# MICROBIAL DIVERSITY IN RIVER WATER AND SEDIMENT FROM SULINA AREA

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**Abstract.** Research on the bacterial diversity of the Danube waters from the flowing or stagnant area, somewhat adjacent to Sulina branch and port, is a study case integrated with traditional ecological research, contributing to a more complex characterization of the permanent or accidental populations from these areas. The differences or similarities with respect to the benthic microbiota and the one present in the water mass, as well as the abiotic and biotic characteristics of the delta channels and lakes, correlated with the seasonal climatic variations or generated by the extreme or deficient pluviometric regime, constitute extremely complex and interesting study material that will be able to supplement the results of the research undertaken so far.

**Keywords:** bacterial diversity, benthic microbiota, sediments samples.

**Abstract. Diversitatea microbiană a apei şi a sedimentelor din zona Sulina.** Cercetările privind diversitatea bacteriană a apelor Dunării, din zona curgătoare sau stagnantă, limitrofă într-o oarecare măsură braţului şi portului Sulina, constituie un caz de studiu, integrat cercetărilor ecologice deja cu tradiţie, contribuind la o mai complexă caracterizare a populaţiilor de organisme, permanente sau accidentale din aceste areale. Diferenţele sau similitudinile în ceea ce priveşte microbiota bentonică şi cea prezentă în masa apei, cât şi caracteristicile abiotice şi biotice ale apelor lacurilor de deltă şi ale canalelor, corelate cu variaţiile climatic sezoniere sau generate de regimul pluvial extrem sau deficitar, constituie un material de studiu extrem de complex şi de interesant care va putea să completeze rezultatele cercetărilor întreprinse până în prezent.

**Cuvinte cheie:** diversitate bacteriană, microbiotă bentonică, probe de sediment.

## INTRODUCTION

The studies undertaken to fully characterize the biodiversity of the Danube River, supplement the projects of mapping and distribution of rare and protected species of plants and animals, in the enhanced interest of protecting the environment, not only in the Danube Delta Biosphere Reserve, but anywhere where accurate knowledge of fish, ornithological, botanical and microbial groups is required (KIRSCHNER et al., 2009). In this respect, we are working on the application of the most appropriate measures required by specialists from very diverse but related fields, thus justifying the interest of carrying out studies on the microbiota in the delta area, adjacent to Sulina Ecological Station, a research point belonging to the Institute of Biology Bucharest, under the aegis of the Romanian Academy.

The present paper is part of a series of three studies, based on the analysis of the samples taken in September 2016 from the Delta lakes Puiu, Rosu and Rosulet, as well as from the related linking channels. The papers are subject to presentations at international scientific events organized by the University of Pitesti and by the County Museum of Oltenia during 2017. Thus, at the Scientific Symposium Current Trends in Natural Sciences organized in Piteşti in April 2017, the paper "Highlighting of some physiological bacterial groups isolated from the Danube Delta Biosphere Reserve, Rosu, Roşuţ and Puiu lakes" was presented, which signals of the main physiological groups of microorganisms present in the water and benthos samples taken from several delta points where previous environmental analyses were performed.

In the other two papers brought to the attention of specialists and of those interested in the microbial diversity of the Danube Delta, we present a physico-chemical characterization of both water and benthic soil samples on the one hand, and an interpretation of the presence of certain bacterial physiological groups (PĂCEŞILĂ et al., 2008), thus making a comparison between the microbiota present in the water mass and the one identified in the sediment layer.

At the same time, by isolating and testing bacterial populations from the same analyzed samples, selections of bacterial strains with biotechnological potential were made. These researches on the bacterial cell metabolism and the possible uses of the obtained bioproducts are the subject of the third scientific communication that will be presented this autumn at the international scientific manifestation organized by the Oltenia Museum, Craiova.

## MATERIAL AND METHODS

**Field sampling.** Water samples were collected in September 2016. The analyzed Danube water sampling points were located on Busurca Channel (M3), Împuţita Channel, at the confluence with Rosuleţ Lake (M4), in Roşu Lake (M6), at the mouth of Roşu Lake (M8), and in Mândra backwater (M9).

For the sediment samples, the chosen sampling sites were Împuţita Channel at the confluence with Roşuleţ Lake (M4), Roşu Lake (M6), Musura Gulf (3) and Sulina harbor area.

The samples consisted of 500 mL of column water from each selected station point; some water was filtered through GF / F filters (65 μm) for dissolved forms of nutrients and preserved for further analyses.

