University of Zimbabwe

Speciation and Persistence of Tetracycline Antibiotics in the Aquatic Environment: Characterization in terms of a Linear Rate Model

A thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy in Chemistry

By

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Abstract

The present work aimed at studying the degradation of tetracycline antibiotics in the aquatic environment with a view of arriving at a linear rate model taking into account microbial, photolytic and hydrolytic degradation, as well as adsorption/desorption equilibria. The degradation of the antibiotics was monitored both in river water and sediment of the aquatic microcosm experiments, as well as in control experiments consisting of distilled water over a period of 90 days. Ultrasonic assisted dispersive solid phase extraction was used to extract the antibiotics from water and sediment samples. High performance liquid chromatography coupled to a variable ultra violet detector was used to determine the changes in concentration of antibiotics over the period of 90 days. An initial loss of up to 35% at most, due to adsorption by the sediment was observed in the microcosm experiments soon after charging. Triphasic linear rates attributed to microbial degradation of free and sediment or colloidal particle 1 and 2 adsorbed antibiotic for both water phase and sediment phase of the aquatic microcosm experiments were observed for oxytetracycline, chlortetracycline and tetracycline, while biphasic kinetics attributed to degradation of free, colloidal or sediment particle bound antibiotic were observed for doxycycline. The initial rates of degradation ranged from 1.35 - 3.07 x 10^{-2} µg/g/day (water phase), and 7.90 x 10^{-3} to 4.79 x 10^{-2} µg/g/day (sediment phase). Oxytetracycline exhibited the highest rate of degradation while that for tetracycline was the least. The covered distilled water control experiments for all the antibiotics showed a biphasic degradation pattern attributed to hydrolysis ranging from 2 x 10^{-6} to 5 x 10^{-4}µg/g/day and microbial degradation ranging from 1.8 - 2.7 x 10^{-3} µg/g/day. In the distilled water exposed to natural light experiments, monophasic degradation (6.9 x 10^{-3}µg/g/day) was observed for oxytetracycline, while biphasic degradation was observed for the other antibiotics. Addition of nitrates increased slightly the initial degradation rates for the light exposed distilled water experiments while the rates increased significantly in the microcosm experiments. The slight increase observed in the control experiments consisting of distilled water is attributed to photosensitization by the nitrate ions, which form hydroxyl radicals that further degrade the antibiotics while the huge increase in the microcosm experiments is attributed to increased population of microorganisms, due to availability of nutrients (added nitrates). The addition of nitrates did not affect the subsequent slow degradation rates. This is because the degradation rates depend on the rate of desorption of the antibiotic from colloidal and sediment particle surfaces. A kinetic model taking into account hydrolysis, photolysis, microbial degradation, as well as adsorption/ desorption equilibrium is presented to explain the observed zero order kinetics in the present study

Dedication

Maggie my wife, Nicole my daughter, Worship my son.

Acknowledgement

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List of Abbreviations

A	Antibiotic
AH-C-HPLC-UV	Aluminium hydroxide co-precipitation coupled to high performance liquid chromatography with UV detection
APCI	Atmospheric pressure chemical ionization
C_0	Concentration at time = 0
C_1	Colloidal particle 1
C_2	Colloidal particle 2
CDW	Covered distilled water
C_t	Concentration at time t
CTC	Chlortetracycline
DC	Doxycycline
DFOP	Double first order parallel
DSPE	Dispersive solid phase extraction
DSPM-HPLC-DAD	Dispersive solid phase micro-extraction coupled to high performance liquid chromatography-diode-array detection
EDTA	Ethylene diamine tetraacetic acid
ELISA	Enzyme Linked Immunosorbent Assays

ESI	Electrospray ionization
FAB	Fast atom bombardment
FOCUS	Forum for the coordination of pesticides fate models and their use
FOMC	First order multi-component
HLB	Hydrophilic lipophilic balance
HPLC-DAD	High performance liquid chromatography-diode array detector
HPLC-FL	High performance liquid chromatography fluorescence detector
HPLC-PAD	High performance liquid chromatography with photodiode array detection
HPLC-PD	High performance liquid chromatography polarimetry detector
HPLC-UV	High performance liquid chromatography-ultra violet
HS	Hockey and Stick
IUPAC	International Union of Pure and Applied Chemistry
LC-EC	Liquid chromatography electron capture
LC-MS	Liquid chromatography mass spectrometry

LC-MS-MS	Liquid chromatography tandem mass spectrometry
LDR	Linear dynamic ranges
LOD	limits of detection
LOQ	Limit of quantification
MSPD	Matrix solid phase dispersion
OTC	Oxytetracycline
PSA	Primary and secondary amine
RRLC–MS/MS	Rapid resolution liquid chromatography–tandem mass spectrometry
RW & S	River water and sediment
RW&S&N	River water and sediment spiked with nitrates
SAX	Strong anion exchange
SFO	Single first order model
SPE	Solid phase extraction
TC	Tetracycline
TCs	Tetracyclines
TS	Thermospray
TSPE	Tandem solid phase extraction

UA	Ultrasonic assisted
UV	Ultra Violet
WHO	World Health Organization

CHAPTER 1

1.0 INTRODUCTION

1.1 BACKGROUND TO STUDY

In recent years many studies have shown that chemicals such as antibacterial agents that have historically not been considered as environmental pollutants are widely distributed in the aquatic environment (Batt et al., 2007; Watkinson et al., 2009; Luo et al., 2011; Zhou et al., 2013). Antibacterial agents find their way into the aquatic environment from agricultural, municipal and hospital waste effluents (LaPara et al., 2011; Ibraheem and Andul-Ahad, 2012). A major concern regarding antibacterial agents is that they are continuously released into the aquatic environment. The presence of antibacterial agents in the aquatic environment can lead to adverse effects; for instance they can disturb the nitrogen cycle, if they destroy denitrifying bacteria (Szatmari et al., 2011; Yang et al., 2009).

Drugs administered to humans as therapeutics are released into the aquatic environment via waste water treatment plants. This is because waste water treatment plants in use in many countries are not often designed to remove antibacterial agents (Batt et al., 2006; Gomez et al., 2007; Xu et al., 2007). About 70% of the antibiotic administered to a domestic animal as medication is excreted as parent compound in faeces and urine (Martinez-Carballo et al., 2007; Liu et al., 2009), and find its way into surface and drinking water through runoff or seepage, and direct application of manure in fields and vegetable gardens (Kemper, 2008; Avisar et al., 2009a). Veterinary drugs have been detected in surface waters by a number of researchers (Perret et al., 2006; Batt et al., 2006; Pojana et al., 2011). Meyer et al., (2003) detected chlortetracycline (CTC) and oxytetracycline (OTC) in four surface water samples collected from 6 States in USA, while Watanabe et al., (2010) detected tetracyclines in

ground water samples from USA piggery farms. All these authors implicated veterinary applications as the source of the pollution. Antibacterial agents have been reported to have the propensity to foster the development of multidrug resistance factors in microbes in the environment (Unold et al., 2010; Underwood et al., 2011; Luo et al., 2011). Although antibiotics given to humans as medication are not often the same as those prescribed for farm animals, their structure/activity relationship may be similar enough to cause resistance. Such resistance is transferred to humans through the food chain and drinking water, thereby complicating treatment of diseases (Su et al., 2012; Ma et al., 2012). World Health Organization (WHO) reports that each year there are approximately 440000 cases of multidrug resistance strains (WHO, 2012). Information on excessive use, proper disposal, and policy regulation is not often available in most countries. Hence the need for studies on distribution and fate of antibacterial agents in the aquatic environment cannot be over emphasized.

Tetracyclines (TCs) are widely used as human and veterinary broad spectrum antibacterial agents (Ding and He, 2010; Chen and Huang, 2011). Among the TCs, (OTC), doxycycline (DC), (CTC) and tetracycline (TC), (Figure 1.1), are the most prescribed drugs at a global scale, and therefore, merit special attention. TCs are used as medication and prophylactics. They are also added to animal feeds where they act as growth promoters (Wang and Yates, 2008). Concentration levels ranging from ng L^{-1} to mg L^{-1} have been reported in Europe, America and Asia (Ben et al., 2008; Hoa et al., 2011; Yang et al., 2011; Zhou et al., 2011; Luo et al., 2011; Deo and Halden, 2013).

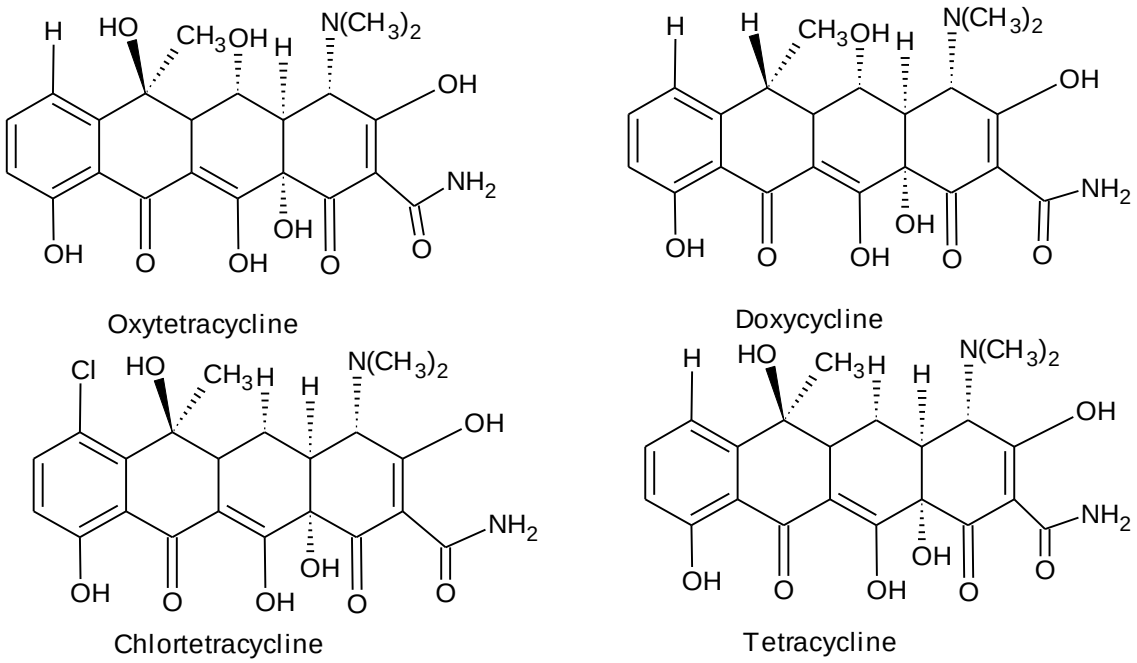


Fig 1.1 Structures of oxytetracycline, doxycycline, chlortetracycline and tetracycline

Persistence of TCs in the aquatic environment has been studied in terms of half-lives according to the first order kinetic model. For this model the half-life of a substance is assumed to be constant despite the prevailing environmental conditions. A review of previous studies (Xuan et al., 2010; Szatmari et al., 2011; Chen et al., 2012) shows that half-lives for tetracycline antibacterials are widely variable contrary to the predictions of true first order kinetic model. Studies conducted by Doi and Stoskopf, (2000) and Xuan et al., (2010) revealed that persistence of TCs vary depending on environmental conditions, such as pH, temperature, soil type and micro-organism population and type. Soil type, pH and temperature affect adsorption and degradation of the antibiotic. TCs have been found to adsorb strongly to soil particles than other antibiotics. TCs consist of three adsorption sites, the phenolic diketone, dimethylamine and tricarbonylamide groups (Chen et al., 2012; Quang and Adams, 2004). This makes them good candidates for studying the effect of adsorption/desorption equilibria on degradation rates. The need to study the effect of

adsorption on to particulate matter on the rate of degradation has been recently highlighted by IUPAC, (2011). This is now the subject of an on-going IUPAC project to quantitatively evaluate the relationship between sorption onto environmental matrices, degradation and molecular structure (IUPAC, 2011). Thus, the aim of the present work was to study the speciation and persistence of OTC, DC, CTC and TC in the aquatic environment with the aim at arriving at a kinetic model of the degradation of the antibiotics that takes into account sorption onto colloidal and sediment particles.

The traditional approach for studying the persistence of antibiotics in the environment consist of spiking the appropriate environmental compartment with the antibiotic and then collecting samples periodically to determine the amount of substance remaining in the compartment at the time of sampling. The concentration of the antibiotic that remains at any given time is then plotted as a function of time to yield a degradation curve. More often the persistence curve resembles a first order decay curve. Therefore, the fate of most organic substance in the environment has been studied in terms of the first order kinetic model. The FOCUS group suggest several other models in situations where Single first order (SFO) fails and these include the first order multi-component (FOMC), the double first order parallel (DFOP), the Hockey and Stick (HS) and the bi-exponential model (Boesten et al., 2006). All these models share the same characteristic that is they assume first order degradation kinetics thus persistence is described in terms of half-lives.

TCs have been shown to degrade through photolytic, hydrolytic and microbial degradation (Wen et al., 2009; Xuan et al., 2010; Migliore et al., 2012), and these are all multi step processes, which cannot be described fully using the first order kinetic model. Multistep processes are best described using steady state approximation. Unlike in first order kinetic

model, where the rate of loss of the parent compound depends on the concentration (C) of the antibiotic remaining at any given time dP/dt = -kC, a steady state is characterised by a constant rate of loss of the parent substance or a constant rate of formation of products (P); i.e. dP/dt = k, which is in line with zero order kinetics (Daniels and Alberty, 1961). In the present study the approach in studying the kinetics of degradation of tetracycline antibiotics in the aquatic environment involved first establishing whether the degradation process involved a steady state or not. Once that has been achieved the next step involved proposing a plausible mechanism followed by applying the steady state approximation to model the results observed from the experiments. The overall aim was to make the final rate equation consistent with the experimentally observed constant rate of degradation.

The loss in the TCs concentration after a given time was computed and plotted as a function of time. This approach was previously employed for the analysis of herbicides, paraquat (Zaranyika and Nyoni, 2013), glyphosate (Zaranyika and Nyandoro, 1993), organophosphate insecticides, fenamiphos, chlorpyrifos and pirimiphos-methyl (Zaranyika and Mlilo, 2012) and organochlorine insecticides endosulfan I and II (Zaranyika et al., 2010). Two linear profiles were obtained for the sediment and water phase and these were attributed to microbial degradation of which each linear portion corresponds to the plateau in Michaelis-Menten curve, when the concentration of the substrate is in excess of the concentration of microorganisms that can bind and degrade the pesticide. Presence of fast and slow degradation rates was attributed to the degradation of free and colloidal or sediment particle adsorbed pesticide. A model taking into account microbial degradation of free and colloidal and sediment particle bound antibiotic was proposed. The proposed model leads to linear rates that are directly proportional to microbial population and inversely proportional to the concentration of adsorbing particles or adsorption sites in the medium. The model, thus,

predicts variable degradation rates, depending on the type and population of microorganisms and adsorbing sites.

1.2 AIMS

- To investigate speciation and persistence of TC antibiotics in the aquatic environment.

1.3 OBJECTIVES

- To study the distribution and degradation of

  (i) Oxytetracycline (OTC),

  (ii) Doxycycline (DC),

  (iii) Chlortetracycline (CTC) and

  (iv) Tetracycline (TC) in the aquatic environment using microcosm and distilled water control experimental setups.

- Based on the results obtained, to propose kinetic models consistent with the kinetics of the experiments.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 TETRACYCLINES: STRUCTURE AND CHEMICAL PROPERTIES.

TCs are broad spectrum antibacterial agents produced by a group of microorganisms called *actinomyces* (Kummerer, 2009, Lopez-Penalver et al., 2010). TCs are used to treat diseases both in humans and domestic animals. TCs consist of bacteriostatic activity against Gram positive and Gram negative bacterial (Sarmah et al., 2006). They are the drugs of choice in many countries (Sarmah et al., 2006; Kummerer, 2009). In humans TCs are used to treat infections such as, gonorrhoea, pneumonia, trachoma, urinary tract infections and cholera (Kummerer, 2006). TCs are also used as prophylactics for prevention of malaria or treatment of complicated malaria when prescribed with quinine (Kummerer, 2009). As veterinary medicine TCs are used to treat infections caused by mycoplasmas and chlamydia and to prevent infections (Fritz and Zuo, 2007). TCs are often used as hydrochloride salts in most medicines. These antibacterials work by interfering with the ability of bacteria to synthesize proteins. TCs prevent the attachment of anaminoacyl-tRNA to the 30s ribosomal accepter and prevent incorporation of a new amino acid to the peptide chain (Daghrir and Drogui, 2013).

TCs consist of an octahydrotetracenecarboxamide skeleton. These compounds are derivatives of the polycyclic naphthacenecaboxamide (Fig 2.1).

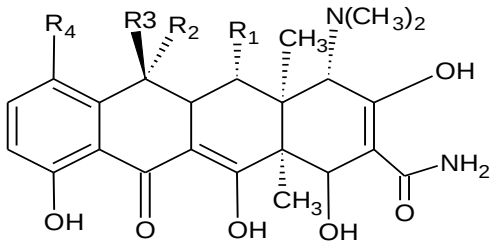


Fig 2.1. The four rings of the basic structure of tetracyclines.

TCs differ from one compound to another chemically at positions 5, 6 and 7 by variation of substituents at these positions, (see Fig 2.2):

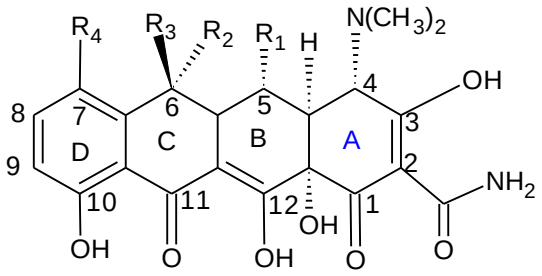


Fig 2.2. Variation positions of tetracyclines antibacterials.

The complicated ring structure and multiple functional groups make TCs amphoteric. Three pK_a values for TCs have been reported previously corresponding to the phenolic diketone, dimethylamine and tricarbonylamide groups (Chen et al., 2012; Qiang and Adams, 2004). TCs can interchange between a cation, an anion or a dianion or a zwitterion depending on the pH of the environment. The rings A, B, C and D form two separate resonance structures; hence, they are chromophores that give two major absorption bands in the range 250 -300 and 340-380 nm in the TCs spectra. The A ring gives the 250-300 nm band only, while the B, C and D rings give both bands (Schneider et al., 2003). Generally tetracyclines are more stable in acidic than in alkaline medium (Loftin et al., 2008). TCs undergo reversible epimerization on C_4 position to form 4-epi-tetracyclines. Iso-tetracyclines are formed in alkaline conditions via nucleophilic attack at the hydroxyl group of C_6. TCs possess a strong tendency to complex metals, soil particles, proteins and organic matter (Kay et al., 2005; Li et al., 2010). Previous studies have shown that the A and the B, C and D rings are the complexing sites. The increased tendency to complex particulate species plays an important role in affecting their fate and distribution in the aquatic environment.

2.2 OCCURRENCE OF TCs IN THE AQUATIC ENVIRONMENT.

The excreted drugs enter the aquatic environment via routes shown in Fig 2.3. Drugs taken in by humans as medication enter the sewer system through urine and faeces and enter sewage treatment plants. The drugs end up in surface and drinking water, due to incomplete removal (Boxall et al., 2003; Halling-Sørensen et al., 2005; Xu et al., 2007). TCs applied as therapeutics in the domestication of cattle, pigs and poultry in intensive livestock units are poorly metabolized and are excreted in manure and urine (Kummerer, 2009). TCs enter the aquatic environment indirectly through the application of manure as fertilizers in fields. Part of the antibiotics can be adsorbed onto soil particles, while another portion is transported through runoff into rivers, dams and lakes. Major factors determining the intensity of surface water contamination includes number of animals kept in an area, frequency of treatment and level of application of animal manure for fertilization purposes in fields. TCs residues released in the manufacturing process and by municipal and hospital sewage system ultimately enter surface waters, since current waste water treatment systems are often not designed to remove antibiotics (Xu et al., 2007). Other minor routes of entry of TCs into the aquatic environment include emissions from the air and through the disposal of unused medicines and containers.

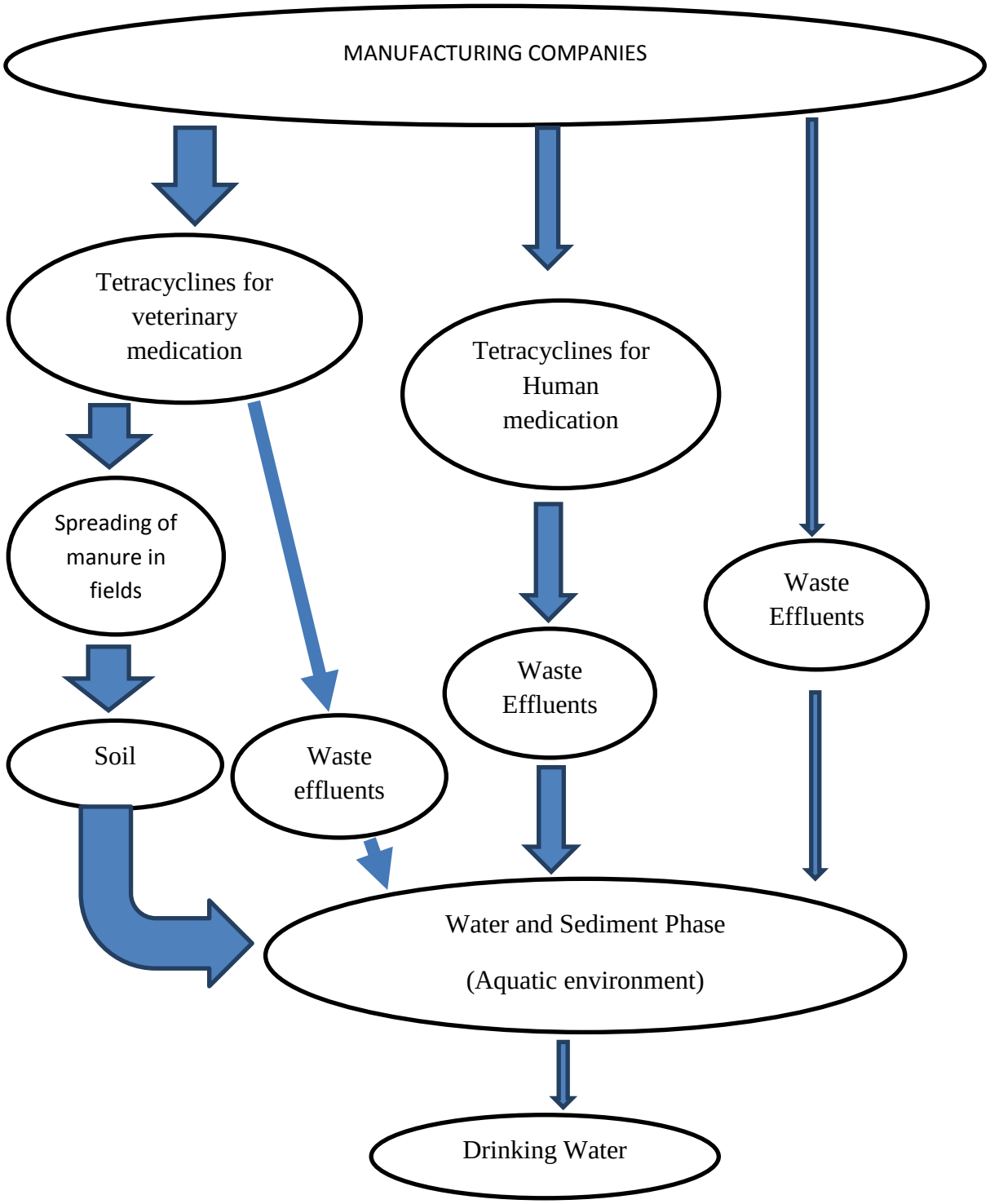


Fig 2.3 Tetracyclines emission routes into the aquatic environment (Modified from Boxall et al., 2003 and Xie et al., 2011)

Table 2.1 Levels of tetracyclines detected in different environmental samples

Sample	Antibacterial agent	Level	Reference
Drinking water	TC, OTC, CTC	0.07 - 1.34 µg/L	Kolpin et al., (2002)
Drinking water	OTC, TC	50-100 ng/L	WHO, (2012)
Drinking water	OTC, TC, CTC	15-75 ng/L	Ye et al., (2007)
Drinking water	TC	50 ng/L	Daff, (2005)
River water	OTC	2 mg/L	Nakata et al., (2008)
River water	TC	1.9 µg /L	Kolpin et al., (2002)
River water	OTC, CTC, TC	2.2 and 2.1ng/L	Jia et al., (2009)
River water	OTC, TC	10 ng/L	Luo et al., (2011)
River sediment	TC, OTC	10.3-72.9 µg/kg	Wei et al., (2014)
River sediment	TC, OTC, CTC	10.3-72.9 µg/kg	Ok et al., (2011).
River sediment	TC, OTC	81.7-232 µg/kg	Yang et al., (2011)
River Sediment	OTC, TC	5 ng/kg	Luo et al., (2011)
Swine urine	OTC, TC	5 - 24 mg/L.	Hamscher, (2000)
Farming soil	OTC, TC	0,1-4mg/kg	Hamscher, (2000)
Farming soil	TC	0.3 mg/kg.	Hamscher et al., (2002)
Farming soil	OTC	199µg/kg	Hamscher et al., (2002)
Sludge	TC, OTC	117-168 µg/kg	Zhou et al., (2013)

TC antibacterials have been detected in surface, ground, waste and drinking water (Lindsey et al., 2001; Tamtam et al., 2008; Karthikeyan and Meyer, 2006; Choi et al., 2007; Benotti et al., 2009; Feitosa-Felizzola and Chiron, 2009), (see also Table 2.1). Although most levels

reported are below the minimum inhibitory concentration of most microorganisms, they have been found to have the propensity to induce microbial resistance (Beausse, 2004; Luo et al., 2011; Suzuki and Hoa, 2012).

2.3 FATE OF TETRACYCLINES IN THE AQUATIC ENVIRONMENT

The fate of TCs in the aquatic environment has been a topic of interest to several researchers (Halling-Sørensen et al., 2002; Boxall et al., 2002; Kay et al., 2004; Unold et al., 2010). Jodeh and Awartani, (2011) studied the fate and mobility of OTC and DC in soil columns. Greater mobility was observed for DC than for OTC. TCs have been reported to mainly degrade abiotically on the assumption that they are bacteriostatic therefore most studies report photochemical and hydrolytic degradation (Eichhorn and Aga, 2004). No degradation was observed in marine aquaculture sediment over a period of 180 days by Samuelsen et al., (1989). TCs are highly sensitive to UV light (Oka et al., 1989; Olack and Morrison, 1991; Doi and Stoskopf, 2000; Xuan et al., 2010). Hardness of water and pH were observed to affect photochemical degradation rates. Hydrolytic degradation was observed to be predominant in neutral solution, while photodegradation of the antibiotics in natural environment may be hampered by particulate matter or increased by humic acid (Xuan et al., 2010). Microbial degradation of TCs in soil, waste waters, animal manure and marine sediment has also been reported (Kim et al., 2005, Maki et al., 2006). Halling-Sørensen et al., (2003) observed hydrolytic and photolytic degradation of OTC in soil interstitial water. Figure 2.4 shows the common transformation/degradation products reported in literature (Pena et al., 1998; Halling-Sørensen et al., 2003, Xuan et al., 2010; Khan et al., 2010). TCs can form epi- TCs by isomerization reactions and anhydro- TCs by loss of a water molecule from the carbon 6 position. The anhydro- TCs have been observed to be more toxic than their

parent compounds therefore; studies; on the fate of degradation products are also important. OTC forms additional transformation products, α-apo-OTC and β-apo-OTC by a nucleophilic attack (Arikan et al., 2006). This is because it has an additional hydroxyl group at the C_5 position. In addition to its epimers, DC form metacycline (Injac et al., 2007).

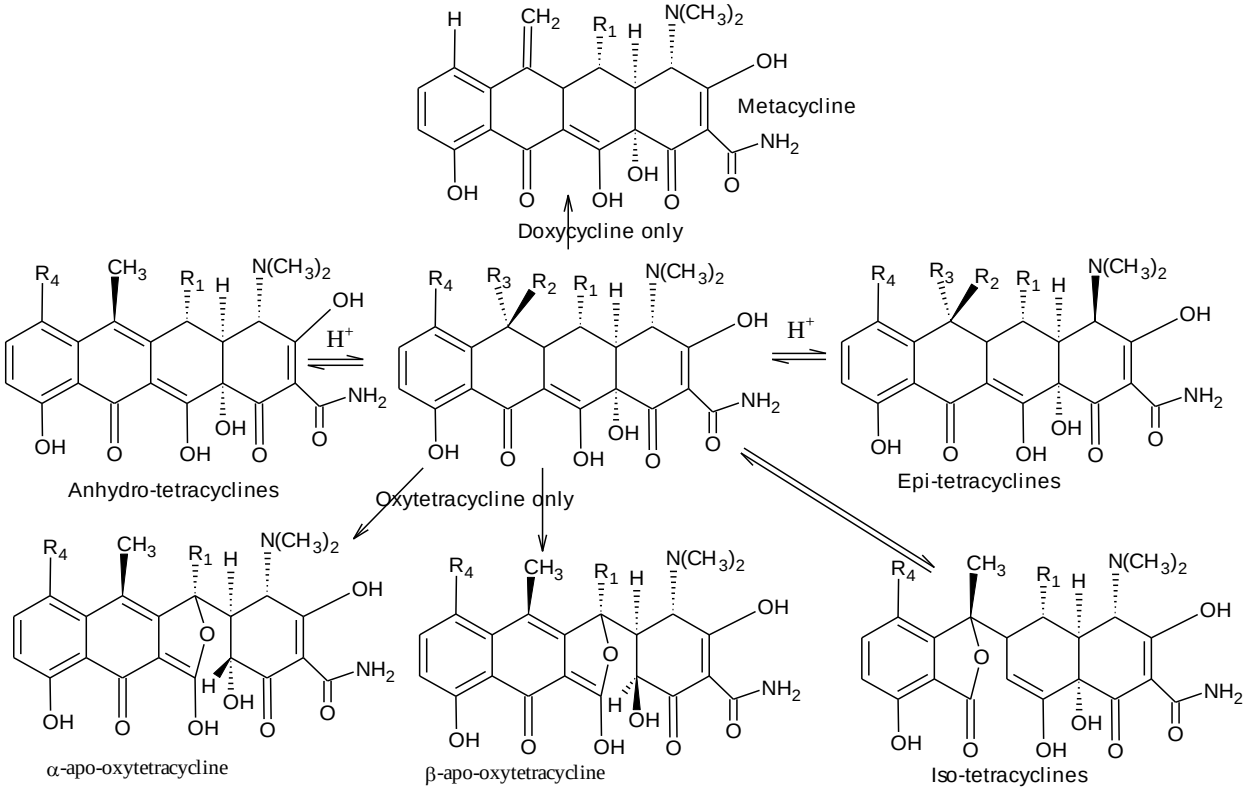


Fig 2.4 Reported transformation/degradation products of oxytetracycline, doxycycline, chlortetracycline and tetracycline (modified from Søeborg et al., 2004; Injac et al., 2007; Xuan et al., 2010).

Degradation of antibiotics in the environment depends on a number of factors. Apart from the physicochemical properties that are structure, concentration and solubility, the degree and kinetics of degradation are to a large extent influenced by adsorption onto particulate material (Tolls, 2001). Adsorption of the antimicrobial agent onto soil, sediment or humic material effectively protects it from degradation (HallingSørenson et al., 2002). Degradation processes have been reported to be affected by environmental conditions such as temperature, rainfall,

humidity and soil type. Sarmah et al., (2006); Loftin et al., (2008) and Wang and Yates, (2008) reported increased degradation of OTC when temperature was increased to 40^{o}C. The degradation was also found to be faster in aerobic than in anaerobic conditions. Ingerslev et al., (2001); HallingSørensen et al., (2003) and Li et al., (2010) reported a higher degradation rate of OTC in surface water in aerobic conditions than in anaerobic conditions. In similar studies conducted by Loftin et al., (2008) degradation of TCs was found to be affected by pH and temperature.

2.4 SORPTION OF TETRACYCLINE ANTIBIOTICS

TC antibiotics are water soluble and ionise, depending on the pH of the environment. Clay and humic substances are the major soil components where adsorption of TCs occur (Gu and Karthikeyan, 2008). They have been reported to adsorb through various sorption mechanisms, including hydrophobic partitioning, surface complexation, electrostatic attraction, cationic exchange, cationic bridging and hydrogen bonding (Tolls, 2001; Thiele-Bruhn, 2003). TCs exhibit multiple ionic states (Qiang and Adams, 2004; Zhao et al., 2011). Generally TCs are zwitterionic over most of the pHs (3-8) found in natural aquatic environments. Cationic and zwitterionic forms interact greatly with clays (Chang et al., 2012), sediment (Figueroa and Mackay, 2005) and dissolved organic matter (Gu et al., 2007). Batch experiments conducted by Figueroa et al., (2010) revealed that TCs can be removed from aqueous media by clay and silicates fractions. Desorption from these sites can be achieved through the use of aluminium chloride (Teixido et al., 2012). This shows the importance of cationic exchange mechanisms in the sorption of TCs. Presence or absence of metal cations enhanced or inhibited sorption of tetracyclines to clay particles (Tanis et al., 2008). Humic acid inhibited sorption onto clay particles through masking available sorption

sites (Pils and Laird, 2007). Sorption onto soils and marine sediment was found to be greater for OTC than its counterpart TCs (Rabolle and Spliid, 2000).

2.5. MICROCOSM EXPERIMENTS

Several experimental set ups have been employed previously to model the aquatic environment. Most of these set ups were based on a pesticide protocol suggested by the Organisation for Economic Cooperation and Development Guide 308 (Rose and Pendersen, 2005). Loftin et al., (2008) used 500 mL amber glass vials filled with 200g of sediment and 300 mL of lake water. The water/sediment system were then spiked with an antibiotic and homogenized. Samples were immediately taken and periodically after for 100 days. Similarly, Kalsch, (1999) used glass aquaria consisting of 3 cm of sediment and 80 L of water. Incubation was allowed for 209 days. The experiments were then exposed to sunlight. Samples were collected periodically from the aqueous phase. Other studies employed even large scale reactors (Brian et al., 2004). A facility comprising of 30 artificial ponds that would carry 12000L of water was used by Brian et al., (2004). The bottoms of the ponds were covered with plastic trays consisting of sediments. Ronnefahrt, (1997) constructed a pond consisting of 15 cm of sediment and 800L of water. The aquarium was located in a greenhouse maintained at 20^{o}C using a lighting system. Samples were collected regularly from the water phase and the upper most part of the sediment. Zaranyika and co-workers, (1993-2013) used 100 and 80 L transparent plastic tanks consisting of 2 kg of sediment and river water to model water/sediment systems. The system was then spiked with pesticides, stirred to homogenise and samples were taken immediately from the water and sediment phase. The experiments were allowed to run for 90 days.

2.6 DEGRADATION KINETIC MODELS

The traditional approach to studying the persistence of antibiotics involves spiking the antibiotic into a portion of the environment. Samples are collected periodically to determine the amount of substance remaining at any given time. The concentration remaining is then plotted as a function of time to give a degradation curve. The curve is then used to deduce the degradation kinetics. Persistence kinetic models for antibiotics reported in previous studies include, first order/single first order (SFO), variability adjusted first order model, first order multi component (FOMC), double first order in parallel/ bi-exponential (DFOP), first order two component (FOTC) and first order sequential biphasic (Hockey and stick) (Boesten et al., 2006). It is important to note that the more complex the degradation pathway is, the more complex the type of kinetics and the more information the model requires for adequate predictive estimation. An easy to interpret model that can give a sensible description of the proposed pathway and decline curves is often preferred. Thus, the single first order kinetic model has been the model of choice in many studies.

2.6.1 Single first order kinetic model (SFO)

The single first order kinetic model assumes that the number of degrading molecules is small relative to the number of microorganisms and enzymes or the number of water molecules (in the case of hydrolysis). Therefore, the rate dC/dt at any time is directly proportional to the concentration of the antibiotics remaining in the system. Thus

$$Rate = \frac{d[C]}{dt} = -k[C] \quad \text{Differential form} \qquad 2.1$$

$$\ln\left(\frac{C}{C_o}\right) = -kt \quad \text{Integrated form} \qquad 2.2$$

where C_o is the initial concentration, while C is the concentration at time t. K is the rate constant. A plot of ln C/C_o against time (t) gives a straight line. Thus this has been used widely to fit empirical data to the SFO model. A higher value of R^2, (close to 1) obtained by applying regression analysis is then used to justify the reason to fit the data to the SFO kinetic model.

The integrated form can be rearranged as follows

$$[C] = C_o e^{-kt} \qquad 2.3$$

A plot of C/C_o against t yields a decay curve of the shape as shown in Fig 2.5. Thus persistence of antibiotics has been studied in terms of the first order kinetic model.

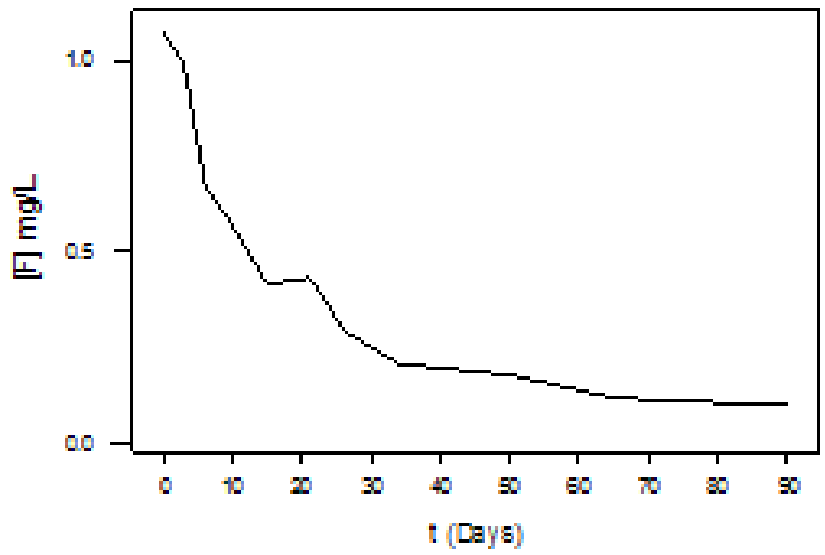


Fig 2.5. A plot of change in concentration against time (Zaranyika and Mlilo, 2012)

$$\ln\left(\frac{C}{C_o}\right) = -kt \qquad 2.4$$

If the initial concentration is decreased by one half and substituting this value in equation 2.4 yields equation 2.5

$$t_{\frac{1}{2}} = \frac{0.693}{k} \qquad 2.5$$

Thus, the time for a decrease in concentration of the antibiotic by a certain amount is constant throughout the course of the experiment and is independent of the initial concentration. Therefore $t_{1/2}$ is constant throughout the experiment and this makes $t_{1/2}$ values easy to interpret.

Sometimes degradation fails to follow SFO kinetics. A fast initial decrease in a pharmaceutical concentration may often be followed by a slow decline, a bi-phasic pattern of degradation. This is because only a fraction of the molecules in aqueous phase may be available for degradation (Scow, 1993). This fraction often decreases with time, due to slow diffusion and sorption processes (Pignatello et al., 2006). Thus the rate of degradation of the molecules at a later stage of the experiment may be decreased. Furthermore, the environment (terrestrial or aquatic) is a spatially variable medium such that the rate of degradation will also be variable throughout the medium (Gustafson and Holden, 1990). For real environmental studies, changes in temperature or moisture often affect rates of degradation and cause deviations from first-order kinetics (e.g. rates of degradation may decrease in winter, due to cooler temperatures, or in summer as a result of drier conditions). Such limitations of SFO model calls for development of other kinetic models. A suitable model should be able to describe the biphasic (slow and fast) pattern of degradation, explains why variable half-lives are often obtained in persistence studies (Table 2.2), explain why the persistence of antibiotics is affected by pH, soil type and presence of organic matter. The pH affects microbial population capable of degrading TCs, while soil type and presence of organic matter, not only affects the microbial population, but also affects the sorption capacity of the medium.

2.6.2 The First-Order Multi-Compartment

The First-Order Multi-Compartment model (FOMC) (Gustafson and Holden, 1990) was obtained by dividing the concerned environment into a number of sub-compartments, each having a different first order rate constant. If the distribution of these rate coefficients is represented by a location parameter β, then this results in a simple analytical equation with few parameters, (Eqns 2.6 and 2.7).

$$C = \frac{C_o}{\left(\frac{t}{\beta} + 1\right)^{\alpha}} \qquad 2.6$$

Where

C = Total concentration of antibiotic present at time t

C_0 = Total concentration of antibiotic applied at time $t=0$

α= Shape parameter which is given by coefficient of variation of rate constant values

β = parameter of location

$$t_{\frac{1}{2}} = \beta\left(2^{\frac{1}{\alpha}} - 1\right) \qquad 2.7$$

The major drawback of this model is its dependence on the researcher to define the location parameters.

2.6.3 The hockey-stick model

The hockey and stick kinetic model involves two sequential first-order degradation curves. The concentration of the antibiotic declines with an initial first order rate constant k_1. At a certain point in time (the breakpoint t_b), the rate constant changes to a different value, k_2. For this bi-phasic pattern, the rate constant k_1, is often greater than k_2. The major drawback is that $t_{1/2}$ value for the overall decline of the antibiotic can only be calculated from k_1 if it is reached

before the breakpoint. If the $t_{1/2}$ value is calculated from k_2 the slow later stage of decline, it will be longer. The integrated forms of the hockey-stick model are shown in eqns. 2.8 and 2.9.

$$[C] = C_o e^{-k_1 t} \qquad \text{for } t < t_b \qquad 2.8$$

$$[C] = C_o e^{-k_1 t_b} e^{-k_2 (t - t_b)} \qquad \text{for } t > t_b \qquad 2.9$$

2.6.4 The Double-First-Order in Parallel model (bi-exponential kinetics)

In this model there are no analytical equations to calculate endpoints for a degradation pattern. These are determined by an iterative technique through the use of the Excel goal-seek function (Boesten et al., 2006). Alternatively, the $t_{1/2}$ can be obtained from a table of calculated concentrations for the time at which it has decreased by ½ the initial fitted value. End points in bi-exponential degradation kinetics are not calculated from individual rate constants.

