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EFFECT OF SOIL CONTAMINATION WITH COPPER ON ITS ENZYMATIC ACTIVITY AND PHYSICOCHEMICAL PROPERTIES

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ABSTRACT

The objective of this study was to determine the effect of soil contamination with copper (0 – 1200 mg kg^{-1} of soil) on its enzymatic activity and physicochemical properties. An attempt was undertaken to establish a possibility of the application of actinomycetes spores *Streptomyces odorifer* and *Streptomyces viridis* in detoxication of such soils. A pot experiment was conducted in two types of soil: leached brown soil formed from light loamy sand and leached brown soil formed from light loam. The experimental plant was spring barley.

The results of the experiment showed that the activity of dehydrogenases, urease, acid phosphatase, alkaline phosphatase, the value of potential biochemical index of soil fertility, exchange bases sum, total exchange capacity, base saturation of soils value and pH of soil were significantly negatively correlated with the level of soil contamination with copper and positively correlated with the yield of spring barley. The negative effect of copper on the activity of dehydrogenases, urease, alkaline phosphatase and on potential biochemical index of soil fertility was generally less significant in heavier soil than in lighter soil. The negative effect of copper on soil enzymatic activity was slightly decreased by soil inoculation with *Streptomyces odorifer* and *Streptomyces viridis* spores. This positive effect of actinomycetes was more significant in light loam than in light loamy sand.

Key words: soil contamination with copper, activity of dehydrogenases, urease, acid and alkaline phosphatase, physicochemical properties.

INTRODUCTION

Accumulation of heavy metals, including copper, in soil upper layers is a very dangerous phenomenon as it induces the disturbance of soil homeostasis [25]. Excessive amounts of heavy metals affect soil biological properties and may change its basic physicochemical properties, which in consequence results in partial or even total fertility loss [12]. Soil microorganisms and enzymatic activity are influenced by highly toxic heavy metals. Their toxicity depends on soil granulometric composition, reaction, organic matter content and sorptive capacity [19]. Soil contamination with heavy metals induces the moderation of many biological processes. The count and species diversity of macro- and microorganisms and enzymatic activity change [4, 27, 29].

High toxicity of heavy metals for soil organisms makes us look for effective methods which may be used for reclamation of soils contaminated with different xenobiotics. Microorganisms are of significant importance for the transformation of heavy metals [21]. Actinomycetes are characterised by relatively high adaptability to increasing concentrations of heavy metals in soil solution [5].

In relation with this, the research was conducted to determine the effect of soil contamination with copper on its enzymatic activity and physicochemical properties and also to verify a possibility of the application of actinomycetes spores: *Streptomyces odorifer* and *Streptomyces viridis* in detoxication of such soils.

MATERIALS AND METHODS

Vegetative experiments were conducted in two types of soil: leached brown soil formed from light loamy sand with pH 5.6 in water solution KCl of 1 mol KCl dm^{-3} concentration, hydrolytic acidity (Hh) -18.0 mmol (H^+) kg^{-1}, total exchange bases (S) – 65.6 mmol(+) kg^{-1} and organic carbon content (C_{org}) – 6.0 g kg^{-1} and leached brown soil formed from light soil with pH 5.9 in 1 mol KCl dm^{-3}, hydrolytic acidity (Hh) – 14.3 mmol (H^+) kg^{-1}, total exchange bases (S) – 81.0 mmol(+) kg^{-1} and organic carbon content (C_{org}) – 8.9 g kg^{-1}.

The experiment was carried out in 4 replications in a greenhouse, in plastic pots filled with 3.2 kg of soil which prior to the experiment was mixed with mineral fertilizers containing the following quantities of elements recalculated as pure components in mg kg^{-1} of soil: N – 120 [$CO(NH_2)_2$], P – 75 [KH_2PO_4]; K – 120 [KH_2PO_4 + KCl], Mg – 40 [$MgSO_4 \cdot 7H_2O$], 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 following doses of copper in the form of $CuSO_4 \cdot 7H_2O$ in mg kg^{-1} (soil): 0, 400, 800 and 1200 were applied to such prepared soil in certain objects. Mineral fertilisers and copper were applied into soil as water solutions.

