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Application of biofilm community structure analysis for assessing the impact of a stormwater system on the aquatic environment

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Abstract
Introduction and Objective. Industrial, agricultural and construction development has brought improvements in living conditions, but have also increased the amount of pollution in the environment. Atmospheric precipitation collects pollutants from urban surfaces, which then end up in stormwater systems, contaminating surface waters. These pollutants are also linked to the similar effects of agriculture, as biogenic pollutants originate from over-fertilized crops. Contaminated surface water forces flora and fauna to adapt to new conditions, and affecting the structure and extent of ecosystems. Monitoring the environment with bio-indication methods is important because it enables identification of the areas in need of protection, in an inexpensive and environmentally harmless way. The aim of this study was to evaluate the possibility of using biocenotic indices to assess the impact of a stormwater system on the aquatic environment.
Materials and Method. Bio-indicative studies were conducted on periphyton sampled at 4 points on the Bystrzyca River in Lublin, eastern Poland, under the influence of stormwater discharge and 1 reference point localized before the stormwater system outflow. The quantitative data concerning the number of chosen algae species was analyzed using indices for the examination of community structure.
Results. Considered the indices, i.e. taxonomic richness, Shannon, MacArthur, Menhinick and McIntosh were calculated, evaluated, and shown in various types of graphs showing the fluctuation of indices at measurement points.
Conclusions. The use of bioindication and classic biocenotic indices allowed for the description, analysis of changes in the periphyton biocenosis under the influence of point source stormwater discharges, and linking measurements from tested samples with environmental conditions and biodiversity in the analyzed study sites and periods.

Key words
surface water, periphyton, bioindication, aquatic environment, community structure, stormwater system, entropy-based indices

INTRODUCTION

Urbanization, as well as industrial and agricultural development have led to an increase in pollution in aquatic ecosystems [1]. Rainfall flushes impervious surfaces and transports anthropogenic pollutants, including heavy metals, organic substances, and suspended solids, into water receivers [2]. Stormwater flowing through sewage systems significantly alters the physicochemical parameters of rivers, affecting the amount of dissolved oxygen, the concentration of nutrients, and the level of toxic substances [3].

The long-term impact of stormwater runoff on aquatic ecosystems can lead to their degradation, disruption of self-purification processes, and a decline in biodiversity [4]. In the context of the growing problem of water scarcity and increasing anthropogenic pressure, it is necessary to develop effective methods for monitoring the quality of the aquatic environment that will allow for the rapid detection of changes in river ecosystems [5].

Bioindication constitutes one of the most effective tools in the monitoring of water quality – a method based on the analysis of the presence and abundance of indicator organisms that respond to changes in the chemical and

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physical composition of the aquatic environment [6]. Bioindicator organisms comprise both macroorganisms, such as benthic macroinvertebrates, and microorganisms, including periphyton and phytoplankton, which are particularly sensitive to pollution from storm sewers [7, 8].

Bioindication allows for the assessment of water quality without the need for costly physicochemical analyses, and enables the tracking of long-term changes in the ecosystem [9]. The stability of indicator organism populations and their response to changing environmental conditions can provide important information on the impact of point source stormwater discharges on receiving waters [10].

Analysis of the structure of aquatic biocenoses is based on the use of biocenotic indicators which allow for a quantitative assessment of changes in ecosystems. The Shannon index and the MacArthur index are commonly used to determine the biological diversity and stability of organism communities [6, 8]. These indices take into account both the abundance and proportions of individual taxa, which allows assessment of the degree of ecological disturbance resulting from human activity [11].

Previous bioindication studies have mainly focused on macroscopic organisms, such as lichens, benthic invertebrates and fish. There is a research gap in the use of microorganisms, especially those that form biofilms (periphyton), which can be sensitive bioindicators of environmental changes. The microbiome of aquatic ecosystems is dynamic and responds to changes in a short time, which may provide a basis for the development of more precise water quality indicators.

OBJECTIVE

The aim of the study is to determine the impact of stormwater drainage on the Bystrzyca River in Lublin, eastern Poland, by applying bioindication methods based on the analysis of biocenotic indicators describing the structure of communities of selected organisms forming periphyton. The study refers to the structure of periphyton communities at various measurement points and the extent of changes occurring in the biocenosis under the influence of rainwater.

MATERIALS AND METHOD

The analysis of the structure of aquatic biocenoses uses biocenotic indicators that allow for a quantitative assessment of biodiversity, the proportions of individual species, and the uniformity of their distribution in the studied environment. The study uses a number of indices that provide comprehensive information on the structure of periphyton communities and the dynamics of changes occurring under the influence of storm water discharges.

