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Original article

Assessment of Microelements Content in Organic Soft Albanian Wheat Genotypes[1]

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Abstract

The increase of micro-element content in wheat seed to reduce human malnutrition is a challenge for all agronomists during plant breeding. The objective of the current study was to assess the micro-element content as Cu, Fe, Mn, Cd, Zn and Cr in 20 accessions and 10 lines of soft wheat grown under organic farming system in Albanian region. The Cu, Fe and Zn contents were determined by flame atomic absorption spectrometry (FAAS) and the Mn, Cd and Cr contents were determined by electrothermal atomic absorption spectrometry (ETAAS). The obtained results showed significant variations in micro-element contents in different wheat grains genotypes. Higher levels of Cu, Zn, Fe and Mn (6.79; 46.42; 66.78 and 34.87 mg/kg, respectively in wheat lines) were observed in the present study. These values were higher compared to data reported in previous studies which are performed in the conventional farming system. The concentrations of Cd as a potential toxic element were below the EU limits in all the analyzed samples. The present study showed that the analyzed wheat samples could be considered as a valuable source of micro-elements in human diet..

Keywords: Concentration, Food requirement, Genotype, Microelement, Soft wheat.

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INTRODUCTION

In many micronutrient-deficient regions, wheat is the dominant staple food, making up to 50% of the diet, and therefore, it has a particularly important role in daily energy intake, especially in the developing world (Cakmak et al. 2010b). Among the minerals, some of them are essential to human (Cu, Ca, Fe, K, Mg), essential to plants and one or more animal species, however, not for humans (As, Cd, Ni and others), some of them are toxic (Hg), some of them are non-essential that can used in therapeutic dosages (Al, Ba) (Tchounwou et al. 2012). However, micronutrients may become harmful when their ingestion rates are too high. On the other hand, microelements are very important for human health and are required in adequate amounts for the healthy function of the human body. The deficiency of specific minerals may lead to various chronic diseases (Branca et al. 2002). Fe, Zn and Mg deficiencies may cause health problems in pregnant women and infants. Approximately two - thirds of all deaths of children are associated with micronutrient deficiencies (Caballero, 2002). Zn is known to have an important role either as a metal component of enzymes or as a functional, structural or regulatory co-factor of a large number of enzymes (Grotz and Guerinot, 2006). Zn deficiency is manifested through a variety of health problems which can be lethal if left untreated. The main factors that affect the amount of Zn in soil are pH, carbonate content, organic matter, soil texture and interactions between Zn and other microelements, such as Fe (Bukvic et al., 2003). The amount of Zn in plants directly correlates with plant growth, crop yield, nutitional quality and Zn toxicity in crop plants is very rare (Cakmak, 2008). Moreover, Zn concentration in some primitive wheat and wild wheat, is higher than in cultivated cultivars, indicating gratpotencial for breeding genotypes with high capacity to accumulate Zn (Cakmak et al., 2004; Chatzav et al., 2010). Also, Cu plays an important role as a constituent of enzyme systems and is involved in metabolic processes in the body. Cu deficiency can result in anemia, since insufficient copper levels induce poor iron absorption and reduce numbers of blood cells. Pb and Cd have become highly toxic metallic elements. The exposures of both of these elements during childhood cause anemia, abdominal pain, neurological and adverse development effects, kidney damage, hypertension and changes in vitamin D metabolism (Kraljević-Balalić et al., 2009). Chromium (Cr) is a naturally occurring element in the environment and in most foodstuffs. According to several studies, the main factors that affect the total Cr concentration in plant tissues is the soil contamination (Cary and Kubota, 1990; Tanee et al., 2015). Dynamic development of the socioeconomic relation in Albania has been followed by a constant process of the modern development and improvement of growing wheat and flour production. Among the numerous purposes of wheat breeders, the important task is creation varieties with high ability to accumulate certain nutrients in grain, considering fact that for human wellbeing is necessary at least 22 mineral elements (Graham et al., 2007). In the breeding program intensive selection of genotypes with increased mineral concentration on the base of exploitation of genes for essential micronutrients is necessary for improving the quality of crop foods.

