Pollution 2022, 8(3): 1038-1048
DOI: 10.22059/POLL.2022.337948.1341

RESEARCH PAPER

Complex application of Microbiological Characteristics in Bottom Sediments and Biochemical parameters of Mussel *Mytilus galloprovincialis* (Lam.) for Assessing the Ecological state of Marine Coastal Areas

Ekaterina Skuratovskaya[1*], Yuliya Doroshenko[2]

[1] Department of Ichthyology, A.O. Kovalevsky Institute of Biology of the Southern Seas of RAS, Nachimov av., 2, Sevastopol, Russian Federation 299011
[2] Department of the Marine Sanitary Hydrobiology, A.O. Kovalevsky Institute of Biology of the Southern Seas of RAS, Nachimov av., 2, Sevastopol, Russian Federation 299011

Received: 24.01.2022, Revised: 26.03.2022, Accepted: 01.04.2022

Abstract
Comparative analysis of microbiological indicators (heterotrophic and hydrocarbon-oxidizing bacteria) in bottom sediments and biochemical parameters (level of oxidized proteins (OP) and lipid peroxidation (LPO), superoxide dismutase (SOD), catalase (CAT), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities) in hepatopancreas of mussel *Mytilus galloprovincialis* (Lam.) from three Sevastopol bays - Laspi, Kazach'ya, Streletskaya (the Black Sea) was carried out. The results obtained allowed to identify certain differences between the studied areas and characterize their ecological state. The maximum abundance of heterotrophic and hydrocarbon-oxidizing bacteria was found in the most polluted Streletskaya Bay (95,000 and 250 cells/g respectively), the minimum - in the conventionally pure Laspi Bay (2,500 and 1.5 cells/g respectively). Parameters of prooxidant-antioxidant system (level of OP and LPO, SOD and CAT activities), as well as ALT activity in the hepatopancreas of mussels from Streletskaya Bay were found higher as compared to those in other tested areas. Most significant differences (more than twofold) in SOD activity, LPO content and basic ketone forms of OP levels were found between Laspi and Streletskaya bays. The results obtained indicate the severe pollution and less favorable living conditions for aquatic organisms in Streletskaya Bay, as well as demonstrate the high level of biochemical adaptation of mussels to complex environmental pollution. The studied parameters can be applied in the ecological monitoring of the coastal sea water areas.

Keywords: oxidative stress, antioxidant system, mussel *Mytilus galloprovincialis*, bacteria, environmental pollution.

INTRODUCTION

Systematic nearshore marine waters pollution by toxicants arouses ever growing concern of public and the scientists since the intensive supply of pollutants via different effluents, industrial wastes, sewages, agricultural and urban discharges (Mironov and Alyomov, 2018). Waste water toxic substances accumulate in bottom sediments, causing physico-chemical changes of waterbodies that adversely affect the biota. Besides, extensive exploitation of the coastal areas in conjunction with the technogenic contamination can lead to depletion of the biological resources and deterioration of their state. It affects not only the quality of the coastal environment but directly influences such spheres as fisheries, tourism and recreation (Rudneva

* Corresponding author Email: skuratovskaya@ibss-ras.ru

et al., 2016; Luk'yanova and Korchagin, 2017; Chesnokova et al., 2020; Sigacheva et al., 2021).

In this respect one of the suchlike regions is Sevastopol area (the Black Sea) which is considered as the collector of different pollutants. Raw wastewaters as well as impure industrial and domestic wastes are regularly discharged in its waters. As a result in its coastal zones there are constantly emerging spots with high concentration of toxicants in the bottom sediments: heavy metals, organochlorine and organophosphorous compounds, petroleum hydrocarbons and radionucleids (Malahova et al., 2018; Tikhonova et al., 2018; Soloveva et al., 2019). These pollutants are absorbed and accumulated by hydrobionts inducing metabolic processes of reorganization and intoxication (Nemova et al., 2014; Luk'yanova and Korchagin, 2017).

Considering all those mentioned, the observation of waterbody and biota contamination appears to be the main task of the ecological monitoring. However, currently applied methods of physico-chemical analyses do not allow to evaluate the state of marine coastal ecosystems as they reflect the situation directly during the period of collecting samples and do not permit to identify all those known, thus stimulating search for unknown pollutants of the marine environment (Nemova et al., 2014; Rudneva et al., 2016; Luk'yanova and Korchagin, 2017).

So nowadays to conduct the nearshore complex assessment of the ecological state along with the traditional physico-chemical methods, there are widely used bioindicaton technologies that more accurately show the quality of the environment and the reaction of hydrobionts to all negative environmental impacts. Application of such approach requires selection of the bioindicator species and the informative indices (biomarkers) according to which the degree of the impaired biological functions occurred under conditions of the multifactoral anthropogenic pressure can be estimated (Nemova et al., 2014; Rudneva et al., 2016; Luk'yanova and Korchagin, 2017; Asif et al., 2018; Polyaka et al., 2020).

Microorganisms due to high ecological plasticity explained by the unique physiological, biochemical and genetic characteristics tend to respond quickly to changes of the environment and the influence of stressors serving as indicators of the environmental pollution (Parmar et al., 2016). The methods of the microbial indication allow to reveal and control the emergence of the pollutants in water much earlier than the irreversible toxic effects occur in hydrobionts. Abundance of the microorganisms appears as the indicator of the extent of water pollution (Sumampouw and Risjani, 2014; Zolkefli et al., 2020). In parallel, bottom invertebrates and their communities are also the susceptible indicators of water environmental contamination. Structural and functional characteristics of zoobenthos appear to be a perspective element in the monitoring system enabling to identify the marine habitats pollution (Mironov, 1988; Sukharenko et al., 2017; Alyomov, 2021).

