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Role of microorganisms of the aquatic environment in the formation of the ecological and sanitary state of water bodies

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Due recent years, humanity has faced the problem of lack of clean water for its needs, so the main goals of science are to increase the efficiency of natural resources that would not impair the quality of water. Chemical and biological methods have traditionally been used to assess water quality, but biological methods are more efficient because they are less costly and more informative. Biological control of water quality has a number of advantages over chemical and physical methods, since the grouping of living organisms mirrors all changes in the ecological state of the aquatic environment, while responding to a complex of various factors and pollutants. The method makes it possible to assess the consequences of both permanent and volley contamination. One of the most important biological methods for assessing the ecological state of reservoirs is the assessment of water quality by microbiological indicators, which is the first and most informative component of biota that responds to the allochthonous introduction of organic substances. Microorganisms are involved in optimizing the conditions of the aquatic environment, namely in the building of hydrological and gas regimes and in the self-cleaning of reservoirs, which ultimately determines the quality of water. Therefore, the assessment of the mechanisms of microbiocenosis formation makes it possible to determine the sanitary state of reservoirs by ensuring the functioning of the ecosystem with a bacterial link. The introduction of new technologies should not bring or form any threats to the environment, health and safety of human life, which is why an important task today is to study the impact of microorganisms on the formation of the ecological and sanitary state of aquatic ecosystems.

Key words: microorganisms, aquatic environment, water classes, bioindication, sapidity zones, heterotrophic bacteria.

Problem statement and analysis of recent research. It is known that the disposal and transformation of organic matter formed in the process of photosynthesis are carried out by microorganisms. They adapt quickly to the environment, and their short life cycle allows to trace the change in the structure of the population and its dynamics and the deviation of some metabolic processes under the influence of organic matter. One of the most important biological methods for assessing the ecological state of reservoirs [24, 26–30] is the assessment of water quality by microbiological indicators, since they are the first to respond to the alochtone of the introduction of organic substances [1].

As we know, water pollution is the cause of various diseases, so today the main goal is to determine the sanitary and epidemiological threats and the risks associated with them. The main source of pathogenic microorganisms of the aquatic environment is the inflow of wastewater [25]. The factors of changes in the aquatic environment can be anthropogenic or natural. Today, it is difficult to find a reservoir that does not suffer pollution as a result of human activity. The deterioration of the water quality of natural water bodies is an extremely serious problem for Ukraine. In the most of rivers and lakes receive insufficiently treated effluents from industrial enterprises, domestic effluents from cities and villages, effluents from livestock farms, etc And here's the result: not only can we not drink water from most of our reservoirs without prior multi-stage water preparation, but swimming in them is sometimes dangerous to health. This is necessary not only to state the fact that water is clean or polluted, but also to develop a set of measures by local authorities and communities to improve the environmental situation on water bodies [2, 23].

For today, it is extremely important to increase the efficiency of natural resources without deteriorating the quality of the aquatic environment as a complex of abiotic and biotic factors necessary to obtain a high-quality food resource, namely, the microbial population is involved in optimizing the conditions of the aquatic environment, namely in the formation of hydrological, gas regimes and in the self-cleaning of reservoirs, which ultimately determines the quality of water [3, 21, 25].

The aim of the study is to analyze the role of microorganisms of the aquatic environment in the formation of the ecological and sanitary state of reservoirs.

Material and methods of research. Chemical and biological methods are used to assess water quality, the latter being more widely used because they are less costly and more informative.

Sanitary and microbiological assessment of water is carried out according to the following indicators: oxidability – the amount of oxygen dissolved in water; the total number of microorganisms is the number of colonies that grew by MPA with 1 ml of water at a temperature of 37 °C for 24 hours; coli-titer is the smallest volume of water (in milliliters), in which one E. coli is found; coli-index is the number of E. coli per 1 liter of water. Normal values for drinking water:Coli index ≤3 (in 1 liter); Coli titer ≥300; Microbial number ≤100 [4].

When determining water quality by microbiological indicators, the coli-titre and coli-index are calculated. The coli-titer of water is measured by the minimum amount of water (ml), in which Escherichia coli bacteria are found, and the coli-index by the number of these bacteria contained in 1 liter of the test water. These indicators are determined by the titration (fermentation) method or by the method of membrane filters. With the titration method, the isolation and identification of bacteria on nutrient media is carried out. When using the membrane filter method, water is first filtered using a Bunsen flask with a Seitz funnel with a membrane filter mounted in it and water is run through filters using a vacuum pump, which are then placed on the surface of the nutrient medium in Petri dishes and, after incubation in a thermostat, the number of colonies that have grown, typical of E. coli bacteria, is counted, and then identified using biochemical tests. Sanitary and microbiological control of drinking water quality. Sanitary and microbiological study of drinking water consists of determination of total microbial count (TMC), the number of Enterobacteria, spores of sulfite-reducing Clostridia and Coliphages [5, 6].

