Journal of Elementology ISSN 1644-2296

Stetsiuk A., Kuzminova N., Niemiec M. 2021.
Distribution of mercury in the liver and gills of the scorpion fish (Scorpaena porcus Linnaeus, 1758) from the Sevastopol Bays.
J. Elem., 26(2): 507-517. DOI: 10.5601/jelem.2021.26.2.2145

RECEIVED: 19 April 2021
ACCEPTED: 30 May 2021

ORIGINAL PAPER

DISTRIBUTION OF MERCURY IN THE LIVER AND GILLS OF THE SCORPION FISH (*SCORPAENA PORCUS* LINNAEUS, 1758) FROM THE SEVASTOPOL BAYS*

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Abstract

The condition of marine organisms mirrors the impact that natural or anthropogenic factors have on them, hence results of an analysis of the mercury content in fish, for example, can help to assess the pollution of sea water with this element. In this study, organs of 86 fish caught in four Sevastopol bays: Karantinnaya, Streletskaya, Kruglaya and Balaklavskaya, were analyzed. Scorpion fish (*Scorpaena porcus* Linnaeus, 1758) was chosen for research because it is a rather inactive fish, staying close to the sea bottom, which can help to evaluate the degree of mercury pollution in the Sevastopol bays. The purpose of the work was to investigate the distribution of mercury in the liver and gills of Black Sea scorpion fish of different sex and age. Mercury in samples was determined by atomic absorption spectrometry (AAS). The study showed that the concentration of mercury in the liver exceeded the concentration of mercury in the gills in fish from all the bays studied. The highest concentration, exceeding the legal limit (500.00 ng g^{-1}), was recorded only in the liver of one female (752.6 ng g^{-1}) and the liver of one male from Streletskaya Bay (571.4 ng g^{-1}). In addition, the average concentration for females and males was approximately the same: 168.4 ng g^{-1} and 169.2 ng g^{-1}, respectively. The absolute concentration of mercury in the liver of all scorpion fish from Streletskaya Bay varied in the range 22.73–752.6 ng g^{-1}, with an average of 167.7 ng g^{-1}. In the bays of Karantinnaya, Kruglaya and Balaklavskaya, the concentration of mercury in the fish liver did not exceed the permissible level, varying from 22.18 to 395.8 ng g^{-1}. The highest content of mercury in the liver of *Scorpaena porcus* from Streletskaya Bay may be associated with he developed infrastructure and shipping, which pollute the water area.

Keywords: mercury, scorpion fish (*Scorpaena porcus* Linnaeus, 1758), liver, gills, the bays of Sevastopol.

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* The work was funded by a state grant for the Institute of Biology of the Southern Seas 'Molismological and biogeochemical basis of the homeostasis of marine ecosystems' (№ 075--007-44-21-00, registration number 121031500515-8).

INTRODUCTION

Mercury and mercury compounds are highly toxic substances with the ability to bioaccumulate in aquatic organisms. Atmospheric deposition, domestic and industrial wastewater are major sources of waterborne metals, including mercury (Jezierska 2001). Although atmospheric emissions dominate global inputs, direct mercury releases to terrestrial and aquatic ecosystems because of industrial processes affect many sites (Steward et al. 2011). Inorganic mercury presented in aquatic sediments undergoes bacterial conversion to methylmercury compounds, which are bioaccumulated in the aquatic food chain, reaching the maximum concentration in predatory fish (Thomas 1997, Petrenko et al. 2010). Fish are bioindicators of water pollution and an important link in the xenobiotic intake in human organism through the food chain (Niemiec et al. 2019). Although the toxic effects of Hg depend on the chemical forms of mercury, all forms of mercury can damage the central nervous system (Harada 1995). In marine fish, mercury accumulates through digestive tract, skin and gills. The liver as an organ involved in redistribution, detoxication and transformation of pollutants is a «target» for inorganic mercury (Yamashita et al. 2005, Marsalek et al. 2007). The mercury impact on the liver leads to some disturbance in and degeneration of pancreatic tissue, stretching of mesenteric vessels and production serous fluid (Kendall 1977).

