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The Potential of Micro-Dictum Preparation in Surface Water Reclamation Subject to Strong Anthropogenic Pressure

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Abstract: The aim of this research was to analyze the potential of e micro-dictum preparation containing compositions of beneficial microorganisms using this product in surface water reclamation. The experiments were carried out in 2016. The scope of this research included the analysis of the physical and chemical properties of a solid preparation; tests of the microbiological parameters of micro-dictum; an analysis of the spread of microorganisms in the aquatic environment; a study of water quality with the solid preparation; and tests of the formulation in real conditions and its potential in the reclamation of surface waters. Tests on the produced formulation were carried out in the laboratory in containers and under real conditions. Laboratory tests have shown that the analyzed preparation may introduce certain amounts of nitrogen and phosphorus into the water. However, they are not important in the case of water reclamation. Analyses of the micro-dictum preparation showed that the content of lactic acid bacteria in the center of the ball is lower compared to the outer layers. The results describing an increase in the number of lactic acid bacteria correlate with a decrease in pH and oxygen dissolved in the water with the preparation. The tests showed no negative impact on changes in the physical and chemical properties of water at the site of application. Changes in physical parameters were recorded, in particular dissolved oxygen and pH at the bottom, where the greatest microbiological activity occurred.

Keywords: micro-dictum; water reclamation; microorganism preparation; freshwater ecosystem

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1. Introduction

Climate change, which causes extreme weather events such as heavy rainfall or droughts, is exacerbating the degradation of aquatic ecosystems. As temperatures rise, the rate of degradation of water bodies accelerates. This is primarily linked to increased pollution levels, reduced biodiversity, and the emergence of invasive organisms or species that were previously absent in these ecosystems. Therefore, there is a need to adapt reculti-vation methods to the amount of anthropopressure and extreme weather conditions. Water is a resource crucial for sustaining life on Earth and also ensures economic development. Unfortunately, many water bodies around the world are polluted and require reclamation to be safe for various uses. It is estimated that about 80% of wastewater generated globally is discharged into rivers and seas without treatment, posing a significant threat to human health, other living organisms, and ecosystem functioning. There is a scarcity of global data on the number of water bodies requiring reclamation. However, according to a 2023 UN report, approximately 2 billion people worldwide lack access to safe drinking water, and 3.6 billion people do not have access to safe sanitation services [1]. The UN stresses the need for close cooperation and better protection of water resources, emphasizing that access to water should be a right, not a commodity.

According to estimates, around 70% of rivers and lakes in China are polluted. Half of Chinese cities have heavily polluted groundwater, and one-third of the country is affected by acid rain [2–4]. For years, pollution was caused by Western corporations that located their factories in China. In Poland, 99.4% of river waters and 77% of lake waters are in

poor conditions [5] and do not meet the standards required by the European Union's Water Framework Directive [6]. Poland has one of the lowest water availability rates in Europe.

Water consumption worldwide has been increasing by about 1% annually since the 1980s. This growth is driven primarily by population growth, industrial and agricultural development, and changing consumption patterns. According to a UNESCO report [7], water demand will continue to grow at a similar rate until 2050. Currently, over 2 billion people live in countries experiencing high water scarcity, and around 4 billion people face severe water shortages for at least one month each year. Water management encompasses the management of surface water and groundwater, as well as water reclamation and reuse. In 2016, 29 out of 48 Asian countries (Asia–Pacific) did not have sufficient water due to low availability and excessive groundwater exploitation. Water scarcity is exacerbated by the effects of climate change, leading to more frequent and intense natural disasters. In Latin America and the Caribbean, millions of people lack access to adequate drinking water sources. Another issue is the lack of proper infrastructure for waste disposal and wastewater management. Many of those deprived of services live in suburban areas on the outskirts of cities [7].

In 2015, three out of five rural residents in Sub-Saharan Africa had access to at least a basic water supply, but only one in five had access to at least basic sanitation facilities. Around 10% of the population still drank untreated surface water, and many poor rural residents, especially women and girls, spent significant amounts of time collecting water. Rural inhabitants of Sub-Saharan Africa make up about 60% of the total population. Over half of the population growth expected by 2050 (1.3 billion out of 2.2 billion globally) will occur in Africa, increasing the demand for water in this part of the world [7].

Global water resources and access to available water are decreasing every year, partly due to its deteriorating quality. The loss of water bodies with good quality, hydromorphological changes, increased pollutants, and the spread of invasive species are issues affecting both developing and developed countries [8]. A poor water quality has a direct impact on human health, increasing health risks. Diseases such as cholera and schistosomiasis remain widespread in many developing countries, where only a very small fraction (in some cases less than 5%) of wastewater is treated before being released into the environment [9].

During the implementation of the Millennium Development Goals (MDGs) from 2000 to 2015, the proportion of the global population with access to basic drinking water services increased from 81% to 89% [8]. Nutrient loads remain one of the most widespread forms of water pollution, with most nutrient emissions coming from agriculture. It is predicted that nutrient emissions into surface waters will increase in most regions, with the largest problems occurring in South and East Asia, parts of Africa, and Central and Latin America. In the near future, rapidly growing cities will become the main sources of nutrient emissions [10].

In 2015, 181 countries were able to provide access to drinking water for over 75% of their populations. Of the 159 million people still using contaminated drinking water directly from surface sources, 58% lived in Sub-Saharan Africa [11].

Due to the problem of degradation of surface waters around the world, caused by anthropopressure and climate change, there is an urgent need for their reclamation. This is the process of restoring the natural properties of an aquatic ecosystem that has been disrupted by human activity. Surface water pollution leads to ecosystem degradation and loss of biodiversity. Microorganisms can play an important role in the reclamation process by improving the quality of surface waters [12–14]. Microorganisms, such as bacteria and algae, can break down organic pollutants, including oils, fats, and pesticides, through biodegradation. Moreover, some species of microorganisms, such as cyanobacteria, can bind heavy metals, helping to remove this type of contamination from water. Microorganisms are also used to remove petroleum-based pollutants, both in water and, for example, from soil. Bacteria can transform mineral oils or gasoline into less toxic organic compounds.

Thus, microorganisms play a crucial role in the reclamation of surface waters [15]. Their use can help eliminate a wide range of pollutants, which may contribute to restoring

the natural state of aquatic ecosystems. There are many species of microorganisms used in water reclamation or for improving the parameters of bottom sediments. One such group is nitrifying bacteria, such as *Nitrosomonas europaea*, *Nitrosococcus oceani*, *Nitrosospira briensis*, and *Nitrobacter winogradskyi*, which are used to remove ammonia and nitrites from water. These bacteria convert ammonia and nitrites into nitrogen compounds that are less harmful to the environment [16–19]. Another group used to improve aquatic ecosystems comprises denitrifying bacteria (*Pseudomonas stutzeri*, *Pseudomonas aeruginosa*, *Paracoccus denitrificans*, *Alcaligenes faecalis*). These bacteria use nitrates as an oxygen source, converting them into molecular nitrogen [20–22].

To remove phosphorus compounds from water, polyphosphate-accumulating organisms (PAOs) (such as *Candidatus Phosphoribacter*, *Candidatus Accumlibacter* phosphatis, *Candidatus Dechloromonas*, *Candidatus Accumulimonas*, *Microlunatis phosphovorus*, *Pseudomonas* spp., and *Paracoccus denitrificans*) or other phosphorus-absorbing bacteria (*Acinetobacter johnsonii*, *Bacillus cereus*, *Bacillus thuringiensis*, and *Pseudomonas fluorescens*) are used. These organisms can remove phosphorus by binding it within their cells or converting it into compounds that are easier to eliminate from water [23,24]. Heterotrophic bacteria (such as *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Saccharomyces boulardii*, *Enterococcus faecium*, and *Staphylococcus aureus*) are also used in water reclamation. They aid in the removal of organic pollutants from water, such as fats, oils, proteins, carbohydrates, or acrylamide. These bacteria can break down such compounds into less harmful substances [25–28].

In addition to bacteria, algae (e.g., *Chlorella vulgaris*, *Scenedesmus obliquus*, *Spirulina platensis*, and *Nannochloropsis* sp.) or consortia such as the algal–algal consortium, the microalgae–bacteria consortium, or the microalgae–fungi consortium can also be used in reclamation [29–31]. Algae are indispensable organisms for aiding the removal of nitrogen compounds, phosphorus, and metallic elements from water. They can absorb these compounds and store them in their cells. Fungi, on the other hand, can be used to remove heavy metals or polycyclic aromatic hydrocarbons from water or soil (e.g., *Penicillium chrysogenum*, *Aspergillus niger*, *Trichoderma viride*, and *Rhizopus stolonifer*) [32,33].

The general aim of this research was to analyze the potential of a micro-dictum solid preparation containing compositions of beneficial microorganisms for use in surface water reclamation. The specific objectives of this research were the following: (a) to analyze the physical and chemical properties of the solid preparation containing compositions of beneficial microorganisms (micro-dictum); (b) to test the microbiological parameters of the micro-dictum; (c) to analyze the spread of microorganisms in the aquatic environment; (d) to study the water quality with the solid formulation; and (e) to conduct real-world tests of the preparation and evaluate its potential in surface water reclamation.

2. Materials and Methods

2.1. Tests of the Physical and Chemical Parameters of the Micro-Dictum

The micro-dictum preparation consisted of ceramic clay (sterile) (70% of the ball), beet molasses, wheat bran, water in appropriate proportions, and microbial mother cultures. All ingredients needed to be properly mixed so that the preparation would be homogeneous and a small ball could be formed from it (Figures 1 and 2). Then, the balls were fermented and dried at a temperature of approximately 20 °C for 5 days. The average weight of the raw ball was 300 g; however, after drying, it was 180 g. A ball shape is the most ergonomic because it ensures a uniform dissolution process and a slow but uniform release of microorganisms, which is important for the efficiency of the reclamation process.

Separately, for each of the 5 macro-dictum balls, analyses were performed for the concentration of total nitrogen (TN) (Kjeldahl titration method), total phosphorus (TP) (MP-AES spectrometry method after microwave digestion), and the pH value (potentiometric method) in samples from whole balls.

Figure 1. Cross-section of micro-dictum balls.

Figure 2. Micro-dictum balls ready for use.

2.2. Tests of the Microbiological Parameters of the Micro-Dictum

Microbiological tests for 5 selected preparation balls distinguished them into individual layers: ball surface up to 1 cm, depth up to 2–3 cm, and center of the ball (Figure 3). The following biological parameters were analyzed:

(a) Number of lactic acid bacteria on MRS culture medium with the addition of 0.1% cyclohexamide solution (plate method), incubation in relatively anaerobic conditions at 37 °C for 48–72 h (test procedure based on PN-EN ISO 15214:2002);
(b) Number of aerobic microorganisms on PCA (plate count agar) culture medium (plate method), under incubation conditions of 28 °C for 5–7 days (PN-EN ISO 4833:2004 + Ap1:2005);
(c) Number of fungi on DRBC culture medium (plate method), under incubation conditions of 24 °C for 5–7 days (PN-EN ISO 21527-2:2009).

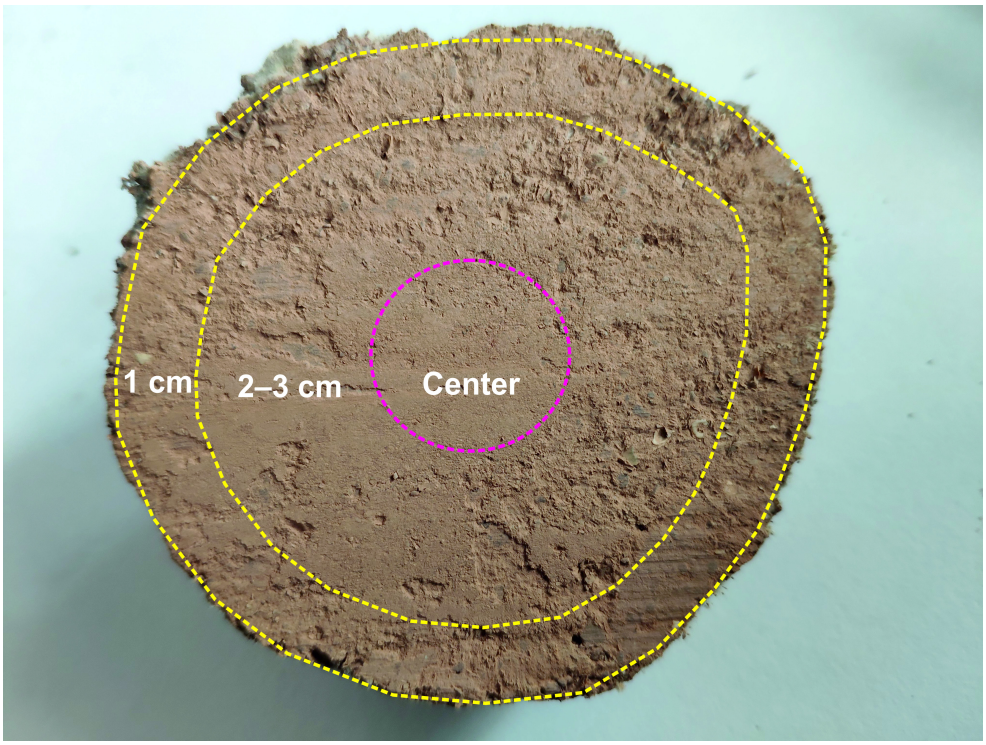

Figure 3. Visualization of a cross-section of a micro-dictum preparation for the purpose of examining the number of microorganisms.

