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Letter to Editor

Enzymatic Activity of Soil Contaminated with Triazine Herbicides

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Abstract

This paper, on the basis of a 5-year study (1996-2000), attempts to answer the following question: do the changes in enzymatic activity observed in an apple-tree orchard at a prolonged (20-year) application of triazine herbicides indicate the degradation processes in soil? In the soil of herbicide-triggered fallow land, the activity of the enzymes under scrutiny was subject to a significant inhibition. Among the enzymes analyzed, the activity of phosphatases was the most sensitive indicator for soil contamination with triazine herbicides. In the light of the obtained results, the criticism of the application of triazine herbicides in orchard-related cultivation is justified. It is recommended that they be replaced with other methods of weed control. However, that requires further research into the activity of enzymes for other types of soils used intensively for orchard-related purposes.

Keywords: apple orchard, triazine herbicides, enzymatic activity, contamination of soil

Introduction

The traditional system of soil cultivation in apple-tree orchards, based on the common use of triazine herbicides, has a positive effect on production and is justified economically, although it may add to the adverse changes effected in the soil environment [1].

Unlike for most agricultural plants, due to the lack of crop rotation in orchards, the same method of soil management is applied for several or several tens of years. The effects of the applied agrotechnical measures on the properties of soil may be heightened over time. Prolonged maintenance of herbicide-triggered fallow land between tree rows results in an ongoing accumulation of herbicides and the products of their decomposition in soil, thus becoming one of the ecological factors having a specific effect on soil biocenosis [2, 3].

All the transformations of nutrients occurring in soil are stimulated by the enzymes that condition their conversion into forms available to plants and micro-organisms. Enzymes are frequently referred to as markers of soil environment purity [4]. Monitoring of the pedosphere using the methods based on enzymatic tests enables a complex assessment of the changes in the soil environment under the influence of anthropogenic factors [5].

In this paper, on the basis of a 5-year study (1996-2000), an attempt was made to answer the following question: do the changes in enzymatic activity observed in an apple-tree orchard at a prolonged (20-year) application of triazine herbicides indicate the degradation processes in soil?

Materials and Methods

The research was carried out in the years 1996-2000 in an apple-tree orchard (Jonathan variety) located at the south-east border of the city of Lublin, Poland (51° 14' N; 22° 34' E). The orchard was established on non-uniform soil developed from silt on marl (Haplic Luvisol). The or-

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chard was planted in the spring of 1979 and ever since herbicide-triggered fallow land had been maintained there in tree rows, in a strip about 1.5 m wide, together with mown grass in between the rows. The herbicides were applied as follows: in 1996-98 in April – Azoprim (atrazine) 3 $kg \cdot ha^{-1}$, in May – Roundup Ultra (Glyfosat) 4 $dm^{-3} \cdot ha^{-1}$ plus Chwastox extra (MCPA) 2 $dm^{-3} \cdot ha^{-1}$; in 1999-2000 in April – Azotop (simazine) 4 $kg \cdot ha^{-1}$, in May – Roundup 3 $dm^{-3} \cdot ha^{-1}$, plus ammonium sulfate approximately 12 $kg \cdot ha^{-1}$, and Dual 720 EC (metolachlorine) 1 $dm^{-3} \cdot ha^{-1}$. Since 1980 the orchard had been used for fertilization experiments by the Faculty of Horticulture at the Agricultural University of Lublin.

The following sites were selected for study: herbicide-triggered fallow land in tree rows and grass strip (Sod) in between the rows – with mineral fertilization of the entire area at a dose of N 150, K_2O 300, and P_2O_5 75 $kg \cdot ha^{-1}$, and herbicide-triggered fallow land in tree rows and grass strip (Sod) in between the rows – without mineral fertilization.

Each year, fertilizers were spread in a single dose in the form of ammonium nitre (34%), potassium salt (60%), and triple superphosphate. Tree protection against diseases and pests was carried out according to current recommendations for apple-tree orchards for commercial production.

