REPORT

Title Biotransformation of pharmaceuticals and personal care products (PPCPs) at an effluent land application site
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Primary PI Deborah L. Carr

Other PIs Todd A. Anderson

Abstract
Biological degradation rates of estrogen compounds and common pharmaceutical and personal care products (PPCPs) were examined in soils with a long history of exposure to these compounds through wastewater effluent and in soil not previously exposed. Biological degradation rates over 14 d were compared under aerobic and anaerobic conditions. Autoclaved soils were used as controls for chemical and physical interactions between the tested compounds and the soil matrix. Estrogen compounds including estrone, β-estradiol, estriol, and 17α-ethinylestradiol exhibited rapid degradation by soil microorganisms in both aerobic and anaerobic conditions. The most rapid degradation rates for estrone, estriol, and 17α-ethinylestradiol occurred in pre-exposed soil under aerobic conditions; half-lives calculated under these conditions were 0.6 d, 0.7 d, and 0.8 d, respectively. Unexposed soil showed similar or slightly longer half-lives than pre-exposed soil under aerobic conditions. β-estradiol was the exception; in all treatments, degradation in unexposed soil resulted in a shorter half-life (2.1 d versus 2.3 d). Anaerobic soils exhibited high biological degradation of estrogens as well. Half-lives of all estrogens ranged from 0.7 d to 6.3 d in anaerobic soils. Triclosan degraded faster under aerobic conditions with half-lives of 5.9 d and 8.9 d in exposed and unexposed soil. Under anaerobic conditions, triclosan half-lives were 15.3 d in unexposed and 28.8 d in exposed soil. Ibuprofen showed the least propensity toward biological degradation compared to the other chemicals tested. Biological degradation of ibuprofen was only observed in the unexposed soil; a half-life of 41.2 d was determined under anaerobic conditions and 121.9 d under aerobic conditions. Interestingly, the unexposed soil exhibited a greater ability under anaerobic conditions to biologically degrade all tested compounds than soil with previous exposure to PPCPs.

Problem and Research Objectives

Several recent media accounts have focused attention on prescription, over the counter drugs, and personal care products showing up in the nation's drinking water sources. While PPCPs have been identified in the environment for several decades (Kummerer, 2001), the fates and persistence of these compounds are not well known. These compounds are considered micropollutants and can be found in wastewater and surface and groundwater near wastewater discharge areas. They can include birth control hormones, antibiotics, antimicrobials, pain relievers, insect repellants, and caffeine to name only a few. In general these are compounds with known pharmacological actions in humans and animals and, although quantities of these contaminants in wastewater may be

low, they are continuously present and constitute a constant exposure. In most cases, no regulatory limits have been set for these compounds and their discharge from WWTPs.

Natural and synthetic estrogens have become one of the emerging contaminants of concern because of their ability to disrupt the endocrine system and their potential to cause long-term impacts to wildlife and human health (Lee and Liu, 2002; Hermanowicz and Wozei, 2002; Norris and Carr, 2006). Investigations into the removal of endocrine disrupting chemicals (EDCs) from water in WWTPs have shown that bacteria in activated sludge systems can reduce concentrations of natural and synthetic estrogens and their breakdown products (Hermanowicz and Wozei, 2002; Andersen *et al.,* 2003). Sorption to sludge particulate matter and biodegradation appear to be the most important removal processes for EDCs in wastewater treatment systems, though microbial populations vary in their ability to degrade estrogens (Layton *et al.,* 2000). While studies have confirmed the ability of EDCs to partition to solids in WWTPs (Gomes *et al.,* 2004), less than 3 percent of the estrogenic activity was found in the sludge (Körner *et al.,* 2000) and only 5 percent of the estrogens were adsorbed onto digested wastewater sludge (Anderson *et al.,* 2003). This implies a significant fraction may be susceptible to microbial degradation.

Several studies have detected antibiotics such as ciprofloxacin (Alder *et al.,* 2000) in WWTP effluents at ng/L to µg/L concentrations. According to a study by the U.S. Geological Survey (Kolpin *et al.,* 2002), out of 31 antibiotics and antibiotic metabolites measured 17 were detected, indicating a strong need for information regarding the fate and transport of antibiotics through waste treatment plants and in the environment.

Many PPCPs other than antibiotics and EDCs have been detected in surfacewater and wastewater effluents. Triclosan, an antimicrobial disinfectant found in a wide array of personal care cleansers and household cleaning products and plastics, has been identified as one of the most commonly occurring chemicals in effluent fed surfacewaters (Kolpin *et al.,* 2002; Focazio *et al.,* 2008). Ibuprofen, a non-steroidal anti-inflammatory drug (NSAID) has been identified as the third most popular drug in the world both by sales and use. It is one of the most common PPCPs occurring in the aquatic environment and has been shown to persist up to 6 months in groundwater aquifers (Drewes *et al.,* 2003).

As water supplies become more limiting (especially in the arid Southwest) and water re-use practices increase, PPCPs in municipal water supplies and the level of effluent treatment become an important human and environmental health issue. Land application as a tertiary treatment for wastewater is an efficient method for many communities because of its low cost and as an alternative to potable water for agriculture and maintaining green spaces in the community. To our knowledge, information about the fate of PPCPs contained in secondary effluent once they are applied to a soil environment is non-existent. Complex soil structure including varying aggregate and soil pore sizes provide multiple niche environments and support a large and varied microbial community (Beare *et al.,* 1995). Difficulty in isolating and culturing the vast majority of these organisms inhibits our understanding of the scope and rates of microbially mediated biochemical processes that may occur within the soil profile (Buckley and Schmidt, 2003). This study attempted to broadly address potential microbial soil environments and their contributions to degrading PPCPs.

