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GENOTYPE SPECIFICITY OF WINTER WHEAT IN ZINC AND COOPER ACCUMULATION IN GRAIN

SUMMARY
To ensure safe food production, cultivar specificity of winter wheat with respect to mineral nutrition was studied in order to determine genotypic differences in the accumulation of certain microelements (Zn and Cu). Soil properties, plant species and genotypes were found to be major factors affecting the uptake of microelements by wheat plants. NPK and lime treatments resulted in a decrease in Zn content, but the decrease was not below the concentrations that would make the plants suffer from nutrient deficiencies. Conversely, as regards Cu in NPK + CaCO₃ and NPK + CaCO₃ + manure treatments, Cu deficiency can lead to certain morphological and anatomical changes in some cultivars.

Keywords: microelements, wheat, accumulation, cultivar specificity

INTRODUCTION
Wheat is the staple food of more than half of the world’s population. The nutritive value of grain makes it the most important farm product used in the diet of more than half of the world’s population. Therefore, wheat grain should contain sufficient levels of microelements (Fe, Mn, Zn and Cu).

Metals have an impact on human health in many ways. Some elements, such as Cu, Mn, and Zn, are essential micronutrients with a human requirement of no more than a few milligrams per day. However, micronutrients may become harmful when their ingestion rates are too high. Deficiencies, excesses, or imbalances in the supply of inorganic elements from dietary sources can have an important deleterious influence on human health (Škrbić and Onjia, 2007; WHO, 1996).

The amount of element ingested by human is directly related to alimentary habits and their content in foodstuffs. Microelement concentrations in foodstuffs depend on soil characteristics, such as content of organic matter, pH, and clay mineralogy, which can affect the bioavailability of elements. Besides

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Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.
environmental pollution, a matter of concern is the addition of chemical products as fertilizers, fungicides, insecticides and herbicides to crops.

In the last few decades cereal products, and among them wheat-based products, have received considerable attention in view of their potential role in transport of toxic microelements into the human diet.

Since 1973, As, Cu, Cd, Fe, Hg, Pb, and Zn have been considered by the joint FAO/WHO Codex Alimentarius Commission to be potentially toxic for human diet, with As (as arsenite) being carcinogenic (Ybanez & Montoro, 1996).

Genotype as an autonomous biological and agronomic entity is among crucial factors in both quantitative and qualitative terms. In other words, the yield and quality of wheat grain produced are highly cultivar dependent. Cultivar as a factor of critical importance has been dealt with by many authors (Borojević, 1983; Mladenov et al., 2007; Denčić et al., 2003, 2007, 2010).

The knowledge of element concentrations in wheat, as well as in other cereals, can provide important information on the impact of the use of chemical products in crops and on levels of the environmental pollution.

**MATERIAL AND METHODS**

Research was conducted at the experimental field of the Small Grains Research Centre in Kragujevac on very acidic soil (pH in KCl=4.26) involving six wheat cultivars: 'Takovčanka', 'Studenica', 'KG-56', 'Lazarica', 'KG-100' and 'Pobeda'. The following treatments were applied: unfertilised Control; N (75 kg/ha) + CaCO₃ (2 t/ha); NPK (500 kg/ha) + CaCO₃ (2 t/ha); NPK (500 kg/ha) + CaCO₃, (2 t/ha) + cattle manure (35 t/ha).

Plants were harvested at full maturity, the grain was separated from the straw and grain microelement concentration was determined by AAS method after dry burning of plant samples.

**RESULTS AND DISCUSSION**

Through its biological, physical and chemical properties, soil affects plant growth and development and, hence, plant productivity and yield. Arable lands in Serbia are increasingly becoming acid, resulting in poor productivity and a limited choice of field crops to be grown. Since the present research was conducted on an acidic soil, it focused on detailed monitoring of the effects of fertilisers on some major chemical indicators of soil fertility (Table 1) and microelement uptake by wheat plants.

The research was conducted on very acid soil (pH in water-5.61; pH in KCl-4.20). The combined use of NPK, nitrogen and lime prevented further acidification and degradation of this soil. Treatments with NPK and lime, and NPK, manure and lime reduced the soil active and substitutional acidity. The highest decrease in acidity i.e. increase in pH was found in the combined treatment with mineral, organic and lime fertilisers. In this treatment, soil pH increased by 0.24 units (in H₂O) i.e. by 0.49 pH units in KCl, in the 0-20 cm soil layer. The soil used for the research had low levels of humus and readily
available phosphorus, but fertilisation had a positive effect in increasing this parameter.

