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BIOLOGICAL PROPERTIES OF SOIL CONTAMINATED WITH THE HERBICIDE APYROS 75 WG

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Abstract

The objective of the study has been to determine the effect of the herbicide Apyros 75 WG on counts of various aerobic microorganisms, activity of soil enzymes and yields of spring wheat.

For this purpose, a pot experiment was carried out in a greenhouse. Samples of soil used for the trials represented loamy sand. Having mixed the soil samples with mineral fertilizers, doses of the herbicide were added and the soil was placed in plastic pots. The lowest herbicide dose was the optimum dose recommended by the producer, and the two other doses were 10- and 100-fold higher. The experiment was conducted in two series: I – unsown soil, and II – soil under spring wheat.

It has been determined that Apyros 75 WG disturbs soil's homeostasis, as it disrupts multiplication of some microbial groups, inhibits the activity of soil enzymes and depresses the yield of spring wheat, even if applied in a recommended dose. Among the soil enzymes, dehydrogenases and urease were the least tolerant to the effect of the herbicide, whereas alkaline phosphatase proved to be the most tolerant one. The vulnerability of microorganisms to soil pollution with the herbicide can be arranged in the following decreasing order: ammonifying bacteria > *Pseudomonas* > copiotrophic bacteria > oligotrophic bacteria > nitrogen binding bacteria > spore-forming oligotrophic bacteria > *Arthrobacter* > cellulolytic bacteria > *Actinomyces* > fungi. Growing spring wheat had a positive effect on the counts of microorganisms and activity of soil enzymes.

Key words: Apyros 75 WG, herbicide, activity of enzymes, soil microorganisms.

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BIOLOGICZNE WŁAŚCIWOŚCI GLEBY ZANIECZYSZCZONEJ HERBICYDEM APYROS 75 WG

Abstrakt

Celem badań było określenie wpływu herbicydu Apyros 75 WG na liczebność różnych grup drobnoustrojów tlenowych, aktywność enzymów glebowych oraz plonowanie pszenicy jarej. Badania przeprowadzono w wazonach w hali wegetacyjnej. W doświadczeniu wykorzystano próbki gleby o składzie granulometrycznym piasku gliniastego. Glebę po wymieszaniu z nawozami mineralnymi zanieczyszczono herbicydem i umieszczono w plastikowych wazonach. Najniższa dawka herbicydu była dawką optymalną, zalecaną do stosowania przez producenta, a kolejne były 10 i 100-krotnie wyższe. Badania prowadzono w dwóch seriach: I – gleba nieobsiana i II – gleba obsiana pszenicą jarą.

Stwierdzono, że Apyros 75 WG narusza homeostazę gleby, gdyż nawet w dawce zalecanej przez producenta zakłóca namnażanie niektórych grup drobnoustrojów, hamuje aktywność enzymów glebowych oraz zmniejsza plon pszenicy jarej. Najmniej odpornymi enzymami na działanie herbicydu są dehydrogenazy oraz ureaza, a najbardziej odporna jest fosfataza alkaliczna. Wrażliwość drobnoustrojów na zanieczyszczenie gleby herbicydem Apyros 75 WG jest następująca: bakterie amonifikacyjne > *Pseudomonas* > bakterie kopiotroficzne > bakterie oligotroficzne > bakterie immobilizujące azot > bakterie oligotroficzne przetrwalnikujące > *Arthrobacter* > bakterie celulolityczne > promieniowce > grzyby. Na liczebność drobnoustrojów oraz aktywność enzymów korzystnie wpływa uprawa pszenicy jarej.

Słowa kluczowe: Apyros 75 WG, herbicyd, aktywność enzymów, drobnoustroje glebowe.

INTRODUCTION

Plant protection chemicals improve and protect crop yields but they can also have an adverse effect on many ecosystems (Cupples, Sims 2007, Shen et al. 2005, Sřrensen et al. 2003, Wg et al. 2005). Their effect on soil environment depends mainly on the type of active substance, application rates as well as the oxidation-reduction potential of soil, soil content of organic substance, physicochemical properties of soil, temperature, moisture, pH, sorptive capacity, grain-size distribution, and counts of bacteria, *Actinomyces* and fungi (Awasthi et al. 2000, Sánchez et al. 2004). Irrespective of the above factors, biocides can migrate from soil to groundwater and foodstuffs, posing threat to humans and animals (McDonald et. al. 1999, Zhang et al. 2006). Widespread use of pesticides and herbicides in agriculture makes it necessary to investigate their influence on natural environments. Thus, monitoring the effects produced by plant protection chemicals, which may involve inhibition of a series of biological processes and consequent disorders in the enzymatic activity of soil or multiplication of microorganisms, can provide us with a reliable measure of the unwanted side-effects (Bending et al. 2006, Braschi et al. 2000, Kucharski et al. 2006, Wyszkowska 2002a, 2002b, Wyszkowska, Kucharski 2004).

