

Jan KUCHARSKI^{1*}, Małgorzata BAĆMAGA²
and Jadwiga WYSZKOWSKA³

DEHYDROGENASE ACTIVITY AS AN INDICATOR OF SOIL CONTAMINATION WITH HERBICIDES

AKTYWNOŚĆ DEHYDROGENAZ JAKO WSKAŹNIK ZANIECZYSZCZENIA GLEBY HERBICYDAMI

Abstract: The effect of soil contamination with the following herbicides: Harpun 500 SC, Faworyt 300 SL, Akord 180 OF and Mocarz 75 WG on the activity of soil dehydrogenases was estimated in a laboratory and pot experiment, in which dehydrogenase activity was determined repeatedly in soil (loamy sand) samples. The herbicides were applied to soil at the manufacturer's recommended doses, and at doses that were 10-, 50-, 100-, 150- and 200-fold higher than recommended. An attempt was also made to alleviate the negative influence of herbicides on dehydrogenases by the addition of bentonite in the amount of 60 g kg⁻¹ d.m. of soil.

It was found that all analyzed herbicides inhibited the activity of soil dehydrogenases. The adverse impact of herbicides was positively correlated with the level of soil contamination, and their inhibitory effect on dehydrogenases was observed over the entire experimental period (112 days) and decreased at a very slow rate. Dehydrogenase activity proved to be a good indicator of the degree of soil contamination with herbicides. Bentonite enhanced the inhibitory effect of herbicides on dehydrogenases.

Keywords: herbicides, soil contamination, dehydrogenase activity, bentonite

The soil environment accumulates a wide variety of synthetic (man-made) chemical substances. The continuous introduction of excessive quantities of xenobiotics into the soil may lead to numerous undesirable changes including the disturbance of the biological balance of soil, which in turn alters the total number of living organisms and the enzymatic activity of soil [1–3]. The group of xenobiotic substances comprises also plant protection chemicals, including herbicides. Apart from their beneficial influence, herbicides can also cause negative sideeffects leading to a decrease in soil fertility [4,

¹ Department of Microbiology, University of Warmia and Mazury in Olsztyn, pl. Łódzki 3, 10–727 Olsztyn, email: jan.kucharski@uwm.edu.pl

² Department of Microbiology, University of Warmia and Mazury in Olsztyn, pl. Łódzki 3, 10–727 Olsztyn, email: m.bacmaga@uwm.edu.pl

³ Department of Microbiology, University of Warmia and Mazury in Olsztyn, pl. Łódzki 3, 10–727 Olsztyn, email: jadwiga.wyszkowska@uwm.edu.pl

5]. Although herbicides are usually designed to be biodegradable, they may nevertheless pose a threat to the natural environment [6]. The adverse impact of herbicides on the soil environment is dependent primarily on the dose applied, frequency of use, environmental persistence, the physicochemical properties of soil, temperature, moisture content, pH and sorption capacity [7]. The toxic effects of herbicides are most often reflected in changes in the quantitative and qualitative composition of microorganisms and in the enzymatic activity of soil [8–13]. Apart from microbial counts, also enzymatic activity is a good measure of the biological activity of soil, since it provides information about changes taking place under the influence of external factors [14–18]. One of the most commonly applied indicators of soil biological activity is the activity dehydrogenases which are most susceptible to biocides [4, 19]. Dehydrogenase activity is also an indirect indicator of soil microbial biomass. Dehydrogenases belong to the group of intracellular enzymes which actively catalyze the oxidation of organic compounds found in the cells of microorganisms. In addition, dehydrogenases represent the class of oxidoreductases, which means that their activity is related to the process of soil microbial respiration [20], and it is affected by soil type and changes in the soil profile depth. These enzymes are also considered to be sensitive indicators of soil quality and key biomarkers of alterations in soil metabolism caused by anthropogenic factors [21]. The analysis of dehydrogenase activity is particularly recommended when newly developed herbicides are to be introduced to agricultural practice.

In view of the above, the objective of this study was to determine the effect of soil contamination with new generation herbicides, Harpun 500 SC, Faworyt 300 SL, Akord 180 OF and Mocarz 75 WG, on the activity of soil dehydrogenases.

