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Effective microorganisms-based bioremediation for improving water and sediment quality in Ponjavica Nature Park (Serbia): A case study of sustainable aquatic ecosystem restoration

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ABSTRACT

Anthropogenic pressures are increasingly degrading protected freshwater ecosystems, particularly eutrophic systems affected by sediment accumulation. Although Effective Microorganisms (EM) have been proposed as an eco-friendly bioremediation approach, field-based evidence from protected freshwater systems under real management constraints remains limited. This study evaluated the effectiveness of EM-based treatment in improving microbiological and physicochemical water and sediment quality in Ponjavica Nature Park (Serbia), a protected eutrophic wetland exposed to long-term anthropogenic pressures. Water and sediment samples were collected before and after EM application to assess changes in key microbiological indicators and organic pollution parameters. The results showed measurable improvements in water quality following EM treatment. Total coliform counts decreased by up to 50% (5,860–6,440 MPN/100 mL), faecal coliforms (*Escherichia coli*) fell below detection limits (<100 MPN/100 mL), intestinal enterococci decreased to < 40 MPN/100 mL, and aerobic heterotrophic bacteria declined slightly. Biochemical oxygen demand (BOD_5) remained consistently below 3.0 mg O_2/L, indicating effective microbial degradation of organic matter, while chemical oxygen demand (COD) ranged from 66.9 to 98.9 mg O_2/L, with localised increases associated with sediment disturbance. Overall, water quality classification improved at several monitoring sites according to national standards. Rather than proposing EM as a substitute for conventional remediation technologies, this study demonstrates its potential to provide environmentally acceptable short-term improvements in water quality in protected freshwater systems where financial, technical, and ecological constraints limit engineering interventions. The applied approach offers a transferable framework for similar eutrophic and sediment-impacted aquatic environments.

Introduction

Water is a vital resource for the survival of all living organisms, essential to human health, biodiversity conservation, and ecosystem sustainability. However, of the total water reserves on Earth, 97.5% is saltwater, while only 2.5% comprises freshwater sources such as rivers and lakes. Alarmingly, only 0.8% of global water volume is directly accessible for human use (Mishra, 2023). In this context, safeguarding the quality of the limited available freshwater supplies has become a global priority. According to the World Health Organization, improving

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water quality management is one of the most effective strategies for public health protection and is a key component of Sustainable Development Goal 6 (SDG 6) – "Ensure availability and sustainable management of water and sanitation for all," particularly within Targets 6.1 and 6.3, which focus on access to safe water and pollution reduction (World Health Organization, 2024).

Water pollution may have natural origins (e.g., geological leaching, alluvial deposits), but under modern conditions, it is predominantly anthropogenic, stemming from activities such as industrial production, agriculture, transportation, and municipal wastewater discharge (Akhtar et al., 2021). Depending on its nature, pollution can be classified as:

- chemical pollution, including heavy metals, pesticides, pharmaceuticals, petroleum derivatives, and other synthetic compounds (Sankhla, 2019);
- biological pollution, encompassing pathogenic microorganisms (bacteria, viruses, parasites), as well as organic waste from households and industry. This type of contamination can trigger a wide spectrum of diseases, from gastrointestinal infections and dermatological conditions to serious zoonoses such as cholera (Lin et al., 2022). Parasites such as Giardia and Cryptosporidium cause severe digestive disorders, particularly in children (Hamilton et al., 2018);
- microplastic pollution, a growing global issue affecting not only oceans but also terrestrial and freshwater systems. Microplastics are detected in significant concentrations and may exert cumulative toxic effects on aquatic organisms and potentially on human health (Kirstein et al., 2021);

Eutrophication results from the excessive input of nutrients, notably phosphates and nitrates, into aquatic systems, leading to algal overgrowth and a significant decline in dissolved oxygen levels (Badamasi et al., 2019). Algal blooms may arise from nutrient runoff from agricultural land, inadequate wastewater treatment, and stormwater discharge, particularly in urban areas (Mishra, 2023). This phenomenon impairs the aesthetic quality of water, reduces biodiversity, and can lead to the emergence of toxic cyanobacteria that bioaccumulate in higher trophic levels of the food chain, including humans (Mishra, 2023).

Sediment pollution involves the accumulation of sludge layers in lakes and rivers, reducing water depth and degrading habitats for benthic and planktonic communities (Kong et al., 2018). Increased organic matter content in sediments, combined with elevated summer temperatures, can stimulate microbial proliferation, raise concentrations of toxic metabolites, and further degrade water quality, especially in enclosed or slow-flowing water systems (Zhang et al., 2021).

In this context, integrated analysis of chemical, biological, and physical pollutants forms the basis for timely identification of risks to ecosystems and public health, as well as for guiding effective remediation measures grounded in sustainable biotechnological approaches.

The European Union has adopted a comprehensive water management framework through the Water Framework Directive (European Parliament and Council of the European Union, 2000), based on principles of integrated and sustainable water resource management aimed at achieving good ecological and chemical status for all water bodies (Voulvoulis et al., 2017). In the Republic of Serbia, this field is regulated by the Law on Waters (Official Gazette of the Republic of Serbia, No. 30/2010 and 93/2012), which addresses river basin management, water classification, and source protection. According to current legislation, waters are classified into four classes and two subclasses (IIa and IIb) based on microbiological, physico-chemical, and biological parameters (Table 1). The classification is determined by indicators such as biochemical oxygen demand (BOD), chemical oxygen demand (COD), pH, suspended solids, and the presence of coliform bacteria, and applies to surface water, groundwater, and lake water bodies.

Microbiological contamination of surface and groundwater represents one of the most serious indicators of water quality degradation (Khan & Gupta, 2020). Indicator bacteria such as total coliforms, *Escherichia coli*, and intestinal enterococci are used to assess microbiological safety. Concurrently, BOD and COD are used to evaluate the degree of organic loading and the presence of biodegradable and oxidisable substances in the water (Young & Lipták, 2018). Elevated values of these parameters indicate organic pollution, the risk of eutrophication, and potential formation of anaerobic conditions in sediments (Li et al., 2023).

In slow-flowing or stagnant water bodies, especially those within protected natural areas, the accumulation of sediment and sludge constitutes an increasing ecological and management challenge. A high content of organic matter in sediments, coupled with elevated temperatures, can result in intense microbial activity, deterioration of water quality, and loss of ecological functions of aquatic ecosystems.

