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ENZYMATIC ACTIVITY IN SOIL CONTAMINATED WITH THE AURORA 40 WG HERBICIDE

Aurora 40 WG is a new generation herbicide for controlling dicotyledonous weeds. Its effect on the biochemical properties of soil has not been investigated to date. The aim of this study was to determine the effect of soil contamination with the Aurora 40 WG herbicide on the activity of dehydrogenases, urease, acid phosphatase and alkaline phosphatase. Soil samples with the granulometric composition of loamy sand and sandy clay loam were used in a pot experiment. The lowest herbicide dose was that recommended by the manufacturer, and the successive doses were 2-, 4- and 40-fold higher than the recommended dose. In selected treatments, soil was mixed with finely ground spring barley straw and basalt meal. The experiment was carried out for 50 days, in two series, in unsown soil and in soil sown with spring barley. Soil samples were analyzed on experimental day 25 and 50. The obtained results indicate that the Aurora 40 WG herbicide did not modify the enzymatic activity of soil. The biochemical activity of soil was largely determined by the date of analysis, the addition of barley straw and basalt meal, soil type and soil use. The investigated enzymes were marked by higher activity levels in sandy clay loam than in loamy sand. Barley sowing had a generally positive effect on the enzymatic activity of soil, excluding alkaline phosphatase whose activity was higher in unsown treatments. The addition of finely ground spring barley straw also enhanced the biochemical properties of soil, while no such effects were demonstrated by basalt meal.

1. INTRODUCTION

Man-made organic compounds are one of the main sources of pollution in the natural environment [1–3]. Herbicides play an important role in this group of compounds due to their extensive use, environmental persistence and toxic properties [4]. Herbicides belong to various chemical groups, which renders them highly effective in weed control and contributes to high quality and quantity of crops [5]. Uncontrolled farming practices may contribute to the spread of herbicides to various ecosystems, posing a threat for living organisms in those habitats, mainly microbes [6, 7]. Activity of soil enzymes is a reliable indicator of soil conditions and a valuable source of information

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on soil changes induced by xenobiotics, including herbicides. The enzymes produced by soil-dwelling microbes and plants play a key role in the environment because they actively participate in the process of circulation of organic matter [8, 9].

To minimize the adverse environmental impact of those substances and to increase the effectiveness of farming practices, attempts are made to modify the form of the applied products and to minimize their dosage [10]. Aurora 40 WG is a new-generation herbicide whose effect on the soil environment has not yet been investigated. The product is supplied by the FMC Corporation of Philadelphia, USA. The active ingredient is carfentrazone-ethyl at the concentration of 40%. The dose recommended by the manufacturer is 50 g·ha^{-1}. Carfentrazone-ethyl, an active substance from the aryl thiazolinone group, is used to control dicotyledonous weeds in cereal crops [11]. Similarly to sulfentrazone, it inhibits protoporphirynogen oxidase activity in chlorophyll, which leads to lipid oxidation and causes changes in the cell membrane [12]. Carfentrazone-ethyl reaches the chloroplast and exhibits herbicidal activity under the influence of light, thus necrotizing plant tissue [11, 13]. As a result of photochemical reactions in the soil, the end product of carfentrazone-ethyl is chloropropionic acid which demonstrates acidic properties that may pose a threat for soil-dwelling microbes [11, 14].

The objective of this study was to determine the effect of Aurora 40 WG herbicide (the active substance is carfentrazone-ethyl) on the enzymatic activity of soil.

2. MATERIALS AND METHODS

A greenhouse experiment was carried out to investigate the effect on the Aurora 40 WG herbicide on the enzymatic activity of soil. Air-dried soil samples of 3 kg each were placed in polyethylene pots. Two types of soil from the humus horizon, classified as proper brown soil developed from loamy sand and sandy clay loam, were used in the study. Soil of the first type comprised 72% of sand, 7% of silt and 21% of silt and clay fractions with pH_{KCl} of 7.00, hydrolytic acidity of 16.05 mmol·kg^{-1}, total exchangeable bases of 75.00 mmol·kg^{-1} and organic carbon content of 7.05 g· kg d.m. soil. Soil of the second type (57% sand, 22% silt and 21% silt and clay fractions) was characterized by the following parameters: pH_{KCl} of 7.00, hydrolytic acidity of 14.55 mmol·kg^{-1}, total exchangeable bases of 196.00 mmol·kg^{-1} and C_{org} content of 14.30 g·kg^{-1}.

