New Materials, Compounds and Applications
Vol.8, No.3, 2024, pp.440-449
https://doi.org/10.62476/nmca83440

STUDY OF THE MICROBIOLOGICAL PARAMETERS OF IRRADIATED SAMPLES AT HOVSAN COASTAL AREA OF THE CASPIAN SEA

Narmin Mammadova Tahir[1,2*], **Muslum Gurbanov Ahmad**[2],
Ulviye Guliyeva Aydin[2]

[1]State Oil Company of Azerbaijan Republic (SOCAR), Baku, Azerbaijan
[2]Institute of Radiation Problems, Ministry of Science and Education, Baku, Azerbaijan

Abstract. Biological organisms can threaten water quality and cause waterborne diseases. Because testing for each pathogenic organism is very expensive and time consuming, coliform bacteria and E. coli from environmental and animal sources serve as good pathogen indicator organisms. This study focused on the evaluation of water quality in microbiologically purified waste from the tap and Hovsan coastal areas of the Caspian Sea via gamma-ray monitoring. Radiation technology allows for the possibility of combined water purification, including chemical and biological treatment; therefore, this technology is more suitable for water disposal. Before irradiation, the concentrations of total microbes (at 22°C and 37°C), E. coli, heterotrophic bacteria and total coliform bacteria were monitored. The samples were then irradiated with different doses of gamma radiation. In this context, the application of gamma radiation for the microbiological treatment of wastewater, domestic tap water and water from the Hovsan coastal area of the Caspian Sea, which can be applied to minimize the effect on the human body, was investigated.

Keywords: *E. coli, total microbial count, total coliform, fecal coliform, adsorbed dose, wastewater, N/D (not detected).*

Corresponding Author: *Narmin Mammadova, State Oil Company of Azerbaijan Republic (SOCAR); Institute of Radiation Problems, Ministry of Science and Education, Baku, Azerbaijan,*
e-mail: n.memmedova1987@gmail.com

Received: *23 July 2024;* ***Accepted:*** *16 October 2024;* ***Published:*** *10 December 2024.*

1. Introduction

The Caspian Sea is situated at the crossroads of Europe and Asia, spanning the coastlines of five countries: Azerbaijan, Iran, Kazakhstan, Russia and Turkmenistan. Approximately 60% of the wastewater that enters the Caspian Sea ultimately reaches the Volga River. Additionally, the Kura and Ural Rivers contribute to the contamination of the Caspian Sea. The municipal and industrial wastewater generated in Armenia and Georgia flows into the Caspian Sea through the Kura River. The Caspian Sea is subjected to a multitude of forms of pollution, including chemical, bacteriological and physical contamination. The underlying causes of this environmental issue can be attributed to a lack of compliance with environmental regulations governing the search, extraction and transportation of hydrocarbon resources in the sea. Additionally, the introduction of various pollutants, including industrial waste and river water and the discharge of

How to cite (APA):
Mammadova, N.T., Gurbanov, M.A., & Guliyeva, U.A. (2024). Study of the microbiological parameters of irradiated samples at Hovsan coastal area of the Caspian. *New Materials, Compounds and Applications, 8*(3), 440-449 https://doi.org/10.62476/nmca83440

domestic wastewater into the Caspian have contributed to the deterioration of the marine environment. The sea is currently devoid of any form of treatment.

When developing international standards, the following must be considered:

In developing international and domestic standards for water quality assessment, it is essential to control water quality by indicators. The absence of pathogenic microorganisms in water must be guaranteed. Norms must take into account regional conditions of formation, the composition of water supply sources, the methods applied for water treatment and transportation (Zagainova *et al*., 2020). Directives of European countries define fecal contamination, i.e., the presence of total coliforms and fecal coliforms, including Klebsiella and *E. coli*, as indicators of recent fecal contamination and as the main indicators for determining the quality of water for various purposes (http://www.doh.wa.gov/CommunityandEnvironment/DrinkingWater/Contaminants/Colifor).

Total coliform, fecal coliform and *E. coli* concentrations are all indicators of drinking water quality. The total coliform group is a large collection of different kinds of bacteria. Fecal coliforms are a type of total coliform that mostly exist in feces. *E. coli* is a subgroup of fecal coliform bacteria. When a water sample is sent to a laboratory for analysis, total coliform bacteria are tested. In the event that total coliform bacteria are present, the sample will also be tested for either fecal coliform or *E. coli*, depending on the laboratory testing method employed (https://doh.wa.gov/community-and-environment/drinking-water/contaminants/coliform; Darija *et al*., 2016).

