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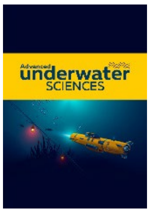

Heavy Metal Levels and Human Health Risk Assessment in Some Fish Species Caught from Kuşadası Bay (Aegean Sea)

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Abstract

The determining of Cu, Zn, Cd and Pb levels in muscle tissue and human health risk assessment of some consumable fish species (Engraulis encrasicolus, Sardinella aurita, Sphyraena sphyraena, Pagellus erythrinus, Mugil cephalus and Mullus barbatus), caught from Kuşadası Bay in 2016-2017 hunting season were aimed. Metal analysis was performed using Inductively Coupled Plasma Mass Spectrophotometry (ICP-MS) in tissues prepared by the dry weight method. Metal level in tissue varied depending on the species, metal and season, Cu and Zn were highest in S. sphraena and E. encrasicolus in winter, while Pb was determined in M. cephalus in spring. The lowest and highest Cu, Zn and Pb levels in tissues were detected 0.80-5.82, 13.09-41.60 and 0.20-1.30 µg g-1 ww, respectively. Cd level in all fish samples was found below the permissible level of ICP-MS. Tissue metal levels were determined within acceptable limits according to the Turkish Food Codex FAO/WHO standards. The intake rate limits and human health risk assessment based upon non-carcinogenic and cancer risk effect specified no negative impacts of investigated fish consumption.

1. Introduction

Fish have caused continuity in the human diet in the historical process, due to their high availability and easy hunting. In addition, have high protein content, low carbohydrate content, fat-soluble vitamins, minerals, unsaturated fatty acids such as EPA (Eicosa Pentaenoic Acid) and DHA (Docosa Hexaenoic Acid) ensure the continuity of consumption (Ersoy and Çelik 2009). The increase in the human population, industrial and technological development, and the agricultural applications have increased the participation of domestic, industrial and agricultural wastes in the hydrosphere, which is the main receiving environment, and caused pollution (Cicik 2003). Pollutants, including metals uptake by aquatic organisms transfered into the upper trophic level caused aquatic ecosystems to rapidly lose their food storage feature, as well as adverse effects of human and environmental health (Çiftçi et al. 2015).

Determination of metal levels in muscle tissue, which is the main consumable part of fish, is very important for both fish and public health (Türkmen et al. 2009). Metals such as Cu and Zn function at low concentrations as cofactors in various enzymes that function in metabolic events, in energy production, connective tissue development, impulse transmission, growth, development, neurotransmission and cell communication (Duran et al. 2015), while the high concentrations' cause tissue accumulation, damage, inflammation of the skin, loss of taste and smell senses, and disorders in metabolism and respiratory events (Zeitoun and Mehana 2014). The others, such as Pb and Cd, are toxic even at very low concentrations and have been found to cause disorders in the circulation, excretion, digestive system and cardiovascular system in humans (Jarup 2003).

Fish meat is often recommended by dietitians because it is healthier than other food sources in terms of both content and digestibility (Fuentes-Gandara et al.

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2018; Borowska-Korezak et al. 2021). The American Heart Association reported that consuming fish at least twice a week reduces the risk of heart disease (Zhang et al. 2008). Although the consumption of fish is important in terms of balanced nutrition and health, pollution in aquatic ecosystems poses a significant threat to both aquatic organisms and public health (Malik et al. 2014; Olmeda et al. 2013).

Kuşadası is a coastal town located in the center of the Aegean Sea, where tourism, agriculture and fishing activities take place at a high intensity. The marina due to boat and cruise traffic, and secondary residences on the coast due to cultural and religious tourism have increase the pressure on the marine ecosystem. Kuşadası Bay contains economical importance fish species. Fish and other aquatic products are widely consumed in the region as they are common elements of cuisines with different cultures.

In this study, it was aimed to determine the health risk assessments of metals (Cu, Zn, Cd and Pb) in the muscle tissues of widely consumed fish (E. encrasicolus, S. aurita, S. sphyraena, P. erythrinus, M. cephalus and M. barbatus) in Kuşadası Bay.

2. Method

2.1. Study Area

Anchovy, sardine, barracuda, red sea bream, gray mullet and red mullet species, which live in different parts of the water column, have different dietary habits and are widely consumed as food in the region, were used as material in the research. Fish samplings were made monthly, 10 of each species, between September 2016 and May 2017, and the fish were obtained from a commercial firm in Kusadasi on the first day they were caught.

