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The effect of carfentrazone-ethyl on soil microorganisms and soil enzymes activity

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Abstract: The aim of this study was to determine the effect of carfentrazone-ethyl (CE) doses of 0.265, 5.280, 10.560, 21.180, 42.240 μg kg^{-1} soil DM on fungi, Acnomycetes, organotrophic bacteria, total oligotrophic bacteria and spore-forming oligotrophic bacteria, and on the activity of dehydrogenases, catalase, urease, alkaline phosphatase, acid phosphatase, arylsulfatase and β-glucosidase. Carfentrazone-ethyl had a stimulating effect on total oligotrophic bacteria and organotrophic bacteria, but it inhibited the growth of *Azotobacter*, fungi, spore-forming oligotrophic bacteria and Actinomycetes. The analyzed substance modified the structure of soil microbial communities, and it induced the most profound changes in fungi. The highest values of the colony development (CD) index and the eco-physiological (EP) index were observed in organotrophic bacteria. The optimal dose of carfentrazone-ethyl stimulated the activity of dehydrogenases, catalase, urease, alkaline phosphatase, acid phosphatase and β-glucosidase, but it had no effect on arylsulfatase. The highest doses of the analyzed substance inhibited the activity of dehydrogenases (reduction from 11.835 to 11.381 μmol TPF), urease (reduction from 0.545 to 0.500 mmol $N\text{-}NH_4$) and arylosulfatase (reduction from 0.210 to 0.168 mmol PNP). Dehydrogenases were most resistant to CE, whereas acid phosphatase and arylsulfatase were least resistant to the analyzed compound.

Introduction

The progressing development and modernization of agriculture has increased the demand for crop protection products, including herbicides (Barchańska et al. 2013, Milošević et al. 2004). The majority of chemical agents are not harmful to the soil environment. They are degraded to harmless products such as water and carbon dioxide which do not affect soil microorganisms (Milošević et al. 2004). Some crop protection products may have toxic impacts on non-target organisms, including soil microbes (Pešaković et al. 2011, Sebiomo et al. 2011). The use of chemical agents in agriculture can also affect the mineralization of organic compounds in soil (Kucharski et al. 2009). In crops where chemical protection products are not used correctly, pesticide residues are found in harvested plants (Łozwiecka and Kaczyński 2011). Soil microbes play an important role in the biogeochemical cycle and the degradation of soil pollutants, including herbicides (Truu et al. 2008). Microorganisms are also a vital source of soil enzymes (Acosta-Martínez et al. 2007, Knight and Dick 2004), whereas the flora and fauna play a less significant role in enzyme production (Singh and Kumar 2008). Intracellular and extracellular enzymes participate in a variety of biochemical processes in soil, they respond rapidly to soil contamination and are effective indicators of soil quality (Acosta-Martínez et al. 2007, Knight and Dick 2004). Dehydrogenases are found in live cells, and they are robust indicators of soil microbial activity (Sebiomo et al. 2011). The effect of herbicides on soil microorganisms varies depending on the active ingredient and the applied dose (Shing and Ghoshal 2010).

Carfentrazone-ethyl (CE) is the active ingredientin Aurora 40 WG, a new-generation herbicide which has been available in Poland since 2008. Carfentrazone-ethyl (CE) is the active ingredient in the Aurora 40 WG herbicide manufactured by the FMC Corporation of Philadelphia, USA. Aurora 40 WG contains carfentrazone-ethyl ((RS)-2-chloro-3-[2-chloro-5-(4-difluoromethyl-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol)-4-fluorophenyl] propionate) at a concentration of 40%. The optimal CE dose is 0.264 μg per kg of soil, which corresponds to 0.660 μg of the Aurora 40 WG herbicide per kg soil DM. CE belongs to the aryl thiazolinone group (FMC Corporation 2010). CE reaches the chloroplast, inhibits protoporphyrinogen oxidase activity and necrotizes plant tissue (Han et al. 2007). CE is transformed into chloropropionic acid as the result of photochemical reactions in soil (Koschnick et al. 2004). Environmental concentrations of CE were estimated at 0.0031 μg kg^{-1} ($PEC\ soil_{20d}$). The effect of CE on aquatic organisms has been well documented (Glomski et al. 2006), whereas its influence on soil microbes remains poorly investigated. The objective of this study was to determine the effect of carfentrazone-ethyl (CE) on the abundance and diversity of soil microorganisms, and the activity and resistance of soil enzymes.

