INVESTIGATION OF LEAD, CADMIUM, ARSENIC AND MERCURY IN SOME SEAFOOD FROM BLACK SEA AND MARMARA SEA BY ICP-MASS SPECTROMETRY

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Abstract

Over the last 200 year our planet has been under a threat due to industrilization and environmental pollution. Accordingly, all the species living in our environmentally polluted planet is potentially a majör source of health risk. This situation is also directly leading to making the seafood and other species of waterborne is contaminated with the industrial pollutants such as heavy metals. The contaminated foods are suitable for the dissemination of these carcinogenic chemicals through the consumers. Heavy metals are the members of the third or higher rows of the periodic scale. In the present time, there are many heavy metals more than 60 such as lead, cadmium, chromium, cobalt, copper, nickel, mercury and zinc. The objective of this study was to analyze Pb, Cd, Hg, As in a total of 150 seafoods (10 shrimp, 10 calamari, 10 octopus, 10 mussel, 10 acorn, 10 mackerel, 10 coral, 10 sea bream, 10 whiting, 10 sole, 5 horse mackerel, 5 anchovy, 5 sardine, 5 bluefish, 5 gray mullet, 5 turbot, 5 red mullet, 5 coral, 5 bream, 5 butts fish) from Marmara Sea (n=75) and Black sea (n=75) by using Inductively Coupled Plasma-Mass Spectrometer ICP-MS according to the criterias by both Turkish Food Codex Declaration About Maximum Limits of Contaminants in Food Products (Declaration No: 2008/26) and NMKL 186 International method. The results revealed that the lead levels in both horse mackerel (0,385 ppm) and mullet (0,387 ppm) , and the mercury levels in both red mullet (1,707 ppm) and sea bream (1,098 ppm) from the Black sea were found to be higher, whereas the lead levels in both sardine (0,417 ppm) and butts fish (0,843 ppm) from Marmara Sea were higher than the upper limits as declared by Turkish Food Codex. In the mussels collected from Marmara Sea, the cadmium levels were found to be nearly close to the upper limit, which was 1 ppm. Also, it was provided that all the samples contained arsenic. However, no comment was performed since the Turkish Food Codex does not indicate any upper limit for itself. In Conclusion, this study provided that the seafoods collected from both Black sea and Marmara Sea significantly included heavy metals, including lead, mecury, cadmium, and arsenic. This situation leads to a significant foodborne health riskfor the public health and the Turkish consumers.

Keywords: *heavy metal, fish, blacksea, cadmium, arsenic, mercury, lead, ICP-MS*

1. Introduction

The environmental levels of chemicals have risen steadily with human population growth, urbanization, and continued worldwide industrialization. All these toxic and inevitable polutants adversely affect the species, threat the human health, and lead to a harmful situation which the nature is not able to overcome [1]. The heavy metals are considered to be extremely toxic elements that have negative impacts on chemical and physiological processes especially in aquatic organisms and humans. There is raising concern regarding the undesired health effects of different metals. Thus, their control, uptake, bioaccumulation, storage and elimination are important for the human health [2].

Heavy metal toxicity can produce serious chronic conditions such as autism, chronic fatigue syndrome, depression, multiple sclerosis, and other

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serious disorders [3]. Zinc, copper, chromium, iron and manganese are essential to body function in small amounts. But, if these metals accumulate in the body in higher concentrations, then serious damages can develop. The major heavy metals associated with poisoning of humans are lead, mercury, arsenic and cadmium. Heavy metal poisoning may occur as a result of industrial exposure, air or water pollution, and foods [4]. Excessive accumulation of dietary heavy metals such as Cd, Cr, and Pb in the human body may be a result of consumption of fishes and other seafoods [5-7]. Seafood is a valuable food in the human nutrition because it consists of protein, fats, vitamins, and minerals. Therefore, seafood has numerous health benefits. For example, recent studies have indicated that regular consumption of seafood can decrease the risk of heart attack, stroke, obesity, and hypertension. Seafood also provides essential nutrients such as omega-3 fatty acids [8].

