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Soil response to chemicals used in a field experiment**

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A b s t r a c t. The effect of chemicals (Reglone 200 SL and Elastiq 550 EC) on soil microorganisms and their enzymatic activity was estimated. The study was conducted in a field experiment which was set up in the split-block design and comprised three treatments. Soil samples were taken six times, twice in each year of study. The results showed that the application of chemicals generally had no negative effect on the number of soil microorganisms. The application of Reglone 200 SL caused an increase of proteolytic and ureolytic activity and affected the activity of dehydrogenases, acid and alkaline phosphatases in the soil. The soil subjected of Elastiq 550 EC was characterized by lower activity of dehydrogenases, protease, urease and alkaline phosphatase.

K e y w o r d s: soil, microorganisms, enzyme activity, Reglone 200 SL, Elastiq 550 EC

INTRODUCTION

The application of herbicides in agriculture is a rapid and effective method of not only weed control but also improvement of yield quality and limitation of seed loss. However, the treatment of crops with chemicals stays not without an effect on the natural environment (Chowdhury *et al.,* 2008). Degradation of pesticides in a soil system depends on their chemical and physical properties as well as on their interactions with the biotic and abiotic components of soil. Soil, as a dynamic system, is subject to permanent changes. These changes are caused by growing crops and soil physical, biological and chemical properties (Sławiński *et al.,* 2012; Szatanik-Kloc, 2012). Chemical compounds applied under field conditions are transformed under the influence of biological and non-biological processes into one or more products of such transformations. The transformations described are conducted on the pathways of various mechanisms by physical, chemical and biological factors in which microorganisms play a significant role. The mechanisms of transformation include oxidation, hydrolysis, reduction, conjugation and are catalysed by various enzymes, and the result of all those is the formation of usually less bioactive products (Chowdhury *et al.,* 2008).

Defining the populations of various microbial groups is used for the estimation of the biological status of soil environment, *eg* soils affected by anthropogenic factors (Caldwell, 2005; Sławiński *et al*., 2012; Szatanik-Kloc, 2012). In the cultivation of certain plants, *eg* rapeseed, an important issue is non-uniform ripening of pods, which may result in increased levels of seed losses during harvest. Therefore the application of a desiccant (such chemicals as Reglone 200 SL and Elastiq 550 EC are very often used for rapeseed crops) causing the drying of the green parts of plants contributes to achieving a better uniformity of the state of ripeness of the whole crop stand. Drying significantly reduces the level of seed losses related to seed shedding.

Reglone 200 SL is a chemical from the group of desiccants and its primary objective is drying of aboveground parts of plants containing chlorophyll. Composition of the chemical includes a biologically active substance called diquat ion (Fig. 1), a compound from the group of pyridyls. It is also used in crop weed control. Reglone 200 SL belongs to the group of pyridyl herbicides considered as inhibitors of photosynthesis in plants. Herbicides from this group have

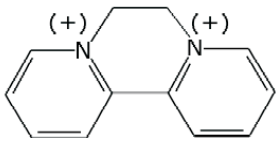

Fig. 1. Structural formula of diquat.

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an ability of capturing electrons from the photosynthetic chain of electron transport in PS I (Photosystem I) and, as a result, the formation of reactive forms of oxygen (RFT) in cells, causing oxidative stress in plants, is observed (Popova *et al.,* 2003).

Elasitiq 550 EC is a chemical applied in the form of a concentrated emulsion before the harvest of *eg* rapeseed. The chemical bonds the pods, preventing excessive seed shedding prior to and during the harvesting. The composition of the chemical includes two biologically active substances – synthetic latex and alkoxylated alcohol. When applied in plant crops, Elastiq 550 EC forms a thin layer of latex which dries rapidly and forms a semi-permeable membrane on the plants. The Elastiq 550 EC contributes to transpiration of plants, but prevents penetration of water into tissues, significantly reducing seed shedding before and during harvest.