The integrated form is

$$[C] = C_1 e^{-k_1 t} + C_2 e^{-k_2 t} \qquad 2.10$$

where

[C] = Total concentration of chemical present

C_1= Concentration of chemical applied to compartment 1 at time t=0

C_2= Concentration of chemical applied to compartment 2 at time t=0

k_1 = Degradation rate constant in compartment 1

k_2 = Degradation rate in compartment 2

The major drawbacks of this model are the complex calculations involved such that the whole process is reduced to curve fitting. Because of this very few studies applied this model in predicting the persistence of antibiotics in the environment.

2.6.5 The availability-adjusted model

If some of the target antibiotic is adsorbed onto soil, manure, or other substances in the environment, the degradation rate is reduced, because the adsorbed molecules are unavailable for degradation. If the ratio of non-adsorbed to the total concentration of the target compound at time *t* is *ì*, then the first order rate integrated form becomes;

$$C = C_o e^{-k'' / a}(1 - e^{-at}) \qquad 2.11$$

Where *a* is a constant called the unavailability coefficient and is non-negative.

k′′= kξ and ξ = is the fraction of the non-adsorbed amount in the total amount of the target compound at *t* = 0

The $t_{1/2}$ is then calculated by the relationship

$$t_{\frac{1}{2}} = -\frac{1}{a}\ln(1 - \frac{0.693a}{k''}) \qquad 2.12$$

All the models discussed above have one thing in common. They follow the half-life model that assumes that the reverse reactions of elementary reactions are negligible (Benson, 1960). Elementary processes are sometimes reversible reactions; sometimes rates of forward and reverse reactions are equal (equilibrium systems).

Antibiotics have been reported to undergo microbial, hydrolytic and photolytic degradation (Maki et al., 2006; Xuan et al., 2010) and these are complex reactions. Only elementary reactions can be characterized by their molecularity or order of reaction, and the adjectives “unimolecular”, “bimolecular”, etc, may not have meaning for complex reactions, such as hydrolysis, photolysis and microbial degradation, which involve a sequence of many elementary steps (Castellan, 1971; Atkins and Paula, 2006). A study of persistence data reported in literature (Table 2.2) shows that for most, if not all, TCs $t_{1/2}$ persistence data are

highly variable, whereas in true first order kinetics, a constant value should be obtained for the $t_{1/2}$ of any given substance, irrespective of the actual environmental conditions prevailing(see eqns 2.4 and 2.5). For kinetic models for studying the persistence of organic substances in the environment to be of any predictive value, they should take into consideration spatial, temporal and climatic changes, as well as microbial binding equilibrium and sorption/desorption equilibrium involving particulate matter (Wania and Mackay, 1999; Zaranyika and Nyandoro, 1993; Zaranyika et al., 2010). Thus it is the aim of the present study to study the degradation of the TCs in terms of a model that takes into account variation in environmental conditions.

Zaranyika and Nyandoro, (1993) and Zaranyika et al., (2010) have studied the persistence of several pesticides in the aquatic environment using microcosm experiments designed to simulate as closely as possible actual aquatic environmental conditions. Control experiments were conducted in distilled water under sunlight conditions. Zaranyika and co-workers found that the curves obtained for the water phase and the sediment phase could be resolved into two linear portions. Fig 2.6 shows typical curves obtained for paraquat for the microcosm experiments, as well as the controls.

Table 2.2 Half-lives reported in literature

Antibacterial	Environment	Half-life (days)	Reference
OTC	Surface water	42–46	Ingerslev et al., (2001)
OTC	Deionized water	0.26-7	Doi and Stoskopf, (2000)
OTC	Humic water	46-36	Doi and Stoskopf, (2000)
OTC	soil interstitial water	2-270	Halling-Sørensen et al., (2003)
OTC	buffered solutions	15-120	Xuan et al., (2010)
OTC	Sea sediment	419	Björklund et al.,(1990)
OTC	Marine sediment	7.3	Hektoen et al., (1995)
OTC	Marine sediment	60.4	Pouliquen et al., (1992)
OTC	Fish farm sediment	70	Jacobsen and Berglind, (1988)
OTC	Marine sediment	32- 64	Samuelsen, (1989)
TC	Buffered distilled water	0,26-67.9	Loftin et al., (2008)
TC	Soil	55-105	Winckler and Grafe, (2001)
CTC	Soil	21-24	Carlson and Mabury, (2006)
CTC	Soil	25-58	Halling-Sørensen et al., (2002)
CTC	Manure	4.39-86.6	Bao et al., (2009)
CTC	Compost	1-3	Arikan et al., (2008)
DC	Soil	4.5-76.3	Szatmari et al., (2011)

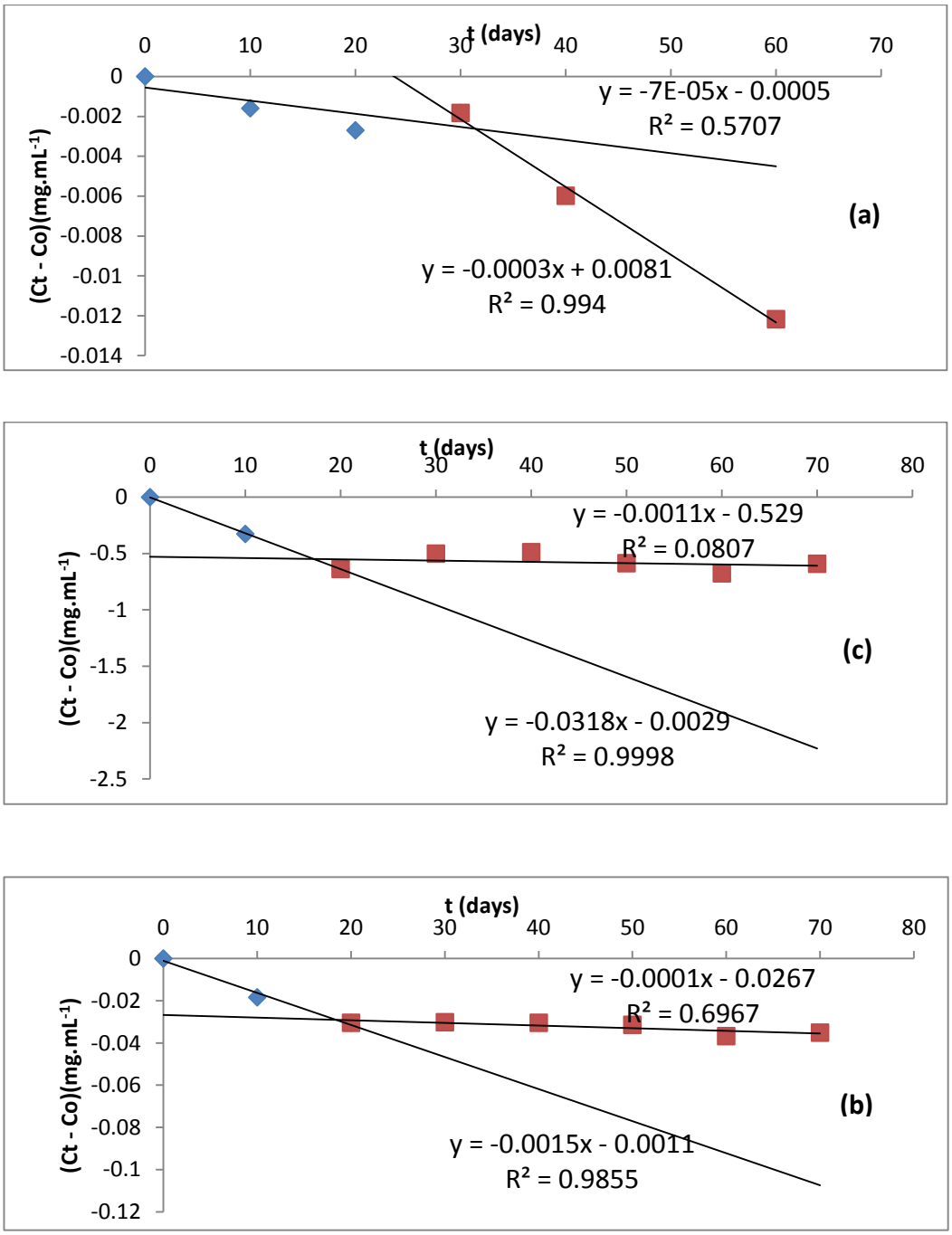


Fig 2.6. Rates of degradation of paraquat in (a) distilled water, (b) river water, and (c) sediment (Adapted from Zaranyika and Nyoni, 2013).

The degradation of paraquat, in distilled water was experimentally observed to proceed via an initial slow rate of 7 x 10^{-5} mg $mL^{-1}day^{-1}$ during the first 35 days, followed by a faster rate of 3 x 10^{-4} mg $mL^{-1}day^{-1}$ for the rest of the study period. Zaranyika and Nyoni, (2013) suggested that the only reasonable explanation for the results observed was that the initial slow rate of 7 x 10^{-5} mg $g^{-1}day^{-1}$ in the distilled water control experiment was due to a combination of chemical and /or photochemical degradation, while the subsequent fast degradation was due to microbial degradation as a result of contamination from the air.

Zaranyika and Nyoni, (2013) attributed the increase in the rate of degradation in the microcosm experiment to microbial degradation. It is generally agreed that free pesticide in solution is available for microbial degradation, (Weber and Coble, 1968; Ogram et al., 1985, Wu et al., 2011) hence, Zaranyika and Nyoni, (2013) attributed the initial fast rate of degradation in the water phase to microbial degradation of the dissolved free pesticide in solution. It has been reported that microorganisms only bind pesticides in the desorbed state (Wu et al., 2011; Shankar et al., 2013) hence, Zaranyika and Nyoni, (2013) attributed the subsequent slow rate of degradation in the water phase to microbial degradation of dissolved colloidal particle adsorbed pesticide. The initial fast rate in the sediment was attributed to microbial degradation of dissolved free pesticide in sediment pore water. The subsequent slow rate of degradation in the sediment phase was attributed to microbial degradation of sediment-phase colloidal particle adsorbed pesticide or sediment particle adsorbed pesticide.

2.6.6 Proposed microbial degradation kinetic model.

Zaranyika and Nyoni (2013) proposed a photolytic kinetic model shown in Table2.3 to account for the observed linear rates in distilled water and showed that the linear rate of degradation is given by eqn 2.13

$$-\frac{dP}{dt}=\left(\frac{k_p k_\phi}{k_f+k_q[Q]+k_p}\right)[P]_o=k'_\phi[P]_o=k''_\phi \quad (2.13)$$

where k''_ϕ is the zero order rate constants for the photolytic degradation, and P denotes the pesticide molecule, and the subscript *o* denotes initial concentration.

For microbial degradation, Zaranyika et al., (2010) proposed the kinetic model shown in Table 2.4. The proposed model involves Steps1a (binding of the pesticide molecule by microbial organism), Steps1b (adsorption by colloidal particles in the water phase and sediment phase of the experiment), and Steps 1c (adsorption by sediment particles in the sediment phase). Steps 1a to 1c occur simultaneously as the pesticide is introduced into the experimental microcosm. Step 2 (binding and metabolism by enzymes) takes place inside the microorganism, following binding of the pesticide by the microorganism. Two basic assumptions were made in arriving at the model proposed in Table 2.4: (a) that binding of adsorbed pesticide molecules by the microorganism occurs only in the desorbed state (Wu et al., 2011; Shanker et al., 2013) and (b) that the rate at which the microorganisms bind the pesticide molecules is greater than the rate at which the pesticides undergo desorption from the colloidal and sediment particles. These assumptions are based on the fact that it is generally agreed that free pesticide in solution is available for microbial degradation (Shanker et al., 2013)

Table 2.3 Photolysis of pesticides in the aquatic environment: Proposed kinetic model (Zaranyika and co-workers 2012 and 2013)

Step	Reaction	Rate constant	Process
1	$P^A + h\nu \rightarrow P^*$	k_ϕ	Light absorption
2	$P^* \rightarrow P + h\nu(\Delta E)$	$k_{-\phi}$	Radiative or collisional relaxation
3	$P^* + Q \rightarrow P + Q^*$	k_q	Quenching (Q = quencher)
4	$P^* \rightarrow D^B$	k_P	Photolysis.

[A]P = pesticide; [B]D = degradation products.

Table 2.4 Microbial degradation of pesticide, P, in the aquatic environment: Proposed kinetic model (Zaranyika and Mlilo, 2012)

Step[A]	Reaction (Water Phase) [B]	R/ const	Reaction (Sediment phase)[B]	[C]R/const
1(a)	$P + M \rightarrow PM$	k_1	$P + M \rightarrow PM$	k_1
	$PM \rightarrow P + M$	k_{-1}	$PM \rightarrow P + M$	k_{-1}
2	$PM + E \rightarrow PE$	k_2	$PM + E \rightarrow PE$	k_2
	$PE \rightarrow P + E$	k_{-2}	$PE \rightarrow P + E$	k_{-2}
	$PE \rightarrow D + E$	k_3	$PE \rightarrow D + E$	k_3
1(b)	$P + nC_1 \rightarrow P(C_1)_n$	k_4	$P + mC_1 \rightarrow P(C_1)_m$	k_5
	$P(C_1)_n \rightarrow P + nC_1$	k_{-4}	$P(C_1)_m \rightarrow P + mC_1$	k_{-5}
1(c)			$P + zS \rightarrow P(S)_z$	K_6
			$P(S)_z \rightarrow P + zS$	k_{-6}

[A]Step 1(a) = Binding by microorganism; 2 = Binding and degradation by enzyme; 1(b) = Adsorption by colloidal particles; 1(c) = Adsorption by sediment particles. [B]P = pesticide; M = microorganism; PE = pesticide-enzyme complex; E = enzyme; D = degradation products; PM = microbial-bound pesticide; C_1 = colloidal particle, type 1; $P(C_1)_n$ = pesticide-colloidal-particle complex; S = sediment particle. $P(S)_z$ = pesticide-sediment-particle complex; [C]R/const = rate constant.

According to the proposed kinetic model (Table 2.4), at least 2 speciation forms (dissolved free pesticide and dissolved colloidal particle adsorbed pesticide) are expected in the water phase of the microcosm experiment represented by Steps 1(a) and 1(b), assuming only one

type of colloidal particle is present. This is consistent with the 2 linear rates of degradation observed experimentally for the water phase, (Fig. 2.6 and Table 2.5). When 2 or more colloidal particle types are present, then more than 2 speciation forms can be expected. Similarly, at least 3 speciation forms (sediment phase dissolved free pesticide, sediment particle adsorbed pesticide and sediment phase colloidal particle adsorbed pesticides) are expected in the sediment phase. However, only 2 linear rates of degradation were observed for the sediment phase. The authors suggest that this is because desorption from the larger sediment particles was faster than the rate at which the microorganism can bind the pesticide molecules, hence there will be no difference between the rate of degradation of pesticide dissolved in pore water (sediment phase dissolved free pesticide) and that of pesticide adsorbed to the larger sediment particle. The authors suggested that pesticide dissolved in sediment pore water exists in dynamic equilibrium with pesticide adsorbed to surfaces of sediment particles, and therefore attributed the fast rate of degradation in the sediment phase to degradation of the sediment particle adsorbed pesticide.

2.6.7 Microbial degradation rate equations.

Microbial counts in natural waters ranging from 5×10^5 to 1.4×10^8 bacteria mL^{-1} were reported by Wommack et al., (1992), Hennes and Suttle, (1995) and Zweifel and Hagstron, (1995). Taking dimethoate as an example, the concentration of 149.0 μgmL^{-1} used by Zaranyika and Nyandoro, (1993) in the microcosm experiments under review amounts to 3.9×10^{17} molecules mL^{-1}, to give a pesticide molecular number density to microbial count (or number density) ratio of 3.6×10^{10}, i.e., [P] >> [M].On the basis of the kinetic model in Table 2.4, and taking into account the fact that the pesticide molecular number density is in excess of the microbial number density, Zaranyika and co-workers were able to show that the rates of degradation for the dissolved and colloidal particle adsorbed speciation forms in the water

phase of the microcosm experiment are given by eqns. 2.14 and 2.15, whereas the rate of degradation of the dissolved form in the sediment pore water is given by eqn. 2.16, and the rates of degradation of the sediment particle and colloidal particle adsorbed pesticide in the sediment phase are given by Eqns. 2.17 and 2.18, respectively

$$-\frac{d[P]}{dt}=\left(\frac{k_1}{k_{-1}+k_2}\right)\left(\frac{k_2k_3}{k_{-2}+k_3}\right)[M]_{S(W)}=k_E[M]_{S(W)}=k'_{E(W)} \tag{2.14}$$

$$-\frac{d[P]}{dt}=\left(\frac{k_1}{k_{-1}+k_2}\right)\left(\frac{k_2k_3}{k_{-2}+k_3}\right)\left(\frac{k_{-4}}{k_4}\right)[M]_{S(W)}=k_{C1(w)}[M]_{0(W)}=k'_{C1(W)} \tag{2.15}$$

$$-\frac{d[P]}{dt}=\left(\frac{k_1}{k_{-1}+k_2}\right)\left(\frac{k_2k_3}{k_{-2}+k_3}\right)[M]_{S(S)}=k_E[M]_{S(S)}=k'_{E(S)} \tag{2.16}$$

$$-\frac{d[P]}{dt}=\left(\frac{k_1}{k_{-1}+k_2}\right)\left(\frac{k_2k_3}{k_{-2}+k_3}\right)\left(\frac{k_{-5}}{k_5}\right)[M]_{S(S)}=k_{C1(S)}[M]_{S(S)}=k'_{C1(S)} \tag{2.17}$$

$$-\frac{d[P]}{dt}=\left(\frac{k_1}{k_{-1}+k_2}\right)\left(\frac{k_2k_3}{k_{-2}+k_3}\right)\left(\frac{k_{-6}}{k_6}\right)[M]_{S(S)}=k_S[M]_{S(S)}=k'_{S(S)} \tag{2.18}$$

where [P] and $[M]_S$ denote concentration of the pesticide and the steady state concentration of microorganisms (or microbial count), respectively.

Eqn. 2.15 can be resolved into a product of 2 factors, a microbial factor (= $k[M]_w$) and an adsorption factor (k_{-4}/k_4), thus:

$$-\frac{dP}{dt}=k_W[M]_W\left(\frac{k_{-4}}{k_4}\right) \tag{2.19}$$

where k_w is now the rate constant for microbial degradation in the water phase (W). Eqn2.17 and 2.18 can be similarly resolved, thus:

$$-\frac{dP}{dt} = k_S[M]_S\left(\frac{k_{-5}}{k_5}\right) \quad (2.20)$$

$$-\frac{dP}{dt} = k_S[M]_S\left(\frac{k_{-6}}{k_6}\right) \quad (2.21)$$

where K_S is now the rate constant for microbial degradation in the sediment phase (S).

Equations 2.14 and 2.16 show that degradation of the free dissolved pesticide depends only on the microbial factor. The kinetic model proposed by Zaranyika and co-workers (2010, 2013) is thus able to resolve between the contribution of microbial degradation and adsorption to the observed rate of degradation of pesticides in the aquatic environment.

2.6.8 Pesticides adsorption onto colloidal and sediment particles: Apparent equilibrium constants for adsorption and desorption.

In eqn2.15 and 2.17, k_{-4}/k_4 and k_{-5}/k_5 are in fact the inverse of the adsorption/desorption equilibrium constants by colloidal particles in the water phase of the microcosm experiment. Likewise, eqn 2.18 is the inverse of the adsorption/desorption equilibrium constant by colloidal and sediment particles in the water phase of the microcosm experiment. By assuming that the density of microorganisms, $[M]_w$, is constant in eqn.2.14 and 2.16, Zaranyika and co-workers were able to calculate the values of k_{-4}/k_4 and k_{-5}/k_5 by dividing eqns. 2.15 and 2.17 by eqn. 2.14 and 2.16 to arrive at the apparent adsorption/desorption equilibrium constant. Similarly for the sediment, the value of k_{-6}/k_6 was obtained by dividing eqn. 2.18 by eqn. 2.16.

2.7 ANALYTICAL METHODS FOR QUANTIFYING TCS

Methods for quantification of TCs residues in the aquatic environment continue to be developed and have been the object of study in several investigations (Pena et al., 1998; Lindsey et al., 2001; Zhu et al., 2001; Löffler, and Ternes, 2003; Halling-Sørensen et al., 2003; Ng and Linder, 2003; Snow et al., 2003; The reporter SUPLCO, 2004; Ben et al., 2008; Jia et al., 2009; Yang et al., 2010; Shafrir and Avisar, 2012). Table 2.5 summarizes analytical techniques that have been used to quantify TCs in the environment. Liquid chromatography-mass spectrometry or tandem mass spectrometry (LC-MS/MS-MS) is becoming the method of choice in many environmental studies. This is because it has the advantage of improved sensitivity and the ability to provide compound structure. LC-MS methods for the analysis of TCs appearing in literature employ the following ionization techniques; particle beam, fast atom bombardment, thermospray, atmospheric pressure chemical ionization and electrospray ionization. Among these electrospray is the most used method (O`Connor and Aga, 2007). A problem inherent in the analysis of TC antibiotics using liquid chromatography is the interaction with residual silanol groups in the C_{18} columns. This often results in peak tailing and analyte recovery inconsistency. This problem has been solved by the addition of complexing agents such as oxalic acid, citric acid or EDTA in the sample and mobile phase (Table 2.5). Although this solved the problem unfortunately the complexing agents may accumulate in the capillary interface or skimmer of the electrospray ionization or atmospheric pressure chemical ionization source. Plugged capillaries and signal loss usually is the result. In an effort to reduce clogging some researchers have suggested the use of elevated nebulizer probe temperature such that the complexing agents decomposes in the atmospheric pressure chemical ionization interface (O`Connor and Aga, 2007). This modification allows prolonged analysis without severe signal loss. Unfortunately, this set up is only amenable to

electrospray instrumentation with off-axis or orthogonal spray sampling and such instruments are not widely available, due to increased expense. Studies utilizing LC-MS techniques in the analysis of tetracyclines in environmental samples have increased steadily over the years. Loftin et al., (2008) used reverse phase liquid chromatography with an Agilent 1100 series LC-MS to analyse TCs antibacterials in aqueous solutions. The flow rate was 0.360 mL/min and the column and oven temperature were set at 30°C. A gradient elution was used with mobile-phase A, consisting of aqueous 0.3% formic acid solution and mobile-phase B, consisting of 100% methanol. The mobile phase were varied over 25 min with a 5-min post-column equilibration. Mass fragmentor voltage was optimized at 190 V.

Tandem mass spectrometry (LC MS-MS) has also been used in the analysis of TCs in river water and sediment (Shafrir and Avisar, 2012; Zhou et al., 2011; Richard, (2010); Jia et al., 2009; Löffler and Ternes., 2003, Halling-Sørensen et al., 2003; Zhu et al., 2001). Lower detection limits were often achieved (Table 2.5). Yang et al., (2010) analysed TCs in river sediment using rapid resolution liquid chromatography–tandem mass spectrometry (RRLC–MS/MS) equipped with electrospray ionization source switched in the positive mode. Limit of detection ranging from, 0.08 μg/kg to 4.2 μg/kg were achieved. The major advantage of RRLC is that it provides better sensitivity, resolution and shorter analysis time (Díaz-Cruz and Barceló, 2007).

Other HPLC methods reported in the literature incorporate fixed or variable ultraviolet (UV) detection (Jiang et al., 2015; Patyra et al., 2014, Shafrir and Avisar, 2012; Chen et al., 2011; Ooishi and Tosa, 2010; Xuan et al., 2010; Ng and Linder, 2003; Pena et al., 2000, Doi and Stoskopf, 2000; Choo, 1994; Pouliquen et al., 1992). Because of its affordability HPLC coupled to UV detection is the most commonly applied technique for routine analysis of TCs.

Blackwell et al., (2004) reported a very sensitive method based on HPLC coupled to UV detection for the analysis of veterinary antibiotics utilizing a strong anionic exchange cartridge in tandem with a polymeric cartridge. Other studies used HPLC with fluorescence detection (Pena et al., 1998). Although fluorescence is more specific than UV detection and is less affected by interference from sample matrix, it requires derivatization (Pena et al., 1998). The drawback with derivatization is that it increase time and cost per analysis. Usually stability of derivatives is limited, and therefore they must be analysed immediately. This adds a time constraint to the analytical technique, therefore, studies utilising this technique in environmental studies are very few. Detection methods such as electrochemical (Simon, 2005) and polarimetric detection (Ng and Linder, 2003) have also been used however studies employing these techniques are also still few.

The best method for environmental analysis is the one that makes it possible to achieve lower detection limits. Ng and Linder, (2003) carried out a comparative study of laser-based polarimetric with UV detection and obtained detection limits that were comparable to those obtained with UV detection. The relative standard deviation for the integrated peak areas for polarimetric detection was found to be 1.7%, compared to 3.1% for UV detection. This is because the polarimeter responds only to optically active material therefore, provides a degree of selectivity. UV detection responds to any substance with a chromophore that is active at that given wavelength. This results in a less precise quantitation. The huge response per mass injection inherent in polarimetric detection results in a better precision for the analysis of TC antibacterials. The major drawback of polarimetric detection is the problem of increased baseline drift, due to temperature variation at the most sensitive scale therefore it has not yet received wide acceptance as MS/MS-MS and UV based detection methods.

Enzyme-Linked Immunosorbent Assays (ELISAs) have also been used in the analysis of TCs in aquatic environment (Daff, 2005). Studies utilizing ELISA are limited despite its low cost and rapid analysis. ELISA use has been limited to screening purposes only due to its inability to distinguish between individual tetracycline analogues. Also in the environment compound of interest exist in an array of other organic molecules that might interfere. The interferences caused by such compounds cannot be completely resolved (Daff, 2005).

Tetracycline antibiotics were also analysed using a UV spectrophotometer (Lunestad and Goksayr, 1990; Jodeh and Awartani, 2011). The major drawback of using UV spectrophotometers without a separation technique is that the method cannot resolve interferences due to degradation products and other matrix components.

Table 2.5 Analytical methods for analysing tetracyclines in sediment and aqueous phase

Compound (s)	Mobile phase	Determination	LOD	References
TC, OTC	1% formic acid in HPLC water (pH 2.3) and 1% formic acid in acetonitrile	HPLCMS-MS	0.56 -7,60 ng/g	Shafrir and Avisar, (2012)
TC	deionized water with 0.1 % formic acid, acetonitrile	LC MS	-	Izbicki and Quinn, (2011)
CTC, DC, OTC, TC	acetonitrile and 5 mM oxalic acid	RRLC MS-MS	0.05 - 0.12 ng/g	Zhou et al., (2011)
TC, OTC	Acetonitrile and 5 mM oxalic acid.	RRLC MS-MS	0.08 - 4.2 μg/kg.	Yang et al., (2010)
CTC, DC, OTC, TC	eluent A , acetonitrile–methanol (1:1, v/v),eluent B, HPLC grade water 0.1% formic acid as eluent B.	HPLCMS-MS	-	Gros et al., (2010)
CTC, OTC, TC	0.3% formic, methanol	LC-MS	-	Loftin et al., (2008)
DC, TC	-	LC MS-MS	8 -15 ng/g	Chenxi et al., (2008)
TC, OTC	Acetonitrile and 5 mM oxalic acid.	LC MS-MS	0.03 - 0.1 μg /L	Jia et al., (2009)
TC	water acidified with 0.3% formic acid and	LC-ESI-MS	-	Kim et al., (2005)

	acetonitrile.			
OTC,EOTC, AOTC	Methanol, formic acid and milliQ-water	HPLCMS-MS	-	Halling-Sørensen et al., 2003
DC,TC, OTC, TC	water, 5% formic acid, acetonitrile, and methanol (23:40:25:12)	HPLCMS/MS	0.20 - 0.28 ug/ L	Zhu et al., 2001
CTC, OTC, TC	-	LC MS	0.06 – 0.11 ng/g	Snow et al., (2003)
TC, OTC, DC, CTC	Mobile phase A contained 10 mMammoniumformate in 90/10 water/methanol with 0.3% formicacid. Mobile phase B contained 10 mM ammonium formate with 0.5% formic acid in MeOH.	LC MS	-	Lindsey et al (2001)
TC	acetic acid at 0.01M in a 75 : 25 (v/v) water/methanol	HPLC UV	-	Ooishi and Tosa, (2010)
OTC	acetonitrile and water (pH adjusted to 3 using H_3PO_4)	HPLC-UV	-	Xuan et al., (2010)
TC, OTC	68% (v/v) 0.1 M oxalic acid ammonium, 27% (v/v) N,N-dimethylformamide, 5% (v/v) 0.2 M diammonium phosphate in high purity water	HPLC-UV	-	Wen et al., (2009)
CTC, TC, OTC,DC	1:1.5:7.5 methanol–acetonitrile, oxalic acid. pH 2	HPLC UV	-	Ng and Linder, (2003)
TC, ETC	-	HPLC UV	34-42ug/ L	Kühne et al., (2000)
	0.001M Na_2EDTA, 0.05M citric acid, 0.013M tri-sodium citrate and 0.1M potassium nitrate	HPLC-UV	-	Coyne et al., (2001)
OTC	5% methanol, 10% acetonitrile,and 85% aqueous 0.01 M oxalic acid.	HPLC UV	0.05 ug/ml	Doi and Stoskopf, (2000)
OTC	1:1.5:7.5 methanol, acetonitrile,0.01 M oxalic acid pH 3.5	HPLC UV	0.01ug/ g	Choo, (1994),
TC	-	HPLC-UV	0.5 µg/g,	Hektoen et al., (1995)
OTC	acetonitrile : 0.02 M orthophosphoric acid solution, pH 2.3 (24 *:76* v/v);	HPLC-UV	0.05 ug/ g	Pouliquen et al., (1992)
OTC		HPLC-UV		Samuelsen, (1989)
TC, OTC	15% methanol-25%	HPLC-DAD	-	Chen and Huang, (2011)

	acetonitrile-60% 0.01 mol/L oxalic			
TC, ETC, ATC	Oxalic acid solution (pH 2.0; 0.01 M) and 20–40% of acetonitrile.	HPLC-FL	-	Pena et al., (1998)
CTC, TC, OTC,DC	1:1.5:5 methanol–acetonitrile, oxalic acid. pH 2	HPLC PD	0.05 ug/ml	Ng and Linder (2003)
TC	N/A	ELISA	-	Daff, (2005)
CTC, DC, OTC, TC	55:45 mixture of 0.18 M trifluroacetic acid (adjusted to a pH of 2 with ammonium hydroxide and high purity grade methanol	LC-EC	5 μg/ L	Simon, (2005)
CTC, TC	N/A	UV	-	Meyers and Smith, (1962)
DC	N/A	UV	-	Jodeh and Awartani, (2011)
OTC	N/A	UV	-	Lunestad and Goksayr, (1990)

- Not described, N/A not applicable.

2.8 SAMPLE PREPARATION TECHNIQUES FOR TETRACYCLINES

In order to design an effective strategy for the extraction technique an understanding of the behaviour of an analyte in its environmental matrix is required. TCs are water soluble however they are highly soluble in organic solvents such as alcohols (O`Connor and Aga, 2007). TCs possess the ability to interact with cationic and anionic sites in the matrix. This gives a daunting task for developing an effective extraction technique. Different extraction techniques continue to be developed and literature reports many of such techniques (O`Connor and Aga, 2007; Yang et al., 2010; Shafrir and Avisar, 2012). The overall aim is to reduce matrix interference so as to improve percentage recovery and reproducibility. In a study conducted by Carvalho et al., (2013) vortex agitation (VA), ultrasonic assisted solvent extraction (UASE) and microwave assisted extraction were compared in the extraction of TCs from sludge and sediment samples. At most the recovery was 25%, which is far below

the US-EPA accepted level of 70-120% (Carvalho et al., 2013). Andreu et al., (2009) used pressurized liquid extraction (PLE) to extract TCs residues from soil samples and recoveries in the range of 70-99% were achieved. As with the case of the Soxhlet extraction (SE), the major drawback of PLE is thermal degradation at the elevated temperatures. Use of elevated temperatures resulted in co-extraction of unwanted matrices, which caused significant difficulties in analyte detection and required sophisticated clean up procedures (Andreu et al., 2009; O`Connor and Aga, 2007). Super critical fluid extraction (SFE) was also employed in previous studies, however, high and reproducible recoveries were not often attained (Jacobsen et al., 2004; Kay et al., 2005; Kim and Carlson, 2006; Lalumera et al., 2004). Although adjusting pH, adding complexing agents such as EDTA, oxalic acid and citric acid to release the antibiotic by interacting with metal cations, ultrasonication and judicial choice of extracting organic solvents (Table 2.6), improved extraction efficiencies, the results were highly variable, 30-125%.

Extraction, clean-up and preconcentration methods based on solid phase extraction have also been employed to reduce matrix interference. Interferences targeted include metal, proteins and humic acids. Solid phase extraction sorbents that have been employed in previous studies include reversed phase C_{18}, Hydrophilic lipophilic balance (HLB), poly-(divenylbenzene-co-N-pyrrolidone) and Strata X (surface modified styrene divenylbenzene). Although recoveries did not improve significantly, the sorbents greatly lowered matrix interferences. HLB, a polymeric sorbent exhibited superior extraction properties (Andreu et al., 2009). This is because HLB does not consist of residual silanol groups that may retain the antibiotics as compared to C_{18} sorbents. Studies employing HLB frequently appear in literature. Tandem solid phase extraction was used for the extraction of TCs. In a study conducted by Blackwell and co-workers (2004) ultrasonic assisted tandem solid phase extracted involving Strong

Anionic Exchange resin (SAX) and HLB resins was used to extract OTC from soil and pig slurry after addition of EDTA and adjusting the pH to 4 using McIlvaine buffer. A recovery of 77% was realised. SAX removed anionic interferences such as, humic acid while TCs being neutral or cationic at pH 4 would only be retained by HLB resin. Ultrasonication affords that extra energy required to dislodge the analyte from its matrix. Yang et al., (2010) and Zhou et al., (2013) used the same method to extract tetracyclines from river sediment and recoveries of 48.2-72.0 and 49.4-125% were realised respectively.

DSPE and MSPD are versatile techniques that have also been applied in the extraction of tetracyclines (Tsai et al., 2009). Recoveries of 97% have been realised when these techniques were used to extract tetracyclines from food samples (Tsai et al., 2009; Oniszczuk et al., 2014). The methods have been observed to be quick, easy to use, cheap, rugged and employ less organic solvents.

As pointed earlier that TCs form complexes with metal cations and organic matter in the aquatic environment, the ease at which TCs can be extracted from natural aquatic samples depends to a large extent by the nature of the complex formed, precisely the strength of the bond formed between the TC molecule and the metal cation or functional groups of the humic acid molecule. The task is further complicated by the fact that TC antibiotics are sensitive to heat such that extraction techniques employing heat such, as Soxhlet and microwave based techniques cannot be used efficiently (Tsai et al., 2009). As per previous discussion, it is important to note that unless the extraction technique provides adequate energy to break the bond between the TC molecule and the metal cation or humic acid, low and variable recoveries will be realised. Ultrasonication provides the energy required to break the bond without subjecting the sample to heat. From the discussion above, SPE gave the best results especially if coupled to ultrasonication.

Table 2.6. Extraction solvents and recoveries of tetracyclines reported in literature

Compound	Extraction solvent	Recovery (%)	References
CTC	McIlvaine buffer with 0.1 M EDTA / disodium phosphate /citric acid	64-112	Shelver and Varel, (2012)
OTC	Methanol /water (60:40) v/v citrate buffer	30-59	Shafrir and Avisar, (2012)
CTC, DC, OTC, TC	citric buffer (pH 3) and acetonitrile (50:50, v/v)	60.0-125	Zhou et al., (2011)
TC,OTC	Acetonitrile, citric acid pH 4 and Na_2EDTA	82-102	Peng et al., (2011)
OTC, TC	Citric buffer, acetonitrile (50:50)v/v	48.2–72.0	Yang et al., (2010)
OTC	Methanol / water (60:20, v/v)	84-102	Xuan et al., (2010)
OTC	Water / methanol (60:20, v/v)	-	Loftin et al., (2008)
CTC, DC, OTC, TC	Methanol, water, $MgCl_2$ pH 8	60-113	Simon, (2005)
TC, OTC	EDTA / McIlvaine buffer	-	Daff, (2005)
CTC, OTC, TC	1M citric acid/acetone with formic acid	103, 108, 99	Aga et al., (2005)
CTC	1M NaCl/1M oxalic acid/ethanol	79	Sassman and Lee, (2005)
OTC	EDTA/citric acid/phosphate	38	Kay et al (2005)
OTC	Methanol/EDTA/McIlvaine Buffer	27–75	Blackwell et al., (2004)
CTC, OTC	Methanol/citric acid buffer	33–78	Jacobsen et al., (2004)
OTC	Methanol/McIlvaine Buffer	81	De Liguoro et al., (2003)
TC, OTC, CTC	Potassium phosphate, citric acid, water	78-90	Zhu et al., (2001)
TC, OTC, CTC	citrate, EDTA, and/or oxalic acid) and pH buffer	89-100	Lindsay et al., 2001
TC	Methanol	-	Pena, (1998)
OTC, TC	Methanol/EDTA/ McIlvaine Buffer	57.5	Pouliquen et al., (1992)

- Not reported

CHAPTER 3

3 PRELIMINARY STUDIES: METHOD OPTIMIZATION AND EVALUATION OF EXTRACTION TECHNIQUES FOR TCs IN RIVER WATER AND SEDIMENT

3.1 INTRODUCTION

Literature reports many techniques for baseline separation, identification, and quantitation of tetracyclines and their degradation products from environmental samples however recoveries, linearity, detection limits and choice of mobile phase varied from one study to another. In such a situation method optimization is of paramount importance. This section of the study was devoted to optimization and evaluation of three extraction techniques; ultrasonic-tandem solid phase extraction, ultrasonic-matrix solid phase dispersion and ultrasonic-dispersive solid phase extraction in the extraction of OTC, DC, CTC and TC from river water and sediment. While tandem solid phase extraction technique has been the method of choice for analysis of TCs in environmental samples (Blackwell et al., 2004; Zhou et al., 2011, 2013) dispersive solid phase extraction and matrix solid phase dispersion techniques have been applied mostly for analysis of TCs in food samples (Tsai and Huang, 2009; Cruz-Vera et al., 2011, Oniszczuk et al., 2014). Recoveries of 97% or more were achieved when TCs were determined in food samples. TCs complex with metals and humic acid in the environment, therefore the ease at which they can be extracted from natural samples depends to a huge extent on the nature and strength of the bonds formed between the TC molecule and humic acid functional groups or metal cations. The use of heat or techniques employing heat is not recommended for tetracyclines because they can be converted to epi-tetracyclines (O`Connor and Aga, 2007). Thus unless the extraction technique provides sufficient energy to break the

bonds between the tetracycline molecule and the matrix, low and variable recoveries will be obtained. Ultrasonication provides the energy required without subjecting the sample to heat.

3.2 MATERIALS AND METHODS

3.2.0 MATERIALS, PREPARATION OF REAGENTS AND METHODS

3.2.1 Materials

Oxytetracycline hydrochloride (95%), tetracycline hydrochloride (98%), chlortetracycline hydrochloride (95%) standards, HPLC solvents (methanol and acetonitrile), Strong Anionic Exchange cartridges (SAX) (3 ml, 500 mg), and hydrophilic-lipophilic balance (HLB) cartridges (6 ml, 200 mg) and disposable filter units (MILLPORE 0.45 and 0.22 µm), primary and secondary amine sorbent material (57738-U-SUPELCO supelclean PSA) were obtained from Sigma Aldrich Darmstadt, Germany. Doxycycline hyclate 99% was obtained from Sigma Aldrich, St Louis-Missouri, USA. Analytical grade orthophosphoric acid, nitric acid, ammonia, sodium hydrogen phosphate, citric acid and disodium ethylene diamine tetra acetate (Na_2EDTA) were obtained from SKYLABS Gauteng, South Africa.

3.2.2 McIlvaine buffer

McIlvaine buffer (pH 4) was prepared by dissolving 21.01 g of citric acid monohydrate, 60.5 g of $Na_2EDTA.2H_2O$ and 44.78 g of $Na_2HPO_4.12\ H_2O$ in 1.63 L of ultrapure water (Pan et al., 2011; Bie et al., 2012). The pH of the prepared buffer was further confirmed using a calibrated pH meter.

3.2.3 Standard stock solutions

Standard stock solutions of antibiotics were prepared at 100 mg L^{-1} by dissolving 100 mg of each standard antibiotic in 1 L volumetric flask with methanol. The solution was then diluted to equivalent volume using methanol. The stock solutions were then stored in a refrigerator. Working standard solutions were prepared from the stock solution by serial dilution with methanol.

