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Accepted Version

Ray, P., Chen, C., Knowlton, K. F., Pruden, A. and Xia, K. (2017) Fate and effect of antibiotics in beef and dairy manure during static and turned composting. *Journal of Environmental Quality*, 46 (1). pp. 45-54. ISSN 1537-2537 doi: <https://doi.org/10.2134/jeq2016.07.0269> Available at <https://centaur.reading.ac.uk/69327/>

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Published version at: <https://dl.sciencesocieties.org/publications/jeq/abstracts/46/1/45>

To link to this article DOI: <http://dx.doi.org/10.2134/jeq2016.07.0269>

Publisher: American Society of Agronomy; Crop Science Society of America; Soil Science Society of America

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1 **Fate and effect of antibiotics in beef and dairy manure during static and turned composting**

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10 **ABSTRACT**

11           Manure composting has general benefits for production of soil amendment, but the  
12 effects of composting on antibiotic persistence and effects of antibiotics on the composting  
13 process are not well-characterized, especially for antibiotics commonly used in dairy cattle. This  
14 study provides a comprehensive, head-to-head, replicated comparison of the effect of static and  
15 turned composting on typical antibiotics used in beef and dairy cattle in their actual excreted  
16 form and corresponding influence on composting efficacy. Manure from steers (with or without  
17 chlortetracycline, sulfamethazine, and tylosin feeding) and dairy cows (with or without  
18 pirlimycin and cephalixin administration) were composted at small-scale (wet mass: 20-22 kg) in  
19 triplicate under static and turned conditions adapted to represent US Food and Drug  
20 Administration guidelines. Thermophilic temperature (>55°C) was attained and maintained for 3  
21 d in all composts, with no measureable effect of compost method on the pattern, rate, or extent of  
22 disappearance of the antibiotics examined, except tylosin. Disappearance of all antibiotics,  
23 except pirlimycin, followed bi-phasic first-order kinetics. However, individual antibiotics  
24 displayed different fate patterns in response to the treatments. Reduction in concentration of  
25 chlortetracycline (71 to 84%) and tetracycline (66 to 72%) was substantial, while near-complete  
26 removal of sulfamethazine (97 to 98%) and pirlimycin (100%) was achieved. Tylosin removal  
27 during composting was relatively poor. Both static and turned composting were generally  
28 effective for reducing most beef and dairy antibiotic residuals excreted in manure, with no  
29 apparent negative impact of antibiotics on the composting process, but with some antibiotics  
30 apparently more recalcitrant than others.

31 **Keywords:** antibiotics, beef and dairy manure, static and turned composting

32 **CORE IDEAS**

- 33 • Antibiotics excreted in their natural forms did not influence manure composting.
- 34 • Antibiotic transformation did not always follow single-phase first-order kinetics.
- 35 • Composting enhanced antibiotic removal from manure, but tylosin was recalcitrant.

36 **INTRODUCTION**

37           Antibiotics are the most commonly used drugs in livestock production and are  
38 administered to treat bacterial infection, prevent disease, or promote growth. According to the  
39 US Food and Drug Administration (FDA), 13.5 million kg of antibiotics were sold in the US for  
40 livestock use in 2011, accounting for about 70% of total antibiotic sales (FDA, 2012; 2014a).  
41 Antibiotics are known to be excreted in feces and urine with up to 90% of administered  
42 antibiotics remaining as parent compound, metabolites, or both (Kemper, 2008). Excreted  
43 antibiotic residues can enter the environment via land application of manure, which is a growing  
44 concern to animal, human, and environmental health. Antibiotics have been shown to persist in  
45 stored manure, soil, and water and, even at subinhibitory concentrations, play a role in  
46 stimulating, selecting, and disseminating antibiotic resistance among bacteria (Beaber et al.,  
47 2004; Gullberg et al., 2011; Kuchta and Cessna, 2009; Kumar et al., 2005; Lamshoft et al.,  
48 2010). Antibiotics in soil may also be taken up by plants and deposited in roots, stems, leaves,  
49 and fruits, making consumption of raw produce a potential contributor to unintended exposure of  
50 humans to antibiotics (Bassil et al., 2013; Dolliver et al., 2007; Kang et al., 2013).

51           Among antibiotics commonly used in the dairy and beef industry, macrolides are  
52 considered “critically important” in human medicine by the World Health Organization (WHO),  
53 while cephalosporins, sulfonamides, and tetracyclines are considered “highly important”  
54 (Collignon et al., 2009). Cephapirin accounts for an estimated 31% of dry cow therapy  
55 administrations in the US (USDA, 2008) and pirlimycin is used to treat ~20% of mastitis  
56 infections (Pol and Ruegg, 2007; USDA/APHIS/VS/CEAH., 2008). Antibiotics are administered  
57 to feedlot cattle via feed or water to prevent diseases, treat respiratory and hepatic disorders, and  
58 improve average daily gain and feed conversion efficiency. Common classes of antibiotics used

59 in beef cattle are tetracycline, macrolides, sulfonamides, and aminoglycosides (Mathew et al.,  
60 2007; Sarmah et al., 2006; USDA, 2000). Tylosin, a macrolide, is fed to cattle on about 20% of  
61 all feedlots, and the combination of chlortetracycline and sulfamethazine is used on 17% of all  
62 feedlots (USDA, 2000).

63         Recycling nutrients to soil by land application of treated manure is considered to be  
64 environmentally-friendly, with guidelines under development to ensure the health and safety of  
65 manure treatments. For example, in the most recent FDA Food Safety Modernization Act (FDA  
66 FSMA) proposed guidelines, a manure treatment processes is acceptable if it can reduce the  
67 number of specified pathogens before land-application (FDA, 2015). However, the efficiency of  
68 FDA-approved manure treatment processes in removal of antibiotics has not been assessed.  
69 Storage of manure in lagoons or pits is one low-cost management approach that has indicated  
70 some success for reducing antibiotics before land application, but long-term storage may not  
71 always be feasible and could require long-distance transport (Boxall et al., 2004; Chee-Sanford  
72 et al., 2009; Kemper, 2008). Anaerobic digestion can also be effective for reducing antibiotic  
73 loads in manure (Arikan et al., 2006), but there are reports that residual antibiotics can disrupt  
74 this sensitive microbial process (Beneragama et al., 2013; Poels et al., 1984; Stone et al., 2009).  
75 Composting is also a process driven by microbiological activity and is a preferred manure and  
76 biosolid management strategy for stabilizing nutrients, reducing mass and volume, killing  
77 pathogens, and reducing odor (Larney and Hao, 2007; Larney et al., 2003; Michel et al., 2004).  
78 Composting has also been used effectively to stimulate biodegradation of chemicals of emerging  
79 concerns such as pharmaceuticals, personal care products, pesticides, and hormones (Bartelt-  
80 Hunt et al., 2013; Büyüksönmez et al., 2000; Ho et al., 2013; Xia et al., 2005). Therefore  
81 composting of manure has been suggested to reduce environmental loading of antibiotics from

82 livestock farms, and has demonstrated success in removing some antibiotics (Cessna et al., 2011;  
83 Dolliver et al., 2008b; Mitchell et al., 2015), but the efficiency is often inconsistent. Dissipation  
84 (rate and extent) of antibiotics during composting has been observed to vary with respect to type  
85 of antibiotics, type of feedstocks (i.e. type of manure and amendments), size of compost pile, and  
86 composting approach (turning vs. no turning) (Cessna et al., 2011; Dolliver et al., 2008b;  
87 Storteboom et al., 2007). Therefore it is difficult to form generalizable conclusions about the  
88 efficiency of composting in reducing or removing antibiotics from livestock manure.