The experimental variables were:

- herbicide doses expressed as a multiple of the recommended dose: 0 – control, 1 – the dose recommended by the manufacturer (0.017 mg·kg^{-1}), and doses 2- (0.033 mg·kg^{-1}), 4- (0.066 mg·kg^{-1}) and 40-fold (0.664 mg·kg^{-1}) higher than the recommended dose,
- soil type: loamy sand and sandy clay loam,

- soil use: unsown soil and soil sown with spring barley,
- the addition of finely ground spring barley straw and basalt meal in the amount of 5 $g \cdot kg^{-1}$,
- the date of soil sampling to determine its enzymatic activity: experimental days 25 and 50.

All treatments were regularly fertilized with macroelements and microelements at the following rates on active ingredient basis per $mg \cdot kg^{-1}$ soil: N – 100 $[CO(NH_2)]_2$, P – 35 $[KH_2PO_4]$, K – 100 $[KH_2PO_4 + KCl]$, Mg – 20 $[MgSO_4 \cdot 7H_2O]$, Cu – 5 $[CuSO_4 \cdot 5H_2O]$, Zn – 5 $[ZnCl_2]$, Mn – 5 $[MnCl_2 \cdot 4H_2O]$, Mo – 5 $[Na_2MoO_4 \cdot 2H_2O]$, B – 0.33 $[H_3BO_4]$. Mineral fertilizers and the herbicide in the form of an aqueous solution were added to the soil, and all components were thoroughly mixed. The experiment was performed in four replications, in two series, in sown and unsown soils. The tested crop was spring barley cv. Orthega – 12 plants per pot. In selected treatments, soil was mixed with finely ground spring barley straw and basalt meal. Basalt meal was supplied by Łużycka Kopalnia Bazaltu Księginki S.A. (basalt mine). The moisture content of soil was maintained at 50% capillary water capacity throughout the growing season with the use of demineralized water.

The granulometric composition of soil was determined by areometric measurements [15]. Soil pH was determined by the potentiometry method in aqueous KCl solutions with the concentration of 1 $mol \cdot dm^{-3}$, hydrolytic acidity (Hh) and total exchangeable bases (S) were determined by Kappen's method [15], and organic carbon content (C_{org}) – by the method proposed by Turin [16]. The activity of the following enzymes was determined twice, on the days of biochemical analysis: dehydrogenases (EC 1.1) – by the method proposed by Lenhard and modified by Öhlinger [17], acid phosphatase (EC 3.1.3.2) and alkaline phosphatase (EC 3.1.3.1) – according to Alef et al. [18], and urease (EC 3.5.1.5) by the method proposed by Alef and Nannipieri [19]. The activity of the investigated enzymes was measured using the Perkin-Elmer Lambda 25 spectrophotometer. The following substrates were applied to determine enzymatic activity: 2,3,5-triphenyl tetrazolium chloride (TTC, P.P.H. Stanlab s.j., Poland) for dehydrogenases, 4-nitrophenylphosphate disodium (PNPNa, ApliChem GmbH, Germany) for phosphatases, and urea (Eurochem Sp. z o.o., Poland) for urease. Enzymatic activity was expressed in millimoles of the product per h and kg d.m. of soil, i.e. dehydrogenases – in micromoles of triphenyl formazan (TFF), phosphatases – in millimoles of 4-nitrophenyl (PNP) and urease – in millimoles of N- NH_4^+.

The results were processed statistically by Duncan's multiple range test and a five-factorial analysis of variance. LSD values for interactions between the experimental factors (herbicide dose×soil type×soil use×the addition of barley straw and basalt meal ×the date of biochemical analysis), standard deviations and correlation coefficients are presented in Tables 1–4. Differences between means were considered significant at $p = 0.05$. Statistical analyses were performed using the Statistica [20] application.

3. RESULTS AND DISCUSSION

The results of the study indicate that soil contamination with excessive quantities of the Aurora 40 WG herbicide had no significant effect on the enzymatic activity of soil. The response of soil enzymes was determined by the date of analysis, the addition of barley straw and basalt meal, soil type and soil use (Tables 1–4).

The activity of dehydrogenases in soil contaminated with Aurora 40 WG was marked by fluctuations throughout 50 days of the experiment (Table 1). The recommended dose did not affect dehydrogenase activity. The application of higher herbicide doses had no influence on the activity of the studied enzymes. On day 25, in treatments sown with spring barley, regardless of soil type, dehydrogenase activity was lower than on day 50, while a reverse trend was reported in unsown treatments. Dehydrogenase activity was also affected by soil type. In sandy clay loam, the activity of dehydrogenases was 1.5-fold higher than in loamy sand. The application of finely ground spring barley straw had a clearly positive effect on dehydrogenase activity which, on average, was stimulated 1.8-fold in loamy sand and 1.2-fold in sandy clay loam in comparison with the control treatment. Basalt meal did not affect the activity of dehydrogenases.

