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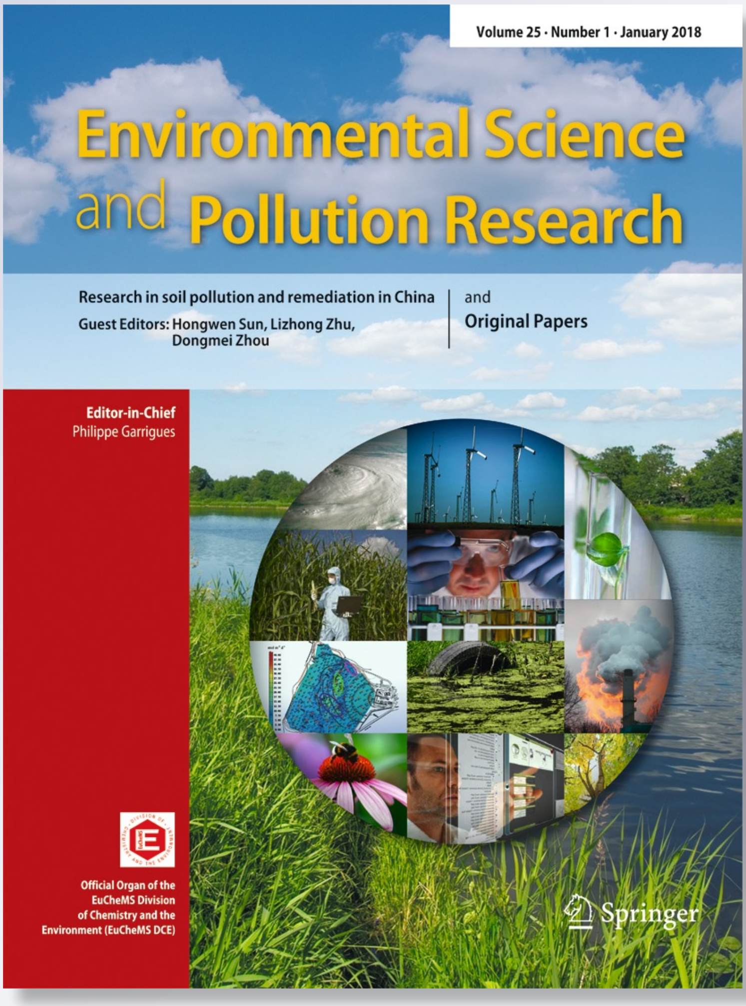

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RESEARCH ARTICLE

Macrophytes as potential biomonitors in peri-urban wetlands of the Middle Parana River (Argentina)

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Abstract The aims of this study were to measure the concentrations of nutrients and pollutants in peri-urban wetlands, to analyze the plant morphology of the most representative macrophyte species, and to determine their potential use as biomonitors. Four wetlands in the Middle Paraná River floodplain evidencing contamination or anthropogenic impact were studied. The studied species were *Typha domingensis* Pers., *Eichhornia crassipes* (Mart.) Solms., *Alternanthera philoxeroides* (Mart.) Griseb., and *Pistia stratiotes* L. Besides, the same plant species from an uncontaminated wetland considered as control were studied. *A. philoxeroides* showed the highest total phosphorus (TP) concentration in leaves throughout the study, while the other species showed a higher TP concentration in roots than in leaves. Since metal concentration in *A. philoxeroides* tissues was always higher than in sediment, further studies focused on its phytoremediation capacity should be carried out. *T. domingensis* exhibited the highest Zn concentrations in roots followed by Pb, and *E. crassipes* presented the highest values of Pb concentrations in roots. The aerial part height of the plants from peri-urban wetlands was significantly higher than that of the plants from the control, while the root length was significantly lower. The root length of *P. stratiotes* showed a negative correlation with soluble reactive phosphorus (SRP) concentration in water. All the root anatomical parameters of *T. domingensis* and *E. crassipes* showed a positive correlation with nitrate and ammonium concentrations in water. The studied macrophytes evidenced a high tolerance, enabling them to grow and survive in peri-urban wetlands that receive pollution from different sources. The use of aquatic and wetland plants as contaminant bioindicators and bioaccumulators in the Middle Paraná River floodplain is completely feasible.

Keywords Aquatic environments · Contaminants · Monitoring · Plant morphology

Introduction

Wetlands are ecosystems frequently impacted by metal contamination caused by human activities such as industrial, rural, and mining exploitation. Macrophytes are a key component in wetland ecosystems. As photosynthetic organisms, they are the main primary producers fixing energy and supplying oxygen for the other ecosystem components. In wetlands all over the world, the metal accumulation in tissues of different plant species have been measured (Bonanno et al. 2017; Cardwell et al. 2002; Coelho et al. 2009; Demirezen and Aksoy 2004; Deng et al. 2004; Kumar et al. 2006; Romero Núnez et al. 2011; Ye et al. 1997). These results are valuable information to be applied in the use of locally available plant species in biomonitoring programs (Bonanno et al. 2017) and in constructed wetlands for the treatment of different effluents (Cardwell et al. 2002).

Wetland plants vary greatly in their degree of metal uptake. According to literature, the range of Cd concentrations in contaminated plants is 5–30 μg g^{-1} (Kabata-Pendias and Pendias

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2000) and the concentration in uncontaminated plants is 1.9 μg g^{-1} (Outridge and Noller 1991). The range of Pb concentrations for uncontaminated freshwater plants is 6.3–9.9 mg kg^{-1} (Outridge and Noller 1991). Ellis et al. (1994) reported that the Zn concentration in *Typha latifolia* L. roots growing in a natural wetland receiving storm water was 0.70 mg g^{-1} d.w. Cardwell et al. (2002) studied 15 macrophyte species in southeast Queensland (Australia) and found that the highest Cu concentration in leaves and in roots were 34 and 1571 μg g^{-1}, respectively. Bonanno et al. (2017) found concentrations of Cr, Cu, Ni, Pb, and Zn in *T. domingensis* roots of 3.73, 16.3, 25.7, 5.67, and 121 mg kg^{-1}, respectively, studying the biomonitoring potential of wetland and marine vascular plants in Sicily (Italy).

