Chapter 243

Bioaccumulation and translocation of metals in *Typha domingensis* (southern cattail) exposed to wastewater in a mesocosm floating wetland

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1 INTRODUCTION

Contaminants posing a great threat to aquatic environments majorly derive from residual waters, agricultural, and mining sites (Hussain et al. 2017; 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 throughout the trophic chain; 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 (Olguín and Sánchez-Galván 2012; Martín-Lara et al. 2014), which requires eco-technologies advancement (Thani et al. 2019). Thus, effective, economically, and ecologically attainable treatments are desirable to prevent metals from entering water bodies (Shahid et al. 2018), which can deplete water quality and threat aquatic ecosystems' health (Mânzatu et al. 2015; Wacławek et al. 2017).

The phytoremediation method has been widely explored in wetlands to remove, reduce, or immobilize metals (Marchand et al. 2010; Soda et al. 2012; Vymazal and Březinová 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 et al. 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 (Mukhtar and Abdullahi 2017; Al-Abbawy et al. 2021; Eid et al. 2020).

Typha domingensis has been extensively studied for the past decade, including bioremediation of metals in contaminated natural environments (Adams et al. 2013; Osma et al. 2014; Bonanno and Cirelli 2017; Bonanno et al. 2017; Bonanno and Vymazal 2017; Mukhtar and Abdullahi 2017; Bonanno et al. 2018; Dube et al. 2019; Saleh Muneera et al. 2019; Viana et al. 2021), and constructed treatment wetlands (Mojiri 2012; Mufarrege et al. 2014, 2015; Hadad et al. 2018; 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 (Oliveira et al. 2018, 2022; 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 metal translocation among roots, leaf base, and leaf apex. Metals analyzed include cadmium (Cd), chromo (Cr), copper (Cu), lead (Pb), and zinc (Zn), a group of metals considered harmful in the aquatic ecosystem in high concentrations (Geist and Hawkins 2016; Kahlon et al. 2018). Our results might enlighten possible green purposes for the macrophytes harvesting biomass (Kushwaha et al. 2015) and for several other purposes such as recreational, household, fodder, fertilizers, or mulch (Bauddh et al. 2017).

2 MATERIAL AND METHODS

Study site

The macrophytes tested here were used in the study carried out by Bauer et al. (2021) assessing the efficiency of a mesocosm in FTW. The *T. domingensis* samples collected for Bauer's study were located in a green area at the same university campus in November 2018. The floating structure was filled with raw wastewater from the Federal University of Rio Grande do Sul (Vale Campus) three times with a hydraulic retention time (HRT) of nine days, in each experiment. The macrophyte units were submitted to the three experiments without replacement, totalizing 27 days of exposure. 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. The 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

Zinc concentration was measured through the flame atomic absorption spectrometry (FLAA) technique, using the spectrometer Perkin Elmer (model 3300), with the results processed through the Perkin-Elmer software. For the other metals (Cd, Cr, Cu, and Pb), the graphite furnace atomic absorption spectrometry (GFAA) technique was used (USEPA 200.7/2001). Tissue samples were weighed (0.50 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 (HNO_3) 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 (US EPA 3052 1996). 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 bioaccumulation in macrophyte samples and Translocation Factor (TF)

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

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

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 for Cd, Cr, Cu, Pb, and Zn in macrophyte tissues were respectively 0.060 µg/g, 0.500 µg/g, 0.600 µg/g, 0.400 µg/g, and 1.700 µg/g.

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

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

Cleaf (base or apex) means the metal concentration found in the plant's leaf base or leaf apex; and *Croot* 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 TF values below 1 suggest deficient translocation to the leaves and its retention by the roots.

Statistical analysis

Statistical analysis verified if metal concentrations in each part of the macrophyte (root, leaf base, and leaf apex) were significative different ($p < 0.05$). All data were checked for normality through the Shapiro-Wilk test and for homogeneity of variances with the Bartllet 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 and descriptive graphical visualization (ggplot2 package) were performed in R Studio.

