

Effects of Composts on Sorption and Dissipation of Tetracyclines in Different Soils of Aligarh District

O P BANSAL

Department of Chemistry, D.S. College, Aligarh 202 001 (U.P.), India

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This study pertains to the investigation of the effects of various composts, farm yield manure (FYM); sewage sludge and poultry manure on the sorption and dissipation of three tetracycline antibiotics viz., chlortetracycline (CTC), oxytetracycline (OTC) and tetracycline (TC) on six agricultural fields' soil samples of Aligarh district. The results of the study revealed that the sorption of TCs increased with the increase in their concentration both in compost amended and unamended soils, denoting that TCs sorption is influenced by the soil properties mainly organic carbon and cation exchange capacity (CEC). The TCs were adsorbed most strongly on composts and their sorption was enhanced significantly when composts were present in soils. This enhancement of sorption might be attributed to the increased organic carbon content and CEC. Sorption followed the compost order sewage sludge > FYM > poultry manure. The adsorptive capacity of soils was in the order: Soil No. 1 > 2 > 3 > 4 > 5 > 6, and was significantly positively correlated with soil organic carbon and CEC. The interaction between TCs and compost might occur via multiple bonding mechanisms including ionic bond between negatively charged organic matter and positively charged TCs and/or hydrogen bonds in between TCs and organic matter. The sorption isotherms of compost amended and unamended soils were best fitted in Freundlich adsorption equation and was 'L' type showing that there is minimum competition of solvent for sites on the soil surfaces. The TCs dissipation followed the first order, and time required for 50% dissociation (DT_{50}) ranged in between 29.1 to 43.4 d and that time required for 90% dissociation (DT_{90}) in between 96.7 to 144.2 d. Soils amended with compost enhanced the stability of TCs and reduced their mobility. The dissipation of TCs in soil environment was slow, indicating that these compounds may persist in soils.

Key Words: Antibiotics; Compost; DT_{50} ; Organic Carbon; Soils

Introduction

The use of antibiotics as veterinary pharmaceuticals has become the integral part of the animal food industry because of their valuable contributions in treating diseases [1], as growth promoters [2], and in improving feed efficiency [3]. The tetracycline's [Tetracycline (TC), Oxytetracycline (OTC) and chlortetracycline (CTC)] are broad spectrum antibiotics widely used in animal production. Tetracyclines contribute approximately 50% of total antibiotics production. After the application to animals, antibiotics will eventually enter the

environment, the major route being the excretion of faeces and urine from medicated animals in live stocks and poultry farms and the subsequent application of contaminated manure as fertilizer in the agricultural land. Thus there is a genuine concern that residual concentration of antibiotics in agricultural soils can easily reach levels equal to that in pesticides if the manure loading for fertilizer increased to kg level per hectare [4].

This widespread contamination of antibiotics in the environment also put human health and sensitive ecosystem at risk, as antibiotics may alter

the composition and diversity of indigenous soil microbial communities and may also cause the formation of antibiotic-resistant organisms in the environment and possibly threatens human health by diminishing the success of antibiotic treatments.

The use of composts derived from source-separated municipal solid waste/animal manure/FYM is now a common agronomic practice throughout the world. Such amendments improve the physico-chemical properties of the soil. Applications of such composts affect the fate and mobility of antibiotics in soil as the addition of compost increases nutrients besides the soil organic matter content [5].

The purpose of the present studies is to examine the sorption of tetracycline on soils, composts and soil/composts mixture using batch experiments as well as the dissipation of TC, OTC and CTC in soils and soil-compost mixture.

Material and Methods

The six soils (1-6) selected for this study were taken from different parts of Aligarh district at plough layer (0-30 cm). All the soils were air dried at room temperature and sieved by passing through a 100 mesh sieve. Their physico-chemical properties were determined by the usual soil laboratory methodology and clay mineralogy by an X-ray diffraction procedure on orientated specimen. Physico-chemical properties, clay mineralogy and classification of soils and different composts are given in Table 1.

Soil Amendment

Soils (1-6) were amended separately with 0, 2.5 and 5 g of organic manure (FYM, sewage sludge and poultry manure) kg^{-1} soil at 60% moisture level of water holding capacity and incubated for 45d at the temperature 25 ± 2 °C.

