

Article

Microalgae Indicators of Charophyte Habitats of South and Southeast Kazakhstan

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Abstract: Charophyte algae is a very sensitive group of organisms occupying Kazakhstan waterbodies. They are distributed throughout the country; however, not enough studies have been conducted, especially in the southern region. Research carried out in 2019–2022 identified 33 habitats of charophyte algae in the south and southeastern regions of Kazakhstan, including 15 new to Kazakhstan. Bioindicators and the statistical analysis of 223 species of nine phyla of microalgae associated with charophytes revealed that the main factors influencing the distribution of algal diversity may be habitat altitude and hydrology. The habitat altitude of about 700 m above sea level was shown to be the boundary between the different diversity distributions. The application of bioindicator methods can expand our knowledge on the ecology of the charophyte species in Kazakhstan. The study of algal diversity in charophyte habitats can serve as a tool for tracking climate change under potential future climate warming.

Keywords: microalgae; charophytes; trophic state; bioindicators; statistic; South and Southeast Kazakhstan



Citation: Sametova, E.;

Jumakhanova, G.; Nurashov, S.;

Barinova, S.; Jiyenbekov, A.; Smith, T.

Microalgae Indicators of Charophyte Habitats of South and Southeast

Kazakhstan. *Diversity* **2022**, *14*, 530.

<https://doi.org/10.3390/d14070530>

Academic Editor: Glenn B. McGregor

Received: 5 April 2022

Accepted: 28 June 2022

Published: 30 June 2022

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1. Introduction

Charophytes are a group of monophyletic, highly evolved benthic macroalgae. This group has received considerable attention since the beginning of systematic botany [1,2]. Charophytes are widely distributed in freshwater lakes, streams, rivers, and wetlands, as well as in brackish to highly saline waters [3]. These species usually form a benthophyte community in lentic meadows or slow-moving streams. Charophytes may occur as monospecific communities or occur together with other microalgae [4]. As pioneer species, they usually start to occupy a new habitat, and are the first to colonize emerging or disturbed water bodies [5]. Since charophytes form in mass such as meadows and have the ability to influence water quality due to their active participation in ecosystem processes, it is important to identify the properties of the habitat in which they occupy a leading position [6,7].

To date, the ecological preferences of certain species of charophyte algae have not been well studied, but there are species whose autecology is discussed quite widely [8]. Some charophyte species are used as indicators for ecosystem status according to the European Water Framework Directive [9] and eutrophication [10].

Data on charophytes in the waters of Kazakhstan, including the Almaty region, can be found in the works of hydrobotanists who conducted research in the 1970–1990s of the last century, namely Dobrokhotova K.V., Kostin V.A., and Shoyakubov R.Sh. [11–14]. Data on the general species composition of charophytes are published in the works of S.B. Nurashov,

E.S. Sametova, E.G. Krupa, and S. Barinova and R. Romanov [15–18]. Some of these species formed dense mats [19–21]. Despite the fact that charophytes have suitable conditions to grow in Kazakhstan, there are still many unstudied rivers, lakes, and ponds. Taxonomic studies of charophyte species in Kazakhstan have a long history [22,23] that confirms that the Kazakhstan environment was favorable for charophytes from Middle Eocene.

Studies of charophyte habitats are more related to the water bodies of northern Kazakhstan [16,24], where 26 species of charophytes were found. In the water bodies of the regions of southern and eastern Kazakhstan, only five species of charophytes were previously found [25–28]. For this, samples were collected for charophytes from fresh and saltwater bodies located in the Kazakhstan deltas of the Ili, Syrdarya, Amudarya rivers, in the Turgai depression, and in the lakes of Burabay, Bilikol, Balkhash, which include 26 species: *Chara*—22, *Nitella*—2, *Tolypella*—1, *Nitellopsis*—1 [11–15]. Based on these publications, a total of 40 species and two forms of charophyte algae have been identified in Kazakhstan.

A biological assessment of water quality according to 10 indicators was carried out. The samples were collected in the protected nature reserve of the Chernaya River [29], the Kegen and Raimbek regions [30], and the Zerenda and Burabay lakes [16,18], taking into account the species-specific preferences of indicator species accompanying charophyte algae.

Charophytes often form a monospecies population such as meadows in unpolluted waters. Their massive meadows often fill a significant part of the reservoir, thus participating in the process of water self-purification. Often, it can be difficult to detect associated microalgae species with water quality, since charophyte plants do not have periphyton in clear waters [4]. On the other hand, information on the ecological preferences of charophyte algae is still insufficient [8]. However, the ecological preferences of microalgae have been studied in Kazakhstan quite well [16,18,29,30]. We carried out an ecological assessment of charophyte habitats using the indicator properties of both charophyte species and microalgae as they represent a unified aquatic community. Moreover, charophytes can be indicators of water properties such as pH and salinity. They are species sensitive to organic pollution and the presence of calcium; however, their indicator categorization has not been determined [8,10]. Autecological data are known regarding organic pollution and trophic status for only a few species of charophytes [31,32]. However, other water properties, such as oxygen availability, organic pollution, temperature, feeding habits of community species, saprobity, organic pollution, trophic status, and water quality class, are categorized and widely applied based on microalgae as indicators of these aquatic environment parameters [33,34]. Thus, the habitat assessment based on the properties of the indicator species of microalgae accompanying the Characeae, where the charophyte species develop in mass, is the identification of the optimum growth conditions for these species. One of the indicators of the optimal environment is organic pollution. The saprobity index reflects organic pollution and is therefore widely used to assess water quality using bioindicator species, mainly microalgae. The saprobity index can reflect not only pollution loads, but also the stage of self-purification of the studied water body. All this leads to the development of the indicator value of charophytes as the edificators of communities and to the need for a systemic approach covering the complex structure of ecological networks, including the accompanying periphyton, identifying the most important species, their topology, and vulnerability that focused on conservation biology [35,36].

The aim of the present work is to identify the preferred habitats of charophyte algae in south and southeastern Kazakhstan and the associated species of microalgae, providing an ecological assessment of known and new habitats with species-indicator properties.

2. Materials and Methods

2.1. Description of Study Area

Algae were collected from rivers, canals, and lakes from three regions from south and southeast Kazakhstan (I—Turkestan regions, II—Zhambyl regions, and III—Almaty regions) (Figure 1) at altitudes between 245 and 3629 m a.s.l. (Table 1).



Figure 1. Location of investigated sites in south and southeast Kazakhstan, 2019–2022.

Table 1. Locations of study sites in south and southeast Kazakhstan, 2019–2022. Asterisk indicates sites sampled for the first time.

