

Some Trace Metals Pollution of Black Sea Anchovy from Crimean Coastal Region (Black Sea and Azov Sea)

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Authors' contributions

This work was carried out in collaboration between all authors. Author IIR conceived of the study, statistical analysis and participated in its design and coordination of the authors contributions. Authors DAB and ENS carried out the heavy metals determinations and helped to draft the manuscript. Author AVZ collected and processed fish samples characteristics. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of the present study was to detect the level of three heavy metals copper, zinc and lead in tissues of commercial fish species anchovy *Engraulis encrasicolus* in six regions of Crimean coastal waters (Black Sea and Azov Sea), and to compare them with the values obtained in various geographical locations of the world.

Study Design: Fish samples were collected from commercial catches at six locations in Black Sea along Crimean coastal area (near the riparian cities Evpatoria, Saky, Sevastopol, Jalta, Alushta, Sudak and Feodosia) and at the region of Arabat Pointer in Azov Sea in spring-summer period 2011.

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Place and Duration of Study: Experimental determinations were provided in the Crimean Experimental Station National Ukrainian Scientific Center of Institute of Experimental Veterinary Medicine, and in the Institute of the Biology of the Southern Seas.

Methodology: Chemical analysis was determined in 5 samples containing 10-15 organisms, assays run in triplicate. Concentrations of Cu, Pb and Zn were measured by atomic absorption method used spectrophotometer S-600 (Ukraine).

Results: Copper level varied from 0.34 to 4.5 mg• kg⁻¹ wet weight, zinc concentration ranged between 0.73 and 4.15 mg• kg⁻¹ wet weight and lead level varied between 0.003 and 3.42 mg• kg⁻¹ wet weight. The concentration of examined trace elements was below than the maximum levels permitted by Ukraine State Standards with the exception of lead, which level was significantly higher in four fish samples collected in western part of Crimea.

Conclusion: The results indicate that the heavy metal pollution of anchovy was higher in western regions of Crimea coastal waters than that in eastern part. The knowledge of differences between concentration of trace metals level in anchovy from examined locations are very important for human health because this fish is highly distributed commercial species in Black Sea and Azov Sea.

Keywords: Heavy metals; bioaccumulation; anchovy; pollution; black sea.

1. INTRODUCTION

Black Sea ecosystem is extensively contaminated with heavy metals released from domestic, industrial, agricultural, navy and maritime transport. The contamination of marine waters influences directly and indirectly on biota. Thus, trace metal analysis of waste waters and biota, including various kinds of food, is very important for human health. Atomic absorption methods are used for the determination of the lowest concentrations of metals in the natural samples [1,2]. Heavy metal pollution may have devastating effects on the diversity of aquatic organisms and especially on fish species, which are the most sensitive to negative effects of chemicals. They accumulate trace elements from water and food, and sometimes their levels are toxic for themselves and for human consumers [3–7]. The data of the trace metals concentration in aquatic organisms especially in fish is very important for assessment the ecological status of coastal waters and the health of field populations [8].

Fish are widely used to evaluate the health of aquatic ecosystems because pollutants transfer via food chain, and they are responsible to accumulate in fish tissues [9]. Heavy metals may alter the physiological status and biochemical characteristics of fish [10]. Contamination of marine environment by heavy metals leads chronic stress in aquatic organisms and disfunction of their metabolism, growth, development, reproduction, change of

populations structure, loss their size and catches decrease [11].

Anchovy *Engraulis encrasicolus* is widely distributed throughout the Black Sea and it is important commercial fish species. Thus the monitoring of heavy metals concentrations in anchovy is the important part of the monitoring of marine ecosystems and evaluation of the quality of seafood. The aim of the present work was to study the level of three heavy metals cooper, zinc and lead in tissues of anchovy, to compare them with the maximum permissible legal levels in Ukraine, to analyze the accumulation of examined trace elements in fish samples from six regions of Crimean coastal waters (Black Sea) and in Azov Sea, and to compare them with the values obtained in anchovy from various geographical locations all over the world.

