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Original Research

Response of Fungi, ß-Glucosidase, and Arylsulfatase to Soil Contamination by Alister Grande 190 OD, Fuego 500 SC, and Lumax 537.5 SE Herbicides

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Abstract

This paper describes the response of fungi as well as β-glucosidase and arylsulfatase to soil contamination with the herbicides Alister Grande 190 OD, Fuego 500 SC, and Lumax 537.5 SE in the following doses: 1 – recommended dose, and doses 20-, 40-, 80-, and 160-fold higher than the recommended one. A laboratory experiment in three replicates was conducted on sandy loam of the pH_{KCl} – 7.0. The results have indicated that counts of fungi increased under the influence of excessive quantities of the tested herbicides. Irrespective of herbicide type, EP decreased while CD increased at higher contamination doses.

With respect to the enzymes, the herbicides were observed to have exerted a negative effect on the activity of arylsulfatase, which was verified by the negative correlation coefficients. However, the activity of β-glucosidase increased after the soil had been enriched with excessive amounts of the herbicides. The RS index for the activity of the enzymes varied, but reached the highest value in soil with Alister Grande 190 OD for both β-glucosidase (0.953) and arylsulfatase (0.567). The contamination of soil with the herbicides caused lasting changes in sandy loam, but the recovery of the enzyme β-glucosidase was faster (the average RL ranged from 0.458 to 0.889). The index of resilience for arylsulfatase was negative, which proves that the adverse effect of all the herbicides on this enzyme was growing.

Keywords: herbicides, soil, counts of fungi, activity of enzymes

Introduction

Changes occurring in a soil environment can be caused by biological factors or induced by human activity. Accumulation of various pollutants in soil often leads to deterioration of soil properties, especially its enzymatic activity. Among the chemical substances that affect soil life are herbicides [1]. Being highly biologically active, herbicides may distort the equilibrium between counts and species of soil microorganisms. The dose of degradation of herbicides in soil depends on both the structure and properties of a given chemical and the ambient characteristics. The most important biological factor that affects the mineralization dose of herbicides is the activity of soil microorganisms [2]. The microbiological decomposition of herbicides in soil is one of the mechanisms that prevents excessive accumulation of these substances in the environment. However, if their decomposition in soil occurs too

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rapidly, the efficacy of herbicides for controlling weeds may suffer [3]. Some herbicides that enter soil can undergo decomposition in just a few days following their application. This process is carried out by microorganisms that secrete enzymes depending on which microbes can use products of their metabolism as a source of nutrients. Microorganisms most rapidly decompose aliphatic and hydroxyl compounds, whereas aromatic compounds undergo transformation at a much slower dose. The last to be decomposed are the substances whose rings contain sulfur, oxygen, and nitrogen [4]. The following microorganisms are capable of degrading herbicides: *Arthrobacter*, *Pseudomonas*, *Bacillus*, *Mycoplana*, *Agrobacterium*, *Corynebacterium*, *Flavobacterium*, *Nocardia*, and *Trichoderma* [2]. Biological assays, such as counts of microorganisms in soil and analyses of soil enzymatic activity, are used to assess the degree of soil contamination with herbicides [5].

The objective of the present study has been to determine the effect of changes occurring in soil under the influence of higher doses of the herbicides Alister Grande 190 OD, Fuego 500 SC, and Lumax 537.5 SE.

Material and Methods

In a laboratory experiment, the effect of the herbicides Alister Grande 190 OD, Fuego 500 SC, and Lumax 537.5 SE on soil biological properties was assessed.

Alister Grande 190 OD is a herbicide marketed in the form of oily suspension to be diluted with water. It is used to control mono- and dicotyledonous weeds on rye, wheat, and triticale fields. This herbicide contains three active substances: diflufenican (180 $g \cdot dm^{-3}$), mesosulfuron methyl (6 $g \cdot dm^{-3}$), and iodosulfuron methyl sodium (4.5 $g \cdot dm^{-3}$). The herbicide became marketable in 2008 and the recommended dose is 0.8-1.0 $dm^3 \cdot ha^{-1}$ [6]. Diflufenican (2',4-difluoro-2-(trifluoromethyl-phenoxy)-nicotinanilid) is a selective herbicide that belongs to phenoxynicotinanilids. It is absorbed by shoots of emerging seedlings [7]. Mesosulfuron methyl (methyl 2-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl] amino]sulfonyl]-4-[[(methylsulfonyl)amino]methyl] benzoate) and iodosulfuron methyl sodium (a monosodium salt of 4-iodo-2-[3-methoxy-6-methyl-1,3,5-triazin-2-yl-)-ureidosulfonyl]benzoate methyl ester) belong to sulfonylurea derivatives. These herbicides are broadly applied to control weeds in cereals. They act as inhibitors of acetylolactate synthase amino acids [8].

