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Propagation of Chlorella Vulgaris and Scenedesmus Obliquus in Dengizkul Lake and determination of protein content in them

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Abstract. The relative differentiation of the protein content in green algae grown in the contours of the Dengizkul reservoir was due to the fact that the level of mineralization of the water of the contours was low at the entrances(mouth) of the lake and high at the contours remote from it. Therefore, when growing green algae with a high protein content of Chlorella vulgaris Beijer. and Scenedesmus obliquus (Turp.) Kütz., the most optimal nutrient medium is the variant in which the juice of poultry manure is mixed with 1,2,3 g / l of water. The recommendations on the breeding of Chlorella vulgaris Beijer. and Scenedesmus obliquus (Turp.) Kütz. suspension grown in pools in semi-productive conditions by feeding fish Hypophthalmichthys molitrix are scientifically substantiated.

1 Introduction

The hydrochemical composition of water in all contours of lake Dengizkul has been studied, algologically pure cells of Chlorella vulgaris and Scenedesmus obliquus have been biotechnologically propagated in these contours, and the content of physiologically active substances of them has been determined. The water of Dengizkul has the favourable conditions for the growth and development of phytoplankton, however, with their intensive reproduction, minerals in the water are contained in insufficient quantities. For this, research was first conducted to find sources of natural raw materials which are rich in organic substances, that is, with a high nitrogen content. To obtain a high amount of green biomass for intensive reproduction of phytoplankton organisms, cattle manure and chicken (grown for meat) manure were used The content of nitrogen oxides and phosphorus in chicken manure is 6-7 times higher than in cattle manure. This circumstance, due to the mineral composition of the water of Dengizkul, made it possible to create a biotechnology for intensive cultivation of algae biomass with a high protein content in the reservoir due to the introduction of chicken manure into the nutrient medium. In our subsequent studies, the total protein content in laboratory-grown chlorella biomass, as well as stenedesmus algae, was studied by applying a solution of chicken manure in various concentrations to the nutrient medium. Chlorella vulgaris Beijer. and Scenedesmus obliquus (Turp.) Kütz., which are grown by mixing 4 g/l of solution of chicken manure with water from all the contours of

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the reservoir, it was found that the protein content in the cells of algae is at a high level, low level at 2 g/l and moderate level at 6 g/l. The protein content in Chlorella vulgaris Beijer. cells was 46%, 48% and 37% when mixing 4 g/l of chicken manure juice in the 1,2,3 rd contours of the reservoir, while the protein content in Scenedesmus obliquus (Turp.) Kütz. cells was 56%, 59% and 54%, respectively [1-24].

Due to climate change in the world, the depletion of natural resources and the deterioration of the environment, providing the population with high-quality food, especially fish, is today's the most significant task. Nowadays, special attention is paid to monitoring the hydrological and hydrochemical state of reservoirs of country, the development of the fishing industry, the efficient use of fish stocks, increasing the natural feed biomass of herbivorous fish in reservoirs using new biotechnological methods and integrated water use. Considering that the main part of the reservoirs of our republic is located on the plains, determining the current hydrochemical state of such lakes and reservoirs, the issues of developing effective biotechnological methods for increasing the amount of natural feed in reservoirs in order to increase the efficiency of growing herbivorous fish remain vital matter. The dengizkul reservoir is located 140 km from the city of Bukhara, 40 km southwest of the center of the Alat district, is a lake bordering the Republic of Turkmenistan. According to scientists, the total area of the Dengizkul reservoir is 35,500 [13] ha, while according to the Metropolskiy in 2020, the lake area was 26,000 ha (Fig.1.) [9].