In the recent years, the levels of heavy metals in fish have been investigated in different parts of the world. Most of these studies concentrated mainly on the heavy metals in muscles, liver, kidneys, heart, gonads, bone, digestive tract and brain [9,10]. Official regulatory institutions of many countries are responsible for inspection of seafood products for heavy metal residues, also including checks for proper labeling and documentation, sensory evaluations (e.g., visual, olfactory), and laboratory screening for other contaminants such as PCBs, toxins, and microbial pathogens [7,11]. Maximum levels have been set for certain contaminants in order to protect public health in the EU in the year 2006 (EU Commission Regulation: 852/2004/EC, 853/2004/EC, 854/2004/EC ve 882/2004/EC), as well as the guidelines set down by the Ministry of Agriculture, Fisheries and Food, the Turkish Food Codex in Turkey as of 2010 [12,13].

In this study, we investigated Pb, Cd, Hg, As in a total of 150 seafoods (10 shrimp, 10 calamari, 10 octopus, 10 mussel, 10 acorn, 10 mackerel, 10 coral, 10 sea bream, 10 whiting, 10 sole, 5 horse mackerel, 5 anchovy, 5 sardine, 5 bluefish, 5 gray mullet, 5 turbot, 5 red mullet, 5 coral, 5 bream, 5 butts fish) from Marmara Sea (n=75) and Black sea (n=75) by using Inductively Coupled Plasma-Mass Spectrometer ICP-MS according to the criterias by both Turkish Food Codex Declaration About Maximum Limits of Contaminants in Food Products (Declaration No: 2008/26) and NMKL 186 International method.

2. Material and Methods

Sampling

During the year 2015, a total of 150 seafoods (10 shrimp, 10 calamari, 10 octopus, 10 mussel, 10 acorn, 10 mackerel, 10 coral, 10 sea bream, 10 whiting, 10 sole, 5 horse mackerel, 5 anchovy, 5 sardine, 5 bluefish, 5 gray mullet, 5 turbot, 5 red mullet, 5 coral, 5 bream, 5 butts fish) from Marmara Sea (n=75) and Black sea (n=75) was collected. The samples were taken to the laboratory in the a sample carry case (JPB, UK) at 4°C. Then, the analysis was started in the same day (Table 1).

Table 1. Sampling data

Ground fish	**Surface fish**	**Others**
Turbot	Bonito	Shrimp
Bluefish	Anchovy	Calamari
Red mullet	Horse mackerel	Octopus
Young blue fish	Sardine	Mussel
Mullet	Atlantic mackerel	
Seabream		
Picarel		
Black bream		
Grey mullet		
Haddock		
Sole		

Sample preparation

The collected samples were initially homogenized using blender (Interscience, Germany). Subsequently, the homogenized sample was burned in a microwave device (CEM, USA) before the analysis in the Coupled Plasma-Mass Spectrometer (ICP-MS). A 0.2-0.5 g of the burned sample was weighed (MS104TS Mettler Toledo, Turkey) in a vessel. Then, 5 ml of Nitric acid (HNO3) (Merck, Turkey) and 2.5 ml hydrogen peroxide (H_2O_2) (Merck, Turkey) were pipetted into the sample, respectively. The mixture was then re-burned in microwave device for 70°C/15 min, 85°C/10 min, 105°C/10 min, 110°C/5 min, 120°C/5 min, and finally 130°C/5 min, respectively. After that, the vessel was allowed for cooling in the room conditions for a while. After cooling, the solution in the burned vessel was transferred to a 25 ml flask. Then, deionized water was poured on to it till the final volume in the flask became 25 ml.

Analysis of Pb, Cd, Hg, and As by ICP-MS

The analysis of Pb, Cd, Hg, and As in the seafood samples was performed by using ICP- MS Agilent 7700X (Agilent, Turkey) according to the Guidelines of NMKL 186 (2007) instructions. Before starting the analysis, calibration blank solution, calibration standards, sample blank, control standards, and control samples were all prepared accordingly. Then, a 10 ml of the solution in the 25 ml flask was put into a 10 ml propylen tube. All the solutions were placed in to the ICP-MS device. The reading was started, completed, and the results were automatically processed by the software.

