Microelements Distribution in Whole Hempseeds (*Cannabis Sativa L.*) and in Their Fractions

MARCELA MIHOC[1] , GEORGETA POP*[1], ERSILIA ALEXA[1], DIANA DEM[2], ANDREA MILITARU[3]

[1] Banat's University of Agricultural Sciences and Veterinary Medicine, Faculty of Agriculture, Timisoara, 119 Calea Aradului, 300645, Timisoara, Romania

[2] National Administration "Romanian Waters", Basinal Administration of Water "Crişuri", 35 I.Bogdan, 410125, Oradea, Romania

[3] University of Medicine and Pharmacy, "Victor Babes", Facultaty of Pharmacy, 2 Eftimie Murgu, 300041, Timisoara, Romania

This study's aim is to be a continuation of the research conducted by Mihoc et al, 2012 to deternine the heavy metal content for the hemp seeds of five romanian varieties by following the distribution of Fe, Mn, Zn, Cu and Ni in the two fractions: hulled hemp seeds and shell as opposed to whole hemp seeds. Microelements content in hemp seeds are: Fe (130-164 mg/kg), Mn (89-108 mg/kg), Zn (42-57 mg/kg), Cu (10-12 mg/kg), Ni (1.6-6.1 mg/kg), Cr (598-877μg/kg) and Mo (265-652μg/kg). The Pb analysis shows a high dispersion of the results with many values under the quantification limit. Therefore the Pb concentration in hemp seeeds belongs to the range of 217-626 μg/kg excepting the Armanca variety. Iron is concentrated in shell, zinc and nickel in hemp heart, while manganese and copper are equally balanced in both the core and the shell.

Keywords: Cannabis Sativa L, microelements, whole seeds, seed fractions: shell, hulled seeds

Hemp (*Cannabis Sativa L.*) belongs to the Cannabaceae family, the Cannabis genus. Hemp fruit is a round achene, consisting of a single seed covered with endosperm and protected by the pericarp. Raw hemp seeds produce a series of derivate products: hulled seeds, hemp flour, protein powder and shell with high-fiber, oil and hemp cake content.

Hemp seeds contain some heavy metals extracted from the soil [1,2]. Iron and manganese are two widespread metals in the soil, therefore they are found in plants taking their food directly out of the natural substrate.

Heavy metals are present in the soil, naturally, in small concentrations or, due to anthropogenic activities, in high concentrations. Plants have a different capacity to absorb, metabolize or transport heavy metals from soil.

Hemp (*Cannabis sativa L.*) is known for its tolerance for elevated heavy metals content soils and even for the ability to extract metals from the soil. Many studies [2-5] report an accumulation of Ni, Pb, Cd, Zn and Cr in hemp which makes the plant to be considered in soil phytoremediation processes. As a consequence, the use of hemp seeds in human or animal food raises questions about the potentially negative effects of the heavy metals content.

The aim of this study was to evaluate the fractions resulted from the hulling of the hemp seeds from the five romanian varieties and the distribution of Fe, Mn, Zn, Ni and Cu in the two fractions (shell and kernel).

Experimental part

Materials and method

Sample preparation

Hemp seed samples were taken from monoecious hemp seed production (Zenit, Diana, Denise) and dioecious (Armanca, Silvana), obtained in 2011 in the area of experimental plots at SCDA Lovrin –Timis, in the south west of Romania. From each sample of monoecious and dioecious seeds hulled seeds, hull and whole seeds were analyzed. The seeds sampling was recorded according the EC Regulation No.333/2007 [6].

Samples of each variety of seed were weighed, then they were used to determine the metal content compared to whole seed. Then another 3 samples of each variety of seed were weighed and were manually husked thus obtaining the two fractions: hulled seed and shell. Each fraction thus obtainined was weighed and then was subject to analysis. During the shelling process and during sample handling contact with metal surfaces or instruments was avoided to minimize the risk of contamination of the samples and alteration of the result.

