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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Assessment of Heavy Metals in Selected Fish Species from Markets in the Black Sea Region of Turkey

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Abstract: The present study examined the heavy metal contaminants in the muscle tissues of various species of fish including *Sarda sarda* (bony fish), *Trachurus trachurus (*Atlantic horse mackerel), *Merlangius merlangus* (whiting fish*)*, *Mullus barbatus* (red mullet), *Sardina pilchardis* (European pilchard), *Thunnus thynnus* (Turkish bluefin tuna), *Belone belone* (Garfish), *Engraulis encrasicolus* (anchovies), *and Scomber scomber* (mackerel) purchased from fish markets across Turkey's Eastern Black Sea region. The contaminants thus found included cobalt (Co): 0.01-0.16, manganese (Mn) 0.11-4.41, chromium (Cr): 0.51-2.30, cadmium (Cd): 0.08-0.10, copper (Cu): 0.26-1.18, lead (Pb): 0.10-0.57, nickel (Ni): 1.07-24.0, and zinc (Zn): 5.46-16.9 ppm. These toxin levels were compared with daily and weekly intake guidelines deemed safe for human consumption, and what was found was that these values were all below outlined levels with the exception of nickel. What can be concluded this that there currently is no hazard to human health with the exception of above normal nickel levels, as found in one species from one station.

Keywords: Fish, fish market, health, heavy metal, risk assessment.

Türkiye'nin Karadeniz Bölgesi'ndeki Balık Pazarlarından Seçilmiş Balık Türlerindeki Ağır Metallerin Değerlendirilmesi

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Öz: Bu çalışma, Türkiye'nin doğu Karadeniz sahillerindekindeki balık pazarlarından satın alınan *Sarda sarda* (Palamut), *Trachurus trachurus* (İstavrit), *Merlangius merlangus* (Mezgit), *Mullus barbatus* (Barbunya), *Sardina pilchardis* (Sardalya), *Thunnus thynnus* (Orkinos), *Belone belone* (Zargana), *Engraulis encrasicolus* (Hamsi) *ve Scomber scomber* (Uskumru) gibi çeşitli balık türlerinin kas dokularındaki ağır metal kirleticileri inceledi. Bu şekilde bulunan kirleticiler arasında kobalt (Co): 0.01-0.16, manganez (Mn) 0.11-4.41, krom (Cr): 0.51-2.30, kadmiyum (Cd): 0.08-0.10, bakır (Cu): 0.26-1.18, kurşun (Pb): 0.10-0.57, nikel (Ni): 1.07-24.0 ve çinko (Zn): 5.46-16.9 ppm'dir. Bu toksin seviyeleri, insan tüketimi için güvenli kabul edilen günlük ve haftalık alım yönergeleriyle karşılaştırıldığında, nikel dışında, belirtilen seviyelerin altında olduğu görülmüştür. Bir istasyondan bir türde bulunan normalin üzerindeki nikel seviyeleri haricinde şu anda insan sağlığı için hiçbir tehlike olmadığı sonucuna varılabilir.

Anahtar kelimeler: Ağır metal, balık, balık marketleri, risk değerlendirmesi, sağlık.

INTRODUCTION

Marine organisms can accumulate heavy metals through respiration, adsorption, and ingestion (Zhou & Sequeria, 2001). Considering that seafood plays a key role in many human diets, many researchers have thus studied metal contamination in various species of fish (Tüzen, 2003; Ikem, & Egiebor, 2005; Minganti et al., 2010; Bilandžić et al., 2011; Türkmen & Dura, 2016; Afonso et al., 2018; Türkmen & Pınar, 2018). Heavy metals flowing into the marine ecosystems can harm both marine organism diversity as well as ecosystems, due to their toxic and accumulative properties (Sipahi et al., 2013; Akkan and Mutlu, 2016). Fish that generally accumulate contaminants from these ecosystems has been widely featured in studies involving food safety (Matta et al., 1999). Three fish markets and one supermarket were selected from Turkey's South Eastern Black Sea coastal region. These areas are densely populated by host of regional fish markets. Since the assessment of the contaminants at these sites is directly tied with regional public health, the present study aims to determine what metal contaminants are present in the in muscles of nine species of fish from the selected markets in question, as well as aims to evaluate whether these species are fit for human consumption by comparing them with the weekly and daily intake guidelines recommended by authorities.

