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DETERMINATION OF HEAVY METALS (Pb, Cd, As AND Hg) IN BLACK SEA GREY MULLET (*MUGIL CEPHALUS*)

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Abstract

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Gray mullet (*Mugil cephalus*) is commercially important marine school pelagic fast moving fish species especially during warm weather. The M. cephalus is an omnivore and usually inshore, entering estuaries and lagoons, like Varna Lake. The aim of the present study was to determine and compare heavy metal contents (Pb, Cd, As and Hg) in edible tissue and gills of grey mullet (*Mugul cephalus*). The fish samples were collected from two different Black sea areas – Varna Lake and Nesebar. The sample preparation was performed by acid microwave digestion with „Multiwave" system in five stages program. Determination of As, Cd, and Pb were carried out on a Perkin Elmer Zeeman 3030 spectrometer with an HGA-600 atomizer, whereas Hg was analyzed by Milestone Direct Mercury Analyzer. Detected levels of As in the studied regions gives exceed those of other analyzed elements. The samples from both regions showed the higher levels of As in edible tissue than gills, especially from Region of Nesebar (1.1 mg/kg w.w.). The results for other heavy metals are several times lower than arsenic and were found in range 0.01–0.12 mg/kg w.w. All studied elements (except As) presented higher amounts from Varna Lake grey mullet compared with Neserbar region samples.

Key words: Heavy metals, Gray mullet, Black Sea

Introduction

Heavy metals are natural trace components of the aquatic environment, but their levels have increased due to industrial, agricultural and mining activities. As a result, aquatic animals are exposed to elevated levels of heavy metals .

The levels of metals in upper members of the food web like fish can reach values many times higher than those found in aquatic environment or in sediments. Thus contamination in the region is an important issue regarding the health of the aquatic animals and in turn, health of the seafood consumers. Gray mullet (*Mugil cephalus*) is commercially important marine school pelagic fast moving fish species especially during warm weather. The *M. cephalus* is an omnivore and usually inshore, entering estuaries and lagoons, like Varna Lake. The habitat of *M. cephalus*, which is an omnivore, is pelagic, usually inshore, entering estuaries and lagoons. While juveniles feed on invertebrates, adults mostly on detritus, bottom algae and small organisms, occasionally on plankton (Yilmaz, 2005).

This study was undertaken to investigate the current heavy metal contamination in edible tissue and gills in gray mullet (*M. cephalus*) from two stations differing in locations – Varna Lake and Nessebar.

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Materials and Methods

Sample preparation

Fresh fishes were purchased from Varna local fish markets in 2010, during fishing season. The species were caught from two fishing areas – Varna Lake (Midle part of Bulgarian Black Sea coast) and Nessebar (South part of Bulgarian Black Sea coast). Specimens of similar body weight and length were selected from all the captured species. Biological characteristics, were determined and noted and body weight (g) was 290.0±5.0 g length (cm), was 35.0± 2.5 cm.

Heavy metals content analysis

All solutions were prepared with analytical reagent grade chemicals and ultra-pure water (18 MΩ cm) was used for all dilutions. HNO_3 was of superb quality purchased from Fluka. All the plastic and glassware were cleaned by soaking in 2 M HNO_3 for 48 h, and rinsed five times with distilled water, and then five times with deionized water prior to use. Stock standard solutions of As, Hg, Cd, and Pb (1000 μg mL^{-1} Titrisol, Merck in 2% v/v HNO_3) were used for preparation of calibration standards.

Fish 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. Arsenic, cadmium and lead were determined 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. Whole data were subjected to a statistical analysis.

Statistical Analysis

The data were analyzed using Graph Pad Prism 5.0 software. T-test was used for calculation of means and standard deviations. One-way ANOVA (nonparametric test) statistical analysis was used to estimate differences between fish species. The significance level was $p<0.05$.

Results and Discussion

Heavy metals content

The data obtained for heavy metals content in analyzed fishes are presented in Table 1.

Table 1

Heavy metal concentration (mg.kg^{-1} wet weight) in grey mullet from Black Sea coast of Bulgaria (mean ± SD)

Location	Varna Lake	Nesebar
	Mercury	
Muscle	0.08 ± 0.01	0.05 ± 0.01**
Gills	0.12 ± 0.02	0.06 ± 0.01***
	Cadmium	
Muscle	0.024 ± 0.002	0.012 ± 0.002***
Gills	0.031 ± 0.003	0.013 ± 0.002***
	Lead	
Muscle	0.07 ± 0.01	0.05 ± 0.01**
Gills	0.08 ± 0.02	0.07 ± 0.01
	Arsenic	
Muscle	0.90 ± 0.10	1.10 ± 0.10**
Gills	0.38 ± 0.02	0.90 ± 0.10***

*** – $p < 0.001$; ** –$p < 0.01$

For species from Varna Lake the metal concentrations decrease in the order: As > Hg> Pb >Cd, whereas samples from Nessebar presented next distribution As > Hg = Pb > Cd. The non-essential elements as Hg and As in gills (Figure 1) and edible tissue (Figure 2) presented significant differences ($p < 0.001$).

