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THE MAIN TYPES OF MICROORGANISMS IN NATURAL AND WASTEWATER OF THE LVIV REGION

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Abstract

The microflora of natural (lake) water and industrial wastewater was investigated and the initial number of microorganisms in these waters was calculated. The main features of microorganisms identified during the process of their identification are indicated. Morphological, physiological and cultural characteristics of microorganisms were investigated by deep seeding of the samples of the investigated waters of the Lviv region on the nutrient media in the Petri dishes and by microscopy of preparations with lifelong staining. The growth pattern of colonies of microorganisms on meat-peptone agar (for bacteria) and wort-agar (for yeast) in a Petri dish is shown. Quantitative and qualitative characteristics of microorganisms were analyzed, revealing the predominant genera of micro-objects in lake and industrial wastewater: *Diplococcus*, *Sarcina*, *Bacillus*, *Pseudomonas* bacteria types, *Oscillatoria* cyanobacteria, and *Saccharomyces* yeasts.

Key words: microorganisms, identification, natural water, wastewater.

1. Problem statement

Due to the sharp increase in the discharge of industrial wastewater into natural waters, these bodies are unable to cope with significant pollution through self-purification, resulting in a decrease in their self-cleaning capacity. As a result, today in Ukraine, there are virtually no surface water sources that can be classified in the first category. Only 15 % of water bodies are classified as categories 1–3 (practically clean), while 60 % are in categories 4–5 (polluted), and 25 % fall into categories 6–7 (polluted and heavily polluted). Consequently, approximately 90 % of water drawn from surface sources and at least 30 % of water from underground sources require purification from harmful impurities and disinfection [1].

The discharge of untreated or insufficiently treated wastewater from chemical and food industry enterprises, municipal sewage systems, livestock complexes, water and rail transport (e.g., the Dnipro, Siverskyi Donets, and the Black Sea near Odesa), are major sources of pollution and deterioration in water quality. Over 60 % of inadequately treated wastewater enters open water bodies, while the remaining 40 % is discharged without any treatment. This situation creates a complex ecological state of the environment, reduces resources of clean drinking water, poses serious health risks for the population in many regions of Ukraine, and leads to high rates of diseases such as intestinal infections and hepatitis, as well as increased exposure to carcinogenic and mutagenic factors. The annual growth in water demand for industrial and agricultural purposes is 4 %, doubling every 20 years.

Drinking water from various sources [2–5] does not meet the standards of regulatory documents based on microbial criteria. Microbial contamination primarily affects the quality of drinking water, which is one of the main causes of widespread outbreaks of acute intestinal infections [1]. Such water poses a potential threat not only to the environment but also to humanity as a whole.

As we can see, the critical state of water resources necessitates the implementation of advanced water treatment technologies that do not cause pollution with organic, inorganic, or biological components when wastewater is discharged into open water bodies, thereby preventing secondary pollution or requiring the improvement of existing water disinfection methods. Therefore, in the context of increasing natural water pollution, choosing the optimal method of purification and disinfection becomes essential. However, to determine the specific method for water treatment and disinfection, it is necessary to establish the quantitative and qualitative composition of microorganisms in the water, which is discussed further below.

2. Analysis of the recent researches and publications

Open water bodies in Ukraine are characterized by high levels of pollution, not only of chemical but also of biological origin. In some cities and even certain regions in the eastern part of Ukraine, deviations in water quality from the norm reach 70…80 % [6]. The contamination of water bodies and violations of water quality standards are largely due to pollutants from industrial enterprises and municipal utilities. Every day, over 10.6 thousand m³ of untreated and inadequately treated wastewater is discharged into water bodies. The situation is most critical in the regions of Eastern Ukraine [6].

Annually, 370 million m^3 of polluted wastewater, or 14 % of the total volume nationwide, flows into the Dnipro River [7], and 6 million m^3 enters the Pripyat River basin [8]. As early as the 1980s, data indicated significant pollution of the Dnipro Reservoir, with

approximately 240 million m^3 of wastewater entering it annually through major side tributaries. It is important to note that over 60 % of Ukraine's population relies on the Dnipro River for water, 15 % use surface water bodies, and only 25 % utilize groundwater [8].

