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Trace Elements Concentrations and Human Health Risk Evaluation for Four Common Fish Species in Sinop Coasts (Black Sea)

Ayşe Gundoğdu[1,a,*], Saniye Türk Çulha[2,b], Fatma Koçbaş[3,c]

[1]Fisheries Faculty, Sinop University, 57000 Akliman Sinop, Turkey
[2]Fisheries Faculty, Izmir Katip Celebi University, 35620 Cigli/Izmir, Turkey
[3]Faculty of Arts and Sciences, Celal Bayar University, 45140 Muradiye/Manisa, Turkey
[*]Corresponding author

A R T I C L E   I N F O

A B S T R A C T

In the study, *Trachurus trachurus*, *Engraulis encrasicolus*, *Merlangius merlangius euxin*us, and *Mullus barbatus* from along the coast of Sinop were analysed for the content of copper (Cu), zinc (Zn), lead (Pb), cadmium (Cd), iron (Fe), nickel (Ni) and aluminium (Al) in the muscle. Zn, Pb and Cd concentrations were determined to be lightly higher than the acceptable rates in fish samples. The provisional tolerable daily and weekly intake of trace metals in our work were all under than the limits set by the Food and Agriculture Organization/World Health Organization, while for Cd, only *M. barbatus* was higher than FAO limits. *E. encrasicolus* and *M. barbatus* had the highest values for the collected total target danger section, but they did not posture a potential hazard within the diet of local residents. For carcinogenic and non carcinogenic risk assessment, the results were lower than the admissible rate of EPA. In the four fish species in the work, the Target cancer risk values of Ni were greater than 10^{-4}, whereas the Target cancer risk values of Pb were smaller than 10^{-6}. According to these results, it is thought that the Ni concentration in fish does pose a carcinogenic risk due to long-term and continuous consumption.

[a] aysegundogdu57@hotmail.com   https://orcid.org/0000-0003-1323-1003   [b] trksanye@gmail.com   http://orcid.org/0000-0003-0380-0858
[c] fatma.1970@gmail.com   http://orcid.org/0000-0002-1053-3455

Introduction

Seafood and fish are an important source of nutrients for the World's growing population in terms of the protein and minerals (essential trace elements), essential fatty acids and specific vitamins. Furthermore, the n-3 fatty acids of polyunsaturated in fish are biologically significant and have been determined to be connected with a reduce risk of cardiovascular disease (Kromhout et al., 1985; Svensson et al., 1995; Han et al., 2000). Depending on the concentrations of trace elements, they can have helpful or deleterious influences on animals, plants and human life (Förstner and Wittmann, 1981). Some trace metals cause highly toxic effects even at very low concentrations in living tissues. For instance, metals taken with nutrition such as arsenic, mercury, lead and cadmium have caused harmful and toxic effects for human health (Stankovic et al., 2011; Stankovic and Jovic, 2012; Ateş et al., 2015). However, some metals only show toxic effects at very high concentrations, they are a natural component of the

ecosystem due to their biological importance (Amundsen et al., 1997; Köse and Uysal, 2008; Tepe et al., 2008; Türkmen et al., 2008a; Türkmen et al., 2008b). When swallowed in large quantities, heavy metals are combine with the body's molecules such as enzymes and proteins, disrupting their functions and structures, and forming stable biotoxic compounds (Duruibe et al., 2007). Researchers have reported that fish and seafood caught in environments contaminated by heavy metals is a threat to healt when consumed by humans (Han et al., 1994; Sipahi et al., 2013; Mok et al., 2015; Javed and Usmani, 2016; Moslen and Miebaka, 2017; Bat et al., 2018). In recent years, researchers have concentrated on the question of whether trace metals are a risk to human health because of the consumption of fish and seafood (Abdallah, 2013; Ahmed et al., 2015; Yılmaz et al., 2016).