The toxic effects of contaminants on aquatic vegetation are usually estimated from changes in some population, biological and physiological parameters (Ellis et al. 1994; Sen and Bhattacharyya 1994; Satyakala and Kaiser 1997; Manios et al. 2003; Maine et al. 2007). However, variations in root structure and root diameter are closely associated with ecological requirements of plants, such as water and nutrient absorption, and may affect the ability of plants to absorb contaminants from water (Ciro et al. 1999; Wahl et al. 2001; López-Bucio et al. 2002; Campanella et al. 2005; Kapitonova 2002). In order to grow in contaminated sites, macrophytes may develop morphological changes in roots (Hadad et al. 2010, 2011; Mufarrege et al. 2010, 2015) showing a high morphological plasticity. López-Bucio et al. (2002) observed that high P concentrations can alter plant growth stimulating aerial biomass production and changing root morphology, as root shortening and CSA increase. Contrarily, a low P availability produces alterations in the root total length and in the formation of lateral roots (Dinkelaker et al. 1995; Bates and Lynch 1996; Borch et al. 1999). In the case of nitrogenous species, NH_4^+ is the preferred form of N absorption for most macrophytes; however, it can be toxic in high concentrations (Britto and Kronzucker 2002). In stress situations by high NH_4^+ concentrations, plants show low growth rate, root shortening, senescence, low shoot frequency, and chlorosis in stems and leaves (Britto and Kronzucker 2002; Tylova et al. 2008; Jampeetong and Brix 2009).

Macrophytes have been studied locally and around the world, in relation to growth, response to contaminants, phytoremediation, and biomonitoring capacity (Lallana 1981; Villar et al. 1998, 1999; Neiff et al. 2001; Cardwell et al. 2002; Hadad and Maine 2007; Coelho et al. 2009; Bonanno et al. 2017). At a local scale, studies carried out in the Middle Parana River floodplain have demonstrated that aquatic plants have shown fast growth, high productivity, and a wide response to floods and pollutant exposition (Lallana 1981; Neiff et al. 2001; Hadad and Maine 2007). Besides, there are numerous local studies focused on the plant responses and the efficiency of accumulation of nutrients and metals in tissues, carried out both, in natural and constructed wetlands, and in greenhouse (Hadad and Maine 2007; Hadad et al. 2006, 2007, 2010, 2011; Maine et al. 2004a, b, 2009, 2013, 2017). However, studies focused on the macrophytes inhabiting the peri-urban wetlands of the Middle Parana River floodplain are non-existent.

The contaminant measurement in different plant tissues, accompanied by contaminant measurements in sediment and surrounding water, is an essential issue in biomonitoring studies. Due to macrophytes have demonstrated a high efficiency in the accumulation of metals and nutrients in tissues and a high tolerance to different contaminants, they could be efficient biomonitors of contaminants in the Middle Paraná River floodplain. The aims of this study were the following:

- to measure the concentrations of nutrients and pollutants in water, sediment, and plant tissues in peri-urban wetlands,
- to analyze the external plant morphology and internal root morphology of the most representative macrophyte species, and
- to determine suitable macrophyte species for their potential use as biomonitors and in constructed wetlands.

Materials and methods

Study sites

Four peri-urban wetlands on the Middle Paraná River floodplain were studied. The peri-urban wetlands are located near the city of Santa Fe, Argentina (Fig. 1). They were called "Rincón," "Arroyo Leyes," "Cayastá," and "RECU." The sampling points in each wetland were Rincón (31° 36′ 20.8″ S; 60° 33′ 49.8″ W), Arroyo Leyes (31° 33′ 54.4″ S; 60° 32′ 59.6″ W), Cayastá (31° 11′ 15.7″ S; 60° 09′ 20.8″ W), and RECU (31° 38.23′ S; 60° 40.42′ W). The sites were selected due to showed evidence of contamination or anthropogenic impact, such as the presence of solid wastes, water runoff, and proximity to roads and populated areas. "Rincon" is swamp that is disconnected from the river due to the existence of a defensive bank. In the vicinity, there are numerous houses and some solid urban waste. "Arroyo Leyes" is a lake that connects to streams and other lakes in periods of flooding. Although there is a growing open dump in the area, it is several meters from the water body. "Cayasta," located to the side of a state road, is an artificial shallow channel that feeds on rainwater. In this site is located a company of production and washing of carrots. "RECU" is a shallow lake located in an urban ecological reserve near Santa Fe city, called "*Reserva Ecológica de la Ciudad Universitaria*" (RECU). This lake has

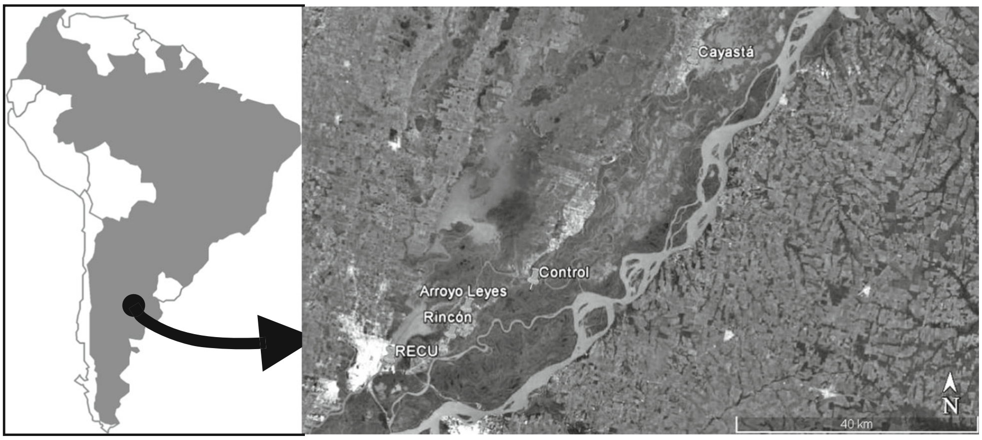


Fig. 1 Location of sampling points in the studied wetlands

no superficial connection with the river, although there are underground connections.

Parallel to the study of these sites, a reference uncontaminated wetland on the Middle Paraná River floodplain (control, 31° 31′ .99″ S; 60° 25′ .19″ O) was studied in order to obtain comparisons of the plant responses (Fig. 1).

Sampling of macrophytes, water, and sediment

Four visits were made to each peri-urban wetland and to the control wetland according to seasons (spring 2014; summer, autumn, and winter, 2015). At each wetland, macrophytes, water, and sediment were collected.

