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ORIGINAL PAPER

Freshwater biodiversity crisis: macroinvertebrates' response along the downstream gradient to receiving effluent from a wastewater treatment plant*

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Abstract

Suburban watercourses receiving sewage effluents often are low-flow and not modified, unlike large rivers to which wastewater is discharged. The impact of chemical pollutants on biodiversity as well as the effectiveness of ecosystem recovery and self-purification are worth careful attention. The aim was to assess the macroinvertebrates' responses to receiving an input from a wastewater treatment plant (WWTP) along the downstream gradient. We studied the variability and recovery pattern of macroinvertebrates in six sections located along a sewage receiving stream continuum. The comparison of chemical parameters showed significant differences in the pH, BOD₅, N-NH₄⁺ and PO₄³⁻. At sites 100, 300 and 500 m downstream of the WWTP, the persistently high amount of pathogenic coliform bacteria testified to the high impact of bacteriological pollution of sewage on the receiver. Based on 18013 macroinvertebrates, non-metric multidimensional scaling showed differences between the assemblages at the sections upstream of the WWTP, and located downstream. Effluent discharge affected the macroinvertebrate, causing a decrease of its quantitative index (abundance) by 7% and qualitative index (EPT) by 26% at the site of the sewage discharge. The recovery pattern was manifested by the gradual increase in macroinvertebrate indices at the sites located downstream from the WWTP, which at distances of 300 m and 500 m from the discharge site attained comparable values to those at the reference sites upstream of the WWTP. The populations of the most sensitive taxa were not fully restored with increasing distance from the WWTP, even at a distance of 500 meters, but instead were replaced by more resistant taxa. More attention should be paid to the impact of wastewater discharge from suburban wastewater treatment plants on receiving watercourse biodiversity and ecological water conditions, especially in the case of small and low-flow sewage receivers, where self-purification processes are difficult.

Keywords: macroinvertebrates, anthropogenic impact, WWTP, water quality, suburban area

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INTRODUCTION

Human impact on aquatic ecosystems can be seen in various aspects and scales, reducing the ecological integrity and biological diversity of lotic ecosystems (Vörösmarty et al. 2010). One of the main adverse effects is the chemical pollution of rivers caused by the influx of inadequately treated wastewater from treatment plants, or even the discharge of untreated wastewater (Sánchez-Morales et al. 2018, Birk et al. 2020). Wastewater treatment plants (WWTPs) are tasked with improving the quality of wastewater before it is discharged into a receiving water body (Wakelin et al. 2008). Limiting the quantity of untreated wastewater discharged into the environment is crucial in highly urbanized areas or locations with high industrial activity, which have received a great deal of attention (Rueda et al. 2002). However, little research has been devoted to the impact of effluents, the rate of self-purification and regeneration of biotic elements in small, low flow receiving watercourses flowing through suburban areas. These areas, due to the close proximity of large cities, are an attractive place to live, offering respite from the noise and bustle of the city. Unfortunately, this also entails increased pressure on the environment and an increase in pollutants released into the water. Moreover, suburban watercourses receiving effluent often have not been strongly transformed, so the effect of chemical pollutants and the efficiency of their self-purifications may not be the same as in the case of large receiving water bodies (Brooks et al. 2006).

The chemical parameters of water discharged from a WWTP have been shown to differ from the quality of the receiving water (Pereda et al. 2020). The differences are mainly due to the large amounts of organic matter and nutrients discharged into receiving waterways. In undisturbed rivers, the nutrient content is low, so that a significant increase in the value of these parameters has a major impact on the community structure of freshwater biota (Arenas-Sánchez et al. 2016). For example, increased nutrient loading can lead to eutrophication, temporary oxygen deficits and disruption of ecosystem stability (Carey, Migliaccio 2009). Moreover, nutrient loads were often more strongly influenced by WWTP effluent than by nonpoint sources (Wąsik et al. 2018). In the case of large rivers, the consequences of an influx of large amounts of biogenic compounds is not always clearly perceptible due to the large volume of water and relatively small fluctuations in the river flow. In small catchments, WWTP effluent may become a dominant contributor to nutrient inputs into aquatic systems, which strongly affects freshwater communities in terms of taxa composition, diversity, and the functional organization of living organisms.

Macroinvertebrates are a common group of freshwater organisms used as indicators of the overall health of an ecosystem (Hering et al. 2006, Kędzior et al. 2022). They are ubiquitous and abundant animals with limited mobility. Moreover, they have a wide range of feeding habits, respond rapidly

to their surrounding environment, and have diverse ecological plasticity (Buss et al. 2015).

The aim of this study was: (i) to determine differences in the chemical water conditions and macroinvertebrate communities over a gradient of distance from a wastewater treatment plant, and (ii) to explain the pattern of an ecosystem's recovery and self-purification in the stream. We hypothesize that WWTP effluents have a negative effect on the community structure of macrofauna.