The application of chemicals, especially those that have the ability of persisting in soil over long periods of time, may lead to their excessive accumulation in the environment and, as a result, to its degradation (Das *et al.,* 2003). Chemical agents of various kinds may cause distinct changes in the enzymatic activity, and its intensity and direction depend on the kind and rate of agent applied, soil type, and duration of impact (Wyszkowska and Kucharski, 2004). Therefore, it is very important to perform the estimations of effects of chemical agents on the biochemical properties of soil, and especially on the activity of soil enzymes, which are very important indicators of the biodiversity of soil (Caldwell, 2005). The enzymatic activity is a parameter which reflects the status of soil environment and can be adopted as an indicator of the total microbiological activity (Alkorta *et al.,* 2003). High activity of soil microorganisms indicates good quality of soils as well as correct functioning of the processes conducted by soil organisms (Wang *et al.,* 2009). It is one of the indices of estimation of the fertility and productivity of soils and permits acquisition of comprehensive knowledge on changes in the soil environment (Janvier *et al.,* 2007) before they can be detected by other soil properties. Measurement of enzymatic activity in soil can be also used for the monitoring of the health status of soils (Alkorta *et al.,* 2003; Janvier *et al.,* 2007) and their contamination with anthropogenic factors.

Chemicals of various kinds, introduced in agricultural cultivations, attract special attention due to their possible toxicity, capacity of accumulation as well as persistence in the environment (Beyer and Biziuk, 2007). In view of the above-mentioned findings, pesticides and other chemical contaminants should be monitored throughout the year to mitigate their unfavourable effects on the environment.

The aim of this paper was to show the effects of Reglone 200 SL and Elastiq 550EC on the total numbers of bacteria and fungi, those with proteolytic capabilities, and on the activity of selected soil enzymes.

MATERIALS AND METHODS

Study on the effects of Reglone 200 SL and Elastiq 550 EC on the microbiological and biochemical properties of soil was conducted in a field experiment. The choice of these chemicals was:

– based on the commonality of their application in rapeseed crops during harvest,
– induced by the absence of literature data on their effects on the microbiological and biochemical activity of soils.

The soil was classified as Mollic Gleysol developed from light silty loam (2-0.5 mm – 65%, 0.05-0.002 mm – 22%, <0.002 mm – 7%). The other basic characteristics of the investigated soil are: 6.1 – pH_{HCl}, 9.8 – organic carbon content, 1.3 – total nitrogen, 0.75 – total phosphorus, 0.09 g kg^{-1} d.m. – potassium, and 10.8 mg kg^{-1} d.m. – copper.

The biologically active substance of Reglone 200 SL is diquat ion, a compound from the group of pyridyls. The amount of the active substance in the chemical is equal to 200 g dm^{-3}. The composition of Elasitiq 550 EC includes two biologically active substances: synthetic latex (carboxylated styrene-butadiene copolymer) – 49.5%, and alkoxylated alcohol – 10%.

A three-year field experiment (2010-2012) was set up in the split-block design at the plant variety evaluation experimental station in Głębokie, Kujawsko-Pomorskie Province (52° 38'41''N, 18° 26'18''E).

In the first year of the experiment all the treatments were sown with winter rapeseed (cv. 'Californium'), in the second year – with sugar beet (cv. 'Lucata'), and in the third one – with spring barley (cv. 'Rubinek'). The choice of the crop (rapeseed) in the first year of the experiment was related to the cultivation of that crop which requires very large outlays on its protection, and thus the application of various chemical agents during its cultivation, including chemicals from the group of desiccants.

In the first year of the experiment, both Reglone 200 SL and Elastiq 550 EC were applied at the rates recommended by the manufacturer. The model of the experiment comprised the following treatments:

1 – control – no chemicals,
2– soil + Reglone 200 SL at the optimum rate (2 dm^3 ha^{-1}),
3– soil + Elastiq 550 EC applied at the optimum rate (1 dm^3 ha^{-1}).

The application of the chemicals was made by means of a knapsack spraying system. In the case of Elastiq 550 EC, the spraying was made 4 weeks and of Reglone 200 SL 10 days before the harvesting of winter rapeseed. In all the treatments the basic tillage operations were performed and the same level of fertilization was applied according to recommendations for the studied crops. The harvesting area of the experimental plots was 12 m^2.

