ENVIRONMENTAL PROBLEMS

Vol. 10, No. 2, 2025

RESULTS OF COMPARATIVE BACTERIOLOGICAL ASSESSMENT OF DRINKING WATER FROM DIFFERENT POINTS OF DELIVERY IN THE CITY OF MYKOLAIV AND POSSIBILITIES OF ADDITIONAL WATER DISINFECTION UNDER FIELD CONDITIONS

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https://doi.org/10.23939/ep2025.02.145

Received: 16.04.2025

Abstract. The provision of quality drinking water is one of the most important conditions for public health, especially in urban areas. Like many other settlements, the city of Mykolaiv faces problems with the quality of water supplied to consumers. This study evaluated some bacteriological parameters of drinking water supplied from different sources in the city and conducted a comparative analysis of the effectiveness of using plant coagulants for disinfection. Water samples were collected from underground sources, artesian wells, the municipal water supply network, and the Bug Estuary. The study was carried out regarding total microbial counts, coliforms, *Escherichia coli*, and enterococci. The results showed the presence of bacterial contamination in some samples; in particular, *E. coli* was detected, indicating fecal contamination of the water. Some of the samples treated by reverse osmosis met sanitary standards. The results emphasize the need to strengthen control over drinking water quality and introduce effective post-treatment technologies. The proposed use of natural coagulants is a promising alternative to traditional chemical treatment methods and can help improve the environmental safety of the water supply.

Keywords: groundwater, microbiological indicators, drinking water, *Escherichia coli*, natural coagulants.

1. Introduction

Globally, more than 2.1 billion people have little or no access to clean drinking water. The latest statistics from the World Health Organization (WHO) showed that another 4.3 billion people live in unsafe sanitation conditions, and the number of these people mainly depends on the region (WHO; UNICEF, 2014). The recent studies highlight the global problem of ensuring clean water (Mukherjee et al., 2019). This problem is acute, especially in developing countries, where 95 % of untreated wastewater is discharged into surface waters (Omarova et al., 2018). The provision of clean and safe water is of paramount importance for human health, and the UN considers it a priority issue, which is Sustainable Development Goal (SDG) number six (United Nations, 2022). In developing countries, population growth, livestock production, and industrialization are putting pressure on safe drinking water supplies (Akoachere et al., 2013). Many people in these regions directly use contaminated water from rivers, shallow wells, and streams for irrigation, domestic use, and drinking, posing significant health risks (Omarova et al., 2018). Microbiologically contaminated water can cause intestinal infections in humans. It indicates insufficient maintenance of the water supply system and control problems (Baye et al., 2021). Failure to comply with basic sanitary standards is often the result of a lack of public awareness. As a result, contaminated water is the leading cause of death from diarrhea and intestinal diseases, especially for young children under five and other vulnerable groups (Prüss-Ustün et al., 2014).

For citation: Hostieva, D., Trokhymenko, G., Chestnykh, Y. (2025). Results of comparative bacteriological assessment of drinking water from different points of delivery in the city of Mykolaiv and possibilities of additional water disinfection under field conditions. *Journal Environmental Problems*, *10*(2), 145–155. DOI: https://doi.org/10.23939/ep2025.02.145

Every year, water quality requirements are increasing, as well as the pollution of natural resources and the impact of climate change, creating new challenges for water supply systems. These existing disinfection methods, such as chlorination, ozonation, and ultraviolet irradiation, are highly effective but have their advantages and disadvantages. Researching new approaches to water treatment and developing more environmentally friendly and cost-effective methods is an important task to ensure a reliable and safe water supply in the future.

Chlorination is one of the most common methods of water disinfection. The advantages of this method are cost and accessibility, as well as powerful antiseptic properties and long-lasting effects. However, this method can also cause pollution of water bodies (Šestopalov et al., 2023).

