Effect of enzymatic activity of diesel oil contaminated soil on the chemical composition of oat (*Avena sativa* L.) and maize (*Zea mays* L.)

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ABSTRACT

The aim of the study was to determine the effect of soil contamination with diesel oil (3, 6, 9, 12 and 24 g/kg soil) on the yield and the content of macroelements in oat and maize cultivated in soil supplemented with nitrogen and sawdust. The correlation between the content of macroelements in the crops and the soil enzymatic activity was also determined. High doses of diesel oil had a definite negative effect on the content of all macroelements in oat, excluding phosphorus. The presence of diesel oil had a favourable effect on the accumulation of most of macroelements in the above-ground parts of maize. Nitrogen application to the soil caused an increase in the content of nitrogen and in the accumulation of calcium and magnesium in the above-ground parts of both crops as well as an increase in sodium content in maize. Sawdust applied to the soil had a considerably lower effect (several to less than 20%) on the content of macroelements in plants. A correlation between the activity of urease and acid and alkaline phosphatase in the soil and the content of macroelements in plants cultivated in diesel oil contaminated soil was observed. This correlation was positive only in the case of alkaline phosphatase activity and phosphorus content in oats.

Keywords: soil contamination with diesel oil; nitrogen application; sawdust; oat; maize; content of macroelements; enzymatic activity

Increasing number of cars boosts the demand for fuel oil that needs to be transported and stored. A failure of pipeline, tanker or cistern transporting fuel oil poses a real threat to the environment. Petroleum-derived products are made up of aliphatic, oleic and naphthenic hydrocarbons (Chi Yuan and Krishnamurthy 1995) as well as of water-soluble aromatic hydrocarbons (benzene, toluene, xylene) that can show potential carcinogenic and mutagenic activity (Krahl et al. 2002). Once in soil, aromatic hydrocarbons − including polycyclic aromatic hydrocarbons (PAH) − are poorly mobile (Galas et al. 1997) and can have a long-term effect on soil, plants or ground waters (Sparrow and Sparrow 1988, Racine 1993, Wyszkowska et al. 2002b). Contaminants penetrating the soil disturb its structure and modify its physico-chemical properties (Iwanow et al. 1994, Sztompka 1999, Caravaca and Rodán 2003). They also affect the biological properties of the soil by modifying the populations of particular microorganisms. This, in turn, affects the soil enzymatic activity (Wyszkowska and Kucharski 2001, Budny et al. 2002, Delille and Pelletier 2002, Wyszkowska et al. 2002a, b, Caravaca and Rodán 2003) and the content of assimilable macro- and microelement forms in it. Under such conditions, the take up of macroelements by plants changes and their content in particular plant organs fluctuates (Wyszkowski et al. 2004). Together with the direct effect of petroleum-derived compounds, it determines the plant growth and development − resulting in reduction of their above-ground and root mass and sometimes in complete withering in initial vegetation phases.

The aim of the study was to determine the effect of diesel oil soil contamination on the yield and macroelements content in oat (*Avena sativa* L.) and maize (*Zea mays* L.) and the correlation between macroelements accumulation in plants and soil enzymatic activity.

MATERIAL AND METHODS

This research was conducted in the vegetation hall of University of Warmia and Mazury in Olsztyn, in polyethylene pots filled with 3.2 kg of Eutric Camisols soil according to WRB (1998) composed of sandy loam (1.0–0.1 mm – 53%; 0.1–0.02 mm – 34%; < 0.02 mm – 13%) originating from an arable humus (top) layer. The experimental soil had the following properties: pH in 1M KCl = 6.0; hydrolytic acidity (HA) = 10.4 mmol(+)/kg; exchangeable cation bases = Ca^{2+}, Mg^{2+}, K^+ and Na^+ (ECB) = 65.0 mmol(+)/kg; cation exchange capacity (CEC)

