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Short research contribution

Bożena ŚNIEG, Janina NOWAK*

Department of Biochemistry, Agriculture University of Szczecin,
Słowackiego 17, 71-434 Szczecin, Poland

*e-mail: jnowak@agro.ar.szczecin.pl (corresponding author)

UREASE ACTIVITY AND ATP CONTENT IN SOIL AND PLANT RELATED TO COPPER CONCENTRATION

ABSTRACT: This study aimed at the assessment of the influence of various $\text{Cu}(\text{NO}_3)_2$ doses added to soil on Cu content in soil and on its influence on the activity of urease and ATP content in soil and in plants of various growth stages. A two-factor pot experiment had been started in 2002 using as test plant – the pea (*Pisum sativum* L.). Soil was taken from 0–30 cm layer of an arable field (light silt loam, 1.2% C content, and neutral reaction). Four doses of copper (II) nitrate (V) as $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ were applied (each in 4 replications) following: I – control (no salt), II – 0.05 mmol kg^{-1} soil, III – 0.50 mmol kg^{-1} and IV – 5.00 mmol kg^{-1} soil. According to six degrees classification of soil contamination by copper, application of 0.05 mmol of copper nitrate per kg of soil increased copper content up to high level of natural content in soil (but still 0° of contamination), after application of 0.5 mmol kg^{-1} – copper content increased to 1° of contamination, 5.00 mmol kg^{-1} Cu^{+2} dose caused high pollution (4° of contamination). Each pot was filled with 2 kg of the treated soil, and 5 pea seeds were planted per pot. The experiment lasted 56 days. Soil moisture was maintained during the experiment at 60% water holding capacity. In the course of the experiment the following growth stages were noted: 2 pairs of leaves stage (day 14th), flowering stage (day 44th), mature stage (day 56th). At those times soil and plant samples were taken to assess copper content in soil (content of total and 1M HCl soluble Cu), urease activity and ATP levels.

A high positive correlation was found between Cu content (total and 1M HCl soluble) in soil and in plants. High Cu content in soil (4° of contamination – high pollution) caused a decrease of urease activity and ATP content in soil. Elevated Cu content in plant caused a distinct inhibition of urease activity in all the analyzed growth stages, and markedly higher content of ATP at the stage of flowering and mature stage of *Pisum sativum* L.

KEY WORDS: urease activity, ATP, Cu in plants and soil

Copper is a heavy metal considered as the vital for living organisms, due to its physiological and biochemical functions. This element has the ability to build complexes of chelate type, and also to change oxidation degree of Cu^{+2} and Cu^+ . In plants copper forms complex compounds with many organic substances, taking part in metabolism and transport of various substances and playing a role in cell respiration and in photosynthesis. Due to the ability to create chelates, copper loses its toxic properties which are connected with free Cu^{+2} ions (Ruszkowska and Wojcieszka-Wykupajtys 1996).

The upper values of natural Cu content in Polish soils are from 15 mg kg^{-1} DW in light acid soils to 40 mg kg^{-1} DW in medium

and heavy soils (Siuta 1995). Because of human activities (industry emission, application of waste deposits, plant protection substances, fertilizers) the content of Cu in soil can markedly increase and surpass the toxic levels. According to Siuta (1995) after IUNG (1992) the contamination of topsoil (0–20 cm) by heavy metals can be divided into 6 degrees (0÷5). This division is related both to heavy metals content and type of soil, and 0° is a natural content (natural amount of Cu in soil presented in this research is 25 mg kg⁻¹ DW), 1° – enhanced amount (50 mg kg⁻¹ DW), 2° – slight pollution (80 mg kg⁻¹ DW), 3° – medium pollution (100 mg kg⁻¹ DW), 4° – high pollution (500 mg kg⁻¹ DW) and 5° – very high pollution (>500 mg kg⁻¹ DW). According to Kabata-Pendias and Pendias (1993) maximal tolerable content of Cu in soil is 100 mg kg⁻¹.

