Acta Aquatica Turcica

E-ISSN: 2651-5474
Home Page: https://dergipark.org.tr/actaquatr
18(2): 208-216, 2022
DOI: 10.22392/actaquatr.1012944

Research Article **Araştırma Makalesi**

Heavy Metal Concentrations and Health Risk Assessment in *Sarda sarda* (Bloch, 1793) Caught in the Turkish Black Sea Coasts

Türkiye'nin Karadeniz Kıyılarında Yakalanan *Sarda sarda*'da (Bloch, 1793) Ağır Metal Konsantrasyonları ve Sağlık Riski Değerlendirmesi

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Received: 21.10.2021 **Accepted:** 22.11.2021 **Published:** 01.06.2022

How to Cite: Verep, B. & Mutlu, T. (2022). Heavy metal concentrations and health risk assessment in *Sarda sarda* (Bloch, 1793) caught in the Turkish Black Sea coasts. *Acta Aquatica Turcica, 18*(2), 208-216. https://doi.org/10.22392/actaquatr.1012944

Abstract: In this study, it was aimed to determine heavy metal levels in muscle tissues of Atlantic bonito samples caught in the Eastern Black Sea Region and to determine their potential to pose a risk to human health. The concentrations of Fe, Zn, Cu, Pb, Mn, and Al have been measured in the edible muscle of Atlantic bonito (*Sarda sarda* Bloch, 1793) from the Trabzon and Rize coasts of the southern Black Sea from September 2020 to December 2020. Cd, Cr, Co, and Ni were not detected in the edible part of *S. sarda* in both coastal areas. These concentrations are lower than the maximum permissible values in European and Turkish regulations. The average weekly intake of heavy metals per body weight value not exceeded the Provisional Tolerable Weekly Intake (PTWI) established. Therefore, it may be concluded that these ten metals should not pose any health threat to the consumers resulting from the consumption of *S. sarda*.

Keywords
- Heavy metals
- Rize ve Trabzon
- Black Sea
- Atlantic Bonito

Özet: Bu çalışmada, Doğu Karadeniz Bölgesinde yakalanan palamut örneklerinin kas dokularındaki ağır metal seviyelerinin belirlenmesi ve insan sağlığı açısından risk oluşturma potansiyellerinin belirlenmesi amaçlanmıştır. Karadeniz'in Trabzon ve Rize kıyılarında avlanan palamutun (*Sarda sarda* Bloch, 1793) yenilebilir kaslarında Fe, Zn, Cu, Pb, Mn ve Al konsantrasyonları ölçülmüştür. Eylül 2020'den Aralık 2020'ye kadar deniz. Her iki kıyı bölgesinde de *S. sarda*'nın yenilebilir kısmında Cd, Cr, Co ve Ni tespit edilmedi. Bu konsantrasyonlar, Avrupa ve Türkiye yönetmeliklerinde izin verilen maksimum değerlerden daha düşüktür. Vücut ağırlığı değerleri başına haftalık ortalama ağır metal alımı, belirlenen Geçici Tolere Edilebilir Haftalık Alım (PTWI) değerini aşmadı. Bu nedenle, *S. sarda* tüketiminden kaynaklanan bu 6 metalin tüketiciler için herhangi bir sağlık tehdidi oluşturmaması gerektiği sonucuna varılabilir.

Anahtar kelimeler
- Ağır metaller
- Rize ve Trabzon
- Karadeniz
- Palamut

1. INTRODUCTION

Heavy metals increase in aquatic environments due to industrial, domestic, and agricultural activities, and they have significant effects on living things due to their toxicity and accumulation feature (Dobaradaran et al., 2010; Akkan et al., 2018; Aktop and Çağatay, 2020; Mutlu, 2021a). Heavy metals mixed into the aquatic environment can not only affect aquatic organisms, but also human health by accumulating heavy metals in their bodies and reaching people through the food chain (Akgün et al., 2007; Yilmaz et al., 2016).

