

## Article

# Environmental Factors Affecting the Efficiency of Water Reservoir Restoration Using Microbiological Biotechnology

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**Abstract:** Aquatic ecosystems are often subject to degradation due to various environmental stressors. The accumulation of an organic sediment layer causes shallowing, algal blooms, and hypertrophy in water reservoirs. The processes of overgrowth and shallowing lead to a reduction in the ecosystem services provided by the reservoir as well as potentially causing the disappearance of the water body. To address these challenges and restore the ecological balance of water reservoirs, effective and sustainable revitalisation methods are essential. In recent years, biotechnological approaches, particularly utilizing microbiological interventions, have emerged as promising strategies for water reservoir revitalization. Microorganisms, with their remarkable ability to degrade pollutants and enhance nutrient cycling, offer great potential in remediating environmental issues in a natural and eco-friendly manner. This article presents the results of a study of 33 Polish reservoirs subjected to reclamation with microbial biopreparations from 2014 to 2023. The results of changes in bottom sediment reduction, water transparency, dissolved oxygen concentration, and water turbidity are presented. Reduction in morphological changes in the fraction of soft organic sediments, an improvement in the oxygen profile of the bottom and surface water layers, and an increase in water transparency were observed after reclamation with the use of biopreparations.

**Keywords:** sustainable revitalisation; effective microorganisms; lakes and ponds; bottom sediments



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## 1. Introduction

Water reservoirs are crucial in sustaining ecosystems and supporting human activities, providing a number of ecosystem services like water supply, flood prevention, recreation, etc. [1,2]. However, lentic aquatic ecosystems are often subject to degradation by environmental stressors, such as pollution, eutrophication, and habitat loss. Human activities have a multidimensional impact on surface water quality. The negative influence is mainly associated with urban agglomerations due to the concentration of residents, manufacturing, and service activities [3]. Effective and sustainable revitalisation methods are essential to address these challenges and restore the ecological balance of water reservoirs [4,5].

In recent years, biotechnological approaches, particularly utilizing microbiological interventions, have emerged as promising strategies for water reservoir revitalization [6,7]. Microorganisms, with their remarkable ability to degrade pollutants and enhance nutrient cycling, offer great potential to remediate environmental issues in a natural and eco-friendly manner [8]. In response to these challenges, increasing attention has been given to the application of microbiological biopreparations, particularly effective microorganisms (EMs), as potential remediation tools [9–11]. Microbiological biopreparations with EMs represent a compelling approach to the remediation of water bodies for their capacity to support natural biodegradation processes and ecosystem restoration. EMs are a unique combination of beneficial microorganisms, including bacteria, yeast, and fungi, which synergistically

interact, exerting a positive influence on the aquatic environment [12]. Numerous reports on the potential benefits offered by these biopreparations, such as water quality improvement, pollution reduction, inhibition of cyanobacterial and algal growth, biodiversity restoration, and revitalization of aquatic ecosystems, emphasize the success and advantages of using such techniques [7,10,13–15]. Be that as it may, the successful application of microbiological biotechnology in water reservoirs heavily relies on understanding and addressing the complex environmental factors that influence its efficiency [15].

As the global need for sustainable water management intensifies, mastering the complexities of microbiological biotechnology in the context of water reservoir revitalization becomes increasingly vital, if we are to comply with the Ecological Assessment of Streams and Reservoirs suggested by the Environment Action Programme 2030 (European Union), protecting, preserving, and restoring ecosystems and biodiversity, and enhancing natural capital [16].

#### *A Brief Overview of the Application Area of the Microbiological Remediation Method for Lakes*

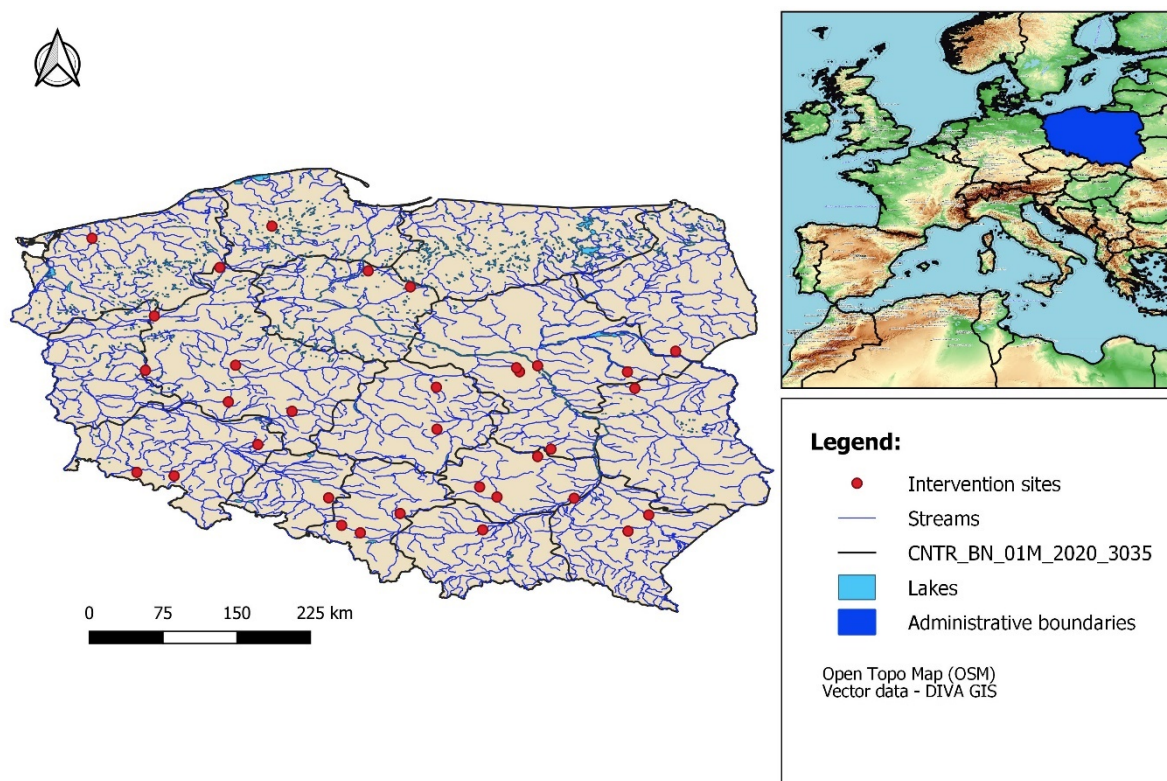
The assessment encompassed 33 water bodies located in Poland, spanning a wide range of geographical and geomorphological regions. The water bodies shared comparable conditions in terms of vegetative seasonal patterns and pathologies within lake ecosystems. The temperate climate surrounding Poland influences the ecosystem's dynamic aquatic vegetative period, governed by a myriad of complex and diverse factors spanning from spring to autumn. It is worthwhile examining in detail the factors that influence duration and intensity, in particular those influencing reservoirs that frequently display uncommon seasonal patterns.

Water temperature plays a fundamental role in the development of aquatic organisms and is one of the main determinants of vegetative seasonal length. Studies by Prejs et al. [17] and more contemporary analyses by Bielczyńska [18], Pelechata et al. [19], and Napiórkowski et al. [20] demonstrated that yearly seasonal temperature variability significantly affects the vegetative period timespan. Water temperature rise during spring stimulates aquatic organisms' activity, driving the onset of the vegetative season; conversely, late summer and autumn cool temperatures decrease aquatic organisms' function and they enter a dormant phase, marking the conclusion of this period [21–23]. The presence of nutrients, such as nitrates and phosphates from many sources, holds essential significance for the duration of the vegetative season. Water enriched with these nutrients can favour vigorous growth in aquatic organisms, which can either shorten or prolong the vegetative season, depending on the extent of nutrient availability [24,25].

Regrettably, the state of water quality in Poland is far from the EU Water Framework Directive's desired objectives. According to the latest research data [26,27], most Polish lakes (approximately 89%) exhibit a low ecological status, and over 90% of water bodies have their chemical conditions rated below good at least once a year. Consequently, there is an urgent need for remedial actions and the elimination of pollution sources to restore ecological balance in Poland's surface waters. The requirement for comprehensive remediation efforts encompassing both small and large water reservoirs necessitates the use of methods and tools characterized by a wide-ranging efficacy, minimal perturbation to the aquatic environment, and sound economic justification. In Poland, only one biotechnological company, ACS Holding (group), engages in large-scale initiatives related to the reclamation of water reservoirs through biological methods. This company exclusively employs biological methods, avoiding chemical approaches, while collaborating with numerous scientific research centres. Since 2014, this consortium of entrepreneurs and water biotechnology experts has successfully executed over 200 microbiological remediation processes for degraded water reservoirs all over Poland.

The aim of this article is to present the results of changes in the reduction in bottom sediments, water transparency, dissolved oxygen concentration (at the bottom and near the surface), and water turbidity for 33 small and large reservoirs, subject to reclamation efforts from 2014 to 2023 (Figure 1). All processes of water reservoir reclamation were carried out

for public entities managed by local government units. Annually, the holding conducts approximately 30 reclamation projects for various water reservoirs in Poland. Sites were selected randomly and according to data monitoring availability for all parameters.



**Figure 1.** Polluted water reservoirs restoration process on the territory of Poland by application of microbiological methods.