MATERIALS AND METHODS

Study area and sampling design

The study was conducted in 2018, along the upper course of the stream Podstyrze-Włosanka, located in the southern Lesser Poland, on the outskirts of Krakow. The stream receives the effluent from the mechanical and biological wastewater treatment plant in Włoszań, with a capacity of $700 \text{ m}^3 \text{ day}^{-1}$, which collects wastewater from the southern part of the municipality Mogilany (population equivalent – PE 7000). The first step of the WWTP's two-step treatment process involves the mechanical treatment on a fine mesh sieve and in a Huber grit trap. The biological treatment step is based on extended aeration, low-loaded activated sludge process with the biological removal of biogenic compounds (BIOCOMPACT technology). The water law specifies the highest permissible pollution rates from the WWTP, amounting to $125 \text{ mg O}_2 \text{ dm}^{-3}$, $25 \text{ mg O}_2 \text{ dm}^{-3}$ and 35 mg dm^{-3} for COD, BOD_5 , suspended solids, respectively (Water Law, 2017). In the case of biogenic substances (total nitrogen and total phosphorus) for the RLM of sewage treatment plants below 9999, their permissible values are not specified.

The stream is the right bank tributary of the Vistula River. Its 8.65 km^2 catchment area is dominated by agricultural landscape (where artificial fertilizers and pesticides can constitute additional threats to stream), with a forested area surrounding the suburban area. The region where the study was conducted has varied geomorphology, with hills of 300-400 m above sea level, and trough-shaped and V-shaped river valleys.

Six sampling sections were selected along the stream continuum receiving the wastewater. The distance between the boundaries of research sections was at least 200 m. Two sections, located respectively 300 m (US_300m) and 100 m (US_100m) upstream of the WWTP input, were designated as reference sites. The third section was located at the outlet of the treatment plant (WWTP). The next three sites were located respectively 100 m (DS_100m), 300 m (DS_300m) and 500 m (DS_500m) downstream of the WWTP outlet. Each section was 100 m in length, except for the site located at the WWTP outlet, where the length was 50 m. In each research

section, 20 sampling sites were selected, where water, microbiological and hydrobiological samples were taken from an area 1m² in surface. The mean flow values (Q) at 100, 300 and 500 m from the wastewater discharge were 45.33, 76.76 and 79.46 L/s, respectively.

Environmental and chemical parameters

In each sampling section, the width, depth and the channel incision were determined. In addition, the percent shares of substrate types (sand, gravel, stones, and silt), macrophytes and the proportion of the most dominant hydromorphological units were calculated. Groups of parameters characterizing the chemical state of the water and microbiological variables were collected 6 times (from May to November 2018) from the sampling sites within each river section. Water temperature, oxygen concentration and pH were measured in field. The following chemical parameters were tested: suspended solids (sum of organic and mineral suspension), acidification, oxygen conditions and organic pollutants (concentration of dissolved oxygen O₂, BOD₅), and salinity concentration of chloride and sulphate, total hardness, sum of calcium and magnesium), as well as biogenic conditions (concentrations of ammonium nitrogen, nitrite nitrogen and orthophosphates).

In addition, the number of coliforms and of *Escherichia coli* bacteria were tested, as typical indicators of the presence of bacteria in the environment (Baird and Bridgewater 2017). Temperature, pH and dissolved oxygen concentration were measured with an ELMETRON CPO-401 multifunction meter. Organic compounds were determined as BOD₅ by the Hg-free method using the OXITOP®. Suspended solids were determined using glass fibre filters. Biogenic compounds were determined by colorimetric methods.

Benthic macroinvertebrate sampling

A total of 240 macroinvertebrate samples were taken during the growing season, using a standard hydrobiological net (500 µm mesh size, 0.625 m² metal frame area). The following macroinvertebrate assemblage parameters were calculated: abundance, taxa number, the Shannon diversity index, dominance. Moreover, the Biological Monitoring Working Party (BMWP_PL) and %EPT were calculated. The BMWP index is commonly used for biomonitoring riverine ecosystems in many countries, including Poland (Roche et al. 2010). It is based on a standardized score system which consists in calculating a score from points attributed to different invertebrate families according to their degree of intolerance to organic pollution. % EPT included combined taxa (Ephemeroptera, Plecoptera, Trichoptera), which are groups recognized as intolerant to pollution, poor water and worse habitat quality (Menció and Boix 2018). A high % EPT index value is expected in healthy lotic environments.

Self-purification of the stream

The following equations were used to assess the overall self-purification capacity of the Podstyrze-Włosanka stream (removal of nutrients):

$$C_i / C_0 = \exp(-KT t) \quad (1)$$

where: C_0 – concentration of wastewater at discharge site, mg L^{-1} ,

C_i – concentration of wastewater at the i -th sampling section, mg L^{-1} ,

KT – empirical coefficient indicating chemical and biological self-purification, day^{-1} ,

t – nutrient uptake time, day.

