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Reducing of oxytetracycline in poultry manure by using the composting technique

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Abstract
The composted poultry litter was taken for the quantitative analysis of Tetracyclines. In this work the relative antibiotic compound included Oxytetracycline and their traces were encountered in the initial poultry litter and in their respective 30 days interval composted samples and the quantification is done by HPLC optimized by the method followed by Feng *et al.,* 2016. From the quantification method the oxytetracycline compound were detected quantitatively to the tune of 216.67 mg kg^{-1} as against 33.97 mg kg^{-1} respectively in the raw poultry litter and composted poultry litter, respectively. It indicated that composting of animal manures is capable of reducing the antibiotic concentration drastically from the raw manures.

Keywords: Poultry litter, oxytetracycline, composting, temperature, oxytetracycline concentration

Introduction
Antibiotics are substances that are used to fight against harmful pathogenic organisms which are of biological origin. Various diseases were treated by administering these substances in human beings, animals and plants. In early 1900s, after the discovery of antibiotics, so many infectious diseases have been removed. Antibiotics react against their counterpart which has the potential to cause disease in living organisms. They are commonly added to animal feed as supplements to enhance the growth of the animals in feedlot conditions (Phillips *et al*., 2004 and Kumar *et al*., 2005) [12]. A sharp rise of antibiotic usage has been a recent concern worldwide. The global average annual consumption of antimicrobials per kilogram of animal produced was estimated 45 mg kg^{-1} and 148 mg·kg^{-1} for cattle and chicken, respectively. Starting from this baseline, the estimation was made that between 2010 and 2030, the global consumption of antimicrobials will be increased by 67% from 63,151 tons to 105,596 tons (Boeckel *et al.,* 2015) [19]. India represents 3% of global antibiotic consumption and is among the top customers around the world, alongside China, the United States, Brazil and Germany (Boeckel *et al*., 2015) [19]. These antibiotics entering the animal as feed additives are ineffectively adsorbed in the gut of the animals and larger part is discharged unaltered in defecation and urine. Animals can excrete up to 75% of an antibiotic dose in feces and up to 90% in urine (Massé *et al.,* 2014) [15].

In addition, Tetracyclines positioned second among anti-infection agents underway and utilization worldwide by 2013, it is the most delivered and as often as possible utilized class of antibiotics in China (Daghrir *et al*., 2013) [5]. Oxytetracycline (OTC) is a common antibiotic and a member of the tetracycline family with awide scope of action, and is widely used in livestock animals including cattle, swine, poultry and fish Sarmah *et al*. (2006) [17]. Oxytetracycline is an expansive range antibiotics broadly utilized for the treatment or counteractive action of disease in poultry production (Loke *et al*., 2003) [13]. In an investigation by Zhao *et al*. (2010) [24], oxytetracycline, another significant individual from TC gathering, was recognized with a relatively high occurrence of 27.8-41.0%. Zhang *et al*. (2008) [23] revealed that oxytetracycline could be estimated in huge buildup levels in pig, chicken and cow compost tests gathered from north Zhejiang Province, with oxytetracycline geometric mean of 8.37, 4.32 and 2.18 mg kg^{-1} for pigs, chickens and dairy animals, respectively. Hu *et al*. (2008) [9] detected oxytetracycline in all animal dung samples investigated at concentrations ranging from 9.7 to 173.2 mg kg^{-1} Karci and Balcioglu (2009) [11] detected oxytetracycline in animal manure samples at concentration ranges of 0.06-0.50 mg kg^{-1}. Carballo *et al*. (2007) [14] reported that the concentration of tetracycline in 30 pig manure samples from Austria was up to 23 mg kg^{-1} and hence create potential risks to the environment. Bewick has reported the antibiotic uptake by vegetable crops by using oxytetracycline fermentation wastes for tomatoes

(*Solanum lycopersicum*) as fertilizers (Kang *et al*., 2013) [10]. Hence there is a potential risk of antibiotics entering into food chain.