Sediment samples: surface sediments samples were also sampled and transported in plastic bags to the lab and preserved at -20 °C until extraction and analysis.

**Measurement of physical parameters.** Depth and transparency were determined using Secchi disc, the redox potential, pH, temperature, conductivity and salinity were measured in the field with a multiparameter WTW 340i, Germany.

**Water chemistry.** Nutrients were determined spectrophotometrically following a modified Berthelot method for N–$NH_4$ (KROM, 1980), Griess-Ilosvay modified method for N-$NO_2$ (KEENEY & NELSON, 1982; TARTARI & MOSELLO, 1997) for N-$NO_3$, P-$PO_4$ and TP.

**Selective growing media.** In order to highlight the main physiological groups of bacteria present in the analyzed water and sediment samples, the following selective cultivation media were used: VL medium (BEERENS, 1954), for anaerobic heterotrophic bacteria, Postgate for reducing sulphate bacteria, Nutrient Broth for aerobic heterotrophic facultative anaerobic bacteria, medium with starch for amylolytic bacteria, Vinogradsky for ferrobacteria, medium for nitrite bacteria, Giltay medium for denitrifying bacteria and 9K medium for iron oxidizing chemolythotrophic bacteria. Serial dilutions were grown on the selective media in triplicates (three repetitions) and incubated at 28 °C for varying lengths of time. The bacterial growth was assessed according to the McReady comparative method.

## RESULTS AND DISCUTIONS

A first step was to establish the physico-chemical parameters of both the water in the sampling points and the collected sediment.

As it can be seen in Tables 1 and 2, parameters such as temperature, conductivity, transparency, pH, $NO_2$, $NO_3$, etc. have been measured.

In the case of water set parameters, it can be observed that there are no major fluctuations between the five sampling points. The measured parameters fall within the set limits for river water. Table 1 presents the physico-chemical analysis of the collected Danube water samples.

Table 1. Physico-chemical analysis of water.

| Parameter/ station | M3 (Busurca Channel) | M4 (Impuţita Channel in Roşuleţ Lake) | M6 (Roşu Lake) | M8 (channel entrance in Roşu Lake) | M9 (Mândra backwater) |
|---|---|---|---|---|---|
| Depth (m) | 2 | 1.1 | 2.7 | 1.6 | 1.7 |
| Transparency (m) | 0.4 | 1.1 | 0.4 | 0.4 | 0.4 |
| T/A | 0.2 | 1.0 | 0.15 | 0.25 | 0.24 |
| Temperature (°C) | 17.5 | 16.2 | 16.9 | 17.1 | 16.9 |
| pH | 7.91 | 8.114 | 8.96 | 8.491 | 8.602 |
| Conductivity (ms/cm) | 421 | 545 | 385 | 386 | 441 |
| Salinity | 0.1 | 0.2 | 0.1 | 0.1 | 0.1 |
| Redox | -56.9 | -56.6 | -110.5 | -90.5 | -92.6 |
| $NH_4$ (mgN/L) | 0.007 | 0 | 0.024 | 0.088 | 0.054 |
| $NO_2$ (mg/L) | 0.003 | 0.001 | 0.001 | 0.009 | 0.001 |
| $NO_3$ (mg/L) | 0.33 | 0.25 | 0.30 | 0.15 | 0.53 |
| DIN (mgN/L) | 0.336 | 0.252 | 0.326 | 0.247 | 0.583 |
| $PO_4$ (µg/L) | 21.25 | 10 | 26.25 | 32.5 | 32.5 |
| Whole P (µg/L) | 30 | 56.25 | 68.75 | 82.5 | 70 |

The physico-chemical parameters of the sediment were assessed using the same method used to establish the physico-chemical parameters of the water. As it can be seen in Table 2, the sediment samples originated from Împuţita Channel and Roşu Lake. Water samples were also collected from the same points. Thus, the temperature, redox and pH parameters were similar for water and sediment samples, while parameters such as salinity, conductivity, increased, showing a double value in the sediment samples as compared to those of the water at the same sampling point. This is due to a higher degree of mineralization in the sediment, which led to increased concentrations of ammonium, nitrite, nitrate, orthophosphate ions.

