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Heavy metals and PCBs level of bluefish (*Pomatomus saltatrix)* from Bulgarian Black sea waters

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Abstract The concentration of some heavy metals (Cd, Mn, Fe, Cu and Pb) and polychlorinated biphenyls (PCBs) were determined in muscle tissue of bluefish *(Pomatomus saltatrix)* collected from the coast of Bulgarian Black Sea. Quantitative determination of the PCBs compounds was performed by gas chromatography–mass spectrometry detection (GC–MS), while the heavy metals were determined by atomic absorption spectrophotometry. The validation of the heavy metal procedure was performed by analysis of standard reference material (DORM-2 Dogfish Muscle). Pb and Cd were under the detection limits for the samples from year 2004. The levels of iron showed the highest value trough the two year period of investigation (from 6.51µg/g up to 7.06 µg/g).

The fourteen congeners of PCB were analyzed including the set of 7 indicators PCBs (IUPAC No 28, 52, 101, 118, 138, 153, 180). PCBs were found in all samples with maximum level in year 2004 (Σ PCBs = 9.1. mg/kg product). The levels of these organochlorines are considered to be comparable to baseline levels.

From an ecotoxicological point of view, the concentrations of heavy metals and polychlorinated biphenyls compounds reflect a comparatively clean and pollution-free environment. These concentrations may be, thus, considered as useful background levels to which to refer for comparison within the Black Sea.

Keywords: Heavy metals, PCBs, bluefish, AAS, GC–MS, Black Sea, Bulgaria

1. Introduction

The world-wide contamination by heavy metals and polychlorinated biphenyls (PCBs) is considered to be of great concern due to their toxic effects on humans and wildlife. PCBs constitute a class of 209 compounds with differential biological activity and toxicity as a result of differences in the number and position of chlorine atoms [1]. Reports from the literature suggest that polychlorinated biphenyls, particularly dioxin-like PCBs, have a complex spectrum of toxicological properties, including chloracne, thymic atrophy, liver damage, immunotoxicity and cancers [2, 3] and have, also, been associated with low birth weights and learning and behavioural deficiencies in children of women who consume large quantities of contaminated fish or are occupationally exposed [4]. The PCBs are fat–soluble and therefore they are accumulated in the lipids and foods containing fats.

Aquatic environmental quality currently receives a great deal of attention [5]. Contamination with heavy metals in aquatic system has been a serious concern for over decades. Heavy metals are introduced in those systems through variety of human activities - industrial waste, agricultural and urban sewage. Under certain environmental conditions, heavy metals may accumulate to toxic concentrations and cause ecological damage [6]. Metals such as copper, zinc, iron, manganese and selenium are essential since they play important roles in biological systems. The essential metals can also produce toxic effects at high concentrations [7].

Fish is the final chain of aquatic food web and an important food source for human. Therefore, heavy metals in aquatic environments are transferred through food chain into humans. A well known fact is that fish muscle is not an active tissue in accumulation of heavy metals [8]. On the contrary liver is a good monitor of water pollution with metals since their concentrations are proportional to those present in environment.

Recently, agricultural and industrial developments as well as increase in population have substantially increased the contamination of

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Bulgarian region of Black sea. The present study reports data on metal (Cd, Mn, Fe, Cu and Pb) and PCB analyses carried out on the muscle tissue of bluefish in order to evaluate its current status of contamination.

Several analytical techniques are available for trace element determination in fish samples such as inductively coupled plasma optical emission spectrometry (ICP-OES), inductively coupled plasma mass spectrometry (ICP-MS), graphite furnace atomic absorption spectrometry (GFAAS), flame atomic absorption spectrometry (FAAS).

Conventional methods for determination of organochlorine compounds in fatty samples usually involve laborious and time consuming clean-up steps, including extraction with organic solvents, acid digestion of extracts followed by adsorption liquid chromatography Most of these analytical techniques use GC-ECD or GC-MS for analytical determination, reaching detection limits at the ng g–1 level in most cases [1, 4].

Bluefish (*Pomatomus saltatrix)* occurs in oceanic and coastal waters. It is a good food fish; marketed mostly fresh, but also dried or salted, and frozen. It is most common along surf beaches and rock headlands in clean, high energy waters. Feed on other fish, crustaceans and cephalopods. Voracious and aggressive. Migrate to warmer water during winter and to cooler water in summer.

