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ASSESSMENT OF NAPROPAMIDE DISSIPATION AND ITS EFFECT ON SOIL ENZYMATIC ACTIVITY

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ABSTRACT

This paper assesses the dissipation of napropamide and its impact on the activity of dehydrogenases, alkaline phosphatase, acid phosphatase, and urease in sandy clay loam. The experiment was carried out on soil samples with organic carbon content of 12.08 $g \cdot kg^{-1}$, total nitrogen content of 0.97 $g \cdot kg^{-1}$, and pH 5.24 with the following variable factors: (a) dose of Devrinol 450 SC formation (containing 450 g of napropamide in dm^3): 0 (control), 0.5, 1, 2, 4, 8, and 16-fold hold of field dose; (b) day of experiment: 1, 7, 14, 28, 56, and 112. The half-life of napropamide ranged from 33.50 to 71.42 days. The use of napropamide at the dose recommended by the manufacturer and at the dose reduced by half appeared to exhibit low toxicity in relation to enzymes determined. In contrast, the application of elevated napropamide doses decreased the values of biochemical parameters of the soil in most cases. The Pearson's correlation coefficients showed statistically significant negative correlation between the content of napropamide residues and the enzymatic activity of the soil.

Keywords: napropamide, soil, dissipation, dehydrogenases, phosphatases, urease

INTRODUCTION

Napropamide ($C_{17}H_{21}NO_2$: *N,N*-diethyl-2-(naphthalen-1-yloxy)propanamide) is used as a pre-emergent herbicide. Chemically, it is a tertiary amide, which has two ethyl groups on the nitrogen atom. This compound has considerable polarity and water solubility [Biswas et al., 2007]. The main mechanism of action of this substance is to inhibit cell division, by blocking the G1 and G2 phases of the mitotic cycle and DNA synthesis. This herbicide reduces the synthesis and activity of plant proteins. In the G1 phase it limits the production of regulatory proteins and the synthesis of different types of RNA. Additionally in the G2 phase it decreases the synthesis of spindle-forming proteins and the plasmid [Di Tomaso et al., 1988].

Dissipation of napropamide in the soil by biotic and abiotic pathways involves cleavage of alkyl groups at the nitrogen atom and subsequent conversion of the amide to carboxylic acid [Donaldson and Miller, 1996].

Photodegradation is the main process of napropamide dissipation in the soil. The microbial degradation in the soil is very slow. However, despite the slow biodegradation of napropamide, the role of microorganisms in the distribution of this compound is significant, as confirmed from the results obtained by Rouchaud et al. [1991]. The 50% dissipation of napropamide in the soil depended on napropamide doses used in the experiment. Other researchers have reported that the 50% dissipation time (DT_{50}), depending on the type of soil and its properties, ranged from 25 to 152 days [Biswas et al., 2007; Guo et al., 2008]. Wauchope et al. [1992] showed that the mean DT_{50} of napropamide is approximately 70 days.

Soil enzymatic activity is a precise measure of the ecological status of soils, considering both the homeostatic capacity of a particular ecosystem and the level of environmental pollution,

which is harmful for living organisms [Bielińska and Mocek 2010]. The enzymatic activity of soils is the result of natural chemical and biochemical processes [Stręk and Telesiński 2016].

The aim of study was to assess the dissipation of napropamide and its effect on the activity of dehydrogenases, alkaline phosphatase, acid phosphatase, and urease in clay soil.

MATERIALS AND METHODS

Soil samples were collected from the topsoil of Drawska Plain (53°38′N, 16°26′E). This soil has been classified as sandy clay loam. The selected chemical characteristics of the soil are as following: pH 5.24, organic carbon (C_{org}) 12.08 g·kg^{-1}, and total nitrogen (N) 0.97 g·kg^{-1}. To consider the spatial heterogeneity, soil samples were collected in triplicates and each replicate consisted of five 10-cm auger cores. Then, the soil samples were pulverized manually and gently, mixed thoroughly, air dried at room temperature, and sieved through a 2-mm mesh to remove stones and plant roots before being used.