2. MATERIALS AND METHODS

2.1 Sampling Sites and Samples

Fish samples were collected from commercial catches at six locations in Black Sea along Crimean coastal area (near the riparian cities Evpatoria, Saky, Sevastopol, Jalta, Alushta, Sudak and Feodosia) and at the region of Arabat Pointer of Azov Sea (Fig. 1) during the cruise of "Comet Lenar" vessel of Comet Galileo Company from May to September 2011. Fish samples were stored at -20°C until chemical analysis.



Fig. 1. Sampling sites in Crimean coastal waters of black sea and Azov sea

2.2. Chemical Analysis

Chemical analysis was determined in 5 samples containing 10-15 organisms, assays run in triplicate.

Metal concentration levels were determined in fish according the method described in [12]. Fish tissues were dried for 9 h at increasing temperature from + 50°C to +450°C with the period of 30 min. Dried samples were digested with concentrated nitric acid. Concentrations of Cu, Pb and Zn were measured by atomic absorption method used spectrophotometer S-600 (Ukraine). Validation of the method was carried out by using the standard reference samples according Ukrainian GOST 11884.15. Zn was determined at the wavelength 213.9 nm, Pb was assayed at wavelength 283.3 nm and Cu was detected at the wavelength 324.7 nm. Concentrations are expressed on a wet weight basis. All determinations were processed in triplicates.

2.3 Statistical Analysis

The results were processed to statistical evaluation by ANOVA. All numerical data are given as means \pm SE [13]. Statistical significant differences were assessed using a Student's *t*-test, the significance level was $P \leq 0.05$. Correlations were calculated by the least-squares method between trace elements concentration used the program CURUEFIT Version 2.10-L.

3. RESULTS AND DISCUSSION

3.1 Results

Measured heavy metals content in tissues of Black Sea and Azov Sea anchovy was below the maximum levels permitted in fish meat in Ukraine (10 for Cu, 1 for Pb, 40 for Zn, $\text{mg} \cdot \text{kg}^{-1}$ wet weight correspondingly), with the exception of lead in fish collected in four locations in the western part of Crimea.

The concentration of examined elements in fish tissues from Black Sea demonstrated regional differences. Copper level in anchovy varied from $0.34 \text{ mg} \cdot \text{kg}^{-1}$ wet weight in Alushta waters to $4.5 \text{ mg} \cdot \text{kg}^{-1}$ wet weight in fish from Saki (Fig. 2). Generally, Cu concentration in fish collected in western part of Crimean waters was significantly higher than in fish from eastern part with the exception of Feodosia. In Azov Sea the level showed intermediate value and estimated as $1.69 \text{ mg} \cdot \text{kg}^{-1}$ wet weight.

Concentration of Zn in fish samples from Black Sea ranged between $0.73\text{-}0.77 \text{ mg} \cdot \text{kg}^{-1}$ wet weight in the regions of Evpatoria and Sevastopol and $4.15 \text{ mg} \cdot \text{kg}^{-1}$ wet weight in Saki (Fig. 3). In Azov Sea the value was $1.18 \text{ mg} \cdot \text{kg}^{-1}$ wet weight. We can't note the general trend of zinc level in fish caught in tested areas and it varied unclearly.