Analysis was set in August and October of 2009, 2010, 2011 with the following six terms: I – immediately after the harvest of winter rapeseed (beginning of August 2009);

II – beginning of October 2009; III – beginning of August 2010; IV – beginning of October 2010; V – beginning of August 2011; VI – beginning of October 2011.

Soil samples for the analyses were taken from the arable soil layer of each plot. 20-30 combined soil samples were taken from each experimental treatment and their total mass was equal to 6 kg. Soil samples were transported in plastic bags with thermal insulation inserts at low temperature. In the laboratory the soil material was thoroughly mixed, dried, and passed through a sieve with 2-mm mesh. Soil subsamples for the microbiological and biochemical analyses were taken from the soil material. Until the completion of all analyses, the prepared soil samples were stored at temperature of 4°C.

Microbial analysis of soil samples included: determination of the total number of bacteria by the plate method on a substrate with the soil solution (Trolldenier, 1995) and of the total numbers of fungi by the Martin method (1950). The determination of the numbers of proteolytic bacteria and fungi was carried out by the plate method on a substrate with gelatine (Trolldenier, 1995). The determination of dehydrogenase activity was done according to the Thalmann (1968) method modified by Alef and Nannipieri (1995). The measurement of protease activity was made using the Ladd and Butler (1972) method modified by Alef and Nannipieri (1995). Urease activity was determined by the Zantua and Bremner (1975) modified method, while acid and alkaline phosphatase activity was measured by the Tabatabai and Bremner (1969) method.

All the results were processed statistically with the use of the analysis of variance (ANOVA). The least significant differences were calculated with the Tukey test at significance level of $\alpha = 0.05$. The statistical analyses were performed using the program STATISTICA 7.1.

RESULTS AND DISCUSSION

The results given in Table 1 present the effects of Reglone 200 SL and Elastiq 550 EC on the total numbers of bacteria and fungi and on the numbers of bacteria and fungi with proteolytic capabilities in the soil. For the most of the terms (II, IV, V and VI) of analyses, higher numbers of bacteria and fungi were recorded in the soil subjected to the effect of Elastiq 550 EC. After the application of Reglone 200 SL, a significant increase in the numbers of fungi was noted for the terms IV and V. A significant decrease in the

T a b l e 1. Total numbers and numbers of bacteria and fungi with proteolytic capabilities in soil contaminated with Reglone 200 SL and Elastiq 550 EC

Treatments	Year of analyses						Average
	2010		2011		2012		
	Terms of analyses						
	I	II	III	IV	V	VI	
	Total numbers of bacteria (cfu 10^8 kg^{-1} d.m. of soil)						
Control	107.427g	35.690b-e	4.535a	28.800b	33.183bcd	36.615b-e	41.042a
Soil + Reglone 200 SL	96.795g	44.526b-e	8.637a	48.462cde	50.978e	31.908bc	46.884ab
Soil + Elastiq 550 EC	48.005cde	77.502f	3.465a	43.447b-e	48.903de	50.278e	45.266b
	Total numbers of fungi (cfu 10^6 kg^{-1} d.m. of soil)						
Control	18.228ab	108.131i	15.697ab	49.778cde	49.261cde	46.030cd	47.854a
Soil + Reglone 200 SL	30.831bc	85.214gh	16.928ab	66.021efg	55.833def	76.934g	55.293b
Soil + Elastiq 550 EC	5.606a	98.072hi	6.929a	48.558cde	71.633fg	54.817def	47.602a
	Numbers of 'proteolytic' bacteria (cfu 10^8 kg^{-1} d.m. of soil)						
Control	28.699c	1.060a	1.046a	3.200a	5.131a	6.277a	7.569b
Soil + Reglone 200 SL	32.982c	18.041b	0.345a	6.321a	2.081a	3.900a	10.612c
Soil + Elastiq 550 EC	1.752a	4.775a	0.693a	3.286a	3.788a	3.492a	2.964a
	Numbers of 'proteolytic' fungi (cfu 10^6 kg^{-1} d.m. of soil)						
Control	10.471f	6.007de	1.380ab	4.978b-e	5.645cde	4.185a-e	5.444a
Soil + Reglone 200 SL	10.755f	17.657g	1.703ab	7.375ef	2.081abc	3.191a-d	7.127b
Soil + Elastiq 550 EC	3.854a-e	16.162g	0.693a	5.476cde	4.477b-e	4.190a-e	5.809a

