Rev. FCA UNCUYO. 2012. 44(2): 47-64. ISSN impreso 0370-4661. ISSN (en línea) 1853-8665.

Remediation of feedlot effluents using aquatic plants

Remediación de efluentes provenientes de feedlots mediante el uso de plantas acuáticas

Pedro Federico Rizzo [1]
Silvana Arreghini [2]
Roberto José María Serafini [2]
Patricia Alina Bres [1]
Diana Elvira Crespo [1]
Alicia Rosa Fabrizio de Iorio [2]

Originales: Recepción: 03/08/2011 - Aceptación: 26/07/2012

ABSTRACT

Feedlots have increased in several regions of Argentina, particularly in the Pampas. The absence of adequate treatments of the effluents produced in these establishments creates serious problems to the society. Phytoremediation can be defined as inexpensive and environmentally sustainable strategy used to remove pollutants by plants. The aim of this study was to evaluate the remediation potential of two macrophyte species (*Eichhornia crassipes* and *Hydrocotyle ranunculoides*) on a feedlot effluent. This effluent was treated with these species for 31 days. Control and macrophyte treatments decreased dissolved inorganic nitrogen (DIN), Kjeldahl nitrogen (Kj N), biological oxygen demand (BOD), chemical oxygen demand (COD), total dissolved salts (TDS), total phosphorus (TP), Pb, Zn and Cr levels. At macrophyte treatments, relatively constant pH levels were kept and decreased EC and TDS values were obtained compared to control, mitigating the release of contaminants and potential greenhouse gases to the atmosphere. Moreover, significant increases in biomass were obtained, being higher in *E. crassipes*. The results allow concluding that the presence of aquatic plants increases the removal rates of nutrients, organic matter and heavy metals from wastewater in approximately 10-17 days for a feedlot effluent with high organic load.

RESUMEN

En diversas regiones de la Argentina, en particular en la región Pampeana, se han incrementado los sistemas de engorde a corral (*feedlots*). La ausencia de tratamientos adecuados de los efluentes producidos por estos establecimientos crea severos problemas para la sociedad. El uso de plantas nativas para la remediación de sistemas contaminados es una tecnología de muy bajo costo y ambientalmente sustentable. El objetivo de este estudio fue evaluar el potencial de remediación de dos especies de plantas acuáticas (*Eichhornia crassipes* e *Hydrocotyle ranunculoides*) sobre un efluente de feedlot. Este efluente fue tratado con estas especies durante 31 días. Tanto el tratamiento control como los tratamientos con macrófitas disminuyen los niveles de nitrógeno inorgánico disuelto, nitrógeno Kjeldahl, demanda bioquímica de oxígeno, demanda química de oxígeno, sales totales disueltas (STD), fósforo total, Pb, Zn y Cr. El uso de macrófitas mantuvo relativamente constante los niveles de pH, disminuyendo CE y STD respecto del control, pudiendo mitigar la liberación de contaminantes hacia la atmósfera. Además se alcanzaron incrementos significativos de biomasa, siendo mayores en *E. crassipes.* Los resultados permiten concluir que la presencia de plantas acuáticas incrementan las tasas de remoción de nutrientes, material orgánico y metales pesados en aproximadamente 10-17 días desde un efluente con alta carga orgánica.

---

1 Laboratorio de Transformación de Residuos. Instituto de Microbiología y Zoología Agrícola. INTA. Las Cabañas y Los Reseros s/n. Castelar, Buenos Aires, Argentina. B1712WAA. prizzo@cnia.inta.gov.ar
2 Cát. de Química Analítica. Facultad de Agronomía. Universidad de Buenos Aires. Av. San Martín 4453. Buenos Aires, Argentina. C1417DSE.

Keywords
intensive livestock systems • effluent • pollution • phytoremediation macrophytes

Palabras clave
sistema de engorde a corral • efluente • polución • fitorremediación • macrófita

INTRODUCTION

The global population growth, the changes in eating habits in developing countries and the use of cereals, sugar, oilseeds and vegetable oils to produce fossil fuel substitutes have generated a steady growth in the global demand for food (http://www.fao.org). In the last decade, the production systems have recorded a shift to continuous cropping and a displacement of the agricultural frontier into traditional farming areas. This has led to the dramatic increase in the intensity of land use resulting in the eutrophication of surface waters (7).

The development of modern agriculture with an improved production efficiency and specialization has led to the separation of livestock and agriculture practices, and to the production of an excess of manure in small areas (17). A daily manure production (5-6% of the animal body weight) is equivalent to almost twice the food it eats (21). These large volumes of manure contain high concentrations of nutrients, organic matter, minerals and pathogens, which, if not provided a proper destination, result in the generation of adverse effects on the environment, local residents and even the production itself. One of the negative aspects of feedlot installation in wet regions is the potential pollution of groundwater, streams and lakes due to the confined animals manure effect (3, 10).

Oxic and anoxic decantation ponds are often used to reduce the pollution load of the effluent in intensive farming systems. However, some studies have reported that the levels found in these effluents generate a high impact on surface waters (7). This leads to the need to improve the quality of water that reaches inland water bodies.

The recovery of diversity in heavily modified areas has a high ecological value. The use of aquatic plants is a strategy for managing contaminated sediments and wastes, which has proven its efficiency in the removal of a wide range of organic substances as well as of nutrients and heavy metals (4, 24). The use of native plants has the added advantage of recovering wildlife habitats. The required residence time is a function of the degradation/removal rates and treatment goals and will vary depending on the specific contaminants. Several authors showed high removal percentages of nutrients and heavy metals in a relatively short period of time (4, 24). *Eichhornia crassipes* (water hyacinth) and *Hydrocotyle ranunculoides* (pennywort) are native floating macrophytes widely distributed in the aquatic systems of Argentina (13). While there are many reports on the purifying action of *E. crassipes* (14), *H. ranunculoides* has been comparatively less studied. Field observation has allowed selecting *H. ranunculoides* and *E. crassipes*, since they are often present in contaminated water bodies in areas associated of highly industrialized areas.

The aims of this study were to evaluate the ability of *H. ranunculoides* and *E. crassipes* and to establish the needed time to remediate a feedlot effluent with a high organic load.

MATERIALS AND METHODS

Collection of plants and wastewater

H. ranunculoides and *E. crassipes* plants were collected from an uncontaminated site located in Escobar city, Buenos Aires, Argentina. Wastewater was obtained from a 10-yard feedlot (50 m x 700 m) located in Mercedes, Buenos Aires. The land slope in the feedlot is 0.1%, which allows the flow of the effluent to a central sedimentation channel. This channel leads to an artificial lagoon inside a cattle breeding establishment (150 m x 100 m x 4 m), from which effluent was collected.