2. Experimental

2.1. Sampling

Bluefish species from two different locations were collected by local fishermen's nets in September 2003, September 2004 and September 2005 (**Fig. 1**). These sampling sites in the coastal waters of Bulgarian Black Sea are Kaliakra (North) and Nesebar (South). All the samples were immediately transported to the laboratory and frozen at −20 °C until analysis. All the equipments used for sample collection, transportation, and preparation, were free from contamination.

This period was chosen because autumn months are considered as the end of the feeding period of the fish species before their further migration.

2.2. Sample treatment

2.2.1. Heavy metal analyses

All reagents used in this study were of analytical reagent grade unless otherwise stated. Double deionised water (Milli-Q Millipore 18.2 MΩ cm^{-1} resistively) was used for all dilutions. HNO_3 was purchased by Merck, Darmstadt, Germany. All laboratory ware 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.

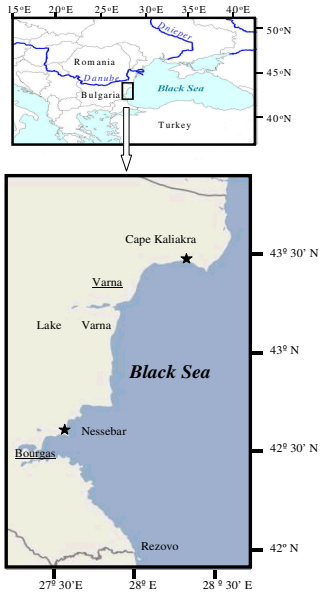

Fig. 1. The map of sampling locations in the Bulgarian Black Sea coast

The total weight (g) and length (cm) were determined prior to analysis. The biometric data of the fish samples are presented in **Table 1.**

Table 1. Biometric data (Mean ± SE) of bluefish (*Pomatomus saltatrix*) from Bulgarian Black Sea Coast

Sample	Sampling Location	Sampling season, year	Habitant	N	Weight (g)	Length (cm)
A	North	Autum 2004	pelagic	4	53.75 ± 4.8	16.1 ± 0.8
B	South	Autum 2004	pelagic	6	51.5 ± 8.8	16.1 ± 1.5
C	South	Autum 2005	pelagic	6	85.8 ± 16.3	20.0 ± 1.3

Special care was taken to prevent metal contamination of the samples by the laboratory equipment. Samples were then dissected to separate muscle and stored in polyethylene plastic bags at -20 ºC until chemical analysis. A portion of the dissected sample visually approximating 1 g was precisely weighed, and then the material transferred to a 100 mL Teflon beaker. Thereafter, 10 mL ultrapure concentrated nitric acid was added slowly to the sample. The Teflon beaker was covered with a watch glass, and heated at 200 ºC on a hot plate for 3 h, until the solution evaporate slowly to near dryness. Two milliliters of 1 N HNO_3 was added to the residue and the solution was evaporated again on the hot plate. By repeating the additional digestion twice, all organic materials in each sample were completely digested. After cooling, 2.5 mL of 1 N HNO_3 was added to digested residue and was transferred to 25 mL volumetric flasks, then diluted to level with deionized water. Before analysis, the samples were filtered through a 0.45 µm nitrocellulose membrane filter. Sample blanks were prepared in the laboratory in a similar manner to the field samples [9]. Metal contents were expressed as mg/kg wet weight. All samples were analyzed three times for Cd, Cu, Fe, Mn and Pb by Atomic Absorption Spectrometer (Varian Model Spectrometer AA-240). Deuterium background corrector was used. Copper, manganese, cadmium, iron and lead were determined in air-acetylene flame. The operating parameters for working elements were set as recommended by manufacture given in **Table 2.**

The atomic absorption signals were measured as a peak height mode against an analytical curve. The analytical measurements were based on peak height. Standard solutions were prepared from stock solutions (Merck, multi element standard).

A Dorm-2 certified dogfish tissue was used as the calibration verification standard. Recoveries between 90% and 110% were accepted to validate the calibration. All specimens were run in batches that included blanks, a standard calibration curve, two spiked specimens, and one duplicate. The results showed good agreement between the certified and the analytical values, the recovery of elements being partially complete for most of them

2.2.2. PCB analyses

Approximately 10.0 g sample was taken from each batch. Sample preparation includes extraction of fat with mixture of n-hexane (dichloromethane (3:1 v/v) after addition of internal standards PCB 30 (Dr.Ehrenstorfer, Germany) and PCB204 (Dr.Ehrenstorfer, Germany). Further clean-up and lipid removal are achieves by using acid and basic modified silica gel multiplayer columns. The sample was concentrated on a rotation vacuum evaporator at temperature 35-40ºC. 0.2 g sample from the extract was taken for analysis.

