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No. 1

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# MICROFAUNA COMPOSITION OF ACTIVATED SLUDGE IN DOMESTIC AND INDUSTRIAL SEWAGE ACTIVATED SLUDGE SYSTEMS

The microfauna in activated sludge deriving from two different wastewater treatment plants has been compared. Investigations were conducted during the period of seven months taking into account the seasonal changes of microfauna. One studied plant received mainly industrial (70%) and some part of domestic (30%) sewage, and the other mainly domestic sewage (90%). The aim was to compare both plants in terms of occurrence and abundance of key group of microfauna. The determination of key groups and taxa along with biodiversity indices showed that they have potential value as indicators of different conditions characteristic for both types of plants. The innovative using of biotic indices in biodiversity studies also provides useful information on plant operating conditions and performance.

#### 1. INTRODUCTION

Wastewater is a mixture of all types of used liquids, colloids and suspended solids, which due to their sources may be classified into main groups: domestic, industrial, agricultural, rainwater, infiltration and industrial cooling waste water. Their biological treatment consists of mineralization of organic matter, removal of biogenic substances and elimination of pathogenic microorganisms [1]. The most common and important method of biological wastewater treatment is activated sludge process. This process uses air and a biological flocks composed of bacteria, protozoans and metazoans. Flock is the basic structural unit of sludge. On its surface, organic matter adsorption occurs. After adsorption on the flock surface, compounds present in wastewater are decomposed and then can be absorbed by the microorganisms. Some of them are used for the growth of biomass [2]. The most important component of flocks are bacteria which execute biological oxidation of organic substrates, nitrification of ammonia, denitrification

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of nitrates and accumulation of phosphorous. Their composition and condition display performance of wastewater treatment plant [3].

Protozoa feed on bacteria and dissolved substances. Their abundance reflects the presence of certain bacteria, condition of sludge and its composition. Hence, their presence, or changes of diversity ratio imply the effectiveness of wastewater treatment plant (WWTP), or signalize activated sludge malfunctioning [3].

The aim of the study was the comparison of performance of two WWTP in Lower Silesia differing in types of sludge received by the analysis of composition of microbial community and flocks structure of activated sludge.

### 2. MATERIALS AND METHODS

*Description of wastewater plants.* WWTP in Wołów is relatively new and was modernized in 2005–2006 because of the planned increase of wastewater load. The WWTP uses two stages of the treatment process, with mechanical and biological blocks. The plant receives domestic wastewater from Wołów (population of 12 000), additionally wastewater is delivered from the town and commune by vacuum trucks. At the time of dry weather, the plant receives hydraulic load of 4000 m<sup>3</sup>/day, which is a mixture of municipal, infiltration and random wastewater. In municipal wastewater, about 400 m<sup>3</sup>/day comes from denim processing industry, 320 m<sup>3</sup>/day from penitentiary, and 10 m<sup>3</sup>/day from both: motor transport and manufacturing industries. At the rainy weather, the plant receives load of 7366 m<sup>3</sup>/day. Mean sludge age for the plant is 15 days.

Table 1

Index	Wastewater inflow	Wastewater outflow	Limit value
BOD5,	185.0	4.0	15.0
COD	463.0	24.0	125.0
Total suspended solids	220.0	7.0	35.0
Total nitrogen	45.0	10.6	15.0
Total phosphorus	7.1	0.2	2.0

Parameters of wastewater entering and leaving Wołów WWTP [mg/dm3]

There are two non-standard technological solutions in Wołów WWTP: each of three chambers of the biological block is aerated, thus risk of nitrification breakdown is reduced (especially in winter when the temperature is low). Aeration of the entire biological block allows simultaneous nitrification and denitrification in all active sludge stages. The second non-standard solution in the wastewater treatment technology of Wołów facility is the lack of a primary sedimentation tank (low value of total suspended solids). The parameters of wastewater entering and leaving Wołów WWTP are shown in Table 1.