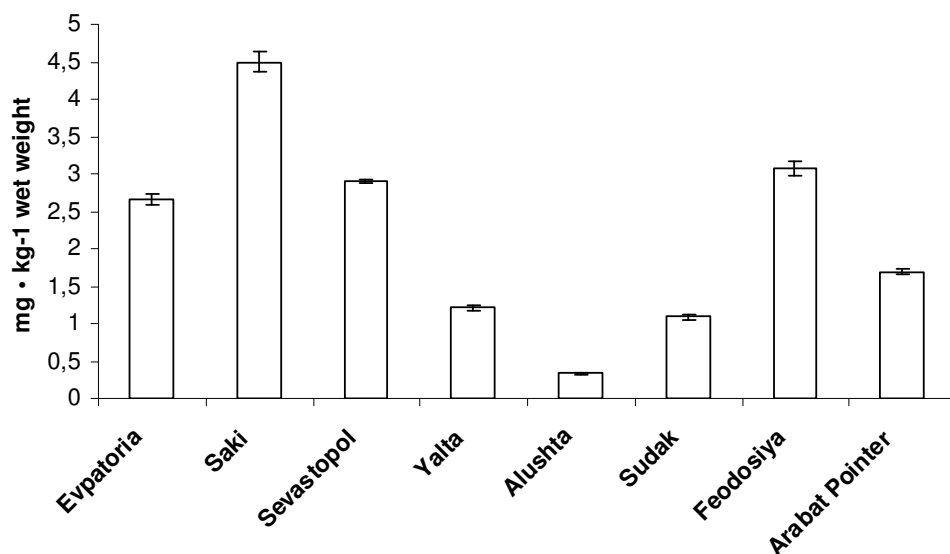


Fig. 2. Cu levels in tissues of black sea anchovy caught in Crimean coastal waters (mean ± SE, n = 5)

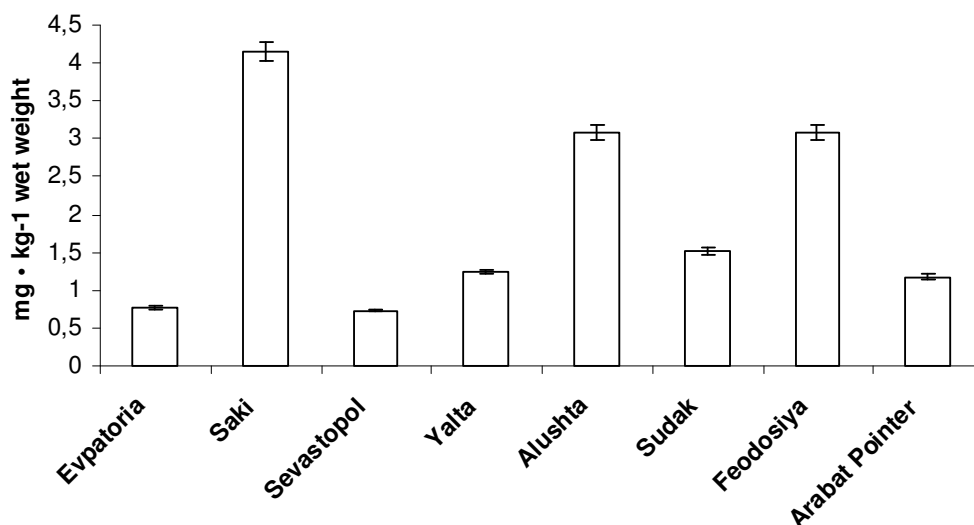


Fig. 3. Zn levels in tissues of black sea anchovy caught in Crimean coastal waters (mean ± SE, n = 5)

The level of Pb ranged between 0.003 mg · kg⁻¹ wet weight in fish collected in Sudak and 3.4 mg · kg⁻¹ wet weight in anchovy caught in Evpatoria and Yalta (Fig. 4). In fish from Azov Sea Pb concentration was low, and the value was comparable with the data obtained in Black Sea fish (0.34 mg · kg⁻¹ wet weight). In the region of Alushta, Sudak, Feodosia and in Azov Sea Pb values were significantly lower than the maximum permissible levels while in the samples

collected in Evpatoria, Saki, Sevastopol and Yalta they were significantly higher. Hence, the trend of Pb level variations in anchovy was the similar as the trend of Cu levels in fish from examined locations.

No relationships were observed between Cu and Pb values, while high correlation was noted between Cu and Zn levels (r=0.63) and

intermediate correlation was shown between Zn and Pb concentration in fish ($r=0.31$).