90% of Turkey's annual production of approximately 500.000 tons of aquatic products is obtained through sea-

fishing, while 75-80% of the production occurs in the Black Sea (TUIK, 2017). Due to its economic importance, the most commonly consumed target species off the coast of Sinop are pelagic and benthic species such as *Trachurus trachurus* (horse mackerel), *Engraulis encrasicolus* (anchovy), *Merlangius merlangius euxinus* and *Mullus barbatus* (red mullet). These fish are frequently consumed by humans due to the high quality of their meat quality and their taste. The aim of the work was to assess of the trace metal values in popular fish (red mullet, anchovy, horse mackerel and whiting) hunted in the coast of the Black Sea. The Potential health risks were also calculated for the trace metals as a result of consuming these fish. The health risk related to the trace metals in fish was assessed using Target Hazard Quotients (THQ) and the estimated purchases was compared with toxicological references. The total THQ (TTHQ) value was also calculated since there was a possibility of exposure to more than one chemical substance and this could increase the health risk. The study also appraised the carcinogenic and non-carcinogenic health risk to humans of fish consumption.

Material and Method

Sampling and Analytical Method

Samples of *Trachurus trachurus;* L.1758, *Engraulis encrasicolus;* L.1758, *Merlangius merlangius euxinus;* N. 1840 and *Mullus barbatus;* L. 1758 were collected from local salesman in Sinop between November 2008 and January 2009. Fish samples were preserved at -21°C till the trace metals concentrations assessment. Total length (cm) and weight (g) of the fish were measured before starting the shredding process. The muscle tissue of all fish was disintegrated and dried after washing with tap water and pure water. They were weighed out and dried in the oven at 105°C. Drying was continued till a permanent weight was achieved. The dried samples were then weighed and stored until analysis. The dried samples were homogenized as a fine powder using a stirrer and grinder, then weighed 0.5 g for analysis. Subsequently, the wet-burning method was performed by adding Hydrochloric acid (37% HCl, Merck): Nitric asid (65% HNO_3, Merck) (3: 1) to the Teflon cups in a CEM MARS-5 microwave (Yap et al., 2004; Turk Culha et al., 2011). Values of copper (Cu), zinc (Zn), lead (Pb), cadmium (Cd), iron (Fe), nickel (Ni) and aluminium (Al) were measured with ICP-AES spectrometry (Inductively Coupled Plasma-Atomic Emission Spectroscopy, Varian Liberty- Series II). Standard solutions were prepared from stock solutions (Merck, multi element standard). Limit values for Cu, Zn, Pb, Cd, Fe, Ni and Al were 1.2, 1.8, 40.0, 2.0, 1.3, 6.5 and 2.0 $\mu g \ g^{-1}$, respectively. The recovery percentages of metals in the samples were determined between 86.49 and 114.0% according to the standard reference data (ERM-C278, muscle tissue). The blank specimen and samples taken from fish were analysed in three replicates by the same method.

Maximum Permissible Levels and Metal Pollution Index (MPI)

The concentrations of trace metals detected in four different fishes were evaluated one by one with the maximum permissible element values in fish determined according to

national and international food standards (FAO/WHO, 1984/1989; USFDA, 1993; WHO, 1995; TFC, 2002; EC, 2006). The metal absorption of each fish was interpreted with the Metal Pollution Index (MPI) calculated by the equation shown below (Usero et al., 1997): where Cn are the average concentration of trace metals (n) in fish muscle tissue (mgkg⁻¹dry weight) used in the study. If this combined index is above 1 the concentrations of trace metals would be considered elevated and ecosystem could be regarded as "polluted" (Teodorovic et al., 2000).

$$MPI = (C1 \times C2 \times \ldots \times Cn)^{1/n}$$

Human Risk Assessment Analysis and Estimated Daily Intake (EDI)

The estimated weekly intake was determined by calculating the respective levels situated in fish muscle given that the rate of consumption in Turkey in 2015 was 8600 g/person/year, equivalent to 23.56 g/person/day (TUIK, 2015). Estimated Daily Intake (EDI) values were determined by multiplying the average concentrations of each metal and the quantity of fish consumed daily. The established EDI and Provisional Tolerable Weekly Intake (PTWI) values for a 70 kg adult were calculated. The EDI was determined using the following equation (Vu et al., 2017).

$$EDI = \frac{(C \times FIR)}{B_W}$$

Where;
C   = Element concentration in fish tissue (mg kg⁻¹)
FIR = Fish consumption rate (23.56 kg/daily)
BW = Body weight (70 kg for adults).