Table 2. Physico-chemical analysis of the sediment.

| Parameter/station | M4 (Împuţita Channel in Roşuleţ Lake) | M6 (Roşu Lake) | Musura Channel | Sulina Harbour |
|---|---|---|---|---|
| Depth (m) | 1.1 | 2.7 | 1.6 | 1.0 |
| pH | 8.149 | 8.639 | | |
| Redox | -64.5 | -94.5 | | |
| Temperature (°C) | 19.3 | 19 | | |
| Salinity | 0.4 | 0.4 | | |
| Conductivity (ms/cm) | 940 | 855 | | |
| $NH_4$ mg/g s.u | 17.76 | 22.48 | 11.17 | 33.96 |
| $NO_2$ mg/g s.u | 0.623 | 0.516 | 0.073 | 0.065 |
| $NO_3$ mg/g s.u | 8.38 | 7.28 | 1.45 | 1.72 |
| $PO_4$ µg/g s.u | 3488.25 | 5892.57 | 1642.47 | 1411.17 |

In the case of sediment samples taken from Musura Channel and Sulina Harbor, the ammonium, nitrite, nitrate, orthophosphate ions quantities were found to be lower in comparison to the sediment samples M4, M6, due to the presence of denitrifying bacteria present in large number in the sediment, depleting the nitrogen compounds from the soil by reducing the nitrates and nitrites. Both sediment and water samples were analyzed from the microbiological point of view, by seeding on specific media of different physiological groups. In this study, we focused on signaling the presence of the dominant physiological groups of microorganisms in both the analyzed water and sediment samples (Tables 3; 4).

Table 3. Limit value number of bacteria/ml water sample.

| | **M3 (Busurca Channel)** | **M4 (Împuţita Channel in Roşuleţ Lake)** | **M6 (Roşu Lake)** | **M8 (channel entrance in Roşu Lake)** | **M9 (Mândra backwater)** |
|---|---|---|---|---|---|
| **No. of cell/ml** | **11 x $10^5$** sulphate reducing bacteria – **15 x $10^8$** iron bacteria | **3 x $10^3$** heterotrophic anaerobic bacteria – **11 x $10^6$** iron bacteria | **9.5 x $10^3$** suphate reducing bacteria; heterotrophic aerobic and facultative anaerobe bacteria; amylolytic bacteria – **11 x $10^4$** heterotrophic anaerobic bacteria | **0.4 x $10^3$** heterotrophic anaerobic bacteria – **11 x $10^7$** nitite bacteria | **0.4 x $10^2$** iron bacteria – **11 x $10^7$** nitite bacteria |

Table 4. Limit value bacterial count/ml sediment sample.

| | **M4 (Împuţita Channel in Roşuleţ Lake)** | **M6 (Roşu Lake)** | **Musura Channel** | **Sulina Harbour** |
|---|---|---|---|---|
| **No. of cell/ml** | **1.5 x 10** Iron-oxidizing chemolithotrophic bacteria – **4.5 x $10^7$** Heterotrophic anaerobic bacteria | **4.5 x 10** Iron-oxidizing chemolithotrophic bacteria – **11 x $10^9$** heterotrophic anaerobic and facultative anaerobic bacteria | **9.5 x 10** Iron-oxidizing chemolithotrophic bacteria – **11 x $10^9$** Denitrifying bacteria | **2.5 x 10** Iron-oxidizing chemolithotrophic bacteria - **14 x $10^8$** Heterotrophic anaerobic bacteria |

It should be noted that in the water samples from the Busurca and Împuţita Channels ferrobacteria are predominant, while in Roşu Lake and in Mândra backwater the nitrite bacteria are preponderant. In the analyzed sediment samples, the bacteria belonging to the anaerobic heterotrophic group predominate, and in Musura Bay the denitrifying bacteria were present.

The comparison between the two sets of samples, water and sediment, reveals that in all four sediment samples there are minimal and relatively constant concentrations of iron-oxidizing chemolythotrophic bacteria. It can be concluded that the sedimentary benthic layer, due to the presence of aerobic facultative anaerobic bacteria, is microbial-active.

The circulating water mass, with a depth of 1.0 to 2.7 meters, varies depending on the Danube water shares at different time intervals, as well as the degree of transparency. It is necessary to periodically analyze the same sampling points in order to observe any variations of the microbiota.

## CONCLUSIONS

The analyzed samples presented a wide variety of physiological groups of the microorganisms.

Ferrobacteria were predominantly present in the mass of water coming from channels with a relative circulating flow, while in the lakes with stagnant water volume there are increased amounts of nitrite bacteria.

Sediment samples taken from depths of 1 down to 2.7 meters show normal benthic microbial activity.

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