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Biochemical Properties of Soil Contaminated by Petrol

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Abstract

In this experiment the effects of lead and lead-free petrol applied at the following doses of: 0, 2, 4 and 6 $cm^3 \cdot kg^{-1}$ of soil on growth and development of triticale and biochemical properties of the soil were studied. For detoxication of petrol organic amendment with barley straw was applied. The experiment was performed in two experimental series with and without triticale cover.

It was found that soil contamination by petrol adversely affected growth and development of tested crops. Barley straw appeared to be ineffective in detoxication of the contaminated soil. Lead and lead-free petrol (irrespective of plant cover and application of straw) adversely affected activity of soil dehydrogenases and urease. Straw application and growing of triticale positively affected biochemical properties of the soil. These positive effects were diminished by petrol, irrespective of the lead addition. Biochemical index of soil fertility calculated on the basis of enzymatic activity and carbon content was negatively correlated with the level of soil contamination by petrol and positively with triticale yield.

Keywords: lead and lead-free petrol, enzyme activity, organic substance

Introduction

The growing threat to the natural environment caused by oil products due to leakage from tanks and pipes, truck tanks, during distribution process as well as by car and railway transport and petrol station is growing [1, 2, 3].

Oil products, including petrol not only modify physico-chemical [2] and biological properties of the soil [4, 5, 6, 7], but also contribute to limitations of the productive ability of arable crops. It is known that these compounds are able to affect the quality of surface and ground water and that these products are potentially dangerous for animal and human health.

Acceleration of the process of reclamation of product soils polluted by oil might be performed by the following methods: soil aeration, optimization of soil moisture [6, 8, 9] and inoculation of the soil by microorganisms [6, 10,11, 12, 13, 14]. Biological methods of pollutant removal are more effective and more environmentally friendly than physicochemical ones. The final result of properly performed bioremedation is the transformation of pollutants into microbial biomass and stabile and not-toxic compounds as: water, CO_2 and in anaerobic conditions CH_4. Such effects might be gained by an increase of the abundance and activity of endogenic microflora which show the capability to degrade oil compounds which could be achieved by application of special microbial inoculations [8].

The aim of presented studies was to determine the biochemical properties of soil contaminated with lead-free petrol and lead petrol 98 and to check the possibility of utilization of organic substances (barley straw) in detoxication of such soils.

Methods

Studies were performed in conditions of cold greenhouse in plastic pots (in 4 replications) containing 2.5 kg of proper brown soil. Soil originated from light loamy sand and showed the following physicochemical parameters: C - 0.75%; pH in 1 M KC1 6.5; hydrolytical acidity (Hh) - 1.16 mmol • 100 g^{-1} of the soil; base sum (S) - 14.1 mmol • 100 g^{-1} of soil; sorption complex capacity (T) - 15.26 mmol • 100 g^{-1} of soil; base saturation (V) - 92.4%). The test crop was triticale cv. Gabo (25 plants per pot). Before filling the pots the soil was mixed with the following doses of mineral nutrients in g • $kg^{-1,}$ of soil: N - 0.15 [$CO(NH,)_2$]; P - 0.1 [K_2HPO_4]; K - 0.15 [K_2HPO_4 + KC1]; Mg - 0.05 [$MgSO_4 \cdot 7 H_2O$]. At the same time lead and lead-free petrol was added to the soil at the following doses: 0, 2, 4 and 6 $cm^3 \cdot kg^{-1}$ of the soil. Aim different doses application of petrol was examination degree of soil contamination on biochemical properties.

Studies were performed in two experimental series with or without amendment with fine grounded barley straw at the dose of 2.4 g • kg^{-1} of soil. Our earlier investigation indicated that barley straw can be used for petrol detoxication.