Table 2. Instrumental analytical conditions of investigated elements

Working conditions	Mn	Cu	Cd	Fe	Pb
Wavelength (nm)	279.5	324.8	228.8	248.3	283.3 and 217.0
Slit width (nm)	0.2	0.5	0.5	0.2	1.0
Lamp current (mA)	5	4	4	5	10
Ar flow (ml/min)	250	250	250	250	250
Acetylene/air ratio	3.5/1.5	3.5/1.5	3.5/1.5	3.5/1.5	3.5/1.5

The analysis of PCBs was performed by Gas Chromatograph Perkin Elmer Autosystem XL coupled with an electron-capture detector and a mass spectrometer detector. A Restek® Rtx-5 column (Macherey-Nagel, Hoerdt, France) 60 m-long with 0.25 mm internal diameter and 0.25 µm film thickness was used. Detector temperature was 310ºC, injector split/splitless at e temperature 250º C. Carrier gas helium at speed 30 cm/min, temperature program: 120ºC, increase by 20ºC/ min up to 320ºC and retention time 15 min.

The parameters of the methods were as following: limit of detection were between 0.1 and 0.3 ng/g for individual congeners, analytical recovery were between 59 and 65%, and variation coefficients – 23-28 %. The fourteen congeners of PCB (IUPAC no 28, 31, 52, 77, 101, 105, 118, 126, 128, 138, 153, 156, 169, 180) were analyzed including the set of 7 indicators (IUPAC no 28, 52, 101, 118, 138, 153, 18)

2.3. Statistical analysis

The whole data from the heavy metal determination and PCB were subjected to a statistical analysis. Student's-test was employed to estimate the significance of values

3. Results and discussion

3.1. Heavy metal contaminations

The results of the analyses for trace metals in bluefish muscle tissues are shown in **Table 3.**

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 (2004) 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 [10] is 0.2 mg/kg fresh weight in fish. According to Turkish Food Codex, the maximum lead level permitted for sea fishes is 0.3 mg/kg [11] while Bulgarian Food Regulation sets this level as 0.4 mg/kg fresh weight (for sea fish0 [12] . There is lack of data about lead levels in that fish species in the literature for the period of investigation. The lead concentration in various Adriatic fish species such as anchovy, anger, hake, mackerel, red mullet and sole were between 5.0 µg/kg f.w and 29.0µg/kg f.w [13]. The lead level of *M.cephalus* and *T.mediterranaeus* caught at three stations in Iskenderun Bay, Turkey range between 0.71 µg metal/g wet weight and 10.87 µg metal/g wet weight [14]. Just recently Tuzen [15] has found 0.87 µg/g in edible part of *P.saltor* from the Turkish side of Black Sea.The experimental data indicate that the highest level of this element was found in the species from Nesebar (South) in year 2005. The average value after three parallel determinations was 0.07 mg/kg w.w. The lowest lead levels were found in the muscle tissues from both samples in year 2004 - under 0.01 mg/kg w.w.) The values obtained from the analyzed samples were below the values reported in the literature and below the level set by various health organizations

Cadmium

The occupational levels of cadmium exposure prove to be a risk factor for chronic lung disease and testicular degeneration [16]. Cadmium could originate from water, sediments and food. Cadmium may accumulate in the human body and may induce kidney dysfunction, skeletal damage and reproductive deficiency [17]. The European Community [10] established the maximum levels permitted of cadmium in a fish as 0.05 mg/ kg fresh weight. Moreover, the Joint FAO/WHO has recommended the provisional tolerable weekly intake (PTWI) as 0.007 mg/kg body weight for cadmium [18]. The maximum Cd level permitted for fish samples are 0.10 mg/kg fresh weight according to Turkish Food Codex [11] and the Bulgarian Food Authority [12]. Cadmium levels in analyzed fish species from year 2004 were below 0.02 mg/kg w.w. for muscle. On the contrary, the level of this element in year 2005 has shown a relatively high value according to FAO (0.07 mg/kg wet weight) but still in the limits set by Bulgarian and Turkish organizations. An explanation of the high concentrations of cadmium may be expected to result from a cephalopod-based diet [19].

