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Estrogen occurrence and persistence in vernal pools impacted by wastewater irrigation practices

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ABSTRACT

Planned beneficial re-use of water has become an increasingly common conservation practice worldwide, sparking questions about the degree of water treatment needed to mitigate negative environmental impacts. Since the early 1980s, as an alternative to surface discharge, the Pennsylvania State University has spray-irrigated all of its treated wastewater effluent via land application onto an environmental setting known as the "Living Filter" site (~245 ha). The impacts of spray irrigation on nearby ephemeral wetlands, known as vernal pools, were explored. The pools gain water from both natural rainfall and spray-irrigation of the University's treated wastewater. The occurrence and persistence of estrogens in three vernal pools were quantified by analyzing > 137 water samples collected from the pools over an eight-week period coincident with the development period of native amphibian larvae. Additionally, dissolved oxygen, oxidation-reduction potential, water level, water temperature, electrical conductivity, pH, and rainfall data were measured continuously throughout the study period within each pool. Further, the treated wastewater effluent was sampled during each weekly spray-irrigation event. Estrone was detected in nearly 100% of the vernal pool samples, with concentrations up to 6.2 ng L^{-1}. Additionally, 17α-estradiol was not detected in the wastewater effluent, but was present in 52% of the vernal pool samples. 17β-estradiol, estriol, and 17α-ethinylestradiol were detected in fewer than 10% of the vernal pool samples. The findings of this research have important implications for management practices that can help protect these critical habitats.

1. Introduction

The presence of emerging contaminants (ECs) in the environment has become a significant concern in recent years, especially in sensitive aquatic ecosystems. Many ECs, such as natural and synthetic estrogens, are known to be relatively widespread in surface water bodies (DeLaune and Moore, 2013; Finlay-Moore et al., 2000; Gall et al., 2011; Goeppert et al., 2014; Kjær et al., 2007; Kolpin et al., 2013; Nie et al., 2015; van Donk et al., 2013), given their presence in municipal wastewater effluent (Andaluri et al., 2012; Desbrow et al., 1998; Nakada et al., 2004; Ternes et al., 1999; Ying et al., 2002). Conventional wastewater treatment processes were not designed to remove estrogens and other ECs, and therefore wastewater effluent discharged to receiving water bodies is an important pathway of estrogens into the environment (Chang et al., 2011; Pal et al., 2010; Ying et al., 2009). The estrogens most commonly found in treated wastewater include the natural estrogens 17β-estradiol (17β-E2), estrone (E1), estriol (E3), and the synthetic form of estrogen 17α-ethinylestradiol (EE2), which is mainly used in various hormonal contraceptive pharmaceuticals.

Once introduced into aquatic environments, the fate and transport of estrogens are affected by their physicochemical characteristics as well as the characteristics of the receiving water body. Studies have reported higher degradation rates of hormones under aerobic conditions compared to anaerobic conditions (Czajka and Londry, 2006; Combalbert and Hernandez-Raquet, 2010; Yang et al., 2010). Reversible transformation of E1 to 17α-E2 and 17β-E2 has been observed under anaerobic conditions of confined animal feeding operations (CAFO) lagoon water (Zheng et al., 2012), and under laboratory-controlled nitrate- and sulfate-reducing conditions (Mashtare et al., 2013a,b). Under reducing conditions, the conversion of 17β-E2 to 17α-

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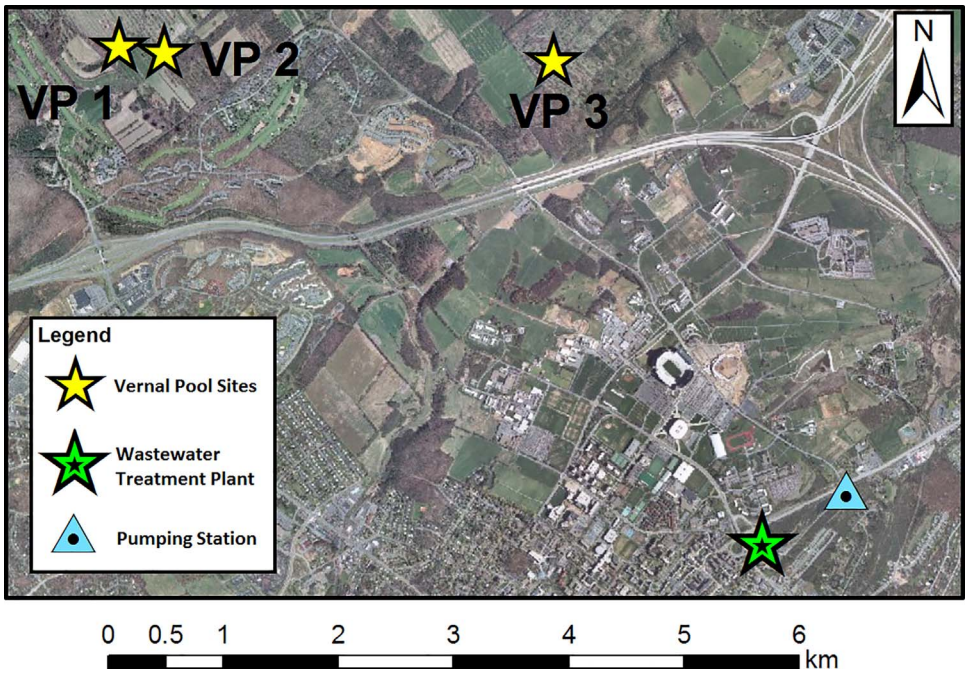

