

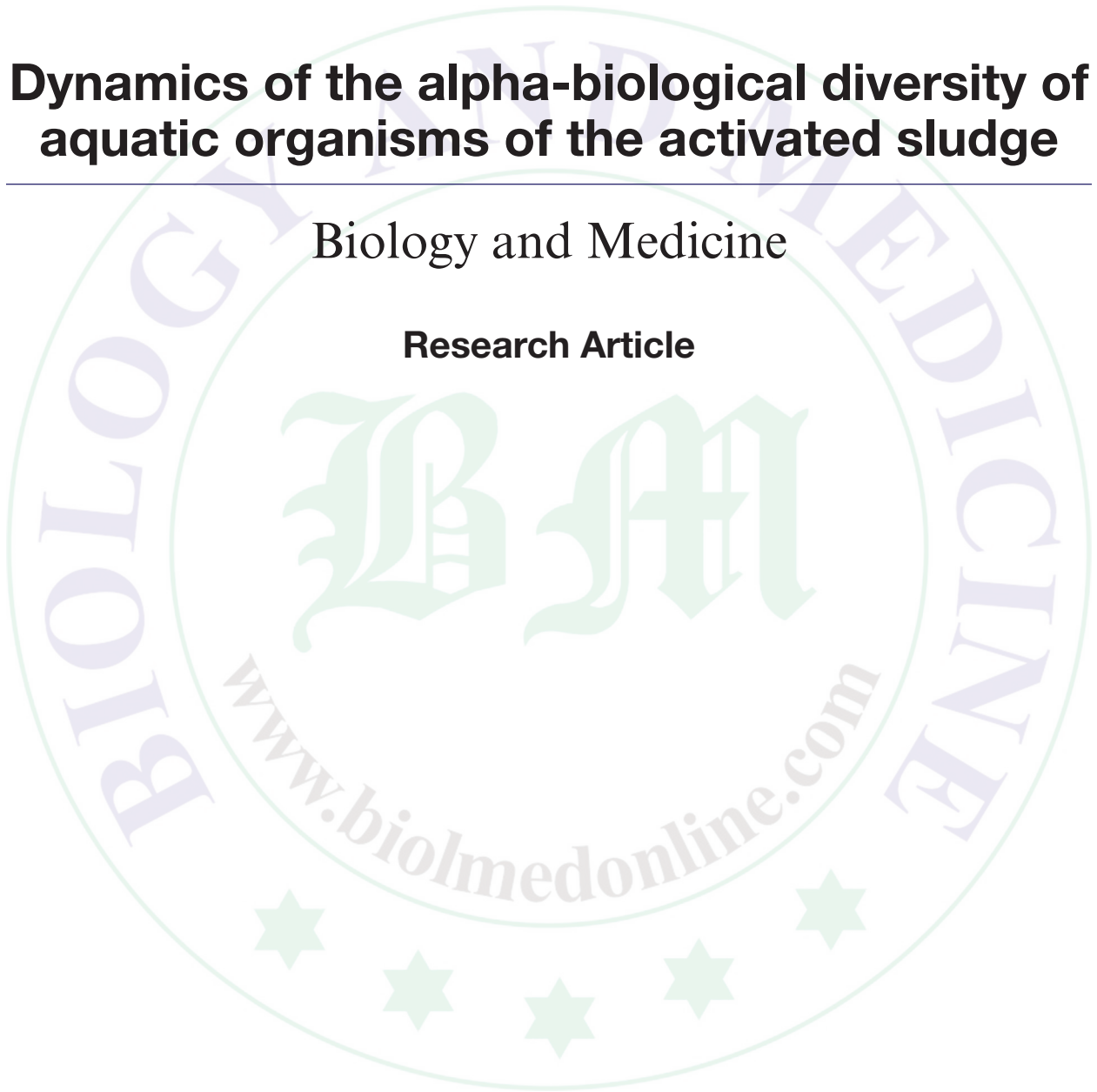
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## Dynamics of the alpha-biological diversity of aquatic organisms of the activated sludge

Leontyev Vyacheslav Vitalyevich\*

Department of Biology and Ecology, Faculty of Biology, Elabuga Institute, Kazan Federal University, Elabuga, 89 Kazanskaya Street, 423607, Russia.