The effect of copper on enzymatic activity and physicochemical properties of soil was analysed in two series. In the first series no microogranisms were added whereas in the second one the effect of increasing copper doses was tested after prior application of cultured *Streptomyces odorifer* (100 x 10^6 kg^{-1} of soil) and *Streptomyces viridis* (42 x 10^6 kg^{-1} of soil) spores into soil. These both *Streptomyces* were the best growing in the laboratory experiment on the contamination medium with copper.

Streptomyces odorifer and *Streptomyces viridis* were derived from own collection of the Department of Microbiology. Cells were cultured on slants at 28°C for 7 days, next a slant with 3 cm^3 sterile, 0.85 % water solution of NaCl was carried out. 60 slants were set into conical flask of 1 dm^3 capacity and after mixing 5 cm^3 of suspension was measured for 1 pot (3.2 kg of soil). The suspension was mixed with soil on the day of experiment setting. *Streptomyces odorifer* and *Streptomyces viridis* culture was prepared on nutrient solution composed of: soluble starch – 10.0 g, casein – 0.3 g, KNO_3 – 2.0 g, NaCl – 2.0 g, K_2HPO_4 – 2.0 g, $MgSO_4 \cdot 7H_2O$ – 0.05 g, $CaCO_3$ – 0.02 g, $FeSO_4$ 0.01 g, agar – 20.0 g, distilled water – up to 1 dm^3, pH – 7.0.

After mixing with fertilisers and copper sulphate soil moisture was maintained at 60 % capillary water capacity for 20 days. Afterwards, cv. Rabel spring barley was sown into pots (15 plants per pot). Spring barley was harvested at the phase of earing. During plant growth (42 days) soil moisture was maintained at a constant level of 60 % capillary water capacity.

Biochemical and physicochemical analyses of soil were conducted on the day of plant sowing, 20 days after trials establishment and on the day of plant harvest. The results of biochemical and physicochemical determinations were given as means for these two terms. Biochemical analyses involved the determination of the activity of soil dehydrogenases (Deh) with TTC substrate [23], urease (Ure) – according to Alef and Nannpieri [1] as well as acid phosphatase (Pac) and alkaline phosphatase (Pal) with Alef *et al.* method [2].

Physicochemical analyses of soil consisted in the determination of pH in KCl water solution of 1 mol dm^{-3} (soil and solution ratio was 1 : 2.5), organic carbon (C_{org}) – with Tiurin method [17], hydrolytic acidity (Hh) and total exchange bases (S) – with Kappen method [18]. On the basis of these determinants, total exchange capacity (T) and base saturation value (V) were calculated according to the formula: T = S + Hh; V = S T^{-1} 100.

Due to the insignificant effect of soil contamination with copper on organic carbon content in soil, these results were not included in this paper. The mean carbon content in 1 kg of leached brown soil formed from light loamy sand was 6.18 g, and in leached brown soil formed from light soil was 8.95 g.

Moreover, the potential biochemical index of soil fertility was calculated on the basis of enzymatic activity and carbon content according to the formula [13]:

$$M_w = (Ure\ 10^{-1} + Deh + Pac + Pal)\ \%\ C.$$

All the laboratory analyses were carried out in three replications. Results were elaborated statistically using Duncan's test and three-factor analysis. Regression equations between plant yield and activity of dehydrogenases, urease, acid phosphatase, alkaline phosphatase and potential biochemical index of soil fertility as well as regression equations and determination coefficient between the level of soil contamination with copper and enzymes activity were calculated. Pearson's simple correlation coefficients between a dose of analysed metal and plant yield, biochemical activity, physicochemical properties of soil was also calculated. Statistical analysis was accomplished with Statistica software [24].

RESULTS AND DISCUSSION

Heavy metals, including copper, infiltrating into soil can modify enzymatic activity of soil by changing its physical and chemical properties [7, 27].

The findings of the experiment conducted proved that the excessive amount of copper as a destructive factor had an inhibitory effect on soil enzyme (Table 1). Mean activity of all enzymes was reduced under the influence of copper in soil both formed from light loamy sand and light loam. Significantly negative correlation coefficients between soil contamination with copper and activity of particular enzymes show that the analysed metal inactivated dehydrogenases, urease, acid phosphatase and alkaline phosphatase.