The presence of Coliphages (bacteriophages that parasite on *E.coli*) is determined in the water of surface sources and drinking water prepared from it, as well as in wastewater. They are indicators of the effectiveness of groundwater protection and drinking water treatment. The study is carried out using the Grazia agar layers method. To titrate the Coliphages, the test water is mixed with molten and cooled feed agar, where the E.coli indicator strain is also introduced. The resulting mixture is poured with a second layer onto a nutrient agar in a Petri dish and after solidification of the medium, the dish is incubated at 37 °C for 24 h. As a result, the indicator culture forms a uniform solid growth, and in the presence of Coliphages, transparent colonies are formed. The result of the study is expressed in colony-forming units (CFU/ml). Standards for drinking water: the number of microorganisms in 1 ml of water – no more than 50, coli-index – no more than 3 bacteria in 1 liter of water. Enterobacteriaceae and thermophilic bacteria should be absent in 300 ml of drinking water, spores of sulfite-reducing clostridia should be absent in 20 ml of water, and coliphages – in 100 ml of water.

Total microbial count (TMC) is the number of mesophilic aerobic and facultative anaerobic bacteria in 1 cc (1 ml) of water, which is determined in all types of water.

1 ml of the test water is introduced into two sterile Petri dishes and poured with molten and cooled feed agar (depth inoculation method). Incubate at 37 °C for 24 h, then at room temperature for another 24 h. Calculate the number of colonies on 2 plates (on the surface and in the depth of nutrient agar) and calculate the arithmetic mean value [8].

To detect mold and yeast fungi, the test water is seeded with 0.5 ml per Sabouraud medium and incubated at room temperature for 3–4 days. The number of colonies is calculated and the arithmetic mean is also calculated. The result of the total microbial number is calculated by summing the average arithmetic number of bacteria, yeasts and molds and expressed in colony-forming units (CFU/ml).

The total microbial count allows to estimate the level of microbial contamination of water, complementing the indicator of faecal contamination, and at the same time to detect pollution from other sources, for example, industrial emissions. A sharp increase in TMC, even within the norm, detected repeatedly, is a signal for finding the cause of pollution. Also, this indicator is indispensable for the urgent detection of massive microbial contamination of unknown origin in drinking water.

When assessing the quality of water, the number of Enterobacteriais determined. The presence of *Enterobacteria* (coliform bacteria) is also determined in all types of water. The term "coliform bacteria" includes bacteria of the *Enterobacteriaceae* family and thermotolerant coliform bacteria.

Enterobacteriaceae bacteria include gram-negative asporogenic sticks that do not have oxidase activity and ferment lactose to form acid and gas at 37 °C for 24–48 hours (or ferment glucose to form acid and gas at 37 °C for 24 hours). Detection of *Enterobacteriaceae* in drinking water indicates a potential epidemic hazard of water use. The presence of bacteria of the genus *Escherichia* in food, water, soil, on the equipment indicates fresh faecal contamination of great sanitary and epidemiological importance. *Citrobacter and Enterobacter* are believed to be indicators of older (several weeks) faecal contamination and therefore have a lower sanitary and indicative value compared to *Escherichia*.

Thermotolerant coliform bacteria have the same characteristics, but additionally ferment the lactose to form acid and gas at 44.5 °C after 24 h. Thermotolerant coliform bacteria quickly die in the external environment, so their detection indicates fresh faecal contamination of water [9].

To determine the presence of Enterobacteriain drinking water, the method of membrane filters is used. The test water (3 samples of 100 ml) is passed through 3 bacterial filters of nitrocellulose, which are then placed on Endo medium and incubated at 37 °C for 24 h. Count the number of lactose-positive colonies identified as coliform bacteria grown on the filters. From 2–3 colonies of red color, swabs are prepared, stained according to Gram and oxidase activity is determined. For this purpose, the filter with the bacterial colonies grown on it is transferred with tweezers, without overturning, onto a patch of filter paper moistened with dimethyl-phenyldiamine. When oxidase is present, the indicator stains the colony blue. 2–3 colonies that did not change the original coloration are seeded in a semi-liquid medium with 0.5 % glucose solution. The crops are incubated for 24 hours at 37 °C. In the presence of gas formation, the number of red colonies on the filter is counted and the circle-index is determined.