Apyros 75 WG belongs to a new generation of herbicides, and its effect on soil environment has not been thoroughly investigated or discussed yet.

Therefore, this study's aim has been to determine how Apyros 75 WG affects counts of many groups of aerobic bacteria, activity of soil enzymes and yields of spring wheat.

MATERIAL AND METHODS

The experiment with 5 replicates was conducted in a greenhouse at the University of Warmia and Mazury in Olsztyn. Samples of soil used for the laboratory trials were collected from proper brown soil created from light loamy sand possessing the following properties: 5.60 pH in 1 mol KCl^{-3}, 13.05 mmol(H^+) kg^{-1} hydrolytic acidity, 5.00 $g \cdot kg^{-1}$ C_{org}, 57.06 mmol(+) kg^{-1} total exchangeable base cations, 70.11 mmol(+)kg^{-1} sorptive complex exchangeable capacity, 81.39% saturation with base cations. Prior to placing in the pots, soil was fertilized with the following macro- and microelements, in $mg \cdot kg^{-1}$ soil (expressed as pure component): 100 N [$CO(NH_2)_2$], 44 P [K_2HPO_4], 83 K [KH_2PO_4 + KCl], 20 Mg [$MgSO_4 \cdot 7H_2O$], 5 Zn [$ZnCl_2$], 5 Cu [$CuSO_4 \cdot 5H_2O$], 5 Mn [$MnCl_2 \cdot 4H_2O$], 5 Mo [$Na_2MoO_4 \cdot 2H_2O$] and 0.33 B [H_3BO_3]. All the mineral fertilizers, except urea, were introduced to soil once before sowing wheat. Urea was added in doses: 1/2 N dose before sowing and 1/2 N dose in the wheat tillering stage.

The experiment's variables were:

1) rates of the herbicide Apyros 75 WG in μm kg^{-1} of soil: 0, 8.9, 89.0 and 890.0;
2) application of the herbicide: soil application (the herbicide was mixed with a whole portion of soil used to fill up one pot prior to placing it in the pot), top-dressing (the herbicide was applied over the surface of soil during the tillering of wheat plants);
3) soil use: unsown soil and soil sown with spring wheat;
4) day of analysis: 14 days after placing the soil in pots (on the day when spring wheat was sown) and on day 73 of the trials (on harvest day).

The lowest rate of the herbicide was the optimum dose recommended by the producer, and the following rates were 10- and 100-fold higher.

Before setting up the experiment, a portion of soil to fill up one pot (3.2 kg) was mixed with the mineral fertilizers and with the herbicide Apyros 75 WG where appropriate. The active substance in Apyros 75 Wg is 75% sulfosulfuron 1-(4,6-dimethoxypyrimidin-2-yl)-3-(2-ethylsulfonylimidazo[1,2-*a*]pyridin-3-ylsulfonyl)urea. Apyros 75 WG is used to control loose silkybent (*Apera spica-venti*) and broad-leaved weeds in winter wheat, spring wheat, winter triticale and potato. This herbicide does not represent a significant risk to human health (class IV).

Having placed the soil in the pots, its moisture content was brought up to 60% of capillary water capacity and maintained at this level over the whole experiment (73 days). During the first 14 days soil was not sown. On

day 14 samples of soils were collected for microbiological and biochemical assays, after which cv. Sakwa spring wheat (12 plants per pot) was sown. The experiment was conducted in two series: I – unsown soil, II – soil under spring wheat. Wheat plants were harvested in the inflorescence stage (on day 59 of the growing season). On the same day soil samples were collected for the second time to perform analyses.

The microbiological assays consisted of determination of counts of the following microorganisms: copiotrophic and spore-forming copiotrophic bacteria on ONTA-HATTORI medium (1983), *Arthrobacter*, *Pseudomonas*, nitrogen binding, ammonifying and cellulolytic bacteria on the medium described in the paper by WYSZKOWSKA et al. (2007), *Actynomyces* on Kuster and Williams medium with nystatin and actidione (PARKINSON et al. 1971) and fungi – on a medium proposed by MARTIN (1950). The microorganisms were grown on Petri plates at 28ºC for 2 (*Azotobacter*) to 21 days (oligotrophic bacteria). Spores of oligotrophic and copiotrophic bacteria were determined on material which was pasteurised at 85ºC for 15 minutes. The count of colony forming units (cfu) was determined using a colony-count method.

The biochemical tests included determination of the activity of: dehydrogenases using a TTC substratum (ÖHLINGER 1996), urease – according to ALEF and NANNPIERI (1998) and acid and alkaline phosphatase – by the method described by ALEF et al. (1998). Activity of dehydrogenases was expressed in cm^3 of H necessary to reduce TTC to TFP; urease – in mg $N\text{-}NH_4$ produced from hydrolysed urea, the phosphatases – in nmol of *p*-nitrophenol (PNP) produced from sodium 4-nitrophenyl phosphate.