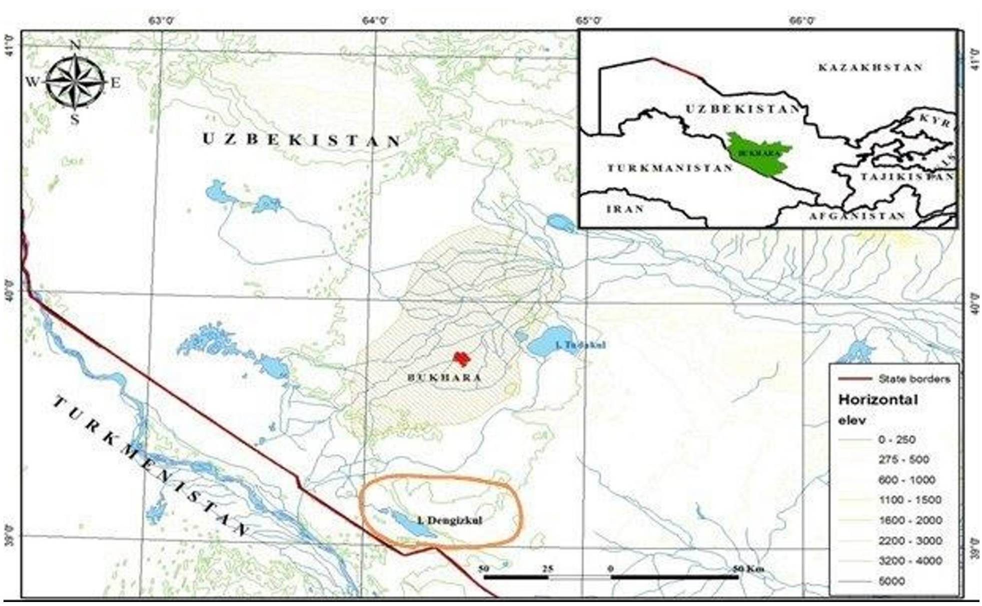

Fig. 1. Dengizkol map.

The area of the lake covered by water as of June 2021 is 25,649 hectares, and the water in the lake has a seasonally changing character in terms of hydrochemical composition. The level of water transparency in the lake ranges from 2.5 – 2.8 meters in January and February, and in summer in July and August this indicator consist of 0.5-1.5 meters. The Dengizkul natural reservoir was created in 2004 on the basis of the processes of privatization and denationalization of property and is a natural reservoir in which fishing activities are carried out, divided into 6 contours [12].

While the waters at the confluence of several reservoirs in reservoir Dengizkul are slightly saline (contours 1, 2 and 3), moderate and strong salinity is observed in the center and at the end of the lake [3].

2 Methods

When collecting hydrobiological data from the Dengizkul reservoir, the methods of such scientists as G.K. Plotnikov, I.G. Radchenko and N.A. Lemez [7, 14, 15] were used. In particular, two different methods were used to collect samples of phytoplankton organisms:

1. Quality;
2. Quantity.

For both methods we used the Apshteyn grid. The brand of the Apshteyn grid is Kapron No.76, the diameter of the water inlet is No.20. A specially made glass bowl was mainly used to quantify microscopic aquatic plants that spread across the reservoir [6, 22, 24]. Samples of phytoplankton organisms were collected from all contours of the Dengizkul reservoir, that is, from areas with a depth of 0.5 m to 1.5 m. The collected samples were placed in a 4% formalin solution for laboratory testing and stored in a dark place away from direct sunlight. In laboratory conditions, phytoplankton were divided into several species based on generally accepted methods of scientists such as V.M.Katanskaya and A.M.Muzafarov. The species of algae isolated from the water of the reservoir were examined using light microscopes of the XDS-3, B-380 brand, and the species composition of aquatic plants was studied using determinants of algae species by such scientists as A.M.Muzafarov, A.E.Ergashev, T.T.Taubayev, S. Keldibekov, V.M. Katanskaya, O.V. Anisimova, P.M. Tsarenko [1, 2, 4, 5, 10, 11, 15-22]. Water analyses were studied in laboratory conditions according to the methods of Y.Y.Lurye and N.S. Stroganov [8, 17]. The Keldal method was used to determine the total amount of protein in the biomass of Chlorella vulgaris Beijer and Scenedesmus obliquus grown by biotechnological methods in all contour waters of the Baikal reservoir [16]. The procedure of the experiment: an accurately measured sample weighing 1.0 g, a catalyst tablet and 25 ml of concentrated sulfuric acid are placed in the combustion flask and burned in the following mode: burned at 500 ° C for 6 hours. At the end of ignition, the sample passes into a transparent colorless state. Then the cooled flask is placed in the drive device. 100 ml of 30% sodium hydroxide solution is infused until the solution automatically turns black. To collect the ammonia gas released from the solution, methyl red is dripped into a solution of 0.1N sulfuric acid through a glass tube.

