HEALTH RISK ASSESSMENT VIA THE CONSUMPTION OF MUSSELS (*MYTILUS GALLOPROVINCIALIS)* FROM THE BOKA KOTORSKA BAY, MONTENEGRO

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PROCENA ZDRAVSTVENOG RIZIKA PUTEM KONZUMIRANJA DAGNJE (*MYTILUS GALLOPROVINCIALIS*) IZ BOKOKOTORSKOG ZALIVA, CRNA GORA

Abstrakt

Sa povećanjem korišćenja morskih plodova u ishrani u poslednjih nekoliko godina, školjke su postale komercijalno važna vrsta u svetu. Zahvaljujući sadržaju proteina, kalcijuma i gvožđa, vitamina, omega-3 masnih kiselina, selena i joda, školjka predstavlja značajnu namirnicu u ishrani ljudi. Međutim s obzirom na veliki kapacitet akumulacije kontaminanata mogu predstavljati i potencijalnu opasnost za ljude. Istovremeno, školjka *M. galloprovincialis* se koristi kao bioindikator zagađenja morske sredine, jer toleriše velike koncentracije zagađivača iz okoline.

Školjka *M. galloprovincialis* je široko rasprostranjena u priobalnim vodama Crne Gore. Uzgaja se na farmama i prvenstveno je namenjena za tržište, ali se i dalje divlje školjke ove vrste prikupljaju za ličnu potrošnju od strane lokalnog stanovništva i turista. U Crnoj Gori trenutno postoji oko 16 farmi školjki i svaka od njih proizvodi od 10 do 50 tona godišnje. Sve farme se nalaze u Bokokotorskom zalivu, gde su okeanografski, fizički, hemijski i biološki uslovi pogodni za uzgoj ove vrste. Iako se uzgoj ove školjke povećao u odnosu na predhodnu deceniju ova grana industrije je i dalje nedovoljno razvijena.

Cilj ovoga rada bio je da se odredi kvalitet školjke *Mytilus galloprovincialis*, odnosno da se proceni eventualni zdravstveni rizik putem konzumiranja ove morske vrste sa više lokacija iz Bokokotorskog zaliva.

Uzorkovanje školjki vršeno je sezonski (zima, proleće i jesen) u 2008. godini. Odabrano je sedam lokacije unutar samog zaliva: Herceg Novi, Perast, Sv Stasija, Opatovo

i Tivat, lokacije sa kojih su uzorkovane divlje školjke, i lokacije Kukuljina i Krašići sa kojih su uzorkovane uzgajane školjke. U svim uzorcima školjki određivane su koncentracije kadmijuma i olova.

Izmerene koncentracije kadmijuma i olova upoređene su sa maksimalno dozvoljenim koncentracijama regulisanim evropskim i crnogorskim regulativama. Koncentracija Cd u svim ispitivanim uzorcima je manja od maksimalne dozvoljene koncentracije propisane evropskom i crnogorskom regulativom za kvalitet školjki. U slučaju olova, lokacija Tivat je jedina lokacija gde srednja vrednost za ispitivane tri sezone prelazi maksimalno dozvoljenu koncentraciju propisanu od strane Crne Gore.

Na osnovu koncentracije za ova dva ispitivana elementa i prosečne nedeljne potrošnje izračunat je nedeljni unos Cd i Pb u ljudski organizam konzumiranjem školjki.

Nedeljni unos kadmijuma i olova ispod je preporučenog maksimalnog limita Svetske zdravstvene organizacije. U najgorem slučaju, uzimajući u obzir maksimalno izmerene vrednosti ispitivanih elemenata, nedeljna količina školjki koju je neophodno uneti u organizam, a koja bi mogla da izazove zdravstvene probleme kod čoveka je 750 g u slučaju kadmijuma i 940 g u slučaju olova.

