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RESEARCH ARTICLE

HEALTH RISK ASSESSMENT OF HEAVY METALS IN SEABREAM (*Sparus aurata*) SAMPLED FROM A PUBLIC MARKET IN TÜRKİYE

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ABSTRACT

There is a dearth of data about public health in the regular evaluation of heavy metal levels in seafood obtained from public market. This study aimed to examine the levels of essential (copper, zinc) and nonessential metals (cadmium, lead) in the tissues of cultured seabream (*Sparus aurata*), which was in public market. For this purpose, seabream samples were collected from the public market. And then, copper (Cu), zinc (Zn), cadmium (Cd), and lead (Pb) metal accumulation levels in tissues of the dissected gill, liver, kidney, and muscle were measured by inductively coupled plasma mass spectrometry (ICP-MS). In order to understand whether the fish posed a risk to public health, risk evaluation formulations (estimated daily intake: *EDI*, hazard coefficients: *HQ*, hazard index: *HI*) were calculated for the muscle tissue. The data showed that Zn, Cd and Pb concentrations were maximal in the kidney tissue of *S. aurata* and Cu concentration were in the liver tissue, while the minimum heavy metal levels were in the muscle tissue. As a result, the maximum levels of heavy metals in the edible tissue of seabream were found as 0.11 mg Cu/kg wet weight, 0.72 mg Zn/kg wet weight, 0.12 mg Cd/kg wet weight, and 0.34 mg Pb/kg wet weight. Maximum values of them do not indicate any health risks as they are lower than the allowable tolerable levels specified by the international *EDI* estimation committees. The examined *HQ* and *HI* indicators were observed below 1 in all seasons. However, it is always possible for heavy metal intake to pose potential risks. For this reason, it is essential that the results of the study be interpreted from different perspectives and taken into consideration by other scientists.

Keywords: *Sparus aurata*, Heavy metals, Accumulation, Health risk assessment, Türkiye

1. INTRODUCTION

It is known that fish consumption has many beneficial and protective properties, especially for heart health [1]. Moreover, regular intake of a rich fish oil supplement has been shown to regulate or alter various metabolic processes connected with lipid metabolism, atherosclerosis, thrombosis, and inflammation [2]. Consumption of fish meat is becoming more important every day because it is easier to digest and contains more protein, minerals, vitamins, essential unsaturated fatty acid and especially polyunsaturated fatty acid (omega-3-PUFA) compared to terrestrial organisms [3,4]. However, marine ecosystems are being polluted by heavy metals day by day, and there is an increase in the bioconcentration levels of heavy metals in the bodies of organisms [5,6]. Some heavy metals such as cadmium, lead and mercury accumulate in tissues even at very low concentrations and cause toxic effects, and these show non-essential properties for organisms [7]. On the other hand, copper-zinc in structural and metabolic events which have biological importance in organisms are needed within homeostatic control limits [8,9]. However, copper and zinc can cause public health problems such as neurotoxicity if the tolerable limits for metabolism are exceeded [10]. In case of exceeding the tolerated limits, heavy metals accumulate in the body and cause neurological disorders such as Parkinson's and Alzheimer's, changes in hematological parameters, growth and development disorders, and various metabolic organ function anomalies, which in turn result in a series of fatal health problems [11]. The World Health Organization recommends regular monitoring of the levels of heavy metals such as copper, zinc, cadmium, lead, mercury and aluminum in fish tissues [12]. Seabream (*Sparus aurata*) is a commercially important fish species, and it is recommended to be monitored in the environmental pollution risk analysis [13,14]. According to the Turkish Statistical Institute

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(TUIK), while the average fish consumption per person in Türkiye was 5.49 kg in 2017, it increased by 11.8% to 6.14 kg in 2018 [15]. The rates of toxic substances in food and their effects on human health have been gaining importance in recent years [16]. To this end, the present study was firstly aimed to evaluate the heavy metal accumulation levels in the tissues of *S. aurata* seasonally and to monitor whether an abnormal situation occurred. Secondly, the study was intended to examine the heavy metal levels in the edible muscle tissue of the fish to evaluate its public health risk level through some risk evaluation calculations such as *EDI* (estimated daily intake), *HQ* (hazard coefficients), *HI* (hazard index).

2. MATERIALS AND METHODS

2.1. Sampling Fish

A total of 72 *S. aurata* were obtained from the fish market of Osmaniye province in the winter, spring, summer, fall and winter seasons of 2018 by random sampling method in the research. The total length and total weight of the collected fish samples were measured on a clean bench that could not cause any contamination. The total length measurement of the samples was carried out with ±0.1 mm scale caliper and the total weight measurement was carried out with a balance with ±0.1g precision (Sartorius BP-310S). Height and weight characteristics of the fish according to the seasons were 68.10 ±5.20 g and 13.30 ±1.10 cm for winter, 70.35±4.90 g and 15.25±1.15 cm for spring, 80.55±5.30 g and 20.30±1.30 cm for summer, 80.90±3.90 g and 28.25±0.90 cm for fall. The fish were then stored in a -20 freezer until the metal analysis. All glass and polypropylene materials to be used in the experiment were cleaned according to appropriate protocols to eliminate the presence of various chemical residues [17].

