Effect of Soil Contamination with Treflan 480 EC on Biochemical Properties of Soil

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Abstract

The aim of this experiment was to study the effect of soil contamination by treflan 480 EC on biochemical properties of soil and on the growth and development of crops (spring rape and white mustard). The tests were conducted on leached brown soil formed from clay slightly dusty sand of 5.8 pH in 1 M KCl, to which the following quantities of treflan 480 EC were added: 0, 1.5, 3.0, 4.5, 6.0, 9.0 and 12.0 $mm^3 \cdot kg^{-1}$ of soil. The biochemical analyses were performed 7 days after the experiment was established and during the flowering of plants.

It was found that treflan 480 EC had a negative influence on the activity of dehydrogenases, urease, acid phosphatase and alkaline phosphatase. Spring rape and white mustard were intolerant to high concentrations of treflan. The yield of crops was negatively correlated with the herbicide rate. Potential biochemical index of soil fertility derived from the activity of dehydrogenases, urease, acid and alkaline phosphatases as well as the content of organic carbon were negatively correlated with the concentration of treflan in soil, but positively correlated with the yield of spring rape and white mustard.

Keywords: treflan 480 EC, enzymatic activity, spring rape, white mustard.

Introduction

One of the side effects of the application of pesticides is that these chemicals accumulate in soil. Pesticides are biologically highly active, therefore they will have an effect not only on the organisms subjected to their activity but also on a number of other organisms present in this environment, many of which are useful in nature. Such side effects are compounded by long degradation times of some pesticides in soil [1]. Persistence of these xenobiotics in soil depends on several factors determining the rate of their disappearance from the environment, of which the chemical structure of the active substance of a preparation, its chemical properties, formation of bonds with other compounds and biodegradability are most significant [2, 3]. An important role is also played by environmental and agricultural factors, including temperature, pH, moisture, soil type, organic matter content, fertilisation and count and activity of soil microorganisms [4, 5, 6].

Degradation of pesticides is catalysed by enzymes excreted by microorganisms, producing in effect some intermediate metabolites, which may have a selective influence on soil microflora. Accurate determination of such modifications occurring during microbiological and biochemical processes is essential for sustaining and regenerating the fertility of soil [7]. An assay of the enzymatic activity of soil, especially the activity of enzymes involved in the conversion of nitrogen, carbon and phosphorus, can be regarded as a good indicator of the effect of pesticides on soil metabolism. For this reason a study has been undertaken to determine the effect of ”treflan 480 EC” on changes in the enzymatic activity of soil.

Methods

The tests were carried out in a pot experiment established in a greenhouse, in plastic pots (in five replications) filled with 3.4 kg leached brown soil from slightly dusty clay sand, which possessed the following characteristics: pH in 1 M KCl – 5.8, Hh – 1.35 cmol (+) · kg^{-1} of soil, C_{org} – 6.0 g , total of bases (S) – 3.80 , sorptive complex capacity (T) – 5.15, base saturation of soils (V) – 73.8%. Against the background of constant fertilisation with macro- and microelements in the amounts calculated as pure elements in mg · kg^{-1} of soil: P – 100 [K_2HPO_4]; K – 150 [K_2HPO_4 + KCl], Mg – 50 [$MgSO_4 \cdot 7H_2O$], Zn – 5 [$ZnCl_2$], Cu – 5 [$CuSO_4 \cdot 5H_2O$], Mn – 5 [$MnCl_2 \cdot 4H_2O$], Mo – 5 [$Na_2MoO_4 \cdot 2H_2O$], B – 0.33 [H_3BO_3], the effect of the herbicide treflan 480 EC, containing trifluraline as an active substance, on the enzymatic activity of soil, and on the growth and development of spring rape cv. Lisonne and white mustard cv. Nakielska. Trifuraline belongs to the group of toluidines, derivatives of aniniline (chemical name: α, α, α-trifluoro-2,6- dinitro-N-N-dipropylotoluidine), and is attributed toxicity grade V. Treflan was applied to soil in the form of aqueous solution prepared from commercially available preparation, in the following concentrations: 0; 1.5; 3.0; 4.5; 6.0; 9.0 and 12.0 in mm^3 · kg^{-1} of soil. Seven days after mixing the fertilisers and treflan with soil, spring rape and white mustard were sown in the pots. Seven plants were left in each pot after emergence. Constant soil moisture (60% of capillary water capacity) was maintained throughout the whole vegetative period of plants (35 days for white mustard and 40 days for rape plants).

