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Investigation of the Presence of Mutagens in the Coastal Part of Lake Senezh near the Highway

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Abstract

The relevance of the study is determined by the need to improve the methods used to assess the presence of mutagens in the coastal parts of natural and artificial reservoirs located in the immediate vicinity of the highway. The purpose of this study was to assess the presence of mutagens in the coastal part of Lake Senezh near the highway. The work carried out relates to the field of genetic ecology of water bodies, therefore, to study the toxicity of water, models that covered two levels of the effects of genotoxicants on hydrobionts were used: genomic and gene. Gene level of exposure was determined by Ames test. According to the results of the study, it is shown that both at the chromosomal and genetic level, after the exposure of test objects in the samples of water from the city Bay, mutations are revealed that genotoxicants enter the water from the highway running along the very shore of the lake and having no stormwater runoff collections. And studies to increase the size of somatid-like particles in the blood of fish also indicate that among these toxicants in the coastal areas of the lake there are carcinogens. The study of water from the central region of the lake showed that no effect of genotoxicants was observed at all genetic levels. The materials of the article will be useful for regional and federal authorities dealing with the presence of mutagens in artificial and natural reservoirs. The described assessment techniques in the coastal part of the analyzed lake can be used when conducting similar studies in other regions.

Keywords: mutagens, genetic ecology of water bodies, carcinogenic substances, Lake Senezh

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INTRODUCTION

In recent decades, a problem related to environmental pollution by mutagens that are common as a result of human activities has arisen. Research in this area is dictated by the concern for the health of present and future generations, for the fate of existing gene pools.

Man as a living organism is in constant interaction with the environment. Intensive pollution of water bodies with harmful substances directly and indirectly affects the entire human body, causing a complex of environmentally caused diseases. Among the possible consequences of human exposure to environmental pollution, malignant neoplasms occupy a special place.

As noted in the paper (Vlastos et al. 2017) «the evaluation of the potential genotoxic effects and the analysis of the physicochemical parameters of lakes' surface water samples is a first step in our effort to evaluate the water quality, in terms of the presence and environmental/human risk of genotoxicants in the studied lake ecosystems».

Water in nature is a complex with dissolved, suspended and colloidal substances, the composition of which varies in place and time, depending on the characteristics of admission, transfer and chemical and biological transformation processes. Organisms, including humans, that consume water with mutagens, will not be exposed to individual substances, but to the whole complex, and the mutagenic effect can therefore be either enhanced due to synergistic action, or

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weakened due to neutralizing interaction. Thus, the traditional approach, when contaminants are evaluated separately for mutagenicity, does not provide a true picture of mutagenicity (Drozdova et al. 2017, Girina et al. 2018). In addition, a complete qualitative and quantitative analysis of the composition of natural or drinking water is very laborious, requires sophisticated equipment and a qualified specialist - genetics to master the techniques. Therefore, the most promising method seems to us is to determine the total mutagenicity of drinking water.

A study was carried out to identify the presence of carcinogenic substances in the coastal areas of the lake, performed using such an indicator as the enlargement of somatidopodobnyh particles in the blood plasma of fish after two weeks of aging in samples of water from lake Senezh, located in the Moscow region.

METHODOLOGICAL FRAMEWORK

To achieve the goal of the study, the Ames test was applied (Ames 1971, Ames et al. 1973, Bernstein et al. 1982). For the Ames test, we acquired two strains of Salmonellatyphimurium TA-98 and TA-100 at the Institute of Genetics and Breeding of Industrial Microorganisms of the State Research Center of the Russian Federation, which then we cultivated in our microbiological laboratory. The Ames test was carried out strictly according to the methodological guidelines for recording gene mutations (Simakov 1998). As a mutagenic substance for positive control, we used the standard mutagen used in a similar kind of research, cyclophosphamide at a concentration of 0.5 mg per dish (Colvin 1999).

The degree of mutagenicity of water from the contaminated area of the lake was judged by the multiplicity of the decrease in the number of revertant colonies in the experiment (grown on minimal medium with the addition of water from the lake) compared to the number of colonies in Petri dishes grown on full-fledged IPA.