Benthic organisms largely subject to the impacts of the chemical contamination as they inhabit pre-bottom areas where different toxicants sink accumulating in the sea beds (Sarker et al., 2016). In food of the benthic organisms the content of toxicants is found increased as they arrive from pre-bottom water layers and soil (Uzbekova and Lutsenko, 2011; Pešić et al., 2018; Talukdar et al., 2022). The dominant species in the macrozoobenthos communities of Sevastopol bays is the mussel *Mytilus galloprovincialis* (Mironov, 1988). Owing to significant accumulation of toxicants in body at low concentrations in marine waters, *M. galloprovincialis* is often used as the bioindicator of marine water pollution by heavy metals, pesticides and other toxicants in different habitats of this species (Vidal-Linan et al., 2016; Sukharenko et al., 2017; Benaly et al., 2017; Ozkan et al., 2017).

To detect the initial changes in metabolism of water organisms, molecular biomarkers allowing determination of negative environmental factors on certain metabolism chains are used. It assists to define the main strategy and peculiarities of structural functional changes in the body when adapting to unfavourable habitats (Nemova et al., 2014; Rudneva et al., 2016; Luk'yanova and Korchagin, 2017). Biomarkers have been also extensively used to provide the connection between external levels of the contamination exposure, internal levels of it in tissues, and early adverse effects in organisms. As such, they are considered as 'early warning' signals that have the potential to detect an effect in target biota prior to one that is being observed at the population, community, ecosystem level. Hence, the use of biomarkers can be a critical line of evidences to understand relationships between stressors and effects on the coastal resources, and to prevent detrimental impacts of contamination on the ecosystem structure and function (Kroon et al., 2017; Luk'yanova and Korchagin, 2017; Chesnokova et al., 2020). The most informative molecular biomarkers are indicators of the oxidative stress (level of lipid and protein peroxidation, DNA damage) and antioxidant (AO) defense (low-molecular AO content, enzyme activities), biotransformation enzyme activities (cytochrome P450, ethoxyresorufin O-deethylase, glutathione-S-transferase), functional state (aminotransferase activities) and neurotoxic (cholinesterase and choline acetyltransferase activities) parameters that were determined experimentally (Cajaraville et al., 2000; Oliva et al., 2014; Vidal-Linan et al., 2016; Benaly et al., 2017; Ozkan et al., 2017; Klimova et al., 2019).

The present article was aimed at studying the ecological state of the coastal areas of the Black Sea (Sevastopol) basing on the biochemical parameters in hepatopancreas of the Black Sea mussel *Mytilus galloprovincialis* and the results of the microbiological monitoring in bottom sediments.

MATERIAL AND METHODS

The research was performed in three bays (the Black Sea, Sevastopol region) with the different levels of pollution in summer, 2020 - Laspi, Kazach'ya, Streletskaya (Figure 1).

The mussels as well as the samples of the bottom sediments for the microbiological analyses were collected simultaneously in all study areas. The sediment samples were transported in the clean containers kept in ice and were processed shortly within 1–2 h after sampling. The mussels were transported to the laboratory in containers with the aerated sea water.

Abundance of heterotrophic (HB) and hydrocarbon-oxidizing (HOB) groups of bacteria was determined in bottom sediments by the limiting decimal dilution method using liquid media. For HB, peptone water was used (Netrusov, 2005; Sonnenwirth and Jarett, 1980). For HOB,

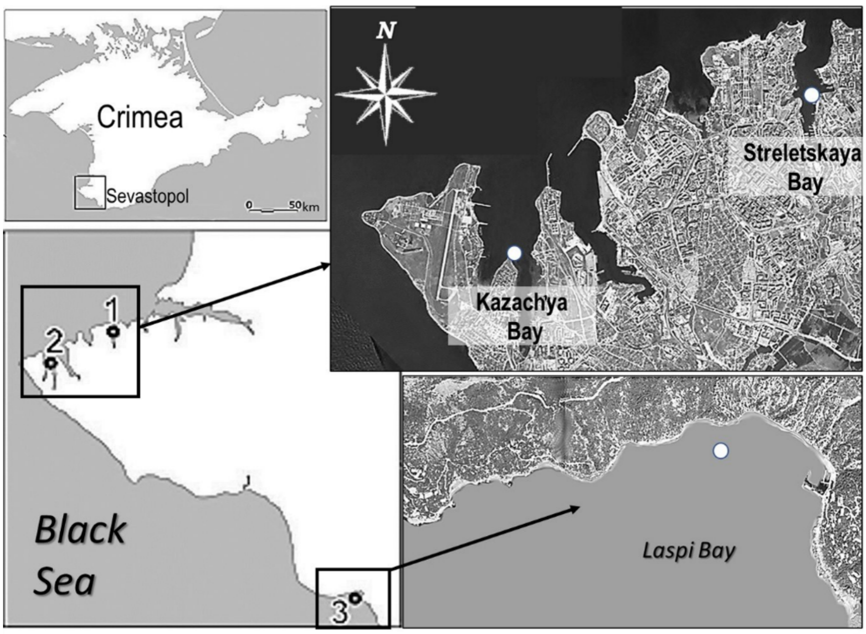

Fig. 1. Study area map showing locations of selected bays:
1 – Streletskaya Bay, 2 – Kazach'ya Bay, 3 – Laspi Bay