MATERIAL AND METHOD

A total of 88 fish specimens from nine commercially valuable fish species, including *Sarda sarda* (SSR, bony fish), *Mullus barbatus* (MB, red mullet), *Engraulis encrasicolus* (EE, anchovies), *Trachurus trachurus* (TTR, Atlantic horse mackerel), *Merlangius merlangus* (MM, whiting fish), *Belone belone* (BB, Garfish), *Sardina pilchardis* (SP, European pilchard), *Scomber scomber* (SS, mackerel) and *Thunnus thynnus* (TT, Turkish bluefin tuna) were collected from three stations in the Turkish provinces of, Ordu (ORD: 40º58'N, 37º54'E), Giresun (GRS: 40º54'N, 38º24'E) and Trabzon (TRZ: 41º0'N, 39º42'E). Of these, *Sardina pilchardis* (SP), *Scomber scomber* (SS) and *Thunnus thynnus* (TT) were canned fish which obtained from a supermarket in Giresun. Approximately 0.5 g of muscle tissue from each specimen was dissected, washed, packed and stored at -18 ºC.

Approximately 0.5 g muscles tissues from each fish were dissected, washed, homogenized, and digested as previously described (Türkmen, et al., 2009). Then the residue was diluted with deionized water in 50 ml volumetric flasks, followed by samples being filtered. The filtered samples were analyzed for their heavy metal levels using ICP-MS as per mg kg^{-1} wet weight. Blanks were prepared in the laboratory in a similar manner to the field samples. Calibration standards were prepared from a multi-element standard. A Dorm-4 certified fish protein was used as the calibration verification standard. To test the differences between sites and fish species, one way ANOVA and following Post hoc test (Duncan) was performed ($p<0.05$).

RESULTS AND DISCUSSION

Table 1 outlines the contamination levels in the muscle tissues. What was revealed was that, across all of the fish species and stations, the metals uncovered were statistically significant in terms of level, with the exception of cadmium ($p<0.05$). The two metals that stood out in terms of their highest level were nickel and zinc. Conversely, the two metals that were deemed to be the lowest in terms of level of cobalt and cadmium. It appears that many researchers have previously reported similar situations (Türkmen & Akaydin, 2017; Carneiro et al., 2011; Gökkuş & Türkmen, 2016; Türkmen & Dura, 2016).

Table 2. Comparison of the findings in present study with other studies.
Tablo 2. Mevcut çalışmadaki bulguların diğer çalışmalarla kıyaslanması.