Fuego 500 SC, in the form of concentrated suspension to be diluted with water, is applied to soil or sprayed over leaves to control weeds on winter oilseed rape fields. It contains the active substance metazachlor in the amount of 500 $g \cdot dm^{-3}$. The manufacturer's recommended dose is 2 $dm^3 \cdot ha^{-1}$. The herbicide entered the market in 2008 [9]. Metazachlor, an active substance found in many herbicides, is absorbed by roots and the hypocotyl of plants, acting as an inhibitor of plant enzymes. This herbicide influences cell division in plant seedlings by inhibiting the biosynthesis of proteins, fatty acids, and lignin [10].

Lumax 537.5 SE is a preparation used to control weeds in maize. It contains three active substances: terbuthylazine (187.5 $dm^3 \cdot ha^{-1}$), mesotrion (37.5 $dm^3 \cdot ha^{-1}$), and s-metolachlor (312.5 $dm^3 \cdot ha^{-1}$). This product became available on the market in 2008 and is applied in recommended doses of 3.5 to 4.0 $dm^3 \cdot ha^{-1}$ [11]. Mesotrion (2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione) belongs to triketones and is used to eradicate mono- and dicotyledonous weeds. It is taken up mainly by leaves but also through roots of plants. It inhibits the activity of the enzyme P-hydroxyfenolpyruvate, which leads to the inhibition of biosynthesis of carotenoids and chlorophyll. As a result, leaves begin to bleach [12]. Metolachlor ((chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) is a substance that belongs to chloroacetamides and is used to control monocotyledons, being absorbed by their germs and the hypocotyl. It acts as a growth inhibitor as it halts the synthesis of chlorophyll, proteins, and lipids. In soil, this substance undergoes biological and chemical degradation [13]. Terbuthylazine (6-chloro-N-(1,1-dimethyl-ethyl)-N′-ethyl-1,3,5- triazine-2,4-diamine) is a selective herbicide that belongs to trazines and acts as an inhibitor of photosynthesis. It is transferred mainly through roots of weeds but also through their leaves, which means that it is applicable during both the pre- and post-emergence period. This substance is degraded by microorganisms. The major metabolite of terbuthylazine is 2-hydroxy-desethyl-terbuthylazine, which is produced through dealkylation and hydroxylation [14].

The experiment was conducted on sandy loam of the pH_{KCl} – 7.0, hydrolytic acidity 8.00 $mmol(+) \cdot kg^{-1}$, exchangeable base capacity 111.00 $mmol(+) \cdot kg^{-1}$, and organic carbon content 7.05 $g \cdot kg^{-1}$.

Prior to the laboratory experiment, the soil was passed through a 2 mm mesh size sieve. 100 g of soil, previously mixed with the herbicides as required, was placed in 150 cm^3 glass beakers each. The herbicides were added to soil in the following doses: 1 – recommended dose, and doses 20-, 40-, 80-, and 160-fold higher. The control treatment consisted of soil without any herbicide. Next, the soil samples were adjusted to moisture content equal to 50% of capillary water-holding capacity by adding distilled water. The material was then incubated in a thermostat for 160 days at 25ºC. On days 20, 40, 80 and 160, counts of fungi were determined after 5 days of incubating the cultures on Martin's medium [15] in serial dilutions; on the same days, the activity of β-glucosidase (EC 3.21.21) and arylsulfatase (EC 3.1.6.1) was determined colorimetrically in a spectrophotometer according to the method described by Alef and Nannipeieri [16]. The activity of β-glucosidase was measured at the wavelength $\lambda = 400$ nm, whereas the wavelength used for determination of the activity of arylsulfatase was 420 nm. When the experiment was started, the soil without herbicides contained $1.603 \cdot 10^7$ $cfu \cdot kg^{-1}$ d.m. of soil; the activity of β-glucosidase in this soil was 0.322 mmol $PNP \cdot kg^{-1}$ $d.m. \cdot h^{-1}$ and that of arylsulfatase was 0.246 mmol $PNP \cdot kg^{-1}$ $d.m. \cdot h^{-1}$ of soil. On days 20 and 80, the colony development (CD) and eco-physiological diversity (EP) indices were determined. The value of the CD index was calculated from the formula by Sarathchandra et al. [17], whereas the EP index was achieved from the formula suggested by De Leij et al. [18]. Determination of the structure of microbial communities was based on the dose of development of cultures on plates with agar medium, counted every 10 days. On set days, microbiological and enzymatic assays were performed, from which average values of counts of fungi and activities of β-glucosidase and arylsulfatase as well as indices of colony development and ecophysiologiocal diversity of fungi were obtained. Additionally, on day 160, the indices of resistance (RS) and resilience (RL) for β-glucosidase and arylsulfatase in soil contaminated with the herbicides were calculated according to the method described by Orwin and Wardle [19]. The results of all the above assays and calculations were subjected to statistical processing with multi-range Duncan's test available in a Statistica software package [20].