Table 1. Size-weight characteristics of mussels *Mytilus galloprovincialis* from three Sevastopol bays (mean ± SEM)

Parameter	Laspi Bay (n=15)	Kazach'ya Bay (n=15)	Streletskaya Bay (n=15)
Length, mm	51.44±0.52	52.11±0.51	51.54±0.93
Height, mm	26.83±0.39	26.74±0.58	29.51±1.22
Depth, mm	19.41±0.42	19.89±0.38	21.92±1.5
Weight, g	10.62±0.54	9.34±0.3	10.34±0.7

n - number of individuals

Voroshilova-Dianova medium was used (Netrusov, 2005). As the only source of carbon and energy, 1% of sterile oil was added to each test tube after inoculation. When preparing the media, the salinity of seawater was taken into account. The most probable number of microorganisms per unit of volume was calculated using the McCrady table (in triplicate) based on a method of variation statistics (Netrusov, 2005).

In the laboratory length (L), height (H), depth (D) and weight (W) of mollusks were measured. For the biochemical analysis 15 mussels of 50-55 mm length from each tested area were selected. Size-weight characteristics of the examined individuals are presented in the Table 1.

Since the hepatopancreas of the mussels neutralizes a wide range of the toxic substances, it was used for the biochemical analysis. The hepatopancreas from the individuals was washed several times by cold 0.85% physiological solution, homogenized and centrifuged at 8,000 g for 15 min at the temperature of 0–4˚C in a refrigerated centrifuge MPW-352 (MPW Med. Instruments, Poland). All biochemical parameters were determined in the supernatants.

The level of oxidized proteins (OP) was analyzed by using method based on the reaction of interaction between the oxidized protein amino-acid residues and 2,4-dinitrophenylhydrazine with the formation of 2,4-dinitrophenylhydrazones (Dubinina et al., 1995). The optical density of the newly formed 2,4-dinitrophenylhydrazones was recorded at the following wavelengths (λ): 356 nm (neutral aldehydes), 370 nm (neutral ketones), 430 nm (basic aldehydes), and 530 nm (basic ketones).

The concentration of the lipid peroxidation (LPO) secondary products - thiobarbituric acid reactive substances (TBARS) was determined by the reaction with thiobarbituric acid (λ = 352 nm) (Stalnaya and Garishvili, 1977).

Superoxide dismutase (SOD) activity was assayed in the nitroblue tetrasolium –phenazine methosulfate – NADH system (λ = 560 nm) (Nishikimi et al., 1972). Catalase (CAT) activity was measured based on the reaction of interaction between hydroperoxide and molybdite ammonium (λ = 410 nm) (Korolyuk et al., 1988).

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined using the standard assay kits OLVEX DIAGNOSTICUM (Russia). The AST activity was analyzed by means of the reaction of interaction between oxaloacetate and 2,4-dinitrophenylhydrazine, while the ALT activity was measured by the reaction of interaction between pyruvate and 2,4-dinitrophenylhydrazine in an alkaline medium (λ = 537 nm).

All determinations were made on spectrophotometer SF-2000 (St. Petersburg, Russia). The biochemical parameters were calculated per mg protein. Total soluble protein concentration was quantified by biuret method using the standard assay kits OLVEX DIAGNOSTICUM (Russia).

The results were also processed statistically. Mean values +/- SEM (standard error of the mean) were established. The significance of the difference between the samples was evaluated by using Mann-Whitney U-test. The difference was found great at the significance level $p \leq 0.05$. The statistical analysis was carried out by using software programs Past 3 and Microsoft Office Excel 2016.

RESULTS AND DISCUSSION

Streletskaya Bay is one of the zones in Sevastopol region that is mostly subjected to anthropogenic pressure (Mironov and Alyomov, 2018; Tikhonova et al., 2018). The reason for this is that the bay's territory is deeply embedded into the shoreline and is extensively exploited as the berthing place for the small boats as well as the port. Likewise it is the warship base that covers the whole eastern coast. In contrast, the western shoreline is occupied by the urban residential buildings. In recent decades there has been a change in quality of the anthropogenic load in this area originating from the intensive construction of the municipal, hotel and tourist resort centers located offshore the coastal strip and the extension of piers for mooring of the small (recreational) boats (Tikhonova et al., 2018). Hence the sediments in Streletskaya Bay accumulate silty shells, sands as well as the black silts. The loosened sediments here are mainly presented by silty-pelitic fractions, exhibiting the highest accumulation capacity resulted in severe contamination of bottom water columns by poisonous pollutants containing organochlorine compounds and petroleum hydrocarbons (Tikhonova et al., 2018; Malakhova et al., 2018). Contaminant pollution of bottom sediments of the bay by heavy metals is largely sustained by such metals as arsenic and zinc. Maximum concentrations of arsenic at the Bay outlet are recorded as 50 mg/kg that is 1.5 half as much as the content of this element found for the shallow sediments in the Black Sea. In the bottom sediments the petroleum hydrocarbon (PH) concentrations have reached 700 mg/100 g. Over the last years, in bottom sediments of the central part of the Bay, the concentrations of polychlorinated biphenyls (PCBs) and dichlorodiphenyl trichloroethane (DDT) have averaged 100 and 51 ng/g dry mass, respectively (Malakhova et al., 2018).