The fractional share of individuals counted in individual species is a parameter expressing the proportion of a given species in relation to the entire biocenosis community in a given sample. This is calculated as the ratio of the number of individuals of a given species to the number of all identified organisms in the sample:

$$\Pi_i = \frac{n_i}{N} \qquad (1)$$

where: n_i – number of individuals in the i-th analyzed species, N – number of individuals in all analyzed species in a given sample.

The Shannon index is one of the most commonly used indicators of biological diversity, which takes into account both species richness (number of species) and the evenness of their distribution [12], calculated according to the formula:

$$H = -\sum_{i=1}^{S^*}(\Pi_i \cdot log_2 \Pi_i), \qquad (2)$$

where: S^*– number of species, Π_i – fractional share of individuals counted in individual species.

A high index value indicates high biodiversity and an even distribution of species abundance, while a low value indicates the dominance of one or more taxa, which may suggest ecological disturbances [8].

Fraction analysis allows for assessment of the dominance of specific species, which may be crucial in research on bioindication and the assessment of water environment quality [7].

Taxonomic richness is the basic indicator of biocenotic diversity, which determines the number of distinct taxa from all analyzed species in a given sample. High taxonomic richness suggests ecosystem stability, while low values may indicate environmental degradation and a decline in biodiversity [8]. This index is calculated as:

$$S^* = \sum_{i=1}^{S} s_i, \qquad (3)$$

where: s_i – occurrence of the i-th species (1 when it is present in the sample, 0 when it is not), S – number of considered species.

The evenness index assesses how evenly individuals are distributed among different species, calculated using the following formula:

$$V = \frac{H}{H_{max}} \qquad (4)$$

where: H – fractional share of individuals counted in individual species (Shannon index), H_{max} maximum value of the Shannon index. V values range from 0 – 1, where 1 represents a perfectly even distribution of all species. A decrease in the index value may suggest that certain species dominate the ecosystem, which may be the result of environmental pollution [8].

The MacArthur index is an indicator showing whether all species in a given sample have similar abundance or whether there is a clear dominance of one or more taxa [8]:

$$E = z^H \qquad (5)$$

where: H – fractional share of individuals counted in individual species (Shannon index), z – base of the logarithm applied (in this study, the base-2 logarithm). The higher the E index value, the more balanced the structure of the biocenosis, which means greater ecosystem stability [8].

Proportionality index is calculated using formula:

$$P = \frac{E}{S^*} \cdot 100, \qquad (6)$$

where: E – MacArthur index, S^* – species richness. This index assesses the proportion of a given species relative to all individuals counted in a given sample. The values of this index allow for identification of the dominant species and their potential impact on the functioning of the entire biological community [8, 11].

The Menhinick index [13] also assesses species richness, but places greater emphasis on therelationship between the number of species and the abundance of individuals. Higher M values suggest greater diversity:

$$M = \frac{S^*}{\sqrt{N}}, \tag{7}$$

where: S^* – species richness, N – total number of individuals.

The McIntosh index is another, less commonly used indicator of species diversity, which shows the proportional number of individuals of different species in a community. This index takes into account the sample size. The higher the McIntosh index value, the greater the species richness in the sample studied [14], and is calculated using the following formula:

$$D = \frac{N - \sqrt{\sum_{i=1}^{s} n_i^2}}{N - \sqrt{N}} \tag{8}$$

The value of D ranges from 0 – 1 and expresses how large the observed diversity is in relation to the maximum possible for a given N. It has the advantage of expressing the observed diversity as a proportion of the maximum absolute diversity for a given value of N and ranges from 0 – if there is only one species, to 1 – if the diversity is maximum [15]. It is assumed that if the number of observations of all species N is equal to 0, the McIntosh index is also 0.

The coefficient of variation, which is a measure of the variability in the distribution of the variable under study, was calculated using the formula:

$$\hat{c}_v = \frac{s}{\bar{x}}, \tag{9}$$

where: s – standard deviation estimator, and $\bar{x}$ – sample mean. It was used to compare the variability of the index values obtained at each biofilm measurement point.

The values of the analyzed biocenotic indicators for all measurement days and biofilm sampling points are presented using boxplots. These types of graphs allow for a graphical representation of data distribution, variability, skewness, and outliers [16]. In the central part of the graph there is a rectangle (box) that covers 50% of the values between the first (Q1) and third quartiles (Q3), i.e., the interquartile range (IQR). The line in the middle of the box represents the median, while the lower and upper boundaries of the box correspond to the Q1 and Q3 quartiles, respectively [17]. The whiskers of the chart extend to a maximum of values in the range Q1 – 1.5 × IQR and Q3 + 1.5 × IQR, while values outside this range are marked as outliers. This approach allows for the identification not only of central values and data dispersion, but also of extremes that may indicate unusual or disruptive measurements [16].

