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ASSESSMENT OF MERCURY CONTENT IN FISH TISSUES FROM SELECTED LAKES IN BULGARIA AND BULGARIAN BLACK SEA

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Abstract

Comparisons are made of mercury concentrations in muscle tissue of three fish species caught in two lake's points (Bourgas Lake and Mandra Lake) with two marine fish species caught from the south part of Bulgaria Black Sea (Nesebbar). The freshwater species are gibel carp, roach and perch. The seawater fish species are sprat and Mediterranean horse mackerel. The total mercury determination was performed by Milestone Direct Mercury Analyzer DMA-80.The results were expressed as mg/kg w.w.

The mean concentration of gibel carp was 0.0162 mg/kg w.w; of roach varies between 0.0273-0.0233 mg/kg w.w (depending on the location); of perch was 0.0273 mg/kg w.w. On the contrary, the mean concentrations of two sea fish species were higher: sprat was 0.12 mg/kg w.w and Mediterranean horse mackerel was 0.16 mg/kg w.w.

Concentrations of mercury were within the limits set from various health organization and are at acceptable levels for human consumption.

Key words: *Fish, mercury, Black Sea, Burgas Lake, Mandra Lake*

1. INTRODUCTION

Contamination with mercury on local, regional and global scale, has become intensively studied in recent years, due to the fact that mercury and its compounds are persistent, toxic, tend to bioaccumulate, and they pose human and ecosystem risks (Kljaković-Gašpic et al., 2006). It is widely recognized that human activities have artificially increased mercury loads in the atmosphere on a local, regional and even hemispheric scale, leading to the contamination of the environment. Population growth and urbanization have contributed to significantly elevated levels of mercury in the atmosphere and it has been estimated that mercury derived from anthropogenic activities in the atmosphere is up to 80% of the total mercury in the atmosphere The enhanced atmospheric deposition of mercury is often the dominant source of mercury to the aquatic systems, which may reflect in fish mercury concentrations (Voegborlo & Akag, 2007).

Since the tragedy of Minamata Bay in Japan most concern has centered on the presence of mercury in fish since seafood is a major source of this element. With the exception of occupational exposure, fish are acknowledged to be the single largest source of mercury to man. Fish accumulate substantial concentrations of mercury in their tissues and thus can represent a major source of this element to humans. This has been a matter of concern since its toxicity was clearly documented. Mercury, particularly in the form of methylmercury, is extremely toxic to marine organisms, wildlife, and man. The main pathway for human exposure to methylmercury is through consumption of fishery products. The likelihood of mercury toxicity from fish consumption has been identified in Peru and some coastal regions of the Mediterranean. In some instances, fish catches have been banned for human consumption because their total mercury content exceeded the maximum limits recommended by the Food and Agriculture/ World Health Organisation (FAO/WHO, 1972). Consequently, extensive surveys have been carried out in a number of countries to evaluate the presence of mercury in the aquatic biota including fish. Mercury also biomagnifies through the food chain; so large predatory fish species tend to have higher levels than non-predatory fish species at lower levels in the food chain. Recently, levels of mercury in fish have been widely reported (Batista et al, 2011; Lacerda et al., 2000; Love, Rush, & McGrath, 2003; Pan & Wang, 2011; Storelli, Giacominelli-Stuffler, &

Marcotrigiano, 2002; Storelli, Stuffler, Storelli, & Marcotrigiano, 2003). However, information on mercury levels in marine organisms from the Black Sea region is unavailable.

Due to the lack of any comprehensive data on the Hg content of fish from the Bulgarian part of the

Black Sea and its adjacent sea basins and the considerable global concern about mercury contamination of commercial and recreational fishery products, a survey of Hg concentrations in different species of fish from the coastal waters of Bulgarian Black Sea and two lake's points which water flows in Bulgarian coast of Black sea has been initiated, in order to determine whether mercury

occurs in marine and freshwater fish from the coastal waters of Bulgaria at concentrations of potential human health concern.

The Black Sea is the world's largest natural anoxic water basin below 180m in depth. It is a closed sea with a very high degree of isolation from the world's oceans, but it receives freshwater inputs from some of the largest rivers in Europe; the Danube, the Dniester, and the Dnieper (Stoichev et al., 2007). For this reason, Black Sea is considered one of the most polluted seas, and the increasing concentration of nutrients in recent years have led to a higher degree of eutrophication. The fishery yield has declined dramatically, and the tourism industry also suffers from serious pollution of the Black Sea.

