RESEARCH PAPER

# HYDROCHEMICAL AND BACTERIAL PROPERTIES OF WATER BODIES OF THE EAST EUROPEAN PLAIN DURING LOW WATER PERIOD

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**ABSTRACT.** This paper is devoted to the study of the chemical and biological properties of river waters and the relationship between them. We examined the hydrochemical and bacterial properties of surface water in 3 waterbodies: the Mezha River, a pond in Zapovedny village (Central Forest Nature Reserve, Tver Oblast) and the lower reaches of the Don River (Rostov Oblast). The biodiversity of bacteria was determined based on their growth on dissolved organic matter (DOM). Among bacterioplankton capable of growing on DOM as the only source of carbon, the predominant species in the Don River were *Pseudomonas* and *Deinococcus*, in the Mezha River – *Pseudomonas* and *Janthinobacterium*, in the pond – *Arcicella*. In terms of sanitary and microbiological indicators, none of the waterbodies complied with the Sanitary Rules and Regulations 1.2.3685-21 for surface waters. The content of most of the studied elements and heterotrophic bacteria in stagnant waterbodies was lower than in flowing streams. The concentration and activity of heterotrophic bacteria in the studied waters correlated positively with the content of biophilic elements in them and negatively with the absence of a current. We showed that there is a strong correlation between bacterial and chemical indicators due to common factors: eutrophication, features of the physical and geographical conditions of the territory, and the presence of a current or animal waste products.

**KEYWORDS:** surface waters, heterotrophic bacteria, DOM, elements, biodiversity

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## INTRODUCTION

There are many papers devoted to the study of sanitary and microbiological indicators (Dolgonosov et al. 2006; Sorokovikova et al. 2013; Lartseva et al. 2015; Obukhova et al. 2017) and geochemical characteristics (Lobbes et al. 2000; Grishantseva et al. 2020; Drozdova et al. 2021; Pakusina et al. 2022) of lowland rivers in Russia. However, only a few of them investigate the relationship between the abundance and properties of microorganisms, their activity and the chemical composition of water (Judd et al. 2006; Tanentzap et al. 2019). Moreover, despite the constant monitoring of sanitary conditions in waterbodies, there is little data on the autochthonous microbiome and its functioning, especially for the rivers of the Russian Federation (Belkova et al. 2003; Kopylov and Kosolapov 2011). At the same time, the role of heterotrophic bacterioplankton in the biogeochemical cycle is extremely high (Fasching et al., 2014; Amado and Roland 2017; Cai et al. 2021; Yang et al. 2021), since it is the most important trophic link in the nutrient network of waterbodies. Previously, Azam et al. (1983) formulated the concept of a "microbial loop", according to which most of the matter created by phytoplankton in the form of intravital secretions and mortmass represented by dissolved organic matter (DOM) serves as food for planktonic heterotrophic bacteria which then become food for larger organisms. The "microbial loop" is an alternative to the well-known phytoplankton-zooplankton-fish pathway for the transfer of matter and energy through trophic networks. The DOM of natural waters differs significantly: in the southern rivers, autochthonous DOM prevails due to their high production, while in the northern rivers, allochthonous DOM originating from the watershed is predominant. In this study, we examined surface water samples from two natural zones: the large steppe river Don (in the area of the Tsimlyansk Reservoir)

and the Mezha River, particularly a section of the river in the taiga zone, a dam on the Mezha River and a small pond in the Mezha River basin (Tver Oblast). Our hypothesis was that the chemical and bacterial properties of surface waters are closely interrelated and this relationship determines the differences in the functioning of waterbodies as aquatic ecosystems. The aim of this study was to identify the relationship between the concentration and activity of various groups of heterotrophic bacteria and the chemical composition of stagnant (river dam, pond) and flowing (rivers) waters of the taiga and steppe zones of the East European Plain, as well as to identify bacterial dominants capable of using DOM as the only source of carbon.

## MATERIALS AND METHODS

*Water sampling* was carried out in mid-August of 2021 at four locations: the Don River, the Mezha River (2 points) and a pond in Zapovedny village (Fig. 1). The characteristics of the studied waterbodies are given in Table 1. For microbiological analyses, water samples were collected into sterile 15 mL flacons and transported in a thermal bag; for chemical analyses, water was collected into 5 L plastic bottles. The sampling was carried out in triplicate and the samples were delivered to the laboratory within 24 h.