Kazach'ya Bay is situated 15 km apart from the city center and according to the hydrochemical indicators, its environmental factors refer to conditionally favourable water bodies (Soloveva et al., 2019). The bottom sediments of Kazach'ya Bay are mainly presented by silted barnacles and sands. Such coarse-grained sediments are defined by good washability and low sorption of the pollutants. Maximum concentrations of PH (140 mg/100g) are accumulated in silts at the head of the Bay where their concentrations were found increased by 1.8 times between 2003 and 2015 years. As regards the central parts and the areas of the estuary, the highest concentrations of PH were observed in 2003 and in the following years were found decreasing (Mironov and Alyomov, 2018). In bottom sediments the pollutants of the DDT group do not accumulate and the concentration of PCBs is 9 ng/g (Malakhova et al., 2018).

Laspi Bay is the large open zone located on the southern coast of the Crimea. The region has the unique recreational characteristics and good perspectives for the development of the marine farms. The estimates of water dynamics in the bay demonstrate the processes of water exchange as quite intensive. The average assessment of water renewal is registered within the period of 5-6 hours (Pankeeva and Mironova, 2019). The concentrations of organochlorine pollutants and petroleum hydrocarbons are found much lower here than in other research areas. The average content of PCBs appears to be 6 ng/g, DDT - 1.3 ng/g (Malakhova et al., 2018). The PH concentrations are 9 mg/100g (Tikhonova et al., 2016). Overall, these evidences confirm the unfavourable ecological state of the bay. Thus we can state that the pollution level of the tested bays varies considerably and is especially pronounced for the bottom sediments.

In our study HB abundance in bottom sediments of Laspi Bay amounted 2,500 bacterial cells per gram of sediments, in Kazach'ya Bay - 25,000 cells/g, in Streletskaya Bay - 95,000 cells/g. The maximum abundance of HOB is registered in Streletskaya Bay - 250 cells/g. In Kazach'ya Bay the abundance of HOB constituted 45 cells/g. In Laspi Bay only trace values (1.5 cells/g) were observed. Biochemical parameters in the hepatopancreas of mussels *Mytilus galloprovincialis* are presented in the Table 2.

Activity of SOD, CAT, ALT as well as the TBARS content in the hepatopancreas of mussels

Table 2. Some biochemical parameters in the hepatopancreas of mussels *Mytilus galloprovincialis* from three Sevastopol bays (mean ± SEM)

Parameter	Laspi Bay (n=15)	Kazach'ya Bay (n=15)	Streletskaya Bay (n=15)
TBARS, nmol$^{-mg\ protein}$	2.92±0.42	4.84±0.35*	6.47±0.7*, **
D_{356}, optical units$^{-mg\ protein}$	0.053±0.004	0.059±0.006	0.069±0.006*
D_{370}, optical units$^{-mg\ protein}$	0.057±0.006	0.058±0.008	0.087±0.01*, **
D_{430}, optical units$^{-mg\ protein}$	0.031±0.004	0.038±0.005	0.052±0.006*
D_{530}, optical units$^{-mg\ protein}$	0.006±0.002	0.012±0.003*	0.014±0.004*
SOD, arbitrary units$^{-mg\ protein \times min}$	4.25±1.04	12.27±2.42*	22.06±0.46*, **
CAT, mkat$^{-mg\ protein}$	0.185±0.03	0.176±0.07	0.326±0.06*, **
ALT, µmol$^{-mg\ protein \times h}$	0.153±0.01	0.095±0.01*	0.195±0.01*, **
AST, µmol$^{-mg\ protein \times h}$	0.098±0.02	0.083±0.009	0.094±0.009

TBARS - thiobarbituric acid reactive substances, D356 - neutral aldehydes, D370 - neutral ketones, D430 - basic aldehydes, D530 - basic ketones, SOD - superoxide dismutase, CAT – catalase, ALT - alanine aminotransferase, AST - aspartate aminotransferase, n - number of individuals, * indicates significant differences when compared to Laspi Bay, ** - to Kazach'ya Bay, p< 0.05

from Streletskaya Bay significantly exceeded the values of the individuals from the other tested bays (p<0.05). The level of OP was observed to be significantly higher in mollusks from Streletskaya Bay as compared to the specimens from Laspi (at all wavelengths) and Kazach'ya (at λ = 370 nm). At the same time, SOD activity, level of TBARS and basic ketones (D530) in mussels from Kazach'ya Bay were recorded as higher than in Laspi Bay (p<0.05) whereas for ALT the reverse trend was noted (p<0.05). No differences in AST activity were found (Table 2).

Currently, huge amounts of household organic matters and petroleum hydrocarbons are discharged into the coastal marine ecosystems. The accumulation of organic substances in bottom sediments can lead to disruption of marine environment self-purification, intensive accumulation of other pollutants, including heavy metals, also development of hypoxia (Kotelyanets et al., 2017; Ovsyaniy and Orekhova, 2019). In this connection microorganisms can indicate these changes. Microbial indication methods are based on the ability of microorganisms to use specific organic compounds as the only carbon source, as well as to survive and multiply in the environment containing high concentrations of toxic pollutants. For example, the number of hydrocarbon-oxidizing bacteria is an indicator of oil pollution, while the heterotrophic bacteria correlate with readily available organic matters (Karetnikova and Garetova, 2009; Mironov and Alyomov, 2018). These microorganisms react quickly to changes in the environment and can be considered as important indicators for assessing the quality of the water bodies (Parmar et al., 2016).

