Original scientific paper DOI: /10.5513/JCEA01/19.2.2051
Journal of Central European Agriculture, 2018, 19(2), p.423-436

Cultivar-specific accumulation of iron, manganese, zinc and copper in winter wheat grain (*Triticum aestivum* L.)

Sortna specifičnost akumulacije gvožđa, mangana, cinka i bakra u zrnu ozime pšenice (*Triticum aestivum* L.)

Jelena MILIVOJEVIĆ[1], Ljiljana BOŠKOVIĆ-RAKOČEVIĆ[2*], Vera ĐEKIĆ[1], Kristina LUKOVIĆ[1] and Zoran SIMIĆ[3]

[1]Small Grains Research Centre, Save Kovačevića 31, 34000 Kragujevac, Serbia

[2]University of Kragujevac, Faculty of Agronomy, Department of Soil and Mechanisation, Cara Dušana 34, 32000 Čačak, Serbia, *correspondence: ljiljabr@kg.ac.rs

[3]University of Kragujevac, Faculty of Science, Institute of Hemistry, Radoja Domanovića 12, 34000 Kragujevac, Serbia

Abstract

To ensure safe food production, cultivar specificity of mineral nutrition in winter wheat was studied in order to determine genotypic differences in the accumulation of several selected microelements (Fe, Mn, Zn and Cu). Soil properties, plant species and genotypes were found to be major factors affecting the uptake of microelements by wheat plants. The application of NPK fertilizers, manure and lime resulted in a decrease in Fe, Mn and Zn contents, but the decrease was not below the concentrations that would make the plants suffer from nutrient deficiencies. In all treatments, the ratio Fe/Mn was <1.5, indicating that iron was not physiologically active, due to which its function was taken over by manganese. The analysis of variance showed highly significant effects of cultivar and fertilization on grain yield and 1,000-grain weight.

Keywords: cultivar specificity, fertilization, microelements, wheat

Sažetak

U cilju proizvodnje zdravstveno-bezbedne hrane, proučavan je uticaj mineralne ishrane kod različitih sorti pšenice kako bi se utvrdile genotipske razlike u akumulaciji određenih mikroelemenata (Fe, Mn, Zn i Cu). Najvažniji faktori koji utiču na usvajanje mikroelemenata od strane pšenice su osobine zemljišta, biljna vrsta i genotipovi. Primena NPK đubriva, stajnjaka i kreča dovela je do smanjenja sadržaja Fe, Mn i Zn, ali smanjenje nije bilo ispod koncentracija koje bi izazvele simptome nedostatka kod biljke. U svim varijantama odnos Fe/Mn je bio manji od 1,5 što ukazuje da gvožđe nije fiziološki aktivno, zbog čega je njegovu ulogu preuzeo mangan. Analiza varijanse je pokazala veoma značajne efekte sorte i đubrenja na prinos zrna i težinu 1000 zrna.

Ključne reči: đubrenje, mikroelementi, pšenica, sortna specifičnost

Introduction

In the last few decades, a huge amount of experimental results on mineral nutrition and fertilization of wheat has been collected. Continuous progress in the selection and creation of new varieties provides a strong incentive to conduct studies on various issues related to mineral nutrition in wheat. Accordingly, with the benefit of new varieties of wheat (which exhibit a range of useful properties, especially a significantly higher yield potential), it has been established that their mineral nutrition requirements are also significantly higher (Jelić et al., 2013; Đekić et al., 2014).

The nutritional 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) (Conti et al., 2000). Metals have an impact on human health in many ways. Some elements, such as Cu, Mn, and Zn, are essential micronutrients, with the human requirement of not more than a few milligrams per day. However, micronutrients may become harmful when their ingestion rates are too high (Reuter and Robinson, 1997). Deficiencies, excesses, or imbalances in the supply of inorganic elements from dietary sources may have a deleterious impact on human health (World Health Organization, WHO, 1996; Škrbić and Onjia, 2007).

The amount of an element ingested by humans is directly related to alimentary habits and its content in foodstuffs. The concentration of microelements in foodstuffs is dependent on soil characteristics, such as organic matter content, pH and clay mineralogy, which can affect the bioavailability of elements. Apart from environmental pollution, adding chemical products such as fertilizers, fungicides, insecticides and herbicides to crops is a matter of serious concern (Škrbić and Onjia, 2007). Fe, Mn, Zn and Cu accumulation in wheat grain is a complex trait dependent on a number of external factors, internal plant mechanisms and their interaction. Traditional plant breeding focuses on high grain yields in wheat, thus leading to the “biological dilution“ of the grain i.e. a decrease in microelement concentrations in the grain and a reduction in genotypic variation in terms of Zn and Fe accumulation in the grain.