2.2. Sample Preparation of Fish and Heavy Metal Analyses

Fish tissues were dissected, then left to dry in an oven (Memmert UFE 500, Germany) at 150°C for 48 hours. After the tissues came to constant weight, they were weighed by an electronic balance with a precision of ±0.001 g (Sartorius CP-2248). The tissues (about 0.5 g) were transferred to the experiment tubes, and a mixture of 2:1 (v/v) nitric acid and perchloric acid (Merck, %65, S.G.; 1.40 and %60, S.G.; 1.53) was added to them, and the tissues were homogenized on the hot plate (Thermo Scientific, 2200, USA) at 120°C for 3 hours [18]. Following the homogenization, the samples were transferred to polypropylene tubes. The tubes were diluted to 5 ml with ultrapure water so that the samples were ready for metal analysis. The samples were stored in the refrigerator at +4ºC until the time of analysis. Metal concentrations (Cu, Zn, Cd, Pb) in the samples were determined with the ICP-MS Agilent 7500ce model spectrometer (Octopole Reaction Systems, Agilent Technologies, Japan). The instrument calibration of metal analyses was performed according to C-5524 Sigma standards (Sigma Chem. Co. St. Louis, USA). Standard solutions of the metals were diluted with ultrapure water (Millipore Elix Milli-Q, resistivity 18.2 MΩ/cm). The TORT 2 (Lobster hepatopancreas) standard reference material was used to check the reliability of the measurements. The validation parameters of analytic techniques are shown in Table 1.

Table 1. The validation parameters of analytic techniques of Cu, Zn, Cd and Pb.

Element	Recovery (%)	Detection limit (µg/kg)	Quantification limit (µg/kg)	Relative standard deviation (%)	R^2
Cu	95.60	0.60	2.01	3.07	0.99
Zn	97.70	2.45	8.27	3.75	0.99
Cd	91.70	0.45	1.55	2.01	0.99
Pb	95.05	0.35	1.20	2.10	0.99

2.3. Risk Assessment Calculations and Statistical Analysis

The estimation of daily intake rate [$(EDI) = (C_{element} \times D_{fish})Bw^{-1}$] equation was used to estimate the daily intake (*EDI*) of metals (mg/kg body weight/day). The abbreviation $C_{element}$ in the formula refers to the metal concentration in the muscle tissue (mg/kg wet weight (ww)), D_{fish} means the daily fish consumption (g/person/day), and *Bw* (kg) represents the average body weight of the population [19,20]. In Türkiye, the annual fish consumptions of an adult person of 70 kg was found to be 5.5 kg. The hazard quotient [$HQ = EDI/RfD$] relation was used to calculate the hazard coefficient (*HQ*). It is stated in the literature that if the *HQ* value is less than 1, it does not constitute a significant risk potential; in contrast, if the *HQ* value is equal to or higher than 1, then it poses a potential risk for the public health. The abbreviation *RfD* in the formulation refers to the oral reference doses for Cu, Zn, Cd, and Pb, which are specified by the risk assessment information system [21] as 0.3, 0.7, 0.001 and 0.004, mg/kg/day, respectively. The *RfD values* were also utilized as the reference values in the present study. The hazard index [$HI = \Sigma HQs = HQ_{Cu} + HQ_{Zn} + HQ_{Cd} + HQ_{Pb}$] formulation was estimated as the sum of the *HQs* for all the heavy metals in the formula. If the *HI* value of metals is less than 1, then it is stated that the health of local fish consumers is safe, whereas, if the *HI* is equal to or greater than 1, then it is stated that it is harmful for public health [22]. Evaluation of the samples studied in triplicate for every season was performed in the Microsoft Office Excel 2010 program. Mean, standard error, minimum and maximum values were calculated for the data which were grouped according to the seasons. Statistical analysis of the data was carried out using the SPSS 20.0 statistical software (IBM Corp., Armonk, NY, USA). Tukey multiple range test was used to determine whether the metal concentrations varied significantly among tissues based on the season [23].

3. RESULTS AND DISCUSSION

Metal accumulation in tissues and organs of aquatic organisms varies depending on seasonal changes [24], species of the organism [25], morphological structure of the organism such as length and weight [26,27], environmental conditions such as physical and chemical condition of the aquatic ecosystem [28]. The arithmetic mean and standard error values of the heavy metal levels in *S. aurata* tissues (gill, liver, kidney and muscle) were calculated according to the seasons, and the variation in seasonal metal levels were examined by calculating the sum of the squares of the deviations of the data from the arithmetic mean. Accordingly, a summarized graphical representation of the arithmetic mean, standard errors and statistical differences of the metals in the tissues was displayed in Figure 1.

The mean annual Cu, Zn, Cd and Pb concentrations in the gill tissue of *S. aurata* sampled in winter, spring, summer and fall were found to be 0.16, 0.72, 0.12 and 0.25 mg/kg ww, respectively. According to the seasons, Cu accumulation in the gill tissue was the most in the spring season, while the accumulation of Zn was the most in the fall season ($P \leq 0.05$). The highest accumulation of cadmium in the gill tissue was observed in the fall season, but no statistical difference was observed between the accumulation averages of cadmium in the fall and winter seasons and the accumulation rates of lead in the spring and summer seasons ($P \geq 0.05$). The heavy metal accumulation in the gill tissue in the fall and winter season was in the form of Zn>Pb>Cd>Cu, while it was determined as Zn>Cu>Pb>Cd and Zn>Cu>Pb≈Cd in the spring and summer seasons, respectively (Figure 1). The mean annual Cu, Zn, Cd and Pb concentrations in the liver tissue were 0.46, 0.90, 0.15 and 0.23 mg/kg ww, respectively. Cu and Pb accumulation in liver tissue was found to be the highest in the spring, while Zn and Cd accumulations showed the highest accumulation in the summer and fall seasons, respectively ($P \leq 0.05$). While the heavy metal accumulation was in the form of Zn>Cu>Cd>Pb in the fall season, it was determined as Zn>Cu>Pb>Cd in other seasons (Figure 1). The mean annual Cu, Zn, Cd and Pb accumulations in the kidney tissue were 0.29, 1.84, 0.30 and 0.47 mg/kg ww, respectively. The Zn accumulation in the kidney tissue showed the highest accumulation in the summer while the highest accumulations of Cu, Cd and Pb were detected in the fall season compared to the other seasons

