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Metabolic and cometabolic degradation of herbicides in the fine material of railway ballast

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Abstract

Microbial degradation of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diuron) and mineralization of 4-chloro-2-methylphenoxyacetic acid (MCPA) were studied in soil samples taken from the ballast layers of three Swedish railway embankments. The degradation of diuron followed first-order kinetics and half-lives ranged between 122 and 365 days. The half-lives correlated strongly with microbial biomass estimated by substrate-induced respiration (SIR; $R = -0.85$; $p < 0.05$) and with the amount of organic matter measured as loss on ignition ($R = -0.87$; $p < 0.05$). Accumulation of the metabolites 1-(3,4-dichlorophenyl)-3-methyl urea (DCPMU) and 1-(3,4-dichlorophenyl) urea (DCPU) was observed in all samples and these were only detectably degraded in the sample with the highest SIR. Addition of ground lucerne straw to the ballast samples stimulated microbial activity and led to increased formation of metabolites, but further transformation of DCPMU and DCPU was not enhanced. Mineralization of MCPA followed growth-linked kinetics and the time for 50% mineralization was 44.5 ± 7.1 days in samples of previously untreated ballast. In samples of ballast that had been previously treated with the herbicide formulation MCPA 750, the time for 50% mineralization was reduced to 13.7 ± 11.3 days. The number of MCPA degraders, quantified using an MPN technique, was clearly increased but highly variable. An average yield of 0.18 cells pg^{-1} of MCPA was estimated from the kinetic data. The yield estimates correlated with the amount of nitrogen in the ballast, indicating that mineralization of MCPA was nitrogen-limited in the railway embankments studied. This has practical implications for weed control using herbicides on railways.

Keywords: Metabolic and cometabolic degradation; Mineralization; MCPA; Diuron; Railway ballast

1. Introduction

Herbicides are used on railways in order to maintain the quality of the track and a safe working environment for railway personnel (Torstensson, 2001). Due to the coarse texture and low organic matter content of railway embankments, there is much concern that application of herbicides to railways may lead to groundwater contamination. Several studies have investigated the leaching of pesticides from railway tracks and, with some exceptions, most of them indicate that the leaching potential is considerable and that concentrations in the groundwater beneath the track may significantly exceed the EU limit for drinking water of 0.1 μg l^{-1} if the application rate is too high (Torstensson, 1983, 1985; Lode and Meyer, 1999; Schmidt et al., 1999; Börjesson et al., 2004; Ramwell et al., 2004; Torstensson and Börjesson, 2004; Torstensson et al., 2005).

While leaching can be substantial from coarse-textured environments such as railways, research indicates that the microbial degradation might be accelerated in these kinds of soils because of the high availability of herbicides to microorganisms (Hassink et al., 1994; Klein, 2002; Strange-Hansen et al., 2004). However, microbial degradation of herbicides is also related to the amount and activity of microorganisms (Anderson, 1984; Torstensson and Stenström, 1986; Voos and Groffman, 1997; Jones and Ananyeva, 2001), and these are both known to be of very limited magnitude on railways (Smith et al., 1981; Cederlund and Stenström, 2004).

If there is concern about herbicide degradation in such a situation, with a high availability of the compound and a

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low number of degrading microorganisms, it would be relevant to try to identify an herbicide that is used as a substrate for microbial growth. Such an herbicide would potentially induce exponential microbial proliferation in the field and, hence, also give an exponentially increasing degradation rate. On the other hand, the degradation rate of an active ingredient that does not support microbial growth would depend on its concentration and on the initial number of degrading microorganisms and their activity.

Diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea] is a substituted phenylurea herbicide used for total weed control in non-crop areas (Tomlin, 2003). In Sweden, the use of diuron was prohibited in 1993 by the Swedish Chemicals Inspectorate but globally it is still one of the main herbicides used for weed control on railways tracks. The long residual activity of diuron is one of the main reasons for its popularity, but is also problematic for environmental reasons. Field studies confirm that diuron can be very persistent and also that it is highly prone to leaching when used on railways (Torstensson, 1983, 1985; Torstensson et al., 2002; Skark et al., 2004). The ability of microorganisms to use diuron as a carbon source for growth does not appear to be widespread. Microbial degradation of diuron in the field is usually very slow and follows first-order kinetics (Hill et al., 1955; Sørensen et al., 2003).