Materials and Methods

Collection of raw materials for composting and sampling

The raw material of the experiment *i.e*., poultry litters were collected from the poultry farm at Sulur. The raw materials collected were analysed for its initial characteristics. By utilizing the poultry litter and the bedding material as source, composting was done aerobically through heap method. A known quantity of the poultry waste as collected along with coir pith is inoculated with *Pleurotus sajor-caju* @ 2 packets per tonne of waste in order to speed up the composting process. This mixer should be placed under shade as heap. The moisture content of the heap should be maintained at 50 to 60%. Periodical turning must be given on 21th, 28th and 35th days of composting. Another two packets of *Pleurotus sajor-caju* is to be added during turning given on the 28th day of composting. Good quality compost will be attained after 90th day of composting. The matured compost with the moisture content of 40% was harvested, stored at room temperature and utilized for further analysis. Regarding the sampling frequency, the samples were drawn in 30 days interval. The nutrient contents and the physico chemical properties of poultry manure are given in table 2.

Chemical and Reagents

Standards of Oxytetracycline were purchased from HiMedia Laboratories Pvt Ltd., EDTA MC-Ilvaine buffer from Ponmani & Co, Methanol (HPLC grade) from Central Drug House (P) Ltd, Acetonitrile (HPLC grade) from Merck Life Science Private Limited, formic acid, HLB Catridge (BVN Instruments), hexane (HPLC grade) from Merck Life Science Private Limited and 0.22µm filters (Sree Sasthaa Scientific Company).

Standard solutions

Stock solutions (1 mg mL^{-1}) of OTC were prepared by dissolving 10 mg in 10 mL of methanol. They were kept in brown glass vials in the freezer (-22 ºC) and were stable at least 5 months. Working standard solutions to the concentration of 0.1 mg mL^{-1}were prepared daily by dilution of stock solutions in methanol to appropriate concentrations. These solutions were prepared immediately before use.

Extraction Procedure for HPLC

The samples were lyophilized and sieved through a 2 mm sieve. The lyophilized animal wastes and manure samples 1g were taken in the centrifuge tubes to which the 10 ml of EDTA-MC Ilvaine buffer solution was added. The samples were subjected to vortexing and sonicating for 30s and 15 min respectively. The samples were centrifuged at 6000 rpm for 15 min and the supernatant were poured off into a glass bottle. The procedure involved the repeating centrifuge once with 10 ml of EDTA-MC Ilvaine buffer solution and twice with 5 ml of 2:2:1 methanol, acetonitrile and acetone solution. The collected supernatant were degreased with n-hexane and passed through the syringe filters. To concentrate the extracts they were subjected to rotary evaporation (70 rpm) maintained at 40 °C which was then passed through the HLB Catridge preconditioned with 5ml of methanol and 10ml of deionized water. The samples are allowed to pass through the catridge and rinsed with 5ml of 25:75 v/v ratios of methanol and water. The elution was carried with the 10ml of 65:35, v/v of methanol and deionized water which was dried and re dissolved in acetonitrile and water (20:80 v/v) were taken for HPLC analysis.

Quantification in HPLC

The antibiotic residues in the animal manure were detected using HPLC using UV-Visible detector. The mobile phase used in this detection of antibiotic chemicals were 0.1% formic acid and acetonitrile in the ratio of 80:20, v/v. the samples were injected with the flow rate of 1ml min^{-1} and the wavelength were set as 290 nm for the detection of compounds.

Results

Properties poultry litter at different stages of composting

The collected poultry litter along with the bedding material is put to compost in the heap method. The initial characters of the poultry litter have been described in the table 1. The results showed the initial poultry litter has Acidic pH while Electric conductivity (EC), Organic carbon (OC), C: N ratio, Total Nitrogen (TN), Total Phosphorus (TP) and Total Potassium (TK) were recorded as 4.36 dS m^{-1}, 35.17%, 27.91, 1.26%, 0.33% and 0.53%, respectively. The initial poultry litter on subjecting to the composting process was observed with the following results, there was a change in the pH from the acidic condition to neutral with the range of 6.62 to 7.56 while the EC, OC, C: N ratio, TN, TP and TK were observed with the range of 4.36 to 3.6 dS m^{-1}, 35.17 to 21.72%, 27.91 to 13.65, 1.26 to 1.89%, 0.33 to 1.09 % and 0.53 to 2.34%, respectively. Highest value of pH (7.94) was recorded in the 60th day compost sample and to that of EC (4.36 dS m^{-1}) was recorded from initial poultry litter. Similarly, OC (35.17%) of the initial sample, C: N ratio (27.91) of the initial sample and TN (1.89%), TP (1.09%), TK (2.34%) of the 90th day sample also recorded the highest value.

