CONTEMPORARY AGRICULTURE / *SAVREMENA POLJOPRIVREDA* 58 (3-4) 44-51, 2009.
UDC: 63(497.1)(051)-„540.2“ ISSN 0350-1205

UDC: 622.34:658.2:634.1

HEAVY METALS CONTENT IN THE SELECTED SOILS AND FRUITS IN MONTENEGRO AND ESTIMATION OF THEIR DAILY INTAKE THROUGH FRUITS CONSUMPTION

SVETLANA ANTIĆ-MLADENOVIĆ, DRAGOJA RADANOVIĆ, JASMINA BALIJAGIĆ, MIODRAG JOVANČEVIĆ AND VLADO LIČINA[1]

SUMMARY: Levels of Pb, Ni, Cr and Cd, in soil and fruits (Rubus idaeus - raspberry and Vaccinium myrtilus - blueberry) at 26 locations in Montenegro were examined. Heavy metals content in the samples was determined by AAS method. In the soils, the content of heavy metals was below pollution threshold, except at one location (Kolašin region). In the berries, heavy metals content was mostly within normal range for plants and guideline values for fruits. The calculated daily intakes of metals through selected fruits consumption are found to be below the recommended tolerable daily intakes proposed by FAO/WHO.

Key words: *Heavy metals, soil, food, contamination, blueberry, raspbery, daily intake.*

INTRODUCTION

The major public concern for quality of food and risk, regarding the consumption of products contaminated by pesticides, heavy metals and/or toxins, has been constantly increasing (D'Mello, 2003). Some trace elements, such as: Cd, Hg, Pb and As, of both natural and anthropogenic sources are related to human health condition. Heavy metals, in general, are not biodegradable, have long biological half-lives and have potential for accumulation in different body organs, leading to unwanted side effects (Jarup, 2003). The general population is exposed to trace elements mainly by ingestion of drinking water and food and by inhalation of air (Kabata-Pendias and Mukherjee, 2007). Food contamination with heavy metals depends on several factors amongst which are soil

Original scientific paper / *Originalni naučni rad*

[1] Dr Svetlana Antić-Mladenović, docent and dr Vlado Ličina, associate professor, Faculty of Agriculture, Belgrade. MSc Jasmina Balijagić, research assistant and dr Miodrag Jovančević, senior scientist, Faculty of Biotechnology, Podgorica, Montenegro.
Corresponding author: Dr Dragoja Radanović, scientific adviser, Institute for Medicinal Plants Research „Dr Josif Pančić“, Tadeuša Košćuška 1, 11 000 Beograd. Tel: 381 11 218 34 20, e-mail: dradanovic@mocbilja.rs

concentration, soil chemical and physical properties, human activities (such as: irrigation with contaminated water, addition of low quality sewage sludge, fertilizers and metal-based pesticides, industrial emission, transport, the harvesting process, storage, and/or at the point of sale) and plant species and cultivars.

Among trace elements, lead (Pb), cadmium (Cd), mercury (Hg), and chromium (Cr) appear to be primarily involved in dietary exposure to chemical contaminants (Gunderson, 1995). Lead and cadmium are among the most abundant heavy metals and are particularly toxic. Excessive content of these elements in food is associated with a number of diseases and are implicated in carcinogenesis, mutagenesis and teratogenesis. On the other hand, nickel and chromium have been recently introduced as essential elements, but can be toxic at high level of exposure. Studies on humans and experimental animals have shown that trivalent chromium has an essential role in the maintenance of normal glucose and fat metabolism in humans and animals, while nickel may have some beneficial effects at low levels of exposure (NAS/IOM, 2003).

There is plenty of information on heavy metals in vegetables and estimations of their daily intake through vegetable consumption worldwide (Kumar et al., 2009; Maleki and Zarasvand, 2008; Radwan and salama, 2006), but there are limited data regarding heavy metals in fruits, particularly blueberry and raspberry. Keeping in view the potential toxicity, persistent nature and cumulative behavior of heavy metals, as well as the increasing consumption of fruits, there is necessity to analyze these food items to ensure that the level of heavy metals meets the international requirements. This study, therefore, presents data on the level of heavy metals (Pb, Ni, Cr and Cd) in selected soils and fruits from Montenegro, as well as the calculated daily intakes of these metals through selected fruits consumption.

