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Diversity and distribution of testate amoebae (Amoebozoa, Rhizaria) in reservoirs, Northeastern Bulgaria

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Abstract*: The aim of the study is to describe the testate amoebae fauna in seven reservoirs in the Northeastern Bulgaria and to investigate the relationship of taxonomic diversity and abundance of these organisms to the some characteristics of the reservoirs. A total of 52 species, varieties and forms belonging to 15 genera were identified in the benthal of the coastal zone. There are significant differences in species richness and abundance between the testacea of different reservoirs. Number of taxa was significantly higher in the Beli Lom (29 species and 12 genera) and Loznitsa (22 species and 13 genera). Most of the reservoirs - Kara Michal, Bogdantsi, Isperih, Lipnik and Brestovene have extremely poor fauna compared to other similar reservoirs. This is confirmed by the low values of the Shannon-Weaver diversity index, which varied between 1.04 and 2.396, as well as by the other indices used to assess the environmental conditions in the reservoirs. The data of the regression analysis showed that a relation between age, surface area and water volume of the reservoirs and the species richness and abundance of testacea is not established.*

Keywords: testate amoebae communities, reservoirs, regression analysis, benthal

Introduction

Reservoirs are one of the main factors that ensure efficient use of water resources of a country. These facilities collect and retain water during wet periods and ensure its balanced use throughout the year. They allow for the collected water to be managed efficiently and the effluent water to be safely taken away. Reservoirs have many uses – they help to control rivers' levels, provide water for irrigation, water supply and electricity production. The reservoir lakes are successfully used for fish farming, sports events and tourism.

Despite their artificial origin, reservoirs are areas of extreme importance for the conservation of biodiversity, as they are inhabited by dozens of plant and animal species. The destruction of natural wetlands during the last century increased the importance of the remaining reservoirs and fish farms and their biodiversity, as they have become the last shelters for wildlife.

At the same time, however, the biodiversity of these artificial ponds is highly endangered. The increased anthropogenic influence and large fluctuations in water levels have a negative impact on the majority of organisms inhabiting the reservoirs. The steep reduction of the water level and, on the other hand, the frequent flooding of reservoir banks hinders the formation of the typical habitats and leads to

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decreased fauna species diversity and to inability to form stable clearly structured communities [1]. With the withdrawal of water, the zoobenthos remain dry and quickly perish. The decomposition of the macro-zoobenthos leads to deterioration of the environment and subsequently to increasing the eutrophication of the reservoir. Some of the threats to biodiversity are related to the cultivation of agricultural areas in the nearby surroundings, as well as with the construction of roads and other facilities nearby.

The construction of a reservoir leads to the total destruction of biocenoses which existed in the place. Then, with the beginning of its functioning they are rebuilt again over a long period of time. Depending on the geographic location, the depth, the tributary area, the volume, the hydrological regime and other parameters of these ponds, their biota is quite variable and diverse [2-4].

This paper presents an assessment of benthic testate amoebae in seven reservoirs located in northeastern Bulgaria, which have not been investigated so far. Our aims are to describe the diversity and distribution of testate amoebae and to investigate the relationship of taxonomic richness and abundance of these organisms to the some characteristics of the reservoirs.

Material and Methods

The present study covers the freshwater testate amoebae fauna of seven reservoirs – Brestovene, Beli Lom, Loznitsa, Kara Michal, Bogdantsi, Isperih and Lipnik, which are located relatively close to each other, in the Northeastern Bulgaria (Table 1, by data of the Public register of dams on the territory of Razgrad District). The region is characterized by plain and hilly relief, with an average altitude of 250-270 m. The climate is temperate continental. It has relatively cold winters (absolute minimum temperature of -28.6° C), cool springs, dry and warm summers (absolute maximum temperature +40.6°C) and prolonged autumns. It is characterized by large temperature variation during the year (in winter months: -10-15°C, and in the summer: +30-35°C) and a normal amount of atmospheric precipitation of 565-786 l/m^2. The average annual amplitude is about 26°C, which is one of the largest for the country [5].