The aim of this study was to evaluate the variability of micro-elements content in different accessions and lines of Albanian wheat grown under organic farming system in order to provide the information for selection of wheat accession with substantial capacity to acquire minerals and to examine the interrelations among mineral concentrations in soft wheat.

Material and Methods

Plant and Soil Materials

Twenty accessions (PKR) and ten lines (PZA) of Albanian wheat analyzed in this study, were grown under organic farming system during the year 2011-2012 and 2012 - 2013 at the Experimental Didactics Economy (E.D.E) of Agricultural University of Tirana (latitude 41°19’39”N, longitude 19°49’08”E; average altitude 89 m). The average of annual precipitation of the region is about 1189 mm and the average of annual temperature is 16°C. The soil reaction was slightly alkaline (pH= 7.80), the humus and nitrogen contents were 2.2% and 0.150%, respectively. The other soil parameters are described in Table 1.

Table 1. General characteristics of soil in the experimental field

CaCO3 (%)	P2O5 (mg/kg)	K (mg/kg)	Na (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Texture Sand (%)	Clay (%)	Silt (%)
8.7	0.74	242	43.7	4812	1081	25.5	44.4	30.1

Chemical Analyses

About 50g of wheat of each grain sample was collected and milled to flour (whole grain) with a laboratory mill (Pulverisette 14) for further analysis. Afterwards, the flour samples were dried and vacuum packed. The samples were digested in a microwave oven according to the previous published procedure (KRISTL et al., 2002). The prepared solutions were diluted to 25 ml with Milli-Q water. All the chemicals used in the sample treatments were of ultrapure grade. The blank solutions were prepared in the same manner as the samples. The concentration of Fe, Cu and Zn were determined by FAAS, whereas the contents of Mn, Cd, Cr were determined by ETAAS. Three commercial reference materials (NIST 1515, NIST 1547 and NIST 1575) were used as quality-control samples. The accuracies were adequate for all of the studied mineral elements (data not presented). Each sample was analyzed in duplicate and the results were expressed as mg/kg of dry weight (DW).

Statistical Evaluation

All measurements were performed with three replications. Descriptive statistical analyses of obtained results was reported as mean and standard deviation (SD). ANOVA was conducted for the comparison of the means. Furthermore, principal component analysis (PCA) was applied successfully to classify and discriminate the different cultivars of wheat. Obtained results were processed using StatSoftStatistica 10.0® software. In order to get a more complex observation of the ranking of wheat quality, standard scores (SS) were evaluated. Min-max normalization is one of the most widely used techniques to compare various characteristics of complex food samples determined using multiple measurements, where samples are ranked based on the ratio of raw data and extreme values of the measurements used. The normalized scores of samples for different measurements when averaged give a single unit less value termed as SS, which is a specific combination of data from different measuring methods with no unit limitation.

Results

Summary of descriptive statistics for microelements content in lines and accessions of soft wheat is presented in Tables 2 and 3. The results for Cd are expressed in μg/kg, whereas the contents for all other minerals are presented in mg/kg on a dry weight basis (DW). Substantial differences in the concentration of the grain minerals were found among years and amongthe genotypes (Tables 2 and 3). Compared with the lines of wheat, accessions showed higher concentration of Cu during the two years of the study 2011-12/2012-13 (5.39 mg/kg, 5.98 mg/kg, respectively) and a high concentration of Zn, especially in the first year of the study (48.99 mg/kg).The lines of soft wheat were detected to have a high average concentration of Fe (66.78 mg/kg), Mn (34.87 mg/kg), Cd (28.39 μg/kg) and Cr (2.05 mg/kg) during the study years, whereas the accessions show a high average concentration for Cu (6.79 mg/kg) and Zn (46.42 mg/kg). Organically grown crops have been found to have 21% more Fe than conventionally grown crops (Rembialkowska, 2007). The ANOVA showed highly significant differences between wheat cultivars for mineral concentration. Trace elements may differ in their distribution in the wheat grain. The differences between genotypes in the concentrations of the mineral elements in whole grain may be due to the variation in kernel size and plumpness through their influence on the proportion of the endosperm to total grain weight (Peterson et al., 1986). The accumulation of micronutrients in wheat involves a plethora of processes, including mobilization from the soil, absorption from the rhizosphere, translocation from roots, remobilization from vegetative tissues and deposition in bioavailable forms in grains, each of which is controlled by numerous genes (Bouis and Welch, 2010). Photosynthetic activity of vegetative tissue is an important factor in determining grain mineral concentration. Dias et al. (2006), found a correlation between the chlorophyll and Fe concentrations.

