Sorption, degradation and transport of veterinary antibiotics in New Zealand pastoral soils

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ABSTRACT

Veterinary antibiotics are used worldwide for prevention and treatment of various diseases of livestock animals. After administration, the majority of antibiotics are excreted in urine and faeces as unchanged parent compound, and/or as their metabolites. With increases in the intensive use of antibiotics in New Zealand agriculture and direct land application of waste as manure, there is concern that excreted compounds could migrate to the receiving environment with potential impact on surface and groundwater. Antibiotic residues in soil could give rise to a variety of ecotoxicological problems and could also confer antibiotic resistance. As a first step towards assessing the risk of these compounds, it is important to investigate their fate and behaviour in the soil environment. The focus of this work was to determine the fate and transport behaviour of selected group of veterinary antibiotics in several New Zealand pastoral soils. A macrolide (TT) and three sulfonamide antibiotics (SMO, SCP and SM) were chosen for this study as they are commonly used overseas as well as in New Zealand.

A simple, yet robust analytical method was developed to detect and quantify TT and the three sulfonamides using high performance liquid chromatography and ultraviolet detection. The limits of detection at signal: noise ratio of 3 were 20.0 μg L^{-1} and 50 μg L^{-1} for all SA's and TT respectively. The average recoveries for all SA's and TT in aqueous matrices ranged from 95 to 105% across the six concentrations investigated. Recoveries from the residual soils were slightly lower for SA's and TT (~ 50 to 60%).

The results of the kinetics studies showed that sorption was rapid in the first few hours of the contact time (0 to 2 h for SA's and 0 to 4 h for TT) and thereafter an apparent equilibrium concentration was achieved slowly. Batch sorption results performed in six different pastoral soils showed that Freundlich isotherms were nonlinear ($N \neq 1$) for most of the compounds. The degree of isotherm linearity (N) for SCP and SM varied between 0.87–1.11 in the six soils. SMO showed a highly non-linear pattern ($N = 0.75$) in just one soil (Manawatu). Isotherms of both TT and SMO were non-linear, with the degree of non-linearity for TT ($N =$ 0.41–0.73) being greater than for SMO (0.88–1.21) in all soils. Concentration-dependent effective distribution coefficient (K_d^{eff}) values for the SMO, SCP and SM antibiotics in the

soils ranged from 0.37 to 4.6 L kg^{-1} and for TT K_d^{eff} ranged from 1.2 to 500 L kg^{-1}. The sorption affinity for all soils followed an order: TT > SCP > SM > SMO. Statistical analysis of sorption data revealed a correlation between the sorption coefficients and soil properties such as % organic carbon, cation exchange capacity and clay content. Sorption of TT onto soils is mostly driven by the cation exchange capacity of the soil whereas for sulfonamides it is primarily due to hydrophobic interactions due to hydrophobic interactions.

On further investigation it was found that sorption of the SMO antibiotic to soils was highly dependent on pH, ionic strength, and organic matter of the soil. Sorption of SMO decreased with the increases in pH, and increased with increasing ionic strength and organic carbon content. A hydrophobic pH-partitioning model linking sorbate speciation with species-specific sorption coefficients describing the pH dependence of the apparent sorption coefficients was successfully used to derive the fraction of each species of SMO that are likely to be present in the environment.

The results from the biochar amended sorption studies showed that certain biochars can be used as a potential tool to remove residues of antibiotics. Soil amended with pine saw dust biochar was found to be the most effective in adsorbing antibiotics. Pine sawdust biochar absorbed 30 times more antibiotics than soil alone. This was attributed to its high surface area, which was four times higher than that of other biochars, or could be due to its high carbon content (91%). The results have shown that biochar applied at the rate of 0.5 to 1% by soil weight could prove to be an effective way to slow down the release of these contaminants to a manageable level thereby reducing the risk of ecotoxicity and antibiotic resistance.

SMO degraded slowly in agricultural soils. The degradation times (DT_{50}) for SMO in Hamilton clay, Te Kowhai and Horotiu soils under non-sterilized conditions were 9.24, 4.3 and 13.33 days respectively. Soil dehydrogenase activities were directly correlated with degradation kinetics of SMO antibiotic. Results from degradation studies have shown that SMO is not likely to persist more than 90 days in all three soils suggesting that natural biodegradation may be sufficient for the removal of these contaminants from the soil. Both the degree of biological activity and temperature of the soil influenced overall degradation. SMO degradation in sterile soils indicated abiotic degradation and abiotic factors such as strong sorption of the antibiotic onto soil components also played a role in the degradation of

SMO in soil. Four kinetic models, a single first-order model (SFO) and three biphasic kinetic models, were applied to fit the observed SMO degradation kinetics data. The results showed that the First-order double-exponential decay (FODED) and first-order two-compartment (FOTC) model was superior to the bi-exponential model (BEXP) and all three biphasic models were superior when compared to the SFO model for describing SMO degradation data. Use of the FOTC model enabled the estimation of the degradation endpoints.

The results from two soil lysimeters indicate that the three sulfonamides were highly mobile and that at field collected soil pH mobility was similar to the mobility of the conservative bromide. The breakthrough curves of the three sulfonamides varied depending on soil type with Hamilton soil showing more retardation than Matawhero soil. For the three sulfonamides studied the order of breakthrough was generally observed to be: SM > SMO > SCP for both Matawhero and Hamilton soils. Residual antibiotic concentrations for SMO and SCP were detected up to depths of 18 cm. The CXTFIT model study described the peak arrival time as well as the maximum concentration of the antibiotic breakthrough curves but showed some underestimation at advanced stages of sulfonamide leaching, especially in the Hamilton soil. Results showed that the sulfonamides have weak sorption affinity and relatively high mobility in soils.

Keywords: Veterinary antibiotic, tylosin, sulfonamides, sorption, degradation, transport, dehydrogenase activity, kinetics, modelling, lysimeter.

PUBLICATIONS/CONFERENCE PAPERS FROM THIS THESIS

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- Srinivasan, P., A. K. Sarmah, and M. Manley-Harris.: *Effect of pyrolysis temperature on the retention capacity of green waste biochar to sorb steroid hormones and veterinary antibiotics.* Presented at the New Zealand Biochar Research Centre Workshop, Massey University, 11th and 12th February, 2010. Palmerston North, New Zealand (**oral paper**). http://www.biochar.co.nz/Files/Biochar%20Workshops.pdf

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ACN = acetonitrile

ACVM = Agricultural compounds and veterinary medicines

ASE= accelerated solvent extractor

BET = Brunauer, Emmett, and Teller isotherm

BEXP = bi-exponential decay

BIC = Bayesian information criteria

BTC = breakthrough curve

CAFO = confined animal feeding operation

CC = corn cob

CDE = convection-dispersion equation

CEC = cation exchange capacity

DAD = diode array detector

DCM = dichloromethane

DDT = dichlorodiphenyltrichloroethane

DHA = dehydrogenase activity

DT = degradation time

FD = fluorescence detection

FODED = first order double-exponential decay

FOTC = first order two-compartment

E1 = estrone

E2 = 17β-estradiol

EDC = endocrine disrupting chemicals

EDS = Energy dispersive spectrometry

ESI = electro-spray ionisation

FTIR = Fourier Transform Infrared

GC = gas chromatography

GW = green waste

HPLC = high-performance-liquid-chromatography

HTT= heat treatment temperature

ICPMS = inductively coupled plasma mass spectrometry

K_d = distribution coefficient

K_d^{eff} = effective distribution coefficient

K_{ow} = octanol-water distribution coefficient

K_f = Freundlich coefficient

LOD = limit of detection

LOQ = limit of quantification

LC = liquid chromatography

MAF = Ministry of agriculture and forestry

MBC = microbial biomass carbon

MC = moisture content

MDL = method detection limit

MeOH = methanol

MWHC= maximum water holding capacity

MRL = maximum residue limit

MS = mass spectrometer(s)

MSE = mean squared error

NZ = New Zealand

NZFSA = New Zealand food safety authority

OC = organic carbon

PAH = poly-aromatic hydrocarbon

PSD = pine saw dust

RMSE= root mean square error

SA = sulfonamide

SCP = sulfachloropyridazine

SDZ= sulfadiazine

SEM = Scanning electron microscope

SFO = single first order

SMO = sulfamethoxazole

SM = sulfamethazine

S/N = signal: noise

SPE = solid phase extraction

SS = subsoil

SSA = specific surface area

STP = Sewage treatment plant

STZ= sulfathiazole

TFA = trifluoroacetic acid

THF = tetrahydrofuran

TPF = triphenyl formazan

TS = topsoil

TT = tylosin tartarate

UV = ultraviolet

VA = veterinary antibiotic

WWTP = wastewater treatment plant

XRD = X-ray diffraction

Chapter 1: Introduction

1.1 Background

The development of veterinary antibiotics (VA) has helped safeguard the health and welfare of livestock for many decades in various parts of the world. Veterinary antibiotics are often used in large amounts for therapeutic and prophylactic purposes. They are also used as growth promoters to increase meat production in many parts of the world. Many of these growth promoters are banned in New Zealand (MAF Food Assurance Authority 2002) as in most of the developed countries like the USA and Europe (Boxall *et al.* 2003; Gao and Pedersen 2005). Veterinary antibiotics play an important role in the agricultural economy, sustainability, and production of more affordable, good-quality food for the consumer. However, in today's world there is increasing pressure on food supplies and the need for more meat and dairy products has led to the intensification of the livestock industry and its operations and consequent increase in the use of antibiotics.

After treatment of animals, the majority of antibiotics are excreted in urine and faeces as unchanged parent compounds, and/or as their metabolites. With increases in the intensive use of antibiotics in New Zealand agriculture and the direct land application of waste, there is concern that excreted compounds could potentially migrate to the receiving environment with potential impact on surface and groundwater (Sarmah *et al.* 2006a). This may give rise to a variety of ecotoxicological problems like antibiotic resistance and toxicity to non-target organisms.

An early review by Boxall *et al*. (2003) proposed negligible risk associated with antibiotics in the environment claiming that their concentration has not yet reached toxic levels in soil and water. However, studies have confirmed that long-term exposure to low doses of antibiotics can lead to development of antibiotic resistant bacteria, which would render many of the existing veterinary antibiotics ineffective (Halling-Sørensen *et al.* 1998; Sengelov *et al.* 2003; Acar and Moulin 2006; Pruden *et al.* 2006). Elsewhere, studies have shown that many veterinary antibiotics could also be toxic and possibly lethal to non-target organisms such as

fish and earthworms (Kummerer 2003; Isidori *et al.* 2005). Bacterial populations isolated from the gut of animals exposed to antibiotics were found to be five times more likely to be resistant to any given antibiotic than those from animals without antibiotic exposure (Sarmah *et al.* 2006a).

A number of studies worldwide have provided evidence of antibiotic occurrence in soils, surface water and ground water (Hamscher *et al.* 2003; Zuccato *et al.* 2005; Perret *et al.* 2006; Luo *et al.* 2011). Once released into the environment through faeces, or by application of manure, veterinary antibiotics can disperse through a variety of soil processes (Thiele-Bruhn 2003; Boxall *et al.* 2004; Sarmah *et al.* 2006a). These processes, which include sorption, leaching and degradation, play a major role in the ultimate fate of released compounds in the soil–water systems. The processes are driven by various physico-chemical properties of the antibiotics, such as their molecular structure, size, shape, solubility, speciation and hydrophobicity (Tolls 2001).

Though in recent times studies on veterinary antibiotics have multiplied from initial exploratory studies to risk assessments of these compounds. There is currently no literature describing the fate of veterinary antibiotics in New Zealand soils although other compounds such as pesticides, and emerging contaminants such as endocrine disrupting chemicals have been studied (Sarmah *et al.* 2005; Close *et al.* 2006; Sarmah and Northcott 2008; Scherr *et al.* 2009a). This thesis investigated the fate and behaviour of selected veterinary antibiotics in the New Zealand environment including soils involving pasture soils.

1.2 Need for this research

New Zealand has a rapidly expanding dairy industry, and well-established beef, sheep, pig and poultry production. Farmlands, pasture and farm animals dominate more than half the country's land surface. Agriculture farming has been outranked by intensive dairy farming mainly due to income generated by direct dairy exports to the rest of the world. The livestock industry is a major driver of New Zealand's economy, contributing 47% of the total exports in 2005-2006, and over 9.5 million head of cows and cattle graze the farmlands and pastures of New Zealand throughout the year (Statistics New Zealand 2008).

New Zealand's livestock population excretes about 40 times more waste than the entire human population of the country (Sarmah *et al.* 2004). Within the Waikato region alone, animal waste is now applied to pasture by 70% of dairy farms, 70% of piggeries, and some poultry farms. New Zealand's poultry industry generates 162 000 tonnes of wet broiler litter and 25 000 tonnes of dry manure per year, and this quantity is steadily growing (Ministry of Agriculture and Forestry 2001). Application of animal waste effluent onto land is a permitted activity in New Zealand and a common practice among the farmers who do not require resource consent as long as they follow the prescribed rules set by the regional council authorities (Sarmah *et al.* 2006b). It is that 80% of total land application of animal excreta occurs directly through animal production.

Thus, given the above scenarios, contamination of surface and groundwater by antibiotic residues due to run-off and/or leaching through soil could potentially occur and eventually further impact on the ecosystems. To date, no information exists on the environmental fate of many of the veterinary antibiotics used in New Zealand animal industries. Also there is no evidence of low levels of antibiotic residue in New Zealand soils which make the regulatory bodies less concerned when compared to other environmental problems such as nutrient, sediment and microbial contamination of waterways or irreversible metal accumulations in soils. Even though the current practices to license and manage these compounds are thought to be sufficient there is pressure on the governmental agencies to more carefully monitor the use and fate of antibiotics. This may come to bear in the form of best practice protocols expected by agents in overseas markets. In many countries, risk assessments of these drugs are carried out to determine the concentration, stability and fate is considered important (Grung *et al.* 2008). Though at present, veterinary antibiotics are not regulated in terms of monitoring their levels in streams, rivers lakes or groundwater, it is possible that New Zealand may adopt this practice in the coming years, thus it is imperative to understand their fate and behaviour (sorption, degradation and transport) in New Zealand soils.

Recent studies on the fate and degradation of a number of pesticides and endocrine-disrupting chemicals in New Zealand soils have shown very different behaviours to those observed in other parts of the world (Sarmah *et al.* 2009). For example, the sorption affinity of many New Zealand soils for a range of pesticides and steroid hormones associated with dairy effluents differs remarkably from those observed in many overseas soils (Sarmah *et al.* 2005; Sarmah

and Northcott 2008). The predominance of volcanically derived New Zealand soils with a high percentage of allophane clay mineral having a high surface area and high organic carbon content strongly influences sorption. Therefore, extrapolation of overseas data on the fate of veterinary antibiotics to New Zealand soils may not be appropriate in the absence of comparative New Zealand specific data because of significant differences in the inherent soil properties that can influence the sorption affinity.

This thesis provides a knowledge base to unify the fate studies of veterinary antibiotics in the environment and improves understanding about the behaviour and effects of commonly used veterinary antibiotics in the New Zealand environment under laboratory conditions. It also provides valuable data for the development of regulatory guidelines for risk assessment purposes.

1.3 Scope of this thesis

Following the general introduction (Chapter 1) a detailed literature review (Chapter 2) summarises the current state of research in this area. Chapter 2 provides an overview of the occurrence, fate and behaviour of veterinary antibiotics in different environmental matrices; mainly soil, manure and wastewater effluents. The review highlights the importance of determining the fate of residues of antibiotics in various environmental media, existing analytical methods and subsequent extraction and clean up techniques. It also gives information on the sales and usage of veterinary antibiotics within the last decade for New Zealand, and reviews its ecotoxicological effects, antibiotic resistance and uptake of antibiotics by plants.

Chapter 3 describes a simple but robust analytical method to separate and detect three sulfonamides and one macrolide antibiotic in soil and aqueous matrices using High Performance Liquid Chromatography with Ultra Violet detection. In order to investigate the sorption of three sulfonamides and a macrolide (TT) antibiotic onto six different New Zealand dairy farm soils, laboratory batch sorption studies were conducted using 5 mM $CaCl_2$ as the mediator solution. The data obtained from these batch sorption experiments were later used to construct Freundlich isotherms and estimate sorption parameters which are discussed in Chapter 4.

Chapter 5 investigates the influence of pH, ionic strength and soil organic carbon content on the sorption of SMO antibiotic onto three different soils. The results of competitive sorption experiments for the SCP-E2 system are also presented in this chapter. In Chapter 6, the possibility of using biochar as an engineered sorbent for antibiotics residues (SMO) has been investigated. Biochars obtained from three different feedstocks were tested for their retention ability. The effect of pyrolytic temperatures on the sorptive properties of biochars were also investigated. This chapter provides insights onto various biochar characterisation techniques such as Fourier transform infrared spectroscopy, scanning electron microscope/energy dispersive X-ray spectroscopy, X-ray diffraction and inductively coupled plasma mass spectrometry.

Chapter 7 investigates the degradation behaviour of SMO antibiotic in three different soils at two different incubation temperatures, two different depths and under sterile and non-sterile conditions. The degradation kinetic data obtained from Chapter 7 were later used to fit a single first order and three different non-linear biphasic models. Excel solver® was used to estimate the degradation end points (DT_{50} and DT_{90}). Statistical analysis for the four models was also performed to evaluate the goodness of fit for the models; these results are presented Chapter 8. In order to study the transport and retardation of three sulfonamides in soils, two different undisturbed soils micro-lysimeters were used to obtain breakthrough curves for these antibiotics and a conservative bromide tracer together with their residual concentration; these results are presented in Chapter 9. CXTFIT code was used to solve the one-dimensional convection-dispersion equation under steady state flow in a homogeneous soil conditions. Finally Chapter 10 presents general conclusions, and suggestions for future research.

1.4 Objectives of this thesis

In New Zealand, even with its well-established livestock industry, there is a general lack of information on the stability, fate and behaviour of veterinary antibiotics in farmland soils. To assess the fate and behaviour of veterinary antibiotics under these conditions, experimental data are needed in order to deduce compound and matrix specific values (e.g. for partitioning, degradation, and mobility) which in turn will improve the risk assessment scheme for these

organic contaminants. The general objective of this thesis is to extend the knowledge of the behaviour of veterinary antibiotics in pastoral soil environments.

The main objectives of this thesis are:

- Review the literature published evaluating the occurrence, detection and fate of veterinary antibiotics in different environmental matrices.
- Develop an analytical method using HPLC-UV/Fluorescence which is capable of quantifying the selected antibiotics in soils and aqueous matrices.
- Investigate the sorption behaviour of selected antibiotics in 6 different dairy farms soils.
- Develop a soil clean-up tool based on biochar made from different feedstock and at different pyrolytic temperature and to study their influence on soil properties and their efficacy to sorb veterinary antibiotics.
- Study the role of pH, ionic strength, soil organic carbon and co-contaminants (steroid hormones) on the sorption of veterinary antibiotic.
- Investigate the degradation kinetics of SMO antibiotic in three different dairy farm soils under varying initial concentration, depth, temperature and sterile regimes in the laboratory.
- Fit the datasets obtained from the degradation experiments with three different biphasic models and then compare them to the simple first order kinetics model.
- Study the transport and retention of 3 different sulfonamide antibiotics using two different soil columns.

Chapter 2: Review of relevant literature on the occurrence, detection and fate of selected veterinary antibiotics

2.1 Scope of review

This chapter provides information on the usage and sales of veterinary antibiotic in New Zealand. It contains a detailed review of the published literature evaluating the occurrence, detection and fate (sorption, degradation and transport) of the selected veterinary antibiotics in different environmental matrices. This chapter also reviews the information available on veterinary antibiotics with respect to its ecotoxicity, plant uptake and antibiotic resistance.

2.2 Veterinary antibiotics and their function

Veterinary antibiotics are widely used to treat various types of diseases and to protect the animals. Some antibiotics are used as growth promoters, often allowing animals to be brought to market faster and at lower cost (Boxall *et al.* 2003). Ever since the introduction of the first antibiotic penicillin almost 50 years ago, antibiotics have made an important contribution to the livestock industry by substantially reducing the number of animals that suffer or die from infectious bacterial diseases (Kummerer 2001). Yeasts and fungal produced antibiotics are commonly used in feed application within the New Zealand livestock industry (Sarmah *et al.* 2006a). The antibiotics used in the New Zealand industry predominantly have a narrow spectrum of activity (Sarmah *et al.* 2006a). A number of drugs and feed additives are approved for use in food-animal agriculture (Ministry of Agriculture and Forestry 1999), the most commonly used antibiotics for veterinary purpose in Europe are shown in Table 2.1.

2.3 Veterinary antibiotics in the New Zealand environment

New Zealand is a small country with numerous lakes, rivers and streams, and a rapidly expanding livestock industry consisting of beef, sheep, pig, and poultry production (Sarmah *et al.* 2006a). Paddocks and farmlands dominate more than half the country's land surface and there has been in recent times an increasing trend for pastoral farming of animals (Scherr

et al. 2009b). With the exception of the intensively housed fed poultry and pig industries, where antibiotics are used in feed, New Zealand raises its large population of ruminant animals on pasture (Sarmah *et al.* 2006a). For many years, wastes from dairy farms in New Zealand were largely treated in oxidation ponds before being discharged into nearby waterways. However, in recent years, the practice of direct application of dairy effluent onto land has become popular among farmers. According to a report by the Ministry for the Environment (1997), the livestock population excretes about 40 times more waste than New Zealand's human population.

Table 2.1: Veterinary antibiotics used in Europe having high potential of entering the environment adapted from Boxall *et al*.(2003).

Veterinary antibiotics that have a high potential of entering the environment **Stars indicate compounds that have been monitored and detected*		
amitraz	enrofloxacin	oxolinic acid*
amoxicillin	fenbendazole	oxytetracycline*
amprolium	flavomycin	piperronyl butoxide
antiseptics	flavophospholipol	poloxalene
baquiloprim	florfenicol	procaine benzylpenicillin
cephalexin	flumethrin	procaine penicillin
chlortetracyline*	ip**	robenidine hydrochloride
clavulanic acid	Ivermectin*	salinomycin sodium
clindamycin	lasalocid sodium	sarafloxacin*
clopidol	levamisole	sulfadiazine
cryomazine	lido/ligocaine HCL	tetracycline*
decoquinate	lincomycin*	tiamulin
deltamethrin	maduramicin	tilmicosin
diazinon*	monesin	toltrazuril
diclazuril	morantel	triclabendazole
dihydrostreptomycin	neomycin	trimethoprim*
dimethicone	nicarbazin	tylosin*
emamectin benzoate*	nitroxynil	
	**ip=immunological products	

About 70% of farm dairies and 70% of piggeries now apply effluent onto land within the Waikato region (Ministry of Agriculture and Forestry 2001). Sarmah *et al*. (2006a) reviewed veterinary antibiotics used in New Zealand and evaluated sales and usage data reported by the Ministry of Agriculture Forestry (1999; 2001). A more recent survey in 2008-09 (Ministry of Agriculture and Forestry 2010) listed the individual active ingredients and the statistical data were combined into antibiotic family groups for reasons of commercial confidentiality. Table

2.2, 2.3 and Table 2.4 show sales in kilograms by active ingredient, approved species, and approved route of administration, respectively (Ministry of Agriculture and Forestry 2007; 2010).

Table 2.2: Sales in kilograms by active ingredient from 2002 to 2009.

Antibiotic Family	**2002/03**	**2003/04**	**2004/05**	**2005/06**	**2006/07**	**2007/08**	**2008/09**
Aminoglycosides	2325	2134	1898	1658	1620	1253	1217
Bacitracin	26579	27264	18057	29528	22757	18820	21733
Cephalosporins	1176	1076	1202	1520	1443	1739	1528
Clavulanic Acid	73	141	184	187	195	190	213
Fluoroquinolones	23	28	27	34	33	40	41
Fusidic Acid	1	2	2	4	4	2	2
Macrolides/Lincosamides	6279	5011	5764	5235	4680	5369	5439
Nitrofurans	168	111	42	10	9	19	6
Nitro-imidazoles	60	105	61	72	146	108	49
Novobiocin	5	6	4	3	2	3	1
Other	57	56	9	461	615	478	336
Penicillins	11065	13708	13765	14979	14676	16383	15552
Sulphonamides / Trimethoprim	2998	4429	4702	5251	5219	4615	5187
Tetracyclines	1509	3458	4298	3923	4788	4002	4492
Virginiamycin	4	28	16	16	16	12	14
Total	**52702**	**57557**	**50032**	**62883**	**56203**	**53031**	**55809**

Source : Ministry of Agriculture and Forestry (2010)

In 1999, animals accounted for about 57% of nearly 93 000 kg of antibiotics used in New Zealand. About 34% of these antibiotics were ionophores, which have a quite distinct mode of action from other groups. Without the inclusion of ionophores, total use of antibiotics in animals accounted for about 47% out of the remaining 75 000 kg (Ministry of Agriculture and Forestry 1999). In 2005-06, sales figures showed nearly 62 833 kg of veterinary antibiotics in use. About 50% of these antibiotics were Bacitracin, which is extensively used in the poultry industry. Other antibiotics such as macrolides/lincosamides, tetracyclines, sulfonamides, penicillins, cephalosporins, aminoglycosides and others contributed to the remaining 50%. Following that year there was 10% decrease in sales reported for the year 2006-07 and from then on the usage was about 55 000 kg for 2007-08 and 2008-09 (Ministry of Agriculture and Forestry 2010).

Table 2.3: Sales in kg by active ingredient by approved species in 2006-07.

Family	Companion	Cattle	Pigs/ Poultry	Multiple-species	Other	Total
Aminoglycosides	30	358	141	854	5	1389
Bacitracin	1	0	22756	0	0	22757
Cephalosporins	353	965	0	126	0	1443
Clavulanic Acid	119	41	0	35	0	195
Fluoroquinolones	18	4	0	12	0	33
Fusidic Acid	4	0	0	0	0	4
Macrolides/Lincosamides	14	118	293	4132	0	4557
Nitrofurans	0	0	6	0	2	8
Nitro-imidazoles	11	0	133	0	3	146
Novobiocin	0	2	0	0	0	2
Other	0	6	599	93	0	698
Penicillins	404	4338	33	8169	0	12945
Sulphonamides/Trimethoprim	16	188	14	2246	2100	4564
Tetracyclines	27	13	0	3464	4	3506
Virginiamycin	0	0	0	0	0	0
TOTAL	**997**	**6033**	**23975**	**19131**	**2113**	**52248**

Table 2.4: Sales (kg) by active ingredient family and approved route of administration 2006-07.

Family	Oral	Injectable	Feed	Water	Intra-mammary	Other	Total
Aminoglycosides	96	629	88	116	450	10	1389
Bacitracin	0	0	22756	0	0	1	22757
Cephalosporins	353	126	0	0	902	63	1443
Clavulanic Acid	150	21	0	0	25	0	195
Fluoroquinolones	16	17	0	0	0	0	33
Fusidic Acid	0	0	0	0	0	4	4
Macrolides/Lincosamides	14	1233	3214	53	45	0	4557
Nitrofurans	0	0	6	2	0	0	8
Nitro-imidazoles	14	0	126	7	0	0	146
Novobiocin	0	0	0	0	2	0	2
Other	0	16	599	0	84	0	698
Penicillins	522	8400	0	33	3964	27	12945
Sulphonamides/Trimethoprim	4243	151	14	0	0	156	4564
Tetracyclines	27	1425	1714	33	132	176	3506
Virginiamycin	0	0	0	0	0	0	0
TOTAL	**5434**	**12018**	**28517**	**242**	**5602**	**437**	**52248**

Source : Ministry of Agriculture and Forestry (2007)

Data collected from the 1999 survey and from a more recent survey in 2009 by the Ministry for Agriculture and Forestry are shown as the percentage contribution of each of these antibiotics to the total (Table 2.1). Total sales in the year 2008-09 had decreased by 11.9% compared with to 2005-06 sales. Most of the fluctuation in sales in this period was driven by sales of zinc Bacitracin. In 2008-09 this antibiotic represents 36% of all antibiotic by weight, 94% of antibiotic usage in the pig and poultry category, and 93% of in-feed and water usage. Sales of sulfonamides have steadily increased since 2002 and a 10% increase in sales was seen from 2007-08 to 2008-09. Sales of macrolide/lincosamides have decreased over the years driven by in-feed sales of tylosin. In 2006-07, tylosin made up 93% of the total quantity of the macrolide/lincosamide group. Approximately 29% of this was in injectable formulations, which have significant use patterns in dairy cattle for treatment of conditions such as mastitis. 71% of tylosin sales, and of the whole macrolide/lincosamide group, are products intended for in-feed and in-water medication (Ministry of Agriculture and Forestry 2007; 2010).

2.3.1 Regulation and legislation in New Zealand

The Agricultural Compounds and Veterinary Medicines (ACVM) group is responsible for the registration of agricultural compounds and veterinary medicines, and monitoring their importation, manufacture, sale and use in conjunction with the Animal Remedies and Pesticides Boards. The ACVM Act (1997) is part of the wider reform of agricultural legislation agreed to by the government of New Zealand in the late 1980's. It controls the agricultural compounds and veterinary medicines used in association with animals and plants. The ACVM Act replaces the Animal Remedies Act 1967, the Stocks Foods Act 1946, the Fertilizers Acts 1960 and 1982, together with the Hazardous Substances and New Organisms (HSNO) Act 1996 and the Pesticide Act 1979. The ACVM group is focused on its core functions, which are primary produce, agricultural security, and animal welfare. The group, headed by the Director-General of agriculture and forestry, also ensures that products are sold with sufficient information so that they can be used safely and appropriately. On its part, the New Zealand Food Safety Authority (NZFSA) conducts routine sampling programme to monitor animals for compliance with the approved maximum residue limit (MRL) (Ministry of Agriculture and Forestry 2007).

Note: During the time leading up this thesis submission, two of the above mention agencies have not existed for some months, so the information is out of date. NZFSA and MAF no longer exists as a separate entity but as a part of the ministry of Primary Industries (MPI).

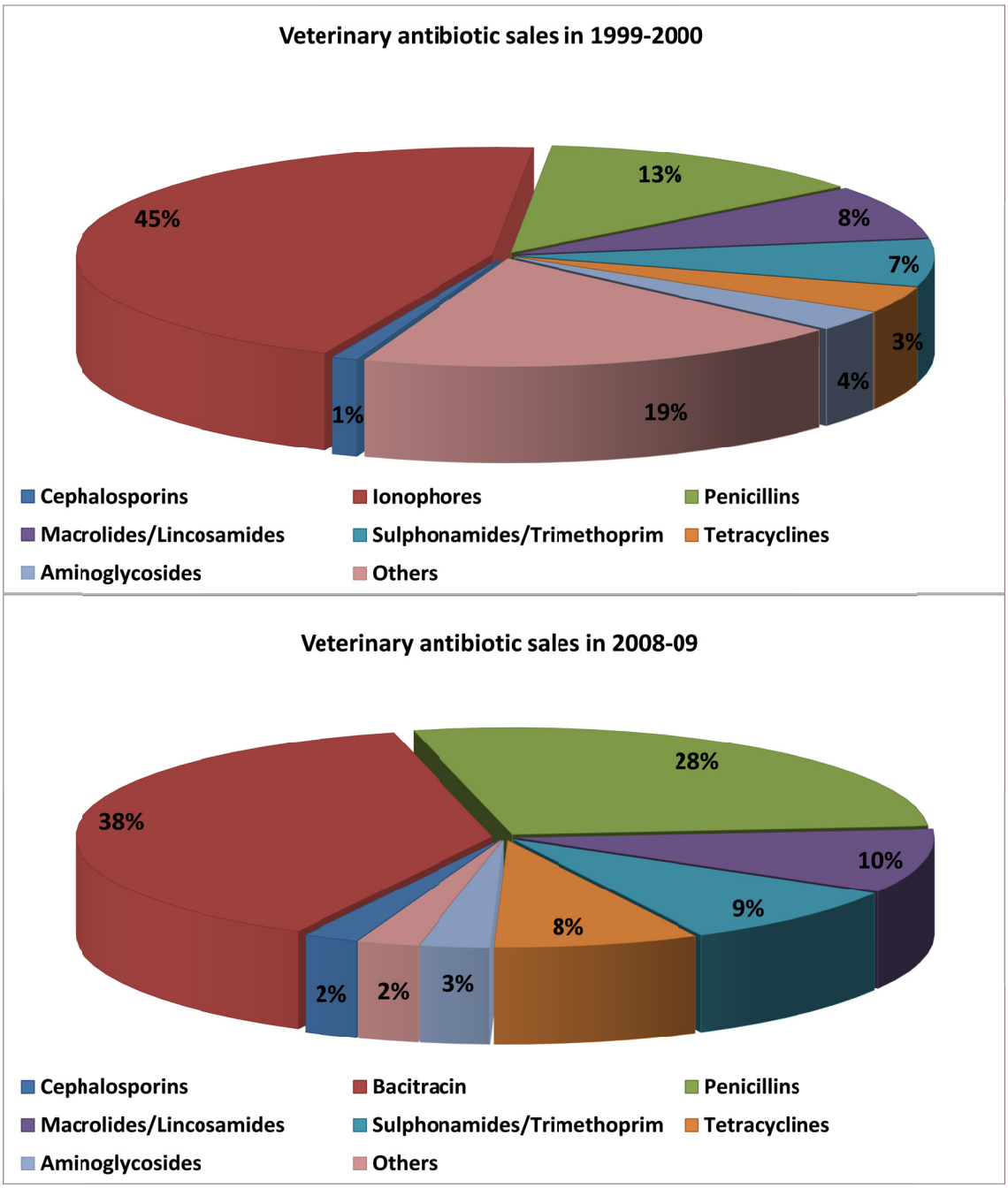
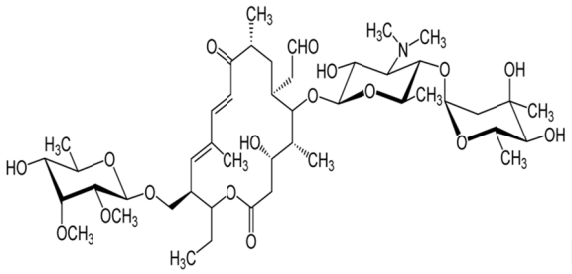


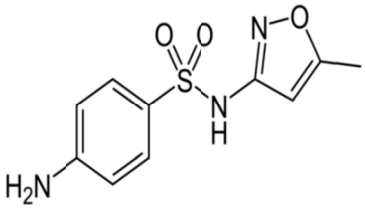
Figure 2.1: Total antibiotic sales (%) for agricultural industries in New Zealand. Amounts indicate percentages of total antibiotics in the years 2000 and 2008-09.

2.4 Selection of veterinary antibiotics for this work

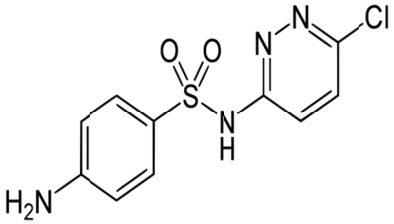
From the high usage antibiotic groups (macrolides and sulfonamides), some important representative compounds were selected: TT, SMO, SCP, and SM. Together they account for nearly 17% of the annual 55 000 kg usage (Ministry of Agriculture and Forestry 2010).



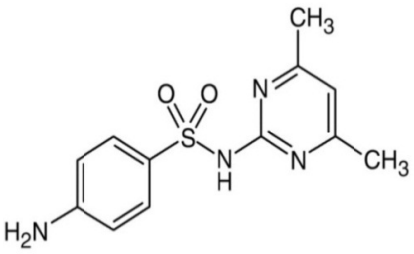
Tylosin (TT)



Sulfamethoxazole (SMO)



Sulfachloropyridazine (SCP)



Sulfamethazine (SM)

Figure 2.2: Molecular structures of the antibiotics used in this study.

Table 2.5: Selected chemical properties of the antibiotics used in the study.

Properties	**TT**	**SMO**	**SCP**	**SM**
Molecular formula	$C_{48}H_{77}NO_{17}$	$C_{10}H_{11}N_3O_3S$	$C_{10}H_9N_4ClO_2S$	$C_{12}H_{14}N_4O_2S$
Molecular weight (g mol^{-1})	917.1[a,b]	253.28	284.72	278.3
Water solubility (mg L^{-1})	5000[a]/6000[d]	600[e]	7000[i]/8235[j]	1500[a]/1900[c]
Vapour pressure (Pa)[k]	2.65×10^{-32}	1.74×10^{-5}	1.77×10^{-7}	9.1×10^{-7}
Log K_{ow}	3.5[a]/3.41[b]	0.89[f,g]	0.31[i]	0. 89[a]/0.80[b]/0.28[g]
pK_{a1} / pKa_2at 25 °C	7.1[b]/7.4[h] 3.3[L] /7.4[L]	1.7[e] / 5.6[e] 1.83[g] / 5.57[g] 1.85[f] / 5.2[f]	1.87[L] /5.5[j]	2.65[a] / 7.65[a] 2.28[g] / 7.42[g]

[a] Tolls (2001); [b] Sarmah *et al*.(2006a); [c] Dolliver *et al*.(2007); [d] Allaire *et al*. (2006); [e] Çalışkan and Göktürk (2010); [f] Hou *et al*. (2010); [g] Figueroa-Diva *et al*. (2010); [h] Kolz *et al*. (2005); [i] ter Laak *et al*. (2006); [j] Lin *et al*. (1997); [k] U.S. Environmental Protection Agency (2012); [L] Babić *et al*. (2007).

2.4.1 Properties of the selected antibiotics

The physico-chemical properties of the antibiotics used in this study are shown in Table 2.5 and their molecular structures in Figure 2.2. TT belongs to the macrolide group, and is composed of a 16-membered highly substituted lactone ring with several sugars attached. It is used as a feed additive to treat digestive infections and also as growth promoter in cows, pigs and poultry (Clay *et al.* 2005). TT is unstable in acidic and alkaline media and relatively stable under neutral pH conditions. TT pK_{a2} (7.1) corresponds to the dimethylamine acquiring a proton, so at pH values below 7 it should exist as tylosin^{+}. Its pK_{a1} is thought to be a simple artefact of the fact that TT is produced as tartarate, so the pKa_1 value of TT (3.31) may correspond to the tartarate moiety (Babić *et al.* 2007). In manure, where the pH is usually neutral to alkaline, the compound is in equilibrium between its neutral and protonated forms and the degree of protonation increases with decreasing pH (Loke *et al.* 2002). TT is water soluble and when in the protonated form has the ability to bind to negatively charged matrices such as soils and manure.

Sulfonamides are used to prevent and treat diseases and as feed additives to promote growth in livestock especially in pig farms (concentrated animal feeding operations). SA's contain a benzene ring substituted with an amine moiety ($–NH_2$), and a sulfonamide group ($–SO_2NH_2$). The amine and sulfonamide groups must be *para* to one another for the SA to possess antibacterial properties. The most commonly used sulfonamides are SMO, SCP and SM (Sarmah *et al.* 2006a). Sulfonamides may be relatively insoluble in water and solubility can range from 0.1 to 8 g L^{-1} and is compound specific within this group (Sarmah *et al.* 2006a). In fact SCP is more soluble than TT (Table 2.5). Although the sulfonamides are amphoteric in nature, they generally function as weak acids in the physiological pH range (Thiele-Bruhn 2003). They contain both a basic amine and an acidic amide group. They are characterised by two pKa values indicating deprotonation of the $–NH_3^{+}$ at a pH of 2 -3 and deprotonation of the $R_1SO_2NHR_2$ moiety at a pH of 5-11 (Ingerslev and Halling-Sørensen 2000). For this reason they are positively charged under acidic conditions, neutral between pH 2.5 and 6 and negatively charged in alkaline conditions. Their transformation would follow a pattern H_2A^{+} < HA (neutral) < A^{-} (Babić *et al.* 2007).

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2.5 Occurrence and exposure pathways for veterinary antibiotics in the environment

Veterinary antibiotics can enter the environment by different pathways ranging from the manufacturing and treatment processes, to the disposal of unused drugs which are sometimes washed off into the sewage system (Kummerer 2001). The most important routes of entry (Figure 2.3) into the environment are likely to be from the direct excretion in urine or faeces from animals onto pasture lands and the deliberate application of the wash-off from livestock animals onto lands (Ingerslev and Halling-Sørensen 2000; Thiele-Bruhn 2003; Sarmah *et al.* 2006a). Inputs from the manufacturing process are unlikely to cause much contamination in the United States and European Union, where manufacture and formulation are subject to tight regulatory controls (Boxall *et al.* 2003). Substances absorbed by an animal can be metabolised but the degree of metabolism will depend on the type of substance, the species treated, and the age and condition of the treated animal. They are mostly excreted as parent compounds, however their metabolites might be also bioactive (Kummerer 2003).

Excretion rates following the passage through the gastro-intestinal tract are likely to be in the range of 40 to 90% for sulfonamides and tetracyclines (Winckler and Grafe 2001). Information on the excretion rate of various antibiotics by livestock is available in the literature (Halling-Sørensen *et al.* 2001; Jjemba 2002; Zuccato *et al.* 2005). For instance, Haller *et al.* (2002) reported that the excretion rates varied even among a single antibiotic substance depending upon, the treated species and the mode of application, as was shown when sulfonamides were administered to pigs (Haller *et al.* 2002). It has been reported that in some cases as much as 80% of the antibiotics administered orally to livestock passes through the animal unchanged (Halling-Sørensen *et al.* 1998; Kummerer 2001; Sarmah *et al.* 2006a). Urinary or fecal excretions can contain the unchanged product, and its metabolites, and these eventually end up in a waste lagoon where they are stored and applied to the field as an organic matter supplement or fertilizer. As a result there is a constant loading of these contaminants into the environment through manure spreading. Often soil and water bodies are the most affected environmental matrices by antibiotic contamination. Another significant source of antibiotics in the environment is their use in aquaculture for fish production where they are directly introduced into surface waters (Samuelsen *et al.* 1994; Hamscher *et al.* 2006). Many of these antibiotic compounds persist in the soils and wastewater unchanged

(Gavalchin and Katz 1994; Kummerer *et al.* 2000), and is not transformed, even after aeration of manure at increased ambient temperature (Winckler and Grafe 2001). However, recently Radke *et al*. (2009) have reported the presence of transformed products in a sediment water effluent.

Antibiotics and their daughter products that are directly exposed to the environment may be transported to the nearby streams, lakes or other aquatic bodies or leach downward through the soil during rainfall (Alder *et al.* 1997; Hirsch *et al.* 1999). A number of antibiotics used for veterinary purposes are often used for humans too (sulfonamides), and if disposed of incorrectly, may finally end up at municipal sewage treatment plants (Tolls 2001). The tendency for veterinary antibiotics to be distributed into the air is negligible because of their low vapour pressure. However, aerosol dust particles from excreta could contribute to the dispersion of these compounds and needs further investigation (Hamscher *et al.* 2003).

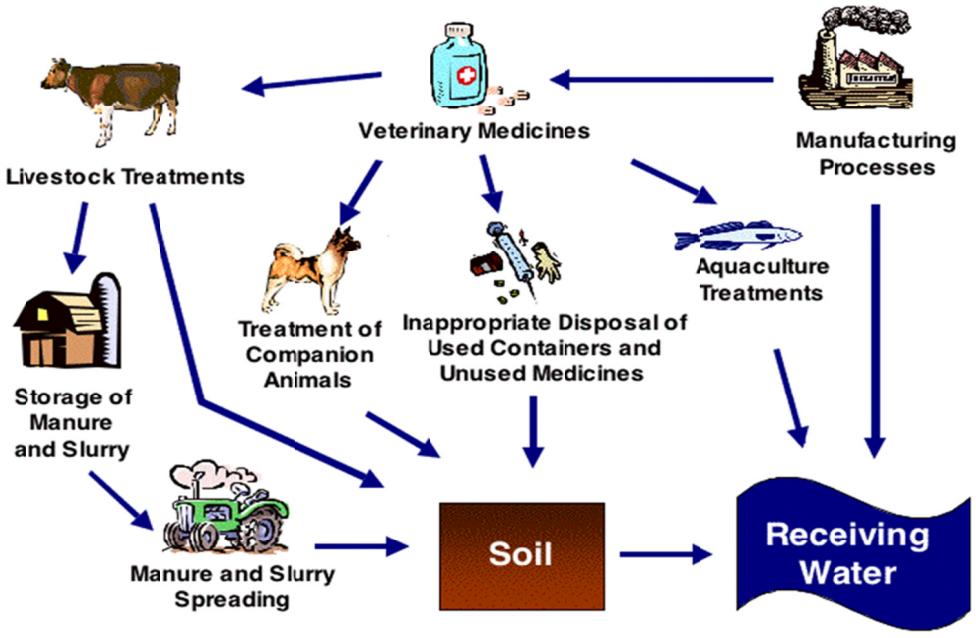


Figure 2.3: Anticipated exposure pathways for veterinary antibiotics in the environment adapted from Boxall *et al*. (2003).

2.6 Detection of veterinary antibiotics in different environmental matrices.

Veterinary antibiotics are considered emerging contaminants along with surfactants, endocrine disrupting chemicals (EDC), pesticides, algal toxins, and pharmaceuticals (Snow *et al.* 2007). Veterinary antibiotics are often found in the environment at parts-per-trillion and parts-per-billion concentrations which are low when compared with other hydrophobic persistent organic contaminants like PCBs, PAHs and DDT (Richardson 2008). This often makes detection of these antibiotic residues difficult, but the recent advances in analytical instrumentation have increased the ability to detect and quantify organic contaminants at trace levels in various environmental matrixes such as soils, manure and water (Haller *et al.* 2002; Diaz-Cruz and Barcelo 2006; Diaz-Cruz and Barcelo 2007; Kim and Carlson 2007a).

Detection of these compounds in different environmental matrices such as soils, sediments, manure and waste water has been challenging owing to the high detection limits of the instruments and interferences from co-eluting compounds as a result of insufficient clean-up steps after extraction. However, new instrumentation, coupled with the development of new extraction and clean-up techniques, provides accurate measurement tools. As a result, a number of recent studies have reported the occurrence of veterinary antibiotics in surface, groundwater, waste-water effluents, soil, manure, and marine sediments at trace concentrations, and these reports are discussed in the following section in detail.

2.6.1 Surface and waste waters

Many recent studies have reported the presence of veterinary antibiotics in pristine river waters. For instance, Kim *et al*. (2009b) detected the presence of antibiotics in surface water from the Mankyung River in South Korea. Similarly, another study accounted for a widespread contamination of macrolides, sulfonamides, and trimethoprim in tropical water along the Mekong delta in Vietnam (Managaki *et al.* 2007). In the same study, the authors also reported the detection of a variety of antibiotics in the Tamagawa River in Japan, for comparison purposes. SM was detected in high concentrations in the Mekong delta, indicating that it is heavily used in agriculture. Another study reported that various hydrological conditions, dense animal, and human population along the river course could be

responsible for the occurrence of antibiotics in the Seine river (Tamtam *et al.* 2008). Gulkowska *et al*. (2007) detected tetracycline, erythromycin, norfloxacin, and trimethoprim in Hong Kong coastal waters.

In Europe, an earlier study by Alder *et al*. (1997) detected residues of SM and other groups of antibiotics in Swiss surface waters, which the authors attributed to runoff from land-applied manure. The concentrations of SM measured in a lake surrounded by intensive animal husbandry were higher than concentrations in the effluent of wastewater treatment plants in the area. This clearly indicates that SM in this case comes from animal manure origin. In the USA, a nationwide survey of pharmaceutical compounds revealed that among numerous other pharmaceuticals, a number of veterinary and human antibiotics were detected in 21 of 139 river water samples at concentrations of up to 0.7 $\mu g\ L^{-1}$ (Kolpin *et al.* 2002). Of these, large proportions were antibiotics used in animals as growth promoters, such as tylosin, tetracyclines, and sulfonamides. The frequency of detection was highest for sulfonamides and lincomycin, followed by tylosin. The concentrations of the individual compounds detected in this study were generally less than 1 $\mu g\ L^{-1}$. Tetracyclines and sulfonamides were detected, with the limit of detection being 0.1–0.3 $\mu g\ L^{-1}$ in soil water samples collected from agricultural land (Hamscher *et al.* 2006). Tylosin was also detected in river waters in Lombardy, Italy (Zuccato *et al.* 2000). Perret *et al*. (2006) detected sulfonamide antibiotics in 3 of 4 river samples at concentration levels up to 0.4 $\mu g\ L^{-1}$ and in 4 samples of bottled mineral water with a maximum concentration of 0.08 $\mu g\ L^{-1}$. Samples from lake surface water and municipal drinking water did not have detectable levels of sulfonamides. Veterinary antibiotics have also been detected in watersheds in the recent past (Kim and Carlson 2006; Arikan *et al.* 2008; Watkinson *et al.* 2009).

Antibiotics have also been the focus of several studies on wastewater effluents; wastewater treatment plants are not optimized for complete removal of the antibiotics, allowing them to re-enter rivers from these discharges following treatment. Antibiotics have been detected in the $\mu g\ L^{-1}$ range in domestic sewage and in the effluent of sewage treatment plants (Ternes 1998; McArdell *et al.* 2003; Miao *et al.* 2004; Ghosh *et al.* 2009). Lindberg *et al*. (2005) used SPE with LC/ESI-MS/MS to measure 12 antibiotics in wastewater treatment plants in Sweden. Sulfamethoxazole and trimethoprim concentrations were similar in the raw sewage and the final effluent, suggesting only partial removal of these antibiotics. Sulfamethoxazole

was detected downstream at concentration of 300 ng L^{-1} in an investigation to determine the efficiency of a wastewater treatment plant (WWTP) in New Mexico. The raw wastewater, treated effluent, and river water both upstream and downstream of the WWTP discharge were analysed using SPE LC/MS/MS (Brown *et al.* 2006a). Elsewhere, six different antibiotics were detected in surface water sites adjacent to a WWTP at concentrations < 1.3 µg L^{-1} with two antibiotics (sulfamethoxazole and tetracycline) detected in ground water wells adjacent to the WWTPs (Karthikeyan and Meyer 2006). Another recent study showed that effluent samples from four wastewater treatment plants in Greece contained trimethoprim and sulfamethoxazole up to 0.4 µg L^{-1} (Botitsi *et al.* 2007).

2.6.2 Agricultural soils, manure and sediments

Low levels of veterinary antibiotics have been detected worldwide in soils and manure and this topic has been reviewed recently (Thiele-Bruhn 2003; Sarmah *et al.* 2006a; Kemper 2008; Snow *et al.* 2008). Residues of sulfonamides and tetracycline antibiotics either as metabolite or parent compound were detected in soil fertilised with liquid manure (Hamscher *et al.* 2005). Pawelzick *et al*. (2004) conducted a survey on the occurrence of various tetracyclines and SM in sandy soils fertilised with liquid manure in North-Western Germany. The authors reported concentrations for the tetracyclines to range 27 µg kg^{-1} to 443 µg kg^{-1}, and 4.5 µg kg^{-1} for SM antibiotic in the top 0-30 cm soil. Kemper *et al*. (2008) failed to detect a wide range of antibiotics in manure and leachate from two conventional and two organic dairy farms in northern Germany, while Aust *et al*. (2008) found SM up to 9.99 µg g^{-1} and chlortetracycline up to 0.4 µg g^{-1} but did not detect tylosin in manure and soil from two Canadian feedlots administering these antibiotics. In Austria, a risk assessment study was carried out to detect pharmaceutical contamination from pigs, chicken and turkey manure and manure fertilised soils. The samples showed the presence of, sulfonamides trimethoprim and fluoroquinolone antibiotics (Martinez-Carballo *et al.* 2007). In Northern Colorado a wide range of antibiotics was tested in water and sediment samples and results showed that the highest concentrations in both media occurred in the winter, when flows and temperatures were low. The highest concentrations of veterinary antibiotics were mainly found downstream of agricultural activity. Pie *et al*. (2006) found tetracyclines (45.7-399.1 µg L^{-1} total) and sulfonamides (3.5-22.4 µg L^{-1} total) in sediments to be highest at sites impacted by urban and agricultural activity.

The study also reported sediment samples to have higher concentrations than water samples of the same site (Kim and Carlson 2007a). Sulfonamide concentrations in manure ranged between 10 μg kg^{-1} and 100 mg kg^{-1} (Pfeifer *et al.* 2002; Jacobsen and Halling-Sørensen 2006; Martinez-Carballo *et al.* 2007), and concentration of 12.4 mg L^{-1} was measured in pig slurry grab samples for the sulfonamide SM (Haller *et al.* 2002). In a soil and drainage water monitoring study after two consecutive years of pig slurry application onto arable land, SCP was detected in soil at concentrations up to 365 μg kg^{-1} (Kay *et al.* 2004). Sulfapyridine was detected at a concentration of 197 μg kg^{-1} in sludge (Gobel *et al.* 2005). In another German study, 90% of samples collected from the dust originating from a pig-fattening farm showed the presence of five groups of antibiotics, including tylosin, various tetracyclines, SM, and chloramphenicol, in total amounts up to 12.5 mg kg^{-1} dust (Hamscher *et al.* 2003).

2.6.3 Groundwater

Veterinary antibiotics have been detected in groundwater in a number of studies (Batt *et al.* 2006; Perret *et al.* 2006; Pojana *et al.* 2011). Meyer *et al*. (2003) detected one or more of these antibiotics, namely chlortetracycline, oxytetracycline, lincomycin, SM, trimethoprim, sulfadimethoxine, and the dehydrated metabolite of erythromycin, in four groundwater samples collected from six states in the USA. Multiple classes of antimicrobial compounds (tetracycline, macrolide, β-lactam, and sulfonamide) were also detected in groundwater samples collected near swine farms in the USA (Watanabe *et al.* 2010). Hirsch *et al*. (1999) reported that out of the 18 antibiotics under study, only residues of sulfonamide antibiotics were detected in four samples collected from an agricultural area in Germany, with two samples showing SM at concentrations of 0.08 and 0.16 μg L^{-1}. The authors attributed the detection of these compounds in the groundwater to veterinary medicine applications. Batt *et al*. (2006) studied the impact of a confined animal feeding operation in Idaho, US, on groundwater from six nearby wells. Two sulfonamide antibiotics, SM and sulfadimethoxine, were found in all wells at concentrations up to 0.22 μg L^{-1} for SM and 0.068 μg L^{-1} for sulfadimethoxine using SPE with LC/MS/MS analysis.

2.7 Analytical methods for analysing veterinary antibiotics in the environment

Veterinary antibiotics are classed as emerging contaminants because release into the environment has occurred for quite some time, but methods for their detection at environmentally relevant concentrations have only recently become available. The analysis of these typically polar contaminants in environmental matrices (water, wastewater, soils and sediments) is particularly challenging because of the low detection limits required, the complex nature of the samples, and difficulty in separating these compounds from interfering co-eluting compounds. New extraction and clean-up techniques, coupled with improvements in instrumental technologies provide the needed sensitivity and specificity for accurate measurement as seen in a number of studies (Jacobsen *et al.* 2004; Blackwell *et al.* 2004a; Blackwell *et al.* 2004b; Diaz-Cruz and Barcelo 2005; Diaz-Cruz and Barcelo 2007). Recently a number of reviews have reported the detection, extraction and trace level analysis of veterinary antibiotics using different analytical instruments (Richardson 2006; Richardson 2008; Richardson 2010). A detailed review of recently developed methods for the analysis of veterinary antibiotics has been included in Chapter 3. However, in this chapter they have been summarised in Table 2.6.

Table 2.6: Selected examples of literature data on extraction of veterinary antibiotics in different environmental matrices. LOD = limit of detection; LOQ = limit of quantitation.

Compound	Sample	Extractant	Column type	Operating conditions	Recovery LOD/LOQ	Reference
Tylosin	Soil	Methanol	Supelco C_{18}, 150 mm × 4.6 mm, 5 µm	ACN:0.2 M tetrabutylammonium hydrogen phosphate: 0.2 M H_3PO_4: water (23:5:5:67), 1 mL min^{-1}, 25°C, 25 µL; UV 280 nm	95% ±14% LOD = NR	Sassman *et al*. (2007)
Tylosin	Manure	Methanol	YMC-Pack ODS-AQ, 250 mm × 4.6 mm, 5 µm	2.25% sodium perchlorate (pH 2.5 with HCl):ACN (60:40), 1 mL min^{-1}, 30°C, 100 µL; UV 290 nm	100.2-106.7% LOQ =1 mg L^{-1}	Loke *et al*. (2002)
Tylosin	Manure, soil	Phosphate buffer / Methanol (1:2)	Phenomenex-ODS, Prodigy 250 mm × 4.6 mm, 5 µm	60% A (2% CH_3COOH in 0.08 M tetra-butylammonium bromide) and 40% B 75% MeOH, 25% ACN, 100 µL; isocratic; UV 282 nm	74%, 82% LOD < 100 µg kg^{-1}; LOQ < 250 µg kg^{-1}	De Liguoro *et al*. (2003)
Sulfonamides	Marine sediment	0.1 M NaOH	ODS-Hypersil C_{18}, 100 mm × 4.6 mm, 3 µm	0.05 M H_3PO_4 /ACN, Gradient; UV 270 nm	LOD = NR 79.4%, 80%	Samuelsen *et al*. (1994)
Sulfonamides	Sewage sludge	Water	Phenomenex ODS2 C_{18}, 250 mm × 4.6 mm, 5 µm	ACN:16.7 mM CH_3COOH (pH 5 with 4 M NaOH) 1 mL min^{-1}, gradient; UV 280 nm	LOD 19-52 µg L^{-1}; LOQ 67-174 µg L^{-1} 98 -106%	Ingerslev and Halling-Sørensen (2000)
Sulfapyridine	Soil	Methanol	RP C_{18}, 250 mm × 4.6 mm, 5 µm	0.01 M H_3PO_4/MeOH, 1 mL min^{-1}, gradient, 22°C, 10 µL; UV/FLD 265 nm	LOD 25 µg L^{-1} Recovery = > 90%	Thiele (2000)

Sulfonamides	Soil	Methanol	Lichrospher RP- C_{18}, 150 mm × 4.6 mm	PO_4buffer/ACN; isocratic 1 mL min^{-1}; UV 265-269	22-89% LOD = NR	Figueroa-Diva *et al*. (2010)
Oxytetracycline, sulfachloropyridazine tylosin	Ground and Surface water	EDTA–McIlvaine (50:50) + Methanol	GENESIS C_{18}, 150 mm × 4.6 mm, 5 µm	ACN/THF/TFA gradient, 1 mL min^{-1}, 20 µL; UV 285 nm	99.9-105%; 71.6 – 94.9% LOD 0.25-0.35 µg L^{-1}	Blackwell *et al*. (2004a)
Oxytetracycline, sulfachloropyridazine tylosin	Soils and pig slurry	EDTA–McIlvaine (50:50) + Methanol	GENESIS C_{18}, 150 mm × 4.6 mm, 5 µm	MeOH/THF/TFA gradient, 1 mL min^{-1}; HPLC-UV and FLD	68-85%; 47-105% LOD = 18-140 µg kg^{-1}	Blackwell *et al*. (2004b)
Sulfonamides and trimethoprim	Animal manure	Ethyl acetate	Nucleosil C_{18}, 125 mm × 3 mm; 5µm	NH_4OAc and ACN, 0.25 mL min^{-1}, gradient, 50 µL; LCMS, +ve ion mode	50-90% ±5 LOQ 0.1 mg kg^{-1}	Haller *et al*. (2002)
Sulfonamides and trimethoprim	Feed premixes	Water	Phenomenex ODS 30% C, 150 mm × 4.6 mm, 5 µm	Gradient, MeOH and NH_4OAc, 20 µL	LOD 0.3 & 0.4 mg L^{-1}	Cancho Grande *et al*. (2001)
Sulfachloropyridazine and tylosin	Soil	$CaCl_2$	Supelco C_{18}, 150 mm × 4.6 mm, 5 µm	ACN:Water: 30:70, 0.7 mL min^{-1}; gradient UV-DAD 265; 230 nm	NR	Laak *et al*. (2006)
Sulfachloropyridazine	Soil, water	EDTA–McIlvaine (50:50) + Methanol	Supelco C_{18}, 150 mm × 4.6 mm, 5 µm	Gradient, 10 mM M H_3PO_4 and 30% ACN; UV 260, 285 nm, 20 µL	LOD 250 ng L^{-1} 99 -105%	Boxall *et al*. (2002)
Sulfathiazole	Clay minerals	0.01M $CaCl_2$	GROM-SIL 120 ODS-3 CP, 3 µm, 60 × 2 mm	Gradient, 0.25 mL min^{-1}; 50 µL; LCMS-ESI	LOQ 0.03 mg kg^{-1}	Kahle and Stamm (2007b)
Tylosin, sulfamethoxazole, sulfachloropyridazine and sulfamethazine	Soil	Dichloromethane, Methanol	Phenomenex C_{18} Luna, 150 mm × 4.6 mm, 5 µm & C_{18} Onyx, 100 mm × 4.6 mm, 5 µm	Isocratic/gradient 1 mL min^{-1}; 20 µL, ACN/THF/TFA; UV-275 & 290 nm	LOD 0.02 & 0.05 µg mL^{-1} LOQ 41.6 & 13.3 µg kg^{-1}	This study

2.8 Fate of veterinary antibiotics in soils

Once in the receiving environment, the fate of veterinary antibiotics is governed by three main processes: they 1) adhere to soil particles (sorption), 2) are broken down by chemical or biological processes (degradation), 3) become mobile and penetrate deep into soils to reach the water table (transport). The tendency for antibiotic to be distributed into the air is negligible because of their low vapour pressure. However, aerosol dust particles from excreta could contribute to the dispersion of antibiotics (Hamscher *et al.* 2003). Figure 2.4 depicts some of the possible dissipation pathways for antibiotic in soils. Since this study investigates the fate of only two groups of antibiotic, only studies relating to these antibiotics have been discussed in this review.

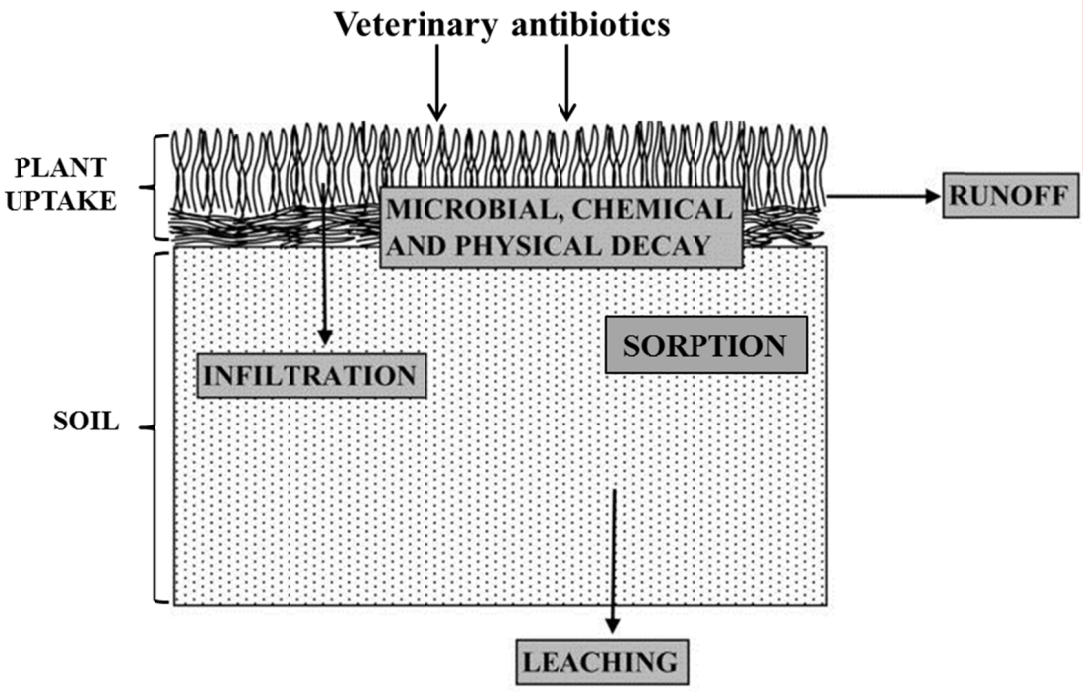


Figure 2.4: Dissipation pathways for antibiotics in soil modified from Magri and Haith (2009).

2.8.1 Sorption

Of all the processes governing the fate of veterinary antibiotics, sorption is by far the most important as it reduces the bioavailability of the material thereby affecting other processes such as degradation and transport (Tolls 2001). Sorption can lead to the material being retained in soil for a long time, thus making the chemical unavailable for the microbes to degrade, and reduces its mobility (Tolls 2001; Sarmah *et al.* 2006a; Teixido *et al.* 2011).

Furthermore, this process can also give rise to antibiotic resistant microbial populations causing health problems in animals and humans due to the failure of antibiotics in medical activities (Halling-Sørensen *et al.* 1998; Sengelov *et al.* 2003). Sorption of organic contaminants to soil need not necessarily be due to the organic matter content of the soil; it is known to be influenced by the sorbent properties such as hydration status, grain size, surface coatings of the grains and surface charge. In addition, factors such as pH, ionic strength, temperature of the solution and the presence of co-solutes can also influence the overall sorption process (Pouliquen and Le Bris 1996; Gao and Pedersen 2005).

Sorption coefficients (K_d) for veterinary antibiotics to soils are known to vary considerably for a given compound in different soils (Holten Lützhøft *et al.* 2000). Sorption of veterinary antibiotics has been extensively studied over the past decade. Many reviews have been published describing the sorption behaviour of veterinary antibiotic in soils and sediments (Tolls 2001; Thiele-Bruhn 2003; Sarmah *et al.* 2006a; Snow *et al.* 2008; Kim *et al.* 2011). The reviews revealed the studies to be diverse in results, and attributed this to the adoption of differing experimental protocols in studies which makes it difficult to compare the derived sorption parameters. Many studies have been conducted on the sorption of macrolides (Table 2.7) and sulfonamides (Table 2.8) in soils. Tylosin has been found to be strongly sorbed to soil particles for example Sassman *et al.* (2007) reported K_d values ranging from 0.0022 to 5.52 L kg^{-1}, while earlier Rabølle and Spliid (2000) reported K_d values ranging from 8 to 128 L kg^{-1} for tylosin in soils.

Sulfonamides tend to be highly mobile and have the potential to leach and contaminate groundwater; K_d values ranged from 0.9 to 1.8 L kg^{-1} for SCP (Boxall *et al.* 2002), 8.3 L kg^{-1} for SM (Clay *et al.* 2005) and 9.1 to 150 L kg^{-1} in lake sediments for SMO (Hou *et al.* 2010). Kahle and Stamm (2007) determined the sorption properties of STZ in sterile manure, compost, and humic acid and observed that STZ sorption was strongly affected by contact time and pH, with sorption continuing after an initial fast sorption period of 1 day (Kahle and Stamm 2007a). Sorption to inorganic sorbents (clay and ferrihydrite) was an order of a magnitude lower than to the organic matter and highly dependent on pH (Kahle and Stamm 2007b). Kurwadkar *et al*. (2007) also found a strong pH dependency for sorption of STZ and SM in three sandy and loamy soils.

Table 2.7: Literature values for partitioning coefficients of tylosin in various environmental matrices.

Matrix	pH	f_{oc} (%)	K_d (L kg^{-1})	K_{oc} (L kg^{-1})	K_f	N	Equilibrium time (h)	Initial concentration (mg L^{-1})	Reference
Sandy soils and sandy loam soils	5.6-6.3	1.1-1.6	8.3-128	550-7990	2.3-7.0	0.8-0.9	24	1.25-25	Rabølle and Spliid (2000)
Soil1: 88% sand, 5% clay, 5% silt	----	1.4	----	----	2.03±0.9	0.67±0.07	24	25	Ingerslev and Halling-Sørensen (2001)
Soil2: 76% sand, 11% Clay, 11% silt	----	1.6	----	----	32.1 ± 13.2	0.67 ± 0.06	24	25	Ingerslev and Halling-Sørensen (2001)
Soil1:clay loam: 34% clay	----	4.4	92	2090	----	----	----	1000	cited from Kolz *et al*. (2005)
Soil2: sandy loam 10.4% clay	----	2.2	66	3000	----	----	----	1000	cited from Kolz *et al*. (2005)
Fresh swine Manure (solid)	----	42	240	571	----	----	24	200	Loke *et al*. (2002)
3 Soils	6.3;6.1;7.1	2.4;2.6;2.7			1155-1385	0.85;0.92;0.93	24	22;44;217	Clay *et al*. (2005)
2 Sands	4.4;6.8	0.36;0.28			7.0-23	0.61-1.3	24	22;44;217	Clay *et al*. (2005)
3 Manures					160-770		24	22	Clay *et al*. (2005)
Swine manure -1:	----	10	38.6-91.2	241-565	39.9-67.7	0.83-1.32	24	1-55	Kolz *et al*. (2005)
Swine manure -2:	----	10	107.5-106	831-827	99.5-182.5	0.85-1.02	24	1-55	Kolz *et al*. (2005)
10 Soils	3.41-7.38	2.2-12.2	10.4-397	----	----	----	48	3.0-7.5	Laak *et al*. (2006)
Sandy loam; Heavy clay	5.7;5.6	3;1.1	----	----	----	----	4-24	3000	Allaire *et al*. (2006)
Soil: clay loam	6.98	3.1	156±8	----	72-102	0.96	48	3.0	Laak *et al*. (2006)
Soil: loamy sand	6.82	2.2	8.9±0.4	----	6.4-7.5	1.07	48	3.0	Laak *et al*. (2006)
Soil: Silty clay, clay, sand (Tylosin)	4.4-7.4	0.36-2.9	2.23-5520	----	0.41-168	0.40-0.68	60	4.5-40	Sassman *et al*. (2007)
Silty clay, clay, sand (Tylosin D)	4.4-7.4	0.36-2.9	547-4745	----	9.98-2560	0.53-0.79	60	4.5-40	Sassman *et al*. (2007)
Silty clay, clay, sand (tylosin A -Aldol)	4.4-7.4	0.36-2.9	597-6520	----	10.9-465	0.53-0.74	60	4.5-40	Sassman *et al*. (2007)
3 Soils	6.0;6.8;8.1	----	65;42;24	----	----	----	24	25	Hu and Coats (2009)
Hog manure	----	----	285	----	----	----	24	25	Hu and Coats (2009)

Table 2.8: Literature values for partitioning coefficients of sulfonamides in various environmental matrices.

Matrix	Sulfonamide	pH	f_{oc} (%)	K_d (L kg^{-1})	K_{oc} (L kg^{-1})	K_f	N	Equilibrium time (h)	Initial concentration (mg L^{-1})	Reference
Silt Loam 1 Silt Loam 2	sulfapyridine	7.0 6.9	1.6 2.4	1.6 7.4	101 308	----	----	----	----	Thiele (2000)
Silt loam	sulfadiazine sulfadimidine sulfanilamide sulfadimethoxine sulfachloropyridazine	7.0	1.6	2.0 2.4 1.7 2.3 10	124 149 106 143 129	----	----	----	----	Thiele-Bruhn *et al*. (2004)
Clay loam	sulfadiazine sulfadimidine sulfaisoxazole sulfathiazole	6.2	3.1	4 2.5 3 1.5	81 97 48 97	----	----	----	----	cited from Thiele-Bruhn (2003)
Clay loam; Sandy loam	sulfachloropyridazine	6.5;6.8		0.9 ; 1.8	----		0.97;0.91	48		Boxall *et al*. (2002)
10 Soils	sulfachloropyridazine	3.41-7.38	2.2-12.2	0.4-34.8	----	----	----	48	1.0-3.0	ter Laak *et al*. (2006)
Soil: clay loam; loamy sand	sulfachloropyridazine	6.98;6.82	3.1;2.2	16.6;8.1	----	2.5;1.5	0.93;0.95	48	3.0	Laak *et al*. (2006)
Sand Soil 1 Soil 2	sulfadimethoxine	6.97 5.03 4.66	0 1.5 2.1	0.4 10.4 25.8	 1195 2148	3.1 2.1 14.5	0.64 1.32 1.10	48	0.05-0.5	Sanders *et al*. (2008)
Silt Loam; Sand	sulfamethazine sulfachloropyridazine	7.5;7.2 7.5;7.2	1.8;0.94 1.8;0.94		---- ----	6.75;4.21 6.11;3.97	0.79;0.75 0.88;0.87	14 14	1-100 1-100	Accinelli *et al*. (2007)
Quartz sand; 4 soils	sulfamethazine	7.0 7.5-8.2	0 5.3-9.2	7.5 16.6-206	---- 228-2241	----	----	168	0.012-1.22	Fan *et al*. (2011)
Lake Sediment	sulfamethoxazole	----	----	9.1-154		21-230	0.11-0.82	168	1-120	Hou *et al*. (2010)
Activated carbon	sulfamethoxazole	2-10	----	----	----	68.89	0.31	5	20; 10	Çalışkan and Göktürk (2010)
4 Soils	sulfamethazine	5.4-8.2	0.1-3.8	0.23-3.77	30.4-639	0.13-4.77	0.52-1.17	24	1.1-22.2	Lertpaitoonpan *et al*. (2009)
Soil	sulfadiazine	6.1-8.0	3.3	----	----	11-78	0.63-1.11	0.75-153	0.044-12.94	Wehrhan *et al*. (2010)
Montmorillonite; Illite; Ferrihydrite	sulfathiazole	----	0.3;0.3;2.7	4; 46;4 19;184;20	----	----	----	24 336	----	Kahle and Stamm (2007b)

2.8.2 Degradation

Both biotic and/or abiotic degradation processes are important components of any antibiotic mass-balance or exposure model, and rates of degradation are required for the risk assessment of these chemicals. Besides the physicochemical properties (structure, concentration and solubility) of the antibiotics, the extent and kinetics of degradation are also considerably influenced by adsorption to soil (Tolls 2001). Fixation of antibiotic compounds to pores of the soil matrix may effectively protect them from biodegradation (Gavalchin and Katz 1994; Halling-Sørensen *et al.* 2002). The degradation process may be affected by environmental conditions, such as temperature, rainfall, humidity (Holten Lützhøft *et al.* 2000; Sarmah *et al.* 2006a) and the nature of soil properties (Loftin *et al.* 2008). For example, when moisture content was increased from 60 to 100% of its field capacity, oxytetracycline (OTC) degradation was found to increase, and increasing temperature also greatly accelerated OTC degradation in manure (Wang and Yates 2008). Sulfadimethoxine degradation was accelerated in laboratory-scale tests with increasing manure content in amended soil and with increasing moisture content (Wang *et al.* 2006a). It was also faster and more complete under aerobic conditions compared with anaerobic conditions (Wang and Yates 2008; Liu *et al.* 2010).

The degradation of oxytetracycline, tylosin, sulfadiazine and streptomycin in surface water was similar or slightly lower under anaerobic conditions compared with aerobic conditions (Ingerslev *et al.* 2001; Halling-Sørensen *et al.* 2003). Loftin *et al.*(2008) reported degradation rates of tetracycline being affected by pH and temperature, although no significant degradation was observed for sulfathiazole, trimethoprim, and tylosin A under similar conditions. This was in contrast to the findings from two separate studies that reported limited influence of pH on the dissipation of sulfadiazine and oxytetracycline (Yang *et al.* 2009b) in soils. These environmental and/or edaphic conditions vary from site to site and time to time, therefore the results obtained from any one particular study is very specific to that particular location, and cannot be extrapolated to a different soil type in another location (Sarmah *et al.* 2009).

Degradation rates also vary significantly across antibiotic types and environmental matrix. A study by Loftin *et al*. (2008) showed that tetracycline degraded relatively rapidly (half-lives from 6 hour to ~10 weeks), while the sulfonamides, tylosin, lincomycin, and trimethoprim

were much more stable in aqueous suspension. Half-lives for chlortetracycline, monensin and tylosin in spiked turkey manure were found to be 1, 17 and 19 days respectively, while SM did not degrade during a laboratory study (Dolliver *et al.* 2008).

SCP dissipated rapidly with DT_{50} and DT_{90} values of 3.5 and 18.9 days, but oxytetracycline was found to be more persistent with DT_{50} and DT_{90} values of 21.7 and 98.3 days (Blackwell *et al.* 2007). Earlier studies involving macrolides and penicillins showed that degradation for both these groups of antibiotics in soil was fast (Gavalchin and Katz 1994). Likewise, tylosin, a macrolide, has been found to have half-lives of 3.3-8.1 days in soil-manure slurries (Ingerslev and Halling-Sørensen 2001). However, half-lives for tylosin were much higher ($t_{½}$ 19 days) in pure manure (Dolliver *et al.* 2008), and even higher in normal agricultural ($t_{½}$ 21-25 days) soils (Sassman *et al.* 2007).

Degradation processes might reduce the potency of these chemicals; however, some degradation products have similar toxicity to their parent compound (Halling-Sørensen *et al.* 2002). Halling-Sørensen *et al.* (1998) proposed a two-step metabolism of parent compound consisting of phase I and phase II reactions. Phase I reactions comprise oxidation, reduction or hydrolysis, and the products are often more reactive and sometimes more toxic than the parent drug. Phase II reactions involve conjugation, which normally results in inactive compounds. Both phase I and phase II reactions change the physical and chemical behaviour of the substance as metabolism renders daughter compounds more water soluble than the parent compounds (Halling-Sørensen *et al.* 1998). The conjugation reactions can be reversible, and the metabolite may turn back to the parent compound in the environment (Halling-Sørensen *et al.* 2002). Thus it is not only the parent compound that should be subject to risk assessment but also the metabolites. In soils, degradation is mainly driven by microbial processes as these antibiotics are susceptible to enzymatic transformation reactions like oxidative decarboxylation and hydroxylation (Al-Ahmad *et al.* 1999) and sulfadimethoxine is known to be possibly demethylated in sediments (Samuelsen 1994).

Several studies have shown that antibiotics usually degrade faster in dung, manure, and soil (Ingerslev and Halling-Sørensen 2000; Halling-Sørensen 2001) compared with water. Recently Yang *et al.* (2012) showed that three sulfonamide antibiotics (SMO, sulfamonomethoxine and sulfamethoxine) were completely decomposed in aqueous phase

and solid activated sludge in < 13 days. Accinelli *et al*. (2007) reported enhanced degradation in soils for SM and SCP when liquid swine slurry was added. Sulfonamides including SMO were completely eliminated during a 5 week anaerobic fermentation of manure (Mohring *et al.* 2009). Sulfonamide degradation was even more rapid and uniform with complete biodegradation occurring from 0.2 to 3 days in activated sludge (Ingerslev and Halling-Sørensen 2000). The rapid degradation in sludge is most likely due to the presence of a high population of microorganisms in manure or sludge resulting in increased biodegradation of antibiotics in soil (Ingerslev and Halling-Sørensen 2001). This could also be due to the co-application of large amounts labile carbon resulting in the co-metabolism of the compound in question (Alexander 2000).

Degradation of most antibiotics is faster and more complete under aerobic than under anaerobic conditions. Degradation of oxytetracycline, tylosin, sulfadiazine, and streptomycin in activated sludge, soil and surface water was similar or slightly lower under anaerobic compared with aerobic conditions (Ingerslev *et al.* 2001; Halling-Sørensen *et al.* 2003). Wang *et al*. (2006) used a modified first-order degradation model to describe the degradation kinetics of sulfadimethoxine in manure and manure amended soil. The authors reported that sulfadimethoxine degraded much faster in non-sterile manure than in sterile manure, and that sulfadimethoxine degradation was accelerated in laboratory-scale tests with increasing manure content in amended soil and with increasing moisture content. The other findings of this study were that the degradation constant decreased with increasing sulfadimethoxine concentration and that storing manure before application along with dilution of more contaminated manure with less contaminated manure resulted in diminished sulfadimethoxine concentration.

Sulfonamides are known to be susceptible to photodegradation (Halling-Sørensen *et al.* 2003). However, under similar conditions no significant abiotic degradation of sulfapyridine was observed (Thiele-Bruhn and Aust 2004). Photodecomposition in water is known to decline with increasing water depth and turbidity (Samuelsen *et al.* 1994). Thus, it can be assumed that photo degradation has no significant effect on the concentration of antibiotics in soils, especially when they are spread onto soils as sludge or slurry. Also processes such as fixation and penetration into voids of the soil solids protect antibiotics from photodecomposition.

Degradation of antibiotics in water can also occur through abiotic processes such as photodegradation and/or hydrolysis. These processes often play an important role in their overall dissipation and elimination in the environment. Except for a few studies there is a dearth of information on the abiotic degradation of sulfonamide antibiotics in aqueous media, and all of these studies show great variation in the degradation rate (Ingerslev *et al.* 2001; Loftin *et al.* 2005; Barber *et al.* 2009; Radke *et al.* 2009). Theile-Bruhn and Peters (2007) reported significant photodegradation of various sulfonamides and tetracyclines in sterile water and soil. The rate coefficients were dependent on soil sorptive properties and varied with antibiotic type. This showed that photo-degradability of antibiotic could play an important role in its environmental fate. Many separate studies have been conducted on the degradation of sulfonamide antibiotics in soils. The information from these studies is summarised in Table 2.9

2.8.3 Transport

Strongly sorbed veterinary antibiotics can accumulate in the top layer of the soil; however, sulfonamides are known to be highly mobile and also known to have low sorption affinity (Tolls 2001; Boxall *et al.* 2002) and thus have the potential to leach to the groundwater (Hirsch *et al.* 1999). They can be quite stable toward degradation in the treated animal, the manure, and the soil and water purification processes (Ingerslev and Halling-Sørensen 2000). In the last decade a number of laboratory (Wehrhan *et al.* 2007; Unold *et al.* 2010; Kurwadkar *et al.* 2011) and field-based (Kay *et al.* 2005a) studies have investigated the transport characteristics of veterinary antibiotics (Rabølle and Spliid 2000; Boxall *et al.* 2002; Hu and Coats 2009). Through field studies in the UK it was demonstrated that weak acids such as sulfonamides and oxytetracycline have high potential to be transported to surface waters (Boxall *et al.* 2002; Kay *et al.* 2004). In contrast, tylosin was not detected in the leachate of a soil column experiment (Hu and Coats 2009).

Table 2.9: Literature on sulfonamide degradation

Sulfonamides	Concentration	Matrix pH / OC%	Conditions	Degradation %	Time DT_{50}, DT_{90} (day)	Reference
Sulfachloropyridazine	35.4 mg L^{-1}	Sandy loam soil		50, 90 (SFO)	3.5 and 18.9	Blackwell *et al*. (2007)
Sulfonamides		Soil	Field condition	50&90%	29 and 89	Kay *et al*. (2004)
Trimethoprim	500 µg g^{-1}	Sewage sludge / 6.5 / 37		50%	22 - 41	Halling-Sørensen (2001)
12 Sulfonamides	250-1000 µg L^{-1}	Activated sludge	6°C, 20°C	50 (LDM)	0.4-4.1(HL)	Ingerslev and Halling-Sørensen (2000)
Sulfadimidine	1.0 µg g^{-1}	loamy sand / 5.6 / 2.3	10°C, 20°C	0.2 / 0.3	64	cited from Thiele-Bruhn (2003)
		clay silt / 6.9 / 1.1		0.3 / 0.7	64	
Sulfadimethoxine	18-270 µmol g^{-1}	Manure/ 8.37/14	25°C, 83% MC	50% (AAFO)	1.4 to 10.2	Wang *et al*. (2006a)
Sulfadimethoxine	10-100 µmol g^{-1}	Manure amended soils	25°C, 20% MC	50% (AAFO)	3 to 11	Wang *et al*. (2006b)
Sulfadiazine	1, 10, 25 mg kg^{-1}	3 soils/ 4.3 to 8.5/ 0.35 to 2.57	25°C, 50% MWHC,	50% (SFO)	2 - 265	Yang *et al*. (2009a)
Sulfamethoxazole Trimethoprim	10 mg kg^{-1}	Soil/4.9/13.5 g kg^{-1}	aerobic, 25°C anoxic, 25°C	50% (SFO)	2,4 days 7,11 days	Liu *et al*. (2010)
Sulfachloropyridazine & Sulfamethazine	1, 10, 100 µg g^{-1} 10 µg g^{-1}	Silt loam/ 7.5/1.8 Sand/ 7.2/ 0.94 swine slurry/7.8/ 20	Non-sterile/sterile 25°C, MC at -33kPa	50% (SFO)	18.6, 21.3 18.6, 21.3 NR, > 10	Accinelli *et al*. (2007)
Sulfamethoxazole& acetyl sulfamethoxazole	2000 µg g^{-1} 3000 µg g^{-1}	Sewage Sludge/6.5/73 Soil/6.9/1.6	20°C, 10 h light/14 h dark	50, 90 50, 90	1,18 1,9	Holtge and Kreuzig (2007)
Sulfamethoxazole, Sulfadimethoxine, Sulfamonomethoxine	100 µg L^{-1}	activated sludge (MLSS of 2.56 g L^{-1})/ pH 7.0	25°C, DO of 3.0 mg L^{-1}	100% (AZK)	12-14	Yang *et al*. (2012)
Sulfamethazine	52 g/ 12 m^3	Turkey manure	Composting, 40% MC	No degradation	No degradation	Dolliver *et al*. (2008)
Sulfadiazine	1, 10, 100 mg kg^{-1}	Soil/ 4.8/1.0% Manure/ 7.5	Manure amendment 10°C in dark	50% (PFO)	1-2 (graph)	Hammesfahr *et al*. (2011)
Sulfapyridine	10 to 1000 µg g^{-1}	Sandy topsoil	25°C& 50% MWHC,	50%	1-3 (graph)	Thiele-Bruhn and Aust (2004)
Sulfamethoxazole & 2 metabolites	20 µg L^{-1}	Water/8.3/ 6.4 mg L^{-1} Sediment/7.0/9.4%	25°C, with sterile controls	50% 50%	8.5-17.2 3.3-25.6 (h)	Radke *et al*. (2009)

Simple first order (SFO); Availability adjusted first order (AAFO); apparent zero order kinetic (AZK); pseudo first order (PFO); Logistic degradation model (LDM). DT_{50} and DT_{90}, are the degradation endpoints for 50, and 90% antibiotic degradation. NR = not reported

Transport of veterinary antibiotic in soils depends on several factors. Chemical properties such as water solubility, dissociation constants, sorption–desorption processes, stability and binding to the soils and the partitioning coefficients at various pH values can all affect the mobility of antibiotics in the soil environment (Tolls 2001; Unold *et al.* 2010; Kurwadkar *et al.* 2011). Other factors that can influence the mobility of veterinary antibiotic are the timing of manure application, as well as prevailing weather conditions and the preferential flow via desiccation cracks and worm channels, as recently demonstrated in a UK field study (Kay *et al.* 2004; Kay *et al.* 2005a).

