Heavy Metal Concentrations in the Muscle Tissues of Seven Commercial Fish Species from Sinop Coasts of the Black Sea

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1. Introduction

Anthropogenic activities such as mining, industry and agriculture lead to elevate the metal concentrations in aquatic environment over the last few decades, which result in adverse effects on not only aquatic animals but also human being via food chain [2,6,21,30]. Among these pollutants, heavy metals constitute a considerable part of the aquatic pollution. Aquatic organisms absorb heavy metals, essential or not, from the surrounding environment [2] and they may reach the food chain through various biochemical processes such as bioconcentration, bioaccumulation and eventually threaten human health via seafood consumption [21,32].

Fisheries is one of the most important food production sectors of supplying protein for human consumption. According to Food and Agriculture Organization, world fisheries production has reached 154 million tons in 2011 [13]. In Turkey, marine fisheries comprise an important part of the total catch. Total marine fisheries production reached a peak level of 277,773t in 2009 and 73% of the total catch was captured in Black Sea [34]. However, it is known that Black Sea receives tons of zinc, lead, mercury, copper, chromium and many other wastes from rivers or direct discharge of industrial wastes without any effective treatment [23]. Therefore, the water quality of the Black Sea has been severely relapsed [12] which also pose a risk for human health via food

chain. Therefore, monitoring the contents of trace metals in marine organisms inhabiting Black Sea, consumed as sea food, is an extremely important issue for human health.

In the present study, heavy metal levels in seven commercial fish species (Twaite shad *Alosa fallax nilotica*, European Anchovy *Engraulis encrasicolus*, Whiting *Merlangius merlangus euxinus*, Bluefish *Pomatomus saltator*, Atlantic Horse Mackerel *Trachurus trachurus*, Red Mullet *Mullus barbatus* and Turbot *Scophthalmus maximus*) from Sinop coasts of the Black Sea were investigated. The fish species chosen are commercially important and considered one of the most prominent sources of fisheries in Black Sea, Turkey [24] and were used for the determination of chromium (Cr), iron (Fe), copper (Cu), zinc (Zn), cadmium (Cd) and lead (Pb) contents in the muscle tissue. In addition, current findings from Sinop coasts were compared to recent data in the Black Sea, Turkey, thus creating a database for further studies.

2. Materials and research methods applied

The Black Sea is a semi-closed sea with a maximum the depth of 2,200 m and it receives significant amount of freshwater from rivers. The salinity is very low due to the fact that the water loss by evaporation is less than the input of freshwater [28]. The most distinctive feature of the Black Sea is the presence of an anoxic layer below 150–200 m, due to the lack of water exchange [25].

Fish samples were collected at a depth of 15–25 m with the help of local fishermen from the coastal waters of Sinop, Turkey on November 2011. A total of randomly selected 12 to 20 fish from each of the seven species were collected within the 2 different stations (Fig. 1). The sampling stations chosen at localities characterised by intensive anthropogenic activities such as shipping. The average weight and length of the fish are summarized in Table 1. The physical and chemical properties of the water at each station were also recorded. The parameters included Secchi depth (m), temperature (°C), electrical conductivity (µS/cm), salinity ‰, total dissolved solids (mg/L), dissolved oxygen (mg/L) and pH, which were measured using a Secchi disk and a YSI 6600-V2 Sonde, respectively.
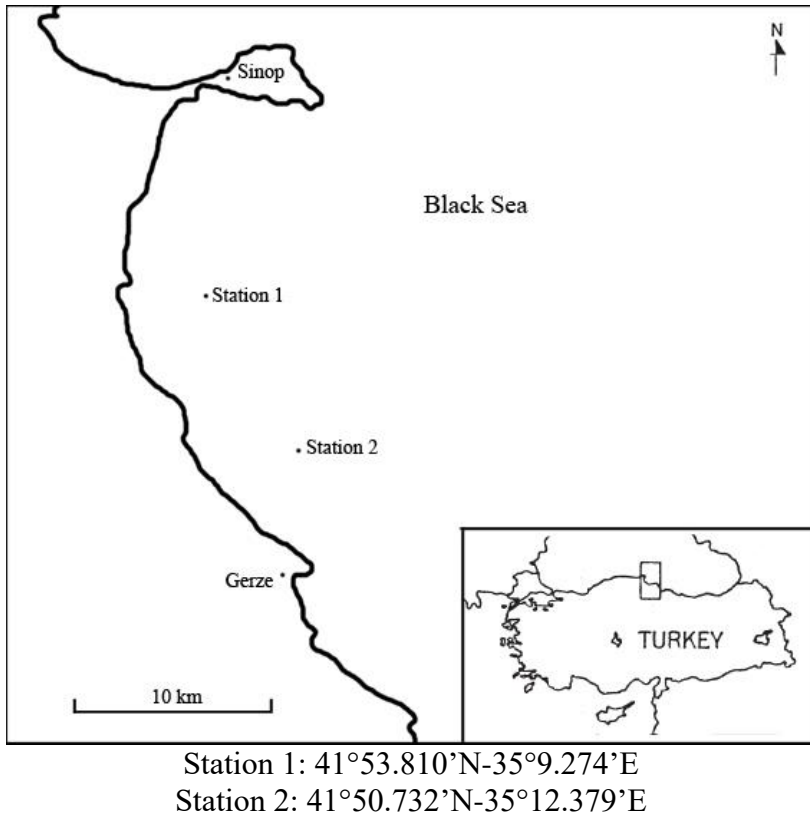
Station 1: 41°53.810'N-35°9.274'E
Station 2: 41°50.732'N-35°12.379'E