The t parameter was calculated according to the following formula (Bagdziunaite, Litvinaitinene 2017):

$$t = L_i W_i D_i n / Q_i \quad (2)$$

where: L – length of i -th sampling section, m,

W_i – average width of i -th sampling section, m,

D_i – average depth of i -th sampling section, m,

n – degree of free flow of water (this was not measured but assumed to be $n = 1$),

Q_i – average stream discharge (i -th sampling section), $\text{m}^3 \text{d}^{-1}$.

The coefficient KT was calculated after transforming formulas 1 and 2:

$$KT = \ln(C_i/C_0) Q / L_i W_i D_i \quad (3)$$

Statistical analysis

The environmental, chemical and bacteriological data were not normally distributed (Shapiro-Wilk test for normality, $p < 0.001$). The Kruskal-Wallis test was performed to verify the statistical significance of the differences between the six stream sections (Statsoft 2013). To analyze the effect of the WWTP on the chemical quality of the receiving stream, statistical grouping of the measurement points was performed. Cluster analysis by the Ward's minimum variance method was used in the calculations, and Euclidean distance was the measure of similarity. The groups distinguished in this manner were used as input variables in the statistical inference on the significance of differences between values of water quality parameters in the sections upstream and downstream of the WWTP.

Non-metric multidimensional scaling (NMDS) was used to test the relationships between the macroinvertebrate communities in the six sampling sections. The significance of differences was tested by PERMANOVA on the Bray-Curtis dissimilarities matrix with 499 permutations of the data (Past v. 3.13 software). Redundancy analysis (RDA) was employed to explore the relationships between chemical parameters, samples and macroinvertebrate taxa (CANOCO software). A generalized linear mixed model (GLMM) was used to model the relationship between macroinvertebrate assemblage indi-

ces and the sampling sections. The assemblage parameters of macroinvertebrates were not normally distributed (Shapiro-Wilk test for normality, $p < 0.001$), so we fitted the model to the Poisson distribution. The Kruskal-Wallis test was used to compare the values of the macroinvertebrates diversity and biotic indices among stream section type. The spatial autocorrelation across all sites within studied distance was tested using SAM v 4.0 (Rangel et al. 2010). We computed Moran's I for environmental and chemical variables to describe their spatial patterns across the study area, and to verify whether spatial autocorrelation was an issue that we should address in the analyses described above.

RESULTS AND DISCUSSION

Effect of wastewater effluent and the recovery of chemical and habitat parameters along the downstream gradient

Habitat parameters of the sampling sections were not spatially autocorrelated except elevation, width and incision only (Moran's I results), and significantly different only in the case of the percent share of stone, silt and macrophytes (Table S1 in supplementary materials). The highest proportion of rock substrate was found in the section located at the outlet of the WWTP. This was intended to stabilize the morphology of the watercourse under the influence of water discharged from the WWTP. Significant variation between the sites was also noted for the percentage of macrophytes, which was the highest in the sections upstream of the WWTP and in the section located 500 m downstream of the outlet.

Comparison of chemical parameters between sampling sections showed significant differences for pH, BOD_5 , $N-NH_4^+$ and PO_4^{3-} and coliform indicator bacteria (Table 1). Our results show that the chemical parameters of the stream are most altered at the site where the outlet of the WWTP is located. This is consistent with the results of other studies and indicates that WWTPs have a negative impact on the chemical conditions of the receiving water body (Souto et al. 2011). Menezes et al. (2015) describe similar problems concerning the removal of impurities introduced with effluent from a municipal WWTP to an urban stream. There are also studies describing the dynamics of nutrient removal in streams (Carey and Migliaccio 2009).

The average concentration of NH_4^+ ions in the water (500 m) downstream of the effluent outlet was 20 times as high as the value upstream. According to the literature, the effectiveness of ammonia nitrogen removal in the wastewater treatment process was not always sufficient (Cruz et al. 2019). A similar increase in pollution was observed in the case of the other biogenic substance – orthophosphates (Table 1). In the water samples from the site of the discharge, PO_4^{3-} was 16 times as high as the value upstream.

Table 1
 Mean values (\pm SD) of chemical parameters for the six sampling sections with the Kruskal-Wallis test and spatial autocorrelation (Moran's I) results