The yield of above-ground parts of plants was determined during the flowering phase. Biochemical analyses of the soil were made on the sowing day, i.e. 7 days after establishing the experiment, and during the flowering phase (after harvest). Biochemical tests comprised the determination of the activity of: soil dehydrogenases (Deh) by Lenhard's method modified by Casidy et al. [8], urease (Ure) – according to Gorin and Chine Chang [9], and acid phosphatase (Pac) and alkaline phosphatase (Pal) – with the method of Tabatabai and Bremener [10]. Tiurin's method [11] was used to determine the content of organic carbon (C_{org}).

Because the herbicide did not modify the content of organic carbon in soil, the latter results are not cited in the paper, and the mean organic carbon content in 1 kg of soil analysed seven days after the application of treflan was 5.2g, after rape harvest – 6.0 g, and after mustard harvest – 5.6g.

Moreover, on the basis of the enzymatic activity and carbon content, a potential biochemical index of soil fertility was computed from the formula:

$$M_w = \left(\frac{Ure}{10} + Deh + Pal + Pac\right) \cdot \%C.$$

All the laboratory analyses were made in three replications. The results were elaborated statistically using Duncan's test. The following were calculated: regression equations between the yield of plants and the activity of dehydrogenases, urease, acid phosphatase, alkaline phosphatase and potential biochemical index of soil fertility; regression equations and determination coefficients between the rate of treflan and activity of enzymes; Pearson's simple correlation coefficients between the rate of treflan and the yield of plants and biochemical activity of soil.

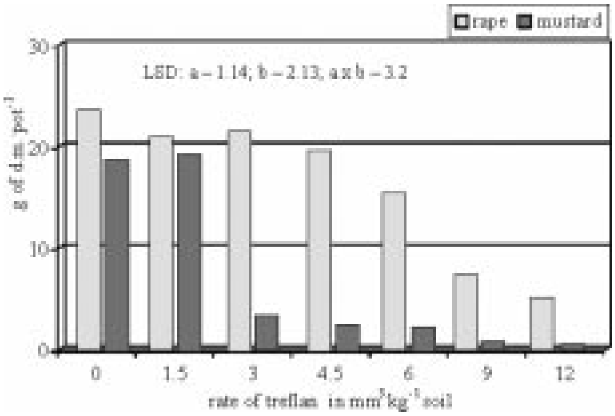

Fig. 1. Effect of treflan on yield of d.m. of plants. a – plant species, b – rate of treflan.

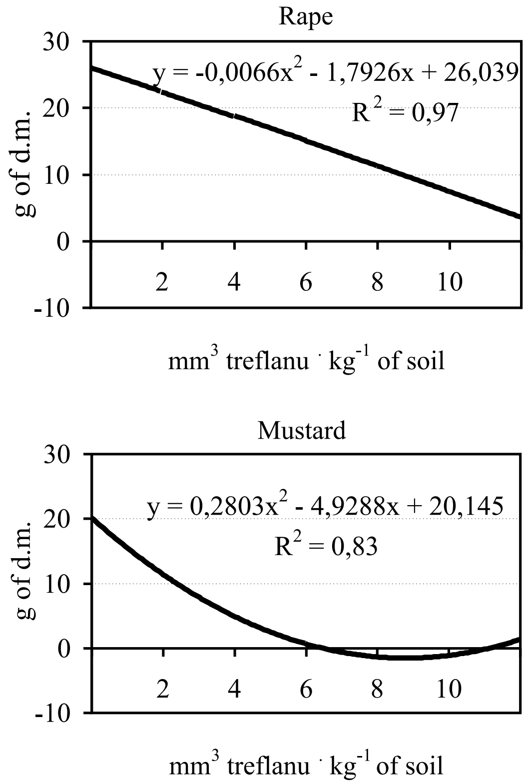

Fig. 2. Regression equations for yield of crops (g d.m. · pot^{-1}).