3.2.4 Cleaning of glassware

In glass apparatus particles are bound together by silanol during the manufacturing process. Silanol is easily combined with TCs by chelation. Because of this all glassware were soaked in 4 M nitric acid, rinsed with detergent and then heated for 2 hours in an oven. The glassware was then cooled, rinsed with methanolic EDTA solution and then, air dried in an oven (Hamscher et al., 2002; Zhou et al., 2011)

3.2.5 Sample collection

River water (2.5 L) and sediment (2 kg) sample portions were collected from the same location in Wayerera River near Bindura University, Zimbabwe (19º 19' 52" South, 42º 21' 52" East). Water samples were collected using pre-cleaned 2.5 L amber glass bottles with Teflon lined caps, while sediment samples were collected using a stainless steel grab sampler (Wei et al., 2014). All the samples were placed in cooler boxes with ice and transported straight to the laboratory, where they were stored in a refrigerator at 5ºC until required for analysis. Physicochemical parameters shown in Table 3.1 were determined using EPA (2001) and APHA (2004) standard methods for analysis of pH, metals, organic matter, nitrates,

cationic exchange and soil clay/sand/loam composition). The results in Table 3.1 show presence of metals, organic matter and clay particles which are major complexing agents for tetracyclines.

3.2.6 Sample preparation: water samples

Ultrapure water and river water samples, 2 L each in replicates of three, were spiked with 0.05, 0.5 and 1 µg mL^{-1} concentrations of antibiotic dissolved in methanol. The samples were vortexed and centrifuged for 1 min at 3000 rpm to separate solid particles from the liquid phase. The liquid phase was then decanted and filtered through 0.45 µm Millipore glass filters.

Table 3.1 Physicochemical parameters of Wayerera river sediment (19º 19' 52'' South, 42º 21' 52'' East).

Property	Mean ± SD n = 3
pH	7.20 ± 0.40
Ca (mg Kg^{-1})	0.16 ± 0.02
Mg (mg Kg^{-1})	0.14 ± 0.05
Fe (mg Kg^{-1})	0.35 ± 0.09
Organic matter content (mg Kg^{-1})	1.20 ± 0.40
Nitrate content (mg Kg^{-1})	0.12 ± 0.01
Cationic exchange capacity (meq L^{-1} Na)	5.58 ± 1.62
Clay (%)	10.30 ± 2.20
Silt (%)	63.60 ± 0.50
Sand (%)	26.10 ± 1.60

3.2.7 Ultrasonic Assisted Tandem Solid Phase Extraction (UA-TSPE)

UA-TSPE was set up according to a previous method reported by (Zhou et al., 2011). Strong anion exchange (SAX) cartridges 3ml (500 mg) and hydrophilic-lipophilic balance (HLB) cartridges 6 mL (200 mg) were set up in tandem. To 2 L of the water sample, 5 mL of Na_2EDTA (0.1 M) and 10 mL of McIlvaine buffer at pH 4 were added and the mixture was ultrasonicated for 15 minutes at 30^oC and centrifuged at 3000 rpm for 10 minutes. Aqueous EDTA was added to chelate metals (Table 3.1) that may interact with the antibiotic of interest. Preconditioning of each cartridge was done with 10 mL of methanol followed by 10 mL of ultrapure water. The supernatants were then passed through the cartridges at a flow rate of 5 mL min^{-1}using a SUPELCO vacuum manifold system connected to a vacuum pump. SAX cartridges were then removed. HLB cartridges were rinsed with 10 mL of ultrapure water to remove weakly bonded impurities and Na_2EDTA and then dried under vacuum for 2 hours. Elution of antibiotics was done with 10 mL of methanol. The methanol eluent was evaporated under vacuum using a Buchi rotary evaporator (Navratilova et al., 2009; Wei et al., 2014) to almost dryness and then re-dissolved in 500 µL of HPLC grade methanol. After filtration through 0.22 µm glass Millipore filters to remove any remaining particulate matter, the extract was placed into amber glass vials and stored in a fridge at 4^oC until required for HPLC analysis. Amber glass vials were employed to prevent photodegradation of TCs.

3.2.8 Ultrasonic Assisted Dispersive Solid Phase Extraction (UA-DSPE)

UA-DSPE was performed following a previous method reported by Cruz-Vera et al., (2011) and Oniszczuk et al., (2014). Separate water samples, 2 L each, were vigorously shaken with 10 mL of acetonitrile in a separating funnel. 5 mL of 0.1 M Na_2EDTA, and 10 mL of McIlvaine buffer (pH 4) were also added to chelate metals present (see Table 3.1).

Magnesium sulphate and sodium chloride 0.5 g each were then added to displace the extraction equilibrium towards the organic phase. The contents were centrifugation at 3000 rpm for 10 min and then the organic portions were transferred into a conical flask followed by adding 40 mg of primary and secondary amine sorbent material (57738-U-SUPELCO supelclean primary or secondary amine (PSA). This sorbent material was added to remove interferences such as humic acid and metals (Table 3.1). The mixture was ultrasonicated for 15 minutes at 30^{o}C and centrifuged at 3000 rpm for 10 minutes. The supernatants were collected and evaporated to almost dryness under vacuum and then re-dissolved in 500 µL of HPLC grade methanol. The contents were filtered through a 0.22 µm Millipore glass filters to remove any particulate matter and then placed into amber glass vials and stored in a fridge at 4^{o}C until required for HPLC analysis. All experiments were conducted in replicates of three.

3.2.9 Ultrasonic Assisted Matrix Solid Phase Dispersion (MSPD)

This was performed following a method reported by Cruz-Vera et al., (2011). Separate water samples (2L each) were shaken vigorously with 10 mL of acetonitrile using a separating funnel followed by adding 5 mL of Na_2EDTA (0.1 M), and 10 mL of McIlvaine buffer prepared at pH 4. Magnesium sulphate and sodium chloride (0.5 g each) were added to facilitate phase separation. The mixture was centrifuged at 3000 rpm for 10 min, and the organic supernatant was transferred to a conical flask and 40 mg of hydrophilic-lipophilic balance (HLB) sorbent was added to trap the analyte on the sorbent leaving interferences in the organic phase. The mixture was ultrasonicated for 15 minutes and centrifuged at 3000 rpm for 10 minutes. The solid layer was collected and packed in a 6 mL polypropylene syringe barrel. Packed polypropylene syringe barrels were washed with ultrapure water to remove loosely held interferences and vacuum dried for 2 hours. Elution of the antibiotics was achieved by adding 12 mL of methanol. The methanol eluate was evaporated to almost

dryness under vacuum and then the contents re-dissolved in 500 µL of HPLC grade methanol. The solutions were filtered through 0.22 µm Millipore glass filters and then placed into 2 ml amber glass vials, and stored in a fridge at 4^{o}C until required for HPLC analysis.

3.2.10 Sample preparation: sediment sample

Dried sediment samples (2 g each) were placed into three separate glass tubes, followed by addition of 1 mL of each standard stock solution (0.05, 0.5 and 1 µg g^{-1}). The contents were mixed by centrifugation and placed in a refrigerator overnight (Zhou et al., 2011; Pan et al., 2011). Ten milliliters of McIlvaine buffer (pH 4) was added into each glass tube and mixed for 1 min. All glass tubes were then centrifuged at 3000 rpm for 10 min. The supernatants collected from each glass tube were then transferred into 250 mL conical flasks. The process of extraction was repeated twice and the supernatants collected from the two extractions were combined. The solutions were diluted to 100 mL with ultrapure water. After filtration through 0.45 µm Millipore filters, clean-up and pre-concentration processes were carried out as described for river and ultrapure water samples. Blank samples, without added antibiotics were also analysed to determine background levels of antibiotics and possible matrix interferences.

3.2.11 HPLC analyses

A Varian HPLC consisting of a Rodyne manual injector, a 20 mL loop and a variable wavelength UV detector (prostar 325) was used for determining the concentration of the antibiotics. The UV variable detector worked remotely, using the Varian Star or Galaxie Chromatography workstation version 6 software. The analytes were separated on a Varian Microsorb MV 1005 packed C_{18} column 250 x 4.6 mm id, 5 µm particle sizes. The separation was performed in an isocratic mode. Mobile phases appearing in literature for analysis of

tetracyclines were tested for the best results (Pouliquen et al., 1992; Doi and Stoskopf, 2000; Snyder, 2010; Navratilova et al., 2009; Zhou et al., 2011). The most efficient mobile phase that managed to base separate tetracyclines and their degradation products consisted of either 240 mL HPLC grade acetonitrile and 760 mL of 0.02 mol $dm^{-3}$of orthophosphoric acid at pH 3 or methanol, acetonitrile and 0.01 M aqueous oxalic acid at pH 3.0 in the ratio 1: 1.5: 7.5 (v/v). The mobile phase containing methanol, acetonitrile and 0.01 M aqueous oxalic acid at pH 3.0 in the ratio 1: 1.5: 7.5 (v/v)was used throughout the analyses. Fresh solutions were prepared, filtered and degassed for every analysis. Column conditions were ambient temperature (22-24 ^{o}C), flow rate 1mLmin^{-1} and injection volume was 10 µL. The detection wavelength was 360 nm which was determined by scanning on a Thermo-fisher UV spectrophotometer GENESYS 10S UV-Vis v4.003 2L9Q129001. Concentration of antibiotics was determined basing on peak area using a calibration curve method generated by regression analysis.

3.2.12 Methodology characteristics

The methods were validated on the bases of an in-house validation procedure following the recommendations of the Commission Decision 2002/657/EC (Pan et al., 2011). Validation parameters such as linearity, specificity, precision, limits of detection (LOD) and limit of quantification (LOQ) were used.

3.2.13(a) Linear dynamic range

The linearity of the methods was determined by analysing eight solutions in the range 0.01-2 µg mL^{-1}. Each concentration was analysed three times. Calibration curves were generated by plotting the analyte peak area against concentration of standard. Table 3.2 show the linear

dynamic ranges (LDR) and regression coefficients (R^2) that were obtained for TC, OTC, CTC and DC.

Table 3.2. Method linearity

Antibiotic	UA-TPSE		UA-DSPE		UA-MSPD	
	LDR (μgmL^{-1})	R^2	LDR (μgmL^{-1}) (μgmL^{-1})	R^2	LDR (μgmL^{-1}) (μgmL^{-1})	R^2
TC	0.01-1.00	0.998	0.01-1.00	0.999	0.01-1.00	0.995
OTC	0.01-1.00	0.995	0.01-1.00	0.997	0.01-1.00	0.999
CTC	0.01-1.00	0.996	0.01-1.00	0.999	0.01-1.00	0.996
DC	0.01-1.00	0.999	0.01-1.00	0.998	0.01-1.00	0.998

LDR-linear dynamic range, R^2 = regression coefficients, UA-TSPE = Ultrasonic assisted tandem solid phase extraction, UA-DPSE = Ultrasonic assisted dispersive solid phase extraction, UA-MSPD = Ultrasonic assisted matrix solid phase dispersion.

3.2.13(b) Limit of detection (LOD) and limit of quantification (LOQ)

In analytical chemistry LOD and LOQ are terms used to describe the smallest concentration of an analyte that can be reliably measured by an analytical procedure. LOD is taken as the lowest concentration of an analyte in a sample that can be detected, but not necessarily quantified under the stated conditions of the test, and is given by (Shrivastava and Gupta, 2011);

$$LOD = 3.s/S \quad (3.1)$$

where s is the standard deviation of y-residuals and S is the slope of the calibration curve. The LOQ is the lowest concentration of an analyte in a sample that can be determined with

acceptable precision and accuracy under the stated conditions of test, and is given by (Shrivastava and Gupta, 2011):

$$LOQ = 10s/S \quad (3.2)$$

The results obtained are shown in Tables 3.3 and 3.4. The results were also confirmed by analysis of successive decreasing concentrations.

Table 3.3 Method limit of detection (LOD) (ng mL^{-1}) and limit of quantification (LOQ) (ng mL^{-1}) in spiked river water

Compound	LOD (ng mL^{-1})			LOQ (ng mL^{-1})		
	UA-TPSE	UA-DSPE	UA-MSPD	UA-TPSE	UA-DSPE	UA-MSPD
TC	22.75	11.60	11.97	55.43	36.00	56.22
OTC	20.34	11.55	11.80	93.12	35.05	35.80
DC	11.58	11.53	15.80	35.00	35.10	35.06
CTC	21.10	11.66	18.30	35.55	37.20	51.92

UA-TSPE = Ultrasonic assisted tandem solid phase extraction, UA-DPSE = Ultrasonic assisted dispersive solid phase extraction, UA-MSPD = Ultrasonic assisted matrix solid phase dispersion,

Table 3.4. Method limit of detection (LOD) (ng g^{-1}) and limit of quantification (LOQ) (ng g^{-1}) in spiked river sediment

Compound	LOD (ngg^{-1})			LOQ (ngg^{-1})		
	UA-TPSE	UA-DSPE	UA-MSPD	UA-TPSE	UA-DSPE	UA-MSPD
TC	18.85	12.70	20.78	35.00	56.10	53.40
OTC	20.00	12.93	21.33	30.72	45.94	45.25
DC	11.82	11.60	21.00	37.00	55.74	55.16
CTC	16.52	12.10	19.70	51.56	36.10	41.90

UA-TSPE = Ultrasonic assisted tandem solid phase extraction, UA-DPSE = Ultrasonic assisted dispersive solid phase extraction, UA-MSPD = Ultrasonic assisted matrix solid phase dispersion.

Table 3.5. Precision analysis

Method	Analyte	Mean concentration (µg mL^{-1}) ± RSD (%) n = 5
UA-TPSE	TC	0.45 ± 7.54
	OTC	0.46 ± 8.97
	DC	0.47 ± 5.41
	CTC	0.46 ± 5.20
UA-DSPE	TC	0.47 ± 4.62
	OTC	0.46 ± 3.57
	DC	0.46 ± 5.42
	CTC	0.45 ± 10.50
UA-MSPD	TC	0.47 ± 4.32
	OTC	0.46 ± 10.14
	DC	0.46 ± 11.61
	CTC	0.45 ± 10.10

RSD = Relative standard deviation UA-TSPE = Ultrasonic assisted tandem solid phase extraction, UA-DPSE = Ultrasonic assisted dispersive solid phase extraction, UA-MSPD = Ultrasonic assisted matrix solid phase dispersion.

3.2.13(c) Precision and Selectivity

Precision was evaluated by analysing doped sediment samples with 0.5 µg $g^{-1}$of antibiotic five times in a day and calculating the intraday relative standard deviations (Table 3.5). The relative standard deviation was then evaluated using the EPA, (2001) method performance verification scale; 0-10 % very precise, 10-15 % precise and 15-20 % acceptable. Selectivity was assayed by extracting and analysing blank river water and sediment from 10 different

sources. It was then determined by assessing peak purity and presence of interfering peaks (Fig 3.1) in the retention time windows of the antibiotics (Madureira et al., 2010)

3.3 RESULTS AND DISCUSSION

3.3.0 METHOD VALIDATION PARAMETERS

3.3.1 Linear dynamic range

Calibration curves for each method generated by plotting analyte peak area versus concentration were linear in the range 0.01-1µg ml^{-1}see Table 3.2. Linear regression coefficients (R^2) were in the range 0.995 to 0.999. All R^2 values are above 0.995 showing good linearity.

3.3.2 Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ obtained for the three extraction techniques are shown in Tables 3.3 and 3.4. LOD of the spiked river water was in the range 11.53-22.75 $ngmL^{-1}$, and LOQ was in the range of 35.00-93.12 ng mL^{-1} while sediment LOD and LOQ was in the range 11.60-21.33 and 30.72-56.10 ng g^{-1} respectively. All the three techniques gave LOD and LOQ results that are comparable to data reported in previous studies using other techniques (see Table 3.6)

Table 3.6. Linear dynamic range and Limit of detection of ultrasonic assisted tandem solid phase extraction, ultrasonic assisted dispersive solid phase extraction, ultrasonic assisted matrix solid phase dispersion and other methods appearing in literature

Method	Antibiotic	Linear dynamic range/ng mL^{-1}	Limit of detection (ng mL^{-1})	Sample Matrix	Reference
DSPM-HPLC	Tetracycline, doxycycline	2-50	0.7-3.2	Water	Tsai et al., (2009)
On-line-SPE-HPLC	Tetracycline, chlortetracycline	5-1000	1.5-8.0	Water	Zhenzhen et al., (2013)
EA-IL-DLLME-HPLC	Tetracycline, doxycycline, chlortetracycline, metacycline	10-500	0.46-0.97	Deionized water	Dongli et al., (2014)
AH-C-HPLC	Oxytetracycline, Tetracycline, chlortetracycline	5-50	81.7-115	Water	Yang et al., (2013)
UA-TSPE-HPLC	Oxytetracycline, Tetracycline, doxycycline, chlortetracycline	10-1000	11.58-22.75	River water	The present study
UA-TSPE-HPLC	Oxytetracycline, Tetracycline, doxycycline, chlortetracycline	10-1000	11.82-20.00	River sediment	The present study
UA-DSPE-HPLC	Oxytetracycline, Tetracycline, doxycycline, chlortetracycline	10-1000	11.53-11.66	River water	The present study
UA-DSPE-HPLC	Oxytetracycline, Tetracycline,	10-1000	11.60-12.93	River sediment	The present study

	doxycycline, chlortetracycline				
UA-MSPD-HPLC	Oxytetracycline, Tetracycline, doxycycline, chlortetracycline	10-1000	11.80-18.30	River water	The present study
UA-MSPD-HPLC	Oxytetracycline, Tetracycline, doxycycline, chlortetracycline	10-1000	19.70-21.33	River sediment	The present study

EA-IL-DLLME-HPLC-UV = ethyl acetate-ionic liquid dispersive liquid-liquid micro extraction high performance liquid chromatography coupled to variable wavelength UV detector, AH-C-HPLC-UV = Aluminium hydroxide co-precipitation coupled to high performance liquid chromatography with UV detection, DSPM-HPLC-DAD = dispersive solid phase micro-extraction coupled to high performance liquid chromatography-diode-array detection, on line HPLC-PAD= online solid phase extraction coupled to high performance liquid chromatography with photodiode array detection

3.3.3 Precision and Specificity

Precision as a parameter for quality control was estimated by calculating relative standard deviation for 5 replicate samples (Table 3.5.) Computed relative standard deviations obtained in the present study for all techniques (see Table 3.5) are all in the very precise to precise range 3.57-11.61(EPA, 2001). Chromatograms recorded for all the methods, Figs 3.1a-e were free of interfering peaks in the antibiotics retention windows both in the spiked and blank samples. Peak purity as assessed by the Varian Star or Galaxie Chromatography Workstation version 6 software showed that levels of purity for all peaks were equal to or greater than

99%. The retention times for oxytetracycline, tetracycline, chlortetracycline and doxycycline were 2.4 ± 0.3, 2.8 ± 0.1, 3.3 ± 0.7 and 7.6 ± 0.4 minutes respectively.

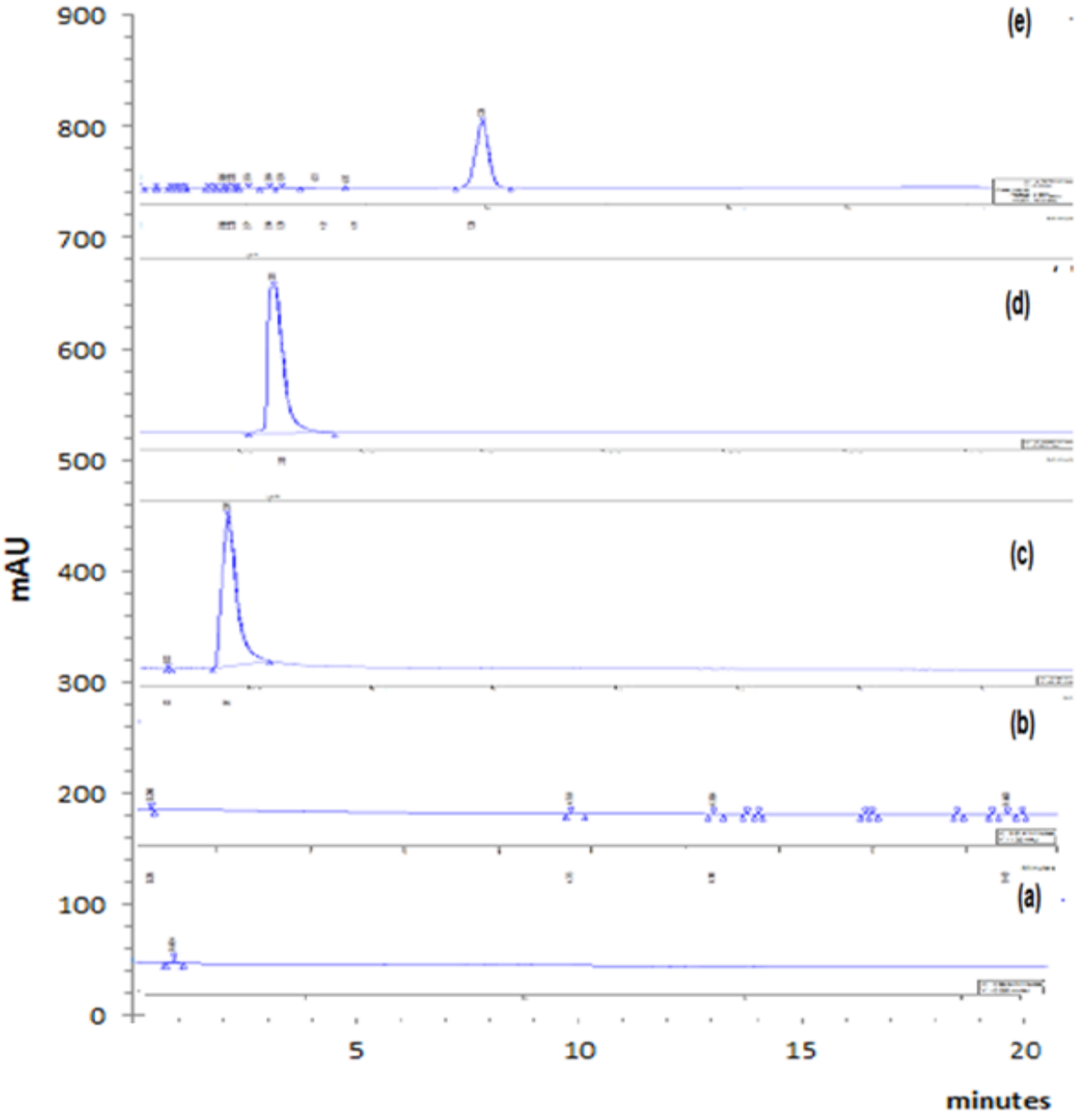


Fig 3.1. HPLC chromatograms for the analysis of (a) blank river water, (b) blank sediment sample, (c) oxytetracycline (d) chlortetracycline (e) doxycycline in river sediment. mAU = milliabsorbance unit

The efficiency of the extraction methods in minimizing humic acid absorption was determined by comparing results from the analysis of blank river water with and without applying solid phase extraction. Chromatograms obtained for the analyses are shown in Fig 3.2.

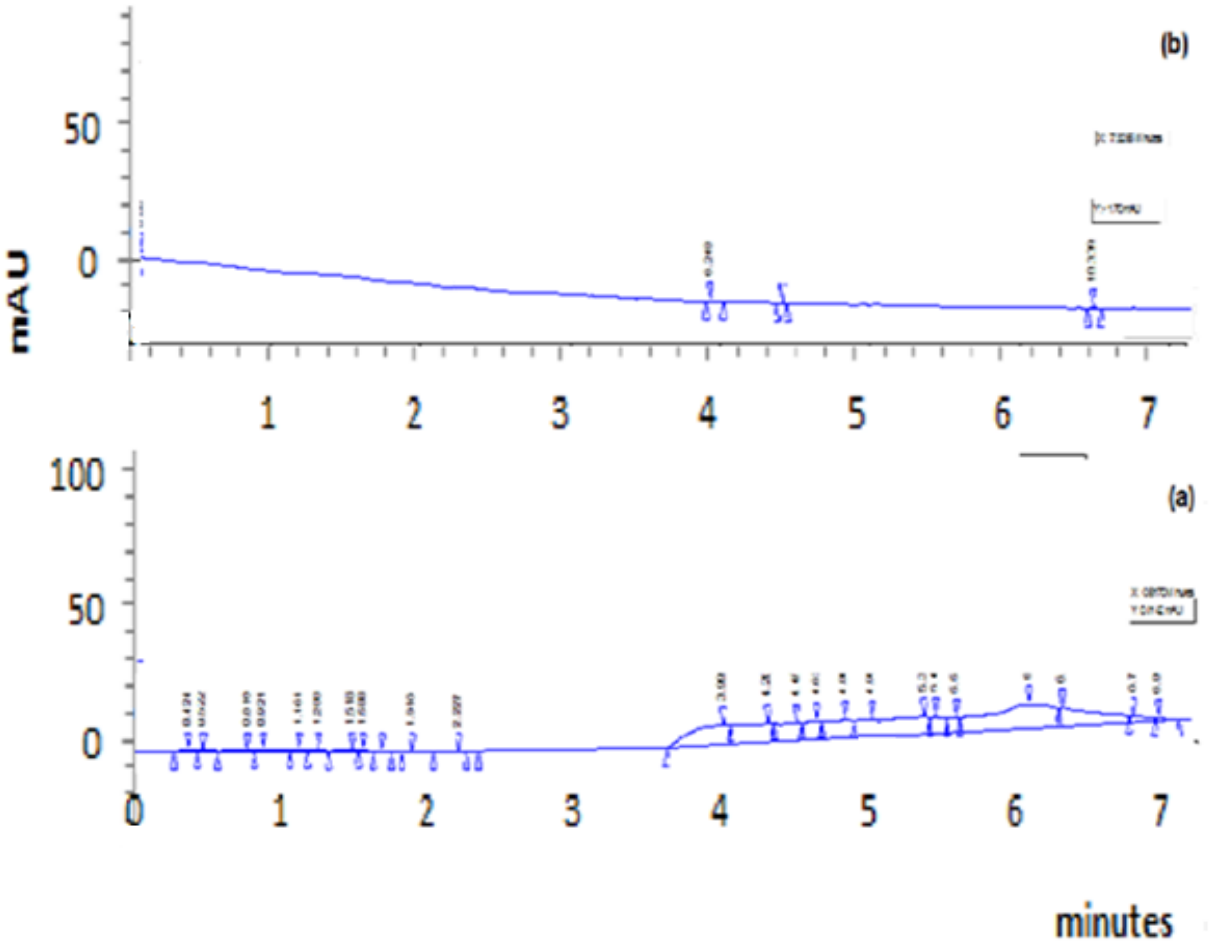


Fig 3.2. HPLC chromatograms for the analysis of blank sediment samples (a) without employing solid phase extraction (b) employing ultrasonic dispersive solid phase extraction

The difference in the baseline shift between chromatograms in Fig 3.2(a) and (b) is related to absorption of humic substances present in the sediment samples (see Table 3.1). The baseline shift disappeared when solid phase extraction was applied (see Fig 3.2 b). Solvent change

over and use of matrix trapping sorbents (primary and secondary amine) was responsible for reducing the concentration of humic substances therefore removing to a greater extent matrix noise. Primary and secondary amine sorbent material effectively removed matrix interferences and enhancement in previous studies (Boscher et al., 2010; Zhen Ru et al., 2011). In a similar study SAX was applied to alleviate matrix effects through adsorptive removal of anionic interferences. SAX improved precision (relative standard deviation was reduced from 6.6% to 2.2%) when it was used in the determination of chlortetracycline from swine waste waters (Pan et al., 2011). Recoveries improved to 98.8%.

3.3.4 Percentage recoveries

The objectives of solid phase extraction are the removal of interfering matrix components, improving recoveries and detection limits. Percentage recoveries of the three solid phase extraction techniques for ultrapure water, river water and sediment at three different spiking concentrations 0.01, 0.5 and 1 µg $mL^{-1}$are shown in Table 3.7-3.9. All the three extraction techniques yielded high recoveries in the range 92.13-99.62%. Zhou et al., (2011) report recoveries in the range of 49.4-125 for tetracyclines extracted from river sediment. In a study conducted by Lindsey et al., (2001) the mean recovery was 98 ± 12%. Jia et al., (2009) achieved recoveries within the range 64-113%. In a study conducted by Zhu et al., (2001) recovery from fortified lagoon water ranged from 86-110%. Zhenzhen et al., (2013) reported recoveries in the range of 81.70-96.45% when they extracted tetracyclines from water using aluminium hydroxide co-precipitation coupled to high performance chromatography. Dongli et al., (2014) used ethyl acetate ionic liquid dispersive liquid-liquid micro-extraction to extract tetracyclines from tap, lake and spring water and obtained recoveries in the range 62.6-109.6%. Manual shaking for 2 minutes and ultrasonication at 4300 rpm for 20 minutes

was observed to increase extraction efficiency. Manual shaking and ultrasonication provide the required energy to dislodge tetracycline molecules from the matrix.

Table 3.7. Extraction recoveries of antibiotics from 2L ultrapure water ($\bar{x} \pm RSD$, n = 3)

Spiked concentration (μgmL^{-1})	Method	Percentage recoveries for			
		TC	OTC	CTC	DC
	UA-TSPE	95.31 ± 4.56	96.81 ± 8.64	96.22 ± 6.22	94.99 ± 11.47
0.05	UA-DSPE	97.88 ± 5.77	98.91 ± 3.10	97.33 ± 3.39	98.05 ± 9.01
	UA-MSPD	96.33 ± 9.92	96.94 ± 3.80	95.90 ± 6.72	97.09 ± 11.90
	UA-TSPE	96.03 ± 5.77	96.21 ± 6.13	96.89 ± 7.08	96.09 ± 5.90
0.50	UA-DSPE	98.01 ± 3.11	99.62 ± 6.15	98.12 ± 7.21	97.96 ± 5.13
	UA-MSPD	96.56 ± 8.97	96.23 ± 8.64	96.07 ± 3.27	96.33 ± 8.96
	UA-TSPE	95,97 ± 9.97	97.13 ± 8.61	96.34 ± 3.54	95.33 ± 6.66
1.00	UA-DSPE	98.09 ± 7.21	98.85 ± 6.13	97.89 ± 8.17	98.23 ± 6.67
	UA-MSPD	97.01 ± 5.57	97.84 ± 9.11	96.12 ± 5.98	96.33 ± 7.71

$\bar{x}$ = mean, RSD = relative standard deviation, n = number of replicates, UA-TSPE = Ultrasonic assisted tandem solid phase extraction, UA-DPSE = Ultrasonic assisted dispersive solid phase extraction, UA-MSPD = Ultrasonic assisted matrix solid phase dispersion. TC = tetracycline, OTC = Oxytetracycline, CTC = chlortetracycline and DC doxycycline.

Carvalho et al., (2013) obtained improved recoveries when tetracyclines were extracted using ultrasonic assisted extraction and solid phase than when ultrasonication alone, Tetracycline presented at most a recovery of 27 % when ultrasonication was employed alone against 70-90 % for ultrasonication and solid phase extraction. Ultrasonication was performed for 15 minutes using an ultrasonic bath at a controlled temperature of 40^{o}C.Differences in extraction recoveries were as a result of different solvents, methanol-formic (96:4), methanol-hydrochloric acid (1:1), methanol-water (95:5), methanol-acetone (95:5) and methanol-acetonitrile (99:1) and dispersing sorbents employed. Methanol-formic (96:4), methanol-hydrochloric acid (1:1) gave extracts with strong matrix interferences. In the present study ultrasonic assisted dispersive solid phase extraction significantly yielded higher recoveries for almost all the antibiotics in both ultrapure, river water and sediment (see Table 3.7-3.9).Recoveries ranged from 97.13-99.62 % for ultrasonic assisted dispersive solid phase extraction while that for ultrasonic assisted tandem solid phase extraction ranged from 94.99-97.75 %. Percentage recovery for ultrasonic assisted matrix solid phase dispersion ranged from 92.13-97.84 %. Ultrasonic assisted dispersive solid phase extraction yielded higher recoveries because it involved dispersing a primary secondary amine sorbent. Primary and secondary amine sorbent has an excellent retention power for anionic compounds such as humic acid and proteins. At the pH of 4 used in the present study tetracyclines exist mainly as neutral or cationic forms therefore will not be retained strongly by primary and secondary amine. Dispersing the sorbent ensured maximum contact with matrix such as humic acid and metals which may complex the antibiotic and reduce extraction efficiency. The results also show that the recoveries were independent of spiking concentration for all the three methods. Percentage recoveries for the river water and sediment are similar to recoveries obtained for

ultra-pure water (Table 3.7-3.9), further substantiating the robustness of the methods to reduce matrix interference in the extraction of the antibiotics.

Table 3.8. Extraction recoveries of antibiotics from 2 L river water sample ($\bar{x} \pm RSD$, n =3)

concentration (μgmL^{-1})	Method	Percentage recoveries for			
		TC	OTC	CTC	DC
	UA-TSPE	96.22 ± 8.89	97.04 ± 5.44	97.75 ± 9.17	97.13 ± 10.00
0.05	UA-DSPE	98.00 ± 8.84	98.42 ± 5.64	97.98 ± 6.77	98.22 ± 7.15
	UA-MSPD	96.22 ± 3.77	96.40 ± 6.33	97.07 ± 6.67	97.03 ± 4.67
	UA-TSPE	97.11 ± 7.45	97.14 ± 6.12	97.13 ± 11.23	97.62 ± 4.72
0.50	UA-DSPE	98.22 ± 5.57	98.96 ± 6.13	98.07 ± 6.12	98.44 ± 8.36
	UA-MSPD	96.81 ± 5.72	97.30 ± 5.66	96.89 ± 6.92	96.86 ± 7.71
	UA-TSPE	96.98 ± 6.15	97.50 ± 5.44	97.05 ± 8.90	97.55 ± 7.71
1.00	UA-DSPE	98.11 ± 3.45	98.85 ± 6.80	98.01 ± 10.13	98.19 ± 8.99
	UA-MSPD	96.39 ± 5.77	97.39 ± 11.31	97.22 ± 3.79	96.04 ± 3.07

$\bar{x}$ = mean, RSD = relative standard deviation, n = number of replicates, UA-TSPE = Ultrasonic assisted tandem solid phase extraction, UA-DPSE = Ultrasonic assisted dispersive solid phase extraction, UA-MSPD = Ultrasonic assisted matrix solid phase dispersion. TC = tetracycline, OTC = Oxytetracycline, CTC = chlortetracycline and DC doxycycline.

Table 3.9. Extraction recoveries of antibiotics from 2g river sediment ($\bar{x} \pm RSD$, n =3)

Spiked concentration ($\mu g g^{-1}$)	Method	Percentage recoveries for			
		TC	OTC	CTC	DC
	UA-TSPE	96.23 ± 5.18	96.22 ± 9.64	96.39 ± 4.19	97.12 ± 5.75
0.05	UA-DSPE	97.98 ± 6.65	96.39 ± 4.19	98.11 ± 9.81	98.14 ± 11.01
	UA-MSPD	95.89 ± 7.99	96.08 ± 7.86	95.94 ± 8.84	96.13 ± 3.99
	UA-TSPE	97.03 ± 5.55	95.89 ± 11.20	96.89 ± 11.90	96.33 ± 5.66
0.50	UA-DSPE	98.12 ± 2.11	98.05 ± 7.75	98.24 ± 6.77	98.13 ± 7.71
	UA-MSPD	96.97 ± 9.97	97.12 ± 3.37	92.13 ± 1.89	96.88 ± 4.49
	UA-TSPE	96.81 ± 8.88	96.37 ± 6.64	96.85 ± 7.72	96.82 ± 8.99
1.00	UA-DSPE	98.10 ± 3.77	97.97 ± 5.44	98.07 ± 11.22	98.19 ± 3.22
	UA-MSPD	97.02 ± 10.97	96.98 ± 7.97	93.99 ± 6.68	96.70 ± 8.29

$\bar{x}$ = mean, RSD = relative standard deviation, n = number of replicates, UA-TSPE = Ultrasonic assisted tandem solid phase extraction, UA-DPSE = Ultrasonic assisted dispersive solid phase extraction, UA-MSPD = Ultrasonic assisted matrix solid phase dispersion. TC = tetracycline, OTC = Oxytetracycline, CTC = chlortetracycline and DC doxycycline.

3.3.5 Method Application

Ultrasonic assisted dispersive solid phase extraction gave better recoveries therefore it was further tested in the analysis of oxytetracycline and doxycycline in real environmental samples (surface and treated drinking water samples) collected from different areas in Bindura and Harare. Samples were collected from storm drains, sewage discharge points and 30 meters away from sewage discharge points. Oxytetracycline and doxycycline was detected

in storm drains and sewage discharge points. No detections were observed in areas 30 meters away from discharge or sewage points. An interlaboratory study of the liquid chromatography analysis of oxytetracycline and doxycycline was conducted. Samples were analysed in the Bindura University chemistry and Varichem laboratories, Zimbabwe. No significant difference was found in the amounts of oxytetracycline and doxycycline analysed in each laboratory. Average recoveries for both laboratories were above 90%.

3.4 CONCLUSION

In the present study three methods for extraction of tetracyclines from river water were optimized and validated in terms of linearity, detection limits, precision, selectivity and extraction recovery. The three techniques are comparable in terms of matrix effects reduction and detection limits. Percentage recoveries for all the techniques were above 90%. Dispersive solid phase extraction exhibited superior extraction efficiency therefore this method was adopted for kinetic studies. The results also revealed that using sorbents that lower matrix interferences such as humic acid and metals, coupling with ultrasonication and performing solvent change over makes it possible to obtain high and consistent recoveries.

CHAPTER 4

4 DEGRADATION KINETICS EXPERIMENTS

4.0 METHODOLOGY

4.1 MATERIALS

Standards, oxytetracycline (95%), 4-epi-oxytetracycline (97%), α-apo-oxytetracycline (97%), β-apo-oxytetracycline (95%), doxycycline hyclate (99%), metacycline (97%), 4-epi-doxycycline (97%), 6-epi-doxycycline (98%), chlortetracycline (97%), 4-epi-chlortetracycline (95%), iso-chlortetracycline (95%), epi-anhydro-chlortetracycline (95%),tetracycline (98%), 4-epi-tetracycline (98%), and anhydro-tetracycline (95%), HPLC grade solvents (methanol and acetonitrile), 57738-U-SUPELCO supelclean primary and secondary amine sorbent material, a polymerically bonded ethylene diamine-N-propyl phase consisting of both secondary and primary amines and MILLPORE (0.45 µm)glass and nylon disposable sample filter units were obtained from Sigma Aldrich (Germany). Orthophosphoric acid, nitric acid, oxalic acid, disodium ethylene diamine tetraacetate (Na_2EDTA), citric acid and sodium hydrogen phosphate were of analytical grade and were purchased from SKYLABS, Gauteng, South Africa. River water (8 X 80L) and sediment (8 X 2 kg) were collected from Wayerera River, Bindura, Zimbabwe (19^o 19' 52" South 42^o 21' 52" East).

4.2 EQUIPMENT

4.2.0 DISPERSIVE SOLID PHASE EXTRACTION EQUIPMENT

4.2.1 Centrifuge

A Thermo Scientific Sorvall Legend XT benchtop centrifuge 4 x 1000 mL, maximum speed 15200 rpm equipped with a 60 minute timer was used to separate suspended substances from aqueous solutions.

4.2.2 Ultrasonicator and pH meter

Bransonic CPXH 1800H-E heated ultrasonic bath equipped with a 99 minute digital timer, continuous ultrasonic operation and can be heated to a temperature of 69^{o}C was used for ultrasonic extraction of antibiotics and for degassing mobile phase. Hanna benchtop pH meter model H2210 1-4 Cashel road, Wirral, Merseyside UK was used to measure pH of solutions and microcosm and control experiments. The pH meter was calibrated using buffers at pH 4 and 10 supplied by the manufacturer

4.2.3 Rotary evaporator

A Buchi Rotary evaporator R-300, Mumbai 400055, Maharashtra, India was used for preconcentration of samples under vacuum.

4.3 PREPARATION OF STANDARD SOLUTIONS

Stock solutions, 1 mg mL^{-1}solutions were prepared by weighing 10.0 mg of the standard antibiotic and dissolving in 10 mL of HPLC grade methanol. Working standard solutions were prepared by diluting suitable aliquot of stock solutions with HPLC grade methanol.

4.4 MICROCOSM EXPERIMENTS

The experiments were designed in line with a previous method reported by Zaranyika and co-workers, (2010, 2013) with slight adjustments. River water (2 x 80 L each) and distilled water (3 x 80 L each) were transferred into separate 80 L white plastic tanks purchased from Mega Pak private limited company, Harare, Zimbabwe. Two kilograms of sediment each were added into the vessels with river water and the levels marked. Physicochemical parameters of the sediment were determined using EPA (2001) and APHA (2004) recommended standard methods for analysis of environmental samples and results are shown in Table 4.1. Ammonium nitrate was added into one tank containing river water and sediment to yield a concentration of 2 mg mL^{-1} and, in another tank containing distilled water. One tank containing distilled water only was completely covered with aluminium foil to prevent light from getting inside, however making sure that that free circulation of air was not hindered. Two other tanks containing distilled water, one spiked with 2 mg mL^{-1}ammonium nitrate and another without, were left exposed to natural light. All the tanks were then spiked with standard OTC that was dissolved in 1 mL methanol so as to achieve a final concentration of 1.2 μg mL^{-1} in each tank. The tanks were then stirred thoroughly to distribute the antibiotic. The contents were allowed to settle and samples were then taken immediately. Perforated transparent polythene was used to cover the top of the tanks and then they were left outside in a safe place close to the department of chemistry of the Bindura University of Science Education. Thereafter, samples were collected periodically for a period of 90 days, each time compensating for evaporation by adding distilled water 24 hrs prior to collecting samples. A stainless scoop was used to collect sediment samples from the bottom of the tanks each time ensuring minimum agitation. Similar experiments were repeated for DC, CTC and TC each

time making the concentration of the antibiotics in the tanks to be 1 μg mL^{-1}. The temperature and pH of each tank were recorded before each sampling and the results are shown in Table 4.2.

