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Estimation of THQ and potential health risk for metals by comsumption of some black sea marine fishes and mussels in Bulgaria

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Seafood is an essential component of the world population's diet being an important source of proteins, vitamins and unsaturated essential fatty acids. Despite this, seafood products are a commodity of potential health concern as they can be contaminated with a wide range of environmentally chemicals among which are heavy metals.

Human exposure to As, Cd, Hg, Cu, Cr, Mn, Fe, Ni, Zn and Pb trought consumption of three marine fish species (European anchovy, garfish and leaping mullet) and mussels was estimated by evaluation of target hazard quintet (THQ), target risk (TR), and hazard (HI) indexes.

The heavy metals analysis show that Zn and Fe are with maximum values in comparison to the other elements. The results from this study show that the THQ for the toxic and essential elements understudied are less than 1; signified that a daily exposure at this level is unlikely to cause any adverse effects during a person lifetime. Additionally, HI of each trace element were also lower than 1 suggesting that these pollutants perhaps pose no hazard to local residents. The TR values were between 10^{-6} and 10^{-4} mean that there is no important cancer risks associated with the consumption of the marine fish species subject to this study.

***Keywords**: heavy metals, marine fishes, mussels THQ, HI, Black Sea*

INTRODUCTION

Contamination of many ecosystems, including aquatic one, arise from both antropogenic sources of pollution and natural weathing. Among the serious chemical pollutants are heavy metals which are toxic, persistent and not easily degradable. The major source of human exposure to heavy metals is via food web. Humans require, for the proper function of most body processes, adequate levels of some essential elements such as magnesium, manganese, selenium, chromium (III), copper, cobalt, iron, and zinc. However, humans may be exposed to harmful non-essential elements such as arsenic, lead, mercury, cadmium, and nickel mainly through consumption of fresh and processed foods such as marine fishes and molluscs.

Fish is widely consumed in many parts of the world by humans because it has high protein content, low saturated fat and also contains omega fatty acids known to support good health [1]. Fish and other aquatic life forms such as mussels are constantly exposed to chemicals in polluted and contaminated waters [2]. Fish and marine mussels have been found to be good indicators of heavy metal contamination in aquatic systems because they occupy different trophic levels [3]. Cantillo found good agreement among the data sets of metal concentrations in mussels, validating the use of mussels in metal monitoring [4].

The heavy metal pollution may cause acute and chronic effect to human [5]. Several methods have been proposed for estimation of the potential risks to human health of heavy metals in fishes [6]. The risks may be divided into carcinogenic and non-carcinogenic effects [7]. Risk assessment is one of fastest method which is need to evaluate the impact of the hazards on human health and also need to determine the level of treatment which are tend to solve the environmental problem that occur in daily life [7]. Today the methods, which are used the most, are based on calculation on Target Hazard Quotients (THQ), Hazard Index (HI) and Target Risks (TR).

The aim of this study was 1.) to determine the concentration of As, Cd, Hg, Cu, Cr, Mn, Fe, Ni, Zn and Pb in three marine fish species (European anchovy, garfish and leaping mullet) and mussels caught from Bulgarian Black Sea coast; 2.) to evaluate the human exposure to As, Cd, Hg, Cu, Cr, Mn, Fe, Ni, Zn and Pb through consumption of these three marine fish species and mussels by estimating the hazard quintet (THQ), target risk (TR), and hazard (HI) indexes; 3.) to compare the data obtained for the marine species with those of black sea mussel samples.

EXPERIMENTAL

Sampling, sample preparation and storage

The fishes used in this study were European anchovy (*E. encrasicolus*), garfish (*Belone belone*), leaping mullet (*Chelon saliens*). Samples of fish and wild Black Sea mussels (*M. galloprovincialis*) were randomly acquired in local fishermen market from cities across the coastal waters of Bulgarian Black Sea. These sampling sites of Bulgarian Black Sea

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coast are Varna, Varna Lake, Pomorie, and Kavarna. All the fish species were sampled from spring and fall of the year 2016.