Then complete neutralization of the resulting solution is carried out using 0.1 N sodium hydroxide solution until the equivalence point of automatic weighing “Titrion-pH” is reached in the titrator. In parallel, a control experiment is conducted in which distilled water is used instead of a sample. The total amount of nitrogen is calculated by the formula: N%=(VH2SO4-VNaOH)*0.14/g. N%= total amount of nitrogen, %; VH2SO4= volume of 0.1 N sulfuric acid solution, ml; VNaOH = 0.1 N sodium hydroxide solution, ml; g = sample weight

3 Results and discussion

The results of chemical analysis of water samples taken from different points of the 6 contours of the Dengizkul reservoir show that the degree of mineralization of the water of the contours differs sharply from each other. This is explained by the fact that the degree of their water supply is different. The highest indicator of the degree of mineralization was detected in a water sample from contours 5 and 6 of lake Dengizkul. According to this, the total mineralization in contour 5 was 19.5 g/l, and in contour 6 was 20.0 g/l. It is noted that this mineralization occurs mainly due to chlorides and sulfates. In the 5th contour of the lake, the content of chlorides was 11.3 g/l, sulfates-6,128 g/l, in the 6th contour of chlorides-11.5 g/l, sulfates-6.3 g/l. During the chemical analysis of the samples obtained, it was found that the quantitative indicators of the remaining compounds: ammonium, nitrite, Nitrate and bicarbonates are in small amounts.

In order to increase the development of phytoplankton in the Dengizkul resrvoir, the formation of large volumes of biomass and their use as feed for fish farmimg, research was carried out to determine the composition of the nutrient medium by the chemical composition of minerals in the reservoir. To do this, research was first conducted to find sources of natural raw materials rich in organic substances, that is, with a high nitrogen content. It is known from the literature that animal manure contains a large amount of organic compounds, and nitrogenous compounds formed during the decomposition of these substances in water are absorbed by algae and synthesize protein substances. This, in turn, serves as a natural protein-rich food source for herbivorous fish in the lake.

In our experiments, taking into account the large number of livestock and poultry farms in the republic, cattle manure and poultry (grown for meat) manure were used. Poultry raised for meat is fed with feed with an unchanged composition under special conditions and in accordance with the established procedure. As a result, organic minerals in bird droppings do not change throughout the year (Table 1).

In the data given in the table, the content of nitrogen and phosphorus oxides in poultry manure is 6-7 times higher than in cattle manure. This circumstance, due to the mineral composition of Dengizkul water, makes it possible to create a biotechnology for intensive cultivation of algae biomass with a high protein content in the reservoir due to the introduction of bird droppings into the nutrient medium.

Table 1. Seasonal changes in the chemical composition of cattle and poultry manure.

№	Season	Type of manure	Mineral composition					
			N_2	K_2O	CaO	Mg	P_2O_5	H_2SO_3
1	Winter	Cattle manure	0,25	0,14	0,42	0,11	0,16	0,04
		Poultry manure	1,61	0,89	2,43	0,72	1,51	0,42
2	Spring	Cattle manure	0,31	0,14	0,52	0,11	0,13	0,06
		Poultry manure	1,60	0,88	2,42	0,71	1,50	0,41
3	Summer	Cattle manure	0,45	0,19	0,58	0,08	0,13	0,06
		Poultry manure	1,63	0,91	2,43	0,74	1,54	0,45
4	Autumn	Cattle manure	0,27	0,11	0,36	0,12	0,18	0,05
		Poultry manure	1,59	0,87	2,41	0,71	1,49	0,4

Chlorella vulgaris ва *Scenedesmus obliquus*(Turp.) Kütz. cells were planted in laboratory conditions, prepared from 2, 4 and 6 grams of bird droppings per 1 liter of water taken from the contours of lake Dengizkul, and their development and reproduction were observed. The development and reproduction of green algae was determined daily using

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Goryaev's camera under a microscope. The experiments were tested for 6 days, and at the end of the experiment, the amount of biomass was determined (Table 2).