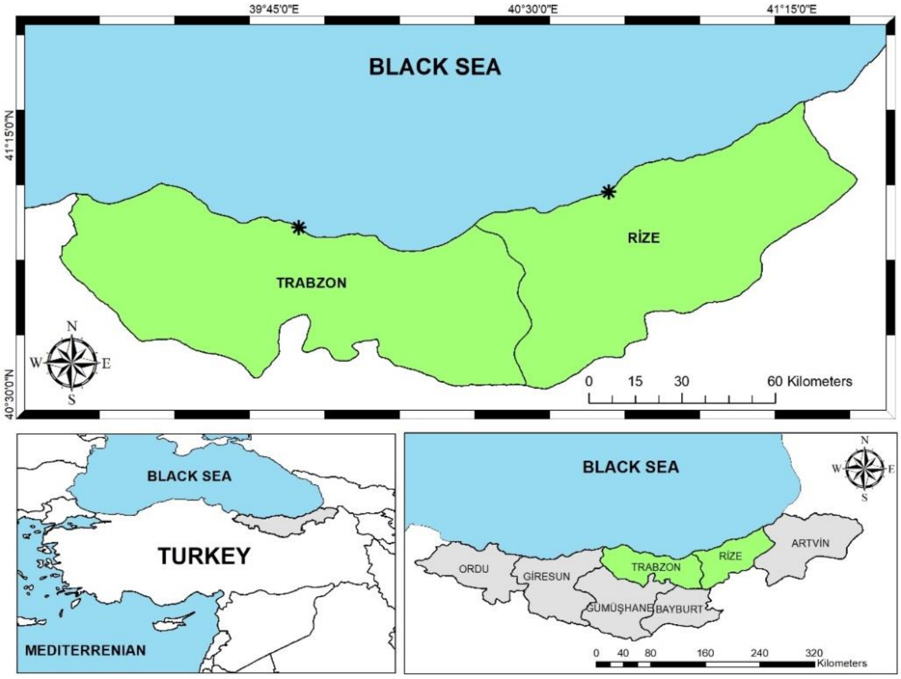

Figure 1. The study area (Trabzon and Rize coasts).

Although bivalves fed by filtering water are primarily used in monitoring heavy metal pollution in aquatic ecosystems, heavy metal research in fish located at the top of the food pyramid has been increasing in recent years since they are the main food sources of humans. There is a similar trend in heavy metal pollution research in the Black Sea region where the study was conducted. However, it is seen that research on bottom fish living close to the seabed or sediment layer has been intensified recently. On the other hand, it is necessary to monitor and reveal the risk status of pelagic species that migrate for food and reproduction. Because the Black Sea is under the effect of freshwater containing pollutant loads carried by many streams from the precipitation basin of 6 different countries surrounding it.

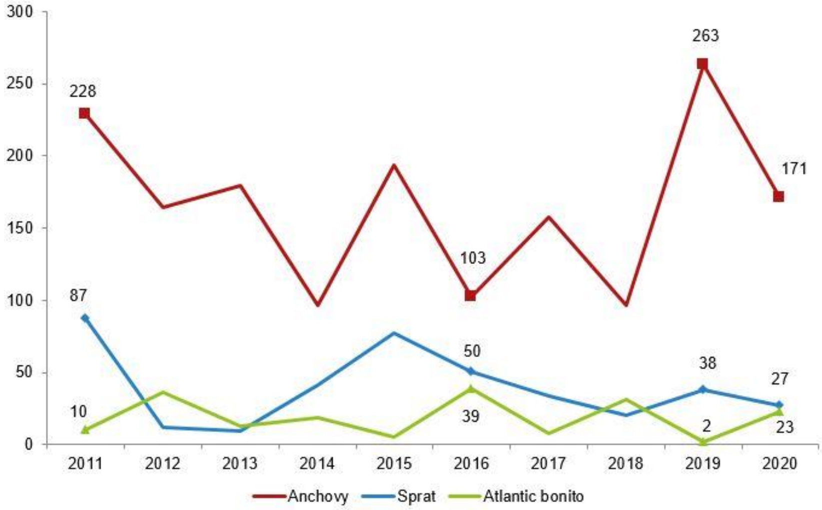

Figure 2. The most captured sea fish, 2011-2020 (Thousand tonnes) (TSI, 2020).