The experiment was performed on soil with or without triticale cover. This plant was chosen for study because we want to examine all corns, and triticale is one of them. During plant growth (49 days) humidity on the level of 60% of capillary water holding capacity was maintained. After harvesting triticale at the stage of heading, yield was determined and in the soil with and without plant cover activity of the following enzymes was determined: soil dehydrogenases (Deh) according to Lenhard's method modified by Casidy et al. [15], urease (Ure) - according to Gorina and Chine Changa [16] and acid (Pac) and alkaline (Pal) - phophatases according to Tabatabai and Bremener's method [17].

Content of organic carbon (C_{org}) according to Tiurin's method was also determined [18].

Because the studied oil products did not modify organic carbon content in the soil, results of measurements of C content are not presented. Average C content in soil contaminated with lead-free petrol was - 0.76%, in amended with straw - 0.80% and in soil contaminated with lead petrol C content amounted to 0.75% and 0.81%, respectively.

On the basis of soil enzymatic activity and C content the coefficient of biochemical potential soil fertility was calculated using the following formula:

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

All results are presented after statistical analysis according to Dundan's test.

Results and Discussion

Our results indicated that tested oil compound appeared to be extremely toxic to triticale plants because the lowest dose (2 $cm^3 \cdot kg^{-1}$ of the soil) of lead-free and lead petrol significantly inhibited growth and development of the tested crop, which was seen as yield reduction by 14.4% and 13.5%, respectively (Fig.l and 2). Adverse effects of studied compounds on plant yield was related to the level of soil pollution. The highest amount of petrol caused 11 and 9.9 times reduction of triticale yield for lead-free and lead petrol, respectively. Straw introduced to the soil not only did not increase yield in the control treatment but also stimulated toxic effects of two tested oil products. This might be a result of too intense nitrogen immobilization in treatment with straw amendment which was indicated by an increased number of microbes [19] and yield reduction in the conditions of straw application in control pots without petrol contamination. Lack of available nitrogen and phosphorus in oil contaminated soils is one of the reason of lowered yielding potential [9].

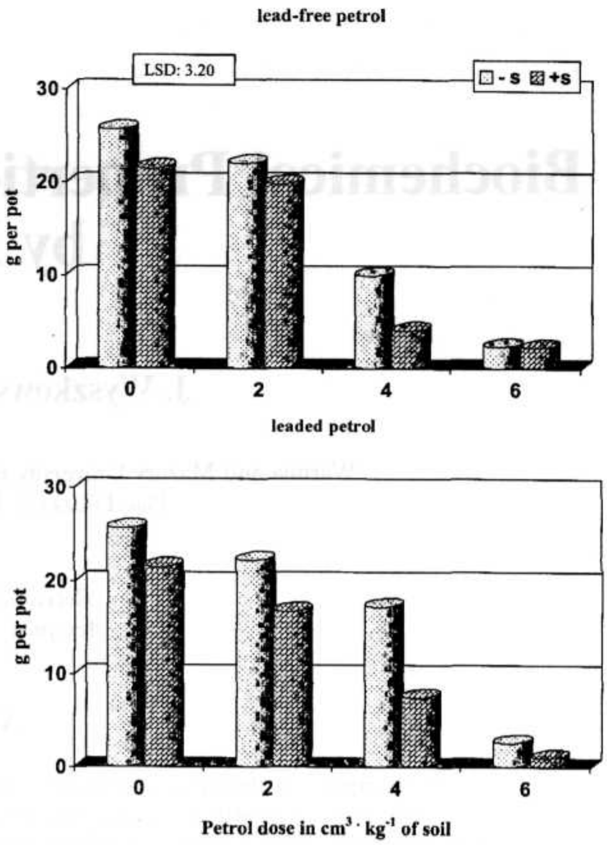

Fig. 1. The effect of petrol on triticale yield

- s: soil without straw applied

+ s: soil with straw applied

Our results confirm that soil contamination by oil products negatively affects crop production by decreasing yield [7, 9,12]. Plant response to soil contamination by oil products is related not only to the kind of soil, its pH, and water-air relations, but also to crop species. In the experiment described by Rytelewski et al. [9] on plots contaminated by diesel oil cereals (spring barley, cereal-legume mixture, winter rye) died out in different stages a few weeks after emergence, whereas potatoes did not even start growth.