Conventional remediation techniques such as dredging, sediment removal, or chemical oxidation are often associated with high financial costs, ecological disturbance, and limited feasibility in protected natural areas. These constraints have driven increasing interest in biologically based, low-impact alternatives that can support ecosystem restoration without major physical alteration. The technology of Effective Microorganisms (EM) represents such an approach, utilising the synergistic action of beneficial bacteria, yeasts, and other microorganisms to enhance organic matter degradation, reduce pathogen loads, and stabilise microbial communities in aquatic ecosystems.

EM applications have been reported to reduce sediment, improve microbial balance, increase dissolved oxygen levels, and mitigate unpleasant odours. Although the effects of EM-based bioremediation are often described as short- to medium-term and highly dependent on site-specific conditions, its practical value lies in providing rapid and environmentally acceptable improvements in water quality, particularly in small, eutrophic, and hydromorphologically constrained water bodies where large-scale engineering interventions are not feasible. In this context, EM-based solutions are consistent with the principles of sustainable resource management and align with the objectives of the United Nations Sustainable Development Goals, including Target 12.4 (environmentally sound management of chemicals and waste) and Goal 13 (climate action), by supporting ecosystem restoration without introducing additional chemical pollution.

Within this framework, Ponjavica Nature Park, a Category III protected natural area located in the municipality of Pančevo, serves as a

Table 1
Macrolocations A and B – treatment by injection/infusion method.

Macrolocation	Sampling site	Treatment method	Injection depth (cm)	EM applied (L)	Water sampling – Before	Water sampling – After (6 days)	Sediment sampling –
A	A1	Sediment injection	30	450	Yes	Yes	Monthly (4 months)
A	A4	Sediment injection	40	450	Yes	Yes	Monthly (4 months)
B	B3	Sediment injection	30	360	Yes	Yes	Monthly (4 months)
B	B8	Sediment injection	30	270	Yes	Yes	Monthly (4 months)

relevant case study. This watercourse, approximately 13 km long, has experienced significant siltation over recent decades. Data from 2006 indicate that the lake contained over 1,350,000 m^3 of sediment. Despite formal protection, Ponjavica is subject to numerous anthropogenic pressures, including eutrophication, organic pollution, water stagnation, and the presence of invasive species (Jaćimović et al., 2022). Biological and physicochemical parameters indicate low ecological value (Karadžić et al., 2013; Jovanović et al., 2016). While existing studies have primarily focused on environmental status assessment, limited attention has been given to evaluating active, field-based remediation options capable of addressing sediment accumulation and microbiological degradation in protected freshwater systems. This identified gap forms the basis for the present study, which evaluates the applicability and environmental acceptability of EM-based bioremediation under real ecological, regulatory, and operational constraints. Based on the above, the aim of this study was to evaluate the effectiveness of treating the lake ecosystem with a preparation based on effective microorganisms, focusing on: (1) reducing the volume of sediment and sludge, (2) improving microbiological and physicochemical water quality parameters, and (3) assessing the potential of EM technology as a sustainable solution for the restoration of protected aquatic ecosystems. In this way, the study provides novel field-based evidence supporting biologically based water revitalisation strategies, demonstrating the practical applicability of EM technology for improving water quality in protected, sediment-impacted freshwater systems, in alignment with the objectives of the 2030 Agenda for Sustainable Development.

Materials and methods

Study area

The field experiment was conducted in Ponjavica Nature Park, a designated Category III protected natural area situated in the municipality of Pančevo, South Banat District, Serbia (Fig. 1). The park includes a slow-flowing stream, Ponjavica, extending approximately 13 km in length, with an average width of 20–30 m and a water depth ranging from 0.5 to 1.5 m, depending on seasonal hydrological conditions. The surrounding landscape is characterised by a mosaic of agricultural land, riparian vegetation, and alluvial deposits, which contribute to diffuse nutrient input, particularly during periods of intense rainfall and runoff. In addition to non-point source pollution, organic matter accumulation and limited water circulation contribute to sediment buildup and microbiological loading. According to the national surface water classification system, parts of the Ponjavica stream are designated as Class II waters. However, field observations and water quality monitoring data suggest progressive eutrophication, siltation, and microbial contamination during the summer months. The site was selected for this study based on its:

1. protected status, making conventional dredging unfeasible;
2. visible sediment accumulation;
3. previous assessments indicating low contamination by heavy metals or toxic substances; and
4. potential for eco-biotechnological restoration using biological agents.

Fig. 1. Geographic location of Ponjavica Nature Park (44°43′36″ N, 20°47′25″ E) showing macro-locations A and B with sampling sites along the 13 km watercourse between Omoljica and Banatski Brestovac.

Two macro-locations were designated for treatment:

1) macro-location A: open water zone with visible surface exposure and minimal aquatic vegetation;
2) macro-location B: semi-vegetated zone with partial macrophyte coverage.

Each macro-location included two monitoring points (A1, A4; B3, B8) used for pre- and post-treatment sampling. The field study was conducted in Ponjavica Nature Park during the summer monitoring period, from June to September 2024. This period was selected to capture post-treatment water quality responses and the short- to medium-term stability of sediment characteristics under field conditions.

Sampling

Water sampling was performed according to standard protocols for representative assessment of surface water quality (Voulvoulis et al., 2017). Samples were collected at three characteristic points along the Ponjavica stream, selected based on accessibility, degree of visible siltation, and representativeness of the upstream–downstream gradient.

At each location, the following samples were collected:

a) water samples for microbiological and physicochemical analyses,
b) sediment samples from the riverbed, using a telescopic sediment probe.

Samples were collected in sterile glass or polypropylene bottles (500 mL) with screw caps, previously autoclaved at 121 °C for 15 min. All sampling procedures were carried out in accordance with ISO standards for surface water and sediment monitoring (International Organization for Standardization (ISO), 2006; International Organization for Standardization (ISO), 2023; International Organization for Standardization (ISO), 2024; International Organization for Standardization (ISO), 2014; International Organization for Standardization (ISO), 2017).