89         While there is no report on the effect of composting on dissipation of cephalosporin and  
90 lincosamide antibiotics in dairy manure, there are some reports on the dissipation of macrolide,  
91 sulfonamide, and tetracycline in beef manure during composting. However, the majority of  
92 studies used beef manure spiked with antibiotics to evaluate the efficiency of composting in the  
93 removal of antibiotics. To better understand real-world conditions, consideration of actual  
94 excreted antibiotics is ideal. There is also a paucity of information about the effect of composting  
95 approach on disappearance of antibiotics in manure, with benchmarking against recent FDA  
96 guidelines of particular interest. Therefore, the objective of this study was to determine the effect  
97 of static and turned composting of beef and dairy manures, collected during peak excretion  
98 following antibiotic administration, on the disappearance of cephalosporin, lincosamide,  
99 macrolide, sulfonamide, and tetracycline antibiotics. Of further interest was the effect of  
100 antibiotics and type of manure on efficacy of the composting process.

## 101 **MATERIALS AND METHODS**

### 102 **Animal Experiment and Manure Collection**

103         To generate manure for composting experiments, nine healthy yearling Hereford steers  
104 (body weight:  $341 \pm 35$  kg) were selected for homogeneity of body weight, housed in individual



105 pens and adapted to a grain-based diet gradually over 28 days. None had a history of antibiotic  
106 treatment. After the diet adaptation period, the steers were fed a basal diet containing corn silage  
107 (45%) and non-medicated or medicated grain mix (55%) for seven days and offered free choice  
108 water. Three steers were fed chlortetracycline plus sulfamethazine at 350 mg of each  
109 antibiotic/steer d<sup>-1</sup> and three steers were fed tylosin at 11 mg kg<sup>-1</sup> feed. The three remaining  
110 steers were fed the basal diet containing non-medicated grain mix. The steers were fed a  
111 restricted amount of feed (~9 kg dry weight) to ensure complete consumption of antibiotic doses.  
112 Total collection (feces and urine) was conducted from d 3 to 7 post-treatment and manure from d  
113 3 (when peak excretion of antibiotic resistance genes was expected) was used for the composting  
114 experiment. Manure from control steers served as control beef manure.

115 To generate dairy manure, six healthy, peak lactation dairy cows and three cows at the  
116 end of their current lactation cycle were used. Three peak lactation cows were treated  
117 therapeutically with pirlimycin (intramammary dose typical for clinical mastitis; two doses of 50  
118 mg each, 24 h apart) and three end of lactation cows received cephalosporin (intramammary dry cow  
119 therapy; single dose of 300 mg into each of four quarters). The three remaining healthy lactating  
120 cows were used as negative controls with no antibiotic treatment. Experimental cows were  
121 selected for homogeneity of body weight and stage of lactation, and none had received antibiotic  
122 treatment in the current lactation.

123 All cows were offered free choice water and *ad libitum* total mixed ration and were  
124 housed in tie stalls (1.25 × 2.25 m) throughout the study. After 24 h of acclimation period, the  
125 cows were treated with the assigned antibiotic. Total (24 h) collection of feces and urine was  
126 conducted on d 3 post treatment. Feces and urine from 3 cows of each treatment were  
127 composited and mixed to achieve homogeneous dairy manure. Manure from cephalosporin and

128 pirlimycin treated cows were mixed on wet weight basis (1:1, w/w) to get composited dairy  
129 manure containing both antibiotics. Manure from control cows contained no antibiotics and  
130 served as control dairy manure.

### 131 **Composter Set up**

132 Compost tumblers [71 cm (L) × 64 cm (dia.)] were used in this experiment. The  
133 composters were equipped with 20 holes to facilitate natural aeration and placed in a temperature  
134 controlled room (average room temperature: 27°C). Four different types of manure 1) dairy  
135 control, 2) dairy antibiotics (cephapirin and pirlimycin), 3) beef control, and 4) beef antibiotics  
136 (chlortetracycline, sulfamethazine, and tylosin) were composted using either static or turned  
137 composting methods. Raw materials used to prepare compost mixtures were dairy or beef  
138 manure, alfalfa hay, mulch (pine bark), and sawdust with proportions set to achieve a C:N ratio  
139 of 25-30 and moisture content of 55 to 65%. Dairy manure was mixed with alfalfa hay, mulch,  
140 and sawdust at a ratio of 5:1:3.3:1.5 (w/w, wet weight basis). Beef control and antibiotic manures  
141 were mixed with alfalfa hay, mulch, and sawdust at a ratio of 5:1:3.8:2 and 5.5:1:3.8:1.5 (w/w,  
142 wet weight basis). It may not be a standard practice to use alfalfa hay and pine bark as raw  
143 materials in commercial compost facilities, but the presence of these materials is not uncommon  
144 in dairy or beef farm waste, given that alfalfa hay and sawdust are commonly used feed  
145 ingredients and bedding materials, respectively. It is assumed that the addition of these high  
146 lignocellulosic materials may prolong the persistence of organic matter (OM) during short-term  
147 small-scale composting and thus may reduce antibiotic degradation by providing more sorption  
148 sites on OM for antibiotics (Lynch and Wood, 1985; Zhang et al., 2012).

149 Each manure type × composting approach combination was replicated 3 times. Static  
150 composters were not turned after initial mixing and loading into the composters. Static

151 composters were aerated using an air pump (Model: DOA-P704-AA, GAST, MI) at a flow rate  
152 of 0.1 CFM. The pump was on for 5 min every hour during the thermophilic phase and then for 1  
153 min every hour during the mesophilic phase. Turned composters were turned four times daily  
154 during the thermophilic phase, and once daily during the mesophilic stage. The composters were  
155 insulated (R 21 Double reflective Insulation, Reflectix, Markleville, IN), except for the holes.  
156 Temperature was monitored by placing two temperature sensors at the depth of 7.5 and 22.5 cm  
157 and recorded every 15 min using HOBO temperature data loggers (HOBO UX120-006M, Onset  
158 Computer Corp., Bourne, MA).