Materials/Methodology

Site Description

The Lubbock land application site (LLAS), a component of the Lubbock wastewater treatment plant (LWWTP) was the site of interest for this study. The LLAS receives effluent after secondary treatment from the LWWTP and has been in continual operation since 1915. LLAS soils are classified as Friona loam of the Estacado series. The soil is a slightly alkaline brown clay loam of weak granular structure, with permeability of 1.5 to 5.0 cm/hr (USDA, 2007). Depth to the water table exceeds 200 cm with a moderately high to high hydraulic capacity (9-28 μm/s). Sampling occurred from exposed soils – soils under an irrigation pivot receiving effluent, and from unexposed soil – soil at the same site but never having been irrigated. The two sample sites have very similar soil characteristics which are summarized in Table 1.2. The reduced cation exchange capacity (CEC) in the exposed soil (22.9 meq/100 g) versus 36.7 meq/100 g CEC in the unexposed soils may reflect effluent nitrogen levels. Soil pH in the unexposed soil was 7.9 and is typical for soils of the Friona Loam series (USDA, 2007). The soil pH of 7.3 in the exposed soil may indicate high nitrate levels and a degree of nitrogen saturation. The effluent exposed soils have less than half the soluble salts content as the unexposed soils due to leaching of the salts through frequent irrigation of secondary treated effluent at a rate of 3-4 cm/week (M. Gonzales, LWWTP, personal communication).

Soil Collection

Samples were collected from the top 5-18 cm topsoil from three locations within the irrigation pivot area (exposed) or outside the irrigation range (unexposed). The soils were mixed to form a composite of wastewater effluent exposed or unexposed control soil, and stored in the dark at 4ºC until needed. Prior to experimental set-up, the soils were coarse sieved (4.0 mm) to achieve a degree of homogeneity while maintaining meso- and micro-aggregate soil structure. Soil aliquots to serve as abiotic controls were autoclaved twice for 1 hour, at 24 h intervals, to ensure that the microbial community was killed.

Experimental design

Ten grams of soil was added to 40 mL clear glass vials with Teflon® silica septa in polypropylene lids. Soil samples were spiked with 25μg/mL micropollutant standard. Sterile Milli-Q water was added to achieve 30% field capacity v/w (Table 1.2). The headspace of each vial was sparged with either compressed, breathing quality air (aerobic) or compressed nitrogen ultra-high purity (anaerobic) equal to five volume replacements. Triplicate samples were incubated in the dark at 22 ºC under aerobic or anaerobic conditions. Killed controls under aerobic and anaerobic conditions were also monitored in triplicate for each chemical. All efforts were made to maintain starting oxygen conditions by allowing a slight positive pressure in the vials and incubating them upside down. Upon opening the vials for extraction, vials maintained a slight positive pressure, confirming that gas exchange had not occurred. Samples were extracted after incubation periods of 0, 3, 7 and 14 d with 20 mL high-performance-liquid chromatography (HPLC)-grade acetonitrile (ACN) (Fisher Scientific, Suwanee, GA)

(Golet, 2003). Preliminary studies indicated 98 % recovery of E1, E2 and EE2 compounds, 52 % of E3, 96 % of ibuprofen, and 93 % of triclosan by this method. Extracts were filtered through 0.45 μm nylon filters (Whatman International Inc., Florham, NJ, USA) into 2 mL amber glass HPLC auto-sampler vials, sealed with PTFE/rubber septa and stored frozen until analysis.

Test Chemicals and Analysis

Estrogens

Three natural steroid estrogens, estrone (E1), β-estradiol (E2) and estriol (E3), and synthetic steroid estrogen, 17α-ethinylestradiol (EE2) were used in this study. All estrogens were HPLC grade (> 98%) and obtained from Sigma Aldrich (St. Louis, MO, USA). Spiking solutions were made up in 100 percent ACN at 25 μg/mL. Stock solutions at 100 mg/L were made for each chemical and diluted for analytical standards using HPLC grade ACN. Estrogens from soil extractions were determined by reverse phase HPLC equipped with Chemstation analytical software (HP series 1100, Hewlett-Packard, Avondale, PA, USA). Sample injection volume was 50 μL, eluent flow was 0.8 mL/min through a C-18 column (Alltech Platinum 250 X 4.6 mm, 5 μm, Deerfield, IL, USA), and detection wavelength was 200 nm. An acetonitrile:water mobile phase was used for analysis, but the ratio was adjusted for each compound; E1 was 85:15, E2 and EE2 was 80:20, and E3 was 50:50 ACN: water. Reporting limit was 0.02 μg/g soil

Triclosan

Triclosan as HPLC-grade (Irgasan) (EC 3380-34-5) was obtained from Sigma Aldrich (St. Louis, MO, USA). The solution for initial spiking was made up in 100 percent ACN at 25 μg/mL. A stock solution of 100 mg/L was diluted for analytical standards using HPLC-grade ACN. Standards and extracted samples were analyzed by reverse phase HPLC with a sample injection volume of 25 μL, 1.0 mL/min eluent flow, through a C-18 column (Alltech Platinum 250 X 4.6 mm, 5 μm, Deerfield, IL, USA), with detection at λ = 200 nm. The mobile phase was 80:20 ACN:water. Reporting limit was 0.02 μg/g soil.

Ibuprofen

HPLC-grade ibuprofen (EC 51146-56-6) was obtained from Sigma Aldrich (St. Louis, MO, USA). The solution for initial spiking was made up in 100 percent ACN at 1.5 μg/μL. A standard stock solution at 100 mg/L was diluted for analytical standards using HPLC-grade ACN. Standards and extracted samples were analyzed by reverse phase HPLC with a sample injection volume of 50 μL, 0.8 mL/min eluent flow through a C-18 column (Alltech Platinum 250 X 4.6 mm, 5 μm, Deerfield, IL, USA), and detection at λ = 200 nm. The mobile phase was 90:10 MeOH:0.04M H_3PO_4. Reporting limit was 0.1μg/g soil.

Ciproflaxacin

HPLC-grade ciprofloxacin (EC 85721-33-1) was obtained from Sigma Aldrich (St. Louis, MO, USA). The solution for initial spiking was made up in ACN with 0.1 % acetic acid at 1.5 μg/μL. A standard stock solution at 100 mg/L made up in ACN plus 0.1 % acetic acid was diluted for analytical standards using HPLC-grade ACN. Standards and extracted samples were analyzed by reverse phase HPLC with a sample injection volume of 25 μL, 0.8 mL/min eluent flow through a C-18 column (Alltech Platinum 250 X 4.6 mm, 5 μm, Deerfield, IL, USA), and detection by fluorescence with excitation λ =

278 nm and emission λ = 445 nm. The mobile phase was 50:50 ACN:5 mM mono-phosphate buffer KPO_4 at pH 3. Reporting limit was 0.02 µg/g soil.