*Brzeg Dolny WWTP*. Because of the dynamic development of the PCC Rokita SA, the Central WWTP at Rokita Chemical Plant in Brzeg Dolny is constantly undergoing modernization and rapid expansion. It is a three-stage wastewater treatment plant based on physical, chemical and biological processes such as sedimentation, mixing, flotation, averaging, coagulation, filtration, and biological processes. It receives municipal wastewater from the PCC Rokita and communal wastewater from Brzeg Dolny. The average daily hydraulic load of sewage entering the facility is 15 000 m<sup>3</sup>/day. Concentrations of pollutants and the amount of sewage entering the treatment plant fluctuate significantly. Raw sewage contains a number of specific impurities that are hardly biodegradable or toxic. The parameters of wastewater entering Brzeg Dolny WWTP are shown in Table 2.

#### Table 2

Index	Param ei	eters of wa ntering WW	stewater VTP	Mean daily parameters of wastewater entering the biological block			
	Mean	Minimal	Maximal	Minimal	Maximal		
Volume, m <sup>3</sup> /day	15 000	11 540	20 820		-		
pH	_	2.1	12.2	8.5	10.5		
COD, mg/dm <sup>3</sup>	700	270	4 100	104	895		
Phenols, mg/dm <sup>3</sup>	-	0.16	200	0.09	32		
Chlorides, mg/dm <sup>3</sup>	5 657	1 518	13 530	2 046	8 250		
Total suspended solids, mg/dm <sup>3</sup>	298	60	2 200	40	280		
Sulfates, mg/dm <sup>3</sup>	_	_	_	248	924		
Total dissolved substances, mg/dm <sup>3</sup>	-	-	-	5 121	16 704		

Parameters of wastewater entering Brzeg Dolny WWTP

Sampling and examination. Samples of activated sludge were collected once a month from August to March (excluding December), in the mornings of subsequent days. For the credibility of the results, samples were collected directly from aeration chamber (well mixed and oxygenated) at the same place (at middle-length of the aeration chamber).

For microscopic examination, activated sludge was taken always fresh, thus some of its properties such as composition and proportion of species would be independent of time because of altered trophic and physical conditions, especially in the case of very loaded wastes. The bottles for specimens were filled two-thirds with sludge and delivered in the same day to the laboratory to avoid oxygen deficiency and temperature changes. Observations were performed under the microscope Olympus CX31.

Three biodiversity indices such as the Shannon–Wiener diversity index (H'), Simpson index (D) and Margalef index (d) [4] have been employed:

• Shannon–Wiener diversity index

$$\bar{H}' = -\sum \frac{N_i}{N} \log_2 \frac{N_i}{N}$$

Simpson index

$$\overline{D} = 1 - \frac{N_i(N_i - 1)}{N(N - 1)}$$

Margalef index

$$\overline{d} = \frac{S-1}{\log_e N}$$

where: S – number of all species, N – number of all individuals,  $n_i$  – number of individuals of i species.

#### 3. RESULTS

#### 3.1. COMPOSITION OF ACTIVATED SLUDGE ORGANISMS FROM THE STUDIED WASTEWATER PLANTS

In Wołów WWTP, during the studied period of 7 months, 42 taxa of ciliates were identified and few other higher invertebrates were also recorded (rotifers, nematodes and tartigrades) and flagellates <20 µm as well. We found altogether 30 species of Ciliata, including 5 species of free-swimming ciliates, 7 of crawling ciliates and 17 of attached ciliates, 4 species of naked amoebas and 3 testate amoebas. The dominant group of Protozoa was attached ciliates (42%), then accordingly: crawling ciliates (17%), free swimming ciliates (12%), naked amoebas (10%), testate amoebas (7%). We recorded presence of flagellates, rotifers, nematodes and tardigrades as well (Table 3). Among ciliates, the most frequent were: *Litonotus lamella* (100% of samples), *Vorticella convallaria* (100% of samples), *Vorticella microstoma* (100% of samples), *Vorticella striata* (100% of samples), *Arcella vulgaris* (100% of samples), *Pyxidicula* sp. (100% of samples), *Aspidisca cicada* (87% of samples) and *Epistylis coronata* (87% of samples).

