HEAVY METALS IN WATER, SEDIMENTS AND MARINE FISHES FROM BULGARIAN BLACK SEA

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Abstract

In this study, the ecosystem marine water-sediment-biota was investigated and the pollution was assessed. The concentrations of eight elements were determined in marine water, sediments and four fish species collected from Black Sea (Varna), Bulgaria during 2013. Marine water recorded the highest concentrations of Zn (15-22 μg/L), As (1.1–1.2 μg/L) and Pb (0.7-0.8 μg/L) while Zn (31-52 μg/g), Pb (21-29 μg/g) and Cu (20-34 μg/g) and show the highest concentrations in sediments. Water and sediments showed similar spatial distribution patterns for the highest mean values of the different metals.

In the analysed fish species, the highest concentration of the metals Cu and Zn were found in *Trachurus Mediterrneus* (0.42 mg/kg w.w) and in *Sprattus Sprattus* (12.7 mg/kg w.w), respectively while the heavy metals As and Hg were found with maximum values in *Pseta Maxima* (3.99 mg/kg w.w and 0.08 mg/kg w.w respectively). The results from this study were compared with our data for a previous period (2004-2006) and they show decrease in the levels of heavy metal.

Key words: fishes, marine water, sediments, Black Sea, heavy metals

1. **INTRODUCTION**

Fish has been acknowledged as an integral component of a well balanced diet, providing a healthy source of energy, high-quality proteins, vitamins and a wide range of other important nutrients [1]. Moreover, fish is a significant source of omega-3 polyunsaturated fatty acids (PUFAs) whose benefits in lowering the risk of coronary heart disease and contributing to normal neurodevelopment in children have been widely recognized [2].

In contrast to the potential health benefits of dietary fish intake, the chemical pollutants contained in these products have emerged as an issue of concern, particularly for frequent fish consumers [3]. In this regard, heavy metals contamination is a worldwide-recognized public health hazard because these pollutants are widespread in the environment, including marine ecosystems, from either natural or anthropogenic sources [4]. As a consequence, they can be accumulated by marine organisms through exposure to metals present in water and sediments or in the food chain. Thus, diet comprises the main route of exposure to these elements in the general population [5].

The presence of trace metals in aquatic systems originates from the natural interactions between the water, sediments and atmosphere with which the water is in contact. The concentrations fluctuate as a result of natural hydrodynamic chemical and biological forces. Man, through industrialisation and technology, has developed the capacity to alter these natural interactions to the extent that the very waters and the aquatic life therein have been threatened to a devastating point.

Different aquatic organisms often respond to external contamination in different ways, where the quantity and form of the element in water, sediment, or food will determine the degree of accumulation [6,7]. The region of accumulation of heavy metals within fish varies with route of uptake, heavy metals, and species of fish concerned. Their potential use as biomonitors is therefore significant in the assessment of bioaccumulation and biomagnification of contaminants within the ecosystem. Many dangerous chemical elements, if released into the environment, accumulate in the soil and sediments of water bodies The lower aquatic organisms absorb and transfer them through the food chain to higher trophic levels, including fish [8]. The concentrations of heavy metals in the organs of fish are determined primarily by the level of pollution of the water and food [8]. Under certain conditions, chemical elements accumulated in the silt and bottom sediments of water bodies can migrate back into the water, i.e. silt can become a secondary source of heavy metal pollution [8].

The Black Sea is considered as one of the most polluted seas in the world since it is closed sea and with anoxic waters (below 180 m). Moreover, it receives freshwater inputs from some of the largest rivers in Europe (the Danube, the Dniester, and the Dnieper) which also contribute to its high degree of eutrophication and persistent pollutants [9, 10].

The aim of this study was to determine the heavy metals concentrations (Cr, Ni, Cu, Zn, As, Cd, Hg and Pb) in water, sediments and in muscle tissues of four marine fish species (*Trachurus Mediterrneus, Sprattus Sprattus, Pseta Maxima* and *N. melanostomus*) from Bulgarian Black Sea coast. The results obtained from this study would provide information for background levels of metals in the water, sediment and fish species of Black Sea, Bulgaria, contributing to the effective monitoring of both environmental quality and the health of the organisms inhabiting this ecosystem.

