# RESPONSE OF MAIZE (ZEA MAYS L.) TO SOIL CONTAMINATION WITH COPPER DEPENDING ON APPLIED CONTAMINATION NEUTRALIZING SUBSTANCES

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#### Abstract

Copper is an essential trace element in living organisms, but its excess interferes with metabolic transformations in plant, animal and human cells. The uptake of copper from contaminated soils can be regulated, for example, by soil liming or application of other substances which enhance soil adsorption capacity and Cu binding in the substrate. The purpose of this study has been to assess the response of maize to soil contamination with copper depending on the applied neutralizing substances. The tested factors were increasing concentrations of Cu in soil: 0, 200, 400 and 600 mg Cu kg<sup>-1</sup> dm, and soil application of mineral (lime, loam and zeolite) and organic (manure, peat and bark) neutralizing substances.

Soil contamination with copper within the range of 200 to 600 mg kg $^{-1}$  of soil caused reduction in maize yields. Positive influence such as alleviation of the harmful effect of copper contamination was demonstrated by lime and manure, which enhanced yields of maize, especially in the series polluted with 200 and 400 mg Cu kg $^{-1}$ . The other soil amending substances, especially peat added in amounts of 400 and 600 mg kg $^{-1}$  of soil, caused a considerable depression in maize yields. A linear dependence has been demonstrated between the concentration of Cu in maize plants and the content of Cu in the substrate, with the root content of Cu being on average six-fold higher than in aerial organs. Mineral soil amendments significantly decreased the BTC index in maize compared to organic substances, and lime as well as pine bark decreased the BCF index to 2.33 and 1.67 versus the value of 4.21 found in the control treatment without any neutralizing substances. The uptake of copper depended on the volume of yield and - to a lesser degree - on the concentration of Cu in plants. The uptake of copper by plants was the highest in treatments

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contaminated with a rate of 200 mg Cu  $\rm kg^{-1}$ , which was the consequence of higher yields from that treatment than from the plots polluted with 400 or 600 mg Cu  $\rm kg^{-1}$  of soil.

Key words: copper, BTC, BCF, BAC, phytotoxicity, neutralizing amendments.

## REAKCJA KUKURYDZY (ZEA MAYS L.) NA ZANIECZYSZCZENIE GLEBY MIEDZIĄ W ZALEŻNOŚCI OD ZASTOSOWANYCH DODATKÓW NEUTRALIZUJĄCYCH

#### Abstrakt

Miedź to niezbędny w funkcjonowaniu organizmów żywych pierwiastek śladowy, jednak jej nadmiar objawia się zakłóceniem metabolizmu roślin, zwierząt oraz ludzi. Pobranie miedzi z gleb zanieczyszczonych można regulować m.in. stosując zabieg wapnowania oraz inne substancje wpływające na zwiększenie sorpcji i wiązanie Cu w glebie. Celem badań była ocena reakcji kukurydzy na zanieczyszczenie gleby miedzią oraz zastosowane dodatki neutralizujące. Czynnikami były rosnące stężenia Cu w glebie: 0, 200, 400 i 600 mg Cu kg<sup>-1</sup> s.m. oraz doglebowe dodatki neutralizujące: mineralne (wapno, ił i zeolit) oraz organiczne (obornik, torf i kora).