To assess public health risks, the EDI and PTWI intakes (mg kg⁻¹) were Compared with the PTWI recommended by the Joint FAO/WHO Expert Committee of Food Additives (2004; 2007; 2010), Nasreddin et al. (2010) and EFSA (2011).

Target Hazard Quotients (THQ), Total Target Hazard Quotient (TTHQ) and Target Cancer Risk (TR)

The health risks for the Turkish consumer from consuming *T. trachurus*, *E. encrasicolus*, *M. merlangus* and *M. barbatus* from the Black Sea were detected based on the target hazard quotients (THQ). To determine the risk to human health from consuming fish contaminated with trace metals, the THQ is calculated according to the Region III Risk-Based Concentration Table of the United States Environmental Protection Agency (USEPA, 2018). This method is an indicator of the non-carcinogenic risk level from exposure to trace metals. To calculate THQ, the equation shown below was taken into consideration (Han et al.,1998; Chien et al., 2002; Storelli, 2008):

$$THO = \frac{(E_F \times E_D \times F_{IR} \times C)}{(RfD_o \times W_{ab} \times T_a)} \times 10^{-3}$$

$$TTHQ = THQ1 + THQ2 + \ldots THQn$$

According to Bannett et al. (1999), E_F is the exposure frequency (365 days/year), the duration of exposure (E_D) is the mean lifetime (70 years). The food ingestion rate (F_{IR})

for Turkish consumers is 23.56 g/day (TUIK 2015). Kumar et al. (2013) determined that C is the metal concentration (mg kg^{-1}), RfD$_o$ is the oral reference dose (mg kg^{-1}day^{-1}), W$_{ab}$ is the average body weight (70 kg) and T$_a$ is the average exposure time for noncarcinogens (365 days/year × ED, supposing 70 years in this work). The oral reference doses (RfD$_o$) for Cu, Zn, Pb, Cd, Fe, Ni and Al have been suggested as 0.04, 0.30, 0.002, 0.001, 0.70, 0.02 and 1.00 (mg kg-^{1}day^{-1}) respectively (FAO/WHO: 2004, 2007, 2010; Nasreddine et al., 2010; EFSA, 2011; USEPA, 2018). The total THQ (TTHQ) was determined as the sum of the THQ of the trace metal in each fish. To appreciate the total potential health risk posed by more than one fish, the THQ values of every fish areadded up and this becomes the hazard index (HI). For detecting the total potential health risk of more than one metal, the TTHQ may be calculated as the aggregate of the THQs of each element (USEPA, 2018). The hazard index (HI) to the human population owing to exposure to trace metals (Cu, Zn, Pb, Cd, Fe, Ni and Al) exposure throughfish consumptionwas calculated as follows (Zheng et al., 2007; Ahmed et al., 2016; Javed and Usmani, 2016; Mol et al., 2017):

$$HI=TTHQ_{Cd}+TTHQ_{Cu}+TTHQ_{Pb}+TTHQ_{Ni}+$$
$$TTHQ_{Zn}+TTHQ_{Fe}+TTHQ_{Al}$$

Target cancer risk (TR) is used to demonstrate the carcinogenic risk. Risk evaluations are based upon suppositions. The method used to assess TR is given inthe USEPA Region III Risk-Based Concentration Table (USEPA, 2018). TR was calculated by the following equation:

$$TR=\frac{(E_F \times E_D \times F_{IR} \times C \times CPS_o)}{(W_{ab} \times T_C)} \times 10^{-3}$$

E$_F$, E$_D$, F$_{IR}$, C and W$_{ab}$ are described in the above mentioned THO equation. CPSo is the carcinogenic potencyslope, oral (mg kg^{-1} bw^{-1}day^{-1}). Tc is the average time for carcinogens (365 days/year × 70 years). The CPSo table values of Cu, Zn, Cd, Fe and Al have not yet been disclosed by USEPA. That's why their carcinogenic effects have not been calculated. Since CPSo values for Ni and Pb were known, and TR values were determined when these elements were taken. Their CPSo values are the slope calculated as constant by USEPA (2018) and this is shown in Table 5.

