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METALS BIOACCUMULATION AND TRANSLOCATION IN *Typha domingensis* (SOUTHERN CATTAIL) EXPOSED TO A REAL DOMESTIC EFFLUENT IN A MESOCOSM FLOATING WETLAND

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Abstract: In this study, we aimed to quantify metals bioaccumulation in *Typha domingensis* exposed to urban wastewater in a mesocosm floating wetland and to investigate their translocation from roots to leaves' base and apex. Macrophytes were exposed to three batches of raw wastewater for 27 days. Six plants were selected for analysis, resulting in 18 tissue samples (6 roots, 6 leaf base, and 6 leaf apex). We measured cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), and zinc (Zn) concentration and compared them among the different parts of the plant. Biomass gain and root length were also measured. The results showed only Cu and Zn concentrations above the detection limit, presenting higher loads in roots over leaves and no significant difference between leaf base and leaf apex. Both Cu and Zn presented translocation factors below 1 for *leaf (base or apex)/roots*, displaying phytostabilization characterized by poor metal translocation. Overall, most plants' roots decreased or remained the same length throughout the experiments, suggesting a negative impact posed by the wastewater and metals entrapment in the roots. Our research confirms *T. domingensis*' potential to bioaccumulate metals, exhibiting phytostabilization and phytoextraction, which allows safe pruning practices and application in ecological restoration practice.

Resumo: Neste estudo, nosso objetivo foi quantificar a bioacumulação de metais em *Typha domingensis* exposta a um efluente urbano em um *wetland* flutuante em mesocosmos e investigar a translocação desses metais das raízes para a base e o ápice das folhas. As macrófitas foram expostas a três lotes de águas residuais brutas por 27 dias. Seis plantas foram selecionadas para análise, resultando em 18 amostras de tecido (6 raízes, 6 bases de folhas e 6 ápices de folhas). Medimos as concentrações de cádmio (Cd), cromo (Cr), cobre (Cu), chumbo (Pb) e zinco (Zn) e as comparamos entre as diferentes partes da planta. O ganho de biomassa e o comprimento da raiz também foram avaliados. Os resultados mostraram apenas concentrações de Cu e Zn acima do limite de detecção, apresentando cargas mais altas nas raízes do que nas folhas e nenhuma diferença significativa entre a base e o ápice das folhas. Tanto Cu quanto Zn apresentaram fatores de translocação abaixo de 1 para folhas (base ou ápice)/raízes, mostrando fitoestabilização caracterizada pela translocação deficiente de metais. De modo geral, as raízes da maioria das plantas diminuíram ou permaneceram com o mesmo comprimento durante os experimentos, sugerindo um impacto negativo causado pelas águas residuais e pelo aprisionamento de metais nas raízes. Nossa pesquisa confirma o potencial da *T. domingensis* para bioacumular metais, exibindo fitoestabilização e fitoextração, o que permite práticas de poda seguras e aplicação em práticas de restauração ecológica.

Palavras-Chave – Macrófitas, Fitorremediação, Tratamento de águas residuais

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INTRODUCTION

Contaminants posing a great threat to aquatic environments majorly derive from residual waters, agricultural, and mining sites [Zhang *et al.* (2018)]. Among these pollutants, metals are particularly dangerous because of their non-degradable nature [Muhammad *et al.* (2009)]; bioaccumulation in the environment; and potential harm to aquatic organisms chronically or acutely [Gall *et al.* (2015)]. Most conventional techniques relying on chemical and physical steps are yet costly and environmentally unsafe [Martín-Lara *et al.* (2014)], which requires eco-technologies advancement. Thus, effective, economically, and ecologically attainable treatments are desirable to prevent metals from entering water bodies [Shahid *et al.* (2018)].

The phytoremediation method has been widely explored in wetlands to remove, reduce, or immobilize metals [Vymazal and Brazinová (2016)]; an affordable technique suitable especially in developing countries or economically disadvantaged regions [Compaore *et al.* (2020)]. The system applies fast-growing macrophyte species with sharp bioaccumulation aptitude to reduce pollution [Compaore (2020)]. Biological features that enable these plants to rapidly expand biomass and persist in harsh environments play an important role in diminishing contaminants from effluents as their uptake occurs more efficiently [Liu *et al.* (2016)]. Hence, one frequent choice of macrophyte to investigate phytoremediation potential has been *Typha domingensis*, an emergent macrophyte with its proven ability to survive in contaminated medium and to perform bioaccumulation [Al-Abbawy *et al.* (2021)].

