Metals in Wild and Cultured *Dicentrarchus labrax* (Linnaeus, 1758) from Fish Markets in Sinop: Consumer’s Health Risk Assessment

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Abstract

Concentrations of Cd, Hg, Pb, As, Al, Cu, Fe, and Zn were determined in the muscles of wild and farmed European seabass in Sinop markets between September and December in 2020, using inductively coupled plasma mass spectrometry after microwave digestion. In the study, iron (Fe), zinc (Zn), aluminum (Al), and copper (Cu) were found higher than the other metals both in wild and cultured *Dicentrarchus labrax*. These are essential elements, but excess amounts act as a poison. Arsenic (As) concentration was higher than cadmium (Cd), mercury (Hg), and lead (Pb) both in wild and cultured *D. labrax*. The estimated maximum total dietary intakes of these eight metals from both wild and farmed European seabass were below the maximum acceptable daily intake values set by the Turkish Food Codex and European Union Regulation. Results showed that according to metal amounts, consumption of *D. labrax* had no threat to consumers’ health. The target hazard quotient (THQ) revealed that harmful health impacts may not occur. Furthermore, risk index (RI) indicated that there may have a lower risk of developing cancer in the future who have been exposed to Pb and As through fish intake. Although the fish are not overly contaminated, the metal level is rising. Increased amounts of heavy metals in fish in different areas could be due to an increase in farm inflow water, domestic sewage, and a number of other anthropogenic sources, all of which should be looked into further. Precautions should be made to safeguard this fish from metal contamination and to reduce the risk to human health.

Keywords Heavy metals · Wild and culture · ICP-MS · *Dicentrarchus labrax* · Consumer health risk

Introduction

The Black Sea, an inland sea, has unique features in the world. It can be easily affected by all kinds of natural disasters and negativities. It receives contaminants carried by rivers not just from countries bordering the Black Sea, as well as from a wide range of European countries [1]. Contaminants can enter marine ecosystems through a variety of natural and human-caused sources, which include industrial or domestic effluents, pesticide and inorganic fertilizers, rainwater, landfill leaching, shipping and harbor activities, atmospheric deposition, and geological erosion of the lithosphere [2–8]. The most important of these contaminants are metals. Metals can be beneficial or toxic to biota and human life depending on their level. Several of these metals are hazardous to live creatures even at trace amounts, while others are physiologically essential but poisonous at high concentrations [9–11]. Metals mix with biomolecules in the body, such as proteins and enzymes, to generate persistent bio-toxic substances, mutilating their properties and inhibiting their bioreactions, when taken in significant amounts. Metals can build up in the edible tissues of fish, which are frequently found at the top of the aquatic food webs [1, 10].

The ever-increasing human population has been increased the demand for food supplies. The demand for fish products has increased due to their high protein content and being cheaper than red meats. The true value of fish in the food system stems not only from its high protein content but also from the two types of omega-3 polyunsaturated fatty acids, namely eicosatetraenoic acid (EPA) and docosahexaenoic acid (DHA). Omega-3 (n-3) fatty acids are necessary for...
proper growth since they reduce cholesterol levels and the risk of cardiovascular disease, stroke, and early birth. Fish also contains vitamins and minerals that are beneficial to the body [12].

Although the amount of fishing in the seas has fluctuated since the 2000s, it has tended to decrease in recent years. On the other hand, there is an increase in the amount of fish farmed. The fishing production amount of marine fish in Turkey in 2019 was 374,726 tons. On the other hand, the amount of aquaculture production in the seas is 256,930 tons. It is predicted that the amount in aquaculture will exceed the amount in fishing very soon. Turkey has made significant progress in marine aquaculture in the last 10 years. It is thought that the geographical location of Turkey has a great impact on this development in terms of fish production. European seabass is a cultured fish of great importance for the Mediterranean region, having high-quality meat in the seas of Turkey. Recently, its cultivation has been increasing in the Black Sea. European seabass is in the top rank among the most cultivated species in Turkey. The production amount of European seabass by aquaculture in 2019 is 137,419 tons [13].