Based on the results of the microbiological analysis, the studied bays can be arranged in the following sequence according to increasing level of pollution in bottom sediments: B. Laspi → B. Kazach'ya → B. Streletskaya. The results obtained are consistent with the literature data on the content of petroleum hydrocarbons (PH) and chloroform-extractable substances (CES) in bottom sediments of the studied areas. Thus, the maximum concentrations of PH were noted in the bottom sediments of the Streletskaya Bay – from 105 to 700 mg/100 g. In the Kazach'ya Bay the content of PH was 60 mg/100 g. In the Laspi Bay the minimum values were noted - 9 mg/100 g. The CES concentration in B. Laspi was 37 mg/100 g, B. Kozach'ya - 23.4 mg/ 100 g, B. Streletskaya - from 200 to 1,560 mg /100 g (Tikhonova et al., 2016; Tikhonova et al., 2018; Soloveva et al., 2019).

Earlier, the ecological state of the coastal areas of Sevastopol was also assessed by using the main chemical and microbiological indicators. The highest abundance of heterotrophic and hydrocarbon -oxidizing bacteria was registered in sediments of the most contaminated stations - in the apical parts of the Kruglaya, Artilleriyskaya and Yuzhnaya bays. Low abundance of the microorganisms was noted in Kazach'ya Bay, characterized by relatively low levels of chemical pollution and high biodiversity (Tikhonova et al., 2018). A direct correlation between the abundance of hydrocarbon-oxidizing bacteria and the intensity of seawater oil pollution was shown by other researches (Litvinova et al., 2011; Novikov, 2005; Schuka and Volodkovich, 2015). To assess oil pollution in coastal waters of the Baltic Sea and the Kara Sea, the number of hydrocarbon-oxidizing bacteria was determined. The average values of HOB in the southeastern part of the Baltic Sea were: in winter - 10-100 cells/mL; in spring - 10-1,000 cells/mL; in summer and autumn - 10-10,000 cells/mL. In coastal waters of the Kara Sea the number of hydrocarbon-oxidizing microorganisms obtained 100 cells/mL. In all cases, large number of HOB was found in waters with elevated concentrations of petroleum hydrocarbons (Schuka and Volodkovich, 2015). Microbiological studies in Kola Gulf showed direct correlation between the number of hydrocarbon-oxidizing microorganisms and the level of petroleum hydrocarbons pollution (Litvinova et al., 2011).

Mussel *Mytilus galloprovincialis* is a sedentary active filtrator and bioaccumulator of mineral and organic pollutants. Excessive accumulation of toxicants in body tissues induces oxidative stress caused by reactive oxygen species (ROS) and leads to lipid and protein peroxidation, DNA damage. It also changes and inhibits activity of different enzymes, causes cell damage resulting in apoptosis. An increase of lipid and protein peroxidation in hydrobionts tissues is a universal indicator of oxidative stress under the impact of certain toxicants and complex pollution of water areas. Oxidative damage may be minimized by antioxidant defense mechanisms through low molecular weight compounds and enzymes which protect the cell against cellular oxidants and repair systems, preventing the accumulation of oxidatively damaged molecules. Antioxidant enzymes CAT and SOD play a vital role in maintaining cellular homeostasis, protecting cellular damage from harmful effects of ROS (Rudneva et al., 2016; Benaly et al., 2017; Kroon et al., 2017; Ozkan et al., 2017).

In our research an increase in the TBARS and oxidized protein levels in the hepatopancreas of mussels from the most polluted Streletskaya Bay can indicate an enhance of free radical processes and the development of oxidative stress (Table 1). At the same time, an increase in the activity of the key antioxidant enzymes SOD and CAT against the significantly high levels of oxidized proteins and lipids can demonstrate a compensatory nature of the adaptive response to pollution, aimed at reducing the intensity of free radical oxidation. Results of the present study showed that intensification of oxidative processes in hepatopancreas of *M. galloprovincialis* from the polluted area led to activation of OA protective mechanisms due to the increase of enzyme activities.

Similar responses of prooxidant-antioxidant system were observed in the tissues of bivalves under conditions of high pollution of the aquatic environment (Ozkan et al., 2017; Sukharenko et al., 2017; Klimova et al., 2019). The increase in TBARS content, SOD and CAT activities were found in gills and digestive gland of mussel *M. galloprovincialis* from most polluted area (Aliaga) compared to control one (Urla) in the Aegean Coast of Turkey (Ozkan et al., 2017). Significantly high values of LPO and CAT activity were observed in tissues of *Dreissena polymorpha* inhabiting polluted sites in comparison with conventionally pure area in the Rybinsk Reservoir (Russia) (Klimova et al., 2019). A significant increase in LPO as well as CAT, SOD, glutatione reductase (GR) and glutatione-S-transferase (GST) activities was observed in the hepatopancreas of *Dreissena polymorpha* affected by fuel oil compared to the control group. A similar LPO growth and modulation of CAT, SOD, GR, GST activities were determined in the hepatopancreas of the mussel *Mytilus galloprovincialis* collected in the Kerch gulf polluted with resins, hydrocarbons and asphaltenes compared to the conventionally pure waters of Donuzlav lake (the Black Sea) (Sukharenko et al., 2017).

