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Principal Component Analysis of Trace Elements in Serbian Wheat

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Trace elements (Cu, Fe, Pb, Hg, Cd, As, Mn, Zn) were analyzed quantitatively in 14 wheat samples collected from fields in all Serbian growing regions, harvested in 2002. Microelements were determined according to an atomic absorption spectrophotometric method. Principal component analyses (PCA) were performed on data matrices consisting of contents of trace elements in wheats (columns) and all Serbian wheat-growing regions (rows). It was found that four principal components account for 87.2% of the total variance in the data. The plot of component loadings showed significant groupings for concentration of some microelements. The component scores indicated the similarities among the Serbian wheat-growing regions. The loading plot reveals that there is no need to measure all of the variables to achieve the same classification. It is enough to measure one variable per group. Naturally, this conclusion is valid only within the limits of the present study of wheat grain samples from different parts of Serbia.

KEYWORDS: Trace elements; wheat; principal component analysis

INTRODUCTION

Human health is directly affected by the environment. The number and amount of toxic substances entering the environment have increased due to the expansion of transportation activities in the past few decades. The contamination of soil by metallic elements and/or their aerial deposition is likely to result in a corresponding contamination of harvested crops and the food consumers eat (*1*−*3*).

Therefore, there is international concern about human intake of microelements. There are three groups of mineral elements interesting to food technologists and scientists and nutritionists (*4*, *5*): those essential in the diets of humans (Cu, Ca, Fe, Mg, etc.); those essential to one or more species and plants but not currently known to be essential for humans (As, Cd, Ni, etc.); and those known only for their toxicity or therapeutic use (Al, Ba, Hg).

It should be stated that everything is toxic—only the dose makes something not toxic. The boundary separating the essentiality of an element from toxicity depends on the concentration of the element and its quantity of dietary intake. Therefore, the ability of trace elements to cause harmful effects, both through deficiency and through excess, separates them from other known toxicants in foods and highlights the importance of the danger they present to food safety.

Since 1973 As, Cu, Cd, Fe, Hg, Pb, and Zn have been considered to be potentially toxic in the human diet by the joint FAO/WHO Codex Alimentarius Commission, with As (as arsenite) being carcinogenic (*6*). Intake of relatively low doses of these elements over a long period can lead to malfunction of organs and chronic toxicity.

Serbian legislation (*7*) sets maximum levels for only four elements (As, Pb, Hg, and Cd) in wheat grain. The maximum established concentrations for As, Pb, Hg, and Cd are 1, 0.4, 0.05, and 0.1 mg/kg of dry matter, respectively.

In a number of countries a survey of toxic elements in wheat grain has been carried out (*1*, *2*, *8*−*14*).

This study could be considered as a typical example of the role of a food technologist working on the minimization of food contaminants by identifying the levels of microelements in wheat grains.

Furthermore, the aim of the present work was to apply chemometric techniques to characterize the wheats from the different growing regions of Serbia (geographic origin) on the basis of microelement contents. Moreover, we would like to decrease the number of measured quantities while preserving the same level of characterization, that is, to determine which investigated microelements are responsible for an appropriate classification.

EXPERIMENTAL PROCEDURES

Wheat Grain Samples. Fourteen wheat grain samples of the harvest of 2002 representing all wheat-growing regions in Serbia were investigated. Grain samples were collected within the framework of the yearly monitoring of wheat quality during the harvest period by the National Center for Cereal Technology—Novi Sad.