Experimental design

The plants were placed into ponds with nutritive medium for 34 days for acclimatization. Then, viable individuals with similar size and shape were selected and placed into a container with 90 liters of wastewater. Two treatments were carried out (T_H: with 800 g of fresh weight of *H. ranunculoides*; and T_E: with 800 g of fresh weight of *E. crassipes*) and compared with control treatment without plants (T_C). All treatments were carried out in triplicate. The experiment was carried out in a greenhouse between 19 - 25. 5°C .

Water samples from each container were collected at 0, 3, 7, 10, 14, 17 and 31 days from the beginning of the experiment. The water lost by absorption and/or evapotranspiration processes was compensated daily with distilled water to prevent the concentration contaminant effects.

Then, the solution was homogenized and a composite sample (three subsamples) was collected from each container.

Physical-chemical analysis of water

Water electrical conductivity (EC), pH and temperature were measured at each sampling date. Water samples were filtered through Whatman GF/C filters and concentrations of soluble reactive phosphorus (SRP), nitrate plus nitrite (here informed as $N\text{-}NO_3^-$), ammonium ($N\text{-}NH_4^+$), calcium and magnesium were determined according APHA-AWWA-WPCF (2).

The concentration of dissolved inorganic nitrogen (DIN) was calculated as the sum of ammonium, nitrate and nitrite. Suspended matter (SM) was estimated by gravimetry, and digestion of these solids with sulphochromic solution was used to determine particulate organic carbon (POC) (9).

Chemical oxygen demand (COD), Kjeldahl nitrogen (Kj N) and total phosphorus (TP) were determined according to Page *et al*. (18). Biochemical oxygen demand (BOD_5) was determined at 0, 17 and 31 days from the beginning of experiment.

Concentrations of Cu, Cr, Zn and Pb were determined through acid mineralization and atomic absorption spectrophotometry, only at the beginning and at the end of the experiment.

Total dissolved solids (TDS) were calculated using the following formula (6):

$$\mathbf{TDS\ (mg.L^{-1}) = EC\ (\mu S.\ cm^{-1})\ x\ 0.64}$$

Percentage of nutrient removal (%R) was calculated using the formula (25, 26):

$$\mathbf{\%R = ((C_i - C_f)\ x\ 100)/C_i}$$

where:
C_i and C_f are the initial and final concentrations of nutrients in the water respectively.

To determinate the improvement in the quality conditions of the effluent, we based on "Wastewater quality guidelines for agriculture use" established by FAO (20). To complement this, we used an argentine resolution, which establishes the maximum allowed physico - chemical levels of the effluent to discharge to a surface water bodies and soil (1). The table shows the wastewater quality guidelines established by FAO and AGOSBA Resolution.

Table. Initial physical-chemical characteristics of wastewater and wastewater quality guidelines for agriculture use (WQGA) established by FAO and AGOSBA Resolution 336/03.
Tabla. Características físicas y químicas iniciales del efluente, y niveles guía de calidad de efluentes para uso agrícola establecidos por la FAO y la Resolución AGOSBA 336/03.

Variable[a]	Initial physico-chemical characteristics of wastewater	WQGA (FAO) Degree of restriction on agricultural use			AGOSBA Resolution Discharge limits for:	
		None	Slight to moderate	Severe	Surface water bodies	Soil
pH	7.8		6.5 - 8		6.5 - 10	6.5 - 10
EC	1.78	< 0,7	0.7 - 3	> 3	-	-
TDS	1140	< 450	450 - 2000	> 2000	-	-
Kj N	113	-	-	-	≤ 35	≤ 105
$N\text{-}NH_4^+$	44	-	-	-	≤ 25	≤ 75
$N\text{-}NO_3^-$	1.76	< 5	5 - 30	> 30	-	-
POC	17.3					
COD	985	-	-	-	≤ 250	≤ 500
BOD_5	291	-	-	-	≤ 50	≤ 200
TP	64	-	-	-	≤ 1	≤ 10
SRP	45					
Cu	0.024		≤ 0.2		≤ 1	-
Cr	0.017		≤ 0.1		≤ 2	-
Pb	0.044		≤ 5		≤ 0.1	-
Zn	0.124		≤ 2		≤ 2	≤ 1

a Units: EC ($mS.cm^{-1}$); TDS, nitrogen forms, carbon and phosphorus forms, BOD_5, and heavy metal concentrations ($mg.L^{-1}$).

a Unidades: EC ($mS.cm^{-1}$); sales totales disueltas, formas de nitrógeno, carbono y fósforo, DBO_5, y concentraciones de metales pesados ($mg.L^{-1}$).

Plant analysis and biomass estimation

Plants were collected both at the beginning and at the end of the experiment. Plants were washed with tap water and deionized water and separated into roots, stems and leaves. Plant material was oven-dried at 70°C. Each part of the plant was ground into powder with a blender. One gram of plant tissue was digested with HNO_3 and $HClO_4$ (2:1).

At the end of the experiment, plant fresh weight was obtained and relative growth rate (RGR) was estimated from the equation proposed by Harper (11):

$$\mathbf{RGR = (\ln W_f - \ln W_i)/ \Delta t}$$

where:
W_i and W_f denote initial and final fresh weight respectively
Δt represents the experimental period (31 days)

Analysis of experimental data

The variables measured at the end of the experiment (pH, electrical conductivity, SRP, nitrate, ammonium, SM, POC, COD, Kj N, TP, heavy metals and biomass) were examined for normal distribution using the Lilliefors test.

Data that did not follow a normal distribution were $\log_{10}$- transformed and their distributions were re-examined. All variables were tested for homogeneity of variances using Levene's test and examined by one-way ANOVA. Tukey's HSD test was used to reveal whether treatments were significantly different. All statistical analyses were performed using GENSTAT 7.1 (8). Differences are reported as significant at $p < 0.05$.