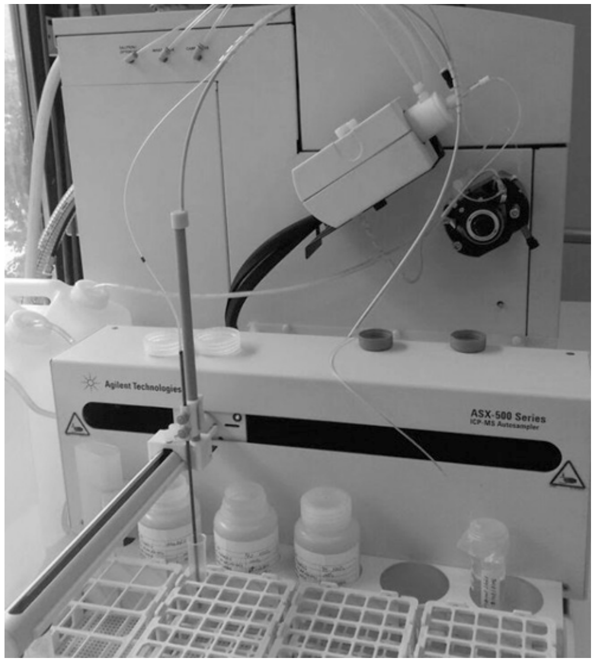

Figure 1. ICP-MS device

3. Results

In this study, a total of 150 seafood samples from Marmara Sea (n=75) and Black sea (n=75) was analysed for the presence of Pb, Hg, Cd and As by using ICP-MS device according to the Guidelines of NMKL 186 (2007) procedures.

Heavy metal levels in the analyzed seafood samples from Black Sea

The Hg level in red mullet (1.707 ppm>1 ppm) and black bream (1.098 ppm>0,5 ppm), and the Pb level in Horse mackerel (0.358 ppm>0.3 ppm) and mullet (0.387 ppm>0.3 ppm) were found to be higher than the limits declared by the Turkish Food Codex. It was also determined that all the fish samples from Black Sea included As higher than the limit given by the China (0.15 ppm).

Also, Hg was not detected in the other seafoods such as shrimp, calamari, octopus, and mussel samples, while the average levels of Cd, Pb, and As were found as 0.245 ppm, 0.347 ppm and 32.932 ppm, respectively. These data were seen to be lower than the upper limits of Turkish Food Codex, excluding the As level given as 0.15 ppm by China.

Heavy metal levels in the analyzed seafood samples from Marmara Sea

The Pb level in sardine (0.417 ppm>0.3 ppm) and picarel (0.843 ppm>0.5 ppm) were found to be higher than the limits declared by the Turkish Food Codex. It was also determined that all the seafood samples from Marmara Sea included As higher than the limit given by the China (0.15 ppm).

However, Hg was not detected in the other seafoods such as shrimp, calamari, octopus, and mussel samples, while the average levels of Cd, Pb, and As were found as 0.384 ppm, 0.440 ppm and 30.824 ppm, respectively. Only mussel samples contained Cd close to the upper limit by the Turkish Food Codex as 1 ppm.

Overall results

The results, higher than the upper limits of the Turkish Food Codex and China, belonging to the levels of heavy metals present in the analyzed seafoods were tabulated in Table 2 and Figure 2. Accordingly,

Table 2. The levels exceeding the upper limits in the analyzed seafoods

Origin	Ground/ Surface	Type	Hg (ppm)	Cd (ppm)	Pb (ppm)	As (ppm)
Black Sea	Ground fish	Red mullet	1.707	-	-	All above Chineese limit 0.500 ppm
		Mullet	-	-	0.387	
		Black bream	1.098	-	-	
	Surface fish	Horse mackerel	-	-	0.358	
Marmara Sea	Ground fish	Picarel	-	-	0.843	
	Surface fish	Sardine	-	-	0.417	
	Others	Mussel	-	1.000	-	

