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The Evaluation of Heavy Metal Accumulation in Whiting Fish (*Merlanguis merlangus euxinus* Nordmann, 1840), a Local and Economic Species of the Central Black Sea Region

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ABSTRACT

The present study aimed to determine the heavy metal cadmium (Cd), chromium (Cr), nickel (Ni), lead (Pb) and mercury (Hg) accumulations in muscle tissues, gills, gonads, liver, and other visceral organs (liver) of whiting fish (*Merlanguis merlangus euxinus* Nordmann, 1840) samples obtained by hunting from the Sinop (Black Sea) coasts. The mean cadmium value in muscle tissue, which concerns public health, was found to be 1.07±0.02 $\mu g.kg^{-1}$, the chromium value was found to be 1.48±0.07 $\mu g.kg^{-1}$, the nickel value was found to be 1.77±0.14 $\mu g.kg^{-1}$, the lead value was found to be 5.38±0.29 $\mu g.kg^{-1}$ while the mercury value was determined only in the gill and could not be determined in organs, and since it was below the limit values declared by international organizations for all metals, it was determined that it would not create adverse effects for human consumption. Also, examining the order of accumulation of heavy metals in tissues and organs from highest to lowest, it was sorted as liver> visceral organ> gill> muscle> gonad for cadmium; visceral organ> gill> muscle> liver> gonad for chromium; visceral organ> gill> liver> muscle> gonad for nickel; gill> visceral organ> liver> muscle> gonad for lead, while the mercury accumulation was detected only in the gill. Also, the data obtained as a result of the analyses made in the gonad is an important determination in terms of the effects of heavy metals on the reproduction of the species, as well as on the continuity of the population. Since the values determined in the other analyzed tissues were below the limit values reported by international organizations, the results showed that they do not pose any threat to human health in terms of workplace and time, type, and determined heavy metals.

Türk Tarım – Gıda Bilim ve Teknoloji Dergisi, 9(4): 683-688, 2021

Orta Karadeniz Bölgesi Yerel ve Ekonomik Bir Tür olan Mezgit Balığındaki (Merlanguis merlangus euxinus Nordmann, 1840) Ağır Metal Birikiminin Değerlendirilmesi

MAKALE BİLGİSİ

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ÖZ

Çalışmada, Sinop (Karadeniz) kıyılarından avcılık yolu ile elde edilen mezgit balığı (*Merlanguis merlangus euxinus* Nordmann, 1840) örneklerinin kas dokusu, solungaç, gonad, karaciğer ve diğer içorganlarda (karaciğer) ağır metallerden kadmiyum (Cd), krom (Cr), nikel (Ni), kurşun (Pb) ve cıvanın (Hg) birikim konsantrasyonlarının belirlenmesi amaçlanmıştır. Çalışmada halk sağlığını ilgilendiren bölüm olan kas dokuda ortalama kadmiyum değerinin 1,07±0,02 $\mu g.kg^{-1}$, kromun 1,48±0,07 $\mu g.kg^{-1}$, nikelin 1,77±0,14 $\mu g.kg^{-1}$, kurşunun 5,38±0,29 $\mu g.kg^{-1}$, cıvanın ise sadece solungaçta belirlendiği onun haricindeki organlarda ise belirlenemediği, tüm metallerde uluslararası kuruluşlar tarafından bildirilen sınır değerlerinin altında olduğundan insan tüketimi için olumsuzluk oluşturmayacağı belirlenmiştir. Ayrıca çalışmada, ağır metallerin doku ve organlarda birikim sıralamaları incelendiğinde, kadmiyumda karaciğer> içorgan> solungaç> kas> gonad; kromda içorgan> solungaç> kas> karaciğer> gonad; nikelde içorgan> solungaç> karaciğer> kas> gonad; kurşunda solungaç> içorgan> karaciğer> kas> gonad, cıvada ise sadece solungaçta olduğu tespit edilmiştir. Ayrıca gonad organında yapılan analizler sonucunda elde edilen veriler ağır metallerin türün üremesine aynı zamanda popülasyon devamlılığındaki etkileri bakımından önemli bir tespit olmuştur. Analizi yapılan diğer dokularda belirlenen değerler, uluslararası kuruluşlar tarafından bildirilen sınır değerlerinin altında olduğundan, çalışma yeri ve zamanı, tür ve belirlenen ağır metaller açısından insan sağlığı üzerine şimdilik herhangi bir tehdit oluşturmadığını göstermiştir.