Typha domingensis has been extensively studied for the past decade, including bioremediation of metals in contaminated natural environments [Osma *et al.* (2014); Bonanno and Cirelli (2017); Bonanno *et al.* (2017); Bonanno and Vymazal (2017); Bonanno *et al.* (2018); Saleh Muneera *et al.* (2019); Viana *et al.* (2021)], and constructed treatment wetlands [Mufarrege *et al.* (2014); Mufarrege *et al.* (2015); Hadad *et al.* (2018); Hadad *et al.* (2020); Compaore *et al.* (2020); Maine *et al.* (2021); Mufarrege *et al.* (2021)]. Nonetheless, experiments with floating treatment wetlands (FTW) using *T. domingensis* to bioremediate metals with real wastewater [Bauer *et al.* (2021)] are still incipient; although the ones using synthetic solutions are recently expanding [Soudani *et al.* (2022)]. Hence, our study goals are (1) to quantify metals bioaccumulated hydroponically by *T. domingensis* exposed to raw urban wastewater and (2) to investigate their translocation among roots, leaf base, and leaf apex. Metals analyzed were cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), and zinc (Zn), a group considered harmful in the aquatic ecosystem in high concentrations [Kahlon *et al.* (2018)]. Our results might enlighten possible green purposes for the macrophytes harvesting biomass, and for other purposes such as recreational, household, fodder, fertilizers, or mulch [Baudhdh *et al.* (2017)].

METHODOLOGY

Study site

The macrophytes tested were collected in a green area at the Federal University of Rio Grande do Sul (Vale Campus) in November 2018. The floating structure was filled with the university's own raw wastewater three times with a hydraulic retention time (HRT) of nine days. The macrophyte units were exposed for a total of 27 days. Effluent's composition was similar to urban residual waters and metals were measured [Bauer *et al.* (2021)] considering possible chemical discharges from the university laboratories.

***Typha domingensis* sampling and preparation process**

After the overall experiment duration, six macrophytes were selected and divided into root, leaf base (15 cm), and leaf apex (15 cm), summing 18 samples. Every sample was cleaned with distilled water to remove any extra organic material attached to the roots and leaves. Different tissue parts of each macrophyte were reserved in clean paper bags to be dehydrated at 60°C for 96 hours. After dehydration, samples were manually ground to a fine powder using a Willey-type knife mill (MA 048 model, Marconi), being submitted to further dehydration for 24 hours in an industrial oven to remove any liquid mass left in the organic material.

Analytical methods for metal concentration

Metal concentration was determined through atomic absorption spectrophotometer according to the United States Environmental Protection Agency 3052 (US EPA 3052, 1996). Tissue samples were weighed (0.5 g of dry material) in an analytical weighing balance (Sartorius BP 210 S) and saved in Teflon tubes. The plant parts (roots, leaf base, and leaf apex) were digested with 4 mL of distilled water and 3 mL of nitric acid (HNO₃) in the digester (model CEM II MARS6), where the samples were submitted to a temperature of 190°C for 20 min and cooled down for 15 min. Subsequently to the digestion process, the samples were filtered on filter paper, transferred to 50 mL volumetric flasks, and diluted with mili Q water. Metal concentration was quantified according to the Flame Atomic Absorption method (FAAS - Flame Atomic Absorption Spectrometry). The results were processed through the Perkin-Elmer software.

Metal bioaccumulation in macrophyte samples and Translocation Factor (TF)

Each metal concentration in the samples was determined based on the following expression:

$$Result = \frac{(SM - B) * VF * DF}{M} \quad (1)$$

SM: reading measurement signal (µg/L);

B: reading measurement signal of white solution (µg/L);

VF: volumetric flask (L);

DF: samples dilution factor (calculated by the ratio between the volumetric flask volume and the sample volume, in the case of total metals DF=1, since the flask volume was 50 mL and the sample volume is 50 mL);

M: mass (g). Detection limits in macrophyte tissues for Cd, Cr, Cu, Pb, and Zn were respectively 0.060 µg/g, 1.00 µg/g, 0.600 µg/g, 0.400 µg/g, and 1.700 µg/g.