Each of the fish was dissected separately, muscle tissue samples were taken and placed in polyethylene bags and stored in a deep freezer set at -20 °C until analysis. A total of 540 fish were analyzed in the study. Since the heavy metal level in fish varies depending on size and weight, fish of similar size and weight were used in the study (Table 1).

Table 1. Length and weight of the fish used as materials and their habitats

	n	Total length (cm)	Weight (g)	Habitat
		$\bar{X} \pm s\bar{x}$	$\bar{X} \pm s\bar{x}$	$\bar{X} \pm s\bar{x}$
E. encrasicolus	90	12.45±1.09	10.10±3.31	Pelajik
S. aurita	90	13.74±1.11	20.08±2.34	Epipelajik
S. sphyraena	90	31.87±3.98	121.50±8.58	Pelajik
P. erythrinus	90	14.79±0.92	64.19±3.60	Bentopelajik
M. cephalus	90	16.47±1.23	49.00±1.68	Bentopelajik
M. barbatus	90	13.28±0.68	27.40±2.01	Demersal

Frozen tissue samples were carried out in the laboratory of the Fisheries Faculty of Mersin University in cold chain and prepared for analysis at the end of the hunting season. Heavy metal levels in muscle tissue determined by Durmuş et al. (2018). The experimental tissues were dried at 150 oC for 72 hours to reach constant weight. After the tissue dry weights were determined they were digested in nitric acid/percloric acid mixture (2/1: v/v) at 120 °C for four hours. Then digested samples transferred to polyethylene tube and their volume were completed to 10 ml with bidistiled water. The same procedures were applied to the blank and IAEA samples. Tissue metal levels calculated over dry weight were converted to wet weight values considering the moisture rate accepted for a significant part of the species and compared with previous studies (Cresson et al. 2017; Giraldo et al. 2017; Hislop et al. 1991; Payne et al. 1999). IAEA-407 obtained from fish tissue homogenates was used as reference material (IAEA, 2003). Recovery and correction values of control samples prepared from fish tissue homogenates for IAEA were determined as 96.90%, 98.02%, 96.15% and 95.50% for Cu, Zn, Pb and Cd, respectively. The formula of the linear regression equation was obtained from the absorbance values of the standard concentrations and this formula was used to determine the tissue concentration of each metal. The absorbance values of the standards for each metal were reread after every 10 readings to check that the device was working properly. All measurements were repeated 3 times. The validation parameters of the analytical method are shown in Table 2. SPSS v.16.0 (IBM. Corp. Armonk, NY, USA) package program was used in the analysis of the data, and homogeneity of variance was checked before starting the statistical analysis. One-way ANOVA test was applied to the data and the results found significant ($P<0.05$) were reanalyzed with the post-hoc test (SNK= Student Newman Keul's) to determine the difference between the groups.

Table 2. Validation parameters of the analytical method

Metals	Measured Value Concentration mg kg^{-1}	Certificated Values %95 Confidence interval (mg kg^{-1})	LOD ng g^{1}	LOQ ng g^{-1}	Recovery %95	RSDr %	R^2
Cu	3.29	3.20-3.36	0.33	2.31	96.90	3.099	0.9997
Zn	67.98	66.3-67.9	2.38	7.12	98.02	2.925	0.9998
Pb	0.12	0.10-0.14	0.79	1.85	96.15	1.260	0.9993
Cd	0.189	0.185-0.193	0.79	1.92	95.50	2.195	0.9999

2.2. Risk Analysis In Terms of Human Consumption

Determining the possible risks of pollutants on human health is generally an event to determine or predict the harmful effects of toxic substances on health (Jawed and Usmani, 2016). Parameters such as Estimated Daily (EDI) and Weekly (EWI) Metal Intake, Target Hazard Ratio (THQ), Hazard Index (HI) and Cancer Risk (CR) are widely used to determine the harmful effects of heavy metals on human health (USEPA, 1989). Factors such as metal intake, exposure time, average body weight and oral reference dose affect these parameters significantly (USEPA, 1989; Harmanescu et al. 2011). In this study, EDI, EWI, THQ and CR were examined among the mentioned parameters. EDI, EWI, THQ and CR values were calculated using the following mathematical formulas in case the investigated species were consumed.