From each selected site, soil samples for the study were collected in three repetitions, from the A-horizon, from the layer 0 – 20 cm, in the middle or towards the end of May each year. In order to increase the comparability of the results obtained in particular years of the study, soil samples for enzymatic analyses were collected in the period when the soil was in the state of dynamic balance that maintained the course of biochemical processes in that environment within the limits of moderate intensity.

In the analyzed soil samples, the activities of the following enzymes were determined: dehydrogenases [6], phosphatases [7], urease [8], and protease [9]. In addition, total organic carbon content [ISO 14235], the content of available phosphorus forms [ISO 11263], and pH value in 1 $mol \cdot dm^{-3}$ KCl [ISO 10390] were determined.

The differences between mean values were checked using t-test and the significance of the results using Analysis of Variance at $\alpha = 0.05$ (ANOVA).

Table 1. Selected chemical properties of investigated soil.

Site	Years	pH KCl	C [$g \cdot kg^{-1}$]	P [$mg \cdot kg^{-1}$]
Herbicide fallow + NPK	1996	3.8	8.30b	112.3d
	1997	3.6	8.22b	111.9d
	1998	3.6	7.98a	111.5d
	1999	3.5	7.90a	111.2d
	2000	3.4	7.85a	110.8d
Sod + NPK	1996	4.5	9.80c	79.6c
	1997	4.5	9.76c	79.2c
	1998	4.3	9.72c	79.3c
	1999	4.1	9.80c	78.6c
	2000	4.0	9.75c	78.4c
Herbicide fallow (without NPK)	1996	4.3	8.64c	49.8b
	1997	4.2	8.52b	49.5b
	1998	4.0	8.40b	49.6b
	1999	3.9	8.23b	48.7b
	2000	3.7	8.12b	48.2b
Sod (without NPK)	1996	5.5	10.21d	33.5a
	1997	5.6	10.14d	33.8a
	1998	5.5	10.30d	33.4a
	1999	5.6	10.24d	32.9a
	2000	5.6	10.28d	32.8a

Differences between values in the column followed by the same letter (a, b, c…) are not significant.

Results

The pH value of the soil in the orchard was different with regard to the mode of its maintenance (Table 1). The soil under grass was characterised by higher pH values than the soil maintained in the herbicide triggered fallow land. Those differences ranged from 0.2 to 2.2 pH unit in 1 mol·dm^{-3} KCl. The highest acidity was noted in the fertilized soil of herbicide-triggered fallow land (strong acid reaction), and the lowest in the soil under grass without fertilization (slightly acid reaction). The application of herbicides and a high level of mineral fertilization effected in the increase of soil acidity over the years.

The mode of soil management in the orchard differentiated in a significant way in the contents of total organic carbon and available phosphorus forms in soil (Table 1).

The content of $C_{org.}$ in the soil under the grass strips was higher by about 20% than in the soil maintained as herbicide-triggered fallow land. The detrimental effect of herbicide-triggered fallow land on the content of that element in soil was connected with the lack of fresh organic matter influx. The highest content of $C_{org.}$ characterized the soil under grass without fertilization and the lowest content was stated in the fertilized soil of herbicide-triggered fallow land. Attention is drawn by the fact that in the Ap-horizon of the soil the content of organic carbon was relatively low (after calculation, the humus content ranged from 1.35% in the soil of fertilized herbicide-triggered fallow land to 1.77% in the soil under grass (without fertilization).

The soil was characterized by high phosphorus content (Table 1). In Ap-horizons, the content of available phosphorus ranged from 32.8 to 112.3 mg·kg^{-1}. Intensive fertilization with superphosphate over many years had a significant effect on the accumulation of phosphorus in the soil of fertilized sites. Within the analyzed fertilized sites, the application of herbicide-triggered fallow land effected in an increase of the content of available phosphorus forms in soil. The lower content of available phosphorus in the soil under grass than in the soil maintained as herbicide-triggered fallow land was the effect of an intensive uptake of that element by grass roots. The highest content of that phosphorus form characterized the fertilized soil of herbicide-triggered fallow land, and the lowest content was found in the soil under grass without fertilisation.