In order to compare the distributions of microorganism abundance in samples from each group, the Kruskal-Wallis test was performed. This test is a non-parametric test used to compare 3 or more independent groups, to determine whether they come from the same distribution [18]. It is a non-parametric alternative to one-way analysis of variance (ANOVA) and is used when the data do not meet the assumptions of normality or equal variance required for ANOVA. The test involves ranking all data from all groups, summing-up the ranks for each group, and calculating a test statistic (H) which is then compared to a chi-square distribution. The test statistic is given by the formula:

$$H = \frac{12}{N(N+1)} \sum_{i=1}^{k} \frac{R_i^2}{n_i} - 3(N+1) \tag{10}$$

where N – the number of observations in all compared groups, k – the number of groups, n_i – size of the i-th group and R_i – sum of ranks in the i-th group.

The study was conducted on the Bystrzyca River in Lublin, on a section in the an area of al. Józefa Piłsudskiego south of the city centre The Bystrzyca, a tributary of the Wieprz, flows through the city from west to northeast, and its length within the boundaries of Lublin is approximately 70 km. The river receives both rainwater and treated domestic and industrial wastewater from the Hajdów Municipal Wastewater Treatment Plant on the north-eastern outskirts of the city [19].

In the south-eastern part of Lublin, the river flows through an artificially created dam reservoir – the Zemborzycki Reservoir, which plays an important role in water retention and flood protection. The reservoir, with an area of 278 ha and a depth of 2–4 m, suffers from eutrophication, characterized by massive cyanobacterial blooms and high concentrations of nitrogen and phosphorus [20].

Samples were collected at 5 measuring points located along the Bystrzyca River. The points were selected to take into account the impact of stormwater drainage on the aquatic ecosystem:

- Point 1 – approximately 50 m before the storm water discharge, served as a reference point, reflecting the conditions before the discharge.
- Point 2 – directly at the storm sewer discharge point, allowed for the assessment of the impact of storm water at the moment of its introduction into the river.
- Point 3 – approximately 50 m downstream from the discharge point, behind the 700-lecia Lublina bridge, near the mouth of the second storm sewer.
- Point 4 – 60 m below point 3, allowed for the analysis of the spread of pollutants.
- Point 5 – 190 m from point 4, was the furthest monitoring point, allowing for the determination of the broader impact of pollutants on the aquatic biocenosis.

The same environmental conditions were maintained at each point, including distance from the shore (40 cm), hydraulic conditions and light exposure.

The research material consisted of periphyton biofilm growing on basic microscope slides placed in the river at a depth of 15 cm. The slides were weighted appropriately and remained in the water for 2 weeks, after which they were replaced with new ones to maintain the continuity of the colonization process. The samples collected from the sites were transported to the laboratory of the Faculty of Environmental Engineering at the Lublin University of Technology, where they were immediately analyzed under a microscope at 10 times magnification of the main lens. The research was conducted from 23 April 2022 – 8 April 2023, in a 2-week cycle, resulting in a total of 20 measurement series.

Observations were made for 50 fields of view of each sample, documenting them digitally using the OptaView program. During the study, over 6,000 photographs were taken, which were analyzed and the results of counting archived in Excel. During the analysis, a total of 6,872 microorganisms were observed and treated as indicators.

The bioindicative analysis focused on 12 indicator species of algae, including diatoms and green algae:

- Diatoms: *Achnanthes lanceolata, Cyclotella comta, Nitzschia acicularis, Pinnularia microstauron, Rhoicosphenia curvata, Synedra acus, Synedra ulna, Tabellaria flocculosa*.
- Green algae: *Microspora amoena, Pediastrum duplex, Scenedesmus quadricauda, Ulothrix tenuissima*.

These bioindicators were selected based on their previous use in studies assessing the water quality of the Bystrzyca River, where their effectiveness in determining pollution levels and the impact of human activity on the ecosystem was confirmed [6, 21].

RESULTS AND DISCUSSION

The analyzed biological membrane samples showed clear differences in species richness between individual measurement points, the locations of which were shown in Figure 1. The results are presented in graphs (Fig. 2). Particularly noticeable is the decrease in biocenotic indicators – both the number of taxa and their relative abundance – at Point 2, located directly at the storm sewer discharge site. This may indicate the impact of pollutants transported by rainwater, which introduce various substances into the environment: mineral suspensions, heavy metals, nutrients, and organic residues [9, 22]. At subsequent points, as the distance from the discharge point increased, the values of the indicators returned to their initial levels and, in some cases, even increased.

