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FOSPHATASE ACTIVITY AND ATP CONTENT IN SOIL AND PLANT RELATED TO COPPER (II) NITRATE (V) CONCENTRATION IN SOIL

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ABSTRACT

The paper presents the results of a pot experiment on phosphatase activity and ATP level in plant and in soil induced by diversified doses of copper (II) nitrate (V). Five combinations of copper (II) salt were applied; each combination was conducted in 4 identical series. Water solutions of copper (II) nitrate (V) were introduced into 5 soil samples in the following doses: no salt introduced (control), 0.05 mmol kg⁻¹, 0.50 mmol kg⁻¹, 5.00 mmol kg⁻¹, 50.00 mmol kg⁻¹ of soil for the samples from 1 to 5, respectively. Size grading of the soil experimented was typical for light silty clay. Sown Pea *Pisum sativum* L. of Amethyst variety served as a test plant. Enzymes activities and ATP contents in both soil and plant samples were analysed in various developmental stages of Sown Pea, namely at the two-leaves, blooming and green legume stages. Copper (II) nitrate (V) introduced into soil (apart from 0.05 mmol kg⁻¹ dose) had an inhibiting effect on the acid phosphate activity both in soil and in plant. Similar effect was observed for the alkaline phosphatase in soil, while in plant at the two-pairs of leaves stage (14th day) for all combinations with the applied salt copper (II) admixture, the enzyme activity was lower than in the control samples, i.e. with no admixture of copper (II) nitrate (V). At the time of blooming (44th day) the studied enzyme was found to have been activated in all the cases in comparison to its activity in the control samples. In the last examined stage of plant development, namely green legume phase (56th day of the experiment) the level of alkaline phosphatase was comparable with the enzyme activity in the control samples. The highest level of ATP in soil was observed on the 1st day of the experiment, just after the pea *Pisum sativum* L. seeds had been sown, while the lowest ATP content was determined on 44th day of the experiment, at blooming stage when the highest values for ATP content was noted in the plants growing on the control substrate to which 0.05 mmol kg⁻¹ of copper (II) salt was introduced. ATP content in the plants growing on the substrate with copper (II) salts concentrations of 0.50 mmol kg⁻¹ and 5.00 mmol kg⁻¹ was rising with time. The highest of all

the applied in the reported experiment concentrations of copper (II) nitrate (V), i.e. 50.00 mmol·kg⁻¹, prevented the tested plant from growing. The determined total and soluble copper content in the soil samples and overall amount of copper in the plants provided data for calculating correlation between the examined specimens. The obtained correlation values indicate that the content of studied forms of copper both in soil and in plant and the activity of the enzymes under examination are highly inter-dependable. Significantly lower correlation values were obtained for the relation between the ATP level and the content of the studied copper forms in soil and in plant.

Key words: asoil, phosphatase activity, ATP.

INTRODUCTION

Dynamics of phosphorus transformations, one of important metabolic processes that occur in soil, and phosphatase activity is a reliable soil fertility and its productivity indicator [20,22]. Living organisms require continuous supplies of energy, which prior to its consumption, is partially accumulated in an easily available form, namely – adenosinetriphosphate (ATP). ATP is a direct donor for unbound energy in biological systems, and its level is tightly related to the development and the current state of the organism. The compound is also phosphatase sensitive. Therefore, ATP provides an ideal molecule to measure both contents and activity of organisms living in soil [12,27].

Enzymatic activity and intensity of metabolic transformations depend both on physical and chemical properties of soil and on living organism participating in the processes in question [6,25]. Due to such participation microorganisms are reported to play an important role in maintaining soil fertility and its biological productivity [16,17].

Excessive introduction of heavy metals, copper in particular, into the natural environment can very soon disturb the right path of biochemical processes taking place in soil [21,23]. The remark remains equally valid for the soil itself and the plants grown upon. Higher heavy metal content contributes also to altering the reaction of the soil solution making it distinctly more acid. The resulting soil conditions are disfavoured to enzymes actions, since they affect detrimentally nutrition elements accumulation in plants, which in turn directly affects enzymes activity in plants [8].

The presented research has been aimed at determining the dependence between plant and soil phosphatase activities as well as to analyse ATP content in soil and plant in relation to copper concentration for specific soil combinations.