To assess the ecological state of coastal marine areas, along with prooxidant-antioxidant system parameters, it is recommended to study the activity of aminotransferases (ALT and

AST) characterizing the state of the main metabolic pathways in an organism. ALT and AST catalyze the mutual conversion of amino acids and α-ketoacids by transferring amino groups, therefore, changes in their activity lead to a violation of carbohydrate and protein metabolism. Aminotransferases are highly sensitive to the influence of natural and anthropogenic factors and take part in the organism responses to environmental pollution (Mohite et al., 2011; El-Khayat et al., 2015; Rudneva et al., 2016). In our studies ALT activity was significantly higher in the hepatopancreas of mussels from most polluted Streletskaya Bay, indicating the activation of transamination processes between alanine and α-ketoglutaric acid. The revealed features against significantly high levels of oxidized proteins in specimens from B. Streletskaya (Table 2), could be referred to the compensatory rearrangement of protein metabolism in the direction of substrate support for the reactions of gluconeogenesis and amino acid synthesis. An increase in aminotransferase activities in the hepatopancreas of *M. galloprovincialis* living in the most polluted water area compared to conventionally pure areas was found by other researchers (Sigacheva et al., 2021). With prolonged exposure to heavy metals (cadmium and zinc), AST and ALT activities in the hepatopancreas of green mussel *Perna viridis* had been rising (Mohite et al., 2011). In tissues of freshwater mollusks genera *Planobris, Physa, Biomphalaria* from waters with high level of pollution an increase in the aminotransferase activities and glucose content was also noted. The authors explained the revealed features by the intensification of gluconeogenesis, which is necessary for the energy support of mollusk tissues under anthropogenic impact (El-Khayat et al., 2015).

CONCLUSION

Thus a comparative analysis of microbiological indicators in bottom sediments and biochemical parameters in the hepatopancreas of *M. galloprovincialis* from three Sevastopol bays (the Black Sea) allowed to identify certain differences between the tested areas and characterize their ecological state. The maximum abundance of heterotrophic and hydrocarbon-oxidizing bacteria was found in the most polluted Streletskaya Bay, the minimum - in the conventionally pure Laspi Bay. Significant increase in the parameters of prooxidant-antioxidant system (level of oxidized proteins and lipid peroxidation, SOD and CAT activities), as well as ALT activity in the hepatopancreas of mussels from Streletskaya Bay compared to the corresponding parameters in Kazach'ya and Laspi bays was found. The results obtained indicate the severe pollution and less favorable living conditions for aquatic organisms in Streletskaya Bay, as well as demonstrate the high level of biochemical adaptation of mussels to complex environmental pollution. The studied parameters can be applied in the ecological monitoring of the coastal sea water areas.

GRANT SUPPORT DETAILS

The present research was carried out within framework of the State assignment of the IBSS on the theme " Patterns of formation and anthropogenic transformation of biodiversity and bioresources of the Azov-Black Sea Basin and other areas of the World Ocean" (№ 121030100028-0) and the theme "Molismological and biogeochemical foundations of the marine ecosystems homeostasis" (№ 121031500515-8).

CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

REFERENCES

Alyomov, S. V. (2021). Seasonal and interannual dynamics of macrozoobenthos communities in Sevastopol bay (the Black sea) at the beginning of the XXI century in areas with different pollution levels. Proceedings of the T.I. Vyazemsky Karadag scientific station - Nature Reserve of the Russian Academy of Sciences, 1(17); 3-16. (In Russ.)

Asif, N., Malik, M. F. and Chaudhry, F. N. (2018). A review of on environmental pollution bioindicators. Pollution, 4(1); 111-118.

Barinova, S. and Krupa, E. (2017). Bioindication of ecological state and water quality by phytoplankton in the Shardara reservoir, Kazakhstan. Environment and Ecology Research, 5(2); 73-92. (In Russ.)

Benali, I., Boutiba, Z., Grandjean, D., de Alencastro, L. F., Rouane-Hacene, O. and Chèvre, N. (2017). Spatial distribution and biological effects of trace metals (Cu, Zn, Pb, Cd) and organic micropollutants (PCBs, PAHs) in mussels Mytilus galloprovincialis along the Algerian west coast. Mar. Poll. Bull., 115; 539-550.

Cajaravillea, M. P., Bebianno, M. J., Blasco, J., Porte, C., Sarasquete, C. and Viarengo, A. (2000). The use of biomarkers to assess the impact of pollution in coastal environments of the Iberian Peninsula: a practical approach. The Science of the Total Environment, 247; 295-311.

Chesnokova, I. I., Sigacheva, T. B. and Skuratovskaya E. N. (2020). Comparative Analysis of Hepatic Biomarkers of Black Sea Scorpion fish Scorpaena porcus Linnaeus, 1758 from Sevastopol Water Areas (the Black Sea) with Different Pollution Levels. Water Resources, 47(3); 486-490.

Dubinina, E. E., Burmistrov, S. O., Khodov, D. A. and Porotov, I. G. (1995). Oxidative modification of human serum proteins, method for its determination. Problems of Medical Chemistry, 41(1); 24-26. (In Russ.)

