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TOXIC METALS IN *Engraulis encrasicolus* (LINNAEUS, 1758) FROM THE COASTAL WATERS OF SINOP IN THE BLACK SEA

ABSTRACT

The aim of this study is to determine the amounts of As, Cd, Hg and Pb in the muscle tissue of *Engraulis encrasicolus* caught from Sinop coasts of the Black Sea. Metal analysis was performed by ICP-MS. Pb showed the highest concentration followed by As, Hg and Cd. The presence of four toxic metal levels was determined in the edible tissues of *E. encrasicolus* however, in quantities even below the allowable limits set by the Turkish Food Codex (TFC), EU Commission Regulation and other international standards. As has the highest concentration (0.13 mg/kg wet wt.) among the toxic metals as shown in Figure 3 which followed by Pb and Hg with levels of 0.09 and 0.055 mg/kg wet wt. The highest Cd level was 0.033 mg/kg wet wt. The total of Target Hazard Quotient values for each element was less than 1, and it was concluded that anchovy consumption was safe for human health.

Keywords: *Engraulis encrasicolus*, Black Sea, Sinop Shores, Estimated Daily Intake, Target Hazard Quotient

1. INTRODUCTION

In the marine ecosystem, toxic metals are accumulated by fish either directly from water or through their diet or both. However, concentration of bioaccumulation of metals depends on the bioavailability of each element in the surroundings, storage, excretion mechanisms and ingestion rate. Non-essential toxic metals such as cadmium, arsenic, mercury and lead are of no benefit to living organisms and have a detrimental effect. Toxic metal levels in fish can counteract their beneficial effects leading to adverse effects of heavy metals on human health including serious threats such liver damage, cardiovascular diseases, renal failure and even death. Fish is important source of protein, having rich contents of unsaturated fatty acids, essential minerals and vitamins which are essential in people health [1]. The current heavy metal resources of the Black Sea are discussed in one of the recent reviews [2]. Pollution loads carried not only by the countries that have a coast to the Black Sea but also from Europe, especially the large rivers such Danube flowing into the Black Sea [3], affect this unique ecosystem [4] negatively (Figure 1). Sinop is one of the smallest cities on the Black Sea coast and does not have an industry. Sinop city has been depend on fishing as the main livelihood for the coastal fishermen [5]. Anchovy is the most commercial fish species in the Black Sea [6]. It is a preferred fish by humans. They are consumed as frying, steaming, baking, grilling, stuffed rice and so on.

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Figure 1. Under the various pollution effect of the Black Sea Basin [2]

The aim of this study is to determine the concentration of As, Cd, Hg and Pb in the edible tissues of *Engraulis encrasicolus* caught in Sinop coasts of the Black Sea and if the metal levels are within the permissible limits set by the Turkish Food Codex (TFC) [7 and 8] EU Commission Regulation [9 and 10], Georgian Food Safety Rules [11] and GAIN Report Russian Federation [12]. In addition, health risks of these toxic metals were consumed by people who consumed anchovy.

2. RESEARCH SIGNIFICANCE

In this investigation, concentrations of four toxic heavy metals (As, Pb, Hg and Cd) in *Engraulis encrasicolus* from Sinop coasts of the southern Black Sea and calculate indices of human health risk related to the consumption of its edible tissues.

3. MATERIALS AND METHODS

In this study, anchovy samples that were caught during the 2017 fishing season in Sinop coasts (Figure 2) were purchased from fishing markets. Total lengths of specimen were recorded from the fish samples and rinsed in clean water. The anchovies were between 8 and 12cm in length. Fresh fish specimens were dissected using a sharp plastic knife, removing edible tissues of the fish and sealed in a plastic bag. A total of 100 individuals were analyzed, 25 for each sampling month. All samples were put deep frozen at -21°C till their analysis. Metal analysis in anchovy was performed using m-AOAC 999.10- ICP/MS method by accredited Environment Industrial Analysis Laboratory Services Trade Company (TÜRKAK Test TS EN ISO IEC 17025 AB-0364-T). The method for determination of elements, used acid, standard reference material, wet digestion was used by European standard method with number EN 15763. The limits of detection used for analysis of arsenic, cadmium, mercury and lead were 0.05, 0.03, 0.05 and 0.05, respectively.