Chemical water disinfectants are an important tool in water disinfection from pathogenic microorganisms and water treatment. Among the most commonly used disinfectants are chemical compounds of chlorine, iodine, and potassium permanganate. These disinfectants have their specific application and mechanisms of action, high efficiency in the microbiological aspect, but can hurt the environment (Musee et al., 2023). A particular danger is the formation of disinfection by-products (trihalomethanes, chlorites, chlorates, etc.) that can harm human health (Golfinopoulos et al., 2024). Ozonation is one of the conventional methods of disinfection (Pyatkovskyy et al., 2024). Ozone is used as a powerful oxidant to kill microorganisms. This method is safer because it does not form toxic residues and decomposes quickly after water treatment. Another advantage is the improvement of the organoleptic properties of water after treatment. However, like most methods, ozonation has some disadvantages. The biggest ones are cost and energy consumption, as this method requires special equipment to generate ozone.

Among the modern alternative methods of water disinfection, the method of ultraviolet irradiation can also be distinguished. This method demonstrates high efficiency both as a separate method of water treatment and in combination with other methods. In combination with chlorination, ultraviolet radiation helps to reduce the concentration of organochlorine compounds in water, which helps to reduce environmental pollution (Cemenov et al., 2016).

The use of ultrasonic treatment together with UV irradiation can increase the disinfection efficiency, especially in cases of high-water turbidity or a significant concentration of suspended particles. At the same time, the effectiveness of this combination depends on the specific processing conditions, and the increased power consumption may limit its practicality (Jin et al., 2013).

To date, chemicals such as Aquatabs and Dutrion, which contain sodium dichloroisocyanurate and chlorine dioxide, respectively, are widely used for water disinfection in domestic conditions (Kgabi et al., 2014). These drugs are highly effective in killing bacteria, viruses, including cholera vibrio and E. coli. An important aspect of using these tablets is to follow the instructions and take the correct dosage, so to ensure sustainable use and maximum effectiveness, it is necessary to educate the population on the proper use of these drugs, as well as to ensure their availability and acceptability.

Biological water treatment methods use microorganisms to remove pollutants through metabolic processes. These methods can effectively treat a wide range of contaminants, including organic matter, heavy metals, and inorganic compounds (Hasan et al., 2020; Jyoti et al., 2022). Various microorganisms, such as bacteria, fungi, yeasts, and algae, are used in bioremediation processes due to their ability to bind, concentrate, and modify toxic species (Coelho et al., 2015). Biosorption is a particularly effective mechanism for the removal of heavy metals, providing high efficiency and environmental friendliness (Jyoti et al., 2022). The advantages of biological methods include environmental safety, sustainability, and lower long-term costs compared to the conventional chemical methods (Hasan et al., 2020; Jyoti et al., 2022). However, these methods also face challenges such as dependence on environmental conditions and longer processing times (Hasan et al., 2020).

Natural coagulants are an alternative to chemical disinfectants and modern, but costly, methods of water purification (Trokhymenko & Chestnykh, 2024; Atreya et al., 2023; Varkey, 2020). In developing countries, as well as in conditions of military conflicts and emergencies, there is a need to find alternative natural methods of drinking water purifycation (Yefanova et al., 2022). Recent studies have shown the effectiveness of using plants such as Morínga oleiféra, Agave americana, Carpobrotus acinaciformis, Austrocylindropuntia subulate, and Senicio as natural coagulants for the treatment of wastewater and natural waters from pathogenic microorganisms and fungi (Sabadash et al., 2021). Ecotoxicity was absent or minimal in some cases (Zakaria et al., 2022;

Abd-Elhalim, 2022; Yamaguchi et al., 2021). The use of natural coagulants can contribute to the durability of water treatment plants, as they produce less sludge than chemical coagulants, do not pose a threat to the environment, and are therefore a safer alternative (El Bouaidi et al., 2022).

The environmental and economic benefits of using natural coagulants are attracting interest in their more detailed study, with a view to their future use in rural areas, developing countries, and in military conflicts, where access to chemical purifiers may be limited or non-existent.

In many areas of Mykolaiv, groundwater is the main source of drinking water for the population, as it is believed to have lower levels of impurities and be less susceptible to pollution, as well as more resistant to climate variability than surface water (Lapworth et al., 2023). However, groundwater quality is affected by climate, slope, drainage conditions, water-rock interaction, and anthropogenic activities (Motlagh et al., 2020). Factors such as stormwater runoff, animal waste leakage into the environment, agricultural practices, industry, energy production, mining, wastewater, and the infiltration of inadequately treated domestic wastewater can deteriorate groundwater sources (Conboy & Goss, 2000).

