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Vadose Zone Transport of Natural and Synthetic Estrogen Hormones at Penn State's "Living Filter" Wastewater Irrigation Site

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Abstract

The increase in endocrine-disrupting compounds in the environment has generated research focused on the behavior of these compounds in natural soil and water ecosystems. To understand how estrogens behave in the soil environment as a result of 25+ yr of wastewater irrigation, soils from Penn State's "Living Filter" wastewater irrigation site were extracted and analyzed for two natural estrogens (17β-estradiol and estrone) and one synthetic estrogen (17α-ethynylestradiol). Soil estrogen concentrations were compared for two independent variables: type of land cover and sampling time. Soils were sampled from cropped and forested land areas, and soils were sampled 2 d and 3 wk after a single 12-h effluent irrigation event. A nonirrigated control site was sampled to provide natural background data. For 17β-estradiol, the nonirrigated mean concentration was 0.68 ± 0.11 ng cm^{-3}, and the irrigated values, including samples from both land areas and time frames, ranged from 0.99 ± 0.11 to 1.82 ± 0.69 ng cm^{-3}. For estrone, the nonirrigated mean concentration was 2.36 ± 0.22 ng cm^{-3}, and the irrigated values, including samples from both land areas collected and time frames, ranged from 2.18 ± 0.20 to 6.24 ± 3.14 ng cm^{-3}. The 17α-ethynylestradiol nonirrigated mean concentration was 0.47 ± 0.40 ng cm^{-3}. The irrigated values, including samples from both land areas and time frames, ranged from 0.25 ± 0.06 to 1.37 ± 0.39 ng cm^{-3}. This study found that time of sampling, land cover, and irrigation can affect estrogen concentrations in soils, resulting in levels that exceed natural background and require improvements in management practices.

THE PRESENCE OF pharmaceuticals and endocrine-disrupting compounds (EDCs) in the environment has increased over the last half century, and one group of EDCs, estrogenic compounds, has been found in surface waters across the United States (Kolpin et al., 2002). The known entrance pathways for estrogens into the environment include surface water runoff from manure-amended fields (Shappell et al., 2010), wastewater treatment plant effluent discharge into streams (Andersen et al., 2003; Barel-Cohen et al., 2006), leaky septic systems (Wilcox et al., 2009), land application of biosolids (Langdon et al., 2014), combined sewer overflow events (Phillips et al., 2012), and leaching of estrogens from agricultural storage lagoons (Arnon et al., 2008). When male fish come into contact with estrogens found in wastewater effluent, they synthesize vitellogenin, a protein in female fish used to produce yolks. This feminization response occurs at low concentrations (ng L^{-1}). It causes hermaphroditism in male fish, and it can destroy the fish population (Purdom et al., 1994; Kidd et al., 2007). Studies have also shown that an increase in environmental estrogen exposure can affect endocrine and reproductive processes in vertebrates, including humans (Dickson et al., 1986; Dickerson and Gore, 2007).

Previous studies have looked at how manure and wastewater enter and affect the environment. Baronti et al. (2000) and Andersen et al. (2003) looked at estrone, 17β-estradiol, and 17α-ethynylestradiol concentrations in sewage treatment plant effluent. Both studies found that the estrogen hormones were biologically reduced during treatment, and outlet effluent concentrations, including all three estrogens, ranged from 0.4 to 1.4 ng L^{-1}. Some of the factors that affect the outlet effluent concentrations include influent concentrations, treatment technologies, and type of estrogen hormone. Barel-Cohen et al. (2006) quantified estrone, 17β-estradiol, estriol, and ethynylestradiol concentrations along sections of the Lower Jordan River in Israel. Specific sampling locations were chosen that included tributary inputs containing fish pond effluent, agriculture runoff, and sewage effluent. Results were used to determine what effect these inputs

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Abbreviations: EC, electrical conductivity; EDC, endocrine-disrupting compound; LOD, limit of detection; MTBE, methyl tert-butyl ether; SPE, solid phase extraction; TC, total carbon; TN, total nitrogen; TOC, total organic carbon.

have on estrogen concentrations in the river. Estradiol and estrone were present along the river (0.9–9.4 ng L^{-1}), and ethynylestradiol concentrations in 70% of the samples taken from the Lower Jordan were greater than the reported lowest observed effect level value of 1 ng L^{-1}. To understand manure inputs, Hutchins et al. (2007) measured estrogen concentrations (estrone, 17β-estradiol, 17α-ethynylestradiol, and conjugates) in large animal waste lagoons. Swine lagoons had the highest concentrations of estrogens (1000–21,000 ng L^{-1}), and beef lagoons had the lowest concentrations of estrogens(22–24 ng L^{-1}).

Arnon et al. (2008) identified leaching as a potential issue from single-stage earthen unlined dairy cattle waste lagoons. This study measured estrogens as deep as 32 m below the lagoon at concentrations >50 ng kg^{-1} dry soil. Even though numerous studies have reported that estrogens are present in the environment and have effects on organisms, little research has been done on field soils that have been amended with animal waste or irrigated with human effluent (Finlay-Moore et al., 2000; Duran-Alvarez et al., 2009; Mahjoub et al., 2011; Langdon et al., 2014).

Consequently, this study looked at effluent irrigated soils. Two natural estrogens, 17β-estradiol and its metabolite estrone, and one synthetic estrogen, 17α-ethynylestradiol, were studied in the soils from the irrigation site. All three estrogens are hydrophobic, with reported aqueous solubilities ranging from 4.8 to 13.0 mg L^{-1} and log K_{ow} values ranging from 3.10 to 3.94, depending on the specific estrogen (Lai et al., 2000). Sorption studies were conducted to determine each estrogen's log K_{oc} value specific to the silt loam soil found at the Living Filter wastewater irrigation field site. Measured log K_{oc} values ranged from 3.03 to 3.17 (Woodward, 2010), which suggests that estrogens have a high affinity for organic carbon in the soil. This range in log K_{oc} values is comparable to that reported by other studies (Lee et al., 2003; Casey et al., 2005; Hildebrand et al., 2006).