The study was carried out in June 2017. For the fauna analysis benthic samples were taken from 5 different localities (stations) from the coastal zone of each reservoir at a depth from 0.5 m – a total of 35 samples.

The samples of benthos were fixed with 4% formaldehyde. Prior to laboratory testing, the samples

Table 1. Coordinates and basic parameters of the reservoirs.

Reservoirs	Coordinates (reservoir centers)	Year completed	Reservoir surface, km^2	Reservoir volume, m^{3}x10^3
Brestovene	43°45'26.0"N 26°36'28.0"E	1956	0.05	130
Beli Lom	43°23'09.9"N 26°40'57.5"E	1963	3.5	25500
Loznitsa	43°22'16.9"N 26°37'14.2"E	1963	0.06	600
Kara Michal	43°35'20.7"N 26°50'23.7"E	1970	0.404	1500
Bogdantsi	43°37'25.4"N 26°49'45.0"E	1969	0.281	260
Isperih	43°41'14.7"N 26°49'20.8"E	2000	0.206	320
Lipnik	43°35'23.9"N 26°32'26.0"E	1959	0.088	480

were thoroughly stirred and a certain amount (1 cm^3) was extracted with an automatic pipette, and the found specimens were counted.

Identification of the testate amoebae is based on the taxonomic guides on separate taxons [6-14].

The following indices were used to analyse the structure of the testacean communities and to assess the environmental conditions in the reservoirs [15]:

- Index for concentration of domination (D), calculated by the formula: $D = \Sigma (n_i /N)^2$, where n_i is the assessment of significance of every species (number of specimens); N – total assessment of significance (total number of specimens).
- Berger-Parker dominance index (B): ratio of the number of specimens of the dominant species to the total number of specimens.

When these two indicators have values close to 0 indicate, this means that the dominance is distributed among many species, i.e. the conditions in the given biotope are favorable, and when they

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come closer to 1 it shows that only a few species dominate, i.e. in the given biotope there are limiting factors that inhibit the development of a large number of species.
- Margalef index (d) of species variety, calculated by the formula: d = S – 1 / ln N, where S is the number of species; N – total number of specimens.
- Shannon-Weaver diversity index (H), calculated with the formula: $H = - \Sigma (n_i / N) \ln (n_i / N)$, in which n_i is the significance of each species, and N – the overall estimation of significance.

The indicators d and H are in positive correlation with each other and are inversely proportional to D and B.

Significant differences were found between data from different reservoirs using Student's t-test for variables ($p < 0.05$). The relationships between testate amoebae diversity and abundance and some parameters of the reservoirs were explored with regression analysis. The data analysis was performed using STATISTICA and PAST program [15, 16].

Results and Discussion

A total of 529 testate amoebae specimens, belonging to 52 taxa (including species, varieties and forms) and 15 genera were identified in the reservoirs. All the found taxa are reported for first time for the investigated reservoirs. The list of observed taxa and their distribution in the reservoirs are presented in Table 2.

The taxonomic composition of the testate amoebae communities, established in the surveyed reservoirs, is represented mainly by eurybionts with cosmopolitan distribution, typical of the fauna in many other ponds, lakes and reservoirs [17-29]. The highest species diversity is in the genera *Difflugia* (18 species), *Centropyxis* (9) and *Arcella* (6), which are dominants. The remaining 12 genera are presented with 1 to 3 species.

The spread of testate amoebae in the different reservoirs is uneven (Table 2, Fig. 1). Species richness was significantly higher in samples from Beli Lom Reservoir compared to that in the reservoirs Brestovene, Kara Michal, Bogdantsi, Isperih and Lipnik (t-test, Table 3), also in samples from Loznitsa Reservoir in comparison with the reservoirs Brestovene and Bogdantsi, and in the reservoirs Kara Michal, Isperih and Lipnik compared with samples from Bogdantsi.

