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DETERMINATION OF HEAVY METALS IN BLACK SEA *MYTILUSGALLO PROVINCIALIS* AND *RAPANA VENOSA*

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ABSTRACT

Shellfish living in seas can accumulate heavy metals and thus serve as excellent passive biomonitors. Concentrations of arsenic (As), cadmium (Cd), mercury (Hg), lead (Pb) and manganese (Mn) in two kinds of shellfish, *Rapana venosa* and *Mytilus galloprovincialis* were determined. Samples were collected at three coastal sites along the Bulgarian Black Sea, including one mussel farm. Shellfish tissues were subjected to microwave-assisted acid digestion followed by appropriate atomic absorption spectrometry (AAS) (Flame AAS for Mn, Electrothermal AAS for Cd, Pb and As). Concentration of total mercury was determined by Direct Mercury Analyzer. Levels of metals varied within species. The results clearly indicated that the concentrations of As exceeded the maximum permissible levels (MLPs) of 2,0 mg/kg according to the Bulgarian Food Codex (2004).

Key words: heavy metals, atomic absorption spectrometry, *Rapana venosa*, *Mytilus galloprovincialis*, Bulgarian Black Sea

INTRODUCTION

Black Sea is the world's largest natural anoxic water basin below 180 m in depth. It is a closed sea with very high degree of isolation from the world's oceans, however, it receives freshwater inputs from some of the largest rivers in Europe such as the Danube, the Dniester, and the Dnieper (12,13). Black Sea is considered as one of the most polluted seas, and recently increasing concentration of nutrients has led to a higher degree of eutrophication.

Heavy metal pollution of the marine environment has been long recognized as a serious environmental concern (2,14). Marine organisms, especially shellfish, are capable of accumulating the metals from the environment in which they live. Heavy metals can be accumulated by marine organisms thought a variety of pathways (18). Over the last few decades the marine environment has been contaminated by persistent pollutants of agricultural and industrial origin. Heavy metal contamination has been identified as a concern in coastal environment, due to discharges from industrial waste, agricultural and urban sewage. Heavy metal levels are known to increase dramatically in marine environment mainly through anthropogenic activities. Shellfishes are good indicators of the long term monitoring of metal accumulation in the marine environment.

There are scanty data about heavy metal pollution in shellfish from the Bulgarian Black Sea coast for the last twenty years. The aim of this study was to determine the levels of lead (Pb), cadmium (Cd), arsenic (As), mercury (Hg) and manganese (Mn) in Black Sea mussel (*Mytilus galloprovincialis*) and rapana (*Rapana venosa*).

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MATERIAL AND METHODS

Black mussel (*Mytilus galloprovincialis*) is a natural biofilter that inhabits tidal areas attached to rocks. *Rapana venosa* is a predatory sea snail that has entered the Black Sea in the middle of the last century and feeds mainly on black mussels.

Sampling

Shellfish samples were obtained at three sites along Bulgarian Black Sea coast such as Varna, Kranevo (shellfish farm) and Krapetz in spring 2011. The collected mollusks were depurated in filtered seawater for approximately 24 h before being transported to the laboratory with ice freezing. The soft tissues of mollusks were excised by stainless steel scalpel blades and then thoroughly rinsed with Milli-Q water to remove extraneous impurities. Total soft shellfish tissue was taken for analysis. After sufficient homogenation by a blender, the samples were kept at -18 °C until analysis. Special care was taken to prevent metal contamination of the samples by the laboratory equipment.

Analytical procedure

All the solutions were prepared with analytical reagent grade chemicals and ultra-pure water (18 MΩ cm) was used for all of them. HNO_3 of superb quality was purchased from Fluka. All the plastic and glassware were cleaned by soaking in 2 M HNO_3 for 48 h. They were rinsed five times with distilled water and then five times with deionised water prior to use. Stock standard solutions of As, Hg, Cd, Mn

and Pb (1000 мg mL^{-1} Titrisol, Merck in 2% v/v HNO_3) were used for preparation of calibration standards.
Shellfish tissues were dissected and thoroughly washed with MQ water. To assess the total metal contents, microwave-assisted acid digestion procedure was carried out. Microwave digestion system 'Multiwave', 'Anton Paar' delivering a maximum power and temperature of 1000 W and 300°C, respectively, and internal temperature control was used. Reactors were subjected to microwave energy at 800 W in five stages program. Determination of Mn was performed by flame atomic absorption spectrometry on a Perkin Elmer Zeeman 1100 B spectrometer with an air/acetylene flame. The instrumental parameters were optimized in order to obtain maximum signal-to-noise ratio. As, Cd and Pb were estimated by electrothermal atomic absorption spectrometry on a Perkin Elmer Zeeman 3030 spectrometer with an HGA-600 atomizer. Pyrolytic graphite-coated graphite tubes with integrated platforms were used as atomizers. Pd as $(NH_4)_2PdCl_4$ was used as modifier for ETAAS measurements of As and Cd. Total mercury was determined by Milestone Direct Mercury Analyzer DMA-80. Samples were analyzed in triplicate. All the data were subjected to a statistical analysis. Student's *t*-test was applied to estimate the significance of values.