Soil fertility is affected in the biochemical processes among the activity of soil enzymes (Myśków *et al.* 1996, Wyszowska and Kucharski 2003). Both the enzyme activity (Trasar-Cepeda *et al.* 2000, Caravaca *et al.* 2002), and ATP content (Kennicutt 1980) can be considered as the indicators of environment pollution. According to Domsch *et al.* (1983) side-effects (i.e. growth or activity inhibition) of agrochemicals on populations and functions of soil microorganisms can be divided into three categories: the first – would cause side-effects up to 30 days (*normal*), the second (*tolerable*) – in which side-effects disappear in 60 days, and the third (*critical*) – side-effects persist longer than 60 days. To calculate the critical ecological dose (ED₅₀) of cadmium in soil, Moreno *et al.* (2001) used the activity of dehydrogenases, urease, and ATP content. Many authors stressed the inhibition of urease caused by copper: if the concentration of Cu was high, the inhibition could exceed 50% and last for a long time (Tabatabai 1977, Frankenberger *et al.* 1983; Nowak *et al.* 1999, Marzadori *et al.* 2000, Nowak *et al.* 2001, Kucharski and Wyszowska 2003).

The content of Cu in soil affects its level in plants. According to Kabata-Pendias (1996) the range of 5–30 mg·kg⁻¹ DW is considered as physiological amount of copper in plants. In plants from non-polluted

sites cuprum content is within 2–9 mg·kg⁻¹ DW. Papilionaceous plants often contain elevated amounts of Cu. The elevated Cu content can be caused either by increased uptake from soil or directly from atmospheric dust (Strączyński and Andruszczak 1996). Studies done by Chłopecka (1994) indicated that increased amount of copper caused a decrease of plant yield. An indicator of plant vigour and growth ability is among others the content of ATP, which is connected both with the species of the plant and external factors (Gorm and Madsen 1981, Sobczyk *et al.* 1985).

This study was aimed at the assessment of the influence of various copper doses added to soil on the content of Cu in soil and further on the activity of urease and content of ATP in soil and test plant – pea (*Pisum sativum* L.) in various stages of growth. The pea (*Pisum sativum* L.) is the annual plant, which is able to accumulate elevated amounts of cuprum, and different forms of nitrogen, therefore it can serve as the good test plant.

A two-factorial experiment was started in 2002, using complete random pot study. As test plant pea (*Pisum sativum* L.) was used. The soil samples were taken from 0–30 cm layer of an arable field. It was a light silt loam of neutral pH, containing 1.2% of organic carbon and high content of available potassium and magnesium (Bogda *et al.* 1990). Four different doses of copper (II) nitrate (V) were added to soil, each treatment was repeated four times. Soil was passed through a 2 mm sieve, and divided into four portions of 8 kg each. The soil was treated with water solutions of Cu(NO₃)₂·3H₂O as follows: control, 0.05 mmol kg⁻¹ soil, 0.50 mmol kg⁻¹ and 5.00 mmol kg⁻¹. Addition of 0.05 mmol of copper salt to 1 kg of soil, slightly increased copper amount in soil, and according to previously mentioned classification, it is upper level of natural content in soil – 0° level of contamination, addition of 0.50 mmol caused elevation of copper content up to 1° of contamination, and addition of the highest dose – 5.0 mmol classifies the soil as 4° – the high polluted. Pots were filled to ¼ height with basalt chippings for drainage, and with 2 kg of the pre-treated soil. Pea (*Pisum sativum* L.) was sown, 5 seeds per one pot, and that day was 1st day of experiment. Soil moisture