Site	Basin	Name	North	East	Altitude (m)
1	I	* Canal Dostyk	41°00'31.80"	68°12'40.43"	245
2	I	Syrdarya River	41°02'16.79"	68°30'49.94"	418
3	I	Karatausky nature reserve Kizhi, source Karakuz	43°51'07.48"	68°32'14.65"	971
4	I	* Sharbulak River	41°46'19"	69°24'10"	650
5	I	* Merki River	42°54'11.09"	73°09'51.17"	676
6	II	* Theris River	42°39'59"	70°48'05"	953
7	II	Mynaral River	45°24'49"	73°40'51"	343
8	II	Chu River	43°16'05"	74°12'13"	533
9	II	* Karabalta River	43°12'01"	74°0'36"	520
10	II	Kakpatas River	43°21'13"	74°24'48"	561
11	II	* Dam Copa	43°21'13"	74°28'45"	636
12	II	Aksu River	43°11'53"	74°3'48"	751
13	III	* Ili River, Canal Arystan	45°32'29"	74°52'42"	341
14	III	* Ili River, Canal Zhidely	45°33'00"	74°53'42"	341
15	III	* Canal Bakanas	44°52'50.37"	76°10'13.98"	389
16	III	Lake Yubelejnoe	43°20'31"	76°42'02"	696
17	III	Lake Sorbulak	43°38'01"	76°36'29"	618
18	III	* Talgar River	43°41'50"	77°15'25"	394
19	III	Ostemir pond	43°38'52"	77°15'48"	523
20	III	* Kaskelen River pond	43°46'27"	77°4'53"	488
21	III	Ili-Kapchagaiplatinum	43°55'7.49"	77°5'49.31"	475
22	III	Kaskelen River	43°47'03"	77°7'47"	623
23	III	* Lake Kaiyndy	42°59'05.58"	78°27'54.79"	1865
24	III	* Karkara River	42°50'57.64"	79°13'57.98"	2062
25	III	* Mynzhylky River	42°44'15.8"	79°16'53.7"	3000
26	III	* Sartasu River	42°37'14.49"	79°19'18.61"	3629

Table 1. *Cont.*

27	III	* River Kegen	43°00'27.64''	79°15'13.23''	1821
28	III	Charyn River	43°52'49.40''	79°27'13.56''	512
29	III	* Ulken-Kokpak River	42°36'06.68''	79°50'42.41''	1836
30	III	* Tekes River	42°50'37.1''	80°03'07.5''	1766
31	III	* Narynkol River	42°43'24.86''	80°08'06.54''	1831
32	III	* Tentek River	45°16'31.99''	80°73'75.03''	2338
33	III	Alakol Lake	46°40'77.51''	81°45'71.19''	347

Charophyte algae were collected in the Turkestan region (I) (south Kazakhstan) from five rivers, in three of which charophytes were recognized for the first time (Table 1, Figure 2a). Charophytes in Zhambyl region (II) were collected from seven sites, in three of which charophytes were found for the first time (Table 1, Figure 2b). Charophyte algae in the Ili River basin in Almaty region (III) of southeast Kazakhstan were collected from 21 sites, in 14 sites of which charophytes were found for the first time (Table 1, Figure 2c,d).



Figure 2. Sites sampled for the first time in south and southeast Kazakhstan, 2019–2022: The Merki River (a); the Copa Dam (b); Canal Arystan of the Ili River (c); Kaskelen River pond (d).

The regional climate changes from mild warm, temperate in the west to cold, and temperate in the east. In winter, there is more rainfall than in summer. The average annual temperature decreases from west to east from 13.2 °C to 8.6 °C, and annual precipitation increases from 502.4 mm to 511.8-mm [37–39].

2.2. Sampling and Laboratory Study

Temperature, conductivity, and pH of the water were measured during sampling with a Hanna Waterproof Portable pH/Temperature meter HI-9813-5. GPS coordinates of the sampling points were defined with Garmin GISMAP 64.

Overall, 75 microalgae samples of microperiphyton and microphytobenthos were taken from submerged plants and stones by scraping and were fixed in 3% neutral formaldehyde solution. Phytoplankton samples were taken with a 20 µm mesh plankton net and fixed at the site with a 3% neutral formaldehyde solution. Charophyte algae were harvested

with scrapers or anchors and collected by hand at the site. The samples were dried in situ and placed in the herbarium. The wet samples were transported in an icebox to the Institute of Botany and Phytointroduction (Almaty) for microscopic studies. Samples were separated for study at the Institute of Botany and Phytointroduction (Kazakhstan), at the Institute of Evolution, University of Haifa (Israel), and partly separated for study at the Arkansas State University Beebe (USA). For species identification in Kazakhstan, an MBS-9 stereomicroscope (Scopia, Russian Federation) and a MicroOptix light microscope (MicroOptix, Inc., Austria) were used. The sizes of all algae species were measured using an eyepiece-micrometer and photographed using a Motic BA-400 (Motic Asia, Hong Kong, China) microscope camera.

The processing of algal material in Israel was carried out according to generally accepted methods. Diatom slides were prepared using the peroxide technique [40]. All slides of non-diatom and diatom algae were identified with a Leica DM2500 (Leica Microsystems EMEA, United Kingdom) light microscope under 400–1000× magnification and photographed by Omax 9.0 MP USB Digital Camera.

International handbooks were used to identify algae [41–47]. All taxa names are currently accepted taxonomically, according to the Algaebase.org website [48].

All species were recorded and ranked according to their relative abundance in the sample using the species frequencies six-score scale [40] (Table 2).

Table 2. Species frequencies scale according to [40].

Score	Visual Estimate	Cell Numbers of Plankton per L	Cell Numbers of Periphyton per Slide (20 × 20 mm)	Cell Number of Each Species, %
1	Occasional	1–10 ³ cell L ⁻¹	1–5 cells per slide	<1
2	Rare	10 ³ –10 ⁴ cell L ⁻¹	10–15 cells per slide	2–10
3	Common	10 ⁴ –10 ⁵ cell L ⁻¹	25–30 cells per slide	10–40
4	Frequent	10 ⁵ –10 ⁷ cell L ⁻¹	1 cell over a slide transect	40–60
5	Very frequent	10 ⁶ –10 ⁷ cell L ⁻¹	Several cells over a slide transect	60–80
6	Abundant	More than 10 ⁷ cell L ⁻¹	One or more cells in each field of view	80–100

The calculation of saprobic indices was carried out according to the Pantle–Buck method in Sládeček’s modification [49]. Saprobity indices were obtained for each algal community as a function of the number of saprobic species and their relative abundances:

$$S = \sum_{i=1}^n (s_i h_i) / \sum_{i=1}^n (h_i) \quad (1)$$

where S is the index of saprobity for algal community (unitless), s is species-specific saprobity index, and n is the cell density of each species (Table 1).

The BioDiversity Pro 2.0 program was used for similarity calculation. Correlation network and multiple regression analyses was performed in JASP on the botnet package in R Statistica [50]. Canonical correspondence analysis of species and environmental variables’ relationships was performed with the CANOCO Program 4.5 [51]. A heat map was constructed in the ExStartR program [52]. Bioindicator analysis was performed with species-specific ecological preferences of the revealed algae and cyanobacteria [32–34].