## 2. Materials and Methods

Lake reclamation using biopreparate application involves road transportation to the application site in volumes appropriate to the reservoir reclamation plan (ranging from a couple to several dozen cubic meters) (Figure 2).



**Figure 2.** Biopreparations (for water and sediments) transported on the banks of Ozanna Lake before the application process.

The application of biopreparations can begin when the water temperature exceeds 5 °C, though the optimal temperature range is 10–25 °C, within this range, microorganisms awaken from a dormant state and initiate active metabolism in the new aquatic

environment. The cocktail of biopreparates also stimulates the growth of autochthonous microorganisms and colonizes the aquatic macrophyte root radicular zone. The application of biopreparations for the reduction in soft organic sediment fractions is carried out with gentle flow injector streams, so as not to disturb the bottom sediments and cause increased turbidity in the water, thus reducing the efficiency of the process.

Water surface application is conducted with high-flow injector streams, thereby ensuring good mixing in water and improved oxygenation (stimulating better adaptation of consortia to the new critical environment). Biomixtures were intensively introduced into the macrophyte zone, which provides a very safe area for their development and protects against the negative effects of sunlight and UV radiation (Figure 3). The application is carried out by several technical groups in predesignated quadrants of the reservoir.



**Figure 3.** Application process of biopreparation in Ozanna Lake (intensive saturation of macrophyte zone).

Before commencing the biopreparation application process, a comprehensive monitoring of water quality parameters is conducted with the participation of the project's inspectors.

The main types of biopreparations used were primarily ODO-1 for sediment treatment and ODO-2 for reduction in organic pollutants in surface water.

ACS ODO-1 biopreparation: comprises water, a blend of lactic acid bacteria, phototrophic bacteria, yeast, ecological molasses derived from sugar cane, fermented wheat bran, and minerals. In addition, the micro-level ingredients of this biopreparation include phytosterols (such as sitosterol and taraxasterol), phytohormones, triterpenes (like lupeol, betulin, and betulinic acid), flavonoids (including hyperoside, quercetin, and kaempferol), ellagic acid, pyrocatechic acid, brevofolin (an ellagic acid derivative), vitamins (C, PP, P, B3, B5, B8, B11, B1, B2, A, E, and F), and tannins.

ACS ODO-2 (aqua 2) biopreparation: comprises water, sugar cane molasses, and effective microorganisms, including the primary strains of effective microorganisms *Lactobacillus casei*, *Lactobacillus plantarum* (at a concentration of  $5.0 \times 10^6$  cfu·mL<sup>-1</sup>), and *Saccharomyces cerevisiae* (at a concentration of  $5.0 \times 10^3$  cfu·mL<sup>-1</sup>).

### 2.1. Water Monitoring

Measurements were conducted at multiple points within each of the analysed reservoirs, following the established research plan, before commencing the reclamation process, and in the subsequent season. The research included a minimum of 6 measurement points. In the case of interconnected reservoirs, 6 measurement points were utilized for each reservoir. Monitoring studies were conducted at corresponding times in both seasons (before

revitalization and after). Water quality was monitored during rain-free periods as well as during periods of algal blooms. Samples were collected in the morning hours. The measured parameters and methods/tools used for water monitoring are shown in Table 1.

**Table 1.** Measurement methods applied in the study of selected water quality parameters.

The Measured Parameters	Methods/Tools
Dissolved oxygen 2014–2018	By portable multi-function meter CX-401 with oxygen galvanic oxygen sensors COG-1 (made by Elmetron)
Dissolved oxygen 2014–2018	By portable multi-function meter HQ 1130 with a 5-m cable and oxygen sensor (probe) (made by Hach)
Transparency	Measurement by Secchi disc
Sediment layer	With the application of geodetic staff, an endoscopic camera with its light source, and a visual monitor connected to the camera via a USB cable—sediment thickness readings were displayed on a metric scale on the geodetic staff
Turbidity	By portable turbidity meter 2100Q (made by Hach)
Chlorophyll-a	Spectrophotometric method according to the PN-86 C-05560/02 standard [28]

The data were averaged for each lake, and comparative statistical analyses were performed for the selected sites using the statistical significance analyses of differences with SPSS 25 software for the examined water quality parameters before reclamation, and in the subsequent vegetative season after bioremediation treatments. Statistical non-parametric Wilcoxon paired *t*-tests were employed to verify data significance.

## 2.2. Environmental Parameters of Investigated Water Reservoirs

Most of the analysed water reservoirs are classified as shallow, with only three cases considered to have a moderate depth (Table 2). The Pniowiec Dam Reservoir and Niskie Lake have zones where a thermocline is present (Table 2). The others belong to polymictic reservoirs, which respond rapidly and vigorously to changes in water trophic status and the influence of water pollution. These reservoirs represent the whole territory of Poland (Figure 1), with the exception of the mountainous regions.

**Table 2.** Characterization of water bodies subjected to microbiological bioremediation process in Poland.

No.	Water Reservoir	Location	Date of Restoration	Surface Area (m <sup>2</sup> )	Volume (m <sup>3</sup> )	Maximum Depth (m)	Average Depth (m)	Water Flow Types	Flow Velocity	River Tributary
1	Pniowiec Dam Reservoir	Rybnik	2018–2019	885,000	4,800,000	12	5.4	exorheic reservoir	slow	Ruda
2	Niskie Brodno Lake	Brodnica	2022–2023	823,420	5,681,595	18.2	6.9	exorheic reservoir	slow	Brodniczka
3	Kórnickie Lake	Kórnik	2022–2023	741,669	2,225,007	6	3	exorheic reservoir	slow	Kórnicki canal
4	Błędno Lake	Grójec	2022–2023	700,000	1,330,000	5.6	1.9	exorheic reservoir	slow	Obra-Szarka
5	Bugaj Lake	Piotrków Trybunalski	2017–2018	520,000	936,000	4	1.8	exorheic reservoir	slow	Wirzejka
6	Pasternik Lake	Starachowice	2021–2022	420,000	840,000	4	2	exorheic reservoir	slow	Kamienna

Table 2. Cont.

No.	Water Reservoir	Location	Date of Restoration	Surface Area (m <sup>2</sup> )	Volume (m <sup>3</sup> )	Maximum Depth (m)	Average Depth (m)	Water Flow Types	Flow Velocity	River Tributary
7	Muchawka Dam Reservoir	Siedlce	2014–2015	400,000	600,000	5	1.5	endorheic reservoir	very slow/no flow	Muchawka
8	Łokacz Lake in K.W.	Krzyż Wielkopolski	2022–2023	382,241	1,146,722	3.4	3	exorheic reservoir	slow	Człopica
9	Mrożyczka Dam Reservoir	Głowno	2019–2020	380,000	608,000	3.3	1.6	exorheic reservoir	slow	Mroga
10	Siemiatycze Dam Reservoir	Siemiatycze	2016–2017	274,000	548,000	5.1	2	exorheic reservoir	slow	Kamionka and Mahomet
11	Tarpno Lake	Grudziądz	2021–2022	270,000	1,512,000	5.8	5.6	exorheic reservoir	slow	Trynkowa
12	Studzinniczo Lake	Studzinniczo, Bytów	2022–2023	210,623	1,474,363	20	7	exorheic reservoir	slow	Studnica
13	Iżanka Reservoir	Iża	2020–2021	185,447	148,358	3	0.8	exorheic reservoir	slow	Iżanka
14	Ożanna Lake	Kuryłówka	2020–2021	181,245	253,743	3.7	1.4	exorheic reservoir	slow	Złota
15	Zimna Woda Lake	Łuków	2020–2021	180,000	270,000	3	1.5	endorheic reservoir	very slow/no flow	Krzna Południowa
16	Pinczów Dam Reservoir	Pińczów	2022–2023	115,000	218,500	3.5	1.9	exorheic reservoir	slow	Nida canal
17	Warszawa city pond	Warsaw	2022–2023	108,807	321,091	5	2.5	exorheic multi reservoirs	slow	Wystawowy Canal–Vistula
18	Śmieszek Lake	Żory	2019–2020	95,000	190,000	2.7	2	exorheic reservoir	slow	Ruda
19	Bąk Lake	Okonek	2018–2019	65,000	97,500	3	1.5	endorheic reservoir	very slow/no flow	Czarna
20	Leśny Lake	Sosnowiec	2021–2022	50,913	101,826	2.5	2	endorheic reservoir	no flow	No-name water canal
21	Pruszków city pond	Pruszków	2020–2021	30,998	46,496	1.95	1.5	endorheic reservoir	very slow/no flow	Utrata
22	Oleśnica city pond	Oleśnica	2021–2022	28,408	42,612	2	1.5	endorheic reservoir	very slow/no flow	Oleśnica
23	Ożarów Mazowiecki city pond	Ożarów Mazowiecki	2020–2021	13,314	19,972	2	1.5	endorheic reservoir	very slow/no flow	City canal
24	Strzelce Opolskie city pond	Strzelce Opolskie	2020–2021	12,289	12,289	1	1	endorheic reservoir	no flow	groundwater-fed ponds
25	Jędrzejów Dam Reservoir	Jędrzejów, Świętokrzyskie	2021–2022	11,636	23,272	3	2	endorheic reservoir	very slow/no flow	Brzeźnica
26	Kaczeńcowa city pond	Kraków County	2017–2018	8200	11,480	2	1.4	endorheic reservoir	very slow/no flow	groundwater-fed ponds–Młynówka

Table 2. Cont.