Material and methods

The study consisted of two experiments, a laboratory experiment and a pot experiment. Both experiments were designed to determine the effect of four herbicides, Harpun 500 SC, Faworyt 300 SL, Akord 180 OF and Mocarz 75 WG, on dehydrogenase activity. The characteristics of the tested herbicides are presented in Table 1. The experimental materials comprised samples of soil classified under natural conditions as typical brown soil developed from loamy sand, with the following physicochemical properties: $\text{pH}_{\text{KCl}} - 6.5$, hydrolytic acidity $- 8.25 \text{ mmol}(+) \text{ kg}^{-1}$, sum of exchangeable cations $- 78 \text{ mmol}(+) \text{ kg}^{-1}$, organic carbon content $C_{\text{org}} - 6.3 \text{ g kg}^{-1}$.

The laboratory experiment was performed in three replications. 100 cm^3 beakers were filled with 100 g of air-dried soil. Variable experimental factors were as follows: I – herbicide type: Harpun 500 SC, Faworyt 300 SL, Akord 180 OF, Mocarz 75 WG; II – herbicide dose: 0 – control, 1 – manufacturer's recommended dose, doses 50-, 100-, 150- and 200-fold higher than recommended; III – soil incubation time (days): 28, 56, 84 and 112. Soil was mixed with herbicides in beakers and moisture content was brought to 60 % of the capillary water capacity with the use of deionized water. Samples were incubated at a temperature of 25 °C for a specified period of time (experimental factor III) and dehydrogenase activity was determined.

Table 1

Characteristics of the herbicides used in the study

Herbicide	Active ingredient		Recommended dose [dm ³ ha ⁻¹] [kg ha ⁻¹]	Recommended dose [mm ³ kg ⁻¹] [μg kg ⁻¹]
	Name	[%] [g kg ⁻¹]		
Harpun 500 SC	isoproturon	500 g	2.5 dm ³	0.83 mm ³
Faworyt 300 SL	chloryralid	300 g	0.35 dm ³	0.12 mm ³
Akord 180 OF	phenmediphan desmediphan ethofumesate	60 g 60 g 60 g	5 dm ³	0.16 mm ³
Mocarz 75 WG	tritosulphuron dicamba	25 % 50 %	0.2 kg	6.66 μg

The pot experiment was performed in five replications. Polyethylene pots were filled with 3 kg of soil. The first two experimental factors, ie the type and dose of herbicides, were identical as in the laboratory experiment, while the third factor – time of analysis – was limited to 25 and 50 days. The fourth variable experimental factor was bentonite dose: 0 and 60 g kg⁻¹ d.m. of soil. Before filling the pots, soil was mixed with an appropriate dose of herbicides and with mineral fertilizers. Spring barley cv. Rabel (12 plants), spring rape cv. Sponsor (12 plants), oat cv. Kasztan (12 plants) and carrot cv. Kalina (5 plants), were sown in pots containing soil contaminated with the herbicides Harpun 500 SC, Faworyt 300 SL, Mocarz 75 and WG Akord 180 OF, respectively.

In the experiment with spring rape, spring barley and oat, fertilization levels were as follows [mg kg⁻¹ soil]: N – 100 (CO(NH₂)₂), P – 35 (KH₂PO₄), K – 100 (KH₂PO₄ + KCl), Mg – 20 (MgSO₄ · 7H₂O), Cu – 5 (CuSO₄ · 5H₂O), Zn – 5 (ZnCl₂), Mn – 5 (MnCl₂ · 4H₂O), Mo – 5 (Na₂MoO₄ · 2H₂O) and B – 0.33 (H₃BO₄). In the experiment with carrot, fertilization levels were almost identical as in the experiments with cereals and spring rape, only the rates of P and K were increased to 44 mg kg⁻¹ and 120 mg kg⁻¹, respectively.

Over the experimental period soil moisture content was maintained at a stable level equal to 60 % of the capillary water capacity. On day 25 and 50 soil samples were assayed for dehydrogenase activity. Both in the laboratory and pot experiment the activity of these enzymes was determined by the Lenhard method modified by Öhlinger et al [22], and it was expressed in [cm³ H₂ kg⁻¹ d.m. soil d⁻¹].

