Fate and Transport of 17β-Estradiol beneath Animal Waste Holding Ponds

Lori A. Duncan,* John S. Tyner, John R. Buchanan, Shawn A. Hawkins, and Jaehoon Lee

Abstract

Concentrated animal feeding operations typically store livestock waste in clay-lined ponds. Although these ponds are regulated to include a liner with a small hydraulic conductivity to limit leaching, previous studies have traced surface and groundwater contamination from such regulated animal waste ponds. This research examined the transport of 17β-estradiol (E2) and its primary metabolite, estrone (E1), through soil liners using field- and laboratory-based studies. Additionally, a potential engineering solution to limit hormone transport—applying biochar to new pond liners to act as a retardant—was studied. Soil cores 80 cm in length were collected beneath a mature dairy waste pond and analyzed for moisture content and hormone concentrations. Unsaturated conditions and E2 concentrations of 4 to 250 ng g⁻¹ were detected beneath the waste pond. In the laboratory portion of the study, hand-packed columns of sand or clay were subjected to infiltration by a 2.3-m head of dairy waste. A subset of the hand-packed sand columns was amended with powdered biochar to test its ability to retard E2 and E1. For 3 mo, column leachate was analyzed for hormone concentrations, and at the conclusion of the study E2 and E1 concentrations in the soil were measured. In the 44 d after sealing, the clay, sand, sand with a thin layer of biochar, and sand mixed with a biochar amendment leached a total of 0.54, 1.3, 0.09, and 0.45 μg of E2, respectively. The biochar amendments to the hand-packed columns considerably minimized E2 in the leachate.

Dairy waste at concentrated animal feeding operations (CAFOs) is typically stored in soil-lined holding ponds. These ponds seal rapidly when animal waste is placed in them (Miller et al., 1985; Chang et al., 1974; DeTar, 1979; Cihan et al., 2006; Tyner et al., 2006; Tyner and Lee, 2004). The Agricultural Waste Management Field Handbook (NRCS, 2009) suggests a sealing credit of a half order of magnitude less than that of the pond liner to predict its leakage rate. The research over the last few decades describes a wholly different set of boundary conditions: one in which a rapidly formed seal limits the flux, almost regardless of the underlying soil type, and in doing so causes the underlying soil to desaturate to the point that its unsaturated hydraulic conductivity is equal to the flux (Tyner and Lee, 2004; Tyner et al., 2006; Cihan et al., 2006). Even with infiltration being limited by soil liners and subsequent sealing, studies (Peterson et al., 2000; Arnon et al., 2006) observed concentrations of estrogens in groundwater near CAFOs and speculated that the estrogen had moved via infiltration through the soil profile.

Steroidal hormones, such as natural estrogens, are excreted by female mammals and can contaminate surface and groundwater. Specifically, 17β-estradiol (E2) and its metabolite estrone (E1) are prevalent in dairy waste and have been found to leach into groundwater (Arnon et al., 2008). Both E2 and E1 are considered to be endocrine-disrupting chemicals because of their developmental and carcinogenic effects in reptiles, amphibians, and, in some studies, humans (Herman and Kincaid, 1988; Sumpter and Jobling, 1995; Dickson et al., 1986; Nakamura, 1984). Natural and synthetic estrogens have different capabilities of disrupting natural hormone processes in the body, most commonly referred to as estrogenic potency. More than 95% of the total estrogenic potency from excreted natural hormones by humans and livestock can be attributed to E2 and E1 (Khanal et al., 2006). Many studies, including Routledge et al. (1998), have shown that E2 concentrations in the 1 to 10 ng L⁻¹ range have adverse health effects on aquatic life and that exposure to a combination of E2 and E1 was more detrimental than either hormone by itself.