The macrophyte species sampled in each site were *Typha domingensis* Pers. (Rincón), *Eichhornia crassipes* (Mart.) Solms. (Arroyo Leyes), *Alternanthera philoxeroides* (Mart.) Griseb. (Cayastá), and *Pistia stratiotes* L. (RECU). They were chosen according to their abundances and plant cover. The same macrophyte species were sampled in the unpolluted wetland. Macrophytes were collected by hand and placed in plastic bags. Between 10 and 15 individuals of each species were collected. Water temperature and conductivity were measured in situ using an OHAUS ST10C-B portable conductivity meter. Dissolved oxygen (DO) and pH were also measured in situ with a Hanna HI 9146 portable meter and an Orion pH-meter, respectively. Water samples were collected in triplicate at each point and kept at 4 °C.

Surface sediment samples were collected using a 3-cm diameter PVC corer at a depth range of approximately 10–20 cm according to the sediment of the different sites, and stored at 4 °C until analysis.

Plant study

The external morphology of collected plants was described by measuring aerial part height and root length. The internal root morphology was studied in spring 2014, and in summer-autumn 2015. For this study, sections—approximately 30 mm long—were cut from the middle of the root and stored in formaldehyde 4% for 48 h. Then, the root sections were immersed in ethanol 70% for their preservation. For anatomical measurements, the main roots were taken at random and cross-sectioned by hand applying the technique reported by D' Ambrogio de Argüeso (1986). For each plant species, 30 root sections were analyzed by light microscopy. The values of cross-sectional areas (CSAs) of the whole root, stele, and metaxylem vessels were calculated, and the number of metaxylem vessels per section was counted.

Chemical analysis

A previous multi-elemental screening was carried out in the samples of water, tissues, and sediment by inductively coupled plasma emission spectroscopy (ICP), Shimadzu ICPE-9000 (data not shown). Then, physicochemical characterization of water and determination of total phosphorus (TP) and metal concentrations in plant tissues and sediment were carried out.

Water temperature and conductivity were measured in situ using a YSI portable conductivity meter. Dissolved oxygen (DO) was measured with a Horiba OM-14 portable meter. pH was determined in situ using an Orion portable meter. The physicochemical water characterization was performed according to APHA (2012). For the determination of metals in water, samples were treated according to EPA method 200.2 (USEPA 1994) and analyzed by atomic absorption spectrometry (APHA 2012). SRP was determined by the colorimetric molybdenum blue method (Murphy and Riley 1962). In the case of total phosphorus (TP), non-filtered water samples were digested with sulfuric acid-nitric acid. SRP was determined in the digested samples (Murphy and Riley 1962).

At the laboratory, the collected material from plants was sorted into aerial parts, submersed parts (roots of floating species) or below-ground parts (rhizomes and roots of rooted species). Plants were washed with distilled water and rinsed thoroughly. Plants and sediment were oven-dried at 70 °C for 48 h (APHA 2012). Dried plant (leaves and roots) and sediment samples were ground, sieved, and digested with a mixture of HNO_3 (1 + 1) and HCl (1 + 4) (USEPA 1994) for chemical measurements. For the determination of metals, digested samples were analyzed by atomic absorption spectrometry (APHA 2012). Besides, in the same samples, SRP was determined according to Murphy and Riley (1962).

QA/QC

Analytical grade reagents, Milli-Q water, and certified standard solutions were used. All glassware was washed with 2 M HNO_3. Replicate analyses of the samples showed a precision of typically less than 5% (coefficient of variation). Detection limits for water ($\mu g\ L^{-1}$) and macrophyte tissues and sediment ($\mu g\ g^{-1}$) were Cr (2), Cu (2), Ni (2), Pb (2), Zn (3), and P (5).

Statistical analysis

Two-way analysis of variance (ANOVA) was used to determine whether significant differences existed in aerial part height and root length among samplings in peri-urban wetlands and the control wetland. The normality of residuals was checked with Shapiro-Wilk's test, and the homoscedasticity of variances was checked applying Bartlett's test. Tukey's test was used to differentiate means where appropriate.

Since root morphology parameters (CSA of roots and number of vessels) did not show a normal distribution, non-parametric tests and box-and-whisker plots were performed using the median as central trend measure

Table 1 Physicochemical parameters measured in water and TP and metals measured in sediment. The results are presented as minimum and maximum values measured along the study. Values in parentheses are the detection limits of the method

Parameter	Rincón	Arroyo Leyes	Cayastá	RECU	Control
Water					
Temperature (°C)	14.4–20.2	15.8–23.7	15.1–23.5	16.6–23.1	15.2–23.6
Dissolved oxygen (mg/l)	5.5–8.7	6.67–8.5	6.5–10.8	4.7–8.14	5.1–8.3
pH	6.9–7.67	6.77–7.93	6.9–8.27	6.9–7.5	6.8–7.61
Conductivity (μS/cm)	558–1070	135.7–158.0	290–379	1047–1608	121–142
Alkalinity (mg/l)	141.4–254.1	46.7–73.7	152.7–178.8	160.5–192.7	45.2–70.6
CO_3^{2-} (mg/l)	ND (0.5)	ND (0.5)	ND (0.5)	ND (0.5)	ND (0.5)
HCO_3^- (mg/l)	172.5–310	56.9–89.9	186.4–218.1	195.8–235.1	47.9–78.4
Total hardness (mg/l)	150.4–351.5	73.4–130.6	111.2–173.9	158–428.4	54.3–104
Ca (mg/l)	38.3–107.3	10.7–16.6	30.7–43.2	38.5–147.6	9.81–15.2
Mg (mg/l)	13.1–20.2	8.7–21.1	5–17.5	14.5–25.8	4.8–19.8
Fe (mg/l)	ND (0.05)–0.74	0.08–7.72	ND (0.005)–0.11	ND (0.05)–0.008	ND (0.05)–0.81
SO_4^{2-} (mg/l)	58.4–128.6	ND (3.0)–24.1	8–34.2	109.5–444.7	ND (3.0)–22.1
Cl^- (mg/l)	75.4–130.7	6.9–17.8	10.3–17.8	147.2–160.2	5.7–15.6
NO_3^- (mg/l)	1.8–4.3	1.5–1.9	1.1–4.7	3.2–3.5	0.9–2.3
NO_2^- (mg/l)	ND (0.002)	ND (0.002)	ND (0.002)	ND (0.002)–0.135	ND (0.002)
NH_4^- (mg/l)	0.93–1.89	0.52–1.26	0.53–1.25	0.76–1.04	0.48–0.83
TP (mg/l)	0.405–0.871	0.072–0.284	0.894–1.33	0.137–0.571	0.056–0.276
SRP (mg/l)	0.05–0.358	0.005–0.03	0.789–1.03	0.08–0.342	0.005–0.045
Sediment					
Cr (mg/g d.w.)	0.006–0.009	0.005–0.006	ND (0.002)–0.009	0.006–0.022	ND (0.002)
Cu (mg/g d.w.)	0.033–0.093	0.020–0.316	0.020–0.086	0.024–0.077	ND (0.002)
Ni (mg/g d.w.)	0.004–0.005	ND (0.002)–0.005	ND (0.002)–0.003	0.001–0.008	ND (0.002)
Pb (mg/g d.w.)	0.020–0.147	0.019–0.055	ND (0.002)–0.137	0.045–0.065	ND (0.002)
Zn (mg/g d.w.)	0.081–0.139	0.037–0.363	0.091–0.197	0.051–0.124	0.030–0.060
TP (mg/g d.w.)	0.382–1.272	0.086–0.258	0.081–0.249	0.710–0.761	0.075–0.242

