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Performance of a system of natural wetlands in leachate of a posttreatment landfill

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Abstract Phytoremediation is an emerging technology in landfill leachate posttreatment. The evaluation of a system composed of three natural wetlands. The wetlands vegetation cover was monitored during 2 years by estimating the coverage area of the macrophytes. Chemical analyzes of the effluent were conducted monthly. The monitoring and identification of macrophytes indicated that the vegetation structure was represented by four species of higher relative cover: *Pistia stratiotes* L. (water lettuce), *Echinochloa polystachya* (Kunth) Hitchc. (creeping river grass), *Eichhornia crassipes* (Mart.) Solms (water hyacinth) and *Alternanthera philoxeroides* (Mart.) Griseb. The system of natural wetlands had an average efficiency of 75 % for biochemical oxygen demand, 63 % for chemical oxygen demand, 84 % for ammoniacal nitrogen, 89 % for total nitrogen and 70 % for phosphorus. The concentrations of heavy metals in the roots, as well as in the branches of *E. crassipes* and *E. polystachya,* lead us to the conclusion that such species perform phytoextraction for Cd and Pb accumulating the metals in the biomass. The results show that this is a viable alternative that can be associated with forms of conventional treatment of leachate, such as the treatment with aerobic and facultative ponds.

Keywords Solid waste · Environmental pollution · Remediation · Phytoremediation · Macrophytes

Introduction

The leachate generated in landfills, if not collected and treated properly, poses an environmental risk due to its high pollution load (Jones et al. 2005; Renou et al. 2008; Cheng and Guo 2014). Its characteristics depend on the amount and nature of the waste, geomorphology and weather conditions of the location where the landfill operates. These characteristics include high levels of chemical oxygen demand (COD), biochemical oxygen demand (BOD), total and volatile solids, ammoniacal nitrogen (AN) and total (TN), phosphate (P) and heavy metals (Jones et al. 2005; Renou et al. 2008).

Due to its high concentration of organic and inorganic materials, leachate requires treatment prior to its release into a receiving water body in order to avoid or mitigate environmental pollution. However, landfills often do not treat leachate within acceptable levels of release as per the parameters of the environmental legislation in effect, which requires posttreatment. Therefore, to attain acceptable purification levels before leachate is released into the environment, there is a need to find new treatment alternatives.

The development of alternative systems that are efficient and inexpensive is a challenge faced by sanitary landfill managers. A promising alternative for posttreatment of the leachate is the use of natural wetlands, also known as marshes, river floodplains and swamps. These systems stand out among self-purification processes because they are constantly or seasonally flooded areas and are influenced during periods of low rainfall or drought (Frank et al. 2010). Zhang et al. (2010); Bialowiec et al. (2012) reported

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that the phytoremediation system associated with a wetland brings promising results regarding the reduction of contaminants.

The use of such systems in the posttreatment of leachate comprises a sustainable practice because during development of macrophytes, pollutants are removed and the biomass produced can later be used for power generation. The efficiency of the removal of organic and inorganic pollutant load in these systems is closely related to the amount of bio-available nutrients, the primary production rate, and the ability of plants to survive the environmental conditions imposed, the life cycle of plants and their strategies for degradation, or immobilization of pollutants. These areas also have high rates of primary productivity of macrophytes throughout the year.

Given that not all plants can develop in contaminated environments, the first step for the use of phytoremediation is to identify species, which, besides being suitable to local conditions, are tolerant to contaminants (Marques et al. 2011). The next step, according to Marques (2005), is to evaluate the capacity of the plant to promote decontamination.

Within this perspective of alternative treatment, this study was conducted with the objective of evaluating a system composed of wetlands used in the posttreatment of leachate and phytoremediator strategies used by plants in the wetlands. To do so, we identified the structure of the vegetation represented by the area of seasonal coverage of macrophytes present in the three wetlands, the structural organization of macrophytes with higher coverage area: *Pistia stratiotes* L., *Echinochloa polystachya* (Kunth) Hitchc. and *Eichhornia crassipes* (Mart.) Solms., and evaluated the contents of Ni, Cr, Zn, Pb, Cu and Cd in the leachate and plant biomass, as well as their relationship with the leachate treatment efficiency and chemical analysis of the leachate for a period of 2 years.

Materials and methods

Area of study

The study was conducted at the Caximba landfill, located in the city of Curitiba, Paraná state, Brazil, at coordinates 25°62′73.88″S and 49°33′42.38″W. The total area of the landfill is 1,015,000 m^2, and the waste disposal area is 439,540 m^2. The landfill started operating in 1989 and was closed in October 2010. On average, it received 2,400 t of municipal solid waste (MSW) per day from the city of Curitiba (1.7 million inhabitants) and from 17 neighboring cities.

Curitiba is in the Iguaçu River basin, and the landfill is located on the right bank of that river, covering two distinct sire lithology features: recent sediments (alluvial deposits) and sediments from the alteration of older crystalline rocks. The local soils developed from unconsolidated sediments have medium to clay texture with clay content varying from 300 to 700 kg/g (Zanello et al. 2009). The sediments of the Iguaçu River consist predominantly of clay and silt (MINEROPAR 2004).

Three natural wetlands surrounding the landfill (Fig. 1) have been used in the posttreatment of the leachate since February 2011. The first wetland contains a greater diversity of macrophytes and receives the treated leachate from the landfill, at an average flow of 24 m^3/h. It covers an area of about 15,424 m^2, with average depth of 1.6 m, volume of 17,969 m^3 and water retention time of approximately 31 days. The second wetland has the largest water surface area, at about 35,724 m^2, average depth of 1.3 m, volume of 47,870 m^3, and water retention time of roughly 84 days and *E. crassipes* as the dominant species. The third wetland is totally covered by macrophytes, and the dominant species is also *E. crassipes*. It covers an area of about 19,993 m^2, with average depth of 1 m, volume of 19,923 m^3 and water retention time of approximately 35 days. The treated leachate from this wetland flows into the Iguaçu River.

In the wetlands, some local adjustments have been made to avoid contamination by the leachate to adjacent areas and for leachate supply. The areas were shaped with gaps so that the effluent flow is continuous. The embankments or slopes were strengthened to prevent floodwaters from the Iguaçu River from returning to the wetlands.

Identification and seasonal monitoring of macrophyte coverage (phytosociology)

The development of macrophytes in the three natural wetlands was monitored by means of a phytosociological survey during the four seasons of the year from January 2011 to December 2012. For the phytosociological survey, two parallel and permanent transversal transects were established in each natural wetland. On these transects, ten times the visual coverage of each species in an area of 1 m^2 were estimated. The average coverage of the species was defined using the Braun-Blanquet scale (1979), to determine degrees of horizontal coverage: from 1 to 10 % (average degree 5 %); from 10 to 25 % (average degree 17.5 %); from 25 to 50 % (average degree 37.5 %); from 50 to 75 % (average degree 62.5 %); and from 75 to 100 % (average degree 87.5 %). After obtaining the respective degrees of coverage, phytosociological parameters were estimated for each species present: VC: coverage value (%) and CR: relative coverage of species *i* (%):

$$VC = 100 \times (AC/AT); \quad (1)$$

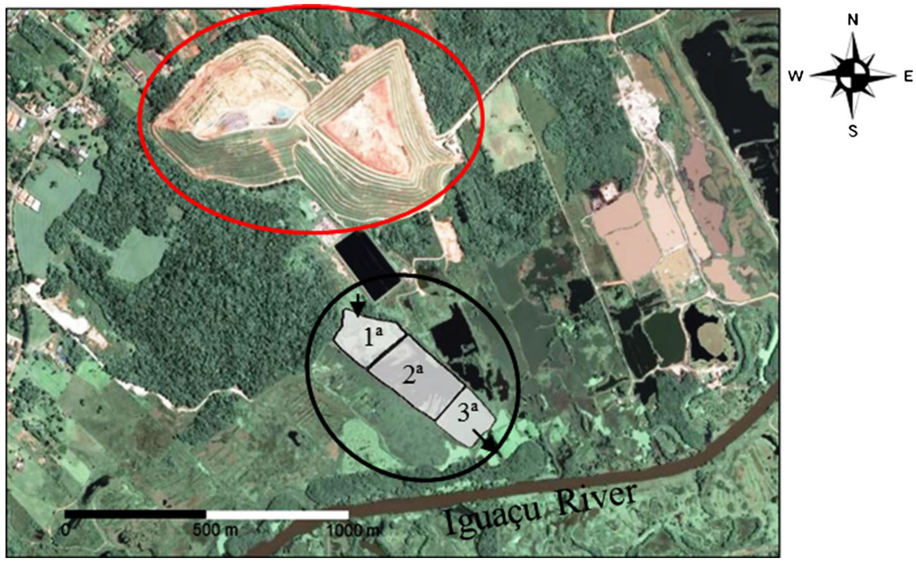

Fig. 1 Orthophoto of Caximba Sanitary Landfill, Curitiba, PR, Brazil. *Red* highlights waste disposal area and *black* the three natural wetlands (first, second and third); leachate flowing into first wetland; leachate exiting third wetland into Iguaçu River

$$CR = 100 \times \left(AC/\sum AC\right); \quad (2)$$

where VC: coverage value of species i in the portion (%); AC: area covered by species i (m^2); AT: total area sampled (20 m^2); CR: value of relative coverage of species i (%).