Twenty-nine species (55.8% of all found species) belonging to 12 genera were found in Beli Lom Reservoir. Eight species were reported only from that reservoir – *Arcella discoides v. foveosa*, *A. hemisphaerica f. undulata*, *A. hem. v. intermedia f. undulata*, *Difflugia bryophila*, *D. globulosa*, *D. tenuis*, *Hyalosphaenia punctata*, *Pontigulasia rhumbleri*, while in the other reservoirs the number is considerably smaller. Typical aquatic genera *Difflugia* (11 species) and *Arcella* (5 species) are predominant, and make up 55.2% of the identified species in this reservoir. Loznitsa Reservoir is characterized by similar taxonomic diversity – 22 species (42.3% of all found species), belonging to 13 genera. However, only 3 species were found only in this reservoir – *Corythion pullchelum*, *Phryganella acropodia* and *Plagiopyxis intermedia*. The predominant are representatives of the genus *Centropyxis* (6 species), which constitute 27.3% of all species found in the reservoir. The reservoirs Isperih, Kara Michal, Lipnik and Brestovene have a smaller species and generic diversity. In them were found 17 species (32.7% of all species found in the reservoirs), belonging to 5 genera, 16 species (30.8% of all species) belonging to 8 genera, 14 (26.9% of all found species), and 9 (17.3% of all found species) from 6 genera respectively. In Isperih, Kara Michal and Lipnik predominated the typical aquatic representatives of the genera *Difflugia* and *Centropyxis*, which constituted respectively 70.6%, 62.5% and 64.3% of the species

Table 2. List of testate amoebae taxa and number of specimens in different reservoirs.

Taxa	Reservoirs						
	Brestovene	Beli Lom	Loznitsa	Kara Michal	Bogdantsi	Isperih	Lipnik
Arcella *discoides* Ehrenberg, 1843							1
A.disc. v. scutelliformis Playfair, 1918		4	2				1

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A.disc. v. foveosa Playfair, 1918		2					
A.hemisphaerica Perty, 1852		5		1			
A.hem. f. undulata Deflandre, 1928		28					
A.hem.v. intermedia f. undulata Deflandre, 1928		25					
Centropyxis *aculeata* Ehrenberg, 1838	1	2	2				
C. aerophila Deflandre, 1929		1	5			3	3
C. aer. v. sphagnicola Deflandre, 1929		1	12	1		16	11
C. constricta (Ehrenberg, 1841) Deflandre, 1929					1		
C. deflandriana Bonnet, 1959						5	
C. ecornis Ehrenberg, 1841				2			
C. hirsuta Deflandre, 1929			1	1	1		1
C. platystoma Penard, 1890			2			2	
C. sylvatica Deflandre, 1929			1			1	
Corythion *pullchelum* Penard, 1890			1				
Cyclopyxis *ambigua* Bonnet & Thomas, 1960						10	
C. eurystoma Deflandre, 1929	6		24	2		9	2
C. kahli Deflandre, 1929		2	2				
Difflugia *acuminata* Ehrenberg, 1838						1	
D. ampullula Playfair, 1918				1			
D. bryophila (Penard, 1902) Jung, 1942		1					
D. decloitrei Godeanu, 1972	1	1					1
D. elegans Penard, 1890							1
D. glans Penard, 1902		1					1
D. globularis (Wallich, 1864) Leidy, 1877	1					1	3
D. globulosa Dujardin, 1837		2					
D. gramen Penard, 1902		1		2	2	99	
D. lacustris (Penard, 1899) Ogden, 1983		3		1		1	
D. lobostoma Leidy, 1879		1				1	
D. minuta Rampi, 1950	3	1		1			
D. penardi Hopkinson, 1909						1	
D. pristis Penard, 1902				2			1
D. pulex Penard, 1902		2		1			
D. sarrisa Li Sun Taï, 1931	4	2		5			1
D. schurmanni van Oye, 1932			1			3	
D. tenuis (Penard, 1890) Ogden, 1983		1					
Difflugiella *oviformis* Bonnet &Thomas, 1955		3	6				
Euglypha *rotunda* Wailes, Penard, 1911		3	19				
Hyalosphaenia *papilio* Leidy, 1874				11			
H. punctata Penard, 1891		1					
Microchlamys *patella* (Clap, Lach.,1885) Cockerell, 1911	2	2	4				
Phryganella *acropodia* (Hert., Less.,1874) Hopkinson, 1909			1				
P. hemisphaerica Penard, 1902			3			1	1
Plagiopyxis *declivis* Thomas, 1955		1	11	1		7	4
P. intermedia Bonnet, 1959			1				
P. minuta Bonnet, 1959						1	
Pontigulasia *rhumbleri* Hopkinson, 1909		1					
Schonbornia *viscicula* Decloitre, 1964	1		5	2			
Tracheleuglypha *acola* Bonnet & Thomas, 1955		22	4				
Trinema *enchelys* (Ehrenberg, 1838) Leidy, 1878	1	21	3	2			
T. lineare Penard, 1890		4	21				