($P \leq 0.05$). The heavy metal accumulation sequence in the kidney tissue was observed as Cd>Cu>Zn>Pb in the winter, Zn>Pb>Cd>Cu in the spring, and Zn>Pb>Cu>Cd in the summer and fall (Figure 1).

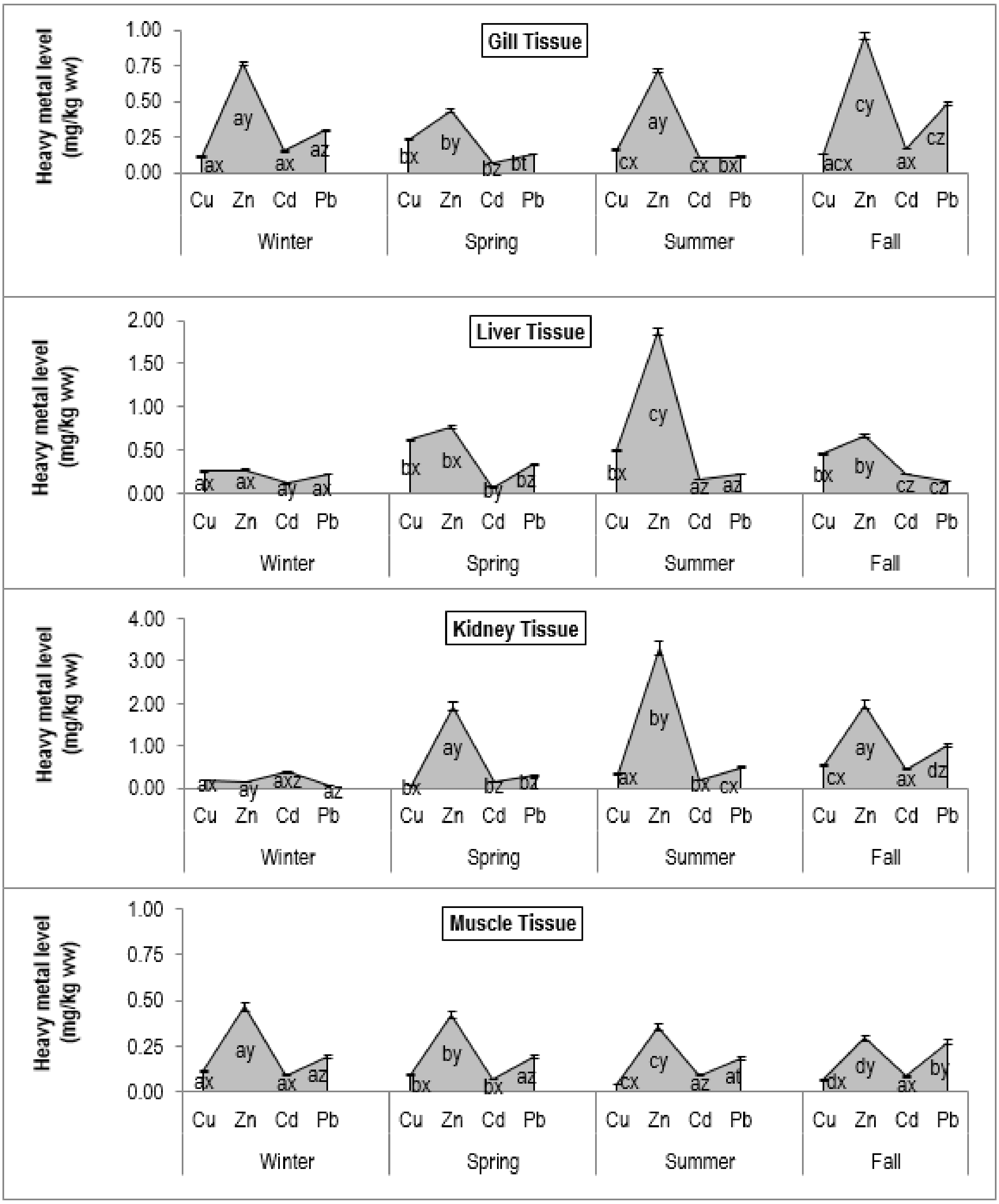

Figure 1. Mean and standard error of heavy metal concentrations in gill, liver, kidney and muscle tissue of *S. aurata* in different seasons. Letters x, y, z and t show differences among Cu, Zn, Cd, Pb metals; a, b, c and d among winter, spring, summer, fall seasons.

The mean annual Cu, Zn, Cd and Pb concentrations in the muscle tissue of *S. aurata* were found to be 0.07, 0.38, 0.08, 0.21 mg/kg ww, respectively. Cu and Zn accumulations in the muscle tissue were determined to be the highest in the winter season, while the highest accumulation of Pb was identified in the fall season ($P \leq 0.05$). Cadmium showed the highest accumulation in the summer season, while statistical difference for the cadmium accumulation was observed only between the spring and summer ($P \leq 0.05$). The heavy metal accumulation of the muscle tissue showed Zn>Pb>Cu>Cd sequence for winter, spring and fall, while it was Zn>Pb>Cd>Cu for summer (Figure 1). Among the heavy metals examined in 10 species including *S. aurata*, zinc was the most detected metal [29], and similarly, the presence of zinc was found at the highest level in our study.

