Environment Protection Engineering

Vol. 40 2014 No. 4

DOI: 10.5277/epe140402

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RESPONSE OF MICROORGANISMS AND ENZYMES TO SOIL CONTAMINATION WITH THE HERBICIDE SUCCESSOR T 550 SE

The study has been made to determine the effect of soil contamination with a new generation herbicide on the abundance and diversity of microorganisms, activity of soil enzymes and their tolerance to the herbicide Successor T 550 SE. Herbicide disturbed the biological balance of soil as expressed by the numbers and diversity of microorganisms and enzymatic activity. The recommended dose of herbicide significantly stimulated the growth of total oligotrophic and organotrophic bacteria as well as actinomycetes, depressed the activity of fungi and had no effect on oligotrophic sporulating bacteria and *Azotobacter*.

1. INTRODUCTION

More intensive agricultural production involves higher consumption of pesticides, including herbicides [1]. Plant protection chemicals used according to the manufacturer's recommendations should not interfere with the metabolism of crops or soil. However, when used excessively and incorrectly, herbicides can evoke unpredictable changes in the soil environment with such consequences as changes in the activity of soil-borne microbes [1, 2]. Another risk is posed by application of several chemicals over a short period of time, each of which can affect other than target organisms. Use of herbicides can increase or lower the biomass of microorganisms, or else leave it unchanged. The actual change in microbial biomass depends on the quality and quantity of the applied preparation [2, 3].

The biggest change in the number of soil microorganisms and soil enzymes activity can be observed directly after application of the herbicide into the soil [4].

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Degradation of herbicides is a complex process, in which microorganisms play a role [2], Herbicides undergo transformation through hydrolysis, oxidation and photolysis, which proceed with the participation of such microorganisms as *Arthrobacter*, *Agrobacterium*, *Bacillus*, *Corynebacterium*, *Flavobacterium*, *Nocardia, Pseudomonas* and *Trichoderma*. Fungi are most tolerant to unfavourable environmental conditions. Soil microorganisms can decompose – partly or completely – soil-borne herbicides. The ability of microorganisms to metabolize chemical substances depends on the structure and bioavailability of the latter. Microorganisms more readily decompose aliphatic and hydroxyl compounds but need most time to disintegrate compounds whose rings contain sulphur, oxygen and nitrogen. Intermediate products formed during degradation of an active component, play an important role as well. Frequently, they are more toxic than the initial material [5, 6].

Changes in the soil environment caused by herbicides can be assessed, for example, by analyzing the response of microorganisms [1] and enzymatic activity [2–4] to these substances. The knowledge gained from such analyses about the effect of herbicides on soil metabolism is extremely valuable for soil protection and management.

Sucessor T 550 SE, is produced by Stähler International GmbH & Co. KG, is a new generation herbicide whose effect on the microbial activity and soil enzyme has not yet been investigated. It contains two active components: pethoxamid (2-chloro-N--(2-ethoxyethyl)-N-(2-phenylprop-1-enyl-2-methyl)acetamide), a compound in the group of chloroacetamides, and terbuthylazine (2-tert-buthylamine-4-chloro-6-ethyl-amine-1,3,5-triazine), a compound in the group of triazines. 1 dm^3 of herbicide contains 300 g of pethoxamid and 250 g of terbuthylazine. The predicted environmental concentration in soil (PECsoil20d) is: 0.009 $mg \cdot kg^{-1}$ of petoxamid and 0.069 $mg \cdot kg^{-1}$ of terbuthylazine. The herbicide became commercially available in 2008. Pethoxamid is a relatively new active component, inhibiting the biosynthesis of fatty acids. The herbicide is used to control mono- and dicotylenous weeds on maize plantations [7]. The manufacturer's recommended dose is 4 $dm^3 \cdot ha^{-1}$ (1.33 $mm^3 \cdot kg^{-1}$) of soil.

Thus, the present study has been undertaken so as to determine the effect of soil contamination with a new generation herbicide on counts and diversity of microorganisms, activity of soil enzymes and their tolerance to Successor T 550 SE.