Zanieczyszczenie gleby Cu w ilości od 200 do 600 mg kg<sup>-1</sup> gleby powodowało spadek plonowania kukurydzy. Pozytywny wpływ na łagodzenie skutków szkodliwego działania miedzi wykazały wapno oraz obornik, poprawiając plonowanie roślin, głównie w odniesieniu do gleby zanieczyszczonej 200 i 400 mg Cu kg<sup>-1</sup>, pozostałe dodatki, szczególnie torf stosowany w glebie zanieczyszczonej 400 i 600 mg Cu kg<sup>-1</sup>, przyczyniły się do znacznego obniżenia plonu. Wykazano liniową zależność między zawartością Cu w roślinach kukurydzy a zawartością Cu w podłożu, przy czym zawartość Cu w korzeniach była średnio ponad 6 razy wyższa niż w części nadziemnej. Dodatki mineralne istotnie wpłynęły na wskaźnik BTC w kukurydzy w stosunku do zastosowanych dodatków organicznych. Wapno i kora sosnowa wpłynęły na obniżenie wskaźnika BCF do wartości 2,33 i 1,67 w stosunku do wartości 4,21 w obiekcie kontrolnym – bez dodatków. Pobranie miedzi zależało od wielkości plonu, w mniejszym stopniu od zawartości Cu w roślinach. Na obiektach zanieczyszczonych 200 mg Cu kg<sup>-1</sup> – wyżej plonujących – średnia wielkość pobrania była istotnie wyższa niż na obiektach o bardzo dużej zawartości miedzi – 400 i 600 mg Cu, ale dużo niżej plonujących.

Słowa kluczowe: miedź, BTC, BCF, BAC, fitotoksyczność, dodatki neutralizujące.

#### INTRODUCTION

Progress in our civilization is invariably associated with environmental pollution. The contamination of the natural environment is mainly caused by emission of metal-bearing dusts, which are eventually deposited on the surface of earth. Other sources of trace metals are industrial waste, municipal sewage and wastewater, fertilizers and plant protection chemicals (Bowszys et. al 2009, Wołoszyk et al. 2009). In Poland, soil contamination with heavy metals is detectable locally, mainly in industrialized areas, although in some locations near sources of emission, soils can be heavily polluted (Rosada 2007, Dopierala 2009, Szulc, Kobierski 2010). The content of Cu in Polish soils

ranges from 0.2 to 725.0 mg Cu kg<sup>-1</sup> d.m. and the average is 6.50 mg Cu kg<sup>-1</sup>. Near metal smelting plants, e.g. the Głogów Copper Smelter (Poland), the concentration of Cu is 1440 mg Cu kg<sup>-1</sup> on average (Karczewska et al. 2012), and contamination rates detected near metal smelters in other parts of the world range from 510-9700 mg Cu kg<sup>-1</sup> – Sudbury (Canada), 1400--3700 mg Cu kg<sup>-1</sup> – Coniston (Canada) (CCME 1997), 11 600-14 200 mg Cu kg<sup>-1</sup> – Lubumbashi (DR-Congo) (NARENDRULA et al. 2012). Soil contamination with copper can also result from inadequate and repetitive application of plant protection chemical preparations. Copper-based fungicides used in viniculture until the end of the 19th century (the Bordeaux mixture  $CuSO_4 + Ca(OH)_2$ ) as well as the ongoing application of copper compounds such as Cu(OH)<sub>2</sub> CuCl<sub>2</sub>, CuSO<sub>4</sub> 3Cu(OH)<sub>2</sub>, Cu<sub>2</sub>O, Cu(OH)<sub>2</sub> have led to serious accumulation of Cu in soil of grapevine plantations in France, Brazil, Croatia and Spain. In these countries, average concentrations of Cu in the 0-20 cm soil layer are 332, 665, 700 and 560 mg Cu kg<sup>-1</sup> respectively (Komarek et al. 2010). Copper-based fungicides are also applied for protection of other crops (Schramel et al. 2000, Li et al. 2005, Komarek et al. 2009). Trace elements are not biodegradable (Antoniadis et al. 2007) and in soil can be dissolved, chelated, adsorbed by humus, bound by soil minerals, uptaken by plants or titrated with other compounds. Persistence of copper bonds depends on the soil pH, quantity and type of other mineral components, redox potential, soil adsorption capacity and content of organic matter (Dube et al. 2001, USEPA 2002). One possible method which alleviates the harmful effect of heavy metals consists in soil amendment with neutralizing substances (NWACHUKWU, PULFORD 2008) that bind copper into insoluble metal and mineral or metal and organic forms, which - under favourable conditions - may remain in soil for a long time as harmless compounds (Zolnowski et al. 2009). Such soil amending substances include zeolites, loam, lime, ground tree bark, manure and peat (NWACHUKWU, PULFORD 2008, KOMAREK et al. 2009, Najmowicz et al. 2010). Methods for immobilization of metals in soil remain the only available and economically viable way to rehabilitate contaminated soils, which enable restoration of biological life and plant cover on degraded land (Karczewska, Kabała 2010). High concentrations of heavy metals in soil may lead to their plant concentrations higher than normal, which in turn can cause their increased transfer to subsequent links in the trophic chain, where they can accumulate and produce lasting harmful effects.

