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RESEARCH ARTICLE

BIOLOGICAL DISSIPATION OF TETRACYCLINE IN SOILS UNDER DIFFERENT CONDITIONS

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Abstract

The dissipation kinetics of a widely-used veterinary antibiotic tetracycline (TC) was investigated in the laboratory in six different soils under aerobic and anaerobic conditions. The results showed that the dissipation of TC in soils followed first order reaction kinetics and dissipation rates decreased with increasing concentration of TC. The dissipation of TC was faster under aerobic conditions than under anaerobic conditions. The $t_{½}$ values for TC dissipation under aerobic conditions ranged from 41.2 to 56 days for non- sterile treatments and 95.6 to 130 days for sterile treatments, while under anaerobic conditions the half lives of TC ranged in between 57 and 71 days for non-sterile soils and in between 100 and 144 days for sterile soils, suggesting that microbes can degrade TC in agricultural soils. Strong sorption of TC by soil components, dependent on soil nature, soil pH and soil organic matter, also affects the dissipation of TC.

Keywords: Tetracycline; Soil contamination; Sorption; dissipation; Microbes.

Introduction

Veterinary antibiotics are used in large amounts for therapeutic and prophylactic purposes as well as growth promoters (Sim *et al*., 2011; Aminov, 2009). Antibiotics are specifically designed to control bacteria in human or animals and help to protect their health. Tetracyclines are broad spectrum antibiotics widely used as growth promoters in modern animal husbandry. Tetracycline administered in humans and animals undergo minimal or no metabolism and are excreted in urine and manure in an either unaltered or as metabolites some of which are still bioactive (Sarmah *et al*., 2006), which makes them potentially hazardous to bacteria and other organisms in the environment. These antibiotics are released in the terrestrial environment via the application of animal manure and biosolids containing excreted antibiotics to agricultural land as fertilizer (Diaz-Cruz *et al*., 2006; Kemper, 2008). Antibiotics can also be introduced to the agricultural land through irrigation with sewage waste water (Herklotz *et al*., 2010; Kinney *et al*., 2009).

A survey of literature denotes that there have been some studies regarding biological degradation of antibiotics in soils and factors influencing the degradation. The chemical concentration, soil moisture, temperature and soil physico-chemical properties are the major factors which influence degradation of antibiotics in soils (Laak *et al*., 2006; Parolo *et al*., 2008; Figueoa *et al*., 2004).

Some studies are there on degradation of tetracycline in manure and manure- amended soil (Wang and Yates, 2008) but so far, there has been no study on the influence of redox condition and soil type on the dissipation of tetracycline in soils. So, it was considered useful to investigate dissipation of tetracycline in six different soils of India under different redox conditions at different tetracycline concentrations.

Materials and Methods

Six types of surface soils (0-25 cm depth), which had never been applied with tetracycline were collected from different parts of India. These soils were air dried, crushed and grounded to pass through <70 mesh sieve, then stored at 4^0C before use. The physicochemical properties of these soils are given in Table 1.

Biodegradation

In order to study the biodegradation of tetracycline in soils the experiment was conducted in two sets. In first set of experiments the redox and soil type experiments were conducted on six soils by taking five g of soil in several glass stoppered tubes. These glass stoppered tubes were spiked with 10 mg kg^{-1} tetracycline in methanol separately. These tubes were left in a luminar flow cabin for complete evaporation of methanol, simultaneously sterile controls were performed for each treatment by autoclaving the soils in the glass tubes at 121 ± 2^0C for 20 min. in two days (preliminary studies indicated no viable bacterial existence after this treatment). Aspectic techniques were followed during the spiking processes, including sterilization of glassware and pipettes and operations were performed inside the laminar cabin.