ST.	SP.	Chromium	Manganese	Cobalt	Nickel	Copper	Zinc	Cadmium	Lead
ORD	TTR	0.63 ± 0.13^{a}	0.28 ± 0.02^{a}	0.03 ± 0.01^{a}	5.10 ± 2.58^{ab}	0.63 ± 0.06^{abcd}	6.66 ± 0.56^{ab}	0.09 ± 0.00^{a}	0.16 ± 0.01^{a}
	EE	0.55 ± 0.01^{a}	0.59 ± 0.09^{a}	0.03 ± 0.00^{a}	1.62 ± 0.08^{a}	0.55 ± 0.26^{abcd}	16.9 ± 4.26^{b}	0.09 ± 0.01^{a}	0.19 ± 0.04^{a}
	MM	0.55 ± 0.05^{a}	0.21 ± 0.01^{a}	0.02 ± 0.00^{a}	2.42 ± 0.94^{a}	0.26 ± 0.02^{a}	8.26 ± 0.48^{ab}	0.09 ± 0.00^{a}	0.16 ± 0.02^{a}
	SSR	0.54 ± 0.01^{a}	0.16 ± 0.03^{a}	0.05 ± 0.03^{a}	1.29 ± 0.02^{a}	0.77 ± 0.29^{abcd}	6.30 ± 0.58^{ab}	0.09 ± 0.00^{a}	0.14 ± 0.20^{a}
	MB	0.62 ± 0.04^{a}	4.41 ± 2.01^{b}	0.10 ± 0.06^{ab}	2.66 ± 0.50^{a}	0.33 ± 0.03^{ab}	8.55 ± 0.56^{ab}	0.08 ± 0.00^{a}	0.13 ± 0.01^{a}
GRS	SSR	0.57 ± 0.03^{a}	0.23 ± 0.06^{a}	0.01 ± 0.00^{a}	1.07 ± 0.00^{a}	1.16 ± 0.28^{cd}	7.21 ± 0.69^{ab}	0.09 ± 0.00^{a}	0.14 ± 0.02^{a}
	MB	0.74 ± 0.14^{a}	0.41 ± 0.07^{a}	0.04 ± 0.01^{a}	5.76 ± 2.43^{ab}	0.39 ± 0.02^{abcd}	8.30 ± 1.15^{ab}	0.10 ± 0.01^{a}	0.18 ± 0.05^{a}
	EE	2.30 ± 1.09^{b}	1.13 ± 0.25^{ab}	0.16 ± 0.08^{b}	21.2 ± 6.83^{b}	1.03 ± 0.26^{abcd}	14.1 ± 2.18^{ab}	0.10 ± 0.01^{b}	0.32 ± 0.06^{a}
	TTR	0.53 ± 0.01^{a}	0.20 ± 0.03^{a}	0.02 ± 0.00^{a}	1.08 ± 0.01^{a}	0.54 ± 0.08^{abcd}	7.29 ± 0.92^{ab}	0.09 ± 0.00^{a}	0.13 ± 0.01^{a}
	BB	0.61 ± 0.03^{h}	0.86 ± 0.20^{a}	0.04 ± 0.01^{a}	1.10 ± 0.03^{a}	0.47 ± 0.12^{abcd}	11.0 ± 1.70^{ab}	0.09 ± 0.00^{a}	0.33 ± 0.11^{a}
	MM	0.51 ± 0.03^{a}	0.23 ± 0.01^{a}	0.04 ± 0.02^{a}	2.53 ± 0.65^{a}	0.26 ± 0.04^{a}	6.41 ± 0.52^{ab}	0.09 ± 0.00^{a}	0.31 ± 0.14^{a}
TRB	SSR	0.70 ± 0.06^{a}	0.13 ± 0.00^{a}	0.02 ± 0.00^{a}	4.75 ± 1.38^{ab}	0.79 ± 0.13^{abcd}	8.17 ± 0.77^{ab}	0.09 ± 0.00^{a}	0.13 ± 0.01^{a}
	EE	0.77 ± 0.13^{a}	0.63 ± 0.13^{a}	0.04 ± 0.01^{a}	3.97 ± 0.72^{a}	1.18 ± 0.27^{d}	15.1 ± 2.64^{ab}	0.10 ± 0.00^{a}	0.33 ± 0.12^{a}
	BB	0.51 ± 0.03^{a}	0.13 ± 0.02^{a}	0.02 ± 0.01^{a}	1.22 ± 0.03^{a}	0.37 ± 0.06^{abc}	9.65 ± 0.89^{ab}	0.10 ± 0.00^{a}	0.25 ± 0.08^{a}
	TTR	0.52 ± 0.03^{a}	0.17 ± 0.03^{a}	0.03 ± 0.00^{a}	1.25 ± 0.01^{a}	0.60 ± 0.06^{abcd}	8.57 ± 0.67^{ab}	0.09 ± 0.00^{a}	0.55 ± 0.39^{a}
	MM	0.51 ± 0.05^{a}	0.45 ± 0.16^{a}	0.04 ± 0.01^{a}	1.60 ± 0.32^{a}	0.69 ± 0.32^{abcd}	9.42 ± 3.29^{ab}	0.10 ± 0.00^{a}	0.57 ± 0.44^{a}
	MB	0.49 ± 0.03^{a}	0.48 ± 0.11^{a}	0.03 ± 0.00^{a}	1.24 ± 0.03^{a}	0.32 ± 0.05^{a}	7.92 ± 1.48^{ab}	0.09 ± 0.00^{a}	0.17 ± 0.03^{a}
SMK	SP	0.88 ± 0.07^{ab}	1.48 ± 0.10^{ab}	0.04 ± 0.01^{a}	4.87 ± 0.45^{ab}	1.14 ± 0.07^{bcd}	16.9 ± 1.64^{b}	0.09 ± 0.00^{a}	0.13 ± 0.03^{ab}
	SS	0.63 ± 0.04^{a}	0.17 ± 0.04^{a}	0.01 ± 0.00^{a}	5.03 ± 0.01^{ab}	0.54 ± 0.04^{abcd}	5.46 ± 0.10^{a}	0.08 ± 0.00^{a}	0.10 ± 0.01^{a}
	TT	0.77 ± 0.02^{a}	0.11 ± 0.01^{a}	0.02 ± 0.01^{a}	4.41 ± 0.02^{ab}	0.45 ± 0.04^{abcd}	7.37 ± 0.02^{ab}	0.10 ± 0.01^{a}	0.27 ± 0.14^{a}