The study of the presence of carcinogens in water samples from the contaminated areas of Lake Senezh was carried out using Brachydaniorerio, in which fluorescent microscopy of living blood revealed the presence of large somatids. Brachydaniorerio is an indispensable universal object for the study of carcinogenic and anticarcinogenic compounds (Degtyareva et al. 2000, Dyachenko 2003, Ilinsky et al. 1988, Solovykh et al. 2017, Sudarikov et al. 2017, Yashnov 1969).

Their use is advantageous in experiments, when making experiments, because of cost savings, since the cost of one fish is many times cheaper than a rat or mouse, which were used previously to conduct research in the field of cytogenetics and oncology. At the same time, zebrafish have many genes that are similar to the genes of humans and other mammals, including the p53 suppressor gene, which is responsible for eliminating reborn cancer cells from the body (Oren 2003).

RESULTS AND DISCUSSIONS

Assessment of the Presence of Mutagenicity of Water Samples from the Coastal Part of Lake Senezh

Determination of the mutagenic activity of water samples taken from the contaminated part of Lake Senezh, where a motorway runs along the shore, showed the following: the greatest difference in the growth of salmonella colonies was found in experiments with a minimal environment that contained water taken from the polluted coastal points indicated in the study as points 1 and 2. This phenomenon was detected both on the TA-98 strain, and on the TA-100 Salmonella strain.

The study of water samples taken in the city beach area showed that the ratio of the number of salmonella colonies growing on the minimum environment to the control does not exceed 2.5 times, and, consequently, the mutagenicity of water that is observed in the lake's sections of the nearby highway detected.

The results of the studies to determine the possible mutagenic activity of water samples near the road and in the central region of the lake by the Ames test are presented in **Table 1**.

Thus, only when using the T-98 strain in samples taken from the coastal zone, where the highway passes along the coast, we obtained results indicating the presence of weak mutagenicity in water.

In samples of coastal water taken in December, in the area of the city beach, the Ames test did not reveal mutagenicity, which is apparently due to a decrease in the flow of combustion products of motor fuel into the water due to the remoteness of the road. In the central part of the lake, no detection of genotoxicants leading to gene mutations is noted.

Thus, as a result of the work to identify the presence of genotoxic substances in the coastal water of Lake Senezh, where the Timonovskoye Highway passes near (points 1,2), it was found that by mid-autumn high

Table 1. A study on the presence of mutagenicity of water samples from the coastal part of Lake Senezh, where the highway runs along the shore, near the city beach and the central part of the lake (the average number of colonies per Petri dish is given)

Strain Salmonella typhimurium	Experiment	Control	0/K	Control with mutagen	Control on a full-fledged IPA
Coastal water near the highway on October 6 (3 rep.)					
TA-98	15	**5**	3	363	345
TA-100	12	**7**	1,7	486	320
Water from the area of the city beach on December 10 (3 repetition.)					
TA-98	6	**3**	2	363	345
TA-100	6	**4**	1,5	486	320
Water from the central part of the lake on October 6 (3 repetition.)					
TA-98	8	**6**	1,3	363	345
TA-100	5	**7**	0,7	486	320

levels of pollutants accumulate in the water, which can show weak mutagenic effects and cause even gene mutations (Stribling and Davie 2005).

In water samples taken in the city beach area in the first decade of December, there is a lack of genotoxicity, which is apparently due to the removal of the highway from this part of the lake, although pollution by other toxicants in this part of the lake is distinct.

In addition, the concentration of genotoxicants in the water, apparently, decreases due to the partial self-purification of the reservoir. Therefore, in water samples taken in the coastal area near the city beach in mid-December, the mutagenic properties of the water are not detected by the Ames test.

In water samples taken in the central zone of the lake, in the middle of autumn, the presence of substances that could cause gene mutations is not observed.

A comparative analysis of the revealed genotoxic properties of water from the lake by the Ames test with the earlier work on the detection of chromosomal aberrations on polytene chromosomes of chironomids suggests that the pollution of the lake areas where the highway passes along the shore is more pronounced at the chromosomal level.

Most likely, vehicle exhaust gases from the nearby highway bring 3,4-benzpyrene to the lake (Volk et al. 2003). This is characterized by a kind of genetic effect of this genotoxicant, disrupting the synthesis of M-RNA and gene expression in aquatic organisms.

