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ORIGINAL PAPER

THE IMPACT OF COPPER ON CATALASE ACTIVITY AND ANTIOXIDANT PROPERTIES OF SOIL UNDER AMARANTH CULTIVATION

Barbara Skwaryło-Bednarz[1], Anna Krzepiłko[2], Marzena S. Brodowska[3], Ryszard Brodowski[4], Marta Ziemińska-Smyk, Joanna Onuch, Barbara Gradziuk[5]

[1]Department of Plant Protection
[2]Department of Biotechnology Human Nutrition and Science of Food Commodities
[3]Department of Agricultural and Environmental Chemistry
[4]Department of Environmental Engineering and Geodesy
[5]Department of Economics and Management
University of Life Sciences in Lublin, Poland

Abstract

The effects of various doses of copper (0, 50, 150, 300, 450 mg kg^{-1} of soil) on the activity of catalase and total antioxidant capacity (TAC) in soil under the amaranth cv. Aztec were evaluated in a pot experiment. The activity of catalase increased in fresh and air dry soil for all objects during the growing period of amaranth (soil sampling deadlines – June, August and October). Higher activity of catalase was observed in fresh soil samples than in air dry ones. It has been shown that increasing doses of copper applied (50, 150, 300, 450 mg kg^{-1}) contributed to a reduction in the activity of catalase in fresh and air dry soil samples in the test months in relation to the control object. The highest catalase activity was observed in objects without the application of Cu, whereas the lowest catalase activity was affected by the highest dose of Cu. An increase in total antioxidant capacity under amaranth cultivation was caused only by the first dose of copper (50 mg kg^{-1}). The application of increasing doses of copper, greater than 50 mg kg^{-1}, resulted in progressive reduction of the mean value of total antioxidant capacity, which in any case was lower than the value for the control object. The mean value of total antioxidant capacity increased during the growing period, regardless of the applied dose of copper. The statistical analysis showed high significant negative correlations between a dose of Cu and the catalase activity in fresh and air dry soil samples in the test months as well as between a dose of Cu and the value of its total antioxidant capacity.

Keywords: amaranth, copper, catalase activity, total antioxidant capacity.

Marzena S. Brodowska, PhD DSc, Department of Agricultural and Environmental Chemistry, University of Life Sciences in Lublin, Akademicka 15, 20-950 Lublin, Poland, e-mail: marzena.brodowska@up.lublin.pl

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INTRODUCTION

Initial studies confirm that amaranth can be grown in areas contaminated with heavy metals and other chemical compounds, where the plant-soil homeostasis is affected, which can lead to chemical degradation of soil environment (Ogunkunle et al. 2013). It is common knowledge that heavy metals occur in soils in different forms and quantities. The majority of heavy metals are crucial for many biochemical reactions to proceed properly in soil. However, excessive accumulation of these elements in soil poses a serious threat because it disturbs the biological processes that occur in soil (Wyszkowska et al. 2005, Oliveira, Pampulha 2006, Solanki et al. 2011, Bartkowiak, Lemanowicz 2014). This influences the diversity of organisms that inhabit the soil as well as the enzymatic activity of the soil (Wyszkowska et al. 2005, Bielińska, Mocek-Płóciniak 2009, Symanowicz et al. 2014). Different studies confirm that excessive accumulation of heavy metals in soil has a toxic effect on the activity of soil enzymes (Renella et al. 2005, Khan et al. 2007, Wyszkowska et al. 2010). The results obtained by Bandick and Dick (1999) and Kunito et al. (2001) revealed that heavy metals bind with amino acids and inactivate extracellular enzymes, which leads to a decrease in the number of microorganisms that produce enzymes.

Enzyme activity is considered to be a soil quality indicator and can be used to determine the degree of environmental pollution with heavy metals and their impact on living organisms (Khan et al. 2007, Bartkowiak, Lemanowicz 2014).The monitoring that employs methods based on enzymatic tests allows for comprehensive evaluation of changes which occur in the soil environment (Shaw, Burns 2003).