No.	Water Reservoir	Location	Date of Restoration	Surface Area (m <sup>2</sup> )	Volume (m <sup>3</sup> )	Maximum Depth (m)	Average Depth (m)	Water Flow Types	Flow Velocity	River Tributary
27	Stawek pond in Gryfice	Gryfice	2021–2022	6866	27,466	9	4	endorheic reservoir	very slow/no flow	No-name water canal
28	Ostrów Wielkopolski city pond	Ostrów Wielkopolski	2022–2023	5900	8849	2	1.5	endorheic reservoir	very slow/no flow	No-name water canal
29	Gedymin pond	Szczawno-Zdrój	2021–2022	5445	8167	2	1.5	exorheic reservoir	very slow/no flow	Szczawnik
30	Baranów Sandomierski Palast pond	Baranów Sandomierski	2022–2023	4718	9435	2	2	endorheic reservoir	very slow/no flow	No-name water canal
31	Krobia community pond	Gmina Krobia	2021–2022	4009	5124	1.5	1.5	endorheic multi reservoirs	very slow/no flow	No-name water canal
32	Mysłakowice Palast pond	Mysłakowice	2021–2022	3640	5460	2	1.5	exorheic reservoir	very slow/no flow	Łomnica
33	Pond at Łañcut Castle Park	Łañcut	2022–2023	3121	4682	2	1.5	exorheic reservoir	very slow/no flow	No-name water canal

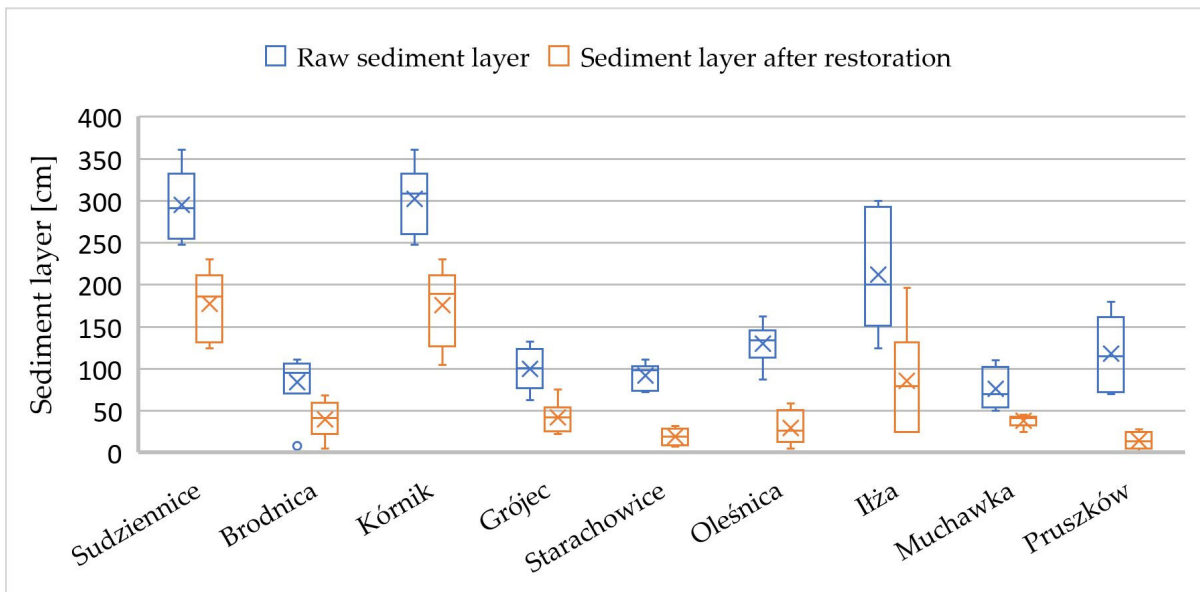
### 3. Results and Discussion

#### 3.1. Reduction in Soft Organic Sediment Fractions (SOSF)

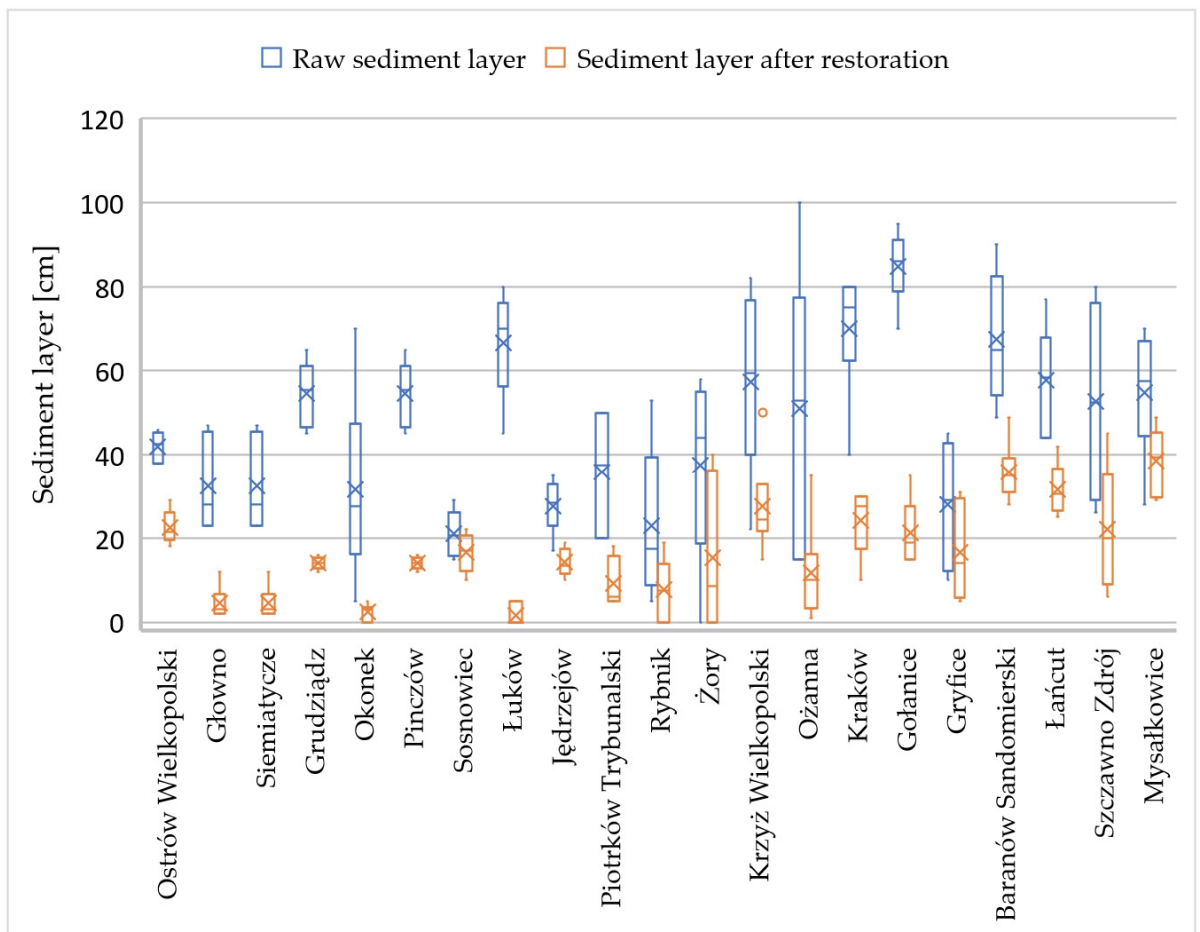
The sedimentation process of suspended particles, predominantly consisting of organic fractions, leads to an increase in bottom sediments during the spring–summer and autumn periods in eutrophicated and highly polluted reservoirs [29–32]. The rise in lake trophic levels, coupled with intense algal blooms, intensifies the summer sedimentation processes, resulting in significant increments in organic fractions, thereby affecting the variability in sediment thickness [31]. In nine of the assessed reservoirs, a layer of accumulated organic sediment fractions exceeding 1 m in thickness was observed (Figure 4). These reservoirs were characterized by pronounced algal blooms and hypertrophy due to the inflow and accumulation of significant nutrients in the water. In the remaining surveyed reservoirs, the layers of organic sediments did not exceed 1 m in thickness, albeit still representing a considerable burden for these water bodies. The biological decomposition of organic material in the bottom layer, once dissolved oxygen is depleted, occurs under strongly oxygen-deprived conditions through fermentation, generating a range of toxic substances and environmental nuisances for such degraded lakes [33].

All rehabilitated reservoirs, prior to treatment, exhibited odours and other environmental and social nuisances that hindered their proper exploitation and exhibited an overall negative impact on the quality of aquatic ecosystems. In all studied reservoirs, a successive reduction in soft organic fractions in bottom sediments was observed in the vegetative season following the application of directional biomixtures (Figures 4–6).

In reservoirs where the accumulated thickness of organic sediment fractions (SOSF) exceeded 1 m, there was a pronounced dynamic reduction observed in subsequent seasons during corresponding research periods, clearly observed in Gotanice, Tuków, and Baranów (Figure 4). The reduction processes were slightly slower in water bodies with sediment layers less than 1 m thick, namely, Sosnowiec, Jędrzejów, and Rybnik (Figure 5).