The aim of this study has been to assess the response of maize to soil contamination with copper and to determine the effectiveness of copper immobilization in soil by using mineral (lime, loam and zeolite) and organic (manure, peat and tree bark) neutralizing soil amendments.

#### MATERIAL AND METHODS

A pot experiment was set up in 2011, in a greenhouse at the University of Warmia and Mazury in Olsztyn. The experiment was designed according to the method of randomly selected blocks with four replicates. There were two groups of factors: 1) incremental contamination of soil with copper: 0, 200, 400 and 600 mg Cu kg<sup>-1</sup> of soil, which was simulated by adding to soil aqueous solution of copper (II) sulphate (CuSO<sub>4</sub> 5H<sub>2</sub>O; produced at the POCh in Gliwice), and 2) neutralizing substances: lime in an amount equivalent to 1 Hh, i.e. 8,48 g  $CaCO_3$  pot<sup>-1</sup>, loam, natural zeolite  $\varnothing$  1-2.5 mm (SUBIO EKO Polska, Sp. z o.o.), cattle manure (P.P.H.U. CDN Ireneusz Cal), peat (Athena Bio-Produkty Sp. z o.o.) and pine bark (fraction Ø 2-5 mm) (Hollas Sp. z o.o.) 3% of soil mass per pot, i.e. 240 g pot<sup>-1</sup>. NPK fertilization was:  $2.17~{\rm g}$  N as ammonia,  $\rm CO(NH_2)_2,~0.6~{\rm g}$  P as  $\rm KH_2PO_4,~1.25~{\rm g}$  K as  $\rm KH_2PO_4$  $(0.75~{\rm g~K})$  and  ${\rm K_2SO_4}$   $(0.5~{\rm g~K})$ ,  $0.18~{\rm g~Mg~as~MgSO_4}$  and  $0.25~{\rm mg~B~as}$ H<sub>3</sub>BO<sub>3</sub> pot<sup>-1</sup>. Typical brown soil developed from poor loamy sand class IVb and collected from the Ap horizon was used in the experiment. In the Polish soil valuation system, the soil belongs to good rye complex. In the FAO WRB (World Reference Base for Soil Resources) taxonomy (FAO 2006), the soil was classified as Cambisol - Brown Soils. The basic properties of soil are specified in Table 1.

 $\label{thm:constraint} \mbox{Table 1}$  Some physicochemical properties of soil used in experiment

Soil type	pH KCl	pH H <sub>2</sub> O	Hydro lytic acidity (cmol kg <sup>-1</sup> )	Alka- line cations (cmol kg <sup>-1</sup> )	Cation excha- nge capacity CEC (cmol kg <sup>-1</sup> )	Total base satura- tion (%)	Salinity (µS cm <sup>-1</sup> )	N total (g kg <sup>-1</sup> )	C org. (g kg <sup>-1</sup> )	C:N ratio
Cambisols - brown soil	4.50	5.70	2.84	5.47	8.31	65.83	71.60	0.76	5.55	7.30

The experiment was set up in Kick-Brauckmann pots, by filling each pot with 8 kg of soil passed through a  $\oslash$  1 cm mesh sieve. During the vegetative growth of plants, the soil moisture was maintained at the level of 60% full water capacity. Maize (*Zea mays* L.) cv. San (Hodowla Roślin Smolice Sp. z o.o.) was grown for green matter and harvested 100 days after sowing. After harvest, the yield of aerial parts and roots (after washing off the soil residue and drying) was determined. Samples were dried at 65°C, ground and wet digested according to the EPA Method 3052 (Microwave Assisted Acid Digestion of Siliceous and Originally Based Matrices) (EPA 1996), using microwave heating with an appropriate microwave system (MARS-5,