The initial studies prove that amaranth absorbs the excess of toxic elements from the soil to build its biomass (Ogunkunle et al. 2013), thus preventing heavy metal bioaccumulation. Moreover, this plant improves biological activity of the soil. The studies confirm that seed amaranth cultivated in the climatic-soil conditions found in the south-east of Poland has a stimulating effect on the development of soil micro flora and enzymatic activity (Skwaryło-Bednarz, Krzepiłko 2009*a*). The evaluation of soil enzymatic activity is often referred to as an indicator of biochemical and microbiological activity of soil (Niemi et al. 2005).

The previous studies (Skwaryło-Bednarz 2012) proved that the activity of dehydrogenases in the soils where amaranth is cultivated is one of the most popular indicators of enzymatic activity of soils contaminated with heavy metals and non-contaminated ones. There are also other soil enzymes whose activity in contaminated and non-contaminated soil where amaranth is cultivated have not been thoroughly studied yet. It seems interesting to determine the activity of catalase enzyme. Catalase is an intracellular enzyme involved in the metabolism of oxidoreductase bacteria (Garcia-Gil et al. 2000). It decreases the effect of oxidative stress (Bartkowiak, Lemanowicz

2014). The activity of catalase in soil depends on the content of organic matter, biomass, absorption of O_2, emission of CO_2, and on the activity of dehydrogenases, glycosidase amidase and phosphodiesterase (Riffaldi et al. 2002, Dinesh et al. 2004). The results obtained by Bartkowiak and Lemanowicz (2014) revealed that the activity of catalase in soil decreased when the depth of soil profiles increased.

One of the innovative methods for the determination of soil properties is the assessment of total antioxidant capacity (TAC), which allows the user to determine the intensity of the oxidation and reduction processes taking place in the soil. Humic acids are the most important antioxidant substances in soils (Rimmer 2006, Cardelli et al. 2012, Saviozzi, Cardelli 2014). The total antioxidant capacity of soil also depends on the species of plants cultivated. Different chemical composition of their root secretions may stimulate or hinder the development and activity of the soil microflora, and hence the total antioxidant capacity of soils (Skwaryło-Bednarz, Krzepiłko 2009*b*). The macronutrient fertilization also affects the total antioxidant capacity of soils (Skwaryło-Bednarz, Krzepiłko 2009*b*). The determination of total antioxidant capacity in soils in different condition, i.e. contaminated with heavy metals (copper), seems to be interesting.

The aim of the study was to evaluate the influence of soil contamination with increasing doses of copper on the activity of catalase and total antioxidant capacity (TAC) in soil cropped with amaranth cv. Aztek.

MATERIAL AND METHODS

The experiment was carried out in plastic pots holding 3.5 kg of soil each, and set in three replications. The pots were filled with silt loam having pH = 6.4 marked in solution of KCl at the concentration of 1 mol dm^{-3}. The content of total copper in soil was 13.0 mg kg^{-1} of soil and the content of organic carbon equalled 32.2 g kg^{-1} of soil. Nowadays, the permitted content of total copper in such soils in Poland is 30.0 mg kg^{-1} of soil (Regulation of the Minister of the Environment 2002). Before *Amaranthus cruentus* L., cv. Aztek (a Polish cultivar with pink and red flowers planted for seeds) was sown, uniform fertilization with macroelements was applied in the following doses per pure element (g kg^{-1} of soil): N – 0.25, P – 0.10, K – 0.10. The soil was fertilized twice with N in the form of ammonium nitrate, before sowing and during the intensive growth period. P and K fertilizers were applied before sowing as Polifoska fertilizer and potassium chloride.

The variable factor in the experiment comprised doses of copper in mg kg^{-1} of soil: 0, 50, 150, 300, 450 ($CuSO_4 \cdot 5H_2O$). The choice of copper doses was made according to the sequence given by Wyszkowska et al. (2009), which characterizes the strength of the limiting influence of copper, where a dose of copper causes a 50% decrease in the activity of dehydrogenases.