After the phytosociological survey, the three selected species for the evaluation of the structural organization were represented by the larger coverage on the wetland system: *E. polystachya* and *P. stratiotes* were collected in the first wetland, and *E. crassipes* in the second wetland.

Analysis of the structural organization of the plants and their relationship with the treatment

For analysis of the structural organization, six specimens of each species were chosen randomly. From each individual, samples were obtained of the roots starting 3 cm from the apex. The samples were fixed in the field in FAA 70, formaldehyde, acetic acid and ethanol 70 % (Johansen 1940) and then conserved in 70 % ethanol (Berlyn and Miksche 1976).

Permanent slides were prepared from the root samples. The material was first embedded in glycol methacrylate (JB-4), according the vendor's specifications (Polysciences Inc.). The sections were cut in a rotary microtome (Leica RM2125), with thickness of 7 µm, and were stained with 0.05 % toluidine blue, in 0.1 M phosphate buffer (pH 6.8), according to the method described by O'Brien et al. (1965). The slides were mounted with synthetic resin (Entelan®). The sections were analyzed and photographed with a photomicroscope (Olympus-BX41), and the images were captured by the Image Pro-Plus software, with the scales obtained under the same conditions.

Chemical analyses of the leachate

The leachate samples were collected from the natural wetlands monthly, at four points: (1) entrance to the first wetland; (2) outlet of the first wetland; (3) outlet of the second wetland; and (4) outlet of the third wetland.

Analyses of pH, dissolved oxygen (DO), alkalinity, BOD, COD, AN, TN, P, nitrate, nitrite and heavy metals (Cu, Cr, Zn, Pb, Cd e Ni) were conducted in accordance with the Standard Methods for Examination of Water and Wastewater (APHA 1998).

Calculation of the efficiency of posttreatment of the leachate

The efficiency of the leachate treatment was calculated by the method proposed by Kadlec and Knight (1996), which permits measurement of the percentage of mass removed [M_r (%)], according to the following equation:

$$M_r\,(\%) = \frac{100(m_1 - m_2)}{m_1} \quad (3)$$

where m_1 is the mass of the pollutant in the sample on entering the first natural wetland, and m_2 is the mass of the pollutant in the sample on exiting the third natural wetland.

Collection and analysis of heavy metals in plants

For the analysis of heavy metals (Ni, Cr, Zn, Pb, Cu and Cd), the collection of the macrophytes *E. polystachia* and *E. crassipes* took place between December 2010 and January 2012, always at the end of autumn, winter, spring and summer. For each species, six individuals were collected using the wetland system. After the collection, the samples were treated according with Malavolta et al. (1997). The samples were opened according with Carneiro et al. (2006). Afterward, the samples were analyzed regarding their quantities in the spectrometry of plasma emission with optical detection. Brand of equipment: Perking Elmer, model: Optima 3000.

Statistics analysis

The data on the amount of heavy metals in leachate and plants as well as its chemical patterns have been analyzed based on the *t* test in the Statistical Package for the Social Sciences (SPSS) Statistics. Numbers starting from $p < 0.05$ were qualified as considerable difference. The test had as aim verifying the following: (1) if the amount of heavy metals and average concentration of DBO, DQO, NA, NT and P in leachate are equivalents when compared with entrance and outlet of the system, and (2) if the average concentration of metals in plants are equivalents when compared with the species *E. polystachya* and *E. crassipes*.

Results and discussion

Identification and seasonal monitoring of macrophyte coverage (phytosociology)

From the seasonal monitoring and identification of the macrophytes present in the natural wetlands, it was found that the vegetation structure was mainly represented by four species, which presented the highest rates of relative coverage, namely *P. stratiotes* L. (water lettuce), *E. polystachya* (Kunth) Hitchc (creeping river grass), *E. crassipes* (Mart.) Solms (water hyacinth) and *Alternanthera philoxeroides* (Mart.) Griseb (Table 1).

Structural organization of the plants with greatest coverage area

Pistia stratiotes has roots in cross section composed of a uniseriate epidermis and a cortex divided into an external, median and internal part. Inside the epidermis, there is a unistratified exodermis, not lignified and not suberized (Fig. 2a). The median cortex contains ample intercellular spaces, corresponding to the aerenchyma, well-developed tissue occupying about 60 % of the root cross section and a variable number of cells arranged radially that terminate with three layers of cells without mutual spacing near the endodermis (Fig. 2a, d). The cells of the endodermis have sparse Casparian strips. The pericycle is formed by parenchymatic cells (Fig. 2d). The protoxylem is organized in a circle, inside of which there are large metaxylem vessel elements. The phloem alternates with the vessel elements, forming a polyarch structure (Fig. 2a).

The root cross section of *E. polystachya* presents a uniseriate epidermis, and inside the epidermis, there are 1–2 exodermis strata. A well-developed aerenchyma region comes next, occupying approximately 75 % of the root cross section, with a variable number of cells arranged radially that terminate with a layer of cells without mutual spacing near the endodermis (Fig. 2b). The endodermis cells have a U-shaped reinforcement, but the passage cells do not have this reinforcement in the walls (Fig. 2e). The central cylinder is compact and fairly uniform, and is surrounded by an endodermal sheath. The pericycle is formed by parenchymatic cells (Fig. 2b, e). The protoxylem is organized in a circle, inside of which are 8–9 large metaxylem vessel elements arranged in the same form. The phloem alternates with the vessel elements, forming a polyarch structure. The innermost region contains a medullary parenchyma (Fig. 2b).

The root cross section of *E. crassipes* presents a uniseriate epidermis, inside of which there are 3–4 exoderm strata. Next comes the aerenchyma region, occupying approximately 45 % of the cross section, with a variable number of cells arranged radially that terminate with a layer of cells without mutual spacing near the endodermis (Fig. 2c). The endodermis cells have sparse Casparian strips. The pericycle is formed by parenchymatic cells (Fig. 2c, f). The protoxylem is organized in a circle, inside of which are five large metaxylem vessel elements. The phloem alternates with the vessel elements, forming a polyarch structure (Fig. 2c).

Table 2 presents a comparison of the main characteristics of the roots of *P. stratiotes*, *E. polystachya* and *E. crassipes*. All three species share the presence of an aerenchyma, so they require supply of O_2 to the roots, since all of them develop in aquatic environments.

Treatment efficiency of wetlands system

The first wetland receives the leachate after conventional treatment at the landfill (aerobic pond followed by facultative pond). On average, this leachate treated by the conventional system has the following chemical characteristics: pH 8.23; DO 3.65 mg/L; temperature 22.5 °C; BOD 122 mg/L; COD 1,702 mg/L; AN 1,136 mg/L; TN 1,136 mg/L; nitrate 227 mg/L; nitrite 280 mg/L; P 12 mg/L and alkalinity 2,467 mg CaCO3/L.

Table 1 Seasonal phytosociological profile of the macrophytes species into system of natural wetlands employed in the posttreatment of sanitary landfill leachate

	First wetland															
	2011								2012							
	Summer		Autumn		Winter		Spring		Summer		Autumn		Winter		Spring	
	VC%	CR%	VC%	CR%	VC%	CR%	VC%	CR%	VC%	CR%	VC%	CR%	VC%	CR%	VC%	CR%
Macrophytes species																
P. stratiotes L.	46.20	88.99	10.25	16.61	–	–		–		–	–	–	–	–	–	–
S. auriculata Aubl.	0.10	0.07	–	–		–	–	–	3.65	5.16	–	–	–	–	–	–
E. polystachya (Kunth) Hitchc.	4.10	7.98	50.10	81.19	43.00	72.83	45.00	62.65	60.14	84.96	62.85	100.00	67.80	78.93	58.25	82.74
C. difformis L.	1.40	2.76		–	–	–	–	–	–	–	–	–	–	–	–	–
H. bonariensis Mathias & Constance	0.10	0.16	–	–	0.70	11.14	0.10	0.05	–	–	–	–	0.85	0.99	–	–
Undetermined species 2	0.30	0.03	0.20	0.32	0.10	0.13	–	–	–	–	–	–	–	–	–	–
A. philoxeroides (Mart.) Griseb.	0.10	0.01	0.03	0.04	0.90	15.56	2.70	37.25	7.00	9.89	–	–	17.25	20.08	12.15	17.26
Undetermined species 1	–	–	0.06	0.10	0.10	0.34	0.10	0.05	–	–	–	–	–	–	–	–
Undetermined species 3	–	–	1.05	1.70	–			–	–	–	–	–	–	–	–	–
Undetermined species 4	–	–	0.03	0.04		–	–	–	–	–	–	–	–	–	–	–