Heavy metals in the contaminated aquatic ecosystem lead to bioaccumulations in various organs and structural units of fish from the food web or in the aquatic environment over time [30]. In a previous study, it was reported that there were significant differences between the metal bioaccumulation levels in fish tissues ($P \leq 0.05$), and that the metal accumulation was higher in the liver and gill organs than in other organs [30,31]. Since metabolic rates of large internal organs such as the liver and kidney are higher than other organs, the heavy metal accumulation in these organs increases in parallel. The negatively charged surface of the gill tissue in fish tends to bind to many positively charged metals [32]. Heavy metals enter aquatic organisms through two metabolic routes: the gills and muscle tissue. Therefore, metal concentrations in these tissues and organs are mostly reflected as an indicator of the ambiance in the aquatic ecosystem [33]. In environmental monitoring studies of heavy metal pollution, various concerns may be stated about the frequency and persistence of being affected by contaminated heavy metals due to the consumption of the muscle tissue of fish. However, if the muscle tissue compared to other soft organs does not have an active structure in the metal accumulation, this situation may be related to low metal binding proteins [34]. This situation is explained in many studies by the fact that the muscle tissue of the fish has less metabolic activity compared to other organs, together with less heavy metal accumulation [31, 35]. The high concentrations of heavy metal determined in the liver tissue of aquatic organisms are due to its high metallothionein production, which has made it an important storage organ as an indicator of chronic metal exposure [31]. In aquatic ecosystems, the entrance of heavy metals to the structure of organisms in the food chain occurs in two ways: the first is directly supplied to the organism through the digestive tract, while the other is by external means such as the muscles and gills [33]. The direct route of uptake of heavy metals from the aquatic ecosystem is more effective because the gills are the main target organ of heavy metal poisoning in fish [36]. The orders of annual metal accumulations according to tissues of the seabream were observed as kidney>liver>gill>muscle for both Zn and Cd, liver>kidney>gill>muscle for Cu, and kidney>gill>liver>muscle for and Pb, in the current study (Figure 1).

In this study, the minimum and maximum levels of metals in the muscle tissue differed between the following ranges, respectively; 0.01-0.11 mg Cu/kg ww, 0.25-0.72 mg Zn/kg ww, 0.05-0.12 mg Cd/kg ww and 0.18-0.34 mg Pb/kg ww (Table 2). Average Cu, Zn, Cd and Pb concentrations in the seabream muscle samples taken from public aquaculture fish in Osmaniye province were found to be far below the maximum levels allowed for fisheries by international organizations (Table 3). Heavy metal samples in the seabream samples obtained from the coastal locations of the Black Sea [29] and the Mediterranean Sea in Türkiye (Table 3) had higher content than the heavy metal levels of the samples in this study. These values in the present study were within the limits set by the Turkish Food Codex Commission Regulation [37]. In a previous study, it was reported that the average values of heavy metals such as total As, Cd, Cu, Pb and Zn in farmed fish were lower than in the wild fish, and the Cd concentration was higher in farmed fish [38]. Table 3 partly shows that the cultured fish in this study differed from wild species.

The *HI* index of heavy metals in wild and farmed seabream sampled from the Algerian coast was determined to be less than one [38]. It has been stated that the consumption of farmed fish *Dicentrarchus labrax* and *S. aurata* analyzed from fish farms in different locations of the

Mediterranean does not pose a serious threat to human health and the fish are in a consumable condition [39]. The international legal limits and indices of trace elements (Ag, Al, As, Ba, Bi, Cd, Cr, Cu, Fe, Li, Mn, Mo, Pb, Sb, Se, Sn, U, V, Zn) in muscle tissues of cultured seabream examined from the Corsican Coasts of the Northwestern Mediterranean were determined to be below the levels that could pose a risk to human health. It was also reported in this study that some elements (As etc.) found in wild *S. aurata* should be consumed in moderation considering their carcinogenic risks [40]. These results in the literature are consistent with the current study with *S. aurata* (Figure 2).

Table 2. Minimum and maximum metal concentrations in muscle tissue of *S. aurata* in different seasons.

Heavy Metals (mg/kg ww)	Winter min-max	Spring min-max	Summer min-max	Fall min-max
Cu	0.11-0.12	0.01-0.02	0.03-0.06	0.01-0.02
Zn	0.35-0.58	0.25-0.72	0.26-0.43	0.29-0.31
Cd	0.08-0.10	0.07-0.07	0.05-0.12	0.07-0.11
Pb	0.18-0.20	0.19-0.20	0.16-0.21	0.24-0.34