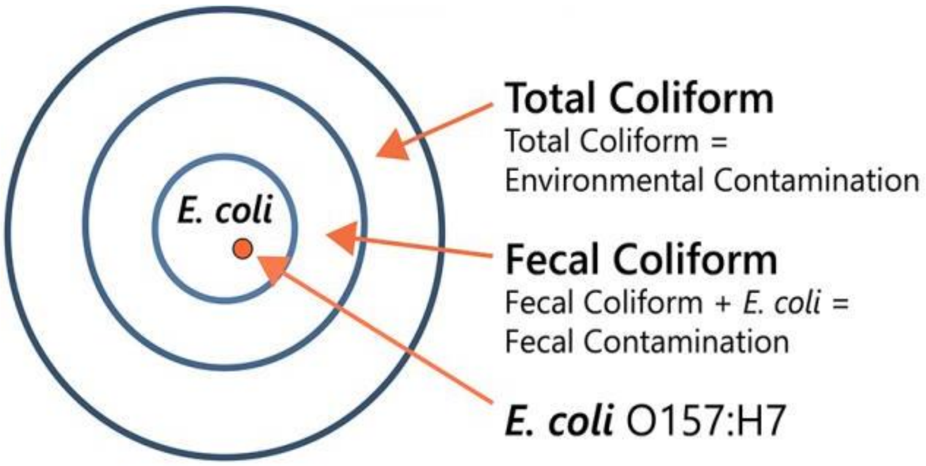

Figure 1. Microbiological indicators for water assessment
Source: Darija et al. (2016)

The presence of total coliform bacteria in drinking water may indicate natural environmental contamination or the potential introduction of pathogens. Fecal coliform bacteria, a subgroup of coliform bacteria, are found in the intestines and feces of humans and animals. The presence of these bacteria in water may signify fecal contamination and an increased risk of disease-causing pathogens. Among fecal coliform bacteria, *E. coli* is a subgroup of bacteria, most of which are harmless, but some strains may cause illness. The presence of *E. coli* in drinking water may indicate recent fecal contamination and a greater opportunity for pathogens. It is therefore important to identify and eliminate the source of these bacteria to safeguard public health (Darija *et al*., 2016).

The assessment of the microbiological quality of the Caspian Sea water showed that the level of bacteria in the sea water at different stations did not significantly change. However, the analysis of the samples showed that at some points, there was excess microbial contamination, which is dangerous for human health due to the possible presence of pathogenic microorganisms. Therefore, it is necessary to regularly monitor the level of microbial contamination in seawater and comply with safety and water quality standards to ensure public safety. The study also revealed a significant association between the fall and spring seasons and between the fall and summer seasons, although no differences were found between the summer and winter seasons. It is important to note that fecal coliform levels were greater in the fall than in the other seasons (Shahryari *et al*., 2020).

The Caspian Sea has been and continues to be polluted by domestic wastewater discharged from Border States, especially from the Volga (Russia) and Kura Rivers from the territory of the Republic of Azerbaijan, oil refineries located in the coastal zone and complex wastewater generated as a result of activities related to oil production at sea. These toxic components are released either from the high seas by wind or directly from residential areas to coastal areas. As a result, the waters of the Caspian coast are subject to biological pollution and there are serious risks to people and biodiversity when they are in contact with seawater. The use of radiation technologies allows for the simultaneous chemical and biological treatment of these waters and there are various technologies for neutralizing contaminated wastewater.

The main indicators of chemical water pollution are heavy metals, phenols, synthetic surfactants, oil, other carbon-organic compounds, nitrates and nitrites and bacteriological indicators (*Escherichia coli*, shingles) (Mammadova *et al*., 2023; Akhmedzadeh *et al*., 2019; Karimov *et al*., 2024; Mammadova *et al*., 2021; Idrisov *et al*., 2018). Notably, bacteriological testing of the water was carried out on *E. coli* bacteria. Every year before the beach season (May-September), the quality of the coastal water located in the Hovsan settlement in the Caspian Sea is assessed. The results of the monitoring carried out by the State Sanitary Control Authorities and representatives of the Caspian Administration of Complex Ecological Monitoring ETSN show that the quality of water on the beaches of the Caspian Sea has improved (ISO 9308-2, 2012; MUK 4.2.1884-0403, 2004; Sakovich & Bezmaternykh, 2005). The use of seawater has improved as a result of the significant investments made by the organisations involved in waste management in the Caspian Sea. To prevent pollution of the Caspian Sea from small local sources, the "System of Environmental Protection of the Caspian Sea" was created by installing 17 local modular treatment stations along a length of 86 km on the northern coast of the Absheron Peninsula. Because of the population density in the Absheron region, all discharges of untreated sewage into the Caspian Sea are planned to be stopped. To this end, water-polluting substances and objects have been identified in this area. Thus, if microorganisms were previously detected via seawater quality monitoring after 2010, the results of joint monitoring conducted by state sanitary control bodies, specialists of the Ministry of Ecology and Natural Resources and employees of local institutes and organizations showed positive dynamics in terms of water quality indicators**.**

