Assessment of wastewater treatment plant effluent impact on the ecosystem of the river on the basis of the quantitative development of ciliated protozoa characteristic of the aeration tank

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ABSTRACT

The work is devoted to the task of simplifying the assessment of the effect of effluents from treatment facilities on the river hydrobiocenosis. The studies were carried out on the mountain river Uzh (Uzhgorod, Ukraine). Our approach to assessing the impact of waste treatment facilities on the river receiver is based on the estimate of the similarity of species composition and quantitative characteristics of populations of organisms from the aerotank and from the river. It is shown that the quantitative development of populations of species of ciliates from the aeration tank is a good indicator for assessing the degradation of organic matter coming with wastewater. The use of qualitative and quantitative characteristics of the protozoa from the wastewater treatment plant as a criterion for assessing the quality of the environment in the area of wastewater discharge showed their representativeness and effectiveness. The use of a limited number of species makes it possible to conduct an express assessment of the effect of effluents on receiving reservoirs for specialists working with activated sludge in the laboratories of treatment facilities.

Key words | activated sludge, assessment, effluents, environmental impacts

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HIGHLIGHTS

- Species of ciliated protozoa from treatment plants turned out to be sensitive indicators of the interaction of river biocenosis and wastewater.
- The use of qualitative and quantitative characteristics of the protozoa from the wastewater treatment plant as a criterion for assessing the quality of the environment in the area of wastewater discharge showed their representativeness and effectiveness.
- The use of a limited number of species makes it possible to conduct an express assessment of the effect of effluents on receiving reservoirs for specialists working with activated sludge in the laboratories of treatment facilities.

INTRODUCTION

The negative impact of point sources of pollution is a significant problem for surface waters (Smith 2003; Bernhardt & Palmer 2007; Sutton *et al.* 2011; Grant *et al.* 2012). Effluents from wastewater treatment plants exist everywhere, so the evaluation of their impact is important. Traditionally, the impact of wastewater was evaluated by studying the chemical composition of water. In this case, standard sanitary norms adopted in specific countries are used. To assess the influence of effluents usually parameters such as the nitrates, nitrites and ammonium, phosphorus, oxygen content in water, oxidation, biochemical oxygen demand, and several other parameters are measured (Rueda *et al.* 2002; Gücker *et al.* 2006). Also evaluation based on biological indicators of water quality before and after discharge expressed as numerical indices is used (Madoni & Zangrossi 2005; Drury *et al.* 2013). For example, popular are the indices of saprobity and biodiversity (Madoni & Zangrossi 2005; Babko *et al.* 2016; Pliashechnyk *et al.* 2018). Certain complexity to estimate the influence of the effluents is due to water flow, causing effects of local influence to possibly be significantly offset downstream. Such a shift is largely determined by the speed of the river, its type (mountainous, lowland), the characteristics of bottom (sandy, muddy, or rocky), and a number of other factors, including hydromorphological characteristics of rivers.

The modern surface water quality assessment system in Europe is based on such biological indicators as species composition and abundances of aquatic vegetation, benthic invertebrates, species composition, abundances, and age structure of fish populations (European Commission 2000). The variety of valuation approaches is caused by the ambiguity of responses from hydroecosystems and difficulties in standardizing the criteria (Smith 2003; Woodcock & Huryn 2005; Izagirre *et al.* 2008; Sanchez-Perez *et al.* 2009; Bernot *et al.* 2010; Sutton *et al.* 2011; Cabrini *et al.* 2013). This is largely due to the fact that there are many factors of influence. This can be, for example, mechanical disturbance of natural riverbeds, such as straightening, deepening, removal of alluvium, change in flow direction, etc. (Aristi *et al.* 2014). Various chemical pollutants, such as non-toxic dissolved nutrients or toxic substances cause different responses of hydroecosystems (Smith 2003; Sutton *et al.* 2011; de Castro-Catala *et al.* 2014; Aristi *et al.* 2015). However, the idea that in water systems it is not so much the quality of water as the quality of the biological system that supports it is justified and becomes generally accepted (European Commission 2000).

There is a problem of standardization of approaches to assess how much the effluent from wastewater treatment plants affects the river and how much the river is able to neutralize the effect. In this context to choose the appropriate criteria for such assessments is of importance. In fact, important is that and what we compare when assessing such impact. Rivers flowing through urban areas and agricultural lands are exposed to many negative factors so to isolate the impact of wastewater treatment facilities can be difficult. Macroinvertebrates and plants not always can be used as indicators, for example, in places of chronic pollution, where conditions limit the presence of certain indicator organisms. E.g. if the turbidity is high, photosynthetic organisms cannot be used as indicators effectively, or when pollution exceeds the tolerance limits of macrobenthos species, other groups of indicator species are needed. In this case, microorganism based assessment may be most effective; for this, protozoa are widely used (Foissner *et al.* 1991, 1992, 1994, 1995; Sladecek 1973). Particularly, benthic ciliates have been proposed as biological indicators of organic pollution in rivers (Cairns 1969; Madoni Zangrossi 2005). At the same time, the use of protozoa as indicators for assessing water quality and the level of influence of effluents is also fraught with a number of difficulties, the main of which is the difficulty of identifying them and the huge potential diversity (Foissner 2016). We proposed a way to make bioindication of the impact of wastewater on the river ecosystem more accessible for practical use. Therefore, the evaluation on the basis of the protozoa involves protozoological professional training, without which it is essentially not feasible, or at least not very effective and reliable. We propose here to simplify this task based on the fact that most of the treatment plants in their laboratories have specialists who analyze the quality of activated sludge also on the basis of composition of the protozoa. We have proposed an approach to the assessment of the impact of wastewater for which is sufficient the level of competence of these professionals.

MATERIALS AND METHODS

Samples were taken from the aeration tank in Uzhgorod municipal wastewater treatment plant and from the River Uzh between 02.2016 and 02.2018. In total, 236 samples were taken from the Uzh River. Samples were taken in the first and last 10 days of each month. At the same time,

samples were taken from the aeration tank of the waste-water treatment plant of Uzhgorod city. Samples from the aerotank were taken in three places: at the beginning of the aerotank, in its middle part, and at the exit to the channel leading to the secondary sedimentation tank. Data collected from those places of the aerotank were averaged, considering them as an integral sample, reflecting the state of the protozoa community at the time of sampling. A total of 72 samples of activated sludge were analyzed. The placement of sampling sites is shown in Figure 1.