was maintained at 60% water holding capacity. Soil and plant samples were taken at the following growth stages of pea: two pairs of leaves (14th day), flowering (44th day) and green pod (56th day). The samples were analyzed for copper content, urease activity and ATP content. Soil samples were also analyzed for Cu total and soluble in 1 M HCl, and at 1st and 56th day also pH in H₂O and in 1 M KCl was assessed. The content of Cu in soil and plant material was assessed by emission spectrometry with excitation of induction-coupled argon plasma (ICP-OES), using an Optima 2000 DV, Perkin Elmer apparatus. Urease activity in soil was measured by the method of Bonmati *et al.* (1991), using 3% urea solution as substrate. The enzyme activity was expressed as the amount of $\mu\text{g N-NH}_4^+$ formed in 1 g of dry soil per 1 hour ($\mu\text{g N-NH}_4^+ \text{ g}^{-1} \text{ DW soil h}^{-1}$). Urease activity in plant was assessed according to Hofmann (1963), using 10% urea solution, and calculated per 1 g fresh matter per 1 hour ($\mu\text{g N-NH}_4^+ \text{ g}^{-1} \text{ FW plant h}^{-1}$). The analyses were done using Lambda Bio, Perkin Elmer apparatus. ATP content in soil and plant was done by bioluminescence in the enzymatic system Luciferin-Luciferase (L/L), using the Lumat LB 9507 (Berthold). Soil ATP was measured following Maire (1982, 1984) method, modified by Margesin (1996) and calculated per 1 g dry soil ($\mu\text{g ATP g}^{-1} \text{ DW soil}$). To assess ATP content in plant the method of John (1970) was used and expressed as $\mu\text{mol ATP g}^{-1} \text{ FW plant}$.

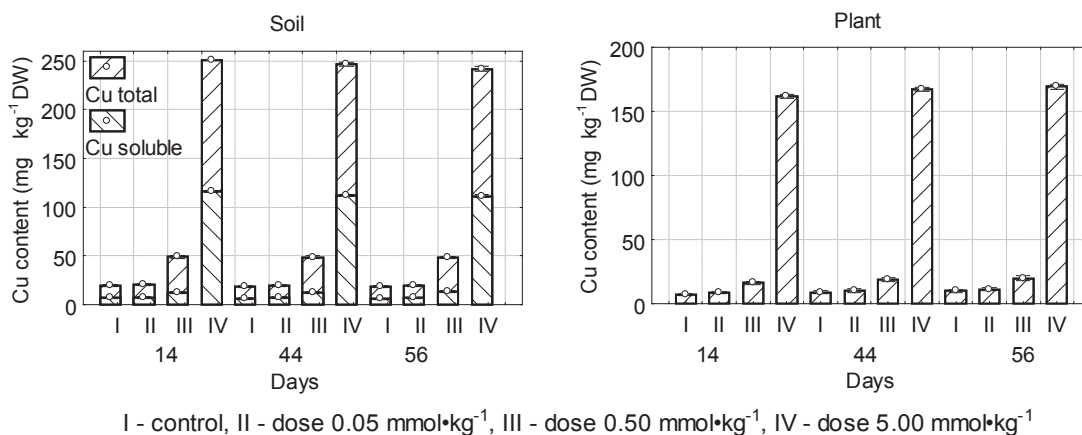
Statistical analysis of the results was done using the two-factor analysis of variance, complete randomization (Rudnicki 1992). Using the Tukey test LSD at $\alpha = 0.05$ was calculated and presented graphically on the figures. Correlations between the dates were also calculated and presented as coefficients in the Table 1.

Addition various doses of copper nitrate to soil had no influence on its reaction, $\text{pH}_{\text{H}_2\text{O}}$ was equal to 6.68 ± 0.08 , and that in KCl was lower 6.55 ± 0.09 .