Table 1. Effect of soil contamination with copper on enzymatic activity in 1 kg of soil d.m.

Cu dose mg kg^{-1} of soil	Dehydrogenases (cm^3 H_2 d^{-1})		Urease (mg N-NH_4 h^{-1})		Phosphatase (mmol PNP h^{-1}) acid		Phosphatase (mmol PNP h^{-1}) akaline		M_w	
	IIs	II	IIs	II	IIs	II	IIs	II	IIs	II
Control – without actinomycetes										
0	2.77	3.65	9.10	11.67	2.30	2.63	1.02	0.87	4.16	7.56
400	0.45	0.72	5.25	5.66	1.50	1.76	0.59	0.54	1.85	3.27
800	0.15	0.24	4.40	4.01	1.25	1.12	0.58	0.36	1.45	1.86
1200	0.05	0.07	4.19	3.77	0.89	1.03	0.30	0.31	0.98	1.62
Average	0.85	1.17	5.73	6.28	1.48	1.63	0.62	0.52	2.11	3.58
r	-0.85	-0.86	-0.88	-0.89	-0.97	-0.95	-0.94	-0.95	-0.91	-0.90
Streptomyces viridis										
0	2.38	4.27	8.22	9.77	2.00	2.58	1.10	0.86	3.97	7.59
400	0.25	0.60	5.50	4.70	1.69	2.32	0.69	0.61	2.00	3.59
800	0.13	0.30	3.76	4.38	1.40	1.79	0.50	0.50	1.48	2.66
1200	0.05	0.05	3.52	3.80	0.96	1.16	0.25	0.39	1.02	1.74
Average	0.70	1.30	5.25	5.66	1.51	1.96	0.63	0.59	2.12	3.89
r	-0.82	-0.84	-0.94	-0.85	-1.00	-0.99	-0.99	-0.98	-0.93	-0.93

Table 1. cont.

Streptomyces odorifer										
0	2.86	4.20	8.61	10.73	2.04	2.69	1.07	1.03	4.24	7.95
400	0.25	0.47	5.08	5.86	1.64	2.15	0.54	0.53	1.83	3.32
800	0.10	0.31	3.33	4.45	1.31	1.52	0.36	0.44	1.33	2.46
1200	0.05	0.08	2.65	3.95	1.11	1.57	0.33	0.38	1.11	2.22
Average	0.82	1.26	4.91	6.25	1.52	1.98	0.57	0.59	2.13	3.99
r	-0.81	-0.82	-0.95	-0.91	-0.99	-0.93	-0.90	-0.89	-0.89	-0.87
LSD*	a – 0.08; b – 0.06; c – 0.07; a x b – 0.12; a x c – 0.14; b x c – 0.10; a x b x c – 0.20		a – 0.37; b – 0.26; c – 0.32; a x b – 0.53; a x c – 0.65; b x c – 0.46; a x b x c – 0.92		a – 0.09; b – 0.06; c – 0.08; a x b – 0.13; a x c – 0.16; b x c – 0.11; a x b x c – 0.22		a – 0.04; b – 0.03; c – 0.03; a x b – 0.05; a x c – 0.07; b x c – 0.05; a x b x c – 0.10		a – 0.21; b – 0.16; c – 0.18; a x b – 0.31; a x c – 0.37; b x c – 0.26; a x b x c – 0.53	

M_w - potential biochemical index of soil fertility.
*LSD for: a – Cu dose, b – actionomyces application, c – kind of soil, n.s. – non-significant.
lls - light loamy sand, ll - light loam

Copper activity was determined by contamination level, type of soil and inoculation with actinomycetes spores (Table 1). Even the lowest dose (400 mg Cu kg^{-1} soil), in the series without inoculation, irrespective of the soil type, caused reduction of dehydrogenases activity by 82%, urease by 47%, acid phosphatase by 34% and alkaline phosphatase by 40% on average in comparison to control object (not contaminated). According to Kieliszewska-Rokicka [10] and Trazar-Cepeda *et al.* [26] dehydrogenases are very susceptible to anthropogenic stress factors and therefore they turned to be the most responsive to soil contamination with copper. Own research shows that dehydrogenase activity under the influence of 1200 mg Cu kg^{-1} was inhibited by 98% both in light loamy sand and light loam. The effect of copper on other enzymes was less toxic, their reduction in light loamy sand and light loam amounted to: urease by 54% and 68%, acid phosphatase by 62% and 61%, alkaline phosphatase by 71% and 64%, respectively.

