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ScienceDirect
Chemosphere 66 (2007) 664–671
CHEMOSPHERE
www.elsevier.com/locate/chemosphere

Microbial aspects of the interaction between soil depth and biodegradation of the herbicide isoproturon

Gary D. Bending *, M. Sonia Rodriguez-Cruz [1]

Warwick HRI, University of Warwick, Wellesbourne, Warwick CV35 9EF, UK

Received 22 June 2006; received in revised form 28 July 2006; accepted 28 July 2006
Available online 22 September 2006

Abstract

Factors controlling change in biodegradation rate of the pesticide isoproturon with soil depth were investigated in a field with sandy-loam soil. Soil was sampled at five depths between 0–10 and 70–80 cm. Degradation rate declined progressively down the soil profile, with degradation slower, and relative differences in degradation rate between soil depths greater, in intact cores relative to sieved soil. Neither the maximum rate of degradation, or sorption, changed with soil depth, indicating that there was no variation in bioavailability. Differences in degradation rate between soil depths were not associated with the starting population size of catabolic organisms or the number of catabolic organisms proliferating following 100% degradation. Decreasing degradation rates with soil depth were associated with an increase in the length of the lag phase prior to exponential degradation, suggesting the time required for adaptation within communities controlled degradation rates. 16S rRNA PCR denaturing gradient gel electrophoresis showed that degradation in sub-soil between 40–50 and 70–80 cm depths was associated with proliferation of the same strains of *Sphingomonas* spp.

Keywords: Pesticide; Leaching; Biodegradation; Bioavailability; Sub-soil; *Sphingomonas* spp.

1. Introduction

The major environmental concern arising from pesticide use is the capacity of pesticides to leach from soil and contaminate water resources (Kookana et al., 1998). The amount of pesticides leaching through soil reflects the interaction of degradation and sorption processes in both top-soil and sub-soil (Fomsgaard, 1995). Many pesticides are degraded by cometabolism in which degradation follows first order kinetics, with the organisms responsible apparently showing no capacity to proliferate following degradation of the compound. Other pesticides are degraded by growth-linked metabolism, in which organisms responsible for biodegradation have adapted to use the pesticide as an energy and nutrient source, resulting in cell proliferation and an increase in degradation rate over time (Aislabie and Lloyd-Jones, 1995).

Degradation rates of pesticides are usually assumed to decrease down the soil profile (Fomsgaard, 1995). However, in some instances degradation rates of pesticides susceptible to both cometabolic and growth-linked degradation can be greater in sub-soil than in top-soil. The precise relationship between top- and sub-soil degradation rate can vary between different compounds at single sites, and at different sites for individual compounds (Di et al., 1998; Karpouzas et al., 2001; Mills et al., 2001). The reasons for contrasting patterns of degradation rates in sub- and top-soil are unclear.

A number of counteracting biotic and abiotic factors could be important for determining differences in pesticide degradation rate through the soil profile. The size of the microbial community, which decreases with soil depth (Fomsgaard, 1995) could determine both the availability of suitable microbial strains and genetic elements for adaptation to enable pesticide degradation, the survival of

* Corresponding author. Tel./fax: +44 24 76575057.
E-mail address: gary.bending@warwick.ac.uk (G.D. Bending).
[1] Present address: Institute of Natural Resources and Agrobiology (CSIC), Department of Environmental Chemistry and Geochemistry, Salamanca 37008, Spain.

0045-6535/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2006.07.099

adapted strains and the extent to which the activities of adapted strains are limited by competition. Sorption, which may reflect bioavailability (Jensen et al., 2004), is closely linked to organic matter content, and declines with soil depth. A range of environmental factors such as oxygen and temperature, which can affect microbial growth rates and pesticide degradation, also change with soil depth (Fomsgaard, 1995; Vink and van der Zee, 1997; Williams et al., 2003).

