# Morphological Response of Typha domingensis to an Industrial Effluent Containing Heavy Metals in a Constructed Wetland

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Abstract Typha domingensis had become the dominant species after 2 years of operation of a wetland constructed for metallurgical effluent treatment. Therefore, the main purpose of this study was to investigate its ability to tolerate the effluent and to maintain the contaminant removal efficiency of the constructed wetland. Plant, sediment, and water at the inlet and outlet of the constructed wetland and in two natural wetlands were sampled. Metal concentration (Cr, Ni, and Zn) and total phosphorus were significantly higher in tissues of plants growing at the inlet in comparison with those from the outlet and natural wetlands. Even though the chlorophyll concentration was sensitive to effluent toxicity, biomass and plant height at the inlet and outlet were significantly higher than those in the natural wetlands. The highest root and stele cross-sectional areas, number of vessels, and biomass registered in inlet plants promoted the uptake, transport, and accumulation of contaminants in tissues. The modifications recorded accounted for the adaptability of T. domingensis to the conditions prevailing in the constructed wetland, which allowed this plant to become the dominant species and enabled the wetland to maintain a high contaminant retention capacity.

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The construction of artificial wetlands for wastewater treatment has been developing fast over the last decades and currently it represents an accepted and increasingly common treatment alternative. Constructed wetlands were mainly used for nutrient and organic matter retention in domestic and municipal sewage, storm water, and agricultural runoff (Hammer [1989](#page-8-0); Moshiri [1993;](#page-8-0) Kadlec and Knight [1996](#page-8-0); Vymazal et al. [1998](#page-9-0); Kadlec et al. [2000](#page-8-0)). The choice of plants is an important issue in constructed wetlands, as they must survive the potentially toxic effects of the effluent and its variability. Common reeds (Phragmites australis [Cav.] Trin.), cattails (Typha spp.), bulrushes (Scirpus spp.), and reed canary grass (Phalaris arundinacea L.) have been used for both domestic and industrial wastewater treatment (Shepherd et al. [2001;](#page-9-0) Mbuligwe [2005](#page-8-0); Vymazal [2005;](#page-9-0) Vymazal and Krópfelová [2005\)](#page-9-0).

Bahco Argentina S.A., a metallurgic industry, constructed a free water surface wetland to treat the wastewater from the whole factory (sewage and industrial effluents) in Santo Tomé, Santa Fe, Argentina). The effluent presents a high pH and conductivity and contains Cr, Ni, and Zn. The wetland proved to be very efficient in metal retention (Maine et al. [2006](#page-8-0)). An assemblage of locally common macrophytes was transplanted. Eichhornia crassipes (Mart.) Solms. covered most of the surface for almost 1 year, followed by a receding stage that extended more than 6 months. Since then, only the emergent macrophyte Typha domingensis Pers. has been the dominant species.

Typha genus is one of the most widely used plants in constructed wetlands (Gersberg et al. [1986](#page-8-0); Jenssen et al. [1993](#page-8-0); Ellis et al. [1994](#page-8-0); Merlin et al. [2002;](#page-8-0) Hadad et al. [2006](#page-8-0); Wang et al. [2008;](#page-9-0) Maine et al. [2009\)](#page-8-0). Most studies of Typha species are aimed at assessing removal efficiencies, without taking into account its morphological response to

pollutants. Variations in root structure and root diameter are closely associated with ecological requirements of plant species, such as water and nutrient uptake, and may affect the ability of plants to absorb contaminants from water. This is key knowledge not only for understanding macrophyte behavior but also for optimizing effluent depuration using constructed wetlands.

