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Effects of liquid swine manure on dissipation of 17β-estradiol in soil

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ABSTRACT

17β-estradiol (E2), a natural estrogenic hormone, degrades within hours and bind strongly to soils and sediments; however, estrogens are frequently detected in the environment at concentrations that impact water quality. Colloidal (COC) and dissolved (DOC) organic carbon may enhance the persistence and mobility of E2. Soil batch experiments were used to identify the persistence and sorption of radiolabeled E2 dissolved in solutions of (i) COC/DOC derived from liquid swine manure and (ii) $CaCl_2$. Estradiol disappeared from the aqueous phase before 7 d in the $CaCl_2$ solution, yet persisted throughout the duration of the 14 d experiment in the liquid manure solution. There was also concomitant formation of estrone (E1; a metabolite of E2) as E2 dissipated in sterile batch experiments, which was attributed to abiotic oxidation. The liquid manure solution appeared to interact with the estrogen and/or oxidation reaction sites, reducing E2 degradation. Furthermore, the liquid manure solution reduced E2/E1 binding to the soil surface resulting in more E2/E1 in the aqueous layer compared to the $CaCl_2$ solution. Ultrafiltration results of liquid manure indicated that ~1/3 of E2 was associated with COC, which may be responsible for the reduced degradation and sorption of E2 in the liquid manure solution.

1. Introduction

Exogenous estrogens in the environment have the potential of disrupting the endocrine systems of sensitive organism at low concentrations [1,2]. Although laboratory studies indicate estrogenic hormones are short-lived [3–5] and immobile [5,6], they have been detected at relatively high frequencies and concentrations in field surface waters [7]. Certain organic carbon (OC) fractions can contribute to facilitating the transport and persistence of some immobile and/or labile contaminants within the soil environment (e.g. [8–12]). The strong association of estrogenic compounds with dissolved (DOC) and colloidal (COC) organic carbon fractions [13,14] suggests that they may have a major influence on the fate and transport of hormones. Indeed, several field studies have found correlations between E2 detections and DOC and COC in soil leachate [15,16] and rivers [17].

A known source of estrogenic hormones in surface waters is poultry and livestock manure [18,19]. Compared to other animal feeding operations, manure from swine (*Sus scrofa domesticus*) gestation and farrowing facilities has some of the greatest reported potential to produce and contribute natural estrogenic compounds to the environment [20–22]. For example, Raman et al. [22] calculated ratios of estrogen to macronutrient content (N, P, and K) in various manures, and found ratios in liquid swine manure (i.e., excrete manure/urine mixture with >95% moisture content) were 4 times greater than those of manure from lactating dairy cows. Hutchins et al. [23] also indicated that >95% of estrogens in liquid swine manure are aqueous free (i.e. deconjugated and dissolved), which is greater than other animal manure sources they measured. This may be a reflection of typical swine housing, where slotted floors allow immediate transfer of feces and urine to pits where deconjugation of the sulfated and glucuronidated estrogens occur by fecal bacteria.

Estrogenic hormones will preferentially associate with DOC/COC fractions in soil and sediments [14,24,25], indicating that DOC/COC may play a significant role in their fate and transport. Holbrook et al. [14] found that 60% of aqueous 17β-estradiol (E2), the primary natural estrogenic hormone, will associate with COC derived from wastewater. Holbrook et al. [14] defined COC as the size fraction that passes through a 1.5 μm filter, but is retained by a 1000 Da ultrafilter. The DOC was defined as the fraction that passes through the 1000 Da filter [14]. By conventional definitions: the particulate OC would be the fractions retained by a 0.45 μm membrane filter, while the <0.45 μm filtrate contains DOC and COC fractions. The OC fraction between 0.45 μm and 1000 Da is commonly accepted as the colloidal and DOC as below 1000 Da [14,26,27].

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Liquid swine manure is a complex mix of different particle sizes, but nearly 50% of the organic matter in liquid manure is present as DOC and COC [28], which is potentially the most mobile fraction. Liquid manure is injected within the top 10 cm of soil as a common agronomic practice to improve soil fertility and health that results from the nutrient and organic components of the manure. Kjaer et al. [29] detected the estrogens, estrone (E1; the primary oxidized metabolite of E2) and E2, in subsurface tile drainage beneath a field that received swine liquid manure by surface injection. 17β-Estradiol and E1 were measured in the tile drainage (average depth of 1 m) up to 11 months after the liquid manure had been applied [29].

The processes that lead to estrogen persistence and mobility in the field environment are not well understood. This lack of understanding is especially true in the context of manure management, where there is the potential for high estrogenic concentrations and strong association of estrogens with mobile OC fractions. The purpose of this study was (i) to measure the effect of COC/DOC derived from swine liquid manure, on the sorption and persistence of E2 in soil, and (ii) to identify the relative distribution of E2 + E1 within the OC fractions.

2. Experimental

The soils used in this study came from the surface (0–15 cm) and subsurface (15–30 cm) of a Garborg loamy fine sand (*Sandy mixed frigid Typic Endoaquoll*), which were obtained from southeastern North Dakota, USA. These soils were obtained from the same locations of previous field [16,30] and laboratory [5,31,32] experiments. The soils were air-dried and ground to pass through a 2 mm sieve and were stored frozen until the batch experiments commenced. The main difference between the two horizons was the organic matter content and electrical conductivity (Table 1).

The solutions used for both the batch and ultrafiltration studies were 0.45 μm-filtered liquid manure and 0.01 M $CaCl_2$. The liquid manure was obtained from a farmstead within 3 km of where the soil was collected. The liquid manure was obtained from a storage pond that received slurry manure drained from subfloor pits beneath a series of enclosed pens [16,30]. The liquid manure was filtered through a 0.45 μm Whatman filter to remove particulates before its use in the experiments. The liquid manure was characterized for chemical and physical parameters by Servi-Tech Laboratories (Hastings, NE) using their Lagoon Analysis package (Table 2).