Materials and methods

Soil

A laboratory experiment was carried out on samples of soil with the granulometric composition of sandy loam and pH of 7.0, containing sand (72%), silt (7%) and clay (21%) fractions. The analyzed soil had the following parameters: pH_{KCl} – 7.0, hydrolytic acidity – 8.00 mmol(+)kg^{-1}, total exchangeable bases – 111.00 mmol(+)kg^{-1}, organic carbon content 7.05 g kg^{-1}, total nitrogen content – 0.86 g kg^{-1}. The examined soil was classified as EutricCambisol based on the World Reference Base of Soil Resources (2006).

The following microbial counts were determined on the first day of the experiment (CFU kg^{-1} soil DM): total oligotrophic bacteria – $12.527 \cdot 10^9$, spore-forming oligotrophic bacteria – $3.232 \cdot 10^8$, organotrophic bacteria – $12.855 \cdot 10^9$, *Azotobacter* – $4.929 \cdot 10^4$, Actinomycetes – $12.270 \cdot 10^9$ and fungi – $16.035 \cdot 10^6$.

Enzymatic activity (kg^{-1}DM h^{-1}) was determined at: dehydrogenases – 16.154 µmol TPF, urease – 0.567 mmol $N\text{-}NH_4$, catalase – 0.222 mol O_2, acid phosphatase – 1.072 mmol PNP, alkaline phosphatase – 1.781 mmol PNP, arylsulfatase – 0.245 mmol PNP and β-glucosidase – 0.322 mmol PNP.

Experimental design

The experiment was performed in three replications. 150 cm^3 glass beakers were filled with 100 g of air-dried soil passed through a sieve with 2 mm mesh size. Soil samples were contaminated with CE doses (µg kg^{-1}) of: 0.264 (recommended dose), 5.280 (20-fold higher than the recommended dose), 10.560 (40-fold higher), 21.180 (80-fold higher) and 42.240 (160-fold higher). Uncontaminated soil served as the control treatment. The moisture content of soil was brought to 50% of capillary water capacity. Soil samples were incubated at 25°C for 160 days. On experimental days 20, 40, 80 and 160, selected treatments were terminated, and soil samples were subjected to microbiological and biochemical analyses. A separate series of soil experiments was carried out in three replications for each of the above experimental days. In the study, the results concerning the number of microorganisms and activities of enzymes are presented as the average of all determination terms.

Determination of microbial counts

The counts of oligotrophic bacteria were determined in the Onta and Hattori medium (1983) diluted 1:100, organotrophic bacteria – in the Bunt and Rovira medium (1955), bacteria of the genus *Azotobacter* – as described by Fenglerowa (1965), fungi – in the Martin medium (1950), and Actinomycetes – in the Küster and Williams medium with the addition of antibiotics nystatin and actidione (Parkinson et al. 1971). Plates were kept in a thermostat set to 28°C throughout the entire incubation period. The number of colony forming units (CFU) was determined with a colony counter: *Azotobacter* – after 3 days, fungi – after 5 days, Actinomycetes and organotrophic bacteria – after 7 days, total oligotrophic bacteria and spore-forming oligotrophic bacteria – after 21 days. Microbial counts were determined on the above dates in 9 replications.

Biodiversity of soil microbes

The biodiversity of organotrophic bacteria, Actinomycetes and fungi was determined on the experimental days 20 and 80. Diluted soil suspensions were incubated in Petri dishes in 9 replications, colony growth rates were monitored and colonies were counted over a period of successive 10 days. The plates were incubated at 28°C. The results were used to determine the colony development (CD) index based on the formula proposed by Sarathchandra et al. (1997):

$$CD = [N_1/1 + N_2/2 + N_3/3...N_{10}/10] \text{ x } 100$$

N_1, N_2, N_3,...N_{10} – proportions of microbial colonies identified on days 1, 2, 3,...10.

The eco-physiological (EP) index was calculated in accordance with the formula proposed by De Leij et al. (1993):

$$H' = - \sum(p_i \text{ x } \log 10 p_i)$$

p_i – share of individuals of the i^{th} species in the community relative to the total number of individuals in the community.