Table 1. The effects of fertilizers on chemical properties of the soil

| Soil properties | Fertilisation variants |
|-----------------|------------------------|
|                 | Control | NPK | N+CaCO₃ | NPK+CaCO₃ | NPK+CaCO₃+manure |
| pH (H₂O)        | 5.61    | 5.61| 5.61    | 5.75      | 5.85            |
| pH (KCl)        | 4.20    | 4.20| 4.20    | 4.40      | 4.69            |
| Humus %         | 2.17    | 2.41| 1.85    | 2.87      | 3.45            |
| Azot %          | 0.12    | 1.15| 0.13    | 0.15      | 0.17            |
| P₂O₅ mg/100g    | 1.3     | 1.6 | 1.4     | 3.5       | 5.8             |
| K₂O mg/100g     | 22.4    | 28.5| 26.0    | 30.2      | 31.2            |

Zinc in wheat grain. Zinc is an essential element which, if accumulated in elevated amounts, may become toxic. Zinc plays a very important role in plant growth and development, it is involved in many enzymatic reactions and is vital for chlorophyll and carbohydrate synthesis. Its deficiency results in reduced grain yield and quality. Moreover, zinc is important in human nutrition. Zinc is a trace element in the human body, but importantly, it occurs in almost all cells in the body. Zinc interacts with more than 100 enzymes, taking part in the metabolism of protein, carbohydrates, fats and nucleic acids and strengthening the immune system. Noteworthy, zinc contributes to the development of T-lymphocytes that prevent infections. Zn deficiency is manifested through a variety of health problems which can be lethal if left untreated.

The results (Table 2.) showed significant variation in grain Zn content in all winter wheat genotypes analysed. Zinc content of plants is dependent on the biological properties, soil characteristic of the plant and the content of available forms of this element in the soil. Plant species and genotypes within the species exhibit significant differences in Zn accumulation and uptake. Eid et al. (1996) determined that IRT1 membrane proteins are relatively specialised and can serve for Zn transport, and ZIP proteins in cereals play an important role in Zn uptake.

As shown by the analysis of variance (LSD-test), grain Zn content was the highest in ‘KG-56’ and lowest in ‘Takovčanka’.

The comparison between the control and the fertilisation treatments revealed that the Zn content of winter wheat grain was affected by mineral nutrition. The highest content was obtained in the control (pH=4.2 in 1N KCl) i.e. on very acid soils, and ranged from 26.00 to 39.9 mg/kg, giving an average of 34.78 mg/kg. These values are considered normal for most food crops (Kabata-Pendias, 2011). As determined by previous research, grain Zn content of wheat grown in Serbia ranged from 26.6 to 43.3 mg/kg, averaging 32.0 mg/kg (Škrbić and Onjia, 2007).

The phosphorus applied through NPK fertiliser had an adverse effect on grain Zn content in wheat either due to its fixation or its reduced uptake by the plant resulting from the antagonistic effect between phosphorus and zinc.
Milojević et al. (2011) found that available phosphorus has a negative correlation \( (r = -0.783^{**}) \) with zinc content in plant samples. The antagonism between the two elements is largely based on the chemical reaction in the root zone. Smilde et al. (1974) found that the Zn-P antagonism cannot be accounted for only by mutual immobilisation; but rather that the interaction is, mostly, a physiological characteristic of plants. Furthermore, the use of lime, through which \( \text{Ca}^{2+} \text{ and } \text{Mg}^{2+} \) as limestone are added, inactivates Zn in the soil due to the competition between \( \text{Ca}^{2+} \text{ and } \text{Zn}^{2+} \) on root surface, thus leading to reduced Zn translocation from the straw to the grain.

Table 2. Concentration of Zn in the wheat grain (µg/g)

| Varieties | Control | NPK | N+CaCO\(_3\) | NPK+CaCO\(_3\) | NPK+CaCO\(_3\) + manure | Average |
|-----------|---------|------|---------------|----------------|--------------------------|---------|
| Takovčanka | 26,0    | 26,9 | 31,7          | 19,8           | 22,7                     | 25,42   |
| Studenica  | 33,7    | 29,7 | 32,3          | 22,2           | 24,9                     | 28,56   |
| KG-56     | 39,6    | 27,7 | 41,4          | 28,2           | 27,5                     | 32,88   |
| Lazarica  | 34,3    | 26,3 | 30,7          | 23,6           | 19,8                     | 26,94   |
| KG-100    | 39,9    | 29,6 | 32,0          | 23,8           | 26,4                     | 30,30   |
| Toplica   | 35,2    | 30,3 | 33,5          | 21,8           | 25,2                     | 29,13   |
| Average   | 34,78   | 28,38| 33,60         | 23,18          | 24,42                    | 28,87   |

LSD

| 0.05 | 0.72511 | 0.79432 | 1.77616 |
| 0.01 | 0.96476 | 1.056847| 2.36318 |

A-fertilization variants, B-varieties, AB-interaction

Copper in wheat grain. Copper is an essential microelement for humans, animals and plants. It plays an important role as a constituent of enzyme systems, and is involved in metabolic processes in the body. Cu deficiency in crops is a widespread and serious problem not only in Europe, but also worldwide. Wheat is among crops susceptible to Cu deficiency. Cu deficiency symptoms include leaf chlorosis and necrosis, death of apical shoots, wilting, leaf roll and decay of young leaves. Cu is poorly mobile in plants as it is strongly bound to nitrogen and protein. During the growing season, growth retardation and, hence, reduced yield can result.

