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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Toxic Metal Contents and Potential Human Health Risk Assessment in Cultured Trout Sampled from Samsun Fish Market [*]

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Abstract: This study carried out to investigate the potential health hazards of four hazardous metallic elements via trout consumption. Twenty specimens of big trout were purchased between March and June 2022 from Samsun Fish Market. The metallic elements were assessed by the contamination level of non-essential arsenic (As), lead (Pb), mercury (Hg), and cadmium (Cd) in the edible tissues of big trout. The results didn't show any significant differences between months. The lowest metal level was Cd, followed by Hg Pb and As. The mean As, Pb, Hg and Cd concentrations were found to be 0.060, 0.023, 0.005 and 0.001 mg/kg wet wt., respectively, which was far below the both national and internationally permissible values. The findings of the assessment of potential dangers to human health revealed that the indices for estimated weekly intake (EWI) and target hazard quotient (THQ) are below the specified welfare standards. Additionally, there was no potential carcinogenic risk to human health because the total THQs, hazard index (HI) and the total carcinogenic risk (TCR) index fell below the thresholds. In conclusion, it was determined that the consumption of *Oncorhynchus mykiss* taken from Samsun Fish Market did not cause any health problems.

Keywords: Hazard index, toxic metals, *Oncorhynchus mykiss*, target hazard quotient.

Samsun Balık Halinden Alınan Kültür Alabalıklarında Zehirli Metal İçerikleri ve Potansiyel İnsan Sağlığı Risk Değerlendirmesi

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Öz: Bu çalışma, alabalık tüketimi yoluyla dört tehlikeli metalik elementin, potansiyel sağlık tehlikelerini araştırmak için yapılmıştır. Mart ve Haziran 2022 tarihleri arasında Samsun Balık Hali'nden 20 adet iri alabalık örneği satın alınmıştır. Metalik elementler, büyük alabalıkların yenilebilir dokularındaki esansiyel olmayan arsenik (As), kurşun (Pb), cıva (Hg) ve kadmiyumun (Cd) kirlenme düzeyi ile değerlendirilmiştir. Sonuçlar aylar arasında önemli bir fark göstermemiştir. En düşük metal düzeyi Cd, ardından Hg, Pb ve As olmuştur. Ortalama As, Pb, Hg ve Cd konsantrasyonları sırasıyla 0,060, 0,023, 0,005 ve 0,001 mg/kg yaş ağırlık olarak bulunmuş ve bu hem ulusal hem de uluslararası izin verilen değerlerin çok altında kalmıştır. İnsan sağlığına yönelik potansiyel tehlikelerin değerlendirilmesinin bulguları, tahmini haftalık alım (EWI) ve hedef tehlike katsayısı (THQ) endekslerinin, belirtilen sağlık standartlarının altında olduğunu ortaya koymuştur. Bununla beraber, toplam THQ'lar, tehlike indeksi (HI) ve toplam karsinojenik risk (TCR) indeksi eşik değerlerin altına düştüğü için insan sağlığı açısından potansiyel kanserojen riski yoktu. Sonuç olarak Samsun Balık Hali'nden alınan *Oncorhynchus mykiss* tüketiminin herhangi bir sağlık sorununa yol açmadığı belirlenmiştir.

Anahtar kelimeler: Hedef tehlike katsayısı, toksik metaller, *Oncorhynchus mykiss*, tehlike indeksi.

INTRODUCTION

Especially in the Black Sea, with the permission of trout farming and the increase in production as a result, consumption is also increasing. For the people living on the Black Sea coast, fish consumption has a special place in their diet. Along with other nutrients necessary for cell growth and cognitive function, important fatty acids, needed amino acids, and minerals, fish meat also protects against heart disease.

However, Samsun coasts of the Black Sea are frequently contaminated and harmed as a result of industrial discharges, urbanization, and extensive agricultural activities (Bat et al., 2018). Metal contamination is a major concern because they are continuous bio-accumulative toxic environmental pollutants that could be transferred to people through fish consumption once they enter the food chain. Essential metals play a notable part in biological systems and typical biological processes in living organisms including fish. However, non-essential metals and metalloids such as As, Pb, Hg and, Cd derived from both natural and anthropogenic sources are toxic substances and their presence in much quantities can reason critical health issues, they enter the marine environment and herewith the food chain by bio-magnification to in the end reach the human.

In this sense, the first thing that comes to mind is fish in pollutants that can be consumed directly and reach humans through the food chain (Bat, 2014 and 2017; Bat and Arici, 2018). *Oncorhynchus mykiss* (Walbaum, 1792) is a preferred species in both toxicological (Verep et al., 2007a; Gündoğdu et al., 2009; Terzi and Verep, 2011; Beşli et al., 2016; Verep et al., 2016) and metal accumulation (Verep et al., 2007b; Varol et al., 2017; Bat et al., 2018; Yardım and Bat, 2020; Bat et al., 2021; Öztürk, 2022; Öğretmen, 2023) studies. Today, *O. mykiss*, a popular species whose aquaculture is increasing rapidly in the Black Sea, is consumed with pleasure. It is not only consumed in cities with a coast on the Black Sea, but also exported to many countries. Therefore, it is essential to monitor the health risks of toxic metals to consumers in commercial fish.

