World Academy of Science, Engineering and Technology
International Journal of Animal and Veterinary Sciences
Vol:18, No:7, 2024

Rivers Drain Impact on the Black Sea Coastal Line Biocenosis within the Greater Sochi Area Assessed by Bioassay Method

Tatiana L. Gorbunova

Abstract**—**The research is dedicated to the study of the polluted river inflow impact on the Black Sea coastal marine environment within the watercourse's plumes in the Greater Sochi area applying bioassay methods with using freshwater and marine microalgae. River waters were analyzed using microalgae *Chlorella vulgaris* Beijer and sea waters were tested with marine diatoms *Phaeodactylum tricornutum* Bohlin. Experiments included algae cells abundancy growth assessments in acute (24 hours), sub-acute (72 hours) and chronic (168 hours/7 days) tests. The increase in algal cell growth rates compared to the control in the summer period was detected as a consequence of the recreational activities intensification during the tourism seasonal peak. Most of the analyzed samples demonstrated a significant effect of algae cells growth stimulation compared to the control. It is established that under the impact of contaminants carried by river's drain to the sea, the capacity of the coastal marine ecosystem is partially capable to compensate its effect on the coastal biocenosis, but the general trends of the impact processes remain constant.

Keywords**—**Algae abundance growth, bioassay, microalgae, modeling.

I. Introduction

THE area of the research was established as a prominent subtropical resort in southern Russia, located along the North-Eastern Black Sea coast. The town attracts a large number of tourists and, in connection with this, experiences a significant anthropogenic pressure, which is increasing in summer season [1].

Moreover, the Greater Sochi area has a wide river network, which, in conjunction with the coastal marine zone, forms a unified ecological system where the massive migration and transformation of suspended solid, colloidal and dissolved material from terrestrial sites to the sea and from the water column to the bottom sediments occur [2]. Therefore, specifically in the estuarine zones, a particular contact zone of marine and river waters is formed [3].

To assess the complex influence of various impact factors delivered to the marine coastal environment through rivers inflow, this work employed biotesting methods utilizing unicellular planktonic microalgae. As representatives of the primary trophic chains level, these organisms provided a valuable tool to detect both the toxic and stimulating pollutants effects and to predict the development of the aquatic organisms of subsequent food levels [4], [5].

This research aims to utilize autotrophic organisms occupying the initial level of aquatic biocenosis' food chain as biomarkers to assess the complex influence of various impact factors delivered to the marine coastal environment through river inflow. The assessment will consider the watershed's mouths and their plumes zones as a unified ecological system, highlighting the interconnectedness of the river-sea environment.

II. Materials and Methods

Water samples collected from the mouths of the Psezuapse, Sochi, Kherota and Mzymta rivers located within the Greater Sochi area, as well as their plume waters at their inflows into the Black Sea (approximately 50 m from the coastal line), were examined to detect toxicity or stimulating effect using bioassay methods. The sampling was conducted in the spring-summer period (from April to August) in 2022. The locations of the sampling stations are presented in Table I.

TABLE I
THE SAMPLING STATIONS` LOCATION OF THE RIVERS AND THEIR PLUMES

Sampling station	Locations of the sampling stations
Mouth of Psezuapse River	43°54'12.3"N 39°20'22.3"E
Mouth of Sochi River	43°35'23.4"N 39°43'03.8"E
Mouth of Kherota River	43°26'26.7"N 39°54'36.0"E
Mouth of Mzymta River	43°25'43.2"N 39°56'10.8"E
Psezuapse River's plume	43°53'56.6"N 39°19'58.6"E
Sochi River's plume	43°34'59.7"N 39°42'50.8"E
Kherota River's plume	43°26'22.2"N 39°54'19.3"E
Mzymta River's plume	43°25'02.9"N 39°55'18.6"E

River water biotests were carried out using microalgae *Chlorella vulgaris* Beijer according to the methodological recommendations of [6], while sea water bioassays were carried out according to the method proposed by [7] using *Phaeodactylum tricornutum* Bohlin.

The culture of *C. vulgaris* is received from the Institute of Plant Physiology named after Dr. Timiryazev (Moscow, RF) and was grown on Tamiya medium. Diatoms *P. tricornutum*, isolated from the Black Sea plankton at the Department of Ecological Physiology of Algae, FIC InBSS, was grown on Goldberg's medium in Kabanova's modification.