Lead

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

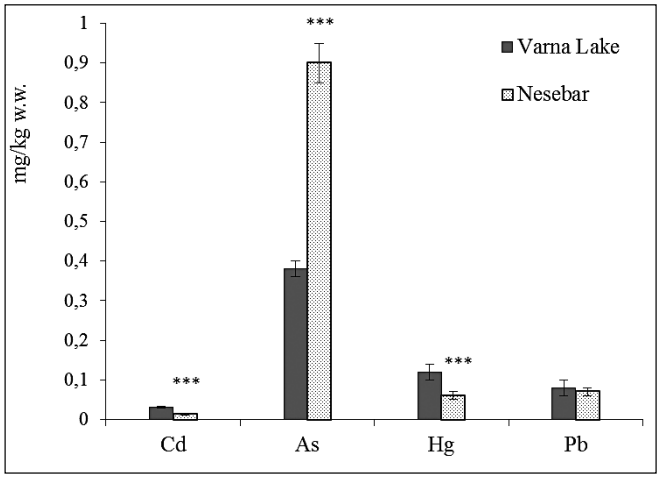

Fig. 1. Comparison of heavy metal contents in gills

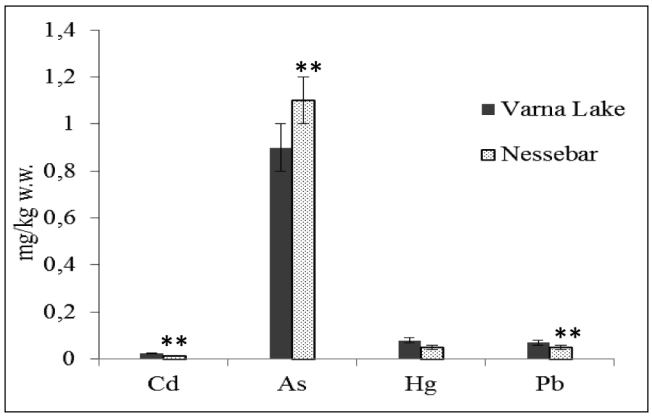

Fig. 2. Comparison of heavy metal contents in edible tissue

(2004) Expert Committee on Food Additives establishes a provisional tolerable weekly intake (PTWI) for lead as 0.3 mg.kg^{-1} body weight. European Community (No 1881/2006) and Bulgarian Food Codex (No 31/2006) set maximum permitted level for Pb in fish of 0.4 mg.kg^{-1}w.w. In present study, the higher concentration of lead was measured in gills in grey mullet from Varna Lake – 0.08 mg kg^{-1} w.w., whereas the edible tissue from this location presented 0.07 mg kg^{-1} w.w.

Tuzen et al. (2003) reported for fish species from the middle Black Sea lead levels in the range of 0.22–0.85 mg kg^{-1}ww. Uluozlu et al. (2007) were investigated lead contents of fish species from Black and Aegean seas and were found values in range of 0.33–0.93 mg kg^{-1} for edible fish tissue. In current research, Bat et al. (2012) presented three times higher concentrations for Pb (0.023 mg.kg^{-1} ww) for golden mullet from Sinop region (Turkey Black Sea coast) compared to our results. In general, analyzed species showed significantly lower Pb concentration in comparison to species from middle Black Sea and Aegean Sea. Highest Pb levals were obtained in gills in grey mullet from Varna Lake.

Cadmium

Cadmium is a non-essential, highly toxic and ecotoxic metal. The occupational levels of Cd exposure prove to be a risk factor for chronic lung disease and testicular degeneration. Cadmium could originate from water, sediments and food and may accumulate in the human body as may induce kidney dysfunction, skeletal damage and reproductive deficiency (WHO/FAO, 2004). The European Community (No 1881/2006) and Bulgarian Food Regulation recommended the maximum levels permitted for Cd in sea fish as 0.05 mg.kg^{-1} w.w. Moreover, the Joint FAO/WHO has recommended the provisional tolerable weekly intake (PTWI) as 0.007 mg.kg^{-1} body weight for this element. The obtained concentrations of Cd were highest in gills from Varna Lake grey mullet (0.031 mg.kg^{-1}ww) compared to edible tissue (0.024 mg.kg^{-1}ww) and Nessebar samples ($p < 0.001$) (Table 1). Bat et al. (2012) was determined a similar levels for Cd (0.025 mg.kg^{-1}) in edible tissue for mullet from Sinop region (Turkey Black Sea coast) compared to our results, as well as Nisbet et al. (2010) was detected for Cd in *Spicara smaris* and *Engraulis encrasicolus* (0.035 μg/g), from middle Black Sea. We can summarize that analyzed species from both regions showed significantly lower Cd concentration in comparison to same species monitored in middle and Southeast Black Sea.