Table 2. The microelements content in 10 lines of soft wheat

Year		Cu	Zn	Fe	Mn	Cd	Cr
2012	Average	5.39	42.78	61.87	35.35	30.98	1.91
	St. dev.	0.54	3.73	8.16	2.65	13.87	0.81
	Min.	4.14	36.42	49.08	31.61	17.64	0.48
	Max.	5.91	50.59	74.81	39.44	62.62	3.69
	Var.	0.29	13.92	66.59	7.04	192.41	0.66
2013	Average	5.98	45.52	71.70	34.40	25.80	2.18
	St. dev.	0.62	4.28	8.43	2.73	4.49	1.23
	Min.	5.31	40.53	54.24	31.16	19.50	0.27
	Max.	7.07	53.48	80.41	39.69	31.67	3.96
	Var.	0.38	18.28	71.10	7.43	20.12	1.51

[1] St. dev. - standard deviation, Min. - minimum, Max. - maximum, Var. - variation

Table 3. The micro elements content in 20 accessions of soft wheat

Year		Cu	Zn	Fe	Mn	Cd	Cr
2012	Average	6.81	49.00	55.63	33.83	18.60	0.82
	St. dev.	0.62	8.18	10.96	5.90	7.94	0.87
	Min.	5.84	35.19	34.17	22.12	11.54	0.05
	Max.	8.44	62.81	75.67	43.19	46.85	3.54
	Var.	0.39	66.94	120.10	34.79	63.11	0.76
2013	Average	6.76	43.84	53.42	33.97	18.69	1.00
	St. dev.	0.51	3.24	10.62	3.51	8.47	0.79
	Min.	5.98	39.64	34.90	26.58	9.05	0.12
	Max.	7.94	50.69	69.29	38.80	42.58	3.73
	Var.	0.26	10.51	112.80	12.31	71.74	0.62

[1] St. dev. - standard deviation, Min. - minimum, Max. - maximum, Var. - variation