## 159 **Sampling and Analysis**

### 160 *Raw Materials and Compost Properties*

161 Sub-samples of raw materials were collected and analyzed for moisture, total C, total N,  
162 and pH. Compost samples were collected on day 0, 4, 7, 14, 21, 28, and 42. Because of the  
163 heterogeneous nature of compost, samples were collected from several locations at different  
164 depths, and then composited and mixed. Two sets of sub-samples were collected, with one set  
165 immediately frozen at -80°C and freeze-dried (for antibiotic analysis), and another set frozen (-  
166 20°C) to evaluate compost characteristics (moisture, total C and total N). Additional samples  
167 were collected on d 0 and 42 and stored frozen for ash analysis or used to measure pH and EC  
168 immediately. Detailed sample analysis plan can be found in supplemental materials (Sample  
169 Analysis).

### 170 *UPLC-MS/MS Quantification of Antibiotics*

171 Freeze-dried samples of dairy and beef compost were respectively analyzed for  
172 cephalosporin and pirlimycin using the methods described previously (Ray et al., 2014a; b) and for  
173 sulfamethazine, tylosin, chlortetracycline, and tetracycline using a method modified from

174 published methods (Jacobsen et al., 2004). Freeze-drying of a sample prior to extraction to remove  
175 water interference is a common protocol used to target total recovery of organic compounds in solid  
176 environmental samples for analysis (Jacobsen and Halling-Sørensen, 2006; Khairnar et al., 2012).  
177 Freeze-dried compost samples were extracted using methanol: phosphate buffer (70:30, v/v) or  
178 methanol: McIlvaine buffer (50:50, v/v) and extracts were clarified using solid phase extraction  
179 (SPE). Clarified extracts were analyzed using UPLC-MS/MS (Agilent 1290 UPLC coupled with  
180 Agilent 6490 Triple Quad tandem mass spectrometry) for antibiotics. Detailed antibiotic  
181 quantification is available in supplemental materials.

## 182 **Statistical Analysis**

183 All data were analyzed using the GLIMMIX procedure in SAS (SAS Institute Inc., Cary,  
184 NC) with composter (n = 3) as the experimental unit. The effects of manure type, composting  
185 approach, day of composting, and their interactions on compost properties were evaluated using  
186 a mixed statistical model which included manure type and composting method as fixed effects  
187 with day as a repeated factor and composter as random variable. Data from day 0 were used as a  
188 covariate in the model. Antibiotic concentration and reduction data were analyzed using a mixed  
189 model with composting approach as a fixed effect and day as a repeated factor. The effect of  
190 composting approach on antibiotic half-life and dissipation rate constants was evaluated using a  
191 mixed model with composting method as a fixed effect. Means of main effects were separated  
192 using a multiple comparison test following the Tukey-Kramer method. Data were reported as  
193 least square means and standard errors, and statistical significance of difference was declared at  
194  $P < 0.05$ .

## 195 **RESULTS AND DISCUSSION**

### 196 **Temperature**

197           Thermophilic temperature ( $\geq 55^{\circ}\text{C}$ ) was achieved and maintained for 3 d in all composts,  
198 as recommended by US FDA Food Safety and Modernization Act (FSMA) guidelines (FDA,  
199 2014b). Thermophilic phase duration was not influenced by manure type (dairy or beef),  
200 antibiotic content (with or without antibiotics) or composting approach (static or turned; Fig. 1).  
201 With or without antibiotics, the temperature during static composting of dairy manure reached  
202  $\geq 55^{\circ}\text{C}$  by d 2 of composting, continued to increase for the next 24 h to attain peak temperature,  
203 and then gradually declined below  $55^{\circ}\text{C}$  by d 5. Lack of any negative influence of residual  
204 antibiotics in manure on the temperature profiles during composting confirms that microbial  
205 activity and exothermic processes during composting were not compromised. This is similar to  
206 the observation when composting swine and poultry manure, where the presence of antibiotic  
207 residues did not influence temperature profiles (Hu et al., 2011). The temperature profile during  
208 static composting of beef manure (with or without antibiotics) was similar to that during static  
209 composting of dairy manure, but thermophilic temperature was achieved on the 4<sup>th</sup> d of beef  
210 manure composting. Temperature in beef compost gradually increased for the next 36 h to reach  
211 peak temperature and declined below  $55^{\circ}\text{C}$  by day 7.

212           The temperature profile during turned compost of dairy or beef manure with or without  
213 antibiotics followed the same pattern observed during static composting of the respective manure  
214 type. A similar lack of effect of manure type (poultry vs. swine) on temperature profiles during  
215 composting was reported by others (Bao et al., 2009; Hu et al., 2011).

216           Turning the compost did not extend the duration of the thermophilic phase, which was  
217 not expected based on some previous reports (Cáceres et al., 2006; Derby et al., 2011). The  $\sim 3$  d

218 thermophilic phase achieved for turned composting in this study would not meet the criteria for  
219 turned composting (15 d at 55°C) in the recent FSMA recommendation (2011). Therefore, the  
220 present study provides insight into the effect of turning itself as a more high-intensity manure  
221 management approach, benchmarked against static-composting achieving FSMA standards. Lack  
222 of an extended thermophilic phase during turned composting in this study was likely due to the  
223 relatively small size of the composters, which was necessary to compare a variety of manures in  
224 a replicated and head-to-head fashion. During composting of dairy manure with sawdust, high  
225 temperature (>40°C) in larger windrows was maintained longer than in smaller windrows  
226 (Tirado and Michel, 2010). The effect of compost size on temperature evolution was also  
227 observed during a small scale (5 kg dry wt. of manure) composting of broiler manure with hay  
228 (Ho et al., 2013).

### 229 **Physico-chemical Parameters**

230 Moisture content was influenced by the interaction of manure type and composting  
231 approach ( $P < 0.05$ ; Supplemental Table S1). Average moisture content across all sampling days  
232 (59 to 61%) did not differ between manure types during turned composting and was consistent  
233 with a range reported to be optimal for biodegradation during composting (Richard et al., 2002).  
234 Static composted beef manure with no antibiotics had lower moisture content compared to static  
235 composted dairy manure with no antibiotics (48 vs. 56%;  $P < 0.05$ ). The interactions of manure  
236 type by day and composting approach by day also influenced the moisture content ( $P < 0.05$ ;  
237 Supplemental Table S1). Moisture content in dairy compost did not vary with time, but beef  
238 control compost was wetter on day 0 than other sampling days. Moisture content was not  
239 influenced by composting approach from d 0 through 4, but static compost was drier than turned  
240 compost thereafter. Moisture content did not vary substantially in turned compost throughout the

241 entire study period, but initial moisture content (d 0 and 4) in static compost was higher  
242 compared to all subsequent sampling days. This more uniform moisture profile in turned  
243 compost is in agreement with the results of a swine manure composting experiment and could be  
244 attributed to the turning process (Derby et al., 2011).