Activated sludge of Brzeg Dolny WWTP was dominated by ciliates: 9 genera of attached ciliates, 3 of crawling ciliates and 2 of free swimming ciliates which constituted: 53%, 19% and 13% of total sludge organisms accordingly, with predomination

# of *Epistylis coronata* (100% of samples), *Vorticella microstoma* (100% of samples) and *Opercularia minima* (100% of samples). Other taxa consisted of small (<20 $\mu$ m) flagellates (Table 4).

#### Table 3

		Mon	thly al	oundan	ce of s	tudied	organi	sms
No.	Organisms/group	in Wo	łów W	WTP	activat	ed sluc	lge [sp	/cm <sup>3</sup> ]
		VIII	IX	Х	XI	Ι	II	III
	Free-sv	vimmin	g cilia	tes		1		
1	Colpidium sp.	_	20	50	20	<10	_	_
2	Litonotus lamella	20	20	40	70	<10	30	<10
3	Plagiocampa rouxi	<10	<10	_	<10	_	_	20
4	Prorodon teres	_	20	<10	20	_	_	40
5	Pseudocohnilem–bus pusillus	40	_	20	20	-	_	60
	Crav	vling c	iliates					
6	Aspidisca cicada	320	260	280	120	20	-	40
7	Aspidisca lynceus	80	60	60	-	-	-	20
8	Chilodonella cucullulus	20	<10	<10	-	_	-	20
9	Chilodonella uncinata	80	20	30	60	-	<10	<10
10	Euplotes affinis	50	40	70			30	20
11	Euplotes moebiusi	١	١	<10	1	-	<10	I
12	Trachelophyllum pusillum	30	<10	30	40	<10	<10	<10
	Atta	iched ci	iliates					
13	Acineta tuberosa	<10	١	20	I	-	١	I
14	Carchesium polypinum	80	80	60	I	-	١	<10
16	Epistylis chrysemydis	I	1	1		20	1	
17	Epistylis coronata	80	20	30	140	20	<10	I
18	Opercularia articulata	<10	<10	I	I	-	20	<10
19	Opercularia minima	1	20	20	90	-	<10	<10
20	Sphaerophrya magna	-	20	<10	40	-	-	-
21	Tokophrya infusionum	_	-	<10	<10	_	20	-
22	Tokophrya lemnarum	1	I	<10	<10	<10	20	<10
23	Vorticella alba	<10	20	<10	30	<10	<10	30
24	Vorticella aquadulcis	20	<10	I	40	<10	١	<10
25	Vorticella campanula	<10	1	20	20	20	20	30
26	Vorticella communis	60	<10	<10	20	-	١	I
27	Vorticella convallaria	140	160	130	140	220	180	160
28	Vorticella microstoma	180	160	210	220	120	140	160
29	Vorticella similis	١	١	<10	<10	20	20	<10
30	Vorticella striata	40	20	60	40	80	20	140
	F	lagella	tes					
31	Small <20 µm	<10	40	50	30	40	20	20
32	Large >20 μm	40	20	60	40	20	<10	<10

Composition of microfauna of activated sludge in Wołów WWTP

## Table 3

	Nal	ked am	oebas					
33	Amoeba albida	-	<10	50	80		30	60
34	Amoeba gattula	<10	-	20	30	30	<10	30
35	Ameba verrucosa		<10	20	60	40	120	100
36	Cochlipodium granulatum	60	160	180	190	310	220	120
	Tes	tate am	oebas					
37	Arcella vulgaris	220	80	170	120	100	140	120
38	Euglypha tuberculata	80	240	450	320	80	40	40
39	Pyxidicula sp.	840	400	210	360	120	240	220
	Multicel	lular in	vertebr	ates				
40	Rotifers	20	20	40	20	20	<10	
41	Nematoda	_	_	<10	-	_	<10	-
42	Tardigrada		-	<10	-			