Discussion

Cd is highly toxic non – essential heavy metal and it does not have a role in biological process in living organisms. Cd poisons in human could lead to anemia, renal damage, bone disorder and cancer of the lungs (Edward et al., 2013). However, in all samples of grain (lines and accessions) the Cd concentration was below the EU limit, which is 0.12 mg/kg (Stefanovic et al., 2008). Also, in this study, the Cd concentrations were substantially lower than the concentration presented by Sękara et al. (2005) (0.24 mg/kg). Low Cd concentration in grain was likely caused by different soil pH; an almost neutral soil reaction, resulting in low Cd availability (Uprety et al. 2009; Konvacevic et al. 2011) as in our study. This fact allows us to confirm that the analyzed wheat flours are safe concerning this toxic metal. In wheat flour, the content of Cd is usually very low (Gonzales et al., 2001). The main characteristic of this element is that is not biodegradable and is able to accumulate in the organism, causing therefore serious problems to the human health (Bernard, 2008). Cr is an essential element required for normal sugar and fat metabolism. Cr (III) and its compounds are not considered a health hazard, while the toxicity and carcinogenic properties of Cr (VI) have been known for a long time (Kalagbor et al., 2014). The concentration of Cr in accessions of soft wheat was lower compared with the concentration reported by Jelic et al which ranges from 0.4- 2.9 mg/kg. Possible reasons for a different concentration of Cr could be the different soil characteristics, as the availability of Cr is high in acid soil (Jelic et al., 2009) and the genotype of wheat. In the present study a high mean level of most of the minerals was found as compared to previous studies (Table 4). Spiegel et al. (2009), had evaluated the mineral concentrations under organic conditions and Ryan et al. (2004) under inorganic conditions. Thus, the high concentration of minerals in this study might be attributed to the organic farming system used. However, comparative studies have shown insignificant differences in grain concentration of micro- and risk elements of wheat produced in organic and inorganic farming systems (Hejman et al., 2013). However, the specific genotype can be the central factor to consider in an organic farming system. This study showed the positive effect of producing wheat with a high level of mineral concentration by the use of an organic farming system in combination with the selected genotype. We calculate the percentages of recommended daily intakes (RDIs) based on an average consumption of 200 g of whole grain per day. Furthermore, the calculations were based on statistics from FAO and the values for recommended intake for adults, according to DGE as shown in table 4. The values calculated for Cu and Zn were exceptionally high and approached 76% and 88% for the lines and 91% and 93% for accessions, respectively, and the contributions of Fe and Mn in recommended daily intake are more than 100% for lines and accessions of wheat. These values need to be considered more as orientational values because they are based on the assumption of 100% absorption of individual minerals. According to Liu et al. (2007), the absorption of Zn, Fe, Cu and Mn can be reduced by the content of phytate, activity of endogenous phytate (EC 3.1.3.26) and tannins.

Table 4. Comparison of mineral concentration (mg/kg) in the present study with previous sudies

Lines of wheat							
	In our study	Spiegel et.al 2009	Ryan et al. 2004	Jelic et al.,2009 (mg/kg^{-1})	Present study (mg/day)	Recommended intake (mg/day) according to DGE 2001	The percentage of the recommended intake from flour consumption 200g/person/day
Cu	5.68	3.9	n.a	-	1.1	1.5	76
Zn	44.15	23.9	35.0	-	8.8	10	88
Fe	66.78	31	18	-	13.4	10	>100
Mn	34.87	36.9	41	-	6.9	5	>100
Cr	2.05	-	-	2.12	0.4	1.5	27
Cd	28.39	-	-	0.38	5.7	70[a]	4[b]
Accessions of wheat							
Cu	6.79	3.9	n.a	-	1.4	1.5	91
Zn	46.42	23.9	35.0	-	9.3	10	93
Fe	54.53	31	18	-	10.9	10	>100
Mn	33.90	36.9	41	-	6.8	5	>100
Cr	0.91	-	-	2.12	0.2	1.5	12
Cd	18.67	-	-	0.38	3.7	70[a]	5[b]

[a] For Cd this values is the provisional tolerable daily intake (PTDI) (μg/kg). [b] For Cd this value is the percentage (%) of the tolerable intake. This study and the study from Spiegel et al. 2009 were performed under organic conditions, whereas Ryan et al.2004 and Jelic et al,. 2009 were carried out under inorganic conditions.

Today, over three billion people are afflicted with micronutrient malnutrition and the number are increasing (Welch et al., 1997; WHO, 1999). One of the reason of micronutrient deficiency is the low concentration in cereals (Hurrel, 2000). This research indicate that genotype and organic farming can influence in the increasing in significant level the concentration of Fe, Zn and Mn. The two-way ANOVA test showed the significant effects of the independent variables (lines or accessories and year) to the metal content and which of the metal contents were significantly affected by the varying treatment combinations (Table 5). This analysis revealed that the content of Cu is affected by the year in wheat lines, statistically significant at $p<0.05$ level. The content of Fe is impacted by the year ($p<0.01$ level) and the type of wheat line ($p<0.01$ level). The contents of Cu, Fe, Cd and Cr in wheat accessions are affected by the type of the accession, statistically significant at $p<0.01$ level, while the content of Mn is affected by accession type, at $p<0.05$ level. The contents of Zn and Cr in wheat accession are impacted by year, statistically significant at $p<0.05$ level.

