Potravinarstvo Slovak Journal of Food Sciences
vol. 11, 2017, no. 1, p. 20-25
doi: https://dx.doi.org/10.5219/693
Received: 1 December 2016. Accepted: 18 January 2017.
Available online: 31 January 2017 at www.potravinarstvo.com

ISSN 1337-0960 (online)

COPPER CONTENT IN CEREALS GROWN IN THE MODEL CONDITION

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ABSTRACT

The consumption of cereals in Slovakia but also worldwide is increasing by every year. From 30000 to 50000 tons of mercury circulates thought the biosphere that gets into the atmosphere degassing of the earth's crust and world oceans. Trace element as copper is one of the most efficient antioxidants in the body, often referred to as an element of beauty. It acts as a powerful catalyst for many enzymes and vitamins, through which already small amounts affects many activities in the body (strengthens immunity, reduces levels of histamine). It accumulates in the barley, beans, cucumber, nuts or milk and so on. The aim of our work was the evaluation of transfer of mercury from sludge to edible part of chosen cereals. The objectives were achieved in simulated conditions of growing pot experiment. We used agricultural soil from the location of Výčapy – Opatovce for the realization of the experiment. The sludge, which was added at various doses, was taken from Central Spiš area from locality of Rudňany near the village where minedironore that contains mainly cooper and mercury during last few decades was. We used three types of cereals: barley (*Hordeum sativum* L.) variety PRESTIGE, spring wheat (*Triticum aestivum* L.) variety ISJARISSA and oat (*Avena sativa* L.) variety TATRAN. The length of growing season was 90 days. From the obtained results of two years can be concluded that the accumulation of cooper by seed follows barley < oat < wheat. Even though that the barley is characterized by the highest accumulation of cooper in the seeds, the content did not exceed the maximum level specified by The Codex Alimentarius of the Slovak Republic (CA SR). The results shows that the suitable cultivation of the cereals in localities, which are contaminated with heavy metals, especially by cooper, that the high content of cooper in soil do not pose a risk of accumulation of the metal into the cereal grain.

Keywords: heavy metals; cereals; contamination; copper; seeds

INTRODUCTION

Soil pollution by heavy metals is a global environmental problem as it has affected about 235 million hectares of arable land worldwide **(Bermudez et al., 2012)**.

Copper is one of the most common metal contaminants in terrestrial surface ecosystems. It can originate from smelters and from the use of copper fungicides **(Helling et al., 2000)** or from the use of pig slurry as fertilizer. Copper is rarely the only heavy metal present in contaminated land. Most often, copper contamination occurs in combination with a mixture of other different heavy metals such as zinc, lead and cadmium **(Holmstrup et al., 2011)**.

The continuous use of fungicides has caused copper (Cu) accumulation in soils, which represent a major environmental and toxicological concern. Despite being an important micronutrient, Cu can be a potential toxicant at high concentrations since it may cause morphological, anatomical and physiological changes in plants, decreasing both food productivity and quality **(Brunneto et al., 2016)**.

Copper is a micronutrient necessary for normal plant growth and development; however, its deficiency and redundancy result in some defects in plant metabolism, especially photosynthesis. Plants are evolved to counterattack the adverse effects of copper by developing protective mechanisms, one of which is exclusion of copper ions from the cells by sequestration, which is a kind of isolation Cu from cellular components. The other way is reduction of ion uptake by roots. When the roots are exposed to excess copper, then detoxification strategies such as metal chelation and transport and activation of signal mechanisms, hormones, proteins, and antioxidant system are induced **(Salgam et al., 2016)**.

Excess Cu can affect important physiological processes in plants and cause problems in plant growth and development. Cu taken from the soil must be transported, distributed, and compartmentalized within different tissues and organelles for healthy plant growth and development **(Habiba et al., 2015)**. On the other hand, excessive Cu is characterized by a reduced plant biomass, leaf chlorosis, inhibited root growth, bronzing, and necrosis. The effect of Cu toxicity is largely on root growth and morphology, which has particular importance for the whole plant. Because water and nutrients enter plants by the roots, any defect or malformation of the roots creates problems for plant growth and development **(Marschner, 2011)**.

Generally, vegetable plants are more sensitive for metal toxicity than crop plants with lower toxicity thresholds **(Bo et al., 2015)**.