*Dicentrarchus labrax* L., 1758, which is a solitary and carnivorous species, is fed with crustaceans, small shrimps such as crangonid, small amphipods such as *Gammarus* in its young period, and fish such as sardines, anchovies, rockfish, as well as crabs and shrimps in its adult period. Fish take up metals from their food and their environment. On the other hand, fish farms use seawater for the cultivation of marine species and use pelleted feed. Farmed fish may absorb dissolved metals from their feeding meals and ambient water, resulting in minor accumulation in various tissues and evoking contamination levels at quality standards.

European seabass, which has very tasty and high-quality meat, is consumed willingly by the public. In Sinop, which is a fishing city, European seabass always finds a place at fish-selling stalls. The habit of people eating fish in Sinop is higher than in many regions of Turkey.

Because the accumulation of pollutants in fish muscles is widely accepted, the current study is concerned with metal concentrations in fish muscles as they are also the most consumed component by consumers. Additionally, fish store substantial quantities of metals in their tissues, making them a key dietary source of these metals for humans. These ratios may surpass permissible levels in the muscle of such fish in contaminated areas. In this sense, we conduct health risk assessments by detecting the amount of the metals in muscle tissues of both wild and cultured *Dicentrarchus labrax* were measured, and their potential risk was calculated using many indicators such as estimated daily intake (EDI), estimated weekly intake (WDI), target hazard quotient (THQ), hazard index (HI), and risk index (RI).

### Materials and Method

The city of Sinop (Turkey) is known as the fishing city. Since there is no industry, fishing comes to the fore. In recent years, the number of fish farmed has approached the amount obtained by fishing. The Ministry has given permission by expanding the aquaculture areas in certain parts of the Sinop coast.

### Collection of Fish Samples

A total of 12 wild and 12 cultured whole fresh European seabass in Sinop (Turkey) markets were purchased between September and December 2020. The fish were cleaned in deionized water, packaged in polyethylene bags, and maintained frozen at $-21^\circ C$ till metal analysis. In this investigation, it was studied in accordance with the Directive on the Protection of Animals Used for Scientific Purposes of the European Parliament and Council [14].

### Metal Analysis

Plastic and glassware were washed and immersed in a 10% (v/v) HNO$_3$ solution overnight. Then, these were cleansed with deionized water and then dried before use. All of the acids used were from Merck in Germany and were of the best standard.

The fish were thawed and dissected before analysis. Each fish’s dorsal side muscle tissues were extracted without skin and homogenized. Bernhard’s [15] technique was used to prepare muscle tissues of the fish samples for analysis. Accredited laboratories employed ICP-MS (Agilent Technologies / 7700X ICP-MS Systems) to assess metals in fish tissues utilizing TURKAK Test TS EN ISO/IEC 17025 and EN 15763 in-house method DMS 19236, which was developed from AOAC (Association of Official Analytical Chemists) 999.10, to quantify elements using a microwave digestion system (Milestone SK10), and certified reference material (Lobster TORT-2). The accuracy of elements in the certified reference material varied within 10%. The limits of detection (LOD) for Cd, Hg, Pb, As, Al, Cu, Fe, and Zn were 0.004136, 0.002434, 0.03223, 0.03705, 2.114, 0.07025, 0.7099, and 1.351 ppb, respectively. All analyses were performed in triplicate, and the mean values were used to analyze the data. Results are given in mg/kg wet weight.
Calculation of the Daily and Weekly Intakes of Metals

It is critical to analyze daily metal intake from edible tissues of fish and compare it to the estimated daily intake (EDI) level given by different accredited worldwide organizations for health security under normal consumption habits. This can be obtained simply by multiplying the concentration of each metal in the edible tissues of the European seabass by the average daily quantity of fish consumed per capita.

To evaluate the level of exposure to fish diet, daily and weekly intakes were computed based on the concentration of metals in the edible tissues [16]. The following equation was used to calculate the estimated daily intake (EDI) of metals for consumers in (mg/kg/day):

\[
\text{EDI} = \frac{C_{\text{metal}} \times W_{\text{fish}}}{BW}
\]

where \(C_{\text{metal}}\) is the metal amount in European seabass samples (mg/kg, on a wet wt. basis); \(W_{\text{fish}}\) is the daily average fish consumption in this region (g/day); and \(BW\) is the consumer’s body wt. (kg).