Water sampling was conducted twice, before and after the application of the EM-based treatment, with a six-day interval between sampling events, in accordance with the experimental design (Table 1). Sediment samples were collected as part of a precautionary environmental safety assessment, before and after EM treatment, at monthly intervals over a four-month period. This sampling design allowed evaluation of the temporal stability of sediment-bound heavy metals and verification that the EM-based intervention did not induce metal mobilisation. Heavy metal concentrations in sediment were determined using the validated laboratory method DM 124, with reference to national sediment quality threshold values defined in the Regulation on limit values of pollutants in surface and groundwater and sediment and deadlines for their achievement (Official Gazette of the Republic of Serbia, No. 50/2012).

Meteorological conditions during sampling were recorded, as they may influence microbial activity and organic matter degradation in the aquatic environment. Sampling was conducted under stable summer weather conditions, characterised by predominantly sunny days and average ambient temperatures of approximately 28 °C.

Study design and treatment

The experimental treatment of the lake ecosystem was carried out using a preparation based on Effective Microorganisms (EM), which consists of a consortium of lactic acid bacteria, photosynthetic bacteria, yeasts, and actinomycetes (Çelebi et al., 2023). The preparation and application of the EM solution included the following steps:

1. Activation of the EM solution following a standardised protocol consistent with the manufacturer's recommendations, as previously described and applied by Ćurčić et al. (2026).
2. Application of the activated solution to the water body at a dose of 1 L/m^2 of sediment surface area, conducted over one day using a manual sprayer from a boat.

The study area was divided into two macro-locations based on vegetation cover and hydromorphological characteristics to evaluate the effectiveness of the treatment:

- Macro-location A: open water zone with minimal aquatic vegetation,
- Macro-location B: partially vegetated shoreline zone.

At each macro-location, two sampling points were established for paired measurements before and four weeks after treatment, resulting in a total of four reference points: A1 and A4 within macro-location A, and B3 and B8 within macro-location B. Treatment effectiveness was evaluated based on paired before–after changes in microbiological and physicochemical parameters.

The EM solution was injected at a depth of 30 cm into the sediment at each treatment point. The applied volume was 450 mL per point in macro-location A and 360 mL per point in macro-location B. Sampling included both water and sediment, and all analyses were conducted in accordance with ISO standards. Although untreated zones were present within the study area, systematic control-site data suitable for formal comparative analysis were not collected due to hydrological connectivity and operational constraints.

An overview of treatment and sampling points is shown in Table 1.

Laboratory Analyses

1. Microbiological analyses included enumeration of:
   - Total coliforms (International Organization for Standardization (ISO), 2012),
   - Faecal coliforms and *Escherichia coli* (International Organization for Standardization (ISO), 2012),
   - Intestinal enterococci (International Organization for Standardization (ISO), 2000),
   - Aerobic mesophilic heterotrophic bacteria (International Organization for Standardization (ISO), 1999).
2. Physicochemical analyses of water samples included the following parameters:
   - pH value,
   - Temperature,
   - Biochemical Oxygen Demand (BOD$_5$) (International Organization for Standardization (ISO), 2019),
   - Chemical Oxygen Demand (COD) (International Organization for Standardization (ISO), 2002),
   - Sediment: visual assessment, bulk density, organic matter content (by loss on ignition, International Organization for Standardization (ISO), 2025).

Laboratory equipment and reagents

The following materials were used for laboratory procedures:

BOD$_5$ determination: allylthiourea (2 mg/L final concentration), standardised inoculum, sterile pipettes and Petri dishes, thermostatic incubator, magnetic stirrer.

COD determination: potassium dichromate ($K_2Cr_2O_7$) 0.25 N, silver sulfate (Ag_2SO_4), concentrated sulphuric acid (H_2SO_4, specific gravity $\sim$ 1.84 g/cm^3), ferroin indicator, burettes and titration equipment, electric hot plate, fume hood, safety gloves, and protective goggles.

All analyses were conducted in an ISO/IEC 17025-accredited laboratory (International Organization for Standardization / International Electrotechnical Commission, 2017).

Statistical analysis

Statistical analysis was performed in accordance with the study design and applied separately to (i) microbiological and physicochemical parameters of water and (ii) heavy metal concentrations in sediment.

For microbiological and physicochemical water parameters, statistical evaluation was limited to descriptive analysis and paired pre–post comparisons at each sampling location. As only one measurement before and one after EM treatment was available per site, inferential statistical testing was not applied. Treatment effects were therefore expressed as percentage change between pre- and post-treatment values, calculated for each parameter and location as:

$$\text{Reduction (\%)} = [(\text{C\_before} - \text{C\_after}) / \text{C\_before}] \times 100,$$

where C_before represents the concentration measured before EM application and C_after the concentration measured after treatment.

For sediment heavy metals, descriptive statistics were used to summarise concentration levels and their variability over the four-month monitoring period. As repeated measurements were available for both macrolocations across multiple sampling campaigns, differences between macrolocations A and B were assessed using a paired-sample *t*-test.

Statistical analyses were performed using Python (SciPy library) for water quality parameters and Minitab Statistics (version 17) for sediment heavy metals. The level of statistical significance was set at $p < 0.05$.

Results

Microbiological analysis

The results of microbiological analyses for the collected samples are presented in tables 3 and 4. To facilitate interpretation of these findings, Table 2 presents the water quality classification by microbiological parameters based on threshold values defined in the decree on limit values of pollutants in surface and groundwater and sediments and deadlines for their achievement in accordance with the Law on Waters of the Republic of Serbia (Official Gazette of the Republic of Serbia, No. 30/2010 and 93/2012). This legal framework classifies water bodies into four classes and two subclasses (IIa and IIb), based on microbiological, physicochemical and biological indicators.

