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UNDESIRABLE METALS CONTENT IN WHEAT OF DIFFERENT WHEAT VARIETIES

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Selected samples of the different wheat varieties grown in the local region were tested for the content of undesirable metals in wheat grains, bran and flourdetermined by atomic absorption spectrophotometry. The results obtained show significant variations in undesirable metals content in different wheat grains varieties. These results were compared to the maximum values allowed by the pertinent regulations.

KEYWORDS: Undesirable metals, wheat, varieties, atomic absorbtion spectrophotometry

INTRODUCTION

Wheat is one of the most important foodgrains in the world. Apart from containing nutrients (high level of vitamins, minerals and cellulose fibers) wheat grain also contains a number of elements (Cu, Zn, Fe, Ni, Mn) vital to our biological functions, but hazardous to our health in high concentrations (1-3). It also contains some toxic elements (As, Pb, Hg, Cd) which CERCLA Priority List (4) from the year 2003 rated as the first, second, third and seventh in toxicity. There are three groups of minerals of interest to the food industry, science and nutrition specialists:

(a) essential to people (Cu, Ca, Fe, K and Mg)

(b) essential to plants and one or more animal species however not for humans (As, Cd, Ni, and others) and

(c) toxic or used in therapeutic dosages (Al, Ba, Hg).

Still, it would be important to emphasize that they are all toxic and what makes them non-toxic are the amounts (dosages) themselves. The fine line between the essential and toxic is relative to the amount and intake through food.

The quality of bread, pastries and other prouducts depends primarily on the quality of the flour as basic ingredient, that is the quality of the wheat variety as basic raw material (5, 6).

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The quality of wheat depends on the genotype, agricultural and environmental conditions as well as the genotype and environment interaction. The quality of wheat is greatly influenced by the genotype, and the genetic potential of a variety determines the diversity of the future use (for bread and pasta production, confectionary products and animal feed).

The undesirable metals originate from contaminated air and soil. These metals can get into the soil if crop protection chemicals containing such metals are used (7, 8). Excessive use of organic and chemical fertilizers, especially phosphorus-based ones, air sediments causes soil contamination.

The air in the industrial areas is one of the main sources of polution. The undesirable metals are absorbed by plants from the soil and in certain cases through the leaves. These elements are more concentrated in the root than in the above-ground parts (9, 10).

Dynamic development of the socio-economic relations in our country has been followed by a constant process of modern development and improvement of growing wheat and flour production. It has been of economical immportance from many aspects, most of all in view of food supply to the population as well as for the Republic strategic planning. Each citizen in the Republic consumes in average 350 g of bread a day. Considering that bread is consumed on daily basis, the emphasis should be put on its healthy production. If there are undesirable matters in bread, there is a risk of jeopardizing people's health. This may be prevented by way of constant quality control. In ideal circumstances, we should be able to meet all the requirements of our consumers' refined tastes. It goes without saying that huge efforts have been made in the field of refinement, but due attention should be paid to the environmental protection, to decrease as much as possible the presence of the undesirable metals (11-13).

The key problem tackled in this research is the analysis of 36 wheat samples divided into 5 sub-samples according to the variety.

EXPERIMENTAL

The content of undesireable metals was analysed in the variety Evropa (III tecnological group) - 6 samples; variety Renesansa (II technological group) – 9 samples, variety Pobeda (II techmological group) - 12 samples, variety Ljiljana (II tech. group) - 3 samples, and variety Pesma (upgrading variety) - 6 samples. This division into technological groups was made according to the data found in the relevant literature (13).

The samples were ground in the laboratory mill Buhler MLU 202, comprising three coarse grinding rolls and three milling rolls, thus producing three fractions of coarse grinding, i.e. coars ground flour, and three milled fractions, i.e. milled flour. Flour is produced trough homogenization of all six fractions flour, by-products being shorts and bran.

The Fe content was determined by flame atomic absorption spectrophotometry whereas Pb, Cd, Zn and Cu were assayed by flameless atomic absorption spectrophotometry (Perkin Elmer, model 5000), using electrothermal atomizer (HGA 400) and background emission correction (D2 lamp). The working conditions were as suggested by manufacturer: wave lengths 283.3, 228.8, 324.7 and 213.9 nm which were used for the analysis of Pb, Cd, Cu and Zn, respectively. Temperatures were: for Pb 1800ºC, Cu 2300ºC, Cd 1600ºC, Zn 2100ºC. Injected volume of the sample was 20 μl. The undesirable metal

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content was determined by the method of calibration curve based on the standard metal solutions. Detection limits of the instrument (instrument signal higher than the average values for the instrument noise three times the standard deviation for the instrument noise) were as follows: 0.003 μg/l for Fe; 0.001 μg/l for Zn; 0.005μg/l for Pb; 0.003 μg/l for Cd; 0.02 μg/l for Cu. Each of the tests was done in duplicate. Tests were conducted in argon atmosphere with the deuterium emission source.

The data were then processed by appropriate mathematical-statistical methods. The analysis was conducted in three stages: testing of the similarity or differences hypotheses and testing the significance of the difference.