245         Manure type and composting approach did not influence total carbon (TC) concentration  
246 (Supplemental Table S). The average concentration of TC (averaged across all manure types and  
247 composting approaches) decreased sharply (3.22% of initial concentration) within the first 4 days  
248 of composting, overlapping with the thermophilic phase, and then decreased gradually for the  
249 next 38 days. The concentration of total nitrogen (TN) was influenced by composting approach  
250 (Supplemental Table S1). Static compost had higher concentrations of TN compared to turned  
251 compost (2.11 vs. 2.04%), suggesting greater TN loss as ammonia during the turning process  
252 (Cook et al., 2015; Tirado and Michel, 2010). Loss of TC was more rapid than volatilization of  
253 ammonia, as indicated by increasing TN concentration from d 0 through d 14. This was followed  
254 by a phase of decline in TN until d 42. A similar temporal pattern of TN concentration change  
255 was observed during composting of poultry (Ho et al., 2013) and cattle manure (Michel et al.,  
256 2004; Parkinson et al., 2004). In the current study, temporal variation in some physico-chemical  
257 parameters such as total P and K (Supplemental Table S1), pH, EC, and ash content was  
258 observed, but there was no major influence of antibiotic residues or composting method  
259 (Supplemental Table S2). Overall, temperature and physico-chemical data indicate that the  
260 presence of antibiotic residues in the manure did not have any major negative influence on the  
261 composting process.

## 262 **Transformation Patterns: Beef Antibiotics**

263 Initial (d 0) concentrations of chlortetracycline in static and turned compost derived from  
264 antibiotic-treated steers were 1,198 and 675 ng g<sup>-1</sup> dry compost, respectively. The transformation  
265 of chlortetracycline was rapid during first 2 weeks of composting (Fig. 2). In static compost,  
266 chlortetracycline concentration was reduced by 33 and 60% of its initial concentration after 4 and  
267 14 d of composting, respectively (Supplemental Table S3). Turned composting was effective in  
268 removing chlortetracycline by 54 and 73% of d 0 concentration after 4 and 14 d, respectively.  
269 After 2 weeks, removal was relatively slower in both static and turned composting with  
270 chlortetracycline concentration reduced by 71% and 84% of the initial concentration after 42-d  
271 composting. The initial concentrations of tetracycline in antibiotic-static and antibiotic-turned  
272 compost were 96.9 and 81.6 ng g<sup>-1</sup> dry compost, respectively.

273 Although tetracycline was not intentionally fed in this study, it was detected in the feces  
274 of antibiotic-fed steers over a range of 91.1 to 102 ng g<sup>-1</sup> dry manure, likely indicating that  
275 tetracycline was present as an impurity in antibiotic mix used to prepare the medicated grain. The  
276 transformation of tetracycline followed a temporal pattern similar to that of chlortetracycline  
277 (Supplemental Fig. S1). By day 4 of composting, the reduction in tetracycline concentration was  
278 28 and 19% of the initial concentration for static and turned compost, respectively (Supplemental  
279 Table S3). In static and turned compost, the extent of reduction in tetracycline concentration  
280 after 14 d of composting was 57 and 45% of the initial concentration, respectively. Relatively  
281 slower transformation after 14 d resulted in 63 and 66% removal of tetracycline in static and  
282 turned compost, respectively.

283 Initial (d 0) concentrations of sulfamethazine in static and turned antibiotic beef compost  
284 were 1200 and 992 ng g<sup>-1</sup> dry compost, respectively. The transformation pattern for



285 sulfamethazine was similar to those observed for chlortetracycline and tetracycline  
286 (Supplemental Fig. S1). Removal of sulfamethazine was >90% of initial concentration in static  
287 and turned compost after 14 and 7 d of composting, respectively (Supplemental Table S3).  
288 However, relatively slower transformation after 2 weeks resulted in only 1 and 5% additional  
289 decline in sulfamethazine concentration during static and turned composting, respectively. By  
290 the end of composting (d 42), over 95% of sulfamethazine was removed in all compost. By  
291 contrast, Dolliver et al. (2008a) did not observe any transformation of sulfamethazine during 35-  
292 d composting of turkey litter, which could have been affected by lack of microbial adaptation or  
293 a strong adsorption effect preventing biological transformation. However, our results were  
294 consistent with a recent study examining the transformation of sulfamethazine in turned vessel  
295 composting of beef manure (Amarakoon et al., 2016), where 93 to 99% of the initial  
296 sulfamethazine concentration in fortified and excreted manure was transformed after 30 d.

297       The initial concentrations of tylosin in static and turned antibiotic beef compost were 49.3  
298 and 36.1 ng g<sup>-1</sup>, respectively. The mean concentration of tylosin increased in the first week and  
299 then declined in both compost types (Fig. 2). Similarly, the concentration of tylosin in spiked  
300 turkey manure increased during compost (Dolliver et al., 2008b). Deconjugation of conjugated  
301 tylosin or transformation of metabolites to their parent compound (tylosin) during composting  
302 might have contributed to the increase in tylosin concentration. In the current study, the  
303 concentration of tylosin in static and turned compost increased by 138 and 356% of the initial  
304 concentration and reached a peak (116 and 161 ng g<sup>-1</sup>, respectively) by 7 days. The removal of  
305 tylosin after 14 d of static composting was 43% of tylosin concentration observed on d 7, while  
306 the removal in turned compost was 79% of d 7 concentrations (Supplemental Table S4). In static  
307 and turned compost, the tylosin concentration was reduced by 63 and 81%, respectively, relative

308 to their d 7 concentration, after 4 weeks of composting. The concentration of tylosin in finished  
309 static and turned compost (45.7 and 23.1 ng g<sup>-1</sup>) was comparable to their initial concentration.  
310 Overall reduction in chlortetracycline, tetracycline, sulfamethazine, and tylosin after 42-d  
311 composting ranged from 71 to 84, 66 to 72, 97 to 98, and 62 to 86%, respectively.