Assessment of the Presence of Carcinogens in Water Samples from Contaminated Areas of Lake Senezh

We used potassium dichromate (a standard toxicant used in aquatic toxicology), which has both mutagenic and carcinogenic properties (Napalkov 1989, Simakov 2011) as a genotoxic substance for comparative analysis of the effect of polluted water from a lake on somatoid-like particles in the blood of fish

The blood cells and luminous somatoid-like particles in the blood plasma were studied. Somatids glow green, which indicates the content of DNA in them.

On the preparation, blood plasma with microparticles containing DNA was studied simultaneously with the morphological features of the nuclei of erythrocytes. When using acridine orange DNA fluoresce green and RNA red. In our case, the red glow of somatoid-like particles is not observed.

Fluorescent analysis (Gauglitz and Vo-Dinh 2003, Joseph 2006, Lakowicz 1999, Rendell 1987, Sharma and Schulman 1999, Zwinkels and Gauthier 1999) of fish blood using acridine orange (Kasten 1967), allows detecting DNA containing particles and exclude artifacts, other microparticles in the blood that do not contain nucleic acids.

A comparative morphological analysis with somatids in human blood (blood pictures obtained on a somatoscope by Canadian researchers) shows that the morphology of the particles we observe in the blood of fish is similar to the morphology of somatids in human blood (**Fig. 1**).

The action of a genotoxic substance is directly related to the amount of somatid in the blood plasma. However, there is a difference. Somatoids in the blood plasma of fish in large quantities appear only under the action of a carcinogenic substance, while in humans they may be present in large quantities even in a normal state.

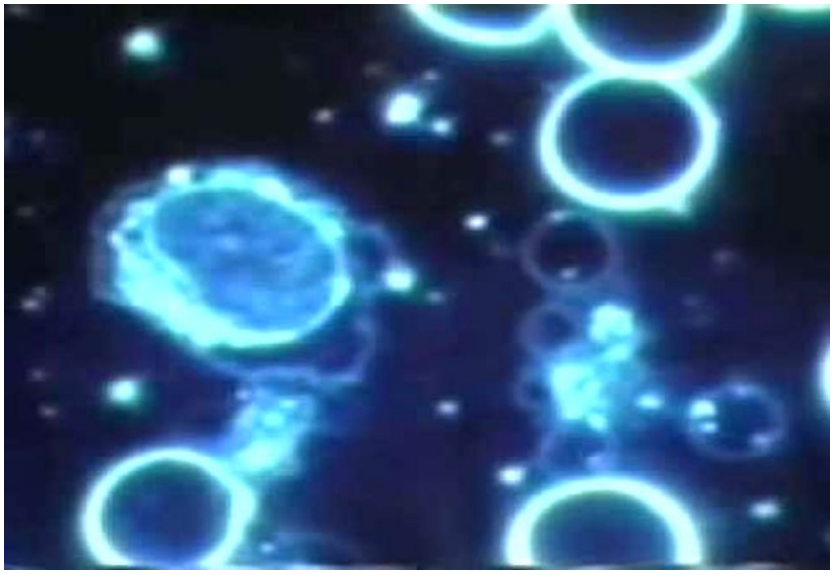

Fig. 1. Somatids in human blood (the type of live blood in a somatoscope) (by morphology and size, they are identical to somatoid-like particles in Brachydaniorerio blood)

