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Assessment of cadmium, mercury and lead contents of frozen Eurorean sea bass (*Dicentrarchus labrax* L., 1758) and gilthead sea bream (*Sparus aurata* L., 1758) fillets from Turkey

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ABSTRACT

In this study, trace metals [cadmium (Cd), mercury (Hg) and lead (Pb)] consentration of 76 pieces of frozen European sea bass (*Dicentrarchus labrax* Linnaeus-1758) and gilthead sea bream (*Sparus aurata* Linnaeus-1758) fillets, produced and marketed in Turkey, were determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) after microwave damp burning process, and results obtained were assessed in terms of public health. This study was conducted from June 2010 to July 2011. At the end of study, maximum heavy metal levels for sea bass and sea bream fillets were determined as 256.50, 216.22 µg/kg for Cd; 414.79, 338.46 µg/kg for Hg ve 1047.61, 147.14 µg/kg for Pb, respectively. At the end of the study, the levels of cadmium (for 3 samples) and lead (for 1 samples) were higher than the recommended legal limits of the European Union for human consumption. It was detected that the fillets which were analyzed was good quality from the point of cadmium, mercury and lead contents.

Keywords: European Sea Bass Fillet; Gilthead Sea Bream Fillet; Cadmium; Mercury; Lead; Turkey

1. INTRODUCTION

The effects of heavy metals on human health and the environment is of great interest today, especially for aquatic products [1]. Toxic elements can be very harmful even at low concentration when ingested over a long time period. The essential metals can also produce toxic effects when their intake is excessive [2]. Levels of trace elements including cadmium, mercury and lead in fish and fishery products from many areas in Turkey have been reported [2,3]. In many countries, significant alterations in industrial development lead to an increased discharge of chemical effluents into the ecosystem, leading to damage of marine habitats. Heavy metal discharged into the marine environment can damage both marine species diversity and ecosystems, due to their toxicity and accumulative behaviour [4]. Heavy metals are present in the aquatic environment where they bio accumulate along the food chain. Accumulation occurs in the tissues of aquatic animals and may become toxic for fish and also for people when it reaches a substantially high level [5].

Fish has been the main supply of cheap and healthy protein to a large percentage of the world's population [6]. The proper human diet should satisfy the requirements for energy and nutritive components including: essential polyunsaturated fatty acids, exogenous amino acids being the component of standart proteins, mineral components, fat and water-soluble vitamins [7]. In addition to the key nutrients mentioned, fish can accumulate substantial concentrations of heavy metals in their tissues. Fish is therefore a product for which suitable measures should be taken to provide chemical monitoring of the risks derived from its consumption [8]. High metal concentrations in food can provoke serious health hazards in humans (**Table 1**).

Turkey is surrounded by four different seas with 8333 km long coastal line and fishing is one of the biggest income sources for the country. The four seas around Turkey each reflect a different ecological characters, for instance salinity is 18 per thousand in the Black Sea, 23 per thousand in the Marmara Sea, 32 per thousand in the Aegean Sea and 38 per thousand in the Mediterranean Sea. The aim of this study was to determine ranges of toxic trace metals (Cd, Hg and Pb) in the fisheries products (frozen European sea bass and gilthead sea bream fillets) produced and marketed in Turkey.

Table 1. Potential health hazards of cadmium, mercury and lead.

Element	Potential health hazards	References
Cd	Kidney damage, testicular tumors, renal dysfunction, hypertension, arteriosclerosis, growth inhibition, chronic diseases of old age, and cancer	[9,10]
Hg	Minamata disease, cardiovascular disease, sensorimotor symptoms, memory loss in adult, late talking and late walking in children/infants, decreases rate of fertility in both males and females, birth of abnormal offsprings, decreases overall immunity of the body, cancer	[11-13]
Pb	Delays in physical or mental development in infants, slight deficits in attention span and learning abilities in children, kidney problems, high blood pressure, anemia, muscle paralysis	[9,14]

2. MATERIALS AND METHODS

2.1. Samples Collection

Total seventy six composed European sea bass and gilthead sea bream fillet samples were collected ramdomly from İzmir which is a port city in Turkey to assess the levels of cadmium, mercury and lead. This study was conducted from June 2010 to July 2011.

2.2. Reagents

Double deionized water (ELGA water purification system, 18.0 MΩ cm resistivity) was used for all diluons. All reagents used were of analytical grade (nitric acid, 65% Suprapur Merck; hydrochloric acid, 30% Suapur Merck). Multi-element calibration solutions of all investigated elements were prepared daily by dilution of 10 mg/L mix element standard stock solution (AccuTrace MES-21-1) and 10 mg/L mercury standard stock solution (AccuTrace MES-21-HG-1).

2.3. Sample Preparation (Microwave Digestion)

European sea bass and gilthead sea bream fillets were homogenized throughly in a laboratory blender (Waring trade marker) with stainless steel cutters. For each homogenized samples, 0.5 g homogenate (wet weight) was weighed and placed in polytetrafluorethylene (PTFE) vessel with 5 mL of 65% nitric acid. Material was then subjected to a microwave program (**Table 2**). The temperature of each vessel was rised to 190˚C in approximately 10 minutes and remained at 190˚C for 15 minutes. Digest was finally made up with 2% nitric acid, 0.5% hydrochloric acid solution to 50 mL in acid washed standard flasks and then placed in 50 mL polypropylene centrifuge tubes.