In the last few decades, cereal products and, among them, wheat-based products have received considerable attention with respect to their potential role in the food chaintransport of toxic microelements into human diet. Since 1973, As, Cu, Cd, Fe, Hg, Pb and Zn have been considered by the joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Codex Alimentarius Commission to be potentially toxic for human diet (Ybanez and Montoro, 1996).

The objective of this study was to evaluate the effect of liming, humification and NPK fertilization on the grain yield and quality of winter wheat grown on an acid vertisol.

Knowledge of the concentrations of elements in wheat and other cereals can provide important information on the impact of fertilization, pesticides and other chemical products on crops, as well as on the environment.

Material and methods

Field trial and plant material

Research was conducted at the experimental field of the Small Grains Research Centre in Kragujevac (44° 22′ N, 20° 56′ E, 173-220 m a. s. l.), Šumadija region, Central Serbia, on a strongly acidic vertisol (pH in KCl 4.2). The region has a temperate continental climate with an average annual temperature of 11.6 °C and total rainfall of about 600 mm.

Data in Table 1 for the wheat growing season 2011/2012 clearly indicate that weather conditions in the experimental year did not differ from the long-term average typical of the Kragujevac region.

The trial was carried out during the wheat growing season 2011/2012, and included six wheat cultivars: ‘Takovčanka’, ‘Studenica’, ‘KG-56’, ‘Lazarica’, ‘KG100’ and ‘Toplica’. A randomized complete block design was used, with three replications in a split-plot arrangement. Plot size was 100 m^2 (10x10 m). The preceding crop was maize. Seeding was performed at an optimum date, on 10 October 2011, at a rate of 700 germinating seeds per m^2. Weed control was achieved using recommended herbicides and rates based on the spectrum of weeds present. The other practices used were standard, except irrigation.

Table 1. Temperature and rainfall in the growing season 2011/2012, and in the 1981-2010 reference period, for the Kragujevac location

Year	Months										
	X	XI	XII	I	II	III	IV	V	VI	VII	
	Mean temperature (^{o}C)										Average
2011/2012	10.4	3	4.4	0.6	-3.9	8.3	12.9	16	22.9	25.6	10.02
1981-2010	11.9	6.4	2.1	0.9	2.3	6.6	11.7	16.7	20	21.9	10.05
	Total rainfall (mm)										Sum
2011/2012	33.3	2	45.3	95.4	60.4	6.3	74.6	87.3	57.8	35.4	497.8
1981-2010	48.9	49.5	45.8	37.9	37	42.3	53.9	58.7	76.4	57.7	508.1

The following treatments were applied: 1. unfertilized control; 2. NPK (500 kg*ha^{-1}); 3. N (75 kg*ha^{-1}) + $CaCO_3$ (2 t*ha^{-1}); 4. NPK (500 kg*ha^{-1}) + $CaCO_3$ (2 t*ha^{-1}); 5. NPK (kg*ha^{-1}) + $CaCO_3$ (2 t*ha^{-1}) + cattle manure (35 t*ha^{-1}). The fertilizers applied were complex NPK fertilizers (8:24:16) and ammonium nitrate as a nitrogen fertilizer (containing 34.4% of N_{TOT}). Winter wheat was fertilized with an organic fertilizer i.e. cowshed manure having on average N_{TOT}-0.5%, P_2O_5-0.3%, K_2O-0.6%, organic matter-25%, C:N ratio 18:1 (Larney et al., 2006). Lime fertilizer ("Njival Ca") is finely ground lime rock (grit 0.0-0.1 mm). Chemically, it is pure carbonate with 98.5% $CaCO_3$ and 1% $MgCO_3$ i. e. 55.3% recalculated CaO and 0.5% recalculated MgO. Manure and lime were applied in early October 2011 during deep primary tillage. The whole amount of the mineral fertilizers was applied at seedbed preparation. The crop was harvested at full maturity.

Soil and plant analysis

Before and after trial establishment, soil samples were collected from a depth of 0-30 cm for the following analyses: soil pH was measured at a 1:2.5 ratio of soil to distilled water and 1N KCl; humus content was determined by oxidation with $KMnO_4$ solution (according to Kotzman); total nitrogen was estimated by Kjeldahl's analysis (Gerhardt Vapodest); available phosphorus and potassium were evaluated by ammonium-lactate AL-extraction with 0.1 M NH_4-lactate and 0.4 M CH_3COOH, according to Egner-Riehm (P was analyzed spectrophotometrically by the phospho-vanadate colorimetric method and K was determined by flame photometry).