Samples of about 0.1g of hulled seeds, shell or whole seeds were mineralized with 10mL nitric acid (HNO3) 65% with low metal content (Ultrapur Merck). Digestion to bring in the solution the solid sample of seed and seed fractions, was carried out using the Berghof Speedwave MWS-2 microwave by the 3050B EPA method[7], using the program presented in the table 1.

Digested samples, cooled at room temperature, were passed through a paper filter of cellulose acetate with a pore size of 0.45 μm. Filtrates containing acid metals extract from seed samples were collected in 25 mL flasks and were brought to final volume with double distilled water.

The microelements determination

The metal content determination was performed in the laboratory of the Basinal Administration of Water "Crişuri, with atomic absorption spectrophotometers (Thermo M Series and Unicam Solaar 969).

The acid extract was analyzed for the following metals: Fe, Mn, Zn, Cu, Ni, Cr, Pb and Mo. The metal content of the extract was determined using two different techniques, depending on the metal concentration to be determined. Thus for Fe, Mn and Zn air-acetylene flame AAS was used and for Cu, Ni, Cr, Pb and Mo by graphite furnace.

By increasing the temperature based on a program, the sample is dried, pyrolysed and atomized. The dry phase is carried out at 105 and at 120 °C ensuring the drying of the sample in 40 s for Pb Ni Cr, 50 s for Mo and 70 s for Cu. The ash phase needs different temperatures depending on the element: Pb-700°C (20s), Ni-1000°C (20s), Cr-1200°C (20s), Mo-1800°C (20s). The inert gas used to release the vapors

* Tel.: 0256 277007

Table 1
TEMPERATURE USED FOR SAMPLE DIGESTION

The stages of the digestion process / Parameters	1	2	3
T [°C]	140	160	175
POWER [%]	80	85	90
TIME [SEC]	5	5	20

Table 2
GRAVIMETRIC ANALYSIS OF HEMP SEED AND ITS FRACTION

	Variety	Number of seed	Whole seed weight , g	Hulled seed weight, g	Shell weight, g	Percentage hulled seed %	Percentage shell %
1	**Zenit**	**8**	0.1208	0.0846	0.0309	70.0	25.6
2	**Zenit**	**8**	0.1661	0.0987	0.0605	59.4	36.4
3	**Zenit**	**8**	0.1395	0.0810	0.0565	58.1	40.5
	Mean , %					**62.5**	**34.2**
	Standard deviation, %					**6.6**	**7.7**
4	**Diana**	**8**	0.1650	0.1006	0.0587	61.0	35.6
5	**Diana**	**8**	0.1477	0.0866	0.0586	58.6	39.7
6	**Diana**	**7**	0.1427	0.0871	0.0542	61.0	38.0
	Mean , %					**60.2**	**37.7**
	Standard deviation, %					**1.4**	**2.1**
7	**Denise**	**7**	0.1037	0.0554	0.0464	53.4	44.7
8	**Denise**	**7**	0.1268	0.0770	0.0421	60.7	33.2
9	**Denise**	**7**	0.1065	0.0614	0.0445	57.7	41.8
	Mean , %					**57.3**	**39.9**
	Standard deviation, %					**3.7**	**6.0**
10	**Armanca**	**7**	0.1429	0.1075	0.0364	75.2	25.5
11	**Armanca**	**8**	0.1718	0.1147	0.0571	66.8	33.2
12	**Armanca**	**8**	0.1947	0.1297	0.0635	66.6	32.6
	Mean , %					**69.5**	**30.4**
	Standard deviation, %					**4.9**	**4.3**
13	**Silvana**	**8**	0.1911	0.1086	0.0702	56.8	36.7
14	**Silvana**	**9**	0.2974	0.1635	0.1277	55.0	42.9
15	**Silvana**	**7**	0.1358	0.0904	0.0421	66.6	31.0
	Mean , %					**59.5**	**36.9**
	Standard deviation, %					**6.2**	**6.0**
	Content of hulled seeds, %					**61.8**	**+/-4.5**
	Shell content, %					**35.8**	**+/-5.2**