EDI (μg/day/70 kg body weight) =CxFIR

EWI (μg/week/ 70 kg body weight) = EDIx7 days

C(Concentration)=Mean metal concentration in muscle tissue (μg g-1 w.w.)

FIR (Fish Consumption Rate) = Daily fish consumption amount per capita in Turkey 20g (FAO 2005)

THQ= (EFxEDxEDI/ATxRfDxBW)x 10-3

CR = (EFxEDxEDIxCSF/ATxBW) x10-3

EF; (Frequency of exposure to a person who eats fish seven times a week, 365 days/year) (Traina et al. 2019), ED; Time of exposure (age 70 as adult), BW; Body weight is taken into account as the average weight of an adult in Turks is 70 kg. RfD; Orally, the reference dose is mg/kg/day. HORSE; mean time for non-carcinogenic substances (365 days/yearxED), CSF; Cancer Slop Factor mg/kg/day. The parameters and their values used in the risk analysis are given in Table 3.

Table 3. Parameters and values used in health risk analysis

Factor	Statement	Unit	Value	References
FIR	Daily Consumption Amount	g/person/day	20	FAO 2005
BW	Average Body Weight	kg	70	Karayakar et al. (2022)
EF	Frequency of Exposure	day/year	365	Karayakar et al. (2022)
ED	Influence Time	year	70	Karayakar et al. (2022)
AT	Average Time	day	25550 (EDxEF)	Gu et al. (2017)
RfD	Average Time	mg/kg/day	$4E^{-02}$ (Cu), $3E^{-01}$(Zn), $1E^{-03}$ (Cd), $4E^{-03}$ (Pb)	USEPA (2018)
CSF	Cancer Slop Factor	mg/kg/day	$8.5E^{-03}$ (Pb), $6.3E^{-00}$ (Cd)	Traina et al. (2019)

3. Results

Metal levels determined in mussle tissues of E. encrasicolus, S. aurita, S. sphyraena, M. cephalus, P. erythrinus, M. barbatus caught from Kusadası Bay in 2016-2017 hunting season (Table 4). The Cd level in tissue could not be determined because it was below the detection limit of ICP-MS. In the autumn, winter and spring seasons, a correlation was found between metals in terms of concentration in the muscle tissues of the species, such as Zn>Cu>Pb (Table 4). Cu (5.82 μg g-1 ww) was found the highest level in S. sphyraena, and Zn (41.60 μg g-1 ww) in E. encrasicolus in winter while the highest Pb level (1.30 μg g-1 ww) was detected in M. cephalus in spring (Table 4).

Table 4. Heavy metal levels (μg g-1 w.w.) determined in muscle tissues of fish

Season	n	Experimental Species	Metals			
			Cu $\bar{x} \pm s_x$*	Zn $\bar{x} \pm s_x$*	Pb $\bar{x} \pm s_x$*	Cd $\bar{x} \pm s_x$*
Autumn	30	*E. encrasicolus*	1.54±0.23 ^{as}	39.44±2.18 ^{bs}	DA ^{cs}	DA
	30	*S. aurita*	1.26±0.22 ^{as}	26.06±6.05 ^{bt}	0.44±0.22 ^{ct}	DA
	30	*S. sphyraena*	0.80±0.66 ^{as}	21.15±3.01 ^{bt}	0.21±0.10 ^{ct}	DA
	30	*M. cephalus*	0.94±0.08 ^{as}	39.69±6.01 ^{bs}	0.28±0.10 ^{ct}	DA
	30	*P. erythrinus*	0.97±0.15 ^{as}	32.64±3.99 ^{bs}	0.34±0.17 ^{ct}	DA
	30	*M. barbatus*	0.96±0.22 ^{as}	41.38±2.67 ^{bs}	0.21±0.10 ^{ct}	DA
Winter	30	*E. encrasicolus*	3.79±0.90 ^{as}	41.60±8.41 ^{bs}	0.70±0.37 ^{cs}	DA
	30	*S. aurita*	2.70±0.68 ^{as}	30.72±2.95 ^{bt}	0.41±0.28 ^{cs}	DA
	30	*S. sphyraena*	5.82±0.58 ^{at}	13.09±1.19 ^{bx}	0.26±0.15 ^{cs}	DA
	30	*M. cephalus*	3.28±1.45 ^{as}	27.84±3.21 ^{bt}	0.48±0.35 ^{cs}	DA
	30	*P. erythrinus*	1.97±0.24 ^{ax}	18.94±2.55 ^{by}	0.20±0.15 ^{cs}	DA
	30	*M. barbatus*	2.89±1.31 ^{as}	26.63±4.89 ^{bt}	0.74±0.27 ^{cs}	DA
Spring	30	*E. encrasicolus*	5.28±0.85 ^{as}	41.26±3.46 ^{bs}	DA ^{cs}	DA
	30	*S. aurita*	3.16±0.53 ^{at}	32.30±1.42 ^{bt}	0.46±0.23 ^{ct}	DA
	30	*S. sphyraena*	2.90±0.78 ^{at}	19.96±4.50 ^{bx}	0.98±0.54 ^{ct}	DA
	30	*M. cephalus*	3.44±1.40 ^{at}	25.63±2.43 ^{bx}	1.30±0.49 ^{ct}	DA
	30	*P. erythrinus*	3.03±1.54 ^{at}	20.11±3.84 ^{bx}	0.92±0.15 ^{ct}	DA
	30	*M. barbatus*	4.41±0.93 ^{as}	37.87±2.07 ^{bs}	0.78±0.19 ^{ct}	DA