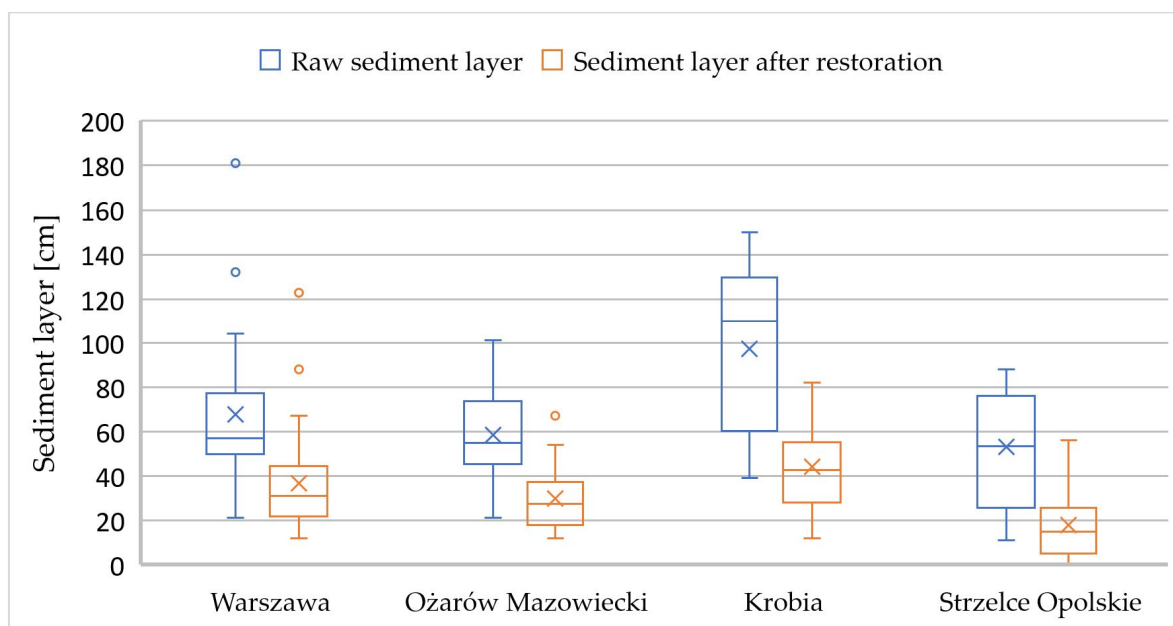


**Figure 4.** Reduction in soft organic sediment fractions in reservoirs with a primary layer exceeding 1 m (○—indicate outliers, x—indicate average value).



**Figure 5.** Reduction in soft organic sediment fractions in reservoirs with a primary layer below 1 m (○—indicate outliers, x—indicate average value).





**Figure 6.** Reduction in soft organic sediment fractions in interconnected small reservoirs (○—indicate outliers, x—indicate average value).

An analysis of the distribution of results demonstrated a highly satisfactory decomposition process, with, in most cases, a twofold or greater reduction compared to the previous season before the start of the rehabilitation efforts. Additionally, the interconnected reservoirs in the analysed areas exhibited a highly satisfactory decrease in SOSF (Figure 6). Due to the local conditions, the individual interconnected ponds had varying thicknesses of bottom sediment layers, with the initial reservoirs having thicker sediment layers and the final ones significantly thinner. This is evident in the distribution of SOSF results, both for the original layers and post-rehabilitation (Figure 6).

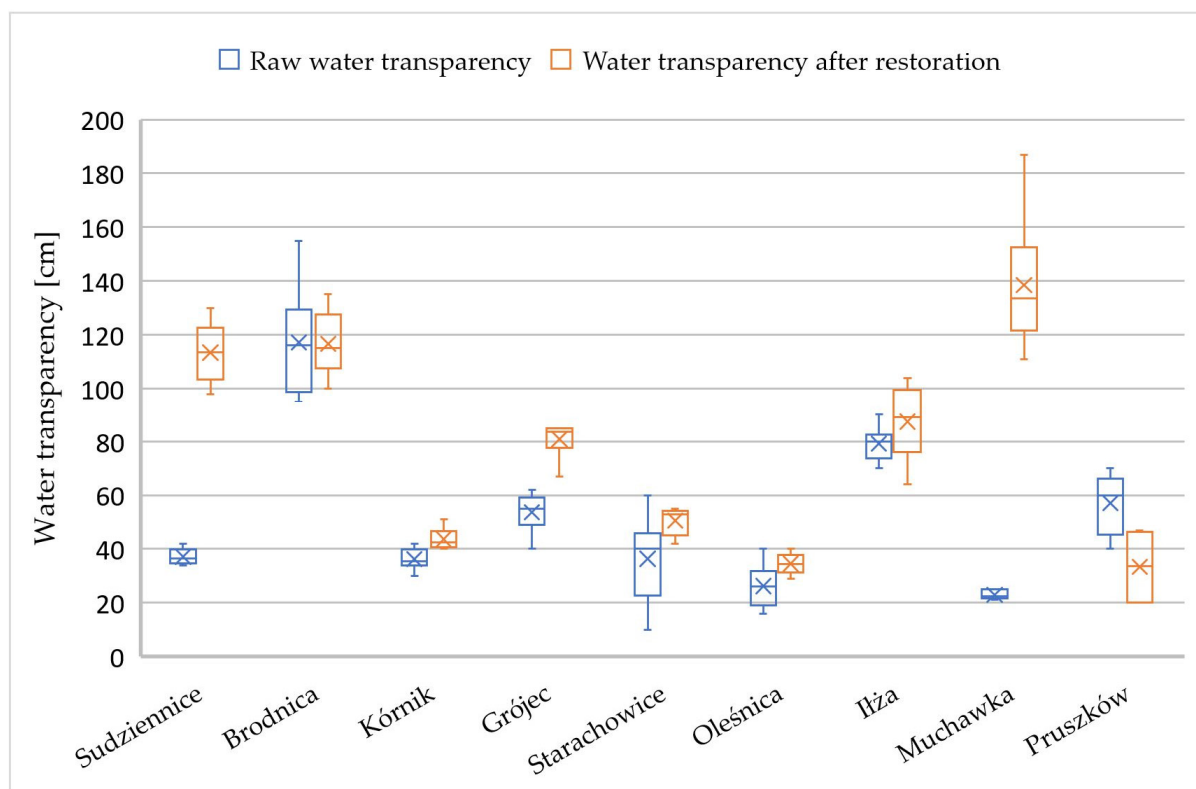
Observations conducted after the application of biopreparations to rehabilitate water bodies revealed morphological changes in SOSF. Accumulated primary layers were characterized by significantly compact and dense consistency. Following the introduction of biopreparations, a gradual softening of these fractions was observed, as they were further hydrated and more easily carried away by the flowing water. In reservoirs where these fractions reached thicknesses of 2 to 3 m (Studniczno, Iłżanka, Kórnik reservoirs—Figure 4), an additional factor enhancing the dynamics of their reduction could have been erosion, especially in the case of flow-through lakes. Besides the microbiological remediation method, the only effective and rapid removal of sediment from silted and polluted lakes was by mechanical sediment removal processes [34–37]. However, mechanical methods are extremely costly and invasive for the aquatic environment, making them nearly impossible to implement in the case of large water reservoirs. Both draining a lake and removing sediment with a bulldozer and using bucket- or hydraulic-type dredges is destructive to aquatic ecosystems [38]. Other ecological remediation methods are not as effective in reducing SOSF. Biological remediation methods oriented to the development of submerged vegetation or the forming of buffer emergent vegetation in the nearshore zone of the reservoir can even contribute to increasing the sediment layer [39,40]. The reduction dynamics characteristic of other methods are not as effective when compared to this biotechnological method based on microbial consortia utilizing SOSF as a source of organic carbon [10]. Changes in the bottom sediment layer outside the park pond in Sosnowiec exhibited statistically significant differences in both periods before reclamation and in the subsequent season after bioremediation treatments were conducted (Table A1, Appendix A). Watercourses supplying the Leśny pond in Sosnowiec continued to supply a substantial amount of sediment, contributing to turbidity, during the reclamation period, disrupting the purification

processes. Since this reservoir is non-flowing, the excessive accumulation of introduced sediments had a detrimental impact on the rate of their reduction.

