Applied Soil Ecology 66 (2013) 8–18

Contents lists available at SciVerse ScienceDirect

Applied Soil Ecology

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

Short-term effects of the herbicide napropamide on the activity and structure of the soil microbial community assessed by the multi-approach analysis

Mariusz Cycoń[a,*], Marcin Wójcik[b], Sławomir Borymski[c], Zofia Piotrowska-Seget[c]

[a] Department of Microbiology and Virology, Medical University of Silesia, Jagiellońska 4, 41-200 Sosnowiec, Poland
[b] Department of Ecotoxicology, Institute of Industrial Organic Chemistry, Doświadczalna 27, 43-200 Pszczyna, Poland
[c] Department of Microbiology, University of Silesia, Jagiellońska 28, 40-032 Katowice, Poland

ARTICLE INFO

Article history:
Received 11 March 2012
Received in revised form 9 January 2013
Accepted 27 January 2013

Keywords:
Herbicide napropamide
Enzyme activities
Nitrogen transformation
Microbial community structure
PLFA
Biodegradation

ABSTRACT

Napropamide is one of the most commonly used herbicide in agricultural practice and its continuously application poses a potential risk for non-target soil microorganisms. Therefore, the objective of this study was to assess the impact of napropamide, applied at the field rate (FR, 2.25 mg kg^{-1} of soil) and 10 times the FR (10*FR, 22.5 mg kg^{-1} of soil) on soil microorganisms. To ascertain this impact, substrate-induced respiration (SIR), dehydrogenase (DHA), acid and alkaline phosphatases (PHOS-H and PHOS-OH), urease (URE) activities and changes in concentrations of NO_3^- and NH^{4+} ions were determined. In addition, numbers of total bacteria and bacteria involved in soil nitrogen transformation were enumerated. A phospholipid fatty acid (PLFA) method was used to assess changes in the structure of soil microbial communities. Results showed negative effect of napropamide applied at the FR for SIR, the number of total bacteria, DHA, both PHOS, and URE at the beginning of the experiment. In 10*FR treated soil, a decreased activity of SIR, DHA, PHOS-OH and PHOS-H was observed over the experimental period. Nitrifying and N_2-fixing bacteria appeared to be the most sensitive to napropamide. The concentration of NO_3^- decreased in both napropamide-treated soils, whereas the concentration of NH_4^+ on day 28 in soil with 10*FR was 5 times higher than in the control. Analysis of the PLFA profiles showed that napropamide decreased the biomass of total, bacterial and fungal PLFAs on day 1, while at the end of the experiment in the soil treated with FR dosage of napropamide biomass of total, Gram-negative bacteria and fungi was significantly higher than those observed in the control. A principal component analysis of the PLFAs showed that napropamide application significantly shifted the microbial community structure on days 1 and 14. The degradation kinetics data showed that napropamide degradation by soil autochthonous microorganisms was relatively slow. The results indicated that a broad spectrum of analyze gives a better insight into the true effects of napropamide on soil microorganisms than the single assays.

1. Introduction

Currently different herbicides with a wide range of physico-chemical properties and mode of actions are used to protect crops against unwanted weeds in agricultural practice. One of the most commonly used herbicide is napropamide [*N*,*N*-diethyl-2-(1-naphthalenyloxy)propanamide] belonging to the amide herbicide family. It is used to control many grasses and broadleaf weeds in fruit and vegetable crops, field-grown tobacco as well as ornamentals. Napropamide is easily transferred from soil to plant tissue where it can accumulate and exert its toxic effect (Biswas et al., 2007). Its main mode of action is the inhibition of DNA synthesis and cell division by blocking progress in G1 and G2 of the cell cycle. This herbicide also reduces the synthesis and activity of plant proteins that are probably involved in the regulation of mitosis (DiTomaso et al., 1998). In field studies napropamide exhibited moderate to high persistence, with half-life ranging from 24 d to 131 d (EPA, 2005). Napropamide is susceptible to photodegradation which is responsible to a great extent for the loss of this herbicide in soil and water. It absorbs solar light and transforms rapidly following the first-order kinetics with a time of 3–4 d to reach 50% of the initial concentration in loamy sand soil.

In order to estimate the relation between pesticide treatment and soil microbial parameters many methods have been used over the last decades. The simplest involved the single-process tests such as measurement of substrate induced respiration (SIR) and nitrogen transformation. These parameters were adopted by OECD (Organization for Economic Co-operation and Development) and

* Corresponding author. Tel.: +48 515 270 972; fax: +48 32 364 15 02.
E-mail address: mcycon@sum.edu.pl (M. Cycoń).

0929-1393/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.apsoil.2013.01.014

Table 1
General characteristic of the soil used in the experiment.

Parameter	Value
Sand (2000–50 μm) (%)	86.0 ± 2.7
Silt (<50–2 μm) (%)	11.0 ± 2.4
Clay (<2 μm) (%)	3.0 ± 0.5
Density (g cm^{-3})	1.2 ± 0.2
$pH_{(in\ water)}$ (1:5)	6.6 ± 0.3
Cation exchange capacity (CEC) (cmol + kg^{-1})	12.9 ± 1.7
Water holding capacity (WHC)(%)	32.4 ± 2.8
C_{org} (%)	1.0 ± 0.2
N_{tot} (%)	0.09 ± 0.03
Microbial biomass (mg kg^{-1} d.w.)	668.0 ± 34.2

The values are the means of three replicates with the standard deviation which was within 5% of the mean.