The content of Cu in soil and plants varied (Fig. 1). The total Cu content in soil decreased with time. The content of soluble Cu both in control soil and that treated with $0.05 \text{ mmol kg}^{-1} \text{ Cu(NO}_3)_2$ was similar (about $6\text{--}7 \text{ mg kg}^{-1} \text{ DW}$). In soil treated with $0.50 \text{ mmol kg}^{-1} \text{ Cu(NO}_3)_2$, content of soluble Cu was similar during experiment (about $12\text{--}13 \text{ mg kg}^{-1} \text{ DW}$) and in soil with 5.00 mmol of copper salt content of soluble Cu was high in 14th day ($116.20 \pm 0.90 \text{ mg kg}^{-1} \text{ DW}$) and decrease to a small value in 44th and 56th day (respectively 112.10 ± 1.42 and $111.50 \pm 1.20 \text{ mg kg}^{-1} \text{ DW}$). A similar situation occurred in plants. Plants from control soil and soil treated with $0.05 \text{ mmol kg}^{-1} \text{ Cu(NO}_3)_2$ contained similar amount of Cu ($7.29 \pm 0.59 \text{ mg kg}^{-1} \text{ DW}$ in control and $8.96 \pm 0.80 \text{ mg kg}^{-1} \text{ DW}$ in combination with 0.05 mM at 14th day and respectively $9.92 \pm 1.00 \text{ mg kg}^{-1} \text{ DW}$ and $11.36 \pm 1.20 \text{ mg kg}^{-1} \text{ DW}$ at 56th day). Plants from combination with 0.50 mmol salts contained from $16.20 \pm 1.00 \text{ mg kg}^{-1} \text{ DW}$

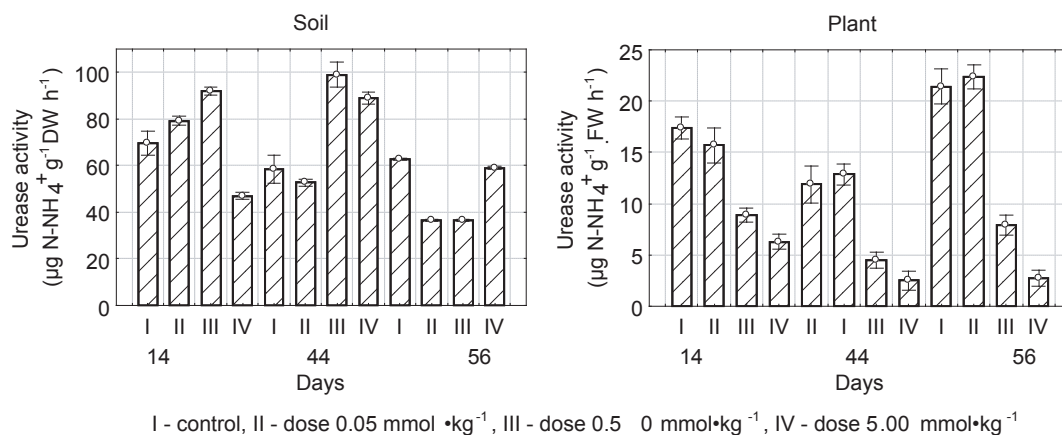
Table 1. Significant correlation coefficients r ($n = 12$, $P \leq 0.05$) between urease activity, ATP content and Cu content in soil and plant (*Pisum sativum*)

	Day of experiment	Dose of $\text{Cu(NO}_3)_2$	Cu total, content in soil	Cu soluble, content in soil	Cu content in plant
Dose of $\text{Cu(NO}_3)_2$			1.00	1.00	1.00
Cu total, content in soil		1.00		1.00	0.99
Cu soluble, content in soil		1.00	1.00		1.00
ATP content in soil	0.93				
Urease activity in plant		-0.70	-0.71	-0.67	-0.66