3. Results

We identified 33 habitats supporting the growth of charophytes, 15 of which were examined for the first time. Charophytes were often found as monospecies clumps. Microalgae communities forming around charophyte plants were found in 15 habitats. Monospecific charophyte meadows were represented in all other sites. The average measured pH, temperature, and conductivity, and known data from previous studies for some water

bodies for oxygen, BOD, and color from the National Monitoring Reports [53–56], are shown in Table 3.

Table 3. Average data for environmental variables of water in studied sites of south and southeast Kazakhstan, 2019–2022. Data for dissolved oxygen, BOD, and odor were taken from the references [53–56].

Basin	Site	Altitude, m a.s.l.	Temperature, °C	pH	O ₂ , mg L ⁻¹	BOD, mg O ₂ L ⁻¹	Pt/Co Color Degree	Index S	No. of Species
Basin I	4	650	27	7.00	-	-	-	2.07	35
Basin II	6	941	12	7.00	-	-	-	1.91	21
	8	739	36	7.24	10.50	3.85	10.0	1.85	27
	10	561	30	7.00	-	-	-	1.87	46
	12	751	30	7.39	11.85	6.76	12.5	-	37
Basin III	13	341	22	7.33	11.85	0.87	5.5	1.72	20
	14	341	24	7.33	11.85	0.87	5.5	2.11	32
	15	396	24	7.00	-	-	-	1.61	40
	16	808	10	6.00	-	-	-	1.73	25
	17	632	35	7.75	-	-	-	2.00	12
	20	488	8	6.50	-	-	-	1.59	37
	21	475	21	7.44	12.10	1.09	5.5	1.96	31
	24	1900	4.5	7.43	12.15	1.43	6.0	1.73	16
	25	3000	14	7.00	-	-	-	-	13
	29	1836	4.5	7.00	-	0.65	0.0	1.26	45
	30	1766	3	7.76	11.35	0.85	6.0	1.72	36
	33	347	22	7.50	-	-	-	1.74	10

Water quality parameters changed with altitude (Figure 3). Because the graphs are organized by site altitude, the trend lines plotted for each of the variables can reflect the effect of altitude on the distribution pattern and, in this case, suggest that temperature, species richness, BOD, and color are negatively correlated to site altitude, but that oxygen increases slightly, although pH and S index remain virtually unchanged. The index saprobity S that reflected organic pollution in the aquatic object, on average, fluctuated across a narrow range, and shows middle polluted waters within Class 3 of water quality. Some sites such as the Ulken-Kokpak River were organically clear with S = 1.26 (Class 2) at an altitude of about 1900 m a.s.l.; however, some sites, such as Ili River, Canal Zhidely (S = 2.11), at an altitude of 341 m a.s.l., were organically polluted.

This distribution highlights habitat altitude as an important property of charophyte communities. Although the general patterns in Figure 3 reveals a decrease in both variables, temperature, and species richness, with an increase in habitat altitude, a detailed analysis of the relationships between these two indicators reveals more subtle relationships and shows mutually opposite dynamics at some stations. That is, at some stations, as the temperature rises, the number of species in the community declines, and vice versa, as rich communities were found at lower water temperatures at the station. Multiple Regression calculation for altitude as independent variables and dependent variables presented for all stations (temperature, water pH, index S, and no. of species) confirms that altitude can play a regulatory role for these parameters with a negative correlation ($b^* = -0.68$; $p = 0.0049$). The dynamics of these environmental indicators suggest that, firstly, this data does not sufficiently characterize the habitats of charophytes, and, secondly, the altitude of the habitat, as an indicator of the environment, should be included as an important attribute when analyzing the identified species' composition and ecological preferences of microalgae species.

Analysis of the phytoplankton and phytobenthos samples revealed 223 species of algae from eight phyla (Table 4 and Table S1). Diatoms made the greatest contribution to the composition of communities (Table 4). Charophyte algae had the second highest contribution in many communities. Among the most abundant species of the diatoms were *Epithemia gibba* in Lake Alakol, *Cymbella turgidula* in Kakpatas River, *Nitzschia fonticola*

in Canal Bakanas, and *Achnanthydium minutissimum*, which developed in masses in the Ulken-Kokpak River, along with *Spirogyra* sp. and other filamentous charophytes. In the Canal Bakanas and in the Chu River, cyanobacteria genus *Merismopedia* was also abundant.

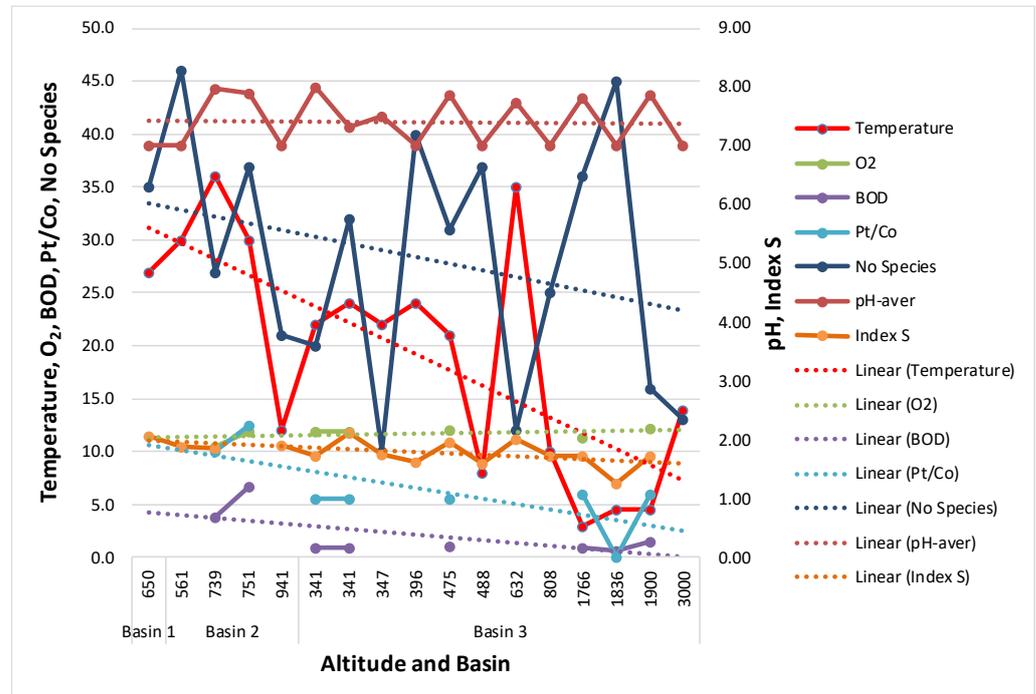


Figure 3. Dynamics of environmental and biological variables along the altitudinal gradient of study sites. Dashed lines are linear trend lines.

Table 4. Species richness in taxonomic phyla, number of indicator species, altitude, and saprobity index S at sampling stations in South and Southeast Kazakhstan ¹.