Results and Discussion

Plants give an accurate, albeit indirect picture of the changes occurring in the microbiological and biochemical properties of soil. Our study has revealed that spring rape

Table 1. Effect of treflan on activity of dehydrogenases in soil (cm^3 H_2 · kg^{-1} · d^{-1}).

Rate of treflan [mm^3 · kg^{-1} of soil]	Before sowing	After harvest of		$\bar{x}$
		rape	mustard	
0	1.74	1.04	1.24	1.34
1.5	1.47	0.95	1.21	1.21
3.0	1.47	0.44	0.30	0.74
4.5	1.52	0.74	0.21	0.82
6.0	1.44	0.40	0.20	0.68
9.0	1.42	0.26	0.20	0.63
12.0	1.42	0.42	0.20	0.68
$\bar{x}$	1.50	0.61	0.51	0.87
LSD*	a – 0.04; b – 0.06; a x b – 0.11			

a – date of analysis; b – rate of treflan.

Table 3. Effect of treflan on activity of acid phosphatase in soil (mmol PNP · kg^{-1} · h^{-1}).

Rate of treflan [mm^3 · kg^{-1} of soil]	Before sowing	After harvest of		$\bar{x}$
		rape	mustard	
0	1.15	1.09	1.15	1.13
1.5	1.12	1.00	1.13	1.08
3.0	1.13	0.89	1.06	1.03
4.5	1.05	0.93	0.55	0.84
6.0	1.08	0.89	0.46	0.81
9.0	1.07	0.87	0.45	0.80
12.0	1.06	0.84	0.46	0.79
$\bar{x}$	1.09	0.93	0.75	0.93
LSD*	a – 0.01; b – 0.02; a x b – 0.04			

* designations under Table 1.

Table 2. Effect of treflan on activity of urease in soil (mg $N\text{-}NH_4$ · kg^{-1} · h^{-1}).

Rate of treflan [mm^3 · kg^{-1} of soil]	Before sowing	After harvest of		$\bar{x}$
		rape	mustard	
0	8.44	4.94	6.43	6.60
1.5	8.80	4.97	6.53	6.77
3.0	7.09	4.92	9.12	7.04
4.5	8.32	4.68	9.23	7.41
6.0	8.42	3.01	8.08	6.50
9.0	8.07	2.57	5.97	5.54
12.0	7.57	2.91	5.73	5.40
$\bar{x}$	8.10	4.00	7.30	6.47
LSD*	a – 0.22; b – 0.34; a x b – 0.34			

* designations under Table 1.

Table 4. Effect of treflan on activity of alkaline phosphatase in soil (mmol PNP · kg^{-1} · h^{-1}).

Rate of treflan [mm^3 · kg^{-1} of soil]	Before sowing	After harvest of		$\bar{x}$
		rape	mustard	
0	0.79	0.61	0.75	0.72
1.5	0.70	0.66	0.84	0.73
3.0	0.64	0.60	0.84	0.69
4.5	0.66	0.59	0.73	0.66
6.0	0.63	0.54	0.75	0.64
9.0	0.63	0.54	0.73	0.63
12.0	0.63	0.45	0.71	0.60
$\bar{x}$	0.67	0.57	0.76	0.67
LSD*	a – 0.01; b – 0.02; a x b – 0.03			

* designations under Table 1.

and white mustard were not tolerant to high concentrations of treflan. The toxic effect produced by the herbicide depended on its concentration in soil and on plant species (Fig. 1 and 2). Treflan raised the yield only when applied at the lowest dosage (1.5 mm^3 · kg^{-1}) to white mustard. Higher rates of the herbicide correlated negatively with the yield of rape (r=-0.98**) and mustard (r = -0.77). Symptoms of toxicity such as chlorosis of new leaves and necrosis of some plants became specially evident on soil sown with white mustard, where plants in the objects treated with the highest rates of treflan (9 and 12) ceased to grow as early as at the stage of seedlings.