Among the western regions of Ukraine, Lviv and Ternopil regions have reported the highest pollution levels [6]. While Lviv is relatively well-supplied with water resources, the current state of these resources is concerning, primarily due to the discharge of inadequately treated wastewater into open water bodies. However, the pollution of open waters in Lviv is mainly caused by water blooming, decaying vegetation, and ongoing contamination with solid household waste.

This is far from an exhaustive list of direct pollution sources affecting open water bodies through industrial and domestic waste, but it is sufficient to grasp the catastrophic state of water resources. When assessing the risk level of water for human health, depending on the nature of undesirable contaminants, microbial pollution plays the most critical role. The risk of diseases from microbial contamination is significantly higher (up to 100000 times) than from chemical contamination of various origins. Pathogenic microorganisms are responsible for serious diseases that claim 2.2 million lives annually. Therefore, the identification of microorganisms and their concentration per unit volume of water is one of the key factors in selecting a water purification method.

3. Statement of the problem and its solution

The task of the presented research is the following:

– to conduct a microbiological analysis of water samples from open water bodies and wastewater in the Lviv region;

– to determine the quantitative and qualitative composition of microorganisms to identify the dominant microflora present.

3.1. Materials and methods

The essence of the method for counting the number of microorganisms in 1 cm^3 of the investigated water consists of determining the total number of microorganisms capable of growing on meat-peptone agar (MPA) (for bacteria) or wort agar (WA) (for yeasts) in Petri dishes. The composition of MPA is as follows: meat extract (1 dm^3), peptone (10 g), and agar (15 g); the composition of WA consists of malt wort (1 dm^3) with a dry matter content of 6…8 % and agar (2 %).

The total number of microorganisms in 1 cm^3 of water is determined through the following steps: preparation of dilutions [9]; inoculation onto solid nutrient medium in Petri dishes [10]; and counting colonies on the nutrient medium [9].

When analyzing a water sample, it must be diluted before inoculation, based on the anticipated number of microorganisms in 1 cm^3, which is determined by deep plating of the investigated water in Petri dishes [10]. Since the study samples included water from open water bodies in various settlements of the Lviv region and wastewater from various industries in Lviv, one dilution was prepared for natural waters (due to their low initial count) and three dilutions for wastewater. The process of preparing dilutions and introducing the inoculum into the nutrient medium is described in detail in [9].

The number of microorganisms in 1 cm^3 of the studied natural water from open bodies ranged from 10^2 to 10^3 CFU/cm^3, while for wastewater, it ranged from 10^4 to 10^5 CFU/cm^3. The volume of nutrient medium for inoculation was 12…15 cm^3 and was cooled to 45…48°C. This was carefully poured into a Petri dish under sterile conditions, followed by mixing with circular motions.

After closing the Petri dish lid, the nutrient medium with the inoculum was thoroughly mixed by gently rotating the dish to ensure even coverage of the bottom. The dishes were then transferred to an incubator. An electric dry-air thermostat TS–80M–3 was used for cultivating microorganisms.

For the quantitative determination of microorganisms, the number of colonies that grew on the Petri dishes was counted, based on the premise that each colony developed from a single cell. The obtained results must be recalculated to the initial water sample, considering the dilution using the formula:

$$N = a \cdot 10^n, \quad (1)$$

where a is the number of colonies that grew in the dish;

n is the dilution factor.

Experimental data were obtained based on the arithmetic mean counts from three parallel inoculations of water samples.

Additionally, the morphological, cultural, and physiological properties of the microorganisms were determined, and fixed preparations of the cells were made using safranin as a dye [11]. Live preparations of the investigated waters were prepared with live staining of microorganisms as follows: a drop of the investigated water was mixed with a drop of the dye solution (methylene blue) on a microscope slide, followed by covering with a coverslip and subsequent microscopic examination [12].