Table 2. Cultivation of green algae in a solution of poultry manure in laboratory conditions.

№	Place of water sampling	Algae of the experiment	Manure juice g/l	Number of cells, mln/ml						Wet biomass g/l
				1 st day	2nd day	3rd day	4th day	5th day	6 th day	
1	Dengizkul 1 st contour	*Chlorella vulgaris* Beijer.	2	2,0 ±0,06	3,8 ±0,08	7,0 ±0,10	16,8 ±0,26	28,4 ±0,38	35,0 ±0,47	1,9 ±0,03
			4	2,0 ±0,03	4,1±0,09	8,5±0,13	21,0±0,29	36,5±0,44	42,5±0,54	2,3±0,08
			6	2,0,±0,04	3,9±0,07	8,3±0,12	18,6±0,27	33,2±0,42	39,4±0,49	2,1±0,06
		Scenedesmus obliquus (Turp.) Kütz.	2	2,0,±0,02	3,5±0,08	8,0±0,11	20,4±0,30	31,5±0,41	39,0±0,49	2,2±0,07
			4	2,0,±0,03	4,2±0,09	9,0±0,14	22,0±0,31	37,5±0,48	46,0±0,57	2,6±0,09
			6	2,0,±0,04	3,8±0,07	8,6±0,13	21,0±0,30	36,5±0,46	43,0±0,55	2,4±0,08
2	Dengizkul 3 rd contour	*Chlorella vulgaris* Beijer.	2	2,0,±0,05	3,8±0,08	8,4±0,14	17,2±0,27	31,4±0,40	37,5±0,48	2,0±0,03
			4	2,0,±0,06	4,2±0,09	9,5±0,116	22,5±0,32	38,5±0,49	44,8±0,54	2,4±0,07
			6	2,0,±0,03	3,9±0,08	9,0±0,15	21,5±0,31	36,5±0,46	42,6±0,53	2,4±0,08
		Scenedesmus obliquus(Turp.) Kütz.	2	2,0,±0,04	3,8±0,07	8,6±0,13	21,6±0,29	30,5±0,40	40,0±0,50	2,2±0,05
			4	2,0,±0,04	4,4±0,11	10,2±0,17	24,0±0,34	39,4±0,38	48,5±0,59	2,7±0,10
			6	2,0,±0,05	4,3±0,10	9,8±0,16	23,4±0,33	37,5±0,48	45,5±0,55	2,5±0,09
3	Dengizkul 5 th contour	*Chlorella vulgaris* Beijer.	2	2,0,±0,03	4,0±0,08	10,7±0,15	22,3±0,31	33,0±0,42	40,0±0,50	2,2±0,04
			4	2,0,±0,03	4,1±0,09	11,8±0,17	26,1±0,37	38,1±0,50	45,5±0,56	2,4±0,05
			6	2,0,±0,04	4,0±0,09	11,4±0,17	25,3±0,35	36,4±0,45	43,5±0,53	2,3±0,04
		Scenedesmus obliquus(Turp.) Kütz.	2	2,0,±0,03	3,9±0,08	10,6±0,17	22,3±0,26	31,4±0,42	42,5±0,51	2,3±0,04
			4	2,0,±0,05	4,5±0,09	12,3±0,18	26,1±0,37	40,3±0,53	49,5±0,61	2,7±0,11
			6	2,0,±0,03	4,3±0,09	10,7±0,17	24,1±0,34	38,3±0,51	46,5±0,56	2,6±0,10

According to the data given in the table, high efficiency was observed when applying 4 grams of poultry manure along all contours. In particular, in contour 1, the number of *Chlorella vulgaris* Beijer. cells in 2 grams of poultry manure was 35 million/ml, in 4 grams of poultry manure -42.5 million/ml, in 6 grams of poultry manure -39.4 million/ml, in circuit 3, the number of cells was 37.5 million/ml in 2 grams of poultry manure, in 4 grams of poultry manure 44.8 ml/ml, in 6 grams of poultry manure 42.6 million/ml, in contour 5 the number of cells was observed 40.0 ml/ml in 2-gram poultry manure, 45.5 ml in 4-gram poultry manure and 43.5 ml/ml in 6-gram poultry manure. However, in all experiments with 6 grams of poultry manure, there was a decrease compared to 4 grams of poultry manure.