Results and Discussion

The research results demonstrated than the herbicides affected the biological activity of soil. The actual effect depended mainly on the type of herbicide and its dose (Tables 1-9).

Soil contamination with the herbicides had a positive effect on multiplication of fungi, the relationship being verified by the positive correlation coefficients (Table 1). The highest count of fungi was observed in soil contaminated with Lumax 537.5 SE ($3.849 \cdot 10^7$ cfu·kg^{-1} d.m. of soil); in turn, the lowest count was detected in soil treated with Fuego 500 SC ($3.024 \cdot 10^7$ cfu·kg^{-1} d.m. of soil). Application of elevated doses of the herbicides led to an increase in counts of fungi, but their highest growth was observed when the dose of a herbicide added to soil was 20-fold higher than recommended by the manufacturer. Alister Grande 190 OD added to soil in such an amount raised the count of fungi by 1.5-fold versus the control, Fuego 500 SC 1.4-fold, and Lumax 537.5 SE 1.7-fold.

It was found that the herbicides significantly affected the diversity of the analyzed microbial communities (Table 2). The highest EP of fungi, on average 0.637, was determined in soil with Lumax 537.5 SE, while the lowest one (0.622) in soil treated with Alister Grande 190 OD. It also was observed that the value of EP decreased after excessive quantities of a herbicide, irrespective of its type, had been introduced to soil. The highest herbicide dose (160-fold higher than the recommended one) depressed the value of EP relative to the control by 30.59% (Alister Grande 190 OD), 31.32% (Fuego 500 SC), and 23.22% (Lumax 537.5 SE).

The present experiment demonstrated varied influence of the herbicides on the value of the colony development index (Table 3). The value of the CD index for fungi in soil contaminated with the herbicide Alister Grande 190 OD was on average 32.564, with Fuego 500 SC – 32.149, and with Lumax 537.5 SE – 32.016. The application of Alister Grande 190 OD in a dose 80-fold higher than recommended increased the value of the CD index by 38.29%. In respect of the other two herbicides, Fuego 500 SC and Lumax 537.5 SE, fungi grew most vigorously when these preparations had been added to soil in doses 20-fold higher than recommended. The value of CD for these herbicides was 35.29% and 37.67% higher than the control, respectively.

The herbicides introduced to soil also modified the activity of β-glucosidase (Table 4). In general, these preparations when applied to soil in doses 160-fold higher than the recommended ones stimulated the activity of β-glucosidase. The herbicides Alister Grande 190 OD and Lumax 537.5 SE added in such quantities raised the activity of this enzyme by 1.2-fold compared to the control. However, the highest activity of β-glucosidase was detected in soil with the herbicides Alister Grande 190 OD and Lumax 537.5 SE

Table 1. Counts of fungi in soil contaminated with herbicides, 10^7 cfu·kg^{-1} d.m. of soil.

Herbicide dose*	Alister Grande 190 OD	Fuego 500 SC	Lumax 537.5 SE
0	2.458	2.458	2.458
1	3.485	3.116	4.193
20	3.799	3.513	4.242
40	3.681	3.103	4.129
80	3.496	2.972	4.169
160	3.277	2.984	3.904
average	3.370	3.024	3.849
r	0.119	0.022	0.253
$LSD_{0.01}$**	a – 0.166 b – 0.234 ab – 0.405		

*0 – control, not contaminated with herbicides, 1 – a dose recommended by the manufacturer, doses higher than the recommended one by 20-, 40-, 80-, and 160-fold

** $LSD_{00.1}$ for: a – type of herbicide, b – dose of herbicide, r – correlation coefficient

Table 2. Eco-physiological diversity (EP) index for fungi in soil contaminated with herbicides.