2. MATERIAL AND METHODS

Soil characteristics. The experiment was run under laboratory conditions with the use of soil with the texture of sandy loam (Table 1).

Experimental design. The experiment was conducted with three parallel replications, in 150 cm^3 glass beakers, each containing 100 g of air-dry soil passed through a 2 mm mesh sieve. Next, the soil received one of the following doses of the herbicide

as aqueous suspension: 1 – the technological dose, and 20-, 40-, 80- and 160-fold higher doses than recommended by the manufacturer. Soil with no herbicide added served as the control. Once the soil samples had been thoroughly mixed with the herbicide, their soil moisture content was increased to 50% of capillary soil moisture by adding demineralised water. Afterwards, the soil samples were incubated in an incubator for 20, 40, 80 and 160 days at 25 °C.

Table 1

Selected physicochemical properties of soil used in experiment

Granulometric composition [%]			C_{org}	N_{total}	pH_{KCl}	*Hh*	*S*	*T*	*V* [%]
Sand	Silt	Clay	[g·kg^{-1}]	[g·kg^{-1}]		[mmol (+)·kg^{-1}d.m. soil]			
72	21	7	7.05	0.86	7.00	8.00	111.00	119.00	93.28

Hh – hydrolytic acidity, *S* – base exchange capacity, *T* – total sorptive capacity, *V* – saturation with base cations, C_{org} – organic carbon, N_{total} – total nitrogen.

Numbers of microbes. Numbers of microorganisms were obtained by plating soil samples after 20, 40, 80 and 160 days of incubation. The microorganisms were counted in the following way: oligotrophic bacteria on Onta and Hattori medium diluted 100-fold [8], organotrophic bacteria according to Bunt and Rovira [9], bacteria of the genus *Azotobacter* according to Fenglerowa [10], actinomycetes on Kuster and Willimas medium with added nystatin and actidione [11] and fungi on Martin glucose-peptone medium [12]. The paper presents means of the results obtained on four dates of assays. Microorganisms were incubated in an incubator at 28 °C. Numbers of colony forming units (cfu) of the genus *Azotobacter* were determined after 3 days for bacteria, fungi after 5 days, organotrophic bacteria and actinomycetes after 7 days and oligotrophic total and oligotrophic sporulating bacteria after 21 days. Colonies from nine parallel cultures were counted.

Moreover, in 9 consecutive replications, appropriate solutions of incubated soils were placed on Petri plates with selective medium so as to determine numbers of colonies of organotrophic bacteria, actinomycetes and fungi achieved on 10 subsequent days. These data were then used to calculate the colony development index (*CD*) according to the model by De Leij et al. [13] and ecophysiological index (*EP*) from the formula suggested by Sarathchandra et al. [14]. Numbers of colony forming units were determined with a colony counter.

Enzyme activities. On incubation days 20th, 40th, 80th and 160th, the activity of the following enzymes in soil was determined: alkaline phosphatase [EC 3.1.3.1], acid phosphatase [EC 3.1.3.2], arylsulphatase [EC 3.1.6.1], β-glucosidase [EC 3.2.1.21], dehydrogenases [EC 1.1], catalase [EC 1.11.1.6] and urease [EC 3.5.1.5]. All determinations had 9 replications. Dehydrogenases were determined according to Öhlinger

[15], and the remaining enzymes – according to the protocol described by Alef and Nannpieri [16]. The results presented in this paper are means for the four determination dates.

The substrates used for determination of activity of particular enzymes were 2,3,5-triphenyltetrazolium chloride (TTC) for dehydrogenases, H_2O_2 for catalase, urea for urease, 4-nitrophenylphosphate disodium for acid and alkaline phosphatase, potassium-*p*-nitrophenylsulfate for arylsulphatase, and *p*-nitrophenyl-β-D-glucopyranoside for β-glucosidase. The activity of dehydrogenases was expressed in μmol triphenyl formazan (TPF)·kg^{-1} d.m.·h^{-1}, catalase in mol O_2·kg^{-1} d.m.·h^{-1}, urease in mmolN-NH_4·kg^{-1}·h^{-1}, and acid phosphatase, arylsulphatase and β-glucosidase in mmol *p*-nitrophenol (PNP)·kg^{-1} d.m.·h^{-1}. All the enzymes except catalase were determined on a Perkin-Elmer Lambda 25 spectrophotometer. Activity of dehydrogenases was measured the wavelength of $\lambda = 485$ nm, β-glucosidase at $\lambda = 400$ nm, arylosulphatase at $\lambda = 420$ nm, and urease, acid phosphatase and alkaline phosphatase at $\lambda = 410$ nm.