Wastewater is discharged into the Uzh River down-stream the city of Uzhgorod. The investigated section of the river is influenced by dispersed pollution sources from the city, so the stations above the treated wastewater discharge were not considered as 'clean', but only as such that did not experience the influence of wastewater, only as a reference point, control. In the studied area, the bottom is mainly stony and silty; flow velocity ranges from 1 to 2 m/s. During floods and high water caused by heavy rains, the flow velocity rises to 4–6 m/s.

Samples of river bottom sediments were analyzed. At stations located 50 and 250 m above the treated water inflow, the bottom is rocky and slightly silty. At the confluence of the runoff (station 0) and at stations 50 and 100 m

downstream, a rocky bottom with a large amount of silt. At a station 250 m upstream the runoff, a rocky bottom mixed with silted sand. At the station 300 m downstream the runoff, the bottom is rocky, the amount of silt deposits is insignificant, close to that at stations located 50 and 250 m above the treated wastewater discharge.

Samples of bottom sediments from the river were taken using a 100 mL syringe with a plastic tip up to 0.5 m long. The diameter of the inlet of the tip is 4 mm. At each point, three samples were taken in triplicate with a volume of 100 mL. At each station, temperature, pH, and O_2 content were measured using a HACH HQ40d portable multimeter. The content of nitrogen species was determined using a HACH DR2800 spectrophotometer. The averaged results of hydrochemical analyzes are presented in Table 1.

After sampling, the samples were transported to the laboratory where they were placed in the refrigerator. Sample processing was performed immediately after sampling. Samples were processed according to the procedure described in Foissner Berger 1996, Babko *et al.* 2016, and Pliashechnyk *et al.* 2018. Population abundance was estimated by counting in 25 μl subsamples extracted using a micropipette, as described by Madoni (Madoni 1994). The number of repeated counts was 5 for samples of activated sludge and
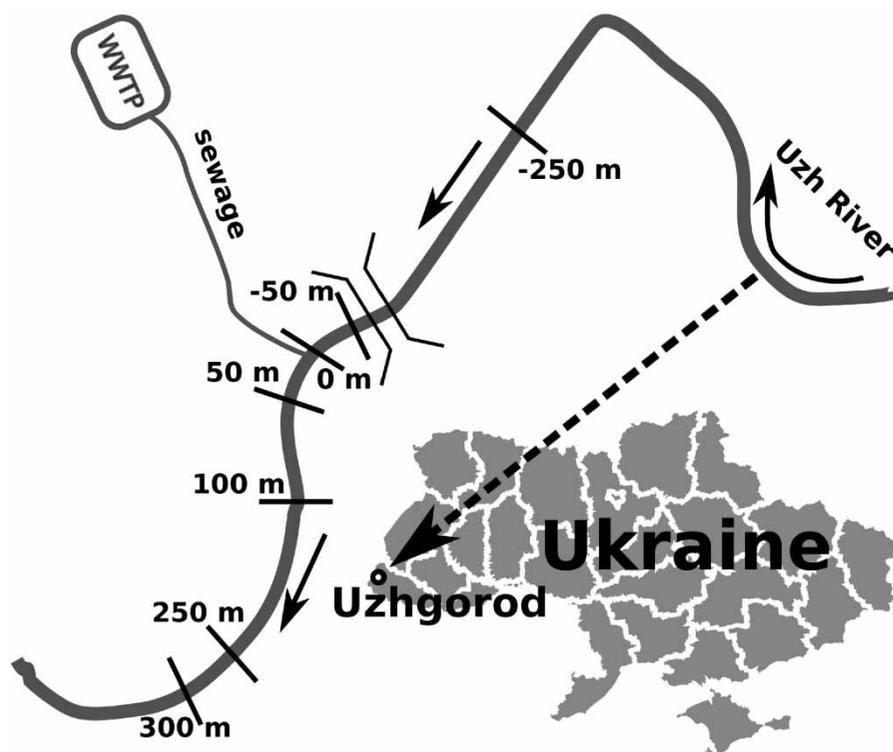

Figure 1 │ Scheme of the research area and the location of sampling stations. The direction of the river flow is shown by solid arrows. A dashed arrow indicates the location of the study area on the map. WWTP – Uzhgorod municipal wastewater treatment plant.

