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Distribution of Zn, Cu, Pb and Cd in the Tissues and Organs of *Psetta maxima* from Sinop Coasts of the Black Sea, Turkey

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Abstract In the present study, turbot *Psetta maxima* were selected and analyzed for the Zn, Cu, Pb and Cd concentrations in the muscles, livers and gonads. The results revealed that Zn concentrations were the highest followed by Cu; Pb and Cd being the lowest in the tissues (muscle) and organs (gonads and liver). The liver metal concentrations showed the highest values, whereas the muscle concentrations were the lowest. A comparison between the concentration of each metal found in males and females found significant differences between the sexes for all metals. The liver concentrations of Zn, Cu, Pb and Cd were in the range of 46.89-57.21, 15.96-29.51, 1.75-4.09 and 0.19-0.44 mg/kg wet weight, respectively. The gonad concentrations of Zn, Cu, Pb and Cd were in the range of 37.81-44.11, 6.66-11.32, 0.21-0.34 and 0.14-0.27 mg/kg wet weight, respectively. The muscle concentrations of Zn, Cu, Pb and Cd ranged 18.56-35.33, 1.81-6.11, 0.07-0.21 and 0.016-0.046 mg/kg wet weight, respectively. The values obtained were compared the Turkish Food Codex and Commission Regulation (EC) maximum permitted levels in food. The present study demonstrated that estimated daily and weekly intakes of selected metals via consumption of fish were below the Permissible Tolerable Daily Intake (PTDI) and Provisional Tolerable Weekly Intake (PTWI) values established by FAO/WHO.

Keywords Black Sea, Turbot, Zinc, Copper, Cadmium, Lead

1. Introduction

The Black Sea, located approximately between latitudes of 41° to 46°N and longitudes of 28° to 41.5°E, is a nearly enclosed and isolated environment, has suffered from severe ecological changes since the 1970s. It has experienced one of the worst environmental degradations of the world oceans for 30-40 years. The environmental crisis and subsequent dramatic changes in the ecosystem and its resources were a direct consequence of anthropogenic pollution due to an enormous increase in nutrients and pollutant load from rivers discharging into the Black Sea, uncontrolled industrial and municipal wastewater inputs around the periphery, dumping of wastes into open parts of the sea, and accidental and operational releases of oil[1,2]. Anthropogenic factor has become the basic one not only in the pollution of the Black Sea but also in considerable changes of its biological ecosystem. Heavy metal pollution of the Black Sea has also become an important problem because of urbanization and industrialization processes and intense agriculture activities[3-5]. The compounds of heavy metals such as zinc, copper, lead and cadmium appear to be dangerous contaminants having a direct toxic effect on the aquatic organisms and through the food chain process eventually threaten the health of human.The first two metals are essential in trace quantities, but are markedly toxic, whilst lead and cadmium are non-essential element.

The studies related to heavy metals in marine coastal areas involve monitoring a series of metals in water, sediment and marine species[6,7] which are used for environmental assessment. Because of its commercial significance and the relation to human health, the regional and national administrations show a clear tendency to monitoring marine organisms including bottom fish. Therefore, it is important to monitor heavy metal in fish.

The Black Sea was one of the most productive seas, pelagic and benthic organisms recording a remarkable abundance. The shallow marine coastal zones of the coast of Sinop Peninsula provide nursery areas, identified as essential fish habitat, for a wide variety of commercial fish species, particularly flatfish. In the present study *P. maxima* were monitored. They are a unique group of marine fish widely distributed throughout the world, usually found in a broad range of shallow-water habitats. Turbot is a benthic flatfish species that lives in contact with the sediment and feed on small invertebrates and fish.

The aims of the present study were to:

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1- Determine if zinc, copper, lead and cadmium were present in *P. maxima* from Inceburun, northernmost area of Turkey, of Sinop coastal waters of the Black Sea,

2- Describe if the concentrations of these heavy metals were significantly different among the tissues (dorsal muscle) and organs (gonads and liver),

3- Determine if the concentrations of Zn, Cu, Pb and Cd were significantly different between male and female of *P. maxima*,

4- Compare the concentrations of any Zn, Cu, Pb and Cd present with the guidelines set down the Turkish Food Codex and Commission Regulation (EC) for the safe consumption limits of fish.

2. Material and Method

2.1. Sampling

P. maxima is an important commercial fish in the Black Sea regions. Catches of the turbot from Turkish waters in 2009 were 383 tons[8]. The material were sampled in 2009, by gill-nets at the depth of 70-80 m from Inceburun, northernmost area of Turkey during fishing season from the Black Sea. Figure 1 shows fish sampling area.