El-Khayat, H. M. M., Hamid, H. A., Gaber, H. S., Mahmoud, K. M. A. and Flefel, H. E. (2015). Snails and Fish as Pollution Biomarkers in Lake Manzala and Laboratory A: Lake Manzala Snails. Fisheries and Aquaculture Journal, 6(4); 1-9.

Karetnikova, E. A. and Garetova, L. A. (2009). Bacterioplankton and bacteriobentos of the Amur estuary and adjacent water areas in the summer of 2006. Oceanology, 49 (3); 409-417. (In Russ.)

Klimova, Y. S., Chuiko, G. M., Gapeeva, M. V., Pesnya, D. S. and Ivanova, E. I. (2019). The Use of Oxidative Stress Parameters of Bivalve Mollusks Dreissena polymorpha (Pallas, 1771) as Biomarkers for Ecotoxicological Assessment of Environment. Inland Water Biology, 12(2); S88-S95.

Korolyuk, M. A., Ivanova, L. I., Mayorova, I. G. and Tokarev, V. E. (1988). Method for determination of catalase activity. Laboratory working, 1; 16-19. (In Russ.)

Kotelyanets, E. A., Gurov, K. I., Tikhonova, E. A. and Soloveva, O.V. (2017). Some geochemical indicators of sea bottom sediments in coastal waters under the influence of antropogenous factor (using Kazachya Bay, Sevastopol, as an example). Bulletin of Udmurt University, 27(1); 5-13. (In Russ.)

Kroon, F., Streten, C. and Harries, S. (2017). A protocol for identifying suitable biomarkers to assess fish health: A systematic review. PLoS ONE, 2(4); e0174762.

Litvinova, M. Yu., Ilyinsky, V. V. and Peretrukhin I. V. (2011). Quantitative assessment of heterotrophic bacterioplankton in the water of the northern and middle knees of the Kolskiy Bay. Fundamental research, 7; 203-206. (In Russ.)

Luk'yanova, O. N. and Korchagin, V. P. (2017). Integral biochemical index of the state of aquatic organisms under polluted conditions. Biology Bulletin, 4(2); 203-209.

Malakhova, L. V., Skuratovskaya, E. N., Malakhova, T. V., Boltachev, A. R. and Lobko, V. V.(2018). Organochlorine compounds in scorpion fish Scorpaena porcus Linnaeus, 1758 in the Sevastopol marine area (The Black sea): spatial distribution and biological response. Marine Biological Journal, 3(4); 51-63. (In Russ.)

Mironov, O. G. (1988). Mussels as an element of the hydrobiological treatment of polluted sea waters. Water resources, 5; 104-111.

Mironov, O. G., Alyomov, S. V. (Eds.) (2018). Sanitary and Biological Studies of the South Western Crimea Coastal Waters At the Beginning of XXI Century. (ARIAL, Simferopol)

Mohite, V. T., Lahir, Y. K., Pathare, M., Agwuocha, S. and Mane, U. H. (2011). Study on the effects of exposure of sub lethal dose of cadmium and zinc on the enzymatic activity in the tissues of green mussel – Perna viridis (L) from Ratnagiri coast, Maharashtra. Journal of ecophysiology and occupational health, 11(3-4); 131-140.

Nemova, N. N., Mecheryakova, O. V., Lysenko, L. A. and Fokina, N. N. (2014). Assessment of the state of aquatic organisms by their biochemical status. Proceedings of KarRC RAS, 5; 18-29. (In Russ.)

Netrusov, A. I. (Eds.) (2005). Workshop on microbiology. (Moscow: Academia Press)

Nishikimi, M., Rao, N. A. and Yagi, K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochim. Biophys. Res. Commun, 46(2); 849-854.

Novikov, Yu. V. (Eds.) (2005). Ecology, environment and person. (Moscow: FAIR-Press) (In Russ.)

Oliva, M., Gravato, C., Guilhermino, L., Galindo-Riano, D. M. and Perales, A. J. (2014). EROD activity andcytochrome P4501A induction in liver and gills of Senegal sole Solea senegalensis from a polluted Huelva Estuary (SW Spain). Comp Biochem Phys C., 166; 134-144.

Ovsyaniy, E. I. and Orekhova, N. A. (2019). Accumulation of organic matter in bottom sediments of Kazach'ya Bay (Black Sea) as a result of anthropogenic load. Meteorologiya i Gidrologiya, 5; 85-93.

Ozkan, D., Dagdeviren, M., Katalay, S., Guner, A. and Yavaşoğlu, N. Ü. K. (2017). Multi-Biomarker Responses After Exposure to Pollution in the Mediterranean Mussels (Mytilus galloprovincialis L.) in the Aegean Coast of Turkey Bull. Environ. Contam. Toxicol., 98; 46-52.

Pankeeva, T. V. and Mironova, N. V. (2019). Spatial distribution of macrophytobentos with due account for the landscape structure of the bottom of Laspy Bay (Black Sea). Bulletin of Udmurt University, 29(1); 111-123.

Parmar, T. K., Rawtani, D. and Agrawal, Y. K. (2016). Bioindicators: the natural indicator of environmental pollution. Frontiers in Life Science, 9(2); 110-118.