MATERIAL AND METHODOLOGY

The experiment was performed on soil samples collected from ornohumus horizon (0-30 cm) black soils of Gumieniecka Plain. The soil originated from a cultivated field of a size grading determined as light silty clay (42% of sand, 32% of silt, and 26% illuvial parts), the humus content between 1.2 and 1.8%, slightly acid or neutral reaction, highly available phosphorus abundance, and average to high available potassium and magnesium abundance. The soil was classified as arable land, and belong to II, IIIa and IIIb bonitation class for arable lands [2].

The experiment was set up as one factor experiment in a complete randomisation arrangement and carried out in 2001 vegetation season. The tested plant was Sown Pea */Pisum sativum L./* of Amethyst variation. Five combinations of copper (II) nitrate (V) doses were applied, each one repeated in 4 series. Water solutions of [Cu (NO₃)₂·3H₂O] were introduced into particular soil samples, in the following amounts: no salt (control), 0.05, 0.50, 5.00, and 50.00 mmol per kg of soil for the samples from 1 to 5, respectively. Constant soil wetness was maintained, at the level of 60% m.p.w. over the entire experiment.

The pots were filled up to 1/3 of their height with crushed basaltic stone as drain. Each pot was then filled with a 2 kg soil sample of a given copper (II) nitrate (V) concentration, into which 5 pea seeds */Pisum sativum L./*. In the vegetation period the plant and the soil from all the combinations were sampled at the following developmental stages 'two pairs of leaves', blooming, and green legumes which corresponds to 14th, 44th and 56th day of the experiment to analyse activity of the soil and plant phosphatase and ATP content. For soil samples additional activity measurements were performed for both acid and alkaline phosphatase as well as ATP content on the 1st day of the experiment. On the 1st and 56th day soil samples were analysed to determine the total and soluble copper content. At the last development stage of the plant samples were collected, dried and their copper content was determined.

A calorimetric method was applied to determine activities for both soil phosphatases according to Tabatabai&Bremner [26] and Eivazi&Tabatabai [3] and Margesin's modifications [14] with a Zeiss Jena spectrophotometer at wavelength $\lambda=400$ nm. Soil phosphatase activities were given in μg of p-nitrophenol amount per gram of soil dry mass and 1 hour incubation time [$\mu\text{g p-Np} \cdot (\text{g}_{\text{soil d.m.}} \cdot \text{h})^{-1}$]. For both plant phosphatases calorimetric measurements were conducted at wavelength of 415 nm, with the same spectrometer as earlier. Strained plant homogenate was used to determine activities for both phosphatases according to the Bessey's-Lowry's method (cited as in Klyszejko-Stefanowicz [10]). The obtained results were then recalculated as Bessey's units, i.e. the number of p-nitrophenol mmoles released by the enzyme per 100 g of fresh mass of leaves in 30 minutes.

ATP content in the soil material was determined according to Margesin [15] method. Diluted soil extracts were determined by means of bioluminescence method in a luciferin-luciferase (L/L) enzymatic system by using a Lumat LB 9507 luminometer manufactured by Berthold. ATP content is given as the amount of ATP in μg per soil dry mass [$\mu\text{g ATP} \cdot \text{g}^{-1}_{\text{soil d.m.}}$].

To determine ATP content for plants a bioluminescence method was applied with enzymatic L/L system and the luminometer mentioned above. The John's [7] method was applied to prepare the extract. ATP content in the plant tissue is given as ATP content in μmol per 1 gram of fresh mass of plants [$\mu\text{mol ATP} \cdot \text{g}^{-1}_{\text{plants d.m.}}$].

All the copper content data for the studied soils and plants was obtained with an Optima 2000 DV apparatus manufactured by Perkin Elmer, which employs inductively coupled plasma optical emission spectroscopy (ICP-OES).