Table 3. The mean heavy metal concentration (mg/kg w.w ±SD) in the tissues of bluefish (*Pomatomus saltatrix*) from Bulgarian Black Sea coast.

Sample	Pb	Cd	Cu	Mn	Fe
A	< 0.01	< 0.02	1.46 ± 0.09	0.03 ± 0.003	6.79 ± 0.32
B	< 0.01	< 0.02	1.45 ± 0.09	0.05±0.005	7.06 ± 0.30
C	0.07 ± 0.007	0.07 ± 0.007	1.34 ± 0.13	0.14 ±0.01	6.51 ± 0.65

Copper

Copper is an essential nutrient for all vertebrates and some lower animal species. Several abnormalities have been observed in copper-deficient animals, including anemia, skeletal defects, degeneration of the nervous system and etc. There are a number of important copper-containing proteins and enzymes, some of which are essential for the proper utilization of iron. Organ meats, especially liver, are the richest sources of copper in the diet, followed by seafood, nuts, and seeds. Additional contributions to intake may come from adventitious sources, such as copper-containing fungicides sprayed on agricultural products [20].

The copper concentration found in this study was between 1.34 up to 1.46 mg kg^{-1} wet weight. Copper in the literature range from 2.26 μg metal/g d.w. muscle tissues from *Mullus Barbatus* up to 6.15 μg metal/g d.w. for muscle tissues from *Caranx crysos* caught in Mediterranean Sea waters [21], 0.12-14.7 ppm dry weight for muscle of the Eastern Mediterranean fishes (Israel) [22]. Canli et al. indicated that tissues metal concentration of Cu varies between 2.19 μg metal/g d.w up to 4.41 μg metal/g d.w for different fish types [23]. An FAO/WHO Expert Committee concluded that no deleterious effects can be expected in humans whose copper intake is 3.5 mg/kg body weight/ week [24]. The maximum copper level permitted for sea fishes is 10 mg/kg according to Bulgarian Food Authority [22] and 20 mg/kg according to Turkish Food Codex [21]. Unfortunately the literature lacks results of copper accumulation in bluefish. Our values were lower than the values from the literature and those set by the health organizations.

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. A number of manganese-activated enzymes play important roles in the metabolism of carbohydrates, amino acids, and cholesterol. In humans, demonstration of a manganese deficiency syndrome has been less clear. Signs of manganese deficiency include impaired growth, impaired reproductive function, skeletal abnormalities, and altered carbohydrate and lipid metabolism [25]. Dairy products, meat, fish, and poultry are among the richest dietary sources of manganese [20].

The minimum and maximum manganese levels observed were 0.03 mg/kg w.w. in the samples from year 2004 and 0.14 mg/kg w.w. for samples in 2005. Manganese content in the literature have been reported in the range of 1.56-3.76 μg /g d.w. in fish samples of the middle Black Sea (Turkey) [26], 0.69-3.56 μg /g d.w in different fish species from Turkish Black Sea coast [27], 0.10-0.99 mg/kg w.w. in seafood from Marmara, Aegean and Mediterranean seas in Turkey [28]. According to both FAO [29] and Bulgarian standards [12] there is no information on the carcinogenity of manganese. The US National Academy of Science [30] recommended 2.5-5 mg day manganese and the World Health Organization [31] recommended 2-9 mg day for an adult. Regarding this RDA the intake of Mn in our samples is below the above values.

Iron

Iron is a constituent of hemoglobin, myoglobin, and a number of enzymes and, therefore, is an essential nutrient for humans [32]. In addition to these functional forms, as much as 30 % of the body iron is found in storage forms such as ferritin and hemosiderin (mainly in the spleen, liver, and bone marrow), and a small amount is associated with the blood transport protein transferring. Iron availability may be enhanced by consumption of foods containing ascorbic acid. Iron is widely distributed in food supply: meat, eggs, and fish sources.

The lowest and highest iron levels in fish were 6.51 mg/kg in the samples from Nesebar in autumn 2005 and 7.06 mg/kg in the samples from the same place in autumn 2004. Iron content in the literature have been reported in the range of 0.82-27.4 μg/g dry weight in fish species from Iskenderun Bay [33], 9.52-32.4 μg/g dry weight in fish samples of the middle Black Sea (Turkey) [34], 32.2-129.0 μg metal/g d.w. in fish tissues from the Northeast Mediterranean Sea [21], and 12.6-70.2 μg/g dry weight in fish samples from the Western Indian Ocean [34]. The US National Academy of Science [20] recommends a RDA for iron in elderly women and men — 10 mg/day. There is no information about the maximum permissible iron concentration in fish tissues in Bulgarian standards [12]. Our iron concentrations were generally in agreement with the literature

3.2. PCB concentrations

Concentrations of individual PCB and total PCBs in bluefish (*Pomatomus saltatrix*) are shown in **Table 4**, expressed in nanograms per gram of fat and milligram per kilogram fresh weight (f.w).