Within the period of the study, a slight decrease was noted in the contents of $C_{org.}$ and available P in soil (Table 1). Significant differences noted in the fertilized soil of herbicide-triggered fallow land related to $C_{org.}$ In the years 1996-97, the content of that element was significantly higher than in the years 1998-2000.

The mode of soil management in the orchard also differentiated its enzymatic activity in a significant way (Table 2).

The activity of all the enzymes in the soil from the grass strips was significantly higher than in the soil maintained as herbicide-triggered fallow land. The most substantial lowering of enzymatic activity occurred in the soil of fertilized herbicide-triggered fallow land. The highest enzymatic activity characterized the soil of the grass strip without mineral fertilization, where the activity of the enzymes was several times higher than in the fertilized soil of herbicide-triggered fallow land. The differences observed were dependent on enzyme type. A particularly negative effect of the application of herbicide-triggered fallow land was revealed for the activity of phosphatases. The activities of dehydrogenases, phosphatases, urease, and protease in the soil under grass were significantly higher, about 4-, 3-, 2-, and 1.5-times respectively, than in the soil maintained as herbicide-triggered fallow land.

The application of herbicide-triggered fallow land and a high level of mineral fertilisation effected in the lowering of the enzymatic activity of soil over the years. The activities of all the enzymes noticed in 2000 were significantly lower than in 1996. This phenomenon was not observed for the soil from the grass strip without fertilization (Table 2).

Discussion

The obtained results showed a negative effect of herbicide-triggered fallow land on the enzymatic activity of the soil studied. The influx of fresh organic matter to the soil, limited by the multi-year application of herbicides, was definitely a significant factor in lowering the activity of the analyzed enzymes. The presence of carbon substrates induces and stimulates biosynthesis of the enzymes by soil micro-organisms [10-12]. It should be emphasized that fresh organic matter not only stimulates metabolic activity of micro-organisms but also has a positive effect on the rate of pesticide decomposition [13-15], which plays an important role in soils used for orchard-related purposes (due to intensive chemical protection).

Another cause for the lowering of soil enzymatic activity in herbicide-triggered fallow land in tree rows may have been the increased death rate for micro-organisms due to intensive chemical plant protection, characteristic of orchards [16, 17]. The influence of herbicides on the activity of soil enzymes depends on their chemical properties and their concentrations in soil, physico-chemical properties of soil, and type of enzyme [2, 18-20].

Particularly detrimental to biological activity of the soil appeared to be the application of the high level of mineral fertilization simultaneously with chemical weed control. Perrin-Ganier et al. [21] observed that the addition of nitrogen and phosphorus inhibited the decomposition rate of isoproturon in the soil. The study by Krutz et al. [22] showed that Roundup Ultra inhibited biodegradation of atrazine in the soil.

A substantial decrease in the level of the parameters of soil biochemical activity in fertilized herbicide-triggered fallow land is most probably the result of the complex effect of the factors discussed above (pesticides and organic matter) heightened by intensive NPK fertilization. The accompanying decrease in pH_{KCl} was an additional cause of the strong lowering in soil enzymatic activity for that site. Studies by many authors [4, 23, 24] showed that an increase in the concentration of hydrogen ions in soil has a negative effect on its enzymatic activity. According to Frankenberger and Johanson [23], lowering the soil enzymatic activity due to an increase in its acidity is the effect of the destruction of hydrophobic, ionic, and hydrogenic bonds within the active centre of enzymatic protein. According to some authors [25, 26], prolonged exposure to application of simulated acid rains had no effect on biological parameters of the soil, which indicates that micro-organisms adapt to low pH over time.

Among the enzymes under study, the highest sensitivity to the application of herbicide-triggered fallow land was noted for the activity of phosphatases. While studying the impact of pesticides on biological soil processes, Burrows and Edwards [18] showed that phosphatases were most susceptible to the use of high doses of herbicides. The results obtained by Tan et al. [27] also confirmed the sensitivity of phosphatases to anthropogenic stressors.