x̄±s_x :Arithmetic mean ± standard error, n: Number of sample

*SNK = Data shown with different letters indicate significant difference at p<0.05 level. Letters a, b, and c are used to show difference between metals and s, t, x and y among species.

The minimum and maximum Cu, Zn and Pb levels in fish muscle were determined as 0.80-5.82, 13.09-41.60 and 0.20-1.30 μg g-1 w.w. respectively. National and international organizations such as FAO/WHO, USEPA, the European Union Commission and the National Food Codex Commission of the Ministry of Agriculture and Forestry of the Republic of Turkey have determined the maximum acceptable limits of heavy metals in foods and limited their levels in nutrients (Table 5). In this study, it was determined that the average Cu, Zn and Pb levels in fish muscle, were rather below the acceptable upper limits determined by national and international organizations (Table 5).

Table 5. Acceptable upper limits of some heavy metals in muscle tissues of fish determined by national and international organizations.

National and International Organizations	Fish	Metal (μg g^{-1} w.w.)				References
		Cu	Zn	Pb	Cd	
Turkish Food Codex		20	50	1.0	0.1	Turan et al. 2009
FAO/WHO		30	40	0.5	0.5	FAO/WHO 1989
England		20	50	2.0	0.2	MAFF (2000)
European Union		20	50	1.0	0.2	Türkmen et al. (2009)
Canada		100	100	-	-	Turan et al. 2009
Hungary		60	80	-	-	Turan et al. 2009
Australia		100	150	-	-	Turan et al. 2009
	E. encrasicolus	3.53	40.76	0.23	-	
	S. aurita	2.37	29.69	0.43	-	
	S. sphyraena	3.17	18.06	0.48	-	
	M. cephalus	2.55	26.24	0.68	-	
	P.erythrinus	1.99	23.89	0.48	-	
	M. barbatus	1.47	35.29	0.57	-	

Results from this study, the estimated daily and weekly heavy metal (Cu, Zn, Pb) uptake levels were rather below the tolerable levels in case of consumption of the mentioned fish species (Table 6). Therefore, the consumption of the examined species as food does not pose any risk to human health.

Table 6. Estimated weekly and daily metal uptake in case of consumption of fish species

Metal	Cu	Zn	Pb	Cd
TWI[a]	3500	7000	25	7
TWI[b]	245000	490000	1750	490
TDI[c]	35000	70000	250	70
EWI (EDI) *E. encrasicolus*	739.2(10.6)	5824(832)	98 (14)	-
EWI(EDI) *S. aurita*	442.4(63.2)	4522(646)	644(9.2)	-
EWI(EDI) *S. sphyraena*	814.8(116.4)	2961(423)	137.2(19.6)	-
EWI(EDI) *M. cephalus*	481.6(68.8)	5556.6(793.8)	182(26)	-
EWI(EDI) *P. erythrinus*	424.2(60.6)	4569(652.8)	128.8(18.4)	-
EWI(EDI) *M. barbatus*	617.4(88.2)	5824(832)	109.2(15.6)	-

a;Tolerable weekly intake (μg/week/kg/body weight)
b; Tolerable weekly intake for a 70 kg person (μg/week/70 kg body weight)
c;Tolerable daily intake (μg/day/70 kg body weight)
EWI; Estimated Weekly Intake (μg/week/70 kg body weight)
EDI; Estimated Daily Intake (μg/day/70 kg body weight)