The experiments were carried out in triplicate under laboratory conditions, with the following variable factors: (a) dose of Devrinol 450 SC formulation (containing 450 g of napropamide in dm^3): 0 (control), 0.5, 1, 2, 4, 8, and 16-fold hold of field dose; (b) days of experiment: 1, 7, 14, 28, 56, and 112 days. The 1-kg soil samples were adjusted to 60% maximum water holding capacity, and they were incubated at a temperature of 20°C.

The individual doses of the formulation were converted into 1 kg of soil, considering the soil layer with a thickness of 10 cm. The details are presented in Table 1.

During the experiment, the content of napropamide residues and the activity of soil enzymes in the samples was measured six times (on day 1, 7, 14, 28, 56, and 112) from each repetition in three subsequent replications.

The napropamide residues were extracted from soil using a solvent mixture of acetonitrile:water (3:1 v/v) according to the procedure of Guo et al. [2008] and were determined by high performance liquid chromatography (HPLC, a Perkin Elmer model Series 200) equipped with UV detector (220 nm). The column used was Kinetex 150 × 4.6 mm (1.7 μm). The mobile phase was acetonitrile:water (4:1 v/v) at a flow rate of 1.0 cm^3 min^{-1}. A 20 mm^3 aliquot of each sample was injected each time for residue analysis. The representative retention time of napropamide was 1.89 min and recovery was 93.45%. Napropamide content was calculated from the calibration curve. The obtained results in mg·kg^{-1} soil were then calculated as a percentage of napropamide residues relative to the amount applied to the soil.

Experimental data were matched by regression equations, obtaining fading curves in the form:

$$y = pe^{qt} \quad (1)$$

where: y – napropamide residue in relation to the applied dose (%),
t – time of experiment (days),
e – basis of natural logarithm,
p, q – estimated curve parameters.

Based on the obtained curve equation, the half-life of napropamide was calculated according to the formula:

$$DT_{50} = [ln(50) - ln(p)]/q \quad (1)$$

where: DT_{50} – half-life of napropamide,
p, q – estimated curve parameters.

The soil enzyme activities were determined spectrophotometrically using UV-1800 spectrophotometer produced by Shimadzu. The activity of dehydrogenases (EC 1.1.1.x) was measured using the method described by Thalmann

Table 1. Doses of preparation Devrinol 450 SC and its active substance napropamide used in experiment

Doses	The amount of Devrinol 450 SC		The amount of napropamide introduced (mg·kg^{-1})
	(dm^3·ha^{-1})	(mm^3·kg^{-1})	
0.5 FD	1.20	0.78	0.35
1 FD	2.40	1.56	0.70
2 FD	4.80	3.12	1.40
4 FD	9.60	6.24	2.80
8 FD	19.20	12.48	5.60
16 FD	38.40	24.96	11.20

FD – field dose

[1968], the alkaline phosphatase (EC 3.1.3.1) and acid phosphatase (EC 3.1.3.2) activities were assayed according to the procedure of Tabatabai and Bremner [1969], and the urease (EC 3.5.1.5) activity was determined as described by Kandeler and Gerber [1988].

The results of the studies were determined statistically using a statistical software package Statistica v. 13.1 (Statsoft, Inc.). The significance of the observed differences was verified using a one-way analysis of variance followed by the post-hoc Tukey's HSD test. Differences with a *P* value of < 0.05 were considered as significant. Moreover Pearson's linear correlation coefficients between napropamide residues and enzyme activities were calculated.

RESULTS

The dissipation of napropamide in the soil depended on the doses of herbicide. Herbicide residues were not detected in soil treated with napropamide at the doses of 0.5 FD, 1 FD, and 2 FD on the last day of the experiment. But, in soil with herbicide at doses of 4 FD, 8 FD, and 16 FD, contents of napropamide residues were reported at the level of 3.19%, 13.19%, and 21.88%, respectively.

Analysis of the calculated 50% dissipation time (DT_{50}) showed that the time of persistence of napropamide in the soil increased with increase in the dose of herbicide. The lowest values of DT_{50} for the dose of 0.5 FD – 33.50 of day and the largest for 16 FD – 71.42 of day were noted (Table 2).