2.1. Batch experiments

2.1.1. Aqueous phase

For both the batch and ultrafiltration studies, various concentrations of radiolabeled (^{14}C) E2 (American Radiolabeled Chemicals, St. Louis, MO) were first dissolved in an ethanol stock solution, which was then added to the batch vials in 10 μL injections to obtain initial solution concentrations. Masses of 1.6 g of soil were added to 10 mL clear glass vials, then 8 mL of solution (i.e., liquid manure or $CaCl_2$) were added to the vials. Vials were then sealed with Teflon septa and metal crimp caps. Experimental controls contained aqueous phase without soil. After solutions were added all batch vials were sterilized by exposing them to a $^{137}CsCl$ Gamma Irradiator (Model #M38-4 Gammator, 1970) for 16 h at 8.5 kGy. Post-irradiation, ^{14}C stock solutions were injected through the septums to deliver 0.7, 0.3, 0.07, and 0.01 μCi in 10 μL of ethanol. The final ^{14}C concentrations were 0.088, 0.038, 0.009, and 0.001 μCi mL^{-1}, which corresponded to 433, 186, 43, and 6 μg L^{-1}. The batch studies were conducted in triplicate. Although the concentrations of ^{14}C-labeled E2 were above typical levels found in manures that have been applied to agricultural fields [22,23], these concentrations were necessary to ensure detection of radioactivity in samples.

Table 1
Physical and chemical properties of the surface and subsurface Garborg soil used in this study.

Depth cm	Sand g kg^{-1}	Silt g kg^{-1}	Clay g kg^{-1}	OM[a] g kg^{-1}	pH	CEC[b] cmol(+) kg^{-1}	EC[c] dS m^{-1}
0–15	73.8	14.6	11.6	21.4	7.6	10.9	0.2
15–30	74.0	15.1	10.6	16.0	7.5	11.3	1.07

[a] OM: organic matter.
[b] CEC: cation exchange capacity.
[c] EC: electrical conductivity.

Table 2
Physical and chemical parameters of the <0.45 μm fraction of the liquid manure used for the batch and ultrafiltration studies.

Parameter	Concentration
Total N (mg L^{-1})	162
Organic N(mg L^{-1})	12
Ammonium N (mg L^{-1})	150
Nitrate–N (mg L^{-1})	<1
Magnesium (mg L^{-1})	3
Sodium (mg L^{-1})	30
Zinc (mg L^{-1})	<1
Iron (mg L^{-1})	4
Manganese (mg L^{-1})	<1
Copper (mg L^{-1})	<1
Electrical conductivity (dS m^{-1})	12.6
Phosphorus (mg L^{-1})	6
Potassium (mg L^{-1})	67
Sulfur (mg L^{-1})	11
Calcium (mg L^{-1})	17
Boron (mg L^{-1})	2
Moisture (%)	99.4
Total solids (mg L^{-1})	600
Organic matter (mg L^{-1})	300
Ash (mg L^{-1})	300
Carbon to nitrogen ratio	1:1
pH	7.4[a]

[a] $CaCl_2$ solution pH 7.4.

All batch vials were placed onto a rotating drum (360°/5 s) and aqueous aliquots were sampled at 0.17, 0.33, 1, 2, 3, 7, and 14 d. At each sampling time, batch vials were removed, centrifuged (380 × g for 20 min), sampled using a syringe, and placed back onto the rotating drum. Aqueous samples were taken using a clean syringe that was inserted twice into a vial to obtain (i) a 100 μL sample for analysis of total radioactivity assay by liquid scintillation counting (LSC), and (ii) a 150 μL for thin layer chromatography (TLC) to detect E2 metabolites. The 100 μL aliquots were added to 5 mL of Ecolite scintillation fluid (88247505, MP BioMedicals, Solon, OH) and then analyzed by LSC. The 150 μL aliquot samples were concentrated on a centrifugal rotary evaporator (SpeedVac, Savant Instruments, Farmingdale, NY) to approximately 10 μL and then spotted onto a reverse-phase TLC plate (RP-18F 266013, Analtech, Newark DE) along with standards (E1 and E2). The solvent ratio was 48:2 chloroform to methanol. Radioactivity on plates was analyzed using a TLC System 2000 Imaging Scanner (Bioscan, Inc., Washington, D.C.). Verification of the TLC data was supported using liquid chromatography tandem mass spectrometry analysis with preparation and analysis of samples as described by Thompson et al. [16].

2.1.2. Soil extraction

In addition to the batch vials, seven additional vials were created of the lowest concentration (0.001 μCi mL^{-1}, chosen for cost considerations) of ^{14}C-labeled E2. One vial at each time point (seven total vials) was removed from the experiment, injected with 216 μL of formaldehyde (2.7%) to inhibit or stop biological transformations of E2, and frozen (−4.4 C°) until further analysis. Later, the seven vials were thawed, centrifuged at 380 × g for 10 min, and the supernatant decanted, placed in a separate vial, and refrozen (−4.4 C°). The remaining soil in the vials was washed with 3 mL of nanopure water, sonicated for 30 min, centrifuged at 380 × g for 10 min, and supernatant decanted into a 10 mL polypropylene plastic bottles. This soil washing procedure was repeated twice using nanopure water, and performed a third time using acetone. Independent extractions were collected in separate bottles and stored frozen. After the soil had been washed, it was then air-dried for 5 d at 25 °C. Aliquots (100 μL) of each of the soil extracts were assayed for ^{14}C using LSC. The air-dried soil was mixed with a sterile spatula and six aliquots of 0.1 g soil were combusted in a tissue oxidizer (Packard Model 307 Oxidizer; Downers Grove, IL) to allow the calculation of non-extractable ^{14}C. During the high temperature combustion analysis, the non-extractable ^{14}C was converted $^{14}CO_2$ and trapped in a sodium hydroxide solution. The ^{14}C was then quantified in the sodium hydroxide solution using LSC analysis.

