Ecotoxicology and Environmental Safety 137 (2017) 143–148

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Constructed wetlands for the removal of metals from livestock wastewater – Can the presence of veterinary antibiotics affect removals?

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ARTICLE INFO

Keywords:
Wastewaters
Green technology
Remediation
Enrofloxacin

ABSTRACT

The presence of emergent antibiotics, in livestock wastewater may affect constructed wetlands (CWs) performance in the removal of other pollutants. The main objective of this study was to evaluate the influence of two antibiotics commonly used in livestock industry, enrofloxacin and ceftiofur, on metal removal by CWs.

Microcosms (0.4 m × 0.3 m × 0.3 m), simulating CWs, were constructed with *Phragmites australis* to treat livestock wastewater spiked or not with 100 μg/L of enrofloxacin or ceftiofur (individually or in mixture). Wastewater was treated during 20 one-week cycles. After one-week cycle wastewater was removed and replaced by new wastewater (with or without spiking). At weeks 1, 2, 4, 8, 14, 18 and 20, treated wastewater was analysed to determine the removal rates of metals (Zn, Cu, Fe and Mn) and of each antibiotic. At weeks 1, 8 and 20 portions of the plant root substrate were collected and metals determined. At the end of the experiment metal levels were also determined in plant tissues.

Removal rate of Fe from wastewater was 99%. Removal rates of Cu and Zn were higher than 85% and 89%, respectively, whereas for Mn removal rates up to 75% were obtained. In general, no significant differences were observed through time in the removals of the different metals, indicating that the systems maintained their functionality during the experimental period. Antibiotics did not interfere with the system depuration capacity, in terms of metals removals from wastewater, and ceftiofur even promoted metal uptake by *P. australis*.

Therefore, CWs seem to be a valuable alternative to remove pollutants, including antibiotics and metals, from livestock wastewaters, reducing the risk the release of these wastewaters might pose into the environment, although more research should be conducted with other antibiotics in CWs.

1. Introduction

Constructed wetlands (CWs) are engineered systems designed and built to mimic the biological, chemical and physical processes that occur in natural wetlands (Zhang et al., 2014). These processes include sorption, sedimentation, photolysis, hydrolysis, volatilization, plant uptake and accumulation, plant exudation, microbial degradation, filtration, precipitation and adsorption, removing pollutants from contaminated waters in a more controlled environment (Garcia-Rodríguez et al., 2014; Wu et al., 2014).

CWs can be used to reduce the load of pollutants, such as nutrients, metals or organic matter, present in different types of wastewaters, including livestock wastewaters (e.g. Meers et al., 2005, Meers et al., 2008). In fact, CWs have successfully treated wastewaters contaminated with metals from acid mine drainage, metallurgy, tannery, swine production, landfill leachates and domestic effluents (Arivoli et al., 2015) and references therein). Although the efficiency for metal removal varied with the plant species (Vymazal and Březinová 2015) and with the substrate composition (Allende et al., 2011), as well as with the metal itself (Marchand et al., 2010), removal rates as high as 90% have been reported (e.g. Vymazal, 2005; Morari et al., 2015).

CWs systems have also recently been used to remove the so-called pollutants of emerging concern, including pharmaceutical compounds. In fact, several review articles on removal of pharmaceuticals from wastewaters by CWs have recently been published (e.g. Verlicchi et al., 2013; Li et al., 2014). Although most of the work has been for urban wastewaters, some studies have already shown CWs potentialities for the removal of pharmaceuticals, including antibiotics, from livestock wastewaters (e.g. Hussain et al., 2012; Carvalho et al., 2013; Hsieh et al., 2015). The more complex matrix of livestock wastewaters when

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http://dx.doi.org/10.1016/j.ecoenv.2016.11.021
Received 18 March 2016; Received in revised form 24 November 2016; Accepted 25 November 2016
Available online 19 December 2016

compared with that of urban wastewaters makes these wastewaters more difficult to treat. These wastewaters can have high loads of organic matter, solids and nutrients, as well as pharmaceutical compounds such as antibiotics. However, pollutants of emerging concern, including antibiotics, can be harmful for both microorganisms and plants, which are key players in CWs removal processes. For example, there is a growing body of evidence documenting a reduction of microbial diversity in soils contaminated with antibiotics (Jechalke et al., 2014). In addition, exposure of plants to pharmaceuticals, including antibiotics, may influence, for example, plant development, due to phytotoxicity (Carvalho et al., 2014). Thus, the presence of pollutants of emerging concern, namely antibiotics, in livestock wastewaters may affect CWs performance for the elimination of other pollutants, such as metals. This subject needs to be investigated.

Therefore, the aim of this work was to evaluate the influence of two antibiotics commonly used in livestock industry, enrofloxacin and ceftiofur, alone or in a mixture, on the removal of metals from livestock wastewaters by CWs.