Means followed by the same letter are not significantly different according to Tukey test (p = 0.05).

numbers of bacteria was observed after the application of both chemicals on term I. The statistical analysis of the results obtained during the three-year experiment showed that Reglone 200 SL caused a significant increase in the numbers of fungi in the soil.

The highest total number of fungi was found in the soil subjected to the effect of Reglone 200 SL for term VI of analysis (76.93 cfu 10^6 kg^{-1}d.m. soil), while in the case of Elastiq 550 EC, a significant increase of the total number of fungi was noted for term V. A significant decrease in the number of fungi took place in the soil with Elastiq 550 EC on term I and in that with Reglone 200 SL on term II. Mean values of the results demonstrated that Reglone 200 SL caused a significant increase in the number of soil fungi.

In the period between terms III and VI the numbers of 'proteolytic' bacteria after the application of both Reglone 200 SL and Elastiq 500 EC oscillated at a level similar to that of the control treatment. Over the whole period of the experiment, the mean numbers of 'proteolytic' bacteria were significantly higher in the soil with the herbicide Reglone 200 SL, whereas Elastiq 500 EC caused a significant decrease in the numbers of bacteria with proteolytic capabilities.

The determination of the numbers of fungi with proteolytic capabilities revealed a significant increase in their numbers for term II in both soils subjected to the effect of Reglone 200 SL and in that with Elastiq 550 EC. Mean values of the results showed that Reglone 200 SL caused a significant increase in the numbers of fungi with proteolytic capabilities.

The results given in Table 2 present the effects of Reglone 200 SL and Elastiq 550 EC on the activity of dehydrogenase, protease, and urease.

The dehydrogenase activity in the soil affected by Reglone 200 SL and Elastiq 550 EC remained at a very low stable level during the period of the experiment. The mean activity of dehydrogenases in the soil after the application of Elastiq 550 EC was significantly lower than in the treatment with Reglone 200 SL that was related to a very low activity of the enzyme (0.461 mg TPF kg^{-1} d.m. day^{-1}) for term II.

After the application of Reglone 200 SL and Elastiq 550 EC the soil was characterized by a significantly higher proteolytic activity than that in the control treatment for term II. A significant decrease in the proteolytic activity up to zero level was observed in the soil with Elastiq 550 EC for term III. For the three subsequent terms of analyses the activity of protease occurred at a level similar to that in the control treatment. Mean values of the results showed that Reglone 200 SL caused a significant increase of protease activity of the soil.

There were significant variations in the ureolytic activity during the experiment. The urease activity of soil with Reglone 200 SL was higher than that in the soil of

T a b l e 2. Dehydrogenase, protease and urease activity in soil contaminated with Reglone 200 SL and Elastiq 550 EC

Treatments	Year of analyses						Average
	2010		2011		2012		
	Terms of analyses						
	I	II	III	IV	V	VI	
	Dehydrogenase activity (mg TPF kg^{-1} h^{-1})						
Control	0.480a	1.574abc	1.278abc	1.761bc	0.612a	0.930abc	1.106a
Soil + Reglone 200 SL	0.962abc	1.849a	0.564a	1.441abc	1.002abc	1.140abc	1.160a
Soil + Elastiq 550 EC	0.515a	0.461a	0.507a	1.187abc	1.232abc	0.673ab	0.762b
	Protease activity (mg tyrozine kg^{-1} h^{-1})						
Control	6.217de	1.880abc	2.539abc	2.688abc	1.293ab	2.905a-d	2.920a
Soil + Reglone 200 SL	4.743cde	19.104f	2.539abc	2.827abc	2.440abc	4.269b-e	5.987b
Soil + Elastiq 550 EC	2.404abc	7.071e	0.000a	3.399bcd	1.121ab	2.713abc	2.785a
	Urease activity (mg N-NH_4 kg^{-1} h^{-1})						
Control	6.040ab	15.422cd	6.052ab	7.703abc	3.561a	6.776abc	7.592a
Soil + Reglone 200 SL	9.585abc	23.397d	9.243abc	8.263abc	5.759ab	6.151abc	10.399b
Soil + Elastiq 550 EC	5.575ab	12.986bc	5.103ab	7.240abc	5.683ab	5.129ab	6.952a