Herbicide dose*	Alister Grande 190 OD	Fuego 500 SC	Lumax 537.5 SE
0	0.827	0.827	0.827
1	0.623	0.627	0.600
20	0.582	0.570	0.599
40	0.563	0.590	0.585
80	0.563	0.584	0.578
160	0.574	0.568	0.635
average	0.622	0.628	0.637
r	-0.492	-0.511	-0.272
$LSD_{0.01}$**	a – 0.021 b – 0.029 ab – 0.051		

*explanation see Table 1.

Table 3. Colony development (CD) index for fungi in soil contaminated with herbicides.

Herbicide dose*	Alister Grande 190 OD	Fuego 500 SC	Lumax 537.5 SE
0	25.411	25.411	25.411
1	33.156	32.636	34.069
20	33.318	34.380	34.983
40	34.087	34.030	33.611
80	35.140	34.377	33.041
160	34.271	32.059	30.979
average	32.564	32.149	32.016
r	0.506	0.269	0.038
$LSD_{0.01}$**	a – 0.679 b – 0.960 ab – 0.960		

*explanation see Table 1.

Table 4. Activity of β-glucosidase in soil contaminated with herbicides, mmol PNP·kg^{-1} d.m.·h^{-1}.

Herbicide dose*	Alister Grande 190 OD	Fuego 500 SC	Lumax 537.5 SE
0	0.470	0.470	0.470
1	0.556	0.505	0.534
20	0.581	0.496	0.578
40	0.569	0.475	0.568
80	0.569	0.470	0.558
160	0.564	0.459	0.566
average	0.552	0.479	0.546
r	0.375	-0.703	0.470
$LSD_{0.01}$**	a – 0.005 b – 0.008 ab – 0.014		

*explanation see Table 1.

applied in doses 20-fold higher than the recommended ones. There, the activity of this soil enzyme was 0.581 and 0.578 mmol PNP·kg^{-1} d.m.·h^{-1}, respectively. As for the herbicide Fuego 500 SC, the activity of β-glucosidase was the highest (0.505 mmol PNP·kg^{-1} d.m.·h^{-1}) when the recommended dose of this preparation had been introduced to soil.

Soil contamination with the herbicides inhibited the activity of arylsulfatase, the effect that was confirmed by the negative coefficients of the correlation between doses of the herbicides and activity of arylsulfatase (Table 5). The highest activity of this enzyme was detected in soil with Alister Grande 190 OD (0.183 mmol PNP·kg^{-1} d.m.·h^{-1}), and the lowest – in soil contaminated with Fuego 500 SC (0.157 mmol PNP·kg^{-1} d.m.·h^{-1}). Irrespective of the type of herbicide, the activity of arylsulfatase decreased as the herbicide doses increased. The strongest inhibitory effect was produced by a dose 160-fold higher than the recommended one, as it lowered the activity of this enzyme by 25.24% in soil with Alister Grande 190 OD, 41.90% in soil with Fuego 500 SC and 36.67% in soil with Lumax 537.5 SE. Of the three tested herbicides, the strongest negative influence on the activity of arylsulfatase was produced by Fuego 500 SC.

It has been demonstrated that the resistance of β-glucosidase in herbicide-contaminated soil depended on the dose and type of herbicide (Table 6). The average RS value in soil with Alister Grande 190 OD was 0.897, with Fuego 500 SC – 0.790 and with Lumax 537.5 SE – 0.900. The most severe negative changes were observed in soil with Fuego 500 SC, where the value of the RS index fell by 27.17% after the dose 160-fold higher than recommended of the herbicide had been introduced. An analogously high dose of Lumax 537.5 SE depressed the resistance index by 13.55%. Slightly different values were obtained in the soil treated with Alister Grande 190 OD. When a dose 160-fold higher than recommended had been added, the resistance index rose from 0.802 to 0.953.

In our experiment, the resistance of arylsulfatase in soil with the herbicides was varied (Table 7). The average values of RS for arylsulfatase in soil polluted with Alister

Table 5. Activity of arylsulfatase in soil contaminated with herbicides, PNP·kg^{-1} d.m.·h^{-1}.

Herbicide dose*	Alister Grande 190 OD	Fuego 500 SC	Lumax 537.5 SE
0	0.210	0.210	0.210
1	0.215	0.175	0.198
20	0.186	0.161	0.194
40	0.174	0.143	0.172
80	0.153	0.132	0.142
160	0.157	0.122	0.133
average	0.183	0.157	0.175
r	-0.832	-0.817	-0.931
$LSD_{0.01}$**	a – 0.003 b – 0.004 ab – 0.008		

*explanation see Table 1.

Table 6. Resistance (RS) index for β-glucosidase in soil contaminated with herbicides.

