OH&RM ONE HEALTH & RISK MANAGEMENT

VOL. 5, ISSUE 4
2024

BIOLOGICAL SCIENCES

OPEN ACCESS

THE IMPACT OF MICROALGAE CHLORELLA VULGARIS AND SCENEDESMUS QUADRICAUDA ON THE GROWTH PARAMETERS OF THE CILIATE PARAMECIUM CAUDATUM

Elena ROSCOV, Ion TODERAS, Laurentia UNGUREANU, Daria TUMANOVA

Institute of Zoology, Moldova State University, Republic of Moldova

Corresponding author: Elena Roscov, e-mail: elena.roscov@sti.usm.md

DOI: 10.38045/ohrm.2024.4.04 CZU: 582.263:593.171.4

Keywords: *microalgae, Chlorophyta, Ch. vulgaris, S. quadricauda, P. caudatum, population size, reproduction rate.*

Introduction. *An important aspect of research in the field of microbial ecology is understanding the interactions between organisms, particularly the influence they have on one another within aquatic ecosystems. Green microalgae (phylum Chlorophyta) represent a diverse group of photosynthetic organisms, playing a significant role in ecological cycles. Investigations have focused on evaluating the impact of two microalgae species, Chlorella vulgaris and Scenedesmus quadricauda, on the growth parameters of the natural population of Paramecium caudatum, making it a suitable indicator for assessing the influence of these organisms on the food chain.*

Material and methods. *The research was based on the work of Kokova, V. (1982), and Likhachev, S.V. (2020). The productivity of the ciliates was determined by their division rate, according to Zaika V.E. (1983) and Spinei L. (2009). Experiments were conducted over intervals of 24-144 hours, using microalgae solution concentrations at 0.1, 0.5, 1, and 10 mg/L.*

Results. *For Chlorella vulgaris the lower doses generated a significant stimulatory effect on P. caudatum, resulting in notable increases in both number and reproduction rate.*

In contrast, for Scenedesmus quadricauda, lower doses also had a pronounced stimulatory effect, while higher doses yielded varied results, including both increases and decreases in parameters.

Conclusions. *Both strains of microalgae demonstrate stimulatory potential on the natural population of P. caudatum, particularly at lower doses.*

Cuvinte-cheie: *microalge, Chlorella vulgaris, Scenedesmus quadricauda, Paramecium caudatum, efectivul numeric, rata de reproducere.*

IMPACTUL MICROALGELOR CHLORELLA VULGARIS ŞI SCENEDESMUS QUADRICAUDA ASUPRA PARAMETRILOR DE CREŞTERE A CILIATEI PARAMECIUM CAUDATUM

Introducere. *Un aspect important al cercetărilor în domeniul ecologiei microorganismelor constă în înţelegerea interacţiunilor dintre organisme, în special influenţa pe care o au acestea asupra ecosistemului acvatic. Microalgele verzi (Filumul Chlorophyta) reprezintă un grup divers de organisme fotosintetice, care au un rol semnificativ în ciclurile ecologice. Astfel, investigaţiile au fost orientate spre evaluarea impactului a două specii de microalge, Chlorella vulgaris şi Scenedesmus quadricauda, asupra parametrilor de creştere a populaţiei naturale de Paramecium caudatum.*

Material şi metode. *Ca suport metodologic au servit cercetările propuse de cercetătorii Kokova V. (1982) şi Likhachev S.V. (2020). Productivitatea infuzoriilor a fost determinată după viteza dividerii lor, conform cercetătorilor Zaika V. E. (1983) şi Spinei L. (2009). Experimentele s-au desfăşurat în intervale de timp de 24 - 144 ore, utilizând concentraţiile soluţiilor de microalge de 0,1; 0,5; 1 şi 10 mg/l.*

Rezultate. *Pentru Chlorella vulgaris s-a constatat că dozele mai mici au generat un efect stimulativ semnificativ asupra P. caudatum, cu creşteri notabile ale numărului şi ratei de reproducere. În cazul Scenedesmus quadricauda, dozele mai mici au avut un efect stimulator pronunţat, în timp ce dozele mari au dus la rezultate diferenţiate, inclusiv creşteri şi scăderi ale parametrilor.*

Concluzii. *Concluziile indică faptul că ambele microalge au un potenţial stimulator asupra populaţiei naturale P. caudatum, în special dozele mai mici.*

INTRODUCTION

Green algae (Chlorophyta) belong to the group of photosynthetic organisms known chlorophyta, which are green in color due to the presence of chlorophyll. With their ability to harness the solar energy and convert it into food, green algae play a crucial role in the planet's energy cycle. Important species in this class are *Chlorella vulgaris Beijerinck 1890* and *Scenedesmus quadricauda Chodat 1926*, which are studied for their photosynthetic properties, high nutrient content, bioactive compounds and potential applications in various fields (1, 2, 3).

A significant aspect of research in microbial ecology is understanding the interactions between organisms, particularly their influence on others within aquatic ecosystems. *Paramecium caudatum Ehrenberg, 1833*, a widely distributed ciliate protist, serves as a valuable model organism for such studies due to its well-documented biological characteristics and sensitivity to environmental changes (4).

Thus, the general development mechanisms of the entire ecosystem can be uncovered, and the interactions between populations can be analysed (5). In addition to investigating industrial cultivation possibilities for autotrophic microalgae, considerable attention is also given to establishing methods for producing large biomass quantities of heterotrophic invertebrate organisms (6).

The purpose of this work was to investigate the interactions between green microalgae from the phylum Chlorophyta, specifically or particularly *Chlorella vulgaris* and *Scenedesmus quadricauda* species, and their effect on the population of *Paramecium caudatum* in a controlled culture media (nutrient medium). Assessing the impact of these green algae on the behavior, reproduction, and other biological aspects of the ciliate will contribute to a deeper understanding of aquatic resource dynamics and the potential benefits or risks associated with the presence of these microorganisms in aquatic ecosystems.

This study provides a specific example of the interactions between certain microorganisms, contributing to a broader understanding of ecological relationships (3).

MATERIAL AND METHODS

The study focused on the ciliate *Paramecium caudatum*, from which separate clonal specimens were obtained for subsequent research through binary fission. *Saccharomyces cerevisiae* yeast served as their food source. Mass cultures and individual lines were used in experiments, obtained by isolating a single specimen from a culture in full development. Cultivation was conducted in micro-aquariums, following classical methods proposed by K. Kokova, V. (1982) (7); Likhachev, S.V., (2020) (8) with daily counting of cells. An optimal culture medium was created for the paramecia, including chemical composition and environmental conditions. During the cultivation of protozoa, significant attention was paid to the studies of trophic conditions, as well as the qualitative composition of their food and nutritional intensity. From each individual, a line of descendants was established, and parameters such as growth rates, lifespan, reproduction, and natural population size were monitored according to Zaika V.E (1983) (9); Spinei L. (2009) (10). These parameters were determined by counting the number of divisions per day for each line over a period of six days and data analysis.

