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RELATION BETWEEN SELECTED HEAVY METAL CONCENTRATIONS IN WHEAT FROM GROWING REGIONS OF SERBIA

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Abstract. The content of heavy metals in representative wheat samples taken from all important wheat growing regions of Serbia was analysed. Concentration of 8 trace elements in 14 wheat samples was determined by atomic absorption spectrophotometric technique. The obtained results were compared with the allowed maximum concentration given by the National Regulation and with literature published data. The data were interpreted using multiple linear correlation analysis.

Keywords: wheat, heavy metals, atomic absorption spectroscopy, multiple linear correlation.

AIMS AND BACKGROUND

Microelements are essential components of plant metabolism and often accumulate in seeds. They are important in plant growth and development either as essential nutrients or through their effect on enzyme systems. The mineral contents of cereal grains are affected by a number of factors including soil, climate and cultural practices. Genotypical and environmental effects influence wheat mineral contents. Generally, heavy metals are usually not taken up by crop plants except in small quantities because of their limited (Mn, Zn, Ni, Cu, Fe, Mo) or absent (Pb, Hg, Cr, Cd) physiological relevance[1].

The objective of this study was quantitative determination of heavy metals in wheat grain samples representative for various wheat growing regions (districts) of Serbia, harvested in 2002. The following eight elements were analysed: Cu, Mn, Zn, Fe, As, Pb, Cd, and Hg. Due to interpreting the data the Pearson correlation matrix was computed.

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EXPERIMENTAL

Plant sampling. The representative samples of wheat grain were collected from 14 important wheat growing regions of Serbia (Fig. 1), during the harvest in 2002.

Chemical analysis. The wheat grain samples were dried to constant mass at 105°C, then were grounded and homogenised. 0.5 g subsample was dissolved with 10 ml of concentrated nitric acid and heated. After dissolution, 10 ml of concentrated perchloric acid were added and heated until the formation of nitrous fumes stopped. The solution was placed in a 50-ml volumetric flask made up to volume with deionised water Milli-Q™ type (18 μS).

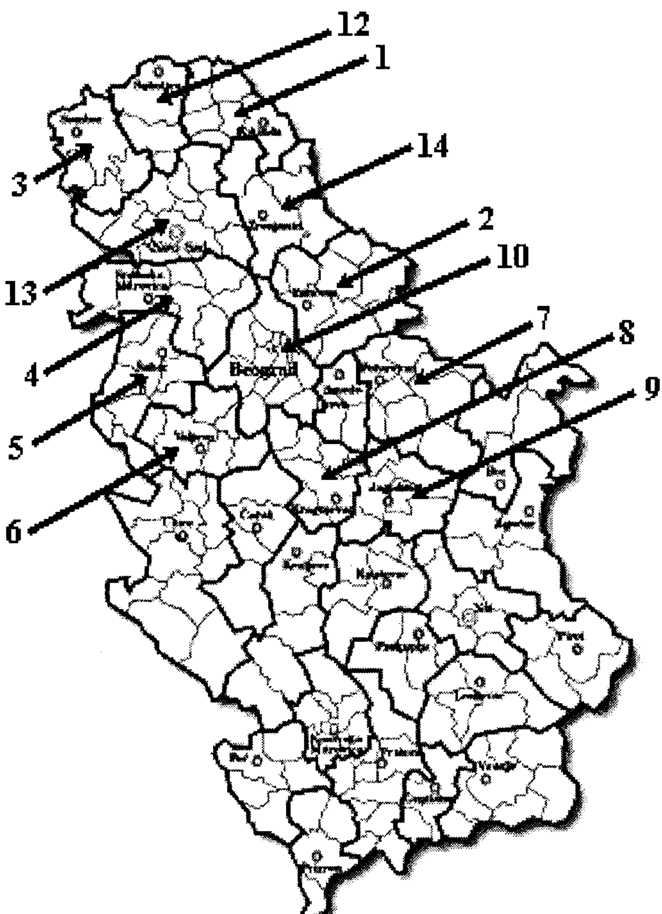

Fig. 1. Districts in Serbia for which representative samples of wheat grains harvested in 2002 were analysed (number and names of districts are given in Table 1)

Samples were analysed depending on the type of elements and their concentration by a Perkin–Elmer atomic absorption spectrometer (AAS) model 5000. Software HG Graphics II (Perkin–Elmer, Norwalk, CT, USA) was employed for spectrum acquisition and data processing.