Station	13	14	33	15	21	20	10	17	4	8	12	16	6	30	29	24	25
Altitude	341	341	347	396	475	488	561	632	650	739	751	808	941	1766	1836	1900	3000
Bacillariophyta	8	12	6	21	14	21	21	5	19	15	30	16	15	19	35	13	8
Charophyta	3	5	4	6	3	8	7	3	3	4	4	4	2	6	7	3	3
Chlorophyta	5	6	0	3	9	1	14	2	5	2	0	3	2	2	2	0	0
Cyanobacteria	4	9	0	8	4	4	3	2	8	5	3	2	2	7	0	0	1
Miozoa	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0
Ochrophyta (Chrysophyceae)	0	0	0	0	0	2	0	0	0	0	0	0	0	1	0	0	0
Ochrophyta (Xanthophyceae)	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0
Euglenozoa	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1
No. Species	20	32	10	40	31	37	46	12	35	27	37	25	21	36	45	16	13
Index S	2.11	1.72	1.74	1.61	1.96	1.59	1.87	2.00	2.07	1.85	-	1.73	1.91	1.72	1.26	1.73	-
Habitat																	
B	5	14	6	16	9	19	15	6	14	14	21	12	7	16	24	10	6
P-B	12	13	3	18	16	11	20	4	19	11	11	12	12	13	18	6	6
P	2	2	0	2	4	1	6	1	1	1	0	0	0	2	0	0	1
Temperature																	
cool	0	0	0	0	0	2	0	0	0	0	2	0	1	0	4	1	0
temp	3	7	3	13	12	11	14	2	13	10	8	11	8	15	16	8	2
eterm	2	1	0	1	1	0	1	0	2	0	0	1	1	2	3	0	2
warm	0	1	0	1	0	0	1	0	0	1	0	0	1	1	2	0	0

Table 4. Cont.

Oxygen																		
aer		0	2	0	3	2	1	1	0	2	1	1	0	0	1	1	0	0
str		0	0	0	1	0	5	0	0	1	2	2	1	0	0	3	0	0
st-str		11	14	7	19	17	16	24	5	21	13	12	15	12	20	26	10	6
st		1	3	1	4	2	5	6	1	1	3	2	3	4	1	3	1	2
Watanabe																		
sx		0	0	1	3	1	5	3	0	3	2	5	3	4	5	9	3	1
es		5	8	4	11	10	9	11	1	11	7	8	9	7	9	16	7	3
sp		1	2	0	3	2	0	4	1	3	2	0	1	2	1	3	1	0
Salinity																		
hb		1	1	0	1	0	3	2	0	1	0	2	0	1	2	4	1	0
i		6	9	4	21	17	16	22	3	18	17	17	16	11	18	24	9	7
hl		3	5	1	5	4	4	5	2	8	1	3	2	2	2	4	1	0
mh		0	3	1	0	0	0	0	1	0	0	1	0	0	1	3	0	1
hlnbnt		0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
eh		0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
pH																		
acb		1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
acf		1	1	0	0	1	1	0	1	1	0	3	0	0	1	1	0	0
ind		5	5	1	10	7	6	14	0	11	13	8	5	7	10	18	5	4
alf		4	9	4	16	11	15	15	5	13	6	14	12	10	12	18	8	4
alb		2	1	1	2	0	2	1	0	2	2	0	1	0	3	1	0	0
Autotrophy-Heterotrophy																		
ats		1	1	1	4	3	9	6	2	4	5	5	2	2	4	13	1	3
ate		4	6	3	12	8	8	8	1	8	5	7	11	8	11	16	8	1
hne		1	4	1	4	1	2	5	2	5	2	3	2	2	2	2	2	2
hce		1	1	1	0	2	1	2	0	2	2	0	1	2	1	0	1	0
Trophy																		
ot		0	1	0	1	1	2	1	0	2	2	3	0	1	1	5	1	0
om		0	1	2	7	1	4	5	1	4	4	2	1	2	5	8	0	1
m		1	6	0	2	0	2	2	0	0	2	2	3	0	4	3	4	1
me		3	0	3	7	5	10	5	0	8	7	0	4	2	8	6	0	1
e		7	13	3	11	12	6	15	5	15	5	7	6	8	4	9	4	3
o-e		1	2	2	3	1	3	2	2	0	2	5	2	0	2	1	2	1
he		1	1	0	0	1	0	2	1	1	1	0	1	1	1	0	1	0
Class of Water Quality																		
Class 1		1	0	0	1	0	2	0	0	0	0	4	0	0	0	5	0	0
Class 2		2	6	5	15	9	16	17	4	11	11	12	13	6	15	21	7	5
Class 3		7	14	2	12	13	5	14	1	10	7	5	7	6	10	10	5	5
Class 4		1	4	2	3	4	4	3	1	6	3	2	1	4	2	1	1	0
Class 5		0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0

¹ Note: Habitat: P—planktonic, P-B—plankto benthic, B—benthic. Temperature: cool—cool water, temp—temperate temperature, eterm—eurythermic, warm—warm water inhabitants. Oxygenation and water moving (Oxygen): st—standing water, st-str—low streaming water, str—streaming water, aer—aerophiles. Halobity degree (Salinity): i—oligothalobes—indifferent, hl—halophiles, hb—halophobes, mh—masohalobes, hlnbnt—halobiontes, eh—euhalobes. Acidity (pH): alf—alkaliphiles, ind—indifferents; acf—acidophiles, alb—alkalibiontes, acb—acidobiontes. Organic pollution indicators according to Watanabe (D): sx—saproxenes, es—eurysaproxenes, sp—saprophiles. Nitrogen uptake metabolism (Aut-Het): ats—nitrogen—autotrophic taxa, tolerating very small concentrations of organically bound nitrogen; ate—nitrogen—autotrophic taxa, tolerating elevated concentrations of organically bound nitrogen; hne—facultatively nitrogen—heterotrophic taxa, needing periodically elevated concentrations of organically bound nitrogen, hce—facultatively nitrogen—heterotrophic taxa, needing elevated concentrations of organically bound nitrogen. Trophic state (Tro): ot—oligotraphentic; om—oligo-mesotraphentic; m—mesotraphentic; me—meso-eutraphentic; e—eutraphentic; o-e—oligo-eutraphentic; he—hypereutraphentic. “-” property is unknown.

To identify the environmental factors most strongly influencing the formation of algae communities in the studied charophyte habitats, a comparison of the species composition was carried out. A similarity tree was constructed for those sites where microalgae were identified (Figure 4); two clusters were obvious. Cluster 1 included only three communities

similar at the level of 48% and belonging to habitats at altitudes between 800 and 1900 m. Cluster 2 had five communities at a similarity level of 40% and included habitats at altitudes between 500 and 700 m. The rest of the communities were highly individual and did not form a separate cluster. They were included in the tree at lower levels of similarity. Thus, it turned out habitat altitude is the most significant environmental factor that affects the distribution of the species composition of charophytes.