The authors' own studies and the reports reported by other researchers suggest that petroleum compounds [12], heavy metals [13], residues [14] and pesticides [7, 15, 16] modify the enzymatic activity of soil, and the extent of their influence varies, depending on the type of compound. Pesticides may inhibit [7, 17], have no effect or even stimulate soil enzymatic activity [18].

The results obtained hereby prove that treflan may determine the enzymatic activity of soil. The herbicide modified the activity of all the examined soil enzymes (Tab. 1-4). The effect of treflan on the enzymatic activity of soil was correlated with the concentration of the xenobiotic, date of analysis and species of the crop (Tab. 2). In general, treflan had an inhibitory effect on dehydrogenases (Tab. 1), urease (Tab. 2), acid phosphatase (Tab. 3) and alkaline phosphatase (Tab. 4). Activity of dehydrogenases, acid and alkaline phosphatases, both in the analysed soil before rape and mustard were sown and after they were harvested, was significantly negatively correlated with the concentration of treflan. The effect was confirmed by Pearson's simple correlation coefficients between the rate of treflan and soil enzymatic

Table 5. Pearson's simple correlation coefficients between the rate of treflan and the yield of crops versus the microbiological activity of soil.

	Treflan	Yield	Deh	Ure	Pac	Pal	Mw
before sowing							
Treflan	1.00		-0.61**	-0.34	-0.48*	-0.74**	-0.15
Deh	-0.61**		1.00	0.25	0.11	0.77**	0.39
Ure	-0.34		0.25	1.00	-0.08	0.38	0.21
Pac	-0.48*		0.11	-0.08	1.00	0.11	0.20
Pal	-0.74**		0.77**	0.38	0.11	1.00	0.35
Mw	-0.15		0.39	0.21	0.20	0.35	1.00
rape							
Treflan	1.00	-0.98**	-0.76**	-0.84**	-0.83**	-0.89**	-0.85**
Yield	-0.98**	1.00	0.77**	0.88**	0.79**	0.86**	0.85**
Deh	-0.76**	0.77**	1.00	0.74**	0.85**	0.60**	0.96**
Ure	-0.84**	0.88**	0.74**	1.00	0.65**	0.74**	0.83**
Pac	-0.83**	0.79**	0.85**	0.65**	1.00	0.70**	0.91**
Pal	-0.89**	0.86**	0.60**	0.74**	0.70**	1.00	0.71**
Mw	-0.85**	0.85**	0.96**	0.83**	0.91**	0.71**	1.00
mustard							
Treflan	1.00	-0.77**	-0.74**	-0.34	-0.85**	-0.65**	-0.91**
Yield	-0.77**	1.00	0.99**	-0.29	0.81**	0.47*	0.80**
Deh	-0.74**	0.99**	1.00	-0.33	0.82**	0.46*	0.78**
Ure	-0.34	-0.29	-0.33	1.00	0.05	0.28	0.20
Pac	-0.85**	0.81**	0.82**	0.05	1.00	0.76**	0.93**
Pal	-0.65**	0.47*	0.46*	0.28	0.76**	1.00	0.77**
Mw	-0.91**	0.80**	0.78**	0.20	0.93**	0.77**	1.00

Correlation coefficient significant at * $p < 0.05$; ** $p < 0.01$; $n = 21$

Table 6. Regression equations and determination coefficients between the rate of treflan and activity of enzymes.