Besides, on 20th day of the experiment, values of the resistance index (*RS*) for particular enzymes to contamination with the herbicide were determined, and their resilience (*RL*) was measured on 160th day. The *RS* and *RL* were calculated from the formulas defined by Orwin and Wardle [17].

Statistics. The studies were carried out as two-factors as in randomized block design. The results of microbiological and biochemical assays were processed statistically with the two-factor ANOVA analysis of variance using Statistica software [18] and applying Duncan's multiple interval test at the significance level $p = 0.01$. This paper provides average scores of the terms and LSD for herbicide dose. Additionally, Pearson's correlation coefficient was calculated for each dose of the herbicide in function the enzymatic activity and numbers of microorganisms.

3. RESULTS

3.1. MICROBE NUMBERS

The results of the experiment suggest that the herbicide Successor T 550 SE significantly modified microbiological properties of soil. The actual impact of pethoxamid and terbuthylazine on counts and diversity of microorganisms largely depended on the degree of soil contamination (Figs. 1–3). The numbers of microorganisms are given in Table (total oligotrophic bacteria, oligotrophic sporulating bacteria, or-

ganotrophic bacteria, *Azotobacter,* actinomycetes and fungi) in the soil at the onset of the experiment.

Table 2

Numbers of microorganisms (cfu) in 1 kg d.m. of soil
at the onset of the experiment

Olig	$Olig_p$	Org	Az	Act	Fungi
12.527×10^9	3.232×10^8	12.855×10^9	4.929×10^4	12.270×10^9	1.603×10^7

Olig – oligotrophic bacteria, $Olig_p$ – oligotrophic sporulating bacteria, Org – organotrophic bacteria, Act – actinomycetes, Az – *Azotobacter*.

Soil contamination with Successor T 550 SE significantly stimulated the multiplication of total oligotrophic, oligotrophic sporulating and organotrophic bacteria as well as actinomycetes (Fig. 1).

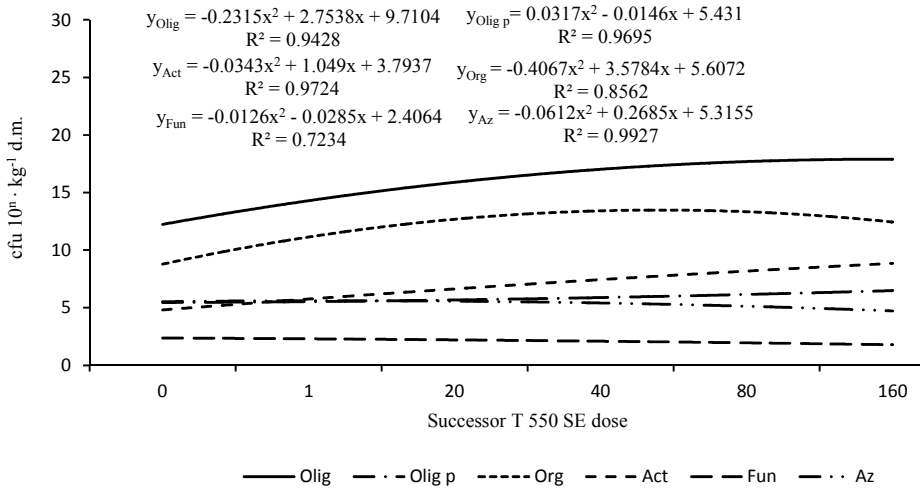

Fig. 1. Numbers of microorganisms in soil contaminated with the herbicide Successor T 550 SE (cfu·kg^{-1} d.m. of soil): Olig – oligotrophic bacteria (10^9); $Olig_p$ – oligotrophic sporulating bacteria (10^8), Org – organotrophic bacteria (10^9), Act – actinomycetes (10^9), Fun – fungi (10^7), Az – *Azotobacter* (10^4), 0 – control sample, 1 – manufacturer's recommended dose, doses 20-, 40-, 80-, 160-fold higher than recommended

