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# THE CONTENT OF POLYPHENOLS AND CHOSEN HEAVY METALS IN CHICKPEA SEEDS (*CICER ARIETINUM* L.) AFTER MICRONUTRIENTS APPLICATION INTO SOIL

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**ABSTRACT**

The aim of work was the investigation of the input of chosen heavy metals into chickpea and polyphenols accumulation in seeds cultivated after Zn and Cu application into soil in model conditions. The soil used in the pot trial was uncontaminated. Chickpea (cv. Slovák) was harvested in full ripeness. With increased Zn (Cu) doses applied into the soil (0-500 and 0-100 mg.kg$^{-1}$ for Zn and Cu, respectively), the strong statistical positive relationship between soil Zn (Cu) content and Zn (Cu) amount in seeds of chickpea was confirmed. Despite of high Zn (Cu) doses applied into the soil in model conditions, in all variants the determined Zn and Cu amount in chickpea was below the maximal allowed content in foodstuffs. Chickpea cv. Slovák accumulated high amounts of Pb and Cd (in all variants higher than hygienic limits). The contents of other tested heavy metals (Zn, Cu, Ni, Cr) were lower than hygienic limits. With increased Zn doses applied into the soil the polyphenols contents determined in seeds of chickpea were increased. With increased Cu doses applied into the soil the polyphenols contents determined in seeds of chickpea were decreased. The strong statistical relationship between soil Zn content and polyphenols amount (R=0.911) as well as the antioxidant capacity values (R=0.992) was confirmed. The moderate statistical linear negative relationship between soil Cu content and polyphenols content (R= - 0.671) and increased antioxidant capacity values in seeds of chickpea was confirmed.

**Keywords:** chickpea, metal contamination, zinc, copper

## INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an annual grain legume that is extensively cultivated for human consumption. Chickpea is a good source of carbohydrates (low glycaemic index) and protein, together constituting about 80% of the total dry seed mass **(Chibbar *et al.*, 2010)** in comparison to other pulses. Chickpea also contains important minerals (i.e. Ca, Mg, Zn, K, Fe and P) and vitamins (i.e. thiamine and niacin) **(Zia-Ul-Haq *et al.*, 2007)**, and is relatively free from antinutritional factors **(Muzquiz, Wood, 2007)**. Chickpea, similar to other legume seeds, must be soaked and/or cooked before consumption, a procedure that improves the flavor and palatability of the food product and increases its nutritional bioavailability by inactivaving antinutritional factors **(Chau *et al.*, 1997)**. Seeds of chickpea are helpful source of zinc and folate. Zinc is the main micronutrient in the soil that limits chickpea productivity **(Ahlawat *et al.*, 2007).** In general, each tonne of chickpea grain removes 38 g of Zn from the soil **(Ahlawat *et al.*, 2007)**. Among micronutrients, Zn deficiency is perhaps the most widespread **(Roy *et al.*, 2006)** and is common among chickpea-growing regions of the world.

The antioxidant capacity and antiproliferative effects of legumes are associated with the presence of phenolic compounds **(Dong *et al.*, 2007)**. Phenolic cpmpounds are known to exhibit a range of biological activities, including antibacterial, antioxidant and antiinflammatory properties **(Kamatou *et al.*, 2010)**. In studies with chickpea it was found that isolated hulls from a colored chickpea line contain large amounts of polyphenols and flavonoid compounds that exhibit high levels of antioxidant activity **(Segev *et al.*, 2010)**. On the other hand, common chickpea varieties with beige-colored seeds have low levels of total phenolic content, total flavonoid content and antioxidant activity determined by ferric reducing ability of the plasma **(Segev *et al.*, 2010)**. This variation in both seed coat color and antioxidant activity maked colored chickpea a strong potential model for studies of functional foods. Chickpea also contains isoflavones such as daidzein and genistein. In addition, high levels of genistein hexoside were detected recently in raw chickpea flours **(Aguilera *et al.*, 2011)**.

In our work, chosen micronutrients zinc and copper were added to the soil to reduce the intake of other heavy metals, especially of Cd or Pb - these toxic metals are present in the soil above the hygienic limit on most territory of the Slovak Republic - as well as to its consecutive accumulation in chickpea seeds and influence on total polyphenols creation and antioxidant activity of chickpea.

## MATERIALS AND METHODS

### Plant material

The cultivar of chickpea (*Cicer arietinum* L.) (cv. Slovak) was harvested in full ripeness and obtained from the Research Centre of Plant Production in Piešťany (Slovakia).