Enterococcus bacteria are normal intestinal residents, but are excreted in the external environment in a smaller amount than *E. coli.* Enterococci are more likely to die in water and soil. As a rule, they do not reproduce in these objects, which allows them to be considered as an indicator of fresh faecal contamination. The presence of enterococci is considered an additional indicator of faecal contamination of water and other objects. However, their isolation requires more complex media in preparation and they grow more slowly.

Proteus bacteria live in both human and animal gut (*P.mirabilis*) and rotting residues (*P.vulgaris*). The presence of proteins in environmental objects indicates their contamination with decomposing substrates and extremely unfavorable sanitary conditions [10].

Also, in the environmental assessment of water, studies are carried out to identify spores of sulfite-reducing clostridia. Spores of sulfite-reducing clostridia are more resistant to decontamination and adverse environmental factors than other indicator bacteria. This feature is of particular importance in the evaluation of primary chlorination, since it inactivates almost all indicator bacteria. Detection of clostridia in water before entering the distribution network indicates insufficient purification and that decontamination-resistant pathogenic microorganisms are not likely to have died during purification. To determine the clostridium, the water under study is introduced into the molten and cooled medium of Wilson-Blair. The medium contains thiosulfate (hyposulfite) and a colourless iron salt. As a result of the germination of spores, the reproduction of clostridia and their reduction of sulfite, iron sulfide is formed, which gives the environment a black color [11].

Results and discussion. Traditionally, qualityof water is determined by chemical and bacteriological methods. Biological control of water quality has a number of advantages over chemical and physical methods, since the groups of living organisms mirror all changes in the ecological state of the aquatic environment, while responding to a complex of various factors and pollutants [12]. The method allows to assess the consequences of both permanent and salvo contamination, since it averages the "contamination effect" in time. The most common is the system of assessment of the ecological state of the reservoir and water quality, which is based on the study of the qualitative and quantitative composition of the types of indicators, that is, bioindication of fresh water. During biomonitoring, information about the state of the ecosystem ofthe water body is accumulated, changes occurring in it are detected, and measures are developed to improve its ecological state. To bioindicate the quality of the environment, those types of bioindicators living in a rather small range of environmental conditions are selected. In the case of organizing and conducting environmental monitoring of the state of the reservoir, the use of bioindicators usually provides more valuable information than the assessment of pollution by chemical methods or special devices that determine only individual factors of pollution. Instead, bioindicator species respond to a complex of pollutants or general changes in external conditions. Various hydrobionts are used for biotesting – algae, microorganisms, invertebrates, fish. The most popular objects are juvenile *forms of* planktonic crustacean filters *Daphnia magna, Ceriodaphnia affinis*. For example, a seven-day test on daily young ceriodaphnia *Ceriodaphnia affinis* allows for a shorter period of time (7 days) than on *Daphnia magna* (21 days) to give a conclusion about chronic water toxicity [13, 14].

An important condition for the correct conduct of biotesting is the use of genetically homogeneous laboratory cultures, because they undergo sensitivity testing, are held in special laboratory conditions provided by the standards, which ensure the necessary reproducibility of research results, as well as maximum sensitivity to toxic substances.

Each group of organisms can be used as bioindicators of the state of the environment, but it is very important that the method is relatively cheap and fast. Therefore, the most extensive use is made of microbiological studies, since the earliest and most effective indicator that responds to the introduction of organic matter into the aquatic environment is the number of heterotrophic microorganisms Natural reservoirs are very different in terms of the quality of water, which is conventionally divided into several classes. I class of water quality – very clean, water of similar quality is mainly noted in mountain rivers and lakes, where the impact of humans on nature is still extremely small. II class of water quality – pure, the amount of nutrients in the water increases, but the oxygen regime remains quite favorable. There is a high species diversity of algae, clams, crustaceans, larvae and insects. The overgrowth of immersed plants is predominant, which are spread over significant areas of the water area. III class of water quality – contaminated. In such waters, the content of biogenic elements, organic matter, is significantly increased, as a result of which the bioproductivity of the reservoir increases dramatically. The consequence of this is the emergence of such a phenomenon as the "flowering" of water due to the mass development of microscopic algae, first of all blue-green. Class IV water quality – dirty, this class includes very silt water with poor oxygen regime, frequent phenomena of suffocation and low transparency of water. V class of water quality – very dirty. Determined in reservoirs where the concentration of dissolved oxygen is extremely low (less than 10 %), and the bottom sediments contain hydrogen sulfide. Aquatic plants and bottom macroinvertebrates are usually absent or very rare [15].