Chemical parameters	US_300m	US_100m	WWTP	DS_100m	DS_300m	DS_500m	Kruskal-Wallis H test (<i>p</i> -value)	Spatial autocorrelation	
								Moran I	p
pH	7.97 \pm 0.03	7.96 \pm 0.05	7.52 \pm 0.11	7.76 \pm 0.14	7.82 \pm 0.10	7.87 \pm 0.08	<0.001	-0.294	0.733
O ₂ (mg dm ⁻³)	7.57 \pm 1.66	7.87 \pm 1.31	6.007 \pm 1.28	7.71 \pm 1.07	6.71 \pm 1.52	7.48 \pm 1.96	0.285	-0.284	0.799
BOD ₅ (mg O ₂ dm ⁻³)	2.5 \pm 2.07	2.16 \pm 1.60	26.67 \pm 27.72	10.33 \pm 10.56	12.17 \pm 9.37	9.33 \pm 7.26	0.027	-0.417	0.435
Suspended solids (mg dm ⁻³)	30.27 \pm 21.16	23.77 \pm 23.77	38.26 \pm 18.16	30.13 \pm 27.14	22.73 \pm 14.09	32.3 \pm 24.70	0.812	-0.521	0.176
Organic suspension (mg m ⁻³)	21.73 \pm 14.42	20.57 \pm 14.92	14.7 \pm 36.51	21.3 \pm 12.25	15 \pm 6.07	22.3 \pm 17.47	0.814	-0.683	0.122
Mineral suspension (mg m ⁻³)	8.87 \pm 6.42	6.03 \pm 3.61	16.77 \pm 10.07	15.43 \pm 13.88	9.4 \pm 39.50	11.9 \pm 38.95	0.409	-0.538	0.122
N-NH ₄ ⁺ (mg dm ⁻³)	0.29 \pm 0.09	0.28 \pm 0.09	15.63 \pm 31.67	4.32 \pm 8.84	3.56 \pm 7.43	4.13 \pm 7.93	0.042	-0.372	0.571
N-NO ₂ (mg dm ⁻³)	0.23 \pm 0.16	0.18 \pm 0.10	0.18 \pm 0.16	0.13 \pm 0.08	0.15 \pm 0.08	0.14 \pm 0.07	0.863	0.071	0.293
PO ₄ ³⁻ (mg dm ⁻³)	0.15 \pm 0.13	0.17 \pm 0.10	2.64 \pm 1.63	1.17 \pm 1.26	1.14 \pm 1.25	1.21 \pm 1.11	<0.001	-0.390	0.471
Conductivity (μ S cm ⁻¹)	810.85 \pm 109.52	805.48 \pm 114.96	952.15 \pm 478.55	604.77 \pm 254.50	740.9 \pm 152.49	738.05 \pm 147.14	0.052	-0.420	0.405
Cl ⁻ (mg dm ⁻³)	95.08 \pm 31.92	99.26 \pm 25.80	122.90 \pm 43.22	86.26 \pm 47.06	80.95 \pm 25.30	81.5 \pm 27.00	0.549	0.142	0.199
Total alkalinity (mval dm ⁻³)	3.9 \pm 0.33	4.25 \pm 0.89	4.78 \pm 1.10	3.67 \pm 0.93	3.82 \pm 0.61	3.92 \pm 0.66	0.203	-0.133	0.807
SO ₄ ²⁻ (mg dm ⁻³)	8.51 \pm 3.18	7.48 \pm 2.50	9.70 \pm 3.98	9.53 \pm 5.56	9.88 \pm 6.22	10.41 \pm 7.18	0.931	-0.056	0.566
Total hardness (mg CaCO ₃ dm ⁻³)	283.33 \pm 22.73	290.8 \pm 31.69	277.5 \pm 19.94	262.5 \pm 34.31	265.00 \pm 32.56	270.00 \pm 22.14	0.751	-0.036	0.455
Coli form bacteria (CFU 100 ml ⁻¹)	2.28 10 ⁴ \pm 1.86 10 ⁵	2.35 10 ⁴ \pm 1.25 10 ⁵	1.10 10 ⁴ \pm 2.64 10 ⁵	4.38 10 ⁴ \pm 2.85 10 ⁵	4.13 10 ⁴ \pm 3.49 10 ⁵	4.22 10 ⁴ \pm 3.23 10 ⁵	0.006	0.270	0.113
Escherichia coli (CFU 100 ml ⁻¹)	4.00 10 ⁴ \pm 7.90 10 ⁵	3.00 10 ⁴ \pm 5.43 10 ⁴	2.93 10 ⁴ \pm 4.33 10 ⁵	1.87 10 ⁴ \pm 3.13 10 ⁴	1.93 10 ⁴ \pm 2.09 10 ⁵	5.37 10 ⁴ \pm 9.18 10 ⁴	0.086	-0.371	0.745

The average concentration of orthophosphates measured 500 m downstream of the WWTP was also higher than the value upstream. The BOD₅ value upstream of the WWTP was low, on average 2.5 mg O₂ dm⁻³ (and 2.17 mg O₂ dm⁻³ (US_300m and US_100m respectively). At the site of the effluent discharge, BOD₅ averaged 26.67 mg O₂ dm⁻³, while downstream of the WWTP it decreased to 9.33 mg O₂ dm⁻³. It has been reported in a number of studies that intense human activities resulting from discharge of organic pollutants into streams lead to an increase in nutrient levels and in biological oxygen demand, which in turn affects the distribution and abundance of benthic invertebrates (Armiro, Keke 2017).

In the bacteriological assessment, the persistently high levels of bacteria at the points downstream from the WWTP are indicative of the substantial influence of bacterial contamination of wastewater on the water of the small, suburban stream receiving it (Corsi et al. 2021).

The cluster analysis performed for the chemical parameters distinguished two groups of variables (Figure 1).