ND not detected

Table 2 Ranges (min.–max.) of metal and TP concentrations (mg/g d.w.) in tissues of plants collected at the wetlands. Values in parentheses are the detection limits of the method

Plant species	Leaves	Roots	Leaves	Roots	Leaves	Roots
	TP		Cr		Cu	
T. domingensis (Rincón)	2.05–2.78	1.15–4.36	ND (0.002)	ND (0.002)–0.008	ND (0.002)	0.036–0.072
E. crassipes (Arroyo Leyes)	1.14–1.79	3.15–3.93	ND (0.002)	ND (0.002)–0.003	ND (0.002)	ND (0.002)–0.046
A. philoxeroides (Cayastá)	4.41–5.45	3.09–3.94	ND (0.002)	ND (0.002)	ND (0.002)	0.05
P. stratiotes (RECU)	1.01–1.31	0.923–2.27	ND (0.002)	ND (0.002)–0.005	ND (0.002)	0.028–0.031
	Ni		Pb		Zn	
T. domingensis (Rincón)	ND (0.002)	ND (0.002)–0.008	ND (0.002)	0.029–0.089	ND (0.003)	0.056–0.209
E. crassipes (Arroyo Leyes)	ND (0.002)	ND (0.002)–0.004	ND (0.002)	0.029–0.107	ND (0.003)	0.029–0.059
A. philoxeroides (Cayastá)	ND (0.002)	ND (0.002)	ND (0.002)	0.05	ND (0.003)	0.133
P. stratiotes (RECU)	ND (0.002)	ND (0.002)–0.001	ND (0.002)	0.031–0.032	ND (0.003)	0.043–0.094

ND not detected

and interquartile range (25 and 75%) as its variability measure. The Kruskal-Wallis test was applied to check the differences among the morphometric parameters measured in roots among the different seasons and the control.

Correlations were carried out using Pearson coefficient between biological parameters and water nutrient concentrations. In all comparisons, a level of $p < 0.05$ was used.

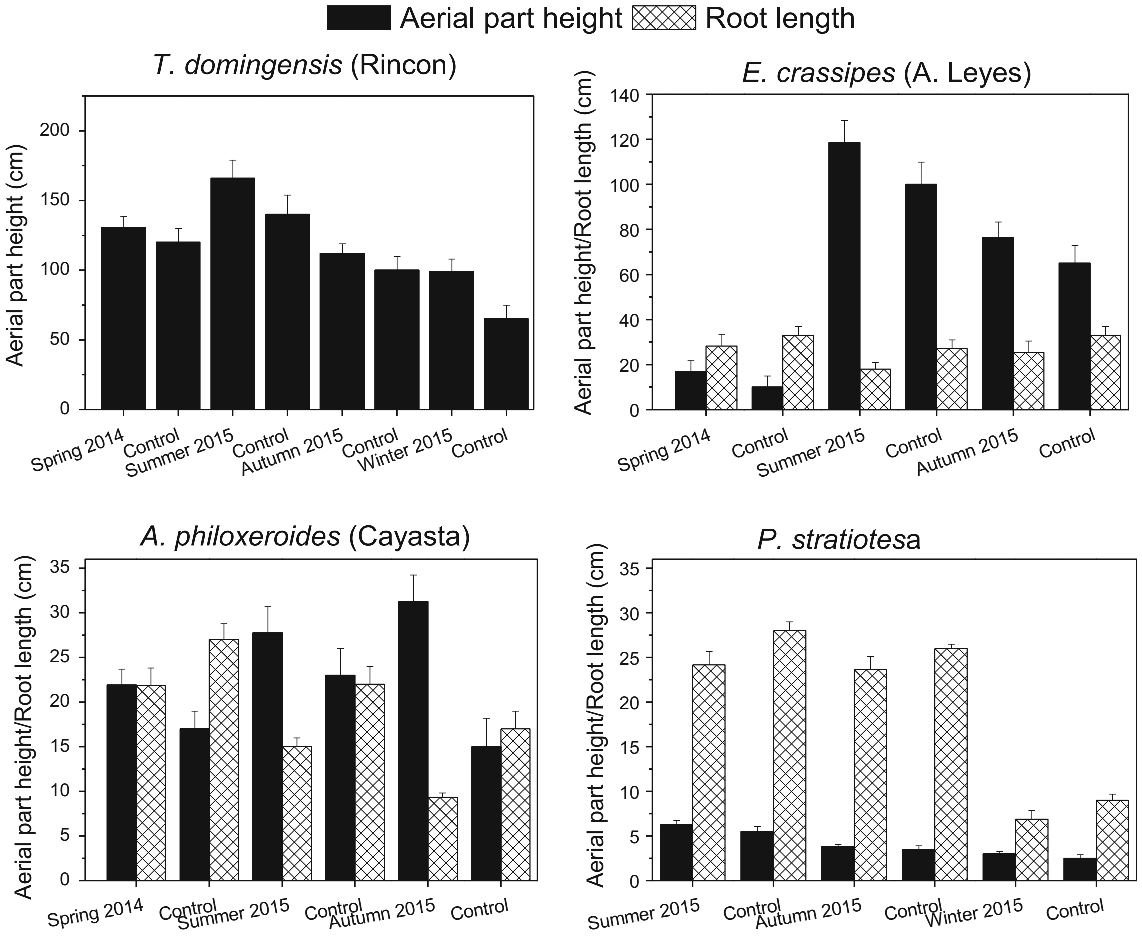


Fig. 2 Aerial part height and root length of the studied macrophytes in each site (in *T. domingensis* only aerial part height was measured)

Results and discussion

In water of the peri-urban wetlands, the highest conductivity was registered in RECU, while the lowest conductivity was registered in Arroyo Leyes (Table 1). The highest conductivity measured in RECU was due to this site showed the highest concentrations of SO_4^{2}, Cl^{-}, and total hardness. This site is the most urbanized and it receives storm water. There were not statistical significant differences between the conductivity of Arroyo Leyes and the measured in the control. Cayastá and Rincón showed wide ranges in the concentration of nitrates. Nitrite concentrations were under the detection limits of the analytical method, except in winter in RECU. The highest concentrations of ammonium were recorded in Rincón. The highest TP and soluble reactive phosphorus (SRP) in water were found in Cayastá, and the lowest ones in Arroyo Leyes. In the control site, all concentrations were significantly lower than that of the peri-urban wetlands (Table 1). Metal concentrations in water in all studied wetlands were below the detection limits of the method.