Bioassays and algae cultures' sensitivity checks were performed under standardized conditions, outlined in the methodological recommendations. Algal cell counts were

T. L. Gorbunova is with the Federal Research Centre the Subtropical Scientific Centre of the Russian Academy of sciences, Sochi, Russian Federation (phone: +7 988 2841570; e-mail: tatianashaw@ mail.ru).

performed using a Goryaev chamber at four designated time points: 0 hours (baseline), 24 hours (to assess acute toxicity), 72 hours (to assess sub-acute toxicity), and 7 days (to assess chronic toxicity).

The coefficient of algae cell abundance growth was calculated using:

$$Kgr = Nt \div Nn \quad (1)$$

where Nt - initial cells count, determined at the beginning of the experiment in control and tested water; Nn – cell count, calculated after n time of the experiment exposure (24 hours day, 72 hours, 7 days).

Calculations of the deviation degree proportion of the Kgr value of the tested samples experiment from the control sample were carried out according to:

$$Xk = [(Kexp. - Kcont.) \div Kcont.] - 100\% \quad (2)$$

where Xk is the deviation of the microalgae cell abundance growth factor, %, Kexp. is the microalgae cell number growth factor in the experiment, Kcont. is the coefficient of microalgae cell abundance growth in the control.

A 25% variation (suppression or stimulation) in the algae abundance growth coefficient compared to the control samples prepared according to the methodological instructions served as the criteria of water samples toxicity.

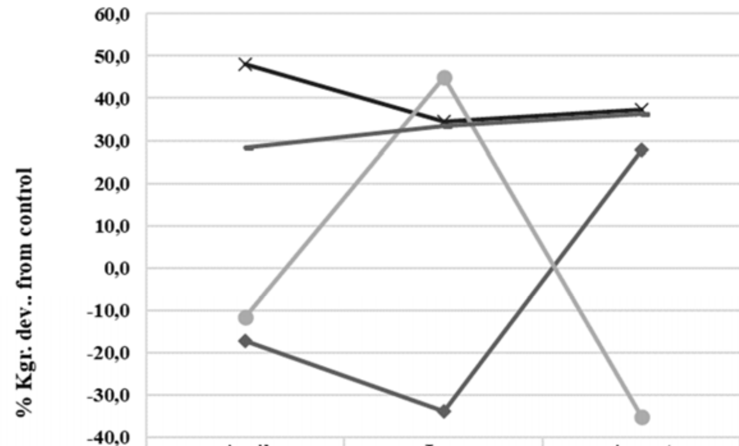
The significance of deviations in the cell abundance growth coefficient between the analyzed sample and the control was assessed using Student's test at P = 0.95 and n = 9. A significant positive deviation from the control indicates the inhibitory effect of toxicants on algae, while a significant negative deviation suggests a stimulating effect of the tested water on the test organisms, indirectly suggesting the presence of organic substances in the water. Experimental data were processed using mathematical modeling with the three-dimensional interpolation methods application through the software "gnuplot" (version 5.4).

III. Biotesting the River Water

No significant deviations from the control were observed in any river samples after 24 hours of exposure, indicating the absence of acute toxicity in the tested waters. The most significant deviations of the algae abundance growth coefficients values from the control were detected within the sub-acute experiment (72 h) in the Sochi River samples in April with the effect of cell growth stimulation (Fig. 1). However, in the chronic experiments (7 days) magnitude of deviation from the control in the same samples decreased (Fig. 2).

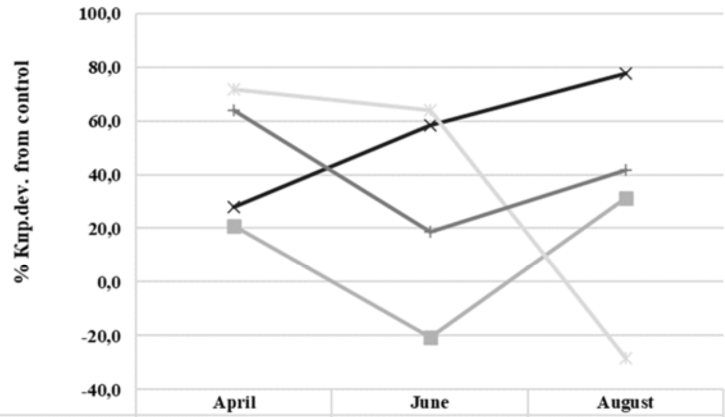
At the same time, in the Sochi River mouth samples taken in June and August, significant stimulatory effect was enhanced in the chronic experiment compared to sub-acute exposure time (Figs. 1 and 2). All samples of the Kherota River mouth demonstrated significant deviations from the control in regards to algal growth; however, in April, a significant stimulatory effect was observed only in the chronic experiment. In June, samples from this station displayed a significant effect of algae growth stimulation, while in August, inhibition of cells abundance growth was observed in both sub-acute and chronic experiments (Figs. 1 and 2).

