Potential water quality impacts originating from land burial of cattle carcasses

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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 is not well understood. Typically, on-farm burial pits are constructed without liners to prevent percolation of leachate 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 (electrical conductivity, COD, TOC, TKN, TP, and solids) as well as some antimicrobials and steroid hormones for a period of 20 months. High concentrations of conventional contaminants were detected in leachate collected from the field burial pits. In addition, 17β-estradiol and monensin were also observed at maximum concentrations of 20,069 ng/L and 11,980 ng/L, respectively. Estimated mass loading of total steroid hormones and veterinary pharmaceuticals were determined to be 1.84 and 1.01 µg/kg of buried cattle carcass materials.

Keywords

Cattle carcasses, leachate, burial, anaerobic decomposition, organic contaminants, steroids, veterinary pharmaceuticals

Introduction

Production of cattle and calves in the United States is approximately 100 million head per year over the past 60 years [1] with a reported retail equivalent value of 79 billion dollars in 2011 [2, 3]. 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[4]. A 5-year retrospective cohort study from Loneragan *et al* [5] 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.

Conventional livestock disposal methods include 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 [6]. On-farm burial is a method preferred by animal producers due to the limited infrastructure requirements and economic benefits [7].

Few studies have documented the impacts of animal mortality burial on groundwater quality. To date, investigation of groundwater quality impacts due to animal carcass disposal have focused largely on poultry carcass disposal and have investigated only a limited number of conventional 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 [8-10]. Variability in the results from these studies is partially due to variation in local soil texture, background water quality, groundwater flow direction, and water table depth. Ritter and Chirnside [10] 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/L N and 77.6 mg/L N, respectively and the maximum concentration of chloride was reported to be 209 mg/L. Slightly increased nitrate concentrations (i.e. increases of 2 mg/L N from the median nitrate concentration) was found by Hatzell [8] in west-central Suwannee County in Florida at a location with a chicken carcass disposal pit. No obvious effects of the disposal pit on other targeted water quality parameters were determined. Both Ritter [10] and Myers [9] detected fecal pathogens, though at low concentrations (generally <20/100 mL for most samples), in groundwater samples obtained near poultry disposal pits. However, on site waste disposal practices, such as uncovered litter stockpiles, were thought to have a higher impact on groundwater quality than the pit itself [8, 9]. In a related study [11], groundwater samples were collected and analyzed for conventional 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 (1,527 mg/L) and chloride (109 mg/L) were detected in the monitoring wells installed within one meter of the burial site. It suggested that complete decay in lightly loaded burial trenches with well-drained soils may take two years or more.

Even more limited is information on leachate 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* [12] reported average leachate concentrations of ammonia-N (3,294 mg/L), alkalinity (9,400 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 m^3 of leachate was generated. A field study to investigate 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[13].Significant amount of ammonium-N (12,600 mg/L), alkalinity (46,000 mg/L as bicarbonate), chloride (2,600 mg/L), sulfate (3,600 mg/L), potassium (2,300 mg/L), sodium (1,800 mg/L), phosphorus (1,500 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 by animal disposal pits since most of the on-farm mortality pits in the U.S. are unlined. However, no previous studies examined the characteristics of naturally produced leachate from animal burial pits. In the United States, animals are routinely administered steroid hormones and antibiotics 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.

To better understand the quality of naturally-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 conventional parameters as well as pharmaceuticals and steroid hormones.

Materials and Methods

Carcass burial pits were constructed at the University of Nebraska-Lincoln Agricultural Research and Development Center near Mead, Nebraska. Dimensions of the burial pits are provided in Figure 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 10.16 cm gravel and 15.24 cm structural sand (Figure S1). Approximately 1400 kg of beef cattle carcasses were placed in each of 3 replicate pits and the pits were backfilled with native soil and compacted. The top of each pit was graded at a 20:1 slope to minimize ponding on the pit surface.

All carcasses were obtained from an operating commercial beef cattle feedlot. Carcasses used in this project were routine mortalities and were younger than 30 months of age. Carcasses were placed in the pits one or two days after death.

Site monitoring and sampling. Local temperature and precipitation data were obtained from a weather station operated by the High Plains Regional Climate Center (Figure S1& S2). Leachate was sampled by submerging a double stage 12V DC purge pump (Geotech Environmental Equipment Inc., Denver, CO) into the leachate reservoir. At each sampling event, all leachate was pumped out of the reservoir. Leachate sampling was conducted biweekly for the first two months and monthly for the following 18 months, for a total of 20 months of sampling. 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.

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 hours 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).