Adsorption Studies

Adsorption experiments were conducted by taking soil samples (1 g) (unamended and amended with organic material at 60% moisture level) in a large number of stoppered conical flasks adding various amounts of antibiotic solutions (0-15 mL of $50 \mu\text{g mL}^{-1}$ in $0.01 \text{ mol L}^{-1} \text{ CaCl}_2$) and making up the

volume to 100 mL with distilled water. The suspensions were shaken for 30 h at 25 ± 2 °C (preliminary studies indicated that equilibrium was attained within <27 h) followed by centrifugation.

For the dissipation studies 5 g soil samples (unamended and amended with organic material at 60% moisture level) were taken in a large number of stoppered conical flasks. These flasks were spiked with 100 mg kg^{-1} tetracycline in methanol separately and were aerated by opening the lid of tube and shaking the tubes daily during the incubation period. The flasks were capped and left in an incubator at 25 ± 1 °C after 0, 1, 7, 14, 21, 28, 35, 42, 49 and 56 days of treatment the samples were taken out and TCs were extracted and analyzed immediately to determine the concentration.

Extraction and Analysis of Tetracyclines

The TCs were extracted from the soils and soil-compost mixtures samples by solid phase extraction method [6]. 1 g of soil sample was taken in a centrifuge tube, 15 ml of extraction buffer [0.1 M Mellvaine buffer ($\text{Na}_2 \text{HPO}_4$ and citric acid); 0.1 M EDTA and methanol 25:25:50 v/v] was added to it. The tubes were mixed with a vortex for 30 sec and then placed in an ultrasonic bath for 10 minutes, followed by centrifugation at 1200 g for 15 min. The supernatant was decanted into a glass bottle. The soil residues were extracted two more times and supernatants were combined and diluted to 400 mL with water, the pH of solution was adjusted to 2.8 using phosphoric acid. The three antibiotics were extracted from the solution by solid phase extraction, using an Isolute strong anion exchange cartridge to remove interfering humic materials in tandem with a hydrophilic-lithophilic-balanced cartridge to extract the compounds. The cartridges were preconditioned with methanol and buffer, after washing the extracts were eluted with $2 \times 1 \text{ mL}$ of methanol. A flow rate of 10 mL min^{-1} was used for the extractions. The extracts were stored at -20 °C till the completion of analysis.

The concentration of TC, OTC and CTC in soil and water extracts were analyzed by HPLC using an Agilent 1100 system with an octadecilsilan column

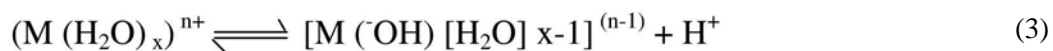
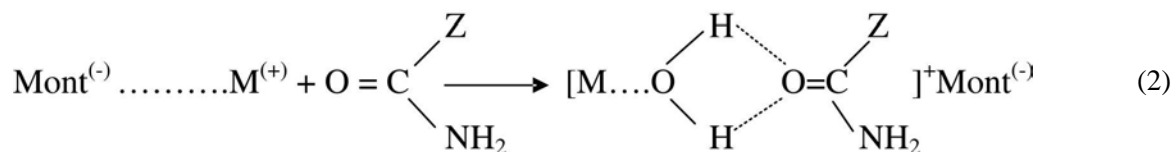
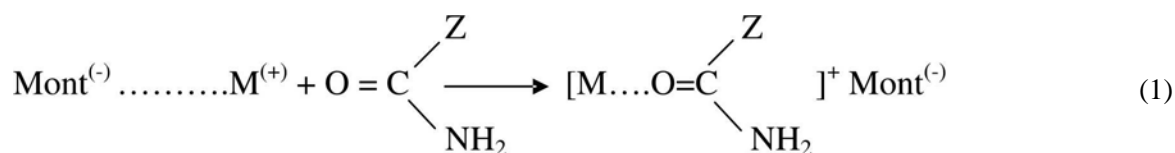
(50mmx4mmx3um, AQ-YMC), TC, OTC and CTC were analyzed simultaneously. A gradient elution was carried out over 20 min with 0.1% formic acid in acetonitrile (Solvent A) and 0.1% formic acid in water (Solvent B). The initial percent of Solvent A was 5%, which was then increased to 30% from 0 to 7 min and remained at 30% from 7 to 8.5 min. The percentage of Solvent A was returned to 5% from 8.5 to 10 min and remained at 5% from 10 to 12 min. The flow rate was maintained at 0.70 mL min⁻¹ throughout the analysis and simultaneous detection of TC, OTC and CTC was performed at 360 nm. Retention times of OTC, CTC and TC were 6.4, 17.3 and 9.6 min. respectively. The minimum limit of detection was 0.5µg kg⁻¹ soil.