Apart from microbial biomass the biochemical potential index of soil fertility might be its enzymatic activity [20]. The proper index of microbial capability to rapid degradation of hydrocarbons is activity of dehydrogenases [21]. In the presented experiment lead-free as well as

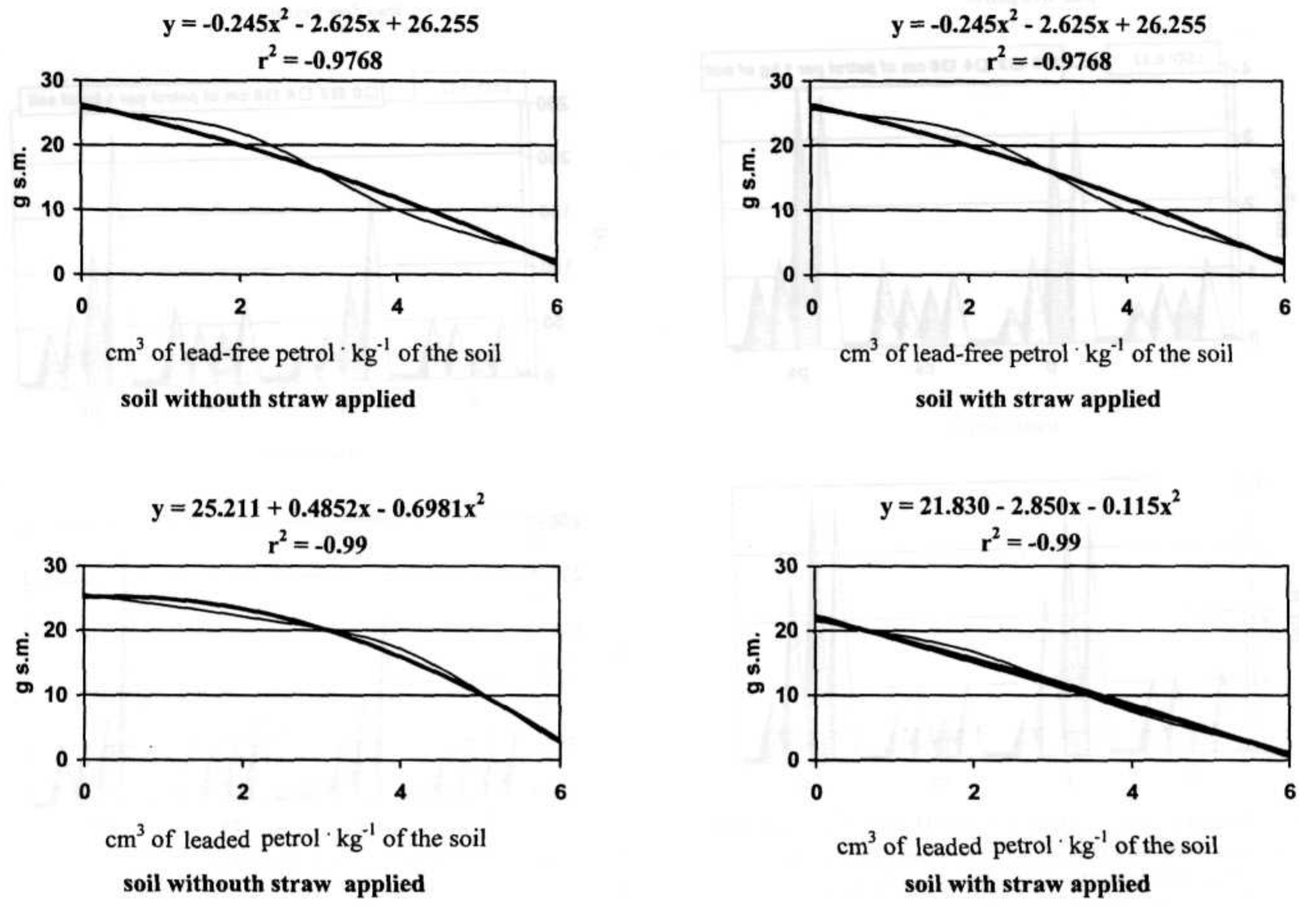

Fig. 2. Regression equations of triticale yield (g of DM • pot $^{-1}$).