The strains of green microalgae from the phylum Chlorophyta were obtained from Laurentia Ungureanu, a corresponding member of the ASM (Academy of Science of Moldova), research professor, and doctor habilitatus in biological sciences, as well as from Daria Tumanova, PhD in biological sciences, in the laboratory of Hydrobiology and Ecotoxicology at the Institute of Zoology. The strains were patented and deposited in the Non-pathogenic Microorganism National Collection at the Institute of Microbiology and Biotechnology, Technical University of Moldova. The microalgae were cultivated in standardized media under controlled conditions of temperature, light, and nutrients, following the methods described by Wasser S.P. et al. (1989) (11). The algal biomass was separated from the growing medium by filtration and centrifugation.

Test groups were divided based on the species of microalgae (*Chlorella vulgaris*, *Scenedesmus quadricauda*) and their concentrations, subjected to a time interval of 24 to 144 hours. Concentrations of microalgal solutions of 0.1 mg/L, 0.5 mg/L, 1 mg/L, and 10 mg/L were prepared. Control groups were also included to assess the accuracy of the procedures and confirm the results.

Quantitative analysis was performed in 10 replicates at a temperature of 25°C, over 24-144

hours using the binocular microscope MBC-9 with ocular magnification of 14x.

Appropriate statistical methods were employed to assess the significance of differences between groups (Statistica 7.0, Excel 2007, Biostat). The t-Student test (significance test) was used to test for a statistically significant difference between the values obtained in the experimental and control groups. Interpretation of P values for the significance test indicates the level of statistical significance, thus *P<0.05 (statistical correlation is significant (S, 95% confidence)), **P<0.01 (statistical correlation is significant (S, 99% confidence)), ***P<0.001 (statistical correlation is highly significant (HS, 99.9% confidence)) and P>0.05 (statistical correlation is insignificant, IS).

RESULTS

The study investigated the sensitivity of the ciliate *Paramecium caudatum* to cultures of *Chlorophyta* microalgae, represented by the species *Chlorella vulgaris* and *Scenedesmus quadricauda*, as well as to microalgal culture liquids. The focus was on evaluating the response of the test organism to various concentrations (0.1, 0.5, 1, and 10 mg/L) and different time intervals (24, 48, 72, 96, 120, and 144 hours).

Evaluating the impact of different concentrations of *Chlorella vulgaris* over a 24-hour interval revealed statistically significant effects at concentrations of 0.1 and 10 mg/L. Specifically, the concentration of 0.1 mg/L showed a significant negative effect on population dynamics, resulting in a decrease of 43.33%, while the concentration of 10 mg/L resulted in a more pronounced decrease of 60.00% (fig. 1).

The reproduction rate of *Cw* at concentrations of 0.1 and 10 mg/L exhibited significant reductions of 31.84% and 50.84%, respectively. Medium concentrations of 0.5 and 1 mg/L also had a negative impact, but the effects were weaker, suggesting only a slight, insignificant reduction in *Nt* and *Cw* (tab. 1, fig. 1).

Table 1. Experimental results for the tested cultures with the microalgae *Chlorella vulgaris* as food for the ciliates *Paramecium caudatum.*

Experimental groups	N	Chlorella vulgaris							
		Sample size (Nt) M±ES	Difference compared to the control			Repro duction rate (Cw) M±ES	Difference compared to the control		
			d	%	t_d		d	%	t_d
					24 h				
Control	*10*	*6.00±0.67*				*1.79±0.11*			
0.1 mg/L	*10*	*3.40±0.55*	*-2.60*	*43.33*	*3.00***	*1.22±0.18*	*-0.57*	*31.84*	*2.70***
0.5 mg/L	*10*	*4.90±0.53*	*-1.10*	*18.33*	*1.29*	*1.59±0.12*	*-0.20*	*11.17*	*1.23*
1 mg/L	*9*	*5.89±1.10*	*-0.11*	*1.83*	*0.09*	*1.77±0.17*	*-0.02*	*1.12*	*0.10*
10 mg/L	*10*	*2.40±0.17*	*-3.60*	*60.00*	*5.21****	*0.88±0.07*	*-0.91*	*50.84*	*6.98****
					72 h				
Control	*9*	*109.00±18.69*				*1.56±0.05*			
0.1 mg/L	*10*	*157.60±35.38*	*+48.60*	*44.59*	*1.21*	*1.69±0.09*	*+0.13*	*8.33*	*1.26*
0.5 mg/L	*10*	*178.40±18.80*	*+69.40*	*63.67*	*2.62***	*1.73±0.03*	*+0.17*	*10.90*	*2.92***
1 mg/L	*10*	*187.40±30.00*	*+78.40*	*71.93*	*2.22**	*1.74±0.05*	*+0.18*	*11.54*	*2.55**
10 mg/L	*10*	*136.00±24.71*	*+27.00*	*24.77*	*0.87*	*1.64±0.08*	*+0.09*	*5.77*	*0.95*
					120 h				
Control	*10*	*199.40±27.14*				*1.06±0.04*			
0.1 mg/L	*10*	*354.90±80.94*	*+155.50*	*77.98*	*1.82*	*1.17±0.06*	*+0.07*	*6.60*	*0.97*
0.5 mg/L	*10*	*437.30±101.26*	*+237.90*	*119.31*	*2.27**	*1.22±0.06*	*+0.11*	*10.38*	*1.53*
1 mg/L	*10*	*301.30±28.72*	*+101.90*	*51.10*	*2.58***	*1.14±0.02*	*+0.09*	*8.49*	*2.01**
10 mg/L	*9*	*223.33±44.06*	*+23.93*	*12.00*	*0.46*	*1.08±0.05*	*0.00*	*0.00*	*0.00*
					144 h				
Control	*10*	*160.30±15.78*				*0.85±0.02*			
0.1 mg/L	*10*	*343.90±45.68*	*+183.60*	*114.54*	*3.80****	*0.97±0.03*	*+0.12*	*14.12*	*3.33****
0.5 mg/L	*10*	*460.50±32.87*	*+300.20*	*187.27*	*8.23****	*1.02±0.01*	*+0.17*	*20.00*	*7.60****
1 mg/L	*10*	*285.60±42.72*	*+125.30*	*78.17*	*2.75***	*0.94±0.05*	*+0.09*	*10.59*	*1.67*
10 mg/L	*9*	*222.78±36.18*	*+62.48*	*30.98*	*1.58*	*0.90±0.05*	*+0.05*	*5.88*	*0.93*