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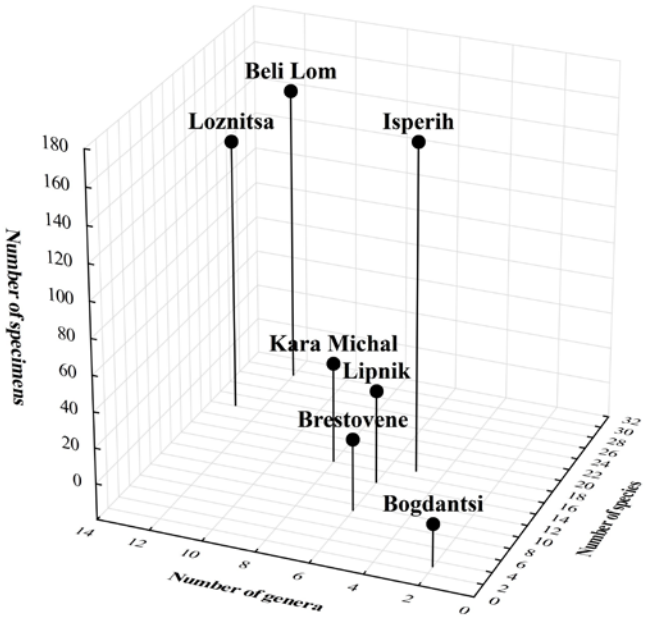

Fig. 1. 3D scatterplot of number of specimens against number of species and number of genera in different reservoirs.

Table 3. Significance (p values) of species richness (above diagonal) and number of specimens (below diagonal) among the different reservoirs.

Reservoir	Brestovene	Beli Lom	Loznitsa	Kara Michal	Bogdantsi	Isperih	Lipnik
Brestovene	1.00000	0.00003***	0.00357**	0.07027	0.08320	0.08817	0.19961
Beli Lom	0.01081*	1.00000	0.18043	0.01092*	0.00000***	0.03832*	0.00428**
Loznitsa	0.00376**	0.82936	1.00000	0.24302	0.00002***	0.30166	0.08817
Kara Michal	0.22313	0.02940*	0.02376*	1.00000	0.00045***	0.83716	0.64189
Bogdantsi	0.06590	0.00375**	0.00203**	0.01378*	1.00000	0.00057***	0.00352**
Isperih	0.16392	0.87413	0.77196	0.21172	0.03406*	1.00000	0.49662
Lipnik	0.39901	0.02579*	0.00869**	0.81751	0.03136*	0.19735	1.00000

Significance level: *** p < 0.001, ** p = 0.001-0.01, * p = 0.01-0.05

identified in the reservoir. The number of species found only in them was 5 – *Centropyxis deflandriana, Cyclopyxis ambigua, Difflugia acuminata, D. penardi* and *Plagiopyxis minuta*, 3 – *Centropyxis ecornis, Difflugia ampullula* and *Hyalosphaenia papilio* and 2 – *Arcella discoides* and *Difflugia elegans* respectively. The Brestovene Reservoir is dominated by the representatives of the genus *Difflugia* (4 species), which make 44.4% of the species found in the reservoir. No taxa were reported only in samples from this reservoir. Bogdantsi is characterized by the smallest taxonomic diversity with only 3 species (5.8% of all found species) belonging to 2 genera – *Difflugia* (2 species) and *Centropyxis* (1 species).