The macrophyte's capability to translocate heavy metals throughout its system, from roots to leaf parts, was estimated by the equation:

$$TF: Cleaf (base or apex) / Croot \quad (2)$$

Leaf (base or apex) means the metal concentration found in the plant's leaf base or leaf apex; and *Root* means the metal concentration detected in roots [Padmavathiamma and Li (2007)]. Values above 1 indicate efficient translocation of the metal from the roots to the aerial part [Pandey *et al.* (2019)], while values below 1 suggest deficient translocation to the leaves and its retention by the roots.

Statistical analysis

Statistical analysis confirmed if metal concentrations in each part of the macrophyte (root, leaf base, and leaf apex) were significantly different ($p < 0.05$). All data were checked for normality through the Shapiro-Wilk test and for homogeneity of variances with Bartlett test. Although both metals (Cu and Zn) data set presented normal distribution, only Cu showed homogeneity. Thus, for this element, a One-Way ANOVA was performed followed by Tukey's test; and for Zn, the non-parametric Kruskal-Wallis test was applied followed by the Pairwise Mann-Whitney-Wilcoxon test. All statistical analyses were performed in R Studio, as the data graphical visualization.

RESULTS

Metal bioaccumulation and translocation factor

From the five metals analyzed, Cu and Zn were the only metals found above the detection limit in *T. domingensis* tissues. Copper and Zn found in the roots exceeded significantly ($p = 0.003$) the concentrations in the plant's aerial parts (leaf base and leaf apex). Copper concentration found in macrophyte's parts was: $16.67 \pm 3.29 \mu\text{g/g}$ (43.5%) in roots; $10.83 \pm 1.66 \mu\text{g/g}$ in leaf base (28.2%); and $10.84 \pm 2.51 \mu\text{g/g}$ in leaf apex (28.3%). For Zn, the metal distribution was: $116.54 \mu\text{g/g} \pm 33.18$ in roots (63.4%); $38.48 \pm 13.20 \mu\text{g/g}$ in the leaf base (20.9%); and $28.86 \pm 4.32 \mu\text{g/g}$ in the leaf apex (15.7%).

Figure 1. Copper (a) and Zinc (b) concentration among roots, leaf base, and leaf apex. Significant differences were found between roots and leaves ($p = 0.003$), but not between leaf base and leaf apex ($p > 0.05$)

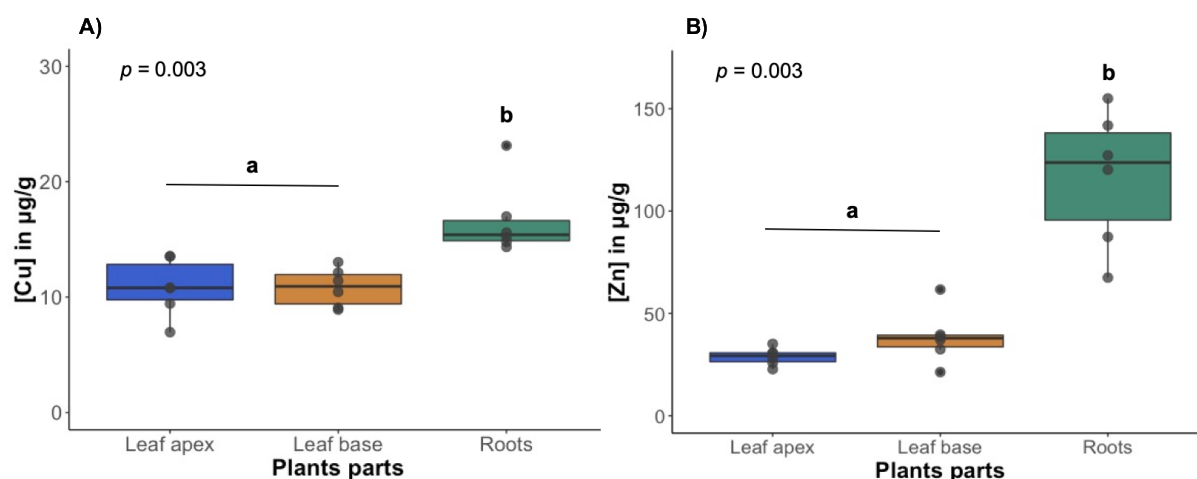


Table 1. Copper and zinc concentrations present in each plant sample (root, leaf base, and leaf apex) of the six macrophytes analyzed and the mean metal concentration and standard deviation (SD).