Herbicide dose*	Alister Grande 190 OD	Fuego 500 SC	Lumax 537.5 SE
1	0.802	0.898	0.930
20	0.808	0.894	0.935
40	0.975	0.746	0.914
80	0.947	0.760	0.915
160	0.953	0.654	0.804
average	0.897	0.790	0.900
r	-0.597	0.826	0.794

*explanation see Table 1.

Table 7. Resistance (RS) index for arylsulfatase in soil contaminated with herbicides.

Herbicide dose*	Alister Grande 190 OD	Fuego 500 SC	Lumax 537.5 SE
1	0.863	0.755	0.677
20	0.828	0.559	0.502
40	0.689	0.544	0.239
80	0.232	0.323	0.217
160	0.223	0.390	0.152
average	0.567	0.514	0.357
r	0.904	0.705	0.507

*explanation see Table 1.

Table 8. Resilience (RL) index for β-glucosidase in soil contaminated with herbicides.

Herbicide dose*	Alister Grande 190 OD	Fuego 500 SC	Lumax 537.5 SE
1	0.786	0.766	0.916
20	0.812	0.669	0.925
40	0.967	0.358	0.900
80	0.936	0.268	0.897
160	0.945	0.227	0.795
average	0.889	0.458	0.887
r	-0.595	0.770	0.817

*explanation see Table 1.

Table 9. Resilience (RL) index for arylsulfatase in soil contaminated with herbicides.

Herbicide dose*	Alister Grande 190 OD	Fuego 500 SC	Lumax 537.5 SE
1	-0.248	-0.592	-0.459
20	-0.169	-0.526	-0.444
40	-0.209	-0.561	-0.620
80	-0.558	-0.494	-0.626
160	-0.595	-0.352	-0.555
average	-0.356	-0.505	-0.541
r	-0.241	0.519	-0.568

*explanation see Table 1.

Grande 190 OD and Fuego 500 SC were on a similar level, reaching 0.567 and 0.514, respectively. In contrast, the treatments that received the preparation Lumax 537.5 SE were found to have the lowest value of the RS index (0.357). This value was 1.6-fold lower than in the soil with Alister Grande 190 OD and 1.4-fold lower than in the treatments with Fuego 500 SC.

In this experiment, the herbicides caused changes in the analyzed soil (Table 8). Irrespective of the type of herbicide, the value of the RS index for β-glucosidase was positive in all the treatments. The average values of the RS index in soil contaminated with the particular herbicides were as follows: 0.889 for Alister Grande 190 OD, 0.458 for Fuego 500 SC, and 0.887 for Lumax 537.5 SE. β-glucosidase proved to be most sensitive to the herbicide Fuego 500 SC, which, when applied in a dose 160-fold higher than the recommended one, depressed the RS value by 70.36%. In turn, the herbicide Alister Grande 190 OD added in the same dose raised the value of RS from 0.786 to 0.945.

The herbicides contributed to some changes in sandy loam soil, which was evidenced by all values of resilience for arylsulfatase being negative (Table 9). The highest average value of the RL index was observed in soil with Alister Grande 190 OD (-0.356), lower in soil with Fuego 500 SC (-0.505), and the lowest in soil with Lumax 537.5 SE (-0.541). Among the three tested herbicides, the most severe adverse changes were caused by Lumax 537.5 SE.

By participating in the cycling of elements, soil microorganisms are among the most essential biological factors that shape soil properties, thus affecting soil fertility. The development and activity of soil microorganisms can be measured with an aid of appropriate indices, including microbial counts [3]. Chemical pollutants that permeate soil generate strong effects on soil parameters. Herbicides that have an impact on the development of microorganisms, thus influencing the dynamics of biochemical processes run by microbes, are among such chemical contaminants [21, 22].