### Soils samples

In the pot experiments the soil from locality Cakajovce (Slovak village located near to Nitra) was used. The soil was characterized by low supply of humus and the neutral soil reaction suitable for the legume cultivation. The used soil was characterized also by high content of potassium and phosphorus as well as by a very high content of magnesium. The soil used in the pot trial was uncontaminated. Only determined Cd content was on the level of limit value given by **Law N$^o$ 220/2004** (valid in the Slovak Republic) for the soil extract by aqua regia as well as Pb content on the level of critical value given by **Law N$^o$ 220/2004** (valid in the Slovak Republic) for the relationship between soil and plant. The values were far below threshold values proposed.

### The experiment

The experiment was based on four replications in each variant. Five kilograms of soil was thoroughly mixed with a sand (1kg) and togheter were submitted into plastic bowl-shaped pots with foraminated bottom (average: 20 cm and height: 25 cm). The solution of $ZnSO_4.7H_2O$ was added to each pot to correspond to 0 (control, A variant), 40 (B variant), 250 (C variant), and 500 (D variant) mg Zn.kg$^{-1}$ of soil.

The solution of $CuSO_4.5H_2O$ was added to each pot to correspond to 0 (control, A variant), 20 (B variant), 50 (C variant), and 100 (D variant) mg Cu.kg$^{-1}$ of soil. Individual doses were chosen by Decision of the Ministry of Agriculture of Slovak Republic Nº531/1994-540 about the maximum exposure levels of risky compounds in the soil – hygienic limit (B variant), half dose of analytically significant contamination (C variant), analytically significant contamination (D variant).

**Analytical methods**

The chickpea seeds were decomposited with using of $HNO_3$ by microwave digestion in instrument MARS X-PRESS. The solutions were analyzed by flame AAS (AAS Varian AA Spectr DUO 240 FS/240Z/UltrAA). Gained results in mg.kg$^{-1}$ of dry mater were evaluated according to the **Food Codex of the Slovakia** valid in the Slovak Republic as well as according to **Commission Regulation 1881/2006**.

***Total polyphenols***

Total polyphenol content (TP) was determined using Folin-Ciocalteau reagent (FCR) according to **(Lachman *et al*., 2003)**. The solutions were measured at 765 nm using Shimadzu spectrophotometer (710, Shimadzu, Kyoto, Japan). The total polyphenols content was calculated as gallic acid equivalents (GAE) in milligrams per kilogram of dry matter.

***The antioxidant activity*** (AA) was determined using free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to **(Brand-Williams *et al*., 1995)**. The solutions were measured at 515 nm in a Shimadzu spectrophotometer (Shimadzu, Kyoto, Japan). Results were calculated as % inhibition of DPPH.

***Statistics***

All statistical analyses were carried out using the statistical software Statistica 12.0 (Statsoft, USA). Each analysis was done in six repetitions. Descriptive data analysis included mean, standard deviation. Mean comparisons between heavy metals content were done by the LSD-test, $P<0.05$.

**RESULTS AND DISCUSSION**

Two experiments were realised as the pot trials in the vegetation cage with the aims to investigate the effect of addition of increasing rates of selected micronutrients (zinc, copper) into the soil and its consecutive accumulation in chickpea seeds on total polyphenols and antioxidant activity.

**The evaluation of heavy metals content**

The graded Zn doses applied into the soil in the model conditions resulted in increased Zn content in seeds of chickpea harvested in the stage of full ripeness are presented in Table 1. The strong statistical relationship between soil Zn content and Zn amount in seeds of chickpea was confirmed (R= 0.889). In all variants the Zn content in seeds of chickpea was lower than hygienic limit. The determined Pb and Cd contents in control seeds were higher than maximal allowed amounts given by the legislative (by 1200% and 90%, respectively). The Zn, Cu, Pb and Ni contents were increased, the Cd content was decreased and the Cr content was only slightly changed. The strong positive statistical relationship between soil Zn content and Cu, Pb and Ni amount in seeds of chickpea was confirmed (R= 0.799, R= 0.880, R= 0.951, respectively). The negative linear statistical relationship between soil Zn content and Cd amount in seeds of chickpea was confirmed (R= - 0.774). Despite of decreased Cd content even in D variant the determined Cd amount in chickpea seeds was by 20% higher than hygienic limit.