It is worth underlining that the apparent inhibition of the activity of phosphates in soil maintained as herbicide triggered fallow land was closely connected with a heightened content of available phosphorus (Table 1). In many studies [4, 28, 29], it was shown that the excess of available phosphorus forms inhibits the synthesis of phosphatases. The increased phosphorus content in the soil of the analyzed site may also have been a factor that limited the decomposition of the herbicides applied for many years. It was noted that triazines create bonds with PO_4^{3-} that are resistant to microbiological degradation [30].

The high activity of the enzymes analyzed in the soil under grass results from the effect of rhizosphere [31-34]. Plants release into soil root excretions which support the metabolism of micro-organisms [32-34]. Lynch and Whipps [35] showed that the quantity of organic C released by plants to the rhizosphere may amount to 40% of the total dry mass produced by the plant, thus generating a substantial increase in the activity of enzymes. Rhizosphere micro-organisms have the ability to degrade triazines in the soil [32]. In this study, the positive effect of grass on soil enzymatic activity was the most evident for phosphatases. Also the studies by

Table 2. Enzymatic activity of investigated soil (dehydrogenases – $cm^3\ H_2 \cdot kg^{-1} \cdot d^{-1}$, phosphatases – mmol $PNP \cdot kg^{-1} \cdot h^{-1}$, urease – mg $N\text{-}NH_4^+ \cdot kg^{-1} \cdot h^{-1}$, protease – mg $tyrosine \cdot g^{-1} \cdot h^{-1}$).

Site	Years	Dehygrogenases	Phosphatases	Urease	Protease
Herbicide fallow + NPK	1996	2.9b	44.2b	16.7b	8.9b
	1997	2.4a	35.6a	14.9a	7.8a
	1998	2.1a	30.6a	13.7a	6.8a
	1999	1.8a	26.3a	10.2a	6.2a
	2000	1.6a	18.5a	9.1a	5.1a
Sod + NPK	1996	5.2c	98.3d	27.4c	13.5c
	1997	4.8c	87.6d	25.8c	12.9c
	1998	4.5c	78.1c	24.3c	11.5c
	1999	4.1c	73.4c	22.5c	9.3b
	2000	3.4b	67.2c	18.6b	8.1b
Herbicide fallow (without NPK)	1996	4.3c	50.8b	18.2b	11.2b
	1997	3.8b	45.3b	17.6b	10.8b
	1998	3.4b	41.4b	16.0b	9.7b
	1999	2.9b	37.2b	15.3a	8.5b
	2000	2.1a	32.3a	12.7a	6.7a
Sod (without NPK)	1996	6.4d	120.4e	34.6d	15.8d
	1997	6.5d	118.7e	33.5d	16.2d
	1998	6.1d	110.8e	31.9d	15.1d
	1999	6.3d	112.3e	34.2d	15.9d
	2000	6.2d	117.5e	33.8d	16.4d

Differences between values in the column followed by the same letter (a, b, c…) are not significant.

Burns [36] prove that enzymatic activity, in particular the activity of phosphatases, is very high in soil in the root layer of cultivated crops. According to Hedley et al. [37], the activity of phosphatases in the rhizosphere is heightened together with the increase in the deficit of available phosphorus due to the uptake of that element by plant roots.

Conclusions

1. In the soil of herbicide-triggered fallow land, the activity of the enzymes was subject to a significant inhibition. Among the enzymes analyzed, the activity of phosphatases was the most sensitive indicator for soil contamination with triazine herbicides.
2. The decrease of soil biochemical activity in herbicide-triggered fallow land was accompanied by negative changes in its chemical properties, which indicates the usefulness of selected enzymatic tests in the assessment of soil environment status.
3. The negative effect of triazine herbicides on the activity of the enzymes catalyzing the most essential processes of soil organic matter transformation continued to deepen over the years. It certifies that a prolonged application of herbicide-triggered fallow land may lead in time to a humus deficit in soil.
4. In light of our results, the criticism of the application of triazine herbicides in orchard-related cultivation is probably justified. However, it requires further research into the activity of enzymes for other types of soils used intensively for orchard-related purposes.

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