MATERIAL AND METHODS

The soil and berries (Rubus idaeus - raspberry and *Vaccinium myrtilus* - blueberry) samples were collected at 26 locations in Northern Montenegro (regions: Bjelasica mountain, southern hillside of Lisa mountain, Pljevalja, river Lim valley and river Tara valley) from the end of July to the end of August 2008, during maturity period.

Blueberry samples were labeled by the letter B and ascending numbers. Raspberry samples were labeled by the letter M and ascending numbers. After collection, fruit samples were frozen up to -18°C and brought to the laboratory. Before the analyses, fruits were defrosted, then oven-dried (80ºC) for 15 hours, and grounded to powder. Heavy metals content in the plant material was measured by AAS, after digestion in acids: HNO_3 and $HClO_4$, with the addition of H_2O_2. Detection limits were: 0.006 μg Pb ml^{-1}; 0.003 μg Ni ml^{-1}; 0.004 μg Cr ml^{-1} and 0.005 μg Cd ml^{-1}.

Soil samples were collected from the depth of 30 cm by digging an adequate hole close to each sampling plant. For shallow soils, sampling depth was up to parent material. After collection, samples were labelled to correspond to the berries samples, then placed in polythene bags, brought to the laboratory, air-dried (20ºC), and sieved through a 2 mm stainless-steel mesh. Pseudo-total metal concentrations in soil samples were quantified by atomic adsorption spectrophotometry (Varian SpectrAA 2002 FS), in the acetylene/air flame, after digestion using HNO_3 conc. + H_2O_2 (US EPA Method

3050). Detection limits, calculated as analytic concentration greater than three times the standard deviation, obtained after eight measurements of the blank solution, were 0.007 μg Pb ml^{-1}; 0.003 μg Ni ml^{-1}; 0.005 μg Cr ml^{-1} and 0.007 μg Cd ml^{-1}.

The results obtained are presented as the average value and standard deviation derived from three replicants of each sample.

RESULTS

Pseudo-total content of heavy metals: Ni, Cr, Pb, and Cd, in the soils (Table 1) was below pollution threshold at all locations, except Ni and Cr concentrations at M14 (152.9 mg kg^{-1} and 133.2 mg kg^{-1}, respectively).

Table 1. Heavy metals in the soils (mg kg^{-1})

Locality	Label	Ni	Cr	Pb	Cd
		Blueberry			
Bjelasica	B1	9.2±0.24	13.3±0.28	39.6±1.10	1.11±0.01
	B2	18.0±0.33	13.7±0.22	39.4±0.09	0.25±0.01
	B3	9.9±0.26	10.3±0.21	66.1±1.13	0.26±0.02
	B4	13.6±0.42	14.5±0.31	61.7±0.08	0.41±0.02
	B5	3.0±0.12	6.4±0.17	34.1±0.69	0.05±0.004
Lisa	B6	-	-	-	-
	B7	7.4±0.18	8.5±0.22	60.1±1.10	< d.l.
	B8	4.3±0.12	9.3±0.35	36.8±0.78	< d.l.
Bihor	B9	20.0±0.52	20.0±0.39	43.6±0.56	0.05±0.003
	B10	2.1±0.11	5.3±0.16	43.3±0.62	< d.l.
Pljevlja	B11	-	-	-	-
Average		9.72	11.28	47.20	0.355
		Raspberry			
Lim riv. valley	M1	25.9±0.32	19.1±0.52	32.1±0.55	0.05±0.005
	M2	28.5±0.24	22.9±0.44	32.5±0.42	< d.l.
	M3	28.7±0.31	17.1±0.27	27.7±0.33	< d.l.
	M4	27.0±0.28	20.6±0.37	31.4±0.39	< d.l.
	M5	30.4±0.36	20.2±0.51	26.8±0.51	< d.l.
Bjelasica	M6	25.6±0.17	41.5±0.45	88.6±1.98	2.92±0.09
	M7	18.0±0.29	13.7±0.34	39.4±0.64	0.25±
	M8	27.0±0.22	21.3±0.38	68.9±1.73	< d.l.
	M9	13.6±0.19	14.5±0.23	61.7±0.98	0.41±0.03
Bihor	M10	-	-	-	-
	M11	-	-	-	-
Tara riv. valley	M12	50.2±0.25	45.1±0.55	69.8±1.13	0.15±0.008
	M13	54.2±0.31	48.2±0.48	60.0±1.23	0.05±0.002
Kolašin	M14	152.9±0.71	133.2±1.32	24.7±0.85	< d.l.
Pljevlja	M15	-	-	-	-
Average		40.17	34.49	46.99	0.64
Max. allowable conc.*		20-60	50-200	20-300	1-5

Source: * - Bowen, 1979.

