



Management of antibiotic residues from agricultural sources: Use of composting to reduce chlortetracycline residues in beef manure from treated animals

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ABSTRACT

Chlortetracycline (CTC) is one of only ten antibiotics licensed in the U.S.A. for use as growth promoters for livestock. The widespread use and persistence of CTC may contribute in development of antibiotic-resistant bacteria. The objective of this study was to determine the effect of composting on the fate of CTC residues found in manure from medicated animals. The effect of CTC residues on composting was also investigated. Five beef calves were medicated for 5 days with 22 mg/kg/day of CTC. Manure samples collected from calves prior to and after medication were mixed with straw and woodchips, and aliquots of the subsequent mixtures were treated in laboratory composters for 30 days. In addition, aliquots of the CTC-containing mixture were incubated at 25 °C or sterilized followed by incubation at 25 °C and 55 °C (composting temperature). The presence of CTC did not appear to affect the composting process. Concentrations of CTC/ECTC (the summed concentrations of CTC and its epimer ECTC) in the composted mixture (CM) and sterilized mixture incubated at 55 °C (SM55) decreased 99% and 98% (from 113 µg/g dry weight (DW) to 0.7 µg/g DW and 2.0 µg/g DW), respectively, in 30 days. In contrast, levels of CTC/ECTC in room temperature incubated (RTIM) and sterilized mixture incubated at 25 °C (SM25) decreased 49% and 40% (to 58 µg/g DW and 68 µg/g DW), respectively, after 30 days. Concentrations of the CTC metabolite, *iso*-chlortetracycline (ICTC), in CM and SM55 decreased more than 99% (from 12 µg/g DW to below quantitation limit of 0.3 µg/g DW) in 30 days. ICTC levels in RTIM and SM25 decreased 80% (to 4 µg/g DW) in 30 days. These results confirm and extend those from previous studies that show the increased loss of extractable CTC residues with increased time and incubation temperature. In addition, our results using sterile and non-sterile samples suggest that the decrease in concentrations of extractable CTC/ECTC at 25 °C and 55 °C (composting temperature) is due to abiotic processes.

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1. Introduction

The use of antibiotics has become integral to the livestock production industry as growth promoters and therapeutic agents. The use, fate and effects of veterinary antibiotics have been reviewed by Sarmah et al. [1]. Both the total and relative amounts of antibiotics used for human and agricultural purposes are not well documented and estimates are controversial [2,3]. According to Animal Health Institute (AHI), antibiotics used in animal feeding in the United States (U.S.) have increased from nearly 91 ton in

1950 to 9900 ton (including 3000 ton of tetracyclines) in 2004 [3] and 60–80% were used for non-therapeutic purposes [2]. In the European Union, 5000 ton of antibiotics (70% for non-therapeutic purposes) were used in 1999 for veterinary therapy [1]. Studies have shown that between 17–76% of antibiotics administered to animals are excreted via urine and feces in unaltered form and as metabolites [4,5]. Land application of manure as a supplement to fertilizer is a common practice in many countries. It is therefore likely that when animal manure is applied to agricultural fields, antibiotic residues can find their way into the receiving environment [6].

Chlortetracycline (CTC) is an antibiotic that is widely used in livestock production because it is active against a broad range of gram-positive and gram-negative bacteria [7]. 4-*epi*-Chlortetracycline (ECTC) is an epimer of CTC and *iso*-chlortetracycline (ICTC) is an isomer of CTC (Fig. 1). Elmund et al.

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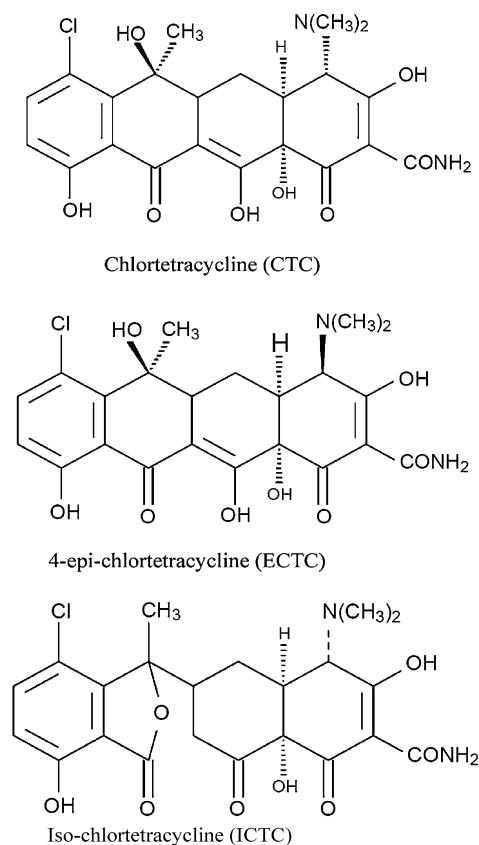


Fig. 1. Chemical structures of CTC, its epimer (ECTC) and isomer (ICTC).

showed that approximately 75% of ingested CTC was recovered in cow manure [8].