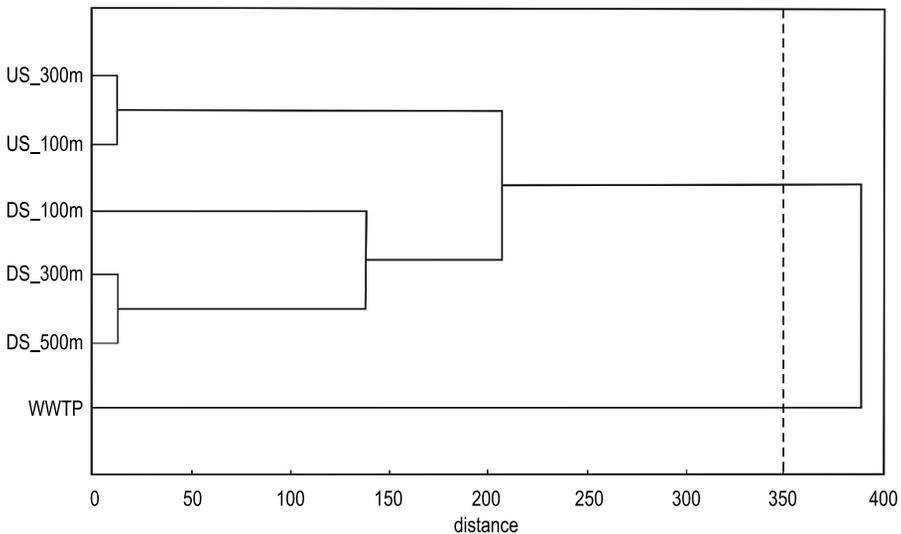


Fig. 1. Classification of sampling sites with chemical parameters through a hierarchical cluster analysis, using the Ward's method and Euclidean distance as a similarity measure

The first group comprised two smaller clusters, the first of which consisted of the measurement points upstream of the WWTP, while the second comprised the sites downstream. The second group included the site at the WWTP outlet. The linkage distance was estimated at 350. This indicates the pronounced effect of the treatment plant on chemical parameters, and also shows that the conditions are not restored over the gradient marked out for the study relative to the reference sections (Rueda et al. 2002).

Effect of wastewater effluent and the effectiveness of macroinvertebrate assemblage recovery along the downstream gradient

In total, 18013 specimens belonging to 30 taxa of aquatic macroinvertebrates were collected. NMDS and PERMANOVA showed differences in the benthic macroinvertebrate community depending on the sampling section (Figure 2, Table S2 in supplementary materials).

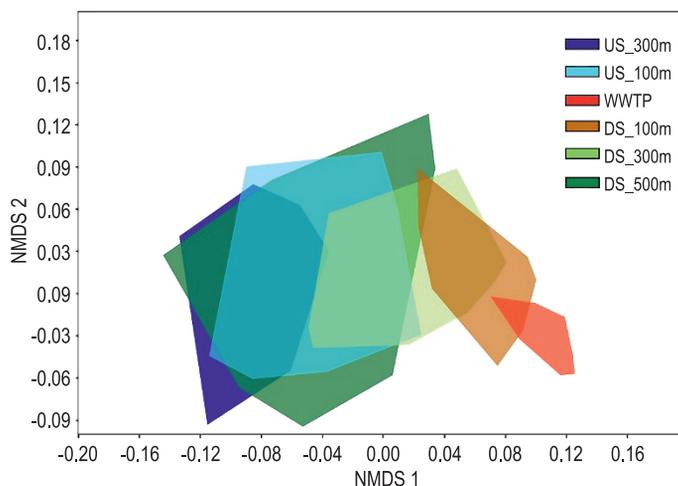


Fig. 2. NMDS for macroinvertebrate communities

The macroinvertebrate assemblages inhabiting the location at the site of the effluent discharge differed significantly from those in the sections upstream and downstream of the WWTP (Ortiz et al. 2005, Englert et al. 2013). According to Cortes et al. (2002) and Hamada et al. (2002), distribution and assemblages of benthic macroinvertebrates may be attributed to the small-scale variability in a variety of water quality parameters. However, Chatzinikolaou et al. (2006) highlighted that pollution and excessive nutrient enrichment from anthropogenic sources, in particular sewage and solid waste, can affect habitat quality and benthic macroinvertebrates changing their community structure and composition (Sharma et al. 2012). This impact can be also visible in freshwater fauna's reproductive cycles and food chain dysfunction (Adakole, Annune 2003).

The RDA distinguished groups of macroinvertebrates arranged over gradients of chemical variables (Figure 3).