Table 1 | Chemical parameters in water of the Uzh River at sampling stations

	Stations and seasons						
	− 250 m	− 50 m	0 m	50 m	100 m	250 m	300 m
	Winter						
Temperature, °C	2.12 ± 0.41	2.50 ± 0.54	2.18 ± 0.12	2.50 ± 1.40	2.60 ± 1.70	1.54 ± 1.27	1.14 ± 0.59
pH	9.90 ± 0.36	10.10 ± 0.16	10.14 ± 0.76	9.70 ± 0.26	8.50 ± 0.52	10.70 ± 1.50	9.61 ± 0.41
O_2, mg l^{-1}	13.20 ± 0.41	13.26 ± 0.34	9.54 ± 2.07	11.90 ± 0.70	11.90 ± 1.10	11.70 ± 1.14	12.37 ± 1.45
$N\text{-}NH_4$, mg l^{-1}	1.00 ± 0.30	1.74 ± 0.23	2.32 ± 0.29	2.14 ± 0.10	1.86 ± 0.16	1.48 ± 0.22	1.04 ± 0.19
$N\text{-}NO_3$, mg l^{-1}	0.80 ± 0.15	0.84 ± 0.08	1.08 ± 0.21	1.08 ± 0.06	0.99 ± 0.07	0.89 ± 0.07	0.79 ± 0.12
	Spring						
Temperature, °C	–	9.65 ± 7.43	13.58 ± 3.89	11.30 ± 4.30	8.70 ± 6.47	9.18 ± 6.20	9.46 ± 7.50
pH	–	10.40 ± 0.49	8.24 ± 0.54	8.20 ± 0.45	8.60 ± 0.37	8.80 ± 0.30	8.46 ± 0.31
O_2, mg l^{-1}	–	12.23 ± 1.46	6.70 ± 1.44	8.10 ± 1.50	10.90 ± 1.36	10.16 ± 2.10	10.89 ± 2.18
$N\text{-}NH_4$, mg l^{-1}	–	1.06 ± 0.19	1.94 ± 0.19	1.36 ± 0.29	1.40 ± 0.30	1.24 ± 0.16	0.98 ± 0.18
$N\text{-}NO_3$, mg l^{-1}	–	0.76 ± 0.10	0.98 ± 0.30	0.80 ± 0.17	0.82 ± 0.08	0.75 ± 0.14	0.64 ± 0.09
	Summer						
Temperature, °C	22.10 ± 2.20	23.20 ± 2.78	20.13 ± 0.69	20.40 ± 0.90	22.30 ± 0.80	22.10 ± 0.78	21.90 ± 1.25
pH	9.30 ± 0.64	8.60 ± 0.63	8.62 ± 0.44	9.00 ± 0.60	9.40 ± 0.43	9.10 ± 0.55	8.93 ± 0.77
O_2, mg l^{-1}	9.54 ± 0.58	7.63 ± 0.95	6.20 ± 0.46	6.90 ± 0.30	7.70 ± 0.60	8.05 ± 0.67	7.63 ± 0.57
$N\text{-}NH_4$, mg l^{-1}	2.54 ± 0.48	2.36 ± 0.32	3.58 ± 0.40	3.20 ± 0.23	3.06 ± 0.32	2.94 ± 0.19	2.52 ± 0.18
$N\text{-}NO_3$, mg l^{-1}	0.62 ± 0.11	0.71 ± 0.09	0.90 ± 0.24	0.83 ± 0.10	0.80 ± 0.16	0.74 ± 0.10	0.69 ± 0.06
	Autumn						
Temperature, °C	8.50 ± 0.37	7.41 ± 0.72	12.45 ± 1.51	8.40 ± 2.70	6.02 ± 2.30	7.70 ± 1.30	7.36 ± 1.30
pH	10.40 ± 1.33	10.40 ± 0.50	10.20 ± 0.90	9.40 ± 0.40	9.80 ± 0.35	9.14 ± 0.42	9.20 ± 0.54
O_2, mg l^{-1}	10.90 ± 0.66	10.46 ± 0.82	6.84 ± 0.62	6.80 ± 1.80	10.90 ± 0.28	9.80 ± 0.96	10.85 ± 0.86
$N\text{-}NH_4$, mg l^{-1}	2.68 ± 0.40	2.74 ± 0.27	4.38 ± 0.34	3.98 ± 0.12	3.38 ± 0.53	3.28 ± 0.27	2.98 ± 0.21
$N\text{-}NO_3$, mg l^{-1}	0.75 ± 0.12	0.79 ± 0.17	1.26 ± 0.17	0.94 ± 0.10	0.95 ± 0.11	0.69 ± 0.14	0.78 ± 0.18

11 for bottom sediments. In most cases, ciliates have been identified *in vivo*. If necessary, cells were stained using 1% methyl green or silver nitrate (Foissner 1991). Species identification is based on Warren 1986, 1987, Foissner Berger 1996, and Saprobic assessments were performed using saprobic values from Sladecek (Sladecek 1973), edited by Foissner *et al.* (Foissner *et al.* 1991, 1992, 1994, 1995).

Data on the species abundances were processed using R Version 3.6.2 (R Core Team 2019) with the package tidyverse (Wickham *et al.* 2019). Plots were produced with the R package ggplot2 (Wickham 2009).

RESULTS AND DISCUSSION

The complexity of the processing of samples of protozoa, including ciliates, is one of the main obstacles to their widespread use as indicators of the state of ecosystems and the quality of water treatment (Foissner 2016). At the same time, they are one of the best bioindicators of the state of the aquatic environment, since representatives of the Ciliophora are widespread in the whole variety of conditions provided by the aquatic environment (including anaerobic ones) (Fenchel 1987; Finlay Esteban 1998; Corliss 2002).

For example, during the work devoted to assessing the influence of municipal pollution on the ciliated community in the Lyna River (Hul 1987), 130 species of ciliates were identified in order to compare the species composition of these organisms in the river sections up and downstream the point of discharge of polluted water into the river. Madoni & Zangrossi (2005) indicated that 89 species of ciliates were identified in similar studies of the composition of organisms in a river subjected to organic contamination. These examples demonstrate that assessment based on

protozoans requires professional protozoological training, without which it is essentially impracticable, or at least not very effective and reliable. Similar data were obtained in the course of our research. In the studied section of the river, which is affected by effluents from the wastewater treatment facility, 88 species of ciliated protozoa were identified. In the aeration tank, 40 species were recorded over the same period, while almost half of the species identified in the aeration tank were found only occasionally. About 20 species were systematically found in activated sludge, which were used in the subsequent analysis.

Most researchers show the integral effect of settlements on the river based on changes in the species composition and quantitative representation of all species present in the studied sections. Under this approach, research is quite time-consuming. We propose here to simplify this task based on the fact that the variety of species of protozoa is relatively small in wastewater treatment plants (Madoni 1994), and their ecological requirements are the most studied and can be easily refined if necessary. Also, at most treatment facilities in their laboratories there are specialists who analyze the quality of activated sludge, including on the basis of the composition of protozoa. We proposed an approach to assessing the impact of wastewater on rivers, for which the level of competence of these specialists is sufficient.

Our approach to assessing the impact of waste treatment facilities on the river receiver is based on the estimate of the similarity of species composition and quantitative characteristics of populations of organisms from the aerotank and from the river. It is assumed that the greater is the influence of wastewater on river, the more close they are by the composition and the amount of nutrients, oxygen content, and species composition. Species composition and abundances of organisms in treatment plants in this case is taken as a reference point. To assess the impact of water treatment facilities on the river it is necessary to study not the entire structure of the river communities but only populations of species represented in the wastewater treatment plant. Accordingly, the high similarity of the species composition of the river and wastewater treatment plant is indicative of the significant influence of wastewater and vice versa. Such an approach will also make it possible to assess how far downstream the runoff affects the river ecosystem and, accordingly, how quickly the influence of the runoff is neutralized by the river.

We studied how the ciliate populations characteristic of the aeration tank develop in the river. As an indicator of the conditions in the river, we considered the comparison of species composition, quantitative development, and biomass of protozoa from the aeration tank and from bottom

sediments in the river, as well as the saprobity index calculated on their basis.

Organisms of activated sludge exist in conditions of a fairly stable level of organic pollution and oxygen regime. Their species composition is quite limited, stable, and predictable (Madoni 1994; Serrano *et al.* 2008). Populations of the same species may exist in the river if favorable conditions are found there. A limited set of species simplifies the analysis of the situation in the river, making it less technically difficult and less time-consuming.

Under river conditions, depending on its self-cleaning potential, organic pollution can be quickly processed or in contrast accumulated, forming a plume zone. The length of this plume zone, therefore, depends on the level of energy subsidies introduced by drains, and the ability of the hydrobiocenosis to utilize these subsidies (Aristi *et al.* 2015). The species disappearance, the change in their abundance and biomass can be a reliable indicator demonstrating the resistance of the river biocenosis to the effects of organic pollution from the wastewater treatment plant.