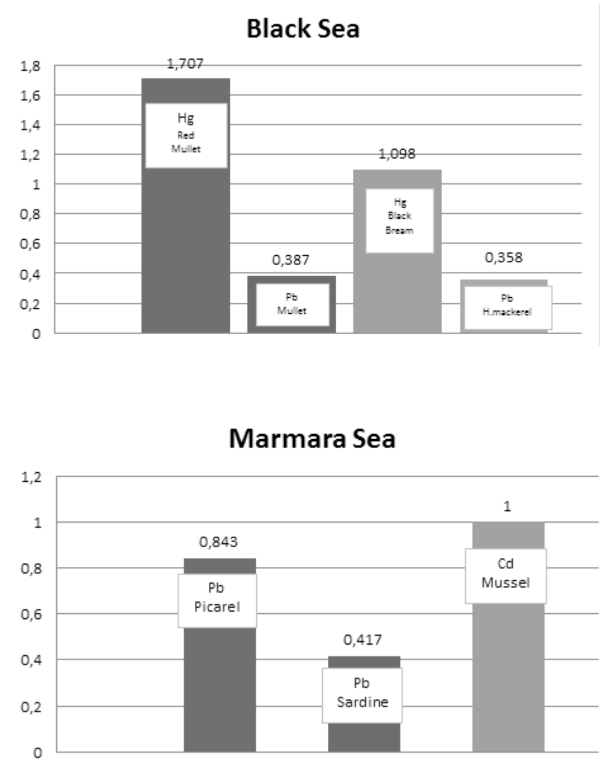

Figure 2. Comparison of overlimit results of the seafoods from Black Sea and Marmara Sea

4. Discussion and Conclusions

In this study,we investigated the presence of major heavy metals such as Hg, Pb, Cd and As in a total of 150 seafood samples from Marmara Sea (n=75) and Black sea (n=75) by using ICP-MS device according to the Guidelines of NMKL 186 (2007) procedures.

The results revealed that Hg level in red mullet (1.707 ppm>1 ppm) and black bream (1.098 ppm>0,5 ppm), and the Pb level in Horse mackerel (0.358 ppm>0.3 ppm) and mullet (0.387 ppm>0.3 ppm) from Black Sea, and that in sardine (0.417 ppm>0.3 ppm) and picarel (0.843 ppm>0.5 ppm) from Marmara Sea were found to be higher than the limits declared by the Turkish Food Codex. All the fish samples were found to be contaminated with As over the upper tolerable limit as 0.15 ppm by China. The other seafoods such as shrimp, calamari, octopus, and mussel were free of Hg, and had low levels of Pb and Cd except for the mussel samples from Marmara Sea (1 ppm). The

As levels in all other seafoods were significantly higher than the upper limit set out by China.

Heavy metals are significant environmental pollutants and their toxicity is an increasing issue significant for ecological, evolutionary, nutritional and environmental reasons. Various public health measures are undertaken to control and prevent metal toxicity at various levels, such as occupational exposure, accidents and environmental factors. Thus, metal toxicity is dependent on the döşe and duration of exposure, i.e. acute or chronic. [14].

Arsenic (As) is a protoplastic poison. It affects primarily the sulphydryl group of cells causing malfunctioning of cell respiration, cell enzymes and mitosis[15-17]. Lead (Pb) creates toxicity in living cells in the organisms following ionic mechanism and that of oxidative stress [18]. Mercury (Hg) is extremely toxic element and bioaccumulative[18]. Cadmium (Cd) is the seventh most toxic heavy metal as per ATSDR ranking [19,20].