For the purpose of this study the Serbian territory was subdivided into its 14 administrative wheat-growing regions. Regions were in turn subdivided into provinces. Within each province having a significant wheat production, samples of grains were collected in different

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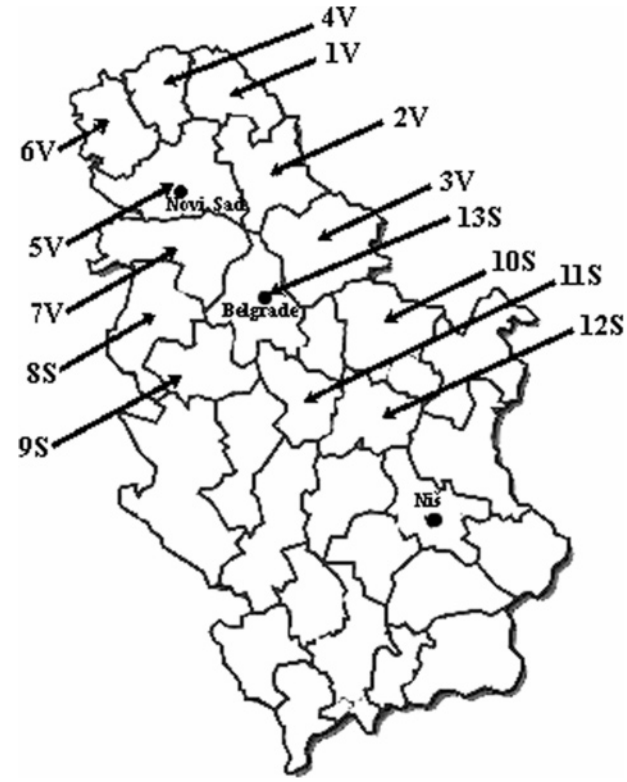

Figure 1. Origins of the samples.

Table 1. Wheat-Growing Regions in Serbia and the Number of Wheat Grain Samples Collected from All Representative Fields in the Regions

sample	region	no. of samples	sample	region	no. of samples
1V	northern Banat	33	8S	Mačva	30
2V	central Banat	31	9S	Kolubara	26
3V	southern Banat	34	10S	Braničevo	29
4V	northern Bačka	35	11S	Šumadija	25
5V	southern Bačka	39	12S	Pomoravlje	28
6V	western Bačka	36	13S	Belgrade	25
7V	Srem	40	14S	Bijeljina	22

representative fields located in the whole provincial territory during the harvest period in the following way:

• Samples from the fields representative for one province of the region were collected during a day and mixed to form the daily samples of the representative fields.

• Subsamples from the daily samples of the representative fields obtained during harvest were mixed to form the individual sample of the province.

Individual samples from the provinces of one region, each weighing ∼1 kg, were pooled together to obtain the test samples of the regions. Therefore, 14 test samples (coming from the total of 433 samples) obtained in the described way represented the average quality of the wheat grain cultivated in each of the 14 administrative Serbian growing regions.

The selected representative fields of the province accounted for the majority of wheat cultivated in Serbia, and for each of them the relative number of samples [according to the national legislation (*15*)] was proportional to its percentage of the national production. The origins of the samples are presented in **Figure 1**.

The wheat-growing regions of Serbia are listed in **Table 1** with the number of samples collected per each region.

Analytical Methods. Aliquots from the pooled test grain samples were manually cleaned and milled in a laboratory mill (Bühler MLU 202) to obtain a fine wholemeal flour. Flours were stored in clean polyethylene flasks at 5 °C pending analysis.

Table 2. Content of Microelements in Representative Wheat Grain Samples from 14 Regions of Serbia Harvested in 2002

sample	Cu, mg/kg	Fe, mg/kg	Pb, μg/kg	Hg, μg/kg	Cd, μg/kg	As, μg/kg	Mn, mg/kg	Zn, mg/kg
1V	5.6	165	352.6	<0.1	30.2	123.1	48.2	33
2V	7.64	99.3	74.8	<0.1	23.5	<20	88.4	44.3
3V	5.76	155	291	<0.1	30.9	162.4	47.2	30.1
4V	4.12	67.4	157	53.5	5.7	41.2	53	29.6
5V	3.83	51.7	286	<0.1	2.4	52.6	38.3	26.6
6V	6.11	61.1	378	<0.1	27.5	91.6	49.5	31.2
7V	5.86	67.1	213	<0.1	24	79.9	44.7	30.4
8S	4.91	105	119	<0.1	13.9	147	46.6	32
9S	5.48	70.1	233	<0.1	36.6	60.9	56.1	36.5
10S	6.27	63.6	1099	<0.1	50.6	<20	45.8	41.6
11S	5.27	53.1	295	<0.1	252	158	50	32.1
12S	4.71	56.5	186	27.9	13.1	158	48.7	34.6
13S	3.61	54.2	826	<0.1	4.1	<20	37	27.6
14S	4.98	60.9	609	57.6	21.6	<20	59.8	34.7

Dry matter determinations were performed for each sample on separate 10 g aliquots by oven-drying to constant mass at 105 °C.

