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Assessment of the Factors and Consequences of the Presence of Genotoxicity of the Coastal Waters of Lake Senezh

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Abstract

The development of modern methods of assessing the factors and effects of pollution of artificial and natural water bodies, changes in their ecological status in recent years is becoming increasingly important due to the growth of environmental threats. The purpose of this study was to assess the presence of genotoxicity of the coastal waters of Lake Senezh due to the gross impact of pollutants (the possible appearance of deletions, or fragmentation of chromosomes). To achieve the objectives of this study, the authors of the article used a model object - salivary glands of the larvae of Chironomus (Chironomus plumosus), which are widely used in laboratory studies and contain giant chromosomes in the nuclei of cells. The focus was on the research of short-term and cost-effective of test system "in vitro", mainly bacterial. The authors of the article conducted a study of the genotoxicity of coastal water from Lake Senezh, and the coastal zone of which revealed the presence of a genotoxicant. According to the results of the study, the authors of the article concluded that, based on the revealed rearrangements of polytene chromosomes, it can be sun up that genotoxicity is present in the coastal part of the lake, which may further increase due to accumulation of pollutants. The necessity of an integrated approach in assessing the existing and potential genetic risk of genotoxicity in artificial and natural water bodies is substantiated. The materials of the article will be useful for governments at the regional and federal levels governing environmental activities in the territories of artificial and natural water bodies. The described methods for assessing the factors and consequences of the presence of genotoxicity of coastal waters can be used when conducting similar studies in other regions.

Keywords: genotoxicity, coastal waters, ecological state, freshwater ecosystems, Lake Senezh

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INTRODUCTION

Recently, the number of research papers on various issues of genotoxicity has increased (Barescut et al. 2005, Durnev and Lapitskaya 2012, Ivanova 2017, Lan et al. 2018, Marchenko et al. 2015, Nazarenko et al. 2016, Rassolov 2015, Simonyan et al. 2016, Viana et al. 2018, Zemskova 2016), as well as the pollution of artificial and natural water bodies (Solovykh and Kolchugina 2018), including effects on freshwater ecosystems (Atkinson et al. 2017, Blabolil et al. 2017, Brett et al. 2017, Castello and Macedo 2016, Castillo et al. 2018, Feld et al. 2016, Jean-Nicolas et al. 2017, Jenny et al. 2015, Mazeika et al. 2016, Nikiforov-Nikishin et al. 2018, Pinto et al. 2018, Schmeller et al. 2018, Schofield et al. 2018, Simakov 2011, Vergolas 2015).

The authors of this article conducted a study of the genotoxicity of the coastal waters of Lake Senezh, which is located in the Moscow region. The lake is an artificial flow pond. This is one of the oldest reservoirs near Moscow. The lake area is 8.51 km^2, and the catchment area is 69.2 km^2.

Despite the fact that Lake Senezh is used only for recreational fishing and recreation in the summer, for

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the period of research (from spring to autumn), allochtonous microflora and enterobacteria are already released in the water area. This indicates the intervention of the anthropogenic factor (pollution by household wastewater at certain points) and the unfavorable state of the reservoir. The process of water self-purification by the fall is not completed, so the numerical values of different bacterial groups are close.

METHODOLOGICAL FRAMEWORK

In order to visualize differential activity under the action of mutagens and antimutagens, when conducting research it is necessary to have cells with chromosomes, where polytenium reaches several thousand n. Such giant cells with polytene chromosomes exist, and they have been studied for a long time by geneticists and oncologists (Harms and Chen 2005, Hirvenoja and Michailova 1998, Layed and Norman 1998, Lehmann 1984, Michailova et al. 1996).

To indicate the environmental consequences of changes in the characteristics of Lake Senezh, samples were constantly collected to determine the qualitative and quantitative composition of the relevant microflora and its functional activity in water and fish. Along with this, the authors of this study evaluated the nature of the self-cleaning ability of the reservoir in order to develop measures to prevent the threat of their local eutrophication and change the epizootic status.

To achieve the objectives of this study, the authors of the article used a model object salivary glands of the larvae of Chironomus (Chironomus plumosus), which are widely used in laboratory studies and contain giant chromosomes in the nuclei of cells (Benner 1988).

Under the influence of pollutants, products of combustion of automobile fuel, rubber particles from tires, the mineral oils which are washed away after the journey violations of differential activity of genes, both on genetic, and at the epigenetic level were established, that is at regulation of a transcription of genetic information. These abnormalities in gene expression can also be tracked by changing the morphology of polytene chromosomes and by the intensity of m-RNA synthesis, especially in the Balbiani rings of the 4th chromosome (Simakov 1998, 2007).