Table 1: Characteristic of poultry litter during different stages of composting process

Parameter	Initial Poultry litter	30th day	60th day	90th day
pH	6.62	7.11	7.94	7.56
EC (dS m^{-1})	4.36	4.1	3.84	3.6
OC (%)	35.17	33.103	28.96	21.724
C:N Ratio	27.91	22.01	18.99	13.654
TN (%)	1.26	1.504	1.525	1.891
TP (%)	0.33	0.65	0.82	1.09
TK (%)	0.53	1.32	2.06	2.34

Temperature influence on composting process

The results showed that the evolution of the temperature within the composting pile that occurs in three major phases: heating phase, thermophilic phase, and cooling phase. The temperature in the compost pile was recorded at 5 days interval. Initially the temperature of the compost increased rapidly and reached a maximum of 52 ºC, as a consequence of the heat generated as a result of biodegradation of the composting feedstock. The composting pile achieved thermophilic temperature (>50 ºC) on day 5 of the composting cycle where as on later days the temperature found to be gradually decreased after which the cooling phase started due to the depletion of compostable organic matter.

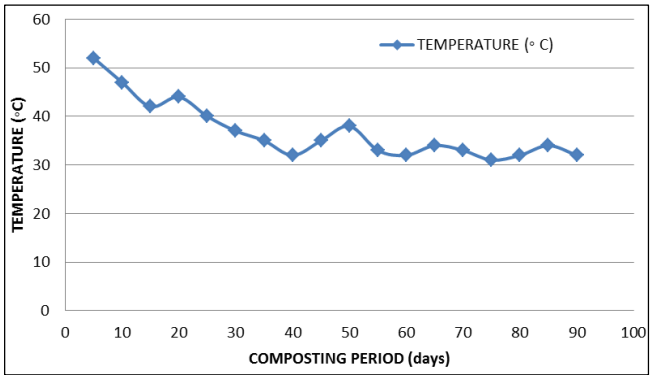

Fig 1: Graph representing temperature during various stages of composting

Detection of OTC in manure sample

Initially the concentration of OTC in the raw poultry litter was observed to be 216.67 mg kg^{-1}. On subjecting the raw materials to composting they showed decreasing trend of concentrations in the manure samples. During the composting process OTC concentration were observed as 120 mg kg^{-1}, 70.49 mg kg^{-1} and 33.97 mg kg^{-1} in the 30th, 60th and 90th day of manure samples respectively. The concentration of OTC in the samples has decreased 55.38% on 30th day from the initial poultry excreta, 32.53% decrease on 60th day and 15.678% decrease on the 90th day of composting process. The decrease in the concentration in the manure samples may be due to thermophilic stage through which the samples have to undergo during the composting process. However, at the end of the 90th day of composting experiment, the OTC concentration 33.97 mg kg^{-1} still remained. Details of the changes in OTC concentrations in the initial sample and during the composting process were presented in fig. 2, 3, and 4.

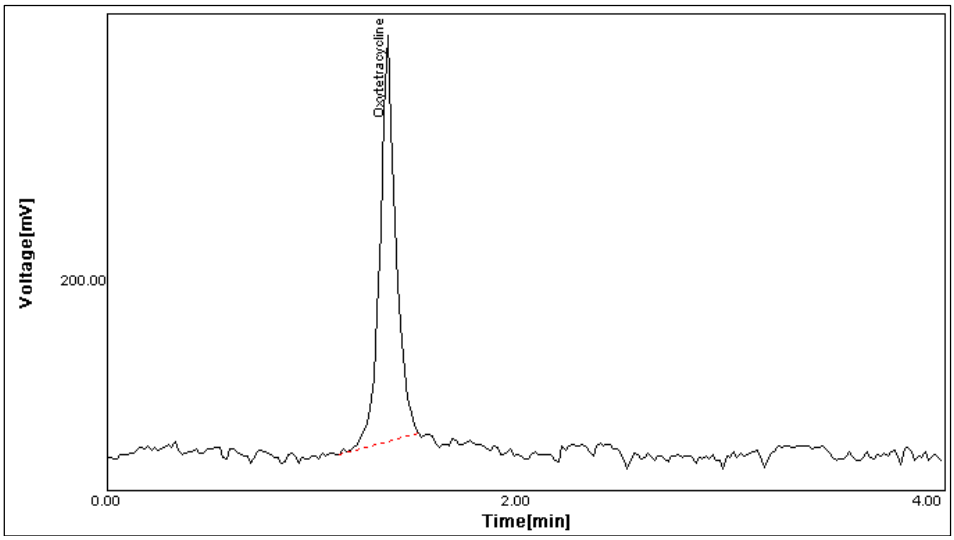

Fig 2: Showing OTC standard peak with the with RT 1.36; Area [mV*s]-976.28; Area % - 100; Height (mV)-183.514; Height % - 100