According to Kucharski [13], Myśków [20] and Trazar-Cepeda *et al.* [26] more information about biochemical transformations in soil can be obtained taking into account a higher number of enzymes. Therefore, the soil fertility biological indicator allowing for activity of dehydrogenases, urease, acid phosphatase, alkaline phosphatase and organic carbon content was figured out in the paper. Similarly to individual enzyme activity, it was modified by soil contamination with copper degree, type of soil and *Streptomyces* application to soil. Adverse copper effect on soil enzymes activity resulted in reduction of potential biochemical index of soil fertility. Irrespective of soil type and inoculation applied its volume lowered under the effect of 400 mg Cu kg^{-1} by 55%; 800 mg Cu kg^{-1} by 68%; 1200 mg Cu kg^{-1} by 75%.

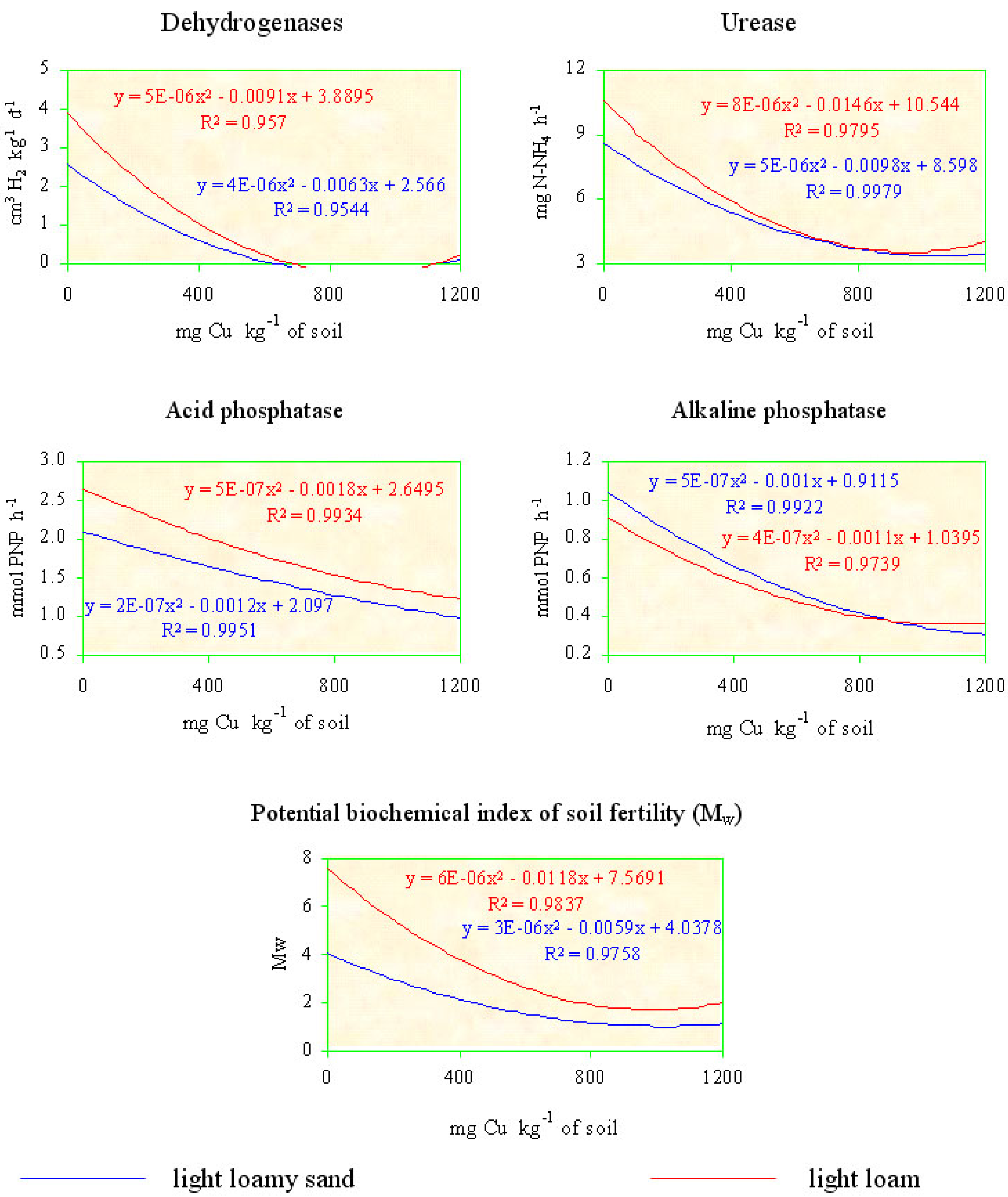
Own previous research [14, 16] and the findings of other authors [6, 9, 27] showed negative effect of copper on the activity of all the analysed soil enzymes. Effect of copper and other heavy metals is related to the level of soil contamination. Enzyme activity can be stimulated when the content of heavy metals in soil only slightly exceeds natural value but it is inhibited under the influence of their excessive amount [19, 22]. In this situation heavy metals act as typical inhibitors of soil enzymatic and microbiological activity and change its biological balance [6, 27].

