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Effect of water quality on the spatial distribution of charophytes in the Peshawar Valley, Khyber Pakhtunkhwa, Pakistan

by

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Abstract

The presented research was conducted in 2018–2019 in the Peshawar Valley, Pakistan, to study for the first time the effect of water quality on the spatial distribution of charophytes. A total of six taxa of charophytes were found at 41 sites in the Peshawar Valley along the banks of seven rivers, 16 streams and two wetlands: *Chara braunii* C.C.Gmelin, *C. connivens* Salzmann ex A.Braun, *C. contraria* A.Braun ex Kützing, *C. globularis* Thuiller, *C. vulgaris* Linnaeus, and *Nitellopsis obtusa* (Desvaux) J.Groves. *Chara vulgaris* was the most abundant species, followed by *C. globularis*, and *C. contraria*. Water pH, electrical conductivity (EC), total dissolved solids (TDS), salinity and dissolved oxygen (DO) were within the permissible limits for Pakistan, while water temperature, oxidation reduction potential (ORP) and resistivity showed deviations. Canonical Correspondence Analysis (CCA) revealed that DO affected *Chara vulgaris*, pH and resistivity affected *C. braunii, C. connivens* and *C. globularis*, temperature and ORP affected *C. contraria* and *Nitellopsis obtusa.* Furthermore, CCA showed that TDS, EC, and salinity had no effect on the spatial distribution of *Chara contraria*, *C. vulgaris* and *Nitellopsis obtusa*. *Chara contraria* and *Nitellopsis obtusa* should be protected under VU (Vulnerable) status (IUCN) along with their habitats.

Key words: charophytes, spatial distribution, water quality, statistical mapping, CCA, Peshawar Valley, Pakistan

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1. Introduction

Charophytes are part of the large group Charophyta (Guiry & Guiry 2021), which includes five different classes, but this work covers only the diversity of the family Characeae from the order Charales. It is a group of monophyletic, highly evolved benthic macroalgae. They occupy an important position in the tree of life as modern terrestrial plants descend from this group and therefore have received considerable attention since the beginning of systematic botany (Domozych et al. 2016). Charophytes are distributed throughout the world except Antarctica. They are invasive species of freshwater lakes, streams and rivers; several species are also found in brackish to highly saline waters, while others are found in wetlands (Shepherd et al. 1999). They usually occur as benthophytes and form meadows in lentic or slow slowing waters. Charophytes usually form monospecific communities or occur together with other microalgae (Pełechata & Pełechaty 2010). As pioneer species, charophytes are the first to colonize emerging or disturbed water bodies (Wade 1990).

Charophytes include both extant and fossil members (Feist et al. 2005), with the former grouped in one extant family, i.e. Characeae with six genera and 690 species (Guiry 2012). *Chara* and *Nitella* are widely distributed and relatively species-rich genera, while the other genera – *Lamprothamnium*, *Tolypella*, *Lychnothamnus* and *Nitellopsis* – are represented by only a few species (Wood & Imahori 1965). Only 30 species of charophytes have been recorded so far in Pakistan (Faridi 1955, 1956; Pal et al. 1962; Sarim 1991; Aisha & Shameel 1995; Sarim & Ayaz 2000; Langangen & Leghari 2001).

Water is an essential component of life and the only habitat for charophytes. Physical and chemical components of water quality significantly affect the spatial distribution of aquatic organisms, especially charophytes. The diversity, distribution, and growth of charophytes are mostly affected by water temperature, pH, salinity, electrical conductivity, total dissolved solids, and the level of nutrients (Dąmbska 1964; Krause 1981; Blindow 1992; Haas 1994; Blindow & Langangen 1995; Pełechaty et al. 2004; Gąbka et al. 2007; Boszke & Bociąg 2008; Caisova & Gąbka 2009; Becker et al. 2016).

