

## Detection of Oxytetracycline and Chlortetracycline Residues in Agricultural Fields in Turkey

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### ABSTRACT

Tetracycline residues in the soil were investigated in this study due to their wide use in veterinary medicine and fertilization of agricultural fields with manure containing antibiotics. Soil samples were characterized by using various physicochemical parameters. Solid phase extraction and high performance liquid chromatography were used to detect oxytetracycline and chlortetracycline residues in the soils. The mean recovery rates of oxytetracycline and chlortetracycline were 51.07±27.66% and 62.12±17.05%, respectively. The concentrations of tetracyclines detected in soil samples were ranged from 0.019 mg kg<sup>-1</sup> to 0.144 mg kg<sup>-1</sup>.

**Key Words:** Oxytetracycline, chlortetracycline, agricultural soil.

### INTRODUCTION

Veterinary antibiotics are used to treat infectious disease and as growth promoters in livestock. They can reach to the environment and exert their potential adverse effects on organisms when manure is applied to agricultural field as fertilizer (Thiele-Bruhn 2003, Sarmah et al. 2006, Wang et al. 2006, Kummerer 2009). In addition, the number of antibiotic resistance genes increases in the soil after manuring, since manure stemmed from a farm operation with low or regular use of antibiotics can contain a high diversity of antibiotic resistance genes and antibiotics (Schmitt et al. 2006, Cengiz et al. 2010).

Tetracyclines (TCs) deserve special importance among various classes of antibiotics, since they are commonly used in veterinary practices in many countries as well as in Turkey. As a result, TCs can contaminate soils and due to their sorptive characteristics they can remain in soil along time after treatment. The aim of this study is to detect oxytetracycline (OTC) and chlortetracycline (CTC) residues in agricultural fields that they are irregularly fertilized with both cattle and poultry manure.

### MATERIALS AND METHODS

#### Soil sampling

Soil samples were collected from eight agricultural lands located in the South Marmara sub-region of Turkey. The amount of manure applied to the agricultural lands investigated in the present study was approximately 30 tones ha<sup>-1</sup>. Soil was sampled from specifically marked areas (Table 1) at depths of 10-20 cm below the soil surface. All field samples were air-dried and passed through a 2 mm-sieve, and composite samples were prepared by mixing an equal amount of discrete subsamples and kept at 4°C prior to experiments. The pH, texture and organic carbon (OC) content of soil samples were determined by using potentiometric (in 1 M KCl) (Forster 1995), hydrometric (Bouyoucos 1962) and Walkley-Black Methods (TSE 1990), respectively. Furthermore, concentrations of sodium, potassium, calcium, magnesium, chromium, copper, nickel, zinc and iron determined by atomic absorption spectrometry (AAS, AAnalyst 300, Pelkin Elmer).

**Table 1.** Description of the agricultural fields

Sample ID	Manure Type for Fertilization	Fertilization Date/ Sampling Date	Sampling Location	
			Latitude (N)	Longitude (E)
E1	Poultry	November 2005/ June 2006	40°17'32	027°53'46
E2	Poultry	November 2005/ June 2006	40°17'05	027°54'12
K1	Cattle	August 2004/ June 2006	40°11'58	028°25'11
K2	Cattle	September 2005/ June 2006	40°10'21	028°21'47
F1	Poultry and Cattle	September 2005/ June 2006	40°14'50	028°52'24
F2	Poultry and Cattle	August 2004/ June 2006	40°14'11	028°52'16
T1	Poultry	September 2005/ June 2006	40°21'58	028°42'20
T2	Poultry	September 2005/ June 2006	40°21'59	028°43'14

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### **Determination of OTC and CTC in soil samples**

All antibacterial standards used in the analysis were purchased from Riedel-de Haën. The chemicals used for the extraction of TCs from soil samples and the solvents used for HPLC analysis of extracts were of analytical grade and purchased from Merck and Sigma-Aldrich.