2.3. Study of the Spread of Microorganisms from a Bioreparation—Containers in and Outside the Laboratory

The study of the spread of microorganisms from the micro-dictum preparation was conducted on 6 randomly selected balls between 16 March 2016, and 18 April 2016. The balls were placed in 6 basins with dimensions of 0.6 m × 0.6 m × 0.3 m. The basins were filled with lake water from a body of water whose ecological status was classified as good (according to the Water Framework Directive—WFD)—Lake Przykona. Water with low microbiological activity, i.e., devoid of or with a small number of microorganisms, both pathogenic and those which were part of the micro-dictum preparation, was a guarantee of conducting reliable tests. The preparation was placed in the center of each basin. Samples for microbiological testing were taken at distances of 10 cm, 20 cm, and 30 cm from the preparation. Five basins were located in the laboratory (stable conditions—constant temperature of 20 °C). Samples from these 5 basins were collected after 24 h and 48 h and on days 3, 4, 5, 6, and 34. One basin was placed outside the building to assess the potential impact of atmospheric elements on the incubation of microorganisms from the micro-dictum preparation (natural conditions). Samples from basin 5 were taken after 24 h and on days 6, 14, and 34 of incubation. Additionally, a microbiological analysis of the water was conducted before adding the solid preparation, as well as a control sample analysis on days 14 and 34. No lactic acid bacteria were detected in the water before the solid preparation was added.

The microbiological parameters analyzed in the water in the basins were the following:

(a) Number of lactic acid bacteria on MRS + Actidione culture medium with the addition of 0.1% cycloheximide solution, incubated under relatively anaerobic conditions at 37 °C for 2–3 days, with pH 5.5;
(b) Number of aerobic microorganisms on PCA (plate count agar) culture medium, with incubation conditions of 28 °C for 5–7 days;
(c) Number of fungi on DRBC culture medium, with incubation conditions of 24 °C for 5–7 days.

The experiment was conducted until the number of microorganisms stopped increasing or began to decrease. Additionally, on days 1, 2, 3, 4, 5, 6, and 7 of the experiment, measurements of parameters such as pH, dissolved oxygen content, and electrical conductivity were taken. After the seventh day, analyses were conducted weekly. A total of 16 measurements were taken between 15 March and 19 April 2016. The basins were covered with a non-woven fabric to prevent evaporation while allowing air to pass through. Since evaporation from the basins containing the micro-dictum preparation was minimal, the water level was replenished to the original level (gently pouring water along the sides). The water in the basins was replenished with distilled water halfway between analyses (not on the day of analysis or immediately afterward, to allow the water to stabilize).

2.4. Micro-Dictum Solubility with Forced Water Circulation

This study also included analyses related to the solubility of the preparation in forced water circulation and the spread of selected microorganisms under conditions of increased water movement. The experiment with forced water circulation was intended to simulate the water flow that takes place in streams. The experiment was to answer whether the preparation would be suitable for use in flowing rivers or, possibly, in flow-through reservoirs. The microbiological parameters in the water were examined, similar to the stable basins, including the number of lactic acid bacteria, the number of aerobic microorganisms, and the number of fungi, using the methods described in the previous section. A device that put the water in motion was used, consisting of a container with a cross-section of 0.2 m. Water from Lake Przykona (a post-mining lake with good ecological status) was used for this study. The mixing speed of the device was 100 rpm/min. After mounting the rotor in the container and placing the micro-dictum ball inside, microbiological tests of the water were carried out once a week. The container with the mixer was placed in the laboratory. The temperature inside the building was stable at approximately 20 °C.

2.5. Study of Water Quality in Containers with the Biopreparation

In the water selected for the experiment, initial sample analyses were conducted. The physical parameters measured included the following: pH, dissolved oxygen, electrolytic conductivity, and chemical parameters, such as total nitrogen, ammonium nitrogen, nitrate nitrogen, nitrite nitrogen, total phosphorus, and phosphates. The physical parameters were measured between 15 March and 19 April 2016, with a total of 16 measurements taken for each parameter. The chemical parameters, from the nutrient group, were analyzed four times: on the first day before filling the containers with water (15 March), and then on 22 March, 31 March, and 19 April. The tests were conducted on filtered samples, except for total nitrogen and phosphorus, where both filtered (f) and unfiltered (nf) water samples were analyzed. The following parameter concentrations were examined:

- P-PO_4 [mg/L^{-1}]—spectrophotometric method;
- Total phosphorus [mg/L^{-1}]—atomic emission spectrometry method after microwave digestion;
- Total nitrogen [mg/L^{-1}]—spectrophotometric method;
- N-NH_4 [mg/L^{-1}]—spectrophotometric method;
- N-NO_3 [mg/L^{-1}]—spectrophotometric method;
- N-NO_2 [mg/L^{-1}]—spectrophotometric method.

All analyses of the chemical compounds in water and the preparation used an Agilent 4210 MP-AES spectrometer (Santa Clara, CA, USA) and an LC-MS liquid chromatography system with an atomic mass detector, based on a triple-quadrupole detector (QQQ) and conventional DADs and FLDs (Agilent Technologies, Santa Clara, CA, USA).

2.6. Tests of the Preparation in Real Conditions

In addition to laboratory tests, since 2016, experiments have been conducted on the constructed SED-BIO system. The system was located on the Gnieźnieńska Struga stream, which is subject to strong anthropopressure from various factors related to the functioning of the city, but also agricultural pressure in the source section of the river. The last few years have seen enormous pressure from weather conditions in the region. Strong winds and heavy rainfall, caused by climate change, caused massive material and environmental damage in this area. The SED-BIO system's task is to control the processes of sedimentation and decomposition of excess deposited organic sediments and limit the inflow of nutrients (NP) to the waters of Lake Jelonek, located in the city of Gniezno (WielkopolskaVoivodeship). The SED-BIO system consists of two sections (Figure 4):

- Section A—located on an existing water reservoir (approximately 0.74 ha), situated on the Struga Gnieźnieńska stream, about 150 m from Lake Jelonek;
- Section B (between the reservoir and Lake Jelonek)—located on the Struga Gnieźnieńska stream, situated at a distance of 100 to 120 m from the lake (section length approximately 20 m, width approximately 8 m).

Both sections are composed of separate segments:

- Section A—3 segments: sedimentation and microbiological activity, phosphorus sorption, and plant biofilter with a denitrifying bed;
- Section B—4 segments: sedimentation and microbiological activation zone, biogeochemical barrier for phosphate binding, denitrification zone, and biofiltration zone.

In the mineral filters, limestone and blast furnace slag—chunky, air-cooled, with a fraction size of 30–70 mm—were used as substrates, arranged in layers. The filters were replaced annually. In 2020, in Section B, instead of the standard filter, BioKer was used (patent application no. P.420265). This is a biological filter based on ceramic aggregate. The aggregate is coated with a multilayer biopolymer along with nano- and microparticles of calcite combined with microorganisms from the PAO group (phosphate-accumulating organisms). Detailed information about the SED-BIO system can be found in the publication by Kupiec et al. [34].

In this study, data from two periods—2016 and 2022, during the months of May to November—were used. Water samples were collected once a month in 2016 at 7 points, and in 2022 at 4 points (1, 2, 5, 7). The most important sampling points were those related to microbiological activity in the segments where the micro-dictum preparation was applied (points 2 and 5). Physical parameters such as temperature, pH, dissolved oxygen content, and electrolytic conductivity were analyzed. Chemical parameters were also examined, focusing on phosphorus compounds as substances which determine the eutrophication process:

- PO_4 [mg/L^{-1}]—spectrophotometric method;
- Total phosphorus [mg/L^{-1}]—atomic emission spectrometry method after microwave mineralization.

In the environmental studies, an anion chromatograph from DIONEX ICS 6000 (Thermo Scientific, Bremen, Germany), a multi-parameter meter 556 MPS (YSI) (Yellow Springs, OH, USA), and a Hanna HI 9829-00042 water quality meter (Hanna Instruments, Woonsocket, RI, USA) were used.

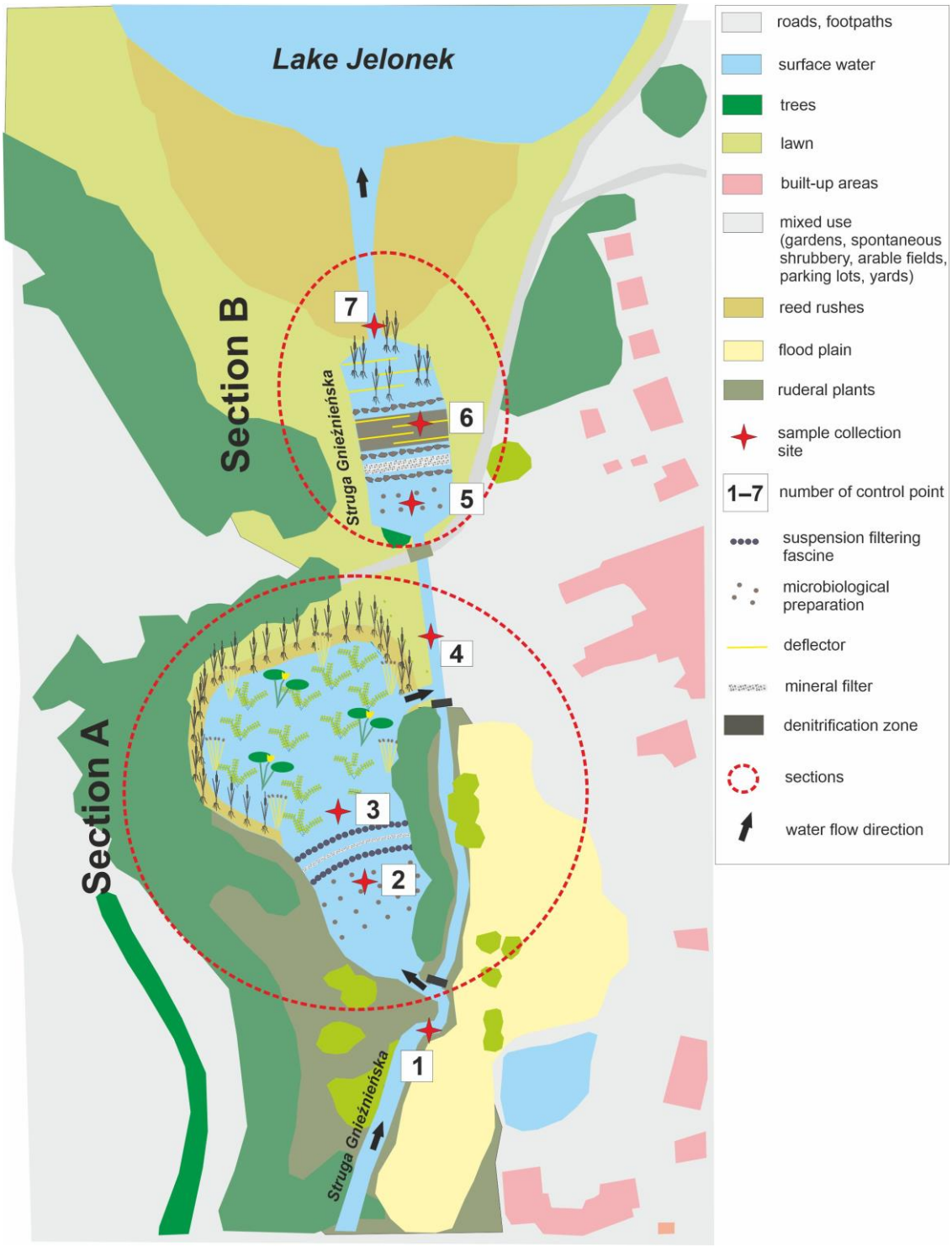

Figure 4. Location of sample sites in the SED-BIO system.

Analysis of Physical Parameters at the Water Surface and Bottom in Sedimentation Zones

Physical parameters (Temp., pH, EC, and DO) were also compared at the water surface and near the bottom, where microbiological activity was the highest in Section A, within the sedimentation zone of the SED-BIO system. The depth in this area varied between 1.0 and 1.2 m. In the case of Section B, the sedimentation zone was quite shallow, up to 0.5 m, so similar measurements were not conducted here. This study was performed during the growing season between June and October.

3. Results and Discussion

3.1. Results of Physical and Chemical Parameters Tests of the Micro-Dictum

Tests of the chemical composition of the balls show that the amount of nitrogen is approximately 1% and the amount of phosphorus is less than 2 g/kg in each ball (Table 1). The biogenic substances present in the preparation can therefore be gradually released into the water from the dissolving ball. Some of them may be absorbed by microorganisms present in the ball. The phosphorus introduced with the preparation can be used in the initial period of multiplication by the microorganisms contained in the micro-dictum.

Table 1. Physical and chemical parameters of the micro-dictum preparation.

Ball Number	pH [a]	Total N [%] [b]	Total P [g/kg] [c]
1	5.45	0.92	1.79
2	5.40	1.12	1.81
3	5.46	1.03	1.89
4	5.47	0.96	1.85
5	5.40	1.32	1.93
Mean	5.44	1.07	1.85

[a] potentiometric method; [b] Kjeldahl titration method; and [c] method of atomic emission spectrometry after microwave mineralization.

3.2. Results of Testing the Microbiological Parameters of the Micro-Dictum

Microbiological analyses of the balls revealed that the content of microorganisms in individual balls may vary, and the quantitative composition of microorganisms inside the ball is smaller compared to the outer layers (Table 2). In the case of lactic acid bacteria, the average number in the surface layer was 15.5 times greater than in the central part of the ball. The number of aerobic microorganisms in the outer layers was 12.4 times higher than in the central parts of the balls, while the number of fungi in the layer up to 1 cm from the surface of the ball was twice as high. A decrease in the number of microorganisms was observed as early as in the 2–3 cm layer of the preparation. For lactic acid bacteria, this decrease was approximately 24%, for aerobic microorganisms 91%, and for fungi 62%.

Table 2. The number of microorganisms in the cross-section of the micro-dictum ball.

