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FROM DIRECT OBSERVATION TO ENVIRONMENTAL DNA: A COMPARATIVE ANALYSIS OF BIODIVERSITY ASSESSMENT METHODS

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Introduction

Recent advances in environmental DNA (eDNA) have transformed biodiversity monitoring, enabling non-invasive detection of species in diverse ecosystems. It is well-known that biodiversity is the foundation of the health and stability of ecosystems, through the contribution and benefits essential to human survival and well-being. The variety of species, genetic structure and ecosystems ensures resilience against environmental change, supports crucial ecosystem services and underpins economic and cultural activities. The literature demonstrates a surge in eDNA-based research across aquatic, terrestrial, and atmospheric environments, with freshwater, marine, and brackish systems being prominent focal points. eDNA is increasingly applied for conservation, restoration, invasive species surveillance, and forensic investigations, providing sensitive and cost-efficient alternatives to traditional survey methods. Our woks aimed to present the specific diversity of microorganisms of an urban freshwater system through eDNA-based and traditional methods.

Materials and methods

For the assessment of specific diversity in this case study, the analysis focused exclusively on microbiological quality indicators of a freshwater system. Dambovita River - which crosses Bucharest, and on the course of which several types of ecosystems are encountered, including urban ecosystems, but also due to a number of issues that target the degradation of this aquatic system (e.g. water pollution, waste dumped into the river, concrete banks, etc) was the case study. Seven sampling stations along Dambovita River were selected (2 upstream stations in the spring sector (*Group 1*, G1), and five stations in the urban area (*Group 2*, G2), four station in Bucharest and one station at the confluence with the Arges River. The sampling campaign was in the spring of 2025. For the metabarcoding analysis, eDNA was extracted from freshwater samples after concentration on different types of membrane filters (polyethersulfone, Sterivex, glass fiber, mixed cellulose esters, Nucleopore). A slightly modified protocol of DNeasy Blood&Tissue kit was used for eDNA extraction. DNA concentration was detected using Clariostar microplate reader (BMG Labtech, Germany). Only DNA samples with a 260/280 absorbance ratio greater than 1.8 were selected for sequencing, ensuring adequate DNA purity. Downstream library preparation and high-throughput sequencing was performed by Novogene (Munich). Specific diversity analysis by traditional methods targeted the quantitative determination of total coliform bacteria and *Escherichia coli* as well as enterococci,

which were performed in accordance with SR EN ISO 9308-2, and SR EN ISO 7899-2, respectively. Organic load (measured as chemical oxygen demand, COD and biochemical oxygen demand, BOD) as well as nutrients were detected using in force standard methods.

Results and conclusions

The analysis of biotic community diversity through eDNA-based methods in the urban aquatic system revealed a higher relative abundance of bacterial taxa belonging to the phylum *Pseudomonadota* in the spring sector (G1—Group 1). Pseudomonadota phylum (formerly Proteobacteria) comprises a highly diverse group of organisms, including environmentally important bacteria involved in nutrient cycles and bioremediation in soil and freshwater ecosystems, as well as photogenic species such as Escherichia coli and Salmonella spp., which are major causes of diseases. In this case, this pattern may be related to the specific conditions of the upper river section, including high oxygen levels, inputs of soil-derived microorganisms, and low anthropogenic disturbance, which favor the colonization of mineral substrates by biofilm-forming *Pseudomonadota* species.

Downstream, in the urban area, (G2—Group 2), changes in physicochemical conditions resulted in a lower relative abundance of *Pseudomonadota* and an increased contribution of other taxa, such as *Actinomycetota*. Species-level sequencing analysis also indicated anthropogenic influence through the detection of *Homo sapiens* DNA in the urban river section (Figure 1).

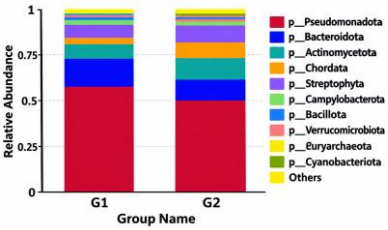


Figure 1 - The barplot of relative abundance

Similarly, quantitative analyses of microbiological quality indicators by traditional methods indicated the presence of total and faecal coliforms, *E. coli*, and enterococci species, particularly at the point where the river enters the urban area and further downstream (G2), and less in the spring sector (G1). The density of total coliform bacteria recorded a value higher than 80 x 10^3 MPN/100 mL where the watercourse enters the city, 19 x10^3 MPN/100 mL in the central area of the city and over 20 x 10^3 MPN/100 mL in the downstream area. Elevated concentrations of organic load expressed as COD and BOD as well as increased nutrients levels (e.g. total nitrogen), were recorded at the same sampling sites (data not shown). The same pattern was observed for the other microbiological indicators, with densities several orders of magnitude lower than those of total coliform bacteria. Overall, we consider that these methods should work complementary and not in competition, depending on the studies objectives.

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