CEM Corporation USA). Concentration of Cu was determined by flame atomic absorption spectroscopy (FLAAS) (VARIAN SpectrAA – FS240, Varian Inc. Australia). The content of copper was calculated per absolutely dry matter, determined at  $105^{\circ}$ C. The biological transfer coefficient for copper BTC – 1, biological concentration factor BCF – 2, and biological accumulation coefficient BAC – 3, were determined (Tukura et al. 2012).

$$BTC = \frac{\text{metal concentration (mg kg}^{-1} DM) \text{ abveground part of plant}}{\text{metal concentration (mg kg}^{-1} DM) \text{ in roots}}$$
 (1)

$$BCF = \frac{\text{metal concentration (mg kg}^{-1} DM) \text{ in roots}}{\text{metal concentration (mg kg}^{-1} DM) \text{ in soil}}$$
(2)

$$RAC = \frac{\text{metal concentration (mg kg}^{-1} DM) \text{ aboveground part of plant}}{\text{metal concentration (mg kg}^{-1} DM) \text{ in soil}}$$
(3)

The uptake of copper by plants was expressed in mg of copper taken up from 1 kg of soil. The results were processed statistically with Anova at the level of significance of  $\alpha = 0.05$ , using a Statistica v. 9.0 software package (StatSoft 2009). The correlation between the analyzed factors was established using a simple linear correlation model, with the Microsoft Excel programme (Microsoft 2002).

#### RESULTS AND DISCUSSION

The tested soil contamination with copper caused a significant, linear decrease in the mass of roots and aerial matter yield produced by maize, except the rate of 200 mg Cu kg<sup>-1</sup> of soil, which stimulated the roots yield, raising it by 13.80 g DM pot<sup>-1</sup> relative to the control and by 28.8 g versus the manure-amended pots (Table 2). No such effect was observed in respect of the aerial mass yield in the control treatment without soil amending substances. Out of the tested amendments, lime and manure are noteworthy as they raised the yield of root dry matter from pots contaminated with 200 mg Cu kg<sup>-1</sup> of soil by 16.50 and 15.63 g DM pot<sup>-1</sup>. In this experiment, copper contamination was observed to have inhibited the growth of maize roots, which became thicker and shorter. A similar response of plants to Cu contamination of soil has been described by Rossi et al. (2004), Szulc, Kobierski (2010) and Singh et al. (2007). Crops can be improved when soil is amended with substances which can arrest copper. Such substances include composts made from plant waste, coir and tree bark or, less often used, bone meal and peat (NWACHUKWU, PULFORD 2008). However, organic soil amendments do not always produce same effects on chosen heavy metals. In a study completed by Ciecko et al. (2001), application of charcoal or compost with lime or in a series without liming did not improve yields of triticale.

Table 2

Effects of soil pollution with copper on the dry matter weight of roots and aboveground part of maize