With the outbreak of armed conflicts that led to significant damage to water supply networks and water pollution, the city of Mykolaiv began to turn to alternative sources of drinking water.

There are several main types of bottled drinking water in the city. One of them is water from CHP (combined heat and power) plants, which undergoes a multi-stage treatment, including mechanical filtration and chemical treatment per the sanitary standards of DSanPiN 2.2.4-171-10 (Odiyo & Makungo, 2018). Drinking water vending machines are also common, providing purified water through reverse osmosis and ultraviolet disinfection following the requirements of DSTU. Additionally, the water from the well must meet drinking water quality standards, even if it is used without its treatment.

All of these types of water have characteristics and advantages that influence consumers' choices in the face of constant uncertainty about water quality.

In this regard, it is important to note that water quality problems are not only theoretical or global issues, but also have specific manifestations at the local level. For example, before February 2022, Mykolaiv already had problems with water and water supply, and during the war, the situation became critical. The quality of water from the Dnipro River and tap water in Mykolaiv, in terms of total hardness, alkalinity, calcium, magnesium, and dry residue, met regulatory requirements after treatment, except for a fluoride deficit in the water supply and chlorine levels. The city's water supply system included sedimentation tanks and rapid filters, which allowed for high-quality water treatment, regardless of its initial quality (Safranov et al., 2011). After passing through the filters, the water was fed into clean water tanks (CWTs), where it was disinfected with liquid chlorine (Malovanyy et al., 2021).

Water is supplied to the city through five water pipelines, where it is distributed to consumers (Safranov et al., 2011). Water from artesian wells intended for domestic drinking purposes contained contamination levels that exceeded the permissible standards for drinking water supply to the population (Safranov et al., 2016). Despite this, it was used both for technical needs and for drinking without additional water treatment.

During the active hostilities in Kherson and Mykolaiv regions, a serious problem with water supply arose due to damage to the water pipeline and then the inability to use water from the Dnipro River. Residents of the city were forced to use salty estuarine water first, which led to corrosion and destruction of main and in-house water pipes (Trokhymenko et al., 2024). Currently, water quality can vary due to the characteristics of the Ingulets River, from which water is currently supplied, and it can only be used for technical needs. It is currently impossible to predict whether this water will continue to be suitable even for domestic needs. In the face of constant uncertainty about the quality of the supplied water, there is a general need for a detailed analysis of its microbiological composition. As well as bacteriological analysis of water from the points of delivery.

The study of water microbiocenosis depends on the identification of sanitary pathogens, such as the bacterial group of Escherichia coli (BGB), E. coli, enterococci, enteroviruses, and coliphages. These microorganisms are indicators of fecal contamination and the epidemiological safety of water. Their detection allows for assessing serious contamination and health risks (Some et al., 2021). The relationship between the sanitary quality of river water and external sources of urban runoff pollution is shown in detail in Fig. 1.