For aerobic dissipation studies, the soil moisture level in each glass tube was adjusted to 50% of maximum water holding capacity and the glass tubes were aerated by opening the lid of tube and shaking the tubes daily during the incubation period. Anaerobic conditions were created by adding about 10 mL of sterile water (2.5 cm) in each tube and sealing the lid. All glass tubes were capped and left in an incubator at 25 ± 1^0C after 0,1,7,14,21,28,35,42,49 and 56 days of treatment the samples were taken out and tetracycline was extracted by adding 10 mL of CH_3OH: H_2O (9:1 ratio) and by centrifuging for 15 min at 13000 rpm. The tetracycline in supernatant was estimated as: The concentrations of TC in extracts were analyzed by HPLC using an Agillent 1100 system with an octadecilsilan column (50mmx4mmx3um, AQ-YMC). 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 0.70 mL min^{-1} throughout the analysis and detection of TC was performed at 360 nm. Retention time of TC was 9.6 min. The minimum limit of detection was 0.5ug kg^{-1} soil.

Microbial activity in the aerobic conditions was studied after 7, 14, 28, 42 and 56 days of spiking by counting the bacterial numbers in soil including in non-spiked soils.

In the second set of experiments glass tubes containing 5 g of soils were spiked with 10, 20 and 50 mg kg^{-1} of tetracycline in methanol and dissipation studies under aerobic conditions were made as discussed earlier.

All the experiments were performed in triplicate.

Results and Discussion

Influence of soil type on dissipation of tetracycline in six different soils under aerobic and anaerobic conditions were conducted with an initial concentration of 10 mg kg^{-1} soil. The data are recorded in Fig. 1. An examination of Fig. 1 showed that the tetracycline concentration in non-sterile soils decreased from 8.44±0.22 to 3.08±0.16 mg kg^{-1} soil during the 56 days incubation period; the % dissipation was 50.6% for soil 1; 58% for soil 2; 61% for soil 3; 51% for soil 4; 59% for soil 5 and 50% for soil 6. The half lives ($t_{½}$) period calculated on the basis of first order kinetics for non-sterile soils were 55, 47.3, 41.2, 53.4, 42.5 and 56 d for soils 1, 2, 3, 4, 5 and 6 respectively. In sterile control soils under aerobic conditions losses of tetracycline has also been observed (Fig. 1) and % dissipation were in the range 25.1 to 31.1 for all the studied six soils, suggesting that losses of tetracycline in sterile soils were due to physiochemical processes. The losses due to biological processes were calculated to be 21.6% for soil 1; 30.3 % for soil 2; 29.8% for soil 3; 25.8% for soil 4; 27.3% for soil 5 and 22.4%for soil 6. To support these inferences microbial activity in soils was estimated by counting soil bacterial numbers after 7, 14, 28, 42 and 56 days of incubation. The results of microbial population are given in Table 2. An examination of Table 2 denote that the bacterial populations in non-spiked soils were in the order soil 3> soil 5> soil 2> soil 4> soil 1> soil 6 and bacterial population decreased significantly in spiked soils in comparison to non- spiked soils and

Table 1.. Selected Physical and Chemical properties of the soils used

Soil	Location	Organic Matter (%)	Organic Carbon (g/ kg soil)	Clay %	Sand %	Silt %	pH (1:2.5)	CEC (cmol (p+) /kg)
S_1	Bangalore	1.24	7.2	19.4	30.4	50.2	6.4	6.5
S_2	Aligarh	1.75	10.1	13.4	38.4	48.2	8.8	11.4
S_3	Kota	3.40	19.7	46.2	11.2	42.6	7.2	30.6
S_4	Jhansi	1.48	9.6	28.2	47.2	24.6	7.5	23.7
S_5	Doiawala	2.30	13.3	20.6	25.2	54.2	5.9	19.5
S_6	Ludhiana	0.78	4.5	30.2	31.2	38.6	8.4	15.2

Table 2. Bacterial Counting ($CFUg^{-1}$ soil X 10^6) at different sampling times in 10 mg kg^{-1} tetracycline treated soils and in untreated soils