Rabølle and Spiild (2000) demonstrated that a stronger adsorbing antibiotic such as tylosin was retained at different depths depending on the soil properties and the risk of soil water/groundwater contamination by tylosin was low. It is assumed that significant transport of antibiotics like tylosin is restricted to fast preferential and macropore flow or is facilitated by co-transport with mobile colloids like dissolved organic matter (DOM) with facilitated transport or preferential flow within the cracks of a structured silt loam. SSCP was not leached through a sandy soil, whereas in a structured clay soil rapid preferential transport into drainage water was observed within 7 days (Boxall *et al.* 2002). Davis *et al*. (2006) showed that the veterinary antibiotics tylosin and erythromycin had the highest affinity for sediment transport; monensin and sulfonamides had the highest run-off loss; while tetracyclines had the lowest. Field investigations revealed that point sources caused ground water contamination resulting from transport of antibiotics through soil as determined in the vicinity of manure lagoons (Campagnolo *et al.* 2002). A diffuse contamination of surface water by antibiotic leaching from agricultural soils has also been reported (Arikan *et al.* 2008). In contrast, antibiotics were only detected in a small number of ground water samples from regions with intensive livestock production (Hirsch *et al.* 1999). There are many studies on the transport of sulfonamides antibiotic in soils, which are summarised as Table 2.10.

Table 2.10: Summary of results from column, lysimeter and field studies with veterinary antibiotics

Study substance	Sample	Concentrations/ % recovery	Reference
Macrolide	Soil	50 µg kg^{-1}	Halling-Sørensen *et al*. (2005)
Tylosin	Soil	ND	Blackwell *et al*. (2007)
	Soil	ND	Kay *et al*. (2004)
	Leachate	ND	Blackwell *et al*. (2007)
	Drainage water	ND	Kay *et al*. (2004)
	Overland flow water	ND	Kay *et al*. (2005b)
	Leachate	ND	Kay *et al*. (2005b)
	Leachate	0.27 ng mL^{-1}	Hu and Coats (2009)
	Soil	ND	Hu and Coats (2009)
	Soil	50-85%	Jeong *et al*.(2012)
Sulfonamides			
Sulfadiazine	Soil	0.8 µg kg^{-1}	Boxall *et al*. (2005)
	Overland flow water, grass	27.60%	
	Overland flow water, arable	2.50%	
	Leachate	4.10%	Kreuzig and Holtge (2005)
	Overland flow water	0.57%	Burkhardt *et al*. (2005)
	Surface water	4.13 µg L^{-1}	Boxall *et al*. (2005)
	Leachate	18 to 83%	Wehrhan *et al*. (2007)
	Leachate	95-97%	Unold *et al*.(2009)
	Leachate	0.08 to 56.7 µg L^{-1}	Aust *et al*. (2010)
Sulfachloro-pyridazine	Run-off	25.9 µg L^{-1}	Blackwell *et al*. (2007)
	Drainage water	613 µg L^{-1}	Kay *et al*. (2004)
	Overland flow water	416 µg L^{-11}	Kay *et al*. (2005b)
	Leachate	0.77 µg L^{-1}	Kay *et al*. (2005b)
	Leachate	0.78 µg L^{-1}	Blackwell *et al*. (2007)
Sulfadimidine	Overland flow water	2.10%	Burkhardt *et al*. (2005)
Sulfathiazole	Overland flow water	1.11%	Burkhardt *et al*. (2005)
	Leachate	34-75%	Kurwadkar *et al*. (2011)
Sulfamethazine	Soil	2 µg kg^{-1}	Hamscher *et al*. (2005)
	Groundwater	0.24 µg L^{-1}	Hamscher *et al*. (2005)
	Leachate	502005)	
	Leachate	50-90%	Kurwadkar *et al*. (2011)
	Leachate	69-99.7%	Fan *et al*. (2011)
	Leachate	41-97%	Strauss *et al*. (2011)
	Surface run-off	ND	Kim *et al*. (2010)
Sulfamethoxazole	Leachate	56-92%	Strauss *et al*. (2011)
	Leachate	100%	Chen *et al*. (2011)
Sulfadimethoxine	Leachate	38%-86%	Strauss *et al*. (2011)
	Leachate	0.08 to 56.7 µg L^{-1}	Aust *et al*. (2010)

2.9 Ecotoxicological effects of veterinary antibiotics

Veterinary antibiotics are designed to affect a wide variety of bacteria found in animals. This also makes them potentially hazardous to other such organisms found in the environment. The residues of antibiotics and their potentially active metabolites may facilitate antibiotic resistance, harm non-target organisms and interfere with sensitive ecosystems. The toxic effects of the antibiotic residues in the environment until recent were largely unknown. However due to the risk assessment processes, data on the toxicity of these substances to fish, daphnids, algae, microbes, earthworms, plants, etc have become available.

A wide range of data is available on the toxicity of many veterinary antibiotics to these organisms. Their effects on soil and other organisms including plants have been studied extensively under controlled laboratory conditions (Migliore *et al.* 1996; Baguer *et al.* 2000; Halling-Sørensen 2000; Halling-Sørensen 2001; Halling-Sørensen *et al.* 2003; Boxall *et al.* 2006; Kinney *et al.* 2008; Liu *et al.* 2009; Kim *et al.* 2009a; Kotzerke *et al.* 2010; Santos *et al.* 2010). A number of studies have investigated the toxic effects of veterinary antibiotics on aquatic species (DiDelupis *et al.* 1992; Migliore *et al.* 1997; Wollenberger *et al.* 2000), most of which used a concentration range of mg L^{-1}. For example Wollenberger *et al*. (2000) studied the acute and chronic toxicity effects of some commonly used antibiotics on the freshwater crustacean *Daphnia magna*. The acute toxicity (48 h EC_{50} values), for sulfadiazine (SDZ) was found to be 221 mg L^{-1}; chronic toxicity was found at much lower levels at 13.7 mg L^{-1}. Similarly Halling-Sorensen (2000) reported EC_{50} values of tylosin to be 1.38 mg L^{-1} for *Selenastrum capricornutum* (green algae). It was also reported that freshwater cyanobacteria were more sensitive with corresponding EC_{50} values being 0.034 mg L^{-1}.

In a more recent study Isidori *et al.* (2005) investigated the ecotoxicity of six antimicrobials (erythromycin, oxytetracycline, sulfamethoxazole, ofloxacin, lincomycin, and clarithromycin) on 14 non-target organisms. Macrolides (lincomycin, erythromycin, and clarithromycin) were the most harmful to the aquatic environment. They determined that the acute toxicity was in the mg L^{-1} range and the chronic toxicity was in the μg L^{-1} range. Algae were determined to be the most sensitive species studied with EC_{50} values ranging between 0.002 and 1.44 mg L^{-1}. In soil, much higher EC_{50} values were calculated for oxytetracycline, tylosin and SCP of 50, 30 and 75 mg kg^{-1}dry soil, respectively (Vaclavik *et al.* 2004). Average effective dose values (ED_{50}) were 47.6 mg kg^{-1} soil for sulfadiazine and 25.4

mg kg^{-1} soil for chlortetracycline (Thiele-Bruhn 2005). In another separate study, Thiele-Bruhn and Beck (2005) reported the ED_{10} values ranging from 0.003 to 7.35 mg kg^{-1} soil for sulfapyridine and oxytetracycline depending on the antibiotic compound and its soil adsorption.

Agricultural pharmaceuticals that are naturally excreted by livestock animals are also known to influence microbes in soil as their presence can change the microbial community of the soils. However, their effects on microbes are sensitive to soil properties (Zielezny 2008). Another study by the same author found that sulfadiazine significantly affected soil respiration in the presence of glucose while chlortetracycline did not (Zielezny *et al.* 2006). Earthworms have been used as anthropogenic waste indicators to study the bioaccumulation of pharmaceuticals from agricultural soil amended with biosolid or swine manure (Kinney *et al.* 2008). Some studies have gone one step further to show the negative impact of sulfadiazine contaminated manure applied to soil on the denitrifiers in the gut of earthworms (Kotzerke *et al.* 2010). Kotzerke *et al* (2008) observed reduced microbial activity (as measured by cell respiration) in soils amended with manure containing sulfadiazine (SDZ), whereas pure manure increased activity. Another study amended two soils with manure and SDZ at much lower doses and observed decreases in total phopholipid fatty acids and bacteria: fungi ratios (Hammesfahr *et al.* 2008). In contrary bacterial growth in soils was only inhibited at a high tylosin dose of 1500 mg kg^{-1} (Demoling and Baath 2008).

Only one study was carried out in New Zealand to measure the potential non-target effects of residues of antimicrobials in water (Schallenberg and Armstrong 2004). This report showed that water collected from a lake receiving water from an agricultural drain displayed variable, concentration-dependent antimicrobial activity on aquatic bacteria. As the study was a preliminary case study, the results were not consistent over time.

A review by Boxall *et al.* (2003) compared the available ecotoxicity data on standard organisms for commonly used veterinary antibiotics with available monitoring data from water, soil, and dung samples. The authors reported that the environmental concentrations were more than an order of magnitude lower, suggesting acute environmental effects were unlikely. These results were also supported by three other separate studies, while DiDelupis

et al. (1992) showed that antibiotic effects against algae and daphnids have been reported at surprisingly low concentrations (5–100 μg L^{-1}), Wollenberger *et al.* (2000) reported toxic levels of antibiotics for microorganisms, bacteria and micro-algae present in the environment to be 2–3 orders of magnitude lower than the toxic values for higher trophic levels. Kim and Carlson (2007b) evaluated the aquatic toxicity of six sulfonamides and trimethoprim; the authors pointed out that the acute median lethal concentrations for marine bacterium, freshwater invertebrate and fish were in mg L^{-1}range and these antibiotics did not pose a real threat to these species. Sarmah *et al*. (2006a) in his review criticises about majority of studies available in the literature as they were undertaken at higher than environmentally relevant concentrations and were performed for a short duration. Also parameters such as temperature, pH, and time duration often influence the results of a toxicity investigation, sometimes by orders of magnitude (Koller *et al.* 2000).

2.10 Antibiotic uptake and effects on plants

Veterinary antibiotics often end up on arable land through manure application and even directly through excretal inputs from grazing animals. This is another area of concern as bioaccumulation of these residues and its potential could enter the food chain, resulting in indirect toxicity (Kummerer 2004). The uptake of veterinary antibiotics by plants is known to vary considerably and often depends on soil properties, antibiotic groups, and plant species (Migliore *et al.* 1996). To date only a few studies exist on the effect of the antibiotics on the uptake by plants from manure-amended soils (Boxall *et al.* 2006; Dolliver *et al.* 2007; Ferro *et al.* 2010; Li *et al.* 2011)

On uptake, the growth of plants was found to be reduced, as plants appear to be sensitive to a variety of antibiotic groups (Migliore *et al.* 1998). Sulfamethoxine antibiotic was noticed in the roots and stems of certain plant species, at dose levels (13 to > 2000 mg kg^{-1}), The authors found that the source of this phyto-toxicity was bioaccumulation in the plants and that the presence of sulfadiamethoxione in the soil at a concentration of 300 mg L^{-1} significantly inhibited the growth of crop plants and weeds. Also bioaccumulation was found to be higher in the roots than in the stems (Migliore *et al.* 1995,1997,1998).

Dolliver *et al.* (2007) evaluated the plant uptake of a sulfonamide-class antibiotic, SM, in corn, lettuce, and potato grown in a manure-amended soil. SM concentrations in plant tissue reportedly increased with corresponding increase of SM in manure even though the total accumulation of SM in plant tissue after 45 days of growth was less than 0.1% of the amount applied to soil in manure. Highest plant tissue concentrations were found in corn and lettuce, followed by potato. Similarly Grote *et al.* (2004) demonstrated the uptake of SDZ and chlortetracycline in wheat from manure-fertilised soil. The authors reported concentrations for chlortetracycline and SDZ, which were 1.1 mg kg^{-1} and 0.5 mg kg^{-1} respectively. Another study by Kummerer (2004) reported translocation of ^{14}C-sulfadimidine from soil into maize plants declined from 15 to 3% after 32 days of aging in soil, and the transfer from roots to shoots was less than 0.04% of the total radiolabel. A detailed review by Jjemba (2002) provides useful information on the effect of antibiotics on plants. The author discusses the possibility of the antibiotics undergoing ageing and getting suppressed in the soils even before the plant uptakes them and concludes that the negative impacts on plants could occur only at concentrations that are unlikely to occur on fields.

Although plant uptake of antibiotics could reduce yield and expose consumers of plants to low levels of veterinary antibiotics, they could also be used in phytoremediation. Studies have also investigated the potential to use plants to remediate antibiotics from manure amended soils (Dolliver *et al.* 2007; Ferro *et al.* 2010). Ferro *et al*. 2010 evaluated the ability of plants to remove sulfonamides present in soils and waters; as the concentration of sulfonamides found in soils and waters was below the concentration used initially, the authors suggest that barley plants may be of use in the remediation of soils contaminated with sulfonamides.

2.11 Antibiotic resistance

The rapidly increasing incidence of resistance associated with veterinary antibiotic usage is now recognized worldwide as a serious threat and as a result of this, antibiotic growth promoters used in intensive livestock production have been banned in many countries (Boxall *et al.* 2003). Many studies suggest there is a possibility that resistance genes could potentially occur in the environment as a result of the direct use of antibiotics in animal production, and

groundwater may be a likely source of antibiotic resistance in the food chain (Kummerer 2003; Kummerer 2004). Kummerer (2004) describes the resistance problem in environmental compartments such as waste water, surface water, ground water, sediments and soil. Samuelsen *et al.* (1994) reported findings of sediment bacteria which were resistant to various antibiotics used as feed additives in fish farms. In Europe, van den Bogaard *et al.* (2000) also reported the presence of bacteria resistant to a range of antibiotics in the faeces of pigs in the Netherlands and Sweden. Seveno *et al.* (2002) reported the occurrence and transfer of antibiotic resistant genes in the environment and concluded that consumption of tainted food was the main transmission pathway of drug resistance. However in contrast to other studies, Halling-Sorensen *et al.* (2005) showed that though there was an initial increase in the level of both chlortetracycline and tylosin-resistant aerobic bacteria in the manure-amended field soil, it seemed to decline over time to the same level as had been observed at the beginning of the experiment.

Sulfonamide resistance genes were recently found in nearby dairy lagoon water and irrigation ditches and agriculturally impacted river sediments (Pruden *et al.* 2006). Using polymerase chain reaction methods, Pei *et al*. (2006) detected sulfonamide and tetracycline resistance genes at all sites, with gene concentrations significantly higher at the agricultural & urban influenced sites than at the pristine site. The same authors later tested the effects of aerobic and anaerobic dairy lagoon water treatment on different antibiotic resistant genes namely tetracycline, sulfonamide and macrolide in the laboratory. Responses varied for each gene type, depending on temperature and aerobic conditions (Pei *et al.* 2007). Sulfonamide resistance genes were detected in shrimp ponds, a city canal, and fish ponds receiving wastewater from swine farms in Vietnam (Hoa *et al.* 2008). Elsewhere during manure storage, sulfonamide resistance genes increased exponentially through 60 day but decreased greatly thereafter (Heuer *et al.* 2008). Jindal *et al*. (2006) investigated antibiotic use and resistance in swine farm waste treatment systems at a number of Illinois concentrated animal feeding operations (CAFOs) with varying antibiotic use practices. Resistances to tetracycline and macrolide antibiotic classes were found on all farms, including an organic farm with no current antibiotic usage, and were detected in lagoons, and soil samples. Mackie *et al.* (2006) found four common tetracycline resistance genes in wells nearby to two separate swine farms in Illinois, tetracycline resistance was detected in groundwater impacted by swine the CAFOs.

Chapter 3: Analysis of veterinary antibiotic in soils

3.1 Introduction

The analysis of these typically polar contaminants and their metabolites at trace levels in different environmental matrices, such as water, wastewater, soils and sediments, is particularly challenging because of the complex nature of the samples, and the difficulties associated with the separation of these compounds due to the interferences caused by co-eluting compounds (Diaz-Cruz and Barcelo 2005; Koester and Moulik 2005; Richardson 2008). Analytical methods for the quantitative analysis of sulfonamides, macrolides and many other antibiotics are mostly based on high performance liquid chromatography (HPLC), which offers a reliable and repeatable analytical tool for separating these contaminants from environmental samples. HPLC has been used for the analysis of antibiotic residues with single wavelength UV detection (Blackwell *et al.* 2004a; Blackwell *et al.* 2004b; Gao and Pedersen 2005; Malintan and Mohd 2006; Lester *et al.* 2008; Lara *et al.* 2009; Mouamfon *et al.* 2010), fluorescence detection (Thiele-Bruhn *et al.* 2004; Blackwell *et al.* 2004b; Peng *et al.* 2008; Camacho-Munoz *et al.* 2009), and diode array detection (Cancho Grande *et al.* 2001; Peng *et al.* 2008; Camacho-Munoz *et al.* 2009), and with more sensitive but more expensive mass spectrometric detectors (MS) (Kolz *et al.* 2005; Abuin *et al.* 2006; Brenner *et al.* 2011). Extensive sample pre-treatment and analyte derivatization makes GC–MS a rather time consuming method (Hirsch *et al.* 2001), and GC-MS has rarely been used for the analysis of these compounds. Various methodologies that are found in the literature are summarised in Table 2.6 for comparison with the method described here.

Recent reviews have summarised the development of a wide variety of analytical methods for the detection, extraction, and measurement of veterinary antibiotic in different environmental matrices (Thiele-Bruhn 2003; Sarmah *et al.* 2006a; Nikolaou *et al.* 2007; Snow *et al.* 2008). For the analysis of environmental residues the procedure usually requires the development of extraction and clean-up techniques, or enrichment of the substance, coupled with appropriate instrumental technologies that can provide the needed sensitivity, and specificity for accurate measurement. However, for laboratory fate studies (e.g. sorption, degradation and transport) involving these compounds, spiked samples are often used for extraction and subsequent

analysis by HPLC techniques. Spiked samples are at concentrations higher than the concentrations at which these compounds are likely to be found in the environment. While studies involving antibiotic sorption to manure require additional clean up before analysis (Clay *et al.* 2005), soil sorption samples usually require only filtration through 0.45 μm or 0.2 μm filter paper (ter Laak *et al.* 2006; Accinelli *et al.* 2007). Therefore, studies involving fate and transport of antibiotics in environmental media such as soils or sediments do not require sophisticated extraction techniques or high sensitivity and accuracy, and can be accomplished with conventional HPLC with UV or fluorescence detection.

The objective of this study was to develop a simple, yet robust, analytical method capable of detecting and quantifying three sulfonamides –SMO, SCP and SM – and a macrolide TT in aqueous (calcium chloride solution and soil leachate) and solid (agricultural soils) matrices using HPLC in combination with UV and fluorescence detection. The method has been optimized and validated to yield acceptable recoveries, and evaluated in terms of its reproducibility, linearity of response, limit of detection, and limit of quantification using three agricultural soils that were devoid of antibiotic residues. The method was used to investigate soil sorption and degradation studies performed under controlled laboratory conditions using the above compounds.

3.2 Materials and methods

3.2.1 Chemicals

Antibiotics TT, SMO, SCP, SM of > 99% purity (Figure 2.2), and calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$ > 99% purity) were obtained from Sigma Aldrich, Australia. Acetonitrile (Mallinckrodt ChromAR, ≥ 99.8% purity), dichloromethane (Mallinckrodt UltimAR, ≥ 99.9% purity) and methanol (Mallinckrodt ChromAR, ≥ 99.9% purity) were obtained from Biolab Scientific Ltd, New Zealand. HPLC grade de-ionised water was obtained from an arium® 61316 high performance reverse osmosis system (Sartorius Stedim Biotech GmbH, Germany). Nitrogen gas oxygen free (gas code 152) was purchased from BOC (New Zealand).

3.2.2 Soils

Six topsoils (0-5 cm) and three subsoils (30-40 cm) representing different farming areas of New Zealand were selected for this study. The topsoils used in this study were archived soils, while the subsoils were freshly collected from different sites within Ruakura Agresearch. The fresh soils were air-dried at room temperature, sieved to < 2 mm and stored until use at 4^{o}C. The soils vary, in their pH, organic matter content and particle size distribution as shown in Table 3.1. OC content of the soils were analysed using a Leco CNS2000 Analyzer which utilises the Dumas dry combustion principle (Nelson and Sommers 1996). The specific surface area (SSA) was estimated from the clay percentage, organic matter content, and clay mineral composition of the soils using SSA values for single mineral components and organic matter as cited by Hedley *et al.* (2000). The CEC of the soils was measured based on ammonium acetate method described by Blakemore *et al.* (1987). A full description of the soil and the methods used to determine the physico-chemical properties can be found elsewhere (Hewitt 1998).

Table 3.1: Selected properties of soils used in the study.

Soils	pH 1:2 H_2O	pH 1:2.5 $CaCl_2$	OC (%)	CEC ($cmol_c\ kg^{-1}$)	Sand %	Silt %	Clay %	SSA (m^2g^{-1})
Matawhero silt loam	6.1	4.3	2.1	15.4	11.0	62.0	27.0	6.0
Te Kowhai silt loam	6.7	5.1	5.0	21.7	9.0	54.0	37.0	19.7
Te Kowhai SS	5.7	5.5	0.5	n.a.	12.3	62.8	24.9	n.a.
Hamilton clay loam	5.8	4.8	4.0	17.2	19.0	51.0	30.0	22.3
Hamilton SS	5.1	4.1	0.8	n.a.	13.4	40.3	46.2	n.a.
Horotiu silt loam	5.7	5.4	8.2	28.2	34.0	48.0	17.0	19.7
Horotiu SS	6.6	6.0	1.7	n.a.	47.0	33.9	29.0	n.a.
Manawatu sandy loam	5.1	5.4	3.3	9.7	87.0	11.0	2.0	13.6
Gibson sandy loam	6.9	5.2	1.1	7.6	45.0	41.0	14.0	n.a.

[#]SS = subsoil; n.a.= not available

3.2.3 High performance liquid chromatography (HPLC)

Analysis of the three SA's and TT was performed on a Dionex Summit high performance liquid chromatography (HPLC) system comprising a Dionex solvent rack SOR-100, a Dionex

thermostatted column compartment TCC-100, a Dionex ASI-100 automated sampler, a Dionex P680 LP-pump, a Dionex UVD 170U detector, and a RF 2000 fluorescence detector. The UV lamp was a L2D2 deuterium lamp (Hamamatsu Photonic, K.K., Japan) and the injection syringe was a Gastight® from Hamilton Bonaduz AG (#1725, 0.25 mL, Switzerland). An Onyx® Monolithic C_{18} column (100 mm × 4.6 mm, Phenomenex®, Australia) was used for the TT, and chromatography of the three SA's was performed on a Luna® 5µ RP-C_{18} (150 mm × 4.6 mm, Phenomenex). All columns were operated with a Security Guard™ column cartridge holder equipped with a C_{18} cartridge (4 mm, Phenomenex®, Australia).

The optimal detection wavelength was determined by preparing stock solutions of TT, SMO, SCP, and SM, at 10 µg mL^{-1} each, in methanol. A Shimadzu UV 160A UV-Visible Recording Spectrophotometer (Shimadzu, Japan) was used to measure the UV absorbance of the solution in the range from 200 to 300 nm in a full-scan mode. An elution scheme similar to Blackwell *et al*.(2004a) was modified for chromatography of these antibiotics as single analytes. An isocratic method was used with a mobile phase consisting of acetonitrile: trifluoroacetic acid (0.05%): tetrahydrofuran in ratio of 22.5:68:9.5 (TT), 40:55:5 (SMO), 32:63:5 (SCP) and 31:64:5 for SM (v/v) at a flow rate of 1 mL min^{-1}, and an injection volume of 20 µL. For the gradient method, at the start of the analysis, the mobile phase consisted of 5% ACN, 90% TFA, and 5% THF; and this composition was maintained for 4 min. The composition changed linearly between 4 and 10 min to give a composition of 37.5% ACN, 57.5% TFA and 5% THF. Between 10 and 12 min the composition changed linearly back to its initial composition of 5% ACN, 90% TFA, and 5% THF and remained like this until 13 min (Figure 3.8). The limits of detection (LOD) were calculated on a signal to noise ratio of 3 to 1. LOD for TT was 0.05 µg $mL^{-1}$and for the three sulfonamides it was 0.02 µg mL^{-1}. For SMO, the LOD was lowered to 0.002 µg mL^{-1} by using a fluorescence detector (excitation and emission wavelength were 272 and 340 nm respectively) in tandem with UV detection. Standard linearity was assessed in the concentration range of 0.1 to 1.0 µg mL^{-1} and 1.0 to 20 µg $mL^{-1}$and quantification was achieved by using external standards prepared from serial dilution of the stock solution.

3.2.4 Direct analysis /extraction from aqueous matrices

Extractions of TT, SMO, SCP and SM were performed by spiking these antibiotics into aqueous solutions (5 mM $CaCl_2$) as part of batch sorption studies conducted on six different New Zealand dairy farm soils. Aqueous solutions of the antibiotics in 5 mM $CaCl_2$ were prepared at concentrations of 2.5, 5, 10, 15, 25 and 35 μg mL^{-1} for TT; and 1.5, 3, 5, 7.5, 10 and 15 μg mL^{-1} for the three sulfonamides by adding appropriate amounts of methanolic stock solutions (1000 mg L^{-1}). An aqueous solution containing the antibiotics (30 mL) was placed in a 35 mL Kimax glass centrifuge tube into which soil (2 g) had been accurately pre-weighed. The centrifuge tubes were closed with plastic caps with Teflon septa, and placed onto an end-over-end shaker and equilibrated for 24 h followed by centrifugation at 1750 × g (10 min). After centrifugation, 1 mL of the supernatant was carefully decanted into a HPLC vial and analysed by HPLC-UV/ fluorescence detection.

In order to study the degradation of the three sulfonamides in sterile and non-sterile deionised water, 50 mL of water was added into a 100 mL Schott bottle, and spiked with an appropriate amount of the antibiotics to yield a nominal concentration of 0.5 μg mL^{-1} of the 3 sulfonamides together. At regular intervals replicate samples (0.5 mL) were pipetted into a vial for direct analysis by HPLC. Liquid-liquid extraction was performed for the measurement of the sulfonamides concentration in the leachate following the laboratory soil column transport study for two soils. For this, a leachate volume of 20 mL was extracted with 10 mL of DCM, and an aliquot of 1.75 mL of each solvent extract was evaporated to dryness under N_2, and reconstituted in 0.5 mL of methanol for analysis.

3.2.5 Extraction from soil residues

Extraction of the antibiotics from soil was performed in samples obtained during a series of batch sorption studies (6 soils), degradation (3 topsoil and 3 subsoil), and for residual concentration analysis from post-soil column transport experiment using 2 soils. For sorption studies six topsoils (0-10 cm) were freshly collected from different dairy farming regions of the North Island of New Zealand, sieved to below 2 mm and air dried. Approximately 2 g of each soil was weighed into glass centrifuge tubes, and equilibrated (24 h) with 5 mM $CaCl_2$ solution containing the spiked antibiotic. After equilibration, the supernatant was carefully decanted and the residual antibiotic in the soil was extracted with 10 mL methanol (TT) and

10 mL of DCM (sulfonamides) by shaking the tubes for 12 h. Following shaking, 1 mL of aliquot of each solvent extract was evaporated to dryness under N_2, reconstituted in methanol (0.5 mL) and analysed by HPLC/UV detection.

During the degradation study the extraction recovery was assessed by the analysis of SMO antibiotic for the 0^{th} day sample. Three soils from different depths (0-10 cm, 30-40 cm) were freshly collected, sieved (2 mm), and were adjusted to 60% of the maximum water holding capacity (-33 kpa), and separately incubated at 25^oC and 7.5^oC. Part of the degradation study also included autoclaved controls, which was accomplished by autoclaving the soils (121^oC, 103 kPa for 15 min). Duplicate soil samples (5 g) for a set time interval were placed in 35 mL Kimax centrifuge tube, and an appropriate amount of SMO stock solution was spiked to obtain an initial concentration of 0.5 mg kg^{-1}. At selected time intervals samples were extracted with DCM (10 mL) and shaken for 12 h in an end-over-end shaker. The DCM extract (~1.5 mL) was pre-concentrated under a gentle stream of nitrogen, reconstituted in methanol (0.5 mL), and immediately analysed using HPLC-UV/Fluorescence detection.

3.2.6 Soil recovery test

An isocratic elution scheme was used to conduct soil recovery tests for TT in Matawhero and Te Kowhai soils at four different initial spiked concentrations (0.2, 0.5, 2, and 5 mg kg^{-1}), and SMO in Horotiu, Te Kowhai, and Hamilton soils at three different spiked concentrations (0.05, 0.2, and 0.5 mg kg^{-1}). Also a gradient elution method was used to study the recovery and reproducibility of all three sulfonamides in six soils (Horotiu, Te Kowhai and Hamilton top and subsoils).

The rationale for using different spiking levels was to investigate the capability of the method to extract the antibiotics from these soils, as the extraction efficiency of any given method can be dependent on the type and nature of media from which the chemicals are to be extracted. Since the soils selected in this study possess organic carbon content of as low as 1% to as high as >8%, with varied clay-mineralogical characteristics along with high surface area. Sorption affinities for these soils for these antibiotics are likely to be significantly different, and could contribute to significant differences in extraction recoveries.

3.3 Results and discussions

The UV absorbance spectra of the individual antibiotic standards for the wavelength ranging from 150 to 400 nm are shown in Figure 3.1. Figure 3.2 shows the graph of wavelength against peak height for the 3 sulfonamides obtained from running the standard mixture at different wavelength using different channels in the UV detector. The maximum absorbance for all 3 sulfonamides occurred between 265 and 275 nm, which was in agreement with the available literature (Figueroa-Diva *et al.* 2010). SMO had a second maximum at 210 nm but this was not used because of potential interference by methanol. For TT the maximum was 290 nm, which was in agreement with the earlier findings of Hu and Coats (2009), who reported the absorbance maximum to be 290 nm, while Sassman *et al*. (2007) reported it to be 280 nm. Loke *et al*. (2002) used 203 nm as the detection wavelength for TT, which would be less than optimum.

A plot of wavelengths against peak height for a mixed standard of the 3 sulfonamides revealed the maximum peak height for SMO, SCP, and SM as at wavelengths of 275, 270 and 241 nm respectively. Lertpaitoonpan *et al*. (2009) reported the detection wavelength for SM to be 254 nm, while Boxall *et al*. (2002) reported the detection wavelength for SCP to be 285 nm. More recently, Çalışkan and Göktürk (2010), and Hou *et al*. (2010) reported the detection wavelength for SMO to be 268 nm and 265 nm respectively. In the present study, < 5% change in peak height was observed for the SCP and the SM standards when the wavelength was changed to 275 nm.

Given the contrasting values for wavelengths used in the literature (Table 2.6), and to maintain uniformity for all measurements, a wavelength of 275 nm was selected for the three sulfonamides in our study. The excitation and emission wavelength for SMO was determined by injecting a standard directly into the fluorescence detector and running a full scan (200 to 900 nm) for excitation and emission modes. The maximum excitation occurred at 276 nm and maximum emission occurred at 340 nm (Figure 3.3), a finding similar to that reported by Thiele-Bruhn and Aust (2004).

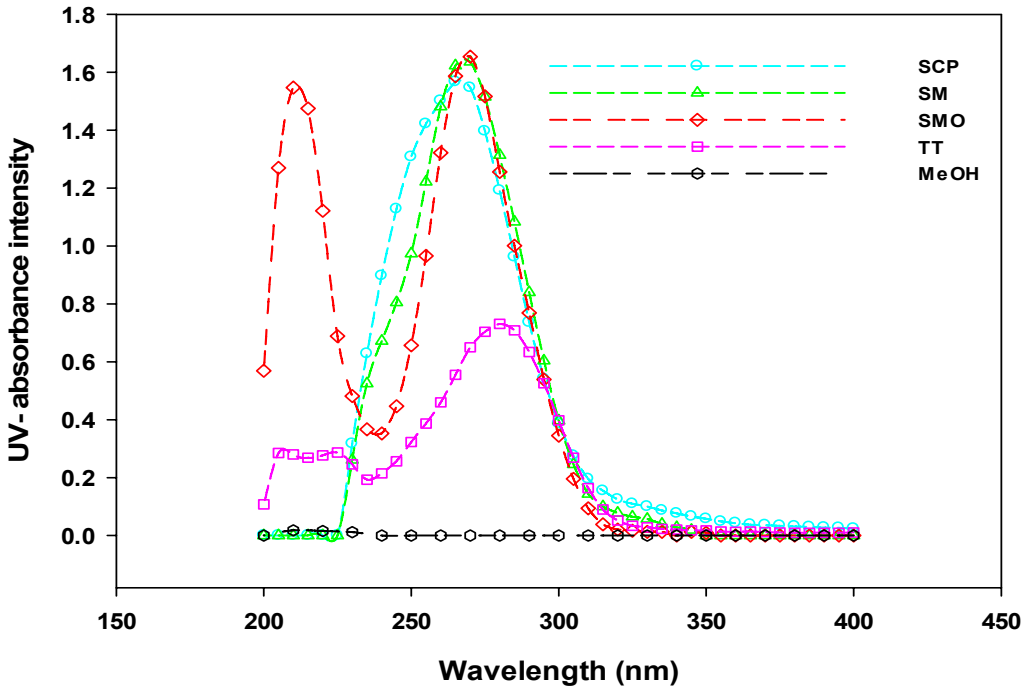


Figure 3.1: UV-absorbance spectrum of TT, SMO, SCP and SM standard (10 mg L^{-1} in methanol).

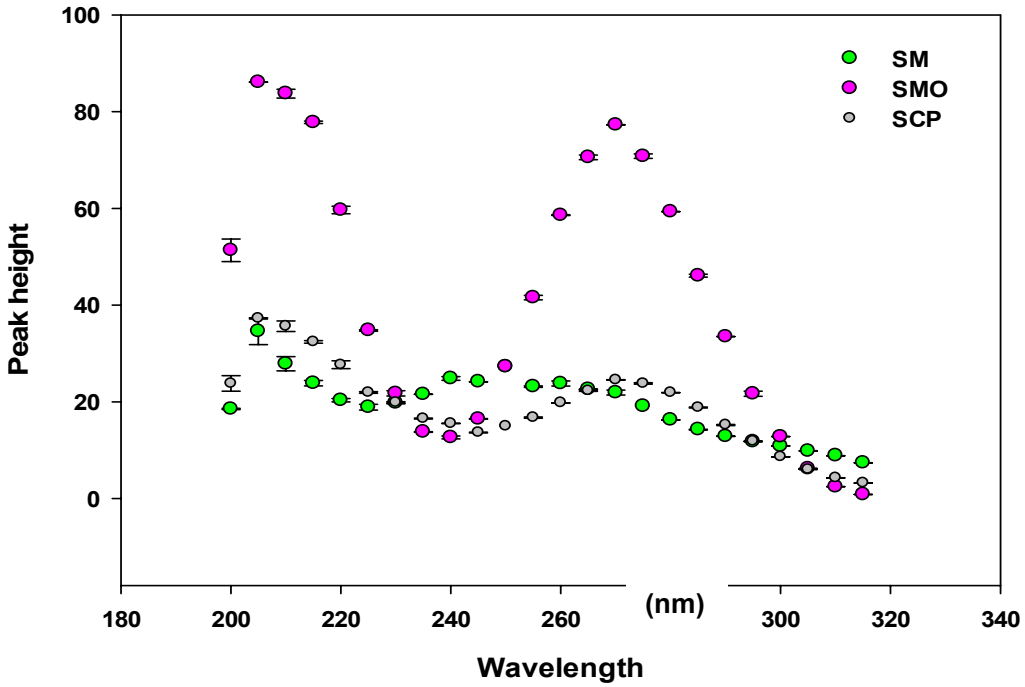


Figure 3.2: UV absorbance spectrum for SMO, SCP and SM (10 mg L^{-1} in methanol) using a multi-channel UV detector.

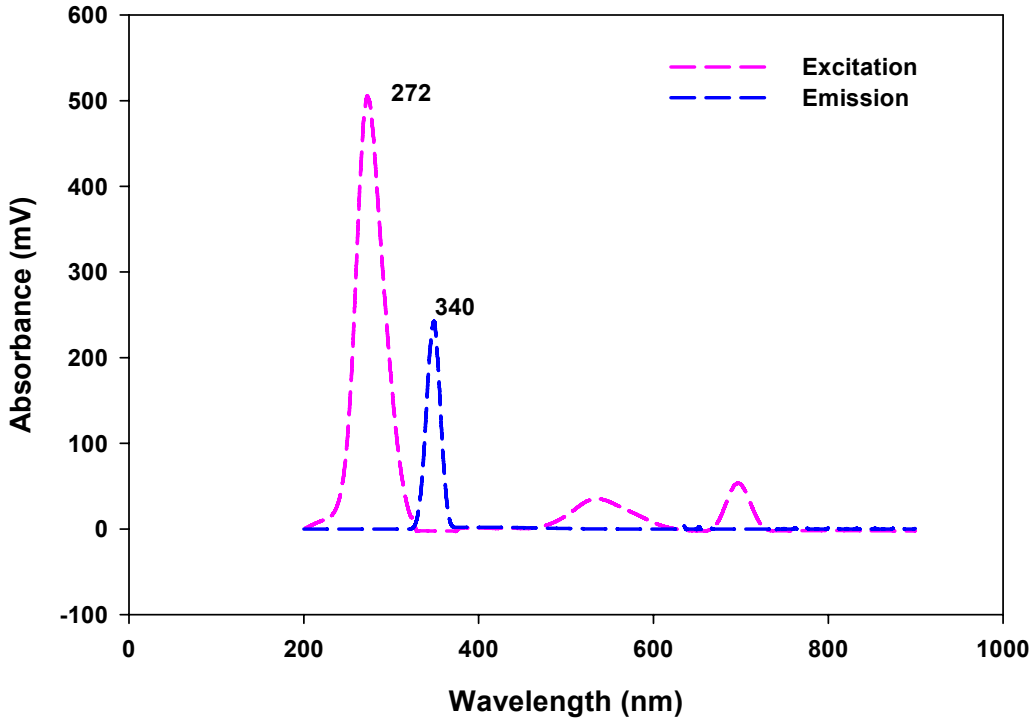


Figure 3.3: Fluorescence-absorbance spectrum of SMO standard (10 mg L^{-1}).

3.3.1 Compound elution/separation

An elution scheme similar to Blackwell *et al*. (2004a) was modified for chromatographic determination of the three sulfonamides using an isocratic run on a Luna 5µ RP-C_{18} column, and for TT an Onyx monolithic C_{18} column. The pH of the mobile phase was maintained at 2.3 by using 0.05% TFA in water. The mobile phases, injection volume, and flow rate were optimized. The final mobile phase consisted of ACN: TFA (0.05%): THF in the ratio 22.5:68:9.5 for TT, 40:55:5 for SMO, 32:63:5 for SCP and 31:64:5 for SM (v/v). A flow rate of 1 mL min^{-1} and an injection volume of 20 µL were used for both columns, and the column temperature was maintained at 22^{o}C. The optimized isocratic runs gave elution of TT, SMO, SCP and SM at 2.5, 3.0, 3.6, and 2.6 minutes irrespective of matrix (Figures 3.4 to 3.8). Isocratic runs were adequate for the sorption/degradation studies with a single analyte as it meant a reduction in runtime and consequently a higher sample throughput. This elution scheme avoids the use of buffers and therefore eliminates the possibility of the formation of precipitate and the time taken for system clean-up following the analysis.

A gradient elution scheme was employed to measure the three sulfonamides simultaneously (Figure 3.8). The flow rate, injection volume, and column temperature were kept similar to the isocratic run. Separation of SM, SCP, and SMO was achieved on a Luna 5µ RP-C18 column at 6.0, 8.8 and 9.5 minutes respectively with a total run time of 13 minutes. This gradient scheme was used to analyse the samples collected during a study involving degradation kinetics of sulfonamides in aqueous matrix (water), analysis of leachate concentrations, and soil residues after a soil column transport experiment performed under controlled laboratory conditions.

The standard chromatograms (Figures 3.4a to 3.8a) showed that both the isocratic and the gradient system yielded a distinct separation of the four target compounds and the matrix or mobile phase influence was negligible. Blank matrix runs and mobile phase runs were regularly conducted for quality assurance, and to identify possible additional retention mechanisms that might occur on analytical columns as a result of high sample throughput and/or guard column failure. Soil blank runs showed (Figure 3.4b to Figure 3.8b) that there was no interference from co-eluting peaks, which indicated little interference.

3.3.2 Standard linearity of response and detection limits

The responses for the standards for all four compounds were linear in the range of 0.01 to 1.0 µg mL^{-1} (n = 6), and 1.0 to 20 µg mL^{-1} (n = 6), respectively. The correlation coefficients for the standard curves were > 0.998 during the entire course of the experimental studies, and deviation in the correlation coefficient was a good indicator of the accuracy of standard preparation. The limits of detection at an injection volume of 20 µL and S/N (signal: noise) ratio of 3:1 for these compounds using UV detection were 50 µg $L^{-1}$and 20 µg $L^{-1}$for TT and all three sulfonamides respectively.

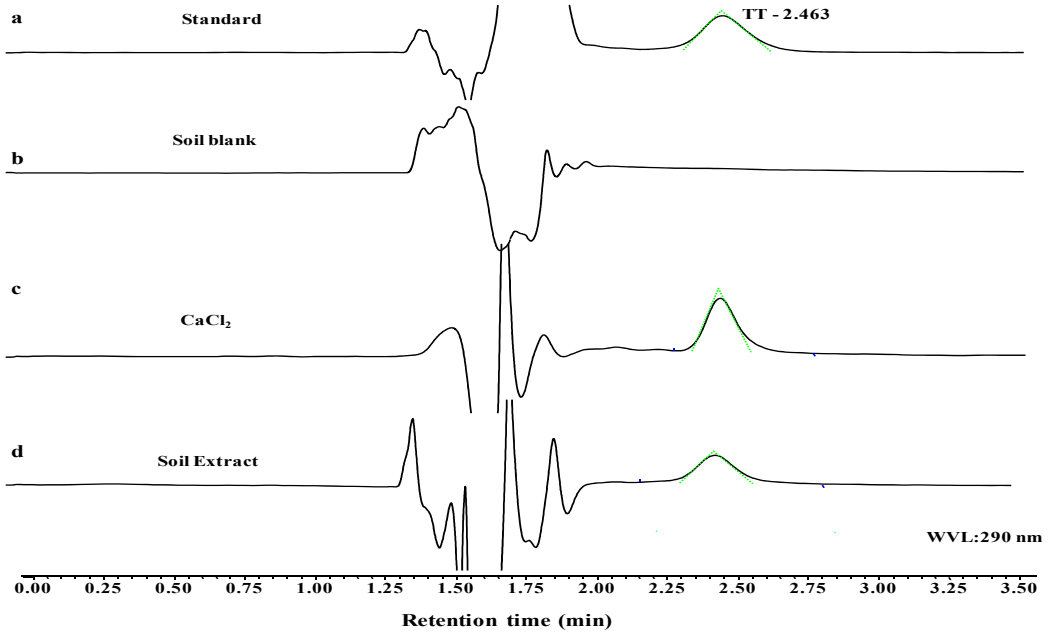


Figure 3.4: Sample HPLC chromatograms for TT (a) standards at 2 mg L^{-1}, (b) Hamilton clay soil blank, (c) $CaCl_2$ supernatant from sorption study, and (d) Hamilton clay soil extract from sorption study separated on a monolithic C_{18} column (onyx®, 100 mm × 4.6 mm) using UV detection at 290 nm wavelength.

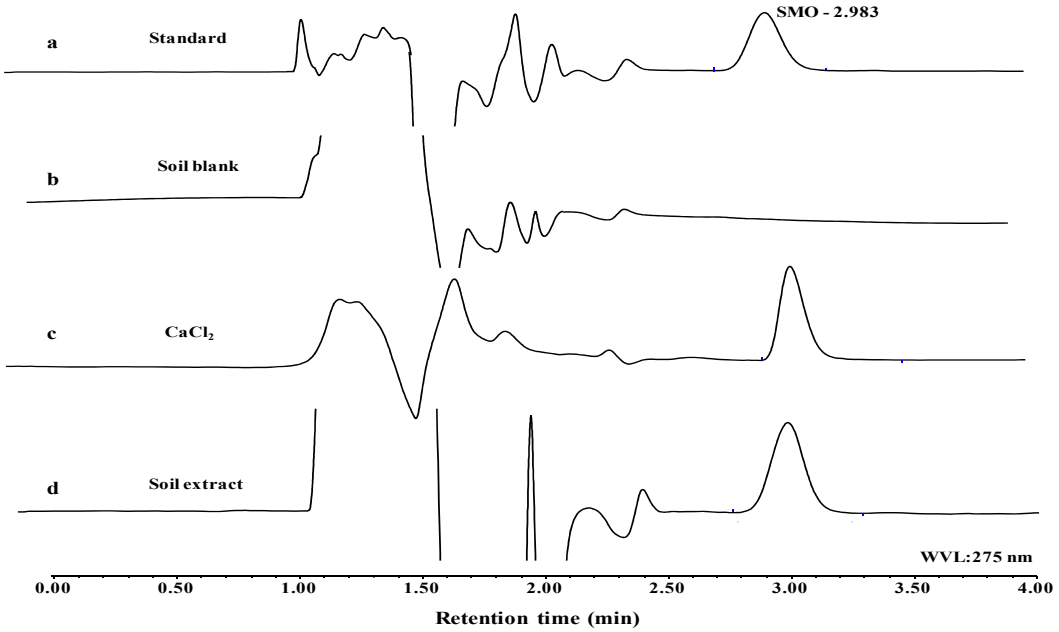


Figure 3.5: Sample HPLC chromatograms for SMO (a) standards at 0.5 mg L^{-1}, (b) Hamilton clay soil blank, (c) $CaCl_2$ supernatant from sorption study, and (d) Hamilton clay soil extract from sorption study separated on a C_{18} column (Luna®, 150 mm × 4.6 mm) using UV detection at 275 nm wavelength.

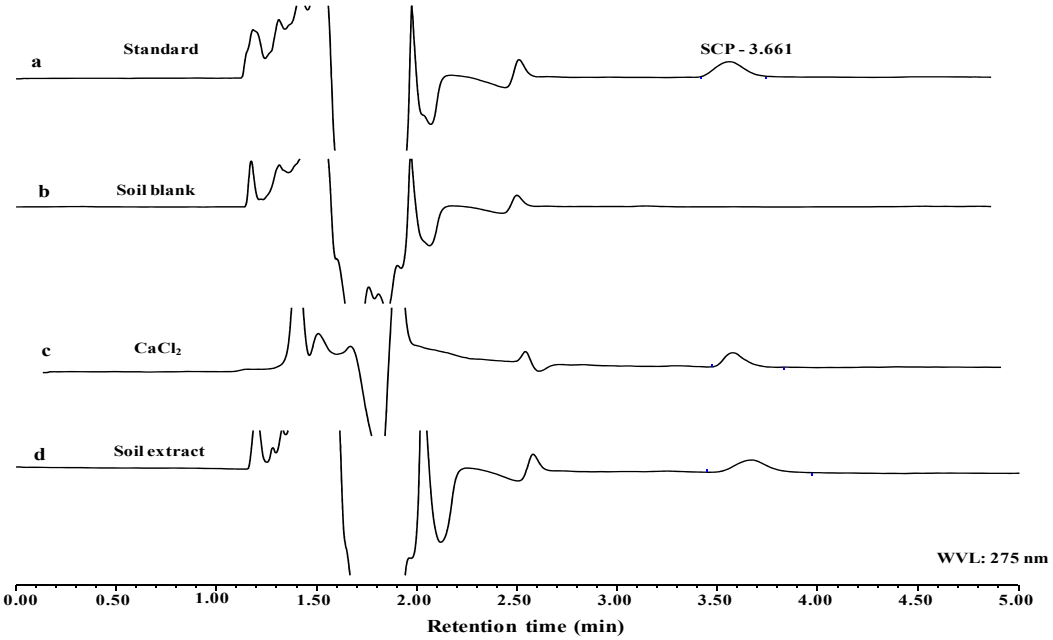


Figure 3.6: Sample HPLC chromatograms for SCP (a) standards at 0.5 mg L^{-1}, (b) Hamilton clay soil blank, (c) $CaCl_2$ supernatant from sorption study, and (d) Hamilton clay soil extract from sorption study separated on a C_{18} column (Luna®, 150 mm × 4.6 mm) using UV detection at 275 nm wavelength.

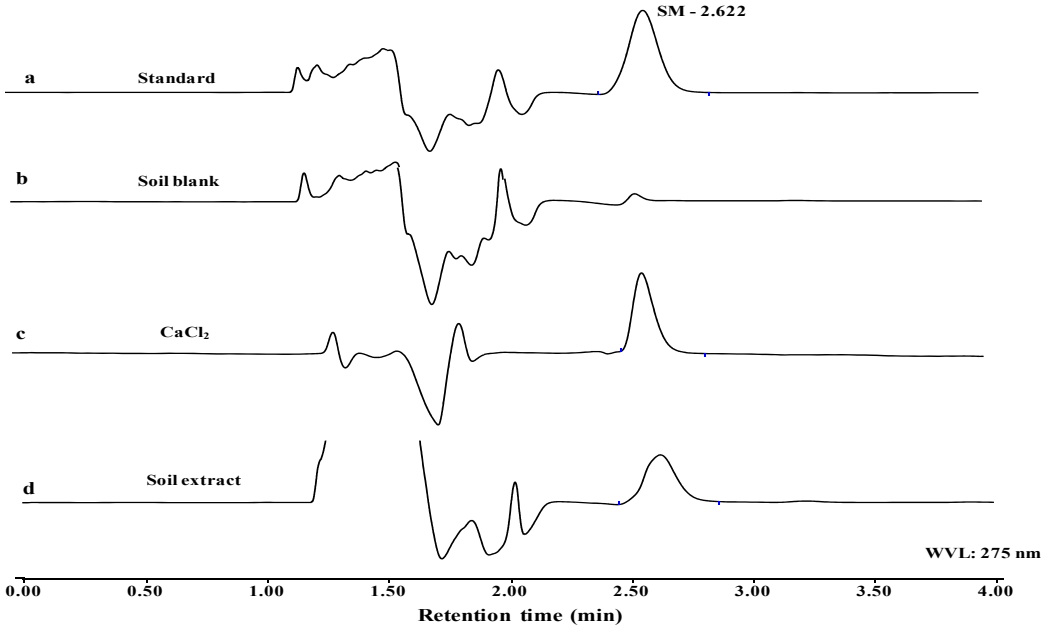


Figure 3.7: Sample HPLC chromatograms for SM (a)standards at 3.0 mg L^{-1}, (b) Hamilton clay soil blank, (c) $CaCl_2$ supernatant from sorption study, and (d) Hamilton clay soil extract from sorption study separated on a C_{18} column (Luna®, 150 mm × 4.6 mm) using UV detection at 275 nm wavelength.

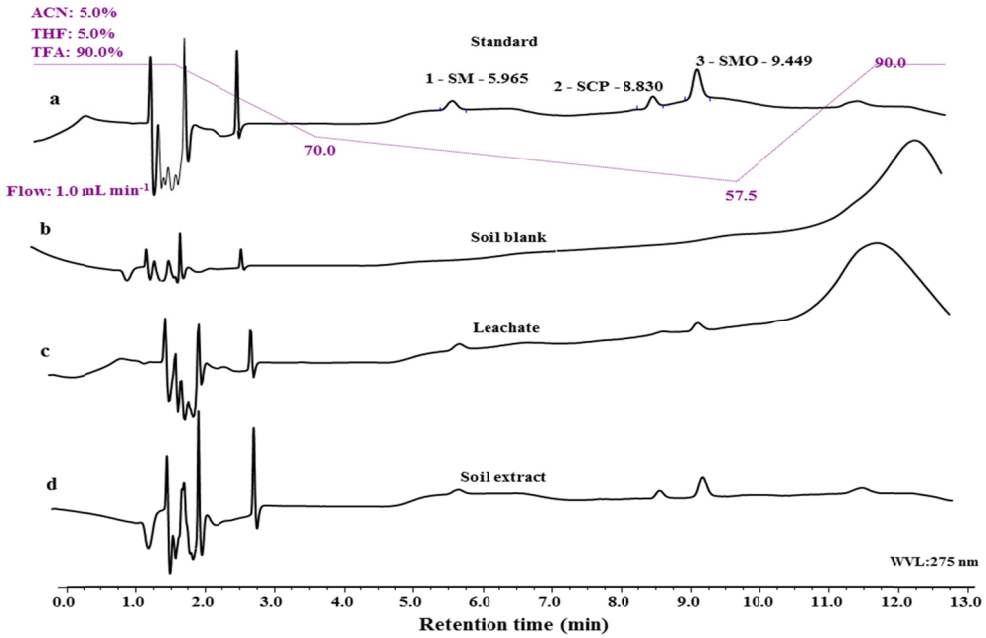


Figure 3.8: Sample HPLC chromatograms for the sulfonamides (a) standards at 0.5 mg L^{-1}, (b) Horotiu soil blank, (c) $CaCl_2$ leachate from transport study, and (d) Horotiu soil extract from sorption study separated on a monolithic C_{18} column (Luna®, 150 mm × 4.6 mm) using UV detection at 275 nm wavelength. Dotted lines indicated the changes in the mobile phase composition of 0.05% TFA relative to ACN.

3.3.3 Recovery from aqueous matrices

The aqueous phases were measured directly without the need for any extraction and clean up techniques. The matrix effect from $CaCl_2$ was negligible as shown in the chromatograms (Figure 3.4c to Figure 3.8c). Several earlier studies involving sorption of TT and SAs have also used direct analysis of aqueous phases. (Thiele-Bruhn *et al.* 2004; Accinelli *et al.* 2007; Sassman *et al.* 2007). In general, the recoveries of TT, SMO, SCP and SM from spiked 5 mM $CaCl_2$ solution ranged from 95% at 15 mg L^{-1} to 105% at 0.5 mg L^{-1}. A similar recovery range was also reported by Boxall *et al*. (2002) for SCP. The method detection limits were about an order of magnitude higher than the detection limits yielded from LC-MS methods (Haller *et al.* 2002), and therefore may not be suitable for detection of antibiotic residues in environmental samples. However, the method described in the present study, employing both isocratic and gradient elution schemes, was suitable to obtain data for laboratory-scale investigations investigating sorption, degradation, and transport of antibiotics in soils.

3.3.4 Extraction from soil

The chromatograms from soil extract are shown in the Figure 3.4d toFigure 3.8d. The extraction of antibiotics from soils constitutes a challenge that is due to the possible formation of bound residues in soils leading to low extraction recovery. These bound residues could be attributed to irreversible sorption or very slow desorption (Hamscher *et al.* 2005), cation exchange interactions (Richter *et al.* 2009), or formation of covalent linkages between amine moieties and organic matter functional groups (Bialk *et al.* 2005). In addition, these compounds often degrade in natural soils thus altering their soil concentrations, which could in turn affect their recovery. This problem can be overcome by sterilizing the soil; however, techniques such as autoclaving or treatment with sodium azide are known to change soils physico-chemical properties, thus leading to bias in results in relation to recovery of the compounds. Gamma irradiation, on the other hand, could avoid these limitations; however, this was not attempted in the present study.

3.3.5 Reproducibility, accuracy and precision test

Acceptable recoveries were obtained for both TT and SMO at different spiking concentration levels in 2 soils using an isocratic elution scheme. TT recoveries ranged from 77.5 to 100.7% for Te Kowhai, and 58 to 95% for Matawhero soils. Recoveries for SMO in 3 soils ranged from 67 to 106%. The results showed a general trend of decreased recovery with increases in the spiked concentration level (Table 3.2), contrasting with the earlier findings of low recovery reported for TT at low spiked levels (Blackwell *et al.* 2004b). A plausible explanation could be the formation of more bound residues at higher spiked concentrations, which often require greater volume of solvent to extract the compounds. SMO recovery was poor but still > 70% in Horotiu soil. This soil in particular is volcanically derived, and has a high percentage of allophane, an alumino-silicate clay mineral with high specific surface area, and high organic carbon content exhibiting high sorption affinity (Sarmah *et al.* 2008). The precision, when compared with the calibration accuracy was slightly poorer for both compounds at lower spiking levels.

The gradient elution scheme was also used to test the recovery of a sulfonamides mixture spiked at 0.5 mg kg^{-1} in 6 different soils and the results are shown in Table 3.2c. Recoveries for SMO, SCP and SM ranged from 53 to 97%, 43 to 83%, and 37 to 55.5% respectively

across all soils, with recoveries for SM being the least compared with SMO and SCP in all soils. The relatively lower recovery of SM could be attributed to the formation of permanently bound residues and the tendency among these antibiotics to sorb on active sites of soil, and make it harder for these compounds to be extracted by DCM. Recovery for SMO at a concentration 0.05 mg kg^{-1} for three topsoils measured using the isocratic method was higher compared with the SMO recoveries measured with the gradient method, and for this reason the isocratic scheme was chosen to validate the method for sorption and degradation studies. The gradient scheme was used to investigate degradation only in aqueous samples, and to analyse leachate concentrations from soil column transport studies where soil extraction was deemed unnecessary.

3.3.6 Recoveries during the sorption study

Table 3.3 shows the combined recovery from the soil and aqueous phase during the study of sorption of TT, SMO, SCP and SM to six different soils. Overall, recoveries from sorption studies were found to be acceptable with values ranging from 63 to 101% (TT), 89 to 95% (SMO), 71 to 94% (SCP), and 82 to 97% (SM) respectively in the six soils. The lowest recoveries for TT in Matawhero and Te Kowhai soils were 63% and 70% respectively, while for SCP lower recoveries were recorded in four soils. Residual soil recovery by methanol extraction for TT residue yielded 46-99% recovery across the six soils studied, while DCM extraction for sulfonamides gave lower yields at 43-59% (SMO); 33-80% (SCP) and 46-55% for SM (Table 3.3). Employing a second extraction did not increase the overall recoveries much, only accounting for an overall increase in residual recovery by < 5% and < 2% for TT and sulfonamides respectively. These recoveries, however, were higher when compared with an earlier study that reported residual recoveries of 22-89% for four sulfonamides (Figueroa-Diva *et al.* 2010)

3.3.7 Recoveries during degradation study

The extraction scheme employed during the degradation experiment resulted in acceptable recoveries ranging from 72 to 88% for SMO in three top (0-10 cm) soils and sub-soils (30-40 cm) as summarised in Table 3.4. The choice of the solvent is an important step in the extraction procedure, and is dependent on the analyte or analyte mixture. The solvent must be able to remove the target compounds from the matrix and minimize the co-extraction of other

matrix components. Mixtures of low and high polarity solvents generally provide more efficient extractions than single solvents if analytes with a wide range of polarities are extracted. The most common extraction solvent described in the literature for the sulfonamides group is ethyl acetate (Haller *et al.* 2002; Pfeifer *et al.* 2002; Hamscher *et al.* 2005; Kreuzig and Holtge 2005) and methanol (Accinelli *et al.* 2007). Some authors prefer a mixture of methanol/water (Gobel *et al.* 2005), methanol/citric acid (Jacobsen *et al.* 2004) or acetonitrile (Sørensen and Hansen 2002) as a single solvent.

In this study conventional liquid extraction was used and a number of pure solvents were tested as extraction solvents, namely methanol, acetonitrile, water, hexane, ethyl acetate, dichloromethane and acetonitrile/water (70: 30). For TT, methanol and acetonitrile gave similar extraction yields, and methanol was chosen as it was most cost effective. The use of DCM instead of ethyl acetate resulted in 4-fold higher recoveries of sulfonamides in this study. Conventional liquid extraction is often used for the extraction of these antibiotics from environmental samples (Haller *et al.* 2002; Pfeifer *et al.* 2002; Hamscher *et al.* 2005; Kreuzig and Holtge 2005). However, in the last few years new extraction techniques such as pressurized liquid extraction (PLE), also known as accelerated solvent extraction (ASE), microwave-assisted extraction, and ultrasonic extraction have been increasingly used (Jacobsen *et al.* 2004; Blackwell *et al.* 2004b; Gobel *et al.* 2005; Forster *et al.* 2008).

The method detection limits (based on a signal to noise ratio of 3:1) are presented in Table 3.5. The present method detection limits are higher than those reported by other studies (Thiele-Bruhn *et al.* 2004; Blackwell *et al.* 2004a; Sassman *et al.* 2007). These studies used MS detectors, which are more sensitive than conventional UV detectors for analysing very low concentrations i.e. lower LOD. Furthermore these past studies used sophisticated techniques for extraction, such as an accelerated solvent extractor or a microwave extractor, which were not available for the present work. Such new techniques are often needed to analyse environmental samples, which demand addition clean-up. However, the conventional liquid extraction technique described here gave method detection limits that were certainly suitable for the study of spiked samples, which can be used to study sorption, degradation and transport of these antibiotics in soils. Given that a large number of samples must be analysed, the application of SPE cartridges to these studies would be expensive, and, moreover, would be unnecessary due to the high concentrations involved.

Table 3.2: Recovery test (a) TT, (b) SMO both isocratic, and (c) for three sulfonamides using the gradient elution (0.5 mg kg^{-1}). The averaged values along with their standard errors are reported here, with TS = topsoil, SS= subsoil.

(a)

	Te Kowhai		Matawhero	
Conc (mg kg^{-1})	Avg (%)	Error (%)	Avg (%)	Error (%)
0.2	100.7	2.1	94.6	4.4
0.5	95.5	6.8	82.3	2.2
2	86.1	0.6	58.8	2.6
5	77.5	2.5	67.0	2.9

(b)

Soil	Conc (mg kg^{-1})	Avg (%)	Error (%)
Te Kowhai	0.05	106.8	4.2
	0.2	90.8	2.1
	0.5	73.1	2.0
Hamilton	0.05	95.9	5.2
	0.2	105.0	3.8
	0.5	83.6	1.0
Horotiu	0.05	78.1	7.8
	0.2	67.2	2.0
	0.5	70.1	1.0

(c)

	SMO		SCP		SM	
Soil	Avg (%)	error (%)	Avg (%)	error (%)	Avg (%)	error (%)
Horotiu-TS	74.2	11.2	70.5	1.0	55.5	1.3
Te Kowhai-TS	70.4	12.9	82.5	1.0	51.7	4.0
Hamilton-TS	96.7	3.6	47.5	1.5	52.7	1.1
Horotiu-SS	86.0	2.0	43.7	3.9	37.4	0.7
Te Kowhai-TS	71.5	2.4	56.3	5.3	50.7	1.9
Hamilton-TS	53.2	3.7	51.0	2.9	49.4	4.9

Table 3.3: Summary of sorption recovery for TT, SMO, SCP and SM.

Soils	TT		SMO		SCP		SM	
	*OSR%	#RSR%	OSR%	RSR%	OSR%	RSR%	OSR%	RSR%
Matawhero	70.3	49.5 (2)	94.1	42.3	93.4	51.8	81.5	50.2 (2)
Te Kowhai	63.0	50.8	90.6	45.2	93.8	80.2	87.4	55.9 (2)
Hamilton	92.0	51.2	91.9	57.6	71.2	28.6 (2)	90.4	54.2
Horotiu	93.0	46.9	89.4	55.1	71.1	35.7 (2)	85.9	50.2
Gibsons	100.6	96.9(2)	92.9	42.5	72.5	33.3 (2)	96.7	46.3
Manawatu	96.8	68.9	94.9	58.3	76.8	40.6 (2)	96.3	54.2

*OSR= overall sorption recovery (extraction of both liquid and solid phases); #RSR = Residual sorption recovery (extraction of only solid phases); numbers within bracket indicate # of extractions

Table 3.4: SMO recovery during the degradation experiment. TS = topsoil; while SS = subsoil.

Soil	Recovery %	Stdev	Error %
Horotiu-TS	78.0	13.3	5.4
Te Kowhai-TS	71.7	8.4	3.4
Hamilton-TS	88.3	9.6	3.9
Horotiu-SS	83.9	11.0	4.5
Te Kowhai-TS	88.5	6.2	2.5
Hamilton-TS	88.5	12.0	4.9

Table 3.5: Method detection limits (µg kg^{-1}) for TT, SMO, SCP and SM extracted from all agricultural soils under study.

	TT	SMO	SCP	SM
Isocratic	41.6	13.3	13.3	13.3
Gradient	--	16.2	9.1	13.3
Fluorescence	--	3.3	--	--

3.4 Summary

A simple but robust analytical method was developed for extraction of TT and three sulfonamides from aqueous samples and soils. An isocratic elution scheme was developed to detect and quantify a macrolide (TT) and three SA's as single analytes using a mobile phase consisting of acetonitrile, trifluroacetic acid (0.05%, pH 2.3) and water and UV detection at 275 nm. A separate gradient method to detect all three sulfonamides in one mixture was also developed using a Luna C_{18} column. Both methods gave short retention times, and high sample throughput with minimum solvent use. The isocratic and gradient methods have been successfully implemented to investigate the sorption, degradation, and transport of these antibiotics in aqueous systems and soils in succeeding chapters. Employing methanol and dichloromethane as extraction solvents for TT and SA's gave excellent recoveries from soil residues. The extraction method is cost effective because it does not employ any extraction buffer or any solid phase extraction clean up techniques. Low extraction recoveries for certain soils can be attributed to the formation of permanently bound residues. The method detection limits were higher when compared with other studies involving the analysis of antibiotics in soils; however, its application seems appropriate in experiments involving spiked samples in soils and sediments or laboratory scale (sorption degradation and transport) studies, which do not necessarily require the low detection limits needed for environmental samples. The effectiveness of the extraction and analytical technique outlined in this chapter may vary significantly, depending on the soil type. Many New Zealand soils (including those used in this study) are among a subset of notably acidic soils. It is unclear how well the extraction approach might work on more alkaline and globally typical soils.

Chapter 4: Sorption behaviour of one macrolide, and three sulfonamide antibiotics in six New Zealand pasture soils

4.1 Introduction

To date, no published studies are available in New Zealand on the occurrence of antibiotic residues in its soils, lakes, and groundwater. However antibiotic residues have been detected in various parts of the world in environmental media such as soils, surface water and ground water (Hamscher *et al.* 2003; Zuccato *et al.* 2005; Perret *et al.* 2006; Luo *et al.* 2011). Given the amounts of antibiotic usage in New Zealand, and the fact that most ruminant animals graze the pasture throughout the year, potential exists for the occurrence of antibiotic residues in New Zealand's terrestrial and aquatic eco-systems. Furthermore, frequent use of antibiotics can also give rise to antibiotic resistant microbial populations causing different health problems in animals and humans due to the failure of antibiotics in medical activities (Halling-Sørensen *et al.* 1998; Sengelov *et al.* 2003). If the levels of the antibiotic residues in the receiving environment become high, they could even be toxic to non-target soil and aquatic organisms (Kummerer 2003). Therefore, an understanding of the behaviour of antibiotics in the New Zealand soil environment is of utmost important.

The ability of a soil to retain veterinary antibiotics depends on how well these chemicals sorb onto soil particles, and this tendency is often quantified as the sorption or partition coefficient (K_d), which is useful for predicting their transport potential in the environment and also for the purpose of risk assessment of these chemicals. Sorption plays a major role in the fate of chemicals in the environment including transport and degradation kinetics, and can result in the material remaining in the soil for a long time, and potentially encourage the development of resistant bacteria (Tolls 2001). Sulfonamides and macrolides are the two common classes of antibiotics that are widely used in livestock operation. According to New Zealand Food Safety Authority (NZFSA) both tylosin (a macrolide) and sulfonamide group of antibiotics contribute ~17% of the total antibiotic usage. A number of separate studies have been conducted on the sorption of sulfonamides and macrolides in soils. Tylosin has been found to be strongly sorbed to soil particles (Rabølle and Spliid 2000; Sassman *et al.* 2007); For instance, Sassman *et al.* (2007) reported K_d values ranging from 0.0022 to 5.52 L kg^{-1}, while

an early study by Rabølle and Spliid (2000) reported K_d values of tylosin to range from 8 to 128 L kg^{-1} in a few Danish soils. Sulfonamides, however have very low sorption affinity and tend to be highly mobile with K_d values ranging from 0.9 to 1.8 L kg^{-1} for SCP (Boxall *et al.* 2002), 8.3 L kg^{-1} for SM (Clay *et al.* 2005) and 9.1 to 150 L kg^{-1} for sulfamethoxazole in lake sediments (Hou *et al.* 2010). Sorption of veterinary antibiotics to soils is known to depend on soils properties such as CEC, pH and ionic strength (Ingerslev and Halling-Sørensen 2000). Sometimes mechanisms other than hydrophobic partitioning, such as cation exchange, cation bridging at clay surfaces, surface complexation, and hydrogen bonding, may also play a role in the sorption of veterinary medicines to soils and sediments (Tolls 2001; Sarmah *et al.* 2006a).

The behaviour of other classes of contaminants that are either excreted naturally by livestock animals (hormones) or applied (range of pesticides) to land are well documented in New Zealand soils (Sarmah *et al.* 2005; Sarmah *et al.* 2008; Scherr *et al.* 2009b). However, no information currently exists on the fate of veterinary antibiotics in the New Zealand environment. Results obtained from overseas studies cannot be extrapolated to reflect similar behaviour for the antibiotics in New Zealand conditions. This is primarily due to differences in pedo-climatic conditions, rainfall pattern, rainfall intensity and other temporal and spatial variability of soils including carbon content, mineralogical characteristics (such as clay and silica content) or pH. The predominance of volcanically derived New Zealand soils with a high percentage of allophonic clay mineral having high surface area, and high organic carbon content are known to strongly influence the fate of these organic contaminants (Sarmah *et al.* 2008).

The primary objective of this chapter was to evaluate the sorption affinity of a macrolide and three sulfonamide antibiotics for a range of New Zealand pastoral soils. Batch sorption experiments were conducted using two approaches: a complex solvent extraction scheme and the more traditional mass balance approach to determine the sorption parameters of the selected antibiotics in soils. Since the soils collected for the batch studies had a range of contrasting physico-chemical properties, correlation between soil properties and sorption parameters for the antibiotics were investigated.

4.2 Materials and Methods

4.2.1 Soils

Six archived topsoils (0-5 cm) representative of the dairy farming areas of the North Island of New Zealand with contrasting physico-chemical properties were selected for this study. The soils were freshly collected, air dried at room temperature and sieved to < 2 mm. The major physical and chemical properties of the soils are summarised in (Table 4.1). A full description of the soil and the methods used to determine the physico-chemical properties can be found in **Chapter 3**.

Table 4.1: Selected properties of soils used in the sorption study.

Soils	pH 1:2 H_2O	pH 1:2.5 $CaCl_2$	OC (%)	CEC ($cmol_c\ kg^{-1}$)	Sand %	Silt %	Clay %	SSA (m^2g^{-1})	% oven dry Fe	Mn
Matawhero silt loam	6.1	4.3	2.1	15.4	11.0	62.0	27.0	6.0	---	---
Te Kowhai silt loam	6.7	5.1	5.0	21.7	9.0	54.0	37.0	19.7	---	---
Hamilton clay loam	5.8	4.8	4.0	17.2	19.0	51.0	30.0	22.3	---	---
Horotiu silt loam	5.7	5.4	8.2	28.2	34.0	48.0	17.0	19.7	2.9	0.25
Manawatu sandy loam	5.1	5.4	3.3	9.7	87.0	11.0	2.0	13.6	3.2	0.1
Gibsons sandy loam	6.9	5.2	1.1	7.6	45.0	41.0	14.0	---	2.9	0.1

a=specific surface area was measured by para nitro-phenol (Hewitt 1998)

4.2.2 Kinetics study

Preliminary studies were conducted to determine the appropriate soil to solute ratio used in the batch sorption studies. For this, a single point K_d value for each antibiotic was used and equilibrium concentration (C_{eq}) was calculated for varying aqueous concentrations (C_w) such that these C_{eq} values fell within the detection limit. A soil to solution ratio of 1:15 gave concentrations above detection limit for all combinations. To determine the equilibration time, 5 mM $CaCl_2$ (400 mL) solution was spiked with an appropriate volume of methanolic stock solution of TT, SMO, SCP and SM (1000 mg L^{-1}) to yield aqueous concentrations of 2, 6.5, 2 and 3.5 mg L^{-1} respectively. This solution (30 mL) was added to soil (2 g) taken in a glass centrifuge tube (35 mL), which was pre-weighed and equipped with a Teflon®, lined screw cap.

For every time interval (0.33, 0.8, 1, 2, 4, 8, 12, 24, 36, 48 h) duplicate samples were prepared and the tubes were wrapped with aluminium foil to avoid photodegradation. The samples were then placed into a rotary drum shaker (end-over-end) at room temperature (23 ± 1°C). Duplicate samples at a predetermined time interval were removed from the shaker and the tubes were centrifuged (1750 × g) for 10 minutes. For the sulfonamides 0.5 mL of the supernatant was transferred into an amber glass HPLC vial for direct HPLC-UV analysis. For TT which exhibited high sorption, the supernatant (10 mL) was extracted with DCM (5 mL). The extracted DCM (1 mL) was quantitatively transferred into a HPLC vial, evaporated to dryness under a gentle stream of N_2, reconstituted in methanol (0.5 mL) and analysed using HPLC-UV detection. The sorbed amount C_s [mg kg^{-1}] was estimated by the mass difference between the initial and final concentration as follows:

$$\boldsymbol{C_s = (\, C_i - C_w\,) * V_w/m_s} \qquad \textbf{(4.1)}$$

Where, C_i is the initial solute concentration [mg L^{-1}]; C_w [mg L^{-1}] is the equilibrium solute concentration [mg L^{-1}], V_w is the solute volume [mL]; and m_s is the soil mass [kg], respectively.

4.2.3 Batch sorption studies

A stock solution of TT, SMO, SCP and SM antibiotic at a concentration of 1000 mg L^{-1} was prepared in methanol, covered with aluminium foil and was stored in the dark (4^oC). By adding an appropriate amount of the stock solution separately to 5 mM $CaCl_2$ solution, six different initial aqueous solution concentrations were prepared in duplicate. Batch studies were performed using a similar experimental protocol earlier adopted by Sarmah *et al*. (2008). Duplicate samples of air-dried soils (2 g) were weighed into glass centrifuge tubes (35 mL) with Teflon-lined screw caps. Aliquots (30 mL) of six concentrations of TT (2.5, 5, 10, 15, 25 & 35 mg L^{-1}) and 3 sulfonamide (1.5, 3, 5, 7.5, 10 & 15 mg L^{-1}) were added to the respective tubes. The tubes were wrapped in aluminium foil, placed in the dark to limit degradation due to photolysis and shaken in an end-over-end shaker for 24 h. Solutions containing only the antibiotic but without soil (soil blank), and solution containing only soil in 5 mM $CaCl_2$ without antibiotic (antibiotic blank), served as controls. These treatments were included in all experiments to determine container sorption losses and to check for peaks that might interfere during analysis. Also the mobile phase was analysed separately to check for possible interfering peaks.

4.3 Extraction and analysis

Following equilibration, the tubes were centrifuged (1750 × g, 10 min). In order to determine the aqueous phase concentration for each antibiotic; supernatant (0.5 mL) was immediately analysed by HPLC and UV detection. The remaining supernatant was carefully decanted and the weight of the remaining residual liquid phase was measured gravimetrically. It was assumed that antibiotic concentration in the residual soil was the same as that in the bulk supernatant solution. Due to high sorption affinity of TT to Matawhero soil, liquid-liquid extraction was performed on 10 mL of the supernatant obtained after centrifugation with 5 mL of DCM, and the subsequent steps were similar to the residual soil extraction protocol as discussed in Chapter 3.

4.3.1 Data Analysis and Sorption modelling

Sorption coefficients were modeled using the Freundlich model: $C_s = K_f C_w^N$, where C_s ($\mu g\ g^{-1}$) and C_w ($\mu g\ mL^{-1}$) are the equilibrium sorbed and aqueous phase concentrations respectively, K_f ($\mu g^{1-N} mL^N g^{-1}$) is the Freundlich sorption coefficient and N (dimensionless) is the measure of sorption non-linearity ($N = 1$ represents a linear isotherm). K_f was quantified by the empirical Freundlich equation in the log transformation form: $\text{Log } C_s = \log K_f + N \log C_w$. The amount of antibiotic sorption per unit soil organic carbon, K_{oc}, was determined using the relationship $K_{oc} = K_f$/soil organic carbon content (%).

For organic contaminants whose isotherms are non-linear, the linear partition coefficient (K_d) does not best describe the sorption behaviour as Freundlich coefficients depend on exponent (N) value. While there is no universally accepted threshold value of N that defines sorption non-linearity, it has been suggested that that any isotherm with $1.05 > N > 0.95$ can be considered linear, with caution required in data interpretation as N is sensitive to the concentration range used in the study (Pignatello *et al.* 2006). Due to observed inconsistency in isotherm linearity for the majority of soil-solute combinations, we found that it was difficult to compare K_d or K_{oc} values between solutes or soils as they were concentration-dependent. Therefore, in order to compare sorption across soils, a concentration-dependent effective distribution coefficient ($K_d^{eff} = K_f C_w^{N-1}$) was calculated with an equilibrium concentration of 0.5 mg L^{-1} (Table 4.3). Like-wise, concentration-dependent organic carbon-

normalised partition coefficient values (K_{oc}, mg L^{-1}) were calculated for SMO and SCP for each soil at two different concentrations of 0.5 mg L^{-1} and 5 ng L^{-1} using the equation $K_{oc} = K_f C_w^{N-1} / f_{oc}$, where f_{oc} is the fraction of soil organic carbon. The K_{oc} defined through this approach is equivalent to a single-point OC-normalised distribution coefficient determined at a particular concentration (C_e); however, it is not the same as the slope of the linearized isotherm, as it is commonly defined in the literature (Pignatello *et al.* 2006).

4.4 Results and discussion

4.4.1 Sorption kinetics

Matawhero silt loam soil was chosen as model soil for the sorption kinetics experiment. The soil had neutral pH, high CEC, and average OC values (Table 4.1). The aim of this study was to measure the sorption kinetics of TT, SMO, SCP and SM to determine the equilibrium time, and assess any biotic and abiotic degradation of these compounds which could occur during short term batch sorption experiments. A study by Allaire *et al*. (2006) showed that for sorption experiments shorter than 48 h of contact time the soils need not be sterilized.

The results for the kinetic studies showed that sorption was rapid in the first few hours of the contact time of 0–2 h for sulfonamide and 0–4 h for TT (Figure 4.1); however, apparent equilibrium was attained as evident from plateauing of the curve. Irrespective of the various initial concentrations used in this study, the initial rapid sorption phase was similar for all compounds, indicating that initial phase of sorption was concentration independent. Similar aqueous concentrations after the initial rapid sorption phase suggest no influence of degradation processes or desorption phenomena even after 48 h of contact time. A study by Rabolle and Spliid (2000) showed little difference between partition coefficients for tylosin in non-sterile soils either at 4 h or 24 h. A similar conclusion was reached in a study by Ingerslev and Halling-Sorensen (2001) who observed no significant changes in K_d values for tylosin in sodium azide (used to inhibit microbial degradation) amended soil-manure slurry at 1 h and 24 h. No metabolite formation was observed within the studied incubation times. These outcomes and literature confirmed that the use of sterile soils in the batch sorption studies was not required.

TT sorption in the first few hours was fast, with > 95% of sorption occurring within the first 4 h. However, for sulfonamides a contact time of 8 h was required for the equilibrium to be attained. This observation was, similar to the findings of Figueroa-Diva *et al.* (2010), who observed slow sorption (12–48 h) for sulfonamides. Sanders *et al.* (2008) showed that apparent equilibrium was reached for sulfadimethoxine in three soils after 24 h. This was in contrast with the slow sorption kinetics observed by Kahle and Stamm (2007b) who reported that the sorption affinity for STZ was higher after 14 days than after one day of equilibration time for all investigated soils.

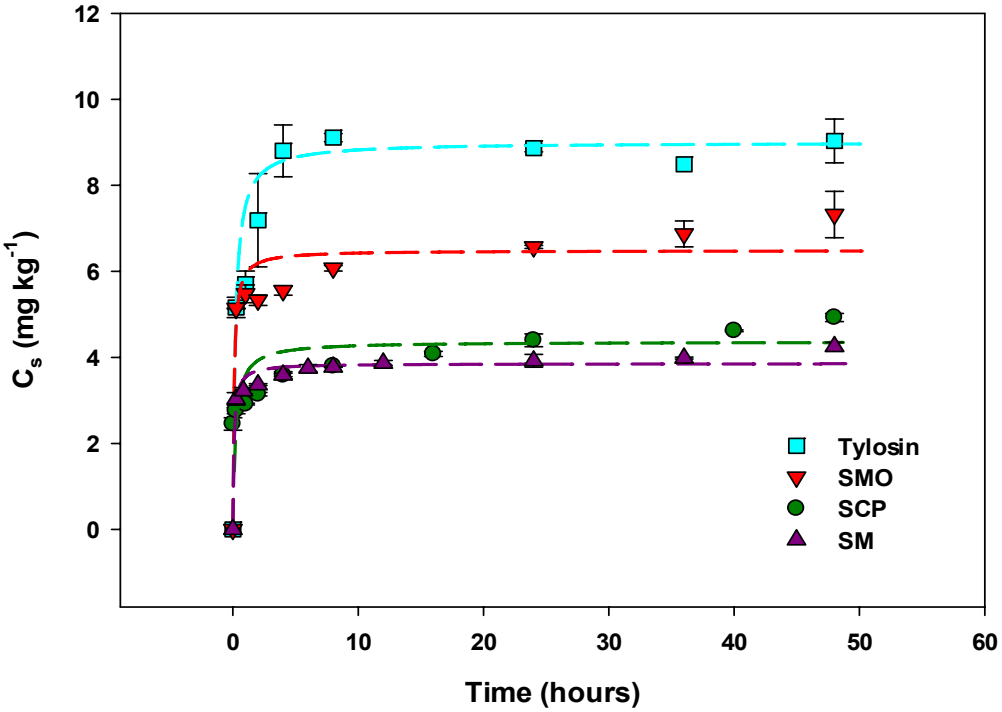


Figure 4.1: Sorption kinetics for TT, SMO, SCP and SM in Matawhero soil. Vertical bars represent standard error of means (n = 2). Bars that are not visible fall within the symbol.