In order to obtain a representative sample at the each location more than 2 kg of mussels and 2 kg of fishes of similar length were collected, placed in plastic bags collected and transported to the laboratory. Total length and weight of the fish sample brought to laboratory after collection were measured to the nearest millimeter and gram before dissection. Only fillets of edible part of each individual were collected and included in the respective composite samples. Approximately 1 g sample of muscle from each fish were dissected, washed with distilled water, weighted, packed in polyethylene bags and stored at -18º C until chemical analysis. The molluscs were cleaned, rinsed and dissected fresh. The soft tissue of the samples was rinsed with Milli-Q water to remove any remaining sand and/or other particles, freeze-dried and homogenized using a mill. About 25–30 mussels from each sampling site were selected, pulverized and analyzed for the trace elements.

Chemical analysis

Wet digestions were performed in triplicate by weighing approximately 1.0 g of the fish and mussel tissues with a mixture of 10 ml HNO_3 (65% Merck, Suprapur) in a microwave digestion system MARS 6 (CEM Corporation, USA) delivering a maximum power and temperature of 800 W and 200 °C, respectively, and internal temperature control, was used to assist the acid digestion process. The digested fish and mussel samples were diluted to 25 ml with Milli-Q water and stored in polyethylene bottles. A blank digest was performed in the same way. The concentrations of As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn in the samples were determined using ICP-OES Spectrometer (Optima 8000, Perkin Elmer, USA) with plasma gas flow – 10 L/min, auxiliary gas flow -0.7 L/min, nebulizer gas flow - 0.2 L/min and axial plasma view. The analyses of total Hg was performed using continuous flow hydride generation inductively coupled plasma - optical emission spectrometry (CF-HG-ICP-OES, Optima 8000, Perkin Elmer, USA) with reducing agent 0.2% $NaBH_4$ prepared in 0.05% (w/v) NaOH. The accuracy of the applied analytical procedure for the determination of trace metals in mussels was tested using SRM 2976 (Mussel homogenate, NIST) certified reference material. The recovery ranges were between 95.3 and 102.6%. A DORM-2 (NRCC, Ottawa) certified dogfish tissue was used as the calibration verification standard. Recoveries between 90.5 and 108% were accepted to validate the calibration.

Statistical analysis

The whole data were subjected to a statistical analysis. Student's- test was employed to estimate the significance of values.

RESULTS AND DISCUSSION

Heavy metal concentration in marine products

The concentration levels of studied heavy metals (As, Cd, Cr, Cu, Hg, Ni, Zn, Pb, Mn, Fe) detected in the analyzed samples from different stations of Bulgarian Black Sea coast are illustrated in Table 1. Among the different metals analyzed lead, cadmium, chromium and nickel are classified as chemical hazards [8].

Seafood could be a major source of total arsenic exposure for man, since it contains mg/kg [9]. Arsenic exists in many forms. Arsenobetaine and arsenocholine are more efficiently accumulated from seawater by the fish and mussel than other chemical forms such as arsenite and arsenate and other organometallic complexes [10]. The lowest mean total arsenic concentration is found in mussel samples (0.094 mg/kg) and the highest mean in leaping mullet (0.218 mg/kg w.w). In the literature the data corresponding to As show values of 0.98±0.07 µg/g in muscle tissues of *Lophius budegassa* from Iskenderun Bay (Turkey) [11] between 0.38 mg/kg w.w in shad (*Alosa pontica*) and 1.1 mg/ kg w.w. in gray mullet (*M. cephalus*) for samples from Bulgarian Black Sea [12] and between 1.67 and 16.37 mg/kg fresh weight in edible part of the *M.galloprovincialis* taken from offshore along the Northern Adriatic coast in Rijeka Bay (Croatia) during different season [13]. The concentration of As in this study was generally low in all the species compared with the data in the literature and world food standards (Table 1).