The root anatomy of plants can be affected by different concentrations of nutrients (Ciro et al. [1999](#page-8-0); Wahl and Ryser [2000](#page-9-0); López-Bucio et al. [2003](#page-8-0)) or heavy metals (Kapitonova [2002](#page-8-0); Hadad et al. [2009\)](#page-8-0). Exposure to a high level of nutrients increases root and metaxylem vessel diameters in poplar (Harvey and van den Driessche [1999](#page-8-0)), Poaceae species (Wahl et al. [2001](#page-9-0)), and E. crassipes (Campanella et al. [2005](#page-8-0)). Regarding the effect of heavy metals, Fe, Mn, Ni, Zn, and Cu produced changes in the structure of the epidermis of vegetative plant organs of Typha latifolia L., Alisma plantago-aquatica L., and Lemna minor L. (Kapitonova [2002\)](#page-8-0). Ni and the combination of  $Cr + Ni + Zn$  caused toxicity in *Pistia stratiotes* L. through a decrease in cross-sectional area (CSA) of roots, stele, metaxylem vessels, and total metaxylem vessels (Mufarrege et al. [2009\)](#page-8-0). Other contaminants, such as synthetic dye compounds (Nilratnisakorn et al. [2007\)](#page-8-0) and petroleum hydrocarbons (Merkl et al. [2005](#page-8-0)), can also change the anatomical features of vegetative organs.

Because T. domingensis showed good adaptation to the constructed wetland at Bahco Argentina S.A., we proposed that this species presents a high morphological plasticity to adapt to different environmental conditions at the same time that it maintains the contaminant removal efficiency of the wetland. The main purpose of this study was to investigate the root anatomical changes undergone by T. domingensis to survive under the conditions of this effluent and to maintain the contaminant removal efficiency of the constructed wetland.

### Materials and Methods

## Study Sites

The wetland was constructed on the grounds of Bahco Argentina metallurgic plant, located in Santo Tomé, Santa Fe, Argentina (31 $\degree$ 40' S, 60 $\degree$ 47' W). The free-water surface wetland was 50 m long, 40 m wide, and 0.3–0.6 m deep, with a central baffle. The baffle doubled the flowpath and resulted in a 5:1 length:width ratio (Fig. [1\)](#page-2-0). Design details are given by Maine et al. ([2006\)](#page-8-0). Wastewater discharge was approximately 100  $m<sup>3</sup>$  day<sup>-1</sup> and the hydraulic residence time ranged from 7 to 12 days. Both wastewater from industrial processes and sewage from the factory were

treated together and were released to the wetland following primary treatment.

Sampling occurred twice during the winter of 2007. The registered range of the environmental temperature was between  $-4$  and  $21^{\circ}$ C. *T. domingensis* plants, water, and sediment were sampled at the inlet and outlet of the constructed wetland and in two noncontaminated natural floodplain wetlands near the constructed wetland showing intermittent contact with the Paraná River branch (Pond 1, 31°38′ S, 60°39′ W; Pond 2, 31°31′ S, 60°30′ S), to obtain parameters for comparison. The natural wetlands are characterized by shallow, stagnant water, muddy sediment, and dense monospecific stands of T. domingensis. The plants used in the constructed wetland were collected from these sites.

Environmental and Biological Analyses

#### **Water**

Conductivity was measured in situ using a YSI 33 portable conductivity meter. Dissolved oxygen (DO) and pH were measured in situ with a Horiba OM-14 portable meter and an Orion pH-meter, respectively. Water samples were collected at each point in triplicate; samples were kept at 4-C and later filtered through Millipore membrane filters  $(0.45 \mu m)$  for nutrient determinations. Chemical analysis was performed following the APHA [\(1998](#page-8-0)).  $NO_2^-$  was determined by coupling diazotation followed by a colorimetric technique;  $NH_4^+$  and  $NO_3^-$  were determined by potentiometry (Orion ion-selective electrodes; sensitivity, 0.01 mg  $L^{-1}$  N; reproducibility,  $\pm 2\%$ ). Soluble reactive phosphate (SRP) was determined by the colorimetric molybdenum blue method (Murphy and Riley [1962\)](#page-8-0).  $Ca^{2+}$ was determined by EDTA titration. Total alkalinity was measured by HCl titration.  $SO_4^2$ <sup>-</sup> was assessed by turbidimetry. Chemical oxygen demand (COD) was determined by the open reflux method and biochemical oxygen demand (BOD) by the 5-day BOD test (APHA [1998\)](#page-8-0). Fe, Cr, Ni, and Zn concentrations were determined in water samples by atomic absorption spectrometry (by flame or electrothermal atomization, according to the sample concentration; Perkin Elmer 5000, USA), following the APHA [\(1998](#page-8-0)).