This study is expected to involve the analysis of mercury in muscle tissues of two different species marine fish caught in the region of Bourgas , Bulgaria (sprat (*Sprattus sprattus*) and Mediterranean horse mackerel (*Trachurus mediterraneus ponticus)* and three freshwater species (gibel carp (*Carassius gibelio*), roach (*Rutilius rutilus*) and perch (*Perca fluviatilis*) from two lake's point situated near the gulf of Bourgas.

2. MATERIAL AND METHODS

2.1. Sampling

The fish samples were caught from the region of Bourgas Lake, Mandra dam and Nessebar (Figure 1). The two marine fishes sprat (*Sprattus sprattus*) and Mediterranean horse mackerel (*Trachurus mediterraneus ponticus)* were from the town of Nessebar in the region of Bourgas, Bulgaria. The two freshwater fish species roach (*Rutilius rutilus*) and perch (*Perca fluviatilis*) are caught from Mandra dam. The other two freshwater fish species roach (*Rutilius rutilus*) and gibel carp (*Carassius gibelio)* are from Bourgas Lake. The fish species were sampled in 2014.

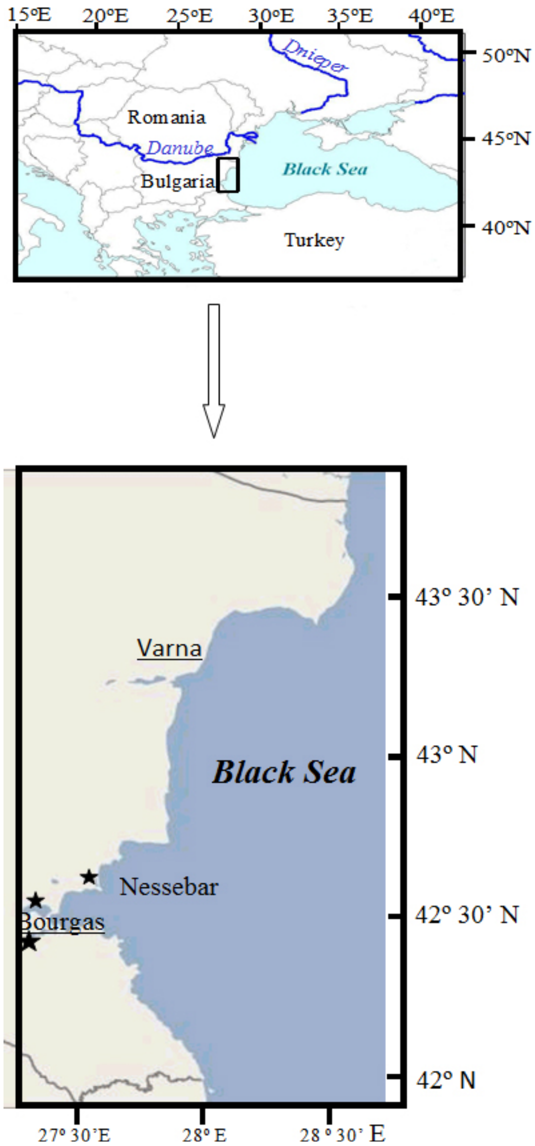


Figure 1. The map of sampling locations in the Bulgarian Black Sea coast

Total length and weight of the sample brought to laboratory on ice after collection were measured to the nearest millimeter and gram before dissection (Table 1). 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.

Sample	**Region**	**N**	**Weight(g) ± *SD***	**Length(cm) ± *SD***
Sprat (*Sprattus sprattus*)	Nessebar	12	52 ± 3	11± 3
Mediterranean horse mackerel (*T.mediterraneus ponticus)*	Nessebar	8	62 ± 4	15 ± 5
Roach (*Rutilius rutilus*)	Mandra dam	4	18.5 ± 2.3	11.1 ± 5.6
Perch (*Perca fluviatilis*)	Mandra dam	3	228 ± 23	22.7±1.1
Roach (*Rutilius rutilus*)	Bourgas Lake	4	17.8 ± 2.1	10.7 ± 1.1
Gibel carp (*Carassius gibelio)*	Bourgas Lake	4	117 ±9	17.9 ± 2.4

Table 1. Biometric data (mean ± SE) of the analyzed fish

2.2. Reagents and standard solutions

All solutions were prepared with analytical reagent grade chemicals and ultra-pure water (18 MΩ cm) generated by purifying distilled water with the Milli-QTM PLUS system. HNO_3 was of suprapur quality was purchased from Fluka®, Germany, 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 deionised water prior to use.