= 75.4 mmol(+)/kg; base saturation (BS) = 86.2%; content of C_{org} = 6.0 g/kg. The experiment was carried out in 4 replications. The experiment involved oat (*Avena sativa* L.) var. Borowik (15 plants per pot) and maize (*Zea mays* L.) var. Reduta as a successive crop (4 plants per pot). The effects of increasing doses of diesel oil applied in the amounts of 3, 6, 9, 12 and 24 g/kg of soil on the above-ground parts and on the chemical composition of the experimental crops were compared. The experimental diesel oil had the content of water – max. 220 mg/kg; content of solid contaminants – max. 24 mg/kg; sulphur content – max. 0.5 mg/kg; density (temp.15°C) – max. 860 kg/m^3 and viscosity (temp. 40°C) – 4.5 mm^2/s (PN-EN 590, 1999). The experiment was performed in two series. In the first one a nitrogen dose 125 mg N/kg of soil was applied before sowing oat and 62.5 mg N/kg foliar application at the blossoming of maize. In the second one it was 125 mg N before sowing oat, 125 mg N/kg foliar application at the blossoming of oat and 125 mg N/kg foliar application at the blossoming of maize. Nitrogen was applied to the soil as $CO(NH_2)_2$. Ground pine sawdust in the amount of 5 g/kg of soil was used to cushion the effect of diesel oil on the plants. Prior to the experiment, the soil was blended with mineral fertilisers and, in respective pots, with diesel oil and pine sawdust. Equal amounts of macro- and microelements were applied to all pots (P – 50 [K_2HPO_4]; K – 90 [K_2HPO_4 + KCl]; Mg – 20 [$MgSO_4.7\ H_2O$]; Zn – 5 [$ZnCl_2$]; Cu – 5 [$CuSO_4.5\ H_2O$]; Mn – 5 [$MnCl_2.4\ H_2O$]; Mo – 5 [$Na_2MoO_4.2\ H_2O$]; B – 0.33 [H_3BO_3] in mg/kg of soil). Oat was harvested at the panicle formation phase. Plant and soil materials were sampled for analyses. After sampling the soil from the pots, it was mixed with 1/2 of the nitrogen dose applied before sowing and a successive crop was sown (maize) (4 plants per pot). Maize was harvested at the tassel formation phase. Constant humidity of 60% of aquatic capillary capacity was maintained during the oat (44 days) and the maize (50 days) vegetation periods.

Plant samples were cut, dried and ground. Next, they were mineralised with 25 cm^3 of concentrated H_2SO_4 with the addition of hydrogen peroxide as a catalyst per 1 g of dry matter. The mineralised samples were transferred into conical flasks; the flasks were replenished with distilled water in the volume of 200 cm^3 and assayed for the content of macroelements. The plant samples were analysed for nitrogen content with the Kjeldahl method (Bremner 1965), phosphorus content with the colorimetric method (Calvell 1955), magnesium content with the atomic absorption spectrometry – AAS (Szyszko 1982) and potassium, sodium and calcium contents with the atomic emission spectrometry – AES (Szyszko 1982). The biochemical analyses of soil included urease activity (Ure) determined with Alef and Nannpieri (1998) method and acid phosphatase (Pac) and alkaline phosphatase (Pal) activity determined with the method described by Alef et al. (1998). Moreover, prior to sowing, the soil was analysed for pH (exchangeable acidity) with potentiometrical method with the use of aquatic solution of KCl at the concentration of 1M KCl/dm^3, hydrolytic acidity – exchangeable H^+ and Al^{3+} (HA) and exchangeable cation bases – Ca^{2+}, Mg^{2+}, K^+ and Na^+ (ECB) with Kappen method and content of organic carbon (C_{org}) with Tiurin method (Lityński et al. 1976). Based on the hydrolytic acidity and exchangeable cation bases, the cation exchange capacity (CEC) and base saturation (BS) were calculated with the following formulas: CEC = ECB + HA, BS = (ECB/CEC) × 100. The results were statistically analysed with Statistica (StatSoft, Inc. 2003) with three-factor variance analysis ANOVA. Regression equations and determination coefficients between the soil enzyme activity and nitrogen and phosphorus contents in plants were also calculated.

RESULTS AND DISCUSSION

The content of macroelements in plants is determined by many factors. The most important are the plant variety and climatic and soil conditions under which plants grow and develop. Any change in these conditions by different factors modifies the content of macroelements in plants. The experimental factors were soil contamination with diesel oil, nitrogen supplementation with the aim to ensure an adequate carbon to nitrogen ratio and the application of sawdust to cushion the effect of petroleum-derived substance on the soil. As expected, their application affected the content of macroelements in plants (Tables 1 and 2). It was largely determined by plant variety. The nitrogen, phosphorus and sodium contents were higher in the above-ground parts of oat than in maize. In the case of potassium and magnesium, the correlation was reverse.