Veterinary pharmaceuticals 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. Steroid hormones were analyzed using either on-line or off-line SPE followed by atmospheric pressure photoionization (APPI) LC-MS/MS using the Quattro Micro. Details of the on-line SPE LC-MS/MS method for steroid hormones are provided elsewhere [14]. A list of 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, 0.45µm 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 µL 2.4 M citric acid. Calibration standards were prepared by fortifying 2.4 M citric acid with analytes (10 to 1,000 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 (1x2 mm) HLB solid-phase extraction cartridge and then eluted with mobile phase for subsequent separation and detection.

Off-line extraction using 200 mg HLB cartridges (Waters Oasis, Milford, MA) was followed for high matrix samples (50 milliliters) collected during the later part of the project. Samples were spiked with surrogate and passed through glass fiber filters (25 mm Whatman GF/F, 0.7 um) and SPE cartridges under vacuum. The cartridges were then washed with 10 milliliters of 10% methanol in water, followed by elution with 0.1% formic acid in methanol. Off-line extraction of water samples for steroid hormones followed a similar protocol as the veterinary pharmaceuticals with elution by 10 milliliter of methanol, followed by evaporation and cleanup. 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 µm Particle Size). Internal standards were added after elution with 3 x 3 milliliters of 50:50 dichloromethane:methanol. Purified extracts were evaporated to dryness and redissolved in 400 µL methanol:water followed by analyses using APPI LC-MS/MS as described for extracts from solids[14].

Veterinary pharmaceuticals were detected using electrospray ionization and selected reaction monitoring (Table S2). A Thermo HyPurity C18 5 µm 2x250mm 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/hr in the desolvator and 30 L/hr in the cone.

Results and Discussion

During the 20 month sampling period, a total of 1.4 m (55 inches) of precipitation (including the equivalent snow melt) was observed (Table S3 & Figure S2).The maximum and minimum air temperatures were found to be 38°C and -24°C, respectively. At a

depth of around 10 cm (4 inches), maximum and minimum soil temperatures were 32°C and -1°C (Figure 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).

Significant leachate production was not observed until after 370 days of decomposition (Figure S4). Prior to this time, less than 300 L of leachate was collected from each pit. 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 2,230 L of leachate. The total volume of leachate produced in each of the three pits was 3,843 L, 7,763 L, and 7,759 L, respectively (Table S3).The most likely explanation for the observed variability may be due to an increased compactive effort that was applied to the soil in pit 1. Another possible explanation may be due to the site topography. Pit 1 was at the highest elevation with pit 2 and 3 located down slope, which may have resulted in increased runoff over the surface of pits 2 and 3.

The measured pH of leachate was initially acidic (Figure 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[15]. 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[15-17]. The dissolved oxygen concentration of the leachate decreased from 7.5 mg/L initially to less than 1 mg/L within the first two weeks of decomposition(Figure 1, panel B). DO levels in the leachate remained less than 3 mg/L for the duration of the study.

High levels of conventional water quality parameters were detected in leachate (Figure 1, panels C through I). The maximum electrical conductivity observed in the leachate was 63,760 µS/cm, 43,960 µS/cm, and 16,460 µS/cm in pits 1, 2 and 3, respectively (Figure 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 (Figure 1, panel D). The maximum observed value of chloride was 2,614 mg/L (Table1 & Figure 1D). Most other conventional 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 be95,333 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 & Figure 1). The majority

of solids measured in the leachate were volatile solids (Figure S5). The occurrence of most conventional contaminants in leachate was detected between 50 and 400 days after the initiation of experiments (Figure 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 to 200 days. The only conventional parameter not exhibiting elevated concentrations was total phosphorus (Figure 1H). The maximum concentration of TP observed in the leachate was 3 mg P/L.

The maximum contaminant concentrations measured in this study were consistent with the results reported by Pratt [13], with the exception of total phosphorus. The maximum chloride concentration reported here (2,614 mg/L) is comparable to that reported in the earlier study (2,600 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 approximate five times larger than the average concentration of ammonium-N (3,294 mg/L) [12] reported in the natural leachate from foot and mouth (FMD) mass burial site in the United Kingdom. We also observed higher peak concentrations of COD in this study (95,333 mg/L) compared with a COD concentration of 20,414 mg/L observed in the natural leachate from FMD disposal sites[12]. Most of the contaminant concentrations reported in the current study compare favorably with a field study investigating freshly produced leachate from a municipal solid waste landfill in Greece in which daily waste deposits contained nearly 50% food wastes [18].

The concentration of total phosphorus detected in this study was substantially different of that detected in other studies. Concentrations of total phosphorus detected in previous study of carcass decomposition remained at approximate 1,200 mg/L [13] 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 [18]. 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 [18], which is close to the level of TP detected in this study. In the present study, the carcasses were surrounded by soil and soil adsorption may be one reason for the low concentrations of phosphorus detected in leachate in the current study.