A subsample of fresh wholemeal flour (∼0.5 g on dry matter basis) was dissolved with 10 mL of concentrated nitric acid and heated under reflux. After dissolution, 10 mL of 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 losses of As and Hg (the range of temperature was from 70 to 85 °C). The solution was placed in a 50 mL volumetric flask and made up to volume with deionized water of Milli-Q type (18 μS).

All of the chemicals used in the sample treatments were of ultrapure grade. The glassware was cleaned prior to use by soaking overnight with 10% v/v HNO_3 (Suprapur, Merck) and rinsed with Milli-Q water.

Samples were analyzed 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) was employed for spectrum acquisition and data processing.

Fe and Zn concentrations were determined by direct aspiration of the aqueous solution into air−acetylene flame AAS. Microelements such as Pb, As, Cd, Cu, and Mn were determined by a flameless atomic absorption spectrophotometer equipped with HGA 400 heated graphite atomizers and deuterium arc background correction. The operating conditions were based on those suggested by the manufacturer and were presented elsewhere (*16*). Total Hg content was analyzed by cold vapor atomic absorption spectrophotometry using a vapor generation accessory.

Quantification was carried out using appropriate calibration curves prepared with standard metal solutions in the same acid matrix. Each measurement was repeated twice and the average taken. The absorption signal of the sample was evaluated after subtraction of the mean value of the blank. The detection limits for these analytical techniques for each element are as follows: 0.003 mg/L for Fe, 0.001 mg/L for Zn, 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. The measured data are presented in **Table 2**. The validity of the applied method was confirmed by participation in an interlaboratory comparison program involving 31 laboratories organized and conducted by the Swiss Agency for Development and Cooperation (*17*). The obtained results fall within ±2 times the standard deviation, which can be considered as good agreement.

Principal Component Analysis (PCA). PCA is a powerful tool for pattern recognition, classification, modeling, and other aspects of data evaluation (*18*−*21*). Also, PCA is a projection method, and dimension reduction of the data can be achieved using a smaller number of principal components than original variables.

In summary, PCA decomposes the original matrix into several products of multiplication into loading (microelements) and score (wheat-growing regions of Serbia) matrices.

Microelements are taken as variables (column of the input matrix) and all wheat-growing regions from Serbia as mathematical−statistical cases (rows of the matrix).

Table 3. Results of Principal Component Analysis for Microelements in Wheat Grain Samples: Varimax Rotated Principal Component Loadings

microelement	PC1	PC2	PC3	PC4
Cu	**0.8677**	0.0543	0.3851	0.0647
Fe	0.1696	0.4675	0.6190	−0.4103
Pb	−0.1182	**−0.9091**	0.1403	0.0227
Hg	−0.0270	0.0812	**−0.8550**	−0.1969
Cd	0.0733	0.0884	0.1092	**0.9453**
As	−0.2940	**0.7250**	0.3925	0.3282
Mn	**0.9195**	0.2099	−0.2416	−0.0622
Zn	**0.9161**	−0.2068	0.0499	0.0461
explained variance	2.6046	1.9700	1.3116	1.0896
proportion of total variance, %	32.6	24.6	16.4	13.6

PCA will show which kinds of microelements and which wheat-growing regions are similar to each other, that is, carry comparable information, and which ones are unique. An assumption was made during the analysis, namely, that microelements express important features of the wheat-growing regions of Serbia.

