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Effect of ciprofloxacin on microbiological development in wetland mesocosms

Kela P. Weber [a,b], *Michael R. Mitzel* [c], *Robin M. Slawson* [c], *Raymond L. Legge* [a,*]

[a] *Department of Chemical Engineering, University of Waterloo, 200 University Avenue W., Waterloo, ON N2L 3G1, Canada*
[b] *Department of Civil and Environmental Engineering, University of Western Ontario, London, Ontario N6A 3K7, Canada*
[c] *Department of Biology, Wilfrid Laurier University, Waterloo, ON N2L 3C5, Canada*

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ABSTRACT

An understanding of how antibiotics and other "emerging contaminants" affect both water treatment systems and natural environments is of growing interest. Ciprofloxacin is a broad-spectrum antibiotic active against both Gram-positive and Gram-negative bacteria and has been extensively used over the past 20 years. The objective of this research was to study the effect of an antibiotic such as ciprofloxacin on the development, function and stability of bacterial communities in wetland systems. Four mesocosm wetlands planted with *Phragmites australis* were initially seeded with activated sludge from a waste water treatment plant and allowed to develop for a 1 week period, after which 2 of the 4 mesocosms were exposed to a ciprofloxacin concentration of 2 μg/mL for a 5 day period. The 4 mesocosms were then monitored for several microbiological and hydrological parameters over the course of 22 weeks. The bacterial community species distribution and catabolic capabilities were assessed via denaturing gradient gel electrophoresis (DGGE) and community level physiological profiling (CLPP), based on carbon utilization. DGGE results indicated that the ciprofloxacin decreased the total number of PCR-amplifiable DNA (bacteria) and the overall diversity of bacterial operational taxonomic units (OTUs). Through CLPP it was shown that the interstitial microbiological community was initially adversely affected by the ciprofloxacin, creating a temporary decrease in the activity and overall catabolic capabilities of the inherent wetland bacterial communities. However, after a 2–5 week recovery period the activities and catabolic capabilities of the bacterial communities exposed to ciprofloxacin returned to levels comparable to those found for bacterial communities not exposed to the ciprofloxacin. These findings suggest that ciprofloxacin exposure may have an adverse effect on the inherent bacterial communities in wetland systems initially reducing their ability to assimilate anthropogenic carbon-based compounds; however, normal functionality may resume after a 2–5 week period. It was also observed that plants in the ciprofloxacin-treated mesocosms did not adapt to the antibiotic presence, instead showing initial browning of above ground parts and eventual die-off. Reduced porosity, evapotranspiration, and overall hydrological mixing in the ciprofloxacin-treated mesocosms was observed.

* *Corresponding author*. Tel.: +1 519 888 4567x36728; fax: +1 519 746 4979.
E-mail address: rllegge@engmail.uwaterloo.ca (R.L. Legge).
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1. Introduction

Emerging contaminants are defined as chemicals or microbiological constituents previously undefined or not recognized as being of concern to human or environmental health (Petrovic et al., 2004). Of particular interest are pharmaceuticals, antibiotics, and antibiotic resistant pathogenic microorganisms, all of which can be exceedingly challenging to identify, remove or inactivate in water. Although this field of research is still quite new, a growing effort into this area has been initiated worldwide (Hotchkiss et al., 2008).

Both natural and constructed wetlands contain a microbiological regime consisting of a complex, dynamic and mixed species microbiological community associated with the wetland substrate which plays a major role in ecosystem health, cycling of nutrients and in the degradation of contaminants (Parkinson and Coleman, 1991; Aelion and Bradley, 1991; Wynn and Liehr, 2001; Truu et al., 2009; Faulwetter et al., 2009; Kadlec and Wallace, 2009; Weber and Legge, 2008). The role of the microbiological regime and the related mechanisms associated with nutrient cycling and contaminant treatment in wetland treatment systems is, however, not fully understood. Research into understanding microbial population density and diversity, both spatially and temporally, will help in understanding natural wetland ecosystem health, stability and robustness, in addition to the ongoing optimization and future design of constructed treatment wetlands (Faulwetter et al., 2009; Weber and Legge, 2009).