The number of colonies of organotrophic bacteria, Actinomycetes and fungi cultured at specific time intervals was determined with the use of the below formula:

$$Ks = (Nx/Nt) \cdot 100$$

Ks – percentage of microbes cultured at specific time intervals,
Nx – number of colonies cultured at two-day intervals counted for 10 days,
Nt – total number of colonies cultured in 10 days.

The colony development (CD) index and eco-physiological (EP) index are presented as the average of the two terms (20 and 80 days).

Determination of enzymatic activity

The activity of the following enzymes was determined in 9 replications: dehydrogenases [EC 1.1], urease [EC 3.5.1.5], catalase [EC 1.11.1.6], arylsulfatase [EC 3.1.6.1], β-glucosidase [EC 3.2.1.21], alkaline phosphatase [EC 3.1.3.1] and acid phosphatase [EC 3.1.3.2]. The activity of dehydrogenase was identified based on the method proposed by Öhlinger (1996), and the activity of the remaining enzymes – according to the procedure described by Alef and Nannipieri (1998).

The following substrates were used for the determination of enzymatic activity: 3% aqueous solution of triphenyltetrazolium chloride (TTC) for dehydrogenases, 4-nitrophenylphosphate disodium for phosphatases, potassium-p-nitrophenylsulfate for arylsulfatase, p-nitrophenyl-β-D-glucopyranoside for β-glucosidase, urea for urease and hydrogen peroxide for catalase.

Extinction was measured with the Perkin-Elmer Lambda 25 spectrophotometer. The activity of alkaline phosphatase, acid phosphatase and urease was determined at a wavelength of 410 nm, arylsulfatase – at 420 nm, β-glucosidase – at 400 nm, and dehydrogenases – at 485 nm. Catalase activity was identified based on its ability to break down hydrogen peroxide in the presence of potassium permanganate.

Enzymatic activity was expressed in kg^{-1} DM h^{-1}, i.e. µmol TPF for dehydrogenase, mol O_2 for catalase, mmol *p*-nitrophenol (PNP) for alkaline phosphatase, acid phosphatase, arylsulfatase and β-glucosidase, and mmol $N\text{-}NH_4$ for urease.

Statistical analysis

The results were processed by ANOVA and Duncan's multiple range test at a significance level of p = 0.01. The coefficients of Pearson's correlation were determined between the CE dose and microbial counts, CD, EP, Ks and enzymatic activity. The average results for four experimental dates were presented. The percentage of the observed variability was calculated using the coefficient η^2 of the two-factor ANOVA analysis. Statistical analyses were performed in the Statistica application (2012).

Results

Microbial counts

The application of CE to soil significantly reduced the abundance of Actinomycetes and fungi (Table 1). The highest CE dose (42.240 μg kg^{-1}) had the most inhibitory effect on *Azotobacter* counts, whereas the optimal dose increased the population size of spore-forming oligotrophic bacteria by 8.3% in comparison with control. The tested substance also stimulated the growth of total oligotrophic bacteria and organotrophic bacteria. The highest counts of organotrophic bacteria (increase by 62.5% compared with the control treatment) were noted after the application of 5.280 μg CE kg^{-1}. The CE dose of 42.240 μg kg^{-1} had the most stimulating effect on total oligotrophic bacteria whose counts increased by 15.3% in comparison with control. With regard to the total number of microorganisms the percentage variation, which corresponds to time, varies between 1.9% (fungi) to 82.0% (*Azotobacter*), dose – from 3.7% (oligotrophic bacteria) to 23.8% (fungi), and the interaction of these factors – from 4.9% (oligotrophic bacteria) to 65.4% (fungi).

Biodiversity of soil microbes

The highest values of the CD index and the EP index were observed in organotrophic bacteria (Table 2). The application of CE significantly increased CD values for fungi. The highest CD values were noted following the application of the optimal CE dose (increase by 30.8% compared with the control treatment). In soil treated with CE doses of 5.280 and 10.560 μg kg^{-1}, the value of the CD index for Actinomycetes was reduced by 4.8% and 4.6%, respectively. Doses of 21.180 and 42.240 μg kg^{-1} led to a significant increase in CD values, by 12.3% and 9.0%, respectively. The addition of CE in doses of 0.264 to 42.240 μg kg^{-1} significantly decreased the values of the EP index of fungi – the lowest value was noted in the treatment with the CE dose of 42.240 μg kg^{-1} (18.9% decrease).