The authors' own studies have shown that soil contamination with herbicides led to changes in counts of fungi. The preparations applied to soil in excessive quantities caused an increase in the counts of these microorganisms. Kucharski and Wyszkowska [23], who ran trials on the preparation Apyros 75 WG, noticed its inhibitory effect on the growth of fungi. However, out of all examined microorganisms (ammonifying bacteria, nitrogen-fixing bacteria, *Pseudomonas*, *Arthrobacter*, copiotrophic bacteria, oligotrophic bacteria and their spores, cellulolytic bacteria, actinomycetes, and fungi) fungi proved to be the most resistant to excessive amounts of the herbicide Apyros 75 WG, hence a dose 100-fold higher than recommended of this preparation depressed the count of fungi by just 5%. Singh and Ghoshal [1] claim that herbicides have some effect on the content of organic matter in soil and therefore affect microorganisms that dwell in it. Following the application of herbicides, the biomass of microorganisms may increase, decrease or remain unaltered. Zabaloy et al. [24] demonstrated that 2,4-dichlorophenoxiacetate introduced to soil in doses from 1 to 10 $mg \cdot kg^{-1}$ raised the activity of soil microorganisms, including fungi. These authors suggest that microorganisms could have used the herbicide as a source of nutrients, which therefore had a positive effect on their development. Similar results were obtained by Crouzet et al. [25] in their studies on soil treated with mesotrion in doses 0.45, 4.5, and 45 $mg \cdot kg^{-1}$. They observed a stimulating effect of the highest tested doses of the herbicide on counts of fungi. Also, Araújo et al. [26] found a positive effect of glyphosate introduced to soil in the amount of 2.16 mg·kg^{-1}. However, Martinez et al. [27], who tested sulfentrazone added to soil in a dose of 0.7 µg·g^{-1}, failed to observe any significant changes in the growth of fungi.

Modifications in the structure of microbial communities caused under the influence of contaminants which enter a soil environment are frequently used in microbiological assays [18]. Determination of the indices of microbial diversity may provide us with information on the type of changes induced by the application of various chemical compounds. Ros et al. [28] observed an adverse effect of atrazine, which they applied in doses of 10, 100, and 1,000 mg·kg^{-1} d.m. of soil, on diversity of soil microorganisms. In the authors' own experiment, the diversity of fungi also depended on the doses of herbicides. The negative influence of plant protection chemicals on diversity of microorganisms and the colony development index also has been observed by Cycoń and Piotrowska-Seget [29].

Enzymatic activity is an appropriate parameter in assessment of modifications in a soil environment induced by various contaminants [30, 31]. Enzymes that belong to hydolases, e.g. β-glucosidase and arylsulfatase, are important in nature owing to their role in the cycling of elements [32]. Arylsulfatase is an enzyme that catalyzes the hydrolysis of organic sulfates [33]. Therefore, it plays an important role in the cycling of sulfur in nature. This enzyme is produced mainly by bacteria and fungi, but it can also be secreted by animals and plants [34]. β-glucosidase is an enzyme that catalyzes glycosidic bonds and participates in the cycling of carbon. This enzyme is essential for decomposition of organic matter and nutrients found in soil, especially for degradation of cellulose [35]. The response of these enzymes to herbicides that enter the soil environment may vary. Herbicides can either stimulate or inhibit enzymes, although a neutral response also is possible [36]. In the authors' own experiment, it was observed that the herbicides had a stimulating effect on β-glucosidase but inhibited the activity of arylsulfatase. While investigating the effect of metalaxyl on biochemical properties of soil, Sukul [32] found a negative effect of this preparation on the activity of arylsulfatase and β-glucosidase. The inhibitory influence of herbicides (cinosulfuron, prosulfuron, thifensulfuron methyl, triasulfuron) on the activity of these enzymes also has been demonstrated by Sofo et al. [37]. Likewise, Tejda [38], who tested glyphosate and diflufenican, noticed a negative effect of these preparations on soil enzymes (dehydrogenase, urease, phosphatase, β-glucosidase and arylsulfatase). Saha et al. [39], in their investigations on the effect of three herbicides (alachlor, butachlor, and pretichlor) on the activity of β-glucosidase, found that the latter increased in soil with the herbicides, same as in the present study. The cited researchers applied the herbicides in doses of 5, 25, and 50 mg·kg^{-1} d.m. of soil. Peruci et al. [40] reported somewhat different results. These authors tested the herbicide rimsulfuron in doses 10- and 100-fold higher than recommended. They observed the biggest decrease in the activity of β-glucosidase after a dose 100-fold higher than recommended had been introduced to soil.

The degree of contamination of soil with different chemical substances can be determined, for example, on the basis of values of resistance and resilience indices [19]. Be'caert et al. [41], who tested 2,4-D in a dose of 36 mg·kg^{-1}, found out that β-glucosidase and arylsulfatase were most sensitive to soil contamination with this herbicide. The experiment presented in this paper showed that β-glucosidase was more resistant to excessive amounts of herbicides than arylsulfatase.

Conclusions

1. Soil contamination with the herbicides Alister Grande 190 OD, Fuego 500 SC, and Lumax 537.5 SE raised counts of fungi in soil.
2. The herbicides caused a decrease in the value of the biological diversity index but increased the colony development index.
3. Application of highest doses of the herbicides to soil had an inhibitory effect on the activity of arylsulfatase, but stimulated the activity of β-glucosidase.
4. The highest resistance index for the enzymes in response to soil contamination with the herbicides was detected in soil samples treated with Alister Grande 190 OD. In respect of the resilience index, the lowest values were recorded in soil with the herbicide Fuego 500 SC.

