Soil Biology & Biochemistry 75 (2014) 64–72

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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

Assessing the impacts of chemical cocktails on the soil ecosystem

J. Horswell [a,*], J.A. Prosser [a], A. Siggins [a], A. van Schaik [a], L. Ying [b], C. Ross [c], A. McGill [d], G. Northcott [e]

[a] Institute of Environmental Science and Research (ESR) Ltd, Kenepuru Science Centre, Porirua, New Zealand
[b] Plant and Food Research Ruakura, Private Bag 3230, Hamilton, New Zealand
[c] Landcare Research, Palmerston North, New Zealand
[d] Landcare Research, Hamilton, New Zealand
[e] Northcott Research Consultants Ltd, 20 River Oaks Place, Private Bag 3200, Hamilton, New Zealand

ARTICLE INFO

Article history:
Received 26 November 2013
Received in revised form
12 March 2014
Accepted 18 March 2014
Available online 13 April 2014

Keywords:
Biosolids
Triclosan
Heavy metals
Co-contaminants
Soil health indicators

ABSTRACT

Little is known about the environmental fate and effect of low levels of co-contaminants that are commonly present in wastes such as biosolids. Lysimeters were established using soils contaminated with Cu or Zn and augmented with triclosan. Triclosan degraded rapidly in the soils, with methyl-triclosan being the major degradation product. However, as metal concentration increased, transformation and biodegradation of triclosan decreased. For some soil health indicators (e.g. sulphatase enzyme), results suggested that general toxicity was increased when metals and triclosan were both present. These preliminary results suggest that co-contaminants can result in a combined effect that is potentially greater than the sum of the individual effects, with additional impacts on the rate and extent of contaminant degradation.

1. Introduction

Triclosan (5-choloro-2-(2,4-dichlorophenoxy) phenol; TCS) is a broad spectrum antimicrobial agent, which is used in a wide variety of personal care products including deodorants, hand soaps, toothpaste, textiles, laundry detergents, antiseptics, shower gels and cleaning agents. Household products containing triclosan are typically discarded into the sewage system. The level of transformation and biodegradation of TCS in waste water treatment plants (WWTPs) varies with operating conditions (Xia et al., 2005). Some studies have shown >90% removal of TCS using activated sludge as secondary treatment (McAvoy et al., 2002; Bester, 2003; Kanda et al., 2003; Sabaliunas et al., 2003; Heidler and Halden, 2007). However, due to the hydrophobic nature (log $K_{ow} = 4.8$) of TCS, it is likely that a significant removal mechanism is sorption onto biosolids (McAvoy et al., 2002; Reiss et al., 2002). Both TCS and its transformation product methyl-TCS (5-chloro-2-(2,4-dichlorophenoxy)-anisole) have been detected in surface waters downstream of sewage treatment plants (Lindstrom et al., 2002; Kookana et al., 2011). The limited data available in the literature suggests that the concentration of TCS in effluents can range from 35 ng L^{-1} to 2700 ng L^{-1} (McAvoy et al., 2002; Reiss et al., 2002; Singer et al., 2002; Sabaliunas et al., 2003; Halden and Paul, 2005). In a study of 19 effluents in Australia, Kookana et al. (2011), found concentrations of TCS ranging from 23 to 434 ng L^{-1}. A similar study of TCS in the influent and effluent of 13 WWTPs in New Zealand found TCS concentrations ranging from 25 to 100 ng L^{-1} in the influent and 4.43–158 ng L^{-1} in treated effluents (Strong et al., 2010). Concentrations of TCS in biosolids have been found to be an order of magnitude higher than in effluents ranging from 0.43 to 133 mg kg^{-1} in the USA, (McAvoy et al., 2002; USEPA, 2009; Cha and Cupples, 2009); from 0.09 to 16.79 mg kg^{-1} in Australia (Kookana et al., 2011) and 1.05–17.23 mg kg^{-1} in New Zealand biosolids (Speir and Northcott, 2006).

A major pathway for the movement of organic contaminants such as TCS to the environment is through the land application of biosolids, a common practice in many countries (Lozano et al., 2012). Triclosan and other broad spectrum antimicrobial chemicals are specifically added to personal care products to prevent their deterioration by microorganisms. TCS targets numerous intracellular and cytoplasmic sites within cells, it may influence the transcription of genes participating in amino acid, carbohydrate and lipid metabolism (Reiss et al., 2009).

* Corresponding author. Tel.: +64 4 9140684; fax: +64 4 9140770.
E-mail address: Jacqui.Horswell@esr.cri.nz (J. Horswell).

http://dx.doi.org/10.1016/j.soilbio.2014.03.013
0038-0717/

It is these features that can unintentionally affect soil and aquatic animals should their habitats become contaminated with such chemicals. In a soil environment, previous studies have demonstrated inhibition of soil processes however, these are often short lived and the microbial community recovers (Waller and Kookana, 2009; Butler et al., 2011). Butler et al. (2011) measured both basal and substrate-induced respiration (SIR) in three soils types and found that TCS inhibited both parameters at concentrations as low as 10 mg/kg (in the loamy sand soil), however, both basal respiration and SIR recovered. Waller and Kookana (2009) also demonstrated TCS inhibited SIR in Australian sandy and clay soils. Their study demonstrated that TCS at concentrations below 10 mg/kg disturbed the nitrogen cycle in some soils (sandy soil; Waller and Kookana, 2009). Harrow et al. (2011) demonstrated changes in microbial numbers and community structure after irrigation of soils with greywater containing TCS. Impacts on microbial community structure were also found by Drury et al. (2013) in artificial streams.