TCs were extracted from the soil samples by solid phase extraction using the method described by Blackwell et al. (2003). Briefly, 1 g of soil was weighed into a centrifuge tube and extraction buffer [0.1 M McIlvaine buffer (Na<sub>2</sub>HPO<sub>4</sub> and citric acid)/0.1 M EDTA/methanol 25:25:50 v/v] was added. The tubes were mixed with a vortex (Nuve, NM110) for 30 sec and then placed into an ultrasonic bath (Bandelin, Sonorex Super RK 510) for 10 min. After centrifugation (Selecta, Meditronic BI-S) at 1200 g for 15 min, the resultant supernatant was decanted into a glass bottle. The soil residue was extracted two more times and the supernatants were combined and diluted to approximately 400 mL with water to reduce methanol content below 2%. The pH of the solution was adjusted to 2.9 using phosphoric acid. Hydrophilic-lipophilic balance (HLB) polymer cartridges (Oasis Waters) and strong anion exchanger (SAX) cartridges (Isolute) were set up in tandem on a vacuum manifold system (Phenomenex). The solid phase extraction cartridges were pre-conditioned with methanol and conditioning/washing buffer (prepared by diluting 3.75 mL of extraction buffer to 100 mL with water and adjusting the pH to 2.9 with phosphoric acid) and loaded with diluted soil extract at 10 mL min<sup>-1</sup>. SAX cartridges were then removed and HLB cartridges were washed sequentially with washing buffer, 0.1 M sodium acetate, distilled water and 20% methanol. HLB cartridges were then air dried for 10 minutes and the extracts were eluted with 1 mL of methanol twice. The extracts were stored at -20°C until analysis.

The soil extracts were analyzed by HPLC using an Agilent 1100 system (Agilent) with an octadecylsilan column (50 mm x 4 mm x 3 µm, AQ-YMC). OTC and CTC were analyzed simultaneously. A gradient elution was carried out over 12 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 0.70 mL/min throughout the analysis and simultaneous detection of OTC and CTC was performed at 360 nm for TCs. The method was validated by spiking soil samples to yield concentrations of 0.2 and 1 mg kg<sup>-1</sup>. Retention times of OTC and CTC were 5.4 and 7.3, respectively.

## **RESULTS**

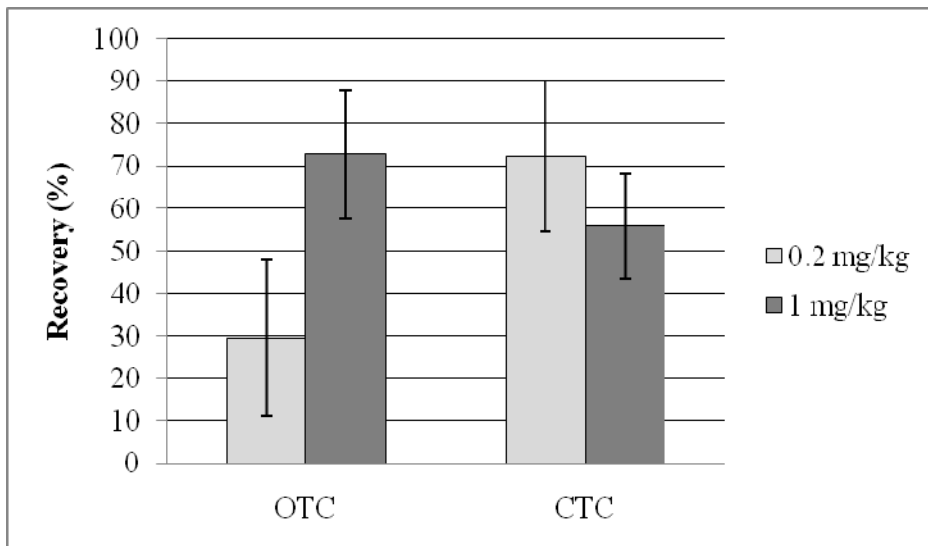
The collected agricultural soil samples were characterized by various parameters. The metal contents of soil samples were given in Table 2 and found range from 0.09 g kg<sup>-1</sup> (Cu and Ni) up to 120.80 g kg<sup>-1</sup> (Fe). The recovery values of these samples were 62.10% (T2) and 73.34% (E1). Texture analysis of them were performed and the results were evaluated according to the international soil textural classification (Soil Survey Division Staff 1993). Soil samples were classified as clay loam (E1 and E2), sandy (K1 and K2), sandy clay loam (F1 and T1), clay (F2), and loamy (T2). The pH values of soil samples were in range of 6.44-7.33. The OC content of soil samples varied from 1.38 to 7.51%.