Plants were harvested at full maturity. The grain was separated from the straw. For the analysis, 1 kg of wheat grain was sampled from each treatment. Aliquots of the pooled composite test grain samples were manually cleaned and milled in a laboratory mill grain (Tecator, UD Corporation Boylder Colorado) to obtain fine whole-meal flour. Flours were stored in clean polyethylene flasks at 5 °C pending analysis. Dry matter determinations were performed for each sample using separate 10 g aliquots by oven drying to constant mass at 105 °C. A sub-sample of fresh whole-meal flour (about 0.5 g on a dry matter basis) was dissolved in 10 cm^3 concentrated nitric acid and heated under reflux. Then, 10 cm^3 concentrated perchloric acid was added and heated until the formation of nitrous fumes stopped. The digestion temperature did not exceed 85 °C to prevent the loss of As and Hg (the temperature range was between 70 and 85 °C). The solution was placed in a 50 ml volumetric flask and made up to volume with Milli-Q^{TM} type deionized water.

Samples were analyzed by a Perkin–Elmer atomic absorption spectrometer (AAS) model 3300/96, MHS-10. Fe, Mn, Zn and Cu concentrations were determined by the direct aspiration of the aqueous solution into an air-acetylene flame AAS. Quantification was carried out using appropriate calibration curves obtained for standard element solutions in the same acid matrix.

Grain yield ($t*ha^{-1}$) was harvested and reported at 14% moisture. Two parameters of grain quality i.e. test weight ($kg*hL^{-1}$) and 1,000-grain weight (g) were analyzed. Thousand -grain weight was determined using an automatic seed counter. Test weight is the weight of a measured volume of grain expressed in kilograms per hectoliter.

Statistical analysis

Based on the results, the usual variational statistical indicators were calculated: average values, standard error, standard deviation and LSD-test. Statistical analysis was made in MSTAT-C statistical computer package (Michigan State University, East Lansing, MI, USA).

Results and discussion

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

The research was conducted on a very acid soil (pH in H_2O 5.6; pH in KCl 4.2). Treatments with NPK and lime, and NPK, manure and lime reduced both active and substitutional soil acidity (Table 2). The highest decrease in acidity i.e. increase in pH was found in the combined treatment with mineral, organic and lime fertilizers. In this treatment, soil pH increased by 0.2 units (in H_2O) i.e. by 0.5 pH units in KCl, in the 0-30 cm soil layer. The soil used for the research had low levels of humus and readily available phosphorus, but fertilization had a positive effect in increasing this parameter.

Table 2. Effects of fertilizers on soil chemical properties

Soil properties	Fertilization treatments				
	Control	NPK	N+$CaCO_3$	NPK+$CaCO_3$	NPK+$CaCO_3$+manure
pH/H_2O	5.6	5.6	5.6	5.7	5.8
pH/KCl	4.2	4.2	4.2	4.4	4.7
Humus (%)	2.17	2.41	1.85	2.87	3.45
Nitrogen (%)	0.12	0.15	0.13	0.15	0.17
P_2O_5 (mg*kg^{-1})	13	16	14	35	58
K_2O (mg*kg^{-1})	224	285	260	302	312

Different fertilization systems and liming were highly effective in increasing grain yield and grain quality parameters in all wheat cultivars (Table 3). The lowest values for grain yield and yield components were obtained in the untreated control. Grain yield and 1,000-grain weight were highest in the combined treatment with lime, manure and mineral fertilizer. These fertilizers reduced soil acidity and increased the efficiency of utilization of certain soil and fertilizer nutrients. The average grain yield was lowest in the unfertilized control (1.71 t*ha^{-1}) and significantly higher in fertilized treatments, ranging from 2.96 t*ha^{-1} (NPK) to 4.46 t*ha^{-1} (NPK+$CaCO_3$+ manure). The results confirmed the report of Jelic et al. (2010) on soil acidity in Central Serbia as a severe problem leading to a significant decline in grain yield and quality of wheat. The tested wheat cultivars showed no statistically significant differences in grain yield, which ranged from the highest in ‘Toplica’ (3.68 t*ha^{-1}) and ‘Takovčanka’ (3.67 t*ha^{-1}) to the lowest in ‘KG 100’ (2.92 t*ha^{-1}).

The various fertilization systems used in the research period showed significant effects on two parameters of grain quality. During the experimental period, thousand-grain weight was highest under NPK+$CaCO_3$+manure treatment (40.91 g), and was higher than NPK+$CaCO_3$ by 2.99 g. The control achieved the lowest average

1,000-grain weight. The average test weight was highest under NPK+$CaCO_3$ (79.82 kg*hL^{-1}), and slightly lower under NPK+$CaCO_3$+manure (79.02 kg*hL^{-1}).