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References

1. SINGH P., GHOSHAL N. Variation in total biological productivity and soil microbial biomass in rainfed agroecosystems: Impact of application of herbicide and soil amendments. Agric. Ecosyst. Environ. **137**, 241, **2010**.
2. ARBELI Z., FUENTES C. L. Accelerated biodegradation of pesticides: An overview of the phenomenon, its basis and possible solutions and a discussion on the tropical dimension. Crop Protec. **26**, 1733, **2007**.
3. DAS A. C., NAYEK H., CHAKRAVARTY A. Soil application of dinitroaniline and arylphenoxy propionic herbicides influences the activities of phosphate-solubilizing microorganisms in soil. Environ. Monit. Assess. **184**, 7453, **2012**.
4. MILOŠEVIĆ N. A., GOVEDARICA M. M. Effect of herbicides on microbiological properties of soil. Proc. Nat. Sci. **102**, 5, **2002**.
5. SHARMA P., SURI C. R. Biotransformation and biomonitoring of phenylurea herbicide diuron. Bioresour. Technol. **102**, 3119, **2011**.
6. http://www.bayercropscience.pl.
7. BENDING G. D., LINCOLN S. D., EDMONDSON R. N. Spatial variation in the degradation rate of the pesticides isoproturon, azoxystrobin and diflufenican in soil and its relationship with chemical and microbial properties. Environ. Pollut. **139**, 279, **2006**.