The presence of both steroid hormones and veterinary pharmaceuticals originating in cattle carcasses was evaluated in this study. In the cattle industry, veterinary antibiotics and steroid hormone implants are typically used for disease prevention and growth promotion. Of the 20 steroid hormones and 17 veterinary pharmaceuticals (Table S1) evaluated, 17β-estradiol, estrone, testosterone, and 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 described 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 testosterone. Estrone was detected starting at approximately day 100 at concentrations of 2,706 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); 3,890 ng/L (pit 2); and 191 ng/L (pit 3).

After the initial observation of 17β-estradiol, the observed began increase with peak concentrations occurring at ~140 days. The maximum observed concentration of 17β-estradiol in the replicate disposal pits was 20,069 ng/L, 3,009 ng/L, and 1,740 ng/L. No consistent trends in estrone, testosterone or monensin occurrence was observed. These compounds were primary detected in leachate between 100 and 320 days except testosterone which was detected from day 50 to 105. The highest level of monensin, estrone, and testosterone were determined to be 11,980 ng/L, 2,706 ng/L, and 235 ng/L, which were all reported in samples collected from pit 1. The concentration of total steroids and total veterinary pharmaceuticals observed in the leachate is presented in Figure 2. Peak values of total steroid hormones and veterinary pharmaceuticals were determined to be 21,255 ng/L and 11,980 ng/L with the highest concentrations of individual compounds being 17β-estradiol and monensin, which is widely used in ruminant animal feed [19].

The levels of these compounds detected in the leachate in this study are much higher than those observed in the effluent of municipal and industrial sewage treatment plants which was considered to be the major source of endocrine disrupting compounds to the aquatic environment, up to 3 orders of magnitude [20-22]. 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 [23]. The occurrence of 17β-estradiol in the leachate with limited detections of 17α-estradiol seem contradictory to previous findings that indicate that beef cattle typically excrete larger amount of 17α-estradiol [23].

By accumulating the mass of contaminants produced in each sampling period, estimated total mass loading for each component was summarized in Table 3. For conventional contaminants, the highest estimated mass loading was COD with an average value of 31.31 g per kg of buried cattle carcass material. Average mass loading of total steroid hormones and pharmaceuticals were determined to be 1.84 and 1.01 μg per kg of cattle carcass, respectively. Concentrations of these components should not vary a lot between animal species (swine, poultry and bovine) based on the available information [13] therefore these number are also valuable for the estimation of contaminants mass loading in disposal pits with swine and poultry. The amount of contaminants loading into the environment could be scaled much larger when unlined animal disposal pits, which is always the case, were enlarged with continuous burial of carcasses as a routine disposal option. More seriously, during the outbreak of foot and mouth disease the number of carcasses could be tremendous. In the 1967 and 2001 outbreak of FMD in Great Britain, a total of 433,987 (211,825 cattle) and 1,281,278 (306,053) heads of animals were disposed of [24]. It would lead to the loading of thousands of tons of contaminants including hundreds of kilograms of hormones and antibiotics to the environment when the worst case happened.

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 residues was found after 31 months of decomposition. Samples of the remaining carcass material was evaluated for composition analysis with methods described previously [17]. A great portion of fat was left in the residues (Table 4) which was consistent with previous finding [17] but with a higher percentage of fat in the dry mass residue, 92.8% versus 62.5%.

The high concentrations of steroid hormones, veterinary antibiotics as well as other conventional contaminants detected in the leachate from cattle carcass disposal is of concern, especially as many on-farm animal carcass disposal sites are not lined. To date, a very small number of studies have investigated the quality of leachate or groundwater associated with animal carcass burial. The potential for adverse water quality impact from on-site carcass disposal practices should be considered.

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Table 1. Conventional water quality parameters in leachate

Parameter	Pit 1			Pit 2			Pit 3		
	Maximum	Minimum	Peak Day[1]	Maximum	Minimum	Peak Day[1]	Maximum	Minimum	Peak Day[1]
EC (µs/cm)	63760	558	245	43960	645	137	16460	793	135
Cl^- (mg/L)	2614	6	140	2214	7	137	2274	1	370
COD(mg/L)	95333	585	245	56083	312	137	24717	268	135
TOC (mg/L)	27158	104	245	20788	56	137	13985	16	99
TKN (mg/L)	14640	2	209	3440	3	101	1363	2	135
TP (mg P/L)	0.84	ND	171	3.06	0.03	71	0.90	ND	406
TS (mg/L)	29060	1467	319	24150	720	168	16705	615	135
TVS (mg/L)	19400	733	319	14395	280	168	9695	190	135
TSS (mg/L)	1240	87	74	1500	144	71	630	88	99

[1]Days of decomposition at which maximum value was detected

Table 2. Steroid Hormones and Pharmaceuticals detected most frequently in leachate

Pit	Compound	Concentration (ng/L)													
1	Day1	**46**	**59**	**74**	**104**	**140**	**171**	**209**	**245**	**284**	**319**	**375**	**411**	**480**	**574**
	17β-Estradiol	**160**	ND[2]	ND	**1501**	**20069**	ND	**11617**	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	**11980**	**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[3]	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

[1]Days of decomposition at which listed compounds were detected.