*Note: * - P<0.05 (S); ** - P<0.01 (S); *** - P<0.001 (HS); P>0.05 (NS)*

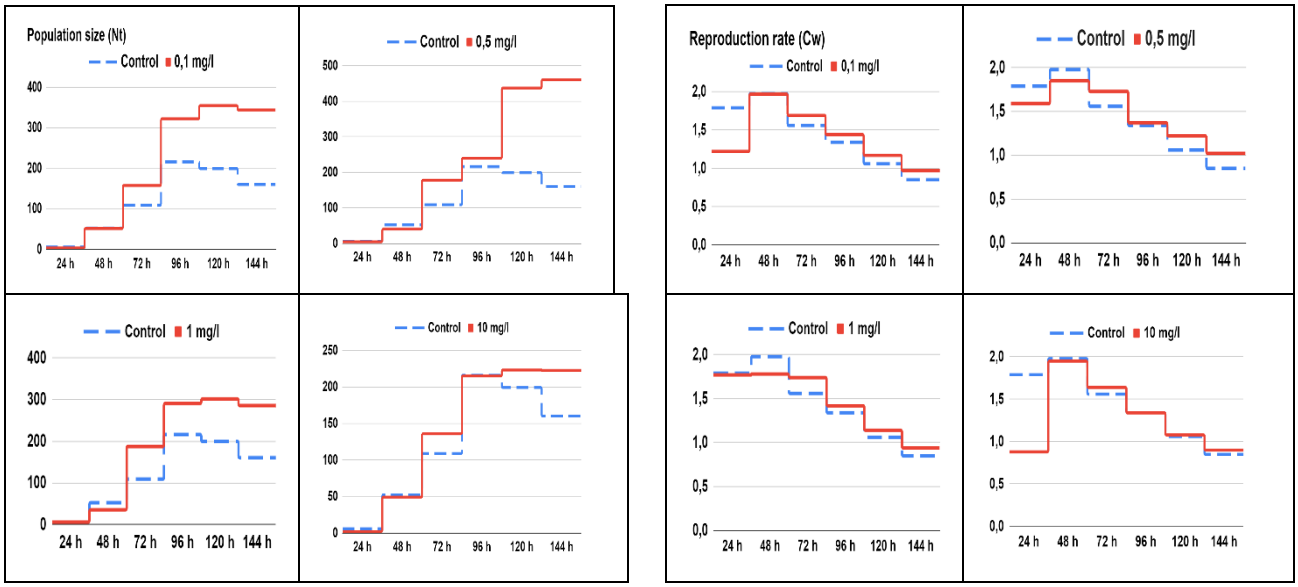

Figure 1. The dynamics of the population size (Nt) and reproduction rate (Cw) of *Paramecium* after administration of 0.1 mg/L, 0.5 mg/L, 1 mg/l, and 10 mg/L of *Chlorella vulgaris* medium over 24…144 hours (ĩ(hours)).

After 144 h incubation, it was observed that at a concentration of 0.1 mg/L, Nt increased significantly by +183.60 with an increase of 114.54% (t_d=3.80; P<0.001), while at 0.5 mg/L, it increased by +300.20, with a substantial increase of 187.27% (t_d=8.23; P<0.001). The statistical correlation is highly significant (HS, 99.9% confidence). Concentrations higher than 1 and 10 mg/L showed positive effects but were statistically insignificant (tab. 1).

Thus, the experimental results demonstrated that the tested concentrations of *Chlorella vulgaris* on the ciliate *P. caudatum* positively influence the abundance (Nt) and reproduction rate (Cw), with these effects increasing at higher concentrations, especially over longer time intervals.

To further investigate the effects of the analyzed test, the solution in which the microalga Chlorella vulgaris was cultivated was introduced into the nutrient medium of the paramecia. It was demonstrated, that after 24 h of incubation, concentrations lower than 0.1, 0.5, and 1 mg/L had significant negative effects, leading to a decrease in Nt and Cw. The deviation is at the 5% probability level when P value <0.05 (S). The concentration of 1 mg/L had the greatest negative impact, with a significant and considerable reduction in Nt, indicated by a difference of -2.40 and a significant percentage decrease of -40.00%, and in Cw indicated by a difference of -0.40 and a significant percentage decrease of -22.35%. The deviation is negatively significant (P<0.001) at a 0.1% probability level. The concentration of 10 mg/L has a nonsignificant positive impact of +0.30 (5.00%), suggesting a possible stimulating effect (tab. 2, fig. 2).

After 72 hours of incubation, the concentration of 0.1 mg/L continues to have a negative influence, but with values closer to the control values, with a difference of only - 2.22 (2.04%). As the concentrations increased, the effects became positive, remaining nonsignificant at 0.5 and 1 mg/L, and significant at 10 mg/L. At the concentration of 10 mg/L, Nt increases by 93.68% (t_d=4.90; P<0.001) and Cw by 14.10% (t_d=4.31; P<0.001) compared to the control. The deviation is statistically significant when the probability (P) is 0.1%. The values decreased after 120 hours, then increased again after 144 hours.

At 144 hours of incubation, the concentration of 0.1 mg/L improved both the population size and reproduction rate, approaching the control level. Concentrations of 0.5, 1, and 10 mg/L generated a significant increase compared to previous intervals, surpassing the control in the case of 1 mg/L concentration by 73.30% and 10 mg/L by 52.42%.