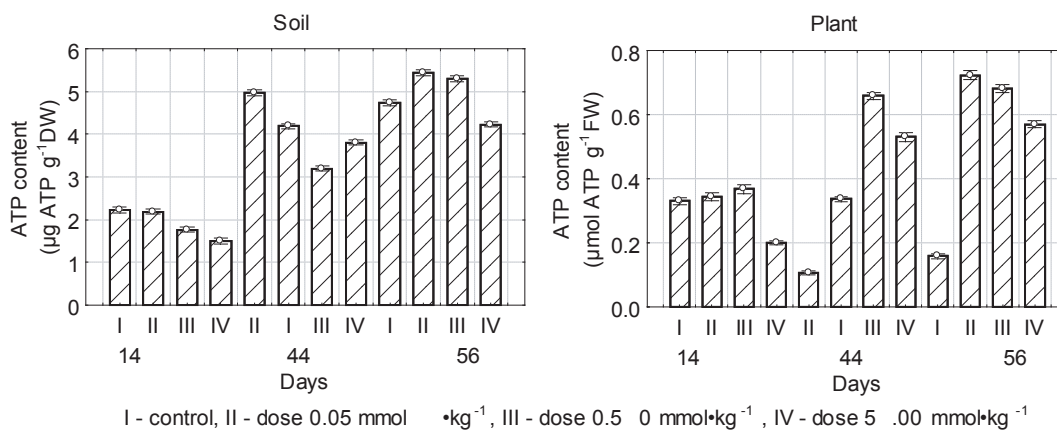
I - control, II - dose 0.05 mmol•kg⁻¹, III - dose 0.50 mmol•kg⁻¹, IV - dose 5.00 mmol•kg⁻¹

Fig. 1. Content of Cu in the soil and in the plant (*Pisum sativum*) after application of different doses of the Cu(NO₃)₂ to soil during 56 days of experiment.



I - control, II - dose 0.05 mmol •kg⁻¹, III - dose 0.5 0 mmol•kg⁻¹, IV - dose 5.00 mmol•kg⁻¹

Fig. 2. Urease activity in the soil and in the plant (*Pisum sativum*) after application of different doses of the Cu(NO₃)₂ to soil during 56 days of experiment.



I - control, II - dose 0.05 mmol •kg⁻¹, III - dose 0.5 0 mmol•kg⁻¹, IV - dose 5 .00 mmol•kg⁻¹

Fig 3. ATP content in the soil and in the plant (*Pisum sativum*) after application of different doses of the Cu(NO₃)₂ to soil during 56 days of experiment.

at 14th day to $19.81 \pm 1.90 \text{ mg kg}^{-1} \text{ DW}$ at 56th day. Plants from soil with 5.00 mmol salt of copper contained high amount of Cu from $161.92 \pm 1.54 \text{ mg kg}^{-1} \text{ DW}$ at 14th day up to $169.27 \pm 1.98 \text{ mg kg}^{-1} \text{ DW}$ at 56th day. A significant correlation was found between the applied Cu dose and the content of total and soluble Cu in soil and plant, and also between the content of total and soluble Cu in soil and its content in plants (Table 1).

The activity of urease in soil varied in the course of the experiment (Fig. 2). At the stage of 2 pairs of leaves (14th day) the activity increased by $70 \mu\text{g N-NH}_4 \text{ g}^{-1}$ in control soil to $79 \mu\text{g N-NH}_4 \text{ g}^{-1}$ at the dose 0.05 mmol $\text{Cu(NO}_3)_2$, and $92 \mu\text{g N-NH}_4 \text{ g}^{-1}$ at the dose 0.50 mmol $\text{Cu(NO}_3)_2$. In soil which had been treated with the highest Cu dose the activity of urease decreased reaching only 67% of the activity in control soil. At flowering stage (44th day) activity of urease in soil treated with the lowest Cu dose decreased by 10% as compared with control, but if the Cu doses had been higher (0.5 and 5.0 mmol) urease activity increased by 69 and 51%, respectively. At the last day of the experiment (mature stage) the urease activity in Cu treated soil decreased (0.05 or 0.50 mmol) by 42%, and in soil treated with 5.0 mmol Cu only by 6%. The differences of urease activity in soil of the various treatments were significant.

The content of ATP in soil increased in the course of the experiment (Fig. 3). In control soil at 14th day it was 2.22, at 44th day – 4.19, and at 56th day was $4.73 \mu\text{g ATP g}^{-1}$. Soil which had been treated with Cu salt, at 14th and 44th day the content of ATP decreased with increase of Cu dose, and at 56th day in soil which had been treated with 0.05 or 0.50 mmol – the ATP content increased by 15 and 12%. If the dose had been 5.00 mmol, ATP content decreased by 10% as compared to control. ATP content in soil differed significantly (Fig. 3).

