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Potential water quality impacts originating from land burial of cattle carcasses

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HIGHLIGHTS

- We investigated water quality impacts of buried cattle carcass leachate.
- Cattle carcass burial produced leachate containing a variety of organic and inorganic contaminants, including nutrients.
- Steroids and pharmaceuticals were also detected in the leachate at high concentrations relative to other wastewaters.

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ABSTRACT

Among the conventional disposal methods for livestock mortalities, on-farm burial is a preferred method, but the potential water quality impacts of animal carcass burial are not well understood. Typically, on-farm burial pits are constructed without liners and any leachate produced may infiltrate into soil and groundwater. To date, no information is available on temporal trends for contaminants in leachate produced from livestock mortality pits. In our study, we examined the concentrations of conventional contaminants including electrical conductivity, COD, TOC, TKN, TP, and solids, as well as veterinary antimicrobials and steroid hormones in leachate over a period of 20 months. Most of the contaminants were detected in leachate after 50 days of decomposition, reaching a peak concentration at approximately 200 days and declined to baseline levels by 400 days. The estrogen 17 β -estradiol and a veterinary antimicrobial, monensin, were observed at maximum concentrations of 20,069 ng/L and 11,980 ng/L, respectively. Estimated mass loading of total steroid hormone and veterinary pharmaceuticals were determined to be 1.84 and 1.01 μ g/kg of buried cattle carcass materials, respectively. These data indicate that leachate from carcass burial sites represents a potential source of nutrients, organics, and residues of biologically active micro-contaminants to soil and groundwater.

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1. Introduction

1.1. Cattle and calf production

Production of cattle and calves in the United States is approximately 100 million head per year over the past 60 years (USDA/NASS Homepage, 2012) with a reported retail equivalent value of 79 billion dollars in 2011 (USDA/NASS, 2011; USDA/ERS Homepage, 2012). The United States Department of Agriculture reports that since the late 1980s, over 2.2 million mortalities occur in cattle and calf production facilities each year on average (USDA/NASS Homepage, 2012). A 5-year retrospective cohort study (Loneragan et al., 2001) investigated 121 cattle feedlots in the United States and found an approximate annual routine mortality rate of 1.3%, suggesting that over 1 million cattle routine mortalities require disposal each year.

1.2. Animal carcass disposal methods

Conventional disposal for deceased livestock includes burial, composting, rendering, and incineration. Burial and composting are attractive disposal options for cattle mortalities due to the costs and regulatory restrictions on rendering and incineration of cattle carcasses (Code of federal regulations, 2010). On-farm burial is a method preferred by animal producers due to the limited infrastructure requirements and minimal disposal costs (Gwyther et al., 2011).

1.3. Impacts of carcass burial on groundwater

Few studies have documented the impacts of carcass burial on groundwater quality. To date, investigations of groundwater quality impacts due to animal carcass disposal have focused largely on poultry carcass disposal and have investigated only a limited number of routinely-measured contaminants, including nutrients, chloride and fecal pathogens. Increased concentrations of ammonia, nitrate, chloride, and fecal pathogens in groundwater have been observed on farms with poultry carcass disposal pits (Hatzell, 1995; Ritter and

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Chirnside, 1995; Myers et al., 1999). Ritter and Chirnside (1995) found the highest concentrations of ammonia and nitrate adjacent to poultry disposal pits in Delmarva Peninsula. Maximum concentrations of ammonia and nitrate detected at this location were 366 mg N/L and 77.6 mg N/L, respectively and the maximum concentration of chloride was reported to be 209 mg/L. In contrast, Myers et al. (1999) reported 39.7 mg N/L as the highest level of nitrate detected in the monitoring wells while almost all water samples contained ammonia-N less than 0.2 mg N/L. Moreover, only slightly increased nitrate concentrations (i.e. increases of 2 mg N/L from the median nitrate concentration) were found by Hatzell (1995) in west-central Suwannee County in Florida at a location with a chicken carcass disposal pit. No obvious effects of the disposal pit leachate on other water quality characteristics were determined. Variability in the results of nutrients observed from these studies is partially due to variation in local soil texture, background water quality, groundwater flow direction, and water table depth. However, the elevated nitrate levels were all above the drinking water standard of 10 mg N/L. Both Ritter and Chirnside (1995) and Myers et al. (1999) detected fecal pathogens, though at low concentrations (generally <20/100 mL for most samples), in groundwater samples obtained near poultry disposal pits. However, other on site waste disposal practices, such as uncovered litter stockpiles, were thought to have a higher impact on groundwater quality than the pit itself (Hatzell, 1995; Myers et al., 1999). In a related study (Glanville, 2000), groundwater samples were collected and analyzed for routinely-measured contaminants near disposal pits containing 28,400 kg turkey mortalities and 6 swine carcasses. Elevated levels of BOD (230 mg/L), ammonia-N (403 mg/L), TDS (1527 mg/L) and chloride (109 mg/L) were detected in the monitoring wells installed within 1 m of one of the study sites. These values were all significantly greater ($p < 0.05$) than mean background concentrations detected in the control monitoring well measured as 6 mg/L, 0.2 mg/L, 658 mg/L, and 8.6 mg/L, respectively. It also suggested that complete decay in lightly loaded burial trenches with well-drained soils may take two years or more.