2.2. *Ultrafiltration*

Volumes of 10 mL of (i) 0.01 M $CaCl_2$, (ii) nanopure water, and (iii) 0.45 μm-filtered liquid manure were spiked with 7 μL of ^{14}C-labeled E2 stock solution (0.001 μCi μL^{-1}) in triplicate, and stirred for 15 min prior to ultrafiltration. Each of the three solutions spiked with ^{14}C-E2 were ultrafiltered using a 1000 Da filter (PLAC02510, Millipore Regenerated Cellulose Ultrafiltration Membrane, Billerica, MA) and a 10 mL Amicon stirred cell (Model 8010; Billerica, MA). The stirred cell ultrafilter was placed under 22 kPa pressure using N_2 gas for up to 4 h (times varied depending on solution being filtered). When ultrafiltration was complete, three 100 μL filtrate aliquots were analyzed for ^{14}C by LSC. The stirred cell was then dismantled and the 1000 Da filter was removed, and placed onto aluminum foil to air-dry at 23 °C for 24 h. The filters were then cut in half (so as not to exceed the mass limits for combustion) and each half was submitted for combustion analysis (described in Section 2.1.2) to quantify the amount of ^{14}C retained on the 1000 Da filter. The ^{14}C measured on the two filter halves was summed. The above ultrafiltration procedure was replicated three times for all three solutions. The 6.35 mm diameter HDPE polypropylene tubing used for output from the Amicon stirred cell was replaced between each run to avoid cross contamination.

3. Results and discussion

3.1. *Persistence*

Degradation of E2 is predominantly a biological process [3,5]; nonetheless, in the present study under sterile conditions E2 was transformed to E1 (Fig. 1). The first-order rates of removal of E2 from the aqueous phase were calculated to be 15.60 d^{-1} (standard error, SE = 2.09) and 18.96 d^{-1} (SE = 1.34) for the surface and subsurface soils with $CaCl_2$ solution, respectively. For the liquid manure solution, the respective rates were 11.69 d^{-1} (SE = 1.96) and 12.85 d^{-1} (SE = 2.72) for the surface and subsurface soils. Photolysis is an abiotic process that could produce E2 from E1 [33,34]. However, previous studies have shown no significant photolysis of E2 in identical batch experiments, perhaps resulting from low transmission of light through the glass vial and batch soil–water solution [32].

Abiotic transformation of E2 to E1 can also be caused by oxidation by soil manganese oxides. Plant available manganese is prevalent in soils in ND [35], and Mn oxides are described in the official soil survey description of the Garborg soil series used in this study [36]. Oxidation occurs when the hydroxyl group at position 17 of the E2 molecule is oxidized to a carbonyl [37,38]. Estradiol oxidation by manganese oxides can be rapid in sterile soil, i.e., 57% and 95% of E2 dissipated by 1 d and 4 d, respectively [37]. Similarly, in the current study, E2 was removed rapidly from the aqueous phase for the $CaCl_2$ solution in the presence of either surface or subsurface soils, and was difficult to detect at 7 d. For the liquid manure solution, however, the E2 persisted in the aqueous layer throughout the duration of the experiment (14 d) for surface and subsurface soils. Loss of compounds from the aqueous phase will be retarded if they are bound to suspended or colloidal particles, which may explain the persistence of E2 in the liquid manure solution with abundant COC/DOC. For example, pesticides bound to suspended (defined as ≥0.7 μm) and colloidal (defined as <0.7 μm) particles in river water will persist compared to aqueous free pesticides [39].

The difference in loss of E2 from the aqueous phase in the presence of liquid manure versus $CaCl_2$ solutions could also result from interactions between DOC/COC and soil manganese oxide reaction sites. Solutes, such as DOC, can potentially reduce the oxidation capacity of soil manganese oxide by adsorbing to Mn oxide reaction sites or competing with reactants (e.g. E2) for reaction locations [40]. Furthermore, the DOC/COC suspended in the aqueous phase could bind to the E2 molecule prohibiting contact with Mn oxide surface reaction sites [38]. Compared to the $CaCl_2$ solution, the more abundant DOC/COC of the liquid manure solution, and its corresponding interactions with Mn oxide might explain the difference in E2 loss between the two solutions (Fig. 1).

Besides degradation, aqueous dissipation or loss can also result from sorption to the solid soil surface. Higher DOC/COC concentrations will reduce soil sorption affinity of E2 [14,17]. The greater dissipation of E2 in the $CaCl_2$ solution, compared to the liquid manure solution, could have resulted from this greater surface sorption. Whereas, the liquid manure solution has greater abundance of DOC/COC that could bind the E2, allowing the E2 to remain in the aqueous layer bound to suspended COC/DOC particles unable to partition to sorption sites on the soil surface.

3.2. *Radioactive recoveries*

3.2.1. *Aqueous phase recoveries*

When interpreting the aqueous radioactivity in this study, the approach of Holthaus et al. [41] was used, where radioactivity was assumed to represent a composite concentration of E2 and E1 (E1/E2). This assumption is based on similarities of E2 and E1 molecular structures, physiochemical properties (e.g., molecular mass, solubility), and sorption parameters. Furthermore, the ^{14}C label at the C-4 position of the A-ring and is maintained in the steroidal structure during transformation of E2 to E1 [41]. For the current study, this assumption proved appropriate because TLC analysis indicated the presence of only E1 and E2.