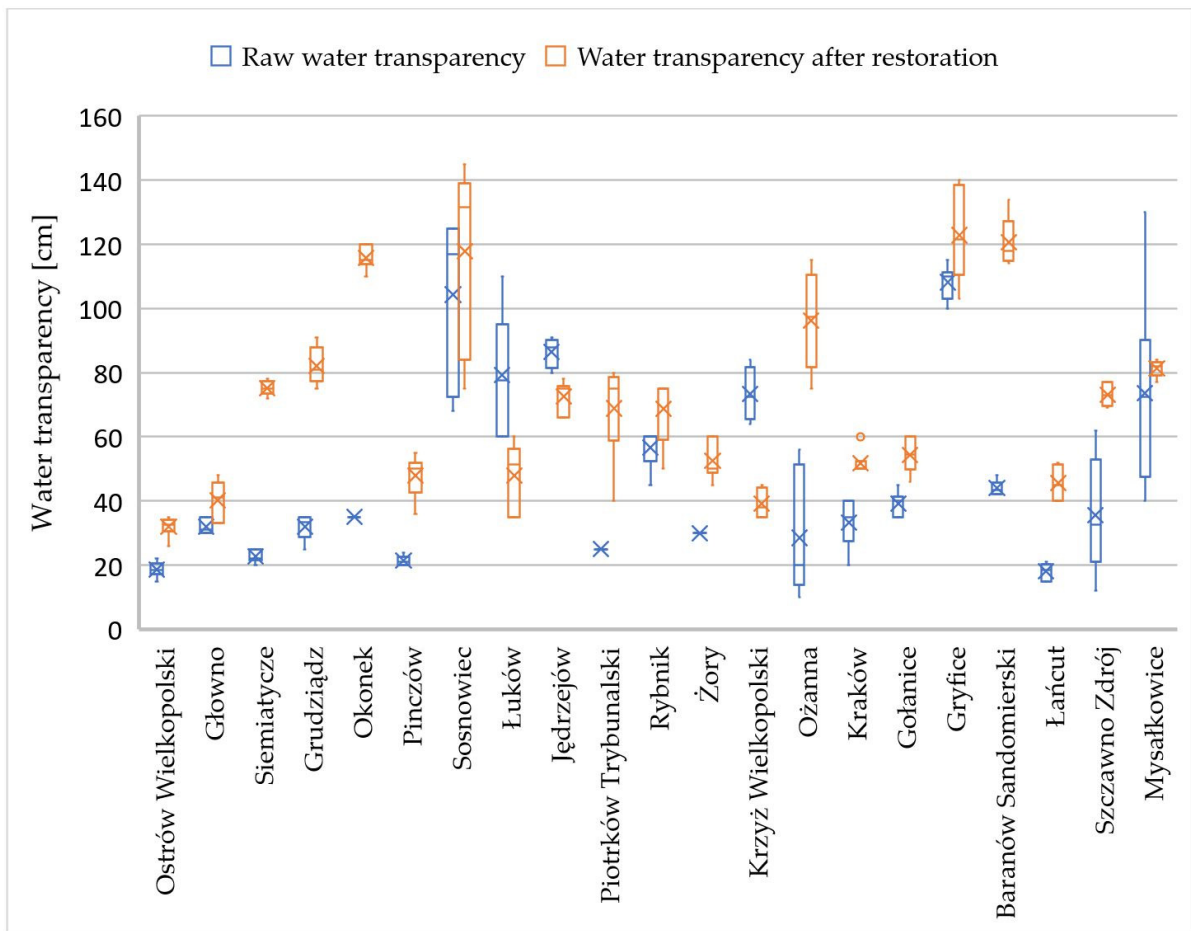
### 3.2. Changes in Water Transparency and Turbidity

Water transparency and water turbidity are inversely proportional phenomena, resulting from a variety of environmental factors. The process of eutrophication and algal blooms leads to a significant decrease in water transparency and a reduction in the penetration of light into the aquatic environment [41–43]. However, water turbidity may result from causes other than the development of planktonic algae, such as heavy rainfall or snowmelt, which result in turbulent flows responsible for persistent turbidity due to an increase in suspended mineral particles in the water [44]. The water of all the rehabilitated reservoirs, prior to treatment, was characterized by significant eutrophication and intense algal blooms during the growing season. In most cases, the predominant form of water turbidity is long-term reconstructed turbidity resulting from the development of planktonic algae.

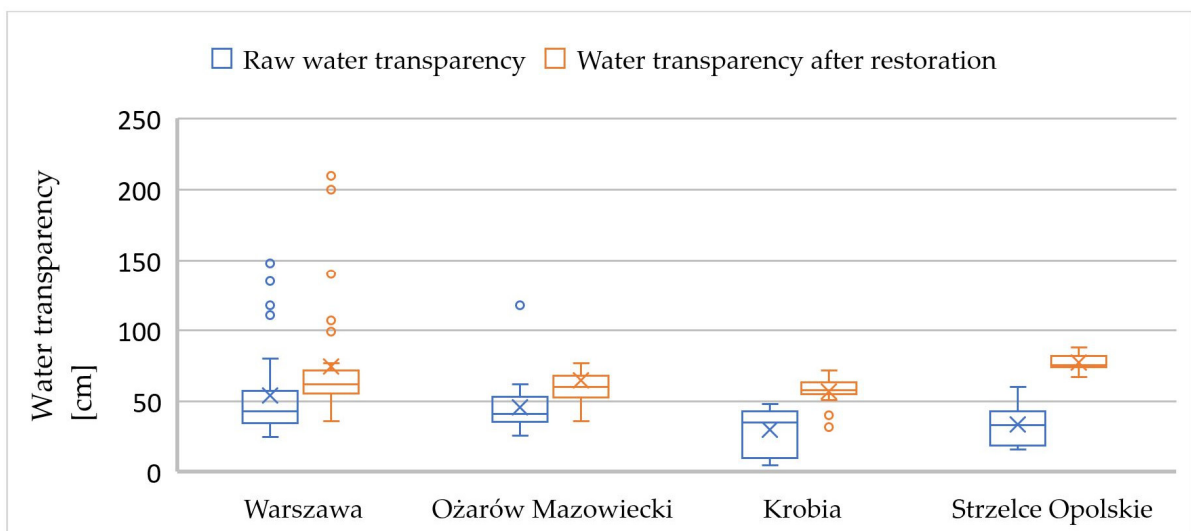
In many of the examined reservoirs, the initial transparency value did not exceed 60 cm, with only a few characterized by a transparency zone of 60 cm or more (Figures 7–9). There is no clear correlation between the thickness of sediment layers and the light penetration zone in the reservoirs subjected to reclamation processes. In the following growing season, a significant improvement in the euphotic zone was observed in most of the examined cases (Figures 7–9), except for the Jędrzejów and Łuków reservoirs (Figure 8). These were cases in which the commission's investigations took place during a rainy period with persistent mineral suspensions affecting water transparency.



**Figure 7.** Changes in transparency in reservoirs with a primary SOSF layer exceeding 1 m (x—indicate average value).



**Figure 8.** Changes in transparency in reservoirs with a primary SOSF layer below 1 m (o—indicate outliers, x—indicate average value).



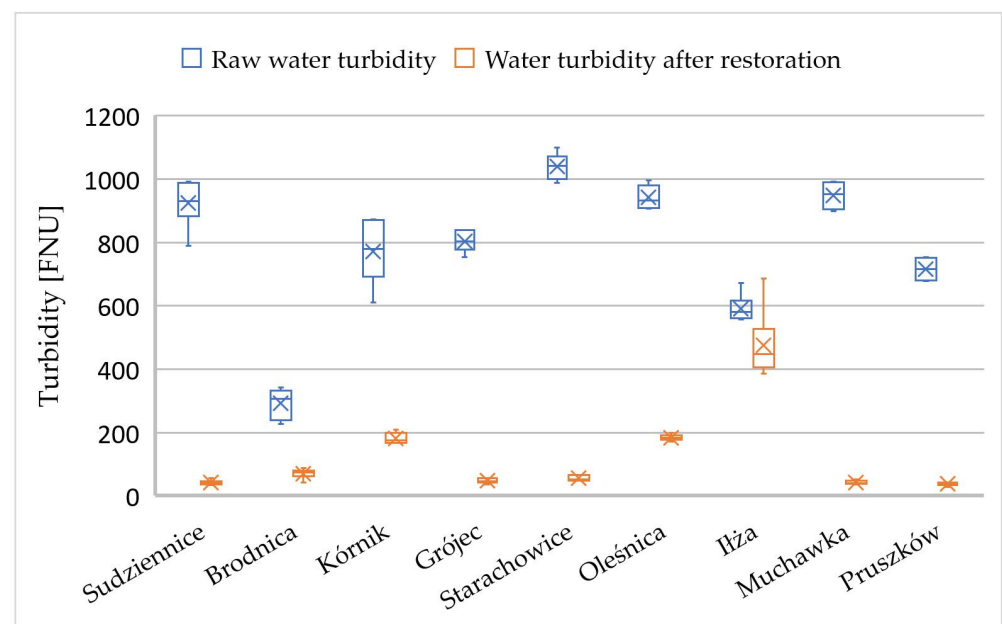
**Figure 9.** Changes in transparency in interconnected small reservoirs (o—indicate outliers, x—indicate average value).

The reservoirs in the towns of Brodnica, Krzyż Wielkopolski, Pruszków, Sosnowiec, and Myszałkowice (Figures 7 and 8) also exhibited low or poor water transparency due to stagnant water conditions and the release of nutrients from the biological degradation of

organic carbon in the sediment layers. In the remaining reservoirs, water flow and a well-developed aquatic macrophyte buffer zone stabilized the supply of nutrients, preventing algal blooms in the subsequent season after reclamation. Although the development of nearshore aquatic macrophyte buffer zones is one of the reservoir restoration methods, the overgrowth of macrophytes may also cause terrestrialization processes in lakes [39,40]. Floating treatment wetlands with macrophytes growing hydroponically on a constructed raft can be pointed out as another method of biological reclamation of reservoirs. This technology is used to remove nutrients, specifically phosphorus, from urban water bodies [44]. The floating treatment wetlands are rather used for long-term phosphorus removal, in reservoirs where it is not possible to introduce emergent or floating vegetation. The technology is time-consuming due to the growth of plants on the raft. Studies indicate that phosphorus removal capacity depends on environmental factors and annually ranges from 0.8 to 7 g-P/m<sup>2</sup> raft [45,46].