#### Sediment

Triplicate samples were collected to a depth of 10 cm using a 4-cm-diameter PVC corer. Each sample was placed in a plastic bag and stored at 4°C. Total phosphorus (TP) was determined after acid digestion with an  $HClO<sub>4</sub>:HNO<sub>3</sub>:HCl$ (7:5:2) mixture followed by SRP determination in the digested samples (Murphy and Riley [1962](#page-8-0)). Cr, Ni, and Zn

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concentrations were determined in the digests by atomic absorption spectrometry (Perkin Elmer 5000 [APHA [1998\]](#page-8-0)). Certified standard solutions were used. The detection limits were 0.3, 0.6, and 9  $\mu$ g g<sup>-1</sup> for Cr, Ni, and Zn, respectively. These determinations were carried out in triplicate in each sediment replica.

# Macrophytes

Plant height was measured prior to harvesting. To estimate dry biomass, T. domingensis stands were sampled with a  $0.50 \times 0.50$ -m square sampler following Vesk and Allaway [\(1997](#page-9-0)) in the inlet, outlet, and natural wetlands. Four replicates were collected at each sampling location. Plants were harvested, washed, separated into aboveground (shoots and leaves) and belowground (roots and rhizomes) parts, and dried at 105°C until a constant weight was reached (Westlake [1974](#page-9-0); APHA [1998\)](#page-8-0).

For P and metal analyses, plants were thoroughly washed with running tap water and rinsed with deionized water to remove any sediment particles attached to the plant surfaces. The aboveground and belowground tissues were then separated and oven-dried (70°C). Dried plant samples were ground and sieved. Tissue samples were digested and P and metals were determined in the same way as in sediment.

Chlorophyll a concentration was determined in triplicate. Chlorophyll was extracted with acetone for 48 h in the dark at  $3-5$ °C. The transmittance percentage of the extracts was read at 645 and 665 nm using a UV–Vis spectrophotometer following Westlake [\(1974](#page-9-0)).

To describe the internal morphology of roots, 50 plants of T. domingensis were collected randomly from each study site (inlet and outlet of the constructed wetland and in the natural wetlands) during the two sample periods, placed in plastic bags, and immediately taken to the laboratory. A section approximately 30 mm long was cut from the middle of the root and stored in 4% formaldehyde. After 48 h, they were immersed in 70% ethanol for preservation. For anatomical measurements, the main roots were taken at random and cross-sectioned by hand applying the tech-nique proposed by D'Ambrogio de Argüeso [\(1986](#page-8-0)). To distinguish cell walls from the background, the material was stained with aniline blue, which stains cellulose blue. Sections were examined by light microscopy  $(x100$  and  $\times$ 400). One root section from each plant (50 sections) from each sampling site was analyzed. The diameters of root, stele, and metaxylem vessels were measured using a micrometric ocular. The formula to calculate the area of a circle was applied to obtain the values of the CSA of the whole root, stele, and metaxylem vessels (Wahl et al. [2001](#page-9-0)). In addition, the number of metaxylem vessels per section was recorded and the total metaxylem CSA was calculated by adding the areas of all the vessels per section.

## Statistical Analysis

One-factor ANOVA was performed to determine if there were significant differences among the sampling points (inlet and outlet of the constructed and natural wetlands) in plant height, chlorophyll concentration, and aerial and aboveground biomass. To determine if there were significant differences among the sampling points for TP concentrations and metals in plant tissues, a two-factor ANOVA (sampling points and plant tissues) was carried out. Duncan's test was used to differentiate means when appropriate. Normality of residuals was tested graphically. Bartlett's test was used to verify that variances were homogeneous (Walpole et al. [1999](#page-9-0)). Because the root internal morphology parameters (root, stele, metaxylem vessels, and total metaxylem CSAs and number of vessels) did not show a normal distribution, nonparametric tests and box-and-whisker plots were performed using medians as central trend measures and interquartile ranges as measures of variability. Kruskal–Wallis analysis was applied to check the differences in anatomical parameters measured in roots from plants at the inlet versus the outlet of the constructed and natural wetlands. When statistically significant differences were found, Wilcoxon's test was used to compare treatments among themselves. In all comparisons a level of  $\alpha$  < 0.05 was used. Calculations were carried out with the Statgraphics Plus 5.0 program (Manugistics, Inc./JDA, Scottsdale, AZ, USA).