2.4. Apparatus

Bergh of speed wave MWS-3 microwave digestion system with DAP 60+ vessels was used to digest European sea bass and gilthead sea bream fillet samples prior to toxic element analysis. Inductively coupled plasma-mass spectroscopy (Agilent 7700×) with auto sampler (Agilent ASX-500) was used to analyze digested samples for total metals. The operating conditions for ICP-MS are shown in **Table 3**.

2.5. Determination of Recovery

Ten homogenized fish muscle tissues were spiked with 1000 µg/kg each metal (cadmium, mercury and lead) for the recovery repeatability test. Ten spiked samples and ten blanks were taken through the microwave digestion procedure. And then, digested spiked samples were analyzed using ICP-MS. Obtained values were given in **Table 4**. The mean recoveries for cadmium, mercury and lead 100.22, 107.11 and 92.82 respectively. Acceptable recovery of >90% - <110% were obtained for studied metals, an indication of good analytical protocol.

2.6. Statistical Analysis

To determine the correlations between the metal concentrations in fish and fish products was performed with Mann-Whitney U Test by using Statistica software program. Any statistical significant difference was not detected for cadmium, mercury and lead between European sea bass and Gilthead sea bream samples ($p = 0.599$; $p >$

Table 2. Microwave burning process.

Process	1	2	3
T (˚C)	160	190	100
Ta (transition time, minute)	5	1	1
Waiting time (minute)	5	15	10

Table 3. ICP-MS operating conditions.

Radio frequency power	1550 W
RF matching	2.1 V
Sample depth	8 mm
Carrier gas	1.05 L/min
S/C temperature	2˚C
Nebulizer type	MicroMist

Table 4. Recoveries of cadmium, mercury and lead from fish muscle tissue.

µg/kg	Sample concentration	Concentration of lead added	Concentration of recovered	% Recovery
Cd	1.08	1000	1003.28	100.22
Hg	7.60	1000	1079.25	107.11
Pb	65.41	1000	988.95	92.82

0.05 for cadmium, p = 0.277; p >0.05 for mercury and p = 0.590; p > 0.05 for lead).

3. RESULT AND DISSCUSSION

In this study, seventy six samples of fillet were analysed for cadmium, mercury and lead. Good recovery was obtained for studied elements (**Table 4**). The mean levels obtained in our study are listed in **Table 5**. Box Whisker plot of measured toxic metal concentrations in fillets are given **Figure 1**. The evaluation of the levels of the cadmium, mercury and lead in the European sea bass and gilthead sea bream fillets was performed based on the maximum levels set forth in Commission Regulation EC 1881/2006 [15]. Public health concerns have focused on the fish fillets the levels of cadmium in only three samples analyzed (one sample European sea bass fillet, two samples gilthead sea bream fillet) was found that might have exceeded the regulatory limits. The concentration of lead in one European sea bass fillet investigated was higher than the recommended legal limits (**Table 6**). In European sea bass and gilthead sea bream fillets analyzed, the mean concentrations of toxic elements decreased as mercury > lead > cadmium.

3.1. Cadmium (Cd)

Cadmium is a non-essential metal, but competes with other essential metallic ions when accumulated in organisms and produces unpredictable changes [16]. Mean cadmium concentrations of the studied fillets were 7.92 µg/kg for European sea bass, 9.88 µg/kg for gilthead sea bream. The highest cadmium content was found in the European sea bass fillet (256.50 µg/kg). 2.63% of samples of gilthead sea bream fillet, 5.26% of samples of European sea bass fillet exceeded the limits of EC 1881/2006 [15]. Mean cadmium concentrations of European sea bass and gilthead sea bream muscle tissues was found as 92 µg/kg and 120 µg/kg, respectively, by Dural *et al.* [17]. In another study, mean cadmium concentrations in the flesh of cultured European sea bass were found 270 µg/kg [18]. Our obtained values were found as lower than these values.

3.2. Mercury (Hg)

Mercury is a known toxicant which is present in the environment as a result of natural processes and anthropogenic activities [19]. Fish accumulate substantial concentrations of mercury in their tissues and thus, can represent a major dietary source of this element to humans [20]. The highest mercury value was detected in the European sea bass fillet (353.36 µg/kg). All examined samples had a mercury content below the permissible level of 500 µg/kg (wet weight of fish) for human consumption recommended by EC 1881/2006 [15]. The highest mercury concentrations measured in our study is lower then that reported by Abreu *et al.* [21], 1700 µg/kg, in European sea bass muscle tissues in Portugal. In a similar way, mean mercury levels (120 µg/kg) were found higher than current study (74.90 µg/kg) in gilthead sea bream muscle tissues in Ligurian Sea, Italy [22].