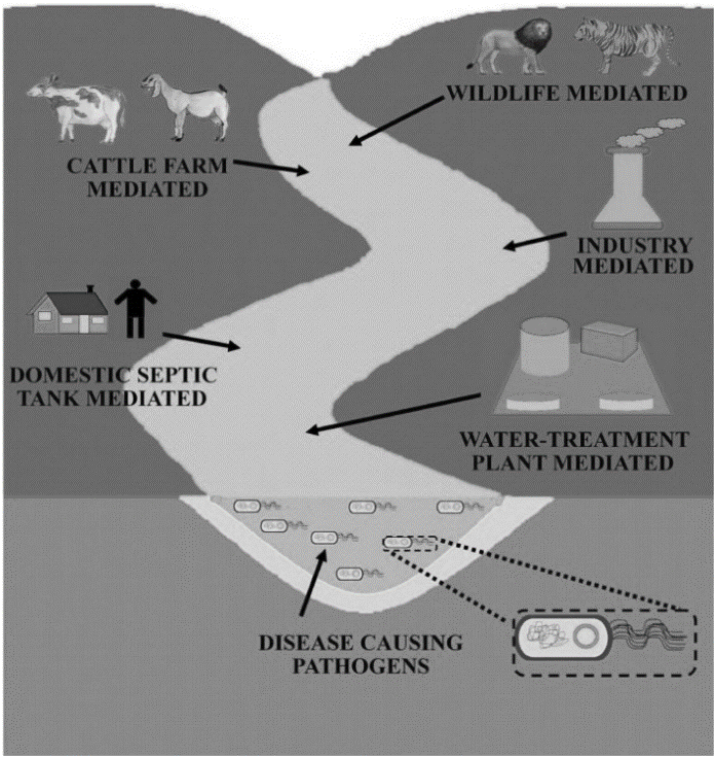

Fig. 1. The relationship between the sanitary quality of river water and external sources of urban runoff pollution (Some et al., 2021)

To ensure the microbiological safety of a drinking water source, numerous barriers are applied at all stages from water intake to the consumer. This includes regular monitoring of water quality, use of modern methods of treatment and disinfection, and prevention of pathogens from entering the source.

However, many drinking water collection points in Mykolaiv lack regular maintenance and water quality control, which leads to potential risks to public health. This is especially critical in a time of armed conflict, when access to quality and safe water is more important than ever. Due to shortcomings in water treatment systems and insufficient attention to regular monitoring, the supplied water may not meet sanitary standards, which in turn requires the implementation of stricter measures to control the quality and ensure the safety of water for the population. Therefore, the area of research related to microbiological control of drinking water quality is extremely relevant.

Analysis of the latest research and publications. The quality of drinking water directly affects human health. Drinking water with physicochemical parameters that deviate from acceptable standards can negatively affect the development of various diseases. One of the key parameters of water quality is the hydrogen index (pH), which determines its acid-base balance. The pH level affects the corrosiveness of water, the speed of chemical reactions, and the toxicity of pollutants. Scientists believe that the optimal pH of drinking water should be close to the pH of human blood, which is 7.5 (Matvijchuk et al., 2021).

In addition to physicochemical indicators, an important aspect of water quality assessment is the microbiological state. Studies show that in many cities of Ukraine, tap water does not meet sanitary and hygienic standards and is unsuitable for raw consumption. This is confirmed by scientific studies (Matvijchuk et al., 2021; Pro zatverdzhdennya metodychnykh vkazivok, 2005).

Sanitary and microbiological control of drinking water determines its epidemic safety following established standards. The main indicator of water pollution is the presence of Escherichia coli, which indicates possible fecal contamination. Ukraine applies a stricter approach to water quality assessment than many other countries: control covers all types of glucose-positive coliform bacteria, not just lactose-positive variants. This approach is reasonable, as lactose-negative coliform bacteria can also enter water and, under certain conditions, multiply in drinking water, which can lead to negative impacts on human health (Matvijchuk et al., 2021).

According to the European Union (EU) Directive 98/83/EEC of November 3, 1998, *E. coli* is defined as the main indic ator of fecal contamination of drinking water (Pro zatverdzhdennya metodychnykh vkazivok, 2005).

The study aim is to assess the microbiological quality of drinking water taken from different sources of water supply in Mykolaiv to identify potential pathogens and determine the level of water safety for human consumption. We also conducted a comparative analysis of the effectiveness of the use of plant coagulants and water disinfection products.

2. Experimental part

The research was conducted at the laboratory of the Admiral Makarov National University of Shipbuilding. Water samples were collected from wells equipped at certain drinking water collection points and from residential buildings with their wells, and water samples were also collected from the Bug Estuary for comparative evaluation of water disinfection products. Samples were collected following Sanitary and Epidemiological Norms 2.2.4-171-10 (DSanPin 2.2.4-171-10**,** 2012), under aseptic conditions in 1000 ml sterile bottles, and then transported to the chemical laboratory for analysis. Before transporting the water samples, the sample bottle caps were protected with aluminum foil to avoid any form of hand contamination and to adhere to aseptic methods. The water samples were collected during April – May 2024, a period characterized by increased precipitation and rising temperatures, which may influence microbial activity and water quality. The samples were assigned identification numbers and source locations. The samples were analyzed within 2 hours after being collected to avoid unpredictable changes in the bacterial population (Saturday & Runyonyozi, 2019).