Table 4.1 Physicochemical properties of Wayerera river sediment (19º 19' 52'' South, 42º 21' 52'' East)

Property	Mean ± SD n = 3
pH	7.2 ± 0.20
Ca (mg Kg^{-1})	0.12 ± 0.02
Mg (mg Kg^{-1})	0.11 ± 0.03
Fe (mg Kg^{-1})	0.25 ± 0.06
Organic matter content (mg Kg^{-1})	1.2 ± 0.30
Total organic carbon (mg/L)	32.9 ± 0.80
Nitrate content (mg Kg^{-1})	0.14 ± 0.02
Cationic exchange capacity (meq L^{-1} Na)	5.55 ± 2.61
Total viable bacterial count (cfu/mL)	1.36 x 10^4
Total viable fungal count (cfu/mL)	1.02 x 10^2
Clay (%)	10.1 ± 2.4
Silt (%)	63.3 ± 0.5
Sand (%)	25.4 ± 1.6

SD = standard deviation

Table 4.2 Average temperature and pH of river and distilled water experiments.

Experiment	Average temperature ^{o}C in,		Average pH in,	
	River water	Distilled water	River water	Distilled water
OTC	27 ± 5	28 ± 3	7.2 ± 0.4	5.5 ± 0.8
DC	26 ± 4	26 ± 3	7.2 ± 0.4	6.6 ± 0.6
CTC	27 ± 3	24 ± 6	7.1 ± 0.6	6.8 ± 0.4
TC	28 ± 4	25 ± 4	7.2 ± 0.3	6.9 ± 0.6

4.5 SAMPLE EXTRACTION, CLEAN UP AND CONCENTRATION

OTC, DC, CTC, TC and degradation products were extracted from water and sediment samples using the optimized UA-DSPE technique as described in section 3.2. Samples were analysed in triplicate.

4.5.1 Water Samples

Water samples (100 mL portions) were centrifuged at 3000 rpm for 15 min at 25 ^{o}C. The supernatants collected were each shaken vigorously with 10 mL of acetonitrile using a separating funnel. Na_2EDTA (5 mL, 0.1 M), and 10 mL of McIlvaine buffer at pH 4 were also added to chelate metals present (Table 4.1). Magnesium sulphate and sodium chloride (0.5g each) were then added to displace the extraction equilibrium towards the organic phase. After centrifugation at 3000 rpm for 10 min at 25 ^{o}C, the organic supernatants were transferred into a conical flask. SUPELCO-U- 57738 supelclean primary and secondary amine sorbent material was then added to remove interferences, such as humic acid. The analyte of interest remained in the organic phase. The mixture was subjected to

ultrasonication for 15 minutes at 30 ^{o}C and centrifuged at 3000 rpm at 25 ^{o}C for 10 minutes. The supernatants were collected and evaporated to dryness under vacuum and, then redissolved in 500 μL of HPLC grade methanol. The contents were filtered through a 0.45 μm Millipore glass filters to remove any particulate matter and, then placed into amber glass vials and stored in a fridge at 5 ^{o}C until required for HPLC analysis.

4.5.2 Sediment Samples

Portions of sediment samples (2 g each) were centrifuged to remove excess water and the antibiotic of interest extracted as follows. Each glass tube was added 10 mL of McIlvaine buffer at pH 4 and was mixed for 1 minute and, then centrifuged at 3000 rpm for 15 min at 25 ^{o}C. The supernatants in each glass tube were transferred into 250 mL flasks and kept under refrigeration. The process of extraction was repeated twice and the supernatants collected from the two experiments were combined and diluted with ultrapure water to a final volume of 100 mL. The contents were shaken vigorously with 10 mL of acetonitrile in a separating funnel. Na_2EDTA (5 mL, 0.1 M), and 10 mL of McIlvaine buffer at pH 4 were added to remove by chelation any metals that were not removed in the first step. The extraction process was then carried out as described for water samples in section 4.5.1.

4.6 HPLC ANALYSIS

4.6.1 Choice of UV-Vis detection wavelength

The detector was set at 360 nm. (The wavelength at maximum absorbance was determined using a UV-Vis instrument, GENESYS 10S UV-Vis v4.003 2L9Q129001 Themofisher scientific, USA) (see Fig 4.1).

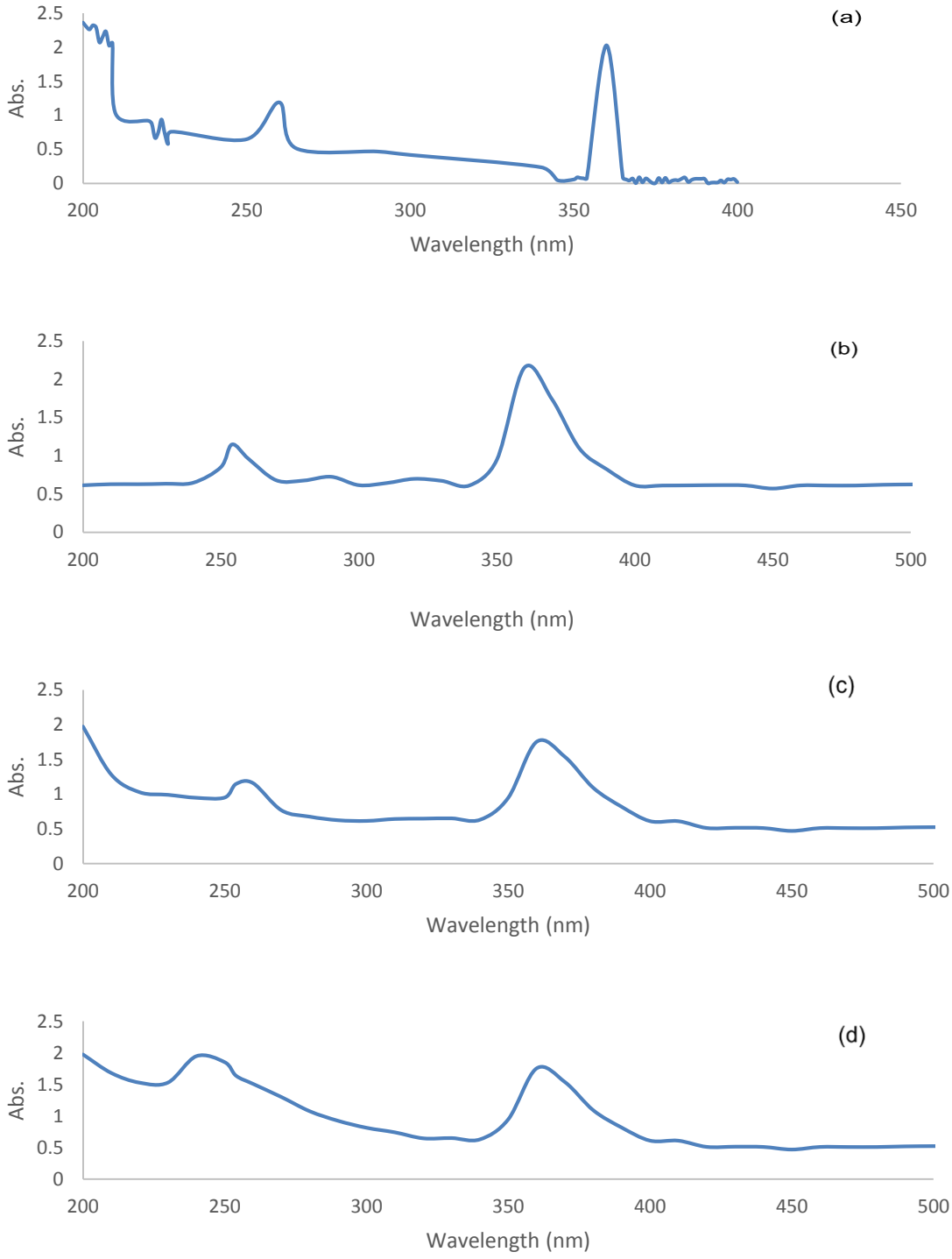


Fig 4.1 UV spectrum (a) oxytetracycline and (b) doxycycline(c) chlortetracycline and (d) tetracycline

4.6.2 Analysis

The analysis for antibiotics and metabolites was performed on a Varian HPLC equipped with a 20 µL loop Rodyne manual injector and a UV Vis variable wavelength detector, Prostar 325. The detector was controlled remotely by the Varian Star or Galaxie Chromatography Workstation version 6 software. All HPLC separations were carried out using a C_{18} (Varian Microsorb MV 1005) packed column (250 x 4.6 mm id, 5 µm particle size). The mobile phase was made by mixing methanol, acetonitrile and 0.01 M aqueous oxalic acid in the ratio of 1:1.5:7.5 with pH adjusted to 3.0. The column was maintained at 25 ^{o}C. The flow rate was 1.0 mL min^{-1}. A sonicator was used to mix and remove air bubbles from the mobile phase before HPLC analysis. Sample injection volume was 10 µL. Figures 4.3-4.6 show typical chromatograms obtained for the method. Peak purity was assessed using Varian Star or Galaxie Chromatography Workstation software and was found to be equal or greater than 99 % in all cases, showing that there was no co-elution of peaks. Recoveries of 99.62±5.78 and 98.56 ±6.59 (OTC), 88.66 ± 9.38 and 83.59 ± 5.31 (DC), 98.12 ± 6.13 and 98.84 ± 3.34 (CTC) and 98.22 ± 5.17 and 97.18 ± 8.84% (TC) were obtained when river water and sediment samples spiked at 0.5 µg/mL and 0.5 µg/g respectively were analyzed. OTC, DC, CTC and TC were not detected when blank river water and sediment samples were analyzed, (see Fig 4.7). Recoveries of 98.4% and 99.5% from sediment and seawater respectively were reported previously by Samuelsen, (1989) for OTC. Table 4.3, 4.4, 4.5 and 4.6 show the results obtained for the analysis of OTC, DC, CTC and TC.

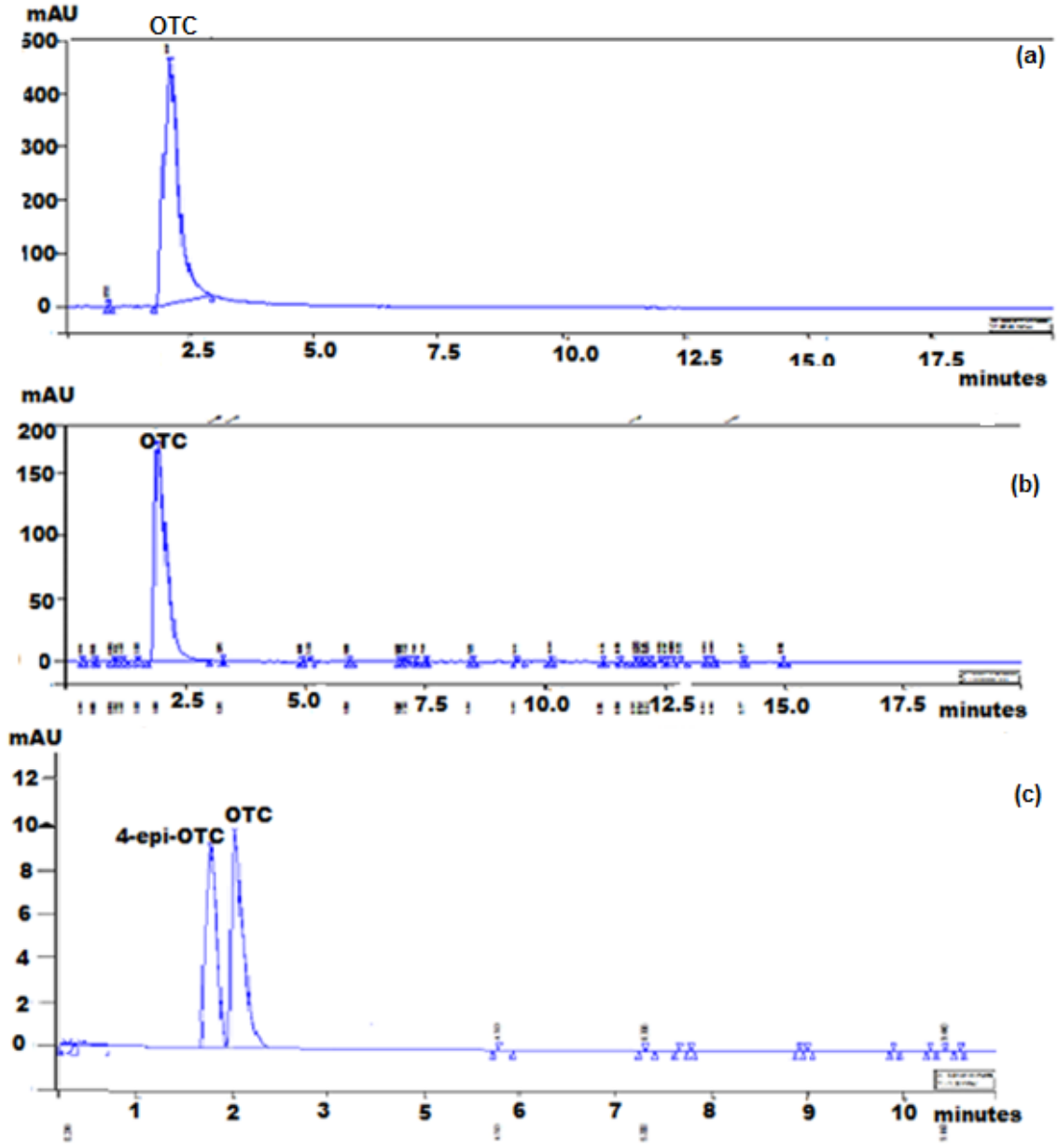


Fig 4.2 High performance liquid chromatography (HPLC) chromatograms for oxytetracycline (a) Covered distilled water experiment on day 48, (b) river water experiment on day zero, (c) river water experiment on day 64, OTC = oxytetracycline, 4-epi-OTC = 4-epi oxytetracycline. mAU = milliabsorbance unit

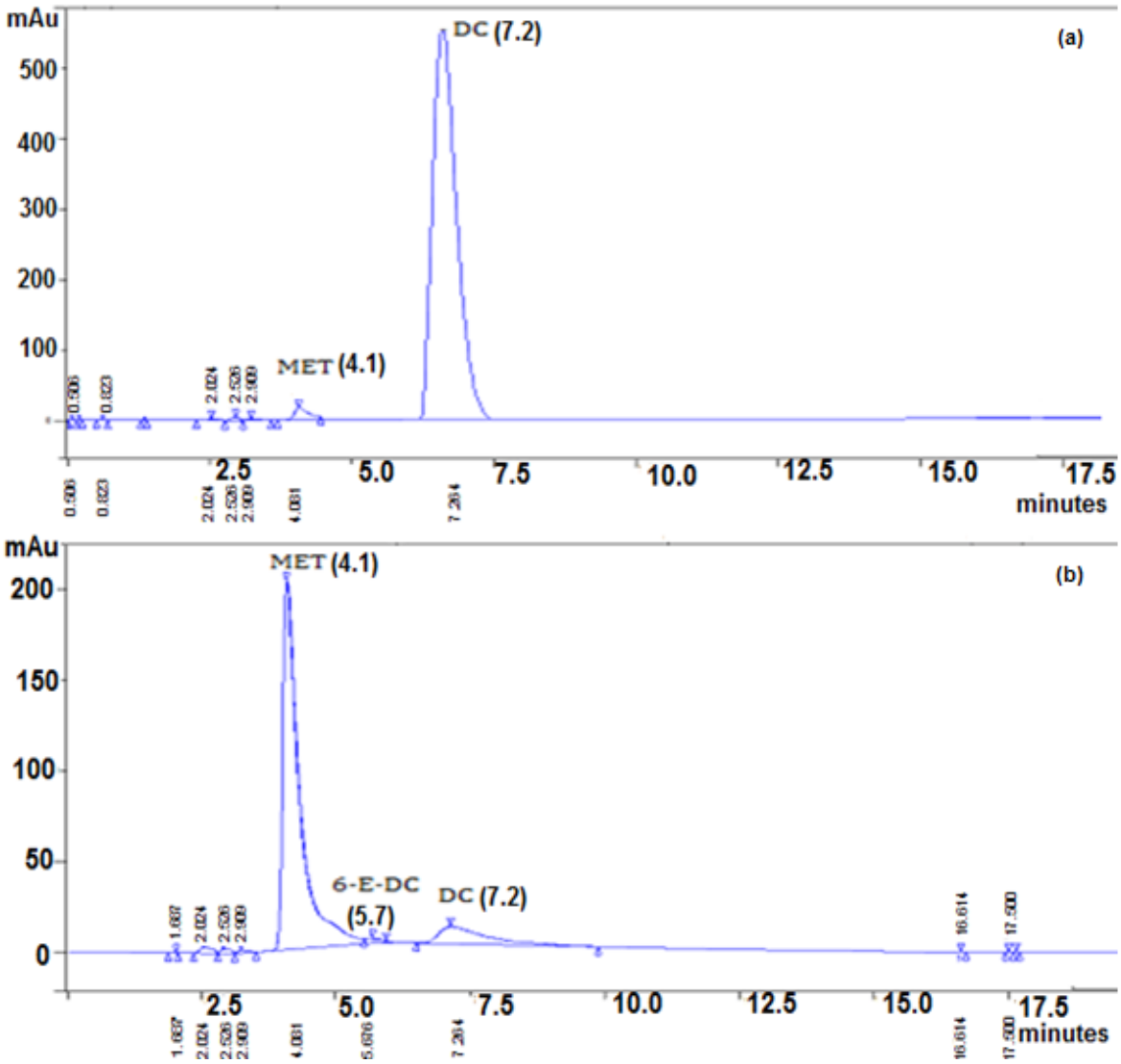


Fig 4.3. Typical high performance liquid chromatography (HPLC) chromatograms for river water experiment on day 3 (a) and 64 (b), MET = metacycline, 6-E-DC = 6-epi-doxycycline, DC = doxycycline. mAU = milliabsorbance units

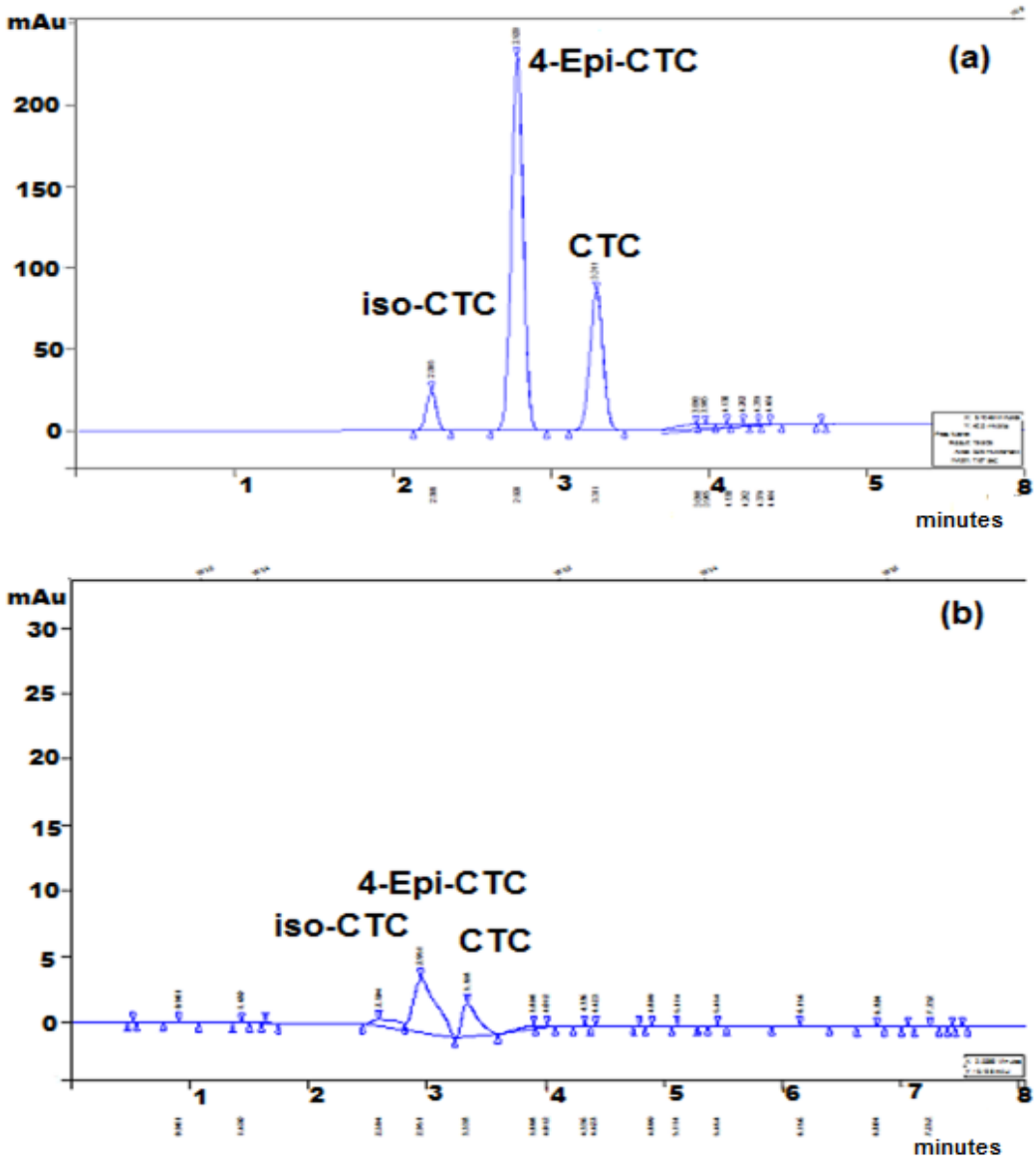


Fig 4.4 Typical HPLC chromatograms for river water experiment on (a) day 26 and (b) day 64, CTC = chlortetracycline, 4-Epi-CTC = 4-epi-chlortetracycline, iso-CTC = iso-chlortetracycline. mAU = milliabsorbance units

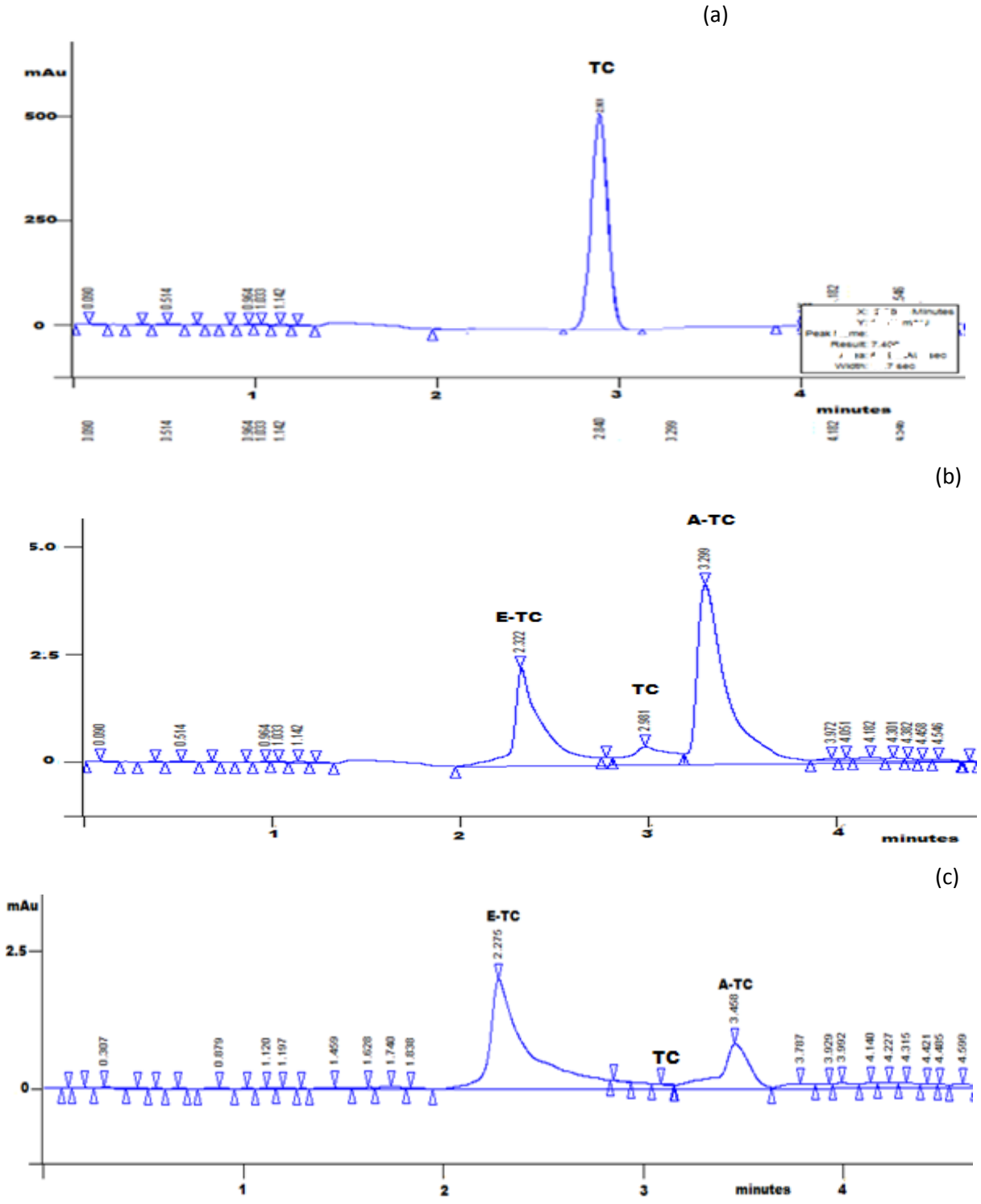


Fig 4.5Typical HPLC chromatograms for river sediment experiment at (a) day 3, (b) day 64 and (c) day 90, TC = tetracycline, E-TC = epi-tetracycline and A-TC = anhydro-tetracycline

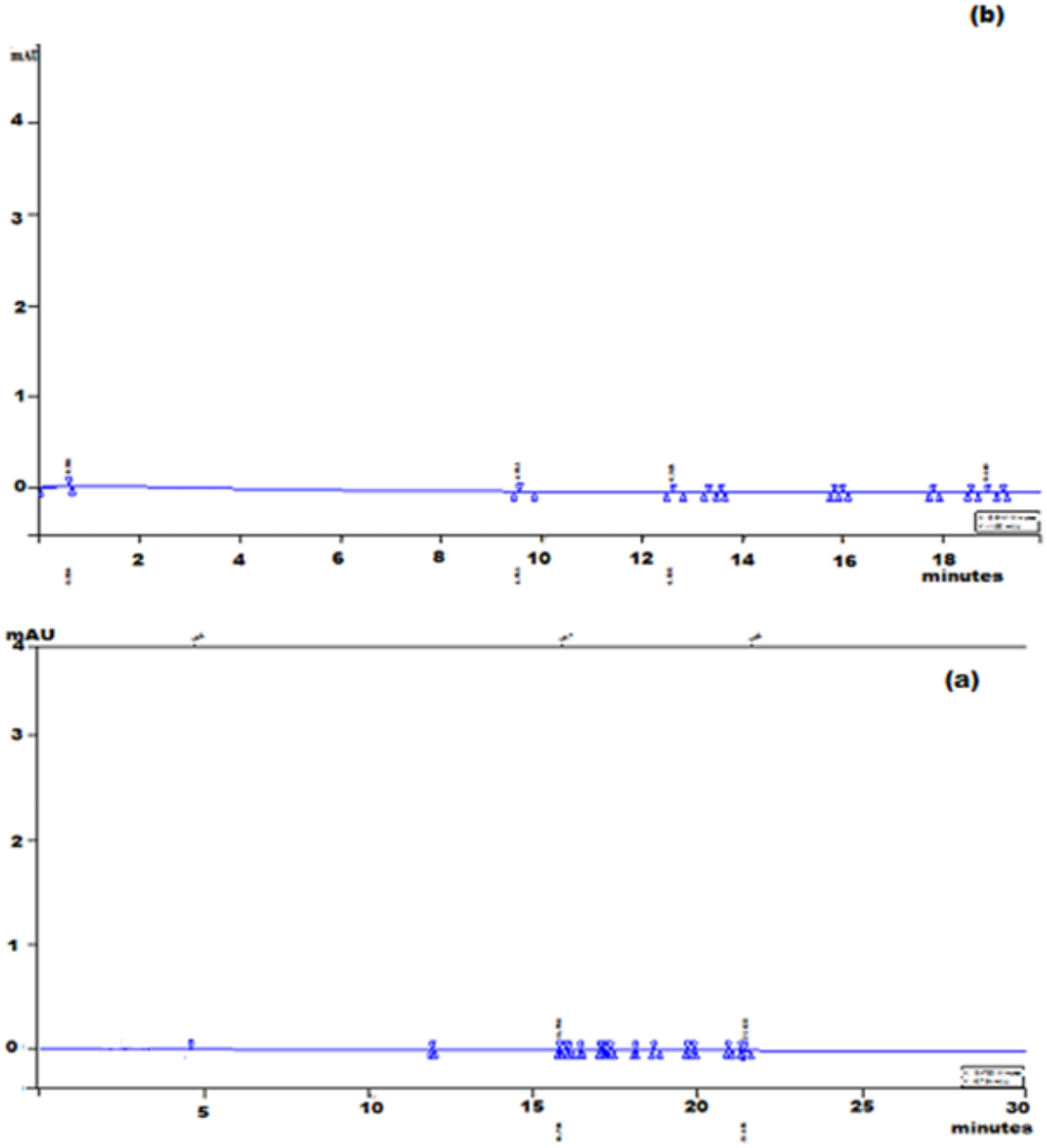


Fig 4.6.Typical HPLC chromatograms for (a) blank river water sample and (b) blank river sediment sample collected from Wayerera River.

Table 4.3. Concentration changes of oxytetracycline in distilled and river water and sediment

Day of sampling	Concentration ($\bar{X}$ ± SD), n = 3						
	CDW(µg/ml)	DWE(µg/ml)	DWN(µg/ml)	RW(µg/ml)	RWN(µg/g)	RS(µg/ml)	RSN(µg/g)
0	0.9936(0.00190)	0.9330(0.01580)	0.9934(0.002670)	0.5029(0.03130)	0.5035(0.03360)	0.4205(0.01409)	0.4204(0.01820)
1	0.9934(0.00219)	0.9650(0.01478)	0.9590(0.003665)	0.4670(0.02490)	0.3950(0.001280)	0.3507(0.002339)	0.3257(0.003134)
3	0.9934(0.002349)	0.9800(0.01320)	0.9530(0.004247)	0.3976(0.01640)	0.3420(0.001918)	0.2795(0.001578)	0.2157(0.02327)
4	0.9935(0.001995)	0.9480(0.01277)	0.9470(0.006512)	0.3602(0.01169)	0.2438(0.007870)	0.2166(0.009600)	0.1503(0.005429)
7	0.9935(0.002069)	0.9380(0.02926)	0.9320(0.003099)	0.2838(0.005730)	0.2372(0.009780)	0.1692(0.001165)	0.1292(0.002756)
8	0.9935(0.002176)	0.9160(0.04220)	0.8990(0.005505)	0.2552(0.009661)	0.2254(0.01730)	0.1776(0.001162)	0.1134(0.007691)
11	0.9935(0.003162)	0.8930(0.01986)	0.8610(0.007456)	0.2519(0.001570)	0.2145(0.001035)	0.1422(0.006210)	0.09750(0.007994)
13	0.9935(0.001258)	0.8580(0.01258)	0.8310(0.002786)	0.2299(0.001484)	0.1954(0.006529)	0.1414(0.001373)	0.09780(0.003780)
15	0.9934(0.003070)	0.8620(0.004418)	0.8270(0.002746)	0.2157(0.001990)	0.1864(0.001661)	0.1145(0.007080)	0.08730(0.007777)
18	0.9834(0.01206)	0.8410(0.01037)	0.7860(0.006035)	0.2019(0.001247)	0.1682(0.001684)	0.1109(0.008588)	0.07560(0.001806)
20	0.9834(0.003077)	0.8190(0.02795)	0.7670(0.005113)	0.1909(0.007420)	0.1608(0.007493)	0.1084(0.001071)	0.07560(0.002698)
26	0.9637(0.002110)	0.7920(0.01595)	0.7280(0.006075)	0.1107(0.007293)	0.0987(0.007088)	0.05680(0.0001711)	0.03160(0.001931)
34	0.8977(0.003575)	0.7020(0.02582)	0.6690(0.003689)	0.09870(0.007030)	0.0887(0.004997)	0.03890(0.0002960)	0.02490(0.0005264)
64	0.8482(0.001588)	0.5701(0.03410)	0.4840(0.003762)	0.06980(0.001838)	0.0598(0.003667)	0.02870(0.0001485)	0.02490(0.001915)
72	0.8186(0.005504)	0.4813(0.004675)	0.3691(0.008363)	0.04870(0.002727)	0.0387(0.003356)	0.02850(0.0001761)	0.02460(0.001225)
90	0.8016(0.005764)	0.3490(0.002496)	0.1960(0.01168)	0.03220(0.002181)	0.0222(0.002493)	0.02800(0.0001435)	0.01420(0.001319)

Table 4.4. Persistence of doxycycline in distilled and river water and sediment

Day of sampling	Concentration ($\bar{X}$ ± SD), n = 3						
	CDW(µg/ml)	DWE(µg/ml)	DWN(µg/ml)	RW(µg/ml)	RS(µg/g)	RWN(µg/ml)	RSN(µg/g)
0	0.9960(0.003135)	0.9980(0.001016)	0.9870(0.003292)	0.7720(0.004379)	0.2250(0.001377)	0.7700(0.01806)	0.2230(0.01365)
1	0.9940(0.002614)	0.9870(0.003686)	0.9840(0.002131)	0.7370(0.002425)	0.2020(0.004310)	0.6700(0.003753)	0.1770(0.01171)
3	0.9930(0.002219)	0.9720(0.002524)	0.9780(0.002207)	0.6580(0.004000)	0.1960(0.006584)	0.5760(0.01376)	0.1500(0.01016)
4	0.9940(0.002850)	0.9780(0.002888)	0.9720(0.003503)	0.6560(0.001545)	0.1780(0.001209)	0.5020(0.02571)	0.1250(0.001976)
7	0.9920(0.002070)	0.9670(0.002106)	0.9680(0.002043)	0.5540(0.003108)	0.1650(0.004946)	0.4650(0.01464)	0.0930(0.002303)
8	0.9840(0.003814)	0.9570(0.002902)	0.9700(0.003870)	0.5350(0.002426)	0.1520(0.001048)	0.4520(0.02264)	0.0720(0.002752)
11	0.9850(0.003548)	0.9590(0.002307)	0.9570(0.003200)	0.5120(0.002168)	0.1320(0.009488)	0.4320(0.02943)	0.0690(0.002058)
13	0.9760(0.003531)	0.9580(0.002960)	0.9430(0.002480)	0.4730(0.002213)	0.1280(0.005074)	0.4280(0.01001)	0.0630(0.003181)
15	0.9660(0.001949)	0.9360(0.001884)	0.8900(0.002885)	0.4720(0.002827)	0.1240(0.006759)	0.3970(0.001704)	0.0600(0.002203)
18	0.9670(0.002893)	0.8970(0.002014)	0.8880(0.002312)	0.4600(0.002865)	0.1170(0.006239)	0.3870(0.005475)	0.0590(0.002621)
20	0.9510(0.003550)	0.8790(0.002900)	0.8370(0.002846)	0.4290(0.002398)	0.1180(0.006680)	0.3780(0.001974)	0.0580(0.002513)
26	0.9420(0.003681)	0.7890(0.001699)	0.7970(0.001892)	0.3930(0.002098)	0.1140(0.003290)	0.3560(0.001819)	0.0440(0.003256)
34	0.9160(0.002518)	0.6920(0.002135)	0.6930(0.003548)	0.3800(0.002128)	0.1030(0.002222)	0.2990(0.001835)	0.0370(0.003248)
48	0.9020(0.003810)	0.5580(0.001307)	0.5400(0.003343)	0.3040(0.001975)	0.0770(0.002164)	0.2300(0.001456)	0.0280(0.002303)
64	0.8630(0.001878)	0.4080(0.009200)	0.3890(0.002166)	0.2470(0.001318)	0.0560(0.002596)	0.1660(0.008484)	0.0170(0.001847)
72	0.8710(0.002330)	0.3610(0.001126)	0.3570(0.001821)	0.2020(0.008451)	0.0380(0.003504)	0.1200(0.007126)	0.0120(0.001311)
90	0.8280(0.002046)	0.2130(0.009350)	0.1920(0.004026)	0.1320(0.003523)	0.0130(0.001064)	0.0130(0.001296)	0.0100(0.001002)

Table 4.5 Concentration of CTC in distilled and river water and sediment.

Day of sampling	Concentration ($\bar{X}$ ± SD), n = 3 (CTC)						
	CDW(µg/ml)	DWE(µg/ml)	DWN(µg/ml)	RW(µg/ml)	RS(µg/g)	RWN (µg/ml)	RSN(µg/g)
0	0.9960(0.005114)	0.9780(0.003157)	0.9470(0.02951)	0.6220(0.002433)	0.3350(0.001724)	0.6170(0.004039)	0.3430(0.01909)
1	0.9940(0.003149)	0.9670(0.002117)	0.9440(0.01976)	0.5470(0.001199)	0.3120(0.007318)	0.4870(0.002775)	0.2870(0.001628)
3	0.9930(0.002658)	0.9520(0.003171)	0.9480(0.002961)	0.5280(0.01652)	0.2560(0.008010)	0.4560(0.003063)	0.2150(0.01444)
4	0.9940(0.002101)	0.9680(0.004935)	0.9420(0.002984)	0.5010(0.01114)	0.2280(0.007116)	0.4020(0.001168)	0.1690(0.001135)
7	0.9920(0.003068)	0.9670(0.002259)	0.9380(0.002710)	0.4140(0.01426)	0.2050(0.004420)	0.3350(0.002954)	0.1630(0.001105)
8	0.9940(0.001997)	0.9570(0.002318)	0.9370(0.002938)	0.4050(0.008732)	0.2120(0.003785)	0.3020(0.002319)	0.1580(0.001126)
11	0.9850(0.003623)	0.9590(0.002872)	0.9370(0.002044)	0.4020(0.01256)	0.2020(0.010607)	0.2820(0.002508)	0.1590(0.001298)
13	0.9860(0.004120)	0.9580(0.002997)	0.9330(0.002938)	0.3630(0.007746)	0.1980(0.008935)	0.2680(0.002656)	0.1430(0.001288)
15	0.9860(0.002104)	0.9360(0.002340)	0.9180(0.003249)	0.3720(0.001534)	0.1840(0.009381)	0.2470(0.01020)	0.1260(0.003544)
18	0.9770(0.003168)	0.9470(0.003909)	0.9080(0.003031)	0.3460(0.008009)	0.1770(0.008480)	0.2270(0.01089)	0.1090(0.001600)
20	0.9610(0.003524)	0.8890(0.001787)	0.8770(0.002223)	0.3190(0.01122)	0.1680(0.003558)	0.2180(0.001704)	0.1080(0.002835)
26	0.9580(0.004890)	0.8690(0.002549)	0.8430(0.002952)	0.2830(0.009952)	0.1540(0.009528)	0.2060(0.001837)	0.0940(0.001473)
34	0.9560(0.002973)	0.8220(0.003028)	0.8130(0.002547)	0.2680(0.006248)	0.1430(0.0011429)	0.1990(0.001591)	0.0870(0.002733)
48	0.9220(0.001855)	0.7580(0.002348)	0.6840(0.002118)	0.2540(0.001056)	0.1370(0.007779)	0.1830(0.001244)	0.0760(0.002410)
64	0.8930(0.003560)	0.7080(0.02919)	0.6090(0.002843)	0.2280(0.009072)	0.1260(0.007071)	0.1710(0.001571)	0.0680(0.001317)
72	0.8810(0.004019)	0.6610(0.01546)	0.5570(0.02346)	0.2120(0.008272)	0.1180(0.007290)	0.1620(0.001639)	0.0630(0.001613)
90	0.8380(0.004729)	0.5630(0.003447)	0.4660(0.002372)	0.2020(0.006308)	0.1030(0.008156)	0.1410(0.01584)	0.0540(0.001388)

Table 4.6. Concentration of tetracycline in distilled and river water and sediment

Day of sampling	Concentration ($\bar{X}$ ± SD), n = 3 (TC)						
	CDW(µg/ml)	DWE(µg/ml)	DWN(µg/ml)	RW(µg/ml)	RS(µg/g)	RWN(µg/ml)	RSN(µg/g)
0	0.9880(0.001085)	0.9850(0.001247)	0.9810(0.001072)	0.7960(0.008851)	0.6350(0.01840)	0.7880(0.01238)	0.6230(0.01692)
1	0.9830(0.006110)	0.9870(0.001107)	0.9640(0.002808)	0.7670(0.001678)	0.6320(0.008482)	0.7170(0.008095)	0.6070(0.01292)
3	0.9880(0.003087)	0.9820(0.001146)	0.9680(0.002032)	0.7280(0.002788)	0.5560(0.012409)	0.6560(0.01111)	0.4050(0.008056)
4	0.9870(0.001646)	0.9840(0.001085)	0.9620(0.003004)	0.7010(0.001796)	0.5280(0.006245)	0.6220(0.01799)	0.3520(0.01012)
7	0.9840(0.001137)	0.9830(0.001092)	0.9680(0.003738)	0.6840(0.002141)	0.4050(0.009027)	0.5450(0.009751)	0.2630(0.009098)
8	0.9840(0.002137)	0.9820(0.002314)	0.9570(0.001090)	0.6680(0.001427)	0.3620(0.007853)	0.5220(0.005843)	0.2580(0.003447)
11	0.9850(0.001202)	0.9790(0.003063)	0.9490(0.009628)	0.6220(0.007044)	0.3420(0.008608)	0.4620(0.005443)	0.2190(0.004813)
13	0.9860(0.001113)	0.9780(0.002398)	0.9460(0.002109)	0.6130(0.001948)	0.3180(0.008910)	0.4680(0.005607)	0.2030(0.004507)
15	0.9810(0.002271)	0.9760(0.003886)	0.9510(0.002970)	0.5720(0.006591)	0.2940(0.004742)	0.4470(0.006525)	0.1860(0.003334)
18	0.9710(0.001522)	0.9670(0.002882)	0.9280(0.004453)	0.5220(0.001743)	0.2770(0.006036)	0.4270(0.007101)	0.1490(0.004294)
20	0.9720(0.002266)	0.9660(0.001808)	0.9070(0.003021)	0.5090(0.001483)	0.2680(0.007032)	0.3880(0.009502)	0.1180(0.002888)
26	0.9660(0.001110)	0.9250(0.002010)	0.8660(0.002315)	0.4330(0.001261)	0.2140(0.004577)	0.3660(0.008195)	0.0840(0.003230)
34	0.9520(0.001075)	0.8920(0.002791)	0.8270(0.01784)	0.3980(0.01273)	0.1830(0.004500)	0.2790(0.01055)	0.0670(0.001155)
48	0.9220(0.02026)	0.8580(0.002333)	0.7640(0.01153)	0.3040(0.008266)	0.1470(0.003399)	0.2030(0.006557)	0.0560(0.002085)
64	0.8930(0.001904)	0.7880(0.002105)	0.6990(0.002022)	0.2380(0.007120)	0.1190(0.004424)	0.1750(0.006998)	0.0480(0.001761)
72	0.8810(0.009679)	0.7610(0.008871)	0.6330(0.01325)	0.2290(0.004790)	0.1080(0.004015)	0.1620(0.003984)	0.0330(0.009631)
90	0.8510(0.002444)	0.6930(0.001483)	0.9520(0.002708)	0.2120(0.003854)	0.0830(0.003422)	0.1410(0.003062)	0.0310(0.008629)

4.7 MICROBIAL COUNTS

The standard American Public Health Association (APHA) pour plate procedure (APHA, 2004) was applied in the determination of the total viable count of microorganisms in river water at days 6 and 26. Fig 4.7 show the presence of resistant strains of microorganisms.