Time (Days)	Soil 1		Soil 2		Soil 3		Soil 4		Soil 5		Soil 6	
	Non-spiked	spiked	Non-spiked	spiked	Non-spiked	spiked	Non-spiked	spiked	Non-spiked	spiked	Non-spiked	spiked
7	3.1±1.4	3.0±1.2	10±1.6	8±1.9	64±10	12±4.3	8.2±2.4	6.3±2.1	34±6	8.2±1.2	2.3±1.2	2.8±1.1
14	4.6±1.6	2.6±1.2	16±2.6	6±1.5	85±12	10±2.4	12.4±3.0	5.1±1.2	44±7	7.1±1.0	3.1±1.2	2.3±1.2
28	6.4±1.2	2.1±0.8	20±2.1	4.8±1.3	94±14	8±2.0	14.3±2.4	4.7±1.3	50±7.8	6.20±0.9	3.4±1.3	2.0±0.8
42	5.4±1.1	1.8±0.7	18±2.0	4.3±1.1	80±10	8.5±1.1	12.7±2.1	4.2±1.2	42±6.5	5.6±0.7	3.1±1.1	1.9±0.7
56	5.1±0.9	1.6±0.7	16.2±1.6	4.0±1.0	75±8	8±1.0	11.8±1.7	3.9±1.1	38±3.9	4.9±0.8	2.8±0.9	1.7±0.8

Table 3. First order kinetic parameters for the dissipation of oxamyl spiked with 100 mg kg^{-1} soil.

Soil	Aerobic nonsterile			Aerobic sterile			Anaerobic nonsterile			Anaerobic sterile		
	k (mg kg^{-1} d^{-1})	R^2	$t_{½}$ d	k (mg kg^{-1} d^{-1})	R^2	$t_{½}$ d	k (mg kg^{-1} d^{-1})	R^2	$t_{½}$ d	k (mg kg^{-1} d^{-1})	R^2	$t_{½}$ d
1	0.0192±0.0004	0.98	36	0.0071±0.0002	0.97	98.9	0.0126±0.0004	0.95	55	0.0057±0.0002	0.96	122
2	0.0195±0.0003	0.97	35.5	0.0073±0.0003	0.97	95.4	0.0132±0.0003	0.97	52.5	0.0060±0.0003	0.97	115
3	0.0204±0.0005	0.97	33.9	0.0079±0.0002	0.99	87.5	0.0146±0.0004	0.96	47.6	0.0071±0.0002	0.98	97
4	0.0199±0.0004	0.99	34.7	0.0070±0.0003	0.99	97.2	0.0139±0.0004	0.98	49.5	0.0063±0.0003	0.99	109
	0.0201±0.0005	0.96	34.4	0.0076±0.0004	0.97	91.2	0.0142±0.0004	0.97	48.6	0.0066±0.0004	0.97	105