# **Results**

The chemical characterization of influent and effluent of the constructed wetland and the parameters measured in the water of the natural wetlands are reported in Table [1.](#page-4-0) Contaminant concentrations in the influent were highly variable. The efficiency of the system was demonstrated by a decrease in concentration and variability of the parameters of the effluent. Table [2](#page-4-0) reports TP, Cr, Ni, and Zn concentrations in sediment of the constructed and natural wetlands. The inlet sediment showed significantly higher concentrations than the outlet and the natural wetlands. Cr and Ni concentrations in leaves and roots were significantly higher in the inlet plants of the constructed wetland, in comparison with the concentrations recorded in the plants at the outlet and in the natural wetlands (Fig. [2\)](#page-4-0). At the inlet, Ni presented the lowest concentration in leaves, denoting a limited translocation to leaves. Zn concentrations in roots of the plants growing at the inlet of the constructed wetland were significantly higher than those in roots of the outlet plants. However, Zn concentrations in leaves did not show significant differences between inlet and outlet plants. Although the Zn concentration in the influent and effluent remained below analytical detection limits, Zn tissue concentrations suggest that influent and effluent contained Zn that could only be detected in plants. Zn concentrations in leaves and roots of the plants of the natural wetlands were significantly lower than those recorded in plants of the constructed wetland. The bioaccumulation factor (BF) was calculated as the ratio of metal concentration in roots:sediment. In addition, the translocation factor (TF) was expressed as the ratio of the metal concentration in leaves:roots to show metal translocation properties from roots to shoots. BFs were 0.181, 0.247, and 0.857 and TFs were 0.152, 0.030, and 0.197 for Cr, Ni, and Zn, respectively. TP concentrations in leaves and roots of

the inlet plants were the highest. There were no significant differences in root TP concentrations among plants of the outlet, Pond 1, and Pond 2. However, leaf TP concentration of the outlet plants was significantly higher than that of Pond 1 and Pond 2 plants, indicating higher translocation. In all cases, the TP concentration was significantly higher in leaves than in roots.

There were no significant differences in above- and belowground dry weight and plant height between inlet and outlet plants of the constructed wetland, but these values were significantly higher in comparison with those obtained in the natural wetlands (Fig. [3a](#page-5-0), b). Chlorophyll concentrations in inlet plants were significantly lower than those in outlet plants and in natural wetland plants (Fig. [3c](#page-5-0)). Root, stele, and total metaxylem CSAs of the inlet plants were significantly higher than those of the outlet and natural wetlands plants (Fig. [4a](#page-5-0), b, c, respectively). Root and total metaxylem CSAs (Fig. [4](#page-5-0)a, c, respectively) were not significantly different between plants from the outlet of the constructed wetland and plants from Pond 2. Metaxylem vessel CSA was not significantly different between plants from the inlet and those from the outlet of the constructed wetland (Fig. [5a](#page-6-0)). The highest metaxylem vessel CSA was obtained in Pond 2. The number of vessels was significantly higher in inlet plants compared with those at the outlet and in the natural wetlands (Fig. [5](#page-6-0)b). To show the root structure obtained in each site and to establish comparisons among them, representative light microscopy images of the plants at the inlet and outlet of the constructed and natural wetlands are shown in Fig. [6](#page-7-0).

## Discussion

Metal concentrations in sediment and in plant tissues measured in the inlet of the constructed wetland look comparatively low compared with reported toxicity thresholds in the literature. According to Kabata-Pendias and Pendias ([2000\)](#page-8-0), total metal in sediment in the ranges of 0.070–0.40 mg  $g^{-1}$  dry weight (dw) Zn would be considered toxic to plants and the toxic level of Zn in plants is up to 0.230 mg  $g^{-1}$  dw (Borkert et al. [1998](#page-8-0)). Ellis et al. [\(1994](#page-8-0)) reported one order of magnitude larger Zn concentration in T. *latifolia* roots (0.70 mg  $g^{-1}$  dw) in a natural wetland utilized for stormwater treatment in London compared with what was observed in the roots of inlet plants (Fig. [2c](#page-4-0)). Gibson and Pollard ([1988\)](#page-8-0), Fakayode and Onianwa [\(2002](#page-8-0)), and Cardwell et al. [\(2002](#page-8-0)) observed a positive growth of wetland plants growing in sediments with Zn concentrations of 16, 0.30, and 0.51 mg  $g^{-1}$  dw, respectively. Manios et al. ([2003\)](#page-8-0) compared chlorophyll concentrations in T. latifolia exposed to metal mixtures of