There is a growing concern about the potential impact of antibiotic residues on the environment because persistent antibiotic residues may lead to development of antibiotic-resistant bacteria [9]. Hamscher et al. detected CTC in cattle manure slurry and pig manure slurry at concentrations of 11 $\mu\text{g}/\text{kg}$ and 1435 $\mu\text{g}/\text{kg}$, respectively [10]. CTC has been shown to be quite persistent in soil systems by the same researchers. They reported average concentrations of 9.5 $\mu\text{g}/\text{kg}$ CTC in the upper 10 cm of the soil that had been manured with animal slurry 2 days before sampling. The concentrations of CTC, ECTC and ICTC in pig faeces were 281,000 $\mu\text{g}/\text{kg}$, 157,000 $\mu\text{g}/\text{kg}$, and 67,000 $\mu\text{g}/\text{kg}$, respectively after 10 days of CTC medication [11]. CTC has been detected in water samples at the maximum concentrations of 0.03 $\mu\text{g}/\text{L}$ [12], 0.16 $\mu\text{g}/\text{L}$ [13], and 0.69 $\mu\text{g}/\text{L}$ [14] and in sediments at concentrations between 2 $\mu\text{g}/\text{kg}$ dry weight (DW) and 10 $\mu\text{g}/\text{kg}$ DW [12].

Treatment of manure containing CTC may assist in reducing the amount of this compound that is ultimately released into the environment. Manure can undergo further treatment with anaerobic digestion and/or composting processes before land application. Manure composting is a well-described approach for stabilization of nutrients and reduction of pathogens and odors [15]. In addition, composting has been shown to be effective in reducing relatively persistent organic compounds such as pharmaceuticals and personal care products (PPCPs) [16,17], chlorophenol [18] 2,4,6-trinitrotoluene [19,20], diazinon [21], pyrene and simazine [22], 17 β -estradiol and testosterone [23]. Our recent study has also shown that composting is also an effective treatment for reducing oxytetracycline (OTC) levels in manure [24]. The objective of this study was to determine the effect of composting on CTC residues

in manure from medicated calves. In addition, the effect of CTC residues on composting process was also investigated.

2. Methods

2.1. Animal medication and sample collection

Five male and female beef calves, 5–7 months old and ranging from 190 kg to 350 kg in body mass, were kept in individual pens at the Beltsville Agricultural Research Center, Beltsville, Maryland, USA. Pens were scraped clean daily, after which approximately 2 kg of sawdust was scattered on the floor of each pen as bedding material. After a two-week acclimatization period for the animals, the manure–sawdust mixture from each pen was collected, pooled, mixed, and the mixture was stored at 4 °C until later use as the “unmedicated” manure. The calves were then medicated for 5 days at 22 mg/kg body mass per day of CTC (a standard dosage in agricultural practice; [25]) by ingestion of the daily ration containing CTC as a feed additive. Feed consisted of a mixture of unmedicated/medicated beef creep pellet (31%), corn silage (43%) and grass silage (26%). Medicated grain was given to the animals prior to other constituents in order to insure complete consumption of the CTC dose. Medicated manure–bedding mixtures collected on the fifth day of medication (when CTC levels were expected to be the highest) were combined and used in laboratory composting experiments.