Statistical Analysis of Data

Minitab 16.0 statistical package program was used to analyze the results obtained statistically. The suitability of the data to normal distribution was tested by Anderson-Darling test. Homogeneity of variances between fish species was tested using Levene's statistic test. In cases where the distribution was not normal, nonparametric Kruskal-Wallis test was applied. The significance test of homogenous groups was evaluated with the Tukey-HSD test following the one-way Variance Analysis (ANOVA). It was concluded that the P results obtained (P>0.05) were not statistically significant. Correlation matrix analysis was used to determine whether there was a correlation between Cu, Zn, lead, Cd, Fe, Ni and Al concentrations obtained

from muscle tissue. Statistical significance was given when the correlation values between the metals of the fishes were P≤0.001, P≤0.01, P≤0.05 and except for P>0.05.

Results and Discussion

Concentrations of Trace Metals

In the study, the body length and weight of each of the 806 fish were determined. The mean values and standard deviation of weight and length measurements were 29.13±1.11 g and 13.30±0.18 cm (*T. trachurus*); 10.25±0.27 g and 11.17±0.10 cm (*E. encrasicolus*); 29.62±1.84 g and 14.90±0.30 cm (*M. merlangus euxinus*); 19.67±0.87 g and 11.33±0.16 cm (*M. barbatus*). The results of one-way analysis of variance with the metal levels in the muscles of the fish are shown in Table 1. Although each metal concentration (Cu, Zn, Fe and Al) showed statistically significant differences between the four fish samples (P<0.05), the statistical differences between the concentrations of Pb, Cd and Ni in the same fish were not significant (P>0.05). The variations that were expressed in the different standard deviations that occurred in individual metals were the results we expected. The results of the Anova test showed the complex relationship between environmental concentrations of metals and bioaccumulation in fish. Trace metals of industrial activities or anthropogenic origin may cause contamination of fish muscle in water. Environmental and anthropogenic sources are responsible for the different amounts of bioaccumulation of these metals (Jovic et al., 2012). According to Cai et al. (2017) the most likely reason for the different amounts may be related to the fish having different capacities to accumulate metals, as much as how much the water environment has been affected by its environment in previous years (Mutlu et al., 2012). The sequential order of the levels of the trace metals achieved from the muscle tissues of four different fish were Zn>Fe>Al>Cu>Ni>Pb>Cd. The results showed that the mean concentrations were higher for Zn (25.06 mg/kg) and Fe (23.06 mg/kg). The higher concentrations of these metals were not surprising, owing to fact that they are essential elements in fish nutrition. Mol et al. (2017) found similar values of Zn concentration in red mullet. Similar trace metal results were seen in study Jezierska and Witeska (2006) and Javed and Usman (2011).

In many studies on this subject, it has been shown that the amount of trace metal in the muscle of fish varies according to the fish species and there is a statistically significant correlation (Mohammadnabizadeh et al., 2013; Monroy et al., 2014). While *T. trachurus* and *E. encrasicolus* are the pelagic species used in this study, *M. merlangus euxinus* and *M. barbatus* are benthic fish species. The maximum levels of metal accumulation in each species were identified in *M. barbatus* for Pb and Cd; in *M. merlangus euxinus* for Cu and Al; in *E .encrasicolus* for Zn, Fe and Ni. When the maximum values of the metals were compared, it is determined that the level of metals was higher in the benthic fish than in the pelagic fish (Yi et al., 2011; Hosseini et al., 2015). The degree of absorption of metals by fish depends on the depth of the body of water in which they live, as well as on the species of fish. Sediment at the base of the aquatic system is very dense in terms of heavy metal content (Dalman et al., 2006; Liu et al., 2015). The MPI was calculated to compare the total metal concentrations in