Pešić, V., Gadawski, P., Gligorović, B., Glöer, P., Grabowski, M., Kovács, T., Murányi, D., Płóciennik, M. and Šundić, D. (2018). The diversity of the Zoobenthos communities of the Lake Skadar/Shkodra basin. (In Pešić, V., Karaman, G. and Kostianoy A. G (Eds.). The Skadar/Shkodra Lake Environment, Handbook of Env. Chem. (pp. 255-293). Switzerland AG: Springer)

Polyaka, Yu. M., Demchuk, A. S., Sharova, A. N., Gubelit Yu. I. and Berezina N. A. (2020). Hydrocarbon-Oxidizing Bacteria in the Digestive System of Fish as an Indicator of Coastal Pollution. Doklady Biological Sciences, 491; 71-74.

Rudneva, I. I., Skuratovskaya, E. N., Chesnokova, I. I., Shaida, V. G. and Kovyrshina, T. B. (2016). Biomarker response of Black Sea scorpion fish Scorpaena porcus to anthropogenic impact (In A. Kovács & P. Nagy (Eds.), Advances in Marine Biology., 1 (5, pp. 119-147). New York: Nova Sci. Publs)

Sarker, J., Tanmay, M. H., Rahman, F., Patwary, M. S. A. and Rima, N. N. (2016). Assessment of coastal water pollution in Greater Noakhali-Bangladesh. J Coast Zone Manag, 19(2); 1-7.

Schuka, T. A. and Volodkovich, Yu. L. (2015). Study of the processes of microbial destruction of oil pollution and the experience of monitoring the spread of hydrocarbon-oxidizing microorganisms in the southeastern parts of the Baltic and Kara Seas. Problems of ecological monitoring and modeling of ecosystems, 26(1); 180-204. (In Russ.)

Sigacheva, T. B., Chesnokova, I. I., Gostyukhina, O. L., Kholodkevich, S. V., Kuznetsova, T. V., Andreenko, T. I., Kovrigina, N. P., Gavruseva, T. V., Kirin, M. P. and Kurakin, A. S. (2021). Assessment of recreational potential of Sevastopol bays using bioindication methods. South of Russia: ecology, development, 16(1); 151-167. (In Russ.)

Soloveva, O. V., Tikhonova, E. A., Klimenko, T. L., Skrupnik, G. V. and Votinova, T.V. (2019). Organic Substances of Sea Bottom Sediments in Conditions of the Coast Urbanization (for Example Kazachiya Bay, the Black Sea). Oceanology, 59(2); 234-242. (In Russ.)

Sonnenwirth, A. C. and Jarett, L. (1980). Gradwohl's clinical laboratory methods and diagnosis, eighth ed. Library of congress cataloging in publication data (USA).

Stalnaya, I. D. and Garishvili, T. G. (1977). Method for determination of malondialdehyde using thiobarbituric acid. (In Modern methods in biochemistry (pp. 66-68). Moskva: Meditsina.) (In Russ.)

Sukharenko, E. V., Nedzvetsky, V. S. and Kyrychenko, S. V. (2017). Biomarkers of metabolism disturbance in bivalve molluscs induced by environmental pollution with processed by-products of oil. Biosystem Diversity, 25(2); 113-118.

Sumampouw, O. J. and Risjani, Y. (2014). Bacteria as indicators of environmental pollution: review. International Journal of Ecosystem, 4(6); 251-258.
Talukdar, D., Basumatari, D. and Rahman, S. (2022). Monitoring Nature's Kidneys with the aid of Macrobenthic Assessment: A case study in the city Wetlands of Guwahati, India. Pollution, 8(1); 45-56.
Tikhonova, E. A., Burdiyan, N. V., Soloveva, O. V. and Doroshenko, Yu. V. (2018). The estimation of the Sevastopol bays ecological state on basic chemical and microbiological criteria. Ecology, Environment and Conservation, 24(4); 1574-1584.
Tikhonova, E. A., Kotelyanets, E. A. and Volkov, N. V. (2018). Characteristic of pollution of the bottom deposits in the coastal area of Sevastopol on the example of the Streletskaya bay (The Black sea) Ecological safety of coastal and shelf zones of sea, 1; 74-80. (In Russ.)
Tikhonova, E. A., Kotelyanets, E. A. and Soloveva, O. V. (2016). Assessment of the level of pollution of bottom sediments of the Crimean coast of the Black and Azov Seas. Principles of the Ecology, 5(21); 56-70. (In Russ.)
Uzbekova, O. R. and Lutsenko, E. S. (2011). Study of the activity of mussels Mytilus edulis littoral of the Gulf of Kola according to microbiological indicators. Advances in current natural sciences, 8; 73-75.
Vidal-Linan, L., Bellas, J., Soriano, J. A., Concha-Grana, E., Muniategui, S. and Beiras, R. (2016). Bioaccumulation of PCB-153 and effects on molecular biomarkers acetylcholinesterase, glutathione-S-transferase and glutathione peroxidase in *Mytilus galloprovincialis* mussels. Environmental Pollution, 214; 885-891.
Zolkefli, N., Ramli, N., Mohamad-Zainal, N. S. L., Mustapha, N. A., Yusoff, M. Z. M., Hassan, M. A. and Toshinari, M. (2020). Alcaligenaceae and Chromatiaceaeas pollution bacterial bioindicators in palm oil mill effluent (POME) final discharge polluted rivers. Ecological Indicators, 111; 106048.