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Heavy Metal Levels in the Black Sea Sprat (*Sprattus sprattus*)

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ABSTRACT

The aim of this study is to evaluate the levels of eight heavy metals (aluminium, arsenic, copper, zinc, mercury, iron, cadmium and lead) in the muscle tissue of sprat (Sprattus sprattus) caught at Sinop and Samsun coasts of the Black Sea during fishing season in 2013 and 2014. Metal analyses in fish were performed using ICP-MS (Inductively Coupled Plasma – Mass Spectrometer). The levels of Al, Hg, Cd and Pb in edible tissues of sprat were below the limit of detections. Metal levels in sprat from Samsun coasts were found to be higher than those caught at Sinop coasts. The concentrations of the studied heavy metals were far below the established values by the Turkish Food Codex and Commission Regulation (EC). Therefore, their contribution to the total body burden of these heavy metals can be considered as negligibly small.

Keywords: *Heavy metals, Sprattus sprattus, Sinop, Samsun, Black Sea, Provisional Tolerable Weekly Intake, Marine Strategy Framework Directive.*

INTRODUCTION

Rapid population growth, urbanization and industrialization in recent years, together with the human impact on nature, consumption of resources and production of wastes brought up a lot of environmental issues. Water, soil and air contaminations accompanied depletion of natural resources by human activities within this period. Therefore, pollution in natural resources is among the most important environmental issues to be combatted today. Pollution in aquatic environments is a popular and intensively studied topic as it covers both the life of humans and the aquatic organisms. Contamination in aquatic environments is a direct result of domestic and industrial discharges to the receiving water body. Pollutants may also reach to the aquatic ecosystem by erosion and floods if the soil is contaminated with pesticides, and synthetic or organic fertilizers. Consequently, oceans and all sea waters which constitute a great majority of the ecosystem have been receiving environments eventually. Heavy metals are among the most significant pollutants which cause pollution in marine ecosystems. In spite of the fact they are found at trace levels in marine environments, their concentrations and bioaccumulations in organisms are varied. The term "heavy metal" covers all the metals and metabolites on earth.

In natural waters, heavy metals are found as free ions, inorganic and organic compounds and also as absorbed forms on particle materials. As a result of several physical and chemical conditions, they may become ionic and toxic even if they had been settled down in the sediment or absorbed. These metals are of considerable importance in marine ecosystems since they cause environmental pollution and they may be toxic even in very low concentrations to marine organisms and eventually to humans at the top of the food chain. Several heavy metals (copper (Cu), zinc

(Zn) and iron (Fe)) are found at trace amounts and they are essential for the life of organisms. On the other hand, heavy metals such as mercury (Hg), cadmium (Cd), lead (Pb), arsenic (As) and aluminium (Al) are toxic even if they are found at trace levels. Toxic metals particularly accumulate in visceral organs and notably result in digestive and nervous system disorders, but also cause cancer and even mortality.

Hazards arising due to heavy metal pollutions have been the focus of many scientific studies since similar cases are reported in many countries including European Union that border seas. Marine Strategy Framework Directive (MSFD) published as the 2008/56/EC directive of the European parliament council on 17 June 2008 under the subject of Marine Environment Policies has been developed to protect and restore the ecological quality and integrity of estuaries, and coastal and offshore marine ecosystems. MSFD supports an ecosystem-based management approach, considering all the pressures on marine ecosystem with a regional point of view. The target of the directive is to achieve a Good Environmental Status (GES) in European Union marine waters by 2020. GES is an environmental status of ecologically diverse, dynamic, clean, healthy and productive marine waters (MSFD, 2008). The concept of environmental status evaluates the structure and functionality of marine ecosystems, natural physiographic, geographic and climatic factors together with physical and chemical conditions due to human activities in the subject area. MSFD creates a framework using 11 qualitative descriptors for the development of marine strategies to achieve GES in marine environments by 2020. Descriptor 9 asserts that 'contaminants in fish and other sea food for human consumption do not exceed levels established by Community legislation or other relevant standards' (MSFD, 2008).