Aquaculture in Türkiye waters increased by 11.9% in 2021. In 2021, 335644 tons of aquaculture production took place in the seas and 136042 tons in inland waters. The most important fish species grown in inland waters was *O. mykiss* with 134174 tons. While the production of *O. mykiss* in the seas was 18182 tons in 2020, it increased to 31509 tons in 2021 and the change was observed as 73.3% (TÜİK, 2022). This ratio is expected to increase further in the coming years.

In our study, it was goaled to determine the levels of four toxic metals in this species, purchased from Samsun Fish Market, one of the largest fish markets in the region, and to evaluate the possible health risks in consumers.

MATERIAL AND METHOD

Total of twenty specimens of *O. mykiss* were purchased Samsun Fish Market between March and June 2022. The length of the purchased trout was between 50-55 cm and their weight was between 3-3.2 kg. Fish were washed with distilled water in the laboratory and their muscle tissues in the dorsal were dissected with sterile plastic tools. Then each fish meat sample was placed in zippered plastic bags and stored at -21 degrees Celsius until metal analysis. To prevent any potential contamination, acid-cleaned laboratory supplies were used throughout sample preparation. The dorsal tissues of the fish were processed for analysis according to Bernhard's procedure (1976). Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was used to determine the metal concentrations.

Toxic metal analysis in *O. mykiss* was done by an approved Environment Industrial Analysis Laboratory Services Trade Company (TÜRKAK Test TS EN ISO IEC 17025 AB-0364-T) handling the m-AOAC 999.10-technique after Microwave Digestion. The European Standard EN 15763 was used. The method's sensitivity was determined using the detection limits established for the ICP/MS, which were 0.001 g/L for As, Pb, and Cd and 0.01 g/L for Hg.

Health Risk Assessment: To evaluate the risk to human health associated with the farmed *O. mykiss* consumption, the Estimated Daily Intake (EDI), the Estimated Weekly Intake (EWI), the Target Hazard Quotient (THQ), the Hazard Index (HI) and lifetime cancer risk (CR) were calculated for As, Pb, Hg and, Cd. The EDI was estimated according to the equation (1) considering the studied metals content in *O. mykiss* muscle and the daily fish consumption.

$$EDI = \frac{Cm \times DFC}{Bwt.} \quad (1)$$

Where:

EDI is reported in mg/kg wet wt.; DFC is the daily fish consumption (mg/kg wet wt.); the daily fish consumption is accepted as 13.36 g/day (BSGM, 2022) and the Bwt. is the mean body weight (70 kg) in Türkiye.

The THQ was calculated using the equation (2).

$$THQ = \left[\frac{(EFr \times ED \times FIR \times Cm)}{(Rf.Do. \times BW \times ATn)}\right] \times 10^{-3} \quad (2)$$

Where:

EFr = The exposure frequency (set as 365 days $year^{-1}$),

ED = The average lifetime duration (70 years for the Turkish people),

FIR = The fish ingestion rate (18.36 g/person/day) in Türkiye (BSGM, 2022),

Cm = The metal concentration in the *O. mykiss* sample (mg/kg wet wt.),

Rf. Do.= The reference oral dose of As, Hg and Cd as follows: 3×10^{-4}, 3×10^{-4} and 1×10^{-4}, (mg/kg-day), respectively (The Risk Assessment Information System (RAIS, 2022); Regional Screening Level (RSL) Summary Table (TR=1E-06, HQ=1) (US EPA, 2022). The Rf. Do. value for Pb is not included in the most recently published The Risk Assessment Information System and Regional Screening Level (RAIS, 2022; US EPA, 2022). It has been evaluated and complied with in this way in many recent publications (Bat et al., 2021 and 2022a,b).

BW = The average body weight of the adult consumer (70 kg for Turkish people),

ATn = The average exposure time for non-carcinogens (365 days/year × 70 years).

The Hazard Index (HI) is the sum of the HQs for toxic As, Hg and Cd, which was estimated by the equation (3):

$$HI=\sum THQs= THQ_{(As)} + THQ_{(Hg)} + THQ_{(Cd)} \quad (3)$$

General doses calculated using HI less than 1 are often regarded as appropriate since they are unlikely to cause any noncarcinogenic adverse health consequences over the course of a lifetime of exposure.