Fig. 1. Map of Penn State University's Wastewater Treatment Plant and Pumping Station, which pumps treated wastewater to the Living Filter, where the three vernal pool study sites (VP 1, VP 2, and VP 3) are located.
(Aerial Image. Source: http://pasda.psu.edu)

E2 was observed in swine and poultry lagoons (Hutchins et al., 2007), and the nitrification of activated sludge in batch experiments led to conversion of 17β-E2 to E1 under alternating aerobic and anaerobic conditions, with faster rates of transformation observed for the aerobic environment (Dytczak et al., 2008). Persistence of 17α-E2 and EE2 under anoxic conditions was also observed (Dytczak et al., 2008).

Research on the impacts of nutrients in agricultural runoff on aquatic systems and organisms has expanded to include the impacts of ECs. In agricultural landscapes, atrazine, a pesticide known for its endocrine disrupting activity, can reach vernal pool habitats through various pathways, with agricultural runoff being the most common (Storrs and Kiesecker, 2004). Abnormalities in aquatic organisms have been observed when exposed to the natural estrogens 17β-E2, E1, E3, and the synthetic estrogen EE2. For example, feminization of male fish has been attributed to estrogens detected in surface water downstream of wastewater treatment plant discharge, with male fish producing vitellogenin, a protein normally synthesized by female fish (Kidd et al., 2007). Shappell et al. (2010) observed feminization of male fathead minnows when exposed to 17β-E2, reporting 17β-E2 to be eight to nine times more potent than 17α-E2. Feminization of male rainbow trout was also observed when exposed to 17β-E2, reporting 17β-E2 to be two to three times more potent to male rainbow trout than E1 (Thorpe et al., 2003).

However, the presence and potential impacts of estrogens in vernal pools have not yet been studied. Higher than normal occurrence of intersex traits in amphibians has been observed in vernal pools impacted by human sources of wastewater (Smits et al., 2014). While Smits et al. (2014) did not examine the presence of ECs in the vernal pools, the detection of caffeine suggested that human wastewater, potentially from leaking septic tanks, was reaching these pools. Additionally, EE2 has been shown to disrupt the mating success of male *Xenopus laevi* (African clawed frog) by reducing the proportion of their mating calls at concentrations as low as 0.296 ng L^{-1}, which is in the range of environmentally relevant estrogen concentrations (Hofman and Kloas, 2012). Clearly, more information on estrogens and other emerging contaminants in vernal pools is needed to properly assess potential contributions to the global amphibian decline.

This study sought to assess the impacts of spray irrigation of treated wastewater on estrogens in surface waters of nearby depressional, temporary wetlands. These vernal pools gain water from both natural rainwater and the spray-irrigated treated wastewater. They typically contain shallow water in the winter and spring, and may dry out during the summer and fall seasons. Vernal pools are important habitats for various amphibian species (Wiggins et al., 1980), including *Rana sylvatica* (wood frog), a native frog in Pennsylvania (Rowe et al., 2009). Amphibians rely on vernal pools to breed and metamorphose (Pough and Wilson, 1977), and are often used as indicators of ecosystem integrity, given their permeable eggs, gills and skin (Dunson et al., 1992). Reducing conditions in vernal pools, particularly during the March – June period when seasonal decrease of dissolved oxygen levels occurs (Carrino-Kyker and Swanson, 2007), can potentially lead to persistence and reversible transformation of estrogens (Zheng et al., 2012). The goal of this study was to quantify how various water quality parameters in three vernal pools impacted by treated wastewater irrigation would influence the occurrence, transformation, and persistence of estrogens. The objectives were to: (i) measure various physical and chemical parameters within the vernal pools, including dissolved oxygen (DO), oxidation-reduction potential (ORP), pH, electrical conductivity (EC), water temperature, and water level; (ii) compare concentrations of estrogens 17α-E2, 17β-E2, E1, E3 and EE2 in the treated effluent and in the vernal pool water; (iii) identify parameters that affect the transformation and degradation of estrogens in the vernal pools; and (iv) examine the persistence of the different estrogens under aerobic/reduced oxygen/anaerobic conditions present in the vernal pools. The study period for sampling of the vernal pools was chosen to coincide with the development period of amphibian larvae native to the Central Pennsylvania region, although frogs (spring peepers) were found in only one of the selected study sites. The relevance of the findings for best management practices that can help protect critical vernal pool habitats and breeding amphibians were explored.

2. Materials and methods

2.1. Study site

This research was conducted at the Pennsylvania State University's Living Filter site, located approximately 3.2 km from the University's main campus in Centre County, Pennsylvania. This 245 ha site has deep (> 30 m) soils overlying bedrock and has been spray-irrigated with the University's treated wastewater since the mid 1960s, with full-scale