Depth	Value	Number of Lactic Acid Bacteria	Number of Aerobic Microorganisms	Number of Fungi
		CFU/mL		
1 cm	mean	2,518,000	470,000,000	150,000,000
	min.	150,000	200,000,000	80,000,000
	max.	9,300,000	870,000,000	290,000,000
2–3 cm	mean	1,919,200	44,200,000	57,560,000
	min.	32,000	11,000,000	2,800,000
	max.	9,200,000	120,000,000	160,000,000
Center	mean	162,200	38,000,000	74,300,000
	min.	5000	30,000,000	1,500,000
	max.	520,000	70,000,000	140,000,000

3.3. Results of Research on the Spread of Microorganisms from a Biopreparation

3.3.1. Containers in the Laboratory

Water for the six containers was delivered in a single bulk tanker. In five containers, prior to the application of the micro-dictum preparation, analyses were conducted for the presence of aerobic microorganisms and fungi. The analyses showed that the number of aerobic microorganisms ranged from 2.5×10^3 to 1.3×10^4. In the case of fungi, their numbers ranged from 2.2×10^1 to 3.3×10^2 (Table 3). Control sample studies conducted

after 14 days showed a slight increase in the number of aerobic microorganisms and a decrease in the number of fungi. After 34 days, a decrease in the number of aerobic microorganisms and a further decline in the number of fungi were observed in the control samples (Table 3). This was most likely due to the depletion of available nutrients.

Table 3. Microbiological analyses of water from Przykona Lake (before adding the ball).

Container Number	Number of Aerobic Microorganisms [CFU/mL]	Number of Fungi [CFU/mL]
Container no. 1	2.5×10^3	1.6×10^2
Container no. 2	1.1×10^4	2.3×10^1
Container no. 3	8.6×10^3	2.4×10^2
Container no. 4	1.3×10^4	3.3×10^2
Container no. 5	1.0×10^4	2.2×10^1
Mean	9.0×10^3	1.6×10^2
Water control sample analyzed after 14 days of incubation	1.7×10^5	3.0×10^1
Water control sample analyzed after 34 days of incubation	9.0×10^4	1.6×10^1

The experiment demonstrated that the processes occurring after the application of the micro-dictum preparation are very rapid. From the fourth to the sixth day of incubation, there was a stabilization of lactic acid bacteria (LAB) at a level of 10^5. The analysis conducted on day 34 already showed a decline in the number of LAB to a level of 10^3 (Table 4). The results describing the dynamic increase in the number of lactic acid bacteria correlate with the decreasing pH value. A complete or almost complete depletion of oxygen was also observed after seven days of the experiment By analyzing the correlation between the number of bacteria, pH, and dissolved oxygen (DO), it can be concluded that the LAB concentration at the level of 10^5 remained stable for another 2 days, after which it began to decrease. A similar correlation was observed on day 34 of incubation—the decrease in the number of lactic acid bacteria coincided with an increase in and stabilization of the pH.

Table 4. Average number of microorganisms depending on the distance from the preparation and the incubation time.

Sample Collection Distance from the Micro-Dictum	Incubation Time	The Number of Lactic Acid Bacteria	Number of Aerobic Microorganisms	Number of Fungi
[cm]		CFU/mL		
10	24 h	4.5×10^1	2.4×10^6	2.1×10^3
20		5.5×10^1	2.8×10^6	2.4×10^3
30		3.2×10^2	1.3×10^6	2.4×10^3
10	48 h	1.2×10^4	10.3×10^6	4.8×10^3
20		4.4×10^2	6.8×10^6	3.0×10^3
30		6.8×10^3	11.6×10^6	5.4×10^4
10	3 days	8.7×10^3	2.3×10^6	9.0×10^3
20		1.1×10^4	2.5×10^6	3.7×10^3
30		6.1×10^4	3.1×10^6	1.1×10^4
10	4 days	8.7×10^4	2.9×10^6	3.1×10^3
20		8.2×10^4	2.7×10^6	2.6×10^3
30		7.7×10^5	4.0×10^6	3.0×10^3
10	5 days	4.0×10^5	2.2×10^6	1.3×10^4
20		1.2×10^5	2.8×10^6	4.4×10^3
30		9.6×10^5	3.4×10^6	1.0×10^4

Table 4. *Cont.*

Sample Collection Distance from the Micro-Dictum	Incubation Time	The Number of Lactic Acid Bacteria	Number of Aerobic Microorganisms	Number of Fungi
[cm]		CFU/mL		
10	6 days	4.3×10^5	3.3×10^6	2.9×10^3
20		4.5×10^5	3.3×10^6	4.6×10^3
30		4.7×10^5	3.5×10^6	3.5×10^3
10	34 days	1.5×10^3	1.0×10^6	5.4×10^2
20		1.5×10^3	9.5×10^5	3.2×10^2
30		1.5×10^3	1.7×10^6	3.4×10^2

In the case of aerobic microorganisms, a significant increase in these organisms was observed after 48 h of incubation at all three distances—up to 10, 20, and 30 cm (Table 4). The lowest number of aerobic microorganisms was recorded on day 34 of incubation. In most cases, a higher number of aerobic microorganisms was observed at a distance of 30 cm from the preparation compared to 20 cm, as well as 10 cm. The same trend was observed for fungi until the 5th day of incubation. Regarding lactic acid bacteria, their numbers in the 30 cm zone from the preparation were higher than in the central zone (20 cm) from the 2nd to the 6th day of the experiment. By day 34, the number of bacteria was identical at all three distances from the micro-dictum preparation.

Throughout the experiment, a decrease in water transparency was observed, along with the appearance of suspended particles and surface scum (Figures 5–8). This was likely the result of multiplying microorganisms, as well as their metabolites. Additionally, substances contained in the preparation (clay, molasses, and bran), which serve as carriers for microorganisms, may have been released from the balls.

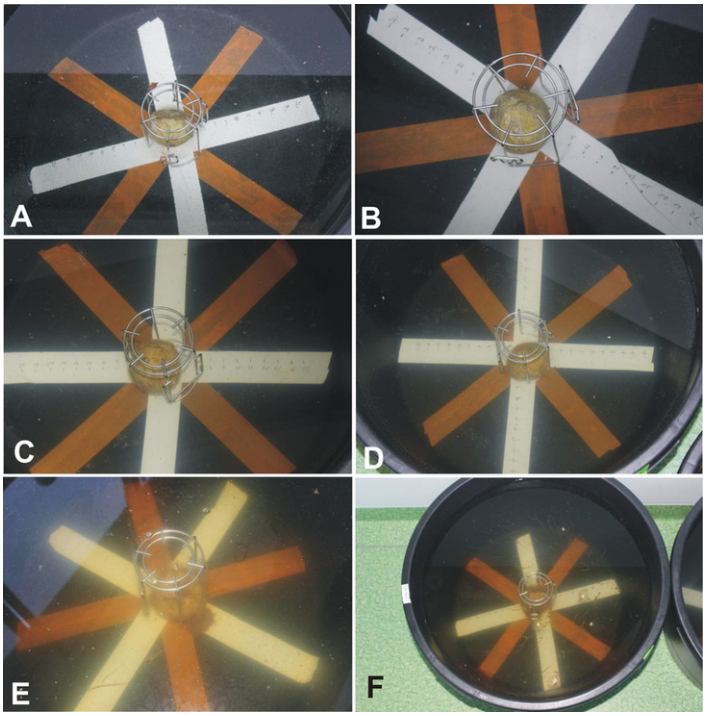

Figure 5. Physical changes in water in selected containers with the preparation: (**A**)—beginning of the experiment (container 1); (**B**)—beginning of the experiment (container 2); (**C**)—after 24 h of incubation (container 3); (**D**)—after 24 h of incubation (container 4); (**E**)—after two days of incubation (container 5); and (**F**)—after two days of incubation (container 1).

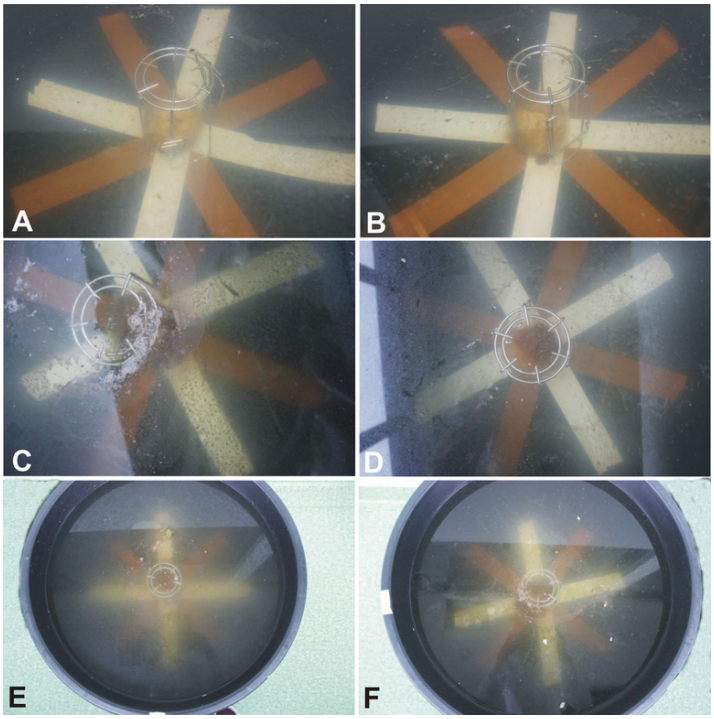

Figure 6. Physical changes in water in selected containers with the preparation: (**A**)—after three days of incubation (container 2); (**B**)—after three days of incubation (container 3); (**C**)—after four days of incubation (container 1); (**D**)—after four days of incubation (container 4); (**E**)—after five days of incubation (container 5); and (**F**)—after five days of incubation (container 1).

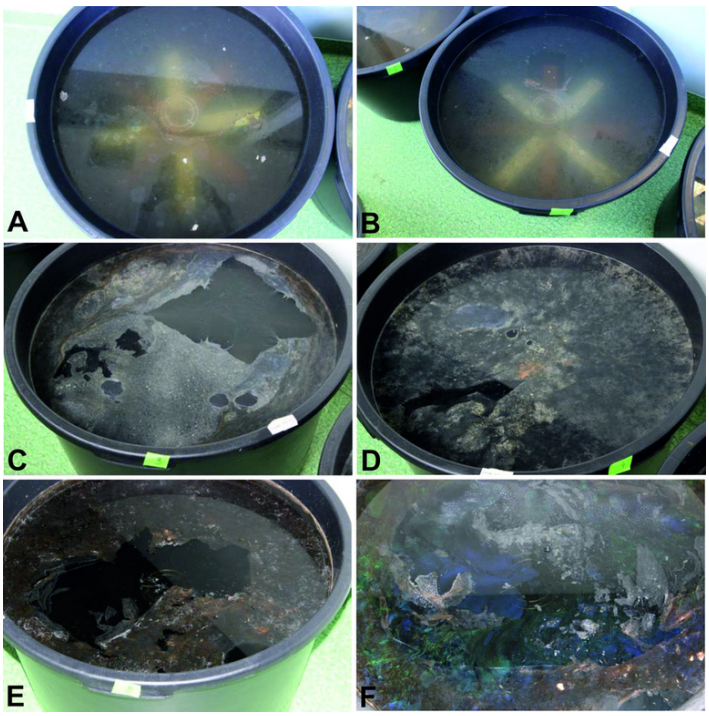

Figure 7. Physical changes in water in selected containers with the preparation: (**A**)—after six days of incubation (container 1); (**B**)—after six days of incubation (container 2); (**C**)—after sixteen days of incubation (container 3); (**D**)—after sixteen days of incubation (container 4); (**E**)—after thirty-four days of incubation (container 5); and (**F**)—after thirty-four days of incubation (container 1).