Other studies have shown that contact times of up to 48 h (Boxall *et al.* 2002) and 72 h (Figueroa-Diva *et al.* 2010) were required for equilibration to be achieved for sulfonamides. In the present study, for TT, < 5% decrease in the relative concentration was observed from 8 to 48 h of contact time. However, < 10% increase in the relative concentration was observed for SA's from 12 to 48 h. Therefore, 24 h was chosen to be appropriate and the batch equilibrium studies were carried out under conditions at or approaching equilibrium (pseudo-equilibrium) time for SA's and TT. This contact time was the same as that which was earlier used by Allaire *et al.* (2006). The contact times for the other 5 soils were assumed to be

similar to that of Matawhero soil, and all isotherms were constructed using an apparent equilibration time of 24 h.

4.4.2 Recovery

Quantitative recovery of TT, SMO, SCP and SM obtained from 5 mM $CaCl_2$ solution over the concentration range ranged from 95% at 15 mg L^{-1} to 105% at 0.5 mg L^{-1}. Overall sorption recoveries ranged from 63 to 101% (TT), 89 to 95% (SMO), 71 to 94% (SCP), and 82 to 97% (SM) in 6 soils respectively (Figure 4.2). Methanol extraction for TT residue yielded 46 to 99% recovery from all six soils. DCM extraction for sulfonamides yielded soil phase recovery of 43–59% for SMO, 33–80% for SCP, and 46–55% for SM (Figure 4.3).

Analysis of the sample chromatograms showed no evidence of a metabolite peak (analytical methodology could not detect metabolite) corresponding to concurrent loss of SMO, suggesting the low recovery in six soils was the result of the portion that was non-extractable with the organic solvent rather than degradation. Low extraction efficiency from soil residues often leads to underestimation of sorption in which case the mass balance approach is preferable (Kolz *et al.* 2005; Figueroa-Diva *et al.* 2010). These past studies computed sorbed antibiotic concentrations by the difference between the initial aqueous concentration (C_i) and the final aqueous concentration (C_w) after equilibrium.

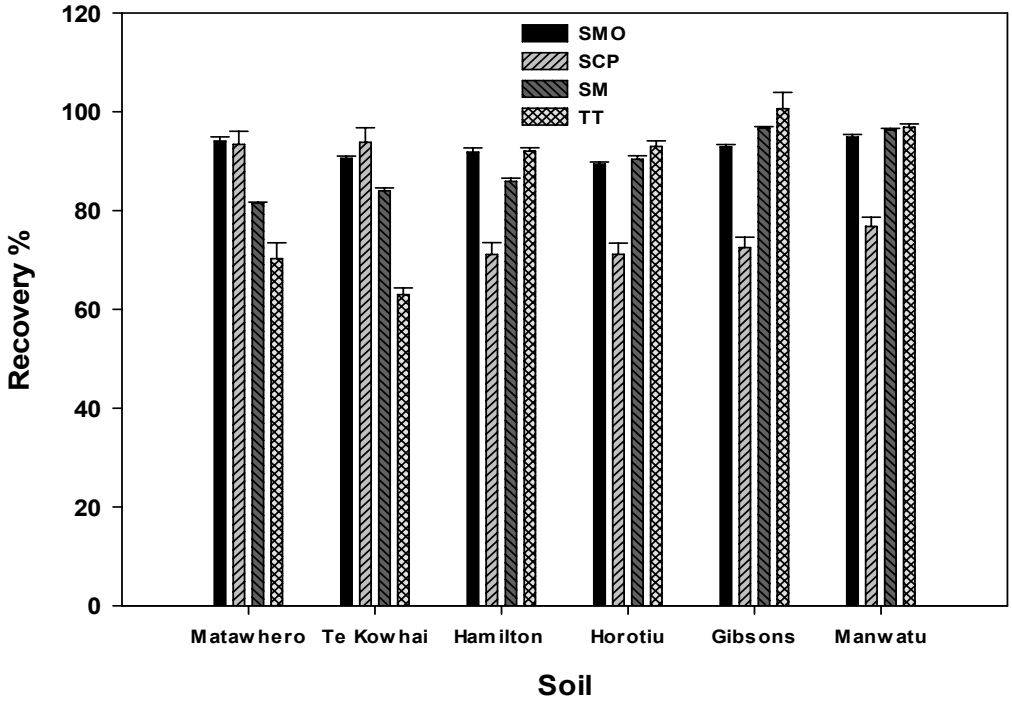


Figure 4.2: Recoveries from the sorption study from both aqueous matrices $CaCl_2$ (5 mM) and residue soil at six different initial concentrations. Average values and relative standard deviations are shown.

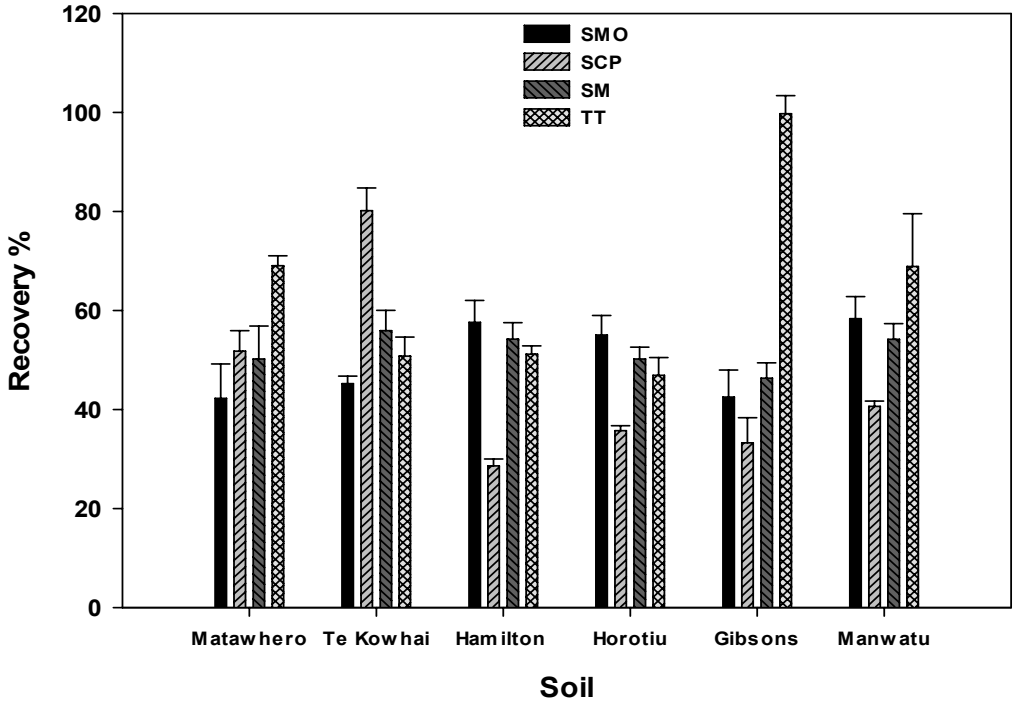


Figure 4.3: Recoveries from the sorption study only from the residue soil at six different initial concentrations. Average values and relative standard deviations are shown.

4.4.3 Sorption isotherms

Plots for sorption isotherms of TT, SMO, SCP, and SM constructed from both the techniques are shown in Figure 4.4 and Figure 4.5. The model derived sorption parameters for each soil and compound are summarised in Table 4.2 and Table 4.3, along with their *N* and R^2 values. The Freundlich model could describe the isotherms constructed from both the schemes (by difference/mass balance approach, and extraction technique).The isotherms constructed using extraction datasets produced a better fit than the isotherms produced using data obtained from the mass balance approach (difference between the initial and final solution concentration), as indicated by the high coefficient of determinant (R^2) values. However, given that the mass recoveries extracted were low, and no metabolite was formed during sorption equilibration, it was more accurate and appropriate to construct the isotherms using concentration values obtained by difference technique than the datasets obtained from extraction technique. Henceforth only isotherms constructed by using difference technique have been discussed in detail. However, for the purpose of comparison, isotherm plots were constructed from both the techniques.

4.4.4 Variation in sorption parameters

Based on the pKa and soil pH data from Table 3.1, the expected net charges for each antibiotic under the pH values of the soils would be as follows: TT would be present as a cation (TT^+) under all conditions. SCP and SMO would be present as anions (SCP and SMO would be present as anions (SCP^-, SMO^-) except in the case of SMO in Manawatu soil- where the neutral form would dominate. SM would always be present in neutral on average and therefore more lipid soluble. Among the sorbates examined, K_d^{eff} for TT ranged from as low as 1.6 in Manawatu soil to 756 L kg^{-1} in Matawhero soil, while for the three sulfonamides it ranged from 1.37 to 13.54 L kg^{-1} across the 6 soils investigated. The general trend was that isotherms for all four compounds were highly non-linear (Table 4.3). For TT isotherms exhibited highly nonlinear behaviour except in Manawatu soil with *N* value = 1, while *N* values for TT varied between 0.38 and 0.71 for the other 5 soils.

For the sulfonamides there was a marked difference in K_d^{eff} values across all soils consistent with the soils OC content (Table 4.1). Values for *N* varied between 0.40 and 0.96 for SMO, 0.76 and 0.93 for SCP, and ranged from 0.50 to 1.08 for SM in the six soils.

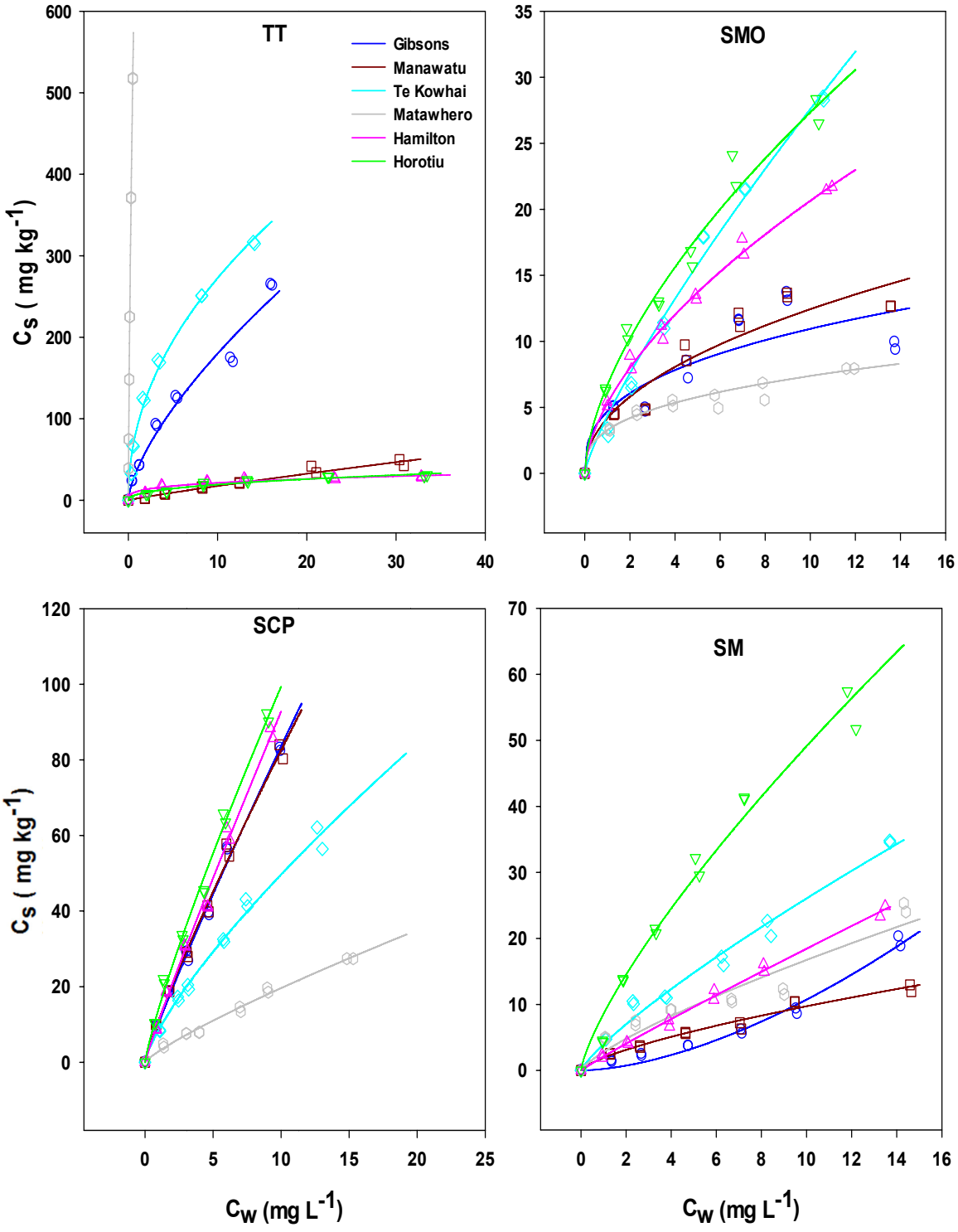


Figure 4.4: Multiple-concentration batch sorption isotherms for TT, SMO, SCP and SM obtained by the difference approach. Symbols represent measured data, while solid lines represent Freundlich model fits.

Table 4.2: Summary of sorption parameters for TT, SMO, SCP and SM derived from the multiple-concentration isotherms constructed using the difference (mass balance) approach for six soils.

Soils	TT								SMO							
	K_d	R^2	K_f	N	R^2	$^{\#}K_d^{eff}$	Log K_{oc}^{a}	Log K_{oc}^{b}	K_d	R^2	K_f	N	R^2	$^{\#}K_d^{eff}$	Log K_{oc}^{a}	Log K_{oc}^{b}
Matawhero	1150.00	0.95	851.14	0.71	1.00	756.13	4.70	6.16	1.40	0.90	2.37	0.75	1.00	2.83	2.13	3.40
Te Kowhai	26.17	0.71	80.02	0.56	0.96	66.88	3.34	5.55	2.91	0.97	3.26	0.96	0.99	3.21	1.83	2.02
Hamilton	1.16	-0.19	8.82	0.38	0.84	6.86	2.53	5.63	2.20	0.69	5.44	0.56	0.94	4.55	2.27	4.48
Horotiu	1.13	0.28	4.78	0.57	0.82	4.02	1.89	4.03	3.09	0.83	6.75	0.60	0.95	5.75	2.03	4.01
Gibson	16.75	0.91	42.14	0.59	0.99	35.76	3.23	5.25	1.12	0.23	4.16	0.42	0.72	3.29	2.28	5.17
Manawatu	1.53	0.95	1.63	1.00	0.96	1.63	2.17	2.19	1.27	0.49	3.85	0.51	0.86	3.16	2.69	5.13

Soils	SCP								SM							
	K_d	R^2	K_f	N	R^2	$^{\#}K_d^{eff}$	Log K_{oc}^{a}	Log K_{oc}^{b}	K_d	R^2	K_f	N	R^2	$^{\#}K_d^{eff}$	Log K_{oc}^{a}	Log K_{oc}^{b}
Matawhero	1.87	0.92	3.27	0.76	0.96	2.96	2.27	3.48	1.66	0.84	4.94	0.50	0.80	4.03	2.52	5.02
Te Kowhai	5.07	0.99	8.01	0.80	0.99	7.38	2.27	3.27	2.61	0.96	5.15	0.69	0.95	4.55	2.10	3.64
Hamilton	9.53	0.99	14.49	0.83	0.99	13.54	2.61	3.45	2.92	0.89	5.34	0.74	0.96	4.80	2.20	3.52
Horotiu	10.59	0.98	10.98	0.93	0.99	10.66	2.15	2.51	5.08	0.93	5.98	0.96	0.96	5.89	1.87	2.07
Gibson	8.76	0.82	10.11	0.87	0.80	9.60	2.52	3.16	1.45	0.90	0.83	1.08	0.95	0.85	1.37	0.97
Manawatu	8.60	0.98	11.47	0.85	1.00	10.79	3.06	3.82	0.96	0.91	2.00	0.68	0.98	1.76	2.36	3.95

K_d^{eff} is the concentration dependent effective sorption distribution coefficient ($K_d^{eff} = K_f C_w^{N-1}$) using lowest aqueous equilibrium solution concentrations of C_w = 0.5 mg L^{-1}; *concentration dependent OC-normalized sorption coefficient calculated using $K_{oc} = K_f C_w^{N-1} foc^{-1}$ at $C_w = 0.5^a$ mg L^{-1} & 5^b ng L^{-1}; K_d^{eff} in L kg^{-1} and K_f in mg $^{1-N}$ L ^{N} kg^{-1}

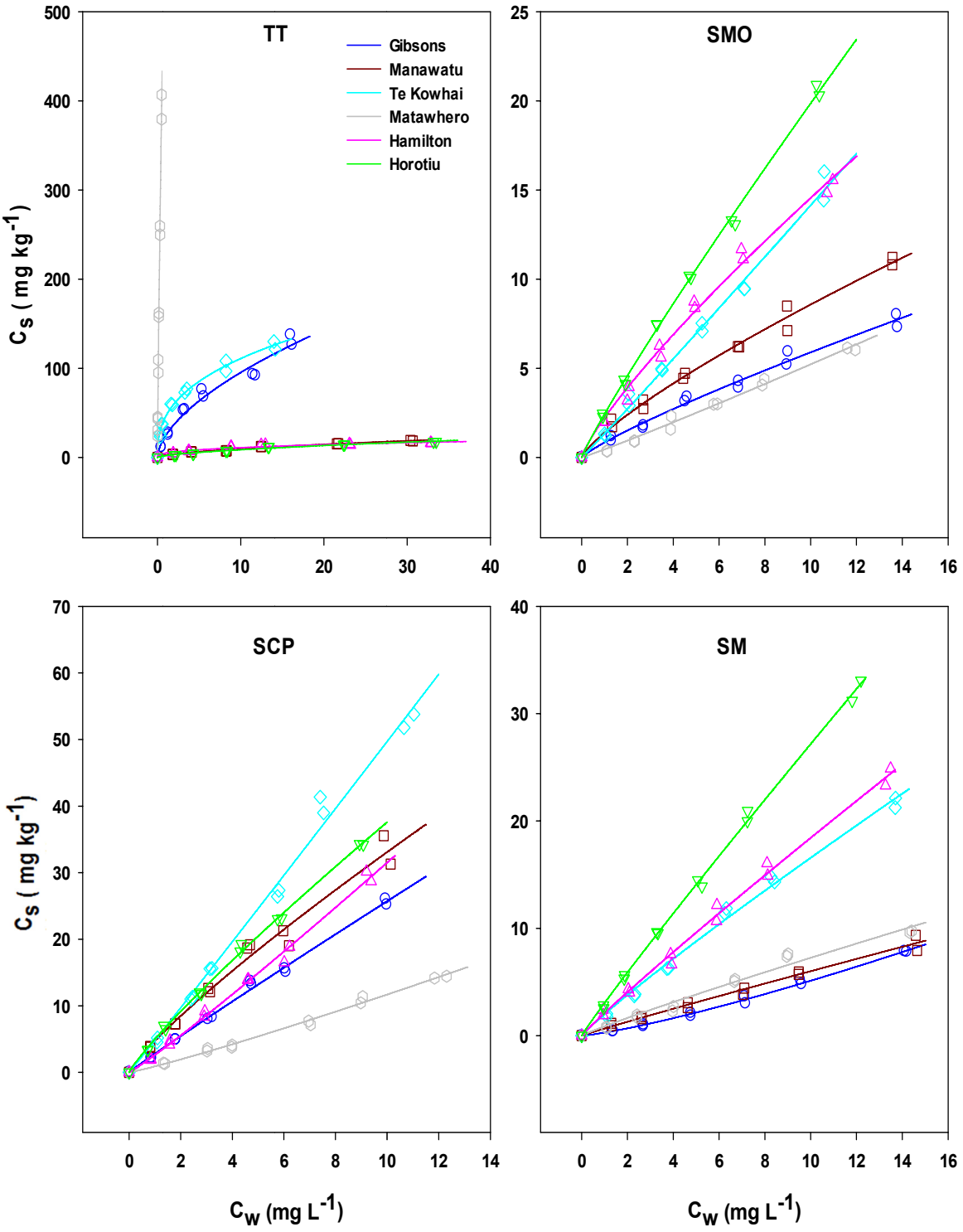


Figure 4.5: Multiple-concentration batch sorption isotherms for TT, SMO, SCP and SM obtained by the solvent extraction approach. Symbols represent measured data, while solid lines represent Freundlich model fits.

Table 4.3: Summary of sorption parameters for TT, SMO, SCP and SM derived from the multiple-concentration isotherms constructed using the extraction scheme for selected soils.

Soils	TT								SMO							
	K_d	R^2	K_f	N	R^2	$^{\#}K_d^{eff}$	Log K_{oc}^{a}	Log K_{oc}^{b}	K_d	R^2	K_f	N	R^2	$^{\#}K_d^{eff}$	Log K_{oc}^{a}	Log K_{oc}^{b}
Matawhero	842.63	0.97	631.68	0.74	1.00	496.05	4.56	5.88	0.52	0.99	0.34	1.21	0.99	0.37	1.14	0.08
Te Kowhai	10.67	0.43	44.59	0.40	0.98	25.84	3.13	6.11	1.41	0.99	1.35	1.02	0.99	1.36	1.43	1.32
Hamilton	0.64	0.21	3.76	0.45	0.92	2.28	2.14	4.87	1.52	0.97	2.00	0.88	0.99	1.90	1.74	2.36
Horotiu	0.61	0.86	1.58	0.72	0.98	1.21	1.37	2.79	2.04	0.99	2.51	0.89	1.00	2.40	1.52	2.04
Gibson	14.86	0.82	41.62	0.60	0.98	28.89	3.70	5.69	0.59	0.97	0.81	0.86	0.98	0.77	1.91	2.61
Manawatu	0.70	0.84	2.34	0.62	0.98	1.64	1.97	3.89	0.86	0.95	1.50	0.75	0.98	1.36	1.73	2.99

Soils	SCP								SM							
	K_d	R^2	K_f	N	R^2	$^{\#}K_d^{eff}$	Log K_{oc}^{a}	Log K_{oc}^{b}	K_d	R^2	K_f	N	R^2	$^{\#}K_d^{eff}$	Log K_{oc}^{a}	Log K_{oc}^{b}
Matawhero	0.80	0.98	0.97	1.08	0.99	1.00	1.64	1.23	0.78	0.96	1.10	0.99	0.97	1.10	1.72	1.76
Te Kowhai	4.76	0.99	4.54	1.03	0.99	4.60	1.95	1.79	1.70	0.98	1.97	0.94	0.99	1.92	1.61	1.92
Hamilton	3.06	0.99	2.52	1.11	0.99	2.63	1.77	1.23	1.85	0.99	2.19	0.92	0.99	2.12	1.76	2.16
Horotiu	3.95	0.99	4.74	0.91	1.00	4.57	1.79	2.24	2.74	0.99	2.91	0.98	1.00	2.88	1.56	1.68
Gibson	2.08	0.87	2.61	1.01	1.00	2.62	2.37	2.34	0.52	0.97	0.27	1.27	1.00	0.31	1.32	-0.03
Manawatu	2.42	0.96	4.55	0.87	0.99	4.31	2.18	2.85	0.60	0.98	0.77	0.88	0.98	0.73	1.40	1.98

$^{\#}K_d^{eff}$ is the concentration dependent effective sorption distribution coefficient ($K_d^{eff} = K_f C_w^{N-1}$) using lowest aqueous equilibrium solution concentrations of C_w = 0.5 mg L^{-1}; *concentration dependent OC-normalized sorption coefficient calculated using $K_{oc} = K_f C_w^{N-1} foc^{-1}$ at $C_w = 0.5^a$ & 0.5^b ng L^{-1}; K_d^{eff} in L kg^{-1} and K_f in mg $^{1-N}$ L ^{N} kg^{-1}

The linearity was best at 0.96 for SMO in Te Kowhai and SM in Horotiu soil. Sorption of SM in Gibson soil showed significant deviation from linearity, with $N > 1$, implying that percent change in sorption (slope) with increasing C_w is greater at the higher concentration range. Overall, the *N* values obtained in this study fell within the range reported in the literature. Similar nonlinear sorption isotherms have been reported for tylosin and sulfonamides under neutral pH conditions (Ingerslev *et al.* 2001; Clay *et al.* 2005; Lertpaitoonpan *et al.* 2009; Figueroa-Diva *et al.* 2010; Wehrhan *et al.* 2010) in contrast to some studies which reported linear isotherms (Boxall *et al.* 2002; ter Laak *et al.* 2006). The reasons for non-linear isotherms in batch sorption studies involving soils have been well-documented (Pignatello and Xing 1996). These types of non-linear behaviour for hydrophobic contaminants have been attributed to the broad range of interaction energies, as well as to the high degree of variability in those energies from one sorbent to another (Weber *et al.* 1992).

In this present study, the soil properties such as texture, soil organic carbon content, CEC and soil pH were different across the soils investigated. As a result the sorption affinity of the antibiotics varied from soil to soil, and also with the antibiotic type. For TT^+, the sorption potential in Matawhero soil (2.1% OC), which has a high CEC value, was 200-fold greater than that for Horotiu soil (8.1% OC). This can be attributed to the role of cation exchange in TT^+ sorption as all 6 soils had a pH below the pKa of TT (7.1). Matawhero and Horotiu soils both have high CEC, however, the high OC content of Horotiu must inhibit cation exchange. Te Kowhai, which has a lower CEC and a lower OC content than Horotiu gives a higher K_f or for TT. This, coupled with fast sorption kinetics, indicates a low probability of TT leaching through Matawhero and Te Kowhai soils and contaminating the aquifer. While TT could be non-mobile and sorptive in soils like Matawhero, Te Kowhai and Gibson, it could behave differently in Hamilton, Manawatu and Horotiu soils where the compound can become mobile. However, potential exists for dissolved organic-matter facilitated transport, or colloid facilitated-transport along with other nutrients or by preferential flow (Kay *et al.* 2005a).

It is conceivable that the presence of more divalent cations in Matawhero and Gibson soils as indicated by the ICPMS results for soils (Table 12.1) could possibly lead to enhanced TT sorption. Recently, it was shown that divalent cations at low concentrations enhanced the sorption of ibuprofen and ketoprofen onto silica, and trivalant cations (Al^{3+} and Fe^{3+}) enhanced it even further (Bui and Choi 2010). A closer look at the XRD data for the soils

(Figure 12.3) reveals an intense presence of quartz (Q), feldspar (F) and calcite (K) in Matawhero, Gibson, and Manawatu soils; while the peaks from Hamilton, Horotiu and Te Kowhai were less intense. This greater mineral composition of the bulk soil could have contributed to the increased sorption affinity of TT to Matawhero and Gibson soil.

For the three sulfonamides, the K_d^{eff} values ranged from 1.37 to 13.54, the lowest being for SM and highest for SCP antibiotic across all soils. Horotiu soil (OC 8.2%) gave the highest K_f values for all 3 sulfonamides, while Matawhero soil and Gibson with an OC content of around 2.1 and 1.1% (Table 4.1) gave the lowest. Horotiu soil is volcanically derived, and has a high content of allophane (an amorphous hydrous alumino-silicate clay mineral with a high surface area) of 28% (Sarmah *et al.* 2008). This may contribute to the overall sorption mechanisms for the antibiotics onto this soil and may be responsible for higher K_d^{eff} values (Table 4.2). The sorption parameters obtained in this study are similar to those obtained in various overseas studies (Tables 2.–2.8). For instance, the results obtained in the present study were in agreement with some earlier findings of Drilla *et al*. (2005), who found K_d values of SMO in two soils with varying organic carbon and clay content as 0.23 L kg^{-1} (0.4% OC and 45% clay) and 37.6 L kg^{-1} (7.1% OC and 15% clay content). Overall, sorption of sulfonamides to the six soils investigated in the present study increased with increases in OC content. Recent studies have reported the influence of soil organic carbon on the sorption of sulfonamide antibiotic (Lertpaitoonpan *et al.* 2009; Figueroa-Diva *et al.* 2010). Kahle and Stamm (2007a) reported sorption to inorganic sorbents (clay) to be an order of magnitude lower than that to organic matter at neutral pH. Sulfonamides at soil pH tend to be mostly in neutral and anionic form. While the neutral species are readily adsorbed on to the organic matter, the ionic form is readily taken up by negatively charged clay particles. Therefore these findings lend further support that sorption of sulfonamide antibiotic to soils is primarily due to hydrophobic partitioning mediated by soil organic carbon, and partially due to anion exchange to negatively charged soil particles.

Concentration-dependent average log K_{oc} values for the three sulfonamides based on the lowest equilibrium concentration of 0.5 mg L^{-1} ranged from 1.37 (SM) to 3.07 (SCP). Based on the log K_{oc} values obtained in this study, of the three sulfonamides, SM seemed to possess the highest leaching potential, and SCP the lowest. The average log K_{oc} value for TT (2.98) was 0.5 log units higher than SCP (2.47). When an environmental concentration of 5 ng L^{-1}

was used, log K_{oc} values for TT doubled, however for the sulfonamides the increase was not substantial making them mobile and a potential threat to surface and ground water than TT which showed more sorptive tendency.

In comparison with parameters obtained with the difference technique, K_d^{eff} values obtained from the extraction scheme were 2 to 3 times lower for TT and SA's, and the isotherms were mostly non-linear for TT sorption and mostly linear for sulfonamide sorption (Table 4.3). It is noteworthy that for Gibson soil, K_f values obtained by both schemes for TT were the same i.e. 42.2 mg $^{1-N}$ L ^{N} kg^{-1}, this was because of the ~100% recovery from the soil residue. In this study significant differences in K_f values were obtained by measuring only the solution phase, and by solvent extraction of the solid phases. For example, Table 4.2 shows that the log K_{oc} values for TT, SMO, SCP and SM and in all the six soils were higher than that which was observed when solvent extraction was done. Another interesting observation was the increases in the sorption linearity in the soils when sorption was estimated by measuring the solvent extracted solid phase. It is clear from this study that differences in experimental protocol could potentially contribute to the significant disparity in values reported for K_d and N from various studies in the past.

4.4.5 Correlation between soil properties and sorption affinity

In this study no correlation was seen for TT sorption to soil parameters such as CEC and %OC. For SM however good correlation was obtained to CEC, % OC and % clay (Figure 4.6). The findings were similar to a previous study by ter Laak *et al*. (2006). When similar datasets from ter Laak *et al*. (2006) were plotted so that a correlation between the soil properties (Figure 4.7), it was observed that none of these individual soil properties could explain the variation in sorption coefficients for TT, while SCP showed positive correlation to OC% of the soil. This emphasis the fact that sorption of antibiotics to soil is complex due to their ionisable functional groups, which increase their binding ability to soil particles. This could be due multiple mechanisms such as hydrogen bonding, electrostatic interactions, ionic exchange, and hydrophobic interactions may play a role in sorption process. This was, however, in contrast to the findings reported by Sassman *et al.* (2007), who observed good correlation of tylosin sorption with % clay and SSA of soils. More recently, Fan *et al*.(2011) also reported that sulfonamide sorption was well correlated with % OC, % sand and % silt in

a number of US soils. The authors reported difficulty in distinguishing the effects of pH from the effects of soil OM on sorption. However, other studies (Boxall *et al.* 2002; Thiele-Bruhn *et al.* 2004) have shown that the sorption of SA's dramatically decreased as soil pH increased suggesting the role of pH contributing to the relationship between OM and sorption of sulfonamides in their study.

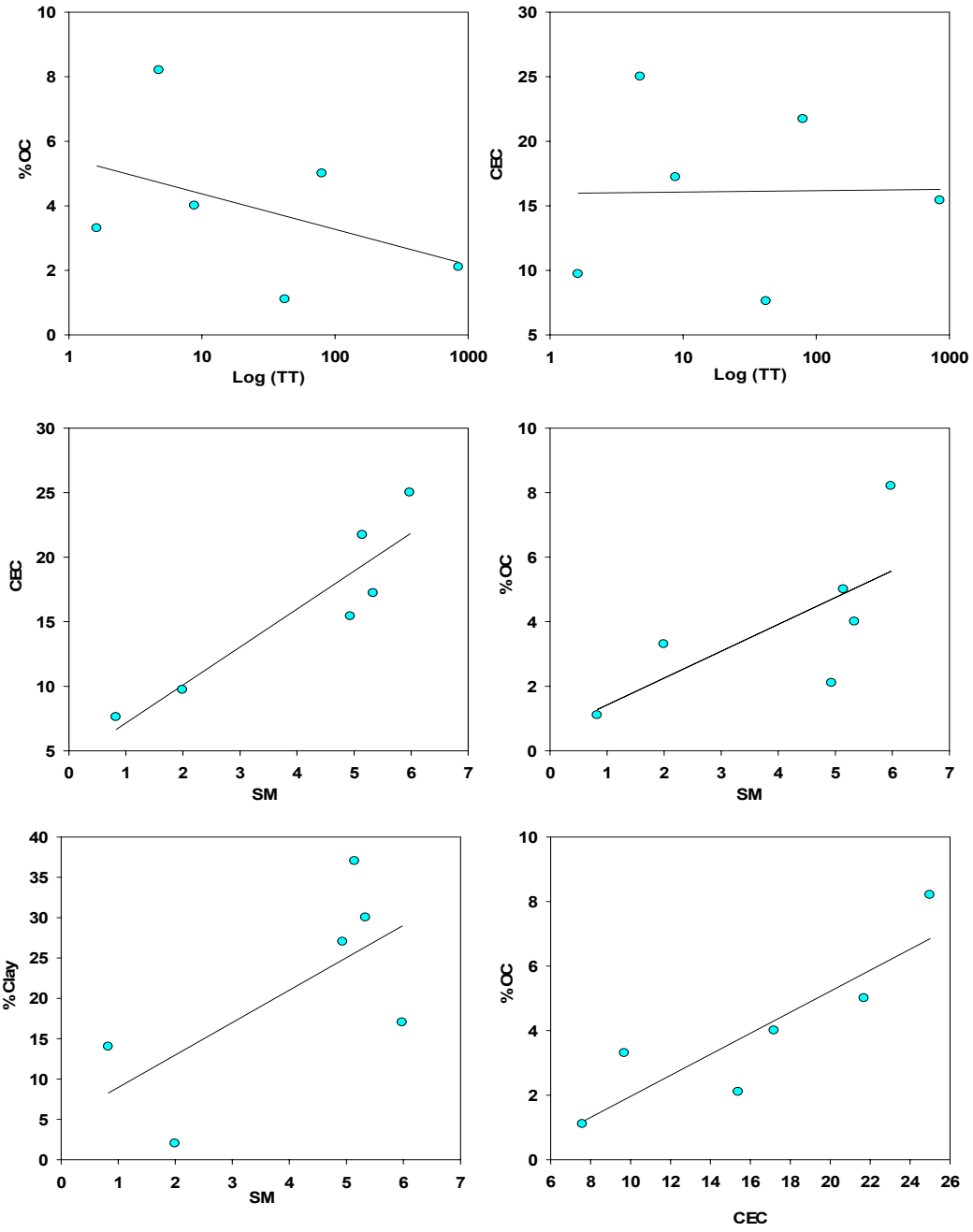


Figure 4.6: Scatter plots showing relationship between for soil properties and sorption affinity. #Lines represent linear regression.

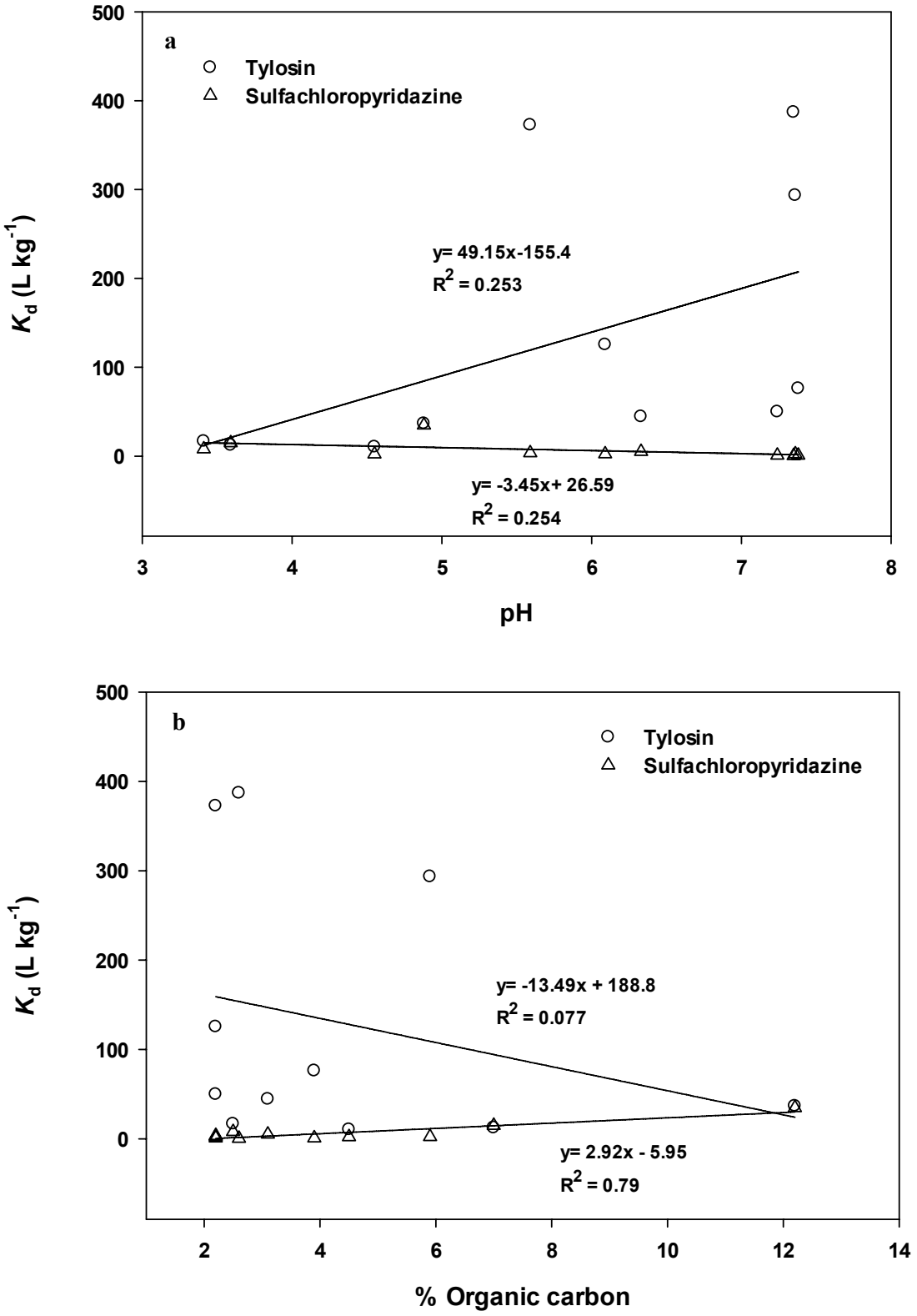


Figure 4.7: Correlation between soil properties pH (a) and % OC (b) and sorption affinity, data obtained from ter Laak *et al*. (2006).

4.4.6 Statistical analysis of sorption data:

Fan *et al*. (2010) reported sorption of sulfonamides to be strongly associated with the physio-chemical properties of the medium such as pH, ionic strength, cation exchange capacity; and that these properties might be related to each other. Since the one-to-one variable scatter plots provided little information. A simple correlation analysis of the data where the soil variables for pH, OC, CEC and clay were taken from Table 4.1 and K_f data from Table 4.2 has been illustrated by the Pearson's correlation matrix (Table 4.4). The correlation matrix shows that %OC and CEC are positively correlated (Figure 4.6). Sorption of SM increased with both CEC and OC while SCP sorption showed a rather weak correlation with OC and non to CEC. K_f value for SMO also showed a strong positive correlation to CEC. TT negatively correlated with SMO/SCP/SMO and its sorption is not influenced by either OC or CEC. Figure 4.9 shows that a relationship between LTT and CEC does not exist. It must be noted that failing to take log of TT values would give a pseudo 2-point line and the impression of a relationship between TT and CEC based on unduly high R-value.

Table 4.4: Results of the Pearson's correlation analysis results. The significant scenarios are highlighted in bold.

	pH	OC	CEC	Sand	Silt	Clay	SSA	TT	LTT	SMO	SCP	SM
pH	1											
OC	-0.325	1										
CEC	-0.080	**0.914**	1									
Sand	-0.557	-0.137	-0.51	1								
Silt	0.555	0.110	0.49	-0.976	1							
Clay	0.519	0.136	0.47	-0.947	0.855	1						
SSA	0.100	**0.646**	0.50	-0.096	-0.012	0.237	1					
TT	0.046	-0.357	-0.07	-0.396	0.476	0.256	**-0.882**	1				
LTT	-0.139	-0.176	0.03	-0.293	0.261	0.325	**-0.851**	0.824	1			
SMO	-0.412	**0.731**	0.52	0.178	-0.153	-0.221	**0.699**	-0.647	-0.685	1		
SCP	-0.154	0.221	-0.01	0.286	-0.334	-0.199	**0.851**	-0.868	-0.851	0.735	1	
SM	0.154	**0.601**	**0.86**	-0.847	**0.837**	0.773	0.326	0.208	0.161	0.286	-0.098	1

Apart from a weak positive correlation to % sand and % silt, pH does not seem to be an influential variable in this study. Although pH is an important variable for antibiotic sorption in soils its range for soils in this study were such that SCP and SMO should be present as anions (except SMO in Manawatu soil-neutral) and SM should be always neutral. TT should always be TT^+. There is not enough variation in soil pH values to see any shift from one form

to another. The correlation matrix also shows that %clay is positively correlated to SM sorption, while SSA is positively correlated to SMO and SCP, it is negatively correlated to TT sorption. Principal component analysis was performed on the selected data based on the correlation matrix shows the same thing in a different way.

Table 4.5: Results of the principal component analysis.

Component Matrix

	Component					
	1	2	3	4	5	6
OC	.895	.303	-.315	-.003	.088	.000
CEC	.808	.580	-.029	-.071	.072	.000
LTT	-.508	.798	-.125	.299	-.020	.000
SCP	.554	-.774	.158	.234	.122	.000
SM	.611	.653	.445	.017	-.043	.000
SMO	.906	-.337	-.130	.079	-.208	.000

Extraction Method: Principal Component Analysis. 6 components extracted.

Factor 1 in Table 4.5 (which accounts for the largest portion of the explained variability) indicates that OC, CEC, SMO, SCP and SM all track with each other. Factor 1 also shows again that TT does not the same behaviour as the other antibiotics as it has a negative score. A chemical reason that could explain the difference between these groups could be explained by their pK_a (acid constant) values, TT at soil pH is expected to be present as TT+ whereas the other antibiotic are likely to anionic or neutral.

4.4.7 Methodological differences

An extensive literature examination on the sorption of veterinary antibiotics in agricultural soils employing batch equilibration techniques revealed that most studies provided diverse results. Sarmah *et al.*(2006a), in their review, noted that the variety of experimental protocols adopted in many sorption- related studies often make the comparison difficult between studies. The estimated sorption parameters were found to vary with the initial solution concentrations, sterilised and non-sterilised soils, varied equilibration times and the technique of estimating sorption.

Most of the sorption studies conducted in the recent past followed the traditional method of estimating sorption parameters by using the mass balance approach (Boxall *et al.* 2002; Loke *et al.* 2002; Clay *et al.* 2005). This approach results in sorption values obtained from the difference between the applied initial aqueous solution concentration and the concentration measured in the solution phase alone after equilibration (i.e. the mass of applied antibiotic not found in solution phase was assumed to be sorbed to the soils). Some recent reviews have criticized the above approach suggesting the values obtained without extracting both the liquid and solid phases with appropriate solvents could lead to over estimation and may not represent the true sorptive process (Thiele-Bruhn 2003; Sarmah *et al.* 2006a). However, this approach can only become important in a situation where daughter product/s is likely to be concomitantly formed during the sorption process.

Few studies have also reported extracting both the liquid and solid phases with appropriate solvents (Thiele-Bruhn *et al.* 2004; Sassman *et al.* 2007; Figueroa-Diva *et al.* 2010). Estimating sorption coefficient values by such an approach can produce results for compounds that can be subjected to microbial degradation or surface-induced abiotic degradation (Thiele-Bruhn 2003; Sarmah *et al.* 2006a). The use of short equilibration times or long contact time can also result in differences in K_f values. Because of these differences it is often not possible to compare results or not practical to generalize findings reported in various literature. Therefore, any comparisons among studies should be treated with caution and these differences need to be considered in batch equilibration sorption studies. It is for this reason that the sorption parameters in the present study were estimated using both the difference and extraction scheme so that some comparison could be possible.

4.5 Summary

The sorption potential for three sulfonamides namely SMO, SCP and SM and a macrolide, TT onto six New Zealand dairy farm soils were investigated. Kinetics studies showed that the sorption was rapid in the first few hours of the contact time (0–2 h for sulfonamide and 0–4 h for TT) and thereafter apparent equilibrium was reached as evident from the plateauing of the curve for both group of antibiotics. The data from the difference scheme were used to estimate sorption parameters as the technique of employing solvents (methanol and DCM) to extract the residual phase concentrations yielded poor recoveries.

Sorption was particularly high for TT in Matawhero soil, while sulfonamide sorption was very low in all soils consistent with previous studies. Sorption of TT and SA's in all the six selected soils followed the order: TT > SCP > SM > SMO. Values for K_d^{eff} for SMO, SCP and SM antibiotics in the soils ranged from 0.88 to 14.49 L kg^{-1} while it ranged from 1.63 to 851.14 L kg^{-1} for TT. Non-linear behaviour was observed for all the four antibiotics in different soils. Due to analytical constraints, the concentrations used in this study were significantly higher than would normally be encountered in the environment; however, the sorption estimate *(*K_d^{eff}) values in this study do provide a basis for evaluation of leaching risk from these soils. Under realistic conditions, where concentrations of antibiotics are expected to be at ng L^{-1} level, the sorption process is likely to be very slow and greater sorption capacities may be exhibited in soils (Tolls 2001; Sarmah *et al.* 2006a).

Statistical analysis of the sorption data revealed good correlation between the sulfonamide sorption parameters to soil properties such as OC, CEC, and clay content. Adsorption of TT to soil did not correlate to the cation exchange capacity of the soil. Sulfonamides sorption on the other hand are highly pH dependant, typically at soil pH (4.5–6.5) they exist partially as anionic and partially as neutral species. This can make them mobile in the environment or in situations when they are present in manure. The sorption of neutral species depends on hydrophobic partitioning and is influenced by the organic matter of the soil. Given the moderate to stronger sorption affinity of TT for New Zealand soils, vertical mobility would be limited within the soil profile as demonstrated in packed soil column studies (Hu and Coats 2009). SA's, however, due to their low sorption affinity have the potential to penetrate to the deeper soil profile as demonstrated by a few packed soil column studies (Aust *et al.* 2010; Unold *et al.* 2010). This could potentially be a risk to the groundwater under conditions conducive for leaching such as during heavy rainfall. In general, sorption of these contaminants onto soil is also likely to be pH dependent, and could vary considerably when manure is used as soil amendment. As manure pH can be higher than normal field soils, more work is warranted to understand sorption behaviour of these antibiotics under realistic field situations especially in soils amended with manure.

Chapter 5: Effect of pH, ionic strength, organic carbon and presence of co-contaminants on the sorption of sulfonamide antibiotics

5.1 Introduction

Given the heightened concern about the potential antibiotic residues migrating to the aquatic bodies in livestock grazing areas of New Zealand, understanding their fate in pasture soils has recently become an important issue (Srinivasan *et al.* 2010). In order to develop management practices to minimise potential ecological risks of these compounds, understanding their fate in the environment is a logical first step. Obtaining information on the quantification of sorption capacity of veterinary antibiotics in pasture soils would enable prediction of their transport in the aquatic environment as well as their bioavailability to microorganisms in soils (Hamscher *et al.* 2005).

Sorption of organic contaminants to soils is often influenced by sorbent properties such as hydration status, grain size, surface coatings of the grains, and surface charge, etc. In addition, factors such as pH, ionic strength, temperature of the solution and the presence of co-solutes can also influence the overall sorption process (Pouliquen and Le Bris 1996; Gao and Pedersen 2005). The influence of pH and ionic strength on the sorption of veterinary antibiotics (sulfonamide, tylosin and ciprofloxacin) to soils has been previously reported (Laak *et al.* 2006; Kahle and Stamm 2007b; Lertpaitoonpan *et al.* 2009; Vasudevan *et al.* 2009). At pH values greater than the pK_a, these compounds, undergo pH-dependent speciation, and become charged species; thus changing their physico-chemical properties, including their sorption affinity. Ionic strength, on the other hand, can influence the sorption of ionisable antibiotics by changing the interfacial potential and competing for exchange sites (Laak *et al.* 2006). For risk assessment purposes it is important to know to what extent pH and ionic strength influence sorption of these chemicals to soil.

Sulfonamide antibiotics possess two functional groups that can be easily ionised; these compounds in general undergo a change in their molecular structure when the pH of their aqueous solution changes (Bajpai *et al.* 2000). They are known to be highly persistent and

mobile, with the ability to leach through soil and into the groundwater (Boxall *et al.* 2003). The fate of sulfonamides in soils, groundwater and surface water is known to be dependent on sorption affinity and solubility (Blackwell *et al.* 2007). Previously reported partition coefficients (K_d) of sulfonamide antibiotics were found to vary with texture and soil properties (Boxall *et al.* 2002; Thiele-Bruhn and Aust 2004; Kurwadkar *et al.* 2007; Kahle and Stamm 2007b; Fukahori *et al.* 2011). Two separate studies conducted on the sorption of sulfonamide with manure addition gave contrasting K_d values; Boxall *et al*. (2002) reported a decrease in K_d for SCP, whereas Sukul *et al*. (2008) found a significant increase in sorption of SDZ, with manure addition in soils. Gao and Pedersen (2005) studied the sorption of SM antibiotic to clay minerals as a function of pH, ionic strength and exchangeable cations; and the authors reported that K_d values decreased with an increase in the pH, ionic strength and surface charge density, consistent with a cation exchange mechanism.

Veterinary antibiotics are likely to co-occur with other antibiotics or contaminants from other classes (Monteiro and Boxall 2009). In the pasture environment veterinary antibiotics excreted by grazing animals are likely to encounter other contaminants such as a range of pesticides, naturally excreted steroid hormones and agricultural fertilisers (Kolpin *et al.* 2002). This can lead to additivity, antagonism, synergism, and eventual interactive effects on terrestrial and aquatic organisms, and hence a possible increase or decrease in the effects of a particular compound in the ecosystem (Kolpin *et al.* 2002; Sarmah *et al.* 2006a). Only a few published studies are available in the literature on the potential interactions between veterinary antibiotics and other contaminants emphasizing the persistence and bioavailability aspect (Chun *et al.* 2005; Accinelli *et al.* 2006). Steroid hormones such as 17β-estradiol (E2), and its primary metabolite estrone (E1), are naturally excreted in the faeces and urine of dairy cows and other livestock animals (Sarmah *et al.* 2006a). Agricultural antibiotics and natural estrogens often occur in urine simultaneously (Chun *et al.* 2005), so it is possible that dairy cows or beef cattle or other livestock animals such as pigs, or poultry treated with antibiotics can excrete unmetabolised antibiotic together with steroid hormone.

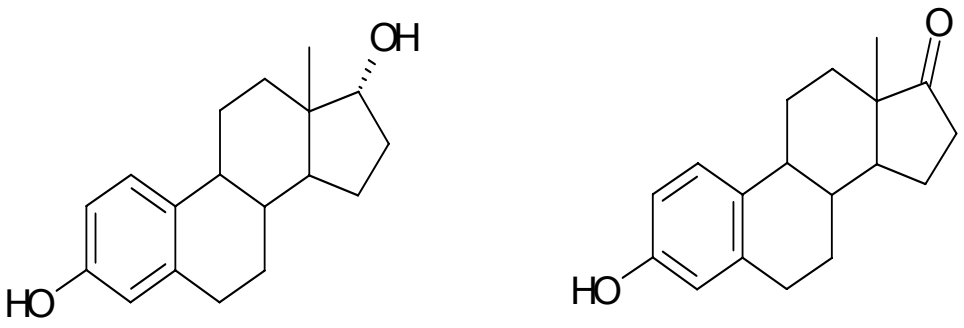
Although the environmental fate of a veterinary antibiotic in soil is often estimated by determining its persistence and sorption affinity, there is a dearth of information on antibiotic-hormone interaction, and its influence on the overall sorption process. No studies have so far been attempted to elucidate the role natural estrogens might play on the overall

sorption behaviour of sulfonamide antibiotics in pasture soils. SMO and SCP antibiotic were chosen for this study as little information is available on the role of pH, ionic strength and organic carbon on their sorption affinity to soils. Therefore the main objective of this study was to investigate the effect of pH, ionic strength and organic carbon on the sorption capacity of SMO antibiotic to selected New Zealand pasture soils. The secondary objective of this study was to determine the sorption behaviour of SCP antibiotic in the presence of a natural steroid hormone, 17β-estradiol in Hamilton soil.

5.2 Materials and methods

5.2.1 Chemicals

SMO, SCP, E2 (> 98% purity), E1 (> 99% purity) (Figure 5.1, Table 5.1), and calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$ > 99% purity) were obtained from Sigma Aldrich, Australia. Acetonitrile (Mallinckrodt ChromAR, ≥ 99.8% purity), dichloromethane (Mallinckrodt UltimAR, ≥ 99.9% purity), methanol (Mallinckrodt ChromAR, ≥ 99.9% purity), concentrated hydrochloric acid (HCl) and potassium hydroxide (KOH) were obtained from Biolab Scientific Ltd. New Zealand. HPLC grade deionised water was obtained from an onsite Arium® 61316 high performance reverse osmosis system (Sartorius Stedim Biotech GmbH, Germany). Oxygen free nitrogen gas (gas code 152) was purchased from BOC (New Zealand).



17β-estradiol (E2) **Estrone (E1)**

Figure 5.1: Molecular structure of the steroid hormones used in this study.

Table 5.1: Physiochemical properties of the chemicals used in the study

Properties	**SMO**	**SCP**	**E2**	**E1**
Molecular formula	$C_{10}H_{11}N_3O_3S$	$C_{10}H_9N_4ClO_2S$	$C_{18}H_{24}O_2$	$C_{18}H_{22}O_2$
Molecular weight (g mol^{-1})	253.28	284.72	272.4	270.4
Water solubility (mg L^{-1})	600[a]	7000[d]/8235[e]	1.51[h]	1.30[h]
Vapour pressure (Pa)*	1.74×10^{-5}	1.77×10^{-7}	3×10^{-8}	3×10^{-8}
Log K_{ow}	0.89[b,c]	0.31[f]	3.10, 4.0[i]	3.43, 3.13, 3.3[i]
pK_a or pK_b at 25°C	1.7[a]& 5.6[a] 1.83[c]&5.57[c] 1.85[b]/5.2[b]	5.5[e]	10.3–10.8[i]	10.3–10.8[i]
Melting point (°C)	167-169	205.6[g]	171[i]	259[i]

[a] Çalışkan and Göktürk (2010); [b] Hou *et al*. (2010); [c] Figueroa-Diva *et al*. (2010); [d] Tolls (2001) ;[e] Lin *et al*. (1997); [f] Laak *et al*. (2006); [g] U.S. Environmental Protection Agency (2012); [h] Shareef *et al*. (2006); [i] cited by Scherr (2009)

5.2.2 Soils

Four topsoils (0–5 cm) with contrasting organic carbon (OC) content, fine earth particle size distribution, pH, and cation exchange capacity (CEC) were selected (Table 4.1). Of the four soils, three soils (Te Kowhai silt loam, Hamilton clay loam, and Horotiu silt loam) were from the Waikato dairy farming areas, while Matawhero silt loam was from the Gisborne region of the North Island. The soils were collected fresh, air dried, sieved (< 2 mm), and stored at 4°C before study. A full description of the soils and the methods used to determine their physico-chemical properties can be found elsewhere (Hewitt, 1992; Bruce, 1997).

5.2.3 Batch sorption studies

Stock solutions of each target compound (SMO and SCP antibiotics) at a concentration of 1000 mg L^{-1} were prepared by dissolving appropriate amounts of each active ingredient in high performance liquid chromatography (HPLC) grade methanol. Solutions were stored in the dark (4°C) in amber glass bottles covered with aluminum foil. By mixing an appropriate amount of the stock solution separately, six initial aqueous solution concentrations (SMO) in

duplicate were prepared in $CaCl_2$ solution. In the experimental protocol involving the construction of batch sorption isotherms, three distinct parts were involved: pH adjustment, ionic strength adjustment, and spiking steroid hormone 17β-estradiol (E2) of two known concentrations in the presence of SCP antibiotic in a selected model soil (Hamilton clay loam). SCP was chosen in preference to the SMO antibiotic as the former seemed to exhibit higher sorption affinity to soils than SMO in a preliminary batch sorption experiment (Srinivasan *et al.* 2010).

5.2.4 Adjustment of pH

For the first part of the experiment, appropriate amounts of 0.1 M KOH and 1 M HCl were added to 0.005 M $CaCl_2$ to achieve target pH values of 2, 3, 4, 5.5, 6.5, 7.5, and 8.5. Preliminary experiments were conducted to determine the amount of KOH and HCl needed for the pH adjustments. To account for the neutralizing capacity of soils the pH of the aqueous solution was measured before and after equilibration.

5.2.5 Adjustment of ionic strength

Calcium chloride ($CaCl_2$) as mediator solution was prepared by adding the appropriate amount of $CaCl_2.2H_2O$ to deionised water to obtain three different molar concentrations (0.005, 0.05 and 0.2 M), which corresponded to 0.01, 0.1, 0.4 M in ionic strength for cations. The ionic strength was calculated using the following equation.

$$\mathrm{I} = \frac{1}{2}\sum_{i=0}^{n} m_i \, v_i^2 \qquad (5.1)$$

Where, I is the ionic strength (mg L^{-1}); m_i concentration of the i^{th} ion (mg L^{-1}) and v_i, is the charge of the i^{th} ion. The mediator solutions were later used to prepare six different aqueous concentrations of the SMO antibiotic. The initial conductivity of the soil suspension without any $CaCl_2$ electrolyte addition, at a soil to water ratio of 1:15 was 0.12, 0.07 and 0.16 mS cm^{-1} for Horotiu, Te Kowhai and Matawhero soil respectively. The initial conductivities of the $CaCl_2$ mediator solution were 1.68, 11.70 and 34.7 mS cm^{-1} for 0.005, 0.05 and 0.2M respectively. Post equilibration supernatants are likely to contain other cations apart from Ca^{2+}, however, Ca^{2+} is often the most abundant and important ion in agricultural soils

(Spostio 1989; Laak *et al*. 2006). Following, post equilibration (24 h), the final conductivity of the soil solution reached close to the initial conductivity of the mediator solutions (± 0.4 mS cm^{-1}).

For the second part of the experiment, appropriate amounts of $CaCl_2$ were dissolved in deionised water to get 0.005 M, 0.05 M and 0.2 M $CaCl_2$ solution and the six initial aqueous solution concentrations were prepared using these as mediator solution at soil pH. The conductivities of the soil, $CaCl_2$ and the equilibrated supernatant were also measured. To investigate the co-contaminant effect on the sorption of SCP antibiotic in a model soil, six initial aqueous concentrations of SCP in 0.005 M $CaCl_2$ were prepared in duplicates. An appropriate amount of 17β-estradiol (E2) stock solution was added to each set of initial aqueous concentrations containing the SCP antibiotic to yield a concentration of 0.075 mg L^{-1} and 3 mg $L^{-1}$of E2 respectively.

5.2.6 Batch sorption protocol

The batch sorption protocol followed here was the same as that used for SMO and SCP in Chapter 4. Six initial aqueous solution concentrations of the antibiotic were prepared in duplicates in 0.005 M $CaCl_2$ solution. Appropriate amounts of 0.1 M KOH and 1 M HCl were added to 0.005 M $CaCl_2$ to achieve pH values of 2, 3, 4, 5.5, 6.5, 7.5, and 8.5. Similarly, appropriate amounts of $CaCl_2$ were dissolved in de-ionised water to get three different molar concentrations of 0.005 M, 0.05 M and 0.2 M $CaCl_2$ solution, and the six initial aqueous solution concentrations were prepared using these molar concentrations as mediator solution at soil pH. For all experiments, a 24 h equilibration time was used for batch sorption studies.

5.2.7 HPLC analysis for SMO and SCP

The protocol for the analysis of SMO in aqueous phase along with the isocratic HPLC elution scheme used were similar to those reported in Chapter 4.

5.2.8 17β-Estradiol (E2) and estrone (E1) extraction from soil samples

After centrifuging and decanting of the supernatant, the residual soil remaining in the tube was extracted with DCM (10 mL). Tubes containing solution and the soil with DCM were

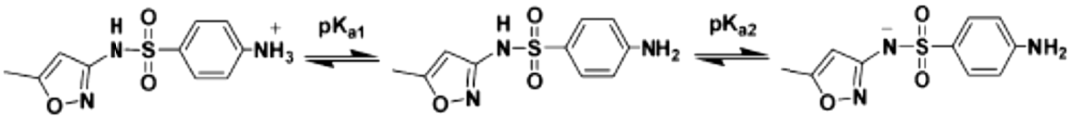
shaken for 12 h, and an aliquot (1 mL) of extract from each phase was evaporated to dryness under gentle stream of nitrogen, and reconstituted in 70:30 methanol: water (1 mL). Analysis was carried out by high performance liquid chromatography with UV detection (201 nm) on a Phenomenex Onyx Monolithic C_{18} column (100 × 4.6 mm), using a mobile phase of acetonitrile, and acetonitrile: water (47:53 (v/v)), at a flow rate of 1.0 mL min^{-1}, and an injection volume of 50 µL. Both E2 and E1 were separated using a single isocratic elution, with a total run time of 4.5 minutes. Detailed experimental protocol for extraction and analysis of E2 and E1 can be found elsewhere (Sarmah *et al.* 2010).

5.2.9 Data analysis and Sorption Modelling

Details on the estimation of sorption coefficients and Freundlich model used here have been described in detail in Chapter 4**.**

5.2.10 Hydrophobic pH modelling

A model linking sorbate speciation with species-specific sorption coefficients that can describe the pH dependence of the apparent sorption coefficients has been extensively studied (Gao and Pedersen 2005; Laak *et al.* 2006; Kurwadkar *et al.* 2007). This model is often referred to as the Hydrophobic Partitioning Model based on the concept developed in Snoeyink and Jenkins (1980) and Schwarzenbach *et al*. (2003). The model has been useful for implementation in the assessment of sorption of many ionisable compounds including pesticides (Fontaine *et al.* 1991; Spadotto and Hornsby 2003) and antibiotics such as tetracycline (Sassman and Lee 2005), sulfonamides (Laak *et al.* 2006; Kurwadkar *et al.* 2007; Lertpaitoonpan *et al.* 2009) and ciprofloxacin (Vasudevan *et al.* 2009). Sulfonamide antibiotics possess two ionisable functional groups that are relevant to environmental pH ranges. Taking SMO as an example, the cationic species (SMO^{+}) dominates at low pH values; the neutral species (SMO^{0}) which is also its principal form, dominates at pH values between pK_{a1} and pK_{a2} and the anionic species (SMO^{-}) is the main form at higher pH values (Figure 5.2).



pH< 1.73 1.73 < pH < 5.5 pH > 5.5

Figure 5.2: pH dependent speciation of sulfamethoxazole antibiotic adapted from Dodd and Huang (2004)

SMO behaves as weak acid, therefore it occurs as neutral and ionised species both in aqueous solution and sorbed on soils. The pH-dependent speciation of this compound is determined by its pKa values and this speciation affects the sorption of sulfamethoxazole. Of the total SMO in solution at any given concentration C_w, the mass fractions present as cation (α_+), neutral (α_0) and anion (α_-) can be determined by aqueous pH and the compound's pKa (Figure 5.3), and can be written as follows (Kurwadkar *et al.* 2007).

$$\alpha_+ = 1/(1 + 10^{(\mathrm{pH-pKa1})} + 10^{(2\mathrm{pH-pKa1-pKa2})}) \quad (5.2)$$

$$\alpha_0 = 1/(1 + 10^{(\mathrm{pKa1-pH})} + 10^{(\mathrm{pH-pKa2})}) \quad (5.3)$$

$$\alpha_- = 1/(1 + 10^{(\mathrm{pKa2-pH})} + 10^{(2\mathrm{pH-pKa1-pKa2})}) \quad (5.4)$$

Where α_+ and $\alpha_0$and α_-represent the mole fraction of cationic, neutral and anionic species respectively. The observed overall sorption coefficient $K_{d'}$of SMO at a particular pH is given by the following equation:

$$K_{d'} = K_{d+} * \alpha_+ + K_{d0} * \alpha_0 + K_{d-} * \alpha_- \quad (5.5)$$

Where K_{d+}, K_{d0}andK_{d-} are the sorption coefficients of the cation, neutral and anionic species. It is also possible that SMO may exist in zwitterionic form but the contribution of this species was considered to be negligible, and this was recently demonstrated in a study by Gao and Pedersen (2005), where the authors observed that sorption of zwitterionic species of SM did not contribute significantly to its overall sorption (Gao and Pedersen 2005; Figueroa-Diva *et al.* 2010). Sorption coefficients for each species were estimated by fitting the overall sorption coefficients using equation 6.5 for each soil-pH combination by nonlinear regression analysis of experimental *versus* modeled $K_{d'}$ value using MS Excel 2010 ® solver.

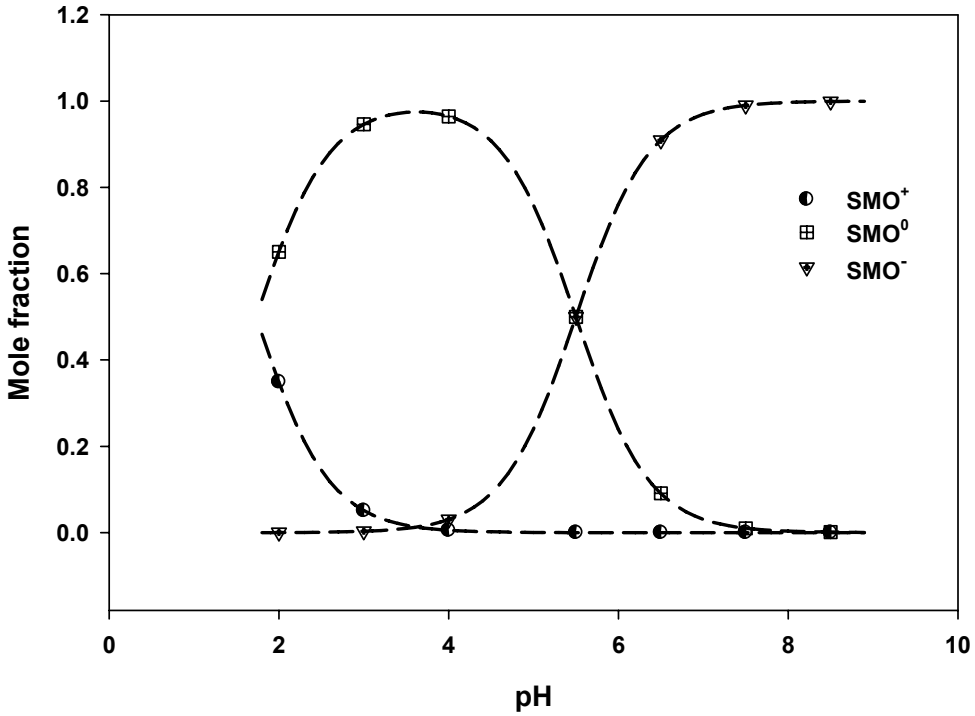


Figure 5.3: Mole fraction of cationic (SMO^{+}), neutral (SMO^{o}) and anionic (SMO^{-}) present at different pH for the antibiotic SMO calculated from equations (5.1–5.3).

5.3 Results and discussion

5.3.1 Sorption isotherms

Figure 5.4 shows the sorption isotherms constructed for SMO in three different soils together with the Freundlich model fits at varying pH. The calculated values of K_d, Freundlich coefficients (K_f) and exponent (N) for SMO are summarised in Table 5.2. In general, all the isotherms observed for Matawhero and Horotiu soils over the pH range studied were found to be highly non-linear, with N values ranging anywhere from 0.33 to 0.85 for Matawhero soil, 0.73 to 1.21 for Te Kowhai soil, and 0.74 to 0.88 in the case of Horotiu soil. Kurwadkar *et al*. (2007) reported similar non-linear isotherms for SM and STZ in three different soils over a range of pH. Another study by Lertpaitoonpan *et al*. (2009) reported both non-linear as well as linear isotherms for SM in five soils ranging in pH from 5.5 to 9. Recently Schwarz *et al*. (2012) also reported non-linear sorption behaviour for three sulfonamides namely

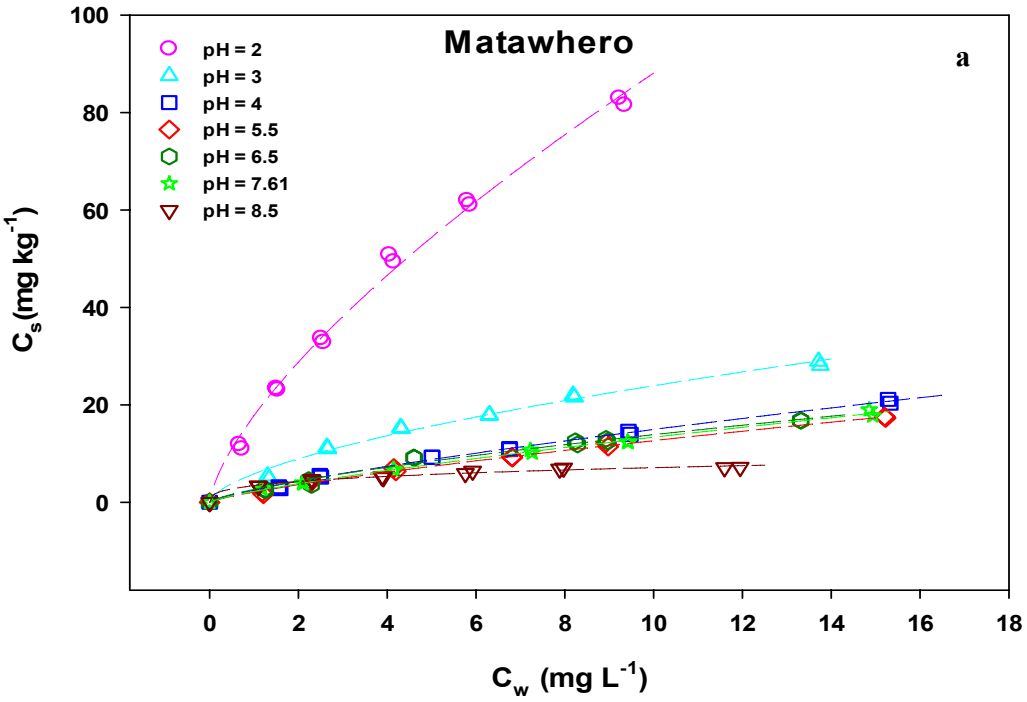
sulfanilamide, sulfapyridine and sulfadimethoxine on soil organic matter and humic acid. The K_d values obtained in our study were similar to those found in study by Holtge and Kreuzig (2007) and Drilla *et al*. (2005). While Holtge and Kreuzig (2007) reported K_d values for sulfamethoxazole in arable land soil and grassland soil to be 1.3 and 2.9 L $kg^{-1}$and the sorption behaviour for sulfamethoxazole was found to increase with K_d values of 10.7 and 13.6 L kg^{-1} with the addition of test manure.

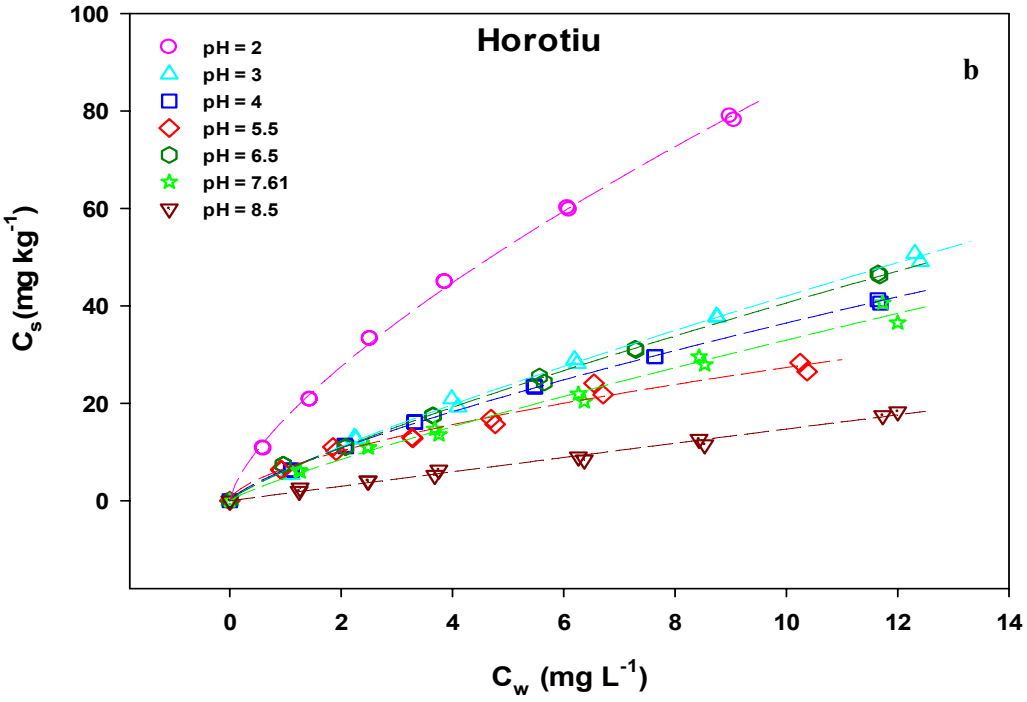
Drilla *et al*. (2005) determined K_d for SMO to be 0.2 L kg^{-1} in a soil of low organic carbon (0.4%) and high clay content (43%). As no published data are available on the variation of K_d values for SMO under a similar pH range to that used in this study, it is difficult to compare the K_d values obtained from the present study. However, given that most sulfonamides are known to behave in a similar manner some comparison is worth attempting. Overall, the measured values of K_d for SMO within the studied pH range of 2 to 8.55 across all soils ranged from 1.24 to 10.30 mg L^{-1}. All the three soils exhibited the highest values for both K_d and K_f value at pH 2 amongst the three soils, The K_d values obtained in this study were similar to those reported in the literature for SDZ (Sukul *et al.* 2008) and SM antibiotic (Lertpaitoonpan *et al.* 2009). The former reported K_d values of 0.07–5.12 L kg^{-1} at concentrations ranging between 0.3 and 20 mg L^{-1}, with soils having 0.1-3.8% OC and pH values ranging from 8.5 to 5.5.

Table 5.2: Summary of sorption parameters for SMO derived from the multiple-concentration isotherms for three soils at pH ranging from 2 to 8.55.

Soil								
	Matawhero							
pH	K_d	R^2	K_f	N	R^2	$^{\#}K_d^{eff}$	Log K_{oc}*	
							0.5 mg L^{-1}	5 ng L^{-1}
2.0	9.95	0.92	16.22	0.76	0.99	19.14	2.96	4.16
3.0	2.43	0.82	4.86	0.71	0.97	5.94	2.45	3.90
4.0	1.47	0.96	3.34	0.33	0.97	5.31	2.40	5.74
5.5	1.40	0.90	2.37	0.75	1.00	2.83	2.13	3.40
6.75	1.38	0.93	2.24	0.80	0.98	2.56	2.09	3.07
7.51	1.31	0.96	2.28	0.82	0.99	2.58	2.09	2.98
8.55	1.24	0.96	1.82	0.85	0.98	2.02	1.98	2.72
	Te Kowhai							
2.0	10.3	0.93	17.31	0.73	1.00	20.94	2.62	4.00
3.0	4.66	0.97	6.75	0.83	1.00	7.60	2.18	3.03
4.0	3.82	0.99	4.68	0.84	1.00	5.24	2.02	2.83
5.5	3.65	0.99	4.32	0.92	1.00	4.56	1.96	2.34
6.75	3.46	0.99	4.21	0.91	1.00	4.48	1.95	2.40
7.51	3.28	0.98	3.26	0.96	0.99	3.35	1.83	2.02
8.55	2.91	0.97	2.59	1.21	0.96	2.23	1.65	0.59
	Horotiu							
2.0	9.62	0.93	16.03	0.74	1.00	19.21	2.37	3.68
3.0	4.27	0.98	5.70	0.88	0.99	6.20	1.88	2.49
4.0	4.19	0.98	6.99	0.75	0.99	8.33	2.01	3.27
5.5	3.86	0.96	6.75	0.60	0.98	8.88	2.03	4.01
6.75	3.79	0.95	6.21	0.78	1.00	7.09	1.94	3.02
7.51	3.36	0.97	6.10	0.79	1.00	7.19	1.94	3.00
8.55	3.09	0.83	5.07	0.81	0.99	5.80	1.85	2.82

$^{\#}K_d^{eff}$ is the concentration dependent effective sorption distribution coefficient ($K_d^{eff} = K_f C_w^{N-1}$) using lowest aqueous equilibrium solution concentrations of C_w = 0.5 mg L^{-1}; *concentration dependent OC-normalized sorption coefficient calculated using $K_{oc} = K_f C_w^{N-1} foc^{-1}$ at C_w = 0.5 mg L^{-1} and 5 ng L^{-1}; K_d^{eff} in L $kg^{-1}$and K_f in mg $^{1-N}L^N kg^{-1}$


Matawhero
a
pH = 2
pH = 3
pH = 4
pH = 5.5
pH = 6.5
pH = 7.61
pH = 8.5
C_s (mg kg^{-1})
C_w (mg L^{-1})
0
20
40
60
80
100
0
2
4
6
8
10
12
14
16
18


Horotiu
b
pH = 2
pH = 3
pH = 4
pH = 5.5
pH = 6.5
pH = 7.61
pH = 8.5
C_s (mg kg^{-1})
C_w (mg L^{-1})
0
20
40
60
80
100
0
2
4
6
8
10
12
14

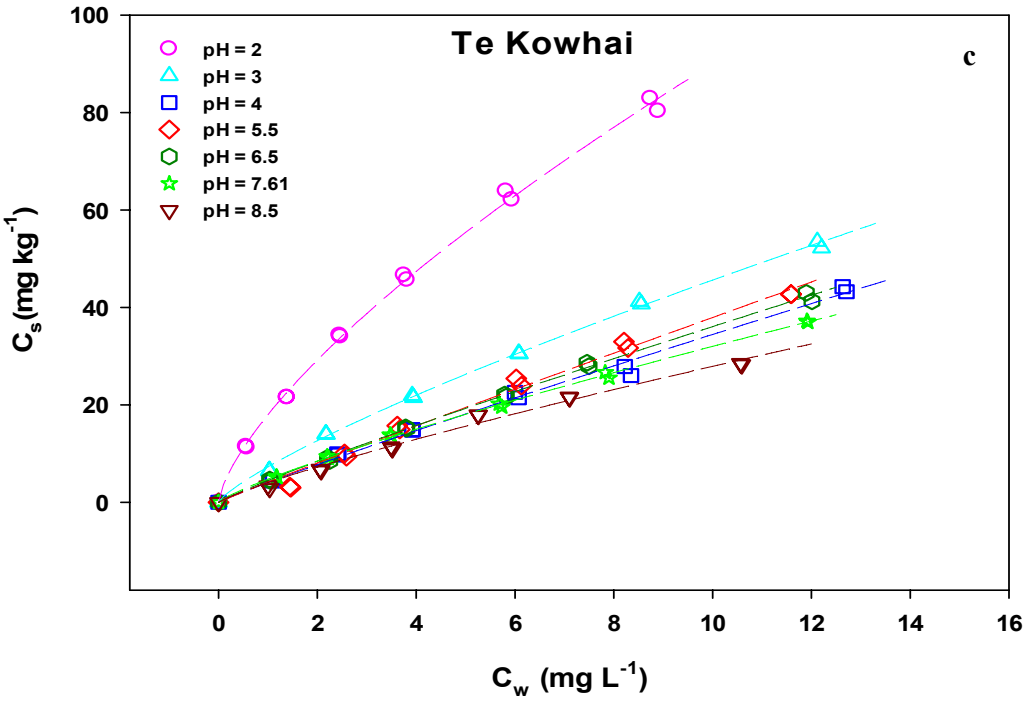


Figure 5.4: Multiple-concentration batch sorption isotherms (24 h contact time) for SMO in (a) Matawhero, (b) Horotiu and (c) Te Kowhai soils under varying pH. Symbols represent measured values while dashed lines represent Freundlich model fits.

Highest K_d values for SMO in all three soils were observed at a pH 2, which is close to pK_{a1}, (Table 5.1). This was expected, at a pH SMO is approximately 50% in the cationic form and the remaining as neutral form. Therefore, there would be sorption because of SMO^+ attraction to charged negative soil particles as well as due to sorption due to soil organic matter. When the pH of the mediator solution was increased to 3, a marked decrease in the K_d was noted for all three soils. The neutral species predominating at this pH would not sorb to negatively charged soil particles. Further increasing the pH up to 5.5 decreased the sorption affinity of SMO considerably. At this pH, sorption would be mainly influenced by soil organic matter. Between pH 5.5 and 8.55, the experimental sorption coefficients seemed to indicate a slight decrease due to limited sorption affinity of SMO^-. Similar to the findings of Kurwadkar *et al.* (2007), Lertpaitoonpan *et al.* (2009), and Schwarz *et al.* (2012), the present study also revealed non-linear sorption dependency for SMO upon the pH of the solution that is *N* varied with solution pH. Lertpaitoonpan et al. (2009) reported increase in *N* values with an increase in solution pH (up to pH 8) followed by a slight decrease in *N* value at pH 9 for all

soils under study. In this present study, such trend of increased *N* values with increasing solution pH was observed in Te Kowhai soils; the other soils were more variable.

Concentration dependent effective distribution coefficient (**K_d^{eff}**) values of SMO ranged from 2.02 to 19.14 for Matawhero soil, 2.23 to 20.94 for Te Kowhai, and 5.80 to 19.21 L kg^{-1} for Horotiu soil across the pH range used in the study. As with the K_d and K_f values, K_d^{eff} values were the highest at a pH of 2 for all the three soils, with Horotiu being the highest. It was observed that at neutral and higher pH range, OC content of the soil enhanced the concentration dependent effective sorption coefficients (Table 5.2). Horotiu soil had the highest K_d^{eff} value at neutral pH compared to Te Kowhai and Matawhero soil. This could be because Horotiu soil apart from having high OC content is known to be volcanically derived with an allophane (an alumino-silicate clay mineral) content of 28% (Sarmah *et al.* 2008). This may contribute to the overall sorption mechanism of SMO to Horotiu soil and could be responsible for its high K_d^{eff} values. However, at the lower pH range, the effect of OC content on the soils sorption affinity seems to be nullified by the pH dependent CEC effect.

Concentration-dependent average log K_{oc} values for SMO based on the lowest equilibrium concentration of 0.5 mg L^{-1} ranged from 1.98 to 2.96 for Matawhero, 1.65 to 2.62 for Te Kowhai and 1.85 to 2.37 for Horotiu soil over the pH range (high to low). When an environmental concentration of 5 ng L^{-1} was used the Log K_{oc} values increased slightly except when N >1 (Te Kowhai soil, pH = 8.55), Table 6.2. Highly non-linear behaviour was observed for Matawhero and Te Kowhai soils under certain pH values. This could be attributed to a greater percentage of clay, high Fe and Mn content and finer texture of soil (Sarmah *et al.* 2008). Throughout the pH range investigated the 3 soils had similar very low log K_{oc} values suggesting that SMO could become mobile under conditions conducive to leaching during high rainfall or storm events, and may potentially migrate into the ground water.