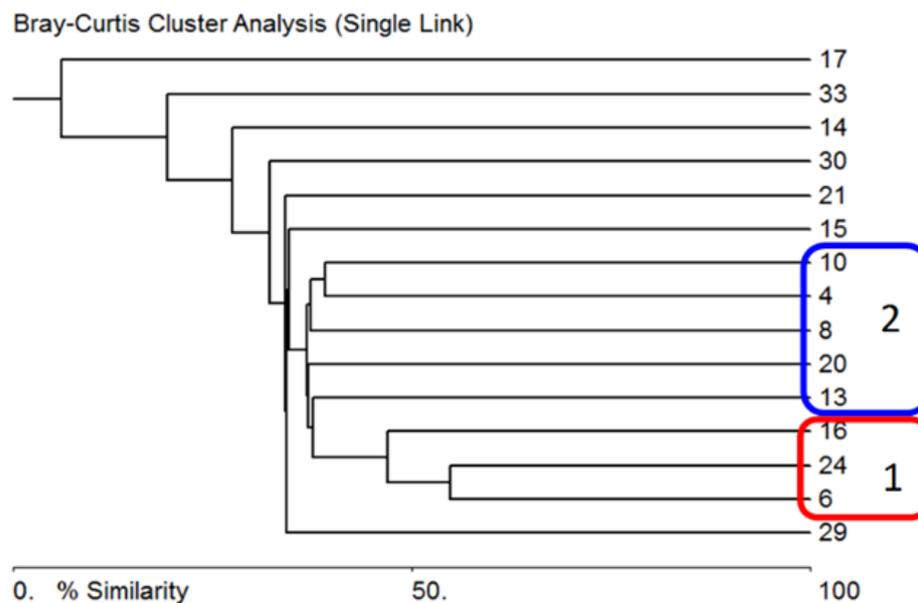


Figure 4. Bray–Curtis dendrogram of the similarity of species composition in the communities of the studied sites of South and Southeast Kazakhstan, 2019–2022. Cluster 1, red outline, highland sites, Cluster 2, blue outline, middle altitude sites.

To verify the altitude conclusion, a JASP correlation network plot was constructed based on Table 4, which considers both the taxonomic proportions of communities and the indicative characteristics of species, showing preferences for environmental properties. The communities were divided into main basins (I, II, III), showing that the whole set is divided into two clusters (Figure 5). Cluster 1 includes communities belonging to all three basins, while Cluster 2 includes communities from only basins II and III. The unifying parameter for the clusters was altitude, while belonging to the basin was not significant. Thus, Cluster 1 included habitats whose altitude was from 341 to 751 m (low), and Cluster 2 united communities of sites at an altitude of 632–3000 m (high). Thus, the influence of environmental conditions associated with habitat altitude was manifested at about 700 m a.s.l.

All identified species turned out to be indicators of eight properties of the environment and habitats (Table 4). Based on the results of the calculations and constructions made above (Figures 4 and 5), the distribution histograms of the species composition and indicator groups for each environmental parameter were compiled according to altitude. The phyla species numbers are not related to the altitude gradient (Figure 6a). This reflects the response of communities to local conditions. The species-rich communities are at an altitude of about 560 and 850 m. However, the percentage histogram (Figure 6b) reflects a marked increase in the proportion of diatoms with altitude, as well as a decrease in other non-charophytes.

Indicators of temperature revealed a dominance of temperate species; however, the mesothermic species are present in both the lowest habitats of the Balkhash part of region II (Ili River) and at an altitude of 3000 m in the shallow warm areas of the Mynzhylky River in region III (Figure 7a). This indicates that hydrology also plays a role in relation to temperature.

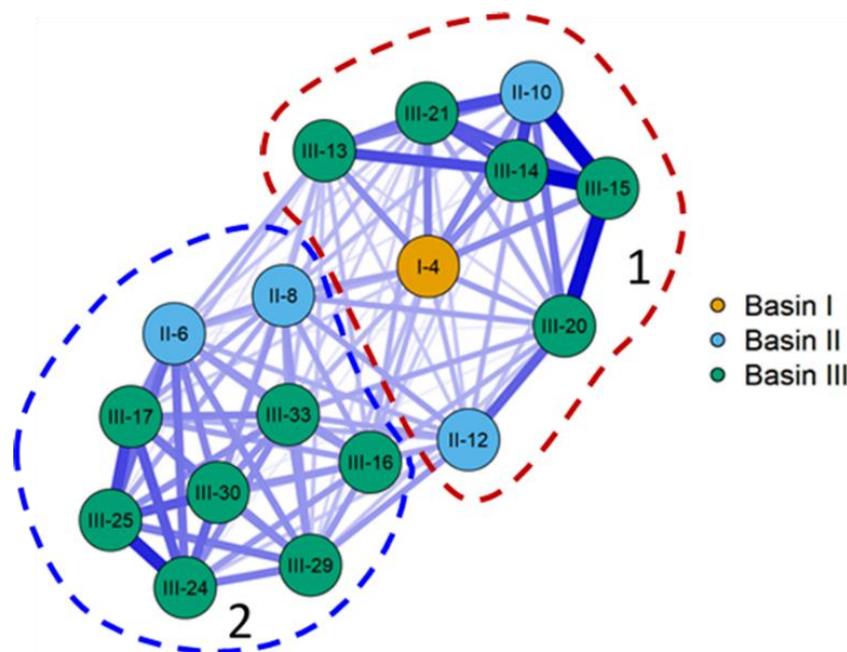


Figure 5. JASP correlation networks plot for diversity of indicator species in the communities of the studied sites of South and Southeast Kazakhstan, 2019–2022. Cluster 1, red outline, lowland sites, Cluster 2, blue outline, highland sites.

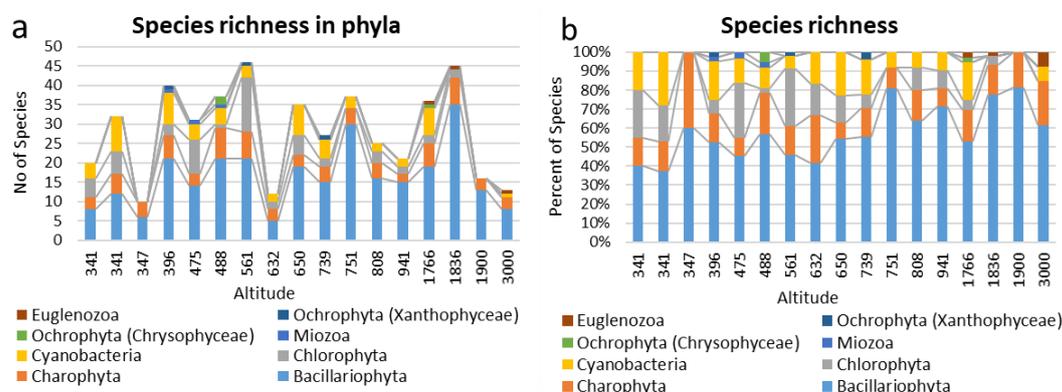


Figure 6. Dynamic of species richness in taxonomic phyla in the communities of the studied sites. Number of species in phyla (a); percent of species in phyla (b).