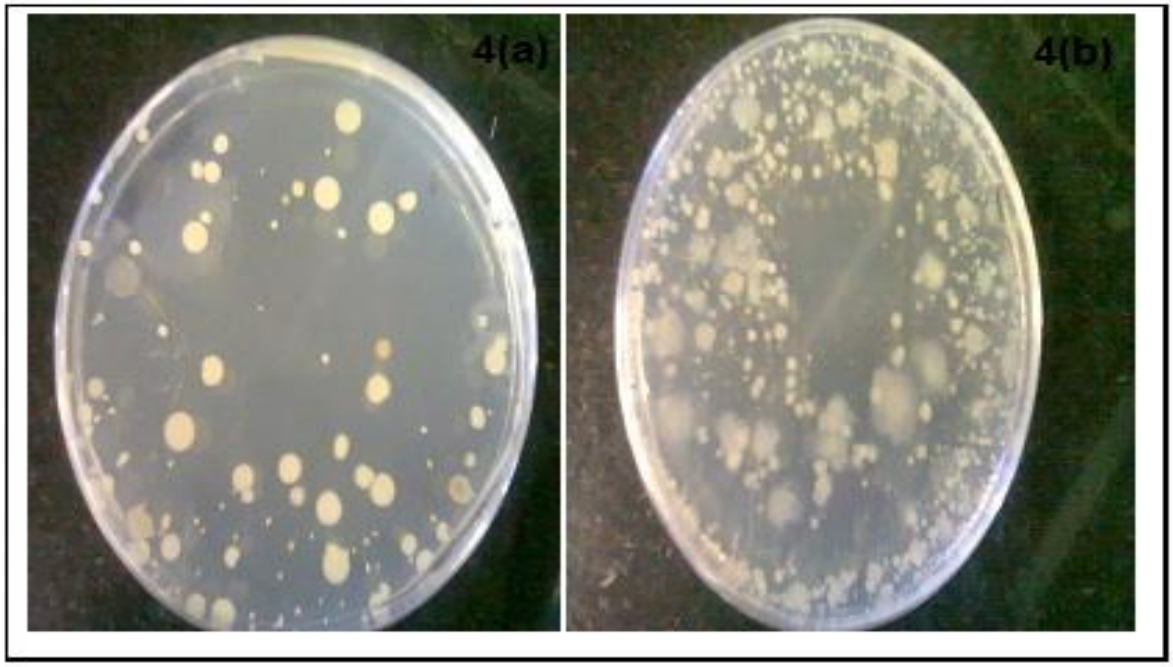


Fig 4.7 Total viable count of microorganisms in river water at day 6 (4a) and 26 (4b) respectively performed by the pour plate procedure (APHA, 2004).

CHAPTER 5

5.0 KINETIC STUDIES: RESULTS AND DISCUSSION

5.1 PERSISTENCE AND DEGRADATION PRODUCTS.

5.1.1 Oxytetracycline

Figure 5.1.1 shows graphically the changes in concentration of OTC in distilled and river water and sediment under dark, natural light, without and with nitrate conditions. The degradation was slowest in the covered distilled water experiment giving a 2% removal after 20 days, as compared to 19% and 62% for exposed distilled water and river water experiments respectively for the same period. It can also be seen that adding nitrates to the exposed distilled water and river water and sediment increased the degradation rate of OTC. Figure 5.1.2 shows graphically the concentration changes for degradation products of OTC that were detected in the study period. Detection of the degradation products was performed by applying a method reported by Xuan et al., (2010) and the Chinese Pharmacopeia, (2005). The degradation products were identified by matching their retention times with those of standards and by adding internal standards into selected samples. The results show that 4-epi-oxytetracycline was detected after 30 days while β-apo- OTC was detected after 20 days. The degradation products could not be detected as from day zero. This is because their concentration was below the detection limit of the method. The results also show that the degradation products undergo further degradation under the experimental conditions.

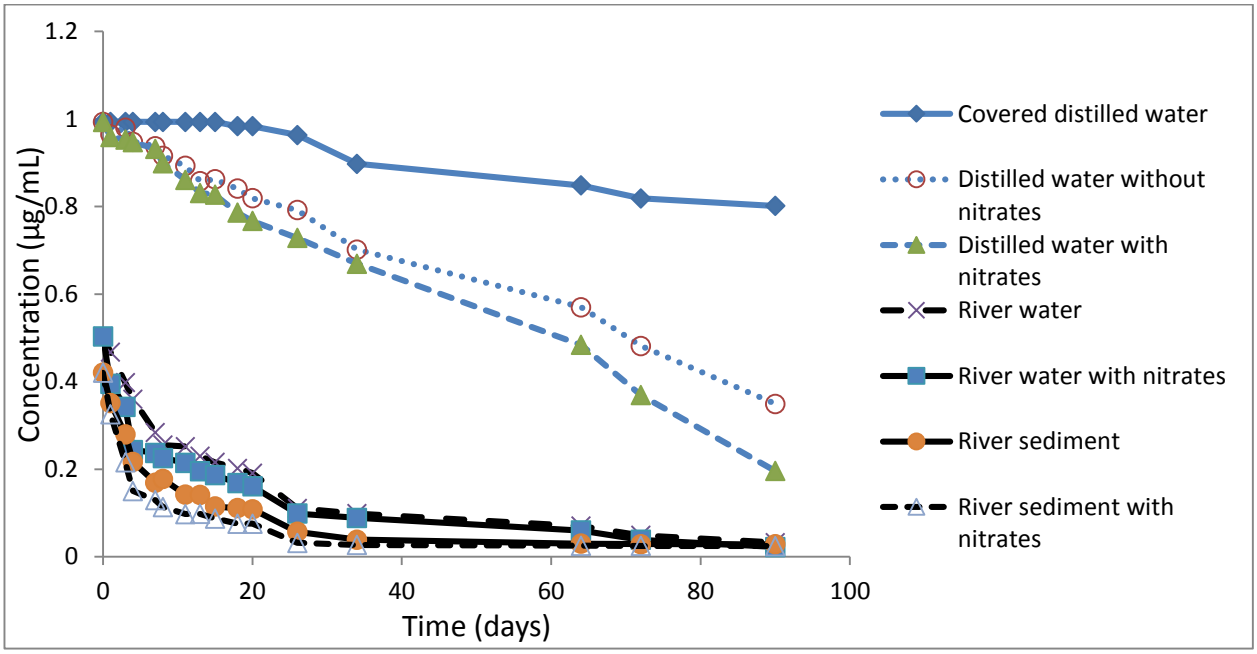


Figure 5.1.1.Concentration changes of oxytetracycline in distilled and river water and sediment experiments

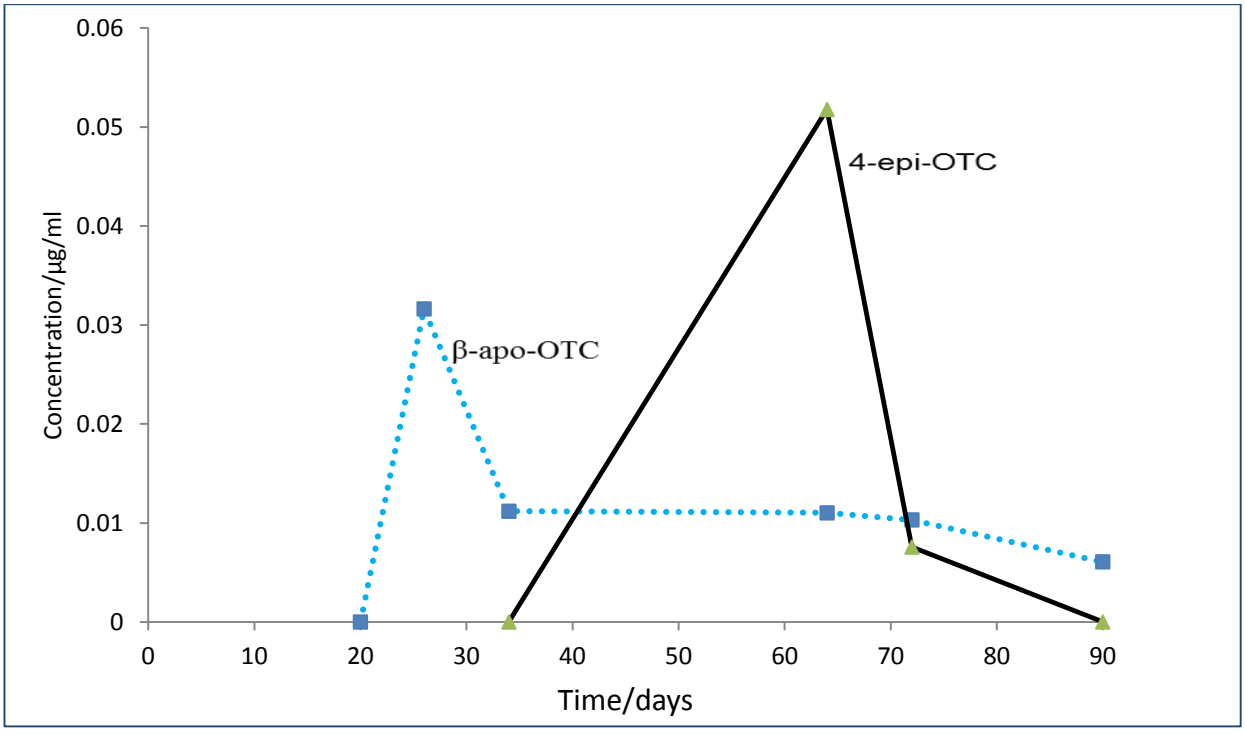


Fig 5.1.2 Concentration changes of 4-epi-oxytetracycline (4-epi-OTC) and β-apo-oxytetracycline (β-apo-OTC) in the river water experiment

5.1.2 Doxycycline

Figure 5.1.3 show graphically the changes in concentration of DC in distilled water control experiments and river water and sediment in dark, exposed to natural light, with and without nitrates conditions. At the beginning the concentration of DC decreased slowly in distilled water experiments while the concentration decreased rapidly as from day one in river water and sediment. Under analytical conditions employed in the present study, doxycycline and two widely reported degradation products metacycline and 6-epi-doxycycline were separated (see Fig 4.3 and 5.1.4). The changes in concentration of metacycline (MET) and 6-epi-doxycycline (6-E-DC) over the 90 day period are shown in Fig 5.1.5. The degradation product 6-epi-DC was present at a lower concentration than MET throughout the experiment. The concentration of the transformation products increased initially up to 0.39 µg mL^{-1} (MET) and 0.02 µg mL^{-1} (6-epi-DC) and decreased thereafter showing that they also undergo degradation.

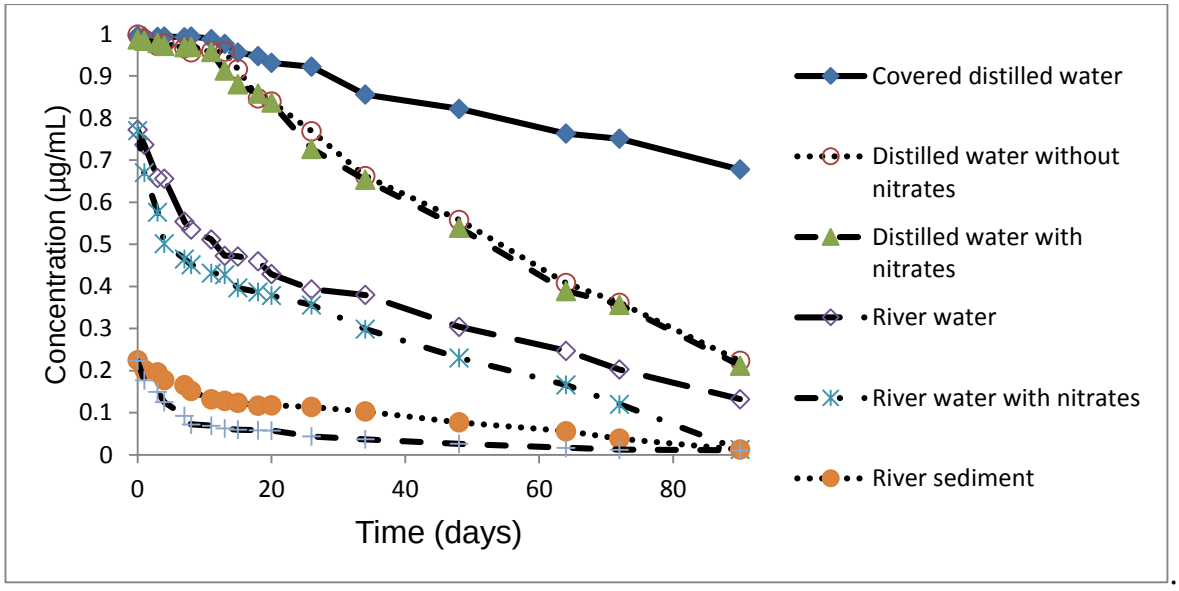


Figure 5.1.3 Changes in concentration of doxycycline in distilled water, river water and sediment.

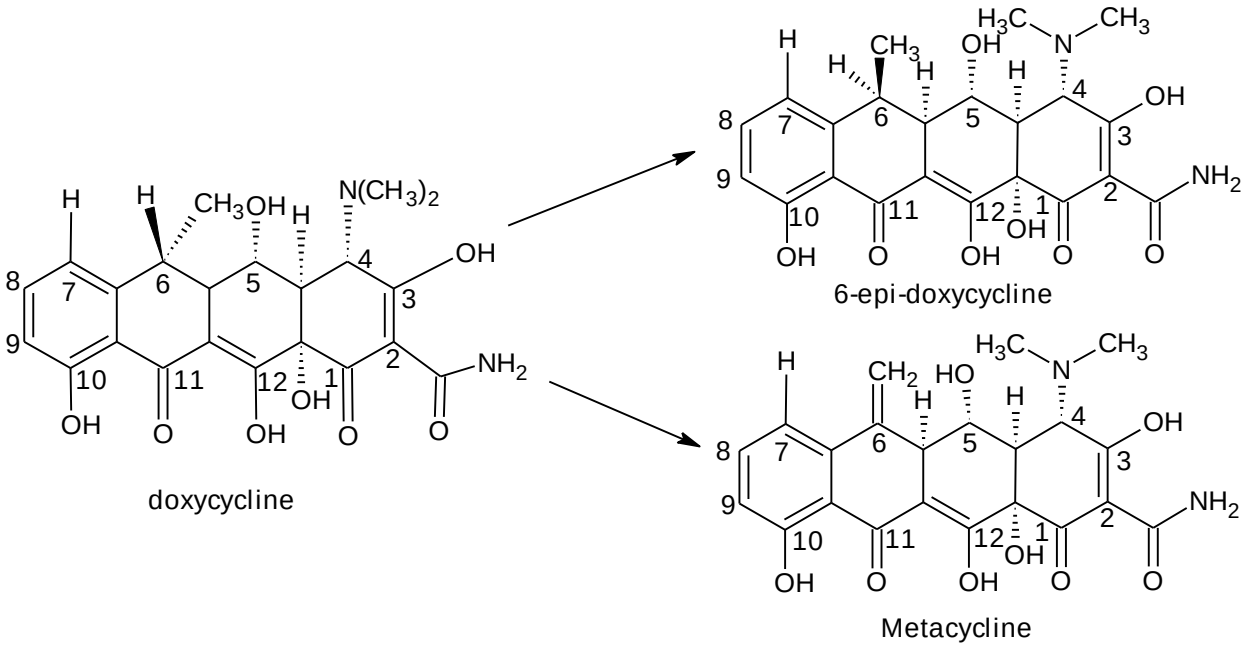


Fig 5.1.4.Transformation scheme for doxycycline to metacycline and 6-epidoxycycline (Injac et al., 2007).

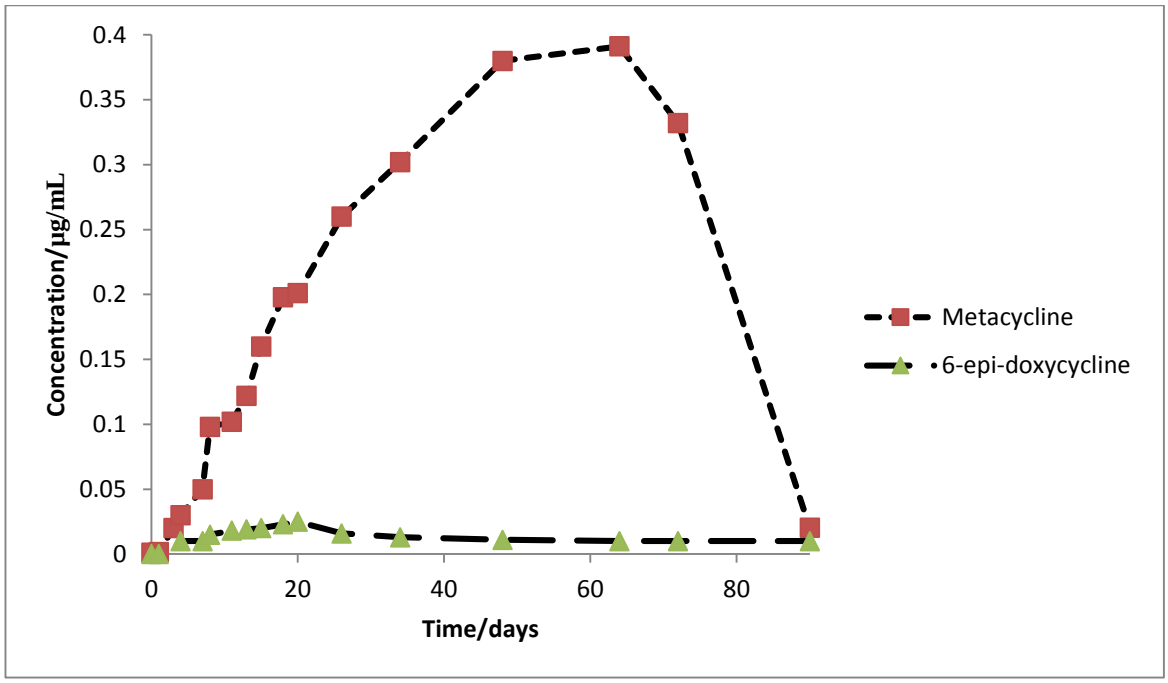


Fig 5.1.5. Changes in concentration of metacycline (MET) and 6-epi-doxycycline (6-E-DC) during the study period.

5.1.3 Chlortetracycline

Changes in concentration of CTC in distilled water, river water and sediment are shown graphically in Figure 5.1.6. From day 1 to 15 the concentration of CTC decreased slightly in the distilled water control experiments while in river water and sediment the concentration decreased rapidly as from the beginning. Iso-CTC, 4-epi-CTC, and 4-epi-iso-CTC and anhydro- CTC are degradation products of CTC which have been identified previously by Loftin et al., (2008). Two of the degradation products, 4-epi- CTC and iso- CTC were also identified in the present study and their concentration changes are shown in Fig 5.1.7. The concentration of iso- CTC was lower than 4-epi- CTC throughout the experiment. Chemical structures of CTC and two of its degradation products are shown in Fig 5.1.8.

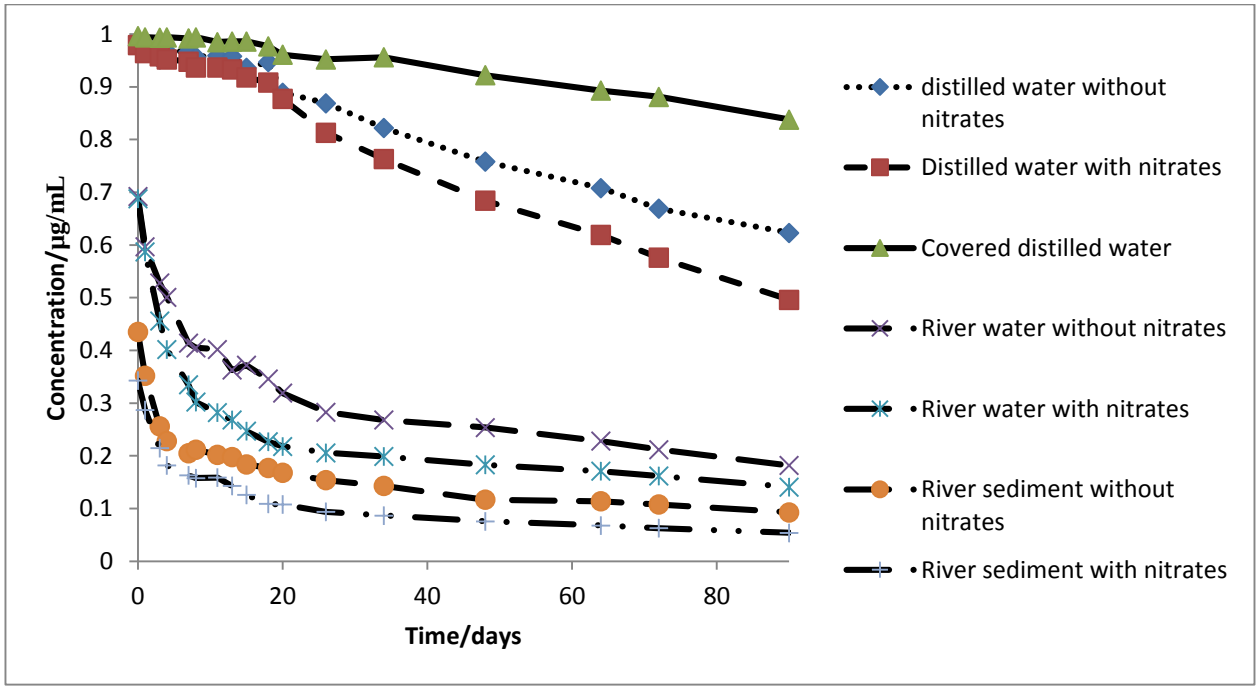


Fig 5.1.6 Changes in concentration of chlortetracycline in distilled water, river water and sediment.

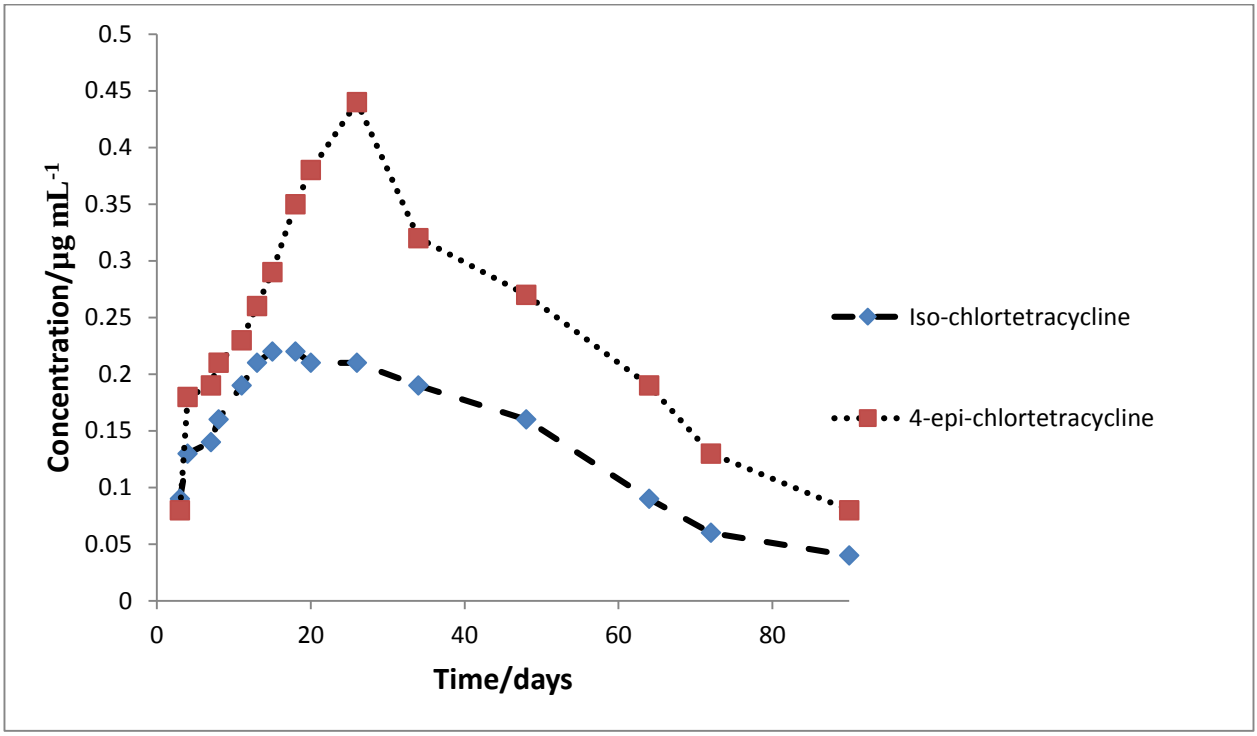


Fig 5.1.7 Concentration changes of iso-chlortetracycline and 4-epi-chlortetracycline in river water

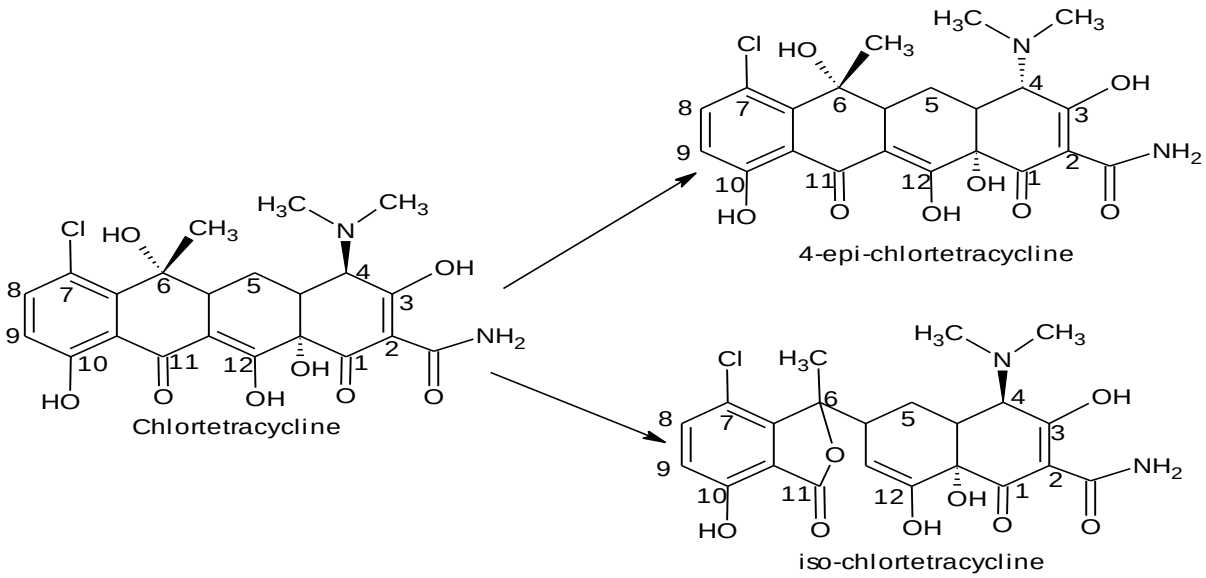


Fig 5.1.8 Transformation scheme for chlortetracycline to 4-epi-chlortetracycline and iso-chlortetracycline (Loftin et al., 2008).

5.1.4 Tetracycline

Concentration changes of TC in the dark, exposed to light with or without nitrates experiments are shown in Fig 5.1.9. The concentration decreased in a similar pattern as for OTC, DC and CTC. The concentration decreased slowly in the distilled water control experiments until day 15 while the concentration decreased rapidly in river water and sediment as from day 1. Fig 5.1.10 shows changes in concentration of the major degradation products of TC reported by Loftin et al., (2008) that were also detected in the present study. Degradation products were identified using a method reported by Loftin et al., (2008). Standard degradation products mixes were used for confirming TC degradation products by standard addition in selected samples. The concentration increased at the beginning and then decreased thereafter, showing that the transformation products also undergo degradation. There were very little changes in concentration between day 8 and 26 for both 4-epi-tetracycline and anhdro- TC possibly because of fewer numbers of microorganisms that could degrade 4-epi- TC and anhdro- TC. Fig 5.1.10 shows that anhydro-tetracycline degrades rapidly as compared to 4-epi-tetracycline.

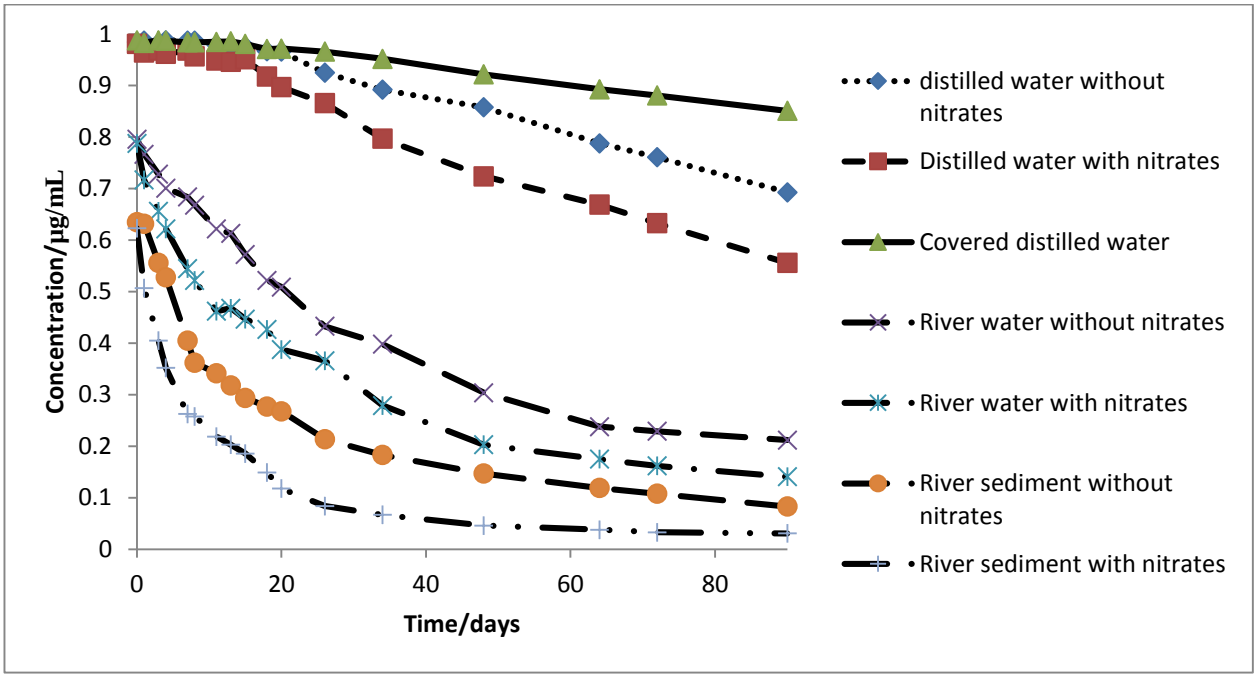


Fig 5.1.9. Concentration changes of tetracycline in distilled water control experiment and river water and sediment experiment

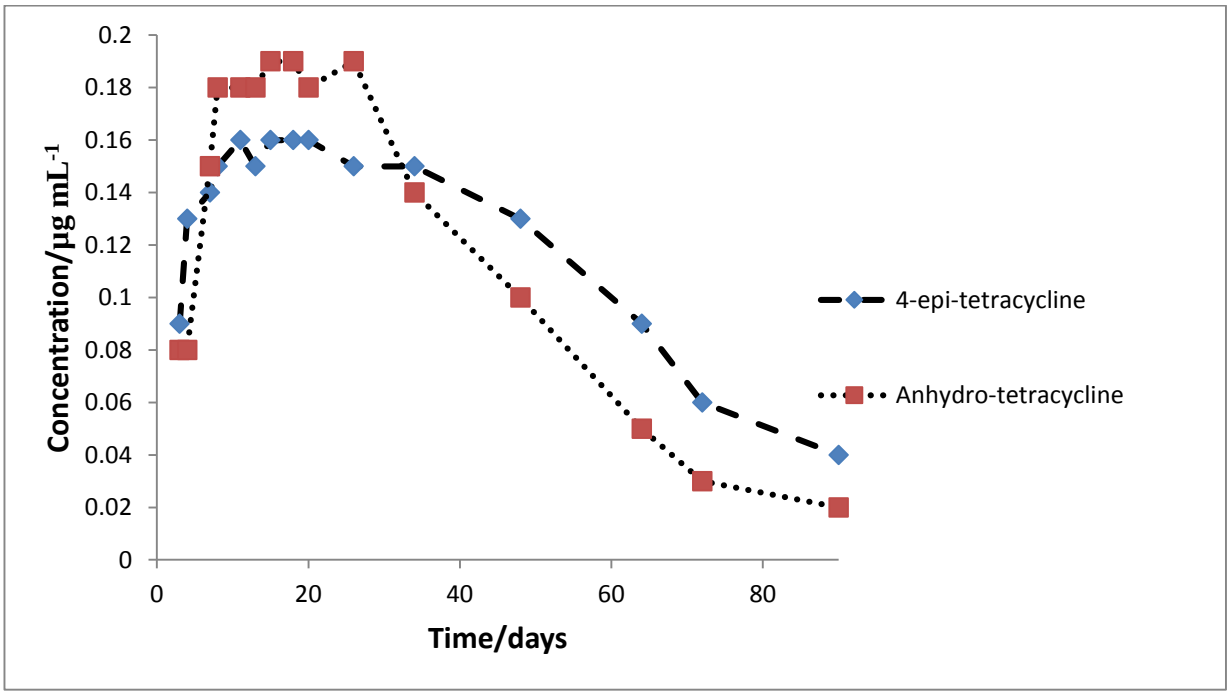


Fig 5.1.10 Concentration changes of 4-epi-tetracycline and anhydro-tetracycline in river water

5.2 Rates of degradation.

5.2.1 Rates of degradation in the distilled water control experiments.

The rates of degradation were arrived at by plotting the loss in antimicrobial concentration (C_t-C_o, where C_o = initial concentration and C_t = concentration at time t) as a function of time in accordance to zero order kinetics in Figures 5.2.1 to 5.2.4, using data from Tables 4.3 to 4.6.

Figs. 5.2.1(a), 5.2.2(a), 5.2.3(a) and 5.2.4(a) show that the (C_t- C_o) versus t curve for the degradation of OTC, DC, CTC and TC under dark conditions in distilled water can be resolved into two linear portions: a very slow linear rate of degradation up to 12, 8, 11 and 5 days for OTC, DC, CTC and TC respectively, followed by a relatively faster linear rate of degradation subsequent to that. The low R^2 values in Figs 5.2.1a, 5.2.3a and 5.2.4a were further tested by inspecting variability of residuals using the Dixon` s Q-test at 95% confidence interval. Results of the test showed that there are no outliers. This shows that one can still draw important conclusions about how changes in the rate are associated with changes in time. The respective rates of degradation, given by the slopes of the linear portions of the curve, are summarized in Table 5.2.1.

Table 5.2.1 Rates of degradation ($\mu g g^{-1} day^{-1}$) of tetracycline antimicrobials in the distilled water control experiments.

Antimicrobial	Dark conditions		Sunlight exposed		Degradation mechanism and rates	
	Initial	Subsequent	Initial	Subsequent	Hydrolysis	Photolysis
OTC	2.0×10^{-6}	2.7×10^{-3}	6.9×10^{-3}	6.9×10^{-3}	2.0×10^{-6}	6.9×10^{-3}
DC	5.0×10^{-4}	1.9×10^{-3}	2.9×10^{-3}	9.8×10^{-3}	5.0×10^{-4}	2.9×10^{-3}
CTC	3.0×10^{-4}	1.8×10^{-3}	1.5×10^{-3}	5.1×10^{-3}	3.0×10^{-4}	1.2×10^{-3}
TC	1.0×10^{-4}	1.8×10^{-3}	6.0×10^{-4}	3.8×10^{-3}	1.0×10^{-4}	5.0×10^{-4}

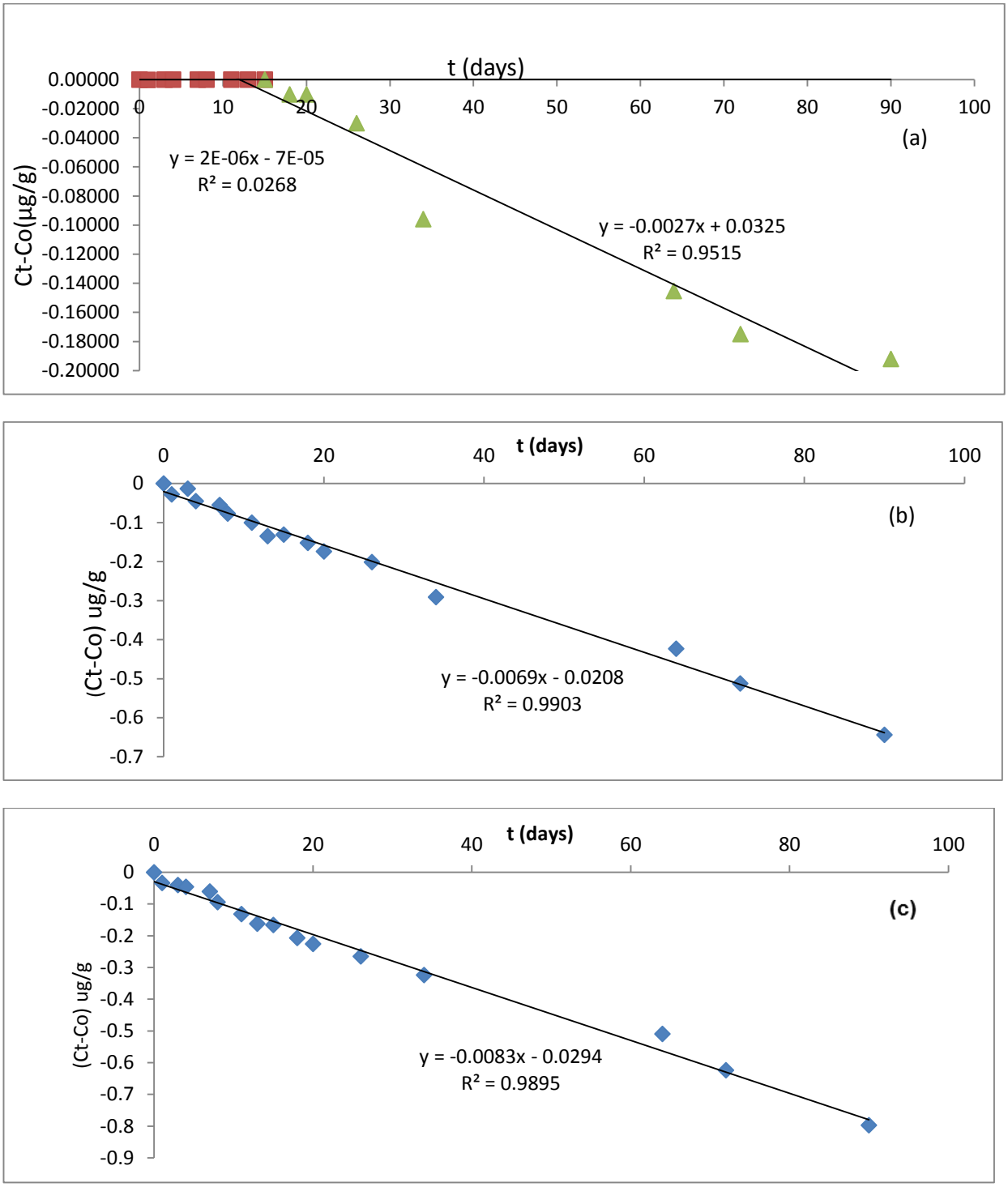


Fig 5.2.1. Oxytetracycline degradation rates in (a), covered distilled water experiment (b), distilled water exposed to light experiment and (c) distilled water containing 2 mg mL^{-1} nitrate experiment.