312         Parallel to the composting study, beef antibiotic manure was stored at 4°C for 42 d, which  
313 resulted in relatively less reduction in chlortetracycline, tetracycline, sulfamethazine, and tylosin  
314 (59, 22, 50, and 47%, respectively). Thus, the transformation of antibiotics could be contributed  
315 by abiotic and biotic process associated with composting, both of which rely largely on  
316 temperature. The observations here were consistent with the corresponding temporal pattern  
317 during composting. During composting, thermophilic conditions (>55°C) were attained and  
318 maintained for 3 d, and then gradually reduced to a range of 30-40°C by d 14. Arikan (2008)  
319 reported transformation of chlortetracycline due to abiotic process(es). With a higher  
320 temperature, the frequency of molecular collision increases and more molecules hold energy to  
321 overcome the barrier for reaction activation. Also, microbial activity is strongly temperature-  
322 dependent, with the slowdown of transformation observed here consistent with trends in  
323 reduction of overall heterotrophic bacterial plate counts (data not shown). A correlation between  
324 temperature and transformation of chlortetracycline and tetracycline was reported by Loftin et al.  
325 (2008). Increasing temperature greatly accelerated the transformation of chlortetracycline in both  
326 manure and soils (Zhang and Zhang, 2010). In the present study, significant differences ( $P <$   
327 0.05) in transformation were observed in the second phase between different composting  
328 approaches. In turned compost, transformation of antibiotics continued even after the  
329 temperature reached steady-state, while the antibiotics were relatively stable in static compost.  
330 Given that there was no difference in temperature at steady-state between static and turned

331 composting, this suggests that biotic processes might have played a role in the removal of  
332 sulfamethazine during the second phase. In particular, oxygen availability is an important factor  
333 for biodegradation (Ali et al., 2013). It is possible that more oxygen was supplied using the  
334 turned approach and resulted in increased microbial activities during the second phase of turned  
335 composting.

### 336 **Transformation Kinetics: Beef Antibiotics**

337 Transformation of the four beef antibiotics followed a bi-phasic pattern, except for  
338 tylosin in static compost (Fig. 2, Supplemental Fig. S1). Each phase followed first order kinetics,  
339 with distinct transformation rate constants (Table 1; Supplemental Table S5) and half-lives  
340 (Table 2; Supplemental Table S6) noted for each phase. Transformation rate constants did not  
341 differ between static and turned compost, except in the case of tylosin (Table 1). The  
342 transformation rate constant for tylosin was higher during the first phase of turned composting  
343 compared to static composting (0.223 vs. 0.047 d<sup>-1</sup>). Overall, transformation rate constants in the  
344 first phase were higher than those in the second phase, consistent with a general slow-down of  
345 transformation after 2 weeks (Table 1).

346 Other than sulfamethazine, the half-lives of beef antibiotics were not influenced by  
347 composting approach (Table 2). The half-life of sulfamethazine during the second phase of static  
348 composting was higher compared to turned composting (73.7 vs. 21.5 d; Supplemental Table  
349 S6). Overall, the half-lives of antibiotics in the first phase were significantly shorter compared to  
350 those in the second phase, consistent with their higher transformation rate constants (Table 2).  
351 During static composting, the half-life of sulfamethazine for the first phase was shorter compared  
352 to the second phase (2.03 vs. 73.7 d<sup>-1</sup>; Supplemental Table S6).

353 In this study, the half-lives of chlortetracycline in the first phase (8.68 and 6.12 d for  
354 static and turned compost, respectively) were comparable to a half-life of 8.2 d reported for  
355 composting of swine manure (Arikan, 2008). However, Dolliver et al. (2008b) observed a  
356 relatively shorter half-life of 1 d during composting of turkey manure. The difference in  
357 degradation rates might be due to either the activities of microbes or abiotic factors, both of  
358 which rely on the environmental factors such as temperature. In the current study, half-lives of  
359 sulfamethazine observed in first phase (2.03 and 2.78 d for static and turned, respectively) were  
360 comparable to those reported in swine manure-amended soils under aerobic conditions (1.2-6.6  
361 d<sup>-1</sup>) (Lertpaitoonpan et al., 2015). A short half-life of 1.4 d for sulfadiazine, a structurally similar  
362 sulfonamide, was also noted during composting of broiler manure (Ho et al., 2013) . Relatively  
363 faster dissipation of sulfadiazine was also observed during composting of swine manure  
364 (complete removal within 3 days) (Selvam et al., 2012). Dolliver et al. (2008b) did not observe  
365 any degradation of sulfamethazine during 35-day composting of turkey litter, with the  
366 persistence of sulfamethazine likely due to the lack of microbial adaptation or a strong  
367 adsorption effect preventing biodegradation. Reported half-lives of tylosin ranged from less than  
368 2 to 30 d (Dolliver et al., 2008b; Ho et al., 2013; Ingerslev and Halling-Sorensen, 2001; Lee et  
369 al., 2001; Loke et al., 2000). In the current study, the half-life of tylosin was 18 d, which was  
370 comparable to a half-life of 19 d during composting of turkey manure spiked with tylosin  
371 (Dolliver et al., 2008b).

### 372 **Transformation Patterns and Kinetics: Dairy Antibiotics**

373 Cephapirin was present in d 0 compost samples, but was not detected thereafter. Initial  
374 concentrations of cephapirin in static and turned dairy compost were 11.0 and 14.2 ng g<sup>-1</sup> dry  
375 compost, respectively. Rapid disappearance of cephapirin was not surprising considering the

376 instability of cephalosporins at high temperature. In aqueous solution, degradation of cephalosporins at  
377 37°C was 40% after 24 h (Berendsen et al., 2009). Pirlimycin was detected in d 0 compost  
378 samples at comparatively higher concentrations (154 and 109 ng g<sup>-1</sup> dry compost for static and  
379 turned compost, respectively) and, as observed generally for beef antibiotics, its reduction was  
380 not influenced by composting approach (Supplemental Table S4). In static and turned compost,  
381 the reduction in pirlimycin concentration was 32 and 48% of initial concentrations, respectively,  
382 by d 4. The decline in pirlimycin concentration was almost 70% of initial concentration by d 7 of  
383 composting. In both static and turned compost, the removal of pirlimycin was more than 90% of  
384 initial concentration after 14 days and was almost complete by 42 days (99.8 and 99.9% of initial  
385 concentration for static and turned compost, respectively). While near complete removal was  
386 achieved during composting, only 55% reduction in pirlimycin concentration was observed after  
387 42-d storage of dairy antibiotic manure at 4°C.

