Soil Biology & Biochemistry 52 (2012) 64–74

Contents lists available at SciVerse ScienceDirect

Soil Biology & Biochemistry

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

The effects of copper on microbial activity and the degradation of atrazine and indoxacarb in a New Zealand soil

K.A. Dewey [a], S.K. Gaw [a,*], G.L. Northcott [b], D.R. Lauren [b], S. Hackenburg [a,1]

[a] Department of Chemistry, University of Canterbury, Private Bag 4800, Christchurch 8140, New Zealand
[b] Plant and Food Research, Ruakura, Private Bag 3230, Waikato Mail Centre, Hamilton 3240, New Zealand

ARTICLE INFO

Article history:
Received 27 July 2011
Received in revised form
1 April 2012
Accepted 2 April 2012
Available online 3 May 2012

Keywords:
Copper
Atrazine
Indoxacarb
Microbial biomass
Enzyme activities

ABSTRACT

Copper is present in a range of fungicides as well as in some animal manures and biosolids that are applied to agricultural soils as fertilisers. Elevated and increasing levels of copper in agricultural soils are of worldwide concern. Copper is toxic to soil microorganisms and has been reported to reduce the ability of soil microorganisms to degrade pesticides. A glasshouse study was undertaken to determine if copper inhibited the degradation of atrazine and indoxacarb in soil. A fine sandy loam agricultural soil was fortified with copper at five concentrations over a concentration range of 0–1000 mg/kg copper, then field-aged for 6 months prior to treatment with either indoxacarb or atrazine at a rate of 2 mg/kg. The soils were sampled twice at intervals based on published half-lives. The samples were analysed for a range of parameters including total and bioavailable copper, urease and phosphatase activity, ergosterol and either indoxacarb or atrazine and its degradation products. The soil microbial biomass and enzyme activities decreased with increasing copper concentration ($p < 0.05$). There were no significant differences in soil atrazine and indoxacarb concentrations between the copper levels. At sampling time two, the concentrations of hydroxyatrazine in treatments containing the three highest copper concentrations were significantly greater ($p < 0.05$) than for the control soil. Our results indicate that copper does not inhibit the first step of indoxacarb and atrazine degradation, but may affect degradation of secondary metabolites like hydroxyatrazine in soil.

1. Introduction

Synthetic organic compounds are widely used as pesticides (herbicides, fungicides and insecticides) throughout the world in primary production. It has been estimated that less than 0.1% of pesticides reach the targeted organisms with the remainder available to interact and exert effects in the receiving environment. Adverse effects of pesticide applications include soil and water contamination and exposure of non-target organisms. The persistence of a pesticide is a key determinant of its environmental fate and potential adverse effects (Sarmah et al., 2004). Degradation is the primary process affecting pesticide persistence. Degradation mechanisms for pesticides in soil include both microbial degradation, and chemical degradation processes such as hydrolysis, oxidation–reduction, and photolysis. Factors that determine the extent of degradation include key soil properties such as pH and organic matter content, the structure of the pesticide, the composition and biological activity of the soil microbial community and the presence of co-contaminants (Sarmah et al., 2004).

While pesticides are commonly applied concurrently or used on land that has previously received applications of pesticides, the majority of data reporting pesticide persistence in soil is derived from trials solely investigating the active ingredient of interest. Only a limited number of studies have investigated the impacts of trace element co-contamination on the degradation of pesticides in agricultural soils. Copper has been reported to inhibit the microbial degradation of several pesticides including coumaphos (Jindal et al., 2000) thiobencarb (Gunasekara et al., 2005), DDT (Gaw et al., 2006), cypermethrin and cyhalothrin (Liu et al., 2007) and glyphosate (Kim et al., 2011). Conversely, the presence of heavy metals has also been shown to enhance degradation of glyphosate (Kools et al., 2005).

Soil microorganisms can be adversely affected by trace element contamination. These effects include inhibition of enzyme activities, reduced biomass, alteration of the microbial community

* Corresponding author. Tel.: +64 33642818; fax: +64 33642110.
E-mail addresses: sally.gaw@canterbury.ac.nz (S.K. Gaw), Grant.Northcott@plantandfood.co.nz (G.L. Northcott), Denis.Lauren@plantandfood.co.nz (D.R. Lauren), sch506@york.ac.uk (S. Hackenburg).
[1] Present address: Department of Chemistry, University of York, Heslington, York YO10 5DD, United Kingdom.

0038-0717/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2012.04.009

diversity and composition and reduced degradation of organic contaminants (Gaw et al., 2006; Merrington et al., 2002; Wang et al., 2009). Trace elements can inhibit microbial enzymes directly involved in the biodegradation processes of organic contaminants as well as enzymes involved in general metabolism. Trace elements including copper and zinc can inhibit enzyme activities by either: 1) reducing the production of enzymes through toxic effects to the microorganisms; 2) combining with the active protein groups of the enzyme; 3) complexing with the substrate required by the enzyme; or 4) reacting with the enzyme–substrate complex (Wang et al., 2009).

Copper is a common ingredient present in a range of fungicides and is often present in some animal manures and biosolids that are applied to agricultural soils as fertilisers. Elevated and increasing levels of copper in agricultural soils are of concern both in New Zealand and internationally (Bunemann et al., 2006; Gaw et al., 2006). Copper based fungicides are among the most toxic pesticides to soil microorganisms (Bunemann et al., 2006) and they have been widely used in agriculture over a number of decades.