	Second wetland															
	Summer		Autumn		Winter		Spring		Summer		Autumn		Winter		Spring	
	CR%	VC%	CR%	VC%	CR%	VC%	CR%	VC%	CR%	VC%	CR%	VC%	CR%	VC%	CR%	VC%
Macrophytes species																
E. crassipes (Mart.) Solms	55.00	100.00	70.00	100.00	70.00	100.00	82.00	91.11	58.65	83.37	56.15	100.00	–	–	–	–
A. philoxeroides (Mart.) Griseb.	–	–	–	–	–	–	0.80	8.89	11.60	16.63	–	–	–	–	33.50	100.00

	Third wetland															
	Summer		Autumn		Winter		Spring		Summer		Autumn		Winter		Spring	
	CR%	VC%	CR%	VC%	CR%	VC%	CR%	VC%	CR%	VC%	CR%	VC%	CR%	VC%	CR%	VC%
Macrophytes species																
E. crassipes (Mart.) Solms	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	79.35	79.35	86.50	92.13	15.34	76.7	78.40	78.40
A. philoxeroides (Mart.) Griseb.	–	–	–	–	–	–	–	–	6.95	6.95	–	–	1.46	16.00	11.00	10.60
E. polystachya (Kunth) Hitchc.	–	–	–	–	–	–	–	–	13.70	13.70	13.50	7.87	3.20	7.30	10.60	11.00

VC coverage value (%), *CR* relative coverage (%)

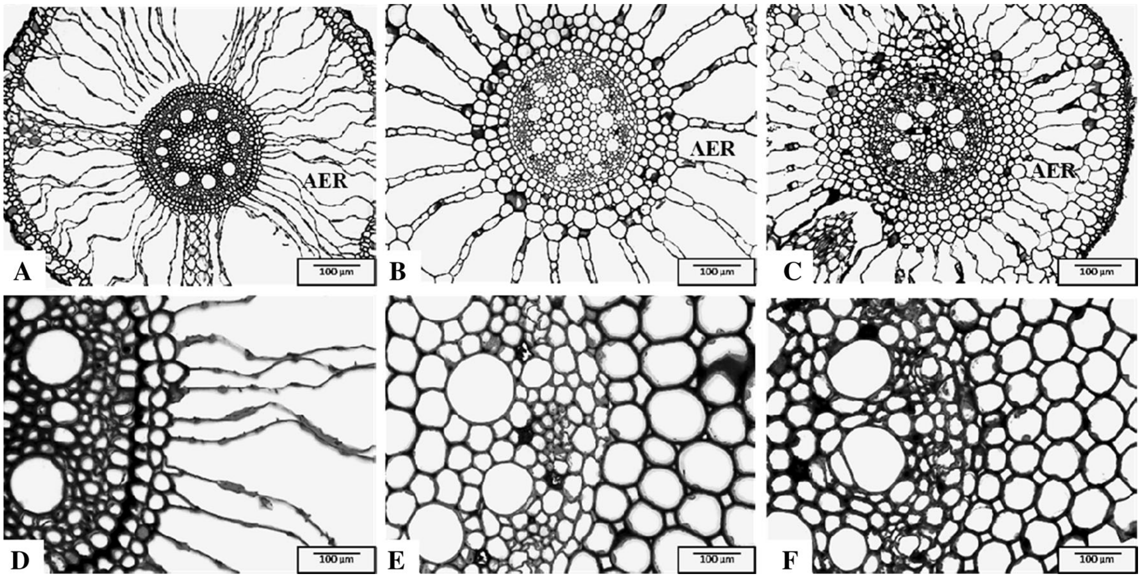

Fig. 2 Structural organization of the roots of the macrophytes with the largest coverage area in the wetlands of the Caximba sanitary landfill, Curitiba, Brazil. **a–c** General view of the cross section of the root of *P. stratiotes* L., *E. polystachya* (Kunth) Hitchc. and *E. crassipes* (Mart.) Solms, respectively. **d–f** Detail of the central cylinder of the root of *P. stratiotes*, *E. polystachya* and *E. crassipes*, respectively. *AER* aerenchyma

Table 2 Comparison of the main anatomical characteristics of the roots of *P. stratiotes* L., *E. polystachya* (Kunth) Hitchc. and *E. crassipes* (Mart.) Solms

Charactersitics	*P. stratiotes*	*E. polystachya*	*E. crassipes*
Epidermis	Uniseriate	Uniseriate	Uniseriate
Exodermis	3–4 strata	1–2 strata	3–4 strata
Aerenchyma	Well developed (60 %)	Well developed (75 %)	Well developed (45 %)
Endodermis	Sparse Casparian strips	Cells with "U" reinforcement	Sparse Casparian strips
Pericycle	Uniseriate	Uniseriate	Uniseriate
Xylem and phloem	Alternating, polyarch structure	Alternating, polyarch structure	Alternating, polyarch structure
Medula	Absent	Present	Absent

As for performance of the system in the posttreatment of the leachate, a reduction of the pollutant load in all chemical parameters analyzed was observed (Table 3).

The treatment efficiency presented considerable difference ($p < 0.05$) between system entrance and outlet in the biggest majority of patterns in all seasons. Only the DBO did not present such difference in the winter 2011 and spring 2012 (Table 3).

The system studied performed well over 2 years, considering that average treatment efficiencies were 75 % for BOD, 63 % for COD, 84 % for AN, 89 % for TN and 70 % for phosphorus (Table 3). Shildar and Sharma (1980) showed that *P. stratiotes* reduced BOD up to 85 % and COD by 61 % in ponds that receive various pollutants. Ciria et al. (2005), in a study of constructed wetlands planted with *Typha latifolia* in wastewater treatment, concluded that the presence of macrophytes increases system performance regarding BOD and AN. Zimmels et al. (2006) reported that *P. stratiotes* and *E. crassipes* reduce and maintain fairly low levels of BOD (5–7 mg/L) and COD (40–50 mg/L). Nivala et al. (2007), upon evaluating the treatment of leachate by constructed wetlands with *T. latifolia*, reported average efficiency of 92 % for BOD and 46 % for COD.

Justin and Zupancic (2009) studied a constructed wetland planted with *Phragmites australis* in the pretreatment of leachate and reported treatment efficiency of 41 % for COD, 65 % for BOD, 42 % for AN, 35 % for TN and 38 % for P. Chiemchaisri et al. (2009) studied the removal efficiency of organic matter and nitrogen in a constructed

Table 3 Median ± standard deviation and removal efficiency (%) during the 2011–2012 for biochemical oxygen demand (BOD), chemical oxygen demand (COD), ammoniacal nitrogen (AN), total nitrogen (TN) and phosphorus (P) in the system of natural wetlands employed in the posttreatment of the sanitary landfill leachate

	2011											
	Summer			Autumn			Winter			Spring		
	Influent	Effluent	(%)	Influent	Effluent	(%)	Influent	Effluent	%)	Influent	Effluent	(%)
Chemical parameters												
BOD (mg/L)	29.03 ± 0.47	7.10 ± 3.63	–	174.00 ± 142.80	132.20 ± 10.20	–	89.41 ± 57.76[a]	12.95 ± 9.04[a]	86	102.63 ± 23.83[a]	25.44 ± 10.56[b]	76
COD (mg/L)	1,497.48 ± 75.18	91.94 ± 62.89	–	1,766.50 ± 432.30	164.3 ± 105.10	–	1,786.11 ± 428.62[a]	293.09 ± 44.12[b]	83	1,616.73 ± 372.11[a]	496.04 ± 49.46[b]	68
AN (mg/L)	452.63 ± 14.88	4.71 ± 5.25	–	556.40 ± 38.50	29.60 ± 9.80	–	530.95 ± 44.59[a]	42.26 ± 14.00[b]	92	485.23 ± 94.52[a]	73.59 ± 11.24[b]	85
TN (mg/L)	991.27 ± 37.79	7.74 ± 5.85	–	1,443.20 ± 600.40	48.8 ± 17.90	–	1,407.29 ± 452.89[a]	100.42 ± 47.20[b]	92	786.18 ± 139.89[a]	100.61 ± 16.72[b]	87
P (mg/L)	NM	NM	–	3.60 ± 6.20	0.60 ± 0.10	–	7.32 ± 6.30[a]	1.10 ± 0.58[a]	61	13.89 ± 2.96[a]	1.99 ± 0.03[b]	85
	2012											
	Summer			Autumn			Winter			Spring		
	Influent	Effluent	(%)	Influent	Effluent	(%)	Influent	Effluent	(%)	Influent	Effluent	(%)
Chemical parameters												
BOD (mg/L)	72.61 ± 25.31[a]	23.25 ± 14.76[b]	69	104.24 ± 25.2[a]	25.13 ± 11.18[b]	74	103.66 ± 11.02[a]	22.14 ± 7.82[b]	78	266.72 ± 127.52[a]	89.24 ± 40.20[a]	65
COD (mg/L)	1,591.51 ± 121.76[a]	583.52 ± 31.36[b]	63	1,579.52 ± 129.66[a]	737.38 ± 79.67[b]	53	1,730.38 ± 317.39[a]	828.67 ± 97.34[b]	51	2,120.08 ± 550.76[a]	879.78 ± 219.24[b]	62
AN (mg/L)	448.89 ± 36.45[a]	65.93 ± 4.38[b]	85	506.04 ± 26.1[a]	78.72 ± 12.16[b]	84	499.91 ± 59.13[a]	86.86 ± 2.44[b]	82	635.81 ± 35.46[a]	81.93 ± 3.54[b]	78
TN (mg/L)	1,004.29 ± 41.90[a]	96.60 ± 5.99[b]	90	1,213.10 ± 184.10[a]	120.53 ± 24.33[b]	90	1,106.43 ± 37.81[a]	142.99 ± 28.21[b]	87	1,262.92 ± 21.53[a]	131.71 ± 12.09[b]	89
P (mg/L)	14.04 ± 0.78[a]	2.32 ± 0.74[b]	84	12.67 ± 1.63[a]	4.13 ± 0.90[b]	66	12.64 ± 0.13[a]	4.63 ± 0.22[b]	63	11.53 ± 3.44[a]	5.45 ± 0.71[b]	59