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Introduction

The Black Sea has historically been one of the most biological productive regions in the world. According to investigations, these biological speciality losses year by year with the effects of pollution in Black Sea (Bat et al., 2009). It is known that heavy metals originating from various sources also have important effects on the Black Sea ecosystem. Heavy metals are natural trace components of the marine environment, but their levels have increased due to domestic, industrial, mining and agricultural activities (Bakan and Büyükgüngör, 2000; Altas and Büyükgüngör, 2007; Bat et al., 2012). The pollution levels of the aquatic environment by heavy metals can be estimated by analyzing water, sediments and marine organisms. The levels of heavy metals in marine organisms are often higher than in other constituents of marine environment because their ability to concentrate heavy metals from their habitat and it is important to know the changes in metal levels in marine ecosystem (Bat et al., 1998).

Kizilirmak and Yesilirmak, the two most important rivers of Black Sea Region, and a lot of big and little industries (food, cement, fertilizer, pesticides, resin, plastic, textile, cigarette manufacturing) exist in the Middle Black Sea Region of Turkey. Most of these factories have no treatment plant and they have potential to create local pollution problem (Altaş and Büyükgüngör, 2007). The two important iron and steel factories of Turkey exist in the western part of the Black Sea region. On the other hand, the eastern part of the Black Sea Region has no important industrial factories, but only hazelnut facilities, floor manufacturing and fish-oil factories. Besides small industrial activities, pulp and paper factory present in this region is one of the important industries. Heavy metals in marine environment causes by especially discharge of industrial pollutants (Bat et al., 2009).

Pollutants from metals and other wastes are of primary importance among the chemical pollutants since they can be from a wide variety of sources, causing widespread pollution, are resistant to environmental conditions, always have an effect on biological systems, and can easily enter the food chain and accumulate in living creatures (Tepe et al., 2006; Türkmen, 2011; Mutlu, 2019). The public health problems that arise when people are exposed to certain chemicals and especially heavy metals are increasing day by day. Heavy metals such as Hg, Pb, Cd, Co are toxic metals. As organisms continue their development in natural environments where these substances are scarce, they do not have a mechanism to eliminate their toxic effects (Parlak, 1985; Türkmen, 2011; Uncumusaoğlu and Mutlu, 2019). In aquatic organisms that form a ring of the biological cycle and are also consumed as an important protein source (fish, crustaceans, algae), the effects of increasing heavy metal pollution must be investigated, the ecological balance must be preserved and the level of contaminants contained in these organisms for the consumer must be determined and measures must be taken on time according to the results (Türkmen, 2011; Mutlu and Uncumusaoğlu, 2017; Mutlu and Uncumusaoğlu, 2018). Especially heavy metals such as cadmium (Cd), mercury (Hg), lead (Pb), and chromium (Cr) tend to accumulate as they cannot be excreted by natural physiological mechanisms in living bodies that they enter through food chains and show toxic effects when they exceed certain limit values in the organism (Vural, 1996; Yılmaz Bayrak, 2016).

In the present study, heavy metals cadmium (Cd), chromium (Cr), nickel (Ni), lead (Pb), and mercury (Hg) accumulation concentrations were determined in muscle tissue, gonad, liver, other visceral organs, and gills of whiting fish (*Merlanguis merlangus euxinus*) samples obtained by hunting from the Sinop coasts.

Materials and Method

The present study was carried out in Sinop coasts (in the Black Sea), located in the north of Turkey (Figure 1). Whiting fish was obtained from fishermen who hunt on the shores of Sinop in September-October-November-December in 2012 and January-February-March-April-May-June-July and August in 2013.