The results were processed statistically by Duncan's test. A statistical analysis was performed with the use of Statistica software [23].

Results and discussion

In the laboratory experiment the activity of dehydrogenases in soil not contaminated with weed killers was dependent on the duration of the experiment (Table 2). In control treatments the highest and the lowest activity of these enzymes was observed on day 28 and 112, respectively. On day 112 dehydrogenase activity was 2.2-fold lower than on day 28.

Table 2

Dehydrogenase activity in soil contaminated with herbicides as dependent on herbicide type and dose, and time of analysis, $\text{cm}^3 \text{H}_2 \text{kg}^{-1} \text{d.m. d}^{-1}$ (laboratory experiment)

Herbicide dose*	Time of soil incubation [days]			
	28	56	84	112
Harpun 500 SC				
0	3.57 ± 0.08	3.23 ± 0.05	3.05 ± 0.05	1.74 ± 0.04
1	3.56 ± 0.07	2.83 ± 0.06	2.43 ± 0.06	1.73 ± 0.07
50	3.73 ± 0.08	2.74 ± 0.05	2.40 ± 0.08	1.46 ± 0.05
100	3.83 ± 0.12	2.02 ± 0.14	2.06 ± 0.10	1.32 ± 0.08
150	3.83 ± 0.12	1.88 ± 0.06	1.84 ± 0.10	1.22 ± 0.04
200	3.72 ± 0.11	1.76 ± 0.10	1.61 ± 0.10	0.91 ± 0.07
r	0.60	-0.95	-0.97	-0.95
Faworyt 300 SL				
0	3.57 ± 0.08	3.23 ± 0.05	3.05 ± 0.05	1.74 ± 0.04
1	1.46 ± 0.07	2.47 ± 0.07	2.14 ± 0.07	1.69 ± 0.04
50	1.43 ± 0.06	2.45 ± 0.09	2.11 ± 0.10	1.59 ± 0.06
100	1.38 ± 0.05	2.38 ± 0.05	2.05 ± 0.11	1.55 ± 0.08
150	1.36 ± 0.07	1.74 ± 0.12	2.07 ± 0.10	1.51 ± 0.09
200	1.36 ± 0.04	1.70 ± 0.06	1.98 ± 0.09	1.48 ± 0.04
r	-0.64	-0.94	-0.70	-0.93
Akord 180 OF				
0	3.57 ± 0.08	3.23 ± 0.05	3.05 ± 0.05	1.74 ± 0.04
1	1.92 ± 0.05	2.34 ± 0.07	2.32 ± 0.08	1.78 ± 0.11
50	1.67 ± 0.04	1.62 ± 0.07	1.61 ± 0.08	1.81 ± 0.05
100	1.67 ± 0.04	1.25 ± 0.07	1.44 ± 0.06	1.72 ± 0.07
150	1.67 ± 0.07	1.16 ± 0.09	1.39 ± 0.04	1.64 ± 0.09
200	1.68 ± 0.09	1.07 ± 0.06	1.11 ± 0.03	1.26 ± 0.09
r	-0.65	-0.85	-0.86	-0.83
Mocarz 75 WG				
0	3.57 ± 0.09	3.23 ± 0.05	3.05 ± 0.05	1.74 ± 0.04
1	2.21 ± 0.03	2.14 ± 0.05	1.76 ± 0.03	1.59 ± 0.06
50	2.11 ± 0.05	2.15 ± 0.07	1.65 ± 0.04	1.58 ± 0.06
100	2.03 ± 0.07	2.15 ± 0.08	1.27 ± 0.10	1.34 ± 0.05
150	2.00 ± 0.09	2.12 ± 0.08	1.19 ± 0.07	1.29 ± 0.05
200	1.77 ± 0.06	1.93 ± 0.09	1.17 ± 0.02	1.28 ± 0.10
r	-0.77	-0.72	-0.82	-0.94
LSD _{0.01} **	<i>a</i> - 0.01; <i>b</i> - 0.02; <i>c</i> - 0.01; <i>a</i> · <i>b</i> - 0.03; <i>a</i> · <i>c</i> - 0.03; <i>b</i> · <i>c</i> - 0.03; <i>a</i> · <i>b</i> · <i>c</i> - 0.07			

* 0 – control sample (not contaminated with herbicides); 1 – manufacturer's recommended dose; doses 50-, 100-, 150- and 200-fold higher than recommended;

** LSD for: *a* – herbicide type; *b* – herbicide dose; *c* – time of analysis;

r – coefficient of correlation.