2.2. Composting experiments

Composting studies were conducted in four self-heating laboratory composters for 30 days [26]. Each composter is comprised of a covered, double walled, insulated tank; an air-tight cylinder; an inner screen mesh cylinder (8.4 L capacity) that contains the material to be composted; a heater, and a differential temperature control system (Fig. 2). Continuous compressed air was supplied and regulated by a rotameter. The maximum temperature in the cylinders and the temperature differential between the cylinders and the insulated tank were set at 55 °C and 2–3 °C, respectively. A portion of the pooled manure–bedding mixture collected from calves on the fifth day of medication was mixed with straw (chopped into less than 1 cm) and hardwood woodchips (sieved through 1 cm mesh size) in a ratio of 3:1:1 (v/v; woodchips:straw:manure) and 3.5 kg aliquots of the resulting mixture were loaded into each of two cylinders and placed into duplicate composters of 1 and 2 (medicated composters). An identical mixture of unmedicated manure–bedding (collected from animals prior to CTC medication), straw, and woodchips was loaded into other two cylinders and placed into remaining separate duplicate composters of 3 and 4 (unmedicated composters). The characteristics of medicated and unmedicated manure–straw–woodchip mixtures are shown in Table 1. The sampling dates for each composter were: day 0, 2, 7, 14, 21 and 30. At each time point, the composting mixture to be sampled was unloaded from the composter cylinder into a bucket, mixed prior to removal of 100 g samples, and the remaining material was reloaded into the cylinder and respective composter.

Composter temperatures were recorded every 15 min using a Omega OM-220 datalogger (Omega Engineering, Inc., Stamford, CT). Carbon dioxide evolved from each composter was trapped using 6N NaOH trap solutions that were changed daily. Carbon dioxide levels were determined by titration using 0.2 M H_2SO_4 and phenolphthalein as an indicator after precipitation of carbonate with BaCl_2 [27]. For moisture determination, compost samples were dried for 24 h at 105 °C. The total C and N contents of samples

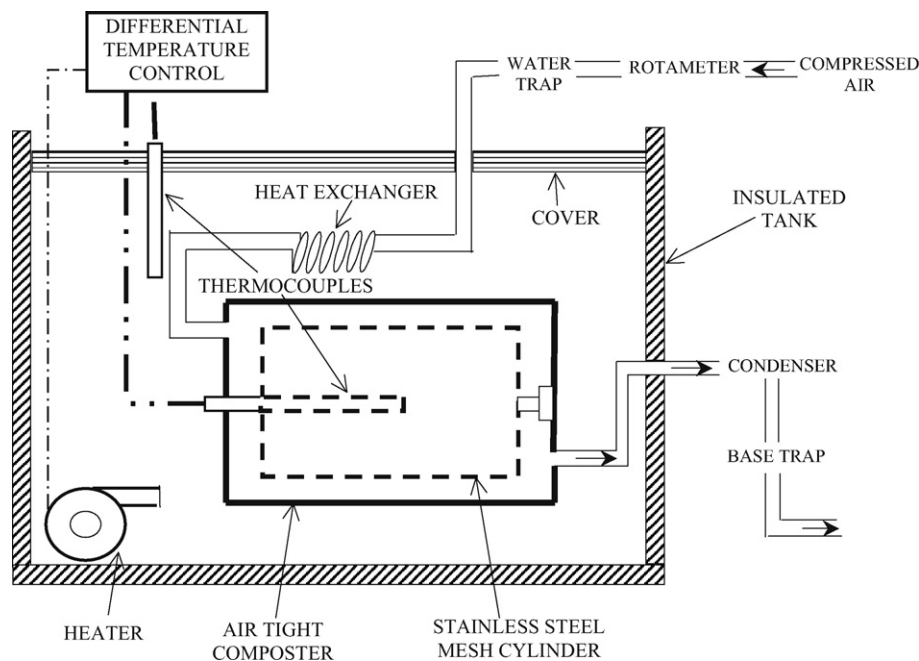


Fig. 2. Schematic illustration of the composting system.

were determined using the catalytic tube combustion method and a Vario Max CNS Macro Elemental Analyzer with a TCD detector (Elementar Americas, Inc., Mt. Laurel, NJ). Total phosphorus content was determined by block digestion and flow injection analysis (Lachat Instruments, Milwaukee, WI). pH, electrical conductivity and volatile solids were analyzed according to protocols listed in test methods for the examination of composting and compost [28].

The statistical significance of CTC's effect on cumulative CO₂ evolution during composting was determined with the repeated measures ANOVA procedure using SAS Proc Mixed [29]. Significant differences were determined at the $p < 0.05$ level of significance.

Removal of compounds was assumed to follow first-order kinetics. A rate constant, k , was determined as the slope of the curve calculated by linear regression. The half-life, $t_{1/2}$, was then calculated as $t_{1/2} = \ln(2)/k$.