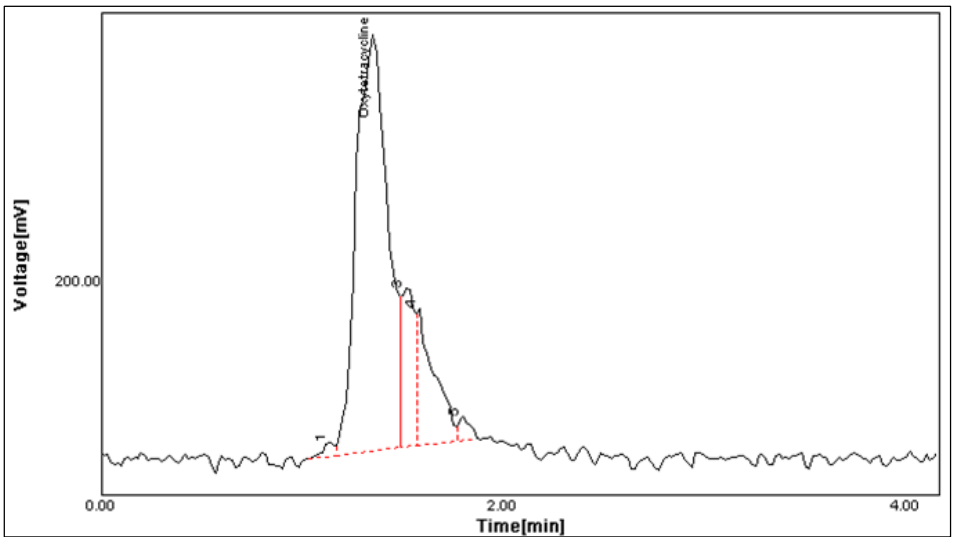

Fig 3: Showing PL excreta intial sample with RT 1.36; Area [mV*s]-2115.34; Area % - 72.42; Height (mV)-196.176; Height % - 55.70

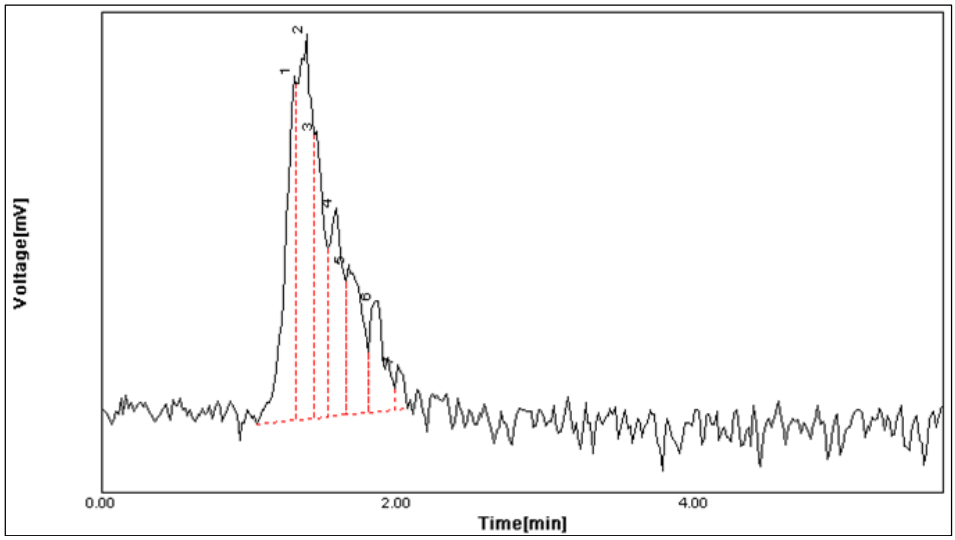

Fig 4: PL 90 with the RT 1.36; Area [mV*s]-331.691; Area % - 27.37; Height (mV)-53.2008; Height %-25.17