In this paper we report the results of a glasshouse experiment that was undertaken to determine the effects of copper on the degradation of pesticides in a NZ soil. Atrazine and indoxacarb (Fig. 1) were chosen as experimental compounds as both are registered for use and widely used in New Zealand. The pre-emergent herbicide atrazine (2-chloro-4-(ethylamino)-6-(isopropylamino)-1,3,5-triazine) is commonly used in New Zealand agriculture. Due to its widespread use internationally and recent evidence of endocrine activity, there is increasing concern regarding the use and subsequent persistence of atrazine in the environment (Jablonowski, 2011). The selection of atrazine provided the advantage of having a well established degradation pathway(s), and it a range of primary and secondary metabolites to investigate.

Indoxacarb ((S)-methyl-7-chloro-2,5-dihydro-2-[[(methoxycarbonyl)[4-(trifluoromethoxy)phenyl]amino]carbonyl]-indeno-[1,2-e][1,3,4]- oxadiazine-4a(3H)-carboxylate) is a member of the new class of oxidiazine insecticides used to control Lepidoptera insects in fruit, vegetable and cotton crops. Indoxacarb is increasingly used in New Zealand despite limited data being available on its environmental fate.

The objective of the study reported in this paper was to determine if copper inhibited the degradation of atrazine and indoxacarb in soil. Soils fortified with copper were spiked with either atrazine or indoxacarb. Sub-samples of these soils collected over the duration of the study were analysed for either indoxacarb or atrazine and its degradation products. A range of soil microbial properties were measured as indicators for potential adverse effects of copper on the soil microbial community as pesticides in soil can be degraded by both biotic and abiotic processes. Soil microbial biomass was selected to provide a measure of microbial abundance. Phosphatase enzyme activity was selected as a suitable parameter as it has previously been shown to be a sensitive indicator of the detrimental effect of copper contamination on microorganisms in New Zealand soils (Speir et al., 2007). Urease activity was selected to provide a measure of the ability of the soil microorganisms to catabolise the two nitrogen containing pesticides (atrazine and indoxacarb) as a nitrogen source (Shapir et al., 2007). Fungi can play a key role in degradation of synthetic organic compounds in soil (Harms et al., 2011) and ergosterol was selected as a surrogate measure of soil fungal biomass. This paper presents and discusses the significance of the experimental results, and the effect of copper as a co-contaminant on the microbial degradadation of indoxacarb and atrazine in soil.

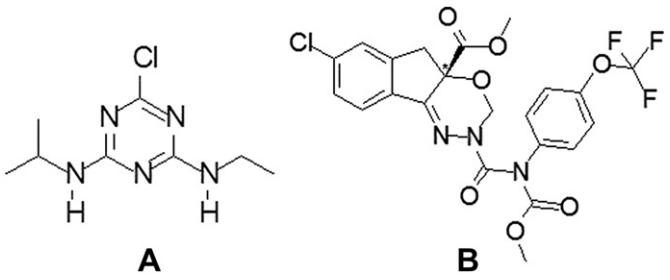

Fig. 1. Structures of (A) atrazine and (B) indoxacarb. * indicates a chiral centre.

2. Materials and methods

2.1. Sample collection and preparation

A Templeton sandy loam bulk soil was collected (approx. 130 kg) from the Lincoln University Dairy Farm (New Zealand; 43°38′40″S, 172°28′7″E; 60% sand, 30.7% silt and 9.3% clay), from a nominal depth of 15 cm below the grass line. The site had received one off applications of bentazone and diazinon 8 years prior to this study. Approximately 130 kg of soil was collected and transported to the University of Canterbury (Christchurch, New Zealand). The bulk soil was sieved (<5 mm) and homogenised by hand with a clean shovel. Soil moisture content was determined at 105 °C. The concentration of copper in the prepared soil was measured as described in Section 2.6.

2.2. Copper fortification and aging

The soil was fortified with $CuSO_4$ at final rates that ranged from 0 to 1000 mg kg^{-1} and field-aged for 6 months prior to spiking with pesticides. These copper concentrations are consistent with the range previously measured in New Zealand horticultural soils (Gaw et al., 2006). The atrazine and indoxacarb treatment rate of 2 mg kg^{-1} was chosen as a compromise between field application rates and method detection limits. The atrazine treatment rate was consistent with the field application rate (750 g ha^{-1}) (AGPRO, 2008), while the indoxacarb treatment rate was 6.5 times the field application rate of 400 g ha^{-1} (Du Pont, 2005).

Fortification solutions were prepared by dissolving weighed quantities of analytical grade $CuSO_4 \cdot 5H_2O$ in Milli-Q water (18 MΩ). For each copper level, 25 kg (DW equivalent) of the bulk soil was spread out to a depth of ca. 2 cm on a plastic-covered surface. Each 25 kg batch of soil was hand sprayed with 1 L of copper fortification solution. During fortification the soil was turned over by hand after every application and the direction of spraying and turnover varied regularly to ensure even soil copper concentrations. The soils were fortified in increasing order of copper concentration to provide final concentrations of 0, 100, 250, 500 and 1000 mg Cu kg^{-1} soil (Cu-1, -2, -3, -4 and -5, respectively).

The copper fortified soils were transferred into commercial black polypropylene worm bins ("soil aging bins"). These bins consisted of a lower bin to collect leachate and an upper bin to retain the copper fortified soil. A layer of 10 mm size plastic garden mesh followed by a layer of nylon 250 μm mesh was laid over the drainage holes in the upper bin to retain the soil and prevent it passing into the lower section. Prior to the addition of the copper fortified soil the soil aging bins were pre-leached overnight by soaking twice with distilled water. The soil aging bins were stored outside for 6 months (194 d total) to age the copper fortified soils under field conditions. The soil aging bins were covered with netting to prevent disturbance by animals. Each bin was bunded to collect any leachate overflow. Leachate levels were monitored