Table 5. ANOVA calculation of microelements content in 10 lines of soft wheat, and in 20 accessions of the soft wheat

		Lines				*Accessions*			
		Intercept	Year	Name	Error	Intercept	Year	Name	Error
DOF		1	1	9	9	1	1	19	19
Cu	SS	46.12	1.73	4.54	1.49	187.63	0.02	11.27	1.07
	F	277.97	10.43	3.04		3339.45	0.32	10.56	
	p	0.00	0.01*	0.06**		0.00	0.58	0.00+	
Zn	SS	3206.58	37.53	203.13	86.71	11730.86	265.95	824.35	647.20
	F	332.84	3.90	2.34		344.38	7.81	1.27	
	p	0.00	0.08**	0.11		0.00	0.01*	0.30	
Fe	SS	5417.33	482.75	969.03	270.17	13387.93	49.22	4172.11	253.05
	F	180.46	16.08	3.59		1005.21	3.70	16.49	
	p	0.00	0.00+	0.04*		0.00	0.07**	0.00+	
Mn	SS	2633.96	4.46	60.86	69.35	4538.57	0.21	631.12	263.77
	F	341.81	0.58	0.88		326.93	0.01	2.39	
	p	0.00	0.47	0.58		0.00	0.90	0.03*	
Cd	SS	2615.37	134.32	869.51	1043.28	1370.05	0.08	2391.95	170.27
	F	22.56	1.16	0.83		152.88	0.01	14.05	
	p	0.00	0.31	0.60		0.00	0.93	0.00+	
Cr	SS	5.46	0.35	9.60	9.96	1.65	0.32	24.88	1.27
	F	4.93	0.31	0.96		24.60	4.85	19.57	
	p	0.05	0.59	0.52		0.00	0.04*	0.00+	

+Significant at $p<0.01$ level, *Significant at $p<0.05$ level, **Significant at $p<0.10$ level,.
DOF – degrees of freedom, SS - sum of squares, F - F- test, p - p – value.

Standard score analyses could be used as a reference for developing strategies for improving the genotype characteristics (regarding the mineral contents). Scores above 0.7 stand for a high standard of mineral content in soft wheat lines. The best overall SS values in year 2012's were observed for the lines PZA 5, PZA 6 and PZA 9 (SS=0.74), while the best standard scores in year 2013's were noticed for PZA 8 and PZA 9 (recorded SS values were 0.70 and 0.71, respectively), Fig. 1. The dashed line in Fig. 1 represents the overall SS limit of 0.7; samples located above this line should be considered as optimal regarding the mineral content of samples.