Water samples were collected from various types of sources in Mykolaiv, including municipal taps, water distribution points, and vending machines (CHP). The type of water source for each sampling point is specified in the text and illustrated in Fig. 2. Table 1 presents the bacteriological water quality indicators for each sampling point.

Fig. 2. Location of drinking water points in Mykolaiv

Fig. 3 shows some points of drinking water extraction in the city, including CHPs and drinking water points. Some of them have equipped platforms, but others appear to be disorganized, which may contribute to water pollution. It is evident that residents actively use these sources, which emphasizes the importance of monitoring their quality.

This situation points to the need for modernization of water supply systems and regular monitoring.

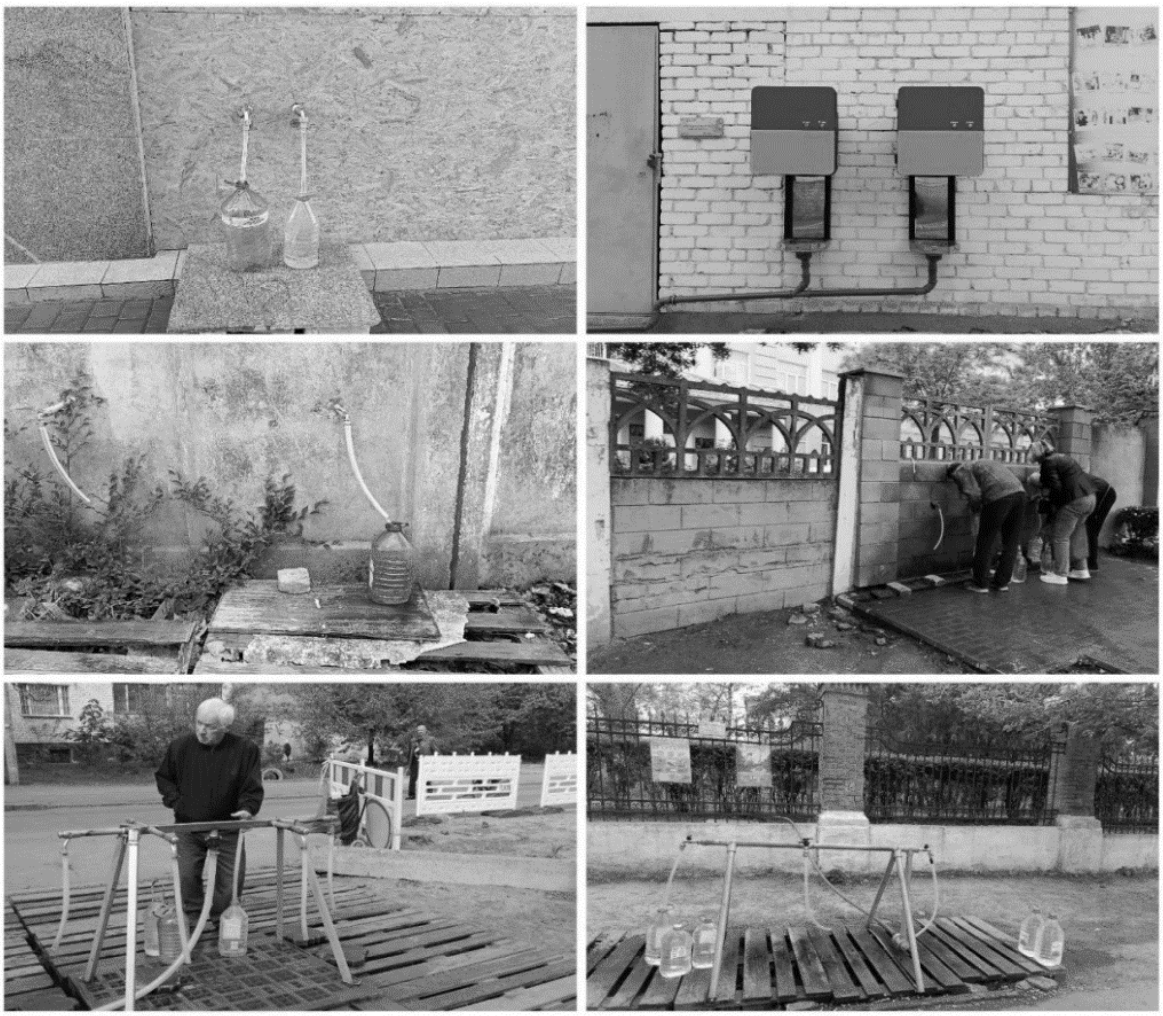

Fig. 3. Water sampling locations

The total microbial contamination and coli-index were determined by bacteriological methods according to the established methodology. To assess the level of bacterial contamination, the nutrient media Compact Dry AQ and Compact Dry EC (Nissui Pharmaceutical Co. Ltd., Japan) were used. For inoculation, 1 ml of the water sample was dripped into the middle of the Compact Dry plate. The sample automatically and evenly diffused into the sheet and transformed the dried sheet into a gel within seconds. The cap was then placed on the plate, and the necessary information was recorded in a note. The plate was inverted with the cap and placed in an incubator at 37 ± 1 °C for 24 ± 2 hours. After incubation, the number of colored colonies under the plate was counted using white paper to facilitate counting. The last step was to determine the arithmetic mean of the two Petri dishes to ensure the reliability of the results.

The study also included an assessment of the effectiveness of water treatment methods in different parts of the city, which allowed us to identify problem areas and propose appropriate measures to improve the quality of the water supply.

In the city of Mykolaiv, reverse osmosis systems are used in automatic water filling points, which allows for high water quality standards. However, the

main problem is the need to regularly replace filters and membranes. Due to wear and tear and biofouling, the system may become less efficient, increasing the risk of water contamination. Therefore, it is important to regularly check the condition of the filters and replace the membranes on time to maintain the high quality of treated water.