***Isolation of culturable bacteria.*** Isolation of bacteria was carried out using the dilution method onto the following agar media: nutrient agar (Himedia, M001), MacConkey agar with crystal violet and 0.15% bile salts (Himedia, M081), bile esculin agar with sodium azide (Himedia, M493), Ashby agar with mannitol (Himedia, M706), medium for the isolation of iron bacteria (Himedia, M622), peptone-yeast extract-glucose (PYG) agar (10 g/L peptone, 5 g/L yeast extract, 2 g/L glucose, 15 g/L agar), copiotrophic (oligotrophic) agar (Semenov et al. 1991) (2.5 g (25 mg/L) glucose, 0.2 g/L casein hydrolysate, 0.5 g/L $MgSO_4 \times 7H_2O$, 0.5 g/L $KH_2PO_4$, 0.06 g/L $Ca(NO_3)_2$), as well as agar with the DOM of a waterbody as the only source of carbon. For the latter medium, we used sterile water from a waterbody (filtered through a 0.2 μm filter) mixed with the mineral base of the 2x Czapek-Dox medium. Incubation on PYG, copiotrophic/oligotrophic agar, Ashby medium, and agar for the isolation of iron bacteria was carried out at 28 °C. Colony counting was conducted after 5 days of incubation. Incubation on MacConkey agar and bile esculin agar was conducted at 37 °C, colony counting was conducted after 1.5 days. Incubation on nutrient agar was conducted at 22 °C and 37 °C and colony counting was conducted after 5 and 1.5 days, respectively. All experiments were performed in triplicate.

***Identification of bacteria:*** Bacterial strains were identified based on the 16S rRNA gene sequences as described previously in Belov et al. (2018). The resulting sequences were checked for chimeras using the DECIPHER 2.20.0 program (Wright et al. 2012) and deposited into GenBank under accession numbers OM763864-OM763869.

***Comprehensive structural and functional approach.*** To study the bacterial complex of the waterbodies, we used a comprehensive structural and functional approach described by Yakushev (2015). The approach is based on the analysis of the total concentration of bacteria cells that have grown on a selection of liquid nutrient media containing biopolymers (or Tween 20) as the only carbon source after their inoculation with water from the studied waterbodies. The concentration of

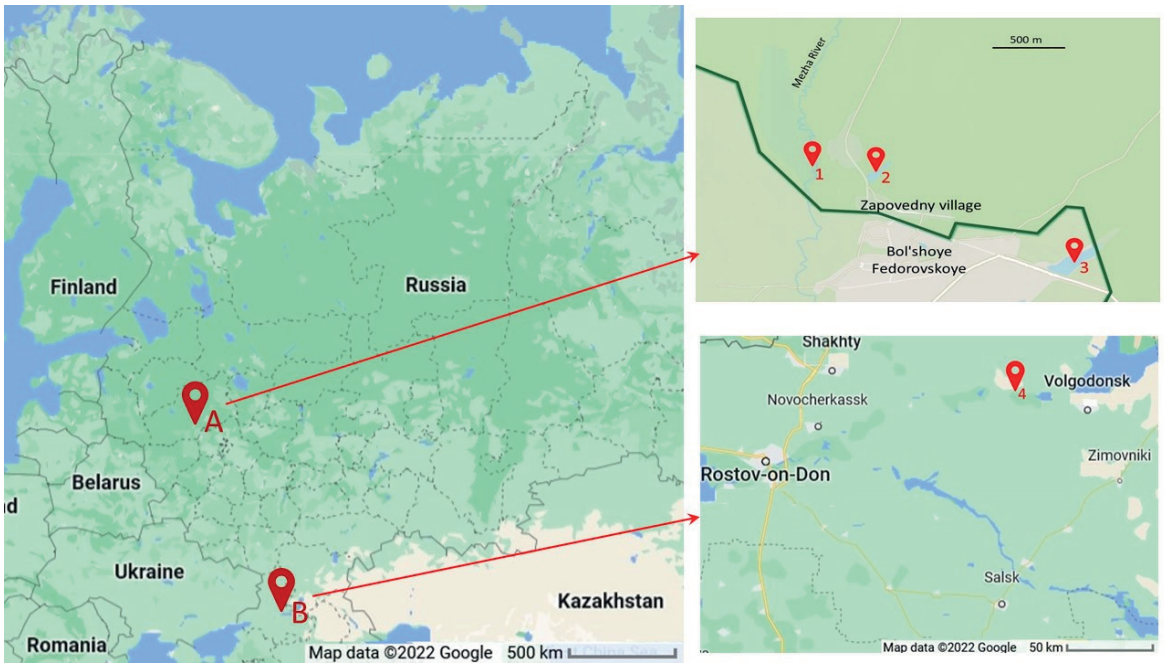


**Fig. 1. Sampling scheme: 1 - the Mezha River, 2 - the pond, 3- the Mezha River ('Old dam') (Tver region – A), 4 - the Don River (Rostov region - B)**

**Table 1. Characteristics of sampling points**

| Object | Geographical coordinates | River length, km/surface area, $m^2$ | Water temperature, °C | Air temperature, °C |
|---|---|---|---|---|
| Don River | 47.55898N, 41.99808E | 1870/ | 26 | 32 |
| Mezha River (taiga zone) | 56.45525N, 32.96316E | 259/ | 15 | 15 |
| Mezha River ('Old dam') | 56.45006N, 32.98901E | /25000 | 19 | |
| Pond in Zapovedny village ('Prudka') | 56.45637N, 32.97012E | /7000 | 16 | |

bacteria in nutrient media was determined by measuring the dynamics of their optical density at 620 nm in 96-well culture plates. The approach was modified for water objects as follows: (i) the number of media was reduced to 8 (media with chitin, cellulose, xylan, agarose, keratin, inulin, dextran, Tween 20); (ii) 100 µL of water from a waterbody and 100 µL of a liquid medium with a polymer at a concentration of 5 g/L were added to the wells of 96-well culture plates; (iii) changes in the concentration of bacteria in liquid media were registered over 260 h; (iv) the intensity of the bacterial community growth on polymers was characterized using the area under the growth curve as an integral parameter.