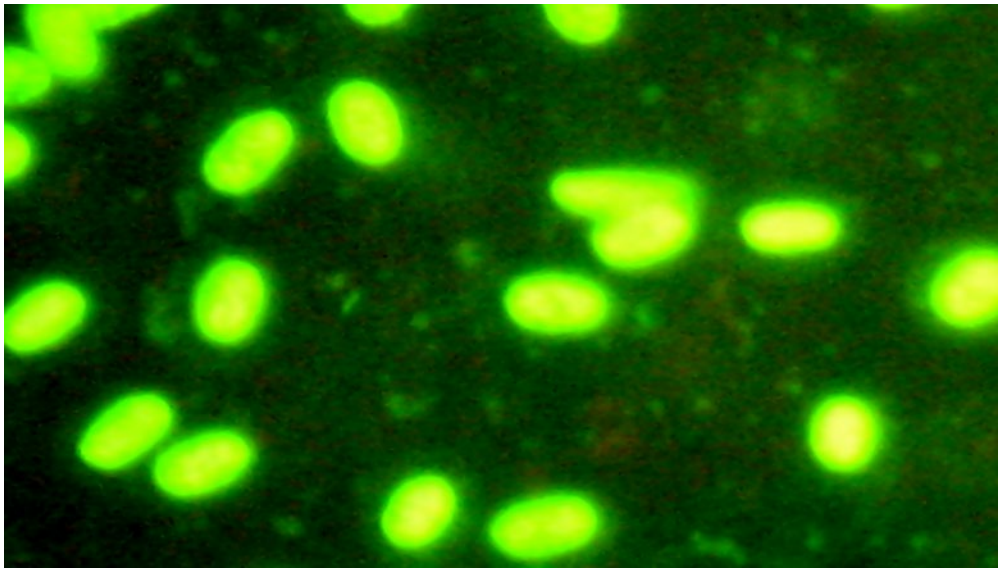

Fig. 2. Somatoid-like particles in danio blood plasma after a 15-day stay in the coastal water of the lake, from the zone where the highway goes along the coast

Analysis of the fish contained in the water from the City Bay shows that they also have somatoid-like particles in the blood plasma. Most likely, this is due to the weak carcinogenic effects of water from that part of the lake, where the road is close.

Somatid-like particles in the blood plasma in this case are found in smaller quantities than under the action of the standard carcinogen, and they glow less intensely. A picture of the blood of fish after 15 days in water is presented in **Fig. 2**.

The presence of DNA in somatoid-like particles, which we detected in the blood of fish kept in polluted water near the road with acridine orange, indicates that the assumption that the somatoid is derived from mitochondria most likely corresponds to reality. Essentially, they induce cancer and its metastases.

In this case, with an increase in the size of about two times, they may indicate the presence in the water of carcinogenic substances entering the water from the exhaust gases of motor vehicles and hydrocarbons, washed away from the roadway near the passing highway.

Thus, as a result of tests to detect somatid-like particles carrying DNA in the blood plasma of laboratory fish, it was possible to develop a new environmental test for the presence of carcinogenic

substances in the burned fuel of cars, as well as oil products and rubber residues washed away in the roadway to the lake.

This study shows how dangerous the road with heavy traffic is for the reservoir, especially if it does not have protected areas and exclusion zones. During the design and construction of the Timonovskoye Highway, all SNIPs related to environmental supervision were violated and most regulatory documents were not taken into account.

Examination of the blood of fish in water samples taken from the coastal zone of the lake, near the road passing along the shore, shows that somatid-like particles can be found in the plasma, which are formed by the action of carcinogenic pollutants.

In fish that were in the water from the central zone of the lake, no large somatoid like particles were found in the blood plasma. The picture of their blood was close to the control, and corresponded to the blood of fish kept in aquariums with tap water.

Thus, we can conclude that the pollutants entering from the highway to the coastal areas of the lake contain carcinogenic substances. Samples taken from the central zone of the lake do not cause the formation of large somatoid-like particles in the blood plasma of fish, which indicates self-purification of water from mutagenic and carcinogenic compounds, most likely due to higher aquatic vegetation developed in the coastal zones of the lake.

CONCLUSION

According to the results of all conducted researches it is proved that both at the chromosomal and genetic level, after the exposure of test objects in the samples of water from the city Bay, mutations are detected, indicating that genotoxicants enter the water from the highway running along the very shore of the lake and having no stormwater collections. Studies to increase the size of somatoid-like particles in the blood of fish also indicate that among these toxicants there are carcinogenic substances in the coastal areas of 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. Most likely, water self-purification occurs in areas of the lake, which are remoted from the shore, since coastal aquatic vegetation plays an important role in this process.

According to the results of the study, the authors of the article justified certain conclusions, as well as the need to implement the following recommendations.

1. It is recommended to preserve the belt of higher aquatic vegetation in the coastal part of the lake, as a natural biofilter (Chaudhary et al. 2003), which promotes the purification of wastewater entering the lake from highways and industrial plants.

2. A 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.

3. The best way out of this situation would be to block the road for urban and private vehicles and to build a small section of the road parallel to the highway that goes directly to the dam to get to Timonovo village.

4. It is necessary to install trays for collecting storm water from the road and diverting them to the bioplato at the dam of this lake, which should be built during the specified environmental protection measures.

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