Biosolids contain a suite of contaminants including heavy metals, which can be present at concentrations significantly higher than organic compounds, primarily because they are not degraded by WWTP processes. Among the most prevalent of heavy metals in sewage are copper (Cu) and zinc (Zn), originating from both industrial and domestic sources (Smith, 1996). Copper and Zn therefore are the elements most likely to limit the amounts of treated sludge that can be applied to land (Smith, 1996). Heavy metal contaminants, present a risk to biological components of soil systems because of their persistence, toxicity to soil organisms and impairment of biological functions (Brookes and McGrath, 1984; Giller et al., 1998; Speir et al., 2007). What remains unknown is whether low concentrations of numerous compounds in biosolids combine to produce synergistic/antagonistic/additive ecotoxicological effects on ecosystems (Daughton and Ternes, 1999; Daughton, 2003; Dorne et al., 2007).

As in other countries, current risk assessment procedures in New Zealand are reductionist, focussing on the fate and effects of individual chemicals and/or defined classes of chemicals in isolation from other contaminants present in biosolids. As a result, there is incomplete information about the environmental fate and effects of the complex cocktail of components in biosolids (e.g. nutrients, biological and chemical contaminants). Taking a first step to a holistic understanding of the toxicological effects and impacts of complex mixtures of contaminants is challenging, but is critical to assessing the potential risks that chronic low-level exposure may present to the environment.

In this study we investigated the fate and effects of TCS in combination with copper or zinc in soil. We selected TCS as a model organic antimicrobial chemical to investigate the potential effects it may elicit on soil microbial activity and function in combination with the heavy metals Cu and Zn which are biologically active and found at high concentrations in wastes such as biosolids.

An added complication to understanding the interaction of complex mixtures of contaminants in biosolids was illustrated by Speir et al. (2003, 2007) who postulated that responses of sensitive soil health indicators (such as enzymes) to potentially toxic elements can be masked by the effects of added organic matter (such as biosolids). Thus, in this study we avoided these confounding effects by using field soils historically contaminated with copper or zinc salts, and amending these with TCS spiked at 5 mg kg^{-1} and 50 mg kg^{-1}, in the absence of biosolids. The degradation dynamics of TCS in the presence of increasing concentrations of heavy metals was measured, as well as changes in the soil microbial community. Effects of the co-contaminants on soil enzymes (phosphate and sulphatase), biomass and respiration, Most Probable Number *Rhizobium*, and the activity of sensitive microbial biosensors (Lux) were measured as well as terminal restriction fragment length polymorphism (T-RFLP) bacterial community analysis.

2. Materials and methods

2.1. Experimental background and sample site

The soil was obtained from an existing field trial, on a Horotiu sandy loam (a Vitric Orthic Allophanic Soil, Vitric Hapludand) at Ruakura, Hamilton, New Zealand (175° 19′ E, 37° 47′ S) (established April 2007). Soil physiochecmial properties are show in Table 1.

The trial comprised 30 plots (1 m^2), set out in two fully-randomised replicate blocks. Each block contained seven plots amended with Cu, seven plots amended with Zn and a common unamended control plot. Prior to amendment, the top 100 mm of soil over the entire 1 m^2 plot was removed and sieved (6.5 mm) to break up large clumps and remove vegetation (pasture species), then thoroughly mixed in a concrete mixer. Sulphate salts of Cu ($CuSO_4$) or Zn ($ZnSO_4$) were added to the mixing soil to raise metal concentrations and a basal dressing of superphosphate, $(NH_4)_2SO_4$ and KCl was also mixed in at the same time. The amended soil was returned to its plot and packed down to its original field density. The plots were sown with a ryegrass/inoculated clover pasture seed mix. In 2010, three years after the trial was set up, approximately 15 kg of soil was collected from 11 of the plots to a depth of 10 cm sieved (2 mm) and stored at 4 °C until required. The concentrations of Zn and Cu in the soils used for the current experiment are presented in Table 2.

2.2. Lysimeter construction

Lysimeters were established in duplicate, with 33 treatments resulting in a total of 66 lysimeters. Each treatment contained a combination of varying concentrations of TCS and one of the heavy metals. Specifically, a range of five metal concentrations (Cu 101, 187, 478, 1083 and 2944 mg kg dry $soil^{-1}$; Zn 186, 283, 632, 1224 and 2235 mg kg dry $soil^{-1}$; Table 1). The range of metals were chosen in order to obtain high enough Cu and Zn concentrations in soil to sufficiently inhibit soil health indicators measured in this study (e.g. biomass and respiration) and allow accurate determination of dose response curves (covering previously determined EC_{50} values for Cu and Zn, Speir et al., 2007). The metal spiked soils were combined with either 0, 5 (low) or 50 (high) mg kg^{-1} TCS. Three control treatments containing no (or only background levels of) heavy metals, combined with 0, 5 or 50 mg kg^{-1} TCS were also included in the study.

The lysimeters were constructed from 15 cm lengths of PVC pipe (ø 13 cm). The base of the lysimeters contained a layer of gravel to retain the soil and assist drainage. Field moist sieved soil (1 kg) was adjusted to 65% water-holding-capacity (WHC) and spiked with TCS to obtain a final concentration of 0, 5 or 50 mg kg^{-1} TCS. The spiking procedure was adapted from Brinch et al. (2002) to minimise the impact of organic solvents upon soil microbes. Briefly, 250 g field moist soil was distributed evenly on the base of a large shallow glass container. Either 3.56 mg or 35.57 mg of triclosan (for the 5 and 50 mg kg^{-1} spiked soils respectively) was weighed into a glass vial and mixed with 20 mL of acetone. Using disposable glass Pasteur pipettes, the TCS-acetone solution was dripped evenly over

Table 1
Selected physiochemical properties of the Horotiu sandy loam.

Total carbon (%)	Total Nitrogen (%)	pH (H_2O)	CEC (cmol + kg^{-1})
11.6	1	5.4	39