Different letters indicate significant differences by *t* test ($p < 0.05$)

NM not measured

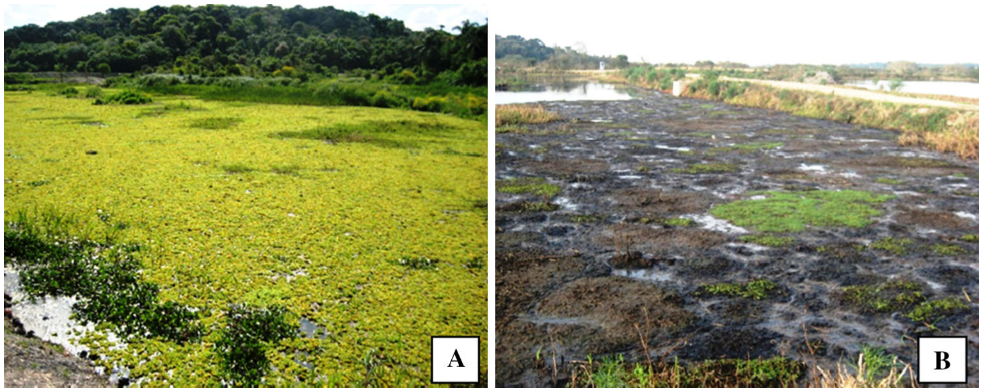

Fig. 3 First natural wetland. **a** Summer of 2011 with the presence of *P. stratiotes* and *E. polystachya*. **b** Spring 2011 with the formation of sludge due to the *P. stratiotes* degradation

wetland with *Typha angustifolia* that presented average removal between 71 and 98 % for BOD and between 43 and 46 % for TN. Souza et al. (2013) used *Myriophyllum aquaticum* in the treatment of polluted waters and demonstrated its potential application in phytoremediation, with treatment efficiency of 75 % for BOD, 67 % for COD and 93 % for P.

In 2 years of study, the wetland system adapted to the conditions imposed, i.e., changes in seasonality, meteorological conditions, pollutant and leachate flow. In the second year of the study, the system did not show a great change in terms of performance. There was a change of BOD from 65 to 78 %, COD from 51 to 63 %, AN from 78 to 85 % and TN from 87 to 90 % (Table 3; Figs. 3, 4). The results show stabilization of the system, which may be related to the fact that wetlands have self-depuration processes, and develop vegetation adapted to the conditions imposed by the environment. In these systems, the water, biota and sediments form a balanced ecosystem with nutrient recycling. According to Hill and Payton (1998) and Manios et al. (2000), the performance of wetlands is not affected by climate change in the different seasons of the year.

The results showed that the organic matter in the leachate degraded along the system, that is, the system's average efficiency in terms of BOD and COD was 75 and 63 %, respectively (Table 3; Figs. 3, 4). The treatment efficiency decreased gradually in the winter and spring (2011) and summer (2012) seasons, likely due to the wetlands adaptation to leachate pollutant load (Figs. 3, 4). The results show that after the leachate entered the wetlands, *P. stratiotes* showed signs of toxicity, followed by death, and was gradually replaced by *E. polystachya*, the species that currently dominates the area (Fig. 5a, b).

COD efficiency continued to decline in the autumn and winter of 2012 (Table 3; Figs. 3, 4), which may be related to the death of all *E. crassipes* individuals in the second wetland and consequent return of nutrients to the system (Table 1; Figs. 3, 4). According to Jing et al. (2001), the death of plants consumes oxygen, which causes a decrease in COD removal efficiency. This is corroborated by this study, where the lowest COD efficiency was 51 % in the winter of 2012 (Table 3; Fig. 4). According to Martinez et al. (2003), if the environment where macrophytes live is not handled properly, many of the nutrients incorporated in the biomass return to the water due to the decomposition process. Therefore, in order to make pollutant removal more effective, plants should be collected at the end of each growing season so that nutrients do not return to the environment.

As a result of the nutrient capture in wetlands, large volumes of biomass are produced (Tanner 1996; Billore et al. 1999; Souza et al. 2013). However, in the winter months with the death of plants, the translocation of nutrients from the aerial part and rhizomes takes place, resulting in an increase of nutrients in the water (Armstrong and Beckett 1992).

Several aspects may have contributed to the removal of the organic load of the leachate, among them aerenchyma and reserve tissue of oxygen present in the structure of the macrophytes. We observed that the two macrophyte species have a well-developed aerenchyma in the leaves and roots, in *E. polystachya* composing about 75 % of the total root volume (Table 2; Fig. 2b) and 45 % in *E. crassipes* (Table 2; Fig. 2c). The intercellular spaces in the aerenchyma of both species compose a continuous system from the leaves to the roots. Previous studies have indicated that the parenchyma of wetland plants can compose up to 60 %

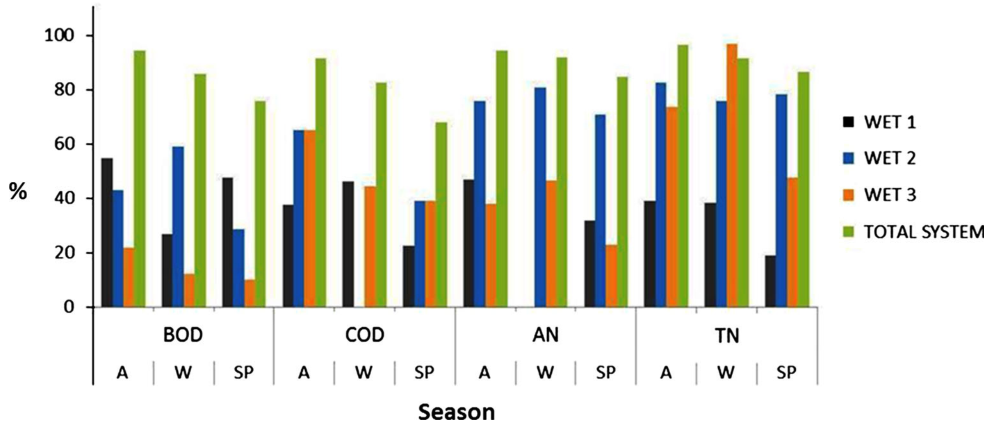

Fig. 4 Seasonal efficiency of posttreatment (%) per wetland and wetland system to parameters biochemical oxygen demand (BOD), chemical oxygen demand (COD), ammoniacal nitrogen (AN) and total nitrogen (TN) in 2011. *WET* wetland, *A* Autumn, *W* winter, *SP* spring

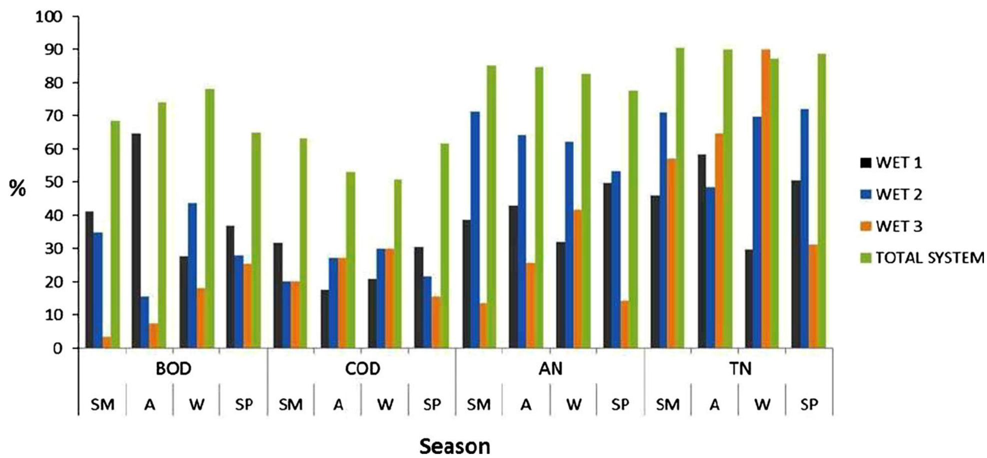

Fig. 5 Seasonal efficiency of posttreatment (%) per wetland and wetland system to parameters biochemical oxygen demand (BOD), chemical oxygen demand (COD), ammoniacal nitrogen (AN) and total nitrogen (TN) in 2012. *WET* wetland, *A* Autumn, *W* winter, *SP* spring

of the root volume (Bedford et al. 1991; Chen et al. 2002; Li et al. 2011).