The regression equation is non-linear, and it was as following:

$$Y = A - BX + CX^2 \quad (1)$$

where Y and X are the corresponding element concentrations ($\text{mg} \cdot \text{kg}^{-1}$ wet weight), A, B and C are the coefficients. The relationship between Cu and Zn levels was the following $Y = 3.57 - 2.42X + 0.57X^2$. The link between Zn and Pb levels was $Y = 5.37 - 3.82X + 0.69X^2$.

Thus, Cu and Zn levels in anchovy were significantly lower than the permissible legal standards in Ukraine, while Pb concentrations were higher in fish samples collected in western part of Crimea which were more polluted than the samples from eastern part.

3.2 Discussion

The discussion of the results obtained in present study will consider three main points:

- Biological and toxicological significance of examined heavy metals;
- Regional variations of heavy metals concentration in anchovy and their relations to

ecological status of the sampling area;

- Comparison of the trace elements concentration in the tissues of Black Sea anchovy and samples from different geographical locations of the world.

3.2.1 Biological and toxicological role of tested trace metals

Among tested elements lead is non-essential element while Cu and Zn are essential. Many enzymes contain Zn and Cu. Because they are essential elements their concentrations could be regulated in the organism and thus their levels cannot be used in biomonitoring purposes [14] while Pb could successfully apply to the evaluation of marine environment. On the other hand essential elements Cu and Zn in high concentrations can be harmful for marine animals and for people [15,16].

Copper is essential element and at low concentrations it is an important component of many enzymes, catalyzing the oxidative, reducing and hydrolytic metabolic processes. Its concentration is relatively stable in fish tissues. However, high level of copper leads to intoxication in fish which results in disbalance of tissue respiration, damage of mineral and nitrogen metabolism [17,18].

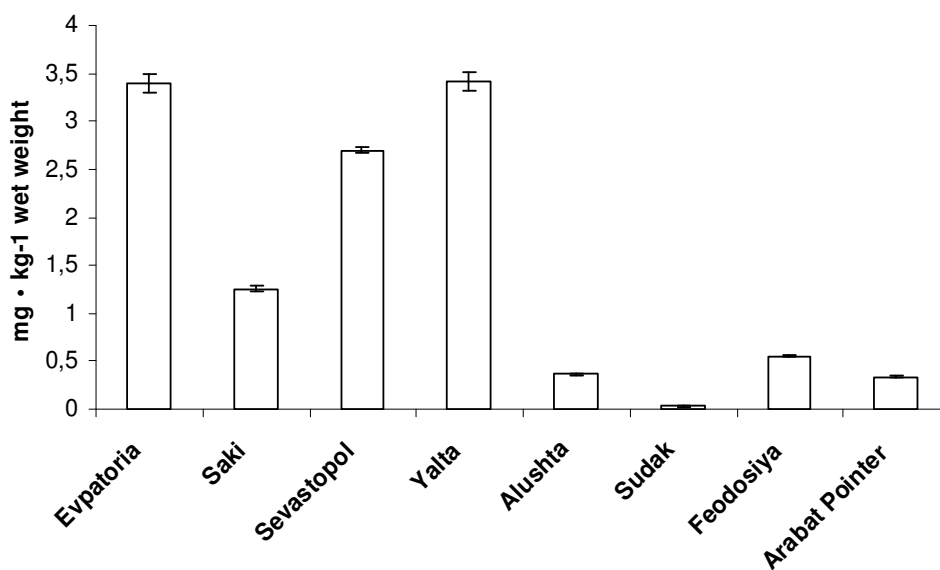


Fig. 4. Pb levels in tissues of black sea anchovy caught in Crimean coastal waters (mean \pm SE, n = 5)

Copper is a common pollutant in surface waters, and its toxicity is largely attributable to its cupric (Cu^{2+}) form. Cu has been introduced into the marine environment with the sewage of industry (mining, electroplating, paint and pigment textile, chemical industries) and agricultural effluents (pesticides). Complex form of copper is biologically unavailable, but living organisms may absorb some copper in the environment. In the unpolluted water, copper may be less than 5 $\mu\text{g/L}$ [19]. While acute effects may be death, chronic effects cause reduction of growth, shorter lifespan, reproductive problems, loss fertility and behavioral changes. The toxicity of copper to aquatic organisms varies with the physical and chemical conditions of the water [20].