In general, during the year, 26 species of ciliated protozoa were identified in the aerotank. Next, we monitored in the river these species from the aeration tank. The results of the number of species from a sewage treatment plant in the river, covering all seasons, at stations before and after runoff are shown in Figure 2. Changes in the number of species demonstrate three important points. Firstly, at stations above the confluence, the number of species in common with treatment plant is insignificant; the species composition of the river differs significantly from that of the aerotank. Secondly, it can be seen that the number of species from the aeration tank in the river decreases with distance from the point of wastewater inflow. Thirdly, as was shown in a previous work (Pliashechnyk *et al.* 2018), in which the entire composition of the ciliates was analyzed in this section of the river, the greatest similarity in species composition to the aero tank was observed not at the point of inflow of wastewater, but at a slight distance from it, 50 m below. Thus, the energy subsidy is mainly used at a fairly short distance downstream, and with the distance the influence of runoff is obviously reduced, which is expressed in a decrease in the number of species from the aeration tank observed in bottom sediments. If we talk about seasonal changes, it should be noted that, in spring, summer, and autumn, the number of species from the treatment plant in the river is noticeably less than in the treatment plant itself, including at the station 50 m below the drain, where conditions are most favorable for species from the aeration tank (Figure 3(a)). In winter, at a point 50 m below the
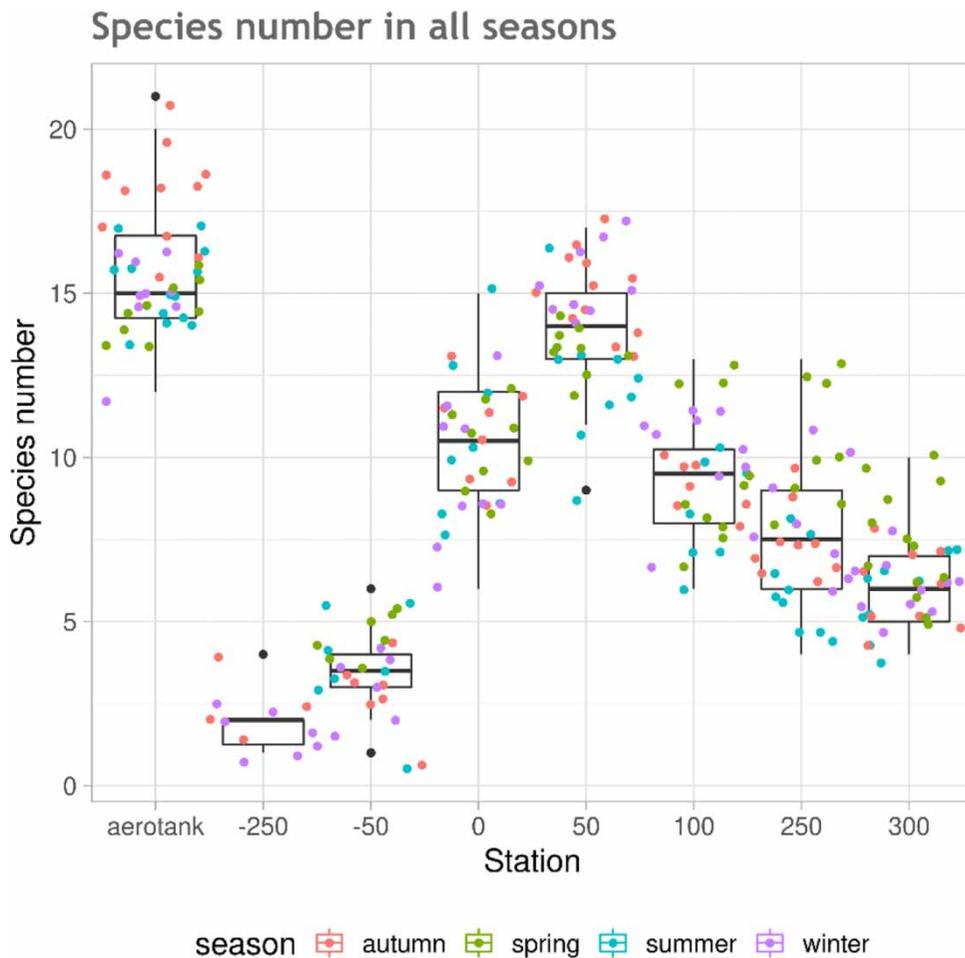
Figure 2 | Species number at stations studied on the Uzh River and at aeration tank of Uzhgorod municipal wastewater treatment plant. Numbers on the x-axis correspond to the distance from wastewater discharge point upstream (negative values) and downstream (positive values).

wastewater inflow, the composition of the aeration tank and the composition of ciliates in the bottom sediments was almost identical, and then the number of species common to the river and the aeration tank decreased more slowly than in other seasons (Figure 3(b)).

For a more complete understanding and assessment of the effect of wastewater on a water body and the ability of river biocenosis to resist the influence, it is important not only the number of common species, but also the quantitative development of populations as well as the intensity of energetic processes stimulated by the additional energy coming with wastewater. In this context, interesting results are demonstrated by the distribution of population abundance and its relationship with population abundance in the aerotank.

The species abundances from the aeration tank reached maximum in the area from the place where the wastewater inflows into the river until station 100 m below. Unlike the number of species, the abundances reach their maximum values not at a distance of 50 m, but directly at the place where the wastewater inflows. At the same time, at the station 50 m below the discharge, both the species composition and the quantitative development of aeration tank's species are as close as possible to the species composition of the aeration tank in all seasons (Figure 4).