European Union countries strictly monitor the concentration of Cd in marine environment since this element is very toxic, easily transported in the air. The maximum permissible value for fish (0.05 mg/kg w.w) and mussels (1.0 mg/kg w.w) do not exceed the values obtained in this study (Table 1). Levels of Cd reported in the literature vary between 0.012 mg/ kg w. w for muscle tissues of gray mullet and 0.015 mg/kg w.w for atlantic bonito caught from the waters of Black Sea [12], between 0.25- 1.16 µg/g w.w. in the soft tissue of green-lipped mussel *P. viridis* collected from coastal waters off Peninsular Malaysia [14] and between 0.10 mg/g d.w and 0.48 mg/g d.w. in *Alosa caspia*, Engraulis encrasicholus, *Trachurus trachurus*, *Sarda sarda* and *Clupea sprattus* of the middle Black Sea (Turkey) [15].

Table 1: Mean concentrations (mg/kg w.w) and standart deviation of heavy metals for each species (N is the number of analyzed fish and mussel species)

	European anchovy *(E.encrasicolus)* *N=12*	**Leaping mullet** *(C.saliens)* *N=3*	**Garfish** *(Belone belone)* *N=6*	**Guidelines** (in mg/kg w.w)			**Wild Black Sea mussels** *(M.galloprovincialis)* *N=20*	**Guidelines** (in mg/kg w.w)	
				BFC *(2004)*	**EC** *(2006)*	**FAO** *(2003)*		**BFC** *(2004)*	**EC** *(2006)*
As	0.134±0.020	0.218±0.103	0.128± 0.029	**5.0**	**---**	**---**	0.094±0.014	**2.0**	
Cd	0.013±0.0005	0.014±0.001	0.048±0.002	**0.05**	**0.05**	**0.05**	0.018±0.002	**---**	**1.0**
Cr	0.023±0.005	0.024±0.003	0.048±0.004	**0.3**	**---**	**---**	0.013±0.001	**---**	**---**
Cu	0.210±0.020	0.169±0.021	0.218±0.022	**10**	**---**	**---**	0.088±0.001	**30**	**---**
Fe	5.078±0.800	1.556±0.264	1.496±0.166	**---**	**---**	**---**	1.949±0.085	**---**	**---**
Hg	n.d*	0.123±0.011	0.096±0.005	**0.50**	**0.50**	**---**	0.023±0.006	**---**	**0.50**
Mn	0.328±0.056	0.166±0.0127	0.256±0.026	**---**	**---**	**---**	0.241±0.002	**---**	**---**
Ni	0.025±0.003	0.022±0.0418	0.029±0.026	**0.5**	**---**	**---**	0.034±0.016	**---**	**---**
Pb	0.082±0.0015	0.088±0.0110	0.160±0.036	**0.30**	**0.20**	**---**	0.017±0.061	**---**	**1.50**
Zn	3.044±0.144	1.282±0.0340	5.365±0.590	**50**	**---**	**---**	2.111±0.019	**200**	**---**

*n.d-under limit of detection

**BFC-Bulgarian Food Codex, EC-European Commission, FAO- Food and Agriculture Organization

Of particular concern is Cr which is considered as a heavy metal and pollutant, but at the same time as a microelement, whose biologically usable form plays an essential role in glucose metabolism [11]. Chromium together with arsenic and nickel are considered as hazardous elements by the USFDA [16] even though not covered by EC regulations for fish and other aquatic products. Chromium was detected in all the samples-mussel has less concentration of Cr than fish samples (0.013 mg/kg w.w for *M.galloprovincialis* and between 0.023 and 0.048 mg/kg for *E.encrasicolus, C. saliens and B.belone). Usero et al*. [17] found that Cr concentration in common sole vary between 0.015 and 0.031 mg/kg from salt marshes on the southern Atlantic coast of Spain; between 2.0 and 4.2 mg/kg d.w. in the sampled mussels from five sites on the Montenegrin coastal area (southeastern Adriatic Sea) [18]; and between 0.03 and 0.07 mg/kg f.w. in five most consumed marine fishes from Bulgarian Black Sea Coast [19]. Since there is no MPL for Cr in food set by various organizations it can be concluded that the level of Cr in analyzed samples is in accordance with the literature data.