In the first contour *Scenedesmus obliquus*(Turp.) Kütz. cells at the end of the experiment contained 39.0 million/ml in 2 grams, 46.0 million/ml in 4 grams, 43.0 million/ml in 6 grams, 40.0 million/ml in 2 grams of contour 3, 48.5 million/ml in 4 grams, 45.5 million/ml in 6 grams, 42.5 million/ml in 2 grams of contour 5, 49.5 million/ml in grams, 46.5 million/ml in 6 grams, an increase in cells was detected.

It is known from the literature that the amount of protein in the resulting biomass varies depending on the composition of the nutrient medium used in the biotechnology of intensive algae cultivation. The protein content in *Chlorella vulgaris* Beijer. is up to 52.8%, and the protein content in *Scenedesmus obliquus* (Turp.) Kütz. is up to 68.7 [23-25].

Because of this, in our subsequent studies, we studied the total protein content in the biomass of *Chlorella vulgaris* Beijer. and *Scenedesmus obliquus* (Turp.) Kütz. algae grown in laboratory conditions by applying poultry manure juice in various concentrations to the nutrient medium. The results of the experiment are presented in 2nd and 3rd pictures.

Chlorella vulgaris Beijer. cells grown by mixing 4 g/l of poultry manure juice with waters of all contours of the Dengizkul reservoir showed a high protein content, but low result in 2 g/l and average in 6 g/l.

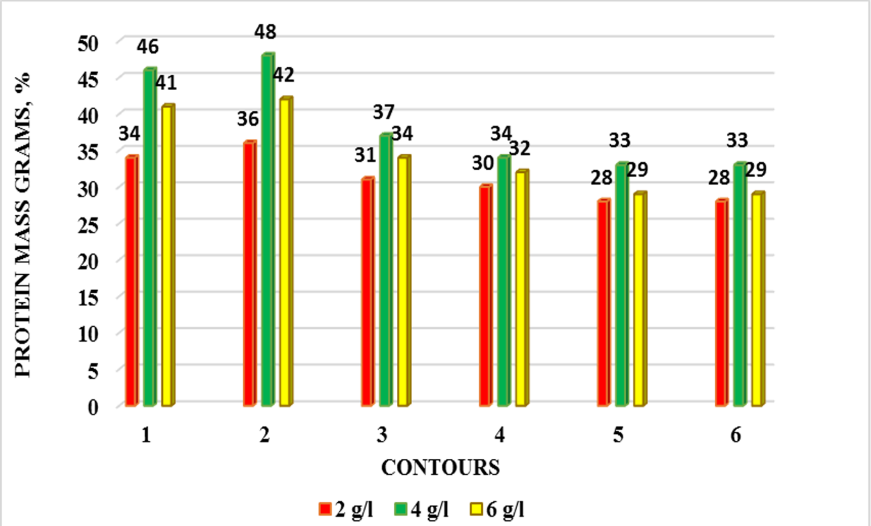

Fig. 2. Total protein content in *Chlorella vulgaris* Beijer. Grown in the Dengizkul reservoir (%).

When comparing the contours of the lake, it was found that the protein content in *Chlorella vulgaris* Beijer. cells in contours 1,2,3 is 46%, 48% and 37%, and in contours 4,5,6-34%, 33% and 33%. We can cite the fact that the greatest productivity falls on contours 1 and 2.

The protein content in *Scenedesmus obliquus* (Turp.) Kütz. cells grown by mixing 2,4,6 g/l of poultry manure juice in the waters of all the contours of the lake was studied in the laboratory. Results: cells grown by mixing 4 g/l of poultry manure juice in contours 1,2,3 had a high protein content, i.e. 56%, 59% and 54%, respectively. It was noted that at 2 g / l it is 44%, 46% and 42%, and at 6 g / l -51%, 52% and 48%. For the remaining contours of the watershed, the decrease in this indicator is shown in Figures 2 and 3.