Regarding sediment, multi-elemental ICP analysis detected the presence of Cr, Cu, Ni, Pb, and Zn. Then, the quantification of these metals was carried out by atomic absorption spectrometry. Ni and Cr showed the lowest sediment concentrations (Table 1). Rincón and Cayastá presented the highest Pb concentrations in sediment. The highest concentrations of Cu and Zn were found in Arroyo Leyes. RECU did not show high metal concentration in sediment. The highest TP concentration in sediment was measured in Rincón and RECU. In the control wetland, all metal concentrations in sediment were below the detection limits of the method, with exception of Zn. The TP concentration in sediment of the control did not show statistically significant differences than that of the measured in Arroyo Leyes.

According to Kabata-Pendias and Pendias (2000), Zn and Cu sediment concentrations in the studied wetlands were within toxic range (70–400 μg g^{-1} Zn, 60–125 μg g^{-1} Cu). Also, high sediment concentrations of Pb in Rincón and Cayastá were found. However, macrophytes sampled at these sites showed tolerance to these metals.

Metals were not detected in water; however, they were retained in sediment and in plant roots, in agreement with what was reported by Romero Núnez et al. (2011) in tropical wetlands. In most samplings, a higher concentration of metals in sediment in relation to plant tissues was observed (Table 2).

A. philoxeroides showed the highest P concentration in leaves during all the study period (Table 2), being the TP tissue concentrations in the control significantly lower.

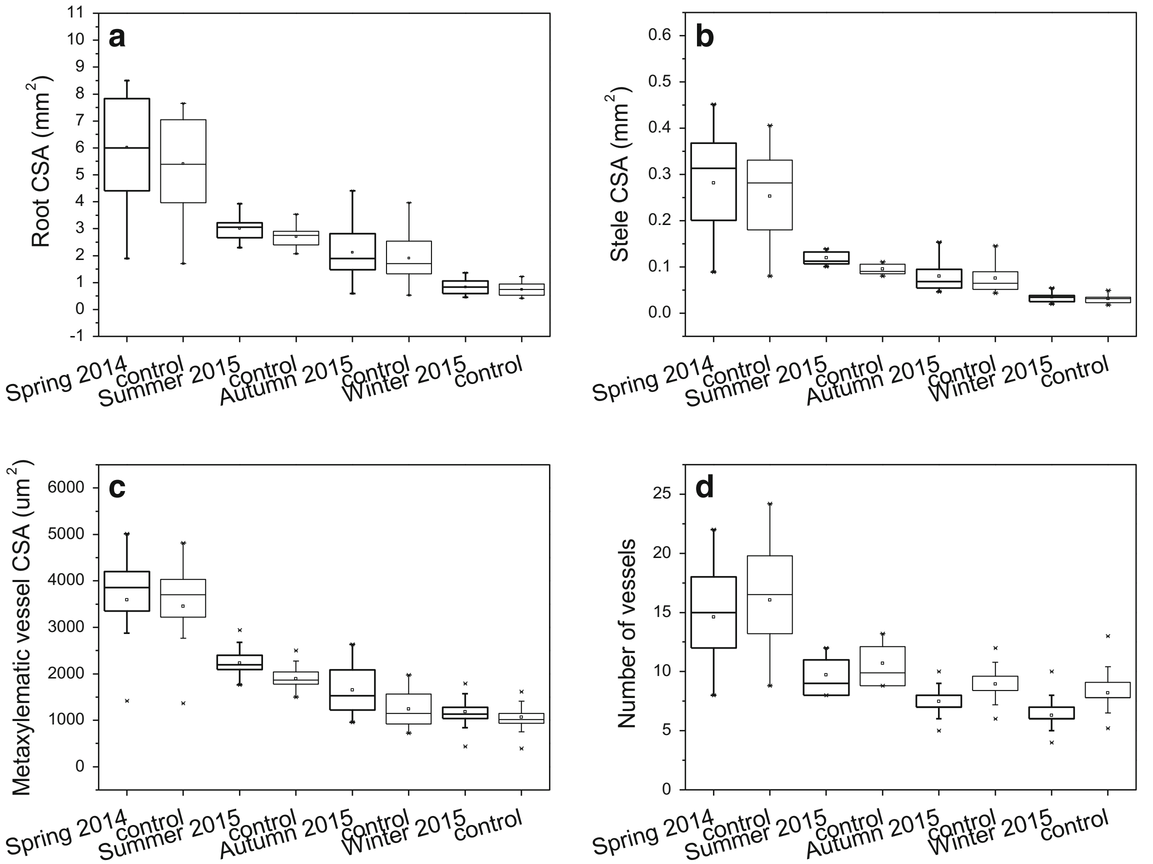


Fig. 3 Box-and-whisker plots of cross-sectional areas (CSAs) of root (**a**), stele (**b**), and metaxylematic vessels (**c**), and number of vessels (**d**) of *T. domingensis* obtained in different seasons. Different letters represent statistically significant differences among seasons

A. philoxeroides was sampled in the site that showed the highest SRP and TP in water (Table 1), indicating an effect of the characteristics of the surrounding water on the P concentrations of the plant tissues. The other species showed significantly higher TP concentrations in roots than in leaves.

Metal concentrations in leaves were below the detection limits (Table 2). In roots, Cr and Ni showed the lowest concentrations. *T. domingensis* (Rincón) exhibited the highest Zn concentrations in roots followed by Pb. *E. crassipes* (Arroyo Leyes) presented the highest values of Pb root concentrations. Metal concentrations in tissues of the plants from the control wetland were below the detection limits, while TP concentrations were significantly lower than that of the peri-urban wetlands.