The phenomenon of oxygen release from the roots through the aerenchyma to the rhizosphere is called radial oxygen loss. It is an active physiological process in wetland plants that can be related to their ability to adapt to flooding (Stottmeister et al. 2003; Lai et al. 2012) and to remove nutrients (Sorrell and Brix 2003; Sasikala et al. 2009). Therefore, *E. polystachya* and *E. crassipes* present high radial oxygen loss, since they have a large number of aerenchyma in the roots (Table 2). This loss causes the concentration of oxygen to be greater in the rhizosphere, diminishing along it (Kirk et al. 1993; Van Bodegom et al. 2001), forming aerobic–anaerobic gradients. This is a benefit for the growth of aerobic, anaerobic and facultative microorganisms, which act to remove nutrients.

Several articles, such as those of Brix (1997), Jones et al. (2005) and Zhang et al. (2010), have mentioned the importance of the aerenchyma in the process of rhizodegradation, a phytoremediation strategy in which oxygen is released by the roots in the rhizosphere, favoring the development of aerobic microorganisms that are involved in the degradation of organic matter.

In a study by Ciria et al. (2005) with constructed wetlands, BOD removal occurred due to biological and physical processes such as sedimentation and biological degradation, primarily by bacteria adhered to the roots of plants. In this study, *E. crassipes*, *A. philoxeroides* and *E. polystachya* presented a biofilm adhered to the roots. This has been reported by several authors in studies of organic pollutant removal by wetlands (Armstrong and Beckett 1992; Ansolla and Fernandez 1995; Tanner et al. 1995; Brix 1997; Naylor et al. 2003; Ciria et al. 2005; Leto et al. 2013; Ansola et al. 2014). For Manios et al. (2003), the presence of emergent macrophytes is not the only factor responsible for the performance of the system; physical

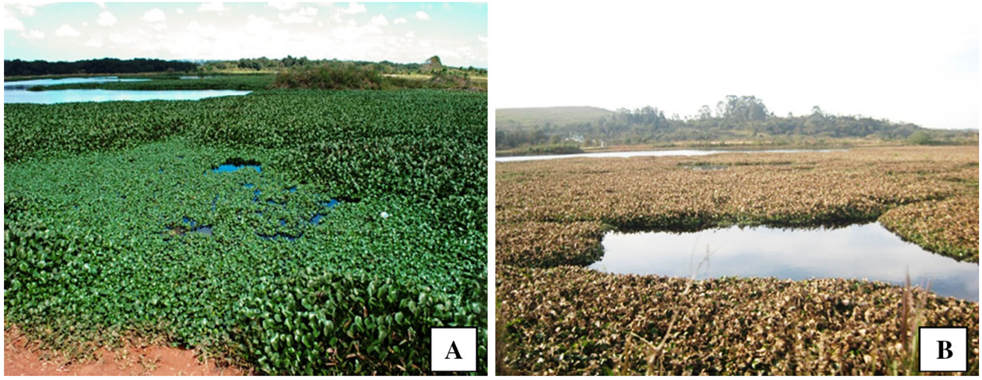

Fig. 6 Second natural wetland. **a** Summer of 2011 with the presence of *E. crassipes*, **b** fall 2012 with the presence of *E. crassipes* showing signs of toxicity

processes such as filtration and sedimentation also lead to COD reduction.

By the end of autumn 2012, *E. crassipes* covered more than 56 % of the area in the second wetland (Table 1; Fig. 6). After the death of its population, sludge formed on the surface due to biomass degradation, favoring the emergence of *A. philoxeroides*, which in the spring of 2012 had already covered about 33 % of the area. These results demonstrate that plants exhibit a set of characteristics probably fixed throughout the evolution of these species and constitute a determining factor for their colonization, such as the presence of aerenchyma in the anatomical structure and tolerance to survive in an environment with various pollutants and scarcity of oxygen.

The average total efficiency of the treatment was 84 % for AN, 89 % for TN and 70 % for P, respectively. All plant species use different forms of nitrogen during their growth, development and biomass allocation (Wang and Li 2011; Zhaia et al. 2013). This process is known as phytoextraction. According to Granato (1995) and Jones et al. (2005), the use of aquatic plants in phytoremediation is explained by the intense absorption of nutrients through foliar and roots uptake used in their growth. According to Ciria et al. (2005), in wetlands nutrient removal occurs through plant nutrient capture by the microorganisms present in the roots, which transform nutrients, especially N, into inorganic compounds (NH_4^+ and NO_3^-) that are directly available to plants and by physical processes, such as sedimentation and filtration.

According to Gumbricht (1993), Brix (1994) and Morris et al. (2009), plants in natural wetlands are very productive, and considerable amounts of nutrients can be used for the formation of biomass. The capture capacity of emergent macrophytes is approximately 30–150 kg/P/ha/year and 200 to 2,500 kg/N/ha/year. Brix (1997) reported that *E. crassipes* had high potential to capture nutrients (350 kg/P/ha/year and 2,000 kg/N/ha/year).

The concentration of AN changed between entering and exiting the system (Table 3; Figs. 3, 4). According to Manios et al. (2002), the removal efficiency of AN is due to the important role of aquatic plants in the translocation of oxygen from the aerial parts to the roots, which facilitates the process of nitrification. For Greenway and Woolley (1999), higher nutrient removal occurs in wetlands by a combination of emergent macrophytes, free floating and submerged, in which higher concentration of N occurs on leaves and stems, and higher concentration of P occurs in roots and rhizomes. Kadlec and Zmarthie (2010) studied a wetland with 0.85 ha of cattail for leachate treatment with 180 days of hydraulic retention and concluded that the natural system has great potential in the control of pollutants, showing 99.5 % efficiency for AN.

Ciria et al. (2005), in a study of constructed wetlands with *T. latifolia* in wastewater treatment, concluded there are no seasonal differences when observing the removal of pollutants, except in the case of P, which showed a higher removal efficiency in the summer. This result agrees with that obtained in this study, in which the highest removal efficiency of P was 84 % in the summer of 2012 (Table 3; Fig. 5).

The removal of nutrients has a close relationship with the plant's growth (Karathanasis et al. 2003; Preussler et al. 2007; Cheng et al. 2009; Liang et al. 2011), biomass

Table 4 Annual average concentration (mg/L) of Cu, Zn, Cr, Ni, Pb and Cd in the leachate at the entrance and outlet of the wetlands system in 2011 and 2012

Leachate of the wetlands system				
2011			2012	
Metals	Entrance	Outlet	Entrance	Outlet
Copper (mg/L)	0.02 ± 0.01[a]	0.01 ± 0.01[b]	0.04 ± 0.03[a]	0.03 ± 0.03[a]
Zinc (mg/L)	0.37 ± 0.20[a]	0.11 ± 0.11[b]	0.45 ± 0.12[a]	0.32 ± 0.22[a]
Chromium (mg/L)	0.20 ± 0.11[a]	0.03 ± 0.03[b]	0.23 ± 0.07[a]	0.08 ± 0.02[b]
Nickel (mg/L)	0.23 ± 0.11[a]	0.09 ± 0.05[b]	0.22 ± 0.05[a]	0.22 ± 0.03[a]
Cadmium (mg/L)	0.01 ± 0.01	ND	0.01 ± 0.01	ND
Lead (mg/L)	0.02 ± 0.01	ND	0.03 ± 0.02[a]	0.01 ± 0.02[b]

Different letters indicate significant differences by *t* test ($p < 0.05$)

ND not detectable

production (Greenway and Woolley 2001) and root morphology (Kyambadde et al. 2004; Cheng et al. 2009).

The results show that *E. crassipes* (Table 1; Fig. 6) was the species with the largest area of coverage in the system during the period of the study. This species grows in moist areas and in nutrient-rich water, and tolerates a wide range of pH, temperature and nutrients adaptation. The ideal pH for the growth of water hyacinth is 6-8, and temperature tolerance is from 1 to 40 °C, while the growth rates increase with the amount of nitrogen available (Heard and Winterton 2000). This species is very productive, showing a mean annual productivity of 50 t/ha/year of dry biomass (Abbasi and Ramasamy 1999).