For infants in age group of 1, children age group of 10, and adults, the study used 13, 27, and 41 g/day, respectively. Weights are taken as 10, 30, and 70 kg for infants, children, and adults [17]. Estimated weekly intakes (EWI) were derived from EDI multiplied by 7 as shown in the formula below.

\[
\text{EWI} = \text{EDI} \times 7
\]

Consumer’s Health Risk Assessment

The target hazard quotient (THQ) was used to measure the health risks associated with fish consumption by Sinop residents. The following equation describes the method of evaluating risk using THQ as defined in the United States Environmental Protection Agency Region III risk-based concentration table [18–21].

\[
\text{THQ} = \frac{\text{EDI}}{\text{Rf.D.}} \times 10^{-3}
\]

The Rf. D. is the oral reference dose of metal (mg/kg day), 1.0E + 00 for Al, 3.0E – 04 for As, 1.0E – 03 for Cd, 4.0E – 02 for Cu, 7.0E – 01 for Fe, 3.0E – 04 for Hg, and 3.0E – 01 for Zn. Reference dose was not given for Pb, but oral slope factor (SFO) value was given as 8.5E – 03 mg/kg day. For As, the SFO value is given as well as the RFD value. The SFO value for As is 1.5E + 00 mg/kg day [22, 23].

The risk index (RI) was calculated using the following formula:

\[
\text{RI} = \text{EDI} \times \text{SF}
\]

The RI is considered negligible if the RI is < 10^{-6}, allowable or bearable if the RI is 10^{-6} < RI < 10^{-4}, and important if the RI is > 10^{-4}.

The THQs are summed together to generate the hazard index (HI):

\[
\text{HI} = \text{THQ}_{\text{Cd}} + \text{THQ}_{\text{Hg}} + \text{THQ}_{\text{As}} + \text{THQ}_{\text{Al}} + \text{THQ}_{\text{Cu}} + \text{THQ}_{\text{Fe}} + \text{THQ}_{\text{Zn}}
\]

If the HI is less than 1.0, it is doubtful that there will be evident negative consequences, whereas an HI more than 1.0 suggests the likelihood of negative consequences. When the HI exceeds ten (10), the risk is considered high, persistent, or even severe [1, 24, 25].

Statistical Analysis

The data were analyzed with the SPSS v.21 statistics software. Calculations were made using Microsoft Excel 2019. Statistical differences in the metal levels between wild and cultured European seabass were compared by the Mann–Whitney \(U\), or Student’s \(t\)-tests, where appropriate (\(p = 0.05\)) [26].

Results and Discussion

Zn had the highest quantities, whereas Cd had the lowest (Fig. 1). The mean (± Std Deviation) of Cd (0.015 ± 0.002), Hg (0.025 ± 0.007), and Pb (0.042 ± 0.005) values (mg/kg wet wt.) in European seabass was lower than the recommended limits of Cd < 0.05, Hg < 0.50, and Pb < 0.30 mg/kg wet wt. by European Union Commission Regulation and Turkish Food Codex [27, 28]. Similarly, the As, Cu, and Zn values averaged 0.11 ± 0.02, 0.25 ± 0.07, and 16.87 ± 4.18 mg/kg wet wt. in European seabass, respectively. These results were found to be 9.1, 80, and 3 times less than the recommended 1.0, 20, and 50 mg/kg wet weight values [29]. In the present study, Al amounts ranged from 0.11 to 0.19 mg/kg wet wt. Al is the third most abundant component in the crust of the earth and has numerous applications in human life. Natural sources, water used in meal preparation, feedstuffs, and tools used in cooking all contribute to the presence of Al in the availability of food. It is pointed out that Al levels in foodstuffs can range from 0.1 to 20 mg/kg [30]. In this study, Fe values were found between
4 and 10 mg/kg wet wt. Council of Europe [30] pointed out that Fe is existing in most foods. It has been stated that many foods have Fe concentrations of up to 150 mg/kg and the recommended amount of Fe intake is 10–15 mg/day [30]. However, Cd, Hg, Pb, As, Al, Cu, Fe, and Zn in wild and cultured European seabass from Sinop markets were within the safe limits.