4-chloro-2-methylphenoxyacetic acid (MCPA) is an arylalkanoic acid widely used for post-emergence control of broad-leaved weeds (Tomlin, 2003). Degradation of MCPA usually follows growth-associated kinetics, indicating that MCPA is used as a substrate for microbial growth (Audus, 1951). Hence, the dissipation of MCPA in agricultural soils is often rapid, at least after the first time of its use (Kirkland and Fryer, 1966; Torstensson et al., 1975).

The objectives of this study were to characterize microbial degradation of the herbicides diuron and MCPA in fine materials of railway ballast, to investigate the relationship between microbial biomass and activity and degradation rates and to determine the extent to which adaptation of the railway microflora occurs in response to repeated applications of MCPA.

2. Materials and methods

2.1. Chemicals

Certified standards of 3-(3,4-dichlorophenyl)-1,1-dimethyl urea (diuron, 97.7% purity), 1-(3,4-dichlorophenyl)-3-methyl urea (DCPMU, 99.5% purity), 1-(3,4-dichlorophenyl) urea (DCPU, 99.7% purity), 3,4-dichloroaniline (DCA, 99.0% purity) and MCPA (99.0% purity) were purchased from Dr. Ehrensdorfer GmbH (Augsburg, Germany), while 4-chloro-2-methylphenoxy-[1-^{14}C]-acetic acid (^{14}C-MCPA, 57 mCi mmol^{-1}) was purchased from Amersham Biosciences. Formulations used for the field treatments were BASF MCPA 750 (MCPA dimethylamine salt, 750 g a.i. l^{-1}) from BASF AB and Roundup Bio (glyphosate isopropylamine salt, 360 g a.i l^{-1}) from Monsanto. All other chemicals and solvents used for the study were of analytical grade.

2.2. Diuron degradation study

Soil was sampled in 2002 from the surface layer of two railway tracks between Mora-Älvdalen (61°00′N, 14°28′E; denoted 'Mora' in the text) and Nässjö-Vetlanda (57°28′N, 15°03′E; denoted 'Vetlanda' in the text). Both tracks were regularly treated with diuron until 1992, but since then only glyphosate and imazapyr have been used. All samples were sieved (<4 mm) and stored at 4 °C. The sampling sites have been previously described by Cederlund and Stenström (2004) and some basic characteristics of these soils are presented in Table 1. Samples were pooled on the basis of their substrate-induced respiration (SIR)-values so that soils (900 g wet wt.) of 'high' (A), 'intermediate' (B) and 'low' (C) microbial biomass were obtained for each track. A fraction of each soil (100 g) was contaminated with diuron (12 mg) dissolved in acetone (5 ml) (procedure adopted from Brinch et al., 2002). When the acetone had evaporated the contaminated fraction was mixed thoroughly with the rest of the soil to obtain an approximate diuron concentration of 15 μg g^{-1} dry wt. (a realistic in-field concentration). The samples were incubated in aerated plastic jars in the dark at 20 °C for 460 days. The water content was adjusted to and kept at 60% of the water holding capacity (WHC) of the soils throughout the experiment by the addition of deionized water. Duplicate samples (10 g) for HPLC analysis were taken regularly to monitor the concentration of diuron and its metabolites. The SIR and basal respiration were measured on duplicate samples (40 g) on days 8, 194 and 393 of the incubation.

An additional experiment was carried out in which the microbial activity of the soil was stimulated by the addition of ground lucerne straw (5 mg g^{-1} dry wt. soil). The soil was incubated as described above and concentrations of diuron and metabolites were monitored for 239 days. The SIR and basal respiration were measured on days 10, 44, 91 and 187.

2.3. Analysis of diuron

The soil samples (10 g) were extracted for 60 min with methanol (25 ml). The samples were then centrifuged at 1500 rev min^{-1} for 10 min (Sorvall T6000D, Lambda Polynom AB, Sweden) and filtered (OOH Whatman; 11 cm) before HPLC analysis. The HPLC analyses were performed using an UV detector G1314A, a pump G1311A and an autoinjector G1329A (Agilent Technologies AB; 1100 Series; Sweden) equipped with a Zorbax SB-C18 column (12.5 × 4.6 mm, 5 μm; ChromTech AB, Sundbyberg, Sweden). The eluent was a gradient of acetonitrile–water, 10–100% for 11 min, followed by 2 min equilibration