In Figure 5(a) are shown population density data at stations in all seasons. It can be seen from the figure that the influence of the treatment plant is maximum at the place of wastewater discharge. Stable energy subsidies ensure the maximum development of protozoa. At a station 50 m lower, the situation is close to that in the active sludge of an aerotank, and downstream the conditions in the river gradually become less favorable for the development of species from the aerotank, their numbers approaching those in the river above the effluent discharge.
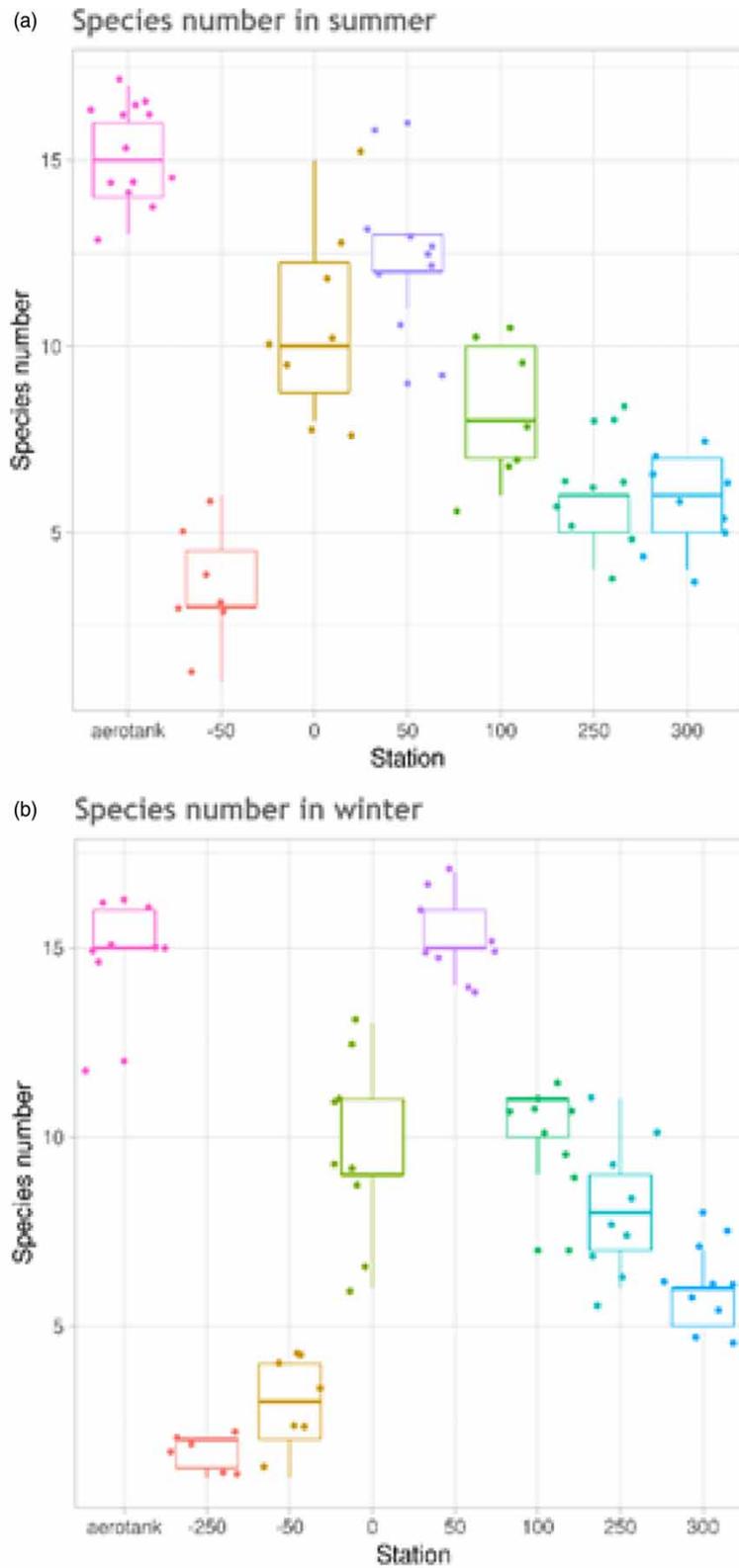