As expected, essential metals were detected higher than toxic metals. Metals such as Cd, Hg, Pb, As, Al, Cu, Fe, and Zn have no recognized beneficial effects and are well known for their harmful effects on human health, whereas metals like Cu, Fe, and Zn are nutritionally vital metals, but their toxic effects on human health begin when they are present at high amounts [31]. These metals can be introduced into the environment by human actions or natural sources. Many studies have been carried out to evaluate the levels of these metals in diverse fish species all over the world [32]. Monthly changes in trace metal content in European seabass can be caused by different environmental and biological factors, such as water physicochemical parameters, physiological and ecological characteristics, and fish habits and diets.

**Fig. 1** Toxic and essential elements in muscles of wild and cultured *Dicentrarchus labrax* (mg/kg wet wt.) from Sinop markets between September and December in 2020
Cd and Pb levels were recorded significantly high in wild European seabass than cultured European seabass, while As and Fe levels were recorded significantly higher in cultured European seabass compared to wild European seabass ($p < 0.05$). Hg, Al, Cu, and Zn levels in the wild and cultured European seabass are not found significant differences ($p > 0.05$). Wild European seabass is usually fed with crustaceans and small pelagic fish; however, cultured ones are fed mainly with pellet feeds.

When compared to other studies, the toxic elements (Cd, Hg, As, and Al) found in this study were generally low. The highest Cd value (3.09 mg/kg wet wt.) was found in European seabass bought from Trabzon fishing markets [33]. The Hg levels in Table 1 are remarkably similar to those obtained in other investigations. Pb levels were highest in European seabass sampled from Dardanelles [34], followed by those in retailers of Kayseri [35], Paradeniz Lagoon in Mersin [36], and from fish farms in the Aegean Sea [37]. Arsenic is one of the least studied elements and the highest value was obtained from the fish found in Trabzon markets [33], followed by the fish caught in Bafa Lake of Aydın-Muğla [38]. Cu, Fe, and Zn values were determined in wild and farmed fish sampled from the Aegean Sea [37]. The fact that the results of this study are quite low may be stated as the lack of human activities due to the closure and restriction of travel due to COVID-19 in 2020. Continuity of studies is considered very important to arrive at a definitive conclusion.

In Table 1, the level of the metals in *D. labrax* determined in the present study is compared to those found in other studies.

### Health Risk Taxation from Fish Intake

Based on the metal content in the fish and the amount intake, toxic metals in fish may constitute a health risk to consumers. The term “hazard” refers to the process of identifying the toxicological qualities of a material. Tolerable intake is a term that is commonly used to define safe amounts of consumption; it can be represented in either estimated daily intakes (EDI) or estimated weekly intakes (EWI). In this investigation, the EDIs and EWIs are estimated and shown in Table 2. For babies, children, and adults, intake estimations were reported as mg/kg body weight/daily or weekly.

According to the guidelines, the metal consumption from fish muscle tissues was substantially lower and had no negative impacts on the consumers. Because of their toxic effects and accumulation in biota, determining metal amounts in commercial fish species has gotten a lot of attention in many nations in the region and globally. This attention was motivated by a need to ensure the safety supply while limiting the danger to public health.

### Table 1 Comparison of measured metal amounts in *D. labrax* with values taken from the literature as ppm (LOD=limit of detections)