The main goals of this research were to quantify estrone, 17β-estradiol, and 17α-ethynylestradiol concentrations in the top 1 m of the soil profile at Penn State's "Living Filter" system and to determine whether estrogens have accumulated at the site. This site has received 25+ yr of wastewater irrigation. After undergoing primary and secondary treatment, 100% of the university's wastewater is spray-irrigated at the Living Filter onto cropped and forested areas and on grasslands (Parizek et al., 1967; Richardson 2010). Soil cores were collected from the cropped and forested areas 2 d and 3 wk after a scheduled 12-h effluent application. This study will provide details on (i) how land cover differences could influence estrogen transport through the soil profile and (ii) the persistence of hormones in the environment after long-term (25+ yr) exposure to a point source.

Materials and Methods

Field Site and Sampling

Penn State's Living Filter is a wastewater spray irrigation operation located approximately 3.2 km from The Pennsylvania State University campus (University Park, PA). This study was conducted at the Astronomy site (Fig. 1). The Astronomy site consists of three different land cover areas. One area is used to grow crops, one area is forested, and one area is covered with grass. Soils were sampled from an irrigated cropped area and an irrigated forested area in late September and early October (Fig. 1). The cropped area was planted with wheat (*Triticum aestivum* L.) in early October after a corn silage harvest, and the forested area consists of mature hardwood trees. The sample sites are all Hagerstown silt loam soil.

Before selecting soil sampling locations, wastewater application depths were measured using rain gauges. Along two irrigation laterals (10/2 and 4/6), rain gauges were set up in 2-m increments perpendicular to the lateral sprinkler heads. The laterals were turned on for a 12-h period. Wastewater application depths were recorded for each rain gauge, and the depths were plotted against the rain gauge distance from the sprinkler heads to determine the distance at which maximum application occurred. The maximum application depth was achieved at a specified distance (7.9 m). All soil cores taken in this study were sampled perpendicular to the sprinkler heads at this distance (Fig. 2).

For the year 2011, leading up to the irrigation for this experiment, lateral 10/2 was run 11 times, and lateral 4/6 was
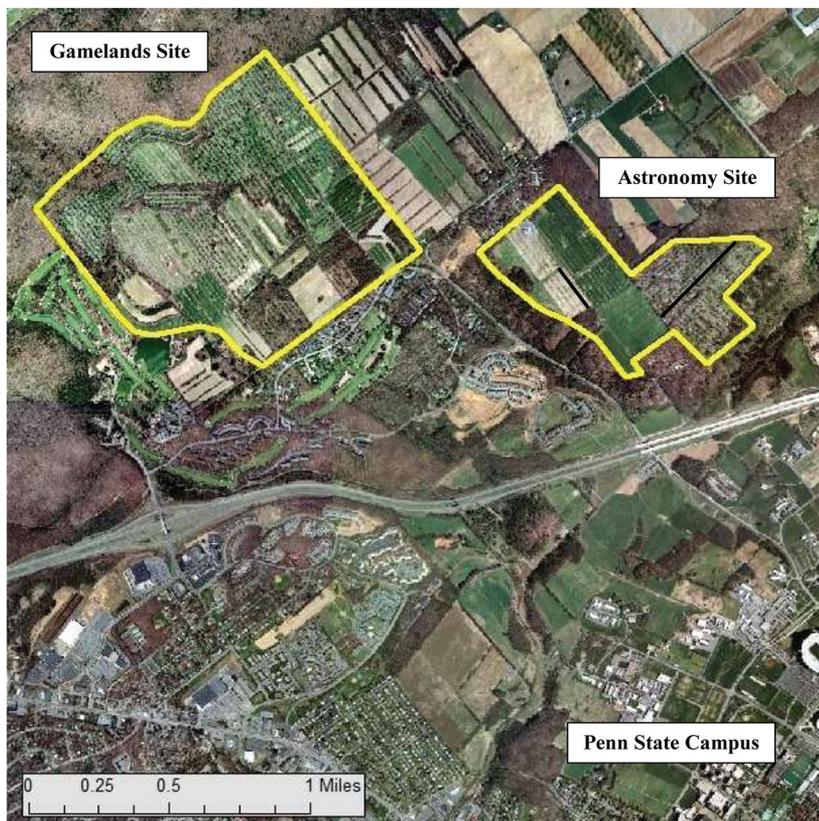

Fig. 1. Location map of the two wastewater irrigation sites, Gamelands and Astronomy, operated by The Pennsylvania State University. Lines marked on the Astronomy site represent the two spray laterals: 10/2 (cropped) and 4/6 (forested).
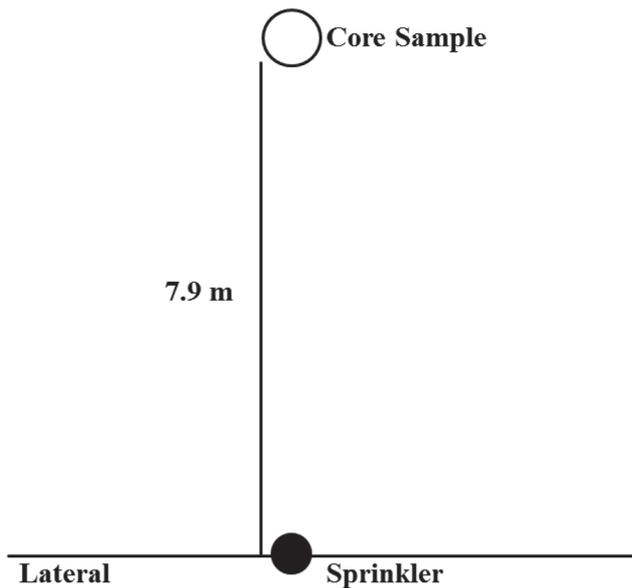
Fig. 2. Core sampling location in relation to the irrigation lateral and sprinkler head.