Water is not an environment conducive to the reproduction of pathogenic microbes, for which natural biotopes are human and animal organisms. The viability of pathogenic bacteria is affected by concomitant, competitive flora (antagonist microbes, phages, the simplest, algae), as well as temperature, insolation, various chemicals, etc [16, 17].

However quantitative and qualitative ratios in biocenoses are unstable and change under the influence of various factors, that is, they change in sapidity. The term "saprosity" (from Greek *Sapros* – rotten) denote a set of features of the reservoir, including the composition and amount of microorganisms in the water, which contains organic and inorganic substances in certain concentrations. As you know, sapidity is the ability of aquatic organisms to live in water containing different amounts of organic substances. The processes of self-purification of water in reservoirs occur sequentially and continuously, with a gradual change in biocenoses. Polysaprobic, mesosaprobic and oligosaprobic zones are distinguished. The probability zones are also determined by microbiological indicators:

Polysaprobic zones (heavily polluted areas) contain large amounts of organic substances that are easily decomposed, and are almost completely devoid of oxygen. Microbial biocenosis of similar zones is especially rich, but the species composition is limited by anaerobic bacteria, fungi, actinomycetes. The number of bacteria in 1 ml of water in the polysaprobic zone reaches a million or more. Very many saprophytic microflora, well-developed heterotrophic organisms: filamentous bacteria (Sphaerotilus); sulfurous bacteria (Beggiatoa, Thiothris); bacterial zoogles (Zoogloearamig era); the simplest − infusorias (Paramecium putrinum, *Vorticella putrina*); colorless hookworms; oligochaetes (Tubifex *tubifex, Polytomauvella water)*.

Mesosaprobic zones (zones of moderate pollution) are characterized by the dominance of oxidative and nitrification processes. The quality composition is varied. These are mainly nitrifying, obligate aerobic bacteria, as well as species *Clostridium, Pseudomonas, Mycobacterium, Streptomyces, Candida,* etc The total number of microorganisms is hundreds of thousands in 1 ml. Alpha mesosaprobe zone – aerobic decomposition of organic substances begins, ammonia, carbon dioxide is formed, oxygen is scarce, hydrogen sulfide and methane are absent, the number of saprophytic bacteria is determined by tens and hundreds of 1 ml, individual organisms develop in the mass such as bacterial zooglions; filamentous bacteria; fungi; from algae – oscillatorium, stigeoclonium, chlamydomonas, euglena. Beta mesosaprobe zone – there are no unstable organic substances, there is complete mineralization, saprophytes are thousands of cells in 1 ml, and their number sharply increases during the period of plant death, the content of oxygen and carbon dioxide fluctuates depending on the time of day, during the day excess of oxygen, carbon dioxide deficiency and at night vice versa.

Oligosaprobic zones (clean water zones) are characterized by a finished self-cleaning process, a small content of organic compounds and the end of the mineralization process. Water is distinguished by a high degree of purity. The number of bacteria is from 10 to 1000 in 1 ml of water [88]. These are practically pure water bodies. There is no flowering, the content of oxygen and carbon dioxide does not fluctuate.

Pathogenic microorganisms entering reservoirs are quite abundant in polysaprobic zones, gradually die in mesosaprobic zones and are practically not detected in oligosaprobic zones [18].

Our previous studies have shown that when assessing the water quality of fish ponds according to the ecological and sanitary classification when applying fertilizers, the water quality ranged from the class "clean", the discharge "sufficiently clean" to the class "contaminated", the discharge "conditionally contaminated – very contaminated", which corresponds to the β-oligosaprobic – λ-mesosaprobic zone. A comparative analysis of the average seasonal numbers of heterotrophic bacteria when fertilizing showed an increase of 42 % and 37 % compared to the control. Significant fluctuations in environmental and sanitary indicators of water quality when applying fertilizers indicate that microflora is a highly informative biological component of the aquatic ecosystem, which quickly responds to the application of both traditional and new fertilizers in fish farming. The assessment of the water quality of fish ponds according to the ecological and sanitary classification showed that fertilizers lead to the intensification of the development of bacterioflora and heterotrophic microorganisms, but does not lead to a deterioration in water quality [19].