[2]ND: not detected. Detection limit of steroids and pharmaceuticals were 2 ng/L and 20 ng/L, respectively.

[3]NA: not available. No samples were collected during the sampling date.

Table 3. Estimated total mass loading of contaminants

Pit	Cl^- (mg/kg)	COD (g/kg)	TOC (g/kg)	TKN (g/kg)	TP (mg/kg)	TS (g/kg)	TSS (g/kg)	TVS (g/kg)	TSH[1] (µg/kg)	TVP[2] (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

[1]TSH: total steroid hormones

[2]TVP: total veterinary pharmaceuticals

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

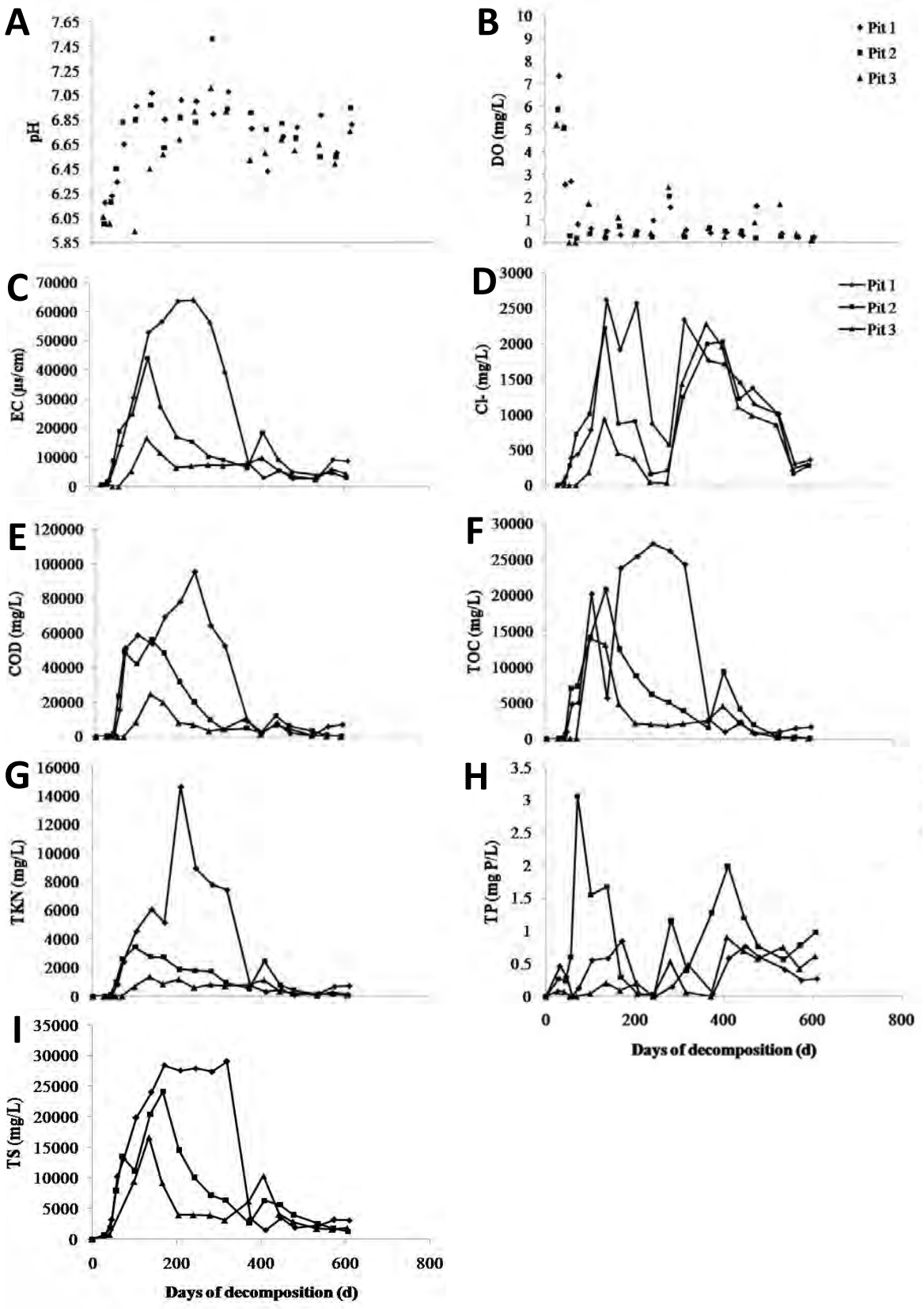


Figure 1. Trends of conventional contaminants in the cattle carcass leachate during land burial decomposition. (A) pH; (B) Dissolved oxygen; (C) Electrical conductivity; (D) Chlorides; (E) Chemical oxygen demand; (F) Total organic carbon; (G) Total Kjeldahl nitrogen; (H) Total phosphorus; (I) Total solids.