Discussion

The present study clearly shows that change in the physico chemical parameters were similar to that of other research carried on poultry manure composting process. The pH of the compost varied from acidic to alkaline, EC of the composting material showed the decreasing trend and it was found to be higher in the initial sample. The OC of the composted material were found to decrease due to carbon material used as a source of food by microbes in the degradation process. The C: N ratio decreased due to the degradation of carbon material in composting process. The TN, TP and TK has increased and recorded the highest percentage of 1.89%, 1.09% and 2.34% on 90th day of composting process. The results furnished in table 1. proved that the OTC present in the manure samples has not influenced the physico chemical properties of the manure.

The degradation of oxytetracycline during composting treatment may be due to higher biological activity as a result of elevated temperatures. Rose *et al*. (1996) found that oxytetracycline rapidly degraded in heated sterile water, indicating abiotic degradation processes. In their study, oxytetraycline half-life was 2, 15, and 120 min in water at 100, 80, and 62 °C, respectively. Hence, temperature has a great influence on reducing the concentration of OTCs in the composting material.

OTC in the initial poultry litter and manure samples were detected in the HPLC with the rapid extraction using EDTA-MC Ilvaine buffer solution. The retention time of OTC was obtained 1.36 minutes for the elution of OTC. The detection and quantification limit of OTC by HPLC-DAD was found to 0.01 and 0.05 mg/L irrespective of the matrices analyzed.

The average recoveries for OTC from initial poultry litter and manure samples were 80.00 per cent across different concentrations. Since the replication standard deviation was less than 2.0 per cent and an average recovery was more than 80 per cent for OTC, the present method of analysis using HPLC-DAD by employing EDTA-MC Ilavine buffer solution as extractant was found to be sensitive enough. Adequate resolution of all OTC peaks was achieved within a relatively short time. Hence, the extraction and chromatographic procedures are useful for screening and quantification of the OTC in samples. The OTC concentration obtained in the chromatographic fashion reveals that there is a decreasing trend of the OTC concentration in the manure samples. The OTCs degradation depends on various environmental factors, including temperature, moisture and redox conditions as well as biological factors (Doi *et al.*, 2000; Dolliver *et al.*, 2008**;** Wang *et al.*, 2008 and Yang *et al.*, 2009) [6, 7, 20, 22]. Temperature is an important factor that could influence OTCs degradation (Doi *et al.*, 2000) [6]. In steer manure, OTC degradation was accelerated by increasing moisture and temperature under aerobic conditions, and thermal degradation became noticeable at high temperatures (Wang *et al.*, 2008) [20]. During the pig manure-rice straw composting process, the temperature in the composting pile was maintained at 50 to 65 ◦C for about 12 days. Therefore, high temperatures could significantly accelerate degradation of TCs (Chai *et al.*, 2016) [3].

Yang *et al*. (2009) [22] found that the half-life of OTC in soil under aerobic conditions were 29-56 days for non-sterile soil and 99-120 days for sterile soil. And in a recent study, it was discovered that microbial action is a major process that results in the degradation of TCs in swine wastewater (Chang *et al.*, 2014) [4]. As reported in several investigations, composting has been identified as a feasible and effective way to reduce the environmental impact of antibiotics in manure (Ho *et al.*, 2011; Selvam *et al.*, 2012 and Bao *et al.*, 2009) [18, 2]. During pig manure composting, degradation of three tetracyclines CTC, OTC and TC predominately took place in the thermophilic stage (>50 ºC) of the composting process (Wu *et al.*, 2011) [21].

Conclusion

The results of this study have shows that the raw chicken manure samples studied were contaminated with oxytetracycline and this antibiotic contamination might be due to it being used as a feed additive for therapeutic purposes and for improving growth. However, the composting undergoing thermophilic process has substantially reduced the quantity of antibiotic in the final composted samples. The concentration of 33.97 mg kg^{-1} of OTC was recorded at the end of the composting process. It is notable that poultry litter as a raw application to the land can cause contamination of OTC which may have the direct influence on the soil microbes, plants and also to the humans, where as composting the poultry litter can lessen the adverse effect creating to the environment. Future work should establish the optimum period and composting technique necessary for complete degradation of OTC in chicken manure as well as identify the

antibiotic resistant and degrading microorganisms involved in the thermo composting.

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