Whiting is a local species of the Black Sea and is an economical species that are commonly consumed by the people of the region (Figure 2). The present study aimed to determine the heavy metal accumulation concentrations in different tissues and organs of fish. The whiting fish selected for the study is a species that can be found in any season, and it was preferred because it would be an important pollution indicator since it is a sea bottom fish.

Collection of Samples and Preparation for Analysis

Whiting fish samples (n=1450) were obtained monthly from Sinop (Black Sea) coasts between September 2012 and August 2013. After their biometric data were obtained, the samples of muscle, gill, gonad, visceral organ (except for liver), and liver of each month were removed. All the separated tissues and organs were mixed and homogenized and packed in plastic bags in three replications and stored in a deep freezer (-20°C) until the time of analysis. After the samples of fish tissue and organs were washed and cleaned, their weights were measured. To determine the heavy metal concentrations in the muscle tissues which is especially the consumed part of the fish, reproductive organ, gonad, liver which include findings on the metabolic functions of fish, visceral organs (except for liver) and gills, cadmium (Cd), chromium (Cr), nickel (Ni), lead (Pb) and mercury (Hg) analyses were carried out.

Heavy Metal Analyses

The samples were first ground sterile in an Isolab grinder and homogenized. The samples were incinerated using a Microwave Combustion System (Milestone Ethos). Then, 0.5 g of the sample was weighed and 10 ml HNO_3 (67%v/v) was added. The temperature program has been set for incineration by tightly closing the microwave containers. According to the temperature program procedure determined for the sample to be analyzed, the temperature was raised to 200ºC for the first 15 minutes at 45 bar pressure, then it was kept constant at 200ºC for 15 minutes. At the end of the process, the solutions were left to cool to room temperature. Then, the sample solutions were completed with ultra-pure water.

The multi-element standard stock solution (Merck, Germany) provided for ICP-OES was used in the preparation of calibration standards. The standard stock solution differs according to the target elements. The standard solution suitable for analysis was chosen. The prepared samples and calibration solutions were analyzed in the SpectroBlue brand ICP-OES device. Metal concentrations were measured in three replicates using the ICP-OES device, then the mean values and the standard deviations of these measurements were calculated.

Statistical Analysis

The differences between heavy metal concentration values of different tissues and organs were evaluated by one-way ANOVA and Duncan multiple comparison tests. Also, the relationship levels between heavy metal concentrations of whiting fish were evaluated by the correlation analysis. The IBM SPSS 21 statistics software was used for statistical calculations. All data are given as mean ± standard error. The level of significance was taken as $P<0.05$.

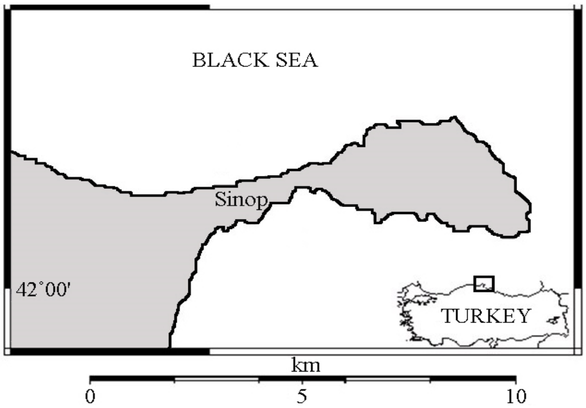

Figure 1. The image of the sampling area

Figure 2. Whiting fish (*Merlanguis merlangus euxinus*) and the organs of fish used in the study.

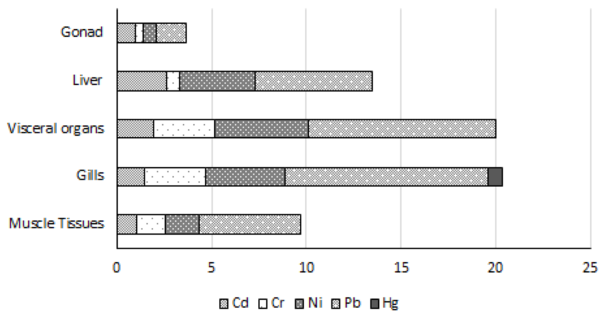

Figure 3. The mean Cd, Cr, Ni, Pb, Hg values in tissues and organs ($\mu g.kg^{-1}$).