Data analysis

Sample concentrations at various time points were normalized as percent of the day 0 concentrations and plotted by treatment on a scatter diagram. Best fit regression curves were fit to the scatter diagrams and analyzed by ANCOVA in R version 2.7.2.

Principal Findings

Loss of target compounds in the following results section has been interpreted to be due to biological processes in the soil because there was no loss of compound over time observed in the samples that contained killed soil resulting from autoclave sterilization.

Estrogens

Estrone

Estrone exhibited biological degradation under all experimental conditions as indicated by no loss of compound over time in the killed (autoclaved) controls (Figure 1). Biological degradation was most rapid under aerobic conditions. Half-lives calculated from best-fit regression equations were 0.6 d and 1.1 d in exposed and unexposed soil, respectively. Half-lives under anaerobic conditions were slightly longer: 6.3 d in exposed and 3.4 d in unexposed soil (Table 1). The calculated half-life under aerobic conditions was half that of unexposed soil. That finding was reversed under anaerobic conditions, where the unexposed soil exhibited a shorter half-life for estrone than in exposed soil. Regression lines of biodegradation rate for each treatment were significantly different from each other ($p < 0.001$) by ANCOVA analysis.

β-Estradiol

Biological degradation was responsible for more than 96% loss of β-Estradiol in 14 d under both aerobic and anaerobic conditions and regardless of prior exposure to wastewater effluent (Figure 2). Calculated half-lives from biodegradation (regression fit) under aerobic conditions were 2.3 d and 2.1 d in exposed and unexposed soil, respectively. Anaerobic conditions resulted in half-life calculations of 1.9 d in exposed soil, and 1.6 d in unexposed soil (Table 1). β-Estradiol exhibited slightly shorter half-lives in unexposed soil than in soil with prior exposure. All treatments regressions were significantly different ($p < 0.001$) by ANCOVA analysis.

Estriol

There was no loss of estriol in killed control treatments except under anaerobic conditions in the exposed soil where an 18% loss was seen over the 14 d incubation. Rapid loss of estriol was seen in all live treatments over the 14 d incubation period (Figure 3). Calculated half-lives by regression fit were 0.7 d for aerobic conditions in both exposed and unexposed soil and under anaerobic conditions in unexposed soil.

Anaerobic conditions in exposed soil resulted in a significantly higher ($p < 0.001$) half-life of 1.7 d (Table 1).

17α-Ethinylestradiol

Biological degradation of *17α*-Ethinylestradiol under aerobic conditions was significantly faster ($p < 0.001$) than biological loss under anaerobic conditions. No loss of 17*α*- Ethinylestradiol was observed in the killed controls (Figure 4). The half-life of *17α*- Ethinylestradiol was calculated as 0.8 d in aerobic soil. Anaerobic soil conditions resulted in half-lives of 3.0 d in exposed soil and 2.0 d in unexposed soil (Table 1). Regression analysis indicated significant differences between the exposed and unexposed anaerobic treatments ($p < 0.001$) though not between the aerobic exposed and unexposed treatments

Triclosan

Biological degradation of triclosan occurred in all live treatments over the 14 d study. There was no significant loss of triclosan in the killed controls (Figure 5). Biotic degradation of triclosan was fastest in exposed soil under aerobic conditions, with a 5.9 d half-life. Unexposed soil under aerobic conditions resulted in a half-life of 8.9 d for triclosan. The half-life of triclosan under anaerobic conditions was 15.3 d in unexposed soil and 28.8 d in effluent exposed soil (Table 1). Regressions analysis by treatment (ANCOVA) showed significant differences between all treatment conditions on degradation rate ($p < 0.001$).

Ibuprofen

Ibuprofen only exhibited biological degradation in unexposed soils (Figure 6). Exposed soils under both aerobic and anaerobic conditions exhibited no significant differences between killed controls, thus no half-lives could be calculated by regression. Over the 14 d study only 21% or less ibuprofen was degraded biologically. In the unexposed soils half-lives were calculated as 121.9 d under aerobic soil conditions and 41.2 d in anaerobic soil (Table 1). The rates in unexposed soils were shown to be significantly different ($p < 0.001$ and $p < 0.05$) between aerobic and anaerobic conditions, and aerobic from exposed and killed controls.

Ciproflaxacin

No degradation data were obtained for ciprofloxacin under aerobic and anaerobic conditions.