The presence of diesel oil in the soil had a strong effect on the content of macroelements both in oat and maize (Tables 1 and 2). In the series without sawdust, with first nitrogen dose and with low diesel oil doses (3–6 g/kg of soil) the content of nitrogen, potassium and sodium in the above-ground parts of oat increased by 33%, 25%, 13%, respectively. Further increases in diesel oil doses had a definite negative effect on the content of all macroelements in oat, excluding phosphorus, for which a reverse correlation was observed. Upon the application of 24 g of diesel oil per 1 kg of soil, the nitrogen and sodium contents decreased particularly in oat, by 64% and 82%, respectively, when

Table 1. Content of nitrogen, phosphorus and potassium in plants (g/kg dry matter)

Diesel oil dose (g/kg of soil)	Oat (*Avena sativa* L.)				Maize (*Zea mays* L.)			
	without sawdust		with sawdust		without sawdust		with sawdust	
	N dose 1	N dose 2	N dose 1	N dose 2	N dose 1	N dose 2	N dose 1	N dose 2
Nitrogen (N)								
0	14.07	17.43	11.93	18.45	8.41	11.58	9.33	12.97
3	14.32	22.99	16.42	22.56	9.34	12.59	6.03	11.30
6	18.72	24.13	15.81	25.47	12.46	9.98	6.81	10.07
9	6.91	21.32	11.52	19.04	9.04	10.53	6.20	9.90
12	5.62	14.65	8.14	20.98	6.86	9.98	7.11	8.36
24	5.11	12.61	6.64	17.74	6.84	7.44	6.85	8.41
Average	10.46	18.86	11.74	20.71	8.83	10.35	7.06	10.17
	14.82		16.23		9.59		8.61	
	15.52				9.10			
r	–0.704**	–0.675**	–0.774**	–0.393	–0.522*	–0.918**	–0.327	–0.848**
LSD	a = 0.65**, b = 0.65**, c = 1.13**, a × b = n.s., a × c = 1.60**, b × c = 1.60**, a × b × c = 2.26**				a = 0.27**, b = 0.27**, c = 0.47**, a × b = 0.38**, a × c = 0.66**, b × c = 0.66**, a × b × c = 0.93**			
Phosphorus (P)								
0	5.48	5.77	5.27	5.96	3.44	3.22	4.99	4.39
3	6.54	5.69	7.58	5.72	3.40	3.09	4.08	3.18
6	6.95	6.22	7.42	6.01	3.66	3.32	4.12	3.10
9	6.73	6.57	8.18	6.40	4.21	3.44	4.66	3.68
12	6.27	6.58	7.77	6.40	4.19	3.52	4.60	3.18
24	6.57	6.49	7.74	6.60	3.80	3.45	4.68	3.57
Average	6.42	6.22	7.33	6.18	3.78	3.34	4.52	3.52
	6.32		6.76		3.56		4.02	
	6.54				3.79			
r	0.365	0.725**	0.550*	0.858**	0.481	0.701**	0.150	–0.232
LSD	a = 0.25**, b = 0.25**, c = 0.43**, a × b = 0.35**, a × c = 0.60**, b × c = 0.60**, a × b × c = 0.85**				a = 0.11**, b = 0.11**, c = 0.19**, a × b = 0.15**, a × c = 0.26**, b × c = 0.26**, a × b × c = n.s.			
Potassium (K)								
0	17.19	15.79	15.97	15.83	11.95	8.61	12.39	10.07
3	18.79	15.63	19.26	15.77	11.53	9.59	14.66	11.73
6	21.45	14.26	20.94	16.84	14.28	14.27	20.94	13.68
9	12.35	14.29	15.81	17.38	19.80	16.93	18.81	16.63
12	13.34	14.27	12.87	14.21	29.03	17.46	28.59	19.03
24	13.30	12.87	10.01	12.92	22.26	27.04	28.01	26.77
Average	16.07	14.52	15.81	15.49	18.14	15.65	20.57	16.32
	15.30		15.65		16.90		18.45	
	15.47				17.67			
r	–0.596*	–0.941**	–0.783**	–0.725**	0.692**	0.989**	0.857**	0.998**
LSD	a = 1.23**, b = 1.23**, c = 1.45**, a × b = 1.32, a × c = 1.72**, b × c = 1.72**, a × b × c = 2.21**				a = 0.49**, b = 0.49**, c = 0.85**, a × b = n.s, a × c = 1.20**, b × c = 1.20**, a × b × c = 1.70**			