Explanations as in Table 1.

control treatment. A significant increase in urease activity to 23.40 mg N-NH_4 kg^{-1}d.m. h^{-1} was observed for term II. However, Elastiq 550 EC had no significant effect on the ureolytic activity of the soil. Mean values of the results demonstrated that Reglone 200 SL caused a significant increase in urease activity of the soil.

The activity of acid phosphatase was subjected to variations during the experiment (Fig. 2). The highest significant increase of acid phosphatase activity, compared to that in the control treatment (32.84 mg PNP kg^{-1} d.m. h^{-1}), was observed for term II after the application of Reglone 200 SL (53.78 mg PNP kg^{-1} d.m. h^{-1}) and Elastiq 550 EC (56.267 mg PNP kg^{-1} d.m. h^{-1}). Reglone 200 SL caused a significant decrease in the activity of acid phosphatase on terms IV and V. The activity of that enzyme remained at a level similar to that in the control treatment for the other terms of analyses.

The results of the present studies showed that the activity of alkaline phosphatase was also subject to variations during the period of the experiment (Fig. 3). Reglone 200 SL, applied according to the recommendations of the manufacturer, caused a significant increase in the activity of that enzyme for terms II, IV and V, while Elastiq 550 EC

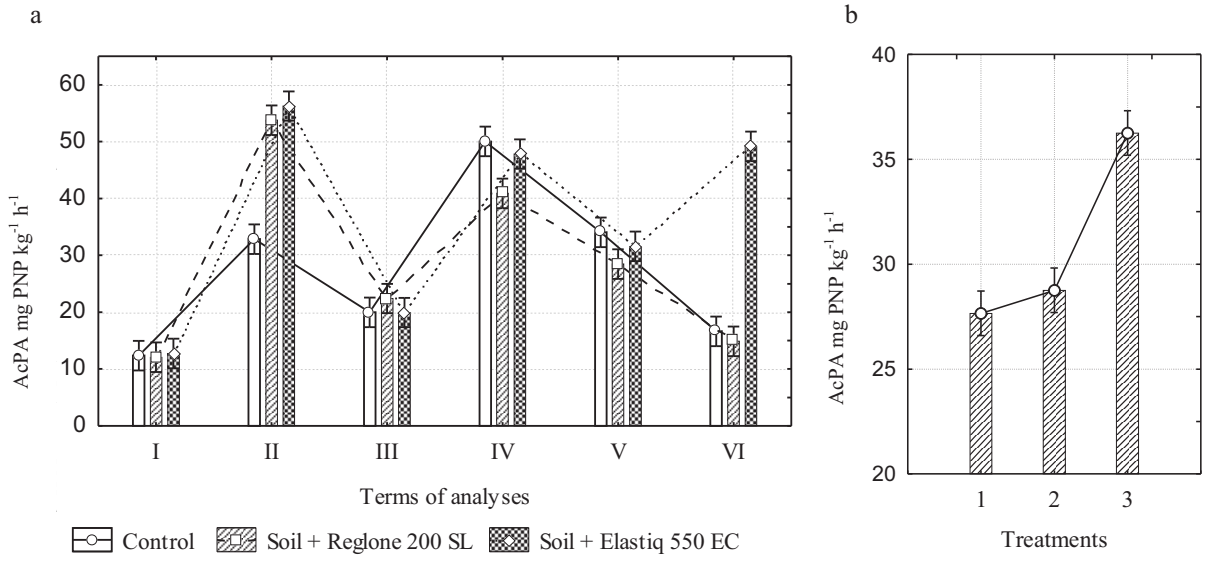

Fig. 2. Acid phosphatise activity (AcPA): a – in soil contaminated with Reglone 200 SL and Elastiq 550 EC, b – mean values of acid phosphatise activity. 1 - control soil, 2 - soil + Reglone 200 SL, 3 - soil + Elastiq 550 EC. Vertical bars denote 0.95 confidence intervals.