Microbes contributing to degradation of xenobiotics in top-soil have traditionally been investigated using enrichment methods (e.g. Sørensen et al., 2001), although the catabolic organisms obtained using these procedures may not reflect those organisms acting *in situ* (Newby et al., 2000). Little is known about the nature of catabolic communities acting in sub-soil. Furthermore, catabolic strains obtained from top-soil by isolation, or communities characterised using *in situ* molecular profiling techniques, have typically been investigated in soil samples derived from single environmental locations (e.g. Cullington and Walker, 1999; Sørensen et al., 2001; Singh et al., 2003). The extent to which there is spatial variability in the structure of catabolic communities, at both the field and landscape scale, has received little attention.

Bending et al. (2003) found that catabolism of the herbicide isoproturon was associated with proliferation of *Sphingomonas* spp. in a transect over a high pH (7.0–7.5) area of a field, but within a low pH (pH 6.0–6.5) area located just 50 m away, it appeared that different strains were involved in catabolism. Within agricultural fields, vertical changes in soil properties with soil depth are much greater than the horizontal spatial variability in soil properties which can occur within top-soil (Rodriguez-Cruz et al., 2006). Clearly, such changes in soil physico-chemical and biological properties with soil depth could result in the selection of different catabolic communities between top- and sub-soil, although this has not previously been investigated.

The aims of the current study were to investigate the interactions between catabolic communities, physico-chemical properties and pesticide degradation through the soil profile. Experiments were performed to address the following questions: 1. Are changes in pesticide bioavailability with soil depth associated with changes in the rate of biodegradation and its kinetics? 2. Does change in biodegradation rate with soil depth reflect differences in the size of the initial catabolic community, or the extent to which the catabolic community is able to proliferate? 3. Is there vertical spatial variability in the nature of communities contributing to pesticide catabolism?

2. Materials and methods

2.1. Pesticides and pesticide treatment history

Studies focussed on the herbicide isoproturon (3-(4-isopropylphenyl)-1,1-dimethylurea). Isoproturon is a member of the phenylurea group of herbicides, and together with related compounds are frequently detected as contaminants of groundwater and surface freshwater in Europe (Sørensen et al., 2003). Sampling occurred in Long Close field on the farm at Warwick HRI, Wellesbourne, Warwickshire, UK. The soil is a sandy loam of the Wick series (Whitfield, 1974), and the field had a history of isoproturon use (1999, 2001).

2.2. Soil collection

Soil was collected from five depths at three sampling locations. Three pits (A–C) separated by 60 m were excavated to 1 m depth using a mechanical digger, in February 2003. One side of each pit was further excavated using a surface sterilised trowel, so that the face was free of loose soil. Soil was collected from 0–10, 20–30, 40–50, 60–70 and 70–80 cm depth using two methods. 1. From each depth approximately 2 kg soil was collected using a trowel and placed into a polythene bag. The trowel was surface sterilised with ethanol between the collection of each soil sample. Soil was spread onto clean polythene bags and left on the bench overnight to reduce moisture content, before being sieved (<3 mm) using surface sterilised sieves. 2. In order to maintain the physical and microbiological integrity of the soil, further samples were taken using intact 10×5 cm pre-sterilised stainless steel cores. Two cores were obtained at each depth from each hole by hammering the core horizontally into soil. Following removal, the top and bottom of each core were sealed with parafilm.

2.3. Analysis of soil characteristics

In the pre-sieved soil, total organic matter, microbial biomass-N, dehydrogenase activity and pH were measured by procedures described in Bending et al. (2006). Clay, sand and silt content were determined according to Day (1965).

2.4. Pesticide application

For pre-sieved soil, commercial isoproturon formulation (Atlas Crop protection, Doncaster, UK) was dissolved in distilled H_2O and added to single 300 g fw portions of soil from each location to provide 5 mg pesticide kg^{-1} soil, and further H_2O was added to bring the water holding capacity to 40%. Each soil was mixed thoroughly by hand, and then further mixed by passing through a <3 mm sieve five times. Each soil was transferred to a sterile polypropylene container which was loosely capped and incubated at 15 °C. Moisture content was maintained by the addition of sterile distilled water as necessary (usually once each week).

In the case of the two soil cores collected from each sampling location, four 250 μl aliquots of the commercial formulation of isoproturon in water were injected centrally at 2 cm depths to give a final concentration of 5 mg kg^{-1} soil. The soil cores were sealed base and top with parafilm and incubated vertically at 15 °C in the dark.