A significant improvement in water quality was observed across several microbiological parameters following EM treatment, as shown in Tables 3 and 4 and Figs. 2–5. In particular, total coliforms (TC) showed reductions of up to 50% at some sampling sites. For example, at site A1, the concentration dropped from 13,140 to 6,440 MPN/100 mL, while at A4 it decreased from 10,810 to 9,080 MPN/100 mL (approximately 16% reduction). Similarly, samples from vegetated zones (B3, B8) also showed substantial decreases: for B3, from 6,020 to 4,260 MPN/100 mL (29%), and for B8, from 10,140 to 5,860 MPN/100 mL (42%). Regarding faecal coliform bacteria (E. coli), no measurable effect of the EM treatment was observed, as concentrations remained consistently below the detection threshold of <100 MPN/100 mL across all sampling sites, both before and after treatment. Although these values comply with class I water quality, the lack of detectable change suggests that either the initial contamination level was already low or the analytical method's sensitivity limited the ability to quantify reductions. Intestinal enterococci (ENT) levels also dropped drastically – for example, at site A1 from 940 to <40 MPN/100 mL. At all other sites, levels decreased below quantifiable limits post-treatment. Aerobic heterotrophic bacteria (HPC) counts decreased in all samples as well, for instance from 1,418 to 755 CFU/mL at site A4, indicating an overall reduction of microbial load in the aquatic environment. These results support the effectiveness of EM-based bioremediation in improving selected microbiological aspects of water quality in both open and vegetated zones of the Ponjavica stream, supporting its application in similar freshwater ecosystems. Based on the microbiological results obtained after the EM treatment, water quality improved significantly across all sites (Tables 3 and 4).

Table 2
Water quality classification by microbiological parameters.

Parameter	Unit	Class I	Class II	Class III	Class IV	Class V
Total coliform bacteria	MPN/100 mL	5×10^2	10^3	10^4	10^6	$>10^6$
Fecal coliform bacteria (E. coli)	MPN/100 mL	10^2	10^3	10^4	10^5	$>10^5$
Intestinal enterococci	MPN/100 mL	2×10^2	4×10^2	4×10^3	4×10^4	$>4 \times 10^4$
Aerobic heterotrophic bacteria	CFU/mL	5×10^2	10^4	10^5	$7{,}5 \times 10^5$	$>7{,}5 \times 10^5$

Legend: MPN/100 mL-Most Probable Number per 100 ml; CFU/mL-Colony Forming Units per milliliter; * – Water quality classes are defined according to the Serbian Regulation on limit values of pollutants in surface and groundwater and sediment (Official Gazette of the Republic of Serbia, No. 50/2012), which is aligned with the principles of the EU Water Framework Directive (2000/60/EC).

Categorisation of water quality according to national legislation (Law on Waters and the related Decree on threshold values) showed that E. coli and intestinal enterococci concentrations were consistently within class I, with E. coli values remaining below the analytical detection limit throughout the study period. Aerobic heterotrophic bacteria (HPC) values corresponded to class II, indicating moderate levels of organic matter degradation. Total coliform bacteria (TC) levels placed the water within class III, which, although not pristine, denotes acceptable environmental quality for surface waters. This post-treatment categorisation confirms that the EM-based intervention was effective in shifting the overall microbial status of the water towards higher quality classes, contributing to improved ecological conditions and compliance with national water quality standards.

Physicochemical analyses

The results of physicochemical analyses are summarised in Table 6 and Fig. 6, while water quality classification criteria for the relevant parameters are provided in Table 5, in accordance with the Decree on Limit Values of Pollutants in Surface and Groundwater and Sediments and Deadlines for Their Achievement, as defined by the Law on Waters (Official Gazette of the Republic of Serbia, No. 30/2010 and 93/2012). The evaluated parameters included Biochemical Oxygen Demand (BOD_5) and Chemical Oxygen Demand (COD), which serve as key indicators of organic pollution in aquatic environments.

To facilitate interpretation of treatment effects, graphical representations of the results before and after the EM treatment are shown in Figs. 5 and 6, respectively, for BOD_5 and COD concentrations across sampling points (A1, A4, B3, and B8).

Water quality classes for physicochemical parameters are defined according to the Serbian Regulation on limit values of pollutants in surface and groundwater and sediment (Official Gazette of the Republic of Serbia, No. 50/2012), which represents the national implementation of the EU Water Framework Directive (2000/60/EC).

A significant reduction in BOD_5 concentrations was observed at all sampling points following the application of the EM-based treatment. Before treatment, BOD_5 values ranged from 4.2 to 8.5 mg O_2/L, corresponding to class III or IV water. After treatment, all measured BOD_5 values fell below 3.0 mg O_2/L, meeting or exceeding the criteria for class II water quality. These results indicate a marked improvement in the biological condition of the water, particularly at sites A1 and A4 (surface water), as well as B3 and B8 (vegetated zones), where the reduction reached up to 50%. The observed decrease in BOD_5 reflects enhanced microbial decomposition of organic matter, leading to reduced oxygen demand in the aquatic environment. During treatment, effective microorganisms oxidise organic compounds into carbon dioxide and water.

Table 3
Microbiological results before and after EM treatment.

Location	TC		Reduction (%)	FC (E. coli)		ENT		Reduction (%)	AHB		Reduction (%)
	Before	After		Before	After	Before	After		Before	After	
A1	$13{,}14 \times 10^3$	$6{,}44 \times 10^3$	51,0	$<10^2$	$<10^2$	$9{,}4 \times 10^2$	$<4 \times 10^1$	95.7	$1{,}018 \times 10^3$	$9{,}64 \times 10^2$	5.3
A4	$10{,}81 \times 10^3$	$9{,}08 \times 10^3$	16,0	$<10^2$	$<10^2$	$9{,}64 \times 10^2$	$<4 \times 10^1$	95.9	$1{,}418 \times 10^3$	$7{,}55 \times 10^2$	46.8
B3	$6{,}02 \times 10^3$	$4{,}26 \times 10^3$	29.2	$<10^2$	$<10^2$	4×10^1	$<4 \times 10^1$	–	$7{,}36 \times 10^2$	$7{,}55 \times 10^2$	+2.6
B8	$10{,}14 \times 10^3$	$5{,}86 \times 10^3$	42.2	$<10^2$	$<10^2$	3×10^2	$<4 \times 10^1$	86.7	$1{,}064 \times 10^3$	$9{,}73 \times 10^2$	

TC – total coliforms, FC – faecal coliforms (E. coli), ENT – intestinal enterococci, AHB – aerobic heterotrophs (Köhl), $<10^2$ – below detection limit; Microbiological indicators can be interpreted in relation to EU bathing water assessment frameworks (European Environment Agency, 2021). Results are reported as MPN/100 mL; therefore, comparisons with EU criteria, commonly reported as CFU/100 mL, should be considered indicative.

Table 4
Water quality categorisation after EM treatment by parameter and location.