| Sampling area                  | Wet/dry wt | Metals | References |
|-------------------------------|------------|--------|------------|
|                               |            | Cd     | Hg         | Pb          | As | Al | Cu | Fe | Zn |
| Ordu (Perşembe)               | Dry        | 0.24   | -         | <0.05       | -  | -  | 1.01| 30 | 25.7 | [39] |
| Aegean Sea (Güllük Bay)       | Dry        | <0.01–0.04 | -       | <0.02–0.4   | -  | -  | <0.1| <0.5–7.2 | [40] |
| Adana (Çamlık Lagoon)         | Dry        | 0.08   | -         | -           | -  | -  | 5.62| 82.65 | [41] |
| Aegean Sea (from fish farms)  | Wet        | -      | -         | -           | -  | -  | 24.70| 2.83 | [42] |
| Aegean Sea (from fish farms)  | Wet        | -      | -         | -           | -  | -  | 25.77| 2.89 | [43] |
| Aegean Sea (from fish farms)  | Wet        | 0.3    | -         | 1           | -  | -  | 3.9 | 51.2 | 45.1 | [36] |
| Agean Sea (wild)              | Wet        | 0.20   | -         | 0.80        | -  | -  | 3.00| 63.10| 43.60 | [36] |
| Dardanelles                   | Dry        | 0.19   | 0.03      | 20.00       | -  | 37 | 3.00| 41.00| <0.01 | [34] |
| İskenderun Bay (from fisherman)| Wet       | 0.03   | -         | 0.48        | -  | -  | 1.06| 33.3 | 10.7 | [44] |
| Mediterranean Sea (İskenderun Bay) | Wet | 0.14 | - | 0.27 | - | - | 1.98| 0.70| 2.92 | [45] |
| Adana (Yelkoma Lagoon)        | Wet        | 0.10   | -         | 0.19        | -  | -  | 0.71| 28.90| 6.01 | [46] |
| Mersin (Paradeniz Lagoon)     | Wet        | 0.67   | -         | 1.02        | -  | -  | 0.83| 42.20| 8.53 | [36] |
| Sinop (from fish farms)       | Dry        | 0.00   | <0.001    | 0.04        | -  | -  | <0.106| <0.38| 8.40 | [47] |
| Aydn-Muğla (Bafa Lake)        | Dry        | 0.05   | 0.08      | 0.23        | 0.24| 2.58| 0.61| -    | -    | [38] |
| Trabzon (from fish markets)   | Wet        | 3.09   | <LOD      | 0.29        | 0.61| 8.6 | 9.36| -    | -    | [33] |
| Kayseri (from retailers)      | Wet        | 0.85   | -         | 1.07        | -  | 1.01| 12.8| -   | -    | [35] |
| Sinop (from markets)          | Wet        | 0.01   | 0.03      | 0.06        | -  | -  | 0.42| 9.20 | -    | [48] |
| Malatya (from local bazaars)  | Wet        | -      | -         | -           | -  | -  | 3.12| 9.41 | -    | [49] |
| Sinop markets wild            | Wet        | 0.017  | 0.03      | 0.047       | 0.09| 0.14| 0.22| 5.2  | 16.7 | This study |
| Sinop markets cultured        | Wet        | 0.012  | 0.02      | 0.038       | 0.13| 0.16| 0.27| 8.5  | 17   | This study |
According to the directorate General of Fisheries in the Turkish Ministry of Agriculture, the average quantity of fish consumed per person annually in Turkey is 6.7 kg, which is calculated as 18.36 g per person per day. However, this data does not reflect the truth as it shows the average value obtained by dividing the consumed part of the total fish amount produced and caught that year by the whole population. However, some regions of Turkey do not have the habit of eating fish. Fish consumption is generally higher in coastal cities such as Sinop. In addition, infants, children, and adults consume different amounts of fish. Calculations were made according to the values found in the present study, where infants, children, and