Soil contamination with herbicides had an adverse effect on dehydrogenase activity, which was found to vary over time (Table 2). At the beginning of the experiment (day 28), the herbicide Harpun 500 SC applied at doses 50- and 200-fold higher than recommended stimulated dehydrogenase activity. When applied at the highest dose (200-fold higher than recommended), this herbicide increased dehydrogenase activity by 4 %, as compared with the control treatment. Only the manufacturer's recommended dose did not cause significant changes in the activity of these enzymes. On day 56 the optimum dose of the herbicide decreased dehydrogenase activity by 12 %, while the dose 200-fold higher than the optimum dose – by as much as 46 %. On day 84 and 112 the adverse effect of Harpun 500 SC on dehydrogenases was similar as on day 56, as confirmed by negative coefficients of correlation between herbicide dose and enzymatic activity.

The second of the tested herbicides, Faworyt 300 SL, decreased dehydrogenase activity even when applied at the recommended dose: by 59 % on day 28, by 24 % on day 56, by 30 % on day 84 (Table 2). The negative impact of this dose was not observed only on day 112 of the experiment. Dehydrogenase activity was adversely affected by higher doses of this herbicide during the entire experiment, although their effect diminished considerably over the soil incubation period.

Dehydrogenases were also sensitive to Akord 180 OF (Table 2). On experimental day 28 all analyzed doses of this herbicide decreased dehydrogenase activity approximately twofold, in comparison with the control treatment. The highest dose of Akord 180 OF reduced dehydrogenase activity threefold on day 56, 2.7-fold on day 84 and 1.4-fold on day 112.

Mocarz 75 WG was also found to be a strong inhibitor of dehydrogenases (Table 2). The inhibitory effect of this herbicide on dehydrogenase activity was reported following the application of both the manufacturer's recommended dose and increased doses. In soil contaminated with the highest dose of Mocarz 75 WG (200-fold higher than recommended), dehydrogenase activity was inhibited by 38 % on day 28, by 40 % on day 56, by 62 % on day 84 and by 26 % on day 112.

All tested herbicides strongly inhibited the activity of dehydrogenases also in the pot experiment (Table 3). Their adverse impact was observed with respect to both increased and recommended doses. In this experiment Faworyt 300 SL exerted the slightest negative effect. Harpun 500 SC applied at the lowest dose decreased enzymatic activity by 37 % on average on day 25 and by only 8 % on day 50. In treatments contaminated with a dose 200-fold higher than recommended, dehydrogenase activity decreased 2.8-fold and 2.5-fold on day 25 and 50 respectively, compared with the control sample.

Akord 180 OF and Mocarz 75 WG, applied at the recommended dose, decreased dehydrogenase activity on average by 44 % and 50 % respectively, regardless of the time of analysis and bentonite addition. Higher doses of the above herbicides had an even greater negative influence on dehydrogenases. The adverse impact of the tested herbicides on dehydrogenase activity was enhanced by the addition of bentonite which was also found to be a strong inhibitor of these enzymes under the experimental conditions.

Table 3

Dehydrogenase activity in soil contaminated with herbicides as dependent on herbicide type and dose, time of analysis and bentonite dose, $\text{cm}^3 \text{H}_2 \text{kg}^{-1} \text{d.m. d}^{-1}$ (pot experiment)