In addition, it is possible to follow the manifestation of large mutations in the chromosomes by the occurrence of heterogeneous sites and the appearance of asynapses. Homologous chironosome chromosomes are conjugated (paired), and when deletions and other mutations occur in one of their conjugates under the influence of mutagenic substances, they lead to the appearance of heterogeneous regions and the formation of fork-like chromosomes. It can be expected that in water samples from uncontaminated areas of the lake, the number of asynapses in polytene chromosomes will be less common, and the morphology of chromosomes will be similar to that found in control individuals.

Thus, it is possible to answer the questions whether the differential activity remains at the gene and epigenetic level after the ingress of pollutants from the road into the water (Reutova et al. 2016).

The revealed features of the effect of polluted water on chromosomes of chironomid larvae provide an answer to the question whether genetic reprogramming always occurs in cells that mutagens and carcinogens can cause (Stupak and Stupak 2012). In addition, it is also possible to make a comparative assessment of the effects of water from polluted and clean, more separated from the shore areas of the lake, and to determine the degree of pollution of coastal areas near the road by genotoxicants.

RESULTS AND DISCUSSIONS

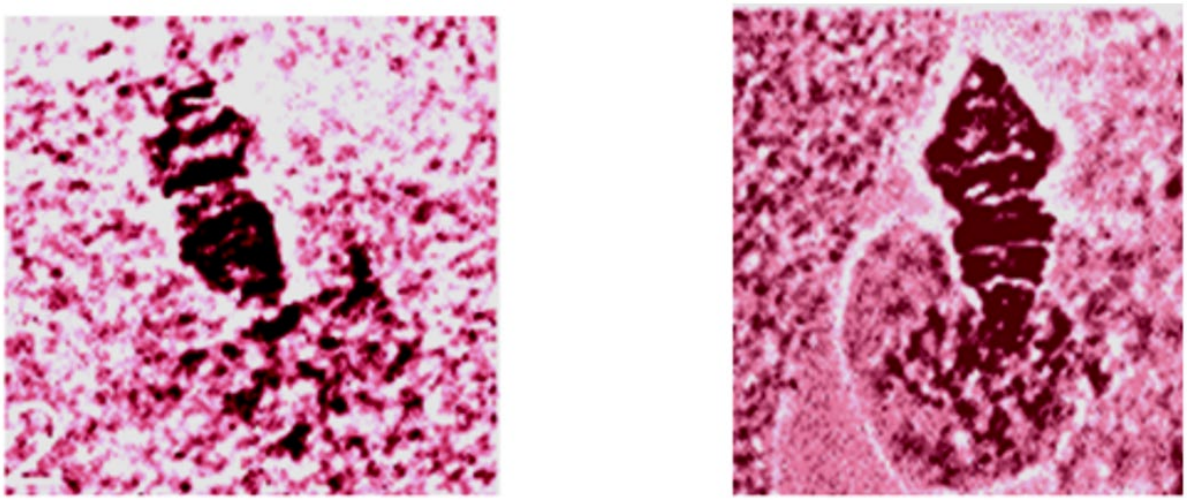
Assessment of the Genotoxicity of the Coastal Waters of Lake Senezh, its Main Causes and Consequences

As a result of the study in the coastal zone, the presence of a genotoxicant was identified. Under the influence of a genotoxicant in polluted coastal water in the third chromosome of chironomid larvae asynapses arise, and the chromosome takes on a Y-shaped appearance. The fourth doubled chromosome under the influence of pollutants completely diverges into two homologous chromosomes. There are no similar aberrations in the karyotype of the salivary glands of chironomid larvae contained in the water from the central zone of the lake.

The data obtained in the course of the study allow making an unequivocal conclusion that the greatest pollution of the lake is observed in the areas along which the road passes (Timonovskoye Highway). This is one of the main sources of pollution of the lake, along with the influence of urban runoff, which is more difficult to eliminate than the road (**Fig. 1**).

One of the peculiarities of water pollution of the lake by motor transport is that the main pollutants are related to oil products and their combustion products, among which a significant place is occupied by 3,4-petrolpyrene, which is washed off by drains directly to

Fig. 1. Toxicants of petrochemical nature on the coast of Lake Senezh



A B

Fig. 2. The state of the fourth chromosome of chironomid larvae in assessing water pollution in the lake
A - The zone of chromosomes of the "lamp brush" type (at the bottom of the fourth chromosome is the Balbiani K1 ring) with the content of larvae in the water taken in the lake near the highway, m-RNA synthesis is inhibited;
B - Balbiani Ring with pronounced m-RNA synthesis and clearly defined border (at the bottom of the chromosome), on the fourth chromosome of the chironomid larvae contained in the water from the central part of the lake.