2.3. Sample digestion

Fish samples are thoroughly washed with MQ water. The fish specimens were dissected and samples of fish fillets quickly removed and washed again with MQ water. To assess the total metal content, 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 to assist the acid digestion process. (Table 2):

Microwave digestion system "Multiwave", "Anton Paar" Acid mixture	
HNO_3	3.0 cm^3
Temperature (max)	300 ^oC
Pressure (max)	75 bar
Quartz vessels	HQ 50
Sample amount	0.5 g
Final volume	10 ml

Table 2. Microwave digestion system general parameters

Reactors were subjected to microwave energy at 800 W in five stages program described below (Table 3):

Step	**Initial power(W)**	**Time (min)**	**Final power (W)**	**Fan**
1	100	5	600	1
2	600	5	600	1
3	600	5	800	1
4	800	15	800	1
5	0	15	0	3

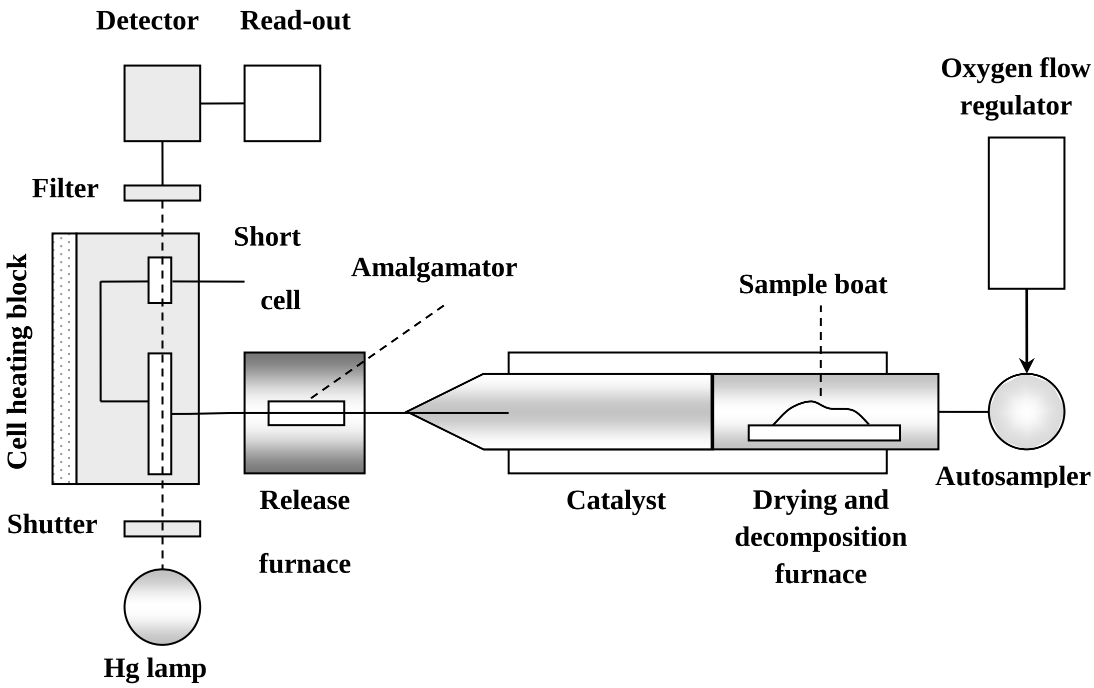
Table 3. Microwave digestion system operational parameters

An ICP-MS spectrometer (Agilent 7700x; Agilent Technologies, Tokyo, Japan) based on quadrupole mass analyser and octapole reaction system (ORS 3) equipped with concentric nebuliser and Ni cones served for quantitative analysis of mercury for the freshwater samples. The operating conditions of ICP-MS are shown in Table 4.