Application of napropamide at the doses of 0.5 FD and 1 FD mainly did not induce significant changes in the activity of dehydrogenases. Only on day 7, in soil treated with 1 FD, decrease in activity of dehydrogenases by 7.83% (compared to control) and on days 14 and 112 in soil with 0.5 FD, increase in this enzyme activity by 6.77% and 10.23%, respectively (compared to control), were reported. Herbicide addition at higher doses caused inhibition of dehydrogenases activity during the whole experiment. Furthermore, this effect increased with the increase of napropamide doses. The lowest activities of dehydrogenases was observed in soil treated with 16 FD on day 7 (Figure 1A).

The activity of alkaline phosphatase in soil treated with napropamide at the doses of 0.5 FD and 1 FD was significantly higher than the control only on day 1, and this stimulation was 68.97% and 12.23%, respectively. But, in later days of experiment, this enzyme activity was mainly inhibited. Application of herbicide at higher doses significantly decreased the alkaline phosphatase activity in soil, compared to control (Figure 1B).

Acid phosphatase activity was significantly increased only on day 28 in soil treated with napropamide at the dose of 0.5 FD (17.14%). Application of herbicide at the dose of 1 FD did not cause significant changes in the enzyme activity during the whole experiment. In samples containing herbicide at the dose of 2 FD significant, decrease in the acid phosphatase activity was observed only on day 1 and 7 (9.59% and 9.65%, respectively, compared to control). Treatment with higher napropamide doses caused decrease in this enzyme activity on all days of measurements. This inhibition was in the range 9.19–14.71%, 9.85–37.14%, and 15.28–56.82% for doses 4 FD, 8 FD, and 16 FD, respectively (Figure 1C).

Urease activity in soil with napropamide at the dose of 0.5 FD was significantly increased from day 1 to day 56 (6.66–18.56%, compared to control). Field dose of the herbicide did not cause significant changes in urease activity. However, application of napropamide at higher doses caused significant decrease in the urease activity during the whole period of experiment (except for 2 FD on day 1). Moreover, this effect increased with the increase of herbicide dose (Figure 1D).

Table 2. Half-life of napropamide in soil and estimated curve parameters

Napropamide doses	Parameters		R^2	DT_{50} (days)
	p	q		
0.5 FD	101.25	-0.021	0.93	33.50
1 FD	99.12	-0.016	0.96	42.89
2 FD	99.57	-0.014	0.98	49.60
4 FD	94.92	-0.011	0.94	58.34
8 FD	91.28	-0.009	0.97	65.36
16 FD	100.12	-0.010	0.97	71.42

Based on the calculated Pearson's linear correlation coefficients, there was a significant negative correlation at $P < 0.05$, between the napropamide residue contents and activities of all enzymes. The strongest correlation was found between the herbicide residue contents and the urease activity (r = -0.92). Analysis of the relationship between the activity of each enzymes in the soil individually showed that there was significant positive correlation at $P < 0.05$ (Table 3).