the muscles of four different fish species (Table 1). The following order was seen: *E. encrasicolus* (1.75)>*M. merlangus euxinus* (1.65)>*M. barbatus* (1.63)>*T. trachurus* (1.46). According to the data obtained from the study, the metal absorption of benthic fish is higher than other fish species, which is related to the amount of metal and the form of metal (ion, compound, dissolved etc.) presence in water. Besides, the amount of MPI determined in fish muscles is significantly associated with fish species has indicated in research (Li et al., 2013; Idris et al., 2015; Cai et al., 2017).

Table 1. Trace metal concentrations determined in muscle tissues of fish samples (µg g^{-1} dry weight; $X \pm sx$)

Species	N	MPI	Cu Min-max	Zn Min-max	Pb Min-max	Cd Min-max	Fe Min-max	Ni Min-max	Al Min-max
T. trachurus	162	1.46	0.66±0.02[a] 0.63-0.70	25.63±1.43[b] 20.48-35.03	0.32±0.03[a] 0.05-0.70	0.17±0.06[a] 0.09-0.29	19.47±0.36[a] 15.32-21.15	0.74±0.05[a] 0.66-0.96	1.07±0.05[a] 0.91-1.05
E. encrasicolus	296	1.75	1.09±0.06[b] 0.75-1.53	36.6±1.85[c] 28.05-48.68	0.30±0.02[a] 0.05-0.59	0.15±0.02[a] 0.05-0.19	28.30±0.79[b] 24.42-34.43	0.87±0.03[a] 0.64-1.52	1.12±0.03[a] 1.02-1.49
M. merlangus euxinus	186	1.65	1.39±0.09[b] 0.98-1.59	25.09±1.79[b] 18.64-30.43	0.18±0.02[a] 0.02-0.41	0.15±0.04[a] 0.08-0.24	21.50±1.73[a] 18.22-31.23	0.70±0.03[a] 0.56-0.78	2.33±0.13[b] 1.47-4.15
M.barbatus	162	1.63	0.92±0.06[ab] 0.77-1.24	12.93±0.59[a] 10.71-14.54	0.44±0.04[a] 0.14-0.82	0.19±0.03[a] 0.06-0.29	25.12±1.24[a] 20.25-39.94	0.76±0.01[a] 0.61-0.88	1.62±0.10[ab] 1.14-2.80

[a,b,c]Different letters in the same column show statistical differences (P<0.05), N: Number of fish, MPI: Metal Pollution Index

The correlation coefficients of the metals are shown in Table 2. Although the correlation between Al and Fe was positive, the relationship between aluminum and other metals (Cd, Ni, Pb and Zn) were negative. However, these relationships were not statistically significant when compared with P>0.05. While Cd's relationship with both Pb and Ni was positive, the relationship between Cd and other metals (Cu, Fe, Zn) were negative and not statistically significant (P>0.05). The correlation between Cu and both Fe and Zn was both positive and statistically significant (P≤0.05). Fe was also associated strongly and positively with Ni (r=0.363) and Zn (r=0.387) at P≤0.001. There was a negative correlation between Pb and Zn (r=0.220) at P≤0.05. Uluturhan and Kücüksezgin (2007) and Gundogdu et al. (2016) thought that there was a powerful positive correlation of Zn with Cu and Cd on account of the fact that these trace metal arrive from the identical resources. The interplays and similar accumulation behavior of elements in fish may be described by the positive correlation between the different metals identified in their muscle tissues. (Kojadinovic et al.,2007). Metal density in water column is several times lower than sediments (Mendil and Uluozlu, 2007). Trace metal may accumulate on the surface of the sediment. However, they may be dissolved and transferred to the water column due to the water movements and solvent effect of the water. *M. barbatus* is a ground-fish and is in close communicate with the sediment. Therefore, it has more trace metal accumulation than other fish (Tabinda et al., 2013).