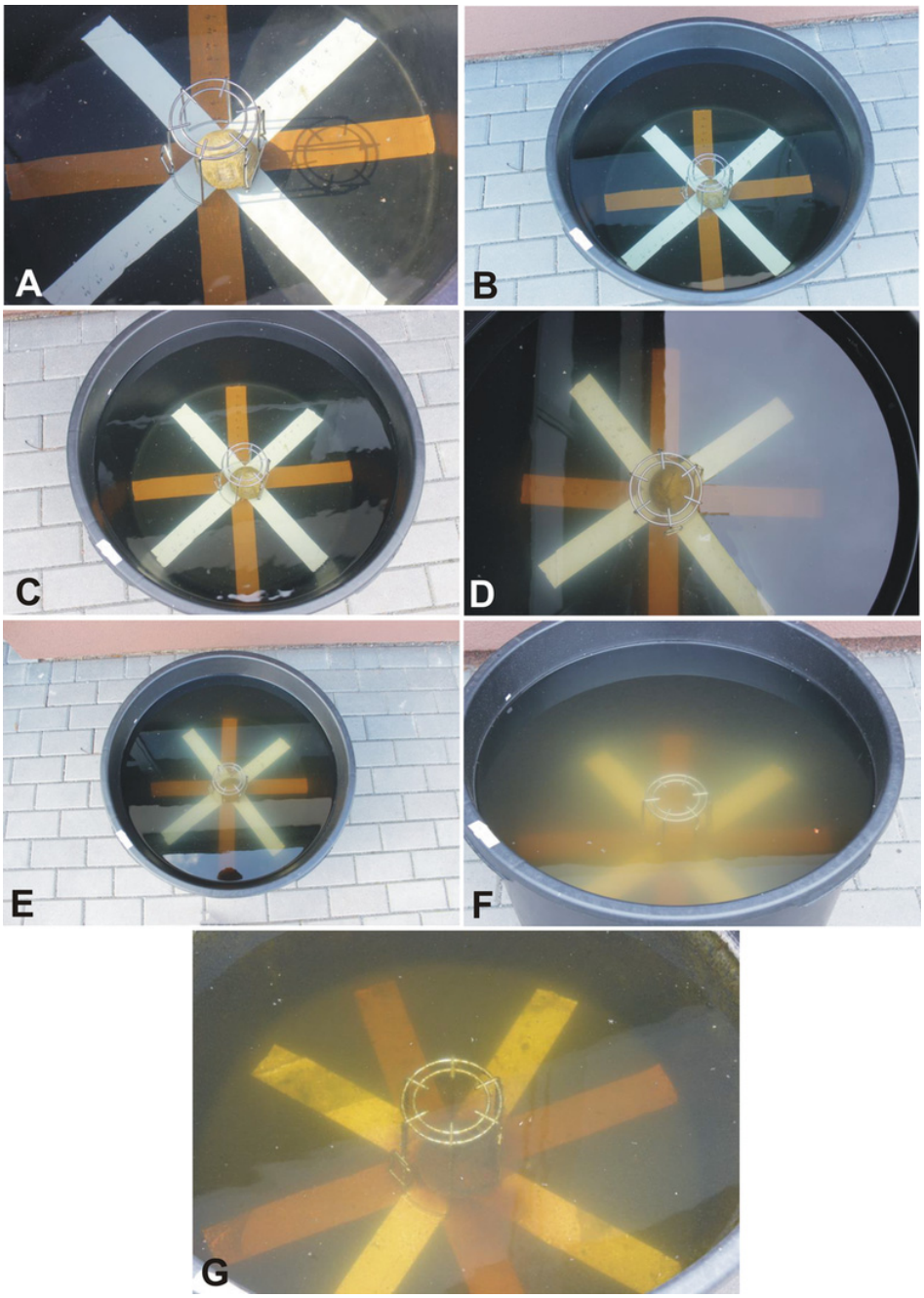

Figure 8. Physical changes in the water in container no. 6 with the preparation located outside the building: (**A**)—after 24 h of incubation; (**B**)—after two days of incubation; (**C**)—after three days of incubation; (**D**)—after five days of incubation; (**E**)—after six days of incubation; (**F**)—after sixteen days of incubation; and (**G**)—after thirty-four days of incubation.

3.3.2. Container Outside the Laboratory

When incubating the microorganisms contained in the micro-dictum preparation kept outside the building, the number of lactic acid bacteria reached its maximum value of 10^3 after a 14-day incubation period (Table 5). The following analysis (34th day of incubation) showed a decrease in the number of microorganisms. There was a noticeable correlation between the development of these bacteria and the water temperature. Due to the low ambient temperature during this period and, thus, limited biological activity, such rapid changes in dissolved oxygen concentrations in the water were not observed, especially in the early days of the experiment, as was the case in experiments conducted at room tem-

perature. The results obtained also suggest that microorganisms show certain preferences regarding their spread within the container. The highest densities were observed near the dissolving ball and at the container walls, while the lowest abundance was recorded in the water column between the ball and the edge of the vessel.

Table 5. Average microorganism count depending on the distance from the preparation and the incubation time (for the container outside).

Sample Collection Distance from Micro-Dictum	Incubation Time	Number of Lactic Acid Bacteria	Number of Aerobic Microorganisms	Number of Fungi
[cm]		CFU/mL		
10	24 h	<1	1.8×10^4	2.0×10^2
20		<1	2.2×10^4	6.0×10^2
30		<1	3.8×10^4	7.0×10^2
10	6 days	<1	1.9×10^6	4.0×10^4
20		<1	2.5×10^6	6.4×10^4
30		<1	3.2×10^6	8.0×10^4
10	14 days	1.3×10^3	9.3×10^6	7.0×10^2
20		1.4×10^3	6.6×10^6	1.0×10^2
30		1.2×10^3	6.9×10^6	6.0×10^2
10	34 days	1.7×10^2	6.0×10^4	9.3×10^1
20		1.1×10^2	6.0×10^4	8.4×10^1
30		7.6×10^1	4.0×10^4	8.3×10^1

The number of aerobic microorganisms, in contrast to lactic acid bacteria, had increased by the 6th day and continued to rise until the 14th day of this study (Table 5). By the 34th day of the experiment, the number of aerobic microorganisms had decreased. The number of fungi increased until the 6th day of the study, after which it began to decrease to values lower than at the beginning of the experiment. Up until the 6th day of incubation, the abundance of aerobic microorganisms and fungi was highest in the area farthest from the preparation (30 cm), while on the 14th and 34th days of the study, the highest abundance was recorded closest to the preparation (10 cm).

In the case of dissolved oxygen (DO) concentrations, it can be observed that, despite a decrease in oxygen content, similar to what occurred in the containers in the laboratory, the oxygen content began to increase in the final stage of the experiment under external environmental conditions. This might have been influenced by a milder course of microbiological processes due to the lower water temperature in the container placed outside the laboratory building. In the outside container, lower electrical conductivity was also recorded compared to the containers inside the laboratory.

3.4. Biopreparation Solubility with Forced Water Circulation

The experiment was conducted for 6 days. On the 10th day of incubation, a decision was made to finish the experiment due to the release of very intense odors from the container. Analyzing the results concerning the microorganism counts, an increase in the number of aerobic microorganisms and fungi was observed, peaking on the fifth day compared to the first day of incubation. On the 6th day, a decrease in these microorganisms was recorded (Table 6). In the case of lactic acid bacteria, their count increased significantly on the fifth day compared to the first day of incubation, from 10^1 to 10^6. The increase in these bacteria continued on the 6th day of incubation. As the experiment progressed, the visual and sensory properties of the water also changed. Water transparency decreased, a scum formed on the water surface, and the intensity of the foul odor increased (Figure 9).

Table 6. Number of microorganisms depending on the incubation time in a container with forced water circulation.

Incubation Time	Number of Lactic Acid Bacteria	Number of Aerobic Microorganisms	Number of Fungi
	CPU/mL		
24 h	4.7×10^1	8.5×10^7	1.4×10^5
48 h	6.8×10^3	2.0×10^7	1.0×10^5
3 days	8.2×10^5	1.0×10^8	5.8×10^5
4 days	1.0×10^6	1.3×10^8	1.0×10^4
5 days	7.2×10^6	4.1×10^8	1.3×10^6
6 days	1.2×10^7	1.5×10^8	7.5×10^5

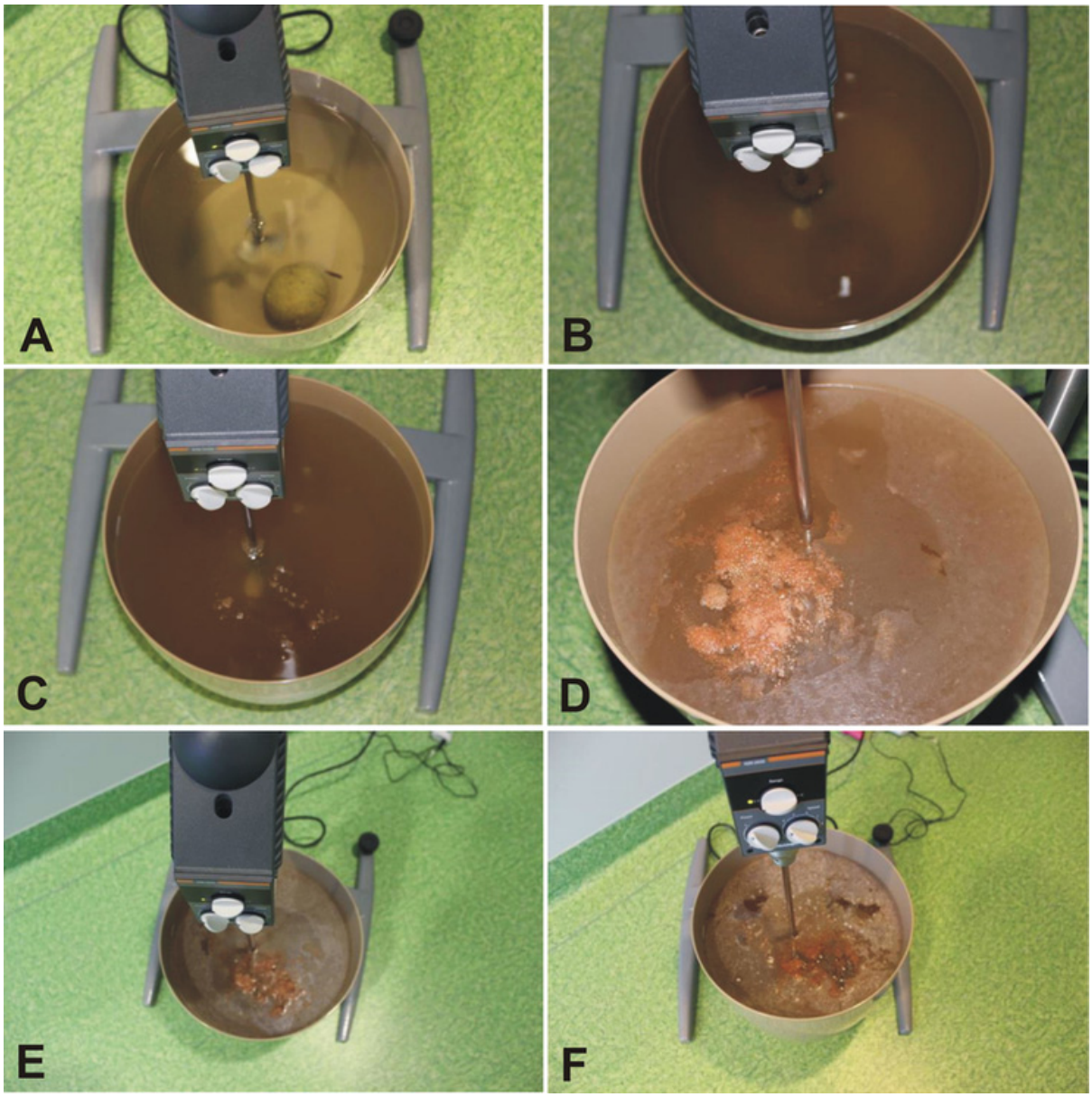

Figure 9. Physical changes in water in a container with forced circulation and the preparation placed in it: (**A**)—beginning of the experiment; (**B**)—after 24 h of incubation; (**C**)—after two days of incubation; (**D**)—after four days of incubation; (**E**)—after five days of incubation; and (**F**)—after six days of incubation.

3.5. Results of Water Quality Tests in Containers with the Biopreparation

3.5.1. Physical Parameters—Comparison of Containers in the Laboratory and Outside the Building

The temperature analyses indicated significant differences between the water in the containers located in the laboratory and that situated outside the building, reaching up to 18 °C (Figure 10A). Temperature is one of the factors determining the development of microorganisms. It may also affect the process of reclamation—primarily its efficiency and duration. The pH showed similar trends for the studied containers. However, certain differences were observed on the 4th, 5th, 7th, 11th, and 12th days of the experiment (Figure 10B). On the 4th and 5th days, the pH in the container was lower than in the laboratory, while, on the other days (7th, 11th, and 12th), the pH of the water was higher. However, the differences in pH did not exceed 0.5 units. Electrolytic conductivity showed no significant differences between the containers until the 8th day of the experiment. Starting from the 10th day, a higher conductivity was observed in the containers located in the laboratory (Figure 10C).

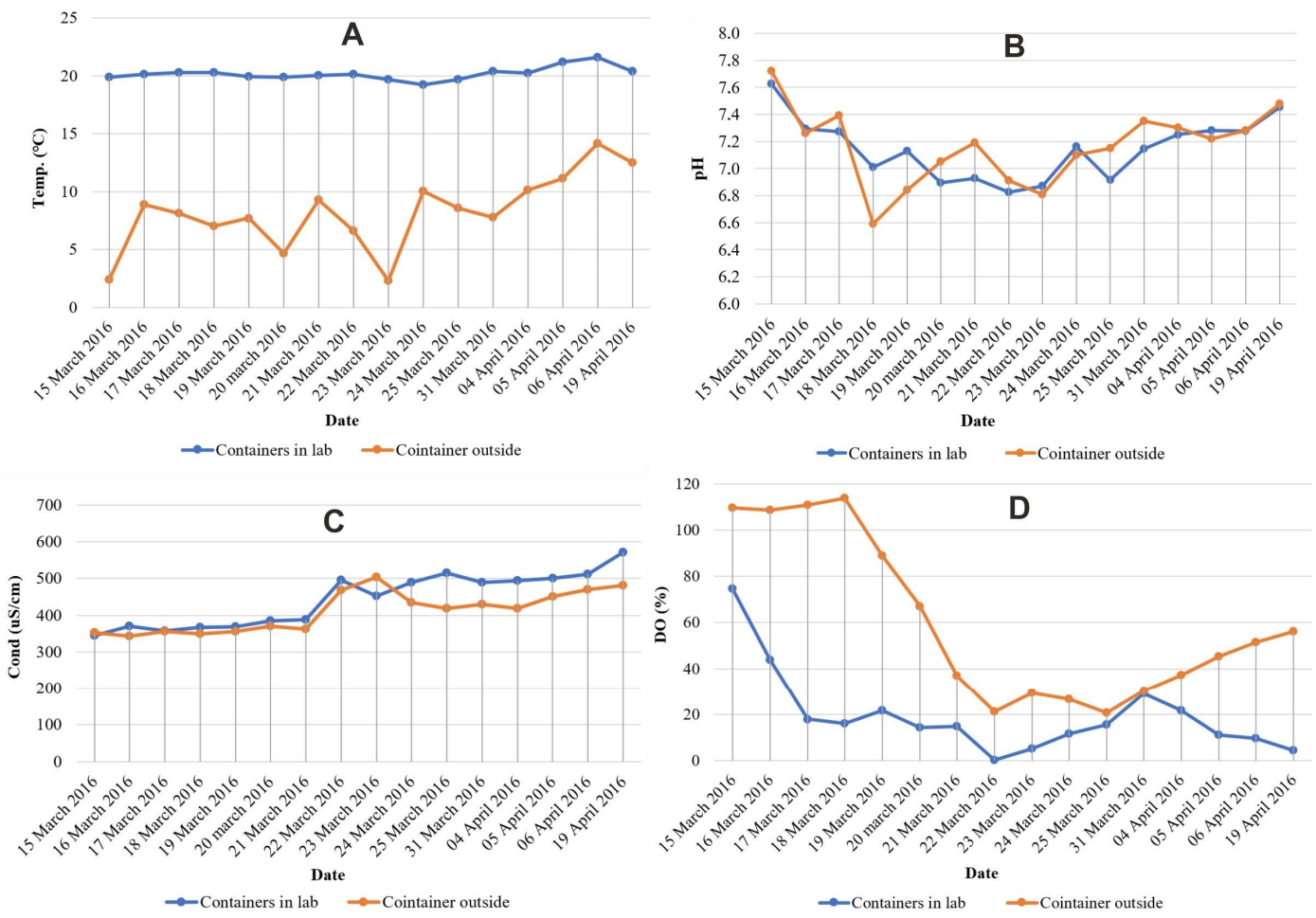

Figure 10. Comparison of trends in changes in physical parameters of water to which micro-dictum balls were applied: containers in the laboratory vs. outdoor container: (**A**)—temperature; (**B**)—pH; (**C**)—electrolytic conductivity; (**D**)—dissolved oxygen.