Human impact and consequent environmental changes have led to a progressive decline in the abundance, occurrence and diversity of charophytes over the past decades (Eriksson et al. 2004; Romanov 2009). Some of them have become rare or even extremely rare (Blaženčicć et al. 2006; Helcom 2013). Scientists in different countries study the effects of water quality on the spatial distribution of charophytes, their diversity and ecology (Blindow 2000; Bornette & Arens 2002; Sviridenko & Sviridenko 2003; Torn et al. 2004; Blaženčić et al. 2006; Romanov & Kipriyanova 2010; Romanov & Barinova 2012; Bolpagni et al. 2013; Pukacz & Pełechaty 2013; Noedoost et al. 2015; Torn et al. 2015; Vesić et al. 2016; Blaženčić et al. 2018). However, research on the effects of water quality on the spatial distribution of charophytes are yet to be undertaken in Pakistan.

The Peshawar Valley is an important geographical region in the upper Indus basin in Pakistan. Although this is a hydrologically rich area, no comprehensive research on the effects of water quality on the spatial distribution of charophytes has been carried out here so far. To address this, it was decided to investigate the spatial distribution of charophytes in the Peshawar Valley located in the province of Khyber Pakhtunkhwa (Pakistan) and to determine the major environmental factors governing it.

2. Materials and methods

2.1. Study area and selection of sampling sites

The Peshawar Valley is a geographically important location in the Khyber Pakhtunkhwa province of Pakistan. The valley is located between 33.45′ and 34.30′N latitude and 71.22′ to 72.50′E longitude, at an altitude of about 345 m. The valley is girdled by mountains except its eastern side, where the Indus River forms a natural boundary. The center of the valley is a broad plain, generally level, but with occasional hills and rocky protuberances. The Peshawar Valley has a rich hydrography, including many rivers, streams and wetlands (Fig. 1). The Kabul River collects water from the streams and rivers of the Peshawar Valley and drains it into the Indus River near Attock (Government of Pakistan 1998). All water bodies in the Peshawar Valley were surveyed for the spatial distribution of charophytes and water quality effects, however, the studied algae were found at only 41 sites along the banks of seven rivers, 16 streams and two wetlands (Fig. 1). The geospatial location of each sampling site was recorded using a Garmin GPS Navigator (Table 1).

2.2. Collection of specimens, preservation and laboratory processing

Charophyte specimens were collected at each sampling location within a 100 m radius by hand and using grapnels. Collected specimens were immediately preserved in situ with 5% FAA (formalin, acetic acid,