The seeds were sown on 28 May 2010. After sprouting, 3 plants were left in each pot. The experiment covered the whole growth period of *Amaranthus*. 5 soil samples were taken (into plastic bags) from each pot in order to obtain a composite sample. The soil was carefully mixed, and then sifted through a 2 mm mesh sieve. The catalase activity in fresh soil samples and air dry ones (to indicate the effect of biological and chemical properties in relation to chemical properties) was measured at the same time and presented in cm of emitted oxygen in 1 min by 1 g of soil.

The soil was taken from each composite sample for analysis of the activity of catalase. The activity of catalase was marked in June, August and October. The study included determination of the activity of catalase with the Beck's modified method (Brauner, Bukatsch 1987). To this end, 1.0 g of fresh or air dry soil was weighed and then placed in an Eykman tube to determine the activity of catalase. The next stage of the analysis comprised an addition of 25 cm^3 of 3% H_2O_2. After a specified time, the height of the resulting oxygen column was measured. A unit of the activity of catalase determined this way was cm min^{-1} g^{-1}.

Soil extract for determination of total antioxidant capacity (TAC) was prepared according to the procedure described by Baran (2000).

Determination of total antioxidant capacity was made with the Rice-Evans and Miller method (1994). The method uses ABTS+ (2,2-azinobis--(3-ethylbenzothiazoline-6-sulfonic acid radical) as an indicator substance. The ABTS+ solution is green. Adding antioxidants causes discoloration of ABTS+. The decrease of absorbance after a short time of mixing is a measure of the content of some antioxidants i.e. some vitamins and glutathione. Proteins and some substances having antioxidant properties react more slowly with ABTS+. A decrease of absorbance after 30 min is a measure of the total content of all antioxidants in the sample.

In this study, the soil extract was added to ABTS + solution. The decrease of absorbance was measured at a wavelength of 414 nm after 30 minutes. A standard curve was used to read the trolox concentration corresponding to the change in absorbance in the sample. Trolox ($C_{14}H_{18}O_4$) is an organic chemical compound, soluble in water, used in biological or biochemical processes to reduce stress or damage caused by oxidation. Total antioxidant capacity corresponding to the 1cm^3 of extract prepared from 1 g of soil was expressed in μM of the trolox equivalent (μM trolox cm^{-3} g^{-1} of soil).

In this paper, the correlation coefficients were calculated. The Tukey's test with a 5% of error risk was used for calculating the least significant difference. The statistical analyses were supported by the Enterprise Guide 4.2 (SAS 9.2) programme.

RESULTS AND DISCUSSION

A higher activity of catalase was observed in fresh soil than in air dry soil throughout the experiment. The activity of this enzyme in the soil in all the objects gradually increased during plant growth period (Tables 1 and 2).

Table 1

The activity of catalase in fresh soil in the experimental months in relation to contamination of the soil with copper (mean values)

Cu dose (mg kg^{-1} soil)	Catalase activity (cm min^{-1} g^{-1})		
	June	August	October
0 (control object)	2.47	2.58	2.98
50	2.45	2.56	2.85
150	2.30	2.10	2.12
300	1.50	1.64	1.68
450	0.74	0.76	0.78
$LSD_{0.05}$ Cu dose 0.72 month 0.08 Cu dose x month 0.57			

Table 2

The activity of catalase in air dry soil in the investigated time periods in relation to soil contamination with copper (mean values)

Cu dose (mg kg^{-1} soil)	Catalase activity (cm min^{-1} g^{-1})		
	June	August	October
0 (control object)	2.25	2.28	2.47
50	2.15	2.19	2.32
150	2.04	2.08	2.10
300	1.30	1.44	1.52
450	0.60	0.68	0.72
$LSD_{0.05}$ Cu dose 0.62 month 0.07 Cu dose x month 0.49			

The investigation reveals that the highest mean values of catalase activity in fresh soil in the analysed months (June, August, October) were observed in control objects, where no copper was applied (Table 1). It was observed that the mean value of catalase activity in the consecutive months of the analysis decreased alongside with the increase in the content of copper in the soil – 50, 150, 300, 450 mg kg^{-1} (Table 1). A similar correlation was observed when the activity of catalase in air dry soil was determined (Table 2).