Variable	Before sowing	After harvest of	
		rape	mustard
Deh	$y = 0.003x^2 - 0.059x + 1.659$ $R^2 = 0.37$	$y = 0.008x^2 - 0.158x + 1.065$ $R^2 = 0.58$	$y = 0.018x^2 - 0.300x + 1.309$ $R^2 = 0.55$
Ure	$y = -0.001x^2 - 0.040x + 8.354$ $R^2 = 0.58$	$y = 0.009x^2 - 0.332x + 5.350$ $R^2 = 0.71$	$y = -0.069x^2 - 0.711x + 6.539$ $R^2 = 0.12$
Pac	$y = 0.0009x^2 - 0.019x + 1.150$ $R^2 = 0.23$	$y = 0.002x^2 - 0.045x + 1.065$ $R^2 = 0.69$	$y = 0.008x^2 - 0.168x + 1.270$ $R^2 = 0.72$
Pal	$y = 0.002x^2 - 0.037x + 0.767$ $R^2 = 0.55$	$y = 0.0007x^2 - 0.006x + 0.631$ $R^2 = 0.79$	$y = 0.0004x^2 - 0.002x + 0.796$ $R^2 = 0.42$

activity (Tab. 5), and by the regression equations and determination coefficients between the rate of treflan and activity of enzymes (Tab. 6). Dehydrogenases were the most susceptible of all the soil enzymes to the effect of herbicide. The highest rate of treflan (12 $mm^3 \cdot kg^{-1}$ of soil) depressed the activity of these enzymes in soil prior to sowing by 59.6% and 83.9% relative to the control objects, which were not treated with the herbicide. Inhibitory effect of the pesticide on the activity of dehydrogenases was confirmed by Strzelec [15, 16], who claimed that it was correlated with the type of soil. It was stronger in lighter than in heavier soil. According to Beck