\*Corresponding author

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### Abstract

The aim of this study was to analyze the dynamics of alpha-aquatic biodiversity in the activated sludge process of biological wastewater treatment. Aspects of the dynamics of alpha-diversity community aeration Elabuga District treatment facilities (northeast of Republic of Tatarstan, Russia) were considered. The high species richness of aquatic organisms of activated sludge in the continuing long-term dynamics and falling within the optimal values under the influence of industrial waste water demonstrates the effectiveness of biological treatment of sewage treatment plants

**Keywords:** Water treatment plant; activated sludge; aquatic animals; alpha diversity; species abundance; evenness.

### Introduction

At wastewater treatment plants, there are constant recurring and *ad hoc* changes in both the quality and quantity of treated wastewater and the treatment mode. The structure and population of the community of the activated sludge adequately reflect the environmental conditions of the habitat. Cultivation of activated sludge in the confined space of aeration tanks, in the circumstances of oxygen abundance and relatively high trophic level (load of organic pollutants), and significant anthropogenic stressing (load of industrial pollutants) leads to formation of a unique community that is significantly different from that of natural ecosystems.

Without going into the constructional and technological characteristics of the whole cycle, we need to note that the biological treatment of water takes place in aeration tanks and secondary sedimentation tanks, in the process of which the slurry, colloidal, and dissolved contaminations are removed from the wastewater. Biological wastewater treatment is based on the principle of biochemical oxidation of contaminants with the biocenosis of activated sludge. Protozoa and bacteria of activated sludge as a result of their vital functions perform biochemical

oxidation of contaminants, followed by sorption, assimilation, and intracellular oxidation in the nitrification process. Nitrification is the process of oxidation with air oxygen of the ammonium nitrogen to nitrites and nitrates by nitrifying microorganisms. In the aeration tanks, the “working” circulating activated sludge mixing with the new portions of clarified wastewater forms a sludge mixture, in which the actual process of biochemical treatment takes place in the presence of atmospheric oxygen. The aerated sludge mixture is supplied from the aeration tanks to the secondary sedimentation tanks designed to separate the activated sludge from the fluid being treated. As a result of the complete biological treatment, mineralization of organic substances to carbon dioxide and water occurs [1].

Activated sludge is an amphoteric colloidal system. The elemental chemical composition of active sludge has the formula –  $C_{54}H_{212}O_{82}N_8S_7$ . The dry substance of activated sludge contains 70-90% of organic and 10-30% of inorganic materials. In addition to living organisms, sludge contains a substrate – a variety of solid residues, to which microorganisms are attached. Externally, activated sludge is lumps and flakes of 3-150  $\mu m$  in size and high surface area – about 1200  $m^2$  per 1  $m^3$  of sludge. The activated sludge

community is represented with bacteria, protozoa, molds, yeast, actinomycetes, insect larvae, crustaceans, algae, etc. Dissolved organic contaminants are removed from the wastewater by bacteria that are in a dispersed state, and the liquid clarification takes place in subsequent phases, wherein bacteria transform to the state of zoogloaeas. Bacteria are represented by such types as pseudomonas, bacillus, nitrobacter, nitrosomonas, etc. 1 m<sup>3</sup> of sludge contains  $2 \times 10^{14}$  of bacteria. Active sludge contains four groups of protozoa: sarcodic, flagellate, ciliate infusoria, and suctorians, which absorb large amounts of bacteria thus controlling their optimal number. They contribute to the deposition of sludge and clarification of wastewater in the secondary sedimentation tanks. The nitrification stage accompanied by the high concentration of oxygen dissolved in the water is always associated with the development of a large number of rotifers that feed on bacteria and protozoa. A stable system of matured good activated sludge in a normally operating aeration tank is characterized by predominance of sedentary ciliates with mandatory presence of free-floating forms. The composition of the biocenosis of sludge depends on the presence and concentration in the wastewater of various organic substances. With high content of organic substances in the wastewater, heterotrophic bacteria predominate, and while the amount of nutrients decreases, the number of predatory protozoa grows [2].