Table 2. Correlations between trace metal contents in muscle tissues in four fish

	Al	Cd	Cu	Fe	Ni	Pb
Cd	-0.119					
Cu	0.385***	-0.222				
Fe	0.196	-0.054	0.245*			
Ni	-0.169	0.117	-0.010	0.363***		
Pb	-0.102	0.179	-0.289**	0.151	-0.125	
Zn	-0.114	-0.053	0.228*	0.387***	0.327***	-0.220*

P>0.05, *P≤0.05, **P≤0.01, ***P≤0.001

Risk Assessment of Human Health

The trace metal results obtained in the study were compared with the consumable limit levels of the different organizations mentioned in Table 3. The maximum Cu levels permitted for fish are 10, 20 and 30 mg kg^{-1} according to the FAO /WHO (1989), TFC (2002) and EC (2006) limits, respectively. Cu values were found to be lower than the literature values. The highest concentration of Zn was measured at 48.68 µg g^{-1} in *E. encrasicolus*. This concentration was above the FAO (1983) and FAO/WHO (1989) limits, but the legal limit of the TFC was below 50 µg g^{-1} (TFC, 2002). Pb was detected in almost all the samples and the highest concentration (0.82 µg g^{-1}) was detected in *M. barbatus*; however, the value was below the limits of 1.5 µg g^{-1} (EC, 2006). When the results of the study are compared with the standard limit values, it is clear that Cd in all species studied except *E. encrasicolus* was above the TFC (2002 and EC (2006) limits, and that Pb was below the FAO (1983), FAO/WHO (1989) and EC (2006) limits. The highest concentration of Fe was measured as 39.94 µg g^{-1} in *M. barbatus*, whereas the highest Fe concentration of average values was 28.30 ± 0.79 µg g^{-1} in *E.encrasicolus*. This concentration was well below the legal limit of 80 µg g^{-1}and 100 µg g^{-1} (FAO/WHO, 1984; 1989). Ni was detected in all fish samples analyzed in a range of 0.56–0.88 µg g^{-1} dry weight. The highest concentration was

detected in *M. barbatus*. Ni values in all of the fish were very under the legal limit of 80 µg g^{-1} (USFDA, 1993). In the study, the highest aluminum concentration was determined in *M. merlangus euxinus* (4.15 µg g^{-1}). Comparison could not be made since there is no limit value for Al.

Dietary Intake of Trace Metals

The daily and weekly intake of metals in Sinop was calculated from the consumption of seafood by an adult individual (Table 4). In this study, all trace metal intake through fish consumption in Sinop was determined to be below the daily and weekly intake limits. Similarly, in studies carried out by Turkmen and Dura (2016) and Mol et al. (2017) indicated that EWI and EDI values of red mullets caught in the Black Sea were below the consumable limit. Bat and Arıcı (2016) explained that the trace metal ratios of the Atlantic acorn captured in the Black Sea were below the specified PTWI values.

Table 3. Comparison of trace metal concentrations and international standards in fish (µg g^{-1}).

Organization	Cu	Zn	Pb	Cd	Fe	Ni	Al	Reference
FAO	30	30	0.5-2	0.5	-	-	-	FAO (1983)
EC	-	-	1.5	0.05	-	-	-	EC (2006)
WHO	30	-	2	-	-	-	-	WHO (1995)
USFDA	-	-	-	-	-	70-80	-	USFDA (1993)
FAO/WHO	30	40	0.5	0.5	100	-	-	FAO/WHO (1989)
FAO/WHO	30	50	-	2	80	-	-	FAO/WHO (1984)
Turkish	20	50	0.30	0.05-0.1	-	-	-	TFC (2002)

FAO: Food and Agriculture Organization, EC: European Commission, WHO: World Health Organization, USFDA: Food and drug administration (US), TFC: Turkish food codex.

Table 4. The estimated daily (EDI) and weekly (PTWI) intakes (mg/kg) for *T. trachurus, E. encrasicolus, M. merlangus euxinus* and *M. barbatus* from Black Sea, consumed by adult people in Sinop.

