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**INFLUENCE OF CONTAMINATION OF SOIL
WITH COPPER ON THE ACTIVITY OF DEHYDROGENASES
IN AREAS WHERE AMARANTHUS IS CULTIVATED**

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NA AKTYWNOŚĆ DEHYDROGENAZ POD UPRAWĄ AMARANTUSA**

Abstract: The pot experiment investigated the influence of various dosages of copper (0, 20, 40, 80, 120 mg · kg⁻¹ of soil) on the activity of dehydrogenases in the soils in which amaranthus, Rawa variation, was cultivated. The pot experiment revealed that the highest activity of dehydrogenases could be observed when no fertilization with Cu was applied, and the lowest activity of dehydrogenases occurred with the highest dosage of Cu in the experimental pot. An increase in the activity of dehydrogenases was found in the control objects and objects where the lowest dosage of copper was applied in the investigated period (June–August–October). Addition of increased dosages of copper resulted in the decrease of the activity of dehydrogenases in the investigated months, as related to the control objects. Significant high negative correlations was found between the dosage of Cu that was applied and activity of dehydrogenases in the investigated months.

Keywords: amaranthus, activity of dehydrogenases, copper

Amaranthus is a universal plant [1], and is grown mainly to obtain seeds that are rich in nutrients. It can also be used for energy purposes. A growing interest in cultivating Amaranthus in Poland, especially the south-eastern part of the country, encourages studies that can estimate the influence of this plant on enzymatic activity of soil environment. Initial studies suggest that amaranthus has a stimulating effect in development of soil microflora and enzymatic activity [2]. Such a beneficial influence of amaranthus on biological properties of soil environment can be used in studies, and then in attempts to improve the activity of soils that have been degraded by excessive accumulation of heavy metals, namely: copper. It should be noted that contamination of soil with copper leads to bioaccumulation of this element, and affects plant – soil homeostase, which can lead to degradation of soil environment [3].

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The aim of the study was to evaluate the influence of contamination of soil with growing dosages of copper on the activity of dehydrogenases in soil environment in areas where amaranthus, Rawa variety, is cultivated.

Material and methods

The experiment was carried out in plastic pots which capacity was 3.5 kg in three replications. The pots were filled with silt loam (pH 6.6) marked in solution of KCl with concentration $1 \text{ mol} \cdot \text{dm}^{-3}$. Before amaranthus, Rawa variation, was sown, uniform fertilization with macroelements was applied in the following dosages counted for pure element [$\text{g} \cdot \text{kg}^{-1}$ of soil]: N – 0.25, P – 0.10, K – 0.10.

Variable factors in the experiment were dosages of copper [$\text{mg} \cdot \text{kg}^{-1}$ of soil]: 0; 20; 40; 80; 120; (in the form of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$). The seeds were sown on 26.05.2008. After sprouting 3 plants were left in each pot. The experiment spread over the whole vegetation period of amaranthus. 5 soil samples were taken (into plastic bags) from each pot in order to obtain the mean sample. The soil was carefully mixed, and then sifted through a sieve with 2 mm holes. Soil was taken from each mean sample for analysis of the activity of dehydrogenases. Activity of dehydrogenases was determined in June, August, and October. The study included marking the activity of dehydrogenases with Thalmann's method [4]. 5 g portions of soil were put into 100 cm^3 flasks, and then 5 cm^3 of 1 % Triphenyltetrazolium chloride (TCC) was added which had earlier been dissolved in Tris-HCl buffer with pH 7.4. The soil samples were incubated for 96 hours at temperature of $30 \text{ }^\circ\text{C}$, then 20 cm^3 of methanol was added to each flask. Next, the samples were shaken out for five minutes and filtered. The filtrate was marked spectrophotometrically at wavelength $\lambda = 485 \text{ nm}$.

Results and discussion

Enzymatic activity of soils is considered to be a sensitive indicator of both favourable and unfavourable changes that take place in soil environment [5, 6]. Evaluation of dehydrogenase activity in soil is of particular importance. In many papers, the activity of dehydrogenases in soil is often referred to as total microbiological activity of soil environment [7].

Moreover, enzymatic activity of soil, and especially the activity of dehydrogenases, can be considered as an indicator of contamination with heavy metals [8]. Excess of heavy metals, such as copper, is harmful because they contaminate soils and decrease biochemical activity of soil, and are also harmful for plants, animals, and humans [9]. Cultivation of plants that are used as food in soils with higher contents of heavy metals or contaminated soils is not advisable. However, energy plants are often cultivated in such soils. Planting amaranthus in areas that are contaminated with heavy metals can decrease contamination with heavy metals.

The investigation that was carried out reveals, that the highest values of dehydrogenase activity in the analysed months (June, August, October) were found in control objects, where no copper was applied (Table 1). It was observed that the value of

dehydrogenase activity in the consecutive months of the analysis decreased alongside with the increase in the contents of copper in soil (Table 1).