It should be noted that from the 52 species, varieties and forms found, none of them were there present in all the reservoirs studied.

There is a statistically significant difference in the number of specimens between the reservoirs Beli Lom and Loznitsa on the one hand, and the reservoirs Brestovene, Kara Michal, Bogdantsi and

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Lipnik on the other, and also between the Bogdantsi Reservoir and the reservoirs Kara Michal, Isperih and Lipnik (t-test, Table 3).

The received results about distribution of testate amoebae in different reservoirs are confirmed of the values for indices used to analyse the species structure of the testacean communities and to estimate the ecological conditions in researched reservoirs, presented on Table 4.

The index for concentration of domination (D) and Berger-Parker index (B) have the lowest values in the reservoirs Beli Lom and Loznitsa, which shows that they have the most favorable conditions for supporting testate amoebae. These indices have significantly higher values for the reservoirs Bogdantsi and Isperih. In Bogdantsi the number of species is extremely low and is almost equal to the number of specimens found. Although a relatively large number of species and specimens were found in Isperih Reservoir, most species have small numbers. The large number of specimens is due to only one species - *D. gramen*.

Contrary to D and B, Margaleff diversity index (d) and Shannon-Weaver index (H) are highest for the reservoirs Beli Lom and Loznitsa, which once again proves that the conditions in them are suitable for the development of testate amoebae. These two reservoirs are characterized by great species diversity, and most of the species are represented with approximately equal number of specimens. For the other reservoirs and especially Bogdantsi, Isperih and Brestovene, d and H have significantly lower values, indicating that the conditions in them are the most unfavourable. Bogdantsi and Brestovene are characterized by very low species diversity, whereas in Isperih the dominance is concentrated in one to several species, and the main part of the species are with a small number of specimens.

We have traced some parameters of the reservoirs (Table 1, Figs. 2, 3), such as the age, surface area and water volume, whose impact on the testate amoebae diversity has not yet been studied. Many authors have analyzed the influence of the age and size of the lakes on the species richness of different systematic group organisms and their results are very contradictory [30-35]. Hobaek et al. [30] indicated a slight, but significant, positive effect of the lake area on zooplankton richness and Hessen et al. [31] established that the species richness was inversely correlated with the lake surface area. Frisch et al. [32] observed that the most of the copepod and cladocerans assemblages are strongly positively related to size of ponds in south-west Spain and surface area is one of the factors structuring species composition of both groups. According to Dodson et al. [33] the age of the artificial lake did not have a significant effect on zooplankton species richness, while land use had a highly significant adverse effect. Vadeboncoeur et al. [34] showed that there is no correlation between age of the lakes and species richness for invertebrates, but their diversity is positively related to the surface area. Comparing lakes older and younger than 30 years Mailand [35] indicated significantly higher species richness and average abundance of benthic gastropods in lakes older than 30 years.

The data of the regression analysis showed that a relation between age of the studied reservoirs and the species richness and abundance of testacea is not established (Fig. 2). Most of the reservoirs, including Beli Lom and Loznitsa, characterized by the highest taxonomic diversity and abundance have functioned as water basins for about 50-60 years. They were built in the same period of 1956-1970. Only Isperih Reservoir is newer, it has been functioning for 18 years, but it is characterized by relatively high species diversity and has the greatest abundance.

Similar results were obtained for the surface and water volume of the reservoirs (Fig. 3). Of all studied dams only Beli Lom has significantly higher surface and volume. The resulting data do not explain the significantly high number of species and specimens in Loznitsa and Isperih, which are the third and fifth largest respectively, as well as the fact that the Brestovene Reservoir is the smallest, but doesn't have the smallest testate amoebae fauna. Reservoirs Kara Michal and Lipnik are characterized by similar taxonomic richness and abundance, but are very different in size (Table 1).