leaded petrol modified activity of all investigated soil enzymes. Effects of petrol treatments on activity of dehydrogenases (Fig. 3), urease (Fig. 4) acid (Fig. 5) and alkaline (Fig. 6) phosphatases were related to the level of contamination. Oil products irrespective of plant cover and straw amendment negatively affected the activity of dehydrogenases and urease. Activity of mentioned enzymes in the soil was negatively correlated with concentration of pollutants. Application of the highest dose of lead-free and lead petrol (6 $cm^3 \cdot kg^{-1}$ of soil) resulted in reduction of dehydrogenanes activity 6.5 and 5.3 times, whereas urease 5.7 and 5.5 times, respectively, in relation to control soil. More resistant to pollution appeared to be phosphatases comparing with dehydrogenases and urease. Petrol given at doses of 4 cm^3 or 6 $cm^3 \cdot kg^{-1}$ of soil inhibited activity of acid phosphates. The only exception was soil with triticale cover, where lead petrol at a dose of 4 $cm^3 \cdot kg^{-1}$ of soil did not inhibit activity of this enzyme. Activity of acid phosphatase was related more to straw amendment than to plant cover. Straw addition to the soil stimulated activity of acid phosphatase but did not fully overcome results of petrol contamination.

Activity of alkaline phosphatase contrary to acid phosphatase was stimulated by triticale as by straw addition to the soil. Distinctly lower activity of this enzyme was noted for treatment without straw and without triticale cover. Alkaline phosphatase showed an interesting pattern of response to petrol because in treatments with bare soil incubated in the same conditions as for soil with triticale cover petrol stimulated its activity. The only exemption was treatment with lead petrol at the dose of 6 $cm^3 \cdot kg^{-1}$ of soil, which significantly inhibited activity of alkaline phosphatase.

In soil with triticale without straw addition, lead-free petrol at doses from 2 to 6 $cm^3 \cdot kg^{-1}$ of the soil did not adversely affect alkaline phosphatase, whereas leaded petrol inhibited activity of this phosphatase only when it was applied at the dose of 6 $cm^3 \cdot kg^{-1}$ of the soil. A distinct increase of alkaline phosphatase activity was found in the soil with plant cover in the series with straw addition but in conditions of higher activity significantly adverse effects of petrol contamination were revealed. The higher the dose of lead-free or leaded petrol applied, the higher inhibition of alkaline phosphatase activity was found.

Triticale cover irrespective of straw addition to the soil positively affected the number of soil microflora analyzed [19] but also stimulated activity of all tested soil enzymes excluding acid phosphatase. It might be the result of favourable effects of root secretions on microbiological and biochemical soil properties as well as positive effects of root system on soil aeration which could play an important role under conditions of petrol contamination. This positive impact of triticale on the activity of dehydrogenases, urease and alkaline phosphatase was overcome by petrol