Heavy metal studies have also been conducted in our country for many years (see Bat, 2014). The Black Sea, one of a few inland seas on earth, is connected to another inland sea, Sea of Marmara, through a narrow strait and then to the Aegean Sea. Therefore, its ability to clean and renew itself is limited as a result of partial circulation. Sakarya, Kızılırmak, Yeşilırmak Rivers from the south (Turkey), the second major European river Danube from the west (Romania) and the third major European river Dnieper (Ukraine), the fourth major European river Don (Russia) and Dniester (Ukraine), from the north carry millions of tons organic material and other wastes to the Black Sea basin. These pollutants from almost one third of the whole land area of continental Europe, which is an area including major parts of seventeen countries, thirteen capital cities and about 170 million people drainage into the Black Sea (Köse et al., 2013). However, the Black Sea is the most valuable sea of Turkey in terms of fisheries and holds more than 80% of production by fishing (TUIK 2015). The Black Sea still faces pollution stress in recent years due to overfishing and shipping activities, mining facilities, discharge of toxic materials and domestic wastes, and introduction of pollutants through rivers (Polikarpov et al., 2004; Mironescu, 2008).

The sprat has been most abundant and commercially important fish species in the western and northern Black Sea (Panayotova, 2001; Nikolsky, et al. 2009a, 2009b; 2012). The sprat stock in the Black Sea is dominated by young fish. Quantity of caught sprat in the Turkish Black Sea was 9.764 tons in 2013 and 41.647,9 tons in 2014 (TUIK, 2015). After anchovy, sprat has the highest rate of catch of sea fish in 2014.

A very few studies addressed the heavy metals levels in sprat from Turkish Black Sea coast (Bat et al., 2012). Recent studies of heavy metal levels in the fishes are more common on the Bulgarian (Stancheva et al., 2013; 2014; Peycheva et al., 2015; Stoyanova et al., 2015) and Romanian (Oros and Gomoiu, 2012) coasts.

The aim of the present study were (1) to evaluate the levels of eight heavy metals (aluminium, arsenic, copper, zinc, mercury, iron, cadmium and lead) in the muscle tissue of sprat (*Sprattus sprattus*) caught from Sinop and Samsun coasts of the Black Sea during fishing season in 2013 and 2014 and (2) to compare the concentrations of heavy metals present with the guidelines set down the Turkish Food Codex (TFC) and Commission Regulation (EC) for the safe consumption limits of fish. Values obtained were compared with national and international standards as to the public health and the protection of biotopes.

Materials and Methods

This study has been conducted during the 2013-2014 fishing seasons to examine the heavy metal concentrations in sprat sampled along Samsun and Sinop coasts located on the Turkish Black Sea (Figure 1). A mean randomized sample of fish (about 100 individuals) was selected from every catch, which usually contained specimens of 80 to 120 mm in length. Fish samples were then labelled, they were preserved using ice and transported to the main laboratory. All the samples were stored at -21^{0}C prior to pre-treatment and analysis.

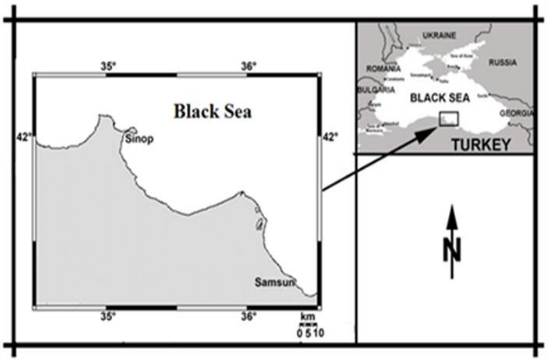

Figure1. *The Black Sea map showing Samsun and Sinop coasts of fish sampling.*

Heavy Metal Analysis

Heavy metal analysis in sprat have been performed by ÇEVRE Food Analysis Laboratory Environmental Industrial Analysis Industry and Trade Inc. using ICP-MS and application of m-AOAC 999.10 (AOAC: Association of Official Analytical Chemists; reference number TÜRKAK Test TS EN ISO IEC 17025 AB-0364-T) method. The limits of detection used for analysis of Al, As, Cu, Zn, Hg, Cd, Pb and Fe were 0.5, 0.05, 0.5, 0.5, 0.05, 0.03, 0.05 and 0.5, respectively.