The cereals are probably the most important source of food for humans and feed for animals. Consequently, the low level of contamination can affect the health of consumers. Chemical contamination can occur from growing of cereals to their processing and storage **(Alldrick, 2012)**.

The cereals are the most common crops that are grown on arable land of EU. The fifty percent of cereal production in Southern Europe consists of wheat and then barley and maize. Other cereals as oat and rye are grown to a limited amount **(Finch et al., 2014)**.

Cereals contain various types of proteins, including water-soluble albumins, saline-soluble globulins, alcohol-soluble prolamins, and insoluble glutenins. The absolute and relative amounts of these protein types vary considerably among cereals, as do their digestibility and immunogenicity. The prevalence of allergies to cereals is low, although no exact frequencies are known. Most commonly mentioned, and described in this review, are allergies to wheat, maize and rice, of which occupational bakers' asthma has the highest economic impact **(Gilissen, Van der Meer and Smulders, 2014)**.

All cereals are strachy foods and contain protein that does not meet the essential aminoacid balance required by growing infants. They are considered a good source of energy, most B vitamins, and dietary fiber when consumed as whole grains **(Serna Saldivar, 2016)**.

The cereals and cereal products are the main sources of carbohydrates in food for humans and feed for animals. Cereal grains are an important source of energy and nutrients in the form of protein, fat, fiber, minerals and vitamins **(Beverly, 2014)**.

The cereals are as well as the most important source of fructans in our daily diet. Nowadays are hotly discussed and compared a lot of different cereals in the terms of fructans structure. Their degradation during processing of food is considered as a potential health benefit. Recent published data suggest that they may also have a prebiotic effect **(Verspreeta et al., 2015)**.

The cereals and cereal bran obtained a significant position as a functional food. They are a source of carbohydrates (arabinoxylan, beta – glucan), phenolic acids (ferulic acid), flavonoids (anthocyanins), oil (γ-oryzanol), vitamins, carotenoids, folates and sterols. Their physico – chemical properties makes them a necesssary ingredient for food fortification. The bran of rice, wheat, oat, barley, millet, rye and corn contain a huge amount of health-promoting ingredients. The anti-atherogenic, anti-hypertensive and hypoglycemic properties were verified. Further, it was found the effect against oxidative stress. They reduce insulin resistance prevent the risk of obesity by inducing the feel of fullness **(Patel, 2015)**.

Recent large-scale epidemiological studies have shown that regular consumption of whole grain cereals can reduce the risk of heart disease and certain cancers by 30 percent. One of the factors that increase the functionality of foods is theso-called in digestible resistant starch **(Duchoňová and Šturdík, 2010)**.

MATERIAL AND METHODOLOGY

The aim of our work was the evaluation of transfer of mercury from sludge to edible part of chosen cereals. The objectives were achieved in simulated conditions of growing pot experiment. We used agricultural soil from the location of Výčapy – Opatovce for the realization of the experiment. The sludge, which was added at various doses, was taken from Central Spiš area from locality of Rudňany near the village where was mined iron ore that contains mainly copper and mercury during last few decades. We used three types' of cereals: barley (*Hordeum sativum L.*) variety PRESTIGE, spring wheat (*Triticum aestivum L.*), variety ISJARISSA and oat (*Avena sativa L.*) variety TATRAN. The length of growing season was 90 days.

Before the establishment growing pot experiment we performed all necessary analyses in soil and sludge. We determined soil reaction, content of nitrogen by Kjeldahl method, phosphorus content, potassium and magnesium contentby Mehlich II solution. Subsequently, we determined the content of heavy metals in the acid mixture HNO_3 and HCl (decomposition by aqua regia) by AA Swith Varian AA240FS (Australia). For analysis of each element, we used the multi-element standard Sigma Aldrich (Germany).

To every one of all tested pots was weighed 5 kg of soil with 1 kg of silica sand, while the bottom of the container was filled with a small drainage layer of gravel. In each pot was applied the calculated dose of sludge.