Table 3. Average values for the characteristics of the tested winter wheat cultivars

Traits	Cultivar	Control		NPK		N+$CaCO_3$		NPK+$CaCO_3$		NPK+$CaCO_3$ + manure	
		X	S	X	S	X	S	X	S	X	S
Grain yield (t*ha^{-1})	Takovčanka	1.98	0.437	3	0.547	3.78	0.926	4.08	0.742	5.52	0.83
	Studenica	1.52	0.284	2.67	0.615	3.41	1.17	3.7	1.393	4.63	1.126
	KG 56	1.28	0.268	2.85	0.661	3.54	0.395	4.16	1.194	4.46	0.787
	Lazarica	1.71	0.404	3.31	0.516	3.46	0.416	4.87	1.139	4.38	0.673
	KG 100	1.6	0.293	2.68	1.434	3.31	1.176	3.68	1.567	3.33	1.598
	Toplica	2.17	0.812	3.24	0.744	4.22	0.152	4.35	0.683	4.42	0.206
	Average	1.71	0.488	2.96	0.732	3.62	0.751	4.14	1.064	4.46	1.045
1,000 grain weight (g)	Takovčanka	29.77	0.723	32.43	0.351	33.1	1.513	35.3	2.152	38.23	1.626
	Studenica	33.17	0.153	35.3	1.015	35.23	0.252	38.03	2.06	42.08	0.575
	KG 56	33.23	1.159	35.33	1.677	35.12	1.418	40.33	1.305	43.33	1.563
	Lazarica	29.97	0.493	31.37	1.274	31.2	1.442	34.63	1.93	37.27	0.551
	KG 100	34.17	0.924	38.27	1.305	36.2	1.216	39.7	1.572	41.77	1.305
	Toplica	34.37	0.850	35.97	0.351	35.9	0.4	39.53	2.079	42.77	1.266
	Average	32.44	2.04	34.78	2.529	34.46	2.058	37.92	2.765	40.91	2.586
Test weight (kg*hL^{-1})	Takovčanka	81.03	0.833	80.63	1.007	80.37	1.405	80.63	0.833	80.1	0.4
	Studenica	80.23	2.053	79.43	0.462	80.77	0.833	81.57	0.611	81.1	0.529
	KG 56	78.33	1.919	76.73	3.516	77.93	1.543	77.95	2.002	76.98	0.462
	Lazarica	81.7	0.693	80.1	1.442	79.57	1.222	80.83	0.305	81.23	0.115
	KG 100	77.72	0.503	77.13	1.828	76.98	1.155	78.48	3.302	77.15	3.383
	Toplica	74.4	0.133	77.67	1.47	75.25	1.743	79.43	1.007	78.62	1.429
	Average	78.9	2.734	78.62	2.211	78.48	2.309	79.82	1.951	79.02	2.201

X- the average value per replication; S- standard deviation

Also, the ANOVA (Table 4) indicated very highly significant effects of cultivar and 1,000-grain weight (F_{exp}= 6.62578***) and test weight (F_{exp}=17.35135***). A number of authors (Đekić et al., 2013; Jelić et al., 2013) underlined that 1,000-grain weight is a cultivar-specific trait, with considerably higher variations being observed among genotypes than among treatments or environmental factors. Nevertheless, the highest values for 1000-grain weight and grain yield were obtained in NPK+$CaCO_3$+manure treatment, in support of Jelić et al. (2013) who reported an increase in grain yield with increasing 1,000-grain weight.

The results indicated that grain yield and yield components significantly improved after combined mineral and organic fertilization. The integrated use of organic and mineral fertilizers is useful in improving crop yields, soil pH, and N, P and K availability in soils. Apart from its positive effect on wheat grain yield, organic fertilizer (manure) is beneficial in improving soil structure. It enhances root development, facilitates nutrient uptake, prevents loss of fertilizer nutrients, promotes their binding and assists in their gradual release as part of continuous plant nutrition throughout the growing season (Jelic et al., 2013; Đekić et al., 2014). However, concerns for the environment and natural resources demand harmonization between further increases in wheat production and soil fertility preservation.

The interaction of grain yield, 1,000-grain weight, test weight and cultivar x fertilization interaction did not show statistical significance (P>0.05).