Ključne reči: *školjka, kadmijum, olovo, nedeljni unos*

INTRODUCTION

With the increase in the consumption of seafood in recent years marine mussels have become commercially more important seafood species worldwide (Stankovic et al., 2011). The mussel *M. galloprovincialis* is widely distributed in the coastal waters of Montenegro, where mussels are cultivated on farms for the market, while wild ones are still hand-collected for personal consumption. Consumption patterns for mussels and aquaculture products have increased in recent years in the Mediterranean region and also in Montenegro. Cultivation of mussels along the Montenegrin coast has been increasing, 150 tones/per year in last decade (FAO, 2007), particularly in the Boka Kotorska bay due to the good natural conditions, but it is still underdeveloped.

Mussels are important species, both economically and ecologically. This type of shellfish is commercially important seafood since it provides a good source of proteins, Ca and Fe, some vitamins, omega-3 fatty acids, selenium and iodine for human consumption (Dahl et al., 2010). Also they are available throughout the year, reasonably tolerant to environmental change and pollution; they have good net accumulation capacities making them an excellent metal biomonitoring agent (Stankovic et al., 2011).

Pollutant trace elements have the ability to bioconcentrate in mussels directly from the water, bioaccumulate and biomagnify in the food chain, causing higher trophic organisms to become contaminated with high concentrations of chemical contaminants (Suseno et al., 2010). The bioaccumulation of these metal concentrations in marine organisms depends on many factors, either environmental, such as metal concentrations in sea water, temperature, salinity, dissolved oxygen, pH or purely biological *i.e.,* species, tissues, organs, feeding conditions (Sunlu, 2006).

Trace elements, such as Cd and Pb can be present in food either naturally or as a result of human activities, such as mining, irrigation, energy extraction, agricultural practices, incineration, industrial emissions and car exhausts. These metals are very toxic and their absorption and toxicity depends on dose and, among other diet constituents, on the intake

of essential metals through diet. These elements have no known bio-importance in human biochemistry and physiology and their intake, even at very low concentrations, can cause toxic effects, because they tend to accumulate in the human body over time.

Cd, a metal with high toxic effects, which is strongly bioaccumulated in mussels, has an elimination half-life of 10–30 y and accumulates in the human body, particularly the kidney. Cd may act as an acute and chronic type of poison. Over time, Cd can accelerate osteoporotic process, since a high calcium dose can inhibit Cd absorption. The reverse situation - the inhibition of calcium absorption by Cd - has also been reported. This interaction is of special importance because of the suggested role of Cd in the development of bone softening due to decalcification, a characteristic of ltai-itai disease (Han et al., 2000).

Absorbed Pb is bound to erythrocytes in the blood and initially distributed to the liver, kidney and heart, where it preferentially binds to cell membranes and mitochondria (Widmeyer et al., 2004). Most forms of Pb are then distributed and stored in the bones. Pb is known to cause both acute and chronic adverse effects in the hematopoietic, nervous, gastrointestinal and renal systems (Widmeyer et al., 2004). Acute poisoning causes gastrointestinal colic, often resulting in mortality, while chronic poisoning causes anemia due to a decrease in the hemoglobin levels leading to organ damage in one or all of the four above-mentioned systems (Stankovic et al., 2011).

The aims of this study were to evaluate levels of Cd and Pb in relation to the maximum limits prescribed by national / international regulations, *i.e.*, to investigate whether the concentrations of these metals were within the permissible limits, thus rendering these mussels acceptable for human consumption.

MATERIALS AND METHODS

The mussel samples were collected seasonally (spring, fall and winter) from seven sites in Boka Kotorska bay, Montenegro: Herceg Novi, Perast, Sveta Stasija, Opatovo, Tivat, Kukuljina and Krasici during the year 2008. From each site in each season, pooled samples of 25–30 mussels, similar in length were selected, cleaned and rinsed with deionized water, dissected fresh and the soft tissue was rinsed with Milli Q water. All samples were measured before freezing at –10 ºC. The freeze-dried samples at – 40 ºC for 48 hours were weighed, homogenized to a fine powder and stored until analyses. The average water content of the soft mussels tissues were: 87.7 % (winter), 81.8 % (spring) and 79.8 % (fall).