run 32 times. Each run lasted approximately 12 h and distributed 12 cm ha^{-1} of effluent to the soil. For future data interpretation, it may be important to consider that more effluent was distributed on the forested soils before this experiment. The scheduled irrigation for this study occurred on 20 Sept. 2011. While the irrigation laterals were running, four 1-L effluent samples were collected in amber glass jars, two from the cropped lateral (10/2) and two from the forested lateral (4/6) sprinkler heads. The irrigation laterals were turned on at 6:00 PM. Effluent samples were collected one time only, 1 h after the laterals were turned on. Laterals remained on until 6:00 the next morning. Irrigation was applied one time only over this 12-h period and was then discontinued for the remaining 3 wk of the study (20 Sept.–11 Oct. 2011).

The irrigation laterals are above-ground pipes that run across each land cover type. Each lateral has around 10 sprinkler heads, approximately 20 m apart. Five sample locations, specific to five different sprinkler heads along each lateral, were chosen, and samples were collected 2 d after (22 Sept. 2011) and 3 wk after (10 Oct. 2011) the 12-h irrigation that started the study. Estrogens degrade rapidly in the laboratory. Therefore, the initial 2-d sampling was chosen to capture some of the estrogens present in the soil as a result of the 12-h effluent irrigation event. The estrogens should degrade within the first few days. The 3-wk sampling was done to see if estrogens remained in the soil, indicating that degradation was not as rapid as literature studies would suggest. Two days after irrigation, three cores were sampled at each of the five locations using a Giddings hydraulic probe: (i) a 120-cm-long soil core taken for conventional soil physical and chemical property analyses, (ii) a 120-cm-long core taken for estrogen analysis, and (iii) a 15-cm-long core from the soil surface also taken for estrogen analysis. Because surface soil extractions in the laboratory were conducted in 2-cm increments, the 15-cm-long core was collected to ensure that there was enough mass of soil to run through an extraction procedure. Three weeks after irrigation, a second set of estrogen cores was collected at the same five sample locations: (i) a 120-cm-long

core and (ii) a 15-cm-long core both taken for estrogen analysis. All of the sample locations chosen were topographic high points to limit the effects of lateral flow and runoff into the sampling area.

Initially, nonirrigated control sites were identified and sampled at the Living Filter. However, after conducting a drift experiment, results showed that these sites were receiving effluent input from spray drift. For this reason, an off-site nonirrigated control site was sampled to provide background estrogen concentrations. The control site used was The Russell E. Larson Agricultural Research Farm located approximately 16 km southwest of University Park. According to the land use history of the area, the following agricultural sources could not have affected the control site: manure applications, biosolid applications, and/or runoff from livestock facilities. The soil at the control site was mapped as the same soil series (Hagerstown) as the irrigated site. Three 10-cm cores were collected at the nonirrigated control site for estrogen analysis.

Once collected, irrigated and nonirrigated soil cores were retained in their sampler liners, and the liners where immediately capped and placed in dark trash bags. Samples were stored in a walk-in refrigerator (4°C) within 8 h of sample collection until processed. All samples were processed within 1 wk after collection.

Laboratory Analyses

Soil Properties Analysis

The 120-cm soil physiochemical properties' cores from the Living Filter and the 10-cm cores from the control site were described, subsampled, and analyzed for percent total carbon (%TC), percent total nitrogen (%TN), electrical conductivity (EC), and pH. The following subsampling depth increments were used when applicable: 0 to 2, 2 to 4, 4 to 6, 6 to 10, 10 to 20, 20 to 30, 30 to 40, 40 to 80, and 80 to 120 cm. The control soil was analyzed at Penn State's Agricultural Analytical Services Lab. For the Living Filter soil cores, subsamples for physical/chemical properties were placed in open Ziploc bags and air dried. Once dried, subsamples were sieved through a 2-mm mesh screen in preparation for EC and pH analyses. Additional subsamples were sieved through a 0.25-mm mesh screen in preparation for organic carbon and nitrogen analyses. The Thomas (1996) method was used to determine pH. Thomas (1996) uses a water extraction with a 1:1 soil to water ratio (mass basis). The Rhoades (1996) method was used to determine EC. Rhoades (1996) uses a water extraction with a 1:2 soil to water ratio (mass basis). All water used was ultrapure deionized (18 MΩ). Both parameters were measured using a Thermo Scientific Orion Star A215 pH/conductivity meter (Fisher Scientific). The finer fraction (<0.25 mm) was analyzed using a Carlo Erba CHNS-O Elemental Analyzer (EA1110, LECO) for %TC and TN. The method used to prepare and analyze soils with this machine comes from the Carlo Erba manual (CE Instruments, 1996). Preliminary work identified that no pretreatment step was needed to account for inorganic carbon in the soils sampled; TC was equated to total organic carbon (TOC).

Chemicals

Estrone (≥99%), 17β-estradiol (≥98%), and 17α-ethynylestradiol (≥98%) were purchased from Sigma-

Aldrich. Solvents included LC-grade methanol (MeOH), HPLC-grade methyl tert-butyl ether (MTBE) (Sigma-Aldrich), and HPLC- and LC/MS-grade acetonitrile (Fisher Scientific). Other reagents used in this study were sodium azide (NaN$_3$; ≥99.5%) (Sigma-Aldrich) and ammonium hydroxide (NH$_4$OH; trace-metal grade) (Fisher Scientific). All water used was ultrapure deionized (18 MΩ).