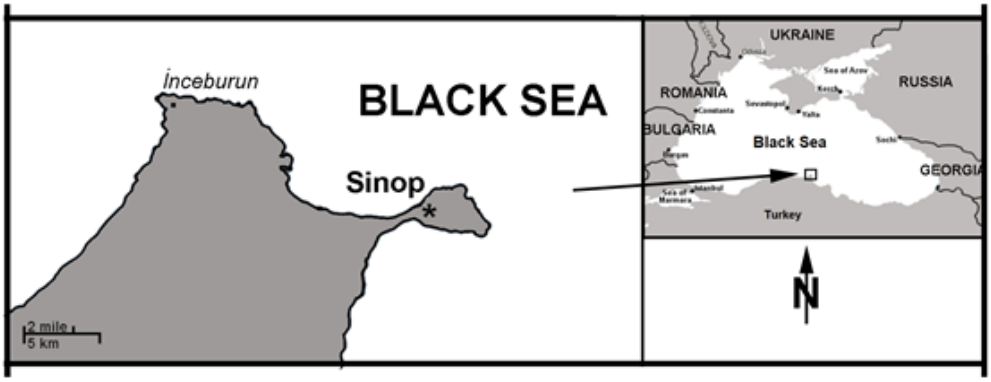

Figure 2. The study area, Sinop coasts of the Black Sea

3.1. Assessments Target Hazard Quotient (THQ) of Heavy Metals in Anchovy

Hazard from metals entering owing to ingestion may be defined using a THQ as the rate of the estimated daily intake (EDI) mg/kg of body wt. and the reference dose (RfD mg/kg.). The THQ was computed by using the equation below [13]:

$THQ=EDI/RfD$

The EDI depends on both the metal amount and the quantity of consumption of anchovy. The EDI of metals was calculated using the equation below:

$EDI=C_{metal} \times W_{anchovy}/Bw$

Where: C_{metal} is the metal levels in anchovy; $W_{anchovy}$ represents the daily mean consumption of fish; Bw is the body weight of an adult (kg). $W_{anchovy}$ represents the daily mean ingestion of fish is given as: 0.013 for infants, 0.027 for children and 0.041 for adults (kg/day); Bw is the body weight of 10 kg for infants, 30 kg for children and 70 kg for adults [14]. According to the Risk Assessment Information System (RAIS) and [16] U.S. Environmental Protection Agency (USEPA)the Rf. D for As, Hg, Cd and Pb are 0.0003, 0.0003, 0.001 and 0.0035mg/kg/day, respectively [15 and 16].

The sum of THQs calculated for each element gives TTHQs.

$TTHQ=THQ(As)+THQ(Pb)+THQ(Hg)+THQ\ (Cd)$.

If TTHQ>1.0, so the EDI of a certain element overruns the RfD, pointing out that there is a possible hazard associated with that element. Statistical analysis was performed using the SPSS, version 21. One-way ANOVA with post hoc test analyses based on Tukey was used to compare the difference between the concentrations. P-value of <0.05 or less was considered statistically significant [17].

4. RESULTS AND DISCUSSION

Results showed that As has the highest concentration (0.13mg/kg wet wt. in October) among the toxic metals as shown in Figure 3 which followed by Pb and Hg with levels of 0.09 and 0.055mg/kg wet wt. in December. The highest Cd level was 0.033mg/kg wet wt. in October. One-way analysis of variance showed that there were significance differences ($p<0.05$) in the amounts of four toxic metals in the edible tissues of anchovy.