			and aboves	ground par	t of marze					
Soil pollution with copper $(mg Cu kg^{-1})$	Objects									
	without amen- dments	lime	clay	zeolite	FYM	peat	pine bark	Average		
Weight of roots (DM g pot $^{-1}$ )										
0	46.95	98.63	60.10	51.53	47.58	72.08	58.28	62.16		
200	60.75	77.25	48.25	26.25	76.38	68.35	42.25	57.07		
400	4.25	5.78	5.18	3.70	5.88	2.20	4.13	4.45		
600	1.60	1.80	1.80	1.93	1.28	1.93	2.53	1.84		
$\overline{x}$	28.39	45.87	28.83	20.85	32.78	36.14	26.80	31.38		
$R_2$	0.64*	0.80**	0.84**	0.83**	0.54*	0.79**	0.81**	0.75**		
LSD $_{\alpha=0}$	LSD $_{\alpha=0.05}$ for Cu soil pollution = 5.31; for amendments = 7.03; for interaction = 14.06									
		Yield	of aerial p	art of maiz	ze (DM g p	$ot^{-1}$ )				
0	168.15	166.46	152.09	156.60	175.48	160.98	163.85	163.37		
200	145.84	147.61	107.98	74.86	149.53	83.34	101.85	115.86		
400	18.92	18.96	20.85	15.32	21.19	3.97	18.23	16.78		
600	3.40	3.30	3.88	2.69	2.62	3.04	3.42	3.19		
$\overline{x}$	84.08	84.08	71.20	62.37	87.21	62.83	71.84	74.80		
$R_2$	0.83**	0.87**	0.89**	0.89**	0.84**	0.83**	0.66*	0.83**		
LSD $_{\alpha=0.05}$ for increasing Cu soil pollution = 13.21; for amendments = 17.47; for interaction = n.s.										

<sup>\*</sup> determination coefficient  $R_2$  significant for  $\alpha \leq 0.05$ ;

Manure introduced to soil may have a stimulating effect on mobility of metals, possibly because it contains nitric compounds, which are often physiologically acidic (Sienkiewicz et al. 2009). The concentration of copper in maize plants was varied and depended on the analyzed part of the plant and the degree of soil contamination with copper (Table 3). Roots contained on average 1661.38 mg Cu  $\rm kg^{-1}$  DM in the series without neutralizing additives and the aerial organs had 299.02 mg Cu  $\rm kg^{-1}$  DM.

Application of lime depressed the concentration of copper in plant roots by an average of 771.37, zeolite by 225.83 and loam by 113.12 mg Cu kg<sup>-1</sup> DM of roots. Among the tested organic soil amendments, pine bark caused a significant decrease of the concentration of Cu in roots by 1141.68 mg kg<sup>-1</sup> DM, while manure decreased it by 461.91 mg Cu kg<sup>-1</sup> DM compared to the average concentration found in the non-amended series.

<sup>\*\*</sup> determination coefficient  $\tilde{R}_2$  significant for  $\alpha \leq 0.01$ ; n.s. – not significant; n=8

 $\begin{tabular}{ll} Table 3 \\ Effects of soil pollution with copper on the copper content in roots \\ and above ground part of maize \\ \end{tabular}$ 

				ground par						
Soil pollution with copper (mg Cu $kg^{-1}$ )	Objects									
	without amen- dments	lime	clay	zeolite	FYM	peat	pine bark	Average		
Copper content in roots (mg kg <sup>-1</sup> DM)										
0	19.18	17.93	31.00	53.50	11.00	12.20	14.75	22.79		
200	176.28	117.85	276.20	266.20	143.78	171.38	156.53	186.89		
400	1292.65	525.08	1242.35	898.75	1006.25	2542.40	643.18	1164.38		
600	5157.40	2899.17	4643.50	4523.75	3636.85	6080.00	1264.35	4029.29		
$\overline{x}$	1661.38	890.01	1548.26	1435.55	1199.47	2201.49	519.70	1350.84		
$R_2$	0.79**	0.74**	0.80**	0.75**	0.81**	0.88**	0.93**	0.81**		
LSD $_{\alpha=0.0}$	LSD $_{\alpha=0.05}$ for Cu soil pollution = 60.58; for amendments = 80.14; for interaction = 160.28									
	C	Copper con	tent in aer	ial part of	maize (mg	$ m kg^{-1}~DM)$				
0	15.30	6.05	10.08	8.30	8.50	5.38	8.00	8.80		
200	10.80	10.93	9.30	11.35	23.03	52.50	9.85	18.25		
400	64.33	20.83	32.58	27.78	49.38	510.20	118.00	117.59		
600	1105.65	483.60	735.20	571.43	330.05	1398.40	280.13	700.64		
$\overline{x}$	299.02	130.35	196.79	154.71	102.74	491.62	103.99	211.32		
$R_2$	0.63*	0.62*	0.62*	0.63*	0.70**	0.86**	0.86**	0.70**		
LS	LSD $_{\alpha=0.05}$ for increasing Cu soil pollution = 10.26; for amendments = 13.57; for interaction = 27.15									