Similarly to dehydrogenases, urease activity also fluctuated throughout the experiment (Table 2). It reached the highest level on experimental day 25, and then decreased significantly. The highest dose of Aurora 40 WG (40-fold higher than recommended) stimulated urease activity by 6.1% in loamy sand, while a 4.0% drop in urease activity was reported in sandy clay loam. The addition of straw had a stimulating effect on urease activity regardless of soil type. Basalt meal also enhanced urease activity, but to a much lower extent than straw. Basalt meal was more effective in medium loam treatments where urease activity levels were 1.2-fold higher than in loamy sand. Urease activity was also dependent on soil type and soil use. Higher levels of enzymatic activity were determined in sandy clay loam than in loamy sand. Regardless of soil type, urease activity was higher in treatments sown with spring barley.

Contrary to dehydrogenases and urease, the activity of acid phosphatase (Table 3) was highest on day 50. The application of the studied herbicide at the dose 40-fold higher than the recommended dose enhanced the activity of acid phosphatase by 4.2% in loamy sand and by only 0.7% in sandy clay loam on average. The addition of straw stimulated the activity of acid phosphatase 1.3-fold in loamy sand and 1.2-fold in sandy clay loam. In comparison with the control treatment, basalt meal increased the activity of acid phosphatase by 3.5% in sandy clay loam, and decreased by 2.1% in loamy sand. The granulometric composition of soil had a significant effect on the activity of acid phosphatase which was higher in sandy clay loam than in loamy sand. Treatments sown with spring barley were also characterized by higher levels of acid phosphatase activity.

Table 1

Dehydrogenase activity in soil contaminated with the Aurora 40 WG herbicide [μmol TFF·kg^{-1} d.m.·h^{-1}]

Herbicide dose [mg·kg^{-1}]	Loamy sand				Sandy clay loam			
	Date of analysis (days)							
	Sown treatments		Unsown treatments		Sown treatments		Unsown treatments	
	25	50	25	50	25	50	25	50
Control								
0.000	3.93±0.08	4.93±0.12	2.48±0.04	1.55±0.02	4.68±0.09	7.25±0.06	4.22±0.03	3.83±0.05
0.017	3.59±0.11	4.70±0.03	3.17±0.08	1.87±0.01	4.50±0.08	8.28±0.03	4.28±0.06	2.75±0.05
0.033	3.64±0.10	4.76±0.09	2.57±0.01	1.88±0.05	4.27±0.13	6.24±0.06	3.88±0.04	2.93±0.11
0.066	3.09±0.14	4.63±0.07	3.05±0.07	1.84±0.03	4.15±0.05	7.84±0.01	4.15±0.09	2.91±0.10
0.664	2.73±0.19	4.57±0.07	3.21±0.07	2.10±0.02	3.99±0.05	6.03±0.04	2.90±0.07	3.78±0.04
Average	3.40±0.09	4.72±0.08	2.90±0.05	1.85±0.03	4.32±0.08	7.13±0.04	3.89±0.06	3.24±0.07
r	–0.83	–0.50	0.55	–0.83	–0.72	0.04	–0.97	–0.25
Straw								
0.000	5.87±0.13	6.98±0.04	5.55±0.06	3.51±0.04	7.40±0.16	7.23±0.08	4.77±0.05	3.36±0.06
0.017	6.75±0.11	6.38±0.09	5.67±0.10	3.73±0.06	6.97±0.20	7.08±0.10	5.18±0.22	3.66±0.03
0.033	6.67±0.08	7.26±0.07	5.84±0.02	3.20±0.01	7.25±0.06	6.14±0.09	6.05±0.07	3.66±0.05
0.066	6.49±0.11	6.06±0.23	5.45±0.01	3.18±0.05	6.59±0.06	6.35±0.05	4.64±0.07	3.98±0.02
0.664	4.80±0.04	5.75±0.10	5.56±0.02	3.43±0.01	5.18±0.08	4.35±0.04	4.19±0.05	3.85±0.05
Average	6.12±0.09	6.48±0.11	5.61±0.04	3.41±0.03	6.68±0.08	6.23±0.07	4.97±0.09	3.71±0.04
r	–0.89	–0.67	–0.22	0.01	–0.96	0.73	–0.62	–0.34
Basalt meal								
0.000	3.52±0.03	4.43±0.07	2.94±0.05	2.23±0.03	3.87±0.13	6.77±0.02	3.40±0.08	3.09±0.08
0.017	2.44±0.04	4.62±0.02	2.47±0.05	1.78±0.05	3.97±0.08	6.64±0.05	3.93±0.06	3.37±0.01
0.033	2.95±0.03	4.71±0.06	2.73±0.03	1.79±0.02	3.80±0.12	6.76±0.08	4.20±0.07	3.38±0.03
0.066	3.34±0.02	4.59±0.10	2.69±0.01	1.80±0.05	3.79±0.09	6.53±0.11	3.44±0.02	3.27±0.03
0.664	3.35±0.03	3.51±0.04	2.42±0.05	2.14±0.04	3.78±0.13	7.33±0.04	3.53±0.06	3.72±0.02
Average	3.12±0.03	4.37±0.06	2.65±0.04	1.95±0.04	3.84±0.11	6.81±0.06	3.70±0.06	3.37±0.03
r	0.30	–0.97	–0.63	0.42	–0.48	0.34	–0.27	–0.84
$LSD_{0.05}$*	*a* – 0.07, *b* – 0.04, *c* – 0.04, *d* – 0.06, *e* – 0.04, *ab* – 0.10, *ac* – 0.10, *bc* – 0.06, *ad* – 0.12, *bd* – 0.08, *cd* – 0.08, *ae* – 0.10, *be* – 0.06, *ce* – 0.06, *de* – 0.08, *abc* – 0.14, *abd* – 0.17, *acd* – 0.17, *bcd* – n.s., *abe* – 0.14, *ace* – 0.14, *bce* – 0.09, *ade* – 0.17, *bde* – 0.11, *cde* – 0.11, *abcd* – 0.25, *abce* – 0.20, *abde* – 0.25, *acde* – 0.25, *bcde* – 0.16, *abcde* – 0.35							