When the aqueous radioactivity was expressed as a fraction of original radioactivity, or relative radioactivity ($n = 12$; i.e., four initial concentrations and three replicates), then the relative aqueous radioactivity for the individual experimental controls (e.g. surface/subsurface, $CaCl_2$/liquid manure) followed the same trends through time regardless of initial radioactivity applied (Fig. 2). For the $CaCl_2$ solution, the aqueous radioactivity was significantly different (probability ≤0.05) for surface than subsurface soils at two measurement times (0.17 and 1 d), which can be attributed to the hydrophobic sorption of E2/E1 to soils [42]. Specifically, there would be greater sorption of E2/E1 in soil with greater OC content, such as surface compared to subsurface soil (Table 1). For the liquid manure solution, however, there was no difference in aqueous radioactivity for the surface and subsurface soils. Furthermore, the liquid manure solution aqueous concentrations of E2/E1 were

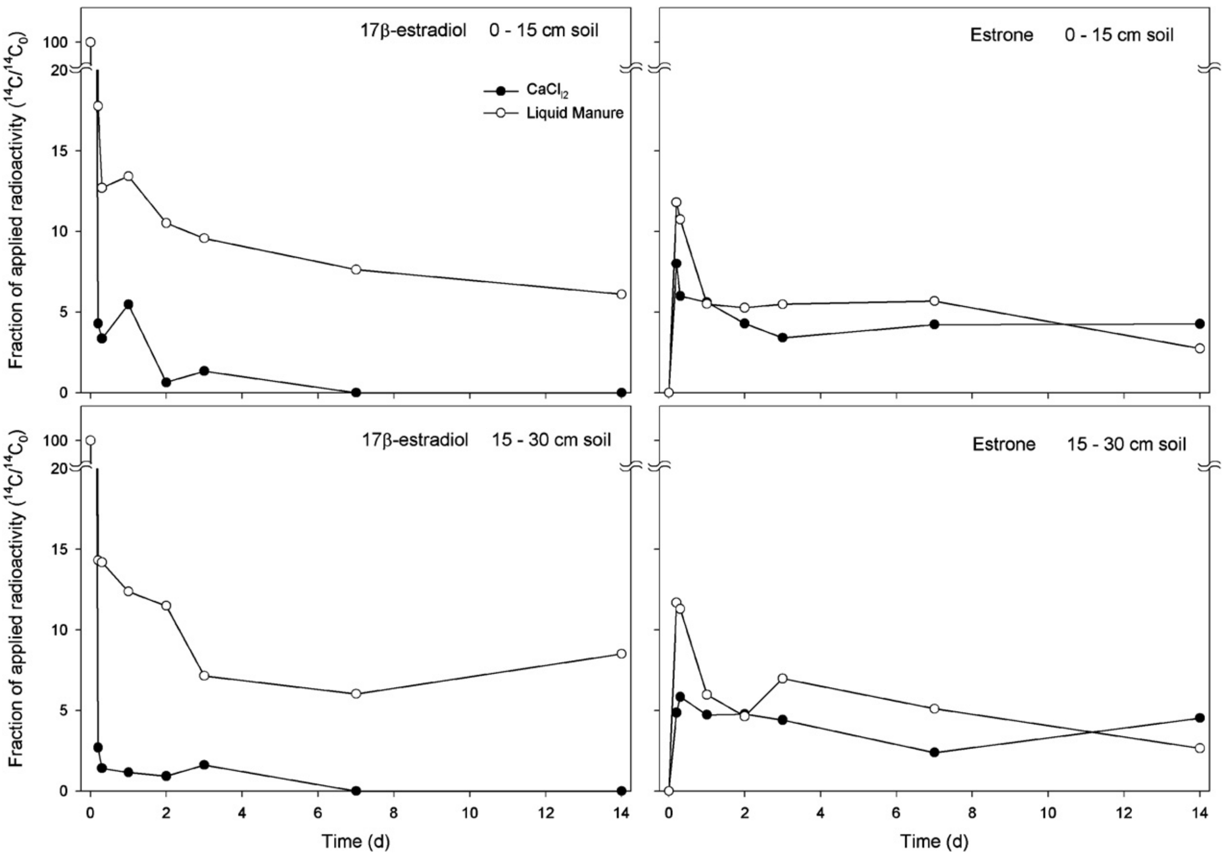

Fig. 1. Fractional recovery of radioactivity in aqueous phase as 17β-estradiol or estrone in calcium chloride ($CaCl_2$) and liquid manure solutions, in the presence of surface and subsurface soils (identified by TLC). The relative concentrations of the four initial concentrations were calculated and each data point represents the average of these four relative concentrations at the various measurement times.

significantly greater (probability ≤0.05) than the $CaCl_2$ solutions (Fig. 2) at the end of 14 d. The hydrophobic sorption to the solid soil was apparently inhibited by the more abundant DOC/COC of the liquid manure solutions. These results show E2 can associate with suspended DOC/COC fractions, in agreement with previous reports [14], reducing E2/E1 available for binding to soil surfaces, and thereby increasing its potential for transport in soil/water systems.

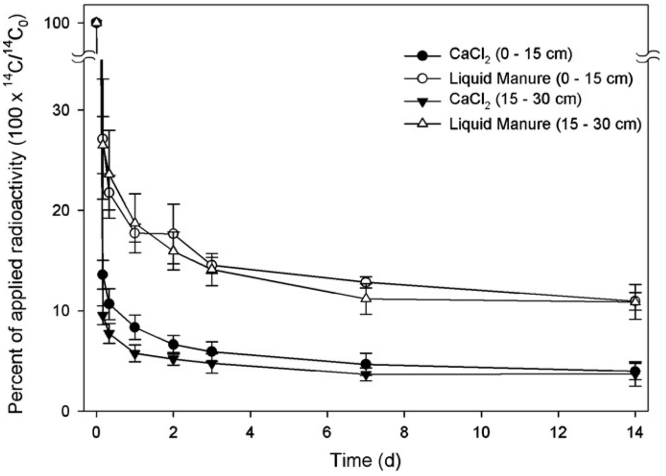

Fig. 2. The average percent of the applied radioactivity recovered from the aqueous phase through time for the calcium chloride solution ($CaCl_2$) and liquid manure solution for the surface and subsurface soils. The error bars represent standard deviations of the % applied radioactivity for the four initial concentrations and three triplicate reaction vials ($n = 12$).