3 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) (**Fig. 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 ($p > 0.05$)). Copper concentration found in macrophyte's parts was: 16.67 ± 3.29 µg/g in roots; 10.83 ± 1.66 µg/g in leaf base; and 10.84 ± 2.51 µg/g in leaf apex. For Zn, the metal distribution was: 116.54 µg/g ± 33.18 in roots; 38.48 ± 13.20 µg/g in the leaf base; and 28.86 ± 4.32 µg/g in the leaf apex (**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)). 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. Considering leaf base/root and leaf apex/root, TFs for Cu 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

Wet biomass and root growth measurements are described by Bauer et al. (2021). Macrophytes samples were weighted and then pruned by the end of each HRT. Overall, macrophyte samples selected for this study 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 (**Fig. 2.** Average wet biomass gain (A) and root length (B) at the beginning (t0) and the end (t27) of the experiment).

Fig. 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 ($p > 0.05$)

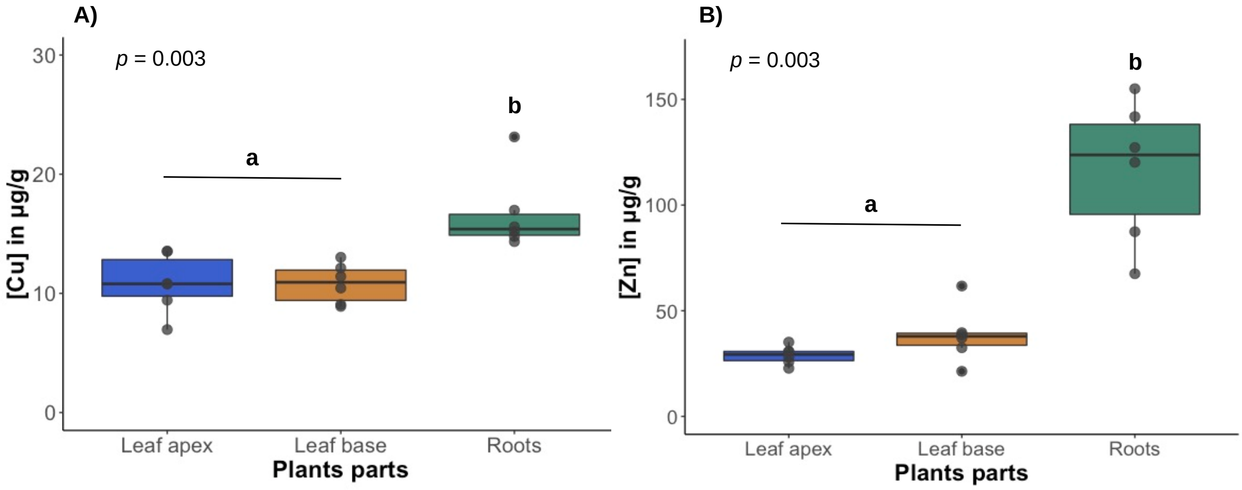
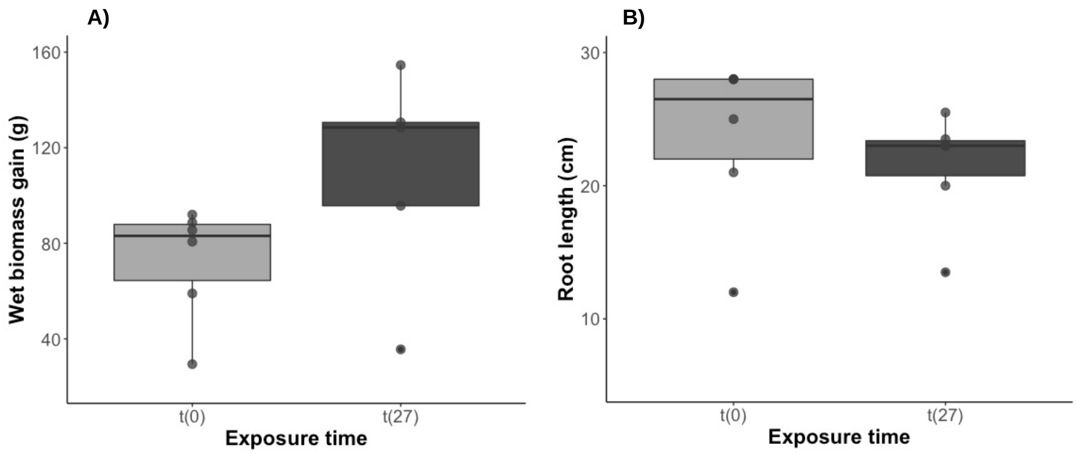


Table 1. Copper and zinc concentrations present in each plant sample (root, leaf base, and leaf apex) of the six macrophytes analyzed and the mean metal concentration and standard deviation (SD).