Fe and Zn concentrations were determined involving direct aspiration of the aqueous solution into air-acetylene flame AAS. Heavy metals like Pb, As, Cd, Cu, and Mn were determined by flameless atomic absorption spectrophotometer equipped with HGA 400 heated graphite atomisers, and deuterium arc background correction. The operating conditions were based on those suggested by the manufacturer. The analyses were conducted in an argon atmosphere using a deuterium light source. The wavelengths for Pb, As, Cd, Cu and Mn analyses were 283.3, 193.7, 228.8, 324.7 and 279.5 nm, respectively. The atomising temperature was 1800°C (Pb), 2300°C (As and Cu), 1600°C (Cd) and 2200°C (Mn). The injected sample volume were 20 μl. Each recording was repeated three times. Total mercury content was done by cold vapour atomic absorption spectrophotometry using mercury hydride system MHS-1.

Quantification was carried out using appropriate calibration curves prepared with standard metal solutions in the same acid matrix. The detection limits for these analytical techniques for each element are: 0.003 mg/l for Fe, 0.001 mg/l for Zn and 0.05 μg/l for Pb, 0.2 μg/l for As, 0.003 μg/l for Cd, 0.02 μg/l for Cu and 0.001 μg/l for Mn and Hg.

Data analysis. Descriptive statistical analysis and multiple linear correlation were done through using MS Excel.

RESULTS AND DISCUSSION

Wheat grain analysis. Contents of examined heavy metals in wheat samples representative for 14 districts in Serbia harvested in 2002, are presented in Table 1.

Grain concentrations of elements concerned, especially of Hg and As, varied widely between sites. The maximum levels of Cd, Pb, Fe, Mn, Cu, and Zn were up to 105, 15, 3, 2, 2, and 2 times higher than their minimum levels of wheat grain elements studied, respectively. The most frequently occurring pattern for wheat is Fe > Mn > Zn > Cu > Pb > As > Cd > Hg.

Descriptive statistics of the raw set are summarised in Table 2. In the statistical calculations half of the detection limit value was substituted for sample results that were below the detection limit[2].

Table 1. Content of heavy metals in wheat grain samples representative for 14 districts in Serbia harvested in 2002

No of sample	Cu (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	Fe (mg/kg)	Pb (µg/kg)	Hg (µg/kg)	Cd (µg/kg)	As (µg/kg)	District
1	5.60	33	48.2	165	353	<0.1	30.2	123	Northern Banat
2	5.76	30.1	47.2	155	291	<0.1	30.9	162	Southern Banat
3	6.11	31.2	49.5	61.1	378	<0.1	27.5	91.6	Western Backa
4	5.86	30.4	44.7	67.1	213	<0.1	24.0	79.9	Srem
5	4.91	32	46.6	105	119	<0.1	13.9	147	Macva
6	5.48	36.5	56.1	70.1	233	<0.1	36.6	60.9	Kolubara
7	6.27	41.6	45.8	63.6	1099	<0.1	50.6	<20	Branicevo
8	5.27	32.1	50	53.1	295	<0.1	252	158	Sumadija
9	4.71	34.6	48.7	56.5	186	27.9	13.1	158	Pomoravlje
10	3.61	27.6	37	54.2	826	<0.1	4.1	<20	Belgrade
11	4.98	34.7	59.8	60.9	609	57.6	21.6	<20	Bijeljina
12	4.12	29.6	53	67.4	157	53.5	5.7	41.2	Northern Backa
13	3.83	26.6	38.3	51.7	286	<0.1	2.4	52.6	Southern Backa
14	7.64	44.3	88.4	99.3	74.8	<0.1	23.5	<20	Middle Banat

Table 2. Descriptive statistics of trace elements concentration in the analysed wheat grain samples (n=14)

Statistic measure	Cu (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	Fe (mg/kg)	Pb (µg/kg)	Hg (µg/kg)	Cd (µg/kg)	As (µg/kg)
Mean	5.30	33.2	50.95	80.7	366	9.97	38.3	79.6
Median	5.375	32.05	48.45	65.35	288.5	0.05	23.75	70.4
Standard deviation	1.06	4.97	12.3	37.2	289	20.7	62.9	60.5
Skewness	0.355	1.07	2.29	1.61	1.65	1.88	3.45	0.20
Range	4.03	17.7	51.4	113.3	1024	57.55	249.6	152.4
Minimum	3.61	26.6	37	51.7	74.8	0.05	2.4	10
Maximum	7.64	44.3	88.4	165	1099	57.6	252	162.4

It can be clearly observed that the contents for Cu and As in grain from various districts were distributed normally, but the values of the other examined elements were positively skewed, i.e. Fe, Pb, Hg, Cd, Mn, and Zn contents differed markedly between grain samples harvested in various districts.