3.2.2. Sorbed phase recoveries

Radioactivity in the sorbed phase was recovered in three pools, which were (i) exchangeable or water extracted, (ii) potentially exchangeable or acetone extracted, and (iii) irreversibly bound (Fig. 3). The partitioning of E2/E1 into potentially extractable and non-extractable or irreversibly bound sorption sites is consistent with previous studies [32]. Irreversible sorption of organic compounds in the soil OC has been accounted for by diffusion, physical entanglement, and/or covalent binding [43,44].

The acetone extract and combusted fractions of radioactivity were greater for all $CaCl_2$ solutions compared to liquid manure solutions (Fig. 3). The association of these hormones with the suspended COC/DOC resulted in less sequestering of the presumed E2/E1 to irreversible sorption sites (^{14}C quantifiable, but structural identification not possible), which could increase E2 availability and mobility in soil. This result is consistent with field lysimeter studies that have suggested DOC derived from soil and/or manure can facilitate hormone transport [45]. The facilitated transport of E2 was observed through soil monoliths and was attributed to preferential flow paths and/or by association with swine manure organic matter [15]. Significant correlations between DOC and E2 detections in field lysimeter and well samples near a swine farm suggested DOC enhances estrogen mobility and persistence [16].

The average mass recovery for all experiments was approximately 75%, with individual mass recoveries ranging from 60% to 120% for the various time points and experimental conditions (e.g., surface/subsurface soil, $CaCl_2$/liquid manure solution, initial concentrations). Low mass recoveries may be caused by the hydrophobic nature of E2 and/or E1 where E2/E1 could have precipitated onto experimental equipment, such as the glassware, thus reducing mass recoveries. Also, incomplete combustion of the soil

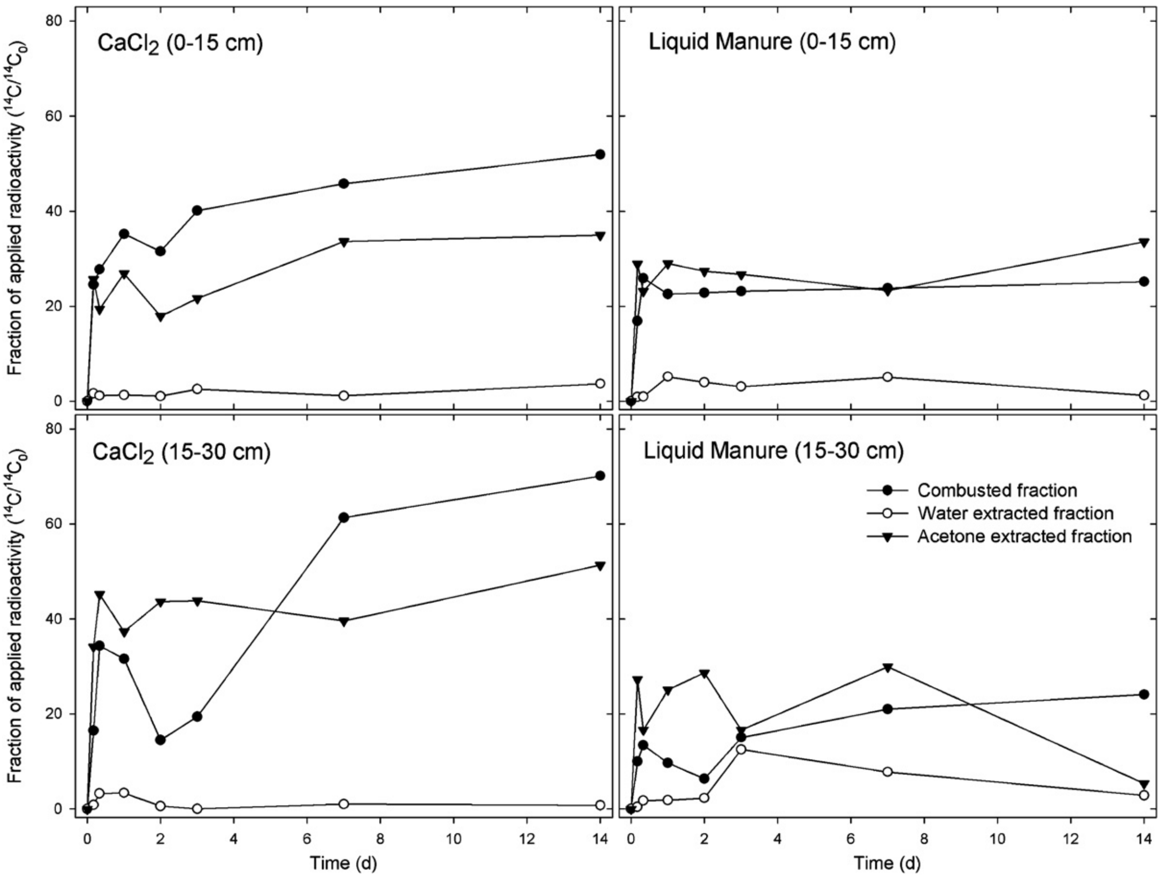

Fig. 3. The radioactivity recovered from the sorbed phase by combustion, water extraction, and acetone extraction through time.

during the soil combustion analysis could result in low mass recoveries [5]. There were only two measurement points where the mass recoveries were greater than 100%. This overestimation of mass recovery may have resulted from adding background disintegrations per minute (dpms) from the LSC analysis towards the actual ^{14}C, which occurs at the lower detection limits of the LSC.