RESULTS AND DISCUSSION

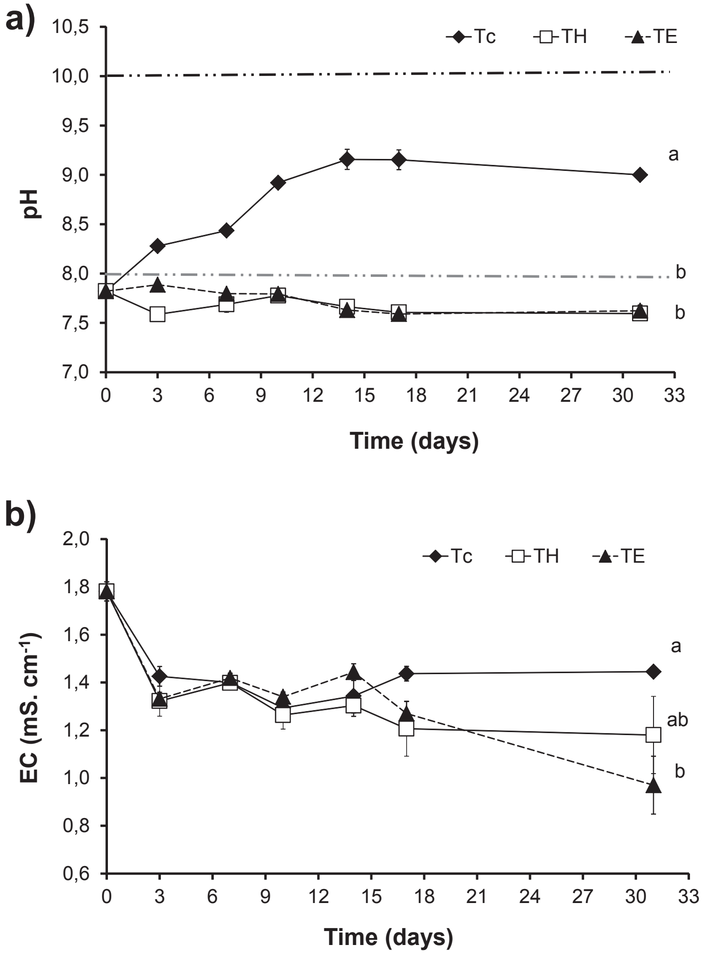
Initial physical-chemical characteristics of wastewater

Initial pH of wastewater was slightly alkaline (table, page 50) . Electrical conductivity was moderate and TDS revealed slightly saline condition, corresponding to a slight to moderate degree of restriction (Wastewater quality guidelines for agriculture use - FAO). Suspended matter was higher than uncontaminated rivers of Buenos Aires (5). Nitrogen and phosphorus levels, BOD_5 and COD were higher than the limits allowed for discharges to surface waters (1). Organic nitrogen was the main form of nitrogen. These characteristics were similar to those reported by García and Iorio (7) for another feedlot effluent of Buenos Aires. The levels of heavy metals found in wastewater were lower than the limits allowed for discharges to surface waters (1).

Water physical-chemical variations

Electrical conductivity and pH

In treatments with plants (*i. e.*, T_H and T_E), pH remained almost constant over time and near neutrality (7.6 ± 0.1), without significant differences between treatments at the end of experiment (figure 1, page 52). After 10 days, an increase in one unit of pH was observed in T_C and alkaline conditions were observed at the end of the experiment (9.0 ± 0.1). These values were higher than those recorded in T_H and T_E ($p < 0.01$). The algal bloom observed in T_C after 10 days could explain these alkaline conditions. Sajn Slak *et al*. (22) also found that treatments using aquatic macrophytes show a higher buffer capacity than those using algae.



Different letters denote significant differences between treatments at the end of the experiment.
The parallel lines to the x axis indicate the maximum pH value allowed by the Wastewater quality guidelines established by FAO (grey) and AGOSBA Resolution (black).

Letras distintas denotan diferencias entre tratamientos al término del ensayo.
Las líneas paralelas al eje x indican el valor máximo de pH permitido por los niveles guía de efluentes establecidos por FAO (gris) y por la Resolución AGOSBA (negro).

Figure 1. Mean ± standard deviation of pH (a) and EC (b) over time.
Figura 1. Media ± desvío estándar de pH (a) y CE (b) en el tiempo.

Electrical conductivity decreased over time in all treatments (figure 1b, page 52), which was higher in T_C than in T_E at day 31 ($p < 0.05$). The removal percentages of TDS were higher in treatments with plants than in T_C during all time, being higher in T_H than T_E only between 7 and 17 days (figure 2a). Sooknah and Wilkie (25) also found a markedly decrease on EC in anaerobic effluents treated with *E. crassipes* and *H. ranunculoides* compared with algal treatment. Aquatic plants are considered more tolerant to high salinity than terrestrial ones. Besides, its high biomass yield could require higher salt absorption in relation with algal treatments.

Suspended Matter and Particulate Organic Carbon

Initial SM concentration was 214 $mg.L^{-1}$. After 7 days, SM showed a remarkable decrease in all treatments remaining relatively constant (figure 3a, page 55) and reaching more than 70% of removal (figure 2b) until day 31. Although no significant differences in SM were found among treatments at the end of the experiment, the percentage of removal of SM in T_C was higher than that in the treatments with plants from day 14 (more than 90%). Before that, the highest removal was found in T_E between days 7 and 10 (87%) and in T_H between days 10 and 14 (86%) (figure 2b).

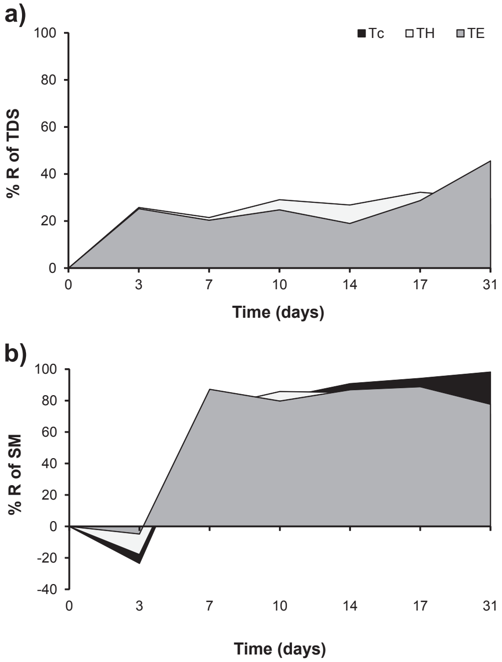


Figure 2. Removal percentages of total dissolved solids (a), suspended matter (b), Kjeldahl nitrogen (c), total phosphorus (d) and organic matter expressed as COD (e) for each treatment over time.

Figura 2. Porcentajes de remoción de sólidos totales disueltos (a), material en suspensión (b), nitrógeno Kjeldahl (c), fósforo total (d) y materia orgánica expresada como DQO (e) para cada tratamiento durante el transcurso del ensayo.