The results from the determinations of Cd and Pb are presented as mean values of one pooled sample with 5 replicates of approximately 0.5 g from each station in each season. Approximately 0.5 g of the soft tissue was digested with a mixture of concentrated HNO_3 and H_2O_2 in a High Microwave Digestion System (CEM CORPORATION, MDS-2100). The digested samples were diluted to 25 ml with Milli Q water containing 1.0 % HNO_3. The analyses for Pb and Cd were performed using Graphite Furnace Atomic Absorption Spectrometry (Perkin-Elmer, 4100ZL, with Zeeman background correction). The accuracy of the applied analytical procedure for the determination of heavy metals in mussels was tested using SRM 2976 (Mussel homogenate; NIST) certified reference material.

RESULTS AND DISCUSSION

The mean concentrations of Cd and Pb in the mussel *Mytilus galloprovincialis* from the year 2008 are shown in Table 1.

Mean Cd concentrations in mussels from the Boka Kotorska bay ranged from 0.28 to 0.40 mg kg^{-1}. The highest mean value of Cd concentration in mussel samples (0.40 mg kg^{-1}) was obtained from Perast site (Table 1). All samples contained cadmium below the maximum level fixed by the European Commission Decision (Commission regulation (EC), 2006) and Montenegrin regulation (Montenegrin Food Regulation, 2002), Table 1.

The highest mean lead concentration was found in mussels from Tivat site (1.20 mg kg^{-1}) which is above the maximum level fixed by the Montenegro (Montenegrin Food Regulation, 2002), but below the maximum level fixed by the European Commission Decision (Commission regulation (EC), 2006). The same case is with the maximum measured Pb concentrations for sites Perast, Opatovo and Kukuljina. The maximum Pb concentration was measured in mussels from site Tivat, and it was the only sample that had level of Pb above the maximum level fixed by the European Commission Decision (Commission regulation (EC), 2006).

Table 1. The mean and range concentrations of Pb and Cd (mg/kg wet weight) in the soft tissue of mussels collected from seven sites in the year 2008

Location	Nature	WB	Cd (mg/kg)	Pb (mg/kg)
Herceg Novi	wild	wet	0.31 (0.18-0.37)	0.55 (0.46-0.67)
Perast	wild	wet	0.40 (0.22-0.65)	0.76 (0.32-1.31)
Sv Stasija	wild	wet	0.33 (0.23-0.42)	0.39 (0.29-0.56)
Opatovo	wild	wet	0.35 (0.27-0.44)	0.86 (0.49-1.39)
Tivat	wild	wet	0.36 (0.26-0.51)	1.20 (0.77-1.86)
Kukuljina	cultivated	wet	0.29 (0.24-0.37)	0.58 (0.29-1.10)
Krasici	cultivated	wet	0.28 (0.21-0.32)	0.42 (0.23-0.68)
Permissible limits by Montenegrin Food regulation (2002)		wet	1.0	1.0
EU (2006) Comm. Regulation (EC) No. 188/2006		wet	1.0	1.5

In this study the Provisional Tolerable Weekly Intake (*PTWI*) was used for the calculation of the metal concentration levels of concern associated with mussel consumption in the study area. The weekly intake of Cd and Pb through mussels consumption has been calculated by using average values for consumption of 46.8 grams per person per day (GEMS/FOOD Regional Diets, 2003) and Cd and Pb concentrations, Table 2.

Based on the mean, maximum and minimum concentrations of Cd and Pb, the consumption of 46.8 g of mussels on a daily basis or 327, 6 g/person per week from all sites

are below the *PTWI* value for Cd (7 μg kg^{-1} bw $week^{-1}$) and Pb (25 μg kg^{-1} bw $week^{-1}$), (FAO/WHO, 2004),Table 2. In the worst case, taking into account the maximum measured concentration, weekly intake of mussels must be larger than 0.75 kg and 0.94 kg to exceed the *PTWI* values for the Cd and Pb, respectively (Table 2).