Senezh in those areas where the road goes directly along the lake shore.

Considering these circumstances, a study was conducted mainly of water from the polluted areas of the lake adjacent to the road, and water samples taken from the central zone of the lake.

First of all, cyclical hydrocarbons produced by the combustion of automotive fuel, which can lead to the formation of malignant tumors in fish and humans, were detected in this water.

One of the distinguishing features of malignant tumors is the violation of the differential activity of genes and the appearance of polytene chromosomes in the nuclei of cells (Degtyareva et al. 2000). The appearance of polytene chromosomes in tumor cells can be considered as one of the signs of malignant cell degeneration.

The authors of the article succeeded in revealing that the impact of water from the coastal areas of the lake adjacent to the highway to the polytene chironosome chromosomes was primarily reflected in the regulation of gene activity at the level of transcription of genetic information.

In addition, control samples show how m-RNA synthesis proceeds (most likely it is a nucleoprotein). When exposed to a genotoxicant, chromosomes appear like "lamp brushes", the puffing process is not disturbed, however, m-RNA synthesis is inhibited, since there are few synthesized granules on the opened DNA chains, and the nucleoprotein does not form a total mass with a clearly visible border (**Fig. 2, a**).

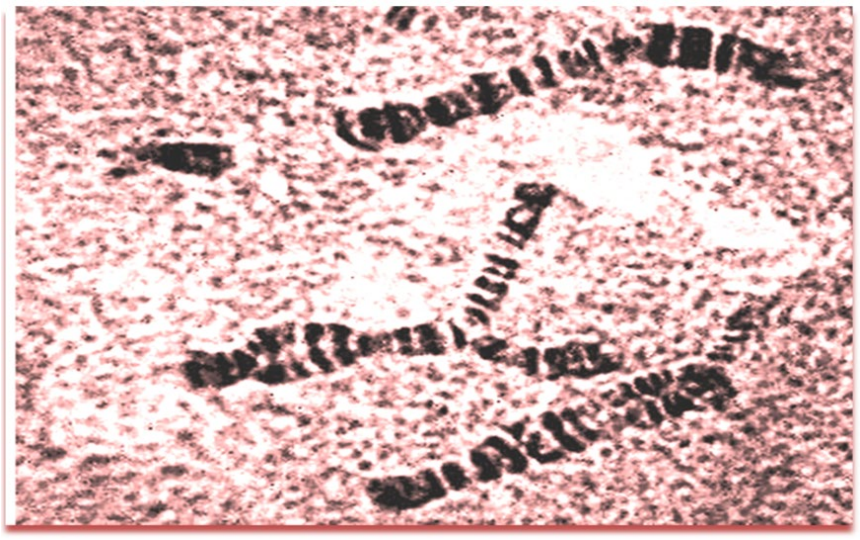


Fig. 3. Asynapses in the second and third chromosomes in the karyotype of the larvae of Chironomus plumosus after a 7-day stay in the polluted coastal water of the lake. (20 x 15). One can see Y-shaped chromosomes with dispersed homologs

The chromosome structure of the chironomid larvae contained in the water from the central part of the reservoir was similar to the picture noted in the control. The presence of m-RNA granules is clearly marked in the loose threads of the chromosomes of the Balbiani ring, and the mass border of the synthesized nucleoprotein is visible. Thus, it was concluded that the water from the coastal zone of the lake contains toxicants that do not affect the formation of the Balbiani rings, but disrupt the expression of genes at the transcription level.

Self-purification of water in the reservoir, and, possibly, the belt of higher coastal aquatic vegetation, contributes to the decomposition of genotoxicants entering the lake from the highway, and protects the middle of the reservoir from the ingress of genotoxicants. This is revealed by the absence of disturbances in the transcription of genetic information, which could lead to the cell degeneration during chironomid metamorphosis.

The karyotypes of chironomid larvae with partial asynaps on the third chromosome are presented in **Fig. 3**.

Studies show that, due to the formation of heterogeneous regions, the second chromosome partially dispersed and became Y-shaped; the fourth chromosome, in which the homologs were bound only in the area of the Balbiani ring, completely dispersed into two conjugates (only one of them is clearly visible on the preparation).

Thus, due to the formation of asynapses in the polytene chromosomes of chironomid larvae, it is possible to judge the presence of genotoxicants in water, in which large quantities of products of combustion of automobile fuel and pollutants washed away from the road fall.