Table 1

Activity of dehydrogenases in the investigated months depending on contamination of soil with copper (mean values)

Cu dose [mg · kg ⁻¹ of soil]	Dehydrogenases activity [mg TPF · kg ⁻¹ · day ⁻¹]		
	June	August	October
0 (control object)	9.5	17.8	25.5
20	9.4	17.4	24.5
40	8.8	14.0	9.0
80	7.0	12.2	6.8
120	4.4	6.2	6.5

Throughout the experiment, it was determined, that the activity of dehydrogenases gradually increased only in soil from control objects and soil in which the lowest dosage of Cu had been applied. Application of higher dosages of Cu (40 mg · kg⁻¹, 80 mg · kg⁻¹, and 120 mg · kg⁻¹) increased the activity of dehydrogenases in June–August, and then it decreased from August to October (Table 1).

The activity of dehydrogenases in June in the control objects was 9.5 mg TPF · kg⁻¹ · day⁻¹. When 20 mg · kg⁻¹ of Cu was applied, the activity of dehydrogenases was only 1.1 % lower than in the control objects. Application of dosages of Cu into the soil (40 mg · kg⁻¹ Cu, 80 mg · kg⁻¹ Cu, and 120 mg · kg⁻¹ Cu), caused a decrease of the activity of dehydrogenases by 7.4 %, 26.3 % and 53.7 %, respectively (Table 1).

The activity of dehydrogenases in August in the control objects was 17.8 mg TPF · kg⁻¹ · day⁻¹. It was 2.2 % higher when compared with the first dosage that had been applied, and 21.3 %, 31.5 % and 65.2 % higher for the consecutive, increased dosages (Table 1).

The activity of dehydrogenases in October in the control objects was 25.5 mg TPF · kg⁻¹ · day⁻¹, and it was the highest value that was recorded. A slightly lower activity of dehydrogenases was observed in the objects where the lowest dosage of Cu had been applied – 24.5 mg TPF · kg⁻¹ · day⁻¹, which was by 3.9 % lower than in the control objects. Significantly lower activity of dehydrogenases was observed in the objects where 40 mg · kg⁻¹ Cu had been applied (64.7 % decrease), 80 mg · kg⁻¹ Cu (73.3 % decrease), and 120 mg · kg⁻¹ Cu (74.5 % decrease), as compared with the control objects (Table 1).

The investigation clearly reveals that application of copper into the soil, especially in the highest dosage used in the experiment, causes a decrease in the activity of dehydrogenases in the soil. Similar correlations were observed by Nowak et al [10]. The authors claimed, that when contamination of soil with heavy metals increased, the activity of dehydrogenases significantly decreased. Negative effects of copper in the activity of dehydrogenases were also observed by Kucharski and Hlasko-Nasalska [11].

The statistical analysis that was carried out also revealed significant negative correlation between the dosage of copper that had been applied and the activity of dehydrogenases in the soil where amaranthus was cultivated (Table 2).

Table 2

Correlation coefficients between the dosages of Cu that had been applied [$\text{mg} \cdot \text{kg}^{-1}$ of soil] and the activity of dehydrogenases in the soil where amaranthus was cultivated, at $p = 0.01$

Cu dose [$\text{mg} \cdot \text{kg}^{-1}$ of soil]	Dehydrogenases activity [$\text{mg TPF} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$]		
	June	August	October
	-0.974	-0.976	-0.850

Conclusions

1. The highest activity of dehydrogenases in the pot experiment was found in the object where no Cu fertilisation had been used.

2. A successive increase in the activity of dehydrogenases was observed in the control objects, and the objects where the lowest dosage of Cu had been applied in the experimental period (June–August–October).

3. Application of higher dosages of copper (40, 80, 120 $\text{mg} \cdot \text{kg}^{-1}$) caused a decrease in the activity of dehydrogenases in the investigated months, as compared with the control objects. It was observed, that the objects where dosages of Cu had been applied showed an increase in the activity of dehydrogenases in June–August, and a decrease in this activity in August–October.

4. Significant negative correlation between the dosage of copper that had been applied and the activity of dehydrogenases in the soil were observed in the investigated months.

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Abstrakt: W doświadczeniu wazonowym oceniano wpływ różnych dawek miedzi (0, 20, 40, 80, 120 mg · kg⁻¹ gleby) na aktywność dehydrogenaz w glebie pod uprawą szarłatki odmiany Rawa. W warunkach przeprowadzonego doświadczenia wazonowego najwyższą aktywnością dehydrogenaz charakteryzowały się obiekty bez zastosowanego nawożenia Cu, a najniższą obiekty z najwyższą dawką tego mikroelementu. Stwierdzono wzrost aktywności dehydrogenaz w obiektach kontrolnych oraz wraz z zaaplikowaną najmniejszą dawką miedzi w badanym okresie (czerwiec–sierpień–październik). Dodanie większych dawek miedzi przyczyniło się do zmniejszenia aktywności dehydrogenaz w badanych miesiącach w porównaniu z obiektami kontrolnymi. Stwierdzono, że obiekty z tymi dawkami wykazują wysokie istotne ujemne zależności pomiędzy zaaplikowaną dawką Cu a aktywnością dehydrogenaz w badanych miesiącach.

Słowa kluczowe: amaranthus, aktywność dehydrogenaz, miedź