Sample	Metal	Root (µg/g)	Leaf base (µg/g)	Leaf apex (µg/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

Fig. 2. Average wet biomass gain (A) and root length (B) at the beginning (t0) and the end (t27) of the experiment.



4 DISCUSSION

Among the five 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 concentrations (Fairbrother et al. 2007), as they participate in plant reproduction, and compose enzymes (Laghlimi et al. 2015). The former is involved in photosynthesis and a range of physiological processes (Laghlimi et al. 2015); while the latter forms proteins (Marschner 2012) and cell membranes; and participates in DNA transcription, prevention of stress (Laghlimi et al. 2015), and metabolic activities (Bonanno 2013).

Zinc detected in the wastewater presented a slight reduction (although non-significant) after macrophytes treatment, 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 high Cu concentration was undetected in the wastewater, this element load in *T. domingensis* roots and leaves (16.6 µg/g and 10.8 µg/g, respectively) exceeded the determined levels for unpolluted environments ([Cu] < 8.4 µg/g) (Kabata-Pendias 2011), as expected.

Typha domingensis efficiently compartmentalized both micronutrients in roots with poor translocation to the aerial parts (TF < 1). The majority of data available regarding Cu bioaccumulation by the genus typha agrees with our findings (e.g. Phillips et al. 2015; Bonanno 2013; Bonanno et al. 2017, 2018; Bonanno and Vymazal 2017; Bonanno and Cirelli 2017; Dube et al. 2019). However, this element concentration in all macrophyte parts was lower than the range considered toxic for plants (25 - 40 µg/g) (Chaney 1989), allowing the plant to take advantage of this micronutrient for biomass gain. 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. Because our experiment was performed during summer, the photosynthetic activity probably demanded more Cu as well.

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; Adams et al. 2013; 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 referred for a range of contaminants (Bonanno et al. 2017; Hadad et al. 2018; Oliveira et al. 2018). Toxic levels of metals might diminish the plant's capability to produce chloroplasts and essential proteins (Al-Janabi et al. 2020), impacting photosynthetic activity. 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 concentrate most of this micronutrient in its radicular system.

Zinc presence majorly in macrophyte's roots as well as under its toxic level was also identified for *T. domingensis* in previous studies. Bonanno and Cirelli (2017) found a similar result with this plant species growing naturally in a wetland receiving residual waters. Zinc concentration in root and leaf were respectively 118 μg/g (0.118 mg/g) and 38.8 μg/g (0.038 mg/g) in Spring, and 122 μg/g and 35.4 μg/g in Autumn; in both seasons TFleaf/root were above 1 (0.33 and 0.29, respectively). Another study from Bonanno et al. (2018) found 0.104 mg/g of Zn in roots and 0.065 mg/g in leaves of *T. domingensis* with a TFleaf/root = 0.66. The same pattern can be observed for the macrophyte in question sampled at the two sites Acquicella and Morello investigated by Bonanno and Vymazal (2017).

Detrimental impacts were 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 (Barceló and Poschenrieder 1990) along with the high nutrient concentration detected in the raw wastewater (Bauer et al. 2021). Root plasticity is an important morphological process though to adjust metals and nutrient absorption, keeping *T. domingensis* less susceptible to their influences (Wahl et al. 2001; Hadad et al. 2010; Oliveira et al. 2022). The literature discusses that root diameter can suffer variation to fulfill macrophyte's requirements to control contaminants' uptake from water (Maine et al. 2021). For instance, for metals such as Cd, *and T. domingensis* might attempt to control negative effects by enlarging roots aerenchyma and cortical cells (Oliveira et al. 2022). We visually verified an increase of lateral roots throughout experiments that could be related to macrophytes' response to wastewater's holistic hazardous impacts.

The concentration of nutrients encountered in *T. domingensis* reflexes its potential for both phytostabilization and phytoextraction (Hadad et al. 2018). Ecological management actions require knowledge about 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. Also, root maximum development exposed to a range of different metal concentrations might be further explored to allow scientists and managers to choose the right macrophyte for floating wetlands.

5 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 concentrations of metals.

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