In the samples of Mzymta River, a significant stimulating effect of algae cells growth compared to the control was observed in practically all sub-acute (72 h exposition) experiments (Fig. 1). In chronic experiments, however, fluctuations of the of algae cells growth against control coefficients were observed in that sample; and in samples taken in July, deviations from the control after 7 days of exposure declined to statistically insignificant values (Fig. 2).



	April	June	August
Psezuapse River, mouth	-17,2	-33,9	27,9
Sochi River, mouth	48,1	34,4	37,3
Kherota river, mouth	-11,7	44,9	-35,2
Mzymta river, mouth	28,4	33,5	36,4

Fig. 1 Algae (*C. vulgaris*) growth coefficients deviation from control in sub-acute experiments in the rivers samples biotests



	April	June	August
Psezuapse River, mouth	20,9	-20,5	31,4
Sochi River, mouth	27,9	58,2	77,6
Kherota river, mouth	71,9	63,8	-28,3
Mzymta river, mouth	63,8	18,8	41,7

Fig. 2 Algae (*C. vulgaris*) growth coefficients deviation from control in chronic experiments in the rivers samples biotests

Samples of the Psezuapse River mouth demonstrated significant deviations from the control values in June in the sub-acute experiments with the effect of inhibiting cell abundancy growth, and in August, both in sub-acute and chronic experiments, the effect of stimulation was displayed (Figs. 1 and 2).

Only samples of two rivers demonstrated significant deviations from the control values with the effect of algae abundance inhibiting: the Psezuapse River mouth in sub-acute experiments in June and the Kherota River mouth in August both in the sub-acute and chronic experiments (Figs. 1 and 2). This response of the biomarkers indicates the toxic substances influence to the development microalgae in these watercourses. However, the fact that in the Psezuapse River samples their toxic properties disappear during an extended exposure of the chronic test (7 days) suggests the possible transformation of the toxicant in the aquatic environment over the period of time to the substance without toxic effect. Toxic properties of the Kherota River samples were retained in the chronic experiment, but the cell abundancy growth deviation from the control level differed.

IV. Biotesting the Black Sea Coastal Waters in the Rivers' Plumes

Analyzing the river drains' impact on the coastal marine waters, the simultaneous influence of other factors on their quality, such as storm water and household unorganized sewerage effluents from the adjacent territories, should not be excluded.

In a series of samples there was detected a significant deviation from the control (filtered through the filter "Blue Ribbon" and pasteurized conditionally clean seawater served as a control for testing sea water samples) primarily with the effect of cell abundance growth stimulation. Significant inhibitory effects were observed only in August samples, collected from the Kherota River plume, in the chronic experiment.

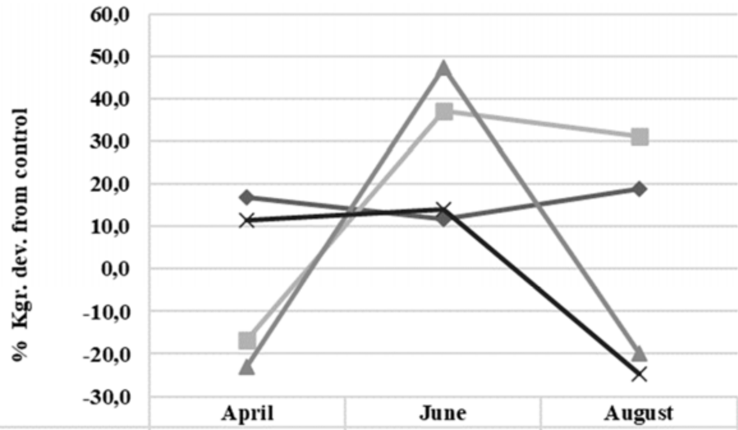
In sub-acute experiments (72 h), minor suppression of algal abundance growth was also observed in several samples: Sochi and Kherota Rivers in April and August, and Mzymta River in August. However, no significant deviations from the control were detected in these experiments (Fig. 3).