Failure to comply with these requirements can lead to ineffective water treatment and an increased risk to consumer health.

Many apartments in Mykolaiv have additional water treatment systems, such as carbon filters, reverse osmosis systems, and ultraviolet disinfectors. These systems can significantly improve water quality, but their effectiveness depends on proper maintenance. At the same time, the lack of regular replacement of filters and membranes can lead to the accumulation of microorganisms, which negatively affects water quality. Therefore, users of these systems must be informed about the need for regular maintenance, replacement of filter elements and membranes, and maintenance of these installations.

The study also conducted a comparative analysis of the effectiveness of using nature coagulants, namely Morínga oleiféra seeds, and Micropure and Aquatabs for disinfecting natural water from the Bug Estuary.

Natural coagulant was prepared by seeds pounding in ovenware. Powdered seeds were mixed with ethanol. Then 5 ml of this extract was selected and put into Petrie dish, weighted (weight was fixed) and left to thorough drying. After drying dish was weighted again. Consequently, the quantity of Moringa oleifera seeds active component was determined it's 0.0042 g/ml. For the test's necessary concentrations (0.1 and 0.05 mg/cm^3) were selected and added into water solutions (river water). Next, the total microbial number was determined according to the method translated above and the processing efficiency was calculated.

It was decided to include Micropure and Aquatabs in the study because they can be used in emergencies and conditions of military conflict.

3. Results and discussion

The results of the bacteriological study of drinking water in Mykolaiv revealed bacterial contamination at certain sampling points. The analysis of water sources taken from different wells showed an excess of the permissible norm of *E. coli* at two sampling points – a water vending machine (point 10) and a dispensing point (point 8). The rest of the water samples met the regulatory requirements. Table shows the results of the bacteriological analysis, which reflect the presence of pathogens in the water samples, as well as their compliance with regulatory requirements.