1.4. Leachate quality from carcass burial pits

Even more limited is information on leachate water quality from animal burial sites. To our knowledge, only two studies have reported data describing the quality of leachate produced from animal burial. MacArthur et al. (2002) reported average leachate concentrations of ammonia-N (3294 mg/L), alkalinity (9400 mg/L), BOD (12,700 mg/L), and COD (20,414 mg/L) on a burial site with food-and-mouth disease mortalities of mixed species. In addition, a total of 4000 m³ of leachate was generated. A field study investigating leachate quality was conducted with poultry, bovine, and swine carcasses buried in separate pits and isolated from the surroundings with a sealed 40 mil polyethylene liner (Pratt and Fonstad, 2009). Elevated levels of ammonium-N (12,600 mg/L), alkalinity (46,000 mg/L as bicarbonate), chloride (2600 mg/L), sulfate (3600 mg/L), potassium (2300 mg/L), sodium (1800 mg/L), and phosphorus (1500 mg/L), and relatively lesser amount of iron, calcium, and magnesium were present in leachate samples. These data provide important information on the potential for groundwater contamination from animal disposal pit leachate since most of the on-farm mortality pits in the U.S. are unlined. No previous studies have examined the characteristics of naturally produced leachate from animal burial pits.

1.5. Presence of steroid hormones and veterinary pharmaceuticals in wastes from animal production environments

Animals in the United States routinely receive steroid hormone and antibiotic supplements as growth promotants and to prevent disease. Currently, no information is available on the potential for release of these compounds in leachate from animal carcass burial sites. However, these compounds have been detected in wastes

from animal production facilities. In a concentrated animal feeding operation (CAFO) with animal manure application located near Lansing, MI, monensin and amprolium were detected frequently ranging from 1 to 189 ng/L and 6 to 288 ng/L in surface water, and 0.004 to 0.5 µg/kg and 0.03 to 0.26 µg/kg in top soils (Song et al., 2010). 13 pharmaceutical compounds and 4 steroid hormones were also detected in lagoon wastewater and adjacent groundwater underlying selected beef cattle and swine CAFOs in Nebraska (Bartelt-Hunt et al., 2011). Among the targeted pharmaceuticals, monensin was detected most frequently and the maximum level was measured as 13,000 ng/L in lagoon wastewater. Detected steroid hormones including estrone, testosterone, 4-androstenedione, and androsterone ranged from 30 to 3600 ng/L in lagoon wastewater and 30 to 390 ng/L in groundwater. These data indicated that steroids and veterinary pharmaceuticals originating from animal production wastewater can infiltrate to shallow groundwater.

1.6. Objective

To better understand the chemical composition of rainfall-produced leachate after burial of cattle carcasses, a two-year field study was performed by burying cattle mortalities in lined pits with a leachate collection system. The objectives of this study were to determine temporal trends in leachate generation and contaminant concentrations in the leachate, including both routinely-measured parameters as well as veterinary pharmaceuticals and steroid hormones.

2. Materials and methods

2.1. Carcass burial pit description

Carcass burial pits were constructed at the University of Nebraska-Lincoln Agricultural Research and Development Center near Mead, Nebraska. The surface area of each disposal pit was approximately 120 m² (12 m by 10 m) and depth of the pit was approximately 5 m. Pits were constructed with 1:1 side slope. Details of the pit dimensions are provided in Fig. S1. The pit was lined with a 40-mil PVC liner. A 445 L PVC reservoir was placed beneath the liner and was connected to a perforated HDPE pipe for leachate collection. The leachate collection system and bottom liner were covered by 10 cm gravel and 15 cm structural sand (Fig. S1). Approximately 1400 kg (5 to 7 head) of beef cattle carcasses was placed in each of 3 replicate pits and the pits were backfilled with native soil and compacted with a tapping machine right after the carcasses were placed. Soil compaction was evaluated by soil density test. For each pit, 5 to 6 soil sampling sites were selected and soils were sampled during backfilling at one-inch increments from a depth of 4 inches to 10 inches below the ground surface. Compaction testing was conducted with a nuclear gauge and a confirmation test for density was performed using a sand cone apparatus. The top of each pit was graded at a 20:1 slope to minimize ponding on the pit surface.

2.2. Source of materials

All carcasses were obtained from an operating commercial beef cattle feedlot. Animals at the feedlot received growth promotants as feed additives containing monensin, trenbolone acetate, and estradiol. Additionally, florfenicol, cefiofur, and enrofloxacin were used in the feedlot hospital. Carcasses used in this project were routine mortalities from the hospital and were younger than 30 months of age. Carcasses were placed in the pits one or two days after death.