3.2. Results and Discussion

In 1994, more than 1.5 billion m^3 of polluted wastewater was discharged annually from urban sewage systems into natural water bodies according to the results of [13]. The reduction in anthropogenic load and water use during the 1990s and 2000s did not improve water quality. In 2006, over 2 billion m^3/year of wastewater was entering Ukraine's water bodies [14], while in 2010, approximately 2.6 billion m^3/year of domestic and industrial wastewater, containing around 8 million tons of various pollutants, was reported [1]. By 2018, this figure had risen to 2.9 billion m^3/year of contaminated wastewater. Anthropogenic pressure on the hydrosphere has significantly increased over time according to average statistical data.

Natural water is a multi-component dynamic system consisting of gases, mineral and organic substances present in truly dissolved, colloidal, and suspended states, as well as various microorganisms [8, 15]. Therefore, natural water samples for analysis were collected in sterile glass containers from the following locations in the Lviv region: the city of Sudova Vyshnya, the city of Horodok, the village of Domazhyr,

the village of Myklyashiv, and from Lviv (Navariya Lake) for comparison purposes.

The initial microbial load of the studied natural waters, representing their quantitative composition of microorganisms, is shown in Table 1.

Wastewater is a complex heterogeneous system that contains organic and inorganic impurities in insoluble, colloidal, and dissolved forms [8]. In addition, wastewater is contaminated with various pathogenic microorganisms, among which representatives of the genera Salmonella, Shigella, Vibrio, Leptospira, as well as Enterococcus faecalis, Pseudomonas aeruginosa, Aeromonas hydrophila, Mycobacterium, and others are most frequently encountered.

The studied wastewater samples were taken from the "Halychpharm" factory and the private brewery "Kumpel" in Lviv, with the initial microbial load (quantitative composition of microorganisms) presented in Table 2.

Water samples from open water bodies were collected during the summer period (June to July). The depth of sampling was 100 mm from the water surface, at a distance of 1 m from the shore. Samples of industrial wastewater were taken from a continuous flow. For each water body, five samples were collected, and the obtained microbial counts were averaged per unit volume of water.

Each dilution was inoculated in three parallel Petri dishes using the deep inoculation method on nutrient media and placed in an incubator for the cultivation of microorganisms at a temperature favorable for their growth. Different groups of microorganisms grow at different rates; therefore, the Petri dishes containing MPA were kept in the incubator at 35±0.5°C for 2…3 days, which is typical for bacterial colony growth, and at 30±0.5°C for 4…5 days for yeast colony growth. The cells of microorganisms multiply, and their mass increases to form colonies visible to the naked eye (Figure 1). As shown in Figure 1, the colonies differ in shape and size and exhibit varying pigmentation, indicating their belonging to different genera of microorganisms. The growth characteristics of colonies on nutrient media (MPA and WA) in Petri dishes, along with microscopic examination of the studied water samples, allow for the determination of the genus characteristics of the microorganisms.

The microorganisms identified in natural and wastewater, along with the number of cells in dominant quantities, are presented in Table 3. The quantitative results of the detected microorganisms from different water sources are expressed as percentages (Table 3).

The identified microflora in open water bodies, found in significant quantities per unit volume of water, has a negative impact when used in the national economy, particularly for domestic applications. Special attention should be paid to the detected cyanobacteria, which pose a potential threat to fish farming by decreasing the concentration of dissolved oxygen in the water, leading to fish mortality.

Table 1 – Initial number of microorganisms (NM_0) of natural water in settlements of Lviv region

Water Bodies	NM_0, CFU/cm^3
Lake (Sudova Vyshnya)	820…2090
Lake (Horodok)	2200…2340
Lake (Domazhyr)	2100…2600
Lake (Myklyashiv)	2450
Lake Navariya (Lviv)	1800…2050

Table 2 – Initial number of microorganisms (NM_0) of industrial wastewater from Lviv city

Water Bodies	NM_0, CFU/cm^3
Wastewater from "Kumpel" Brewery	110000
Wastewater from "Halychpharm" Pharmaceutical Factory	38600

Table 3 – Quantitative and qualitative composition of microflora in different water sources of Lviv region