Water quality indicators

Indicator	Permissible limits (DSanPin 2.4-171-10)	Unit	Tap water				Distribution point					Vending machine	
Sampling point			1	2	3	4	5	6	7	8	9	10	11
Total Microbial Count	< 50	CFU/cm^3	0	0	46	2	0	0	0	400	0	0	0
Total Coliforms	Absent (A)		A	A	A	A	A	A	A	A	A	A	A
E-coli			A	A	A	A	A	A	A	32	A	27	A
Enterococci			A	A	A	A	A	A	A	A	A	A	A

These data emphasize the need for urgent ongoing monitoring and control of drinking water quality at the point of delivery.

Thus, reverse osmosis is one of the most effective methods of water purification, which allows for reducing the content of pathogenic microorganisms, heavy metals, and other impurities. This is because reverse osmosis membranes are highly selective, allowing only water molecules to pass through, while all other impurities, including bacteria and viruses, remain on the membrane.

However, in addition to such high-tech methods, there are natural alternatives to water treatment. A comparative analysis of the use of plant coagulants and

preparations for disinfecting natural water has shown that the result of treatment with a natural coagulant, namely Moringa oleiféra seeds in various forms, is significantly different. The bacteriological study showed that the use of Moringa oleiféra seed powder led to continuous growth, but the seed extract demonstrated strong antiseptic properties. The study of the extract was conducted in two stages. The first results showed an efficacy of 99 %. However, subsequent studies showed lower results, which amounted to 50–70 % Figs. 4–5. This may be due to several factors. First, the analyses were conducted in different years, and the seeds were from different suppliers, which could affect the chemical composition of the components and the concentration of active ingredients. Thus, there is a need to continue researching the process of water treatment using plant coagulants to obtain the most effective coagulant concentration and stable, statistically reliable results.

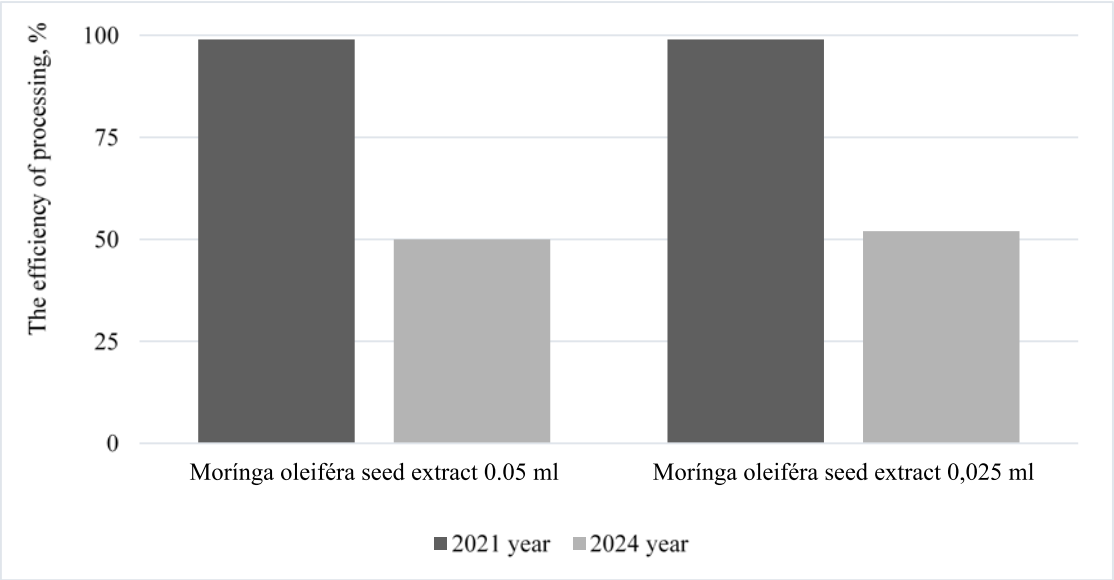

Fig. 4. Comparative analysis of the use of Moringa oleiféra seed extract in 2021 and 2024

