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Original Research

The Effect of Chlorpyrifos and Teflubenzuron on the Enzymatic Activity of Soil

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Abstract

The aim of our study was to determine changes in soil quality, evaluated throughout the analysis of selected soil enzyme activities (dehydrogenases, urease, alkaline phosphatase, acid phosphatase, and catalase) after the application of the insecticides chlorpyrifos and teflubenzuron. Chlorpyrifos is a phosphoorganic insecticide and a cholinesterase inhibitor. Teflubenzuron is a benzoyl urea derivative that inhibits chitin synthesis.

The pot experiment involved the two types of insecticides at different doses and sampling of two types of soil. The results of the experiment indicate that both insecticides modified the biochemical parameters of soil. Dehydrogenases proved to be most sensitive to the presence of xenobiotics in soil. Their activity decreased at increasing levels of soil contamination for each of the insecticides. In comparison with teflubenzuron, chlorpyrifos reduced the activity of dehydrogenases, urease, and alkaline phosphatase to a greater extent.

Keywords: activity of soil enzymes, insecticides, soil contamination

Introduction

Arable soils have been subjected to various types of cultivation treatment for many years, including pest control [1]. None of the chemical substances applied in the past decades have the features of an ideal pesticide. An ideal pesticide should eliminate only selected organisms, it should be biodegradable, and it should not contaminate groundwater [2]. Pesticide treatments are applied several times during the growing season, so their effect on the environment [1], including soil as the place of pesticide accumulation, should be analyzed. Soil is the habitat of microorganisms responsible for the circulation of elements in the natural environment and the preservation of soil fertility. The presence of toxic substances in the soil affects the life cycle of microbes, which has a direct or an indirect effect on their activity, soil fertility, and the cultivated crops [3].

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The biochemical properties of soil are commonly used as an indicator of soil quality and the effect exerted by toxic substances [4]. Changes in the enzymatic activity of soil are a sensitive parameter illustrating changes in soil quality and the activity of soil microorganisms related to nutrient metabolism [5]. Soil enzymes are increasingly often regarded as an early indicator of changes in soil quality, resulting from cultivation and standard farming practices [6]. According to Singh and Kumar [7], the most frequently analyzed soil enzymes are: dehydrogenases, catalase, phosphatase, amylase, cellulase, protease, urease, and others. The majority of soil enzymes are produced by soil-dwelling bacteria and fungi, while only a small fraction is excreted by other organisms, i.e. plants and animals.

As noted by Gianfreda et al. [8], soil enzymes respond quickly to changes in natural and anthropogenic factors that affect soil. There exists a vast body of research investigating the response of soil enzymes to environmental pollution with heavy metals [9], petroleum hydrocarbons [10], and

pesticides. Chlorpyrifos, produced since 1965 [11], is an ingredient of numerous commercial products. The effects of chlorpyrifos have been thoroughly researched, but a few studies investigated the influence of this insecticide on soil enzymatic activity. The impact of teflubenzuron has not been analyzed in detail, either. Studies into the effects of the above xenobiotics on the enzymatic activity of soil, which serves as a microbiological indicator of its quality [12], will expand our knowledge of the impact of plant protection chemicals on soil quality.

The objective of this study was to evaluate the effect of soil contamination with chlorpyrifos and teflubenzuron on the activity of the following soil enzymes: dehydrogenases, urease, alkaline phosphatase, acid phosphatase and catalase.

Materials and Methods

Two insecticides were investigated in the experiment. The active ingredient of the first insecticide was chlorpyrifos – O,O-diethyl-O-(3,5,6-trichloro-2-pyridyl)phosphorothioate. In this study, chlorpyrifos, a phosphoorganic insecticide, was administered in the form of a commercially available product, Dursban 480 EC, supplied by Dow AgroSciences LLC. The chlorpyrifos content of the product was 480 $g \cdot dm^{-3}$. Chlorpyrifos is used to control pests affecting sugar beets, fodder beets, tobacco, orchard plants, vegetables, ornamental plants, and in forests. It affects the plant at surface and deep levels. Chlorpyrifos is a cholinesterase inhibitor.