Table 2. Weekly intake of Cd and Pb based on max, min and mean obtained value

		Cd	Pb
Provisional tolerable weekly intake (μg kg^{-1} bw $week^{-1}$)		7	25
	Location	Average consumption (327, 6 g/person per week)	
Weekly intake based on maximum value (μg kg^{-1} bw $week^{-1}$)	H.Novi	1.73	3.14
	Perast	3.04	6.13
	SvStasija	1.97	2.62
	Opatovo	2.06	6.51
	Tivat	2.39	8.70
	Kukuljina	1.73	5.15
	Krasici	1.50	3.18
Weekly intake based on minimum value (μg kg^{-1} bw $week^{-1}$)	H.Novi	0.84	2.15
	Perast	1.03	1.50
	SvStasija	1.08	1.36
	Opatovo	1.26	2.29
	Tivat	1.22	3.60
	Kukuljina	1.12	1.36
	Krasici	0.98	1.08
Weekly intake based on mean value (μg kg^{-1} bw $week^{-1}$)	H.Novi	1.45	2.57
	Perast	1.87	3.56
	SvStasija	1.54	1.83
	Opatovo	1.64	4.02
	Tivat	1.68	5.62
	Kukuljina	1.36	2.71
	Krasici	1.31	1.97
Amount of mussel per week required to exceed limit (kg)	Based on minimum	2.72	7.61
	Based on maximum	0.75	0.94

CONCLUSION

Concentrations found for Cd and Pb indicate that the investigated mussels from Boka Kotorska bay pose no health risk to seafood consumers. Based on FAO/WHO recommended safe limit, Cd and Pb contents were within the permissible range established for safe human consumption.

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REFERENCES

Commission regulation EC (2006): No 1881/2006, setting maximum levels for certain contaminants in foodstuffs, Official Journal of European Union, L 364, 5-24.

Dahl, L., Molin, M., Amlund, H., Meltzer, M.H., Julshamn, K., Alexander, J., Sloth, J.J. (2010): Stability of arsenic compounds in seafood samples during processing and storage by freezing. Food Chemistry 123, 3, 720-727.

FAO (2007): Fisheries and Aquaculture Department, National Aquaculture Sector Overview, Montenegro. http://www.fao.org/fishery/countrysector/naso_montenegro/en.

FAO/WHO (2004). Joint Expert Committee on Food Additives. Summary Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA1956-2003). Food and Agriculture Organization of the United Nations and the World Health Organization, ILSI Press International Life Sciences Institute.

GEMS/FOOD regional diets, Food Safety Department World Health Organization, 2003, Geneva, Switzerland.

*Han, B.C., Jeng, W.L., Hung, T.C., Ling, Y.C., Shieh, M.J., Chien, L.C. (*2000): Estimation of metal and organochlorine pesticide exposures and potential health threat by consumption of oysters in Taiwan. Environmental Pollution 109, 1, 147-156.

Montenegrin Food Regulation (2002): Legislation on maximum permitted level of pesticides, heavy metals and other toxic substances, hormones, antibiotics and mycotoxins in food, Sluzbeni list SRJ, 5, 67–85.

Stankovic, S., Jovic, M., Stankovic, R.A., & Katsikas, L. (2011): Pollutant trace elements in the Mediterranean mussel *Mytilus galloprovincialis* as seafood: risks to human health. Book Series: Sustainable Agriculture, in Environmental Chemistry, Volume 2, (E. Lichtfouse, E., Hamelin, M., Navarrete, M., Robert, D. eds.), Springer, New York, USA, in press.

Sunlu, U. (2006): Trace metal levels in mussels (*Mytilus Galloprovincialis* L. 1758) from Turkish Aegean sea coast. Environmental Monitoring and Assessment 114, 1-3, 273-286.

Suseno, H., Pws, S.H., Budiawan, B., Wisnubroto, D.S. (2010): Effects of concentration, body size and food type on the bioaccumulation of Hg in farmed tilapia Oreochromis mossambicus. Australian Journal of Basic and Applied Sciences 4, 5, 792-799.

Widmeyer, J.R., Crozier, E.D., Moore, M.M., Jurgensen, A., Bendell-Young, L.I. (2004): Role of Leptothrix discophora in Mediating Metal Uptake in the Filter-Feeding Bivalve Mytilus trossulus (edulis). Environmental Science and Technology 38, 3, 769-774.