

Microbiology of Contaminated Water

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Received October 11, 2021; revised and accepted November 1, 2021

Abstract: Microbiological methods for identifying the status of water in modified anthropogenic zones are the most accurate techniques that reflect the actual condition of water bodies. This work aims to perform a comparative analysis of hydrochemical and microbiological indicators in 20 small rivers of the Surgutsky and Oktyabrsky districts of Khanty-Mansi Autonomous Okrug of the Russian Federation. The research was conducted in 2018-2019 in Surgutsky and Oktyabrsky districts using water samples collected in spring, summer and autumn from 20 small rivers. The neutral and mildly alkaline values of pH were the highest in summer (7.6). Maximum ammonium ion concentrations of up to 0.5 mg per 1 dm³ were registered during spring when the snow was melting. No differences in total microbial accounts between 2018 and 2019 were noted in the Surgutsky district ($p \geq 0.05$). However, there were differences between rivers. Thus, rivers No. 4, 5, and 7-10 accounted for the minimum in both years (microbial count is 10^1 - 10^2), while rivers No. 1-3 had 10-12 times higher microbial count ($p \leq 0.001$, 10^3). For river No. 6, the values were 4 times higher compared with rivers No. 1-3 ($p \leq 0.01$, 10^4) and 30-40 times higher compared with rivers No. 4, 5, and 7-10 ($p \leq 0.0001$, 10^{1-2} versus 10^4). The pollution in 2018-2019 can be considered constant. Rivers No. 18 and 19 in the Oktyabrsky region were the most contaminated, with microbial accounts 5 to 10 times higher than others ($p \leq 0.001$, 10^{2-5} versus 10^1). River No. 12, with a microbial count of 2-5 thousand per 1 ml in 2018, reached 42-45 thousand per 1 ml in 2019 ($p \leq 0.001$), a value higher than in the constantly polluted rivers No. 18-19 ($p \leq 0.05$ with 2019). Microflora was consistent during the two-year study. It is explained by the fact that the number of bacteria is determined by water temperature, indicators of dissolved O₂ concentration, and indicators of organic compound concentration. The cluster analysis revealed 16 genera of bacteria and 3 mold fungi in the waters of 20 rivers studied. The Oktyabrsky district has the most significant number of very polluted rivers, whereas there are a lot of relatively clean rivers. The Surgutsky district is characterized by very clean rivers, along with moderately polluted rivers. Compared with the Oktyabrsky district, the rivers in the Surgutsky district are cleaner in microbiology because of lower anthropogenic pressure. Bacterial contamination can be reduced if the release of organic matter to waste water is lower, thereby substantially reducing the role of ammonifying bacteria in the microbiome.

Key words: Bacterial pollution, microflora, small rivers, total microbial count.

Introduction

One of the most important water quality determinants is microflora, particularly pathogenic microorganisms (Dauda et al., 2019). Furthermore, water quality is not only essential for human survival and various needs but also important to safeguard the integrity and functioning of various ecosystems (Cai et al., 2013, Castine et al., 2013).

Surface water pollution can generally be divided into two directions. The first includes naturally occurring pollutants that enter the water from rock and soil following the minerals' leaching (Zhong et al., 2011). Leaching usually occurs through meltwater and rainwater, which may contain essential impurities of chemically active substances (in industrial areas), as well as suspended solids of microorganisms, including pathogens. Anthropogenic sources include effluents from industrial plants, factories, domestic waste, and landfills and effluents from agricultural complexes like field fertilisers, insecticides, and herbicides (Abia et al., 2016; Chigor et al., 2013). Another important source of microbes is dust, which penetrates the water, as well as air borne micro-particles of internal combustion products, which can adsorb microbes on their surfaces. Agricultural waste (faeces and animal residues) and synthetic detergents are an important source of rapid microbial growth (Kebede et al., 2020).

The chemical and microbiological composition of city waters results from interactions between natural and anthropogenic sources of pollution (Bo et al., 2017).