Parameter	Value
RF power (W)	1550
Sampling depth (mm)	8
Cool gas flow rate (l/min)	14.95
Auxiliary gas flow rate (l/min)	0.9
Nebulizer gas flow rate (l/min)	1.09
He flow rate (ml/min)	4.3
Energy discrimination voltage (V)	5
Nebulizer pump speed (rps)	0.1
Chamber temperature	2°C

Table 4. ICP-MS conditions

Determination of Hg for the marine fish samples was perform by Milestone Direct Mercury Analyzer DMA-80 (Figure 1).



Sample size	Drying time at 300°C	Decomposition Time	Waiting time
0.020-0.060 g	60 s	180 s	60 s

Figure 2. Total mercury determination in fish by DMA-80

A Dorm-2 certified dogfish tissue was used as the calibration verification standard. Recoveries showed good agreement between the certified and the analytical values.

2.4. Statistical analysis

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

3. RESULTS AND DISCUSSIONS

The common roach, perch and gilber carp were selected for monitoring biaccumulation of total mercury in dam and lake ecosystems. The roach (*Rutilius rutilus*) mostly inhabits waters that are somewhat vegetated, because larval and young fish are protected by the vegetation and the mature fish can use it for food. The common roach eats plant material, bottom-dwelling invertebrates, and plankton. Perch (*Perca fluviatilis*) are carnivorous fish most commonly found in small ponds, lakes, streams, or rivers. These fish feed on smaller fish, shellfish, or insect larvae. The gibel carp *(Carassius gibelio*) is a member of the family Cyprinidae, which includes many other fish, such as the common carp, and the smaller minnows. They are omnivorous and feed on plankton, invertebrates, plant material and detritus. All thus fish species are suitable for monitoring of aquatic ecosystems (Houserova et al, 2006)

Sprats (*Sprattus sprattus*) are highly selective in their diet and are strict zooplanktivores that do not change their diet with fish size, but include only zooplankton in their diet. They eat various species of zooplankton in accordance to changes in the environment, as temperature and other such factors affect the availability of their food. Mediterranean horse mackerel (*T.mediterraneus ponticus)* is pelagic, summer spawning fish with Mediterranean origin and landings in front of the Bulgarian Black Sea coast occur during spring and autumn. Information on the diet of *Trachurus trachurus* (L. 1758) in the Portuguese coast indicates that it is main diet comprises of crustaceans (Cabral and Murta, 2002).

Levels of mercury in the muscle of the analyzed fish species from coastal waters of Bulgarian Black Sea are shown in Figure 3. The summarized results of this study are expressed as means (mg/kg) wet weight.