2. Methodologies

2.1. Microcosms experiments

Experiments were carried out in controlled conditions in microcosms simulating CWs. Small-scale process experiments allow to fully control the experimental conditions when intending to study the influence of specific variables, such as, in the present case, the presence of antibiotics in wastewater. Microcosms were planted with *Phragmites australis*, one of the plants more frequently used in CWs (Stottmeister et al., 2003). Two antibiotics commonly used in Portuguese livestock industry for therapeutic purposes were chosen: enrofloxacin (Enr) and ceftiofur (Cef). These antibiotics belong to different families (fluoroquinolone (Enr) and cephalosporin (Cef) families) and present different physical-chemical properties. Considering that more than one antibiotic can be present in wastewaters and that either antagonistic or synergetic effects can occur, the influence of a mixture of the two antibiotics on CWs performance was also assessed.

2.1.1. Sampling

Livestock wastewater (after being treated in two lagoons (one anaerobic and another aerobic)) was collected every week in a pig farm. The wastewater was used as collected or spiked with one or both antibiotics. This wastewater already contained significant amounts of different metals (see Section 3).

P. australis plants were collected in Lima River (NW Portugal) with the sediment attached to their roots to preserve plants' rhizosphere. In the laboratory, sediment was removed and mixed with river sand (in a 1:2 proportion) to prepare the plants roots bed substrate into which plants were transplanted (each microcosms had ca. 80 plants). This mixing was carried out to increase the substrate porosity.

A few plants and roots bed substrate were set aside to determine initial metal levels. These samples were called field plants and field substrate.

2.1.2. Microcosms' assemblage

Microcosms (0.4 m × 0.3 m × 0.3 m), simulating CWs, were constructed to treat livestock wastewater not spiked (Control) or spiked with 100 μg/L of Enr or with 100 μg/L of Cef. Another set of microcosms was prepared in which the wastewater was spiked with a mixture of Enr and Cef, each antibiotic with a concentration of 100 μg/L (Mix). This concentration, although relatively high, has already been found in wastewaters effluents (Babić et al. 2010). Three replicates per variable were constructed. All CWs microcosms had three layers: the first one with gravel (4 cm), the second one with lava rock (2 cm) and the third layer with the plants roots bed substrate (11 cm height) described above. These CWs systems were based on previously used ones (Carvalho et al., 2013).

All microcosms had a tap at the bottom and worked in batch mode. The wastewater was poured on top, to percolate through the different layers of the solid matrix (matrix water saturation of ca 100%, with water just below the surface, ca. 1 L of wastewater), and drained out through the tap when necessary, simulating a sub-surface vertical flow CW. In this type of CWs wastewater flows under the surface of the planted bed, with the water percolating through the substrate.

2.1.3. Microcosms operation

The livestock wastewater was treated during 20 one-week cycles. At the beginning of each week, new wastewater (with or without spiking with veterinary antibiotics) was added. Wastewater was daily recirculated to prevent the formation of anaerobic areas. After each one-week cycle, treated wastewater was removed and replaced by new wastewater (with or without spiking with the selected antibiotics), allowing to simulate a hydraulic retention time commonly used in CWs. This continuous addition of wastewater into the CWs systems also allowed simulating the cumulative effect of adding continuously a new load of pollutants to the system, which is the situation in full scale real CWs systems. This continuous load of pollutants can lead in the long term to accumulation of pollutants in the systems, which might affect CWs performance along time.

At the end of weeks 1, 2, 4, 14, 18 and 20 the wastewater treated by the CWs was collected for analysis of metals (naturally present in the livestock wastewater) and to estimate their removal rates in the presence and absence of antibiotics. At the same time aliquots (ca. 5 g) of plants roots bed substrate were also collected from each CW microcosm.

Microcosms were kept under greenhouse conditions, subjected to environmental temperature variations (21–25 °C) and to natural light exposure, along 20 weeks (May to October 2014), after which they were dismantled.

After dismantling, plants were separated from the plants roots substrate, washed and put to dry until constant weight. Then plants were separated into roots, rhizomes, leaves and stems. All substrate samples were lyophilized.

2.2. Samples analysis

All reagents were pro analysis or equivalent. All material was washed with deionised water (conductivity < 0.1 μS cm^{-1}), immersed in nitric acid solution (20% v/v) for 24 h, washed again with deionised water and dried in a clean oven.

Samples of initial and treated wastewater were filtered (nitrate cellulose filters, 0.45 μm porosity) and acidified (with 1% nitric acid) before direct analysis.

Plants roots substrate and plant tissues (dry samples) were digested with concentrated nitric acid (HNO_3) and 30% hydrogen peroxide (H_2O_2) solution (only for plant tissues). Digestions were carried out in a high-pressure microwave system (Ethos, Milestone) in closed Teflon vessels.

Metals (Cd, Cu, Fe, Mn, Ni, Pb, Zn) concentrations were measured in an atomic absorption spectrophotometer with flame atomization (AAS-F-PU 9200X, Philips), as described before (Almeida et al., 2004). A calibration curve obtained with aqueous standard solutions of different metal concentrations (0–3 mg/L) was used. These standard solutions were prepared from 1000 mg/L stock standard solutions of each metal.

Antibiotics concentrations in treated wastewaters and plants roots bed substrate were determined by high-performance liquid chromatography (HPLC), after an ultrasonic extraction (only for solid matrix) and a pre-treatment by solid phase extraction (SPE) as described before (Carvalho et al., 2013).