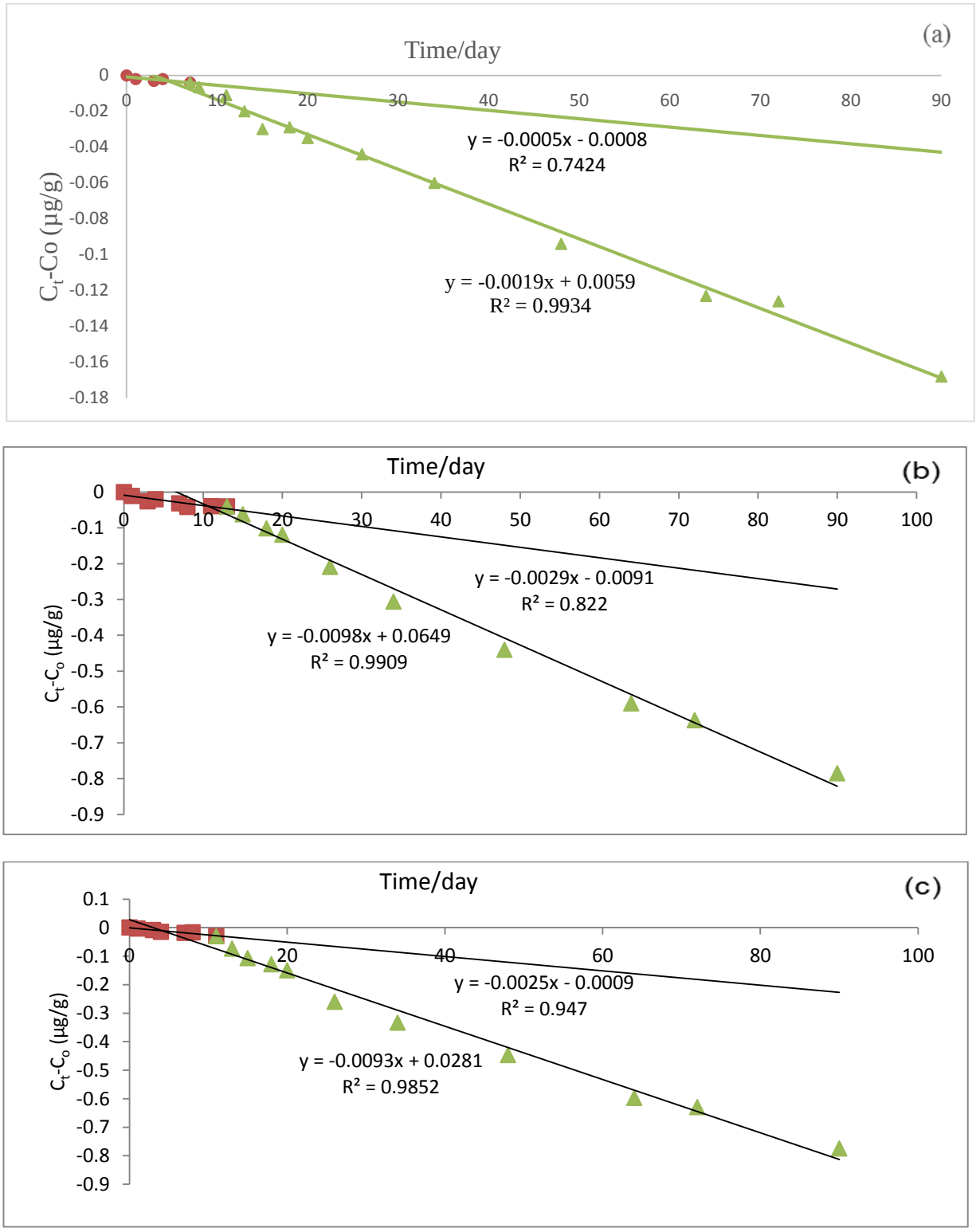
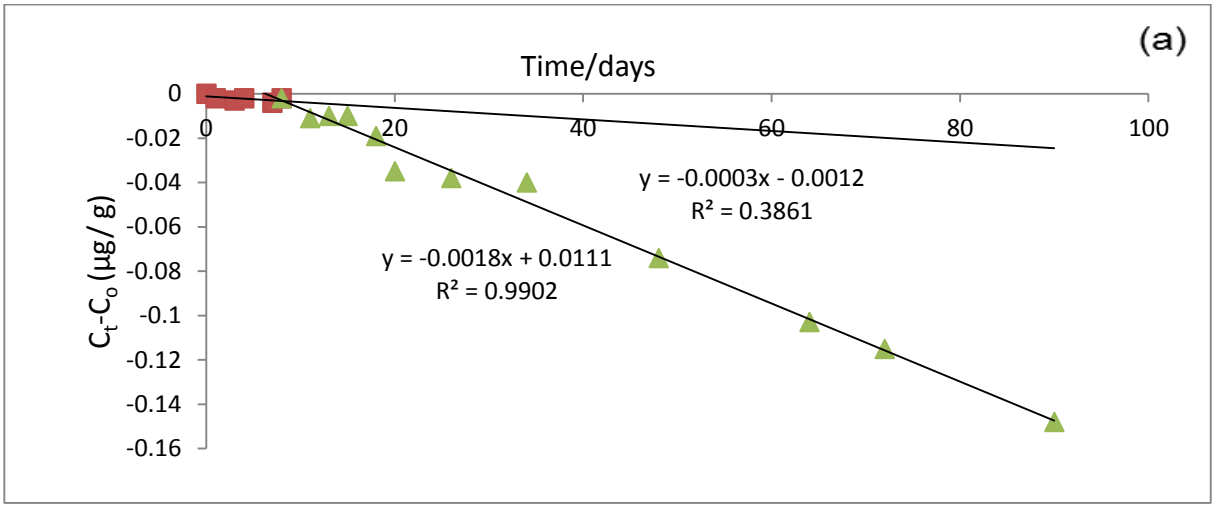
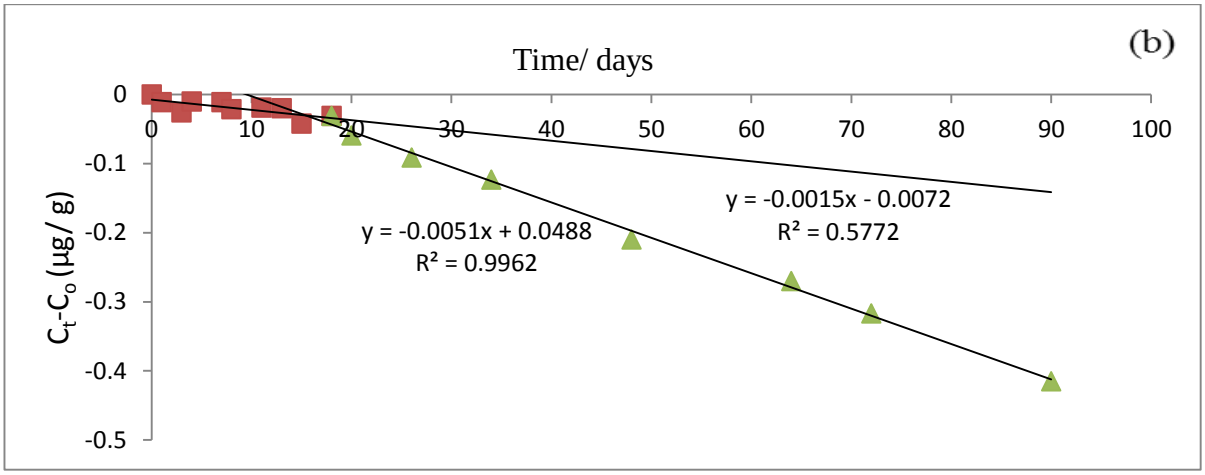


Fig 5.2.2. Doxycycline degradation rates in (a), covered distilled water experiment (b), distilled water exposed to light experiment and (c) distilled water containing 2 mg mL^{-1} nitrates experiment.





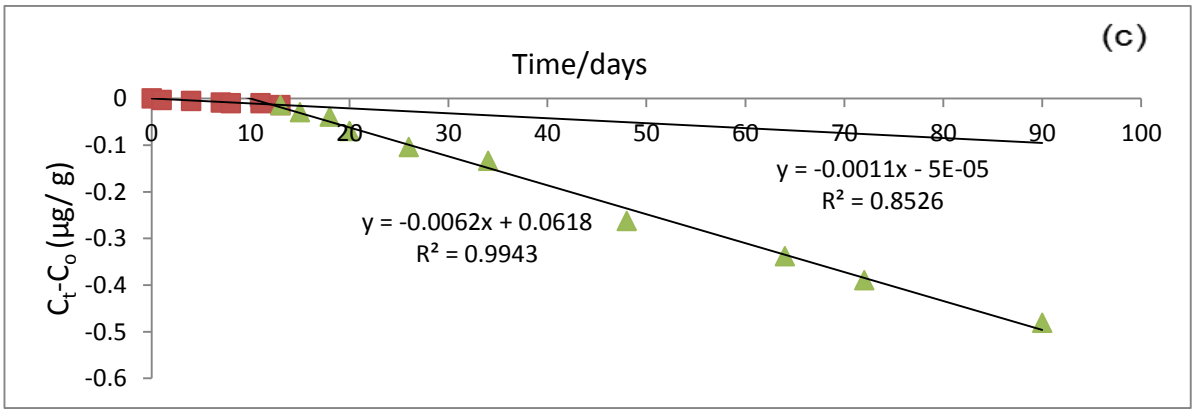
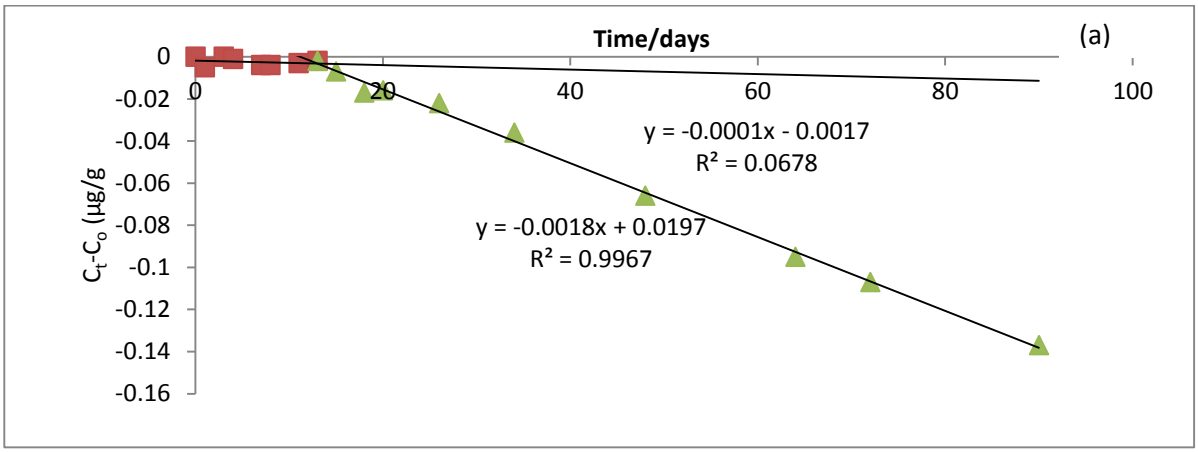
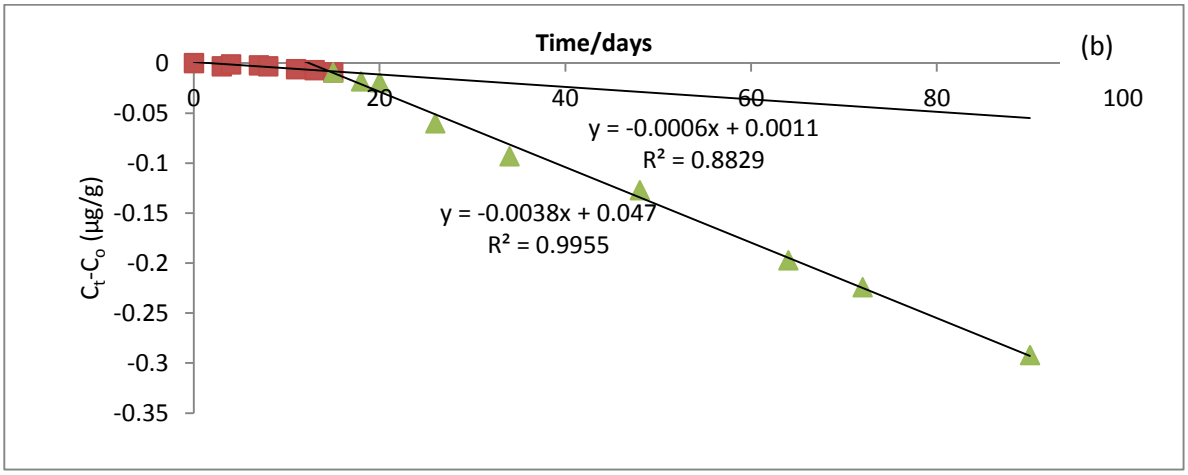


Fig 5.2.3 Chlortetracycline degradation rates in (a) covered distilled water experiment, (b) distilled water exposed to light experiment and (c) distilled water containing 2 mg mL^{-1} nitrate.





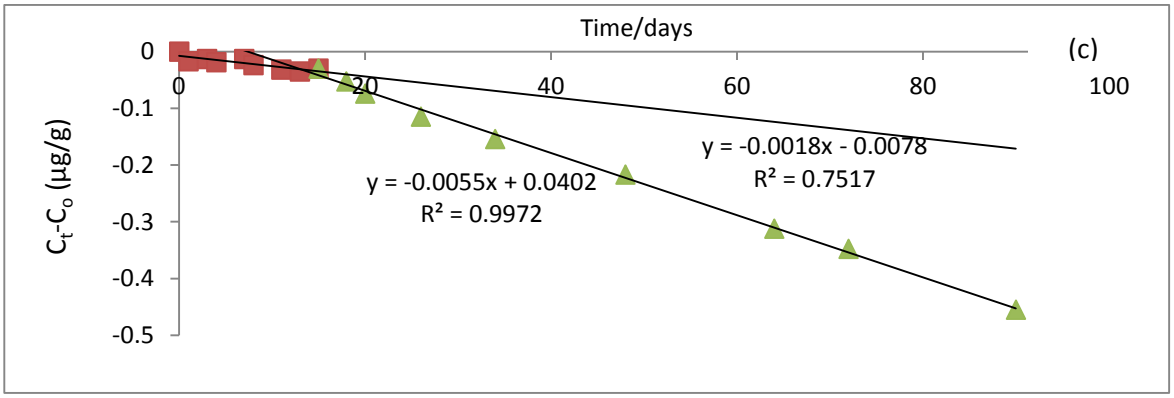


Fig 5.2.4 Degradation rates of tetracycline in (a) covered distilled water experiment, (b) distilled water exposed to light experiment and (c) distilled water containing 2 mg mL^{-1} nitrate experiment.

In designing the distilled water control experiments, two basic assumptions were made that is, (i) since distilled water was virtually sterile, microbial degradation of the antibiotic as a result of re-colonization from the atmosphere would require a lag phase to allow for microbial multiplication and adaptation, (ii) since hydrolysis and photolysis do not require a lag period therefore, they could be resolved from biodegradation if its contribution is significant. Therefore no preservative was added to stop microbial activity. It was also assumed that such a preservative, if added, could compete with the antibiotic for adsorption sites in the system, thereby altering the experimental conditions. On the basis of these assumptions, the slow degradation rate in the dark control experiment was therefore attributed to hydrolysis, while the subsequent fast rate was attributed to a combination of hydrolysis and microbial degradation.

Figs. 5.2.1(b), 5.2.2(b), 5.2.3(b) and 5.2.4(b) show the (Ct- Co) versus t curves for the degradation of OTC, DC, CTC and TC respectively in distilled water exposed to sunlight. It is apparent that, whereas the curves for DC, CTC and TC can be resolved into two linear portions as before, the curve for OTC consists of a single linear rate of degradation of 6.9 x 10^{-3}μg/g/day. For DC, CTC and TC the initial slow rate is attributed to a combination of hydrolysis and photolysis, so that the difference between the initial linear rates of degradation in (a) and (b) represent the increase in the rate of degradation due to photolysis. For OTC the single linear rate of degradation in control experiment (b) suggests minimal microbial contamination or re-colonization of the experimental set-up, so that the difference between this single linear rate of degradation in (b) and the initial slow rate of degradation in (a) represents the contribution from photolysis. The contributions from hydrolysis and photolysis to the observed rates of degradation are summarized in the last columns of Table 5.2.1

Figs. 5.2.1(c), 5.2.2(c), 5.2.3(c) and 5.2.4(c) show the (C_t-C_o) versus t curves for the degradation of OTC, DC, CTC and TC respectively in distilled water spiked with nitrate ion and exposed to sunlight. Table 5.2.2 show the effect of addition of nitrate on the rates of degradation. Except for TC, data in Table 5.2.2 shows that addition of nitrates has no effect on the initial rate of photolysis of OTC, DC and CTC. On the other hand the data in the same table shows that, except for DC, addition of nitrates increases the subsequent rate of degradation of OTC, CTC and TC by 20%, 22% and 45% respectively. The increase in the degradation rate on the addition of nitrates may be due to an increase in the rate of photochemical degradation, as a result of sensitization by the nitrate ion (Jeong et al., 2010; Niu et al., 2013), or to an increase in the rate of microbial degradation as a result of the increase in nutrients for microbial growth and multiplication.

Table 5.2.2. Effect of adding nitrates on rate of degradation of tetracycline antimicrobials in distilled water exposed to sunlight.

Antimicrobial	Initial slow rate			Subsequent fast rate		
	Before	After	$\frac{After}{Before}$	Before	After	$\frac{After}{Before}$
OTC	6.9×10^{-3}	2×10^{-6}	2.9×10^{-4}	6.9×10^{-3}	8.3×10^{-3}	1.20
DC	2.9×10^{-3}	2.5×10^{-3}	0.86	9.8×10^{-3}	9.3×10^{-3}	0.95
CTC	1.5×10^{-3}	1.1×10^{-3}	0.73	5.1×10^{-3}	6.2×10^{-3}	1.22
TC	6×10^{-4}	1.8×10^{-3}	3	3.8×10^{-3}	5.5×10^{-3}	1.45

5.2.2 Rates of degradation in the microcosm experiments.

Figs. 5.2.5 to 5.2.8 show the (Ct-Co) versus t curves for the degradation of OTC, DC, CTC and TC respectively in the microcosm experiments. Figs. 5.2.5 to 5.2.8 show that, except for DC, the curves can be resolved into three linear portions: very fast linear rates of degradation

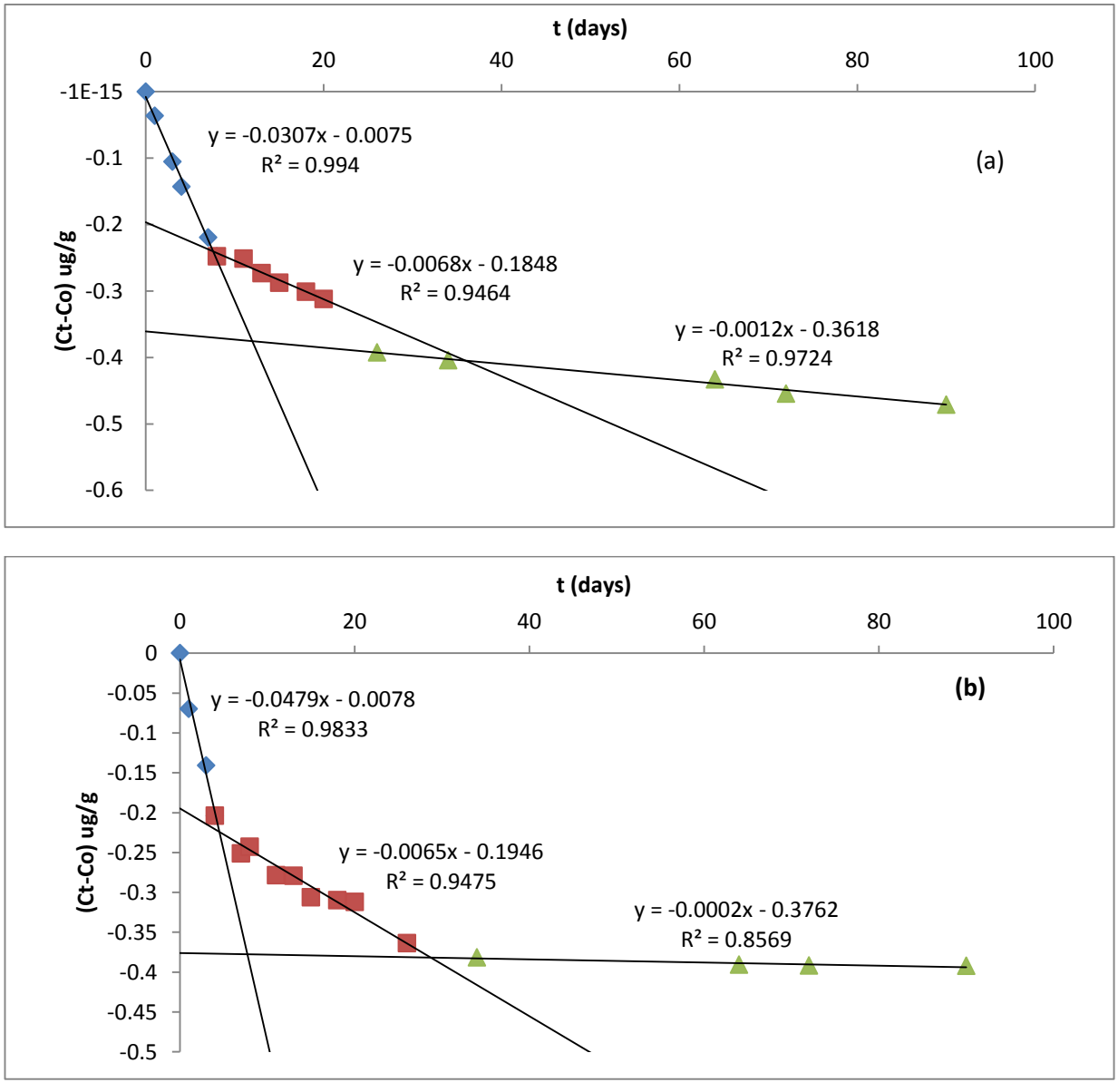


Fig 5.2.5 Oxytetracycline degradation rates in (a) river water experiment and (b) river sediment experiment.

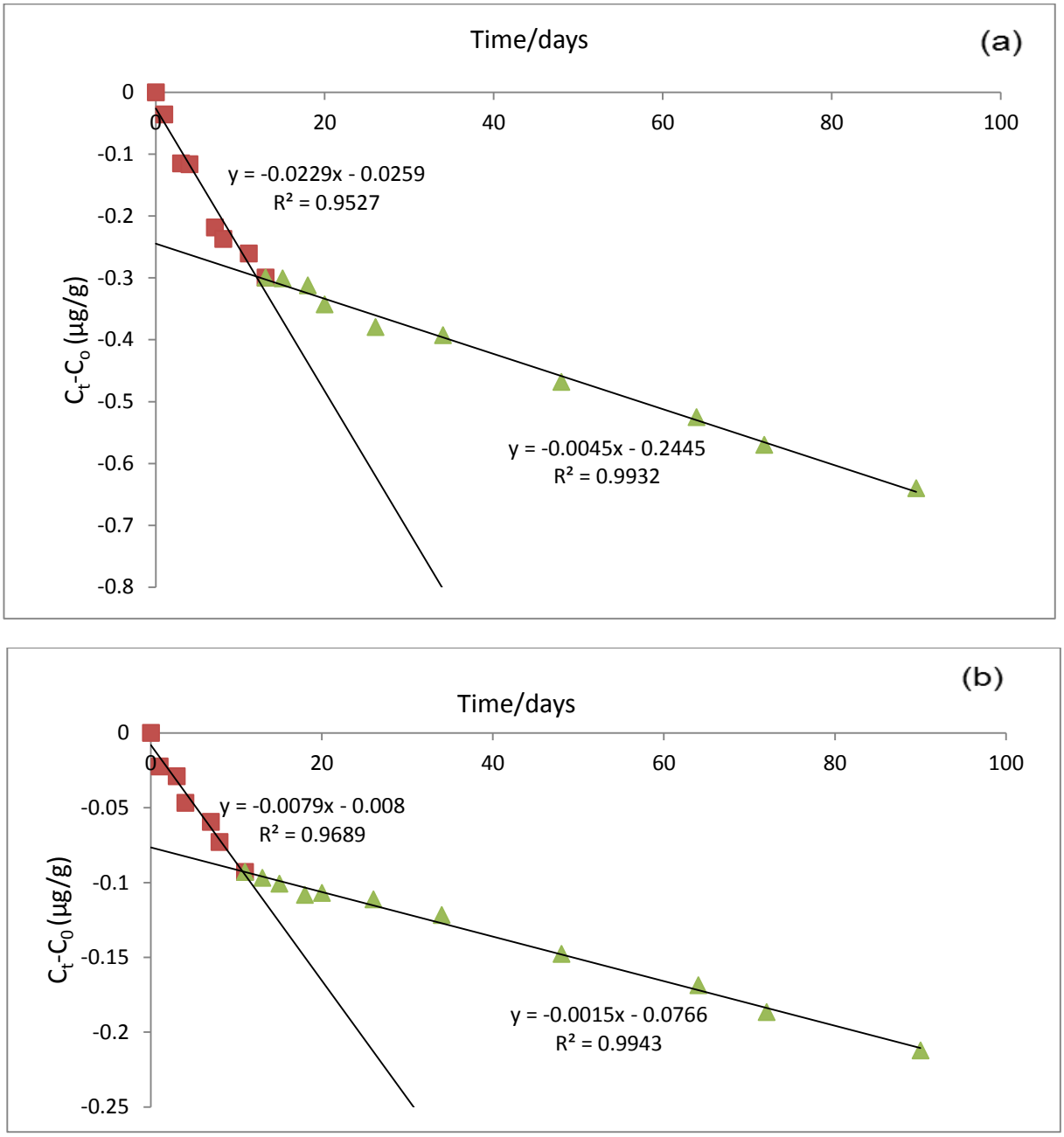


Fig 5.2.6. Doxycycline degradation rates in (a) river water experiment and (b) river sediment experiment.

of 1.35 x 10^{-2} to 3.07 x 10^{-2}µg/g/day up to about 25 days depending on the antimicrobial, followed by a relatively slower linear rates of degradation of 4.5 x 10^{-3} to 7.0 x 10^{-3}µg/g/day

between 8 and 60 days, and 1 x 10^{-3} to 1.3 x 10^{-3}µg/g/day subsequently in the water phase; very fast linear rates of degradation of 7.9 x 10^{-3} to 4.8 x 10^{-2}µg/g/day, followed by a relatively slower linear rates of degradation of 1.5 x 10^{-3} to 7.5 x 10^{-3}µg/g/day between 5 and 45 days, and 2 x 10^{-4} to 1.5 x 10^{-3}µg/g/day subsequently in the sediment phase. The respective rates of degradation are given by the slopes of the linear portions of the curves, and are summarized in Table 5.2.3.

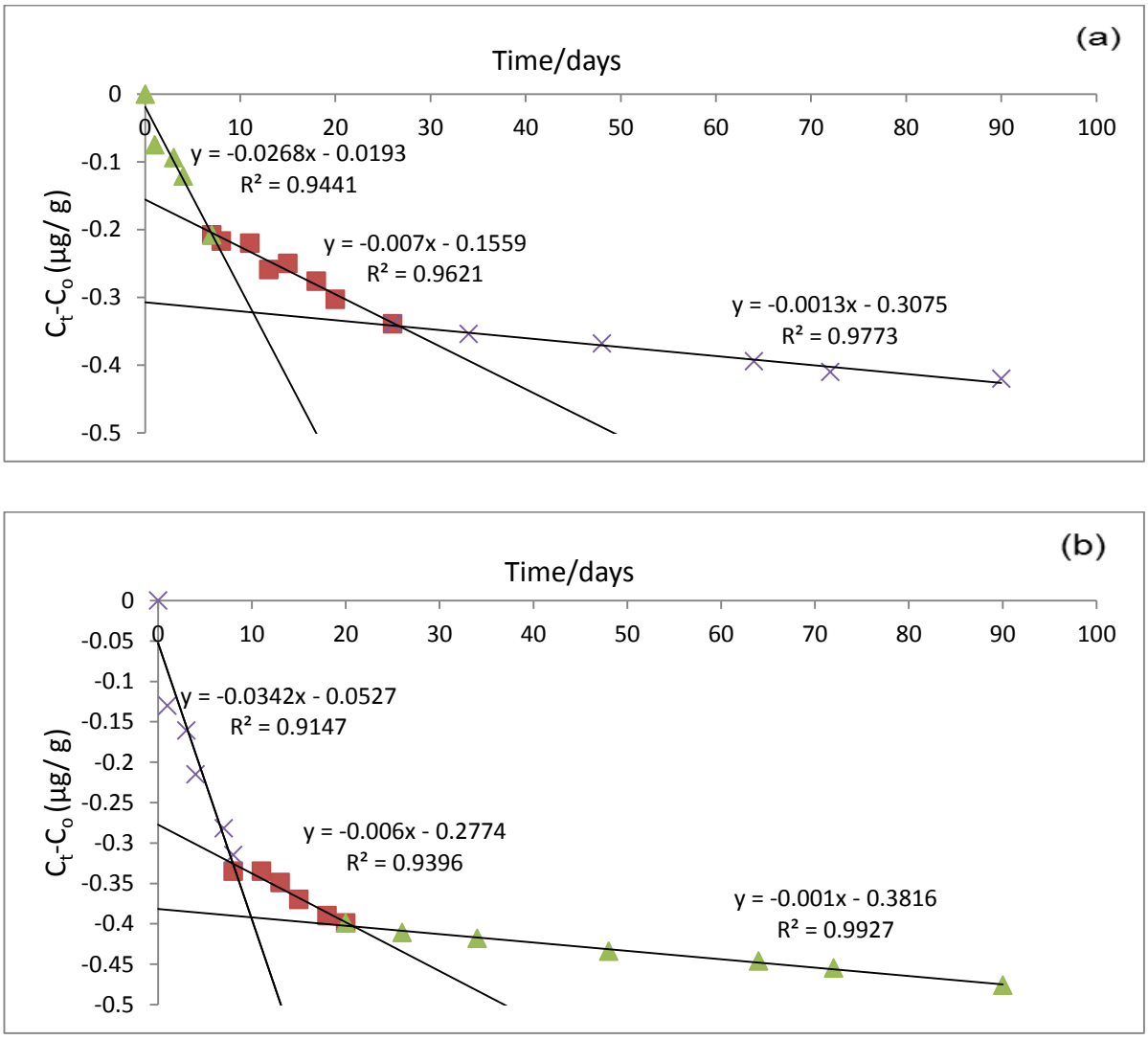


Fig 5.2.7 Chlortetracycline degradation rates in (a) river water experiment and (b) river sediment experiment

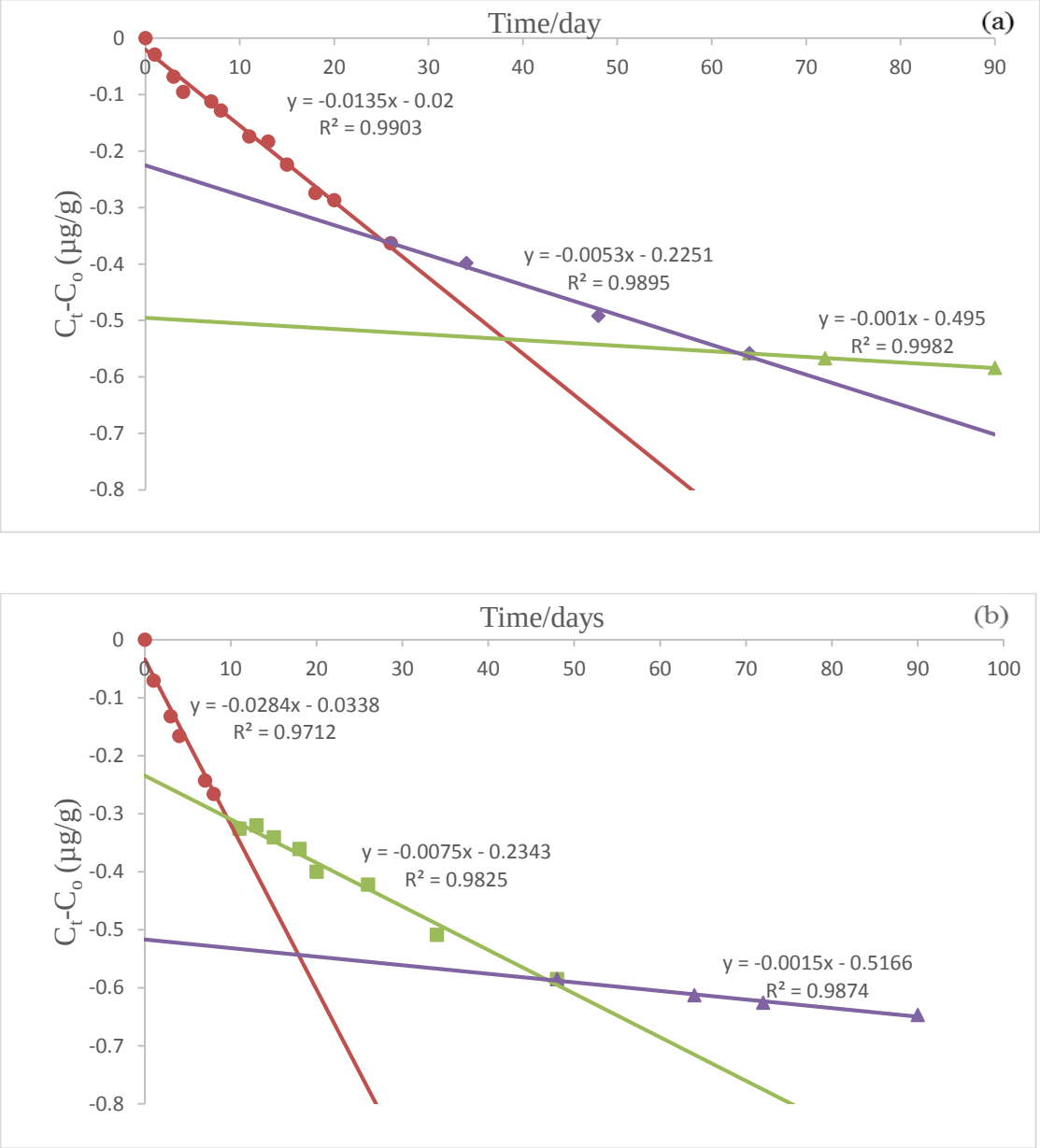


Fig 5.2.8 Degradation rates of tetracycline in (a) river water experiment and (b) river sediment experiment

Table 5.2.3 Rates of degradation ($\mu g g^{-1} day^{-1}$) in the microcosm experiments (without added nitrates)

Antimicrobial	Rate of degradation in water phase			Rate of degradation in sediment phase		
	Initial	Intermediate	Final	Initial	Intermediate	Final
OTC	3.07×10^{-2}	6.80×10^{-3}	1.20×10^{-3}	4.79×10^{-2}	6.50×10^{-3}	2.00×10^{-4}
DC	2.29×10^{-2}	4.50×10^{-3}		7.90×10^{-3}	1.50×10^{-3}	
CTC	2.68×10^{-2}	7.00×10^{-3}	1.30×10^{-3}	3.40×10^{-2}	6.00×10^{-3}	1.00×10^{-3}
TC	1.35×10^{-2}	5.30×10^{-3}	1.00×10^{-3}	2.84×10^{-2}	7.50×10^{-3}	1.50×10^{-3}
Speciation*	FIS(WP)	CPA(WP)	CPA(WP)	FIS(SPW)	CPA(SP)	CPA(SP).
Degradation mechanism	Hydrolysis Photolysis Microbial	Microbial	Microbial	Microbial	Microbial	Microbial

* FIS(WP) = free in solution (water phase), CPA(WP) = colloidal particle adsorbed (water phase), FIS(SPW) = free in solution (sediment pore water), CPA(SP) = colloidal particle adsorbed (sediment phase).

Comparison of the rates of degradation of OTC in the water phase of the microcosm experiment without added nitrates to the rates of OTC degradation in the distilled water control experiments, shows that the fast rate of degradation of 3.07×10^{-2}μg/g/day in the water phase in the microcosm experiment, is 444% greater than the rate of degradation of 6.9×10^{-3}μg/g/day in the sunlight exposed distilled water control experiment without added

nitrate. This marked increase in the degradation rate of OTC is attributed to the increased microbial population/count in the microcosm experiment. If we assume that microorganisms only bind antimicrobial molecules in their desorbed state (Wu et al., 2011), then the slow rates of degradation in the water phase of the microcosm experiment can then be explained by assuming that the rate of desorption of antimicrobial molecules adsorbed by colloidal particles is much slower than the rate at which microorganisms bind free antimicrobial molecules in solution, i.e., the rate of desorption becomes rate limiting. A similar argument can be used to account for the slower rates in the sediment phase.

The initial fast rates of degradation in the water phase are attributed to a combination of hydrolysis, photochemical and microbial degradation of free antimicrobial in solution. The intermediate slow rates of degradation in the water phase are attributed to antimicrobial degradation of colloidal particle adsorbed antimicrobial. As explained above, the difference in the degradation rates of free antimicrobial and adsorbed speciation forms arises from slow rate of desorption of the antimicrobial. The existence of two slow rates in the water phase points to the adsorption of the antimicrobial to two different types of colloidal particles in the water phase. Similarly, the existence of two slow rates of degradation in the sediment phase suggests the adsorption of the antimicrobial by two different types of colloidal particles in the sediment pore water, or two different types of sediment particles, or adsorption to colloidal particles and sediment particles simultaneously.

Fig. 5.2.9-5.2.12 shows the (Ct- Co) versus t curve for the degradation of the TC antibiotics in the microcosm experiment with added nitrate. As with Fig. 5.2.5-5.2.8, the curves can be resolved into three linear portions for OTC, CTC and TC and, two linear portions for DC. Table 5.2.4 shows that the addition of nitrate increases the fast rate of degradation in the

water phase but has no effect on (a) subsequent slow rates of degradation in the water phase, and (b) degradation rates in the sediment phase. This observation suggests that degradation is mainly microbial, so that increase in microorganism count on addition of nitrates has no effect on the degradation of colloidal and sediment particle adsorbed antimicrobial, for which the rate of desorption is rate limiting.