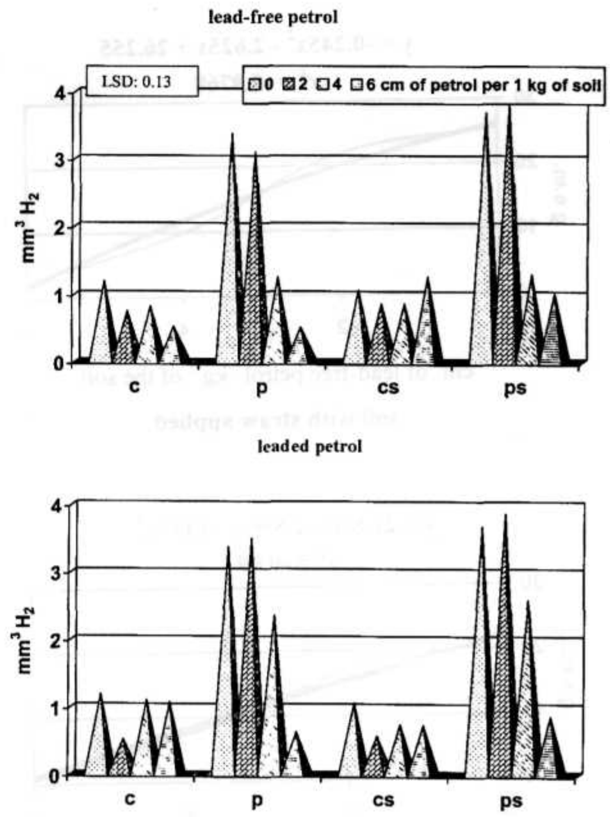

Fig. 3. Activity of dehydrogenases (mm^3 H_2) per 1 g of soil DM
c - soil not sown with triticale without straw fertilization p - soil sown with triticale without straw fertilization cs - soil not sown with triticale with straw fertilization ps - soil sown with triticale with straw fertilization

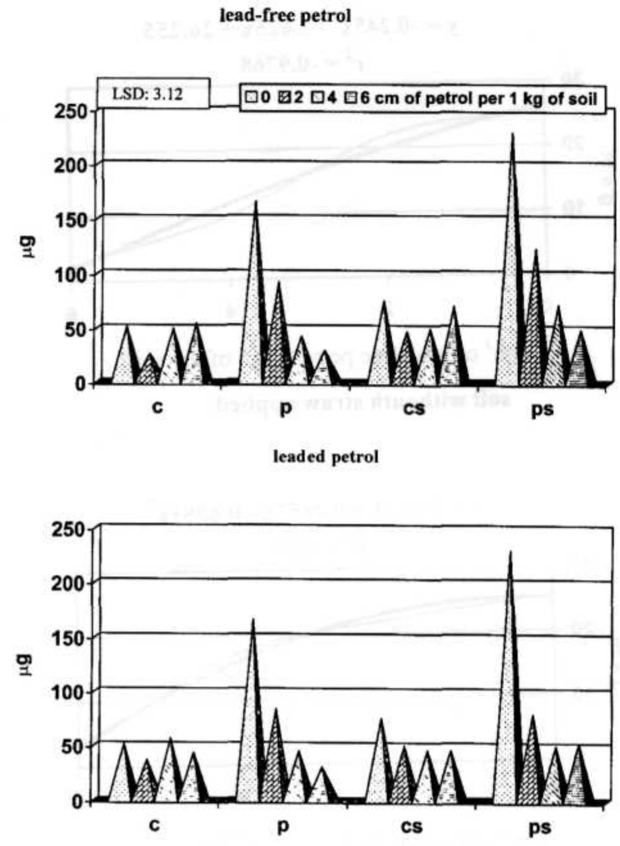

Fig. 4. Activity of urease µg hydrolized urea • h^{-1}) per 1 g of soil DM
* explanations as in Fig. 3

contamination (especially at the dose of 6 cm^3 • kg^{-1} of soil). Negative correlation between level of soil contamination and relation of enzyme activity in soil with and without triticale cover was noted (Table 1).

Soil amendment with barley straw increased enzyme activity of the soil (Table 2). Reduction of the ratio of enzyme activity (below 1) in soil with addition of barley straw to soil without straw was observed only in the case of soil

Table 1. Ratio of enzymatic activity in 1 g of soil DM between soil with to soil without plant cover.