Scorpaena belongs to the dominant coastal species of the marine biocenosis off the coast of Crimea. This fish leads an inactive life and does not make long migrations, which makes it a convenient object for assessing mercury pollution of some Black Sea bay in and near Sevastopol. At the same time, *Scorpaena porcus*, as a predator, accumulates in its body more mercury than organisms which are below it in the food chain, so it is a good biological indicator of marine pollution owing to high sensitivity to very small differences in Hg concentrations in both seawater and plankton (Monteiro et al. 1991). This is due to the fact that the concentration of mercury increases through the food chain and accumulates in biological tissues in higher level than in the water column (Buffoni et al. 1982). Therefore, predatory fish, as a food product, can be dangerous to humans. Mercury concentration tends to positively correlate with body size and age: it is higher in long-lived species compared to short-lived ones, in larger and older individuals compared to young individuals of the same species (Svobodova et al. 1999, Sonesten 2003, Mcintyre, Beauchamp 2007).

Most of the research on mercury concentrations in fish concern freshwater fish. Moreover, the available literature reports about the mercury concentration in marine fish mainly concern mainly muscles, although the level of this element in other organs, especially in the liver, can be higher (Petrenko et al. 2010). Marine organisms, like fish, absorb mercury through the gills, from which it directly enters the bloodstream, but it can also be absorbed through the skin and then spread passively through the bloodstream (Bat 2017). There have been papers published in last decade that describe investigations of toxic elements, including Hg, in fish from the Bulgarian and Turkish coast of the Black Sea (Alkan et al. 2012, Makedonski et al. 2015, Mol et al. 2017, Bat, Arici 2018, Bat et al. 2018,). Data on the concentrations of mercury in the gills and liver of the Black Sea fish of the Sevastopol bays are scarce. It was interesting to consider the content of mercury in the gills and liver of fish since these are organs that responsible for the primary input of metal and its accumulation, accordingly. This is important for understanding the mechanisms of response (accumulation and detoxification) of fish tissues to chemical pollution of water systems.

The aim of this analysis is to assess the mercury concentration in the liver and gills of Black Sea scorpion fish, as well as the influence of sex and age on the accumulation of mercury in the organs studied.

MATERIALS AND METHODS

The liver and gills of a scorpion fish composed the material for the study. The fish were caught in such bays of Sevastopol city as Karantinnaya, Streletskaya, Kruglaya (Omega) and Balaklavskaya, in November – December 2019 (Figure 1). Total (TL) and standard (SL) lengths, weight, sex and maturity of the gonads were determined for each individuals. The gonads of *Scorpaena porcus* were at maturity stage II (rest). The age was analyzed using otoliths.

Next, sampling and sample preparation were carried out for the liver and gills to study the content of mercury. Sample preparation included sample decomposition with a mixture of acids (10 ml ½H_2SO_4 and 5 ml HNO_3 per sample) and thermal processing.

A total of 172 samples were prepared (86 liver samples and 86 gill samples). The content of mercury in the samples was analyzed according to the method of atomic absorption spectrophotometry (AAS) using a mercury analyzer “Hiranuma-1” (from the Japanese company Hiranuma-1sangyoco. LTD). AAS is based on resonant absorption of light by free atoms of an element arising from transmission of a light beam through a layer of atomic vapor. The method is based on the oxidation of mercury contained in the sample, in a divalent ion in an acidic medium, its reduction in the metal form and determination with an atomic absorption spectrophotometer. The equipment was calibrated using a solution of pure mercury in 1-molar HNO_3. The amount of mercury was determined at a wavelength 253.7 nm. The maximum sample weight should not exceed 3 g. The concentration of mercury in analyzed samples are reported in ng per 1 g wet weight.

First, blank calibration was performed (100 ml of distilled water + 5 ml of H_2SO_4 (1: 1)), then another calibration using a series of calibration solu-