Intake Levels Calculation

The average heavy metal weekly intake was calculated according to the following formula:

Heavy metals intake level = average heavy metal content X consumption of fish per person/ body weight. The annual quantity of fish consumed is 6.3 kg / person in 2013 and 5.4 kg / person in 2014 (TUIK, 2015), which is equivalent to 17.3 and 14.8 g/day for Turkey, respectively. The body weight of adult person is 70 kg. Consumption of fish varies between regions and coastal areas are 25 kg per person (Köprücü, 2007). Sarıözkan (2016) stated that the average target for fish consumption should be 10 kg / person in Turkey. This value is based on the present study.

Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Data were analysed by ANOVA at α= 0.05. Comparison of means was performed by Duncan test and difference was considered significant at $p < 0.05$ (Zar, 1984). IBM SPSS Statistics version 21 software is used for statistical analysis.

RESULTS AND DISCUSSION

The Black Sea sprat caught at the south coast of the Black Sea, during fish seasons in 2013 and 2014 were analysed for Al, As, Cu, Zn, Hg, Cd, Pb and Fe. Al, Hg, Cd and Pb were not detected in the edible part of sprat from both Samsun and Sinop coasts of the southern Black Sea. They were all below the limits of detections (see in Material and Methods). The concentration of examined metals in fish tissues from the Black Sea demonstrated regional differences. The concentrations of As, Cu, Zn and Fe in sprat are presented in Figs. 2, 3, 4 and 5.

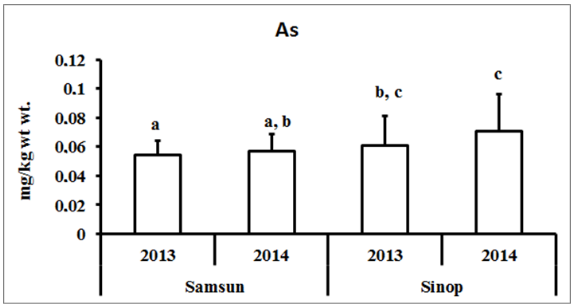

Figure2. *The means with standard deviations (vertical line) of As concentrations (mg/kg wet wt.) in the edible tissues of Sprattus sprattus collected from Samsun and Sinop coasts of the Black Sea during fishing season in 2013 and 2014. a, b, c= The same letters above the vertical bars indicate the values are not significantly different (P>0.05).*

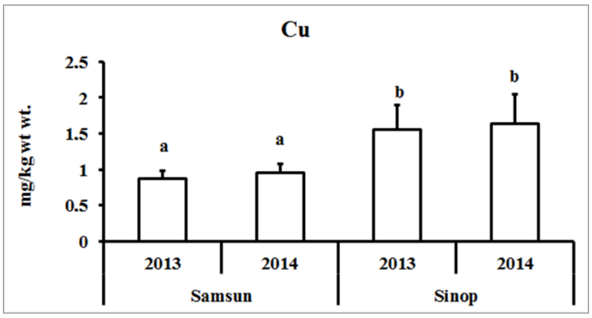

Figure3. *The means with standard deviations (vertical line) of Cu concentrations (mg/kg wet wt.) in the edible tissues of Sprattus sprattus collected from Samsun and Sinop coasts of the Black Sea during fishing season in 2013 and 2014. a, b= The same letters above the vertical bars indicate the values are not significantly different (P>0.05).*

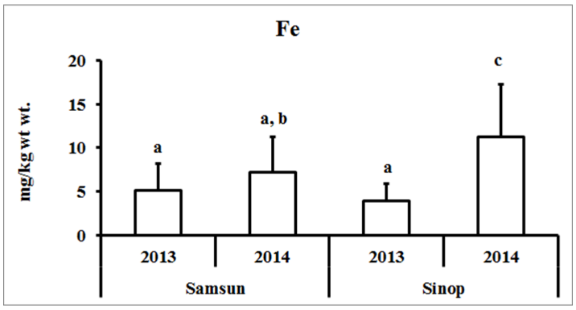

Figure4. *The means with standard deviations (vertical line) of Fe concentrations (mg/kg wet wt.) in the edible tissues of Sprattus sprattus collected from Samsun and Sinop coasts of the Black Sea during fishing season in 2013 and 2014. a, b, c= The same letters above the vertical bars indicate the values are not significantly different (P>0.05).*