Petrol dose (cm^3 · kg^{-1} soil)	Dehydrogenases (mm^3 H_2)		Urease (µg hydrolized urea · h^{-1})		Phosphatase (µmol p-nitrophenol) acid		Phosphatase (µmol p-nitrophenol) alkaline	
	–Pb	+Pb	–Pb	+Pb	–Pb	+Pb	–Pb	+Pb
soil without straw applied								
0	2.83	2.83	3.24	3.24	0.93	0.93	1.47	1.47
2	4.13	6.73	3.60	2.30	1.05	1.00	1.57	1.42
4	1.52	2.14	0.85	0.80	1.13	1.07	1.27	1.41
6	0.98	0.59	0.53	0.70	0.98	0.87	1.22	1.10
soil with straw applied								
0	3.47	3.47	3.05	3.05	0.86	0.86	1.79	1.79
2	4.42	6.77	2.59	1.59	0.99	0.76	1.82	1.47
4	1.51	3.50	1.44	1.09	1.14	0.88	1.35	1.03
6	0.81	1.18	0.70	1.15	1.09	0.90	1.26	1.58

– Pb: lead-free petrol; + Pb: leaded petrol

Table 2. Ratio of enzymatic activity in 1 g of soil DM between soil with and without straw applied

Petrol dose ($cm^3 \cdot kg^{-1}$ soil)	Dehydrogenases (mm^3 H_2)		Urease (µg hydrolized urea $\cdot h^{-1}$)		Phosphatase (µmol p-nitrophenol) acid		Phosphatase (µmol p-nitrophenol) alkaline	
	–Pb	+Pb	–Pb	+Pb	–Pb	+Pb	–Pb	+Pb
0	1.04	1.04	1.41	1.41	1.31	1.31	1.39	1.39
2	1.21	1.10	1.44	1.07	1.12	1.18	1.07	1.15
4	1.04	0.97	1.32	0.94	1.11	1.12	1.15	1.13
6	2.17	0.94	1.43	1.34	1.07	1.03	1.13	1.02

* Explanation as in Table 1.

contaminated by lead petrol at the dose of 4 and 6 $cm^3 \cdot kg^{-1}$ of the soil for dehydrogenases and 4 $cm^3 \cdot kg^{-1}$ of the soil for urease.

Relationships between the level of soil contamination by oil products and activity of dehydrogenases and urease which were found in the presented studies were reported earlier [22]. Galas et al. [21] reported that the level of dehydrogenases activity was related to a high extent to concentrations of hydrocarbons in the medium. Matachowska-Jutsz et al. [6] during studies on activity of amylases, proteases and dehydrogenases in soil polluted by oil fractions compared to the control soil (without xenobiotics and modifiers), found a decrease in the activity of studied enzymes.

On the basis of microbiological and biochemical properties of the soil its fertility might be determined [20, 23]. Presented in this paper the coefficient of biochemical fertility (Mw), which takes into account the activity of dehydrogenases, urease, acid and alkaline phosphatase and content of organic carbon reflected adverse effects of petrol on triticale yield (Table 3). In soil with plant cover irrespective of the straw amendment Mw was negatively correlated with the level of soil contamination. Statistical analysis showed significant correlation between value of Mw and yield of triticale (Fig. 7). Values of correlation coefficients for lead-free petrol were 0.997 and 0.965 for soil without and with straw amendment, whereas for lead petrol respective values were as follows: 0.964 and 0.994.