The following data set was analyzed: the concentrations of the following microelements (**Table 2**) (columns of the input matrix) were ordered as variables: Cu, Fe, Pb, Hg, Cd, As, Mn, and Zn. Half of the detection limit value was substituted for sample results that were below the detection limit (*9*). Fourteen wheat-growing regions from Serbia were arranged in rows of the input matrix. The notations were 1V−7V for 7 wheat-growing regions from Vojvodina (northern Serbia) and 8S−14S for 7 from central Serbia (**Table 1**). First, the data were mean-centered (column means were subtracted from each matrix element). Then each matrix element was divided by the standard deviation of the respective column. In this way, the correlation matrix was established. Thus, the data were centered to zero mean and scaled to unit variance. The obtained 8 × 14 matrix was investigated by PCA.

The algorithm of PCA can be found in the standard chemometric articles and textbooks (*22*, *23*).

RESULTS AND DISCUSSION

The contents of examined microelements in representative wheat samples collected from fields in all of the Serbian growing regions in 2002 are presented in **Table 2**.

The ranges of average values for wheat samples harvested in 2002 were as follows: Cu, 4−8 ppm; Fe, 52−165 ppm; Pb, 75−1099 ppb; Hg, <0.1−58 ppb; Cd, 2−252 ppb; As, <20−162 ppb; Mn, 37−88 ppm; and Zn, 27−44 ppm. 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 in wheat grain samples studied, respectively. The most frequently occurring pattern for wheat is Fe > Mn > Zn > Cu > Pb > As > Cd > Hg. Also, it should be stressed that in some instances the contents of Pb (samples 10S, 13S, and 14S), Cd (sample 11S), and Hg (samples 4V and 14S) were higher than the ones set by Serbian legislation (*7*).

The number of factors retained in the model for proper classification of the data from **Table 2** were determined by application of Kaiser's rule (*24*). This criterion retains only principal components with eigenvalues >1. Therefore, four components having eigenvalues >1 were used for further analysis.

PCA yields four PCs explaining 87.2% of the total variance in the data. Loading values (i.e., correlation coefficients) >0.7000 (concentration of microelements) are marked throughout **Table 3** in boldface type.

The loadings express how well the new abstract principal components correlate with the old variables. The first new abstract principal component, PC1, correlates well with Cu, Mn, and Zn contents of the wheat grain samples. The second

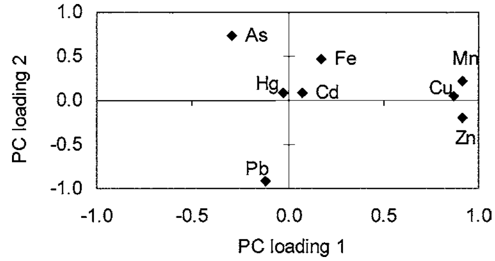

Figure 2. Varimax rotated principal component loadings (similarities of microelements), loading 1 versus loading 2.

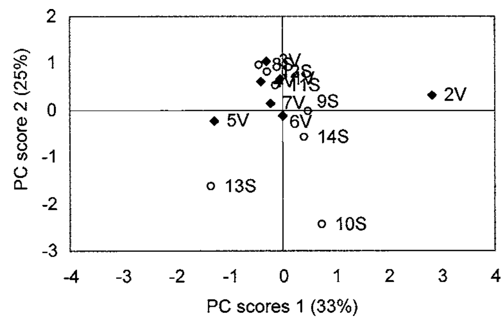

Figure 3. Rotated principal component scores (similarities of wheat-growing regions), score 1 versus score 2. (The explained variances are in parentheses.)

component, PC2, correlates with Pb and As. Pb correlates with the new PC negatively. The third one correlates with Hg negatively and the fourth one with Cd. Iron fails to load significantly on retained PCs.

Figure 2 shows the first two PCs loadings against each other. The correlation between Cu and Zn (or Mn) in relation to the data is unambiguous and significant. There is no need to measure and evaluate all of the variables to achieve the same classification of wheat samples in further studies. Copper carries almost the same information as Mn and Zn and has low loading on PC2 (close to 0). Therefore, it could be concluded that this element has no significant influence on the attained classification of the wheat samples. **Figure 2** also suggests that Hg and Cd are not needed to achieve the same classification, because their loadings are close to 0, but these elements have significant influence on PC3 and PC4, respectively, and they could not be omitted from further studies, especially because their toxicity is known.