Results and Discussion

Heavy Metal Values of Fish Tissues and Organs

The heavy metal (Cd, Cr, Ni, Pb, Hg) analysis results determined in different tissues and organs of whiting in the study are given in Table 1 and Figure 3.

Examining the results of the heavy metal analysis in tissues and organs of haddock fish, it was found that cadmium concentration was 1.07±0.02 $\mu g.kg^{-1}$ in the muscle tissues, 1.43±0.02 $\mu g.kg^{-1}$ in the gill, 1.94±0.03 $\mu g.kg^{-1}$ in visceral organs, 2.65±0.54 $\mu g.kg^{-1}$ in liver, and 0.94±0.05 $\mu g.kg^{-1}$ in gonad (Figure 4). Examining the average values of cadmium accumulation of haddock, it was determined that the order was liver> visceral> gill> muscle> gonad. Also, it was determined that the cadmium value (1.07±0.02 $\mu g.kg^{-1}$) determined in muscle tissue was below the limit values declared by international organizations, therefore it will not create adverse effects for humans' consumption.

The mean chromium concentration was 1.48±0.07 $\mu g.kg^{-1}$ in muscle tissue, 3.23±0.19 $\mu g.kg^{-1}$ in the gill, 3.25±0.11 $\mu g.kg^{-1}$ in visceral organs, 0.68±0.10 $\mu g.kg^{-1}$ in the liver, and 0.42±0.19 $\mu g.kg^{-1}$ in the gonad (Figure 5).

Examining the mean values, it was determined that the chromium accumulation order was visceral organ> gill> muscle> liver> gonad. Also, it was determined that the chromium value (1.48±0.07 μgkg^{-1}) determined in muscle tissue was below the limit values reported by international organizations, so it would not cause adverse effects for human consumption.

The mean nickel value in the muscle tissue was found to be 1.77±0.14 $\mu g.kg^{-1}$; 4.21±0.09 $\mu g.kg^{-1}$ in the gill, 4.95±0.27 $\mu g.kg^{-1}$ in visceral organs, 3.99±0.96 $\mu g.kg^{-1}$ in liver and 0.69±0.31 $\mu g.kg^{-1}$ in gonads (Figure 6). In the present study, it was determined that the nickel accumulation order was visceral organ> gill> liver> muscle> gonad. Also, it was determined that the nickel value (1.77±0.14 $\mu g.kg^{-1}$) determined in muscle tissue was below the limit values declared by international organizations, therefore it will not cause adverse effects for human consumption.

The mean lead value in the muscle tissue was 5.38±0.29 $\mu g.kg^{-1}$, 10.72±0.29 $\mu g.kg^{-1}$ in the gill, 9.88±0.20 $\mu g.kg^{-1}$ in visceral organs, 6.16±0.34 $\mu g.kg^{-1}$ in the liver, and 1.57±0.65 $\mu g.kg^{-1}$ in the gonad (Figure 7).

It was determined that the lead accumulation order was gill> visceral organ> liver> muscle> gonad. Also, it was determined that the lead value (5.38±0.29 $\mu g.kg^{-1}$) determined in the muscle tissue was below the limit values declared by international organizations, so it will not cause adverse effects for human consumption.

It was determined that mercury was undetectable in the muscle tissues, visceral organs, liver, and gonads whereas it was determined to be 0.75±2.42 $\mu g.kg^{-1}$ in the gills.

Correlation Analysis of Heavy Metal Values in Fish Tissues and Organs

The heavy metal values (Cd, Cr, Ni, Pb) determined as a result of the analyses performed in different tissues and organs (muscle, gill, visceral organs, liver, gonad) of the whiting fish were evaluated by the correlation analysis and the formed matrix is given in Table 2.