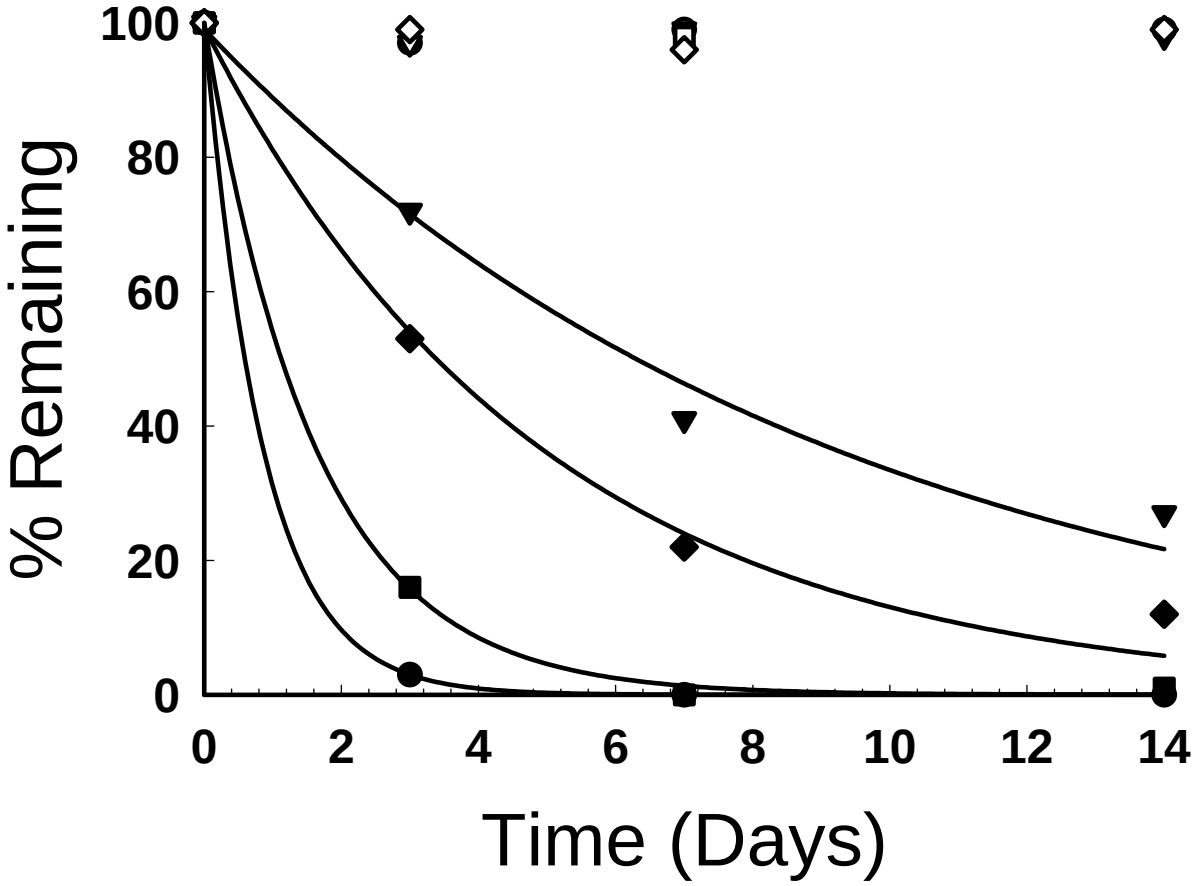

Figure 1. Aerobic and anaerobic degradation of estrone in soil.
Data points represent triplicate measured values. Aerobic effluent exposed (●), aerobic unexposed (■), anaerobic effluent exposed (▼), anaerobic unexposed (♦). Killed controls are represented by open symbols. Vertical bars represent ± standard error of the means (n=3). Bars not visible fall within the dimensions of the symbols. Lines represent best fit regression equations.

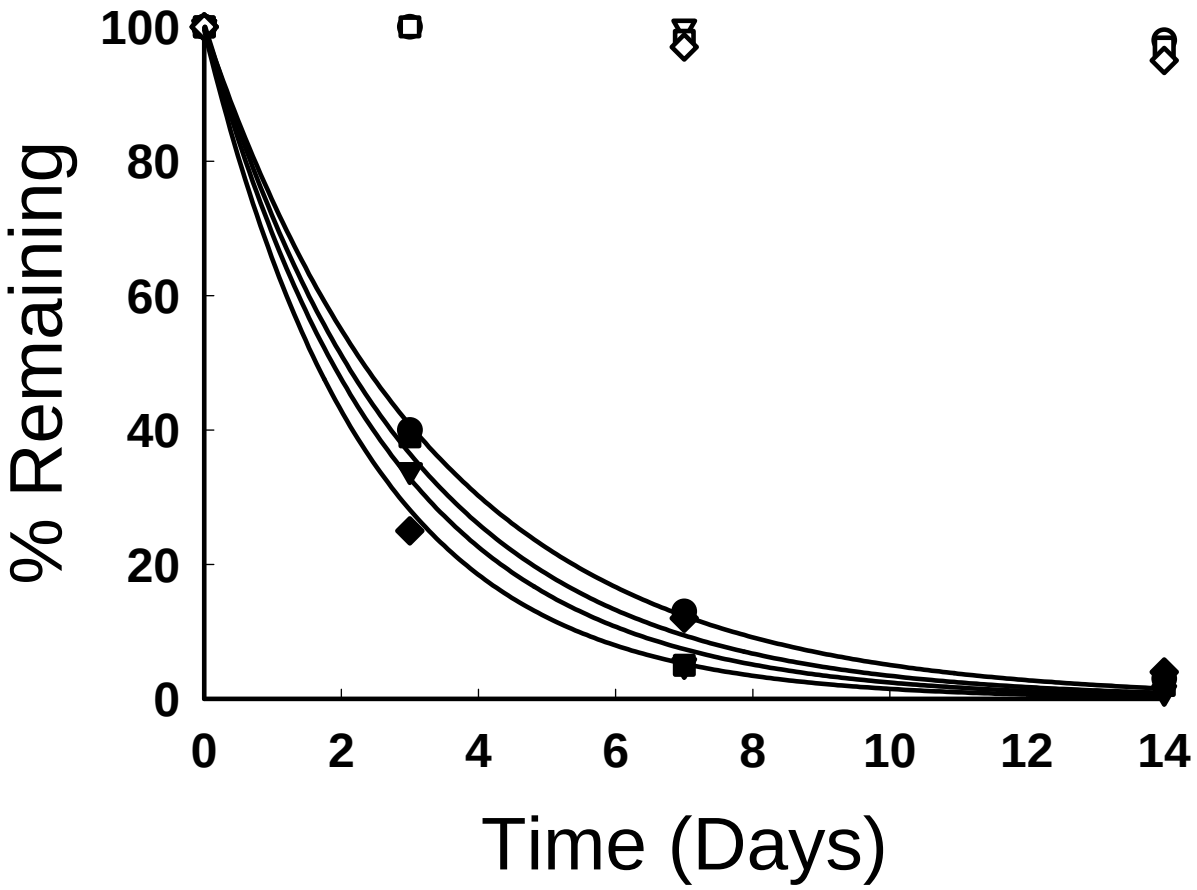

Figure 2. Aerobic and anaerobic degradation of β-estradiol in soil.
Data points represent triplicate measured values. Aerobic effluent exposed (●), aerobic unexposed (■), anaerobic effluent exposed (▼), anaerobic unexposed (♦). Killed controls are represented by open symbols. Vertical bars represent ± standard error of the means (n=3). Bars not visible fall within the dimensions of the symbols. Lines represent best fit regression equations.