The mean values of the metal concentrations determined in the muscle tissues of the examined species were used to determine the THQ and CR values, and the results showed that the THQ was below 1.0 and the CR values were within the acceptable range in terms of public health (Table 7).

Table7. THQ and CR values according to the mean metal levels (μg/g w.w.) in the muscle tissues of the examined species

Species		Metal		
		Cu	Zn	Pb
E. encrasicolus	THQ	$37.8x10^{-3}$	$377x10^{-3}$	$50x10^{-3}$
	CR	-	-	$1.7x10^{-6}$
S. aurita	THQ	$22.5x10^{-3}$	$307x10^{-3}$	$32x10^{-3}$
	CR	-	-	$1.1x10^{-6}$
S. sphyraena	THQ	$41.57x10^{-3}$	$201x10^{-3}$	$70x10^{-3}$
	CR	-	-	$2.3x10^{-6}$
M. cephalus	THQ	$24.57x10^{-3}$	$377x10^{-3}$	$92x10^{-3}$
	CR	-	-	$3.15x10^{-6}$
P. erythrinus	THQ	$21.64x10^{-3}$	$310x10^{-3}$	$65x10^{-3}$
	CR	-	-	$2.23x10^{-6}$
M. barbatus	THQ	$31.5x10^{-3}$	$374x10^{-3}$	$55x10^{-3}$
	CR	-	-	$1.89x10^{-6}$

4. Discussion

Fish has a healthy food source for human, so the determination of heavy metal levels in fish have become the focus of attention, especially after the Minemata disaster, which occurred as a result of the consumption of methyl-mercury-containing fish in Japan (Castro-Gonzalez and Mendez-Armenta 2008). If the excretion of heavy metals from the body is negligible compared to the intake, it causes accumulation in tissues. This accumulation is tried to be regulated by activating homeostatic mechanisms. However, insufficiency of homeostatic mechanisms due to the increase in intake results in acute toxicity (Javed and Usmani 2017).

Heavy metal accumulation in fish tissues varies depending on length and weight, as well as various factors (Canpolat and Calta 2003). It was investigated whether there was a relationship between tissue metal concentration and length in *M. barbatus, Solea vulgaris* and *Diplodus annularis* in the Izmir Bay (Küçüksezgin et al. 2002). A negative correlation was found between Cd, Cr, Cu, Fe, Pb and Zn levels in liver, gill and muscle tissues and fish size in different fish species in the Northeastern Mediterranean (Canlı and Atlı 2003). So that, the same size and weight of fish were used as material in order to minimize the effect of length and weight factors on accumulation in present study.

The accumulation show an alteration also depend on the species. The muscle tissue levels of Cu, Zn, Pb and Cd in 10 different fish species sampled from the Sinop coast of the Black Sea were determined and it was found that *Sprattus sprattus* contained metals at higher levels than other species (Bat et al. 2012). Another research findings emphasised that, the muscle Cd, Fe, Pb, Zn, Cu, Mn, Ni, Cr, Co and Al levels in *Saurida undosquamis*, *Sparus aurata*, and *M. barbatus* sampled from Iskenderun Bay determined vary depending on the species (Türkmen et al. 2005). In this study, Cu, Zn and Pb tissue levels in fish caught from Kuşadası Bay differed between species, and the highest levels of Cu, Zn and Pb were detected in *S. sphyraena*, *E. enceasicolus* and *M. cephalus*, respectively. It is possible that this distinction between species in terms of metal levels may be due to different habitats, feeding habits and metabolisms of the species. The distinction between species in terms of tissue metal levels may be due to varies of its habitats, feeding habits and metabolisms.