388         It is likely that that disappearance of pirlimycin during composting involved both biotic  
389 and abiotic process. Pirlimycin was transformed to its nucleotide adducts by microflora in dairy  
390 cow feces (Hornish et al., 1992). In addition to abiotic degradation, adsorption of pirlimycin to  
391 organic matter (such as humic acid) might have also contributed to reducing the concentration of  
392 pirlimycin as adsorption sites were generated during composting (Hartlieb et al., 2003). Most  
393 likely such adsorption would be strong and irreversible, given that a strong solvent extraction  
394 method was employed in this study. Since composting is an aerobic process, oxidation of  
395 pirlimycin to pirlimycin sulfoxide and pirlimycin sulfone should also be considered as a  
396 plausible explanation for reduction. The concentrations of pirlimycin in the final product of static  
397 and turned composting were 0.26 and 0.06 ng g<sup>-1</sup> dry compost, respectively. The transformation  
398 of pirlimycin in both static and turned compost followed first-order kinetics (Fig. 3), with no

399 significant difference in transformation rate constants between static and turned compost (Table  
400 1). Similarly, the half-life of pirlimycin was not influenced by composting approach, with values  
401 of 4.67 and 4.41 d for static and turned compost, respectively (Table 2).

## 402 **Limitations and Suggested Further Studies**

403 It is important to note that the present study focused on the fate of parent antibiotic  
404 compounds fed to the cattle. It is likely that many antibiotics are transformed into metabolites  
405 that retain antimicrobial activity. Isochlortetracycline has been reported to be the primary  
406 metabolite during degradation of chlortetracycline in swine manure (Shelver et al., 2012), while  
407 tylosin B and D were believed to be major and the minor degradation products of tylosin A (the  
408 type of tylosin analyzed in this study), respectively (Loke et al., 2000). Almost complete  
409 dissipation of sulfamethazine observed in the current study might be partly or completely due to  
410 its transformation into the metabolite N<sup>4</sup>-acetylsulfamethazine (Grant et al., 2003). Therefore, it  
411 cannot be assumed based on the present study that loss of antimicrobial activity is equivalent to  
412 the dissipation of the parent compound.

413 Phase partitioning and bioavailability could also affect the residual antimicrobial activity  
414 in the compost with time. In this study we employed a bulk extraction approach to recover the  
415 total residual parent compound. However, this approach may not represent the bioavailable  
416 fraction of antibiotics because compost is rich in organic carbon (OC), which can bind to  
417 antibiotics and influence their activity. Hydrophobic antibiotics are more likely to partition to  
418 organic matter, with water-OC partition coefficients ( $K_{OC}$ ) for sulfamethazine and tylosin  
419 reported to range from 82-208 and 553-7990 L kg<sup>-1</sup>, respectively (Sarmah et al., 2006). Given  
420 that the concentration of OC in soils is very low (1 to 6%) compared to compost ( $\approx$ 50%),  
421 extrapolation of soil  $K_{OC}$  values to compost predicts availability of sulfamethazine and tylosin in

422 the range of 0.6 to 1.6% and 0.01 to 0.2%, respectively. However, when normalizing to OC, the  
423 effect of hydrophilic interactions (e.g., as a result of ionic functional groups) is not taken into  
424 account and such predictions of availability may not be accurate.

425 It is also important to acknowledge that small-scale composting is not a perfect  
426 representation of full-scale because parameters such as heat accumulation and loss, moisture,  
427 aerobic or anaerobic conditions, and substrate compaction vary with the scale of composting  
428 (Petiot and Guardia, 2004). The overall effect of smaller scale tends to be accelerated reaction  
429 rates. Therefore, while the general patterns reported in this study at small-scale are expected to  
430 translate to full-scale, the precise rates of antibiotic transformation may differ. For example,  
431 Dolliver et al. (2008) reported slightly slower rate of chlortetracycline dissipation in full-scale  
432 composting compared to smaller scale vessel composting. In contrast, first-order degradation rate  
433 constants of organic micro-pollutants were not different between bench-scale and full-scale  
434 composting (Sadeh et al., 2015). In future large scale composting experiment should be  
435 conducted where metabolites of antibiotics in addition to parent compounds should be  
436 quantified. In addition to total extracted antibiotics any effect of composting on bioavailable  
437 fraction of antibiotics should be monitored.

#### 438 **Conclusions and Implications for Composting Manure with Antibiotics**

439 Overall temperature profile, physico-chemical properties, and temporal patterns of  
440 nutrient concentrations were not influenced by manure type and indicated that presence of  
441 antibiotics did not negatively influence the process of composting. While the static compost  
442 condition achieved federal time  $\times$  temperature guidelines for pathogen reduction, the turned  
443 condition did not achieve the recommended extended thermophilic stage, which is likely related  
444 to the small-scale employed in this study. Under the conditions of this study, the transformation

445 of antibiotics was not strongly affected by static versus turned composting; both static and turned  
446 compost resulted in complete removal of cephalosporin, lincosamide, and sulfonamide  
447 antibiotics while removal of tetracycline antibiotics ranged from 66 to 84%. Removal of tylosin  
448 was poor over the 42 d of composting. The transformation of all antibiotics, except lincosamide  
449 followed, bi-phasic first-order kinetics. Antibiotic transformation rates generally decreased from  
450 first to second phase, corresponding to the shift in thermophilic to mesophilic conditions. Overall  
451 it is concluded that composting is promising for the reduction of downstream impacts of  
452 antibiotics from livestock to crops and the environment, but future studies should verify that the  
453 benefits carry over to metabolites and verify rates at full-scale.

454



455 **ACKNOWLEDGEMENTS**

456           This study was supported by NIFA Competitive Grant no. 2014-05280 from the USDA  
457 National Institute of Food and Agriculture. The authors appreciate the assistance of Courtney  
458 O'Haro, Elizabeth Fazio, and Hosanna Nystrom in conducting animal experiments to collect  
459 manure for composting. The authors thank Robert Williams, Giselle Guron, Christy Teets,  
460 Courtney O'Haro, Elizabeth Fazio, and Hosanna Nystrom for their help with composter set up  
461 and sample collection.

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660 Fig. 1. (A) Temporal pattern (28 days) of temperature during static composting of dairy control,  
661 dairy antibiotic, beef control, and beef antibiotic manure. (B) Temporal pattern (28 days) of  
662 temperature during turned composting of dairy control, dairy antibiotic, beef control, and beef  
663 antibiotic manure. Dairy and beef control: No antibiotic in manure; Dairy antibiotic: Manure  
664 from cows after intramammary infusion of cephalosporin and pirlimycin at 1200 mg and 100 mg per  
665 cow, respectively. Temperature data from only 28 days is presented because temperature was  
666 similar to ambient temperature after 28 days.

667 Fig. 2. Dissipation kinetics of (A) chlortetracycline and (B) tylosin during static and turned  
668 small-scale composting of beef manure. Manure was collected from steers fed chlortetracycline  
669 sulfamethazine each at 350 mg d<sup>-1</sup> and tylosin at a daily dose of 11 mg kg<sup>-1</sup> feed.