Indicators of water mobility and dissolved oxygen saturation are largely reflected in the dominance of slow flowing waters, making this environmental characteristic the main indicator of optimal habitat for charophytes (Figure 7b). Water salinity decreased slightly with altitude (Figure 7c), suggesting the impact of arid conditions that increase salinity in lower-lying habitats. Water pH also decreased with altitude, as evidenced by the gradual loss of alkalibiontes (Figure 7d).

An important characteristic of habitat conditions is the presence of mixotrophic algae—organisms which can utilize inorganic and organic substances, switching to heterotrophy if nutrition through photosynthesis is suppressed. In the studied communities, the proportion of mixotrophs (hne + hce) decreases from 40% in lower elevation habitats to 7% at an altitude of about 1900 m, then increased again in the highest mountain habitats (Figure 8a), which indicates potential stressful conditions for charophytic communities at the maximum altitude. Habitat trophic level indicators show a positive trend (Figure 8b) and a decrease in the proportion of eutrophic species with altitude, except for the last two high mountain communities, where mixotrophs also increased their presence.

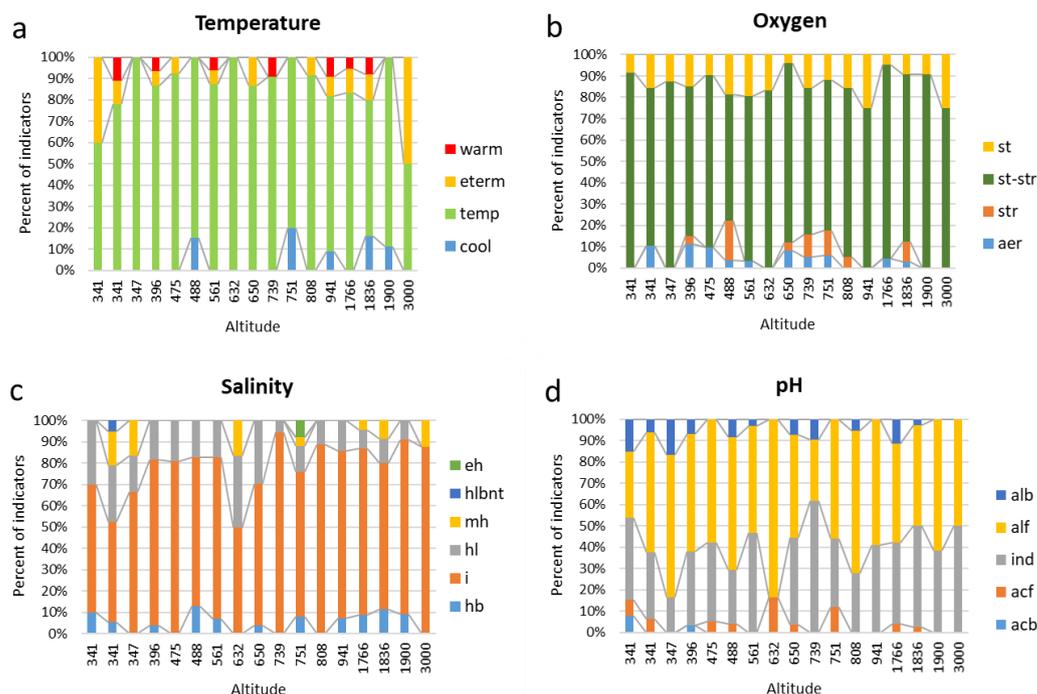


Figure 7. Dynamics of indicator species percentage in the communities over the study sites: (a) temperature indicators (cool—cool water, temp—temperate temperature, eterm—eurhythmic, warm—warm water inhabitants); (b) oxygenation and water moving indicators (st—standing water, st-str—low streaming water, str—streaming water, aer—aerophiles); (c) salinity indicators (i—oligohalobes—indifferent, hl—halophiles, hb—halophobes, mh—masohalobes, hlbnt—halobiontes, eh—euhalobes); (d) water pH indicators (alf—alkaliphiles, ind—indifferents; acf—acidophiles, alb—alkalibiontes, acb—acidobiontes).

Diatoms that are indicators of organic pollution show self-purification with increasing habitat altitude, that is, saprophiles decreased, while saproxens, on the contrary, increase their share in communities (Figure 8c). The water quality class was determined by the value of the saprobity index calculated for each community (Table 4). With an increase in habitat altitude, the proportion of species in class 2 increases, while species of classes 4 and 5 decrease and disappear from the communities (Figure 8d).

The cumulative effect of the impact on the charophyte communities of their environmental parameters can be seen on the heatmap (Figure 9), which shows the general “taxonomic and indicator face” of the communities. Thus, communities of sites 15, 10, and 29 are similar and different from 14, 21, 4, and 12 in terms of the predominance of species composition and groups of indicators.

The final step in identifying the main environmental factors affecting charophyte communities was the Canonical Correspondence Analysis (CCA) (Figure 10). The CCA triplot was built on data in Tables 3 and 4. The analysis included environmental parameters such as habitat altitude, water temperature, and pH, and the saprobity index *S* reflecting organic pollution, the species richness of the community, and the number of charophyte species in the community as independent variables; however, chemical variables known only for some stations were not used. Number of species in the taxonomic phyla was used as dependent variables. The results showed that the altitude of the habitat had an impact on the species composition at sites 24 and 29 (Cluster 1). Cluster 2 reflected the impact of the maximum number of charophyte species on the species composition of site 20, where Chrysophytes were present. Cluster 3 included communities dominated by green algae and reflected the combined effect of temperature and organic pollution. However, no relationship was found between water pH and the total species richness of communities with the number of species in individual phyla.