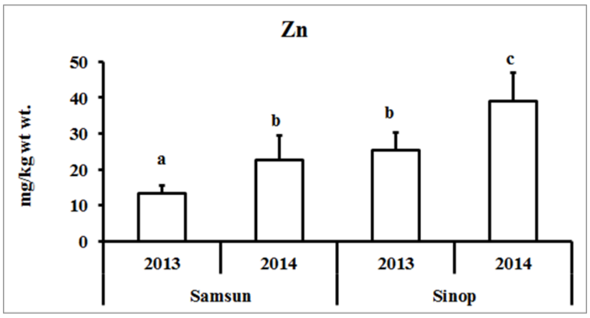

Figure5. *The means with standard deviations (vertical line) of Zn concentrations (mg/kg wet wt.) in the edible tissues of Sprattus sprattus collected from Samsun and Sinop coasts of the Black Sea during fishing season in 2013 and 2014. a, b, c= The same letters above the vertical bars indicate the values are not significantly different (P>0.05).*

Relatively low concentrations of As, Cu, Zn and Fe were measured in Samsun although these two cities are close to each other. However the metals in sprat from both sampling sites are lower than the permitted levels. Legal thresholds are not available for essential metals in Commission Regulation. However, in the edible muscle tissues of fish the average Zn and Cu concentrations were well below the maximum tolerance levels (50 µg/g wet wt. and 20 µg/g wet wt., respectively) for human consumption established by compared with the Turkish Food Codex and the Food Safety of Fish Product (TFC, 2002; MAFF, 1995). Like Zn and Cu, Fe is essential metals since they play virtual roles in biological systems as well. There is also no information about maximum As levels in fish in the Commission Regulation (EC, 2006). As concentrations ranged from 0.054±0.01 mg/kg wet wt. in sprat of Samsun coasts to 0.071±0.025 mg/kg wet wt. in sprat of Sinop coasts in the present study were considerably lower than the maximum level (1.0 mg/kg wet weight) set by Turkish Legislation (Anonymous, 1995).

Unfortunately, detailed studies of heavy metal levels in sprat along the southern Black Sea coasts yet exist except Bat et al. (2012). Our results were lower than those in sprat. Bat et al. (2012) found that mean concentrations of Cd, Pb, Cu and Zn in sprat were 0.07, 0.264, 6.79 and 41.83 mg/kg wet wt., respectively. Again, Stancheva et al. (2013) were investigated Pb, Cd, As and Hg contents of sprat from Nessebar (South part of Bulgarian Black Sea coast) during fishing season in 2010 and were found values with mean of 0.08 ± 0.02, 0.005 ± 0.001, 0.73 ± 0.05 and 0.12 ± 0.02 mg kg^{-1} wet wt. for sprat tissue. In the present study we couldn't determine Pb, Cd and Hg concentrations in sprat. Even they presented more than ten times higher concentrations for As (0.73 ± 0.05 mg kg^{-1} wet wt.) for sprat from the Bulgarian Black Sea coasts compared with the present results (0.061± 0.007 mg kg^{-1} wet wt.).

In an another study of Stancheva et al. (2014) Cd, Ni, Cr, As, Hg Cu, Fe, Mn, Pb and Zn levels in sprat were determined from the Bulgarian Black Sea coasts between February and November in 2010. They found that Cd (0.005±0.001), As (0.73± 0.04), Hg (0.12±0.02) and Pb (0.08±0.02) levels as mg kg^{-1} wet wt. were higher than those in the present study and Fe levels were low. However Cu (1.40±0.08 mg kg^{-1} wet wt.) and Fe (9±1 mg kg^{-1} wet wt.) concentrations were similar to those in the present study (Cu ranged from 0.87±0.11 to 1.63±0.41 mg kg^{-1} wet wt.).