Parameter	A1	A4	B3	B8	Post-treatment values	Water Quality Class
TC	6,440	9,080	4,260	5,860	$<10^3$MPN/ 100 mL	Class III
FC (E. coli)	<100	<100	<100	<100	$<10^2$ MPN/ 100 mL	Class I
ENT	<40	<40	<40	<40	<40 MPN/ 100 mL	Class I
AHB	964	755	755	973	$<10^3$ CFU/mL	Class II

Legend: TC – total coliforms, FC – faecal coliforms (E. coli), ENT – intestinal enterococci, AHB – aerobic heterotrophs (Köhl); Post-treatment categories are defined according to national legislation (Official Gazette of the Republic of Serbia, No. 50/2012). For international context, categories may be indicatively discussed with reference to EU bathing water assessment guidance (European Environment Agency, 2021).

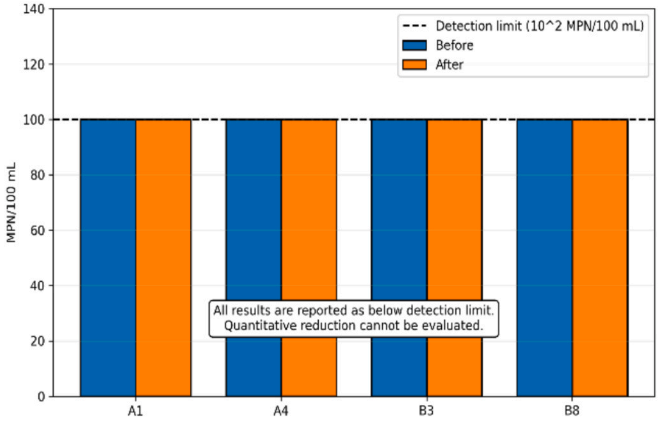

Fig. 3. Faecal coliform bacteria (E. coli).

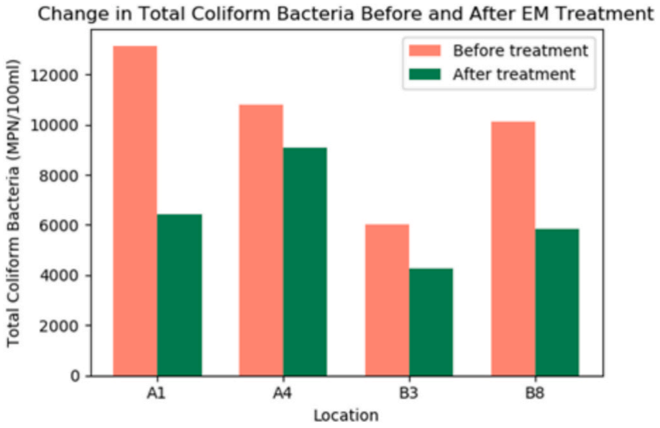

Fig. 2. Total coliform bacteria before and after EM treatment.

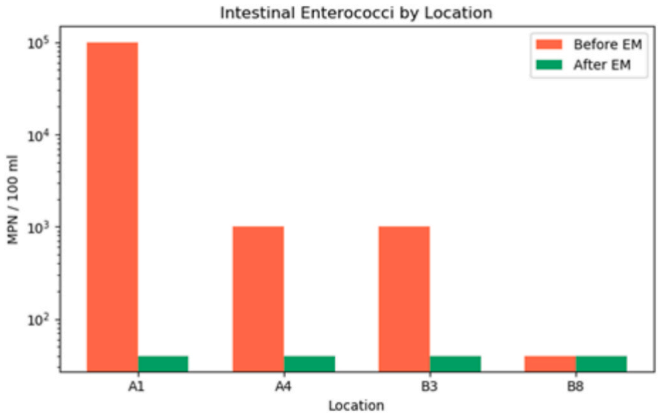

Fig. 4. Intestinal enterococci.

Once most biodegradable matter is metabolised, oxygen demand diminishes, as shown by lower BOD_5 values.

COD values showed a more heterogeneous trend. While COD concentrations at B3 and B8 declined after treatment (e.g., from 67.7 to 53.3 mg O_2/L), indicating successful reduction of total organic load, values at A1 and A4 showed a slight increase (from 67.9 to 98.9 mg O_2/L and from 60.3 to 77.4 mg O_2/L, respectively). This was likely due to a higher dose of EM solution applied at these points and deeper injection into sediment layers, which triggered more intense microbial degradation of sediment-bound organic material. This process may have mobilised additional oxidisable compounds, temporarily increasing COD values. Moreover, the physical disturbance of the sediment could have released inorganic particles (e.g., carbonates, salts, sand granules), contributing to the elevated chemical oxygen demand.

Despite the transient COD increase at some sites, the overall results support the effectiveness of the EM-based treatment in reducing the total organic burden. The substantial and consistent decrease in BOD_5 across all sampling locations demonstrates that the microbial agent facilitated improved water quality by enhancing the breakdown of biodegradable organic matter. This finding confirms the potential of EM applications for the restoration of water bodies impacted by organic pollution.

Threshold values according to the Regulation on limit values of pollutants in surface and groundwater and sediment (Official Gazette of the Republic of Serbia, No. 50/2012, Annex 3) are provided for reference only. No formal regulatory classification of sediment quality was performed.

Descriptive statistics of heavy metal concentrations in sediment collected at macrolocations A and B during four sampling campaigns are presented in Table 7. Overall, the concentrations of the analysed toxic elements showed low variability and no evident temporal trends throughout the four-month monitoring period.