The other insecticide was teflubenzuron, a benzoyl urea derivative, 1-(3,5-dichloro-2,4-difluorophenyl)-3-(2,6-difluorobenzoyl), applied in the form of a commercial preparation, Nomolt 150 SC, at 150 $g \cdot dm^{-3}$. This substance shows gastric activity, and it is used to control the earliest larval stadia of pests affecting farming crops, orchard plants, vegetables, ornamental plants, and forests. The product is used for plant spraying and watering, and seed dressing. Teflubenzuron eliminates pests by inhibiting chitin synthesis [13]. The insecticides were added to soil samples on the day the pot experiment was started at the following single doses: 0.05, 0.5, 5.0, 50, and 500 per $mg \cdot kg^{-1}$ d.m. soil on active ingredient basis.

Two types of soil collected from the humus horizon (0-20 cm) at the Experimental Station of the University of Warmia and Mazury in Tomaszkowo were used in the study: brown soil with the granulometric composition of loamy sand and brown soil with the granulometric composition of sandy loam. Soil characteristics are presented in Table 1. Soil texture was determined by the Cassagrande method modified by Prószyński [14]. Soil acidity in a water solution of KCl (1 $mol \cdot dm^{-3}$) was determinated with a potentiometer. Hydrolytic acidity and exchangeable bases was determined with Kappen's method, and the content of organic carbon with Tiurin's method [14].

The experiment was carried out in the greenhouse of the University of Warmia and Mazury in Olsztyn. Soil samples weighing 3.2 kg each were mixed with mineral fertilizers and the investigated insecticides, and were placed in polyethylene pots. Soil without the addition of insecticides served as a control sample. The following mineral fertilizers were applied, in terms of pure ingredients ($g \cdot kg^{-1}$ soil): macronutrients – 0.12 N [$CO(NH_2)_2$], 0.05 P (KH_2PO_4), 0.12 K (KH_2PO_4+KCl), and 0.025 Mg ($MgSO_4 \cdot 7H_2O$); micronutrients ($mg \cdot kg^{-1}$ of soil) – 5.0 Zn ($ZnSO_4 \cdot 7H_2O$), 5.0 Cu ($CuSO_4 \cdot 5H_2O$), 5.0 Mn ($MnCl_2 \cdot 5H_2O$), 5.0 Mo ($Na_2MoO_4 \cdot 2H_2O$), and 0.33 B (H_3BO_3).

Table 1. Physicochemical properties of soil used in the experiment.

	Loamy sand	Sandy loam
Sand (%) 2.0-0.05 mm	78.0	54.0
Silt (%) 0.05-0.002 mm	15.0	27.0
Clay (%) >0.002 mm	7.0	19.0
pH_{KCl}	6.6	6.7
C_{org} $g \cdot kg^{-1}$	8.50	8.85
Exchangeable bases ($mmol^{+} \cdot kg^{-1}$)	146.33	166.33
Hydrolytic acidity ($mmol^{+} \cdot kg^{-1}$)	10.50	9.69

On the first day of the experiment, 15 seeds of spring rapeseed cv. Huzar were sown in each pot. After germination (day 5), 12 plants were left per pot. Each treatment had three replications. Since soil samples were collected on two different days, replications were run for each of those days separately. Throughout the entire experiment, the moisture content of soil was maintained at 60% capillary water capacity with the use of demineralized water. Soil samples were collected on experimental day 10 (date I) and 50 (date II) for biochemical analyses. Each sample comprised soil collected from three pots, across the entire soil depth, by the Egner-Riehm method. A 300 gram sample was collected from each pot. Soil sampled in three replications was thoroughly mixed and placed in plastic bags. The pots from which soil was sampled were removed on day 10 of the experiment. The samples were stored at 4ºC. All biochemical analyzes were performed in three replications.

