Science of the Total Environment 479–480 (2014) 284–291

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Dissipation of sulfamethoxazole in pasture soils as affected by soil and environmental factors

Prakash Srinivasan [a,b], Ajit K. Sarmah [a,*]

[a] Department of Civil & Environmental Engineering, Faculty of Engineering, The University of Auckland, Private Bag 92019, Auckland, New Zealand
[b] Landcare Research, Private Bag 3127, Hamilton, New Zealand

HIGHLIGHTS

- Sulfamethoxazole dissipation was a combined effect of biotic and abiotic factors, with microbes being the major contributors.
- SMO dissipation rate in soils was independent of initial spiked concentration.
- Phospholipid fatty acid analysis was indicative of higher bacterial presence as compared to fungal community.
- Sulfamethoxazole is unlikely to persist more than 5–6 months in pasture soils at either depth.

ARTICLE INFO

Article history:
Received 9 January 2014
Received in revised form 5 February 2014
Accepted 5 February 2014
Available online 22 February 2014

Keywords:
Sulfamethoxazole
Dissipation
Kinetics
Dehydrogenase
PLFA

ABSTRACT

The dissipation of sulfamethoxazole (SMO) antibiotic in three different soils was investigated through laboratory incubation studies. The experiments were conducted under different incubation conditions such as initial chemical concentration, soil depth, temperature, and with sterilisation. The results indicate that SMO dissipated rapidly in New Zealand pasture soils, and the 50% dissipation times (DT_{50}) in Hamilton, Te Kowhai and Horotiu soils under non-sterile conditions were 9.24, 4.3 and 13.33 days respectively. During the incubation period for each sampling event the soil dehydrogenase activity (DHA) and the variation in microbial community were monitored thorough phospholipid fatty acid extraction analysis (PLFA). The DHA data correlated well with the dissipation rate constants of SMO antibiotic, an increase in the DHA activity resulted in faster antibiotic dissipation. The PLFA analysis was indicative of higher bacterial presence as compared to fungal community, highlighting the type of microbial community responsible for dissipation. The results indicate that with increasing soil depth, SMO dissipation in soil was slower (except for Horotiu) while with increase in temperature the antibiotic loss was faster, and was noticeable in all the soils. Both the degree of biological activity and the temperature of the soil influenced overall SMO dissipation. SMO is not likely to persist more than 5–6 months in all three soils suggesting that natural biodegradation may be sufficient for the removal of these contaminants from the soil. Its dissipation in sterile soils indicated abiotic factors such as strong sorption onto soil components to play a role in the dissipation of SMO.

1. Introduction

An estimated 9000 tonnes of antibiotics is annually used in the livestock industry by the US, 5000 tonnes by the European Union, and 6000 tonnes by China. After administration, a high proportion (30–90%) of the antibiotics is excreted by livestock animals in unchanged form and/or sometimes as metabolite/s (Sarmah et al., 2006). Occurrences of antibiotic residues are common in many parts of the world and have been detected in environmental media such as soils, surface water, and ground water (Hamscher et al., 2003; Luo et al., 2011; Perret et al., 2006; Zuccato et al., 2005). Although the use of antibiotics in livestock industry in New Zealand (NZ) is not as widespread as in many other parts of the world, intra-mammary injectable antibiotics dominate the dairy industry (Srinivasan et al., 2013). According to the New Zealand Food Safety Authority, the sulfonamide group contributes ~17% of total antibiotic usage, and is a common class of antibiotics widely used in livestock industries in NZ. Free pasture grazing by millions of cattle is common in many parts of NZ, and dairy industry has been also expanding at a rapid rate especially in South Island of NZ. Because of direct excretal inputs by grazing animals and permitted activity such as land-application of animal waste by farmers, there is a concern that antibiotic residues may be entering the environment and could potentially impact the aquatic and terrestrial ecosystems.

* Corresponding author at: Department of Civil & Environmental Engineering, Faculty of Engineering, Private Bag 92019, Auckland 1142, New Zealand. Tel.: +64 9 9239385; fax: +64 9 3737462.
E-mail address: a.sarmah@auckland.ac.nz (A.K. Sarmah).

http://dx.doi.org/10.1016/j.scitotenv.2014.02.014
0048-9697/

In the last decade several studies have been conducted on the biodegradation of sulfonamide antibiotics such as sulfadiazine (SDZ), sulfamethazine (SMZ), and sulfachloropyridazine (SCP) in soils under diverse laboratory conditions (Accinelli et al., 2007; Fan et al., 2011; Halling-Sørensen et al., 2003; Kreuzig and Holtge, 2005; Thiele-Bruhn and Peters, 2007; Wang et al., 2006b; Yang 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. Limited studies on sulfonamide degradation in soils have shown that 50% dissipation half-life (DT_{50}) values for sulfonamides ranged from as low as 1 day to 2 weeks under varied initial concentration and temperature (SI. Table 1). These studies are not appropriate to compare each other because of the differences in their experimental approaches and objectives. Some of the major findings from previous studies which focused on degradation of SMO or other compounds within the same group in soils suggest that sulfonamides may be more persistent than would be predicted from laboratory controlled studies (Bialk et al., 2005). Studies conducted by Accinelli et al. (2007) found that high initial concentration (100 mg kg^{-1}) did not affect the dissipation rate of SMZ and SCP suggesting that at environmental concentrations (ppb or ppt level) there would be little or no effects. Elsewhere, SMO and trimethoprim showed higher dissipation than tylosin in soils, owing to greater sorption potential for the latter in soils (Liu et al., 2010). 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., 2009).