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Table 4. Indices for structure of the communities in different reservoirs.

Indices	Reservoirs						
	Brestovene	Beli Lom	Loznitsa	Kara Michal	Bogdantsi	Isperih	Lipnik
D	0.175	0.1185	0.1049	0.1373	0.375	0.3941	0.1641
B	0.3	0.1944	0.1832	0.3056	0.5	0.6111	0.3438
H	1.947	2.578	2.567	2.396	1.04	1.558	2.219
d	2.67	5.634	4.308	4.186	1.443	3.145	3.751

Our results show that the testate amoebae fauna in the reservoirs can be very different and specific. The water basins studied are located relatively close to each other, at almost the same altitude, most of them are built at the same time (except for Isperih) and have similar hydrographic characteristics (with the exception of Beli Lom, which is larger). However, they differ significantly in terms of taxonomic richness and abundance of testate amoebae.

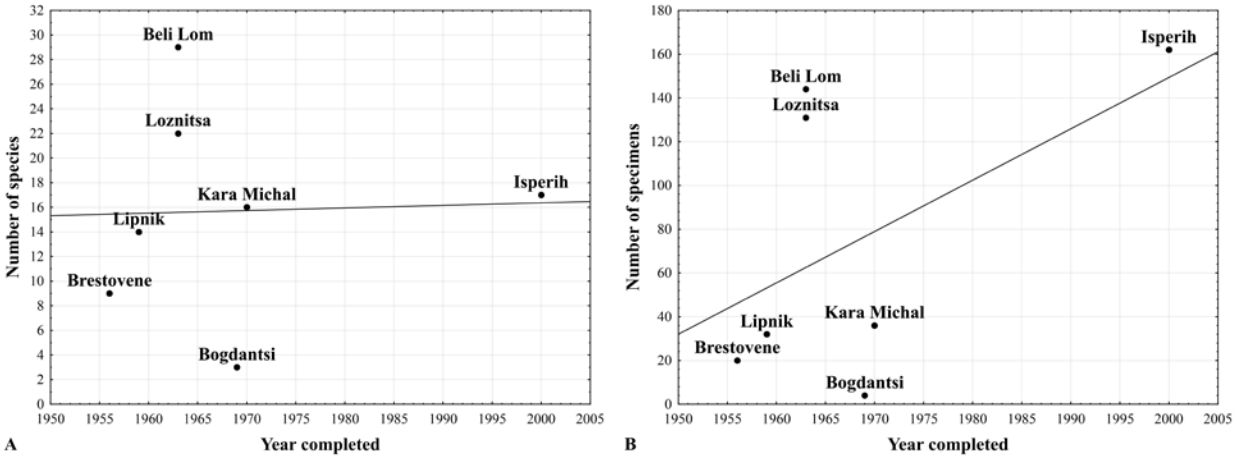
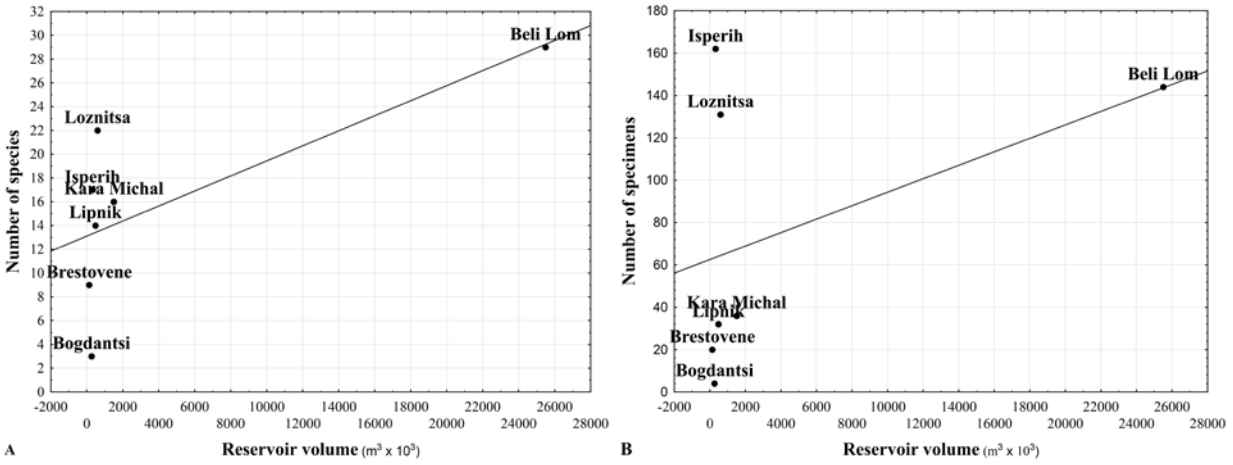