**Table 2.** Heavy metal levels of soil samples listed with mean values and standard deviations

<b>Sample ID</b>	<b>K (g kg<sup>-1</sup>)</b>	<b>Ca (g kg<sup>-1</sup>)</b>	<b>Mg (g kg<sup>-1</sup>)</b>	<b>Cr (g kg<sup>-1</sup>)</b>	<b>Cu (g kg<sup>-1</sup>)</b>	<b>Ni (g kg<sup>-1</sup>)</b>	<b>Zn (g kg<sup>-1</sup>)</b>	<b>Fe (g kg<sup>-1</sup>)</b>
<b>E1</b>	19,53	20,29	19,06	0,31	0,13	0,22	0,41	106,05
<b>E2</b>	14,63	21,02	12,68	0,36	0,19	0,14	0,36	58,45
<b>K1</b>	29,08	3,37	69,28	1,88	0,11	0,98	0,34	108,00
<b>K2</b>	28,73	14,39	88,28	1,87	0,17	1,24	0,40	104,35
<b>F1</b>	18,18	8,89	20,03	1,24	0,17	0,37	0,34	31,50
<b>F2</b>	17,18	5,99	26,46	1,70	0,09	0,70	0,22	62,65
<b>T1</b>	13,33	63,12	18,61	0,22	0,20	0,09	0,26	42,70
<b>T2</b>	14,98	37,47	171,48	2,99	0,25	2,73	0,32	120,80
<b>Mean</b>	19.46	21.82	53.24	1.32	0.16	0.81	0.33	79.31
<b>SD*</b>	6.17	19.88	55.16	0.98	0.05	0.88	0.06	34.28

\*:Standard Deviation

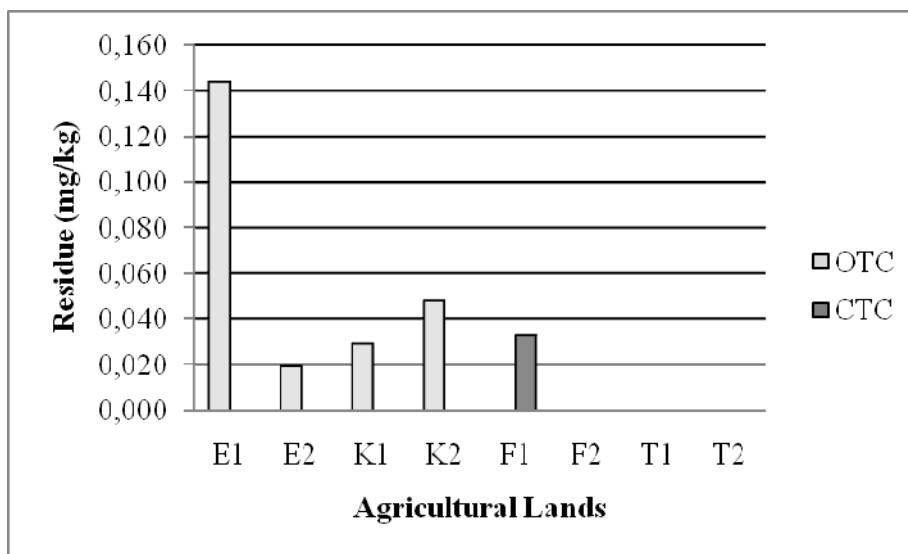
Before the analysis of antibiotics in soil samples recovery studies have been performed to evaluate data about the occurrence of TC antibiotics in the soil. For this purpose extractions were performed in triplicate and the collected data were used to calculate recoveries which are represented in Figure 1. The limits of detection were calculated based on a signal-to-noise ratio greater than 3 and those for OTC and CTC were 0.012 and 0.065 mg kg<sup>-1</sup>, respectively.