In the reservoirs that showed improved transparency, there was also a trend of reduced summer sedimentation rates. A strong inverse correlation was observed between transparency and water turbidity results. In five cases (Table A2), statistical analyses did not allow for such conclusions to be drawn for the majority of the examined reservoirs. Statistically significant differences in water transparency improvement were clearly demonstrated in the season following the microbiological bioremediation processes. The research results were influenced by local weather conditions or lake parameters that disrupted natural transparency or slowed down the improvement process. Sediment analysis for these reservoirs with disturbed euphotic zones nonetheless indicated that biological decay processes are effective in most cases (Table A1).

Reservoirs that exhibited increased turbidity levels in the subsequent season after reclamation also had a poorer euphotic zone. In these cases, these relationships are closely intertwined (Figures 10 and 11). Turbidity in most cases was induced by planktonic algae, though when employing microbiological reclamation methods, the improvements in the studied parameters become evident in the following season. Enhancing the euphotic zone plays a crucial role in the development of aquatic organisms and achieving the appropriate ecological diversity for this type of ecosystem [47]. The improvement of the euphotic zone and the reduction in turbidity constitute the second important criterion achieved using such biopreparations in the reclamation process of degraded water reservoirs.



**Figure 10.** Changes in turbidity in reservoirs with a primary SOSF layer exceeding 1 m (x—indicate average value).

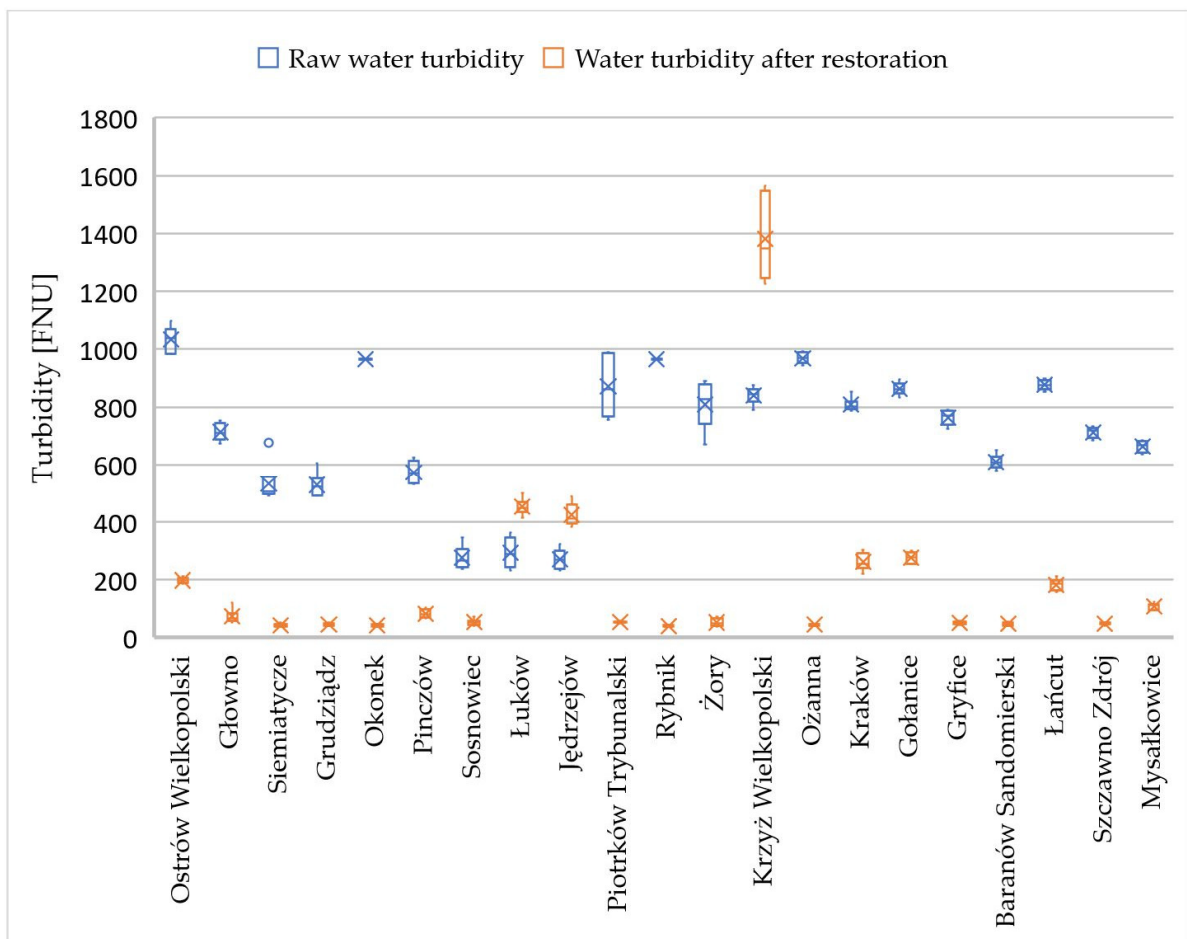


Figure 11. Changes in turbidity in reservoirs with a primary SOSF layer below 1 m (○—indicate outliers, x—indicate average value).

Changes in water turbidity level (FTU) and dissolved oxygen concentration near the bottom (Figure 12) in almost all cases showed statistically significant differences (Table A3). Improvements in these parameters in the subsequent vegetative season after bioremediation treatments were demonstrated in nearly all reservoirs at 33 locations.

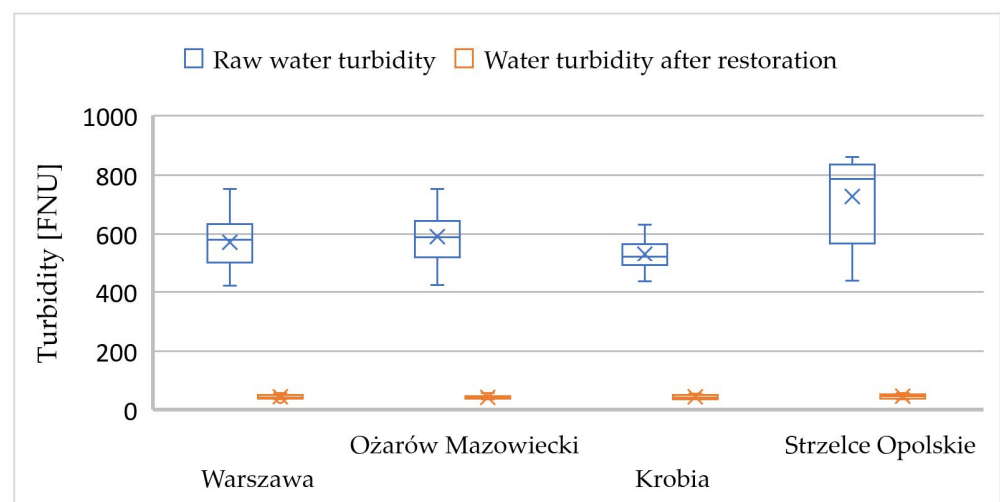
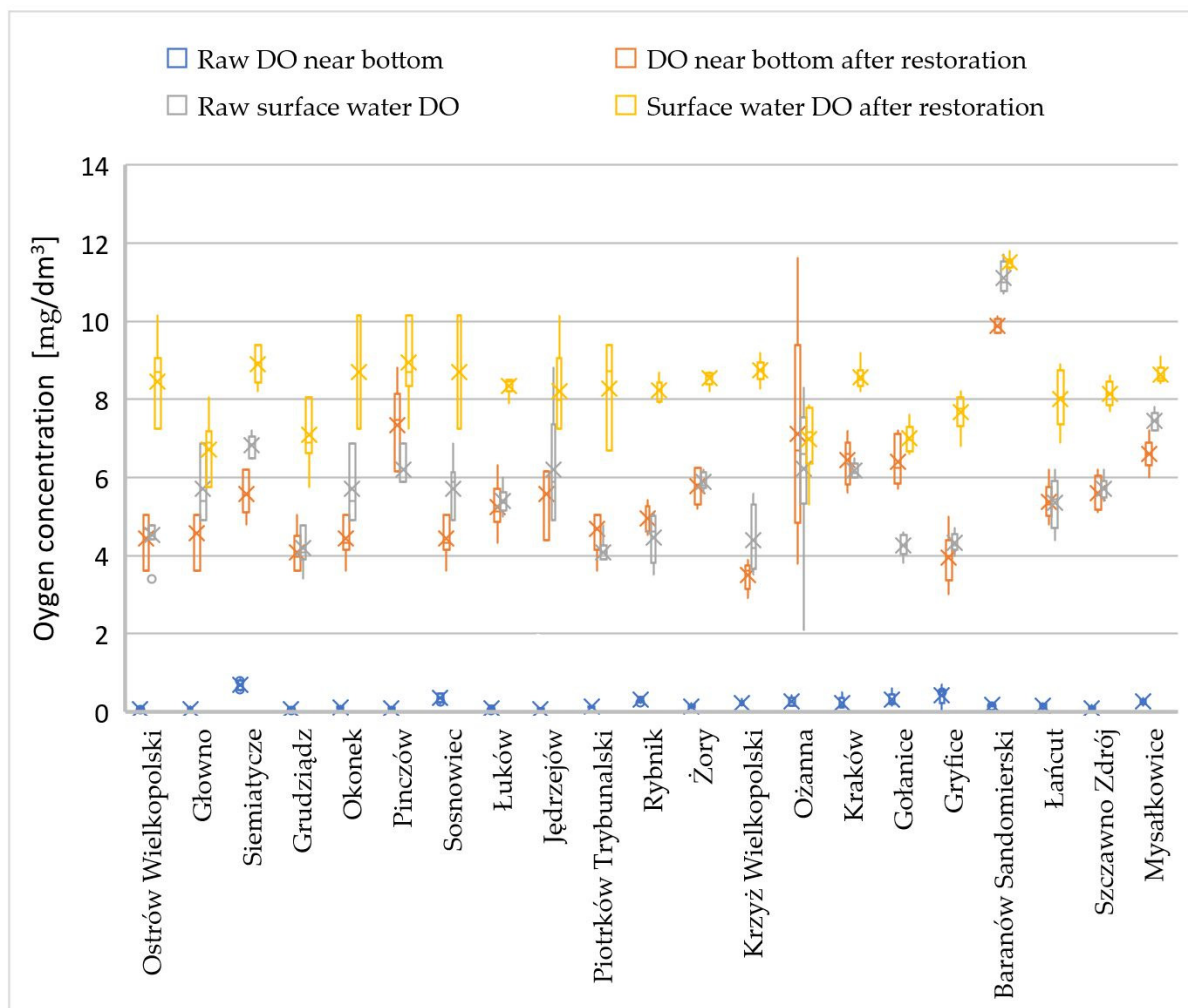