The most significant values of the cell abundance growth coefficients deviations from the control with the effect of cell growth stimulation were observed in sub-acute experiments with samples collected from the Sochi and Kherota rivers plumes in June (Fig. 3).

Also, chronic experiments (7 days) with the plume samples of the Sochi (August) and Kherota (April) rivers demonstrated significant deviations from the control values of algae cells growth (Fig. 4). The magnitude of algae growth coefficient deviations from the control in chronic experiments in comparison to sub-acute experiments data for samples from Kherota river plum (August) had significantly increased. However, the samples taken from Mzymta river plume during the same month demonstrated changes in character of influence on the biomarkers: if in the sub-acute experiment (72 h) insignificant inhibitory of algae cells abundance was observed (Fig. 3), in the chronic experiment (7 days), the noticeable effect of algae growth stimulation was detected (Fig. 4).

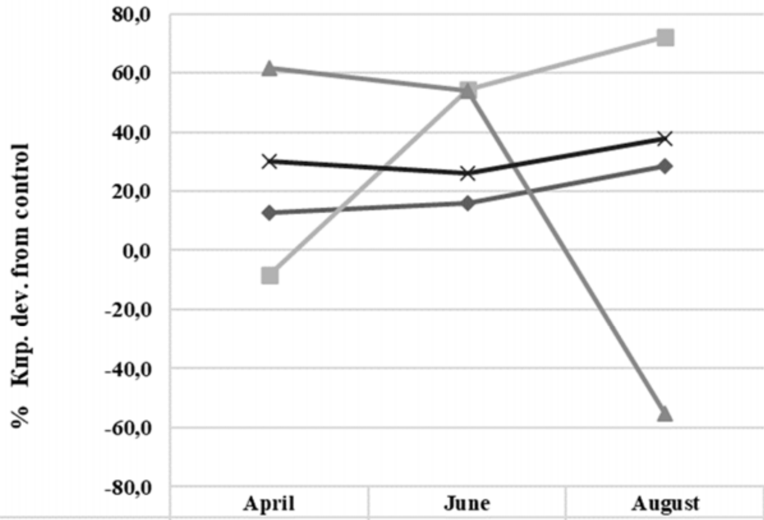
Throughout the research period, 25% of coastal seawater samples collected in the rivers plumes zones were identified as having significant deviations from the control within the sub-acute experiments (Fig. 5). All these samples demonstrated the effect of cell abundance growth stimulation which could be caused by both excessive nutrient content and trace concentrations of inductors of natural genesis, technogenic or secondary pollution, such as phenols, oil products, ions of certain metals, etc. [8]-[11].

At the same time, during chronic biotesting, some samples, which previously had no significant effect on algae growth, showed significant deviations from the control with the effect of inhibiting cell growth (8%), which indicates a change in the toxic properties of pollutants through processes occurring in the aquatic environment over time [12]. This reduced the proportion of non-toxic samples to 76% (Fig. 6).



	April	June	August
Psezuapse River plum	16,9	11,8	18,9
Sochi River plum	-16,7	37,1	31,2
Kherota River plum	-23,0	47,5	-19,8
Mzymta River plum	11,4	14,2	-24,8

Fig. 3 Algae *(P. tricornutum*) growth coefficients deviation from control in sub-acute experiments in the rivers plumes samples tests



	April	June	August
Psezuapse River plum	12,9	16,1	28,3
Sochi River plum	-8,4	54,3	72,1
Kherota River plum	61,5	54,0	-55,2
Mzymta River plum	30,2	26,2	37,7

Fig. 4 Algae (*P. tricornutum*) growth coefficients deviation from control in chronic experiments in the rivers plumes samples biotests

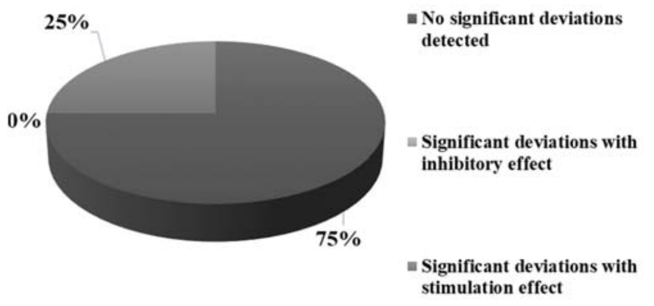


Fig. 5 Characteristics of the rivers plums waters impact to marine microalgae in sub-acute experiments