2.3. Room temperature incubation of CTC-containing mixture and incubation of sterilized CTC-containing mixture at 25 °C and 55 °C

Duplicate 50 g aliquots of the CTC-containing manure–straw–woodchip mixture (described above) were placed in separate 250 ml flasks, and incubated at room temperature (25 ± 2 °C) for 30 days (referred to hereafter as the RTIM). In order to determine the effect of abiotic processes on CTC levels, a 250 g aliquot of

the CTC-containing manure–straw–woodchip mixture was placed in a 500 ml flask, sealed with a rubber stopper and sterilized by irradiation (3 Mrads for 395 min from a Co⁶⁰ source at the University of Maryland Radiation Facilities, College Park, MD). The effect of sterilization was checked by bacterial plate counts and no microorganisms were found. Duplicate 50 g aliquots of the sterilized mixture were placed in separate sterile 250 ml flasks and incubated at 25 °C (referred to hereafter as SM25). Additional duplicate 50 g aliquots of the sterilized mixture were placed in two different sterile 250 ml flasks. These flasks were placed in the insulated tank of Composter 2 (medicated composter) so that they were incubated at the composting temperature of 55 °C (hereafter referred to as SM55). The sampling dates for RTIM and SM25 were: day 0, 7, 14, 21 and 30. An additional sample on day 2 was taken from the SM55. At each time point, 2 g samples (1 g for CTC, ECTC and ICTC analyses and 1 g for moisture content determination) were collected from the flasks.

2.4. Analysis of CTC, ECTC and ICTC

Compost subsamples were extracted in duplicate for CTC, ECTC and ICTC analyses using the method described by Capone et al. [30]. Briefly, 1 g subsamples were extracted three times with 3 ml of 0.1 M Na₂EDTA–Mcllvaine buffer by vortexing for

Table 1

Characteristics of medicated and unmedicated manure–straw–woodchip mixtures prior to treatment (mean \pm standard error)

Constituent	Medicated manure–straw–woodchip mixture ^a	Unmedicated manure–straw–woodchip mixture ^b
pH	8.3 \pm 0.1	8.4 \pm 0.2
Moisture content (%)	68.3 \pm 0.3	68.8 \pm 0.4
Electrical conductivity (mS/cm)	2.9 \pm 0.3	2.6 \pm 0.2
C (% wet basis)	13.9 \pm 0.2	12.8 \pm 0.4
N (% wet basis)	0.6 \pm 0.1	0.5 \pm 0.1
C/N	25.5	24.0
Volatile solids (% of dry matter)	93.3 \pm 0.2	93.1 \pm 0.3
Total phosphorus (% wet basis)	0.20 \pm 0.02	0.16 \pm 0.04

^a Used in medicated composters (composters 1 and 2).

^b Used in unmedicated composters (composters 3 and 4).

30 s followed by sonication for 5 min in a 100 W sonication bath (Bronson Ultrasonics, Danbury, CT). After each extraction, the extracts were subjected to centrifugation ($500 \times g$, 5 min, 5°C), the supernatants pooled, again subjected to centrifugation ($1650 \times g$, 20 min, 5°C), filtered through Whatman glass microfibre (grade GFB) filter paper, and passed through Waters 60 mg HLB (hydrophilic-lipophilic balance) Oasis[®] cartridges (Waters Corp., Milford, MA) after the cartridges had been prewashed with 5 ml methanol and 10 ml 0.1 M Na_2EDTA -McIlvaine buffer. After the extracts were loaded, the cartridges were flushed with 20 ml distilled water, followed by sample elution using 8 ml of 0.01 M methanolic oxalic acid. The eluents were concentrated under a flow of N_2 to a volume of 0.5 mL. Distilled water (0.5 ml) was added to each concentrated eluent, the resulting mixture was vortexed for 30 s, and then transferred to 2 mL amber autosampler vials. Demeclocycline (20 μg) was added to each vial as an internal standard prior to analysis by LC/MS/MS.