The growth rates (Ks) of soil microorganisms in specific time intervals significantly affect their quality (Table 3). The growth rates of the analyzed microbial colonies were determined by the degree of soil contamination with CE. The most profound changes were observed in the first two days of the experiment when 31.3% of organotrophic bacteria, 26.6% of Actinomycetes and 13.6% fungi appeared in uncontaminated soil. In treatments contaminated with CE, the abundance of organotrophic bacteria ranged from 29.9% to 42.4%, Actinomycetes – from 22.3% to 36.2%, and fungi – from 19.6% to 18.2%. Different results were reported in 4-day-old cultures: the number of developed fungal colonies reached 51.9% in uncontaminated soil and 77.2–84.2% in soil contaminated with CE, the number of Actinomycetes colonies – 63.6% and 57.0–70.0%, respectively, and organotrophic bacteria – 69.0% and 62.7–75.6%, respectively. The addition of CE exerted the strongest effect on fungal colonies, which persisted until day 10.

Enzymatic activity

CE had a varied effect on soil enzymatic activity (Table 4). It stimulated the activity of catalase, alkaline phosphatase and acid phosphatase, as demonstrated by positive coefficients of correlation between the CE dose and the activity of the above enzymes. Negative correlations were observed for urease, dehydrogenases, arylsulfatase and β-glucosidase. The optimal CE dose induced a significant increase in the activity of dehydrogenases (3.1%), catalase (10.1%), alkaline phosphatase (50.0%), acid phosphatase (10.2%) and β-glucosidase (3.8%), relative to uncontaminated soil samples. The CE dose of 42.240 μg kg^{-1} inhibited the activity of dehydrogenases (by 3.8%), urease (8.3%) and arylsulfatase (20.0%). The above dose stimulated the activity of catalase (by 15.1%), alkaline phosphatase (13.9%) and acid phosphatase (8.1%).

Table 1. Average microbial counts (cfu kg^{-1} soil DM) in soil contaminated with carfentrazone-ethyl (CE)

CE dose μg kg^{-1}	Olig 10^9	Olig p 10^8	Org 10^9	Act 10^9	Fun 10^7	*Az* 10^4
0.000	12.492	5.501	8.098	7.697	2.458	5.511
0.264	13.401	5.957	12.891	5.486	2.171	5.707
5.280	13.010	5.371	13.162	5.860	2.109	5.724
10.560	13.966	4.743	10.184	6.492	2.146	5.759
21.180	13.559	4.105	10.050	6.506	2.145	5.112
42.240	14.406	4.030	10.440	6.970	2.117	4.886
$\bar{x}$	13.472	4.951	10.804	6.502	2.191	5.450
r	0.847	-0.884	-0.160	0.252	-0.465	-0.879
$LSD_{0.01}$	0.404	0.285	0.389	0.345	0.237	0.285

r – coefficient of correlation
Olig – oligotrophic bacteria
Olig p – spore-forming oligotrophic bacteria
Org – organotrophic bacteria
Act – Actinomycetes
Fun – fungi
*Az – Azotobacte*r

Table 2. Average values of the colony development (CD) index and the eco-physiological (EP) index in soil contaminated with carfentrazone-ethyl (CE)

CE dose µg kg^{-1}	CD			EP		
	Org	Act	Fun	Org	Act	Fun
0.000	34.584	29.694	25.411	0.814	0.810	0.827
0.264	38.917	29.056	33.236	0.792	0.843	0.725
5.280	39.309	28.258	33.037	0.806	0.816	0.681
10.560	41.682	28.339	32.056	0.802	0.846	0.721
21.180	35.025	33.355	32.414	0.855	0.781	0.687
42.240	34.468	32.356	32.789	0.848	0.759	0.671
$\overline{x}$	37.331	30.176	31.490	0.820	0.809	0.719
r	-0.456	0.737	0.364	0.800	-0.823	-0.623
$LSD_{0.01}$	2.595	1.226	2.462	0.025	0.030	0.076

* Refer to the legend for Table 1.