Fate and transport mechanisms of E2 have been studied extensively for saturated groundwater systems (Fan et al.,...
Materials and Methods

Field Core Sampling

The University of Tennessee manages a Dairy Research Farm in Lewisburg, TN. This farm maintains 175 lactating Jersey cattle that are only administered antibiotics or hormones when it is medically necessary. The research farm includes a waste pond that has functioned continuously since 1977 and is periodically pumped empty, with the contents being sprayed onto local fields as a fertilizer. After the pond was emptied, a small electric jackhammer was used to drive galvanized-steel Shelby tubes (7.6 cm diameter, 91 cm long) approximately 80 cm deep into the clay liner of the pond. After driving the tubes into the liner, a chain was attached to the tops of the tubes, and a Hi-Lift jack was used to extract the cores, which were immediately capped. To minimize soil disturbance, the cores were collected approximately 0.5 m apart from one another. Boreholes were back-filled with bentonite.

The soil cores were immediately dissected in the field (Fig. 1). Core samples were collected from 0 to 80 cm in 10-cm increments during the first field sampling date (Nov. 2010) and, to increase sampling intensity, at depths of 4, 8, 12, 16, 25, 35, 45, 55, 65, and 75 cm during the second field sampling (Sept. 2011). The moisture contents of the samples were measured gravimetrically (D2216, ASTM) (ASTM Standard, 2010) by oven drying at 105°C for 24 h to provide a soil moisture profile of each core. At each depth increment, additional subsamples were collected from the interior of the core for subsequent E2 and E1 analysis. These 5-g subsamples were acidified in the field with 2 mol L⁻¹ H₂SO₄ to a pH of 2 in glass vials and placed in a 4°C refrigerator for no more than 2 wk (Raman et al., 2004).

Hand-Packed Soil Column Experiments

A hand-packed soil column study was conducted to simulate waste pond conditions with various soil liner types. All soil columns had a diameter of 7.6 cm and were 10.2 cm in length, with a 2.3-m head of liquid waste in a 2.5-cm diameter pipe placed atop the soil columns. The treatments included three hand-packed sand columns, three hand-packed clay columns, three hand-packed mixed sand and biochar columns, and three hand-packed sand columns with a layer of biochar approximately 2.5 cm from the bottom (Fig. 2).

The sand and clay were moistened to their optimum moisture contents (11 and 19%, respectively) and hand-packed with 3.5-cm lifts using 25 blows of a standard proctor hammer (D698, ASTM) (ASTM Standard, 1998). Approximately half of each lift was redisturbed before adding the subsequent lift to avoid the formation of compacted layers. Biochar was added to two sets of columns to evaluate the effectiveness of biochar at attenuating the transport of E2 and E1. The biochar (Biochar Solutions, Inc.) feedstock was pine tree chips and was in powdered form...
approximately 2 mg L\(^{-1}\) to further increase the specific surface area and thus the sorptive capacity. Biochar was applied at 1% w/w to six sand columns. Three columns contained a single layer (~0.5 cm) of biochar approximately 2.5 cm from the bottom, and three treatments were mixed uniformly by hand. A PVC cap with a small drain hole and coarse sand was placed on the base of each soil column to allow for leachate collection.

To simulate waste pond conditions, a 2.3-m head of dairy waste (~3% total solids) was maintained atop the soil columns throughout the experiment. Once the leachate flux from the columns approached \(10^{-6}\) cm s\(^{-1}\), we assumed the seal atop the soil had fully developed. Unspiked dairy waste (without additional E2) was used until the seal developed (~30 d), and then spiked waste was used for the remainder of the experiment so that leachate concentrations were large enough to be analyzed. The waste originally contained an average of 9 ± 13 µg L\(^{-1}\) E2 and was spiked to approximately 56 ± 43 µg L\(^{-1}\) E2 with a stock solution.