Many traditional and modern methods are used to purify water; in many cases, chlorine is used as a disinfectant. This leads to water contamination with other organic pollutants and as a result, an additional purification process is needed. UV light sources are the least expensive, but they have limitations. Traditional methods should be used in conjunction with radiation technologies. Therefore, when purifying water with radiation technologies, chemical purification is complemented by microbiological purification. The experimental results demonstrate that radiation technology can be effectively used to treat large volumes of wastewater.

The application of radiation technologies for purifying contaminated water is complex and allows for biological purification, as well as for purifying water from toxic chemical compounds. The processes of chemical water purification using ionizing radiation have been widely studied (Mammadova *et al*., 2023; Akhmedzadeh *et al*., 2019; Mammadova *et al*., 2021). Given the promising results of radiation technologies for biological treatment, identifying the optimal parameters of the treatment process would be beneficial. In addition to the presence of toxic compounds, the Caspian Sea is affected by wastewater discharged into the Volga River and coastal settlements. Monitoring has revealed the presence of various types of bacteria and petroleum hydrocarbons (crude oil, oil refining waste, etc.) that form during oil production at sea.

E. coli is a type of bacteria that is widely used as a model microorganism in genetic and microbiological research, as well as in modern biotechnology (for example, for insulin, enzymes, vaccines and fuel production). The amount of intestinal bacteria in the water is indicated by the *E. coli* titer or the *E. coli* index. The coliform-titer is the small amount of the examined water, from which it is possible to find intestinal bacteria with the appropriate method. The lower the *E. coli* titer is, the more polluted the water is. The *E. coli* index is the number of intestinal bacteria in 1 liter of water. In clean water, the *E. coli* titer is at least 100 and the *E. coli* index is no more than 10.

The main objective of this study was to assess the impact of gamma radiation on the number of heterotrophic bacteria, *Escherichia coli* and total coliform bacteria, as well as on the number of fecal coliform bacteria, in tap water samples collected from the Hovsan coastal area of the Caspian Sea and on the biochemical treatment of the wastewater of the oil refinery named after H. Aliyev.

2. Materials and methods

2.1. Study area and sampling

Microbiological tests were conducted in the "Complex Research Laboratory" of the Ecology Department of the "Ecol Engineering Service Closed Joint Stock Company" by standard methods. The tests were carried out on heterotrophic bacteria, *Escherichia coli* and total coliform bacteria, as well as fecal coliform bacteria. The experiments were carried out with common microorganisms. The samples were taken from the Hovsan coastal area of the Caspian Sea and from domestic tap water. The methods for separating and evaluating coliform bacteria and *E. coli* were performed according to the "MUK 4.2.1884-0403 (2004) guidelines standard" (ISO 6222, 2018).

The SimPlate for HPC, US EPA method was used to carry out the analysis of heterotrophic microorganisms. The results were then viewed under a UV lamp and the illuminated cells were counted, recorded and calculated using a SimPlate table. We conducted an experiment in which we irradiated water samples from the Hovsan coastal area of the Caspian Sea with MRX-Gamma-25 Co60 isotope installation using different doses at a rate of 0.07 Gy/sec.

3. Results and discussion

3.1. Bacteriological analysis of initial samples taken from the Hovsan coastal area of the Caspian Sea

The results were calculated after a specific, step-by-step growth process. The results clearly showed that 96 CFU/ml of microorganisms were found in tap water and 600 CFU/ml in a water sample taken from the Hovsan coastal area of the Caspian Sea (Figure 2).