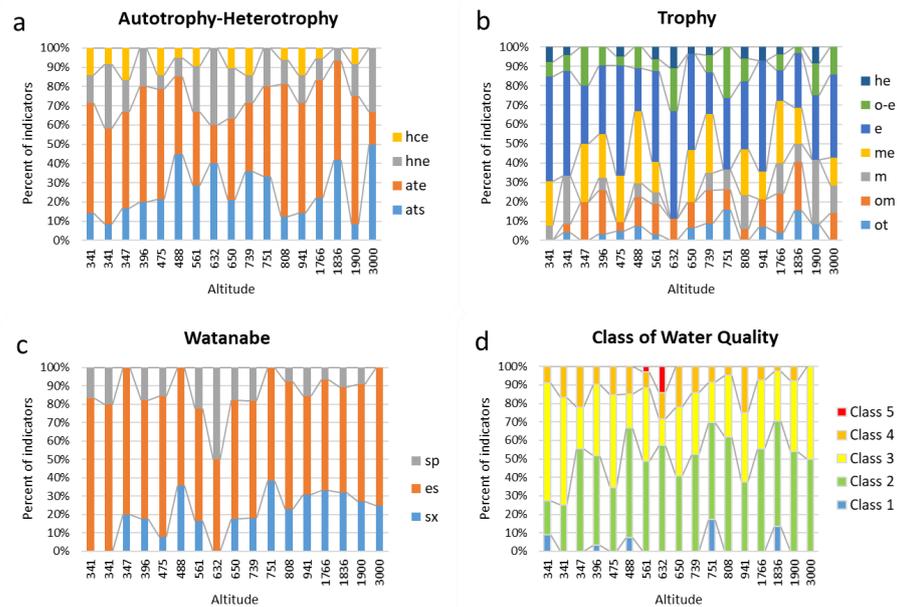


Figure 8. Dynamic of indicator species percentage in the communities across the study sites: (a) nutrition type indicators (ats—nitrogen–autotrophic taxa, tolerating very small concentrations of organically bound nitrogen; ate—nitrogen–autotrophic taxa, tolerating elevated concentrations of organically bound nitrogen; hne—facultatively nitrogen–heterotrophic taxa, needing periodically elevated concentrations of organically bound nitrogen, hce—facultatively nitrogen–heterotrophic taxa, needing elevated concentrations of organically bound nitrogen); (b) trophic state indicators (ot—oligotraphentic; om—oligo-mesotraphentic; m—mesotraphentic; me—meso-eutraphentic; e—eutraphentic; o-e—oligo-eutraphentic; he—hypereutraphentic); (c) organic pollution diatom indicators (sx—saproxenes, es—eurysaprobies, sp—saprophytes); (d) class of water quality indicators.

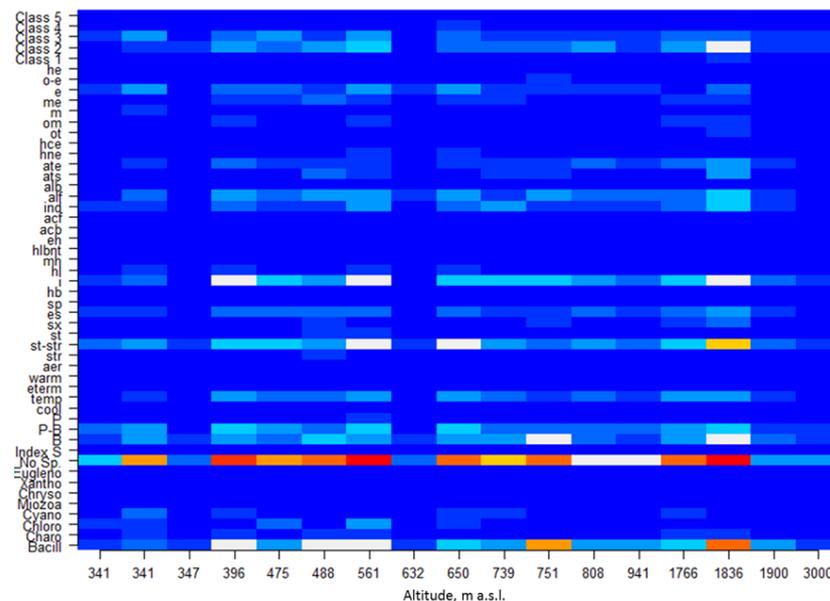


Figure 9. Heat map for species richness in phyla, index of organic pollution S, and species number in the groups of indicators in the communities over the studied sites at South and Southeast Kazakhstan, 2019–2022. Abbreviations in the y-axis and station numbers in the x-axis are the same as in Table 3. Sampling stations are in order of its altitude increasing. The color of the cells varies from white to blue then to red according to the proportion of the number in the entire distribution.

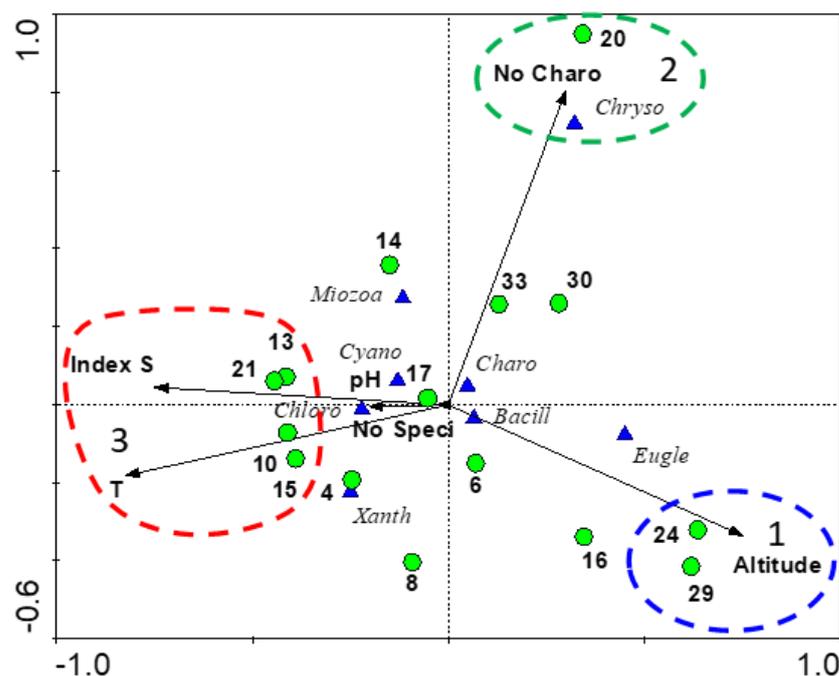


Figure 10. CCA plot of the relationships between environmental variables, index of organic pollution S, and species number in taxonomic phyla and number of charophyte species in community (blue arrows).

4. Discussion

The wide distribution of charophyte algae makes them useful as water quality indicators [8]. However, for many species, there is insufficient knowledge of their autecology. Charophyte algae usually form monospecific clumps [4], with absolute dominance in algal communities. However, since indicator properties are far from known for all species, it is necessary to consider the microalgae accompanying them for which the indicator properties are widely known [32]. Combining water quality analysis for charophyte macroalgae and their cohabitants is especially important for previously unstudied habitats. Despite the fact that, in Kazakhstan, charophytes find favorable conditions for development [22] over a long geological time [23], and the fact that quite a number of publications are devoted to their study [19–21,30,57–66], many potential charophyte habitats remain unexplored.

In the course of our work, 33 charophyte habitats were identified in south and south-east Kazakhstan, and 15 of them were studied for the first time. Indeed, some of the communities were monospecific. However, for 15 habitats, cohabitant microalgae were revealed. A total of 223 species from eight phyla were identified, and all of them were indicators of various water or habitat properties.

This helped us to characterize the properties of the waters of hitherto unexplored habitats in the south of Kazakhstan and in the mountains, for which chemical and physical indicators have not been determined. The indicator properties of the microalgae associated with mass-forming charophytes made it possible to characterize all 33 studied habits. With the help of bioindicator properties of microalgae and statistical programs that reveal the relationship between the composition of communities and external factors, it was possible to identify some habitat preferences of charophyte communities, including altitude, slow currents, average oxygen saturation, slightly alkaline pH, low salinity, and low organic pollution. The habitat altitude of about 700 m a.s.l. was shown to be a diversity boundary associated with an increase in the salinity of water with a decrease in the altitude of the habitat; this indicates the influence of the arid conditions of south Kazakhstan. Climate data support this conclusion, which shows that, in the three studied areas, the average annual air temperature increases from east to southwest [37–39]. Thus, the study of algae diversity in charophyte habitats is beneficial for tracking climate change.