***Chemical analysis of water.*** The content of anions was determined using ion chromatography on a Dionex ICS-1100 chromatograph. The content of metals and metalloids was determined using inductively coupled plasma optical emission spectrometry on an Agilent 5110 ICP-OES instrument. The content of the ammonium ions was determined using spectrophotometry with the indophenol blue dye formed as a result of the reaction of ammonium ions with sodium hypochlorite and sodium salicylate in the presence of sodium nitroprusside at a wavelength of 655 nm. The content of dissolved organic carbon and total nitrogen was determined using a LiquiTOCtrace analyzer (Elementar, UK).

***Statistical data processing.*** Statistical data processing was carried out using the Statistica 8 program. The arithmetic mean and standard error values of the studied parameters were calculated and a correlation analysis was carried out to preliminarily establish the relationship between the chemical and bacterial indicators of waterbodies. To identify the most significant correlations, we carried out a multiple linear regression analysis of the indicators that showed strong correlation during the correlation analysis. To evaluate the relationship between the chemical and bacterial indicators of waterbodies in general, we used the principal component analysis (PCA). The reliability of the principal components was determined based on the cross-validation data, the values of the Kaiser criterion and the scree plot. The analysis was performed separately for bacterial culture chemistry data and for the comprehensive method. In the case of the comprehensive method and chemical indicators, the principal component analysis was first carried out for all indicators, and then only for those that had a correlation coefficient with the principal components (PC) greater than 0.7 in order to improve the accuracy of the analysis and exclude the influence of non-informative indicators.

## RESULTS AND DISCUSSION

***The sanitary and microbiological state of waterbodies*** was assessed using 3 indicators: the concentration of enterococci in water (the medium with esculin), the concentration of total coliform bacteria (TCB, the MacConkey medium) and the ratio between the number of colonies grown on nutrient agar at 22 °C and the number of colonies grown at 37 °C (self-purification index, Sanitary and Epidemiological Guidelines 4.2.1884-04). Based on these three indicators, the waterbodies were ranked according to the increasing level of pollution as follows (the self-purification index is inversely proportional to pollution): the Mezha River (dam area), the pond, the Mezha River (stream), the Don River (Fig. 2, Table 2). According to enterococci and TCB indicators, none of the objects complied with the Sanitary Rules and Regulations 1.2.3685-21 for surface waters for any of the possible uses (recreation, sport, and water supply). The high values of sanitary indicators in the Don River can be explained by the discharge of sewage into the river and the intensification of agriculture (the inflow of sewage into the river with farm animal waste) (Zhuravlev et al. 2010), while in the Mezha River the high abundance of TCB and enterococci can be explained by the activity of beavers (Skinner et al., 1984) and waterfowl (Standridge et al. 1979; Moriarty et al. 2011). In the dam area, sedimentation of suspended matter and colloids takes place, which can also reduce the abundance of the considered microorganism groups (Kepkay 1994). A similar series can be observed for the total abundance of bacteria grown on nutrient agar at 37 °C and on the Ashby agar. When bacteria were grown on the DOM of waterbodies, nutrient agar at 22 °C and PYG, the abundance of microorganisms in stagnant water (the Mezha River near the dam and the pond) was lower than in flowing water. Perhaps, this is due to the feeding activity of planktonic filter-feeding crustaceans, which leads to a reduction in bacterioplankton (Richardson and Mackay 1991). A large concentration of autochthonous microbiome in the Mezha River compared to the Don River was clearly seen through the growth of microorganisms on polymers (Table 3): for all the medium options, except for Tween 20, the growth was more intense in the Mezha River than in the Don River. There was also a trend of weaker growth of microbial complexes in conditionally stagnant waterbodies compared to flowing watercourses (chitin, cellulose, agarose, Tween 20).