Heavy metal content in both blueberry and raspberry samples (Table 2), expressed on dry weight bases, is within normal range for plant tissue. Significantly higher Ni content was found only in blueberry at location B8.

Table 2. Heavy metals in the fruits (mg kg- 1 d.w.)

Locality	Label	Ni	Cr	Pb	Cd
		Blueberry			
Bjelasica	B1	1.0±0.02	0.7±0.04	1.9±0.11	0.10±0.002
	B2	1.5±0.07	0.9±0.04	3.2±0.23	0.16±0.003
	B3	1.5±0.06	1.2±0.08	1.6±0.11	0.11±0.001
	B4	3.1±0.09	0.2±0.03	3.9±0.18	0.16±0.002
	B5	1.5±0.04	0.8±0.05	3.9±0.21	0.16±0.002
Lisa	B6	1.5±0.10	0.9±0.05	8.4±0.57	0.11±0.001
	B7	1.3±0.08	1.4±0.03	3.3±0.14	0.11±0.002
	B8	51.2±0.56	1.1±0.06	4.5±0.16	0.64±0.01
Bihor	B9	2.0±0.11	1.1±0.05	4.1±0.21	0.10±0.001
	B10	1.6±0.10	1.0±0.07	2.4±0.13	0.05±0.003
Pljevlja	B11	1.6±0.09	1.2±0.06	10.5±0.38	0.11±0.002
Average		6.169	0.9618	4.323	0.1645
		Raspberry			
Lim riv. valley	M1	9.9±0.29	0.3±0.02	2.7±0.12	0.43±0.003
	M2	-	-	-	-
	M3	3.9±0.14	0.3±0.02	6.0±0.40	0.27±0.002
	M4	6.4±0.18	0.4±0.03	2.8±0.22	0.38±0.002
	M5	9.6±0.29	0.8±0.06	3.0±0.21	0.48±0.005
Bjelasica	M6	0.05±0.002	0.4±0.04	2.1±0.31	0.11±0.002
	M7	6.0±0.41	0.2±0.01	3.0±0.18	0.38±0.001
	M8	1.9±0.05	0.4±0.02	2.3±0.17	0.32±0.001
	M9	4.1±0.10	0.8±0.09	3.6±0.20	0.59±0.007
Bihor	M10	1.7±0.08	0.6±0.01	2.4±0.16	0.11±0.002
	M11	3.2±0.11	0.8±0.07	2.8±0.13	0.32±0.004
Tara riv. valley	M12	2.7±0.13	0.7±0.03	4.4±0.25	0.21±0.001
	M13	-	-	-	-
Kolašin	M14	10.0±0.39	0.6±0.04	2.55±0.19	0.27±0.002
Pljevlja	M15	3.6±0.26	0.9±0.03	3.5±0.26	0.48±0.003
Average		4.849	0.5492	3.188	0.3346
Normal range in plants*		0.02 -5	0.03-14	0.2-20	0.1-2.4

Source: * - Kabata-Pendias and Mukherjee, 2007.

Estimation of heavy metal intake requires information on the levels of metals in food and the amount of food consumed (NAS/IOM, 2003). Calculation of an estimated daily intake of heavy metals in this study is based on the average metal content in the berries expressed on the fresh matter basis. In order to obtain average metal concentrations in fresh berries, the average concentrations of metals in dry matter were divided by 10,

based on the assumption that berries contain at least 90 % of water. Therefore, if a person consumes 200 g of fresh blueberry per day, they will then ingest 123.38 μg Ni day^{-1}, 19.24 μg Pb day^{-1}, 86.45 μg Cr day^{-1}, 3.29 μg Cd day^{-1}; or 96.98 μg Ni day^{-1}, 10.98 μg Pb day^{-1}, 63.77 μg Cr day^{-1} and 6.69 μg Cd day^{-1} by consumption of 200 g of fresh raspberry (Table 3).