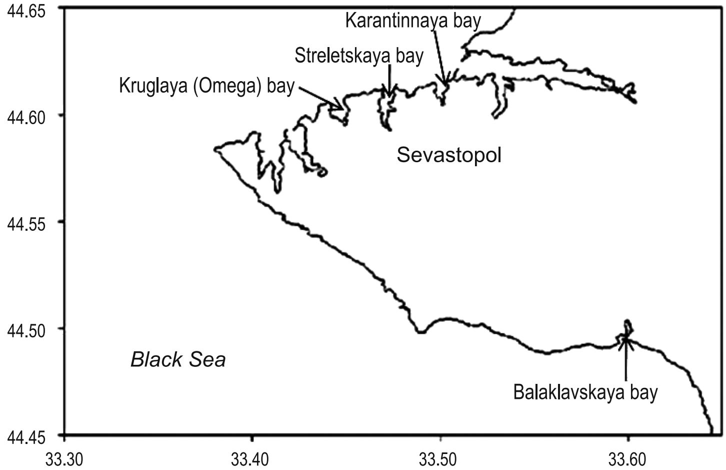

Fig. 1. Map of the study area and location of sampling points

tions was done: 0.2; 0.4; 0.6; 0.8; 1 μg dm^{-3} (10 repetitions for each concentration). The sensitivity of the Hiranuma-1 mercury analyzer is 0.01; detection limit – 0.5 ng dm^{-3} of mercury with high sensitivity 1/1000 of the standard 0.5 μg dm^{-3}. Results of chemical analyses were calculated statistically and presented as mean±standard error of the mean (M±SEM).

RESULTS AND DISCUSSION

Figure 2 shows average concentrations of mercury in organs of *S. porcus* caught in the different bays. The concentration of mercury in the liver was significantly higher than in the gills of fish from all the bays. The concentration of mercury in the gills and liver had same distribution in the following order: Streletskaya Bay > Kruglaya Bay > Karantinnaya Bay > Balaklavskaya Bay (Figure 2*a*).

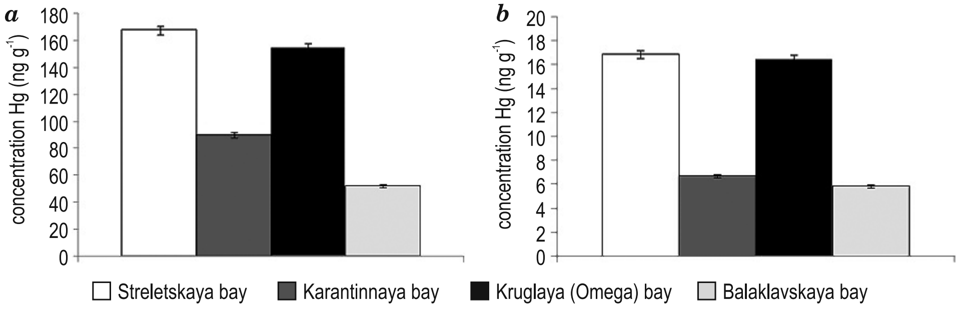

Fig. 2. Total concentration of mercury in the liver (*a*) and gills (*b*) of scorpion fish (on the wet weight basis) from different bays of Sevastopol (M±SEM)

In Streletskaya Bay, the concentration of mercury in the fish liver varied in the range 22.73-752.6 ng g^{-1}, with an average value of 167.7 ng g^{-1}; for fish from Kruglaya Bay, the content of Hg was within 48.10-395.8 ng g^{-1} with an average of 155.2 ng g^{-1}; the results for fish from Karantinnaya Bay were in the range 22.18-258.9 ng g^{-1} with an average value of 90.18 ng g^{-1}. During the study, the fish caught in Balaklavskaya Bay had an average liver content of mercury at 52.17 ng g^{-1} within the range of 27.84-129.4 ng g^{-1}.

The mercury concentration in gills of scorpion fish from Streletskaya Bay varied in the range 2.46-129.8 ng g^{-1}, with an average of 16.87 ng g^{-1} (Figure 2*b*). In Kruglaya Bay, the concentration of mercury in fish gills ranged within 8.053-26.61 ng g^{-1}, with an average value of 16.45 ng g^{-1}. The mercury content in gills of fish from Karantinnaya Bay varied from 1.954 to 28.85 ng g^{-1}, with an average value of 6.631 ng g^{-1}. Fish from Balaklavskaya Bay had a concentration of mercury in gills from 1.740 to 12.61 ng g^{-1}, with an average concentration of 5.816 ng g^{-1}.

It was previously noticed that scorpion fish from Streletskaya Bay (in contrast to other water areas of the Sevastopol city) in most age groups had the lowest values of body weight, but increased values of the hepatosomatic index, which indicates adaptive responses of fish to a chronic effect of unfavorable living conditions (Timofeev et al. 2020).

An additional explanation for the elevated mercury accumulation in fish from this bay is the predominance of the "fish component" in the diet of the *S.porcus* (Timofeev et al. 2020), which proves the intake and cumulative effect of mercury through food chains.