Figure 3 │ Species number at stations studied on the Uzh River and at aeration tank of Uzhgorod municipal wastewater treatment plant in summer (a) and winter (b). Numbers on the x-axis correspond to the distance from wastewater discharge point upstream (negative values) and downstream (positive values).
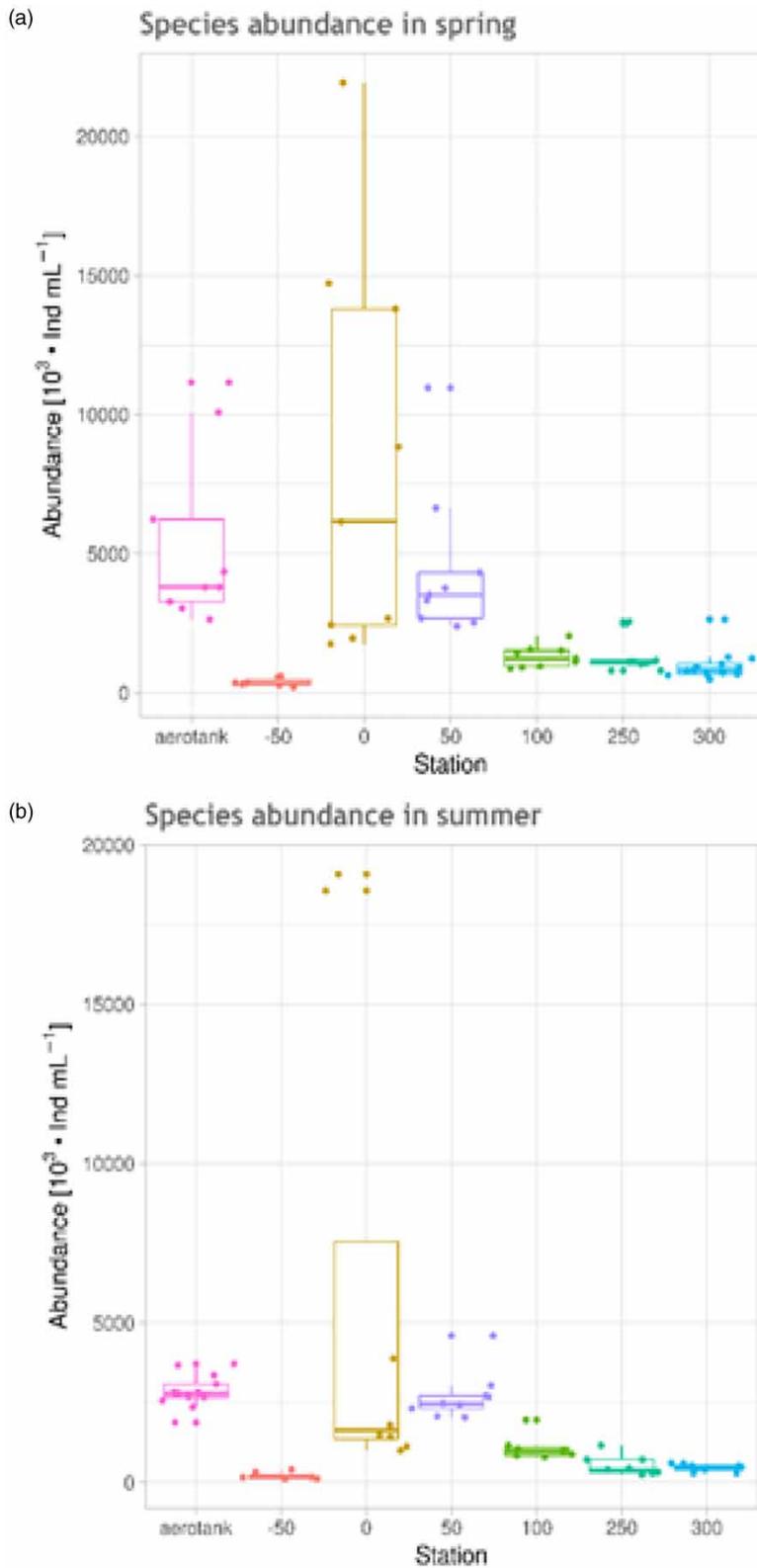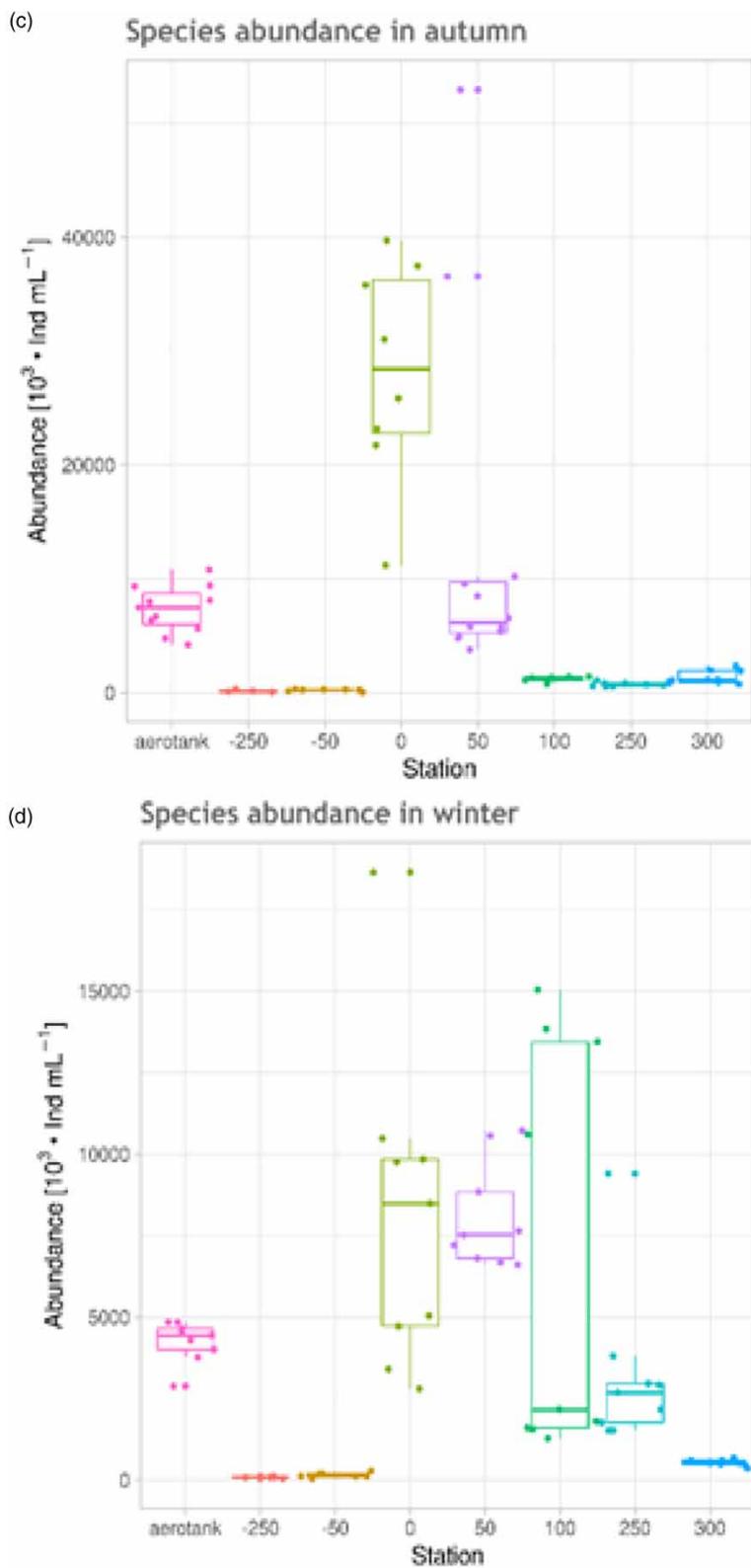
Figure 4 │ Species abundance at stations studied on the Uzh River and at aeration tank of Uzhgorod municipal wastewater treatment plants in spring (a), summer (b), autumn (c), winter (d). Numbers on the x-axis correspond to the distance from wastewater discharge point upstream (negative values) and downstream (positive values). *(continued.).*
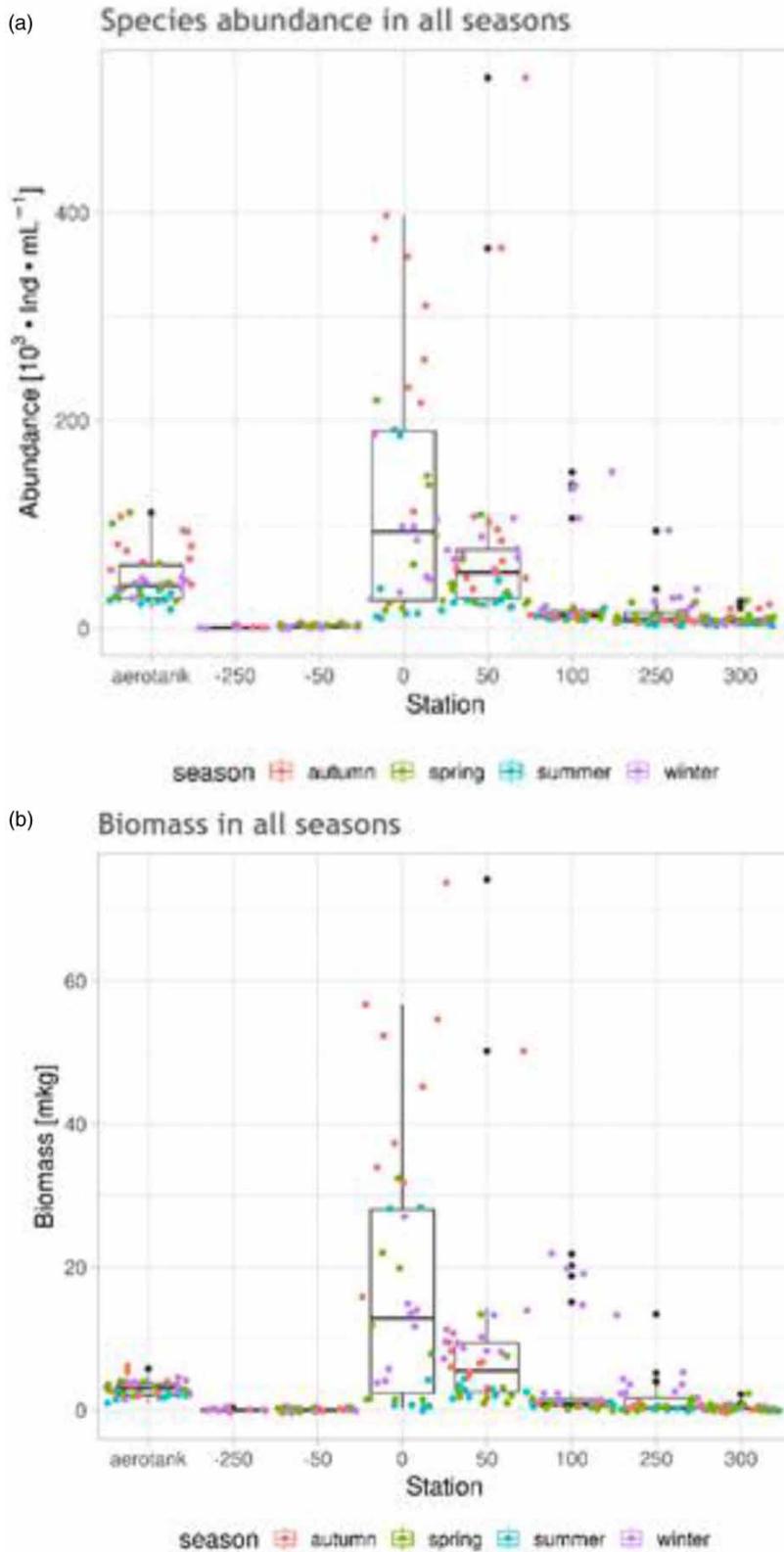
Figure 4 | Continued.