The activities of all enzymes, except for catalase, were determined with a Perkin-Elmer Lambda 25 spectrophotometer [9]. Dehydrogenase activity was determined by the Öhlinger method [15]. The applied substrate was a 3% aqueous solution of 2,3,5-triphenyl tetrazolium chloride (TTC). Soil samples and the substrate were incubated at 37ºC for 24 hours. The produced triphenyl formazan (TFF) was determined spectrophotometrically at a wavelength of 485 nm. Triphenyl formazan concentrations were expressed as mmol $TFF \cdot kg^{-1}$ d.m. $soil \cdot d^{-1}$. Urease activity was analyzed by the method proposed by Alef and Nannipieri [16]. The applied substrate was urea (10% aqueous solution). Soil samples with urea and citrate buffer (pH 6.7) were incubated at 37ºC for 24 h. The $N\text{-}NH_4$ content after reaction with Nessler's reagent was determined spectrophoto-

metrically at a wavelength of 410 nm. $N\text{-}NH_4$ concentrations were expressed as mmol $N\text{-}NH_4 \cdot kg^{-1}$ d.m. $soil \cdot h^{-1}$. The activity of acid phosphatase and alkaline phosphatase was determined using the method proposed by Alef et al. [17]. The applied substrate was a 0.1 M p-nitrophenyl phosphate solution (pH 6.5 for acid phosphatase and pH 11 for alkaline phosphatase). The quantity of p-nitrophenol (PNP) obtained during 1 hour of soil sample incubation at 37ºC was determined spectrophotometrically at a wavelength of 410 nm. Phosphatase activity was expressed as mmol $PNP \cdot kg^{-1}$ d.m. $soil \cdot h^{-1}$. Catalase activity was determined by the method described by Alef and Nannipieri [16]. The reaction substrate was an aqueous solution of H_2O_2. The soil and the substrate solution were shaken for 20 minutes. The quantity of the resulting O_2 was determined using potassium permanganate. Catalase activity was expressed as mol $O_2 \cdot kg^{-1}$ d.m. $soil \cdot h^{-1}$.

The results were processed statistically by Duncan's multiple range test and a four-factorial analysis of variance. Correlation coefficients were determined between the insecticide dose and enzyme activity. Statistical analysis was performed using Statistica [18].

Results and Discussion

Soil enzymes are a sensitive indicator of unique biological and biochemical interactions in soil resulting from anthropogenic, agronomic, chemical and weather changes [7]. In this experiment, soil contamination with insecticides modified the biochemical parameters of the soil. Even the lowest insecticide doses affected soil enzyme activities. However, the effects were positive or negative, depending on the enzyme tested and the applied xenobiotic dose (Fig. 1).