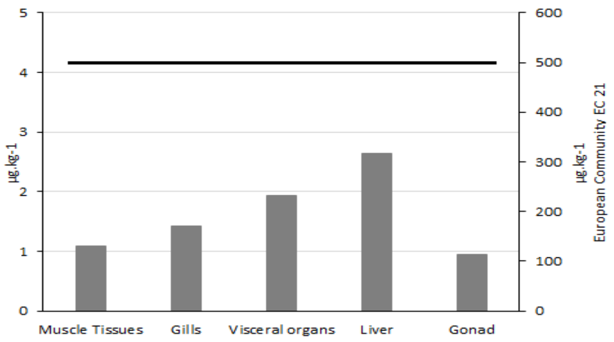

Figure 4. Distribution of Cd concentration in organs

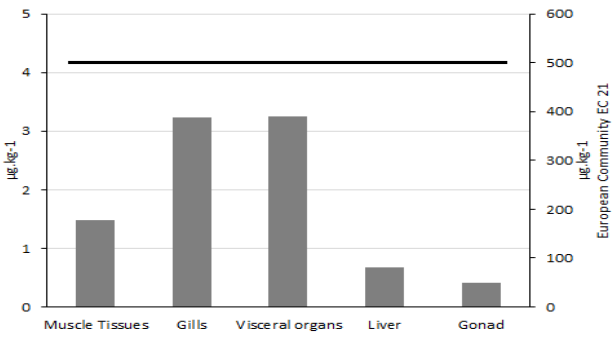

Figure 5. Distribution of Cr concentration in organs

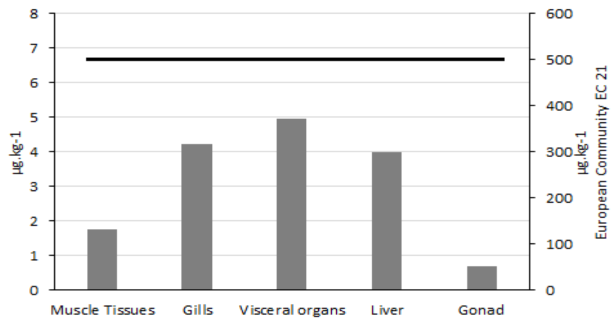

Figure 6. Distribution of Ni concentration in organs

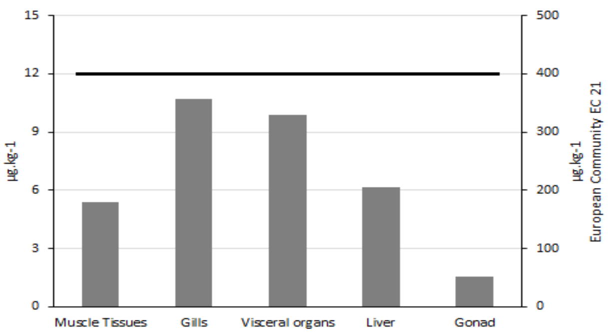

Figure 7. Distribution of Pb concentration in organs

Examining Table 2, it was determined that there were no relationships between cadmium and chromium ($P>0.05$), a weak relationship with lead, and a strong relationship with nickel ($P<0.05$). A strong correlation was found between chromium and nickel, and a very strong correlation with lead ($P<0.05$). There was a strong relationship between nickel and lead ($P<0.05$). In the study, it was seen that the highest correlation levels were between chromium and lead (0.926).

As a result of the correlation analysis performed on the consumed muscle tissue, a very strong (0.879) relationship was found between cadmium and nickel (0.6) whereas a weak or very weak relationship was found in other analyses.

Conclusion

The present study aimed to determine to what extent the tissues and organs of the haddock, which is a local species and is among the most consumed fish, are affected by heavy metal pollution. Accordingly, heavy metal accumulations in the muscle tissue, gonad, liver, other visceral organs, and gills of fish were examined. In the study, the accumulation in other vital organs of the fish besides the consumed muscle tissue was determined. It was also determined in which tissues and organs the heavy metals accumulated more. Also, the data obtained as a result of the analysis of the gonads of whiting fish was an important determination in terms of the effects of heavy metal accumulations on the reproduction of the species, as well as on the continuity of the population.