Inhibitory effect of soil contamination with copper on its enzymatic activity observed in the study may result from indirect effect of copper on the number of microorganisms [15] and from its direct effect on enzymes as the negative effect of heavy metals on proteins is well known [21].

The intensity and profile of copper toxic effect on enzyme activity was also related to soil inoculation with *Streptomyces* spores (Table 1). The application of *Streptomyces odorifer* increased the activity of dehydrogenases, alkaline phosphatase and value of potential biochemical index of soil fertility both in leached brown soil formed from light loamy sand and that formed from light loam but not contaminated with copper. However, *Streptomyces viridis* stimulated only dehydrogenases activity in light loam and alkaline phosphatase in light loamy sand which was changed by application of copper sulphate weakening inoculations influence. Adverse copper effect on soil biochemical properties was only partially mitigated by actinomycetes addition to soil. *Streptomyces odorifer* and *Streptomyces viridis* spores positive effect was more evident in heavier soil (light loam) than in lighter soil (light loamy sand). Results obtained in the previous research [28] indicated that inoculation consisting of *Streptomyces* application did not also level the negative effect of contamination with chromium especially with its higher doses (80 and 120 mg Cr(VI) kg^{-1} soil).

Type of soil was a very important factor modifying soil enzymatic activity. Negative copper effect on the activity of dehydrogenases, urease, alkaline phosphatase and on the potential biochemical index of soil fertility was generally weaker in heavier soil – light loam than in lighter soil – light loamy sand (Fig. 1). In the series without inoculation dehydrogenases activity was higher in heavier than in lighter soil by 48% and soil potential biochemical index of soil fertility by 63% on average. These values amounted to 88% and 80% respectively in the objects enriched with *Streptomyces viridis* and 101% and 88% in the objects enriched with *Streptomyces odorifer*. Lower toxic copper effect on soil enzyme activity in leached brown soil formed from light loam than in leached brown soil formed from light loamy sand can be the result of soil different physicochemical properties because sorption of this metal was probably more intensive in light loam of richer sorption complex.

Fig 1. Inter-realtionship between soil contamination with copper and soil enzymes activity and potential biochemical index of soil fertility (Mw)



Soil contamination with copper, applied as copper sulphate, resulted not only in changing soil biochemical balance but also influenced its physicochemical properties which are important factors indicating growth and development of plants as well as heavy metals accumulation in their tissues [3]. Irrespective of soil and applied actinomycetes type exchange bases sum, total exchange capacity, base saturation of soils degree and soil pH were negatively and hydrolytic acidity positively correlated with this metal dose (Table 2). Exchange bases sum, total exchange capacity and base saturation of soils, unlike hydrolytic acidity, were higher in light loam than in light loamy sand both in non-vaccinated and vaccinated with *Streptomyces odorifer* and *Streptomyces viridis* soils. Jasiewicz and Antonkiewicz [8] in their study claim that heavy metals can also acidify soil.