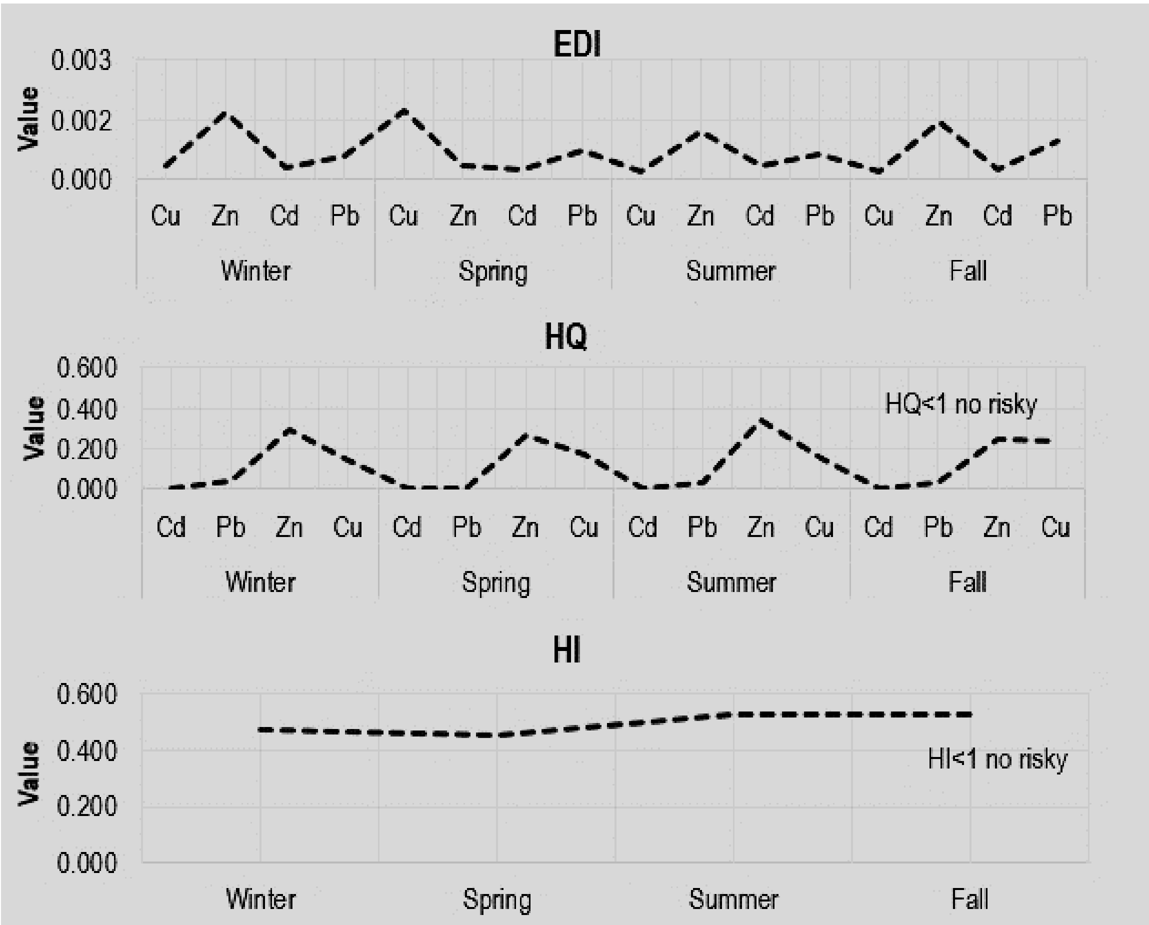

Figure 2. Public health risk values of heavy metals in *S. aurata* from public market in Osmaniye (Türkiye).

Table 3. The maximum limits of heavy metals accepted by international organizations in fishery products and comparison of heavy metal's concentrations in muscle of *S. aurata* with other studies (BDL: Below Detection Limit)

	Heavy Metal (mg/kg ww)				
International organizations	**Cu**	**Zn**	**Cd**	**Pb**	**Reference**
U.S. Food and Drug Administration	150	100	0.20	1.50	[41]
World Health Organization	30	100	1	2	[42]
Turkish Food Codex	20	50	0.50	0.30	[37]
Other studies					
Tuzla Lagoon (Wild fish)	0.55	-	0.12	1.11	[31]
Beymelek Lagoon (Wild fish)	4.31	7.09	-	-	[43]
Kayseri Province (Market fish)	0.90	33.80	1.24	BDL	[44]
Tirana Province (Wild fish)	-	-	0.35	0.10	[45]
Iskenderun Bay (Wild fish)	6.23	14.35	1.25	3.83	[46]
Elazığ Province (Market fish)	1.19	6.27	0.18	0.44	[47]
Aegean Sea (Cultured fish)	BDL	4.99	BDL	BDL	[48]
Ionian Sea (Cultured fish)	BDL	7.02	BDL	BDL	[48]
Adriatic Sea (Cultured fish)	0.39	4.70	0.01	0.10	[49]
Aegean Sea (Cultured fish)	0.36	2.12	-	-	[35]
Hurmabogazı Lagoon (Wild fish)	1.33	11.1	0.13	0.52	[50]
Çamlık Lagoon (Wild fish)	0.88	4.92	0.64	0.45	[51]
Tuzla Lagoon (Wild fish)	0.39	6.91	0.30	0.32	[51]
Corsica Mediterranean Sea (Wild fish)	0.20	3.55	0.002	0.006	[40]
Corsica Mediterranean Sea (Cultured fish)	0.49	4.34	0.004	0.013	[40]
Croatian (Market fish)	0.94	-	9.4	-	[52]
South Adriatic-Montenegro (Wild fish)	0.17	-	<0.02	<0.1	[53]
Cassidaigne canyon (Wild fish)	-	-	BDL	-	[54]
South Italy (Wild fish)	0.17	0.17	0.04	0.08	[55]
Mediterranean Sea (Wild fish)	0.29	4.13	0.002	0.004	[38]
Mediterranean Sea (Market fish)	0.07	0.38	-	-	[56]
Maximum value detected, (Public market-Cultured fish)	0.11	0.72	0.12	0.34	In this study