2.3. Site monitoring and sampling

Local temperature and precipitation data were obtained from a weather station operated by the High Plains Regional Climate Center

(Figs. S2 & S3). Leachate was sampled by submerging a double stage 12 V DC purge pump (Geotech Environmental Equipment Inc., Denver, CO) in the leachate reservoir. During each sampling event, all leachate was pumped out of the reservoir so that subsequent samples were characteristics of new material. Leachate sampling was conducted biweekly (once every two weeks) for the first two months and monthly for the following 18 months, for a total of 20 months of sampling starting on May 17th, 2010 and ending on December 16th, 2011. Composite leachate samples were collected and delivered on ice to the Water Science Laboratory in (Lincoln, NE) or the Environmental Engineering Laboratory at Peter Kiewit Institute (Omaha, NE). Total leachate volumes were also determined.

2.4. Analytical methods

Temperature and dissolved oxygen (DO) of leachate were measured on site with a portable DO meter (YSI 550DO). Leachate pH was measured in the laboratory using a pH meter (Oakton pH 510 series) calibrated with standards at pH 4, 7, and 10 before each use. Other parameters measured in this study include chemical oxygen demand (COD), total organic carbon (TOC), electrical conductivity (EC), chloride, total phosphorus (TP), total Kjeldahl nitrogen (TKN), solids, steroid hormones, and veterinary pharmaceuticals. Chlorides were analyzed with a chloride combination ion selective electrode (Denver Instrument, Denver, CO). For COD analysis, leachate samples were digested in pre-prepared COD digestion tubes (Hach Company, Loveland, CO) and then heated to 150 °C for 2 h followed by colorimetric determination at 620 nm. TP and TKN were measured with EPA method 365.1 and 351.2 (colorimetric) on an AQ2 autoanalyzer (Seal Analytical, Mequon, WI). TOC was analyzed by wet oxidation (Standard Method 5310D) on an OI analytical Model 1010 TOC Analyzer. Solids were analyzed following standard methods (Standard Method 2540).

Seventeen veterinary pharmaceuticals including monensin, ractopamine, tylosin, sulfonamides and tetracyclines were analyzed by either on-line or off-line solid phase extraction (SPE) followed by liquid chromatography tandem mass spectrometry on a Waters Quattro Micro triple quadrupole mass spectrometer (LC-MS/MS) with electrospray ionization (Snow et al., 2003; Zhu et al., 2001). Twenty steroid hormones including 17 α -estradiol, androsterone, estradiol, estrone, and testosterone were analyzed using either on-line or off-line SPE followed by atmospheric pressure photoionization (APPI) LC-MS/MS. Details of the on-line SPE LC-MS/MS method for steroid hormones are provided elsewhere (Snow et al., 2013a). A list of all steroid hormones and veterinary pharmaceuticals included in these methods is given in Table S1.

For on-line extraction and analysis of veterinary pharmaceuticals, 30 mL of water sample was syringe-filtered (Whatman glass fiber GDX, 25 mm, 0.45 μ m pore size), weighed directly into a 40-mL vial, spiked with internal standards and surrogates, and thoroughly mixed with 20 mL reagent water and 500 μ L 2.4 M citric acid. Calibration standards were prepared by fortifying 2.4 M citric acid with analytes (10 to 1000 ng/L) and treated in an identical manner as samples. During analysis each solution was extracted with a Spark Holland Symbiosis on-line solid extraction system using a Waters (1 \times 2 mm) HLB solid-phase extraction cartridge and then eluted with mobile phase for subsequent separation and detection.

As the project progressed, the total solids, nutrient and organic concentrations in leachate sample increased substantially leading to analytical problems and matrix issues. Because of the changing complexity of the matrix, off-line extraction using 200 mg HLB cartridges (Waters Oasis, Milford, MA) was followed during the last half of the monitoring study. Off-line extraction permitted additional extract cleanup for steroid hormones which would not be possible with on-line SPE. A measured volume of sample (50 mL) was spiked with surrogate and passed through glass fiber filters (25 mm Whatman

GF/F, 0.7 μ m) and SPE cartridges under vacuum. The cartridges were then washed with 10 mL of 10% methanol in water, followed by elution with 0.1% formic acid in methanol. Pharmaceutical extracts were concentrated and analyzed as described below.

Extraction of samples for steroid hormones followed a similar protocol as the veterinary pharmaceuticals with elution by 10 mL of methanol, followed by complete evaporation and cleanup using Florisil™. Evaporated extracts were dissolved in 50:50 dichloromethane:hexane, dried using anhydrous sodium sulfate, and purified using normal phase chromatography on Florisil™ SPE cartridges (Waters 3 cc Vac Cartridge, 500 mg Sorbent per Cartridge, 50–200 μ m particle size). Internal standards were added after elution with 3 \times 3 mL of 50:50 dichloromethane:methanol. Purified extracts were evaporated to dryness and redissolved in 400 μ L methanol:water followed by analyses using APPI LC-MS/MS as described for solid extracts (Snow et al., 2013).