Filtration and Extraction Procedure

Effluent Samples. The four 1-L effluent samples were filtered through a 0.7-µm glass fiber filter (Pall Corp.) and run through the solid phase extraction (SPE) procedure described below.

Soil Samples. Soil cores used for estrogen analyses from the irrigated and nonirrigated control sites were subsampled in the same increments as the soil property cores. One by one, subsamples of soil were removed from the core and mixed to create homogeneity. A 50-g portion was taken out of each mixed subsample for estrogen extraction. This 50-g portion was placed into an amber glass jar with 50 mL of NaN$_3$ solution (250 mg L^{-1} NaN$_3$). Each amber jar containing soil and NaN$_3$ was stored in the freezer (−18 to −20°C) until all of the soil cores had been processed (within 1 wk). After processing, soils in the freezer were extracted.

Amber jars were removed from the freezer, and soils were equilibrated to room temperature. Once at room temperature, 100 mL of a 50/50 MeOH/water solvent extractant was added to each jar. The soil and extractant were shaken for 1 h on a rotary shaker and then centrifuged for 1 h at 1500 rpm. Thirty milliliters of supernatant was taken, filtered through a 0.7-µm glass fiber filter (Pall Corp.), and processed through the SPE procedure described below.

Solid Phase Extraction Procedure

Filtered effluent samples and field soil extraction solutions were cleaned further and concentrated using an Oasis HLB Plus (Waters Corp.) cartridge. The SPE method used was a modified version of the procedure provided in the Waters Application Notebook (Waters Corp., 2008). Cartridges were preconditioned with 5 mL of MTBE, 5 mL of MeOH, and 5 mL of water, followed by a loading phase of the extraction solution (30 mL). Cartridges were then washed with 5 mL of a 40% MeOH and water solution and 5 mL of water. Finally, the cartridge was eluted with 6 mL of a 90% MTBE and 10% MeOH solution. For each phase, precondition, load, wash, and elute, a flow rate of 1 to 2 mL min^{-1} was used. The eluent was collected and evaporated to dryness under a gentle stream of nitrogen gas. Samples were then redissolved in 1 mL of MeOH for LC-MS-MS analysis. The total recovery percentages using this method were 59, 42, and 65% for estrone, 17β-estradiol, and 17α-ethynylestradiol, respectively.

Instrumentation

Soil extracts and effluent samples were analyzed for the estrogens of interest using an LC-MS-MS system consisting of a Shimadzu SIL-HTc autosampler fitted with two Shimadzu LC-10AD vp pumps. The column used was an XTerra MS C18 column (2.5 µm; 2.1 × 30 mm) (Waters). The MS-MS used for quantification was a MicroMass Quattro micro API (Waters). The mobile-phase solvents were 0.6% NH$_4$OH in acetonitrile (solvent A) and 0.6% NH$_4$OH in water (solvent B) operated using a gradient method. The initial mobile phase started with 10% solvent A and 90% solvent B. Over the first five minutes, the gradient ramped up linearly to 90% solvent A and 10% solvent B. This was held for 2 min. From 7 min to the end of the run, the gradient dropped back down to 10% solvent A and 90% solvent B. The operating mode was electrospray negative. Flow rate was set at 0.25 mL min^{-1}, and an injection volume of 20 µL was used for each sample. Results of these conditions yielded retention times of 5.18, 5.34, and 5.37 min for 17β-estradiol, 17α-ethynylestradiol, and estrone, respectively. External standard calibration curves were generated using peak areas and known standard concentrations. Peak area outputs from samples were used against calibration curves to estimate sample concentrations. The measured limit of detection (LOD) values were 0.98, 1.3, and 1.6 µg L^{-1} for estrone, 17α-ethynylestradiol, and 17β-estradiol, respectively.

Statistical Analysis

Output from the LC-MS-MS (µg mL^{-1}) was adjusted to account for the SPE concentration step. The SPE process concentrates the sample by 30. A back calculation is done to convert the LC-MS-MS measured concentration back to the lower, actual field soil concentration. The field soil concentrations are presented as total estrogen concentration (ng cm^{-3} of dry soil). The word "total" refers to the extraction step. The concentration of each hormone extracted represents what was sorbed onto the soil and what was in the soil solution. For each estrogen, at each depth increment, total concentration values were averaged across all N samples (max. $n = 5$). These mean total concentration values, for each depth up to 80 cm, were used to create Fig. 3 through 6.

For statistical analyses, the total estrogen concentration values (ng cm^{-3} of dry soil) were weighted for the top 10 cm of soil in each soil core (0–2, 2–4, 4–6, and 6–10 cm). These weighted concentration values were used to conduct all statistical analyses. Possible outlier values were left in for the scope of this study. It was not clear whether the high values were a result of the field sampling location or analysis, and for this reason, all outliers were left in the data set.

Typically, environmental estrogen levels are low, and this is a major challenge for hormone research. To analyze environmental data, a clear method for dealing with samples that fall below the instrumentation's LOD is necessary. In this study, when LC-MS-MS output concentrations were below detection limit, the LOD/$\sqrt{2}$ was used. The estrogen-specific LOD values are reported above. Figures 3 through 6 graphically indicate an LOD range for the three estrogens.