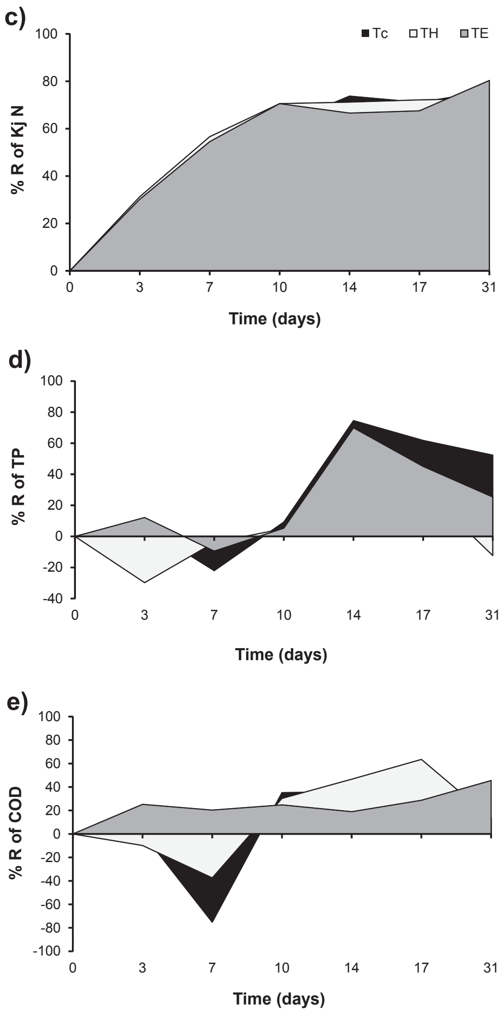
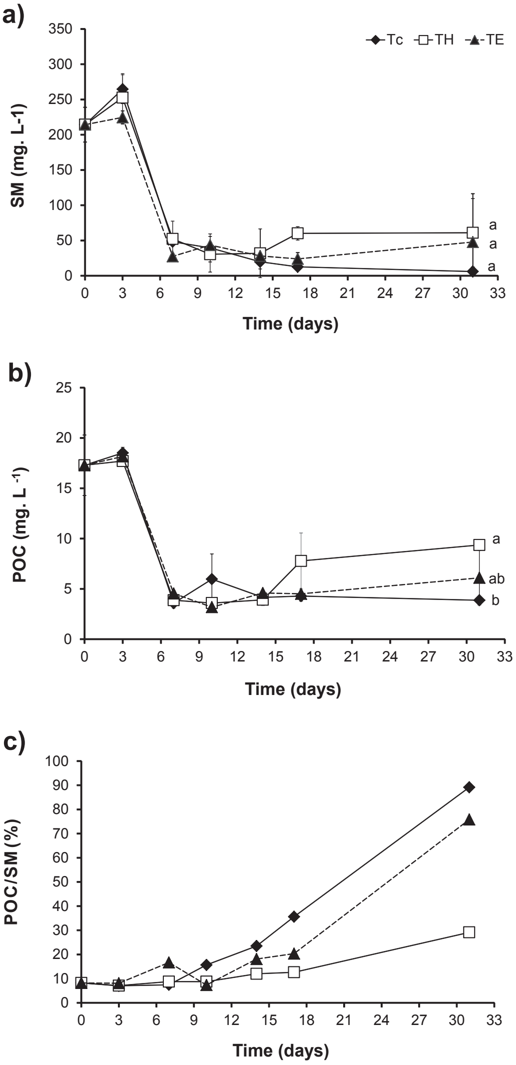


Figure 2 (cont.). Removal percentages of total dissolved solids (a), suspended matter (b), Kjeldahl nitrogen (c), total phosphorus (d) and organic matter expressed as COD (e) for each treatment over time.

Figura 2 (cont.). Porcentajes de remoción de sólidos totales disueltos (a), material en suspensión (b), nitrógeno Kjeldahl (c), fósforo total (d) y materia orgánica expresada como DQO (e) para cada tratamiento durante el transcurso del ensayo.

Since SM is a complex mixture of inorganic and organic particles, the analysis of POC can provide interesting information. High POC/SM ratios are frequently associated with anthropogenic wastes, which are a major source of organic matter and nutrients. The behavior of POC over time was similar to that of SM (figure 3b, page 55). However, although SM concentration decreased in all treatments, the POC/SM ratio suggests that particulate matter was enriched in organic carbon (figure 3c, page 55).

This could be due to the algal development observed only in T_C and to the detachment of plant material in the other treatments.



Different letters denote significant differences between treatments at the end of the experiment.

Letras distintas denotan diferencias significativas entre tratamientos al término del ensayo.

Figure 3. Variation in a) suspended matter, b) particulate organic carbon and c) particulate organic carbon / suspended matter ratio over time (mean ± standard deviation).
Figura 3. Variación en a) sólidos suspendidos, b) carbono orgánico particulado, y c) relación carbono orgánico particulado/sólidos suspendidos en el tiempo (media ± desvío estándar).

Suspended matter constitutes an important indicator of turbidity in natural waters and it is mainly comprised of organic and inorganic materials granulometrically classified as slime and clays. Suspended matter has a high specific surface area and can be essential to determine the mobility and behavior of several chemical species, especially those relatively insoluble.

From an environmental point of view, several heavy metals and organic contaminants can be exchanged between solid and aqueous phases through adsorption/desorption processes or by the formation of inner and outer sphere complexes, promoting their removal from the water column. However, the association between some organic pollutants from feedlot wastewater, such as hormones and antibiotics, with SM could facilitate the transport to surface water bodies (22). Thus, the high removal rates obtained in these treatments could mitigate the action of SM as a vector of contaminants.