**Figure 1.** Recovery rates of tetracyclines in investigated soil samples

The OTC recovery at 0.2 mg kg<sup>-1</sup> spiking level was significantly lower than the CTC recovery value at the same spiking level and this value increased up to 72.72% at 1 mg kg<sup>-1</sup> spiking level. Furthermore, the CTC recovery at 0.2 mg kg<sup>-1</sup> spiking level was slightly higher than the value at 1 mg kg<sup>-1</sup> spiking level and this value decreased from 72.29% to 55.95%.

Eight different soil samples were subjected to TC analysis. While OTC was detected in four different samples (E1, E2, K1 and K2) CTC was detected only in one sample (F1). OTC concentrations were ranged from 0.019 mg kg<sup>-1</sup> to 0.144 mg kg<sup>-1</sup> and CTC concentration was 0.073 mg kg<sup>-1</sup> (Figure 2).



**Figure 2.** Concentrations of the antimicrobial compounds detected in investigated soil samples

## DISCUSSION

Sorption of TCs to metals and organic matter ligand groups is expected to be greatest around pH 8 (MacKay and Canterbury 2005). Therefore, the extraction of samples at acidic condition yields high efficiency. In addition, due to the relative stability of TCs in acidic media and the salt formation potential in acidic conditions as well as alkaline pH (Halling-Sorensen et al. 2002) it is expected that their extraction can be enhanced by forming complexes with metals may be also responsible for their sorption. However, in this study metal ion content in the extraction solution was not evaluated. A correlation between the some metals and recovery values of OTC and CTC in the soil and manure samples were determined in our previous studies (Karci and Balcioglu 2009, Cengiz et al. 2010). Indeed, OTC recovery value (62.10%) of T2, which has the highest total metal content, was respectively lower than other agricultural lands (E1, E2, K1, F1 and T1), but not the lowest. In addition, CTC recovery value (41.81%) of K2, which has the second highest total metal content, was the lowest among all values calculated for CTC at 1 mg kg<sup>-1</sup> spiking level.

In various studies TCs are commonly detected antimicrobial compounds in animal manure and soil samples and the concentrations of TCs in agricultural fields varied from 0.06 to 0.307 mg kg<sup>-1</sup> depending on agricultural practices and soil physicochemical properties (Hamscher et al. 2002, Karci and Balcioglu 2009, Cengiz et al. 2010). In accordance to the previous studies, in this study OTC was detected at considerably high concentration in the 50% of the collected samples. The technical guidance documents in support of the European registration procedure for human and veterinary medicines demand no risk assessment for substances with an exposure level below a certain trigger value for soil that is 0.100 mg kg<sup>-1</sup> (Montforts 2005). Although, OTC concentration measured in the agricultural land E1 was found to exceed the trigger value, toxic effect on terrestrial organisms or Pollution Induced Community Tolerance (PICT) is unexpected. Since, the higher concentrations have been reported for toxic effects and PICT (at least 7 mg kg<sup>-1</sup> and above) (Wollenberger et al. 2000, Schmitt et al. 2006).

However, the potential adverse effects of TCs on organisms obviously can emerge after soil contamination with these compounds. Esiobu et al. (2002) determined that the rate of antibiotic (penicillin, tetracycline and streptomycin) resistant bacteria in soil increased up to 70% after manuring. This indicates that TCs exert selective pressure for development and maintenance of antibiotic resistance in soil and pose a direct hazard to public health (Rysz et al. 2004). Therefore, it can be suggested that the OTC and CTC residues (0.019-0.144 mg kg<sup>-1</sup>) analysed in soil samples of the present study may cause emergence of resistance in microorganisms against to this group of antibiotics.

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