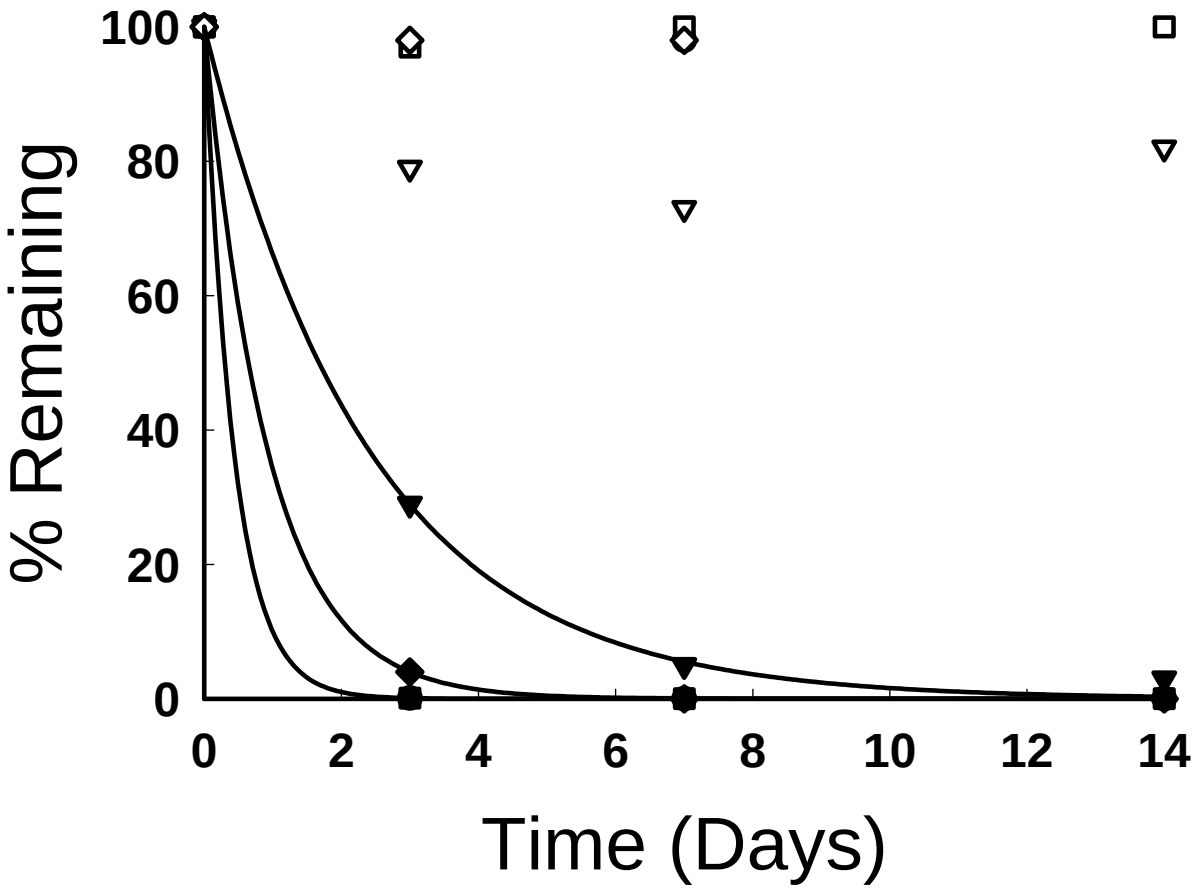

Figure.3. Aerobic and anaerobic degradation of estriol in soil.
Data points represent triplicate measured values. Aerobic effluent exposed (●), aerobic unexposed (■), anaerobic effluent exposed (▼), anaerobic unexposed (♦). Killed controls are represented by open symbols. Vertical bars represent ± standard error of the means (n=3). Bars not visible fall within the dimensions of the symbols. Lines represent best fit regression equations.