Table 4. Analysis of variance of the tested parameters (ANOVA)

Effect of cultivar on the traits analyzed				
Traits	Mean sqr Effect	Mean sqr Error	F(df1,2) 5.84	p-level
Grain yield (t*ha^{-1})	1.4	1.658	0.844	0.522
1,000-grain weight (g)	73.063	11.027	6.625***	0
Test weight (kg*hL^{-1})	47.584	2.742	17.351***	0
Effect of fertilization on the traits analyzed				
Traits	Mean sqr Effect	Mean sqr Error	F(df1,2) 4.85	p-level
Grain yield (t*ha^{-1})	21.432	0.712	30.086***	0
1,000-grain weight (g)	199.111	5.825	34.179***	0
Test weight (kg*hL^{-1})	5.107	5.268	0.969	0.428
Effect of the cultivar x fertilization interaction				
Traits	Mean sqr Effect	Mean sqr Error	F(df1,2) 20.6	p-level
Grain yield (t*ha^{-1})	0.381	0.765	0.497	0.957
1,000-grain weight (g)	1.558	1.644	0.947	0.533
Test weight (kg*hL^{-1})	3.23	2.421	1.334	0.194

The present study showed considerable variation in Fe concentration in wheat grains (Table 5). The highest average Fe concentration was found in the grains of cvs. ‘KG 100’ and ‘KG 56’ and the lowest in ‘Takovčanka’ and ‘Toplica’. Fertilization had a significant effect on Fe content in the wheat grain. Fe levels were highest in the control (50.45 µg*g^{-1}) and lowest under NPK+$CaCO_3$+manure treatment (37.37 µg*g^{-1}). Previous studies reported the range of 17 to 50 mg*kg^{-1}

and the average of 31 mg*kg^{-1} for grain Fe content in wheat grown on different continents (Kabata-Pendias, 2011).

In the control, the low pH of the medium (pH=4.2 in 1N KCl) increased the uptake and concentration of Fe in the grains of the tested cultivars. Under the combined treatment with mineral (NPK), organic (manure) and lime ($CaCO_3$) fertilizers, Fe content ranged from 24.6 to 47.3 µg*g^{-1}, indicating a decrease in Fe uptake by plants with increasing soil pH. Moreover, this treatment gave the highest grain yield and, accordingly, biological dilution of this element occurred. The increase in soil pH and good aeration, along with the presence of P, led to the oxidation of physiologically active Fe^{2+} to less active Fe^{3+} and precipitation of Fe(III) salts. The Fe-P interaction in plants and soil resulted in $FePO_4{\cdot}2H_2O$ precipitation. Furthermore, P anions compete in plants for Fe-binding sites and prevent Fe uptake and translocation in plants (Jelić et al., 2011). The other treatments also showed a decrease in Fe content in wheat grains, and the values were almost identical - about 40 µg*g^{-1}.

Table 5. Concentration of Fe in the wheat grain (µg*g^{-1})

Cultivars	Control	NPK	N+$CaCO_3$	NPK+$CaCO_3$	NPK+$CaCO_3$ +manure	Average
Takovčanka	39.4	35	50.5	32.1	41.1	39.62
Studenica	54.9	41.7	37.8	38.4	38.6	42.28
Kg 56	50.4	40.2	55	37.1	37.7	44.08
Lazarica	48.3	43.6	43	36.1	34.9	41.18
Kg 100	54.1	48	38	58.7	24.6	44.68
Toplica	55.6	39.2	21.6	39	47.3	40.54
Average	50.45	41.28	40.98	40.23	37.37	42.06

LSD	A	B	AB
0.05	0.98222	1.07597	2.40595
0.01	1.30685	1.43158	3.20112

A – fertilization treatments, B – cultivars, AB – interaction

As shown in Table 6, grain Mn concentration in winter wheat grown on very acidic soil varied depending on genotype and mineral nutrition. ‘KG 56’ had the highest average levels (49.9 µg*g^{-1}) and ‘Takovčanka’ had the lowest values (41.04 µg*g^{-1}). The average grain Mn content in the control was about 53.32 µg*g^{-1} (ranging from 46.9 to 64.2 µg*g^{-1}). The lowest content was in NPK + $CaCO_3$ + manure treatment (36.38 µg*g^{-1}), and these values are considered normal for most food crops (Kabata-Pendias, 2011). The present results are in agreement with previous studies by Škrbić and Onjia (2007) who reported an average grain Mn content of 48.00 mg*kg^{-1} (range 37 to 88 mg*kg^{-1}) for wheat grown in Serbia. Reuter and Robinson (1997) indicate that the optimal content of Mn in wheat, in the phase of two leaves, is 200 mg*kg^{-1}, and in the grain, in phase of technological maturity, 21-34 mg*kg^{-1}.