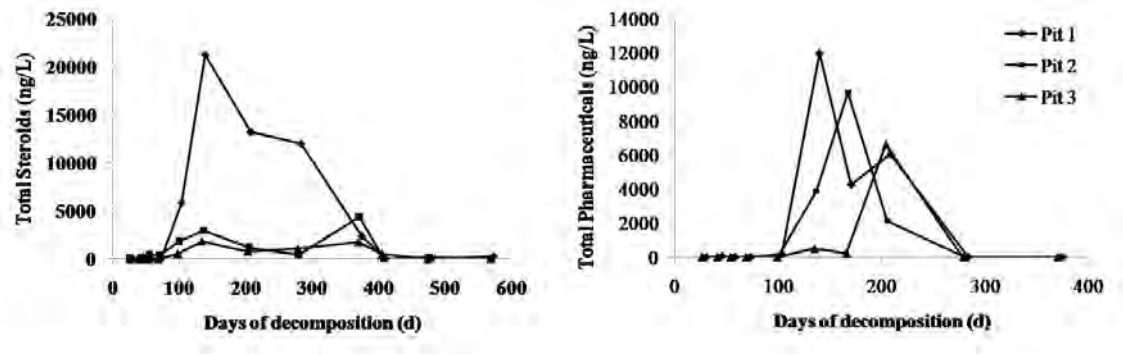


Figure 2. Total steroid hormones and veterinary pharmaceuticals detected in cattle carcass leachate during land burial

Table S1. Steroid hormones and veterinary pharmaceuticals analyzed

Steroids Hormones	Veterinary Pharmaceuticals
11-KetoTestosterone	Virginiamycin
17α-Hydroxyprogesterone	Tylosin
4-Androstenedione	Tiamulin
17α-Estradiol	Tetracycline
Androstanedienedione	Sulfathiazole
Androsterone	Sulfamethoxazole
17α-trenbolone	Sulfamethazole
α-Zearalanol	Sulfamethazine
α-Zearalenol	Sulfamerazine
17β-estradiol	Sulfadimethoxine
17β-trenbolone	Sulfachloropyridazine
β-Zearalanol	Ractopamine
β-Zearalenol	Oxytetracycline
Epitestosterone	Monensin
Estriol	Lincomycin
Estrone	Erythromycin
Ethynyl Estradiol	Chlortetracycline(Total)
Melengesterol Acetate	
Progesterone	
Testosterone	

Table S2. Veterinary pharmaceuticals measured with selected reaction monitoring transitions, cone voltages, collision energies, and expected retention times

Compound	CAS number	Formula	MW (g mol^{-1})	Parent Ion (m/z)	Product Ion (m/z)	Cone Voltage (V)	Collision Energy (eV)	Retention time (min)
Chlortetracycline	57-62-5	$C_{22}H_{23}ClN_2O_8$	478.88	478.9	444	28	20	12.84
Erythromycin	114-07-8	$C_{37}H_{67}NO_{13}$	733.93	734	158	30	30	14.78
Lincomycin	154-21-2	$C_{18}H_{34}N_2O_6S$	406.538	407	126	38	25	10.85
Monensin	17090-79-8	$C_{36}H_{62}O_{11}$	670.871	688.1	635.15	22	17	22.04
(Sodium adduct)				693.1	675.1	50	38	22.04
Oxytetracycline	79-57-2	$C_{22}H_{24}N_2O_9$	460.434	460.9	425.9	25	20	11.66
Ractopamine	90274-24-1	$C_{18}H_{23}NO_3$	301.38	302.2	164.15	18	16	11.07
Sulfachloropyridazine	80-32-0	$C_{10}H_9ClN_4O_2S$	284.72	285	155.95	24	15	12.41
Sulfadimethoxine	122-11-2	$C_{12}H_{14}N_4O_4S$	310.33	311.05	155.95	28	20	13.81
Sulfamerazine	127-79-7	$C_{11}H_{12}N_4O_2S$	264.305	265.1	155.95	28	16	11.33
Sulfamethazine	57-68-1	$C_{12}H_{14}N_4O_2S$	278.33	279.1	155.95	30	18	11.93
Sulfamethizole	144-82-1	$C_9H_{10}N_4O_2S_2$	270.333	271.05	155.95	24	13	10.85
Sulfamethoxazole	723-46-6	$C_{10}H_{11}N_3O_3S$	253.279	254.1	155.95	23	15	12.41
Sulfathiazole	72-14-0	$C_9H_9N_3O_2S_2$	255.319	256.05	155.95	25	14	10.85
Tetracycline	60-54-8	$C_{22}H_{24}N_2O_8$	444.435	444.9	410.05	23	19	11.50
Tiamulin	55297-95-5	$C_{28}H_{47}NO_4S$	493.742	493.9	191.9	32	24	14.40
Tylosin	1401-69-0	$C_{46}H_{77}NO_{17}$	916.10	916.9	174.2	50	35	14.78
Virginiamycin M1	11006-76-1	$C_{28}H_{35}N_3O_7$	525.6	526	355.1	24	18	17.04