4. CONCLUSION

The present study focused on checking the quality of seabream fish in terms of the copper, zinc, cadmium and lead contents. Considering the protein starvation all around the world, seabream is considered a delicious and economically valuable fish. The quality indicators examined in this study showed that seabream is a safe seafood product for consumption given that the calculated data indicators (*HQ* and *HI*) were below 1 for all seasons (Figure 2). As far as we know, there is still no known scale indicator for the content of heavy metals studied in fish in many countries. Hence, a basic comparison was made by taking into consideration the evaluations in our country. However, when dietary regimens and individual morphological structures are taken into account, what is not a risk for one country may pose a risk for another. Therefore, the acceptable levels of heavy metals should be considered according to the references specified by the Food and Drug Administration (FDA) and the World Health Organization. With this regard, it was concluded that Cu, Zn, Cd and Pb levels in fish species were lower than the maximum levels, but further studies are needed to monitor the course of heavy metals over time.

CONFLICT OF INTEREST

The author stated that there are no conflicts of interest regarding the publication of this article.

REFERENCES

[1] Kris-Etherton PM, Harris WS, Appel LJ. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Arterioscler Thromb Vasc Biol 2003; 23(2): 20-30. DOI:10.1161/01.ATV.0000038493.65177.94.

[2] Wang YY, Zhang JX, Tian T, Gao MY, Zhu QR, Xie W, Fu LM, Wang SK, Dai Y. Dietary patterns in association with the risk of elevated blood pressure, lipid profile and fasting plasma glucose among adults in Jiangsu Province of China. Nutr Metab Cardiovasc Dis 2022; 32(1): 69-79. DOI:10.1016/j.numecd.2021.09.004.

[3] Antonijevic B, Jankovic S, Curcic M, Durgo K, Stokic E, Srdic B, Tomic-Naglic D. Risk characterization for mercury, dichlorodiphenyltrichloroethane and polychlorinated biphenyls associated with fish consumption in Serbia. Food Chem Toxicol 2011; 49(10): 2586-2593. DOI:10.1016/j.fct.2011.06.078.

[4] Khalili Tilami S, Sampels S. Nutritional value of fish: lipids, proteins, vitamins, and minerals. Rev Fish Sci Aquacult 2018; 26(2): 243-253. DOI:10.1080/23308249.2017.1399104.

[5] Storelli MM. Potential human health risks from metals (Hg, Cd, and Pb) and polychlorinated biphenyls (PCBs) via seafood consumption: estimation of target hazard quotients (THQs) and toxic equivalents (TEQs). Food Chem Toxicol 2008; 46(8): 2782-2788. DOI:10.1016/j.fct.2008.05.011.

[6] Taş EÇ, Filipuçi I, Çakır DT, Beyaztaş S, Sunlu U, Toğulga M, Özaydın O, Arslan O. Heavy metal concentrations in tissues of edible fish (*Mullus barbatus* L.,1758) from Çandarlı Bay (Turkey). Fresenius Environmental Bulletin 2011; Volume 20 – No 11 : 2834-2839.

[7] Türkmen M, Türkmen A, Tepe Y, Töre Y, Ateş A. Determination of metals in fish species from Aegean and Mediterranean seas. Food Chem 2009; 113(1): 233-237. DOI:10.1016/j.foodchem.2008.06.071

[8] Amundsen PA, Staldvik FJ, Lukin AA, Kashulin NA, Popova OA, Reshetnikov YS. Heavy metal contamination in freshwater fish from the border region between Norway and Russia. Sci Total Environ 1997; 201(3): 211-224. DOI:10.1016/S0048-9697(97)84058-2.

[9] Couture P, Rajotte JW . Morphometric and metabolic indicators of metal stress in wild yellow perch (*Perca flavescens*) from Sudbury, Ontario: a review. J Environ Monit 2003; 5(2): 216-221. DOI:10.1039/B210338A.

[10] DPHHS U. Agency for Toxic Substances and Disease Registry. Toxicological Profile for Zinc. Atlanta, GA: US Department of Public Health and Human Services, 2005.

[11] Isangedighi IA, David GS. Heavy metals contamination in fish: effects on human health. J Aquat Sci Mar Biol 2019; 2(4): 7-12.

[12] Chapman D. Water quality assessments: a guide to the use of biota, sediments and water in environmental monitoring. Boca Raton, Florida, USA: CRC Press, 2021.

[13] Bakiu R, Santovito G, Hoda A, Shehu J, Durmishaj S, Irato P, Piccinni E. Metallothionein (MT): a good biomarker in marine sentinel species like sea bream (*Sparus aurata*). Albanian J Agric Sci 2013; 12(2): 247.

[14] Benhamed S, Guardiola FA, Martínez S, Martínez-Sánchez MJ, Pérez-Sirvent C, Mars M, Esteban MA. Exposure of the gilthead seabream (*Sparus aurata*) to sediments contaminated with heavy metals down-regulates the gene expression of stress biomarkers. Toxicol Rep 2016; 3: 364-372. DOI:10.1016/j.toxrep.2016.02.006

[15] Turkish Statistical Institute. Fisheries Statistics. 2019. https://www.tuik.gov.tr/Home/Index. Accessed July 22, 2022.