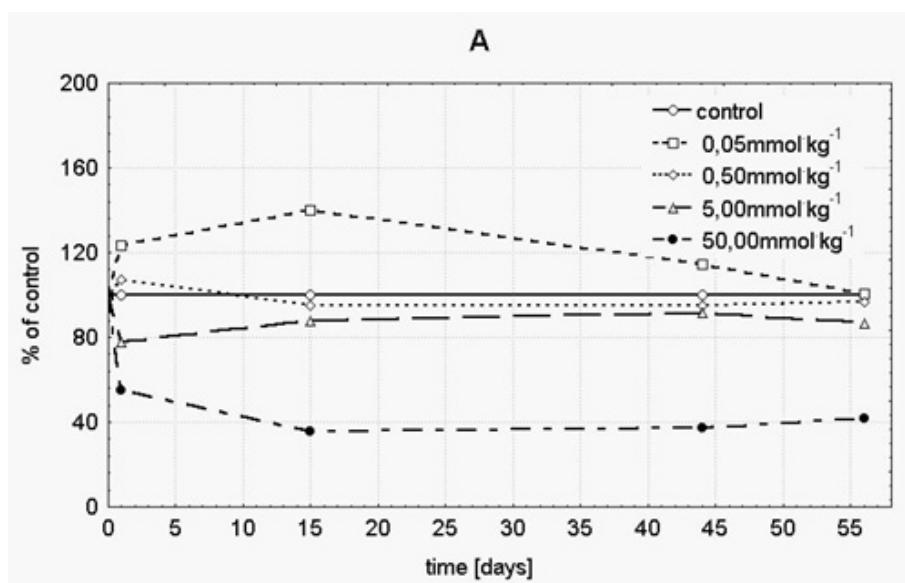
Another element of the complex studies was to determine the reaction of the examined soil samples in H_2O and KCl at the dates quoted above. Values for pH were found by means of electrometric method with a Mera Tronik pH-meter (N517 type).

Statistical analysis of the obtained data was performed with an Microsoft Excel 7.0 PL. sheet; at the determined significance level of $\alpha=0.05$ [24] for a correlation factor (R^2). For the studied soil enzymes and ATP contents first measurements were performed on the 1st day of the experiment. Turkey's test was applied to calculate least significant difference (LSD) at the significance level $\alpha=0.05$.

RESULTS AND DISCUSSION

The processed graphically experimental results are presented as plots in [Figure 1](#) (A and B), [Figure 2](#) (A and B) and [Figure 3](#) (A and B). [Table 1](#) contains data on pH, while the correlation analysis data are presented in [Tables 2](#) and [3](#).

Fig.1. Acid phosphatase activity in soil (A) and in plant (B)



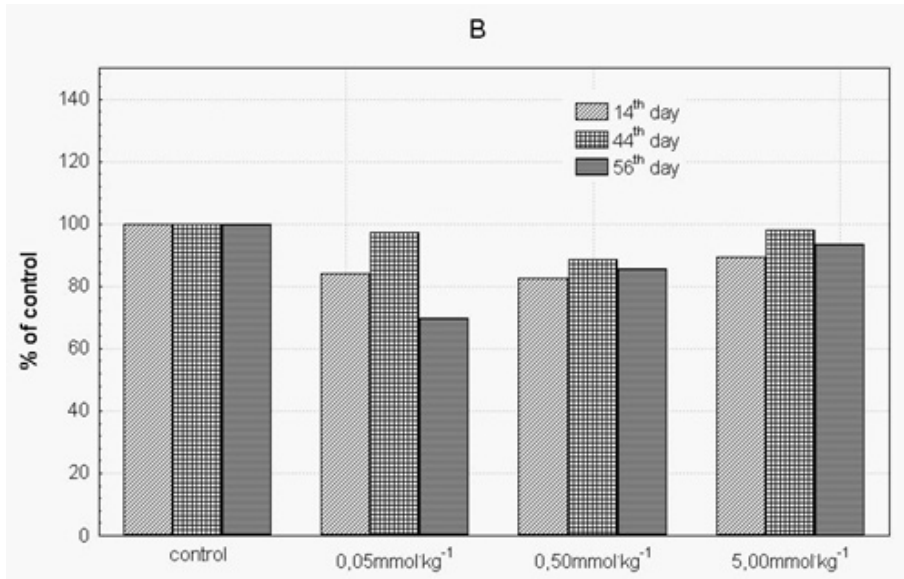


Fig.2. Alkaline phosphatase activity in soil (A) and in plant (B)