RESULTS AND DISCUSSION

Concentrations were expressed in mg/kg wet weight (mg/kg w.w.). The experimental concentrations are summarized in Table 1 given as mean±SD.

Table 1. Trace elements in shellfish (mean±SD) in mg/kg w.w.

Sample	Sampling site	Mn	As	Cd	Pb	Hg
Rapana venosa	Krapetz	0,26±0,02	4,17±0,37	0,005±0,001	0,32±0,03	0,11±0,02
Rapana venosa	Varna	0,48±0,04	2,20±0,20	0,008±0,002	0,12±0,01	0,08±0,01
Mytilus galloprovincialis	Varna	1,24±0,10	2,70±0,24	0,044±0,005	0,11±0,01	0,08±0,01
Mytilus galloprovincialis	Kranevo (shellfish farm)	1,74±0,14	2,07±0,19	0,090±0,01	0,18±0,01	0,32±0,02

Lead

Lead is toxic to humans, with the most deleterious effects on the hemopoietic, nervous, reproductive systems and the urinary tract.

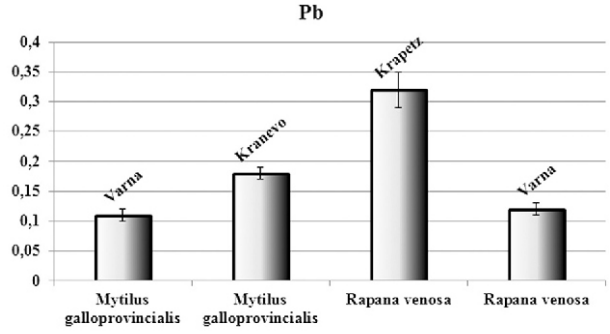

Fig. 1. Pb concentrations in shellfish samples

The Joint FAO/WHO (2004) Expert Committee on Food Additives establishes a provisional tolerable weekly intake (PTWI) for Pb as 0,025 mg/kg body weight (7). European Community and Bulgarian Food Codex set maximum permitted level for Pb in shellfish of 1,5 mg/kg w.w. (4,10). Pb concentration in shellfish samples is shown on Fig. 1. It is highest in *Rapana venosa* from Krapetz - of 0,32 mg/kg w.w. The values obtained from the analyzed samples were below those reported in the literature available and below the standard established by various health organizations.

Cadmium

Occupational Cd exposure levels prove to be a risk factor for chronic lung disease and testicular degeneration. Cadmium could originate from water, sediments and food. It may accumulate in the human body and induce kidney dysfunction, skeletal damage and reproductive deficiency (1).

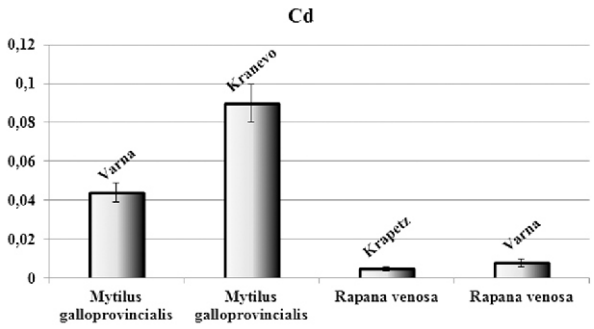

Fig. 2. Cd concentrations in shellfish samples

The highest Cd concentration (0,09±0,01 mg/kg w.w.) was obtained for *Mytilus galloprovincialis* from Kranevo (Fig. 2) while the lowest values were detected in *Rapana venosa* from Krapetz (0,005±0,001 mg/kg w.w.). The European Community (4) established the maximum levels permitted for cadmium in shellfish as 1,0 mg/kg w.w. Moreover, the Joint FAO/WHO has recommended the provisional tolerable weekly intake (PTWI) for Cd of 0,007 mg/kg b. w. (7).

Arsenic

Arsenic, a naturally occurring element, is a worldwide contaminant that is found in rock, soil, water, air and food. Arsenic is a highly toxic element. It is present in inorganic and organic forms. Chronic exposure to inorganic As may cause severe damage in the peripheral and central nervous system. Humans can be exposed to As through the intake of

food and drinking water, however, for most people, the major exposure source is the diet, mainly fish and seafood (3). A long- term As exposure has been related to some types of cancer. In this study, As concentration ranged from 2,07±0,19 mg/kg w.w. in *Mytilus galloprovincialis* from Kranevo up to 4,17±0,37 mg/kg w.w. for *Rapana venosa* from Krapetz (Fig. 3).