Heavy metal toxicity, albeit depending on the dose, the route of exposure, and the type of heavy metal, may change due to various factors such as the age, gender, genetics, and nutritional status of the living thing exposed. Due to their high toxicity, cadmium (Cd), chromium (Cr), lead (Pb), and mercury (Hg) is among the priority heavy metals that are important for the health of humans and other living things. These metallic elements are considered to be systemic toxic substances since they cause multiple organ damages even at low levels of exposure. The accumulation of heavy metals in the ecosystem (water-soil-plant-animal) causes a higher level of toxicity and undesirable consequences for living organisms (Bogut et al., 2000; Piskorova et al., 2003) Heavy metals are classified as vital and non-vital according to their degree of participation in biological processes. Those that are defined as vital must be present in certain concentrations in the structure of the organism, and since these metals participate in biological reactions, they must be regularly ingested through foods. For example, copper is an indispensable part of red blood cells and many oxidation and reduction processes in animals and humans (Bigersson et al., 1988). On the other hand, non-vital heavy metals can cause health problems by affecting the psychological structure even in the case of exposures at very low concentrations (Duffus et al., 1996).

Parsons (1999), in their study on many fish species with different trophic levels and habitats in the food chain, determined that the benthic species contained higher levels of metals than the pelagic species. It has been known that a significant part of the metal in the water is concentrated in suspended particles and bottom sediments (Kirby et al., 2001; Kalay et al., 2004). The measurement of high metal concentrations in crustacean species fed by bottom filtration is explained by the metal content in the sediment (Topçuoğlu et al., 2002). Therefore, whiting fish, which is a benthic species, was chosen as the subject in the study.

In many studies on the accumulation of heavy metals in the tissues and organs of fish, it was observed that the highest metal accumulation was in the liver and gill tissues whereas the lowest was in the muscle tissues (Köse, 2007; Yılmaz and Doğan 2007; Erdoğrul and Erbilir, 2007; Tekin-Özan and Kır, 2008; Türkoğlu, 2008; Alhas et al., 2009; Mol et al., 2010; Kır and Tumantozlu, 2012). Since the edible part of fish is muscle tissue, toxic metals accumulated in this tissue can easily pass to humans.

Table 1. Cd, Cr, Ni, Pb, Hg values in tissues and organs ($\mu g.kg^{-1}$)

Tissue/Organs	Cd	Cr	Ni	Pb	Hg
Muscle Tissues	$1.07 \pm 0.02^{b,v}$	$1.48 \pm 0.07^{c,v,y}$	$1.77 \pm 0.14^{b,y}$	$5.38 \pm 0.29^{b,z}$	ND
Gills	$1.43 \pm 0.02^{c,u}$	$3.23 \pm 0.19^{d,v}$	$4.21 \pm 0.09^{c,y}$	$10.72 \pm 0.29^{c,z}$	0.75 ± 2.42^{t}
Visceral Organs	$1.94 \pm 0.03^{d,u}$	$3.25 \pm 0.11^{d,v}$	$4.95 \pm 0.27^{c,y}$	$9.88 \pm 0.20^{c,z}$	ND
Liver	$2.65 \pm 0.54^{e,y}$	$0.68 \pm 0.10^{b,v}$	$3.99 \pm 0.96^{c,y}$	$6.16 \pm 0.34^{b,z}$	ND
Gonad	$0.94 \pm 0.05^{a,z}$	$0.42 \pm 0.19^{a,y}$	$0.69 \pm 0.31^{a,z}$	$1.57 \pm 0.65^{a,z}$	ND

*There is a statistical difference between the values expressed with different letters in the same column (a, b, c) and the same row (v, y, z) ($P<0.05$). ND: Not Detected