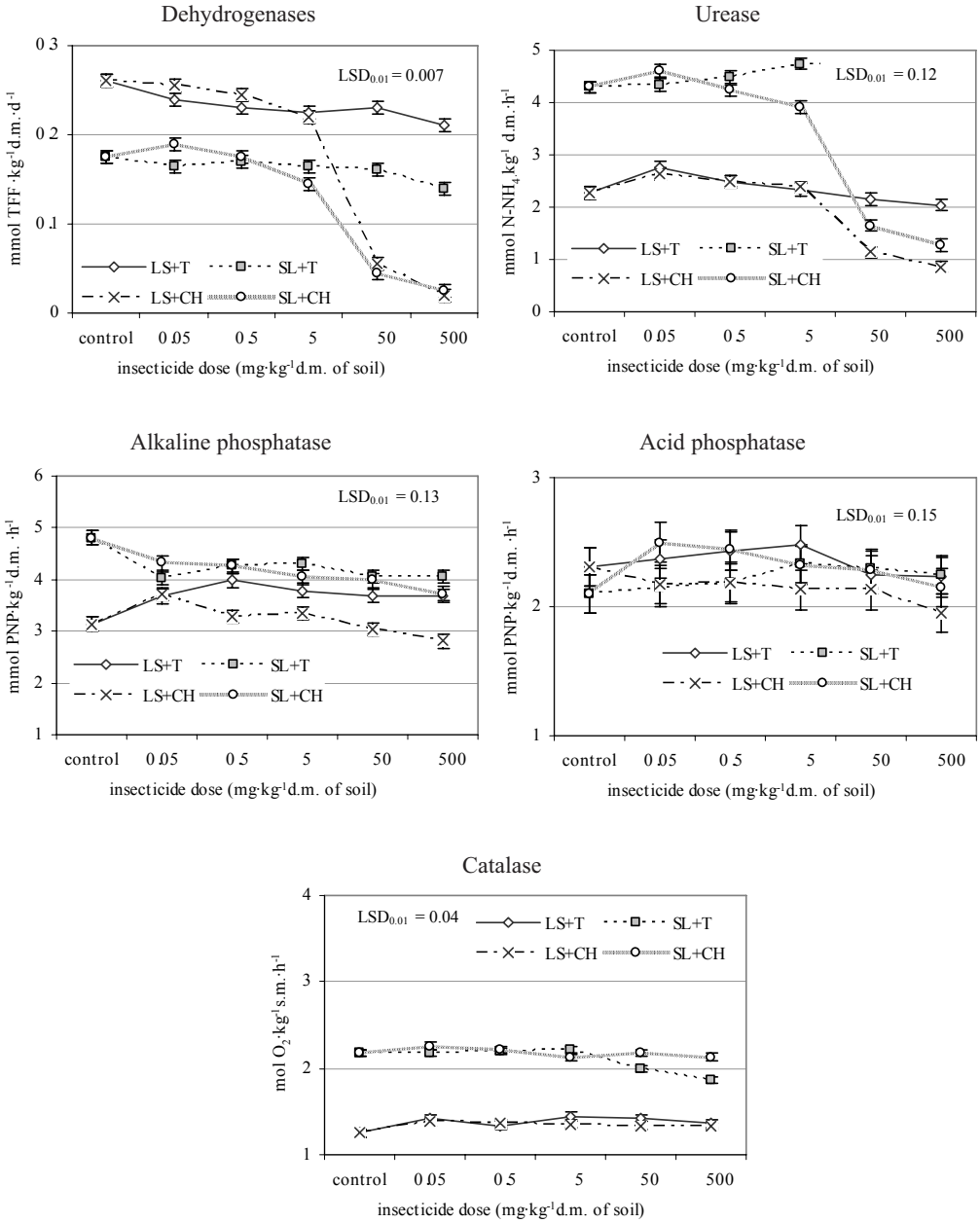

Fig. 1. The effect of insecticide dose and soil type on soil enzymatic activity.
LS – loamy sand, SL – sandy loam, CH – chlorpyrifos, T – teflubenzuron, LSD – least significant differences

Table 2. Activity of dehydrogenases in soil contaminated with insecticides (mmol TFF·kg^{-1} s.m.·d^{-1}).

Insecticide dose (mg·kg^{-1} d.m. of soil)	Soil type			
	Loamy sand		Sandy loam	
	Sampling			
	day 10	day 50	day 10	day 50
control	0.21±0.01	0.31±0.01	0.15±0.01	0.20±0.00
teflubenzuron				
0.05	0.22±0.00	0.26±0.00	0.14±0.00	0.19±0.00
0.5	0.21±0.00	0.25±0.00	0.15±0.00	0.19±0.00
5	0.21±0.01	0.24±0.00	0.14±0.00	0.19±0.00
50	0.21±0.00	0.25±0.00	0.14±0.00	0.18±0.00
500	0.21±0.01	0.21±0.00	0.10±0.02	0.18±0.00
r	-0.15	-0.88*	-0.39	-0.70*
chloropirofos				
0.05	0.21±0.00	0.30±0.00	0.14±0.01	0.24±0.00
0.5	0.21±0.00	0.28±0.00	0.14±0.01	0.21±0.01
5	0.19±0.01	0.25±0.00	0.10±0.01	0.19±0.00
50	0.06±0.00	0.05±0.00	0.05±0.00	0.04±0.01
500	0.01±0.00	0.03±0.02	0.03±0.00	0.02±0.00
r	-0.88*	-0.90*	-0.92*	-0.81*
$LSD_{0.01}$	0.01			