*LSD values for: *a* – herbicide dose, *b* – soil type, *c* – soil use, *d* – type of neutralizing substance, *e* – date of analysis, significant at $p = 0.05$; n.s. – not significant, *r* – coefficient of correlation, ± – standard deviation.

The activity of alkaline phosphatase varied over time (Table 4), and it reached the highest level on day 50. Aurora 40 WG did not exert a negative effect on alkaline phosphatase. The application of the highest dose of the analyzed herbicide (40-fold higher than recommended) increased alkaline phosphatase activity by 8.5% in loamy sand and by 3.5% in sandy clay loam, in comparison with the control treatment.

Table 2

Urease activity in soil contaminated with the Aurora 40 WG herbicide
[mmol N-NH_4·kg^{-1} d.m.·h^{-1}]

Herbicide dose [mg·kg^{-1}]	Loamy sand				Sandy clay loam			
	Date of analysis (days)							
	Sown treatments		Unsown treatments		Sown treatments		Unsown treatments	
	25	50	25	50	25	50	25	50
Control								
0.000	0.24±0.01	0.17±0.01	0.27±0.02	0.14±0.01	0.89±0.03	0.92±0.01	0.80±0.01	0.74±0.01
0.017	0.25±0.01	0.17±0.01	0.27±0.01	0.13±0.01	0.97±0.02	1.13±0.01	0.74±0.02	0.59±0.01
0.033	0.31±0.01	0.18±0.01	0.26±0.01	0.12±0.01	0.98±0.03	0.96±0.01	0.80±0.02	0.64±0.01
0.066	0.37±0.01	0.16±0.01	0.25±0.05	0.16±0.01	0.89±0.05	0.92±0.01	0.75±0.01	0.74±0.01
0.664	0.30±0.01	0.17±0.01	0.24±0.01	0.15±0.01	0.91±0.03	0.91±0.02	0.77±0.03	0.60±0.02
Average	0.29±0.01	0.17±0.01	0.27±0.02	0.14±0.01	0.93±0.03	0.97±0.01	0.77±0.02	0.66±0.01
r	0.16	0.26	–0.94	–0.67	–0.18	–0.43	–0.02	0.37
Straw								
0.000	0.50±0.02	0.27±0.01	0.46±0.01	0.23±0.01	1.52±0.04	1.19±0.01	1.38±0.02	0.96±0.01
0.017	0.59±0.01	0.27±0.01	0.51±0.07	0.24±0.01	1.65±0.03	1.16±0.02	1.41±0.04	0.98±0.01
0.033	0.60±0.03	0.27±0.01	0.51±0.02	0.39±0.01	1.48±0.03	1.00±0.01	1.60±0.02	1.08±0.01
0.066	0.64±0.02	0.23±0.01	0.50±0.02	0.16±0.01	1.39±0.02	1.17±0.01	1.38±0.02	1.23±0.01
0.664	0.72±0.04	0.21±0.01	0.50±0.02	0.22±0.01	1.54±0.02	1.06±0.01	1.39±0.02	1.08±0.02
Average	0.61±0.02	0.25±0.01	0.50±0.03	0.20±0.01	1.51±0.03	1.12±0.01	1.43±0.02	1.07±0.01
r	0.82	–0.82	0.08	0.19	0.07	–0.46	–0.23	0.32
Basaltmeal								
0.000	0.37±0.03	0.19±0.01	0.23±0.01	0.17±0.01	0.98±0.03	0.96±0.01	0.85±0.01	0.76±0.01
0.017	0.36±0.03	0.19±0.01	0.23±0.02	0.16±0.01	1.06±0.02	0.96±0.01	0.87±0.01	0.68±0.01
0.033	0.32±0.01	0.18±0.01	0.22±002	0.15±0.01	0.94±0.05	1.16±0.01	0.92±0.02	0.81±0.01
0.066	0.34±0.04	0.16±0.01	0.19±0.02	0.15±0.01	0.98±0.02	1.10±0.01	1.10±0.01	0.70±0.01
0.664	0.35±0.02	0.16±0.01	0.18±0.01	0.13±0.01	1.30±0.05	1.28±0.01	0.86±0.09	0.81±0.01
Average	0.35±0.03	0.18±0.01	0.21±0.02	0.15±0.01	1.05±0.03	1.09±0.01	0.92±0.03	0.75±0.01
r	–0.09	–0.66	–0.78	–0.85	0.95	–0.65	–0.23	–0.28
$LSD_{0.05}$*	*a* – 0.004, *b* – 0.002, *c* – 0.002, *d* – 0.003, *e* – 0.002, *ab* – 0.05., *ac* – 0.005, *bc* – 0.003, *ad* – 0.006, *bd* – 0.004, *cd* – 0.004, *ae* – 0.005, *be* – 0.003, *ce* – 0.003, *de* – 0.004, *abc* – 0.008, *abd* – 0.009, *acd* – 0.009, *bcd* – 0.006, *abe* – 0.007, *ace* – 0.007, *bce* – 0.005, *ade* – 0.009, *bde* – 0.006, *cde* – 0.006, *abcd* – 0.012, *abce* – 0.010, *abde* – 0.012, *acde* – 0.012, *bcde* – 0.009, *abcde* – 0.018							