Additional analysis of the number of microorganisms at Point 1 (reference point) and Point 2 (discharge point) carried out on selected days – 12 August and 20 September 2022 – confirms a significant decrease in both the number and diversity of organisms in the immediate vicinity of the storm sewer outlet. On 12 August, despite relatively low rainfall in the two preceding weeks (a total of 28.6 mm), 13 mm of rainfall was recorded on the day of sampling, which could have led to the release of sediments and pollutants from the sewage pipes. At Point 2, the lowest values of species richness and number of individuals were recorded at that time.

A similar situation occurred on 20 September – during the 2 weeks preceding the sampling, rainfall was significantly lower (14.7 mm), and no rainfall was recorded during the 4 days preceding the measurement. Despite the lack of heavy rainfall, the abundance and diversity of microorganisms at Point 2 was also significantly lower than at Point 1, which may indicate the accumulation of previously deposited pollutants in the sewage system, and their periodic flushing out even with lower rainfall.

Figure 3 shows the distribution of microorganisms at the 5 measurement points throughout the study period. The data is presented in the form of a boxplot, which shows the median, interquartile range, minimum and maximum values (excluding outliers), and the mean.

Figure 1. Location of biological membrane sampling points for the research in Lublin. *Source:* geoportal.lublin.eu

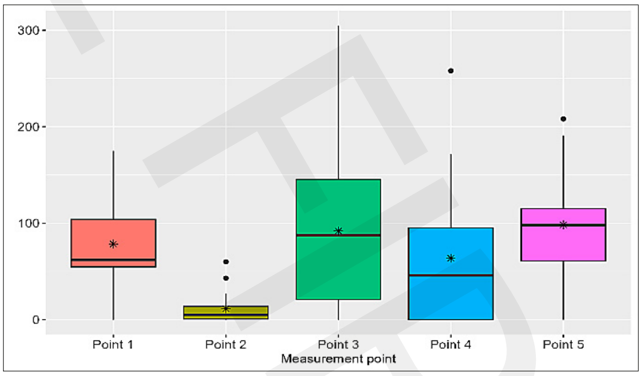

Figure 3. Microorganism counts at sampling points throughout the study period.

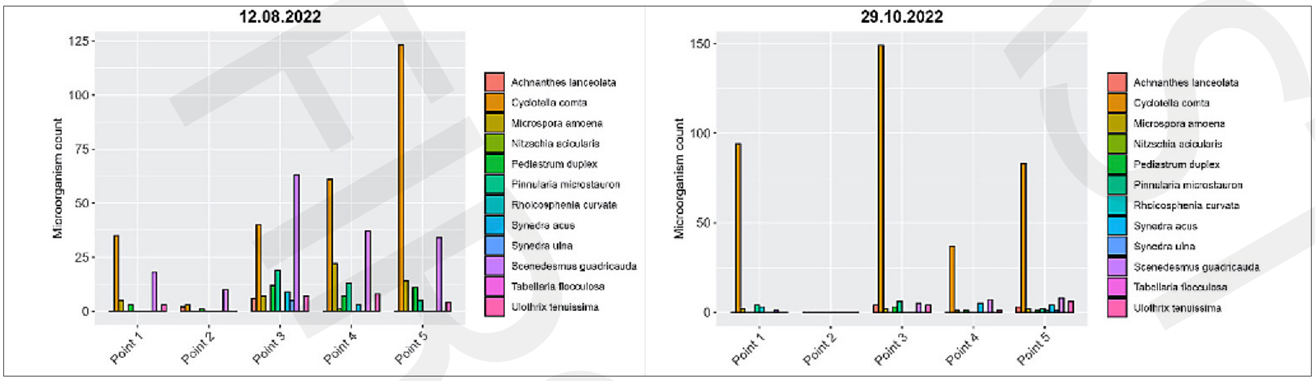

Figure 2. Microorganism counts at test sites on 12 August 2022 and 29 October 2022

The greatest variability in the number of microorganisms was recorded at point 3, which may indicate high environmental dynamics at this location, probably caused by temporary inflows and variability in the physicochemical parameters of the discharged rainwater. In contrast, the lowest median abundance and relatively low variability were recorded at sampling point 2, which is characterized by stable, albeit poor conditions for the growth of microorganisms. This may be due to the direct impact of sewage discharge.

The highest maximum values appeared at points 1 and 3, indicating the occurrence of incidental, strong increases in the population of certain taxa.