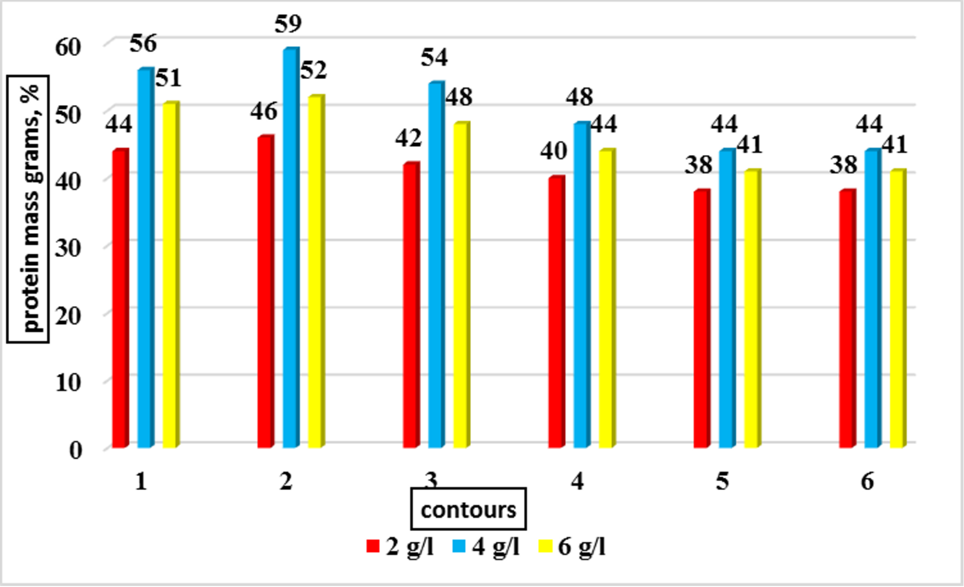

Fig. 3. Total protein content in *Scenedesmus obliquus* (Turp.) Kütz. grown in the Dengizkul reservoir.

4 Conclusion

The relative differentiation of the protein content in green algae grown in the contours of the Dengizkul reservoir was due to the fact that the level of mineralization of the water of the contours was low at the entrances(mouth) of the lake and high at the contours remote from it. Therefore, when growing green algae with a high protein content of *Chlorella vulgaris* Beijer. and *Scenedesmus obliquus* (Turp.) Kütz., the most optimal nutrient medium is the variant in which the juice of poultry manure is mixed with 1,2,3 g / l of water.

The recommendations on the breeding of *Chlorella vulgaris* Beijer. and *Scenedesmus obliquus* (Turp.) Kütz. suspension grown in pools in semi-productive conditions by feeding fish Hypophthalmichthys molitrix are scientifically substantiated.