3.3. Aqueous and sorbed phase distributions

While E2 and E1 were the only radiolabeled compounds detected in the aqueous phase, a large fraction of the radioactive dose was irreversibly sorbed to soils. No chemical composition of the irreversibly sorbed or water and acetone extractions of radioactivity could be performed. In the case of the extractions, this was due to insufficient radioactivity in the samples for TLC analysis. Therefore, the development of independent isotherms for E2 and E1 was impossible due to this lack of quantifiable and qualifiable E2 and E1 sorbed to the soil surface. However, using the approach of Holthaus et al. [41], explained in Section 3.2.1, E2 and E1 were assumed to have identical sorption properties. Furthermore, the amount of E2 and E1 bound to the soil surface was determined by mass balance difference between the applied radioactivity and radioactivity recovered in the aqueous phase. Linear sorption isotherms were developed and sorption partitioning coefficients (K_d) were determined for composite E2 and E1 (Fig. 4). The $\log K_{oc}$ (=log 10(K_d/OC)) values derived from the K_d values for the $CaCl_2$ (average = 2.89; min. = 2.31; max. = 3.39) and liquid manure (averages = 2.52; min. = 2.05; max. = 2.99) solutions agreed well with previously reported $\log K_{oc}$ values for soil [42,46]. The fit of the linear isotherms to the data were acceptable as indicated by high coefficients of determination (r^2), where values ranged from 0.98 to 1.00. The K_d values for the liquid manure solution were the same for both the surface soil and subsurface soil indicating that the DOC/COC derived from the liquid manure minimized the sorption potential to soil regardless of soil OC content. The DOC/COC within the liquid manure hindered the E2/E1 sorption potential to the soil surface, which decreased the K_d.

3.4. Ultrafiltration

Ultrafiltration results were used to determine whether the radiolabeled E2 was preferentially bound to the DOC or COC fraction of

Table 3
Ultrafiltration results showing the average fraction of radioactivity recovered in the filtrate (dissolved organic carbon (DOC)), on the filter (colloidal organic carbon (COC)), and total recovered.

Solution	Average radioactivity recovered in filtrate (<1000 Da)	Average radioactivity recovered on filter (≥1000 Da)	Average total radioactivity recovered
Liquid manure	0.56 (0.11)[a]	0.33 (0.08)	0.89 [0.71–1.08][b]
$CaCl_2$	0.74 (0.03)	0.06 (0.06)	0.80 [0.73–0.89]
Nanopure water	0.99 (0.03)	0.03 (0.01)	1.02 [0.98–1.06]

[a] Values in parenthesis are standard deviations.
[b] Values in brackets are ranges.

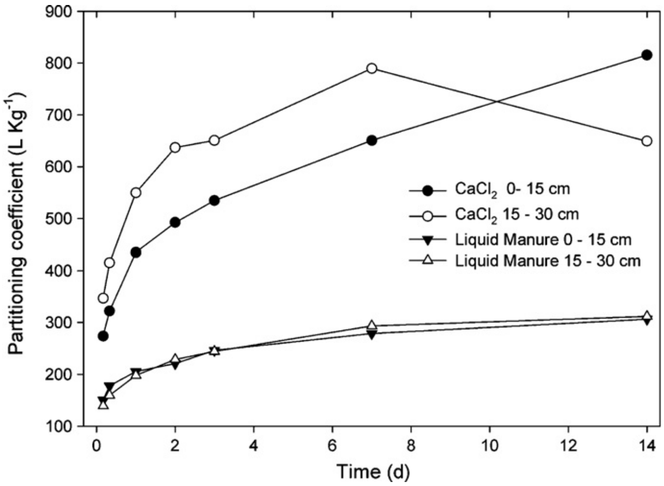

Fig. 4. The linear sorption-partitioning coefficient calculated through time for the two solutions and for the surface and subsurface soils.

aqueous solutions. A third of the radioactivity was measured in the COC fraction for the liquid manure solution, compared to little or no radioactivity recovered in this fraction for the $CaCl_2$ and nanopure solutions (Table 3). The results indicated the COC associated with the ^{14}C-E2 in the liquid manure solution and could be responsible for greater persistence and/or lower sorption of ^{14}C-E2 compared to the $CaCl_2$ solution. Low mass recoveries reported in Table 3. may have resulted from the low water solubility of E2 and its precipitation onto experimental equipment (e.g. glassware, tweezers). For example, if the E2 precipitated onto the glassware and was not recovered using a solvent rinse, then the precipitated E2 would reduce the calculated mass recovery. Additionally, there may have been a “salting out” effect of the $CaCl_2$ in the water solution [47], where the $CaCl_2$ would attract water molecules thereby decreasing the number of water molecules available to interact with the hydrophobic organic molecule, E2. The salting out effect would then facilitate the precipitation of E2 from solution onto glassware, and thereby reducing its mass recovery.

4. Conclusion

The purpose of this study was to observe the abiotic effects of liquid manure on the fate and transport properties of estrogen. It was demonstrated that in the presence of liquid manure, estrogens (E2 and E1) will persist longer in the aqueous phase as a result of reduced potentials to degrade and/or sorb to soil. These results are important, because in the context of manure management, liquid manure-derived DOC/COC could potentially extend the aqueous persistence and thus the mobility of estrogens in the environment. Nonetheless, further study is needed to identify the effects of liquid manure-derived DOC/COC on E2 degradation and sorption under natural, not sterile, conditions. Fecal bacteria are effective in degrading E2 [48], but it is unknown whether this biodegrading potential is reduced when estrogens are bound to DOC/COC. Until similar experiments are conducted under natural conditions, full environmental implications cannot be determined. Additionally, although the association of estrogen with DOC/COC can potentially facilitate its transport and persistence, it is also important to identify whether the DOC/COC reduces the biological/toxicological effects of estrogen, or whether estrogens can be released from DOC/COC. Presumably, the association of estrogen with DOC/COC will affect the ability of the estrogen to bind to estrogen receptors in the endocrine system, and decrease its potential for environmental harm.