5.3.2 Effect of pH

The effect of pH in an environmental system where ionic or polar species of an organic chemical adsorb on a charged surface is of great practical and environmental significance. In the present study, the pH of the aqueous supernatant was measured for all three soils before

and after equilibration, and the change in pH values was < 0.2 units in each case, The pK_a values of SMO are 1.83 and 5.57 (Figueroa-Diva *et al.* 2010). This means that for 1.83 ≤ pH ≤ 5.57 the non-ionised neutral species with some contribution from cationic species would be dominant, while the anionic (deprotonated) form of sulfamethoxazole is prevalent at alkaline pH. The lowest and highest experimental pH values of 2 and 8.55 were close to $pK_{a1}$and > pK_{a2} of the compound. Therefore, the cationic form SMO would likely to exist in greater proportions at pH 2 while the anionic form would predominate at pH 6.5, 7.5 and 8.5 (Figure 5.3). In order to understand the influence of pH on the sorption of sulfamethoxazole to three different New Zealand dairy farm soils, sorption coefficients (K_d and K_{oc}) of SMO were plotted as a function of pH (Figure 5.5). All three soils displayed a decrease in sorption when the pH was increased and SMO sorption was the highest at pH 2 for all the three soils. An increase in pH from 2 to 8.5 for Matawhero soil resulted in 8 fold lower (from 9.95 to 1.24) K_d values for SMO. Varying the pH of the mediator solution causes sulfamethoxazole to exhibit different functionalities with different modes of adsorption. The increased sorption of SMO with decreasing pH can be explained by the formation of positively charged SMO cations which are electrostatically attracted to negatively charged soil surfaces. The trend observed in this study was similar to those observed in past studies (Boxall *et al.* 2002; Laak *et al.* 2006; Kurwadkar *et al.* 2007; Lertpaitoonpan *et al.* 2009).

Another trend which is very evident in this study was that in the pH-range where the neutral species dominates (pK_{a1}< pH < pK_{a2}) sorption is relatively insensitive to pH-variations, findings similar to that reported by Gao and Pedersen (2005) for SM. A plausible explanation is that for pH < pK_{a2} (5.5) of the compound, hydrophobic partitioning with organic matter tends to dominate due to the non-ionised nature of SMO (Lertpaitoonpan *et al.* 2009). However, sulfonamides primarily exist as anions in the soil– water systems (Koskinen *et al.* 2006), which would suggest that most of the SMO would be associated with the soil surface by means of cation bridging, and minor contributions from van der Waals force (Thiele-Bruhn *et al.* 2004). Tolls (2001) reported that ionic interactions, rather than hydrophobic interactions, control the sorption of antibiotics in soils. Similar observation was made by Fan *et al*. (2011) for SM sorption and transport in soils. The authors suggested that transport and high mobility of SM may be due to the reversible interaction between the soil surface and anionic SM. Similarly, Laak *et al*. (2006) demonstrated only a weak sorption tendency of SCP anion (SCP^-) to negatively charged soil surfaces. The authors attributed the more mobile

nature of SCP^- to this, as the anion is more prone to electrostatic repulsion than the neutral species. Richter *et al.*(2009) observed that the sorption of STZ (pKa_2 7.2) to dissolved humic acid was mainly due to cation exchange from low pH to pH ~ 7.7. In the present study at high pH, however, the lower sorption of SMO (pKa_2 5.57) may be attributed to poor sorption of neutral and repulsion of anionic species of SMO.

5.3.3 pH modelling

The experimental and model derived partition coefficients partition coefficients (K_d, K_{oc}) for SMO in a particular soil were plotted as a function of pH (Figure 5.5)**.** The model derived values for each species are summarised in Table 5.3. The experimental data were fitted well by the model with R^2 values ranging from 0.98 to 1.00 across all soils. The model was also used to fit literature data on sulfonamide sorption (Figure 5.6) with R^2> 0.95. The model derived sorption coefficients for the cationic species (K_{d+}) were an order of magnitude higher, followed by neutral (K_{d0}), and anionic (K_{d-}) species. This indicates that the cationic form has the strongest sorption affinity to negatively charged clay followed by the neutral species to the soil organic matter in soils. K_{d+} for Matawhero soil was high compared to other two soils (Table 5.3), and this could be possibly due to a higher prevalence of negatively charged soil sites.

No literature values exist in order to compare K_{d+} for SMO directly. However, the values for K_{d+} obtained in this study are lower than those obtained in earlier studies for another sulfonamide, SM (Gao and Pedersen 2005; Kurwadkar *et al.* 2007), but similar to the K_{d+} values obtained for SM in several US agricultural soils (Lertpaitoonpan *et al.* 2009). The higher K_{d+}values obtained in the present study could be attributed to the pH range used in this study. Typically soil pH varies between 5.5 and 6.5, and in these conditions the fraction of cationic species of SMO (0.2-0.0%) would be expected to be small, and sorption by cationic exchange would not play a significant role (Gao and Pedersen 2005); at these pH, SMO is likely to exist in neutral (50–10%) and anionic form (50–90%) and sorption to soil OC would retard mobility somewhat. The lack of anionic exchange sites on soil along with the higher water solubility of the anionic species would mean these are poorly absorbed (Kurwadkar *et al.* 2007).

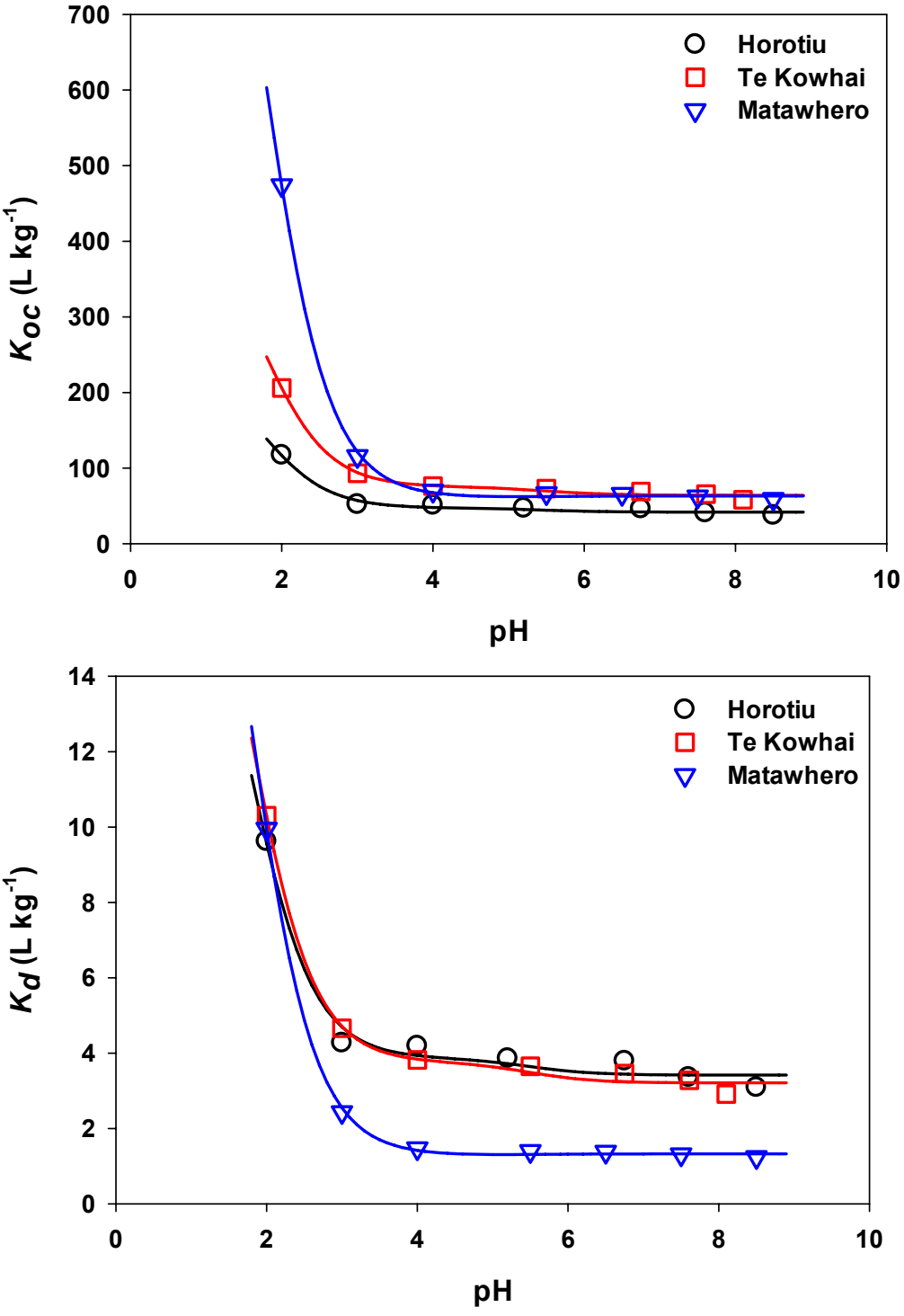


Figure 5.5: Experimental and modelled sorption coefficients K_d (above) and K_{oc} (below) for SMO antibiotic describing its pH dependent behaviour in three soils. Symbols represent measured values and lines represent the model fit.

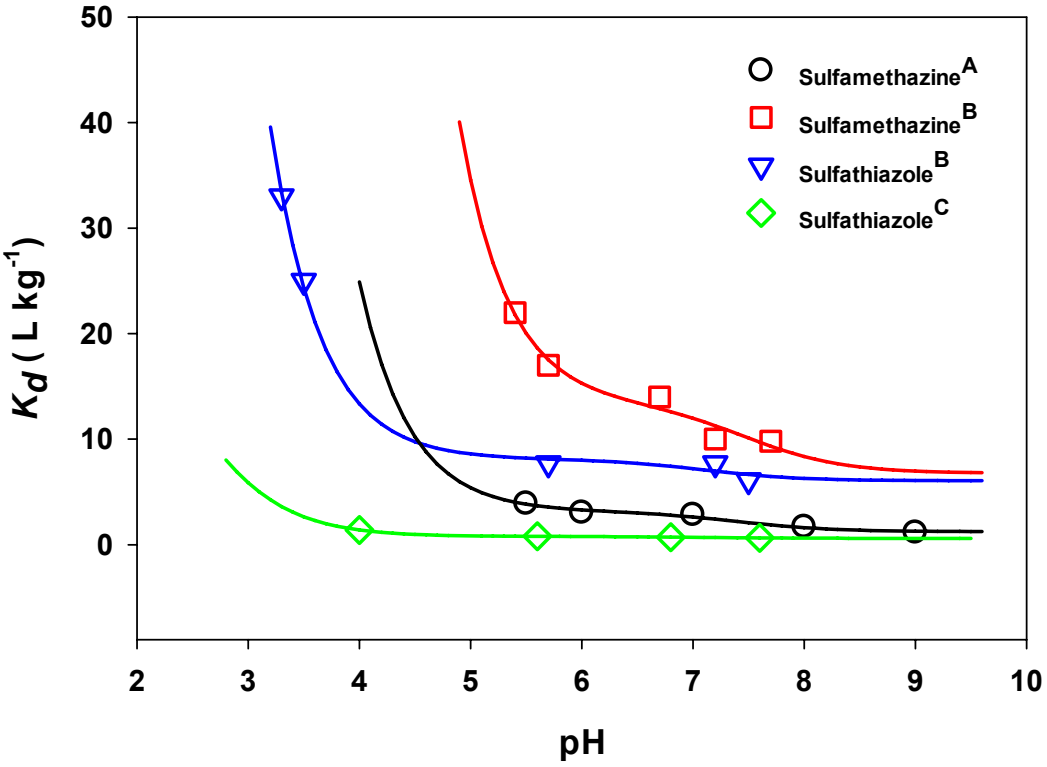


Figure 5.6: Hydrophobic partition model fitted for different literature data on sulfonamide sorption. Symbols represent the literature data while the lines represent the model fit.

[A] Lertpaitoonpan *et al*.,(2009); [B] Kurwadkar *et al*., (2007); [C] Kahle and Stamm, (2007b)

Table 5.3: Partition coefficients estimated by the model for cationic (K_{d+}), neutral (K_{d0}) and anionic (K_{d-}) species of antibiotic SMO in three soils and with its correlation factor (R^2) values.

Soil	K_{d+}	K_{d0}	K_{d-}	R^2
Horotiu	20.18	3.86	3.42	0.98
Matawhero	26.04	1.29	1.33	1.00
Te Kowhai	22.46	3.76	3.21	1.00

5.3.4 Effect of ionic strength

Past studies have shown that ionic strength can affect the sorption/degradation of antibiotics in soils (Laak *et al.* 2006; Loftin *et al.* 2008) and silica (Bui and Choi 2010) by changing the interfacial potential and through competition by solvent ions for ion-exchange sites (Brownawell *et al.* 1990). Antibiotic sorption to solid matrix is also known to increase with the prevalence of divalent and trivalent cations (Bui and Choi 2010), and to their respective oxide formation (ter Laak *et al.* 2006). Most soil surfaces carry a net negative charge, and when ionic strength increases, Ca^{2+} replaces H^{+} ions. Since there are two positive charges, it is possible for SMO^{-} to attach to calcium and therefore bind, which is referred to as cation bridging. In the present study the effects of varying ionic strength on the sorptive behaviour of SMO antibiotic in three agricultural soils have been investigated.

The shape of the Freundlich sorption isotherms for SMO in three soils under varying ionic strengths reveals that the soils behaved differently with increased ionic strength. A justifiable explanation regarding the apparent variation in sorption affinity to these soils could not be offered, however; inherent sorbent properties such as composition of various clay minerals and the associated high surface areas (Table 4.1) for the soils, and the type and the nature of organic matter present may be responsible for this. The isotherms were highly non-linear for Matawhero and Horotiu soils but were more linear for Te Kowhai soil at higher $CaCl_2$ concentration, Figure 5.7, as evidenced by the corresponding N values, Table 5.4. Also a plot of K_d vs. ionic strength, Figure 5.8 shows the different effect of ionic strength on the sorption of SMO antibiotic in the three soils**.** For all three soils when the molar concentrations of the mediator solution was increased from 0.005 to 0.2 M, the K_d^{eff} values for all three soils increased except for Te Kowhai soil at 0.1 M, with Matawhero soil exhibiting a threefold increase in the K_d^{eff} values, whereas increases in the K_d^{eff} values for Te Kowhai and Horotiu soils were minimal (Table 5.4).

For Matawhero soil, sorption of SMO antibiotic increased as ionic strength increased, this could be attributed to the afore-mentioned cation bridging. Another possible explanation could be due to the occurrence of a “salting out” effect causing a decrease in the solubility of SMO antibiotic in the salt solution so that it precipitates in the soil. This phenomenon, which was noted only in Matawhero soil was also reported in some earlier studies (Spongberg and

Ganliang 2000; Ureña-Amate *et al.* 2005). Similar behaviour has been observed in soil sorption studies involving other sulfonamide antibiotics such as SCP (Laak *et al.* 2006), and ketoprofen antibiotic onto silica (Bui and Choi 2010). For example from a sorption study of SCP on clay loam soil, Laak *et al*., (2006) observed that SCP sorption increased by a factor of 2 when the $CaCl_2$ concentration was increased from 0.006 to 0.2 M. In contrast, the addition of electrolytes has been found to reduce the amount of SMO sorbed onto natural zeolite (Farias *et al.* 2011). A plausible explanation for the increase in sorption is that, at a typical environmental soil pH, which is usually neutral to slightly acidic, most of the SMO in soil solution, would exist in neutral and anionic form. The reason why sorption increases with increasing ionic strength is that soil acts like a cation exchange resin. Normally, all the negative sites on the soil are occupied by H^+. Raising the concentration of Ca^{2+} (or Al^{3+}) displaces H^+ and effectively makes the surface acquire a positive charge, which can bridge anions of SMO. Increased cation content would therefore increase K_{d}-.

A recent study has shown that divalent cations at low concentrations were found to enhance the sorption of drugs such as ibuprofen and ketoprofen onto silica, while trivalent cations (Al^{3+} and Fe^{3+}) enhanced sorption further (Bui and Choi 2010). In contrast another study reported that at intermediate pH values there was significant contribution from the zwitterionic form of SM and the involvement of proton exchange phenomena which facilitated interactions (Teixido *et al.* 2011).

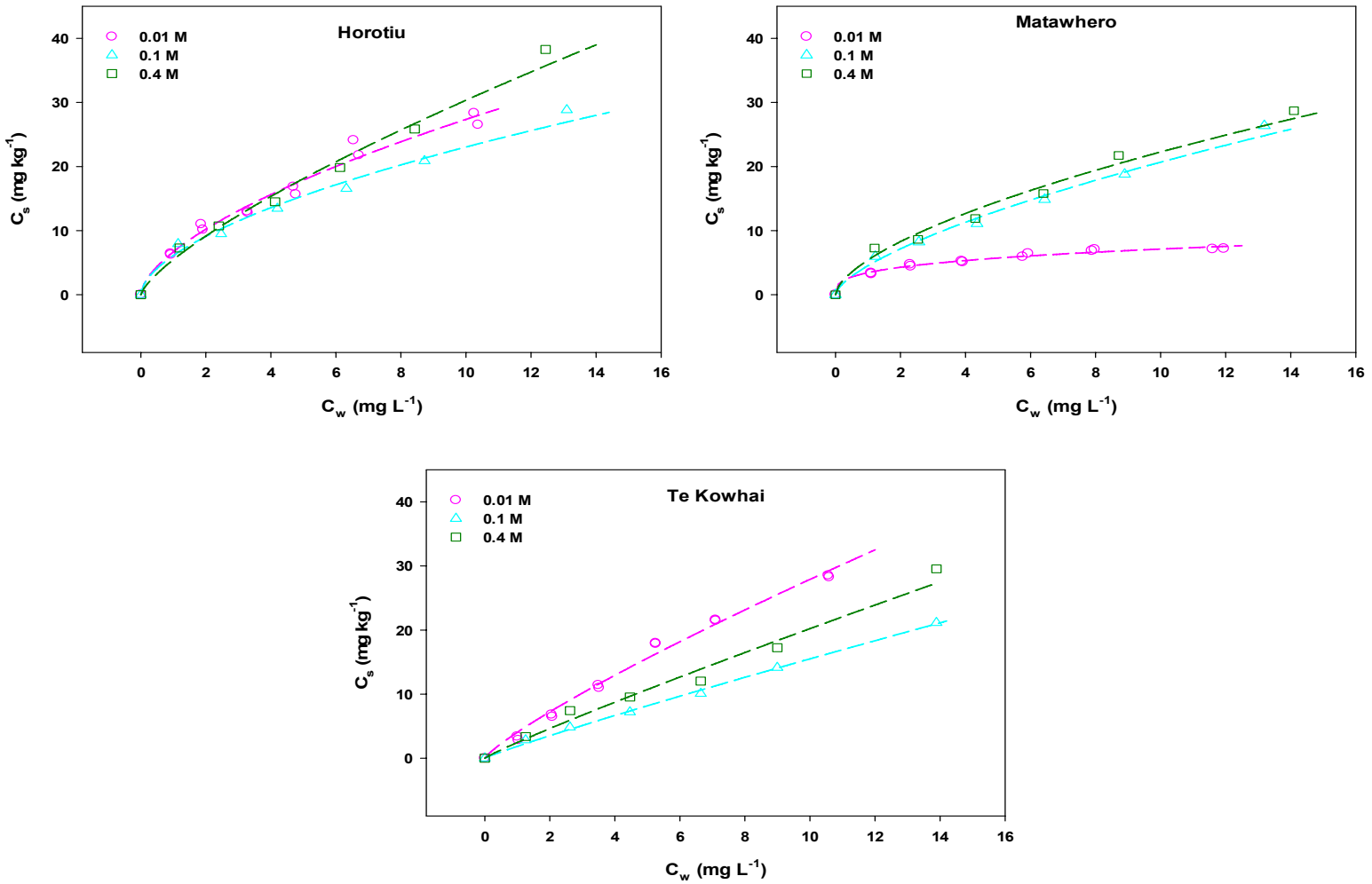


Figure 5.7: Sorption isotherms for SMO for three soils under three different ionic strengths. Dashed lines represent Freundlich model fits

Table 5.4: Summary of sorption parameters for SMO antibiotic in the three selected soils with varying ionic strength.

Soil					Matawhero			
Ionic Strength (M)	K_d	R^2	K_f	N	R^2	$^{\#}K_d^{eff}$	Log K_{oc}* 0.5 mg L^{-1}	5 ng L^{-1}
0.01	1.40	0.90	2.37	0.75	1.00	2.83	2.13	3.40
0.1	2.18	0.93	5.33	0.58	0.95	7.14	2.53	4.63
0.4	2.27	0.91	5.67	0.59	0.95	7.55	2.56	4.63
					Te Kowhai			
0.01	3.65	0.99	3.26	0.96	0.99	3.35	1.83	2.02
0.1	1.71	0.97	3.02	0.75	0.98	3.59	1.86	3.10
0.4	2.02	0.98	2.90	0.83	0.98	3.27	1.82	2.68
					Horotiu			
0.01	3.86	0.96	6.75	0.60	1.00	8.88	2.03	4.01
0.1	2.39	0.97	5.97	0.69	0.98	7.38	1.95	3.48
0.4	3.16	0.99	6.55	0.54	0.96	9.05	2.04	4.37

$^{\#}K_d^{eff}$ is the concentration dependent effective sorption distribution coefficient ($K_d^{eff} = K_f C_w^{N-1}$) using lowest aqueous equilibrium solution concentrations of C_w = 0.5 mg L^{-1}; *concentration dependent OC-normalized sorption coefficient calculated using $K_{oc} = K_f C_w^{N-1}$ foc^{-1} at C_w = 0.5 mg L^{-1}& 5 ng L^{-1}; K_d^{eff} in L $kg^{-1}$and K_f in mg $^{1-N}L^N kg^{-1}$

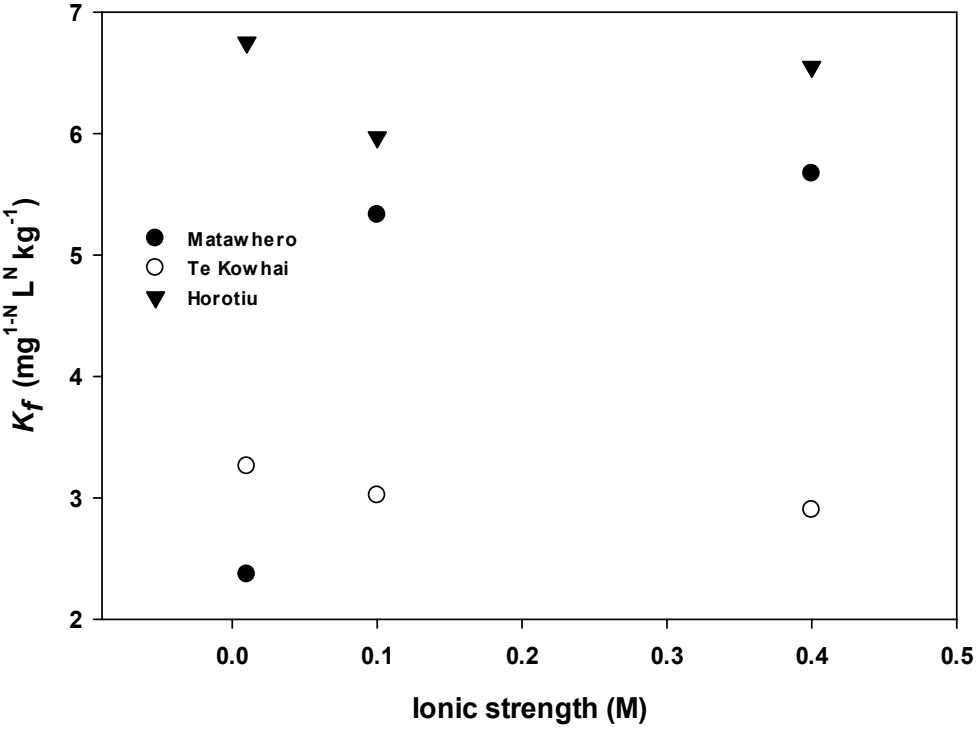


Figure 5.8: Effect of ionic strength on the sorption of SMO antibiotic.

5.3.5 Effect of organic carbon

In order to study the influence of soil organic carbon on the sorption of SMO antibiotic, its K_d values were plotted against the corresponding % OC of the soils used in the study (Figure 5.9). For the pH range used, Horotiu with the highest OC of 8.3% gave the highest sorption compared to Te Kowhai (5%) soil with Matawhero soil (2.1%) as the least sorptive. The results were similar to that obtained by Drilla *et al*. (2005) who determined K_d values for SMO in two soils with varying organic carbon levels, the soil with 0.4% OC and 45% clay yielded a K_d value of 0.23 L kg^{-1}, while for the soil with 7.1% OC and 15% clay it was higher (37.6 L kg^{-1}). Recent studies have shown the influence of soil organic carbon on the sorption of sulfonamide antibiotic (Lertpaitoonpan *et al.* 2009; Figueroa-Diva *et al.* 2010). Tolls (2001) reported that the prediction of log K_{oc} based on the hydrophobicity parameter log K_{ow} leads to significant underestimation. Therefore, for comparison purposes, the sorption coefficients were normalised with OC content to obtain K_{oc} values.

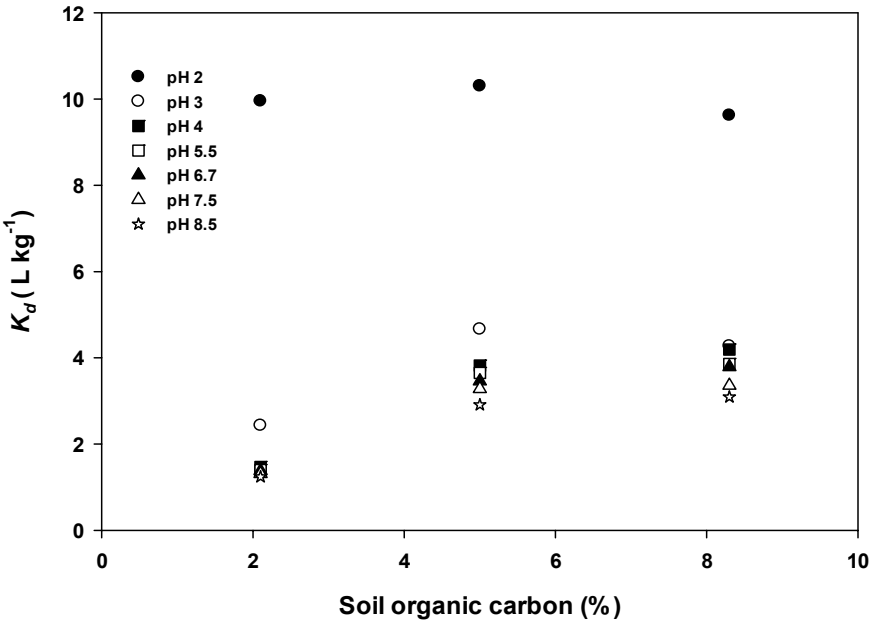


Figure 5.9: Sorption coefficients (K_d) of sulfamethoxazole as a function of pH versus soil OC%.

The log K_{oc} values for all the three soils in this study at any particular pH were more or less similar and they ranged from 2.01 to 2.40 at pH 4, and 1.98 to 1.54 at pH 8.5 for the three

soils investigated. Similar log K_{oc} values that do not vary with pH changes indicate the influence of OC on sorption of organic compounds (Lertpaitoonpan *et al.* 2009). As shown in Figure 5.9, the log K_{oc} value for Matawhero soil at a pH of 2 was the highest (consistent with the low OC content for Matawhero soil) compared with the other two soils. Also at pH = 2 the large amount of SMO^+ would be able to bind to the high clay content and this difference would be less marked at higher pH values where cations are not the major species. The clay: OC ratios for Matawhero, Te Kowhai and Horotiu soils were 12.9, 7.3 and 2.1 respectively. In this study Matawhero soil has low OC and high clay content, and thus it is conceivable that at low pH sorption of SMO^+ to clay surface will become the dominant process causing a rise in K_{oc} value.

Schwarz *et al*. (2012) investigated the sorption of the sulfonamide antibiotics sulfanilamide, sulfadimethoxine, and sulfapyridine to model soil organic matter. Sorption was stronger to humic acid and the authors attributed this to the more complex structure and functional group diversity of humic acid (Schwarz *et al.* 2012). Results from another study also showed that organic matter played an important factor in the sorption of sulfamethoxazole and sulfapyridine to soil (Wu *et al.* 2012). Gao and Pedersen (2005) showed that the sorption of SM to clay surfaces depended on its speciation and clay surface charge density. These authors also suggest that for soils with high clay content and low OC, the sorption to clay surfaces may become the dominant contributor to the K_{oc}. Another study by Kahle and Stamm (2007b) showed that sorption of STZ to inorganic sorbents (clay and ferrihydrite) was an order of magnitude lower than that to organic matter at neutral pH. Therefore, although the role of organic carbon content has a strong influence on the sorption of sulfonamide antibiotic, other qualitative factors such as clay type and minerals may also influence the overall sorption process.

5.3.6 Presence of co-contaminants

To assess the effect of co-contaminants such as the naturally released hormone 17β-estradiol (E2) and its metabolite estrone (E1), on the overall sorption behaviour of SCP, batch sorption studies were performed using a model soil, and the associated sorption isotherms of SCP are presented in Figure 5.10. Isotherms were best described by the Freundlich model, and the sorption parameters (K_f, K_d, and N along with R^2 values) are summarised in Table 5.5.

Examination of sorption data reveals a subtle reduction in sorption affinity of SCP for soil samples equilibrated with E2 at both concentrations compared those containing no E2 (control sample). About 10% and 30% decrease in SCP (K_f) sorption were observed when E2 was added at a level of 0.075 mg L^{-1} and 3 mg L^{-1}respectively. The presence of E2 also influenced the degree of linearity of the SCP isotherms. The control isotherm (with SCP alone) showed a highly nonlinear trend; however, the SCP isotherm in the presence of E2 showed a reduced degree of nonlinearity as supported by the *N* values (Table 5.5). The percentage of E2 and E1 extracted from the soil residue for any given SCP initial spiked concentration was more or less similar meaning E2/E1 sorption was independent of the SCP concentration (Figure 5.11**)**. Small deviations in E2% could be attributed to the formation of its metabolite E1.

Table 5.5: SCP sorption coefficients determined in soil equilibrated with solution containing two different 17β-estradiol (E2) concentrations.

Soil	Hamilton Clay							
Treatment	K_d	R^2	K_f	*N*	R^2	$^{\#}K_d^{eff}$	Log K_{oc}*	
							0.5 mg L^{-1}&	5 ng L^{-1}
SCP-Control	7.78	0.95	11.04	0.73	0.99	13.30	2.52	3.87
SCP-E2(0.075 mg L^{-1})	7.54	0.98	9.88	0.79	0.99	11.46	2.46	3.53
SCP- E2 (3 mg L^{-1})	7.34	0.99	7.90	0.95	0.99	8.21	2.31	2.59

$^{\#}K_d^{eff}$ is the concentration dependent effective sorption distribution coefficient ($K_d^{eff} = K_f C_w^{N-1}$) using lowest aqueous equilibrium solution concentrations of C_w = 0.5 mg L^{-1}; *concentration dependent OC-normalized sorption coefficient calculated using $K_{oc} = K_f C_w^{N-1} foc^{-1}$ at C_w = 0.5 mg L^{-1} and 5 ng L^{-1}; K_d^{eff} in L $kg^{-1}$and K_f in mg $^{1-N}L^N kg^{-1}$

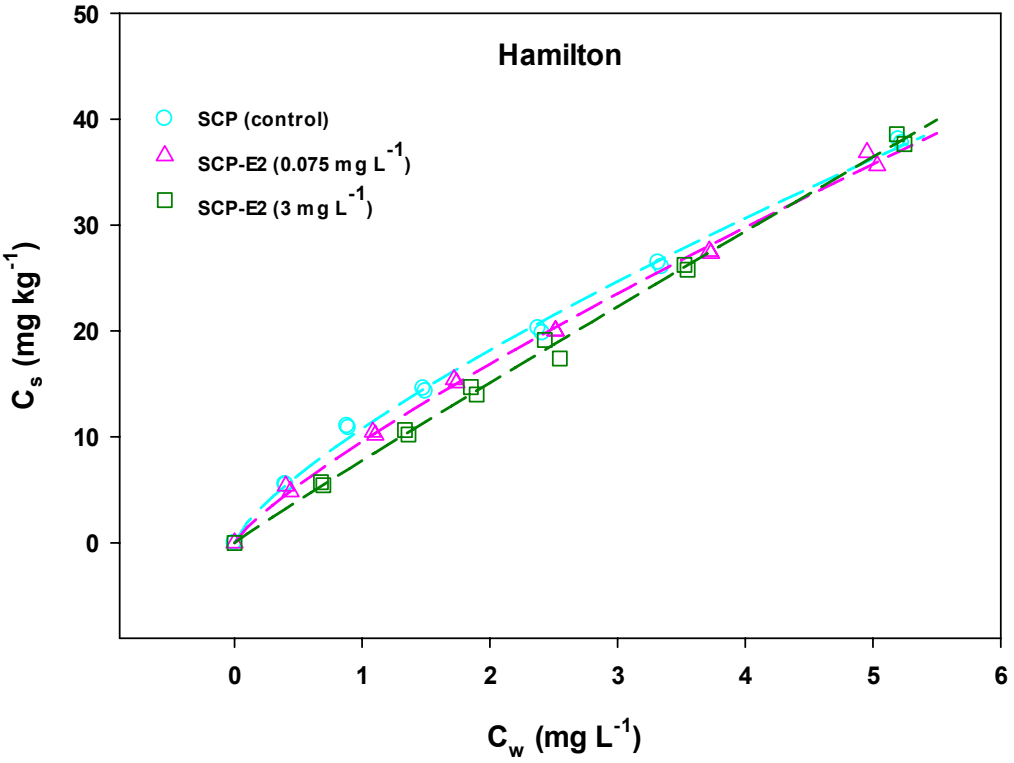


Figure 5.10: Multiple-concentration batch sorption isotherms for SCP constructed using data obtained from equilibration with soil and solution containing two different 17β-estradiol (E2) concentrations and SCP alone (control).

Given that SCP possesses higher solubility than E2, and given the high initial concentrations used for SCP in the batch studies involving E2, one would expect that high concentrations of E2 would affect the sorption of SCP onto soil. However, in this case low concentrations of E2 also inhibited SCP sorption to some extent. This could be either attributed to the different physico-chemical properties of the compounds (Table 5.1) or to different sorption mechanisms (Tolls 2001; Scherr 2009). There is a lack of information on studies related to co-contaminant effects on sulfonamide sorption behaviour in soils. However, Chun *et al.* (2005) reported an increase in the sorbed concentration of E2 and decrease in E1 concentrations compared to the control (2 mg kg^{-1}) when spiked into soils containing 2 mg $kg^{-1}$and 20 mg $kg^{-1}$of SM antibiotics. In that study the formation of E1 during the equilibration time made it difficult to study the influence of SM on E2 concentrations. Accinelli *et al*. (2006) studied the effect of SM on metolachlor sorption in soils equilibrated with 6 and 60 µg L^{-1} of SM and reported decreased sorption affinity (1.11 and 1.18 mg $^{1-1/n}mL^{1/n}g^{-1}$) for SM when compared to a control treatment (1.22 mg $^{1-1/n}mL^{1/n}g^{-1}$).

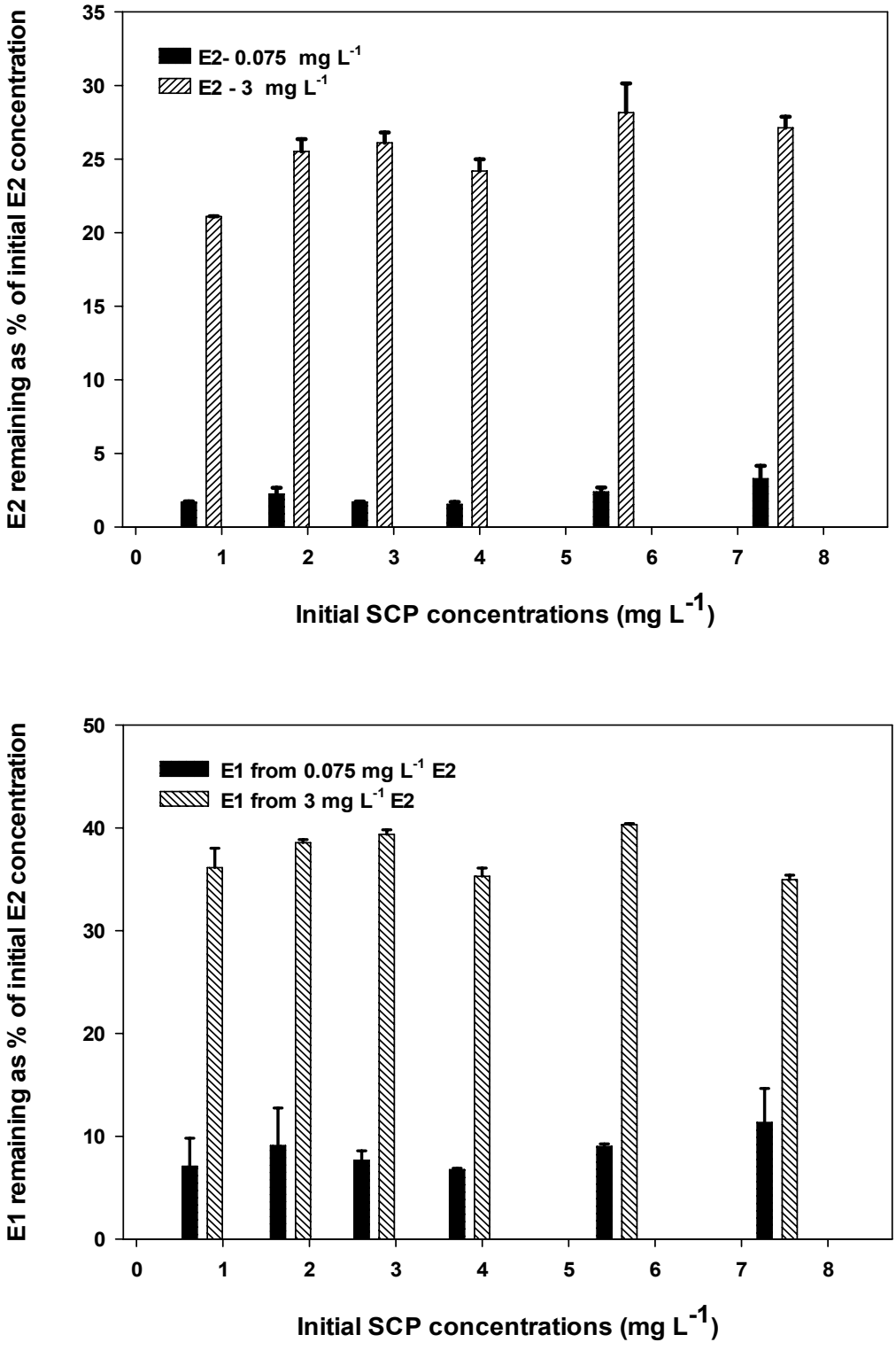


Figure 5.11: Diagram showing the percentage of E2 (above) and its metabolite E1 (below) present in six different initial SCP concentrations. Error bars show deviation of the replicates.

In the present study no comparisons can be made as there are no existing literature data in regard to SCP sorption on the presence of other co-contaminants. However, it is evident from the present study that E2 and its degradation product E1 seemed to have more sorptive affinity to soil than SCP, and readily occupied the active sorption sites.

The present study attempted to evaluate the competition between ionic or relatively polar SCP and non-ionic, relatively non-polar E2. It is important to note that even when it is not ionised SCP is far more polar than E2 and therefore less likely to bind to non-polar soil carbon residues. For non-ionic compounds, the sorption mechanism is based on hydrophobic partitioning, and is mainly driven by the organic carbon content of the soil (Li and Yang 2010). Sulfonamide sorption on other hand is governed by the pH; even though it behaves as a Lewis base at neutral soil pH, its sorption affinity is also mainly driven by hydrophobic partitioning (Lertpaitoonpan *et al.* 2009) and partially (cationic species) by electrostatic force of attraction to clay (Gao and Pedersen 2005). Thus, it is very likely that the presence of non-ionic E2 inhibited SCP sorption to soils at normal soil pH. Li and Yang (2010) investigated the sorption behaviour of 17β-estradiol (E2) on marine sediments treated in the presence of bisphenol A, cationic surfactant, nonionic surfactant and anionic surfactant. The authors concluded that, the sorption behaviour of E2 to marine sediments without the addition of surfactant was mainly related to organic carbon content of sediments. The presence of cationic and non-ionic surfactants enhanced the adsorption capacity of E2 of marine sediments. In contrast, the presence of BPA and anionic surfactant inhibited the adsorption capacity of E2 (Li and Yang 2010).

Another study by Yu and Huang (2005) examined the competitive sorption between 17α-ethynylestradiol (EE2) and two aromatic hydrocarbon compounds, phenanthrene and naphthalene, by three sediments. While the effect of naphthalene on EE2 sorption was insignificant, the competitive effect on the sorption of EE2 by phenanthrene was very significant at low EE2 concentrations. Shariff (2012) studied competitive sorption between of pairs of herbicides, including the effect of atrazine on the adsorption behaviour of metolachlor which is a nonionic herbicide, and propanil on the adsorption behaviour of 2,4-D (2,4- dichlorophenoxyacetic acid) an anionic herbicide. The K_f values for the adsorption process varied between 0.079 and 2.282 mL g^{-1} and 0.058 and 0.720 mL g^{-1} for metolachlor/atrazine and 2, 4-D/propanil respectively. The authors observed that the sorption

affinity of the herbicides decreased with increasing solution concentration. The study also tested a nonionic surfactant for its desorption potential and this was found to be fairly effective with removal of more than 65% sorbed pair competitive herbicides.

Elsewhere, Conkle *et al.*(2010) investigated the competition between three fluoroquinolone antibiotics – ciprofloxacin (CIP), ofloxacin (OFL) and norfloxacin (NOR) and their sorption to a surrogate Louisiana wastewater treatment wetland soil. The K_d values obtained in their study indicated that there is competition between these three compounds for sorption sites. The findings of the various studies conducted in the recent past thus suggest that the fate and transport of emerging pollutants such as a veterinary antibiotic could be affected in the presence of other pollutants in terrestrial systems. In the present study it was found that a high concentration of E2 inhibits SCP sorption and that E2 being non-ionic has a higher adsorption potential than SCP. In the presence of E2, SCP desorption potential could increase and could be dependent upon E2 concentration.

5.4 Summary and environmental significance

From this study it can be inferred that the sorption affinity of sulfonamide antibiotic (SMO) investigated in this study is strongly governed by soil pH and ionic strength. Sorption of SMO decreased with increases in soil pH and there was correlation with the increased ionic strength and organic carbon of the soil. In general, sorption of sulfamethoxazole antibiotic in soil is influenced by the properties of the chemical itself such as the ionisation constant (pK_a), hydrophobicity and soil solution characteristics such as pH, ionic strength, cations present, and the OC content of the soil. These variations can be significant when manure and fertilisers are added to soil. Manure addition can change the pH of the soil usually increasing it above that of normal field soils. In the presence of the manure, which is alkaline in nature, sorption affinity might further decrease because the compound is anionic and thus more mobile in the environment. Similarly fertilizer addition can lead to pH variation, and also increase the ion content in the soil, i.e. varying divalent and trivalent cation composition. It is important to study antibiotic fate under these varying conditions.

Veterinary antibiotics and steroid hormones are likely to co-exist in manure and agricultural soils. Their coexistence can easily affect the ultimate fate of these antibiotics in soils. The effect of natural steroid hormone E2 on the sorption affinity of SCP antibiotic for a dairy

farm soil (Hamilton) revealed that the presence of E2 in the soil can reduce SCP sorption. SCP antibiotic has low sorption affinity and is known to be highly mobile in soils, this implies that in the presence of steroid hormones, SCP could possess significant threat with its potential to leach through the soil profile and eventually contaminate aquifer or groundwater. Thus the co-solute effect in studies dealing with the fate (sorption, persistence and transport) of antibiotics should be considered in order to obtain greater understanding about these antibiotics, fate in real field situations. These experiments were carried out in the laboratory; therefore more work is warranted to understand their sorption behaviour under realistic field situations especially in soils amended with manure, fertilisers, and in the presence of other contaminants in soil.

Chapter 6: Use of biochar as an engineered sorbent for the uptake of veterinary antibiotics

6.1 Introduction

Biochar is a biomass-derived carbonaceous material produced when any form of biomass is burnt without oxygen at temperatures generally between 350^{o}C and 700^{o}C in a process known as pyrolysis (Van Zwieten *et al.* 2010a). Biochars can be produced from a wide variety of biomass material including forestry, crop residue, paper mill sludge, and poultry waste (Singh *et al.* 2010a). Biochar can have a significant influence on the mobility and retention of chemicals in soils. Laboratory, field studies, and traditional farming practices suggest deliberately added biochar can impact on soil fertility, crop production, and availability of nutrients (Lehmann 2007; Kookana *et al.* 2011). Biochar addition to soil might be an effective technique for the removal of residues of veterinary antibiotics and other nutrients associated with faecal and urinary excretion by grazing animals. This may potentially help reduce the risk these compounds can pose to the receiving environment; however, prior knowledge of the ability of the materials to remove contaminants of interest is essential. In the recent past, various types of biochars from a range of biomass have been produced, and the ability of these biochars to remove contaminants such as metals, pesticides and veterinary antibiotics has been documented, e.g. see Bailey *et al*. (1999), Cao *et al*. (2009), and Teixido *et al*. (2011).

Numerous published studies are also available on the ability of various types of biochar for sorbing organic chemicals (Burgess *et al.* 2004; Cornelissen and Gustafsson 2004; Hua *et al.* 2009; Graber *et al.* 2011; Uchimiya *et al.* 2011a), and some authors have described it as a 'supersorbent' (Lohmann 2003; Koelmans *et al.* 2006). Several other environmental benefits can also be obtained from using biochar as a soil amendment. Since it is high in organic matter, it can be added to soils thus improving soil structure and fertility of degraded soils; it can improve cation exchange capacity and reduce aluminium toxicity to plants; it can also improve water retention leading to high crop productivity. It helps to increase crop productivity sequester carbon and reduce greenhouse gas emissions; and in mitigating nutrient leaching and increasing biological activity etc. (Chan *et al.* 2007; Lehmann 2007;

Glaser *et al.* 2009). In New Zealand biochar is mostly seen as an effective waste management solution, whereby organic waste previously composted or applied onto land is instead converted into biochar and energy.

In the recent past biochar amended soils have been studied for sorptive behaviour for pesticides, fungicides, EDC's and heavy metals (Yu *et al.* 2006; Sarmah *et al.* 2010; Wang *et al.* 2010; Ji *et al.* 2011; Karami *et al.* 2011; Sun *et al.* 2011; Marin-Benito *et al.* 2012). However not many published work exists on the potential use of biochar as a soil amendment to remove antibiotic residues. Presence of antibiotic residues in soils could lead to ecotoxicity to non-target organisms, reduce risk of antibiotic resistance and plant uptake. For example, Kotzerke *et al* (2008) observed reduced microbial activity (as measured by cell respiration) in soils amended with manure containing SDZ. Another study amended two soils with manure and SDZ at much lower doses and observed decreases in total phopholipid fatty acids and bacteria: fungi ratios (Hammesfahr *et al.* 2008). Soils spiked with swine manure and SDZ showed higher levels of sul genes after 2 months compared to the control (Heuer and Smalla 2007). SM concentrations in plant tissue reportedly increased with corresponding increase of SM in manure (Dolliver *et al.* 2007). An increased sorption to soil would mean reduced mobility and transport, a direct result of which there would be a reduced risk of ecotoxicity to non-target organisms and antibiotic resistance.

Also the effect of biochar on soil properties can be significantly influenced by feedstock source and pyrolysis conditions (Chan *et al.* 2008; Gaskin *et al.* 2008). For example, biochar produced from nutrient-poor feedstocks may have limited soil fertility (Singh *et al.* 2010a). Only a few investigations have examined the differences in properties of the biochars produced from different feedstocks (Ozcimen and Ersoy-Mericboyu 2010; Singh *et al.* 2010a) and at different pyrolysis temperatures (Gaskin *et al.* 2008; Wannapeera *et al.* 2011; Uchimiya *et al.* 2011b; Kloss *et al.* 2012).

A number of studies have been conducted to determine how the chemical and structural composition changes as a function of pyrolysis temperature affect the sorptive capacity of the char to retain compounds like PAHs, heavy metals, catechol, etc. (Chen *et al.* 2008; Uchimiya *et al.* 2011b). It is postulated that the sorptive capacity of a biochar is likely to be influenced by surface properties of the biochar and the chemical composition of the char

including its pH (Uchimiya *et al.* 2011b). Recently, Teixido *et al*. (2011) reported unconventional adsorption behaviour of the veterinary antibiotic SM on a charcoal when investigated as a function of concentration, pH, inorganic ions, and organic ions and molecules. In most cases, the underlying mechanisms relating to the improvement of soil structure due to biochar additions or the retention capacity of various types of biochar for organic contaminants/nutrients have not been clearly understood. Fundamental knowledge on how biochar reacts with various compounds and with soil biological constituents is important to engineer them to meet specific environmental applications, such as removal of antibiotic residues from soils.

The main aim of this study was to minimize offsite migration, thereby reducing the ecotoxicological effects of residual antibiotic and their potential to confer antibiotic resistance. Also the effects of feedstock, and pyrolysis temperatures on the sorptive properties of soil amended with biochar. Therefore the retention ability of a selected New Zealand dairy farm soil (Matawhero loam) amended with biochar obtained from three different feedstocks (corn cob, pine sawdust and green waste) and at three different pyrolytic temperatures (350^{o}C, 450^{o}C, 550^{o}C Green waste only) to a veterinary antibiotic (sulfamethoxazole) was investigated in detail. Laboratory batch sorption studies were carried out using a complex solvent extraction scheme in order to derive the partitioning coefficients for sulfamethoxazole. Biochars were characterised using inductively coupled mass spectrometry, Fourier transform infrared spectroscopy, Scanning electron microscopy /Energy dispersive X-ray spectroscopy and X-ray diffraction techniques.

6.2 Materials and methods

Matawhero silt loam soil (0–5 cm) representative of the dairy farming region of Hawke's Bay in the North Island of New Zealand was collected, air-dried, and sieved (2 mm). A full description of the soil and the methods used to determine the physico-chemical properties can be found elsewhere (Hewitt 1998).

Biochars used in this study were produced from 3 feedstock namely 1) Green waste (GW) at 3 prepared at three different pyrolytic temperatures–350^{o}C, 450^{o}C and 550^{o}C using a slow-pyrolysis technique; 2) corncob (CC) biochar was produced by a flash carbonization

technique; and 3) pine sawdust (PSD) biochar was produced by slow pyrolysis at 700^{o}C. GW biochars were obtained from Pacific Pyrolysis, Australia; corn cob biochar from Hawaii Natural Energy Research Institute; and pine sawdust biochar from Lakeland Steel Ltd., New Zealand.

6.2.1 Biochar characterisation

The biochars were homogenized and ground to < 2 mm for most of the analyses. The pH/EC (PHM62 standard pH meter calibrated with pH 4 and 7 buffer solutions and "In House" conductance meter with a cell constant K= 0.69 cm^{-1} of the biochars were measured using standard laboratory protocol, which consisted of char and water (1:5) shaken for 24 h in a rotary drum shaker. The samples were centrifuged and the supernatant taken for pH/EC analysis. The C, H, N, S, O, and ash content of the biochars (proximate analysis) were determined at the Campbell microanalytical laboratory in Dunedin (New Zealand). Exchangeable cations were determined using ICPMS and specific surface area for chars was measured by BET nitrogen adsorption isotherm method. All five biochars were characterised using a range of techniques as described below.

6.2.1.1 Scanning Electron Microscope (SEM)

Powdered samples were placed on aluminium stubs using carbon tape and electron micrographs were obtained using a Hitachi S-4700 and ion sputter (E-1030) field emission SEM equipped with an X-ray analyser. Biochar particle structure and surface topography were obtained at three different resolutions (150×, 600× and 3000×). At 3000×, resolution EDX was carried out to obtain the elemental composition for the biochars

6.2.1.2 X-Ray Diffraction (XRD)

A Phillips X'Pert MPD system equipped with a Cu monochromator and a MiniProp detector was used to obtain XRD data. The generator was set at 40 kV and 40 mA, programmable divergence slit (PDS), at 0.1 mm, and programmable receiving slit (PRS) at 0.2 mm. Scans were run over the 2θ range of 2.5–70^{o}, using a step size of 0.02^{o}, and a scan speed of 0.5 s step^{-1}. The minerals were identified by comparing 2θ values to ICDD-PDF mineral database.

6.2.1.3 Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR spectra were obtained using a Spotlight 200 FT-IR Microscope-Spectrometer Software: Spotlight 200. One part of each biochar was mixed with ten parts of KBr powder with mortar and pestle, and pressed between two metal disks at a pressure of 10–11 tons to obtain a thin KBr pellet. The pellet was then scanned in the reflectance mode in the range 4000 – 750 cm^{-1}.

6.2.1.4 ICPMS

ICPMS was carried out according to US EPA standard method 200.2. The sample (1g) was digested (1 h, 70^{o}C) with aqueous HNO_3 (4 mL, 1:2 dilution) and HCl (10 mL, 1:5 dilution). After digestion the sample was made up to 100 ml with water, centrifuged and diluted 1:5 before analysis. The samples were analysed as replicates, as a check on precision and accuracy of the results. In-house quality control standards and a standard river water reference material (SLRS-5) were also analysed at the beginning and end of the sample measurements.

6.2.2 Batch sorption studies

The batch sorption protocol followed here was the same as that used for SMO in **Chapter 4**. Duplicate samples of air-dried soils (2 g) amended with the three types of biochar at two levels (0.5% and 1.0% of soil mass) were weighed into glass centrifuge tubes (35 mL) with Teflon-lined screw caps. The amount of biochar added to the soil was based on biochar application rates of 5 and 10 t ha^{-1}, assuming a bulk density of 1000 kg m^{-3}, and an incorporation depth of 10 cm in the field. Aliquots of (30 mL) of SMO with six initial concentrations (1.5, 3, 5, 7.5, 10 and 15 mg L^{-1}) prepared in background electrolyte solution of 0.005 M $CaCl_2$ were added to the respective tubes. The tubes were wrapped in aluminium foil, placed in the dark to limit degradation due to photolysis and shaken in an end-over-end shaker (24 h). Preliminary investigation using biochar amended Matawhero soil had shown that 24 h was sufficient for the apparent equilibrium to take place.

6.2.3 Extraction and analysis

The protocol for the analysis of SMO along with the HPLC elution scheme used are the same as those reported in Chapter 3.

6.2.4 Data Analysis and Sorption Modelling

Details on the estimation of sorption coefficients and Freundlich model used here have been described in detail in Chapters 4.

6.3 Results and discussion

6.3.1 Analysis of the biochars from three different biomass sources

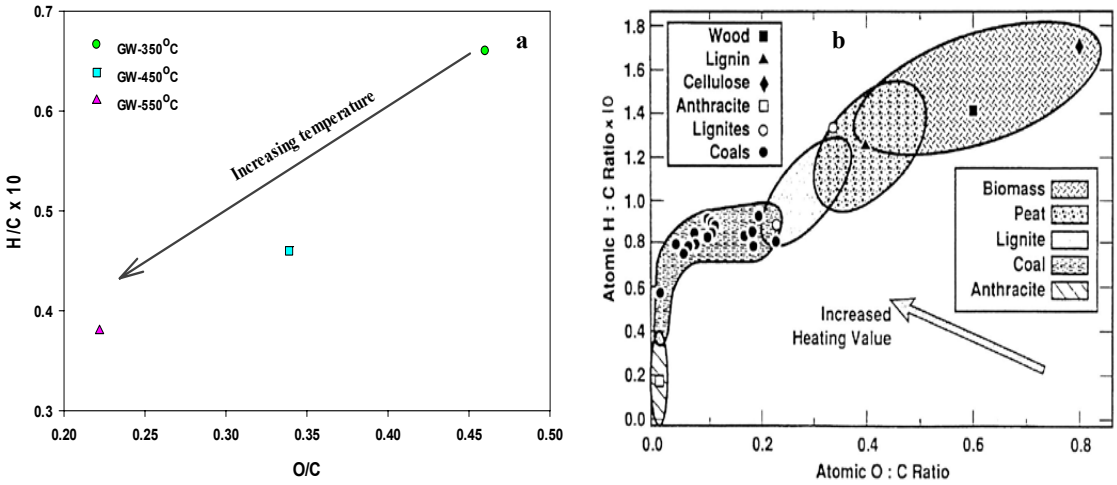
The results of the proximate analysis, pH and EC for the biochars are summarised in Table 6.1. The biochars were analysed for C, H, N, S and ash content (ultimate analysis). The fixed C content for PSD, CC and GW-550 ^{o}C biochar were 91%, 82.2% and 75% respectively. The total C content increased with the heat treatment temperature. Amounts of C observed in different types of biochars are a function of many factors such as the heat treatment temperature, residence time, moisture content of the biomass, and most importantly, the source of biomass (Sarmah 2011). The total N content was the highest for CC biochar with 1.51% by weight followed by GW biochar with 0.32% and PSD biochar at 0.11%. Generally, wood biochars have higher total C content and lower total N than vegetative and forestry chars (Singh *et al.* 2010a), this is evident for PSD biochar. The total S content for all 3 biochars was < 0.03 weight%. Table 6.1 also shows that high temperature chars very highly aromatised resulted in lower molar O/C and H/C ratios.

The pH values for all the biochars varied from near neutral to highly alkaline, varying significantly among feedstocks ranging from 5.3 to 9.7. Higher treatment temperature increased final pH for all the biochars. The EC values of the biochar were different for the feedstocks used in the study. The high EC value for CC biochar and low for GW biochar could be attributed to exchangeable cations in them; especially the cation K. Cation K content for CC biochar was highest at 123.6 cmol kg^{-1}, and lowest for GW biochar at 9.5 cmol kg^{-1}. PSD biochar, which is a woody biochar, had the highest SSA value of 795 m^2g^{-1}, followed by CC and GW biochars (which are vegetative and forestry chars) with SSA values 186 and 153 m^2 g^{-1}.

6.3.2 Stability of GW biochars: Van Krevlin diagram

The proximate analysis for the three types of GW biochars results are also tabulated in Table 6.1. With the increase in the pyrolysis temperature of GW biochar from 350^{o}C to 550^{o}C a general trend began to emerge. The pH, EC, Total C, CEC, exchangeable cations, SSA, and ash content increased with the increase in pyrolytic temperature. Only the total N content and total H decreased with increase in the pyrolysis, while the total S content for all 3 GW biochars was < 0.03%. The stability of biochars is often assessed through changes in their elemental composition that is C, H, O and N and their associated ratios (O/C and H/C) as shown in Table 6.1. The molar ratios of O/C and H/C are used to measure the degree of aromaticity and maturation, which is indicative of long-term stability of the biochar (Spokas 2010), and these ratios are often plotted as a Van Krevlin diagram (Figure 6.1). The O/C and H/C molar ratios of the 3 GW biochars were plotted (Figure 6.1a) against each other and compared with the data from the combustion handbook (Figure 6.1b), which shows the evolution of biomass into coal. The biochars do not fall on the same evolutionary line as coal but follow the same general trend of declining O/C and H/C ratios with increasing severity of treatment. These declining ratios indicate increasing aromaticity.

Recently, Spokas (2010) suggested that when the molar ratio of O/C is > 0.6, the biochar would possess a half-life of <100 years, when the ratios are between 0.2 and 0.6, the half-life would be between 1000 and 100 years respectively, and if the O: C ratio is < 0.2, the resulting biochar would possess a half-life > 1000 years. Table 6.1 shows that all the 3 types of GW biochar had low O/C and H/C ratios, with GW-550^{o}C having the lowest values for O/C and H/C of 0.21 and 0.04 respectively. On the basis on O/C molar ratio, GW-550^{o}C biochar would be more stable and would be able to persist in soil for a long period of time than the other two biochars, although even the 450^{o}C could be expected to persist for several centuries. However, more work is warranted, especially long-term field trials to check the persistency of these biochars under a realistic field situation.



***Source*: (**http://www.handbook.ifrf.net/handbook/cf.html?id=23**)**

Figure 6.1: H/C and O/C ratio (Van Krevlin diagram) for (a) GW biochars and other (b) solid fuels.

6.3.3 Biochar characterisation

Scanning electron micrographs of biochars (Figure 6.2) show that all three biochars (CC, PSD, GW) retain the physical form of their biomass precursor and are highly macroporous. This is particularly evident in the high resolution (3000×) micrographs for CC and GW biochars. In contrast, SEM micrographs of PSD shows a material having a smooth, glassy, elongated appearance, lacking the visible porosity observed in the other two biochars. The pore formation could be attributed to escaping volatiles during high temperature decomposition. It is important to note that the pores observed through SEM micrographs would not necessarily contribute much to the overall specific surface area (SSA) of the material. For instance, although both the CC and GW biochars appear to be highly porous as compared with PSD biochar, the SSA of PSD biochar is actually the highest (Table 6.1), presumably due to its higher pyrolysis temperature. The EDX spectra indicate the elements present in the sample on weight basis. C and O were the two most abundant elements found in all biochar in varying proportions. Traces of Ca, K, Al, Mg and Si were also found but in very small proportions. Woody biochars are known to have higher total C content (Singh *et al.* 2010a), this is clearly evident from the C peak of the PSD biochar in EDS spectra (Figure 12.7). SEM-EDX provides qualitative morphological and elemental information, which is necessary, especially when biochar is used for agronomic benefits.

XRD analysis of the biochars (Figure 6.3) gave identical broad diffractograms that are indicative of an amorphous material having some degree of short-range order. Each of the chars gave identical broad spectra, suggesting that peak shape is not only affected by the degree of crystallinity but also by crystal size, with diffraction peaks becoming increasingly broad with reduced crystal size. The initial low angle scattering peak maxima varies for the carbons and may provide some evidence of a regular macroporous structure for the biochars, reinforcing the SSA results for each biochar type (Table 6.1).

The change in initial low angle base line intensity at 24.5 and 42^{o} with biochar types representing two broadened peak may indicate the early presence of a poor crystalline structure and carbon rich phase in these samples. These types of chars are often known as turbostratic chars, which are dominated by disordered graphitic crystallites (Uchimiya *et al.* 2011b). Biochar derived from corncob or other agricultural wastes are known to have low initial intensities compared to carbons derived from pure sugars (Bourke *et al.* 2007). When the XRD diffractograms (Figure 6.3a) for the three kinds of biochars were compared with synthetic graphite, there was little resemblance of the broad featureless peaks of the three biochars to the sharp intense peaks associated with synthetic graphite (Azargohar and Dalai 2006). The well-defined synthetic graphite peak appears at a higher angle compared to the analogous peak maxima associated with the CC, PSD, and GW biochars. Furthermore, the small peak around 54° (2θ) displayed by synthetic graphite is absent in all diffractograms, suggesting that the graphitic regions in carbonized biochar are comparable in size to the carbon's micropores (Bourke *et al.* 2007).

Table 6.1: Chemical properties of biochar used in sorption studies *specific surface area was measured by BET nitrogen adsorption; # measured by ICPMS.

Biochar	pH	Total C	Total N	Total O	Total H	O/C	H/C	EC	Exchangeable cations# cmol(+) kg^{-1}				CEC	Ash	SSA*
	(water)	(%)	(%)	(%)	(%)			(mS cm^{-1})	Ca	Mg	K	Na	cmol(+) kg^{-1}	(%)	(m^2g^{-1})
Green waste-350 °C	5.3	65.2	0.23	30.00	4.28	0.46	0.07	0.17	10.4	4.8	3.8	2.7	26	1.04	3
Green waste-450 °C	5.8	71.8	0.31	24.32	3.28	0.34	0.05	0.09	18.5	18.3	6.5	8.3	85	1.51	3
Green waste-550°C	8.4	75.4	0.32	16.75	2.83	0.21	0.04	0.18	25.4	38.1	9.5	11.9	151	1.61	153
Corncob > 600 °C	9.5	82.2	1.51	6.08	2.15	0.08	0.03	4.63	2.4	15.7	123.6	1.56	145	6.05	186
Pine sawdust-700 °C	9.7	90.9	0.11	6.10	1.31	0.07	0.01	1.57	26.9	18.2	25.47	1.35	148	1.58	795
Matawhero soil	6.6	2.1	0.20	-	-	-	-	0.33	37.9	20.4	8.2	2.5	129	-	6

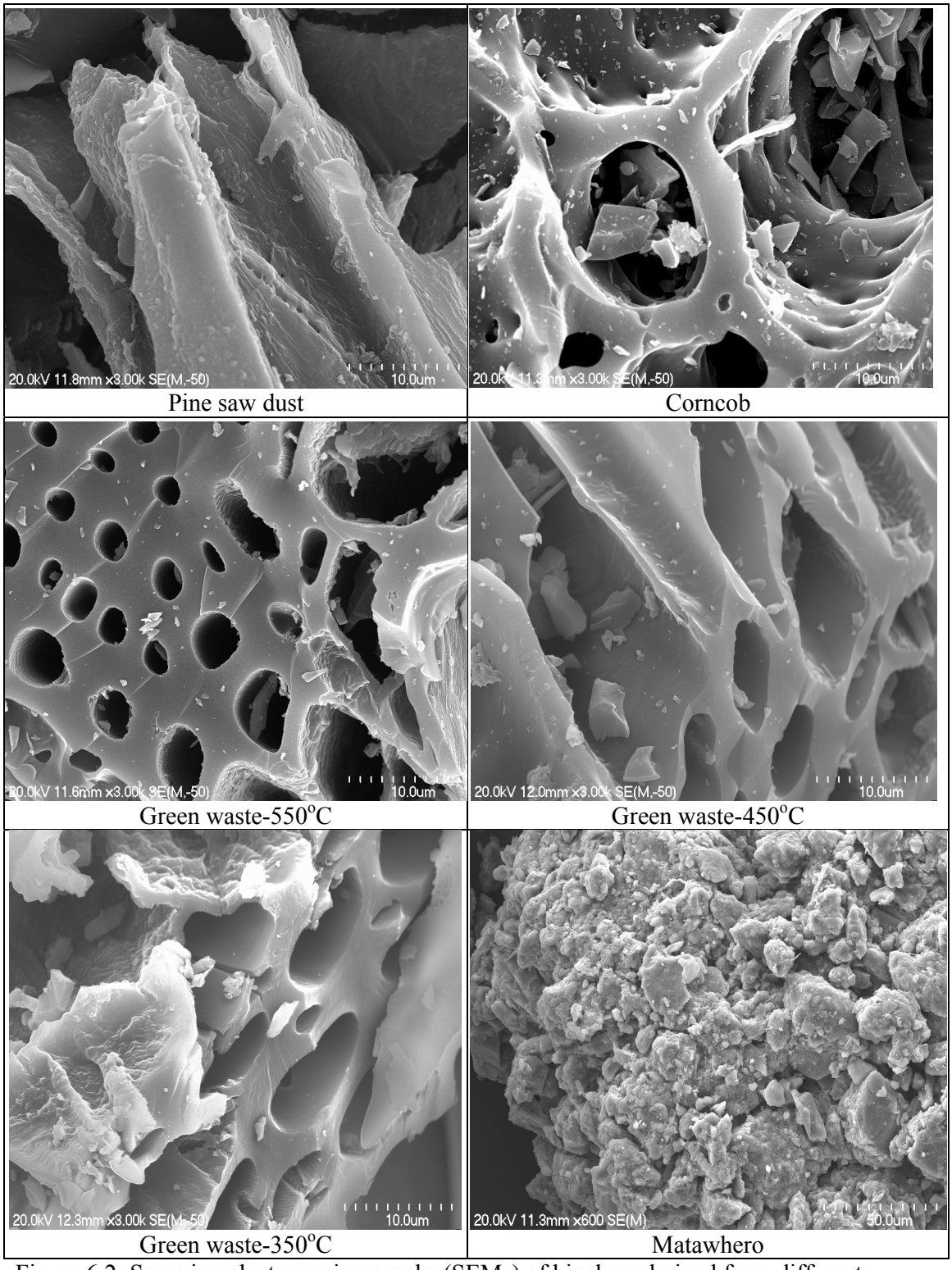


Figure 6.2: Scanning electron micrographs (SEMs) of biochars derived from different feedstocks along with Matawhero soil at (3000×).

The XRD diffractograms for the 3 types of GW biochar were identical (Figure 6.3b) and indicate that their biomass sources were similar, and were not influenced by the varying pyrolysis temperatures. The chars made at 350^{o}C and 450^{o}C chars had higher intensity counts when compared with the 550^{o}C GW biochar. There are no plausible explanations for this; except that it could be attributed to experimental variations as XRD analysis for 550^{o}C GW biochar was done on an earlier date than the others. This means that they could have different background intensity. There was no indication of minerals phases in any of the biochars similar to the finding of Azargohar and Dalai (2006), and Singh *et al.* (2010b) who reported the presence of minerals like whewellite and calcite in *Eucalyptus saligna* wood biochar prepared at 400^{o}C and 500^{o}C respectively. Singh *et al*. (2010b) also found that biochars made at 550^{o}C from cow manure and poultry litter showed the presence of quartz, kaolinite, hematite and sylvite.

FT-IR spectra of CC, PSD and GW biochar and the Matawhero soil are presented in Figure 6.4. The spectra for PSD and CC biochar offered very little information, indicating that they were fairly well aromatized. As the charring temperature rises organic groups were progressively pyrolysed to carbon, and IR absorptions for the residual organic functional groups disappeared to the extent that they became buried in the rolling baseline preventing detailed interpretation.

Since PSD and CC biochars were prepared at higher temperatures compared with GW biochar, their infrared spectra showed no OH signal at 3600 cm^{-1}. As the temperature increases the amount of water (if any) would decrease and OH groups will be reduced via increased dehydration. A signal for the hydroxyl group (–OH) was observed only for GW biochars. Among the GW biochars, GW-350^{o}C was notably different. Spectra for GW-450^{o}C and 550^{o}C were similar with identical signals in the aromatic region. Again due to the low absorbance signals it was difficult to interpret the surface functional groups present in the char. However in general FT-IR absorption bands for aliphatic CH–OH occur at 3600–3200 cm^{-1}; C-O band of phenols (1100–1000 cm^{-1}), carboxylic acid (3000–2500 cm^{-1}), alkynes (2260–2100 cm^{-1}), nitriles C≡N (2277 cm^{-1}), aliphatic C–H (1470–1455 cm^{-1}) and alkenes (1000–675 cm^{-1}). The C=O absorption band of esters typically occur at 1730-1745 cm^{-1}, aldehydes and ketones at around 1700 cm^{-1} and amides at 1680-1670cm^{-1}.

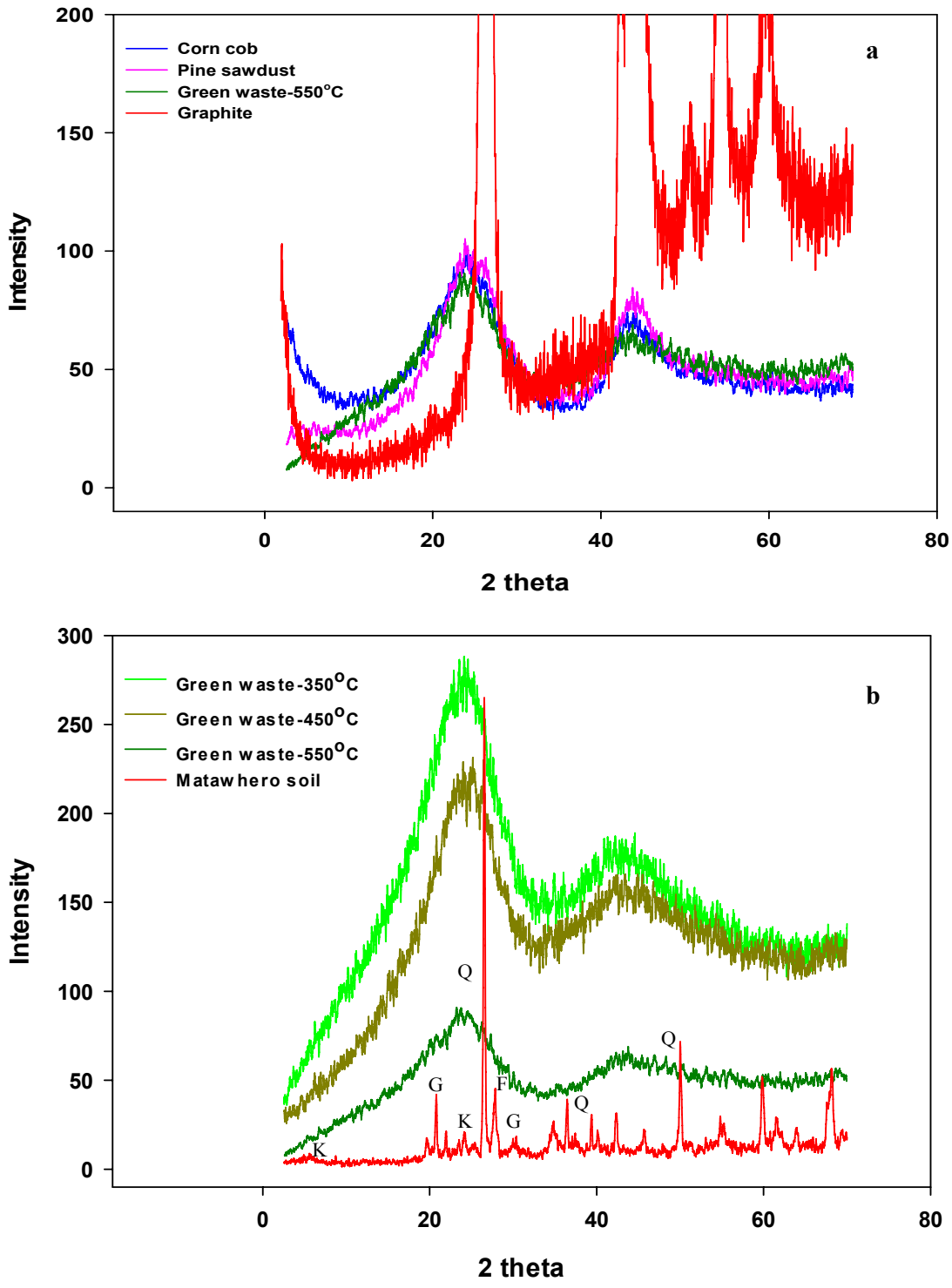


Figure 6.3: X-ray diffraction spectra of (a) CC, PSD and GW-550°C compared with synthetic graphite and (b) for 3 types of GW biochar with Matawhero soil.

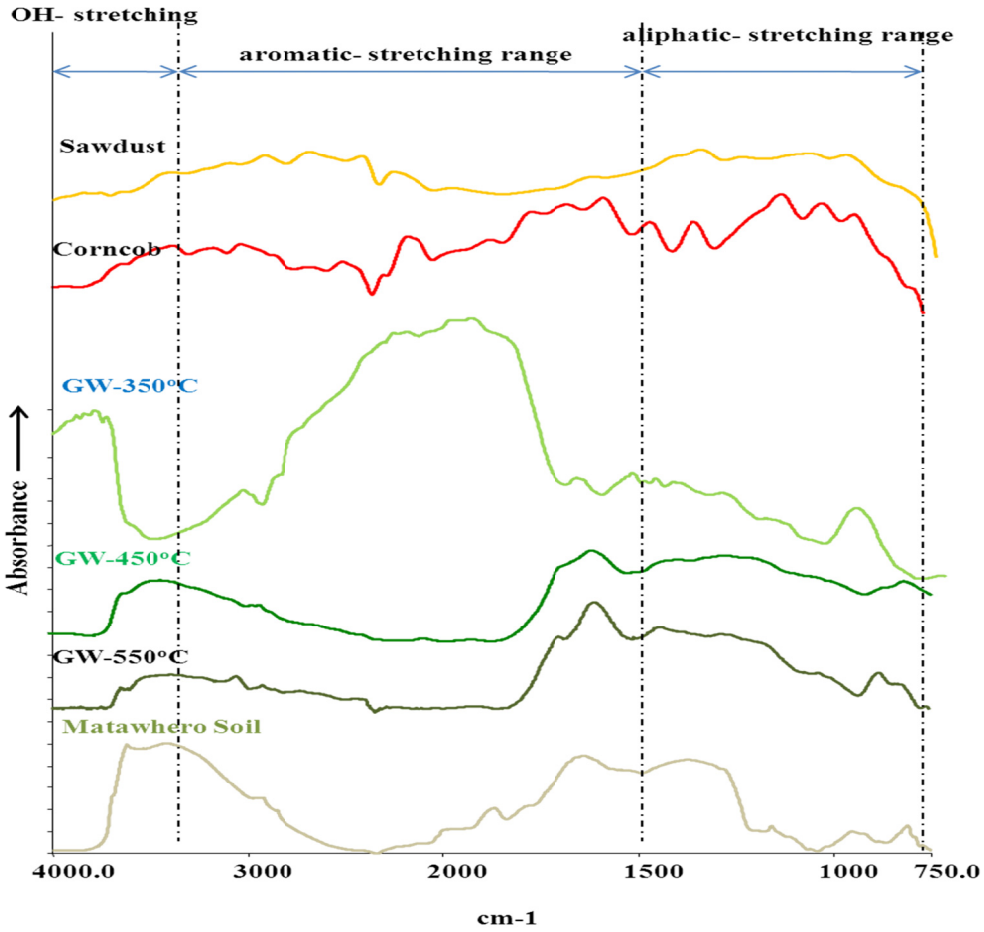


Figure 6.4: Fourier transform infrared spectra of CC, PSD and GW biochars together with Matawhero soil.

The low absorbance signal could have been avoided with the use of ATR-FT-IR (Attenuated Total Reflection-Fourier Transform Infrared) which is often used to measure powder samples (including chars) directly. However, this instrument was not available for the current work. In a separate study conducted on the same biochars, Sarmah *et al.* (2010) recently reported difficulties in discussing the results of standard solid-state ^{13}C NMR analysis of both the CC and PSD biochars and attributed this as common problem for high-temperature chars. The authors reported very poor quality cross polarisation spectra for PSD and CC biochar with only 3% NMR observability (i.e. 97% of potential signal was not detected), making interpretation difficult. However, 92% observability was reported for GW-550^{o}C, with CP spectrum is dominated by the aromatic C resonance centred at ~130 ppm and two small alkyl peaks at ~15 and ~35 ppm (Figure 6.5).

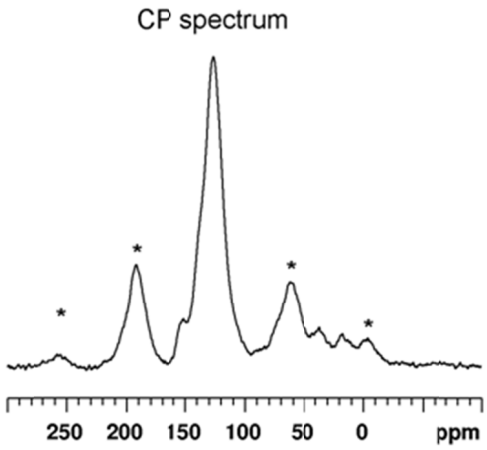


Figure 6.5: Solid-state ^{13}C CP NMR spectra of the GW biochar adapted from Sarmah *et al*. (2010). Spinning sidebands (SSBs) are marked with an asterisk (*).

Understanding the surface functional groups present in the biochar is very important, as these are often responsible for a range of processes that take place during the interaction of biochar with soil particles (Sarmah *et al.* 2010). For instance, biochar with surface carboxylic acid groups, when mixed with soil can improve the cation exchange capacity of soil, which governs the nutrient-holding capacity in soils. The presence of aromatic groups in some biochars (PSD) can be also be beneficial as an effective sorbent for neutral forms of organic compounds such as pesticides, polyaromatic hydrocarbons (PAHs), veterinary antibiotics and steroid hormones.

ICPMS analysis of the biochars showed the presence of various heavier elements in all biochars (Table 6.2). Hg content was lowest for PSD biochar, and ranged from 0.01to 0.07 mg kg^{-1}. Levels of Cr, Cu Cd and Pb were considerably higher in PSD and GW biochar compared to the CC biochar. However, GW biochar had the highest level of As among all biochars with a value of 3.36 mg kg^{-1}. Both GW and PSD biochars showed similar elevated levels of Cr, which is expected for biochar derived from vegetative or woody material (Sarmah 2011).

The concentration of heavy metals in PSD and GW biochars were relatively higher when compared with CC biochar, which had the lowest levels of Cr, Co, As, Ag, Pb and U. However, the levels of most heavy cations in all 5 biochars were well within the threshold limits set by the International Biochar Initiative (2011). Only the Zn level in CC biochar (588

mg kg^{-1}) was higher than the guideline limit (200 mg kg^{-1}). It is important to note that for GW biochar when the treatment temperature was increased from 350^{o}C to 550^{o}C, the cation concentrations increased. This is due to a decrease in mass at higher temperatures, resulting in increased ash concentrations. With the exception of cobalt, both copper and zinc levels were, however, below the level at which these elements occur naturally in the soil. The ICPMS analysis for lighter elements present in the 5 biochars and Matawhero soil are shown in Table 6.3. The results obtained in this study were in a similar range and comparable to other studies on biochar for example van Zwieten *et al*. (2010b).

There is a possibility that biochars themselves can be a source of some classes or organic (PAH's) and inorganic contaminants (heavy metals) depending on feedstock and pyrolysis condition (Kookana *et al.* 2011). Apart from the inorganic load, an increasing aromaticity may in some cases may be linked to higher concentrations of persistent polycyclic aromatic hydrocarbons. The cause of concern is even greater for biochars, which are produced from waste material e.g. municipal solid waste, sewage sludge, or industrial waste (Sarmah 2011) where high levels heavy metals are likely to be present. Therefore is very important to determine the the potential of contaminants to be present in pyrolysis products and the importance of careful choice of waste as a feedstock.

Table 6.2: ICPMS analysis of heavier elements in all 5 biochars and Matawhero soil (mg kg^{-1}).

	Ni	Cr	Co	Cu	Zn	As	Se	Ag	Cd	Hg	Pb	U
GW-350^{o}C	2.25	5.73	0.38	9.84	62.70	2.45	0.01	0.07	0.07	0.07	2.54	0.02
GW-450^{o}C	9.29	11.23	2.21	18.64	93.90	2.97	0.23	0.04	0.11	0.05	2.68	0.13
GW-550^{o}C	19.66	14.76	4.35	31.13	109.90	3.36	0.02	0.03	0.24	0.05	3.25	0.17
CC	4.80	1.19	0.09	20.24	587.80	0.02	0.00	0.01	0.09	0.03	1.17	0.00
PSD	7.53	15.66	22.46	46.88	148.20	0.35	0.08	0.04	0.35	0.01	3.72	0.01
Matawhero soil	27.84	19.66	8.56	27.29	116.40	31.78	0.72	0.29	0.31	0.27	19.85	0.47

Table 6.3: ICPMS analysis of lighter elements and hydrated oxyanions in all 5 biochars and Matawhero soil (mg kg^{-1}).

	Na	Mg	K	Ca	Al	Mn
GW-350 °C	630	582	1477	2070	381	70
GW-450 °C	1916	2227	2535	3710	2629	126
GW-550 °C	2748	4632	3710	5079	5532	150
Corn cob	360	1917	48335	489	116	32
PSD	311	2222	9961	5392	478	403
Matawhero soil	577	4703	1886	8718	9932	1637

6.3.4 Soil characterisation

Figure 6.2 shows scanning electron micrograph of Matawhero to be spherical particles, which at this level appear non-porous, compared to the biochars. This was particularly evident in the high-resolution (3000×) micrographs for the soil. The SSA for the soil was considerably lower than the biochar (Table 6.1). Quantitative analyses by EDS indicated the presence of major elements (Si, C, Al, Fe, Mg, Ca, Na, K), which were measured again qualitatively using ICPMS.

X-ray diffraction patterns for the soil (Figure 6.3b) indicate an overwhelming dominance of quartz and feldspar and calcite. However, since some minerals give much stronger diffraction signals than others, it is difficult to determine the composition of the bulk soil as strength of the XRD peaks is not usually a direct indication of abundance. The XRD was also not indicative of any presence of clay minerals like vermiculite, chlorite, and illite peaks, which tend to occur below a 2θ of 10°.

The FT-IR spectrum of soil is presented in (Figure 6.4). Various bands in the spectra representing stretches due to alcohol and phenol –OH (3600–3200 cm^{-1}; 1100–1000 cm^{-1}), amides C=N (1632 cm^{-1}), aliphatic C–H (1470–1455 cm^{-1}, alkenes (1000–675 cm^{-1}) and ester C=O (1700 cm^{-1}) were observed for Matawhero soil. The distinct bands at around 2920 and 2850 cm^{-1}in the soils represent aliphatic C-H or aliphatic CH_2 vibrations. Quantitative ICPMS analyses showed the presence of common lighter elements (Al, Fe, Mg, Ca, Na, and

K) in the soils. The relative amount of elements present in Matawhero soil was higher compared with the biochars except for K and these are summarised in Table 6.2 and Table 6.3. The results obtained for soil analysis were in a similar range to those established in long-term regional (Taylor 2010).