There is clear separation of macroinvertebrates and samples from the sections upstream of the effluent discharge, at the site of the discharge, and downstream of the discharge (Figure 3). The significant chemical and bacteriological variables explaining the macroinvertebrate variation were pH ($p=0.002$), $N\text{-NO}_2^-$ ($p=0.002$), total hardness ($p=0.021$), suspended solids

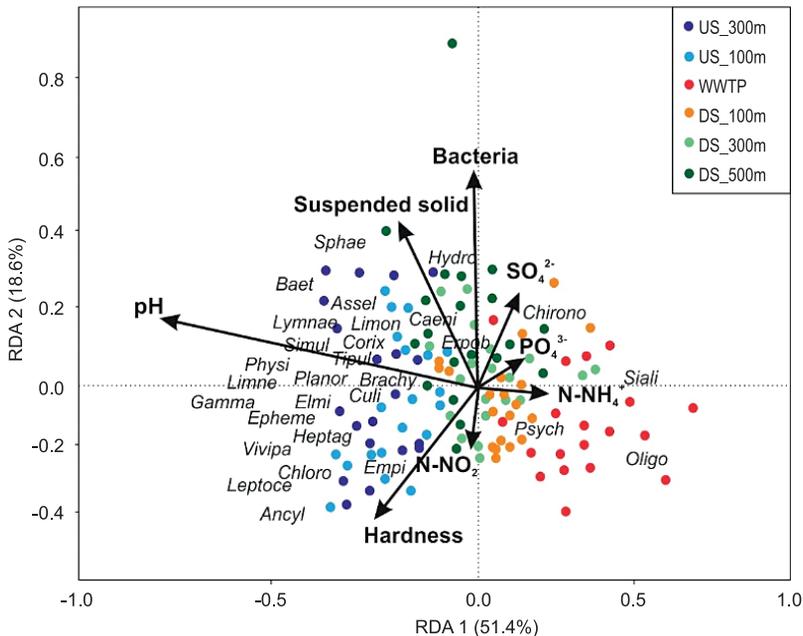


Fig. 3. Redundancy analysis (RDA) plot of freshwater macroinvertebrate communities in response to gradients of chemical variables

($p=0.025$), SO_4^{2-} ($p=0.030$), PO_4^{3-} ($p=0.038$), N-NH_4^+ ($p=0.041$) and coliform bacteria ($p=0.052$). The first group, which was positively correlated with pH, N-NO_2 and total hardness, including Baetidae, Limonidae, Corixidae, Tipulidae, Simuliidae, Planorbidae, Limneidae, Brachypteridae, Gammaridae, Elmidae, Ephemeroidea, Viviparidae, Heptagenidae, Leptocentridae and Chloroperlidae. These taxa were mainly characteristic of sites located upstream of the WWTP and are more sensitive to disturbances (Menció, Boix 2018). The other side of the diagram shows taxa characteristic of the water at the site of the discharge: Oligochaeta, Sialidae and Psychocidae. Ghani et al. (2018) showed that Oligochaeta are especially numerous in highly polluted urban rivers. Hence the differences in taxonomic composition are due to the effect of contamination or transformation of the environment, which leads to an exchange of taxa and the loss of the least resistant ones (Quanz et al. 2021). The third group comprises taxa from the sites located downstream of the WWTP, which were positively correlated with sulphate content, suspended solids, and the presence of coliforms (Hydropsychidae, Sphaeridae and Chironomidae). We suspect that despite the persistent elevated chemical parameters describing organic and biogenic substances, the preservation of varied habitats and a similar average water flow ensured suitable environmental conditions for diverse groups of macrofauna (Beisel et al. 2000). Similar results were reported by Mor et al. (2019), who found that wastewater inputs did not lead to the homogenization of community

composition among downstream sites. Hawkins et al. 2015 highlighted that environmental disturbances can raise the beta diversity of macroinvertebrate assemblages, which we observed in our results.

In the case of an analysis of invertebrate assemblage parameters, statistically significant differences were noted for taxonomic diversity, abundance and taxonomic richness of macroinvertebrates depending on the sampling section (Table S3 in supplementary materials). The highest average abundance was noted at the sites upstream of the WWTP and those located the furthest downstream (DS_300 m and DS_500 m). As regards the number of taxa and taxonomic diversity, the distribution of means was similar, but this value did not return to the initial values (Figure 4).