The activity of catalase in fresh soil in June in the control objects was

2.47 cm min^{-1} g^{-1}. When 50 mg kg^{-1} of Cu was applied, the activity of catalases was only 0.8% lower than in the control objects. Application of doses of Cu into the soil (150 mg kg^{-1} Cu, 300 mg kg^{-1} Cu, and 450 mg kg^{-1} Cu), caused a decrease of the activity of catalase by 6.9%, 39.3% and 70.0% respectively (Table 1).

The activity of catalase in fresh soil in the control objects in August was 2.58 cm min^{-1} g^{-1}. It was 0.8% higher than in the pots to which the first dose that had been applied, and 18.6%, 36.4%, 70.5% higher for the subsequently increasing doses (Table 1).

The highest activity of catalase was observed in fresh soil in October, when it was 2.98 cm min^{-1} g^{-1}. Lower activity of catalase was observed in the objects where the smallest dose of Cu had been applied, there it equalled 2.85 cm min^{-1} g^{-1}, being 4.4% lower than in the control objects. Significantly lower activity of catalase in fresh soil, as compared to the control objects, was observed in the objects where 150 mg kg^{-1} Cu (28.9% decrease), 300 mg kg^{-1} Cu (43.6% decrease), and 450 mg kg^{-1} Cu had been applied (73.8% decrease) – Table 1.

According to Wyszkowska et al. (2006), decomposition of hydrogen peroxide in fresh soil sample is triggered by organic compounds that have antioxidant effects and mineral effects, such as heavy metal oxides, ions of metals belonging to transient groups – Fe^{2+}, Cu^{1+}, and microorganisms that contain the catalase enzyme. Therefore, the rate of hydrogen peroxide decomposition in a fresh soil sample is in most cases higher than in air dry soil (Gulser, Erdgan 2008), which is proved by the results reported in this paper.

The activity of catalase in dry soil in the control objects in June was 2.25 cm min^{-1} g^{-1}. When 50 mg kg^{-1} of Cu had been applied to soil, the activity of catalases was by 4.4% lower than in the control objects. Application of the higher doses of Cu into soil (150 mg kg^{-1} Cu, 300 mg kg^{-1} Cu, and 450 mg kg^{-1} Cu), caused a decrease in the activity of catalase by 9.3%, 42.2% and 73.3%, respectively (Table 2).

The activity of catalase in dry soil in the objects with Cu fertilization was 2.28 cm min^{-1} g^{-1} in August, being 3.9% higher than after the first dose of Cu that had been applied, and then 8.8%, 36.8% and 70.2% higher in response to subsequently higher doses of copper (Table 2).

The highest activity of catalase was observed in dry soil in October, at the end of amaranth's growing period, when it reached 2.47 cm min^{-1} g^{-1}. Lower activity of catalase was observed in the objects where the smallest dose of Cu had been applied. There it equalled 2.32 cm min^{-1} g^{-1} and was by 6.1% lower than in the control objects. The activity of this enzyme in air dry soil decreased alongside with an increase in the doses of copper, as compared to the objects with no Cu fertilization. Lower activity of catalase in fresh soil, as compared to the control objects, was observed in the objects where 150 mg kg^{-1} Cu (15.0% decrease), 300 mg kg^{-1} Cu (38.5% decrease), and 450 mg kg^{-1} Cu had been applied (70.9% decrease) – Table 2.