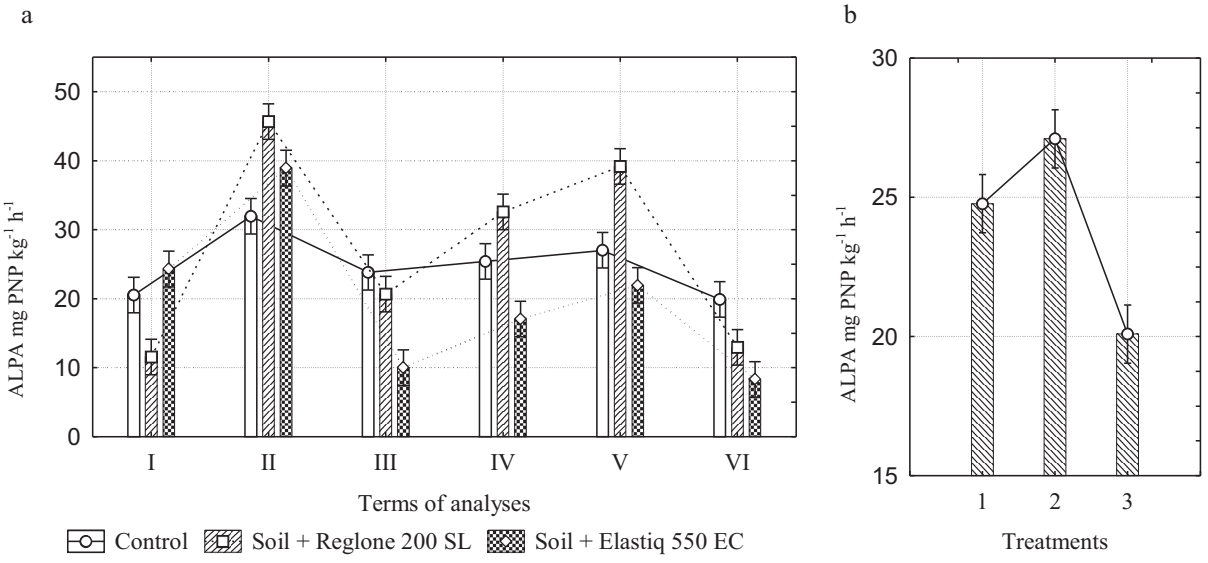

Fig. 3. Alkaline phosphatase activity (ALPA): a – in soil contaminated with Reglone 200 SL and Elastiq 550 EC, b – mean values of alkaline phosphatase activity. Explanations as in Fig. 2. Vertical bars denote 0.95 confidence intervals.

contributed to a significant increase in the activity of alkaline phosphatase only for term II. A significantly lower activity of that enzyme in most of the terms (III, IV, V and VI) of analyses was observed in the soil affected by Elastiq 550 EC. Mean values of the results over the whole period of the experiment showed that Reglone 200 SL caused a significant increase in the activity of alkaline phosphatase, while Elastiq 550 EC significantly reduced the activity of this enzyme.

The study showed that the reaction of the soil varied in the particular analyses, as illustrated in Table 3. During the experiment, Reglone 200 SL and Elastiq 550 EC caused an increase of soil pH as compared to that of the control treatment. The value of pH was 6.5 after the application of Reglone 200 SL and 6.3 after that of Elastiq 550 EC.