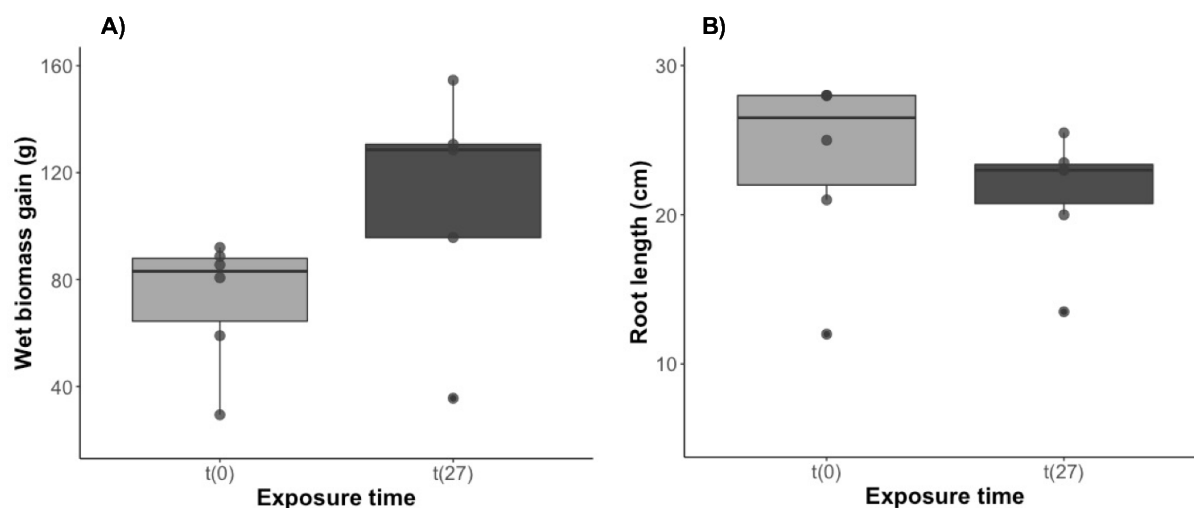
Sample	Metal	Root ($\mu\text{g/g}$)	Leaf base ($\mu\text{g/g}$)	Leaf apex ($\mu\text{g/g}$)
1	Cu	14.77	9.06	9.43
2	Cu	15.60	11.42	10.82
3	Cu	16.98	8.90	6.96
4	Cu	14.34	13.03	13.51
5	Cu	23.13	10.44	13.51
6	Cu	15.20	12.13	10.77
	Mean	16.67	10.83	10.84
	SD	3.29	1.66	2.51
1	Zn	87.40	32.49	30.79
2	Zn	155.05	37.15	35.15
3	Zn	67.70	61.70	30.63
4	Zn	141.85	21.33	22.76
5	Zn	120.22	38.63	27.98
6	Zn	127.24	39.60	25.85
	Mean	116.54	38.48	28.86
	SD	33.18	13.20	4.32

Metal concentration comparing leaf base and leaf apex was not statistically different for Cu ($p = 1.000$) or Zn ($p = 0.093$), although leaf apex presented slightly lower metal bioaccumulation than leaf base for both metals. Translocation factors for Cu considering leaf base/root and leaf apex/root were 0.67 and 0.66, respectively; and for Zn, 0.38 and 0.27, showing entrapment of Cu and Zn in the belowground organ.

Wet biomass gain and root development

Macrophytes samples were weighted and then pruned by the end of each HRT. Overall, macrophyte samples presented an average wet biomass gain of 45,81 g, ranging from 72.55 g to 118.36 g after 27 days exposure period. On the contrary, root length showed an average decrease of -2.25 cm, ranging from 23.6 cm at the beginning of the study to 21.4 cm at the end.

Figure 2. Average wet biomass gain (a) and root length (b) at the beginning (t0) and the end (t27) of the experiment).



DISCUSSION

Among the five trace metals quantified in macrophyte tissues, only Cu and Zn exceeded detection limits, which correlates to their crucial role as micronutrients for the plant's physiologic functions and development [Kabata-Pendias (2011)], contrary to Cd, Cr, and Pb. Bonanno *et al.* (2018) also found the micronutrients Cu, Zn, and Mg in higher concentrations than non-essential components, since plants facilitate their uptake and resist absorbing and storing other elements [Kabata-Pendias (2011)]. Copper and Zn are both essential to macrophyte's nutrition in small loads, as they participate in plant reproduction, and compose enzymes [Laghlimi *et al.* (2015)].