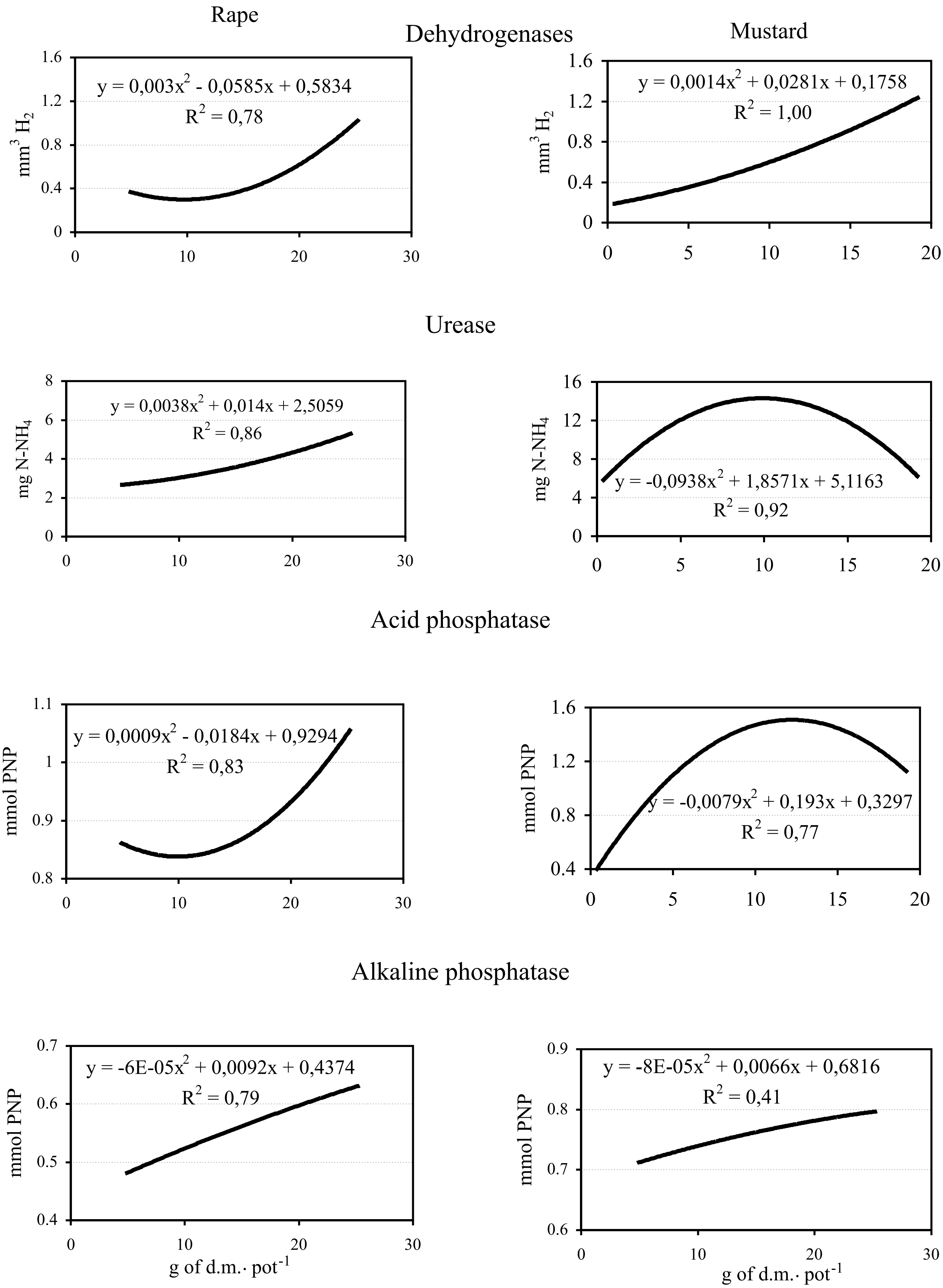

Fig. 3. Relationship between the yield of plants and soil enzymatic activity in 1 kg of soil.

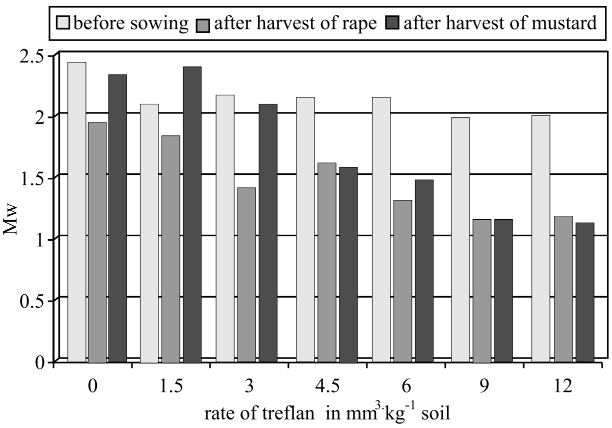

Fig. 4. Effect of treflan on potential biochemical soil fertility index (Mw).

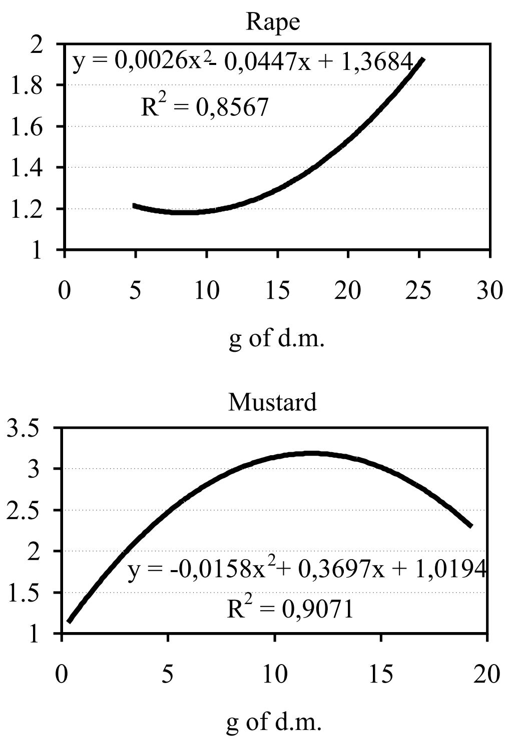

Fig. 5. Regression equations between yield of crops (g d.m. · pot^{-1}) and potential biochemical soil fertility index.