and fumes from inside the cuvette from oxygen was argon with a flow of 0.2L/min. The time of atomization for each studied element was set to 3 s. Based on the data from the spectrophotometer's cook book and the optimizations which were carried out, the temperature of atomization for each metal is Pb-1600°C, Ni -2500°C, Cr-2500°C, Mo-2750°C, Cu -2400°C. The clean phase usually has a temperature higher with 100 degrees than the atomization phase but it depends on the matrix : Pb-2200°C (3s), Ni-2600°C (3s), Cr-2600°C (3s), Mo-2850°C (3s) and Cu-2800°C (3s). Determining Pb and Cr implies the use of $Mg(NO_3)_2$ as a matrix modifier and for Cu of NH_4NO_3. The determination of Ni and Mo is performed without the addition of matrix modifiers.

Equipment calibration was performed using standard monoelement solutions *CertiPUR®*, traceable NIST, solutions in HNO_3 2-3% with the concentration of 1000 mg/L metal. For the preparation of the reagents and of the standard solutions double distilled water was used. The correlation coefficients of the calibration curves were in the range of 0.995-0.999. Quantification limits (LOQ) of the methods used in the laboratory, set for a coefficient of variation of long-term repetability of 10%, are: Fe (0.06mg/L), Mn (0.025mg/L), Zn (10μg/L), Pb (1.0μg/L), Cu (1,0μg/L), Cr (0.6μg/L), Ni (1.5μg/L) and Mo (1.0μg/L).

Statistical analysis

Results are presented as means ± standard deviation (SD) of triplicate measurements. The results of the present study were processed by ANOVA one-way and the least significant difference test, in order to compare the mean values of the investigated parameters.

Results and discussion

Gravimetric analysis of hemp seed and its fraction

Gravimetric analysis of the fractions of seeds resulted from shelling and from the preparation phases for the metal analysis are presented in table 1. The hulled seed fraction is between 53.4 and 75.2% with an average of 61.8%, also the shell with an average of 35.8% has a gap between 25.5 and 44.7%. The separation operation is accompanied by losses of 2.4% (table2).

Analysis of microelements content

The microelements analysis was done on three parallel samples for each variety and for each fraction. For the series of samples obtained from whole hempseeds, the results of the analysis are presented in table 3.

The graphic analysis of the results obtained (fig. 1) produces a grouping of the analyzed metals in three groups.

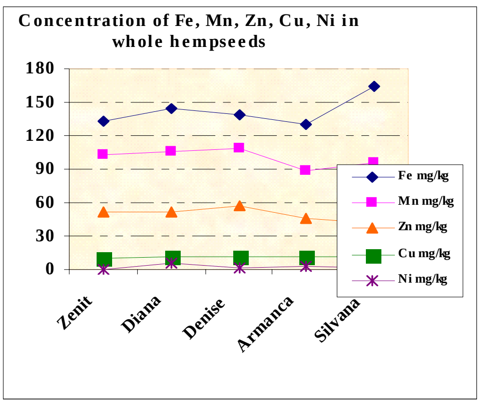

Fig. 1. Concentration distribution of Fe, Mn, Zn, Cu, Ni depending on varieties

Variety	Fe mg/kg	Mn mg/kg	Zn mg/kg	Cu mg/kg	Ni mg/kg	Cr µg/kg	Mo µg/kg	Pb µg/kg
Zenit	133	103	52	10.3	6.0	598	296	626
Diana	145	106	52	10.9	6.1	799	386	217
Denise	139	108	57	11.8	2.1	877	652	248
Armanca	130	89	46	11.9	3.5	856	265	<LOQ*
Silvana	164	96	42	12.0	1.6	821	569	343
min	**130**	**89**	**42**	**10**	**1.6**	**598**	**265**	**<LOQ***
max	**164**	**108**	**57**	**12**	**6.1**	**877**	**652**	**343**
mean	**142**	**100**	**50**	**11.4**	**3.9**	**790**	**421**	**358**
Standard deviation	**22**	**8**	**7**	**1.6**	**2.0**	**271**	**188**	**168**
Standard deviation %	**16**	**8**	**14**	**14**	**52**	**34**	**42**	**90**

