. Plant Physiology and Biochemistry. 172:87-100.

Singh, B.S.M., D. Singh and N. Dhal. 2021. Enhanced phytoremediation strategy for sustainable management of heavy metals and radionuclides, p 100176.

Tabelini, D.B., J.P.P. Lima, A.C. Borges and A. Aguiar. 2023. A review on the characteristics and methods of dairy industry wastewater treatment in the state of Minas Gerais, Brazil. Journal of Water Process Engineering. 53:103779.

Taiz, L. et al. 2017. Plant physiology and development. Artmed, Porto Alegre, Brasil. 858 p.

Team, RC 2021. R: A language and environment for statistical computing Ed. R.F.f.S. Computing, Vienna, Austria.

Tedesco, M. J., C. Gianello, C.A. Bissani, H. Bohnem and S.J. Volkweiss. 1995. Análises
de solo, plantas e outros materiais. Universidade Federal do Rio Grande do Sul Porto
Alegre. 175 p.

Wan, X.-M., M. Lei and T. Chen. 2016. Cost–benefit calculation of phytoremediation technology for heavy-metal-contaminated soil. Science of The Total Environment. 563

Wang, X., C. Feng, L. Tian, C. Hou, W. Tian, B. Hu, Q. Zhang, Z. Ren, Q. Niu, J. Song, D. Kong, L. Liu, Y. He, L. Ma, C. Chu, S. Luan and L. Li. 2021. A transceptor–channel complex couples nitrate sensing to calcium signaling in Arabidopsis. Molecular Plant. 14:774-786.

Welz, B. and M. Sperling. 1999. Atomic absorption spectrometry. Wiley-VCH, Weinheim. 941 p.

Williams, L.E., J.K. Pittman and J.L. Hall. 2000. Emerging mechanisms for heavy metal transport in plants. Biochimica et Biophysica Acta (BBA) - Biomembranes. 1465:104-126.

Wu, D., M.A.-O. Saleem, T. He and G. He. 2021. The Mechanism of Metal Homeostasis in Plants: A New View on the Synergistic Regulation Pathway of Membrane Proteins, Lipids and Metal Ions. Membranes. 11

Xia, P., L. Ma, Y. Yi and T. Lin. 2021. Assessment of heavy metal pollution and exposure risk for migratory birds- A case study of Caohai wetland in Guizhou Plateau (China). Environmental Pollution. 275:116564.

Yan, A., Y. Wang, S.N. Tan, M.L. Mohd Yusof, S. Ghosh and Z. Chen. 2020. Phytoremediation: A Promising Approach for Revegetation of Heavy Metal-Polluted Land. Front Plant Sci. 11:359.

Zhang, Z., X. Chen, X. Qin, C. Xu and X. Yan. 2023. Effects of Soil pH on the Growth and Cadmium Accumulation in Polygonum hydropiper (L.) in Low and Moderately Cadmium-Contaminated Paddy Soil. Land. 12

Zhao, P., M. Ma, Y. Hu, W. Wu and J. Xiao. 2022. Comparison of international standards for irrigation with reclaimed water. Agricultural Water Management. 274:107974.

Zhu, Y., Y. Wang, X. He, B. Li and S. Du. 2023. Plant growth-promoting rhizobacteria: A good companion for heavy metal phytoremediation. Chemosphere. 338:139475.