Maximum concentration of Cu was detected in the sample of *B.Belone* (0.218 mg/mg w.w) and the minimum in wild black Sea mussel (0.088 mg/ kg w.w). In the literature the cocnentration of this essentil element vary between 4.9 mg/kg and 17.2 mg/kg in Mediterranean blue mussels from five sites on the Montenegrin coastal area (southeastern Adriatic Sea) [18]; up to 17.2 mg/kg in *Mugil cephalus* from Bulgarian section of Black Sea (Sinemoretz) [20] and between 1.3 and 4.54 mg / kg d.w for the muscle of *Dicentrarchus labrax* from The Bay of Güllük in Southeastern Aegean Sea (Turkey) [21]. The maximum copper level permitted for marine fishes is 10 mg/kg (30 mg/kg for mussel) according to Bulgarian Food Authority [22]. Our values were lower than the values from the literature and the ones stated by the Bulgarian Food Authorities.

The minimum and maximum iron levels observed were 1.496 mg/kg w.w in *B. Belone* and 5.078 mg/kg w.w in *E. encrasicolus*. Iron contents in the literature have been reported in the range of 0.82– 27.35 μg/g d. w in fish species from Iskenderun Bay, Northern East Mediterranean Sea, Turkey [23], 9.52– 32.40 μg/g d.w. in fish samples of the middle Black Sea (Turkey) [24], 7.46– 40.1 μg/g in seafoods from Aegean, Marmara and Mediterranean seas in Turkey [25]. There is no information about maximum iron levels in fish samples in Bulgarian standards [22] but the values from this study are within the data in the literature.

Mercury (Hg) is one of the most hazardous pollutants in the environment. It can exist in various forms like marine products are rich to methylmercury. The purpose of this study was focused on total mercury determination. The maximum level was found in mussel samples (0.023 mg/kg w.w) and minimum level-for garfish (0.0960 mg/kg w.w). For *E. encrasicolus* the concentrations was under limit of detection. In a study of ten different fish species from the Black Sea, Turkey it was found that the total Hg level vary between 25 mg/kg in *S. sarda* and 84 mg/kg in *M. merlangus* [15], between 0.99 and 14.79 mg/ kg d.w. whole soft tissue of mussels collected at 14 locations along the eastern Adriatic coast [26] and between 0.04 and 0.08 mg/kg w. w in the muscle tissues of the anchovy, mackerel, red mullet and picarel from the Adriatic Sea, Croatia [27]. However, mean metal levels in the analyzed marine samples were below the maximum permissible value indicated by the European Community [28] and the Bulgarian legislation (0.5 mg /kg w.w) [22].

There is no MPL for Mn set by EU [28] or Bulgarian food legislation [22] but in the literature it was found that the concentration of this element vary between 76 μg/g and 9.10 μg/g for ten different fish species from the Black Sea, Turkey [15] between 1.06 and 3.76 mg/g d.w. in *A. caspia*, *E. encrasicholus*, *T. trachur*us, *S. sarda* and *C. sprattus* from Black Sea [24]. The results from current study are less than that values for both fish and mussel samples.

Nickel and lead are two chemical elements considered as priority pollutants according to Water Frame Directive [28]. Fishes and other mollukans accumulate these elements from the surrounding environment. According to Bulgarian Food Codex the level of Ni and Pb in fish and mussels should not be over 0.5 mg/kg w.w and 0.30 mg/kg w.w, respectively. As it can be seen from Table 1 the results from this study are within these limits. Moreover, Pb concentration was in the range of 0.03 mg/kg w.w and 0.09 mg/kg w.w for three fish samples from from salt marshes on the southern Atlantic coast of Spain while Ni concentration – between 0.08 mg/kg w.w and 0.33 mg/kg w.w [17]. In our previous study of seven most consumed Bulgarian fish species collected from north-east coast of Black Sea, the concentrations of Pb ranged between 0.03 mg/kg w.w and 0.08 mg/kg w.w while Ni – between 0.008 mg/kg w.w and 0.028 mg/kg w.w [12]. For bivalve species from Sea of Marmara in Turkey, the concentration of Ni was significantly higher in *D. frunculus* than in *C. gallina* in the winter (1.556 mg/kg), summer (3.633 mg/kg), and autumn

(1.503 mg/kg) and the maximum contents of Pb were 1.342 mg/kg for *C. gallina* [29].