* LOQ –limit of quantification

Table 3
METAL CONTENT IN WHOLE HEMPSEEDS

Variety	Fe	Mn	Zn	Cu	Ni
			mg/kg		
Zenit	135	124	72	10.5	7.2
Diana	103	124	65	13.2	8.6
Denise	155	121	92	11.0	3.3
Armanca	124	85	68	7.5	3.6
Silvana	117	87	63	9.1	2.3
Min	**103**	**85**	**63**	**7.5**	**2.3**
Max	**155**	**124**	**92**	**13.2**	**8.6**
Mean	**127**	**108**	**72**	**10.3**	**5.0**
Std. deviation	**20**	**20**	**11**	**3.2**	**2.7**
Std deviation %	**15**	**19**	**12**	**22**	**56**

Table 4
HULLED HEMPSEEDS METALS CONTENT

Thereby, there is the group of iron and manganese with values of over 100 mg/kg, the group of metals whose values are situated between 10 - 100 mg/kg (Zn, Cu) and the group of the microelemnts whose values are situated under 1 mg/kg, Cr, Mo and Pb which can't even be shown on the same graphic. Nickel has a separate position which although has values between 1-10 mg/kg, it seems that it has a variability which depends on the variety of hemp (table 3).

This grouping is mainly due to the natural spread of the studied elements in the soil. Thus iron and manganese are main constituents of the soil and of parental rocks, being situated on the fourth (Fe) and twelfth (Mn) places in the clark [8]. The placement of Zn ahead of other elements with smaller clark is due to the hemp high capacity of accumulating zinc, as well as to the higher content of zinc in soil in comparison to the rest of the studied elements [2,9,10]. By studying the variation of Fe, Mn, Zn and Cu from a variety to another, a quasiuniformity can be observed. The limited study does not allow that because of the small decrease of the metal content in the Armanca variety to jump to the conclusion that the Armanca variety has the lowest metal content.

The analysis of the fraction representing the core produced the values in table 4 and for the fraction representing the shell the results are in the table 5.

From the study of the values shown in tables 4 and 5, a growth of the standard deviation can be observed, together with a decrease in the metal concentration regardless of the analyzed fraction. In comparison with the standard deviation of the values for the whole seeds (table 3), a larger variation in the determination of the metal content in fractions than in whole seeds can be seen (altough this is not the case for nickel which has a special place at the border of the domains). This appears normal because the separation in fractions requires many operations.

Iron is a microelement with a catalytic role and a dynamic role. His lack produces the yellow coloration of the young leaves and then their whitening. Iron in moderate quantities facilitates the production of red blood cells and energy. The iron concetrations in the soil of the experience are significantly higher than for other metals [2].

The iron concentrations from the seed samples analized is in the 130-164mg/kg range, with an average of 142 mg/kg. From the iron analysis based on the varieties, as it is suggestively represented in figure 1, the conclusion that resulted is that there is a random repartition of the iron content which is not based on the plant type: dioecious or monoecious. This and the large values of the iron concentration, produce a standard deviation of 16% for all the values. Except for the Denise variety a high content of iron in shell can be observed as opposed to the hulled seeds (table 4 and 5). The light fraction which represents 35.8% has an average iron content of 158 mg/kg, while core, the major component (61.8%) in the whole seed, has an average concentration of 127 mg/kg.