2. MATERIALS AND METHODS

Water, sediment and fish samples were collected two times (from February to November 2013) from one region of Bulgarian Black Sea coast (Varna region) . The sampling bottles were pre-conditioned with 5% v/v HNO_3 (Fluka, Buchs, Switzerland) and later rinsed thoroughly with ultra-pure water (18 MΩ cm) generated by purified distilled water with a Millipore Milli-Q Gradient A-10 water purification system (Bedford, MA). At each sampling site, the polyethylene sampling bottles were rinsed at least three times before sampling was done. Pre-cleaned polyethylene sampling bottles were immersed about 10 cm below the water surface. About 0.5 L of the water samples were taken at each sampling site. Samples were acidified with 10% HNO_3 (Fluka, Buchs, Switzerland), placed in an ice bath and brought to the laboratory. The samples were filtered through a 0.45 μm micropore membrane filter and kept at 4 °C until analysis. The samples were analyzed directly.

Sediment samples were collected using grab sampler from two sites. Samples were transported to the laboratory and air-dried in the laboratory at room temperature. Once air-dried, sediment samples were powdered and passed through 160 μm sieve. The samples packed in polyethylene bags and stored below −20°C prior to analysis. Sediments samples were weighed placed into the digestion bombs with 10 mL of HNO_3/HCl (1:3 v/v) and digested in a microwave digestion system. Sediments analysis was carried out according to the procedure described earlier [12].

A total of 34 samples of Mediterranean horse mackerel (*Trachurus Mediterrneus*), sprat *(Sprattus Sprattus)*, turbot (*Pseta Maxima)* and Black sea goby (*N. Melanostomus)* were collected with nets by local fishermen. 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):

Table 1. Biometrics data (mean ± SD) of fish from the coastal waters of the Bulgarian Black Sea

Sample	**Sampling season, year**	**Habitant**	**N**	**Weight (g)± *SD***	**Length (cm)± *SD***
Mediterranean horse mackerel (*Thrachurus mediterraneus ponticus*)	Autumn 2013	pelagic	12	23.0±1.2	13.1±0.8
Sprat (*Sprattus spratus*)	Spring 2013	pelagic	9	10.8±5.3	9.7±1.4
Black Sea Goby (*Neogobius melanostromus*)	Spring 2013	demersal	10	69.1±6.8	16.3±1.0
Turbout (*Psetta Maxima*)	Autumn 2013	demersal	3	1950±30	29.5±1.3

For small species (i.e. sprat and horse mackerel), the entire edible part of each individual was included for preparation of composite sample. However, for bigger species (i.e. goby and turbot) fillets of edible part of each individual were collected separately and included in the respective composite samples. Approximately 1.0 g sample of muscle from each fish were dissected, washed with distilled water, weighted, packed in polyethylene bags and stored at -18 °C until chemical analysis.

To assess the total metal contents in muscle tissues of the analyzed fish species, microwave assisted acid digestion procedure was carried out using the parameters in Table 2:

Table 2: Microwave digestion system general parameters

Microwave digestion system “Multiwave”, “Anton Paar” Acid mixture	
HNO_3	6.5 cm^3
Temperature (max)	300 ^oC
Pressure (max)	75 bar
Quartz vessels	HQ 50
Sample amount	1 g
Final volume	10 ml

Multiwave™ 3000 Microwave Sample Preparation System (PerkinElmer/Anton-Paar) delivering a maximum power and temperature of 800 W and 300 °C, respectively, and internal temperature control, was used to assist the acid digestion process. Reactors were subjected to microwave energy at 800Win five stages shown in Table 3:

Table 3: Microwave digestion system operational parameters

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

Determination of Cu and Zn: Flame atomic absorption spectrometric (FAAS) measurements were carried out on a Perkin Elmer (Norwalk, CT, USA) Zeeman 1100 B spectrometer (Überlingen, Germany) with an air/acetylene flame. The instrumental parameters were optimized in order to obtain maximum signal-to-noise ratio.