The physical meaning of PC1 could be attributed to metabolic processes in wheat. Also, it is interesting to stress that microelements without known physiological relevance in wheat are distributed along PC1 in accordance with their decreasing toxicity defined by the 2003 CERCLA Priority List (*25*): As > Pb > Hg > Cd.

Microelement contents of wheat from all of the growing regions differ among themselves because of numerous factors including soil, climate, agrochemical practices, and proximity of industrial centers.

Figure 3 shows that according to the first two PCs the majority of the wheat-growing regions in Serbia are similar to each other. The points in anomalous positions 2V, 5V, 10S, and 13S are outliers: the wheat sample from region 2V has the highest Cu, Mn, and Zn contents, whereas sample 5V has the lowest contents of Fe, Cd, and Zn; wheat samples of regions 10S and 13S have the highest content of Pb.

On the second score plot (**Figure 4**), again the similarities among wheat-growing regions in Vojvodina and in the central part of Serbia are confirmed. The exceptions are wheats with

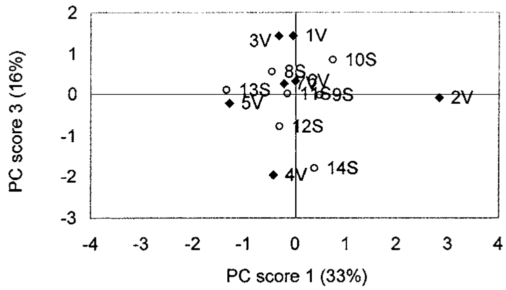

Figure 4. Rotated principal component scores (similarities of wheat-growing regions), score 1 versus score 3. (The explained variances are in parentheses.)

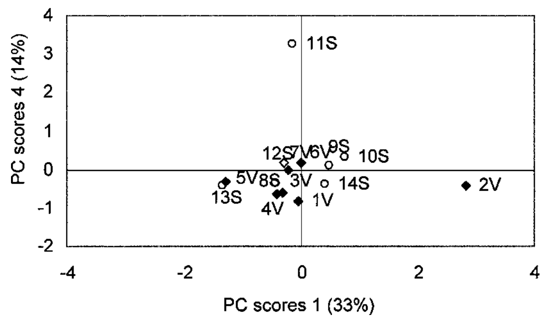

Figure 5. Rotated principal component scores (similarities of wheat-growing regions), score 1 versus score 4. (The explained variances are in parentheses.)

the highest level of Hg from regions marked by 4V and 14S, and, again, sample 2V due to the maximum observed contents of Cu, Mn, and Zn.

The fourth principal component (PC4) classifies the wheat grain samples according to the Cd level, and only one point is separated from the others considering this element. The outlier point in **Figure 5** is sample 11S, with the maximum Cd content. Point 2V stands out because of its high Cu, Mn, and Zn contents.

Score plots show similarities among wheat-growing regions in Serbia that could be attributed to the factors influencing the soil quality. Namely, it is known that soils in all regions used for wheat-growing belong to calcereous type (alluvial soil) (*26*, *27*) with high pH values, which can effectively decrease metal bioavailability for plants.

On the other hand, the outliers that have been already mentioned in previously presented score plots (**Figures 3−5**) could be explained by the proximity of industrial centers and busy motorways (10S, 13S), excessive or inadequate application of plant protection agrochemicals with Zn or phosphatic fertilizers containing Cd (2V, 5V, 11S), and use of organomercury fungicides (4V, 14S).

It could be concluded that PCA is able to point out the outliers among the wheat samples from all the growing regions according to grain trace element contents. The loading plots reveal that there is no need to measure all of the variables to achieve the same classification, that is, Cu carries comparable information to Mn and Zn and has little influence on the attained wheat classification. Naturally, all of these statements are valid within the scope of the investigation; wheats from regions other than Serbia might behave differently.

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