Notably, cadmium (Cd) and mercury (Hg) concentrations were

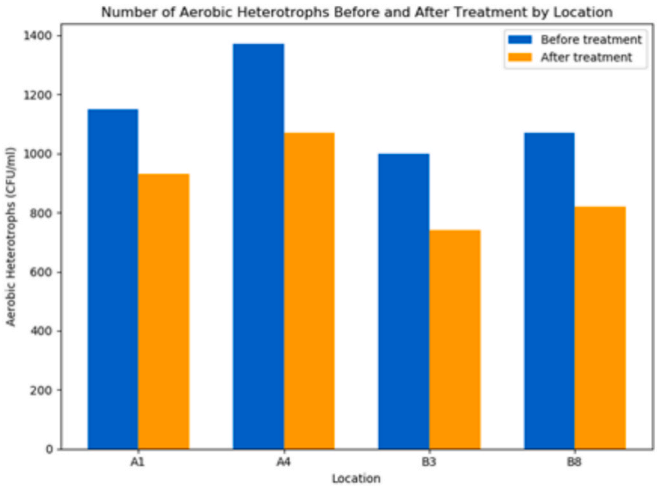

Fig. 5. Number of aerobic heterotrophs (Köhl).

below the limits of detection in all sediment samples from both macrolocations, indicating the absence of measurable contamination by these elements during the study period. The applied analytical methods had detection limits of 0.80 mg/kg for Cd and 0.25 mg/kg for Hg. In addition, lead (Pb) concentrations at macrolocation B were consistently below the limit of detection (5.0 mg/kg) across all sampling campaigns, while measurable Pb levels were detected only at macrolocation A.

Cobalt (Co) and nickel (Ni) were detected in sediment samples from both macrolocations, with descriptive statistics indicating differences in their concentration ranges between groups A and B. It should be noted that in a single sediment sample collected at macrolocation B, the concentration of nickel (Ni) reached 59.14 mg/kg, exceeding the maximum allowable concentration of 44 mg/kg, whereas Ni concentrations in the remaining samples were within the prescribed limits. Consequently, Co and Ni represented the main contributors to the observed variability in sediment metal profiles, while Cd, Pb, and Hg did not contribute to temporal or spatial variability due to concentrations remaining below analytical detection limits.

Discussion

The primary aim of this study was to evaluate whether an effective microorganisms (EM) preparation could improve the microbiological and physicochemical quality of a eutrophic, sediment-impacted small watercourse under field conditions. Using ISO-standardised analytical methods, we documented measurable post-treatment improvements in key microbial indicators, including total coliforms, *Escherichia coli*, intestinal enterococci and heterotrophic plate counts, as well as reductions in BOD_5 at both open-water and vegetated sites. These observed changes should be interpreted within the context of EM-based bioremediation as a supportive, short-term intervention rather than a replacement for conventional remediation technologies. Overall, the results indicate a measurable improvement in water quality following EM application under field conditions.

Although water quality in this study was formally assessed using the Serbian legal framework (Official Gazette of the Republic of Serbia, No. 50/2012), post-treatment results were additionally contextualised using internationally recognised approaches to improve comparability. Microbiological indicators (E. coli and intestinal enterococci) were discussed with reference to EU bathing water assessment guidance (Guidelines for the assessment under the Bathing Water Directive, 2021), while physicochemical parameters were interpreted within the ecological status concept of the EU Water Framework Directive (2000/60/EC). Where appropriate, WHO guideline principles were used to

Table 5
Water quality classification – physicochemical parameters.

Parameter	Unit	Class I	Class II	Class III	Class IV	Class V
Biochemical Oxygen Demand (BOD_5)	mg O_2/L	– (or below detection)	–	7	25	>25
Chemical Oxygen Demand (COD)	mg O_2/L	<10 (or below detection)	15	30	125	>125

Table 6
Physicochemical results before and after EM treatment.

Location	BOD_5		Reduction (%)	COD		Change (%)
	Before	After		Before	After	
A1	8,5	<3	64.7	67,9	98,9	+45.7
A4	6,3	<3	52.4	60,3	77,4	+28.4
B3	4,2	<3	28.6	67,7	53,3	−21.3
B8	4,8	<3	37.5	66,7	66,9	+0.3

Legend: BOD_5 – Biochemical Oxygen Demand, COD – Chemical Oxygen Demand; Physicochemical parameters are classified according to national regulatory thresholds, which are consistent with the EU Water Framework Directive approach to ecological status assessment (Directive 2000/60/EC). International comparability is therefore ensured at the conceptual level rather than through fixed universal numerical thresholds.

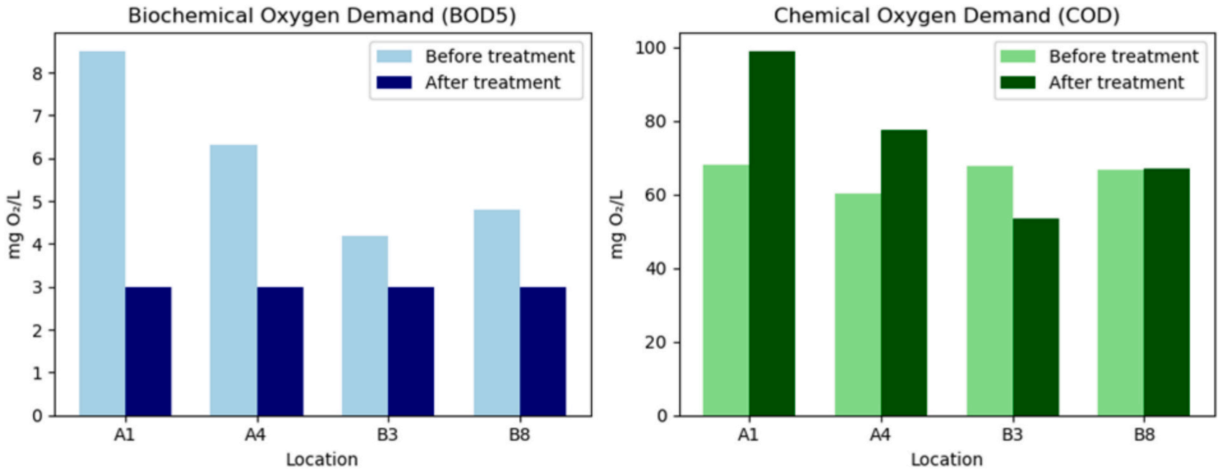

Fig. 6. Biological and chemical oxygen demand of location samples.

Table 7
Descriptive statistics of heavy metal concentrations in sediment at macrolocations A and B during four sampling campaigns.