Determination of As, Cd, Cr, Ni and Pb: Electrothermal atomic absorption spectrometric (ETAAS) measurements were carried out on a Perkin Elmer (Norwalk, CT, USA) Zeeman 3030 spectrometer with an HGA-600 graphite furnace (Table 4). Pyrolytic graphite-coated graphite tubes with integrated platforms were used as atomizers. The spectral bandpass, the wavelengths and instrumental parameters used were as recommended by the manufacture. Only peak areas were used for qualification. Pd as $(NH_4)_2PdCl_4$ was used as modifier for ETAAS measurements of As and Cd.

Determination of Hg was performed by Milestone DMA-80 direct Mercury Analyzer. The sample size was between 0.020 and 0.0060 g, with drying temperature at 300 °C for 60 s, decomposition time for180 s and waiting time of 60 s.

Working standard solutions of metals were prepared with serial dilution of stock standard solutions of each metal containing 1000 mg/L (J.T. Baker, Phillipsburg, NJ, USA). All glassware were previously soaked overnight with 10% nitric acid solution and then rinsed with ultra pure water. The reaction vessels for microwave oven were cleaned before each digestion by using 4 mL of INSTRA-Analyzed nitric acid (70% w/w) (J.T. Baker, Phillipsburg, NJ, USA), heated for 15 min at 600 Wand then rinsed with ultra pure water.

The accuracy of analytical procedure was checked by analyzing the standard reference materials (water: SRM-143d, National Institute of Standards and Technology; sediment: CRM-277, Community Bureau of Reference; fish: DORM-2, (NRCC, Ottawa)). Recovery rates ranged from 79 to 96% for all elements the investigated.

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

3. RESULTS AND DISCUSSION

3.1. Water

The variations of Cr, Ni, Cu, Zn, As, Cd, Hg and Pb, concentrations in coastal water samples from the four stations in the Bulgarian coast of Black Sea are summarized in Table 4.

Table 4. Heavy metal concentrations (mean ± s) in water (μg/L) in various sampling stations

	Stations	Cr	Ni	Cu	Zn	As	Cd	Hg	Pb
1	Krapec /North/	< 0.4	< 0.6	1.1 ± 0.2	18 ± 1	1.5 ± 0.3	0.06 ± 0.02	< 0.05	1.5 ± 0.2
2	Varna Lake	< 0.4	< 0.6	0.9 ± 0.2	22 ± 2	1.1 ± 0.2	0.09 ± 0.02	< 0.05	0.8 ± 0.1
3	Varna /Central/	0.5 ± 0.1	< 0.6	0.6 ± 0.2	15 ± 1	1.2 ± 0.3	< 0.05	< 0.05	0.7 ± 0.1
4	Bourgas /South/	< 0.4	< 0.6	< 0.6	31 ± 2	1.10 ± 0.24	< 0.05	< 0.05	0.34 ± 0.04

The mean Cr concentration ranged between 0.4 and 0.5 μg/L, Ni and Hg were under detection limits, Cu was in the range of 0.6–1.1 μg/L, Zn ranged from 18.00 to 34 μg/L, As concentration ranged between1.10 and 1.50 μg/L, Cd was between 0.05 and 0.09 μg/L, while Pb ranged between 0.34 and 1.5 μg/L. The highest mean values for Cu, As and Pb were observed for Station 1 (Northern part of Bulgarian Black Sea).

Data obtained in the present study are in general comparable to those found in literature. El-Moselhy et al [13] found that the annual mean Cd concentration of water sampled from Suez Canal ranged between 0.15 and 0.18 Ag/l, Pb was in the range 1.84–2.57 μg/L, Cu was in the range 1.16–5.33 μg/L, while Zn ranged from 8.13 to 23.24 μg/L. Begun at al [14] studied Madivala Lakes water of Bangalore, Karnataka, India and the metal concentrations of Cr, Ni, Cd and Pb vary was as follows 0.22– 2.5 μg/L, 1.0– 6.4 μg/L, 1.02–4.9 μg/L, 0.66– 7.2 μg/L, respectively.