Fig. 1. Study area and sampling stations
Rys. 1. Obszar badań i miejsca pobierania próbek

Table 1. Mean length and weight of the fish used in this study
Tabela 1. Średnia długość i waga badanych ryb

Fish species	N	Length (cm)	Weight (g)
A. f. nilotica	16	20.8±4.9	190.2±16.7
E. encrasicolus	20	14.6±2.5	17.8±4.7
M. m. euxinus	20	19.7±5.8	65.4±12.3
P. saltator	18	19.6±5.7	90.2±13.5
T. trachurus	20	15.6±3.4	48.9±8.5
M. barbatus	20	14.9±4.6	54.4±7.9
S. maximus	12	32.6±10.9	604.7±41.6

Fish were rinsed in clean sea water and then placed in plastic bags and transferred to the laboratory on ice and kept frozen at -20℃ until their analysis. Five grams of muscle tissue prepared separately from each fish and were placed in petri dishes and oven dried for 24 h at 105°C. Microwave digestion method was performed to digest samples which involved a mixture of 0.5 g dried fish muscle tissue, 8 ml HNO_3 and 2 ml H_2O_2 in digestion vessels (Berghof Speedwave MWS Four, Germany) and the samples were analyzed in 3 replicates by using Inductively Coupled Plasma Mass Spectrometer (ICP-MS) (Agilent 7500a). All the reagents were of analytical grade and all laboratory glassware were soaked in 10% HNO_3 and rinsed with bidistilled water prior to use.

Statistical analysis of data was carried out using SPSS (version 9.0) statistical package program. All results were expressed as mean±standard deviation (μg/g) of three parallel measurements. One-Way ANOVA and Duncan's post hoc analysis were performed to test the significance of the difference of heavy metal contents among fish species. Results were considered to be significant where *P<0.05*.

3. Results

The mean variations in Secchi depth (m), temperature (°C), electrical conductivity (μS/cm), salinity ‰, total dissolved solids (mg/L), dissolved oxygen (mg/L) and pH were as follows; Secchi depth ranged from 8.5 to 9.2 m, temperature from 15.7 to 17.1 °C, electrical conductivity from 17982 to 22749 μS/cm, salinity from 16.58 to 17.17 ‰, total dissolved solids from 16.22 to 17.96 mg/L, dissolved oxygen from 6.07 to 6.64 mg/L and pH from 8.02 to 8.23.

Mean concentrations and standard deviations for Cr, Fe, Cu, Zn, Cd and Pb in the muscle tissues of fish collected from Sinop coasts in the Black Sea are shown in Table 2 a–f. The heavy metal levels in the muscle tissues of fish in decreasing order were:
Fe>Zn>Cu>Pb>Cr>Cd for *A. f. nilotica*,
Fe>Zn>Cu>Cr>Pb>Cd for *E. encrasicolus*,
Fe>Zn>Cu>Pb>Cr>Cd for *M.m. euxinus*,
Fe>Zn>Cu>Cr>Pb>Cd for *P. saltator*,
Fe>Zn>Cu>Cr>Pb>Cd for *T. trachurus*,
Fe>Zn>Cu>Pb>Cr>Cd for *M. barbatus*,
Fe>Zn>Cr>Cu>Pb>Cd for *S. maximus*.