It is commonly known that copper is a heavy metal which, in small doses, is vital for the proper functioning of live organisms. High doses of copper can be toxic to live organisms. The investigation clearly reveals that application of copper into soil, especially in the highest dose used in the experiment, causes a decrease in the activity of catalases in the soil, despite the fact that catalase is the most resistant enzyme to soil contamination with copper, as demonstrated by Wyszkowska et al. (2009)

The fact that heavy metals, including copper, decrease the activity of soil enzymes has been proved by many authors (Wyszkowska et al. 2005, 2006, Gulser, Erdgan 2008, Wyszkowska, Wyszkowski 2010). The effects of copper on soil enzymes depend on the plant cultivar, type of soil and the way it is used (Wyszkowska et al. 2010). Wyszkowska et al. (2010) in their pot experiments observed that the contamination of soil with copper at doses 150-450 mg kg^{-1} significantly decreases the enzymatic activity of soil, an effect which has also been observed in this study.

Unfavourable effects of copper on enzymatic activity may be due to the indirect toxic influence of copper on microorganism proliferation (Oliveira, Pampulha 2006), and also because of its destructive influence on enzymes (Wyszkowska et al. 2005).

Khan et al. (2007) observed toxic effects of different doses of heavy metals introduced into soil on the activity of catalase. The results of their studies revealed that the extent to wich catalase activity decreased was parallel to the increase in the concentration of heavy metals. They observed higher toxic effects of Cd than of Pb on enzymatic activity of the soil. Higher toxicity of Cd is due to greater mobility of this element and less binding with soil colloids. Different results were obtained by Belyaeva et al. (2005), who observed that the activity of catalase is not clearly depressed by heavy metals.

Szymczak et al. (2011) in their own studies observed a decrease in the activity of catalase alongside with an increase in doses of another heavy metal, namely cadmium, regardless of the time when the analyses were carried out, which was probably influenced by the reaction of the metal with the substrate-enzyme complex, a complex reaction of the substrate or the blockade of catalytically active groups of the enzyme. The research carried out by Szymczak et al. (2011) confirms earlier observations of Liu et al. (2008).

The results obtained by Solanki et al. (2011) carried out on sprouting plants of *Vigna mungo* revealed a decrease in the activity of catalase at the seedling stage. It was also observed that while the activity of catalase decreased, the activity of peroxidase increased. The study also showed that combined effects of copper and zinc are more harmful than the effects of a single metal.

Bartkowiak and Lemanowicz (2014) did not observe any significant effects of heavy metals – Cu, Zn, and Ni (their total form and available form) – present in the soil on the activity of catalase.

The activity of soil enzymes reflects the intensity of biochemical processes

that take place in the soil, although it is often difficult to characterize it because of a great variety of factors shaping the properties of soil environment. Chemical properties of soils determine the activity of soil enzymes (Bartkowiak, Lemanowicz 2014). Soil enzymes are involved in the decomposition of organic matter, reactions that create humus, decomposition of humus, and in production of nutrients available to plants (Mocek-Płóciniak 2010). Bielińska and Domżał (2001) and Wyszkowska et al. (2010) observed that changes in enzymatic activity of soils depend on the type of plants, and even on specific plant cultivars.

The results of numerous studies revealed changes in the enzymatic activity of soils in relation to the depth of a soil profile. Bartkowiak and Lemanowicz (2014) showed an increase in the activity of dehydrogenases in subsurface horizons of the investigated soil profiles. The activity of soil enzymes decreased with the depth of a soil profile, which is due to the specific distribution of humus and soil microorganisms and a decreasing amount of carbon substrates available for microorganisms and enzymes (Bielińska, Mocek-Płóciniak 2009, Skwaryło-Bednarz, Krzepiłko 2009*a*).

The statistical analysis revealed significant negative correlations between the dose of copper that had been applied and the activity of catalase in fresh soil and air dry soil where amaranth was cultivated (Table 3).

Table 3

Correlation coefficient between the doses of Cu and the activity of catalase and between the doses of Cu and TAC in the soil under amaranth cultivation

Cu dose (mg kg^{-1} soil)	Catalase activity (cm min^{-1} g^{-1})		
	June	August	October
	fresh soil		
	-0.976	-0.988	-0.992
	air dry soil		
	-0.980	-0.976	-0.988
	TAC (μM trolox cm^{-3} g^{-1} soil)		
	-0.949	-0.963	-0.971

Correlation significant at $p = 0.01$.