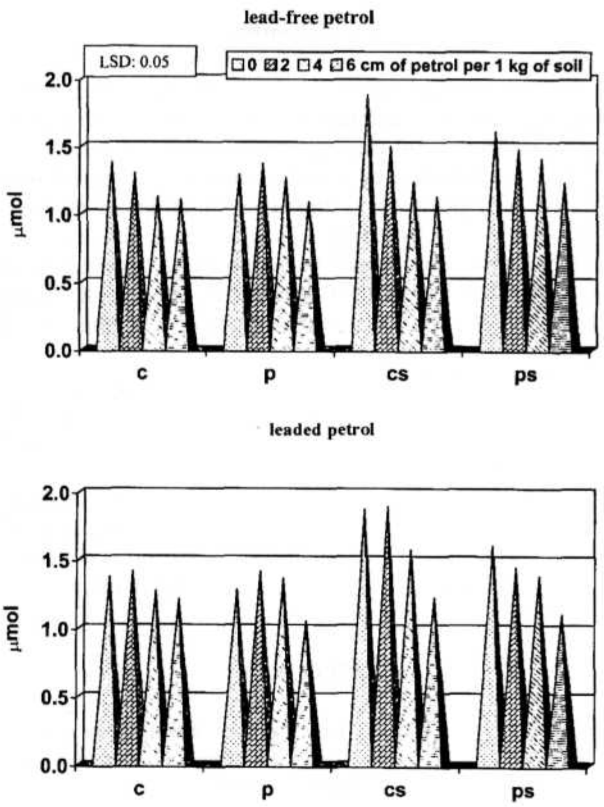

Fig. 5. Activity of acid phophatase µmol p-nitrophenol) per 1 g of soil DM.
* explanations as in Fig. 3.

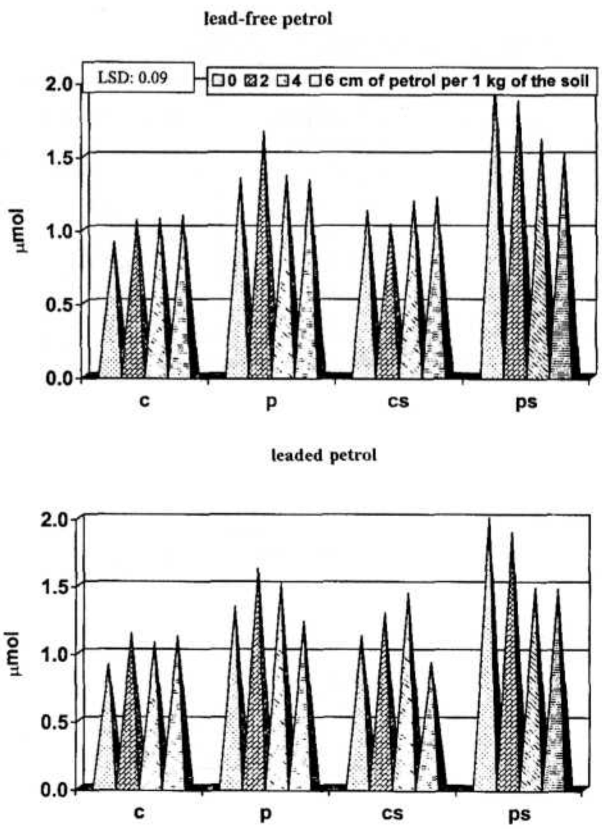

Fig. 6. Activity of alkaline phophatase µmol p-nitrophenol) per 1 g of soil DM.
* explanations as in Fig. 3.