To assess the effect of pollutants on the gene apparatus of water chironomid larvae from the central areas of Lake Senezh, the number of asynapses in the second chromosome was calculated. As a result of this test, it was revealed that the number of chromosomes with asynaps decreased sharply and was only 6% compared with 43% in coastal water. The karyotype of the cells of the salivary glands of the larvae of Chironomus plumosus at the fourth stage of development, located in the cleaner water from the central areas of the lake, is presented in **Fig. 4**.

Thus, as a result of water testing from the coastal part of the lake where the road passes, and water samples from the central part of the lake, it was found that polluted coastal water affects the formation of puffs and increases the formation of asynapses, which indicates the presence of genotoxicants in the water into the lakes with automobile gases.

These violations of polytene chromosomes of chironomid larvae under the influence of pollution, allow to judge the level of impact on the genetic apparatus of aquatic organisms of the studied reservoir. Analysis of cytogenetic preparations of polytene chromosomes shows that the combustion products of automotive fuel entering the lake disrupt the process of

Fig. 4. The karyotype of the cells of the salivary glands of the larvae of Chironomus plumosus in the fourth stage of development after a 7-day exposure to pure water from the central region

transcription of genetic information during protein synthesis.

Impact Assessment of Environmental Pollutants Coming from the Outside on the Aquatic Microbiocenosis of Lake Senezh

According to the results of another research direction of the authors of the article - the ichthyopathological study of fish in the winter period, it was concluded that the identified deviations in all fish in that period were insignificant, therefore they mostly did not affect the structure of the organs.

In the summer period, the picture changes, clinical and morphophysiological studies have revealed pathological changes in the external and internal organs of fish, as indicated by the decrease in indices of individual organs - the liver and intestines (previously presented data).

Identified pathological changes in the internal organs of predatory fish are more associated with parasitic invasions. A large number of parasitic forms in these fish confirms the accumulation of invasive gastropods in the pond - the first intermediate trematodes and the lowest invasive planktonic crustaceans, as well as benthic organisms - tubules and scuds (in cestodes).

In the case of peaceful fish, in the summer period, the appearance of conditionally pathogenic bacteria was noted in the internal organs, which significantly reduced the overall resistance of their body, which also increases the likelihood of their invasion by various parasitic organisms.

Analysis of the material studied showed that at present, external and internal changes in the studied fish occur under the influence of parasitic and bacterial organisms that form and actively circulate through the food chains of the biocenosis in Lake Senezh in the summer.

The results of studying the parameters of heterotrophic self-purification made it possible to establish a decrease in its intensity by the end of the annual observation cycle — the ratio of the number of heterotrophs at different incubation temperatures either flattens or representatives of allochthonous microflora giving rise at 37 ° C begin to dominate over representatives of autochthonous microflora growing at a temperature of 21^0 C.

Perhaps this is due to low temperatures inside the reservoir and an excess of organic matter in the water coming from various sources. Throughout the study, the dominance of uncontrolled forms of bacteria in water samples and rod-shaped forms of bacteria in the microbiocenosis of fish is characteristic, which can serve as an indicator of the intensification of the processes of eutrophication of the reservoir.

The high proportion of bacterial strains with hemolytic activity and mobility determines their pathogenicity and the probability of initiation of an infectious process in fish. Bacterial strains isolated at the end of the summer period, in September and October, fish exhibit high hemolytic activity and mobility.

The upward change in the amount of organic matter in water leads to the growth of bacteria, changes in their qualitative and quantitative characteristics, and the formation of certain groups of bacteria. Microbial communities are sensitive indicators of the state of ecosystems.

The appearance and correlation of indicator bacteria in the microbiocenosis of water and fish reveals the level of bacterial contamination of water bodies, determines the etiologically significant bacteria capable of infecting fish. Increased organic pollution affects the aquatic ecosystem. Therefore, it is relevant and necessary to

conduct complex ecological and microbiological studies of fish and water.

Given the climatic features of the Moscow region, it should be remembered that freshwater ecosystems are slowly recovering and are vulnerable. When the ecological balance is disturbed, irreversible processes of degradation develop.

In the places of sampling with high quantitative indicators, the growth of enterobacteria was observed - bacteria of the group of Escherichia coli, Proteus and representatives of non-fermentative bacteria.

The presence of enterobacteria (intestinal) indicates an increase in the process of local eutrophication and anthropogenic water pollution. Identification of the proteus at specific points indicates the presence of a slightly rotting organic matter in the pond.