Table 2 a. Heavy metal levels in the muscles tissues of fish and comparision of recent data: a (Chromium); b (Iron); c (Copper); d (Zinc); e (Cadmium); f (Lead)
Tabela 2 a. Poziom metali ciężkich w tkankach mięśniowych ryb i porównanie ich z aktualnymi danymi: a (chrom); b (żelazo); c (miedź); d (cynk); e (kadm); f (ołów)

		References							
Chromium (Cr)	**Present study**	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**
A. f. nilotica	0.57±0.12[b]	-	-	-	-	-	-	-
E. encrasicolus	0.87±0.31[a]	1,98	-	0.15	-	0.074	1.12	-
M. m. euxinus	0.63±0.16[b]	0.97	0.19	-	-	0.144	0.86	0.82
P. saltator	0.63±0.18[b]	1.92	-	-	0,18	-	0.82	-
T. trachurus	0.66±0.19[b]	0.95	-	-	-	-	1.74	0.95
M. barbatus	0.62±0.16[b]	1.63	0.15	-	-	1.055	1.35	0.99
S. maximus	1.24±0.38[c]	-	-	-	-	-	1.20	-

Table 2 b.
Tabela 2 b.

Iron (Fe)	Present study	References											
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI[*]	XII
A. f. nilotica	49.66±13.75[c]	-	-	-	-	-	-	-	-	-	16.08	-	33.78
E. encrasicolus	110.86±21.66[a]	95.6	-	35.7	-	18.08	75.7	-	4.87	-	10.45	-	26.06
M. m. euxinus	62.80±19.61[b]	104.0	81.9	-	-	4.48	98.1	27.7	9.04	-	-	57.2	28.84
P. saltator	43.86±10.16[bc]	68.6	-	-	40.3	-	110.0	-	-	21.0	-	421.3	23.81
T. trachurus	48.81±8.36[c]	74.3	-	-	-	-	145.0	36.4	4.28	-	32.40	-	21.17
M. barbatus	34.34±13.12[d]	163.0	21.8	-	-	21.27	53.2	41.4	4.18	-	-	74.3	29.17
S. maximus	48.60±9.06[c]	-	-	-	-	-	36.2	-		-	-	113.3	21.72

Table 2 c.
Tabela 2 c.

Copper (Cu)	Present study	References												
		I	II	III	IV	VI	VII	VIII	IX	X	XI[*]	XII	XIII	XIV
A. f. nilotica	1.99±0.58[c]	-	-	-	-	-	-	-	-	2.93	-	-		-
E. encrasicolus	4.76±1.37[a]	0.95	-	1.12	-	1.96	-	0.69	-	1.96	-	2.73	-	-
M. m. euxinus	1.20±0.31[bc]	1.25	2.90	-	-	1.32	1,8	0.88	-	-	18.54	3.72	0.12	0.91-8.95
P. saltator	1.67±0.61[c]	1.83	-	-	2,97	2.78	-	-	0.58	-	35.6	2.86	-	-
T. trachurus	1.39±0.43[bc]	0.95	-	-	-	0.65	2,4	0.79	-	1.55	-	1.79	-	-
M. barbatus	0.95±0.41[b]	0.98	0.87	-	-	0.96	1,4	0.76	-	-	26.98	3.14	-	0.38-2.71
S. maximus	0.75±0.25[b]	-	-	-	-	0.75	-	-	-	-	26.14	2.13	-	-

Table 2 d.
Tabela 2 d.

Zinc (Zn)	Present study	References												
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI[*]	XII	XIV
A. f. nilotica	15.79±2.64[b]	-	-	-	-	-	-	-	-	-	20.41	-	-	-
E. encrasicolus	72.46±17.77[a]	40.2	-	10.6	-	25.41	38.8	-	3.55	-	18.85	-	26.25	-
M. m. euxinus	16.34±3.83[b]	48.6	12.9	-	-	6.02	65.4	27.7	4.36	-	-	38.47	31.34	8.86-163.2
P. saltator	17.62±3.51[b]	35.4	-	-	15.9	-	93.4	-	-	9.40	-	82.2	25.51	-
T. trachurus	17.35±3.36[b]	37.4	-	-	-	-	52.7	25.7	3.28	-	11.41	-	27.70	-
M. barbatus	17.15±3.78[b]	106.0	10.5	-	-	7.57	75.5	17.8	2.42	-	-	29.79	23.71	1.42-63.29
S. maximus	21.40±5.38[b]	-	-	-	-	-	45.2	-		-	-	170.5	24.83	-

Table 2 e.
Tabela 2 e.