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References

1. O. V. Anisimova, M. A. Gololobova, Kratkiy opredelitel' rodov vodorosley (Universitet, Moscow, 2006)

2. S. B. Buriyev, Kh. K. Esanov, F. K. Shodmonov, "CHRONOS" Mul'tidistsiplinarnyye Nauki **6**, №5(55), 4-7 (2021) https://multidisciplinary.chronos-journal.ru/volume-6-issue-5-55/
3. G. K. Kamilov, B. Karimov, B. Khakberdiyev et al., Vodoyemy Uzbekistana (bez Karakalpaki) i ikh rybokhozyaystvennoye znacheniye («Universitet», Tashkent, 1994)
4. V. M. Katanskaya, Vysshaya vodnaya rastitel'nost' kontinental'nykh vodoyomov. Metody izucheniya (Nauka, Leningrad, 1981)
5. S. Keldibekov, Flora i rastitel'nost' rybovodnykh prudov Chirchik-angrenskogo basseyna (Fan, Tashkent, 1981)
6. A. M. Kobilov, Bukhoro viloyati K͵ora-k͵ir kŭlidagi fitoplankton ҳamda makrofitlarni ŭrganish va ularni balik͵chilikda k͵ŭllash biotekhnologiyasi. Diss. avtoref. ...b.f.f.d.(PhD).Bukhoro, pp. 8-19 (2020)
7. N. A. Lemeza, N.A. Al'gologiya i mikologiya. Praktikum: Mn.: Vysheyshaya shkola, pp. 6-50 (2007)
8. Yu. Yu. Lur'ye, Analiticheskaya khimiya promishlennykh stochnykh vod (Khimiya, Moscow, 1984)
9. M. Mitropol'skiy, Ekspress-otsenka vodno-bolotnykh ugodiy v Uzbekistane. Ozero Dengizkul', p. 26 (2020)
10. A. M. Muzafarov, K. Yu. Musayev, Materialy k flore vodorosley vode khranilishch basseyna reki Zarafshon. Sb. Nauch. tr. TashGU, Tashkent, pp. 235-249 (1961)
11. A. M. Muzafarov, A. E. Ergashev, S. Khalilov, Opredelitel' sine-zelenykh vodorosley Sredney Azii. Kn. 1. Tashkent, Fan, p. 405 (1987)
12. Nauchnyy otchet po khozdogovoru 04/10-11 ot 07 aprelya 2011 goda.«Pasportizatsiya vazhneyshikh rybokhozyaystvennykh vodoyemov Respubliki Uzbekistan kak element ekologicheskogo monitoringa». Tashkent, pp. 3-78 (2011)
13. D. S. Niyozov, X. G. Ġafforov, Balik͵larning ozik͵lanishi. Tashkent. "Dizayn-press", p. 225 (2012)
14. G. K. Plotnikov, T. Yu. Peskova, A. Shkute et al., Sbornik klassicheskikh metodov gidrobiologicheskikh issledovaniy dlya ispol'zovaniya v akvakul'ture. Akademicheskoye Izdatel'stvo Daugavpilsskogo Universiteta. Saule, Moscow, p. 282 (2017)
15. I. G. Radchenko, V. I. Kapkov, V. D. Fedorov, Prakticheskoye rukovodstvo po sboru i analizu prob morskogo fitoplanktona. Mordvintsev, Moscow (2010)
16. Rukovodstvo po metodam kontrolya kachestva i bezopasnosti biologicheski aktivnykh dobavok k pishche. Federal'nyy tsentr gossanepidnadzora Minzdrava Rossii, p. 240 (2004)
17. N. S. Strogonov, Prakticheskoye rukovodstvo po gidrokhimii (Moscow, 1980)
18. T. T. Taubayev, Flora i rastitel'nost' vodoyomov Sredney Azii. Tashkent. Fan (1970)
19. S N. Trifonova, Praktikum po sisteme rasteniy: uchebno-metodicheskoye posobiye. Arzamas, Arzamasskiy filial NNGU (2014)
20. P. M. Tsarenko, Kratkiy opredelitel' khlorokkokovykh vodorosley Ukrainskoy SSSR, p. 208 (1990)
21. A. E. Ergashev, Opredelitel' presnovodnykh vodorosley, p. 126. Tashkent (1992)
22. H. K. Esanov, F. Q. Shodmonov, A. M. Kobilov, American Journal of Plant Sciences **12**, 266-273 (2001) https://www.scirp.org/journal/ajps https://doi.org/10.4236/ajps.2021.122016

23. I. Khozin-Goldberg, C. Bigogno, P. Shrestha, Z. Cohen, Journal of Phycology **38**, No. 5, 991–994 (2002) doi: 10.1046/j.1529-8817.2002.01160.x
24. F. Q. Shodmonov, Dengizko'l suv havzasining baliqlar turlarini aniqlash va ulardan o'txo'rlarini ko'paytirish biotexnologiyasi. Biologiya fanlari bo'yicha falsafa doktori (PhD) ilmiy darajasini olish uchun yozilgan dissertatsiya. Buxoro, pp. 3-128 (2022)
25. N. N. Sushchik, G. S. Kalacheva, N. O. Zhila et al., Russian Journal of Plant Physiology **50**, No. 3, 374–380 (2003) doi: 10.1023/A:1023830405898