The tendency of changes of urease activity in plants (Fig. 2) was similar to that of urease activity in soil, though the rate was greater. At the first sampling (2-pairs of leaves) the activity decreased with the increase of Cu dose – from 17.38 to $6.30 \mu\text{g N-NH}_4^+ \text{ g}^{-1}$, and thus increased the content of Cu in plant. At flowering stage, after a slight increase of the activity caused by 0.05 mmol

dose, a sharp decrease occurred (by 62%) induced by 0.50 mmol and even greater (79%) at the 5.00 mmol dose. During the last sampling (mature stage) the tendency was similar to the previous but stronger. At the dose 0.50 mmol the activity decreased by 37% and at the highest dose – by 87%. The differences of urease activity in plants were significant in the course of the experiment. Urease activity in plants is negatively correlated with the $\text{Cu(NO}_3)_2$ dose, with the content of total and soluble Cu in soil and with the Cu content in plant (Table 1).

The ATP content in plants (Fig. 3) had a definite tendency to increase if 0.05 or 0.50 mmol doses had been applied. At 14th day (two pairs of leaves stage) the increase ATP level was slight – from 0.330 in control to $0.368 \mu\text{mol ATP g}^{-1}$ in combination with 0.50 mmol (about 12%). The 5.00 mmol dose caused about 40% decrease. During flowering stage the content of ATP increased greatly: after 0.05 mmol dose by 215%, after 0.50 mmol by 515%, and the highest dose caused a 395% increase. A similar tendency was found during mature stage (56th day): the 0.05 mmol dose increased the ATP level by 355%, the 0.50 mmol dose – by 329%, and the highest dose – by 258%. The differences were significant during the course of the experiment almost in all experiment combinations.

Introduction to the environment of various substances often causes negative changes of metabolism. Soil pollution by heavy metals directly affects the enzymatic activity, which in turn changes the cycling of elements in soil and plants, among others that of nitrogen. Inhibition of enzymes responsible for the metabolism of nitrogen is often reported. It was found that in one hour after application of heavy metals to soil the activity of urease was changed (Tabatabai 1977, Nowak *et al.* 1999).

Results of our study have shown that after application of various doses of $\text{Cu(NO}_3)_2$ to soil, part of Cu^{+2} ions was transformed into insoluble compounds. On the other hand, the content of both total and soluble forms of Cu had an impact on its content in plants. A very high correlation was between the content of soluble Cu in soil and in plant. Particularly, after soil treatment with the highest dose

of Cu salt, the soil content of soluble Cu increased, so it was that in plants. It confirmed the findings reported by Chłopecka (1994), Kabata-Pendias (1996), Strączyński and Andruszczak (1996).

The activity of urease in soil treated with Cu salt had in our experiment a tendency to increase in the first part of the study period (up to 44th day), then it decreased toward the end of the experiment (56th day). Wyszowska and Kucharski (2003) reported a very high inhibition over 50% caused by large amounts of added Cu salt (over 400 mg Cu kg⁻¹). The content of ATP in soil treated with Cu salt was much lower than in untreated soil, particularly during the first two growth stages of plant. It confirms the results reported by Moreno et al. (2001), who consider the rate of ATP content decrease in soil as an indicator of cadmium pollution. A high content of Cu in plants contributed to changes of urease activity and ATP content. The inhibition of urease activity was very high, almost 90%, which lasted to the end of the experiment. Adapting ecological criteria proposed by Domsch *et al.* (1983) to plants it is an almost critical effect. Application of 5.00 mmol copper salt caused a very high accumulation of Cu in plants and a following inhibition of urease activity. Also during flowering and mature stage increase of ATP content in plants showed that a large amount of Cu had been found.

The following conclusions can be formulated:

1. A high positive correlation has been found between copper content (both soluble and total) in soil and plant.

2. An increased copper content in soil caused a decrease of urease activity and ATP content in soil.

3. High content of copper in plants caused an inhibition of urease activity, a marked increase of ATP content in plants at flowering and mature stage of test plant (*Pisum sativum* L.)

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