Because some E2 was expected to degrade within the 2.3-m standpipe before entering the soil columns, the standpipe was drained every 3.5 d, and the concentrations of E2 and E1 were measured. Shortly thereafter, freshly spiked dairy waste was replaced in the standpipes. The average concentrations in the dairy waste after 3.5 d within the standpipe were 5 µg L\(^{-1}\) E2 and 21 µg L\(^{-1}\) E1. Similarly, Raman et al. (2004) found the concentration of E2 in a dairy waste holding pond to be approximately 2 µg L\(^{-1}\).

Column leachate was collected from the base of the columns in amber bottles housed inside a 0°C freezer. Every 4 d for a period of 6 wk, a 36-mL subsample of leachate from each column was extracted and analyzed for E2 and E1 concentrations. From these data, an E2 breakthrough curve was constructed for each column. At the conclusion of the study, soil was sampled from these data, an E2 breakthrough curve was constructed for each column. At the conclusion of the study, soil was sampled from each column at depths of 0, 5, and 10 cm. Soil samples were extracted and analyzed for E2 and E1 concentrations.

17β-Estradiol and Estrone Analysis

17β-estradiol (≥98% purity) and E1 (≥99% purity) were obtained from Sigma-Aldrich. Stock solutions of E2 were prepared in HPLC-grade methanol, and extractions were performed with HPLC-grade ethyl acetate. Acidified samples were neutralized with the addition of 6 mol L\(^{-1}\) NaOH. Deionized water and ethyl acetate were added to inhibit estrogen separation from the sorbed phase. The samples were then shaken at 200 rpm for 1 h. The supernatant was collected from each sample, which was then re-extracted with ethyl acetate. Each supernatant was combined in a glass vial and dried to a residue with a gentle stream of nitrogen (Colucci et al., 2001; Arnon et al., 2008).

The 2010 field samples were analyzed in the aforementioned manner. In 2011, derivatization of all samples was performed to increase volatility and chromatographic separation (Ding and Chiang, 2003). These samples were derivatized by redissolving the dried extraction residue with 0.5 mL BSTFA + 1% TMCS (Campbell Science) and 0.5 mL hexane. The samples were vortex mixed and heated at 70°C for 1 h to accelerate the reaction and allowed to cool to room temperature immediately before GC/MS analysis. The analysis was performed on a Shimadzu GC/MS system (GC–MS-QP2010). A Shimadzu SHR5×LB (30 m × 0.25 mm × 0.25-μm film) capillary column was used for chromatographic separation in splitless mode using helium at 1 mL min\(^{-1}\) flow rate as the carrier gas. The derivatized samples were injected into the GC/MS system using an AOC-20i auto-injector at 250°C and ion source at 260°C. The oven temperature was set to 80°C (2 min), followed by a 20°C min\(^{-1}\) ramp-up to 250°C and a 5°C min\(^{-1}\) ramp-up to 300°C, then held at 300°C for 5 min. The mass spectrometer was tuned with perfluorotributylamine and used in selected-ion monitoring mode with electron ionization mode (70 eV) to limit the noise of the chromatogram. The selected ion ratios were 416 m/z for E2 (retention time, 16.24 min) and 342 m/z for E1 (retention time, 16.37 min). Instrument detection limits were 15 µg L\(^{-1}\) for E2 and 10 µg L\(^{-1}\) for E1. GCMSolutions 2.4 Shimadzu software was used for system control and data processing.

Results and Discussion

Field Core Soil Moisture Profiles

Average soil moisture values and standard deviations for each depth measured of the waste pond soil profiles from seven cores in 2010 and six cores in 2011 are shown in Fig. 3. The soil moisture profiles from both 2010 and 2011 demonstrated that the soil beneath the pond was unsaturated. The moisture content near the soil surface, several centimeters beneath the seal, was between 65 and 85% saturated. Deeper beneath the seal, the moisture content decreased to approximately 55% saturation and appeared to remain stable throughout the remainder of the 80-cm length of the 2010 and 2011 soil cores.