*Explanations cf. Table 1.

The addition of spring barley straw and basalt meal enhanced the activity of alkaline phosphatase, but the stimulating effect of substances was more pronounced in sandy clay loam than in loamy sand. Similarly to the remaining enzymes, the activity of alkaline phosphatase was affected by soil type and soil use. Its activity was 1.9-fold

higher in sandy clay loam than in loamy sand. Regardless of the remaining experimental variables, the sowing of soil treatments with spring barley lowered the activity of alkaline phosphatase.

Table 3

Acid phosphatase activity in soil contaminated with the Aurora 40 WG herbicide [mmol PNP·kg^{-1}·d.m.·h^{-1}]

Herbicide dose [mg·kg^{-1}]	Loamy sand				Sandy clay loam			
	Date of analysis (days)							
	Sown treatments		Unsown treatments		Sown treatments		Unsown treatments	
	25	50	25	50	25	50	25	50
Control								
0.000	0.80±0.01	1.07±0.01	0.91±0.01	0.99±0.01	1.47±0.01	1.30±0.01	1.33±0.01	1.61±0.01
0.017	0.83±0.01	0.88±0.01	0.97±0.02	0.91±0.01	1.50±0.01	1.47±0.01	1.19±0.01	1.51±0.01
0.033	0.69±0.03	1.06±0.01	0.84±0.01	0.92±0.01	1.47±0.01	1.48±0.01	1.30±0.01	1.38±0.06
0.066	0.92±0.01	1.07±0.01	1.14±0.01	1.04±0.01	1.39±0.01	1.40±0.01	1.49±0.08	1.29±0.01
0.664	0.98±0.01	1.11±0.01	0.95±0.01	0.87±0.01	1.38±0.01	1.58±0.01	1.51±0.01	1.27±0.01
Average	0.84±0.01	1.04±0.01	0.96±0.01	0.95±0.01	1.44±0.01	1.45±0.01	1.36±0.02	1.41±0.02
r	0.72	0.44	0.01	0.15	–0.68	0.35	0.65	–0.40
Straw								
0.000	0.96±0.01	1.60±0.01	1.05±0.01	1.13±0.01	1.16±0.01	1.74±0.01	1.52±0.01	1.57±0.01
0.017	1.21±0.01	1.28±0.01	1.10±0.01	1.26±0.01	1.67±0.01	1.75±0.01	1.65±0.01	1.49±0.01
0.033	1.29±0.01	1.19±0.01	1.19±0.01	1.29±0.01	1.76±0.01	1.76±0.01	1.72±0.01	1.58±0.01
0.066	1.29±0.01	1.24±0.01	0.95±0.01	1.21±0.01	1.78±0.01	1.81±0.01	1.83±0.01	1.49±0.01
0.664	1.26±0.01	1.08±0.01	0.96±0.01	1.30±0.01	1.61±0.01	1.57±0.01	1.77±0.01	1.50±0.01
Average	1.20±0.01	1.28±0.01	1.05±0.01	1.24±0.01	1.60±0.01	1.73±0.01	1.70±0.01	1.53±0.01
r	0.29	–0.62	–0.52	0.54	0.10	–0.02	0.41	0.64
Basalt meal								
0.000	0.79±0.01	1.06±0.01	0.85±0.01	1.08±0.01	1.41±0.01	1.45±0.01	1.69±0.01	1.33±0.03
0.017	0.77±0.01	1.14±0.01	0.85±0.01	0.80±0.01	1.31±0.01	1.46±0.01	1.59±0.01	1.39±0.01
0.033	0.96±0.01	1.24±0.01	1.02±0.01	0.78±0.01	1.27±0.01	1.53±0.01	1.57±0.01	1.24±0.01
0.066	0.70±0.01	1.11±0.01	0.94±0.01	0.91±0.01	1.49±0.01	1.66±0.01	1.49±0.01	1.44±0.01
0.664	0.81±0.01	1.04±0.01	0.83±0.01	1.01±0.01	1.70±0.01	1.77±0.01	1.34±0.01	1.10±0.01
Average	0.81±0.01	1.12±0.01	0.90±0.01	0.92±0.01	1.44±0.01	1.57±0.01	1.54±0.01	1.31±0.01
r	–0.03	–0.54	–0.40	0.36	0.88	–0.20	–0.88	0.02
$LSD_{0.05}$*	*a* – 0.01, *b* – 0.01, *c* – 0.01, *d* – 0.01, *e* – 0.01, *ab* – 0.02, *ac* – 0.02, *bc* – 0.01, *ad* – 0.02, *bd* – 0.01, *cd* – 0.01, *ae* – 0.02, *be* – 0.01, *ce* – 0.01, *de* – *n.s.*, *abc* – 0.02, *abd* – 0.03, *acd* – 0.03, *bcd* – 0.02, *abe* – 0.02, *ace* – 0.02, *bce* – 0.02, *ade* – 0.03, *bde* – 0.02, *cde* – 0.02, *abcd* – 0.04, *abce* – 0.03, *abde* – 0.04, *acde* – 0.04, *bcde* – 0.03, *abcde* – 0.06							

*Explanations cf. Table 1.

Table 4

Alkaline phosphatase activity in soil contaminated with the Aurora 40 WG herbicide [mmol PNP·kg^{-1} d.m.·h^{1})