The literature has showed that the level of As in foods ranges from 0.020 to 0.140 ppm. An adult person may be exposed to 0.050 ppm As a day. The Environmental Protection Agency of the United States of Amerika (EPA) issued some upper limits for As in the year 2006. According to the criteria, this limit in water has been restricted with 0.010 ppm. However, there has been no limit for water in Turkey yet. Similarly, many countries have not set down any limit for major heavy metals in all types of foods. For instance, the limit for As in water in the USA was set as 0.010 ppm while it has not been made for other foods. China has determined the upper limit for As in water as 0.150 ppm. The United Kingdom ruled 1 ppm for As in infant foods (rice products) for those younger than 54 months. In the New Zealand and Austrilia, a STO also known as EOS published a report dated on the year 2012 regarding the upper acceptable limits for As in fish and other seafoods as 2 ppm, respectively (http://ecan.govt.nz/publications/Reports/heavy-metals-fish-shellfish-2012-survey.pdf). Based on this reference value, our fish samples from the Black Sea ranged in between 0.729-68.288 ppm whereas those for the Marmara Sea was in a range of 0.504-65.554 ppm. The highest As levesl were found in shrimp, calamari and octopus samples. Among the analyzed fish samples the highest frequency for As was detected as 30.682 ppm in the red mullet samples only from the Black Sea. As compared to the international upper limits for As, our samples were highly contaminated with As.

Lead metal causes toxicity in living cells by following ionic mechanism and that of oxidative stress [21]. Our study provided that the Pb leves in the samples from Black Sea and Marmara Sea were 0-0.539 ppm and 0-0.843 ppm, respectively. According to our results, the seadfoods from Marmara Sea included Pb higher that Black Sea. Among the analyzed foods, the other seafoods such as shrimp, calamari, octopus and mussel had reasonable Pb levels less than the limits as declarated by Turkish Food Codex, except for As levels as compared to China's limit (0.15 ppm). On the other hand, mullet (0.387 ppm) and horse mackerel (0.385) from Black Sea, and sardine (0.417 ppm) exceed over the tolerable upper limit of Pb (0.300 ppm). Our study revealed that the Marmara Sea was exposed to a much Pb pollution worse than Black Sea. These foods are concern of a public health risk for the consumers.

Our study indicated that the Hg leves in the examined seafoods exhibited interesting differences. Amon the samples from Marmara Sea the Hg levels were ranged in 0 to 0.564 ppm. The average of these measurements were higher than the acceptable level as given by the Turkish Food Codex. The fishes which contained Hg higher than the limits were determined to red mullet (1.707 ppm) and black bream (1.098 ppm). However, any Hg was not detected in shrimp, calamari, octopus and mussel samples. According to the statistical facts issued by American Food and Drug Administration (FDA), our samples had the Hg higher than the limits by FDA and other international authorities.

Our study showed that all the analyzed seafoods did exhibit any potential risk for the consumers because the Cd leves among all the samples were less than the tolerable level as declared by the International authorities, as well as the Turkish Food Codex. The Cd leves in Black sea and Marmara sea were given as 0-0.626 ppm and 0-1 ppm. The highest level was observed as 1 ppm in the mussel samples collected from Marmara sea.

In conclusion, we showed that certain fish samples and other seafoods from Black sea and Marmara sea included harmful heavy metals such as Pb, Cd, Hg and As in a various levels of contamination. Thus, the consumers are under rish if they consume them in excesss amounts.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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Gamze Benlikurt received her B. Sc. in Food Engineering Department. Following that, she completed her M.Sc. degree in Food Safety Department of İstanbul Aydın University in İstanbul, Turkey. In her thesis study, she intestigated the presence of some major heavy metals in fish and other seafoods by using ICP-MS according to the Guidelines by NMKL 186 (2007).

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Prof. Dr. Haydar Özpinar received his veterinary degree from Ankara University in Turkey in 1978. That same year he began to work as research assistant in Istanbul University. Soon after he received a Ph. D. scholarship from German Academic Exchange Program DAAD, and in 1984 he finished his Ph. D. at the Department of Nutrition Physiology of Ludwig Maximilians University in Munich, Germany. After that, he returned to the Department of Nutrition and Nutritional Disease at Veterinary Faculty of Istanbul University. During his scientific career he received quite a few scholarships including those from European Union, DAAD, Alexander von Humboldt Foundation and USA Fulbright. In 2004, he achieved a Fulbright scholarship, and went to University of California Davis, CA, USA. Until 2008, he studied in nutrition, immunology and genetics. He was also involved in a NIH Project. He is currently the director of Institute of Natural and Applied Sciences, a full-time faculty member at Food Engineering Department of Istanbul Aydın University, and also Chair of Food Safety Department.