Heavy Metals	EDI	PTWI	*T. trachurus*		*E. encrasicolus*		*M. merlangus euxinus*		*M. barbatus*	
			EDI*	PTWI*	EDI*	PTWI*	EDI*	PTWI*	EDI*	PTWI*
Cd[a]	0.0575	0.403	0.057	0.399	0.05	0.35	0.05	0.35	0.064	0.448
Cu[b]	35	245	0.222	1.554	0.367	2.569	0.468	3.276	0.31	2.17
Pb[c]	0.25	1.75	0.108	0.756	0.101	0.707	0.061	0.427	0.148	1.036
Ni[e]	0.35	2.45	0.249	1.743	0.293	2.051	0.236	1.652	0.256	1.792
Zn[b]	70	490	8.626	60.382	12.319	86.233	8.445	59.115	4.352	30.464
Fe[b]	56	392	6.553	45.871	9.525	66.675	7.236	50.652	8.455	59.185
Al[d]	20	140	0.36	2.52	0.77	5.39	0.784	5.488	0.545	3.815

PTWI:The established provisional tolerable weekly intake (mg/week/kg body weight); EDI: The estimated provisional tolerable daily Intake (mg/daily body weight); PTWI*: Weekly intake values calculated for a 70 kg adult (mg/week body weight) EDI*: Daily intake values calculated for an adult of 70 kg (mg/daily body weight); [a]FAO/WHO (2010); [b]FAO/WHO (2007); [c]FAO/WHO (2004); [d]EFSA (2011); [e]Nasreddin et al. (2010)

Potency Healthiness Hazard, THQ, HI and TR

Trace elements being able to cause carcinogenic and mutagenic effects in humans over time (Goyer et al., 2003). The International Cancer Research Agency (IARC 2014) reported that it belongs to a group in the Ni, Cd and Pb classification systems and has sufficient evidence of carcinogenic effects on humans. The THQ has been recognized as a useful parameter for the assessment of health risk associated with the consumption of heavy metal-contaminated food (Jezierska and Witeska, 2006; Abdallah, 2013). The admissible guideline value for THQ is 1 (USEPA, 2011). THQ values<1 thus demonstrate that exposure is not as a potential concern. As displayed in Table 5, there were no THQ values for any of the metals greater than 1, suggesting that the local people are be exposed not to a potential health risk from Cu, Zn, Pb, Cd, Fe Ni and Al in fish. Turk Culha et al. (2016) studied the potential human health risks of scorpionfish species caught from the Black Sea and also reported THQ values for trace metals (Al, Cd, Hg, As, Pb, Cu, Ni, U) of below 1 and that there was thus no threat to humans from consumption of scorpionfish. When we look at the results of TTHQs (Table 5), it is determined that fish consumption does not pose a risk for human health due to 1>HI. Mol et al. (2017) reported that the THQ and HI (sum of TTHQ or TDHQ) values were below 1 and that consumption of fish species from the southwest Black Sea might not be hazardous to the consumer with respect to the observed levels of Hg, Cu, Pb, Zn and Cd alone or in combination with each other. Moslen and Miebaka (2017) found that in the *Sarotherodon melanotheron* species exemplified in Nigeria Creek, HI values were<1 for Ni, Cu, Pb and Cd.

TR values were calculated to determine whether there is a carcinogenic hazard. The TR were not calculated because the CPSo table values for Cd, Cu, Zn, Fe and Al were unknown. As CPSo values of Ni and Pbare known, the TR values were calculated by considering these metals together with the diet (Table 6). Although the TR values of Pb were smaller than the limit values specified as a carcinogenic value, Ni concentration waspretty close to the limit value. Since TR values were higher than the admissible limit of 10^{-6}, it shows that over-consumption of fish a long period of time may result carcinogenic effects (USEPA 2018).It has been explained by many researchers that the Pb from the consumption of the fish does not carry the risk of cancer, but that Ni does pose a risk of cancer (Ahmed et al., 2015; Islam et al., 2015; Javed and Usmani, 2016;

