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Macrophytes may not contribute significantly to removal of nutrients, pharmaceuticals, and antibiotic resistance in model surface constructed wetlands

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HIGHLIGHTS

- Shallow model constructed wetlands were used to assess wastewater contaminant removal.
- The presence of aquatic plants did not affect nutrient or pharmaceutical removal.
- The observed pharmaceutical removal rates generally matched calculated rates.
- Photolysis and sorption processes dominated pharmaceutical removal.
- Antibiotic resistance genes rapidly dissipated from the water, but not sediment.

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ABSTRACT

Outdoor shallow wetland mesocosms, designed to simulate surface constructed wetlands to improve lagoon wastewater treatment, were used to assess the role of macrophytes in the dissipation of wastewater nutrients, selected pharmaceuticals, and antibiotic resistance genes (ARGs). Specifically, mesocosms were established with or without populations of *Typha* spp. (cattails), *Myriophyllum sibiricum* (northern water milfoil), and *Utricularia vulgaris* (bladderwort). Following macrophyte establishment, mesocosms were seeded with ARG-bearing organisms from a local wastewater lagoon, and treated with a single pulse of artificial municipal wastewater with or without carbamazepine, clofibric acid, fluoxetine, and naproxen (each at 7.6 μg/L), as well as sulfamethoxazole and sulfapyridine (each at 150 μg/L). Rates of pharmaceutical dissipation over 28 d ranged from 0.073 to 3.0 d^{-1}, corresponding to half-lives of 0.23 to 9.4 d. Based on calculated rate constants, observed dissipation rates were consistent with photodegradation driving clofibric acid, naproxen, sulfamethoxazole, and sulfapyridine removal, and with sorption also contributing to carbamazepine and fluoxetine loss. Of the seven gene determinants assayed, only two genes for both beta-lactam resistance (bla_{CTX} and bla_{TEM}) and sulfonamide resistance (*sul*I and *sul*II) were found in sufficient quantity for monitoring. Genes disappeared relatively rapidly from the water column, with half-lives ranging from 2.1 to 99 d. In contrast, detected gene levels did not change in the sediment, with the exception of *sul*I, which increased after 28 d in pharmaceutical-treated systems. These shallow wetland mesocosms were able to dissipate wastewater contaminants rapidly. However, no significant enhancement in removal of nutrients or pharmaceuticals was observed in mesocosms with extensive aquatic plant communities. This was likely due to three factors: first, use of naïve systems with an unchallenged capacity for nutrient assimilation and contaminant removal; second, nutrient sequestration by ubiquitous

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filamentous algae; and third, dominance of photolytic processes in the removal of pharmaceuticals, which overshadowed putative plant-related processes.

1. Introduction

In the province of Manitoba, Canada, at least 350 rural communities treat their municipal wastewater passively using lagoons (Government of Manitoba, 2004). Lagoons are discharged once or twice per year into surface waters that ultimately flow into Lake Winnipeg, one of Canada's most stressed aquatic ecosystems (Lake Winnipeg Stewardship Board, 2006). Manitoba has water quality standards for discharging effluent from lagoons for total suspended solids (TSS), biochemical oxygen demand (BOD), and fecal coliforms. As of 2011, the province established a nutrient management strategy, setting total nitrogen (TN) and total phosphorus (TP) discharge limits for new and expanding facilities (Manitoba Water Stewardship, 2011). Unfortunately, some sewage lagoons currently release effluent that does not comply with federal and provincial water quality guidelines for mitigation of eutrophication (Carlson et al., 2013; Jones and Armstrong, 2001; Manitoba Water Stewardship, 2011) and may require significant enhancements to meet the new regulations. The issues in Manitoba are also occurring globally, with sewage contributing to eutrophication of surface water bodies (Smith et al., 1999).

In addition to nutrients, many micropollutants (e.g., pharmaceuticals) are discharged in sewage effluent (MacLeod and Wong, 2010). The presence of pharmaceuticals has been well documented in wastewaters and surface waters globally (Verlicchi et al., 2012), and removal by conventional wastewater treatment is generally considered inefficient and inconsistent (Brun et al., 2006; Carlson et al., 2013; MacLeod and Wong, 2010). Although many pharmaceuticals dissipate rapidly in the environment, their patterns of continuous use and therefore discharge result in "pseudo-persistence" in receiving waters and potential for adverse effects on aquatic organisms (Brooks et al., 2006). Effluent-dominated streams seldom contain acutely toxic concentrations of pharmaceuticals, but chronic levels may impair ecosystem function and individual viability over longer periods of time, as observed for example in the Canadian Prairies (Anderson et al., 2013; Carlson et al., 2013; Waiser et al., 2011).