In addition, physiochemical analyses were performed, involving the determination of hydrolytic acidity (Hh) and total base exchangeable cations (S) using Kappen method (MOCEK et al. 1997). From these two values total exchangeable capacity (T) and saturation with base cations (V) were computed using the formulas: $T = S + Hh$ and $V = S \cdot T^{-1}\ 100\%$. Because the physicochemical properties of soil were not significantly correlated with the day of sampling, method of herbicide application or soil use, the results of the above determinations are presented in the paper as means for two days of sampling and independently from the method Apyros 75 WG was applied.

The results were processed statistically by Duncan's multiple-range test, using two- and four-factorial analysis of variance (StatSoft, Inc. ... 2006).

RESULTS AND DISCUSSION

The research proves that soil contamination with Apyros 75 Wg disturbs the microbiological equilibrium of soil (Tables 1-3), although the actual disorders in soil's homeostasis depend on a rate of the herbicide, time period of the herbicide present in soil, soil use, herbicide application method and

Table 1

Count of oligotrophic and copiotrophic bacteria (in 1 kg d.m. of soil)

Herbicide dose Apyros 75 WP	Before spring wheat sowing	Day of analysis		
		after harvest		
		soil use		
		sown		unsown
		g	l	
Copiotrophic total bacteria (cfu 10^{10})				
0	123.7 ± 8.8	124.4 ± 8.8	124.4 ± 1.6	91.0 ± 1.6
Optimum	97.5 ± 1.2	128.3 ± 5.6	102.2 ± 9.7	106.5 ± 4.7
10 x	95.0 ± 7.0	103.2 ± 2.2	92.8 ± 1.6	77.8 ± 4.3
100 x	97.1 ± 4.8	59.1 ± 5.4	86.0 ± 5.4	40.1 ± 2.2
Average	103.3 ± 5.4	103.8 ± 3.4	101.3 ± 3.1	78.9 ± 1.7
r	-0.358	-0.965	-0.666	-0.937
LSD*	a – 3.1; b – 2.2; c – 2.2; d – 2.2; a · b – 4.4; a · c – 4.4; a · d – 4.4; b · c – 3.1; b · d – n.s.; c · d – n.s.; a · b · c – 6.3; a · b · d – 6.3; a · c · d – n.s.; b · c · d – n.s.; a · b · c · d – 8.9			
Sporulating copiotrophic bacteria (cfu 10^{7})				
0	54.5 ± 1.6	19.4 ± 1.1	19.4 ± 1.1	27.6 ± 2.2
Optimum	56.3 ± 4.5	18.6 ± 2.2	21.5 ± 1.9	29.0 ± 2.2
10 x	66.7 ± 2.2	14.3 ± 0.6	15.1 ± 1.1	28.7 ± 2.2
100 x	69.2 ± 4.1	15.1 ± 2.2	17.2 ± 1.1	25.4 ± 1.6
Average	61.6 ± 1.3	16.8 ± 0.4	18.3 ± 0.5	27.7 ± 0.5
r	0.747	-0.554	-0.339	-0.909
LSD*	a – 1.5; b – 1.1; c – 1.1; d – 1.1; a · b – 2.2; a · c – 2.2; a · d – 2.2; b · c – 1.5;b · d – n.s.; c · d – n.s.; a · b · c – n.s.; a · b · d – n.s.; a · c · d – 2.2; b · c · d – n.s.; a · b · c · d – 4.3			
Total oligotrophic bacteria (cfu 10^{8})				
0	30.1 ± 2.2	40.9 ± 1.1	40.9 ± 1.1	15.8 ± 2.2
Optimum	26.9 ± 2.8	44.8 ± 2.2	38.4 ± 2.7	15.1 ± 1.9
10 x	22.9 ± 0.6	25.8 ± 2.2	33.0 ± 2.7	16.1 ± 1.1
100 x	20.1 ± 1.2	16.8 ± 1.2	16.5 ± 0.6	16.5 ± 1.2
Average	25.0 ± 1.4	32.1 ± 0.8	32.2 ± 0.6	15.9 ± 0.7
r	-0.801	-0.831	-0.977	0.727
LSD*	a – 1.2; b – 0.9; c – 0.9; d – n.s.; a · b – 1.7; a · c – 1.7; a · d – n.s.; b · c – 1.; b · d – 1.2; c · d – n.s.; a · b · c – 2.4; a · b · d – 2.4; a · c · d – 1.7; b · c · d – n.s.; a · b · c · d – 3.5			

Sporulating oligotrophic bacteria (cfu 10^7)				
0	17.9 ± 0.6	17.2 ± 1.1	17.2 ± 1.1	19.7 ± 1.6
Optimum	13.3 ± 1.2	16.8 ± 1.6	18.3 ± 1.9	17.2 ± 1.1
10 x	13.3 ± 1.2	15.8 ± 1.2	11.5 ± 0.6	16.8 ± 0.6
100 x	7.9 ± 0.6	12.2 ± 0.6	11.1 ± 0.6	16.1 ± 1.1
Average	13.1 ± 0.6	15.5 ± 0.3	14.5 ± 0.9	17.5 ± 0.3
r	-0.870	-0.984	-0.674	-0.623
LSD*	a – 0.6; b – 0.4; c – 0.4; d – 0.4; a · b – 0.9; a · c – 0.9; a · d – n.s.; b · c – 0.6; b · d – 0.6; c · d – 1.2; a · b · c – 1.2; a · b · d – n.s.; a · c · d – 0.9; b · c · d – n.s.; a · b · c · d – 1.8			