Parameter	Constructed wetland		Natural wetlands	
	Influent	Effluent	Pond 1	Pond 2
pH	8.0(0.8)	7.7(0.2)	7.8(0.1)	7.3(0.1)
Conductivity	3468 (1462)	2175 (194)	185(5)	266(6)
DO $(mg L^{-1})$	0.7(1.5)	0.2(0.3)	5.0(0.2)	8.5(0.3)
BOD (mg $L^{-1}$ )	42.0(16.1)	15.0(4.6)	4.0(1)	3.0(1)
$COD$ (mg $L^{-1}$ )	101(62)	31.0(13.3)	6.0(1)	6.0(1)
SRP (mg $L^{-1}$ )	0.172(0.08)	0.061(0.01)	0.055(0.002)	0.044(0.002)
$NO_3^-$ (mg $L^{-1}$ )	12.0(8.3)	2.5(0.7)	0.45(0.005)	2.0(0.01)
$NO_2^-$ (mg $L^{-1}$ )	0.44(0.64)	0.06(0.05)	0.008(0.001)	0.006(0.001)
$NH_4^+$ (mg $L^{-1}$ )	3.6(2.9)	2.9(1.9)	1.2(0.1)	0.36(0.01)
Alkalinity (mg $L^{-1}$ CaCO <sub>3</sub> )	266 (120)	278 (27)	113.6(0.5)	130.3(0.6)
$SO_4^{2-}$ (mg L <sup>-1</sup> )	1661 (1043)	740 (228)	14.3(0.5)	13.6(0.4)
$Ca^{2+}$ (mg $L^{-1}$ )	182 (118)	88 (25)	11.2(0.2)	10.6(0.1)
Fe (mg $L^{-1}$ )	2.4(0.7)	0.09(0.04)	0.52(0.2)	0.29(0.1)
$Cr (\mu g L^{-1})$	33.0 (23.7)	5.0(1.1)	$ND$ ( $DL = 5$ )	$ND$ ( $DL = 5$ )
Ni (μg L <sup>-1</sup> )	47.0 (41.0)	17.0(5.0)	$ND$ ( $DL = 5$ )	$ND$ ( $DL = 5$ )
$Zn$ (μg $L^{-1}$ )	$ND$ ( $DL = 25$ )	$ND$ ( $DL = 25$ )	$ND$ ( $DL = 25$ )	$ND$ ( $DL = 25$ )

<span id="page-4-0"></span>Table 1 Chemical characterization of the influent and effluent of the T. domingensis-dominated constructed wetland and the natural wetlands: mean (standard deviation)

ND not detected, DL detection level)

Table 2 Total phosphorus (TP), Cr, Ni, and Zn concentrations in inlet and outlet sediment of the constructed wetland and in sediment of the natural wetlands: mean (standard deviation)

Parameter (mg $g^{-1}$ dry weight)	Constructed wetland		Natural wetlands	
	Inlet	Outlet	Pond 1	Pond 2
TP	0.858(0.148)	0.396(0.009)	0.376(0.054)	0.256(0.018)
Cr	0.547(0.051)	0.079(0.052)	0.059(0.013)	0.049(0.011)
Ni	0.401(0.039)	0.079(0.007)	0.056(0.014)	0.052(0.009)
Zn	0.077(0.010)	0.065(0.005)	0.050(0.007)	0.043(0.008)

Fig. 2 a Cr, b Ni, c Zn, and d total phosphorus (TP) concentrations in plant tissues (leaves and roots) of T. domingensis at the inlet and outlet of the constructed wetland and in the natural wetlands. Error bars are ±1 SD