<sup>\*</sup> determination coefficient  $R_2$  significant for  $\alpha \leq 0.05$ ;

The concentration of Cu in roots was the highest in maize plants grown on soil amended with peat: 2201.49 mg Cu kg<sup>-1</sup> DM on average and the maximum concentration of 6080.00 mg Cu kg<sup>-1</sup> DM. on soil polluted with 600 mg Cu kg<sup>-1</sup>. Significant reduction in the concentration of Cu in aerial parts of maize was achieved through enrichment of soil with lime as well as manure or pine bark, where it decreased by 168.67, 196.28 and 195.03 mg Cu kg<sup>-1</sup> DM., respectively. In contrast, the application of peat caused an increase in the content of Cu in aerial parts of maize by 192.6 mg Cu kg<sup>-1</sup> DM. versus the control. Rossi et al. (2004) also demonstrated that the roots of *Brassica napus* accumulated several-fold more copper than the aerial organs, which is in accord with the present results.

<sup>\*\*</sup> determination coefficient  $\tilde{R}_2$  significant for  $\alpha \leq 0.01$ ; n.s. -not significant; n=8

The coefficients of biological transfer (BTC), biological accumulation (BAC) and biological concentration factor (BCF) determined for copper in maize plants depended on the applied neutralizing substances (Table 4).

Table 4  $Effects\ of\ soil\ pollution\ with\ copper\ on\ the\ biological\ transfer\ coefficient\ (BTC),\ biological$ concentration factor (BCF), and biological accumulation coefficient (BAC)  $\,$ 

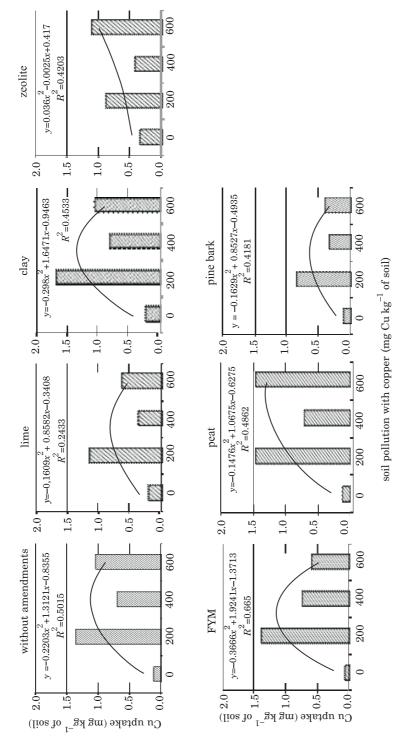
Soil pollution	Objects									
with copper (mg Cu kg <sup>-1</sup> )	without amen- dments	lime	clay	zeolite	FYM	peat	pine bark	Average		
Biological transfer coefficient (BTC)										
0	0.82	0.34	0.32	0.15	0.80	0.44	0.69	0.51		
200	0.06	0.09	0.03	0.04	0.18	0.31	0.06	0.11		
400	0.05	0.04	0.03	0.03	0.05	0.20	0.18	0.08		
600	0.21	0.17	0.16	0.13	0.09	0.23	0.22	0.17		
$\overline{x}$	0.29	0.16	0.14	0.09	0.28	0.29	0.29	0.22		
$R_2$	n.s.	n.s.	n.s.	n.s.	0.64**	0.68**	n.s.	n.s.		
LSD $\alpha =$	LSD $_{\alpha=0.05}$ for Cu soil pollution = 0.10; for amendments = 0.13; for interaction = 0.26									
		Bio	logical con	centration	factor (BC	(F)				
0	2.85	2.06	4.00	5.91	1.81	3.16	0.32	2.87		
200	0.95	0.63	1.56	1.41	1.45	0.97	1.11	1.15		
400	3.77	1.34	3.57	2.37	2.63	7.37	2.26	3.33		
600	9.29	5.28	8.34	7.71	7.15	10.64	2.98	7.34		
$\overline{x}$	4.21	2.33	4.37	4.35	3.26	5.54	1.67	3.68		
$R_2$	0.64**	n.s.	n.s.	n.s.	n.s.	0.74**	0.96**	0.65**		
LSD $\alpha =$	<sub>0.05</sub> for Cu	soil pollut	ion = 0.23	; for amen	dments =	0.30; for i	nteraction	= 0.61		
		Biolog	gical accun	nulation co	efficient (I	BAC)				
0	2.34	0.70	1.30	0.91	1.38	1.40	0.19	1.17		
200	0.06	0.06	0.05	0.06	0.23	0.30	0.07	0.12		
400	0.19	0.05	0.09	0.07	0.13	1.48	0.41	0.35		
600	1.99	0.88	1.32	0.97	0.65	2.45	0.66	1.27		
$\overline{x}$	1.14	0.42	0.69	0.50	0.60	1.41	0.33	0.73		
$R_2$	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.74**	n.s.		
LSD $_{\alpha=0.05}$ for Cu soil pollution = 0.19; for amendments = 0.25; for interaction = 0.51										