[16] Khansari FE, Ghazi-Khansari M, Abdollahi M. Heavy metals content of canned tuna fish. Food Chem 2005; 93(2): 293-296. DOI:10.1016/j.foodchem.2004.09.025

[17] Tuzen M. Toxic and essential trace elemental contents in fish species from the Black Sea, Turkey. Food Chem Toxicol 2009; 47(8): 1785-1790. DOI:10.1016/j.fct.2009.04.029.

[18] Muramoto S. Elimination of copper from Cu-contaminated fish by long-term exposure to EDTA and fresh water. J Environ Sci Health Part A 1983; 18(3): 455-461. DOI:10.1080/10934528309375113.

[19] Griboff J, Wunderlin DA, Monferran MV. Metals, As and Se determination by inductively coupled plasma-mass spectrometry (ICP-MS) in edible fish collected from three eutrophic reservoirs. Their consumption represents a risk for human health? Microchem J 2017; 130: 236-244. DOI:10.1016/j.microc.2016.09.013.

[20] GDFA (General Directorate of Fisheries and Aquaculture). Fisheries Statistics. Republic of Turkey Ministry of Food Agriculture and Livestock, 2018. Accessed May 1, 2022.

[21] The Risk Assessment Information System (RAIS). (2019). University of Tennessee, Available from: https://rais.ornl.gov/index.html.

[22] Li J, Huang ZY, Hu Y, Yang H. Potential risk assessment of heavy metals by consuming shellfish collected from Xiamen, China. Environ Sci Pollut Res 2013; 20 (5): 2937-2947. DOI:10.1007/s11356-012-1207-3.

[23] Sokal RR, Rohlf FJ. Biometry: the principles and practice of statistics in biological research, 3rd edn. New York: WH Freeman and Co, 1995.

[24] Dural M, Göksu MZL, Özak AA. Investigation of heavy metal levels in economically important fish species captured from the Tuzla lagoon. Food Chem 2007; 102(1): 415-421.DOI:10.1016/j.foodchem.2006.03.001.

[25] Türkmen M, Türkmen A, Tepe Y, Ateş A, Gökkuş K. Determination of metal contaminations in sea foods from Marmara, Aegean and Mediterranean seas: twelve fish species. Food Chem 2008; 108(2): 794-800. DOI:10.1016/j.foodchem.2007.11.025.

[26] Damodharan U, Reddy MV. Heavy metal bioaccumulation in edible fish species from an industrially polluted river and human health risk assessment. Fish Aquat Life 2013; 21(1): 19-27. DOI:10.2478/aopf-2013-0003.

[27] Mohammadnabizadeh S, Pourkhabbaz A, Afshari R. Analysis and determination of trace metals (nickel, cadmium, chromium, and lead) in tissues of *Pampus argenteus* and *Platycephalus indicus* in the Hara Reserve, Iran. J Toxicol 2014; Article ID: 576496. DOI:10.1155/2014/576496.

[28] Jezierska B, Witeska M. Metal Toxicity to Fish. Siedlce: Wydawnictwo Akademii Podlaskiej, 2001.

[29] Bat L, Sezgin M, Üstün F, Şahin F. Heavy metal concentrations in ten species of fishes caught in Sinop coastal waters of the Black Sea, Turkey. Turkish J Fish Aquat Sci 2012; 12(5): 371-376. DOI:10.4194/1303-2712-v12_2_24.

[30] Jabeen G, Javed M, Azmat H. Assessment of heavy metals in the fish collected from the river Ravi, Pakistan. Pakistan Vet J 2012; 32(1): 107-111.

[31] Dural M, Göksu MZL, Özak AA. Investigation of heavy metal levels in economically important fish species captured from the Tuzla lagoon. Food Chem, 2007; 102(1): 415-421. DOI:/10.1016/j.foodchem.2006.03.001.

[32] Playle RC. Using multiple metal-gill binding models and the toxic unit concept to help reconcile multiple-metal toxicity results. Aquat Toxicol 2004; 67(4): 359-370. DOI:10.1016/j.aquatox.2004.01.017.

[33] Rajeshkumar S, Liu Y, Zhang X, Ravikumar B, Bai G, Li X. Studies on seasonal pollution of heavy metals in water, sediment, fish and oyster from the Meiliang Bay of Taihu Lake in China. Chemosphere 2018; 191: 626-638. DOI:10.1016/j.chemosphere.2017.10.078.

[34] Squadrone S, Prearo M, Brizio P, Gavienelli S, Pellegrino M, Scanzio T, Guarise S, Benedetto A, Abete MC. Heavy metals distribution in muscle, liver, kidney and gill of European catfish (*Silurus glanis*) from Italian Rivers. Chemosphere 2013; 90(2): 358-365. DOI:10.1016/j.chemosphere.2012.07.028.

[35] Castritsi-Catharios J, Neofitou N, Vorloou AA. Comparison of heavy metal concentrations in fish samples from three fish farms (Eastern Mediterranean) utilizing antifouling paints. Toxicol Environ Chem 2015; 97(1): 116-123. DOI:10.1080/02772248.2014.943226.

[36] Sauliutė G, Svecevičius G. Heavy metal interactions during accumulation via direct route in fish: a review. Zool Ecol 2015; 25(1): 77-86. DOI: 10.1080/21658005.2015.1009734.

[37] Anonymous. Turkish Food Codex Contaminants Regulation Authority Law, 2011. Official Gazette No. 5996, 29.12.2011-28157. Accessed May 2, 2022.