All the chemicals used were of analytical grade and all the experiments were done in three replicates.

Results and Discussion

The sorption isotherms (Fig. 1) showed that the quantity of TCs adsorbed on all matrices increased with increasing TC concentration in equilibrium solution. The slope of the isotherm steadily decreases with the rise in solute concentration as vacant sites become less accessible with the progressive covering of the surface. The curvilinear isotherm suggests that the number of available sites for the adsorption become a limiting factor. The sorption isotherms were convex or 'L' type (Fig. 1) [7]. These kinds of

isotherm arise because of minimum competition of solvent for sites on the adsorbing surface. The adsorption was in the order CTC > OTC > TC. The adsorption of all the three antibiotics followed the order soil 1 > 2 > 3 > 4 > 5 > 6 supporting the hypothesis that clay content, CEC and organic matter plays an important role in sorption of antibiotics. The sorption isotherms for organic manure amended soils (Fig. 1) were non-linear and similar to those unamended soils. Sorption of TCs increased significantly with the addition of composts in soils as composts increase the organic carbon content and CEC of soils. The amount of sorption was directly correlated with the amount of compost added. The sorption of TCs followed the order composts > amended ++ soil > amended + soil > unamended soil. This increase could be related to the sorption of organic matter to the soil by increasing the sorption sites available for adsorption as organic matter enhanced carboxyl and phenolic groups that may interact with polar groups of TCs. The interaction between TCs and organic manure occur via multiple bonding mechanisms including ionic bond between negatively charged organic matter and positively charged TCs and/or hydrogen bonds in between TCs and organic matter [8]. Earlier sorption studies by the author [9] on pure clay indicated that sorption of TCs on clay surfaces occur via cation bridging, ionic bonding and hydrogen bonding as:



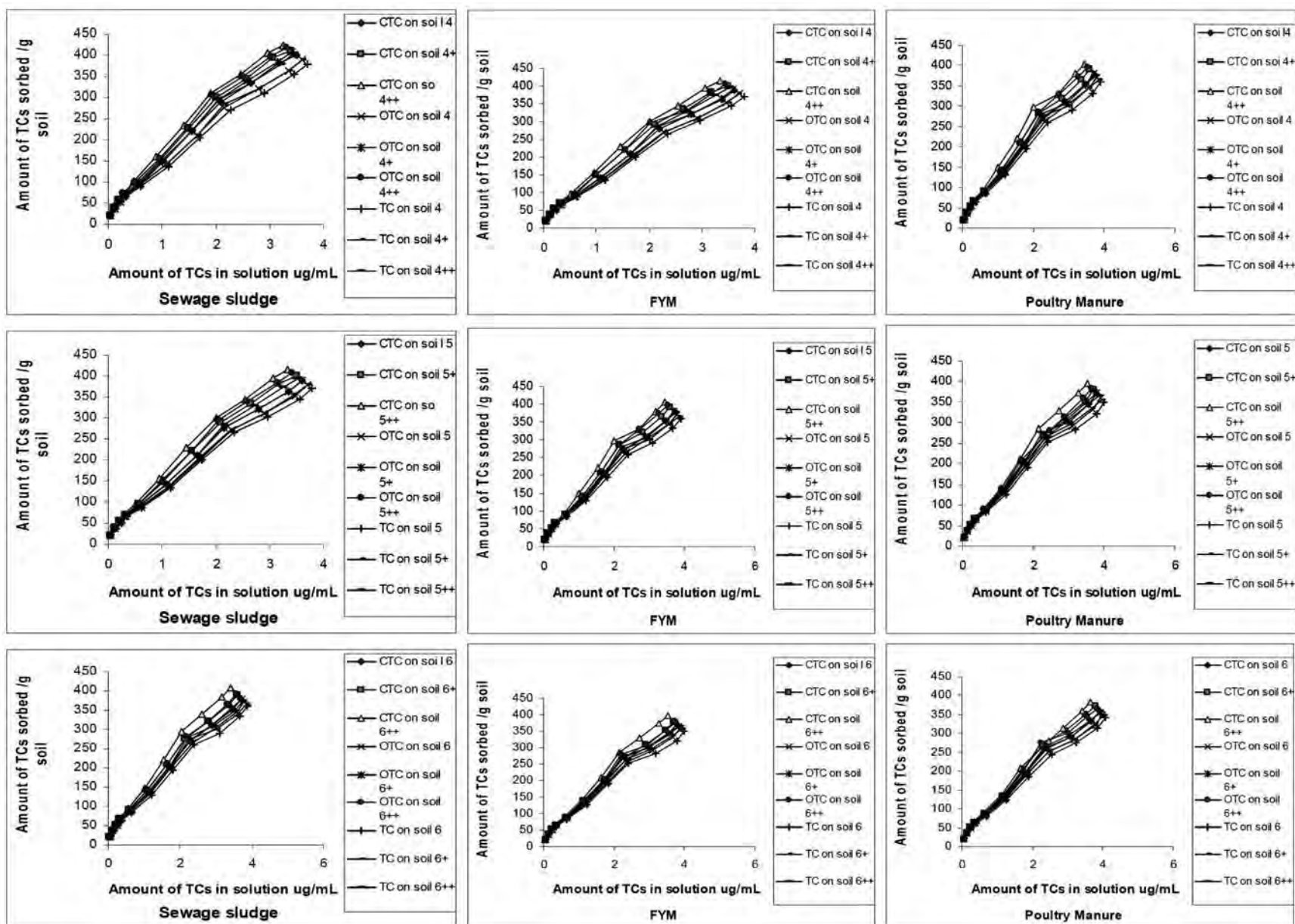
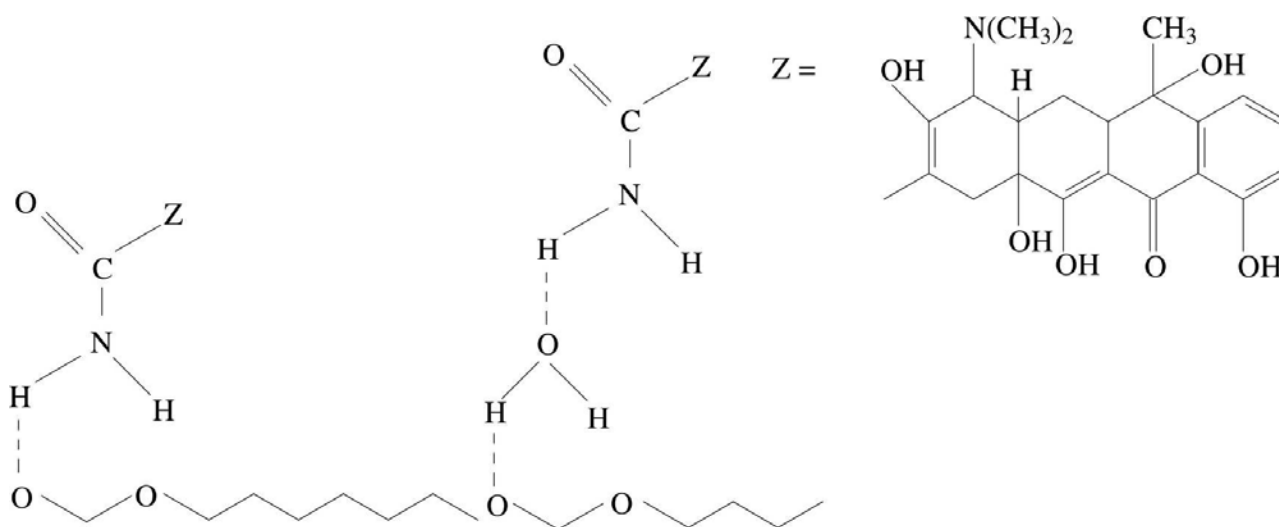
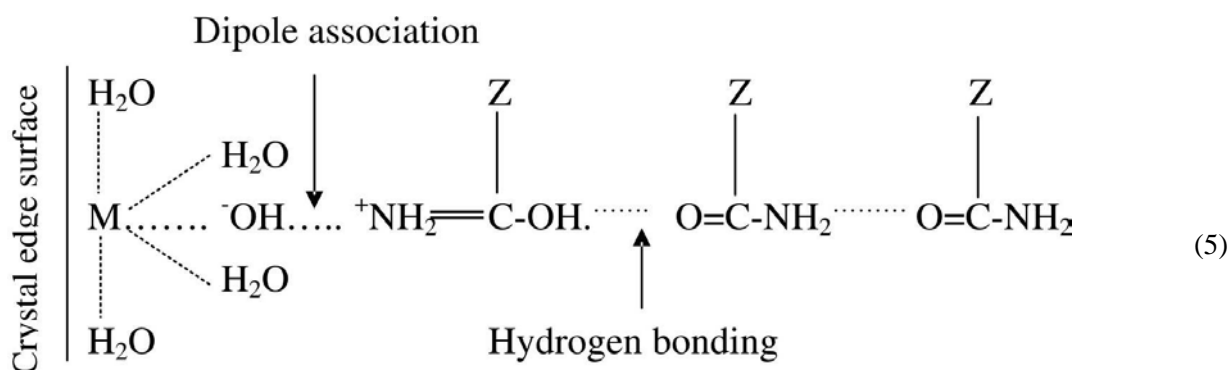
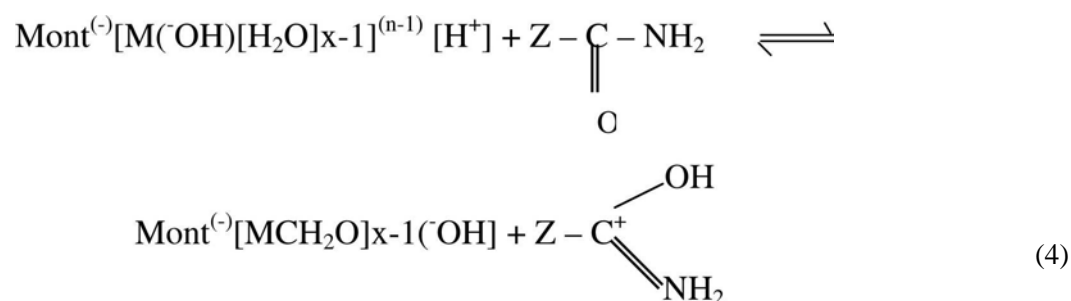