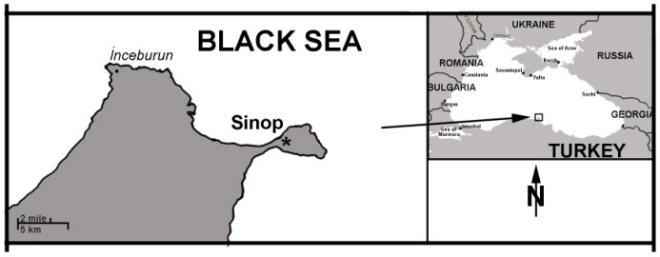

Figure 1. Fish sampling area from Inceburun of Sinop coasts of the Black Sea, Turkey

After capture, fish were placed in clean plastic bags and transported to the laboratory in freezer bags with ice. Total body length (cm) and weight (g) were recorded for fishes. Biometric parameters of *P. maxima* are presented in Table1.

Sampled individuals from each sex were rinsed in clean sea water and then dorsal muscle without skin, gonad and liver were removed. The tissues and organs were thoroughly chopped and mixed and four subsamples of about 80 g taken. All prepared samples were stored deep frozen at -21℃ until their analysis.

Table 1. Biometric data (Mean ± SE) of *Psetta maxima* from Inceburun in Sinop coastal waters of the Black Sea

Psetta maxima	N	Total length (cm) Mean ±SE (Range)	Total weight (g) Mean ±SE (Range)
Male	8	40.38±0.82 (37.5-43.5)	1150±80.09 (900-1500)
Female	8	46.91±0.72 (44.9-49.5)	1988.75±80.43 (1650-2200)

2.2. Chemical Analyses

The samples were thawed and then approximately 20 g of samples were introduced in acid cleaned jars and digested with hot concentrated nitric acid to obtain release of heavy metals. All organic materials in each sample were completely digested. The digests were allowed to cool, filtered through a 0.45 µm Millipore membrane filter, transferred to 50 ml volumetric flasks and made up to mark with 1% nitric acid and diluted with double distilled water to 25 ml. The digestion of each sample was made in triplicate and in all experiences three blanks were also performed in order to check for possible contamination. The digests were kept in plastic bottles and later all determinations were made using Atomic Absorption Spectrophotometer (AAS) (modified from[9]). The values were used to plot a standard curve. The standards and blank were treated in the same way as the real samples to minimize matrix interferences during analysis. Metal contents were expressed as mg kg-1 wet weight. Working calibration standards of all metals were prepared by serial dilution of concentrated stock solutions (Merck, Germany) of 1000 mg/ l. These and blank solutions were also analyzed in the same way as for the digested samples.

The quality of the analysis was assessed by ensuring that blanks were acceptable and also by the inclusion of a Certified Reference Material (CRM) in each analysis. Dogfish muscle and liver (DORM 2 and DOLT 4) as reference materials from National Research Council of Canada was used as the CRM in the present study. These standards were treated and analysed under the same conditions as the samples and recoveries of the metals ranged from 93 to 102%.The results are shown in Table 2

Table 2. Observed and certified values of metal concentrations, as mg/kg, in reference materials (CRM)

Metals	Certified value DORM 2	Observed value*	Recovery (%)	Certified value DOLT 4	Observed value*	Recovery (%)
Zn	25.6 ± 2.3	26.2 ± 2.1	102	116 ± 6	118 ± 2	102
Cu	2.34 ± 0.16	2.29 ± 0.11	98	31.2 ± 1.1	30.2 ± 2.3	97
Pb	0.065 ± 0.007	0.061 ± 0.004	94	0.16 ± 0.04	0.15 ± 0.03	94
Cd	0.043 ± 0.008	0.040 ± 0.006	93	24.3 ± 0.8	22.9 ± 0.8	94

These results showed that all the adopted analytical procedures are accurate and suitable for the analytical task.

2.1. Statistical Analyses

Statistical analysis of data was carried out using Statistica 7.0 statistical package program. A one-way analysis of variance (ANOVA) was performed, followed by Duncan comparisons for the source of statistically significant differences of metal concentrations between tissues and organs and sexes. The paired samples *t*-test was used to compare differences between samples. P-values less of 0.05 were considered statistically significant[10].

3. Results and Discussion

3.1. Metal Concentrations in *Psetta Maxima*

Zn, Cu, Pb and Cd concentrations were determined in edible tissues (dorsal muscle) and organs (gonad and liver) of *P. maxima* from Inceburun, northernmost area of Turkey, of Sinop coastal waters of the Black Sea during fish season in 2009. Heavy metal concentrations found in the tissues and organs of turbot varied considerably depending on the type of the tissue and organs (Figure 2). General metal levels in liver were higher than those in muscle tissue and gonad for both sexes, suggesting that liver is an organ in which metals are stored.