Pharmaceutical compounds were quantified using electrospray ionization and selected reaction monitoring (Table S2). A Thermo HyPurity C18 5 μ m 2 \times 250 mm column provided separation with a mobile phase comprised of 97:3 water/methanol and 3:97 methanol/water each containing 0.1% (v/v) formic acid. LC/MS/MS conditions and transitions were determined and optimized by infusing with concentrated standards. A capillary voltage of 4.0 kV, an extractor of 3 V and an RF lens of 0.1 V were used. The source temperature was 120 °C and the desolvation temperature was 500 °C. The nebulizer flow rate was 700 L/h in the desolvator and 30 L/h in the cone.

2.5. Pit excavation

Disposal pits were excavated at 31 months of decomposition to examine the residues. Samples were sent to Midwest Laboratories (Omaha, NE) for component composition analysis by AOAC (Association of Official Analytical Chemists) official methods 991.36 and LECO 2000 as described previously (Yuan et al., 2012).

3. Results and discussion

3.1. Weather monitoring

During the 20 month sampling period, a total of 1.4 m (55 in.) of precipitation (including the equivalent snow melt) was observed (Table S3 & Fig. S2). The maximum and minimum air temperatures were 38 °C and –24 °C, respectively. At a depth of around 10 cm (4 in.), maximum and minimum soil temperatures were 32 °C and –1 °C (Fig. S3). Over the 20 month study period, the temperature of the leachate immediately after collection ranged from 11 °C to 16 °C (data not shown).

3.2. Leachate production

Substantial leachate production was not observed until after 370 days of decomposition (Fig. S4). Prior to this time, less than 300 L of leachate was collected from each pit. The trend of leachate production was similar between replicate pits, with the majority of leachate production occurring between 370 and 540 d. The maximum leachate volume collected during a single sampling event was 2230 L of leachate. The total volume of leachate produced in each of the three pits was 3843 L, 7763 L, and 7759 L, respectively (Table S3). The final compacted density of the soil ranged between 1585 and 1618 kg/m³ between the three pits. A possible explanation for the differences in leachate production between the pits may be due to the site topography. Pit 1 was at the highest elevation with pits 2 and 3 located down slope, which may have resulted in increased runoff over the surface of pits 2 and 3.