Table 6. Concentration of Mn in the wheat grain (μg*g^{-1})

Cultivars	Control	NPK	N+CaCO$_3$	NPK+CaCO$_3$	NPK+CaCO$_3$ +manure	Average
Takovčanka	46.9	50	40.6	33.5	34.2	41.04
Studenica	54.3	50.4	37.7	36.5	34.8	42.74
Kg 56	64.2	52.8	48.3	43.7	40.5	49.9
Lazarica	47.9	51	40.5	38.2	34.3	42.38
Kg 100	57.2	53.7	43.2	43.7	37.7	47.1
Toplica	49.4	55.3	41.4	35.5	36.8	43.68
Average	53.32	52.2	41.95	38.52	36.38	44.47
LSD	A		B		AB	
0.05	1.09028		1.19434		2.67062	
0.01	1.45062		1.58907		3.55327	

A – fertilization treatments, B – cultivars, AB – interaction

Mineral nutrition was another factor that had an important effect on Mn concentration in the wheat grain. The increased Mn uptake by wheat plants in the control (pH=4.2 in 1N KCl) was an expected result, given that, at low pH values, redox potential is reduced, solubility of Mn compounds is increased and, hence, Mn amounts in mobile fractions (water soluble + exchangeable) are increased (Milivojević et al., 2011b). Furthermore, during the tillage of soils highly deficient in phosphorus, high rates of this macronutrient are applied, leading to Mn^{2+} coupling with orthophosphoric acid to form mono, di- and trivalent salts that produce readily soluble primary Mn phosphates in the presence of high concentrations of H_3O^+ ions on very acidic soils (Milivojević et al., 2013).

Table 7. Ratio of Fe/Mn concentrations in the wheat grain

Cultivars	Control	NPK	N+CaCO$_3$	NPK+CaCO$_3$	NPK+CaCO$_3$ +manure	Average
Takovčanka	0.84	0.7	1.24	0.96	1.2	0.9
Studenica	1.01	0.83	1	1.05	1.13	0.98
Kg 56	0.78	0.76	1.14	0.84	0.9	0.88
Lazarica	1	0.85	1.06	0.96	1.01	0.97
Kg 100	0.94	0.89	0.88	1.34	0.65	0.94
Toplica	1.12	0.7	0.52	1.1	1.28	0.92
Average	0.94	0.78	1.05	1.05	1.02	0.94

As stated above, the total content of Fe and Mn in the wheat grain suggests that wheat plants had a good supply of these microelements. However, the ratio of Fe/Mn concentrations in plants, which was, due to the antagonistic effect, lower than 1.5 in all treatments (Table 7), indicates that iron was not physiologically active, as its function was taken over by Mn, which may have resulted in its deficiency in plants. Moreover, the single-year soil amendment had no substantial effect in terms of improving the Fe to Mn ratio.

The ratio of the two metals (Fe/Mn) in the nutrient solution and plant tissue is more important for plant metabolism than their individual concentrations. The ratio should range from 1.5 to 2.5, which is essential to ensuring safe food production. Values below this range indicate Mn toxicity and Fe deficiency symptoms, whereas those above 2.5 show that toxic effects of Fe are associated with Mn deficiency, which may lead to disorders in the body (Kabata-Pendias, 2011). Moreover, the competition between manganese cations and Fe^{2+} for the same sites of binding to chelate compounds is responsible for the low uptake of Fe and disturbed Fe translocation within the plant. The increase in Mn content in plants most likely promotes the oxidation of Fe^{2+} to Fe^{3+} and, thus, induces Fe deficiency (Kabata-Pendias, 2011).

The results (Table 8) showed significant variation in grain Zn content in all winter wheat genotypes. Grain Zn content was highest in ‘KG 56’ (32.88 µg*g^{-1}) and lowest in ‘Takovčanka’ (25.42 µg*g^{-1}). The content of zinc in plants is dependent on biological properties, soil characteristics and the levels of available forms of this element in the soil. Plant species and genotypes within the species exhibit significant differences in Zn accumulation and uptake. As determined by Eide et al. (1996), IRT1 membrane proteins are relatively specialized and can serve for Zn transport, and ZIP proteins in cereals play an important role in Zn uptake.