Water Sample Source	Identified Microorganisms
Lake (Sudova Vyshnia)	Bacteria: *Bacterium* (16 %), *Pseudomonas* (22 %), *Sarcina* (27 %), *Bacillus** (35 %)
Lake (Horodok)	Bacteria: *Pseudomonas* (26 %), *Staphylococcus* (34 %), *Bacillus** (40 %)
Lake (Domazhyr)	Bacteria: *Sarcina* (9 %), *Bacterium* (15 %), *Bacillus* (21 %), *Pseudomonas** (55 %)
Lake (Myklyashiv)	Bacteria: *Pseudomonas* (24 %), *Bacterium* (32 %), *Bacillus** (44 %) Algae: *Oscillatoria* (99 %)
Lake Navariya (Lviv)	Bacteria: *Streptococcus* (8 %), *Sarcina* (13 %), *Micrococcus* (18 %), *Bacterium** (29 %), *Bacillus** (32 %)
Wastewater from Brewery "Kumpel"	Bacteria: *Bacterium* (11 %), *Micrococcus* (11 %), *Pseudomonas* (18 %), *Sarcina* (25 %), *Bacillus** (35 %), Yeasts: *Saccharomyces* (99 %)
Wastewater from Pharmaceutical Plant "Halychfarm"	Bacteria: *Bacterium* (4 %), *Micrococcus* (10 %), *Streptococcus* (15 %), *Diplococcus* (19 %), *Sarcina* (21 %), *Bacillus** (31 %)

Note: * in dominant quantity.