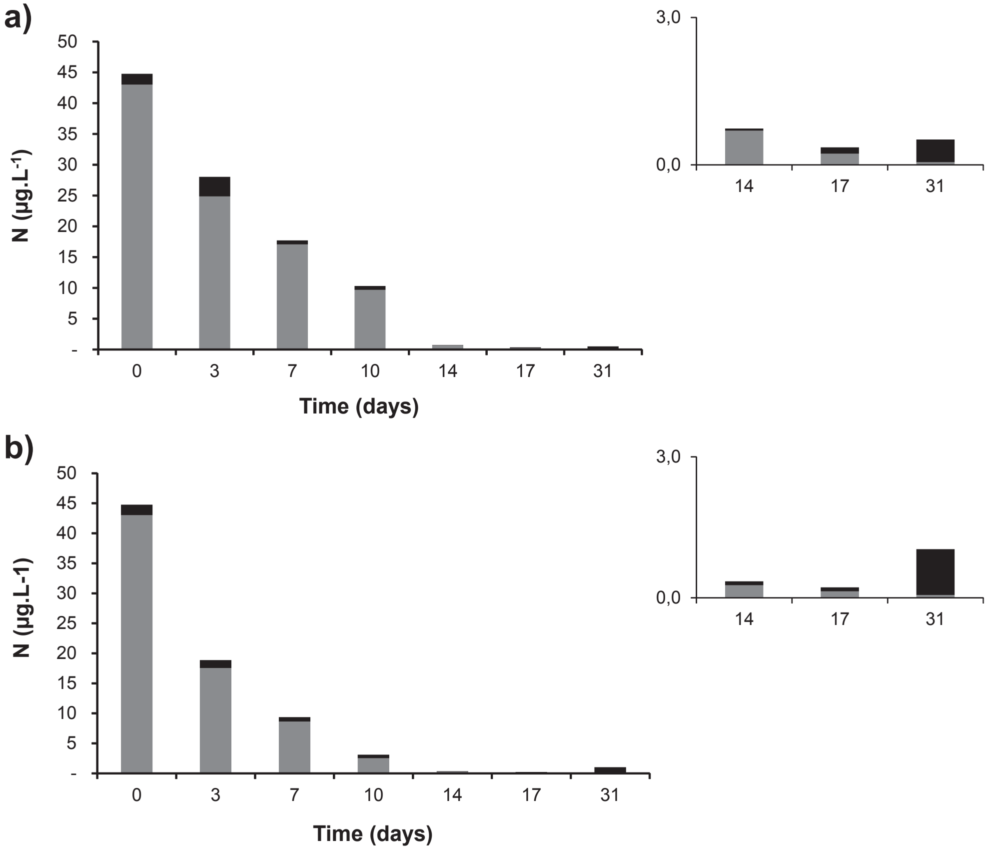
Nitrogen

Initial DIN concentration was 46 $mg.L^{-1}$. The decrease in DIN concentrations was similar in all treatments and especially respond to variation of ammonium levels (figure 4, page 57). From 10th day, the decrease in ammonium concentrations was more pronounced in treatments with aquatic plants and particularly in T_E, although the lowest concentrations were found in T_C at the end of the experiment ($p < 0.05$). Although a decrease in ammonium concentrations was observed over the course of the experiment, the causes of this variation may differ among treatments. In the control treatment, the low concentrations of nitrate and the high ammonium / nitrate ratio (figure 4a, page 57) may indicate that the decrease in ammonium levels cannot be explained only by the oxidation of ammonium to nitrate via nitrification. In contrast, the high pH values, close to 9, recorded from day 10 suggest that nitrogen can be lost not only by absorption by the algal biomass (23) but also by volatilization as ammonia (27) .

In the treatments with aquatic plants, the decrease of ammonium levels was more pronounced than in the control treatment. This could be explained by the absorption and assimilation of nitrogen by aquatic plants, which showed a significant growth in biomass. Ammonium uptake by aquatic plants is widely recognized and it is enhanced compared with the oxidized form (nitrate) (15, 25). In addition, the biogeochemical processes that take place into the water can also explain this. Thus, while the dominant process in T_E seems to be nitrification, due to the high nitrate levels and low ammonium/ nitrate ratio (figure 4c, page 58), the dominant process in T_H seems to be the loss of nitrogen by denitrification and/or the higher uptake by macrophytes, since on day 17 there was a lower level of DIN (figure 4b, page 57) and pH remained near neutrality.

The limiting factor in the process of nitrification in facultative environments is the presence of an accessory surface (*i. e.*: rhizosphere), which allows maintaining an aerobic medium for the appropriate aerobic activity of nitrifying bacteria. The oxygen required for nitrification is supplied by diffusion from the atmosphere and the roots of macrophytes. Several authors state that the presence of floating macrophytes, especially *E. crassipes*, in constructed wetlands increases the levels of dissolved oxygen in the rhizosphere (12, 26). This could explain the higher nitrate levels observed in the treatment with *E. crassipes*.

Vymazal (27) argues that the removal of dissolved inorganic nitrogen is due mainly to biogeochemical processes and that uptake by macrophytes does not represent a significant percentage. The results show a removal of dissolved inorganic nitrogen close to 100% in all treatments after 14 days (figure 4), but could not discriminate the role of aquatic plants in this process. However, if we considered that some loss of nitrogen in T_C could occur by volatilization of ammonia, or even through release of partially reduced species of nitrogen, as NO and N_2O (19), plant treatments could mitigate the release of contaminants and potential greenhouse gases to the atmosphere.

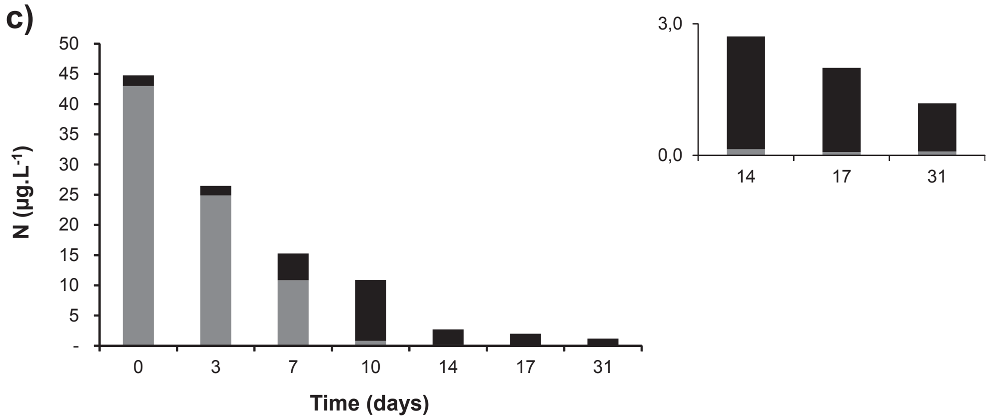


Different letters denote significant differences in DIN among treatments.

Letras distintas indican diferencias significativas en las concentraciones de nitrógeno inorgánico disuelto entre tratamientos.

Figure 4. Dissolved inorganic nitrogen (nitrate -black- and ammonium -grey-) concentrations over time in each treatment: a)T_C, b)T_H, c)T_E. The graffic insert show details from 14 to 31 days.

Figura 4. Concentraciones de nitrógeno inorgánico disuelto (nitrato -en negro- y amonio -en gris-) para cada tratamiento durante el transcurso del ensayo: a)T_C, b)T_H, c)T_E. El gráfico inserto muestra en detalle los días 14 a 31.



Different letters denote significant differences in DIN among treatments.

Letras distintas indican diferencias significativas en las concentraciones de nitrógeno inorgánico disuelto entre tratamientos.