Season is another factor affecting the metal accumulation in aquatic organisms. It was determined that As, Co, Cd, Cr, Cu, Fe, Mn, Ni and Zn levels in the muscle and liver tissues of *Scomber japonicus* sampled seasonally from Antalya Bay, increased in spring and decreased in autumn generally (Aktan and Tekin- Ozan 2012). In this study, Cu, Zn and Pb levels in muscle tissue changed depending on the season, while Cu and Zn were determined the highest in winter season, Pb was determined most in spring. The seasonal difference in metal levels in tissue may be due to changes in the physicochemical properties of seawater.

Fe, Cu, Cr, As, Cd and Pb levels in muscle, gill, brain and liver tissues of *Pelates quadrilineatus, Upeneus moluccensis, Nemipterus randalli, Saurida lessepsianus* sampled from Taşucu region (NE Mediterranean) were investigated and it was found that Cd could have not be detected in all fish tissues, except liver tissue (Karaytuğ et al. 2018). In present study, the Cd level in the muscle tissue of *E. encrasicolus, S. aurira, S. sphyraena, P. erythrinus, M. cephalus* and *M. barbatus* from Kuşadası Bay could be determined under the detection limit of ICP-MS. Cu, Zn, Pb, Cd and Fe levels in the tissues of different fish species sampled from the Yumurtalık shores of Iskenderun Bay in January, April, June and July 2002 were investigated. In the study, it was determined that the highest metal level was found in *Solea solea* sampled in June. There was a relationship between metals in terms of accumulation such as Zn>Cu>Pb>Fe>Cd was established (Çoğun et al. 2006). In this study, which was carried out in the 2016-2017 hunting season, a similar relationship was found between metals in terms of accumulation level in all of the examined species, and it is possible that this may be due to the difference in metal metabolism.

Metal levels in muscle tissue of *S. solea*, *M. barbatus* and *Sardina pilchardus,* sold in supermarkets in Mersin, were determined as Cu; 0.01-1.96, Zn; 1.28-45.95 and Pb; 0.02-1.37 μg g^{-1} ww (Korkmaz et al. 2017). Cu, Zn and Pb levels in muscle tissues of some consumable fish and crustacean species caught from the Northeast Mediterranean coast were found as 0.12-20.62, 14.77-119.01 and 4.26-6.56 μg g w.w. respectively (Külcü et al. 2014). In another research, Cd, Cu, Zn and Pb level in muscle tissue of *E. encrasicolus* and *Spicara smaris* sampled from the Black Sea, Marmara and Aegean Sea coasts of Turkey were found to be 0.01-0.07, 0.21-8.58, 7.12-45.6 and 0.12-0.87 mg/kg w.w. respectively (Turkmen et al. 2008). In this study, Cu, Zn and Pb levels in muscle tissues of six different consumable fish species sampled from Kuşadası Bay were detected as 0.80-5.82, 13.09-41.60 and 0.20-1.30 µg/g w.w. respectively. The results found in present study was similar to the previous research findings. It has been seen that these values are far below the acceptable limits set in foodstuffs for toxic chemicals by various national and international organizations. This indicates that the species examined in the study do not pose any health risks if consumed as food.

The tolerable daily intake of a substance is defined as the daily intake, without any risk, based on body weight. The tolerable daily intake of a metal varies depending on the amount of food consumed and the metal concentration in the food (Ikema and Egieborb 2005). According to FAO (2005), the daily fish consumption of a person weighing 70 kg in Turkey is 20 g, which corresponds to 140 g per week. The muscle tissue Cd, Cu, Pb and Zn levels of 14 different fish species sampled from the Turkish seas were determined and the estimated weekly metal uptake values as a result of their consumption were determined as 4.0-7.4, 3.2-214; 4.6-23 and 113-218 (µg/70 kg body weight) respectively (Ateş et al. 2015). If *Trachurus mediterraneus*, *S. aurata* and *Pegusa lascaris* offered for consumption from Karataş fishing shelter are consumed as food, the estimated daily metal intake levels for Cr, Zn, Pb, Cu, Cd and As were 1.6-5.6, 7.2-11.4, 1.4-2.2, 5.0-7.4 respectively. It has been determined that it varies between 0.1-0.6 and 6.2-9.8 µg/70 kg body weight, and these values are well below the tolerable daily and weekly intake values (Karayakar et al. 2022). The estimated daily intake levels for Cu, Zn and Pb in consumable six different fish species sampled from Kuşadası Bay in present study that were determined between 60.6-88.2, 423-832 and 9.2-26 µg/70 kg body weight respectively. It has been determined that these values are well below the tolerable levels, so their consumption does not pose any risk in terms of health.