Therefore, the sanitary and microbiological assessment of water is conditional and mandatory. Basic sanitary and microbiological methods are aimed at determining the total microbial contamination (total microbial count), detection of pathogenic microorganisms and their metabolites in the studied objects, determining the degree of water pollution. Practical sanitary microbiology uses two main methods of assessing the sanitary and epidemic state of the external environment: direct detection of pathogenic microorganisms and detection of indirect signs of pathogens in the external environment. According to the current regulatory documents, the following are subject to sanitary and microbiological supervision: drinking water (centralized and local water supply, i.e. from water supply, wells, springs and wells); pool water; open water (rivers, reservoirs); wastewater (domestic and fecal, industrial, melt and runoff); purified water for the preparation of medicines; medical and domestic ice.

The microflora of reservoirs are formed by two groups of microorganisms: autochthonous (constantly live and multiply in the reservoir) and allochthonous (get in from outside when contaminated with various sources). Microorganisms that have adapted to living conditions in the water and are regularly found in it can be considered water-specific flora. These include aerobic coca: micrococa, sarcins, *Serratiamarcescens, Bacillus cereus, Bacillus mycoides,* bacteria of the genera *Pseudomonas, Proteus, Leptospira*. The number of microorganisms in open water bodies varies widely: from several tens, hundreds to millions in 1 ml, which depends on the type of water body, the degree of its pollution, the seasons, etc The microflora of reservoirs depends on the substances contained in them and on biocenosis, that is, the species composition and abundance of other living creatures. Yes, phages and the simplest ones in the water destroy bacteria. Microorganisms capable of forming antibiotics cause the death of other bacteria sensitive to these substances.

Water microorganisms play a significant role in the circulation of substances in nature. They break down organic matter to form substrates that use other aqueous microorganisms in food. Biological activity in reservoirs is maximum in the summer-autumn period.

The number of microorganisms in the water of surface runoff in the spring flood period increases to 2.8–3 million in 1 ml. During the flood period, secondary contamination of the water supply network is possible. The microflora of economic and faecal wastewater consists of microorganisms isolated from the intestines of humans and animals, among which there are representatives of normal and opportunistic flora (Escherichia, enterococci, klebsiels, clostridia, fungi of the *genus Candida*, the simplest, etc), but can also be pathogenic – pathogens of intestinal infections (salmonella, shigel, vibrio, yersinia, leptospira, polio, hepatitis A, E viruses, etc). The danger of infection with the latter is particularly great if insufficiently decontaminated wastewater from infectious diseases hospitals enters the reservoirs [20].

Conclusion. To assess the state of water bodies, it is the biological analysis that can be considered the most accurate and convenient. One of the most important biological methods for assessing the ecological state of reservoirs is to assess the quality of water precisely by microbiological indicators, since microorganisms are the first and most informative component of biota that responds to pollution. Maintaining this vector of studying and assessing the sanitary status of water bodies and solving problems regarding the purification of water bodies is the main task of the present.

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Роль мікроорганізмів водного середовища у формуванні еколого-санітарного стану водойм

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Протягом останніх років людство стикнулося з проблемою браку чистої води для своїх потреб, тому основними завданнями науки є підвищення ефективності природних ресурсів, які б не погіршували якість води. Для оцінювання якості води традиційно використовуються хімічні та біологічні методи, проте біологічні методи є ефективнішими, оскільки вони менш затратні та більш інформативні. Біологічний контроль якості води має ряд переваг перед хімічними і фізичними методами, оскільки угрупування живих організмів віддзеркалює усі зміни екологічного стану водного середовища, одночасно реагуючи на комплекс різноманітних чинників і забруднювачів. Метод дає змогу оцінити наслідки як постійного, так і залпового забруднення. Одним із найважливіших біологічних методів оцінювання екологічного стану водойм є оцінювання якості води за мікробіологічними показниками, що є найпершим і найінформативнішим компонентом біоти, який реагує на алохтонне внесення органічних речовин. Мікроорганізми беруть участь у оптимізації умов водного середовища, зокрема у формуванні гідрологічного та газового режимів та в самоочищенні водойм, що в кінцевому результаті і визначає якість води. Тому оцінювання механізмів формування мікробіоценозу дає можливість визначати санітарний стан водойм забезпечення бактеріальною ланкою функціонування екосистеми. Впровадження нових технологій не повинно приносити чи формувати будь яких загроз довкіллю, здоров'ю та безпеці життєдіяльності людини, саме тому важливим завданням сьогодення є вивчення впливу мікроорганізмів на становлення еколого-санітарного стану водних екосистем.

Ключові слова: мікроорганізми, водне середовище, класи води, біоіндекація, зони сапробності, гетеротрофні бактерії.