The coefficient of variation in the number of microorganisms for the period under study is 1.413 for Point 2, i.e., the wastewater discharge point, which indicates high ecosystem instability at this location. This result may be due to irregular inflow of pollutants and variable composition of stormwater. At Point 3 (0.846) and Point 4 (1.102) a decrease in the index can be observed – the increase in value at Point 4 may have been been caused by discharge from another storm sewer located on the opposite river bank. The lowest values of the coefficient occurring at Point 1 (0.568) and Point 5 (0.601) indicate a moderate and relatively stable number of microorganisms. Observations indicate that the greatest variability is observed at the point of sewage discharge, Point 1 was characterized by the greatest ecosystem stability, and the low value at Point 5 indicates the self-cleaning capacity of the river.

Analysis of the number of microorganisms in spatial terms, in relation to the location of the points (including in relation to tributaries and discharges), provides important data for assessing water quality and potential sources of pollution, confirming the usefulness of selected microorganisms as bioindicators in environmental monitoring.

Figure 4 presents variability in the abundance of individual microorganism taxa. Data analysis reveals significant seasonal differences in both total abundance and dominance structure in the samples studied.

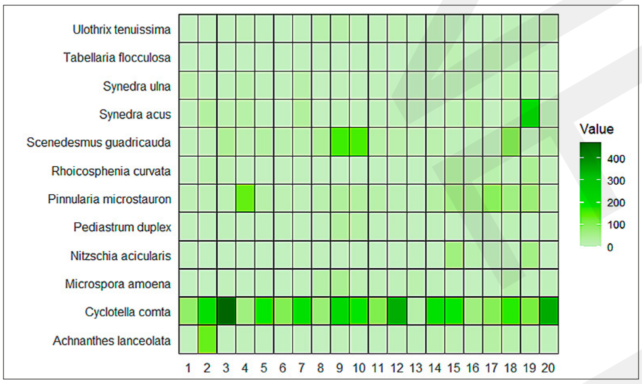

Figure 4. Heatmap of the abundance of individual species in analyzed samples

The dominant organism throughout almost the entire study period was the diatom Cyclotella comta, which was particularly prominent in samples during April, May, July, August, October, and December 2022 (samples: 3, 7, 9, 12, 20). Its high abundance may indicate waters with a moderate trophic status and stable silicon resources [23]. In turn, its presence in winter samples (December-January) suggests tolerance to lower temperatures. From June – October, greater diversity in the composition of microorganisms was observed, including Scenedesmus quadricauda, Pinnularia microstauron, Micraspora amoena, Pediastrum duplex, and Ulothrix tenuissima. This indicates more complex ecological conditions and a seasonal increase in the availability of biogenic substances, especially after rainfall. Between December and February, the abundance of microorganisms decreases significantly (samples 16 and 17), which is consistent with the expected decrease in the metabolic rate of microorganisms under conditions of reduced temperature and reduced light availability. Changes in the structure of dominance and the presence of individual taxa suggest the possibility of using these data for bioindicative assessment of water quality. For example, Pediastrum duplex and Scenedesmus quadricauda are typical of eutrophic waters, while Pinnularia and Rhoicosphenia are sensitive to changes in physicochemical parameters [23].

Analysis of the microorganism abundance at individual points is presented graphically to facilitate interpretation of the results (Fig. 5). The noticeable differences in microorganism abundance at individual points on 12 August 2022, are described above. There was a visible decrease in the number of microorganisms at Point 2 and higher values at Point 3 than at Point 1. The decrease in the values of the indicators at Points 4 and 5 may have been due to the inflow of sewage from the opposite side of the river. These conclusions are confirmed by the values of the indicators: the Shannon index and the MacArthur index – a decrease in values at Points 2 and 4 and a renewed increase at Points 3 and 5.

On 29 October, a significant increase in biodiversity was observed along the river, which may be related to the lack of direct rainfall in the previous days. The number of microorganisms was higher at the points downstream of the discharge than at Point 1, and the MacArthur index confirmed the correctness of the calculations, indicating the highest diversity at the most distant point and the lowest at Point 2.

The differences in biocenotic index values between the measurement points indicate that the selection of study sites was correct. The decrease in the number of species at the wastewater discharge site indicates the impact of stormwater drainage on the river ecosystem. The Shannon index analysis takes into account both the number of microorganism groups and their share in the community.