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Fig. 3 a Plant height (cm), b aboveground and belowground dry weight (g m<sup>-2</sup>), and c chlorophyll concentrations (mg g<sup>-1</sup> dw) of T. domingensis at the different sampling sites. Bars represent standard deviations. Different letters represent statistically significant differences (Duncan test)

increasing concentration, reporting threshold damage for a mixture of Cd, Cu, Ni, Pb, and Zn of 4, 80, 40, 40, and 80 mg  $L^{-1}$ , respectively. Zn content in plant tissue was 0.392 mg  $g^{-1}$  dw in roots and 0.061 mg  $g^{-1}$  dw in leaves. Both tissue and water concentrations were much higher than those reported in inlet plants. On the other hand, Ni content in plant tissues amounted to 0.055 mg  $g^{-1}$  dw in

Fig. 4 Box-and-whisker plot of root (a), stele (b), and total metaxylem cross-sectional area (CSA) of T. domingensis roots at the different sampling sites

roots and 0.028 mg  $g^{-1}$  dw in leaves, slightly lower than in inlet plants (Fig. [2](#page-4-0)b). According to the BF and TF, Zn was the metal that presented a similar concentration between roots and sediment, while Cr and Ni presented significant lower concentrations in roots than in sediment. The studied metals showed a low translocation to leaves, Ni being the metal that presented the lowest value.

Even though low biomass production is typically observed in natural wetlands during winter, the T. domingensis plants

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Fig. 5 Box-and-whisker plot of metaxylem vessel cross-sectional area (CSA) (a) and number of metaxylem vessels per section (b) of T. domingensis roots at the different sampling sites

of the constructed wetland were large and showed little senescence. The aerial biomass of the plants of the constructed wetland was significantly higher than that of the plants of the natural wetlands, due to the fact that the effluent was rich in nutrients. With surplus nutrient, plants shift to proportionally higher aboveground growth (Reddy and Sutton [1984;](#page-9-0) Lallana and Kieffer [1988;](#page-8-0) Neiff et al. [2001\)](#page-8-0). Contrary to the increase in biomass, the chlorophyll concentration decreased in inlet plants. The concentration of this pigment is a better indicator of effluent toxicity than the dry biomass. Chaney ([1993\)](#page-8-0), Burton et al. [\(2004](#page-8-0)), Kolotov et al. [\(2003](#page-8-0)), and Hadad et al. [\(2007](#page-8-0)) reported that chlorophyll concentration in plants is a good toxicity indicator for different metals. Nevertheless, the response depends on each macrophyte. Manios et al. ([2003\)](#page-8-0) observed an increase in chlorophyll hydrolysis due to the accumulation of Ni, Zn, Cd, Cu, and Pb in Typha latifolia L. Maine et al. [\(2004](#page-8-0)) reported that the chlorophyll concentration of P. stratiotes decreased at Cr concentrations in water of  $>1$  mg L<sup>-1</sup>, while Salvinia herzogii De la Sota

did not report significant changes in chlorophyll concentrations up to 6 mg Cr  $L^{-1}$ .

There was a higher TP concentration in aboveground parts than in roots, in agreement with other studies of T. domingensis growing in natural and constructed wetlands (Hadad et al. [2006](#page-8-0); Hadad and Maine [2007](#page-8-0)). Macrophyte leaves possess efficient apoplastic nutrient transfer routes to photosynthetic cells (Barnabas [1988\)](#page-8-0). This fact could explain the higher leaf versus roots P accumulation. Metal concentrations were higher in roots than in leaves, in agreement with studies of floating macrophytes (Delgado et al. [1993;](#page-8-0) Sen and Bhattacharyya [1994;](#page-9-0) Maine et al. [2004](#page-8-0); Paris et al. [2005](#page-8-0); Hadad et al. [2007\)](#page-8-0) and the emergent Typha latifolia L. (Manios et al. [2003\)](#page-8-0). Macronutrients such as P are taken up slowly by roots and then translocated to aerial parts, while toxic elements such as Cr, Ni, and Zn are taken up rapidly and retained in the root system (Maine et al.  $2004$ ; Hadad et al.  $2007$ ; Suñé et al.  $2007$ ). It can be seen that Ni, the most toxic metal (Mufarrege et al. [2009\)](#page-8-0), had the lowest translocation to leaves as a tolerance mechanism. Binding of positively charged toxic metal ions to negative charges in the cell walls of the roots and chelation to phytochelatins followed by accumulation in vacuoles have been invoked as mechanisms to reduce metal transport to aerial parts (Göthberg et al. [2004\)](#page-8-0) and to increase plant tolerance (Poschenrieder et al. [2006](#page-9-0)). T. domingensis achieved a high biomass, indicating tolerance to the contaminants found in the effluent treated in the constructed wetland. Other responses to the effluent included adaptive changes in root structure. The plants growing at the inlet showed the highest root CSA, while the plants in Pond 1 presented the lowest root CSA, which agrees with Ciro et al. [\(1999](#page-8-0)) and Xie and Yu [\(2003](#page-9-0)), who reported a decrease in root CSA as an adaptation to aquatic environments with a low nutrient availability. The root CSA in Pond 2 was significantly higher than the root CSA in Pond 1, probably due to the higher nutrient concentration in Pond 2.