Table S3. Precipitation and leachate volume observed during the sampling period

Pit	Days of Decomposition (d)[1]	Total Leachate Volume (L)	Precipitation (inch)[2]	Precipitation (L)[2]	$V_{Leachate}/V_{Precipitation}$ (%)
1	609	3843	56	175813	2.19
2	606	7763	54	169490	4.58
3	604	7759	54	169303	4.58

[1]Cattle carcasses were buried into 3 pits at different times due to the availability of dead animals.

[2]Precipitation refers to the volume of rainfall that is able to infiltrate into the pits theoretically.

Table S4. Additional hormones and veterinary pharmaceuticals detected in leachate

Pit	Compound	Concentration (ng/L)								
1	**Day**[1]	**46**	**104**	**171**	**209**	**245**	**319**	**375**	**411**	
	17α-Hydroxyprogesterone			229		245				
	4-Androstenedione			33		24	12		3	
	17α-Estradiol		1410							
	17β-trenbolone							228		
	Melengesterol Acetate		5							
	Progesterone		18							
	Tylosin				439					
	Tetracycline	49								
	Sulfamethoxazole		26							
	Sulfachloropyridazine				117					
	Ractopamine		138							
2	**Day**	**56**	**101**	**168**	**206**	**242**	**316**	**372**	**408**	**477**
	17α-Hydroxyprogesterone					22				
	4-Androstenedione	73		11			3		5	
	17α-Estradiol		254							
	17β-trenbolone							3722		
	α-Zearalanol									48
	β-Zearalanol		40							
	Tylosin				62					
	Tetracycline				132					
	Sulfamethazine			394						
	Sulfachloropyridazine		92		115					
	Oxytetracycline				1120					
3	**Day**	**135**	**166**	**204**						
	17α-Estradiol		33							
	α-Zearalanol		74							
	Virginiamycin	212								
	Tiamulin	180								
	Tetracycline			3640						
	Sulfamethazine	99	51	31						
	Sulfachloropyridazine			35						
	Oxytetracycline			2690						

[1]Days of decomposition at which listed compounds were detected.

Blank cells indicated undetectable values. Detection limits for steroids and pharmaceuticals were 2 ng/L and 20 ng/L, respectively.