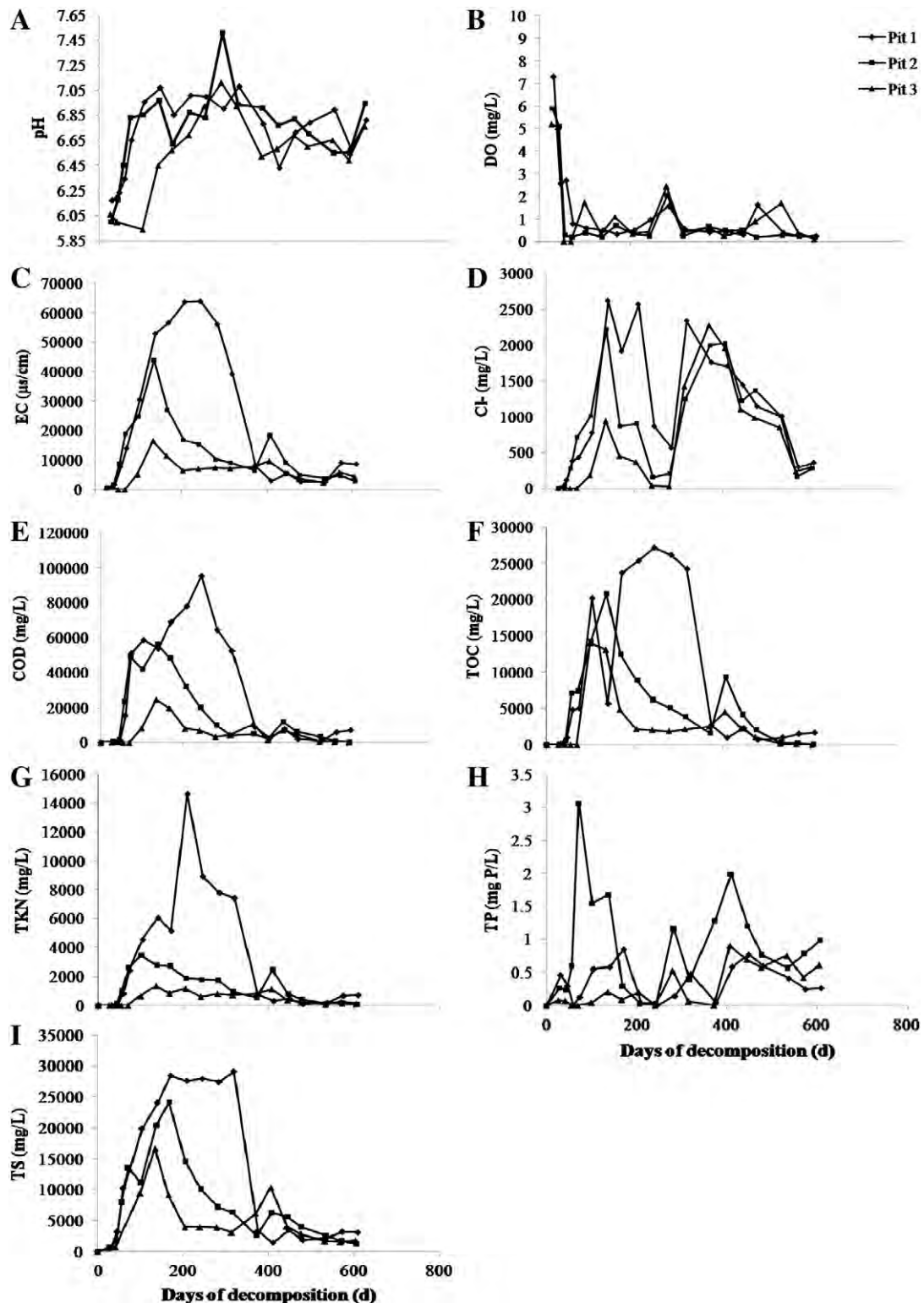


Fig. 1. Trends of conventional contaminants in the cattle carcass leachate during land burial decomposition. (A) pH; (B) dissolved oxygen; (C) electrical conductivity; (D) chlorides; (E) chemical oxygen demand; (F) total organic carbon; (G) total Kjeldahl nitrogen; (H) total phosphorus; and (I) total solids.

3.3. pH and dissolved oxygen in the leachate

The measured pH of leachate was initially acidic (Fig. 1, panel A) with pH < 6 in the first two weeks after burial. This may be attributed to the accumulation of acidic end products of sugar fermentation and the inactive microbiological acid-consuming activity of acetogenic and methanogenic bacteria (Barlaz et al., 1989). Increasing pH was observed

until 120 days of decomposition when the pH remained neutral (pH ~6.8). The observed leachate pH changes in this study investigating anaerobic decomposition of cattle carcasses are consistent with those previously reported in laboratory scale studies of municipal refuse degradation and a previous laboratory scale study of cattle carcass decomposition conducted by the authors (Barlaz et al., 1989; Eleazer et al., 1997; Yuan et al., 2012). The dissolved oxygen concentration of the

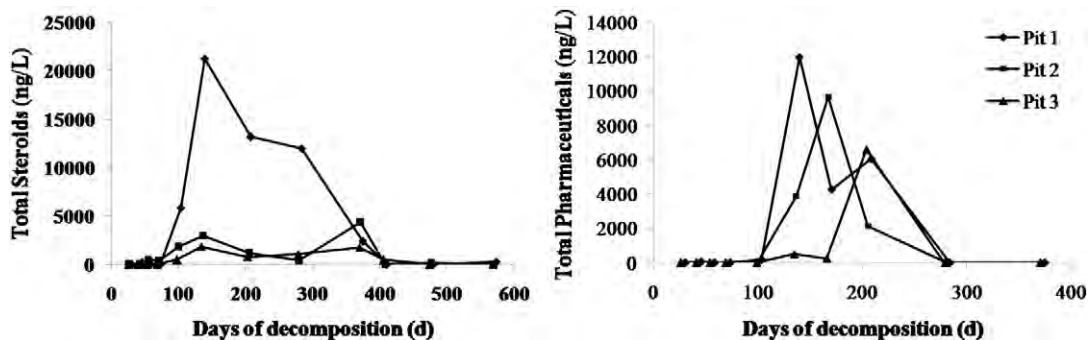


Fig. 2. Total steroid hormones and veterinary pharmaceuticals detected in cattle carcass leachate during land burial.

leachate decreased from 7.5 mg/L initially to less than 1 mg/L within the first two weeks of decomposition (Fig. 1, panel B). DO levels in the leachate remained less than 3 mg/L for the duration of the study.

3.4. Conventional contaminants in the leachate

A lag time of approximately two months was observed for the occurrence of most of the targeted contaminants in the leachate (Figs. 1 and 2). The time required to establish microorganism populations and time required for infiltration of rainfall through soil are likely to be responsible for the delay observed for initiation of carcass decomposition. Compared to the contaminant abundance detected in the first two months, higher levels of conventional water quality parameters and contaminants were detected in leachate (Fig. 1, panels C through I) during the latter part of the study. The maximum electrical conductivity observed in the leachate was 63,760 $\mu\text{s}/\text{cm}$, 43,960 $\mu\text{s}/\text{cm}$, and 16,460 $\mu\text{s}/\text{cm}$ in pits 1, 2 and 3, respectively (Fig. 1, panel C). Two peaks in chloride concentration were observed, with the first peak occurring around day 200 and the second peak occurring around day 400 (Fig. 1, panel D). The maximum observed value of chloride was 2614 mg/L (Table 1 & Fig. 1D). Most other contaminants such as COD, TOC, TKN, and solids displayed similar trends to electrical conductivity. The highest concentrations of these parameters were measured in pit 1 and found to be 95,333 mg/L for COD; 27,158 mg/L for TOC; 14,640 mg/L for TKN; and 29,060 mg/L for total solids (Table 1 & Fig. 1). The majority of solids measured in the leachate were volatile solids (Fig. S5). The occurrence of most conventional contaminants in