The heavy metals: Cu, Zn, Cr, Ni, Cd and Pb were quantified in leachate, and the results showed low concentration in the wetland system entrance, also showing an annual average of, in 2011, 0.02 mg/L of Cu; 0.37 mg/L of Zn; 0.20 mg/L of Cr; 0.23 mg/L of N; 0.01 mg/L of Cd; 0.02 mg/L of Pb; in 2012, 0.04 mg/L of Cu; 0.45 mg/L of Zn; 0.23 mg/L of Cr; 0.22 mg/L of Ni; 0.01 mg/L of Cd; and 0.03 mg/L of Pb (Table 4).

In 2011, when the concentration of metals in leachate in the wetlands system entrance and outlet was compared, there was considerable difference ($p < 0.05$) in the concentration of Cu, Cr, Zn and Ni. Zn was the metal with the highest concentration in the system entrance with 0.37 mg/L. The concentration of Pb and Cd was not detected in the system outlet samples (Table 4).

In 2012, the same comparison showed considerable difference ($p < 0.05$) only in the concentration of Cr and Zn was the metal with the highest concentration, 0.45 mg/L (Table 4).

Our results showed low concentration of heavy metals in leachate. Morais et al (2006) analyzed the leachate in Caximba Sanitary Landfill, Curitiba, PR, Brazil, and detected the following concentrations of heavy metals: 0.28 md/dm of Pb; 0.36 md/dm of Cu; 0.45 md/dm of Cr; 1.06 md/dm of Zn; and 1.43 md/dm of Ni. They attributed the low concentration of metals to the alkaline pH of the leachate. The results revealed that the leachate from Caximba Sanitary Landfill does not pose a contamination threat to the environment regarding the heavy metals studied.

Heavy metals analysis in biomass plants

The results of heavy metals concentration in the root and branches *E. polystachya* and *E. crassipes*, in 2011 and 2012, can be seen in Table 5. Both species concentrated heavy metals in their roots and branches. There was considerable difference ($p > 0.05$) when the concentration of heavy metals between *E. polyslachya* and *E. crassipes* was compared. These results, however, do not express any tendencies concerning season or plant part.

Cu is an element that influences the basal metabolism and the nitrogen in the secondary metabolism of plants. It appears in plants in concentrations between 4 and 20 mg/kg (Larcher 2006). In the present study, *E. polystachya* showed, in its roots, concentrations between 0.29 and 3.03 µg/g and between 0.27 and 1.54 µg/g in its branches. *E. crassipes* had a concentration between 0.86 and 0.25 µg/

Table 5 Median ± standard deviation for concentration (μg/g) of Cu, Cr, Zn, Ni, Pb and Cd in the roots and branches of *E. polystachya* and *E. crassipes* in the summer, autumn, winter and spring and of 2011 and 2012

Metals	Plant part	*E. polystachya*				*E. crassipes*			
		2011				2011			
		Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring
Cooper (μg/g)	Root	0.48 ± 0.01^{b}	2.20 ± 0.06^{a}	0.29 ± 0.02^{b}	1.43 ± 0.03^{a}	0.60 ± 0.04^{a}	0.54 ± 0.14^{b}	0.85 ± 0.28^{a}	0.66 ± 0.28^{b}
	Branche	0.33 ± 0.03^{a}	0.39 ± 0.02^{a}	1.39 ± 0.19^{a}	1.39 ± 0.19^{a}	0.28 ± 0.05^{a}	0.31 ± 0.04^{b}	0.31 ± 0.13^{a}	0.25 ± 0.07^{b}
Chromium (μg/g)	Root	3.31 ± 0.07^{b}	4.34 ± 0.22^{a}	3.52 ± 0.07^{a}	4.53 ± 0.36^{a}	4.45 ± 0.82^{a}	3.65 ± 0.15^{a}	4.63 ± 1.46^{a}	3.40 ± 0.70^{a}
	Branche	2.64 ± 0.39^{a}	3.63 ± 0.32^{a}	4.53 ± 0.36^{a}	1.39 ± 0.19^{b}	3.07 ± 0.20^{a}	3.01 ± 0.24^{b}	2.22 ± 0.53^{b}	2.00 ± 1.11^{b}
Zinc (μg/g)	Root	5.47 ± 0.09^{a}	7.02 ± 0.06^{a}	5.68 ± 0.14^{a}	4.02 ± 0.06^{a}	3.07 ± 0.25^{b}	4.28 ± 1.21^{b}	5.47 ± 1.32^{a}	5.11 ± 2.75^{a}
	Branche	2.54 ± 0.20^{a}	6.98 ± 0.47^{a}	3.63 ± 0.17^{a}	3.34 ± 0.31^{a}	2.93 ± 0.41^{a}	3.39 ± 0.36^{b}	2.52 ± 0.34^{b}	4.05 ± 2.05^{a}
Nickel (μg/g)	Root	ND	0.58 ± 0.01^{a}	0.12 ± 0.01^{b}	0.48 ± 0.02^{a}	0.66 ± 0.21^{a}	0.45 ± 0.02^{b}	0.67 ± 0.26^{a}	0.49 ± 0.24^{a}
	Branche	ND	0.26 ± 0.05^{a}	0.60 ± 0.06^{b}	0.60 ± 0.03^{a}	ND	ND	ND	0.07 ± 0.08^{b}
Lead (μg/g)	Root	ND	ND	ND	ND	5.20 ± 4.64	0.40 ± 0.44	1.69 ± 1.85^{b}	ND
	Branche	ND	ND	ND	ND	ND	ND	ND	ND
Cadmium (μg/g)	Root	ND	0.04 ± 0.00^{a}	ND	ND	0.36 ± 0.32	0.05 ± 0.02^{a}	0.12 ± 0.06^{a}	0.33 ± 0.37
	Branche	ND	ND	ND	ND	ND	ND	ND	ND

Metals	Plant part	*E. polystachya*				*E. crassipes*			
		2012				2012			
		Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring
Cooper (μg/g)	Root	1.03 ± 0.07^{a}	3.03 ± 0.04^{a}	2.24 ± 0.03^{a}	0.81 ± 0.02^{a}	0.68 ± 0.14^{b}	0.79 ± 0.16^{b}	0.86 ± 0.11^{b}	0.52 ± 0.03^{b}
	Branche	1.54 ± 0.21^{a}	0.32 ± 0.05^{a}	0.27 ± 0.04^{a}	0.85 ± 0.08^{a}	1.87 ± 1.77^{a}	0.57 ± 0.26^{a}	0.42 ± 0.18^{a}	0.37 ± 0.02^{b}
Chromium (μg/g)	Root	3.41 ± 0.16^{b}	3.05 ± 0.09^{b}	3.81 ± 0.06^{a}	3.05 ± 0.08^{a}	4.46 ± 0.36^{a}	4.07 ± 0.20^{a}	4.19 ± 0.69^{a}	3.25 ± 0.12^{a}
	Branche	4.22 ± 0.29^{a}	3.14 ± 0.08^{a}	3.12 ± 0.31^{a}	2.59 ± 0.14^{a}	2.80 ± 0.23^{b}	3.46 ± 0.27^{a}	2.88 ± 0.42^{a}	2.66 ± 0.18^{a}
Zinc (μg/g)	Root	3.50 ± 0.07^{a}	5.95 ± 0.07^{a}	6.03 ± 0.04^{a}	2.17 ± 0.17^{b}	3.07 ± 0.25^{b}	4.28 ± 1.21^{b}	5.47 ± 1.32^{a}	5.11 ± 2.75^{a}
	Branche	4.07 ± 0.09^{a}	5.24 ± 0.28^{a}	5.53 ± 0.43^{a}	5.53 ± 0.43	3.77 ± 0.75^{a}	5.51 ± 0.68^{a}	3.41 ± 0.38^{b}	2.99 ± 0.13^{a}
Nickel (μg/g)	Root	0.37 ± 0.00^{b}	0.33 ± 0.02^{b}	0.46 ± 0.04^{b}	0.33 ± 0.04^{a}	0.55 ± 0.09^{a}	0.65 ± 0.12^{a}	0.64 ± 0.12^{a}	0.38 ± 0.04^{a}
	Branche	0.62 ± 0.03^{a}	0.20 ± 0.04^{a}	0.16 ± 0.02^{a}	0.25 ± 0.02^{a}	ND	0.04 ± 0.09^{b}	ND	0.13 ± 0.22^{b}
Lead (μg/g)	Root	ND	ND	0.53 ± 0.01^{b}	0.39 ± 0.01^{a}	ND	ND	2.53 ± 1.77^{a}	ND
	Branche	ND	ND	ND	ND	4.09 ± 3.70	ND	ND	ND
Cadmium (μg/g)	Root	ND	ND	ND	ND	0.02 ± 0.02^{b}	0.06 ± 0.07^{a}	0.18 ± 0.16^{a}	ND
	Branche	ND	ND	ND	ND	0.32 ± 0.30^{a}	ND	ND	ND

Different letters indicate significant differences by *t* test ($p < 0.05$)

ND not detectable

g in its roots and a concentration between 0.62 and 0.16 μg/g in its branches. Therefore, the concentration found in both plants' branches is below the limits of acceptable average for their development.