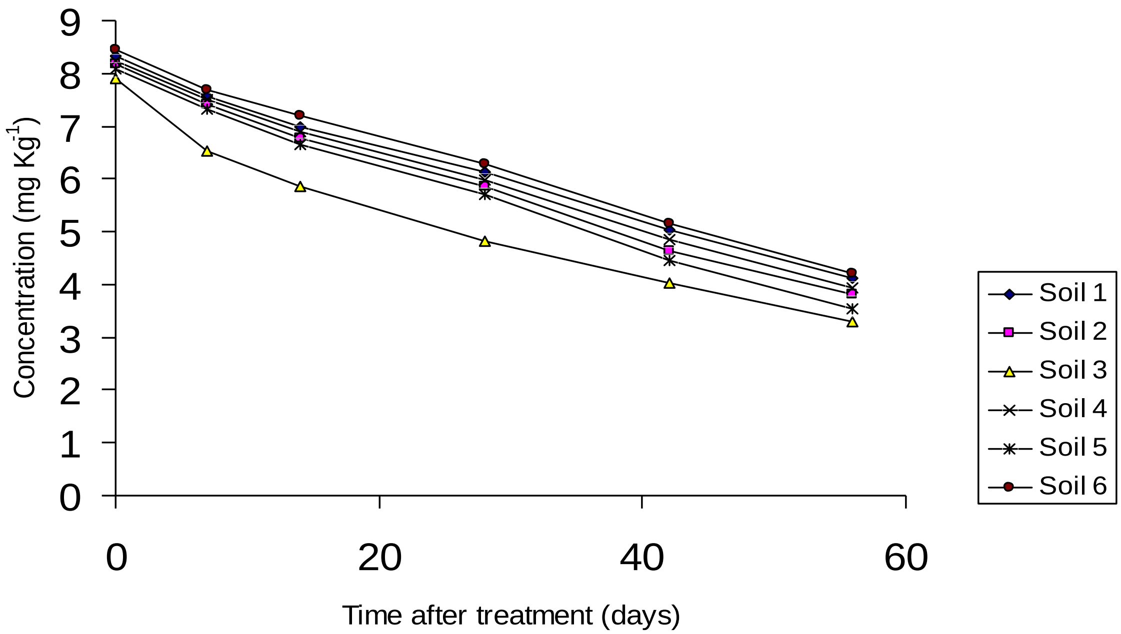

Fig. 1 Tetracycline dissipation in the six soils under aerobic conditions at the spiked concentration of 10 mg kg^{-1}.

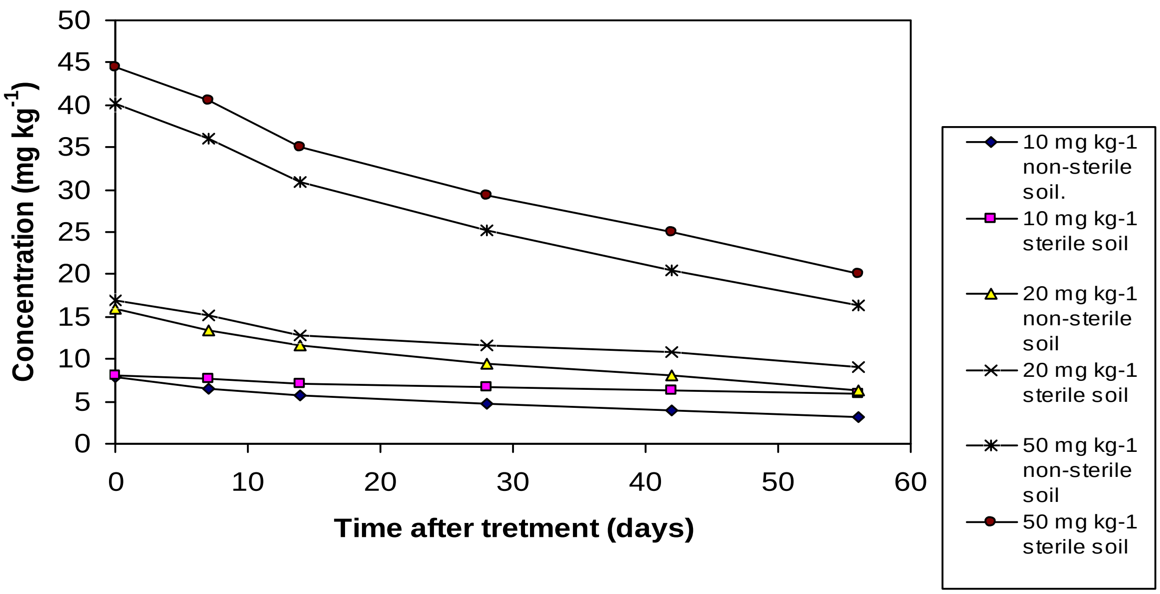

Fig.2 tetracycline dissipation in soil 3 under aerobic conditions at three concentrations (10, 20 and 50 mg kg$^{-1)}$

the effect was minimum in soil 1. The data also denoted that soil 3 contained the most culturable bacteria among all the studied soils. In our study the maximum biological degradation also occurs in soil 3.