Fig. 1: Adsorption isotherms for sorption of CTC, OTC and TC on soils (1 and 6) in compost amended and un-amended soils

The protons released bind the antibiotic molecule to the clay surface by ionic bonding as:



The adsorption equations, Freundlich and Langmuir were used to fit the sorption of TCs in the sorbents. The results showed that Freundlich relationship ($R^2 > 0.88$) can best be used to describe TCs adsorption results on soils and soil/compost of Aligarh district. The linear form of this equation is $\log C = \log K + 1/n \log C_e$, where, C is the amount

(mg kg^{-1}) of TCs adsorbed, C_e is the equilibrium concentration in solution (mg L^{-1}), K ($\text{mg}^{1-1/n} \text{L}^{1/n} \text{kg}^{-1}$) is the Freundlich adsorption coefficient and $1/n$ is a descriptor of isotherm curvature. The values of $1/n$ (Table 2) were less than unity indicating 'L' type of isotherm. The values of K and $1/n$ (Table 2) followed the order $\text{CTC} > \text{OTC} > \text{TC}$ and were

Table 1: Characteristics of soils

S.No.	Place	Taxonomical name	Soil units	Silt %	Clay %	pH (1:2:5)	Organic C %	CaCO ₃ %	CEC (Cmol (P ⁺) kg ⁻¹)	Surface area m ² g ⁻¹	Major clay minerals
1.	Hathras	Entisol	Typic Ustochrept	38.8	16.2	7.8	0.88	6.3	12.8	38.6	Q,I,C
2.	Sikandra Rao	Acidisol	Typic Arigids	52.0	14.0	8.8	0.72	7.6	11.6	36.2	Q,I,C
3.	Datawali	Aridisol	Typic Orthids	37.5	12.0	7.4	0.63	5.8	10.8	30.1	Q,M,I,C
4.	Khair	Aridisol	Typic Orthids	44.9	13.6	7.6	0.56	6.6	10.3	27.2	Q,I,C
5.	Atrauli	Alfisol	Calciorthents	24.9	9.8	8.3	0.44	8.8	9.9	32.1	Q,I,K,C
6.	Bank of Yamuna Tappal	Inceptisol	Calciorthents	27.1	8.5	8.1	0.48	9.0	8.6	25.6	Q,I,K,C
	Sewage Sludge					7.6	26.6		46		
	FYM					7.9	23.3		42		
	Poultry Manure					6.9	20.2		38		

Q = Quartz, I – Illite, M = Montmorillonite, C = Calcite, K = Kaolinite

maximum for composts followed by amended ++ soil > amended + soil > unamended soil. The data of Table 2 also denote that sorption of TCs on different soils were in the order: Soil 1 > 2 > 3 > 4 > 5 > 6, supporting the inferences regarding sorption of TCs on soils. The adsorption of TCs in presence of different composts followed the order sewage sludge > FYM > poultry manure which may be significantly correlated with organic carbon content and CEC of compost.