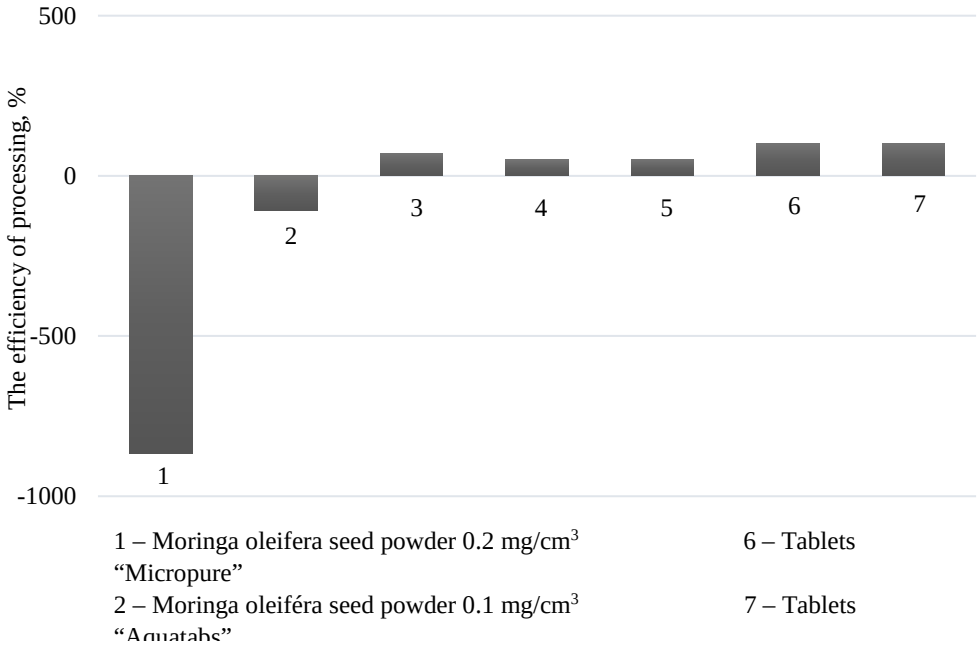

Fig. 5. Efficiency of using plant coagulants and preparations for disinfection of natural water

At the same time, “Micropure” and “Aquatabs” have been proven to be appropriate for use, as they have demonstrated 100 % efficiency in disinfecting natural water from pathogens.

In addition, the economic feasibility of using each method was evaluated. The cost of treating 1 liter of water with a coagulant made from pre-ordered Moringa oleifera seeds was 0.58 UAH, while “Micropure” and “Aquatabs” tablets provide disinfection at a cost of 1.13 UAH/l and 15.02 UAH/l, respecttively. Despite the need to order seeds, the coagulant can be produced at home, potentially making it an affordable alternative in regions with limited access to commercial disinfectants.

4. Conclusions

A study of the bacteriological quality of drinking water in Mykolaiv revealed significant contamination of some water samples taken from alternative water sources. Microorganisms whose concentration exceeds the permissible limits indicate possible secondary contamination of filters or components of the water supply system, which poses a risk to public health. This confirms the need for regular monitoring of water quality and enhanced water treatment measures, as water from alternative sources often does not meet the established sanitary and hygienic requirements before treatment.

Given the results, it is recommended that all water sources be monitored regularly. It is important to develop and implement additional measures to improve water treatment and pollution prevention systems.

The comparison of various methods of additional water disinfection that can be used in regions where active hostilities are taking place and there is no access to clean drinking water has shown that natural coagulants have significant antiseptic properties, but their stable effect requires a clear definition of the effective concentration. Thus, this requires further research to obtain sustainable results.

The prospects of this study are the development of effective methods of water treatment using plant coagulants, such as “Morínga oleifera”, which allows for to reduction of the use of chemicals in water treatment. The use of “Micropure” and “Aquatabs” has shown consistently high results, but strict adherence to dilution instructions is necessary to reduce their toxicity; this is not always possible in field or combat conditions. In contrast, plant-based coagulants are virtually non-toxic and pose minimal risk to human health, making them particularly valuable in emergencies. Improving the quality of drinking water is essential for maintaining public health, especially in modern emergencies such as military conflicts and operations in war zones.

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