The results demonstrated that the cultural media of the microalgae *Chlorella vulgaris* at higher concentrations of 1 and 10 mg/L had a more pronounced effect, positively influencing popu-

Table 2. Experimental results of testing the liquid culture of the microalgae *Chlorella vulgaris* as food for *Paramecium caudatum* ciliates

Experimental groups	N	Liquid culture of the microalgae *Chlorella vulgaris*							
		Population size (Nt) M±ES	Difference compared to the control			Reproduction rate (Cw)M±ES	Difference compared to the control		
			d	%	t_d		d	%	t_d
24 h									
Control	10	6.00±0.67				1.79±0.11			
0.1 mg/L	9	3.89±0.57	-2.11	35.16	2.40*	1.36±0.14	-0.43	24.02	2.42*
0.5 mg/L	8	4.00±0.40	-2.00	33.33	2.56*	1.39±0.11	-0.40	22.35	2.57*
1 mg/L	10	3.60±0.39	-2.40	40.00	3.10**	1.28±0.11	-0.51	28.49	3.28***
10 mg/L	10	6.30±1.64	+0.30	5.00	0.17	1.84±0.26	+0.05	2.79	0.18
72 h									
Control	9	109.00±18.69				1.56±0.05			
0.1 mg/L	9	106.78±13.14	-2.22	2.04	0.10	1.56±0.04	0.00	0.00	0.00
0.5 mg/L	10	120.30±33.65	+11.30	10.37	0.29	1.60±0.10	+0.04	2.56	0.36
1 mg/L	10	142.30±24.59	+33.30	30.55	1.08	1.65±0.06	+0.09	5.77	1.15
10 mg/L	9	211.11±9.23	+102.11	93.68	4.90***	1.78±0.01	+0.22	14.10	4.31***
120 h									
Control	10	199.40±27.14				1.06±0.04			
0.1 mg/L	10	160.10±33.02	-39.30	19.71	0.92	1.02±0.05	-0.04	3.77	0.62
0.5 mg/L	10	208.10±39.40	+8.70	4.36	0.18	1.07±0.05	+0.01	0.94	0.16
1 mg/L	9	147.56±30.85	-51.84	26.00	1.26	1.00±0.08	-0.06	5.66	0.67
10 mg/L	10	169.10±24.88	-30.30	15.20	0.82	1.03±0.03	-0.03	2.83	0.60
144 h									
Control	10	160.30±15.78				0.85±0.02			
0.1 mg/L	10	183.70±27.18	+23.40	14.60	0.74	0.87±0.04	+0.02	2.35	0.45
0.5 mg/L	9	188.56±37.49	+28.26	17.63	0.69	0.87±0.03	+0.02	2.35	0.55
1 mg/L	10	277.80±33.81	+117.50	73.30	3.15**	0.94±0.03	+0.09	10.59	2.50*
10 mg/L	9	244.33±42.26	+84.03	52.42	1.86*	0.92±0.05	+0.07	8.24	1.30

Note: * - P<0.05 (S); ** - P<0.01 (S); *** - P<0.001 (HS); P>0.05 (NS)

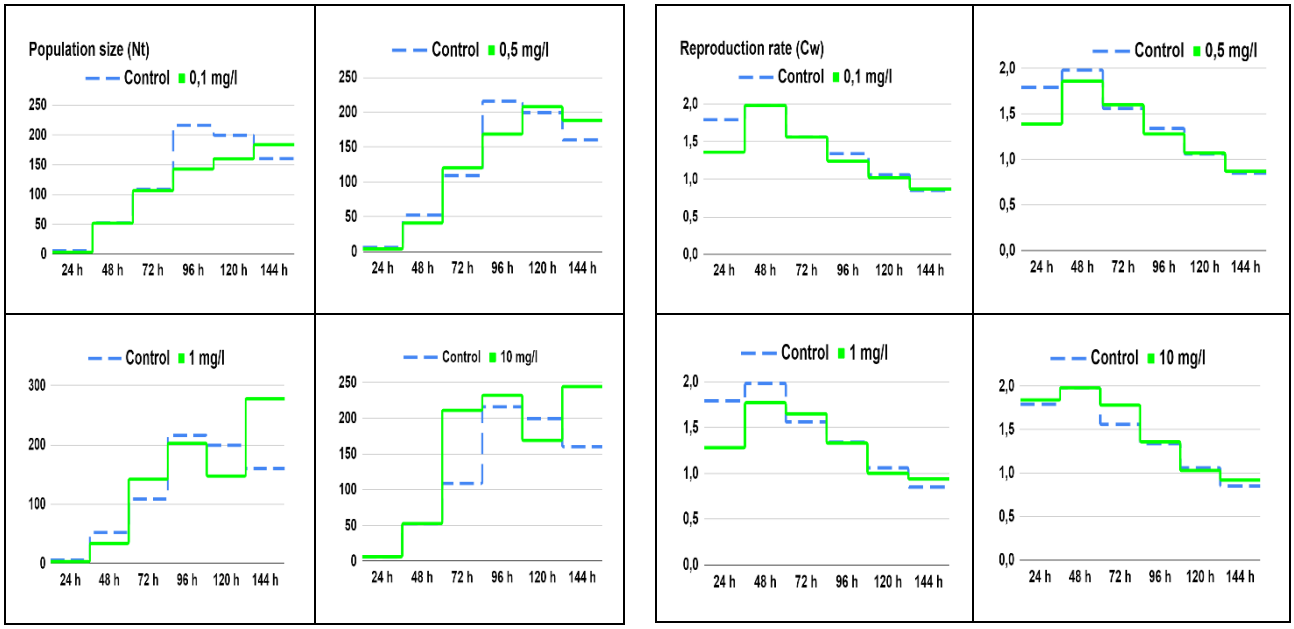

Figure 2. The dynamics of population size (Nt) and reproduction rate (Cw) of *Paramecium* after administration of 0.1 mg/L, 0.5 mg/L, 1 mg/L, and 10 mg/L of the media with microalgae *Chlorella vulgaris* over 24…144 hours (ĩ(hours)).

lation size and reproduction rate at 72, 96, 120, and 144 hours of incubation. In contrast, at 24 and 48 hours, the effects were less consistent than at lower concentrations (0.1 and 0.5 mg/L). Therefore, with the increase in incubation time, the effects of the concentrations became positive.

At 144 hours the concentration of 1 mg/L indicated a significant increase (Nt-td = 3.15, P<0.01 (S); Cw-td = 2.50, P<0.05 (S)), suggesting a stimulatory effect at this concentration and duration. The results at 10 mg/L were more varied, demonstrating a complex influence on the *Paramecium* culture.

The next stage involved adding the microalgae *Scenedesmus quadricauda* to the nutrient medium of *Paramecium*.

After 24 hours of incubation of the experimental samples, it was observed that at 0.1 mg/L, the population size (Nt) of *Paramecium* increased by 14.29%, and the reproduction rate (Cw) increased by 11.20%, indicating a positive influence on reproduction. At 1 mg/L, on the contrary, we observed a highly significant decrease in Nt by 42.86% (t_d=3.54; P<0.001), and Cw decreased by 44.80% (t_d=3.15; P<0.01), suggesting a significant negative influence on reproduction. At 10 mg/L, *Nt* decreased by 46.29% (td=3.54; P<0.001), and *Cw* decreased by 49.60% (td=3.48; P<0.001), with the negative effects becoming more pronounced at this higher concentration. The statistical correlation is highly significant (HS, 99.9% confidence) (tab. 3).