Zn is a micronutrient present in plants in a concentration that ranges between 1 and 400 mg/kg (Larcher 2006). In the present study, *E. polystachya* had, in its roots, a concentration between 2.17 and 7.02 μg/g while its branches had a concentration between 2.54 and 6.98 μg/g. *E. crassipes* band between 0.38 and 5.51 μg/g in its branches. Based on the information above, the concentration found in both plants is within the desirable limits for their growth and development. Cr is found in plants in low concentrations, between 5 and 30 μg/g (Kabata-Pendias and Pendias 2001). In this study, *E. polystachya* showed, in its roots, concentrations between 3.05 and 4.53 μg/g and its branches had it between 1.39 and 4.53 μg/g. *E. crassipes* had a Cr concentration between 3.25 and 4.63 μg/g in its roots and between 2.00 and 3.46 μg/g in its branches. Mishra and Tripathi (2009) studied the removal of Cr and Zn by *E. crassipes* from a solution contaminated with such heavy metals. They explained that the later species is one that keeps Zn and Cr because it presented concentrations between 3,542 and 2,412 mg/L of these metals, respectively. Regardless of treatment concentrations, the root was more effective in accumulating Zn than the branches.

Ni is a micronutrient for plants, and it is found in them in concentrations that range between 0.1 and 5 μg/g. In this study, *E. polystachya* had a root concentration of Ni between 0.12 and 0.58 μg/g and its branches had numbers between 0.16 and 0.62 μg/g. *E. crassipes* showed a root concentration of Ni between 0.38 and 0.67 μg/g, and its branches had numbers between 0.62 and 0.16 μg/g. All concentrations are acceptable for the plants' growth and development. Cd is a nonessential and toxic metal for plants. In the present study, *E. polystachya* presented a Cd concentration of 0.04 μg/g only in its roots. *E. crassipes* had it between 0.02 and 0.36 μg/g in its roots and up to 0.32 μg/g in its branches. Based on the numbers above, authors concluded that *E. polystachya* is a species that accumulates plenty of Cd in its parts.

Pb is a heavy metal which plants do not need and that can be toxic to them in concentrations between 0.03 and 30 μg/g (Kabata-Pendias and Pendias 2001). In the present study, *E. polystachya* had a Pb concentration between 0.39 and 0.53 μg/g only in its roots while *E. crassipes* had it between 0.40 and 5.20 μg/g in its roots and up to 0.59 μg/g in its branches.

The performance of the wetlands system was efficient, demonstrating potential application in the posttreatment of leachate. This performance is related to the presence of emerging and floating macrophytes, which reduces leachate speed, creates better conditions for sedimentation of suspended solids, reduces the risk of erosion and re-suspension and increases the time of contact between the water and the surfaces of plants (Brix 1997). Moreover, the movements of the plants as a result of the action of the wind keep the surface clear, and the growth of the roots within the filtering medium helps to decompose the organic matter. The aerial parts of the macrophytes that are submerged in the water column provide a large surface area for biofilms (Gumbricht 1993; Chappell and Goulder 1994), which are responsible for most of the microbial processing that occurs in wetlands.

The wetlands system includes high vegetation productivity, existence of large surfaces of adsorption of the soil and on plants, presence of aerobic and anaerobic regions, sedimentation and filtration of the soil, nutrient absorption by plants, adsorption of metals in the soil and on plants, nutrient cycling, i.e., characteristics and processes that are able to transform many pollutants into less harmful products, and into nutrients to be used by the biota (Kadlec 1995; Kadlec and Knight 1996).

Conclusion

The performance of the wetlands system was effective in the treatment of the leachate in the 2 years of study and adapted well to changes in seasonality, meteorological conditions and flow of pollutants and leachate.

The results suggest that removal of organic matter and nutrients occurred through the strategies of phytoextraction, phytostimulation and rhizodegradation, respectively. Phytoremediation processes, in addition to promoting posttreatment of the leachate, generate a high production of vegetable biomass, mainly *Eichhornia crassipes*, which was the largest vegetation coverage in the system. The results revealed stabilization of the system, which can be related to the fact that the wetlands presented self-depuration processes and developed vegetation adapted to the conditions imposed by the leachate. The biota, sediment, leachate, precipitation and evapotranspiration make the system a balanced environment with nutrient cycling. The system employed proved to be a viable alternative that can be combined with other leachate treatments.

It is suggested the management of natural wetlands system due to extensive coverage of macrophytes present so that the saturation of the system does not occur and the study of the composition of the plants biofilm to be able to identify the micro-organisms present, as well as the role of each of the degradation of the pollutants.

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References

Abbasi AS, Ramasamy EV (1999) Biotechnological methods of pollution control. Orient Longman, Hyderabad

Ansola G, Arroyo P, Sáenz de Miera LE (2014) Characterization of the soil bacterial community structure and composition of natural and constructed wetlands. Sci Total Environ 473–474:63–71

Ansolla G, Fernandez CLE (1995) Removal of organic matter and nutrients from urban wastewater by using an experimental emergent aquatic macrophyte system. Ecol Eng 5:13–19

Apha-Awwa-Wef (1998) Standard methods for the examination of water and wastewater, 20th edn. American Public Health Association, Washington, DC

Armstrong W, Beckett PM (1992) *Phragmites australis*: venturi and humidity-induced pressure flows enhance rhizome aeration and rhizosphere oxidation. New Phytol 120:197–207

Bedford BL, Bouldin DR, Beliveau BD (1991) Net oxygen and carbon-dioxide balances in solutions bathing roots of wetland plants. J Ecol 79:943–959

Berlyn GP, Miksche JP (1976) Botanical microtechnique and cytochemistry. State University Press, Iowa

Bialowiec A, Davies L, Albuquerque A, Randerson PF (2012) The influence of plants on nitrogen removal from landfill leachate in discontinuous batch shallow constructed wetland with recirculating subsurface horizontal flow. Ecol Eng 40:44–52

Billore SK, Singh N, Sharma JK, Nelson RM (1999) Horizontal subsurface flow gravel bed constructed wetland with *Phragmites karka* in central India. Water Sci Technol 40:163–171

Braun-Blanquet J (1979) Fitossociologia: base para el estúdio de lãs comunidades vegetales. H. Blume ediciones, Madrid

Brix H (1994) Functions of macrophytes in constructed wetlands. Water Sci Technol 29:71–78

Brix H (1997) Do macrophytes play a role in constructed treatment wetlands? Water Sci Technol 35:11–17

Carneiro C, Reissmann CB, Marques R (2006) Comparação de métodos de análise química de K, Ca, Mg e Al em folhas de erva-mate (*Ilex paraguarienses*, St. Hil.). Cerne Lavras 12:113–122

Chappell KR, Goulder R (1994) Seasonal variation of epiphytic extracellular enzyme activity on 2 freshwater plants, *Phragmites australis* and *Elodea canadensis*. Archiv für Hydrobiologie 132:237–253

Chen HJ, Qualls RG, Miller GC (2002) Adaptive responses of *Lepidium latigolium* to soil flooding: biomass allocation, adventitious rooting, aerenchyma formation and ethylene production. Environ Exp Bot 48:119–128

Cheng Y, Guo L (2014) Treatment of municipal landfill leachate using magnetic porous ceramsite carrier. J Water Reuse Desalin 4(2):100–108

Cheng XY, Chen WY, Gu BH, Liu XC, Chen F, Chen ZH, Zhou XY, Li YX, Huang H, Chen YJ (2009) Morphology, ecology, and contaminant removal efficiency of eight wetland plants with differing root systems. Hydrobiologia 623:77–85

Chiemchaisri C, Chiemchaisri W, Junsod J, Threedeach S, Wicranarachchi PN (2009) Leachate treatment and greenhouse gas emission in subsurface horizontal flow constructed wetland. Bioresour Technol 100:3808–3814

Ciria MP, Solano ML, Solano P (2005) Role of macrophyte *Typha latifolia* in a Constructed Wetland for wastewater treatment and assessment of its potential is a biomass fuel. Biosyst Eng 92:535–544

Frank A, Turner G, Iannacone LR, Touchette BW (2010) Ecophysiological responses of five emergent-wetland plants to diminished water supply: an experimental microcosm study. Aquat Ecol 44:101–112

Granato M (1995) Utilização do aguapé no tratamento de efluentes com cianetos. Série Tecnologia Ambiental 5:1–2