Anaerobic dissipation of tetracycline was also studied for all the six soils and results are given in Fig.1. The concentrations of tetracycline in non-sterile studied soils under anaerobic conditions decreased from 8.42±0.24 to 4.12±0.18 mg kg^{-1} soil after 56 days of incubation, while the concentration of tetracycline dissipated from 8.52±0.24 to 6.02±0.16 mg kg^{-1}soil in sterile control soils under anaerobic conditions. The half lives for tetracycline dissipation as calculated from first order reaction kinetics were 57 to 71 days for non-sterile soils (Table 3). From the results it may be concluded that dissipation of tetracycline in soils under anaerobic conditions was slower than under aerobic conditions. As under aerobic conditions, physicochemical processes also played a vital role in dissipation. The results are consistent with the findings of other researchers on some pharmaceuticals and personal care products (Yang et al. 2009; Wu et al. 2008). Comparing with sterile controls, it was found that biological degradation is the dominant process with some contributions from physical and chemical processes.

Soil properties are an important factor that influences tetracycline sorption and dissipation in soil (Jones et al. 2005; Mackay and Canterbury 2005). Though with increase in soil- moisture content the dissipation of antibiotics under aerobic conditions increased (Wang *et al*., 2006; Taylor-Lovell *et al*., 2002), but in present study it was found that rate of degradation of tetracycline in soils saturated with water (2 cm high in the tubes) decreased it might be due to change in redox conditions from aerobic to anaerobic. The % of biological dissipation and bacterial counts in soils are significantly positively correlated with soil organic matter, as soil 3 has maximum organic matter and maximum culturable bacterial number.

These results indicated that soil pH also affects degradation of tetracycline, especially hydrolysis in water (Chen and Huang, 2009). The ionization feature of tetracycline can significantly influence its behaviour including hydrolysis in water and sorption on soil components (Gu and Karthikeyan, 2005; Pouliqen *et al*., 2007). In the present study, the loss of tetracycline due to hydrolysis was limited as previous studies by the author showed that after 33 h of incubation approximately 80-90% of tetracycline was adsorbed by soils. The tetracycline forms strong complexes in soils due to multiple simultaneous interactions between polar and charged functional groups of tetracycline and surface charge sites on the soil via protonation and/or co-ordination of metallic cations on soil clays/humic substance to the carbonyl group of amide of tetracycline. Complexation of tetracycline with cations and minerals in soil make recovery of tetracycline difficult. Therefore, strong sorption was the main cause of low recoveries of tetracycline in the sterile soils during the incubation period.

The half lives period ($t_{1/2}$) for tetracycline in the present study ranged from 41.2 to 56 days in aerobic non- sterile soil and 57 to 71 days in anaerobic non- sterile soil which were higher than reported values for other antibiotics on other soils. The more persistence of tetracycline in studied soils might be due to its stronger tendency to sorb on soil components. It is well known that the sorbed fractions of a chemical are more resistant to degradation than the non- sorbed fractions.

Effect of concentration on dissipation of tetracycline was also studied under anaerobic non- sterile conditions with three tetracycline concentrations 10, 20 and 50 mg kg^{-1} soil on soil 3 and results are given in Fig. 2. The data denoted that with increase in initial concentration of tetracycline dissipation decreased. The concentration of tetracycline during incubation period (56 days) decreased from 7.89± 0.14 to 3.08± 0.18; 15.88± 0.24 to 6.26± 0.14 and 40.2± 0.4 to 16.32±0.40 mg kg^{-1} for the three concentration treatments respectively. The half lives periods as calculated on the basis of first order kinetics were 41.2, 56 and 68 days for the three concentrations respectively. The decreased in dissipation with concentration may be due to decrease in biological processes.

Conclusions

Our study showed that dissipation of tetracycline was influenced by soil properties viz, organic matter, metal ions and soil pH and chemical nature as well as redox conditions. The dissipation of tetracycline in soils under aerobic conditions was faster than anaerobic conditions. Biological processes were the main factors for the dissipation of tetracycline in the soils; due to strong sorption by soils the hydrolysis played a limited effect. As tetracycline forms strong complexes in soils due to multiple simultaneous interactions between polar and charged functional groups of tetracycline and surface charge sites on the soil via protonation and/or co-ordination of metallic cations on soil clays/humic substance to the carbonyl group of amide of tetracycline; recovery of tetracycline from the soils during incubation decreased, which would result persistence of tetracycline in soils.

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