LSD – least significant differences; r – correlation coefficient, * values of the correlation coefficient significant at $p<0.05$.

In addition, the interaction effects were significant, and thus soil enzymatic activity was affected by the type and dose of insecticide, date of sampling, and soil type.

Dehydrogenases were most sensitive to the presence of xenobiotics in soil. Regardless of the date of analysis, dehydrogenase activity decreased significantly with an increase in soil contamination levels in both loamy sand and sandy loam, in comparison with control. The highest teflubenzuron dose (500 mg·kg^{-1} d.m. soil) decreased dehydrogenase activity by 20% in all soil types, while the highest chlorpyrifos dose decreased the investigated enzyme's activity by 92% (loamy sand) and 86% (sandy loam) in comparison with non-contaminated soil. The effect of insecticides on the activity of urease, alkaline phosphatase, acid phosphatase and catalase was determined by the dose of the applied xenobiotic. In many experimental treatments enzyme activity was stimulated, in particular at the lowest xenobiotic doses (Fig. 1).

The findings of other authors suggest that crop protection chemicals modify the biological activity of soil [1, 7, 19-22], which is manifested by changes in enzyme activity levels. In a study by Pandey and Singh [19], the use of chlorpyrifos for seed dressing in doses recommended by the manufacturer led to a drop in the activity of dehydrogenases and alkaline phosphatase. The use of insecticides may deliver various results, depending on the chemical structure of active ingredients and the specific properties of soil. In a 3-year field experiment investigating the effect of acetampirid on soil enzymatic activity, Singh and Kumar [7] noted an increase in dehydrogenase activity and a drop in urease activity. Gundi et al. [21] analyzed the effect of cypermethrin and quinalphos on dehydrogenase activity, a reliable indicator of the respiratory activity of soil-dwelling microorganisms, and observed higher levels of enzyme activity 10, 20, and 30 days after insecticide application in comparison with control. Xiao-Hua Yao et al. [22] did not arrive at conclusive findings as regards the effect of acetampirid applied at a dose of 0.5 to 50 mg·kg^{-1} d.m. soil on catalase activity. Other crop protection chemicals, such as herbicides, also affect the biochemical properties of soil. In a study by Sannino and Gianfreda [20], glyphosat and paraquat stimulated urease activity, while glyphosat had an inhibitory effect on phosphatase activity. In a field experiment carried out by Niemi et al. [1], insecticides decreased the activity of seven enzymes: arylsulphatase, cellobiase, β-glucosidase, β-xylosidase, and α-glucosidase.

The date of biochemical analysis was an important factor affecting the activity of the investigated enzymes (Tables 2-6). The activity of dehydrogenases, alkaline phosphatase, acid phosphatase and catalase was higher on experimental day 50 than on day 10 in both soil types (Tables 2, 4-6). The date of analysis was also correlated with the effect of insecticides and their metabolites on bacteria and fungi, the main source of soil enzymes. Insecticides present in soil for longer periods of time undergo biodegradation. In this experiment, increasing levels of teflubenzuron contamination had a more

Table 3. Activity of urease in the soil contaminated with insecticides (mmol N-NH_4·kg^{-1} s.m.·h^{-1}).