Herbicide dose*	Bentonite dose [g kg^{-1} soil]			
	0		60	
	Time of analysis [days]			
	25	50	25	50
Harpun 500 SC				
0	3.12 ± 0.05	2.65 ± 0.06	1.73 ± 0.06	1.68 ± 0.06
1	3.03 ± 0.03	2.54 ± 0.06	0.59 ± 0.06	1.42 ± 0.06
10	1.57 ± 0.09	1.98 ± 0.06	0.53 ± 0.06	0.96 ± 0.06
50	1.51 ± 0.06	1.51 ± 0.06	0.50 ± 0.06	0.95 ± 0.06
100	1.30 ± 0.03	1.28 ± 0.06	0.49 ± 0.03	0.56 ± 0.06
150	1.28 ± 0.03	1.26 ± 0.06	0.49 ± 0.03	0.55 ± 0.03
200	1.26 ± 0.06	1.20 ± 0.06	0.47 ± 0.03	0.51 ± 0.06
r	-0.75	-0.84	-0.56	-0.88
Faworyt 300 SL				
0	3.12 ± 0.05	2.65 ± 0.06	1.73 ± 0.06	1.68 ± 0.06
1	2.51 ± 0.03	2.76 ± 0.06	1.64 ± 0.06	1.63 ± 0.03
10	2.42 ± 0.06	3.28 ± 0.06	1.57 ± 0.03	1.66 ± 0.09
50	1.98 ± 0.03	3.76 ± 0.09	1.49 ± 0.03	1.94 ± 0.06
100	1.94 ± 0.03	3.14 ± 0.03	1.48 ± 0.03	1.96 ± 0.06
150	1.78 ± 0.07	3.51 ± 0.07	1.47 ± 0.06	2.46 ± 0.03
200	1.60 ± 0.06	3.48 ± 0.07	0.91 ± 0.06	2.44 ± 0.06
r	-0.87	0.59	-0.88	0.93
Akord 180 OF				
0	3.12 ± 0.05	2.65 ± 0.06	1.73 ± 0.06	1.68 ± 0.06
1	1.24 ± 0.06	1.93 ± 0.06	0.44 ± 0.06	1.50 ± 0.03
10	0.96 ± 0.06	1.79 ± 0.03	0.37 ± 0.03	1.25 ± 0.06
50	0.73 ± 0.06	1.71 ± 0.03	0.32 ± 0.03	1.17 ± 0.06
100	0.71 ± 0.03	1.71 ± 0.06	0.34 ± 0.06	1.16 ± 0.03
150	0.68 ± 0.06	1.70 ± 0.03	0.34 ± 0.06	1.00 ± 0.03
200	0.67 ± 0.03	1.64 ± 0.03	0.36 ± 0.03	1.00 ± 0.03
r	-0.59	-0.61	-0.53	-0.87
Mocarz 75WG				
0	3.12 ± 0.05	2.65 ± 0.06	1.73 ± 0.06	1.68 ± 0.06
1	1.22 ± 0.07	1.84 ± 0.03	0.68 ± 0.06	0.89 ± 0.03
10	1.12 ± 0.06	1.66 ± 0.03	0.33 ± 0.03	0.62 ± 0.06
50	0.95 ± 0.06	1.42 ± 0.09	0.30 ± 0.03	0.61 ± 0.06
100	0.95 ± 0.06	1.39 ± 0.10	0.26 ± 0.03	0.43 ± 0.06

Table 3 contd.

Herbicide dose*	Bentonite dose [g kg ⁻¹ soil]			
	0		60	
	Time of analysis [days]			
	25	50	25	50
150	0.74 ± 0.06	1.15 ± 0.06	0.26 ± 0.03	0.42 ± 0.03
200	0.63 ± 0.06	0.96 ± 0.03	0.13 ± 0.06	0.40 ± 0.06
r	-0.69	-0.86	-0.68	-0.74
LSD _{0.05} **	<i>a</i> - 0.02; <i>b</i> - 0.02; <i>c</i> - 0.01; <i>d</i> - 0.01; <i>a</i> · <i>b</i> - 0.04; <i>a</i> · <i>c</i> - 0.02; <i>b</i> · <i>c</i> - 0.03; <i>a</i> · <i>d</i> - 0.02; <i>b</i> · <i>d</i> - 0.03; <i>c</i> · <i>d</i> - 0.02; <i>a</i> · <i>b</i> · <i>c</i> - 0.06; <i>a</i> · <i>b</i> · <i>d</i> - 0.06; <i>a</i> · <i>c</i> · <i>d</i> - 0.03; <i>b</i> · <i>c</i> · <i>d</i> - 0.04; <i>a</i> · <i>b</i> · <i>c</i> · <i>d</i> - 0.08			

* 0 – control sample (not contaminated with herbicides); 1 – manufacturer's recommended dose; doses 10-, 50-, 100-, 150- and 200-fold higher than recommended;

** LSD for: *a* – herbicide type; *b* – herbicide dose; *c* – time of analysis; *d* – bentonite dose; *r* – coefficient of correlation.