# Composition of microfauna of activated sludge in Wołów WWTP

Table 4

Microfauna	composition	of activated	sludge in	Brzeg Dolny V	WWTP
	1		0	8 ,	

		М	onthly	abunda	nce of	studied o	organism	s		
No.	Organisms/group	in Brze	gDolny	's WW	TP act	ivated sl	udge, [sp	o/cm <sup>3</sup> ]		
		VIII	IX	Х	XI	Ι	II	III		
	Fr	ee–swin	nming c	iliates						
1.	Plagiocampa rouxi	220	-	-	-	-	<10	60		
2.	Urotricha armata	20	-	-	—		_			
		Crawlin	ng cilia	tes						
3.	Chilodonella uncinata	-	-	-	-	-	160	40		
4.	Drepanomonas rezoluta	90	<10	-	—		_			
5.	Trachelophyllum pusillum	30	<10	-	—		120	١		
		Attache	Attached ciliates							
6.	Acineta tuberosa	40	<10	-	-	-	<10	<10		
7.	Epistylis coronata	150	180	130	170	210	60	450		
8.	Opercularia minima	220	480	540	330	840	180	520		
9.	Tokophryainfusionum	30	<10	-	-	-	20	20		
10.	Vorticella alba	-	-	<10	<10	1	_			
11.	Vorticella campanula	80	20	_	<10	<10	60	80		
12.	Vorticella convallaria	160	-	-	—	<10	130	80		
13.	Vorticella microstoma	250	120	40	20	40	80	180		
14.	Vorticella striata	_	_	20	<10	<10	_	60		
		Flag	ellates							
15.	Small <20 µm	1000	x	$\infty$	x	1020	1000	950		
16.	Large >20 µm	_	_	_	_	_	_	_		

#### 3.2. ABUNDANCE OF SELECTED ORGANISMS

All representatives of crawling ciliates in activated sludge of Wołów WWTP were much more abundant compared to Brzeg Dolny WWTP. In Wołów, crawling ciliates were also more diversified in taxa and their concentration was highest from August to October. Only in March concentration of crawling ciliates in Brzeg Dolny WWTP was higher than that in Wołów (Fig. 1).



Fig. 1 Abundance of crawling ciliates in both studied plants

Free-swimming ciliates in Wołów WWTP were also more abundant and diversified compared to Brzeg Dolny (with exception of August) (Fig. 2).



Fig. 2. Abundance of free-swimming ciliates in both studied plants.

Attached ciliates in Wołów were less abundant in comparison to those in Brzeg Dolny but they were the most qualitatively diversified group of organisms during this study (17 species) (Fig. 3).



Fig. 3. Abundance of attached ciliates in both studied plants

Testate and naked amoebas were present only in Wołów WWTP. August–November was the period of highest concentration for testate amoebas (1140 specimens/cm<sup>3</sup> in August), while the highest concentration for naked amoebas occurred between November and February (380 specimens/cm<sup>3</sup> in January) (Tables 3, 4). Small flagellates (< 20  $\mu$ m) were the most abundant group in activated sludge of Brzeg Dolny WWTP. Between September and November its concentration was too high to evaluate, while large flagellates were not found, they occurred only in Wołów WWTP (Tables 3-4). Multicellular invertebrates were observed only in Wołów's WWTP, where they were the most abundant in October, its concentration oscillated at the level of 20 specimens/ cm<sup>3</sup> for the rest of the studied period. The results of macroscopic and microscopic studies of sludge from both plants were summarized in Tables 5-6.

Table 5

Observation			l	Montl	1				
Observation	VIII	II	III						
	Macr	oscopic characteristic o	of ac	tivate	d sl	udge			
Colour greenish -brown yellowish-brown									
Odour substrate-like earthy, rotten -1									
Supernatant				clear					
		Characteristic of activ	ated	sludg	ge				
Shape agglom- erates irregular									
Structure		tight				loose			

Results of macroscopic and microscopic observations of activation sludge from Wołów plant

Table	5
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Cohesion		good				poor	
Size	medium						
No. of organic particles		±			+	±	
No. of inorganic particles	±	±	±	H	±	±	±
		Bacteria					
Free-swimming	-	-	-	+	+	+	+
Zooglea	±	±	±	Ŧ	-	-	-
Filamentous category, shape)	2, bent		1,1	oent		3, bent	
Spirillae	±	+	+	+	+	++	++
		Staining					
Neisser	positive		neg	gative	;	positive	
Gram		neg	gativ	e			posi- tive
Sulfur			ve				
Comments	_	occasional bacterial monocolonies	_	_	_	occasional bacterial monocolonies	_