Figure 4 (cont.). Dissolved inorganic nitrogen (nitrate –black- and ammonium – grey-) concentrations over time in each treatment: a)T_C, b)T_H, c)T_E. The graffic insert show details from 14 to 31 days.

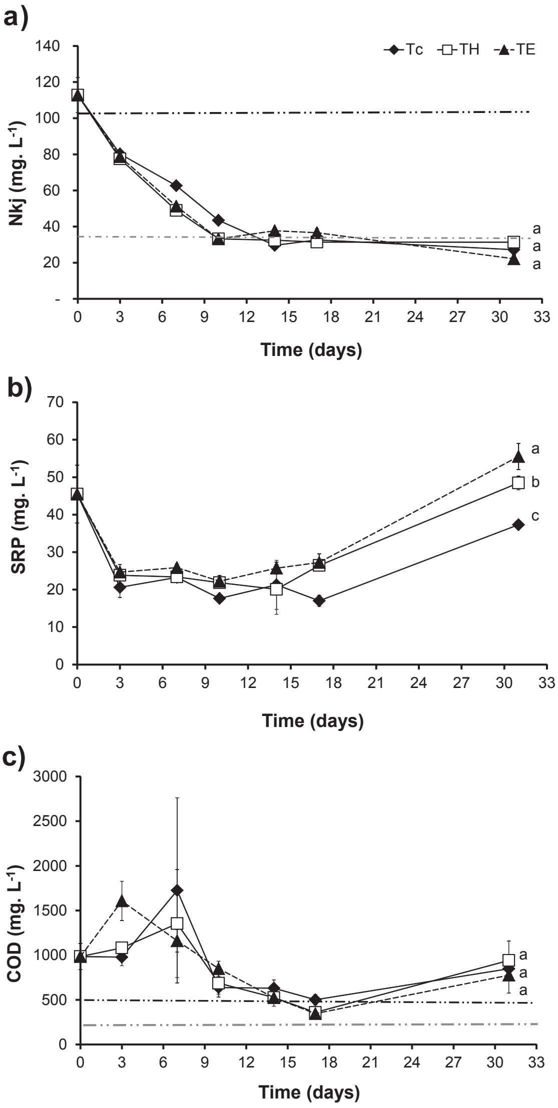
Figura 4 (cont.). Concentraciones de nitrógeno inorgánico disuelto (nitrato, en negro, y amonio, en gris) para cada tratamiento durante el transcurso del ensayo: a)T_C, b)T_H, c)T_E. El gráfico inserto muestra en detalle los días 14 a 31.

All the treatments showed a marked decrease in the levels of Kj N during the first 10 days, reaching in treatments with macrophytes the concentrations suggested by the AGOSBA Resolution (1) (figure 5, page 59). Although no significant differences were found among treatments at 31st day ($p < 0.05$), the highest removal percentage was achieved in T_H and T_E, except at day 14 that was for T_C (figure 2c, page 54).

Phosphorus

Soluble reactive phosphorus (SRP) concentrations decreased until day 17, and subsequently increased to reach levels even higher than the initial ones in T_E and T_H (figure 5b, page 59). At the end of the experiment, SRP concentrations differed significantly among treatments ($p < 0.05$), being $T_E>T_H>T_C$.

From the beginning of the experience until day 10 the lowest removal of total phosphorous (TP) were observed and sometimes an increase in TP concentrations in water were registered (figure 2d, page 54). The highest percentage of TP removal (75%) was obtained on day 14, being T_C the most effective treatment. T_E showed the highest removal on day 14 (70%), whereas T_H showed the highest removal on day 17 (31%). However, none of the treatments reached the values allowed for the discharge to a water body surface, according to the AGOSBA Resolution (1).



The parallel lines to the x axis indicate the maximum concentration allowed for discharge to soil (black) and water bodies (grey) by the AGOSBA Resolution 336/2003.
Different letters denote significant differences between treatments at the end of the experiment.

Las líneas paralelas al eje x indican las concentraciones máximas permitidas para la descarga de efluentes en suelos (negro) y cuerpos de agua (gris) según la AGOSBA 336/2003.
Letras distintas indican diferencias significativas entre tratamientos al término del ensayo.

Figure 5. Concentrations of N Kj (a), SRP (b), and COD (c) for each treatment over time (mean ± standard deviation).

Figura 5. Concentraciones de N Kj (a), PRS (b) y DQO (c) para cada tratamiento en el tiempo (media ± desvío estándar).

The increase in TP observed at the end of the experiment could be due to the detachments of plant biomass, which were able to become mineralized or remain in their organic form through decomposition process.

Different authors have obtained contrasting results assessing the suitability of aquatic macrophytes to remove phosphorus. While some authors have obtained phosphorus removal percentages between 60 and 98% (25, 26), others have achieved removal percentages below 25% (12). However, in all the cases reported, *E. crassipes* was the species with the highest efficiency.

The decrease in SRP concentrations observed in the first stage of the experiment could be due to the adsorption-absorption of inorganic phosphorus by aquatic plants in T_H and T_E and by algae in T_C. Furthermore, in macrophyte systems, the sedimentation of phosphorus associated with SM could contribute to its removal from the water column. On the other hand, the marked decrease in the concentrations of Ca and Mg cations in coincidence with the marked increase in pH recorded after day 10 in T_C (data non shown) could suggest precipitation of insoluble salts. Sajn Slak *et al.* (22) found precipitates of hydroxyapatite at pH levels around 9.5. Therefore, the daily fluctuations in water pH could play an important role keeping the bioavailability of phosphorus in waters with significant concentrations of calcium and magnesium (26).

BOD and COD

During the first seven days of the experiment, an increase in COD was observed in all the treatments (figure 5c, page 59). This could be due to the detachment of plant biomass. In addition, the presence of algae in the T_C could significantly increase the levels of this variable. On 10^{th} day, the levels were below the initial ones. The highest percentages of removal were obtained on day 17, being the treatments with plants the most efficient (near to 65%) (figure 2e, page 54). Although none of the treatments decreased the COD to the maximum levels for discharge to surface water bodies permitted by the AGOSBA Resolution (1), the treatments with aquatic plants showed the nearest levels on day 17 (346-359 mg $O_2.L^{-1}$) and were lower than discharge limit for soils.