Because the pond has been active for 35 yr and the moisture data from 2010 and 2011 are similar, the soil–moisture system appears to be in steady state. Although previous laboratory-based research has shown that the soil beneath earthen animal waste ponds is unsaturated (Miller et al., 1985; Barrington and Madramootoo, 1989; Tyner and Lee, 2004), limited soil moisture data have previously been reported from beneath an active holding pond. The 2010 and 2011 datasets support the consensus that the soil is unsaturated, and water and solute transport mechanisms should be modeled as such.
Field Core Estrogen Profiles

The observed E2 concentrations in the soil beneath the pond in 2010 ranged from below detection limit to 229 ng g\(^{-1}\) near the soil surface (Fig. 4). In 2011, observed E2 concentrations ranged from below detection limit to 48 ng g\(^{-1}\). The large E2 concentrations near the surface from the 2010 sampling date could be attributed to waste being included in the subsample. On average, an E2 concentration of 4 ng g\(^{-1}\) persisted throughout the measured portion of the soil profile. In both the 2010 and 2011 datasets, the metabolite E1 persisted at 1 to 3 ng g\(^{-1}\). The presence of E1 cannot be solely attributed to degradation of E2 within the soil profile because an average concentration of 3.9 ± 1.6 μg L\(^{-1}\) E1 is present in the dairy waste. Figure 4 shows that E2 is persistent in the soil and that minimal degradation is likely occurring beneath the dairy waste pond. Arnon et al. (2008), the one other study that measured E2 concentrations below an active pond, found a concentration of approximately 0.08 ng g\(^{-1}\) throughout the soil profile. In that study, the soil contained a higher percentage of sand and thus was not expected to sorb E2 as well as the clay beneath the pond in this study.

Hand-Packed Soil Column Moisture Profiles

Soil moisture profiles were created for the columns tested by the type of soil and/or biochar amendment (Fig. 5). Similarly to the field cores, the hand-packed columns were unsaturated and had similar moisture contents, with all columns being approximately 50% saturated at the 10-cm depth.

Hand-Packed Soil Column 17\(^{b}\)-Estradiol Breakthrough Curves

Infiltration rates of all soil columns were approaching 10\(^{-6}\) cm s\(^{-1}\) within 60 d. Figure 6 shows that the cumulative infiltration (cm) is linearly correlated with the square root of time (day\(^{1/2}\)) after the seal has been established, as was found by Cihan et al. (2006). This implies that once the seal is established, the seal largely controls the infiltration rate, not the underlying soil's hydraulic conductivity, as was previously thought (Tyner and Lee, 2004).

Figure 7 accounts for the differences in leachate concentrations from the various soil columns by showing the cumulative E2 leached per unit area of the column. The clay columns leached approximately half as much E2 as the pure sand columns. Research has shown that sorption of E2 to soil is highly correlated with TOC (Sangsupan et al., 2006; Ying and...
Although TOC on this clay was not measured, it is generally higher in clay soils than in sandy soils.

The biochar-amended sands leached even less E2 than the clay columns, presumably due to more E2 being sorbed to the biochar, as reported by Sarmah et al. (2010). During the 44-d period shown in Fig. 7, the sand columns leached $2.8 \times 10^{-2}$ μg E2 cm$^{-2}$, and the clay columns leached $1.3 \times 10^{-2}$ μg E2 cm$^{-2}$. The different placement techniques of biochar caused a difference in the amount of E2 leached, with the biochar mix columns leaching $1 \times 10^{-3}$ μg E2 cm$^{-2}$ and the biochar layer columns leaching $0.41 \times 10^{-2}$ μg E2 cm$^{-2}$ in 44 d.

By applying these data to a 0.5-acre pond, a traditional clay liner would leach 263 mg E2 in 44 d. A sand liner would leach 567 mg E2 in 44 d, whereas a sand liner with a uniformly mixed biochar amendment would leach 202 mg E2 and a sand liner containing a single layer of biochar would leach 81 mg E2, even though both application techniques use the same amount of biochar. These data show that a biochar-layered pond liner would likely leach notably less E2 than a traditional clay pond liner.