Cadmium (Cd)	Present study	I	II	III	IV	V	VI	VII	VIII	IX	X	XI[*]	XII	XV
A. f. nilotica	0.071 ± 0.035^{c}	-	-	-	-	-	-	-	-	-	0.35	-	-	-
E. encrasicolus	0.037 ± 0.013^{ab}	0.65	-	0.02	-	0.124	0.27	-	0.025	-	0.18	-	0.035	-
M. m. euxinus	$0.027 \pm 0.^{012ab}$	0.55	0.04	-	-	0.192	0.21	0.18	0.025	-	-	0.355	0.002	<0.02
P. saltator	0.045 ± 0.014^{b}	0.60	-	-	0.03	-	0.23	-	-	0.05	-	0.343	0.025	-
T. trachurus	0.033 ± 0.017^{ab}	0.50	-	-	-	-	0.32	0.22	0.028	-	0.48	-	0.012	-
M. barbatus	0.035 ± 0.018^{ab}	0.45	0.03	-	-	0.208	0.17	0.23	0.023	-	-	0.227	0.020	<0.02
S. maximus	0.021 ± 0.005^{a}	-	-	-	-	-	0.10	-		-	-	0.272	0.022	-

Table 2 f.
Tabela 2 f.

Lead (Pb)	Present study	References											
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI[*]	XIII
A. f. nilotica	0.68±0.31[b]	-	-	-	-	-	-	-	-	-	0.52	-	-
E. encrasicolus	0.41±0.13[a]	0.33	-	0.27	-	0.33	0.30	-	0.78	-	0.39	-	-
M. m. euxinus	0.69±0.34[b]	0.93	0.46	-	-	0.50	0.53	0.46	0.74	-	-	2.18	0.03-1.76
P. saltator	0.40±0.13[a]	0.38	-	-	0.36	-	0.87	-	-	0.55	-	2.25	-
T. trachurus	0.44±0.21[a]	0.68	-	-	-	-	0.82	0.64	0.74	-	0.83	-	-
M. barbatus	0.82±0.34[b]	0.84	0.39	-	-	0.72	0.36	0.40	0.28	-	-	1.27	-
S. maximus	0.42±0.10[a]	-	-	-	-	-	0.28	-	-	-	-	2.72	-

* based on wet weight
I   : Uluözlü et al., 2007 [37]
II   : Tepe et al., 2008 [27]
III   : Türkmen et al., 2008 [30]
IV   : Türkmen et al., 2009 [31]
V   : Turan et al., 2009 [35]
VI   : Tüzen, 2009 [33]
VII   : Durali et al., 2010 [10]
VIII   : Bat et al., 1996 [5]
IX   : Bat and Öztürk, 1997 [4]
X   : Tüzen, 2003 [32]
XI   : Bat et al., 2006 [3]
XII   : Nisbet et al., 2010 [21]
XIII   : Ünsal et al., 1992 [36]
XIV   : Türk et al., 2007 [29]
XV   : Daş et al., 2009 [8]

Among all the metals monitored, Fe was the highest and Cd was the lowest in all of the fish species. The maximum values of Fe, Cu and Zn were recorded in *E. encrasicolus*, while the maximum values of Cr, Cd and Pb were recorded in *S. maximus*, *T. trachurus* and *M. barbatus*, respectively. The minimum values of Cr and Fe were recorded in *M. barbatus*, Cu and Cd in *S. maximus*, Zn and Pb in *M.m. euxinus* and *P. saltator*, respectively.

The results of One-Way ANOVA and Duncan's post hoc analysis were also shown on Table 2 a–f. It was found that, metal levels in the muscle tissue of fish was considered to be significant between fish species ($P<0.05$). The most distinct pattern was observed for Zn; zinc content of the fish was similar in all fish species except for *E. encrasicolus*. While the other monitored metals did not show a marked pattern.