Contamination of small rivers is of particular importance in the microbiological characterisation of water. There are few works in this field (Koffi et al., 2011; Lear et al., 2011), and some characteristics (the ratio of trophic bacteria groups) should be clarified, which can help in determining the relevance of the present work.

Some research works (Ganetal., 2020; Kebede et al., 2020) revealed that water pollution by organic and anthropogenic substances dramatically increases the number of microbes in it. A cumulative effect is manifested in the mutation of some microorganisms, following which non-pathogenic forms of microbes become pathogenic. The introduction of synthetic detergents into the water in large amounts activates pathogenic viruses (Defoirdt et al., 2011). In this regard, the detection of numerous microorganisms using organic substances should be considered an indicator of an unfavourable geochemical environment (Li et al., 2020). Among pathogens, consideration should be

given to *E. coli* bacteria, which can cause epidemics among humans. Such situations often occur in South Asia (Bangladesh, parts of India) and Africa, leading to outbreaks of diseases such as dysentery (Kebede et al., 2020).

This work analyses water samples from 20 small rivers in Khanty-Mansi Autonomous okrug, the Russian Federation. The authors assume that microflora's qualitative and quantitative indicators are determined by some abiotic factors: water temperature and the amount of oxygen and organic matter dissolved in it. This work aimed to carry out a comparative analysis of the hydrochemical and microbiological indicators in the waterways studied. The study's objectives were to (a) collect data on the microbiological and hydrochemical characteristics of water samples and (b) study the impact of human factors on water quality indicators.

Material and Methods

Region of Study

The survey was carried out in 2018-2019 in the Khanty-Mansi Autonomous Okrug-Yugra, the Russian Federation (Figure 1A). Twenty small rivers were chosen for study purposes (Table 1). For convenience,



Figure 1: Geographical coordinates of sampling points.

Table 1: Geographical coordinates of sampling points (20 small rivers)

Small river number	The geographical location of the sampling station	
	North latitude (degrees. minutes. seconds.)	East longitude (degrees. minutes. seconds.)
P1	61.40.31	72.48.45
P2	61.26.17	72.42.02
P3	61.37.49	72.51.50
P4	61.25.01	72.45.19
P5	61.19.35	72.27.19
P6	61.58.03	72.36.19
P7	61.55.42	72.46.32
P8	61.26.24	72.15.22
P9	61.28.50	72.10.28
P10	61.28.37	72.07.59
P11	61.04.46	67.19.34
P12	62.10.45	67.10.31
P13	62.05.57	67.30.08
P14	62.04.00	67.30.35
P15	62.22.24	67.02.28
P16	62.19.40	67.11.44
P17	62.24.51	67.13.27
P18	62.06.10	67.23.37
P19	62.05.57	67.30.08
P20	62.20.03	67.12.07

they are listed in a numbered order without local geographical names.

Points 1-10 correspond to the Surgutsky District and points 11-20 correspond to the Oktyabrsky District (Figure 1B).

Research Methods

Sampling was performed three times: spring, summer, and autumn. Samples were collected from the top water layer, where a sterile container was lowered with a lid that could be opened with a lanyard. As it was lowered to the required depth, the lid was opened, allowing the tank to be filled with water. The following indicators were included water, pH, ammonium ions, and acute toxicity. The latter was measured using the biotesting method, which involves monitoring the effect of toxins on the survival of daphnids. If more than half of the organisms died, the water sample was considered highly toxic. A depth sampling method was used to analyze the water microflora. The sowing was carried out as

follows: Decimal dilutions of water in 10, 100, 1000, 10000 times (1st, 2nd, 3rd, and 4th dilutions) were prepared from selected water samples. Totally 4 test tubes (9 cm³) with sterile water were used. For the 1st dilution, 1 cm³ of the test water with a sterile syringe was put into one of the tubes with sterile water. Each subsequent dilution was prepared with a new sterile syringe by adding 1 cm³ of the previous dilution in sterile water. All works were performed in the area of a spirit flame.