Ahmed et al., 2016). TR values lower than 10^{-6} are noted to show a negligible carcinogenic risk; cancer risks above 10^{-4} are noted to be unacceptable (USEPA 1989, 2010), and risks lying between 10^{-6} and 10^{-4} are generally noted to be in an acceptable range (Fryer et al. 2006). The TR values for Ni in all the fish were higher than the admissible limits of 10^{-6}–10^{-4} founded by USEPA (2018), pointing to a potency healthiness hazard for people consuming the fish. This suggests that the levels of Pb in fish from the Black Sea are safe for human consumption and there may only be a risk of cancer in terms of Ni taken in as a result of continuous and excessive fish consumption. Similar results for Ni have been reported by Bhupander and Mukherjee (2011) and Ahmed et al., 2016).

Table 5. The Estimated Target Hazard Quotients (THQ) for metals caused by consuming *T. trachurus, E. encrasicolus, M. merlangus euxinus* and *M. barbatus* from the Black Sea

Heavy Metals	*T. trachurus*	*E. encrasicolus*	*M. merlangus euxinus*	*M. barbatus*	HI
	THQ<1	THQ<1	THQ<1	THQ<1	HI
Cd[a]	0.057	0.050	0.050	0.064	
Cu[b]	0.006	0.009	0.012	0.008	
Pb[c]	0.054	0.050	0.030	0.074	
Ni[e]	0.012	0.015	0.012	0.013	0.676
Zn[b]	0.029	0.041	0.028	0.015	
Fe[b]	0.009	0.014	0.010	0.012	
Al[d]	0.00036	0.00038	0.00078	0.00055	
TTHQ	0.167	0.179	0.143	0.187	

Table 6. Target Cancer Risk (TR) of trace metals from consumption of four fish species collected from Black Sea, Turkey

	Target cancer risk (TR)				
Heavy metals	CPSo	*T. trachurus*	*E. encrasicolus*	*M. merlangus euxinus*	*M. barbatus*
Pb	0.0085	9.00×10^{-7}	9.00×10^{-7}	5.00×10^{-7}	1.30×10^{-6}
Ni	1.7	4.23×10^{-4}	4.99×10^{-4}	4.01×10^{-4}	4.35×10^{-4}

(CPSo: mg kg^{-1} bw-day^{-1})

Conclusion

Fish, as well as being a source of protein, they also serve as a source of rich polyunsaturated fatty acids and are therefore strongly suggested in diets. In addition to their great health benefits, fish are significant in determining and monitoring the levels of trace elements that may come from the sea as a test animal. Therefore, in the Black Sea region, trace metal values of four fish consumed were evaluated. Zinc, lead and cadmium concentrations were determined to be slightly higher than the acceptable values in fish samples. Cd concentration in all studied except anchovy species was above the TFC (2002) and EC (2006) limits, Pb concentration was below FAO (1983), FAO/WHO (1989) and EC (2006) limits, and Zn concentration was above the FAO (1983) and FAO/WHO (1989) limits. However, the estimated daily (EDI) and weekly (PTWI) intake values for Ni were calculated to be lower than the values given in the USEPA Table. THQ, TTHQ and HI values also remained below 1, pointing no health risk owing to the intake of individual trace metal by consuming one of *T. trachurus, E. encrasicolus, M. merlangus* and *M. barbatus*. According to the data obtained at the end of the study, trace metals present either individually or togetherdo not pose a health hazard. Moreover, the combined effect of the four fish was determined not to be harmful to human health, since the HI was less than 1. In this study, the TR values of Ni calculated in fish were greater than 10^{-4}, whereas the TR values of Pb were smaller than 10^{-6}. These results suggest that there is no risk of cancer from Pb, although there is a high carcinogenic risk for consumption of Ni. Nevertheless, HI, which was the combination value of the trace metals determined in the study, was<1 for consumed and evaluated fish, indicating that there was no potential health risk to human health. Health risks may vary depending on consumption amounts for different populations. Therefore, Therefore, the pollutant levels of caught fish from the dense regions of the industry should be identified, and likely health risks should be detected at specific intervals.

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