4. Discussion of results

Heavy metals are natural trace elements found in the aquatic environment, but domestic, industrial, mining and agricultural activities lead to increase their levels [6]. Discharge of industrial waters polluted with heavy metals into aquatic environment may change aquatic species

diversity and ecosystem due to their toxicity and accumulation. In addition, although some heavy metals such as iron, zinc, copper are considered to be essential since they have a role in many enzyme systems, heavy metals such as lead and cadmium are not believed to be essential for health even in trace amounts [7]. Chronic low level intake of heavy metals via food chain has adverse effects on human beings and other animals because there is no effective mechanism for their elimination from the body [15]. Thus, accurate information on heavy metal concentrations in aquatic ecosystems is needed, especially for the sea food consumed by humans. Since the Black Sea is the main source of marine fisheries is in Turkey and in countries located in the Black Sea basin [34], monitoring the heavy metal levels in marine organisms is an extremely important issue for human health.

The heavy metal levels in various fish species from the Black Sea have been investigated by several researchers [2,6,21,30]. The results for heavy metal levels reported by several researchers and our results are summarized in Tables 2 a–f. Only a few studies monitored the Cr levels in fish from the Black Sea and there were no available data for Cr levels in *A. f. nilotica* in Black Sea. Highest Cr levels were obtained from *S. maximus* and lowest from *A. f. nilotica*. Chromium levels showed a significant difference between fish species (*P<0.05*) and its range in fish muscle tissue was reported as 0.074–1.98 µg/g (Table 2a). It is stated that there is no significant biomagnification of chromium in aquatic food webs [1].

Iron levels in fish showed the highest level of variation both in our and other studies listed in Table 2b and Fe levels were highest in *E. encrasicolus* and lowest in *M. barbatus*. Iron levels in fish showed significant differences between fish species (*P<0.05*) its range was reported between 4.18 and 163.0 µg/g (Table 2b). Copper levels in fish were similar to those listed (Table 2c), except for Bat et al. [3] in which heavy metal levels were given as wet weight. Copper levels based on dry weight were reported as 0.12–3.72 µg/g (Table 2c). In our study Cu levels were highest in *E. encrasicolus* and lowest in *S. maximus*. Zinc levels also showed a high degree of variation among the literature listed in Table 2d and ranged between 1.42 and 163.2 µg/g (dry weight) in the literature. In our study Zn levels were highest in *E. encrasicolus* and lowest in *A. f. nilotica*. Zinc levels in fish were similar in all fish species except for *E. encrasicolus* (*P<0.05*) (Table 2d).

Cadmium levels were reported as >0.10 in most of the studies listed in Table 2e and Cd levels (dry weight) varied among the literature with a range of 0.02–0.65 µg/g. However, in our study highest Cd levels were recorded from *A.f. nilotica* (0.071 µg/g) and lowest from *S. maximus* (0.021 µg/g). Since *A. f. nilotica* is the only fish species in this study that enters freshwaters for reproduction and shad fry live 1–2 years in freshwaters, it is reasonable to conclude that the main source of Cd pollution in Sinop coasts of Black Sea is the rivers that run in to Black Sea. Kilgour [18] stated that bottom-dwelling animals may accumulate Cd. However, in our study Cd levels were not highest in benthic fish species such as *M. barbatus* and *S. maximus*, but in pelagic species such as *A.f. nilotica* and *P. saltator*. Thus it seems that Cd uptake from water is a more important route than uptake from sediment for non-benthic fish. However, it is also possible that *A. f. nilotica* has a higher potential to bioaccumulate Cd.

Lead levels in this study were similar to those listed in Table 2f except for Bat et al. [3] in which heavy metal levels were given as wet weight. The range for Pb levels from fish was reported as 0.03–1.76 µg/g (Table 2f). In our study lead levels were highest in *M. barbatus* and lowest in *P. saltator*. It is stated that Pb levels tend to decrease with increasing trophic levels [11], which was also confirmed in this study; lowest Pb levels was found in *P. saltator* which has a higher position in food chain. It was found that levels of Fe, Cu and Zn were higher than Cd and Pb in each fish species studied. A similar finding was also reported by Wong et al. (2001) [38]. This may simply result from the fact that Fe, Cu and Zn are essential metals while metals such as Cd and Pb are non-essential.