3.2. Sediments

Table 5 shows the distribution of Cr, Ni, Cu, Zn, As, Cd, Hg and Pb in sediment samples from the four stations in the Bulgarian coast of Black Sea

Table 5. Heavy metal cconcentrations (mean ± s) in sediments (µg/g dry wt) in various sampling stations

	Stations	Cr	Ni	Cu	Zn	As	Cd	Hg	Pb
1	Kaliakra /North/	30±2	51±4	81±4	104±5	4.1±0.5	0.07±.01	<0.05	39±3
2	Varna Lake	24±2	44±3	96±6	159±9	8±1	1.5±.02	0.07±0.01	62±4
3	Varna /central/	< 2	11±1	20±2	31±2	4.2±0.7	0.06±0.01	<0.05	21±1
4	Bourgas /South/	20±2	43±3	53±3	87±5	3.9±0.5	0.08±0.01	<0.05	41±3

The mean Cr concentration ranged between 2 and 30 µg/g dry wt, Ni ranged between 11 and 51 µg/g dry wt, Cu was in the range of 20–96 µg/g dry wt, Zn ranged from 31 up to 159 µg/g dry wt, As concentration ranged between 3.9 and 8 µg/g dry wt, Cd was between 0.06 and 1.5 µg/g dry wt, Hg concentration were below detection limits except the sample from Varna Lake, while Pb ranged between 21 and 62 µg/g dry wt. According to data Varna Lake Station exebit maximum concentration in Cu, Zn, As, Cd, Hg and Pb. The local distribution of metals in sediments did not give similar pattern with that found in water, the only exception - the highest Cd levels in station 2.

By comparing the levels of metals determined in this studied with the levels found in the sediments of the northern part of the Gulf of Suez, Red Sea during 1992–1998, it can be observed that the concentrations of Cd and Pb are relatively stable, while the contents of Cu and Zn are higher than that levels [14]. Öztürk et al [15] recorded the following results for the sediments from Avsar Dam Lake in Turkey during 2008 :0.76 ±0.04 mg/kg d.w for Cd; 13.33-14-48 mg/kg d.w for Cr; 23.47- 29.98 mg/kg d.w for Cu; 22743-25268 mg/kg d.w for Fe; 28.25 – 29.99 mg/kg d.w for Ni and 2.44-4.04 mg/kg d.w for Pb. Heavy metals in shallow sediments from the Black Sea, Marmara Sea and Aegean Sea regions of Turkey [16] show the following pattern (in µg/g dry wt): Cd, <0.02-0.9, Cr, 10.8-245; Ni, 2.2-79.2; Zn, 33.9-484.2; Fe , 4.2-29.6; Mn, 206- 2830; Pb, <0.05-84.2 and Cu, 11.5-528 and higher concentrations of Cr and Ni were found at the stations in the Black Sea Rivers. Our results are in good agreement with the data from the literature.

3.3. Marine fishes

Table 6 shows the heavy metal concentrations (mg/kg w.w) of the analyzed fish species collected from the four stations in the Bulgarian coast of Black Sea.

Table 6. Heavy metal cconcentrations (mean ± s) in muscle tissues of different fish species (mg/kg w. w) from four stations in the Bulgarian coast of Black Sea

	Station	Cr	Ni	Cu	Zn	As	Cd	Hg	Pb
Black sea goby /*N.Melanostomus*/	Varna Lake	0.050±0.001	0.028±0.003	0.2±0.01	3.9±0.3	2.460±0.220	0.004±0.001	0.05±0.010	0.06±0.01
Sprat /*Sprattus Sprattus*/	Varna /Central/	0.030±0.001	0.008±0.001	0.3±0.02	12.7±1.0	1.93±0.170	0.005±0.001	0.10±.010	0.03±0.01
Med.horse mackerel /*T. mediterrneus*/	Bourgas /South /	0.040±0.001	0.043±0.004	0.42±0.03	6.4±0.5	1.42±0.130	0.004±0.001	0.09±0.010	0.04±0.01
Turbout / *Pseta Maxima*/	Krapec /North/	0.020±0.001	0.064±0.006	0.19±0.01	4.8±0.4	3.99±0.36	0.005±0.001	0.08±0.010	0.01±0.01