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References

[1] L.K. Irwin, S. Gray, E. Oberdorster, Vitellogenin induction in painted turtle, *Chrysemys picta*, as a biomarker of exposure to environmental levels of estradiol, Aquat. Toxicol. 55 (2001) 49–60.
[2] B. Jalabert, Particularities of reproduction and oogenesis in teleost fish compared to mammals, Reprod. Nutr. Dev. 45 (2005) 261–279.
[3] M.S. Colucci, H. Bork, E. Topp, Persistence of estrogenic hormones in agricultural soils: I. 17β-estradiol and estrone, J. Environ. Qual. 30 (2001) 2070–2076.
[4] M.S. Colucci, E. Topp, Dissipation of part-per-trillion concentrations of estrogenic hormones from agricultural soils, Can. J. Soil Sci. 82 (2002) 335–340.
[5] Z. Fan, F.X.M. Casey, G.L. Larsen, H. Hakk, Persistence and fate of 17β-estradiol and testosterone in agricultural soils, Chemosphere 67 (2007) 886–895.
[6] B.S. Das, L.S. Lee, P.S.C. Rao, R.P. Hultgren, Sorption and degradation of steroid hormones in soils during transport column studies and model evaluation, Environ. Sci. Technol. 38 (2004) 1460–1470.
[7] D.W. Kolpin, E.T. Furlong, M.T. Meyer, E.M. Thurman, S.D. Zaugg, L.B. Barber, H.T. Buxton, Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: a national reconnaissance, Environ. Sci. Technol. 36 (2002) 1202–1211.
[8] G. Chen, M. Flury, J.B. Harsh, P.C. Lichtner, Colloid-facilitated transport of cesium in variably saturated Hanford sediments, Environ. Sci. Technol. 39 (2005) 3435–3442.
[9] H. de Jonge, L.W. de Jonge, O.H. Jacobsen, [C-14]glyphosate transport in undisturbed topsoil columns, Pest Manag. Sci. 56 (2000) 909–915.
[10] J.E. Saiers, J.J. Lenhart, Colloid mobilization and transport within unsaturated porous media under transient-flow conditions, Water Resour. Res. 39 (2003).
[11] L.A. Sprague, J.S. Herman, G.M. Hornberger, A.L. Mills, Atrazine adsorption and colloid-facilitated transport through the unsaturated zone, J. Environ. Qual. 29 (2000) 1632–1641.
[12] K.G. Villholth, N.J. Jarvis, O.H. Jacobsen, H. de Jonge, Field investigations and modeling of particle-facilitated pesticide transport in macroporous soil, J. Environ. Qual. 29 (2000) 1298–1309.
[13] S. Zhao, P. Zhang, M.E. Melcer, J.F. Molina, Estrogens in streams associated with a concentrated animal feeding operation in upstate New York, USA, Chemosphere 70 (2010) 420–425.
[14] R.D. Holbrook, N.G. Love, J.T. Novak, Sorption of 17β-estradiol and 17a-ethinylestradiol by colloidal organic carbon derived from biological wastewater treatment systems, Environ. Sci. Technol. 38 (2004) 3322–3328.
[15] M. Lægdsmand, H. Andersen, O.H. Jacobsen, B.H. Sørensen, Transport and fate of estrogenic hormones in slurry-treated soil monoliths, J. Environ. Qual. 38 (2009) 955–964.
[16] M.L. Thompson, F.X.M. Casey, E. Khan, H. Hakk, G.L. Larsen, T.M. DeSutter, Occurrence and pathways of Manure-Borne 17β-estradiol in Vadose Zone Water, Chemosphere (2009) 472–479.
[17] J. Gong, Y. Ran, D.Y. Chen, Y. Yang, X.X. Ma, Occurrence and environmental risk of endocrine-disrupting chemicals in surface waters of the Pearl River, South China, Environ. Monit. Assess. 156 (2009) 199–210.
[18] L.S. Barber, G.K. Brown, D.W. Kolpin, J.H. Writer, S.D. Zaugg, A reconnaissance for hormone compounds in the surface waters of the United States, in: F.D. Wilde, L.J. Britton, C.V. Miller, D.W. Kolpin (Eds.), Effects of Animal Feeding Operations on Water Resources and the Environment—Proceedings of the Technical Meeting, Fort Collins, Colorado, August 30–September 1, 1999, U.S. Geological Survey Open-File Report 00-204, U.S. Department of the Interior U.S. Geological Survey, Reston, VA, 2000, p. 48.
[19] E.W. Peterson, R.K. Davis, H.A. Orndorff, 17β-Estradiol as an indicator of animal water contamination in mantled karst aquifers, J. Environ. Qual. 29 (2000) 826–834.
[20] D.D. Fine, G.P. Breidenbach, T.L. Price, S.R. Hutchins, Quantitation of estrogens in ground water and swine lagoon samples using solid-phase extraction, pentafluorobenzyl/trimethylsilyl derivatizations and gas chromatography-negative ion chemical ionization tandem mass spectrometry, J. Chromatogr. A 1017 (2003) 167–185.

[21] A. Lorenzen, J. Hendel, K.L. Conn, S. Bittman, A. Kwabiah, G. Lazarovitz, D. Massé, T.A. McAllister, E. Topp, Survey of hormone activities in municipal biosolids and animal manures, Environ. Toxicol. 19 (2004) 216–225.

[22] D.R. Raman, E.L. Williams, A.C. Layton, R.T. Burns, J.P. Easter, A.S. Daugherty, M.D. Mullen, G.S. Sayler, Estrogen content of dairy and swine wastes, Environ. Sci. Technol. 38 (2004) 3567–3573.

[23] S.R. Hutchins, M.V. White, F.M. Hudson, D.D. Fine, Analysis of lagoon samples from different concentrated animal feeding operations for estrogens and estrogen conjugates, Environ. Sci. Technol. 41 (2007) 738–744.