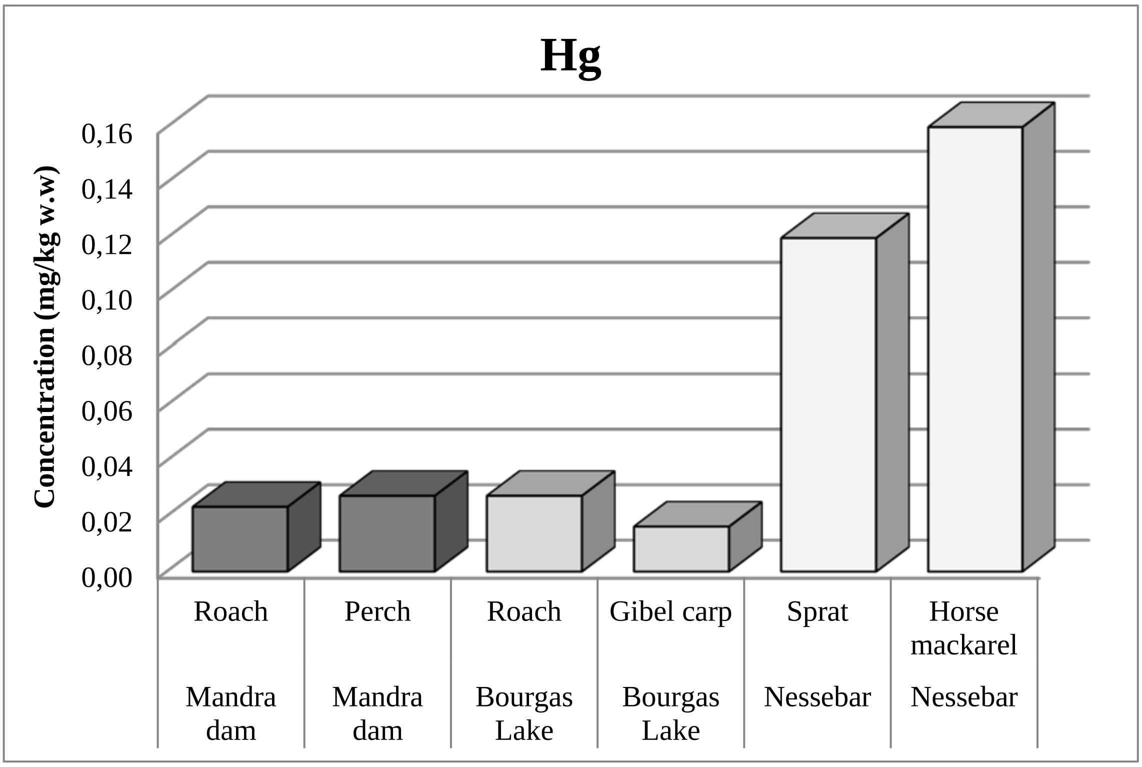


Figure 3. Mercury content of analyzed fish species

Mercury (Hg) is one of the most hazardous pollutants in the environment. It exists in three basic forms: elemental mercury (Hg^0) known as metallic mercury, inorganic mercury compounds (Ino-Hg), primarily mercuric chloride, and organic mercury, primarily methylmercury (Met-Hg) (ATSDR, 1999). Organic forms are more toxic than inorganic (ATSDR, 1999).

All the fish species analysed in this study are consumed by humans. Mercury levels were determined in a total of thirty-five samples, covering two marine fish species and three freshwater fish species. Mercury concentration ranged from 0.0162 to 0.16 mg/kg wet weight depending of the fish species. All the samples had concentration of mercury below the 0.5 mg/kg wet weight limit recommended by the FAO/ WHO (1972) and adopted by many countries (CIFA, 1992, Anonymous, 2004). The PTWI is 5 mg total mercury kg^{-1} body weight (bw) and 3.3 mg methylmercury kg^{-1} bw was reduced to 1.6 mg methylmercury kg^{-1} bw (EFSA, 2009; FAO, 2009) and could be exceeded depending on the species and quantity consumed. Mercury content in fish is considered to be a good indicator of human exposure to organic or methylmercury contamination. That mercury in fish appears to be predominantly in the form of methylmercury has been confirmed by many publications (Al-Majeed & Preston, 2000; Ikem and Egilla, 2008; Emami Khansari et al., 2005). Therefore, diet consisting particularly of fish, could be the main source of exposure to methylmercury in the general population.

The results of this study express in graph 1 show higher levels of mercury accumulated in marine fish species compared to the freshwater fish species. This confirms the assertion that geographical location in addition to other factors like metabolic differences of the various fish species (marine and freshwater) appears to be important with regards to the mercury content of fish; and this is further illustrated by the analysis of fish from different locations (WHO, 1976). Cod fish samples obtained from the strait between Denmark and Sweden, which is heavily contaminated, had values up to 1.29 μg/g wet weight; cod caught in the area of Greenland had values of 0.012 to 0.036 μg/g wet weight, whereas North Sea cod had values in the range of 0.150–0.195 μg/g wet weight (Voegborlo and Akago, 2007). In a study of swordfish from six areas extending from Caribbean Sea to the Grand Banks, significant variations from one area to another were observed in average mercury levels (Voegborlo and Akago, 2007).

Mercury levels in marine fishes from Black Sea region are in the range of 25-84 μg/g w.w Hg for ten most consumed fish species caught in 2008 along the Turkish part of Black Sea (Tuzen, 2009); and under detection limits for fish species from Sinop, Samsun, Fatsa and Ordu region of Black Sea (Nisbet et al, 2010). Mercury concentrations reported in our study are lower by an order of magnitude when compared to values reported in the literature and for other marine areas.

4. CONCLUSION

Mercury levels determined in thirty-five samples covering six species ranged from 0.0162 to 0.16 mg/kg wet weight. All the samples had concentrations of mercury below the FAO/WHO recommended limit of 0.5 mg/kg wet weight. These levels do not therefore constitute any significant health hazard to the general population.

Results from this study supply valuable information about the mercury contents in samples fish species along the Bulgarian coast of Black Sea and its inflows, and consequently indicate no environmental contamination along the coastal areas

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