Results of macroscopic and microscopic observations of activation sludge from Wołów plant

Odour: characteristic, substrate-like, sallow, chemical, rotten, none. Supernatant: clear, cloudy. Shape of flock: circular, irregular, agglomerates. Structure: loose, tight. Cohesion: poor, good. Flock size: big – diameter >500  $\mu$ m, medium – 100–500  $\mu$ m, small <100  $\mu$ m. Number of inorganic particles, organic filaments, zoogleal bacteria: (–) absent, (±) rare, observed occasionally, (+) observed regularly, 5–10 particles or cell per sample), (++) frequent (numerous cells or particles, above 10–15 per sample). The number of free swimming bacteria (–) rare, (+) a few dozens in vision field, (++) hundreds in vision field. Filamentous bacteria: category 0 – nearly total lack of filamentous organisms, category 1 – filamentous organisms rather rare, category 2 – medium number, category 3 – very common, category 4 – abundant. Filaments shape: straight, bent, ball. Spirillae: (–) absent, (±) accidental, (+) 5–10 per sample, (++) above 10–15 per sample.

The three biodiversity indices have been calculated for each month of studies (Table 7). For Wołów WWTP, the Simpson's diversity index had the largest value in March and November (0.93). The Shannon–Wiener and Margalef richness indices reached the highest values in October (2.95 and 4.61, accordingly). Activated sludge from Brzeg Dolny WWTP was the most diversified in August according to all three indices: 0.77 for the Simpson's diversity index, 1.89 for the Shannon index and 1.42 for the Margalef richness index. In Wołów WWTP, the Simpson's diversity index had the highest value in March and November (0.93). The Shannon–Wiener and Margalef richness indices reached the highest values in October (2.95 and 4.61, respectively).

#### Table 6

n	1.	6		•	1 .	•	1	, •	c		1 1	C	р	D 1	D1 /
к	esults	ot	macrosco	nic an	$d m_1$	crosconic	ohsei	rvations	ot.	activation	shinde	tron	1 Brzeg	Dolnt	/ Plant
τ,	courto	U1	macrosco	pic un	u IIII	croscopic		varions	U1	activation	bruug		I DILUS	Domy	1 Iulli

Observation				Ν	lon	th				
Observation	VIII	IX	Х	XI	Ι	II	III			
	Macrosco	opic c	hara	cteristic of activ	ated	l sludge				
Colour	pale yello	w								
Odour	chemical									
Supernatant	claudy									
	Ch	aract	erist	ic of activated sl	udg	e				
Shape	circular									
Structure	loose									
Cohesion	poor									
Size	medium	sma	11	medium						
No. of organic particles	-	±	±	±	±	-	-			
No. of inorganic particles	_	±	±	±	±	±	±			
				Bacteria						
Free-swimming	+	+	+	+	+	+	+			
Zoogleal	+	+	+	±	±	±	±			
Filamen-tous: category, shape	2, straigh	t	2,1	bent	2,	straight	1, straight			
Spirillae	_	-	_	_	_	_	-			
			Sta	ining result						
Neisser	positive	neg	ative	2						
Gram	positive						negative			
Sulfur	positive	neg	ative	2						
Comments	common monocol	bacte onies	rial	bacterial monocolonies	_	bacterial monocolonies	common bacterial monocolonies			

For a detailed legend cf. Table 5.

Table 7

Biotic indices calculated for the plants examined<sup>a</sup>

Indox	VII		IX		Х		Σ	Ι	]	[	Ι	Ι	III	
Index	W	В	W	В	W	В	W	В	W	В	W	В	W	В
Simpson	0.85	0.77	0.90	0.47	0.92	0.43	0.93	0.36	0.89	0.61	0.90	0.67	0.93	0.76
Shanon –Wiener	2.51	1.89	2.69	0.96	2.95	0.81	2.94	0.73	2.54	1.09	2.63	1.60	2.89	1.73
Margalef	3.44	1.42	3.82	1.01	4.61	0.63	3.85	0.77	3.06	0.78	3.73	1.33	3.95	1.28

W - Wołów WWTP, B - Brzeg Dolny WWTP.