When the fish were analyzed separated for their gender, it was noticed that the decreasing of Hg concentrations for females from the bays coincided with the total values of Hg in the liver (Fig. 3*a*) and the gills (Figure 3*b*). However, the average mercury concentrations in the liver and gills of males were distributed differently. The highest concentration of mercury was found in the liver of a female from Streletskaya Bay (752.6 ng g^{-1}). The second highest concentration (571.4 ng g^{-1}) was determined in the liver of a male. Both these values exceeded the maximum permissible concentration (MPC) for marine fish (0.5 mg kg^{-1}).

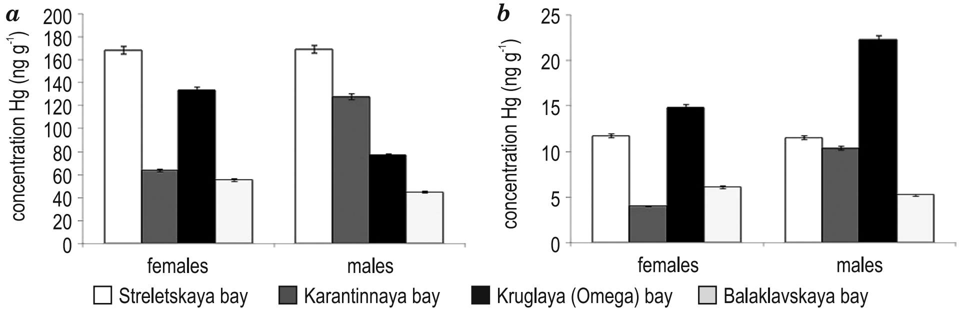

Fig. 3. Concentrations of mercury in the liver (*a*) and gills (*b*) of male and female scorpion fish (on the wet weight basis) from the bays of Sevastopol (M±SEM)

In addition, the average concentrations for females and males were approximately the same: 168.41 ng g^{-1} and 169.2 ng g^{-1}. The absolute concentration of mercury in the liver of all fish from Streletskaya Bay, including immature individuals, varied in the range 22.73-752.6 ng g^{-1}, with an average value of 167.7 ng g^{-1}. In the other bays, the concentration of mercury in the liver did not exceed the MPC, varying from 22.18 to 258.9 in *S.porcus* from Karantinnaya Bay, 48.10 to 395.8 in fish from the Kruglaya Bay, and 27.84 to 129.4 ng g^{-1} in fish from Balaklavskaya Bay.

The concentration of mercury was significantly lower in the gills of fish than in the liver, and varied in the range of 1.74-129.8 ng g^{-1}, not exceeding MPC (Figure 3*b*). The highest value of the average concentration in gills was in a male caught in the waters of Kruglaya Bay (Figure 3*b*).

An investigation carried out on the muscles of the same fish showed that the average concentration of mercury is higher in the muscles of fish from Karantinnaya Bay than from the other bays. At the same time, the concentration of mercury was higher in the muscles of males, regardless of the location. In this study, the mean mercury concentrations in fish from Streletskaya, Kruglaya, Balaklavskaya bays were higher in the liver of females than in males (Fig. 3-a). This result is comparable to the literature data, which describes the need for females to consume more food to replenish energy losses for roe production (Nicoletto, Hendricks 1998). In Karantinnaya Bay, the concentration of mercury was higher in the liver of males than of females.

In the gills of fish from Streletskaya and Balaklavskaya bays, the average concentrations of mercury in male and females were close (Figure 3*b*). In the gills of males from Karantinnaya and Kruglaya bays, they were higher than in females.

The distribution of mercury in the muscles of the same fish occurred in the range of descending: Karantinnaya bay > Streletskaya bay > Balaklavskaya bay > Kruglaya bay. According our earlier data carried out on fish of different species from Martinova and Karantinnaya bays (Kuzminova et al. 2009), the concentration of mercury in the tissues of fish from Karantinnaya bay was also higher, than in others. Moreover, the concentration of mercury in the tissues of scorpion fish exceeded the concentration of mercury in the tissues of other fish (horse mackerel, peacock wrasse, Mediterranean three-barbeled rockling, round goby).