**Hand-Packed Soil Column Estrogen Profiles**

As measured, clay retained the most E2 and E1. The biochar-amended sand columns retained more E2 and E1 than the sand columns. A biochar amendment to clay could possibly enhance the ability to sorb E2 and E1. The E2 profile of the clay columns (Fig. 8) was similar to the 2011 field E2 profile, retaining the most E2 near the liner surface, with an average of 7 ng g$^{-1}$ that decreased to less than 1 ng g$^{-1}$ throughout the remainder of the profile. It is possible that the 0-cm clay samples mistakenly contained a mixture of soil and waste. The sand columns retarded little to no E2; this can also be witnessed in the sand and biochar layer columns where the only depth that E2 was retarded was near the biochar layer, with the 0- and 10-cm depths being pure sand. Concentrations of E2 were approximately 0.3 ng g$^{-1}$ at the surface of the biochar-mixed columns, but it is possible that E2 was sorbed to the biochar strongly such that it was not extractable. Average E1 concentrations were approximately 8 ± 19 ng g$^{-1}$ (Fig. 9).

A mass balance was completed to determine the overall loss of E2 in each hand-packed soil column. This loss could be attributed to degradation of E2 in the waste and within the column and any E2 that was nonextractable from the soil media. Average leachate fluxes and E2 concentrations for each soil column were used to calculate the input and output mass of E2. The total input of E2 was based on average waste concentrations directly above the seal, and the total E2 leached was based on the average leachate concentrations for each soil type. The total amount of E2 within the soil columns was estimated by extrapolating the measured data at 0, 5, and 10 cm across the entire column. Table 1 displays the mass balance for each soil column type. It also shows the amount of E2 not accounted for in each column type. The E2 that was not accounted for with the sand columns can be attributed to degradation. $17\beta$-estradiol will sorb strongly to biochar (Sangsupan et al., 2006; Sarmah et al., 2010), conceivably making it nonextractable with the extraction methods used.
Loss constitutes 17\(^\pm\) SD unless noted otherwise.

| Loss,\% | 71 \(\pm\) 22 | 86 \(\pm\) 7 | 98 \(\pm\) 1 | 95 \(\pm\) 1 |

† Values are mean \(\pm\) SD unless noted otherwise.

‡ Loss constitutes 17\(^\pm\) estradiol that has degraded or transformed or that is nonextractable.

and providing a possible explanation as to why there was more unaccounted-for E2 in the biochar-amended soils.

### Conclusion

The soil beneath the waste pond was unsaturated, being approximately 75\% saturated near the top of the liner and then remaining at approximately 55\% saturation with increasing depth. 17\(^\beta\)-estradiol was found in concentrations of 7 to 150 ng g\(^{-1}\) near the soil surface and remained constant at 4 to 5 ng g\(^{-1}\) throughout the rest of the measured depth. The metabolite estrone was present in smaller concentrations, at an average throughout the rest of the measured depth. The metabolite near the soil surface and remained constant at 4 to 5 ng g\(^{-1}\). The study findings suggest that a biochar amendment would be beneficial to a dairy waste pond liner by limiting leaching 16 and 40\% less than the clay columns, respectively. On average, the biochar mix and biochar layer columns also outperformed the clay columns amended sand columns also outperformed the clay columns.

Soil moisture profiles from the hand-packed soil column study were comparable to the moisture profiles measured from the waste pond. Based on the E2 concentrations of hand-packed columns, both types of biochar amendment greatly improved the efficiency of the sand. On average, the biochar mix and biochar layer columns leached 68 and 77\% less E2 than the sand-only columns, respectively. The biochar-amended sand columns also outperformed the clay columns on average, with the biochar mix and biochar layer columns leaching 16 and 40\% less than the clay columns, respectively. The study findings suggest that a biochar amendment would be beneficial to a dairy waste pond liner by limiting leaching of E2 and E1.