Among the ten metals under study, zinc showed the highest level of accumulation. A similar situation was observed in studies [30]. The concentration of zinc varied from 1.282 (for *L. Mullet*) to 5.365 mg/kg w.w (for garfish). The wild Black sea mussel sample show concentration of 2.111 mg/kg w. The maximum Zn level permitted for fishes is 50 mg/kg and 200 mg/kg for mussels according to Bulgarian Food Codex [31]. Tuzen [15] found zinc concentration to be between 38.8 μg/g in *E. encrasicholus* and 93.4 μg/g in *P. saltor* for samples from Black Sea, between 9.296-25.901 mg/kg for *D. trunculus* from Turkey [32]. The concentration of Zn from current study is within the limits set by various organizations and the data in the literature.

Assessment of health risk

THQs were used to estimate the risks associated with marine fish and mollusk consumption. THQs, calculated by the ratio of exposed heavy metal concentrations to the reference dose concentrations, are used to explain for long-term non-carcinogenic exposure probabilities [33]. A THQ value >1 means that the metal is likely to have adverse health effects. HI from THQs can be expressed as the sum of the hazard quotients [33]. Target cancer risk (TR) was used to indicate carcinogenic risks and it is calculated only for As, Ni and Pb since Cu, Hg, Zn, Mn, Fe, Cd, Cr do not cause any carcinogenic effects. Calculation of THQ, HI and TR is done as follows:

The THQs, HI and TR through consumption of *E. Encrasicolus*, *B.belone*, *C.saliens* and *M. galloprovincialis* from the Black Sea coast separately for males and females are illustrated in Figure 1.

Table 2: Model for estimating various health risks associated with consumption of marine products

TARGET HAZARD QUINTET	HAZARD INDEX	TARGET RISK
$THQ = \frac{(M_C \, . I_R \, . 10^{-3} . EF \, . ED)}{(RfD \, . BWa \, . ATn)}$	HI $= THQ_{As} + THQ_{Cd} + THQ_{Cr} + THQ_{Cu} + THQ_{Fe} + THQ_{Ni} + THQ_{Zn} + THQ_{Pb} + THQ_{Mn} + THQ_{Hg}$	$TR = \frac{(M_C \, . I_R \, . 10^{-3} . CPSo . EF \, . ED)}{(BWa \, . ATc)}$

M_C is the metal concentration in muscle tissues of fish (μg/g)
IR is the mean ingestion rate of fish
EF is the exposure frequency or number of exposure events per year of exposure,
ED is the exposure duration, total for adults
RfD is the reference dose (Cu = 0.04, As = 3×10^{-4}, Zn = 0.3, Ni= 0.02, Cr -3×10^{-3}, Fe = 9×10^{-3}, Cd =13×10^{-3}, Pb = 4×10^{-3}, Mn = 0.14 4 μg/g day),
BWa is the body weight, adult
ATn is the averaging time, noncarcinogens; calculated by multiplying exposure frequency in exposure duration over lifetime (day/year).
CPSo is the carcinogenic potency slope, oral (As = 1.5 and for Ni= 1.7 mg/kg bw-day);
ATc is the averaging time, carcinogens (day/years) and was calculated by multiplying exposure frequency in exposure duration over lifetime

Detected THQ values were below the limit value of 1, which indicates that the heavy metals may not possibly have adverse health effects on a human being by daily average consumption of those three fish species and mussels. In addition to this, HI has been observed that these values were up to 0.25 (for both males and females), meaning the values were below the threshold value of 1. Comparing the TR values with guideline values (10^{-6}-10^{-4}), indicates that marine fishes and mussels from Bulgarian Black Sea coast are safe for human consumption.