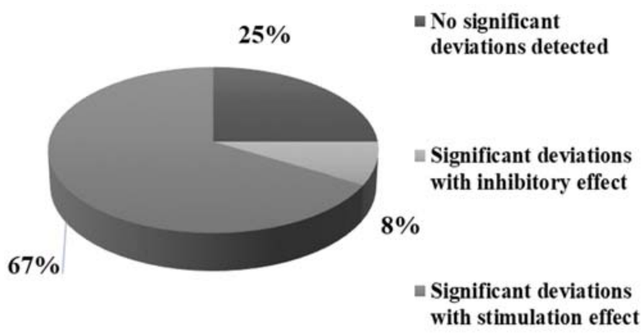


Fig. 6 Characteristics of the rivers plums waters impact to marine microalgae in chronic experiments

V. MATHEMATICAL MODELING

To analyze the impact of rivers drains on the coastal marine zone environment, we applied mathematical modeling with three-dimensional interpolation methods. Fig. 7 reflects an influence of tested river waters on *C. vulgaris* growth rate compared to the control parameters. It demonstrates for the sub-acute experiments a well-defined local maximum (45%) of algae growth coefficient values deviation from the control (Fig. 7 (a)), which is observed in the Kherota River mouth samples collected in June.

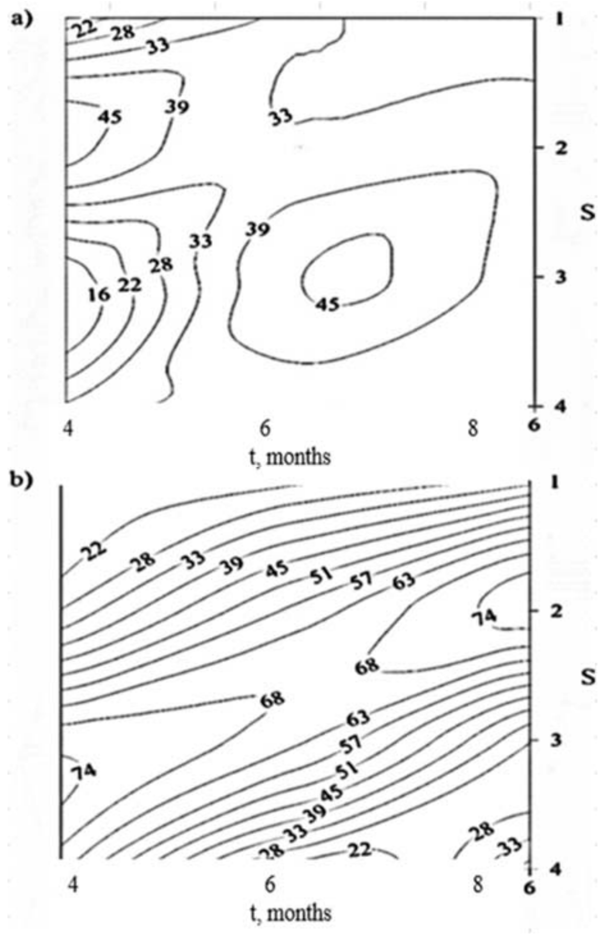


Fig. 7 Dynamic of algae abundance growth coefficients (Kgr) deviations from control (in percent, %) in the rivers' mouths: (a) results of sub-acute tests (72 h); (b) results of chronic tests (7 days); 1 Psezuapse River's mouth; 2 Sochi River's mouth; 3 Kherota River's mouth; 4 Mzymta River's mouth

The second peak of deviation from the control (45%) represents the Sochi River mouth samples, collected in April. And the third, weaker surge (33%) was observed for the samples of the Sochi River collected in August.

However, Fig. 7 (b), which depicts the impact of the river waters on the algae during chronic tests, reveals significant transformation in the local maxima of the algae abundance growth compared to the control over the extended exposure period (7 days).