The highest value of Pb (0.206±0.004 mg kg^{-1}) and Cd (0.053 ±0.002 mg kg^{-1}) were found in sprat samples obtained from the fish market in Varna (Stoyanova et al., 2015) and these values are higher than the sprat caught at the Turkish Black Sea coasts. The results of Zn of the present study (ranged from 13.4±2 to 38.9±8 mg kg^{-1} wet wt.) are similar to those (26.19±0.10) reported by Stoyanova et al. (2015).

Peycheva et al. (2015) also found higher levels of Hg (0.12 mg kg^{-1} wet wt.) in the muscle of sprat from coastal waters of the Bulgarian Black Sea coasts than in the muscle of those (<0.05) caught at the Turkish Black Sea coasts.

Oros and Gomoiu (2012) reviewed available data for the Romanian marine environment on heavy metals available in biota including fish species. They mentioned that heavy metal levels in the dorsal muscle of marine fish varied within wide ranges and mean Cu, Cd, and Pb concentrations for 2001–2011 were 5.08±7.82, 0.67±1.10 and Pb 1.11±1.96 (mg kg^{-1} wet wt.). The highest Cu, Cd and Pb levels in sprat from the Romanian Black Sea waters during 10 years were 10, 2.5 and 3 mg kg^{-1} wet wt., respectively. These values are much more than those in sprat obtained from the Turkish Black Sea waters.

Bustamente et al. (2003) pointed out that difference in heavy metal levels were associated with diet and feeding habits of benthic and pelagic fish species and showed that benthic fish generally accumulated higher levels of heavy metals than pelagic fish. Moreover, it is suggested that mainly plankton consuming fish contain much higher concentrations of some heavy metals than bottom feeding fish (Topping, 1973). Oros and Gomoiu (2012) also suggested that the great metal accumulation in planctonivorous fish such as anchovy and sprat compared with predator fish may be explained on the basis of a higher efficiency of metals assimilated from food. Bat et al. (2012) agree with these conclusions that sprat is a zooplanctonivorous fish and has a high metabolic rate. However, European Food Safety Authority pointed out that Hg content is not associated with the fat content of the fish and therefore is not reckoned with oily fish (EFSA 2015). Similarly Bat et al. (2014) found that Zn was negatively correlated with fat content while no effect of fat content was observed for As accumulation in the Black Sea anchovy. The main food items of sprat are the coldwater copepods such as *Calanus euxinus*, *Pseudocalanus elongatus*, *Oethona nana* etc. (Svetovidov 1964). Sprat usually begins to accumulate lipid reserves in its body during spring and complete it in summer (Shulman, 1974). Lipid reserved in spring and summer declined abruptly in autumn and winter during maturation and spawning, which culminated in January-February (Svetovidov, 1964). The level of lipid accumulation in June-July was used as indicator for the estimation of sprat's nutritional condition (Shulman, 1974). The main components of lipids are fatty acids. Since dietary fatty acids are incorporated into animal lipids with little or no modification of the original structure, fatty acids may be markers of food species which consumed by fish (Sargent et al., 1988). During the feeding period when lipid assimilation much exceeds the expenditure, fatty acid of predator reserve lipids is the most approach to the fatty acid composition of its diet (Shulman, 1974; Shulman et al., 2005; 2007). Our study confirms this assumption.

The heavy metal levels in fish correlate with a number of factors including the size and age of the fish. Larger, older and predatory species contain higher heavy metal levels in their tissues than those in other marine fish (Food Safety Authority of Ireland, 2009). In the specific environmental conditions of the Black Sea, the sprat attains a smaller size with having a short life span and earlier maturation period (Shulman et al., 2005; 2007; 2008). Findings of the present study support this assumption.

From the point of health risks, the allowable weekly intakes were calculated by assets of references for comestible tissues of fishes consumed by people. The annual quantity of fish assumed is 10 kg/person (see Material and Method), which is equivalent to 27.4 g/day for Turkey. The tolerable weekly intake of heavy metals as PTWI (Provisional Tolerable Weekly Intake), are set by the Food and Agriculture Organization/World Health Organization (FAO/WHO) Joint Expert Committee on Food Additives (JECFA). PTWI is the total sum of a pollutant to which a person can be exposed per week over a lifetime without an insufferable risk of health effects (National Academy of Sciences, 1989; WHO 1996; Council of Europe, 2001; FAO/WHO, 2010; EFSA, 2010, 2012a, 2012b).