670 Fig. 3. Dissipation kinetics of pirlimycin in dairy manure during static and turned small-scale  
671 composting. Manure was collected from dairy cows after intramammary infusion of cephalosporin  
672 and pirlimycin at 1200 and 100 mg per cow, respectively.

**Table 1. Transformation rate constants of different antibiotics during static and turned small-scale composting of beef and dairy manure**

	Chlortetracycline	Tetracycline	Sulfamethazine	Tylosin†	Pirlimycin‡
	-----d <sup>-1</sup> -----				
Composting					
Static§	0.049 ± 0.014	0.042 ± 0.007	0.188 ± 0.031	0.047 ± 0.027a	0.154 ± 0.005
Turned§	0.072 ± 0.014	0.030 ± 0.007	0.149 ± 0.031	0.223 ± 0.027b	0.162 ± 0.005
Phase					
First	0.106 ± 0.012a¶	0.052 ± 0.006a	0.316 ± 0.031a	0.223 ± 0.026a	-
Second	0.015 ± 0.012b	0.019 ± 0.006b	0.021 ± 0.031b	0.010 ± 0.026b	-
	<b>P value</b>				
Composting	0.32	0.32	0.43	<0.05	0.34
Phase	<0.05	<0.05	<0.05	<0.05	-
Composting × Phase	0.52	0.12	0.24	-	-

† Effect of composting reflects first phase data and effect of phase reflects turned composting data for tylosin transformation.

‡ Pirlimycin transformation followed single phase first order kinetics.

§ Static and turned are static and turned composting approaches.

¶ Within antibiotic, means followed by different letters are significantly different ( $P < 0.05$ ).

**Table 2. Half-lives of different antibiotics during static and turned small-scale composting of beef and dairy manure**

	Chlortetracycline	Tetracycline	Sulfamethazine	Tylosin†	Pirlimycin‡
-----d-----					
Composting					
Static§	86.9 ± 25.3	26.8 ± 4.88	37.9 ± 4.31	18.0 ± 4.21	4.51 ± 0.13
Turned§	20.4 ± 25.3	27.6 ± 4.88	12.1 ± 4.31	3.32 ± 4.21	4.30 ± 0.13
Phase					
First	7.40 ± 24.8a¶	14.9 ± 3.87a	2.41 ± 4.31#	3.31 ± 23.1a	-
Second	100 ± 24.8b	39.5 ± 3.87b	47.6 ± 4.31	88.9 ± 23.1b	-
	<b>P value</b>				
Composting	0.14	0.91	<0.05	0.07	0.34
Phase	<0.05	<0.05	<0.05	<0.05	-
Composting × Phase	0.14	0.29	<0.05	-	-

† Effect of composting reflects first phase data and effect of phase reflects turned composting data for tylosin transformation.

‡ Pirlimycin transformation followed single phase first order kinetics.

§ Static and turned are static and turned composting approaches.

¶ Within antibiotic, means followed by different letters are significantly different ( $P < 0.05$ ).

# Mean separation is not provided if Composting × Phase is significant.

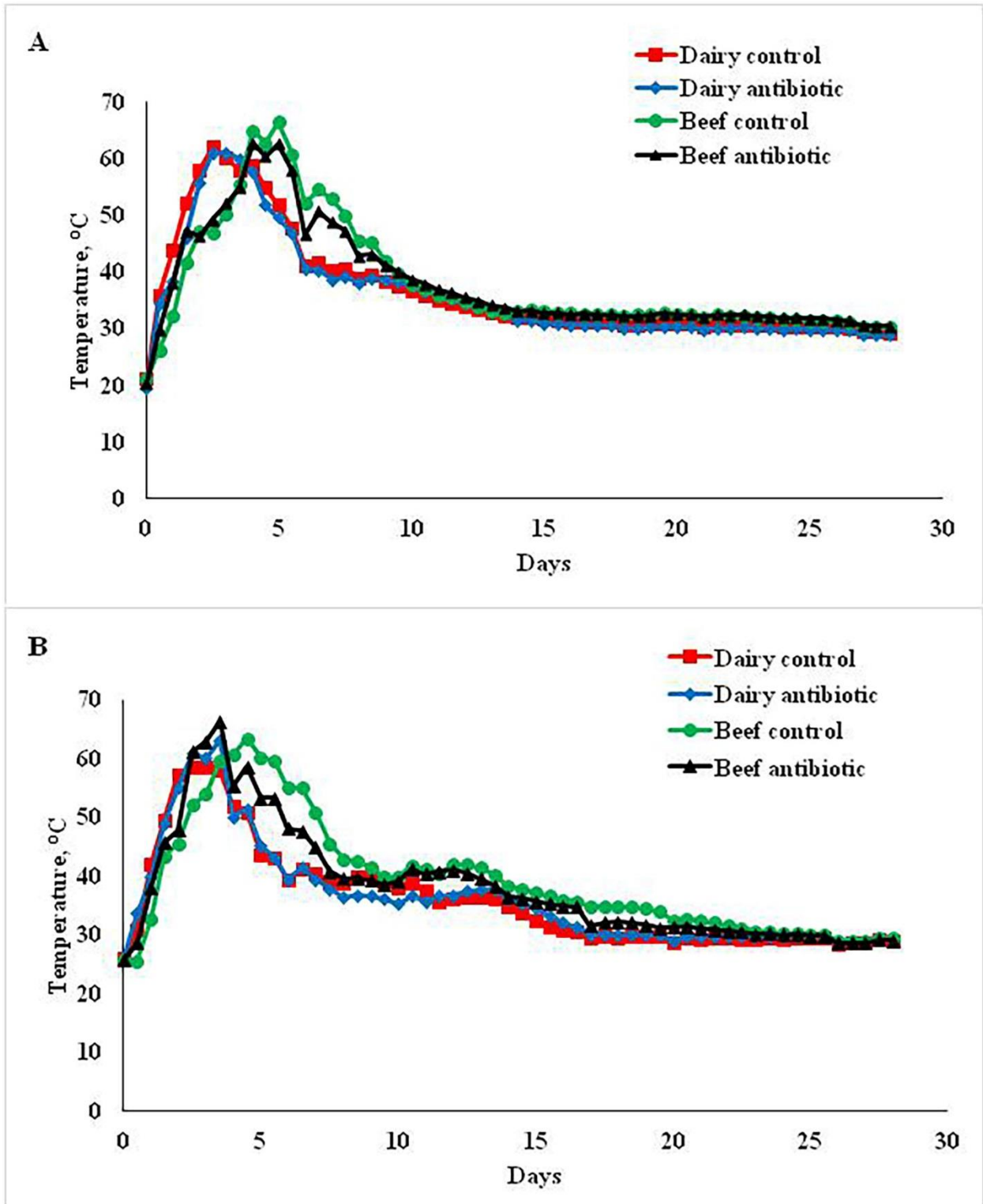


Fig 1.