[*]Vertically, letters *a*, *b* and *c* show statistically significant differences among species ($p<0.05$), [**]ST: Station, SP: Species, ORD: Ordu, GRS: Giresun, TRB: Trabzon, SMK: Supermarket, MB: *Mullus barbatus*, EE: *Engraulis encrasicolus*, TTR: *Trachurus trachurus*, MM: *Merlangius merlangus*, SSR: *Sarda sarda*, BB: *Belone belone*, SP: *Sardina pilchardis*, SS: *Scomber scomber*, TT: *Thunnus thynnus*.

The lowest levels were found in *M. barbatus* from the TRB station for Cr, in *T. thynnus* from the SMK station for Mn, in *S. sarda* from the GRS station for Co and Ni, in *M. Merlangus* from the ORD station for Cu, and in *S.*

scomber from SMK station for Zn, Cd and Pb. On the contrary, the highest levels were found in *E. encrasicolus* from GRS station for Cr, Co and Ni, in *M. barbatus* from ORD station for Mn, in *E. encrasicolus* from TRB station for Cu, in *E. encrasicolus* from ORD station and *S. pilchardis* from SMK station for Zn, in *M. barbatus* and *E. encrasicolus* from GRS station, in *E. encrasicolus*, *T. trachurus* and *M. merlangus* from TRB station and in *T. thynnus* from SMK station for Cd; in *M. merlangus* from TRB station for Pb. Metal contamination in other fish species the literature were shown in Table 2. As can be seen from Table 3, cadmium levels in previous studies were greater than those measured in the present study. Cu, Cr, Co and Pb levels from İskenderun Bay were greater, but others were smaller than those featured in our study (Türkmen et al., 2005). Findings in fish from Turkish seas were greater for Co and Cu, and smaller for Cr, Mn and Ni than the present results, but similar at the same with the present study (Türkmen et al., 2008). Co, Cu and Zn levels in coastal Black Sea species were greater; however Cr and Ni levels were smaller than those of this study. On the other hand, levels of the remaining metals were similar with our findings (Topcuoğlu et al., 2002). Findings from Black and Aegean Seas were greater than ours for Mn and Zn; however for the remaining metals, levels were comparably similar (Uluözlü et al., 2007). Metal levels in the muscle tissues of fish species from United Arab Emirates were compared with this study: Cu and Zn findings were greater, whilst the remaining metals were smaller in terms of level than the present results (Kosanovic et al., 2007). Metal contamination levels in fish species from the South Western Black Sea region were smaller for Cu, Mn and Ni, and greater for the remaining metals when compared with the findings of this study (Türkmen & Dura, 2016).

The daily and weekly intakes for fish species examined in the present study were estimated (Table 3). In Turkey, the average daily fish consumption is about 20 g per person (FAO, 2014), which is also equivalent to 140 g per person per week. The estimated weekly (EWI) and daily intakes (EDI) presented in Table 3 were calculated as previously described (Türkmen & Dura, 2016). The estimated weekly and daily intakes calculated in the present study were below the suggested intake guidelines with the exception of Nickel (FAO/WHO, 2004; EPA, 2014; WHO, 2014).

Table 2. Comparison of the findings in present study with other studies.
Tablo 2. Mevcut çalışmadaki bulguların diğer çalışmalarla kıyaslanması.