leachate was detected between 50 and 400 days after the initiation of experiments (Fig. 1). The maximum and minimum values of all conventional contaminants detected from each replicate disposal pit are summarized in Table 1. Peak concentrations for these constituents occurred at different times, varying from 70 to 400 days of decomposition with most of the peak values observed between 100 and 200 days. The only parameter which did not increase by several orders of magnitude was total phosphorus (Fig. 1H). The maximum concentration of TP observed in the leachate was 3 mg P/L.

The growth of microorganisms and infiltration of rainfall likely varied between pits and among different locations in the same pit during the study period, resulting in varied leachate characteristics (Robinson and Luo, 1991). The maximum contaminant concentrations measured in this study were consistent with related results reported by Pratt and Fonstad (2009) who investigated the quality of pure leachate after two years of decomposition of swine, bovine, and poultry carcasses. Values reported in Pratt study were constant because leachate was retained in the sealed decomposition vessel over the study period. The maximum chloride concentration reported here (2614 mg/L) is comparable to that reported in Pratt study (2600 mg/L). The highest TKN concentrations measured in this study (14,640 mg/L) compared well with ammonium nitrogen concentrations measured in leachate from in-vessel decomposition of bovine (17,300 mg/L), swine (16,900 mg/L), and poultry (18,200 mg/L). The TKN concentrations reported here are approximately five times larger than the average concentration of ammonium-N (3294 mg/L) (MacArthur et al., 2002) reported in the natural leachate from a foot and mouth (FMD) mass

Table 1
Conventional water quality parameters in leachate.

Parameter	Pit 1			Pit 2			Pit 3		
	Maximum	Minimum	Peak day ^a	Maximum	Minimum	Peak day ^a	Maximum	Minimum	Peak day ^a
EC ^b ($\mu\text{s}/\text{cm}$)	63,760	558	245	43,960	645	137	16,460	793	135
Cl ⁻ (mg/L)	2614	6	140	2214	7	137	2274	1	370
COD ^c (mg/L)	95,333	585	245	56,083	312	137	24,717	268	135
TOC ^d (mg/L)	27,158	104	245	20,788	56	137	13,985	16	99
TKN ^e (mg/L)	14,640	2	209	3440	3	101	1363	2	135
TP ^f (mg P/L)	0.84	ND	171	3.06	0.03	71	0.90	ND	406
TS ^g (mg/L)	29,060	1467	319	24,150	720	168	16,705	615	135
TVS ^h (mg/L)	19,400	733	319	14,395	280	168	9695	190	135
TSS ⁱ (mg/L)	1240	87	74	1500	144	71	630	88	99

^a Days of decomposition at which maximum value was detected.
^b EC: electrical conductivity.
^c COD: chemical oxygen demand.
^d TOC: total organic carbon.
^e TKN: total Kjeldahl nitrogen.
^f TP: total phosphorus.
^g TS: total solids.
^h TVS: total volatile solids.
ⁱ TSS: total suspended solids.

burial site in the United Kingdom. We also observed higher peak concentrations of COD in this study (95,333 mg/L) compared with an averaged COD concentration of 20,414 mg/L observed in the natural leachate from FMD disposal sites (MacArthur et al., 2002). Similarity between this study and MacArthur's was that both of them used mono-disposal system and the mortalities were open to surrounding environment. However, the FMD disposal site contained in excess of 400,000 carcasses with the majority species being sheep. Ranges of electrical conductivity (EC: 585 to 63,760 µs/cm), COD (585 to 95,333), TKN (2 to 14,640 mg N/L), and TS (1467 to 29,060 mg/L) concentrations reported in the current study compare favorably with a field study investigating freshly produced leachate from an operating municipal solid waste landfill (EC: 23,000 to 35,500 µs/cm; COD: 44,000 to 115,000 mg/L; TKN: 2023 to 10,558; TS: 32,100 to 77,000 mg/L) in Greece in which daily waste deposits contained nearly 50% food wastes (Tatsi and Zouboulis, 2002).