Boxplots of all analyzed indices in the 5 sampling points are presented in Figure 6. The Shannon index takes into account both the number of microorganisms studied and the share of individual species in the entire ecosystem. Higher values of this index indicate greater biodiversity, which occurs when the analyzed population is larger and more diverse [12]. Lower values, on the other hand, suggest a more homogeneous environment, while values above 3.0 are considered characteristic of well-functioning ecosystems, and values below 1.0 indicate a significant impact of pollution [8]. The averages and medians of the Shannon index for all study sites show that the lowest value was obtained at the sewage discharge site, which is also confirmed by the graphs of other analyzed indices. The coefficient of variation for the Shannon index shows the highest value at Point 2, which confirms the previous statements.

On the basis of the analyses of the McIntosh index values, it can be concluded that the diversity and evenness of organism groups differed between individual measurement points. At points 1 and 3, the median values of this index

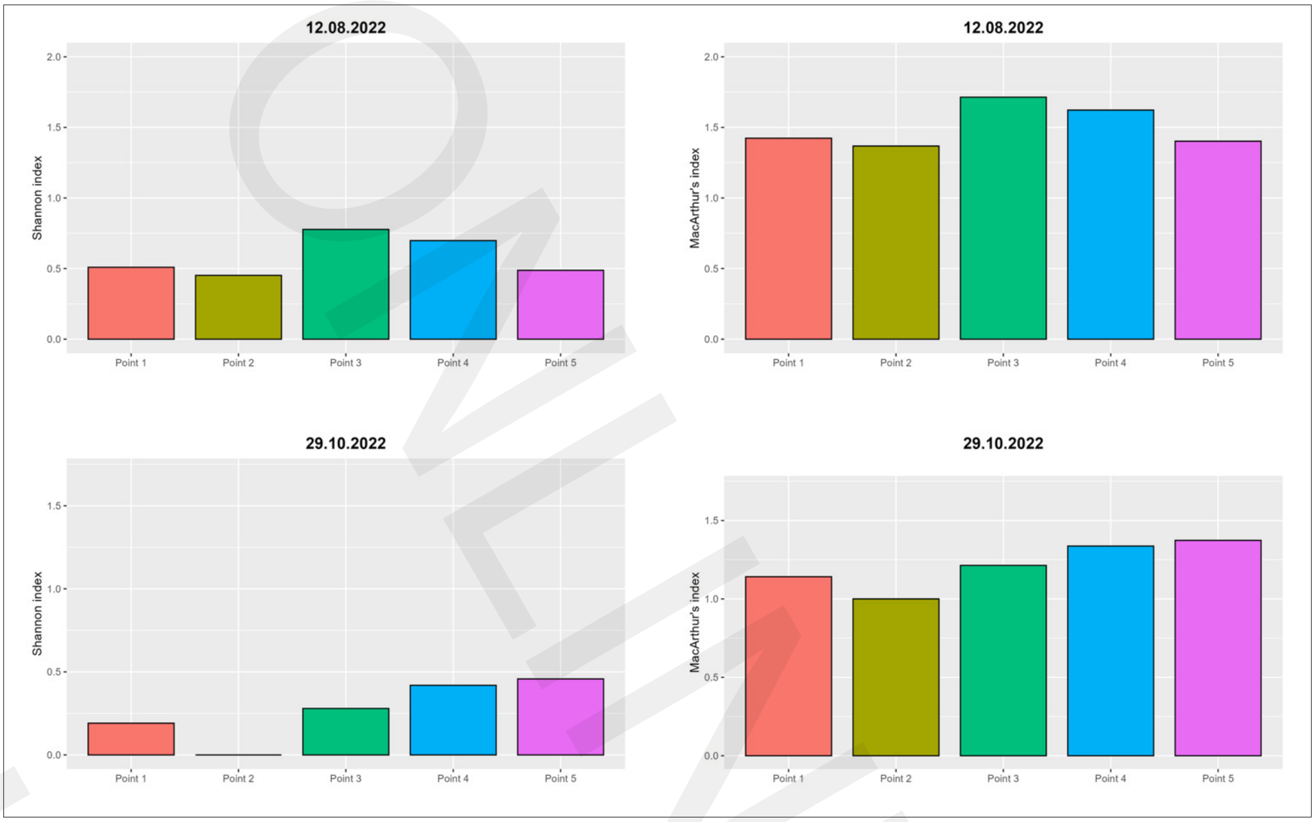

Figure 5. Bar charts of biocenotic indicators (left – Shannon Index, right – MacArthur's Index) based on the abundance of microorganisms for 12 August 2022 and 29 October 2022.