The concentration domain of **Mn** in the studied seed is between 89-108 mg/kg, with an average of 100mg/kg. The very small gap indicates, as in the iron case an almost uniform repartition of manganese in all the five varieties and, with a standard deviation under 10%. By manually shelling the hemp seeds, the separation of the two fractions

Variety	Fe	Mn	Zn	Cu	Ni
			mg/kg		
Zenit	145	98	22	8.9	3.6
Diana	144	115	23	11.4	2.8
Denise	146	120	68	14.1	1.5
Armanca	170	101	40	10.0	4.8
Silvana	187	109	34	7.7	1.4
Min	**144**	**98**	**22**	**7.7**	**1.4**
Max	**187**	**120**	**68**	**14.1**	**4.8**
mean	**158**	**109**	**37**	**10.4**	**2.8**
Std. deviation	**28**	**24**	**20**	**3.6**	**1.7**
Std deviation %	**17**	**22**	**54**	**34**	**59**

Table 5
HEMPSEEDS HUSK METALS CONTENT

is not net. As a result, the Mn concentration registered in the hulled seed and the shell is the same, 108 mg/kg but with standard deviations of 19%, and 22% a lot smaller than for the whole seeds. According to the National Research Council (NRC)[11] the recommended dose of manganese is 5 mg/day/person, provided in this case by approximately 50 g of hemp hard.

The 42-57 mg.kg^{-1} (tab.2) concentration of **Zn** in the whole Romanian hempseeds is comparable to the one in the Turkish seeds (46 -72 mg.kg^{-1}), but higher than the concentrations for the Russian varieties (7.3-17.8 mgkg^{-1}) [12]. The Zn concentrations in the plants raised in this area confirms hemp capacity of depollution through Zn amassment in seeds for plants raised on a soil with 54-64 mg.kg^{-1}[2]. By comparing the zinc repartition in the core and shell fractions, an accumulation in the hulled hempseed can be observed with an average value of 72 mg/kg, in comparison with the lighter fraction of shell with an average content of 37 mg/kg. By analyzing the results from tab. 4 and 5 it can be observed that this difference in the accumulation in the core is larger than for the Zenit and Diana varieties (of about 3 times) than for other varieties, with the accumulation ratio of 1.4-1.8 times.

Copper has a large prevailence in nature, water, air, soil and living organisms. The concentration domain of Cu from the experience soil is between 1-2 mg/kg [9]. Copper is an essential micronutrient for plants in the seeds germination, in supplying water as well as in photosynthesis, but in high concentrations it becomes toxic. Copper deficit produces chlorosis and the wilting of young leaves. Hemp (*Cannabis sativa L.*) is prone to accumulate Cu prefferentially in leaves without afecting the primary liberian fibers [4,13]. The average copper concentration in the whole hemp seeds, monoecious and dioecious, is 11.4 mg/kg, much lower than the critical concentration of 20 mg/kg [14] in plants. The small gap of the experimental values and the standard deviation of 14% emphasize the idea of the copper accumulation in seeds not depending on the variety, only under the influence of this element in the soil (fig 1). The near average concentrations of Copper in the two fractions of seed indicate a balanced distribution of this element in the core and in the shell. The losses produced by the shelling operations bring forth standard deviations higher than the copper concentrations obtained in the two seed fractions (22%-hulled seeds and 34%-shell) as opposed to the whole seed (14%).

Nickel is present naturaly in soil, usually, in low quantities. In the soil of the experiment area the concentration is of 1.27 mg/kg [9]. The nickel ion is easily absorbed by plants and its concentration varies between 0.05-5.0 mg/kg [15-17]. Hemp seeds accumulate nickel in a high range of values 1.6-6.1 mg/kg, higher than the results published in [18] for the turkish varieties (0.55-1.66mg/kg), but lower than the ones found by Eboh (2005)[19] in the hemp grown in Nigeria..