Arsenic

Arsenic is a widely distributed metalloid, occurring in rock, soil, waterand air. General population exposure to arsenic is mainly *via* intake of foodand drinking water. Only a few percent of the total arsenic in fish is present in inorganic form, which is the only form about which a PTWI has been developed by JECFA (WHO/FAO, 2004). Chronic exposure to inorganic arsenic may cause serious impact on peripheraland central

nervous system (Jarep, 2003). In this study the higher concentration of As was measured in edible tissue from Nessebar (1.1 mg kg^{-1} w.w.) and Varna Lake grey mullet (0.9 mg kg^{-1} w.w.), whereas in gills from Varna Lake samples were found lowest concentration (0.38 mg kg^{-1}w.w., $p < 0.001$). The WHO/FAO (2004) and Bulgarian Food Regulation recommended the maximum levels permitted for As in sea fish as 5.00 $mg.kg^{-1}$ w.w. There are limited data about the arsenic content in gray mullet from Black Sea region, in the literature. Falko et al. (2006) was estimate As concentration in 14 edible marine species from Mediterranean Sea and described the fifteen times higher levels of As in red mullet (16.6 $mg.kg^{-1}$ w.w) comparison with our results and WHO/FAO recommendations. Current investigations presented As levels in 12 fish species from Malaysia (Alisa et al., 2012) in range from 0.25 to 6.57 $mg.kg^{-1}$w.w. In general, all of the results obtained are below the maximum As level permitted for fish according to Bulgarian Food Codex – 5.0 $mg.kg^{-1}$w.w. and compared to other fish species from Mediterranean Sea and Malaysian waters.

Mercury

Hg is one of the most toxic elements among thestudied heavy metals and exposure to high level of this element could permanently damage the brain, kidneys and developing foetus (WHO/FAO, 2004). The highest detected values of Hg was in gills of Varna Lake gray mullet (0.12 $mg.kg^{-1}$w.w), whereas in gills in Nessebar samples was measured a twice as lower value (0.06 $mg.kg^{-1}$w.w). Analysis of edible tissue of both region presented lower Hg levels compared to gills. According to Bulgarian Food Codex and European Community, the maximum mercury level permitted for sea fish is 1.0 mg/kg w.w. (1.5). There is insufficient information about the Hg content in gills and edible tissue of grey mullet from Black Sea region in the literature.The highest mercury concentrations measured in our study is lower then that reported by Yabanli et al. (2012) in frozen European sea bass and gilthead sea bream fillets produced and marketed in Turkey. Compared to our results Falko et al. (2006) presented similar concentration for Hg in 14 edible marine species from Mediterranean Sea, whereas Alina et al. (2012) were determined significant higher concentration for Hg in range from 1 to 6.5 mg/kg w.w in 12 fish species from Malaysia. Generally, the results obtained for analyzed species from both observed regions are below the maximum Hg level permitted for fish according to Bulgarian Food Codex – 1.0 $mg.kg^{-1}$w.w. (1) and compared to other fish species from Mediterranean Sea, Malaysian waters and Turkish markets.

Many authors have reported that metal accumulation by liver and gills occur in higher magnitude than muscle. Metal accumulation in each tissue considerably depends upon the accumulation capacity of the tissue. Gill is the main place for gas exchange in fish. In this organ, because of the short distance between blood and surrounding seawater, heavy metals ions may directly taken up from the passing water (Farkas et al., 2003). Safahieh et al. (2011) on the hand, supposed that this metals are also engaged in excretion of metals ion out of the body. Thus, the marked concentration of metals found in this tissue could be related to accumulative capacity of this organ during its role in depuration and absorption of uptaken heavy metals . Another explains for finding high concentration in gill, mucous excretion by this organ. The excreted mucous has affinity to be bound with metal ions. Therefore, even when heavy metals accumulation is low in other tissues it is possible to find considerable amount of metals in this part (Usero et al., 2003; Safahieh et al., 2011).

Conclusion

Heavy metals accumulate in different tissues of mullet fish with different magnitudes. Generally, metals accumulation in muscle was lower than gills. Cd, Hg and As mainly accumulated in gills while the edible tissue presented lower heavy metals concentrations. Fish caught from Varna Lake station closed to the city of Varna and Varna Port was found to containing high level of metals in muscle and gills, except As. The results provide new information on the distribution of these metals in gills and edible tissues of gray mullet. Based on the analyses of fish samples, heavy metal concentrations in fish species both fish samples tested were well within the limits set by the recommendations of European Community (No 1881/2006) and Bulgari-

an Food Regulation and indicate that gray mullets from investigated regions of Bulgarian Black Sea coast are safety for consumers.

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