Table 3. The effect of carfentrazone-ethyl (CE) on the number of microbial colonies (in %) cultured at different time intervals (Ks)

CE dose µg kg^{-1}	Incubation days				
	1–2	3–4	5–6	7–8	9–10
Org					
0.000	31.276	37.750	19.426	9.264	2.283
0.264	36.545	39.005	13.662	7.225	3.563
5.280	36.558	37.153	14.353	9.325	2.610
10.560	42.382	29.802	14.584	8.599	4.632
21.180	29.926	32.810	18.430	14.401	4.433
42.240	30.631	34.109	18.933	10.225	6.101
$\overline{x}$	34.553	35.105	16.565	9.840	3.937
r	-0.432	-0.476	0.497	0.484	0.888
Act					
0.000	26.611	36.968	19.458	12.448	4.515
0.264	23.221	34.953	24.419	12.236	5.171
5.280	23.071	33.895	23.506	14.923	4.605
10.560	22.667	35.602	21.391	14.305	6.036
21.180	36.205	28.636	15.490	15.353	4.317
42.240	32.979	36.979	13.469	10.542	6.031
$\overline{x}$	27.459	34.505	19.622	13.301	5.113
r	0.729	-0.044	-0.862	-0.360	0.467
Fun					
0.000	13.653	38.236	14.571	23.044	10.496
0.264	28.249	53.328	8.370	3.813	6.240
5.280	24.616	59.570	9.626	4.831	1.357
10.560	25.015	52.192	13.814	5.385	3.595
21.180	19.620	64.470	7.661	5.306	2.943
42.240	20.910	63.057	8.536	3.714	3.783
$\overline{x}$	22.011	55.142	10.430	7.682	4.736
r	-0.120	0.667	-0.443	-0.421	-0.410

* Refer to the legend for Table 1.

Enzyme resistance (RS) to soil contamination with CE was determined by principal component analysis (PCA) using standardized data which meets the assumptions of Pearson's correlation (Fig. 1). The first two components for standardized data accounted for 61.66% of total variance. Two homogeneous groups were identified for the first principal component. The first group comprised acid phosphatase and arylsulfatase, which were negatively correlated with the first principal component, and the second group consisted of urease and β-glucosidase, which were positively correlated with the first principal component.The homogeneous group for the second principal component comprised alkaline phosphatase and catalase. Their resistance was positively correlated with the analyzed variable, whereas no correlations were determined between the resistance of alkaline phosphatase and catalase and the first principal component.

The vector representing the original variable for dehydrogenase resistance was located near the x and y coordinates, which indicates that dehydrogenase resistance was not correlated with the level of CE contamination. Only the resistance of acid phosphatase and arylsulfatase was negatively correlated with the CE dose. The distribution of points in four quarters of the plane indicates that the degree of CE contamination can affect the resistance of soil enzymes to the analyzed compound.

Table 4. Average enzymatic activity in kg^{-1} DM per hour in soil contaminated with carfentrazone-ethyl (CE)

CE dose µg kg^{-1}	Deh µmol TPF	Cat mol O_2	Ure mmol $N\text{-}NH_4$	Pal	Pac	Aryl	Glu
				mmol PNP			
0.000	11.835	0.179	0.545	1.748	1.281	0.210	0.470
0.264	12.204	0.197	0.655	1.921	1.412	0.210	0.488
5.280	11.860	0.220	0.651	2.006	1.425	0.220	0.515
10.560	11.665	0.224	0.610	2.052	1.455	0.207	0.502
21.180	11.416	0.219	0.558	2.259	1.401	0.197	0.480
42.240	11.381	0.206	0.500	1.991	1.384	0.168	0.468
$\overline{x}$	11.727	0.207	0.587	1.996	1.393	0.202	0.487
r	-0.837	0.310	-0.721	0.440	0.130	-0.945	-0.446
$LSD_{0.01}$	0.229	0.009	0.023	0.048	0.041	0.007	0.017

Deh – dehydrogenases
Cat – catalase
Ure – urease
Pal – alkaline phosphatase
Pac – acid phosphatase
Aryl – arylsulfatase
Glu – β-glucosidase