By following the dependence of the Ni concentration based on the variety, results a net delimitation in two groups of values obtained for nickel. The first group consists of the Zenit and Diana varieties, with the average values of 6 mg/kg in the whole seeds and with a Ni in core : Ni in shell ratio of 2-3 and the second group consisting of the Denise, Armanca and Silvana varieties, with average values between 1.6-3.5 mg/kg. In this second group the Ni in core: Ni in shell ratio is between 0.8-2.0. Because the Denise variety is monoic and grown in the same area as the Zenit and Diana varieties, the experiment should be continued so that an explanation can be found. A balanced quantity of nickel in organism ensures a good functioning of the liver and pancreas. An intake of nickel higher than 1 mg/day (FDA, 2001)[20] determines toxic effects on the organism, from alergic reactions to lung cancer.

The last group represents Cr, Pb and Mo heavy metals, toxical and with risks for human health. In small quantities Cr and Mo have a pozitive role in the enzimatic processes and in metabolism, but the toxic effect prevails. Characteristical for the results obtained in the experiment are the small quantities and large variability of the results.

Chromium, in different oxidation states and concentrations, produces modifications in the plants germination, in the development of the root, stem and leaves, has a negative impact on the photosynthesis process and in the absorption of minerals from the soil [21]. Plants have an increased process and in the Cr concentrations in soil of 5-100 mg/kg [22-24] even with toxic effects for crop plants. Thus, concentrations of 5-30 mg / kg [25, 26] in plant determines large decreases in yield. In Romania the normalized value of chromium in soil, must be under 30 mg/kg the basis of order 756/1997 [27]. The Cr concentrations from soil, must be under 30 mg/kg the basis of order 756/1997 [27]. The Cr concentrations from the Romanian hemp seed samples are situated in the range of 598-877 μg / kg. After the experiments carried out, a lower Cr value can be observed, of about 600 μg/kg for the Zenit variety, in comparison to the rest of the varieties for which the chromium value is situated around the value of 840 μg/kg with a relative standard deviation of 4%. These values are situated a lot under the ones reported by Eboh (2005) of 15.2±0.25 mg/kg. The close concentrations of chromium in whole hemp seeds from the two areas of the experiment are dependent only of the level of the Cr concentration from the soil and in a small amount of the variety.

Molibden is an essential oligoelement which can be found in the composition of some enzymes with role in plant nitrogen metabolism. The molibden concentration from plants is situated in the range of 500-5000 μg.kg^{-1} [28], and values between 10000-50000 μg.kg^{-1} are, generally, toxic for plants [29]. The Mo concentrations from the Romanian hemp seeds are in the range of 0.26 - 0.65μg.kg^{-1} like the Turkish varieties studied by Kormaz, 2010. The Zenit, Diana and Armanca varieties have an average Mo concentration of 315μg.kg^{-1} representing almost half of the average value (611μg.kg^{-1}) of the Denise and Silvana varieties.

Lead is a powerful pollutant present in the atmosphere, with a bioaccumulation effect. For the soil, the maximum admitted concentration is of 20000 μg/kg according to Order 756/1997[27]. According to the CE regulation nr 1881/2006[30], in Romania, the maximum level of Pb in cereal is of 200 μg/kg. In plant, lead has a negative impact on photosynthesis and may reduce the calcium assimilation. The study performed indicates large variations of the lead content in seeds. To note is also the fact that values under the quantification limit of the analysis method as well as values up to the concentration level of 626 μg/kg (Zenit variety) have been obtained. Because of this the data were not statistically processed and do not allow the establishment of rigorous conclusions.