Atlantic bonito fish is a member of the Scombridae family (*Sarda sarda* Bloch, 1793) and is an epipelagic species. It is distributed in flocks along the Black Sea, Mediterranean, and Atlantic coasts (Collette and Nauen, 1983). This fish species, which is extremely important economically in Turkey, migrates between the Black Sea and the Aegean Sea for breeding and feeding purposes through the Dardanelles and Istanbul Straits (Nümann, 1955). The diet of bonito is formed by small fishes such as anchovies, sardines and sprat, and crustaceans (Campo et al., 2006; Kahraman et al., 2014). In the meantime, fishing is carried out intensively with seine nets, seine nets, and fishing rods. Bonito fish migrates from the Eastern Mediterranean to the Black Sea between May and July to spawn and then migrate to the same region again (Cengiz, 2013; Nümann, 1954). Turkey's waters in 2019 total 374 726 tonnes of sea fish are hunted that 1,578 tons of bonito fish (0.42%) (Figure 1).

It is clear that Atlantic bonito, which is located in the upper layers of the food pyramid in the Black Sea region, can play an important role in heavy metal bioaccumulation. Therefore, in this study, it will be an important issue to investigate heavy metal accumulation in bonito fish in terms of 2 important locations (Trabzon and Rize) on the Southeastern Black Sea coasts.

2. MATERIAL and METHODS

This study was carried out in September 2020 in Trabzon and Rize stations, which are the two most important provinces in terms of fishing in the Eastern Black Sea Region. Atlantic bonito used in this study were sampled from September 2020 to December 2020 directly from local fishing vessels in the southern of the Black Sea (Figure 1).

2.1. Preparation of fish samples before heavy metal analysis

The fish caught were labeled and brought to the laboratory in ice-protected containers the same day, and the length and weight of the samples were determined. The fish samples, which were then washed with distilled water, were dried on filter paper and stored in conditions below -20 °C until analysis after being packed in polyethylene bags.

2.2. ICP-OES Method

ICP is a high-temperature plasma technique, such as 7000-8000 °K, supported by a magnetic field, in which the elements in the sample are atomized and excited. Plasma is called gaseous ion current. In the ICP technique, plasma is created by interacting argon gas with the magnetic field created by the radiofrequency generator, since it is both inert and easily ionizable. ICP-OES is an optical emission spectroscopy technique that uses induction coupled plasma to excite atoms. It is thought that the ICP-OES technique is superior to other optical emission spectroscopy techniques due to its features such as reaching high temperatures, a long retention time of the sample element in the plasma, and atomization and excitation processes can be performed in an inert environment.

In this method, the first step consists of sample preparation. In the second step, the sample in solution is converted into aerosols with the help of a nebulizer. In the third step, the solvent of the sample coming to the plasma is removed and the substances are passed into the gas phase. In the fourth step, substances in the gas phase are separated into atoms and free atoms are obtained in the gas phase. In the fifth step, the gaseous atoms become excited in the plasma, and after a short time, they return to the ground state by emitting a resonance beam. In the last step, the resonance beam formed is detected by the detectors and the measurement is performed. In recent years, the ICP-OES system has been frequently preferred in the metal analysis due to its various advantages. These advantages are; It is possible to reach high temperatures, the retention time of the sample element in the plasma is long, atomization and excitation processes can be carried out in an inert environment, and even very stable compounds can be separated into atoms thanks to the high temperature obtained in the plasma.

In this study, heavy metal analysis in fish was performed using ICP/OES (Inductively Coupled Plasma – Optical Emission Spectrometer) method by Perkin Elmer Optima 7000 DV. EN 15763

European Standard methods were applied. The limits of detection (µg/l) used for analysis of Fe, Zn, Cu, Pb, Mn and Al were 0.5, 0.15, 0.5, 0.5, 0.02, 0.05, 0.78, 0.58, 0.166, 0.5 and 0.05, respectively.