Tha possible contamination of Karantinnaya bay can be confirmed by data about reduced calcium content in fish larvae (Niemiec et al. 2016), because it is known, that a low calcium content is a characteristic of marine organisms living in polluted environments (Lall, Lewiss-Mccrea 2007). High concentrations of xenobiotics in aquatic ecosystems very often result in disturbance of the uptake of macronutrients and microelements by living organisms (Napiórkowska-Krzebietke et al. 2015, Niemiec et al. 2018*a*,*b*, Talić et al. 2020). According to other sources, the mercury acts on the bone cells of fish and affects calcium homeostasis, causing hypercalcemia (Suzuki et al. 2004). Hyperactivation of the MeHg glutamate receptor can increase intracellular Ca^{2+} influx and excess production of active form of oxygen (Farina et al. 2011). Mercury accumulates in the cell in the cytosol mainly (Bose et al. 1993), adversely affecting cellular functions that regulate calcium levels. Increase in calcium content due to its release from intracellular «depot» in associated with the mercury accumulation in the cytosol (Nathanson et al. 2015).

If we compare the concentrations of mercury in organs and muscles, the order of rankings can be described as: liver > gills > muscles. This distribution of mercury in muscles and organs is explained by the alimentary intake of mercury into the fish organism. The liver and gills are a barrier to externally supplied mercury compounds.

No significant relationship between the mercury concentration in the liver and gills versus the age of fish was obtained (Figure 4). The exception was the mercury content in the liver of male scorpion fish from Karantinnaya Bay (Figure 4*b*), with the value of the approximation reliability R^2=0.821.

Such distribution of mercury concentrations depending on the fish age needs further research. It is possible that the accumulation of mercury depends on the size of individuals rather than on the age of scorpion fish

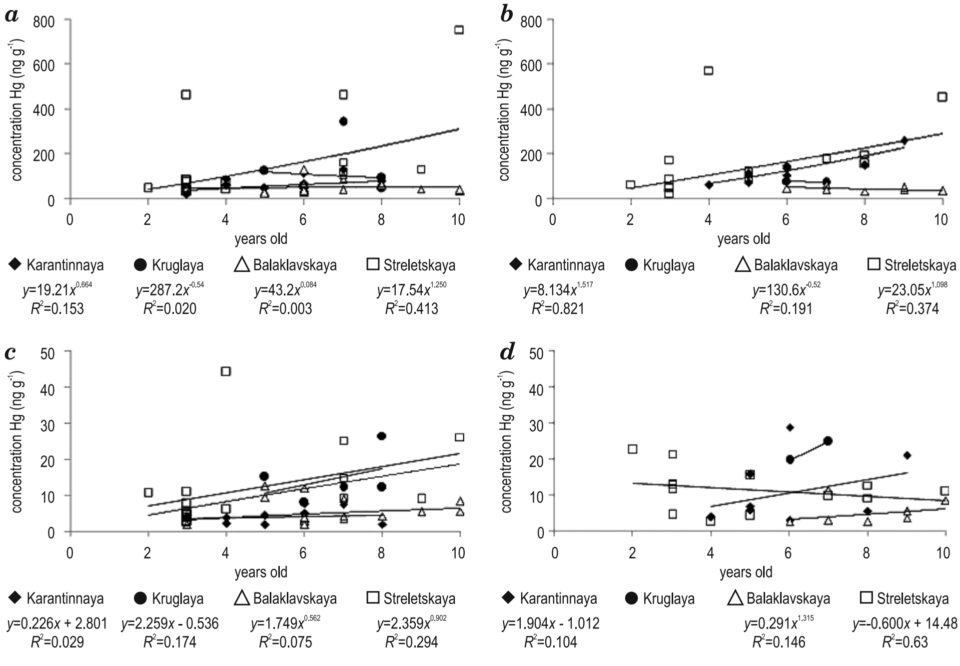

Fig. 4. Concentration of mercury in the liver of females (*a*) and males (*b*), and in the gills of females (*c*) and males (*d*) of scorpion fish (on the wet weight) of different age from the bays of Sevastopol

because this is a long-lived species and the range of sizes of specimens from the same age group is very wide.

The connection between the concentration of mercury in the organs studied and fish mass was also analyzed. These results showed the highest value (R^2=0.5) in the liver of males from Kruglaya Bay. All other data for the liver and gills were quite low, hence these differences were not significant.

In general, there was a very weak relationship between the mercury concentration in the liver of a scorpion fish and the weight of fish (Figure 5). At the same time, it was noted that the increased mercury content in the liver of females from Streletskaya Bay was accompanied by an increase in fish size at R^2=0.442 (Figure 5*c*).