Figure 12. Changes in turbidity in interconnected small reservoirs (○—indicate outliers, x—indicate average value).

### 3.3. Changes in the Concentration of Dissolved Oxygen (DO) in a Water Column

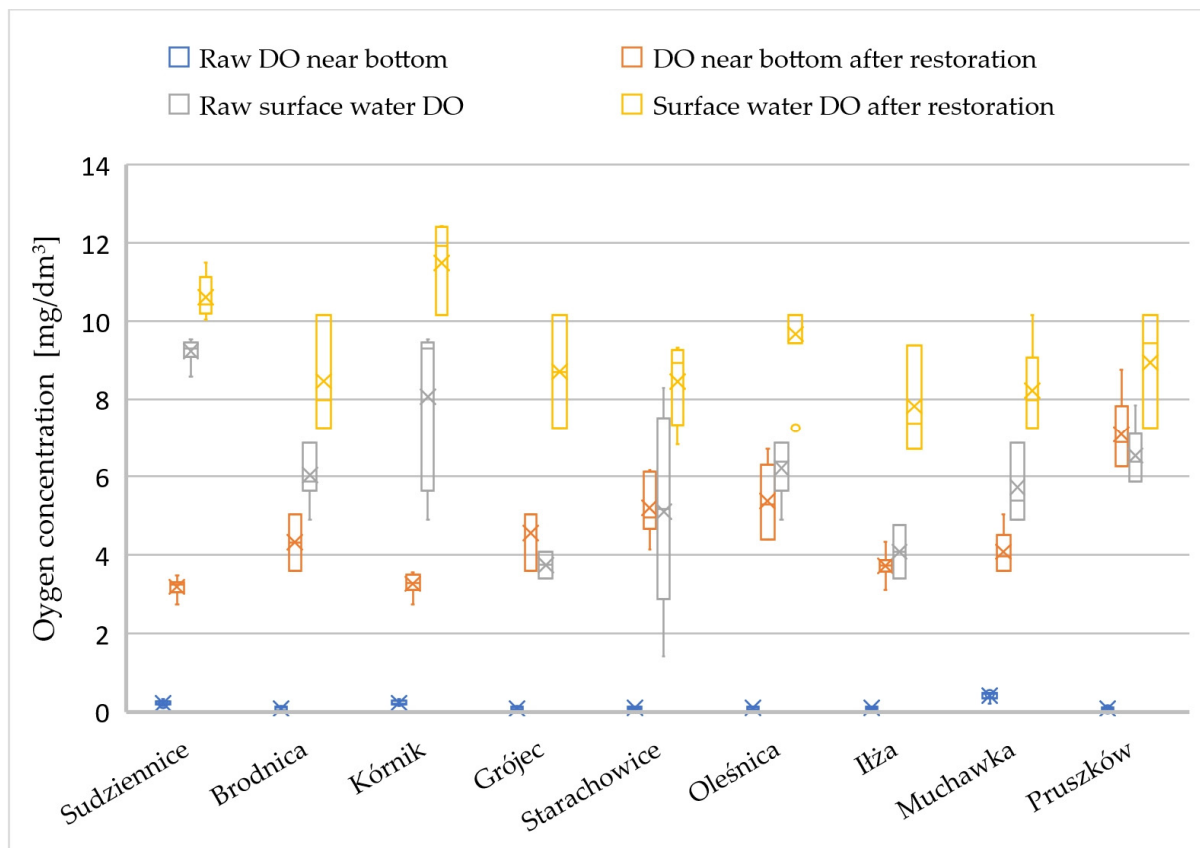
In all examined reservoirs before rehabilitation, a trend associated with a strong hypoxic zone near the bottom was observed. The decomposition of organic matter by autochthonous microorganisms rapidly leads to oxygen depletion in this zone [48]. In all the examined reservoirs, dissolved oxygen (DO) near the bottom approached values close to 0 (Figures 13–15), regardless of whether the surface water zone also had reduced levels of dissolved oxygen or levels appropriate for the specific type of reservoir (Figures 13–15).



**Figure 13.** Changes in DO in reservoirs with a primary SOSF layer below 1 m (○—indicate outliers, x—indicate average value).

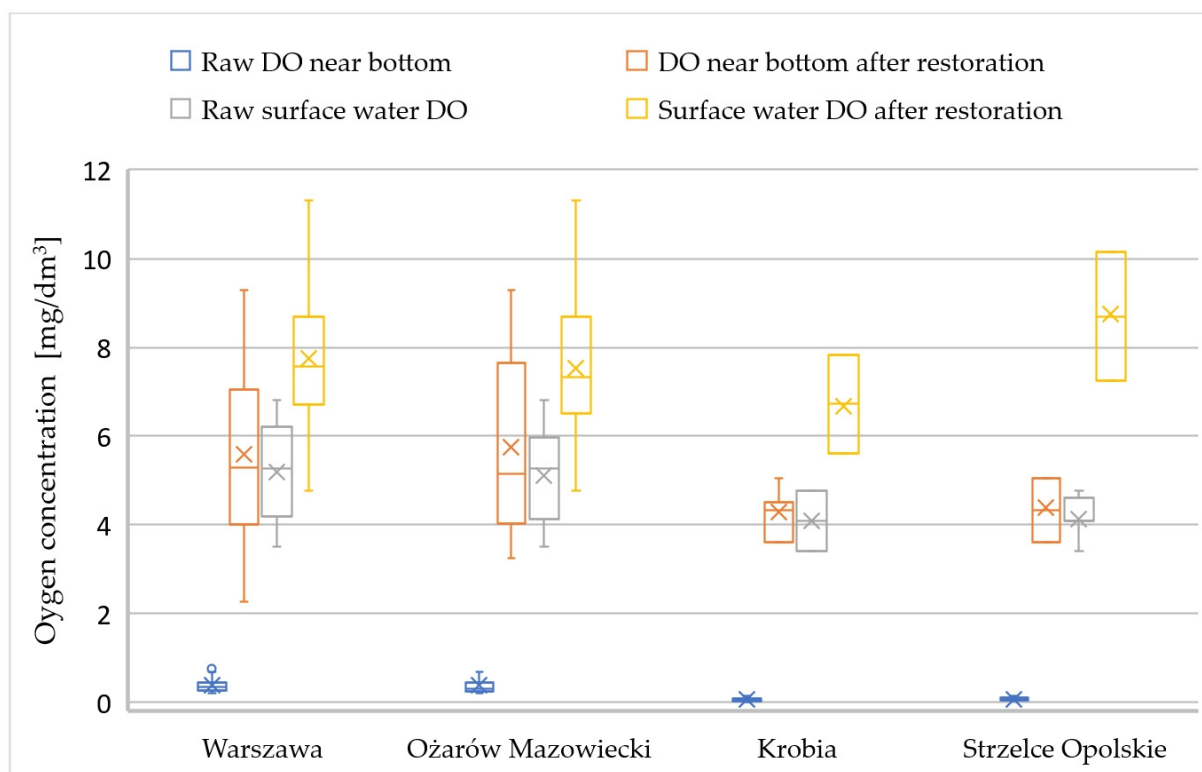
For dissolved oxygen in the surface water layer, statistically significant differences were not observed in two water reservoirs: Głowno (Mrożyczka dam reservoir) and Ożanna dam reservoir (Table A4). In Lake Ożanna, preliminary research conducted shortly after heavy rainfall events and turbulent surface water flows showed non-specific oxygenation in this layer (significant variability in different measurement points). In the Głowno, due to non-specific weather conditions, the dissolved oxygen level during the initial research period was very similar to the concentration after bioremediation treatments. In both periods, the water exhibited very good oxygen conditions in the surface layer.