Table 1 Variants of the experiments.

variants	
A	soil 100 %
B	soil 90 %, sludge 10%
C	soil 80 %, sludge 20 %
D	soil 70 %, sludge 30 %

Determination of heavy metals (by AAS) on the device VARIAN AA 240FS

By analytical weight were weighted 1 – 2 g samples of dried vegetables. Mineralization of samples was in a mixture of distilled water with concentrated nitric acid in a ratio 1:1 .The weighed samples were put into teflon vessels with 5 cm^3 of distilled water with 5 cm^3 of concentrated nitric acid. Closed vessels with a samples were mineralized by microwave digestion unit MARS X-press (USA).

After mineralization were analyzed samples filtered through quantitative filter paper MUNKTELL (Germany) grade 390.84 $g.m^{-2}$ (green) to volumetric flasks (50 cm^3).

Flasks were refilled with distilled water to the mark and after that was the determination of heavy metals by VARIAN AA 240FS (Australia) under the conditions:

Table 2 The process of mineralization – time, temperature (total time 55 minutes).

Stage	Power (W)	Power (%)	Initialization Time (min)	Temp. (°C)	Duration time (min)
Initialization	800	90	15	160	0
Mineralization	800	90	0	160	20
Cooling	–	–	–	–	20

Cd – detection limit - 0.001 $mg.L^{-1}$, sensitivity 0.01 $mg.L^{-1}$
Pb – detection limit - 0.02 $mg.L^{-1}$, sensitivity 0.1 $mg.L^{-1}$
Cu – detection limit - 0.002 $mg.L^{-1}$, sensitivity 0.03 $mg.L^{-1}$
Zn – detection limit - 0.006 $mg.L^{-1}$, sensitivity 0.008 $mg.L^{-1}$
Co – detection limit - 0.005 $mg.L^{-1}$, sensitivity 0.05 $mg.L^{-1}$
Cr – detection limit - 0.003 $mg.L^{-1}$, sensitivity 0.04 $mg.L^{-1}$
Ni – detection limit - 0.008 $mg.L^{-1}$, sensitivity 0.06 $mg.L^{-1}$
Mn – detection limit - 0.003 $mg.L^{-1}$, sensitivity 0.02 $mg.L^{-1}$
Fe – detection limit - 0.005 $mg.L^{-1}$, sensitivity 0.04 $mg.L^{-1}$

Analysis determination has not a deviation more than 3%, the gas flow: air: 13.5 $L.min^{-1}$, acetylene 2.0 $L.min^{-1}$.

For statistical evaluation of results was used a statistical program STATISTICA 6.0 Cz. The results tested on the level of descriptive statistical evaluation, and overall visual indication of the level factor, variability and deviations were expressed as text. We used T-test at the confidence level $p \leq 0.05$.

Table 3 The contents of heavy metals in the soil (decomposition by aqua regia) and comparison of Slovak decree No. 220/2004 Coll. ($mg.kg^{-1}$).

	Cd	**Pb**	**Cu**	**Zn**	**Cr**	**Ni**
soil	0.70	18.2	18.4	55.6	17.2	30.6
220/2004	0.70	70	60	150	70	50

RESULTS AND DISCUSSION

Soil from the locality of Výčapy – Opatovce has not alkaline soil reaction with medium level of humidity. It is characterized by good content of phosphorus and potassium and a high content of magnesium. The contents of heavy metals do not exceed the limit values (Slovak decree No. 220/2004 Coll.).

Sludge from the locality of Rudňany has a strongly alkaline soil reaction. It is characterized by a very low content of phosphorus and potassium. Mercury content (520 $mg.kg^{-1}$) does not exceed the maximum permissible amount (Act No. 188/2003 Coll.).

The highest content of copper in the grain of barley during years 2014 and 2015 was in D Variant. Highest content of copper was 6.60 $mg.kg^{-1}$.

Copper content in seeds of barley in D variant increased 1.5 times in 2014 compared to variant A (soil without the addition of sludge). Increasing amount of sludge that was added into the soil is proportionally reflected in mercury content in seeds of barley.

The content of copper in seeds of barley in all variants was exceeded the maximum levels permitted under Codex Alimentarius of the Slovak Republic.

A statistically significant difference between 2014 and 2015 in copper content in seeds of barley in all variants was not determined.

The highest content of copper in seeds of wheat was in D variant in 2015, where the Cu content in seeds was almost 1.5 times higher than in A variant.