The lowest levels of BOD_5 were recorded on day 17 (T_C: 31.9 mg$O_2.L^{-1}$; T_H: 26.9 mg $O_2.L^{-1}$; T_E: 21.9 mg $O_2.L^{-1}$). These levels slowly increased at day 31 (T_C: 39.4 mg $O_2.L^{-1}$; T_H: 36.4 mg $O_2.L^{-1}$; T_E: 26.4 mg $O_2.L^{-1}$). The removal percentages were 92, 91 and 89% for T_E, T_H and the T_C, respectively being all of them below the maximum permitted by the AGOSBA Resolution (1) for soils and surface water bodies.

Malik (15) showed that the use of *E. crassipes* for the treatment of wastewater rich in nutrients and high levels of BOD is more efficient than that with other species. The higher efficiency in the removal of organic load in macrophyte systems could be due to the better conditions of oxygenation provided by macrophytes. The values of removal obtained in our study are comparable to those reported by other authors (23).

Heavy metals in solution

The initial values of heavy metals recorded in the effluent were below the limits state by AGOSBA Resolution (1) and Wastewater quality guidelines for agriculture use (20), and slightly higher than those reported for an uncontaminated lowland river in the province of Buenos Aires (5). These levels were also markedly lower than the LD_{50} reported by Mohan and Hosetti (16) for floating aquatic plants. However, it should be noted that the effluent used in the present study was pretreated in anaerobic settling ponds, where reducing conditions favor the precipitation of metal sulfides and its removal from the water column.

The levels of Cu (T_C 9 ± 1, T_H 11 ± 1, T_E 11 ± 1 μg.L^{-1}), Zn (T_C 81 ± 6, T_H 36 ± 3, T_E 26 ± 1 μg.L^{-1}) and Cr (T_C 11 ± 3, T_H 11 ± 3, T_E 10 ± 2 μg.L^{-1}) in the water reached at the end of the experiment were significantly lower than initial levels in the three treatments ($p < 0.05$ for Zn and Cu, and $p < 0.01$ for Cr) whereas, Pb (T_C 29 ± 3, T_H 20 ± 6, T_E 30 ± 5 μg.L^{-1}) levels significantly decreased only in the T_H treatment ($p < 0.01$).

The removal of metals observed could be due to physico-chemical (adsorption, complexation and surface precipitation) and biological processes (algal and macrophyte uptake). Although several authors have highlighted the capacity of *E. crassipes* to remove heavy metals from a solution, *H. ranunculoides* has been less studied. Therefore, our results could help to assess the suitability of the latter for remediation and potential use in treatment systems.

Biomass growth and heavy metal absorption

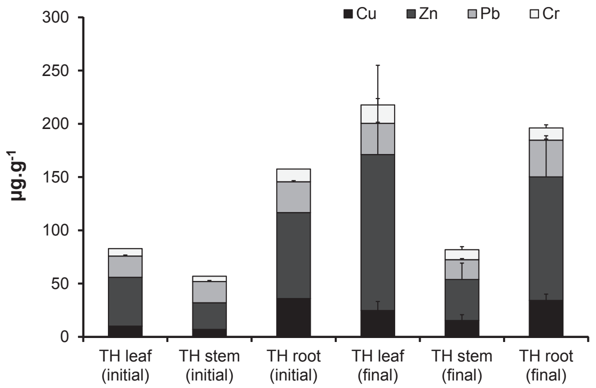
Based on the initial fresh weight of 800 g for both species, a significant increase in biomass was observed after 31 days. *E. crassipes* plants increased their weight by 507% (final biomass: 4070 g), while *H. ranunculoides* increased by 358% (final biomass: 2887 g).

The relative growth rate (RGR) indicates the increase in biomass per unit time and mass. It is mainly determined by the genotype of the species studied, as well as by the environment in which it grows, and is considered a variable of high ecological importance, especially in species whose population density is largely dependent on vegetative propagation. Thus, the greatest increase in biomass recorded by *E. crassipes* (RGR: 0.105 g.g^{-1}.d^{-1}) against *H. ranunuloides* (0.067 g.g^{-1}.d^{-1}) could be due to its great colonizing ability. On the other hand, the optimum temperature range in which the experiment was performed and the environmental nutrient levels would explain the high values recorded for RGR in relation to ones reported in other researches (28). Wilson *et al*. (28) analyzed the factors determining the population growth of *E. crassipes* and noted that, at levels greater than 1 mg N.L^{-1} in water, RGR values are maximum and that this rate can be increased by an order of magnitude in optimal temperature conditions (summer *vs.* winter).

At the beginning of the experiment, root metal concentrations were higher than in other parts of the plant for both species (figure 6, page 62). At the end of the test, the concentrations of metals in the leaves of *H. ranunculoides* increased significantly, resulting in values similar to or greater than those found in the root (figure 6, page 62). Considering the increase in biomass observed during the course of the experiment, *H. ranunculoides* seems to be efficient in extracting metals from the water column.

The behavior of *E. crassipes* was slightly different. At the end of the experiment, the root was the main organ of accumulation of essential metals such as Cu and Zn, although these concentrations were lower than the initial ones (figure 6). These lower concentrations could be the result of a "dilution effect" caused by the increase in biomass. In the case of Cr and Pb , accumulation was also observed in photosynthetic structures (figure 6).

These results suggest that the species studied are suitable for the treatment of effluents from feedlot because of their tolerance to a wide range of environmental conditions, their ability to rapidly produce biomass which allows them to remove potential contaminants and stabilize pH and EC, and their ability to improve the water quality reducing the environmental impact of these farms. Besides, macrophyte treatments have the advantage of accumulating nutrients in biomass, which can then be eventually used as a dietary supplement in animal production systems (12, 15), as a primary source for obtaining energy through biodigestion and organic supplements.



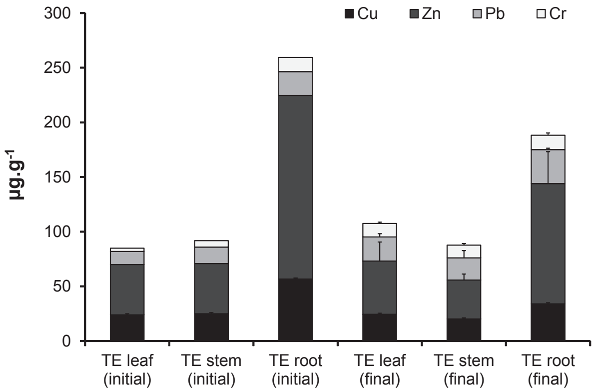


Figure 6. Heavy metal concentrations (µg.g^{-1}) in the different plant parts of *H. ranunculoides* (TH) and *E. crassipes* (TE) at initial and final time.

Figura 6. Concentraciones de metales pesados (µg.g^{-1}) en las diferentes partes de la planta de *H. ranunculoides* (TH) y *E. crassipes* (TE) al inicio y al término del bioensayo.