Chromium contents in the literature have been reported in the range of 0.07–6.46 μg/g dry weight in fish species from Iskenderun Bay, Northern East Mediterranean Sea, Turkey [30] and 0.04-1.75 μg/g in seafood from Marmara, Aegean and Mediterranean seas in Turkey [23]. The lower chromium content was 0.02 mg/kg in *Psetta Maxima* while the highest chromium content was 0.05 mg/kg in /*N.Melanostomus*/. The maximum Cr level permitted for fishes is 0.3 mg/kg according to Bulgarian Food Codex [20]. Our values are under those reported in the literature.

The lowest and highest Ni levels in fish species were 0.008 mg/kg in *Sprattus sprattus* and 0.064 mg/kg in *Psetta Maxima*. Nickel contents have been reported in the range of 0.11-12.9 μg/g dry weight in fish species from Iskenderun Bay [30] and 1.14-3.60 μg/g in fishes from Black Sea, Turkey [22]. The World Health Organization [18] recommends 100-300 μg nickel for daily intake. The maximum nickel level permitted for fishes is 0.5 mg/kg according to Bulgarian Food Codex [20]. The results from this study were below the limits sets by various health organizations and the data in the literature.

The copper concentration found in this study the range of 0.19 in *Psetta maxima* up to 0.42 mg/kg in *Thrachurus mediteranean*. Copper in the literature range from 0.32-6.48 mg/kg for muscle of fish from Marmara, Aegean and Mediterranean seas in Turkey [23] and 0.23 to 9.49 mg/ kg for muscle of fish from Marmara Sea [29]. The maximum copper level permitted for sea fishes is 10 mg/kg according to Bulgarian Food Authority [20] and 20 mg/kg according to Turkish Food Codex [19]. Our values were lower than the values from the literature.

The concentration for zinc reported in the literature range of 9.5-22.9 mg/kg for muscle of fish from the Black Sea coast [21], 16.1-31.4 mg/kg for muscles of fish from Mediterranean sea [31] and 3.51-53.5 mg/kg for species from Aegean and Mediterranean Sea [32]. The minimum and maximum zinc levels in fish in our study were found as 4.8 mg/kg in turbot and 12.7 mg/kg in sprat.The maximum zinc level permitted for fishes is 50 mg/kg

according to Bulgarian Food Codex [20] and Turkish Food Codex [19]. Maximum Zn level in edible parts of fish in this research was found to be below than both the Bulgarian permissible standards and levels reported in the literature.

Arsenic is highly toxic element. Arsenic concentration in this study ranged between 1.42 mg/kg in Mediterranean horse mackerel up to 3.99 mg/ kg in turbot. The Joint FAO/WHO [27] Expert Committee on Food Additives (JECFA) establishes a provisional tolerable weekly intake (PTWI) for inorganic arsenic as 0.015 mg/kg body weigh/week and 0.05 mg/kg body weigh/week for organic-arsenic intakes. The concentration of arsenic reported in fish species from Adriatic Sea ranged of 0.56 to 10.03 mg/kg fresh weight [24]. Tuzen [22] had measured an arsenic concentration in different fish species from Black Sea as follows: 0.15 ±0.01 μg/g for Psetta maxima; 0.27± 0.02 μg/g for Pomatomus Saltator, 0.23 ± 0.01 μg/g for *Mugil cephalus*, 0.14 ± 0.01 μg/g for Sarda Sarda, 0.18± 0.02 μg/g for *Trachurus trachurus* and 0.17± 0.01 μg/g for *Sprattus sprattus*. The concentration of As in this study was generally low in all the species compared with both the data in the literature and world food standards.