The likelihood during the course of a person's lifetime that they would contract cancer as a result of being exposed to a potential carcinogen is known as the carcinogenic risk (CR), following the formula (4):

$$CR = SFO \times EDI \quad (4)$$

Where:

SFO are the cancer slope factors for the Risk Assessment Information System's oral carcinogenic slope factor. SFO values are only available for As and Pb among the studied toxic metals, and these values are given as 1.5 and 8.5×10^{-3} $(mg/kg\text{-}day)^{-1}$, respectively (RAIS, 2022; US EPA, 2022).

The total cancer risk (TCR) was calculated by summing the individual cancer risks of multiple carcinogens following the formula (5):

$$TCR = CR_{As} + CR_{Pb} \quad (5)$$

Where:

The acceptable levels of TCR range are from 10^{-4} to 10^{-6}; TCR values $>10^{-4}$ are unacceptable and those $<10^{-6}$ indicate a negligible risk of cancer an individual's lifetime

Statistical Analysis: Statistical analyses were carried out using the software IBM SPSS 21. The means and standard deviation of values made in triplicates were used to show the experimental results. Wet weight (wet wt.) is used to represent the data as mg/kg. The difference was considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

In our study, the highest measured element in fish muscles was As, followed by Pb and Hg, respectively. The lowest toxic metal is Cd. Figure 1 presents the mean concentrations of the four toxic metals found in the muscle of *O. mykiss.* The obtained values for As, Pb, Hg, and Cd show a nonsignificant difference ($p>0.05$) according to the sampling months (Figure 1).

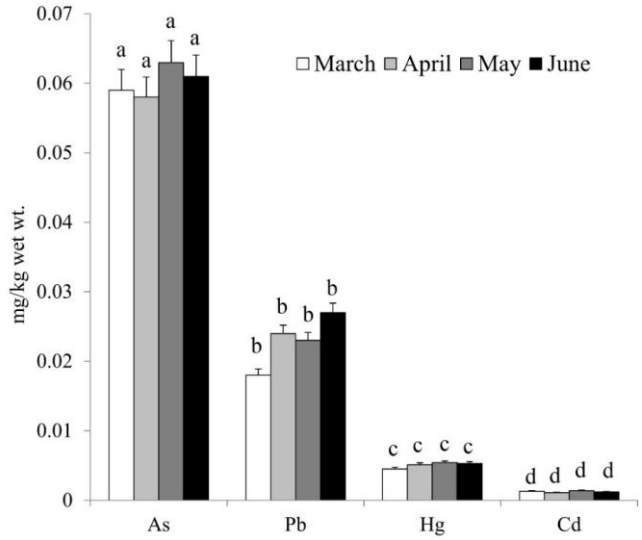

Figure 1. Toxic metals in muscles of *Oncorhynchus mykiss* (mg/kg wet wt.) from Samsun Fish Market in 2022. Vertically, letters a, b, c, and d show a statistically significant relationship among metals and months at $p<0.05$.

These results corroborate previous studies that reported relatively high As levels in fish than those other toxic metals (Bat and Arıcı, 2018). Similarly, As concentration was found higher than Cd, Hg, and Pb both in wild and cultured *Dicentrarchus labrax* (Bat et al., 2022a). In this study, the maximum As concentration was found to be 0.063 mg/kg wet wt., which was far below the internationally permissible values (Table 1). Previously, the United Kingdom levied a restriction of 1 mg/kg wet wt. for As in food, with different limits for varied food categories. These rules were repealed in 2002. At the European Union level, there is no limit set for As in foods, together with fish. Pb is the second abundant toxic metal found in *O. mykiss* analyzed in this study, after As. However, Pb concentration in all the analyzed samples were lower than the minimum level 0.3 mg/kg wet wt. specified by the Turkish Food Codex, the European Union Commission Regulation, and other international standards (Table 1). Hg and Cd levels were very low in fish samples like those reported in most studies (Bat and Arici, 2018; Bat et al., 2022a,b). The eaten of fish containing Hg high

up the acceptable limit may badly affect the central nervous system and the endocrine system. The concentration of Hg in all the samples measured were found to be smaller than the allowable limits set by EU Commission Regulation, Turkish Legislation, and other international standards which is 0.5 mg/kg wet wt. (Table 1). Cd is a toxic metal and it take parts with calcium ion at the enzyme sites of the organism. Cd inclines to have marked nephrotoxic capacities and the high intake may forefront renal and hepatic toxic results. Accordingly, EU Commission Regulation and Turkish Legislation, the maximum admissible limit of the concentration of cadmium is 0.05 mg/kg wet wt. Cd concentrations were found to be lowest in the muscles of all *O. mykiss* samples (between 0.0011 and 0.0014 mg/kg wet wt.) in the present study and were below the legal limits (Table 1).

Table 1. The admissible rates of toxic metals in the fish (mg/kg wet wt.).