All statistics were conducted using SAS 9.3 software (SAS Institute). This study design had three factors, and each factor had two levels. Before the results were analyzed, the values used to analyze each comparison were tested for normality. The p values from two normal tests, Shapiro-Wilk and Kolmogorov-Smirnov, were used to determine normality. If data were normal, an independent t test was used to evaluate the difference in the means between the two independent groups. A *proc ttest* model was used in SAS. Non-normal data were analyzed using a *proc npar1way* model, a Mann–Whitney nonparametric version of the independent t test. If the Shapiro-Wilk and Kolmogorov-Smirnov tests disagreed on normality, then both the t test and
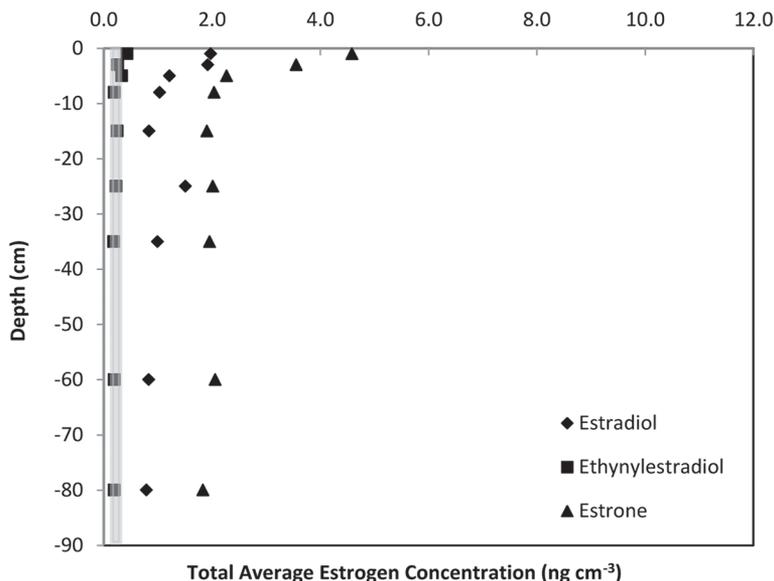
Fig. 3. Average total estrogen concentrations from the cropped irrigated (2 d) sample locations taken at the Penn State Astronomy site (22 Sept. 2011). The gray bar indicates the limit of detection (LOD) range when converted to ng cm⁻³. The LOD values are 0.36, 0.29, and 0.23 ng cm⁻³ for 17β-estradiol, 17α-ethynylestradiol, and estrone, respectively.
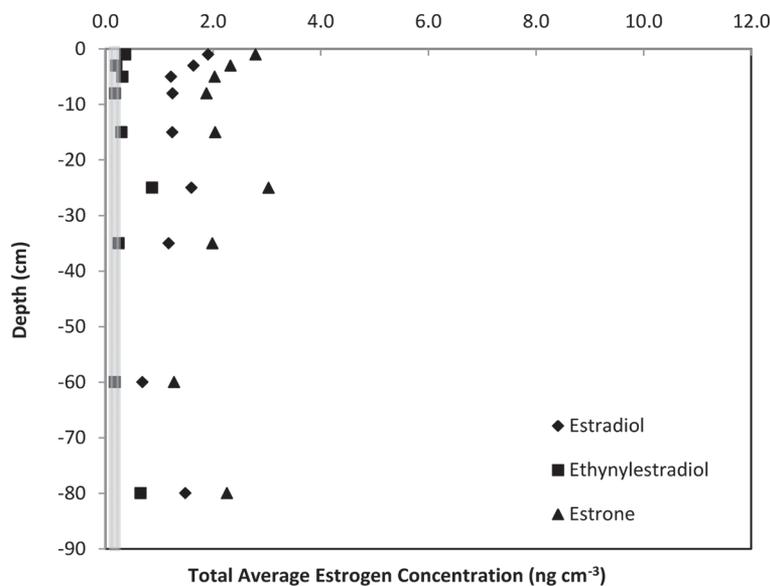

Fig. 4. Average total estrogen concentrations from the forested irrigated (2 d) sample locations taken at the Penn State Astronomy site (22 Sept. 2011).

Mann–Whitney nonparameteric tests were run on the data, and the p values from both tests are reported. All tests were conducted using a significance level of $\alpha < 0.05$.

Results and Discussion

Soil Physical Properties

All of the sampled soil cores were described. The surface horizon was either an A or an Ap. For all of the cores, the A or Ap surface horizon was underlain by a series of Bt horizons (Table 1). Cores were also analyzed for %TOC and %TN. The irrigated forested area had a higher average %TOC at the surface (7.07) than the irrigated cropped area (2.44) (Table 2). Both land uses

exhibited a decrease in %TOC with increasing depth. The nonirrigated control soil had an average %TOC of 1.24. Average %TN results for each land use are also reported in Table 2 and were observed to be similar to %TOC trends. The forested area had a higher average %TN value at the surface than the cropped area (0.53 and 0.22, respectively). Both land cover areas showed a decrease in average %TN with depth.

Environmental variables such as pH and EC reveal information around the charge state of an estrogen compound and water movement in the soil profile. Within each soil core, there was limited variation in pH by depth. For this reason, the pH range (4.8–7.7), including the irrigated and nonirrigated soils, is reported (Table 3). These pH values were below the pKa values (10) for the three estrogens studied. Because the pH values measured in the soil were below the pKa values, it can be assumed that all three estrogens were functioning as nonpolar compounds. Average EC values (1:2, soil to water extract) at the surface were higher in the irrigated soils than in the nonirrigated control soil (928.6, 401.40, and 60 µS cm⁻¹ for the irrigated forested, irrigated cropped, and nonirrigated sites, respectively) (Table 4). Electrical conductivity values in the irrigated soils were greatest at the surface and decreased with depth.

Effluent Analysis

Measured concentrations in the effluent varied for each estrogen. For 17β-estradiol, the average concentration in the effluent was 8.43 ± 3.55 µg L⁻¹. The average estrone concentration was 17.0 ± 8.37 µg L⁻¹, and the average 17α-ethynylestradiol concentration was 6.76 ± 4.49 µg L⁻¹. For all three estrogens, measured concentrations varied between sprinkler heads. The estrogen concentrations measured in the cropped sprinkler heads ranged from 7.78 to 28.71 µg L⁻¹, and the concentrations measured in the forested sprinkle heads ranged from below detection limit to 11.01 µg L⁻¹. These data confirm that even after primary and secondary treatment, estrogens are present in the effluent at concentrations that can affect organisms. Without land application, effluent would be directly discharged into streams. These data highlight one possible benefit of land-based wastewater application: it can be used as a form of hormone removal.