There was no significant ($p > 0.05$) differences between Zn, Cu and Pb levels in gonads of the male and female, but Cd showed less difference in their heavy metal levels but it was a significant difference ($P< 0.05$). Pb recorded their lowest levels in muscle and their highest levels in livers of both sexes. No significant ($p > 0.05$) differences occurred between Pb in muscles and gonads of the both sexes. No significant ($p > 0.05$) differences found between Cd levels in gonad and liver of the female, but males showed significant ($P< 0.05$) difference in Cd level between organs gonad and liver.

The liver metal concentrations showed the highest values, whereas the muscle concentrations were the lowest. A comparison between the concentrations of these metals found in muscle found no significant ($p > 0.05$) differences between the sexes except Zn ($p< 0.05$).

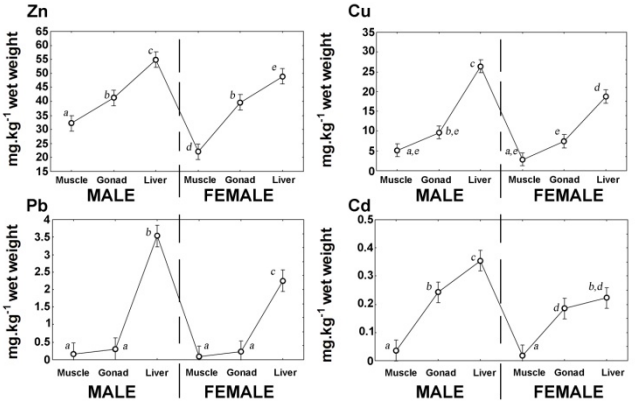

Figure 2. The means with standard deviations (vertical line) of Zn, Cu, Pb and Cd concentrations (µg/g wet wt.) in the dorsal muscle tissues, gonad and liver of *Psetta maxima* from Sinop coastal waters of the Black Sea during fishing season in 2009. (a, b, c, d, e, f = The same letters beside the vertical bars in each graph indicate the values are not significantly different ($p > 0.05$))

The results revealed that Zn concentrations were the highest followed by Cu and Pb. Cd being the lowest in the tissues (dorsal muscle). Metals such as like Zn and Cu are essential for fish metabolism since they play an important role in biological systems, while non-essential metals such as Pb and Cd are toxic even in trace amounts[11]. The essential metals can also produce toxic effects at high concentrations, but their concentrations in aquatic organisms tend to be highly regulated compared to non-essential metals[7,12].

Few data on the heavy metal levels in *P. maxima* from the Black Sea coasts have been investigated (Table 3).

When the metal concentrations were compared among the Black Sea coasts, Zn concentrations were found to be highest in Turkish coast[14]. Cu and Cd concentrations were high in Romanian coastal area[15], suggesting a possible point source of copper and cadmium from these catchment. Nisbet et al.[13] found the highest Pb levels in Turkish coast. Heavy metals levels were found lower than in those other studies (Table 3). It can be said that Sinop coast is a relatively unpolluted marine environment, since almost no industry and only small settlements exist in the surrounding region.

Table 3. Heavy metal concentrations (mg metal kg^{-1} wet wt.) in *Psetta maxima* from the Black Sea coasts. (- : not measured)

Area	Zn	Cu	Pb	Cd	References
Samsun, Sinop, Terme, Fatsa, Ordu	24.83±1.71	2.13±0.21	0.73±0.21	0.022±0.007	13
Turkish Coast	45.2 ± 2.7	0.75 ± 0.05	0.28 ± 0.02	0.10 ± 0.01	14
Romanian coastal area 2001-2006	--	6.1	0.55	0.60	15
Sinop-Male	32.2±2.51	5.18±0.69	0.17±0.04	0.04±0.01	Present Study
Sinop-Female	22.01±4.03	2.84±0.85	0.09±0.02	0.02±0.001	Present Study

3.2. Maximum Permitted Limits of these Metals in Fish

The dorsal muscle concentrations of Zn, Cu, Pb and Cd ranged 18.56-35.33, 1.81-6.11, 0.07-0.21 and 0.016-0.046 mg/kg wet weight, respectively. However, in the dorsal muscle of fish the average metal concentrations were on average well below the maximum tolerance levels for human consumption established by compared the Turkish Food Codex, Commission Regulation (EC) and MAFF (Table 4).