LSD for: a – Apyros 75 WG dose; b– day of analysis; c – soil use; d – herbicide application method; g – herbicide applied to soil before sowing plants; l – herbicide applied to soil surface in the tillering phase of spring wheat

group of microorganisms. Noteworthy is the fact that the herbicide caused changes in the biological properties of soil even when applied in the recommended dose. This effect is implied by modifications in the counts of total copiotrophic, spore-forming oligotrophic (Table 1), ammonifying (Table 2), cellulolytic and *Pseudomonas* bacteria as well as *Actinomyces* (Table 3). The remaining groups of microorganisms (total oligotrophic, nitrogen immobilising, *Arthrobacter*, spore-forming copiotrophic bacteria and fungi) were affected by Apyros 75 WG only when it was applied in the rates 10- and 100-fold higher than the recommended dosage.

The values of the correlation coefficients indicate that the above soil microorganisms differed in their tolerance to the herbicide in soil. According to their vulnerability to the highest herbicide rate, the microorganisms can be ordered as follows: ammonifying bacteria (counts depressed by 49%) > > *Pseudomonas* (by 43%) > copiotrophic bacteria (by 42%) > oligotrophic bacteria (by 38%) > nitrogen immobilising bacteria (by 35%) > spore-forming oligotrophic bacteria (by 34%) > *Arthobacter* (by 32%) > cellulolytic bacteria (by 31%) > *Actinomyces* (by 27%) > fungi (by 5%).

Counts of soil microorganisms were determined by a degree of soil pollution with the herbicide as well as by the time it persisted in soil (Tables 1-3). In soil not sown with wheat, the average number of copiotrophic bacteria and their spores, oligotrophic, ammonifying, nitrogen immobilising bacteria, *Arthrobacter*, *Pseudomonas*, *Actinomyces* and fungi was higher on day 14 of the trials. On day 73 of the experiment, spore-forming oligotrophic and cellulolytic bacteria occurred in higher counts than earlier.

Das et al. (2003) claim that modifications observed as a result of application of herbicides concern mainly changes in the qualitative and quantitative composition of microorganisms and biochemical activity. As a rule, when herbicides are used in compliance with the manufacturer's recommendations,

Table 2

Number of ammonifying, nitrogen immobilizing bacteria and *Arthrobacter* (in 1 kg d.m. of soil)

Herbicide dose Apyros 75 WP	Before spring wheat sowing	Day of analysis after harvest soil use		
		sown		unsown
		g	l	
Ammonifying bacteria (cfu 10^8)				
0	117.2 ± 3.2	169.2 ± 2.7	169.2 ± 2.7	72.4 ± 2.7
Optimum	99.6 ± 5.5	162.0 ± 6.2	150.5 ± 4.3	76.3 ± 4.3
10 x	82.4 ± 5.1	84.2 ± 6.2	140.5 ± 9.6	61.6 ± 4.8
100 x	78.5 ± 4.9	69.5 ± 4.1	139.4 ± 9.1	36.2 ± 3.5
Average	94.4 ± 1.4	121.2 ± 3.9	149.9 ± 4.9	61.6 ± 3.2
r	-0.664	-0.734	-0.571	-0.965
LSD*	a – 2.9; b – 2.0; c – 2.0; d – 2.0; a · b – 4.1; a · c – 4.1; a · d – 4.1; b · c – 2.9; b · d – 2.9; c · d – 2.9; a · b · c – 5.7; a · b · d – 5.7; a · c · d – 5.7; b · c · d – 4.0; a · b · c · d – 8.1			
Nitrogen immobilizing bacteria (cfu 10^8)				
0	70.3 ± 0.6	127.6 ± 8.4	127.6 ± 8.4	16.5 ± 1.6
Optimum	71.3 ± 5.3	138.0 ± 8.6	91.0 ± 5.0	16.5 ± 1.2
10 x	65.9 ± 5.9	68.5 ± 4.3	69.2 ± 2.2	12.5 ± 1.6
100 x	63.1 ± 6.6	62.7 ± 8.4	55.2 ± 4.8	14.0 ± 1.1
Average	67.7 ± 1.3	99.2 ± 2.8	85.8 ± 3.3	14.9 ± 1.1
r	-0.846	-0.690	-0.702	-0.393
LSD*	a – 2.9; b – 2.0; c – 2.0; d – 2.0; a · b – 4.1; a · c – 4.1; a · d – 4.1; b · c – 2.9; b · d – 2.9; c · d – 2.9; a · b · c – 5.8; a · b · d – 5.8; a · c · d – 5.8; b · c · d – 4.1; a · b · c · d – 8.1			
Arthrobacter spp. (cfu 10^9)				
0	48.2 ± 0.8	22.2 ± 2.0	22.2 ± 2.0	8.4 ± 0.3
Optimum	48.0 ± 1.6	21.1 ± 1.4	28.9 ± 1.1	9.7 ± 0.5
10 x	26.3 ± 0.9	22.6 ± 1.4	26.7 ± 1.9	9.3 ± 0.8
100 x	23.7 ± 1.9	22.0 ± 1.6	24.4 ± 2.4	8.4 ± 0.8
Average	36.6 ± 0.4	22.0 ± 0.7	25.5 ± 0.5	9.0 ± 0.3
r	-0.711	0.107	-0.244	-0.534
LSD*	a – 0.8; b – 0.6; c – 0.6; d – 0.6; a · b – 1.1; a · c – n.s.; a · d – n.s.; b · c – 0.8; b · d – 0.8; c · d – 0.8; a · b · c – n.s.; a · b · d – n.s.; a · c · d – 1.6; b · c · d – 1.1; a · b · c · d – 2.2			