Figure 2. Determination of common microorganisms

Both samples were analyzed for heterotrophic microorganisms (Figure 3) and the results were clear: heterotrophic microorganisms were present in the tap water at 40 CFU/ml and 432 CFU/ml in the water sample taken from the Hovsan coastal area of the Caspian Sea. The limit for tap water is 0 CFU/ml, while for the Hovsan coastal area of the Caspian Sea, the limit is 100 CFU/ml (https://nortest.pro/stati/voda/obshhie-koliformnye-bakterii-v-vode.html).

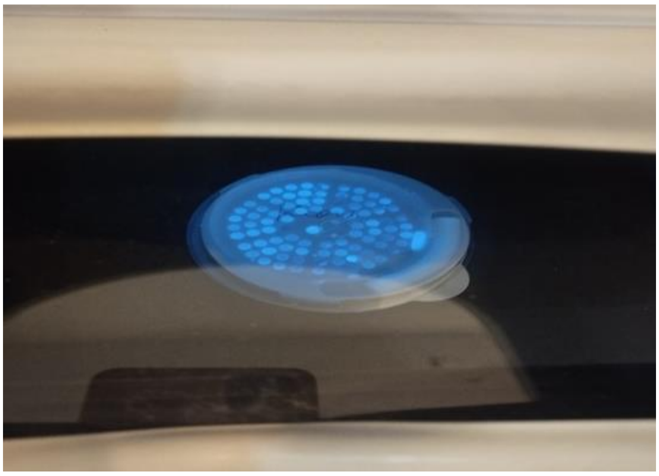

Figure 3. Heterotrophic microorganisms

The bacteria in the yellow unlit slots were total coliforms (35.0±0.5°C) and fecal coliforms (44.5±0.2°C) and the bacteria in the lit slots were *E.* coli. This result is in line with the Kolillera-18 table (g/100 ml). We can therefore conclude that there were no detectable levels of fecal coliform bacteria in the tap water. The total amount of coliform bacteria was determined to be 25.4 CFU/100 ml. A water sample taken from the Hovsan coastal area of the Caspian Sea revealed a total amount of coliform bacteria at 200.5 CFU/100 ml, *E. coli* at 165.2 CFU/100 ml and fecal coliform bacteria at 109.1 CFU/100 ml.

Moreover, the growth of bacteria on the membrane was clearly observed. Once the experiments were completed, a colony counter (Colony Counter SC6 plus, Bio-Cote, R 57000 1124) was used for counting and a colony was prepared. The results are as expected, matching those obtained with the Idexx device.

It is recommended that the allowable concentration limit of *E. coli* bacteria in household tap water be no greater than 0 cfu/100 ml. This also applies to coastal waters located in the Hovsan coastal area of the Caspian Sea. We would like to kindly inform you that the allowable limit concentration of fecal coliform bacteria for tap water is 0 cfu/100 ml. This applies to the Hovsan coastal area of the Caspian Sea. We also determined that the allowable concentration of total coliform bacteria was 0 cfu/100 ml for tap water and 0 cfu/100 ml for the Hovsan coastal area of the Caspian Sea (https://nortest.pro/stati/voda/obshhie-koliformnye-bakterii-v-vode.html).

Figure 4. Total coliform, *Escherichia coli* and Fecal coliform bacteria

3.2. Impact of ionizing radiation on the bacteriological parameters of samples taken from coastal waters located in the Hovsan coastal area of the Caspian Sea

The total microbial count (cfu/ml), *E. coli* concentration (cfu/100 ml), total coliform concentration (cfu/100 ml), fecal coliform concentration (cfu/100 ml) and heterotrophic bacteria concentration (cfu/ml) were determined as microbiological parameters. The results are presented in Table 1.

Table 1. The amount of heterotrophic bacteria changes depending on the absorbed dose

Bacteriological parameters	Unit	D, kGy			
		1,7	**4**	**5,7**	**17,2**
The total microbial count	cfu/ml	48	33	N/D	N/D
E. Coli	cfu/100 ml	N/D	N/D	N/D	N/D
Total coliform	cfu/100 ml	5.3	N/D	N/D	N/D
Fecal coliform	cfu/100 ml	N/D	N/D	N/D	N/D
Heterotrophic microorganisms	cfu/ml	19	N/D	N/D	N/D

Table 1 indicates that the total number of microbes may decrease by 31.2% at an absorption dose of 4 kGy. At a dose of 1.7 kGy, the total amount of coliform bacteria may decrease to zero. The samples were tested and the results indicated that there was no *E. coli* or fecal coliform bacteria present. It seems that the amount of heterotrophic bacteria may decrease from 19 cfu/ml to 0 cfu/ml at doses greater than 1.7 kGy.