Zn concentration in tissues in some macrophytes fell within the range of contaminated plants (100–400 μg g^{-1}) reported by Outridge and Noller (1991). The highest Zn concentration (209 μg g^{-1}) in roots was found in *T. domingensis*. In our study, all plant species showed higher concentrations of Cu than the toxic level (20 μg g^{-1}) reported by Borkert et al. (1998). The metal concentrations measured in the studied macrophytes indicate that there may be internal metal detoxification tolerance mechanisms, in addition to their metal exclusion strategies (Romero Núnez et al. 2011). Several plants tolerate high metal concentrations in sediment since they limit absorption and translocation to the leaves, thus avoiding injuries in this organ and keeping constant and relatively low concentrations in the aerial biomass, independently of the concentration of metal in sediment. Metal concentrations were significantly higher in roots than in leaves, in agreement with the results obtained by other authors (Nilratnisakorn et al. 2007; Hadad et al. 2007, 2011; Kadlec and Wallace 2009; Mufarrege et al. 2010, 2014, 2015; Romero Núnez et al. 2011; Vymazal 2011; Bonanno 2013; Bonanno et al. 2017). The exclusion of metals in root tissues has been suggested as a strategy of tolerance. Tolerance mechanisms consist of metal deposition on the root surface and inside the vacuoles and the root cell walls (Taylor and Crowder 1983; Tangahu et al. 2011).

Metal concentrations in *A. philoxeroides* were significantly higher than that in sediment. There are scarce studies focused on this macrophyte. Rane et al. (2015) reported the phytoremediation capacity of *A. philoxeroides* in cleaning and detoxifying effluents from a textile industry, so this

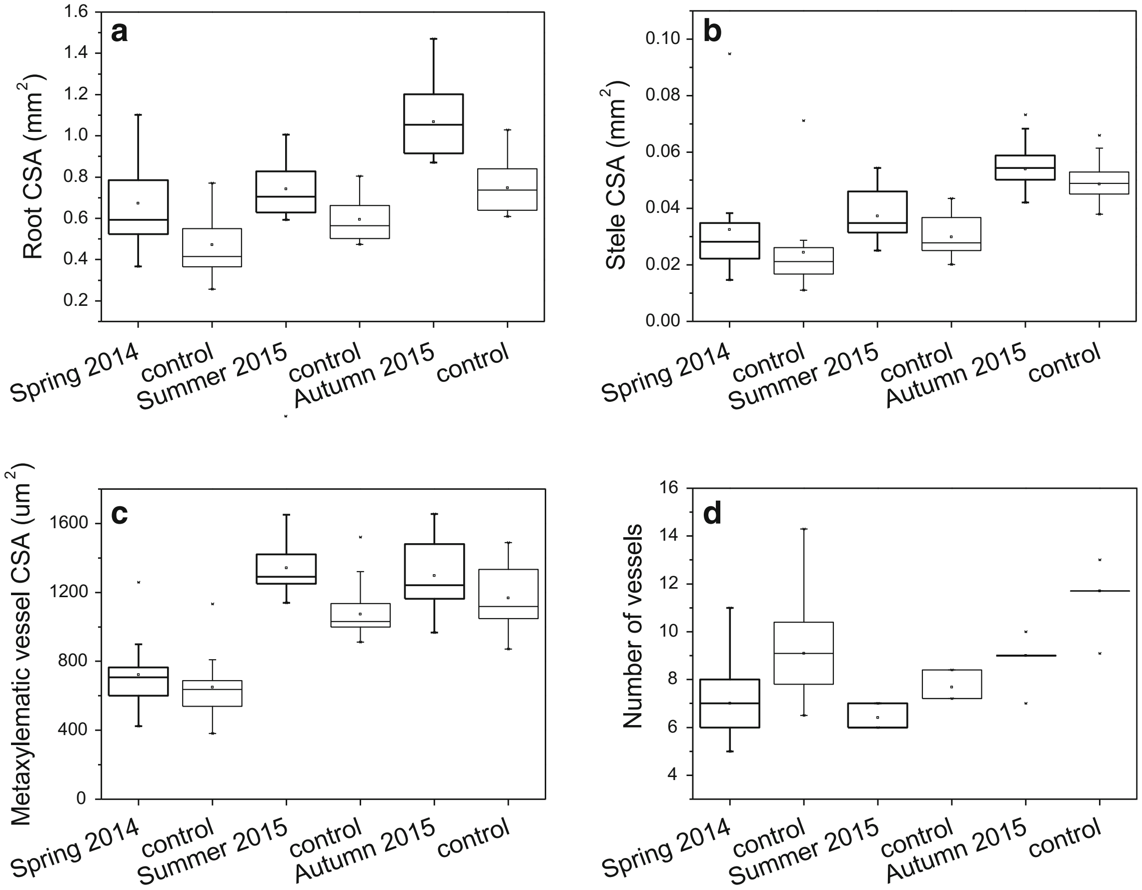


Fig. 4 Box-and-whisker plots of cross-sectional areas (CSAs) of root (**a**), stele (b), and metaxylematic vessels (**c**), and number of vessels (**d**) of *E. crassipes* obtained in different seasons. Different letters represent statistically significant differences among seasons