Significant differences were also noted in the degree of oxygen saturation. A higher level of oxygen saturation was observed in the container situated outside (even by 97.6%), and, during the initial period of the experiment, an increase in oxygen intensity was noticed. It was not until the 5th day of the experiment that a decrease in oxygen saturation in the water was observed. On the 11th and 12th days of the experiment, the oxygen levels were nearly equal between the containers located in the laboratory and those located outside the

building. On the 13th day, the oxygen level in the outside container began to rise while it continued to decline in the laboratory containers (Figure 10D).

The experiment with forced water circulation indicates significant differences in the formation of the studied physical parameters. The results suggest that water movement affects the solubility of the preparation and the development of microorganisms, which determines the course of the reclamation process. Water temperature was more stable in the container with the mixer than in the control (Figure 11A). In the laboratory, the temperature fluctuated slightly between approximately 21 and 22 °C, which primarily affected the water temperature in the containers. The more stable temperature may have been influenced by the mixer agitating the water.

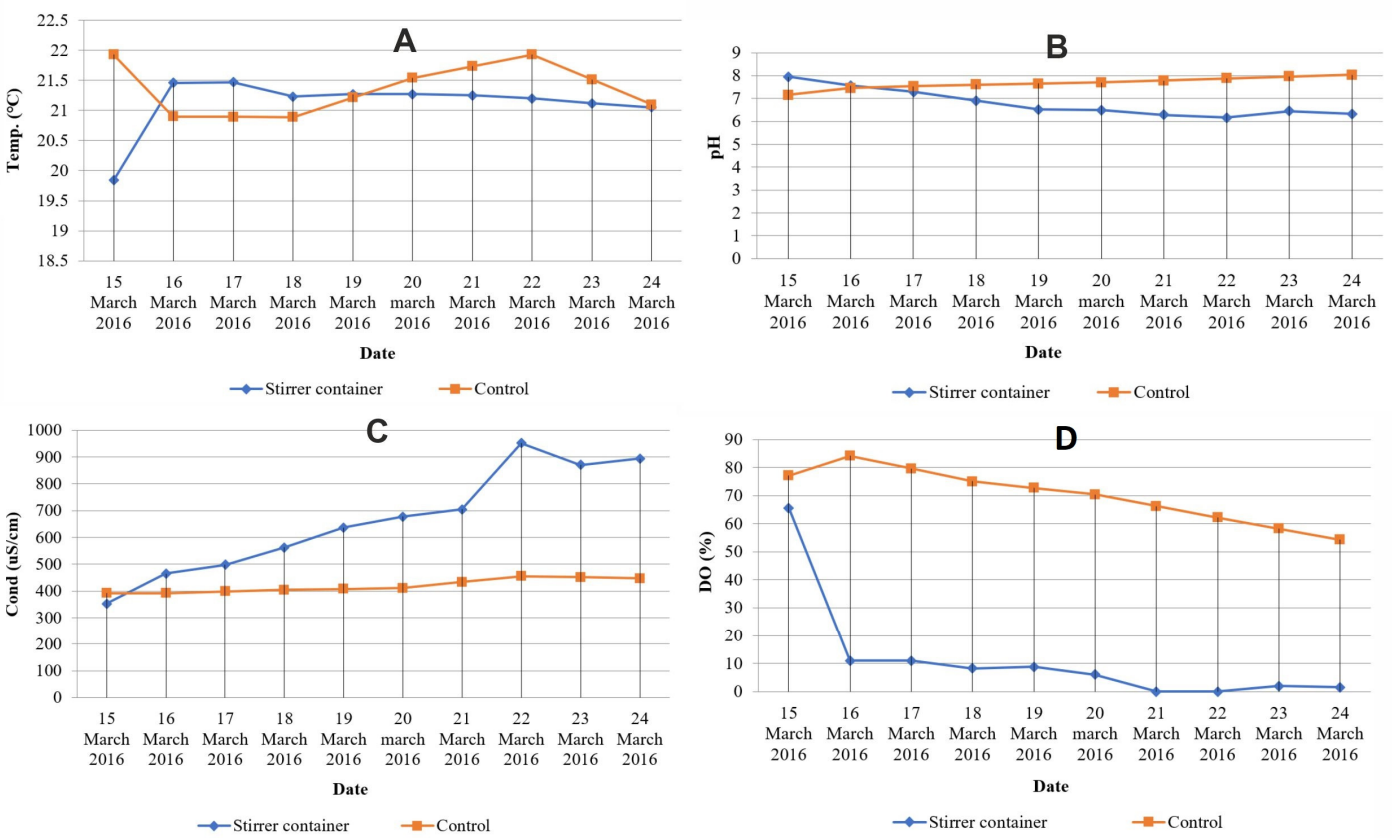

Figure 11. Trends in changes in the physical parameters of water to which micro-dictum balls were applied, in a container with forced water circulation: (**A**)—temperature; (**B**)—pH; (**C**)—electrolytic conductivity; and (**D**)—dissolved oxygen.

Regarding the pH, a constant level was maintained in the control. In contrast, the container with the mixer exhibited a steady decrease in pH by as much as two units (Figure 11B). Electrolytic conductivity in the control remained steady at 392–455 µS/cm, with a slight upward trend. In the container with the mixer, a drastic increase in conductivity was observed from the first day of the experiment. The largest difference between the container with forced water circulation and the control occurred on the 8th day of the experiment, measuring 497 µS/cm (Figure 11C).

Changes in oxygen saturation in the water are the most visible and evident. Already on the second day of observation, significant deoxygenation of the water was noted, decreasing from 65.6% to 11.0% in the container with the mixer. On the 7th and 8th days, complete deoxygenation of the water was observed. In the control, a downward trend in oxygen saturation was also noted. However, this was slower and less significant (from 84.2% to 54.3%) (Figure 11D).

3.5.2. Chemical Parameters

In the analysis of biogenic substance concentrations in five containers set up in the laboratory, a successive increase in the concentrations of ammonium nitrogen, total nitrogen, phosphate phosphorus, and total phosphorus was observed with each measurement from the day on which the preparation was added to the water (Table 7). As shown in Figure 12, the concentrations of N-NH_4, P-PO_4, and TP increased quite uniformly until the 16th day of the experiment. After that, a sharp increase in the concentrations of N-NH_4 and TN was observed. The concentrations of phosphorus compounds continued to rise, but to a much lesser extent than nitrogen compounds.

Table 7. Development of basic indicators in the five analyzed containers, the outdoor container (no. 6), and the control.

Chemical Parameter	Value	Concentrations of Nutrient in Lake Water [1]	Day 7 of the Experiment	Day 16 of the Experiment	Day 36 of the Experiment
P-PO_4 [mg/L^{-1}]	range	0.037	0.20–0.47	0.41–1.12	1.15–2.10
	SD		0.104	0.302	0.365
	container no. 6		0.012	0.021	0.67
	control		0.037	0.053	<0.01
TP (filtered samples) [mg/L^{-1}]	range	0.11	0.32–1.23	0.61–1.68	1.98–2.94
	SD		0.306	0.357	0.353
	container no. 6		0.25	0.41	1.13
	control		0.11	0.09	0.08
TP (unfiltered samples) [mg/L^{-1}]	range	0.11	0.51–1.54	0.95–2.11	2.51–3.17
	SD		0.351	0.401	0.254
	container no. 6		0.39	0.81	1.58
	control		0.11	0.08	0.09
TN (filtered samples) [mg/L^{-1}]	range	0.4	1.2–2.7	1.4–2.5	5.9–7.8
	SD		0.516	0.426	0.615
	container no. 6		1.2	1.1	2.6
	control		0.4	0.3	0.5
TN (unfiltered samples) [mg/L^{-1}]	range	0.4	4.0–5.1	4.1–4.8	12.1–17.1
	SD		0.372	0.224	2.005
	container no. 6		1.8	2.5	4.5
	control		<0.1	0.3	0.5
N-NH_4 [mg/L^{-1}]	range	0.04	0.68–0.84	0.56–1.43	3.85–6.50
	SD		0.064	0.283	0.906
	container no. 6		0.25	0.12	0.389
	control		<0.01	<0.01	0.186
N-NO_3 [mg/L^{-1}]	range	0.23	0.19–0.32	0.15–0.24	0.15–0.21
	SD		0.048	0.032	0.021
	container no. 6		0.23	0.12	0.17
	control		<0.1	<0.1	<0.1
N-NO_2 [mg/L^{-1}]	range	0.004	0.001–0.003	0.004–0.073	0.004–0.005
	SD		0.0009	0.0265	0.0004
	container no. 6		0.009	0.004	0.006
	control		<0.001	0.158	0.002

[1]—Przykona Lake; SD—standard deviation.

An increase in the concentrations of phosphates, total phosphorus, total nitrogen, and ammonium nitrogen was also observed in the container (no. 6) located outside the laboratory building (Table 7). However, the concentrations of both nitrogen and phosphorus compounds were not as high as in the case of the five containers placed in the laboratory at room temperature. This may indicate the influence of temperature on the development of

microorganisms and microbiological processes. The concentrations of nitrate nitrogen and nitrite nitrogen showed a slight decrease.

In the control container, a decrease in the concentrations of phosphates and total phosphorus was noted (Table 7). For total nitrogen and ammonium nitrogen, an increase in the concentrations of these compounds was recorded. Nitrate nitrogen remained at a low level below the analytical range (<0.1). The concentrations of nitrate nitrogen increased on the 16th day of the experiment, after which they significantly decreased again in the final period (Table 7).

It is also worth noting the differences between the filtered and unfiltered samples. In both filtered and unfiltered samples, the concentrations of total phosphorus and total nitrogen increased. Higher concentrations were characteristic of unfiltered samples, indicating the presence of organic suspension in the sample, including microorganisms. The increasing concentrations in the unfiltered and filtered samples indicate the influx of nitrogen and phosphorus into the system from the dissolving micro-dictum preparation. The elements are largely incorporated into the biomass of microorganisms developing in the containers.

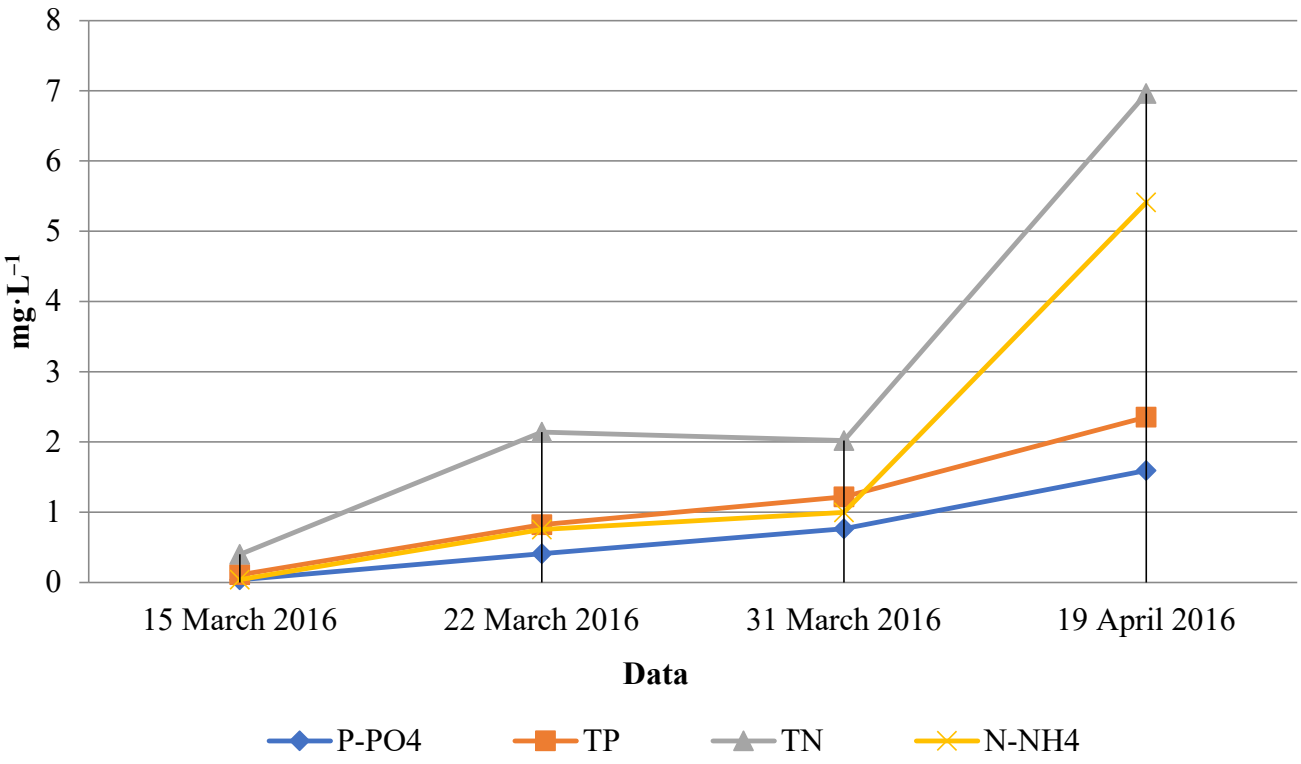

Figure 12. Concentrations of selected biogenic substances in five analyzed containers with the micro-dictum preparation.

