Heavy metal levels in anchovy (*engraulis encrasicolus L.*) from Bulgarian Black Sea coast

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Abstract

Determination of heavy metal levels in fish is a substantial indicator for the pollution degree in a marine ecosystem. The levels of the elements As, Pb, Cd, Hg, Mn, Zn and Al were determined in the total bodies of a commercially valueble pelagic fish *Engraulis encrasicolus* (Linnaeus, 1758) captured from two of the most important fishing municipalities in the western Black Sea. Samples were collected during the fishing season between June and September in 2020 from Varna and Burgas. Metals were determined by using ICP-MS (Inductively Coupled Plasma - Mass Spectrometry). The order of the levels of the heavy metals in the fish samples was Zn > Al > Mn > As > Hg > Pb > Cd from Varna and Zn > Al > Mn > As > Hg = Pb = Cd from Burgas. The experiment results were discussed by comparison with literature values. According to the results heavy metal concentrations in anchovy tissues were within the limits set by the EC regulations.

Key words: heavy metals, fish, anchovy, bioaccumulation, Black Sea

Нива на тежки метали в хамсия (*engraulis encrasicolus L.*) от българското Черноморие

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Определянето на тежки метали в риби е съществен показател за степента на замърсяване на морската екосистема. Елементите As, Pb, Cd, Hg, Mn, Zn и Al бяха определени в цели риби от икономически ценния вид *Engraulis encrasicolus* (Linnaeus, 1758), уловена от два от най-важните риболовни района на Черно Море. Пробите бяха събрани по време на риболовния сезон между юни и септември 2020 г. от Варна и Бургас. Химичните елементи се определят с помощта на ICP-MS (индуктивно свързана плазма - масова спектрометрия). Редът, в който се определи съдържанието на тежките метали в рибните проби, беше Zn > Al > Mn > As > Hg > Pb > Cd от Варна и Zn > Al > Mn > As > Hg = Pb = Cd от Бургас. Резултатите от експеримента бяха обсъдени чрез сравнение с литературни стойности. Нашите резултати показват, че концентрациите на тежки метали в тъканите на хамсия са в референтните граници, посочени в европейското законодтелство.

Ключови думи: тежки метали, риба, хамсия, биоакумулация, Черно море

Introduction

Heavy metal pollution of the aquatic environment is considered as one of the fundamental ecological features resulted mainly by anthropogenic activity. The heavy metals pollution level in sea aquatory is determined by measuring its concentration in water, sediment and organisms from different parts of the food chain (Boran and Altınok, 2010). Bioaccumulation of heavy metals in fish is of ecological concern and because of the consumer health impact (Bosch et al., 2016).

Black Sea (fig. 1) is a large semi-enclosed sea with intense industrial and mining activities along its shores. Heavy metals accumulation remains one of the main environmental problems in the Black Sea (Jitar et al., 2013). It is the result of both natural geological processes and to a greater extent of a human activity (Alloway and Ayres, 1997). Aquatic organisms such as fish accumulate metals to concentrations many times higher than present in water or sediment (Elbeshti et al., 2018).

Black Sea fisheries have been seriously damaged as a result of eutrophication, overfishing and the introduction of alien species, such as *Mnemiopsis* (Aubrey et al., 1996). This leads to social-economical as well as ecological consequences (UNESCO, 2003). The Black Sea hosts large quantities of migratory pelagic species, the most important of which is the anchovy *(Engraulis encrasicholus, L, 1758, subsp. Engraulis encrasicolus ponticus, Alexandrov, 1927*) constituting more than 60% of total catch (Satilmis et al., 2007).

Materials and Methods

Fish samples were collected from commercial catches at two locations in Black Sea along Bulgarian coastal area, Varna and Burgas (Fig. 1) from June to September 2020. Specimens of similar body weight and length were selected. Fish were stored at -20 °C until chemical analysis.

Fish samples (whole body) were thoroughly washed with ultra-pure water (18 MΩ cm) water. The analysis was carried out after homogenization (Vortex homogenizer), followed by

Fig. 1. Black Sea region

microwave assisted acid digestion procedure (ETHOS UP High performance Microwave digestion system, Milestone Inc). After digested with nitric acid an appropriate spectroscopy determination with Inductively coupled plasma mass spectrometry (ICP-MS, Thermo Fisher ™) was performed. The summarized results of this study were presented as mean values (X) (mg/kg) fresh weight ± standard deviation (SD). The data were subjected to a statistical analysis with Student's-test to estimate the significance of values ($p < 0.05$).

Results and Discussion

This study aimed to determine heavy metals (As, Pb, Cd, Hg, Mn, Zn and Al) in *E. encrasicolus* caught in the area of western Black Sea Region.

The results of the tested metals in anchovy are summarized in Table 1. The order of the levels of the heavy metals was Zn > Al > Mn > As > Hg > Pb > Cd from Varna and Zn > Al > Mn > As > Hg = Pb = Cd from Burgas region. In general the results from the northern region of the Bulgarian Black Sea coast (Varna) showed higher heavy metal pollution level compared to southern area (Burgas) for all of the tested elements except for Mn. In both areas the element with highest concentration was zinc, followed by aluminium. But in Varna the determined concentrations of As, Pb and Hg were more significant. In both investigated areas Cd was the element with lowest concentration in anchovy tissue. European Union and Bulgarian legislation set maximum levels of Pb, Hg and Cd less than 0.3, 0.5 and 0.1 mg/kg w wt respectively (EC, 2006; SG, 2004; SG, 2008). Based on it the current results were below the regulated maximum allowable levels.