Increasing amount of sludge that was added to the soil is

Table 4 Copper content in the seeds of spring barley variety Prestige in 2014 and 2015 ($mg.kg^{-1}$) and the comparison of copper content with Codex Alimentaria of the Slovak Republic (CA SR), median n = 4.

	BARLEY	
variants	**2014**	**2015**
A	4.20	4.05
B	5.30	5.70
C	5.45	5.75
D	6.60	5.75
CA SR	**10**	**10**

Table 5 Copper content in seeds of wheat variety Jarissa in 2014 and 2015 ($mg.kg^{-1}$) and the comparison of copper content with Codex Alimentarius of the Slovak Republic (CA SR), median n = 4.

	WHEAT	
variants	**2014**	**2015**
A	2.50	3.00
B	3.25	4.05
C	3.45	4.10
D	3.60	4.40
CA SR	**10**	**10**

Table 6 Copper content in seeds of oat variety Tatran in 2014 and 2015 ($mg.kg^{-1}$) and the comparison of copper content with Codex Alimentaria of the Slovak Republic (CA SR) median n = 4.

	OAT	
variants	**2014**	**2015**
A	3.50	2.45
B	4.55	4.10
C	4.75	4.50
D	5.40	5.45
CA SR	**10**	**10**

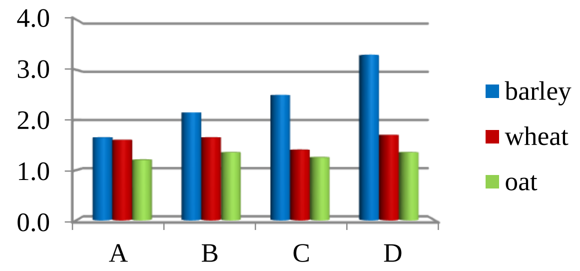

Figure 1 Copper content in aboveground biomass of various cereals in all variants ($mg.kg^{-1}$) that were grown in 2014.

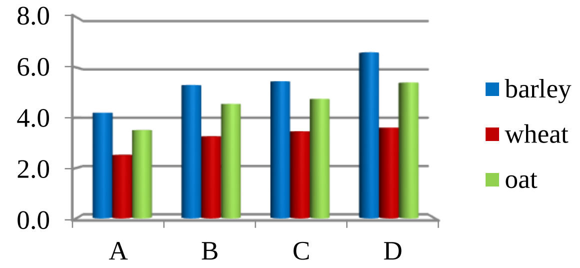

Figure 2 Copper content in seeds of various cereals in all variants ($mg.kg^{-1}$) that were grown in 2014.

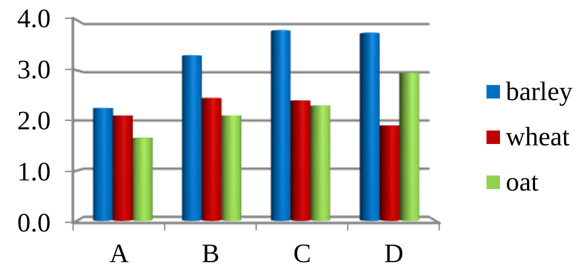

Figure 3 Copper content in aboveground biomass of various cereals in all variants ($mg.kg^{-1}$) that were grown in 2015.

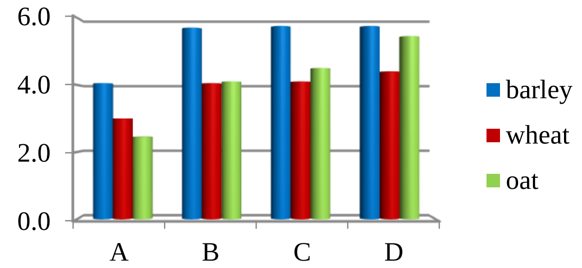

Figure 4 Copper content in seeds of various cereals in all variants ($mg.kg^{-1}$) that were grown in 2015.

proportionally reflected in mercury content in the seeds of wheat. The difference between the highest copper content in seeds of wheat in D variant compared to years 2014 and 2015 was similar as in the case of barley.

The copper content in seeds of wheat in all variants was not exceeded the maximum levels permitted under Codex Alimentarius of the Slovak Republic.

A statistically significant difference between 2014 and 2015 in copper content in seeds of wheat was determined in all variants.