2.5. LC-MS/MS analysis

The analyses of CTC, ECTC and ICTC were performed using LC-MS/MS as previously described [31]. The LC instrument was a Waters 2690 XE (Waters Corp., Milford, MA) separations module with an Xterra MS C_{18} column (150 mm \times 2.1 mm i.d., 5 μm) (Waters Corp., Milford, MA) at 45°C ; the flow rate was 0.25 ml/min and injection volume was 10 μl . A mobile-phase gradient was used to separate the compounds. The solvent compositions were: A, 1% formic acid-methanol (70:30, v/v); B, water, and C, methanol. The solvents were mixed as follows: 0–1 min 50% A, 50% B, 0% C; 1–12 min a linear gradient from the previous settings to 70% A, 0% B, 30% C; 12–20 min 42% A, 0% B, 58% C; 20–22 min 0% A, 0% B, 100% C, 22–25 min isocratic at 0% A, 0% B, 100% C, and 25–27 min, a linear gradient back to initial conditions 50% A, 50% B. The column was allowed to stabilize for 10 min at this setting prior to the next analysis. The total run time was 37 min. Atmospheric pressure ionization-tandem mass spectrometry was performed on a bench-top triple quadrupole mass spectrometer (Quattro LC, Micromass Ltd., Manchester, U.K.) operated in electrospray ionization mode. Acquisition was done in the multiple-reaction monitoring mode (MRM) in electrospray negative (ES⁻). Analyte concentrations were calculated by the internal standard method using demeclocycline as an internal standard [32]. Peak integration and quantitation were performed automatically using MassLynx 3.5 software (Waters Corp., Milford, MA). According to European regulations, CTC levels in foodstuffs of animal origins are calculated as the summed levels of the antibiologically active CTC and ECTC. Therefore, results from CTC and ECTC analyses were summed and are reported as CTC/ECTC values.

2.6. Determination of recoveries

To determine extraction efficiencies, triplicate samples of the unmedicated manure-straw-woodchip mixture were spiked with stock solutions to yield samples containing 1 $\mu\text{g/g}$ and 10 $\mu\text{g/g}$ wet weight of CTC, ECTC, ICTC and extracted as described above prior to LC/MS/MS analysis. The moisture content of the spiked samples was 69%. Thus, the concentrations in the spiked 1 $\mu\text{g/g}$ and 10 $\mu\text{g/g}$ wet weight samples expressed on a dry weight basis were 3.2 $\mu\text{g/g}$ DW and 32 $\mu\text{g/g}$ DW, respectively. Average recoveries of 1 $\mu\text{g/g}$ spikes for CTC, ECTC and ICTC were higher than recoveries of 10 $\mu\text{g/g}$ spikes (Table 2). Average recoveries of CTC, ECTC and ICTC were 72%, 71% and 58%, respectively. The antibiotic concentrations were not corrected for recoveries.

Table 2

Recovery of Chlortetracycline (CTC), 4-*epi*-chlortetracycline (ECTC) and iso-chlortetracycline (ICTC) in unmedicated manure-straw-woodchip mixture

Compound	Recovery (% mean \pm standard error) ^x	
	Spike level ($\mu\text{g/g}$ wet weight) ^y	
	1	10
Chlortetracycline (CTC)	73 \pm 2	71 \pm 2
4- <i>epi</i> -Chlortetracycline (ECTC)	74 \pm 5	67 \pm 3
iso-Chlortetracycline (ICTC)	59 \pm 2	57 \pm 3

^x Values are the means from triplicate samples.

^y The corresponding concentrations in the spiked samples expressed on a dry weight basis are 3.2 mg kg^{-1} and 32 mg kg^{-1} DW.

2.7. Bacterial enumeration

Total heterotrophs, total coliforms and resistant microorganisms in the medicated and unmedicated composted mixture during composting were enumerated by standard dilution plating. Levels of total and CTC-resistant heterotrophs were determined using non-selective Luria Bertani (LB) media and LB media containing 30 $\mu\text{g/ml}$ CTC, respectively, after incubation at 30°C for 48 h. Levels of total and CTC-resistant coliforms were determined using McConkey agar and McConkey agar containing 30 $\mu\text{g/ml}$ CTC, respectively, after incubation at 37°C for 48 h. All analyses were conducted using duplicate samples.

3. Results

3.1. Effect of CTC on composting processes

All composters reached 55°C within 2–3 days following start-up (Fig. 3a). Temperatures remained relatively constant during the following two weeks, then declined to 40 – 45°C until the third week of incubation, and decreased to 20 – 25°C by day 30. The initial rapid increase in temperature and high temperatures observed