In the present study, Hg, Cd, Pb and Al in fish samples were found to be under measurable levels. It can be seen from Table 1 that the estimated EWIs and EDIs of the rest of heavy metals in the present study are far below the recommended PTWIs and/or PTDIs and indicated no toxic effects to the people. In the light of this information, it is possible to say that heavy metal pollution is not found in edible tissues of *S. sprattus* from Samsun and Sinop coasts of the Black Sea according to the calculations based on the highest metal levels.

CONCLUSION

Heavy metal analysis of plankton may result in findings of high levels of metals in these organisms due to the inclusion of suspended particles in sea water called "suspended solids". Zooplankton samples from the Black Sea coast show metal concentrations in detectable levels (Bat et al., 2016). Similarly, water sources of terrestrial origin (rivers) washing down agricultural lands have a role in the transport of heavy metals to the sea. Copper reaching to the sea is located in the water column near the surface at first and then starts to settle down when it combines with sulphur. As already known, sprat is a pelagic fish species found also in the water column near the sea surface. Therefore, it is possible that uptake and bioaccumulation of heavy metals by sprat may be in various pathways.

Table1. *Estimated Weekly Intakes (EWI) and Estimated Daily Intakes (EDI) of Heavy Metals in Edible Tissues of Spratus spratus from Samsun and Sinop Coastal Waters of the Black Sea, Turkey.*

Metals	PTWI[a]	PTWI[b]	PTDI[c]	EWI[d]		EDI[e]	
				Samsun	Sinop	Samsun	Sinop
Fe	5.6	392	56	1.385±0.959	2.148±1.343	0.198±0.137	0.307±0.192
Zn	7	490	70	4.316±0.959	6.138±1.438	0.617±0.137	0.877±0.205
Cu	3.5	245	35	0.182±0.023	0.313±0.079	0.026±0.003	0.045±0.011
As	-	f	f	0.011±0.002	0.014±0.005	0.002±0.0003	0.002±0.0007
Pb	0.025	1.75	0.25	Below Detection Limit			
Cd	0.007	0.49	0.07	Below Detection Limit			
Al	1	70	10	Below Detection Limit			
Hg	0.004	0.28	0.04	Below Detection Limit			

[a]PTWI (Provisional Tolerable Weekly Intake) in mg/week/kg body wt.
[b]PTWI for 70 kg adult person (mg/week/70 kg body wt.)
[c]PTDI (Permissible Tolerable Daily Intake) (mg/day/70 kg body wt.)
[d]EWI (Estimated Weekly Intake) (mg/week/ kg body wt.)
[e]EDI (Estimated Daily Intake) (mg/day/ kg body wt.)
[f]There is no PTWI set for As. The UK previously imposed a limit of 1 mg/kg for arsenic in food with separate limits applicable to certain food categories. These regulations were revoked in 2002.

Bioaccumulation of heavy metals in fish skin is higher than other tissues (Oros and Gomoiu, 2012). Concordant with this finding, several heavy metals can be measured higher due to the fact that removal of skin from muscle tissue is not easy in small pelagic fish species such as sprat.

Since heavy metals are not easily accumulated in lipids (Bat et al., 2014; European Food Safety Authority, 2015), low metal concentrations can be recorded in fish species such as sprat which has high lipid content (Shulman, 1974; Shulman et al., 2005; 2007; Nikolsky et al., 2009a; 2009b), and low levels can also be recorded in fish samples although caught in polluted areas if they have achieved increased fat contents.

The Black Sea sprat constitutes the most massive pelagic fish stock in the Black Sea that consumes considerable part of the mesozooplankton production. Sprat, as a significant component of the upper trophic pelagic layer, represents the “indicator species”, which reflects the features of the Black Sea ecosystem condition in the whole. Food supply is one of the most important characteristic of nutritional condition of sprat populations together with biomass value because feeding is “a connection channel” between the populations and environment (Nikolsky et al., 2009a; 2009b; Shulman et al., 2009a; 2009b). Therefore assessment of the pollution sources and continuous monitoring of heavy metal concentrations in the Black Sea is of extreme importance to take necessary precautions.

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