Soil Analysis

The concentration data used to conduct all statistical analyses are summarized in Table 5.

Irrigation

Results show that wastewater irrigation increases 17β-estradiol concentrations in soils beyond natural background levels. Measured concentrations of 17β-estradiol were higher in the irrigated soils. The nonirrigated 17β-estradiol mean concentration was 0.68 ± 0.11 ng cm⁻³, and the irrigated values, including samples from both land covers and time frames, ranged
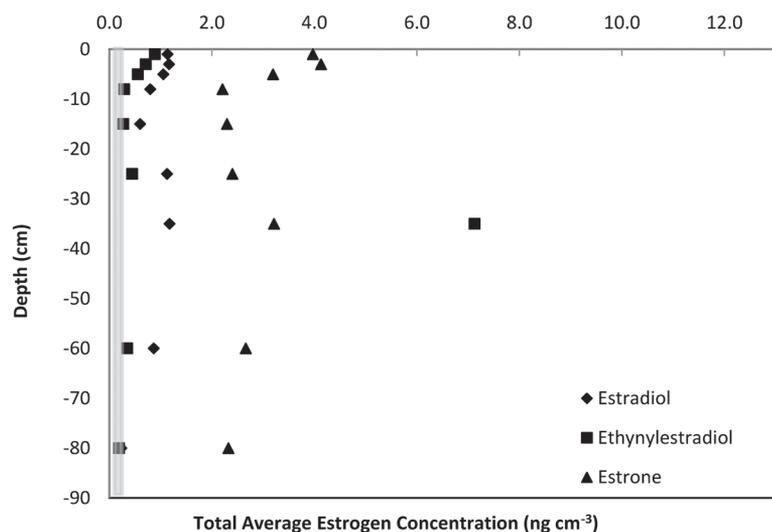
Fig. 5. Average total estrogen concentrations from the cropped irrigated (3 wk) sample locations taken at the Penn State Astronomy site (10 Oct. 2011).
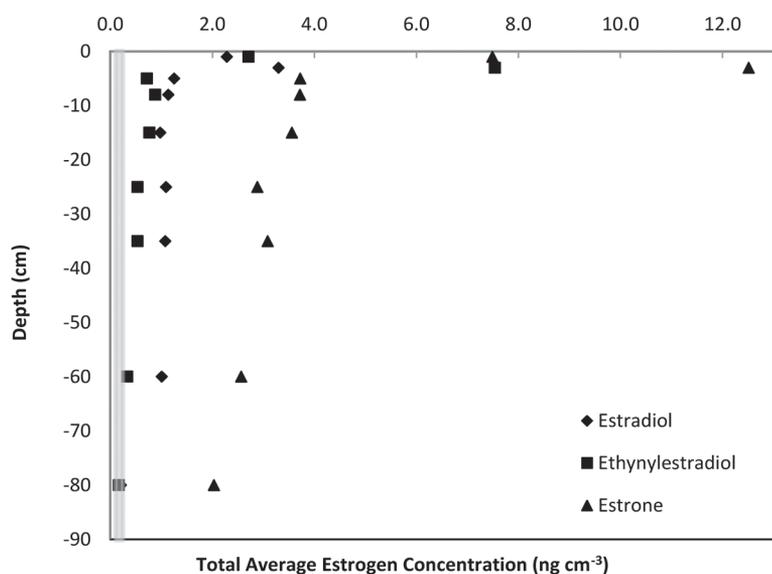

Fig. 6. Average total estrogen concentrations from the forested irrigated (3 wk) sample locations taken at the Penn State Astronomy site (10 Oct. 2011).

from 0.99 ± 0.11 to 1.82 ± 0.69 ng cm^{-3}. Literature laboratory studies have reported that 17β-estradiol degrades within hours to days in aerobic environments (Colucci et al., 2001; Andersen et al., 2003; Lee et al., 2003; Das et al., 2004; Ying and Kookana, 2005). However, these results suggest that long-term irrigation with effluent causes accumulation of 17β-estradiol in the soil profile, and 17β-estradiol does not degrade quite as rapidly as suggested by laboratory studies.

Irrigation affects estrone concentrations as well. Three weeks after irrigation, estrone concentrations differed significantly between the irrigated and nonirrigated soils. The mean estrone concentrations were higher in the cropped and forested irrigated soils than in the nonirrigated soils. The nonirrigated mean concentration was 2.36 ± 0.22 ng cm^{-3}, and the mean concentrations in the irrigated cropped and forested soils were 3.14 ± 0.35 and 6.24 ± 3.14 ng cm^{-3}, respectively. To analyze

the forested irrigated soil data, a *t* test ($p = 0.05$) and a nonparametric Mann–Whitney test ($p = 0.03$) were used.

One possible explanation for the high estrone concentrations in the soils collected 3 wk after irrigation is that 17β-estradiol is degrading into estrone over the timeframe of the study (Colucci et al., 2001; Casey et al., 2003; Hildebrand et al., 2006). However, this process was not supported by the data. In the cropped soils, even though there was a statistical difference in 17β-estradiol between the 2-d and 3-wk soils, there was no statistical difference between the estrone concentrations measured in the soils taken at the two time frames. In the forested soils, there was no statistical difference in 17β-estradiol between the 2-d and 3-wk soils, but there was a difference in estrone concentrations. The results do not support the idea that 17β-estradiol is degrading into estrone, and at this time, there is no reasonable explanation for the high estrone values in the forested soils sampled 3 wk after irrigation.