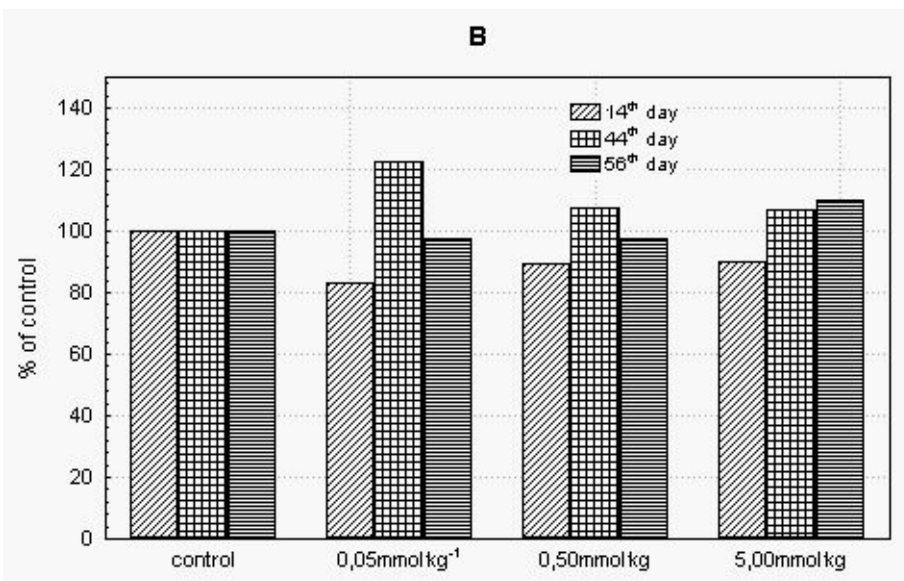
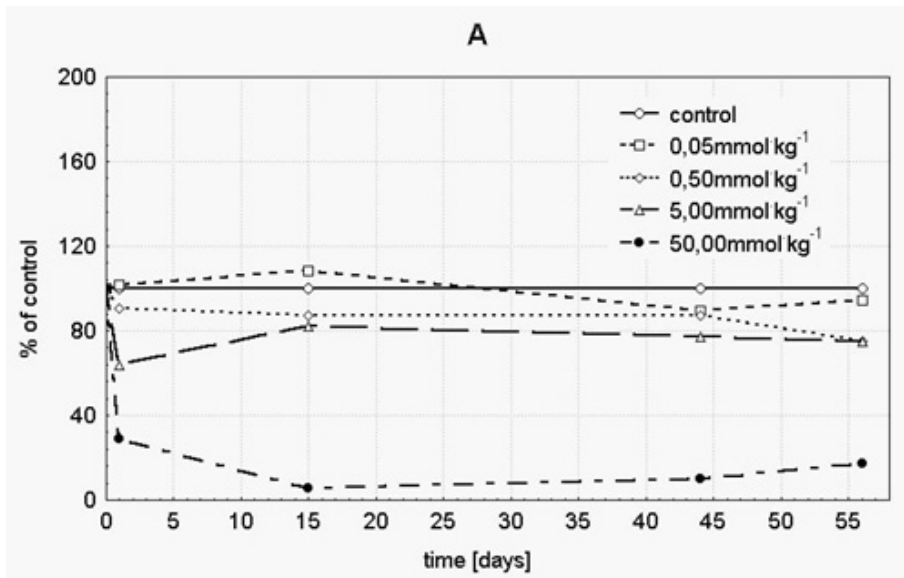
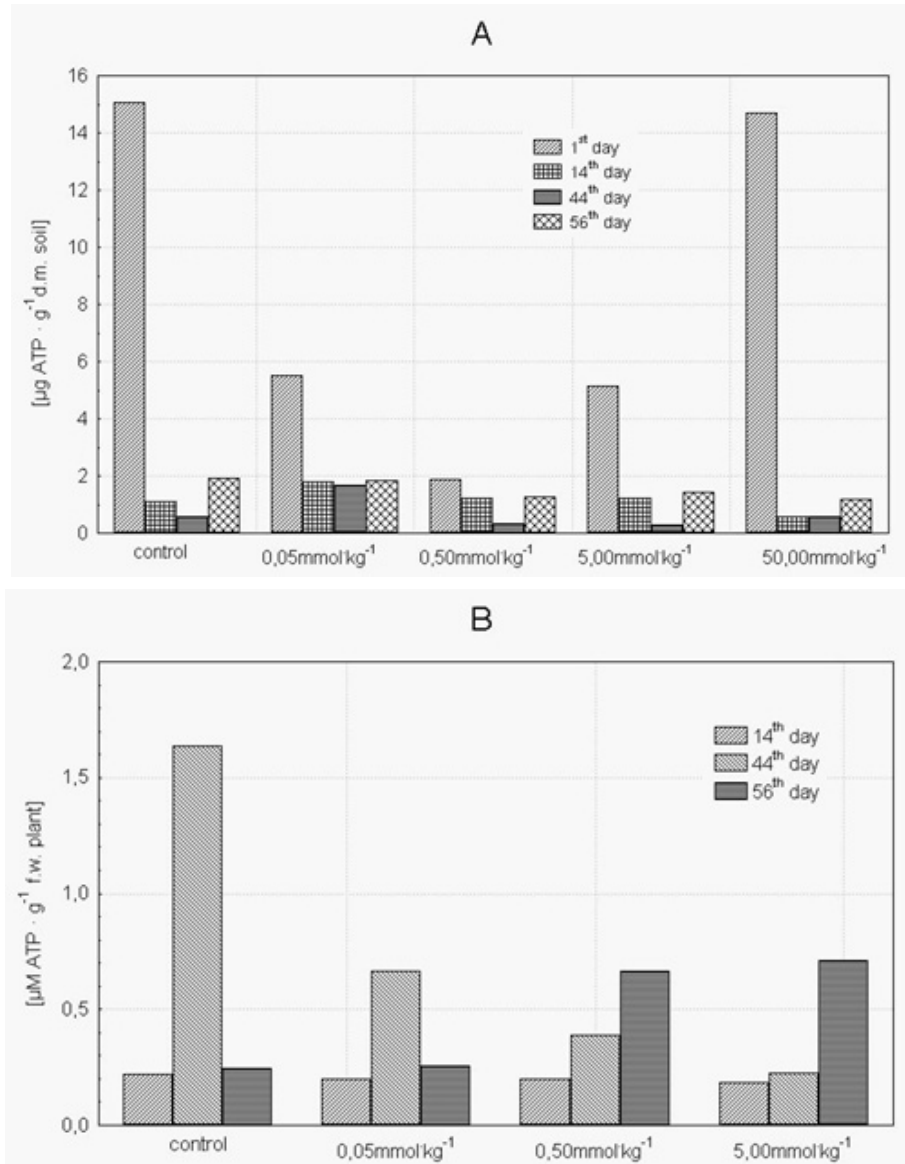