Table 3. Estimated daily intake of heavy metals through consumption of the selected fruits

	Ni	Pb	Cr	Cd
Blueberry				
Average concentration (mg kg^{-1} f.w.)	0.6169	0.09618	0.4323	0.01645
Estimated daily intake (μg day^{-1})	123.38	19.24	86.46	3.29
Raspberry				
Average concentration (mg kg^{-1} f.w.)	0.4849	0.05492	0.3188	0.03346
Estimated daily intake (μg day^{-1})	96.98	10.98	63.76	6.69

DISCUSSION

Heavy metals accumulation in soils is of concern in agriculture due to adverse effects on food quality, plant growth and environmental health. Pseudo-total content of heavy metals: Ni, Cr, Pb, and Cd, in the soils under this investigation corresponds to unpolluted and un-serpentinic sites (Kabata-Pendias and Mukherjee, 2007). High concentrations of both Ni and Cr at the same location – M14 imply to their natural sources, although it does not exclude potentially high accumulation of these elements in plant tissue.

Heavy metal accumulation in the food chain can be highly dangerous to human health. These metals enter the human body mainly through two routes: inhalation and ingestion, and the ingestion being the main route of exposure. Since dietary intake of food may constitute a major source of long-term low-level body accumulation of heavy metals, the detrimental impact becomes apparent only after several years of exposure. Regular monitoring of heavy metals from effluents, sewage, in vegetables and other food is essential for preventing excessive buildup of the metals in the food chain (Islam et al., 2007).

It has been reported that nearly half of the mean ingestion of lead, cadmium and mercury through food is due to plant origin (fruit, vegetables and cereals) (Islam et al., 2007). Taking into consideration that results of the present study are given on the basis of dry weight and that most fruits contain at least 90 % of water, they show low level of contamination of selected fruits with heavy metals, compared with permissible levels given by FAO, WHO and EC. According to Commission regulation (EC) No 1881/2006, maximum level of Pb in berries is set on 0.2 mg kg^{-1} of fresh weight, while Cd level is 0.05 mg kg^{-1} f.w.

In order to establish certain food quality criteria, as well as to assess trace

element risk level, several governmental and private organizations have made a set of recommendations, such as Acceptable Daily Intakes (ADIs) and Provisional Tolerable Daily Intakes (PTDIs) proposed by World Health Organization (WHO), and Reference Dose (RfD) derived by USEPA, which represents an estimate of the daily exposure to which human population may be continually exposed over a lifetime without an appreciable risks of deleterious effects of heavy metals.

Our estimated daily intake of heavy metals by the studied fruits is below limit intakes set by FAO/WHO on the basis of body weight for an average adult (60 kg body weight). PTDI for Pb , Ni, Cr and Cd are 214 μg, 300 μg, 200 μg and 60 μg, respectively (Joint FAO/WHO Expert Committee on Food Additives, 1990). Thus, the consumption of an average amounts (around 200 g) of these foodstuffs does not pose a health risk for the consumer.

For Cr, as essential element for humans and animals, the US National Research Council has recommended a dietary intake for adults of 50–200 μg Cr (III) day–1 (NRC, 1989). It is usually considered that almost all the chromium in food is in the trivalent form (MAFF, 1999). Ingestion of Cr by consumption of an average amount of the fruits from the present study falls within the proposed interval. Consumption of an average amount of blueberry and raspberry studied also corresponds to an average daily intake of nickel (130 μg person $^{-1}$day^{-1}, Kabata-Pendias and Mukherjee, 2007).

CONCLUSION

The present study provides additional data on heavy metals content (Pb, Ni, Cr and Cd) in soils and berries (blueberry, raspberry) from Montenegro and also help in risk assessment of consumer exposure to heavy metals through selected fruits consumption. Soil contamination with heavy metals has not been found, except at one location. Heavy metals content in blueberry and raspberry was mostly within normal range for plant material and within guideline values for fruits. Daily intakes of these metals through selected fruits consumption are found to be below the recommended tolerable daily intakes proposed by Joint FAO/WHO Expert Committee on Food and Additives and may not pose a health hazard to consumers. Nevertheless, regular survey of heavy metals in fruits, as well as in other foodstuff, is recommended in order to evaluate whether any health risk does exist, to ensure food safety and to protect users from food that might be threat to their health.

REFERENCES

BOWEN, H.J.M. Environmental Chemistry of the Elements. Academic Press, London. 1979.