This effect is confirmed by the positive coefficients between dose of the herbicide and numbers of these microorganisms. The highest counts of total oligotrophic (17.994×10^9 cfu·kg^{-1} d.m. of soil) and organotrophic bacteria (13.017×10^9 cfu·kg^{-1}) were observed in the treatments where the herbicide had been applied in a dose 80--fold higher than recommended by the manufacturer, while the highest numbers of oligo-

trophic sporulating bacteria (6.458×10^8 cfu·kg^{-1}) and actinomycetes (9.000×10^9 cfu·kg^{-1}) were found in the samples contaminated with the highest dose of Successor T 550 SE.

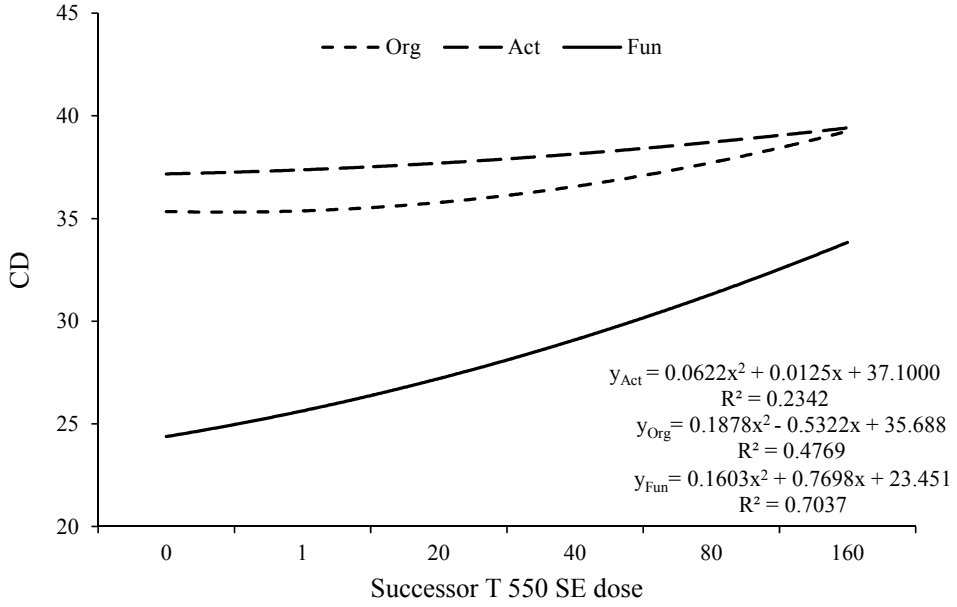

Fig. 2. Colony development index (*CD*) in soil contaminated with the herbicide Successor T 550 SE, for explanation see Fig. 1