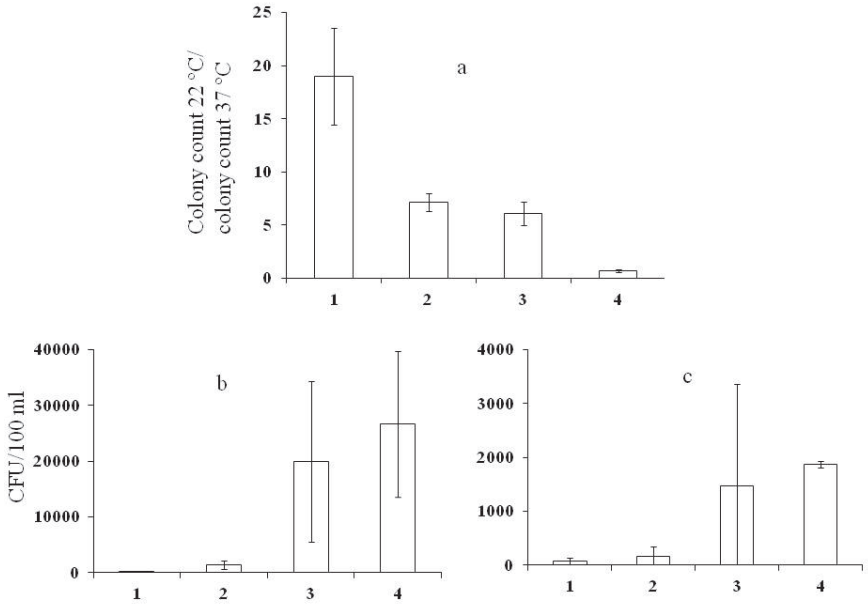


**Fig. 2. The ratio of Colony count at 22°C to Colony count at 37°C (a), the abundance of coliform bacteria (b) and enterococci (c) in (1) the Mezha River ('Old dam'), (2) the pond, (3) the Mezha River and (4) the Don River**

**Table 2. Concentration of culturable bacteria**

| Medium | Concentration of bacteria in waterbodies, CFU/mL | | | |
|---|---|---|---|---|
| | Don River | Dam on the Mezha River | Mezha River | Pond |
| Peptone yeast extract glucose agar | 1,150±321 | 1,133±44 | 9,166±833 | 1,233±120 |
| Ashby's Glucose Agar | 5,000±243 | 783±142 | 3,800±1,200 | 1,066±384 |
| Nutrient Agar 37°C | 1,733±233 | 61±6 | 1,500±75 | 71±3 |
| Nutrient Agar 22°C | 1,250±230 | 1,133±44 | 9,166±833 | 506±29 |
| MacConkey Media | 266±66 | 2.0±0.5 | 199±73 | 14±3 |
| Oligotrophic media | 933±381 | 1,866±550 | 3,333±763 | 2,033±450 |
| Copiotrophic media | 783±202 | 883±600 | 1,666±288 | 1,333±288 |
| Dissolved organic matter | 108,000±80,804 | 63,000±30,512 | 143,000±39,887 | 41,000±34,394 |
| Medium for Isolation of Iron Bacteria | 124,000±27,184 | 70,333±65,574 | 163,33±73,050 | 11,800±6,557 |
| Bile Esculin Azide Agar | 18±0.6 | 0.6±0.6 | 14±5 | 1.6±1.3 |

**Table 3. Growth of bacterioplankton on polymers (comprehensive approach)**

| Liquid medium containing polymer | Area, optical units×h | | | |
|---|---|---|---|---|
| | Don River | Dam on the Mezha River | Mezha River | Pond |
| Keratin | 2,340±780 | 1,820±260 | 4,160±1,300 | 2,600±260 |
| Chitin | 4,160±1,560 | 2,340±1,040 | 5,980±520 | 2,600±260 |
| Cellulose | 598±208 | 390±208 | 1,742±234 | 702±104 |
| Agarose | 192±78 | 75±26 | 465±156 | 286±78 |
| Inulin | 3,900±780 | 2,080±1,040 | 5,200±260 | 4,618±780 |
| Xylan | 2,912±624 | 2,418±780 | 2,418±520 | 1,924±520 |
| Tween 20 | 7,124±520 | 2,080±520 | 6,214±520 | 5,200±780 |
| Dextran-500 | 208±130 | 468±104 | 1,716±442 | 598±156 |

***Species composition of bacteria growing on DOM.*** The dominant bacteria utilizing DOM in the Don River represented the *Pseudomonas* genus with a share of up to 64% of the cultivated bacteria; 25% represented Deinococcus (Table 4). Pseudomonas dominated in the Mezha River as well (70%), while representatives of *Janthinobacterium* comprised the second largest group (20 %). The pond was dominated by the representatives of *Arcicella* (92%). Representatives of these genera are often found in surface waterbodies, and the studied strains had a high similarity of the 16S rRNA gene sequences to those of water strains (Van Horn et al. 2011; Baltrus et al. 2014; Tuohy et al. 2018; Friedrich et al. 2020).

The analysis of Table 5 indicates that there are fundamental differences between the studied waters in terms of chemical indicators. For instance, it shows that the surface and groundwater runoff leads to the enrichment of the rivers with Si, Ca, Mg, Sr, K, and Na compared to stagnant waterbodies (the pond and the dam on the Mezha River), in which suspended and colloidal particles are removed from solution and accumulate in bottom sediments. The water of the more southern region has certain features in the chemical composition: the content of the main inorganic anions and cations in the Don River is much higher than in the waters of the Tver Oblast. Greater content of biophilic elements and