While there have been a number of studies on sulfonamide degradation in manure, sludge amended soils, studies on pasture soils have hitherto been neglected. NZ pasture soils are high in organic carbon content and minerals such as allophane, and these properties have been found to have pronounced effects on the sorption of these compounds which could indirectly influence their degradation behaviour in soils (Srinivasan et al., 2012). Dissipation half-life is an important input parameter required in antibiotic fate modelling exercises and for risk assessment purposes. Sulfamethoxazole (SMO), which belongs to the sulfonamide group of antibiotics, was chosen for this study, as limited information exists about its fate in soil (Holtge and Kreuzig, 2007). Given the varied soil and climatic conditions of NZ, extrapolating degradation data obtained from overseas studies to NZ conditions may not reflect the true nature of SMO degradation behaviour. Although the terms 'dissipation' and 'degradation' have been used interchangeably in the literature, it would be appropriate to use the term 'dissipation' instead of 'degradation' in the work presented here as we did not attempt to identify metabolites formed during the experimental period.

The main objective of this study was to conduct laboratory incubation experiments to investigate the dissipation kinetics of SMO antibiotic in topsoils and subsoils collected from three pasture soils (Te Kowhai, Hamilton and Horotiu). The use of subsoils in this study was necessitated by the marked differences in the values for pH, organic carbon, and microbial biomass of the top and subsoils, which could affect the overall dissipation behaviour of SMO. The incubation conditions were maintained at 60% maximum water holding capacity (MWHC) and with varying initial antibiotic spiked concentrations, different depth profiles, temperature regimes (7.5 °C and 25 °C) and with sterilisation at 60% MWHC. The principal focus was to derive the dissipation times (50%, 90%, and 99%) of SMO under each condition, and compare them to values that were reported in the literature. In order gain a better understanding about the dynamics of the SMO degradation under varied treatment conditions, we also performed phospholipid fatty (PLFA) analysis of samples and discuss our results in relation to the microbial community composition and their effects on the fate of SMO in the selected soils.

2. Materials and methods

2.1. Chemicals

SMO (>98% purity), triphenyl tetrazolium chloride, Tris (hydroxymethyl) aminomethane (TRIS) buffer and triphenyl formazan were obtained from Sigma Aldrich, Australia. Acetonitrile (Mallinckrodt ChromAR, ≥99.8% purity), chloroform, acetone, methanol and dichloromethane (Mallinckrodt UltimAR, ≥99.9% purity) were obtained from Thermo Fischer Scientific Ltd. NZ. High Performance Liquid Chromatography (HPLC) grade deionised water was obtained from an onsite Arium® 61316 high performance reverse osmosis system (Sartorius Stedim Biotech GmbH, Germany).

2.2. Soils

Topsoil and subsoil of three soils (Te Kowhai silt loam, Hamilton clay loam, and Horotiu silt loam) representative of dairy farming areas of Waikato region in the North Island of NZ were collected fresh from two depths (0–10, 30–40), sieved (2 mm), and stored at 4 °C until use. The soil pH was measured using a PHM62 standard pH meter, and organic carbon (OC) content was determined using an IL550 TOC-TN analyser. The microbial biomass carbon (MBC) of the soils was measured by the fumigation extraction method (Wu et al., 1990). The moisture content (MC) of soils was determined gravimetrically at 105 °C and the water content was adjusted to 60% of MWHC. The soil was pre-incubated at 25 °C and 7.5 °C for 2 days before spiking with the antibiotic. These two temperatures were selected based on the typical summer and winter temperatures observed in the regions where the soils were collected from. The soils varied in their pH, OC, clay content and MBC as shown in Table 1. A full description of the soils and the methods used to determine their physico-chemical properties can be found elsewhere (Blakemore et al., 1987).

2.3. Dissipation experiments

For all analyses, destructive soil samples (5 g) were performed to investigate the effect of each factor (temperature, soil depth, sterile vs non-sterile, concentration, and DHA) on SMO dissipation. Overall the experimental protocol in the dissipation experiment involved a total of 36 samples (12 each for temperature, depth and concentration effect) each with 2 replicates. Furthermore, another individual 18 samples (8 for sterile control and 10 for DHA measurement) each with 2 replicates were set up separately. Soil samples (5 g) were placed in 35 mL Kimax centrifuge tubes 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}. The amount of methanol present in the antibiotic solution spiked onto soil was unlikely to have any effect on soil microorganisms as we allowed the methanol to evaporate immediately after spiking inside a fume cupboard. The contents were then thoroughly mixed by vortexing before incubating in the dark at 25 °C and 7.5 °C respectively. The moisture content in each vial was maintained gravimetrically to 60% of its field capacity (−33 kPa) by adding de-ionised water once every 3 days during the experiment, and the tubes were also aerated everyday to ensure a constant oxygen atmosphere. The entire experiment was conducted in closed incubators with temperature control, and wrapping individual tubes with aluminum foil in order to avoid photodegradation. To establish the role of microorganisms in the degradation of the antibiotic, the experiments were also conducted on sterile soils. Sterilisation was achieved by means of autoclaving twice (121 °C, 103 kPa for 30 min). Spiking procedure in sterile control treatment was similar to what was used in non-sterile treatments, except that sterile deionised water was used to maintain the moisture content at 60% of its field capacity during the fortification of soil samples in sterile experiment. All equipment used during sterile treatment was swabbed with