This paper will show the descriptive parameters, average values, standard deviation (SD), minimum and maximum of all values, coefficient of variation (CV), confidence interval, and Kolmogorov- Smirnov test of normal distribution (14).

Multivariate methods and discriminative analysis Manova, as well as the univariante methods, ANOVA were used as mathematical methods for data processing.

The hypotheses for the methods used was done in the following manner:

MANOVA was used to test hypothesis H_1 stating:

H_1 There are no significant differences between the sub-samples for the tested whole.

A_1 There are significant differences between some sub-samples for the tested whole.

Through the discriminative analysis we test the H_2 hypothesis :

H_2 There are no clearly defined borderlines between the sub-samples for the tested whole.

A_2 There is a clearly defined borderline between some sub-samples for the tested whole.

Through ANOVAor Roy test the H_3 hypothesis is being tested :

H_3 There are no significant differences between sub-samples in particular features.

A_3 There is a significant difference between sub-samples in particular features.

RESULTS AND DISCUSSION

The content of undesirable metals was determined in the wheat grain of the different wheat varieties and data are presented in Table 1.

Table l. Undesirable metals content in kernels of different wheat varieties

Sample No.	Sample variety	Fe mg/kg	Cu mg/kg	Zn mg/kg	Pb mg/kg	Cd mg/kg
1	Evropa	29.46-69.26	4.14-5.16	21.83-26.43	1.07-2.33	0.01-0.21
2	Renesansa	28.98-42.29	3.02-3.82	16.01-19.53	0.42-1.57	0.00
3	Pobeda	30.87-51.63	3.93-4.34	20.85-23.13	0.99-1.62	0.00-0.09
4	Ljiljana	25.11-34.97	3.20-3.24	20.26-20.46	0.33-0.33	0.00-0.01
5	Pesma	37.65-39.61	3.70-5.48	18.68-25.47	0.53-2.46	0,00

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The first part shows the centered and dispersed parameters of undesirable metals content in the wheat varieties tested, (Tables 2-6).

The highest deviations from the average values for tested elements were found in the case of wheat varieties Evropa, Pobeda and Renesansa, which is evident from the values of the variation coefficient and standard deviation (Tables 2-4).

In wheat varieties Evropa, Pobeda and Renesansa the highest deviation is for Cd.

Table 2. Centered and dispersed parameters of undesirable metals content in the wheat variety Evropa

	average value	standard deviation	error	min	max	variation coefficient	confidence interval	
Fe	49.36	18.96	7.74	30.52	68.04	38.40	29.46	69.26
Cu	4.65	0.48	0.20	4.17	5.33	10.38	4.14	5.16
Zn	24.13	2.19	0.89	22.15	26.77	9.07	21.83	26.43
Pb	1.70	0.60	0.25	0.99	2.25	35.37	1.07	2.33
Cd	0.10	0.11	0.04	0.00	0.20	108.46	0.01	0.21

Table 3. Centered and dispersed parameters of undesirable metals content in the wheat variety Renesansa

	average	standard deviation	error	min	max	variation coefficient	confidence interval	
Fe	35.63	8.65	2.88	28.65	48.01	24.29	28.98	42.29
Cu	3.42	0.52	0.17	2.70	4.21	15.25	3.02	3.82
Zn	17.77	2.29	0.76	14.53	22.06	12.87	16.01	19.53
Pb	0.99	0.75	0.25	0.00	1.65	75.69	0.42	1.57
Cd	0.04	0.00	0.00	0.00	0.00	-	0.00	0.00

Table 4. Centered and dispersed parameters of undesirable metals content in the wheat variety Pobeda

	average	standard deviation	error	min	max	variation coefficient	confidence interval	
Fe	41.25	16.34	4.72	27.46	68.67	39.61	30.87	51.63
Cu	4.14	0.32	0.09	3.59	4.49	7.70	3.93	4.34
Zn	21.99	1.79	0.52	18.79	24.65	8.16	20.85	23.13
Pb	1.31	0.49	0.14	0.97	2.27	37.91	0.99	1.62
Cd	0.03	0.07	0.02	0.00	0.16	-	0.00	0.09

Table 5. Centered and dispersed parameters of undesirable metals content in the wheat variety Ljiljana

	average	standard deviation	error	min	max	variation coefficient	confidence interval	
Fe	30.04	2.00	1.15	28.04	32.04	6.66	25.11	34.97
Cu	3.22	0.01	0.01	3.21	3.23	0.31	3.20	3.24
Zn	20.36	0.04	0.02	20.32	20.40	0.20	20.26	20.46
Pb	0.33	0.00	0.00	0.33	0.33	0.00	0.33	0.33
Cd	0.04	0.00	0.00	0.00	0.00	-	0.00	0.01

Table 6. Centered and dispersed parameters of undesirable metals content in the wheat variety Pesma

	average	standard deviation	error	min	max	variation coefficient	confidence interval	
Fe	38.63	0.93	0.38	37.17	39.72	2.42	37.65	39.61
Cu	4.59	0.85	0.35	3.55	5.38	18.55	3.70	5.48
Zn	22.08	3.24	1.32	19.04	25.08	14.67	18.68	25.47
Pb	1.50	0.91	0.37	0.66	2.34	61.19	0.53	2.46
Cd	0.03	0.00	0.00	0.00	0.00	-	0.00	0.00

In wheat varieties Ljiljana and Pesma the highest deviations from the average values were found for Cd (Tables 5 and 6).