**Table 4. Percentage of dominant bacteria growing on DOM as the only carbon source**

| Object | Bacterial strain [GenBank accession number] | Percentage, % |
|---|---|---|
| Don River | *Pseudomonas* [OM763864] | 33 |
| | *Pseudomonas* [ OM763866] | 31 |
| | *Deinococcus* [OM763865] | 25 |
| Mezha River | *Pseudomonas* [ OM763868] | 70 |
| | *Janthinobacterium* [ OM763867] | 20 |
| Pond | *Arcicella* [OM763869] | 92 |

better aeration of the Don River with oxygen promotes intensive mineralization of organic matter. In the Mezha River and the pond, the content of organic carbon is 2.3–2.9 times higher than in the Don River. Lower values of the C/N indicator in the Don River indicate a greater predominance of autochthonous organic matter compared to the Mezha River. The hydrological regime of the Central Forest Nature Reserve is significantly affected by swamps, which leads to an increased content of Fe, Mn and Corg in surface waters. An increase in the concentrations of NO3- and NO2- indicates more intense eutrophication of the Mezha River compared to the Don River and stagnant waterbodies due to animal activity.

Thus, it is possible to identify the differences between the studied objects based on the interrelated chemical and bacterial indicators, particularly the features of the physical and geographical conditions of the territory, the presence of a current and eutrophication.

This relationship is confirmed by the correlation between microbiological and chemical indicators (Table 6,7). There is a direct correlation of the CFU of bacteria with the content of biophilic elements (Ca, Fe, K, Mg, P, S, N) and an inverse correlation with the content of organic matter. This can be explained by the fact that the content of biophilic elements in water limits the abundance and activity of bacteria, while DOM, as the main source of nutrition for bacteria, undergoes rapid mineralization. The highest number of correlations between microbiological and chemical indicators is observed for an easily accessible polymer Tween 20, while for a medium with Fe, there is no significant correlation with chemical properties.

Based on Tables 2, 3, and 5, we carried out a multiple correlation analysis which showed the most interconnected chemical and microbiological indicators. These indicators are marked in Table 6 with an asterisk. We found that bacterial indicators are primarily associated with the content of

**Table 5. Concentrations of chemical elements and ions in water (± mean error)**

| Elements and ions | Concentration, mg/L | | | |
|---|---|---|---|---|
| | Don River | Mezha River ('Old dam') | Mezha River | Pond |
| Al | n.d. | 0.082±0.005 | 0.026±0.007 | 0.052±0.006 |
| B | 0.103±0.001 | 0.003±0.001 | 0.016±0.0001 | 0.010±0.0001 |
| Ba | 0.0250±0.0002 | 0.0100±0.0002 | 0.107±0.005 | 0.0250±0.0003 |
| Ca | 32.8±0.2 | 9.1±0.2 | 37.6±0,05 | 17.2±0.1 |
| Co | n.d. | n.d. | 0.0035±0.0001 | n.d. |
| Fe | 0.028±0.003 | 0.88±0.02 | 3.0±0.1 | 1.84±0.06 |
| Cu | n.d. | 0.0006±0.0001 | n.d. | 0.014±0.002 |
| K | 4.99±0.05 | 0.18±0.07 | 1.79±0.01 | 0.747±0,007 |
| Li | 0.0250±0,0001 | n.d. | n.d. | n.d. |
| Mg | 28.1±0,1 | 1.7±0,3 | 9.51±0,04 | 3.19±0,02 |
| Mn | 0.059±0.007 | 0.213±0.009 | 5.5±0.3 | 0.79±0.03 |
| Na | 77.4±0.2 | 2±1 | 4.27±0.02 | 1.32±0.02 |
| P | 0.068±0.006 | 0.032±0.002 | 0.047±0.003 | 0.052±0.001 |
| Si | 2.70±0.02 | 0.49±0.01 | 3.64±0.01 | 1.081±0.02 |
| Sr | 0.690±0.005 | 0.041±0.009 | 0.385±0.002 | 0.065±0.001 |
| F | 0.340±0.002 | 0.052±0.001 | 0.182±0.002 | 0.040±0.001 |
| Ti | n.d. | 0.002±0.001 | n.d. | n.d. |
| Zn | 0.005±0.002 | 0.01±0.001 | 0.002±0.001 | 0.006±0.005 |
| Cl | 94±1 | 0.4±0.2 | 1.91±0.02 | 0.83±0.03 |
| $NO_2$ | n.d. | n.d. | 0.17±0.02 | 0.01±0.01 |
| $NO_3$ | n.d. | n.d. | 0.34±0.07 | 0.09±0.05 |
| $SO_4$ | 131.9±0.6 | 0.8±0.3 | 2.56±0.03 | 0.32±0.03 |
| $NH_4$ | 0.35±0.04 | 0.19±0.04 | 0.510±0.006 | 0.217±0.009 |
| $C_{org}$ | 6.2±0.5 | 17.85±0.09 | 14.1±0.1 | 18.2±0.2 |
| $N_{org}$ | 1.6±0.1 | 1.2±0.1 | 1.1±0.1 | 1.3±0.1 |
| C/N | 3.8±0.2 | 15±1 | 13±1 | 14±1 |

n.d.-not detected

**Table 6. Values of the correlation coefficient between microbiological (microbiological culturing) and chemical indicators. Only indicators with significant correlation are shown**