As shown in Tables 2a–f there were discrepancies among the heavy metal levels reported by different authors, even in the same fish species. It has been showed that the accumulation of heavy metals in a tissue is mainly dependent on concentrations of metal and exposure time, and the other environmental factors such as salinity, pH, hardness and temperature [7,21]. Furthermore, it is also known that the metal accumulation in fish can be species specific [16]. The observed differences between the metal concentrations in fish species may be related to their feeding habits and/or the bioconcentration capacity of each species [14]. It is stated that higher concentrations of the metals are found in bottom feeders as compared to plankton feeders and/or pelagic carnivores [19]. This statement was valid only for Cr levels in *S.*

maximus and Pb levels in *M. barbatus*. However, for the other metals, it was found that metal concentrations were lower in pelagic fish species (*A. f. nilotica*, *E. encrasicolus*, *P. saltator* and *T. trachurus*) when compared to benthic (such as *M. barbatus* and *S. maximus*) and/or bentho-pelagic fish species (such as *M. m. euxinus*). Furthermore, the discrepancies between the studies even for same heavy metal in same fish species seems to be a site spesific issue.

5. Conclusions

The results presented above clearly demonstrate that the Sinop coasts of Black Sea seems to be polluted with Pb and to a lesser extent with Cd. Although, it is known that Pb does not bioaccumulate [11] and the bioavailability of Pb in marine environment is low [17], constant exposure to Pb may cause serious threats to human health via seafood consumption. It was found that *P.maxima* is the most risky fish species for Cr levels, *E. encrasicolus* for Fe, Cu and Zn levels, *A. f. nilotica* for Cd and *M. barbatus* for Pb levels for human consumption. Although, Fe, Cu, Zn and to a lesser extent Cr are essential metals, they can be toxic at chronic exposure to low levels. Furthermore metals like Cd and Pb are non-essential and very toxic even at very low levels [20,22].

Mining and agricultural activities on land may be an important source in the delivery of some metal pollutants in the coastal regions of the Black Sea. However, research on the heavy metal pollution in marine biota of the Black Sea is very limited. Moreover, corresponding data on the pollution state of the Black Sea of Turkey are rare [2] which constitute need for further studies. Since the fish species chosen for this study are of economical importance for all Black Sea, the pollution in Turkish coasts of Black Sea is not a specific problem for Turkey; indeed it is a common concern for all the countries located in the Black Sea basin. Thus preventive measures against pollution should be taken which needs a collaborative approach. Due to the risk of entrance of contaminants such as heavy metals even at low levels to the food chain via marine organisms, it is important to monitor the levels of heavy metals in aquatic environments.

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Stężenia metali ciężkich w tkankach mięśniowych siedmiu gatunków ryb handlowych z wybrzeża Morza Czarnego (Sinop, Turcja)

Streszczenie

W pracy analizowano zawartość metali ciężkich w tkance mięśniowej siedmiu gatunków ryb (*Alosa fallax nilotica, Engraulis encrasicolus, Merlangius merlangus Euxinus, Pomatomus saltator, trachurus trachurus, Mullus Barbatus, Scophthalmus maximus*) z wybrzeży Morza Czarnego (Sinop, Turcja). Badane metale ciężkie to: cynk (Zn), miedź (Cu), żelazo (Fe), chrom (Cr), kadm (Cd) i ołów (Pb). Ponadto, wyniki badań z wybrzeży Sinop porównano z ostatnimi danymi uzyskanymi na obszarze Morza Czarnego w Turcji, tworząc w ten sposób bazę danych dla dalszych badań. Zawartość Fe okazała się najwyższa, podczas gdy zawartość Cd była najniższa w przypadku wszystkich gatunków ryb, spośród wszystkich badanych metali. Stwierdzono, że stężenie Fe, Cu i Zn były wyższe niż Cd i Pb we wszystkich badanych gatunkach ryb. Poziomy Pb były wyższe od dopuszczalnych we wszystkich gatunkach ryb, co wyraźnie wskazuje na to, że wybrzeże Morza Czarnego w okolicach Sinops jest mocno zanieczyszczone ołowiem.

Słowa kluczowe: metale ciężkie, toksyczność, owoce morza
Key words: heavy metals, toxicity, seafood