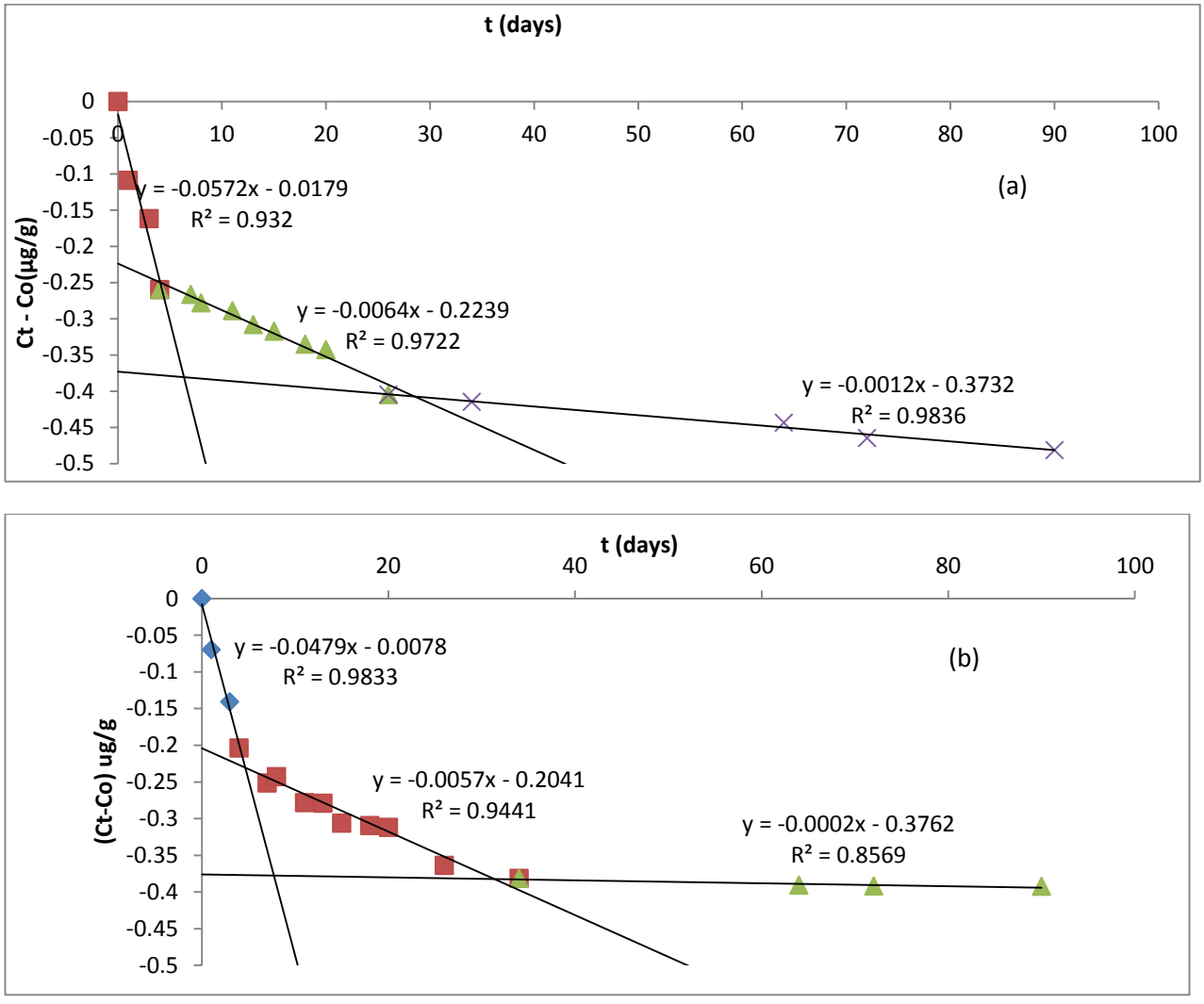


Fig 5.2.9. Oxytetracycline degradation rates in (a) river water experiment containing 2 mg mL^{-1}, and (b) river sediment experiment with 2 mg g^{-1} nitrates

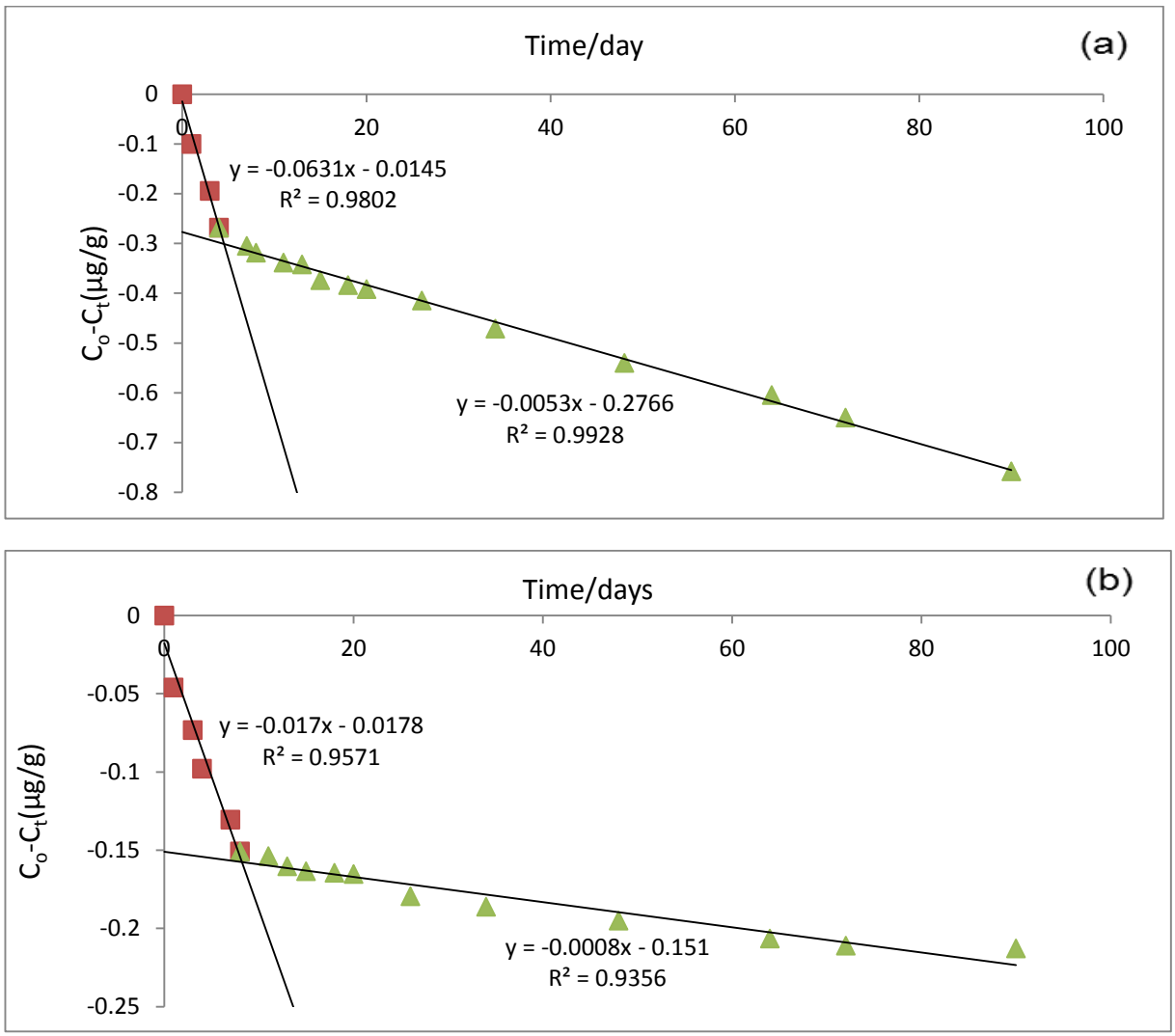


Fig 5.2.10. Doxycycline degradation rates in (a) river water experiment containing 2 mg mL^{-1}, and (b) river sediment experiment with 2 mg g^{-1} nitrates

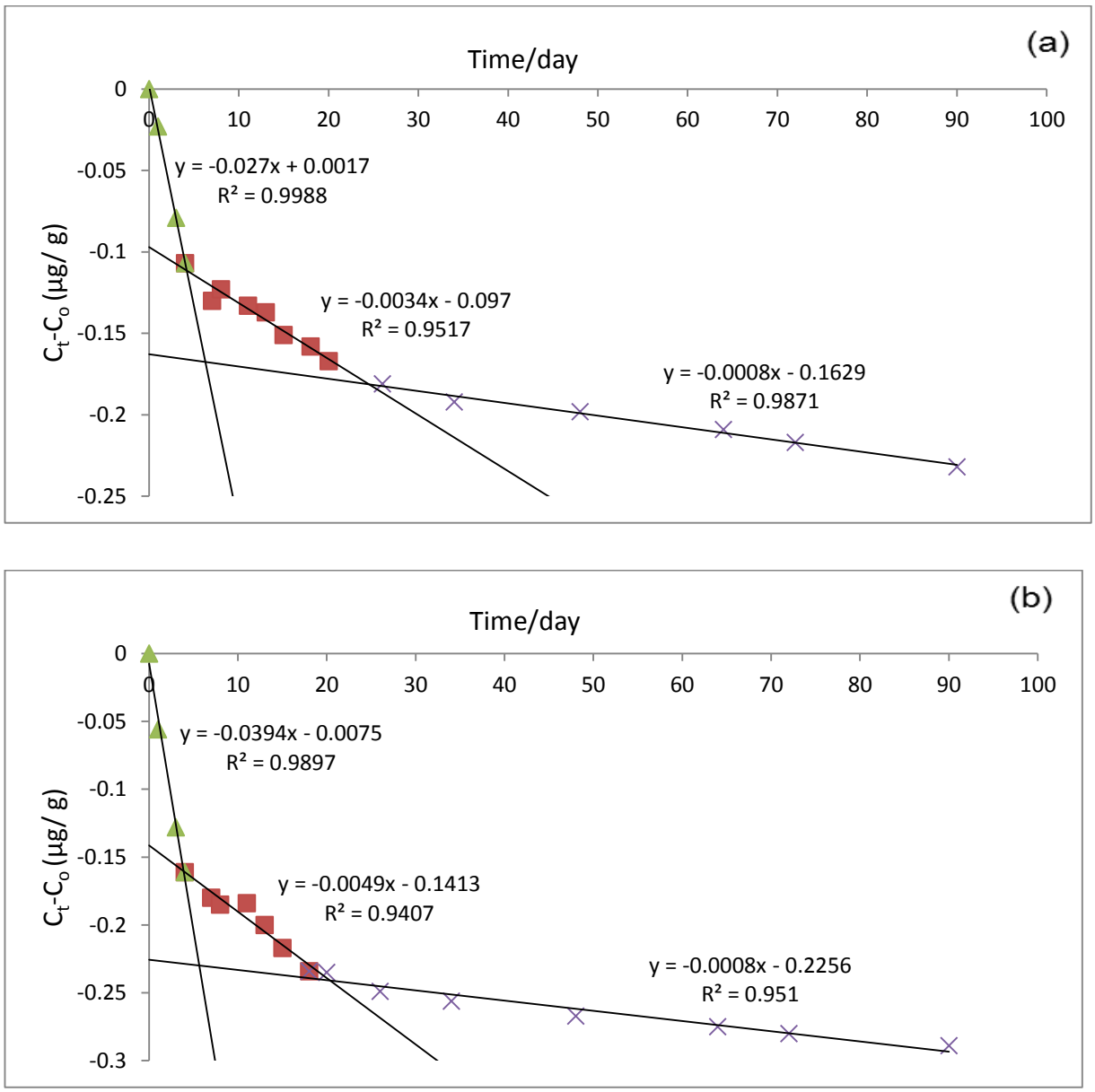
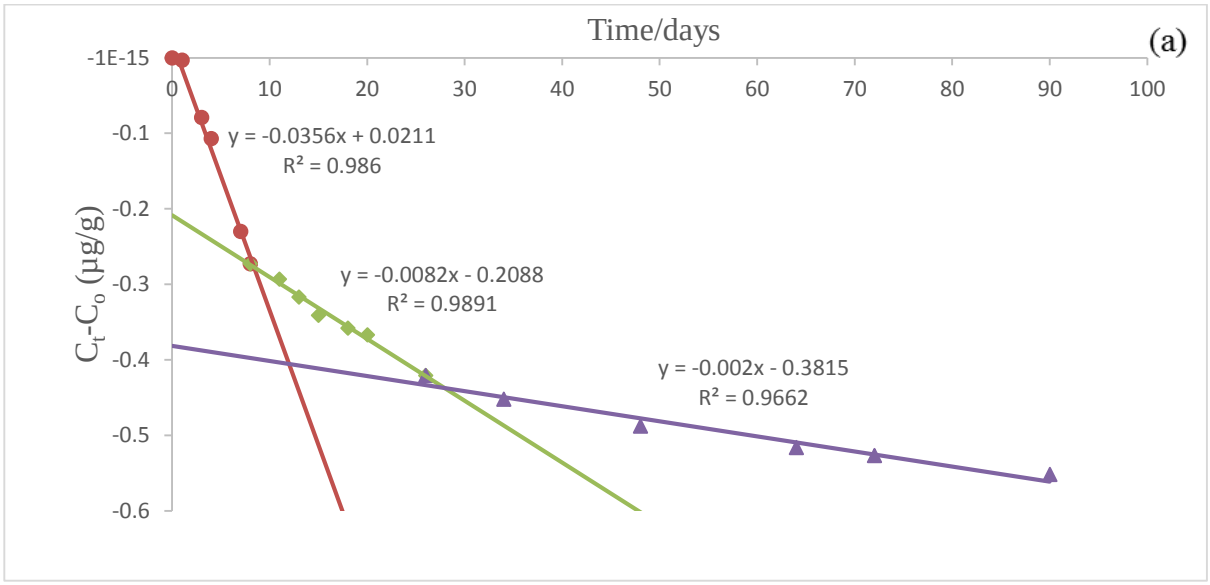


Fig 5.2.11 Chlortetracycline degradation rates in (a) river water experiment containing 2 mg mL^{-1}, and (b) river sediment experiment with 2 mg g^{-1} nitrates



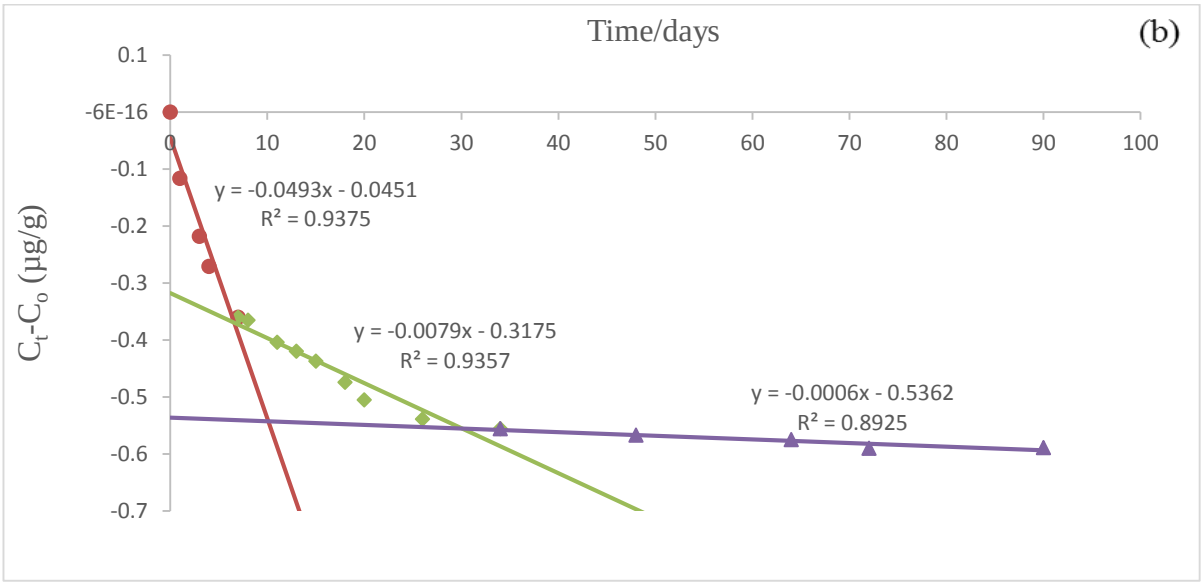


Fig 5.2.12 Tetracycline degradation rates in (a) river water experiment containing 2 mg mL^{-1}, and (b) river sediment experiment with 2 mg g^{-1} nitrates

Table 5.2.4 Rates of degradation ($\mu g g^{-1} day^{-1}$) in the microcosm experiments (with added nitrate)

Antimicrobial	Rate of degradation in water phase			Rate of degradation in sediment phase		
	Initial	Intermediate	Final	Initial	Intermediate	Final
OTC	5.72×10^{-2}	6.40×10^{-3}	1.20×10^{-3}	4.79×10^{-2}	5.70×10^{-3}	2×10^{-4}
DC	6.31×10^{-2}	5.30×10^{-3}		1.70×10^{-2}	8.00×10^{-4}	
CTC	2.70×10^{-2}	3.40×10^{-3}	8.00×10^{-4}	3.94×10^{-2}	4.90×10^{-3}	8.00×10^{-4}
TC	3.56×10^{-2}	8.20×10^{-3}	2.00×10^{-3}	4.93×10^{-2}	7.90×10^{-3}	6.00×10^{-4}
Speciation*	FIS(WP)	CPA(WP)	CPA(WP)	FIS(SPW)	CPA(SP)	CPA(SP)
Degradation mechanism	Hydrolysis Photolysis Microbial	Microbial	Microbial	Microbial	Microbial	Microbial

* FIS(WP) = free in solution (water phase), CPA(WP) = colloidal particle adsorbed (water phase), FIS(SPW) = free in solution (sediment pore water), CPA(SP) = colloidal particle adsorbed (sediment phase).

5.3 MATERIAL BALANCE CALCULATIONS

5.3.1 Oxytetracycline.

Material balance calculations (Table 5.3.1) reveal that of the 0.096 g of OTC that was placed in the distilled water experiment under dark conditions, 0.0165 g (17.2%) was adsorbed on to the walls of the container and 0.0795 g (82.8%) remained in the water. In the river water and sediment experiment (Table 5.3.2), 0.0336g (35.0%) of the total OTC charged into the

experiment was adsorbed onto the sediment, while 0.0402 g (41.9%) remained in the water phase. The remainder of 0.0222 g (23.1%) was adsorbed onto the walls of the container. Sorption of OTC by marine sediment, clay and organic matter was investigated previously by Pouliquin and Le Baris, (1996)

Table 5.3.1.Oxytetracycline material balance calculations: (Distilled water)

Experiment	Phase	Initial Conc. ($\mu g\ mL^{-1}$)	Initial mass ($g\ phase^{-1}$)	Final Conc. ($\mu g\ mL^{-1}$)	Final mass ($g\ phase^{-1}$) (% Loss)
CDW	Water	0.994	0.0795	0.802	0.0642
	Container		0.0165		0.0165
	Deg Loss				0.0153(15.4)
	Total		**0.0960**		**0.0960**
DWE	Water	0.993	0.0794	0.349	0.0279
	Container		0.0166		0.0166
	Deg Loss				0.0550(57.4)
	Total		**0.0960**		**0.0960**
DWN	Water	0.993	0.0795	0.196	0.0157
	Container		0.0165		0.0165
	Deg Loss				0.0634(66.0)
	Total		**0.0960**		**0.0960**

CDW = covered distilled water, DWE = distilled water exposed, DWN = distilled water exposed and spiked with nitrates,

Degradation losses for the 90 days period were 15.9 % (covered distilled water), 57.4% (distilled water exposed to natural light), 66.0% (distilled water exposed to light and spiked with 2 mg mL^{-1} nitrates) (Table 5.3.1), 71.8% (river water and sediment exposed to natural

light) and 73.8% river water and sediment spiked with 2 mg g^{-1} nitrates and exposed to natural light (Table 5.3.2).

Table 5.3.2. Oxytetracycline material balance calculations: (River water and sediment)

Experiment	Phase	Initial Conc. ($\mu g\ mL^{-1}$)	Initial mass ($g\ phase^{-1}$)	Final Conc. ($\mu g\ mL^{-1}$)	Final mass ($g\ phase^{-1}$) (% Loss)
RS&W	Water	0.503	0.0402	0.0332	0.00266
	Sediment	0.420	0.0336	0.0280	0.00224
	Container		0.05496		0.5496
	Deg Loss				0.03832(39.9)
	Total		**0.0960**		**0.0960**
RS&W&N	Water	0.503	0.0402	0.0222	0.00178
	Sediment	0.420	0.0336	0.0142	0.00114
	Container		0.05496		0.05496
	Deg Loss				0.03921(40.8)
	Total		**0.0960**		**0.0960**

RS&W = river sediment and water, RS&W&N = river sediment and water and spiked with nitrates

5.3.2 Doxycycline

Table 5.3.3shows that of the 0.080 g of DC that was charged into the covered distilled water experiment, 0.0004 g (0.5%) was adsorbed on to the walls of the container and 0.0797 g (99.6%) remained in the water phase. In the river water experiments (Table 5.3.4), 0.0005 g (0.63%) was adsorbed by the sediment, while 0.0618 g (77.3%) remained in the water phase and 0.0177 g (22.1%) was adsorbed onto the walls of the containers. The 90 days losses due

to degradation were 16.8% in the covered distilled water experiment, 75.0% in distilled water exposed to natural light experiment, 77.5% in distilled water experiment with added nitrates, 67.6% in river water and sediment experiment and 92.5% in river water and sediment with added nitrates.

Table 5.3.3: Doxycycline material balance calculations: (Distilled water)

Experiment	Phase	Initial Conc. ($\mu g\ mL^{-1}$)	Initial mass ($g\ phase^{-1}$)	Final Conc. ($\mu g\ mL^{-1}$)	Final mass ($g\ phase^{-1}$) (% Loss)
CDW	Water	0.996	0.0797	0.828	0.0662
	Container		0.0004		0.0004
	Deg Loss				0.0134(16.8)
	Total		0.0800		0.0800
DW	Water	0.998	0.0798	0.247	0.0198
	Container		0.0002		0.0002
	Deg Loss				0.0600(75.0)
	Total		0.0800		0.0800
DWN	Water	0.987	0.0790	0.212	0.0170
	Container		0.0010		0.0010
	Deg Loss				0.0620(77.5)
	Total		0.0800		0.0800

CDW = covered distilled water, DW = distilled water exposed, DWN = distilled water exposed and spiked with nitrates

Table 5.3.4: Doxycycline material balance calculations: (River water and sediment)

Experiment	Phase	Initial Conc. (µg mL^{-1})	Initial mass (g $phase^{-1}$)	Final Conc. (µg mL^{-1})	Final mass (g $phase^{-1}$) (% Loss)
RW & S	Water	0.772	0.0618	0.102	0.0082
	Sediment	0.225	0.0005	0.013	0.00003
	Container		0.0177		0.0177
	Deg Loss				0.0541(67.6)
	Total		**0.0800**		**0.0800**
RW&S&N	Water	0.773	0.0618	0.011	0.0009
	Sediment	0.177	0.0142	0.010	0.0009
	Container		0.0040		0.0042
	Deg Loss				0.0740(92.5)
	Total		**0.0800**		**0.0800**

RW & S = river water and sediment, RW&S&N = river water and sediment spiked with nitrates.

5.3.3 Chlortetracycline

Table 5.3.5below show that of the 0.08g of CTC spiked in the covered distilled water control experiments, 0.0004 g (0.5%) was adsorbed on to the walls of the container while 0.0797 (99.5%) remained in solution. In the microcosm experiment (see Table 5.3.6), 0.0554 (69.3%) remained in the water phase while 0.0009 (1.1%) was adsorbed by the sediment and 0.0237 (29.6%) was adsorbed on to the walls of container. The 90 day degradation losses were 10.4% in covered distilled water experiment, 34.5% in distilled water exposed to natural light experiment, 48.3% in distilled water exposed to natural light and spiked with 2 mg mL^{-1} nitrates experiment, 52.8% in river water and sediment experiment and 55.5% in river water and sediment spiked with 2 mg mL^{-1} nitrates.

Table 5.3.5Chlortetracycline material balance calculations (distilled water)

Experiment	Phase	Initial Conc. ($\mu g\ mL^{-1}$)	Initial mass ($g\ phase^{-1}$)	Final Conc. ($\mu g\ mL^{-1}$)	Final mass ($g\ phase^{-1}$) (% Loss)
CDW	Water	0.9960	0.0797	0.8930	0.0714
	Container		0.0004		0.0004
	Deg. Loss				0.0083 (10.4)
	Total		**0.0800**		**0.0800**
DW	Water	0.9780	0.0774	0.6230	0.0498
	Container		0.0026		0.0026
	Deg. Loss				0.0276 (34.5)
	Total		**0.0800**		**0.0800**
DWN	Water	0.9790	0.0783	0.4960	0.0397
	Container		0.0017		0.0017
	Deg. Loss				0.0386 (48.3)
	Total		**0.0800**		**0.0800**

CDW = covered distilled water, DW = distilled water, DWN = distilled water spiked with nitrates

Table 5.3.6 Chlortetracycline material balance calculations (River water and sediment)

RW&S	Water	0.6920	0.0554	0.1820	0.0146
	Sediment	0.4350	0.0009	0.0930	0.0002
	Container		0.0237		0.0237
	Deg. Loss				0.0423 (52.8)
	Total		**0.0800**		**0.0800**
RW&S&N	Water	0.6880	0.0550	0.1410	0.0113
	Sediment	0.3430	0.0007	0.0540	0.0001
	Container		0.0243		0.0243
	Deg. Loss				0.0444 (55.5)
	Total		**0.0800**		**0.0800**

RW & S = river water and sediment, RW&S&N = river water and sediment spiked with nitrate

5.3.4 Tetracycline

Table 5.3.7 shows that of the 0.08 g of TC standard spiked into the covered distilled water 0.0010 g (1.3%) was adsorbed on to the walls of the container and 0.0790 g (98.8%) remained in the aqueous phase. In the microcosm experiment see Table 5.3.8, 0.0637 g (79.6%) remained in the water phase while 0.0014 g (1.75%) partitioned into the sediment phase. The remainder 0.0149 g (18.6%) was adsorbed onto the walls of the container. The 90 day loses were 13.0% in covered distilled water experiment, 29.3% in distilled water exposed to natural light experiment, 42.6% in distilled water exposed to natural light and spiked with nitrates experiment, 59.9% in river water and sediment exposed to natural light experiment

and 66.3% in river water and sediment exposed to natural light and spiked with nitrates experiment see Table 5.3.7 and 5.3.8.

Table 5.3.7Tetracycline material balance calculation (distilled water)

Experiment	Phase	Initial Conc. ($\mu g\ mL^{-1}$)	Initial mass ($g\ phase^{-1}$)	Final Conc. ($\mu g\ mL^{-1}$)	Final mass ($g\ phase^{-1}$) (% Loss)
CDW	Water	0.9880	0.0790	0.8581	0.0686
	Container		0.0010		0.0010
	Deg. Loss				0.0104 (13.0)
	Total		**0.0800**		**0.0800**
DW	Water	0.9850	0.0788	0.6930	0.0554
	Container		0.0012		0.0012
	Deg. Loss				0.0234 (29.3)
	Total		**0.0800**		**0.0800**
DWN	Water	0.9810	0.0785	0.5560	0.0444
	Container		0.0015		0.0015
	Deg. Loss				0.0342 (42.6)
	Total		**0.0800**		**0.0800**

CDW = covered distilled water, DW = distilled water exposed, DWN = distilled water exposed spiked with nitrates,

Table 5.3.8 Tetracycline material balance calculation (River water and sediment)

Experiment	Phase	Initial Conc. ($\mu g\ mL^{-1}$)	Initial mass ($g\ phase^{-1}$)	Final Conc. ($\mu g\ mL^{-1}$)	Final mass ($g\ phase^{-1}$) (% Loss)
RW&S	Water	0.7960	0.0637	0.2120	0.0170
	Sediment	0.6350	0.0014	0.0830	0.0002
	Container		0.0149		0.0149
	Deg. Loss				0.0479 (59.9)
	Total		**0.0800**		**0.0800**
RW&S&N	Water	0.7880	0.0630	0.1410	0.0113
	Sediment	0.6230	0.0014	0.0310	0.0001
	Container		0.0156		0.0156
	Deg. Loss				0.0444 (66.3)
	Total		**0.0800**		**0.0800**

RW & S = river water and sediment, RW&S&N = river water and sediment spiked with nitrates.

5.4 DEGRADATION KINETICS

Figs 5.2.1-5.2.12 reveals that for all the cases investigated, linear rates of degradation were observed. In the covered distilled water control experiments biphasic linear rates were observed, whereas in distilled water control experiments under natural light conditions monophasic linear rates were obtained for OTC. In the river water and sediment experiments triphasic linear rates were obtained except for DC where biphasic linear rates were obtained. Table 5.4.1 shows the proposed mechanisms by which degradation takes place.

Table 5.4.1 Suggested mechanisms for the degradation of the speciation forms of antibiotics inferred from the results of OTC, DC, CTC and TC

Experiment	Rate	Speciation form	Mechanism
CDW	Initial	Free, dissolved	Hydrolysis
	Final	Free, dissolved	Microbial
EDW & EDWN	Initial	Free, dissolved	Photolysis
RWS-WP	Initial	Free, dissolved	Microbial/ photolysis
	Middle	Adsorbed, colloidal particle 1	Microbial
	Final	Adsorbed, colloidal particle 2	Microbial
RW-SP	Initial	Free, dissolved, pore water	Microbial
	Middle	Adsorbed, sediment/colloidal particle 1	Microbial
	Final	Adsorbed, sediment/colloidal particle 2	Microbial

CDW=Covered distilled water, EDW exposed distilled water, EDWN= Exposed distilled water nitrates, RWS-WP = River water-water phase, RW-SP = River water- Sediment phase.

Linear rates of degradation obtained in the present study are consistent with zero order kinetics (Zaranyika et al., 2010; Daniels and Alberty, 1961) or steady state kinetics in which the reaction occurs through the formation of an intermediate transition state complex (Atkins and Paula, 2006). Considering the present experiments under study, it is possible to obtain steady state kinetics considering, (a) bimolecular chemical degradation mechanisms in the presence of an excess reagent, e.g. hydrolysis in the presence of excess water molecules, (b) photochemical degradation via an excited state transition state, and (c) microbial or

enzymatic degradation whereby the constant rate of degradation corresponds to the plateau in the Michaelis-Menten curve.

5.4.1 Degradation kinetics in distilled water under dark conditions

Hydrolysis is represented by steps shown in Table 5.4.2.

Table 5.4.2 Steps in hydrolysis of an antibiotic (A)

Step	Reaction	Rate constant	Process
1	$A + OH_2 \longrightarrow A.OH_2$	k_h	Hydration
2	$A.OH_2 \longrightarrow A + OH_2$	k_{-h}	Dehydration
3	$A.OH_2 \longrightarrow P$	k_p	Hydrolysis (P = products)

A = antibiotic either OTC, DC, CTC or TC

Using steps shown in Table 5.4.2, it can be shown that

$\frac{d[A.OH_2]}{dt} = k_h[A][OH_2] - k_{-h}[A.OH_2] - k_p[A.OH_2] \approx 0$ by applying steady state approximation (5.4.1)

Thus, $[A.OH_2] = \frac{k_h[A][OH_2]}{k_{-h} + k_p}$ (5.4.2)

Using step 3, it can also be shown that

$\frac{dp}{dt} = k_p[A.OH_2]$ (5.4.3)

Substituting for $[A.OH_2]$ into equation 5.4.3 yields equation 5.4.4

$$\frac{dp}{dt} = \left(\frac{k_p k_h}{k_{-h} + k_p} \right) [A][OH_2] \qquad (5.4.4)$$

Considering that $[A]_t = [A]_o - \Delta[A]$ and excess water molecules, equation 5.4.4 becomes

$$\frac{dP}{dt} = \left(\frac{k_p k_h}{k_{-h} + k_p} \right) [A]_t \qquad (5.4.5)$$

where $[A]_t$ = $[A]_o$ – Δ[A] = concentration of an antibiotic at any instant in days, $[A]_o$ is the initial concentration and Δ[A] is the change in [A] per unit time, Δt. If $[A]_o$>>Δ[A], Eqn 5.4.5 reduces to equation 5.4.6

$$\frac{dP}{dt} = \left(\frac{k_p k_h}{k_{-h} + k_p} \right) [A]_o = k_h'[A]_o = k_h'' \qquad (5.4.6)$$

where k_h'' is the zero order rate constant for the hydrolytic degradation under the conditions of covered distilled water control experiment, consistent with Figs. 5.2.1(a) to 5.2.4(a), i.e., k_h'' = 2 x 10^{-6} , 5 x 10^{-5} , 3 x 10^{-4} and 1 x 10^{-4} μgg^{-1}day^{-1}, respectively for OTC, DC, CTC and TC. Hydrolytic degradation is a process that is activation controlled thus in the absence of an external source of energy the rate of degradation becomes very slow, hence these small constant linear rates of hydrolysis obtained for the initial rate of degradation in the distilled water control experiments under dark conditions.

k_h' is the apparent (or pseudo) first order rate constant for the hydrolytic degradation. From Table 5.3.1 to 5.3.4 $[A]_o$ (distilled water covered) = 0.0795 (OTC), 0.0797 (DC), 0.0797 (CTC) and 0.0790 (TC) g spiked in 80 L and Δ[A] = 2 x 10^{-6} (OTC), 5 x 10^{-5} (DC), 3 x 10^{-4}, 1 x 10^{-4}(TC) μgg^{-1}day^{-1}. Therefore

$$k_h' = \frac{k_h''}{[OTC]_o} = \left(\frac{1.9x10^{-3}}{0.0797/80}\right)\left(\frac{\mu g g^{-1} day^{-1}}{gL^{-1}}\right) = 2.3x10^{-11} s^{-1} (2.0x10^{-6} day^{-1})$$

$$k_h' = \frac{k_h''}{[DC]_o} = \left(\frac{1.9x10^{-3}}{0.0797/80}\right)\left(\frac{\mu g g^{-1} day^{-1}}{gL^{-1}}\right) = 2.2x10^{-8} s^{-1} (1.9x10^{-3} day^{-1})$$

$$k_h' = \frac{k_h''}{[CTC]_o} = \left(\frac{1.9x10^{-3}}{0.0797/80}\right)\left(\frac{\mu g g^{-1} day^{-1}}{gL^{-1}}\right) = 3.0x10^{-9} s^{-1} (2.6x10^{-3} day^{-1})$$

$$k_h' = \frac{k_h''}{[TC]_o} = \left(\frac{1.9x10^{-3}}{0.0797/80}\right)\left(\frac{\mu g g^{-1} day^{-1}}{gL^{-1}}\right) = 1.0x10^{-8} s^{-1} (8.6x10^{-4} day^{-1}) \quad (5.4.7)$$

Hydrolysis of tetracycline antibiotics in aqueous solution was studied previously by Xuan et al., (2010), Pouliquen et al., (2007), Doi and Stoskopf, (2000) and Loftin et al., (2008). Xuan et al. reported rate constants ranging from 0.094 ± 0.001 to 0.106 ± 0.003 day^{-1} and a half-life of 6.5 days at 25ºC. The rate of hydrolysis was found to increase with an increase in temperature and pH, whereas presence of Ca^{2+} ions was found to reduce it. From Fig. 5.2.1(a) to 5.2.4 (a) it can be observed that the rate constants reported by Xuan et al. are much too high as compared to the rate of hydrolysis observed in the current investigation. The rate constants reported by Xuan et al. for hydrolytic degradation are closer to the apparent rate constants observed in the current study for microbial degradation in the river water and sediment experiment (Fig. 5.2.5 to 5.3.8). Microbial degradation was also observed after 7-15 days in the hydrolysis experiments, Fig. 5.2.1 (a) to 5.2.4 (a) as a result of microbial contamination from the atmosphere.

5.4.2 Degradation kinetics in distilled water exposed to natural light.

It has been observed that photochemical reactions rates are given by the product of the quantum yield, Φ, and the number of photons absorbed, I_{abs}. The Einstein-Stark law for photochemical equivalence is used to define the quantum yield, i.e.

Φ = (Number of molecules of x formed or decomposed, N_x)/(number of photons absorbed)

Or

$$\Phi = \frac{N_x}{I_{abs}} = \frac{N_x s^{-1}}{(I_{abs})s^{-1}} = \frac{dN_x/dt}{(I_{abs})s^{-1}} \quad (5.4.8)$$

Hence

$$\Phi(I_{abs})s^{-1} = dN_x/dt \quad (5.4.9)$$

Thus the rate of photochemical reactions can also be obtained by determining the rate of formation of photochemical products or loss of the parent substance (Castellan, 1971) The photolysis of an antibiotic can be represented by the steps shown in Table 5.4.3 (Zaranyika and Nyoni, 2013), where k_ϕ is the rate constant for photochemical excitation, k_q is the rate constant for collisional quenching, k_f is the fluorescence rate constant, and k_p is the rate constant for degradation of the excited molecule to give the product P.

Table 5.4.3. Photolysis of an antibiotic A

Step	Reaction	Rate constant	Process
1	$A + h\nu \rightarrow A^*$	k_ϕ	Light absorption
2	$A^* \rightarrow A + h\upsilon$	k_f	Fluorescence
3	$A^* + Q \rightarrow A + Q^*$	k_q	Energy transfer (Q = quencher).
4	$A^* \rightarrow P$	k_P	Photolysis (P = products).

A = antibiotic either OTC, DC, CTC or TC

Using steps shown in Table 5.4.3 for photochemical degradation of antibiotic A in the exposed distilled water experiments, it can be shown that;

$\frac{d[A^*]}{dt} = k_\phi[A] - k_f[A^*] - k_q[A^*][Q] - k_p[A^*] \approx 0$ by invoking steady state approximation

(5.4.10)

Thus $[A^*] = \frac{k_q[A]}{k_f + k_q[Q] + k_p}$ (5.4.11)

Using steps shown in Table 5.4.3 it can also be shown that

$\frac{dp}{dt} = k_p[A^*]$ (5.4.12)

Substituting for $[A^*]$ into equation 5.4.12 gives equation 5.4.13

$\frac{dp}{dt} = \left(\frac{k_p k_\phi}{k_f + k_q[Q] + k_p}\right)[A]_t$ (5.4.13)

Where $[A]_t = [A]_0 - \Delta[A]$, the concentration of antibiotic at any time in days, $[A]_0$ is the initial concentration while $\Delta[A]$ is the change in concentration of the antibiotic at any given time t. If $[A]_0 >> \Delta[A]$ equation 5.4.13 reduces to equation 5.4.14

$\frac{dp}{dt} = \left(\frac{k_p k_\phi}{k_f + k_q[Q] + k_p}\right)[A]_o = \mathrm{k}'_\phi[A]_o = \mathrm{k}''_\phi$ (5.4.14)

where k'_ϕ is the apparent first order rate constant for the photochemical degradation of DC in aqueous solution exposed to sunlight, and k''_ϕ is the zero order rate constant for the

photochemical degradation of antibiotic A in aqueous solution. Considering that both hydrolysis and photolysis occur at the same time in the distilled water under sunlight conditions, the overall degradation rate of the experiment is obtained by summing up eqns. 5.4.6 and 5.4.14,therefore;

$$\frac{dp}{dt}=\left(\frac{k_p k_\phi}{k_f+k_q[Q]+k_p}\right)[OTC]_o \; + \; \left(\frac{k_p k_h}{k_{-h}+k_p}\right)[OTC]_o = k_h'' + k_\phi'' = k_{\phi h}'' = 6.9x10^{-3}\,\mu g g^{-1} day^{-1}$$

$$\frac{dp}{dt}=\left(\frac{k_p k_\phi}{k_f+k_q[Q]+k_p}\right)[DC]_o \; + \; \left(\frac{k_p k_h}{k_{-h}+k_p}\right)[DC]_o = k_h'' + k_\phi'' = k_{\phi h}'' = 2.9x10^{-3}\,\mu g g^{-1} day^{-1}$$

$$\frac{dp}{dt}=\left(\frac{k_p k_\phi}{k_f+k_q[Q]+k_p}\right)[CTC]_o \; + \; \left(\frac{k_p k_h}{k_{-h}+k_p}\right)[CTC]_o = k_h'' + k_\phi'' = k_{\phi h}'' = 1.5x10^{-3}\,\mu g g^{-1} day^{-1}$$

$$\frac{dp}{dt}=\left(\frac{k_p k_\phi}{k_f+k_q[Q]+k_p}\right)[TC]_o \; + \; \left(\frac{k_p k_h}{k_{-h}+k_p}\right)[TC]_o = k_h'' + k_\phi'' = k_{\phi h}'' = 6.0x10^{-3}\,\mu g g^{-1} day^{-1} \qquad (5.4.15),$$

where $k_{\phi h}''$ is the zero order rate constant for the degradation of the antibiotic in aqueous solution exposed to sunlight, consistent with the constant linear rate of degradation obtained for the initial slow rate of degradation in the distilled water experiment under natural light.

Therefore,

$k_\phi'' = (6.9-0.002)10^{-3} = 6.9x10^{-3}\,\mu g g^{-1} day^{-1}$ (OTC).

$k_\phi'' = (2.9-0.5)10^{-3} = 2.4x10^{-3}\,\mu g g^{-1} day^{-1}$ (DC).

$k_\phi'' = (1.5-0.3)10^{-3} = 1.2x10^{-3}\,\mu g g^{-1} day^{-1}$ (CTC).

$k_\phi'' = (0.6-0.1)10^{-3} = 0.5x10^{-3}\,\mu g g^{-1} day^{-1}$ (TC).

From Table 5.3.1 to 5.3.4 $[A]_o$ (distilled water exposed) = 0.0794 (OTC), 0.0798 (DC), 0.0774 (CTC) and 0.0788 (TC) g spiked in 80 L and $k_\phi^{"}$ = 6.9 x 10^{-3} (OTC), 2.4 x 10^{-3} (DC), 1.2 x 10^{-3} (CTC), 5 x 10^{-4}(TC) $\mu g g^{-1} day^{-1}$. Therefore

$$k'_\phi = \frac{k''_\phi}{[OTC]_o} = \left(\frac{6.9x10^{-3}}{0.0794/80}\right)\left(\frac{\mu g g^{-1} day^{-1}}{gL^{-1}}\right) = 8.0x10^{-8}\, s^{-1}(6.9x10^{-3}\, day^{-1})$$

$$k'_\phi = \frac{k''_\phi}{[DC]_o} = \left(\frac{2.4x10^{-3}}{0.0798/80}\right)\left(\frac{\mu g g^{-1} day^{-1}}{gL^{-1}}\right) = 2.7x10^{-8}\, s^{-1}(2.3x10^{-3}\, day^{-1})$$

$$k'_\phi = \frac{k''_\phi}{[CTC]_o} = \left(\frac{1.2x10^{-3}}{0.0774/80}\right)\left(\frac{\mu g g^{-1} day^{-1}}{gL^{-1}}\right) = 1.4x10^{-8}\, s^{-1}(1.2x10^{-3}\, day^{-1})$$

$$k'_\phi = \frac{k''_\phi}{[TC]_o} = \left(\frac{5.0x10^{-4}}{0.0788/80}\right)\left(\frac{\mu g g^{-1} day^{-1}}{gL^{-1}}\right) = 5.0x10^{-9}\, s^{-1}(4.3x10^{-4}\, day^{-1}) \qquad (5.4.16)$$

Photolysis of TCs in aqueous solution has been studied previously by several workers (Doi and Stoskopf, 2000; Xuan et al., 2010; Pouliquen et al., 2007; Hammad Khan et al., 2014; Loftin et al., 2008). Xuan et al., (2010) found photolysis to follow first order kinetics. Photolysis rate constant of 3.61 ± 0.06 day^{-1} was obtained by Xuan et al., (2010). In their study presence of Ca^{2+} ions was observed to enhance the rate of photolytic degradation. The rate constant of 3.61 ± 0.06 $day^{-1}$for photolytic degradation that was reported by Xuan et al. is much higher than the apparent rate constants obtained in the present experiments. In Xuan et al. study the rate constants reported were calculated by assuming first order kinetics. This is probably due to the fact that when the TCs are exposed to ultraviolet radiation in distilled water the rate of photodegradation maybe high such that the assumption made above that $[A]_o >> \Delta[A]$ will no longer holds, and photolysis converts to simple first order kinetics as illustrated in eqn 5.4.14.

5.4.3 Degradation kinetics in the river water and sediment experiments

The initial fast degradation is due to combination of hydrolysis, photolysis, and microbial degradation of the unbound TC antibiotics in solution in the water phase. Using the value obtained for the combination of photolysis and hydrolysis (6.9×10^{-3} (OTC); 2.9×10^{-3} (DC); 1.5×10^{-3}(CTC); 6.0×10^{-4} (TC) μg/g/day), Fig 5.2.1 (b) to 5.2.4 (b) microbial degradation rate is computed as follows,

$(3.07\text{-}0.69)10^{-2}$ $\mu g g^{-1} day^{-1} = 2.38 \times 10^{-2}$ $\mu g g^{-1} day^{-1}$ (OTC).