To determine the number of saprophytic bacteria (total number of bacteria) in 1 cm³ of water, 1 cm³ of the appropriate dilution was placed into three prepared sterile Petri dishes with a sterile syringe and the numbers of teams and dilutions of water (1st, 2nd, 3rd) were indicated. Melted and cooled to 45-50°C meat-peptone-agar (MPA) was poured in cups. Petri dishes remained on the table till the medium solidification. Afterward, the dishes were placed in a thermostat at 37°C for cultivating. The number of *E. coli* bacteria detected in water is an indicator of fecal contamination, which can also be used to determine the level of pollution.

Dishes with some colonies between 30 and 300 are selected from the Petri dishes placed in the thermostat. The dishes are placed in a thermostat for 16 to 18 hours at 37°C. The *E. coli* bacteria grown on Endoagar are visible as dark red colonies with or without metallic sheen, suggesting that colonies lacking metallic sheen are lactose positive. The classical Gram stain method used the microscopic staining of lactose-positive colonies to finally decide whether the dark red colonies belong to the common coliform bacteria. The presence of pink bacilli form cells (Gram-negative) in the stained smears confirms the presence of *E. coli* bacteria in the analysed volume.

A scale of the heterotrophic bacteria number (colony-forming units) was used to evaluate the status of streams in terms of sanitation. According to this scale, 10¹ is a very clean water body, 10¹ to 10² is a clean water body (river), 10² to 10³ is a river with a moderate level of bacterial pollution, 10³ to 10⁴ is a water body polluted with heterotrophic bacteria, 10⁴ to 10⁵ is a dirty water body, and 10⁵ is a very dirty waterbody.

The qualifiers of Bergey were applied to establish the species affiliation of the dominant representatives in terms of the bacteria and fungi abundance forming the mycelium (Hoult et al., 1997).

Statistical Analysis

Statistical analysis was performed using Statistica v. 10 software (Statsoft Inc., USA). The arithmetic mean

and the standard error of the mean were calculated. Ward's cluster analysis methodology was used to aggregate similar and different sampling points. Clusters were chosen based on group bonding distances. The significance of the differences between the groups (samples at different times of the year) was calculated using the Student's *t*-test. The difference was assessed as significant at $p \leq 0.05$.

Results

The maximum pH value (7.6) was recorded during the summer season. Anthropogenic factors accounted for changes in the acidity of rivers. These are manifested by a shift in predominant pH values from slightly acid to slightly alkaline. Due to the spring snowmelt, a high concentration of ammonium ions was also observed (Figure 2).

The peak concentration of ammonium ions (0.5 mg/l dm^3) was seen during the spring season. It can be attributed to intensive snow melting. Ammonium ions have been elevated in surface runoff that enters rivers during flooding. In addition, such high values can be explained by high concentrations of organic matter in the water, which bound dissolved oxygen by oxidation and limited oxygen's ability to participate in nitrification processes leading to the accumulation of reduced nitrogen compounds. Toxicity in hydrobiology refers to the ability of water to cause adverse or pathological effects on river-dwelling animals and plants (hydrobionts), which can also lead to their death. Figure 3 shows the seasonal distribution of toxic water samples from the 20 streams examined.

There was no consistent change in toxicity indicators concerning seasonal dynamics over the 2 years of observation. It can be explained by the fact that each

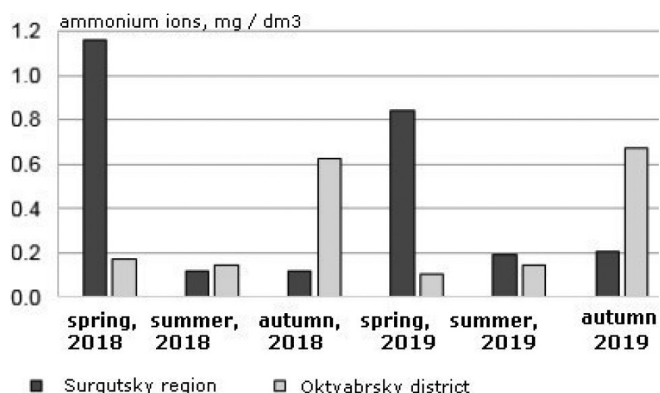


Figure 2: Ammonium ion concentrations between 2018 and 2019 in the regions surveyed.