**Table 1** Heavy metals contents (mg.kg$^{-1}$) in seeds of chickpea cv. Slovak after Zn application into soil

| Var. | Zn | Cu | Ni | Cr | Pb | Cd |
|---|---|---|---|---|---|---|
| A | 31.63 (1.61) | 6.43 (1.38) | 0.70 (0.14) | 0.93 (0.11) | 2.58 (0.20) | 0.19 (0.05) |
| B | 35.60 (0.16) | 5.05 (1.76) | 0.95 (0.36) | 0.80 (0.07) | 2.50 (0.08) | 0.14 (0.04) |
| C | 47.85 (3.55) | 9.65 (1.45) | 1.45 (0.17) | 1.10 (0.14) | 3.00 (0.15) | 0.14 (0.06) |
| D | 49.08 (0.84) | 9.15 (0.57) | 1.65 (0.07) | 0.90 (0.22) | 3.00 (0.22) | 0.12 (0.02) |
| Limit | 50.0 | 15.0 | 3.0 | 4.0 | 0.2 | 0.1 |
| Max. | - | - | - | - | 0.2 | 0.1 |

**Legend:** The results show the mean value and the standard deviation (in parenthesis). Limit value for legumes according to the Food Codex of the Slovakia was shown. Max. value is maximal residue level according to Commission Regulation 1881/2006.

**Wang *et al.* (2010)** determined lower amounts of Cu and a similar Zn content in comparison to our results. By **Khan *et al.* (1998)** observed also zinc concentration in the shoot tissue of chickpea increased with an increase in Zn fertilisation and they confirm that low and high moisture regimes had no effect on critical Zn concentration. **Rutkowska *et al.* (2014)** reported the concentration of active zinc ions ($Zn^{2+}$) in the soil solution for the most part relies upon soil reaction when compared with other investigated physical and chemical soil properties. Higher Zn concentration can be projected in the soil solution of acidic soils when compared to soils with neutral soil reaction.
The graded Cu doses which have been applied to the soil in the model conditions resulted in increased Cu content in seeds of chickpea harvested in the stage of full ripeness are presented in Table 2. The strong statistical relationship between Cu content in the soil and Cu content in seeds of chickpea was confirmed (R= 0.963). In all variants the Cu content in seeds of chickpea was lower than the legislative determined limit value. In control variant only the established Pb content was by 125% higher than maximal allowed amounts given by the legislative regulation. In variants with Cu application the Cu, Ni, Cr and Cd contents were increased, whereas the Zn and Pb contents were decreased and the Zn content was only slightly changed. In D variant (with the highest Cu dose into the soil) the Cd content in chickpea seeds was by 50% higher than the hygienic limit. The positive statistical relationship between soil Cu content and Ni, Cr and Cd amount in seeds of chickpea was confirmed (R= 0.600, R= 0.822, R= 0.980, respectively). The negative statistical relationship between soil Cu content and Pb amount in seeds of chickpea was confirmed (R= - 0.706).

**Table 2** Heavy metals contents (mg.kg$^{-1}$) in seeds of chickpea cv. Slovak after Cu application into soil

| Var. | Zn | Cu | Ni | Cr | Pb | Cd |
|---|---|---|---|---|---|---|
| A | 37.50 (0.12) | 7.35 (0.11) | 1.08 (0.08) | 1.23 (0.13) | 0.45 (0.05) | 0.05 (0.01) |
| B | 37.05 (0.11) | 8.60 (0.10) | 1.63 (0.04) | 1.10 (0.07) | 0.35 (0.11) | 0.04 (0.01) |
| C | 37.88 (0.15) | 9.05 (0.17) | 2.13 (0.23) | 1.23 (0.08) | 0.25 (0.05) | 0.10 (0.01) |
| D | 35.10 (0.12) | 8.85 (0.11) | 1.75 (0.05) | 1.45 (0.11) | 0.30 (0.07) | 0.15 (0,03) |
| Limit | 50.0 | 15.0 | 3.0 | 4.0 | 0.2 | 0.1 |
| Max. | - | - | - | - | 0.2 | 0.1 |

**Legend:** The results show the mean value and the standard deviation (in parenthesis). Limit value for legumes according to the Food Codex of the Slovakia was shown. Max. value is maximal residue level according to Commission Regulation 1881/2006.