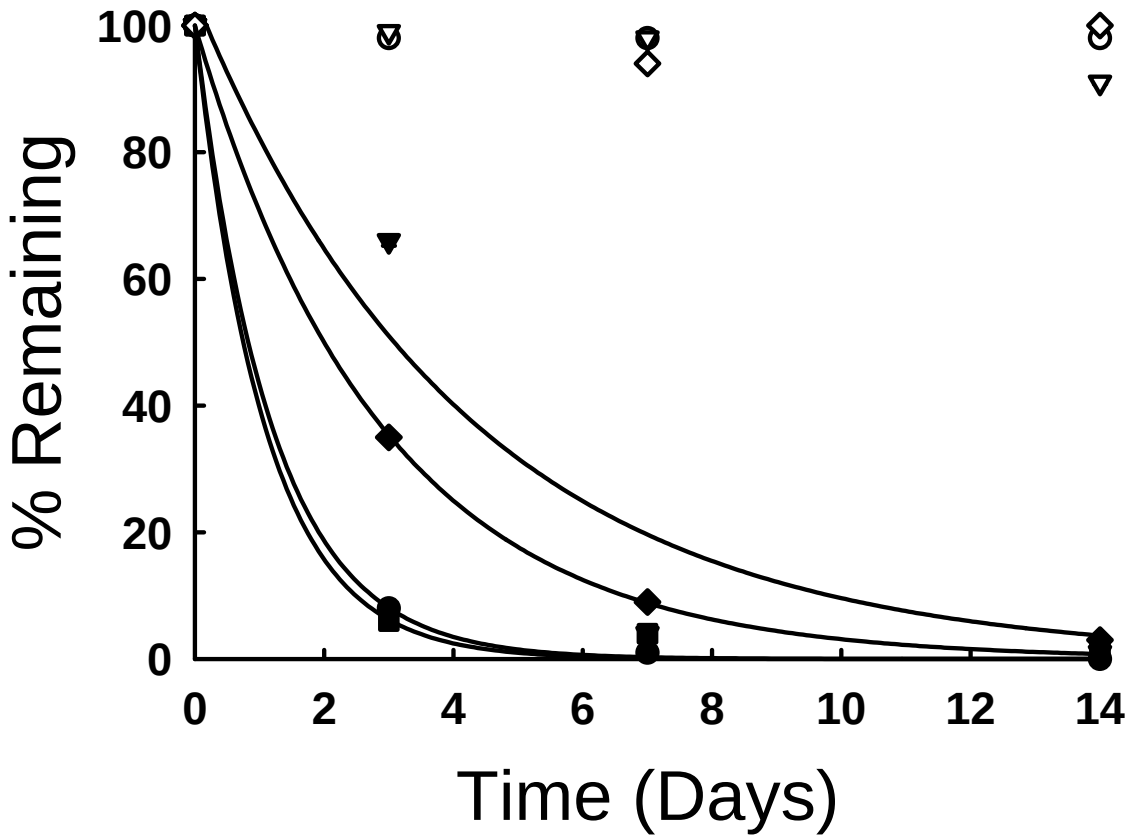

Figure 4. Aerobic and anaerobic degradation of 17α-ethinylestradiol in soil.
Data points represent triplicate measured values. Aerobic effluent exposed (●), aerobic unexposed (■), anaerobic effluent exposed (▼), anaerobic unexposed (♦). Killed controls are represented by open symbols. Vertical bars represent ± standard error of the means (n=3). Bars not visible fall within the dimensions of the symbols. Lines represent best fit regression equations.

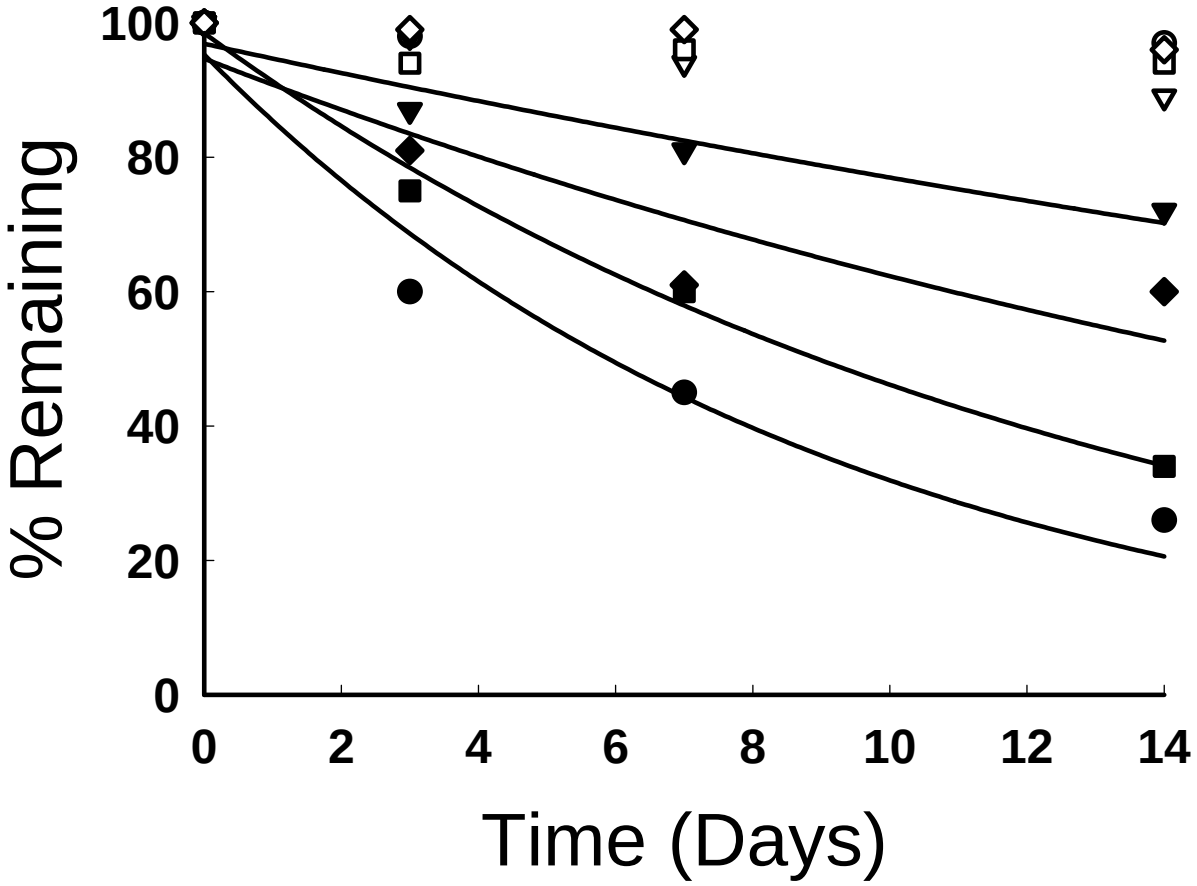

Figure 5. Aerobic and anaerobic degradation of triclosan in soil.
Data points represent triplicate measured values. Aerobic effluent exposed (●), aerobic unexposed (■), anaerobic effluent exposed (▼), anaerobic unexposed (♦). Killed controls are represented by open symbols. Vertical bars represent ± standard error of the means (n=3). Bars not visible fall within the dimensions of the symbols. Lines represent best fit regression equations.