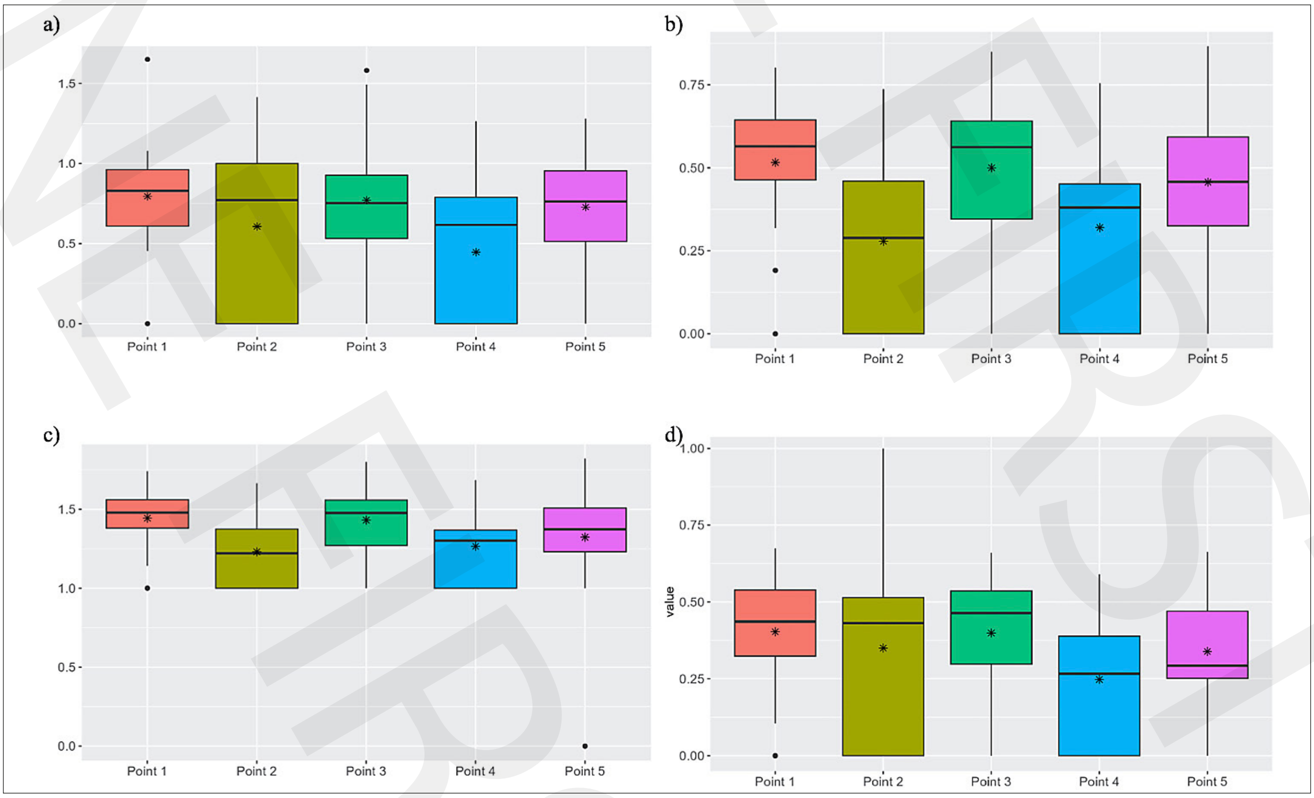

Figure 6. Boxplots of biocenotic indices at sampling points for the entire study period: a) Menhinick index, b) Shannon index, c) MacArthur index, d) MacIntosh index

fluctuated around 0.4 – 0.5, which indicates moderate biodiversity and a relatively even distribution of species in the microorganism structure. These values suggest that there is no clear dominance of one species in these locations, and the share of individual taxa is more balanced. Point 2, despite a median similar to point 1, was characterized by the greatest variability in results – from 0 to nearly 1. This means that at different measurement dates, the species structure there was very unstable: in some cases, there was complete dominance of individual species, while in others, an almost even distribution was observed. Such a large amplitude may indicate a strong influence of environmental factors, in this case anthropogenic, which change the nature of the community over time. At point 4, the index values observed were lower than those at the other measurement points in most cases. The median (approximately 0.25) indicates the dominance of one or a small number of species. This structure suggests a simple species community and a limited ability to evenly utilize available environmental resources. In the context of ecological analysis, this may indicate conditions that are less favourable for most species, or the presence of a limiting factor that caused taxa with high environmental tolerance to predominate. In turn, point 5 was characterized by intermediate values, with a median of approximately 0.35 and moderate variability, suggesting that the community in this location retained characteristics of moderate diversity, albeit with a tendency towards clear dominance of certain species.