The comparison of the results from the present survey with available data for heavy metals bioaccumulation level in anchovy from various areas and periods of the Black Sea allows a current environmental assessment, as well as to analyze the health risk for the consumer (table 2).

To our knowledge there is no recent available data concerning heavy metals accumulation in anchovy tissue from the Bulgarian Black Sea coast.

The analyzed anchovy samples from different points of the Romanian coast identified exceeded levels of lead and cadmium in 2010 based on the allowed levels under the EU legislation and for 2013 the recorded values were well below the maximum allowable level (Galaţchi et al., 2017).

The metal concentrations in *E. encrasicolus* from the Turkish Black Sea coasts in 2013 decreased in the order Zn > Cu > Pb > As > Cd > Hg (Bat et al., 2014). The mean value of Zn and As were found from 11.22 and 0.60 mg/kg wet weight, respectively, which was within the tolerance levels of national and international guidelines and were below Provisional Tolerable Weekly Intake (PTWI) limits set by FAO/WHO (2010). Several heavy metals were not detected (Hg, Cd, Pb, Cu).

Table 1. Heavy metal concentrations (mg/kg wet weight) in total tissues of anchovy from Bulgarian Black Sea coast

Element	Unit	Varna region	Burgas region
As (X ± SD)	mg/kg	0.82 ± 0.08	0.49 ± 0.05
Pb (X ± SD)	mg/kg	0.055 ± 0.006	< 0.05*
Cd (X ± SD)	mg/kg	< 0.05*	< 0.05*
Hg (X ± SD)	mg/kg	0.077 ± 0.008	< 0.05*
Mn (X ± SD)	mg/kg	2.23 ± 0.22	3.91 ± 0.39
Zn (X ± SD)	mg/kg	11.11 ± 1.11	6.30 ± 0.63
Al (X ± SD)	mg/kg	< 10.0*	< 10.0*

**Method detection limits*

***$p < 0.05$*

Table 2. Some heavy metal mean values in anchovy tissue from Black Sea coast (mg/kg, wet wt.)

Heavy metals (mg/kg)	Area	Mean value	Reference
Pb	Romania	0.746	Galaţchi et al., 2017
	Romania	0.092	Galaţchi et al., 2017
	Turkey	0.70	Bat et al., 2013
	Turkey	0.05	Bat and Sezgin, 2015
	Turkey	4.07	Türkmen and Dura, 2016
Cd	Romania	0.358	Galaţchi et al., 2017
	Romania	0.074	Galaţchi et al., 2017
	Turkey	0.27	Tüzen, 2009
		0.02	Bat and Sezgin, 2015
Hg	Turkey	0.055	Tüzen, 2009
	Turkey	< 0.05	Bat et al., 2014
As	Turkey	0.69	Bat et al., 2014
	Turkey	0.65	Bat et al., 2014
Mn	Turkey	0.76	Türkmen et al., 2008
	Turkey	9.1	Tüzen, 2009
Zn	Turkey	17.56	Akaydin, 2014
	Turkey	9.5	Bat et al., 2014
		7.87	Bat et al., 2017

The testing of some commercially and for the ecosystems important fish species from the Black Sea (*Trachurus mediterraneus*, *Engraulis encrasicolus ponticus*, and *Sprattus sprattus)* established that highest concentrations of As, Cd, Co, Cr, Cu, Pb, and Zn were found in *E. encrasicolus ponticus*, whereas the greatest concentrations of Ni were found in *T. mediterraneus*and Mn in *S. sprattus* (Alkan et al., 2016).

The study of Rudneva et al. (2015) has demonstrated that the anchovy samples caught in the area of Crimea coastal (Black Sea and Azov Sea) generally contained low concentrations of Cu and Zn, while some samples collected in western and southern parts of Crimea contained high Pb level.

The summary of the plenty of available data shows the high level of variation, which necessitates intensive and periodic monitoring in different sectors of the Black Sea coast, in order to make a proper assessment of the risk to humans. Zinc is the element with highest availability in most of the reports, and at the same time highly toxic nonessential heavy metals (Pb, Hg, Cd) are generally low in concentration.

It is important to specify that the values of the bioaccumulated metals can exhibited a significant seasonal and regional variation, in which all metals attained their maximum value during summer, while the lowest levels were found during winter. (Turkmen et al., 2008; Bahnasawy et al., 2011). The environmental conditions might play a key role on the processes of uptake, deposition and elimination of the metals by organisms (Copat et al., 2012). Differences in heavy metal concentrations were also associated with the fish species features and biology. Diet and nutritional habits of pelagic species leads to lower concentrations of heavy metals accumulation compared to the benthic fish (Bustamante et al., 2003). Furthermore it should be noted that higher heavy metal levels can be determined in the total fish than in the muscle tissue (Gundogdu et al., 2016). This can be because of the fact that total tissue of the fish includes liver, kidney, digestive tract, etc. and these accumulate heavy metals

easily. For some elements a positive or negative correlation between fat content and metals accumulation degree was established. There were negative correlation between the fat content (%) and the zinc concentrations in anchovy (Bat et al., 2014).

Conclusions

The element with highest concentration in the tested anchovy samples was Zn. In general in northern part of the considered area the metals were with higher values compared to the south. However based on the determined concentrations of the tested heavy metals which were below the maximum levels according to the adopted legislation documents, we can concluded than consumption of anchovy from the Bulgarian Black Sea coast does not pose a hazard health risk.

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