3.6. Characterization of Physical and Chemical Parameters in the Environment

The container tests were a preamble to research in the environment and were intended to provide an answer to the behavioral directions of microorganisms in an aquatic environment and the impact of the preparation on the quality of water subject to recultivation. Container studies do not fully reflect all conditions, both abiotic and biotic, occurring in the environment. Tests in the containers excluded the involvement of any additional phosphorus-consuming organisms. This would be an element disturbing the research, so it could not be used in the container experiment. Organisms that can grow and accumulate phosphorus in the form of intracellular polyphosphate grains include phosphate-accumulating microorganisms (PAOs), which are used in bioremediation [35]. Phosphorus can also be taken up by cyanobacteria or phytoplankton [36,37]. In turn,

phytoplankton is food for higher organisms, e.g., fish or mollusks, which incorporate phosphorus from the diet they consume into their bodies [38,39]. Phosphorus is also taken up in significant amounts by macrophytes [40]. To check the effect of our preparation, taking into account aquatic organisms which consume phosphorus, the experiment was transferred to real conditions.

The microbiological preparation of micro-dictum was used for the reclamation of water flowing into Lake Jelonek via the Gnieźnieńska Struga stream in the city of Gniezno. The SED-BIO system, located on the river and built in 2016, combines biological, physical, and chemical methods aimed at reducing biogenic substances (nitrogen and phosphorus compounds) flowing into Lake Jelonek and supporting the lake's self-purification processes. The system also contributed to improving microbiological biodiversity and enhanced the sanitary quality of the water entering Lake Jelonek [34].

In 2020 (at the turn of July and August), the limestone in section B was replaced with BioKer, a coated expanded clay [41]. The purpose of the BioKer preparation was to adsorb phosphorus from the water. The limestone was also replaced with a new one in section A in September 2020. In 2021, the BioKer in section B was similarly replaced, as well as in 2022.

Environmental research in the SED-BIO system was conducted in such a way as to largely exclude the participation of other organisms in phosphorus absorption. There were no macrophytes in the sedimentation zones that would absorb phosphorus. Lemna minor occasionally appeared in section B. If macrophytes appeared, they were immediately removed. No cyanobacterial or phyto-plankton blooms were observed. The fish population was also small, although they appeared spontaneously in the system.

3.6.1. Physical Parameters at the Water Surface and Bottom in Sedimentation Zones of the SED-BIO System

The results regarding the physical parameters measured at the water surface and bottom, in the sedimentation zone of section A of the SED-BIO system, indicate varying conditions in this shallow body of water (Figure 13). The activity of microorganisms introduced with the micro-dictum preparation likely influenced these conditions. The applied preparation settled at the bottom, where the process of release, multiplication, and colonization of the microsite by the microorganisms began. The differences were particularly noticeable in the pH and oxygen levels in the water. It was evident that these two parameters exhibited values that were lower at the bottom than at the water surface. Microorganisms can modify their habitat by influencing the pH [42,43]. A significant portion of microorganisms develop and grow by consuming oxygen, which is also dependent on the availability of nutrients [44]. The influence of biopreparations on the level of oxygen in the water was noticed by Mazurkiewicz et al. [45]. They subjected a small water reservoir used for recreation—Słoneczko—to a microbiological reclamation process. The authors used two microbiological preparations: The first one was the (a) ACS ODO—1 biopreparation, which contained water, a consortium of lactic acid bacteria, phototrophic bacteria, yeast, ecological molasses from sugar cane, fermented wheat bran, and minerals. The additional ingredients of this biopreparation at the micro-level included phytosterols (sitosterol and taraxasterol), phytohormones, triterpenes (lupeol, betulin, and betulinic acid), flavonoids (hyperoside, quercetin, and kaempferol), ellagic acid, pyrocatechic acid, brevofolin (ellagic acid derivative), vitamins (C, PP, P, B3, B5, B8, B11, B1, B2, A, E, and F), and tannins. The second one was the (b) ACS aqua 2 biopreparation, which contained water, sugar cane molasses, and effective microorganisms, including *Lactobacillus casei*, *Lactobacillus plantarum*, and *Saccharomycces cerevisiae*. However, the preparations were dosed in liquid form. In the case of the pH, the authors noted slight fluctuations between 7 and 7.5.

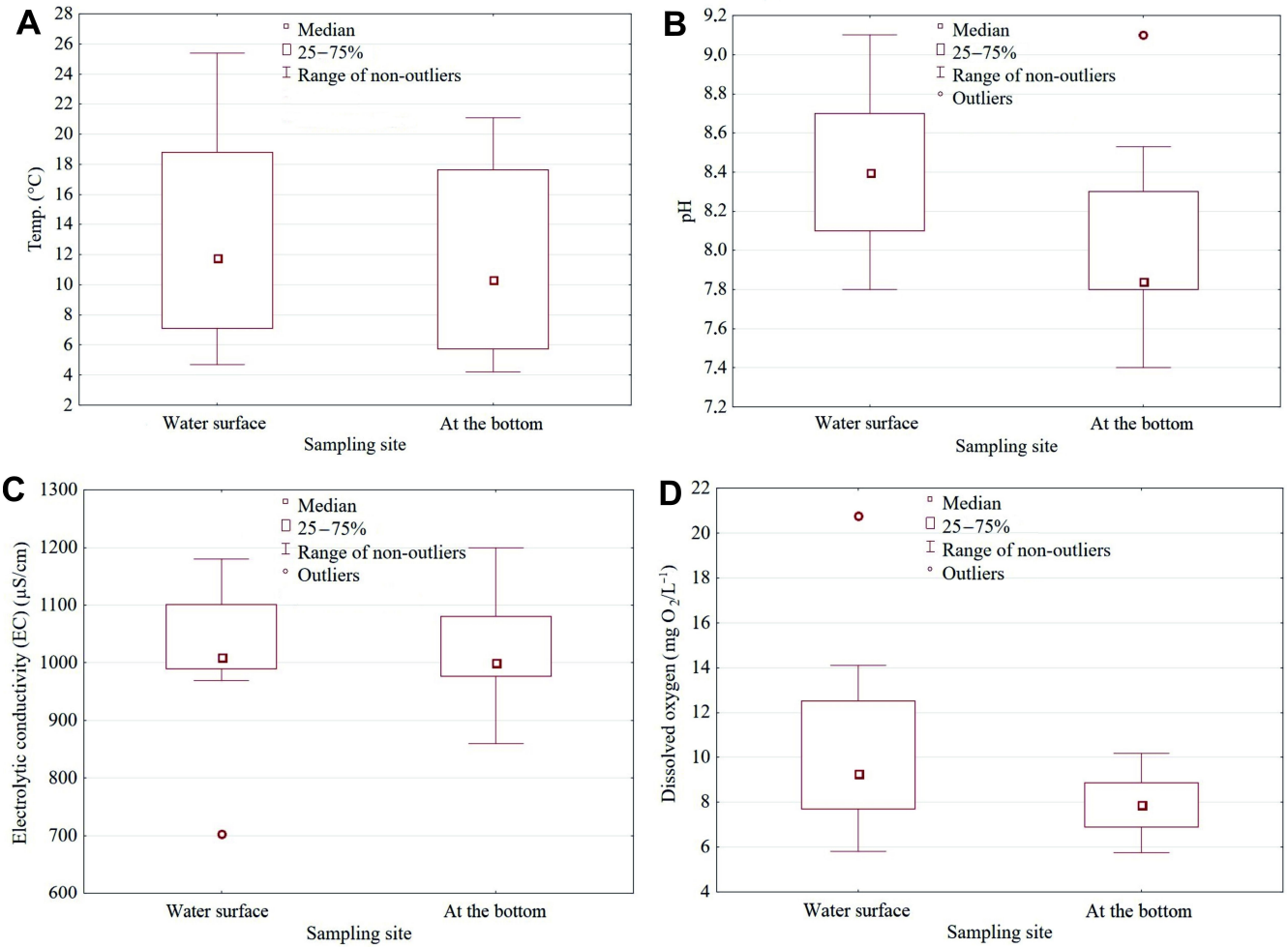

Figure 13. Physical parameters at the water surface and bottom, in the sedimentation zone of the SED-BIO system in section A: years 2016 (**A**,**B**) and 2022 (**C**,**D**).

3.6.2. Chemical Parameters of Water in the SED-BIO System

Phosphorus is one of the most important components stimulating the productivity of aquatic ecosystems, and it also affects the functioning of microorganisms. The majority of significant biogeochemical processes depend on microbial activity. In eutrophic lakes, cyanobacteria, algae, and various heterotrophs (fungi, slime molds, and most bacteria) compete for phosphorus, which can also play a significant role in the remediation of pollutants [46,47].

Analyzing the results regarding phosphate concentrations in 2016 at seven monitoring points, decreasing concentrations of this element can be observed in various segments of the SED-BIO system (Figure 14). In the application points of the preparation, namely points 2 and 5, a reduction in phosphates was recorded at an average level of 0.095 and 0.056 mg PO_4/L^{-1} compared to the previous point. This reduction was 21.4 and 15.1%, respectively. A high reduction in phosphate concentrations of 21.5% was also recorded in point 4, after the water passed through the plant biofilter. Some of the phosphates could therefore be absorbed by the macrophytes planted there. A similar trend was noticed by Mazurkiewicz et al. [45], in their research. The reduction in total phosphorus in point 2 was 6.5%, and, in point 5, it was 2.4% compared to the previous point. In terms of total phosphorus in the 2016 season, a decrease in phosphorus concentration was observed at point 3, followed by an increase in concentrations at points 4 and 5. Subsequently, a decrease in total phosphorus concentrations was again observed at points 6 and 7. The phenomenon of higher total phosphorus concentrations at points 4 and 5 was likely not due to the introduction of a certain pool of phosphorus with the microbiological preparation, as

an increase in phosphorus concentration was not observed in section A. The causes should be sought in the lower buffering capacity of the system in this section (a part of a natural stream with an expanded final section and a small depth of up to 40–50 cm). The backflow from Lake Jelonek also played a significant role, potentially causing the accumulation of organic material in this area.

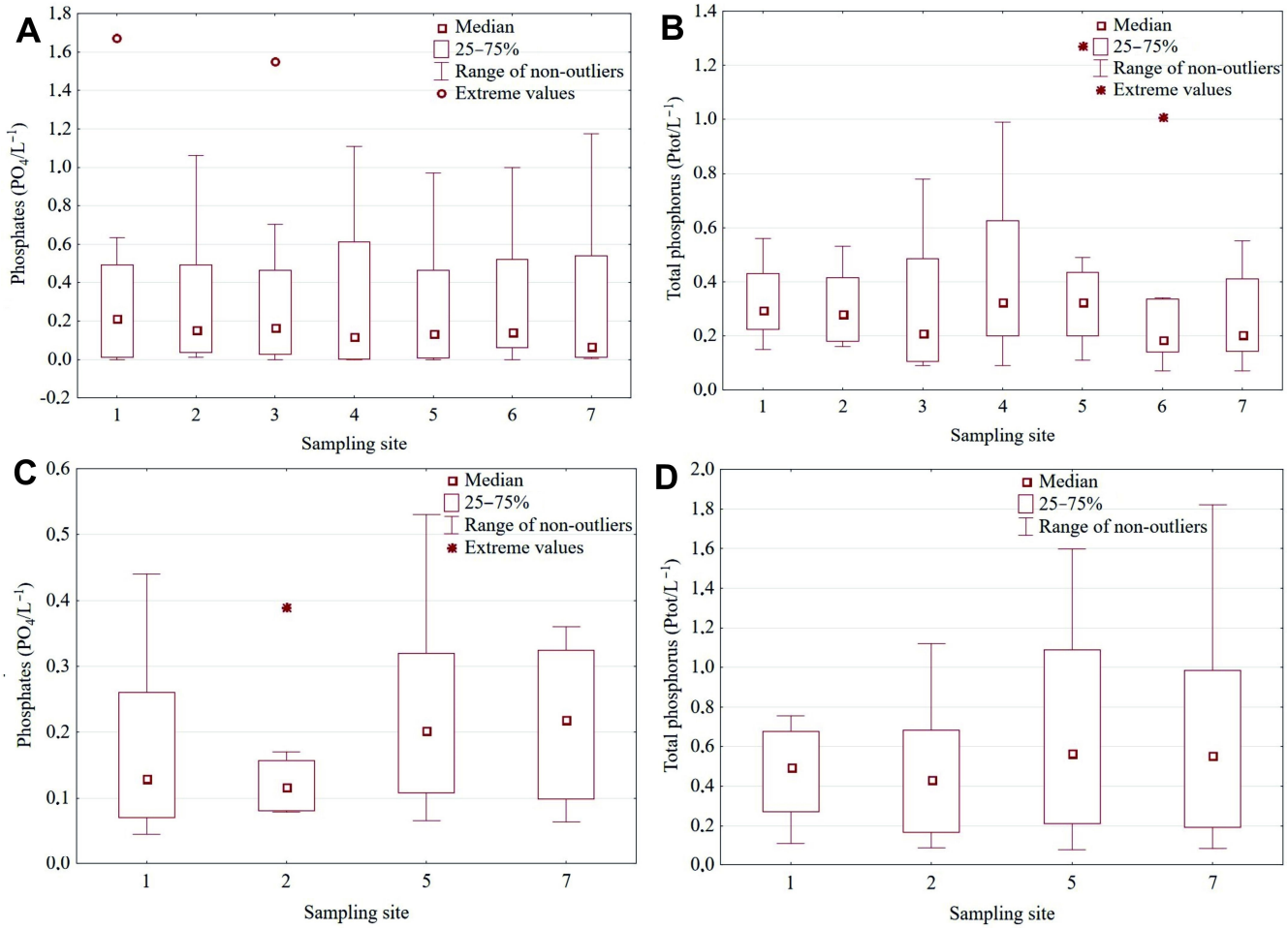

Figure 14. Phosphorus compounds at water sampling points in the SED-BIO system in 2016 (**A**,**B**) and 2022 (**C**,**D**).