The roots of plants growing at the inlet, where water was rich in nutrients, developed the highest total metaxylem CSA. Exposure to an important nutrient supply produces an increase in total metaxylem CSA, which allows a higher transport capacity for further development and maintenance of the aerial part (Wahl et al. [2001](#page-9-0)). Campanella et al. [\(2005](#page-8-0)) observed an increase in root and stele CSA of E. crassipes exposed to sewage in the same constructed wetland. Nutrients contained in sewage, like P, act as signals that can trigger molecular mechanisms, which modify the process of cell division and differentiation within the root (Wahl et al. [2001\)](#page-9-0). These processes have a significant impact on root structure. The responses of the root morphology to nutrients may be modified by regulators of vegetative growth, such as auxin and cytokinins, suggesting that the control of root development could be

<span id="page-7-0"></span>Fig. 6 Optical microscopy image of cross-sectional T. domingensis roots from the inlet and outlet areas of the constructed wetland and from Pond 1 and Pond 2. Ep epidermis, En endodermis, V metaxylem vessels, St stele, Aer aerenchyma. Bar =  $650 \mu m$ 



mediated by changes in the synthesis and transport of hormones upon exposure to a source of nutrients (López-Bucio et al. [2003\)](#page-8-0). The root meristem maintenance is disturbed by physiological and molecular factors as a response to different P concentrations (Desnos [2008\)](#page-8-0). Mufarrege et al. [\(2009](#page-8-0)) evaluated the effects of contaminants of wastewater treated at Bahco constructed wetland (Cr, Ni, and Zn) on the floating macrophyte P. stratiotes and reported that Ni had the greatest toxic effect, in terms of decreasing root anatomical parameters. In our work, plants growing at the inlet showed the highest root anatomical parameters as a response to the wetland effluent toxicity. The morphological changes in T. domingensis observed in this study allowed an increase in the efficiency of the contaminant accumulation in root tissues. In inlet plants, a higher root and stele CSA and a greater number of vessels promote higher uptake and accumulation of metals and transport of P to aerial parts. Wahl and Ryser ([2000\)](#page-9-0) demonstrated that an increase in root and xylem vessel CSA has a positive influence on the hydraulic conductance of roots. When contaminant concentrations increase, coarse roots are an adaptive response to improve contaminant uptake (Ciro et al. [1999](#page-8-0); Xie and Yu [2003](#page-9-0)).

#### **Conclusions**

Trade-offs between plant characteristics that promote resource capture and those that impart stress are crucial in determining the ecological behavior of a species. Root morphological plasticity is an important mechanism of T. domingensis to modify the uptake of nutrients and metals and enable tolerance of contaminants. The studied macrophyte increased the root and stele CSAs and the numbers of vessels as a response to the effluent toxicity. These changes promoted higher contaminant uptake, metal accumulation, and P transport to aerial parts. Even though chlorophyll concentration was sensitive to effluent toxicity, T. domingensis adaptability was also represented in a greater biomass and plant height than recorded for natural wetlands. The observed adaptations allowed this plant to become the dominant species and enabled the wetland to maintain a high contaminant retention capacity.

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