Zinc is an essential element which enters in the organism with food. It involves in more than 20 enzymes, and it plays an important role in the cell division, metabolism, reproduction, and etc. High concentration of Zn in water and in animal tissues causes the decrease of blood pH, growth rate and damage of reproduction process [17]. Zinc has its primary effect on zinc-dependent enzymes that regulate RNA and DNA function. Zinc accumulates in the gills, and the gill epithelium is a primary target site in fish. High levels of Zn suppress tissue respiration leading to death by hypoxia. Zinc pollution also induces changes in ventilatory and heart physiology [10]. Zinc interacts with many chemicals to produce altered patterns of accumulation, metabolism, and toxicity; some interactions are beneficial to the organism, and others are not depending on the organism, its nutritional status, specificity of environmental conditions, other biotic and abiotic factors. The sources of Zn in marine waters may be from geological rock weathering and from anthropogenic activity such as industrial, agricultural and domestic effluents. Low molecular weight proteins called metallothioneins play an important role in zinc homeostasis and in protection against zinc poisoning; zinc is a potent inducer of metallothioneins [9].

The impact of lead is very toxic for marine organisms, because it binds with protein SH-groups and blocked the cell respiration [21]. Toxic role of Pb characterizes its possibility to bind with many anions, SH-groups, phosphates, and etc. High concentrations of Pb suppress protein synthesis, hem and hemoglobin formation, change protein conformation, resulted inactivation of enzymes, molecules aggregation and modification [17]. Pb interacts with blood

proteins and with components of the tissues, where it accumulates.

3.2.2 Regional variations of heavy metals concentrations in anchovy

Our findings demonstrated that the heavy metal levels varied in anchovy collected in examined locations. We could conclude that the samples from western part of the Crimean coastal waters were more polluted than the samples from the eastern part at the case of Pb and Cu, while Zn concentration was high in fish, caught from eastern part. The possible explanations could be the following. Cu is the main component of some pesticides (cuprocsat), which are widely applied in Ukraine agriculture, especially in vineyards located in the western part of Crimea and at the region of Feodosya, while the southern part of Crimea is the recreation area. The main source of Cu in Sevastopol is industry and navy sewage. Index of potential ecological hazard for Sevastopol region is estimated as 50 000 and Cu input is estimated as 32%, value of Pb is estimated as 1%, and Zn is estimated as 3% [22]. Hence, industrial and agricultural wastes containing copper enter into marine environment and accumulate in phyto- and zooplankton, and then heavy metals transfer via food chains to anchovy.

Zn level varied unclearly in anchovy from examined regions, which could be associated with the specificity of the hydrological conditions of the tested locations, and the levels of this element in zooplankton. In both cases Cu and Zn are essential elements and their concentration in fish regulates physiologically [14], and depends on both environmental factors, such as feeding and specificity of physiological, ecological and biological status of the organism.

Pb is toxic element, and its concentration was significantly higher in fish from western and southern part of the Crimean coastal waters as compared with the eastern part. We could propose also that because the western part is agricultural area and the effluents from the fields containing Pb enter into the sea. At the same time city Jalta is the recreation area, and it is a great surprise to indicate high concentration of Pb in anchovy collected from this location. We could propose that this phenomena may have two explanations: the first is that the fish migrate from more polluted area, and the

second one could be associated with high concentration of Pb in the water which comes from atmospheric rains and airborne transportation [8].