Herbicide dose [mg·kg^{-1}]	Loamy sand				Sandy clay loam			
	Date of analysis (days)							
	Sown treatments		Unsown treatments		Sown treatments		Unsown treatments	
	25	50	25	50	25	50	25	50
Control								
0.000	0.51±0.01	2.58±0.03	0.51±0.01	2.10±0.06	1.20±0.01	3.78±0.06	1.21±0.01	5.24±0.01
0.017	0.52±0.01	2.05±0.01	0.49±0.04	2.21±0.03	1.13±0.01	3.78±0.01	1.16±0.04	4.76±0.07
0.033	0.47±0.03	3.00±0.01	0.58±0.01	2.37±0.20	1.23±0.01	4.18±0.34	1.22±0.01	4.95±0.09
0.066	0.49±0.02	3.01±0.01	0.59±0.03	2.69±0.11	1.16±0.02	3.85±0.05	1.21±0.02	4.89±0.03
0.664	0.46±0.01	2.90±0.07	0.40±0.01	2.39±0.08	1.25±0.01	3.51±0.14	1.44±0.05	5.63±0.03
Average	0.49±0.02	2.71±0.03	0.51±0.04	2.35±0.10	1.19±0.01	3.82±0.12	1.25±0.03	5.09±0.05
r	–0.63	0.31	–0.78	0.65	0.65	–0.05	0.98	–0.72
Straw								
0.000	0.73±0.01	4.01±0.10	0.87±0.01	2.71±0.01	1.41±0.01	5.79±0.01	1.62±0.01	6.19±0.08
0.017	0.73±0.01	3.83±0.02	0.77±0.02	3.12±0.02	1.45±0.01	4.66±0.08	1.38±0.01	4.85±0.03
0.033	0.70±0.03	3.95±0.02	0.80±0.02	3.25±0.02	1.45±0.02	4.50±0.14	1.27±0.01	5.59±0.22
0.066	0.87±0.01	3.76±0.07	0.87±0.01	2.30±0.10	1.38±0.01	4.72±0.03	1.34±0.04	6.03±0.08
0.664	0.79±0.01	3.26±0.04	0.67±0.01	2.75±0.01	1.45±0.01	4.99±0.10	1.37±0.08	5.02±0.26
Average	0.76±0.02	3.76±0.05	0.80±0.01	2.83±0.03	1.43±0.01	4.93±0.07	1.40±0.03	5.54±0.13
r	0.24	–0.96	–0.85	–0.16	0.29	–0.07	–0.16	0.83
Basalt meal								
0.000	0.56±0.01	2.86±0.08	0.42±0.02	2.77±0.03	1.36±0.02	4.82±0.03	1.25±0.03	5.24±0.37
0.017	0.53±0.05	2.94±0.03	0.56±0.04	2.05±0.07	1.35±0.01	4.48±0.14	1.22±0.01	5.54±0.10
0.033	0.68±0.02	2.76±0.08	0.67±0.03	2.43±0.15	1.33±0.01	4.56±0.05	1.24±0.02	5.20±0.16
0.066	0.47±0.03	3.01±0.03	0.56±0.02	2.42±0.01	1.36±0.01	5.03±0.02	1.24±0.01	5.62±0.16
0.664	0.68±0.03	2.88±0.02	0.53±0.01	2.38±0.07	1.06±0.01	4.55±0.14	1.06±0.01	5.91±0.02
Average	0.58±0.02	2.89±0.05	0.55±0.02	2.41±0.07	1.29±0.03	4.67±0.08	1.20±0.02	5.50±0.16
r	0.55	–0.03	–0.07	–0.08	–0.99	–0.65	–0.98	–0.08
$LSD_{0.05}$*	*a* – 0.03, *b* – 0.02, *c* – 0.02, *d* – 0.02, *e* – 0.02, *ab* – 0.04, *ac* – 0.04, *bc* – 0.02, *ad* – 0.04, *bd* – 0.03, *cd* – 0.04, *ae* – 0.04, *be* – 0.02, *ce* – 0.02, *de* – 0.03, *abc* – 0.05, *abd* – 0.06, *acd* – 0.06, *bcd* – 0.04, *abe* – 0.05, *ace* – 0.05, *bce* – 0.03, *ade* – 0.06, *bde* – 0.04, *cde* – 0.04, *abcd* – 0.09, *abce* – 0.07, *abde* – 0.09, *acde* – 0.09, *bcde* – 0.06, *abcde* – 0.12							

*Explanations cf. Table 1.