Table 3. Experimental results of testing the culture containing microalgae *Scenedesmus quadricauda* as food for the ciliates *Paramecium caudatum.*

Experimental groups	N	*Scenedesmus quadricauda*							
		Population size (Nt) M±ES	Difference compared to the control			Reproduction rate (Cw)M±ES	Difference compared to the control		
			d	%	t_d		d	%	t_d
24 h									
Control	10	3.50±0.39				1.25±0.11			
0.1 mg/L	9	4.00±0.64	+0.50	14.29	0.67	1.39±0.02	+0.14	11.20	1.25
0.5 mg/L	10	3.10±0.29	-0.40	11.43	0.82	1.13±0.10	-0.12	9.60	0.81
1 mg/L	9	2.00±0.25	-1.50	42.86	3.54***	0.69±0.14	-0.56	44.80	3.15**
10 mg/L	8	1.88±0.24	-1.62	46.29	3.54***	0.63±0.14	-0.62	49.60	3.48***
72 h									
Control	9	90.00±33.64				1.50±0.12			
0.1 mg/L	10	101.00±24.27	+11.00	12.22	0.27	1.54±0.09	+0.04	2.67	0.27
0.5 mg/L	10	71.40±22.96	-18.60	20.67	0.46	1.42±0.13	-0.08	5.33	0.45
1 mg/L	8	51.57±13.34	-38.43	42.70	1.06	1.31±0.09	-0.19	12.67	1.27
10 mg/L	10	49.60±18.09	-40.40	44.89	1.06	1.30±0.12	-0.20	13.33	1.18
120 h									
Control	9	211.11±52.79				1.07±0.06			
0.1 mg/L	10	194.50±35.91	-16.61	7.87	0.26	1.05±0.05	-0.02	1.87	0.26
0.5 mg/L	10	267.00±46.50	+55.89	26.47	0.79	1.12±0.07	+0.05	4.67	0.54
1 mg/L	10	152.33±27.41	-58.78	27.84	0.99	1.01±0.04	-0.06	5.61	0.83
10 mg/L	8	114.50±31.48	-96.61	45.76	1.57	0.95±0.05	-0.12	11.21	1.54
144 h									
Control	9	262.67±23.76				0.93±0.02			
0.1 mg/L	10	203.50±29.23	-59.17	22.53	1.57	0.89±0.04	-0.04	4.30	0.10
0.5 mg/L	10	367.10±34.24	+104.43	39.76	2.51*	0.98±0.02	+0.05	5.38	1.77
1 mg/L	9	261.00±46.70	-1.67	0.63	0.03	0.93±0.03	0.00	0.00	0.00
10 mg/L	9	139.67±36.44	-123.00	46.83	2.83**	0.82±0.05	-0.11	11.83	2.04*

Note: * - P<0.05 (S); ** - P<0.01 (S); *** - P<0.001 (HS); P>0.05 (NS)

After 72 hours of incubation, the low concentration of 0.1 mg/L indicated positive values for Nt and Cw, although these effects were statistically insignificant (tab. 3). Meanwhile, the higher concentrations of 0.5, 1, and 10 mg/L led to a decrease in the studied parameters. The negative effects were less pronounced compared to 48 hours (fig. 3). This suggests a negative influence

of the *Scenedesmus* culture, but a statistically insignificant on the population size and reproduction rate at higher doses.

The concentration of 0.5 mg/L of *Scenedesmus* exposed for 120 h resulted in a non-significant increase in Nt (26.47%) and Cw (4.67%). As time progressed, at 144 h, the concentration of 0.5 mg/L significantly increased the mean Nt value by 39.76% (t_d=2.51; P<0.05) and contributed non-significantly to an increase in Cw by 5.38% (t_d=1.77; P>0.05) compared to the control (tab. 3, fig. 3). This indicates a significant positive effect. From 24 to 96 h, these values were lower, which can be explained by the adaptation period of the ciliates to the addition of *Scenedesmus quadricauda*, after which these indices increased.

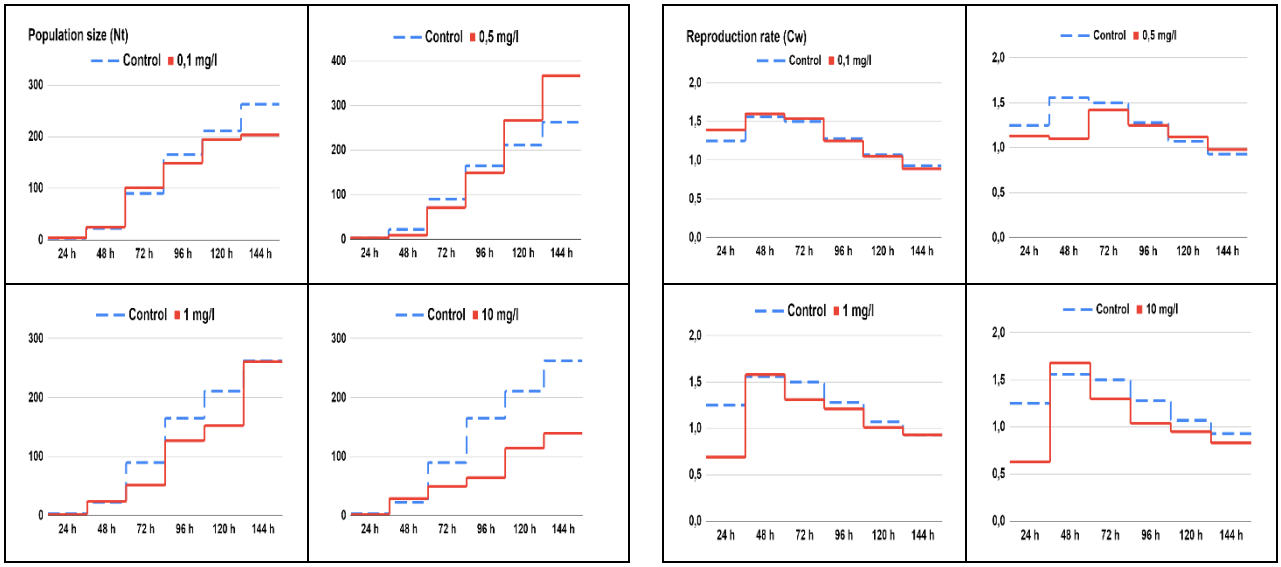

Figure 3. The dynamics of the population size (Nt) and reproduction rate (Cw) of *Paramecium* after exposure to 0.1 mg/L, 0.5 mg/L, 1 mg/L, and 10 mg/L of *Scenedesmus quadricauda* culture for 24...144 hours.