There are a number of bacterial community profiling techniques currently used to characterize wetlands. Some of the more popular molecular methods include denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (TRFLP), and fluorescent *in situ* hybridization (FISH) (see Malik et al., 2008 for a review). Non-molecular techniques include microscopy-based identification, fatty acid methyl ester (FAME) analysis and phospholipid fatty acid (PLFA) analysis, culture-based identification, and community level physiological profiling (CLPP) using BIOLOG™ plates. Two recent studies focused on temporal shifts and changes in the catabolic capabilities of bacterial communities in wetland mesocosms. Weber et al. (2008) studied the temporal response of the resident bacteria in wetland mesocosms in response to a controlled perturbation, whereas Weber and Legge (2010a) studied the temporal development of wetland resident bacteria in mesocosm systems during a developmental period.

Ciprofloxacin is a contaminant of concern as it carries increasing environmental risk with the current pattern of use (Halling-Sørensen et al., 2000). Ciprofloxacin is a broad-spectrum antibiotic active against both Gram-positive and Gram-negative bacteria and has seen extensive use due to its potency (Hooper, 1998). Ciprofloxacin functions by inhibiting DNA gyrase, some topoisomerases and, resultantly, cell division (Drlica and Zhao, 1997). The effect which ciprofloxacin and other antibiotics have on the bacterial communities, in the natural environment and microbiologically mediated water treatment systems, are unclear. Resultant changes in the function of bacterial communities in waterways and water saturated areas such as wetlands are of concern, as bacterial communities play a large role in both nutrient and carbon cycling in the natural environment and in the interactions with macrophytes. The microbial ecology and function of resident treatment-oriented bacterial communities in both conventional and alternative water treatment systems is also of concern, as this could affect the treatment efficiency and stability of such systems reducing effluent water quality.

The objective of this study was to investigate the effect ciprofloxacin has on the development, function and stability of bacterial communities in wetlands. Four mesocosm wetlands planted with *Phragmites australis* were seeded with activated sludge from a waste water treatment plant and allowed to develop for a 1 week period after which 2 of the 4 systems were exposed to a ciprofloxacin. The 4 mesocosms were then monitored for several microbiological and hydrological parameters over a 22 week period to assess changes in the microbiological development and stability, in addition to overall mesocosm hydrological characteristics. The bacterial community was assessed using an overall microbial activity measure, PCR/DGGE and CLPP. Hydrological parameters assessed included porosity, evapotranspiration, and intrinsic mixing measured as the dispersion coefficient associated with a 1D advection-dispersion model.

2. Material and methods

2.1. Experimental design and setup

Several mesocosm studies have recently been used in undertaking a quantitative approach to the study of constructed wetland (CW) systems (Kappelmeyer et al., 2001; Stein et al., 2006; Werker et al., 2007; Weber et al., 2008, 2010). The mesocosm approach has been shown to suitably represent interactions between microorganisms, differing substrates, and contaminants within a complex rhizosphere system (Kappelmeyer et al., 2001; Stein et al., 2006; Werker et al., 2007; Weber et al., 2008; Weissner et al., 2008). Mesocosms cannot be said to completely represent full-scale wetlands, as full-scale wetlands can contain many different hydrological, biological and geochemical sub-environments. The mesocosm is taken to represent a single unit in a full-scale CW or natural wetland system where the geochemical, hydrological, and microbiological characteristics are relatively constant. By controlling different characteristics such as aspect ratios, flow rate, granular media etc., it is possible to create different mesocosms with relatively well controlled hydrological, biological and geochemical environments and used to better understand full-scale CW functionality or natural wetland robustness.

Experiments were started with a 1 week operational period after which 2 of the 4 systems were exposed to ciprofloxacin at a concentration of 2 μg/mL for a 5 d period after which the ciprofloxacin was discontinued. Two mesocosms that were not exposed to ciprofloxacin are identified as “no cipro” and considered as controls, the 2 mesocosms exposed to the ciprofloxacin are identified as “cipro”. During and after the initial exposure period the mesocosms were monitored for changes in several microbiological and hydrological characteristics.