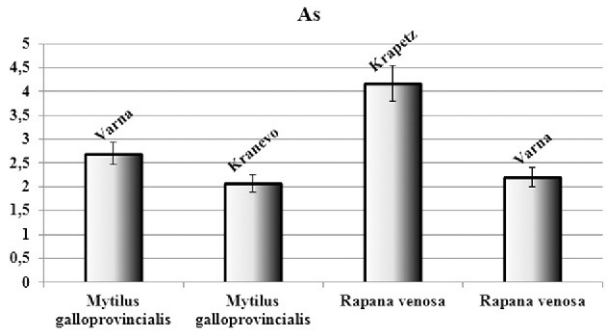

Fig. 3. As concentrations in shellfish samples

There are limited data about As content in shellfish species in the literature available. All the results obtained exceeded the maximum As level permitted for shellfish according to the Bulgarian Food Codex (of 2,0 mg/kg w.w. (10).

Mercury

Mercury is toxic to the developing fetus and considered a possible carcinogen. The primary Hg source in human diet is seafood (5). Our results demonstrated that Hg concentration range was 0,08-0,32 mg/kg w.w. (Fig. 4).

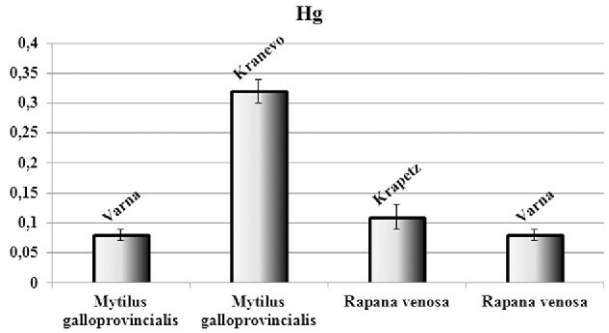

Fig. 4. Hg concentrations in shellfish samples

Mytilus galloprovincialis from shellfish farm showed the highest concentration of 0,32±0,02 mg/kg w.w. According to the Bulgarian Food Codex and European Community, the maximum Hg level permitted for shellfish is 0,5 mg/kg w.w. (4,10). Hg levels in the analyzed samples were lower than legal limits.

Manganese

Manganese is a mineral element that is both nutritionally essential and potentially toxic. Mn plays an important role in a number of physiologic processes as a constituent of multiple enzymes and an activator of other enzymes. Numerous Mn-activated enzymes play important roles in the metabolism of carbohydrates, amino acids, and cholesterol. In humans, demonstration of a Mn deficiency syndrome is insufficiently clarified yet. Such signs include impaired growth and reproductive function, skeletal abnormalities, as well as altered carbohydrate and lipid metabolism (8). Dairy products, meat, fish, and poultry are among the richest dietary Mn sources (11). The minimum and maximum Mn levels observed were 0,26 mg/kg w.w. in *Rapana venosa* from Krapetz and 1,74 mg/kg w.w. in *Mytilus galloprovincialis* from Kranevo mussel farm (Fig. 5).

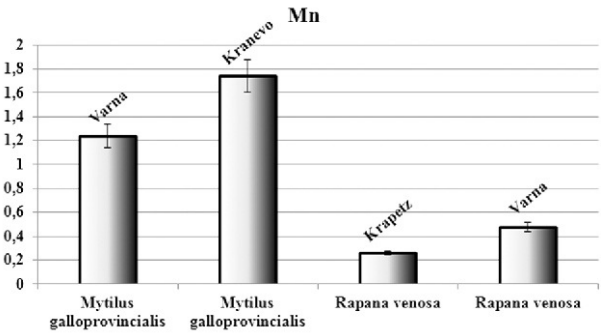

Fig. 5. Mn concentrations in shellfish samples

According to both FAO (6) and Bulgarian standards (10), there is no information about Mn carcinogenicity. The US National Academy of Science (15) recommended Mn of 2,5-5 mg daily and the World Health Organization recommended Mn of 2-9 mg daily for an adult individual (17). Concerning this RDA, Mn intake in our samples is below the above values.

As a whole, Cd, Hg and Mn are established in highest concentrations in *Mytilus galloprovincialis* from Kranevo mussel farm, while As and Pb - in *Rapana venosa* from Krapetz. There is limited information about the heavy metal content in marine shellfish from the Black Sea. The results obtained in this study are, generally, in agreement with or lower than those reported in literature for mussels and rapana from the Black Sea (9) and other seas (16).

CONCLUSIONS

Pb, Cd, Hg and As accumulate in marine organisms and are subject to control in seafood. In this paper, the concentrations of five heavy metals are estimated in two kinds of shellfish such as *Rapana venosa* and *Mytilus galloprovincialis*. They are in the range: 0,11-0,32 mg/kg w.w. for Pb; 0,005-0,044 mg/kg w.w. for Cd; 0,08-0,32 mg/kg w.w. for Hg; 2,07-4,17 mg/kg w.w. for As, and 0,26-1,74 mg/kg w.w. for Mn. All the analyzed elements are within maximum permitted levels established by various health organizations, except for As, which exceeds the maximum permissible level of 2,0 mg/kg w.w. (according to the Bulgarian Food Codex) (1).

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