In the *Paramecium* culture medium, liquid cultures of *Scenedesmus quadricauda* were added at concentrations of 0.1, 0.5, 1, and 10 mg/L. The experimental results showed that after 24 hours of incubation, the concentrations that negatively affected *Paramecium* were 0.1 and 10 mg/L (tab. 4, fig. 4). The *Nt* values decreased by 24.86% (0.1 mg/L) and 26.86% (10 mg/L), and Cw values decreased by 22.40% (0.1 mg/L) and 24.80% (10 mg/L) compared to control values. The significance threshold is P>0.05, so the values were insignificant (IS). The negative effect was more pronounced at the higher dose. At 1 mg/L, Nt increases by 3.71%, and Cw increases by 3.20%. While this indicates a positive effect, it is minimal and nearly equivalent to the control values.

In Table 4, it was observed that after 72 hours of incubation, at a concentration of 0.1 mg/L, Nt decreased by 6.55%, and Cw by 1.33%, demonstrating a negative but minor effect. The concentration of 0.5 mg/L had a positive effect on *Paramecium*, but insignificant, Nt increased by 66.44% (t_d=1.16; P>0.05), and Cw increased by 11.33%. At a concentration of 1 mg/L, Nt significantly decreased by 67.22% (t_d=1.16; P>0.05), and Cw significantly decreased by 24.67% (t_d=2.66; P>0.01(S)). The deviation is significant since t_d (t-Student significance test) is greater than 2.58 with a 99% confidence interval. At 10 mg/L, Nt decreased by 43.09%, and Cw decreased by 12.67%. However, the negative effect was less pronounced compared to 1 mg/L.

After 120 hours, at 0.1 mg/L, Nt increased by 10.37%, and Cw increased by 1.87%. This is a positive but modest effect, stimulating the numerical growth of the *P. caudatum* culture and maintaining a relatively constant reproduction rate. At 0.5 mg/L, there was a significant inhibitory effect, resulting in a decrease in Nt by 21.89% and Cw by 4.67%. The 1 mg/L dose had a more pronounced inhibitory effect, causing a reduction in both parameters, Nt by 24.73%, and Cw by 5.61%. The negative effect is more pronounced compared to 24 hours. Conversely, the

10 mg/L dose had a significant stimulatory effect, contrary to expectations, leading to an increase in both the number and reproduction rate, Nt by 28.46%, and Cw by 4.67%. This effect was positive but statistically insignificant, with a significance threshold of P>0.05 (IS).

After 144 hours the concentration of 0.1 mg/L continued to have a positive but insignificant effect, maintaining a numerical increase and a relatively constant reproduction rate, Nt increased by 9.77%, and Cw increased by 1.08%. Higher concentrations of 0.5 mg/L, 1 mg/L, and 10 mg/L had negative effects on the *Paramecium* (fig. 4). For example, the concentration of 0.5 mg/L had an extremely pronounced inhibitory effect, leading to a decrease in both the number and reproduction rate. Doses of 1 mg/L and 10 mg/L had a more or less moderate, but still inhibitory effect, resulting in a significant reduction in both parameters.

Thus, *Scenedesmus quadricauda* and its culture medium, tested as food for paramecia, significantly influence reproduction, with both positive and negative effects depending on the administered dose and exposure time. Lower concentrations such as 0.1 mg/L and 0.5 mg/L, generally had a stimulative effect on reproduction, while higher concentrations of 1 mg/L and 10 mg/L had varied effects, expressed through increases and decreases in parameters, suggesting a more complex effect.

The research allowed us to assess the degree of ecological plasticity of the natural population of *P. caudatum* in relation to environmental factors, which will serve as a scientific basis for the development of safe monitoring and conservation measures for the species.

Table 4. Experimental results of testing the liquid culture of the microalgae *Scenedesmus quadricauda* as food for *Paramecium caudatum* ciliates.

Experimental groups	N	Liquid culture of the microalgae *Scenedesmus quadricauda*							
		Population size (Nt) M±ES	Difference compared to the control			Reproduction rate (Cw) M±ES	Difference compared to the control		
			d	%	t_d		d	%	t_d
24 h									
Control	10	3.50±0.39				1.25±0.11			
0.1 mg/L	8	2.63±0.45	-0.87	24.86	1.46	0.97±0.18	-0.28	22.40	1.33
0.5 mg/L	10	2.90±0.37	-0.60	17.14	1.12	1.06±0.15	-0.19	15.20	1.02
1 mg/L	8	3.63±0.45	+0.13	3.71	0.22	1.29±0.14	+0.04	3.20	0.22
10 mg/L	9	2.56±0.36	-0.94	26.86	1.77	0.94±0.15	-0.31	24.80	1.67
72 h									
Control	9	90.00±31.73				1.50±0.12			
0.1 mg/L	10	84.10±20.40	-5.90	6.55	0.16	1.48±0.11	-0.02	1.33	0.12
0.5 mg/L	10	149.80±40.55	+59.80	66.44	1.16	1.67±0.09	+0.17	11.33	1.13
1 mg/L	10	29.50±7.54	-60.50	67.22	1.86	1.13±0.07	-0.37	24.67	2.66**
10 mg/L	9	51.22±6.61	-38.78	43.09	1.20	1.31±0.04	-0.19	12.67	1.50
120 h									
Control	9	211.11±52.79				1.07±0.06			
0.1 mg/L	9	233.00±37.80	+21.89	10.37	0.34	1.09±0.03	+0.02	1.87	0.30
0.5 mg/L	10	164.90±31.58	-46.21	21.89	0.75	1.02±0.04	-0.05	4.67	0.69
1 mg/L	10	158.90±33.66	-52.21	24.73	0.83	1.01±0.04	-0.06	5.61	0.83
10 mg/L	10	271.20±47.03	+60.09	28.46	0.85	1.12±0.03	+0.05	4.67	0.75
144 h									
Control	9	262.67±23.76				0.93±0.02			
0.1 mg/L	9	288.33±60.47	+25.66	9.77	0.39	0.94±0.07	+0.01	1.08	0.14
0.5 mg/L	8	174.88±49.72	-87.79	33.42	1.59	0.86±0.06	-0.07	7.53	1.11
1 mg/L	9	240.11±35.87	-22.56	8.59	0.52	0.91±0.03	-0.02	2.15	0.55
10 mg/L	8	215.00±41.60	-47.67	18.15	1.00	0.90±0.03	-0.03	3.23	0.83

Note: * - P<0.05 (S); ** - P<0.01 (S); *** - P<0.001 (HS); P>0.05 (NS)