$(2.30\text{-}0.29)10^{-2}$ $\mu g g^{-1} day^{-1} = 2.01 \times 10^{-2}$ $\mu g g^{-1} day^{-1}$ (DC).

$(2.70\text{-}0.15)10^{-2}$ $\mu g g^{-1} day^{-1} = 2.55 \times 10^{-2}$ $\mu g g^{-1} day^{-1}$ (CTC).

$(1.35\text{-}0.06)10^{-2}$ $\mu g g^{-1} day^{-1} = 1.29 \times 10^{-2}$ $\mu g g^{-1} day^{-1}$ (TC).

The model shown in Table 5.4.4 is proposed for the degradation of TC antibiotics in the river water and sediment. The proposed model involves Step1a (binding of the antibiotic molecule by microbial organism), Steps1b and 1c (adsorption by colloidal particles types 1 and 2 in the water phase of the experiment), and Steps 1d and 1e (adsorption by colloidal and sediment particles in the sediment phase). Steps 1a to 1e occur simultaneously as the antimicrobial is introduced into the experimental microcosm. Step 2 (binding and metabolism by enzymes) takes place inside the microorganism, following binding of the antimicrobial by the microorganism.

Table 5.4.4: Degradation of an antibiotic in the aquatic environment: A proposed kinetic model

Step	Reaction (Water Phase)	R/ const	Reaction (Sediment phase)	R/const
1(a)	$A + M \rightarrow A_B$	k_1	$A + M \rightarrow A_B$	k_1
	$A_B \rightarrow A + M$	k_{-1}	$A_B \rightarrow A + M$	k_{-1}
2	$A_B + E \rightarrow AE$	k_2	$A_B + E \rightarrow AE$	k_2
	$AE \rightarrow A_B + E$	k_{-2}	$AE \rightarrow A_B + E$	k_{-2}
	$AE \rightarrow P + E$	k_3	$AE \rightarrow P + E$	k_3
1(b)	$A + nC_1 \rightarrow A(C_1)_n$	k_4	$A + nC_1 \rightarrow A(C_1)_n$	k_5
	$A(C_1)_n \rightarrow A + nC_1$	k_{-4}	$A(C_1)_n \rightarrow A + nC_1$	k_{-5}
1(c)	$A + yC_2 \rightarrow A(C_2)_y$	k_5	$A + yC_2 \rightarrow A(C_2)_y$	k_5
	$A(C_2)_y \rightarrow A + yC_2$	k_{-5}	$A(C_2)_y \rightarrow A + yC_2$	k_{-5}
1(d)			$A + qS_1 \rightarrow A(S_1)_q$	K_6
			$A(S_1)_q \rightarrow A + qS_1$	k_{-6}
1(e)			$A + zS_2 \rightarrow A(S_2)_z$	k_7
			$A(S_2)_z \rightarrow A + zS_2$	k_{-7}

A = antibiotic, M = microorganism, P = products, subscript B = microbial-bound, C_1 = colloidal particle type 1 and C_2 colloidal particle 2, AC = antibiotic-colloidal particle complex, S = sediment particle, AS = antibiotic-sediment particle complex R/ const. = rate constant

5.4.3 (a) Fast microbial degradation in the water and sediment phase (free antibiotic)

Using the model in Table 5.4.4 it can be shown that;

$$\frac{d[AE]}{dt} = k_2[A_b][E] - k_{-2}[AE] - k_3[AE] \approx 0 \text{ by applying steady state approximation} \quad (5.4.17)$$

Therefore,

$$[AE] = \left(\frac{k_2}{k_{-2} + k_3}\right)[A_b][E] \quad (5.4.18)$$

From step 2 in Table 5.4.4, it can also be shown that;

$$\frac{dp}{dt} = k_3[AE] \quad (5.4.19)$$

Substituting for $[AE]$ into equation 5.4.19 gives equation 5.4.20

$$\frac{dp}{dt} = \left(\frac{k_2 k_3}{k_{-2} + k_3}\right)[A_b][E] \quad (5.4.20)$$

Inside the micro-organism $[E] >> [A_b]$ therefore $[E]$approaches unit and equation 5.4.20 reduces to equation 5.4.21

$$\frac{dp}{dt} = \left(\frac{k_2 k_3}{k_{-2} + k_3}\right)[A_b] \quad (5.4.21)$$

It can also be shown that

$\frac{d[A_B]}{dt} = k_1[A][B] - k_{-1}[A_B] - k_2[A_B][E] \approx 0$ by applying steady state approximation

(5.4.22)

thus

$$A_B = \left(\frac{k_1}{k_{-1} + k_2}\right)[A][M] \quad (5.4.23)$$

Substituting for A_B into equation 5.4.21 results in equation 5.4.24

$$\frac{dp}{dt} = \left(\frac{k_2 k_3}{k_{-2} + k_3}\right)\left(\frac{k_1}{k_{-1} + k_2}\right)[A]_w[M]_w$$

And

$$\frac{dp}{dt} = \left(\frac{k_2 k_3}{k_{-2} + k_3}\right)\left(\frac{k_1}{k_{-1} + k_2}\right)[A]_s[M]_s \quad (5.4.24)$$

Where the subscripts w and s denote concentrations in water and sediment phase respectively.

Assuming $[M]_w$ and $[M]_s$ to be constant and $[A] >> [M]$ in the environment the concentration of the antibiotics therefore approaches unit, thus equation 5.4.24 becomes

$$\frac{dp}{dt} = \left(\frac{k_2 k_3}{k_{-2} + k_3}\right)\left(\frac{k_1}{k_{-1} + k_2}\right)[M]_w = k'_{E(w)}$$

$$\frac{dp}{dt} = \left(\frac{k_2 k_3}{k_{-2} + k_3}\right)\left(\frac{k_1}{k_{-1} + k_2}\right)[M]_s = k'_{E(s)} \quad (5.4.25)$$

Therefore

$$\frac{dp}{dt} = \left(\frac{k_2 k_3}{k_{-2} + k_3}\right)\left(\frac{k_1}{k_{-1} + k_2}\right)[M]_w = k'_{E(w)} = 2.38x10^{-2}\ \mu gg^{-1}day^{-1}\ (OTC)$$

$$\frac{dp}{dt} = \left(\frac{k_2 k_3}{k_{-2} + k_3}\right)\left(\frac{k_1}{k_{-1} + k_2}\right)[M]_w = k'_{E(w)} = 2.01x10^{-2}\ \mu gg^{-1}day^{-1}\ (DC)$$

$$\frac{dp}{dt} = \left(\frac{k_2 k_3}{k_{-2} + k_3}\right)\left(\frac{k_1}{k_{-1} + k_2}\right)[M]_w = k'_{E(w)} = 2.55x10^{-2}\ \mu gg^{-1}day^{-1}\ (CTC)$$

$$\frac{dp}{dt} = \left(\frac{k_2 k_3}{k_{-2} + k_3}\right)\left(\frac{k_1}{k_{-1} + k_2}\right)[M]_w = k'_{E(w)} = 1.29x10^{-2}\ \mu gg^{-1}day^{-1}\ (TC) \qquad (5.4.26)$$

$$\frac{dp}{dt} = \left(\frac{k_2 k_3}{k_{-2} + k_3}\right)\left(\frac{k_1}{k_{-1} + k_2}\right)[M]_s = k'_{E(s)} = 4.79x10^{-2}\ \mu gg^{-1}day^{-1}\ (OTC)$$

$$\frac{dp}{dt} = \left(\frac{k_2 k_3}{k_{-2} + k_3}\right)\left(\frac{k_1}{k_{-1} + k_2}\right)[M]_s = k'_{E(s)} = 7.90x10^{-3}\ \mu gg^{-1}day^{-1}\ (DC)$$

$$\frac{dp}{dt} = \left(\frac{k_2 k_3}{k_{-2} + k_3}\right)\left(\frac{k_1}{k_{-1} + k_2}\right)[M]_s = k'_{E(s)} = 3.40x10^{-2}\ \mu gg^{-1}day^{-1}\ (CTC)$$

$$\frac{dp}{dt} = \left(\frac{k_2 k_3}{k_{-2} + k_3}\right)\left(\frac{k_1}{k_{-1} + k_2}\right)[M]_s = k'_{E(s)} = 2.84x10^{-2}\ \mu gg^{-1}day^{-1}\ (TC) \qquad (5.4.27)$$

where $k'_{E(w)}$ and $k'_{E(s)}$ are the apparent zero-order rate constants for microbial degradation of TC antibiotics in the water phase and sediment pore water respectively corresponding to the observed linear rates of degradation. It can be seen from equation 5.4.27 that $k'_{E(w)}$ and $k'_{E(s)}$ are functions of the antibiotic-microorganism binding equilibrium constant, k_1/k_{-1}. Thus the

magnitude of k_1/k_{-1} will depend on the structure and properties of the antibiotic and microorganism type.

5.4.3 (b) Slow microbial degradation in the water phase (adsorbed antibiotic)

Using steps 1(b) and 1(c) shown in Table 5.4.4 it can be shown that;

$$\frac{d[A(C_1)_n]}{dt} = k_4[A][C_1)]^n - k_{-4}[A(C_1)_n] \approx 0$$

$$\frac{d\lfloor A(C_2)_y \rfloor}{dt} = k_5[A][C_2)]^y - k_{-5}[A(C_2)_y] \approx 0 \text{ by applying steady state approximation} \quad (5.4.28)$$

Thus,

$$[A] = \frac{k_{-4}[A(C_1)_n]}{k_4[C_1]^n}$$

$$[A] = \frac{k_{-5}\lfloor A(C_2)_y \rfloor}{k_5[C_2]^y} \quad (5.4.29)$$

Substituting for $[A]$ into equation 5.4.24 gives equation 5.4.30

$$\frac{dp}{dt} = \left(\frac{k_2k_3}{k_{-2}+k_3}\right)\left(\frac{k_1}{k_{-1}+k_2}\right)\left(\frac{k_{-4}}{k_4}\right)\frac{[A(C_1)_n]_w[M]_w}{[C_1]_w^{\,n}}$$

$$\frac{dp}{dt} = \left(\frac{k_2k_3}{k_{-2}+k_3}\right)\left(\frac{k_1}{k_{-1}+k_2}\right)\left(\frac{k_{-5}}{k_5}\right)\frac{\lfloor A(C_2)_y \rfloor_w[M]_w}{[C_2]_w^{\,y}} \quad (5.4.30)$$

When [C] is in large excess of [A] thus the concentration of [C] and [AC] = 1 and assuming that [M] is constant equation 5.4.30 reduces to equation 5.5.31 and 5.5.32.

$$\frac{dp}{dt} = \left(\frac{k_2k_3}{k_{-2}+k_3}\right)\left(\frac{k_1}{k_{-1}+k_2}\right)\left(\frac{k_{-4}}{k_4}\right)[M]_w = k'_{c_1(w)} = 6.80x10^{-3}\,\mu\text{gg}^{-1}\text{day}^{-1}\,(OTC)$$

$$\frac{dp}{dt} = \left(\frac{k_2k_3}{k_{-2}+k_3}\right)\left(\frac{k_1}{k_{-1}+k_2}\right)\left(\frac{k_{-4}}{k_4}\right)[M]_w = k'_{c_1(w)} = 4.50x10^{-3}\,\mu\text{gg}^{-1}\text{day}^{-1}\,(DC)$$

$$\frac{dp}{dt}=\left(\frac{k_2k_3}{k_{-2}+k_3}\right)\left(\frac{k_1}{k_{-1}+k_2}\right)\left(\frac{k_{-4}}{k_4}\right)[M]_w=k'_{c_1(w)}=7.00x10^{-3}\,\mu gg^{-1}day^{-1}\,(CTC)$$

$$\frac{dp}{dt}=\left(\frac{k_2k_3}{k_{-2}+k_3}\right)\left(\frac{k_1}{k_{-1}+k_2}\right)\left(\frac{k_{-4}}{k_4}\right)[M]_w=k'_{c_1(w)}=5.3x10^{-3}\,\mu gg^{-1}day^{-1}\,(TC) \qquad (5.4.31)$$

$$\frac{dp}{dt}=\left(\frac{k_2k_3}{k_{-2}+k_3}\right)\left(\frac{k_1}{k_{-1}+k_2}\right)\left(\frac{k_{-5}}{k_5}\right)[M]_w=k'_{c_2(w)}=1.20.x10^{-3}\,\mu gg^{-1}day^{-1}\,(OTC)$$

$$\frac{dp}{dt}=\left(\frac{k_2k_3}{k_{-2}+k_3}\right)\left(\frac{k_1}{k_{-1}+k_2}\right)\left(\frac{k_{-5}}{k_5}\right)[M]_w=k'_{c_2(w)}=1.30.x10^{-3}\,\mu gg^{-1}day^{-1}\,(CTC)$$

$$\frac{dp}{dt}=\left(\frac{k_2k_3}{k_{-2}+k_3}\right)\left(\frac{k_1}{k_{-1}+k_2}\right)\left(\frac{k_{-5}}{k_5}\right)[M]_w=k'_{c_2(w)}=1.x10^{-3}\,\mu gg^{-1}day^{-1}\,(TC) \qquad (5.4.32)$$

where C_1 and C_2 are colloidal particle type 1 and 2

5.4.3 (c) Slow microbial degradation in the sediment phase

Using Steps 1(d) and 1(e) (Table 5.4.4) it can be shown that

$$[A]=\frac{k_{-6}\lfloor A(S_1)_q\rfloor}{k_6[S_1]^q}$$

$$[A]=\frac{k_{-7}[A(S_2)_z]}{k_7[S_2]^z} \qquad (5.4.33)$$

Substituting for $[A]$ into equation 5.4.24 gives equation 5.4.34

$$\frac{dp}{dt}=\left(\frac{k_2k_3}{k_{-2}+k_3}\right)\left(\frac{k_1}{k_{-1}+k_2}\right)\left(\frac{k_{-6}}{k_6}\right)\frac{\lfloor A(S_1)_q\rfloor_w[M]_w}{[S_1]_w{}^q}$$

$$\frac{dp}{dt} = \left(\frac{k_2 k_3}{k_{-2} + k_3}\right)\left(\frac{k_1}{k_{-1} + k_2}\right)\left(\frac{k_{-7}}{k_7}\right)\frac{[A(S_2)_z]_w [M]_w}{[S_2]_w^z} \qquad (5.4.34)$$

When [S] is in large excess of [A], the concentration of [S] and [AS] becomes constant and assuming that [M] is constant equation 5.4.34 reduces to equation 5.5.35 and 5.5.36.

$$\frac{dp}{dt} = \left(\frac{k_2 k_3}{k_{-2} + k_3}\right)\left(\frac{k_1}{k_{-1} + k_2}\right)\left(\frac{k_6}{k_6}\right)[M]_s = k'_{s_1(s)} = 6.50x10^{-3}\,\mu\text{gg}^{-1}\text{day}^{-1}\,(OTC)$$

$$\frac{dp}{dt} = \left(\frac{k_2 k_3}{k_{-2} + k_3}\right)\left(\frac{k_1}{k_{-1} + k_2}\right)\left(\frac{k_6}{k_6}\right)[M]_s = k'_{s_1(s)} = 1.50x10^{-3}\,\mu\text{gg}^{-1}\text{day}^{-1}\,(DC)$$

$$\frac{dp}{dt} = \left(\frac{k_2 k_3}{k_{-2} + k_3}\right)\left(\frac{k_1}{k_{-1} + k_2}\right)\left(\frac{k_6}{k_6}\right)[M]_s = k'_{s_1(s)} = 6.00x10^{-3}\,\mu\text{gg}^{-1}\text{day}^{-1}\,(CTC)$$

$$\frac{dp}{dt} = \left(\frac{k_2 k_3}{k_{-2} + k_3}\right)\left(\frac{k_1}{k_{-1} + k_2}\right)\left(\frac{k_6}{k_6}\right)[M]_s = k'_{s_1(s)} = 7.5x10^{-3}\,\mu\text{gg}^{-1}\text{day}^{-1}\,(TC) \qquad (5.4.35)$$

$$\frac{dp}{dt} = \left(\frac{k_2 k_3}{k_{-2} + k_3}\right)\left(\frac{k_1}{k_{-1} + k_2}\right)\left(\frac{k_{-7}}{k_7}\right)[M]_s = k'_{s_2(s)} = 2.00x10^{-4}\,\mu\text{gg}^{-1}\text{day}^{-1}\,(OTC)$$

$$\frac{dp}{dt} = \left(\frac{k_2 k_3}{k_{-2} + k_3}\right)\left(\frac{k_1}{k_{-1} + k_2}\right)\left(\frac{k_{-7}}{k_7}\right)[M]_s = k'_{s_2(s)} = 1.00x10^{-3}\,\mu\text{gg}^{-1}\text{day}^{-1}\,(CTC)$$

$$\frac{dp}{dt} = \left(\frac{k_2 k_3}{k_{-2} + k_3}\right)\left(\frac{k_1}{k_{-1} + k_2}\right)\left(\frac{k_{-7}}{k_7}\right)[M]_s = k'_{s_2(s)} = 1.5x10^{-3}\,\mu\text{gg}^{-1}\text{day}^{-1}\,(TC) \qquad (5.4.36)$$

where $k'_{s_1(s)}$ and $k'_{s_2(s)}$ are microbial degradation apparent zero order rate constants of the sediment particle type 1 and 2 respectively.

5.4.3 (d) Overall rate of degradation of TCs in the aquatic environment

The overall rate of degradation of TC antibiotics in the microcosm experiment therefore is given by combining rates of hydrolysis, and photolysis (eqns 5.4.6 and 5.4.14), microbial degradation in the water phase (Eqns 5.4.25, 5.4.31 and 5.4.32) and microbial degradation in the water phase (Eqns 5.4.25, 5.4.35 and 5.4.36). Thus the rate;

$$\frac{dP}{dt} = \left\{\left(k_h'' + k_\phi''\right) + k'_{E(w)} + k'_{C_1(w)} + k'_{C_2(w)}\right\}_{WP} + \left\{k'_{E(S)} + k'_{S_1(S)} + k'_{S_2(S)}\right\}_{SP} \qquad (5.4.37)$$

where $k_h'' = k_h'[A]_0$ and $k_\phi'' = k_\phi'[A]_0$, and the subscripts WP and SP denote water phase and sediment phase. The brackets indicate that they were not observed in the microcosm experiment as they were masked by the much faster rates of microbial degradation. Under real environmental conditions the observed rate of degradation is determined by the term that is dominant as shown in equation 5.4.37.

5.5 ADSORPTION OF TC ANTIBIOTICS BY COLLOIDAL AND SEDIMENT PARTICLES: APPARENT ADSORPTION-DESORPTION EQUILIBRIA.

In eqns 5.4.31 and 5.4.32, k_{-4}/k_4 and k_{-5}/k_5 are in fact the inverse of the adsorption/desorption equilibrium constants by colloidal particles in the water phase of the microcosm experiment. Likewise in Eqns 5.4.35 and 5.4.36, k_{-6}/k_6 and k_{-7}/k_7 are the inverse of the adsorption/desorption equilibrium constants by particles of sediment respectively in the sediment phase or river water and sediment experiment exposed to natural light. It is reasonable to assume that the population of microorganisms, $[M]_W$ in the water phase remain constant in equations. 5.4.26, 5.4.31 and 5.4.32, hence the values of k_{-4}/k_4 and k_{-5}/k_5 can be found by dividing Eqns. 5.4.31 and 5.4.32 by eqn. 5.4.26. Values of 0.286 and 0.050 (OTC), 0.224 (DC), 0.320 and 0.059 (CTC) and 0.411 and 0.078 (TC) are obtained respectively for k-

$_4/k_4$ and k_{-5}/k_5, to yield apparent adsorption/desorption equilibrium constant of 3.50 and 20 (OTC), 4.46 (DC), 3.13 and 16.95 (CTC) and 2.43 and 12.82 (TC) respectively. Similarly, it is reasonable to assume that the population of microorganisms in the sediment phase, $[M]_s$ is constant in equations. 5.4.27, 5.4.35 and 5.4.36, hence the values of k_{-6}/k_6 and k_{-7}/k_7 can be found by dividing eqns. 5.4.35 and 5.4.36 by eqn. 5.4.27. Values of 0.136 and 0.004 (OTC), 0.031 (DC), 0.176 and 0.026 (CTC), 0.157 and 0.031 (TC) are obtained respectively for k_{-6}/k_6 and k_{-7}/k_7, to give apparent adsorption/desorption equilibrium constant of 7.35 and 250 (OTC), 32.26 (DC), 5.69 and 34.48 (CTC), 6.37 and 32.26 (TC) respectively.

The adsorption of antibiotics by soil and sediment particles is often investigated using the Freundlich adsorption isotherm (Hance, 1965)

$$C_{ads} = K_F C_e^n \qquad (5.5.1)$$

Where K_F is the Freundlich constant, C_{ads} is concentration (mg/mL) of the pesticide adsorbed by the soil/sediment in a colloidal solution and C_e is the concentration of the pesticide in the solution (mg/mL) at equilibrium (Bowman and Sans, 1977). For a given system the Freundlich factor (K_F) is a constant therefore, it may be used to compare the degree of adsorption of different substances onto different soil particles. The term n in the Freundlich isotherm is regarded as a measure of adsorption non-linearity between concentration of solute in solution and that which is adsorbed. One of the major drawbacks of the Freundlich isotherm is that it cannot provide information about the kinetics of the adsorption process, prompting Zaranyika and Mandizha, (1998) to suggest a modified Freundlich isotherm, equations. 5.5.2 and 5.5.3, which can be applied to obtain the apparent adsorption/desorption equilibrium constant because the technique employed cannot resolve between adsorption by colloidal and sediment particles.

$$[X]_{ads} = nK'([X]_e + [SX_n]_w)^n \quad (5.5.2)$$

$$\ln[X]_{ads} = \ln(nK') + n\ln([X]_e + [SX_n]_w) \quad (5.5.3)$$

where*K'* is the apparent adsorption equilibrium constant and $[SX_n]_w$ is the concentration of the colloidal bound fraction in suspension at settling equilibrium. By plotting $\ln[X]_{ads}$ versus $\ln([X]_e + [SX_n]_w)$ the value of *n* and K' can be calculated. A value of K of 111±19 was obtained for armitraz. This value is in good agreement with a value of K of 250 obtained in the present study for oxytetracycline, considering structural differences of armitraz and the OTC. It is important to note that the technique for determining the adsorption equilibrium described in the current study has the added advantage that it can resolve between adsorption by different colloidal and/or sediment particle types.

The adsorption free energy of activation ($\Delta G_{(ads)}$), can be computed from eqn. 5.5.4 (Atkins, 1978; Weston and Schwartz, 1972; Ebbing and Gammon, 2007)

$$-RT\ln K_{ads} = \Delta G_{ads} \quad (5.5.4)$$

Values of ΔG_{ads} calculated for the TC antibiotics colloidal/sediment particle adsorption complexes are shown in Table 5.5. From Table 5.5 it is apparent that the adsorption of TC antibiotics to colloidal in the water phase (for $A(C_1)_n$ and $A(C_2)_y$), is thermodynamically favourable. The formation of sediment phase adsorption complexes $A(S_2)_z$ and $A(S_1)_q$ are also thermodynamically favourable. Since adsorption to colloidal particles in the water phase is thermodynamically favourable, it is reasonable to assume that $A(S_1)_q$ involves colloidal particles in the sediment pore water, whereas the $A(S_2)_z$ adsorption complex involves sediment particles.

The entropy of activation (the measure of the difference in randomness or disorderness of the activation complex and the reacting species is given by equation 5.5.5 (Atkins, 1978)

$$\Delta G = \Delta H - T\Delta S \qquad (5.5.5)$$

Table 5.5. Adsorption/desorption of tetracyclines by colloidal and sediment particles: Apparent adsorption free energy ($\Delta G_{(ads)}$).

Antibiotic	Adsorption complex[A]	K_{ads}	$\Delta G_{(ads)}$ /kJ.mol^{-1}	Adsorption site
OTC	$A(C_1)_n$	4.13	-2.67	Colloidal particle
	$A(C_2)_m$	20	-7.48	Colloidal particle
	$A(S_1)_q$	7.35	-5.00	Sediment- Colloidal particle [A]
	$A(S_2)_z$	250	-14.36	Sediment- Colloidal particle [A]
DC	$A(C)_n$	4.46	-2.88	Colloidal
	$A(S)_z$	32.26	-12.07	Sediment- Colloidal particle [A]
CTC	$A(C_1)_n$	3.13	-2.84	Colloidal
	$A(C_2)_m$	16.95	-7.06	Colloidal
	$A(S_1)_q$	5.69	-4.33	Sediment- Colloidal particle [A]
	$A(S_1)_z$	34.48	-13.89	Sediment- Colloidal particle [A]
TC	$A(C_1)_n$	2.43	-1.85	Colloidal
	$A(C_2)_m$	12.82	-5.35	Colloidal
	$A(S_1)_q$	6.37	-4.12	Sediment-Colloidal particle [A]
	$A(S_1)_z$	32.26	-13.00	Sediment-Colloidal particle [A]

[A] Sediment or colloidal particle

For adsorption processes, the entropy of activation is a measure of the disorderness of the adsorption complex and reactants. As adsorption necessarily results in reduced disorder, the ΔS_{ads} for adsorption is necessarily negative. Hence, ΔG_{ads} data in Table 5.5 suggest that $\Delta H^{\dagger}$ is negative and numerically greater than $T\Delta S^{\dagger}$. In other words, the adsorption of TC antibiotics by colloidal or sediment particles is exothermic, and is enthalpy driven. For physisorption, $\Delta H_{(P)}$ is rarely more negative than about - 25kJ mol^{-1}, whereas for chemisorption, $\Delta H_{(C)}$ is usually more negative, and sometimes much more negative, than - 40 kJ mol^{-1} (Atkins, 1978). This suggests that the adsorption of TC antibiotics by colloidal and sediment particles both in the water phase and sediment phase, involves physisorption, since $\Delta H \geq \Delta G$. ΔG_{ads} for the $A(S_1)_q$ adsorption complex in the sediment phase is close to the ΔG_{ads} values obtained for the colloidal particle adsorption complexes in the water phase of the microcosm experiment, implying that the complex correspond to TCs-colloidal particle adsorption complex in the sediment pore water. ΔG_{ads} for $A(S_2)_z$ complex is significantly higher than values forΔG_{ads} for the colloidal particle adsorption complexes in the water phase, suggesting that $A(S_2)_z$ corresponds to the TCs-sediment particle adsorption complex.

5.6 POSSIBLE WAYS OF CONTROLLING AQUATIC ENVIRONMENT CONTAMINATION AND REMEDIATION STRATEGIES

Equations 5.4.26, 5.4.27, 5.4.31, 5.4.32, 5.4.35 and 5.4.36 define the factors that can be selected and optimized for the removal and mitigation of aquatic contamination by TC antibiotics. Any control measures must be designed to optimize k_1, k_2, k_3, k_{-4}, k_{-5}, k_{-6}, k_{-7}, density and type of microorganisms responsible for the degradation of the antibiotics. Optimizing k_1, k_2 and k_3 will make $k_2 >> k_{-1}$ and $k_3 >> k_{-2}$, so that eqns 5.4.26, 5.4.27, 5.4.31, 5.4.32, 5.4.35 and 5.4.36 reduce to eqns. 5.6.1 to 5.6.6 respectively, thus:

$$\frac{dP}{dt} = k_1[M]_W \qquad 5.6.1$$

$$\frac{dP}{dt} = k_1[M]_S \qquad 5.6.2$$

$$\frac{dP}{dt} = k_1\left(\frac{k_{-4}}{k_4}\right)[M]_W \qquad 5.6.3$$

$$\frac{dP}{dt} = k_1\left(\frac{k_{-5}}{k_5}\right)[M]_W \qquad 5.6.4$$

$$\frac{dP}{dt} = k_1\left(\frac{k_{-4}}{k_4}\right)[M]_S \qquad 5.6.5$$

$$\frac{dP}{dt} = k_1\left(\frac{k_{-6}}{k_6}\right)[M]_S \qquad 5.6.6$$

As k_1 is the rate constant for the binding of TCs by the microorganism, eqns. 5.6.3-5.6.6can be separated into a product of 2 factors, i.e. a microbial factor (= $k_1[M]_w$) and an adsorption factor (k_{-4}/k_4,k_{-5}/k_5, k_{-6}/k_6 andk_{-7}/k_7). Equations 5.6.1 and 5.1.2 show that degradation of the free dissolved TC antibiotic depend only on the microbial factor. Thus microorganisms that can degrade the antibiotics efficiently can be sort and be used to degrade the antibiotics in effluent water before it is discharged into surface waters. These microorganisms can be optimized by providing the necessary nutrients to increase the population. In the current study addition of nitrates increased microbial degradation of TC antibiotics. Few studies have been devoted to this effect. In a study conducted by Wen et al., (2010) enzymes isolated from fungi were used to degrade OTC and TC in water. A 95% degradation of the antibiotics was recorded in 5 minutes. Similarly, Maki et al., (2006) successfully isolated 8 strains of bacteria

from fish farm sediments that could degrade antibiotics up to 42-69% in 21 days. In another study by Meyers and Smith, (1962) microbial degradation was achieved using *Xylaria digitata*. The rate also depends on adsorption/desorption equilibrium even after optimizing the microbial factor. This has been observed in the present work with nitrate fortified experiments which demonstrated that addition of nitrates had no significant effect on the rate of degradation of the adsorbed antibiotics. This is expected because in this system the rate of desorption becomes the limiting step. Thus the rate can only be increased if ways of increasing the rate of desorption is applied. One way is to apply heat. Another way of increasing the rate of degradation for the adsorbed antibiotics is to look for microorganisms that can attack the antibiotic while it is adsorbed. Holding lagoons can also be constructed and filled with soil particulate matter that retains strongly the antibiotic. This has the advantage of reducing the rate of movement of the antibiotic into surface waters. The soil can then be scooped, leached and treated through microbial degradation. A few studies have applied this technique to reduce contamination of aquatic environment. Bansal, (2013) and Barbooti et al., (2012) studied the removal of antibiotics from water by sorption onto soil particles.

5.7 A COMPARATIVE ANALYSIS OF THE DEGRADATION OF OTC, DC, CTC and TC.

The rates of degradation varied as follows OTC > CTC > DC > TC with initial degradation rates of 3.07×10^{-2}, 2.68×10^{-2}, 2.29×10^{-2} and 1.35×10^{-2} μg/g/day respectively. This shows that structure and properties of the antibiotic has an effect on the microbial degradation of the antibiotic. This was demonstrated in eqn 5.4.27. The magnitude of antibiotic-microorganism

binding equilibrium constant k_{-1}/k_1 was demonstrated to depend on the structure and properties of the antibiotic.

While OTC, CTC and TC exhibited triphasic kinetics for the microcosm experiment. DC exhibited biphasic kinetics. DC differs from the other TCs at carbon number 6 where an oxygen atom has been replaced by an H atom. Molecular chemical composition has been demonstrated to have an impact on sorption of TC molecules on clay particles (Avisar et al., 2009b). An oxygen atom is more electronegative than a hydrogen atom which is expressed by a lower pK_{a1} and a higher pK_{a2} values of the tricarbonylamide and phenolic diketone groups of DC molecule, Fig 5.7.1 and Table 5.7 as compared to OTC, CTC and TC. Thus, DC may show a different adsorption pattern than the other TCs. Adsorption of the antibiotic limits its availability to microorganisms (reduce antibiotic-microorganism binding) and reduces microbial activity. The calculated thermodynamic parameters listed in Table 5.5 ranged from -2.67 to -14.36 kJ mol^{-1}. The negative ΔG values indicate attractive interactions between the TC molecules and colloidal and sediment particles (Jiang et al., 2015). The magnitude of the values suggest physical sorption onto colloidal or sediment particles such as cationic exchange and cationic bridging (Turku et al., 2007; Chen et al., 2012; Quang and Adams, 2004). In previous studies that were conducted by Kulshrestha et al., (2004); Chenxi et al., (2009) and Chang et al., (2012), OTC adsorption onto selected montmorillonite clays was observed to increase with decreasing pH 11< 8.7 < 5.0 < 1.5.Such a correlation can be attributed to cationic exchange that is predominant at lower pH values when the TC molecules are positively charged (Fig 5.7.2)

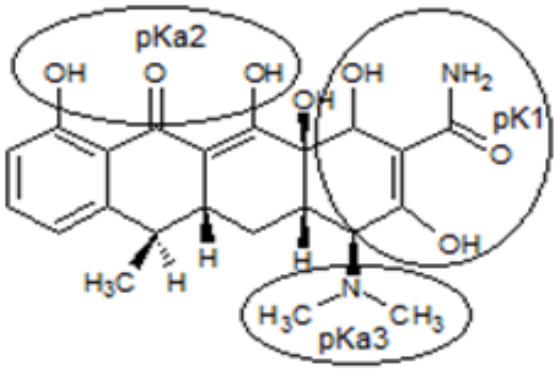


Fig 5.7.1. pK_a sites of doxycycline (Kogawa and Salgado, 2012)

Table 5.7. Pk_a values of TC molecules (Kogawa and Salgado, 2012)

Antibiotic	pK_{a1}	pK_{a2}	pK_{a3}
OTC	3.22	7.46	8.94
CTC	3.33	7.55	9.33
TC	3.32	7.78	9.58
DC	3.02	7.97	9.15

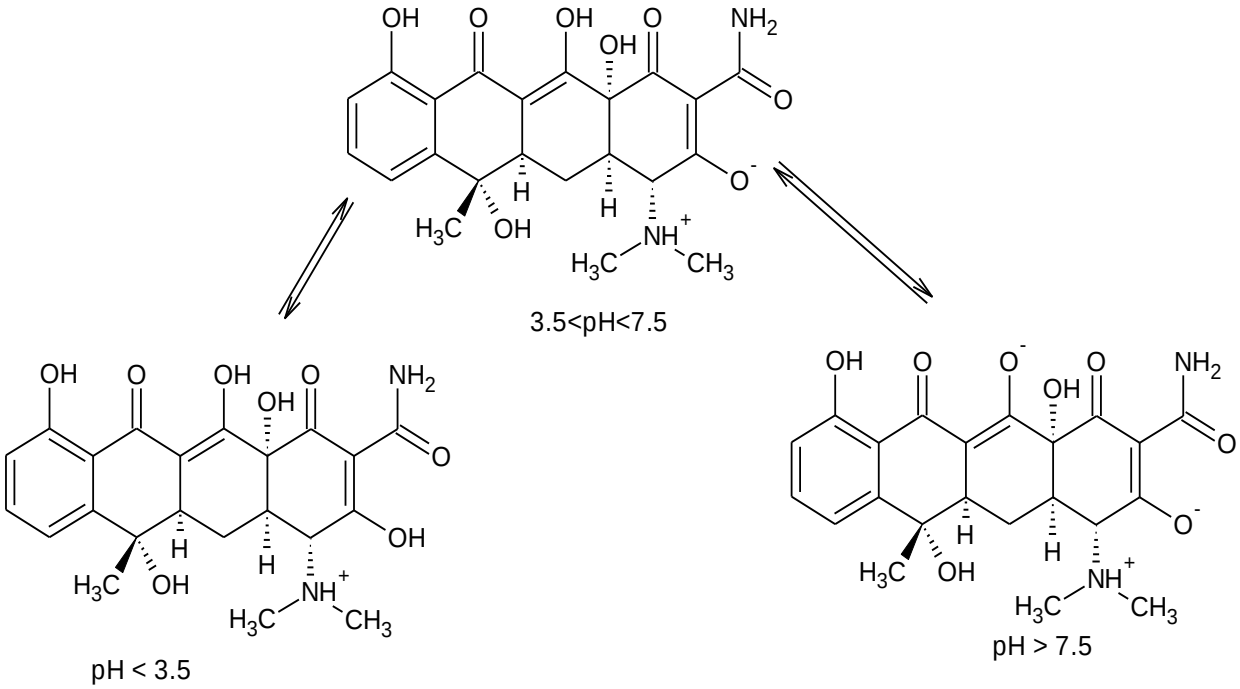


Fig 5.7.2. Different forms of tetracyclines depending on pH (Avisar et al., 2009)

CHAPTER 6

6.1 THESIS CONCLUSIONS

- For all the four antibiotics studied in this study the results show that once they have been introduced into the aquatic environment, they exist as free form in solution of the water phase and pore water and as colloidal or sediment particle 1 and 2 bound. The free form degrades at a faster rate than the bound form.
- The fate of the antibiotic in the environment depends on the path way that is predominant at that juncture. If photolysis is insignificant e.g. in winter or is hampered through screening effect of particulate matter, as the case in the real environment, hydrolysis or microbial degradation becomes the predominant mode of degradation. Photolysis depends on the extent to which sunlight penetrates the water phase. Therefore the depth of the water will determine the extent in which photolysis will contribute to the overall rate of degradation.
- The rates depend on microbial degradation. The rate of microbial degradation primarily depends on type and population of microorganisms. The type and population of microorganisms affect the rate at which microorganisms bind different substances. Population of microorganisms depends on pH, temperature and presence of nutrients.
- Adsorption/desorption equilibrium plays an important role in retaining the antibiotics in the environment. If the rate of desorption is very slow it implies that the antibiotic takes a longer time to degrade. Thus, sediment and soil type affects the rate of degradation.

- Thus the present study has shown that the linear rate model predicts varying rates depending on environmental conditions whereas the exponential model predicts a constant half-life irrespective of the prevailing environmental conditions.
- Of a particular note is the fact that whereas the exponential model predicts that once a substance has been introduced into an environment it will remain there indefinitely, the linear rate model predicts a finite life-time in the environment.

6.2 AREAS OF FURTHER STUDY

The research has focused on a 90 day study of the persistence of TC antibiotics in microcosm experiments with and without nitrates consisting of a single spiked antibiotic. Future studies should aim to;

- Perform studies in winter and summer to assess the effect of temperature on rates of degradation.
- Perform studies involving the effect of other nutrients other than nitrates
- Apply the model to explain the behaviour of other group of antibiotics such, as sulphonamides.
- Apply the model using microcosm studies involving two or more organic molecules since the real environment consists of a plethora of different organic molecules.

7. REFERENCES

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8. APPENDIX

8.1 Publications

Mark F. Zaranyika, **Pamhidzai Dzomba** and Jameson Kugara (2015). Degradation of oxytetracycline in the aquatic environment: a proposed steady state kinetic model that takes into account hydrolysis, photolysis, microbial degradation and adsorption by colloidal and sediment particles. Environmental chemistry; Csiro, 12 (2): 174-188; http://dx.doi.org/10.1071/EN14116, **Thompson Reuter's impact factor 2015 = 3.4**

M. F. Zaranyika, **P. Dzomba**, J. Kugara. Speciation and persistence of doxycycline in the aquatic environment: Characterization in terms of steady state kinetics, Journal of Environmental Science and Health, Part B, Pesticides, Food Contaminants, and Agricultural Waste 12: 1-11.Doi: 10. 1080/03601234.2015.1067101 **Thompson Reuter's impact factor 2015 = 1.4**

P. Dzomba, J. Kugara and M. F. Zaranyika (2015). Extraction of tetracycline antimicrobials from river water and sediment: a comparative study of three solid phase extraction methods. African Journal of Pharmacy and Pharmacology, 9(19): 523-531, doi: 10.5897/ajpp2015. 4341 **Scopus impact factor 0.84**

Dzomba P, Kugara J, Zaranyika M. F. (2015) Characterization of microbial degradation of oxytetracycline in river water and sediment using reversed phase high performance liquid chromatography. African Journal of Biotechnology, 14: 1-9 doi 10.5897/AJB2014.14091. **Scopus impact factor 0.57**

Pamhidzai Dzomba, Mark F. Zaranyika, Jameson Kugara. (2014).Determination of oxytetracycline residues in untreated and treated drinking water in Bindura town by RP-HPLC-UV visible spectrometry after ultrasonic assisted dispersive solid phase extraction (UA-DSPE).World Journal of Pharmaceutical Research 3(2): 1568-1578

P. Dzomba, J. Kugara, M. F. Zaranyika (2014) Analysis of Oxytetracycline and Doxycycline in Surface water sources and treated drinking water in Harare Metropolitan using Ultrasonic Assisted Dispersive Solid Phase Extraction (UA-DSPE)and RP-HPLC-UV Technique. Bulletin of the Environment, Pharmacology. Life Sciences, 3 (3): 100-109

8.2 Conferences

Pamhidzai Dzomba, Mark F. Zaranyika, Jameson Kugara. (2013).Determination of oxytetracycline residues in untreated and treated drinking water in Bindura town by RP-HPLC-UV visible spectrometry after ultrasonic assisted dispersive solid phase extraction (UA-DSPE).1-2 September 2013 SETAC 6th conference Lusaka Zambia. www.setac.org/event/africa-2013

M. F. Zaranyika*, P. Dzomba and J. Kugara. Speciation and persistence of chlortetracycline in the aquatic environment: Characterization in terms of a linear rate law.30-31 July 2015, ANCAP SYMPOSIUM. Makerere University, Uganda. www.ancapnet.org/symposium.11.htm

8.3 Workshops

Use of biomarkers and bio-indicators in the analysis of persistence organic pollutants in the environment. 26-31 August 2013. ANCAP 10th summer school. University of Zambia. Lusaka. www.ancap.org/summer10.htm

Analysis of persistent organic pollutants (POPs) 24 February 2014 Oasis Hotel, Zimbabwe

Process oriented statistical analysis and use of the RZWQM2 model to predict fate and transport of organic molecules in the soil. 27-31 July 2015. ANCAP 11th summer school.Makerere University.www.ancap.org/summer 11.htm

SAMPLES OF PUBLISHED ARTICLES