The ratio of the number of species and population of the systems of aquatic organisms in the activated sludge, their dynamics are an indication of the state of well-being of this ecosystem, and subsequently, the quality of wastewater treatment. Therefore, the aim of our study was to analyze the dynamics of alpha-biological diversity of aquatic organisms in the activated sludge in the process of biological wastewater treatment.

## Methods

The study of the composition and population of aquatic organisms was carried out in the chemical and bacteriological laboratory of the Elabuga district treatment facilities (DTF) at the premises of the Municipal Unitary Enterprise "Elabuzhskij Vodokanal" between 2009 and 2011. The capacity of the DTF is 37,000 m<sup>3</sup>/day. The DTF are designed for full biological treatment of domestic and industrial wastewater from: the residential

development of Elabuga; the household sewage and industrial sewage of enterprises of Elabuga; the industrial sites of the free trade zone. The city of Elabuga is the seventh largest city by population (71,991 people in 2014) in the Republic of Tatarstan of the Russian Federation – a major center of industry in the territory of the "Alabuga" free trade zone (FTZ) of industrial type. The city is located on the right bank of the Kama River, in the southern Taiga subzone (the boreal landscape zone). The climate is relatively wet and cool in summer and moderately cold and snowy in winter (the Prekamsky climatic district). The annual rainfall is 540 mm or more [3].

The DTF include a mechanical wastewater treatment plant; a biological treatment plant; a chlorination plant; a blowing station; a raw sludge and excess activated sludge processing plant; a plant for dewatering sand and stabilized sludge in natural conditions; a storage pond; a household drinking water borehole facility. The product of sewage treatment facilities is the biologically purified and disinfected wastewater, which was discharged into the Kama River.

The samples for analysis were collected on a monthly basis every 1-2 days.

For the study of the microfauna of the activated sludge, the method of "live" drops under a cover glass was used. To determine the types and to count the zooids in each sample, we used counting chambers, a light microscope, and a stereoscope. Identification of the species of aquatic organisms was performed using the determinant of zooplankton and zoobenthos; determinants authored by LA Kutikova [4]; YA Mazey, AN Tsyganov [5].

As a measure of safety assessment of the alpha-diversity of the zooplankton community, we used the Shannon index ( $H'$ ) [6-8], which reflects the abundance of species. The index may rise from 1.5 to 3.5 (very rarely exceed 4.5) due to the increasing number of species, or better evenness of the proportions due to the reducing number of the super predominant, or the growth of the middle-ranking species [9]. To evaluate the numerical ratio of species, we determined in conjugation the index of evenness (heterogeneity) of EC Pielou ( $E$ ) [10], which varies from 0 to 1.  $E = 1$  with equal population of all taxa. The strengthening dominance reduces evenness and diversity. The species diversity may increase despite the increasing dominance if it is connected with the appearance of additional small-number species. The evenness index has the stronger dependence on the number of species.

To test the significance of differences between the sample totals of the values  $H'$ , we used the Student's  $t$ -test, for which we preliminarily calculated the Shannon's index of dispersion ( $V_H$ ) and the number of degrees of freedom ( $df$ ).

## Results and Discussion

In municipal wastewater treatment plants, usually there is no separated treatment of storm, domestic, and industrial wastewater. The main risk to the activated sludge biocenosis is the industrial wastewater containing toxic compounds. Its supply in large quantity and during a long period causes decrease in the dissolved oxygen concentration in the biological system. Activated sludge in comparison with biological biocenoses is much more susceptible to human impacts than to the impact of internal development. The main population of a settled biocenosis in aeration tanks is formed by the species

adapted to the strong regular exposure to toxic agents. Their species composition and abundance ratio may vary somewhat in a volley of discharges to the treatment system and in different seasons of the year. The summer biocenosis of activated sludge in the species composition is somewhat richer than the winter one. However, in large wastewater treatment plants, in terms of hot water supply (25°C), the seasonal changes are less noticeable. The composition of identified species of the main groups of aquatic organisms gives reasons to assess the quality of the effective activated sludge and its treatment capacity.