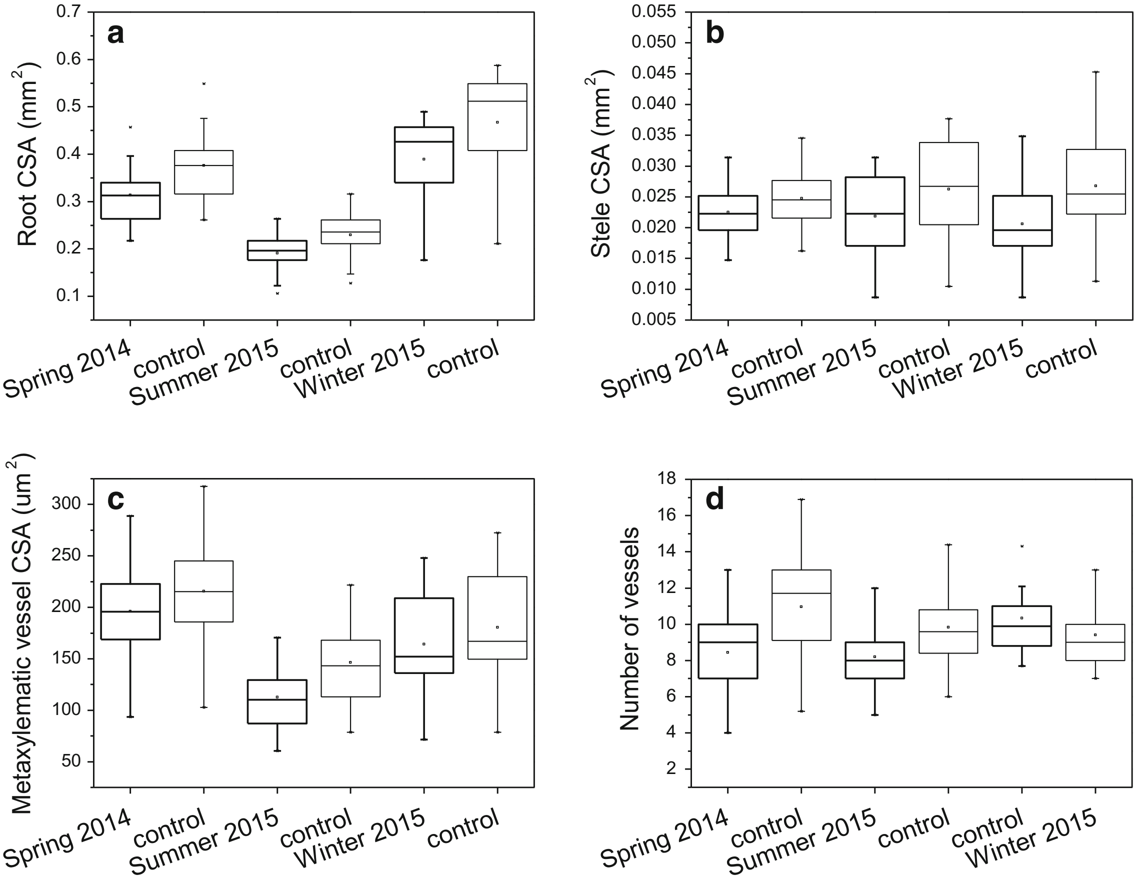


Fig. 5 Box-and-whisker plots of cross-sectional areas (CSAs) of root (**a**), stele (**b**), and metaxylematic vessels (**c**), and number of vessels (**d**) of *A. philoxeroides* obtained in different seasons. Different letters represent statistically significant differences among seasons

species could be proposed for further studies to evaluate its efficiency and possible implementation in treatment wetlands.

In the peri-urban wetlands, the height of aerial parts showed significant differences over time in all species (Fig. 2). *T. domingensis*, *E. crassipes*, and *P. stratiotes* showed the highest values in summer. In *P. stratiotes*, the reduction of aerial parts in winter was accompanied by a significant reduction in root length. *A. philoxeroides* showed an increase of aerial parts in winter. The aerial part height of the plants from peri-urban wetlands was significantly higher than that of the plants from the control, while the root length was significantly lower. The height of *T. domingensis* recorded in this study was similar to the values found by Hadad et al. (2010) in uncontaminated wetlands from the Middle Paraná River floodplain. Mufarrege et al. (2014) reported the tolerance of this species when exposed to an experimental solution of Cr, Ni, and Zn, showing that it can survive and grow in polluted water bodies. In floating macrophytes, leaves show a significant sorption and accumulation of contaminants since they are in direct contact with the water. In emergent species, the submerged parts of leaves can accumulate contaminants efficiently due to their direct contact with water (Mufarrege et al. 2016). Regarding P, it is taken from sediment as phosphate and it can be translocated from roots to aerial parts or vice versa, as it has been demonstrated in laboratory studies (De Marte and Hartman 1974; Eugelink 1998).

The root length of *P. stratiotes* showed a negative correlation with SRP water concentrations ($r = -0.99$; $p < 0.05$). Mufarrege et al. (2010) and Campanella et al. (2005) found the same results for *P. stratiotes* and *E. crassipes*, respectively. These authors concluded that root length depends on the P concentration at which plants were exposed, showing a phenotypic plasticity in response to P. Ciro et al. (1999) and Xie and Yu (2003) reported that plants with thin and long roots could be more efficient in the acquisition of P when this element is scarce that plants with short and thick roots.

Root, stele, and metaxylem vessels CSA, and number of vessels of *T. domingensis* decreased significantly with time (Fig. 3). All the parameters showed a positive correlation with nitrate and ammonium concentrations in water ($r > 0.95$; $p < 0.04$). Root and stele CSAs, and the number of vessels of *E. crassipes* increased significantly from spring 2014 to the next season (Fig. 4). Root and stele CSAs of this species also showed a positive correlation with nitrate and ammonium concentrations ($r = 0.99$; $p = 0.04$; $r = 0.99$; $p = 0.01$, respectively). Campanella et al. (2005) reported that the root CSA of