* explanations under Table 1

Table 3

Number of *Pseudomonas* and cellulolytic bacteria, *Actinomyces* and fungi (in 1 kg d.m. of soil)

Herbicide dose Apyros 75 WP	Before spring wheat sowing	Day of analysis after harvest soil use		
		sown		unsown
		g	l	
Pseudomonas spp. (cfu 10^9)				
0	28.1 ± 1.6	28.5 ± 1.4	28.5 ± 1.4	23.3 ± 1.4
Optimum	26.0 ± 0.8	33.2 ± 2.2	27.2 ± 1.6	15.2 ± 0.3
10 x	24.7 ± 1.6	28.5 ± 1.4	19.9 ± 0.5	15.6 ± 0.5
100 x	18.6 ± 1.2	14.7 ± 0.8	16.3 ± 0.8	11.8 ± 1.9
Average	24.4 ± 0.7	26.2 ± 0.9	23.0 ± 0.7	16.5 ± 0.5
r	-0.961	-0.968	-0.818	-0.677
LSD*	a – 0.8; b – 0.5; c – 0.5; d – 0.5; a · b – 1.1; a · c – 1.1; a · d – 1.1; b · c – 0.8; b · d – 0.8; c · d – 0.8; a · b · c – 1.5; a · b · d – 1.5; a · c · d – 1.5; b · c · d – 1.1; a · b · c · d – 2.2			
Cellulolytic bacteria (cfu 10^6)				
0	11.8 ± 1.1	39.8 ± 2.8	39.8 ± 2.8	84.9 ± 1.1
Optimum	11.8 ± 1.1	33.0 ± 2.7	39.4 ± 2.2	48.4 ± 1.9
10 x	10.8 ± 1.1	21.5 ± 2.2	17.6 ± 0.6	50.2 ± 1.2
100 x	5.7 ± 0.6	18.6 ± 1.2	13.3 ± 0.6	41.9 ± 2.8
Average	10.0 ± 0.4	28.2 ± 1.2	27.5 ± 0.2	56.4 ± 1.1
r	-0.997	-0.712	-0.740	-0.538
LSD*	a – 0.9; b – 0.6; c – 0.6; d – n.s.; a · b – 1.3; a · c – 1.3; a · d – 1.3; b · c - n.s.; b · d – 0.9; c · d – n.s.; a · b · c – 1.8; a · b · d – 1.8; a · c · d – 1.8; b · c · d – 1.3; a · b · c · d – 2.6			
Actinomyces (cfu 10^8)				
0	52.3 ± 3.8	112.2 ± 5.5	112.2 ± 5.5	29.7 ± 1.6
Optimum	41.9 ± 2.8	91.0 ± 4.8	100.0 ± 2.8	34.4 ± 1.9
10 x	39.4 ± 2.5	78.1 ± 2.2	90.7 ± 3.3	33.3 ± 3.2
100 x	41.2 ± 0.6	68.8 ± 4.9	68.8 ± 7.1	31.2 ± 1.1
Average	43.7 ± 1.1	87.5 ± 1.2	92.9 ± 1.6	32.2 ± 1.3
r	-0.349	-0.720	-0.912	-0.278
LSD*	a – 6.5; b – 4.6; c – 4.6; d – n.s.; a · b – 9.2; a · c – n.s.; a · d – n.s.; b · c – 6.5; b · d – 6.5; c · d – 6.5; a · b · c – n.s.; a · b · d – n.s.; a · c · d – n.s.; b · c · d – 9.2; a · b · c · d – 18.4			