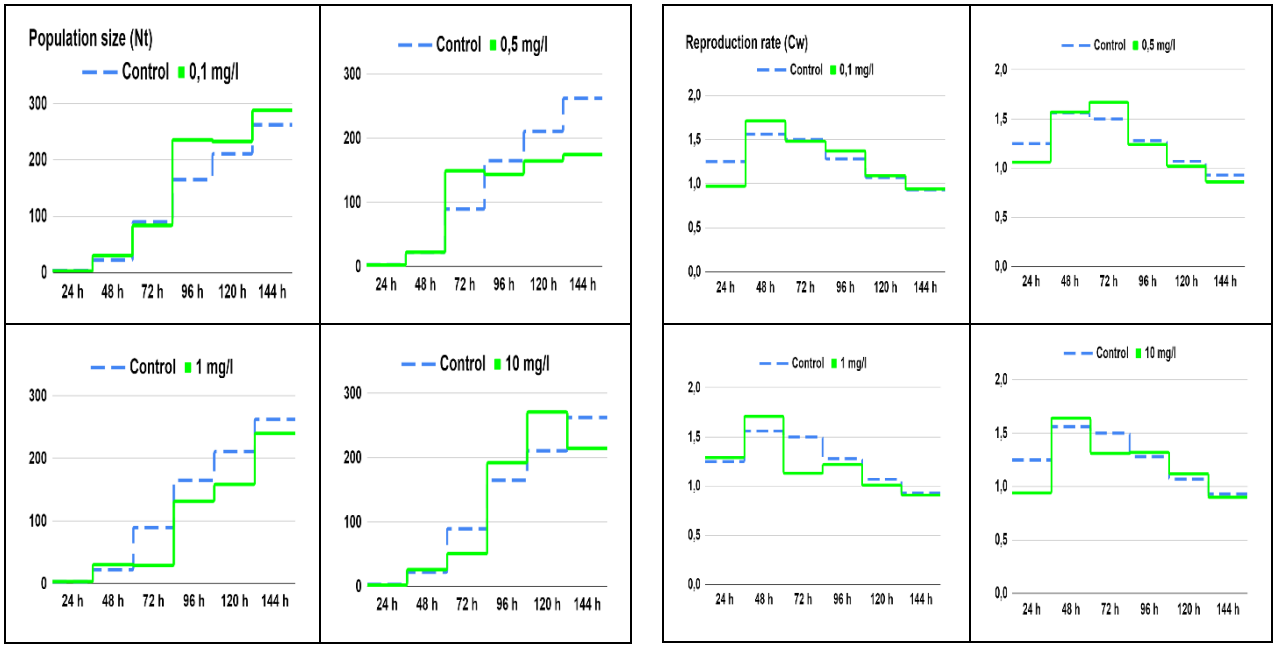

Figure 4. The dynamics of the population size (Nt) and reproduction rate (Cw) of *Paramecium* after exposure to 0.1 mg/L, 0.5 mg/L, 1 mg/L, and 10 mg/L o f *Scenedesmus quadricauda* culture medium for 24...144 hours.

DISCUSSIONS

Microalgae of the phylum *Chlorophyta* play a crucial role in the planet's energy cycle through their ability to harness solar energy and convert it into food. Of the notable species, *Chlorella vulgaris* and *Scenedesmus quadricauda* serve as the foundation of many aquatic ecosystems. Through photosynthesis, they produce oxygen and convert carbon dioxide into organic material, playing a key role in the global carbon cycle. This process sustains algae and generates the primary food source for various aquatic organisms. Thus, these investigations not only contribute to the development of our knowledge of ecological mechanisms, but also emphasize the importance of the conservation and proper management of these fragile environments.

Some studies have developed feeding protocols for bee families at the end of winter, a period of limited foraging, using nutrient paste enriched with biologically active supplements derived from the biomasses of aquatic monocellular microalgae, including *Scenedesmus quadricauda*, *Scenedesmus apiculatus*, and *Oocystis borgei*. The research has shown that feeding bee families enriched with biologically active supplements from the biomass of aquatic microalgae contributes to an increase in the prolificacy of brood – by 7.8-10.3%, in the quantity of brood capped – by 7.7-9.3%, in the bee family's power – by 7, 1-9.3%, family disease resistance – by 1.8-3.8%, bro brood viability in the brood nest – by 1.2-1.7%, amount of accumulated brood in the brood nest – by 15.527.6%, amount of wax grown on combs – by 13.3-36.7% and amount of accumulated honey in the brood nest – by 28.0-38.9% (2).

Additional studies on the use of *Chlorella vulgaris* and *Scenedesmus quadricauda* microalgae have exerted a positive effect on the germination of sugar seed by increasing the efficiency and regularity of this critical process for *Beta vulgaris* seeds. The best results, in germination indices as well as root morphological traits, were reached by using *C. vulgaris* extract at medium concentrations of 1 mg Corg/L and 2 mg Corg/L, while the *S. quadricauda* extract, the concentration effects on germination indices were less evident and differences among concentrations were not significant. Only one concentration of 1 mg Corg/L, had a positive effect (3).

In comparison to previous research, we examined the effects of these two microalgae on the unicellular organism *Paramecium caudatum*, particularly their influence on interactions within an aquatic ecosystem.

Our experimental results indicated that *Chlorella vulgaris* and its medium generally had a significant stimulatory effect on *P. caudatum* during the time intervals of 72 to 144 hours. It is important to note that interpretation may vary depending on the specific context of the experiment and the tested components. Data analysis shows that responses to the tested components are complex and depend on multiple factors, including concentration, time interval, and complex interactions between them.

The microalga *Chlorella vulgaris* showed a more pronounced positive effect compared to its liquid media at time intervals of 120 and 144 hours, overall having a significant stimulating effect on *P. caudatum*.

Lower concentrations (0.1 and 0.5 mg/L) had more pronounced effects, generating significant increases of parameters (Nt and Cw). Higher concentrations (1 and 10 mg/L) also had a stimulatory effect, but with variability in intensity, including an unexpected stimulation at the dose of 10 mg/L. The results demonstrate that *Chlorella vulgaris* may serve as a potential food source for *P. caudatum*.

Microalgae *Scenedesmus quadricauda* and its culture medium, tested as food for paramecia, significantly influence reproduction, with both positive and negative effects depending on the administered dose and exposure time. Lower concentrations, such as 0.1 mg/L and 0.5 mg/L, generally had a stimulative effect on reproduction. Higher doses of 1 mg/L and 10 mg/L had varied effects, expressed through increases and decreases in parameters, suggesting a more complex effect. The exposure time (hours) of 24h, 48h, 72h, 96h, 120h, and 144h plays a crucial role on *P. caudatum* response to their presence.