The existence of similarities or differences between the sub-samples actually proves the hypothesis of similarities or refutes it, thus proving the alternate hypothesis, i.e. it indicates the existence of the differences. In challenging the hypothesis a critical value p is used, which represents the risk to conclusion. If the $p>0.100$, there should be no reason for not accepting the original hypothesis. For refuting the original hypothesis two borderlines of significance were established. In the case of $0.10>p>0.05$ the alternative hypothesis is accepted with the increased risk, and in the case of $p<0.05$ the alternative hypothesis is accepted with the recognition of significant differences (14).

The data of multivariate methods and discriminative analysis are presented in Tables 7-9.

Table 7. The significant difference between the sub-samples in relation to undesirable metals content (MANOVA)

	n	F	p
MANOVA	5	4.039	0.000

n - number of variables (number of elements)
F- Fisher test
p - confidence of the test

As the difference $p = 0.000$ for the undesirable metals content, the alternativee hypothesis is accepted, meaning that there is a significant difference between the 5 varieties (MANOVA test).

Table 8. The significant difference between the wheat varieties in relation to the undesirable metals content (ANOVA test)

ANOVA	F	p
Fe	1.538	0.203
Cu	8.208	0.000
Zn	7.518	0.000
Pb	5.771	0.001
Cd	3.179	0.018

F- Fisher test
p - confidence of the test

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ANOVA or Roy test were used to challenge the hypothesis stating that there are no differences between the subsamples for the tested whole.

As p = 0.000 for copper, the alternative hypothesis A3 is accepted, which means that a significant difference between the 5 varieties was detected. The alternative hypothesis is also accepted for Zn, where p=0.000; as well as for Pb p=0.001 and for Cd, where p=0.018.

Since p =0.203 for iron, there is no reason to reject hypothesis H3, meaning that no significant differences were established for the 5 varieties tested.

Discriminative analysis was used to test hypothesis H2 which states that there are no clearly defined differences between the varieties.

Table 9. The significant differences between the wheat varieties in relation to the undesirable metals content (discriminative analysis)

	n	F	p
DISCRIMINATIVE	5	3.994	0.000

n - number of variables (number of wheat varieties)
F- Fisher test
p - confidence of the test

Taking into account that p = 0.000 for all the five undesirable metals, hypothesis H2 is discarded and the alternate one is accepted, which means that there is a significant difference and a clearly defined borderline between the wheat varieties in relation to the undesirable metals content.

Pb and Cd maximum concentrations allowed in wheat according to the current regulations on the quantities of pesticides, metals, metaloids and other toxic substances, chemotherapeutics, anabolics and other substances that may be found in foodstuffs are 0.4 and 0.1mg per kg of dry matter (15).

In wheat varieties Evropa and Pobeda the content of Fe exceedes the optimum concentration which is 43 - 50 mg/kg, whereas in other varieties a Fe deficit was established.

On average, wheat could have a higher content of Cu since the values found were lower than recommended 4.4 – 10.6 mg/kg. Zn content is quite elevated in all wheat varieties tested compared to the optimum content ranging from 13.5 to 14.0 mg/kg. What is adverse is that the Pb content is increased in comparison to the allowed values in all the varieties with the exception of the Ljiljana variety. Cadmium content is lower than the values prescribed, with the exception of Evropa variety which showed somewhat increased values for Cd content. This can be explained by the presence of the undesirable metals in the soil and excessive use of crop protection chemicals.

CONCLUSION

Based on the results obtained by testing undesirable metals content in different wheat varieties it can be concluded that:

Lead content is increased in all the wheat varieties tested in comparison to the allowed values prescribed by regulations, which is due to the traffic, as well as to the pollution from the industrial areas.

There are variations in susceptibility of wheat varieties to undesirable metals and their presence in the environment and this is something to be taken into account when considering sowing assortment recommended in cases of proximity of pollution sources.

For wholewheat bread production quality and health the control of varieties is of particular importance.

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САДРЖАЈ НЕЖЕЉЕНИХ МЕТАЛА У ПШЕНИЧНОМ ЗРНУ РАЗЛИЧИТИХ СОРТИ ПШЕНИЦЕ

Виолета Ж. Стефановић, Нада К. Филиповић,и Богдан М. Јовановић

На одабраним узорцима пшенице различитих сорти из региона испитиван је садржај непожељних метала у целом зрну пшенице применом атомске апсорпционе спектрофотометрије. Добијени резултати су показали значајност разлике између различитих сорти у односу на садржај непожељних метала у пшеници. Добијени резултати су упоређени са максимално дозвољеним концентрацијама које прописује Правилник о количинама пестицида, метала и металоида и других отровних супстанција, хемиотерапеутика, анаболика и других супстанција које се могу налазити у намирницама.

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