Our results showed that in bluefish in year 2003 the values for PCB congeners were under detection limits for the northern region. On the other hand the south region showed PCB# 126 and 138 with values 0.20 ng/g fat and 0.10 ng/g fat. Our results showed that in bluefish in year 2004 for the north region of Black Sea, PCB # 28, 101, 105 and 126 were the most abundant (20 ng/g fat, 0.20 ng/g fat, 0.20 ng/g fat and 0.30 ng/g fat respectively). The highest PCB concentrations for the south region of the same year were found for PCB 28, which is an indicator one.

PCB 28 was predominant in year 2004 for the North accounting for the main pollution in this year.

Table 4. PCBs concentrations (ng/g fat) in muscle tissue of bluefish

	2003		**2004**	
	North	South	North	South
Fat %	23.5	17.2	17.8	12.5
28*	-	-	20.00	4.70
31	< LOD	-	-	-
52*	-	-	-	0.90
77	-	-	-	-
101*	-	-	0.20	-
105	-	-	0.20	-
118*	-	-	-	-
126	-	0.20	0.30	-
128	-	-	-	-
138*	-	0.10	-	0.70
153*	-	-	-	-
156	-	-	-	0.30
169	-	-	-	-
180*	-	-	-	-
Total PCBs [ng/g fat]	< LOD	0.30	20.70	6.60
Total PCBs [ng/g product]	< LOD	0.002	9.10	3.30

* Target PCBs

Papadopoulos et al. [35] determined the levels of PCDD/ Fs and dioxin-like PCBs in 77 food samples from the Greek market, including fish (wild, 4 samples; aquaculture, 7 samples). The average concentrations of PCDD/Fs were 0.12 and 0.47 pg WHO-TEQ/g fat, for wild and aquaculture fish, respectively. For non-ortho PCBs, average sums were 0.33 and 1.19 pg WHO-TEQ/g fat, for wild and aquaculture fish, respectively.

On the other hand, samples of 12 edible fish species from the Marmara Sea, Turkey, were recently analyzed for PCBs and other organochlorinated compounds [36]. Total PCB

concentrations (sum of 7 congeners) ranged from 63.30 to 508.71 ng/g fat, with an average value of 253.08 ng/g fat.

In Romania, Covaci et al. [37] determined the levels of PCBs in eleven fish species collected in year 2001 from the Danube Delta. The sum of PCBs ranged between 46 ng/g lw and 1416 ng/g lw for the different river fish types. On the other hand, samples of 12 edible fish species from the Marmara Sea, Turkey, were recently analyzed for PCBs and other

We had investigated other fish species for the same period of time and location - 2003 and 2004- and the results were as follows: European sprat (39.8 ng/g fat for North region and 10.9 ng/g fat for South region for year 2003; and 13.8 ng/g fat for North region and 17.3 ng/g for South region for year 2004)

The PCB levels found in the sample here analyzed were lower than those reported in the literature. The results presented as ng/g fat product (0.002-9.10 ng/g product) are also below the data from the literature [38]. We suggest that it is due to the typical way of living of that species and its different feeding habits.

4. Conclusions

Concentration of PCBs has been determined in edible part of bluefish from the Northern and Southern part of Bulgarian Black Sea coast. The PCB level were under detection limit for year 2003 while in year 2004 the main congeners of PCB is number 28 in both locations- South and North (total PCB 6.60-20.7). The concentrations of PCBs in all the collected samples were lower than the levels recommended by the European Union – 200 ng/g lw.

Whatever the source of metals, the results of the present study showed that concentrations of studied metals were into the limits when compared to data obtained from uncontaminated waters. The concentration of some heavy metal such as Fe decreases in bluefish specie for the two year monitoring period.

With specific reference to fish species considered and the element analysis, the data obtained in the study do not show evidence of risk for the consumer, even if the most exposed categories are taken into account. Because PTWI and PTDI values estimated for examined fish and metals were far below the established values by various authorities, it may be concluded that consumption of these species from each seas is not a problem on human health.

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