The certified and measured values of the Reference material used to control the accuracy of the analysis process in the measurements performed in the ICP-OES system in this study are presented in Table 1. Certified reference material (DORM-4; National Research Council Canada, NRCC) was analyzed (n=3) to validate the method for accuracy. It is a fish protein reference material for trace element analysis in fish. While the accuracy of the analysis processes is given separately for each metal, it is seen that it is 89.24 percent on average for all elements (Table 1).

Table 1. Certified, observed values (mg.kg-1) and recoveries (%) of trace metal concentrations in standard reference material for this research.

Metals	**Reference Material Metal concentration (mg/kg) (Dry weight)**		
	Certified	**Observed**	**Recovery (%)**
Cu	154.61	141.02	91.21
Pb	150.55	131.22	87.16
Zn	352.90	315.90	89.52
Fe	2.72	2.50	91.91
Al	1.03	0.86	83.50
Mean			89.24

2.3. Intake Levels Calculation

The average heavy metal weekly intake was estimated according to the following formula (Bat and Arıcı, 2016; Türkmen et al., 2009; Tüzen, 2009):

Heavy metals intake level = average heavy metal content X consumption of fish per person/ body weight

EWI (estimated weekly intake level) values were calculated by multiplying the mean concentrations of each element and the amount of weekly consumed fish. The annual quantity of fish consumed is 6.3 kg/person [TUIK, 2019], which is equivalent to 17.3 g/day for Turkey. The body weight of an adult person is 70 kg.

The health risks for Turkish consumers caused by consuming Atlantic bonito from the Southwest Black Sea were assessed based on THQ. This method offers an indication of the risk level due to the heavy metal exposure. The following equation was used to determine THQ (Han et al.,1998; Chien et al., 2002; Storelli, 2011; Mol et. al., 2018; Mutlu 2021b):

$$THQ = [(EF \times ED \times FIR \times C) / (RFD \times WAB \times TA)] \times 10^{-3}$$

Where EF is the exposure frequency (365 days/year), ED is the exposure duration (70 years, the average lifetime according to Bennett, Kastenberg, & McKone, (1999). FIR is the food ingestion rate (17.3 g/day for Turkish consumers, according to Speedy (2003), C is the determined metal concentration (mg.kg^{-1}), RFD is the oral reference dose (mg.kg^{-1}/day), WAB is the average body weight (70 kg), according to Kumar, Verma, Naskar, Chakraborty, & Shah, (2013), TA is the average exposure time for noncarcinogens (365 days/year x ED, assuming 70 years in this study). The oral reference doses (RFD) for Cu, Zn, Cd, Hg, and Pb (mg.kg^{-1}, day) have been suggested as 4×10^{-2}, 3×10^{-1}, 1×10^{-3}, $1.6 \times 10^{-4,}$ and 4×10^{-3}, respectively (US EPA, 2009).

2.4. Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Data were analyzed by ANOVA at α= 0.05. Comparison of means was performed by Duncan test and the difference was considered significant at $p < 0.05$ [20]. IBM SPSS Statistics version 21 software is used for statistical analysis.

3. RESULTS and DISCUSSION

The Black Sea Atlantic bonito from the south coast of the Black Sea, from September 2020 to December 2020 were analyzed for Fe, Zn, Cu, Pb, Mn, and Al. The mean concentrations and ranges of Fe, Zn, Cu, Pb, Mn, and Al in fish have been presented in Figure 2. The EWI and PTWI values of Atlantic bonito were calculated while THQ values were shown in Table 3. These values were calculated for Turkish consumers.