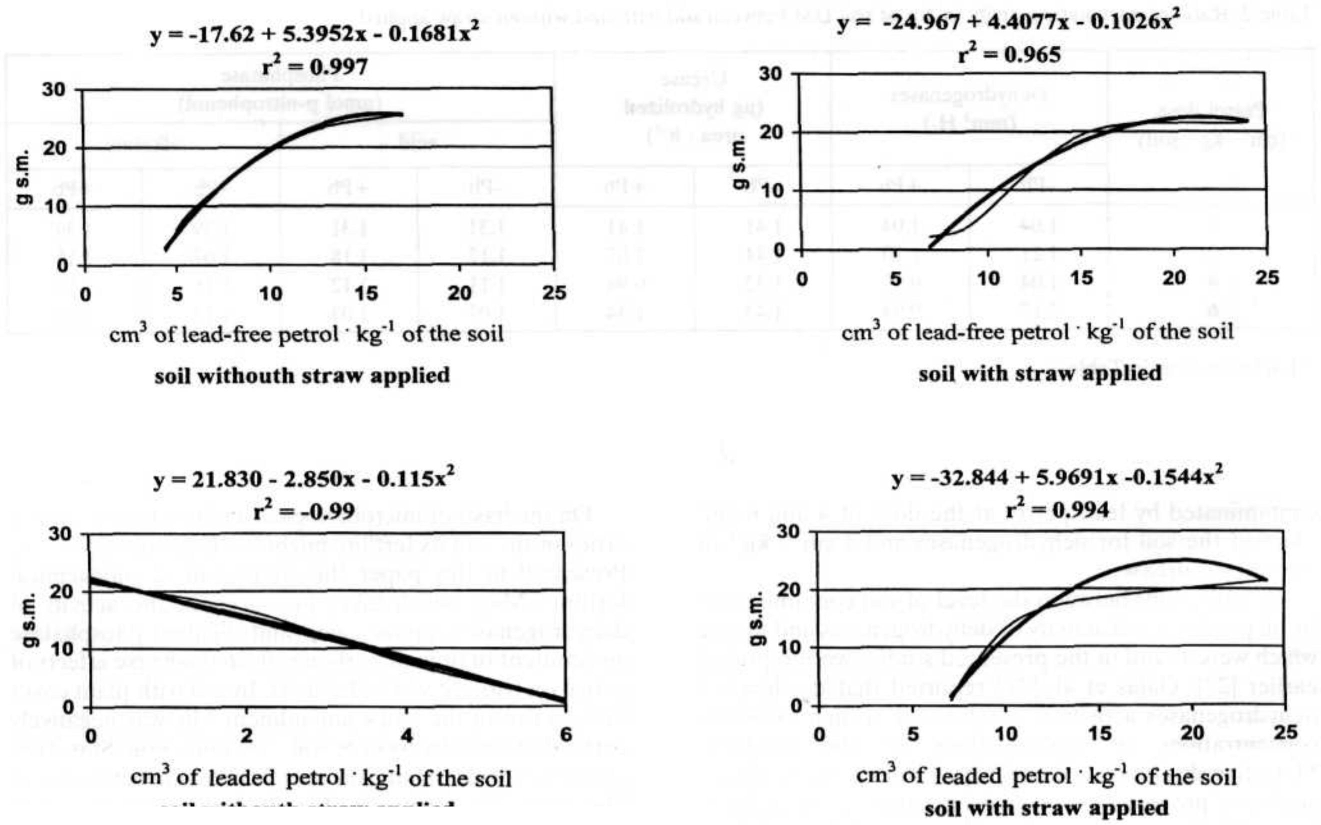

Fig. 7. Realationship between the index of soil fertility and triticale yield.

Table 3. Microbiological index of soil fertility (Mw).

Dose of petrol ($cm^3 \cdot kg^{-1}$ soil)	Lead-free petrol	Leaded petrol
soil without triticale cover and without straw applied		
0	6.47	6.43
2	4.28	5.03
4	6.02	6.79
6	6.18	5.81
$\bar{x}$	5.74	6.02
soil with triticale cover and without straw applied		
0	17.02	16.91
2	11.59	11.17
4	6.12	7.28
6	4.40	4.46
$\bar{x}$	9.78	9.95
soil without triticale cover and with straw applied		
0	9.21	9.38
2	6.45	7.08
4	6.56	6.73
6	8.46	6.05
$\bar{x}$	7.67	7.31
soil with triticale cover and with straw applied		
0	24.04	24.50
2	15.42	12.24
4	9.11	8.44
6	6.89	7.06
$\bar{x}$	13.87	13.06

Conclusions

1. Soil contamination by lead-free and lead petrol negatively affected growth and development of triticale. Barley straw did not diminish toxic effects of petrol.

2. Contamination by lead-free and leaded petrol irre spective of triticale cover and straw amendment adverse ly affected soil dehydrogenases and urease activity.

3. Soil amendment with barley straw and triticale posi tively affected biochemical properties of the soil. These positive effects were diminished by lead-free and leaded petrol.

4. Biochemical index of soil fertility calculated using enzymatic activity and carbon content was negatively cor related to level of soil contamination by lead-free and leaded petrol whereas its positive correlation with triticale yield was found. High values of correlation coef ficients between index of soil fertility and triticale yield (which was found in the conditions of stress) is evidence of its high suitability to estimation of soil fertility.

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