Table 8. Concentration of Zn in the wheat grain (µg*g^{-1})

Cultivars	Control	NPK	N+CaCO$_3$	NPK+CaCO$_3$	NPK+CaCO$_3$ +manure	Average
Takovčanka	26	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	23.8	26.4	30.34
Toplica	35.2	30.3	33.5	21.8	25.2	29.2
Average	34.78	28.42	33.6	23.23	24.42	28.89
LSD	A		B		AB	
0.05	0.72511		0.79432		1.77616	
0.01	0.96476		1.05685		2.36318	

A – fertilization treatments, B – cultivars, AB – interaction

The comparison between the control and the fertilization treatments revealed that the Zn contents of winter wheat grains were affected by mineral nutrition. The highest Zn level was obtained in the control and ranged from 26 to 39.9 µg*g^{-1}, giving an average of 34.78 µg*g^{-1}. These values are in agreement with the results of Khan et al. (2015) who reported a range of 16 to 40 mg*kg^{-1} for Zn in various wheat cultivars. As previously found, the grain Zn content of wheat grown in Serbia ranged from 26.6 to 43.3 mg*kg^{-1}, averaging 32 mg*kg^{-1} (Škrbić and Onjia, 2007). Cakmak (2009) suggested that the average concentration of Zn in the whole grain of wheat (20 to 35 mg*kg^{-1}) should be increased at least by approximately 10 mg*kg^{-1} for a measurable biological impact on human health.

The phosphorus applied through NPK fertilizer 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 (Peck et al., 1980; Milivojević et al., 2011a). Milivojević et al. (2011a) found that available phosphorus was negatively correlated (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. As determined by Smilde et al. (1974), the Zn/P antagonism cannot be accounted for only by mutual immobilization; but rather that the interaction is, mostly, a physiological characteristic of plants. Furthermore, the use of lime, through which Ca^{2+} and Mg^{2+} as limestone are added, inactivates Zn in the soil due to the competition between Ca^{2+} and Zn^{2+} on the root surface, thus leading to reduced Zn translocation from the straw to the grain.

The analysis of the experimental results on copper content in the tested wheat cultivars showed that copper levels in the grain were low i.e. generally below 6 µg*g^{-1} (Table 9). The average Cu values in all fertilization treatments ranged from 3.84 to 4.88 µg*g^{-1}. Grain Cu concentration was highest in wheat cv. 'KG 56' and lowest in 'Lazarica'.

Table 9. Concentration of Cu in the wheat grain (µg*g^{-1})

Cultivars	Control	NPK	N+$CaCO_3$	NPK+$CaCO_3$	NPK+$CaCO_3$ +manure	Average
Takovčanka	5.2	5	4.7	3.2	4.3	4.48
Studenica	4.3	4.1	4.1	3.8	3.1	3.88
Kg 56	5.7	4.5	5.8	4.3	4.1	4.88
Lazarica	4.2	4	4.2	3.7	3.1	3.84
Kg 100	5.7	5	4.7	4.3	4	4.74
Toplica	4.4	4	4.4	4	3.6	4.08
Average	4.92	4.43	4.65	3.88	3.7	4.32
LSD	A		B		AB	
0.05	0.21621		0.2368		0.5296	
0.01	0.28668		0.3151		0.7046	

A – fertilization treatments, B – cultivars, AB – interaction

The significantly highest Cu content in the wheat grain was found in the control, and it ranged from 4.2 to 5.7 µg*g^{-1}, giving an average of 4.92 µg*g^{-1}. The various fertilization systems used during the research period showed that NPK, manure and lime reduced grain Cu concentration in the genotypes tested. Under NPK+$CaCO_3$ and NPK+$CaCO_3$+manure treatments, the average Cu content of wheat grain was 3.88 and 3.7 µg*g^{-1}, respectively, and these values are considered deficient for most crops. These results are in agreement with the findings of previous research reporting the range of 3.6 to 6.3 mg*kg^{-1} and the average of 5.3 mg*kg^{-1} for grain Cu content in wheat grown on soils in Serbia (Škrbić and Onjia, 2007). Cu is required in small amounts (5-20 µg*g^{-1}) for normal plant growth and development, and concentrations below 4 µg*g^{-1} are considered deficient (Jones, 1972). During the growing period, the needs of wheat for Cu are reduced, because the critical values at the beginning of the vegetation period are 4 mg*kg^{-1}, and at the end of the vegetation period 1.3-1.8 mg*kg^{-1} (Reuter and Robinson, 1997).

The results of the present study show that the observed differences in Fe, Mn, Zn and Cu concentrations in wheat grain are due to genetic variations among the cultivars. Furthermore, the decrease in the levels of these elements in treatments employing different fertilization systems and giving increased grain yields indicates the existence of the growth dilution effect.

Conclusion

Different fertilization systems and liming were highly effective in improving grain yield and grain quality parameters in all winter wheat cultivars. The average grain yield was lowest in the unfertilized control (1.71 t*ha^{-1}) and significantly higher in fertilized treatments, ranging from 2.96 t*ha^{-1} (NPK) to 4.46 t*ha^{-1} (NPK+$CaCO_3$+manure).

The content of microelements (Fe, Mn, Zn and Cu) in the wheat grain is dependent on cultivar-specific characteristics and various fertilization systems.