Fig.3. ATP content in soil (A) and in plant (B)



The analysis performed at various stages, regardless the measurement date, proved that the highest concentration [50.00 mmol·kg⁻¹] of all the applied in the experiment doses, significantly affected the acidity of the soil environment; pH in KCl was always higher than in H₂O. For the rest of the soil samples the reaction varied from alkaline to neutral.

Table 1. Reaction of selected soil samples at particular sampling dates

Date of sampling	Copper doses for soil combinations									
	Control		0.05 mmol·kg ⁻¹		0.50 mmol·kg ⁻¹		5.00 mmol·kg ⁻¹		50.00 mmol·kg ⁻¹	
	pH H ₂ O	pH KCl	pH H ₂ O	pH KCl	pH H ₂ O	pH KCl	pH H ₂ O	pH KCl	pH H ₂ O	pH KCl
1 st day	7.80	7.20	7.47	7.10	7.48	7.05	7.31	7.02	4.90	5.04
14 th day	7.69	7.11	7.62	7.10	7.38	7.01	6.98	6.77	4.72	4.92
44 th day	6.97	6.65	7.01	6.88	6.92	6.71	6.67	6.58	4.62	4.88
56 th day	7.00	6.79	6.85	6.59	6.72	6.64	6.52	6.44	4.49	4.65