One of the consequences of antibiotic release into surface waters is the emergence of antibiotic resistance in the environment. This requires development of mitigation strategies to minimize impacts to surface-water quality and receiving populations. Antibiotic resistance genes (ARGs) enter wastewater in bacterial hosts, particularly in urban settings and areas of intensive agriculture (Davis et al., 2006; Mølbak, 2004), as well as from municipal sewage lagoons (Anderson et al., 2013). Resistant bacteria have been detected at high concentrations in agricultural lagoons (e.g., Baquero et al., 2008; Peak et al., 2007; Smith et al., 2004) with elevated concentrations found downstream (e.g., Pei et al., 2006; Pruden et al. 2006) and in groundwater (Chee-Sanford et al., 2001; McKeon et al., 1995). While wastewater treatment plants (WWTPs) can be effective at reducing bacterial loads in receiving waters, the ratio of resistant bacteria to total bacteria may actually be amplified, resulting in elevated resistance traits in the natural environment (Czekalski et al., 2012; Ferreira da Silva et al., 2005; Reinthaler et al., 2003; Zhang et al., 2009a). Previous studies have shown that genes encoding resistance in bacteria disappear quite rapidly in water columns (Engemann et al., 2006, 2008; Knapp et al., 2010a; Zhang et al., 2009b), but can be harbored in peripheral biofilms (Engemann et al., 2008; Knapp et al., 2010b; Zhang et al., 2009b).

A common approach to improve overall effluent quality in a cost-effective manner is the use of a constructed treatment wetland prior to discharge into surface waters (Chen, 2011). In these shallow systems with relatively long residence times, macrophytes, invertebrates, and microorganisms can uptake, metabolize, or sequester pharmaceuticals and nutrients (Brun et al., 2006). There is a clear need to understand and optimize the various processes governing removal of micropollutants, such as pharmaceuticals, in wetlands constructed for wastewater treatment (Hijosa-Valsero et al., 2010), to improve design and management of such systems. Recent work has suggested that macrophyte-dominated microcosms remove pharmaceuticals more efficiently than those lacking this biotic community, at both laboratory-scale (Dordio et al., 2009; Matamoros et al., 2012) and field-scale (Matamoros and Salvadó, 2012; Zhang et al., 2012). However, studies on the efficacy of wetlands to remove pharmaceuticals have generally focused on overall removal efficiency throughout the entire treatment system, with only qualitative discussion of specific attenuation processes that may be involved e.g., the importance of photodegradation for many pharmaceuticals and pesticides (Conkle et al., 2008; Matamoros et al., 2008; Hijosa-Valsero et al., 2010; Matamoros and Salvadó, 2012; Zhang et al., 2012). While characterization of overall removal efficiency is important, little has been reported with respect to quantitative measurement and prediction rates for various dissipation processes (where 'dissipation' refers to general removal from the system when the specific process is uncharacterized or unknown; e.g., photodegradation, abiotic transformation, biotransformation, sorption, etc.). These attenuation processes can all contribute to overall removal of pharmaceuticals both in the dissolved phase and in condensed phases (e.g., plants, sedimenting particles, sediments). They can also occur at rates in the aquatic environment that differ from those in the laboratory (US EPA, 2008). Thus, characterizing the fate processes of these compounds under environmental conditions is valuable in assessing how well such processes can be predicted based on our current understanding of them (Schwarzenbach et al., 2003). Moreover, understanding the factors affecting major removal processes is key to design and operation of treatment systems to optimize removal, e.g., promotion of clear-water conditions to optimize sunlight penetration for photodegradation of organic contaminants (Jasper and Sedlak, 2013).

To address the aforementioned issues, the current study used outdoor mesocosms to simulate a multi-trophic, environmentally relevant, surface wetland ecosystem (Hench et al., 2003) to observe realistic dissipation of pharmaceuticals while incorporating interactions. The disappearance patterns of ARGs in the presence of moderating factors, including macrophytes, exogenous nutrients, and pharmaceutical mixtures, were also investigated. In summary, the main objectives of this study were the following: first, to assess the dissipation of pharmaceuticals and nutrients in surface water wetlands from a simulated single wastewater input in both planted and unplanted mesocosms; second, to determine if dissipation of the target pharmaceuticals could be explained by known sorption and photolytic processes; and third, to quantify the presence and removal of ARGs in these systems.

2. Materials and methods

2.1. Experimental design

Mesocosms were assigned to one of six treatments, which were set up in a randomized complete block design. The treatments were three different waters – control, wastewater (WW), and wastewater plus drugs (WW + D) – and two levels of planting — macrophytes present (M) or not. Details on mesocosm setup and compounds used are provided below.