The main part of the population of the activated sludge biocenosis of the Elabuga DTF contained 37 species, the number and the size of which varied slightly throughout the seasons of the year. Below, we provide the list of species with indication of their belonging only to the major taxonomic categories (Table 1). Here, we do not give any lists of species of algae and fungi.

**Table 1: The species composition of the activated sludge biocenosis of the Elabuga DTF in 2009-2011.**

Seq. No.	Taxon	Trophic group	Habitat
	Phylum Euglenozoa		
	Class Kinetoplastea		
1	<i>Bodo saltans</i> (Ehrenberg, 1838)	P	Bt
	Class Euglenoidea		
2	<i>Euglena gracilis</i> (Klebs, 1883)	M	PI
	Phylum Amoebazoa; Subphylum Lobosa		
	Class Archamoebae		
3	<i>Breviata anathema</i> (G. Walker et al., 2006) (= <i>Mastigamoeba invertens</i> (Klebs, 1893))	P	Bt
	Class Tubulinea		
4	<i>Arcella vulgaris</i> (Ehrenberg, 1830)	P	Bt
5	<i>Centropyxis aculeate</i> (Ehrenberg) (Stein, 1857)	P	Bt
6	<i>Dactylosphaerium polypodium</i> (Ehrenberg)	P	Bt
7	<i>Euglypha ciliate</i> (Ehrenberg) (Leidy, 1879)	P	Bt
8	<i>Saccamoeba limax</i> (Page, 1969)	P	Bt
	Phylum-group Alveolata; Phylum Ciliophora		
	Subphylum Ciliata		
	Class Kinetophragminophora		
9	<i>Amphileptus anser</i> (Ehrenberg, 1838)	Pr	PI, Bt
10	<i>A. claparedei</i> (Stein, 1867)	Pr	PI, Bt
11	<i>Chilodonella uncinata</i> (Ehrenberg, 1835; Kahl, 1931)	B	Bt
12	<i>Colpoda steini</i> (Maupas, 1883)	B-D	Bt
13	<i>Litonotus lamella</i> (Schewiakoff, 1896)	Pr	Pf, Bt
	Class Polihymenophora		
14	<i>Aspidisca costata</i> (Dujardin, 1842)	B	Pf, Bt
15	<i>A. turrida</i> (Ehrenberg, 1838)	B	Pf, Bt

(Continued)

Table 1: (Continued)

Seq. No.	Taxon	Trophic group	Habitat
16	<i>Euplotes affinis</i> (Dujardin, 1842)	B-D	Bt
17	<i>Oxytricha fallax</i> (Stein, 1859)	B	Pl, Bt
18	<i>Stentor roeseli</i> (Ehrenberg, 1835)	B, P	Bt
	Class Peritricha		
19	<i>Epistylis thienemanni</i> (Nenninger, 1948)	B	Pf
20	<i>Opercularia coarctata</i> (Claparede et Lachmann, 1858)	B	Pf
21	<i>Vorticella alba</i> (Fromentel, 1874)	B-D	Pf
22	<i>V. convollaria</i> (Linnaeus, 1757)	B-D	Pf
23	<i>V. microstoma</i> (Ehrenberg, 1830)	B-D	Pf
	Class Suctorea		
24	<i>Podophrya carchesii</i> (Claparede et Lachmann, 1859)	Pr	Pf
25	<i>P. fixa</i> (Quennerstedt, 1867)	Pr	Pf
26	<i>Tokophrya mollis</i> (Bütschli, 1889)	Pr	Pf
	Superphylum Platyzoa; Phylum Rotifera		
	Class Bdelloida		
27	<i>Macrotrachela (Callidina sp.) sp.</i>	B	
28	<i>Philodina roseola</i> (Ehrenberg, 1832)	B	Bt, Pf
29	<i>Rotaria rotatoria</i> (Pallas, 1766)	B	Bt
	Class Monogononta		
30	<i>Cephalodella auriculata</i> (Müller, 1773)	B	Bt, Pf
31	<i>Colurella uncinata</i> (Müller, 1773)	B	Bt, Pf
32	<i>Lepadella ovalis</i> (Müller, 1786)	B	Bt, Pf
33	<i>Monostyla (Lecane) arcuata</i> (Bryce, 1891)	B	Bt, Pf
34	<i>Notommata cyrtopus</i> (Gosse, 1886)	B	Bt, Pf
35	<i>Pleurotrocha petromyzon</i> (Ehrenberg, 1830)	Pz	Bt, Pf
36	<i>Proales daphnicola</i> (Thompson, 1892)	B	Bt, Pf
	Phylum-group Coelomata; Phylum Annelida		
	Class Oligochaeta		
37	<i>Aeolosoma hemprichi</i> (Ehrenberg, 1828)	B-D	Bt