river has an individual load at various times of the year. In 2018, the highest percentage of toxic samples was recorded in autumn (2 times higher than in spring, $p \leq 0.01$). While in 2019, it was observed in summer (2.2 times higher than in autumn, $p \leq 0.01$). The percentage of toxic samples differed by a factor of 0.5 between summers and autumns of 2018 ($p \leq 0.05$). The same is true for the period between spring and autumn of 2019 ($p \leq 0.05$). Toxicity values in the summer of 2018 were slightly higher than in the autumn of 2019, but no significant differences were observed ($p \geq 0.05$).

Furthermore, data for 2018-19 were analysed using the clustering method (Figure 4).

Based on the analytical results, three clusters can be distinguished. The first cluster consists of water bodies with pure water (points 4, 5, 7-10, 14, and 17), with a microbial count of fewer than 1.000 colony-forming units. The second cluster composes clean water bodies (points 11, 13, 15, 16, and 20). In this case,

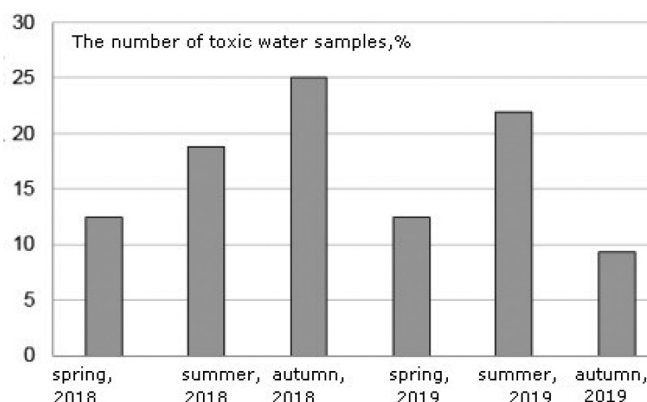


Figure 3: The percentage of toxic water samples obtained from 20 small rivers during spring and autumn seasons (2018-2019).

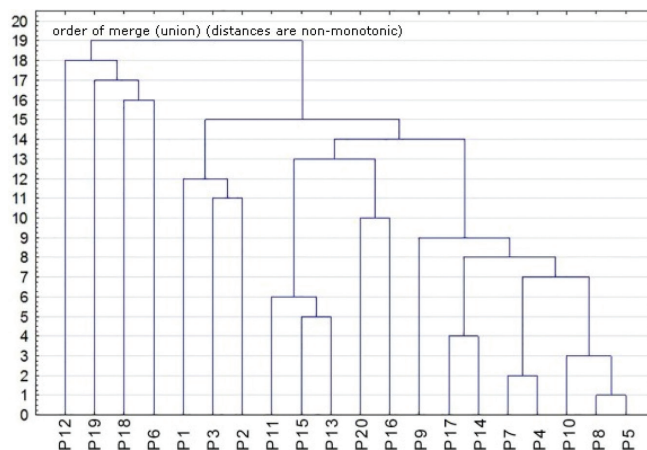


Figure 4: Cluster analysis of samples processed according to microbiological endpoints in 2018-2019.

the microbial count was not greater than 5000 colony-forming units per 1 ml. The third cluster consists of contaminated water bodies with more than 20.000 units per 1 ml (points 6, 12, 18, and 19).

The analysis of microflora in the aqueous samples showed consistency of microorganism abundance dynamics over the study period (Figure 5).