6.3.5 Effect of biochar amendment on sorption

Sorption isotherms for SMO in the three different biochar (by biomass source) amended soils along with the Freundlich model fits are shown in Figure 6.6. Except for GW amended soil, all isotherms were found to be highly non-linear with *N* values ranging from 0.55 to 0.72 and 0.54 to 1.16 at biochar amendment of 0.5% and 1.0% respectively (Table 6.4). However, the amount of biochar added to the soil did not have a marked effect on overall sorption non-linearity. The organic carbon of the biochar-amended soil was estimated based on the amount of biochar added to the soil and the OC of the soil. The total C of the amended soil varied from 2.75 to 3.00% for a 1% addition in biochar and this value was used for calculating K_{oc}.

The effective distribution coefficient (K_d^{eff}) values for SMO at the lowest equilibrium concentration (C_w = 0.5 mg L^{-1}) ranged from 2.48 to 53.5 L kg^{-1} in soil amended with the three types of biochars produced from varying feedstocks. For some biochars, the mass of char present in the soil did not affect the amount of antibiotic sorbed. For example, there was no marked difference between the K_d^{eff} values for SMO for soils treated with 0.5% and 1% CC biochar and GW biochar (Table 6.4). Corresponding log K_{oc} values were similar; 2.53 and 2.68 (CC) and 2.56 and 2.48 (GW) for the two treatments. In contrast, large differences with respect to antibiotic sorbed and amount of biochar added were found for the soil treated with 0.5% and 1% PSD biochar. For instance, more than three-fold increase in SMO sorption was noticeable in soil treated with 1% PSD biochar, as compared to the soil treated with 0.5% PSD biochar (Table 6.4), and the associated log K_{oc} values differed by 0.35 log units.

When comparing the sorption affinity of the soil treated with the three types of biochars with the control (soil only), it was observed that SMO sorption was the highest in the soil amended with 1% PSD biochar, followed by 1% CC biochar amended soil, and least for soil amended with GW biochar. The soil amended with 1% PSD biochar showed a 20-fold increase in sorption affinity for the antibiotic as compared with the control treatment; however, increase

in antibiotic uptake by the soil amended with 1% CC was only 4-fold. Adding 1% GW to Matawhero soil did not improve its sorption affinity, in fact the control, and 1% GW amended soil showed similar K_d^{eff} values of 2.48 and 2.56 L kg^{-1}respectively.

In order to obtain more quantitative information, the values for K_d^{eff} for each treatment were plotted as percent increase in relation to values obtained in the control treatment (Figure 5.6). Addition of 0.5% of PSD biochar caused a dramatic (10 times) increase in the K_d^{eff} value for SMO, while at double the amount of biochar in the soil, uptake of SMO was increased by 20 times (Figure 6.7). A recent study reported K_d values as high as 10^6 L kg^{-1} for veterinary antibiotic SM sorption to charcoal (Teixido *et al.* 2011); however, that study did not involve any soil amendments and experiments were performed in whole biochar.

Table 6.4: Summary of sorption parameters for SMO derived from the multiple-concentration isotherms in Matawhero soil amended with three types of biochar and in soil alone.

Treatment	K_d	R^2	K_f	N	R^2	K_d^{eff}	$Log\ K^*_{oc}$
0.5% CC BC+soil	3.47	0.82	8.55	0.55	0.93	7.11	2.53
0.5% PSD BC+soil	1.55	0.94	28.51	0.61	0.88	24.34	3.05
0.5% GW BC+soil	13.89	0.64	2.86	0.72	1.00	2.56	2.03
1.0% CC BC+soil	5.29	0.74	11.89	0.61	0.96	10.16	2.68
1.0% PSD BC+soil	33.7	0.65	66.99	0.45	0.96	53.53	3.35
1.0% GW BC+soil	1.63	0.95	2.74	0.76	0.99	2.48	1.98
Soil without BC	1.40	0.90	2.37	0.75	1.00	2.83	2.05

K_d^{eff} is the concentration dependent effective sorption distribution coefficient calculated using $K_d^{eff} = K_f C_w^{N-1}$ at C_w = 0.5 mg L^{-1}; *concentration dependent OC-normalised sorption coefficient calculated using $K_{oc} = K_f C_w^{N-1} / foc$ at C_w = 0.5 mg L^{-1}; K_d^{eff} in L kg^{-1} and K_f in mg $^{1-N}$ L ^{N} /kg.

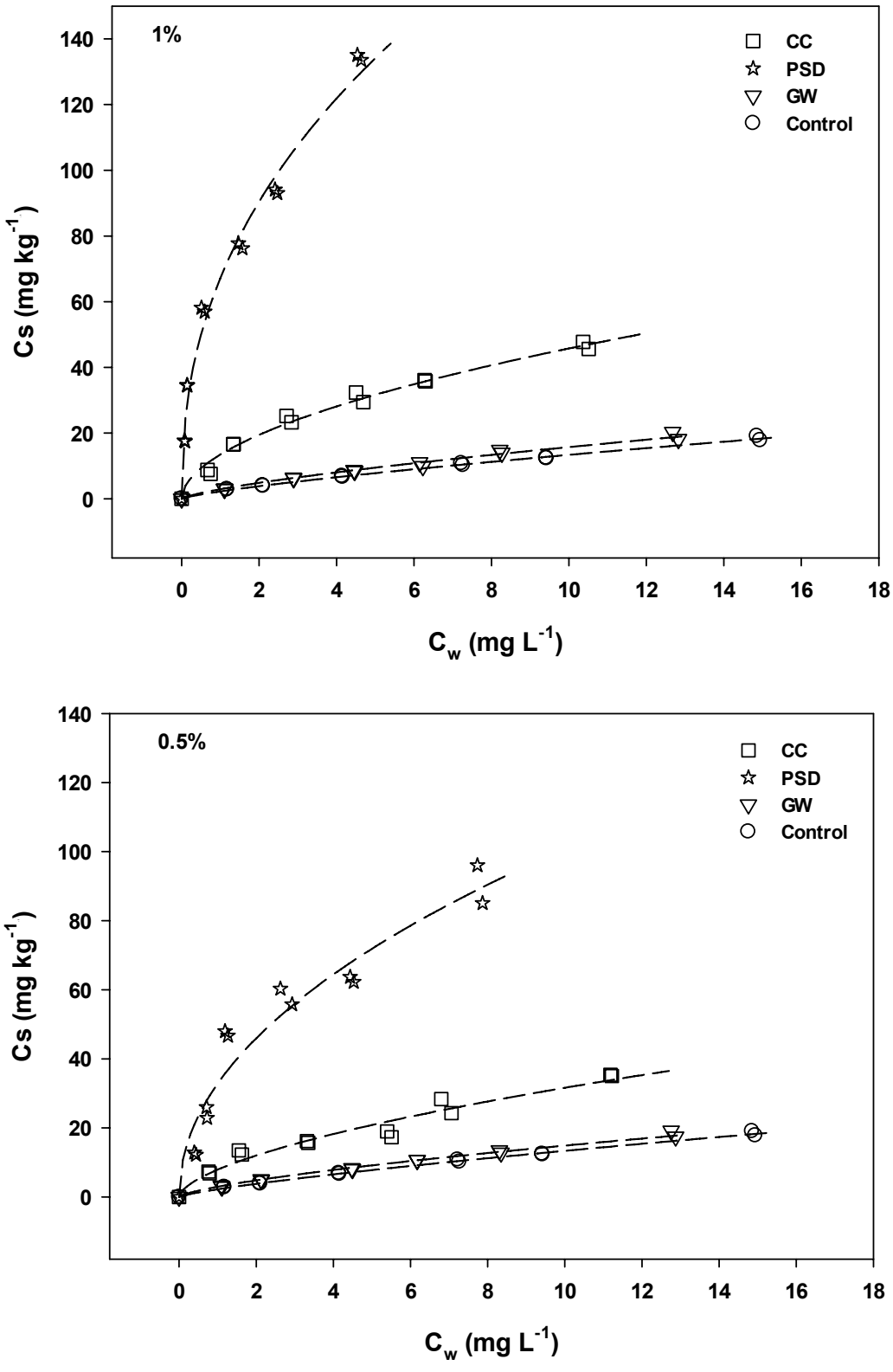


Figure 6.6: Multiple-concentration batch sorption isotherms (24 h contact) for SMO during its equilibration with soil amended with 3 types of biochar at (above) 1% and (below) 0.5% by soil weight. Symbols represent measured values while solid lines represent Freundlich fit.

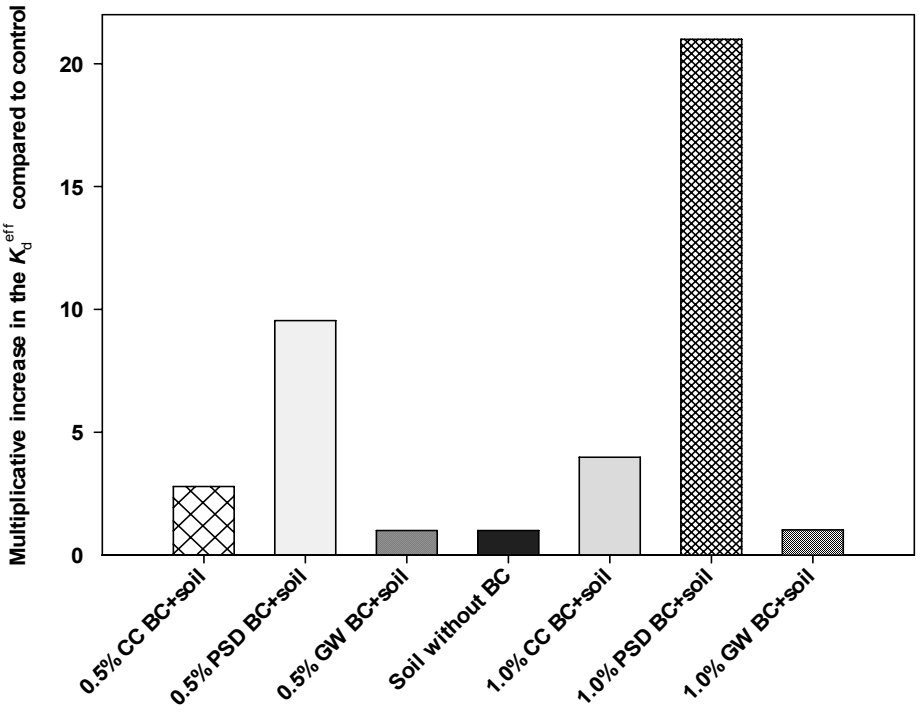


Figure 6.7: Sorptive capacity of different soil amended biochar for SMO antibiotic.

6.3.6 Effect of combustion temperature and type of feedstock on sorption

In order to study only the effect of pyrolytic temperatures on sorption, GW biochar produced by slow pyrolysis at three different pyrolytic temperatures 350^{o}C, 450^{o}C, and 550^{o}C was used. The amendment was 1% by soil weight. Most of the physico-chemical properties albeit % H such as % C, % N, , exchangeable cations, CEC, pH, ash content and SSA of the GW biochars increased with the increases in the pyrolytic temperature. Sorption isotherms for SMO in Matawhero soil amended with the GW biochars produced at the three temperatures were fitted with Freundlich model (Figure 6.8). Overall K_f values for SMO in all 3 types of GW amended biochar were similar to the control (Table 6.5), showing the non-sorptive nature of GW biochars. Addition of biochar to the soil enhanced the linearity of the isotherms compared with the control, which was highly non-linear (N =0.33). The variations in sorption affinity of SMO antibiotic for the Matawhero soil amended with the different types of biochar could be attributed to the differences in the combustion temperature as well as the type of feedstock used to produce these biochars. All biochars were produced from

different feedstocks, using different techniques, and under different conditions and as a result the physico-chemical properties of the biochars varied considerably (Table 6.1).

PSD biochar was produced at a temperature of 700^{o}C using a prototype fast batch pyrolysis, while the production of CC biochar was carried out using a flash carbonization technique that involves high heat treatment temperatures (> 600^{o}C). PSD biochar-amended soil gave increased uptake of SMO antibiotic when compared with soil amended with CC but GW amendment barely changed the sorption behaviour compared with unamended soil. This striking difference in performance of biochars is possibly linked to the heat treatment temperature (HTT) during production. High HTT can cause more volatile matter to be removed, exposing previously inaccessible pores and allowing greater pore accessibility and hence larger surface area (Allen-King *et al.* 2002). The effect the production temperature of char has on the sorption of other organic compounds has been reported previously; James *et al.* (2005) found that sorption of phenanthrene increased in wood chars with increasing production temperatures of the chars. Similarly Bornemann *et al*. (2007) found that charcoals prepared at higher temperatures from grass and wood had a higher uptake of aromatic hydrocarbons.

The effect of the higher HTT is presumably due to increase in surface area, certainly the PSD biochar produced at 700^{o}C had a greater BET surface area when compared with the two other biochars (Table 6.1). Although high production temperature in the carbonization process may result in the formation of cracks and fissures that would further contribute to the specific surface area for the CC biochar (Bourke *et al.* 2007), surface area, pore volume and micro-porosity do not necessarily increase with increasing temperature and this may depend on the feedstock. For instance, there could be a loss of the active constituents within the material and this could result in lower surface area, pore volume and associated micro- porosity (Bornemann *et al.* 2007).

When the heating temperature was increased from 700 to 820^{o}C, the total surface area of wood char prepared from *Betula pendula* actually reduced by nearly seven-fold, along with micro porosity and total porosity (James *et al.* 2005). The surface area of biochars generally increases with the increase in the heat treatment temperature until it reaches the temperature at which deformation occurs, and any further increase would result in reduction in surface

area (Downie *et al.* 2009). Brown *et al*. (2006b) also found that when temperature was increased from 450 to 1000^{o}C, biochars produced from pine at intermediate temperatures (600–750^{o}C) had a much greater surface area. The type of the feedstock used also determines the biochar surface area. For instance, Uchimiya *et al.* (2011b) reported that the BET surface area for cottonseed hull chars was significantly lower when compared to chars from grass and wood produced under the same conditions.

In an attempt to isolate the effect of temperature, the SSA of biochar was investigated using GW biochar obtained a three different pyrolytic temperatures (350, 450 and 550^{o}C). Other treatment conditions however remained the same (i.e. same heating rate, time, equipment used). From Table 6.1 it can be observed that with increasing HTT, the surface area, which was initially very low (3 m^2g^{-1}), remained unchanged at 450^{o}C, but upon increased temperature (550^{o}C) there was a marked increase in the SSA to 153 m^2g^{-1}. Since 550^{o}C was the highest HTT, it is difficult to conclude whether it is the optimum temperature for GW biochar. Further work in this area could examine the effect of different HTT on pine sawdust and corn cob biochars to assess if in fact these were optimized.

Another possible explanation for the variations in antibiotic sorption affinities among the soil amended with the three types of biochars can be linked to the exchangeable cations that are present in the biochars (Table 6.1). Bornemann *et al*. (2007) postulated that a combination of higher cation content of chars (derived from *Phalaris* pasture grass) and low surface area was responsible for reduced sorption capacity for compounds such as benzene and toluene. The authors concluded that when cations are present as salts and in higher amounts, it could lead to the increases in the hydrophilic nature of the char, hindering sorption of organic contaminants on to its surface. The sum of the exchangeable cations in the CC biochar was the highest (~140 cmol (+) kg^{-1}) among the three biochars, followed closely by GW and PSD biochars. Among all treatment, soil amended with PSD biochar gave the highest log K_{oc} values. This was consistent with the higher SSA and lower sum of total cations present in the PSD biochar than the CC biochar. However, despite the lowest values for the total exchangeable cations in the GW biochar, sorption affinity for the antibiotic in GW amended soil was markedly lower than PSD amended soil presumably due to the much smaller surface area and carbon content in the GW biochar. In general, high CEC would be good for retention of antibiotics like tylosin or ones that have a high percentage of cationic form. Further study

is warranted to understand the mechanisms behind the sorption process for this antibiotic, and similar non-polar compounds in soils amended with biochars.

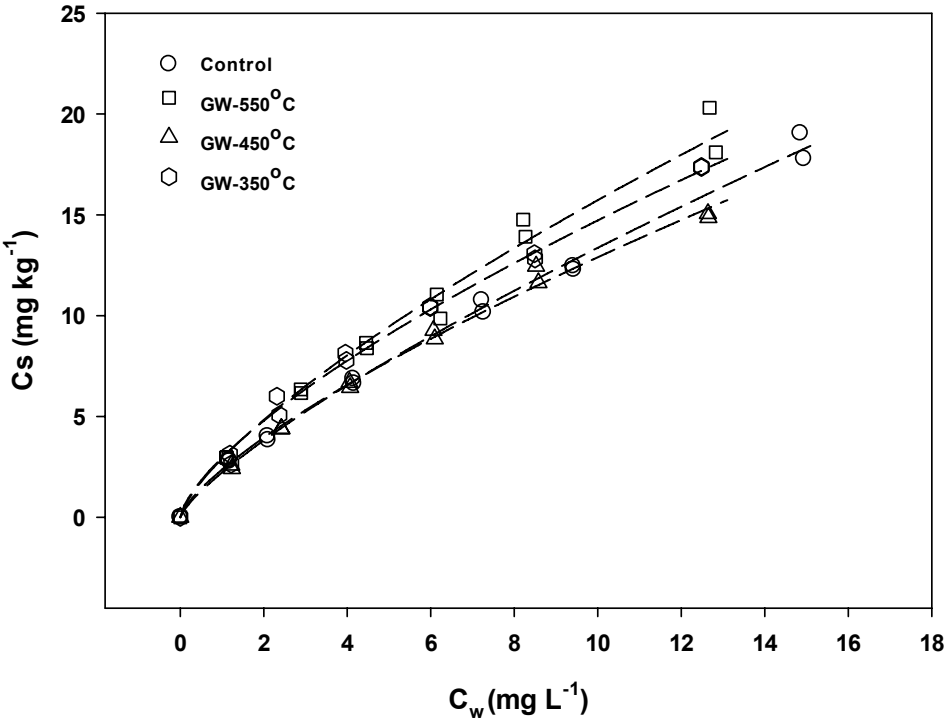


Figure 6.8: Multiple-concentration batch sorption isotherms (24 h contact times) for SMO during its equilibration with soil amended with 3 types of GW biochar at 1% soil weight.

Table 6.5: Summary of sorption parameters for SMO derived from the multiple-concentration isotherms in Matawhero soil amended with 1% GW biochars and in soil alone.

Treatment	K_d	R^2	K_f	N	R^2	K_d^{eff}	Log K^*_{oc}
GW-350°C +soil	1.31	0.94	2.70	0.75	0.98	2.44	2.98
GW-450°C +soil	1.53	0.93	2.18	0.78	1.00	1.99	1.89
GW-550°C +soil	1.63	0.95	2.74	0.76	0.99	2.48	1.99
Soil without BC	1.40	0.90	2.37	0.75	1.00	2.83	2.05

[#] K_d^{eff} is the concentration dependent effective sorption distribution coefficient ($K_d^{eff} = K_f C_w^{N-1}$) using lowest aqueous equilibrium solution concentrations of C_w = 0.5 mg L^{-1}; *concentration dependent OC-normalized sorption coefficient calculated using $K_{oc} = K_f C_w^{N-1} foc^{-1}$ at $C_w = 0.5^a$ mg L^{-1} and 5^b ng L^{-1}; K_d^{eff} in L kg^{-1} and K_f in mg $^{1-N}$ L ^{N} kg^{-1}

6.4 Summary and environmental significance

The effective distribution coefficient (K_d^{eff}) values for SMO varied from 2.48 to 53.5 L kg^{-1} for biochar amended soils. The addition of certain biochar (PSD,CC) resulted increased SMO sorption, meaning decreased mobility and transport. Increasing the antibiotic fixation to soils should also reduce toxicity and availability to soil organisms (microbes, invertebrates and plants). The variations in the pyrolytic temperature had no effect on SMO sorption characteristics. The results of this study demonstrated that biochars made from different feedstocks and under different heat treatment, conditions can possess different behaviour with regard to their sorptive ability for antibiotic. These variations may make the biochars behave differently with soil components thereby affecting its retention capacity.

Readily available feedstock like corncob, green waste and pine saw dust can be easily turned into biochar, which can be utilized as an effective sorbent for antibiotics and other contaminants associated with land-application of effluents. However, control of the production technique is necessary to ensure that the biochar has the required characteristics of large surface area, and low levels of exchangeable cations. Biochar characterisation data reveal that the presence of visible pores in the SEM images, do not always reflect the greater surface area as seen for GW biochar. PSD biochar without distinct porous formation in the SEM images gave the highest log K_{oc} values for antibiotic due to its high carbon content, SSA, and due to the presence of greater aromatic fractions in the char as supported by FT-IR data.

Conversion of biomass into biochar from feedstock that are left behind after agricultural operations seems to be a safe and viable management option as it can not only improve waste management but also protect the environment. While much emphasis is currently placed on seeking environmental solutions for nutrients leaching to lakes and groundwater of New Zealand, it is possible that veterinary antibiotics may one day become regulated compounds, and biochar could potentially be an effective material to remove antibiotic residues from soils. While PSD biochar seems to have the highest sorptive capacity among the three biochars used in this study, the ability and efficacy of PSD biochar to remove nutrients and other inorganic contaminants (heavy metals) must also be tested through laboratory trials. Furthermore, biochar selection as soil amendment for contaminant sequestration must be

made on a case-by case based on characteristics of the biochars, soil or the sorbent properties, as well as the contaminant behaviour in the environment.

Chapter 7: Degradation behaviour of sulfonamides in pastoral soils under varied laboratory conditions

7.1 Introduction

Given the large volumes of antibiotic usage in New Zealand, coupled with direct excretal inputs from grazing animals along with permitted activity such as land application of animal waste, recent years have witnessed a growing concern among the regulatory bodies about the adverse environmental impact from antibiotic residues. Potential exists for the occurrence of sulfonamide and other antibiotic residues in New Zealand's terrestrial and aquatic eco-systems. There are no published studies currently available in New Zealand regarding the occurrence and fate of sulfonamide antibiotic residues in soils, sediments, surface water, lakes or groundwater.

In the last decade several studies have reported on the biodegradation of sulfonamide antibiotics (SDZ, SM, SCP) in soils under diverse laboratory conditions (Halling-Sørensen *et al.* 2003; Kreuzig and Holtge 2005; Wang *et al.* 2006b; Accinelli *et al.* 2007; Thiele-Bruhn and Peters 2007; Yang *et al.* 2009a; Fan *et al.* 2011). Sulfonamide biodegradation has also been investigated in manure, sludge, litter and slurry samples (Ingerslev and Halling-Sørensen 2000; Kreuzig and Holtge 2005; Wang *et al.* 2006a; De Liguoro *et al.* 2007; Dolliver *et al.* 2008; Zarfl *et al.* 2009), as well as in waste water effluents (Miao *et al.* 2004; Karthikeyan and Meyer 2006; Radke *et al.* 2009) and monitored well samples (Barber *et al.* 2009).

Most of the published studies reported in the literature are difficult to compare, as no two studies were similar in terms of the antibiotics investigated, and the experimental conditions and environmental matrices used. For example, De Liguoro *et al*. (2007) studied the fate of sulfadimethoxine in manure and soils as a complete cycle from fresh faeces and bedding to cropland and drainage water. The authors reported the half-life in calf bedding to be 24 hours, while the half-life in stable manure was 64 days; sulfadimethoxine was not found in soil amended with manure. In contrast, SM did not degrade in spiked turkey litter during

composting in three separate treatments (Dolliver *et al.* 2008). The degradation half-lives for the antibiotic monensin and lasalocid in fresh non-sterile soils were found to be < 4 days (Sassman and Lee 2007). Sulfamethoxazole and trimethoprim showed higher degradation than tylosin in soils, owing to greater sorption potential for the latter in soils (Liu *et al.* 2010). Sulfonamides were found to be only partially biodegraded under test conditions in aquatic systems and despite a reduction in concentration, their toxicity could not be reduced (Samuelsen *et al.* 1994; Al-Ahmad *et al.* 1999; Kummerer *et al.* 2000). At a spiked concentration of 10 mg kg^{-1}, SDZ half-lives in aerobic non-sterile soils ranged from 12 to 18 days while it was more persistent in anoxic non-sterile soils with half-lives ranging between 57 and 237 days (Yang *et al.* 2009a). Heuer *et al*. (2008) investigated the fate of SDZ administered to pigs and observed that > 96% of the SDZ and its metabolites were recovered after 10 days in the manure, and the extractable amounts of SDZ decreased exponentially in soil, over time.

Degradation half-life is an important input parameter required in antibiotic fate modelling exercises and for risk assessment purposes. SMO, which belongs to the sulfonamide group of antibiotics, was chosen for this study, as limited information exists about its fate in soil and aquatic media. Data on degradation half-life for SMO are available in the literature (Holtge and Kreuzig 2007); however, given the varied soil and climatic conditions of New Zealand, extrapolation of degradation data obtained from overseas studies to New Zealand conditions is not desirable.

7.2 Objective

The main objective of this study was to conduct laboratory incubation experiments to investigate the degradation kinetics of SMO antibiotic in three different pastoral soils (Te Kowhai, Hamilton and Horotiu). The incubation conditions were maintained at 60% maximum water holding capacity (MWHC) and with varying initial antibiotic spiked concentration, different depth profile, temperature regimes (7.5^{o}C and 25^{o}C) and with sterilization at 60% MWHC. The principal focus was to derive the half-live values for SMO under each condition, and compare them to values that were reported in the literature. The secondary objective was to study the degradation kinetics of the three sulfonamide (SMO, SCP and SM) antibiotics in sterile and non-sterile tap water.

7.3 Experimental protocol for degradation studies

Three soils from different depths (0–10, 30–40cm) were freshly collected, sieved (2 mm) to remove plant roots and gravel, and stored at 4^{o}C until use. The soil pH was measured using a PHM62 standard pH meter, and organic carbon content was determined using an IL550 TOC-TN analyser. The microbial biomass carbon of the soils was measured by the fumigation extraction method (Jenkinson and Powlson 1976; Wu *et al.* 1990). The moisture content (MC) of soils was determined gravimetrically at 105^{o}C and the water content was adjusted to 60% of MWHC, and the soil was pre-incubated at 25^{o}C and 7.5^{o}C for 2 days before spiking with the antibiotic. Sterile soils (controls) were included with sterilization being achieved by means of autoclaving twice (121^{o}C, 103 kPa for 30 minutes)

Duplicate soil samples (5 g) were placed in 35 mL Kimax centrifuge tubes for a set time interval, and appropriate amounts of SMO stock solution (1000 mg L^{-1}) prepared in methanolic solution earlier were spiked onto the soil to obtain an initial concentration of 5 or 0.5 mg kg^{-1}. After allowing the methanol to evaporate, the contents were thoroughly mixed by vortexing before incubation at 25^{o}C and 7.5^{o}C respectively. The moisture content in each vial was maintained gravimetrically to 60% of its field capacity (-33 kPa) by adding water once every 3 days during the span of the experiment, and the tubes were also aerated every day to ensure a constant oxygen atmosphere. The entire experiment was conducted in closed incubators with temperature control, in order to avoid photodegradation. To establish the role of microorganisms in degradation of the antibiotic, the experiments were also conducted on sterile soils. De-ionised water was used during the fortification of soil samples in sterile experiment. All equipment used during sterile treatment was swabbed with methanol, and autoclaved. All handling and operations in relation to the sterile treatment were performed inside a laminar flow cabinet. Additionally, the activity of the microbial community was monitored during the incubation period by measuring the dehydrogenase activity (DHA) of the soils at random sampling times during the entire duration of the experiment.

Before soil incubation studies, preliminary studies were performed to investigate the degradation behaviour of the three sulfonamides (SMO, SCP and SM) in sterile and non-sterile tap water. For this, 50 mL of water was added into a 100 mL Schott bottle, and spiked with an appropriate amount of the three antibiotics to yield a nominal concentration of 0.5 µg

mL^{-1} of each of the three sulfonamides. In order to establish the role of photodegradation, both the bottles were exposed to direct sunlight. At regular time intervals replicate samples (0.5 mL) were pipetted into a vial for direct analysis by HPLC.

7.3.1 Extraction and analysis

Extraction of the antibiotics from soils spiked with the antibiotics involved a destructive sampling technique. At selected time intervals, duplicate samples (5 g) were extracted with DCM (10 mL). The samples were vortexed for 1 minute, followed by 15 minutes of sonication in an ultrasonic bath, and shaken for 12 h in a rotary drum shaker. The tubes were then centrifuged at 1750 g for 5 minutes and the DCM extract (~1.5 mL) was evaporated to dryness under a gentle stream of nitrogen, reconstituted in methanol (0.5 mL), and immediately analysed using HPLC-UV/Fluorescence detection. For the degradation studies in soils, the isocratic elution scheme for SMO used. This gradient scheme was used to analyse the degradation kinetics of three sulfonamides in aqueous matrix (water). The HPLC conditions and detection limits are described in detail in Chapter 3**.**

7.4 Dehydrogenase activity (DHA)

Soil microflora are responsible for the decomposition and conversion of organic substances, aggregation stability and the carbon, nitrogen, sulphur and phosphorus cycles. (Mirás Avalos *et al.* 2007). Dehydrogenase is a cellular enzyme involved in electron transport in the respiratory chain of fungi and bacteria. As dehydrogenase does not exist as an extra- cellular enzyme, any dehydrogenase activity can be taken as a measure of electron transfer activity in living cells (Friedel *et al.* 1994). As any organic residue in soils can serve as a source of carbon and energy to soil organisms, by studying the enzyme activity of the soil it should be possible to determine the potential of soils to biologically transform chemicals and the effects on soil fertility (Kumar 2011).

Determination of DHA is the most common method used to determine soil microbial activity (Ghaly and Mahamoud 2006). The method is based on measuring the DHA using a dye as an electron acceptor. Water-soluble tetrazolium salts are suitable for this purpose as they form water insoluble, coloured formazans that can be measured by spectrophotometry (Friedel *et al.* 1994). The formazan concentration is directly proportional to the community of soil non-

photosynthetic microorganisms. The formazan activation rate can be expressed as activity of soil dehydrogenase. Several authors have reported determination of microbial activity of soils, and have investigated the direct effect of chemicals on microbial communities and the subsequent effect on microbial biomass and degradation on microbial communities. For example, chemicals like heavy metals (Aoyama and Nagumo 1997; Madejón *et al.* 2001; Majer *et al.* 2002), insecticides (Cáceres *et al.* 2009), pesticides (Felsot and Dzantor 1995; Kalam *et al.* 2004; Menon *et al.* 2005; Kalyani *et al.* 2010), fossil fuels (Megharaj *et al.* 2000; Alotaibi and Schoenau 2011), and herbicides (Sebiomo *et al.* 2011) have been investigated using DHA. More recently, emerging contaminants like steroid hormones (Chun *et al.* 2005) and veterinary antibiotics (Boleas *et al.* 2005; Thiele-Bruhn and Beck 2005; Monteiro and Boxall 2009; Gutierrez *et al.* 2010) were also investigated.

7.4.1 Dehydrogenase activity experimental protocol

Each soil (5 g) was added into a 30 mL Teflon centrifuge bottle containing a solution of freshly prepared 2,3,5-triphenyterazolium chloride (TTC) in Tris (hydroxymethyl) aminomethane (TRIS) buffer (1.5%, 3 mL). Samples were vortexed (1 min), homogenized, and incubated (25°C, 24 hours). The TTC was reduced by dehydrogenase enzymes to red water-insoluble triphenylformazan (TPF). After incubation, methanol (20 mL) was added to each sample, tubes were vortexed (1 min), and shaken in a rotary drum shaker for (30 min). The tubes were then centrifuged (1750 g, 5 min) and the absorbance of the clear supernatant were measured at 481 nm and the concentration of the reduced formazan was calculated based on a set of TPF standards (Figure 7.1). Controls without antibiotic and soil were also included and were subtracted from the final DHA reading. Detailed experimental protocol can be found elsewhere (Trevors 1984; Friedel *et al.* 1994; Gong 1997; Ghaly and Mahamoud 2006). Dehydrogenase activity (TPF) ($\mu g\ g^{-1}\ h^{-1}$) was calculated using the following equation:

$$TPF = (C \times V)/odw \times h \qquad (7.1)$$

where C = TPF ($\mu g\ mL^{-1}$) from the calibration curve, V = volume of the solution added (mL), odw = oven-dry weight (g), and h = incubation time (h).

Figure 7.1: DHA samples and measurements using spectrophotometer.

7.5 Single first order exponential decay kinetics

The degradation kinetics for many organic contaminants such as pesticides (Guo *et al.* 2004; Sarmah *et al.* 2009), fumigants (Ma *et al.* 2001), steroid hormones (Scherr *et al.* 2009a) and veterinary antibiotics (Ingerslev and Halling-Sørensen 2000; De Liguoro *et al.* 2003; Dolliver *et al.* 2008; Monteiro and Boxall 2009; Yang *et al.* 2009a; Yang *et al.* 2009b; Liu *et al.* 2010) in the environment often follow simple first order (SFO) rate law. The equation (7.2) below represents the most simple form of the concentration–time relationship, where t is time (days), k_1 is the degradation rate constant (day^{-1}) and M_0 and M_t are the initial and final concentrations respectively, at time t

$$M_t = M_0 exp^{(-k_1 t)} \qquad (7.2)$$

$$\ln M_t = ln\, M_0 - k_1 t \qquad (7.3)$$

Concentration data were analysed assuming the first-order kinetics and using equation 7.3, the degradation parameters were obtained along with the R^2 values. The degradation time for an antibiotic is often computed to determine when 50% (DT_{50}), 90% (DT_{90}), and 99% (DT_{99}) of its initial concentration are lost due to degradation, equations 7.4–7.6.

$$t_{1/2} = DT_{50} = \frac{\ln\{2\}}{k_1} \qquad (7.4)$$

$$DT_{90} = \frac{\ln\{10\}}{k_1} \quad (7.5)$$

$$DT_{99} = \frac{\ln\{100\}}{k_1} \quad (7.6)$$

7.6 Results and discussion

The properties of the 3 topsoils and 3 subsoils used in this study are summarised in Table 7.1**.** The soils vary considerably in their properties such as pH (acidic to near neutral), % OC, moisture content (MC) and microbial biomass (MBC) along with the 60% maximum water holding capacity of the soil (MWHC).

Table 7.1: Selected properties of soils used in the study.

Soils	pH 1:2 H_2O	OC (%)	CEC ($cmol_c\ kg^{-1}$)	Sand %	Silt %	Clay %	SSA (m^2g^{-1})	% MC	MWHC 60%	MBC ($\mu gC\ g^{-1}$)
Horotiu silt loam TS	5.7	8.2	28.2	34	48	17	19.7	49.0	121.2	816
Horotiu silt loam SS	6.6	1.7	--	34	48	17	--	60.9	134.8	584
Te Kowhai silt loam TS	6.7	5	21.7	9	54	37	19.7	23.9	79.6	1126
Te Kowhai silt loam SS	5.7	0.5	--	12.3	62.8	24.9	--	41.4	84.5	536
Hamilton clay TS	5.8	4	17.2	13.7	51	30.4	22.3	23.7	77.6	1724
Hamilton clay SS	5.1	0.8	--	13.4	40.3	46.2	--	23.8	75.5	620

#TS = topsoil; SS = subsoil. -- = not available

7.6.1 Extraction from soil

The extraction of antibiotics from soils constitutes a challenge which is due to the possible formation of bound residues in soils leading to low extraction recovery. These bound residues could be attributed to irreversible sorption or very slow desorption (Hamscher *et al.* 2005), cation exchange interactions (Richter *et al.* 2009), and formation of covalent linkages between amine moieties and organic matter functional groups (Bialk *et al.* 2005). Also these compounds often degrade rapidly in natural soils altering their soil concentrations which could in turn affect their recovery. This latter problem can be overcome by sterilizing the soil;

however, techniques such as autoclaving or treatment with sodium azide are known to change soils physico-chemical properties, thus leading to bias in results in relation to recovery of the compounds (Scherr and Sarmah 2011). Gamma irradiation could avoid these limitations; but was not available for this study and hence was not attempted.

7.6.2 Recoveries during degradation study

The use of DCM as a solvent to extract SMO from the soils resulted in acceptable recoveries ranging from 72-88% in the three topsoils (0-10 cm) and subsoils (30-40 cm), with recovery for SMO being lowest (72%) for the Te Kowhai topsoil (Figure 7.2**)**. The extraction recoveries from manure for sulfamethoxine (another compound within the sulfonamide group) were reported as 89.5% and 91% at two different spiking concentrations (Wang *et al.* 2006a). Comparison of the extraction efficiency between the present study and studies conducted in the past are often difficult as authors have used diverse extraction techniques together with different clean up techniques (discussed in Chapter 3**)**. However, lower recovery could be attributed to the influence of autoclaving, which can change the physical properties of soils such as rearrangement and the stability of the soil aggregates, which could influence the extraction recovery (Scherr and Sarmah 2011). Additionally, during the fortification it is often difficult to ensure uniform distribution of the spiking solution containing the antibiotic onto the soil. Other factors that could have an effect on the overall extraction efficiency may be associated with the solvent to soil ratio used in the study. A multitude of factors may therefore be responsible for the lower recoveries often observed in the extraction of antibiotics or other organic chemicals in soils. The application of solid phase extraction (SPE) cartridges in the extraction technique could have possibly resulted in higher recoveries or a lower limit of detection due to cleaner chromatography. However, given the large number of samples that were to be analysed, and given the high concentrations used in the study this was not attempted. To be consistent throughout the experiment the extractable concentration on day 0, was taken as the initial concentration.

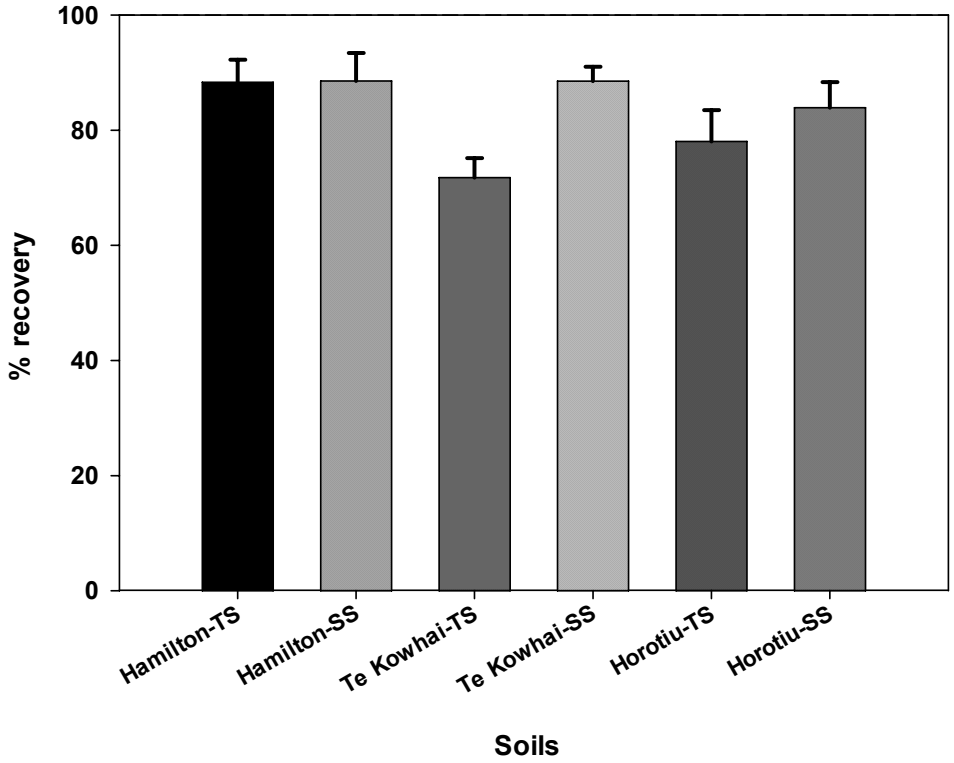


Figure 7.2: SMO recovery during the degradation experiment. TS = topsoil; SS = subsoil.

7.6.3 DHA calibration and analysis

The optimal detection wavelength for TPF (Triphenyl formazan) was determined by preparing stock solutions of TPF at 0, 5, 10, 20, 30, and 50 mg L^{-1}each in methanol. A Shimadzu UV 160A UV-Visible Recording Spectrophotometer (Shimadzu, Japan) was used to measure the absorbance of the solution in the visible range from 375 to 650 nm in a full-scan mode (Figure 7.3). The maximum absorbance for TPF occurred at 481 nm, which was in agreement with the 485 nm reported in literature (Chun *et al.* 2005; Monteiro and Boxall 2009). Standard linearity was assessed in the concentration range of 0 to 40 mg $L^{-1}$and quantification was achieved by using external standards. The correlation coefficients for the TPF standard were >0.996 during the entire study and any deviation in the correlation coefficient was a good indicator of the accuracy of standard preparation (Figure 7.4).

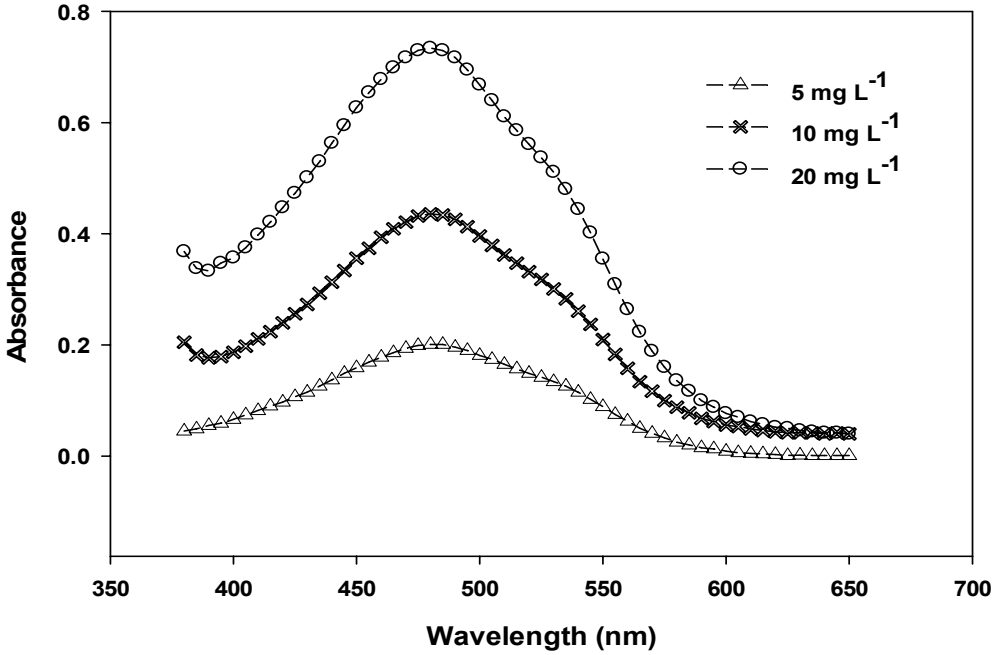


Figure 7.3: TPF absorbance *vs* wavelength.

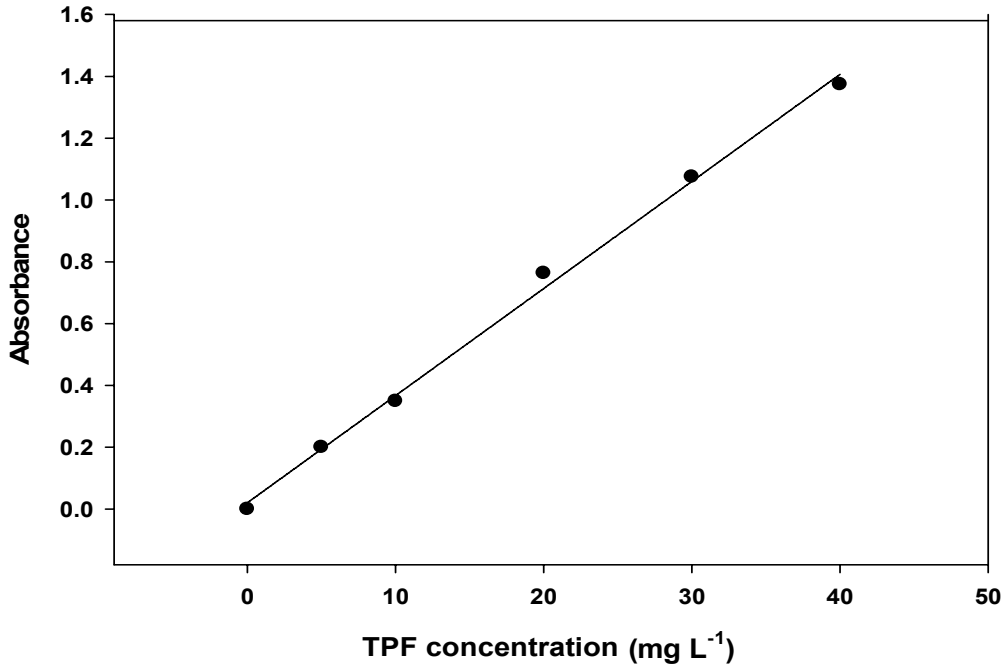


Figure 7.4: TPF calibration curve (at 451 nm in MeOH).

7.6.4 Degradation in water

Figure 7.5 shows the degradation for the three sulfonamides- SMO, SCP and SM in sterile and non-sterile tap water over 60 days. This experiment was carried out as a precursor to the main objective of this chapter. The rate at which these substances degrade in water would help in understanding how they behave in soils. The degradation constants were derived assuming the kinetics of degradation to follow a first order rate-law, however, the degradation pattern showed some deviation from the first order as evident from the coefficient of determinant values (R^2) which ranged from 0.71 to 0.94.

Figure 7.5 shows that the rate of degradation of sulfonamides in water is generally slow and rate of degradation varied considerably among the three sulfonamides. The corresponding values for the rate constants (k_1) and associated DT_{50}, $DT_{90,}$ and DT_{99}values for SMO, SCP and SM are summarised in Table 7.2. In non-sterile conditions, SCP degradation was faster than either SMO or SM, which had similar behaviour. Sterilisation of water had little effect on the overall degradation rate for SCP, and DT_{50} increased only by about 1 week compared to the non-sterile treatment. The effect was even less significant for both SMO and SM between the two treatments. For instance, rate constants for SMO and SM were identical irrespective of whether a treatment was sterile or non-sterile, and this was reflected in the degradation times calculated for each compound. Thus, it can be inferred that abiotic hydrolysis seemed to have dominated the degradation of these groups of compounds in water. This is discussed further below.

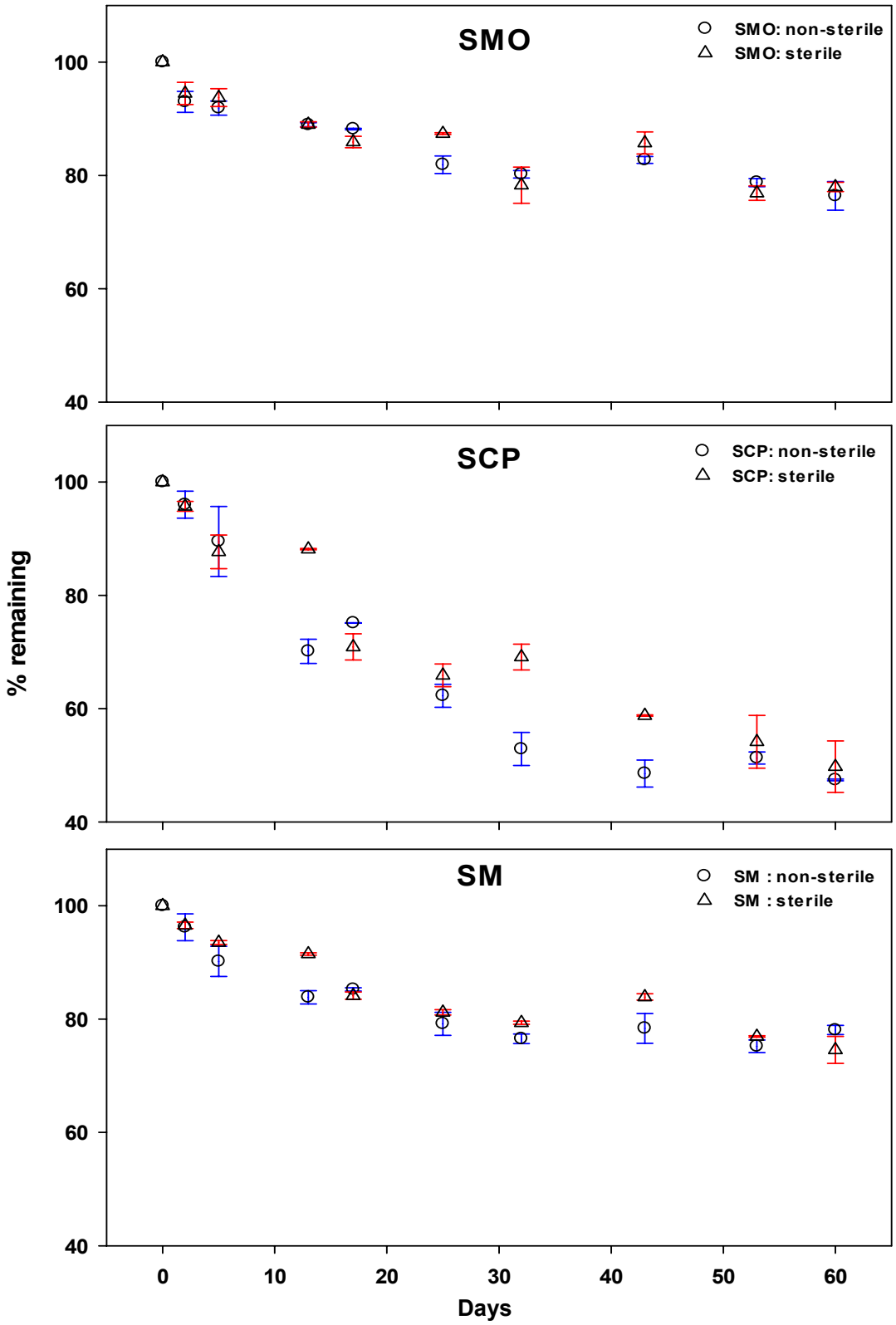


Figure 7.5: Plots of degradation kinetics for three sulfonamides in non–sterile (circle, blue) and sterile (triangle, red) water at initial concentration of 0.5 mg L^{-1}. Error bars show deviation of the duplicate samples.

Table 7.2: First-order rate constant and degradation endpoints for three sulfonamides.

	SMO		SCP		SM	
	Non-sterile	**sterile**	**Non-sterile**	**sterile**	**Non-sterile**	**sterile**
DT_{50} (days)	199.2	201.5	54.6	62.5	182.4	203.9
DT_{90}(days)	661.7	669.4	181.3	207.5	605.9	677.2
DT_{99}(days)	1323.3	1338.7	362.6	414.9	1211.9	1354.5
k_1(days^{-1})	0.0035	0.0034	0.0127	0.0111	0.0038	0.0034
M_0(mg kg^{-1})	0.52	0.51	0.53	0.44	0.61	0.54
R^2	0.88	0.82	0.90	0.94	0.83	0.71

An examination of the data in Table 7.2 indicates that there were no significant changes in the degradation behaviour of the three antibiotics under sterile and non-sterile conditions. This may show that tap water is reasonably sterile with very little or no microbial activity. SCP may be more prone to biotic degradation than SMO, and SM. Loftin *et al.* (2008) suggested that degradation rates of antibiotic in aqueous media may vary significantly across antibiotic types, and are mostly governed by their molecular structure, size and shape, which could make them more or less susceptible to microbial action. In contrast, the results from SMO and SM degradation kinetics clearly indicate that abiotic degradation is principally responsible for the degradation of the compounds. With more than 75% of the initial application remaining at the end of day 70, SMO and SM are more likely to persist in aquatic ecosystems than SCP. Abiotic degradation of antibiotics in water can also occur in processes such as photodegradation and/or hydrolysis (Sarmah *et al.* 2006a; Thiele-Bruhn and Peters 2007). For example, Yang *et al.* (2009a) reported a greater rate of hydrolysis of SDZ in aqueous solution under acidic conditions than neutral or slightly basic conditions suggesting that hydrolysis could play a limited role in the degradation of SDZ.

Many studies report abiotic degradation of sulfonamide antibiotics in aqueous media, and all of these show great variation in the degradation rate (Ingerslev *et al.* 2001; Loftin *et al.* 2005; Barber *et al.* 2009; Radke *et al.* 2009). Radke *et al.* (2009) reported DT_{50} values for SMO in aqueous phase between 8.5 and 17.2 days, whereas other studies have shown that sulfamethoxazole is not readily biodegradable (Al-Ahmad *et al.* 1999) or subject to oxidative photodecomposition in effluent from wastewater treatment plants (Abellán *et al.* 2007; Gonzalez *et al.* 2007; Dirany *et al.* 2011). Abellan *et al*. (2007) studied photo-catalytic

degradation and mineralization of sulfamethoxazole with TiO_2 and UV light and observed 82% degradation of sulfamethoxazole and 23% mineralization in aqueous suspension.
The results obtained in the present study were similar to the findings reported by Thiele-Bruhn and Peters (2007), who found significant photodegradation of various sulfonamides and tetracyclines in sterile water. The authors concluded that the photo-degradability of antibiotic could play an important role in its fate in an aquatic environment. The information available on the biodegradation of antibiotics used for livestock purposes in both surface waters and wastewater effluents is limited. Only a few articles highlight the fate of veterinary antibiotics in aquatic environments (Kemper 2008; Kummerer 2009). A review by Boxall *et al*. (2004) on laboratory biodegradation studies of some commonly used antibiotics (sulfadiazine, sulfamethoxine and oxytetracycline) in livestock animals and fish farms showed significant variation in the reported half-lives, and were often difficult to compare one with another for a single antibiotic due to differences in experimental protocols and laboratory conditions.

In general, no great differences were observed between measured half-lives of sulfonamides in sterile and non-sterile deionised water exposed to light, suggesting limited microbial degradation, and implying that photodegradation is a major contributor in the dissipation of sulfonamide antibiotic in aquatic media. Lam *et al*. (2004) and Lam and Mabury (2005) reported photodegradation and not hydrolysis or biodegradation to be the main cause for degradation of mixtures of antibiotics, which included sulfamethoxazole and trimethoprim in natural waters.

7.6.5 Degradation in soils

In this section the results of degradation kinetics for SMO antibiotic in three different top and subsoils at 60% MWHC (-10 kPa) under varying initial concentration (0.5 and 5 µg kg^{-1}), depth (0–10 cm and 30–40 cm), temperature (25^{o}C and 7.5^{o}C) and under sterile and non-sterile conditions are presented (Figure 7.7 to Figure 7.10). The degradation pattern for SMO under all conditions was indicative of simple first order kinetics and DT_{50}, DT_{90}, and DT_{99} values were estimated from the data in Table 7.3. The degradation parameters and associated endpoints were obtained from the log-transformed plots for SMO antibiotic in non-sterile and sterile soils (Figure 7.6**).** The coefficients of determination (R^2) ranged from 0.80 and 1.00

with the exception of Hamilton clay topsoil where degradation deviated from the first-order kinetic as evident with low R^2 value (0.61) under sterile treatment (Table 7.3). The use of other models to fit degradation data and to calculate the DT_{50} values together with their ability to describe the kinetics of degradation of SMO are dealt with in details in Chapter 8.

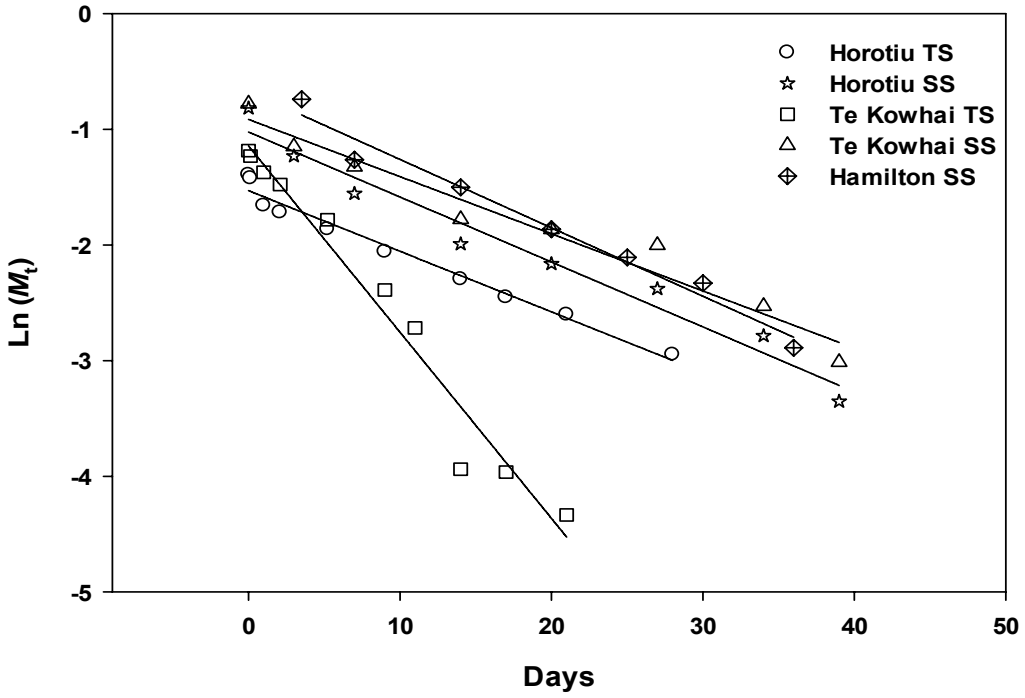


Figure 7.6: Example of log transformed plots for SMO antibiotic in non-sterile soils (25^{o}C & 0.5 mg kg^{-1}) with $R^2 > 0.95$.

7.6.6 Effect of initial concentration

The effect of initial concentration on the degradation behaviour of SMO was studied only in one soil (Hamilton). However, both top and subsoils were included for this experiment, and concentration dependency of SMO degradation was tested under non-sterile and sterile treatments, at 25^{o}C using two initial concentrations of 0.5 mg kg^{-1} and 5 mg kg^{-1}. Table 7.3 provides a summary of the datasets obtained together with the coefficient of determinants (R^2) fits for the single first order kinetics. Even though there was a 15–20% increase in k_1 at lower loadings, because of the disparity arising with the two R^2 value it is difficult to conclude whether or not the degradation was concentration dependent. It was not worthwhile to repeat the experiments for Te Kowhai and Horotiu soils, and the investigation focused on sterile vs. non-sterile soils and temperature effects. According to rate law theory, the first order rate for

the degradation of an organic contaminant in soil or other media should be independent of the initial concentration used (Wang *et al.* 2006a)

Table 7.3: First-order rate constants and degradation endpoints for SMO in three different soils under varying conditions.

		Temperature and initial loading							
		25°C 5 mg kg^{-1}		**25°C 0.5 mg kg^{-1}**		**7.5°C 0.5 mg kg^{-1}**		**Sterile; 25°C 0.5 mg kg^{-1}**	
Soils		TS	SS	TS	SS	TS	SS	TS	SS
Hamilton	**DT_{50}**	11.36	12.38	9.24	11.75	25.39	29.88	11.00	34.66
	DT_{90}	37.75	41.12	30.70	39.03	84.34	99.25	36.55	115.13
	DT_{99}	75.49	82.24	61.40	78.05	168.69	198.50	73.10	230.26
	k_1	0.06	0.06	0.08	0.06	0.03	0.02	0.06	0.02
	M_0	3.10	4.62	0.28	0.39	0.36	0.42	0.40	0.35
	R^2	0.99	0.94	0.84	0.97	0.97	0.91	0.93	0.61
Te Kowhai	**DT_{50}**	NA		4.31	14.15	20.69	20.95	13.00	22.43
	DT_{90}			14.30	46.99	68.73	69.56	43.20	74.52
	DT_{99}			28.60	93.98	137.47	139.13	86.40	149.03
	k_1			0.16	0.05	0.04	0.03	0.05	0.03
	M_0			0.31	0.40	0.31	0.40	0.32	0.40
	R^2			0.97	0.96	0.94	1.00	0.93	0.90
Horotiu	**DT_{50}**	NA		13.33	12.38	23.18	19.69	18.10	22.65
	DT_{90}			44.28	41.12	77.01	65.41	60.12	75.25
	DT_{99}			88.56	82.24	154.02	130.83	120.24	150.50
	k_1			0.05	0.06	0.03	0.04	0.04	0.03
	M_0			0.22	0.36	0.26	0.35	0.37	0.38
	R^2			0.98	0.97	0.91	0.96	0.89	0.88

k_1 is the first order rate constant; M_0 is the initial observed concentration; DT_{50}, DT_{90}, and DT_{99} are the degradation endpoints for 50, 90 and 99% antibiotic degradation; R^2 is the measure of the goodness of fit of the model. TS = topsoil; SS = subsoil. NA= not applicable.

However, the results in the present study indicate that the situation in soil can be more complex given the interaction of multitude of factors such as organic carbon content, clay content, and microbial community composition including the presence of specific degraders, prevailing temperature and water content which could determine the overall degradation

pattern. In general, the SMO degradation rate was found to be rapid irrespective of the soil depth at both initial concentrations, during the initial incubation time.

The DT_{50} values for top and subsoils at 5 mg kg^{-1}were 11.4 and 12.4 days respectively. When the initial spiking concentration was reduced to 0.5 mg kg^{-1} the DT_{50}values reduced to 9.2 and 11.8 days respectively and the degradation constant increased from 0.061 $day^{-1}$to 0.075 day^{-1} for topsoil and 0.056 day^{-1} to 0.059 $day^{-1}$for subsoil. In general for the two treatments, only a subtle difference was evident for the rate constant. Thiele-Bruhn and Beck (2005) showed that the extractability of antibiotic sulfapyridine depended on its initial concentration and that it was most effective at smaller spiking levels. This could be attributed to nonlinear adsorption of the antibiotic to the soils as seen in Chapter 4, and from other studies (Thiele-Bruhn 2003; Thiele-Bruhn and Aust 2004). Similar findings were reported by Wang *et al*.(2006a), who observed that the degradation rate constant decreased with increasing sulfadimethoxine concentration in manure, suggesting that the activity of degrading microorganisms was inhibited at high concentrations. In a separate study Wang *et al*. (2006b) reported decreasing bioactivity of the microorganisms with increases in the initial sulfamethoxine concentrations in manure thereby leading to lessened antibiotic degradation.

A similar trend in the degradation kinetics of 2 fumigants was reported where the observed degradation rate constant decreased with increasing initial concentration (Ma *et al*. 2001). Elsewhere, Yang *et al*.(2009a) varied the initial concentration for antibiotic SDZ from 1, 10 and 25 mg kg^{-1} and found half-life values of 2, 18 and 34 days respectively. The same authors in a different study reported that degradation rates of oxytetracycline decreased with increases in initial concentration, emphasizing the role of initial antibiotic concentration on its overall degradation pattern. The DT_{50}values obtained in this present study were similar to that of Yang *et al*.(2009a) (Table 2.9).

Literature data suggest that SMO degradation kinetics is dependent on the initial concentration and the present study confirms this. In contrast Accinelli *et al*. (2007) observed that the persistence of SCP and SM, (two different sulfonamides) was not affected by the initial antibiotic concentrations. In this study the microbial activity of the soil at both the initial concentrations was considered to be same as indicated by the DHA activity of the

topsoil which was more or less the same at 5 μg g^{-1} h^{-1} TPF at either concentration, while the activity for subsoils was generally low at about 0.5 μg g^{-1} h^{-1} TPF with the exception of few outliers (Figure 7.7). Nevertheless degradation was slightly slower at the higher concentration.

A plausible explanation for this could be that, since the higher initial concentration of antibiotic often involves spiking larger volumes of methanolic stock solution, the stock itself could indirectly inhibit microbial activity or even the high dosage of the antibiotic itself could be lethal to soil microbes (Ma *et al*. 2001), thus lowering the microbial activity of the soil in both cases. It could be plausible that MeOH added here could have acted as a labile substrate. However, this situation is not likely to occur under realistic field conditions, and highlights the shortcomings of laboratory-based fate studies. Also the availability of SMO in the soil for degradation could be a factor affecting its degradation kinetics. Increasing the initial concentration would lead to enhanced sorption and possibly more residues permanently bind to soil thus reducing availability. So the non-availability of the antibiotic along with the toxic effect on the soil microbes may lead to reduced degradation rates at higher initial concentration.

Sulfapyridine and oxytetracycline had no effect on DHA, even at concentrations up to 1000 mg kg^{-1}, which is much higher than typical environmental values (Thiele-Bruhn and Beck 2005). The authors attributed this to the lack of sensitivity of the method or to more specific effects of the compounds on single microbial species that were possibly compensated by the reminder of the microbial community. In the present study, $DT_{50,}$ DT_{90} and even DT_{99} values could be estimated within the length of sampling time of the experiment. Even at a very high concentration of 5 mg kg^{-1} the DT_{99} values for Hamilton clay topsoil and subsoil were 75.5 and 82.2 days. High concentrations of antibiotic are unlikely to occur in field conditions and the findings of the present study would suggest that when present at low concentration, SMO could be easily biodegraded though trace levels of these compounds may persist for a long time especially when there is constant overloading of these contaminants through effluent spreading.

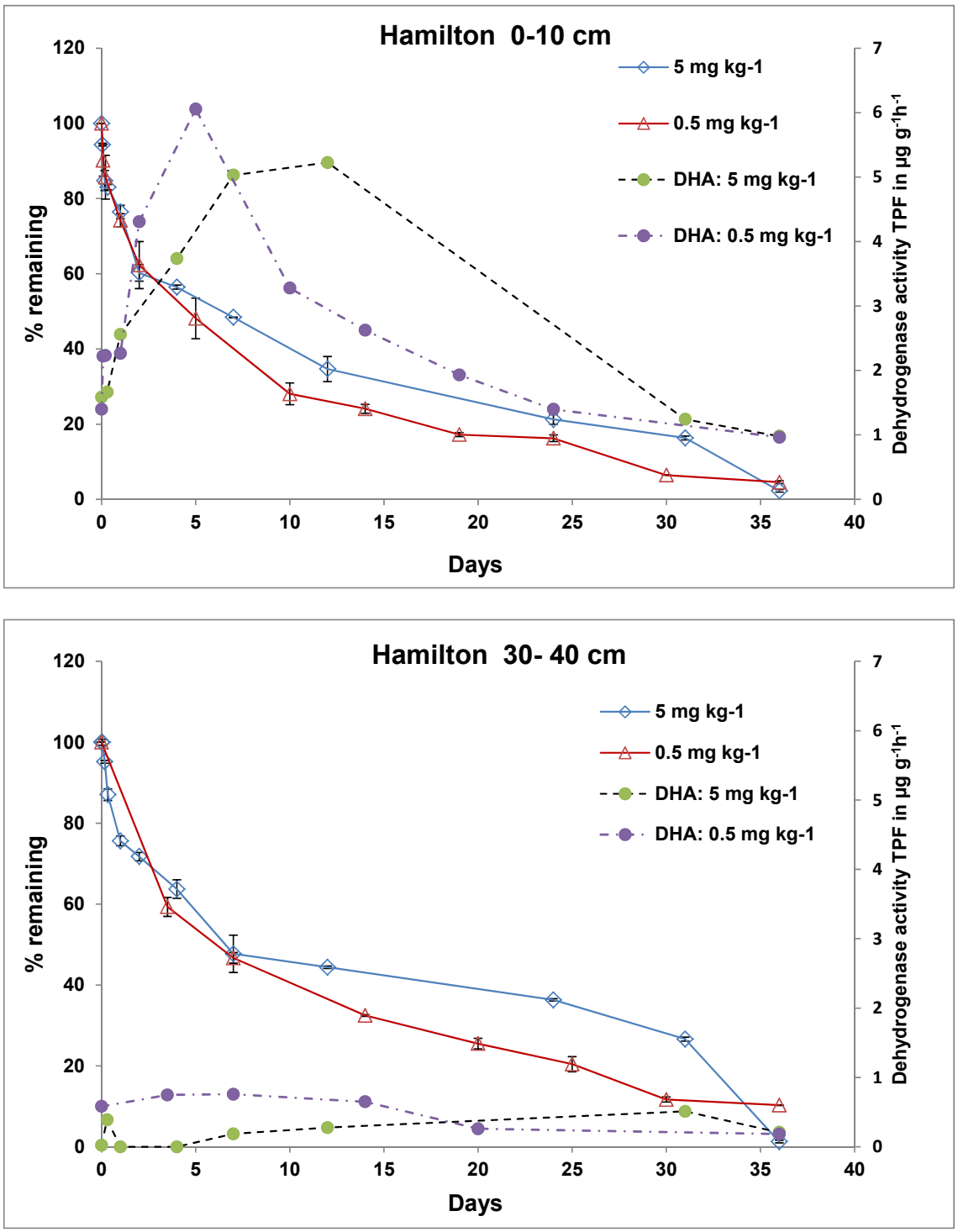


Figure 7.7: Degradation kinetics of SMO as a function of time in Hamilton clay soil (topsoil and subsoil) at initial spiked concentrations of 0.5 mg kg^{-1}(circle, red) and 5 mg kg^{-1} (triangle, blue). Error bars shows deviation of the duplicate samples.

7.6.7 Effect of soil depth

Figure 7.8 shows the general trend in the degradation rate for SMO in the three soils under both soil depths and the associated DHA at each sampling event. A noticeable trend common to all three soils is the rapid disappearance of SMO in all soils irrespective of the soil depth during first few sampling events. Hamilton and Horotiu soils exhibited similar degradation patterns during the incubation period, with ~10% of the initial applied amount still remaining after the last sampling day. SMO degraded at a faster rate in Te Kowhai topsoil with >95% of the applied amount degraded within the half of the incubation period (Figure 7.8).

Soil properties such as OC, clay content, and MBC are known to influence degradation kinetics of organic contaminants (Sarmah *et al.* 2009; Scherr *et al.* 2009a). Sarmah *et al.* (2009) atributed the degradation of pesticide Atrazine in one particular New Zealand soil to the high level of organic carbon (~8%) (Table 7.1). However, no single soil property was found to influence the results obtained in this study. Horotiu soil being allophanic and having a similar level of organic carbon (8.2%) to the previous study had the slowest degradation of SMO antibiotic. There was also no correlation between the rate of degradation and the soils microbial biomass (MBC). For instance, the Hamilton clay loam with the highest MBC value had the smallest degradation endpoints, while the Horotiu soil with the lowest MBC gave largest degradation endpoints. This was in contrast to the findings of Scherr *et al*. (2008) where the authors reported good correlation between MBC and the degradation rates for steroid hormones. In general, the degradation of the SMO antibiotic did not follow a definite ranking order for the topsoils.

For the subsoils, the initial rate of SMO degradation was initially slow compared with the topsoil, especially in Te Kowhai soil; however, only 10% of the SMO remained on the last sampling day. Subsoil properties (Table 7.1**)** of the soils indicate low clay content, OC, and MBC, which are consistent with the low SMO degradation rates except Horotiu soil. The DHA for subsoils in all three soil types was generally low at about 0.5 $\mu g\ g^{-1}\ h^{-1}$TPF. In general, a higher microbial activity in soil suggests a higher rate of degradation of organic chemicals such as pesticides (Kah *et al.* 2007); steroid hormone such as 17β estradiol (Chun *et al.* 2005; Chun *et al.* 2006). In the present study good correlation was obtained in the degradation rate of SMO and the soil bioactivity in topsoils, in contrast to some past studies (Thiele-Bruhn and Beck 2005; Monteiro and Boxall 2009), where no correlation was

observed between the degradation rate and the soil bioactivity (DHA). A combination of reduced microbial activity, decreased organic carbon content, and low microbial biomass in Te Kowhai subsoil are some of the factors in the slower degradation for SMO in Te Kowhai subsoil (Figure 7.8). However, the unusually high rate of degradation in the subsoils for other two soils was found to be similar to the pesticide atrazine study in New Zealand soils (Sarmah *et al.* 2009). The authors reported $t_{1/2}$ values for subsoil to be 121 days, which was ~ 6 weeks less than the topsoil half-life of 165 days. In contrast, another study by Sonia Rodríguez-Cruz *et al*. (2006) reported pesticides showing a decrease in their biodegradation rates with increasing soil depth; this was mainly attributed to reduced microbial activity in deeper soil layers.

Rapid degradation for SMO in Horotiu and Hamilton soils irrespective of the soil depth could be attributed to low sorption affinity of SMO in these soils with sorption coefficients ranging from 0.52 to 2.04 L kg^{-1}(Chapter 4). A recent study by Yang *et al.*(2012) showed rapid biodegradation of three sulfonamides including SMO in activated sludge, while 33–70% degradation of SM happened within 6 hours in a soil column experiment (Fan *et al.* 2011).

The rates of biotic degradation followed the order: Te Kowhai > Hamilton > Horotiu for topsoils, while for subsoils the order was reversed: Horotiu > Hamilton > Te Kowhai. Although a justifiable explanation regarding the apparent variation in the degradation rates could not be offered, inherent soil properties such as composition of various clay minerals and the associated high surface areas (Table 7.1) for the soils, and the type and the nature of organic matter present may be responsible for this variation. The rate constants for the topsoils did not correlate with the MBC of the soils (Table 7.1). Even though Hamilton clay soil had the highest MBC value among the three soils, SMO degradation was slower compared with Te Kowhai soil. High sorption to clay particles could have reduced SMO bioavailability thereby reducing the degradation rate. The DT_{50}values estimated from the log transformation of simple first order kinetics ranged from 4.31 to 3.33 days for the three soils, however; there was little difference between the DT_{50} values in the subsoils (Table 7.3). The DT_{50} and DT_{90} values for SMO obtained in this study were higher in topsoil and subsoil when compared with the values of 1 day and 18 days in an earlier study (Holtge and Kreuzig 2007). Since limited data are available on SMO degradation kinetics in soils, the results obtained in the present study can only be compared with other sulfonamides within the same group. For

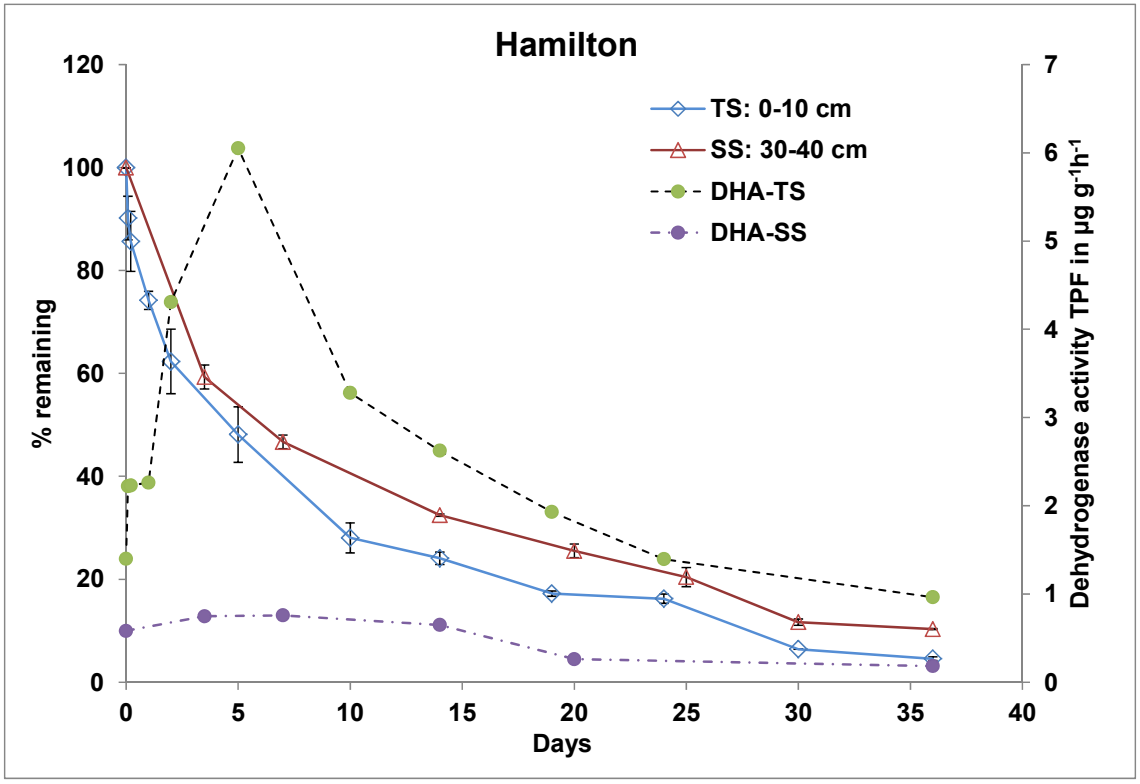
instance, Ingerslev and Halling-Sorensen (2000) found that the biodegradation rate was identical for several sulfonamides in the sludge, which means these compounds may be assessed as a group by studying only a few compounds in applications such as environmental fate assessments. Accinelli *et al*. (2007) investigated the degradation kinetics for two sulfonamides and found half-lives of around 20 days for SM and SCP in silt loam and sandy soils. This was in contrast to the findings of Blackwell *et al*. (2007) who reported a faster degradation rate for SCP with a half-life of only 4 days in a sandy loam soil. Recently the half-life for SDZ in aerobic non-sterile soils was found to range from 12 to18 days (Yang *et al.* 2009a).

Studies conducted in the past have shown that the degradation of antibiotics is a function of a multitude of factors and strongly influenced by soil temperature, moisture content, organic carbon, pH, as well as the microbial activity (Monteiro and Boxall 2009). These properties can significantly vary depending on the study location and prevailing edaphic factors. A plausible explanation for a slower degradation rate for Horotiu topsoil could be linked to the observed rate constants, which were dependent on soil sorptive properties (Thiele-Bruhn and Peters 2007). Horotiu soil is volcanically derived and contains allophane (an alumino-silicate clay mineral), and has the highest % OC of the three soils studied. According to Wang *et al*. (2006a), for soils and manure rich in organic matter a greater proportion of the compound may likely be adsorbed to the soil, thus greatly reducing its bioavailability. Thiele-Bruhn (2003) also reported that sorption and fixation were known to reduce the degradation rate of the antibiotic in the soils, as sorbed compounds remain unavailable for microbes. There was no significant difference in the degradation rate for SMO at either depth at 25^{o}C in Horotiu soil. The soil microbial activity in the Horotiu subsoil was 10-fold lower than the topsoil and in general, reduced levels of organic carbon and low microbial biomass are common characteristics of subsoils (Sarmah *et al.* 2009). Thus, it is conceivable that increased relative degradation rates for Horotiu subsoil compared with the other two subsoils could be associated with a decreased sorption and higher bioavailability for biodegradation. Another possible explanation for the faster degradation in the subsoil despite a lower biological activity as shown by reduced DHA is that degradation might be due to the existence of microbial species, which are more specific in degrading the target compound in the subsoil (Di *et al.* 1998). During field degradation study involving a number of pesticides, increased degradation rates in subsoil samples (25–50 cm) were observed for at least 4 pesticides that

had high sorption coefficients in these soils. The authors pointed out that decreased sorption in the subsoils might have compensated for the lower microbial biomass, leading to a faster degradation.

In the present study, similar degradation rates for top and subsoils could indicate the possibility of abiotic degradation processes such as fixation, photodegradation and/or hydrolysis. Under neutral and slightly basic conditions, hydrolysis was found to play only a limited role in the degradation of SDZ in soils (Yang *et al.* 2009a). In soil and slurry mixtures, sulfonamides are known to be susceptible to photodegradation (Halling-Sørensen *et al.* 2003). However, in a similar matrix, no significant abiotic degradation of sulfapyridine was observed (Thiele-Bruhn and Aust 2004).When present in an aqueous media the photodegradation of antibiotic could play an important role in its environmental fate (Thiele-Bruhn and Peters 2007), however, light penetration in water declines with increasing water depth and turbidity (Samuelsen *et al.* 1994). Furthermore, processes such as fixation and penetration into voids of the soil solids may protect antibiotics from photodecomposition. From these past studies, it can be concluded that hydrolysis and photodegradation had little or no significant effect on the concentration of antibiotics in soils, especially subsoils. One has to be cautious when extrapolating genuine laboratory photo-degradation results obtained for subs oils to expected behaviour in the field it is very likely that a significant part of the degradation seen in the soil experiments is caused by re-equilibration and fixation to stronger sites during the time course of the degradation study. This gradual chemical change could put the still-existing antibiotic residues beyond the reach of the extraction solvent. Similar effect were seen with DDT residues in soils (where a portion of which were still recoverable using base saponification extraction method, but over longer time scale (Gaw *et al*. 2003).

$DT_{50,}$ DT_{90} and even DT_{99} for all the three topsoils occurred within the length of sampling time (40 days). For subsoils, $DT_{90,}$ and DT_{99} values occurred outside the length of sampling time of the experiment (40 days). In general, DT_{50} values for SMO in subsoil increased when compared with the topsoils, which is consistent with the earlier assumption that reduced organic carbon and lower microbial activity at increased depths hinders degradation process. SMO antibiotic did not persist more than 90 days in all three soils, irrespective of the soil depth indicating that natural bio-degradation is sufficient for the removal of these contaminants from the soil.