Insecticide dose (mg·kg^{-1} d.m. of soil)	Soil type			
	Loamy sand		Sandy loam	
	Sampling			
	day 10	day 50	day 10	day 50
control	2.07±0.02	2.47±0.03	6.29±0.16	2.78± 0.04
teflubenzuron				
0.05	2.41±0.01	3.09±0.08	5.82±0.20	2.84±0.04
0.5	2.18±0.05	2.81±0.02	5.76±0.05	3.22±0.05
5	2.08±0.04	2.58±0.11	5.57±0.11	3.91±0.25
50	1.91±0.04	2.38±0.03	5.53±0.06	3.92±0.06
500	1.88±0.06	2.20±0.01	6.08±0.04	2.79±0.04
r	-0.72*	-0.61*	-0.37	0.38
chloropirofos				
0.05	2.63±0.02	2.63±0.07	5.65±0.10	3.55±0.05
0.5	2.71±0.05	2.25±0.04	5.56±0.06	2.90±0.05
5	2.52±0.06	2.23±0.05	4.96±0.09	2.83±0.05
50	1.84±0.05	0.44±0.15	2.48±0.02	0.81±0.05
500	1.68±0.06	0.03±0.01	1.28±0.05	1.25±0.05
r	-0.62*	-0.89*	-0.94*	-0.81*
$LSD_{0.01}$	0.17			

*explanations under Table 1.

Table 4. Activity of alkaline phosphatase in the soil contaminated with insecticides (mmol PNP·kg^{-1} s.m.·h^{-1}).

Insecticide dose (mg·kg^{-1} d.m. of soil)	Soil type			
	Loamy sand		Sandy loam	
	Sampling			
	day 10	day 50	day 10	day 50
control	2.52±0.02	3.76±0.02	3.50±0.09	4.81±0.05
teflubenzuron				
0.05	3.43±0.04	3.90±0.01	3.60±0.06	4.45±0.07
0.5	3.58±0.04	4.38±0.11	3.63±0.02	4.88±0.07
5	3.57±0.00	3.98±0.07	3.54±0.07	5.16±0.06
50	3.52±0.00	3.83±0.01	3.34±0.16	4.75±0.05
500	3.63±0.03	3.73±0.00	3.30±0.07	4.79±0.05
r	0.73*	-0.19	-0.50*	0.36
chloropirofos				
0.05	3.62±0.04	3.82±0.01	3.77±0.10	4.87±0.09
0.5	2.81±0.12	3.75±0.08	3.63±0.05	4.93±0.03
5	2.94±0.01	3.73±0.03	3.59±0.03	4.50±0.00
50	2.91±0.07	3.16±0.09	3.39±0.06	4.57±0.02
500	2.56±0.03	3.06±0.07	3.25±0.06	4.21±0.09
r	-0.35	-0.85*	-0.64*	-0.81*
$LSD_{0.01}$	0.18			

*explanations under Table 1

Table 5. Activity of acid phosphatase in the soil contaminated with insecticides (mmol PNP·kg^{-1} s.m.·h^{-1}).

Insecticide dose (mg·kg^{-1} d.m. of soil)	Soil type			
	Loamy sand		Sandy loam	
	Sampling			
	day 10	day 50	day 10	day 50
control	2.11±0.01	2.50±0.01	1.89±0.02	2.30±0.03
teflubenzuron				
0.05	2.12±0.04	2.62±0.00	1.88±0.02	2.41±0.01
0.5	2.23±0.01	2.63±0.01	1.89±0.01	2.47±0.04
5	2.25±0.01	2.70±0.12	1.94±0.01	2.72±0.03
50	2.22±0.02	2.27±0.12	2.16±0.13	2.44±0.02
500	2.22±0.01	2.24±0.18	2.22±0.03	2.28±0.10
r	0.77*	0.52*	0.52*	-0.11
chloropirofos				
0.05	2.01±0.04	2.32±0.00	2.48±0.07	2.51±0.04
0.5	2.01±0.01	2.36±0.05	2.41±0.11	2.47±0.05
5	2.00±0.01	2.25±0.03	2.33±0.05	2.31±0.02
50	2.02±0.02	2.23±0.04	2.27±0.04	2.28±0.01
500	1.99±0.01	1.90±0.04	2.14±0.03	2.15±0.02
r	-0.38	-0.89*	-0.89*	-0.62*
$LSD_{0.01}$	0.21			

*explanations under Table 1

Table 6. Activity of catalase in the soil contaminated with insecticides (mol O_2·kg^{-1} s.m.·h^{-1}).