3.3. Biochemical analysis of oil refinery wastewater

The methodology states that the total number of microbes in water samples is approximately the number of colony-forming units (CFUs) of microorganisms (CFUs) formed in 1 cm^3 of sample water. The initial samples were analyzed to determine the bacteriological parameters, total number of coliform bacteria and *E. coli*, total microbial count at 22°C and total microbial count at 37°C. The values are 1, 1, 2200 and 1835 CFU/ml, respectively (Akhmedzadeh *et al.*, 2019; https://www.fao.org/faolex/results/details/ru/c/LEX-FAOC183665/).

Table 2. The values of bacteriological parameters during irradiation at 5.7 and 17.2 kGy

Bacteriological parameters	Unit	Dose, kGy	
		5,7	17,2
The total number of coliform bacteria	CFU/100 ml	<1	<1
E-coli	CFU/100 ml	<1	<1
Total microbial count-22℃	CFU/1 ml	<1	<1
Total microbial count -37℃	CFU/ml	<1	<1

Table 2 indicates that microbes were present in the sample. Notably, the remaining bacteriological parameters were below the sensitivity limits of the methods used. The total number of coliform bacteria (CFU/100 ml), *E. coli* (CFU/100 ml) and total number of microbes (CFU/ml) during the irradiation of industrial wastewater at doses of 5.7 and 17.2 kGy were definitively less than unity.

3.4. Biochemical analysis of domestic wastewater

A series of experiments were conducted on the biochemical treatment of wastewater from an oil refinery named after H. Aliyev under the influence of γ-irradiation. The results are presented in the following tables (Tables 3 and 4).

Table 3. Coliform bacteria in domestic wastewater, *E. coli*, total bacteria count (22°C), total bacteria count (37°C)

Bacteriological parameters	Unit	D=0
The total number of coliform bacteria	CFU/100 ml	<1
E-coli	CFU/100 ml	<1
Total microbial count(22 °C)	CFU/1 ml	800
Total microbial count (37°C)	CFU/ml	633

Table 4 shows the values of the bacteriological parameters at 5.7 and 17.2 kGy.

Table 4. Values of the bacteriological parameters during irradiation at 5.7 and 17.2 kGy

Bacteriological parameters	Unit	Adsorbed Dose, kGy	
		5,7	17,2
The total number of coliform bacteria	CFU/100 ml	<1	<1
E-coli	CFU/100 ml	<1	<1
Total microbial count (22^0C)	CFU/1 ml	9	<1
Total microbial count (37^0C)	CFU/ml	<1	<1

3.5. Biochemical analysis of mixed wastewater

The biochemical treatment of mixed waters from the oil refinery named after H. Aliyev was conducted under the influence of γ-irradiation. Table 5 shows the total number of *E. coli*, the total number of microorganisms at temperatures less than 22°C and the total number of microorganisms at temperatures less than 37°C in mixed wastewater samples.

Table 5. Coliform bacteria in domestic wastewater, *E. coli*, total microbial count at 22°C, total microbial count at 37°C in mixed waters from the oil refinery named after H. Aliyev

Bacteriological parameters	Unit	D=0
The total number of coliform bacteria	CFU/100 ml	<1
E-coli	CFU/100 ml	<1
Total microbial count (22^0C)	CFU/1 ml	2000
Total microbial count (37^0C)	CFU/ml	1670
****Coliform bacteria in domestic wastewater, E-coli, total microbial count-22°C, total microbial count (37°C)*		

Biochemical analyses of industrial, domestic and mixed wastewater solutions from the H. Aliyev Oil Refinery revealed that the total coliform bacteria, *E. coli*, total microbial number (220°C) and total microbial number (370°C) were lower. The initial composition of the domestic wastewater showed that the total microbial number (22°C) and total microbial number (37°C) were 800 and 633 CFU/1 ml, respectively. However, when the exposure time exceeded 5.7 kGy, the total microbial number decreased to 9. The total microbial number (22°C) and total microbial number (37°C) in the mixed wastewater decreased from 2000 to 1670.

4. Conclusions

The initial tests indicated that the amount of microorganisms was 0 CFU/100 ml in experiments conducted on tap water taken from seawater and domestic tap water located in the Hovsan coastal area of the Caspian Sea. This was also observed through the growth of bacteria on the membrane. It appears that the amount of heterotrophic bacteria and microorganisms present in samples taken from seawater and domestic tap water located in the Hovsan coastal area of the Caspian Sea may exceed the norm. Even at small doses (1.7 kGy), destruction of microorganisms in water under the influence of gamma-ionizing rays is possible.