Conclusions

In terms of this study's aim, the metal content of the Romanian hemp seeds, with few exceptions, which may be due to the experiment conditions, do not depend on the hemp variety studied. All the hemp varieties studied have a low metal content, situated within the limits of the actual reglementations, which allow and actually recommend them for the alimentary use. The oligoelements (Fe, Mn, Zn, Cu and Ni) are found in concentrations of over 1 mg/kg in whole hemp seeds, and the microelements (Cr, Mo and Pb) determined are under 1 mg/kg. The hulled seed fraction and shell are in a ratio of aproximately 1.6:1. A repartitions of Fe, Zn, Mn, Cu and Ni in the two fractions differently, based on the metal, can be observed. Thus iron is concentrated in shell, whilst zinc and nickel in hulled seeds, whereas manganese and copper have a balanced distribution in the core and in the shell.

References

1. CALLAWAY, J.C., - Hempseed as a nutritional resource, Euphytica , 140, p 65-72, 2004.
2. MIHOC M, POP G, ALEXA E., RADULOV I., Nutritive quality of romanian hemp varieties (Cannabis sativa L.) with special focus on oil and metal contents of seeds, Chemistry Central Journal, 6:122, 2012
3. LINGER P., MÜSSING J., FISHER H., KOBERT J., Industrial hemp (Cannabis sativa L) growing on heavy metal contaminated soil fibre quality and phytoremediation potential, Industrial Crop and products, vol16, No 1, p33-42, 2002
4. BONA E., MARSANO F., MARIA CAVALETTO, BERTA G.,- Proteomic characterization of copper stress response in Cannabis sativa roots, Protomics, Volume 7, Issue 7, p 1121–1130, 2007
5. CARAIMAN COJOCARU, P., MACOVEANU M., Decontamination of polluted soil with cadmium and zinc using greenhouse phytoremediation., Environmental Engineering and Management Journal. , Vol.10, No. 3 ,p 349-355 , 2011.
6. COMMISSION REGULATION (EC) No.333/2007 No - laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffssetting maximum levels for certain contaminants in foodstuffs- Official Journal of the European Union, L 88/29
7. *** USEPA (1996). United States environmental protection agency, method 3050B: Acid digestion of sediments, sludges, soils, & oils, SW-846. Washington, DC: USEPA: [www.epa.gov/osw/hazard/testmethods/sw846/pdfs/3050b.pdf]
8. FORTESCUE J.A.C.– Landscape geochemistry – Retrospect and prospect. Applied Geochemistry, 7, pp. 1-53, 1992 [14] Hossner L. R., Loeppert R.H., Newton R. J., Szaniszlo P.J. and Moses Jr. Attrep. (1998). Literature review: Phytoaccumulation of chromium, uranium, and plutonium in plant systems. Amarillo National Resource Center for Plutonium. Report ANRCP.
9. NEMCSÓK J., HERMESZ E., JANCSO ZS., BĂNEŞ A., HORABLAGA M., BOSTAN C. The pollution of the soils, the underground and surface waters, from Hungarian-Romanian cross-border area, Banat's Journal of Biotechnology, I (2), 2010
10. PENDIAS KABATA A,. MUKHERJEE A. B, -Trace Elements from soil to human, Springer Verlag Berlin Heidelberg, p 187, 197-200, 217-220, 243-247, 285, 2007.
11. *** NATIONAL RESEARCH COUNCIL (NRC) - Recommended dietary allowances. 10th Ed. Washington, D.C. National Academy of Sciences. Pp 241-243, 1989.
12. IVANOVA R, ANGELOVA V, DELIBALTOVA V, IVANOV K, SHAMOV D. Accumulation of heavy metals in fibre crops flax, cotton and hemp .J. Environ. Protection and Ecology, 4, No 1, p 31-38, 2003.