The appearance of bacteria with hemolytic activity (one of the factors of pathogenicity) determines the likelihood of their introduction into the body of fish and the initiation of an infectious process in them, which is noted more clearly in peaceful fish. There was no growth of bacterial flora from pike and perch.

A moderate growth of the bacterial flora during research was noted in the internal organs of roach, crucian carp, and tench. Mostly mobile aeromonads and enterobacteria were identified from these fish. Aeromonas lead to the development of rubella-like disease (aeromonosis) in carp fish, and in conjunction with enterobacteria, they often contribute to the development of the disease of bacterial hemorrhagic septicemia in different fish species.

Outbreaks of disease and death among the studied fish, when conducting this measurement, was not observed. However, clinical and pathological changes in peaceful fish already indicate the development of pathological processes in them.

The analysis data show that bacterial contamination of the internal organs is associated with the temperature factor of the environment and with the physiological state of the fish. A high level of contamination occurs during periods of maximum bacterial content, in particular, aeromonads and enterobacteria (citrobacter) in the aquatic environment.

As a result of the test, it was noted that in larvae that are in coastal water in the fourth chromosome in a significant part of individuals in the Balbiani rings, m-RNA synthesis is suppressed, the number of ribonucleoprotein granules (RNP) is reduced and no clear boundary of the substance synthesized is detected. At the same time, the chironomid larvae contained in the water from the central zone of the lake in the fourth chromosome had little difference from the control measurement.

In chironomids, as one of the most important food objects of fish, chromosomal rearrangements occur (m-RNA synthesis is suppressed and asynapses appear in homologous polytene chromosomes), which leads to a sharp decrease in the food supply of the reservoir under study.

At the time of the study, no more severe effects of polluters were observed (the appearance of deletions or fragmentation of chromosomes), but already from the revealed rearrangements of polytene chromosomes, it is possible to judge about the presence of genotoxicity in the coastal part of the lake, which may further increase due to the accumulation of pollutants and increased movement on Timonovsky highway.

CONCLUSION

In general, it can be concluded that as a result of the tests, it was found that under the influence of the decay products of burning automotive fuel that enters the coastal waters of the lake from the highway adjacent to it, irreparable environmental damage is caused to the lake.

The study of water from the central region of the lake shows that no effect of genotoxicants is observed at all genetic levels. One of the most probable reasons for this is the self-purification of water in areas of the lake remote from the shore. There is every reason to believe that coastal aquatic vegetation plays an important role in this process.

For prompt response to the presence of the genotoxicity of the coastal waters of Lake Senezh identified as a result of this study, according to the authors of the article, it is necessary to carry out a number of reclamation measures aimed at restoring the ecological and epizootic state of Lake Senezh:

– cleaning of the bottom and removal of sludge deposits in places of accumulation of organic substances;

– catching sick fish (with surface swimming) - bream, roach, perch, not less than twice a year (spring, autumn);

– conducting in the places where mollusks accumulate their destruction (withdrawal) by biological or other methods;

– the introduction of black carp, white carp - for the consumption of phytoplankton and loach, consuming benthic organisms and filamentous algae, as ways to combat mollusks;

– carrying out liming in organically polluted areas of the lake during a period of rising temperatures and lowering pH;

– creation of a system of permanent monitoring observations of water bodies and water of Lake Senezh, which should include an assessment of the following parameters:

– hydrochemical, hydrological characteristics;

– modes of surface runoff and possible sources of pollution;

– physiological and morphometric data of fish;

– morphometry of the main aquatic biocenosis objects - representatives of microbiocenotic and parasitic communities, phyto, zooplankton and benthic organisms, representatives of ichthyofauna.

Among the general measures aimed at reducing the genotoxicity of the coastal waters of Lake Senezh, the authors of the article developed a number of recommendations:

1. It is recommended to withstand the first batch after a long absence of rain in the pools for cleaning storm drains with the additional introduction of microorganisms decomposing oil products or the Devoroil preparation for cleaning the urban storm drains.

2. It is recommended to preserve the belt of higher aquatic vegetation in the coastal part of the lake, as a natural biofilter that helps purify wastewater entering the lake from roads and from industrial enterprises.

3. The heavy traffic road passing by the pond - Timonovskoye Highway, designed and built in violation of SNIP, is an extremely dangerous polluter of Lake Senezh. Mutagenic and carcinogenic substances enter the lake from the road with stormwater and through the atmosphere, which makes it dangerous to eat fish and even swim in the lake.

Implementation of the proposed measures will allow reducing, and creating an effective control system, and completely eliminating the genotoxicity of the coastal waters of Lake Senezh.

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