Element	Location	N	Mean ± SD	Min	Max	Target value	MPL	Remediation value
			mg/kg					
Cd	A	16	<LOD			0,80	6,40	55
	B	16	<LOD					
Co	A	16	8,12 ± 1,42	6,0	10,19	–-	–-	–-
	B	16	4,94 ± 0,13	4,90	5,42			
Ni	A	16	43,84 ± 11,70	27,60	59,14	35	44	210
	B	16	19,05 ± 5,56	11,61	26,52			
Pb	A	16	21,65 ± 30,45	<LOD	133,0	85	310	530
	B	16	<LOD					
Hg	A	16	<LOD			0,3	1,6	10
	B	16	<LOD					

N- number of analysed samples; <LOD: Cd < 0.80 mg/kg; Pb < 5.0 mg; Hg < 0.25 mg/kg.

provide broader public health context for indicator organisms, while recognising differences in intended water use (drinking versus surface/recreational waters). For clarity and a more systematic interpretation, the discussion is structured into two subsections addressing (i) Microbiological Indicators and (ii) Physicochemical Parameters.

Microbiological indicators

Our post-treatment findings of marked reductions in intestinal enterococci and total coliforms are consistent with a broad body of literature demonstrating the efficacy of EM in suppressing faecal indicator bacteria (FIB) in both natural and engineered aquatic systems. *Escherichia coli* concentrations remained below the analytical detection limit both before and after treatment and therefore did not allow quantification of treatment-related change. Similar outcomes have been reported in diverse environmental contexts, where EM application frequently achieved over 99% removal of total and faecal coliforms, with E. coli levels often reduced below detection thresholds (Kaur et al., 2024; Besarab & Novik, 2016). Previous study results indicate that EM can be one of the most effective methods for removing unfavourable microorganisms from water – after the application of EM, a reduction in their concentration was observed, consistent with our results (Tomczyk et al., 2024). These results are particularly notable in light of the documented resilience of environmental E. coli strains. As emphasised by Erb et al. (2024), many strains inhabiting aquatic and sediment environments exhibit strong adaptations to extraintestinal niches, including biofilm formation and enhanced stress tolerance, which can hinder their removal by conventional remediation approaches. The observed reductions therefore support the hypothesis that EM may disrupt biofilm-associated persistence mechanisms in addition to exerting competitive and enzymatic suppression. Total coliforms could be present in significantly lower amounts because of a clear reduction after EM treatment; for faecal enterococci, a significant decrease was observed, although values later returned to approximately the previous level but remained within safe limits; and for E. coli (faecal coliforms), there are indications that the overall effect on coliform bacteria is pronounced (Dobrzyński et al., 2022). Evidence from field-based interventions further supports this interpretation. Malaysian river restoration projects employing EM, either as activated solution (EMAS) or in mudball form, have demonstrated substantial reductions in pathogenic microorganisms, improved in situ Water Quality Index (WQI) classifications from Class IV to Class III, and decreased concentrations of ammonia, hydrogen sulphide, and methane (Firdaus & Azman, 2018; Wahid & Azman, 2016). Comparable applications in South African surface waters yielded significant reductions in turbidity and phosphate levels, although ammonia removal was less pronounced, indicating that microbial and chemical responses to EM can vary by parameter (Chooka, 2010). Laboratory-scale experiments reinforce these field observations. Comparative analysis (Shan et al., 2009) showed that the multi-component microbial preparation can significantly enhance the water's self-purification capacity, reduce turbidity, inhibit algal growth, and gradually improve water quality, all at substantially lower costs. Thus, the problem of lake eutrophication can be effectively and sustainably resolved through bioremediation by EM. Also, with this method of EM application, the treated water showed significantly improved quality in terms of microbiological safety. Sitarek et al. (2017) confirm an improvement in water quality in a general sense – both microbiologically and visually. EM inoculation has been shown not only to reduce coliform counts but also to shift microbial community composition towards beneficial taxa such as lactic acid bacteria and photosynthetic bacteria, thereby enhancing biodiversity and aquatic animal health (Çelebi et al., 2023). Decay rates of faecal indicator bacteria (FIB) in aquatic environments are influenced by factors such as sunlight, temperature, predation, and turbidity, with persistence often prolonged in shaded or sediment-rich systems (Korajkic et al., 2019). This underscores the relevance of EM-mediated improvements in our study, as they appear to have overcome environmental conditions that typically favour FIB persistence and sediment-associated regrowth. The persistence of these microbiological benefits depends on both application strategy and site-specific hydrology. Studies emphasise that periodic reapplication and upstream pollution control are critical to sustaining low contamination levels, as untreated inflows can rapidly reintroduce pathogens (Byappanahalli et al., 2006). Liquid EM applications, such as those used here, can provide immediate and pronounced effects, whereas slow-release formulations – such as EM mudballs – may extend the duration of active microbial presence in the water column (Safwat & Matta, 2021; Park et al., 2016). An additional consideration is the role of sediment–water interactions in FIB dynamics. Vegetated zones often serve as bacterial reservoirs, with sediment-associated populations capable of reseeding the water column during disturbance events (Korajkic et al., 2019). The comparable reductions observed across both vegetated and open-water habitats in our case suggest that EM acted effectively at both interfaces, limiting recontamination potential and contributing to more sustained microbial quality improvements. Collectively, these findings align with extensive literature and reinforce the suitability of EM as a low-cost, scalable intervention for controlling microbial contamination in small, eutrophic water bodies – particularly where conventional treatment infrastructure is unavailable or impractical.

Physicochemical parameters

Improvements in physicochemical water quality following EM application have been reported across a range of aquatic systems, with consistent benefits observed in parameters such as biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), and nutrient concentrations. From a management perspective, such changes are relevant as indicators of improved system functionality, even if they do not imply complete remediation of accumulated sediments or organic loads. Zakarya et al. (2025) documented that EM treatment of degraded lake water led to substantial reductions in BOD_5 and ammoniacal nitrogen, moderate COD decreases, and an upgrade in water quality classification according to both the Water Quality Index (WQI) and National Water Quality Standards (NWQS). Similar classification improvements have been achieved in Malaysian rivers and canals following EM application, often associated with a measurable decline in organic and nutrient loads (Firdaus & Azman, 2018; Sharip et al., 2020).

In our study, BOD_5 decreased consistently across all sampling sites, mirroring the magnitude and direction of changes reported by Zakarya et al. (2025) and Firdaus and Azman (2018). The latter achieved high removal efficiencies for ammonia (96%) and phosphorus (100%) in industrial wastewater, while COD reduction remained more modest – an outcome closely aligned with our findings. Such divergence between BOD_5 and COD improvements is frequently observed during early stages of bioremediation, particularly in sediment-impacted systems where organic matter pools contain both readily biodegradable and refractory fractions.