Figure 5 | Species abundances (a) and biomass (b) at stations studied on the Uzh River and at aeration tank of Uzhgorod municipal wastewater treatment plants. Numbers on the x-axis correspond to the distance from wastewater discharge point upstream (negative values) and downstream (positive values).

(a)

(b)

Figure 6 │ Saprobity (Pantle–Buck index) at stations studied on the Uzh River and at aeration tank of Uzhgorod municipal wastewater treatment plants. (a) Calculated on the basis of aeration tank species; (b) calculated on the basis of all occurred species. Numbers on the x-axis correspond to the distance from wastewater discharge point upstream (negative values) and downstream (positive values).
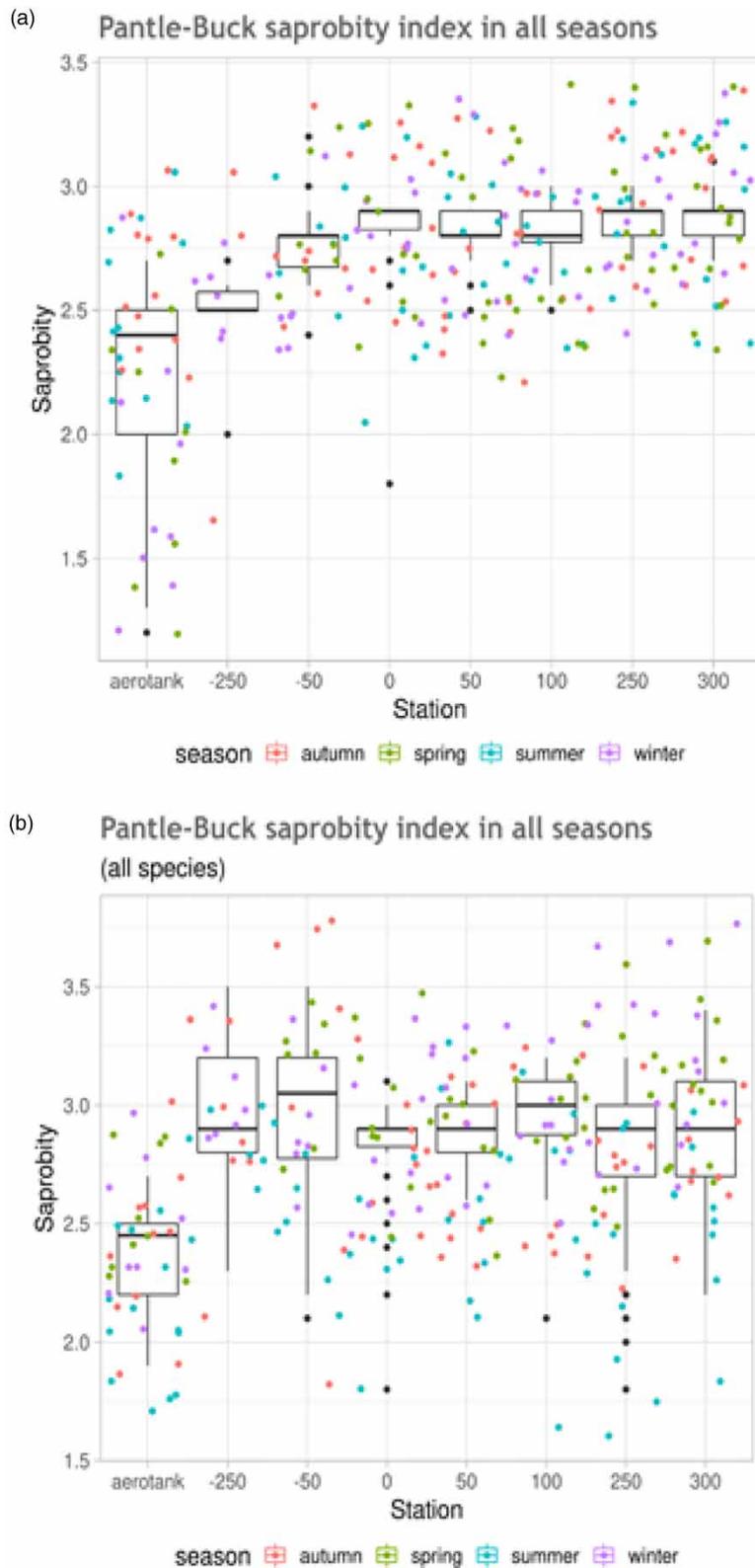
Thus, the Uzh River efficiently utilizes incoming organic matter, coping with the influence of effluents already at the first hundred meters downstream (Figure 5(a)).