The applied in the experiment combinations of copper (II) nitrate (V) were found to diversely affect the soil and plant phosphatase activities. The lowest applied combination, i.e. 0.05 mmol·kg⁻¹ caused activation of the acid phosphatase in the soil throughout the experiment (Fig. 1A). The other combinations in most cases inhibited activation of the studied enzyme. The 0.50 mmol·kg⁻¹ dose caused 8% activation on the 1st day of the experiment, but later it was noted to cause a slight - up to 5% - inhibition for all the acid phosphatase activity measurements that followed. Admixture of copper (II) nitrate (V) into the soil resulted in an inhibitory effect for the phosphatase activity in the plant over the entire experimental period. The inhibition was observed the smallest at the blooming stage, i.e. on the 44th day (Fig. 1B).

Table 2 presents the results of the performed statistical analysis. No significant relation was found between the phosphatase activity in soil and in plant. However, the measurement showed that the studied copper forms in soil and plant are significantly correlated to soil and plant acid phosphatase activity (see Table 3). In most cases the observed influence of various doses of copper (II) nitrate (V) upon the studied enzymes activities was found significant.

The applied combinations of copper (II) salts almost always caused inhibition of alkaline phosphatase in soil (Fig. 2A); the 0.05 mmol·kg⁻¹ dose on the 1st and 14th day of the experiment was found exceptional in this regard. At the beginning of the experiment inhibition of alkaline phosphatase was observed for the plants grown on all the soil combinations (Fig. 2B). The blooming stage (44th day) brought activation of the enzyme in Sown Pea *Pisum sativum* L./ for all the plants grown on all the experimental substrates. At the last of the developmental stages, on the 56th day, in all the plant samples grown on the substrates doped with 0.05 mmol·kg⁻¹ and 0.50 mmol·kg⁻¹ concentrations of copper (II) salt, the activity of the studied enzyme was similar to the level of activity for the plants grown on the control substrate (100%). At the green legume stage, 56th day, the activity was higher than the one observed at the earlier measurement for the plant alkaline phosphatase in Sown Pea *Pisum sativum* L./ grown on the substrate doped with 5.00 mmol·kg⁻¹ of copper (II) nitrate (V).

Performed analysis of correlation (Table 2) proved the relation for alkaline phosphatase in soil and in plant statistically insignificant, though for majority of the cases the content of the studied copper forms are significantly correlated with both plant and soil alkaline phosphatase activity. A significant influence of various copper (II) nitrate (V) upon the activity of the enzymes under consideration was observed.

Diversified combinations of the applied in the experiment doses of copper (II) salt affected the ATP content in the soil and plant samples. On the 1st day of the experiment the highest ATP content was recorded for the control soil and in the soil to which 50.00 mmol·kg⁻¹ of copper (II) nitrate (V) was added. The reported values neared 15 µg·g⁻¹ (Fig. 3A), while the other values did not exceed 6.00 µg·g⁻¹. On the 14th day of the experiment the ATP content was found to decline for all the soil combinations and the recorder values were not higher than 2.00 µg·g⁻¹. The trend was maintained at the next measurement. On the last day a slight increase in the ATP content was observed for all the soil combinations, though the recorded values were not higher than 2.00 µg·g⁻¹. In the tested plant at two-pairs of leaves stage, 14th day, ATP content of 0.30 µmol·g⁻¹ (Fig.3B) was not exceeded. The next stage brought an increase in the compound content that reached the maximum of 1.64 µmol·g⁻¹ ATP in the plant grown on the control sample. At the green legumes stage the level of ATP content for Sown Pea *Pisum sativum* L./ cultivated on the control soil and the soil to which 0.05 mmol·kg⁻¹ of copper (II) salt was applied was similar to ATP content recorded at the two-pairs of leaves stage. For the other plant samples an increase in ATP content to the values of 0.68 µmol·g⁻¹ and 0.74 µmol·g⁻¹ was observed for 0.50 mmol·kg⁻¹ and 5.00 mmol·kg⁻¹ of copper (II) nitrate (V), respectively.