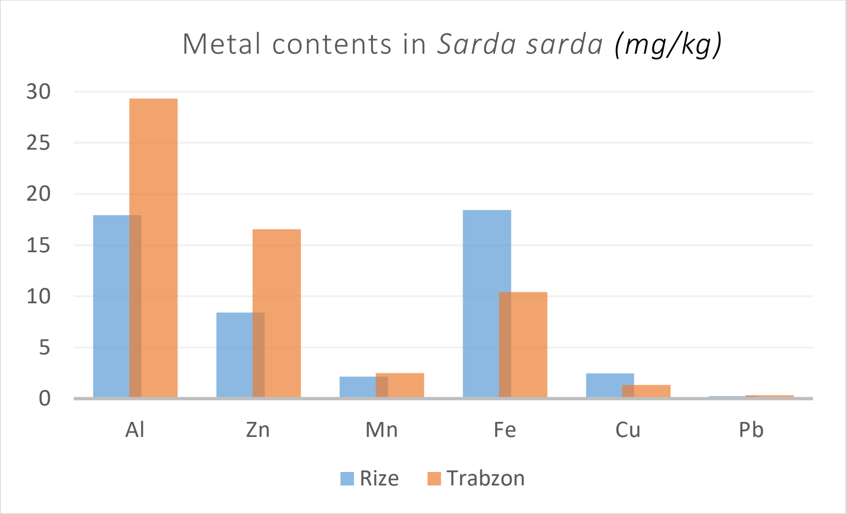

Figure 3. Comparison of the results between Trabzon and Rize stations.

The concentration of examined metals in edible tissues of Atlantic bonito from the southern Black Sea demonstrated regional differences between Trabzon and Rize stations. Mn, Pb, and Cu were detected at low concentrations at similar levels at both stations in the edible part of *S. sarda* in both coastal areas. Figures 3 shows the mean concentrations of heavy metals in the muscle of Atlantic bonito from the Trabzon and Rize coasts of the southern Black Sea, Turkey. The concentrations of measured heavy metals decrease in the order of Pb > Cu > Mn > Zn > Fe > Al in all stations.

In this study, the results indicate that there is a considerable greater accumulation of the metals in muscle tissues of *S. sarda* from Trabzon than those in muscle tissues of fish from Rize coast ($P<0.05$) and that there was a statistically significant difference between the concentrations of the metals (Table 2). On the other hand, when the metal levels accumulating in muscle tissue are evaluated in terms of permissible limits according to international standards, it is seen that the permissible limit values for Pb and Mn are exceeded (Table 2) (Suyatna et al., 2017). For other metals, it is seen that the metal levels in the muscle tissue do not exceed the permissible limit values in terms of Fe, Cu, and Zn. Also, THQ values are given in Table 3. If THQ <1, it means that the consumption of the studied fish species has no adverse effects on human health in light of the current concentrations in the fish (Malakootian et al., 2016).

Table 2. Metal concentration in Atlantic bonito (mg/kg).

Metals	Metal concentrations in *S. sarda*		
	Trabzon	**Rize**	**Permissible limits (mg.kg^{-1})**
Pb	0.33±0.10	0.25±0.18	0.20-0.50
Cu	1.34±0.35	2.46±0.56	<20
Mn	2.49±1.11	2.14±0.75	1.00
Zn	16.56±8.26	8.40±2.69	40
Fe	10.41±10.11	18.42±2.53	100
Al	29.33±26.77	17.94±7.36	-

When the results of this research on heavy metal accumulation in bonito muscle tissues were examined, it was determined that while Pb and Mn exceeded the permissible limit values published by international organizations, this was not the case in terms of Cu, Fe, Zn, and Al values. Although there are not many studies in terms of aluminum, there are many studies on Pb and Mn. While the average Pb accumulation measured in this study was 0.29 ppm, many studies conducted in Turkey and especially in the Black Sea coast yielded results between 0.22 ppm and 0.76 ppm (Tüzen 2003, Uluözlü et al. 2007, Mendil et al., 2010, and Bat et al., 2006). It is seen that in another study on the same species on the Black Sea coast, the results of this study were considerably higher than the results of this study (Bat et al.2016). Therefore, it is seen that the permissible limit values for international standards (WHO, FAO, and EC) are exceeded in most of the studies in the current reviewed literature (Table 4).