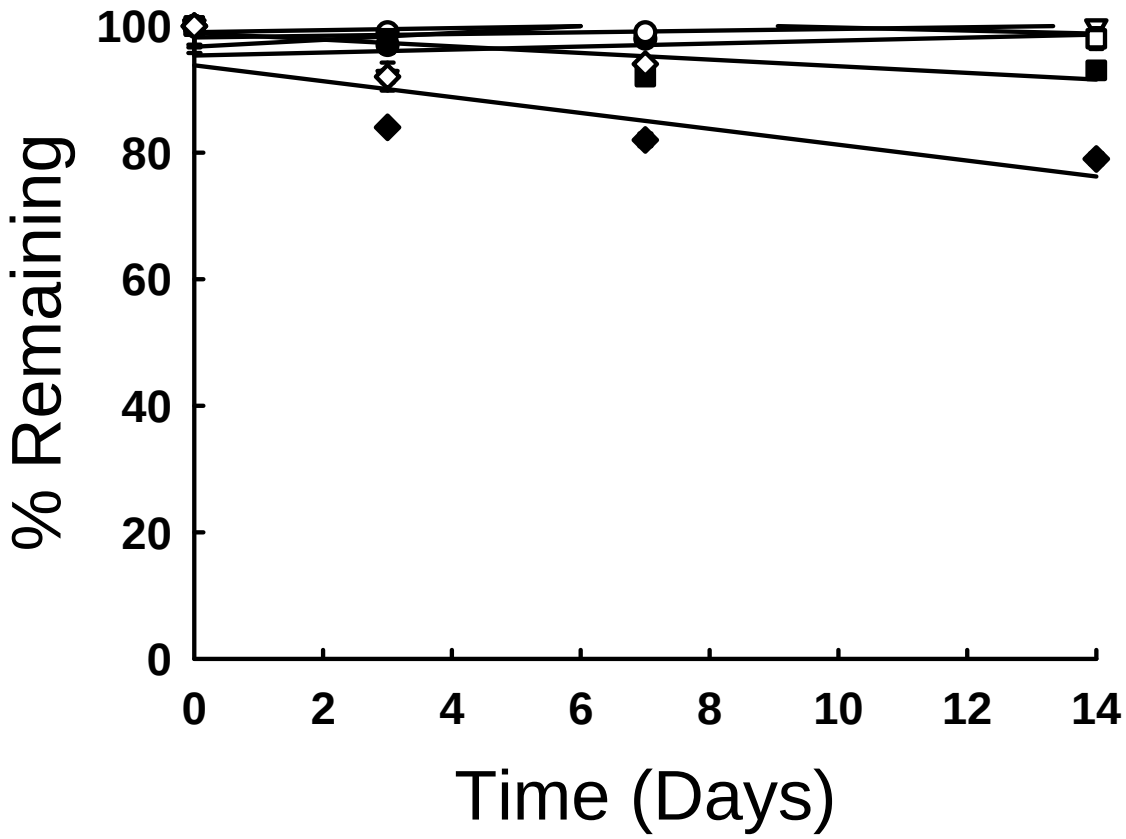

Figure 5. Aerobic and anaerobic degradation of ibuprofen in soil.
Data points represent triplicate measured values. Aerobic effluent exposed (●), aerobic unexposed (■), anaerobic effluent exposed (▼), anaerobic unexposed (♦). Killed controls are represented by open symbols. Vertical bars represent ± standard error of the means (n=3). Bars not visible fall within the dimensions of the symbols. Lines represent best fit regression equations.

Table 1 Calculated half-lives (days) of selected PPCPs in soil under aerobic and anaerobic conditions.

The r^2 for the regression fit is indicated parenthetically.

Treatment	**Calculated Half-life**					
	Estrone	B-Estradiol	Estriol	17α-Ethinylestradiol	Triclosan	Ibuprofen
Aerobic Exposed	0.6 (0.999)	2.3 (0.999)	0.7 (0.999)	0.8 (0.999)	5.9 (0.957)	NC
Aerobic Unexposed	1.1 (0.999)	2.1 (0.996)	0.7 (0.999)	0.8 (0.997)	8.9 (0.992)	121.9 (0.692)
Anaerobic Exposed	6.3 (0.982)	1.9 (0.999)	1.7 (0.999)	3.0 (0.930)	28.8 (0.935)	NC
Anaerobic Unexposed	3.4 (0.991)	1.6 (0.988)	0.7 (0.999)	2.0 (0.999)	15.3 (0.833)	41.2 (0.679)

NC – not calculated

Significance

Estrogens

Under aerobic conditions natural and synthetic estrogen are efficiently degraded by soil microorganisms within 14 d, with half-lives of less than 1 to 2 d. Prior exposure of these soils to these chemicals does not seem to be an important factor in the ability of the endogenous soil microbial community to degrade estrogens. While there were statistical differences ($p<0.05$) between aerobic and anaerobic treatments, the half-lives are short enough to question whether this would be an environmentally significant factor. Apparently a robust anaerobic bacterial community exists in the upper 15 cm of soil as we saw rapid degradation of estrogens under anaerobic conditions as well. In most cases the half-lives under anaerobic conditions were not so different from aerobic half-lives as to be an important factor in the environment. The significant differences we report by comparison of regressions for best fit by ANCOVA relate to the shape of the curves and the high efficiency of the extraction methods which resulted in small standard error of the means. The endpoint measurements of estrogens at 14 days were less than 3 percent of the concentration initially added. Estrone was the exception to this pattern. Under aerobic conditions estrone behaved as the other estrogen compounds, however, under anaerobic conditions degradation was significantly slower and the half-life was 2 to 3 times longer than other estrogens under the same conditions and 3 to 6 times longer than estrone in aerobic conditions. Estrone was the only estrogen tested that had measurable concentrations remaining at the end of 14 days (12-17%) when incubated in an anaerobic environment.

In general, results from this study concur with natural estrogen degradation and removal rates found in other studies. While degradation of estrogens was slower under anaerobic conditions, our observed half-lives from 0.7 d to 6.3 d do not suggest that any of the estrogens tested would be persistent in the soil environment. In a slow rate land application scenario where effluent irrigation occurs rotationally so the soil can maintain unsaturated conditions and return to aerobic conditions, it is unlikely that estrogen degradation would be exceeded by input of new estrogen substrate.

Several studies suggest that 17α-ethinylestradiol is more resistant to degradation under aerobic conditions and resistant under anaerobic conditions in wastewater treatment plants and sediments (Layton *et al.,* 2000; Jürgens *et al.,* 2002;Ying *et al.,* 2003; Braga *et al.,* 2005; Furuichi, *et al.,* 2006). Results presented here suggest that soil environments contain bacterial assemblages capable of rapidly degrading 17α-ethinylestradiol under aerobic and anaerobic conditions. Reports by Coombe *et al.,* (1966) and Shi *et al.,* (2004) suggest that nitrifying bacteria such as *Nocardia sp.* and ammonia oxidizing bacteria like *Nitrosomonas spp.* are capable of rapid degradation of natural estrogens as well as 17α-ethinylestradiol through the oxidation of C4 at the A ring followed by complete mineralization. Both families of these bacterium are well represented in soils; they may be the primary degraders of these compounds including the synthetic estrogen 17α-ethinylestradiol.