Allowed maximum concentrations of As, Pb, Hg and Cd (mg/kg dry matter) in wheat in Serbia[3] are, respectively, 1, 0.4, 0.05 and 0.1. Permissible concentration levels of Pb, Cd, Cu and Zn (mg/kg dry matter) in cereals in Bulgaria[4] are, respectively, 0.5, 0.1, 10 and 40.

Generally, contents of As, Pb, Hg and Cd in wheat grain were below the allowed maximum concentration given by the National Regulation (Table 1), except for Pb in Branicevo, Belgrade and Bijeljina regions; for Hg in Bijeljina and Nothern Backa and for Cd in Sumadija. Level of Mn (Middle Banat) and Fe (Northern and Southern Banat) could be explained by the possible high level of these metals in the soil where wheat was grown. Higher concentrations of Zn (Branicevo, Middle Banat) referring to the Bulgarian Regulation imply the utilisation of Zn containing plant protection agrochemicals. The samples collected close to the urban areas (Belgrade and Bijeljina) showed the high levels of Pb, which could be attributed to the vicinity and the intensity of the traffic, while sample from Branicevo with maximum Pb content was not harvested in the vicinity of urban area and possible explanation of this could be higher natural (background) soil content of Pb in this region. Accumulation of Cd (Sumadija) in crops could be attributed to phosphate fertiliser.

Heavy metals contents in wheat grains taken from different literature sources are presented in Table 3. Levels of trace elements found in this study are generally similar with those reported in literature.

Taking into account contents of heavy metals in polluted area in Bulgaria[4] the authors concluded that higher concentrations of Cu and Zn imply the utilisation of Cu and Zn containing plant protection agrochemicals, while higher contents of Pb and Cd in wheat grain were due to high level of these metals in the soil where plants were grown.

Nan et al.[13] found enhanced level of Cd and Pb in wheat grain samples harvested in Gansu province (China) due to growing on the cultivated land contaminated by waste water irrigation or aerial deposition from local non-ferrous mining and smelting production.

Various environmental effects, such as soil pH, cation exchange capacity, organic matter content, soil total metal content, chemical species of a certain element, etc., influence on crop metal intake. The lower pH and CEC effect the greater plant accumulation of heavy metals. Organic matter can regulate the availability of heavy metals through chelation reactions, in which the metals may form stable 5- and 6-membered ring structures with carboxyl and hydroxyl functional groups of organic aggregates, thus becoming a part of the solid phase that is unavailable to plants[14]. The influence of crop management practices such as nitrogen (N) fertilisation on the mineral concentration of wheat are poorly understood[15-17]. Zebarth et al.[17] found that the increased N application enhanced the average Fe concentration (by 21%) in wheat grains, but had no significant

Table 3. Contents of heavy metals (mg/kg d.m.) in wheat samples – comparison of this work with literature data

Wheat samples	Fe	Mn	Cu	Zn	As	Pb	Hg	Cd
Harvest 2002, Serbia (this work)	51.7-165	37.0-88.4	3.61-7.64	27.6-44.3	<0.02-0.162	0.075-1.10	<0.0001-0.058	0.002-0.252
Harvest 2001, Serbia (Skrbic et al.[5])	22.5-244.1	21.2-42.1	3.15-7.20	22.63-82.64	<0.02-0.330	<0.005-0.197	<0.0001-0.009	0.013-0.076
Vojvodina (Saric et al.[6])	36.3-52.0	23.1-28.0	3.8-4.3	44.1-62.0	0.00073-00362	<0.05-0.06	<0.001	0.0305-0.050
Novi Sad, Vojvodina (Kastori et al.[7])	43.3-55.8	41.7-47.4	4.2-5.1	16.8-30.6	–0.154	–0.304		–0.020
Bulgaria (Bojinova et al.[4])								
polluted area			7.28-11.0	38.1-83.6		0.25-18.5		0.10-1.6
unpolluted area			4.75-7.50	28.3-53.1		0.06-0.40		0.02-0.14
USA (Erdman and Moul[8])			2.6-5.8	17-37				0.042-0.25
USA* (Wolnik et al.[2,9])	17-60	13-67	2.2-8.7	9.3-67		<0.0008-0.716		<0.0017-0.207
UK (McGrath[10])	28.6-74.7	11.0-62.8	2.79-7.53	16.1-55.0				
Netherland* (Wiersma et al.[11])					0.005-0.285	0.03-0.65	<0.0001-0.029	0.02-0.35
Italy (Cubadda et al.[12])	34.5	38.7	4.65	29.5	0.010	0.012		0.014
China (Nan et al.[13])			1.69-10.48	17.12-70.45		0.06-18.49		0.01-1.19