3.3. Lead (Pb)

Lead is a widely distributed environmental poison and, solder used in the manufacture of cans is a source of contamination of food by Pb. Therefore, the monitoring of lead concentration became essential [23]. Lead is a ubiquitous environmental and industrial pollutant that has been detected in almost all phases of environmental and biological systems [24]. The general population is exposed to lead from air and food in roughly equal proportions [25]. Mean cadmium concentrations of the European sea bass and gilthead sea bream fillets were 57.69 and 27.89 µg/kg, respectively. Generally, lead levels of analyzed European sea bass fillet samples (97.37%) were found to be lower than legal limits of EC 1881/2006. Any sea bream fillet samples analyzed did not exceeded EC 1881/2006 limits [15]. The mean lead levels of analyzed European sea bass and gilthead sea bream fillet samples are lower than those reported in the literature (1030 µg/kg for European sea bass, Alasalvar *et al.* [18]; 480 µg/kg for European sea bass, Türkmen *et al.* [26]; 620 µg/kg for gilthead sea bream, Uluozlu *et al.* [1]; 7330 µg/kg for gilthead sea bream, Yılmaz [27].

4. CONCLUSION

The concentration of trace metals in samples is depended on fish species. Some species is accumulated trace metals at high ratio [28]. The consequence of heavy metal pollution can be hazardous to man and it often becomes mandatory to check chemical contaminants in

Table 5. Cadmium, mercury and lead concentrations (μg/kg on a wet weight basis) in frozen European sea bass and gilthead sea bream fillet, number of products investigated (n), arithmetic mean (Mean), standard error (SD).

Product	n	Mean ± SE		
		Cd	Hg	Pb
European sea bass fillet	38	7.92 ± 6.73	97.65 ± 17.26	57.69 ± 28.46
Gilthead sea bream fillet	38	9.88 ± 6.68	74.90 ± 13.43	27.89 ± 7.08

Table 6. The percentage of samples exceeding limits of EC and EC limits.

Element	Sample rate (%) that exceeded of EC 1881/2006 limits		Maximum residue limits (μg/kg) according to EC 1881/2006
	European sea bass fillet	Gilthead sea bream fillet	
Cd	2.63	5.26	50
Hg	*	*	500
Pb	2.63	*	300

*Any samples analyzed did not exceed limits.

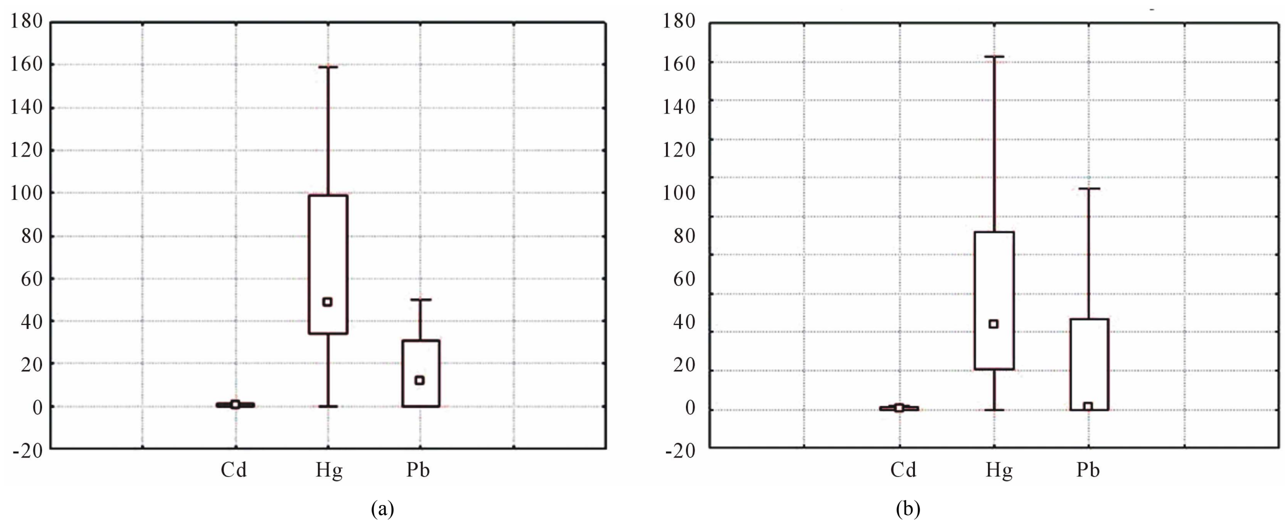

Figure 1. Box whisker plot of concentrations of measured toxic elements in European sea bass filets (a) and gilthead sea bream filets (b) (▫: median, □: 25% - 75%, −: min-max).

foods from the aquatic environment to understand their hazard levels [4]. According to current study results amount of toxic elements in studied muscle tissues (fillets) may vary depending on species. The cadmium, mercury and lead concentrations in the majority of the European sea bass and gilthead sea bream fillets analyzed were convenient within the recommended legal limits for human consumption, except in a few cases. Monitoring of fisheries products in terms of heavy metals is of great importance for public health.

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