References/Regions	Cadmium	Cobalt	Chromium	Copper	Manganese	Nickel	Lead	Zinc
[1]South Eastern Black Sea	**0.08-0.10**	**0.01-0.16**	**0.49-2.30**	**0.26-1.18**	**0.11-4.41**	**1.07-21.2**	**0.10-0.57**	**5.46-16.9**
[2]Black Sea Coasts	<0.02-0.24	<0.05-0.40	<0.06-0.84	1.01-4.54	0.69-3.56	<0.01-2.04	<0.05-0.60	25.7-44.2
[3]Iskenderun Bay	0.34-2.49	0.53-2.85	1.14-3.29	0.64-2.98	0.73-2.64	1.18-8.15	1.33-4.15	1.85-6.33
[4]United Arab Emirates	0.13-2.89	0.002-0.02	0.31-0.73	1.63-24.91	0.12-2.10	-	0.004-0.12	3.71-123
[5]Black and Aegean Seas	0.45-0.90	-	0.95-1.92	0.73-1.83	1.28-7.40	1.92-5.68	0.33-0.93	37.4-106
[6]Turkish Seas	0.02-0.37	0.04-0.41	0.04-1.75	0.32-6.48	0.10-0.99	0.02-3.97	0.33-0.86	4.49-11.6
[7]South Western Black Sea	0.05-0.40	<0.01-0.06	0.02-0.43	0.92-4.13	0.06-1.26	0.14-3.74	0.25-4.58	3.47-32.1

[1]This study; [2]Topçuoğlu et al. 2002; [3]Türkmen et al. 2005; [4]Kosanovic et al. 2007; [5]Uluözlü et al. 2007; [6]Türkmen et al. 2008; [7]Türkmen & Dura, 2016.

Table 3. Comparison of the findings in the present study with recommended intakes.
Tablo 3. Mevcut çalışmadaki bulguların tavsiye edilen alımlarla kıyaslanması.

Metal	PTWI[*]	PTWI[b]	PTDI[c]	EWI[d] (EDI)[e]
Cd	7[a]	490	70	14 (2)
Co	-	-	-	22.4 (3.2)
Cr	-	-	-	322 (46)
Cu	3500[a]	245000	35000	165 (23.6)
Ni	35[g]	2450	350[f]	**3360 (480)**
Mn	980[i]	68600	9800[h]	617 (88.2)
Pb	25[a]	1750	250	79.8 (11.4)
Zn	7000[a]	490000	70000	2366 (338)

[a](FAO/WHO, 2004).
[b]PTWI for 70 kg adult person (µg/week/70 kg body weight).
[c]PTDI, Permissible Tolerable Daily Intake (µg/day/70 kg body weight).
[d]EWI, Estimated Weekly Intake in µg/week/70 kg body weight.
[e]EDI, Estimated Daily Intake in µg/day/70 kg body weight.
[f]WHO recommends a TDI (Tolerable Daily Intake) of 5 µg/day/kg body weight, i.e. 350 µg/day for a 70-kg person (WHO, 2014).
[g]Calculated for a week (µg/week/kg body weight)
[h]EPA recommends a RfD (Reference Dose) of 0.14 mg/day/kg body weight, i.e. 9800 µg/day for a 70-kg person (EPA, 2014).
[i]Calculated for a week (µg/week/kg body weight)
[*]Provisional Tolerable Weekly Intake (PTWI) in µg/week/kg body weight
[**] Mean weekly fish consumption in Turkey is 0.14 kg per person (FAO, 2014).

CONCLUSION

Consequently, the present results provide important information on metal contaminations in fish from the investigated area, and possibly could provide us with a clearer picture of the region's overall environmental contamination situation. Furthermore, these findings can also be used in order to understand the chemical quality of the fish, as well as to evaluate the possible risks that associated with human consumption. As has been previously stated, PTWI and PTDI values in fish muscles were well below the recommended levels by authorities with the exception of nickel (FAO/WHO, 2004; EPA, 2014; WHO, 2014). In turn, the consumption of species from the Central and Western Black Sea regions currently does not appear to pose an overall problem to human health with the exception of nickel for *E. encrasicolus* from station GRS. On the other hand, in the future, the accumulation of nickel and remaining heavy metals in the examined species in this study can pose a possible risk for their consumption, if agricultural, shipping, industrial, and recreational practices in the surrounding Black Sea region is not controlled.

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