The values of Kd (distribution coefficient) [Kd = (X/m) / Ce] were 242.5-48.4; 210-35.3 and 181.7-26.7 for sewage sludge, FYM and poultry manure amended soils respectively, supporting the above inferences. The values of Kd increases with the amendment of organic manure (28-8.2 to 42.5-13 on amending 0-5 g sewage sludge kg⁻¹ soil). The values were minimum in unamended soil No. 6 and maximum in sewage sludge amended soil 1. These results confirm the role of organic matter as the primary adsorbing component for the studied TCs. Similar results are also reported by other workers [10-11].

Preliminary studies denoted that the dissipation of TCs on studied samples followed the first order kinetics, as all the generated regression lines have a coefficient of determination (R²) more than 0.92.

The Equation (1) describes the dissipation kinetics and Equation (2) is used to calculate dissipation half lives.

$$C_t = C_0 \times e^{-(kt)} \quad (1)$$

$$DT_{50} = 0.693/k \quad (2)$$

$$DT_{90} = 2.303/k \quad (3)$$

where, C₀ and C_t are the concentration of analytes at time 0 and time t (days) and k is the first order rate constant determined as the slope value from test substance dissipation curves.

The time taken for dissipation of 50% (DT₅₀) of the CTC in all the treatments were 31.2 to 43.4 d and the time taken for 90% dissipation (DT₉₀) were 103.7 to 144.2 d (Table 3) which were longer than OTC (30.5-42.1 and 101.3-139.9 d respectively), which were longer than TC (30-41.2 and 99.7-136.9 d respectively), denoting that CTC was more stable in the soils and soil compost mixture followed by OTC and TC. An examination of data of Table 3 showed that the rate of degradation followed the soil order S₆ > S₅ > S₄ > S₃ > S₂ > S₁ indicating thereby a role of soil organic matter, CEC and clay content [11-12]. The rate of degradation was inverse of the order of sorption. The DT₅₀ or DT₉₀ increased with

Table 2: Freundlich adsorption constants for sorption of tetracyclines on different soils of Aligarh district in unamended and compost amended soils

Soil compost	Sewage Sludge						FYM						Poultry Manure					
	CTC		OTC		TC		CTC		OTC		TC		CTC		OTC		TC	
	k	1/n	k	1/n	k	1/n	k	1/n	k	1/n	k	1/n	k	1/n	k	1/n	k	1/n
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
	1534± 16	0.925± 0.005	1366± 16	0.910± 0.006	1267± 12	0.895± 0.006	1332± 19	0.910± 0.006	1244± 12	0.895± 0.006	1104± 12	0.885± 0.005	1267± 12	0.885± 0.005	1174± 12	0.875± 0.005	1117± 15	0.865± 0.004
1	1024± 12	0.885± 0.004	936± 10	0.875± 0.006	886± 9	0.865± 0.004	944± 10	0.875± 0.006	900± 10	0.870± 0.004	854± 12	0.860± 0.005	912± 9	0.870± 0.004	866± 12	0.860± 0.005	824±5	0.850± 0.005
1+*	1136± 14	0.895± 0.005	972± 10	0.880± 0.005	922± 10	0.870± 0.005	984± 10	0.885± 0.005	934± 10	0.875± 0.005	884± 8	0.865± 0.004	932± 10	0.875± 0.005	888±8	0.855± 0.004	845±8	0.855± 0.004
1++*	1188± 16	0.900± 0.005	1014± 12	0.885± 0.006	972± 12	0.875± 0.005	1034± 12	0.890± 0.006	986± 12	0.875± 0.005	926± 9	0.870± 0.005	978± 12	0.875± 0.005	922±9	0.860± 0.005	876±9	0.860± 0.005
2	924± 10	0.880± 0.003	808± 8	0.870± 0.005	745± 8	0.860± 0.005	814± 8	0.870± 0.005	762± 8	0.865± 0.005	704± 8	0.855± 0.003	748± 8	0.865± 0.005	720±8	0.855± 0.003	692±8	0.850± 0.003
2+	965± 11	0.885± 0.004	842± 9	0.875± 0.005	808± 8	0.865± 0.004	856± 9	0.875± 0.005	824± 8	0.870± 0.004	756± 8	0.855± 0.003	822± 8	0.870± 0.004	776±8	0.860± 0.003	738±8	0.855± 0.003
2++	1014± 12	0.890± 0.003	872± 10	0.875± 0.005	842± 9	0.865± 0.005	888± 8	0.880± 0.005	854± 9	0.865± 0.005	801± 8	0.860± 0.002	851± 9	0.865± 0.005	808± 8	0.860± 0.002	777±8	0.860± 0.002
3	854± 9	0.865± 0.003	784± 7	0.850± 0.004	734± 7	0.845± 0.003	800± 8	0.855± 0.004	752± 7	0.845± 0.003	688± 9	0.840± 0.003	754± 7	0.845± 0.003	705± 9	0.840± 0.003	668±9	0.830± 0.003
3+	889± 10	0.875± 0.005	827± 8	0.860± 0.005	777± 8	0.850± 0.003	841± 8	0.865± 0.005	792± 8	0.850± 0.003	734± 7	0.845± 0.002	798± 8	0.850± 0.003	754± 7	0.845± 0.002	706±7	0.835± 0.002