Table 2. Effect of soil contamination with copper on physicochemical properties in 1 kg of soil d.m.

Cu dose mg kg^{-1} of soil	pH_{KCl}		Hydrolytic acidity		Total exchange bases		Sorptive capacity		Degree of base saturation %	
			mmol(H^{+}) · kg^{-1}							
	lls	ll	lls	ll	lls	ll	lls	ll	lls	ll
Control – without actinomycetes										
0	6.13	6.23	16.50	14.44	66.25	81.00	79.75	95.44	83.07	84.87
400	5.85	5.93	21.94	18.75	60.00	75.00	76.50	93.75	78.37	80.03
800	5.50	5.40	24.75	23.44	52.75	69.00	74.69	92.44	70.11	74.65
1200	5.23	5.28	19.17	26.44	48.00	64.25	72.75	90.69	65.72	70.81
Average	5.68	5.71	20.59	20.77	56.75	72.31	75.92	93.08	74.32	77.59
r	-1.00	-0.98	0.39	1.00	-1.00	-1.00	-0.99	-1.00	-0.99	-1.00
Streptomyces viridis										
0	5.98	6.50	18.38	14.07	66.75	81.75	80.26	95.82	83.17	85.26
400	5.70	5.50	22.69	20.07	60.25	72.50	78.63	92.57	76.63	78.28
800	5.35	5.20	27.75	23.44	57.50	67.50	80.19	90.94	71.60	74.21
1200	5.25	4.95	20.58	26.63	50.25	57.00	78.00	83.63	64.42	68.29
Average	5.57	5.54	22.35	21.05	58.69	69.69	79.27	90.74	73.95	76.51
r	-0.98	-0.94	0.38	0.99	-0.99	-0.99	-0.59	-0.96	-1.00	-1.00
Streptomyces odorifer										
0	6.08	6.18	19.69	14.25	64.00	80.25	77.50	94.50	82.58	84.79
400	5.85	5.60	21.94	21.01	58.00	70.50	77.69	91.51	74.65	77.05
800	5.63	5.50	23.82	22.32	49.50	66.00	71.44	88.32	69.37	74.75
1200	5.35	4.98	19.74	26.07	47.75	58.75	71.57	84.82	66.82	69.54
Average	5.73	5.56	21.30	20.91	54.81	68.88	74.55	89.78	73.35	76.53
r	-1.00	-0.97	0.13	0.96	-0.97	-0.99	-0.88	-1.00	-0.97	-0.98
LSD*	a – 0.07; b – n.s.; c – 0.06; a x b – 0.11; a x c – 0.13; b x c – 0.09; a x b x c – 0.19		a – 1.06; b – 0.75; c – n.s.; a x b – n.s.; a x c – n.s.; b x c – n.s.; a x b x c – n.s.		a – 2.16; b – 1.53; c – 1.87; a x b – n.s.; a x c – n.s.; b x c – n.s.; a x b x c – n.s.		a – 2.34; b – 1.65; c – 2.02; a x b – n.s.; a x c – n.s.; b x c – 2.86; a x b x c – n.s.		a – 2.34; b – 1.65; c – 2.03; a x b – n.s.; a x c – n.s.; b x c – 2.86; a x b x c – n.s.	

M_w - potential biochemical index of soil fertility.
*LSD for: a – Cu dose, b – actionomyces application, c – kind of soil, n.s. – non-significant.
lls - light loamy sand, ll - light loam

The conducted research indicates strong and important relationship between the activity of dehydrogenases, urease, acid phosphatase, alkaline phosphatase and potential biochemical index of soil fertility and barley yield (Fig. 2, Table 3). It was confirmed by the results obtained from regression equations and highly significant coefficients of determination (from 0.84 to 1.00) calculated for spring barley yield activity of particular enzymes as well as value of potential biochemical index of soil fertility. Activity of analysed enzymes was significantly correlated not only with copper content in soil and plant yield but also with particular enzyme activity and basic physicochemical properties of soil. Conducted study confirms enzyme importance in determination of soil biological condition as they were found more sensitive indicator determining condition of contaminated soil than soil microorganisms [15].