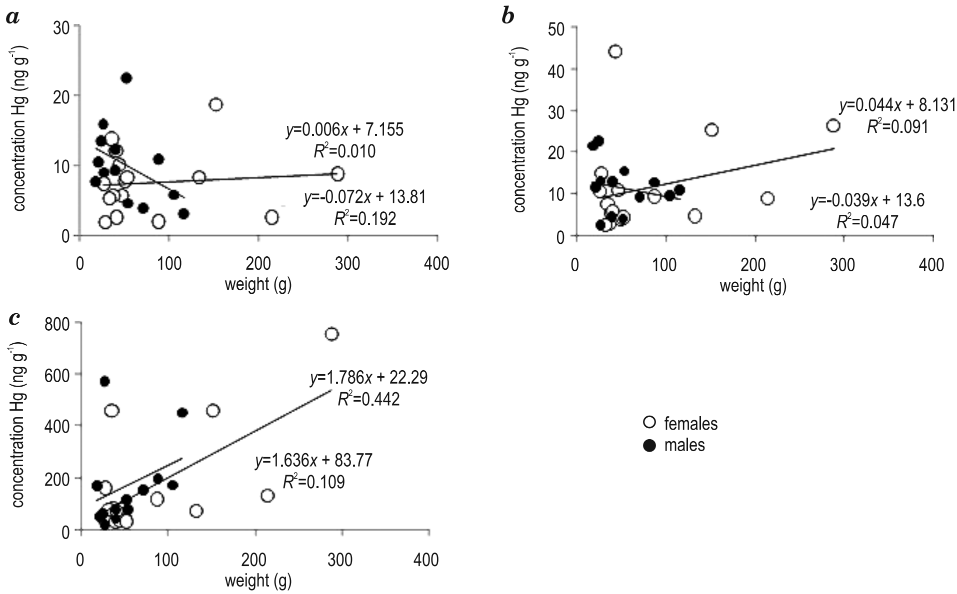

Fig. 5. The concentration of mercury in the muscles (*a*), gills (*b*) and liver (*c*) of females and males of scorpion fish (on the wet weight) with different weight from Streletskaya bay

Based on data indicating the preference of *S.porcus* females to eat fish, and males to consume crustaceans (Kuzminova et al. 2017), differences in the distribution of mercury in the liver of fish of different sexes are possible due to ingested food. It is also known that females accumulate mercury faster than males do (Monteiro et al. 1991). The increased mercury content in the liver of scorpion fish compared to their muscles and gills can be explained by the participation of this organ in detoxification and elimination of mercury from hepatocytes into bile secretion, which requires the formation of a complex of mercury and glutathione reduced (Clarkson, Magos S 2006).

Despite ambiguous results concerning the distribution of mercury in the *Scorpaena porcus* of different sex and age, this is an interesting problem that requires further investigation. It is very important to explain the chain of processes of accumulation and removal of mercury in different organs and tissues.

CONCLUSION

The research results indicate a more significant accumulation of mercury in the liver of *S.porcus* than in the gills and muscles, regardless of the area where the fish lived. There is a positive connection between the content of mercury in livers of males and fish and age (R^2=0.821). Only in two cases out of all liver samples analyzed, the concentration of mercury exceeded the maximum permissible concentration. These were fish from Streletskaya Bay with values 752.63 (female) and 571.43 ng g^{-1} (male).

At the same time, the average concentration for male and female fish were approximately the same: 168.41 ng g^{-1} and 169.24 ng g^{-1}, respectively. The absolute concentration of mercury in the liver of all scorpion fish, including immature individuals, varied in the range 22.73-752.63 ng g^{-1}, with an average value of 167.72 ng g^{-1}. A higher concentration of mercury in the liver and gills of all fish from Streletskaya Bay in comparison with fish organs from the other bays (Karantinnaya, Balaklavskaya and Kruglaya) can be explained by the size characteristics and anthropogenic impact. The shore of this bay is occupied by a shipyard, a fuel pier and a recreational zone, hence there are yachts and boats moving constantly along the bay and polluting the sea water.

The detected concentrations exceeding the MPC may indicate intake of mercury from both the natural and anthropogenic sources. The greatest accumulation of mercury in the liver of *S.porcus* is explained by its barrier properties preventing the penetration of the toxic element into other organs. The above results concerning mercury concentrations in the liver and gills of fish can be used in bioindication studies.

ACKNOWLEDGMENTS

The authors would like to thank the fishermen from the kolkhoz “Put Il’icha” and employees of the Institute of Biology of the Southern Seas of RAS for fish provided as the research material.

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