Hamilton
TS: 0-10 cm
SS: 30-40 cm
DHA-TS
DHA-SS
% remaining
Dehydrogenase activity TPF in µg g-1h-1
Days
0
20
40
60
80
100
120
0
1
2
3
4
5
6
7
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5
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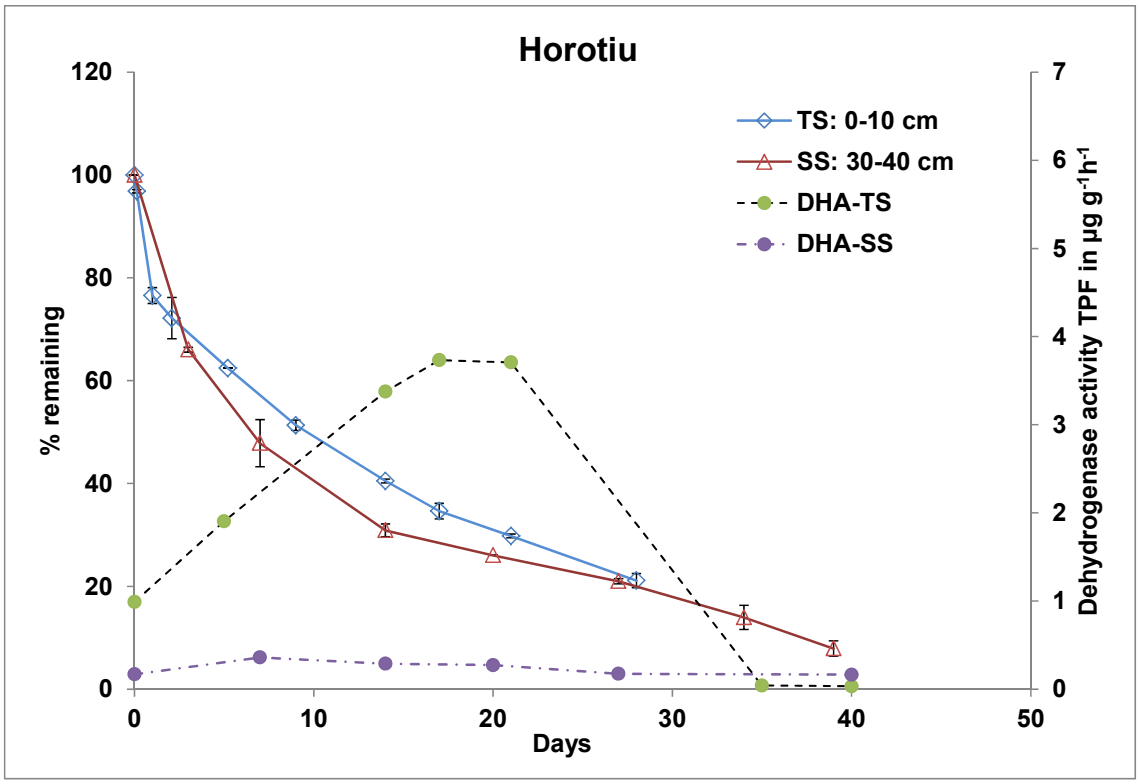
Horotiu
TS: 0-10 cm
SS: 30-40 cm
DHA-TS
DHA-SS
% remaining
Dehydrogenase activity TPF in µg g-1h-1
Days
0
20
40
60
80
100
120
0
1
2
3
4
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6
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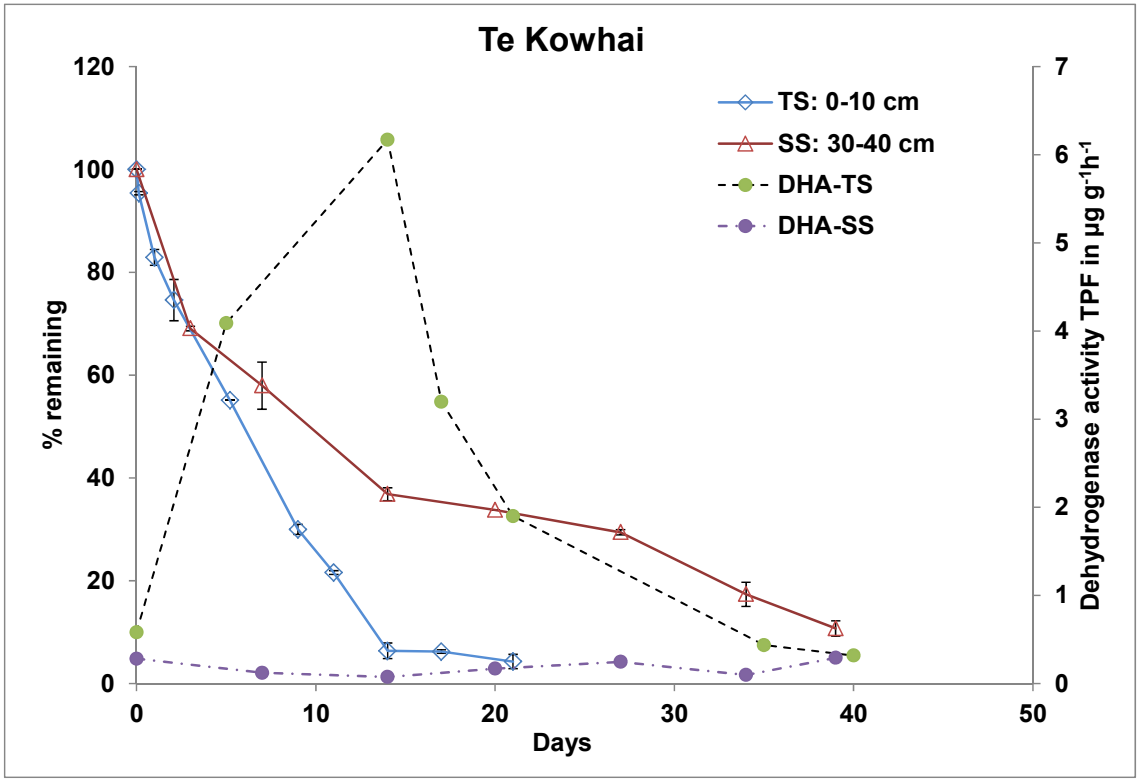


Figure 7.8: Degradation kinetics of SMO as a function of time in Hamilton, Te Kowhai and Horotiu topsoils (blue diamond) and subsoils (red triangle), at initial spiked concentration of 0.5 mg kg^{-1}. Error bars shows deviation of the duplicate samples. The plots also show the DHA activity for topsoils (green dot) and subsoils (purple dots) in the secondary axis.

7.6.8 Effect of incubation temperature

Results of degradation of SMO as affected by the incubation temperature in the soils allowed a relative assessment of the effect of two temperatures in both top and subsoils (Figure 7.9). Summarised datasets show that the degradation rate constants (k_1 day^{-1}) for SMO were higher in all soils incubated at 25°C than at 7.5°C (Table 7.3)**.** For example, in Te Kowhai topsoil at 7.5°C, DT_{99} value for SMO was nearly 5-fold greater than at 25°C. Overall, incubation at reduced temperature resulted in reduced degradation rate for SMO irrespective of the soil depth. Such a result is consistent either with microbial or chemical degradation.

More than 80–90% of the applied antibiotic degraded in the topsoils and subsoils incubated at 25°C in the 20 and 40 days sampling time respectively. However, at 7.5°C it required nearly the whole duration of the experiment (40 days) to reach > 70% degradation in topsoils and

subsoils. A similar reduced degradation rate was observed for other organic chemicals such as pesticide tetbuthylazine (Sarmah *et al.* 2009), and naturally excreted steroid conjugate 17β-estradiol -3- sulphate (Scherr *et al.* 2009a) in New Zealand soils at an incubation temperature of 7.5^{o}C irrespective of the soil depth. Other studies on the temperature effects on antibiotics degradation were mainly focussed on manure and sledges. For example at 6^{o}C, degradation rates of four sulfonamides in activated sludge were found to be three to four times slower than at 20^{o}C (Ingerslev and Halling-Sørensen 2000). Wang *et al.*(2006) reported an increase in the degradation rate constant of the antibiotic sulfadimethoxine from 0.332 to 0.777 day^{-1}in manure when the temperature was increased from 25^{o}C to 40^{o}C. Enhanced oxytetracycline degradation with the rate constant (k) increasing from 0.079 to 0.102 day^{-1} was seen with temperature increment of 15^{o}C to 25^{o}C (Wang and Yates 2008). On further increasing the temperature to 35^{o}C and 45^{o}C, oxytetracycline (OTC) degradation was faster; however, the degradation kinetics deviated significantly from first order kinetics, with the rate of degradation for OTC increased and became linear (zero order) after first two days. Similarly, temperature effects on the degradation rates of three sulfonamide antibiotics were deduced from the Arrhenius equation, and were shown to increase with the increases in temperature (Loftin *et al.* 2008).

In the present study, when incubation temperature was decreased, DT_{50} values increased about two fold for all soils irrespective of the soil depth. The soil microbial activity as measured by the DHA activity indicated little or no activity for soils incubated at 7.5^{o}C when compared to soils incubated at 25^{o}C, thus emphasizing the role of microorganisms in the degradation of SMO antibiotic and the role of temperature in moderating this. During the degradation experiment, the bioavailability of microorganisms was assumed to be constant, meaning that the overall degradation should follow first order kinetics (Wang *et al.* 2006b). However, when the bioactivity is significantly altered by reducing the incubation temperature, the rate constant, which is temperature-dependent, also decreases. It is also possible that with the increase in the temperature, the sorptive capacity of the organic contaminants decreases, thereby increasing the availability of SMO in soils (Fruhstorfer *et al.* 1993). In context to NZ the consequences of cooler soil with typical subsoil temperature of 7.5^{o}C would mean that biodegradation would be slow and leaching may become prominent for veterinary antibiotics like SMO.

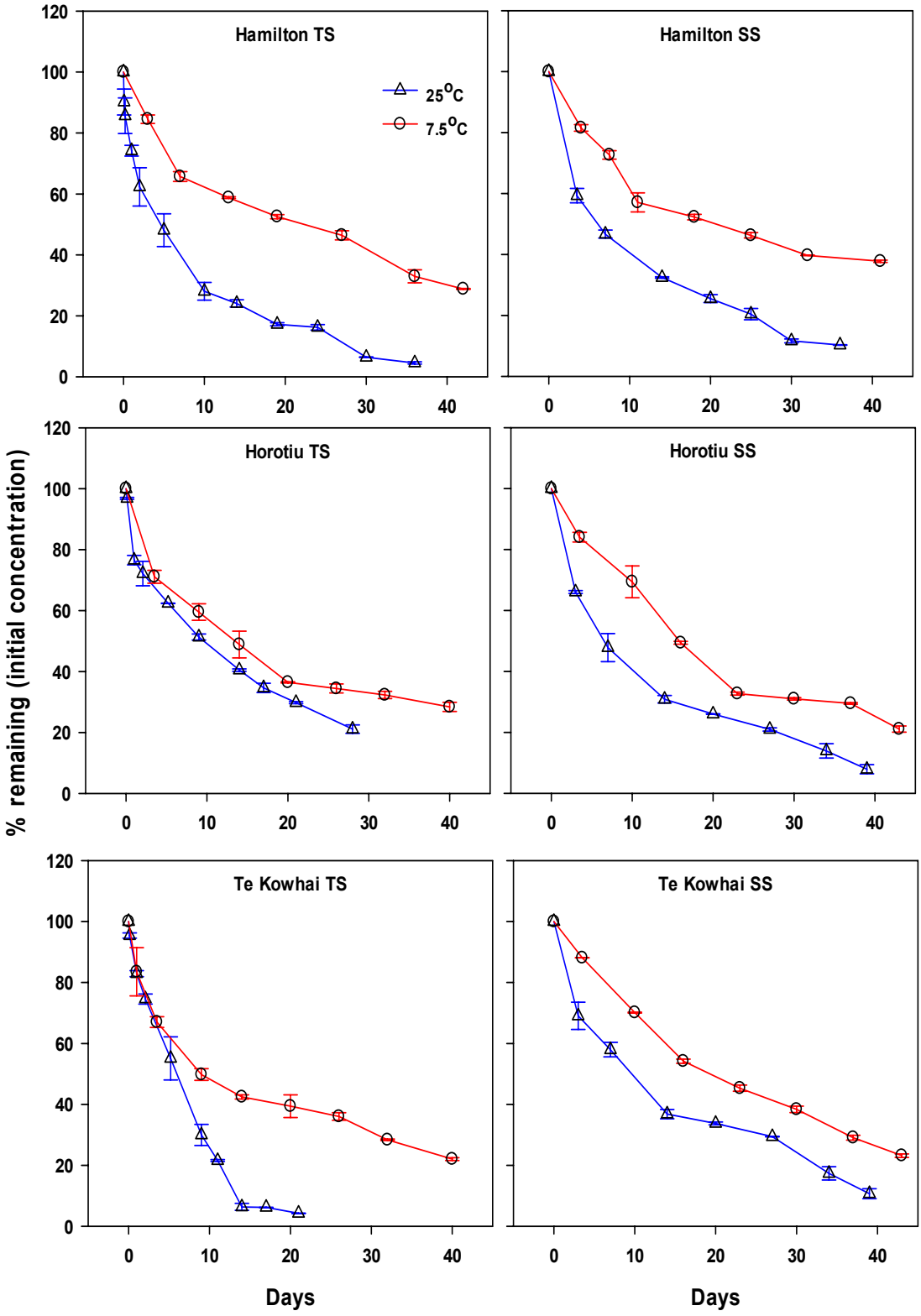


Figure 7.9: Degradation kinetics of SMO in Hamilton, Te Kowhai and Horotiu topsoils and subsoils at 25°C (blue triangle) and 7.5°C (red circle), at initial spiked concentration of 0.5 mg kg^{-1}. Error bars show deviation of the duplicate samples.

7.6.9 Effect of sterilisation

To investigate the relative role of microorganisms in the SMO degradation, soils were sterilised using an autoclave, and Figure 7.10 presents the degradation behaviour of SMO at both soil depths. An examination of data in Table 7.3 reveals that overall, rates of degradation were slower and the associated DT_{50}, DT_{90} and DT_{99} values were higher in sterile soil compared with non-sterile condition in all three soil types, emphasizing the role of microbes in the degradation process. When compared to the non-sterile soils, degradation in sterile soils was slower for all soils except for Horotiu soil where SMO degraded at a rate similar to non-sterile condition (Figure 7.10).

In a separate study both sulfamethoxazole and trimethoprim dissipated more rapidly in non-sterile soil than sterile soil, the rate constants *k* decreased from 0.418 to 0.024 day $^{-1}$, while the DT_{50} values increased from 2 and 29 days respectively in sterile versus non-sterile soil (Liu *et al.* 2010). Similarly, SDZ degradation rate constants were also reported to be higher in three non-sterile soils than sterile soils, with DT_{50}values of 14 and 61 days for one particular non-sterile and sterile soil respectively (Yang *et al.* 2009a). The half-lives for oxytetracycline in soil under aerobic conditions ranged between 29 and 56 days for non-sterile treatments and 99-120 days for sterile treatments (Yang *et al.* 2009b). Sulfadimethoxine also degraded much faster in non-sterile manure than sterile manure (Wang *et al.* 2006b). Slow degradation of oxytetracycline in sterile controls also confirms biodegradation being responsible for major degradation of OTC in manure (Wang and Yates 2008). Biodegradation half-lives for SMO in a river sediment test were between 3.3 and 25.6 h for non-sterile and sterile cases (Radke *et al.* 2009).

Relatively smaller variations in degradation parameters for SMO in Horotiu sterile and non-sterile soil could suggest the reduced influence of soil microbes in this soil. Given the apparent consistency of the results for the non-sterile and sterile treatments, it can be postulated that factors other than biotic processes may have played a role in the degradation of the compound. A close examination of sterile treatment data does suggests some contribution of abiotic processes in the overall degradation of the SMO antibiotic. Under sterile treatment, in all three topsoils and subsoils some abiotic degradation seemed to occur with 99% of the applied antibiotic degrading by 70–140 days for topsoils and 120–360 days for subsoils (Figure 7.10). Several explanations can be offered to support these findings; there

is the possibility chemical hydrolysis, chemical reduction, photolysis, or sorption to soil particles. However, the contribution of each factor in the degradation of SMO is difficult to assess.

Even though all precautions were taken to avoid photolysis, SMO could have degraded as a result of photo catalytic oxidation (Zhou and Moore 1994). This might have occurred while following the experimental protocols (e.g. shaking, pipetting, and centrifuging) where some photolysis could have bound occurred. Chemical hydrolysis is pH dependent, and in acidic or organic soils, hydrolysis seems to be an important factor for degradation of pesticides such as diazinon (Sarmah *et al.* 2004). However, hydrolysis could not explain the decline in SDZ concentrations in sterile soils under aerobic conditions as the degree of hydrolysis is very limited at mildly acidic pH (Yang *et al.* 2009a). The soil pH in the present study ranged from 5.1 to 6.7, and pH measurements of soils before and after autoclaving showed an increase of 0.2–0.4 units, making sterilised soils slightly more alkaline. Although limited abiotic degradation due to hydrolysis was possible, it is highly unlikely, given sulfamethoxazole has no structural features that can be hydrolysed (Loftin *et al.* 2008).

The effect of sterilisation was more evident for subsoils but was less marked for topsoils, which one would expect to be more affected by sterilisation. For example, in Figure 7.8, DHA is markedly lower in subsoil than topsoil, so this large change in the subsoil could well be a physical effect relating to autoclaving rather than to bacteria being destroyed. No microbial activity was observed for sterile soils compared with non-sterile soils as measured by its DHA measurements (Figure 12.8). Even though the autoclaving was monitored by using autoclave tape and sterilisation indicators, the possibility of an artefact during the process of sterilising the soils cannot be ruled out. Autoclaving the soils can alter soil chemistry, and is known to change the physical, chemical, and microbiological properties of the soil due to the high treatment temperature and pressure involved (Fletcher and Kaufman 1980; Wolf *et al.* 1989). For instance, autoclaving the soils has been found to increase the concentrations of dissolved organic carbon dramatically, providing a good environment for those bacterial spores that had survived sterile treatment (Tuominen *et al.* 1994). While studying abiotic transformation of nitrate, Dail *et al.* (2001) observed an increase of 30-fold in dissolved organic carbon in an acid forest soil. Since autoclaving kills the bacteria and not the spores (Nowak and Wronkowska 1987), the one autoclaving done in this study may not

have been sufficient to sterilize the soils. Other sterilisation procedures such as gamma irradiation were not available for this work, while the addition of $HgCl_2$ or NaN_3 was not considered, given that such toxic compounds have been found to increase bacterial activity in sediments after three weeks (Tuominen *et al.* 1994).

Because of the hydrophobic nature of SMO (Table 2.5)**,** there could be an increased sorption affinity due to bonding to the increased amount of organic matter content. This could result in similar irreversible binding in topsoil and subsoil and lesser SMO bioavailability for degradation in soils (Liu *et al.* 2010). Thus it can be hypothesized that sorption had occurred so material was not extractable under the extraction conditions and that autoclaving changed the chemistry to such an extent the sorption process was hindered. It could also be possible that the amide functionalities on residues of the sulfonamide might react with aldehydes present in soil for example reducing sugars to form Amadori compounds (Sheth *et al.* 1990). Some of these reactions may be irreversible and the process of autoclaving might hydrolyse polysaccharides and liberate reducing sugars.

For non-sterile topsoils 99% degradation was achieved between 30 and 90 days and for subsoils between 40 and 95 days, whereas for sterile topsoils it ranged from 75 to 120 days and 150 to 230 days for subsoils. The results also indicate that degradation in non-sterile soils was a result of both biotic and abiotic processes whereas for sterile soils it was due to abiotic process. Abiotic factors such as strong sorption onto soil components also played a role in the dissipation of sulfamethoxazole in soil. Liu et al. (2010), who investigated SMO dissipation in Chinese agricultural soils, drew similar conclusions. The authors also indicated that biodegradation and irreversible binding to soil were responsible for abiotic loss of SMO in the soil. These findings seem to agree with other studies that suggested both biological and chemical processes are responsible for the degradation of organic contaminants (fumigants) in non-sterile media (Ma *et al.* 2001; Guo *et al.* 2004) and only chemical processes are responsible for degradation of pesticides in sterile soils (Sarmah and Close 2009).

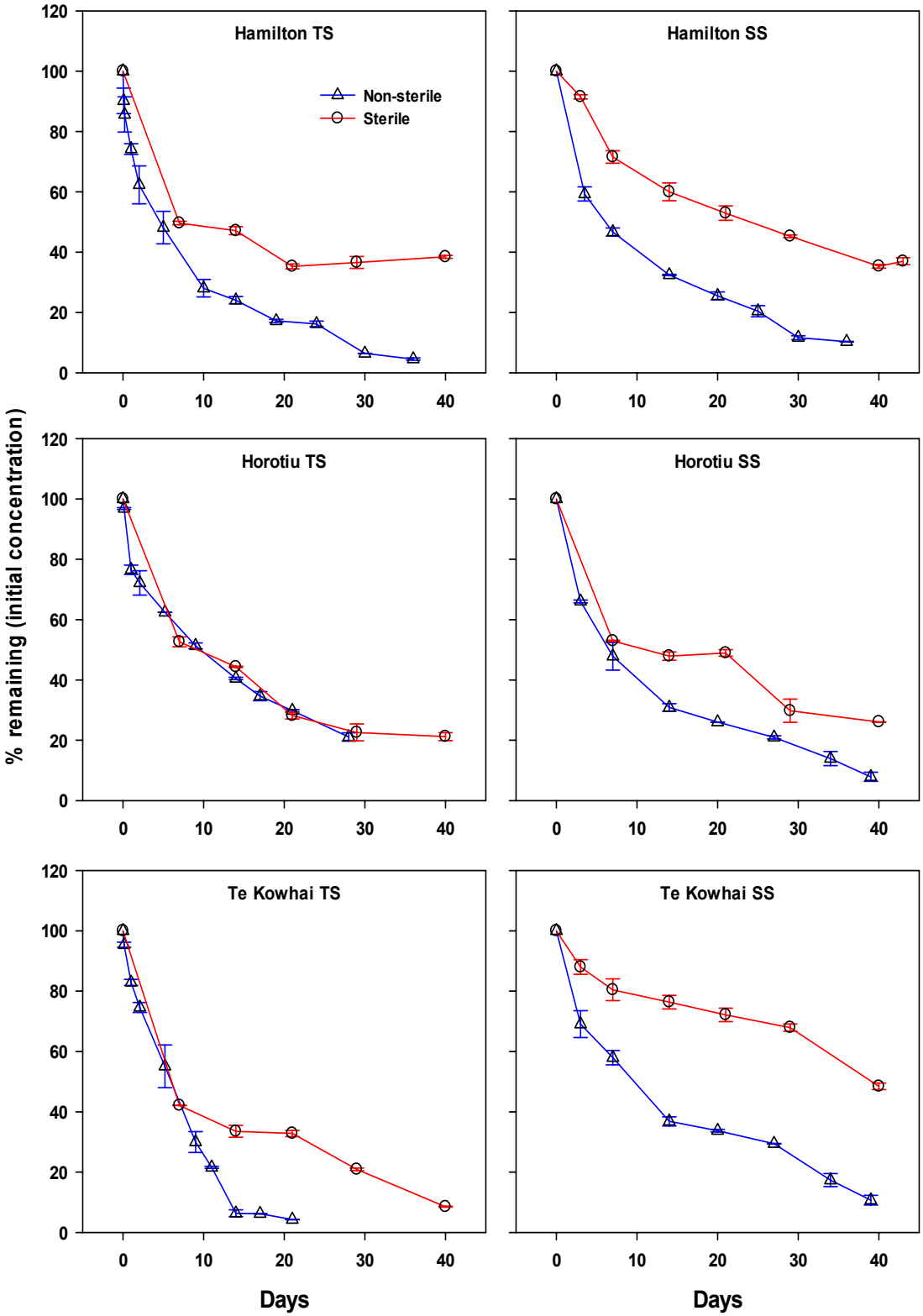


Figure 7.10: Degradation of SMO as a function of time in Hamilton, Te Kowhai and Horotiu topsoils and subsoils under non-sterile (blue triangle) and sterile (red circle) conditions, at initial spiked concentration of 0.5 mg kg^{-1} and incubation temperature of 25°C.

7.7 Summary

The degradation kinetics of three sulfonamides in sterile and non-sterile tap water showed that SCP is slightly more prone to biotic degradation than SMO and SM. However, no significant differences were observed between measured half-lives for sulfonamides in non-sterile and sterile deionised water exposed to light, suggesting photodegradation could play an important role in the dissipation of sulfonamide antibiotics in aquatic media. The degradation rate of a sulfamethoxazole antibiotic in three New Zealand pasture soils varied with initial concentration, soil depth, incubation temperature, and sterilisation. Increased initial spiked concentration resulted in decreased degradation rate constants and consequently increased DT_{50} values. The degradation rate was faster in topsoils when compared with subsoil samples in two of the soils, clearly indicating the prominence in topsoil of soil microbes that strongly influence antibiotic degradation. DHA activities for the topsoils were a unit higher than those obtained for subsoils, suggesting limited microbial activity at increasing depths.

SMO antibiotic does not tend to persist more than 90 days in all three soils at either depth, suggesting that natural biodegradation and chemical degradation is sufficient for the removal of these contaminants from the soil. The degradation rate constants were higher in soils incubated at 25^{o}C than in soils incubated at 7.5^{o}C, which was supported by the measured DHA showing very little or no activity for soils incubated at 7.5^{o}C compared with soils incubated at 25^{o}C. This emphasizes the role of microorganisms in the degradation of SMO antibiotic and that warmer temperatures can enhance biodegradation process. Sterile soils, irrespective of soil depth, exhibit a marked decline in the degradation rate constant compared with non-sterilized soils, and lend further support to the important role of microorganisms in the degradation of the SMO. However, some abiotic degradation occurred in sterile soil, indicating that SMO dissipation in soils was a combined effect of biotic and abiotic degradation. Under laboratory conditions the experimental set-up can be easily altered to suite the research objective; however, under realistic field conditions the degradation rates could be much faster as they could be affected by several factors such as moisture content, temperature, humidity, rainfall, and soil properties simultaneously. The effects of anoxic conditions, varying moisture content, and the addition of manure along with antibiotic persistence in the presence of other contaminants were not investigated in this study.

Also, using only simple first order kinetics to explain the degradation pattern of SMO could lead to biased results; hence, there is a need to incorporate the process of sorption–desorption, biotic and abiotic degradation in a single integrated degradation model. In this study the variation in microbial biomass during the incubation period for each sampling time was not monitored and no attempt was made to isolate the type of microorganisms present in these soils that might be responsible for degrading SMO. Future research in this direction could provide further insights on the possible degradation mechanisms for these compounds in soils.

7.8 Shortcomings of this study

For the degradation samples in water, the glass bottles were made of Pyrex glass and the bottles placed indoors behind window panes. This could have probably filtered out UV light leaving the bottles exposed only to visible and IR light, which would usually not be sufficient energy to initiate photo-degradation.

SMO is known to have two main degradation products, namely N4-acetyl-SMO and SMO-N1-glucuronide (Radke *et al*. 2009). However, analysis of HPLC chromatograms for SMO did not indicate formation of any metabolite during the length of the degradation experiment. It must be noted that with the HPLC method used here, only those metabolites with ultraviolet absorbance at 275 nm and with similar or lower lipophilicity than the sulfonamides would have been detected (Ingerslev and Halling-Sørensen 2000). Therefore, the possible formation of more polar metabolites during degradation of sulfonamides cannot be excluded and either of the two could have been formed and gone undetected. This problem highlights the need for using a mass spectrometer in such cases rather than conventional photosensitive detectors. The development and validation of analytical procedure for known metabolites is also critical as one cannot rely on the chance detection by MS or any other detector, as several factors may preclude the compound ever reaching the detector (extraction, loss during clean-up etc).

8 Chapter 8: Modelling degradation kinetics of sulfamethoxazole antibiotic in three New Zealand agricultural soils under varied laboratory conditions

8.1 Introduction

Degradation is an important process that affects the fate of organic contaminants in the environment. Degradation parameters and associated endpoints for veterinary antibiotics are often evaluated using simple first order kinetics, however, the kinetics of degradation may also exhibit bi-phasic, tri-phasic, and even a logistics pattern, depending on the soil type, or the combination of a multitude of factors. Thus, proper selection and utilization of appropriate mathematical models capable of describing the entire degradation kinetics in water and soil media should be considered in order to predict appropriate degradation end-points.

In Chapter 7, the degradation parameters and endpoints were estimated using simple first order kinetics. Although the coefficient of determinant (R^2) values were acceptable (0.80 - 1.00) for most soils and under varied treatment conditions, some evidence of a biphasic degradation pattern was found for a few particular data sets in which the R^2 values were low. For instance Figure 8.1 shows the kinetics of degradation for SMO in Hamilton clay topsoil (0.5 mg kg^{-1}, 25^{o}C and non-sterile) and Hamilton clay (sterile subsoil) in which biphasic behaviour is clearly reflected. It was evident from Chapter 7 that there was a change in the rate constant during the course of the reaction.

To avoid an underestimation of degradation rates the use of more complicated kinetic models than simple first order (SFO) has been recommended to describe laboratory degradation data and to obtain accurate degradation end-points (Lucas and Jones 2006; Sarmah *et al.* 2008). When an apparent bi-phasic kinetics was considered for some of the treatment conditions, the pattern seemed to dominate the entire degradation process for the SMO antibiotic. Thus the use of simple first order model to describe the kinetics of degradation for SMO would

provide an inaccurate assessment of the degradation end-points for SMO in soils selected for this study.

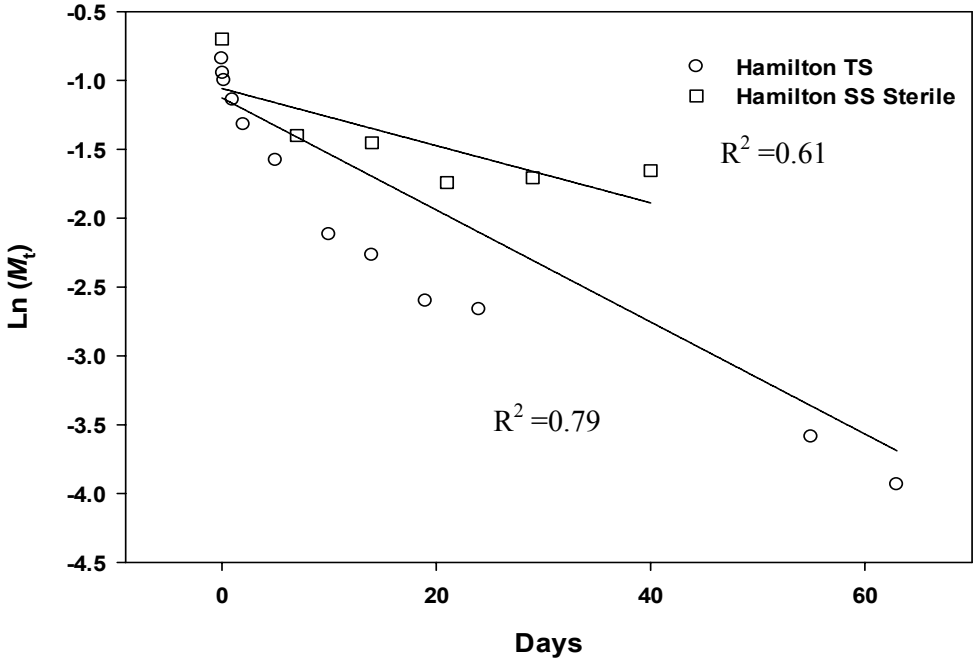


Figure 8.1: Log transformation plots for Hamilton clay topsoil (circle) and sterile subsoil (square) along with their R^2 values.

Therefore three non-linear, bi-phasic models, namely bi-exponential decay (BEXP), first-order double exponential decay (FODED), and first-order two-compartment decay (FOTC) models, were used to fit the observed degradation data for SMO. Corresponding degradation end-points DT_{50} and DT_{90} values for SMO were numerically obtained and compared against those estimated by the simple first-order kinetic (SFO) model. Model selection and evaluation of performance were based on an array of statistical measures such as coefficient of determination (R^2_{adj}), root mean square error (RMSE), chi-square (χ^2) test at 1% significance, Bayesian Information Criteria (BIC), and % model error. The objectives of this study were to test the performance of the models and to investigate the possible mechanism behind the degradation processes. Good fit to any one particular would mean that the model could be used as the basis for future risk assessments involving fate of these antibiotics in similar soils.

8.2 Degradation models

SMO degradation was fitted with a bi-exponential model (BEXP), first-order double-exponential decay (FODED) and first-order two-compartment (FOTC) models, assuming no back conversion, no influence of sorption on the degradation, and no altering because of microbial growth (Scherr *et al.* 2008). These models were selected based on their relative simplicity (three to four parameters) and their potential to provide a good fit to the measured datasets.They have been successfully applied earlier to model aerobic degradation of 4-*n*-nonylphenol and bisphenol-A in groundwater-aquifer material slurry (Sarmah and Rohan 2011), and to study degradation kinetics of estrone-3-sulfate (Scherr *et al.* 2008) and a number of pesticides (Sarmah and Close 2009) in New Zealand soils.

8.2.1 Single first order exponential decay model (SFO)

This model can be represented as in equation 8.1, which is the simplest form of concentration–time relationship, where t is time (h), k_1 is the degradation rate constant (day^{-1}), and M_0 and M_t are the initial and concentrations at time t (Scherr *et al.* 2008):

$$M_t = M_0 e^{(-k_1 t)} \qquad (8.1)$$

8.2.2 Bi-exponential model (BEXP)

According to the BEXP model, degradation takes place in two compartments: in the first compartment rapid degradation is expected to occur within the soil-water phase, where microorganisms have easy access to the compound. In the second compartment, degradation is slow, and the compound is expected to be adsorbed to soil particles with the degradation rate being governed by the slow desorption-diffusion processes (Scherr *et al.* 2008). The equation below describes the concentration–time relationship as the sum of first- and second-order differential rate equations (Sarmah and Rohan 2011).

$$\frac{dM}{dt} = -(k_1 M + k_2 M^2) \qquad (8.2)$$

The above differential equation can be integrated to obtain

$$e^{(-k_1 t)} = \frac{M(k_1 + k_2 M_0)}{M_0(k_1 + k_2 M)} \qquad (8.3)$$

This, on rearrangement, gives the following equation:

$$M = \frac{k_1 M_0}{(k_1 + k_2 M_0)e^{(k_1 t)} - k_2 M_0} \qquad (8.4)$$

Where $k_1$is the first-order rate constant (time^{-1}), k_2 is the second-order rate constant (conc^{-1}time^{-1}), and usually $k_1 > k_2$ (absolute value); M is the concentration at time t, and M_0 is the concentration at t = 0. It is important to note that the integrated rate expression in the above equation is actually not the sum of two exponential terms and that the model is purely empirical, and k values are unique to a specific dataset related to the particular experiment involving a compound (Sarmah and Rohan 2011).

8.2.3 First-order double-exponential decay model (FODED)

This model can be described by the following equation

$$M(t) = M_1(t) + M_2(t) = M_{sol}\, \mathrm{e}^{(-k_1 t)} + M_{sorb}\, \mathrm{e}^{(-k_2 t)} \qquad (8.5)$$

where M_{sol} and M_{sorb} are constants representing compound concentrations initially distributed between two pools (solution phase and sorbed phase) with degradation rate constants of k_1 and k_2, respectively (numerically $k_1 > k_2$). For this model, it is assumed that solution phase material dissipates faster than the sorbed phase material. Researchers have used this model in the past to describe degradation of conjugated steroid hormones such as estrone-3-sulfate in agricultural soils (Scherr *et al.* 2008).

8.2.4 First-order two-compartment model (FOTC)

This model assumes that degradation takes place in two compartments: a 'fast' surface loss in the deposited residue compartment *vs*. a 'slow' degradation loss in the retained residue compartment. It is also assumed that the two loss processes and the transfer process between compartments are first order. This model has been used earlier to describe the degradation kinetics of several commonly used pesticides in agricultural soils (Monteiro and Boxall 2009; Sarmah and Close 2009). For this model, the equations can be written as

$$\frac{dM_1}{dt} = -(k_1 + k_R)M_1 \qquad (8.6)$$

$$\frac{dM_2}{dt} = k_R M_1 - k_2 M_2 \qquad (8.7)$$

where k_R is the retention rate constant of antibiotic transfer between the fast and slow degradation compartments (usually $k_R > 0$), k_1 is the rate constant of the 'fast' compartment, k_2 is the rate constant for the 'slow' compartment, while M_1 and M_2 are the concentrations of the antibiotic in the fast and slow compartments respectively. The total concentration at a time t, in the soil is given by the sum of M_1 and M_2 and is written as:

$$M = (M_1 + M_2) \qquad (8.8)$$

Substituting the values for M_1 and M_2 and solving for M gives

$$M = M_0 \, e^{\{-(k_1+k_R)t\}} + M_0 \frac{k_R}{k_1+k_R-k_2}\{e^{(-k_2 t)} - e^{\{-(k_1+k_R)t\}}\} \qquad (8.9)$$

Except for the SFO model, the rest of the models used describe non-linear degradation kinetics with fast and slow release of the antibiotic with time. Parameters for these models were obtained from the best-fit models using the Solver add-ins tools of MS Excel 2010 ® solver.

8.2.5 Modelling approach

In order to fit the measured data for SMO degradation with the selected models, parameters were optimised according to the recommendations by Boesten *et al*.(2006) by using solver as a modelling tool utilising the Generalized Reduced Gradient (GRG2) non-linear optimization code available within the MS Excel 2010 ®. The dynamic concentrations of SMO were measured in different soils at regular time intervals, under varying laboratory conditions. For each dataset, the models were applied to fit and estimate the degradation parameters. The equations given above were used to predict values of M for the antibiotic, which was used in minimisation of the residuals for each model. The parameters such as M_0, k_1 (SFO); M_0, k_1, k_2 (BEXP); M_{sol}, M_{sorb}, k_1, k_2 (FODED); and M_0, k_1, k_2, k_R (FOTC) were estimated iteratively by minimising the sum of squares of the residuals (SSRes), which is basically the difference between measured and model fitted values. For the iterative algorithm, the initial values for

the parameters were set as follows: the initial spiked concentration for SMO was taken as default M_0 value. However, for other parameters (k_1, k_2, k_R), the initial guess value was by a trial and error approach. It is important not to set the initial guess values for the rate constants to 'zero' in the modelling procedure, allowing the solver to do the required iterations to obtain the best values.

8.2.6 Estimation of DT_{50} and DT_{90} values

The degradation time for an antibiotic is often computed to determine when 50% (DT_{50}) and 90% (DT_{90}) of its initial concentration is lost due to degradation. The equations for SFO and BXEP are in explicit form (analytical solution available), and deriving dissipation time t in terms of other parameters is possible. These derivations are given below.

BEXP model: From equation 8.2, the following equations can be derived

$$(k_1 + k_2 M_0)\, e^{(-k_1 t)} - k_2 M_0 = \frac{k_1 M_0}{M} \qquad (8.10)$$

$$(k_1 + k_2 M_0)\, e^{(-k_1 t)} = M_0 \left(\frac{k_1}{M} + k_2\right) = \frac{M_0}{M}(k_1 + k_2 M) \qquad (8.11)$$

$$e^{(k_1 t)} = \frac{M_0}{M}\left(\frac{k_1 + k_2 M}{k_1 + k_2 M_0}\right) \qquad (8.12)$$

$$t = \frac{1}{k_1} \ln\left\{\frac{M_0}{M}\left[\frac{(k_1 + k_2 M)}{(k_1 + k_2 M_0)}\right]\right\} \qquad (8.13)$$

In equation 8.13**,** *M* represents either 50% or 90% dissipation of the initial spiked concentration of the antibiotic.

FODED and FOTC models. The equations for both FODED and FOTC models have no analytical solutions available, as the models are not in explicit form. An iterative procedure was carried out using the solver tool in Excel. Estimating the values for DT_{50} and DT_{90} for these two models has been described by Sarmah and Close (2009) by the following equation

$$DT_{50} \;=\; M_0 + \ln\left(1 - \frac{50}{100}\right) \text{and } DT_{90} = \; M_0 + \; \ln\left(1 - \frac{90}{100}\right) \qquad (8.14)$$

where M_0 is the initial concentration of the antibiotic at time zero, after logarithmic transformation of equation 8.5 (FODED) and equation 8.9 (FOTC). The right-hand sides of equations 8.14 were taken as 'target cells' in Solver, which were compared with the values obtained using equation 8.5 and 8.9. When the value of devsq was zero or close to zero, DT_{50} and DT_{90} values for the antibiotic were obtained.

8.3 Statistical analysis

A number of statistical indices – coefficient of determination (R^2_{adj}), root mean square error (RMSE), chi-square (χ^2) test at 1% significance, and Bayesian Information Criteria (BIC) – were computed for model comparison and for goodness-of-fit evaluation for a given soil and conditions. All equations, unless specifically cited, have been adapted from Sarmah and Rohan (2011).

8.3.1 Root mean square error (RMSE)

$$\text{RMSE} = \frac{100}{C_{av}}\sqrt{\sum_{i=1}^{n}(M_i - C_i)} \qquad (8.15)$$

where C_{av} is the average of the measured value, M_i is the fitted value, C_i is the measured value, and n is the number of observations. The root mean square error (RMSE) calculates the average difference between fitted and measured data and the value for RMSE should be as close to 'zero' as possible.

8.3.2 Chi-square (χ^2) test

The chi-square test takes into account the deviations between the measured value and the value predicted by the model relative to the uncertainty of the measurements.

$$\chi^2 = \sum \frac{(M_i - C_i)^2}{\frac{err}{100 \times \overline{C_i^2}}} \qquad (8.16)$$

where err = model error, which is the measurement error percentage and is given by the equation below.

$$\text{err} = \sqrt{\frac{1}{\chi^2_{\text{tabulated}}} \sum \frac{(M_i - C_i)^2}{C_i^2}} \qquad (8.17)$$

If $\chi^2 > \chi^2_{\text{tabulated}}$ (at a confidence level of 1%, if otherwise stated), then the model is inappropriate at the chosen level of significance.

8.3.3 Bayesian Information Criteria (BIC)

$$\text{BIC} = s \ln\left(\frac{SSRes}{s}\right) + p \ln(s) \qquad (8.18)$$

where s = number of measurement, SSRes = sum of squares of residuals obtained during data fitting by model, and *p* = number of parameters to be estimated. The assumptions behind BIC are that the model errors are normally distributed. Lower values for BIC indicate a better model fit.

8.4 Experimental datasets

The datasets from Chapter 7 were used in the modelling exercise and results are discussed below.

8.5 Results and discussions

Three biphasic models were selected for comparison to the simple first order kinetic model based on their degree of flexibility and simplicity such as the numbers of parameters needed to be optimized, and their individual ability to fit the measured data within reasonable iterations. The model-fitting procedure suggested that either using measured concentrations in the Y-axis or setting the values as % of the remaining antibiotic did not have any effect on the curve fitting or its trend. However, the values for the solver-optimized parameters were obtained by using observed SMO concentrations. Therefore, in the fitting procedure, values in the Y-axis were set to % of initial applied amount, but in the tabulated data they are represented as mg L^{-1} or mg kg^{-1}.

8.5.1 Degradation of three sulfonamide antibiotics in non-sterile and sterile water

The fitted plots of 4 models (SFO, BEXP, FODED, and FOTC) and the observed concentration data for the three sulfonamides (SMO, SCP, and SM) in non-sterile and sterile deionised water are presented in Figure 8.2-Figure 8.4. The optimized parameters for the models for each sulfonamide antibiotic are summarised in Table 8.1, and the DT_{50} andDT_{90} values and the corresponding statistical indices in Table 8.2. All three biphasic models fitted the degradation data for the three sulfonamides better than the simple first order kinetic model, as supported by the goodness-of-fit statistical indices (Table 8.2**)**.

Table 8.1: Optimised parameters for the models used in modelling the degradation of SMO, SCP, and SM in non-sterile and sterile deionised water.

Parameters		**SMO**		**SCP**		**SM**	
Models		non-sterile	sterile	non-sterile	sterile	non-sterile	sterile
SFO	k_1	0.004	0.004	0.012	0.014	0.004	0.004
	M_0	0.523	0.507	0.447	0.549	0.608	0.545
BEXP	k_1	0.074	0.078	0.048	0.084	0.079	0.159
	k_2	-0.030	-0.031	-0.004	-0.017	-0.038	-0.072
	M_0	0.540	0.523	0.460	0.585	0.634	0.587
FODED	k_1	0.521	0.147	0.002	0.029	0.074	0.071
	k_2	0.003	0.002	0.029	-0.018	0.001	-0.001
	M_{sol}	0.048	0.051	0.219	0.515	0.105	0.149
	M_{sorb}	0.506	0.478	0.240	0.063	0.531	0.436
FOTC	k_1	0.046	0.018	0.152	0.023	0.013	0.018
	k_2	0.500	0.002	0.011	-0.018	0.074	-0.001
	k_r	-0.043	0.145	2.393	0.005	-0.012	0.053
	M_0	0.554	0.529	0.465	0.578	0.636	0.584

k_1 is the first order rate constant (day^{-1}); k_2 is the second order rate constant (L $mg^{-1}day^{-1}$); M_0 is the initial observed concentration (mg L^{-1})

Table 8.2: DT_{50} and DT_{90} (days) values and statistical indices derived during modelling of SMO, SCP, and SM degradation in water under non-sterile and sterile conditions. NP = result not possible.

	End points	**SMO**		**SCP**		**SM**	
Models		non-sterile	sterile	non-sterile	sterile	non-sterile	sterile
SFO	**DT_{50}**	192.6	195.0	48.3	59.9	174.2	189.7
	DT_{90}	639.9	647.8	160.4	199.0	578.6	630.1
	SSRes	0.002	0.003	0.01	0.004	0.005	0.007
	RMSE	3.0	3.5	7.3	5.3	3.8	5.0
	BIC	-88.2	-85.0	-64.8	-74.2	-79.5	-76.2
	χ^2	0.009	0.013	0.043	0.027	0.014	0.022
	Model error	2.12	2.45	4.64	3.71	2.73	3.17
	R^2	0.87	0.83	0.92	0.94	0.83	0.72
BEXP	**DT_{50}**	NP	NP	45.9	64.7	NP	NP
	DT_{90}	NP	NP	NP	NP	NP	NP
	SSRes	0.001	0.002	0.003	0.003	0.002	0.001
	RMSE	2.2	2.9	4.4	5.0	2.6	1.7
	BIC	-93.7	-87.6	-73.9	-74.5	-86.7	-98.7
	χ^2	0.005	0.009	0.019	0.025	0.007	0.003
	Model error	1.59	2.17	3.23	3.66	1.91	1.22
	R^2	0.94	0.89	0.98	0.95	0.93	0.97
FODED	**DT_{50}**	209.8	255.7	43.0	64.7	372.7	NP
	DT_{90}	770.4	950.7	227.9	NP	1543.0	NP
	SSRes	0.001	0.002	0.003	0.003	0.002	0.001
	RMSE	1.9	2.8	4.0	5.0	2.5	1.7
	BIC	-94.2	-86.1	-73.5	-72.0	-84.5	-96.2
	χ^2	0.004	0.009	0.016	0.025	0.007	0.003
	Model error	1.46	2.17	3.09	3.87	1.97	1.28
	R^2	0.95	0.90	0.98	0.95	0.93	0.97
FOTC	**DT_{50}**	210.6	224.2	42.9	57.6	371.8	NP
	DT_{90}	773.4	929.8	203.3	NP	1538.6	NP
	SSRes	0.001	0.002	0.003	0.003	0.002	0.001
	RMSE	1.9	2.8	4.0	5.3	2.5	1.7
	BIC	-94.2	-86.1	-73.5	-70.9	-84.5	-96.2
	χ^2	0.004	0.009	0.016	0.028	0.007	0.003
	Model error	1.46	2.17	3.09	4.08	1.97	1.28
	R^2	0.95	0.90	0.98	0.95	0.93	0.97

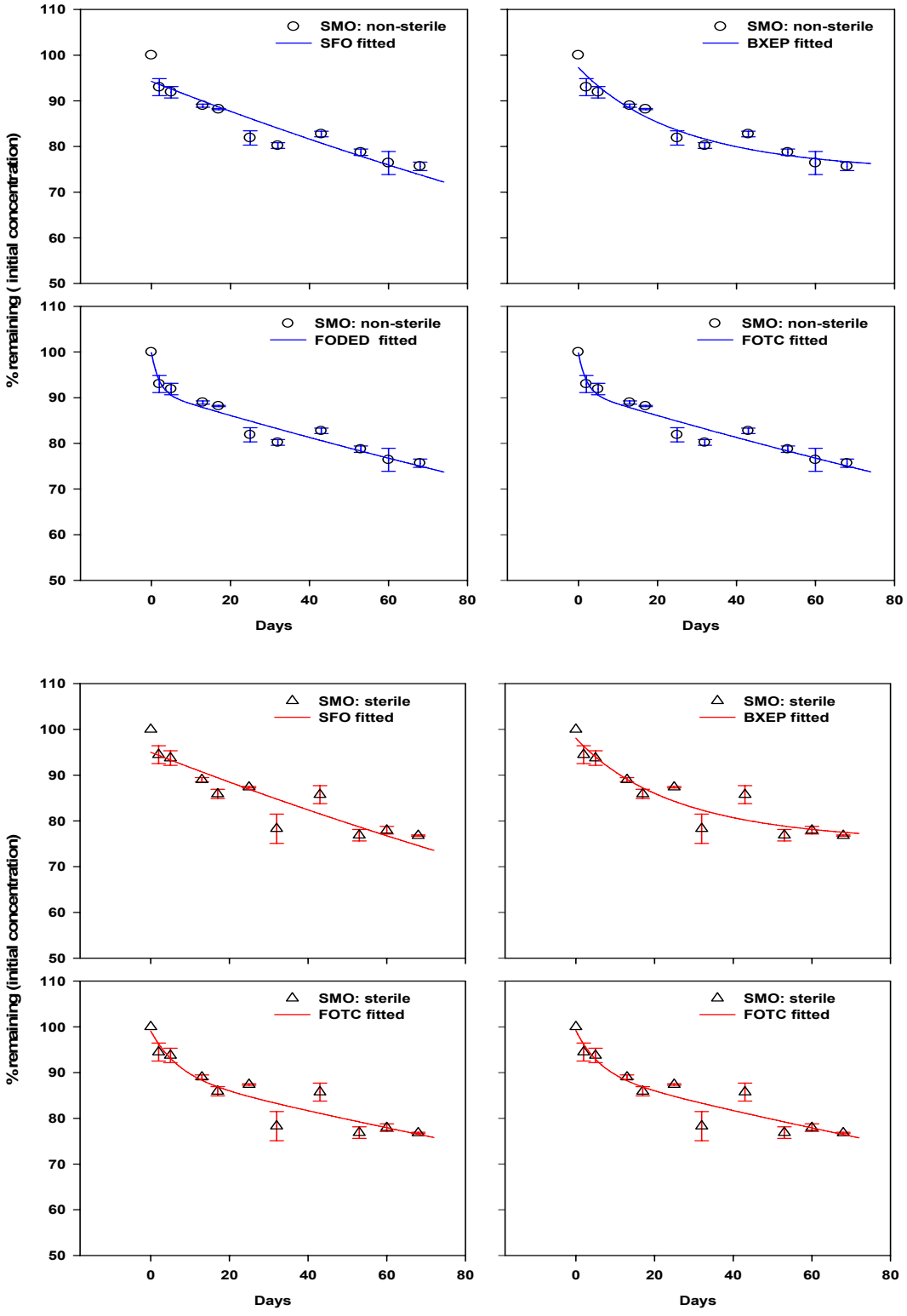


Figure 8.2: Plots of measured concentrations of SMO antibiotic in non-sterile (blue) and sterile (red) de-ionised water as a function of time along with the fit for the SFO, BEXP, FODED, and FOTC models. Vertical error bars represent the range of duplicate results.

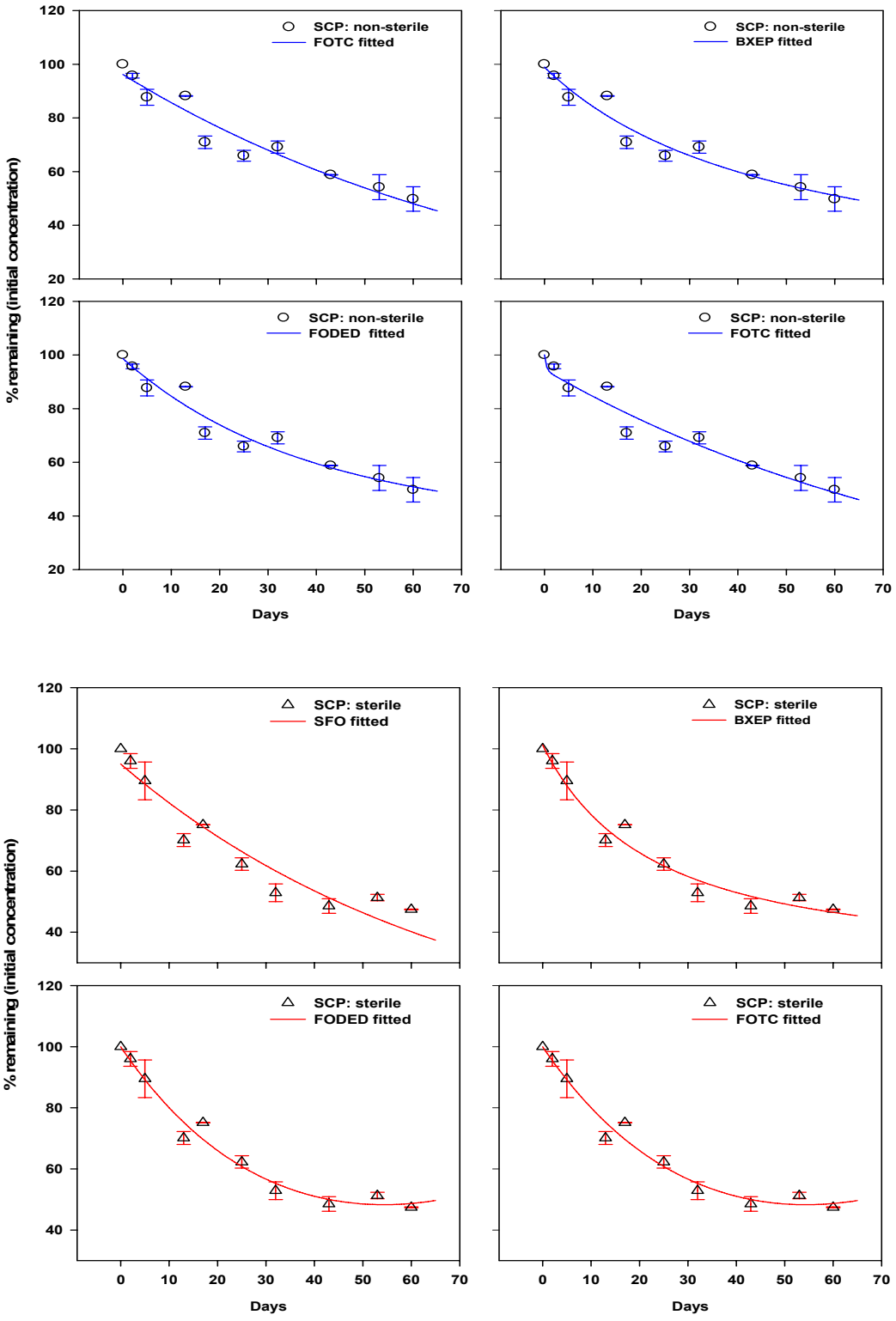


Figure 8.3: Plots of measured concentrations of SCP antibiotic in non-sterile (blue) and sterile (red) de-ionised water as a function of time along with the fit for the SFO, BEXP, FODED, and FOTC models. Vertical error bars represent the range of duplicate results.

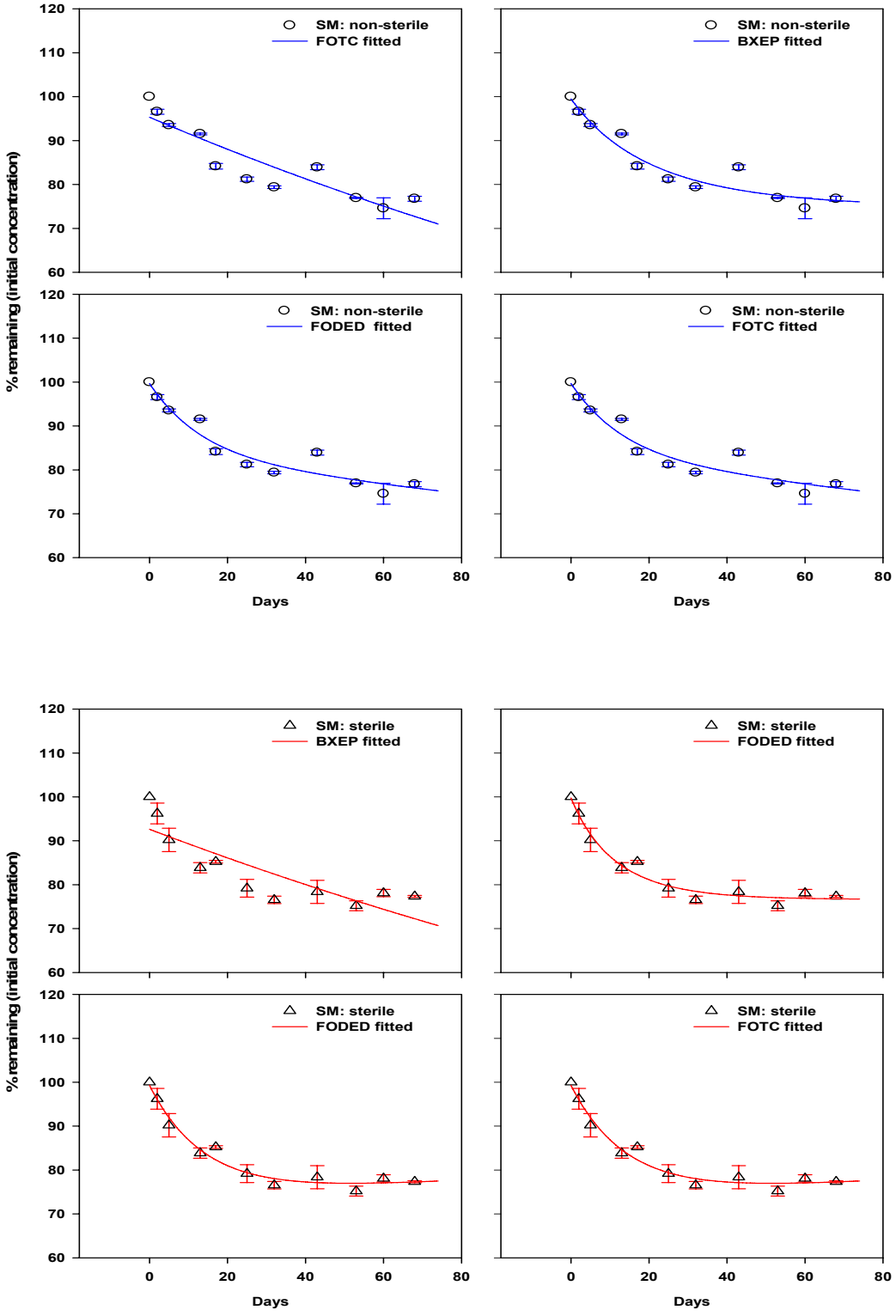


Figure 8.4: Plots of measured concentrations of SM antibiotic in non-sterile (blue) and sterile (red) water as a function of time along with the fit for the SFO, BEXP, FODED, and FOTC models. Vertical error bars represent the range of duplicate results.

A visual examination of the plots indicated a good match between observed and model fitted degradation data under non-sterile and sterile conditions for SMO, SCP, and SM (Figures 8.2-8.4). Good convergence to the solution was achieved for all the three sulfonamides under non-sterile conditions. It is important to note that the SFO fit for the three antibiotics was satisfactory, and it was the only model that could predict the degradation end points (DT_{50}, DT_{90}, and DT_{99}) for all the datasets. However, the SFO model led to underestimation of the initial observed values for the antibiotics when compared with the other models. The models FOTC and FODED investigated here fit the datasets well. The fit for FOTC and FODED models and their convergence to solution were similar for the sulfonamides under non-sterile conditions suggesting that their corresponding DT_{50} and DT_{90} values would be also similar (Table 8.2). The BEXP model could not predict the degradation end points for the three sulfonamides in non-sterile (except DT_{50} for SCP) and sterile water, while FODED and FOTC models failed for SCP and SM under sterile conditions.

Based on the goodness of fit and lowest values for other statistical indices, the FODED and FOTC models were superior in predicting the measured values and degradation endpoints for the three sulfonamides under both non-sterile and sterile (except SM) conditions when compared to BEXP. No solution was possible for SM under sterile conditions.

8.5.2 Degradation of SMO antibiotics in three soils

In order to avoid biased degradation results the data sets in Chapter 7 were also fitted with three different biphasic models, and compared with single first-order exponential decay model. The fitted plots of 4 models (SFO, BEXP, FODED, and FOTC) and the observed data for sulfamethoxazole under varying initial temperature, depth, and under sterile conditions are presented in Figures 8.3-8.5. The optimized parameters from the four model fits together with error estimates, corresponding degradation end-points (DT_{50}, DT_{90}), and the statistical indices used to evaluate the goodness-of-fit for three soils are presented in Tables 8.3-8.8. The selection of the best fit for each data set was based on a suite of statistical indices. The model that had the majority of statistical measures in its favour was selected as the best model to describe the data set.

8.5.2.1 SFO model

The SFO model described the observed degradation data across the soils and temperatures satisfactorily and provided a solution for all data sets (Table 8.3-8.8). However, the goodness of fit (R^2) was far from satisfactory, ranging from 0.79 to 0.99. In one particular data set for Hamilton topsoil under sterile conditions the R^2 value was as low as 0.79.In general, the RMSE values were low, and the model error % was < 15% for most datasets, a threshold value used by Boesten *et al*.(2006). The exception was for the Hamilton and Te Kowhai soils under sterile conditions, where the model error was 16.1 and 15.1% respectively. Also the model predicted value for M_0 deviated considerably from 100% of initial applied amount across most soils and treatments. This deviation over-predicted the values for the rate constants and the corresponding endpoints compared with the biphasic models. For example, in Hamilton soil, where the SFO derived DT_{50} value was found to be 8.9 days, while the biphasic models derived DT_{50}values ranged between 6.4 and 6.9 days.

8.5.2.2 BEXP model

The BEXP model, which divides the degradation pattern into two parallel compartments, fast and slow, did not yield any degradation endpoint for the four data sets. However, applying the BEXP model improved the goodness-of-fit indices (R^2, RMSE, and model-error %) for all data sets (Tables 8.4, 8.6 and 8.8). For instance, at 7.5°C for both Te Kowhai topsoil and Hamilton clay subsoil, no solution was found; however, the BEXP model significantly improved the fit at 25 °C, and this was supported by the low values of statistical indices when compared with the SFO model. These were similar to the findings of Scherr *et al*. (2008) and Herman and Scherer (2006). While the former modelled the degradation and metabolite formation kinetics of estrone-3-sulfate using SFO and BEXP models, the latter investigated the use of four different models including SFO and BEXP to predict pesticide degradation. The authors involved in the two separate studies concluded that the BEXP model resulted in the most accurate predictions overall. Furthermore, their study showed that a BEXP model is rather unlikely to converge when data are well described with the SFO model, which was observed in this case. The BEXP model also showed some inconsistency in the data fitting and this can be judged from the statistical indices and from a comparison with the other models. This can be expected as the BEXP model has only 3 parameters unlike the FODED

and FOTC models which have 4 parameters, so the performance in terms of data fitting would be less accurate.

8.5.2.3 FOTC and FODED models

The fits of FOTC and FODED models for SMO degradation data sets were identical in most cases, and prediction was better than the BXEP model. The FOTC and FODED were very successful in providing solutions for all the three topsoils and subsoils under all conditions investigated. Only a closer look at the statistical indices would indicate subtle differences between each of these models. For example, in Te Kowhai topsoil at 25^{o}C (Table 8.5) FOTC model had better R^2 values and lower statistical indices compared with the FODED model.

8.5.3 Comparison between models based on statistical indices:

The results significantly favoured the usage of biphasic models in all cases over the SFO model. Given that the values for degradation endpoints and half-lives derived from the models were assessed by various statistical measures or goodness of fit, the behaviour of the models to fit the measured values is dependent on the indices. On all occasions the RMSE, SSRes, BIC, and χ^2 values support the choice of using a more complex model in preference to SFO model. The model error % for FOTC and FODED was low compared with BXEP and SFO along with increased goodness-of-fit scores. Their estimates for M_0 were also close to 100% with low standard errors for all data sets. The SFO model's inability to predict the initial measured values of SMO degradation under all conditions correlates with its higher statistical indices values (RMSE, BIC, χ^2 and % model error) as shown in Tables 8.4, 8.6 and 8.8.

Overall, the biphasic (BXEP, FODED, and FOTC) model derived DT_{50} values for SMO were lower when compared with those derived using SFO. DT_{50} values obtained from SFO models were 1 to 4-fold greater than the DT_{50} values obtained by the biphasic models under any given condition for all three soils. It has been postulated that biphasic degradation patterns are the result of reduced microbial activity towards the end of long duration incubation studies (Lucas and Jones 2006; Scherr *et al.* 2008). For instance, Scherr *et al*. (2008) suggests that the association with clay and organic matter particles may allow the enzymes to be

distributed in both the solid and liquid phases of soils permitting degradation in both compartments.

Under sterile conditions, SFO and BXEP models underestimated the measured data at the start of the experiment. The FOTC and FODED models fit for the measured datasets under all conditions were well supported by the statistical indices derived during the fitting procedure. For instance, among the models, FODED gave the best fit of the measured data for SMO in Te Kowhai soil; however, the FOTC model gave low values for the statistical indices with a model error of 3.8%, while displaying the lowest values for other indices such as RMSE (4.7), a BIC value of -41.95, and $\chi^2$of 0.013. This, along with the smallest SSRes (0.002) value, further signifies that the FOTC model was superior to other models in this instance.

Often the experimental protocols adopted and other controlled laboratory conditions may differ considerably between studies. In addition, many studies use modified forms of first-order kinetics and have calculated the half-lives for different sulfonamide antibiotics (Wang *et al.* 2006a; Wang *et al.* 2006b; Yang *et al.* 2009a). Due to these differences, and general lack of previous research work on sulfamethoxazole, no comparison could be made with the available literature information for the biphasic model derived SMO degradation end-points. However, SFO derived degradation endpoints and half-lives for SMO antibiotic in soils have been compared with other available literature (Table 8.8), and have been discussed in detail in Chapter 7**.**

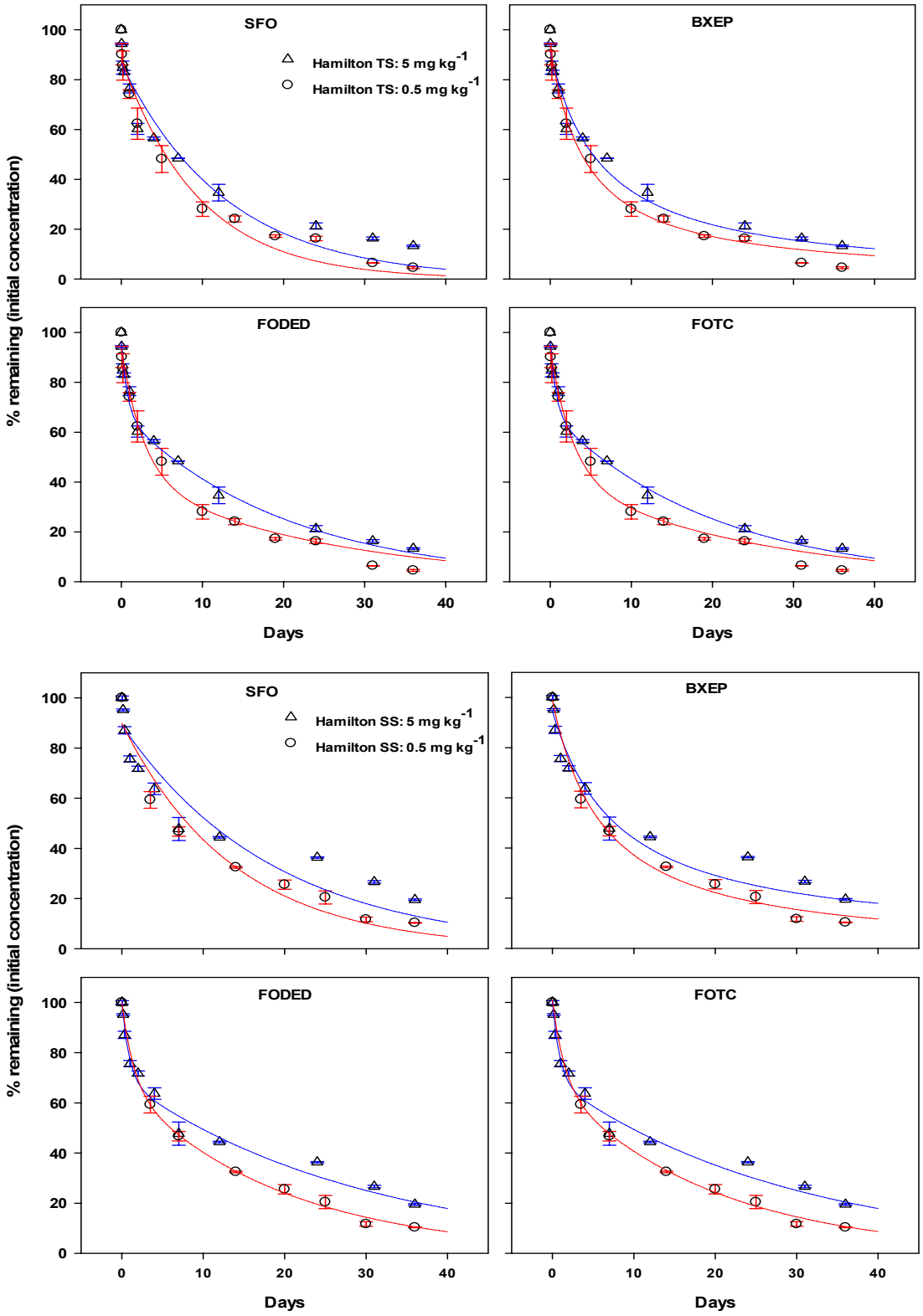
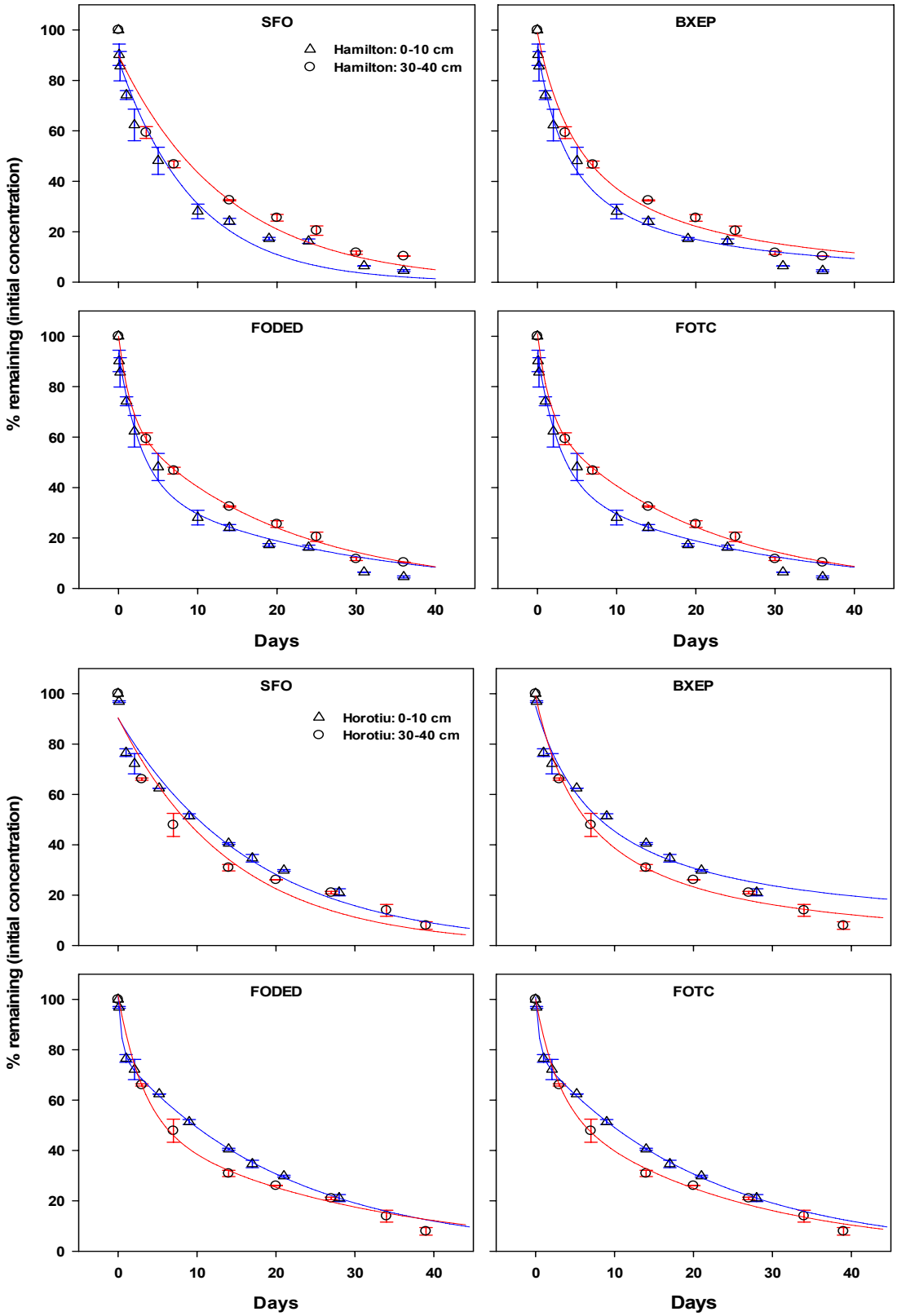


Figure 8.5: Plots of measured residues of SMO antibiotic at varying initial concentration of 0.5 and 5 mg kg^{-1} in Hamilton clay top and subsoils as a function of time along with the fit for SFO, BEXP, FODED, and FOTC models. Vertical error bars represent the range of duplicate results for measured values.


SFO
Hamilton: 0-10 cm
Hamilton: 30-40 cm
BXEP
FODED
FOTC
% remaining (initial concentration)
Days
SFO
Horotiu: 0-10 cm
Horotiu: 30-40 cm
BXEP
FODED
FOTC
% remaining (initial concentration)
Days

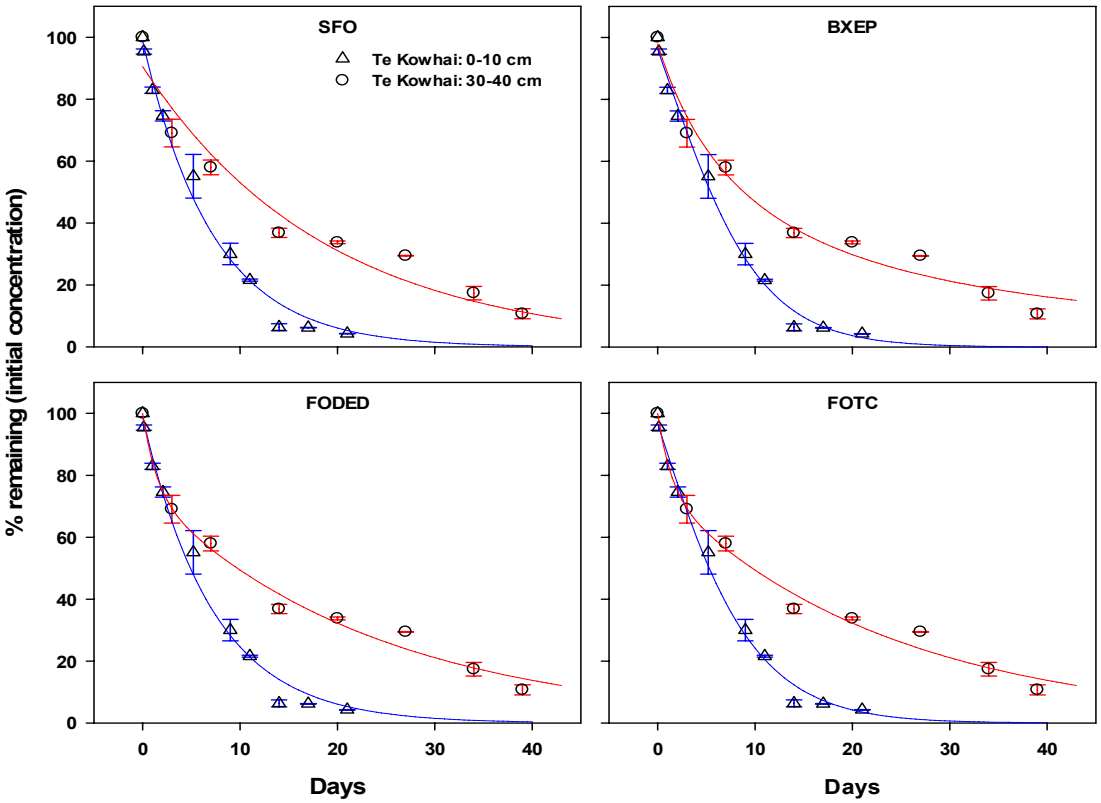
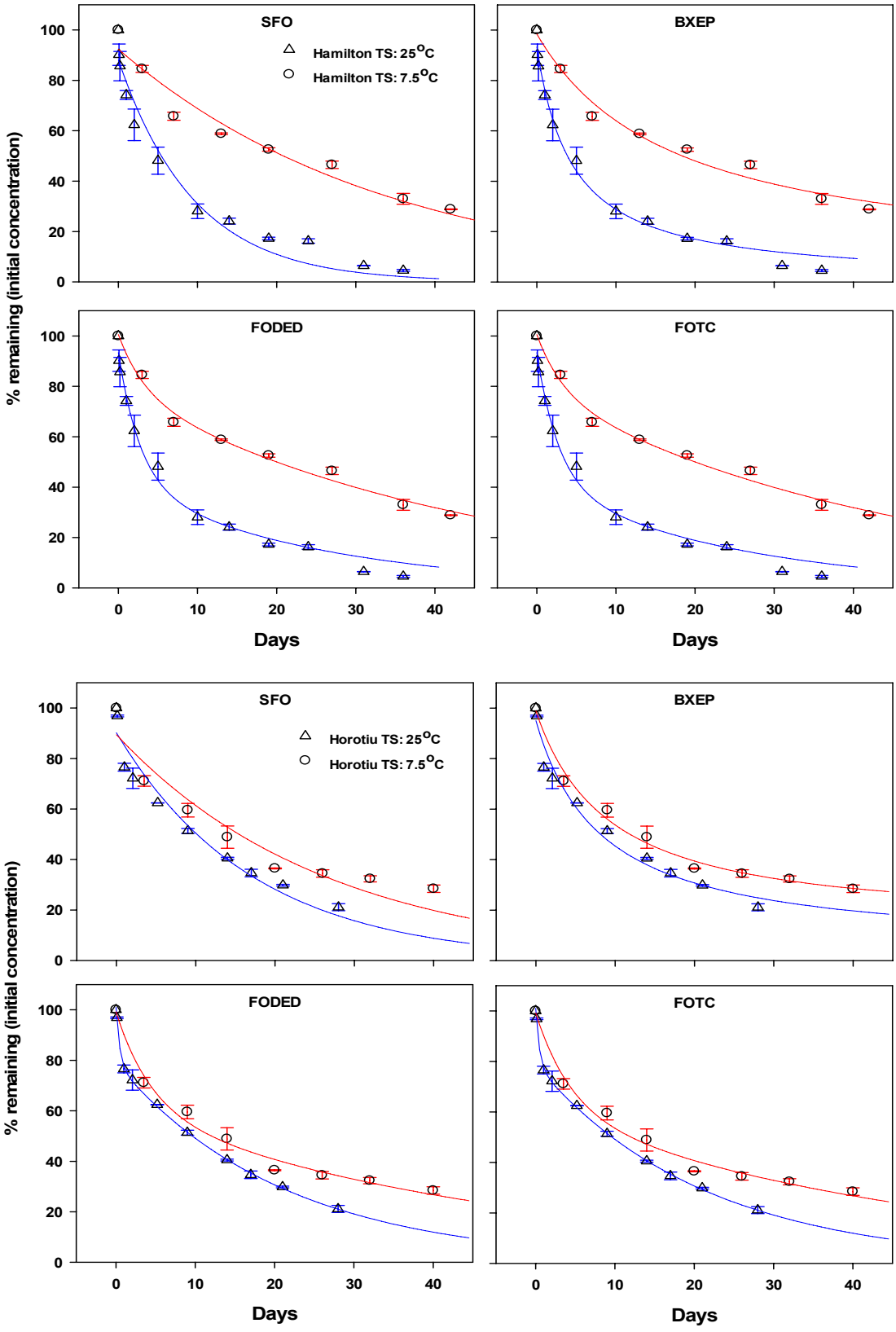
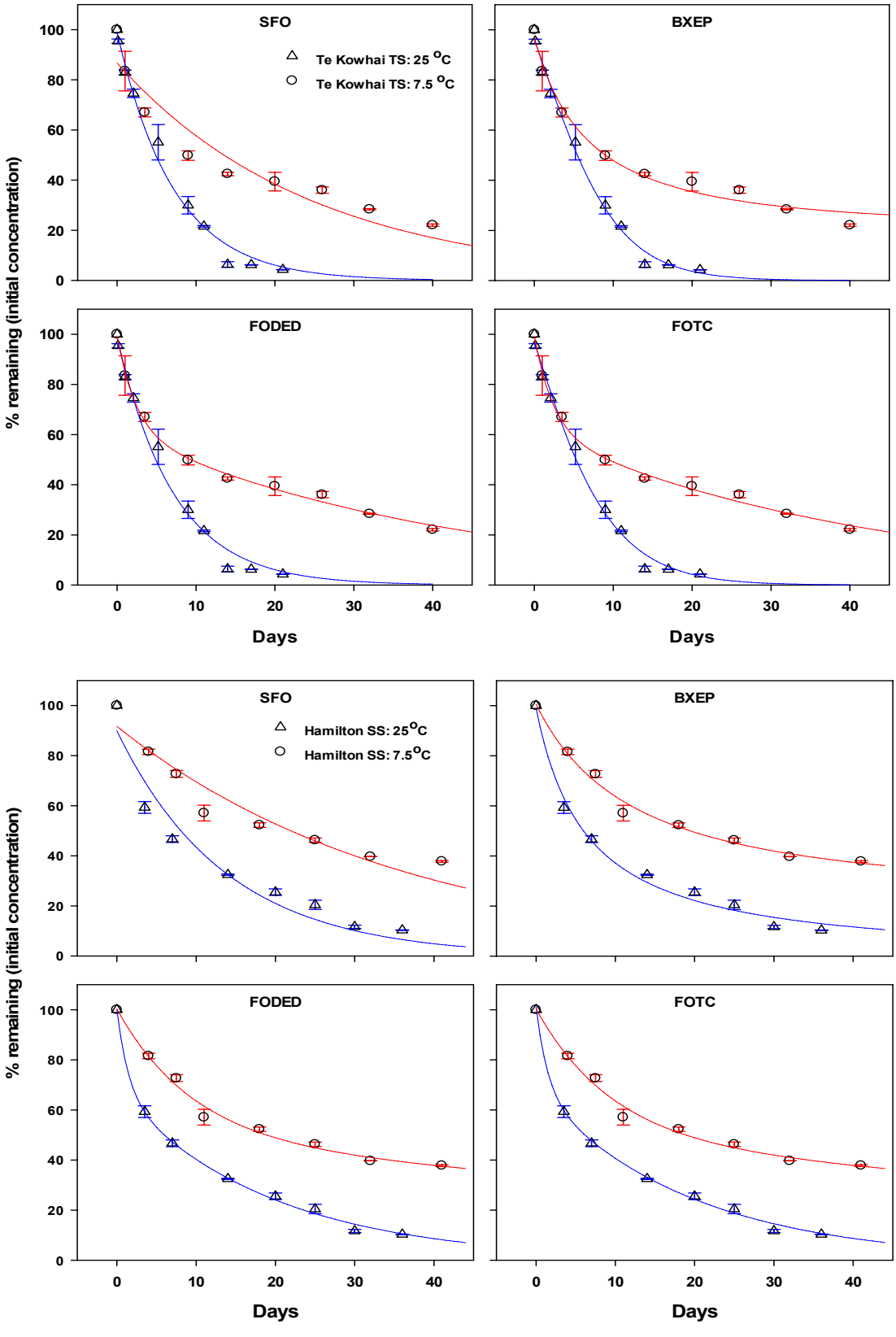


Figure 8.6: Plots of measured residues of SMO antibiotic at different soil depths, at an initial concentration of 0.5 mg kg^{-1} in Hamilton, Horotiu, and Te Kowhai topsoil and subsoil as function of time along with the fit for SFO, BEXP, FODED, and FOTC models. Vertical error bars represent the range of duplicate results for measured values.