It is also important to note the occurrence of extreme weather events. In Gniezno, ever since 2016, heavy rains and winds have occurred in August, causing damage to the system. Along with the rain, a large amount of organic substances flowed down the slopes. Additionally, at the height of the plant biofilter zone, a dry channel was activated, which, in addition to rainwater, also carried unidentified pollutants. The pond section of the system (section A) coped better with the pollutants due to its larger system capacity and slower flow rate. At point 6, in the denitrification zone, a decrease in total phosphorus was again observed. This could have been caused by the uptake of phosphorus by the nitrifying and denitrifying bacteria present in this zone [48,49], but phosphorus could also be absorbed by the denitrifying substrate (lignite). Scientific studies indicate that phosphorus can be bound by organic matter [50–52]. The reduction in phosphorus in this zone could also have been facilitated by the extended flow of water through the installed deflectors, simulating meanders.

In 2022, the system operated under greater pressure. The incoming pollution from the Gnieźnieńska Struga river, as well as the suspension from the combined sewage system and the stormwater system, which discharged wastewater from an ineffective wastewater treatment plant, pollution from settlers after the now-defunct sugar factory, and that from

allotment gardens caused the system to become shallower in many places, resulting in a reduced buffering capacity of the SED-BIO system. This also diminished the efficiency of the mineral filters. This was compounded by extreme weather phenomena such as heavy rains, which caused surface runoff and resulted in the inefficiency of the municipal sewage treatment plant and, thus, the discharge of sewage into the Gnieźnieńska Struga. This increased the level of pollutants flowing into the SED-BIO system. Additionally, strong winds caused backflows from Lake Jelonek into the system. Backflows were also caused by damming water in the lake during states of hydrological drought. The greatest anthropogenic threats on this section of the Gnieźnieńska Struga before it flows into Lake Jelonek are shown in Figure 15.

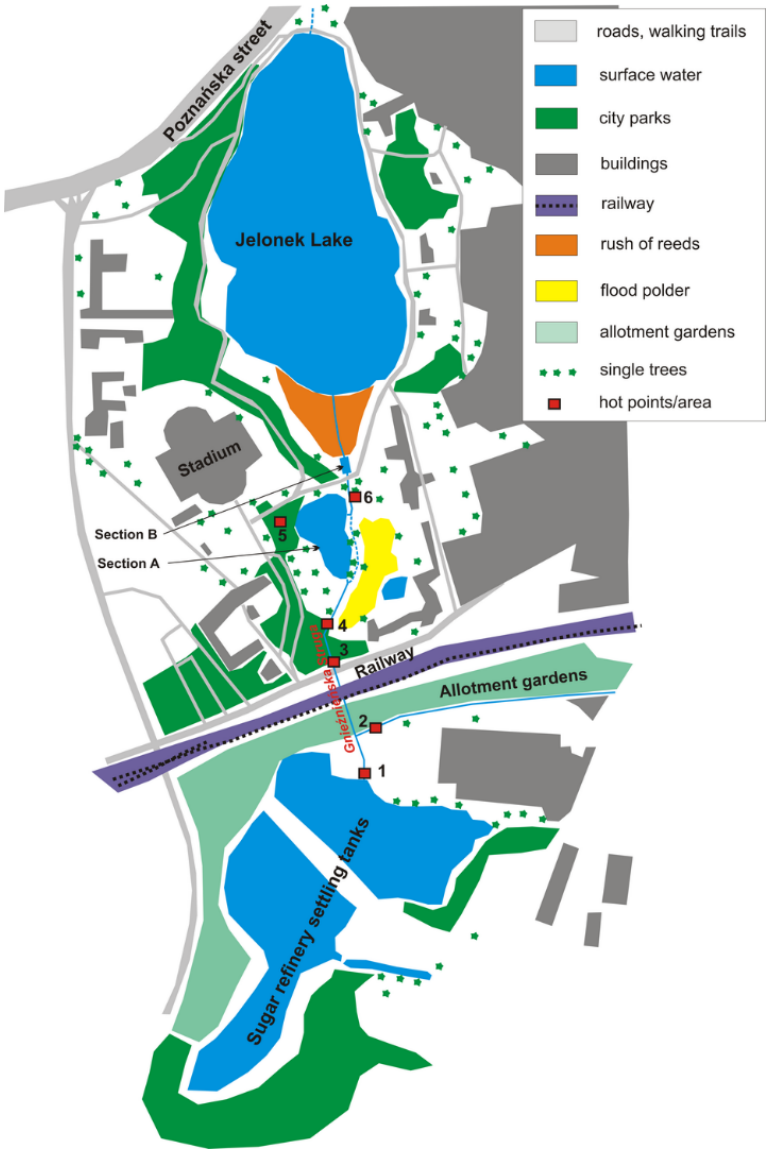

Figure 15. Places of greatest anthropogenic pressure modifying the quality of water in the Gnieźnieńska Struga river: 1—settling tanks of the former sugar factory; 2—allotment gardens; 3—storm sewage system with periodic discharge of municipal sewage from the treatment plant; 4—combined sewage system; 5—temporarily activated storm sewage system; and 6—surface runoff.

In the case of phosphates, it can be observed that, at sample point 2 (section A), the system continued to manage their reduction. Conversely, at point 5 (section B), an increase in phosphate concentrations was observed. The increase occurred from an average value

of 0.18 to 0.21 mg PO_4/L^{-1} compared to point 1, where the inflow entered the system. In terms of total phosphorus, it is evident that the system was less effective in its removal. At sample point 5, the average concentrations of total phosphorus were 0.206 mg/L^{-1} higher than at the inflow to the system (point 1). The concentrations of total phosphorus, similar to those of phosphates, at point 7 in 2022 were higher than at the inflow to the system. There was practically no noticeable reduction in these chemical compounds between points 5 and 7. The exchange of the filter with a mineral substrate for BioKer may have contributed to this, as it did not perform well in a heavily polluted environment and quickly clogged, reducing the active surface area. Another issue was the repeatedly removed suspension that shallowed the system by as much as 70–80% (down to 10–15 cm in depth).

Kupiec at al. [34] describe the operation and efficiency of the system in 2016–2019. During the examined period, the SED-BIO system retained 6.1–38.8% PO_4, 53.1–70.5%, and 4.5–23.6% $N\text{-}NH_4$. On average, annually, in the period of 2016–2019, the system reduced input of nutrient loads to Lake Jelonek by approximately 126.3 kg of PO_4, 2162.6 kg of $N\text{-}NO_3$, and 52.9 kg of $N\text{-}NH_3$.

4. Conclusions

Research on total phosphorus and nitrogen in the micro-dictum preparation indicates that they are present in the preparation at approximately 1% nitrogen and nearly 2 g/kg phosphorus in each pellet. Biogenic substances present in the water can be released by the dissolving product into the aquatic environment. However, no increased concentrations of this substance were observed in the zones of application of microorganisms under real conditions. The pH of the preparation fluctuated around an average level of 5.44.

In chemical studies, an increase in the concentrations of phosphates, total phosphorus, total nitrogen, and ammonium nitrogen in the water with the preparation was observed in all containers throughout the experiment. The concentrations of nitrate nitrogen indicated a slight decrease, while the values of nitrite nitrogen remained at a similar level. In the case of water in the container located outside the building, lower concentrations of phosphates, total phosphorus, ammonium nitrogen, nitrate nitrogen, and total nitrogen were observed compared to the water from the containers inside the building. The increase in the concentrations of these compounds was caused primarily by the small volume of water in the containers relative to the weight of the preparation. Under natural conditions, one ball was used per approx. 1 m^3 of water, and no problems with increasing the concentration of nitrogen and phosphorus compounds were observed.

Microbiological analyses of the micro-dictum preparation showed that the content of lactic acid bacteria in individual pellets may vary, and the quantitative composition of the microorganisms inside the pellet is lower compared to the outer layers.

The studies demonstrated that the activity of microorganisms released from the biopreparation depended on time, temperature, and water movement. The greatest activity of the microorganisms was observed up to the seventh day post application, during which a decrease in oxygen and pH was also evident. Similar processes occur at lower temperatures, though less violently. With forced water circulation, the deoxygenation was much greater, along with an increase in conductivity. This indicated an accelerated metabolism of microorganisms and processes of suspended matter decomposition in the water. This information serves as a basis for determining the method and timing of applying this biopreparation in rivers or water bodies.

Initially, a much longer duration of the micro-dictum pellet decomposition phase and a slow release of microorganisms were assumed; however, the conducted experiment showed that the processes occurring after the application of the micro-dictum preparation were very rapid, especially at room temperature. From the fourth to the sixth day of incubation, there was a stabilization of lactic acid bacteria (LAB) at a level of 10^5. An analysis conducted after the 34th day showed a decrease in the LAB numbers to levels of 10^1–10^3.

The results describing the dynamic increase in the number of lactic acid bacteria correlate with the simultaneously decreasing pH value and dissolved oxygen content.

These parameters also show a decrease in pH up until the sixth day of the experiment and the complete or nearly complete exhaustion of oxygen on the seventh day. Analyzing the correlation between the number of bacteria, pH, and %DO, it can be inferred that the concentration of LAB at a level of 10^5 was maintained for another 2 days before beginning to decline. A similar correlation was evident on the 34th day of incubation—the decline in the number of lactic acid bacteria was associated with the increase in and stabilization of the pH.

In the case of incubating the container outside the building, the number of lactic acid bacteria reached a maximum value of 10^3 after 14 days of incubation. A subsequent analysis (on the 34th day of incubation) showed a decrease in the number of microorganisms. Due to the low ambient temperature and limited biological activity, no changes in the pH values were observed, unlike the experiments conducted at room temperature.

The obtained results also suggest certain preferences of microorganisms regarding their distribution in the basin; the highest densities were observed in close proximity to the dissolving pellet and along the walls of the container, while the lowest numbers were recorded in the water column between the pellet and the vessel's edge. In analyzing the content of biogenic substances, each measurement following the day on which the pellet was placed in the water shows a successive increase in the content of ammonium nitrogen and total phosphorus.

Research conducted in real conditions on a specially constructed SED-BIO system built on the Gnieźnieńska Struga stream, which is a tributary to Lake Jelonek, indicates a high potential of the preparation for use in water reclamation. The studies demonstrated a lack of negative impact regarding changes in the physicochemical properties of the water at the application site. The effective functioning of the preparation requires combined reclamation methods, an appropriate buffering capacity of the system, and protection against extreme weather phenomena. Regular maintenance of the system (e.g., removal of excess accumulated suspended matter) is also important.

The limitations of using this preparation are primarily related to temperature. During cold periods, the activity of microorganisms is much lower, so their efficiency will also be lower. Increased flows may also cause faster dissolution of the preparation and the migration of organisms over long distances. The preparation itself should be properly protected against migration, e.g., with fascines. Extreme weather phenomena such as heavy rains may contribute to increased flows. It is important to design application sites in such a way as to ensure an adequate buffer capacity, especially where heavy rains occur frequently. Changes in physical parameters, particularly oxygen and pH, which are recorded at the bottom, where the greatest microbiological activity occurs, may affect other aquatic organisms. Although a lower pH or oxygen content is a temporary phenomenon, it may affect more sensitive organisms that inhabit the bottom area.

5. Patents

The SED-BIO system is currently subject to patenting procedures (application no. P.422056 for a method for the complex reduction in impurities in flows and the filtering system for the complex reduction in impurities in flows).