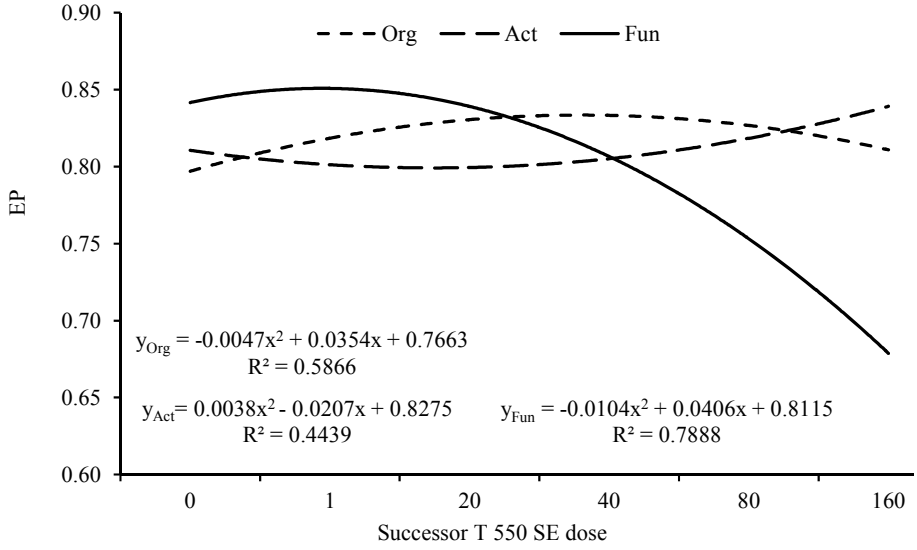

Fig. 3. Ecophysiological index (*EP*) in soil contaminated with the herbicide Successor T 550 SE; for explanations see Fig. 1

In turn, excess of pethoxamid and terbuthylazine introduced to soil in the form of Successor T 550 SE depressed the growth rate of bacteria of the genus *Azotobacter* and fungi. This effect was particularly strong in the case of fungi, whose growth was already by 16% lower versus the control under the manufacturer recommended dose of the herbicide.

Soil contamination with the herbicide produced a relatively small effect on the colony development (*CD*) and ecophysiological (*EP*) indices (Figs. 2, 3). Successor T 550 SE applied in doses from the optimal one to 40-fold higher than permissible left the *CD* and *EP* of microorganisms nearly unchanged. In turn, the *CD* (Fig. 2) of microorganisms isolated from soil contaminated with this herbicide in a dose 160-fold higher than recommended was 17.9% higher for organotrophic bacteria, 11.9% higher for actinomycetes and 26.0% higher for fungi. The *EP* (Fig. 3) of organotrophic bacteria and actinomycetes was only weakly affected by the tested herbicide while the *EP* of fungi significantly decreased in soil samples with the rates of this herbicide 80-fold (by 16.4%) and 160-fold (by 13.9%) higher than recommended.

3.2. ENZYME ACTIVITIES

At the onset of the experiment, the activity of the enzymes was determined (Table 3).

Table 3

Enzyme activity in 1 kg d.m.·h^{-1} of soil at the onset of the experiment

Deh	Cat	Ure	Pac	Pal	Aryl	Beta
[µmol TPF]	[mol O_2]	[mmol N-NH_4]	[mmol PNP]			
16.154	0.222	0.567	1.072	1.781	0.245	0.322

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

Soil contamination with the herbicide significantly modified the activity of soil enzymes (Table 4).

Although most often a negative correlation was detected between the activity of dehydrogenases, urease, acid phosphatase, arylphosphatase and β-glucosidase and the rate of Successor T 550 SE, the impact of the herbicide was not invariable. In fact, both increased and decreased activity of some enzymes was observed. Successor T 550 SE applied to soil in doses from the optimal one to 40-fold higher than the recommended increased rather than decreased the activity of catalase, urease, acid phosphatase and alkaline phosphatase. The highest increase concerned the activity of urease (up to 13.6%) and arylsulphatase (up to 29.5%). In contrast, the activity of dehydrogenases and β-glucosidase decreased linearly as the doses of the herbicide

increased. As a result, in the treatments with pethoxamid and terbuthylazine (the active components of the herbicide) added in the amounts 160-fold higher than recommended, the activity of dehydrogenases decreased by 36.9% and that of β-glucosidase by 6.2%. This highest tested rate of the herbicide also caused a significant decrease in the activity of arylsulphatase (by 21.4%) and urease (by 5.5%).