[38] Lounas R, Kasmi H, Chernai S, Amarni N, Ghebriout L, Hamdi B. Heavy metal concentrations in wild and farmed gilthead sea bream from southern Mediterranean Sea-human health risk assessment. Environ Sci Pollut Res 2021; 28(24): 30732-30742. DOI:10.1007/s11356-021-12864-3.

[39] Kalantzi I, Pergantis SA, Black KD, Shimmield TM, Papageorgiou N, Tsapakis M, Karakassis I. Metals in tissues of seabass and seabream reared in sites with oxic and anoxic substrata and risk assessment for consumers. Food Chem 2016; 194: 659-670. DOI:10.1016/j.foodchem.2015.08.072.

[40] Marengo M, Durieux ED, Ternengo S, Lejeune P, Degrange E, Pasqualini V, Gobert S. Comparison of elemental composition in two wild and cultured marine fish and potential risks to human health. Ecotoxicol Environ Saf 2018; 158: 204-212. DOI:10.1016/j.ecoenv.2018.04.034.

[41] Food and Drug Administration. Fish and fisheries products hazards and controls guidance, 3rd edn. US FDA. Washington, DC: Center for Food Safety & Applied Nutrition, 2001.

[42] Mokhtar MB, Aris AZ, Munusamy V, Praveena SM. Assessment level of heavy metals in *Penaeus monodon* and *Oreochromis spp* in selected aquaculture ponds of high densities development area. Eur J Sci Res 2009; 30(3): 348-360.

[43] Uysal K, Emre Y, Köse E. The determination of heavy metal accumulation ratios in muscle, skin and gills of some migratory fish species by inductively coupled plasma-optical emission spectrometry (ICP-OES) in Beymelek Lagoon (Antalya/Turkey). Microchem J 2008; 90(1): 67-70. DOI:10.1016/j.microc.2008.03.005.

[44] Yıldırım Y, Gönülalan Z, Narin I, Soylak M. Evaluation of trace heavy metal levels of some fish species sold at retail in Kayseri, Turkey. Environ Monit Assess 2009; 149(1): 223-228. DOI:10.1007/s10661-008-0196-7.

[45] Ozuni E, Dhaskali L, Abeshi J, Zogaj M, Haziri I, Beqiraj D, Latifi F. Heavy metals in fish for public consumption and consumer protection. Nat Montenegrina 2010; 9(3): 843-851.

[46] Dural M, Genc E, Sangun MK, Güner Ö. Accumulation of some heavy metals in *Hysterothylacium aduncum* (Nematoda) and its host sea bream, *Sparus aurata* (Sparidae) from North-Eastern Mediterranean Sea (Iskenderun Bay). Environ Monit Assess 2011; 174:147-155. DOI:10.1007/s10661-010-1445-0.

[47] İlhak Oİ, Karatepe P, Özçelik M. Heavy metal levels in some fish species sold at retail in Elazığ. Fırat Univ Med J Health Sci 2012; 26(2): 99-103.

[48] Kalantzi I, Black KD, Pergantis SA, Shimmield TM, Papageorgiou N, Sevastou K, Karakassis I. Metals and other elements in tissues of wild fish from fish farms and comparison with farmed species in sites with oxic and anoxic sediments. Food Chem 2013; 141(2): 680-694. DOI:10.1016/j.foodchem.2013.04.049.

[49] Žvab Rožič P, Dolenec T, Baždarić B, Karamarko V, Kniewald G, Dolenec M. Element levels in cultured and wild sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*)

from the Adriatic Sea and potential risk assessment. Environ Geochem Health 2014; 36(1): 19-39. DOI:10.1007/s10653-013-9516-0.

[50] Türkmen A, Tepe Y, Türkmen M. Determination of metals in tissues of fish species from Hurmabogazı Lagoon. Indian J Geo-Mar Sci 2016; 45 (2): 277-282.

[51] Tepe Y, Türkmen A, Türkmen M. Comparison of heavy metal accumulation in tissues of economically valuable fish species from two nearby lagoons in Mediterranean coastal area. Indian J Geo-Mar Sci 2017; 46(7): 1333-1338.

[52] Bilandžić N, Sedak M, Čalopek B, Đokić M, Varenina I, Kolanović BS, Luburić ĐB, Varga I, Benić M, Roncarati A. Element contents in commercial fish species from the Croatian market. J Food Compos Anal 2018; 71:77-86. DOI:10.1016/j.jfca.2018.02.014.

[53] Antović I, Šuković D, Andjelić S, Svrkota N. Heavy metals and radionuclides in muscles of fish species in the South Adriatic-Montenegro. In RAP Conference Proceedings 2019; 4: 96-102. DOI:10.37392/RapProc.2019.19

[54] Bouchoucha M, Chekri R, Leufroy A et al. Trace element contamination in fish impacted by bauxite red mud disposal in the Cassidaigne canyon (NW French Mediterranean). Sci Total Environ 2019; 690:1626. DOI:10.1016/j.scitotenv.2019.06.474.

[55] Storelli A, Barone G, Dambrosio A, Garofalo R, Busco A, Storelli MM. Occurrence of trace metals in fish from South Italy: Assessment risk to consumer's health. J Food Compos Anal 2020; 90: 103487. DOI:10.1016/j.jfca.2020.103487.

[56] Łuczyńska J, Pietrzak-Fiećko R, Purkiewicz A, Łuczyński MJ. Assessment of fish quality based on the content of heavy metals. Inter J Environ Res Pub Health 2022; 19(4): 2307. DOI:10.3390/ijerph19042307.