Greenway M, Woolley A (1999) Constructed wetlands in Queensland: performance efficiency and nutrient bioaccumulation. Ecol Eng 12:39–55

Greenway M, Woolley A (2001) Changes in plant biomass and nutrient removal over three years in a constructed wetland in Cairns, Australia. Water Sci Technol 44:303–310

Gumbricht T (1993) Nutrient removal processes in freshwater submersed macrophytes systems. Ecol Eng 2:1–30

Heard TA, Winterton SL (2000) Interactions between nutrient status and weevil herbivory in the biological control of water hyacinth. J Appl Ecol 37:117–127

Hill DT, Payton JD (1998) Influence of temperature on treatment efficiency of constructed wetlands. Trans ASAE 41:393–396

Jing SR, Lin YF, Lee DY, Wang TW (2001) Nutrient removal from polluted river water by using constructed wetlands. Bioresour Technol 76:131–135

Johansen DA (1940) Plant microtechnique. McGraw Hill Book, New York

Jones DL, Williamson KL, Owen AG (2005) Phytoremediation of landfill leachate. Waste Manag 26:825–837

Justin MZ, Zupancic M (2009) Combined purification and reuse of landfill leachate by constructed wetland and irrigation of grass and willows. Desalination 246:157–168

Kabata-Pendias A, Pendias H (2001) Trace elements in soils and plants, 3rd edn. CRC Press, Boca Raton

Kadlec RH (1995) Overview: surface flow constructed wetlands. Water Sci Technol 32:1–12

Kadlec RH, Knight RI (1996) Treatment wetlands. Lewis, Boca Raton

Kadlec RH, Zmarthie LA (2010) Wetland treatment of leachate from a closed landfill. Ecol Eng 36:946–957

Karathanasis AD, Potter CL, Coyne MS (2003) Vegetation effects on fecal bacteria, BOD, and suspended solid removal in constructed wetlands treating domestic wastewater. Ecol Eng 20:157–169

Kirk GJD, Begg CBM, Solivas JL (1993) The chemistry of the lowland rice rhizosphere. Plant Soil 156:83–86

Kyambadde J, Kansiime F, Gumaelius L, Dalhammar G (2004) A comparative study of *Cyperus papyrus* and *Miscanthidium violaceum* based constructed wetlands for wastewater treatment in a tropical climate. Water Resour 38:475–485

Lai WL, Zhamg Y, Zhang HC (2012) Radial oxygen loss, photosynthesis, and nutrient removal of 35 wetland plants. Ecol Eng 39:24–30

Larcher W (2006) Ecofisiologia vegetal, 3rd edn. São Carlos, Rima

Leto C, Tuttolomondo T, La Bella S, Leone R, Licata M (2013) Effects of plant species in a horizontal subsurface flow constructed wetland phytoremediation of treated urban wastewater with *Cyperus alternifolius* L. and *Typha latifolia* L. in the West of Sicily (Italy). Ecol Eng 61:282–291

Li H, Ye ZH, Wei ZJ, Wong MH (2011) Root porosity and radial oxygen loss related to arsenic tolerance and uptake in wetland plants. Environ Pollut 159:30–37

Liang MQ, Zhang CF, Peng CL, Lai ZL, Chen DF, Chen Z (2011) Plant growth, community structure, and nutrient removal in monoculture and mixed constructed wetlands. Ecol Eng 37:309–316

Malavolta E, Vitti GC, Oliveira SA (1997) Avaliação do Estado Nutricional das plantas: princípios e aplicações, 2nd edn. Piracicaba, Potafos

Manios T, Millner P, Stentiford EI (2000) Effect of rain and temperature on the performance of constructed reed beds. Water Environ Res 72:305–312

Manios T, Stentiford EI, Millner P (2002) The removal of NH3-N from primary treated wastewater in surface reed beds using different substrates. J Environ Sci Health A 37:297–308

Manios T, Stentiford EI, Millner P (2003) The removal of chemical oxygen demand from primary-treated domestic wastewater in surface-flow reed beds using different substrates. Water Environ Res 75:336–341

Marques M (2005) Phytoremediation. In: KALMAR ECO-TECH'05: waste to energy, bioremediation and leachate treatment, Kalmar, 19–26

Marques M, Aguiar CRC, Silva JJLS (2011) Desafios técnicos e barreiras sociais, econômicas e regulatórias na fitorremediação de solos contaminados. Revista Brasileira de Ciência do solo 35:1–11

Martinez F, Cuevas G, Calvo R, Walter I (2003) Biowaste effects on soil and native plants in a semiarid ecosystem. J Environ Qual 32:472–479

Minerais do Paraná (MINEROPAR) (2004) Atlas geológico do Estado do Paraná. Curitiba. Mineropar, 1 CD-ROM

Mishra VK, Tripathi BD (2009) Concurrent removal and accumulation of heavy metals by the three aquatic macrophytes. Bioresour Technol 99:7091–7097

Morais JL, Sirtori C, Peralta-Zamora PG (2006) Tratamento de chorume de aterro sanitário por fotocatálise heterogênea integrada a processo biologic convencional. Quim Nova 29:20–23

Morris JT, Bernot RJ, Bernot MJ (2009) Nutrient cycling relative to N and C natural abundance in coastal wetland with long term nutrient additions. Aquat Ecol 43:803–813

Naylor S, Brisson I, Labelle MA, Drizo A, Comeau Y (2003) Treatment of freshwater fish farm effluent using constructed wetlands: the role of plants and substrate. Water Sci Technol 48:215–222

Nivala J, Hoo MB, Cross C, Wallace S, Parkin G (2007) Treatment of landfill leachate using an aerated, horizontal subsurface-flow constructed wetland. Sci Total Environ 380:19–27

O'Brien TP, Feder N, McCully ME (1965) Polychromatic staining of plant cell walls by toluidine blue O. Protoplasma 59:368–373

Preussler KH, Maranho LT, Guimarães ATB, Carvalho Filho MAS, Cubas S, Azevedo JAM (2007) Influência do efluente doméstico na folha da macrófita *Cladium mariscus* (L.) Pohl, Cyperaceae. Revista Brasileira de Biociências 5:819–821

Renou S, Givaudan JG, Poulain S, Dirassouvan F, Moulin P (2008) Landfill leachate treatment: review and opportunity. J Hazard Mater 150:468–493

Sasikala S, Tanaka N, WahWah HSY, Jinadasa KBSN (2009) Effects of water level fluctuation on radial oxygen loss, root porosity, and nitrogen removal in subsurface vertical flow wetland mesocosms. Ecol Eng 35:410–417

Shildar MKC, Sharma BM (1980) *Pistia stratiotes* L. in Nigerian waters. Cell Mol Life Sci 36:953–955

Sorrell BK, Brix H (2003) Effects of water vapour pressure deficit and stomatal conductance on photosynthesis, internal pressurization and convective flow in three emergent wetland plants. Plant Soil 253:71–79

Souza FA, Dziedzic M, Cubas SA, Maranho LT (2013) Restoration of polluted Waters by phytoremediation using *Myriophyllum aquaticum* (Vell.) Verdc, Haloragaceae. J Environ Manag 120:5–9

Stottmeister U, Wiessner A, Kuschk P, Kappelmeyer U, Kästner M, Bederski O, Müller RA, Moormann H (2003) Effect of plants and microorganism in constructed wetlands for wastewater treatment. Biotechnol Adv 22:93–117

Tanner CC (1996) Plant for constructed wetland treatment systems - A comparison of the growth and nutrient uptake of eight emergent species. Ecol Eng 7:59–83

Tanner CC, Clayton JS, Upsdell MP (1995) Effect of loading rate and planting on treatment of dairy farm wastewater in constructed wetlands-I. Removal of oxygen demand, suspended solids and faecal coliforms. Water Res 29:17

Van Bodegom P, Goudriaan J, Leffelaar P (2001) A mechanistic model on methane oxidation in a rice rhizosphere. Biogeochemistry 55:145–177

Wang J, Li W (2011) Influence of light and nitrate assimilation on the growth strategy in clonal weed *Eichhornia crassipes*. Aquat Ecol 45:1–9

Zanello S, Melo VF, Wowk G (2009) Mineralogia e teores de cromo, níquel, cobre, zinco e chumbo nos solos no entorno do aterro sanitário da Caximba em Curitiba-PR. Scientia Agraria 10:51–60

Zhaia X, Piwpuana N, Arias CA, Headley T, Brix H (2013) Can root exudates from emergent wetland plants fuel denitrification in subsurface flow constructed wetland systems. Ecol Eng 61:555–563

Zhang BY, Zhebg JS, Sharp RG (2010) Phytoremediation in engineered wetlands: mechanisms and applications. Proc Environ Sci 2:1315–1325

Zimmels Y, Kirzhner F, Malkovskaja A (2006) Application of *Eichhornia crassipes* and *Pistia stratiotes* for treatment of urban sewage in Israel. J Environ Manag 81:420–428