Triclosan

This study demonstrated that triclosan was degraded by microbial populations in soils under both aerobic and anaerobic conditions. The half-life of triclosan in aerobic soil was slightly lower in unexposed soil than in exposed soil (5.9 d vs. 8.9 d). These half–lives are shorter than the 18 d reported by Ying *et al.,* (2007) and by Christensen as reported in Reiss *et al.,* (in press). Another study reported as unpublished (Reiss *et al.,* in press) was a Swiss study

by D. Adam in which triclosan had half-lives ranging from 2.5 d to 10.7 d in aerobic loamy soils. The calculated half-lives for triclosan under aerobic conditions in this study appear to fall in the middle of half-life ranges reported previously.

Ying *et al.,* (2007) reported no degradation of triclosan under anaerobic conditions. In contrast, we observed reasonable rates of microbially mediated degradation and half-lives between 15.3 and 28.8 d in anaerobic soils. Over the 14 d study, between 27 percent and 40 percent of the added triclosan was lost due to microbial activity. Other factors such as nutrient availability, microbial population, and soil structure could influence degradation under anaerobic conditions. By allowing a larger aggregate size in the experimental soils, a greater potential for degradation by fungal hyphae networks was encouraged. Fungal systems, like animal systems, use a different fatty acid synthesis pathway; type I FAS rather than the bacterial type II FAS (Wright, and Reynolds, 2007). Bacteria such as *Staphylococcus aureus* that over express *FabI*, the target of triclosan antibacterial action, may exhibit low level resistance to triclosan (Slater-Radosti *et al.,* 2001), other bacteria with multi-drug efflux pumps actively pump triclosan out of the bacterial cell providing resistance such as seen in *Pseudomonas aeruginosa* (Chuanchuan *et al.,* 2003). Still other bacteria such as *Bacillus spp*. have the *FabK* gene rather than the *FabI*. Both *Bacillus spp*. and *Pseudomonas spp.* are commonly isolated from soils. *Pseudomonas aeruginosa* is a denitrifying chemoheterotroph and a facultative anaerobe. In the absence of oxygen it utilizes nitrate as the terminal electron acceptor during anaerobic respiration. Changing between aerobic and anaerobic respiration occurs within hours for facultative anaerobes (Paul, 2007). The ability to quickly adapt to anaerobic conditions would explain the lack of a lag period in anaerobic degradation rates in our observations.

Ibuprofen

This study observed only slight potential for ibuprofen to undergo biological degradation by soil microbial communities. Only in the unexposed soil could we calculate a half-life for ibuprofen. The most efficient biological degradation occurred under anaerobic conditions when a half-life of 41.2 d was calculated. In aerobic soil, the half-life increased to 121.9 d. Previous studies suggest that ibuprofen is inherently biodegradable (Richardson and Bowron, 1985; Ternes, 1998). This would suggest that ibuprofen would degrade under high bacterial activity found in activated sludge sewage treatment plants, but not necessarily under lower bacterial concentrations found in the environment, including soil. *Nocardia sp.*, a commonly occurring soil actinomycete, has been shown in the laboratory to degrade ibuprofen producing alcohol and ester metabolites (Chen and Rosazza, 1994). Ibuprofen has gram-positive antibacterial (Chowdhury *et al.,* 1996) as well selective antifungal properties (Sanyal *et al.,* 1993; Clausen, 1996). Clausen (1996) demonstrated morphological changes, growth inhibition to fungicidal effects in several brown-rot fungal species, and morphological changes but no inhibitory effects in white rot fungi, both of which are widespread in soils associated with decaying wood and organic matter. Taken together these studies might explain the slow but significant biological degradation we observed in this study. Differences in the exposed and unexposed sites might be due to different microbial community composition.

One of the most surprising observations in this study was the faster degradation rate under anaerobic conditions in the unexposed soils rather than the exposed soils. It is a widely held belief that bacteria capable of anaerobic degradation processes such as de-chlorination, require pre-exposure to the chemical or an adaptation period before active degradation can be measured (Brahushi *et al.,* 2004; Middledorp *et al.,* 2005). This is not the pattern observed in this

study for triclosan, any of the estrogen compounds, or ibuprofen. In all cases, under anaerobic conditions, the unexposed soil produced shorter half-lives. At this time there is not an explanation for this pattern except to propose that soil tillage as part of agricultural crop management in the exposed soil may have lead to a less favorable or less diverse anaerobic bacterial community from that seen in the nearby unexposed soil.

Ciproflaxacin

Degradation of ciprofloxacin in soils was not able to be determined. Although a robust analytical method was developed, we were unable to detect ciprofloxacin in any extracts from the soils. Preparation of ciprofloxacin standards required 0.1 % acetic acid in the ACN solvent to aid in dissolution of the crystallized compound. Initial soil extractions were done with a 2:1 solvent:soil ratio that was used for all other extractions. While extraction efficiency in sand was good (98.04 % ± 1.86), extraction efficiency from the soils in the study was only 4.4 % ± 0.82. Extraction with ACN containing 0.1 % acetic acid resulted in an extraction efficiency of 4.4 % ± 0.66. The soils in this study are somewhat basic with high buffering capacity. These physical characteristics make it likely that a much higher acid concentration would be required to re-solubilize the ciprofloxacin to allow it to be extracted from the soil. An extract with a pH low enough to allow reasonable extraction efficiency would require further treatment and method development before it could be analyzed by HPLC. Further tests for extraction and method development for ciprofloxacin were not pursued at this time. The goal of this study was to examine the fate of pharmaceuticals, in this case ciprofloxacin, once they reach the soil environment. In this study it appears that ciprofloxacin becomes immobilized in these soils and is unlikely to move through the soil profile where it can enter groundwater. The ability of soil microorganisms to degrade ciprofloxacin cannot be addressed in these soils nor should the fate of ciprofloxacin in other more acidic soils be assumed.

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