For a more complete understanding and assessment of the effect of effluents, the total number of species is not enough; their biomass must be taken into account. Biomass most accurately shows the presence or absence of energy subsidies. In this case, changes in biomass and abundances of protozoa go in parallel. As can be seen from Figure 5(b), the biomass both at the station at the place of discharge and at 50 m downstream significantly exceeds not only the biomass of protozoa at stations above the runoff and the rest of the stations, but also on average significantly exceeds the biomass of the ciliated protozoa of activated sludge in conditions of aeration tank. Both the abundance and the biomass of protozoa in the activated sludge and at a station 50 m downstream are close; moreover, at the 50 m point values are even greater than at the point of effluent discharge. Thus, the energy subsidy from the wastewater treatment plant leads to a significant increase in the abundance and biomass of protozoa in bottom sediments at the discharge point. Further, downstream, the abundance and biomass values naturally decrease, approaching the level before the discharge.

Further, determining the level of saprobity is important for assessing the effect of effluents on the river, since saprobity and trophicity are different indicators (Madoni Zangrossi 2005). Calculation of the saprobity Pantle–Buck index based on species from the aeration tank made it possible to estimate the effect of wastewater discharge in a slightly different way. In Figure 6(a) we see that despite the obvious restructuring of the community at the studied stations, judging by the saprobity index, the level of organic pollution in the studied section of the river remains very high. A similar result was obtained by other researchers. Thus, studies on the Lina River showed that the saprobity index upstream of Olsztyn had a value of 2.6 rising to 3 at stations downstream the city. However, these values fit into the water quality range corresponding to the α-mesosaprobic pollution zone and pollution level based on the indicator is not significant (Hul 1987). A close range of values of the saprobity index for the Parma River is given by Madoni (1993): in the sections of the river receiving effluents from the treatment facilities of the city of Parma, the saprobity index was 2.94–3.3; in sections flowing through less populated rural areas, the saprobity index was mostly in the range of 2.49–2.94.

As can be seen from Figure 6 in our studies, the amplitude of fluctuations in the saprobity index in the river was similar: from 2.5 above runoff and up to 3 below. In the vicinity of wastewater discharge and downstream, the index shows steadily high values corresponding to the α-saprobic zone. The fact that the level of saprobity in the aeration tank is lower than in the river is explained by the constant artificial aeration of activated sludge, which leads to the development of populations oriented towards a stable presence of dissolved oxygen. In contrast, in the bottom sediments of the river downstream of the effluents, the systematic intake of organic matter without artificial aeration determines the intensive oxygen consumption by the microbial community and the appearance of ciliate species characteristic of conditions with a high organic matter content and a low oxygen level.

Since one of the research objectives was to search for a simplified procedure for assessing the effect of effluents, and at the same time giving an adequate assessment of the ongoing processes, we compared the values of the saprobity index calculated on the basis of species characteristic of activated sludge with the values calculated on the basis of the traditional approach using all species having established saprobic characteristics Figure 6(b). The values of the saprobity index obtained on the basis of the species of activated sludge show a high similarity to the values calculated on the basis of all species. This gives grounds for asserting that our simplified procedure, based on the use of a set of indicator species from a treatment plant without need for determining the entire species composition of ciliated protozoa, gives quite adequate results.

Thus, the saprobity index showed that the revealed changes in the other characteristics of the assemblage of the protozoa of activated sludge we studied under river conditions are insufficient to confirm that the influence of effluents is limited to a short, mainly 50 m section of the river. It is likely that the biocenosis of the river over the years that it received energy subsidies has changed significantly and even the most remote of the sites we studied, namely 300 m downstream, is still under the influence of effluents. On the other hand, the saprobity index can be suspected of bias or insufficient subtlety of the rating scale. Nevertheless, apparently, this cannot explain everything. Since species from activated sludge were present at all stations, their numbers and abundances reflect the recovery process, its direction, but probably not the result. Apparently this is the dynamically stable state of the system below the discharge point, which is determined by the systemic flow of the effluents delivering additional energy subsidies and, apparently, this situation is quite stable due to the low river resistance in this section. On the other hand, an assessment made by us on the basis of various indicators reveals the fact that the river system dictates the conditions leading to a change in

the other measured by us quantitative indicators, but the saprobity index is too crude to respond to these changes.

These studies revealed two important points. They showed that the assessment of water quality or the resistance of hydrobiocenosis should be carried out using the largest possible set of criteria, since even those relatively few indicators that we studied led to quite different interpretations of the situation. Obviously, the level of organic pollution in the studied area corresponded to the α-saprobic zone. However, within this zone characterized by the same level of organic pollution, judging by the value of saprobity, there are clear changes in the abundances, species composition, and biomass of populations and assemblages of a set of aeration tank's ciliated protozoan species. The amplitude of fluctuations in the number of species, the density of populations and assemblies falls within the limits of the α-saprobity level of pollution and will not change the issue in essence. As an additional source of information for assessing the direction of the processes occurring in the studied area, abundances, number of species, and biomass are very valuable, since they clearly show that the level of organic pollution is objectively reduced, although the saprobity index does not capture this. However, it is obvious that in the end, given these trends, the level of organic pollution will become so low that the river downstream gradually returns to the saprobity zone that existed before the discharge.

CONCLUSIONS

The search for the most adequate and at the same time simple methods for assessing the quality of the environment is an actual area of research.

Species of ciliated protozoa from treatment plants turned out to be sensitive indicators of the interaction of river biocenosis and wastewater.

The use of qualitative and quantitative characteristics of the protozoa from the wastewater treatment plant as a criterion for assessing the quality of the environment in the area of wastewater discharge showed their representativeness and effectiveness.

The use of a limited number of species makes it possible to conduct an express assessment of the effect of effluents on receiving reservoirs for specialists working with activated sludge in the laboratories of treatment facilities.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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