Table 3. Target hazard quotient (THQ) of *Sarda sarda* (ug/kg/bw/day) for the adult.

Metals	Rfd	THQ	
		Rize	**Trabzon**
Cu	40	0.018	0.010
Fe	700	0.008	0.004
Mn	140	0.004	0.005
Pb	4	0.018	0.024
Zn	300	0.008	0.016
TTHQ		**0.055**	**0.058**

Table 4. Literature data and researches on the metal concentration in Atlantic bonito.

Metals	References	Mean metal concentrations (mg /kg)	Statiton
Al	This study	23,63	Trabzon, Rize
	Bat and Arıcı (2016)	ND	Samsun, Sinop
Zn	This study	12,48	Trabzon, Rize
	Tüzen (2003)	11.20±1.44 (dry wt.)	Samsun
	Uluozlu et al. (2007)	48.7 ± 3.7 (dry wt.)	Black Sea
	Mendil et al. (2010)	21.0 ± 2.1 (dry wt.)	Samsun, Ordu, Trabzon, Rize
	Bat et al. (2006)	12.66 (wet wt.)	Sinop
Mn	This study	2,32	Trabzon, Rize
	Tüzen (2003)	1.06±0.27 (dry wt.)	Samsun
	Uluozlu et al. (2007)	2.68 ± 0.22 (dry wt.)	Black Sea
	Mendil et al. (2010)	2.0 ± 0.2 (dry wt.)	Samsun, Ordu, Trabzon, Rize
	Bat et al. (2006)	1.72 (wet wt.)	Sinop
Fe	This study	14,41	Trabzon, Rize
	Tüzen (2003)	9.52±0.81 (dry wt.)	Samsun
	Uluozlu et al. (2007)	73.5 ± 6.3 (dry wt.)	Black Sea
	Mendil et al. (2010)	25.5 ± 2.3 (dry wt.)	Samsun, Ordu, Trabzon, Rize
	Bat et al. (2006)	12.18 (wet wt.)	Sinop
Cu	This study	1,90	Trabzon, Rize
	Tüzen (2003)	1.28±0.14 (dry wt.)	Samsun
	Uluozlu et al. (2007)	0.84 ± 0.05 (dry wt.)	Black Sea
	Mendil et al. (2010)	1.9 ± 0.2 (dry wt.)	Samsun, Ordu, Trabzon, Rize
	Bat et al. (2006)	0.659 (wet wt.)	Sinop
Pb	This study	0,29	Trabzon, Rize
	Tüzen (2003)	0.22±0.04 (dry wt.)	Samsun
	Uluozlu et al. (2007)	0.76 ± 0.05 (dry wt.)	Black Sea
	Mendil et al. (2010)	0.28 ± 0.03 (dry wt.)	Samsun, Ordu, Trabzon, Rize
	Bat et al. (2006)	0.537 (wet wt.)	Sinop

According to the results of this study, when Mn metal values are compared with other studies, it is seen that the values vary between 1.72 ppm and 2.58 ppm and the results are almost 2 times higher than the allowable limit values (Tüzen 2003, Uluözlü et al. 2007, Mendil et al., 2010, and Bat et al., 2006). Although the results of Pb and Mn are high, when the previous studies are evaluated in terms of other metals (Cu, Fe, and Zn) discussed in this study, it is seen that the results are well below the allowable limit values in all the studies examined (Table 3) (Tüzen 2003, Uluözlü et al. 2007, Mendil et al., 2010, and Bat et al., 2006).

FUNDING

No financial support was received in the conduct of this study.

CONFLICT of INTEREST

The authors declare that there are no financial interests or personal relationships that could affect this work.

AUTHOR CONTRIBUTIONS

Editing: BV; Methodology: TM; Performing the experiment: BV, TM; Data analysis: TM; Article writing: BV; Supervision: BV. All authors approved the final draft.

ETHICAL STATEMENTS

Local Ethics Committee Approval was not obtained because experimental animals were not used in this study.

DATA AVAILABILITY STATEMENT

Data used in this study are available from the corresponding author upon reasonable request.

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