Table 4. The tolerable values of Zn, Cu, Pb and Cd in the fish (mg/kg wet weight)

Standards	Zn	Cu	Pb	Cd	References
Turkish Food Codex	--	--	0.30	0.05	16
Commission Regulation (EC)	--	--	0.30	0.05	17
Turkish legislation	50	20	1	0.1	18
The Food Safety (Fishery Product)	50	20	2	<0.2	19

Furthermore, the Provisional Tolerable Weekly Intake (PTWI) value is an estimate of the amount of a contaminant that can be consumed by human over a lifetime without appreciable risk. PTWI is established by the Joint Food and Agricultural Organization for the United Nations (FAO) / World Health Organization (WHO) Expert Committee on Food Additives (JECFA). PTWI values were used in the present study to serve as reference values for safe levels of these metals. Table 5 shows that accepted safe levels of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and Council of Europe for Zn, Cu, Pb and Cd. PTWI values for a 70 kg body weight of an adult person for Zn, Cu, Pb and Cd are.

The dorsal muscle concentrations of Zn, Cu, Pb and Cd in male *Psetta maxima* ranged 29.48-35.33, 4.56-6.11, 0.13-0.21 and 0.025-0.046 mg/kg wet weights, respectively. In female turbot the dorsal muscle concentrations of Zn, Cu, Pb and Cd varied from 18.56 to 27.23, 1.81 to 3.88, 0.07 to 0.11 and 0.016 to 0.020 mg/kg wet weights, respectively.

Estimated Weekly Intake (EWI) and Estimated Daily Intake (EDI) for a 70 kg body weight of an adult person on basis of the present study results were presented in Table 6 and were comprised with standards in Table 5 for assessment of these metals in *Psetta maxima*.

Table 5. Internationally accepted safe levels for Zn, Cu, Pb and Cd (modified from[20])

Metal	Standard	References
Zn	PTWI of 7 mg per kg body weight per week.	21,22
Cu	PTWI of 3.5 mg per kg body weight per week.	21,22
Pb	PTWI of 0.025 mg per kg body weight per week.	22,23,24
Cd	PTWI of 0.007 mg per kg body weight per week.	22,23,25,26

The Joint FAO/WHO Expert Committee on Food Additives established PTWIs for Zn, Cu, Pb and Cd were presented in Table 5, which was equivalent to 490, 245, 1.75 and 0.49 mg/week for a 70 kg adult, respectively. The average daily demersal fish consumption in Turkey is 4 g per person[29]. This is also equivalent to 28 g/week. By using the means of weekly demersal fish consumption in Turkey and the minimum and maximum Zn, Cu, Pb and Cd levels in turbot, weekly intake calculated per person for these metals in dorsal muscles of *Psetta maxima*. As it can be seen from the Table 6, the estimated PTWIs of Zn, Cu, Pb and Cd in the present study is quite below the established PTWIs (Table 5); thereby there is no health threatening concern due to the consumption of edible parts of *Psetta maxima* from Sinop Coastal waters of the Black Sea, Turkey.

The liver concentrations of Zn, Cu, Pb and Cd were in the range of 46.89-57.21, 15.96-29.51, 1.75-4.09 and 0.19-0.44 mg/kg wet weight, respectively. The gonad concentrations of Zn, Cu, Pb and Cd were in the range of 37.81-44.11, 6.66-11.32, 0.21-0.34 and 0.14-0.27 mg/kg wet weight, respectively.

As regards liver concentrations, those of all metals exceed these limits, but turbot livers are not used as food. As regards gonad concentrations, only those of Cd exceed these limits, but turbot livers are also not used for human consumption.

Table 6. Estimated Weekly Intakes (EWI) and Estimated Daily Intakes (EDI) of Zn, Cu, Pb and Cd in dorsal tissue of *Psetta maxima* from Sinop Coastal waters of the Black Sea, Turkey (modified from [27,28])

Metal	PTWI[a]	PTDI[b]	EWI[c]		EDI[d]	
			Male Min. - Max.	Female Min. - Max.	Male Min. - Max.	Female Min. - Max.
Zn	490	70	0.83 - 0.99	0.52 – 0.76	0.12 – 0.14	0.07 – 0.12
Cu	245	35	0.13 – 0.17	0.051 – 0.11	0.019 – 0.024	0.007 – 0.016
Pb	1.75	0.25	0.004 – 0.006	0.002 – 0.003	0.0005 – 0.0008	0.0003 – 0.0004
Cd	0.49	0.07	0.0007–0.0013	0.0004-0.0006	0.0001–0.0002	0.00006-0.00008

[a]PTWI (Provisional Tolerable Weekly Intake) (mg/week/70 kg body weight)
[b]PTDI (Permissible Tolerable Daily Intake) (mg/day/70 kg body weight)
[c]Estimated weekly intake (mg/week/70 kg body weight)
[d]Estimated daily intake (mg/day/70 kg body weight)

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