Although TP levels increase during decomposition, the maximum concentration measured in this study was substantially lower than that reported in other studies. Concentrations of total phosphorus detected in previous study of carcass decomposition remained at approximate 1200 mg/L (Pratt and Fonstad, 2009) for the pure leachate. In the Greece municipal solid waste landfill, level of total phosphorus ranged from 1.6 to 655 mg/L in the leachate collected next to the deposition area (Tatsi and Zouboulis, 2002). However, the range of TP concentration shifted to 1.27 to 19.9 mg/L in the old leachate which stayed at the lowest part of the landfill for several months, subjected to natural attenuation/stabilization but not oxygenation (Tatsi and Zouboulis, 2002), which is close to the level of TP detected in this study. Because the carcasses were surrounded by soil in the present study, soil adsorption is likely one reason for the lower concentrations of phosphorus detected in leachate.

3.5. Steroid hormones and veterinary pharmaceuticals in the leachate

The presence of steroid hormones and veterinary pharmaceuticals in leachate from cattle carcasses was evaluated in this study. In the cattle industry, veterinary antibiotics and steroid hormone implants are widely used for disease prevention and growth promotion. In addition, steroid hormones are naturally produced in animals to regulate reproductive and other functions and continuously excreted into the environment (Shore and Shemesh, 2003). Of the 20 steroid hormones and 17 veterinary pharmaceuticals (Table S1) evaluated, the natural steroid hormones 17β-estradiol, estrone, and testosterone, and one veterinary pharmaceutical (monensin) were detected most frequently in the leachate (Table 2). Eight additional steroid

hormones and nine veterinary pharmaceuticals were sporadically detected in leachate with concentrations presented in Table S4.

As listed in Table 2, 17β-estradiol was detected after 46 days of decomposition at a concentration of 160 ng/L in pit 1. 17β-Estradiol was detected on day 56 at a concentration of 203 ng/L in pit 2 and at day 99 at a concentration of 385 ng/L in pit 3. Testosterone was detected in pit 2 at day 56 at a concentration of 223 ng/L and in pit 1 at 39 ng/L at day 74. No leachate samples from pit 3 contained detectable levels of testosterone. Estrone was detected after day 100 at concentrations of 2706 ng/L (pit 1), 633 ng/L (pit 2), and 77 ng/L (pit 3), respectively. Monensin was not detected until day 140 at concentrations of 11,980 ng/L (pit 1), 3890 ng/L (pit 2), and 191 ng/L (pit 3).

A second 17β-estradiol peak occurred at approximately 140 days and coincided with the maximum observed concentrations of 17β-estradiol of 20,069 ng/L, 3009 ng/L, and 1740 ng/L in each pit. No consistent trends in estrone, testosterone or monensin concentrations were observed, though higher concentrations tended to occur in samples with higher COD, TOC, nutrients, and solid levels (Figs. 1 and 2). These compounds were primarily detected in leachate between 100 and 320 days except testosterone which was detected from day 50 to 105. The highest levels of monensin, estrone, and testosterone were determined to be 11,980 ng/L, 2706 ng/L, and 235 ng/L, which were all reported in samples collected from pit 1 which also produced the highest levels of COD, TOC, nutrients and solid residues. The observation that almost all contaminants from pit 1 exhibited the highest concentrations was probably correlated to the least leachate volume produced in pit 1 due to aforementioned soil compaction and site topography. Peak values of total steroid hormones and veterinary pharmaceuticals (Fig. 2) were 21,255 ng/L and 11,980 ng/L with the highest concentrations of individual compounds being the natural estrogen 17β-estradiol and the ionophore monensin widely used in ruminant animal feed (Butaye et al., 2003).

The levels of organic microcontaminants detected in the leachate in this study are much higher than those observed in the effluent of municipal and industrial sewage treatment plants considered to be major sources of endocrine disrupting compounds to the aquatic environment, by up to 3 orders of magnitude (Desbrow et al., 1998; Spengler et al., 2001; Coors et al., 2003). The occurrence of 17β-estradiol at high concentrations relative to other waste streams characterized previously such as municipal wastewater effluents or lagoon wastewaters is notable considering the potential for endocrine disrupting effects at concentrations in the low ng/L range (Hanselman et al., 2003). The occurrence of 17β-estradiol in the leachate with limited detections of 17α-estradiol appears contradictory to previous findings that

Table 2
Steroid hormones and pharmaceuticals detected most frequently in leachate.

Pit	Compound	Concentration (ng/L)													
1	Day ^a	46	59	74	104	140	171	209	245	284	319	375	411	480	574
	17β-Estradiol	160	ND ^b	ND	1501	20,069	ND	11,617	ND	9932	ND	99	30	43	258
	Estrone	ND	ND	ND	2706	1186	ND	1585	ND	2065	ND	ND	ND	ND	ND
	Testosterone	ND	ND	39	235	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Monensin	ND	ND	ND	ND	11,980	4270	5490	ND	ND	ND	ND	ND	ND	ND
2	Day	43	56	71	101	137	168	206	242	281	316	372	408	477	571
	17β-Estradiol	ND	203	304	781	3009	569	ND	247	ND	303	ND	ND	148	32
	Estrone	ND	ND	ND	633	ND	569	ND	219	ND	315	ND	ND	ND	ND
	Testosterone	ND	223	13	83	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Monensin	ND	ND	ND	ND	3890	9280	710	ND	ND	ND	ND	ND	ND	ND
3	Day	41	54	69	99	135	166	204	240	279	314	370	406	475	569
	17β-Estradiol	ND	NA ^c	NA	385	1363	ND	562	135	699	95	1740	491	ND	47
	Estrone	ND	NA	NA	77	475	ND	201	ND	412	ND	ND	ND	ND	ND
	Testosterone	ND	NA	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Monensin	ND	NA	NA	ND	ND	191	229	ND	ND	ND	ND	ND	ND	ND

^a Days of decomposition at which listed compounds were detected.