The integration of macrophytes into production systems will increase the environmental sustainability. The farmers could implement these technologies to obtain process certifications and thus differentiate themselves in the competitive framework of the new century.

CONCLUSIONS

The presence of aquatic plants in constructed wetlands seems to increase the removal rates of nutrients, organic matter and heavy metals from wastewater from intensive livestock farms.

This goal is achieved without increasing significantly the operational cost or the complexity of the treatment, in a short period of approximately 10-17 days.

Considering that tested plants remove contaminants at different rates, an ecological approach using different species could optimize the remediation process.

REFERENCES

1. AGOSBA. Administración General de Obras Sanitarias de la Provincia de Buenos Aires. AGOSBA 336/2003. (www.gob.gba.gov.ar)
2. APHA - AWWA - WPCF. 1992. Métodos normalizados para el análisis de aguas potables y residuales. Madrid. Ed. Díaz de Santos S. A.
3. Álvarez, A.; D'Elia, M.; Paris, M., Fasciolo, G.; Barbazza, C. 2011. Evaluación de la contaminación de acuíferos producida por actividades de saneamiento y re-uso de efluentes en el norte de la provincia de Mendoza. Revista de la Facultad de Ciencias Agrarias. Uiversidad Nacional de Cuyo. Mendoza. Argentina. 43(1): 19-39.
4. Arreghini, S.; de Cabo, L.; Iorio, A. F. de. 2006. Phytoremediation of two types of sediment contaminated with Zn by *Schoenoplectus americanus*. Int J Phytoremediation 8: 223-232.
5. Arreghini, S.; de Cabo, L.; Seoane, R.; Tomazin, N.; Serafini, R.; Fabrizio de Iorio, A. 2007. A methodological approach to water quality assessment in an ungauged basin, Buenos Aires, Argentina. Geojournal 70: 281-288.
6. Fuentes Yagüe, J. L. 1996. Curso de riego para regantes. Madrid. Ed. Multiprensa. 60 p.
7. García, A. R.; Iorio, A. F. de. 2005. Incidencia de la descarga de efluentes de un feedlot en la calidad de agua del arroyo Morales, Buenos Aires - Argentina. Revista de la Facultad de Agronomía. UBA 25(2): 167-176.
8. GENSTAT Release 7.1. 2003. Published by VSN International, Oxford, UK.
9. Golterman, H.; Clymo, R.; Ohndtad, M. 1978. Methods for the physical and chemical examination of freshwaters. Oxford. Blackwell Sci. Publ. 166 p.
10. Grünwaldt, E. G.; Guevara, J. C. 2011. Rentabilidad del engorde a corral de bovinos de carne en la provincia de Mendoza, Argentina. Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Mendoza. Argentina. 43(2): 21-34.
11. Harper, J. L. 1977. Population biology of plants. London. Academic Press. 900 p.
12. Jianbo, L. U.; Zhihui, F. U.; Zhaozheng, Y. I. N. 2008. Performance of a water hyacinth (*Eichhornia crassipes*) system in the treatment of wastewater from a duck farm and the effects of using water hyacinth as duck feed. J Environ Sci 20(2008): 513-519.
13. Lahitte, H. B.; Hurrell, J. A. 1997. Plantas de la Costa. Editorial LOLA. Argentina. 200 p.
14. Maine, M. A.; Duarte, M. V.; Suñé, N. L. 2001. Cadmium uptake by floating macrophytes. Water Res 35(11): 2629-2634.
15. Malik, A. 2007. Enviromental challenge vis a vis opportunity: The case of water hyacinth. Environ Int 33(1): 122-138.
16. Mohan, B. S.; Hosetti, B. B. 1999. Aquatic plants for toxicity assessment. Environ Res A 81: 259-274.
17. Moreno Casco, J.; Moral Herrero, R. 2007. Compostaje. Madrid. Ed Mundi-prensa S. A. 570 p.

18. Page, A. L.; Miller, R. H.; Keeny, D. 1982. Methods of soil analysis. Part 2. American Society of Agronomy. Soil Science Society of America. Madison, Winsconsin, USA.
19. Pan, T.; Zhu, X. D.; Ye, Y. P. 2011. Estimate of life-cycle greenhouse gas emissions from a vertical subsurface flow constructed wetland and conventional wastewater treatment plants: A case study in China. Ecol Eng 37(2): 248-254.
20. Pescod, M. B. 1992. Wastewater quality guidelines for agriculture use. In: Wastewater treatment and use in agriculture. FAO.
21. Pordomingo, A. J. 2003. Gestión ambiental en el feedlot. Guía de buenas prácticas. INTA Anguil.
22. Sajn Slak, A.; Bulc, T. G.; Vrhovsek, D. 2005. Comparison of nutrient cycling in a surface-flow constructed wetland and in a facultative pond treating secondary effluent. Wat Sci Tech 51(12): 291-298.
23. Sanderson, H.; Laird, B.; Pope, L.; Brain, R.; Wilson, C.; Johnson, D.; Bryning, G.; Peregrine, A. S.; Boxall, A.; Solomon, K. 2007. Assessment of the environmental fate and effects of ivermectin in aquatic mesocosms. Aquat Toxicol 85: 229-240.
24. Serafini, R. J. M.; Arreghini, S.; Iorio, A. F. de. 2007. Could *Sagittaria montevidensis* be used in phytoremediation strategies? In: Environmental change and rational water use. Pág. 195-202. Ed. Scarpati, O. y Jones, J. A. A. Buenos Aires, Argentina.
25. Sooknah, R. D.; Wilkie, A. C. 2004. Nutrient removal by floating aquatic macrophytes cultured in anaerobically digested flushed dairy manure wastewater. Ecol Eng 22(2004): 27-42.
26. Tripathi, B. D.; Upadhyay, A. R. 2003. Dairy effluent polishing by aquatic macrophytes. Water Air Soil Poll 143: 377-385.
27. Vymazal, J. 1999. Nitrogen removal in constructed wetlands with horizontal sub-surface flow - can we determine the key process? In: Nutrient cycling and retention in Natural and Constructed wetlands (Vymazal J, Ed). Backhuys Publishers, Leiden, The Netherlands. p. 1-17.
28. Wilson, J. R.; Holst, N.; Rees, M. 2005. Determinants and patterns of population growth in water hyacinth. Aquat Bot 81:51-67.

Acknowledgements

Funding for this project was provided by the Universidad de Buenos Aires (UBACyT AG055), Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT, PICT 00-356) and Instituto Nacional de Tecnología Agropecuaria (AEGA 1662).