Copper content in oat seeds in D variant (highest addition of sludge) was 2.2 times higher than in A variant in 2015. Compared to 2014, the content of copper in the seeds was almost identical.

The copper content in seeds oat was exceeded the maximum levels permitted under Codex Alimentarius of the Slovak Republic.

In all variants was not a statistically significant difference of copper content in seeds of oat between harvested biomass in years 2014 and 2015.

The following figures show the comparison of copper content in aboveground biomass and in seeds of commodities in different variants.

Increasing of copper content in different variants in the aboveground biomass was determined only in samples of barley. The highest copper content of aboveground biomass was in barley variety PRESTIGE in D variant with value of 3.3 $mg.kg^{-1}$. **Demirevska-Kepovaa et al. (2004)** found the copper content in leaves of barley 20 ±4.33 $mg.kg^{-1}$, which compared with our results of five times greater.

Comparing the copper content of individual variants in biomass of oat and wheat was not significant changes. The lowest accumulation of copper in samples was in aboveground biomass of oat variety TATRAN with value of 1.35 $mg.kg^{-1}$. The copper content in the biomass of oat was 9.5 ±0.3 $mg.kg^{-1}$ in uncontaminated soil **(Moustakas et al., 1994)**.

The copper content in aboveground biomass of cereals in 2014 was in the order barley < wheat < oat.

The graph 2 shows that the copper content of seeds of cereals in B, C and D variants increases with increasing addition of sludge into the soil.

The smallest accumulation of copper by seeds was in spring wheat variety IS JARISSA (2.5 $mg.kg^{-1}$), which is more than 6 times lower than the value measured **Quartacci et al. (2000)**.

In a D variant was 3.6 $mg.kg^{-1}$ of copper content, which is almost half of the amount than was accumulated by barley seeds of variety PRESTIGE (6.6 $mg.kg^{-1}$). **Antolín et al. (2005)** measured copper content in the seeds of barley 3.5 – 4.3 $mg.kg^{-1}$, depending on the addition of the sludge into the soil.

Increasing of copper in different variants in aboveground biomass was determined in samples of barley and oat.

The aboveground biomass of wheat was measured the smallest amount of copper accumulated in D variant with value of 1.9 $mg.kg^{-1}$.

The highest copper content was measured in barley biomass (3.75 $mg.kg^{-1}$) which is almost two times more than copper content in biomass of wheat.

Comparing the results of copper that was accumulated in aboveground biomass and grains of selected crops in charts, it can be concluded that cereal grains have a higher accumulation of copper as their aboveground biomass.

The copper content in biomass of selected cereals was in 2015 in the order as follows barley < oat < wheat.

In Figure 4 is similar situation as in Figure 2, thus increasing doses of sludge into soil and it is also reflected by increasing copper content in seeds of all selected varieties of crops.

The copper content in the seeds oat was 2.45mg.kg^{-1}. **Reith (2009)** measured the copper content in oats 1.1 mg.kg^{-1}, which is half as much as us found quantities.

From the obtained results of two years can be concluded that the accumulation of cooper by seed follows barley < oat < wheat.

Increased copper content in soil of all variants was caused by addition of sludge and had a statistically significant effect on copper content of seeds in all variants of each crop in the level of p <0.05.

The additions of sludge into soil have a statistically significant effect on copper content in biomass of selected varieties of all crops in the level of p <0.05.

CONCLUSION

The results showed that the amount of sludge added in specified amounts into soil increases mercury content in seeds of crops. Although barley was characterized by the highest accumulation of mercury in the seeds. Mercury content was not exceeded the maximum permissible amount permitted under Codex Alimentarius of the SR. From the obtained results in 2014 and 2015 can be concluded that the accumulation of cooper by seeds is in the order barley < oat < wheat. The results showed that the suitable cultivation of the cereals in localities, that are contaminated with heavy metals, especially by cooper with high content of this metal in soil do not pose a risk of accumulation of contaminant in grains. Increasing number of risk metals in soil leads to an increasing content of these metals in crops and subsequently in animal products. This may have adverse effects on people who consume these products.

There are two main reasons why the contamination of the environment with heavy metals causes concern. First, it can reduce the productivity of plants used as human food and animal feed. Second, it affects the quality of agricultural products.

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Acknowledgments:

This work was supported by grant KEGA No. 015SPU-4/2016.

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