Acknowledgments

The authors would like to thank the employees of the H. Aliyev Oil Refinery for their assistance with the sampling of water samples. We would also like to thank EKOL Engineering Services, JSC, Department of Ecology, Complex Research Laboratory and Institute of Radiation Problems of the Ministry of Science and Education.

References

Akhmedzadeh, L.V., Gulieva, U.A., Mamedova, N.T., Guseynova, M.A., Panakhova, A.A. & Gurbanov, M.A. (2019). Radiation-Assisted waste water treatment using nanocatalyst and Fenton's reagent. *Chemical Safety*, 3, 151-159. https://doi.org/10.25514/CHS.2019.2.16010 (In Russian).

Coliform Bacteria in Drinking Water. https://doh.wa.gov/community-and-environment/drinking-water/contaminants/coliform

Common coliform bacteria in water. (2023). https://nortest.pro/stati/voda/obshhie-koliformnye-bakterii-v-vode.html

Darija, V.L., Slaven, J., Arijana, C., Marin, G., Marko, B. & Drazen, L. (2016). Escherichia coli in marine water: Comparison of methods for the assessment of recreational bathing water samples. *Marine Pollution Bulletin*, *113*(1–2), 438-443.

Idrisov, T.S., Gurbanov, M.A. & Gulieva, U.A. (2018). *IV International Conference Actual Scientific and Technical Issues of Chemical Safety*, 34. Moscow. (In Russian).

ISO 6222 (2018). Water quality. After the bacteria were sown in nutrient agar media, the culturable microorganisms were counted. The Moscow Standard in 2020, 10 p.m.

ISO 9308-2 (2012). Water quality - Enumeration of Escherichia coli and coliform bacteria. Part 2: Most probable number method.

Karimov, S., Abdullayev, E., Millet, M. & Gurbanov, M. (2024). Radiolytic degradation of 1, 2, 4-trichlorobenzene (TCB) in some organic solvents by gamma rays: The kinetic properties of complete dechlorination of TCB and its pathway. *Heliyon*, *10*(10). https://doi.org/10.1016/j.heliyon.2024.e31547

Mamedova, N.T., Kulieva, U.A. & Kurbanov, M.A. (2021). Radiation-chemical decomposition of aqueous solution of sodium lauryl sulfate. *Young Researcher Scientific and Practical Journal*, 43-48.

Mammadova, N., Gulieva, U. & Gurbanov, M. (2023). Radiolysis of industrial wastewater in the presence of nano-γ-Al_2O_3 under the influence of γ-radiation. *Proceedings of Azerbaijan High Technical Educational Institutions*, *33*(4), 82-89. https://doi.org/10.36962/PAHTEI33102023-82

MUK 4.2.1884-0403 (2004). Sanitary-microbiological and sanitary parasitological analysis of water from surface water bodies. https://www.russiangost.com/p-159926-muk-421884-04.aspx (In Russian).

Protecting and use of border water flows and international goals of the Convention on Water and Health of the Protocol of the Republic of Azerbaijan.pdf.

Sakovich G.S., Bezmaternykh M.A. (2005). *Physiology and Quantification of Microorganisms*. Yekaterinburg: GOU VPO UGTU-UPI, 28. (In Russian).

Shahryari, A., Safari, H. (2020). Assessment of the microbiological quality of Caspian seawater and the role of physicochemical factors on microbial load. *Journal of Environmental Health and Sustainable Development, 5*(1), 962-970. https://doi.org/10.18502/jehsd.v5i1.2478

State sanitary rules and regulations Hygienic requirements for drinking water intended for human consumption. (2010). Ukraine. https://www.fao.org/faolex/results/details/ru/c/LEX-FAOC183665/

Washington State Department of Healt. Drinking Water Contaminants. http://www.doh.wa.gov/CommunityandEnvironment/DrinkingWater/Contaminants/Coliform

Zagainova, A.V., Trukhina, G.M., Rakhmanin, Y.A., Artemova, T.Z. & Sukhina, M.A. (2020). The rationale for introducing the indices Generalized coliform bacteria and Escherichia coli into the scheme of sanitary and microbiological control of water quality as indices of fecal contamination. *Hygiene and Sanitation*, *99*(12), 1353-1359. https://doi.org/10.47470/0016-9900-2020-99-12-1353-1359