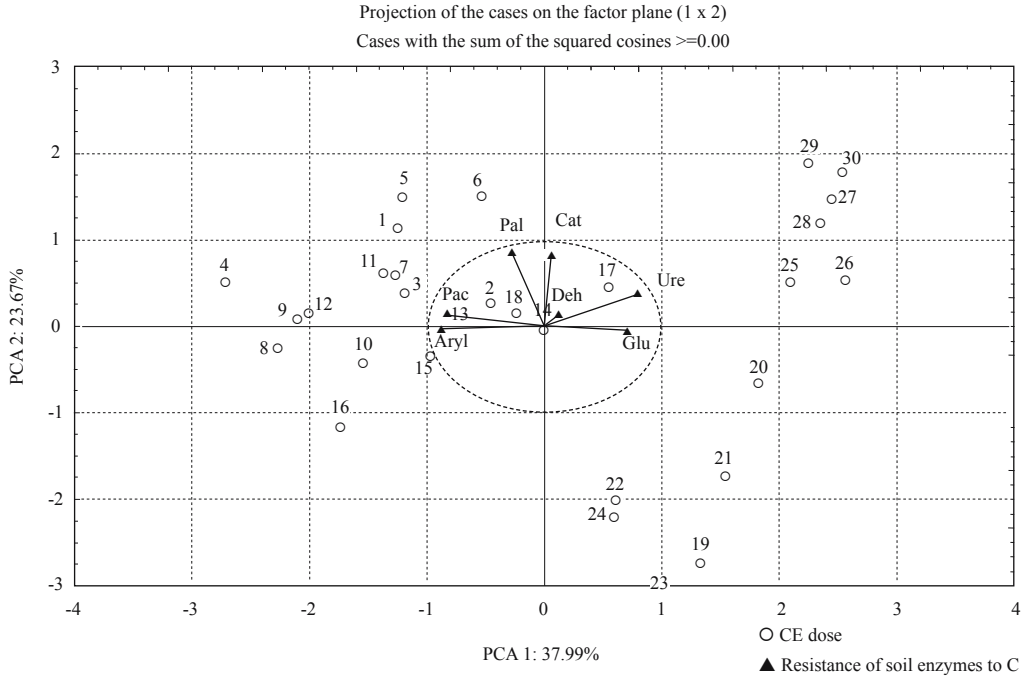

Fig. 1. Resistance of soil enzymes to carfentrazone-ethyl (CE) contamination, determined by PCA

Key: Deh – dehydrogenases, Cat – catalase, Ure – urease, Pal – alkaline phosphatase, Pac – acid phosphatase, Aryl – arylsulfatase, Glu – β-glucosidase; 1–6 (CE dose of 0.264 µg kg^{-1}); 7–12 (CE dose of 5.280 µg kg^{-1}); 13–18 (CE dose of 10.560 µg kg^{-1}); 19–24 (CE dose of 21.180 µg kg^{-1}); 25–30 (CE dose of 42.240 µg kg^{-1})

Although the results described above refer to averages of all terms, when we take into account all the factors examined in the experiment, the highest variation is observed in the duration of the experiment (60.5%), and a much lower in the dose of the herbicide (15.5%). The variability caused by the interaction of these factors was 17.4%.

Discussion

Microbial counts

The results of the present study show that CE had a varied effect on the analyzed soil microorganisms. CE stimulated the proliferation of total oligotrophic bacteria, but in doses several-fold higher than recommended by the manufacturer, it inhibited the growth of spore-forming oligotrophic bacteria. Kucharski and Wyszkowska (2008) analyzed the Apyros 75 WG herbicide containing sulfosulfuron as the active ingredient to demonstrate that doses 100-fold higher than recommended by the manufacturer reduced the abundance of both total oligotrophic bacteria and spore-forming oligotrophic bacteria. Sebiomo et al. (2011) observed that atrazine, primeextra, paraquat and glyphosate reduced the counts of soil bacteria.

Fungal counts in soil contaminated with CE decreased with an increase in the applied dose. Similar observations were made by Sebiomo et al. (2011) in whose study, the application of atrazine, primeextra, paraquat and glyphosate reduced fungal counts in soil. In the work of Kucharski and Wyszkowska (2008), fungal proliferation was inhibited by the Apyros 75 WG herbicide. Contrary results were reported by Crouzet et al. (2010) in a study of mesotrione which had a stimulating effect on fungi when applied in higher doses. In the work of Araújo et al. (2003), a glyphosate dose of 2.16 mg kg^{-1} soil DM stimulated fungal development. Martinez et al. (2008) did not observe significant changes in the abundance of fungi in response to sulfentrazone treatment.