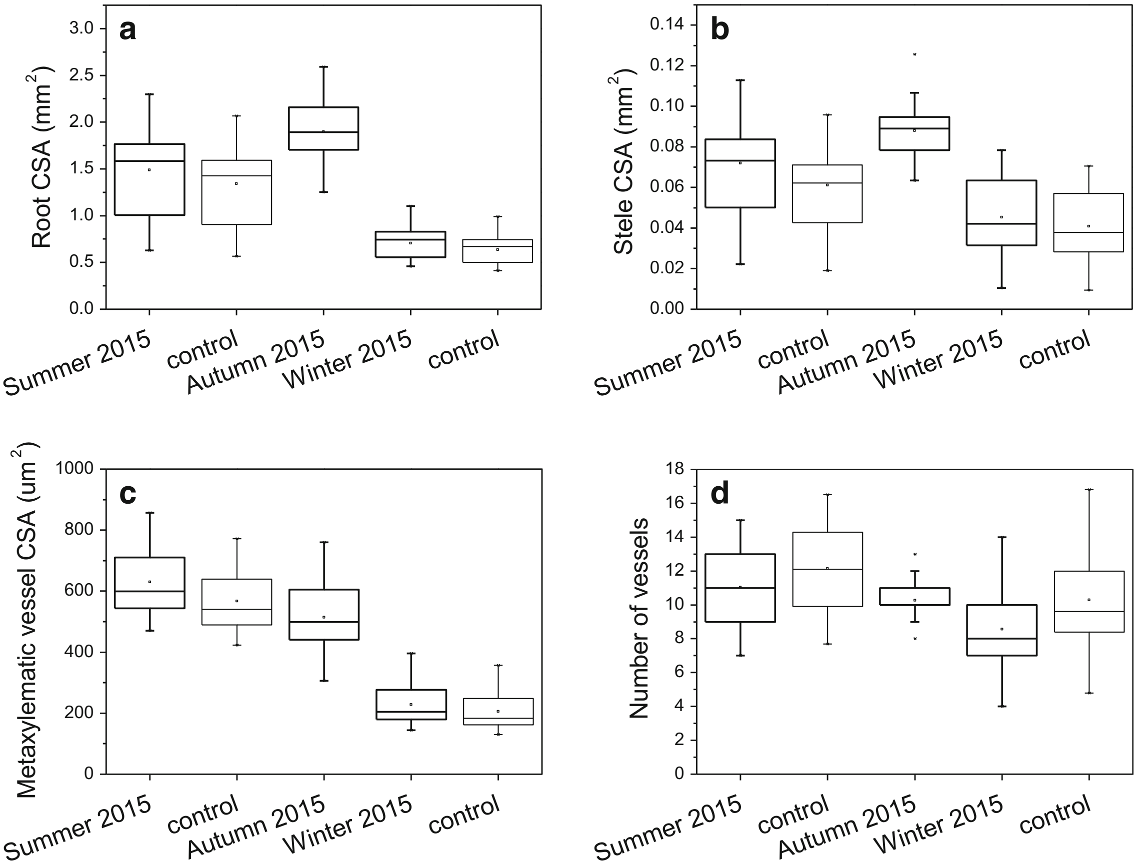


Fig. 6 Box-and-whisker plots of cross-sectional areas (CSAs) of root (**a**), stele (**b**), and metaxylematic vessels (**c**), and number of vessels (**d**) of *P. stratiotes* obtained in different seasons. Different letters represent statistically significant differences among seasons

E. crassipes showed a positive correlation with nutrients in plants growing in a constructed wetland for the treatment of sewage. These authors conclude that the roots in wetlands with high nutrient concentrations in water increase the root CSA. An increase in the CSA of metaxylem vessels in response to high concentrations of nutrients can be understood in terms of the need to increase its transport capacity for further development and maintenance of the aerial part.

The lowest root, metaxylem vessels CSAs and number of vessels of *A. philoxeroides* was found in summer (Fig. 5). Stele CSAs did not show significant differences. In *P. stratiotes*, root, stele, and metaxylem vessels CSAs exhibited significant differences among seasons (Fig. 6). The number of vessels in winter was significantly lower than the number of vessels in summer and autumn. In order to show the root structure obtained in each peri-urban wetland and to establish comparisons among them, representative light microscopy images are shown in Fig. 7. In *T. domingensis*, *E. crassipes*, and *P. stratiotes*, the root, stele, and metaxylem vessels CSAs measured in the control were significantly lower than that of the peri-urban wetlands, while the number of vessels was significantly higher. This indicates the low nutrient availability in the control in comparison with the peri-urban wetlands. The morphological plasticity of roots is an important mechanism to modify the absorption of nutrients and metals, and survive in polluted water bodies (Hadad et al. 2010).

Studying the plant responses and contaminant accumulation in tissues may reveal specific response patterns. This has important implications for pollution control, biomonitoring, and for implementing ecological engineering projects such as constructed wetlands.

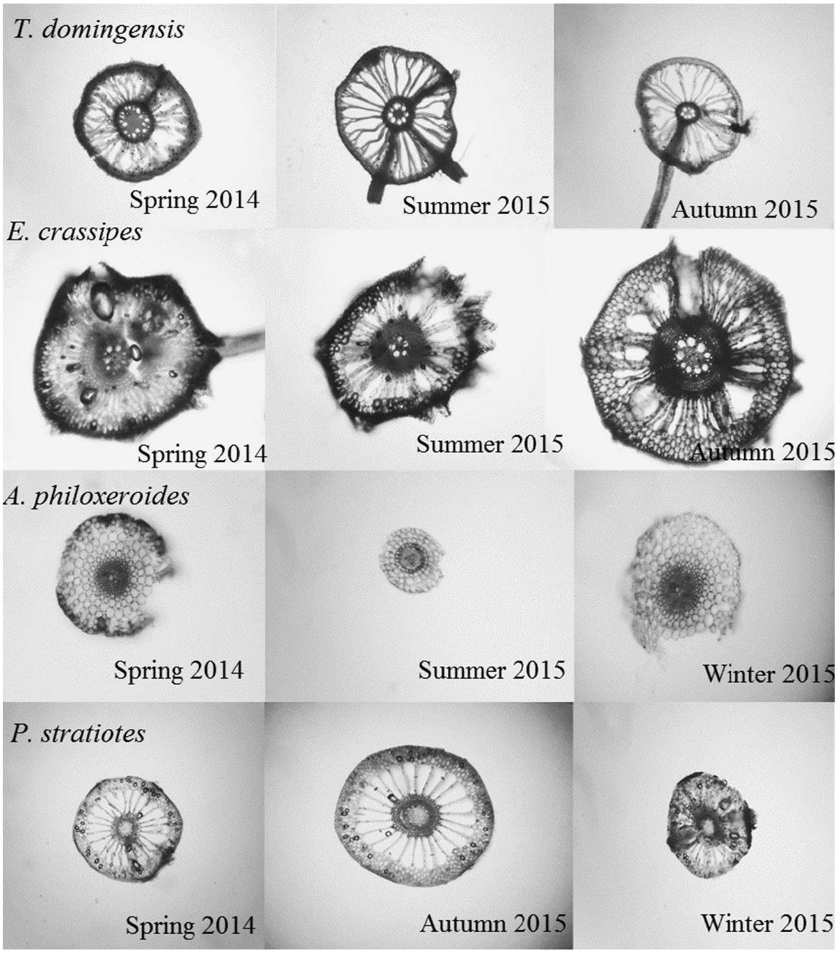
Conclusions

The studied macrophytes evidenced a high tolerance, allowing them to grow and survive in peri-urban wetlands that receive pollution from different sources.

T. domingensis and *E. crassipes* showed an increase of the root CSA in relation to nutrient concentration allowing them to survive in polluted water bodies.

Since metal concentration in *A. philoxeroides* tissues was always higher than in sediment, this species could be used in treatment wetlands.

Fig. 7 Representative light microscopy images of cross-sectional roots of species studied along time



Regarding the plant richness, availability and metal and nutrient accumulation in plant tissues, the use of aquatic and wetland plants as contaminant bioindicators and bioaccumulators in the Middle Paraná River floodplain is completely feasible.

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