Note: B – bacteriophage, B-D – bacteriodetritiphage, M – mixotrophe, P – polyphage, Pr – predator, Pz – parasite; Bt – bentos, Pl – plankton, Pf – periphyton.

Ciliates are the most valuable indicators of the state of activated sludge and the extent of water pollution in the biological wastewater treatment facilities [11-15]. These protozoa as the main consumers of bacteria constantly stimulate the renewal of the bacterial mass and maintain its high biological activity, which is very important for maintaining the treatment process. Ciliates also clean purified water, absorb pathogenic bacteria in the wastewater, and contribute to the formation of sludge flakes. They are both orderlies and biological stimulants of the activated sludge's activity. Attached ciliates are sensitive to oxygen deficiency, overload, poor mixing of the sludge mixture, and the effects of toxicants, and are indicators of high quality treatment.

A similar function is also performed by rotifers. Rotifers have broad ecological plasticity;

some species are resistant to sudden fluctuations in pH and are usually sensitive to oxygen deficiency. Predatory rotifers characterize high quality of treatment, developed nitrification process, good aeration conditions, and satisfactory mineralization of sludge. Oligochaeta are the indicators of deep treatment with nitrification.

The presence of certain species in the activated sludge biocenosis describes its status and quality of water treatment.

Revealed quantitative predominance of attached ciliates (*Epistylis*, *Opercularia*, and *Vorticella*), occurrence of hypotrichs and predatory ciliates (*Litonotus*, *Amphileptus*), suctorians (*Tokophria*, *Podophria*), testate amoebae (*Arcella* and *Centropyxis*), the constant presence of rotifers, the presence of significant quantities of Oligochaeta of the *Aeolosoma* kind is an indicator

of the process of nitrification of activated sludge, when the main part of the organic substrate has already mineralized. The formation of biocenosis of nitrifying sludge indicates not only significant mineralization of organic substances and mineral content of the excess nitrogen ( $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ) but also sufficient oxygen content in the system (4.0-6.0 mg/l).

Periodically, the load on the operation of the aeration tank increased to medium levels. With an average load on the sludge, intensive redox processes occur, aerobic degradation of organic substances takes place, and ammonia and carbon dioxide are produced. The oxygen content is reduced to 2.0 mg/l; no hydrogen sulfide is present. The presence in the substrate of sufficient quantities of organic substances and mineralized products of their aerobic oxidation creates good conditions for forming a community with a wide variety of aquatic organisms with little quantitative dominance of a certain species.

In such activated sludge, both saprozoites (bacteria, fungi, and flagellates) and holozoic organisms (bacteriophages and predators – free-floating and attached infusoria) are present. The number of free bacteria is not large due to the large number of bacteriophages, which determines the transparency of the treated wastewater.

In general, during the years of observation, the permanent aquatic species composition has been recorded, as shown in Table 1, although some species disappeared and reappeared in different seasons. Annual and monthly fluctuations in the species composition of biocenosis of activated sludge and the ratios of the individual species are listed in Table 2 in the form of composite indices of species abundance and evenness.