## 4. DISCUSSION

The basic cause of differences in microbial structure in both plants is the type of sludge received. The plant in Brzeg Dolny receives mainly industrial toxic sewage from the PCC Rokita (70%), such sewage is nearly devoid of bacteria, thus inoculation is

stems

slow. Second studied plant, localised in small town in Wołów, receives mainly domestic sludge (90%) with high load of microorganisms, coming also from soil and water tanks to drainage system which is installed in Wołów.

In the summer time and early autumn (Fig. 1), the number of crawling ciliates is higher in the studied plants with respect to the rest of year. Probably this is a result of temperature variation and abundance of filamentous bacteria in sludge. Inverse relationship has been found between the number of crawling ciliates and filamentous bacteria. When filamentous bacteria are abundant, the number of crawling ciliates significantly declines [5] which was also the case in WWTP in Wołów. The influence of the temperature on the efficiency of activated sludge process is well-known. Temperature influences e.g. dissolved oxygen concentration, substrate solubility, gas exchange among organisms and liquid, speed of biochemical reactions which has an exact impact on the whole biocenosis of activated sludge [6]. For the biocenosis development higher temperatures are favourable. Biernacka [7] observed seasonal changes in microorganisms number in the autumn-winter period of studies and the increase of the population in spring and summer time. The optimum growth of microorganisms in the activated sludge is within the range 26-29 °C which is in accordance with our observations (temperature increase observed in February and March corresponded to increasing number of crawling ciliates). The higher biodiversity of crawling ciliates provides better adaptation to unfavourable conditions in winter (Fig. 1). The crawling ciliates are not abundant in the sludge from WWTP in Brzeg Dolny, which suggests unstable conditions in the plant.

Free-swimming ciliates species are more abundant if the bacteria are more available as they feed on them. The sudden fall of their occurrence in the sludge from Brzeg Dolny plant probably is connected with the high load of toxic substances in September resulting in complete disappearance of this group in sludge. Also the decreasing number of free-swimming ciliates in the plant in Wołów is connected with temperature changes in winter period (Fig. 2).

The attached ciliates are usually the most abundant taxa in the activated sludge process as they exhibit high tolerance to environmental conditions and good resistance to toxicants [5]. These organisms were present in both plants (Fig. 3). The greater abundance of this group was observed in WWTP of Wołów in November and January which could be connected with the increase of wastewater load. They were numerous in the sludge from Brzeg Dolny plant as a result of this plant overload (BOD<sub>5</sub>0.3 kg/(kg·day)). Their higher number in September and complete disappearance of other ciliates at the same time suggest that toxic sewage was delivered to the plant. From December to February the stalks of ciliate were observed (mainly *Epistylis* sp.) pointing to the lack of nutrients and unfavorable conditions in the bioreactor [5]. What is more, the present attached ciliates in Brzeg Dolny were of small sizes which is also connected with the presence of toxicants in the activated sludge process [7]. Numerous occurrence of *Vorticella microstoma* is an indicator of insufficient oxygenation and high load [8]. Its highest abundance was observed in Wołów plant from November to March due to worsening conditions of activated sludge in winter time. Quite high abundance of this species in the plant in Brzeg Dolny is also related to bad quality of the delivered sewage.

On the other hand, the colonies of *Carchesium* sp. and *Epistylis* sp., numerous in the activated sludge in Wołów from September to October suggest good and stable conditions. At the same time high concentration of *Vorticella convallaria* is always connected with good oxygenation, good nitrification and stable conditions. Although we recorded a high number of this species in Wołów plant, it changed from attached to free-swimming forms in January and February which is linked to the lack of nutrients and worsening physical and chemical conditions of activated sludge process.