Insecticide dose (mg·kg^{-1} d.m. of soil)	Soil type			
	Loamy sand		Sandy loam	
	Sampling			
	day 10	day 50	day 10	day 50
control	1.10±0.02	1.40±0.002	2.07±0.003	2.28±0.002
teflubenzuron				
0.05	1.18±0.04	1.66±0.00	2.03±0.04	2.32±0.08
0.5	1.18±0.02	1.50±0.02	2.14±0.03	2.26±0.00
5	1.35±0.02	1.55±0.02	2.21±0.08	2.22±0.02
50	1.32±0.04	1.52±0.00	1.93±0.07	2.05±0.02
500	1.19±0.03	1.53±0.02	1.87±0.03	1.86±0.02
r	0.57*	0.14	-0.43	-0.87*
chloropirofos				
0.05	1.33±0.00	1.43±0.00	2.04±0.03	2.48±0.03
0.5	1.33±0.00	1.38±0.02	2.05±0.03	2.36±0.00
5	1.34±0.00	1.36±0.05	1.99±0.00	2.25±0.00
50	1.33±0.03	1.34±0.02	2.02±0.03	2.34±0.06
500	1.34±0.00	1.32±0.00	2.00±0.05	2.25±0.00
r	0.66*	-0.85*	-0.47*	-0.82*
$LSD_{0.01}$	0.06			

*explanations under Table 1

inhibitory effect on dehydrogenase activity on experimental day 50 than on day 10 (Table 2). Similarly, the negative effect of chlorpyrifos on dehydrogenase activity was not minimized over time.

The effect of the studied insecticides on the remaining enzymes produced less conclusive results on both sampling days (Tables 2, 4-6). All pesticides undergo physical, chemical and biological degradation in soil. The main product of microbiological degradation of chlorpyrifos is 3,5,6-trichloro-2-pyridinol [23]. Pesticide degradation or decomposition is generally a positive phenomenon. Most by-products of the degradation process are biologically inactive, less toxic, and harmless. Nevertheless, some pesticides may decompose into products that are more toxic than the original chemical substance [24]. Although chlorpyrifos and teflubenzuron decomposed in soil over 50 days of the experiment, the by-products of their degradation could exert a powerful effect on microbes, altering their metabolic processes, including enzyme production.

The last factor – soil type – also influenced the activity levels of the studied enzymes. Dehydrogenase activity was significantly higher in loamy sand, while higher levels of urease, alkaline phosphatase, and catalase were noted in sandy loam.

A supporting environment for microorganism development is conditioned by soil type. Soil properties such as granulometric composition, pH and organic matter content also determine enzyme activity. They affect the sorption and desorption of toxic substances, including pesticides [25]. In a study by Cao et al. [26], prometrine sorption was higher in soils with a higher content of organic matter, silt, and clay. Herbicide desorption was also slower in this soil type.

Conclusions

1. In the present experiment, dehydrogenases were the most sensitive indicator of soil contamination with chlorpyrifos and teflubenzuron. Dehydrogenase activity decreased in soil samples contaminated with insecticides at a dose of 0.5 to 500 $mg \cdot kg^{-1}$.
2. In comparison with teflubenzuron, chlorpyrifos reduced the activity of dehydrogenases, urease, and alkaline phosphatase to a greater degree.
3. The activity of dehydrogenases, alkaline phosphatase, acid phosphatase, and catalase was higher on day 50 of the experiment than on day 10.

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