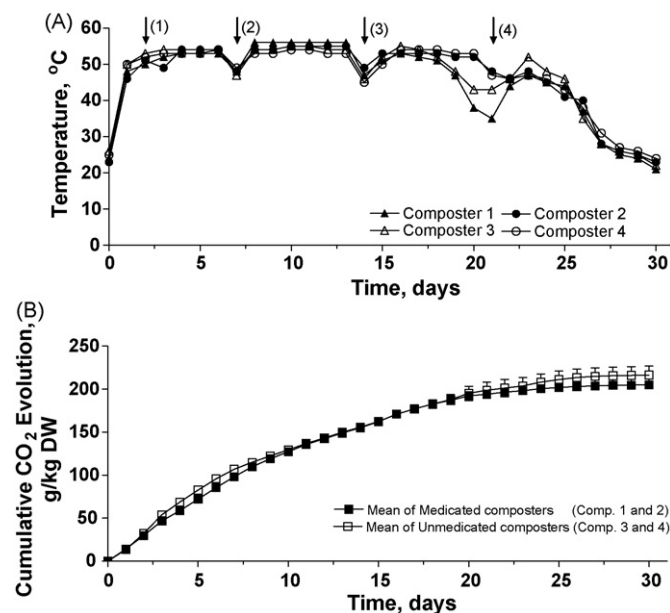


Fig. 3. Comparison of temperature and cumulative CO_2 evolution from composters containing the medicated and the unmedicated manure-straw-woodchip mixtures during composting. Panel A: daily temperature; Panel B: cumulative carbon dioxide evolution. Composters 1 and 2 contain the medicated manure-straw-woodchip mixture. Composters 3 and 4 contain the unmedicated manure-straw-woodchip mixture. Arrows indicate the sampling date.

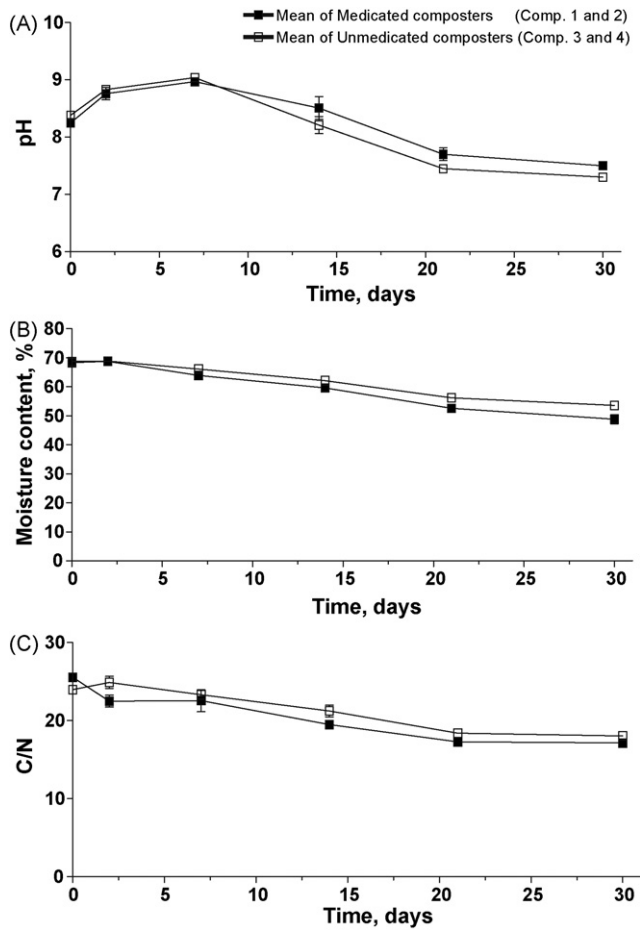


Fig. 4. Changes of pH, moisture content and C/N ratio from composters containing the medicated and the unmedicated manure–straw–woodchip mixtures during composting.

during composting is due to high levels of microbial activity. The mean cumulative carbon dioxide evolution (Fig. 3b) for medicated composters (containing the medicated-manure mixture) and unmedicated composters (containing the unmedicated-manure mixture) were 205 ± 2 g/kg DW and 216 ± 10 g/kg DW, respectively and not significantly different ($p > 0.05$). These values are comparable to those of a previous study in which approximately 150 g/kg DW, 200 g/kg DW and 300 g/kg DW carbon dioxide production were emitted after 30 days thermophilic ($52 \pm 2^\circ\text{C}$) composting of yard waste, grass clippings and food waste, respectively [33]. In addition, there were no significant differences between the medicated and unmedicated composters during composting process with respect to changes in pH, moisture content and C/N ratio (Fig. 4). Overall, our values were typical for composting of manure and similar to those reported for windrow composting of cattle manure composting [34]. These results suggest that levels of CTC in the compost mixtures used in this study did not affect the composting process.