Fungi (cfu 10^6)				
0	43.4 ± 5.9	17.9 ± 0.6	17.9 ± 0.6	23.7 ± 1.9
Optimum	45.9 ± 2.7	17.6 ± 2.2	17.2 ± 1.1	26.9 ± 3.1
10 x	45.9 ± 5.1	15.8 ± 1.6	15.4 ± 0.6	18.6 ± 1.6
100 x	46.2 ± 3.2	15.8 ± 0.6	13.3 ± 0.6	18.6 ± 1.6
Average	45.3 ± 1.8	16.8 ± 0.7	15.9 ± 0.3	22.0 ± 0.6
r	0.497	-0.646	-0.904	-0.612
LSD*	a – n.s.; b – 1.8; c – 1.8; d – n.s.; a · b – 3.6; a · c – 3.6; a · d – n.s.; b · c – 2.5; b · d – 2.5; c · d – 2.5; a · b · c – n.s.; a · b · d – n.s.; a · c · d – n.s.; b · c · d – 3.6; a · b · c · d – 7.2			

* explanations under Table 1

they do not produce any significant influence on the counts of microorganisms or the activity of soil enzymes (Wyszkowska 2002a, Wyszkowska 2004). However, when misused, herbicides can disturb the biological balance of soil, with the disorders being ever more severe as herbicides are resistant to microbial decompositions (Berger 1998, Johnsen et al. 2001, Sřrensen et al. 2003, Wyszkowska 2002b, Wyszkowska, Kucharski 2004). This observation has not been fully verified in our study on Apyros 75 WG, which, when applied according to the manufacturer's recommendations (8.9 μm kg^{-1}), depressed significantly counts of 6 out of 11 analysed groups of microorganisms. This means that the herbicide should be applied with great caution. Araújo et al. (2003) proved that soil pollution with glyphosate increased populations of fungi and *Actinomyces* while depressing counts of bacteria. In the authors' own study it has been discovered that Apyros 75 WG had a stimulating effect only on fungi and only in the objects treated with the optimum dose of the herbicide. In contrast, Apyros 75 WG had a negative effect on *Actinomyces* in all variants of the experiment.

The results of our study prove that the biological activity of soil is conditioned not only by microbial counts but also by the activity of soil enzymes. The herbicide modified the activity of all the analysed soil enzymes (Table 4). The influence of this biocide on the soil's enzymatic activity depended on all the variable factors tested in the experiment. In general, Apyros 74 WG had an inactivating effect on dehydrogenases, urease, acid and alkaline phosphatases. The activity of dehydrogenases, urease, acid and alkaline phosphatases, both in soil analysed before sowing spring wheat and after the harvest, was significantly negatively correlated with the concentration of the herbicide, which is confirmed by Pearson's simple regression coefficients between doses of Apyros 74 WG and the enzymatic activity of soil. Dehdyrogenases and urease proved to be the least tolerant to the herbicide. The highest dose of Apyros 74 WG (100-fold higher than recommended by the manufacturer) depressed the activity of dehydrogenases in soil

Table 4

Activity of enzymes (in 1 kg d.m. of soil)

Herbicide dose Apyros 75 WP	Before spring wheat sowing	Day of analysis		
		after harvest		
		soil use		
		sown		unsown
		g	l	
Dehydrogenases (cm^3 H_2 · d^{-1})				
0	5.34 ± 0.05	14.24 ± 0.19	14.24 ± 0.19	1.35 ± 0.10
Optimum	5.24 ± 0.05	10.87 ± 0.10	8.37 ± 0.10	1.01 ± 0.05
10 x	5.24 ± 0.05	5.29 ± 0.10	2.65 ± 0.05	0.53 ± 0.05
100 x	4.66 ± 0.05	1.88 ± 0.05	2.16 ± 0.05	0.43 ± 0.05
Average	5.12 ± 0.05	8.07 ± 0.12	6.85 ± 0.07	0.83 ± 0.02
r	5.34	14.24	14.24	0.43
LSD*	a – 0.04; b – 0.03; c – 0.03; d – 0.03; a · b – 0.05; a · c – 0.05; a · d – 0.05; b · c – 0.04; b · d – 0.04; c · d – 0.04; a · b · c – 0.07; a · b · d – 0.07; a · c · d – 0.07; b · c · d – 0.05; a · b · c · d – 0.11			
Urease (mg N-NH_4 · h^{-1})				
0	11.52 ± 0.48	10.08 ± 0.48	10.08 ± 0.48	2.16 ± 0.24
Optimum	9.60 ± 0.48	8.40 ± 0.24	9.36 ± 0.24	2.64 ± 0.24
10 x	11.28 ± 0.24	7.44 ± 0.24	7.44 ± 0.24	1.68 ± 0.24
100 x	10.80 ± 0.24	2.16 ± 0.24	7.44 ± 0.24	0.72 ± 0.24
Average	10.80 ± 0.17	7.02 ± 0.25	8.58 ± 0.25	1.80 ± 0.10
r	0.029	-0.968	-0.637	-0.911
LSD*	a – 0.20; b – 0.14; c – 0.14; d – 0.14; a · b – 0.28; a · c – 0.28; a · d – 0.28; b · c – 0.20; b · d – 0.20; c · d – 0.20; a · b · c – 0.39; a · b · d – 0.39; a · c · d – 0.39; b · c · d – 0.28; a · b · c · d – 0.56			
Acid phosphatase (mmol PNP · h^{-1})				
0	1.85 ± 0.07	3.28 ± 0.07	3.28 ± 0.07	2.02 ± 0.03
Optimum	1.95 ± 0.03	3.06 ± 0.02	3.25 ± 0.09	2.14 ±0.02
10 x	1.93 ± 0.02	2.62 ± 0.02	3.08 ± 0.07	2.09 ± 0.03
100 x	1.91 ± 0.01	2.24 ± 0.02	2.92 ± 0.02	1.90 ± 0.02
Average	1.91 ± 0.04	2.80 ± 0.03	3.13 ± 0.07	2.03 ± 0.02
r	0.104	-0.853	-0.888	-0.869
LSD*	a – 0.02; b – 0.01; c – 0.01; d – 0.01; a · b – 0.03; a · c – 0.03; a · d – 0.03; b · c – 0.02; b · d – 0.02; c · d – 0.02; a b · c – 0.04; a · b · d – 0.04; a · c · d – 0.04; b · c · d – 0.03; a · b · c · d – 0.06			