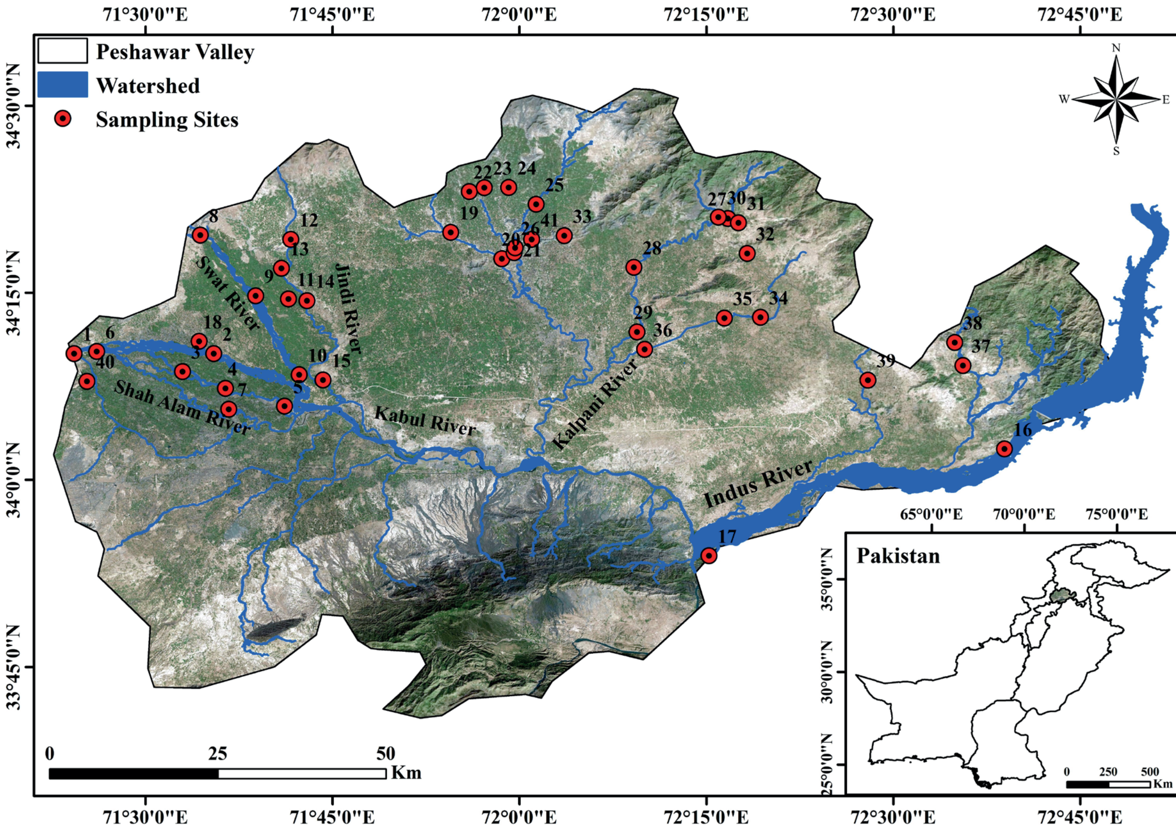

Figure 1

Map of the Peshawar Valley showing the study sites

and alcohol) solution in standard 500 ml specimen jars. Samples were labelled with a site code and stored in cardboard boxes to avoid spoilage (Edler & Elbrächter 2010). Micromorphology of charophytes was studied according to Urbaniak & Gąbka (2014). Specimens were places in Petri dishes and observed under an Olympus SZH stereo microscope using different magnifications (10× to 400×). Taxonomic identification of collected specimens was based on the standard literature of Urbaniak & Gąbka (2014), Wood & Imahori (1964), Pal et al. (1962), Prescott (1962), and Wood & Imahori (1959). To avoid using synonyms, the identified species were checked at www.algaebase.org. The conservation status and rarity of Charophyta species was assessed according to the IUCN criteria (IUCN 2012) using the following scheme:

2.3. Determination of physicochemical properties of water

The main physicochemical parameters of water quality (temperature, pH, oxidation reduction potential, electrical conductivity, resistivity, total dissolved solids, salinity and dissolved oxygen) were measured in situ in parallel with algae sampling at each sampling site using a HANNA HI-98194 multiparameter portable water quality meter (Khuram et al. 2019).

2.4. Statistical analysis and distribution mapping

The map of the study area was prepared in ArcGIS version 10.8 using geospatial coordinates. A new statistical method of environmental mapping was used to visualize the environmental variables and species distribution using Statistica version 12.0 according to parameter values and geospatial coordinates of each site (Barinova, 2017). Canonical Correspondence Analysis (CCA) was used for multivariate direct gradient analysis to assess the effect of water quality on the spatial distribution of charophytes. Charophyta