Copper deficiency can result in anaemia, since insufficient copper levels induce poor iron absorption and reduce numbers of blood cells. In addition, copper deficiency is assumed to cause heart disease and slow down the activity of the nervous system (such as poor concentration). It also reduces the number of white blood cells and, hence, resistance to disease.

The analysis of the experimental results (Table 3) on copper content in the wheat cultivars analysed shows that copper concentrations in the grain are low i.e. generally below 10 µg/g. The average values of Cu content in the wheat cultivars, for all fertilisation treatments, range from 3.76 to 4.88 µg/g. As
determined by the analysis of variance, grain Cu concentration was the highest in wheat cv. 'KG-56' and the lowest in 'Lazarica' and 'Studenica'.

Table 3. Concentration of Cu in the wheat grain (µg/g)

| Varieties  | Control | NPK | N+CaCO₃ | NPK+CaCO₃ | NPK+CaCO₃ + manure | Average |
|------------|---------|-----|---------|-----------|--------------------|---------|
| Takovčanka | 5,2     | 5,0 | 4,7     | 3,2       | 4,3                | 4,47    |
| Studenica  | 4,3     | 4,1 | 4,1     | 3,8       | 3,1                | 3,76    |
| KG-56      | 5,7     | 4,5 | 5,8     | 4,3       | 4,1                | 4,88    |
| Lazarica   | 4,2     | 4,0 | 4,2     | 3,7       | 3,1                | 3,84    |
| KG-100     | 5,7     | 5,0 | 4,7     | 4,3       | 4,0                | 4,74    |
| Toplica    | 4,4     | 4,0 | 4,4     | 4,0       | 3,6                | 5,05    |
| Average    | 5,2     | 4,43| 4,65    | 3,88      | 3,56               | 4,29    |

A-fertilization variants, B-varieties, AB-interaction

The significantly highest Cu content of wheat grain was found in the control (pH=4.2 in 1N KCl) i.e. on very acid soil, and it ranged from 4.2 to 5.7 mg/kg, giving an average of 4.91 µg/g. These values are considered normal for most food crops (Kabata-Pendias, 2011). These results are in agreement with the findings of previous research reporting the range of 3.6 to 6.3 mg/kg and the average of 5.3 mg/kg for grain Cu content in wheat grown on soils in Serbia (Škrbić and Onjia, 2007). Also, under all treatments, the use of phosphorus and lime reduced grain Cu concentration in the genotypes tested. Under NPK+CaCO₃ and NPK+CaCO₃+ humus treatments, the average Cu content of wheat grain was 3.88 and 3.56 µg/g, respectively, and these values are considered deficient for most crops. Jones (1972) found that Cu is required in small amounts 5-20 µg/g for normal plant growth and development, and concentrations below 4.0 µg/g are considered deficient. Therefore, soil improvement practices should involve the use of optimal rates of phosphorus and calcium to prevent Cu deficiency in plants.

**CONCLUSION**

The content of microelements (Zn and Cu) in the grain of wheat cultivars is dependent on cultivar-specific traits, soil characteristic and the content available forms of these elements in the soil. The concentration of these microelements in wheat grain depends on soil pH and P and Ca applied.

Limit values for the content of these microelements in wheat grain were determined, depending on cultivar and mineral nutrition: Zn-25.42 µg/g ('Takovčanka')-32.88 µg/g ('KG-56'); Cu-3.76 µg/g ('Studenica')-4.88 µg/g ('KG-56'). The highest concentration was found in control wheat cultivars (pH=4.26 in 1N KCl) and the lowest under NPK+CaCO₃+manure treatment.
Some wheat cultivars under NPK+CaCO$_3$ and NPK+CaCO$_3$+ manure treatments were found to be deficient in Cu.

The deficiency of Cu for plants in general and wheat in particular leads to certain morphological and anatomical changes. In humans, health problems develop due to the insufficient intake of microelements. These problems include anaemia as the most common disease, growth retardation in children, etc.

Soil improvement practices should involve the use of optimal rates of phosphorus and calcium to prevent Cu deficiency in wheat grain.

ACKNOWLEDGEMENTS

Investigations necessary for this paper are part of the project TP 31054 "Development of new cereals cultivation technologies on acid soils by usage of modern biotechnology", financed by the Ministry of Education and Science of Republic of Serbia

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