Dose 1 = 125 mg N/kg applied to soil before oat sowing + 62.5 mg N/kg foliar application at the maize blossoming

Dose 2 = 125 mg N/kg applied to soil before oat sowing + 125 mg N/kg foliar application at the oat blossoming + 125 mg N/kg foliar application at the maize blossoming

LSD for: a = sawdust application, b = nitrogen dose, c = diesel oil dose

n.s. = non-significant differences at $P = 0.05$, *significant at $P = 0.05$, **significant at $P = 0.01$

r = Pearson's simple correlation coefficient

Table 2. Content of sodium, magnesium and calcium in plants lupine (g/kg dry matter)

Diesel oil dose (g/kg of soil)	Oat (*Avena sativa* L.)				Maize (*Zea mays* L.)			
	without sawdust		with sawdust		without sawdust		with sawdust	
	N dose 1	N dose 2	N dose 1	N dose 2	N dose 1	N dose 2	N dose 1	N dose 2
Sodium (Na)								
0	2.61	2.62	2.80	2.38	0.16	0.42	0.48	0.49
3	2.95	1.06	1.36	1.52	0.16	0.37	0.55	0.31
6	0.45	0.72	0.42	0.75	0.27	0.32	0.27	0.44
9	0.68	0.72	0.42	0.68	0.46	0.47	0.50	0.65
12	0.58	0.92	0.65	0.61	0.45	0.55	0.28	0.60
24	0.48	0.61	0.84	0.58	0.44	0.58	0.40	0.64
Average	1.29	1.11	1.08	1.09	0.32	0.45	0.41	0.52
	1.20		1.08		0.39		0.47	
	1.14				0.43			
r	–0.682**	–0.628**	–0.519*	–0.724**	0.773**	0.787**	–0.824**	0.673**
LSD	a = 0.05**, b = 0.05**, c = 0.08**, a × b = 0.07**, a × c = 0.11**, b × c = 0.11**, a × b × c = 0.16**				a = 0.03**, b = 0.03**, c = 0.05**, a × b = n.s., a × c = 0.07**, b × c = 0.07**, a × b × c = 0.09**			
Magnesium (Mg)								
0	1.72	1.86	1.77	2.03	3.50	3.79	3.70	4.90
3	1.89	1.50	1.63	1.57	3.58	3.47	3.20	4.36
6	1.61	1.49	1.66	1.52	2.87	4.16	3.10	4.30
9	1.17	1.67	1.71	1.57	2.82	4.11	2.92	4.13
12	1.13	1.69	1.64	1.81	3.22	3.70	2.71	4.01
24	1.32	1.56	1.55	1.79	3.33	3.77	2.83	3.53
Average	1.47	1.63	1.66	1.72	3.22	3.83	3.08	4.21
	1.55		1.69		3.53		3.64	
	1.62				3.58			
r	–0.639**	–0.291	–0.794**	0.004	–0.147	0.022	–0.749**	–0.951**
LSD	a = 0.05**, b = 0.05**, c = 0.08**, a × b = n.s., a × c = 0.11**, b × c = 0.11**, a × b × c = 0.16**				a = 0.14**, b = 0.14*, c = 0.24**, a × b = 0.20**, a × c = 0.34**, b × c = 0.34*, a × b × c = 0.48**			
Calcium (Ca)								
0	3.16	4.58	2.63	5.28	2.74	3.54	3.16	4.47
3	2.62	4.31	2.62	4.40	2.60	3.23	2.44	3.21
6	3.14	3.45	2.81	3.77	2.07	3.23	2.59	3.52
9	2.43	3.47	3.15	3.44	2.36	3.54	2.75	3.53
12	2.43	3.46	3.13	3.82	3.70	2.92	3.99	3.36
24	2.43	2.42	3.14	4.18	5.23	4.17	5.26	3.54
Average	2.60	3.62	2.91	4.15	3.12	3.44	3.37	3.61
	3.16		3.53		3.28		3.49	
	3.34				3.38			
r	–0.666**	–0.949**	0.724**	–0.403	0.865**	0.573*	0.871**	–0.366
LSD	a = 0.17**, b = 0.17**, c = 0.29**, a × b = 0.24*, a × c = 0.41**, b × c = 0.41**, a × b × c = 0.58*				a = 0.17**, b = 0.17*, c = 0.30**, a × b = n.s., a × c = 0.42**, b × c = 0.42*, a × b × c = n.s.			