8. ADAMCZEWSKI K. Building up resistance of apera spica-venti by using for long-term sulfonyloureas herbicides. Fragm. Agron. **26**, (2), 7, **2009** [In Polish].
9. http://www.makhteshim-agan.pl
10. MAMY L., GABRIELLE B., BARRIUSO E. Measurement and modeling of glyphosate fate compared with that of herbicides replaced as a result of the introduction of glyphosate-resistant oilseed rape. Pest. Manag. Sci. **64**, 262, **2008**.
11. http://www.syngenta.com.pl
12. PILEGGI M., PILEGGI S. A. V., OLCHANHESKI L. R., DA SILVA P. A. G., MUNOZ GONZALEZ A. M., KOSKINEN W. C., BARBER B., SADOWSKY M. J. Isolation of mesotrione-degrading bacteria from aquatic environments in Brazil. Chemosphere, **86**, 1127, **2012**.
13. SINGH N., KLOEPPEL H., KLEIN W. Movement of metolachlor and terbuthylazine in core and packed soil columns. Chemosphere, **47**, 409, **2002**.
14. CABRERA A., COX L., VELARDE P., KOSKINEN W. C., CORNEJO J. Fate of diuron and terbuthylazine in soils amended with two-phase olive oil mill waste. J. Agric. Food Chem. **55**, 4828, **2007**.
15. MARTIN J. Use of acid rose bengal and streptomycin in the plate method for estimating soil fungi. Soil Sci. **69**, 215, **1950**.
16. ALEF K., NANNIPIERI P. Methods in Applied Soil Microbiology and Biochemistry. Alef K., Nannipieri P. (Eds.), Academic Press. Harcourt Brace & Company, Publishers, London: 350, **1998**.
17. SARATHCHANDRA S. U., BURCH G., COX N. R. Growth patterns of bacterial communites in the rhizoplane and rhizosphere of with clover (*Trifolium repens* L.) and perennial ryegrass (*Lolium perenne* L.) in long-term pasture. Appl. Soil Ecol. **6**, 293, **1997**.
18. DE LEIJ F. A. A. M., WHIPPS J. M., LYNCH J. M. The use of colony development for the characterization of bacterial communities in soil and on roots. Microb. Ecol. **27**, 81, **1993**.
19. ORWIN K.H., WARDLE D.A. New indices for quantifying the resistance and resilience of soil biota to exogenous disturbances. Soil Biol. Biochem. **36**, 1907, **2004**.
20. STATSOFT, INC, STATISTICA, 2010. Data Analysis Software System, version 9.1., <http://www.statsoft.com.>.
21. BAĆMAGA M., BOROS E., KUCHARSKI J., WYSZKOWSKA J. Enzymatic activity in soil contaminated with the Aurora 40 WG herbicide. Environ. Protec. Eng. **38**, (1), 91, **2012**.
22. CHOWDHURY A. PRADHAN S., SAHA M., SANYAL N. Impact of pesticides on soil microbiological parameters and possible bioremediation strategies. Indian J. Microbiol. **48**, 114, **2008**.
23. KUCHARSKI J., WYSZKOWSKA J. Biological properties of soil contaminated with the herbicide Apyros 75 WG. J. Elem. **13**, (3), 357, **2008**.
24. ZABALOY M. C., GARLAND J. L., GOMEZ M. A. Assessment of the impact of 2,4-dichlorophenoxyacetic acid (2,4-D) on indigenous herbicide-degrading bacteria and microbial community function in an agricultural soil. App. Soil Ecol. **46**, 240, **2010**.
25. CROUZET O., BATISSON I., BESSE-HOGGAN P., BONNEMOY F., BARDOT C., POLY F., BOHATIER J., MALLET C. Response of soil microbial communities to the herbicide mesotrione: A dose-effect microcosm approach. Soil Biol. Biochem. **42**, 193, **2010**.
26. ARAÚJO A. S. F., MONTEIRO R. T. R., ABARKELI R. B. Effect of glyphosate on the microbial activity of two Brazilian soil. Chemosphere, **52**, 799, **2003**.
27. MARTINEZ C. O., SILVA C. M. M. S., FAY E. F., MAIA A. H. N , ABAKERLI R. B., DURRANT L. R. Degradation of the herbicide sulfentrazone in a Brazilian typic hapludox soil. Soil Biol. Biochem. **40**, 879, **2008**.
28. ROS M., GOBERNA M., MORENO J. L., HERNANDEZ T., GARCI´A C., INSAM H., PASCUAL J. A. Molecular and physiological bacterial diversity of a semi-arid soil contaminated with different levels of formulated atrazine. Appl. Soil Ecol. **34**, 93, **2006**.
29. CYCOŃ M., PIOTROWSKA-SEGET Z. Changes in bacterial diversity and community structure following pesticides addition to soil estimated by cultivation technique. Ecotoxicol. **18**, (5), 632, **2009**.
30. WANG Q., ZHOU D., CANG L. Microbial and enzyme properties of apple orchard soil as affected by long-term application of copper fungicide. Soil Biol. Biochem. **41**, 1504, **2009**.
31. WYSZKOWSKA J. Effect of soil contamination with Treflan 480 EC on biochemical properties of soil. Pol. J. Environ. Stud. **11**, (1), 71, **2002**.
32. SUKUL P. Enzymatic activities and microbial biomass in soil as influenced by metaxyl residues. Soil Biol. Biochem. **38**, 320, **2006**.
33. KLOSE S., TABATABAI M. A. Arylsulfatase activity of microbial biomass in soils. Soil Sci. Soc. Am. J. **63**, 569, **1999**.
34. LI X., SARAH P. Arylsulfatase activity of soil microbial biomass along a mediterranean-arid transect. Soil Biol. Biochem. **35**, 925, **2003**.
35. ACOSTA-MARTI´NEZ V., ACOSTA-MERCADO D., SOTOMAYOR-RAMI´REZ D., CRUZ-RODRI´GUEZ L. Microbial communities and enzymatic activities under different management in semiarid soils. Appl. Soil Ecol. **38**, 249, **2008**.
36. UZIAK S., STEINBRICH K. Further research into enzymatic activity of cultivated soils treated with herbicides. Pol. J. Soil Sci. **38**, (2), 127, **2005**.
37. SOFO A., SCOPA A., DUMONTET S., MAZZATURA A., PASQUALE V. Toxic effects of four sulphonylureas herbicides on soil microbial biomass. J. Environ. Sci. Health, Part B. **47**, 653, **2012**.
38. TEJADA M. Evolution of soil biological properties after addition of glyphosate, diflufenican and glyphosate +diflufenican herbicides. Chemosphere, **76**, 365, **2009**.
39. SAHA S., DUTTA D., KARMAKAR R., RAY D. P. Structure-toxicity relationship of chloroacetanilide herbicides: Relative impact on soil microorganisms. Environ. Toxicol. Pharmacol. **34**, 307, **2012**.
40. PERUCCI P., VISCHETTI C., BATTISTONI F. Rimsulfuron in a silty clay loam soil: effects upon microbiological and biochemical properties under varying microcosm conditions. Soil Biol. Biochem. **31**, 195, **1999**.
41. BE´CAERT V., SAMSON R., DESCHE^NES L. Effect of 2,4-D contamination on soil functional stability evaluated using the relative soil stability index (RSSI). Chemosphere, **64**, 1713, **2006**.