Table 2. Heavy metal values correlation analysis

Heavy metals	Cd	Cr	Ni	Pb
Cd	*			
Cr	0.054^{b}	*		
Ni	0.794^{a}	0.621^{a}	*	
Pb	0.336^{a}	0.926^{a}	0.792^{a}	*

$^{a}P<0.05$; $^{b}P>0,05$

A similar result was obtained in the present study and it was determined that the highest accumulation of mercury, cadmium, lead, and chromium was in the gills, while nickel was the most accumulated in the liver. Therefore, various organizations such as the Food and Agriculture Organization of the United Nations (FAO), the World Health Organization (WHO), the Environmental Protection Agency (EPA), and the organizations responsible for the food affairs of countries such as the Turkish Food Codex (TGK) determined the limit values of some toxic metals in fish muscles (Kayhan et al., 2009 from Tokatlı et al., 2016). Among the other studies on whiting fish and especially fish sampled from the shores of Sinop, Bat et al. (2006) have reported Pb and Cd concentrations as 0.44 and 0.087 $\mu g.gr^{-1}$, respectively, for the same species collected the shores of Sinop. Bat et al. (2013), determined the Pb and Cd concentrations in whiting fish to be 0.02 $\mu g.gr^{-1}$ and 0.08-0.18 $\mu g.gr^{-1}$, respectively. Nispet et al. (2010), in some fish species sampled from the shores of the Central Black Sea Region (Turkey), determined the seasonal changes in metal concentration in the whole fish and reported the mean Cd, Ni, and Pb concentrations ($\mu g.g^{-1}$) in whiting fish as 0.002, 3.78, and 0.58, respectively whereas stated that the Hg concentration was undetectable. Niemiec et al. (2019) in their study on the concentrations of Cd, Ni, Pb, and Cr in whiting fish ($mg.kg^{-1}$) in Balaklava Bay and Karantina Bay (Black Sea) have reported the Cd, Ni, Pb, and Cr values as 0.369-0.595, 6.958-11.22, 1.050-1.488 and 5.331-5.578 in the muscle tissues, 0.524-0.852, 7.261-9.545, 1.281-1.585, and 5.849-6.284 in the gills, 2.236-3.326, 5.162-5.231, 1.235-1.445 and 2.896-3.125 in the liver, respectively. The researchers argued that heavy metal pollution occurred due to anthropogenic reasons, and the highest concentration was determined for Ni accumulated in the liver, gonad, muscle, and gills. Compared to the aforementioned study, as seen in Figure 3, Pb was the metal determined in the highest concentration in muscle, gill, liver, visceral organ, and gonad. Examining the results obtained in the present study, it was found that the highest accumulation of Mercury, cadmium, lead, and chromium metals were in the gills, while nickel was the most accumulated in the liver. According to the regulations determined by EC (2005), the Pb limit value is 0.2 $mg.kg^{-1}$, the Cd limit value is 0.05 $mg.kg^{-1}$ and the Hg limit value is 0.5 $mg.kg^{-1}$ in all fish and muscle tissues. Also, the study data were compared with the regulations of the Turkish Food Codex (TFC, 2008). The maximum limit values in this regulation for Pb, Cd and Hg are 0.3 $mg.kg^{-1}$, 0.05 $mg.kg^{-1}$, and 0.5 $mg.kg^{-1}$, respectively. According to TFC (2008), it was stated that the limit values are given when the fish is consumed as a whole. The Cd, Cr, Ni, Hg, and Pb levels in the whiting samples in the muscle, gill, liver, other visceral organs, and gonads were found to be lower than the limits of EC (2005) and Turkish Food Codex (2008).

The results obtained from whiting fish, which is an economically important and common seabottom fish, showed that the coasts of Sinop have not reached dangerous levels in terms of heavy metal pollution.

Also, the studies on the examination of the marine effects of all fish species that are economically valuable and available for consumption and heavy metals in seawater and sediments should continue. Although the results of the study do not indicate that heavy metal concentrations are dangerous for human consumption, international precautions should be taken and regular monitoring should be carried out against possible dangers that may increase heavy metal pollution.

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