3.2. Effect of incubation time and temperature on concentrations of extractable CTC/ECTC and ICTC

Concentrations of extractable CTC/ECTC (the summed concentrations of CTC and its epimer ECTC) and ICTC in the manure–bedding mixture (collected on the fifth day of medication) were 208 ± 5 $\mu\text{g/g DW}$ and 33 ± 1 $\mu\text{g/g DW}$, respectively. Roughly 65% of the CTC fed to the calves was recovered in the manure as CTC/ECTC and ICTC. This value is comparable with the previous

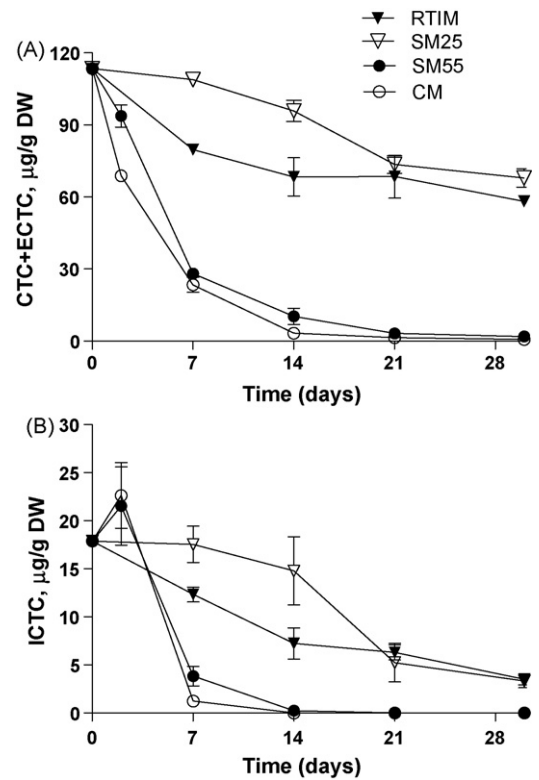


Fig. 5. Extractable CTC/ECTC and ICTC levels as a function of incubation period for the composted mixture (CM), sterilized mixture incubated at composting temperature of 55°C (SM55), room temperature incubated mixture (RTIM), and sterilized mixture incubated at room temperature of 25°C (SM25) (mean + standard error). The initial extractable CTC/ECTC and ICTC concentrations within these mixtures were 113 ± 2 $\mu\text{g/g DW}$ and 18 ± 1 $\mu\text{g/g DW}$, respectively.

study which reported approximately 75% CTC excretion rate for cow manure [8].

The initial concentrations of extractable CTC/ECTC and ICTC in the manure–straw–woodchips mixture to be composted were approximately 50% lower (113 ± 2 $\mu\text{g/g DW}$ and 18 ± 1 $\mu\text{g/g DW}$, respectively) due to the addition of straw and woodchips. The concentrations of extractable CTC/ECTC in the composted mixture (CM) and sterilized mixture incubated at 55°C (SM55) decreased 75–79% (from 113 ± 2 $\mu\text{g/g DW}$ to 23 ± 2 $\mu\text{g/g DW}$ and 28 ± 3 $\mu\text{g/g DW}$, respectively) within 7 days and 98–99% (to 0.7 ± 0.1 $\mu\text{g/g DW}$ and 2.0 ± 0.8 $\mu\text{g/g DW}$, respectively) by the end of the 30-day composting experiment (Fig. 5a). In contrast, levels of CTC/ECTC in room temperature incubated (RTIM) and sterilized mixture (SM25) decreased 40–49% (from 113 $\mu\text{g/g DW}$ to 58 ± 0.1 $\mu\text{g/g DW}$ and 68 ± 4.0 $\mu\text{g/g DW}$, respectively) after 30 days (Fig. 5a). Concentrations of the CTC metabolite, ICTC, in CM and SM55 transiently increased after 2 days then decreased >99% (from 12 $\mu\text{g/g DW}$ to below our quantitation limit of 0.3 $\mu\text{g/g DW}$) after 30 days. ICTC levels in RTIM and SM25 decreased 80% (to 4 $\mu\text{g/g DW}$) after 30 days (Fig. 5b).