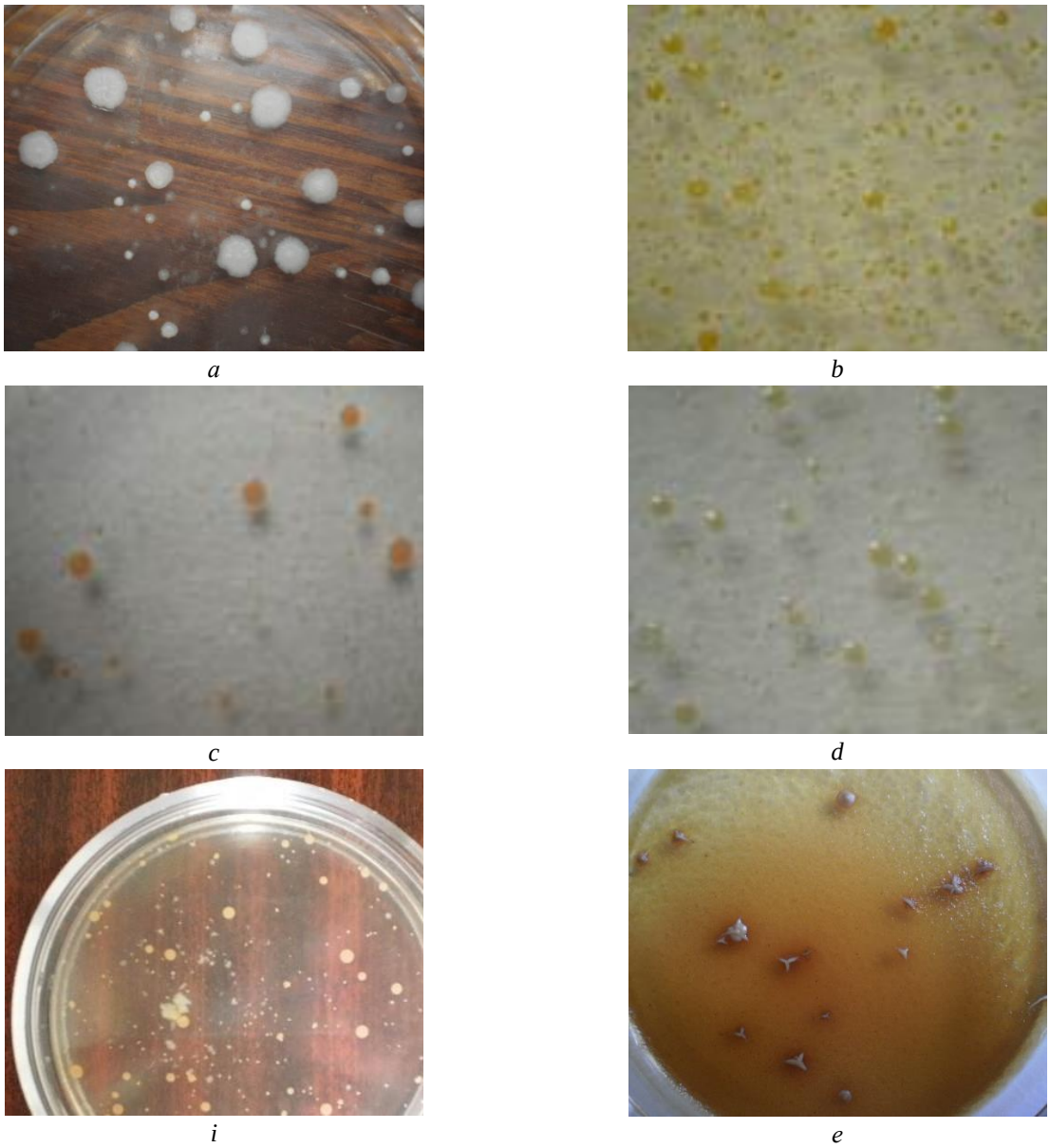

Figure 1 – Growth characteristics of identified bacteria on meat-peptone agar (*a*-*i*) and identified yeasts on wort agar (*e*) in Petri dishes.
Bacterial colonies: *a* – *Bacillus*; *b* – *Sarcina*; *c* – *Diplococcus*; *d* – *Pseudomonas*; *i* – *Bacterium* and *Micrococcus*.
Yeast colonies: *e* – *Saccharomyces*.

Therefore, the identification of aquatic microflora is a crucial factor in selecting an effective method for purifying natural waters and wastewater from specific types of microorganisms. This, in turn, will improve the ecological status of natural water bodies and allow for the discharge of wastewater into open waters with permissible concentrations of microbial contamination.

4. Conclusion.

It was investigated the predominant microflora of natural waters and wastewaters are *Diplococcus*, *Sarcina*, *Bacillus*, *Pseudomonas* bacteria types, blue-green algae of the *Oscillatoria* types, and *Saccharomyces* yeast types. It has been investigated Diplococcus bacteria types are diplococci, spherical, arranged in pairs, belonging to the *Coccaceae* family; *Sarcina* bacteria types are sarcines, spherical in shape, are placed in packages of 8, 16, 32, etc. cocci), non-pathogenic; belong to the family *Coccaceae*; *Bacillus* bacteria types are bacilli, rod-shaped bacteria, sporogenic (forming endospores), diameter of spores smaller than the diameter of the cell; belong to the family *Bacillaceae*; *Pseudomonas* bacteria types are pseudomonads, rod-like; asporogenic; moving; characteristic monopolar polytrichal (lofotrichal) type of plaque; belong to the family *Pseudomonadaceae*; *Saccharomyces* yeast types baker's yeast; stationary; belong to the family *Saccharomycetaceae*.

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Коваль І. З.

ДОМІНУЮЧІ ВИДИ МІКРООРГАНІЗМІВ ПРИРОДНИХ ТА СТІЧНИХ ВОД ЛЬВІВСЬКОЇ ОБЛАСТІ

Досліджена мікрофлора природних (озерних) та виробничих стічних вод та здійснений підрахунок вихідного числа мікроорганізмів в цих водах. Зазначені основні ознаки мікроорганізмів, виявлені під час процесу їх ідентифікації. Шляхом глибинних висівів зразків досліджуваних вод Львівщини на поживні середовища в чашки Петрі та в результаті мікроскопування препаратів з прижиттєвим забарвленням досліджувались морфологічні, фізіологічні та культуральні ознаки мікроорганізмів. Зображено характер росту колоній мікроорганізмів на м'ясо-пептонному агарі (для бактерій) та сусло-агарі (для дріжджів) в чашці Петрі. Проаналізовано кількісні та якісні характеристики мікроорганізмів, що дозволило встановити домінуючі роди мікроб'єктів у озерних та виробничих стічних водах: бактерії типів *Diplococcus, Sarcina, Bacillus, Pseudomonas*, ціанобактерії роду *Oscillatoria* та дріжджі роду *Saccharomyces*.

Ключові слова: мікроорганізми, ідентифікація, природна вода, стічна вода.

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