In the current study, CE also exerted an inhibitory effect on Actinomycetes. A reduction in Actinomycetes counts in soil treated with the Apyros 75 WG herbicide was also reported by Kucharski and Wyszkowska (2008). Araújo et al. (2003) demonstrated that glyphosate applied in a dose of 2.16 mg kg^{-1} soil DM stimulated the growth of Actinomycetes after 32 days of incubation. The stimulating effect of sulfentrazone on Actinomycetes was also noted by Martinez et al. (2008).

According to Milošević and Govedarica (2002), bacteria of the genus *Azotobacter* are highly sensitive to soil contamination and they are effective indicators of pollution caused by crop protection products. In this study, CE had the most inhibitory effect on *Azotobacter* populations when applied in a dose of 42.240 μg kg^{-1}.

Biodiversity of soil microbes

The CD index was determined to illustrate changes in the proportions of slow-growing and fast-growing microorganisms. The EP index describes the biological diversity of microbes in contaminated soil, in this case – in soil treated with CE. This study relied on the ecological concept of r-strategists and K-strategists (life cycle strategies). R-strategists are microbes which proliferate rapidly (24–48 h) in uninhabited environments, and which are sensitive to environmental pollution. K-strategists are slow-proliferating microorganisms which are less susceptible to pollution than r-strategists (Cycoń and Piotrowska-Seget 2009, De Leij et al. 1993). In this study, the value of the EP index decreased with an increase in the CE dose for Actinomycetes and fungi, and organotrophic bacteria were characterized by the highest biodiversity. The value of the CD index was lower for organotrophic bacteria and higher for Actinomycetes and fungi, which could point to higher counts of fast-growing microbes.

The most intensive growth of organotrophic bacteria, Actinomycetes and fungi was reported in the first four days of the experiment, which suggests that the above microorganisms relied on CE as a source of nutrients and energy.

Enzymatic activity

In this study, CE had a varied effect on the activity of soil enzymes. CE stimulated the activity of acid phosphatase and alkaline phosphatase. Similar results were reported by Baćmaga et al. (2012) in whose study, the Aurora 40 WG herbicide applied in doses 2-, 4- and 40-fold higher than recommended by the manufacturer had a positive effect on both phosphatases. However, in a study by Yao et al. (2006) phosphatases were sensitive to soil contamination with acetamipirid. An inhibitory effect of Triflurotox 250 EC, a pesticide containing trifluralin as the active ingredient, on phosphatases was also noted by Wyszkowska and Kucharski (2004). Wyszkowska (2002) reported that the Treflan 480 EC herbicide containing trifluralin suppressed the activity of alkaline phosphatase and acid phosphatase.

In the current experiment, dehydrogenase activity increased in response to the optimal CE dose, whereas the highest dose (42.240 μg kg^{-1}) had an inhibitory effect on the analyzed enzyme. In the work of Baćmaga et al. (2012), Aurora 40 WG did not have a significant influence on dehydrogenase activity. Similar results were reported in the present study where a dose of 10.560 μg kg^{-1} did not inhibit dehydrogenase activity in comparison with control. In an experiment investigating the Apyros 75 WG herbicide, Kucharski and Wyszkowska (2008) concluded that dehydrogenases belong to a group of enzymes which are most sensitive to soil pollution. Similar observations were made by Wyszkowska (2002) in a study of the Treflan 480 EC herbicide.

Baćmaga et al. (2012) reported lower levels of urease activity in sandy clay loam contaminated with the Aurora 40 WG herbicide and an insignificant increase in urease activity in loamy sand. In the present study, only the highest CE dose (42.240 μg kg^{-1}) suppressed urease activity. Sukul (2006) demonstrated that very high doses of metalaxyl inhibited urease activity. The sensitivity of urease to soil pollution was also demonstrated by Kucharski and Wyszkowska (2008) in a study of the Apyros 75 WG herbicide and by Wyszkowska (2002) who investigated the Treflan 480 EC herbicide.

Catalase is an intracellular enzyme which is found in all aerobic bacteria (Trasar-Cepeda et al. 1999) and which decomposes hydrogen peroxide to water and oxygen (García et al. 2007). In this study, CE had a stimulating effect on catalase activity. Yao et al. (2006) did not report significant changes in catalase activity in soil contaminated with acetamipirid.