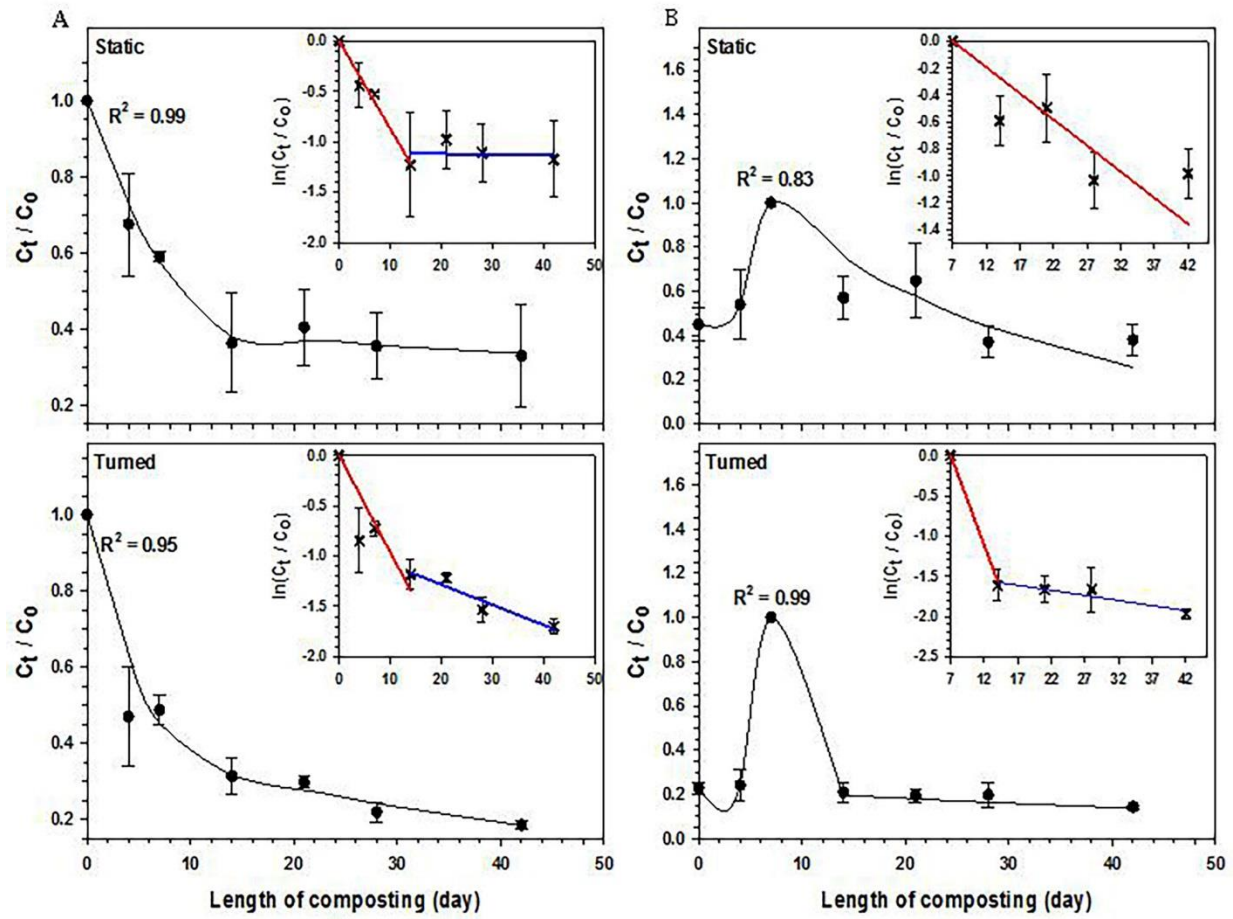


Fig 2.

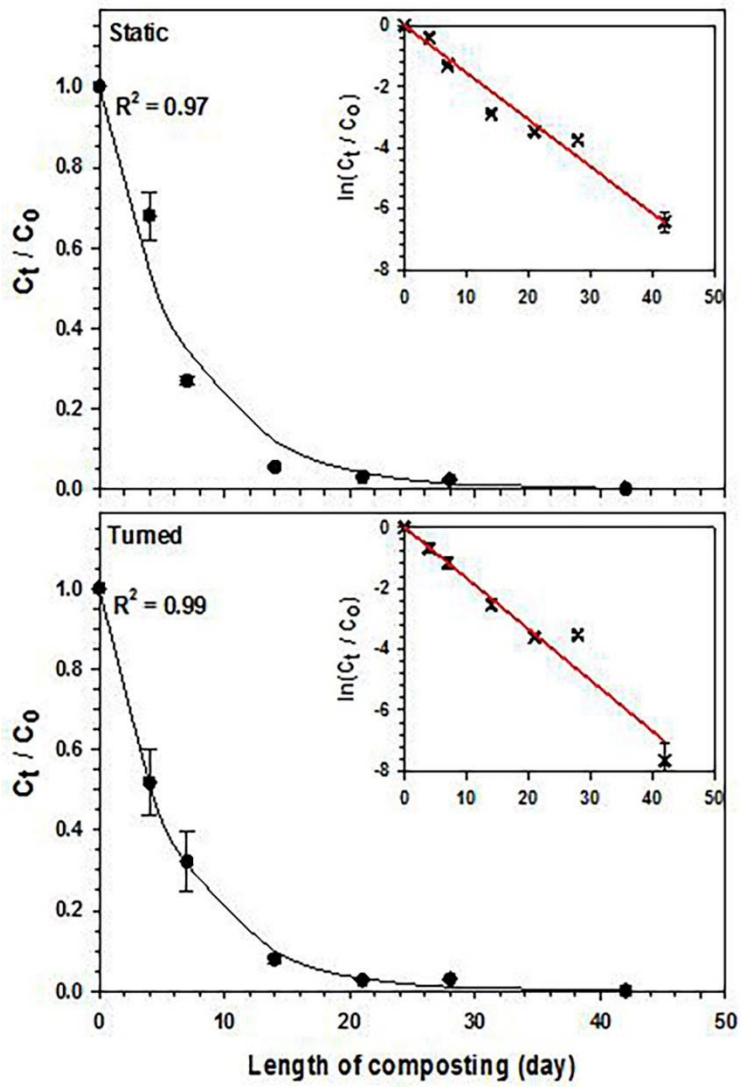


Fig 3.