Alkaline phosphatase (mmol PNP· h^{-1})				
0	0.66 ± 0.01	0.59 ± 0.01	0.59 ± 0.01	0.31 ± 0.02
Optimum	0.63 ± 0.02	0.66 ± 0.01	0.57 ± 0.03	0.28 ± 0.01
10 x	0.62 ± 0.03	0.52 ± 0.01	0.52 ± 0.01	0.25 ± 0.01
100 x	0.58 ± 0.03	0.43 ± 0.02	0.49 ± 0.02	0.21 ± 0.02
Average	0.62 ± 0.01	0.55 ± 0.01	0.54 ± 0.01	0.26 ± 0.02
r	-0.919	-0.863	-0.840	-0.863
LSD*	a – 0.01; b – 0.01; c – 0.01; d – 0.01; a · b – 0.02; a · c – 0.02; a · d – 0.02; b · c – 0.01; b · d – 0.01; c · d – 0.01; a · b · c – 0.03; a · b · d – 0.03; a · c · d – 0.03; b · c · d – 0.02; a · b · c · d – 0.04			

* explanations under Table 1

prior to wheat sowing by 12.6% and after the harvest by up to 86.8% in the variant where the herbicide was applied to soil (mixed with the whole mass of soil in a pot) and by 84.8% in the series where it was applied top dressing during the tillering phase. The herbicide produced the strongest effect on the activity of urease in soil under spring wheat (78.6% decrease) and unsown soil (66.7% decrease) when it was mixed with a whole portion of soil to fill up one pot.

Apyros 75 WG also had an adverse effect on acid and alkaline phosphatases. Its negative influence on these enzymes was particularly evident after the harvest in the objects where it was applied to soil. There, the activity of acid phosphatase under the effect of the highest herbicide rate was 31.8% lower. An analogous decrease in the activity of alkaline phosphatase was 27.5%.

More severe disturbance of the biochemical balance in soil occurred in the objects in which Apyros 75 WG was applied by mixing with the whole mass of soil to fill up one pot than in the variants where top dressing application was performed. In the former case, the optimum dose of the herbicide decreased the activity of dehydrogenases by 23.6%, urease by 16.7%, alkaline phosphatase by 11.6% and acid phosphatase by 6.8%. In the latter series, the analogous percentages were 41.2, 7.1, 2.9 and 1.0.

With respect to the next variable, i.e. the time period over which the herbicide was present in soil, it has been found that the average activity of dehydrogenases and urease was higher on day 14 of the experiment (before sowing spring wheat) whereas the activity of acid phosphatase and alkaline phosphatase was higher on day 73 of the tests (in the series without wheat). The largest differences occurred for dehydrogenases and urease, whose activity before wheat sowing was, respectively, 6.2- and 6.0-fold higher after the harvest. Increased activity of these enzymes can be attributed to a close relationship between the number of live bacterial cells in soil and the rate

of biological decomposition of sulfosulfrone. This conclusion is drawn on the basis of the counts of microorganisms, where up to 80% of the analysed groups of microorganisms were more numerous on day 14 of the experiment.

Negative influence on excessive doses of herbicides on the activity of soil enzymes find confirmation in several reports (MICHALCEWICZ 2004, WYSZKOWSKA 2002a, WYSZKOWSKA, KUCHARSKI 2004, SUKUL 2006, YAO et al. 2006). Significant depression in counts of soil microorganisms and in the soil's enzymatic activity which occurred under the effect of excessively high rates of Apyros 75 WG could have been caused by the fact that herbicide decomposition products can be more toxic than the initial compound (ACCINELEI et al. 2005, WALKER et al. 2001). Such products permeate through plant tissues and destroy cellular structures, causing disorders in metabolism of cells (JOHNSEN et al. 2001, SŘRENSEN et al. 2003). Negative effects produced by Apyros 75 WG on the soil's biological life could also be associated with an indirect influence it exerted on soil organisms by modifying soil's physicochemical properties (Table 5), especially by increasing the soil's acidity.