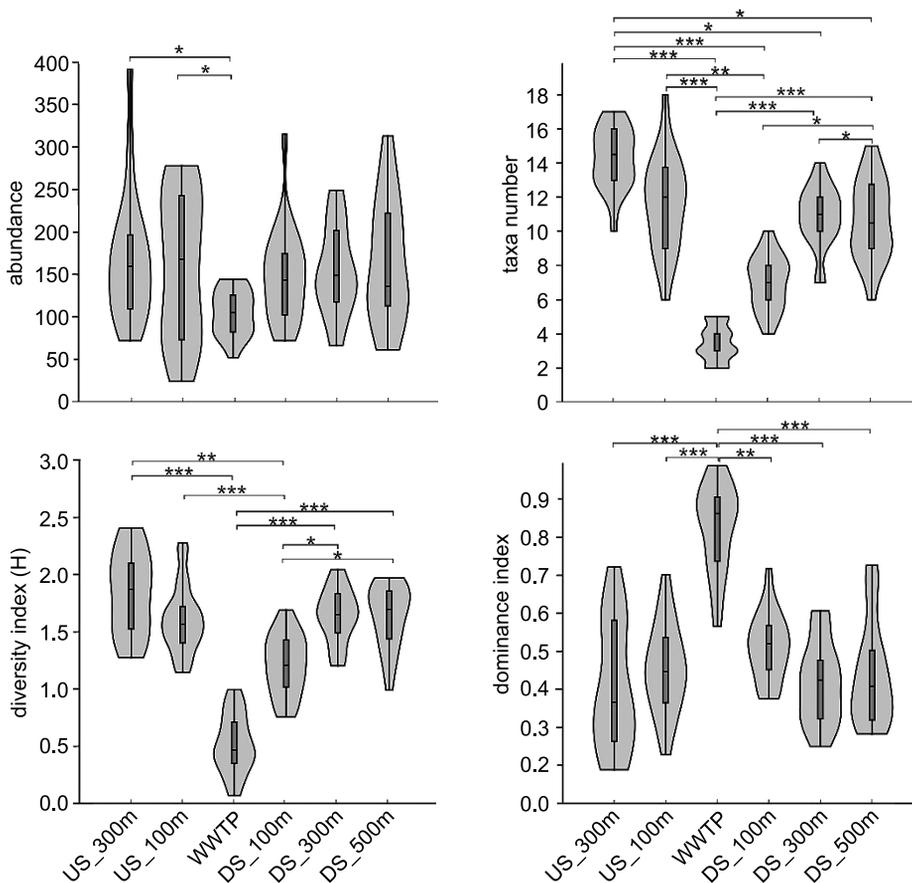


Fig. 4. Violin plots showing total abundance, taxa richness, Shannon-Wiener (H) and dominance index in the 6 analyzed river sections.

The boxplots inside the violins represent the distribution of data with median and lower-upper limits, p values: * < 0.05, ** < 0.01, *** < 0.001

Other studies confirmed our results, highlighting that constant or decreasing numbers of macroinvertebrate taxa are observed along the gradient from upstream to downstream (Elias et al. 2014). Taxa diversity has often been related to the stability of the environment (Kaaya et al. 2015). In this study, the macroinvertebrate communities were subjected to environmental disturbances, which were less predictable and more profound at sites WWTP and DS_100m. In the case of the sections 300 m and 500 m downstream from the WWTP, the diversity index values were similar to those from the sites upstream of the WWTP, which may indicate some recovery of macrofauna assemblages (Figure 4, Ortiz et al. 2005).

The distribution biotic indices at the study sites was analyzed as well (Figure 5). The lowest BMWPPL values were noted at the site of the effluent discharge, with a gradual increase in this parameter observed in the succes-

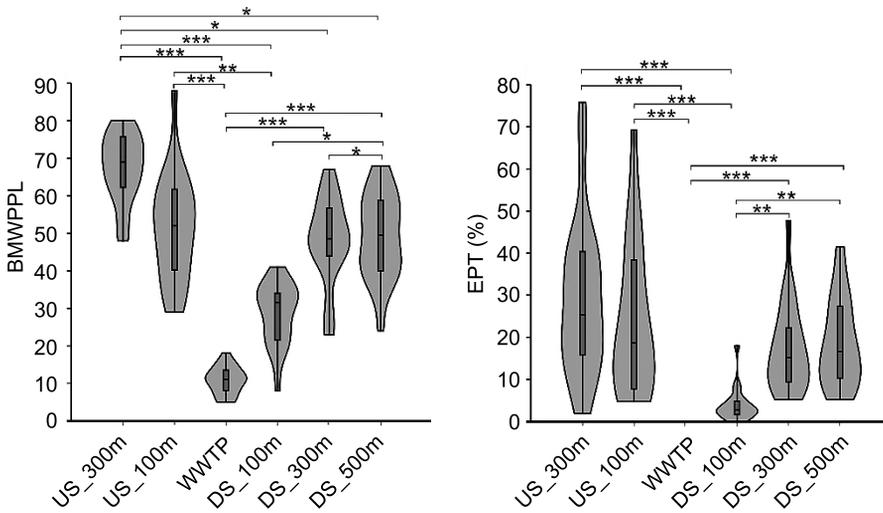


Fig. 5. Violin plots showing BMWPPL and % EPT distribution in the studied sections. The boxplots inside the violins represent the distribution of data with median and lower-upper limits, p values: * <math>p < 0.05</math>, ** <math>p < 0.01</math>, *** <math>p < 0.001</math>

sive sampling sections, until it reached a value similar to that noted at the sites outside the influence of the WWTP (100 m and 300 m upstream).

Moreover, no taxa of the orders Ephemeroptera, Plecoptera or Trichoptera were noted in the assemblages at the WWTP discharge site (%EPT = 0), but with the increasing distance from the WWTP, these sensitive macroinvertebrates were very slowly recolonizing the water body (Menció, Boix 2018).

Self-purification of the stream

Figure 6 shows the values of the empirical coefficient K_T indicating the self-purification of the Podstyrze-Włosanka stream.