To demonstrate the existence of interrelationships between the microorganisms and their biochemical activity in the soil subjected to the effects of Reglone 200 SL and Elastiq 550 EC, the analysis of correlation was performed (Table 4) which has actually proved the existence of such relationships. Among the studied microbial groups, strong positive correlations were found between the total numbers of bacteria and the numbers of bacteria and fungi with proteolytic capabilities, and between the total number of fungi and the number of fungi with proteolytic capabilities. A positive correlation was also observed between the numbers of bacteria and the activity of protease. The number of fungi were positively correlated with the activity of dehydrogenases, protease, urease, acid and alkaline phosphatase, and pH of the soil. The number of fungi with proteolytic capabilities was positively correlated with the activity of protease, urease, acid, and alkaline phosphatase, and with pH of the soil. The statistical analysis demonstrated that the activity of dehydrogenases and protease was positively correlated with the activity of urease, acid and alkaline phosphatase, and with pH of the soil.

The study showed that the chemical agents used in the experiment had a significant effect on the values of the microbiological and biochemical parameters.

Soil is the natural habitat of many different microbial groups. Soil microorganisms play a significant role in the degradation of various kinds of contaminants, including pesticides, thus contributing to the maintenance of suitable quality of the soil (Chowdhury *et al.,* 2008). Determination of populations of various microbial groups is used for evaluating the biological status of the soil environment *eg* the soils influenced by anthropogenic factors (Caldwell, 2005).

The results obtained demonstrate that the chemicals used in the experiment caused a significant increase in the populations of microbial groups. This is probably related to an additional influx of organic matter with the chemicals, having a nutritive effect on the microorganisms. Kaszubiak and Durska (2000) also showed a favourable effect of Oxafun T on the populations of selected groups of microorganisms. An increase in the numbers of microorganisms affected by pesticides was also observed by Das and Mukherjee (2000). Those authors performed an estimation of the effect of such insecticides as HCH, Forat and Fenwalerat on

T a b l e 3. pH of soil contaminated with Reglone 200 SL and Elastiq 550 EC

Treatments	Terms of analyses					
	I	II	III	IV	V	VI
Control	5.9	6.8	5.3	6.4	6.1	6.4
Soil + Reglone 200 SL	6.1	7.0	6.2	6.5	6.5	6.5
Soil + Elastiq 550 EC	5.9	6.9	5.9	6.5	6.4	6.5

T a b l e 4. Correlation coefficients (R) between examined microbial and biochemical parameters

	1	2	3	4	5	6	7	8	9	10
1	-	n.s.	0.74***	0.61***	n.s.	0.29*	n.s.	n.s.	n.s.	n.s.
2		-	n.s.	0.46***	0.42**	0.33*	0.49***	0.59***	0.50***	0.85*
3			-	0.57***	n.s.	0.46***	n.s.	n.s.	n.s.	n.s.
4				-	n.s.	0.76***	0.63***	0.45***	0.50***	0.50*
5					-	n.s.	0.38**	0.30*	0.31*	0.31*
6						-	0.69***	0.35**	0.49***	0.45*
7							-	0.39**	0.55***	0.55*
8								-	0.45***	0.66*
9									-	0.46*
10										-

*, **, ***indicate significance at $p > 0.05$, $p > 0.01$, and $p > 0.001$ level, respectively; n.s.– not significant. 1 – total numbers of bacteria, 2 – total numbers of fungi, 3 – numbers of 'proteolytic' bacteria; 4 – numbers of 'proteolytic' fungi, 5 – dehydrogenase activity, 6 – protease activity, 7 – urease activity, 8 – acid phosphatise activity, 9 – alkaline phosphatase activity, 10 – pH.

selected groups of microorganisms. The results obtained by the authors confirmed microbial growth stimulation by the pesticides. Das *et al.* (2003) conducted a study on the effect of the insecticide Forat and reported on its stimulating effect on the populations of selected groups of soil microorganisms. Our study with Reglone 200 SL and Elastiq 550 EC, applied at the rates recommended by the manufacturer, demonstrated that both chemicals did not have any negative inhibiting effect on the numbers of bacteria, fungi, and fungi with proteolytic capabilities. Cycoń and Piotrowska-Seget (2007) studied the effects of three different pesticides (insecticide, herbicide, fungicide) on the numbers of heterotrophic bacteria as well as of fungi and bacteria participating in nitrogen transformations. They observed that the growth of fungi was stimulated after the application of the herbicide with the active substance linuron. A stimulation of the growth of soil bacteria and actinomycetes, as a result of application of the herbicide Brominal at small rates, was also observed by Omar and Abel-Sater (2001). Moreover, the authors also observed a similar effect of Selectron on the microbial groups. However, the authors do not explain the causes of the effects of the chemical preparations.