Table 1

Study sites in the Peshawar Valley with geographic locations

No.	Site Name	Site Code	Latitude	Longitude
1	Kabul River–Warsak	KRW	34°10′6.96″	71°24′15.84″
2	Kabul River–Haji Zai	KRHZ	34°10′6.96″	71°35′28.32″
3	Naguman River–Niyami	NRN	34°8′40.92″	71°32′58.56″
4	Naguman River–Naguman	NRNA	34°7′19.56″	71°36′25.2″
5	Naguman River–Jalabella	NRJ	34°5′54.6″	71°41′9.6″
6	Shah Alam River–Michni	SARM	34°10″17.04″	71°26′4.56″
7	Shah Alam River–Shah Alam	SARSA	34°5′39.48″	71°36′41.04″
8	Swat River–Munda	SRM	34°19′37.2″	71°34′23.88″
9	Swat River–Dildar Gharhi	SRDG	34°14′44.52″	71°38′50.28″
10	Swat River–Charsadda	SRC	34°8′25.44″	71°42′19.8″
11	Abazai River–Cheena	ARC	34°14′30.12″	71°41′27.96″
12	Jindi River–Tangi Harichand Road	JRTHR	34°19′15.96″	71°41′39.48″
13	Jindi River–Kanewar	JRK	34°16′57″	71°40′54.48″
14	Jindi River–Umarzai	JRU	34°14′21.84″	71°42′57.6″
15	Jindi River–Prang Majoke	JRPM	34°8′0.24″	71°44′14.64″
16	Indus River–Galla	IRG	34°2′29.04″	72°38′55.68″
17	Indus River–Attock Khurd	IRAK	33°53′55.32″	72°15′12.6″
18	Subhan Khwar Stream–Uchawala	SKSU	34°11′6″	71°34′17.76″
19	Jalala Stream–Jalala	JSJ	34°19′50.52″	71°54′29.52″
20	Jalala Stream–Mahabat Khan Koroona	JSMKK	34°17′43.44″	71°58′35.4″
21	Uch Khwar Stream–Umar Abad	UKSUA	34°18′12.24″	71°59′34.8″
22	Bama Kandah Stream–Hathian	BKSH	34°23′6.72″	71°55′59.16″
23	Ghargo Kandah Stream–Spalano Dheri	GKSSD	34°23″25.44″	71°57′10.8″
24	Lund Khwar Stream–Lund Khwar	LKSLK	34°23′26.16″	71°59′8.52″
25	Shamsi Dan Stream–Shamsi Dan	SDSSD	34°22′5.52″	72°1′21″
26	Shamsi Dan Stream–Said Abad	SDSSA	34°18′36″	71°59′39.84″
27	Balar Stream–Hamzakot	BSH	34°20′56.4″	72°16′43.68″
28	Balar Stream–Bakhshali	BSB	34°17′1.68″	72°9′11.16″
29	Balar Stream–Gari Kapura	BSGK	34°11′51.36"	72°9′26.64″
30	Pacha Tangi Stream–Cheena	PTSC	34°21′3.6″	72°15′58.32″
31	Dagi Stream–Hera Wand	DSHW	34°20′36.6″	72°17′34.44″
32	Machi Stream–Machi	MSM	34°18′ 9″	72°18′16.92″
33	Gadar Stream–Katlang Road	GSKR	34°19′33.96″	72°3′35.64″
34	Naranji Stream–Turlandi	NST	34°13′1.56″	72°19′20.64″
35	Naranji Stream–Adina	NSA	34°12′57.96″	72°16′26.76″
36	Naranji Stream–Sim Canal Road	NSSCR	34°10′27.48″	72°10′3.36″
37	Bada Stream–Pabaini	BSP	34°9′10.08″	72°35′35.52″
38	Panjman Stream–Panjman	PSP	34°11′0.24″	72°34′56.64″
39	Badri Stream–Mami Khel	BSMK	34°7′58.8″	72°27′58.68″
40	Warsak Wetland–Peshawar	WWP	34°7′53.76″	71°25′18.12″
41	Jamal Gharhi Wetland–Mardan	JGWM	34°19′16.32″	72°0′59.4″

species richness data along with water quality data were analyzed using CANOCO version 4.5 (Ter Braak and Šmilauer, 2002). Comparative analysis of species abundance of microalgal communities accompanying charophytes was performed using BioDiversity Pro version 2.0.

3. Results and discussion

A total of six taxa of charophytes were isolated and identified from 126 samples, collected from 41 sampling sites along the banks of seven rivers, 16 streams and two wetlands in the Peshawar Valley,