Dose 1 = 125 mg N/kg applied to soil before oat sowing + 62.5 mg N/kg foliar application at the maize blossoming
Dose 2 = 125 mg N/kg applied to soil before oat sowing + 125 mg N/kg foliar application at the oat blossoming + 125 mg N/kg foliar application at the maize blossoming
LSD for: a = sawdust application, b = nitrogen dose, c = diesel oil dose
n.s. = non-significant differences at $P = 0.05$, *significant at $P = 0.05$, **significant at $P = 0.01$
r = Pearson's simple correlation coefficient

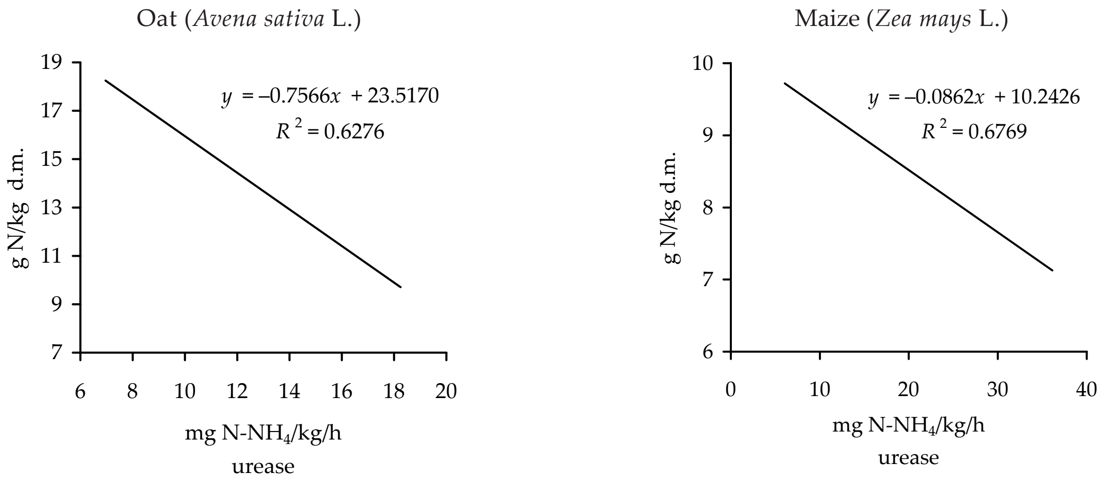

Figure 1. Correlations between the nitrogen content in oat (*Avena sativa* L.) and maize (*Zea mays* L.) and urease activity in soil

compared to the control variant (with no diesel oil). In the parallel series, diesel oil favoured the accumulation of the majority of macroelements in above-ground parts of maize. The sodium content increased almost three times and the potassium and calcium contents increased almost two times in the maize cultivated in the soil contaminated with 24 g of diesel oil per 1 kg of soil.