The application of CE doses in the range of 0.264 to 10.560 μg kg^{-1} increased the activity of β-glucosidase. Saha et al. (2012) observed higher levels of β-glucosidase activity in soil samples treated with alachlor, butachlor and

pretilachlor. The optimal dose of CE had a stimulating effect on arylsulfatase, whereas the highest dose (42.240 μg kg^{-1}) suppressed arylsulfatase activity. In a study by Baćmaga et al. (2012) who investigated the effect of the Aurora 40 WG herbicide in two types of soil, enzymatic activity levels were higher in sandy loam than in loamy sand.

A stable soil system is resistant to changes in environmental conditions induced by natural processes and human activity. Soil stability can be determined based on the resistance and resilience of the soil system (Orwin and Wardle 2004). The index of enzyme resistance (RS) describes the soil's response to contamination (Orwin et al. 2006). In this study, acid phosphatase and arylsulfatase were least resistant to soil contamination with CE, whereas dehydrogenases were most resistant to the analyzed compound. A different reaction of the CE extracellular and intracellular enzymes was observed. Dehydrogenases, as intracellular enzymes, may be a reflection of the current response of microorganisms to carfentrazone-ethyl, however acid phosphatase, as an enzyme, which may be partially adsorbed by soil colloids, is not linked to the actual number of microorganisms. Hence its reaction to CE was positive, which could be due to the protective effect of mineral and organic soil colloids. A similar effect could not be expected in the case of intracellular enzymes – dehydrogenases.

Conclusions

The optimal dose of CE increased the counts of spore-forming oligotrophic bacteria, organotrophic bacteria and bacteria of the genus *Azotobacter* in soil, but it had an inhibitory effect on fungi and Actinomycetes.

The values of indices K_S, CD and EP indicate that CE modifies the structure of soil microbial communities, in particular fungi.

When applied in the optimal dose, CE enhanced the activity of dehydrogenases, catalase, urease, alkaline phosphatase, acid phosphatase and β-glucosidase, but it had no effect on arylsulfatase. The highest CE doses had an inhibitory effect on the activity of dehydrogenases (21.180 μg kg^{-1} and 42.24 μg kg^{-1}), urease (42.240 μg kg^{-1}) and arylsulfatase (21.180 μg kg^{-1} and 42.240 μg kg^{-1}). Dehydrogenases were most resistant to CE, whereas acid phosphatase and arylsulfatase were least resistant to the analyzed compound.

Despite some variations in the properties of microbiological and biochemical properties caused by technological dose of carfentrazone-ethyl, the use of this preparation can be considered safe – it does not cause permanent and lasting changes in these properties.

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Wpływ karfentrazonu etylu na mikroorganizmy i aktywność enzymów glebowych

Streszczenie: W pracy określono wpływ karfentrazonu etylu zaaplikowanego w dawkach 0,265, 5,280, 10,560, 21,180, 42,240 μg kg^{-1}s.m. gleby na grzyby, promieniowce, bakterie organotroficzne, oligotroficzne ogółem i oligotroficzne przetrwalnikujące oraz aktywność dehydrogenaz, katalazy, ureazy, fosfatazy alkalicznej, fosfatazy kwaśnej, arylosulfatazy i β-glukozydazy. W wyniku badań stwierdzono stymulujące działanie karfentrazonu etylu na bakterie oligotroficzne ogółem i bakterie organotroficzne, natomiast inhibicyjne na *Azotobacter*, grzyby, bakterie oligotroficzne przetrwalnikujące oraz promieniowce. Preparat ten zmieniał strukturę zespołu drobnoustrojów. Największe zmiany wywoływał u grzybów. Najwyższe wartości wskaźników rozwoju kolonii (CD) i ekofizjologicznej różnorodności (EP) odnotowano u bakterii organotroficznych. Karfentrazon etylu w dawce optymalnej zwiększał aktywność dehydrogenaz katalazy, ureazy, fosfatazy alkalicznej, fosfatazy kwaśnej i β-glukozydazy, a nie oddziaływał na arylosulfatazę, natomiast najwyższe dawki zmniejszały aktywność dehydrogenaz (obniżenie z 11,835 do 11,381 μmol TPF), ureazy (obniżenie z 0,545 do 0,500 mmol N-NH_4) i arylosulfatazy (obniżenie z 0,210 do 0,168 mmol PNP). Najbardziej opornymi enzymami na działanie KE okazały się dehydrogenazy, a najmniej fosfataza kwaśna i arylosulfataza.