SFO
Hamilton TS: 25°C
Hamilton TS: 7.5°C
BXEP
FODED
FOTC
% remaining (initial concentration)
Days
SFO
Horotiu TS: 25°C
Horotiu TS: 7.5°C
BXEP
FODED
FOTC
% remaining (initial concentration)
Days


SFO
Te Kowhai TS: 25 °C
Te Kowhai TS: 7.5 °C
BXEP
FODED
FOTC
% remaining (initial concentration)
Days
SFO
Hamilton SS: 25°C
Hamilton SS: 7.5°C
BXEP
FODED
FOTC
% remaining (initial concentration)
Days

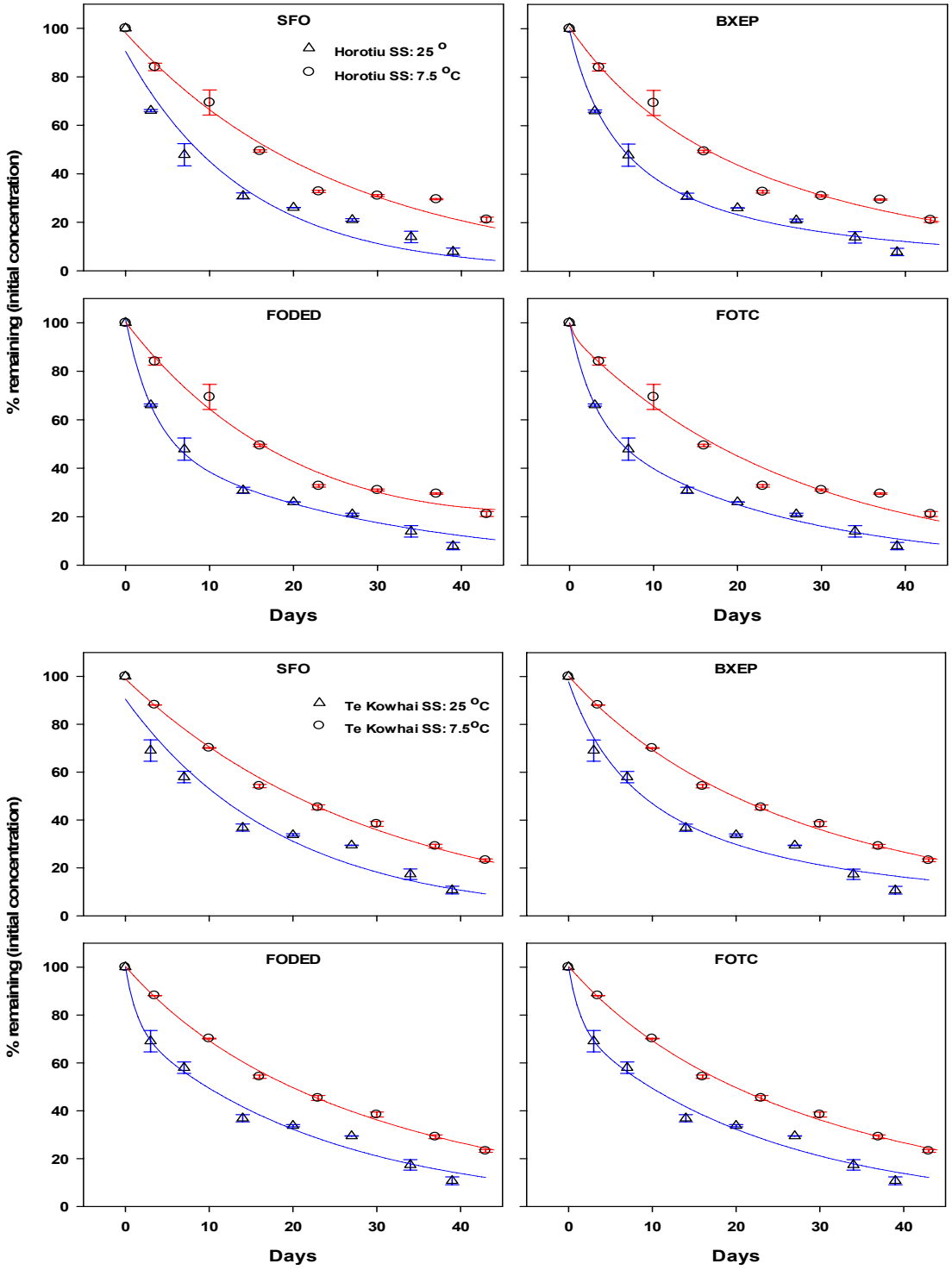
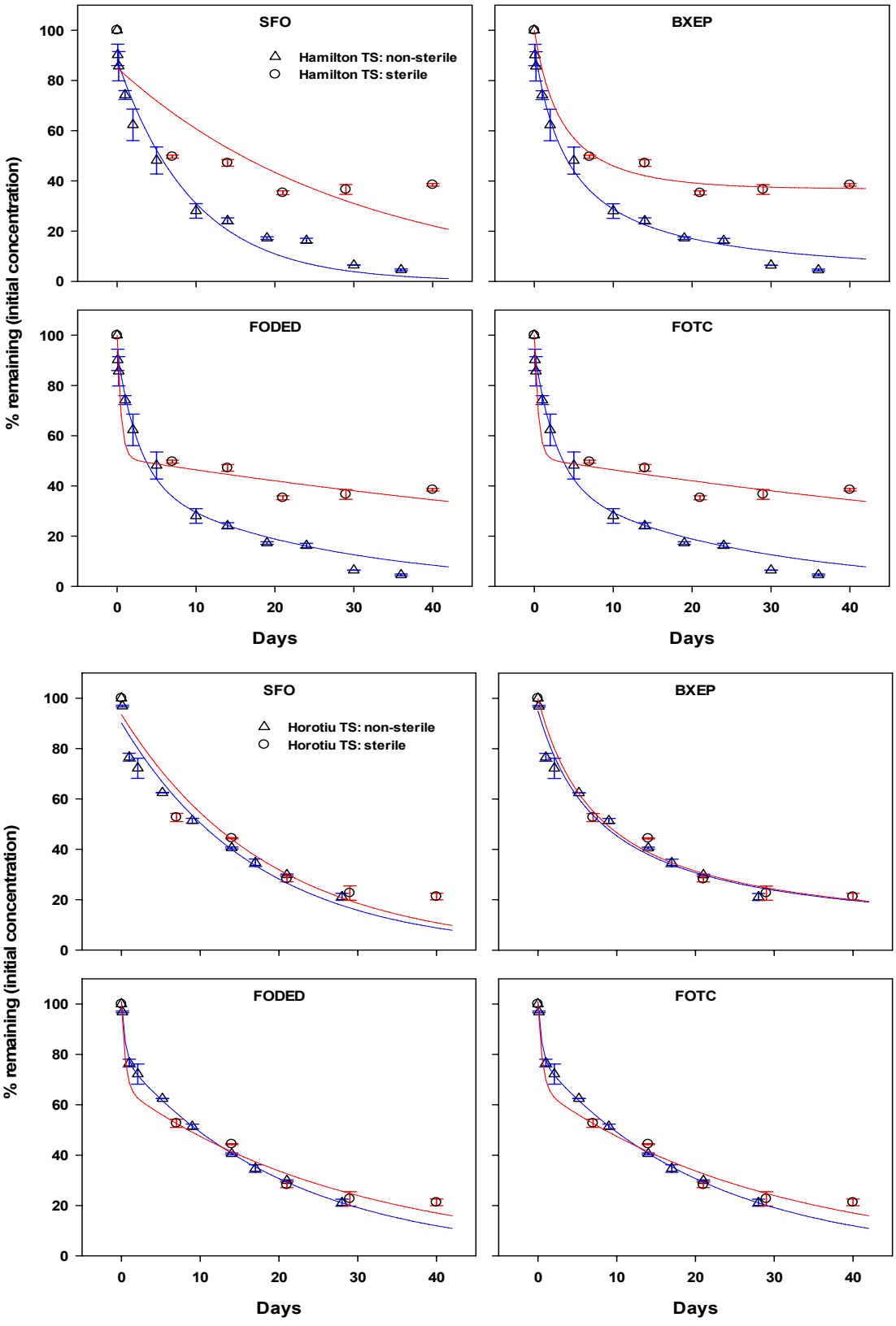
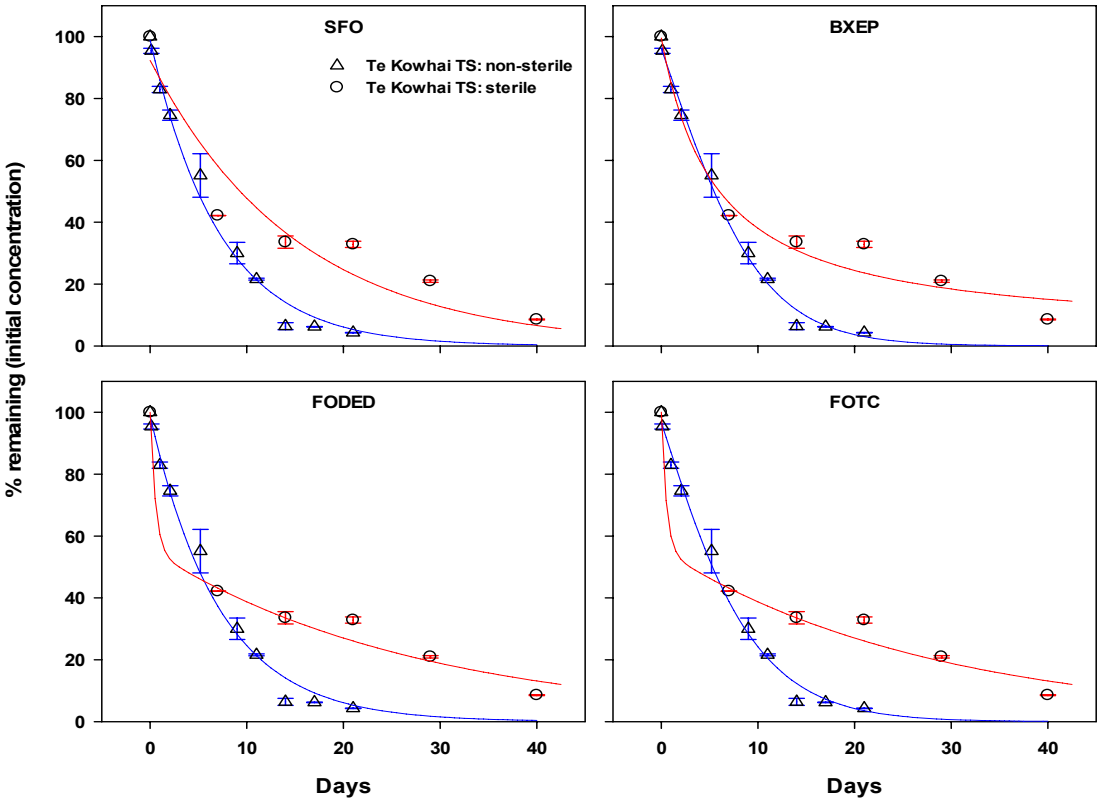
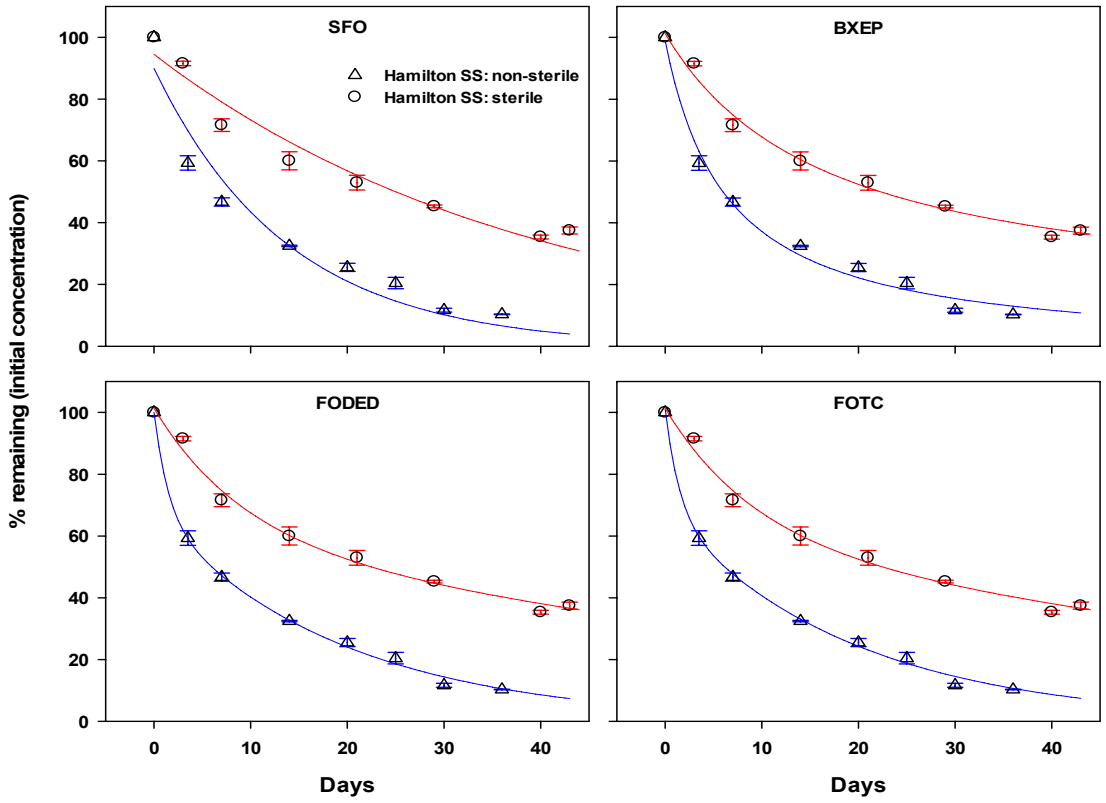


Figure 8.7: Plots of measured residues of SMO antibiotic under different soil temperature regimes, at an initial concentration of 0.5 mg kg^{-1} in Hamilton, Horotiu, and Te Kowhai topsoil and subsoil as function of time along with the fit for SFO, BEXP, FODED and FOTC models. Vertical error bars represent the range of duplicate results for measured values.


SFO
Hamilton TS: non-sterile
Hamilton TS: sterile
BXEP
FODED
FOTC
% remaining (initial concentration)
Days
SFO
Horotiu TS: non-sterile
Horotiu TS: sterile
BXEP
FODED
FOTC
% remaining (initial concentration)
Days


SFO
BXEP
FODED
FOTC
Te Kowhai TS: non-sterile
Te Kowhai TS: sterile
% remaining (initial concentration)
Days
0
20
40
60
80
100
0
10
20
30
40


SFO
BXEP
FODED
FOTC
Hamilton SS: non-sterile
Hamilton SS: sterile
% remaining (initial concentration)
Days
0
20
40
60
80
100
0
10
20
30
40

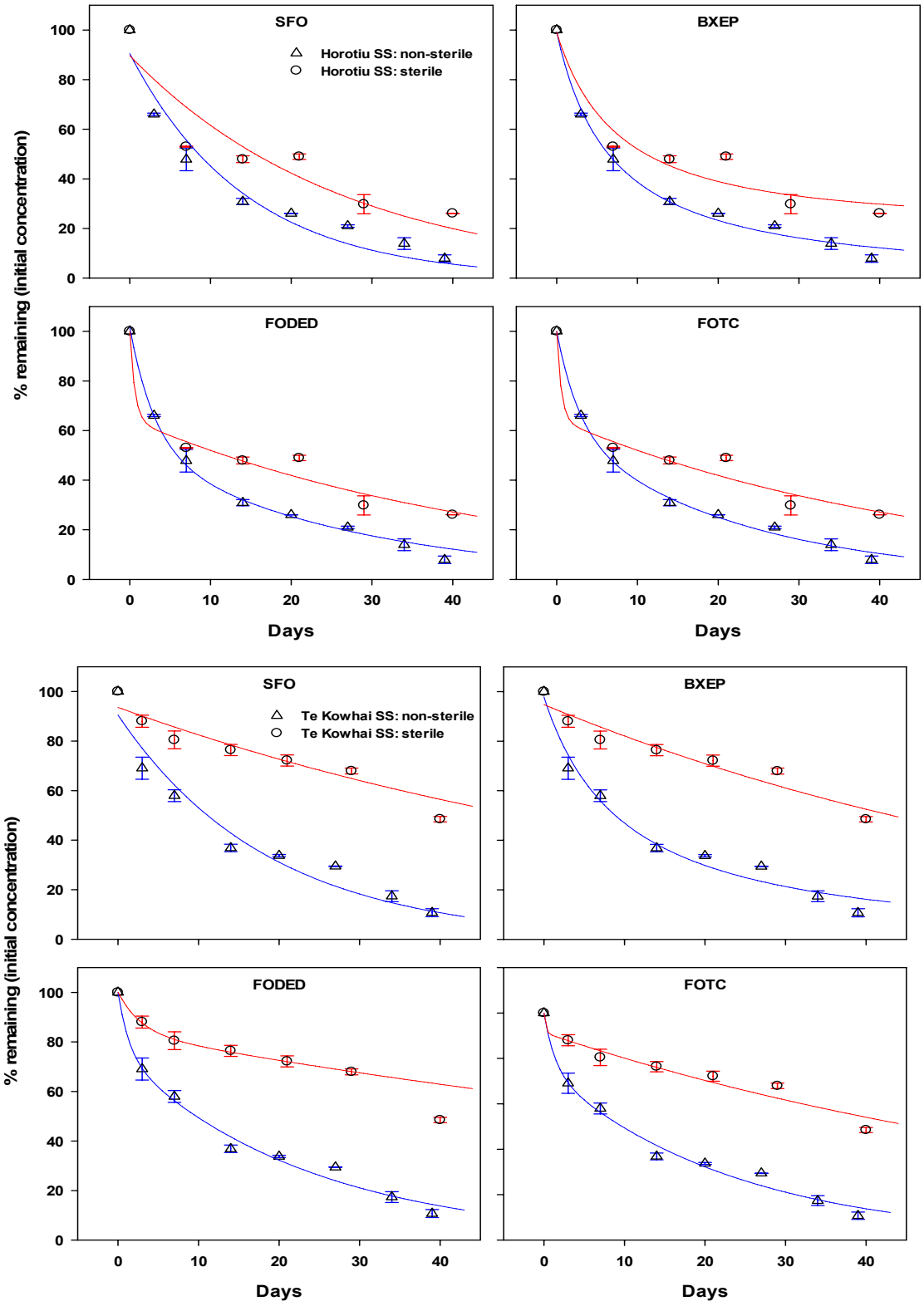


Figure 8.8: Plots of measured residues of SMO antibiotic after soil sterilisation, at an initial concentration of 0.5 mg kg^{-1} in Hamilton, Horotiu and Te Kowhai topsoil and subsoil as a function of time along with the fit for SFO, BEXP, FODED, and FOTC models. Vertical error bars represent the range of duplicate results for measured values.

Table 8.3: Optimised parameters for the models used to fit the degradation kinetics of SMO antibiotic in Hamilton soil under different laboratory conditions.

Hamilton																
Parameters		**25°C; 5 mg kg^{-1}**				**25°C; 0.5 mg kg^{-1}**				**7.5°C; mg kg^{-1}0.5 mg kg^{-1}**				**Sterile; 25°C; 0.5 mg kg^{-1}**			
Models		**TS**		**SS**		**TS**		**SS**		**TS**		**SS**		**TS**		**SS**	
SFO	k_1	0.078	0.004	0.053	0.003	0.104	0.006	0.073	0.002	0.029	0.001	0.028	0.001	0.083	0.000	0.034	0.001
	M_0	3.323	0.027	4.356	0.045	0.379	0.004	0.429	0.009	0.367	0.000	0.445	0.002	0.441	0.008	0.423	0.008
BEXP	k_1	0.044	0.006	0.027	0.006	0.020	0.012	0.008	0.002	0.154	0.003	0.179	0.019	0.490	0.003	0.630	0.009
	k_2	0.004	0.002	-0.005	0.004	0.476	0.117	0.323	0.007	0.005	0.000	-0.023	0.004	-0.037	0.001	-0.115	0.002
	M_0	3.499	0.010	4.627	0.015	0.401	0.009	0.472	0.009	0.391	0.001	0.487	0.008	0.476	0.009	0.497	0.010
FODED	k_1	1.366	0.127	1.094	0.228	1.241	0.409	2.261	0.010	0.311	0.040	0.106	0.001	0.520	0.068	0. 2503	0.032
	k_2	0.049	0.004	0.034	0.002	0.073	0.005	0.051	0.001	0.023	0.001	0.007	0.002	0.046	0.001	0.003	0.000
	M_{sol}	1.102	0.086	1.526	0.149	0.123	0.036	0.162	0.006	0.089	0.001	0.240	0.017	0.179	0.010	0.209	0.011
	M_{sorb}	2.585	0.056	3.362	0.202	0.289	0.030	0.315	0.004	0.311	0.002	0.247	0.024	0.298	0.001	0.288	0.002
FOTC	k_1	0.445	0.063	0.357	0.037	0.387	0.028	1.023	0.025	0.086	0.009	0.058	0.004	0.224	0.025	0.146	0.027
	k_2	0.049	0.004	0.034	0.002	0.073	0.005	0.054	0.001	0.023	0.001	0.007	0.002	0.046	0.001	0.003	0.000
	k_r	0.921	0.064	0.737	0.192	0.854	0.381	2.290	0.010	0.224	0.031	0.049	0.003	0.297	0.669	0.104	0.027
	M_0	3.688	0.030	4.888	0.053	0.412	0.007	0.477	0.011	0.399	0.002	0.487	0.007	0.477	0.011	0.496	0.010

k_1 is the first order rate constant (day^{-1}); k_2 is the second order rate constant (kg mg^{-1}day^{-1}); M_0 is the initial observed concentration (mg kg^{-1})

Table 8.4: DT_{50}, DT_{90}, and DT_{99} (days) values and statistical indices derived during modelling of SMO antibiotic in Hamilton soil under different laboratory conditions . NP = result not possible.

		Hamilton							
		25°C; 5 mg kg^{-1}		**25°C; 0.5 mg kg^{-1}**		**7.5°C; 0.5 mg kg^{-1}**		**Sterile; 25°C; 0.5 mg kg^{-1}**	
Models		TS	SS	TS	SS	TS	SS	TS	SS
SFO	**DT_{50}**	8.7	11.9	6.7	9.5	23.6	25.1	20.6	27.2
	DT_{90}	29.1	39.4	22.4	31.7	78.2	83.3	68.5	90.5
	DT_{99}	58.1	78.8	44.8	63.3	156.5	166.6	136.9	181.0
	SSRes	0.954	2.480	0.008	0.008	0.003	0.006	0.022	0.003
	RMSE	9.7	11.6	9.4	16.9	8.4	9.5	23.9	8.2
	BIC	-25.4	-14.0	-82.8	-51.5	-58.9	-50.9	-30.0	-59.7
	$\chi 2$	0.087	0.093	0.083	0.228	0.056	0.072	0.344	0.053
	Error %	7.01	6.33	6.74	11.66	5.76	6.93	16.09	5.64
	R^2	0.94	0.91	0.97	0.95	0.95	0.92	0.70	0.95
BEXP	**DT_{50}**	6.2	7.9	4.6	6.1	19.0	19.1	5.7	7.6
	DT_{90}	46.5	101.0	32.1	46.8	343.8	**NP**	**NP**	**NP**
	SSRes	0.546	1.631	0.003	0.001	0.001	0.001	0.000	0.001
	RMSE	9.8	12.1	7.3	7.3	5.6	3.5	2.1	5.3
	BIC	-29.6	-16.5	-91.9	-62.8	-52.7	-56.5	-47.7	-46.2
	$\chi 2$	0.116	0.175	0.064	0.043	0.025	0.010	0.002	0.017
	Error %	7.33	8.98	5.41	5.33	4.09	2.56	1.53	3.89
	R^2	0.97	0.94	0.99	0.99	0.98	0.99	1.00	0.99
FODED	**DT_{50}**	6.7	8.9	4.7	6.0	19.7	20.2	5.7	7.4
	DT_{90}	39.6	46.8	27.0	37.1	91.2	231.7	40.2	422.3
	SSRes	0.221	1.014	0.002	0.000	0.001	0.001	0.000	0.001
	RMSE	6.3	9.5	5.8	3.3	4.0	3.4	0.6	5.8
	BIC	-38.0	-19.7	-95.0	-73.3	-58.4	-57.1	-71.1	-43.4
	$\chi 2$	0.047	0.108	0.040	0.009	0.012	0.009	0.000	0.020
	Error %	4.84	7.35	4.47	2.59	3.07	2.61	0.47	4.69
	R^2	0.99	0.96	0.99	1.00	0.99	0.99	1.00	0.98
FOTC	**DT_{50}**	6.7	8.9	4.7	6.0	19.7	18.7	5.7	7.4
	DT_{90}	35.3	46.8	27.0	37.1	91.2	226.0	40.2	422.4
	SSRes	0.221	1.014	0.000	0.000	0.001	0.001	0.000	0.001
	RMSE	6.3	9.5	5.8	3.3	4.0	3.4	0.6	5.8
	BIC	-38.0	-19.7	-95.0	-73.3	-58.4	-57.1	-57.9	-43.4
	$\chi 2$	0.047	0.108	0.040	0.009	0.012	0.009	0.000	0.020
	Error %	4.84	7.35	4.47	2.59	3.07	2.61	0.56	4.69
	R^2	0.99	0.96	0.99	1.00	0.99	0.99	1.00	0.98

Table 8.5: Optimised parameters for the models used to fit the degradation kinetics of SMO antibiotic in Te Kowhai soil under different laboratory conditions.

Te Kowhai													
Parameters		**25°C; 0.5 mg kg^{-1}**				**7.5°C; 0.5 mg kg^{-1}**				**Sterile; 25°C; 0.5 mg kg^{-1}**			
Models		**TS**		**SS**		**TS**		**SS**		**TS**		**SS**	
SFO	k_1	0.139	0.004	0.053	0.000	0.041	0.000	0.034	0.000	0.066	0.003	0.015	0.000
	M_0	0.302	0.002	0.415	0.009	0.335	0.001	0.400	0.000	0.354	0.004	0.441	0.002
BEXP	k_1	0.206	0.037	0.210	0.017	0.429	0.051	0.031	0.004	0.456	0.012	0.015	0.014
	k_2	-0.387	0.226	0.010	0.002	-0.037	0.008	0.026	0.001	-0.010	0.000	-0.001	0.042
	M_0	0.294	0.006	0.449	0.007	0.375	0.003	0.405	0.000	0.382	0.003	0.441	0.009
FODED	k_1	0.139	0.004	0.719	0.364	0.610	0.280	0.082	0.016	1.830	0.024	2.666	0.037
	k_2	0.139	0.004	0.041	0.002	0.025	0.002	0.028	0.003	0.035	0.001	0.011	0.002
	M_{sol}	0.044	0.001	0.124	0.012	0.142	0.004	0.082	0.049	0.168	0.004	0.048	0.024
	M_{sorb}	0.259	0.001	0.335	0.008	0.246	0.014	0.323	0.050	0.215	0.001	0.417	0.012
FOTC	k_1	0.095	0.029	0.802	0.103	0.234	0.093	0.040	0.001	0.890	0.010	0.404	0.175
	k_2	0.215	0.017	0.044	0.001	0.025	0.002	0.028	0.003	0.035	0.001	0.013	0.000
	k_r	0.120	0.046	2.674	0.033	0.376	0.187	0.068	0.040	1.095	0.042	4.042	0.151
	M_0	0.295	0.006	0.459	0.004	0.388	0.010	0.405	0.001	0.384	0.003	0.465	0.012

k_1 is the first order rate constant (day^{-1}); k_2 is the second order rate constant (kg mg^{-1}day^{-1}); M_0 is the initial observed concentration (mg kg^{-1})

Table 8.6: DT_{50}, DT_{90}, and DT_{99} (days) values and statistical indices derived during modelling of SMO antibiotic in Te Kowhai soil under different laboratory conditions. NP = result not possible.

Te Kowhai							
		25°C; 0.5 mg kg^{-1}		**7.5°C; 0.5 mg kg^{-1}**		**Sterile; 25°C; 0.5 mg kg^{-1}**	
Models		TS	SS	TS	SS	TS	SS
SFO	**DT_{50}**	5.0	13.0	17.0	20.5	10.5	54.9
	DT_{90}	16.5	43.1	56.6	68.1	34.9	182.2
	DT_{99}	33.1	86.3	113.2	136.2	69.8	364.5
	SSRes	0.001	0.006	0.007	0.000	0.007	0.003
	RMSE	6.4	13.5	13.9	2.8	22.5	6.1
	BIC	-84.9	-53.3	-52.0	-76.7	-36.9	-53.7
	$\chi 2$	0.041	0.146	0.155	0.006	0.304	0.026
	Model error	4.52	9.33	9.59	1.94	15.13	4.17
	R^2	0.99	0.95	0.91	1.00	0.91	0.95
BEXP	**DT_{50}**	5.7	9.3	9.7	19.7	6.1	46.8
	DT_{90}	15.0	63.3	NA	74.3	75.4	147.7
	SSRes	0.001	0.003	0.001	0.000	0.000	0.003
	RMSE	6.1	9.0	5.9	2.3	2.2	5.5
	BIC	-87.4	-75.2	-73.1	-78.1	-63.3	-49.4
	$\chi 2$	0.038	0.065	0.032	0.004	0.003	0.021
	Model error	4.52	6.59	4.35	1.66	1.58	3.97
	R^2	0.99	0.98	0.98	1.00	1.00	0.92
FODED	**DT_{50}**	5.0	9.7	3.4	19.7	3.0	72.1
	DT_{90}	16.5	47.5	56.0	72.3	47.6	297.8
	SSRes	0.001	0.002	0.000	0.000	0.001	0.000
	RMSE	7.8	6.8	3.0	2.2	8.2	7.2
	BIC	-82.6	-81.1	-83.2	-76.2	-45.5	-82.9
	$\chi 2$	0.061	0.036	0.008	0.004	0.04	0.036
	Model error	6.04	5.24	2.32	1.74	6.61	5.66
	R^2	0.99	0.99	1.00	1.00	0.99	1.00
FOTC	**DT_{50}**	5.5	9.7	9.6	19.7	3.0	46.0
	DT_{90}	15.4	47.5	76.7	72.2	47.6	168.9
	SSRes	0.001	0.002	0.000	0.000	0.001	0.002
	RMSE	6.5	6.8	3.0	2.2	8.2	4.7
	BIC	-86.4	-81.1	-83.2	-76.2	-45.5	-41.9
	$\chi 2$	0.042	0.036	0.008	0.004	0.04	0.013
	Model error	4.98	5.24	2.32	1.74	6.61	3.81
	R^2	0.99	0.99	1.00	1.00	0.99	0.95

Table 8.7: Optimised parameters for the models used to fit the degradation kinetics of SMO antibiotic in Horotiu soil under different laboratory conditions.

Horotiu													
Parameters		**25°C; 0.5 mg kg^{-1}**				**7.5°C; 0.5 mg kg^{-1}**				**Sterile;25°C; 0.5 mg kg^{-1}**			
Models		**TS**		**SS**		**TS**		**SS**		**TS**		**SS**	
SFO	k_1	0.058	0.001	0.070	0.001	0.038	0.003	0.039	0.001	0.054	0.003	0.038	0.002
	M_0	0.224	0.001	0.400	0.000	0.292	0.010	0.344	0.000	0.440	0.006	0.418	0.013
BEXP	k_1	0.526	0.003	0.321	0.062	0.361	0.010	0.081	0.031	0.274	0.007	0.307	0.016
	k_2	-0.009	0.000	0.009	0.012	-0.023	0.000	0.023	0.005	-0.009	0.005	-0.035	0.005
	M_0	0.236	0.001	0.438	0.008	0.322	0.012	0.354	0.005	0.470	0.004	0.461	0.012
FODED	k_1	2.087	0.102	0.481	0.163	0.742	0.590	1.615	0.008	2.085	0.000	1.637	0.017
	k_2	0.047	0.001	0.045	0.009	0.020	0.007	0.038	0.001	0.034	0.002	0.022	0.002
	M_{sol}	0.055	0.006	0.167	0.049	0.128	0.051	0.185	0.157	0.156	0.002	0.165	0.003
	M_{sorb}	0.196	0.002	0.275	0.043	0.197	0.043	0.166	0.162	0.315	0.003	0.301	0.008
FOTC	k_1	0.498	0.063	0.193	0.021	0.250	0.163	0.059	0.003	0.634	0.003	0.662	0.004
	k_2	0.047	0.001	0.045	0.009	0.020	0.007	0.038	0.001	0.034	0.002	0.022	0.002
	k_r	1.589	0.039	0.288	0.142	0.632	0.568	0.847	0.576	1.206	0.002	1.169	0.002
	M_0	0.251	0.004	0.442	0.006	0.325	0.008	0.351	0.005	0.471	0.005	0.466	0.011

k_1 is the first order rate constant (day^{-1}); k_2 is the second order rate constant (kg mg^{-1}day^{-1}); M_0 is the initial observed concentration (mg kg^{-1})

Table 8.8: DT_{50}, DT_{90}, and DT_{99} (days) values and statistical indices derived during modelling of SMO antibiotic in Horotiu soil under different laboratory conditions.

		Horotiu					
		25°C; 0.5 mg kg^{-1}		**7.5°C; 0.5 mg kg^{-1}**		**Sterile; 25°C; 0.5 mg kg^{-1}**	
Models		TS	SS	TS	SS	TS	SS
SFO	**DT_{50}**	11.9	10.0	18.4	17.8	12.9	18.5
	DT_{90}	39.6	33.1	61.2	59.1	42.7	61.3
	DT_{99}	79.2	66.2	122.3	118.3	85.5	122.6
	SSRes	0.002	0.006	0.004	0.002	0.007	0.011
	RMSE	8.6	16.0	12.6	7.6	15.6	17.8
	BIC	-80.4	-53.2	-57.6	-64.3	-37.4	-32.6
	χ^2	0.071	0.205	0.127	0.046	0.147	0.19
	Model error	6.06	11.03	8.69	5.24	10.51	12.93
	R^2	0.95	0.95	0.92	0.98	0.93	0.86
BEXP	**DT_{50}**	9.1	6.5	12.2	16.2	8.7	11.3
	DT_{90}	141.5	48.4	**NP**	69.9	130.0	**NP**
	SSRes	0.001	0.001	0.000	0.001	0.001	0.004
	RMSE	7.1	6.0	4.5	6.6	6.3	11.2
	BIC	-84.5	-66.9	-71.9	-64.4	-46.5	-38.2
	χ^2	0.051	0.028	0.016	0.035	0.024	0.075
	Model error	5.24	4.34	3.29	4.83	4.58	8.14
	R^2	0.98	0.99	0.99	0.98	0.99	0.94
FODED	**DT_{50}**	9.43	5.68	12.28	15.98	8.46	11.81
	DT_{90}	43.55	44.95	88.41	47.87	55.56	86.20
	SSRes	0.000	0.001	0.001	0.001	0.001	0.002
	RMSE	1.4	5.2	4.8	6.0	6.5	7.7
	BIC	-115.0	-67.2	-68.7	-63.8	-44.3	-40.8
	χ^2	0.002	0.021	0.019	0.029	0.025	0.036
	Model error	1.06	4.01	3.76	4.68	5.23	6.25
	R^2	1.00	1.00	0.99	0.99	0.99	0.97
FOTC	**DT_{50}**	9.4	6.2	12.3	17.2	8.5	11.1
	DT_{90}	43.5	40.9	88.4	60.1	55.6	79.8
	SSRes	0.000	0.000	0.001	0.001	0.001	0.002
	RMSE	1.4	4.1	4.8	7.3	6.5	8.1
	BIC	-115.0	-71.0	-68.7	-60.7	-44.3	-40.3
	χ^2	0.002	0.013	0.019	0.043	0.025	0.039
	Model error	1.06	3.15	3.76	5.68	5.23	6.54
	R^2	1.00	1.00	0.99	0.98	0.99	0.97

8.5.4 Box–whisker plots

Box–whisker and residual plots (Figures 8.9–8.11) showed that the residual ranges for all four models were very small, ranging from -0.06 to 0.04, which meant that all four models were good in predicting the SMO dissipation values. However, for the purpose of comparison, FOTC and FODED models were superior in predicting the measured values for SMO under most treatment conditions. For example, for SMO in Hamilton and Horotiu soils, the median of the FOTC and FODED model is in excellent agreement with the zero line, followed closely by BXEP, and SFO model (Figure 8.9–8.10). However, FOTC and FODED model predicted values deviated considerably for the observed value in two cases – one for Hamilton soil at 5 mg kg^{-1} and another for Te Kowhai soil under sterile conditions as noticed by the two large outliers present in the FOTC and FODED model. Under these conditions, Box–whisker and residual plots showed the BXEP model to be a better model than the FOTC and FODED models (Figures 8.10–8.11). However, both models performed equally well when the outliers were removed. These results also support the earlier discussions about the FOTC being the strongest and SFO the weakest models.

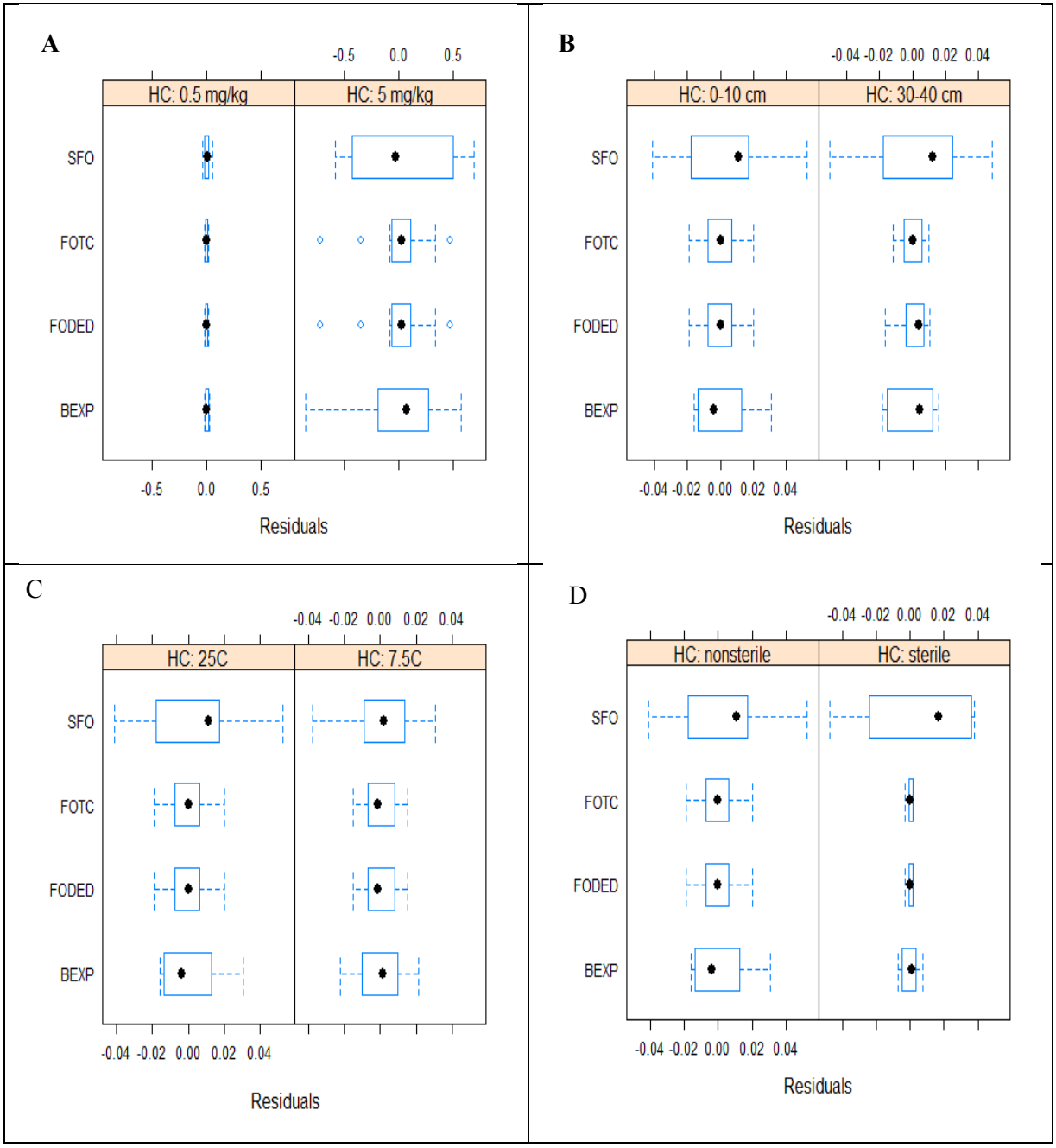


Figure 8.9: Box–whisker plots for the residuals (measured–predicted) of SMO dissipation in Hamilton soil under varying concentration (A), depth (B), temperature (C) and sterilisation (D) for each model. Boxes cover the medial 50% of the data with the median dividing the box. Whiskers range to the largest and smallest points within 1.5 times the interquartile distance, and outliers are shown as circles beyond the whiskers.

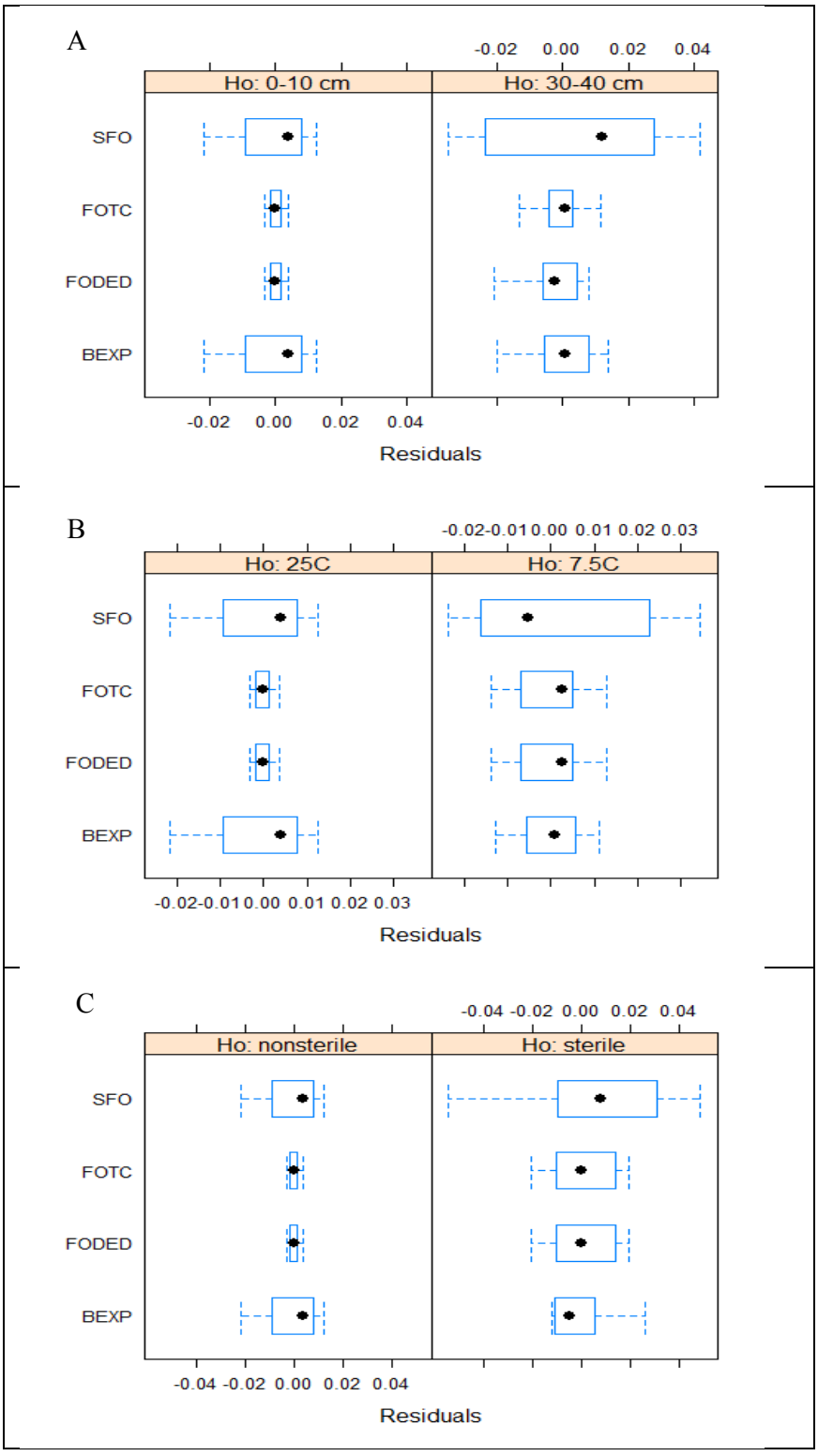


Figure 8.10: Box–whisker plots for the residuals (measured–predicted) of SMO dissipation in Horotiu soil under varying depth (A), temperature (B), and sterilisation (C) for each model.

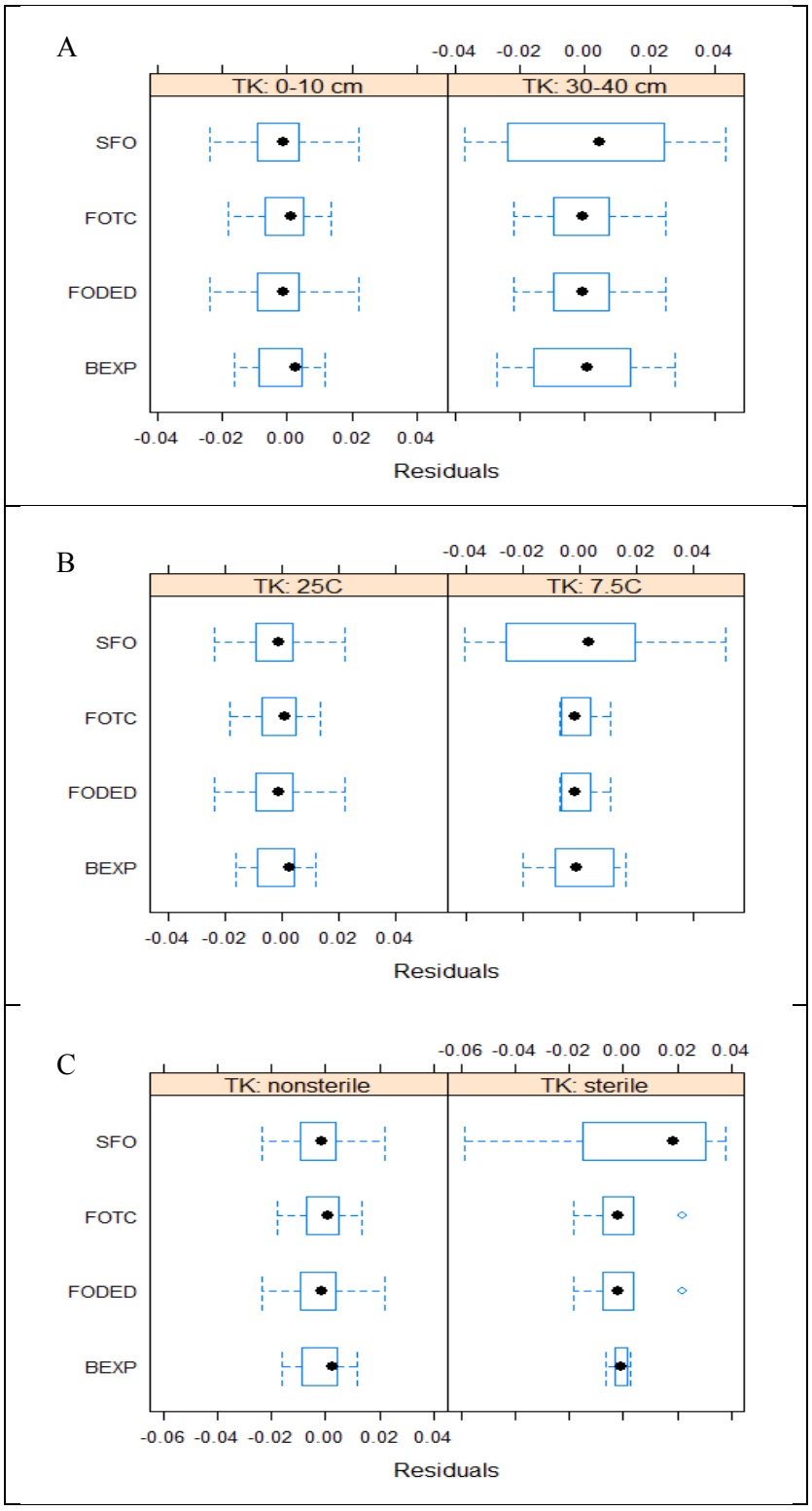


Figure 8.11: Box–whisker plots for the residuals (measured–predicted) of SMO dissipation in Te Kowhai soil under varying depth (A), temperature (B), and sterilisation (C) for each model.

8.6 Comparison with available literature

Biphasic models have been previously used to describe and predict the degradation kinetics of many organic contaminants such as herbicides (Sarmah and Rohan 2011) and steroid hormones (Scherr *et al.* 2008). However, very few published studies are available on antibiotic degradation where a kinetic model, called the "availability-adjusted first-order model" has generally been used. The model is based on the assumption of the availability of target compound during the degradation process, and Wang *et al.* (2006b) used this model to fit measured datasets of sulfadimethoxine degradation in manure-amended soil with resultant $R^2 > 0.99$. However, as this model is a modified form of the first order kinetic equation, and not biphasic in nature, it only accounts for the reduction in degradation due to sorption. In another separate study, apparent zeroth-order degradation kinetics were also found to be useful to describe the degradation of three sulfonamide antibiotic, including SMO in activated sludge (Yang *et al.* 2012). In this case it could be possible that very rapid degradation was due to the abundance of microbes in sewage sludge. This rapid degradation could have resulted in the authors fitting the data with zero order kinetics, where the rate is independent of the concentration of the antibiotic.

Ingerslev and Halling-Sørensen (2000) studied the degradation properties of sulfonamides in activated sludge by fitting the degradtion curves with a logistic model. The authors noticed long lag phases and degradation rates indicating growth of specific degraders. Only one study used a biphasic model namely, a pseudo-first-order rate kinetic model to study the degradation kinetics of SDZ in manure-amended soils (Hammesfahr *et al.* 2011). This model was similar to the BEXP model used in this present study, and the authors reported DT_{50}value for SDZ to be 1–2 days, which was one order of magnitude higher compared to the DT_{50} values (15–20 days) estimated by the BEXP model for SMO degradation in this present study. Antibiotic degradation in soils is known to be slower than in manure, and recent study has shown that sulfadimethoxine degradation could be accelerated in lab-scale tests with increasing manure content in amended soil (Wang *et al.* 2006a). All these studies mentioned above have used different models or different antibiotic or have conducted experiments in different matrix **(**Table 2.9**)**; and as a result a comparison with the present study was difficult.

8.7 Mechanism of biphasic models.

Of the three biphasic models used to fit the observed data, it is worth noting that two of the models are empirical in nature. A mechanistic explanation is not available for BEXP and FODED models, while the two-compartment (FOTC) model can be conceived to have some basis for a mechanistic interpretation of the data (Sarmah and Rohan 2011). The FOTC model provides separate rate constants for each compartment—slow and fast. Sarmah and Rohan (2011) suggest that in the 'fast' compartment it is assumed that compound degradation takes place mainly through surface losses where a combination of physical, photochemical and volatilization processes take place when the compound residues are initially deposited after application. In the 'slow' compartment, degradation is assumed to take place at a much slower rate and is mainly affected by biotic, abiotic and enzymatic hydrolysis. In addition, there is continuous transfer of residues taking place between the two compartments, and their flow is governed by the concentration gradient in each compartment.

While a two compartment (FOTC) mechanism provides an explanation for a non-linear degradation pattern, the possibilities of the involvement of other mechanisms cannot be ruled out. For example, microorganisms may increase or decrease over time, more than two compartments may exist in the soil, and multiple mechanisms of decay may be operating. It is also worth noting that simply fitting the data sets to the model does not guarantee a specific mechanism. This is substantiated by the good fit of the non-mechanistically based models (BEXP and FODED) for the datasets used in the present study. Also due to their slightly different structures and surface charges (from predominantly negative to neutral), the three sulfonamides not necessarily follow the same degradation pathways. The capacity of one model to fit the degradation kinetics data of all the three sulfonamides together may not be a reasonable test to validate the assumption behind that model.

8.8 Summary

The performance of three biphasic degradation models (biexponential, first-order double exponential decay, and first-order two-compartment) was evaluated in comparison with linear simple first order kinetics model. These models were used to describe the degradation kinetics for three sulfonamides in non-sterile and sterile water and only for SMO antibiotic in

three different soils under varying concentration, depth, temperature, and sterile conditions. The fit of each model to the measured values was tested using an array of statistical indices to judge the model's ability to fit the measured datasets. Corresponding 50% (DT_{50}) and 90% (DT_{90}) dissipation times for the antibiotics were numerically obtained and compared against those obtained from using simple first order model. A visual examination of observed and fitted plots as well as the statistical indices showed that all the three biphasic models performed better than the simple first order model. The SMO degradation parameters and endpoints predicted by the FODED and FOTC models were the best, though similar, and they were both better than the biexponential model, and this was supported by the low statistical indices and the box–whiskers residual plots generated for each model. The results were as expected as FOTC and FODED models have 4 parameters compared with the BEXP model, which has only 3 parameters and hence the better fit. These model-derived endpoints have been statically tested for their robustness, and can be used as input parameters often required for risk assessment purposes and in many regulatory modelling exercises. The conclusions can be summarised as follows:

- SMO and SM appear to dissipate more slowly in water than SCP. Sulfonamide degradation in soil was much faster when compared with that in aqueous matrix, with a clear biphasic degradation pattern.
- Overall, FODED and FOTC models were the best models for SMO degradation under all varying conditions.
- Degradation endpoints and parameters predicted by FODED and FOTC were similar, while the BEXP model failed to predict degradation endpoints in most cases.
- The performance of the models used to fit an observed dataset should be evaluated using statistical indices in order to avoid bias in the estimation of the end-points for the compounds.
- Model selection should be based on an understanding of the mechanisms that are contributing to a particular degradation pattern along with the fit of the model with observed data sets.

Chapter 9: Transport and retention of sulfonamide antibiotics in two different soil lysimeters.

9.1 Introduction

Sulfonamides are one of the most commonly used veterinary antibiotics for therapeutic and sub-therapeutic purposes (Sukul and Spiteller 2006). In New Zealand, application of animal wastes containing veterinary antibiotic onto agricultural lands (Sarmah *et al.*, 2006) is a common practice. This, along with the direct excretal inputs from the grazing animals, constitutes a direct source of exposure for antibiotics in the pasture environment. This makes the assessment of its retardation and mobility in soil environment necessary to establish their risk to humans and environment. Strongly adsorbing veterinary antibiotic like tylosin can accumulate in the top layer of the soil (Hu and Coats 2009; Kurwadkar *et al.* 2011); however, sulfonamides are known to be highly mobile and to have low sorption affinity (Tolls 2001; Boxall *et al.* 2002). They have the potential to leach to the groundwater (Hirsch *et al.* 1999) and be transported with the groundwater, drainage water, and surface runoff to surface waters (Kay *et al.* 2005b).

Widespread occurrence of sulfonamides in aquatic and terrestrial environments has been reported in a number studies (Batt *et al.* 2006; Garcia-Galan *et al.* 2011; Kim *et al.* 2011). For example sulfamethoxazole antibiotic, which belongs to this group, was detected at a concentration of 73 μg kg^{-1}in Swiss waste water plants (Diaz-Cruz *et al.* 2006), up to 2.0 μg L^{-1} in STP effluents, and up to 0.48 μg L^{-1} in surface water samples in Germany (Hirsch *et al.* 1999) and at 0.05-0.09 μg L^{-1} in different STP effluents across Europe (Andreozzi *et al.* 2003). Sulfamethoxazole was also detected in groundwater at a concentration of 0.22 μg L^{-1} (Lindsey *et al.* 2001) and in monitoring well samples (Barber *et al.* 2009) and streams (Kolpin *et al.* 2002) in the USA. Therefore, there is a possible exposure pathway for aquatic organisms and also the possibility that these chemicals be may be encountered in drinking water.

Sulfonamides are known be fairly stable in the treated animal, in manure, soil and during water purification processes (Ingerslev and Halling-Sørensen 2000). It is very likely that

sulfonamides (from various sources) may eventually end up in sewage treatment plants and when their rate of biodegradation is low they may even pass through sewage treatment plants as a result of their non-sorptive properties (Ingerslev and Halling-Sørensen 2000).
In the last decade a number of laboratory miscible displacement studies (Wehrhan *et al.* 2007; Unold *et al.* 2010; Kurwadkar *et al.* 2011; Strauss *et al.* 2011) and field-based studies (Kreuzig *et al.* 2005; Kay *et al.* 2005a; Weiss *et al.* 2008) have investigated the transport characteristics of veterinary antibiotics. Through some studies in the UK it was demonstrated that weak acids such as sulfonamides and oxytetracycline have a high potential to be transported to surface waters (Boxall *et al.* 2002; Kay *et al.* 2004). In contrast, tylosin was not detected in soil extracts (Hu and Coats 2009), which was attributed to its rapid degradation (Ingerslev and Halling-Sørensen 2000). A summary of results from laboratory lysimeter and field studies with veterinary antibiotics have been presented in Chapter 2 (Table 2.10)**.**

Recent studies have shown that both physical and chemical non-equilibrium processes contribute to enhanced antibiotic transport (Fan *et al.* 2011; Jeong *et al.* 2012).The transport of veterinary antibiotics in soils depends on several factors such as the chemical properties of the antibiotics, temperature, moisture content, ionic strength (Chen *et al.* 2011), soil charge density (Strauss *et al.* 2011), and contact time (Kurwadkar *et al.* 2011). Chemical properties such as water solubility, dissociation constants, sorption–desorption processes, stability and binding to the soils, and the partitioning coefficients at various pH values can all affect the mobility of antibiotics in the soil environment (Tolls 2001; Unold *et al.* 2010; Kurwadkar *et al.* 2011). Other factors that can influence the mobility of veterinary antibiotics are the timing of manure application, prevailing weather conditions, and preferential flow *via* desiccation cracks and worm channels as demonstrated in a UK field study (Kay *et al.* 2004; Kay *et al.* 2005a).

Although the mobility of pesticides and steroid hormones in New Zealand soils has been previously investigated (Sarmah *et al.* 2005; Scherr 2009), there is currently no information available about the transport of sulfonamide antibiotics in New Zealand soils. Therefore, the main objective of this study was to investigate the transport of three sulfonamide antibiotics (SMO, SCP and SM), in two different undisturbed soil columns under saturated conditions. In a recent study it was observed that the mobility of mixed sulfonamides was not impacted by competitive sorption (Kurwadkar *et al.* 2011), which meant that the mobility of all the

three sulfonamides could be evaluated simultaneously relative to a conservative bromide tracer.

9.2 Materials and methods

9.2.1 Preparation of the soil lysimeters

The soil lysimeters used in this study were made of polyvinylchloride (PVC) with a length of 20 cm and an internal diameter of 7.5 cm. Undisturbed soil cores of Matawhero silt loam and Hamilton clay loam were obtained *in situ* by hand carving from the ground surface. The internal cylindrical surface of the lysimeter casings were coated with petroleum jelly and the casings were slowly pushed into the soil under pressure. When the desired length of core was reached (18 cm) the lysimeters were carefully cut off from the bottom with a sharp metal blade.

Immediately after excavation, both lysimeters were covered with polythene sheet and PVC end caps were attached to prevent water loss. The lysimeters were transported to the laboratory and the bottom of the cores were cleaned and secured with a fly screen (ø 1mm) to allow leachate percolation, and sealed with a PVC cap containing a number of holes that acted as sampling ports for leachate collection. On the outer edge of the cores, molten petroleum jelly was poured slowly to seal the gaps between the PVC column and soil to prevent edge flow during the experiment. The lysimeters were then slowly saturated over a 24 h period by standing the columns in two large trays containing water; this reduced the air trapped and also maintained soil structure (Fan *et al.* 2011). Soil column physical parameters such as porosity, bulk density, water content and the pore volumes for each soil lysimeter are summarised in Table 9.1.

9.2.2 Transport experiment

The soil lysimeters were set up in a constant temperature room (22^{o}C ±2) and transport studies were performed with three sulfonamide antibiotics SMO, SCP, and SM in both soil cores. The mediator solution used for the experiment consisted of 5 mM $CaCl_2$ solution. The experimental setup consisted of a wooden rack on which the saturated lysimeter

was placed. The top open end of the lysimeter was fitted with a tight cylindrical Teflon® compartment equipped with an irrigation head comprised of a reservoir and its lid consisting of 41 hypodermic needles to irrigate the lysimeter uniformly (Figure 9.1). The irrigation head was connected to a peristaltic pump (Cole-Parmer Instrument Company) by means of appropriate tubing and the pump supplied a constant head of 5 mM of $CaCl_2$ solution from a reservoir. The tubes and other accessories of the lysimeter were covered with aluminium foil to avoid photodegradation. Once on the wooden rack, as shown in Figure 9.1, a collecting funnel was attached to the bottom of the soil column. The leachate was then collected into a beaker from which leachate fractions were sampled at regular intervals.

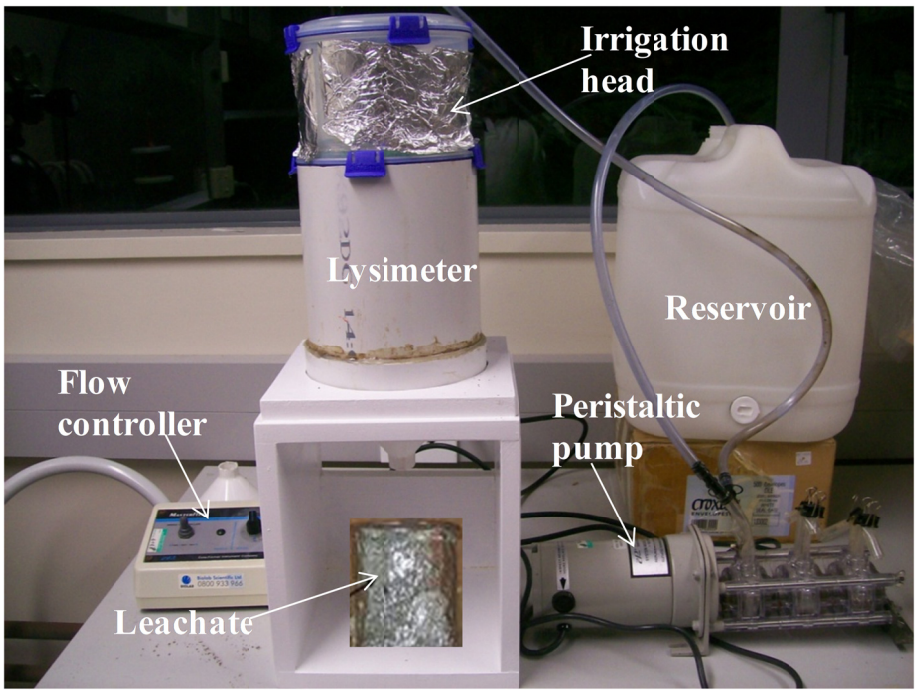


Figure 9.1: Setup for the laboratory soil column transport studies.

One litre of antibiotic solution containing all the three sulfonamides at a concentration of 0.5 mg L^{-1} each was prepared by adding an appropriate amount of methanolic stock solutions (1000 mg L^{-1}) to $CaCl_2$ (0.005M). Also one litre of bromide conservative tracer (KBr 5.35 g L^{-1}) was prepared separately in de-ionised water. Once the lysimeters were completely saturated, flow was established from the top using 0.005 M $CaCl_2$ solution and the flow rate in the controller was set to 7.5 mL min^{-1}. Each lysimeter was irrigated with at least 4 pore volumes (PV) of $CaCl_2$ solution and a constant head was maintained in the compartment, which gave a constant downward flow. Once a steady-state pore velocity was achieved (12.5

mL min^{-1}), a pulse input of bromide ion tracer was applied (1 L) and eluted with the $CaCl_2$ (0.005 M). The leachate was collected at regular time intervals, and each fraction was analysed for the bromide concentration using an ion-selective electrode (Metrohm 6.0502.100, Herisau, Switzerland). The bromide ion breakthrough curve (BTC) was used to identify the physical transport characteristics of each soil column. Once the bromide ion breakthrough curve is obtained, that is, when the bromide concentration becomes zero or consistently low, several pore volumes of the 0.005M $CaCl_2$ solution were flushed through the soil column.

A pulse input consisting of 1 L of the antibiotic solution containing all the three sulfonamides was applied, followed by 0.005 M $CaCl_2$ solution without antibiotic. The leachate was collected in the beaker at different time intervals, and samples of the leachate fraction (1 mL) were taken directly into HPLC vials for measurement. Analysis was performed on an HPLC-UV system using the gradient method described in Chapter 3.

At the end of the experiment, the PVC cap at the bottom of the lysimeters was carefully opened and the soil core was carefully pushed out intact. The soil core was cut into 6 parts (3 cm). The soil from each section was thoroughly mixed by hand and triplicate samples were taken to calculate its water content. Another set of triplicate soil samples (5 g) were placed into 35 mL Kimax centrifuge tubes in order to determine the residual antibiotic concentrations. The samples were extracted with dichloromethane (10 mL) with overnight shaking in a rotary drum shaker. An aliquot DCM extract (1.5 mL) was taken into a HPLC vial and evaporated to dryness under a gentle stream of N_2. The contents were reconstituted with methanol (0.5 mL), vortexed, and analysed by means of HPLC-UV (Chapter 3).

9.2.3 Transport model

The breakthrough curves of the bromide ion, a non-reactive conservative tracer, and the antibiotics were analysed using the physical equilibrium convective dispersive equation (CDE) assuming one-dimensional, steady-state flow in a homogeneous soil (van Genuchten and Alves 1982). The governing equation can be written as

$$\frac{R}{\nu}\frac{\partial C}{\partial t} = \lambda\frac{\partial^2 C}{\partial z^2} - \frac{\partial C}{\partial z} \quad (9.1)$$

where t is time (h), z is distance (cm), λ is dispersivity (cm), ν is the average pore water velocity (cm h^{-1}), C is the bromide concentration (mg L^{-1}), and R is the dimensionless retardation factor that reflects solute sorption. The coefficient R is set to 1 for the bromide tracer because it is assumed that bromide ions have minimal sorption onto soil particles (Fan et al. 2011). Equation 9.1 contains one unknown parameter λ, which was inversely estimated using CXTFIT 2.0 (Toride et al. 1995). The values used to calculate this parameter were the input solution concentration (C_o); the volumetric water content (θ), which was obtained from the average of the six sections; soil bulk density (ρ) (from Pang et al. (2008), 1.16–1.22 g cm^{-1}); and the soil column length (L).The initial condition for this problem assumed that the soil column was bromide-free and is given as:

$$C\ (z, t = 0) = 0\ for\ 0 < z < L \quad (9.2)$$

The upper boundary condition of the soil column is a third-type boundary and is given as:

$$\nu C\ (z, t = 0) - D\frac{\partial C(z=0,t)}{\partial z} = \nu C_0\ (t)\ for\ 0 < t < t_0 \quad (9.3)$$

$$\nu C\ (z, t = 0) - D\frac{\partial C(z=0,t)}{\partial z} = 0\ \ for\ t > t_0 \quad (9.4)$$

and the lower boundary condition of the soil column is given as:

$$\frac{\partial C(z=L,t)}{\partial z} = 0 \quad (9.5)$$

The variable t_0 is the pulse duration (h) and C_0 (t) is the dimensional concentration from time zero to t_0.

The CXTFIT program within the software STANMOD version 2.0 (Simunek *et al.* 1999) was used to obtain the analytical solution for the CDEs by means of inverse fitting of the obtained bromide BTCs. The parameters initially estimated were *v* (pore water velocity), λ (dispersion coefficient). These parameters were later used in the antibiotic BTCs, and *R* (retardation factor) and λ (dispersion coefficient) were estimated. It was assumed that the parent sulfonamide was the only compound present in the leachate, and that transformation products or metabolites were not included in the transport model.

9.3 Results and discussions

9.3.1 Bromide-tracer breakthrough curve

The Bromide-tracer breakthrough curves of the conservative bromide tracer in the two soils are shown in Figures 9.2a and 9.3a. Bromide initiates its breakthrough at approximately 0.85 and 1.05 pore volumes for Matawhero and Hamilton soil, which was expected for a conservative tracer. The maximum amount recovered for the bromide tracer was calculated to be 93% and 94% for Matawhero and Hamilton soil. These results were similar to the findings of Kurwadkar *et al.* (2011), who showed that the breakthrough of bromide conservative tracer was initiated between 1 and 2 pore volumes in 3 US soils with varying physical and chemical properties. This study also reported bromide recoveries to range between 95 and 100%. Scherr (2009), in a hormone transport study, showed that the peak concentration of the conservative bromide tracer in the column effluents occurred within 0.9–1.1 pore volumes for Hamilton soil. In Scherr's study the author also reported significant preferential flow from Hamilton soil; however, in this present study no preferential flow was observed in either of the soil columns, as the maximum breakthrough for the bromide tracer was similar on both occasions. No significant tailing was observed in the bromide BTCs for either soil, which is indicative of a well-saturated soil column.

9.3.2 Antibiotic breakthrough curve

Figures 9.2b and 9.3b shows the breakthrough curves for the three antibiotics in Matawhero and Hamilton soil respectively. The breakthrough for the antibiotics in Matawhero soil occurred between 1.06 to 1.60 pore volumes and between 2.36 to 2.65 pore volumes for Hamilton soil. The maximum amounts recovered in the leachate for SMO, SCP, and SM for Matawhero soils were 65.4%, 78.1%, and 45.4% respectively. For Hamilton soil, however, maximum recoveries for the three antibiotics were much lower and ranged from 17 to 58%.

Recently, Kurwadkar *et al.* (2011) reported the recoveries in the column effluent for SM and STZ to be 50 to 90% and 34 to 75%, respectively. In another study, 69–99.7% of SM, along with its primary metabolite, was recovered in column effluents (Fan *et al.* 2011). Elsewhere, the maximum relative concentrations of SDZ differed as well as the eluted mass fractions, ranging from as low as 8 to 83% after 500 hours of leaching (Wehrhan *et al.* 2007). In a

much earlier study in the UK, from a spiked soil column experiment, Kay *et al*. (2005b) reported SCP concentration of 703.2 μg L^{-1} in rainfall-simulated leachate. Past studies have shown that the transport of SDZ was found to be dependent on the input mass and duration; the mass recovery of SDZ in soil column effluent increased as the solute input duration increased (Wehrhan *et al.* 2007).

In this study the peaks of the breakthrough curves shifted only marginally away from the conservative tracer bromide for Matawhero soil, which exhibited a shift in BTC peak by 0.2 to 0.85 pore volumes whereas for Hamilton soil peaks occurred at 1.6 to 1.9 pore volumes. These results clearly demonstrate that sulfonamides could potentially behave in a similar manner to a conservative tracer dependent on soil type. The slightly late arrival of the antibiotics maxima in Matawhero soil indicates that retardation still played some part in antibiotic transport through the soil column, and this could be attributed to the antibiotic sorption to the soil solids. This effect was much more marked in Hamilton soil.

Recent studies have shown that peak antibiotic concentration in the BTCs can occur simultaneously with bromide breakthrough (Kurwadkar *et al.* 2011). However, in the present study, for Hamilton soil, there was considerable retardation in the antibiotic transport through the soil column. In general, the BTCs for the sulfonamides were broader when compared with the BTCs of the bromide tracer, which is indicative of the influence of the retardation process. Hamilton soil has a greater percentage of clay compared with Matawhero soil, which is loamy by nature. The higher clay content in Hamilton soil could be responsible for increased retardation. Hamilton clay soil has almost twice the organic carbon content of Matawhero soil (Table 3.1), thereby providing a favourable environment for neutral forms of the antibiotic to partition into the organic carbon domain.

Of the three antibiotics studied, SM breakthrough was the quickest in both soils, indicating the highly mobile nature of this compound. SMO and SCP breakthrough was more or less identical in both soils with similar pore volumes. The BTCs observed for the sulfonamides in the present study were similar to BTC at soil pH observed by Kurwadkar *et al.* (2011) for SM and STZ antibiotic. At pH values of 5.1 and 6.1 for both soils, it is likely that all three sulfonamides would exist as 50% neutral and 50% anionic forms making them highly mobile and with little to no retention on the soil column. A visual observation of the BTCs (Figures

9.2 and 9.3) also lends further support to the argument that the sorption and mobility of the sulfonamide group of antibiotics is pH dependent (Lertpaitoonpan *et al.* 2009; Kurwadkar *et al.* 2011). The results obtained in this study correlate well with the findings from batch sorption studies performed on the same set of soils and antibiotics (Chapter 4). All the three sulfonamides were weakly sorbed to the soils, with the sorption capacity of Matawhero soil being lower than that of Hamilton clay soil; therefore it is not surprising to expect some retardation in Hamilton soil. SM, with the lowest K_f values for both soils, had the quickest arrival time (breakthrough), and SCP, with the highest K_f in both soils, had the slowest breakthrough.

9.3.3 Antibiotic retardation

The results of the soil extraction from the sectioned lysimeters are shown in Figure 9.4. For both Matawhero and Hamilton soil, SMO and SCP were detected in each portion of the sectioned core up to the first 18 cm of the column, and were well above the method detection limit (13 μg kg^{-1}); however, no resident concentration of SM was detected at any depth in the entire profile for both the soils, suggesting loss of the compound due to biodegradation in the soil. A laboratory soil column study showed that SM might have penetrated deeper into the soil within 6 h (Fan *et al.* 2011). Kim *et al*. (2010) showed that even though the antibiotic STZ had penetrated into the subsurface no residuals were found for STZ, suggesting this compound might have penetrated even deeper into the soil . In a spiked column experiment, SCP has been found to reach a depth of 20 cm (Kay *et al.* 2005b). No residuals for SDZ were found in a rainfall simulated column study, suggesting this compound might have penetrated even deeper into the soil (Kim *et al.* 2010).

Burkhardt and Stamm (2007) reported the depth distribution of three sulfonamides applied to a loamy grassland soil; with levels of antibiotic concentrations at 30–50 cm depth being equal to the concentration found in the top 5 cm regardless of whether application of antibiotics was in manure or in aqueous solution. In the current study for both soils, the antibiotic concentration profile decreased with depth, indicating that possible preferential flow through cracks, or wormholes did not occur. SCP was found in greater proportion than SMO was at any given depth for either of the soils, suggesting that SCP is more readily retained than SMO; these results again correlate well with the batch sorption results.

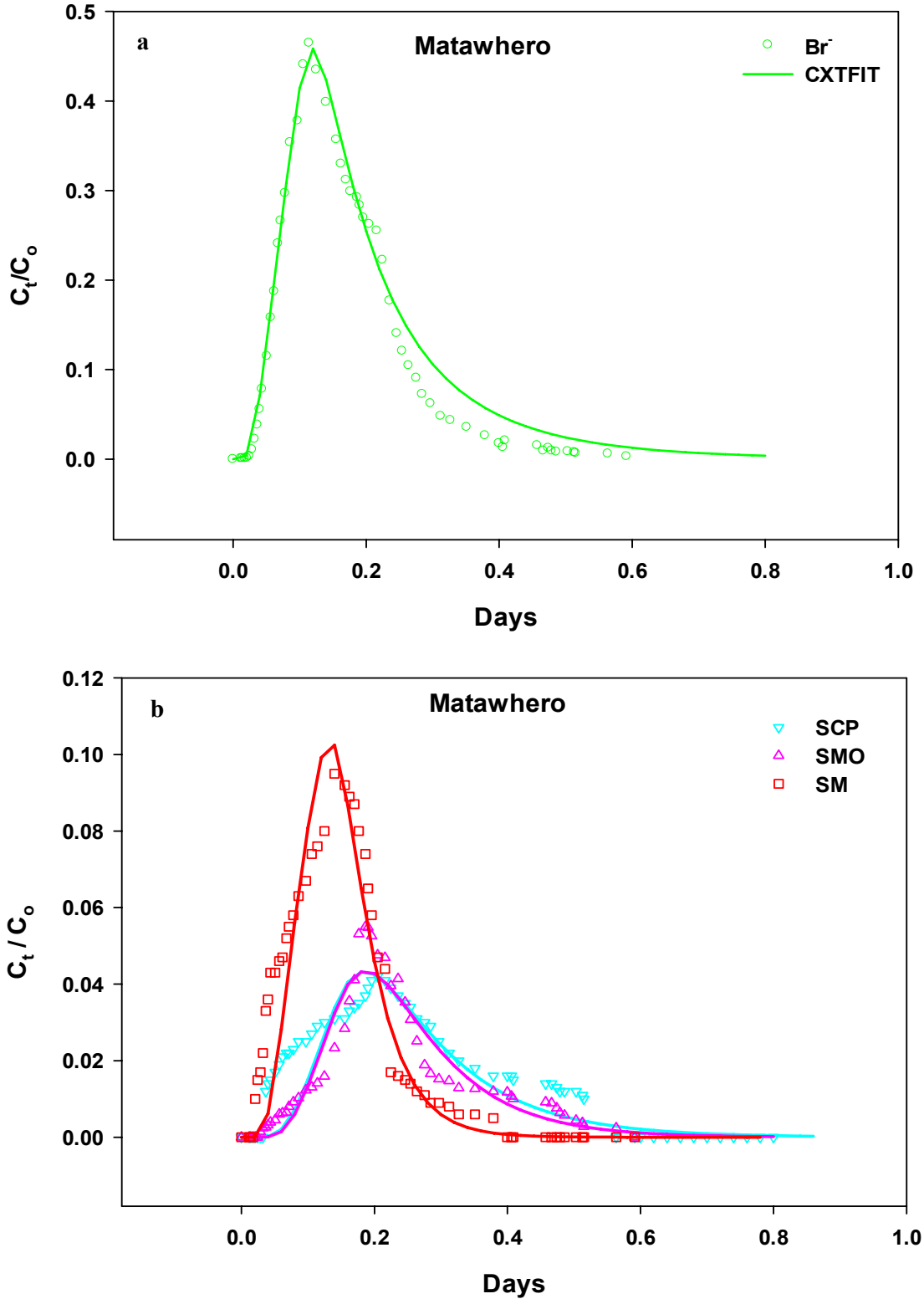


Figure 9.2: (a) Breakthrough curves of bromide and (b) three sulfonamides for Matawhero soil. Symbols represent measured data, while the solid lines indicate the CXTFIT 2 model fits.

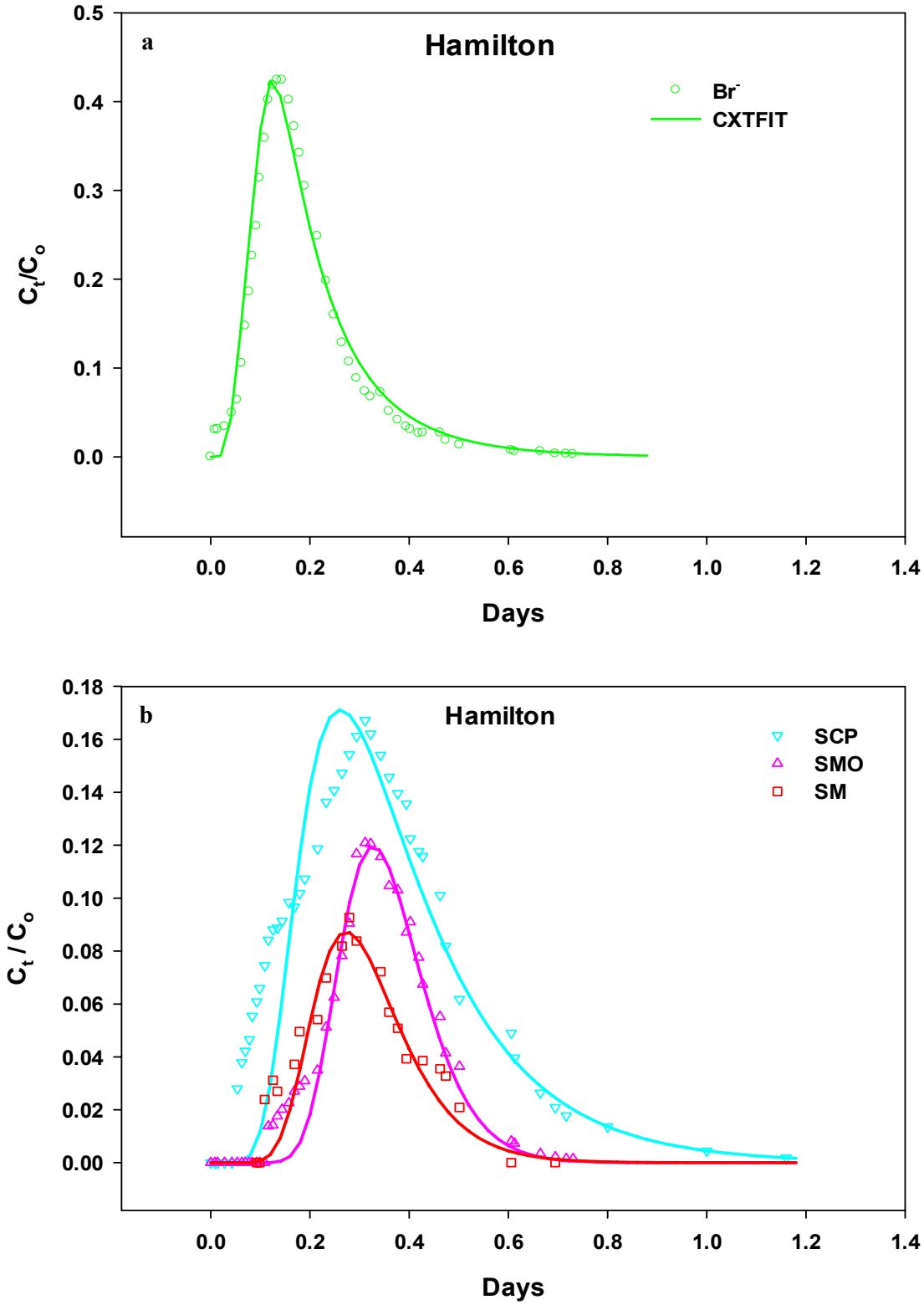


Figure 9.3: (a) Breakthrough curves of bromide and (b) three sulfonamides for Hamilton soil. Symbols represent measured data, while the solid lines indicate the CXTFIT 2 model fits.

The variations in the antibiotic resident concentrations when compared to other reported studies may be due to varying soil properties such as pH, chemical properties and experimental protocol such as column length and pulse input duration (Wehrhan *et al.* 2007; Kurwadkar *et al.* 2011)

9.3.4 Transport modelling

The physical equilibrium model available within the STANDMOD (Toride *et al.* 1995) was used inversely to fit the bromide tracer breakthrough data and to obtain transport parameters ν, and D, respectively. Unlike some recent studies for example Fan *et al.* (2011) and Jeong *et al.* (2012), no tailings and asymmetrical breakthroughs were observed for the bromide BTC's, indicating the absence of physical non-equilibrium conditions during the transport of the antibiotics through the soil columns. The non-sorbing bromide breakthrough curves were all quite symmetrical (Figures 9.2 and 9.3) as expected for a nonreactive solute and were described well (R^2 values of 0.97 and 0.98 for the Matawhero and Hamilton soils respectively) by the model setting *R* equal to 1 (Table 9.1). Recent studies have used a two-site non-equilibrium transport model to describe the breakthrough curves of SM (Fan *et al.* 2011), while Wehrhan *et al*. (2007) showed that a three-site sorption model fitted the breakthrough curves for SDZ the best. Unlike these studies a two-site non-equilibrium transport model did not yield favourable results for either dataset (Matawhero and Hamilton) in the present study. The pore water velocity used in this study was much higher (110–190 cm h^{-1}) compared with the velocities (0.44–0.54 cm h^{-1}) of the study of Wehrhan *et al*. (2007). High pore water velocity is known to limit the contact time of aqueous-dissolved organic contaminant with the sorbed phase (Lee *et al.* 2002), and thus it can be inferred that velocity is the reason why neither the two-site nor the three-site non-equilibrium transport model would fit the data well.

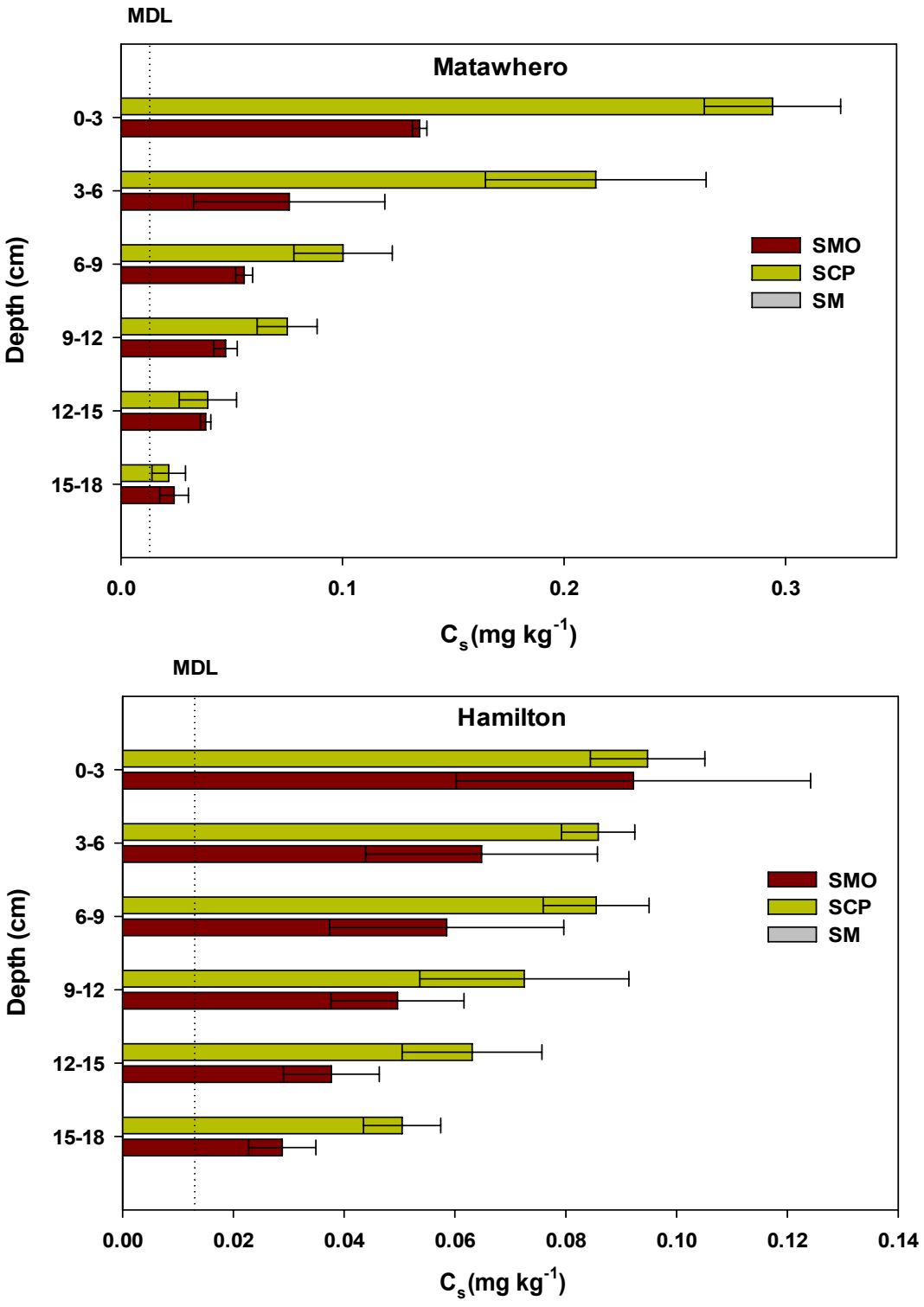


Figure 9.4: Residual antibiotic concentration in the sectioned soil cores respectively. Average values of *n*=3 samples are displayed with one standard deviation. The red line indicates the method detection limit of 0.013 mg kg^{-1}.

