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Short Communication

Potential of constructed wetlands microcosms for the removal of veterinary pharmaceuticals from livestock wastewater

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HIGHLIGHTS

- Evaluation of veterinary drugs removal from wastewater by constructed wetlands (CWs).
- Enrofloxacin (ENR) and tetracycline (TET) removal was studied.
- CWs have potential to mitigate the release of veterinary drugs present in wastewaters.
- Adsorption of the drugs to substrate may be the predominant mechanism for ENR.
- For TET besides adsorption there are signs that degradation is also occurring.

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ABSTRACT

The aim of the present work was to evaluate, at microcosm level, the capacity of constructed wetlands (CWs) to remove veterinary pharmaceutical compounds, from wastewater. Results indicated that CWs have potential to mitigate the release of veterinary drugs, namely enrofloxacin (ENR, a fluoroquinolone) and tetracycline (TET, tetracyclines family). Removal efficiencies of 94% and 98% where achieved for TET and ENR, respectively, when treating pigfarm wastewater effluent doped at 100 $\mu g\ L^{-1}$ drug level, along twelve weeks. Occurrence of adsorption of the drugs to CWs substrate may be the predominant mechanism for ENR, although for TET there are signs that degradation is also occurring.

1. Introduction

Concern on pollution by pharmaceuticals has grown after confirmation of their presence and ability to pseudo-persist in the environment, namely in fresh water resources. However, the main research focus has been on human drugs. Veterinary drugs are a group of emerging compounds that has been extensively used in animal production without almost no control or restriction and has received so far very little attention (Babić et al., 2010). Nevertheless, they are being detected in surface waters in Canada, the USA, Europe and Asia (Hussain et al., 2012), being important to minimize their emissions to the aquatic environment.

The widespread use of some drugs and their generally inefficient removal from wastewaters in waste water treatment plants (WWTPs) are the main reasons for the frequent detection of pharmaceuticals in aquatic bodies. Therefore, there is a growing need for alternative wastewater treatments that can remove pharmaceuticals from waters. Constructed wetlands (CWs) and natural wetland systems can be managed as water quality improving systems representing an alternative or additive low-cost wastewater treatment (Dordio et al., 2010).

Until recently, nitrogen and phosphorus were the primary constituents of concern in wetland systems together with microorganisms removal (Helt et al., 2012). Recently, CWs have started to be researched for the removal of organic micro pollutants, including pharmaceuticals, before the release of wastewater into aquatic systems (Conkle et al., 2008). However, this research has been focused mainly on human pharmaceuticals and on urban wastewaters (Dordio et al., 2009; Hijosa-Valsero et al., 2010; Matamoros et al., 2008) being, in addition, the information limited to only a few compounds. Regarding veterinary drugs, to our knowledge there is a scarcity of studies with only two papers published on this subject. One of the studies focused on the removal of sulfonamides from swine wastewater by a constructed macrophyte floating bed system (Xian et al., 2010). Another, studied ionophores re-

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moval by free water surface CWs (Hussain et al., 2012). Therefore, more research on this topic is in need.

This work aimed to evaluate, at microcosm level, the capacity of CWs to remove two veterinary drugs, enrofloxacin (ENR) and tetracycline (TET), from livestock industries wastewater, which has a matrix much more complex than urban wastewaters. These compounds belong to two different families (fluoroquinolone and tetracyclines), being among those more extensively used in Portuguese livestock industry. Microcosms were planted with *Phragmites australis* a plant that has shown potential for the reduction/elimination of these drugs from wastewaters (Carvalho et al., 2012). While full-scale wetland studies are valuable for assessing overall removal efficiencies, they are complicated by the complexity of interactive processes present in wetlands. Consequently, microcosm scale studies are crucial to differentiate processes, particularly those associated with plants (Reinhold et al., 2010), being the approach selected in this work.

2. Methods

2.1. Plants, support matrix and wastewater collection

Plants (*P. australis*) with shoots and sediment involving its roots were collected at *Lima* river margins (NW Portugal) in April 2012. Sand was collected simultaneously in the river basin (within 1 m of plant stands). At lab, sediment was separated from plant roots, being further mixed with the sand (in 1:1 proportion) and homogenized to prepare the roots' bed substrate for CWs microcosms.

Wastewater was collected in a pig farm, with a capacity of 8000 heads. The wastewater was collected weekly at the exit of the existing WWTP, being the one that was discharged into the environment. Initial wastewater presented pH of 8.04, COD of 1042 mg L^{-1} and 340 mg L^{-1} of particulate matter (PM) being 82% organic PM. Variations along time in wastewater characteristics were considered negligible once pH variation was lower than 0.6 units throughout the study.