According to the findings of other authors, the use of herbicides at the recommended doses does not affect the biological activity of soil [29, 30]. The above observations are consistent with the results of this study where the application of the optimum dose of Aurora 40 WG did not modify the enzymatic activity of soil. Soil contamination with excessive quantities of the analyzed product upsets the soil bal-

ance measured by its enzymatic activity [23]. However, Aurora 40 WG applied in overdose did not disturb soil homeostasis.

The enzymatic activity of soil is a parameter used to diagnose the changes in soil contaminated with various chemical compounds. Most assessments of environmental status are based on activity levels of dehydrogenases, urease, acid phosphatase and alkaline phosphatase due to their widespread occurrence and their significant role in the process of transforming organic matter [24].

Dehydrogenases belong to the group of oxidoreductases which contribute to oxidation of organic compounds and participate in respiratory processes of soil-dwelling microorganisms [25]. The results of this study stand in opposition to the findings of Nweke et al. [26] who reported an inhibitory effect of Atriazine 80 W and Northrin 10 EC herbicides on dehydrogenase activity. Similar observations were made by Kucharski and Wyszkowska [27]. In the present study, the Aurora 40 WG herbicide had no negative impact on dehydrogenase activity, which indicates that the product is environmentally friendly, as dehydrogenases are particularly sensitive to soil contamination. Urease, a hydrolase enzyme which catalyzes the hydrolysis of urea in soil, is susceptible to soil changes induced by herbicides. Urease activity is an indicator of the dynamics of transformations of nitrogen compounds in soil as well as nitrogen availability for plants [28]. Increased herbicide concentrations in soil often inhibit urease activity. The above observations were validated by Kucharski and Wyszkowska [27], while Yao et al. [29] did not report significant changes resulting from herbicide use. In our study, urease activity decreased in medium-heavy loam treated with increased doses of the tested herbicide. The change in urease activity, although small, was statistically significant. Urease activity increased in loamy sand following the application of the highest dose of Aurora 40 WG (40-fold higher than the recommended dose). Yet as demonstrated by the results of this study, herbicides may have both an inhibiting and a stimulating effect on urease activity.