Zinc detected in the wastewater presented a slight reduction (although non-significant), but Cu was undetected throughout the experiments [Bauer *et al.* (2021)], suggesting a Cu concentration only detectable in the macrophytes biomass. Hadad and collaborators (2010) described a similar situation in their study, which found Zn in the plant's tissue, although absent in the effluent pre and post-treatment. Although Cu was undetected in the wastewater, this element load in *T. domingensis* roots and leaves (16.6 $\mu\text{g/g}$ and 10.8 $\mu\text{g/g}$, respectively) exceeded the determined levels for unpolluted environments ($[\text{Cu}] < 8.4 \mu\text{g/g}$) [Kabata-Pendias (2011)], as expected.

Typha domingensis efficiently compartmentalized both micronutrients in roots with poor translocation to the aerial parts ($\text{TF} < 1$). The majority of data available regarding Cu bioaccumulation by the genus typha agrees with our findings (e.g. Bonanno *et al.* (2017, 2018); Bonanno and Vymazal (2017); Bonanno and Cirelli (2017)). This element concentration in all macrophyte parts was lower than the range considered toxic for plants (25 - 40 $\mu\text{g/g}$) [Chaney (1989)], allowing the plant to take advantage of this micronutrient. This reflects in the higher TF found for Cu compared to Zn, since Cu was intensively required as a micronutrient by the aerial parts due to its crucial role in macrophytes photosynthesis [Memon *et al.* (2017)] by both leaf base and apex.

Regarding Zn, we also detected a great load restricted to the root system and scarce translocation to leaves, a result corroborated by many authors [Hadad *et al.* (2010); Osma *et al.* (2014); Mufarrege *et al.* (2015); Bonanno and Cirelli (2017); Bonanno and Vymazal (2017); Bonanno *et al.* (2018); Haddad *et al.* (2018); Maine *et al.* (2021); Soudani *et al.* (2022)]. Zinc compartmentalization in roots suggests a defense mechanism to protect plant parts responsible for

vital functions associated with metabolism, as argued for a range of contaminants [Bonanno *et al.* (2017); Hadad *et al.* (2018)]. As claimed by Borkert *et al.* (1998), for Zn to be considered toxic, its bioaccumulation on plant tissue must be above 0.230 mg/g dry mass, a concentration higher than the one found in this study, even in the roots ([Zn] = 0.116 mg/g). Hence, probably Zn alone did not pose severe harm to the macrophytes, although *T. domingensis* still condensed most of this micronutrient in its radicular system.

Visual and measured impacts were still observed on root length throughout experiments. Growing hydroponically, macrophyte roots became more susceptible to the negative impacts of contaminants, reducing their tolerance compared to plants fixed on sediment [Mufarrege *et al.* (2014)]. Phytostabilization of Cu and Zn might have negatively impacted roots length growth along with the high nutrient concentration detected in the raw wastewater [Bauer *et al.* (2021)]. Literature also discusses that roots diameter can suffer variation to fulfill macrophyte's requirements to control contaminants uptake from water [Maine *et al.* (2021)]. In addition, for other metals such as Cd, *T. domingensis* might attempt to control negative effects by enlarging roots aerenchyma and cortical cells [Oliveira *et al.* (2022)]. Although not properly measured in the present study, we verified visually an increase of lateral roots throughout experiments that could be related to macrophytes' response to wastewater's holistic hazardous impacts.

The concentration of micronutrients encountered in *T. domingensis* reflex its potential for both phytostabilization and phytoextraction [Hadad *et al.* (2018)]. Ecological management actions require knowledge about trace metals behavior in macrophytes used as phytoremediators [Bonnano and Vymazal (2017)], which is crucial to maintaining a healthy function and proper stability of the system. As most researchers analyze the role of sediment together with emergent macrophytes, investigation of the adsorbent and uptake in floating treatment wetlands could be supplemented.

CONCLUSION

The research confirms the *T. domingensis* potential to bioaccumulate metals, displaying both mechanisms of phytostabilization and phytoextraction. Among all metals analyzed, only Cu and Zn were detected on macrophytes tissues, both inefficiently translocated to the aerial parts. Similar to the majority of data in the literature, Zn concentration was extremely higher than Cu within the plant's biomass and it was more restricted to the root system, while Cu presented higher translocation to leaves, which might be due to its role in photosynthesis. Further research on root development hydroponically is encouraged and might provide ecological restoration insights considering *T. domingensis* response to different levels of metals.

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