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Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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References

1. United Nations. *The United Nations World Water Development Report 2023. Partnerships and Cooperation for Water*; UNESCO: Paris, France, 2023. Available online: https://unesdoc.unesco.org/ark:/48223/pf0000384655 (accessed on 2 September 2024).
2. Liu, J.; Zheng, C. Towards Integrated Groundwater Management in China. In *Integrated Groundwater Management*; Jakeman, A.J., Barreteau, O., Hunt, R.J., Rinaudo, J.D., Ross, A., Eds.; Springer: Cham, Switzerland, 2016. [CrossRef]
3. Goldin, W.F. Incentiv Incentives for Change: China es for Change: China's Cadre System Applied t e System Applied to Water Quality. *Wash. Int. Law J.* **2011**, *20*, 399. Available online: https://digitalcommons.law.uw.edu/wilj/vol20/iss2/6 (accessed on 24 August 2024).
4. Zijun, L. *Acid Rain Affects One-Third of China; Main Pollutants Are Sulfur Dioxide and Particulate Matter*; Worldwatch Institute: Washington, DC, USA, 2006. Available online: https://spice.fsi.stanford.edu/docs/water_issues_in_china (accessed on 24 August 2024).
5. Kupiec, J.M. *Wieloaspektowa Ocena Wywieranej Presji Gospodarstw Rolnych na Środowisko*; Publishing House of the Poznań University of Life Sciences: Poznań, Poland, 2023. [CrossRef]
6. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. *Off. J. L* **2000**, *327*, 0001–0073.
7. WWAP (UNESCO World Water Assessment Programme). *The United NationsWorld Water Development Report 2019: Leaving No One Behind*; UNESCO: Paris, France, 2019. Available online: https://unesdoc.unesco.org/ark:/48223/pf0000367306 (accessed on 16 September 2024).
8. UN (United Nations). *Sustainable Development Goal 6: Synthesis Report 2018 on Water and Sanitation*; United Nations: New York, NY, USA, 2018. Available online: www.unwater.org/app/uploads/2018/07/SDG6_SR2018_web_v5.pdf (accessed on 15 September 2024).
9. WWAP (United Nations World Water Assessment Programme). *The United Nations World Water Development Report 2017: Wastewater—The Untapped Resource*; UNESCO: Paris, France, 2017. Available online: https://unesdoc.unesco.org/ark:/48223/pf0000247153 (accessed on 25 September 2024).
10. PBL Netherlands Environmental Assessment Agency. *The Geography of Future Water Challenges*; BPL Netherlands Environmental Assessment Agency: The Hague, The Netherland, 2018. Available online: https://www.pbl.nl/sites/default/files/downloads/pbl-2018-the-geography-of-future-water-challenges-2920_2.pdf (accessed on 2 September 2024).
11. WHO/UNICEF (World Health Organization/United Nations Children's Fund). *Progress on Drinking Water, Sanitation and Hygiene: 2017 Update and SDG Baselines*; WHO/UNICEF: Geneva, Switzerland, 2017. Available online: https://washdata.org/sites/default/files/documents/reports/2018-01/JMP-2017-report-final.pdf (accessed on 28 August 2024).
12. Prasad, R.; Singh, R. Microbial Bioremediation of Aquatic Environment: An Overview. *Res. J. Chem. Sci.* **2015**, *5*, 1–5.
13. Sreedevi, P.R.; Suresh., K.; Jiang, G. Bacterial bioremediation of heavy metals in wastewater: A review of processes and applications. *J. Water Process Eng.* **2022**, *48*, 102884. [CrossRef]
14. Gao, J.; Zhang, Y.; Li, H.; Tao, L.; Li, J.; Li, G. Application of microorganisms in the bioremediation of polluted water: A review. *Environ. Sci. Pollut. Res.* **2017**, *24*, 9092–9105.
15. Yang, Y.; Shi, X.; Ballent, W.; Mayer, B.K. Biological Phosphorus Recovery: Review of Current Progress and Future Needs. *Water Environ. Res.* **2017**, *89*, 2122–2135. [CrossRef]
16. Siripong, S.; Rittmann, B.E. Diversity study of nitrifying bacteria in full-scale municipal wastewater treatment plants. *Water Res.* **2007**, *41*, 1110–1120. [CrossRef]
17. Mellbye, B.L.; Bottomley, P.J.; Sayavedra-Soto, L.A. Nitrite-Oxidizing Bacterium Nitrobacter winogradskyi Produces N-Acyl-Homoserine Lactone Autoinducers. *Appl. Environ. Microbiol.* **2015**, *81*, 5917–5926. [CrossRef]
18. Melki, M.; Isnansetyo, A.; Widada, J.; Murwantoko, M. The significance of water quality parameters on the diversity of ammonia-oxidizing bacteria in the water surface of Musi river, Indonesia. *AACL Bioflux* **2018**, *11*, 1908–1918.
19. Han, D.; Hu, Z.; Li, D.; Tang, R. Nitrogen Removal of Water and Sediment in Grass Carp Aquaculture Ponds by Mixed Nitrifying and Denitrifying Bacteria and Its Effects on Bacterial Community. *Water* **2022**, *14*, 1855. [CrossRef]
20. Carlson, C.A.; Ingraham, J.L. Comparison of denitrification by *Pseudomonas stutzeri*, *Pseudomonas aeruginosa*, and *Paracoccus denitrificans*. *Appl. Environ. Microbiol.* **1983**, *45*, 1247–1253. [CrossRef] [PubMed]
21. Bedzyk, L.; Wang, T.; Ye, R.W. The periplasmic nitrate reductase in *Pseudomonas* sp. strain G-179 catalyzes the first step of denitrification. *J. Bacteriol.* **1999**, *181*, 2802–2806. [CrossRef] [PubMed]
22. Martikainen, P.J. Heterotrophic nitrification—An eternal mystery in the nitrogen cycle. *Soil Biol. Biochem.* **2022**, *168*, 108611. [CrossRef]
23. Wang, J.; Long, Y.; Yu, G.; Wang, G.; Zhou, Z.; Li, P.; Zhang, Y.; Yang, K.; Wang, S. A Review on Microorganisms in Constructed Wetlands for Typical Pollutant Removal: Species, Function, and Diversity. *Front. Microbiol.* **2022**, *13*, 845725. [CrossRef] [PubMed]

24. Habyarimana, J.L.; Juan, M.; Nyiransengiyumva, C.; Qing, T.W.; Yu, C.; Twagirayezu, G.; Ying, D. Critical review on operation mechanisms to recover phosphorus from wastewater via microbial procedures amalgamated with phosphate-rich in side-stream to enhance biological phosphorus removal. *Biocatal. Agric. Biotechnol.* **2022**, *45*, 102484. [CrossRef]
25. Bhakta, J.N.; Munekage, Y.; Ohnishi, K.; Jana, B.B.; Balcazar, J.L. Isolation and characterization of cadmium and arsenic-absorbing bacteria for bioremediation. *Water Air Soil Pollut.* **2014**, *225*, 2150–2159. [CrossRef]
26. Coelho, L.M.; Rezende, H.C.; Coelho, L.M.; de Sousa, P.A.R.; Melo, D.F.O.; Coelho, N.M.M. Bioremediation of Polluted Waters Using Microorganisms. *Adv. Bioremediation Wastewater Polluted Soil* **2015**, *10*, 60770. [CrossRef]
27. Buranasilp, K.; Charoenpanich, J. Biodegradation of acrylamide by Enterobacter aerogenes isolated from wastewater in Thailand. *J. Environ. Sci.* **2011**, *23*, 396–403. [CrossRef] [PubMed]
28. Ayilara, M.S.; Babalola, O.O. Bioremediation of environmental wastes: The role of microorganisms. *Front. Agron. Sec. Plant-Soil Interactions.* **2023**, *5*, 1183691. [CrossRef]
29. Al Bazedi, G.; Ismail, M.M.; Mugwanya, M.; Sewilam, H. Desalination concentrate microalgae cultivation: Biomass production and applications. *Sustain. Water Resour. Manag.* **2023**, *9*, 108. [CrossRef]
30. Zhao, D.; Cheah, W.Y.; Lai, S.H.; Ng, E.P.; Kuan Shiong Khoo, K.S.; Show, P.L.; Ling, T.C. Symbiosis of microalgae and bacteria consortium for heavy metal remediation in wastewater. *J. Environ. Chem. Eng.* **2023**, *11*, 109943. [CrossRef]
31. Gururani, P.; Bhatnagar, P.; Kumar, V.; Vlaskin, M.S.; Grigorenko, A.V. Algal Consortiums: A Novel and Integrated Approach for Wastewater Treatment. *Water* **2022**, *14*, 3784. [CrossRef]
32. Atagana, H.I. Biodegradation of PAHs by fungi in contaminated-soil containing cadmium and nickel ions. *Afr. J. Biotechnol.* **2009**, *21*, 5780–5789.
33. Balaji, V.; Arulazhagan, P.; Ebenezer, P. Enzymatic bioremediation of polyaromatic hydrocarbons by fungal consortia enriched from petroleum contaminated soil and oil seeds. *J. Environ. Biol.* **2013**, *35*, 521–529. Available online: https://www.kau.edu.sa/Files/188/Researches/65402_36751.pdf (accessed on 28 August 2024).
34. Kupiec, J.M.; Bednarek, A.; Szklarek, S.; Mankiewicz-Boczek, J.; Serwecińska, L.; Dąbrowska, J. Evaluation of the Effectiveness of the SED-BIO System in Reducing the Inflow of Selected Physical, Chemical and Biological Pollutants to a Lake. *Water* **2022**, *14*, 239. [CrossRef]
35. Zhang, Y.; Qiu, X.; JLuo, J.; Li, H.; How, S.-W.; Wu, D.; He, J.; Cheng, Z.; Gao, Y.; Lu, H. A review of the phosphorus removal of polyphosphate-accumulating organisms in natural and engineered systems. *Sci. Total Environ.* **2024**, *912*, 169103. [CrossRef]
36. Xiao, M.; Burford, M.A.; Wood, S.A.; Aubriot, L.; Ibelings, B.W.; Prentice, M.J.; Galvanese, E.F.; Harris, T.D.; Hamilton, D.P. Schindler's legacy: From eutrophic lakes to the phosphorus utilization strategies of cyanobacteria. *FEMS Microbiol Rev.* **2022**, *2*, 46. [CrossRef]
37. Aubriot, L.; Bonilla, S.; Falkner, G. Adaptive phosphate uptake behaviour of phytoplankton to environmental phosphate fluctuations. *FEMS Microbiol. Ecol.* **2011**, *77*, 1–16. [CrossRef] [PubMed]
38. Jurkiewicz-Karnkowska, E. Differentiation of Phosphorus Concentration in Selected Mollusc Species from the Zegrzy.ski Reservoir (Central Poland): Implications for P Accumulation in Mollusc Communities. *Pol. J. Environ. Stud.* **2002**, *11*, 355–359.
39. Sugiura, S.H. Digestion and Absorption of Dietary Phosphorus in Fish. *Fishes* **2024**, *9*, 324. [CrossRef]
40. Carrillo, V.; Gómez, G.; Vidal, G. Phosphorus uptake by macrophyte plants in monocultures and polycultures in constructed wetlands for wastewater treatment. *Ecol. Eng.* **2022**, *182*, 106690. [CrossRef]
41. Szubański, K. Łódzki Wynalazek Pomoże Usuwać Zanieczyszczenia Fosforanowe z Wody. PAP—Nauka w Polsce. 2018. Available online: https://naukawpolsce.pl/aktualnosci/news,29580,lodzki-wynalazek-pomoze-usuwac-zanieczyszczenia-fosforanowe-z-wody.html (accessed on 2 September 2024).
42. Ratzke, C.; Gore, J. Modifying and reacting to the environmental pH can drive bacterial interactions. *PLoS Biol.* **2018**, *16*, e2004248. [CrossRef]
43. Lund, P.A.; De Biase, D.; Liran, O.; Scheler, O.; Mira, N.P.; Cetecioglu, Z.; Fernández, E.N.; Bover-Cid, S.; Hall, R.; Sauer, M.; et al. Understanding How Microorganisms Respond to Acid pH Is Central to Their Control and Successful Exploitation. *Front. Microbiol.* **2020**, *11*, 556140. [CrossRef] [PubMed]
44. Riedel, T.E.; Berelson, W.M.; Nealson, K.H.; Finkel, S.E. Oxygen consumption rates of bacteria under nutrient-limited conditions. *Appl. Environ. Microbiol.* **2013**, *79*, 4921–4931. [CrossRef]
45. Mazurkiewicz, J.; Mazur, A.; Mazur, R.; Chmielowski, K.; Czekała, W.; Janczak, D. The Process of Microbiological Remediation of the Polluted Słoneczko Reservoir in Poland: For Reduction of Water Pollution and Nutrients Management. *Water* **2020**, *12*, 3002. [CrossRef]
46. McMahon, K.D.; Read, E.K. Microbial contributions to phosphorus cycling in eutrophic lakes and wastewater. *Annu. Rev. Microbiol.* **2013**, *67*, 199–219. [CrossRef]
47. Rubin, J.A.; Görres, J.H. Potential for Mycorrhizae-Assisted Phytoremediation of Phosphorus for Improved Water Quality. *Int. J. Environ. Res. Public Health* **2020**, *18*, 7. [CrossRef] [PubMed]
48. Hu, J.Y.; Ong, S.L.; Ng, W.J.; Lu, F.; Fan, X.J. A new method for characterizing denitrifying phosphorus removal bacteria by using three different types of electron acceptors. *Water Res.* **2003**, *37*, 3463–3471. [CrossRef]
49. Chen, Y.; Li, S.; Lu, Y.; Zhu, G.; Cheng, H. Simultaneous nitrification, denitrification and phosphorus removal (SNDPR) at low atmosphere pressure. *Biochem. Eng. J.* **2020**, *160*. [CrossRef]

50. Zhang, S.; Huffman, T.; Zhang, X.; Liu, W.; Liu, Z. Spatial distribution of soil nutrient at depth in black soil of Northeast China: A case study of soil available phosphorus and total phosphorus. *Int. J. Soil Sedim. Water* **2014**, *14*, 1775–1789. [CrossRef]
51. Yang, X.; Chen, X.; Yang, X. Effect of organic matter on phosphorus adsorption and desorption in a black soil from Northeast China. *Soil Tillage Res.* **2019**, *187*, 85–91. [CrossRef]
52. Zhao, W.; Xie, X.; He, T.; Zhang, J.; Liu, J. Study on vertical variation characteristics of soil phosphorus adsorption and desorption in black soil region of Northeast China. *PLoS ONE* **2024**, *19*, e0306145. [CrossRef] [PubMed]