Figure 1. Standard score for 10 lines

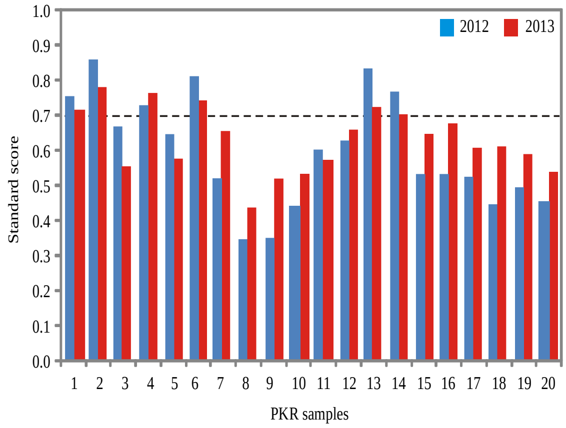

Figure 2. Standard score for 20 accessions

The best standard scores among 20 accessions of soft wheat were observed for samples PKR 1, PKR 2, PKR 4, PKR 6, PKR 13 and PKR 14, for both years. The best results were obtained for PKR 2, and the gained values for SS were 0.86 and 0,78, in year 2012's and 2013's, respectively, Fig. 2. The PCA allows a considerable reduction in a number of variables and the detection of structure in the relationship between measuring parameters and different varieties of wheat that give complimentary information. The number of factors retained in the model for the proper classification of experimental data, in original matrix into loading (wheat cultivars) and score (trace elements content) matrices was determined by the application of Kaiser and Rice’s rule (Bodroža – Solarov et al., 2014). This criterion retains only principal components with Eigenvalues >1. The full autoscaled data matrix consisting of 10 different lines and 20 accessions of soft wheat, during the 2012 and 2013 year, is submitted to the PCA. For visualizing the data trends and the discriminating efficiency of the used descriptors a scatter plot of samples using the first two principal components (PCs) issued from PCA of the data matrix is obtained (Fig. 3).

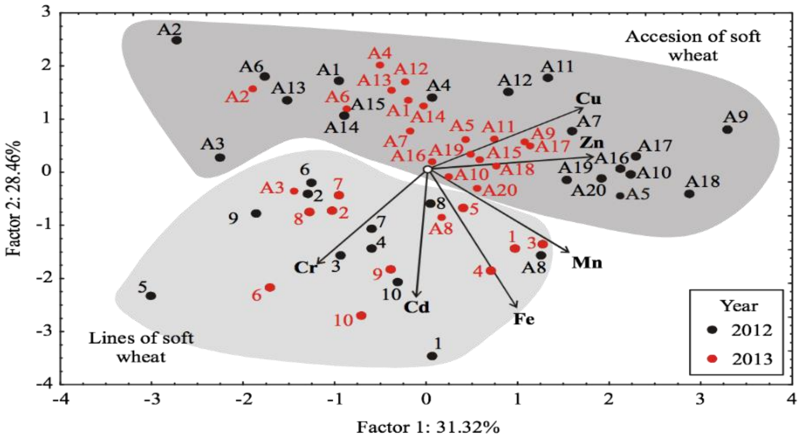

Figure. 3. Biplot for metallic trace element contents in Albanian wheat cultivars

As can be seen, there is a neat separation of different lines and accessions of wheat, according to the applied assays for metallic trace elements content. Different lines of wheat are located at the bottom part of the graphic, while the accession of soft wheat are positioned at the upper part of the graphic.The samples with the augmented mineral content are situated at the right side of the PCA plot, while the samples having the low mineral contents (and also the high SS values) are located at the left side of the graphic. Quality results show that the first two principal components, accounting for 59.78% of the total variability can be considered sufficient for data representation. PC1 correlated well with Cu (variable contributed 26.2% for PC1 calculation), Zn (30.1%), Mn (21.9%) and Cr content (13.1%), while the second component, PC2, correlated with Fe (34.5%), Mn (12.3%) and Cd content (29.7%).

Conclusions

The content of microelements Cu, Fe, Mn, Cd, Zn, Cr in the grain is dependent on the specific genotype and the soil characteristic. The results of this investigation showed that the cultivars growing under organic cultivation have high levels of mineral concentration (Cu, Zn, Fe, Mn). The concentration of Cd in all studied samples was below the EU limits and the concentration of Cr was in the low range (0.27 – 3.96 mg/kg for lines and 0.05 – 3.73 mg/kg for accessions).The two way ANOVA test, show statistically significant differences for elements content between almost all wheat lines /accessions and between years, at $p<0.05$ and $p<0.01$ level; 95%, 91%, confidence limit, respectively. The significant diversity between genotypes suggests the potential to develop cultivars with better ability to accumulate important micronutrients in the grain. In conclusion, we can say that the levels of heavy metals obtained in all wheat samples are within the acceptable range and do not pose any health hazard to the health of their consumers. Albanian wheat samples analyzed in this research are a good source of minerals and trace elements and by using suitable growing conditions, it is possible to improve their quality.

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