2.2. Microcosms setup

Sixteen microcosms were set up in plastic containers (0.4 × 0.3 × 0.3 m) filled with a first 4 cm layer of gravel, a second 2 cm layer of lava rock and finally a 10 cm layer of roots' bed substrate, reaching a total depth of 16 cm. Water level was maintained just above the substrate surface (flooding rate ≈100%). The systems were designed to operate in batch mode having only a tap at the base for sample collection. All microcosms were wrapped in aluminum foil to simulate a real system (no light penetration at substrate level, preventing photodegradation of the compounds). Seven microcosms were planted with *P. australis* (ca. 40 plants per microcosm) and nine microcosms were left unplanted. In two of the unplanted microcosms the substrate was sterilized (7 mol L^{-1} $ZnCl_2$ solution (Teixeira et al., 2012) was circulated along the first conditioning week), to study the drug adsorption capacity of the microcosms support matrix .Two additional unplanted microcosms (one for each drug) were used just filled with gravel and lava rock (total depth of 16 cm) to study the possible influence of the roots' bed substrate.

The set of microcosms was kept under greenhouse conditions, subject to environmental temperature variations (minimum 16 ± 2 °C and maximum 28 ± 8 °C) and environmental light exposure, along thirteen weeks (April to July).

2.3. Microcosms operation and sampling

Microcosms were left to acclimate for one week, adding daily one quarter strength modified Hoagland nutrient solution (Hoagland and Arnon, 1950) to maintain plants at optimum nutritional conditions. The solution was added to all microcosms, including unplanted ones, to ensure that all microcosms support matrixes were in the same conditions. Thereafter, three sets were run in parallel, a set only with wastewater and two with wastewater doped with ENR or with TET (100 μg L^{-1}, a real concentrations previously found in the environment (Babić et al., 2010)). For each drug, three planted, three unplanted and one sterilized microcosms were used. The wastewater was kept in the microcosms for one week (hydraulic retention times normally used in full scale CWs). Microcosms were manually operated for a daily recycle of the wastewater to prevent the development of anoxic areas within the support matrix. The experiment was prolonged for twelve 1-week cycles to evaluate treatment efficiency. For that purpose, each week the microcosms were completely drained and refilled with new doped wastewater. Water evaporation was controlled by addition of deionized water.

Aqueous and solid samples were collected in planted microcosms at week 1, 2, 4, 8 and 12 and only at week 1, 2 and 4 in unplanted ones. The unplanted systems clogged at week 6 and became impracticable to be operated until the 12th week.

Plants fresh biomass was accessed at the beginning and at the end of the experiments, ranging from 902 to 914 g. At the end of the experiments plants were air dried until constant weight and separated into shoots and roots. Plant total biomass increased between 10% and 20%. Roots accounted for ca. 8% of plant total dry weight, rhizomes for ca. 17%, leaves for ca. 19% and stems for ca. 56%.

Liquid samples (250 mL by microcosm) were collected, pH was measured and then samples were acidified with HCl and kept at −20 °C until 24 h prior to being filtered through 0.45 μm pore size membrane filters (Millipore, Ireland), to eliminate suspended solid matter. Afterwards samples were processed for veterinary drugs determination. Additionally, 10 mL of liquid samples of each microcosm were collected and immediately stored at −20 °C for further toxicity screening. Bed root substrates portions were collected in each microcosm in six points to obtain composite samples. Theses samples were then lyophilized in a Christ Alpha 1–4 freeze dryer (B. Braun Biotech International), sieved and kept at −20 °C until further toxicity and veterinary drugs determination.

2.4. Samples characterization

Plant stress indicators were obtained by evaluating the levels of chlorophylls in plants leaves (following Abadía et al. (1984)).

Toxicity in wastewater and sediment elutriates (except those from sterilized microcosms) was measured using ToxScreen, a bioassay where toxicity is estimated through bacterial luminescence of the sample relatively to the test control (luminescent bacterium *Photobacterium leiognathi* (Ulitzur et al., 2002)). Wastewater samples were firstly centrifuged (15 min at 2500 rpm). Sediment elutriates were prepared by agitation (200 rpm) of 2 g of sediment with 8 ml of deionized water followed by centrifugation (30 min at 2500 rpm). Toxicity was measured in terms of relative activity where 100% activity represents 0% toxicity.

For veterinary drugs analysis in wastewater, samples were precleaned/concentrated by solid-phase extraction (Oasis HLB (60 mg, 3 mL)) and separation was performed in a high-performance liquid chromatography (HPLC) Beckman Coulter equipment with a diode array detector (module 128) (Cavenati et al., 2012). Method recovery percentages were 86% and 87% for TET and ENR, respectively, and method overall variability was below 11%. The limits of detection (LODs) in this work (250 mL samples) were 0.4 and 0.2 μg L^{-1} for TET and ENR, respectively.

For solid samples, a similar methodology (unpublished results) was used. Briefly, 2 g of each sediment sample was extracted two