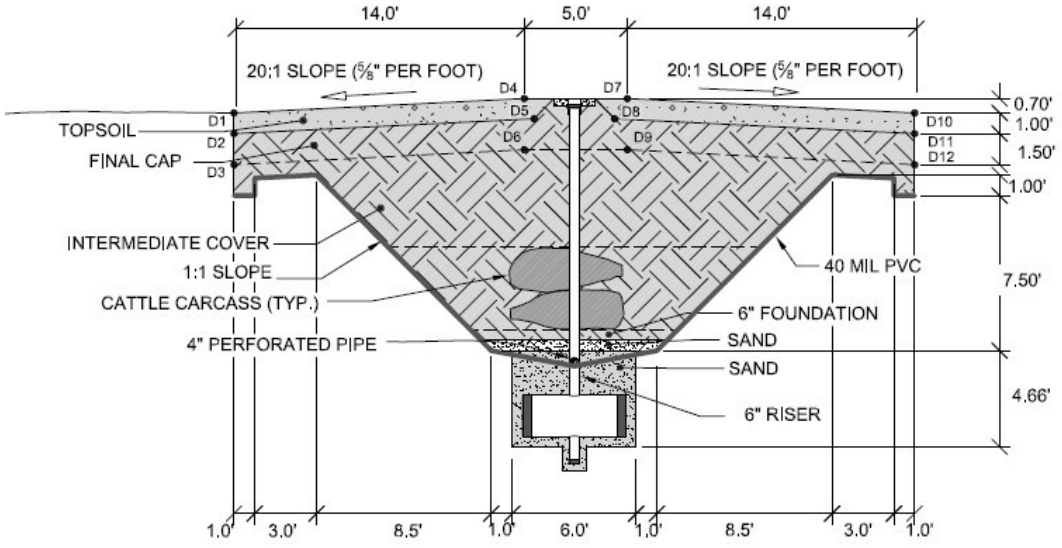


Figure S1. Cross-section of the carcass burial pit

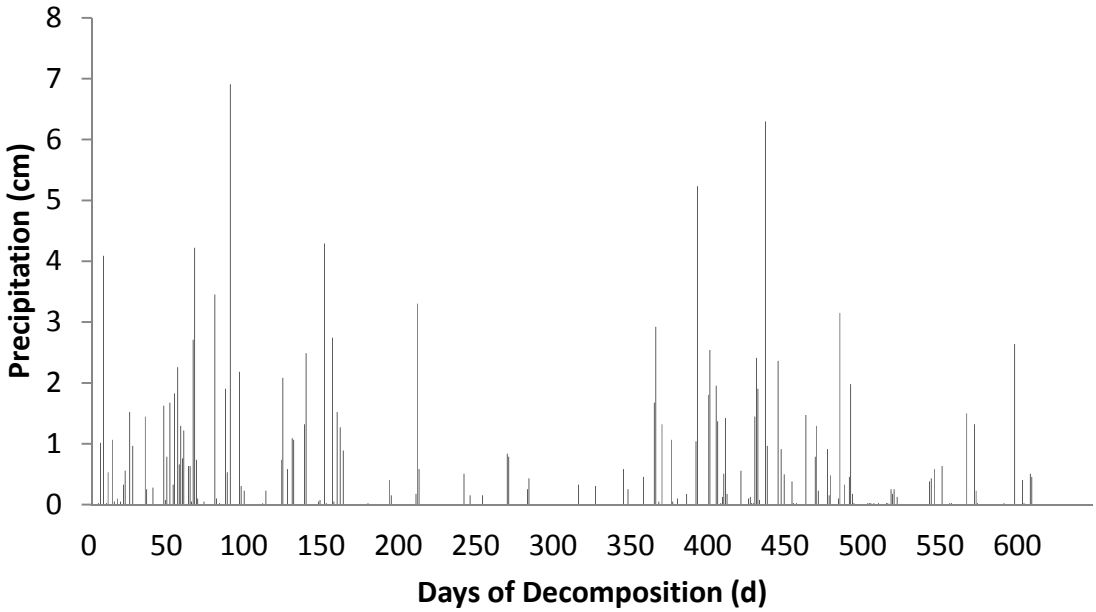


Figure S2. Precipitation during sampling period

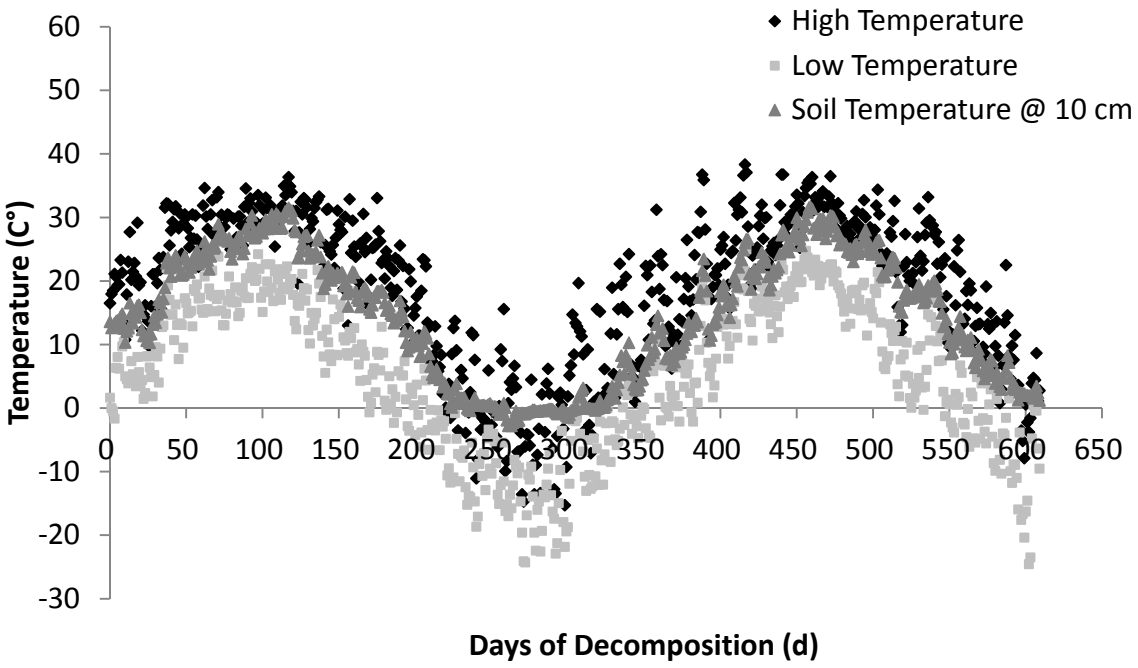


Figure S3. Ambient and soil (at depth of 10 cm) temperature profile

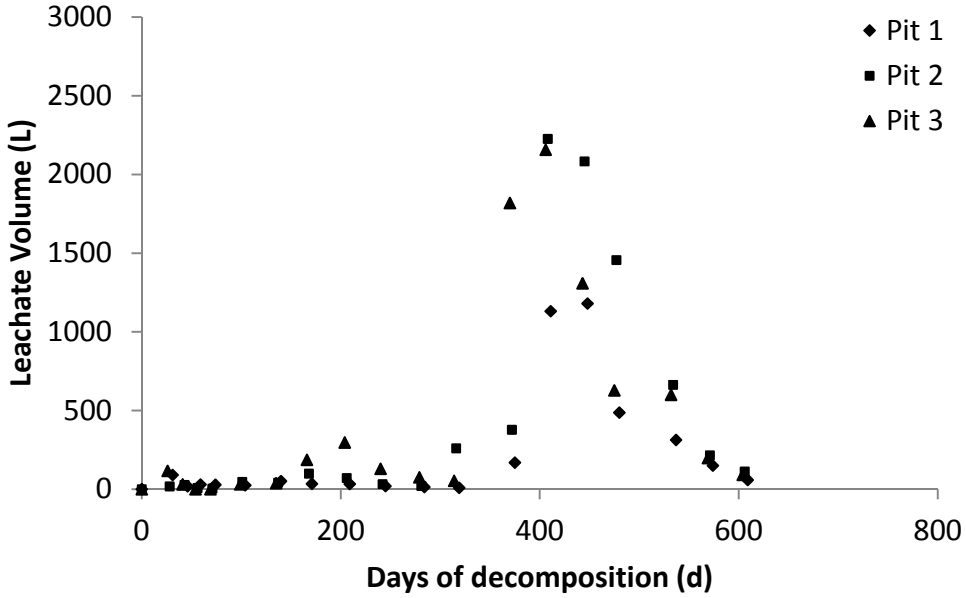