Cases of soil metabolism disorders caused by crop protection chemicals, particularly when present in excessive concentrations, have been also reported by other authors [11, 24–28]. Soil enzymatic activities are reliable indicators of the state of the natural environment [12, 16]. The present study showed that dehydrogenase activity can be considered a good measure of the effect of herbicides (Harpun 500 SC, Faworyt 300 SL, Akord 180 OF, Mocarz 75 WG) on the soil environment, since the activity of these enzymes is usually positively correlated with microbial counts [11]. Changes in individual microbiological and biochemical properties of soil, especially those induced by the application of novel pesticide products, should be closely monitored. According to Singh and Singh [29], these agents act not only on the target organism, but also affect non-target organisms, thus contributing to environmental deterioration.

The results of this study indicate that excessive loads of the herbicides Harpun 500 SC, Faworyt 300 SL, Akord 180 OF and Mocarz 75 WG may disturb the biological balance of soil, and that their toxic effects are difficult to neutralize. The adverse impact of the above herbicides on dehydrogenase activity was observed for a relatively long time, ie for 112 days of the experiment, and the attempt to alleviate their negative influence on dehydrogenases by the addition of bentonite to soil proved unsuccessful.

Conclusions

1. The herbicides Harpun 500 SC, Faworyt 300 SL, Akord 180 OF and Mocarz 75 WG may significantly decrease dehydrogenase activity in the soil environment, particularly when applied in excessive quantities.

2. Bentonite was found ineffective for alleviating the negative impact of herbicides on dehydrogenases, since it proved to be a potent inhibitor of the activity of these enzymes.

3. The inhibitory effect of the tested herbicides on dehydrogenase activity diminished over time, but the process was not dynamic.

4. Dehydrogenase activity may be a good indicator of the level of soil contamination with herbicides.

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AKTYWNOŚĆ DEHYDROGENAZ JAKO WSKAŹNIK ZANIECZYSZCZENIA GLEBY HERBICYDAMI

Katedra Mikrobiologii, Uniwersytet Warmińsko-Mazurski w Olsztynie

Abstrakt: W celu określenia wpływu zanieczyszczenia gleby herbicydami: Harpun 500 SC, Faworyt 300 SL, Akord 180 OF i Mocarz 75 WG na aktywność dehydrogenaz glebowych wykonano dwa doświadczenia: laboratoryjne i wegetacyjne (wazonowe), w których kilkakrotnie określano aktywność dehydrogenaz w glebie (piasku gliniastym). Herbicydy stosowano dogłębowo w dawkach zalecanych przez producenta oraz w dawkach: 10, 50, 100, 150 i 200-krotnie większej. Podjęto także próbę złagodzenia negatywnego oddziaływania herbicydów na dehydrogenazy poprzez dodanie do gleby bentonitu w ilości 60 g kg⁻¹ s.m. gleby.

W wyniku badań stwierdzono, że wszystkie herbicydy hamowały aktywność dehydrogenaz glebowych. Ich negatywne oddziaływanie było dodatnio skorelowane ze stanem zanieczyszczenia gleb, a inhibicyjne działanie na dehydrogenazy utrzymywało się przez cały okres badań (112 dni) i zmniejszało się bardzo powoli. Aktywność dehydrogenaz okazała się dobrym wskaźnikiem oceny stanu zanieczyszczenia gleb herbicydami. Zastosowany bentonit zwiększał inhibicyjne oddziaływanie herbicydów na dehydrogenazy.

Słowa kluczowe: herbicydy, zanieczyszczenie gleby, aktywność dehydrogenaz, bentonit