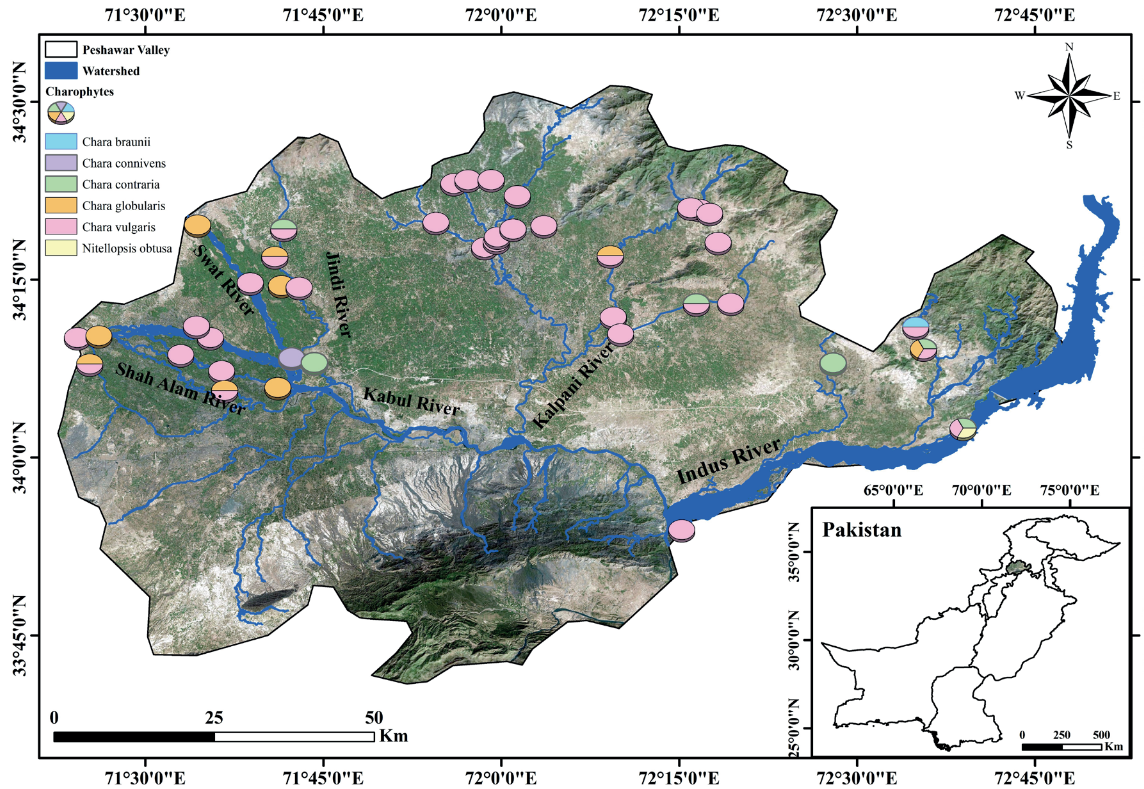

Figure 2

Map of the Peshawar Valley showing the spatial distribution of charophytes

Khyber Pakhtunkhwa, Pakistan. The species list comprises *Chara braunii*, *Chara connivens*, *Chara contraria*, *Chara globularis*, *Chara vulgaris* and *Nitellopsis obtusa* (Table 2). *Chara vulgaris* was the most abundant species, followed by *C. globularis*, and *C. contraria*. *Chara braunii*, *C. connivens* and *Nitellopsis obtusa* were the least abundant species (Fig. 2). The studies by Faridi (1955), Sarim (1991), Aisha & Shameel (1995), Sarim & Ayaz (2000), Langangen & Leghari (2001), Barinova et al. (2016), Khuram et al. (2017, 2019), Mursaleen et al. (2018a,b) also support our results.

Chara braunii C.C.Gmelin 1826

In the Peshawar Valley, *Chara braunii* was found only in the Panjman Stream at the village of Panjman, the Swabi District (Figs 2, 3a). Langangen & Leghari (2001) also reported *Chara braunii* from the village of Nagar at the Karakoram Mountain range, from the town of Khwazakhela and the city of Mangora in the Swat Valley, which supports our results.

Chara connivens Salzmann ex A.Braun 1835

In our study, *Chara connivens* was found only in the Swat River at the city of Charsadda in the Peshawar Valley (Figs 2, 3b). Langangen & Leghari (2001) also reported *Chara connivens* from Lake Kinjhar in the Thatta District, which supports our results.

Chara contraria A.Braun ex Kützing 1845

In the Peshawar Valley, *Chara contraria* was found in the Jindi River at the Tangi Harichand Road, in the Jindi River at the village of Prang Majoke, in the Indus River at the village of Galla, in the Naranji Stream at the village of Adina, in the Bada Stream at the village of Pabaini, and in the Badri Stream at the village of Mami Khel (Figs 2, 3c). Langangen & Leghari (2001) also reported *Chara contraria* from the Kotri pond, Lake Manchar, the village of Rani kot, the city of Sehwan, and the town of Thano bola in the Jamshoro District, which supports our results.