Table 2 contd.

Conrn. of Table 2

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
3++ 10	937± 0.003	0.880± 9	868± 0.003	0.870± 9	826± 0.002	0.860± 9	880± 0.003	0.870± 9	842± 0.002	0.860± 7	780± 0.002	0.850± 9	836± 0.002	0.860± 7	784± 0.002	0.850±	748±7 0.002	0.840±
4 8	788± 0.004	0.850± 6	738± 0.003	0.840± 6	678± 0.003	0.835± 7	744± 0.003	0.845± 6	702± 0.003	0.835± 7	642± 0.003	0.825± 6	714± 0.003	0.835± 7	662± 0.003	0.830±	642±7 0.003	0.825±
4+ 10	832± 0.003	0.860± 7	772± 0.002	0.845± 7	732± 0.002	0.835± 7	780± 0.002	0.850± 7	746± 0.002	0.840± 6	697± 0.002	0.830± 7	748± 0.002	0.840± 7	703± 0.002	0.835±	682±7 0.002	0.830±
4++ 9	856± 0.004	0.865± 7	812± 0.003	0.850± 7	764± 0.002	0.840± 7	821± 0.003	0.855± 7	788± 0.002	0.845± 6	729± 0.004	0.835± 7	774± 0.002	0.845± 6	744± 0.004	0.835±	714±6 0.004	0.835±
5 8	734± 0.002	0.835± 6	672± 0.002	0.830± 6	632± 0.003	0.825± 6	677± 0.002	0.830± 6	644± 0.003	0.825± 6	596± 0.003	0.820± 6	640± 0.003	0.825± 6	602± 0.003	0.820±	584±6 0.003	0.810±
5+ 8	777± 0.003	0.845± 5	712± 0.002	0.835± 5	666± 0.002	0.825± 6	719± 0.002	0.840± 5	686± 0.002	0.830± 5	632± 0.005	0.820± 5	688± 0.002	0.830± 5	652± 0.005	0.820±	618±5 0.005	0.815±
5++ 6	822± 0.004	0.850± 6	762± 0.003	0.840± 6	724± 0.004	0.830± 6	771± 0.003	0.845± 6	736± 0.004	0.835± 5	672± 0.005	0.825± 6	738± 0.004	0.835± 5	678± 0.005	0.825±	634±5 0.005	0.820±
6 6	666± 0.003	0.830± 5	615± 0.002	0.825± 5	585± 0.003	0.820± 5	622± 0.002	0.825± 5	602± 0.003	0.820± 7	556± 0.004	0.815± 5	585± 0.003	0.820± 7	544± 0.004	0.815±	518±7 0.004	0.810±
6+ 8	698± 0.003	0.835± 5	646± 0.004	0.825± 5	624± 0.005	0.820± 5	650± 0.004	0.830± 5	632± 0.005	0.825± 8	578± 0.005	0.815± 5	624± 0.005	0.825± 8	578± 0.005	0.815±	542±8 0.005	0.815±
6++ 7	726± 0.003	0.840± 7	686± 0.003	0.830± 7	654± 0.005	0.825± 7	693± 0.003	0.835± 7	666± 0.005	0.830± 9	606± 0.003	0.820± 7	659± 0.005	0.830± 9	626± 0.003	0.820±	602±9 0.003	0.820±

* + 2.5 g compost, ++ 5 g compost kg⁻¹ soil.