The presence of testate amoebas in the activated sludge from Wołów plant indicates old age of sludge and good oxygenation as well with low content of nitrogen (effective nitrification and denitrification) and low load of sludge in general. Madoni [9] suggested that such genera of testate amoebas like *Arcella* sp., *Euglypha* sp. are very good bioindicators of activated sludge process as they are associated with good oxygenation, extended aeration and good nitrification. On the other hand, the highest abundance of naked amoebas in Wołów plant (maximum in January nearly 400 specimens/cm<sup>3</sup>) indicates worsening conditions in the bioreactor. In Brzeg Dolny plant, no amoeba species was observed resulting from highly unfavourable conditions of activated sludge.

The most abundant group was the Flagellates  $<20 \ \mu\text{m}$ . The dominance of this taxa accompanying by low abundance of ciliates suggest the bad conditions and high load of sludge, low oxygenation and indicates the early stage of microbial community succession. In such conditions flocs are small and sedimentation is not good in the secondary clarifier. Among the flocs free swimming bacteria are abundant which cause cloudiness of sludge [9]. All samples from Brzeg Dolny were cloudy. In the sludge from Wołów plant, spiral bacteria are present, which suggest the removal of nutrients, in Brzeg Dolny they do not occur [5].

All the biotic indices indicate the higher diversity of species in Wołów plant. The Shannon diversity index reaches higher values when the community has a good structure and the abundance is not very important. During the period studied, the values of Shannon index in Wołów plant were higher than scores from Brzeg Dolny plant which confirm the good structure of community in the activated sludge in Wołów plant. The Margalef index takes also into account the rarer taxa. In the study period, it reached higher values in the case of the community from Wołów plant. Simpson's diversity index pays attention to more abundant species in a sample. Thus, the overall value of this index is higher in the case of Wołów plant again.

To summarise, higher diversity of microfauna was recorded in Wołów plant which is related to sewage type. Considering ciliated protozoa, their presence or absence within the system is directly connected with the effluent concentration of organic pollutants

(BOD, SS) [10]. We recorded very diverse community of attached ciliates. The domination and greater biodiversity of attached ciliates always suggest good, stable and healthy performance of activated sludge. If treatment conditions are bad, these ciliates leave their stalks. We recorded attached ciliate Vorticella convallaria in high abundance in Wołów plant, this species is associated with good oxygenation, extended aeration and good nitrification [8–10]. Also the community of crawling and free swimming ciliates was very abundant in Wołów WWTP. According to Curds and Cockburn [11], all the crawling and free swimming ciliates disappear when high load appears. Aspidisca cicada is related to adequate performance and was recorded in Wołów WWTP. Although, among them are also bioindicators like Opercularia sp. which suggest worsening performance of activated sludge [4] which was connected with winter period and low temperature. We found great abundance of Opercularia minima in Brzeg Dolny WWTP, but not in Wołów plant. Testate amoeba Arcella sp. are also very good bioindicators of favorable treatment conditions and this species was recorded in Wołów plant but not in Brzeg Dolny. The presence of rotifers is always connected with the sustained aerobic conditions. The occurrence of higher invertebrates (Nematodes, Tartigrades) is linked also to stable conditions and lack of toxicity. Tardigrades occur only in nitrifying activated sludge systems [8, 9]. These taxa were recorded in Wołów plant.

#### **5. CONCLUSION**

In a plant where domestic sewage predominates, protozoan and metazoan community is more diverse and species are more abundant, we recorded stalked ciliates species, i.e., *Vorticella* sp., *Epistylis* sp., *Carchesium* sp. and crawling ciliates, i.e. *Aspidisca* sp., *Euplotes* sp., *Chilodonella* sp., *Tokophyra* sp. and other higher invertebrates which are good bioindicators for high effluent quality. In the other studied plant which receives mainly industrial sewage, these ciliates and metazoans do not occur or are found in a relatively lower densities. The seasonal changes suggest the influence of temperature on biodiversity fall. A basic microscopic examination can provide a reliable information on plant operating condition and performance. The occurrence and abundance of microfauna could be a key information to assess activated sludge condition.

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