| Chemical indicators | Concentration of bacteria in water taken into account on media | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Peptone yeast extract glucose agar | Ashby's Glucose Agar | Oligotrophic media | Copiotrophic media | Nutrient Agar 37°C | Nutrient Agar 22°C | Dissolved organic matter | MacConkey Media | Bile Esculin Azide Agar |
| Al | -0.25 | **-0.83** | 0.16 | -0.12 | -0.87 | -0.27 | -0.47 | **-0.83** | -0.58 |
| B | -0.24 | **0.73** | -0.6 | -0.4 | **0.71** | -0.19 | 0.25 | 0.68 | 0.59 |
| Ba | **0.97** | 0.4 | **0.74** | 0.61 | 0.54 | **0.96** | 0.55 | 0.37 | 0.42 |
| Ca | 0.66 | **0.81** | 0.23 | 0.29 | **0.91** | 0.68 | 0.62 | **0.78** | 0.7 |
| Fe | **0.81** | -0.12 | **0.86** | 0.72* | -0.02 | **0.76*** | 0.21 | -0.12 | -0.02 |
| K | -0.04 | **0.82** | -0.46 | -0.28 | 0.83 | 0 | 0.35 | **0.76** | 0.67 |
| Mg | -0.06 | **0.81** | -0.48 | -0.3 | 0.82 | -0.01 | 0.35 | **0.76*** | 0.66 |
| Mn | **0.97*** | 0.26 | **0.81** | 0.63 | 0.4 | **0.96*** | 0.49 | 0.24 | 0.32 |
| Na | -0.3 | 0.69 | -0.64 | -0.46 | 0.67 | -0.25 | 0.21 | 0.65 | 0.55 |
| P | -0.13 | 0.56 | -0.45 | -0.18 | 0.55 | -0.13 | 0.16 | 0.44 | 0.5 |
| S | -0.32 | 0.68 | -0.66 | -0.47 | 0.65 | -0.27 | 0.2 | 0.63 | 0.54 |
| Si | **0.75** | **0.78** | 0.32 | 0.34 | **0.9** | **0.77** | 0.64 | **0.75** | 0.67 |
| Sr | 0.19 | **0.88*** | -0.27 | -0.15 | **0.93** | 0.24 | 0.49 | **0.83** | **0.73*** |
| Zn | -0.32 | -0.21 | -0.26 | -0.23 | -0.34 | -0.31 | -0.16 | -0.31 | -0.25 |
| F | 0.13 | **0.87** | -0.32 | -0.21 | **0.91** | 0.19 | 0.48 | **0.82** | **0.72** |
| Cl | -0.32 | 0.68 | -0.65 | -0.46 | 0.66 | -0.27 | 0.19 | 0.64 | 0.55 |
| $NO_3$ | **0.89** | 0.23 | **0.85** | 0.61 | 0.31 | **0.87** | 0.32 | 0.17 | 0.42 |
| $SO_4$ | -0.32 | 0.68 | -0.65 | -0.47 | 0.66 | -0.27 | 0.21 | 0.63 | 0.54 |
| $NH_4$ | **0.82** | 0.68 | 0.52 | 0.52 | **0.83** | **0.84** | **0.71*** | **0.7** | **0.59** |
| $C_{org}$ | 0 | **-0.83** | 0.43 | 0.29 | **-0.86*** | -0.05 | -0.35 | **-0.79*** | -0.69 |
| $N_{org}$ | 0.08 | **0.74** | -0.17 | -0.01 | **0.7** | 0.13 | 0.52 | **0.74** | 0.5 |
| C/N | 0.17 | **-0.77** | 0.53 | 0.33 | **-0.72** | 0.13 | -0.24 | **-0.74** | -0.54 |

Note: Correlation coefficients values that are significant according to the data of correlation analysis (p=0.95) are highlighted in bold; * marks correlation coefficients that are significant according to the data of multiple linear regression

**Table 7. Values of the correlation coefficient between microbiological (comprehensive approach) and chemical indicators. Only indicators with significant correlation are shown**