The activity of phosphatases responsible for the conversion of organic phosphorus into inorganic phosphates that are easily available for plants and soil-dwelling microbes is a reliable indicator of soil changes induced by herbicides. Some researchers have postulated a correlation between phosphatase activity, soil respiration and microbial biomass [30]. The results of this study indicate that the activity of acid phosphatase and alkaline phosphatase was not affected by the tested herbicide. The application of the highest dose of Aurora 40 WG increased the activity of acid phosphatase and alkaline phosphatase. According to Kucharski and Wyszkowska [27], phosphatases are most resistant to the effect of herbicides.

Soil quality and fertility may be enhanced through the application of nutrients which are vital for the growth of plants and soil-dwelling organisms. In the natural environment, nutrients are supplied as a result of rock weathering and organic matter decomposition. The supply of fresh organic matter not only enhances the soil's biological activity and plant productivity, but it also significantly alleviates the adverse

changes in soils contaminated with chemical substances [31]. This group of substances is inclusive of straw which increases the soil content of nutrients being easily available for plants and microbes. Straw may be applied to enhance soil health and fertility [32]. In our experiment, finely ground spring barley straw improved soil fertility and it had a beneficial influence on all analyzed enzymes. The use of basalt meal also minimizes the adverse effects of soil contamination with chemical substances. In the present study, basalt meal had a positive effect on soil enzymatic activity. It stimulated the activity of urease, acid phosphatase and alkaline phosphatase, and had no significant impact on dehydrogenase activity. Similarly to other naturally occurring minerals such as bentonite and dolomite, basalt meal is used in the restoration of degraded soils as a rich source of nutritional elements. The mineral components of basalt meal are gradually released into the soil solution, supplying plants with the essential nutrients. Basalt meal is also characterized by swelling and water absorption capacity, and it may be used to improve the physical and chemical properties of soil [33]. As shown by the results of this experiment, the type of substance is an important consideration. Barley straw was found to be more effective than basalt meal in restoring the balance of soil biochemical processes.

The effect of herbicides on the soil environment is also evaluated based on its granulometric composition. Soils abundant in organic matter are generally less adversely affected by biocides than nutrient-deficient soils. Compact soils are characterized by a higher content of soil colloids which partially neutralize the negative effect of chemical substances on soil enzymes [34]. Soil humic substances absorb pollutants, and they facilitate and speed up herbicide biodegradation [35]. The results of this study validate the above correlation. Higher activity levels of soil enzymes were noted in sandy clay loam than in loamy sand. The organic substances contained in medium-heavy loam could have a favorable influence on the biological properties of soil, thus stimulating soil enzymatic activity.

The biological activity of soil is also determined by the manner of soil use. Vegetation cover may enhance the enzymatic activity of soil, as demonstrated by the results of this study. Barley sowing positively affected the activity of dehydrogenases, urease and acid phosphatase, causing an insignificant decrease in alkaline phosphatase activity. Organic substances produced by plant roots have a beneficial effect on microorganisms, and they contribute to the soil enzymatic diversity [34].

According to many researchers [22, 27] active ingredients of herbicides may affect biochemical processes, enzymatic activity and microbial growth. These changes pose a risk of soil degradation and a decrease in soil fertility. The results of the present study suggest that not all active ingredients found in herbicides have an equally toxic effect on the environment, in particular on soil. The Aurora 40 WG herbicide which contains carfentrazone-ethyl does not distort the soils enzymatic balance, therefore, it can be regarded an environmentally-friendly product. The analyzed herbicide effectively controls dicotyledonous weeds without posing a threat for soil-dwelling organisms.

4. CONCLUSIONS

The Aurora 40 WG herbicide applied in overdose (i.e. at doses 2-, 4- and 40-fold higher than the optimuml dose) had no significant effect on the enzymatic activity of soil, which indicates that it can effectively control dicotyledonous weeds without altering soil environmental conditions.

The activity of soil enzymes was affected by the soil granulometric composition. Enzymatic activity was higher in sandy clay loam than in loamy sand.

The activity of dehydrogenases, urease and acid phosphatase was enhanced in soil sown with spring barley, while alkaline phosphatase showed higher activity in unsown treatments.

Fluctuations in the enzymatic activity of soil were observed throughout the experimental period.

The use of finely ground spring barley straw and basalt meal generally increased the activity of the investigated soil enzymes. Barley straw was a more effective enhancer of soil fertility.

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