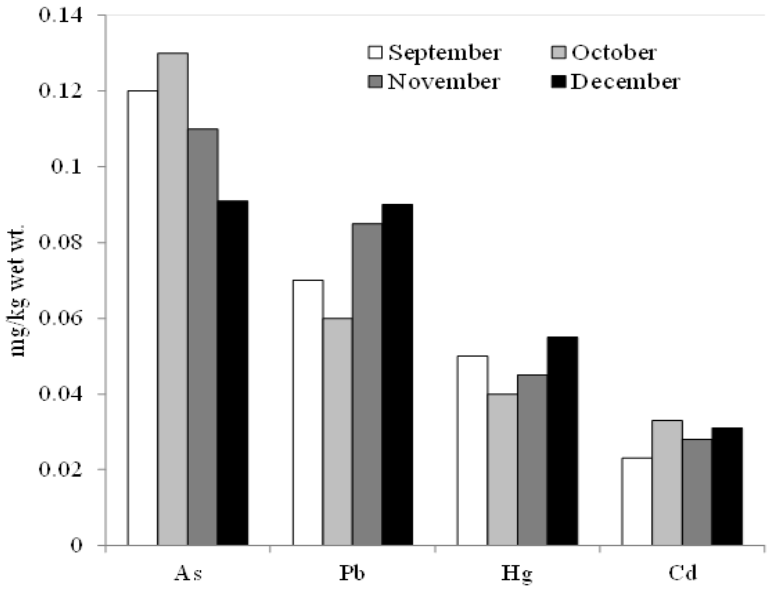

Figure 3. As, Pb, Hg and Cd in the edible tissues of *E. enrasicolus* from the Black Sea coasts in fishing season of 2017

The calculated EDI, THQ and TTHQ values of anchovy from Sinop coasts in the Black Sea for infants, children and adults are presented in Table 1.

Table 1. Estimated Daily Intakes (EDI), Target Hazard Quotients (THQ) and Total Target Hazard Quotients (TTHQ) of toxic metals in edible tissues of anchovy from the Black Sea

Toxic Metals	EDI mg/day/kg body wt.			THQ		
	Infants	Children	Adults	Infants	Children	Adults
As	0.0001465	0.0001014	0.0000660	0.48858	0.33825	0.22013
Pb	0.0000991	0.0000686	0.0000446	0.33041	0.22875	0.14886
Hg	0.0000617	0.0000427	0.0000278	0.06175	0.04275	0.02782
Cd	0.0000380	0.0000263	0.0000171	0.01086	0.00752	0.00489
			TTHQ=	0.89160	0.61727	0.40170

Results of the present study show low metal amounts than the maximum permissible limits in anchovy as recommended by national and international regulations (Table 2), indicates that levels of these metals in the edible tissues of anchovy may not considered toxic and imply to be safe for people consumption as shown on Table 1. Metal amounts may vary among individuals depending on their feeding habits, habitats, age, size and length of the fish and seasons [18]. As, Pb, Hg and Cd are important toxic metals that no play role in biochemical process for fish.

Table 2. The tolerable values of measured toxic metals in the edible tissues of fish (mg/kg wet wt.)

Standards	As	Cd	Pb	Hg
Turkish Food Codex [7]	--	0.1	0.4	0.5
Turkish Food Codex [8]	--	0.05	0.3	0.5
EU Commission Regulation [9]	--	0.05	0.3	0.5
EU Commission Regulation for anchovy [10]	--	0.25	--	--
Georgian Food Safety Rules [11]	2.0	0.2	1.0	0.5
GAIN Report Russian Federation [12]	2.0	0.2	1.0	0.5
Australia and New Zealand Food Standards [13]	2.0	--	0.5	0.5

The UK previously imposed a limit of 1mg/kg for arsenic in food with separate limits applicable to certain food categories. These regulations were revoked in 2002. There is no maximum level set for arsenic in foods at TFCs [7 and 8]and EU regulations [9 and 10] levels. However, the tolerated arsenic values of other international organizations [11, 12 and 13] are shown in Table 2. In this study, the amounts of As, Pb, Hg and Cd, which are toxic in the consumed muscles of anchovy, were found to be below the values approved by national and international organizations [7, 8, 9, 10, 11, 12 and 13]. When the results of this study are compared with other studies, it is seen that toxic metal levels in anchovy are generally lower or in similar to those in recent reviews [19, 20 and 21].

5. CONCLUSION AND RECOMMENDATIONS

The present study also focused on these toxic metal amounts in anchovy muscles since most of the local people include this portion in their daily diet in which for average infants (10 kg body wt.), children (30 kg body wt.) and adults (70 kg body wt.). Calculated THQ values for each toxic metal are given in Table 1. The sum of THQ values obtained from these results was found to be lower than 1 for infants, children and adults. Considering that the weight of the infants is low, it is normal to have high values compared to children and adults. The amount specified by internationally authorized institutions and organizations is the consumption of two portions of fish per week. We believe that paying attention to this will be beneficial for health. Based on the results of the present study four toxic metal levels are present, however, in small quantities. It is known that the muscle is not an active tissue in accumulating metals. Since all these metal amounts are less than the values described by TFC and is considered safe for consumption.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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