<sup>\*</sup> determination coefficient  $R_2$  significant for  $\alpha \leq 0.05$ ; \*\* determination coefficient  $R_2$  significant for  $\alpha \leq 0.01$ ; n.s. -not significant; n=8

Maize was characterized by a relatively low BTC, which means that quite a large portion of absorbed copper stayed in roots. As a rule, plants with BTC >1.0 are said to be hyperaccumulators and can be used for phytoextraction and phytostabilization (Tukura et al. 2012). Lower BTC values than in other series were found in the treatment with zeolite, where the mean BTC was 0.09. This means that peat had a significant effect on arresting metals in roots. A high BTC value was determined for the series without any neutralizers (0.29), and the applied organic soil amendments did not affect the transfer of copper from roots to the upper parts of maize plants. The BCF >1.0 confirms extensive binding of metals, which is a desirable characteristic of plants used for phytoremediation of polluted soils. High BCF informs about the presence of detoxication mechanisms, which lead to sequestration of ions of trace metals, and this in turn prevents translocation of harmful metals of aerial parts of plants (Ghosh, Singh 2005). The tested maize was characterized by a relatively high BCF, which suggests a considerably high uptake of copper and its arrest in roots. Out of the mineral soil amendments, lime caused a decrease in the concentration of Cu in maize (BCF=2.33); organic substances produced diverse effects. Pine bark depressed the biological concentration factor down to 1.67, while peat induced a very high increase in its value - to 5.54 on average, with the highest values of 7.37 and 10.64 in treatments polluted with 400 i 600 mg Cu kg<sup>-1</sup> of soil, respectively.

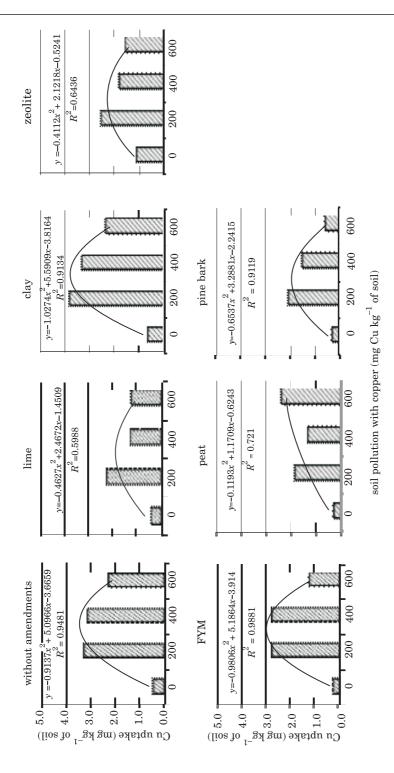
A relatively low BAC indicated high efficiency of lime and pine bark added to soil (0.42 and 0.33, respectively). These neutralizing substances inhibited the transfer of copper from soil to stems and leaves of maize. The highest accumulation coefficient was determined for maize grown on soil with peat (1.41) and without any neutralizers (1.14). The uptake of copper by maize plants (Figures 1, 2) to a large extent depended on the degree of soil contamination with this metal. The highest uptake occurred in the treatments polluted with 200 mg Cu kg $^{-1}$  of soil. In maize grown on soil with higher rates of copper, despite an increase in copper concentration in plant tissues, a significant decrease in the uptake of the metal was observed as a result of the strong depression of maize yield due to the toxicity of copper. Lime, zeolite and bark distinctly depressed the uptake of copper by maize roots compared to the treatments without soil amending substances and with added loam, manure and peat.