Table 4

Activity of enzymes in soil contaminated with the herbicide Successor T 550 SE

Herbicide dose	Deh [μmol TPF $\cdot kg^{-1}$ d.m.$\cdot h^{-1}$]	Cat [mol O_2 $\cdot kg^{-1}$ d.m.$\cdot h^{-1}$]	Ure [mmol N-NH_4 $\cdot kg^{-1}$ d.m.$\cdot h^{-1}$]	Pal	Pac	Aryl	Glu
				[mmol PNP$\cdot kg^{-1}$ d.m.$\cdot h^{-1}$]			
0	11.835	0.179	0.545	1.750	1.281	0.210	0.470
1	11.730	0.187	0.619	1.890	1.309	0.238	0.467
20	11.551	0.179	0.604	1.825	1.325	0.272	0.447
40	11.023	0.187	0.579	1.842	1.279	0.258	0.434
80	9.026	0.184	0.533	1.876	1.255	0.175	0.443
160	7.465	0.180	0.515	1.823	1.248	0.165	0.436
$\bar{x}$	10.438	0.183	0.566	1.834	1.283	0.220	0.449
r	–0.984	–0.156	–0.740	0.110	–0.777	–0.721	–0.693
$LSD_{0.01}$	0.195	0.005	0.021	0.038	0.034	0.008	0.008

0 – control sample, 1 – manufacturer's recommended dose, doses 20-, 40-, 80-, 160-fold higher than recommended, Deh – dehydrogenases, Cat – catalase, Ure – urease, Pal – alkaline phosphatase, Pac – acid phosphatase, Aryl – arylosulphatase, Glu – β-glucosidase, $LSD_{0.01}$ values for herbicide dose, r – coefficient of correlation.

Table 5

Resistance index (*RS*) of enzymes in soil contaminated with the herbicide Successor T 550 SE on 20th day of the experiment

Herbicide dose	Deh	Cat	Ure	Pal	Pac	Aryl	Glu
1	0.965	0.947	0.262	0.884	0.938	0.926	0.972
20	0.942	0.870	0.408	0.868	0.865	0.942	0.858
40	0.950	0.894	0.532	0.963	0.960	0.820	0.730
80	0.755	0.909	0.556	0.829	0.943	0.843	0.947
160	0.852	0.863	0.560	0.821	0.940	0.780	0.920
$\bar{x}$	0.893	0.897	0.464	0.873	0.929	0.862	0.886
r	–0.635	–0.585	0.747	–0.582	0.300	–0.840	0.156

Explanations, see Table 4.

The mean resistance index of the enzymes to soil contaminations with Successor T 550 SE (Table 5) ranged from 0.464 (urease) to 0.929 (acid phosphatase). Excessive

quantities of the herbicide in soil most strongly depressed the *RS* of dehydrogenases, arylsulphatase and β-glucosidase. This critical influence of the herbicide was also reflected by the values of the resilience index (*RS*), which were negative for most of the enzymes (Table 6). In the case of urease and catalase, some tendency towards restored balance could be noticed, as the *RL* values for these two enzymes were positive, except for one treatment.

Resilience index (*RL*) of enzymes in soil contaminated with the herbicide Successor T 550 SE on 160th day of the experiment

Herbicide dose	Deh	Cat	Ure	Pal	Pac	Aryl	Glu
1	–0.028	–0.510	0.924	0.288	–0.132	–0.788	–0.473
20	–0.119	0.360	0.879	0.070	0.035	–0.926	–0.066
40	–0.239	0.306	0.747	–0.723	–0.355	–0.740	0.044
80	0.275	0.388	0.376	–0.154	–0.321	–0.734	–0.620
160	0.225	0.578	0.151	–0.142	–0.122	–0.660	–0.507
$\overline{x}$	0.023	0.224	0.615	–0.132	–0.179	–0.770	–0.324
r	0.703	0.702	–0.972	–0.237	–0.123	0.741	–0.447

Explanations see Table 4.

4. DISCUSSION

Plant protection preparations can cause changes in the microbiological activity of soil. Some preparations result in depressed numbers of microorganisms while other increase their number [3, 19–21]. Such contrary effects of herbicides on soil microorganisms, colony development index and ecophysiological index of bacteria, actinmomycetes and fungi may be due to differences in chemical composition and physical characteristics of pesticides. On the other hand, they may result from different degrees of tolerance of microorganisms to active components of preparations, which for some microorganisms can become an excellent substrate providing them with nutrients and energy [19, 20, 22] and for some can be an unwanted toxic substance [3, 23].