No significant differences were found in the value of total microbial count between years in the Surgutsky district ($p \geq 0.05$). Meantime, there are apparent differences between points. For instance, points 4, 5, and 7-10 exhibited minimum values during both years, while points 1-3 had a total microbial count 10-12 times greater ($p \leq 0.001$). Point 6 stands out. It is the most microbially contaminated water body, with a microbial count 4 times higher than in points 1-3 ($p \leq 0.01$) and 30-40 times higher than in points 4, 5, and 7-10 ($p \leq 0.0001$). It follows that in some waterways, the level of microbiological contamination may vary significantly from year to year due to emissions.

Cluster analysis of the microflora in studied rivers showed the prevalence of organo-heterotrophic microorganisms (Figure 6).

The presence of many saprophytic bacteria suggests an active self-purification process in rivers (Chislock et al., 2013). Moreover, these bacteria indicate a large amount of organic matter (Bo et al., 2017). River microflora is not highly consistent due to the impact of various external factors. However, these are permanently present in the samples among the microorganisms and do not require a high concentration of organic compounds (Chigor et al., 2013). According to the isolate analysis, the microflora of the water samples is dominated by bacteria. Among fungi, mold

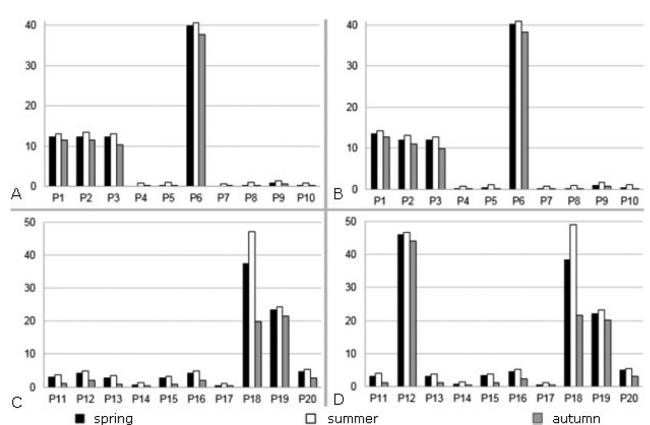


Figure 5: Total microbial count (thousands per 1 ml) during the spring and autumn seasons of 2018-2019 in Surgutsky (A - 2018, B - 2019) and Oktyabrsky districts.

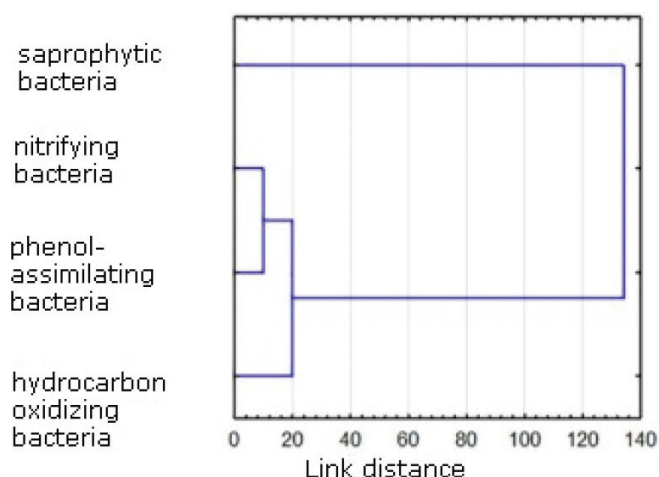


Figure 6: Results of cluster analysis of small river microflora from the Surgutsky and Oktyabrsky districts.

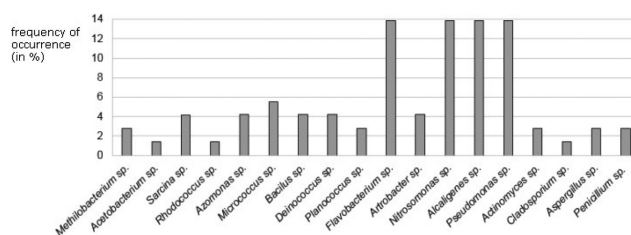


Figure 7: Occurrence rate (%) of bacteria and mold fungi in samples throughout 2 years of study.

fungi from genera *Cladosporium*, *Aspergillus*, and *Penicillium* were found. Figure 7 shows the frequency of microorganisms according to the summation results of two years of the study.