The study of charophyte habitats in Kazakhstan from an ecological point of view is still at an early stage [16,18,30]. Despite this, other habitats are ecologically well studied [67]. Thus, one of the results of our work was the identification of new, hitherto unexplored habitats of charophytes, the list of which can now be expanded. Moreover, the application of bioindication methods can expand our knowledge about the ecology of charophytes.

In densely populated areas, communities containing charophytes attract the attention of researchers. Therefore, in Turkey, the dynamics and diversity of the cohabitants of charophytes and microalgae in the Artabel Lakes Nature Park and high mountain lakes in Rize were described [68,69], and the influence of hydrology on the distribution of their diversity was revealed. Lakes Great Lota and Isikli, inhabited by charophytes, were studied in relation to the dynamics of the diversity of associated microalgae communities [7,70,71]. The communities with a predominance of *Mastogloia* were found to be similar to our slightly saline habitats in the Sorbulak reservoir and the Aksu, Ili, Tekes rivers. Using the indication of charophytic cohabitants, the historical formation of the now highly urbanized basin [72] was traced to the impact of hydrology on such type of communities.

The study of charophyte communities in northern Pakistan in the Kabul River Valley under similar conditions to our study region in terms of climate and anthropogenic pressure [6,73] has revealed that the main regulating factor on the distribution of algal diversity was altitude, similar to our findings. The climatically close region of the eastern Mediterranean was studied in various aspects in relation to charophytes and their distribution according to environmental parameters [74–76]. The influence of climatic parameters, especially altitude, on the distribution of algae, is studied mainly in critical habitats such as high latitude and high mountain habitats [77,78], especially for charophytes [79]. It was found that the impact factor for the microalgae community is the instability of the environment in geologically similar but geographically distant high mountain sites in Central Alaska and in the high Himalayas [77]. At the same time, microclimatic factors such as the meteorological conditions of rain, mist-fog, and clear sunlight accompanying climate change united microalgae communities with the altitude of their habitats [80,81]. The sensitivity of various species to high-latitude and high-mountain habitats has also played a role in patterns of distribution of microalgae communities, where green algae take precedence over cyanobacteria [80]. With increasing altitude starting at 2000 m above sea level, algae have to cope with conditions such as high UV irradiance, alternating desiccation, rain and snow precipitations, extreme diurnal variations in temperature, and chronic scarceness of nutrients [81]. In this case, the capacity to grow heterotrophically gives the preferences for some groups of species with increasing altitudes [81], as we recognized in the current study for mountain rivers in Mynzhylky (3000 m a.s.l.) and Karkara (2062 m a.s.l.). The presence of heterotrophs may indicate a stressed environment that regulates the algae species' distribution [61]. In our case, high mountain riverine communities have similarity in the presence of heterotrophs with the polluted Lake Sorbulak. Distribution of microalgae communities over altitude gradient in climatic stress conditions that were studied in the Ural Mountain reveal similar patterns of the species richness of microalgae decreasing along the altitude gradient [82] from mountain meadow to mountain tundra. The Ural Mountain's distribution demonstrated the positive correlation between the species richness of microalgae and altitude in the forest communities, but a negative correlation in the tundra, which shows the influence of the microclimatic condition on the algae community, such in other stressed habitats [77,80,81]. The increase in charophytes species richness was also revealed with an increase in the habitat altitude [79]. It turned out that hydrology, and especially the altitude of the habitat, play a decisive role in the distribution of the diversity and distribution of the microalgae community and in charophytes such as, for example, in Serbia [83] and throughout the whole world [35]. An important role in this analysis was played by the indicator properties of species associated with charophytes, replacing chemical data, since many desert and mountain habitats in Kazakhstan were difficult to access and therefore chemical samples were extremely limited.

Previous studies of charophyte communities have made it possible to identify such cryptic properties of species as the rarity of their being in scattered habitats, which turned out to be possible to identify with a fairly dense study of the territory, such as in Israel [84], as well as what can be done in Kazakhstan with further studies of charophytes. The data herein presented demonstrate the importance of studying charophyte communities in Kazakhstan, not only in the morphological and ecological methods, but also using modern methods of molecular [8,64,65] and phylogenetic [2] research, which is expected to be performed in the near future. In addition, studies of the communities of charophytes' cohabitants provide an opportunity to contribute to the study and refinement of the known autecology of individual species of charophyte algae [8,85,86].

5. Conclusions

It was possible to identify 33 habitats of charophyte algae in the still little-studied regions of south and southeastern Kazakhstan. Fifteen charophyte habitats were new. Analysis of communities of 223 species of cohabitants from eight taxonomic phyla using statistical methods showed that the factors influencing the distribution of algae diversity are the altitude of the habitat and its hydrological properties, such as the type of the waterbody: river, swamp, lake, canal. The application of bioindication methods can expand our knowledge about the ecology of the charophyte species in Kazakhstan. The study of algae diversity in charophyte habitats can serve as a potential tool for tracking community change due to climate change and future climate warming.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d14070530/s1>, Table S1. Diversity and ecological properties of species inhabitants of charophyte habitats of South and Southeastern Kazakhstan, 2019–2022.

Author Contributions: Conceptualization, E.S. and S.B.; methodology, S.B.; software, S.B.; validation E.S., G.J., S.B., T.S. and S.N.; formal analysis, G.J., S.N., S.B., A.J. and T.S.; investigation, E.S., G.J., S.N. and A.J.; data curation, G.J., S.N., S.B., A.J. and T.S.; writing—original draft preparation, G.J. and S.B.; writing—review and editing, E.S., G.J., S.N., S.B., A.J. and T.S.; visualization, S.B.; funding acquisition, E.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Individual registration number BR10264557 scientific and technical program: “Current flora and plant resources ecological state cadastral assessing (Almaty region) as the scientific basis for effective resource potential management”.

Institutional Review Board Statement: The study was approved by the Institutional Review Board (or Ethics Committee) of RSE on REM “Institute of Botany and Phytointroduction” FWLC MEGNR RK (Protocol No. 3 of 11 March 2022).

Data Availability Statement: Not applicable.

Acknowledgments: We express our gratitude to the General Director Gulnara Sitpayeva (Sitpayeva Gulnara, ORCID ID 0000-0003-4614-6155, Scopus Author ID: 11141957300, 56910146600) and Program Manager Liliya Dimeyeva (Dimeyeva L. ORCID ID 0000-0002-9101-0460, Scopus Author ID—55789522700), mycological laboratories and algologists in support of charophyte research in the region. This work was partly supported by the Israeli Ministry of Aliyah and Integration. We also thankful to Elena Cherniavsky for laboratory support.

Conflicts of Interest: The authors declare no conflict of interest.

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