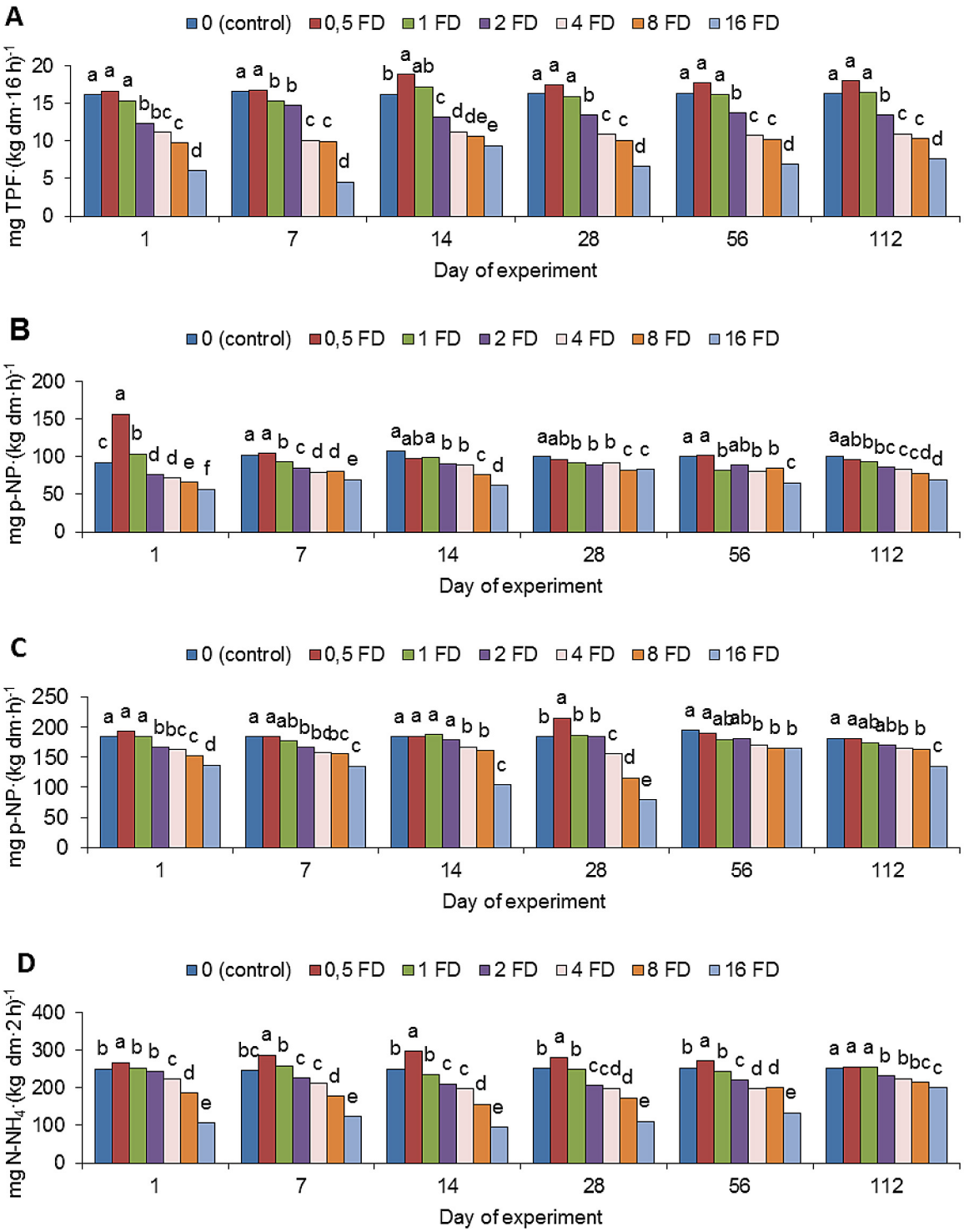

Figure 1. Activity of enzymes: dehydrogenases (A), alkaline phosphatase (B), acid phosphatase (C) and urease (D) in soil treated with napropamide; values denoted with the same letters for each day do not differ statistically at $P < 0.05$

Table 3. Pearson's linear correlation coefficients between napropamide residues and enzyme activities

	NAP	DHA	Pac	Pal	URE
NAP		-0.78	-0.80	-0.64	-0.92
DHA	*		0.73	0.73	0.85
Pac	*	*		0.60	0.83
Pal	*	*	*		0.69
URE	*	*	*	*	

NAP – napropamide, DHA – dehydrogenases, Pac – acid phosphatase, Pal – alkaline phosphatase, URE – urease

DISCUSSION

According to the chemical safety data sheets, the Devrinol 450 SC preparation contains two toxic compounds: napropamide (450 $g \cdot dm^{-3}$) and ethylene glycol (5–10%). Previous studies have shown that ethylene glycol is completely degraded in soil within 3–5 days, without affecting soil microorganisms significantly [McGahey and Bouwer, 1992; Klecka et al., 1993; McVicker et al., 1998]. The mean DT_{50} of napropamide is approximately 70 days [Wauchope et al., 1992]. Therefore, this substance has the greatest impact on the soil environment.