| Metals | Fish Type | EDI (Infants) | EDI (Children) | EDI (Adults) | EWI (Infants) | EWI (Children) | EWI (Adults) |
|--------|-----------|---------------|----------------|-------------|---------------|----------------|-------------|
| Cd     | Wild      | 2.27E-05      | 1.57E-05       | 1.02E-05    | 1.6E-04       | 1.1E-04        | 7.2E-05     |
|        | Cultured  | 1.65E-05      | 1.14E-05       | 7.46E-06    | 1.2E-04       | 8E-05          | 5.2E-05     |
| Hg     | Wild      | 3.99E-05      | 2.76E-05       | 1.80E-05    | 2.8E-04       | 1.9E-04        | 1.3E-04     |
|        | Cultured  | 2.63E-05      | 1.82E-05       | 1.18E-05    | 1.8E-04       | 1.3E-04        | 8.3E-05     |
| Pb     | Wild      | 6.14E-05      | 4.25E-05       | 2.76E-05    | 4.3E-04       | 3E-04          | 1.9E-04     |
|        | Cultured  | 4.94E-05      | 3.42E-05       | 2.22E-05    | 3.5E-04       | 2.4E-04        | 1.6E-04     |
| As     | Wild      | 1.23E-04      | 8.55E-05       | 5.56E-05    | 8.6E-04       | 6E-04          | 3.9E-04     |
|        | Cultured  | 1.72E-04      | 1.19E-04       | 7.76E-05    | 1.2E-03       | 8.3E-04        | 5.4E-04     |
| Al     | Wild      | 1.85E-04      | 1.28E-04       | 8.34E-05    | 1.3E-03       | 9E-04          | 5.8E-04     |
|        | Cultured  | 2.08E-04      | 1.44E-04       | 9.37E-05    | 1.4E-03       | 1.01E-03       | 6.6E-04     |
| Cu     | Wild      | 2.92E-04      | 2.02E-04       | 1.31E-04    | 2.1E-03       | 1.42E-03       | 9.2E-04     |
|        | Cultured  | 3.07E-03      | 2.45E-04       | 1.59E-04    | 2.2E-02       | 1.72E-03       | 1.1E-03     |
| Fe     | Wild      | 6.82E-03      | 4.72E-03       | 3.07E-03    | 4.7E-02       | 3.3E-02        | 2.1E-02     |
|        | Cultured  | 1.10E-02      | 7.65E-03       | 4.97E-03    | 7.7E-02       | 5.3E-02        | 3.4E-02     |
| Zn     | Wild      | 2.17E-02      | 1.50E-02       | 9.81E-03    | 1.5E-01       | 1.05E-01       | 6.8E-02     |
|        | Cultured  | 2.21E-02      | 1.53E-02       | 9.95E-03    | 1.5E-01       | 1.07E-01       | 6.9E-02     |

Table 2 Estimated daily and weekly intake (mg/kg body wt.) of Cd, Hg, Pb, As, Al, Cu, Fe, and Zn in *Dicentrarchus labrax*
adults consume 5, 10, and 15 kg of fish annually, which is 13, 27, and 41 g per day, respectively [17].

Estimating total dietary exposure to inorganic As from food and drinking water using a range of hypotheses, the lower limit on the reference dose for a 0.5% augmented occurrence of lung cancer (BMDL0.5 = benchmark dose lower bound) was measured from epidemiological studies to be 3.0 g/kg body wt./day (2–7 g/kg body wt./day based on the range of assessed total dietary exposure). The Committee determined that the provisional tolerable weekly intake (PTWI) of 15 g/kg body wt. (equal to 2.1 g/kg body wt./day) was no longer adequate because it was close to the BMDL0.5. The preceding PTWI was withdrawn by the Committee [50].

Food and water are the primary sources of non-occupational human exposure to Al, which is naturally found in variable levels in most meals ingested; hence, food is the main contributor. For all Al compounds in food, including additives, JECFA issued a PTWI for Al of 1 mg/kg body wt.; previously established allowable daily intakes and PTWI for its compounds were withdrawn [51].

Assumptions are used in risk assessments. The Risk Assessment Information System (RAIS) describes procedures for calculating the non-cancer risk (THQs) and the goal cancer risk index (RI). The estimated THQs for Cd, Hg, As, Al, Cu, Fe, and Zn and RI for Pb and As to people due to exposure to these from consumption of wild and cultured European seabass from Sinop markets are calculated and presented in Table 3.

HI, the sum of THQs, was found to be smaller than 1 in both wild and cultured European sea bass. This means that consuming these fish does not pose a threat to humans. The maximum HI was seen in the infants’ group, and the lowest was in the category of adults. When it is calculated that 10 kg infants eat 13 g fish per day and 70 kg adults eat 41 g fish daily [17], it can be said that this proportionally is an expected result. Since RI for Pb is < 10⁻⁶, it is considered negligible, and RI for As is 10⁻⁶ < RI < 10⁻⁴, it is considered permissible or bearable. HI and RI values were calculated slightly less in wild European sea basses than in cultured European seabass.

Conclusion

According to the findings of this investigation, metal levels in wild and cultivated European seabass species were well below the permitted standards specified by several international institutions. Furthermore, when assessing varied fish-eating habits, the assessment of THQs undertaken in this study revealed that harmful health impacts may not occur. Furthermore, RI who have been exposed to Pb and As through fish intake may have a lower risk of developing cancer in the future.

Declarations

Conflict of Interest The authors declare no competing interests.

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