[24] J.C. Bowman, J.L. Zhou, J.W. Readman, Sediment-water interactions of natural oestrogens under estuarine conditions, Mar. Chem. 77 (2002) 263–276.

[25] J.L. Zhou, R. Liu, A. Wilding, A. Hibberd, Sorption of selected endocrine disrupting chemicals to different aquatic colloids, Environ. Sci. Technol. 41 (2007) 206–213.

[26] O. Gustafsson, P.M. Gschwend, Aquatic colloids: concepts, definitions, and current challenges, Limnol. Oceanogr. 42 (1997) 519–528.

[27] J.H. Sharp, Size classes of organic carbon in seawater, Limnol. Oceanogr. 18 (1973) 441–447.

[28] M.O. Aust, S. Thiele-Bruhn, K.U. Eckhardt, P. Leinweber, Composition of organic matter in particle size fractionated pig slurry, Bioresour. Technol. 100 (2009) 5736–5743.

[29] J. Kjaer, P. Olsen, K. Bach, H.C. Barlebo, F. Ingerslev, M. Hansen, B.H. Sørensen, Leaching of estrogenic hormones from manure-treated structured soils, Environ. Sci. Technol. 41 (2007) 3911–3917.

[30] W.L. Shelver, H. Hakk, G.L. Larsen, T.M. DeSutter, F.X.M. Casey, Development of an ultra-high-pressure liquid chromatography-tandem mass spectrometry multi-residue sulfonamide method and its application to water, manure slurry, and soils from swine rearing facilities, J. Chromatogr. A 1217 (2010) 1273–1282.

[31] Z. Fan, F.X.M. Casey, H. Hakk, G.L. Larsen, Discerning and modeling the fate and transport of testosterone in undisturbed soil, J. Environ. Qual. 36 (2007) 864–873.

[32] Z. Fan, F.X.M. Casey, H. Hakk, G.L. Larsen, Modeling coupled degradation, sorption, and transport of 17β-estradiol in undisturbed soil, Water Resour. Res. 44 (2008) W08424, doi:10.1029/2007WR006407.

[33] J.M. Jones, T. Borch, R.B. Young, J.G. Davis, C.R. Simpson, Photolysis of testosterone, progesterone, and 17β-estradiol by UVA light, in: Emerging Contaminants of Concern in the Environment: Issues, Investigations, and Solutions, AWRA, Vail, Colorado, 2007.

[34] Y. Zhang, J.L. Zhou, B. Ning, Photodegradation of estrone and 17 beta-estradiol in water, Water Res. 41 (2007) 19–26.

[35] M.E. Schmisek, L.J. Cihacek, L.J. Swenson, Relationships between the Mehlich-III soil test extraction procedure and standard soil test methods in North Dakota, Commun. Soil Sci. Plant Anal. 29 (1998) 1719–1729.

[36] National Cooperative Soil Survey, USA, Garborg Soil Series, 2005, accessed 24 November 2011, https://soilseries.sc.egov.usda.gov/OSD_Docs/G/GARBORG.html.

[37] G.D. Sheng, C. Xu, L. Xu, Y.P. Qiu, H.Y. Zhou, Abiotic oxidation of 17 beta-estradiol by soil manganese oxides, Environ. Pollut. 157 (2009) 2710–2715.

[38] L. Xu, C. Xu, M.R. Zhao, Y.P. Qiu, G.D. Sheng, Oxidative removal of aqueous steroid estrogens by manganese oxides, Water Res. 42 (2008) 5038–5044.

[39] S.B. Lartiges, P.P. Garrigues, Degradation kinetics of organophosphorus and organonitrogen pesticides in different waters under various environmental-conditions, Environ. Sci. Technol. 29 (1995) 1246–1254.

[40] K.a. Barrett, M.B. Mcbride, Oxidative degradation of glyphosate and aminomethylphosphonate by manganese oxide, Environ. Sci. Technol. 39 (2005) 9223–9228.

[41] K.I.E. Holthaus, A.C. Johnson, M.D. Jurgens, R.J. Williams, J.J.L. Smith, J.E. Carter, The potential for estradiol and ethinylestradiol to sorb to suspended and bed sediments in some English rivers, Environ. Toxicol. Chem. 21 (2002) 2526–2535.

[42] L.S. Lee, T.J. Strock, A.K. Sarmah, P.S.C. Rao, Sorption and dissipation of testosterone, and estrogens, and their primary transformation products in soils and sediments, Environ. Sci. Technol. 37 (2003) 4098–4105.

[43] W. Huang, W.J. Weber, Distributed reactivity model for sorption by soils and sediments. 10 relationships between desorption, hysteresis, and the chemical characteristics of organic domains, Environ. Sci. Technol. 31 (1997) 2562–2569.

[44] J.J. Pignatello, B. Xing, Mechanisms of slow sorption of organic chemicals to natural particles, Environ. Sci. Technol. 30 (1996) 1–11.

[45] M. Minamiyama, S. Ochi, Y. Suzuki, Fate of nonylphenol and 17β-estradiol contained in composted sewage sludge after land application, Water Sci. Technol. 57 (2008) 167–174.

[46] F.X.M. Casey, J. Šimůnek, J. Lee, H. Hakk, G.L. Larsen, Sorption, mobility, and transformation of estrogenic hormones in natural soil, J. Environ. Qual. 34 (2005) 1372–1379.

[47] D. Sheehan, Hydrodynamic methods: 7.3.3 precipitation, in: Physical Biochemistry Principles and Applications, John Wiley and Sons, Ltd., Hoboken, NJ, 2009, pp. 284–287 (Chapter 7).

[48] A. Jacobsen, A. Lorenzen, R. Chapman, E. Topp, Persistence of testosterone and 17β-estradiol in soils receiving swine manure or municipal biosolids, J. Environ. Qual. 34 (2005) 861–871.