In our laboratory studies, it was found that the 50% dissipation time of napropamide in sandy clay loam depended on the amount of napropamide, and it ranged from 33.50 to 71.42 of days. The obtained results were similar to the results reported by other researchers [Walker et al., 1985; Biswas et al. 2007; Guo et al., 2008].

According to the literature data, the main pathway for napropamide dissipation in the soil is photodegradation [Cycoń et al., 2013a]. However, Rouchaud et al. [1991] showed that, despite slow biodegradation, the proportion of microorganisms in the napropamide distribution was significant. Han [1995] isolated 22 bacterial strains, both Gram-positive and Gram-negative, capable of degrading this compound. The most biodegradation activity was observed for microorganisms of the genus *Staphylococcus* and *Corynebacterium*. Cycoń et al. [2013b] showed that after the application of napropamide there were changes in the structure of microbial communities and the emergence of bacteria capable of degrading this substance.

Enzymatic tests are often used in the assessment of the impact of pesticides, including herbicides, on soil ecosystems [Płatkowski and Telesiński, 2016]. The basic advantages of biological methods, based on enzymatic assays aimed at evaluating the state of the soil environment, are primarily their capability to express the concise influence of numerous factors and the assessment of parameters that are impossible to determine using a different method, e.g., cellular metabolism elements [Telesiński et al., 2015].

Changes in the enzymatic activity of soils are the earliest signal of changes in the intensity of life processes in the environment [Bielińska and Mocek-Płociniak, 2016]. In soil quality studies, those enzymes that react strongly to stress factors are mainly used and changes in the enzymatic activity is related to the intensity of factors involved [Stręk and Telesiński, 2017].

The results of the experiment have generally demonstrated the rather stimulating effect of half-reduced dose of napropamide (i.e., 0.70 and 0.35 $mg \cdot kg^{-1}$) on activity of dehydrogenases, phosphatases, and urease. Cycoń et al. [2013a], however, have found that the use of napropamide at the dose recommended by the manufacturer exhibited a minor effect on the activity of dehydrogenases, urease, alkaline phosphatase, and acid phosphatase under laboratory conditions.

Application of higher doses of napropamide (1.4–11.2 $mg \cdot kg^{-1}$) in our study caused inhibition of the activity of enzymes determined, which increased with the increase of herbicide concentration. Similar results were obtained by Guo et al. [2008], who analyzed the effect of napropamide on biological properties of the soil under laboratory conditions at doses ranging from 2 to 80 mg kg^{-1}, concluding a decreased activity of urease, invertase, and catalase. The researchers suggest that this phenomenon may have resulted from the toxicity of napropamide in relation to microorganisms. But, they did not demonstrate the effect of the herbicide on the biomass content of living microorganisms. Whereas, Cycoń et al. [2013b] have noted that the introduction of napropamide at doses of 2.25 and 22.5 mg kg^{-1} caused inhibition in the activity of soil dehydrogenases. Our

studies have demonstrated that the inhibitory effect of elevated doses of napropamide was the highest for dehydrogenases.

According to Krzyśko-Łupicka et al. [2015], in their studies for evaluating the effect of herbicides on microorganisms and soil properties, laboratory tests under controlled conditions are preferred, because under field conditions soil microorganisms are more likely to react to environmental changes related to plant vegetation, agrotechnical practice, and climatic conditions than the herbicide alone.

CONCLUSIONS

1. The rate of degradation of napropamide in the soil in laboratory experiment depended on the dose of herbicide; the half-life of this compound ranged between 33.50 and 71.42 days and it increased with elevated dose of napropamide.
2. Effect of napropamide on enzymatic activity of the soil was dependent on the herbicide dose, measurement time, and the type of enzyme.
3. The use of napropamide at the dose recommended by the manufacturer and at a dose reduced by half was found to exhibit low toxicity in relation to the enzymes determined. In contrast, the application of elevated doses of napropamide decreased the values of biochemical parameters of the soil in most cases.
4. Among the enzymes identified, dehydrogenases were the most sensitive to the presence of napropamide in the soil.
5. The values of Pearson's linear correlation coefficients have demonstrated statistically significant negative correlation between the content of napropamide residues and the enzymatic activity of the soil.

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