Fig 2. Inter-relationships between the activity of enzymes in 1 kg d.m. of soil and the potential biochemical index of soil fertility (M_w) and the yield (g per pot) of above-ground parts of spring barley

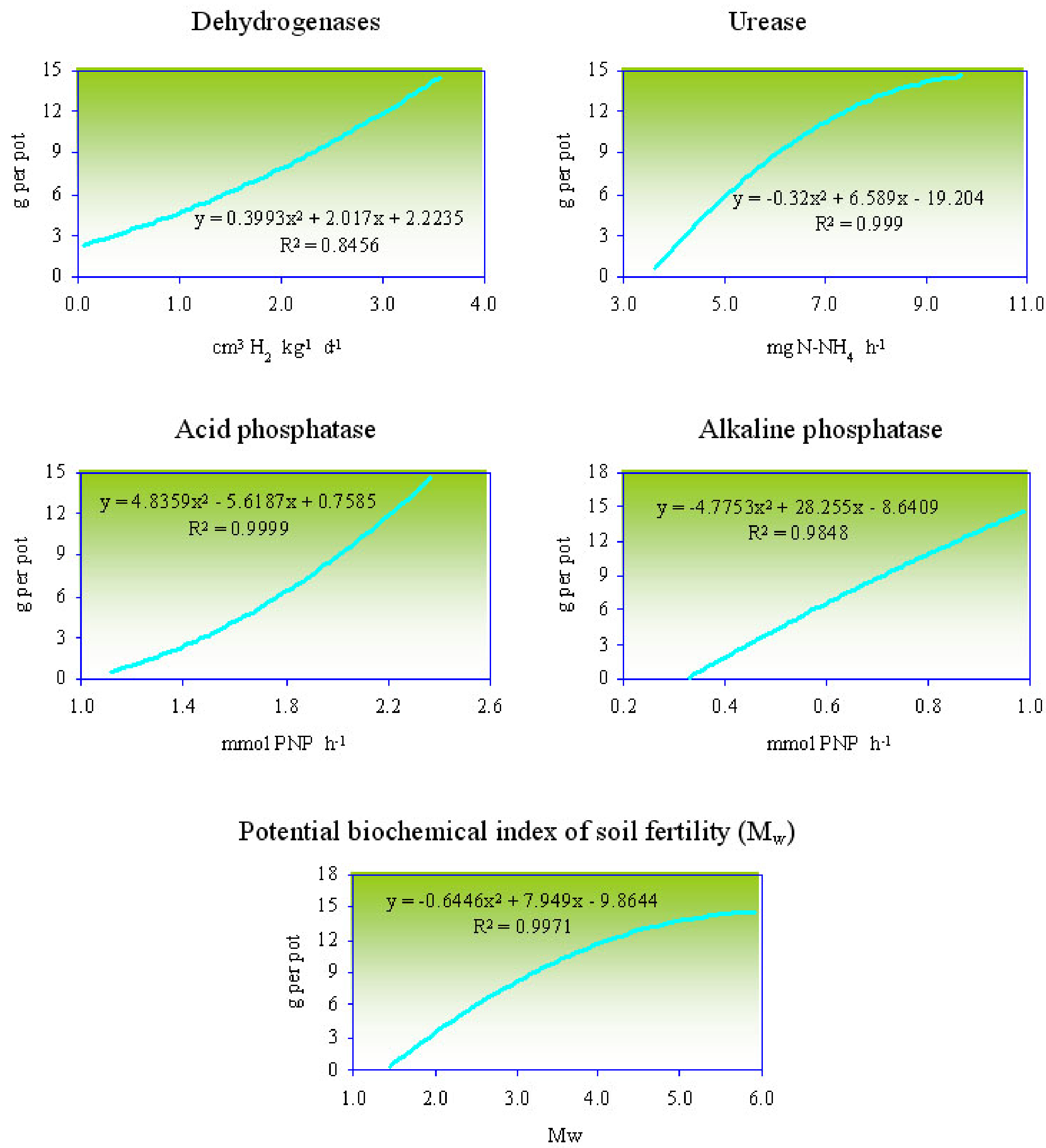


Table 3. Pearson's simple correlation coefficients between spring barley yield and enzyme activity and soil physicochemical properties