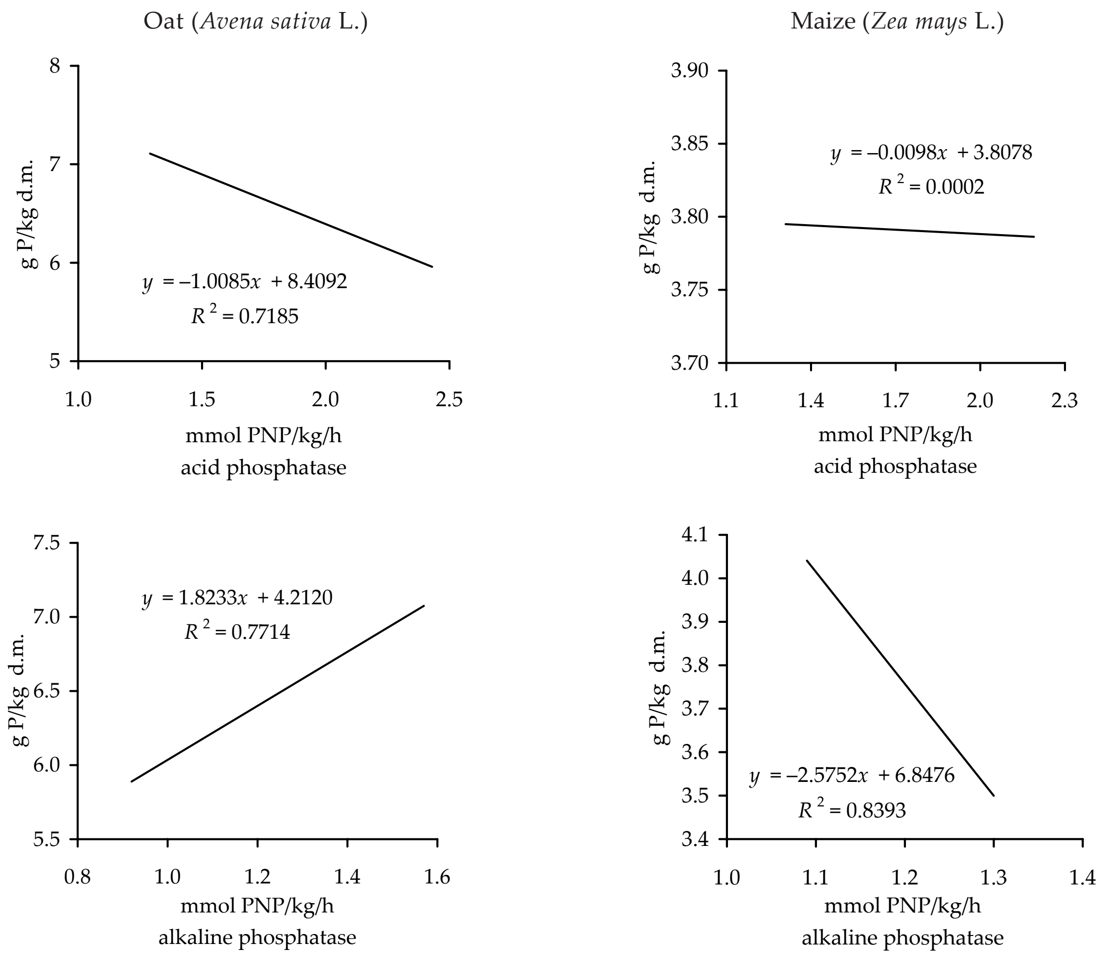

Figure 2. Correlations between the phosphorus content in oat (*Avena sativa* L.) and maize (*Zea mays* L.) and phosphatase activity in soil

The application of additional nitrogen doses to the soil resulted in an increase in its content and in the accumulation of calcium and magnesium in the above-ground parts of both crop species, as well as in the content of sodium in the maize (Tables 1 and 2). This effect was fairly strong, which is evident for the content of nitrogen. In the case of phosphorus and potassium, the correlation was reverse. The effect of nitrogen on the content of the majority of macroelements was stronger in maize than in oat.

The application of sawdust had a significantly weaker effect on the content of macroelements in the experimental plants. This is probably caused by its structure and slow decomposition in soil (Tables 1 and 2). The effect of sawdust was modified by the presence of diesel oil and increased dose of nitrogen in the soil. These changes fell into the range from several to below 20%.