Cadmium is a non-essential, highly toxic metal. The European Community [17] established the maximum levels permitted of cadmium in a fish as 0.05 mg/kg f.w. Moreover, the Joint Food and Agriculture Organization and World Health Organization (FAO/WHO) [18] has recommended the provisional tolerable weekly intake (PTWI) as 0.007 mg/kg body weight for cadmium. The maximum Cd level permitted for fish samples is 0.10 mg/kg according to Turkish Food Codex [19]. The Bulgarian Food Regulation recommends a 0.05 mg/kg f.w. for sea fish [20]. Cadmium levels in analyzed fish species were between 0.004 and 0.005 mg/kg w.w. for muscle. Cadmium concentration in literature has been reported as follow: 0.02-0.24 mg/ kg for gray mullet [21]; from 0.10 μg/g in *Psetta maxima*; up to 0.35 μg/g in *Mugil cephalus*; 0.23 μg/g for *Pomatomus Saltator*; 0.13 μg/g for *Sarda Sarda*; 0.32 μg/g for *Trachurus trachurus*; 0.30 μg/g for *Sprattus sprattus* from the Black Sea, Turkey [22]; 0.02-0.37 mg/ kg for edible part of fishes caught from Marmara, Aegean and Mediterranean seas in Turkey [23] and 0.002- 0.02 mg Cd kg^{-1} fresh weight for species from Adriatic Sea [24]. In the present study, cadmium levels were in good agreement with reported literature data and with the data from the international organizations.

The lowest and highest mercury levels in fish species were found in 0.05 mg/kg in Black Sea goby and 0.10 mg/kg in sprat, respectively. The maximum Hg level permitted for fishes is 0.5 mg/kg according to Turkish Food Codex [19] and Bulgarian Food Codex [20]. The PTWI is 1.6 mg methylmercury kg^{-1} bw [27] and could be exceeded depending on the

species and quantity consumed. Mercury levels in analyzed fish samples were found to be lower than legal limits. In the literature mercury levels in fish samples have been reported in the range of 0.01- 0.50 μg/g in marine fishes in Malaysia [28] and 25-84 μg/kg for fishes from Black Sea [22].

The highest total lead content was found in Black Sea goby(*N.Melanostomus*). The Joint FAO/WHO [18] Expert Committee on Food Additives establishes a provisional tolerable weekly intake (PTWI) for lead as 0.025 mg/kg body weight. Whereas the maximum level of lead in seafood establishes by the European Community [17] is 0.2 mg/kg f.w in fish. According to Turkish Food Codex, the maximum lead level permitted for sea fishes is 0.3 mg/kg [19] while Bulgarian Food Regulation sets this level as 0.4 mg/kg fresh weight (for sea fish) [20]. Lead levels in the literature have been reported in the range of 0.22-0.85 mg kg^{-1} for muscle of fish from the middle Black Sea [26], 0.28 μg/g in *Psetta maxima* and 0.87 μg/g in *Pomatomus Saltator* from the Black Sea, Turkey [22] and between 0.33-0.93 μg/ kg for muscle of fish from Black and Aegean seas [26]. The values obtained from the analyzed samples showed good agreement with values reported in the literature and below the level set by various health organizations.

4. CONCLUSIONS

In summary, the order of heavy metal distribution in water from the four stations in the Bulgarian coast of Black Sea is Zn >As > Cu ~ Pb > Ni > Cr > Cd ~ Hg, in sediments is Zn > Cu > Ni > Pb > Cr >As > Cd > Hg and in marine fishes is Zn >As > Cu > Pb > Hg ~Cr~ Ni > Cd. It can therefore be concluded that sediment and water are good indicators for Zn pollution.

Additionally heavy metals (Cd, Pb, As, Hg, Ni, Cu, , Zn and Cr) were determined in five most consumed Bulgarian fish species collected from Bulgarian coastal of Black Sea. Among the eight metals under study, zinc showed the highest level of accumulation. None the less this value was in the range stated in the literature. The levels of the analyzed elements were in within the recommended legal limits. In the analyzed fishes, there were no health risks in respect to the concentration of cadmium, copper, lead, mercury and other elements' level.

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