In respect of the aerial parts of maize, lime and zeolite as well as peat and pine bark limited the uptake of copper. The amounts of copper removed with harvested parts of maize depended above all on the properties of soil neutralizing substances. Lime was most effective as it enhanced yields, generated a low biological concentration factor and resulted in the lowest uptake of copper. As reported by Sienkiewicz et al. (2009), application of organic substances to soil may be followed by an increase in the uptake of copper, an effect induced by nitric compounds found in such substances which acidify the soil and increase the solubility of metals, thus facilitating their translocation into plants, the event that was also demonstrated in this study.



LSD  $_{a=0.05}$  for increasing Cu soil pollution = 0.09; for amendments = 0.13; for interaction = 0.25 correlation coefficient between soil Cu pollution and Cu uptake r=0.36, ns – not significant correlation

Fig 1. Copper uptake by roots of maize from 1 kg of soil polluted with copper



LSD  $_{a=0.05}$  for increasing Cu soil pollution = 0.15; for amendments = 0.20; for interaction = 0.40 correlation coefficient between soil Cu pollution and Cu uptake r=0.24, ns – not significant correlation

Fig 2. Cu uptake by aboveground parts of maize from 1 kg of soil polluted with copper

#### CONCLUSIONS

- 1. Soil contamination with copper had a significant effect on maize yields. The rate of 200 mg Cu kg<sup>-1</sup> of soil stimulated the growth of maize roots, while the doses of 400 and 600 mg Cu kg<sup>-1</sup> led to reduced root yields. Soil contamination with copper had a significantly negative effect on yields of aerial parts of maize at all the tested rates of contamination.
- 2. Copper contamination significantly raised the concentration of Cu in plants. In roots, it was on average over 6-fold higher than in aerial organs, which indicates that there are certain mechanisms involved limiting the uptake of toxic amounts of copper by maize.
- 3. Mineral contamination neutralizing substances depressed the biological transfer coefficient (BTC) for Cu in maize compared to plants grown on soil with organic soil amendments. The biological concentration factor (BCF) fell down to 2.33 and 1.67 in response to application of lime and, exceptionally, pine bark compared to 4.21 in the control. The BAC in the same treatments decreased down to 0.42 and 0.33 versus 1.14 found in the control treatment, without any neutralizers. The substances introduced to soil evidently inhibited the translocation of copper from soil to maize stems and leaves.
- 4. The uptake of copper by maize depended primarily on the volume of yields and, to a lesser degree, on the concentration of Cu in maize plants. Plants growing on the treatments polluted with 200 mg Cu  $\rm kg^{-1}$  absorbed on average more copper than the ones from pots with soil polluted with 400 and 600 mg Cu  $\rm kg^{-1}$ , despite a very high concentration of Cu in plant tissues in the latter case.
- 5. The smallest average unit uptake of copper by maize, expressed in mg Cu per kg of soil, was demonstrated by plants grown on soil enriched with lime and pine bark, which confirms the claim that these soil amendments belong to substances that most strongly inhibit the uptake of heavy metals by plants.

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