*Values given per wet weight.

effect on Zn and Mn concentrations. Other studies on wheat have also reported increased N application to result in higher concentration of Fe[15,16]. As well it is well known fact that the uptake of some elements by crops correlates with total soil contents of heavy metals. Nan et al.[13] found strong relationship between wheat grain Cd content and total content of Cd, Pb, Zn, and Cu in soil.

Chemical interaction that takes place in soil with alkaline pH, high CEC and high $CaCO_3$ content makes Cd in a bound form and less available for plant uptake. A significant enhancement in the Cd concentrations of maize plants was obtained due to the acidic pH and low CEC soil conditions[14]. Andersson and Nilsson[18] have reported the competition between Cd^{2+} and Ca^{2+} ions (originated from $CaCO_3$) at root surfaces and subsequent formation of $CdCO_3$ due to which there was less Cd uptake from soils. Arsenate is a chemical analogue of phosphate, so they can be taken up by the same plant uptake system[19].

The Pearson correlation matrix was computed to interpret the data. For correlation significance, the criteria values of probabilities ($p<0.05$ and $p<0.01$) are used. It was observed a strong positive correlation between Mn, Zn, and Cu in relation to the data (Table 4), which means that these heavy metals can be considered to be similar variables.

Table 4. The Pearson correlation matrix for heavy metal content in wheat grains

Elements	Cu	Fe	Pb	Hg	Cd	As	Mn	Zn
Cu	1.000							
Fe	0.342	1.000						
Pb	−0.099	−0.236	1.000					
Hg	−0.340	−0.252	−0.028	1.000				
Cd	0.146	−0.147	0.007	−0.201	1.000			
As	−0.027	0.402	−0.483	−0.238	0.363	1.000		
Mn	0.681*	0.141	−0.350	0.168	0.020	−0.280	1.000	
Zn	0.766*	0.076	0.124	−0.046	0.074	−0.298	0.743*	1.000

* Correlation is significant at the 0.01 and 0.05 level (2-tailed)

This also means that there is no need to measure and evaluate all of the variables to achieve the same characterisation in further studies. It is enough to measure one variable per group. Significant correlation for Cu and Zn in wheat grain was also previously found in study of Nan et al.[13] This phenomenon may be due to the metabolic processes of the crop as these elements are essential to plants although they may be toxic beyond their required concentrations.

CONCLUSIONS

The heavy metals have been analysed in average samples of wheat, harvest 2002, that were taken from several parts of Serbia. Generally, it could be concluded that the flour produced from wheat cultivated in Serbia can be used as healthy safe food taking into consideration the levels of heavy metals constituents. In the case of higher contents for trace elements, it would be possible to reduce their concentrations below the allowed content by using proper way of mixing.