Both microalgae, *Chlorella vulgaris* and *Scenedesmus quadricauda*, generally exhibited stimulative effects on *Paramecium caudatum* compared to their culture media.

Lower doses of both microalgae tended to be more effective in stimulating the growth and reproduction of *P. caudatum.*

Higher doses may have varied effects, suggesting the need for careful control of concentrations used in laboratory practices or potential practical applications.

Research on the interactions between microalgae in the phylum *Chlorophyta*, such as *Chlorella vulgaris* and *Scenedesmus quadricauda* and organisms like *Paramecium caudatum*, can provide the necessary understanding in restoring water quality in water pools. Furthermore, exploring the use of these microalgae as a nutritional supplement can bring significant benefits to agriculture and environmental conservation.

CONCLUSIONS

1. In conclusion, lower concentrations of tested *Chlorella vulgaris* culture (except for the 10 mg/L concentration in certain cases) have a positive effect on the natural population of *P. caudatum*, especially over longer time intervals.
2. The tested concentration of 0.5 mg/L *Chlorella vulgaris* in *P. caudatum* food significantly influence the population size and reproduction rate of the ciliate, with these effects increasing over the incubation period, by 119.31% at 120 h and 187.27% at 144 h compared to the control.
3. Higher concentrations (1 and 10 mg/L) of *Chlorella vulgaris* culture medium in *P. caudatum* food positively influence the population size and reproduction rate at 72, 96, 120, and 144 h of incubation, while the effects at 24 and 48 h are less consistent. The concentration with significant stimulating effect was 1 mg/L, generating increases in numerical abundance (by 73.30%) and reproduction rate (by 10.59%) compared to the control.
4. The effects of *Scenedesmus quadricauda* and its culture medium, tested as food for *P. caudatum*, were differentiated based on dose and time interval.
5. Lower doses (0.1 and 0.5 mg/L) generally produced a significant stimulatory effect, resulting in notable increases in both population size and reproduction rate. In contrast, higher doses (1 and 10 mg/L) exhibited varied effects, including both increases and decreases in parameters, indicating a more complex interaction.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

ACKNOWLEDGMENT

The cost for this study were covered by Subprogram 010701: "Evaluation of the structure and functioning of animal world and aquatic ecosystems under the influence of biotic and abiotic factors in the context of ensuring ecological security and the well-being of the population" within the Institute of Zoology, MSU.

REFERENCES

1. Baglieri A, Sidella S, Barone V, Fragalà F, Silkina A, Nègre M, Gennari M. Cultivating *Chlorella vulgaris* and *Scenedesmus quadricauda* microalgae to degrade inorganic compounds and pesticides in water. *Environmental Science and Pollution Research (ESPR).* 2016;23:18165-18174. doi:10.1007/s11356-016-6996-3
2. Cebotari V, Toderaş I, Buzu I, Ungureanu L. Măsuri de ajustare a apiculturii convenţionale la cea organică în Republica Moldova [Measures of converting the conventional beekeeping into the organic beekeeping in the Republic of Moldova.]. *Buletinul Academiei de Ştiinţe a Moldovei. Ştiinţele vieţii*. 2020;3(342):7-28. Available at: https://ibn.idsi.md/ro/vizualizare_articol/130424 (Accessed 06.09.2024).
3. Puglisi I, Barone V**,** Fragalà F, Stevanato P, Baglieri A, Vitale A. Effect of Microalgal Extracts from *Chlorella vulgaris* and *Scenedesmus quadricauda* on Germination of *Beta vulgaris* Seeds. *Plants.* 2020;*9*(6):675. doi:10.3390/plants9060675
4. Chesnokova S, Chugay N. Biologicheskie metody otsenki kachestva ob"ektov okruzhayushchey sredy: ucheb. posobie. [Biological methods for assessing the quality of environmental objects]. ladimir: Izd-vo Vladim. gos. un-ta. 2008. Available at: https://dspace.www1.vlsu.ru/handle/123456789/5105 (Accessed 06.09.2024).
5. Pisman T, Galayda Ya, Shirobokova I. Experimental and mathematical modeling of the consumer's influence on productivity of algae in a model aquatic ecosystem. *Advances in Space Research*. 2005;35(9):521-1527. doi:10.1016/j.asr.2004.12.048
6. Sadchikov A. P. Kul'tivirovaniie vodnykh i nazemnykh bespozvonochnykh: printsipy i metody: ucheb. Posobie. [Cultivation of aquatic and terrestrial invertebrates: principles and methods: textbook.]. MAKS Press. 2009. Available at: https://books.google.it/books?id=0M99kgAACAAJ (Accessed 05.09.2024).
7. Kokova V. Nepreryvnoie kul'tivirovanie bespozvonochnykh. [Continuous cultivation of invertebrates.]. Novosibirsk. «Nauka» Sibirskoie otdeleniie. 1982. Available at: https://shorturl.at/rZS3d (Accessed 05.09.2024).
8. Likhachev S, Pimenova Ye, Zhakova S. Biotestirovanie v ekologicheskom monitoringe: uchebno-metodicheskoie posobiie. [Biotesting in environmental monitoring: educational manual.]. Ministerstvo sel'skogo khoziaistva Rossiiskoi Federatsii, federal'noie gosudarstvennoie biudzhetnoie obrazovatel'noie uchrezhdeniie visshego obrazovaniia «Permskii gosudarstvennyi agrarno-tekhnologicheskii universitet imeni akademika D.N. Prianishnikova». Perm': IPTS «Prokrost». 2020;64–71. Available at: https://shorturl.at/VoBdD (Accessed 04.09.2024).
9. Zaika V. Sravnitel'naya produktivnost' gidrobiontov. [Comparative productivity of aquatic organisms.]. Kiiev: Nauk. Dumka, 1983. https://files.znu.edu.ua/files/Bibliobooks/Domni/0000918.pdf (Accessed 04.09.2024).
10. Spinei L, Lozan O, Badan V. Biostatistica [Biostatistics]. Univ. de Stat de Medicină şi Farmacie „Nicolae Testemiţanu". 2009;186. Available at: https://management.usmf.md/sites/default/files/inline-files/Biostatistica.pdf (Accessed 04.09.2024).
11. Wasser S, Kondrat'yeva N, Masyuk N. i dr. Vodorosli. [Algae.]. Spravochnik. Kiiev: Nauk. Dumka, 1989. Available at: https://shorturl.at/eTAlK (Accessed 04.09.2024).

Date of receipt of the manuscript: 07/06/2024
Date of acceptance for publication: 30/09/2024