The application of diesel oil not only affected the macroelements content in the soil but it also modified the soil biological properties determined by the activity of urease and acid and alkaline phosphatase. The activity of acid and alkaline phosphatase was significantly lower and the activity of urease was stronger in pots containing high doses of diesel oil. Our own experiments indicated a correlation between the activity of urease and acid and alkaline phosphatase in the soil and the macroelement content in the plants cultivated in the pots with diesel oil contaminated soil (Figures 1 and 2). However, only in the case of alkaline phosphatase and phosphorus content in oat was this correlation significant and positive. Reverse correlations were found between following: urease activity and nitrogen content in both crops, alkaline phosphatase activity and phosphorus content in maize, acid phosphatase activity and phosphorus content in the above-ground parts of oat.

Literature usually provides information on the toxic effect of petroleum-derived compounds, including diesel oil, on plants (Sparrow and Sparrow 1988, Iwanow et al. 1994, Amadi et al. 1996, Wyszkowska and Kucharski 2001, Wyszkowski et al. 2004) that was confirmed in the present study. This negative effect results from the effect of petroleum-derived compounds on the soil. They fill the space in the soil and hamper or inhibit the movement of water and air, which causes lumping and worsens its physical, chemical and biological properties. In diesel oil contaminated soil the organic carbon to nitrogen ratio is undesirable due to the presence of hydrocarbons. This limits the reactions of mineral and organic nitrogen compounds in the soil, decreases the intensity of ammonification and nitrification (Iwanow et al. 1994, Amadi et al. 1996, Rimowsky et al. 1998). In organic carbon rich soil, bacteria and fungi develop intensively and absorb macroelements (mainly nitrogen), limiting therefore the availability of these elements to plants (Małachowska-Jutsz et al. 1997, Xu and Johnson 1997). Such a correlation was also found in this experiment. The limited content of nitrogen and other macroelements was reported in oat cultivated in pots where the soil was contaminated with high doses of diesel oil. However, in the above-ground parts of maize this correlation of the contents of the majority of macroelements was reverse. The addition of nitrogen to the soil should then have a positive effect on its availability to plants as it was also confirmed in this experiment. A wide variety of diesel oil effects depending on the plant species was also found by Dimitrov and Mitowa (1998). In the study involving 7 experimental plant species, only 3 species had elevated contents in comparison to the uncontaminated soil and, in the case of phosphorus and calcium, no significant changes in their content in plants cultivated in either uncontaminated or diesel oil-contaminated environment. This research indicates a correlation between the activity of urease and acid and alkaline phosphatase and the content of nitrogen and phosphorus in plants. However, these correlations were strongly modified by the presence of diesel oil, nitrogen and sawdust presence in the soil. In previous experiments, a correlation between the soil enzymatic activity and the content of these macroelements in spring barley was also found (Wyszkowski and Wyszkowska 2004).

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ABSTRAKT

Vliv enzymatické aktivity půdy kontaminované ropnými látkami na chemické charakteristiky ovsa (*Avena sativa* L.) a kukuřice (*Zea mays* L.)

Cílem práce bylo stanovení vlivu kontaminace půdy ropnými látkami (3, 6, 9, 12 a 24 g/kg půdy) na sklizeň a obsah makroelementů v ovsu a kukuřici pěstovaných na půdách s přídavkem dusíku a pilin. Byla také sledována korelace mezi obsahem makroprvků v rostlinách a půdní enzymatickou aktivitou. Vysoké dávky ropných látek prokazatelně negativně ovlivnily obsah sledovaných makroelementů v ovsu kromě fosforu. Přítomnost ropných látek významně ovlivnila akumulaci většiny makroelementů v nadzemní části kukuřice. Aplikace dusíku do půdy působila na zvýšení obsahu dusíku a kumulaci vápníku a hořčíku v nadzemní části obou rostlin a dále na zvýšení obsahu sodíku v kukuřici. Aplikace pilin do půdy měla značně nižší vliv (méně než o 20 %) na obsah makroelementů v rostlinách. Byla stanovena korelace mezi aktivitou ureázy, kyselé a alkalické fosfatázy v půdě a obsahem makroelementů v rostlinách pěstovaných na půdě kontaminované ropnými látkami. Pozitivní korelace byla prokázána pouze mezi aktivitou alkalické fosfatázy a obsahem fosforu v ovsu.

Klíčová slova: kontaminace půdy ropnými látkami; aplikace dusíku; piliny; oves; kukuřice; obsah makroelementů; enzymatická aktivita

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