13. ARRU, L., ROGNONI, S., BARONCINI, M., BONATTI, P.M., PERATA, P. Copper localization in Cannabis sativa L. grown in a copper-rich solution. Euphytica, 140, p 33-38, 2004
14.KHAN M. A., AHMAD IJAZ AND INAYAT UR RAHMAN, Effect of Environmental Pollution on Heavy Metals Content of Withania somnifera, Journal of the Chinese Chemical Society, 54, 339-343, 2007
15. BROWN, P. H., R. M. WELCH, AND E. E. CARY. Nickel: A Micronutrient Essential for Higher Plants. Plant Physiology, 85, p 801–803, 1987
16. BROWN, P. H.. Nickel. In Handbook of Plant Nutrition, edited by A. V. Barker and D. J. Pilbeam, 395–410. Boca Raton, FL: CRC Press Taylor & Francis Group, 2006
17. GUODONG LIU, E. H. SIMONNE, YUNCONG LI - Nickel Nutrition in Plants, Horticultural Sciences, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, http://edis.ifas.ufl.edu
18. KORKMAZ K., METIN KARA S., OZKUTLU F. AND GUL V. - Monitoring of heavy metals and selected micronutrients in hempseeds from North-western Turkey, African Journal of Agricultural Research Vol. 5, No 6, pp. 463-467, 2010
19. EBOH L.O.AND BOYE E. THOMAS - Analysis of Heavy Metal Content in Canabis Leaf and Seed Cultivated in Southern Part of Nigeria, Pakistan Journal of Nutrition 4 (5): 349-351, 2005
20. *** FDA (Food and Drug Administration): Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Report of the Panel on Micronutrients. National AcademyPress, Washington, DC, Food and Drug Administration. Dietary supplements. Center for Food Safety and Applied Nutrition; 2001
21. ARUN K. SHANKER,T, CARLOS CERVANTES, HERMINIA LOZA-TAVERA, S. AVUDAINAYAGAM - Chromium toxicity in plants, Environment International, 31, p 739– 753,2005
22. DAVIDESCU D., Agrochimia. Pregătirea şi aplicarea îngrăşămintelor, Editura Agro-silvică de stat, Bucureşti, 1956
23. HOSSNER L. R., LOEPPERT R.H., NEWTON R. J., SZANISZLO P.J. AND MOSES JR. Attrep. - Literature review: Phytoaccumulation of chromium, uranium, and plutonium in plant systems. Amarillo National Resource Center for Plutonium. Report ANRCP, 1998
24. DAVIES F. T., PURYEAR J. D,. NEWTON R.J. EGILA J.N AND GROSSI J. A. S - Mycorrhizal fungi increase chromium uptake by sun flower plants: Influence on tissue mineral concentration, growth, and gas exchange. J. Plant Nutr. 25, No11, 2389-2407, 2002
25. KHAN M. R. AND KHAN M. M. Effect of Varying Concentration of Nickel and Cobalt on the Plant Growth and Yield of Chickpea , Australian Journal of Basic and Applied Sciences, 4, No 6: 1036-1046, 2010
26. MURAD ALI KHAN, ABDUL WAJID, SUAHAIL NOOR, FAREEDULLAH KHAN KHATTAK, SAEED AKTER, INAZAT UR RAHMAN, Effect of soil Contamination on some heavy metals content of Cannabis sativa, J Chem. Soc. Pakistan, vol 30, No 6, 2008.
27.*** ORDER756/1997 - approving the Regulation on the assessment of environmental pollution issued by the Ministry of Water, Forest and Environmental Protection -Annex.1-values for traces of chemical elements in soil [http://www.epcmediu.ro/system/files/ordin_nr._756_din_3_noiembrie_1997.pdf
28. BĂJESCU I., CHIRIAC A -Distribuţia microelementelor în solurile din România: Implicaţii în agricultură, Editura Ceres, Bucuresti, 1984
29. IYAKA YAHAYA AHMED- Chromium in soils: A review of its distribution and impacts, Continental J. Environmental Sciences, 3, p 13 - 18, 2009.
30.*** COMMISSION REGULATION (EC) No 1881/2006 - setting maximum levels for certain contaminants in foodstuffs- Official Journal of the European Union, L 364/5-24

Manuscript received: 18.02.2013