The axis of the two local maxima runs at an angle close to 40º clockwise. The first such maximum illustrated the impact of Kherota River samples, displaced to the beginning of the observation period (April), with absolute values increasing by 64% (from 45% to 74%). In the second maximum zone, representing Sochi River's mouth, a minimum (22%) is observed with an increased exposure time to 7 days in comparison to the sub-acute test results. Currently, for the sampling station located at the mouth of the Sochi River, the maximum (74%) is observed in August instead of April, as it was observed for the sub-acute experiments.

Fig. 8 displays the dynamic of the coastal marine waters influence in the studied rivers drains plume zones to the sea water algae *P. tricornutum* growth. Fig. 8 (a) indicates that there is one local maximum (45%) of the algae abundance growth coefficient deviation from the control, detected for Kherota River plume waters in June.

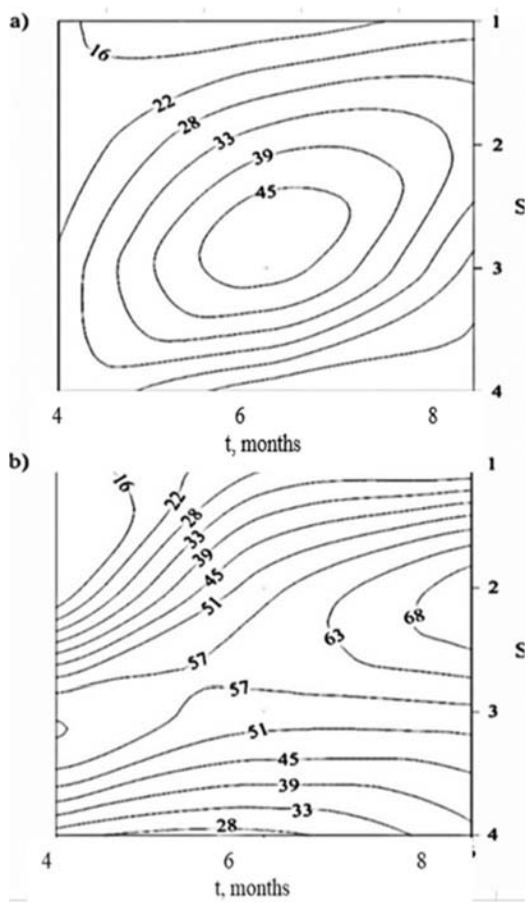


Fig. 8 Dynamic of algae abundance growth coefficients (Kgr) deviations from con8trol (in percent, %) in the marine coastal waters of rivers' plumes: a) results of sub-acute tests (72 h); (b) results of chronic tests (7 days); 1 Psezuapse river's plume; 2 Sochi river's plume; 3 Kherota river's plume; 4 Mzymta river's plume

Compared to the same peak traced for the mouth of this river (Fig. 7), this local maximum has a smoother ascent but a broader distribution area.

Fig. 8 (b) demonstrates that in the coastal marine waters, a local maximum of cell abundance growth deviations from the control is displayed, similar to the analogical parameters, reflected in the graph in Fig. 7 (b). Its general patterns approximate the model which describes the chronic experiments (Fig. 7 (b)). As well, there clearly appears to be a fold axis of the local maximum at an angle close to 40º clockwise, which looks more gradual, that for the scheme, describing the rivers. The maximum that was previously identified (Fig. 7 (a)) becomes part of the ascending folding line of the overall surface response model. The distinct maximum (68%) located in the plume zone of the Sochi River is specific for August. The general maximum values and fold structure level are significantly lower than for the model describing chronic toxicity in the rivers' mouths (Fig. 7 (a)). Thus, it can be assumed that the coastal marine ecosystem capacity partially compensates for the effect of river contamination, but the general patterns of impact processes remain consistent [5].

VI. Conclusion

Samples taken at the rivers' mouth stations, located in catchment areas characterized by intensive urbanization and/or anthropogenic impact, demonstrated significant algal growth rates compared to the control throughout the entire observation period. Notably, certain samples displayed an inhibitory effect on algae, indicating the presence of dissolved toxicants. However, in the majority of cases, there was a stimulating effect compared to the control parameters, conditioned by a complex interplay of natural and anthropogenic factors.

An increase in algae cell growth rates compared to the control in the summer months is a consequence of the recreational and resort activities intensification in the summer period and, therefore, increased anthropogenic pressure within the urban catchment areas of rivers.