Systemic effects may occur when the THQ value is above 1.0. THQ means higher than the reference dose (Copat et al. 2013). In general, Cancer Risk (CR) assessment is performed by metals such as As, Cr, Cd and Pb, which are determined to be carcinogenic by the US Environmental Protection Agency (2018). The US Environmental Protection Agency has determined the average CR levels acceptable for public health as $1x10^{-6}$-$1x10^{-4}$. THQ values for 16 different species of Cd, Cu, Zn, As and Pb sampled from 6 stations determined between Hatay-Samandağ and Mersin-Taşucu in the Northeastern Mediterranean was determined as 5.71 E^{-05}, 1.27 E^{-02}, 8.74 E^{-02}, 1.36 E^{-00} and 2.68 E^{-05} respectively (Korkmaz et al. 2019). It was determined that the THQ values for Cd, Zn, Cu and Pb in *T. mediterraneus, S. aurata* and *P. lascaris* sampled from Karataş were found as 8.5E^{-03} – 1.4E^{-03}, 5.4E^{-04}- 3.4E^{-04}, 2.6E^{-03}-1.8E^{-03} ve 7.8E^{-03}-5.0E^{-03} respectively (Karayakar et al. 2022). In this study, the THQ values for Cu, Zn and Pb in 6 different fish species were ascertained as 2.1E^{-02} – 4.1E^{-02}, 20E^{-02} - 37E^{-02} ve 3E^{-02}-9E^{-02} respectively.

Since some of the heavy metals are carcinogenic, Cancer Risk (CR) values for heavy metals such as As, Cd and Pb have been determined by various organizations. According to the US Environmental Protection Agency, metals with CR values between $1x10^{-6}$ and $1x10^{-4}$ do not pose any health risks (USEPA, 2018). Korkmaz et al. (2019) reported that the CR value for Pb was determined as 2.42E^{-05} in16 different fish species in the Northeastern Mediterranean. In *P. lascaris*, *T. mediterraneus* and *S. aurata*, the CR values for Cd and Pb were stated as 9.0E^{-06}, 2.7E^{-07}, 5.4E^{-05}, 1.7E^{-07} ve 1.1E^{-05}, 1.9E^{-07} respectively (Karayakar et al. 2022). In this study, CR values could not be calculated since muscle tissue of six consumable fish species sampled from Kuşadası Bay in this study Cd levels could not be determined. However, it was determined that the CR values for Pb varied between 3.5E^{-06} and 1.1E^{-06}, and it was determined that the consumption of the mentioned species did not pose any health risks.

5. Conclusion

Cu, Zn, Pb and Cd levels in muscle tissue and its seasonal changes were investigated in *E. engrasicolus*, *S. sphyraena*, *S. aurita*, *P. erythrinus*, *M. cephalus* and *M. barbatus* sampled from Kuşadası Bay. It has been revealed whether the heavy metal levels in the muscle tissues, which constitute the consumable part of the species in question, are suitable for consumption and whether they pose a risk to public health if consumed. The levels of copper, zinc and lead differed between species as well as changing depending on the seasons. While copper and zinc were determined the highest in *E. encrasicolus* is pelagic, the highest amount of lead was determined in *M. cephalus* is benthopelagic. In all examined species, a relationship was found as Zn>Cu>Pb between metals in terms of concentration in muscle tissue. It has been observed that the heavy metal levels determined in the muscle tissues of the fish are far below the acceptable levels determined by national and international organizations in terms of consumption. It has been determined that daily and weekly Cu, Zn and Pb uptake levels are lower than the tolerable daily and weekly metal uptake levels in case of consumption of the mentioned species. It was determined that the consumption of *E. engrasicolus*, *S. sphyraena*, *S. aurita*, *P. erythrinus*, *M. cephalus* and *M. barbatus*, which were caught and offered for consumption from the Kuşadası fish market, did not pose a risk.

Author contributions

The authors declare that they have contributed equally to the article.

Conflicts of interest

There is no conflict of interest between the authors.

Statement of Research and Publication Ethics

Research and publication ethics were complied with in the study.

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