COMMISSION REGULATION (EC) No 1881/2006 2006R1881— EN— 23.07.2008 — 002.001— 1, 2008.

D'MELLO, J.P.F.: Food safety: Contaminants and toxins. CABI Publishing, Wallingford, Oxon, UK, Cambridge, MA. p. 480, 2003.

GUNDERSON, E.L.: FDA total diet study, July1986-April 1991. Dietary intakes of pesticides, selected elements and other chemicals. *J. AOAC Int.* 78:1353-1363, 1995.

ISLAM, E., YANG, X., HE, Z., MAHMOOD, Q.: Assessing potential dietary toxicity of heavy

metals in selected vegetables and food crops. *J. Zhejiang Univ. Sci B*, 8: 1-13, 2007.
JARUP, L.: Hazards of heavy metal contamination. *Br. Med. Bull,* 68:167-182, 2003.
JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES: Summary and Conclusions. In: 53rd Meeting, Rome, June 1-10., 1990.
KABATA PENDIAS, A. and MUKHERJEE, A.B. Trace Elements from Soils to Humans. Springer,-Verlag Berlin Heidelberg, 2007.
KUMAR, A., SHARMA, I.K., SHARMA, A., VARSHNEY, S., VERMA, P.S.: Heavy metals contamination on vegetable foodstuffs in Jaipur (India). *Electronic Journal of Environ., Agric. and Food Chem.,* 8:96-101, 2009.
MAFF (MINISTRY OF AGRICULTURE, FISHERIES AND FOOD).: *Survey of Aluminium, Antimony, Chromium, Cobalt, Indium, Nickel, Thallium and Tin in Food,* The Working Party on the Monitoring of Foodstuffs for Heavy Metals, The Fifteenth Report of the Steering Group on Food Surveillance, The Stationery Office, London, 1985.
MALEKI, A., ZARASVAND, M.A.: Heavy metals in selected edible vegetables and estimation of their daily intake in Sanandaj, Iran. *Southeast Asian J. Trop. med. Publ. Health,* 39:335-340, 2008.
NAS/IOM (National Academy of Sciences/Institute of Medicine). Dietary reference intakes: guiding principles for nutrition labelling and fortification. Food and Nutrition Board, Institute of Medicine, Washington, DC., 2003.
NRC-National Research Council. Recommended Dietary Allowances, 10th edn, National Academy of Sciences, Washington, DC, 1989.
RADWAN M.A. and SALAMA, A.K.: Market basket survey for some heavy metals in Egyptian fruits and vegetables. *Food and Chem. Toxic.*, 44: 1273-1278, 2006.
US EPA Method 3050. U.S. Environmental Protection Agency. Test Methods for Evaluating Solid Waste, Volume IA: 3rd Edition, EPA/SW-846. National Technical Information Service. Springfield, Illinois, USA, 1986.

SADRŽAJ TEŠKIH METALA U ODABRANIM ZEMLJIŠTIMA I VOĆNIM KULTURAMA CRNE GORE I PROCENA NJIHOVOG DNEVNOG UNOSA PUTEM KONZUMIRANJA PLODOVA

SVETLANA ANTIĆ-MLADENOVIĆ, DRAGOJA RADANOVIĆ, JASMINA BALIJAGIĆ, MIODRAG JOVANČEVIĆ AND VLADO LIČINA

Izvod

U radu je ispitivan sadržaj: Pb, Ni, Cr i Cd u zemljištu i plodovima borovnice (*Vaccinium myrtilus*) i maline (*Rubus idaeus*) sa 26 lokacija u Crnoj Gori. Sadržaj teških metala u sakupljenim uzorcima određen je AAS metodom. Sadržaj teških metala u zemljištu bio je ispod praga kontaminacije, izuzev na lokaciji Kolašin. Sadržaj teških metala u plodovima borovnice i maline nalazio se u intervalu normalnih vrednosti za biljke, kao i u okviru predloženih granica za bobičasto voće. Procenjeno je da se dnevni unos teških metala putem konzumiranja plodova nalazi ispod vrednosti dnevnog unosa koji je preporučen kao prihvatljiv od strane FAO/WHO.

Ključne reči: Teški metali, zemljište, hrana, kontaminacija, borovnica, malina, dnevni unos.

Received / *Primljen*: 07.08.2009.
Accepted / *Prihvaćen*: 22.09.2009.