| Chemical indicators | Area under curve describing the concentration of bacterial communities on media with polymers | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Keratin | Chitin | Cellulose | Agarose | Inulin | Xylan | Tween 20 | Dextran-500 |
| Al | -0.23 | -0.34 | -0.32 | -0.34 | **-0.9** | 0.06 | **-0.96** | -0.11 |
| B | -0.11 | -0.19 | -0.18 | -0.13 | **0.74** | -0.08 | **0.8** | -0.39 |
| Ba | **0.79** | **0.97** | **0.94** | **0.82*** | 0.49 | 0.28 | 0.31 | **0.93** |
| Ca | 0.58 | **0.71** | **0.7** | 0.63 | **0.91** | 0.16 | 0.82 | 0.54 |
| Fe | 0.68 | **0.81** | **0.81** | **0.76** | -0.03 | 0.18 | -0.12 | **0.88** |
| K | 0.04 | 0.01 | 0.02 | 0.04 | **0.85** | -0.02 | **0.87*** | -0.2 |
| Mg | 0.02 | -0.02 | -0.02 | 0 | **0.83** | -0.02 | **0.85*** | -0.22 |
| Mn | **0.78** | **0.96** | **0.93** | **0.8** | 0.35 | 0.28 | 0.15 | **0.96** |
| Na | -0.17 | -0.26 | -0.25 | -0.2 | 0.69 | -0.08 | **0.74** | -0.44 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| P | 0.01 | -0.06 | 0.08 | 0.2 | **0.76** | -0.21 | **0.81** | -0.14 |
| S | -0.19 | -0.28 | -0.28 | -0.22 | 0.67 | -0.09 | **0.73** | -0.46 |
| Si | 0.62 | **0.78** | **0.76** | 0.66 | **0.87** | 0.19 | **0.74** | 0.63 |
| Sr | 0.19 | 0.23 | 0.22 | 0.18 | **0.92** | 0.07 | **0.87** | 0.03 |
| Zn | -0.34 | -0.34 | -0.28 | -0.48 | -0.54 | **0.71** | -0.38 | -0.34 |
| F | 0.14 | 0.16 | 0.14 | 0.11 | **0.9** | 0.05 | **0.84** | -0.04 |
| Cl | -0.18 | -0.28 | -0.27 | -0.21 | 0.68 | -0.11 | **0.74** | -0.46 |
| $NO_3$ | **0.83*** | **0.89** | **0.81*** | **0.82** | 0.32 | 0.23 | 0.13 | **0.86*** |
| $SO_4$ | -0.18 | -0.28 | -0.27 | -0.21 | 0.68 | -0.1 | **0.74** | -0.46 |
| $NH_4$ | **0.7** | **0.86*** | **0.75** | **0.72** | **0.72*** | 0.14 | **0.63** | **0.71** |
| $C_{org}$ | -0.06 | -0.03 | -0.03 | -0.02 | **-0.85** | 0.02 | **-0.83** | 0.16 |
| $N_{org}$ | 0.14 | 0.21 | 0.07 | 0.11 | 0.63 | 0.13 | **0.75*** | -0.14 |
| C/N | 0.09 | 0.12 | 0.11 | 0.14 | **-0.71** | -0.01 | **-0.76*** | 0.36 |

Note: Correlation coefficient values that are significant according to the data of correlation analysis (p=0.95) are highlighted in bold; * marks correlation coefficients that are significant according to the data of multiple linear regression

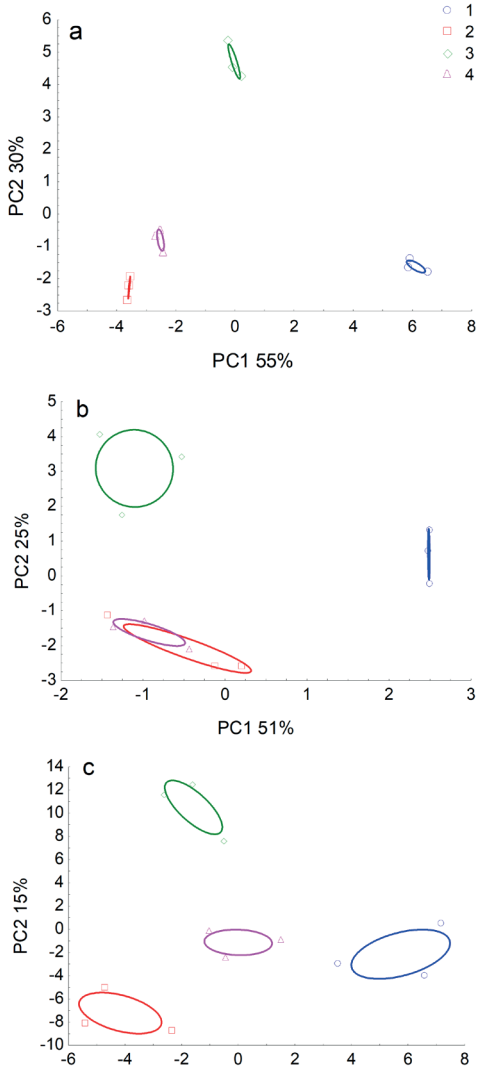


Fig. 3. Relative position of the studied waterbodies in the PC1 and PC2 factor space constructed according to chemical indicators (a), microbiological culturing (b) and the comprehensive approach (c). Correlation ellipses limit the area with p=0.95. 1 – bacterial complex of the Don River, 2 – the dam on the Mezha River, 3 – the Mezha River, 4 – the pond