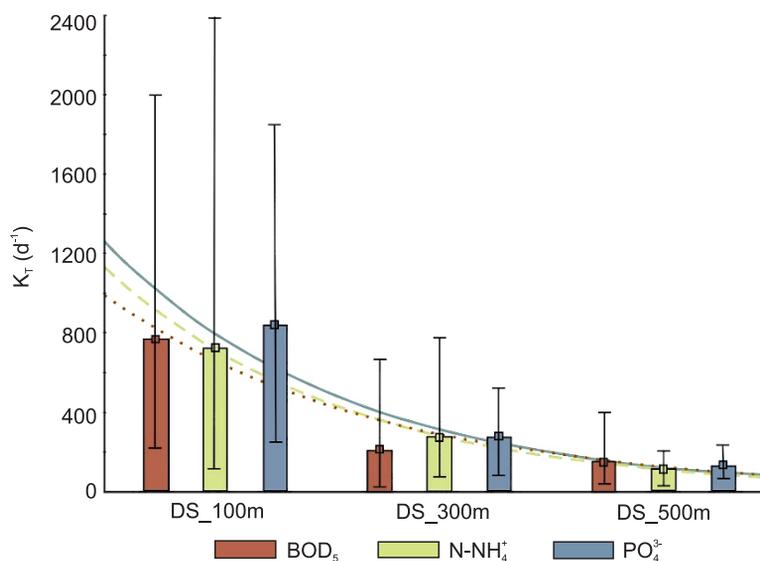


Fig. 6. BOD₅, N-NH₄⁺ and PO₄³⁻ self-purification coefficient in 100 m, 300 m and 500 m from the site of the effluent discharge

The highest degree of self-purification of the stream consisting in the elimination of easily biodegradable organic substances and biogenic substances was noted in the section 100 m from the site of the effluent discharge. This was due to the dilution of the effluent on the one hand and, on the other hand, to biological processes. Natural raising of the water level by the accumulation of large boulders resulted in an increase in the amount of dissolved oxygen (reaeration) due to turbulence in the stream flow. From 300 m to 500 m self-purification of the water stabilized, which is evident in the case of the K_T coefficient for pollution defined as the BOD₅ index. After 900 m from the sewage discharge site, the values of the BOD₅ indicator and the concentrations of N-NH₄⁺ and PO₄³⁻ will be similar to the values of these parameters determined 300 m before the sewage treatment plant (Figure 6). Wastewater inputs in suburban areas are known to influence the nutrients and chemical properties at the receiving sites (Mor et al. 2019, Burdon et al. 2020). The literature contains little information on this subject, which in itself is significant due to the increasing population density in suburban areas (Nasreddine 2021). Research much more often focuses on the effect of large urban wastewater treatment plants on ecosystems, where the rivers receiving the effluent are large and in many cases regulated (West et al. 2006, Drury 2013). In these cases, the rate of self-purification of the water depends on completely different factors than in the case of small receiving watercourses with unaltered hydromorphology (Cheimonopoulou et al. 2011).

CONCLUSIONS

Based on the findings of the study, we would like to emphasize the use of macroinvertebrates as a sensitive indicators for assessing the ecosystem recovery effectiveness in streams impacted by wastewater treatment plant effluents.

In suburban areas, low-flow streams are often receivers of sewage effluents. In such cases, water self-purification can be difficult and biogenic substances or pollutants can influence ecosystems along the downstream gradient. On the other hand, this small and often not transformed hydromorphologically streams are important for maintaining the biological diversity of freshwater as well as riparian ecosystems in these areas.

The results showed significance differences of pH, BOD₅, N-NH₄⁺ and PO₄³⁻ as well as coliform indicator bacteria between sampling sections upstream and downstream of the WWTP.

The highest degree of the stream's self-purification in terms of the elimination of organic substances was noted in DS_100 m section. At 900 m from the sewage discharge site, the values of the BOD₅ indicator and the concentrations of N-NH₄⁺ and PO₄³⁻ will be similar to the values of these parameters determined 300 m before the sewage treatment plant.

The recovery pattern was observed in a gradual increase in macroinvertebrate diversity at distances of 300 m, and 500 m from the effluent output was similar to reference sites. Nevertheless, most sensitive taxa (e.g. Heptageniidae, Caenidae, Ephemeridae, Leptophlebiidae, Chloroperlidae as well as Limnephilidae and Viviparidae) were not fully restored with increasing distance from the WWTP, even at a distance of 500 meters, but instead were replaced by more resistant taxa.

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Author contributions

R.K. – conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, supervision, visualization, writing – original draft preparation, writing – review & editing. E.D. – data curation, formal analysis, investigation, methodology, resources, writing – original draft, writing – review & editing. K. C. – formal analysis, investigation, A.Z. – data curation, formal analysis, writing – original draft, writing – review & editing. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors ensure that they have neither professional nor financial connections related to the manuscript sent to the Editorial Board. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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