In the present study, pethoxamid and terbuthylazine introduced to soil in the form of Successor T 550 SE had a positive effect on counts of oligotrophic bacteria, oligotrophic sporulating bacteria, organotrophic bacteria and actinomycetes, but reduced numbers of fungi and *Azotobacter* cells.

Higher abundance of bacteria in response to excessive amounts of herbicides in soil, analogously to the effect of Successor T 550 SE observed in our study, has been reported by Milošević and Govedarica [1]; lower counts were discovered by Sebiomo et al. [23] in response to atrazine, primeextra, paraquat and glyphosate and by Araùjo et al. [19] after application of glyphosate. According to Milošević and Govedarica [1] bacteria of the genus *Azotobacter* are most sensitive to herbicide and are therefore

a reliable indicator of the soil biological quality. This conclusion is confirmed by Wyszkowska and Kucharski [4] as well as by Kucharski and Wyszkowska [3] who observed smaller populations of these microorganisms under the influence of excessive amounts of herbicides.

An increase in the abundance of actinomycetes due to soil contamination with the herbicide demonstrated hereby is not a sporadic observation because more intensive growth of actinomycetes has also been evidenced by Araujo et al. [19] in soil with glyphosate and by Martinez et al. [22] in soil with sulphentrazone. In turn, the herbicide Apyros 75 WG studied by Kucharski and Wyszkowska [3] and atrazine, primeextra, paraquat and glyphosate analyzed by Sebiomo et al. [23] retarded the growth of actinomycetes in soil.

The literature contains few reports on the negative effect of herbicides on the growth and development of fungi [3]. In this study, among all the examined microorganisms, the growth of fungi was most distinctly depressed by pethoxamid and terbuthylazine. Crouzet et al. [20], who tested soil treated with mesotrione as well as Araùjo et al. [19] in their experiment on glyphosate noticed an increase in numbers of fungi. However, Martinez et al. [22] did not report any significant changes in the growth of fungi in soil treated with sulphentrazone.

Recapitulating, soil microorganisms quickly respond to changes in the environment. For this reason, they are thought to be good indicators of soil quality [2–4]. Soil contamination can result in depressed numbers of some organisms and higher numbers of others. Thus, modifications in structures of microbial assemblages induced by contaminants permeating into the soil environment are often taken advantage of in microbiological research [13].

In the experiment presented in this paper, the herbicide Successor T 550 SE did not have an invariable influence on the diversity of microorganisms in soil. Nonetheless, the colony development index (*CD*) provided much information about changes in mutual proportions between rapidly and slowly growing microorganisms, whereas the *EP* informed us about the ecophysiological diversity of microorganisms which was shaped under the influence by pethoxamid and terbuthylazine. Cycoń et al. [21] claim that an increase in the total number of microorganisms accompanied by the decreased *EP* value may indicate that sensitive species of microorganisms are supplanted by microorganisms more tolerant to stress factors, including herbicides. A similar development was noted in the case of organotrophic bacteria and actinomycetes, whose counts were increasing as the doses of the herbicide increased, and that was most probably a result of their higher metabolic activity at the cost of lysis of cells of other microorganisms more sensitive to the tested herbicide.

The quality of soil is manifested not only by counts and diversity of microorganisms [3, 19–21] but also by the activity of soil enzymes [2–4]. Both inter- and extracellular enzymes may catalyze or mediate biochemical processes such as mineraliza-

tion, cycling of elements, decomposition and production of organic matter in soil and degradation of various types of contaminants, including pesticides [1–4].