(cited in [7]) pesticides may inhibit the activity of dehydrogenases by up to 75%. The study conducted by Furczak and Kościelska [19] showed that in sandy soil, in contrast to clay soil, the activity of dehydrogenases was significantly depressed for 30 days. The authors attributed such an effect to weaker buffering of sandy soil, in which larger quantities of the pesticide may be present in the unabsorbed form, which is biologically active.

Less unambiguous results were obtained concerning the activity of urease, although Nowak [7] claims that urease is one of the soil enzymes which are characterised by high intolerance to the presence of pesticides in soil, as its activity may decline 10 to 90%. In this study, the response of urease to treflan was significantly correlated with the species of the crop. In the objects with white mustard, unlike the series with rape, treflan applied at rates from 1.5 to 6.0 of soil stimulated the activity of urease, but had a inhibitory effect at higher rates (9.0 and 12.0 mm^3 · kg^{-1} of soil).

Treflan inhibited acid phosphatase and alkaline phosphatase. Its effect was correlated with the crop species. Negative influence of the herbicide on acid phosphatase was particularly evident in the soil sown with white mustard, in which the activity of the two enzymes declined by as much as 60% in the objects treated with the highest rate of treflan. On the other hand, the activity of alkaline phosphatase in the objects treated with 1.5 and 3.0 mm^3 · kg^{-1} soil of treflan increased by 12%, while remaining stable in the other variants of the experiment. In the series of analyses of soil prior to sowing and after harvest of rape plants both acid and alkaline phosphatases were negatively correlated with the amount of treflan in soil. Likewise, in the studies of Domsch [20] and Furczak and Kościelska [19] the activity of phosphatases responded strongly to the presence of pest control chemicals.

The activity of all the tested soil enzymes was modified relative to the date of analyses. The activity of dehydrogenases, urease, acid phosphatase and alkaline phosphatase was higher in the soil analysed 7 days after the establishment of the experiment than after harvest.

The statistical analysis showed a positive, highly significant or significant correlation between the activity of dehydrogenases, acid phosphatase, alkaline phosphatase and the yield of both crops as well as between the activity of urease and yield of spring rape (Tab. 5, Fig. 3).

The tests reported in this paper prove that although knowledge of the activity of individual soil enzymes may be sufficient to draw conclusions concerning biochemical changes in soil contaminated with treflan, a more precise insight into the nature and extent of such changes could be attained on the basis of the potential biochemical soil fertility index (Mw), which comprises the activity of dehydrogenases, urease, acid phosphatase and alkaline phosphatase and the content of organic carbon. As a rule, the potential biochemical index of soil fertility thus derived, independently of a series of experiments, tended to decrease at higher rates of treflan concentrations (Fig. 4). It is interesting to notice a highly significant negative correlation coefficient between Mw and a treflan dosage (in the objects after harvest of rape r = -0.89** and mustard r = -0.91**) and a positive correlation coefficient between Mw and the yield of rape (r = 0.85**) and mustard (r = 0.80**; Fig. 5). This observation is confirmed by the regression equations and determination coefficients versus the potential biochemical index of soil fertility (Fig. 5), which seems to prove the latter is a universally applicable measure. The results reported in this paper show that observations of the effect of herbicide on the fundamental biochemical processes in soil may supply a significant amount of information pertaining to soil fertility.

Conclusions

1. Treflan 480 EC had a negative effect on the activity of dehydrogenases, urease, acid phosphatase and alkaline phosphatase.

2. Spring rape and white mustard were not tolerant to high concentrations of treflan. Yield of plants was negatively correlated with the dosage of the herbicide.

3. Potential biochemical soil fertility index calculated on the basis of the activity of dehydrogenases, urease, acid phosphatase and alkaline phosphatase as well as the content of organic carbon was negatively correlated with the concentration of treflan in soil and positively correlated with the yield of spring rape and white mustard.

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