Table 3: Time for dissipation of 50% and 90% (DT_{50} and DT_{90} respectively) of chlortetracycline (CTC), oxytetracycline (OTC) and tetracycline (TC) in different soils and soil/compost mixtures (+ 2.5 g manure, ++ 5 g manure kg^{-1} soil)

Soil	Sewage Sludge						FYM						Poultry Manure					
	CTC		OTC		TC		CTC		OTC		TC		CTC		OTC		TC	
	DT_{50}	DT_{90}	DT_{50}	DT_{90}	DT_{50}	DT_{90}	DT_{50}	DT_{90}	DT_{50}	DT_{90}	DT_{50}	DT_{90}	DT_{50}	DT_{90}	DT_{50}	DT_{90}	DT_{50}	DT_{90}
1	38.5	127.9	37.7	125.3	37	122.9	37.8	125.6	37	122.9	36.4	120.9	37.1	123.3	36.5	121.3	36	119.6
1+	41.5	137.9	40.2	133.5	39.4	130.9	40.3	133.9	39.4	130.9	38.7	128.6	39.4	130.9	38.7	128.6	38.1	126.6
1++	43.4	144.2	42.1	139.9	41.2	136.9	42	139.5	41.2	136.9	40.4	134.2	41.3	137.2	40.4	134.2	39.7	131.9
2	37.1	123.3	36.3	120.6	35.7	118.6	36.4	120.9	35.5	117.9	35.2	116.9	35.6	118.3	35	116.3	34.8	115.6
2+	40	132.9	39	129.6	38.2	126.9	38.7	128.6	38.1	126.6	37.6	124.9	37.9	125.9	37.4	124.3	37	122.9
2++	41.5	137.9	40.2	133.5	39.3	130.6	40	132.9	39.3	130.6	38.5	127.9	39	129.6	38.5	127.9	37.8	125.6
3	35.7	118.6	35	116.3	34.4	114.3	35	116.3	34.2	113.6	33.9	112.6	34.2	113.6	33.6	111.6	33.4	111
3+	37.6	124.9	36.8	122.3	36.1	119.9	36.3	120.6	36	119.6	35.4	117.6	34.5	114.6	35.2	116.9	34.9	115.9
3++	39.1	129.9	38	126.2	37.3	123.9	37.5	124.6	37.1	123.3	36.5	121.3	36.8	122.3	36.2	120.3	36	119.6
4	33.1	110	32.3	107.3	31.8	105.6	32	106.3	31.6	105	31.3	104	31.3	104	31	103	30.8	102.3
4+	35.4	117.6	34.5	114.6	33.7	112	34	113	33.7	112	33.1	110	33.2	110.3	33	109.6	32.5	108
4++	37.8	125.6	36.8	122.3	36	119.6	36.3	120.6	36	119.6	35.3	117.3	35.4	117.6	35	116.3	34.6	115
5	32.0	106.33	31.4	104.3	31	103	31	103	30.7	102	30.4	101	30.2	100.3	30.1	100	30	99.7
5+	33.8	112.3	33	109.6	32.4	107.6	32.4	107.6	32.3	107.3	31.7	105.3	31.5	104.7	31.4	104.3	31.1	103.3
5++	35.4	117.6	34.5	114.6	33.8	112.3	34	113	33.6	111.6	33	109.6	33	109.6	33	109.6	32.4	107.6
6	31.2	103.7	30.5	101.3	30	99.7	30.3	100.7	30.1	100	29.5	98	29.6	98.3	29.4	97.7	29.1	96.7
6+	32.6	108.3	32	106.3	31.4	104.3	31.7	105.3	31.3	104	30.8	102.3	31	103	30.5	101.3	30.2	100.3
6++	33.2	110.3	32.5	108	32	106.3	32.5	108	31.8	105.6	31.3	104	31.7	105.3	31	103	30.6	101.7

increase in amount of compost. The dissipation in different compost amended soils was in the order sewage sludge < FYM < poultry manure which might be significantly correlated with soil organic carbon and CEC which in turn enhances the sorption of TCs [12]. The difference in dissipation times of different antibiotics on same substrates may be attributed to their characteristics and the nature of substrate.

Conclusions

The batch sorption studies of this work demonstrate that the sorption behaviour of TCs in soils is caused by interactions of soils and antibiotics which is related

to soil and antibiotic properties. The results of this study also showed that soil amended with compost enhances the sorption of TCs, reduce their mobility and decrease the environmental risk. The dissipation of TCs in the soil environment was slow denoting that these compounds persist in soils for a longer period.

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