For 17α-ethynylestradiol, results indicate that for the most part irrigation of effluent onto soils does not increase 17α-ethynylestradiol beyond measured background levels. There was one case where the mean 17α-ethynylestradiol concentration was higher in the irrigated soil than in the nonirrigated soil. For the forested soils collected 3 wk after irrigation, the irrigated mean concentration was significantly different from the nonirrigated mean concentration. The irrigated concentration was 1.37 ± 0.39, and the nonirrigated concentration was 0.47 ± 0.40. The irrigated concentration was higher. However, the high value 1.37 ng cm^{-3} could be the result of an outlier effect, and possible outlier values were left in the data set for this study.

Land Cover

Depending on the sampling time, results show that land cover has some effect on soil 17β-estradiol, estrone, and 17α-ethynylestradiol concentrations. The soil cores taken 2 d after irrigation showed no statistical differences in 17β-estradiol concentrations between the two land covers. The cropped and forested areas had similar 17β-estradiol mean concentrations (1.43 ± 0.39 ng cm^{-3} for the cropped area and 1.45 ± 0.07 ng cm^{-3} for the forested area). The cores taken 3 wk after irrigation produced a different result. The mean 17β-estradiol concentration was higher in the forested area (1.82 ± 0.69 ng cm^{-3}) than in the cropped area (0.99 ± 0.11 ng cm^{-3}) at 3 wk. There are key differences in the two land uses: soil organic matter content and in turn sorption potential (Lai et al., 2000; Lee et al., 2003; Casey et al., 2005; Hildebrand et al., 2006), vegetation, and amount of sunlight. The amount and type of vegetation can influence the soil organic matter content and quality and in turn the sorption potential of the area. Differences in vegetative rooting systems can also influence water and hormone transport through the soil profile. Sunlight can also influence measured concentrations; more sunlight leads to increased degradation.

Land cover	Horizon	Depth
		cm
Cropped	Ap1	0–14
	Ap2	14–24
	Bt1	24–30
	Bt2	30–50
	Bt3	50–64
	Bt4	64–73
	Bt5	73–84
Forested	A	0–8
	BE	8–16
	Bt1	16–31
	Bt2	31–57
	Bt3	57–95
	Bt4	95–110
Control	Ap1	0–9
	Ap2	9–10

These physiochemical properties could explain some of the concentration differences seen between the two land covers at 3 wk.

For estrone, only samples collected 3 wk after irrigation showed a statistical difference in concentrations between the two land covers. At 3 wk, the mean estrone concentration was higher in the forested area (6.24 ± 3.14 ng cm^{-3}) than in the cropped area at 3 wk (3.14 ± 0.35 ng cm^{-3}). The samples collected 2 d after irrigation showed no statistical difference in concentrations between the two land covers. The cropped and forested areas had similar mean estrone concentrations (2.90 ± 1.25 ng cm^{-3} for the cropped area and 2.18 ± 0.20 ng cm^{-3} for the forested area).

Differences in 17α-ethynylestradiol concentrations between the two land cover areas were also seen in the samples taken 3 wk after irrigation. The 3-wk samples had higher 17α-ethynylestradiol concentrations in the forested area than in the cropped area. The cropped area had a 17α-ethynylestradiol mean concentration of 0.55 ± 0.13 ng cm^{-3}, and the forested area had a mean concentration of 1.37 ± 0.39 ng cm^{-3}. For this case, the same physical and chemical land cover differences that were described above to explain 17β-estradiol and estrone concentrations could also explain differences seen in measured

17α-ethynylestradiol concentrations. As in the case of estrone, there were no statistical differences between the two land covers in the samples taken 2 d after irrigation. The cropped and forested areas had similar mean 17α-ethynylestradiol concentrations.

Time

Time of sampling after irrigation has some effect on 17β-estradiol, estrone, and 17α-ethynylestradiol soil concentrations. First looking at 17β-estradiol, the cropped and forested areas produced different results when 17β-estradiol was tested over time. For the cropped area, 17β-estradiol concentrations decreased over the 3-wk period. The concentrations measured in the soil cores taken 2 d after irrigation (1.43 ± 0.39 ng cm^{-3}) were higher than the concentrations measured in the cores taken 3 wk after irrigation (0.99 ± 0.11 ng cm^{-3}). Even though the previous section's data suggested that 17β-estradiol does not degrade and return to natural background conditions, these data suggest that there is some degree of degradation occurring between the two sampling time frames. Some degradation of 17β-estradiol between the two time frames is what would be expected according to literature laboratory studies for this compound (Colucci et al., 2001; Andersen et al., 2003; Lee et al., 2003; Das et al., 2004; Ying and Kookana, 2005). For the forested area, there was no significant difference in 17β-estradiol concentrations between the two sampling time frames.

In one case, estrone concentrations changed over the 3-wk period. In the forested soils, the concentrations in the soils collected 2 d after irrigation (2.18 ± 0.20 ng cm^{-3}) were significantly different from the concentrations in the soils collected 3 wk after irrigation (6.24 ± 3.14 ng cm^{-3}); the 3-wk concentrations were higher. For the forested data, a t test ($p = 0.04$) and a nonparametric Mann–Whitney test ($p = 0.02$) were run. Regarding the cropped soils, there were no significant differences in the estrone concentrations between the two sampling time frames.

For 17α-ethynylestradiol, in the cropped and forested land cover areas, 17α-ethynylestradiol concentrations were statistically different in the soils sampled 3 wk after irrigation relative to the 2-d samples. To our knowledge, the only compound that can degrade to 17α-ethynylestradiol is mestranol, another synthetic estrogen used in contraceptives (Ternes et al., 1999). However, mestranol use has declined over time, making this an unlikely

Table 2. Average total organic carbon and total nitrogen of the irrigated cropped and forested soils taken at the Astronomy site (22 Sept. 2011).