The determined contents of Cu, Pb and Zn by **Salama, Radwan (2005)** in lentil and chickpea seeds were many times lower than those determined in our cultivar, and only Cd content determined by these authors was similar to our findings. **Głowacka (2014)** observed an interspecific facilitation between neighbouring plant species. Placement adjacent to the oat strip contributed to higher Cu content in the maize, while placement next to blue lupin increased the content of Fe and Zn. This makes it possible to eliminate or mitigate mineral deficiencies in the plants.

**The total polyphenols content and antioxidant activity evaluation**

Total polyphenols (TP) content and antioxidant activity (AA) determined in chickpea cv. Slovak harvested in the stage of full ripeness are presented in Table 3. The determined values of total polyphenol content ranged from 393 mg $GAE.kg^{-1}$ (Cu application) to 1215 mg $GAE.kg^{-1}$ (Zn application). The determined values of antioxidant activity (as DPPH inhibition) were in interval 4.33% (Cu application) - 11.15 % (Zn application).

**Table 3** Total polyphenols content ($mg.kg^{-1}$) and antioxidant activity (% of DPPH inhibition)

| Variant | Total polyphenol content | | Antioxidant activity | |
|---|---|---|---|---|
| | Zn application | Cu application | Zn application | Cu application |
| A | 742±10$^{a}$ | 672±10$^{a}$ | 10.32±1.60$^{a}$ | 4.80±0.90$^{a}$ |
| B | 1107±58$^{b}$ | 566±31$^{b}$ | 11.15±1.07$^{b}$ | 5.83±0.39$^{b}$ |
| C | 1215±46$^{c}$ | 574±66$^{b}$ | 9.33±1.04$^{c}$ | 4.93±0.80$^{a}$ |
| D | 661±24$^{d}$ | 393±12$^{c}$ | 5.77±1.50$^{d}$ | 4.33±0.53$^{c}$ |

**Legend:** The results show the mean ± the standard deviation. Values in the same column with different letters present significant differences $p < 0.05$.

With increased Zn doses applied into the soil the TP contents established in seeds of chickpea cv. Slovak were increased. The maximal TP content in seeds of chickpea seeds harvested in the stage of full ripeness was found at 250-300 mg Zn applied into 1 kg of the soil while after application of higher Zn doses into the soil a lower TP content in seeds of this legume was observed. The strong statistical relationship was confirmed between Zn content in the soil and TP content (R= 0.911) as well as AA values (R = 0.992) in chickpea seeds.
With increased Cu doses applied into the soil the TP contents determined in seeds of chickpea cv. Slovak were decreased. The moderate statistical linear negative relationship between soil Cu content and TP content in seeds of chickpea cv. Slovak was confirmed (R = -0.671).
With increased Cu doses applied into the soil the AA values determined in seeds of chickpea cv. Slovak were only slightly changed. The moderate statistical linear positive relationship between soil Cu content and AA value in seeds of chickpea cv. Slovak was confirmed (R = 0.621).
Our values of polyphenols and antioxidant activity are although much lower than reported by **Xu, Chang (2007**). However, they are in partial agreement with other authors **(Xu, Chang, 2008; Zia-Ul-Haq *et al*., 2008)**. The differences between results may be attributed to the differences in the sources of the samples.

## CONCLUSION

The graded Zn doses applied into the soil in the model conditions resulted in increased Zn content in seeds of chickpea harvested in the stage of full ripeness. The strong statistical relationship between soil Zn content and Zn level in seeds of investigated chickpea cv. Slovak was confirmed.
With increased Cu doses applied into the soil the Cu contents determined in the fully rippened seeds of chickpea cv. Slovak were increased. The strong statistical relationship between soil Cu content and Cu amount in seeds was confirmed.
Graded Zn doses applied into the soil resulted in statistically significant increased Pb amounts and significantly decreased Cd amount in seeds of chickpea cv. Slovak, whereas inverse relationship for graded Cu doses applied into the soil and those elements was observed (decreased Pb amounts, increased Cd amount). The determined Pb and Cd contents in seeds were higher than maximal allowed amounts given by the legislative. The contents of other tested heavy metals (Zn, Cu, Ni, Cr) were lower than hygienic limits.
In experiment conditions, the total polyphenols contents determined in seeds chickpea cv. Slovak harvested in the stage of full ripeness were increased with soil enrichment with Zn. Moreover, the strong statistical correlation between the content of Zn added into the soil and the antioxidant activity values was confirmed.
Graded Cu doses applied into the soil resulted in increased AA values in seeds of chickpea cv. Slovak harvested in the stage of full ripeness, whereas inverse relationship for TP content was observed.

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