Variable	Yield	Deh	Ure	Pac	Pal	Mw
Light loamy sand						
Yield	1.00	0.92**	0.95**	0.96**	0.93**	0.96**
Deh	0.92**	1.00	0.95**	0.86**	0.92**	0.98**
Ure	0.95**	0.95**	1.00	0.91**	0.96**	0.97**
Pac	0.96**	0.86**	0.91**	1.00	0.94**	0.93**
Pal	0.93**	0.92**	0.96**	0.94**	1.00	0.97**
Mw	0.96**	0.98**	0.97**	0.93**	0.97**	1.00
Hh	-0.53**	-0.61**	-0.57**	-0.37*	-0.41**	-0.54**
S	0.91**	0.81**	0.90**	0.94**	0.93**	0.89**
T	0.56**	0.50**	0.61**	0.64**	0.62**	0.57**
V	0.96**	0.84**	0.92**	0.96**	0.95**	0.92**
pH	0.95**	0.81**	0.87**	0.94**	0.88**	0.88**
C	-0.07	-0.16	-0.27	-0.07	-0.15	-0.12
Light loam						
Yield	1.00	0.93**	0.95**	0.92**	0.96**	0.97**
Deh	0.93**	1.00	0.97**	0.82**	0.95**	0.98**
Ure	0.95**	0.97**	1.00	0.85**	0.95**	0.98**
Pac	0.92**	0.82**	0.85**	1.00	0.93**	0.90**
Pal	0.96**	0.95**	0.95**	0.93**	1.00	0.98**
Mw	0.97**	0.98**	0.98**	0.90**	0.98**	1.00
Hh	-0.98**	-0.91**	-0.92**	-0.90**	-0.94**	-0.94**
S	0.92**	0.84**	0.86**	0.83**	0.87**	0.88**
T	0.77**	0.69**	0.70**	0.67**	0.70**	0.71**
V	0.96**	0.88**	0.90**	0.88**	0.91**	0.92**
pH	0.94**	0.89**	0.89**	0.79**	0.86**	0.90**
C	-0.09	-0.24	-0.12	-0.10	-0.21	-0.18

correlation coefficients significant difference for: ** p=0.01; * p=0.05
Deh – dehydrogenases, Ure – urease, Pac – acid phosphatase, Pal - alkaline phosphatase, M_w - potential biochemical index of soil fertility, Hh – hydrolytic acidity, S – total exchange bases, T –sorptive capacity, V – degree of base saturation, C – organic carbon content

According to Kiss [11], they can even be used for evaluation of the degree of industrial earth evolution as well as for the appraisal of the effectiveness of the reclamation methods applied. This author claims that in comparison to methods based on microbiological parameters, enzymatic methods allow obtaining more synthetic soil evolution indicators because they reflect their past due to enzyme accumulation as humus complexes and also reflect their present biological condition according to their significant role in element circulation.

CONCLUSIONS

1. Activity of dehydrogenases, urease, acid phosphatase, alkaline phosphatase, value of potential biochemical index of soil fertility and exchange bases sum, total exchange capacity, base saturation of soils value and pH of soil were negatively correlated with the level of soil contamination with copper and positively correlated with spring barley yield.
2. Negative copper effect on activity of dehydrogenases, urease, alkaline phosphatase and potential biochemical index of soil fertility was generally weaker in heavier soil (light loam) than in lighter soil (light loamy sand).
3. Soil inoculation with *Streptomyces odorifer* and *Streptomyces viridis* spores only slightly decreased negative copper effect on soil enzymatic activity. Positive effect of actinomycetes was stronger in light loam than in light loam sand.

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