An innovative method for the determination of soil properties is the assessment of total antioxidant capacity (TAC) of soil, *inter alia* under amaranth cultivation. The application of 50 mg Cu kg^{-1} of soil resulted in the highest mean value of the total antioxidant capacity of soil under amaranth cultivation in the test months. The application of higher doses of copper reduced the mean value of its total antioxidant capacity. It has been found that TAC increased in all objects during the plant growing season (Table 4).

In June, the TAC in the pots fertilized with 50 mg Cu kg^{-1} was 188.55 μM trolox cm^{-3} g^{-1} of soil and was higher than in the control object by

Table 4

Total antioxidant capacity (TAC) of the soil in the investigated time periods in relation to soil contamination with copper (mean values)

Cu dose (mg kg^{-1} soil)	TAC (μM trolox cm^{-3} g^{-1} soil)		
	June	August	October
0 (control object)	169.2	177.3	188.1
50	188.6	192.7	199.8
150	152.5	155.9	159.2
300	100.4	106.1	109.8
450	88.5	89.7	91.6
$LSD_{0.05}$ Cu dose 51.5 month 5.6 Cu dose x month 40.6			

11.4%. Higher doses of copper (150, 300 and 450 mg kg^{-1}) reduced the mean value of this parameter by 9.9%, 40.7% and 47.7%, respectively (Table 4).

In August, the mean value of total antioxidant capacity in the control objects was 177.34 μM trolox cm^{-3} g^{-1} of soil. The lowest dose of Cu raised TAC by 8.64% in the comparison with the control object, while the growing doses of copper fertilization decreased TAC by 12.1 to 49.4% (Table 4).

In October, the highest TAC (199.78 μM trolox cm^{-3} g^{-1} of soil) was observed in the object fertilized with the lowest dose of copper. It was higher by 6.2% compared to the control object. The dose of 150 mg Cu kg^{-1} of soil decreased the total antioxidant capacity by 15.3%, while 300 and 450 mg kg^{-1} lowered TAC by 41.6% and 51.3%, respectively (Table 4).

Studies on total antioxidant capacity of soils are mostly carried out in the context of the soil type (Rimmer, Smith 2009) or the impact of organic and macronutrient fertilization (Wang, Lin 2003, Skwaryło-Bednarz, Krzepiłko 2009*b*) on this parameter. Little is known on the relationship between the content of heavy metals in soil and the quantity of antioxidants. Sharma and Agrawal (2005) stated that heavy metals entering from the soil to a plant can affect the level of antioxidants in the plant products. The consumption of these plants can be noxious to human health, which was confirmed by Xiong and Wang (2005).

Our result proved that the amount of antioxidants in the soil is affected by heavy metals, such as copper. The statistical analysis showed significant negative relationships between doses of copper and total antioxidant capacity of tested soils (Table 3).

CONCLUSIONS

1. The pot experiment revealed an increase in the activity of catalase in fresh soil and air dry soil in all the objects throughout the plant growing period of amaranth. Higher activity of catalase was found in fresh soil samples than in air dry ones.

2. The highest activity of catalase was found in the objects where no fertilization with Cu had been applied, and the smallest activity of catalase was observed in the objects where the dose of this microelement was the highest.

3. Application of higher doses of copper (50, 150, 300, 450 mg kg^{-1}) contributed to the decrease in the activity of catalase in soil in the experimental months, as compared to the control objects.

4. The highest mean value of total antioxidant capacity in soil was obtained by the smallest dose of copper (50 mg kg^{-1}) applied. Throughout the study period, the value of this parameter was higher than the value obtained in the soil from the control object. Increasing doses of copper, higher than 50 mg kg^{-1}, resulted in a progressive reduction of the value of total antioxidant capacity, which in any case was lower than the TAC value for the control object.

5. The mean value of catalase activity and total antioxidant capacity increased during the plant growing period, regardless of the copper dose applied to soil.

6. Significantly high negative correlations between doses of Cu and the activity of catalase and total antioxidant capacity in soil in the experimental months were observed.

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