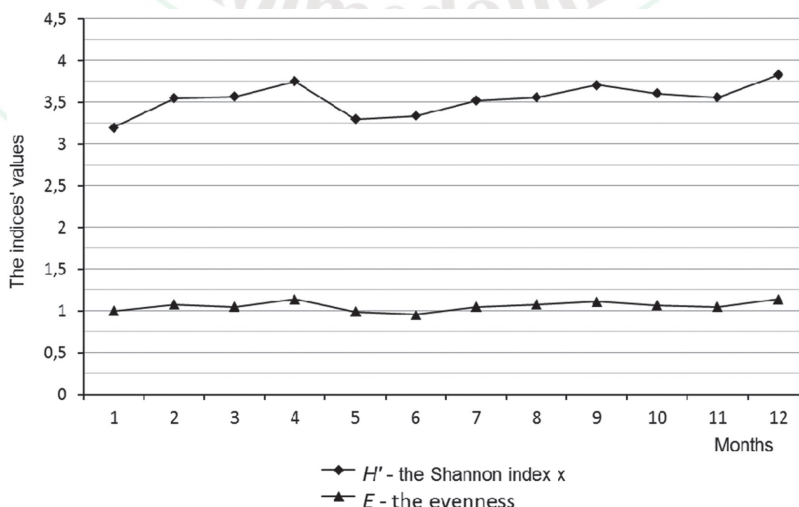
No regular seasonal fluctuations of the indices values have been identified, as in aeration tanks throughout the year a stable temperature of 25°C is maintained. It is obvious that a slight decrease in species abundance is associated

**Table 2: Dynamics of  $\alpha$ -biological diversity of aquatic organisms of the activated sludge in the Elabuga DTF.**

Years	Indices	Months												Average
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
2009	$H'$	3.49	3.63	3.56	3.78	3.19	3.21	3.39	3.55	3.61	3.49	3.56	3.82	3.52
	$E$	1.16	1.17	1.10	1.24	0.99	0.95	1.00	1.12	1.09	1.01	1.07	1.15	1.09
2010	$H'$	3.12	3.49	3.62	3.71	3.43	3.37	3.72	3.50	3.65	3.76	3.48	3.83	3.56
	$E$	0.96	1.03	1.06	1.07	1.00	0.97	1.13	1.07	1.08	1.14	1.02	1.11	1.05
2011	$H'$	3.00	3.53	3.52	3.76	3.27	3.43	3.45	3.64	3.87	3.59	3.64	3.85	3.54
	$E$	0.88	1.04	0.98	1.11	0.99	0.97	1.03	1.05	1.16	1.05	1.06	1.16	1.04

Note:  $H'$  – the values of the Shannon index,  $E$  – the evenness.

**Figure 1: The average annual dynamics of species diversity of aquatic organisms of  $\blacktriangle$   $H'$  – the Shannon index and  $\blacklozenge$   $E$  – the evenness.**



with the influence of wastewater, primarily from enterprises. But despite the increase in industrial production and significant deterioration of the equipment of DTF, the biological treatment technology manages to cope with the increasing load and is able to treat the wastewater to the required level. Right now in the territory of the "Alabuga" free trade zone of industrial type, new sewage treatment plants are being constructed, which will reduce the load on the Elabuga DTF.

Average long-term monthly indices of species abundance and evenness are shown in Figure 1. The values of the latter remain near 1, thus indicating an even proportion of the population of flat majority of aquatic species. The main reason for consistently high species diversity of the activated sludge community is to fill the space with the species capable of rapid population growth in a regular volley of chemical poisoning. As a result of stress impact on the entire association, the diversity increases and dominance decreases, as potential dominants are not able to increase their population and all members of the association in these conditions have equal opportunities.

## Conclusions

Summarizing the above, we can note the following:

1. The fauna of the activated sludge biocenosis of the Elabuga DTF was represented by 37 kinds of aquatic organisms: 2 kinds of flagellates, 6 kinds of amoebas, 18 kinds of ciliates, 10 kinds of rotifers, and 1 kind of annelids.
2. The parameters of  $\alpha$ -biological diversity of the aquatic organisms reflect the high species abundance of the biocenosis and stable annual dynamics, slightly reducing within the optimum level at volleys of wastewater discharges.
3. The rich diversity of aquatic organisms of activated sludge evidences the welfare of the biological system of the aeration tank, the high efficiency of treatment, and the sustainability of the community to the damaging effects of toxic wastewater.

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