Ammonifiers belonging to the genus *Pseudomonas* were found most frequently in water samples. Besides them, autotrophs and heterotrophs belonging to nitrifying bacteria *Nitrosomonas* sp. were dominant in the samples. These bacteria perform nitrification processes in the water bodies under investigation. Depending on their presence, it can be assumed that semi-decomposed residues of organic compounds were involved in the ammonification process in water (Koffi et al., 2011). Among the native microflora, bacteria such as *Flavobacterium*, which live on the fish body's surface, have been discovered. Furthermore, bacteria of the genus *Alcaligenes* have been discovered, which are indicators of feces in the water.

For example, among the 20 streams studied, several groups with different pollution levels, both chemical (ammonium ions) and microbiological, were identified.

Discussion

Based on results for the composition of microorganism species, it was found that there was consistency in species composition and abundance for two years. That is explained by the fact that the number of bacteria is determined by a number of abiotic factors, such as water temperature, indicators of dissolved O₂ concentration in water, and concentration of organic compounds. Cluster analysis showed that organic heterotrophs, in particular 16 bacterial genera and 3 mold genera, predominate and dominate in the waters of 20 rivers studied. These results are similar to those obtained by other authors. However, it was established that fungi could also make up a significant part of the microflora, subject to organo-genic pollution.

Microorganisms are reliable indicators of changes within an ecosystem, including an aquatic ecosystem (Klose et al., 2012). This statement is confirmed by numerous studies and supported by this work. Where chemical analysis methods are inadequate, microbiological studies provide accurate information. Therefore, even with minor changes in chemical concentration, the microflora can be changed by quantitative indicators. For instance, saprophytic bacteria can be detected even when a contaminated water sample is diluted tens of thousands or even hundreds of thousands of times (Lainé et al., 2014).

Furthermore, studies are being carried out to investigate the corrosion of metal pipes and equipment resulting from bacterial activity (Paule-Mercado et al., 2016). High indicators of bacterial concentration per unit volume have been shown to occur when water contamination by anthropogenic and domestic organic compounds intensifies (Rajiv et al., 2012). Bacterial mutations are also known to occur as a consequence of cumulative effect, after which those of their non-pathogenic forms become pathogenic (Zhao et al., 2014). Consequently, if high concentrations of microorganisms are detected, attention must be paid to the fact that the aquatic environment is in a critical state (Jin et al., 2004). This research also confirms that.

In this study, quantitative indicators characterising different groups of bacteria are described. In the future, the studies of large rivers, streams, and water bodies with flowing and non-flowing water from the same region and neighboring regions of the Russian Federation seem relevant to perform a comparative analysis of the sanitary water condition in these water bodies and estimate their microbiological characteristics.

Conclusions

It has been established that variations in hydrochemical indicators across seasons can be associated with different phases of the water regime (snow melting in spring). At the same time, a microbiological study has shown that rivers in both zones can be divided into four cascading systems. The first includes rivers with a total microbial count not exceeding 1.000 colony-forming units per 1 ml of the water sample. The group consists of 8 rivers, 6 of which are located in the Surgutsky district and 2 in the Oktyabrsky district. The group comprises 8 rivers: 6 in the Surgutsky district and 2 in the Oktyabrsky district. Moderately contaminated rivers with microbial counts of up to 20.000 units include 3 rivers located in the Surgutsky district. Finally, the very polluted rivers with a microbial number exceeding 20.000 units are 4 rivers, 3 of which flow in the Oktyabrsky district. Thus, the Oktyabrsky district has the greatest number of extremely dirty rivers, while there are a lot of relatively clean rivers. The Surgutsky district has very clean rivers, but they also have moderately polluted rivers. Generally, the Surgutsky district shows cleaner microbiology in rivers than the Oktyabrsky district due to a lower anthropogenic burden.

Conflict of Interest

The authors declare that they have no conflict of interest.

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