Table 5

Some properties of soil contaminated with herbicide Apyros 75 WG

Herbicide dose Apyros 75 WP	($g \cdot C\ kg^{-1}$ of soil)	pH (1 mol KCl dm^{-3})	Hh	S	T	V (%)
			(mmol(+) kg^{-1} of soil)			
0	5.2 ± 0.1	5.8 ± 0.1	11.8 ± 0.3	54.7 ± 0.7	66.4 ± 0.5	82.3 ± 0.3
Optimum	5.3 ± 0.1	5.7 ± 0.1	12.0 ± 0.3	54.8 ± 0.8	66.8 ± 1.0	82.1 ± 0.3
10 x	5.1 ± 0.1	5.6 ± 0.1	12.7 ± 0.3	54.7 ± 1.3	67.4 ± 1.4	81.2 ± 0.5
100 x	5.2 ± 0.1	5.5 ± 0.1	12.5 ± 0.3	54.1 ± 1.6	66.6 ± 1.7	81.2 ± 0.5
Average	-0.380	-0.995	0.792	-0.892	0.123	-0.880
LSD*	n.s.	0.02	0.3	1.2	1.5	0.4

C – organic carbon content, Hh – hydrolytic acidity, S – total base exchangeable cations, T – total sorptive capacity, V – saturation with base cations, n.s. – non-significant

Furthermore, the unfavourable effect of excessive amounts of pesticides is not restricted to changes in counts of microorganisms or activity of enzymes. Plant protection preparations can also lead to disorders in the growth and development of crops (WYSZKOWSKA, KUCHARSKI 2004, MARTINS et al. 2007). The present study shows that plants can be a good indicator of changes occurring in microbiological and biochemical properties of soil as the test spring wheat proved to be susceptible to high concentrations of Apyros 75 WG (Figure 1). The extent of its toxic impact depended on its concentration in soil and method of application. Although producers of pesticides and herbicides claim that Apyros 75 WG is a selective herbicide producing a system-

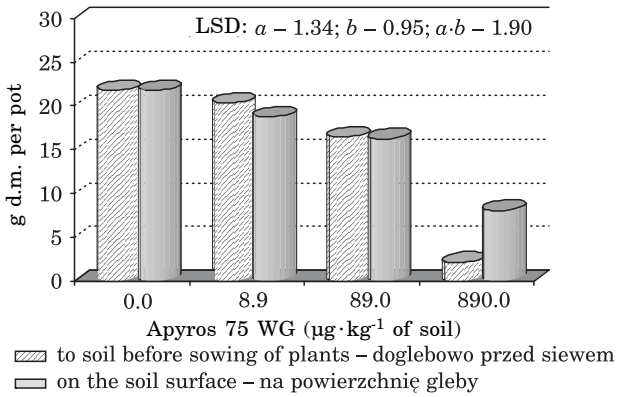

Fig. 1. Spring wheat yield (g d.m. per pot)

ic effect, taken up by leaves and roots of plants and easily transported within whole plants, and that crops are able to easily decompose this herbicide, our tests showed that only optimum doses of Apyros 74 WG, recommended by the manufacturer, did not produce a significant negative effect on the growth and development of wheat plants. Higher rates of this herbicide had a toxic effect on plants, regardless the method of application. Toxic symptoms, such as the browning of leaf blades, chlorosis of new leaves, lost of turgor and, in extreme cases, necrosis of plants, were clearly observable in the objects treated with the highest rate of the herbicide (100-fold above the recommended dosage). When such high doses of Apyros were mixed with soil, necrosis of wheat seedlings occurred in some cases. Phytotoxicity of herbicides has also been indicated by other researchers (URBAN 2000, MARTINS et al. 2007, SUKUL 2006).

CONCLUSIONS

1. Apyros 75 WG disturbs soil's homeostasis as it as it disrupts multiplication of some microbial groups, inhibits the activity of soil enzymes and depresses the yield of spring wheat, even if applied in a recommended dose.

2. Among the soil enzymes, dehydrogenases and urease were the least tolerant to the effect of the herbicide, whereas alkaline phosphatase proved to be the most tolerant one.

3. The tolerance of the microorganisms to the effect produced by the herbicide Apyros 75 WG was as follows: ammonifying bacteria > *Pseudomonas* > copiotrophic bacteria > oligotrophic bacteria > nitrogen immobilising bacteria > spore-forming oligotrophic bacteria > *Arthobacter* > cellulolytic bacteria > *Actinomyces* > fungi.

4. Counts of microorganisms and activity of enzymes are beneficially affected by growing spring wheat on the herbicide polluted soil.

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