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REFERENCES

1. C. ANGLANI: Wheat Minerals. A Review. Plant Foods Human Nutrition, **52**, 177 (1998).
2. K. A. WOLNIK, F. L. FRICKE, S. G. CAPER, G. L. BRAUDE, M. W. MEYER, R. D. SATZGER, R. W. KUENNEN: Elements in Major Raw Agricultural Crops in the United Stated. 1. Other Elements in Lettuce, Peanuts, Potatoes, Soybeans, Sweet Corn, and Wheat. J. Agric. Food Chem., **31**, 1244 (1983).
3. National Regulations: Contents of Pesticides, Metals, Metalloids and Other Contaminants Which Can Be in Foods, **5**, 67 (1992) (in Serbian).
4. P. BOJINOVA, B. GEORGIEV, I. KABAKCHIEV, V. KRASTEVA, L. STANISLAVOVA, H. TCHULDJIAN, G. WELP, G. BRUMMER: Harmonization of the Methods for the Investigation of Heavy Metal Pollution of Soils and the Standardization of the Assessment Criteria for Soil Protection. Report No UBA-FB 96-071 (Ed. K.Terytze). N. Poushkarov Institute of Soil Science and Agroecology, Sofia and Institut fur Bodenkunde, Bonn, 1996.
5. B. SKRBIC, S. CUPIC, J. CVEJANOV: Determination of Heavy Metals in Wheat Samples. Physiology, **12** (4), 12 (2002).
6. M. SARIC, Dj. PSODOROV, M. ZIVKOV-BALOS, Z. MIHALJEV: Processing Quality and Safety of Wheat Originating from Different Growing Regions. Zito-hleb, **29**, 205 (2002) (in Serbian).
7. R. KASTORI, I. MOLNAR, P. SEKULIC, I. ARSENIJEVIC-MAKSIMOVIC: Contents of Essential and Predominantly Toxic Elements in Wheat Grown on Chernozem. In: EKO Conference of Safe Food, September 25-28, Novi Sad, 2002, 251-256 (in Serbian).
8. J. A. ERDMAN, R. C. MOUL: Mineral Composition of Small-grain Cultivars from a Uniform Test Plot in South Dakota. J. Agric. Food Chem., **30**, 169 (1982).
9. K. A. WOLNIK, F. L. FRICKE, S. G. CAPER, G. L. BRAUDE, M. W. MEYER, R. D. SATZGER, E. BONNIN: Elements in Major Raw Agricultural Crops in the United Stated. 1. Cadmium and Lead in Lettuce, Peanuts, Potatoes, Soybeans, Sweet Corn, and Wheat. J. Agric. Food Chem., **31**, 1240 (1983).
10. S. P. McGRATH: The Effects of Increasing Yields on the Macro- and Microelement Concentrations and Offtakes in the Grain of Winter Wheat. J. Sci. Food Agric., **36**, 1073 (1985).
11. D. WIERSMA, B. J. van GOOR, N. G. van der VEEN: Cadmium, Lead, Mercury and Arsenic Concentrations in Crops and Corresponding Soils in The Netherlands. J. Agric. Food Chem., **34**, 1067 (1986).
12. F. CUBADDA, M. BALDINI, M. CARCEA, L. A. PASQUI, A. RAGGI, P. STACCHINI: Influence of Laboratory Homogenization Procedures on Trace Element Content of Food Samples:

An ICP-MS Study on Soft and Durum Wheat. Food Addit. Contam., **18**, 778 (2001).
13. Z. NAN, C. ZHAO, J. LI, F. CHEN, W. SUN: Relation between Soil Properties and Selected Heavy Metal Concentration in Spring Wheat (*Triticum aestirum* L.) Grown in Contaminated Soils. Water Air Soil Pollut., **133**, 205 (2002).
14. V. RAMACHANDRAN, T. J. D'SOUZA: Plant Uptake of Cadmium, Zinc, and Manganese in Soils Amended with Sewage Sludge and City Compost. Bull. Environ. Contam., **61**, 347 (1998).
15. M. M. AGARWAL: Effect of Fertilizer Treatments on the Grain Quality of Wheat. Indian. J. Agric. Res., **10**, 185 (1976).
16. P. W. SYLTIE, W. C. DAHNKE: Mineral and Protein Content, Test Weight, and Yield Variations of Hard Red Spring Wheat Grain as Influenced by Fertilization and Cultivar. Qual. Plant. Plant. Foods Hum. Nutr., **32**, 37 (1983).
17. B. J. ZEBARTH, C. J. WARREN, R. W. SHEARD: Influence of the Rate of Nitrogen Fertilization on the Mineral Content of Winter Wheat in Ontario. J. Agric. Food Chem., **40**, 1528 (1992).
18. A. ANDERSSON, K. O. NILLSON: Influence of Lime and Soil pH on Cadmium Availability to Plants. Ambio, **3**, 198 (1974).
19. A. MEHARG, J. NAYLOR, M. MACNAIR: Phosphorus Nutrition of Arsenate-tolerant and Nontolerant Phenotypes of Velvetgrass. J. Environ. Qual., **23**, 234 (1994).

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