3.2.3 Comparison of heavy metal levels in black sea anchovy with those in other regions and guidelines

Trace elements concentrations in the fillet of Black Sea anchovy were comparable to those reported elsewhere for this fish species [23,24]. Our findings demonstrated that copper and zinc levels in anchovy collected in Crimean coastal areas were significantly lower than the maximum permissible levels both in Ukraine and other international standards, which were presented in (Table 1).

Copper concentration in anchovy caught in Crimean coastal waters were comparable to those reported in Adriatic Sea ($0.4-1.52 \text{ mg} \cdot \text{kg}^{-1}$ wet weight) [25] and Aegean Sea ($0.95 \text{ mg} \cdot \text{kg}^{-1}$ wet weight) [24] and some regions of Black Sea ($0.68 - 1.32 \text{ mg} \cdot \text{kg}^{-1}$ wet weight) [23]. On the other hand the maximum level of Cu concentration in anchovy ($4.5 \text{ mg} \cdot \text{kg}^{-1}$ wet weight) collected in coastal waters of Crimea was significantly higher than the values of fish from other locations in Black Sea.

Opposite, Zn concentration in fillet of anchovy from Ukrainian waters was lower as compared with the level of the samples from Aegean Sea ($40.2 \text{ mg} \cdot \text{kg}^{-1}$ wet weight) [24], and the levels summarized in the review of Bat et al. [23] Probably, feeding conditions were the main reason of low concentration of Zn in anchovy samples examined in this study. Similar trend

of Zn concentration we obtained in Black Sea *Elasmobranchs* caught in Sevastopol region which was significantly lower than the values of fish from other geographical locations (unpublished data).

As we described above, Pb levels in fish collected in western part of the Crimean coastal waters were significantly higher the limits listed both in Ukraine and Turkey standards, and other international standards (Table 1). The similar trend was observed in anchovy caught in the middle part of the Black Sea [24].

Pb level ranged significantly in the tissues of Black Sea anchovy and in some cases the values are of the same concentrations as those measured in anchovy from Adriatic Sea ($0.51-1.16 \text{ mg} \cdot \text{kg}^{-1}$ wet weight) [25], Aegean Sea ($0.33 \text{ mg} \cdot \text{kg}^{-1}$ wet weight) [24] and the regions from Black Sea ($0.06 \text{ mg} \cdot \text{kg}^{-1}$ wet weight) [24]. However, in several examined regions Pb level was significantly greater that those measured in anchovy caught in other geographical locations.

Hence, the levels of heavy metals in anchovy caught in Crimean coastal waters were not uniform and the values depended on region specificity. In several locations the values were very low and lower that the legal levels in Ukraine and other countries while in others they were more higher and could be harmful for consumers. The regional variations of tested heavy metals concentration demonstrated high levels of Cu and Pb in western part characterized intensive anthropogenic impact as compared with the eastern part which was documented several researchers [27].

Table 1. Legal levels of heavy metals in fish according Turkish food codex [24], Ukrainian food standards [12] and other international standards [26]

Country	Maximum permissible levels, $\text{mg} \cdot \text{kg}^{-1}$		
	Pb	Cu	Zn
USA, EPA, 1983	4	120	480
Committee Food Quality Codex, 2001	0,2		
Russian Federation, 1989	1	10	40
Ukraine	1	10	40
Turkey	0.3	20	50
FAO, 1983	0,5	30	30
Official Journal of European Commission, 2003, 2006	0,3		

4. CONCLUSION

The present paper has demonstrated that the examined samples of anchovy caught in coastal waters of Crimea (Black Sea and Azov Sea) generally contained low concentrations of Cu and Zn in fillets, while some samples collected in western and southern parts of Crimea contain high Pb level. The knowledge of differences between concentration of heavy metals level in anchovy from examined locations are very important for human health, because it is highly distributed commercial species in Black Sea and Azov Sea.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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