One notable difference between our results and several prior studies lies in the transient COD increase observed at sites A1 and A4 immediately after treatment. While most literature reports steady COD declines, occasional post-treatment COD spikes have been described (Safwat & Matta, 2021; Park et al., 2016), which have been hypothesised to result from desorption and mobilisation of sediment-bound organics during enhanced microbial and oxidative activity. In the absence of complementary measurements such as total suspended solids (TSS) or dissolved organic carbon (DOC), this interpretation remains hypothetical. In our case, these increases coincided with higher EM dosing and deeper injection, suggesting a plausible association between site-specific application methods and short-term COD trajectories, without necessarily undermining longer-term improvements in organic load. Nutrient reduction trends in our study were also broadly consistent with previous reports. Ammoniacal nitrogen declined markedly post-treatment, in line with the >90% reductions reported by Firdaus and Azman (2018) and Zakarya et al. (2025). Orthophosphate levels decreased but did not reach the near-complete removal observed in some controlled wastewater studies, a difference likely reflecting the continuous inflow of nutrient-rich water in our open-system field conditions. This highlights a key contextual factor: while closed or semi-closed systems can achieve near-total nutrient removal, flowing natural water bodies are more susceptible to re-enrichment from upstream sources. The mechanistic basis for these physicochemical improvements parallels that observed in microbial indicators. EM consortia – comprising lactic acid bacteria, photosynthetic bacteria, actinomycetes, and fermentative fungi – accelerate the decomposition of organic matter through enhanced aerobic and facultative metabolic pathways. Similar acceleration effects have been documented outside aquatic environments; for example, Nursita and Nurwahyuni (2025) found that EM4 increased the decomposition rate and nutrient availability in goat manure–water hyacinth compost. These terrestrial findings reinforce the broader principle that EM promotes rapid organic matter turnover, nutrient cycling, and reduction of oxygen demand across environmental contexts.

Sediment heavy metal analysis was included as a precautionary environmental safety component, complementing the microbiological and physicochemical assessment of water quality. The observed stability of sediment-bound metal concentrations throughout the four-month monitoring period confirms that the EM-based treatment did not adversely affect the chemical integrity of the sediment, thereby supporting its safe application in protected aquatic environments.

The absence of detectable concentrations of cadmium (Cd) and mercury (Hg) in all sediment samples, along with consistently non-detectable lead (Pb) levels at macrolocation B, indicates a generally low level of inorganic contamination in the study area. Although cobalt (Co) and nickel (Ni) were present in sediments from both macrolocations, their concentrations predominantly remained within regulatory thresholds, with a single exceedance of the maximum allowable value for nickel at macrolocation B most plausibly reflecting site-specific geochemical conditions rather than a treatment-related effect. Importantly, none of the analysed metals exceeded the remediation values prescribed by national sediment quality regulations.

Against this background of demonstrated chemical stability, the observed changes in microbiological and physicochemical water quality parameters can be interpreted with greater confidence. Overall, our results show strong agreement with the majority of published studies regarding the direction and relative magnitude of changes in BOD_5, COD, and nutrient concentrations following EM application. Minor deviations – such as transient COD increases and less pronounced phosphate removal – are consistent with differences in hydrological setting, dosing strategy, and system openness. These findings underscore the importance of tailoring EM application protocols to site-specific environmental conditions to maximise and sustain physicochemical benefits. In this context, the primary value of EM application lies in its feasibility and environmental compatibility in protected freshwater systems, where financial, technical, and regulatory constraints often limit the implementation of large-scale engineering interventions.

Limitations of the study

This study has several limitations. (i) The assessment was based on a short post-treatment monitoring period, limiting the evaluation of long-term and seasonal effects. (ii) The number of sampling sites was limited, and only single pre- and post-treatment measurements were available per site, restricting statistical inference to descriptive analysis. (iii) Although untreated areas existed within the study system, systematically collected control-site data suitable for formal comparative analysis were not available. (iv) For some microbiological parameters, particularly *Escherichia coli*, concentrations remained below analytical detection limits before and after treatment, preventing quantitative assessment of treatment-related changes. These limitations indicate the need for future studies incorporating longer monitoring periods, repeated sampling, and, where feasible, comparative reference sites to strengthen causal inference and generalisability.

Conclusion

This study demonstrated that the application of Effective Microorganisms can deliver measurable improvements in the microbiological and physicochemical quality of water in a eutrophic, sediment-impacted small watercourse under field conditions. Post-treatment analyses showed reductions in total coliforms, *Escherichia coli*, and intestinal enterococci, with E. coli concentrations falling below detection limits, alongside consistent decreases in BOD_5, indicating enhanced short-term biodegradation of organic matter. Variations in COD reflected site-specific sediment dynamics rather than uniform treatment effects.

Importantly, these findings should be interpreted within the context of EM-based bioremediation as a supportive and environmentally compatible management tool, rather than as a substitute for conventional remediation technologies. While the effects observed are primarily short-term and influenced by local environmental conditions, the practical value of EM application lies in its feasibility, low cost, and minimal ecological disturbance – particularly in protected or small freshwater systems where financial, technical, and regulatory constraints limit the implementation of large-scale engineering interventions. In this respect, EM-based treatments can contribute to improving water quality and system functionality where alternative options are not readily applicable. To further validate and optimise the observed effects, future research should focus on controlled experimental designs with varied dosing strategies, repeated applications, and multi-seasonal monitoring of ecological, chemical, and microbiological parameters. The inclusion of sediment characterisation and biodiversity assessments would provide a more comprehensive understanding of environmental responses. In addition, pilot studies in other eutrophic or stagnant water bodies are recommended to assess the broader applicability and transferability of this approach.

CRediT authorship contribution statement

Srećko Ćurčić: Validation, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Dragan Milićević:** Writing – original draft, Writing – review & editing. **Vesna Veličković:** . **Milorad Domanović:** . **Aleksandar Peulić:** Validation, Supervision, Software, Methodology, Data curation, Conceptualization. **Dejan Ž. Veljković:** Visualization, Methodology, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

The datasets generated for this study are available from the corresponding author upon reasonable request, due to privacy or ethical restrictions.

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