The evidence of the dissolved substances and their compounds transformation in water over the time were detected through observing changes in the character and magnitude of the tested waters impacts on the algae cells abundance growth in some samples throughout the experiment period: from acute (24 h) and sub-acute (72 h) to chronic (7 days) tests. This phenomenon should be considered while performing an evaluation and forecasting of river drainage impact to the coastal marine environment.

It has been established that under the influence of contaminants carried by rivers inflow to the sea, the capacity of the coastal marine ecosystem is partially capable to compensate its effect on the marine living organisms, but the general trends of the researched processes remain constant. The leveling of the parameters, which describe microalgae growth rate values maxima in sub-acute and chronic experiments, predetermines the anthropogenic effect severity stabilization in the marine coastal ecosystems when polluting agents are introduced into them from outside.

Therefore, based on this work, it is concluded that there is a correlation between contaminated river drain and the characteristics of the coastal marine environment in the watercourses plume zones, as well as the presence of processes occurring in marine biocenoses that partially compensate for the effect of such pollution.

Acknowledgment

The research was supported by the state assignment funding from the Federal Research Centre of the Subtropical Scientific Centre of the Russian Academy of Sciences through project FGRW-2021-0015, 0492-2021-0015.

References

[1] N.K. Gudkova, "Identification of the negative impact factors for aquatic ecosystems in conditions of the resorts extension within Sochi region," Advances in modern natural science, vol. 9, pp. 46 – 51, 2020.

[2] V.V. Gordeev, *Geochemistry of the river - sea system.* Moscow.: Matushkina I.I., 2012, 452 p., ISBN 978-5-94101-261-9.

[3] Y.I. Dzeiris, et. at., "Modern approaches to management of marine recreational territories environment quality (using an example of the Krasnodar area)," *Bulletin of the Altai Academy of Economics and Law*, vol. 11, no 3, pp. 468-476, 2020. DOI: 10.17513/vaael.1450.

[4] R. Altenburger, et. at., "Future water quality monitoring: improving the balance between exposure and toxicity assessments of real-world pollutant mixtures," *Environmental Sciences Europe*, vol. 31, no 1, pp. 2 – 17, 2019. DOI: https://doi.org/10.1186/s12302-019-0193-1.

[5] P. M. Glibert, "Phytoplankton in the aqueous ecological theater: Changing conditions, biodiversity, and evolving ecological concepts," *Journal of Marine Research*, vol. 77, no 2, pp. 83 – 137, 2019. DOI: https://doi.org/10.1357/002224019828474304.

[6] ISO 8692, *"Water quality - Fresh water algal growth inhibition test with unicellular green algae,"*.UK: The British Standards Institution 26 p., February 2012.

[7] P 52.24.690-2006, *"Assessment of toxic pollution of watercourses and reservoirs waters with different salinity and mixing zones of river and sea waters by biotesting methods. Recommendations,"* State Institution "Hydrochemical Institute" of Roshydromet, RF: Rostov-na-Donu, 36p., February 2007.

[8] P. Roslev, et. at., "Relationship between fecal indicators in sediment and recreational waters in a Danish estuary," *Water, Air, and Soil Pollution*, vol. 194, no. 1 – 4, pp. 13 – 21, 2008. DOI: https://doi.org/10.1007/s11270-008-9715-y

[9] Z.Z. Finenko, et. at., *Algae cultivation in laboratory conditions. Microalgae of the Black Sea: problems of biodiversity conservation and biotechnological use*, Sevastopol: Ekosi-Hydrophysics, pp. 186 – 200, 2008.

[10] L.V. Stelmakh, et. at., "Toxicity effect of cooper on two species of marine diatoms microalgae and two species of dinoflagellates," *Ecologica Montenegrina,* vol. 58, pp. 55 – 68, DOI: 10.37828/em.2022.58.6

[11] L. Zakharikhina, E. Vinitskaya E., "Mirroring Land and Sea: The Geochemistry of Coastal Biomarkers," *Ecologica Montenegrina,* vol. 64, pp. 207 – 220, 2023. DOI:10.37828/em.2023.64.6

[12] C. Rishikesh, M.A. Kumar, "Environmental health and risk assessment metrics with special mention to biotransfer, bioaccumulation and biomagnification of environmental pollutants," *Chemosphere,* vol. 302, pp. 134836, 2022. DOI: 10.1016/j.chemosphere.2022.134836.