3.3. Effect of composting on total heterotrophs, total coliforms and CTC-resistant microorganisms

Total heterotrophic bacterial levels (CFU/g DW) in medicated and unmedicated composters gradually declined from 4.8×10^9 ($\pm 1.8 \times 10^8$) and 2.3×10^9 ($\pm 1.6 \times 10^8$) prior to composting to 1.6×10^7 ($\pm 1.0 \times 10^7$) and 2.1×10^6 ($\pm 5.2 \times 10^5$) at day 30, respectively (Fig. 6). Levels of CTC-resistant bacteria decreased in medicated and unmedicated composters from

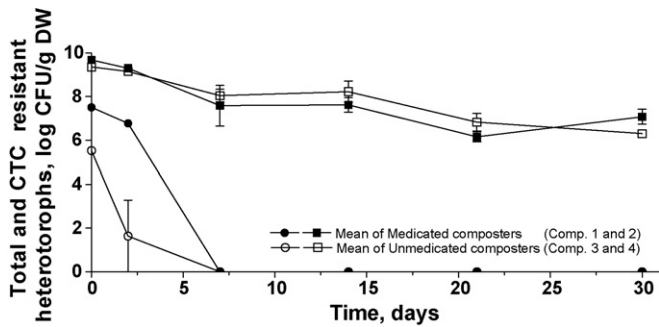


Fig. 6. Total and CTC-resistant heterotrophic bacteria in medicated and unmedicated composters during composting (mean + standard error). Squares and circles show total and CTC-resistant heterotrophs, respectively.

$3.2 \times 10^7 (\pm 8.4 \times 10^2)$ and $3.4 \times 10^5 (\pm 9.9 \times 10^2)$ prior to composting to $7.1 \times 10^6 (\pm 3.8 \times 10^6)$ and $9.4 \times 10^2 (\pm 9.4 \times 10^2)$ within 2 days, respectively and decreased to under detection limit (2×10^2) in 7 days. Levels of total and CTC-resistant coliforms prior to composting were $7.4 \times 10^5 (\pm 5.4 \times 10^2)$ and $2.9 \times 10^5 (\pm 4.3 \times 10^2)$ in medicated composters and $3.6 \times 10^5 (\pm 3.9 \times 10^2)$ and $1.3 \times 10^5 (\pm 2.4 \times 10^2)$ in unmedicated composters, respectively. Both total and CTC-resistant coliforms decreased to under detection limit (2×10^2) within 2 days. Our values compare very well with results from a cattle manure composting study that demonstrated a 10^2 decrease of total heterotrophs and $>10^6$ reduction of total coliforms [34].

4. Discussion

In this study, the calculated half-life values of CTC/ECTC in room temperature incubated sterile (SM25) and non-sterile (RTIM) samples were 36 and 34 days, respectively. The half-life values of CTC/ECTC in sterile (SM55) and non-sterile (CM) samples that were incubated at composting temperatures were 5 and 4 days, respectively. These results showed that concentrations of extractable CTC decreased slowly in sterile and non-sterile samples during incubation at 25 °C and decreased rapidly in sterile and non-sterile samples incubated at 55 °C. The half-life values obtained in our study are comparable to those recently reported in a pilot-scale treatment study characterizing the fate of CTC, OTC, tylosin and monensin in spiked horse manure, and OTC and CTC in dairy and beef feedlot manure [35]. In that study, the half-life value for CTC was 5–8 days in composted horse manure, 6–7 days in composted dairy manure and 13 days in composted feedlot manure.

The fate of tetracyclines in soil, sediments, and in manure has been the subject of numerous studies (reviewed in [1,24,35]). In general, tetracyclines adsorb strongly to organic matter and, consequently, extractable concentrations of tetracyclines and their metabolites decline over time in organic matrices. The temperature dependency of the rate of chlortetracycline disappearance has been previously shown in a soil–chicken faeces mixture by Gavalchin and Katz [36]. They found that 44%, 88%, and 100% of chlortetracycline remained in soil–chicken faeces mixture after 30 days of incubation at 30 °C, 20 °C and 4 °C, respectively. Hartlieb et al. suggested that adsorption sites are generated during composting [22]. They found that 30% of simazine residues became bound within a non-extractable fraction after the thermophilic stage of composting (29 days after the start of composting in their study). In this study, as there was no significant difference in CTC/ECTC and ICTC concentrations from sterile and non-sterile manure samples, decreased concentrations appear to be entirely due to temperature-dependent abiotic processes (adsorp-

tion and/or degradation). In addition, microbial population counts have showed greatly decreased numbers of CTC-resistant organisms after composting of the CTC-containing mixture. This result suggests that non-extractable CTC residues are not bioactive.

This study demonstrates that composting can reduce levels of extractable CTC/ECTC levels in manure more than 98% in 30 days because of abiotic processes. We have no information about the potential desorption of any adsorbed antibiotic residues after land application of the manure. Use of more sensitive microbial bioassays may be one approach to quantify very low rates of antibiotic desorption from composted material.

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