Figure S4. Measured leachate volumes

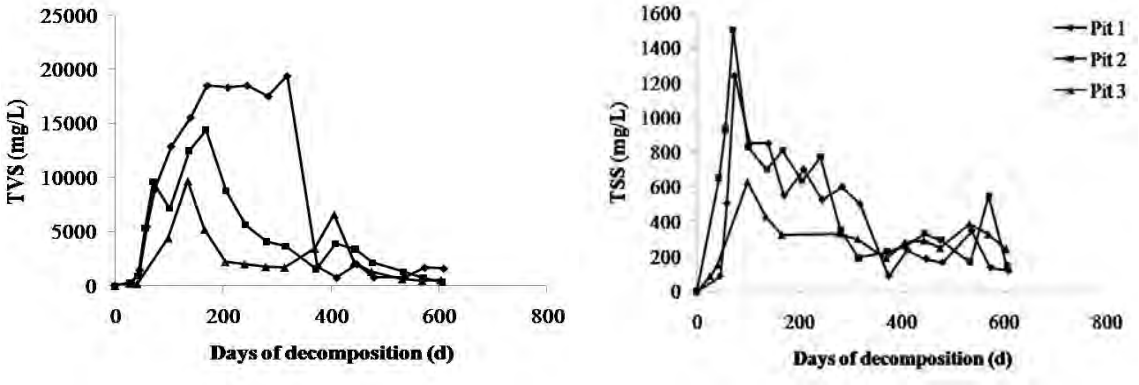
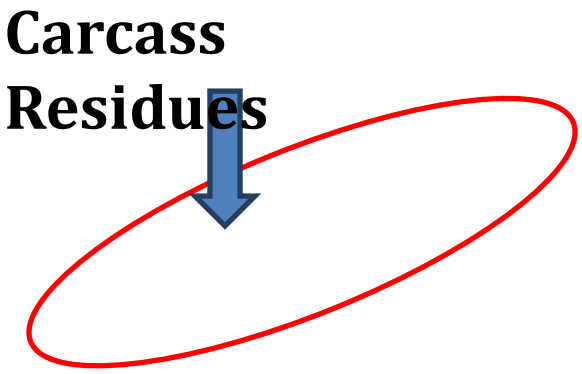


Figure S5. Trends of total volatile solids (TVS) and total suspended solids (TSS) in the cattle carcass leachate during land burial decomposition.

Picture S1

Picture S2 A



Picture S2 B

Picture S3 A


Picture S3 B