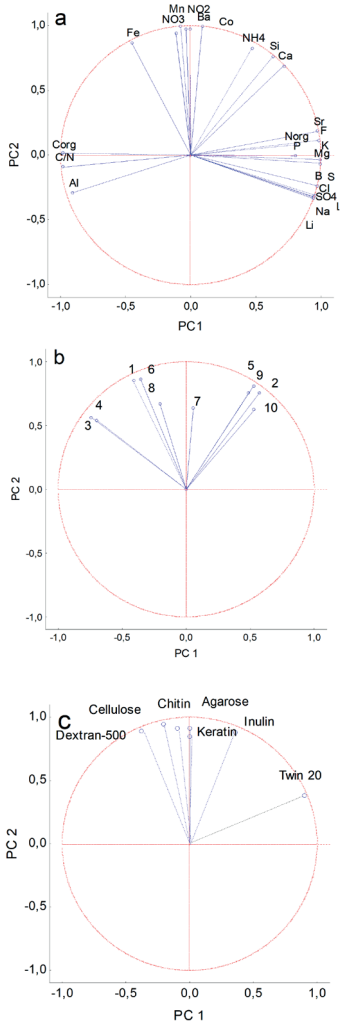


Fig. 4. Values of the correlation coefficient between PC1 and PC2 according to chemical indicators (a), microbiological culturing (b), and indicators of the comprehensive approach (c). 1- Glucose peptone yeast extract agar, 2- Ashby's Glucose Agar, 3- Oligotrophic media, 4- Copiotrophic media, 5- Nutrient Agar 37°C, 6- Nutrient Agar 22°C, 7- Soluble organic matter, 8- Isolation Medium for Iron Bacteria, 9- MacConkey Media, 10- Bile Esculin Azide Agar

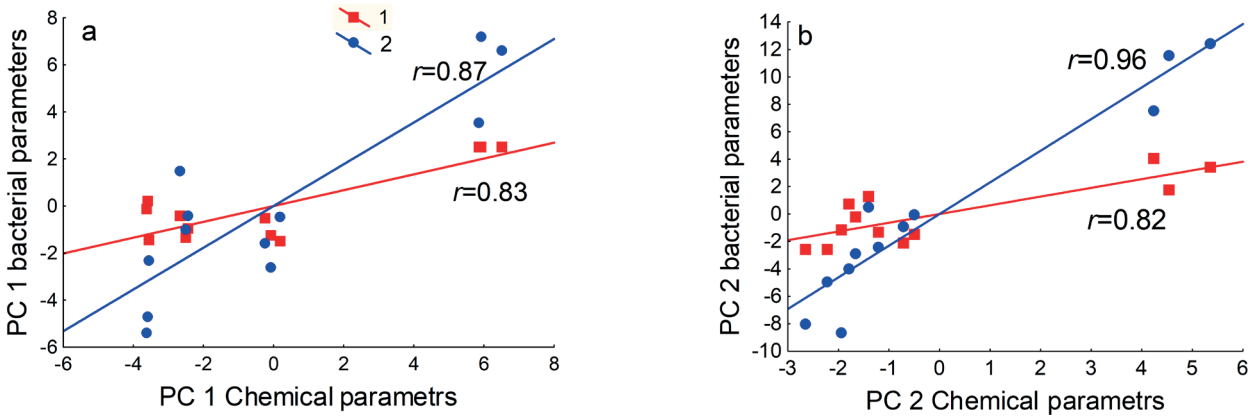


**Fig. 5. Correlation between PC1 (a) and PC2 (b) of the chemical and microbiological indicators. 1 – Bacterial culture on agar plates, 2 – Comprehensive approach**

biophilic elements (Mg, Mn, Fe) and macroelements (N, $C_{org}$). At the same time, only CFU on nutrient agar at 22 °C and the area under the growth curve of the bacterial community on the Tween 20 medium were found to depend on two or more indicators.

The PCA showed that the relative position of the studied waterbodies in the space of PC1 and PC2 calculated from chemical and bacterial indicators are similar (Fig. 3).

Data on the correlation between PCs and the studied water indicators (Fig. 4) fully confirmed the data obtained from the analysis of Tables 2, 3 and 5. This indicates the reliability of the identified patterns and suggests the existence of a general relationship between chemical and bacterial indicators of water. In order to prove this relationship, we examined the correlation between PCs calculated from chemical and bacterial indicators and found that it is strong (Fig. 5).

Thus, either there is a causal relationship between bacterial and chemical indicators, or the same environmental factors affect both chemical and bacterial indicators.

## CONCLUSIONS

Based on the results of the work, we established that differences in the functioning of the studied waterbodies are related to the differences in both hydrochemical and bacterial properties of water. It can be assumed that there is either a causal relationship between the hydrochemical and microbiological indicators of water or an indirect mechanism of correlation through the independent action of the same factors on both hydrochemical and bacterial indicators. We identified three factors that determine the specifics of the studied waterbodies. The most important factor is the presence or absence of current as it affects both bacterial and chemical indicators. The second most important factor is the geochemical features of the region from where water flows into the waterbody. This factor affects the studied hydrochemical indicators. The third most important factor in terms of the impact on the studied waterbodies is eutrophication which affects the concentration of mineral forms of nitrogen in the water, the total concentration of heterotrophic bacterioplankton and sanitary-indicative microorganisms. The influence of seasonal and yearly variation was not examined in this study. Further studies will expand the list of factors affecting water indicators. The established relationships suggest that methods of bacterial bioindication of the hydrochemical properties of water in waterbodies could be developed in the future.

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