CONCLUSIONS

The present study provides new information on the distribution of trace elements in three fish and one wild mussel species from the Black Sea in Bulgaria. Based on the samples analyzed, the edible parts of fish and mussel samples do not carry heavy mineral loads, and the mineral concentrations are below the legal values for fish and fishery products established by various organizations.

Cancerogenic HI did not exceed one and it is assumed that no chronic risks were likely to occur at the site. Calculated average value of carcinogenic risk (TR) of the fishes and mussel under analysis was performed for As, Pb and Ni and indicates that analyzed fish are safe for human consumption. There is no significant difference in the analytical levels, HI and TR for the analyzed heavy metals between marine fishes and mussel samples.

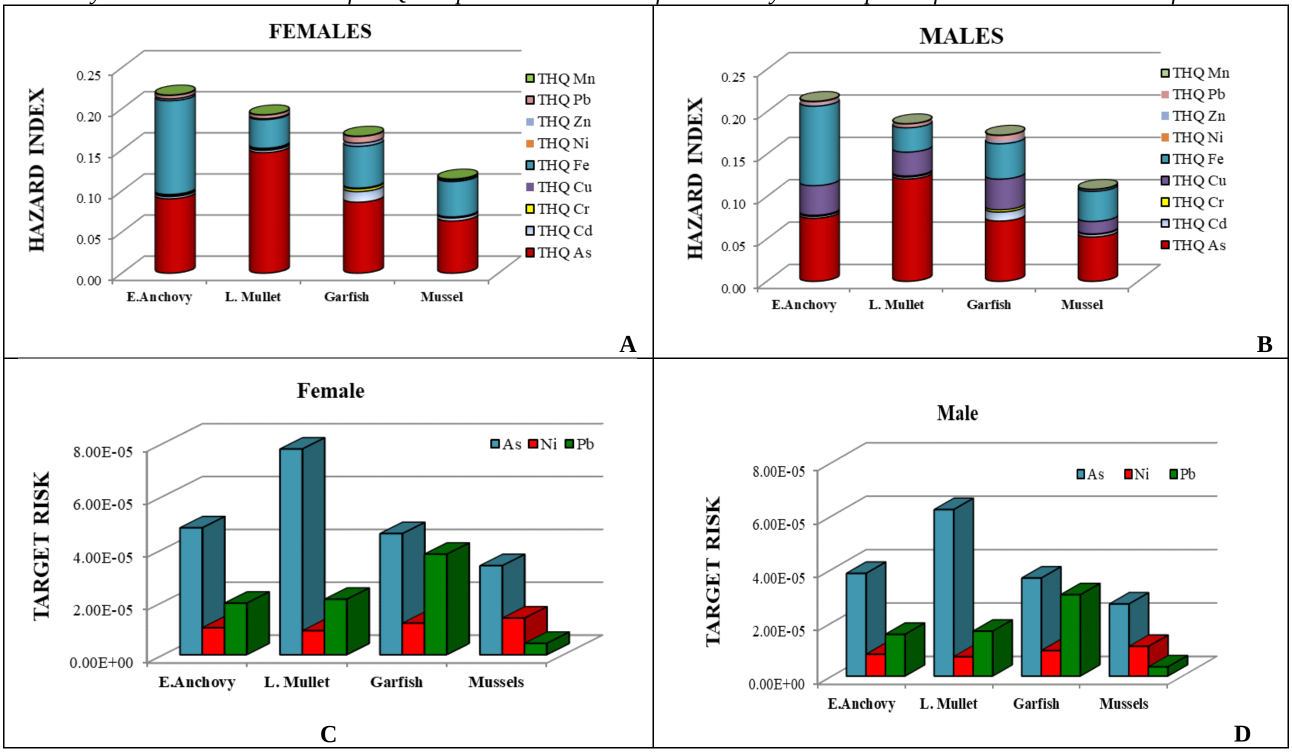

Figure1: Risk values of each metal contaminant of the marine product for females and males: a.) Hazard Index (females); b) Hazard Index (males); c.) Target risk (females); d.) Target risk (males)

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