Fig. 2. Regression analysis showing the relationship between the number of species (A) and number of specimens (B) of testate amoebae and year completed of reservoirs.



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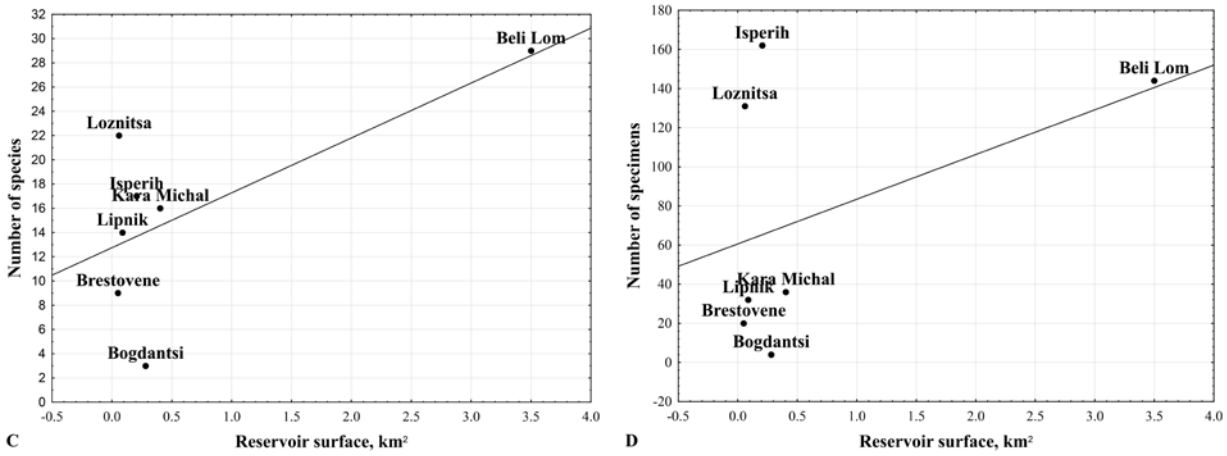

Fig. 3. Regression analysis showing the relationship between the number of species (A, C) and number of specimens (B, D) of testate amoebae and reservoir volume and surface.

Most of the reservoirs – Kara Michal, Bogdantsi, Isperih, Lipnik and Brestovene are characterized by extremely poor fauna compared to other similar reservoirs [22, 25, 27, 29]. This is confirmed by the low values of the Shannon-Weaver diversity index, which varied between 1.04 and 2.396. Past research shows that in "healthy" ponds, where stable and relatively rich testate amoebae fauna develops, the index values are around and above 2.5 [36-38]. The observed differences between the testate amoebae fauna of the reservoirs examined, and the very low taxonomic richness and abundance in some of them may have resulted from various human activities, which in one way or another lead to the pollution of the reservoirs and destruction of their habitats. Further research of the hydro-chemical parameters of reservoirs, the peculiarities of their bottom sediments and other environmental factors is necessary in order to reveal the link between them and the diversity and abundance of testate amoebae communities in the studied area.

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