Depth	Average TOC†			Average TN‡	
	Forested	Cropped	Control§	Forested	Cropped
cm	%				
0–2	7.07	2.44	1.24	0.53	0.22
2–4	5.27	2.17		0.38	0.20
4–6	4.54	1.87		0.32	0.17
6–10	3.36	1.54		0.23	0.14
10–20	1.66	1.16		0.12	0.10
20–30	0.48	0.31		0.05	0.04
30–40	0.20	0.21		0.04	0.04
40–80	0.14	0.17		0.03	0.04
80+	0.08	0.08		0.03	0.03

† Total organic C.

‡ Total N.

§ Percent total organic C from the single control soil analyzed at Penn State's Agricultural Analytical Services Lab.

Table 3. Ranges in pH from the irrigated cropped and forested soils taken at the Astronomy site (22 Sept. 2011) and the measured pH value of the soil sampled at the nonirrigated control site.

Site	Land cover	pH
Irrigated	cropped	5.2–7.5
	forested	4.8–7.7
Nonirrigated	cropped	6.4

Table 4. Average electircal conductivity values by depth for the irrigated cropped and forested soils taken at the Astronomy site (22 Sept. 2011).

Depth	Forested	Cropped	Control†
cm		μs cm^{-1}	
0–2	928.6	401.4	60.0
2–4	553.2	350.6	
4–6	430.2	248.9	
6–10	314.8	212.9	
10–20	145.5	135.2	
20–30	141.5	93.4	
30–40	162.6	106.2	
40–80	210.8	132.6	
80+	146.4	149.7	

† Electrical conductivity from the single control soil analyzed at Penn State's Agricultural Analytical Services Lab.

explanation for the changes seen in 17α-ethynylestradiol over time. Further study is needed to explain this increase.

Conclusions

The goal of this study was to quantify estrogen accumulation and concentrations in different soil profiles at Penn State's wastewater irrigation system, The Living Filter.

Soil %TOC and %TN reflected land cover rather than irrigation effects. Percent TOC values were higher at the surface in the forested soils (7.07) than in the cropped soils (2.44). All %TOC values decreased with depth. Percent TN trends were similar to %TOC: the forested soils had higher %TN at the surface (0.53) than the cropped soils (0.22), and these values decreased with depth.

The compound 17β-estradiol was present at higher concentrations in the irrigated soils than in the nonirrigated control soils. This was true for samples collected at each land cover area and at each time frame after the initial irrigation event. Even though throughout the study 17β-estradiol concentrations exceeded background concentrations, 17β-estradiol did show some degradation between the cropped soils taken 2 d after irrigation and the cropped soils taken 3 wk after irrigation. For soils sampled 3 wk after irrigation, land cover did have an effect on 17β-estradiol concentrations. The forested concentrations were greater than the cropped concentrations.

Estrone concentrations only exceeded background levels in the soils sampled 3 wk after irrigation. This occurred for both the cropped and forested soils. Although both exceeded natural background, the forested soils had higher concentrations than the cropped soils. Estrone could have accumulated in the soils sampled 3 wk after irrigation as a result of 17β-estradiol degradation.

The compound 17α-ethynylestradiol did not vary between irrigated and nonirrigated soils, except for the soil sampled from the forested area 3 wk after irrigation. There were differences seen in 17α-ethynylestradiol concentrations over time and between land covers. At the 3-wk time frame, the forested soils had statistically higher concentrations than the cropped soils. For the forested and cropped areas, 17α-ethynylestradiol concentrations were higher in the soils sampled 3 wk after irrigation than 2 d after irrigation. Future studies will determine the cause of this increase over time and whether the increase is a result of a compound degrading into 17α-ethynylestradiol, a compound mimicking 17α-ethynylestradiol during analysis, and/or simply soil variability.

Three main conclusions can be drawn from this study: (i) 17α-ethynylestradiol does not exceed background, which is good for the site because 17α-ethynylestradiol is one of the more potent estrogens; (ii) 17β-estradiol does exceed background at the site, and being another one of the more estrogenic compounds, future research will need to focus on this compound and its persistence at the site; and (iii) there are inherent properties associated with forested soils that are leading to higher concentrations of estrogens in these soils as compared with the cropped soils.

In terms of limiting estrogen presence in the environment, land-based application of effluent is a more effective management practice than direct stream discharge. Depending on the estrogen compound, the soil can act as a tertiary filter. However, as seen in the case of 17β-estradiol, it appears that effluent irrigation can lead to accumulation in the soil profile. There is no evidence to suggest that the compound is transported through the profile to ground water but rather sorbs to the soil. Overall, effluent irrigation has positive potential benefits for society. It provides an additional source of water and nutrients for crops and the soil. It also aids in filtering out unwanted organics remaining in the effluent that the treatment process does not remove. Knowledge from this experiment increases our understanding of the fate of emerging contaminants in the environment and can be used to strengthen effluent management practices in the future.

Table 5. Depth-weighted concentrations were calculated for the top 10 cm of each soil core.

Site treatment	17β-estradiol		17α-ethynylestradiol		Estrone	
	2d	3w	2d	3w	2d	3w
			ng cm^{-3}			
Irrigated (n = 5)						
Cropped	1.43 ± 0.39†	0.99 ± 0.11	0.28 ± 0.07	0.55 ± 0.13	2.90 ± 1.25	3.14 ± 0.35
Forested	1.45 ± 0.07	1.82 ± 0.69	0.25 ± 0.06	1.37 ± 0.39	2.18 ± 0.20	6.24 ± 3.14
	17β-estradiol		17α-ethynylestradiol		Estrone	
Nonirrigated (n = 3)‡	0.68 ± 0.11		0.47 ± 0.40		2.36 ± 0.22	

† Values are average ± SD of the depth-weighted concentrations for each land cover area and time.

‡ These samples were taken at one time event only after the Living Filter sampling.

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