Acknowledgment

This research is part of Project TP 31054 "Development of new cultivation technologies for cereals on acid soils through modern biotechnology", financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

References

Cakmak, D. (2009) Effect of sulphur fertilization on wheat yield and on nutrient grain content on chernozem in Serbia. Agrochimica, 53 (2), 92-100.

Conti, M.E., Cubadda, F., Carcea, M. (2000) Trace metals in soft and durum wheat from Italy. Food Additives and Contaminants, 17, 45-53. DOI: 10.1080/026520300283577

Đekić, V., Staletić, M., Jelić, M., Popović, V., Branković, S. (2013) The stability properties of wheat production on acid soil. In: Kovačević, D., Proceedings of 4th International Symposium "Agrosym 2013". Jahorina, Bosnia and Herzegovina, 03-06 October 2013, Poljoprivredni fakultet, 84-89.

Đekić, V., Milovanović, M., Popović, V., Milivojević, J., Staletić, M., Jelić, M., Perišić, V. (2014) Effects of fertilization on yield and grain quality in winter triticale. Romanian Agricultural Research, 31, 255-266. DOI: 2067-5720 RAR 2012-255.

Eide, D., Broderious, M., Fett, J., Guerinot, M.L. (1996) A novel iron-regulated metal transporter from plants identified by functional expression in yeast. Proceedings of the National Academy of Sciences, USA, 93, 5624-5628.

Jelic, M., Dugalic, G., Milivojevic, J., Djekic, V. (2013) Effect of liming and fertilization on yield and quality of oat (*Avena sativa* L.) on an acid luvisol soil. Romanian Agricultural Research, 30, 249-258. DOI: 2067-5720 RAR-239.

Jelić, M., Milivojević, J., Trifunović, S., Đalović, I., Milošev, D., Šeremešić, S. (2011) Distribution and forms of iron in vertisol of Serbia. Journal of the Serbian Chemical Society, 76 (5), 781-794. DOI: 10.2298/JSC100619068J

Jones, J.B., Jr. (1972) Plant tissue analysis for micronutrients, In: Mortvedt, J.J., Giordano, P.M., Lindsay, W.L.,eds. (1972) Micronutrients in agriculture. Medison, Wisconsin, 319-347.

Kabata-Pendias, A. (2011) Trace elements in soils and plants. 4th edition. London, New York: CRC, Press Taylor and Francis Group, Boca Ration.

Larney, F.J., Buckley, K.E., Hao, X., McCaughey, W.P. (2006) Stockpiled and composted beef cattle feedlot manure. Journal of Environmental Quality, 35, 1844-1854.

Milivojević, J., Nikezić, D., Krstić, D., Jelić, M., Đalović, I. (2011a) Influence of physical-chemical characteristics of soil on the zinc distribution and availability for plants in vertisols of Serbia. Polish Journal of Environmental Studies, 20 (4), 993-1000.

Milivojević, J., Đalović, I., Jelić, M., Trifunović, S., Bogdanović, D., Milošev, D., Nedeljković, B., Bjelić, D. (2011b) Distribution and forms of manganese in vertisols of Serbia. Journal of the Serbian Chemical Society, 76 (8), 1177-1190. DOI: 10.2298/JSC101229103M

Milivojević, J., Jelić, M, Đekić, V., Simić, Z. (2013) The available contents of heavy metals compared to soil properties in Kragujevac. In: Saljnikov, E., Proceedings of 1st International Congress in Soil Science "Soil-Water-Plant", Belgrade, Serbia, September 23-26 2013, Akademska izdanja.

Peck, N.H., Grunes, D.L., Welch, R.M., MacDonald, G.E. (1980) Nutritional quality of vegetable crops as affected by phosphorus and zinc fertilizer. Agronomy Journal, 72, 528–534.

Reuter, D.J., Robinson, J.B. (1997) Plant analysys an interpretation manual. Australia: CSIRO Publishing.

Smilde, K.W., Koukoulakis, P., van Luit, B. (1974) Crop response to phosphate and lime on acid sandy soils high in zinc. Plant Soil, 41, 445-454.

Škrbić, B., Onjia, A. (2007) Multivariate analyses of microelement contents in wheat cultivated in Serbia. Food control, 18 (4), 338-345. DOI: 10.1016/j.foodcont.2005.10.017

World Health Organization (1996) Trace elements in human nutrition and health. ISBN: 92-4-156173-4 (NLM Classification: QU 130).

Ybanez, N., Montoro, R. (1996) Trace element food toxicology: an old and ever-growing discipline. Critical Reviews in Food Science and Nutrition, 36, 299-320.