However, the available literature presents also examples of a negative effect of pesticides on the numbers of soil microorganisms. Wyszkowska and Kucharski (2004) conducted a study on the effect of the herbicide Chwastox Trio 540 SL on various groups of microorganisms. The authors observed that the application of the herbicide at an optimum rate caused an increase in the numbers of copiotrophic bacteria and actinomycetes, and a simultaneous decrease in the numbers of fungi. Moreover, the authors observed that higher rates of the herbicide *ie* 5- and 10-fold higher than the optimum rate, had negative effects on fungi, cellulolytic, oligotrophic, copiotrophic and spore-forming bacteria, as well as on bacteria from the genus *Azotobacter.* However, the authors do not explain the causes of the effects of the chemical preparations.

Enzymatic activity is a parameter clearly reflecting the status of the soil environment. Our study demonstrated that the herbicide Reglone 200 SL, applied at the optimum rate, had no negative effect on the enzymatic activity of the soil enzymes. The activity of the analysed enzymes in the soil contaminated with the herbicide Reglone 200 SL was generally higher than that of the enzymes in the non-contaminated soil. The study showed that the activity of urease, acid and alkaline phosphatase and protease increased after the application of Reglone 200 SL. An inhibiting effect of the chemical was also observed in relation to the dehydrogenase activity. Sebiomo *et al.* (2011) studied the effect of four herbicides: atrazine, primeextra, paraquat and glyphosate on the activity of dehydrogenases in soil. Based on the obtained results, they concluded that the herbicides caused a considerable decrease in dehydrogenase activity compared to that of the control treatment. Somewhat different results were obtained by Rasool and Reshi (2010). The authors studied the effect of the fungicide Mancozeb on the activity of dehydrogenases and observed an increase in the activity of those enzymes as a result of application of the fungicide. The results of our study showed that the activity of urease increased after the application of the herbicide Reglone 200 SL during the experiment. Wang *et al.* (2009) studied the effect of copper-based fungicides on the biochemical activity of soil. The authors observed an inhibition of urease activity, which was caused by changes in soil acidity. Kucharski *et al.* (2009) reported on a significant decrease in the activity of urease and of acid and alkaline phosphatase as a result of application of Harpun 500 SC at an optimum rate. Rasool and Reshi (2010) observed an inhibition of the activity of urease after the application of the fungicide Mancozeb. Compared to the control treatment, this fungicide caused an increase and a decrease in the activity of alkaline phosphatase after 14 and 28 days of incubation, respectively. The activity of protease after the application of the fungicide increased, especially by the 21st day of incubation.

The observed effects of the chemicals used in the field experiment on the soil microorganisms are most likely connected with the presence of nitrogen and carbon in the composition of the examined substances. Those elements play a crucial role in the biochemical processes taking place in the soil environment, and that is why such chemicals are very often used by the soil microorganisms as a valuable carbon and nitrogen source.

CONCLUSIONS

1. Both Reglone 200 SL and Elastiq 550 EC, applied at the rates recommended by the manufacturer, affected the populations of soil microorganisms.

2. Stimulating effect of the studied chemicals on the numbers of bacteria and fungi, and of 'proteolytic' bacteria and fungi was observed.

3. No negative effect of Reglone 200 SL on the enzymatic activity of soil was observed. Reglone 200 SL stimulated the activity of dehydrogenases, protease, urease, and acid and alkaline phosphatase in soil.

4. Elastiq 550 EC caused a significant decrease in the activity of dehydrogenases and alkaline phosphatase, and a significant increase in the activity of acid phosphatase.

5. High activity of soil microorganisms, indicating good soil quality and availability of nutrients for plants, was observed in soil after the application of Reglone 200 SL.

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