EPA (Environmental Protection Agency) for soil ecotoxicological research and environmental risk assessment. However, such tests do not usually provide adequate data to accurately predict the overall potential environmental hazards of the compounds.

Results of many earlier studies have revealed that continuous and extensive herbicide application affected the soil microbial communities by changing their number (Cycoń and Piotrowska-Seget, 2007; Zhang et al., 2010a), overall microbial activity (Hua et al., 2009; Zabaloy et al., 2008), soil enzyme activity (Sannino and Gianfreda, 2001) and diversity of soil microbial communities (Ratcliff et al., 2006; Zhang et al., 2010b; Mahía et al., 2011; Zabaloy et al., 2012). Since microorganisms are responsible for organic matter decomposition as well as nutrient cycling, herbicide-induced changes in microbial metabolic activity and community structure, they may in consequence affect the soil health and productivity. Moreover, due to close contact with contaminants present in the surrounding soils, microorganisms are expected to be the first and most threatened by pesticide application. Due to their fast response to contaminants, soil microorganisms serve as suitable "biomarkers" since they reflect the potential effects of pesticide treatment and are commonly used in ecotoxicological tests to evaluate the influence of chemicals on soil quality (Filip, 2002).

There is a lack of complete information on the impact of napropamide on the activity and structure of the entire microbial communities. Therefore, in this study we have used broad-spectrum analyses to evaluate the short-term interaction of herbicide and soil microorganisms. To ascertain this impact, the substrate-induced respiration (SIR), the soil enzyme activities, the numbers of total heterotrophic bacteria as well as the numbers of specific groups of bacteria involved in soil nitrogen transformation were determined. Moreover, to assess the alterations in the soil microbial communities, the phospholipid fatty acids (PLFA) approach was used. In addition, changes in the concentrations of ammonium and nitrate ions, indicating the rates of ammonification and nitrification processes were ascertained. Moreover, during the experimental period the rate of napropamide degradation by soil autochthonous microorganisms was also studied.

2. Materials and methods

2.1. Soil

A loamy sand soil that had not been previously treated with napropamide or other pesticides was collected from the top layer (0–20 cm) of grass-covered field that was located within the Pszczyna area of Upper Silesia in southern Poland (49°59′48″N, 18°55′14″E). According to the FAO Soil Classification soil is classified as Orthic Luvisol, and its physico-chemical properties are shown in Table 1. Determination of soil parameters was performed according to the methods described in our previous studies (Cycoń et al., 2012).

2.2. Experimental design and treatments

A herbicide preparation consisting of napropamide (45%) in a soluble concentrate (SC) formulation was used in this study. The experiment had a completely randomized block design with three replications and the following treatments: control and two herbicide rates (5 and 50 mg kg^{-1} of soil), which correspond to 2.25 and 22.5 mg of napropamide per kg of soil, respectively. The lower rate is the recommended field rate (FR) of napropamide, assuming a homogeneous distribution of the herbicide to a depth of 5 cm and a soil density of 1.5 g cm^{-3}. The higher rate of napropamide corresponded to 10 times the FR.

The soil was divided into three portions of equal weight (3000 g) that were placed into plastic pots. Two portions were treated with the above-mentioned rates of the herbicide preparation, and the third portion (control) received an equal volume of water. The water content of the soils was adjusted to 50% of the maximum water holding capacity. The pots were covered with perforated polypropylene sheets and were incubated in the dark at 20 ± 2 °C for 28 d. Throughout the incubation period, deionized water was added to the soil to compensate for any water loss that exceeded 5% of the initial amount added.

Soil samples were periodically removed for the determination of biochemical and microbial properties (on days 1, 14 and 28) as well as for chemical analyses to determine the concentrations of napropamide and its major metabolite (alpha-naphtoxy propionic acid, NOPA) (on days 0, 4, 8, 12, 16, 20, 24 and 28). Sterile soil was used to study the napropamide disappearance rate under abiotic conditions. Soil was sterilized 3 times by autoclaving for 1 h at 121 °C one week prior to the commencement of the experiment to permit the release of the toxic volatile compounds produced.

2.3. Determination of soil respiration

Short-term substrate-induced respiration (SIR) was measured in the soil samples (100 g) after the addition of glucose (2000 mg kg^{-1} dry weight of soil). The total amount of oxygen consumption was determined within 12 h of glucose application using the Sensomat Measurement System (LOVIBOND®, Germany), which measures differences in pressure in a closed system. In this system, during respiration, CO_2 binds to an absorber (45% KOH), and oxygen consumption results in a pressure drop that is proportional to the soil respiration level.

2.4. Determination of enzyme activities

The dehydrogenase activity (DHA) was measured using the method of Alef (1995). Soil samples were suspended in a 2,3,5-triphenyltetrazoliumchloride (TTC) solution and incubated for 20 h at 25 °C. The triphenyl formazan (TPF) product was extracted with acetone and measured photometrically at 546 nm. The acid and alkaline phosphatase activities (PHOS-H and PHOS-OH) were measured using the method of Tabatabai and Bremner (1969). After the addition of a buffered *p*-nitrophenyl phosphate solution (pH 6.5 or 11), the soil samples were incubated for 1 h at 37 °C. The released *p*-nitrophenol (*p*-NP) was extracted and colored with NaOH. The resulting product was measured photometrically at 400 nm. Urease activity (URE) was assayed according to the method of Gianfreda et al. (1994). After the addition of a buffered urea solution (10%, w/v), soil samples were incubated for 5 h at 37 °C. The amount of released ammonium was determined by the Bertholet reaction.