Numerous references [2, 4, 24] suggest that when herbicides applied in optimal doses usually do not affect the biochemical activity of soil. However, when added to soil in excessive quantities, they can modify the activity of soil enzymes. Compared to the above reports, this study presents interesting results because Successor T 550 SE to some extent disturbed the biological balance of soil not only when introduced to soil in doses 20-, 40-, 80- and 160-fold higher than recommended but also when applied as suggested by the manufacturer. Thus, in the treatments with the optimal doses of pethoxamid and terbuthylazine, the activity of urease increased by 14%, arylsulphatase by 13% and alkaline phosphatase by 8%, while the activity of dehydrogenases, catalase, acid phosphatase and β-glocosidase remained quite stable. Obviously, in the treatments with the highest doses of the herbicides, changes in the enzymatic activity were most profound. The enzymes that proved most sensitive to excess of the herbicide (160-fold more than recommended) were dehydrogenases (depressed by 36.9%), arylsulphatases (less by 21.4%), β-glucosidase (by 6.2%) and urease (by 5.5%). Such an effect of the herbicide on soil enzymes confirms many previous studies [2–4, 24]. Wyszkowska and Kucharski [4], who tested Triflurotox 250 EC at doses from 1.5 to 12.0 $mm^3 \cdot kg^{-1}$, demonstrated its adverse effect on dehydrogenases as well as acid and alkaline phosphatases. Apyros 75 WG applied at higher rates also depressed the activity of dehydrogenases and urease, although acid and alkaline phosphatases proved to be more tolerant to this herbicide [3]. Some retardation in the activity of urease in response to metalaxyl was noticed by Sukul [24]. In turn, an experiment conducted by Baćmaga et al. [2] proves that the herbicide Aurora 40 WG had no significant effect on the activity of dehydrogenases, acid phosphatase and alkaline phosphatase, but slightly stimulated the activity of urease in loamy sand and depressed the same enzyme in sandy clay loam. Saha et al. [25] who tested chloroacetanilide herbicides (alachlor, butachlor and pretilachlor), concluded that soil contamination with these preparations caused an increase in the activity of β-glucosidase. Changes in the enzymatic activity of soil induced by the introduction of petoxamid and terbuthylazine as Successor T 550 SE were manifested by the disturbed ecological stability of soil. The soil stability was determined according to the index of resistance of enzymes to the contamination and the index of soil resilience [17]. This research shows that irrespective of the degree of contamination, the mean values of the *RS* allow us to arrange the enzymes in respect of their tolerance to the tested herbicide as follows (from the most to the least tolerant enzymes): acid phosphatase (0.929) > catalase (0.897) > dehydrogenases (0.862) > β-glucosidase (0.886) > alkaline phosphatase (0.873) > arylsulphatase (0.862) urease (0.454). Relatively quick recovery of the activity was demonstrated only by urease (the mean $RL = 0.555$) and catalase ($RL = 0.170$), which was proven by the positive values of the *RL*. However, the influence of the herbicide on the other enzymes, i.e. alkaline phosphatase ($RL = –0.109$), acid phosphatase ($RL = –0.214$),

β-glucosidase and dehydrogenases (*RL* = –0.296) as well as arylsulphatase (*RL* = –0.759), was increasingly destructive. This is evidenced by the negative values of the *RL*.

5. CONCLUSIONS

Soil contamination by the herbicide Successor T 550 SE disturbed the biological balance of soil as expressed by the numbers and diversity of microorganisms and enzymatic activity.

Excess amounts of pethoxamid and terbuthylazine significantly stimulated the growth of total oligotrophic, oilgotrophic sporulating and organotrophic bacteria as well as actinomycetes, but depressed the activity of *Azotobacter* spp. and fungi.

Contamination of soil with the herbicide had a relatively weak effect on the value of the colony development (*CD*) and ecophysiological (*EP*) indices.

The activity of dehydrogenases, urease, acid phosphatase, arylsulphatase and β-glucosidase was negatively correlated with the rate of Successor T 550 SE.

In respect of the tolerance to Successor T 550 SE, the enzymes were ordered as follows (from the most to the least tolerant): acid phosphatase > catalase > dehydrogenases > β-glucosidase > alkaline phosphatase > arylsulphatase > urease.

Changes in the soil metabolism induced by the herbicide were persistent. During 160 days, the activity of one enzyme only, that is urease, was partly restored. In contrast, the negative influence of the herbicide on the activity of alkaline phosphatase, acid phosphatase, β-glucosidase, dehydrogenases and arylsulphatase was increasingly stronger.

ACKNOWLEDGMENTS

The research has been performed as part of the research project funded by the National Centre of Science (NCN) No. N N305 386138.

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