In the light of the performed correlation analysis a strong correlation between the ATP content in soil and in plant was found at the blooming stage (Table 2). The correlation was not significant in all the other cases. Also no correlation was recorded between the copper forms investigated in the experiment and ATP content in soil and plant. Nevertheless, the differences in the ATP content in soil and in plant induced by the various copper(II) nitrate (V) doses were proved to be statistically significant.

Table 2. Correlation coefficients

Sampling Date	Correlation between acid phosphatase activity in soil and in plant	Correlation between alkaline phosphatase activity in soil and in plant	Correlation between ATP content in soil and in plant
14. day	0.41	0.21	0.01
44. day	0.41	0.02	*0.96
56. day	0.36	0.09	0.01

*the result is statistically significant at the significance level $\alpha=0.05$

Table 3. Correlation coefficients between the studied items; significance level $\alpha=0.05$ **B – 1st day of the experiment****E – 56th day of the experiment**

Researched Item	Acid phosphatase activity in soil	Acid phosphatase activity in plant	Alkaline phosphatase activity in soil	Alkaline phosphatase activity in plant	ATP content in soil	ATP content in plant
Soluble in water Cu Content	B -0.83	E -0.76	B -0.91	E -0.83	B 0.56	E 0.60
	E -0.98		E -0.96		E -0.53	
Total Cu content in soil	B -0.84	E -0.95	B -0.91	E -0.87	B 0.56	E -0.54
	E -0.99		E -0.96		E -0.53	
Cu content in plant	E -0.91	E -0.85	E -0.58	E -0.81	E 0.53	E 0.61

Heavy metals compounds introduced into the environment results in decline in soil enzymes activities [20,21,22]. The effect is most pronounced within the first 24 hours following the applied stressogenic factor [21]. Further increase or decrease may be attributed to the enzymes that are produced and released by quantitatively and qualitatively altered microflora [13,18,19]. Phosphatase was reported by others [5] as the least sensitive to stressogens action. The studies on heavy metal ion impact carried out by Ferens and Morawiecka [4] proved phosphatase activity to decline considerably under their action. If concentration of a stressogen factor lies safely below the respected admissible threshold value or only slightly exceeds it, the change is not significant and its range is narrow. However, high doses of toxic substances can dramatically disturb enzymatic processes taking place in soil [20,21,22]. Activity of plant enzymes is tightly related to a developmental stage of the plant. The resented research showed the highest values for activities for both phosphatases were observed at the blooming stage. The reported results of independent experiments performed by Kaczmarczyk et al. [9] confirmed such observations. At the vegetation period of Sown Pea (*Pisum sativum* L.) the differences in growing and development of plants cultivated on particular soil combinations were also recorded. The degree to which plant growth was inhibited and their standard appearance disturbed was found to increase with copper (II) salts concentration in the soil.

The doses of copper (II) nitrate (V) applied in the experiment affected ATP content in soil and in the tested plant. The recorded ATP content was related to developmental stage of the experimental plant, i.e. *Pisum sativum* L.. Lunn and Madsen [11] reported that the highest amounts of ATP were observed when plants growth is the most intense, while at plant maturity stages both dynamics of metabolic processes and ATP content tend to be reduced. Reaction of the substrate is undoubtedly one of the factors that affect the growing rate [1]. Among the papilionaceous plants just lupin/ lupine *Lupinus* L. and saradela have been reported as worth cultivating on acidified soils. In the performed experiment the highest of the applied copper (II) nitrate (V) concentration inhibited the growth of the plant.

CONCLUSIONS

The performed research led to the following conclusions:

- increased copper (II) nitrate (V) content in the soil induced inhibition in soil phosphatase activity; the inhibition of the studied enzymes tend to grow with increased salt concentration;
- in all cases at the blooming phase of Sown Pea (*Pisum sativum* L.) the inhibition of acid plant phosphatase tended to rise slightly (up to 3%) and the alkaline one was activated;
- the highest ATP content were recorded on the 1st day of the experiment, just after seeding Sown Pea (*Pisum sativum* L.);
- in the plant samples the highest ATP contents occurred at the blooming stage for the plants grown on the control substrate and the one into which a dose of 0.05 mmol·kg⁻¹ of copper (II) nitrate (V) was added;
- ATP content in Sown Pea (*Pisum sativum* L.) for plants grown on the other substrates tend to rise with time progressing.

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