^b ND: not detected. Detection limits of steroids and pharmaceuticals were 2 ng/L and 20 ng/L, respectively.

^c NA: not available. No samples were collected during the sampling date.

Table 3
Estimated total mass loading^a of contaminants.

Pit	Cl ⁻ (mg/kg)	COD ^b (g/kg)	TOC ^c (g/kg)	TKN ^d (g/kg)	TP ^e (mg/kg)	TS ^f (g/kg)	TSS ^g (g/kg)	TVS ^h (g/kg)	TSH ⁱ (µg/kg)	TVP ^j (µg/kg)
1	14.42	21.73	6.47	1.99	1.54	10.09	0.64	5.74	1.60	0.69
2	11.17	40.63	27.06	6.49	6.78	29.86	1.65	17.52	1.38	0.91
3	7.94	31.58	15.05	3.83	2.81	33.56	1.40	19.43	2.53	1.44
Mean	11.18	31.31	16.19	4.10	3.71	24.50	1.23	14.23	1.84	1.01
Std err	1.87	5.46	5.97	1.31	1.58	7.29	0.30	4.28	0.35	0.22

^a Total mass loading of each component was calculated as the sum of the leachate volume produced between two sequential sampling dates × corresponding contaminant concentration measured during that sampling period.

^b COD: chemical oxygen demand.

^c TOC: total organic carbon.

^d TKN: total Kjeldahl nitrogen.

^e TP: total phosphorus.

^f TS: total solids.

^g TSS: total suspended solids.

^h TVS: total volatile solids.

ⁱ TSH: total steroid hormones.

^j TVP: total veterinary pharmaceuticals.

indicate that beef cattle typically excrete larger amount of 17α-estradiol (Hanselman et al., 2003).

3.6. Estimated total mass loading of contaminants

The estimated total mass loading for each contaminant is summarized in Table 3. Total carbon as COD had the highest estimated mass loading with an average value of 31.31 g COD per kg of buried cattle carcass material. Average mass loading of total steroid hormones and pharmaceuticals were estimated to be 1.84 and 1.01 µg per kg of cattle carcass, respectively. In general, the potential groundwater impacts from animal carcass disposal are a function of the total number of carcasses as well as local hydrogeologic and precipitation conditions. In the 1967 and 2001 outbreaks of FMD in Great Britain, a total of 433,987 (211,825 cattle) and 1,281,278 (306,053 cattle) animal carcasses required disposal (Scudamore et al., 2002). Assuming an average cattle carcass to be 500 kg, the total mass loading of microcontaminants to groundwater from the 2001 FMD outbreak sources could be as high as 300 kg. Prior to this study, the potential loading of steroid hormones or veterinary pharmaceuticals to the environment from animal carcass burial had not been quantified.

3.7. Carcass residue analysis

All pits were excavated 31 months after burial (picture S1 through S3). Visual observation of the liner during excavation showed it to be intact with no signs of chemical weathering. Very little carcass residue was found after 31 months of decomposition. Samples of the remaining carcass material were analyzed for composition with methods described previously (Yuan et al., 2012). Relative to other dry matters, fat was the major component left in the residues (Table 4) which was consistent with previous results obtained from a laboratory decomposition study of cattle rendering material (Yuan et al., 2012).

Table 4
Composition of cattle carcass residues after 31 months of decomposition.

Component	% of weight
Moisture	25.97
Dry matter	74.03
Crude protein	2.78
Acid hydrolysis fat	68.7
Ash	0.26
Carbohydrate	2.5

4. Conclusion

The measured concentrations of steroid hormones, veterinary antibiotics as well as carbon, nutrients and dissolved solids detected in the leachate from cattle carcass disposal pits are relatively high compared with other agricultural waste sources, such as lagoon wastewater or manure. This is not surprising given that leachate can become highly concentrated from mobilization of chemically labile compounds produced during carcass decomposition. The potential for localized water quality impacts is of concern, especially as on-farm animal carcass disposal sites are typically not lined. To date, a very small number of studies have investigated the chemical composition of leachate or groundwater impacted by animal carcass burial. The potential for adverse water quality impact from on-site carcass disposal practices should be considered when evaluating the occurrence of nutrients and organic microcontaminants in groundwater.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.scitotenv.2013.03.083>.

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