Changes in dissolved oxygen (DO) in the surface layer were variable and mostly dependent on environmental conditions. Deoxygenation was not observed in any of the reservoirs within this zone (Figures 13–15).



**Figure 14.** Changes in DO in reservoirs with a primary SOSF layer exceeding 1 m (o—indicate outliers, x—indicate average value).

The results also indicate an improvement in the surface oxygen profile in the growing season following the application of microbiological biopreparations. The extent of these changes depended on the initial state and the degree of pollution of the respective reservoir (Figures 13–15). However, the changes were not as effective as the increase in DO in the anoxic layer. Due to the continuous sediment decomposition process promoting a considerable oxygen demand zone, the DO levels in the bottom zone remained significantly lower than in the surface zone, which is in contact with atmospheric air [49,50]. Dissolved oxygen serves as a key indicator of eutrophication in freshwater lakes, playing a crucial role in water trophic assessment [50]. Similar to the depth of the euphotic zone, the appropriate DO level provides the necessary conditions for the proper functioning of aquatic ecosystems and the maintenance of biodiversity specific to a given climate and lake type [51]. The microbiological reclamation method, in all examined lakes, demonstrated an improvement in the oxygen profile in both the bottom and surface layers, although not necessarily significant (Figures 13–15).



**Figure 15.** Changes in DO in interconnected small reservoirs (o—indicate outliers, x—indicate average value).

#### 4. Conclusions

Though there has been much debate on the subject, it is clear that the results are generally positive over the one-season analysis period. This is made evident by the fact that general conditions of bottom dissolved oxygen, transparency, and sediment thickness improved considerably after treatment with biopreparates. In addition, there is an increasing emphasis on ecosystem services. This method of reservoir rehabilitation, as an alternative to traditional, technical methods, can improve the conditions of water bodies without destroying ecosystems. Therefore, it can contribute to the proper functioning of aquatic ecosystems and enable them to provide ecosystem services. Despite the very favourable results, the application involves costs which may be considerably reduced if long-term monitoring verifies that no recurrent treatment is necessary, and a healthy bacterial ecosystem is restored.

The most relevant conclusions from the analyses carried out on the 33 water reservoirs subjected to remediation with biopreparations are as follows:

1. After the application of directional biopreparations, a reduction in soft organic fractions in the bottom sediments of all reservoirs was observed in the following growing season. Sediment reduction studies showed a highly satisfactory decomposition process. In most cases, a twofold or greater reduction was observed compared to the season before rehabilitation measures began.
2. Morphological changes in the soft organic sediment fractions were observed after the application of biopreparations for water body restoration. The gradual softening of these fractions helped them to become more hydrated and more easily transported by flowing water.
3. Significant eutrophication and intense algal blooms during the growing season characterised the waters of all rehabilitated reservoirs before the treatment. An improvement in water transparency was shown in the subsequent season after microbial bioremediation.



4. In all studied reservoirs, an improvement in the oxygen profile in both the bottom and surface layers was observed after the application of the microbial restoration method.
5. The use of biopreparations for the reclamation of water bodies is an alternative to mechanical methods, which are extremely expensive and invasive to the aquatic environment. Biopreparation technology can be particularly suitable for large water bodies, where technical methods are almost impossible to implement.

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## Appendix A

**Table A1.** Statistical differences for changes in the bottom sediment layer (SOSF) before and after reclamation, in the case of the studied water reservoirs.

Pair of Variables	Wilcoxon Paired <i>t</i> -Test			
	N Significant	T	Z	<i>p</i>
Sediment layer before and after restoration of reservoir group area *	6	0	2.201398	0.027709
Sosnowiec 1 and Sosnowiec 2	6	1.5	1.886913	0.059173
Rybnik 1 and Rybnik 2	6	1	1.991741	0.0464
Żory 1 and Żory 2	5	0	2.0226	0.043115
Mysłakowice 1 and Mysłakowice 2	6	1	1.991741	0.0464
Warszawa 1 and Warszawa 2	30	0	4.782139	0.000002
Ożar. Maz. 1 and Ożar. Maz. 2	24	0	4.285714	0.000018
Krobia 1 and Krobia 2	18	0	3.723555	0.000196
Strz. Opol. 1 and Strz. Opol. 2	24	0	4.285714	0.000018

\* Ostrów Wielk., Siemiatycze, Głowno, Grudziądz, Okonek, Pinczów, Łuków, Jędrzejów, Piotrków Tryb., Krzyż Wlk., Ożanna, Kraków, Gołanice, Gryfice Garanów Sand., Łańcut, Szczawno Zdr., Studziennice, Bronica, Kórnik, Grójec, Starachowice, Oleśnica, Iłza, Muchawka, Pruszków.

**Table A2.** Statistical differences for changes in the water transparency before and after reclamation, in the case of the studied water reservoirs.

Pair of Variables	Wilcoxon Paired <i>t</i> -Test			
	N Significant	T	Z	<i>p</i>
Transparency before and after restoration of reservoir group area **	6	0	2.201398	0.027709
Głowno 1 and Głowno 2	6	1	1.991741	0.046400
Rybnik 1 and Rybnik 2	6	1	1.991741	0.0464
Gryfice 1 and Gryfice 2	6	2	1.782084	0.074736
Mysłakowice 1 and Mysłakowice 2	6	6	0.943456	0.345448

Table A2. Cont.

Pair of Variables	Wilcoxon Paired <i>t</i> -Test			
	N Significant	T	Z	<i>p</i>
Brodnica 1 and Brodnica 2	6	10	0.104828	0.916512
Starachowice 1 and Starachowice 2	6	1	1.991741	0.0464
Oleśnica 1 and Oleśnica 2	5	1	1.75292	0.079617
Iłża 1 and Iłża 2	6	5	1.153113	0.248865
Warszawa 1 and Warszawa 2	30	38	4.000542	0.000063
Ożar. Maz. 1 and Ożar. Maz. 2	24	27	3.514286	0.000441
Krobia 1 and Krobia 2	17	0	3.621365	0.000293
Strz. Opol. 1 and Strz. Opol. 2	24	0	4.285714	0.000018

\*\* Ostrów Wielk., Siemiatycze, Grudziądz, Okonek, Pinczów, Sosnowiec, Łuków, Jędrzejów, Piotrków Tryb., Żory, Krzyż Wlk., Ożanna, Kraków, Gołanice, Baranów Sand., Łańcut, Szczawno Zdr., Studziennice, Kórnik, Grójec, Muchawka, Pruszków.

Table A3. Statistical differences for changes in the dissolved oxygen (and turbidity) in water near the bottom before and after reclamation, in the case of the studied water reservoirs.

Pair of Variables	Wilcoxon Paired <i>t</i> -Test			
	N Significant	T	Z	<i>p</i>
DO near bottom (and water turbidity) before and after restoration of reservoir group area ***	6	0	2.201398	0.027709
Warszawa 1 and Warszawa 2	30	0	4.782139	0.000002
Ożar. Maz. 1 and Ożar. Maz. 2	24	0	4.285714	0.000018
Krobia 1 and Krobia 2	18	0	3.723555	0.000196
Strz. Opol. 1 and Strz. Opol. 2	24	0	4.285714	0.000018

\*\*\* Ostrów Wielk., Siemiatycze, Głowno, Grudziądz, Okonek, Pinczów, Sosnowiec, Łuków, Jędrzejów, Piotrków Tryb., Rybnik, Żory, Krzyż Wlk., Ożanna, Kraków, Gołanice, Gryfice Garanów Sand., Łańcut, Szczawno Zdr., Mysłakowice, Studziennice, Bronica, Kórnik, Grójec, Starachowice, Oleśnica, Iłża, Muchawka, Pruszków.

Table A4. Statistical differences for changes in the dissolved oxygen in surface water before and after reclamation, in the case of the studied water reservoirs.

Pair of Variables	Wilcoxon Paired <i>t</i> -Test			
	N Significant	T	Z	<i>p</i>
DO surface before and after restoration of reservoir group area ****	6	0	2.201398	0.027709
Głowno 1 and Głowno 2	6	5	1.153113	0.248865
Jędrzejów 1 and Jędrzejów 2	6	1	1.991741	0.046400
Ożanna 1 and Ożanna 2	6	5	1.153113	0.248865
Studziennice 1 and Studziennice 2	6	1	1.991741	0.046400
Warszawa 1 and Warszawa 2	30	0	4.782139	0.000002
Ożar. Maz. 1 and Ożar. Maz. 2	24	0	4.285714	0.000018
Krobia 1 and Krobia 2	18	0	3.723555	0.000196
Strz. Opol. 1 and Strz. Opol. 2	24	0	4.285714	0.000018

\*\*\*\* Ostrów Wielk., Siemiatycze, Grudziądz, Okonek, Pinczów, Sosnowiec, Łuków, Piotrków Tryb., Rybnik, Żory, Krzyż Wlk., Kraków, Kórnik, Gołanice, Gryfice Garanów Sand., Łańcut, Szczawno Zdr., Mysłakowice, Bronica, Grójec, Starachowice, Oleśnica, Iłża, Muchawka, Pruszków.

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