However, using the equilibrium CDE with the boundary conditions set as described earlier, with the assumption of one dimensional steady-state flow in homogeneous soil yielded a good description of the datasets as indicated by the R^2 values (Table 9.1). The CXTFIT model described well the peak arrival time as well as the maximum concentration for the three sulfonamide breakthrough curves but showed some underestimation at advanced stages of sulfonamide (SCP and SMO) leaching, especially in the Matawhero soil. Overall, the CXTFIT model adequately described the measured BTCs, peak arrival time, and the maximum concentration of the three sulfonamides in the final effluent for both soils with contrasting properties. Breakthrough curves for the sulfonamides were not entirely symmetric, with some broadening of peaks when compared with the conservative tracer. These broadened peaks for antibiotics are unlikely to be due to the hydrodynamic characteristics of the soil columns because the bromide tracer exhibited relatively ideal BTCs. The behaviour of the BTCs suggest that sulfonamide transport in both soils may have been retarded (Figures 9.2 and 9.3) to some extent. It was also evident that the BTCs of the antibiotic for the Hamilton soil had much delayed peaks and longer tailing (SCP) than for the Matawhero soil. In addition, there were relatively higher peaks of relative concentration (C/C_o) observed in the Hamilton soil, compared with the Matawhero soil, while the maximum concentration for the bromide and SM occurred at the same time in the case of Matawhero soil.

CXTFIT model parameters were obtained by using ν, and λ values from the bromide BTC, while only R was optimised. The estimated dispersion coefficient along with the estimated pore water velocities for Matawhero and Hamilton data were in good agreement to the experimentally determined values, and small differences can be attributed to the heterogeneous nature of the soil with varying pore sizes affecting the pore water velocity or possibly due to the presence of cracks, or fissures in the soils allowing macropore flow. The retardation factor (R) values obtained with the CXTFIT model for the three sulfonamides for the Matawhero and Hamilton soils are summarised in Table 9.2. The estimated R values of CXTFIT were compared with the experimental R values obtained from the non-linear partition coefficients (K_f) for these antibiotics obtained from the sorption experiment in Chapter 4. The experimental retardation factor is often written as

$$R = 1 + \frac{\rho}{\theta} K_d \qquad (9.6)$$

where R (the dimensionless retardation factor) accounts for fully reversible reactions of a solute species in the soil system (Jeong *et al.* 2012), ρ is the soil bulk density (kg m^{-3}), θ is volumetric water content (v/v), and K_d , the linear sorption coefficient (L kg^{-1}). However, for non-linear sorption coefficients obtained from Freundlich isotherms the equation can be written as

$$R = 1 + \frac{\rho}{\theta} \left(K_f \, \frac{1}{N} \, C^{\frac{1}{N}-1} \right) \qquad (9.7)$$

where K_f is the Freundlich sorption coefficient, C is the concentration of the contaminant (mg L^{-1}) and N is dimensionless and represents the degree of linearity (Sharma and Reddy 2004).

There was a marked difference in the model estimated retardation factors and the values calculated from the K_f values reported in the Chapter 4. These variations could be due to the different experimental protocols adopted. Often the sorption process for organic chemicals in batch experiments tends to occur faster than in a leaching experiment conducted in the laboratory using packed or intact soil columns, and this may be explained by the optimized contact between the continuously agitated (e.g., by shaking) soil and solution in such experiments (Vereecken *et al.* 2011). In the absence of compound-specific (sulfonamides) literature data on retardation factors, a comparison with the R values obtained in the present study is not possible. However, the retardation factors obtained in this study were of a similar order of magnitude to the R values reported for another sulfonamide, STZ with R values ranging from 2.84 to 16.0 in three US agricultural soils having pH values of 5.2 to 7.8 (Kurwadkar *et al.* 2005).

Table 9.1: Soil physical parameters and bromide breakthrough results.

Soil	Porosity	Bulk density (g cm^{-3})	Moisture Content (v/v)	Pore water velocity (cm h^{-1})	Estimated Pore water velocity(cm h^{-1})	R^2	λ (cm)	MSE
Matawhero	0.45#	1.22	0.520	172.3	148.6	0.975	2.416	0.53 E-3
Hamilton	0.51*	1.16*	0.442	110.7	147.5	0.981	1.892	0.42 E-3

assuming similar to a loamy soil from *Pang *et al.* (2008); R^2 is a measure of the relative magnitude of the total sum of squares associated with the fitted equation

Table 9.2: The model-estimated parameters for the deterministic physical equilibrium model fitted to the antibiotic breakthrough data. *R* is the retardation factor; MSE is the mean squared error.

	SA	R^2	MSE	*R* ± SE Estimated	*R* Calculated
Matawhero	SMO	0.905	0.26 E-3	8.20 (0.21)	4.94
	SCP	0. 810	0.58 E-4	7.36 (0.27)	6.24
	SM	0.853	0.14 E-3	2.69 (0.08)	5.35
Hamilton	SMO	0.955	0.78 E-4	12.44 (0.12)	6.40
	SCP	0. 748	0.74 E-3	3.98 (0.12)	24.49
	SM	0.840	0.13 E-3	7.74(0.20)	6.40

9.4 Summary

The results of the soil column studies showed that all three sulfonamides were mobile and exhibited nearly the same conservative behaviour as that of the bromide tracer. The breakthrough curves of sulfonamide varied depending on soil type, and the type of chemical. For the three sulfonamides studied, the order of breakthrough was generally observed to be SMO > SM> SCP for both Matawhero and Hamilton soils. The high mobility of sulfonamide antibiotics can be explained thus, at soil pH ($pH>>pK_{a1}$) they are likely to occur in either neutral form or in anionic form (Ingerslev and Halling-Sørensen 2000; Kurwadkar *et al.* 2011) and in the anionic form, they can attain higher solubility and thus can become highly mobile in the environment (Kurwadkar *et al.* 2005). Therefore, the speciation of sulfonamides significantly affects their tendency to complex with the soil surfaces (Qiang and Adams 2004).

At an environmental pH range (4.5-6.5) like the pH of the two soils reported in the present study, sulfonamide molecules are likely to exist as anions and would exhibit greater mobility. This effect of pH on mobility of antibiotic was also shown in batch experiments where pH dependency on sorption is clearly demonstrated (Chapter 6). In all three soils, a reduction in partition coefficient was observed with the increases in soil pH, as the antibiotic changed its speciation. The CXTFIT model described well the peak arrival time as well as the maximum concentration of the antibiotic breakthrough curves but showed some underestimation at advanced stages of sulfonamide leaching, especially in the Hamilton soil.

From this study it can be concluded that sulfonamides have weak sorption affinity and relatively high mobility in soils. Their transport through soils is of concern after direct excretal inputs from grazing animals or because of effluent application in the field, and thus the potential exists for the antibiotic residues to migrate through the soil profile and cause eventual contamination of surface water and groundwater. In a field scenario, processes such as preferential flow or colloid or dissolved-organic carbon-facilitated transport, could also play an important role in rapid transport of sulfonamides.

Chapter 10: General discussion, conclusion and future research

10.1 General discussion

Chapter 3 presented the development of a method to detect and extract TT, SMO, SCP and SM from aqueous and soil matrices. A solvent extraction scheme was developed to extract these compounds from soil samples followed by an HPLC-UV/Fluorescence detection isocratic method to detect and quantify these four antibiotics, separately. A gradient method was also developed to detect and quantify all three sulfonamide antibiotics simultaneously.

These analytical methods developed were later used to analyse the samples for the following experiments conducted in the laboratory:

- the sorption affinity of TT and SMO, SCP, and SM antibiotic in six different dairy farm soils (Chapter 4);
- the sorption affinity of SMO antibiotic in three different soils with varying pH and ionic strength (Chapter 5);
- the sorption affinity of SMO antibiotic to Matawhero soil amended with biochar produced from different feedstock and temperature in selected dairy farm soils (Chapter 6);
- the sorption affinity of SCP antibiotic in the presence of steroid hormone, 17 β-estradiol (E2) (Chapter 6);
- the degradation behaviour of SMO in three agricultural soils at varying initial concentrations, depths, and incubation temperatures (Chapter 7);
- and the transport and retention behaviour of SMO, SCP, and SM in two different undisturbed soil lysimeters (Chapter 9).

A number of studies are available in the literature on the extraction of range of veterinary antibiotics from different environmental matrices (Haller *et al.* 2002; Blackwell *et al.* 2004a; Blackwell *et al.* 2004b). However, the method developed in the present study to extract the macrolides and sulfonamides from aqueous and soil samples using solvent extraction is novel, robust, and yet simple to use. The method uses dichloromethane as the extraction solvent, and does not employ any extraction buffer or any solid phase extraction clean up technique. Moreover, the method is cost effective and can be easily reproduced in laboratories that do not have access to sophisticated analytical tools.

UV absorption of the TT and sulfonamides was found to be at maximum at 290 and 275 nm, which is in good agreement with previously reported detection wavelengths (Hu and Coats 2009; Figueroa-Diva *et al.* 2010). An Onyx® Monolithic C_{18} column was used for TT, and a Luna 5µ RP-C_{18} for the three sulfonamides. This resulted in good separation of these compounds for both isocratic and gradient elution schemes and resulted in a significant reduction in runtime. The developed HPLC method employed an isocratic as well as gradient system, with the mobile phase consisting of acetonitrile: trifluoroacetic acid (0.05%): tetrahydrofuran. The use of similar mobile phases for separating three different veterinary antibiotics was reported by Blackwell *et al.* (2004a, 2004b). The run time for four antibiotics was < 6 minutes (the isocratic scheme) and < 14 minutes (the gradient run), thus a reduction in run time and consequently a higher sample throughput. This elution scheme avoids the use of buffers and therefore eliminates the possibility of the formation of precipitate and the time taken for system clean-up following the analysis.

The method detection limits in the aqueous method were the same as the instrument detection limits, which are about an order of magnitude higher than the detection limits yielded from LC-MS methods. The limits of detection at an S/N (signal: noise) ratio of 3 were 20.0 µg L^{-1} and 50 µg L^{-1} for all SA's and TT respectively. The method detection limits (based on a signal to noise ratio of 3:1) for all three SA's in the present study were ~ 13 µg kg^{-1} (isocratic run), while it ranged from 9–16 µg kg^{-1} under gradient run. However, with the use of the fluorescence detector, the method detection limit for SMO could be lowered to ~ 3 µg kg^{-1} in all the soils. In contrast, the method detection limit for TT was significantly higher (41 µg kg^{-1}) in all soils.

The average recoveries for all three SA's and TT in aqueous matrices ranged from 95 to 105% across the six concentrations investigated. Similar aqueous recoveries were reported by Boxall *et al*. (2002) for SCP. Recoveries from the soils residues were slightly lower for sulfonamides and tylosin. Recoveries for TT in three soils ranged from 58 to 101% , while for SMO it ranged from 67 to 106%. There was a general trend of decreased recovery, with increases in the spiked concentration level contrasting with the earlier findings of low recovery reported for TT at low spiked levels (Blackwell *et al.* 2004b). Poor recoveries in the present study for certain soils could be due to the formation of more bound residues at higher spiked concentrations, which often required greater volume of solvent to extract the compounds. These recoveries, however, were higher when compared with an earlier study that gave residual recoveries of 22–89% for four sulfonamides (Figueroa-Diva *et al.* 2010), but lower than recoveries for SCP, tylosin and oxytetracycline obtained using ultra sonic extraction (Blackwell *et al.* 2004a).

The method detection limits obtained in the present study are higher than those reported by other studies (Thiele-Bruhn *et al.* 2004; Blackwell *et al.* 2004a; Sassman *et al.* 2007). These studies used MS detectors and sophisticated techniques for extraction, such as an accelerated solvent extractor or a microwave extractor, which were not available during this work. In general, the method may not be suitable for detection of antibiotic residues in environmental samples because of limits of detection (LOD) were too high. However, the method described in the present study, employing both isocratic and gradient elution schemes, was suitable to obtain data for laboratory-scale investigations investigating sorption, degradation, and transport of antibiotics in soils.

Antibiotic residues have been detected in various parts of the world in environmental media such as soils, surface water, and ground water (Hamscher *et al.* 2003; Zuccato *et al.* 2005; Perret *et al.* 2006; Luo *et al.* 2011). Given that about 55 tonnes of antibiotic usage in New Zealand, and the fact that most ruminant animals graze the pasture throughout the year, potential exists for the occurrence of antibiotic residues in New Zealand's terrestrial and aquatic eco-systems. Sulfonamides and macrolides are the two common classes of antibiotics that are widely used in livestock operation. Under New Zealand conditions, no data are available on the fate and behaviour of these antibiotics. These data are essential requirements in the regulatory decision making process and for the risk assessment purposes. This thesis

examined the sorption behaviour of a macrolide (TT), and three sulfonamides (SMO, SCP, and SM) in six different pastoral soils of contrasting properties collected from representative regions in New Zealand.

Batch sorption studies were carried out employing 5 mM $CaCl_2$ as background solution for both the liquid and soil phases. By doing so, a comparison was possible between the complex solvent extraction scheme and the usual mass balance approach to determine the sorption parameters of the selected antibiotics. From a batch sorption study, Allaire *et al.* (2006) concluded that for experiments shorter than 48 h of contact time the soils need not be sterilized. Past studies have reported different equilibration times ranging from 1 h to 24 h for tylosin (Rabølle and Spliid 2000) and 24 h to 72 h for sulfonamides (Boxall *et al.* 2002; Figueroa-Diva *et al.* 2010). In the present study, an apparent equilibrium for TT sorption was attained in the first 8–48 h of contact time while it was between 12 and 48 h for sulfonamides. 24 h was chosen as the appropriate equilibration time for sulfonamides and tylosin. Similar contact times have been used by Allaire *et al*. (2006) to study tylosin and sulfonamide sorption in soils.

The Freundlich model could describe the isotherms constructed from both the schemes better than the Langmuir model could. The extraction isotherms were much better than the isotherms produced using data obtained from the mass balance approach between initial and final solution concentrations. However, given that the mass recoveries extracted were low (65–95%) for all compounds in six soils, and no metabolite was concomitantly formed during sorption equilibration, it was more accurate and appropriate to construct the isotherms using concentration values obtained by difference technique than the data obtained using the extraction technique. The isotherms for TT were non-linear and *N* values varied between 0.38 and 1.00 for all 6 soils. Values for *N* varied between 0.40 and 0.96 for SMO; between 0.76 and 0.93 for SCP; and between 0.50 and 1.08 for SM across the six soils. Similar nonlinear sorption isotherms have been reported for tylosin and sulfonamides under soil pH (4.5–6.5) conditions (Ingerslev *et al.* 2001; Clay *et al.* 2005; Lertpaitoonpan *et al.* 2009; Wehrhan *et al.* 2010). Weber *et al.* (1992) attributed the non-linear behaviour for organic contaminants to the broad range of interaction energies, as well as high degree of variability in those energies from one sorbent to another.

The K_f values for TT ranged from 1.6 to 851 L kg^{-1}, with the sorption affinity to Matawhero soil being the highest, with Manawatu soil exhibiting the lowest value. The sorption potential in Matawhero soil (2.1% OC), with a high CEC value (30 $cmol_c$ kg^{-1}), and was 200 times greater than that for Horotiu soil, despite its high OC content (8.2%). This can be attributed to the role of cation exchange in tylosin sorption as all 6 soils had a pH below the pKa of TT (7.1). For the sulfonamides, Horotiu soil (8.2% OC) gave the highest K_f values for all 3 compounds, while Matawhero soil and Gibsons with an OC content of around 2.1 and 1.1% respectively gave the lowest. Since Horotiu soil is volcanically derived, this high K_f could be attributed to the high allophane (an alumino-silicate clay mineral) content (28%) and high OC of the Horotiu soil (Sarmah *et al.* 2008). Sulfonamides are known to partially exist as neutral and remaining as anionic form at environmental soil pH ranges (Kurwadkar *et al.* 2011). So the neutral species seemed to be readily adsorbed on the organic matter, the anionic form seemed to be mobile. Therefore it can be concluded that at soil pH (4.5–6.5) sorption of sulfonamide antibiotic to soils is primarily due to the hydrophobic partitioning mediated by soil organic carbon and partially due to cation exchange to negatively charged soil particles.

Fan *et al.* (2011) reported sorption of sulfonamides to be strongly associated with the physiochemical properties of the medium such as pH, ionic strength, cation exchange capacity; and that these properties might be related to each other. Sassman *et al.*(2007) also reported good co-relationship of tylosin sorption with % clay and SSA of soils. However, in their study no correlation was obtained between the sorption parameters to any one particular soil property like OC, CEC, and clay content, etc. Similar observation was made by ter Laak *et al*. (2006), who found that none of the individual soil properties could explain the variation of sorption coefficients for tylosin and SCP. In this study statistical analysis of sorption data revealed a correlation between the sorption coefficients and soil properties such as % organic carbon, clay content, cation exchange capacity and pH.

The sorption affinity for all soils followed the trend TT > SCP > SM > SMO. TT is most likely to be strongly retained on soil particles based on its high partition coefficients (K_f), while the sulfonamides with lower K_f values are likely to become mobile, and could potentially contaminate aquatic bodies such as surface or even groundwater.

The sorption of sulfonamide antibiotics onto soils is known to be influenced by pH, organic matter and ionic strength (Laak *et al.* 2006; Kahle and Stamm 2007b; Lertpaitoonpan *et al.* 2009; Vasudevan *et al.* 2009). Batch sorption studies were conducted to investigate the effect of pH, ionic strength and organic carbon on the sorption of SMO antibiotic to three soils representative of New Zealand dairy farming regions. The organic carbon for the three soils studied ranged from 2.1 to 8.2% and the mediator solution ($CaCl_2$) pH ranged from 2 to 8.5. Also the ionic strength of the mediator solution varied from 0.01 M to 0.4 M $CaCl_2$. Sorption of SMO decreased with increases in pH values, and sorption increased with increasing ionic strength and organic carbon content of the soil. All three soils displayed a decreasing trend in SMO sorption when pH was increased, with SMO sorption being the highest at pH 2 for all the three soils. An increase in pH from 2 to 8.5 for Matawhero soil resulted in 9-fold lower K_f values (from 9.95 to 1.24) for SMO. All isotherms observed for Matawhero and Horotiu soils over the pH range studied were found to be non-linear, with *N* values ranging from 0.33 to 0.85 for Matawhero soil, and 0.60 to 0.88 for Horotiu soil. However, for Te Kowhai soil the degree of nonlinearity varied with pH, and was close to unity at pH 7.51 and > unity at pH 5.5. The present study also revealed non-linear sorption dependency for SMO, and sorption was dependent on solution pH, and associated *N* values varied with solution pH. Thus the results of the present study are in agreement with earlier findings reported by other researchers (Kurwadkar *et al.* 2007; Lertpaitoonpan *et al.* 2009).

A hydrophobic pH-partitioning model linking sorbate speciation with species-specific sorption coefficients describing the pH dependence of the apparent sorption coefficients was used to derive the fraction of each species of SMO that are likely to be present in the environment. The cationic form prevails at $pH \leq pK_{a1}$ and, when $pH \geq pK_{a2}$ (6.5, 7.5 and 8.5) the anionic species seems to dominate; however, its sorption affinity to all soils was low. At intermediate pH values, the neutral species of SMO dominates. This was confirmed by regressing the estimated sorption coefficients of cationic, uncharged, and anionic species obtained using the model. The increased SMO sorption with low pH can be explained by the sorption of neutral species of SMO to the organic carbon of the soil along with electrostatic attraction between the cationic forms of SMO to negatively charged soil surfaces. In an anionic form, much less ion exchange or organic partitioning by sorption would be expected on most soils.

On increasing the ionic strength of the mediator solution, the shape of the Freundlich sorption isotherms for SMO in three soils changed. In Matawhero soil, at given SMO concentration at equilibrium, sorption increased strongly as ionic strength increased, which could be attributed to the occurrence of a "salting out" effect causing a decrease in the solubility of SMO antibiotic in the salt solution so that it precipitates in the soil. This phenomenon was also reported in some earlier studies (Spongberg and Ganliang 2000; Ureña-Amate *et al.* 2005). Inherent sorbent properties such as composition of various clay minerals and the associated high surface areas for the soils, and the type and the nature of organic matter present may be responsible for this.

Organic carbon of the soil also enhanced antibiotic sorption considerably. Horotiu, with the highest OC of 8.2%, gave the highest sorption compared with Te Kowhai (5%) soil, while Matawhero soil with OC content of 2.1% exhibited the least sorptive affinity for SMO. Past studies have also reported the influence of soil organic carbon on the sorption of sulfonamide antibiotic (Drillia *et al.* 2005; Lertpaitoonpan *et al.* 2009; Figueroa-Diva *et al.* 2010). The log K_{oc} values for all the three soils at any particular pH were more or less similar, and ranged from 2.01 to 2.40 (pH 4), and 1.98 to 1.54 (pH 8.5) for the three soils investigated. Log K_{oc} values that do not vary with changes in pH values indicate the influence of OC on sorption of organic compounds (Lertpaitoonpan *et al.* 2009).

The present study also investigated the competitive sorption between steroid hormones (17β estradiol) and a selected veterinary antibiotic (SCP), and the results showed that the presence of the steroid hormones did not influence the sorption of veterinary antibiotic to soil considerably. Approximately 10% and 30% decrease in SCP (K_f) sorption was observed when E2 was added at concentrations of 0.075 mg L^{-1} and 3 mg L^{-1} respectively in the soil. The presence of E2 also influenced the degree of linearity of the SCP isotherms. For instance, the control isotherm (with SCP alone) exhibited a highly nonlinear trend; however, the SCP isotherm in the presence of E2 showed a reduced degree of nonlinearity. The competition was between an ionic species of SCP, and non-ionic E2. The sorption mechanism for non-ionic compounds is based on hydrophobic partitioning, and is mainly driven by the organic carbon of the soil (Li and Yang 2010). Sulfonamide sorption, on other hand, is governed by the pH; even though it behaves as a Lewis base at neutral soil pH, its sorption affinity is also mainly driven by hydrophobic partitioning (Lertpaitoonpan *et al.* 2009), and only slightly (cationic

species) by electrostatic force of attraction to clay (Gao and Pedersen 2005). It is very likely that the presence of non-ionic E2 inhibited SCP sorption to soils at normal soil pH. The sorption of sulfonamide antibiotic to the soil seems to be influenced by the properties of the chemical such as pK_a and hydrophobicity, as well as soil solution characteristics such as pH, ionic strength, organic carbon content, and the presence of co-contaminants.

In recent years, biochar amended soils have been studied for sorptive behaviour for pesticides, heavy metals, and EDCs (Yu *et al.* 2006; Wang *et al.* 2010; Ji *et al.* 2011; Karami *et al.* 2011; Sun *et al.* 2011). The use of biochar to soak up unwanted antibiotics residues from the soil is a novelty in this thesis. Biochars obtained from three different feedstocks (corn cob, pine sawdust and green waste) and at three different pyrolytic temperatures (350^oC, 450^oC, 550^oC Green waste) were evaluated to determine the retention ability for a veterinary antibiotic (SMO). Biochars were used as soil amendment and applied at two different rates (5 and 10 tons per hectare) during batch sorption studies. Before using them as soil amendments, the biochars were characterised using inductively coupled mass spectrometry, Fourier transform infrared spectroscopy, scanning electron microscope/energy dispersive X-ray spectroscopy, and X-ray diffraction techniques to determine the physico-chemical properties. Not all biochars when amended with soil enhanced the sorption affinity of the soil towards SMO antibiotic. The addition of certain biochar (PSD, CC) resulted increased SMO sorption, meaning decreased mobility and transport. Increasing the antibiotic fixation to soils should also reduce toxicity and availability to soil organisms (microbes, invertebrates and plants).

Soil-biochar interaction was governed by the biomass source, heat treatment conditions and chemical and structural composition of the chars. High temperature chars showed more enhanced adsorptive potential. SMO sorption was the highest in the soil amended with 1% PSD biochar while for other biochar the rate of biochar application had no effect. The effective distribution coefficient (K_d^{eff}) values for SMO varied from 2.48 to 53.5 L kg^{-1} for biochar-amended soils. The PSD biochar produced at 700^oC showed greater sorption ability than other biochars, which was attributed to its high surface area and high carbon content (91%). Biochars made from green wastes at varying pyrolytic temperatures had no effect on sorptive ability, contrary to woody biochars, which showed high sorptive tendency. In

general, biochar applied at the rate of 10 tons per hectare could prove to be an effective way to reduce the release of these contaminants to a manageable level.

The sulfonamide group of antibiotics is also one of the most widely used, and is excreted in the urine and faeces in unchanged as well as in metabolised form. Literature data on the degradation of the SMO antibiotic in the soil environment are limited (Holtge and Kreuzig 2007; Liu *et al.* 2010). Especially under New Zealand conditions, to date, no published information is available on the behaviour of any sulfonamide group of antibiotics. Available overseas studies on sulfonamides show that degradation rates can vary widely depending on soil properties such as moisture content, organic carbon, pH, temperature, and bioactivity, as well as the properties of the compound.

In this thesis the degradation of SMO antibiotic in three different soils was investigated through laboratory incubation experiments by varying the incubation conditions such as temperature (7.5^{o}C & 25^{o}C), initial antibiotic spiked concentration, and soil depth, and with sterilization at 60% MWHC. DT_{50} values for SMO in Hamilton, Te Kowhai and Horotiu soils under non-sterilised conditions were 9.24, 4.3 and 13.33 days respectively. Based on their estimated first-order half-life values, degradation of SMO in topsoils followed an order: Te Kowhai > Hamilton > Horotiu at 25^{o}C, Te Kowhai > Horotiu > Hamilton > at 7.5^{o}C. Degradation in subsoils followed a slightly different order: Hamilton > Horotiu > Te Kowhai at 25^{o}C, and Horotiu > Te Kowhai > Hamilton at 7.5^{o}C. Similarly, abiotic degradation in sterilised topsoil followed the order: Te Kowhai > Horotiu > Hamilton at 25^{o}C and Horotiu > Hamilton > Te Kowhai at 7.5^{o}C. The degradation rate was faster in topsoils when compared with subsoil samples in two of the soils, clearly indicating the prominence of soil microbes in topsoil, which strongly influenced antibiotic degradation. DHA activities for the topsoils were a unit higher than those obtained for subsoil, suggesting limited microbial activity at increasing depths. For Horotiu soil, however, depth had no effect on the SMO degradation kinetics, which could be attributed to the presence of specific microbial degraders. Similar findings of higher degradation in subsoil than in topsoil were also reported in a field study conducted with various pesticides (Di *et al.* 1998).

In all soils, SMO remained detectable throughout the incubation period at all temperatures. The percent of SMO remaining after 40 days of incubation period was temperature dependent

with values of 10% and 25% of SMO still remaining at 25^{o}C and 7.5^{o}C respectively in the soils. The degradation rate constants were higher in soils incubated at 25^{o}C than at 7.5^{o}C, which was supported by the measured DHA showing very little or no activity for soils incubated at 7.5^{o}C compared with soils incubated at 25^{o}C. Metabolites of SMO have been detected in the effluent samples collected from waste water treatment plant in Germany (Radke *et al.* 2009), confirming SMO to possess metabolites that are formed during degradation in certain media. However, no metabolite was formed in the agricultural soils investigated in the present work.

Under sterile conditions, some abiotic degradation of SMO was observed, especially for the Horotiu soil, which could possibly be due to the autoclaving procedure, which has been shown to change sorption desorption patterns of organic contaminants soils thus affecting the loss of the compound from the media (Lotrario *et al.* 1995). Abiotic loss in the sterile controls suggests the role of chemical processes in the degradation of SMO. This assumption can be supported as there was no correlation between the rate of degradation and the soils microbial biomass (MBC). This was in contrast to the findings of Scherr *et al*. (2008), where the authors reported good correlation between MBC and the biotic degradation rates for steroid hormones in pasture soils.

Antibiotic degradation in soil is also governed by soil sorptive properties, in particular the organic matter content of the soil (Thiele-Bruhn 2005). Determining the effect of a single soil property on the degradation of organic contaminant is often difficult, given the combined effect of soil organic carbon and clay content influencing the sorption and thus directly influencing the process of degradation (Sarmah *et al.* 2009). Microbial biomass, both the degree of biological activity and specific degraders, as well as pH of the soil, could influence overall degradation. In addition, the combined effect of sorption and degradation can also be influenced by the organic carbon content and other mineral constituents present within a particular soil depth, which can have an interactive effect on the overall degradation process (Sarmah *et al.* 2009). The above study, along with the present study, indicates a link between sorption and degradation processes with a general trend of faster degradation associated with weakly sorbed organic contaminants. A comparison between sterile and non-sterile soil suggested that SMO degradation in soils might be due to the combined effect of biotic and abiotic process, with microbes being the major contributors.

In general, SMO degraded more rapidly in all soils than in water, showing the effect of concentration, depth, temperature and sterilization on the degradation rate, which varied among the soils. With increasing concentration and soil depth, degradation was generally slower (except for Horotiu) while with increasing temperature the degradation occurred faster, which was noticeable in all the soils. Overall, SMO antibiotic did not for persist more than 90 days in all three soils at either depth, suggesting that natural bio-degradation is sufficient for the removal of these contaminants from the soil. If degradation for these compounds occurs before they are transported to the deeper soil profile, it is unlikely that residues of SMO antibiotic will persist or accumulate in these soils.

The usage of more complicated kinetic models other SFO has been recommended to describe laboratory pesticide degradation (Herman and Scherer 2006; Lucas and Jones 2006; Sarmah *et al.* 2008). These models avoid an underestimation of degradation rates and can be used to obtain accurate degradation end-points. These models have been successfully applied earlier to model aerobic degradation of 4-*n*-nonylphenol and bisphenol-A in groundwater-aquifer material slurry (Sarmah and Rohan 2011), degradation kinetics of estrone-3-sulfate (Scherr *et al.* 2008), and pesticides (Sarmah *et al.* 2009) in New Zealand soils.

Four kinetic models, SFO and three biphasic kinetic models –BEXP, FODED, and FOTC models – were applied to fit the observed SMO degradation dynamics in order to derive degradation end-points. Even though SFO was appropriate to fit the degradation data of SMO, some of the datasets for the degradation were better described by the biphasic kinetic models. Assuming simple first order kinetics resulted in poor goodness of fit parameters and in underestimation of the initial concentration values. Biphasic models delivered more accurate estimations for degradation endpoints than the SFO model. The model choice was evaluated from its performance and was based on an array of statistical measures such as coefficient of determination (R^2_{adj}), root mean square error (RMSE), chi-square (χ^2) test at 1% significance, and Bayesian Information Criteria (BIC) and % model error.

The BEXP model failed to deliver degradation endpoints for four data sets; this was caused by the iterative procedure that was necessary to estimate these values. The model, however, improved the goodness-of-fit indices in almost all cases, similar to the findings of Scherr *et al*. (2008) and Herman and Scherer (2006). In general, the antibiotic degradation was

successfully predicted by all four models however; the nonlinear biphasic models improved the goodness-of-fit parameters in almost all cases. The fits of FOTC and FODED models for SMO degradation data sets were identical in most cases and prediction was better than the BXEP model. Overall the FOTC and FODED models were found to be superior to the BXEP model, and all three biphasic models were superior when compared with the SFO model for describing SMO degradation data in this study. Despite acceptable fitting results, the simple SFO, BXEP, and FODED models lacked conceptual and mechanistic explanations to describe the mechanisms of degradation of SMO. Though all models were empirical in nature, a conceptual basis and some mechanistic explanations can be found for FOTC model.

Information on the transport and retention behaviour of veterinary antibiotic is important for risk assessment, and several studies in the past have investigated the transport and retention behaviour of range of veterinary antibiotics in soils (Wehrhan *et al.* 2007; Unold *et al.* 2010; Kurwadkar *et al.* 2011; Strauss *et al.* 2011). Under the New Zealand scenario, the transport of organic chemicals such as commonly used pesticides and naturally excreted steroid hormones in pasture soils has been previously investigated (Sarmah *et al.* 2005; Scherr 2009). However, to date there is no published information available about the transport behaviour of sulfonamides in New Zealand soils. In this thesis the transport behaviour of three sulfonamides- SMO, SCP and SM and a conservative bromide tracer (Br^-) was investigated in two undisturbed soil columns collected from the dairy farming regions in the North Island of New Zealand.

Bromide breakthrough maxima occurred at approximately 0.85 and 1.05 pore volumes for Matawhero and Hamilton. Maximum breakthrough for the bromide tracer being similar in both occasions indicated there was no preferential flow observed in either of the soil columns. No significant tailing was observed in the bromide BTCs for either soil, an indication of a well-saturated soil column, and that the soil water is evenly permeable throughout the column length. The breakthrough curves for the three sulfonamides showed that the two soils behaved differently. The three sulfonamides breakthrough curves in Matawhero soil were similar to the conservative bromide tracer. Past studies have also reported peak antibiotic concentration break through to occur simultaneously with bromide break through (Kurwadkar *et al.* 2011). For the Hamilton soil considerable retardation was observed. This delayed peak arrival time (or maxima) highlights the important role of

sorption-related retention processes under saturated flow conditions. Residual antibiotic concentrations for SMO and SCP were detected even in the last core sections at up to depths of 18 cm, while no resident concentration of SM was detected at any depth in the entire profile for both the soils.

A physical equilibrium model available in CXTFIT (Toride *et al.* 1995) was used to inversely fit the bromide tracer breakthrough data. In contrast to some recent studies (Fan *et al.* 2011; Jeong *et al.* 2012), no long tails and asymmetrical breakthrough were seen for the bromide BTCs, indicating the non-existence of physical non-equilibrium. The CXTFIT model described well the peak arrival time as well as the maximum concentration of the sulfonamides in the effluent. However, the model underestimated SCP and SMO leaching at the advanced stage of Matawhero BTCs. The chosen model was limited to deliver information about physical processes only, while the chemical non-equilibrium processes involved were not taken into account. Incorporating both these processes could give a model, that would be better suited to describe antibiotic transport in undisturbed soil media (Fan *et al.* 2011; Jeong *et al.* 2012).

10.2 Conclusion

The fate and behaviour of veterinary antibiotics in New Zealand dairy farm soils is greatly influenced by soil specific properties and the properties of the antibiotic. Their sorption to soil seems to be influenced by chemical properties such as pK_a, hydrophobicity, and soil solution characteristics such clay content, organic matter, pH, ionic strength, and cation exchange capacity of the soil and the presence of other contaminants. Degradation of the sulfonamide antibiotic occurred rapidly in the laboratory incubations experiments, and did not tend to persist more than 90 days in all three soils at either depth, suggesting that natural bio-degradation is sufficient for the removal of these contaminants. Under laboratory conditions, the experimental set-up can easily be altered to suite the research objective; however, under realistic field conditions the degradation rates could be much faster as they could be simultaneously affected by a multitude of factors such as moisture content, temperature, humidity, rainfall, and soil properties. Transport studies indicated that the three sulfonamides were highly mobile at soil pH (4.5–6.5). Residual antibiotic concentrations for SMO and SCP were detected even at depths of 18 cm. Their transport through soils is of concern after direct excretal inputs from grazing animals or by field applications. Their potential to percolate through the soil profile indicates an increased threat to groundwater, surface water and eventually to drinking-water supplies. The knowledge gained from this thesis would be helpful in improving agricultural practices in the future so that soils and waterways are protected from possible antibiotic over-loading in the environment.

Based on the research objectives set out in this thesis, the following key conclusions were drawn:

1. An isocratic elution scheme comprising of acetonitrile, water (0.05% trifluoroacetic acid, pH 2.3) and tetrahydrofuran was developed to separate and detect the selected veterinary antibiotics using a HPLC-UV system. A gradient system comprised of the same mobile phases was also developed to detect and quantify the three sulfonamides in a single run. A dichloromethane (DCM) solvent extraction technique was found to be suitable for the extraction of veterinary antibiotic from aqueous and soil samples. Overall, the method led to significant runtime reductions allowing for higher sample throughput.

2. Sorption of the studied antibiotic varied with antibiotic and soil types. Sorption of TT was very high in Matawhero soil, moderate in Gibson soil, and very low in other investigated soils. Sulfonamides, on the other hand, showed very low sorption affinity to all soils. The governing sorption mechanisms behind tylosin and sulfonamide are postulated to be the cation exchange with the active clay sites and hydrophobic partitioning due to soil organic carbon. Freundlich isotherms were highly non-linear in most cases.

3. Sorption of the sulfonamide antibiotic (SMO) was highly dependent on pH, ionic strength, and the organic carbon content of the soil. Sorption decreased with increases in pH, and also increased with the increasing ionic strength and organic carbon content of soil. A hydrophobic pH-partitioning model linking sorbate speciation with species-specific sorption coefficients describing the pH dependence of the apparent sorption coefficients was successfully used to derive the fractions of each species of SMO that are likely to be present in the environment.

4 Biochar was used as an effective sorbent for antibiotics associated with land-application of effluents. It was observed that biochars made from different feed stocks and under different heat treatment conditions can possess differential sorption behaviour for antibiotic. PSD biochar had the highest sorptive capacity among the three biochars used in this study, being nearly 30-fold greater than the control soil.

5. SMO degraded rapidly in New Zealand agricultural soils. SMO degradation in these soils was a combined effect of biotic and abiotic degradation, with microbes being the major contributors. Both the degree of biological activity and the temperature of the soil influenced overall degradation. Abiotic factors such as strong sorption onto soil components also played a role in the dissipation of SMO in soil.

6. Non-linear biphasic models- BEXP, FODED, and FOTC provided a good description of SMO degradation datasets when compared with SFO model. Although biphasic models like BEXP and FODED lacked conceptual and mechanistic explanations, the FOTC model was found to be the most suitable for obtaining degradation endpoints.

7. Soil column transport studies showed that sulfonamides were highly mobile and exhibited a mobility pattern similar to that of conservative bromide tracer. The mobility of antibiotics varied among soils with different physico-chemical properties and was pH dependent, similar to the recent findings by Kurwadkar *et al*. (2011). The deterministic, physical equilibrium model (CXTFIT) described the peak arrival time as well as the maximum concentration of the antibiotic breakthrough curves well, but showed some underestimation at advanced stages of sulfonamide leaching. The model, however, could not provide information about potential chemical non-equilibrium processes involved in antibiotic transport.

10.3 Future research

Research related to the environmental fate of veterinary antibiotics has increased rapidly, to which this thesis has contributed one peer-reviewed manuscript and two peer-reviewed conference papers. There is a continuous flow of published literature evaluating the occurrence, detection, and fate of veterinary antibiotics, with a particular focus on agricultural systems. These studies provide novel information about specific processes involved in their fate and behaviour in the environment, which are essential from a regulatory and agricultural management perspective. Understanding the occurrence and fate of antibiotics in the environment is also important in order to reduce the impacts of these contaminants on wildlife and humans. Despite recent progress, there are still many gaps in the scientific knowledge about the fate and behaviour of veterinary antibiotics in the pastoral environment, and this thesis provides some recommendations for future research:

- Fate of other veterinary antibiotics need to be investigated with respect to their environmental fate and behaviour. Given bacitracin is one of the most heavily used antibiotics in New Zealand poultry industry accounting for nearly 22 tonnes/year, studies are warranted to investigate their fate in crop lands, where poultry litter is applied by farmers.

- In the environment most antibiotic contamination occurs via urination from animals. The influence of realistic exposure matrices such as urine solutions on the sorption kinetics of veterinary antibiotics have not been studied, which constitutes a need for future research. Fate studies carried out in the laboratory should be kept to a minimum; as much work is needed to understand their sorption behaviour under realistic field situations especially in soils amended with manure, fertilizers and in the presence of other contaminants in soils.

- In this thesis no attempt was made to allow soils mixed with biochar to age before commencing the adsorption experiments or assess the potential effects of presaging on adsorption. Aged biochars could introduce significant changes and could correct for factors like initial water-repellent properties of biochar.

- The effects of anoxic conditions, varying moisture content, and manure amendment along with antibiotic persistence in the presence of other contaminants need to be investigated.

- Measuring the variations in the microbial biomass of the antibiotic spiked soil during the incubation period, along with the isolation of microorganisms present in these soils, could provide new insights into possible degradation mechanisms.

- Recently developed transport models for the organic contaminants that incorporate both physical and chemical non-equilibrium processes could also be applied to study the transport characteristics of veterinary antibiotics. These models could be better suited to predict their transport behaviour in saturated soils.

- Field-based transport experiments could provide better information than laboratory-based studies, and such information would be very important to manage agricultural operations in a manner that protects receiving waters from antibiotic contamination.

- The influence of soil pH on the mobility of veterinary antibiotics needs to be investigated, especially for various ionisable antibiotics.

- Studies should be carried out on the relationship between antibiotic residues and antibiotic resistant bacteria in the environment. The ecotoxicological effects of antibiotic residues on non-target organisms should also be studied.

- More emphasis should be given to exposure modelling and risk assessments studies.

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12 Appendices

12.1 Soil characterisation

Soils were also characterised for their morphological properties using a range of techniques such as scanning electron microscopy (SEM), x-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), inductively coupled plasma mass spectrometry (ICPMS), and energy dispersive x-ray analysis (EDX). A detailed description of the experimental protocols, sample preparation and analysis for these techniques can be found in Chapter 5.

The FT-IR spectra for all the six soils are presented in Figure 12.1. The IR spectrum indicated the surface functional groups present in the soils. The spectra for the soils were similar except for Matawhero soil. For all soils in general various bands in the spectra representing stretches were due to alcohol and phenol–OH (3600–3200 cm^{-1}; 1100–1000 cm^{-1}), amides C=N (1632 cm^{-1}), aliphatic C–H (1470–1455 cm^{-1}) and alkenes C=C, C–H, and CH_2 (1000–675 cm^{-1}). The FT-IR spectrum obtained for the Hamilton soil contained distinct signals representing alcohol–OH (3600 cm^{-1}) at 3640-3550 cm^{-1}, and 908 cm^{-1}alkenes. The FT-IR spectrum of the Horotiu soil contained signals due to alcohol–OH (3600 cm^{-1}), aliphatic hydrocarbon C–H (3050 cm^{-1}), aliphatic CH_2 (2920 cm^{-1}), a much sharper peak for aromatic C=C and C=O (1400–1600 cm^{-1}), alkenes C=C, C–H, and CH_2 (1105 cm^{-1}), and a small peak for aromatic rings at 820 cm^{-1}. A peak for the ester C=O (1700 cm^{-1}) was observed for Matawhero soil. Most of the soils, with the exception of the Matawhero soil, have very similar IR spectra. The Matawhero soil showed absorptions in the 1400–1500 cm^{-1} region that were not present in other soils and lacked the strong absorption at 1000 cm^{-1} that were present in the other soils.

Scanning electron micrographs are shown in Figure 12.2. The macroporous nature of the soils is particularly evident in the high resolution (600 ×) micrographs. It is important to note that the pores observed through SEM micrographs would not necessarily contribute much to the overall specific surface area (SSA) of the sorbent. For instance, the specific surface area for all soils were relatively low as shown in Table 4.1**.**

XRD analysis of the soils (Figure 12.3**)** showed two distinct groupings, with the Matawhero, Gibson and Manawatu soils exhibiting intense peaks, while; the peaks from Hamilton clay, Horotiu and Te Kowhai were less intense. X-ray diffraction patterns showed distinct peaks for quartz (Q), feldspar (F) and calcite (K) The mineral composition of the bulk soil from the peak intensity could be observed as quartz > calcite > feldspar. The XRD was not indicative of presence of clay mineral like vermiculite, chlorite and illite as these peaks occur below a 2θ value of 10^{o} and low angle detection was not monitored.

Quantitative ICPMS analyses of the digested soils **(**Table 12.1**)** gave the major elements (Al, Fe, Mg, Ca, Na, K) present in the soils. The relative amounts of divalent cations (Ca^{2+} and Mg^{2+}) that were present in Matawhero and Gibson soils were higher than for the other soils used in this study and this may have affected sorption capacity by cation exchange which is discussed later.

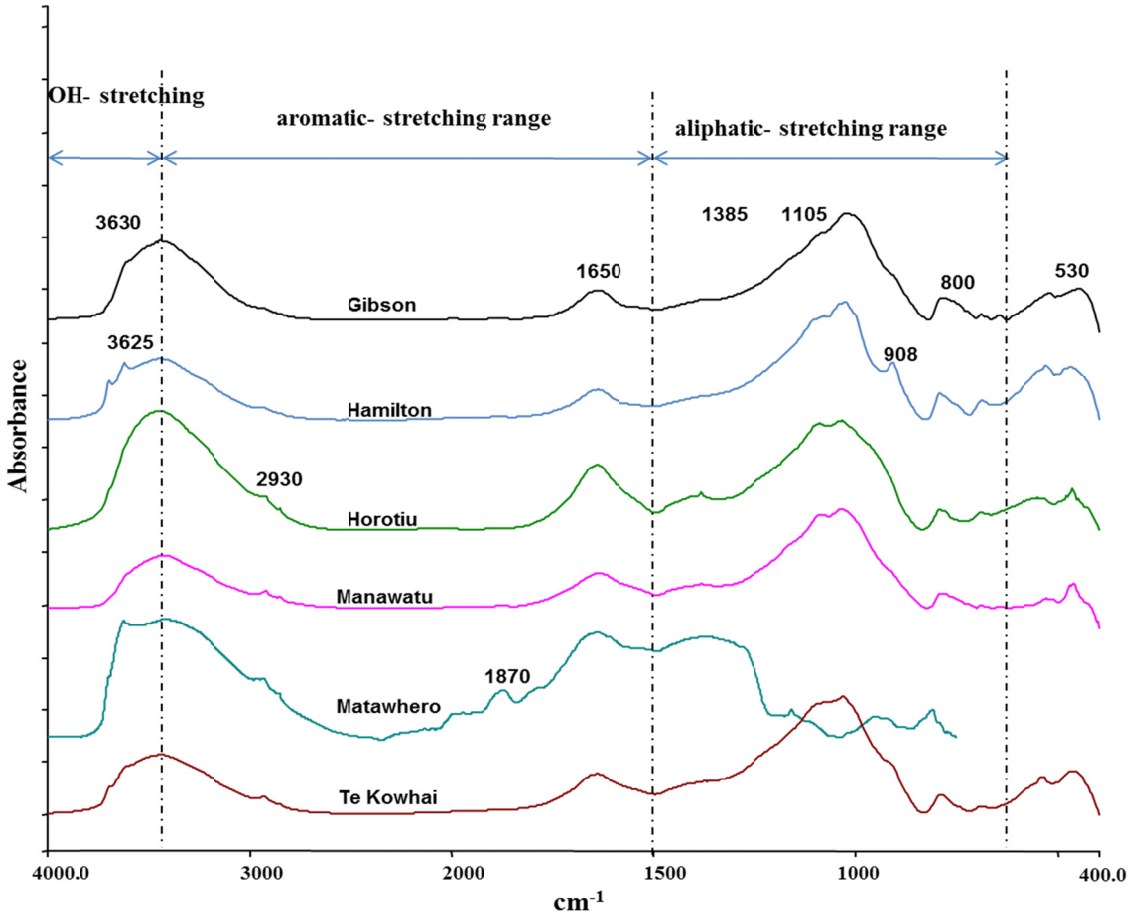
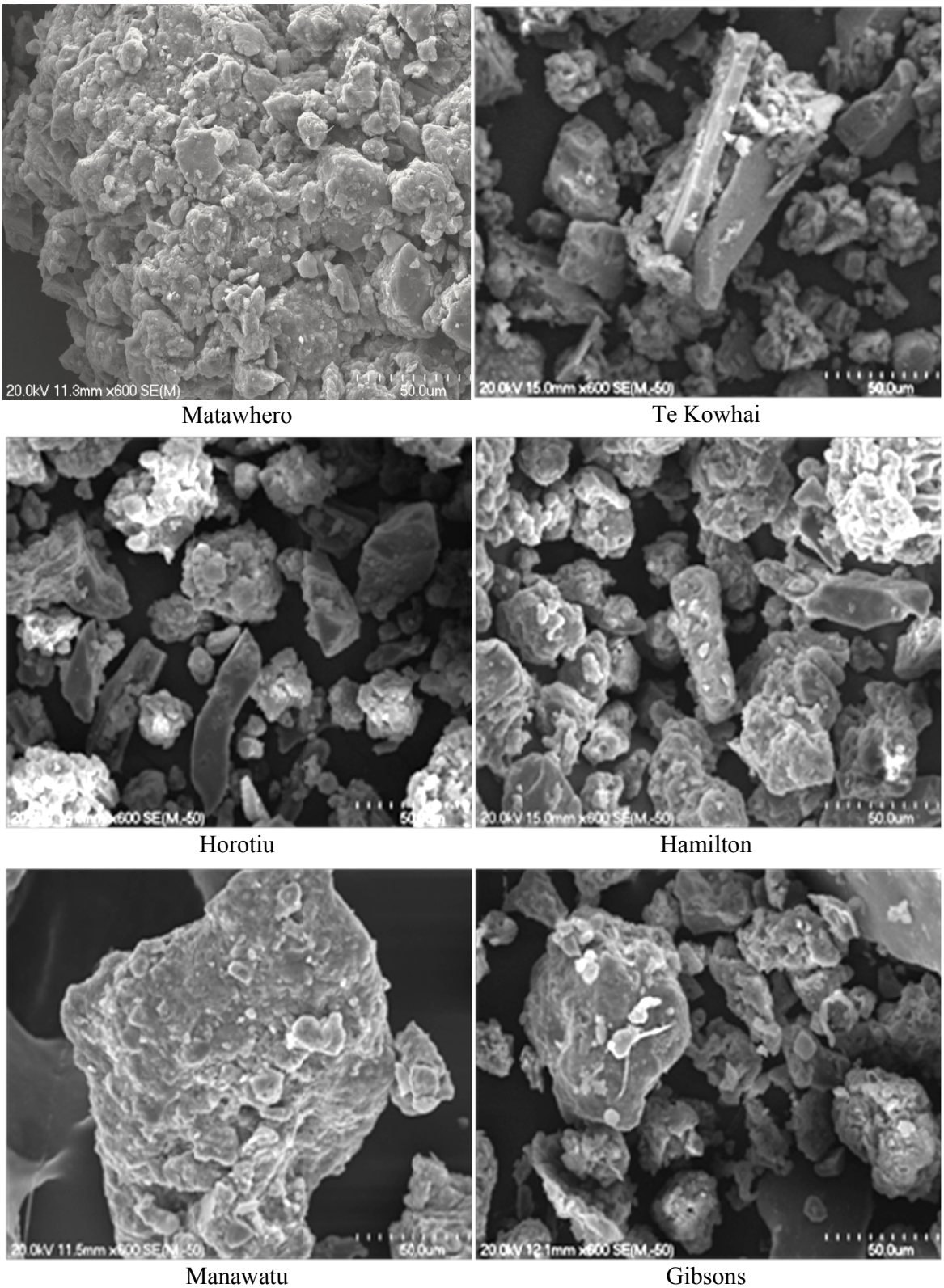


Figure 12.1: Fourier transform infrared spectra of the soils.



Matawhero Te Kowhai

Horotiu Hamilton

Manawatu Gibsons

Figure 12.2: SEM images (600× magnification) of soils.

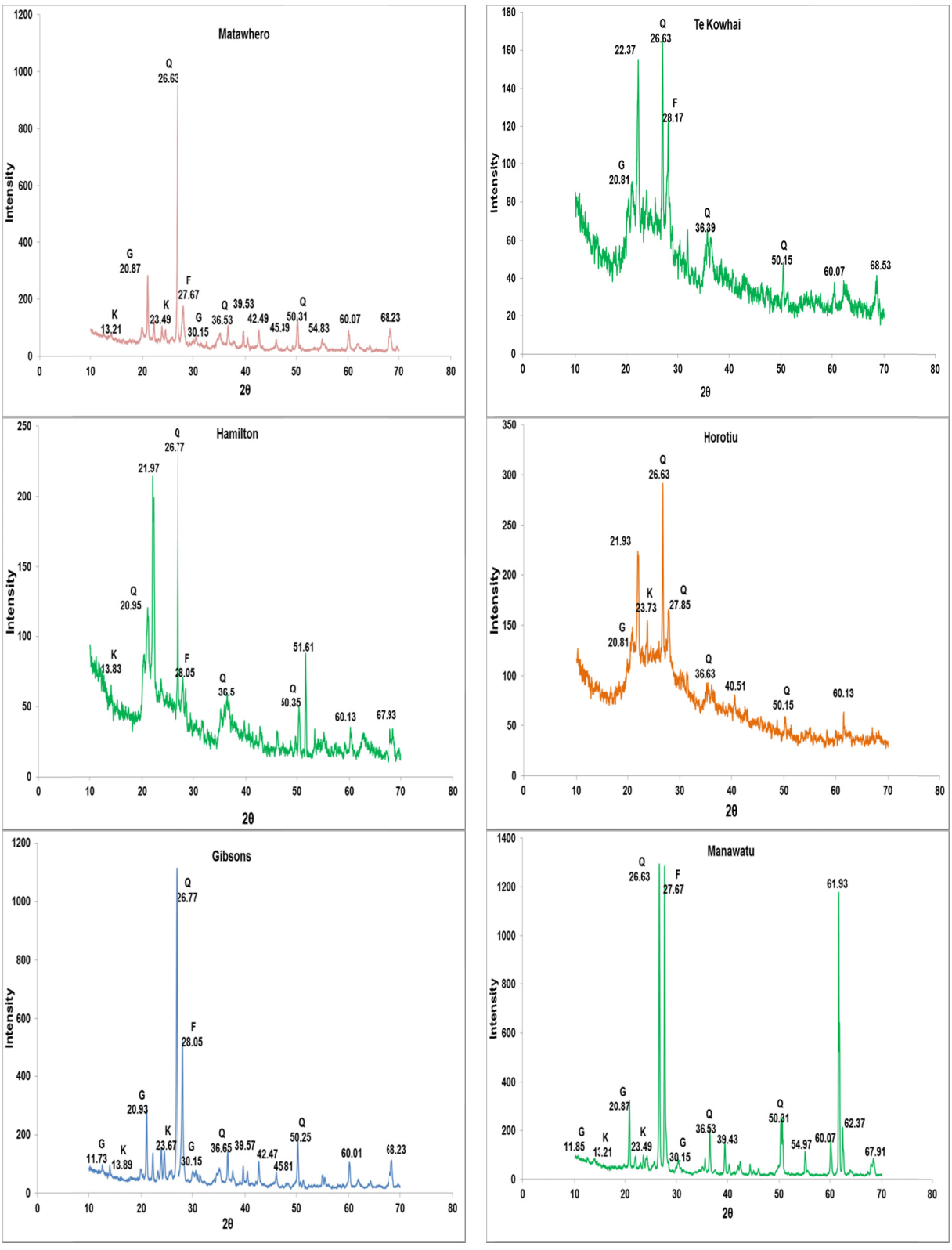


Figure 12.3: X-ray diffraction spectra of the soils.

Table 12.1: ICPMS analysis of cations present in the soils (cmol kg^{-1}).

cmol kg^{-1}	Na	Mg	Al	K	Ca	Fe	Mn	Cu	Zn	Σ cations
Matawhero	2.51	38.71	110.44	4.82	43.59	16.63	0.00	0.02	0.09	216.82
Hamilton	0.54	4.02	205.21	0.71	8.72	26.95	9.01	0.01	0.05	255.23
Horotiu	0.37	4.34	176.23	1.84	15.74	7.53	0.59	0.01	0.04	206.68
Te Kowhai	18.71	2.93	134.05	0.93	14.31	5.83	1.36	0.01	0.07	178.20
Gibsons	5.96	40.49	120.61	4.35	18.64	15.67	0.00	0.02	0.05	205.78
Manawatu	1.50	13.34	80.81	1.22	14.65	9.29	0.37	0.01	0.03	121.22

12.2 Multi-variance analysis

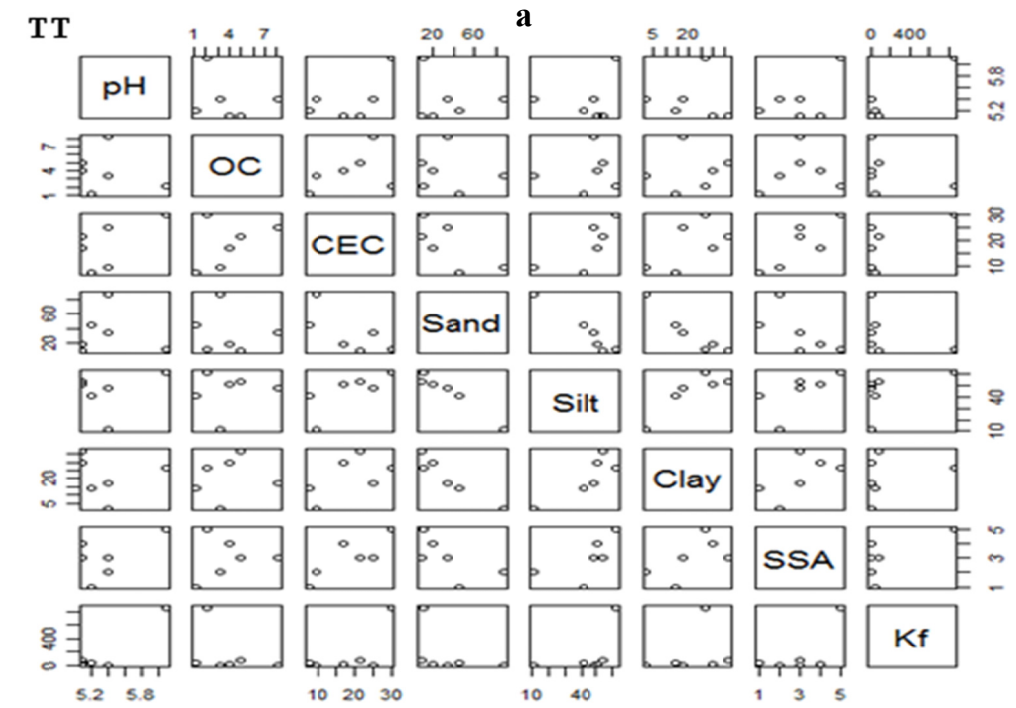
A multi-variance analysis (Figure 12.4**)** of the sorption data was performed using R Gui, with a significance level of 5% (p). Table 12.2 shows the results of the multi-variance analysis. First, there was a need to identify variables (in pairs) which could play a significant role in sorption. Once the variables were identified in pairs, they were then tested as a function of sorption parameter (K_f), and any result that fell within the set confidence level was tested for its interaction. Horotiu soil with high OC content and high CEC had high sorption affinity compared with Matawhero soil, which had low OC content and high CEC. Although soils with high OC and clay content seem to have greater sorption affinity for sulfonamides, the influence of OC content seems to be a dominant factor in determining their overall sorption affinity for soils.

Table 12.2 also shows that the sorption parameters of TT are a function of CEC and % OC, and for SM a function of % OC and % clay (Figure 12.4). These two scenarios were further checked to establish if there were any interactions between the tested soil properties to increase or decrease sorption efficiency of the soil. While the p value for K_f~ CEC* OC (TT) was > 0.05, it was, however, within the limit ($p < 0.05$) for the event K_f~ OC* clay (SM). With a limited number of observations (6) compared to the variables (6), it was difficult to verify the accuracy of the results.

Figure 12.5a clearly indicates that two factors strongly influence the sorption of TT to soils: cation exchange capacity and organic carbon content of the soils. These two factors work in opposition, increasing cation exchange capacity increases sorption, since the pH of all six

soils is less than the TT pKa, whereas increasing organic carbon content decreases sorption. In the case of sulfonamides, SM is strongly influenced by organic carbon content of the soils (Figure 12.5b). The high clay content in Matawhero soil seems to aid in SMO sorption. Although soils with high OC and clay content seem to have greater sorption affinity for sulfonamides, the influence of OC content seems to be a dominant factor in determining their overall sorption affinity for soils.

a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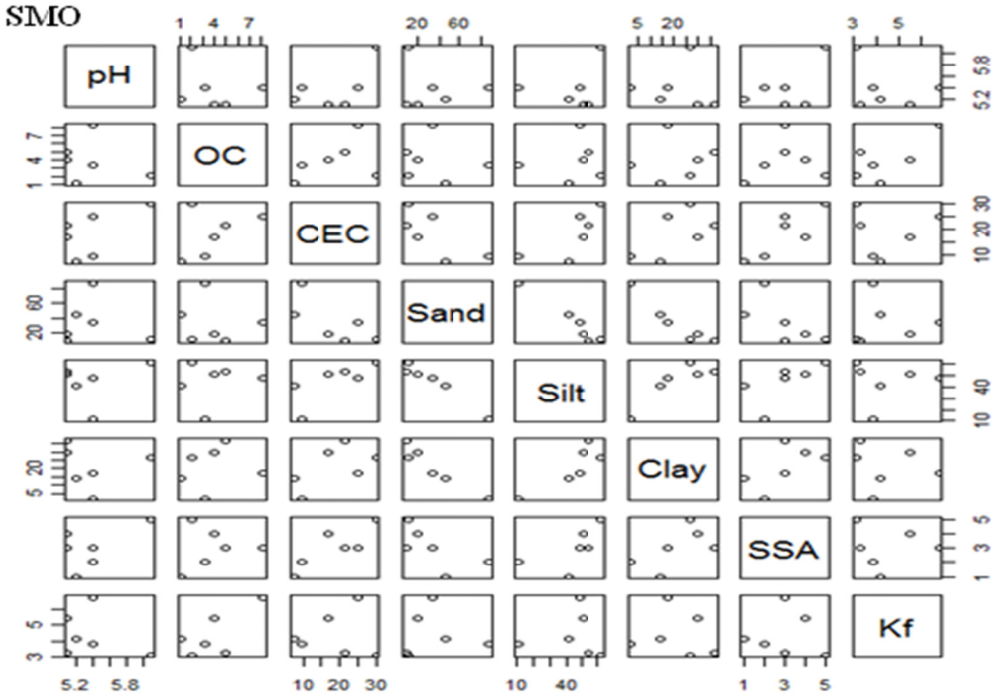
OC
CEC
Sand
Silt
Clay
SSA
Kf


SMO
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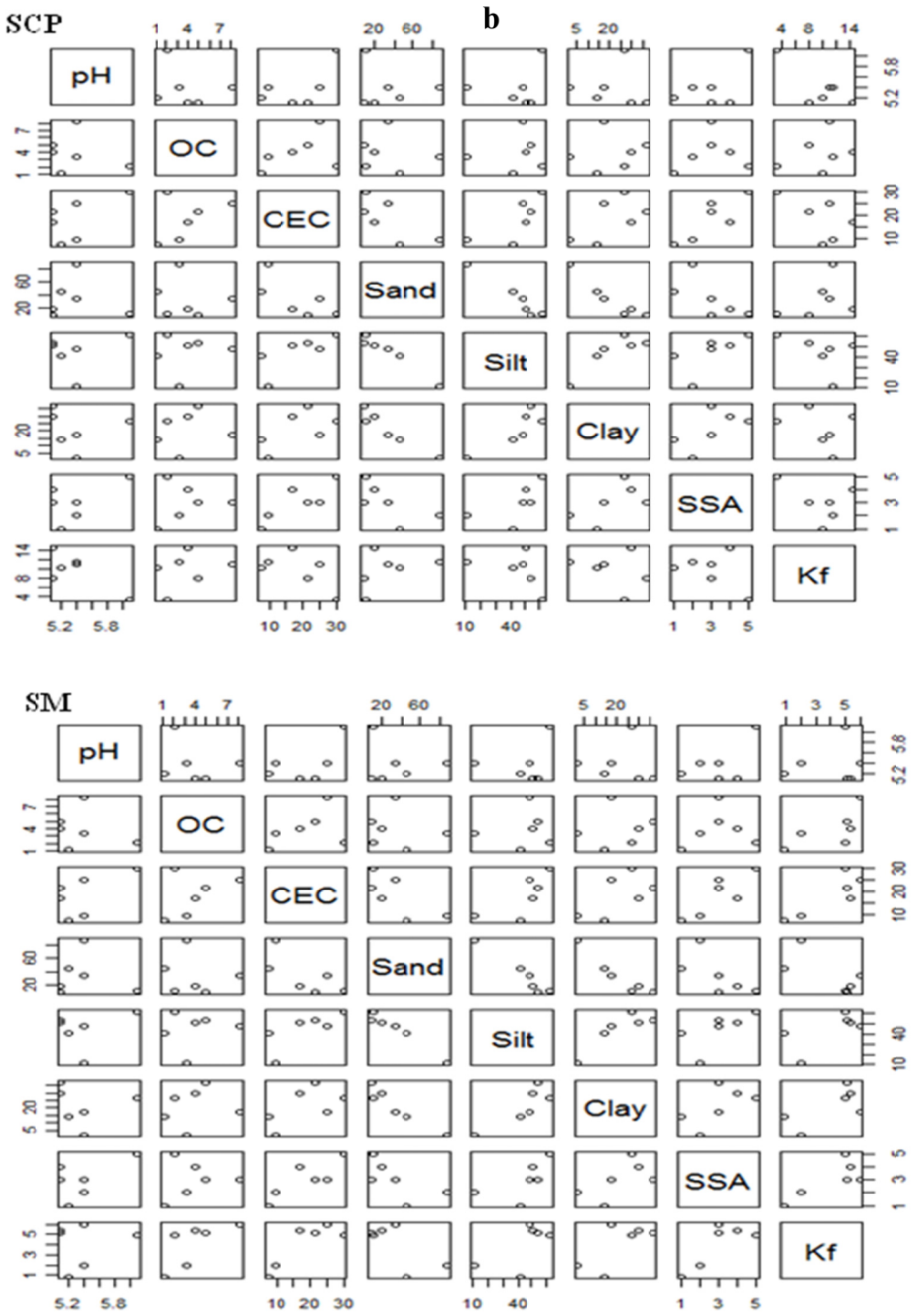

Figure 12.4: Multi-variance plots TT, SMO(a), SCP and SM(b) as a function of six different soil parameters.

Table 12.2: Multi-variance analysis results using R Gui. The numbers (p values) indicating significance level with < 5% being statistically significant. The significant scenarios are highlighted in bold.

		TT	**SMO**	**SCP**	**SM**
1	K_f~ OC+CEC	**0.057; 0.045** **both significant**	0.144; 0.521 both not significant	0.163; 0.093 both not significant	0.183; 0.067 CEC significant
2	K_f~ pH+CEC	0.576; 0.596 both not significant	0.484; 0.627 both not significant	0.234; 0.675 both not significant	0.077; **0.011** **CEC significant**
3	K_f~ pH+ sand	0.219; 0.123 both not significant	0.616; 0.977 both not significant	0.109; 0.418 both not significant	0.892; 0.204 both not significant
4	K_f~ pH+clay	0.147; 0.127 both not significant	0.591; 0.758 both not significant	0.087**;** 0.372 both not significant	0.675; 0.200 both not significant
5	K_f~ OC+pH	0.376; 0.531 both not significant	0.1417; 0.137 both not significant	0.773; 0.138 both not significant	0.138; 0.475 both not significant
6	K_f~ OC+sand	0.1417; 0.137 both not significant	0.176; 0.737 both not significant	0.540; 0.447 both not significant	0.0745; 0.785 both not significant
7	K_f~ OC+clay	0.239; 0.156 both not significant	0.157; 0.559 both not significant	0.580; 0.597 both not significant	0.086; 0.0909 both not significant
8	K_f~ Sand+CEC	0.342; 0.900 both not significant	0.846; 0.849 both not significant	0.939; 0.416 both not significant	0.712; 0.185 both not significant
9	K_f~ Clay+CEC	0.489; 0.637 both not significant	0.712; 0.779 both not significant	0.848; 0.337 both not significant	0.516 ;0.139 both not significant
10	K_f~ Sand+clay	0.346; 0.614 both not significant	0.62; 0.583 both not significant	0.461; 0.575 both not significant	0.707; 0.912 both not significant
11	K_f~ OC*CEC	0.348; 0.153 No interaction		0.469;0.511 No interaction	0.348; 0.153 No interaction
12	K_f~ pH*CEC				0.921;0.537 No interaction
13	K_f~ OC*sand				0.656; 0.167 No interaction
14	K_f~ OC*clay				**0.0584; 0.0632** **Interaction; R^2= 0.96**
15	K_f~ pH*clay			0.845;0.920 No interaction	

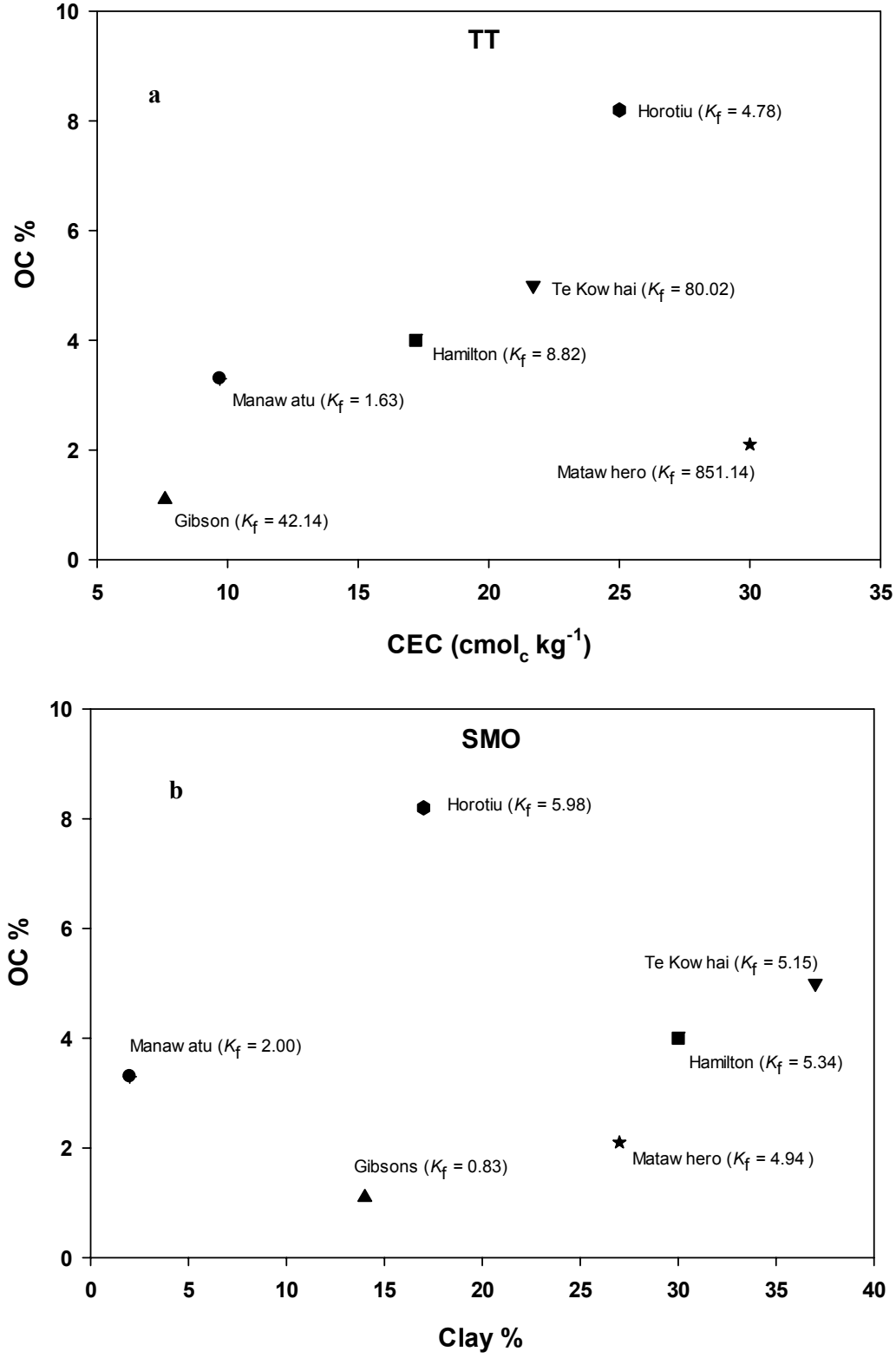

Figure 12.5: K_f values for TT (a) plotted as a function of cation exchange capacity and percent organic carbon; and for SM (b) as a function of clay content and percent organic carbon in six soils.

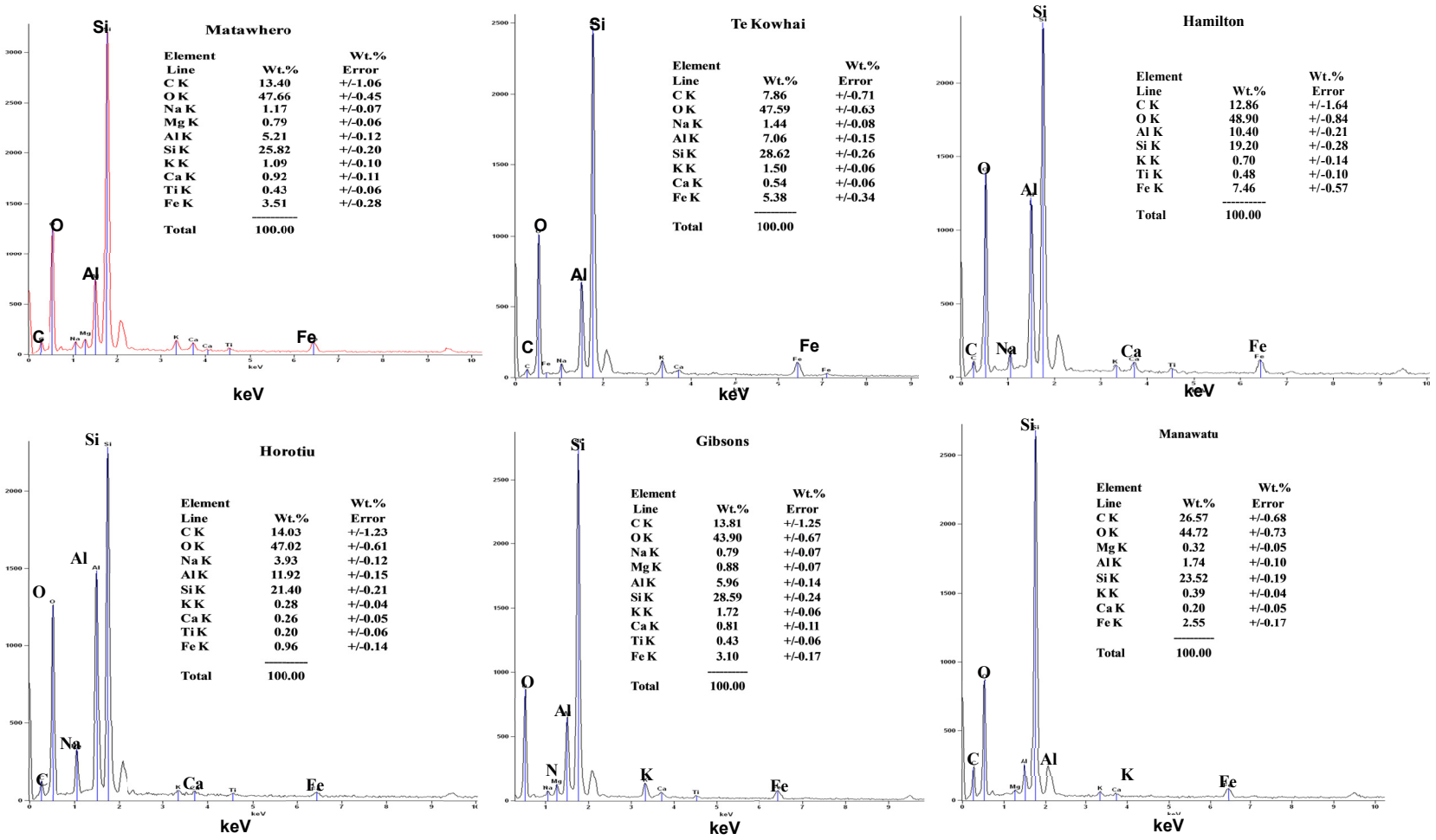

Figure 12.6: EDX spectra of the soils.

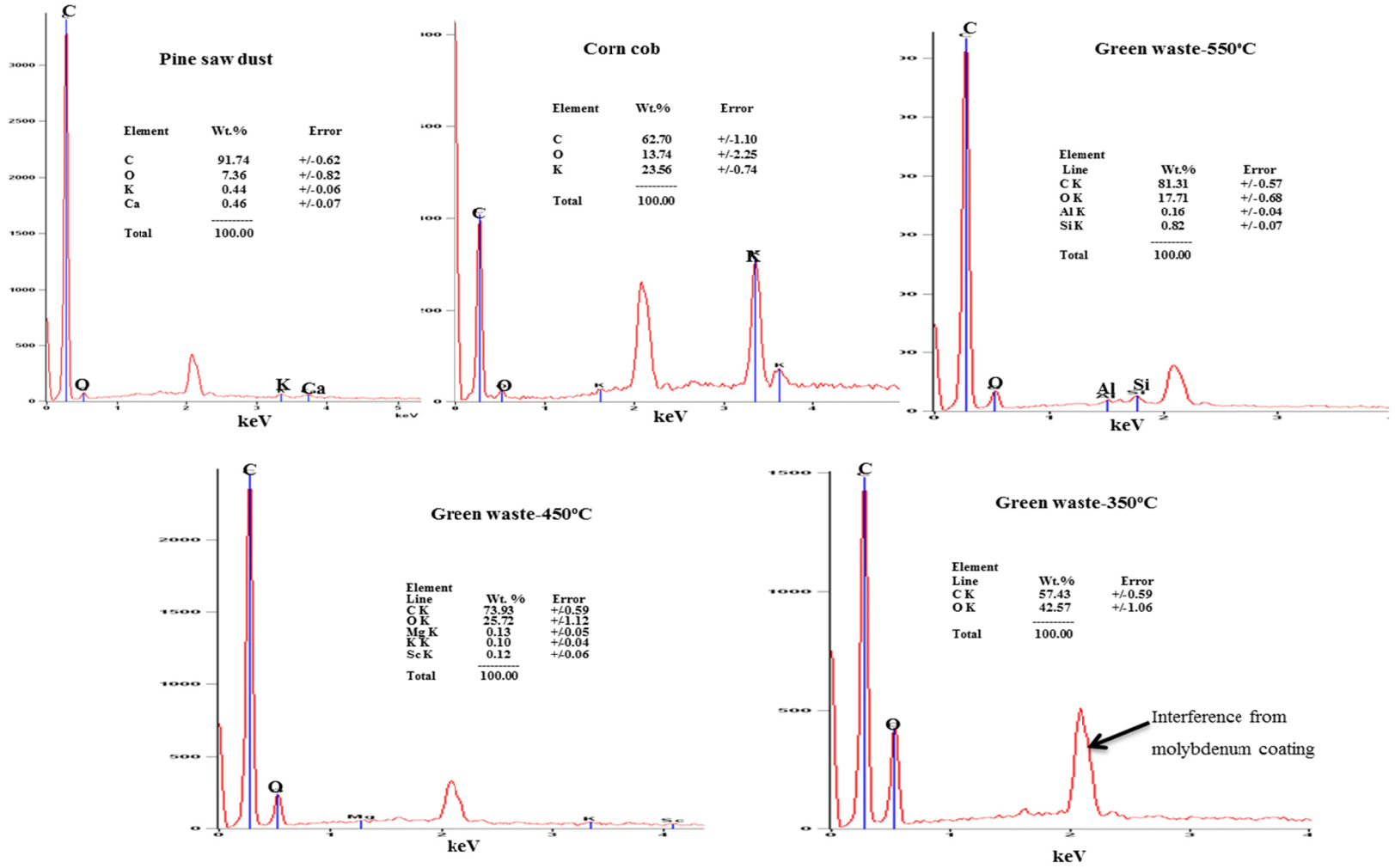

Figure 12.7: EDX spectra of the biochars.

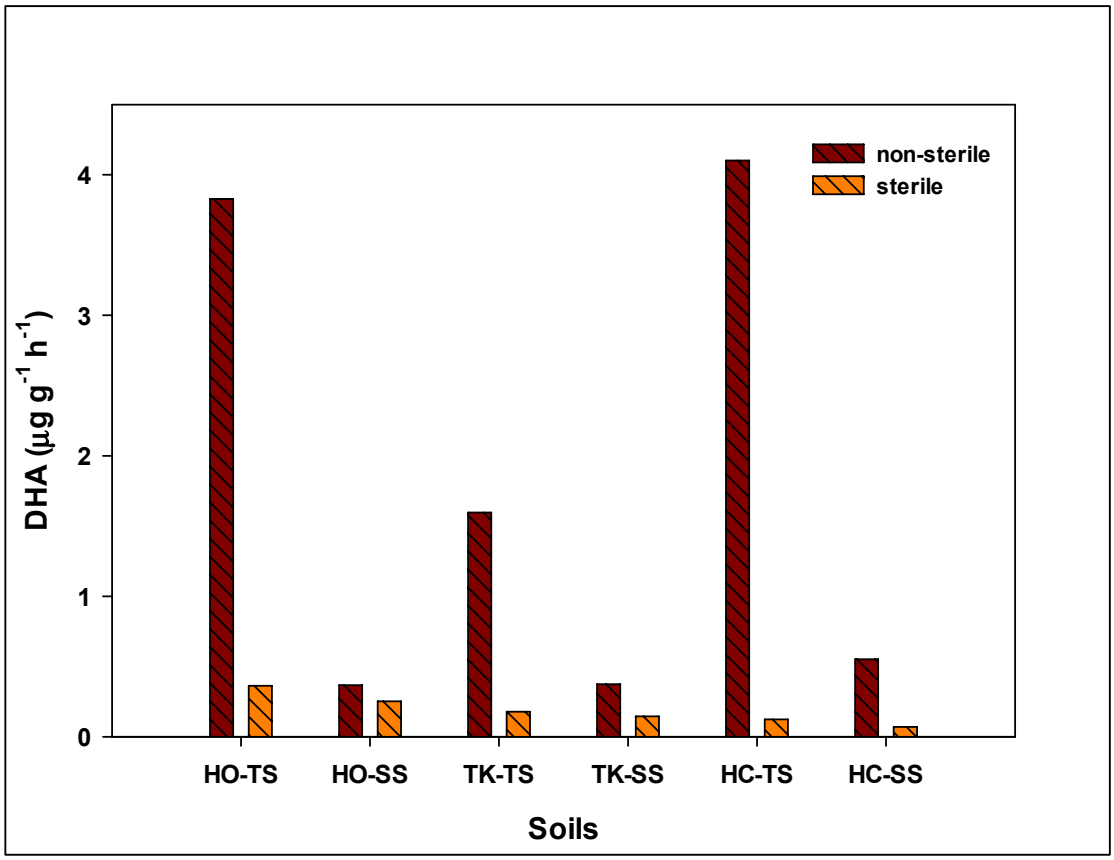

Figure 12.8: DHA activity of sterile and non-sterile soils.