Standards	As	Pb	Hg	Cd
Georgian Food Safety Rules (2001)	2.0	1.0	0.5	0.2
Official Gazette of Republic of Türkiye (2002)	--	0.4	0.5	0.1
GAIN Report Russian Federation (2006)	2.0	1.0	0.5	0.2
Commission Regulation (2006)	--	0.3	0.5	0.05
Official Gazette of Republic of Türkiye (2009)	--	0.3	0.5	0.05

The current study's findings are consistent with previous studies from Sinop fish markets (Bat et al., 2018; Bat et al., 2021), Samsun fish markets (Yardım and Bat, 2020), the Karakaya Dam Reservoir on the Firat River in eastern Anatolia (Varol et al., 2017), and the Fırtına and Güneysu Rivers (Dizman et al., 2017) in edible tissue of *O. mykiss.*

Risk Health Assessment: The mean As, Pb, Hg, and Cd concentrations measured in edible tissue of *O. mykiss* of the present study were used to calculate the estimated daily intake (EDI), estimated weekly intake (EWI), target hazard quotient (THQ), and hazard index (HI) values (Table 2). These parameters admit a sign of the risk health for the presence of these substances in the edible tissues of the fish upon consumption.

Table 2. Risk assessment of the consumers for the toxic metals in edible tissues of *O. mykiss* from Samsun Fish Market.

Metals	EDI	EWI	THQ	CR	TCR
As	1.6×10^{-5}	1.1×10^{-4}	5.3×10^{-2}	2.4×10^{-5}	2.4×10^{-5}
Pb	6×10^{-6}	4.2×10^{-5}	--	5.1×10^{-8}	
Hg	1.3×10^{-6}	9.3×10^{-6}	4.4×10^{-3}	--	
Cd	3.3×10^{-6}	2.3×10^{-6}	3.3×10^{-3}	--	
HI =			6×10^{-2}		

The EDI, EWI, THQ, HI, CR, and TCR were calculated for consumers in Türkiye by taking into consideration an exposure time of 7 per week, an average adult weight of 70 kg and a life expectancy of 70 years of Turkish people. The mean daily fish intake in Türkiye is 13.6 g/person, equal to 95.2 g/person/week. The calculated values were compared to the recommended threshold of Provisional Tolerable Weekly Intake (PTWI) set by the Food and Agriculture Organization of the United Nations and World Health Organization (Table 3).

The EWI amounts, we found in this study are significantly lower than the PTWI values given as a reference by the authorities (Table 3). On the other hand, the EWI values for toxic metals did not exceed the recommended values. HI calculated by the sum of THQs was found to be less than 1, indicating that toxic metal concentrations namely As, Hg, and Cd in edible tissue of *O. mykiss* do not show a hazard effect on human health.

Table 3. Internationally recommended PTWI safe levels for the studied metals.

Metals	Standard	References
As	Withdrawn	FAO/WHO 2011
Pb	PTWI of 0.025 mg/kg body weight average weekly	WHO 2000
Hg	PTWI of 0.004 mg/kg body weight average weekly	FAO/WHO 2011
Cd	PTWI of 0.007 mg/kg body weight average weekly	WHO 2000

The CR was used to estimate the lifetime risk of an individual developing cancer due to exposure to potential cancer-causing agents. The TCR was found as the sum of the CRs. Due to the lack of data regarding the cancer slope factor (RAIS, 2022; US EPA, 2022) and the fact that Hg and Cd are not classified as carcinogens through dietary intake, the CR for these elements was not calculated in the present study. Therefore, both CR and TCR related to the exposure to As and Pb because of the consumption of *O. mykiss* are also given in Table 2. The CR values estimated for As were between the acceptability thresholds (10^{-4}-10^{-6}), which were found to be insignificant ($< 10^{-6}$) for Pb values (Table 2). According to the results obtained, As and Pb are not known to be carcinogenic and do not pose a risk to human health.

The THQs, CR and TCR findings of this study were like those obtained from other cultured fish *Sparus aurata* (Bat et al., 2022b) and *Dicentrarchus labrax* (Bat et al., 2022a) sampled from Samsun and Sinop markets.

CONCLUSION

The results indicated that the As, Pb, Hg, and Cd do not exceed the allowable levels. In terms of consumer health risks, the data suggest that for EWI and HI, the measurements are lower than the established safety values. Moreover, the CR fell below the threshold values. In other words, it was determined that the consumption of *O. mykiss* taken from Samsun Fish Market did not cause any health problems. Due to the increasing consumption and exportation of such a popular fish, it is recommended that monitoring studies be carried out continuously.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest reported in the manuscript.

Ethics approval

All authors followed the ethical responsibilities of this journal.

Animal Welfare Statement

This study was conducted in accordance with the Directive on the Protection of Animals Used for Scientific Purposes of the European Parliament and Council (Directive 2010/63/EU)

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