Figure 7 presents the distribution graphs of microorganism abundance at the 5 measurement points in the form of violin plots. Each violin illustrates the distribution of values for a given point – wider sections indicate a higher density of observations, and in the middle there is a box plot showing the median, quartiles, and typical data range. Analyzing the shape and spread of the violin, it can be seen that points 1, 3, 4 and 5 are characterized by greater variability and higher microorganism counts compared to point 2, where the values are lower and more concentrated. The Kruskal–Wallis test result ($p = 0.0000049$) confirmed that, at the chosen significance level of $\alpha = 0.05$, the difference in the distributions of the number of microorganisms between the analyzed points was highly statistically significant.

Since the early days of civilization, surface waters have been subject to pollution, as water reservoirs and rivers played a key role in the development of human settlements. Today,

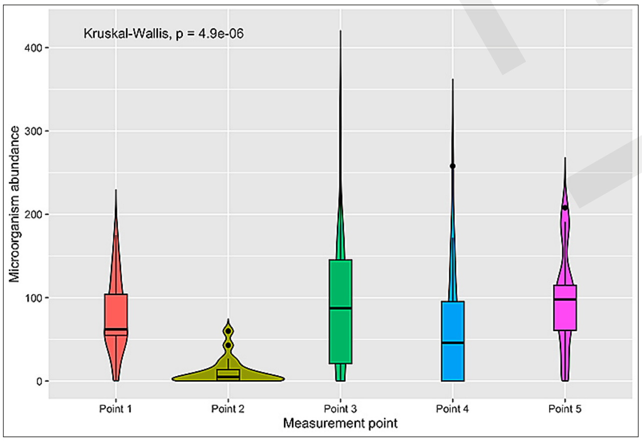

Figure 7. Violin plots of microorganism abundance at sampling points with results of Kruskal-Wallis test

urbanization has led to a significant reduction in green areas in favour of concrete infrastructure, which has disrupted the natural water cycle. Rainwater – instead of seeping into the ground and feeding groundwater – runs off paved surfaces, enters the storm sewer system, and ultimately ends up in a receiving water body, which in the case of the current study is the Bystrzyca River. During this process, rainwater collects pollutants present on urban surfaces, including solid waste, plastics, heavy metals, salts, and organic substances [24].

Under natural conditions, approximately 40% of rainwater evaporates, 50% infiltrates into the ground, and only 10% forms surface run-off. However, excessive urbanization and an increase in impervious surfaces, referred to as 'urban concrete sprawl' [25], leads to a significant increase in surface runoff. Studies show that a 20% increase in surface sealing results in a proportional 20% increase in surface runoff (TU1206-WG2.0–001). In addition, various substances that accumulate in sewage pipes are released rapidly into the receiving water body during heavy rainfall, increasing the risk of water pollution [22].

Previous studies conducted in Lublin confirm the impact of rainwater runoff on the Bystrzyca River. Bioindication analyses have shown that substances transported by storm sewers change the species structure of periphyton, as reflected in the results of studies by Babko et al. [6]. The saprobity index at the wastewater discharge site increased, but the values of this indicator stabilized below the discharge point. Similar relationships were also demonstrated in the research by Kozłowska et al. [21], where the Shannon index was lowest at the discharge point and then gradually returned to its initial values. These differences were confirmed by the MacArthur index, indicating the correct selection of measurement points.

The results presented in the current study, based on subsequent measurements and an extended range of biocenotic indices, led to similar conclusions as those from previous studies, and emphasize the importance of monitoring the impact of stormwater drainage on the river ecosystem.

SUMMARY

- Analysis of biocenotic indicators showed a clear decrease in the diversity of microorganisms at the storm water discharge site, which may indicate a negative impact of the sewage system on the river biocenosis.
- At subsequent measurement points, as the distance from the discharge increased, a gradual increase in biodiversity was observed, indicating the ability of the river to self-purify.
- Compared to the MacArthur index, the Shannon index proved to be more sensitive to changes in the structure of microbial communities, suggesting its greater usefulness in analyzing the impact of sewage systems on the aquatic ecosystem.
- Analysis of the McIntosh index revealed significant differences between measurement points in terms of both the average level of diversity and its stability over time. Analysis of this index is important because it allows for a better understanding of the processes shaping local communities of organisms, and indicates that even within a small area, there may be communities that differ significantly in terms of species structure.

- The low variability in microorganism abundance ($\hat{C}_v < 0.6$) at Point 1 suggests stable environmental conditions in a location not directly exposed to storm water discharge.
- The high coefficient of variation at Point 2 indicates high instability in the abundance of microorganisms, which may indicate the variable, impulsive nature of the impact of storm sewers on the analyzed biocenosis.
- The result of the Kruskal-Wallis test indicated statistically significant differences in the distribution of microorganism counts between measurement points.

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