Chara globularis Thuiller 1799

In our study, *Chara globularis* was found in the Naguman River at the village of Jalabella, in the Shah Alam River at the village of Michni, the Shah Alam River at the village of Shah Alam, the Swat River at the village of Munda, in the Abazai River at the village of Cheena, in the Jindi River at the village of Kanewar, in the Balar

Table 2

Charophyta species and water quality parameters in the Peshawar Valley

Site Code	*Chara braunii*	*Chara connivens*	*Chara contraria*	*Chara globularis*	*Chara vulgaris*	*Nitellopsis obtusa*	Temp.°C	pH	ORP mV	EC, µS cm^{-1}	Resis. Ω-cm	TDS ppm	Sal. PSU	DO mg l^{-1}
KRW	-	-	-	-	+	-	15.84	8.19	67.4	435	1786	197	0.19	3.34
KRHZ	-	-	-	-	+	-	14.9	8.36	88.6	468	2158	240	0.24	4.16
NRN	-	-	-	-	+	-	11.58	8.34	147.3	471	2123	236	0.23	3.12
NRNA	-	-	-	-	+	-	11.94	8.25	28	468	2137	234	0.23	3.82
NRJ	-	-	-	+	-	-	14.07	8.41	86.3	438	2283	219	0.21	4.42
SARM	-	-	-	+	-	-	18.61	8.28	101.3	437	2288	218	0.21	3.48
SARSA	-	-	-	+	+	-	11.66	8.39	−50.8	474	2110	237	0.23	2.27
SRM	-	-	-	+	-	-	13.9	8.66	94.9	243	4115	122	0.12	4.34
SRDG	-	-	-	-	+	-	15.28	7.78	24.6	525	1905	262	0.26	3.91
SRC	-	+	-	-	-	-	16.44	8.39	74.5	308	3247	154	0.15	4.01
ARC	-	-	-	+	-	-	17.44	7.87	63.1	469	2132	234	0.23	3.83
JRTHR	-	-	+	-	+	-	16.42	8.49	68.2	582	1718	291	0.28	2.93
JRK	-	-	-	+	+	-	16.09	8.53	99.1	531	1883	265	0.26	4.39
JRU	-	-	-	-	+	-	15.24	8.07	67	565	1770	283	0.28	4.15
JRPM	-	-	+	-	-	-	14.74	8.2	82.4	549	1821	274	0.27	4.29
IRG	-	-	+	-	+	+	16.54	8.36	131.4	311	3215	156	0.15	3.44
IRAK	-	-	-	-	+	-	10.32	8.25	120.1	643	1555	321	0.32	3.84
SKSU	-	-	-	-	+	-	18.8	8.03	99.3	430	1753	176	0.17	4.02
JSJ	-	-	-	-	+	-	15.71	5.63	236.2	586	2152	297	0.29	3.34
JSMKK	-	-	-	-	+	-	14.79	8.34	63.3	554	1805	277	0.27	4.79
UKSUA	-	-	-	-	+	-	15.03	8.3	78.8	532	1880	266	0.26	4.66
BKSH	-	-	-	-	+	-	18.19	8.15	106.5	547	1828	274	0.27	4.59
GKSSD	-	-	-	-	+	-	16.93	7.8	124.5	581	1721	291	0.28	4.52
LKSLK	-	-	-	-	+	-	17.89	8.18	105.8	258	2175	315	0.31	4.46
SDSSD	-	-	-	-	+	-	15.25	8.45	101.2	296	2256	326	0.32	6.2
SDSSA	-	-	-	-	+	-	14.86	8.38	76.4	477	2096	239	0.23	5.09
BSH	-	-	-	-	+	-	19.37	7.71	85.7	557	1795	278	0.27	3.98
BSB	-	-	-	+	+	-	17.53	8.77	75	506	1976	253	0.25	8.09
BSGK	-	-	-	-	+	-	14.49	8.37	70.2	552	1812	276	0.27	4.59
PTSC	-	-	-	-	+	-	18.5	7.92	60.9	487	2053	244	0.24	3.82
DSHW	-	-	-	-	+	-	15.28	8.24	16.5	238	4202	119	0.11	2.66
MSM	-	-	-	-	+	-	18.53	7.85	78.7	402	2488	201	0.19	3.82
GSKR	-	-	-	-	+	-	18.22	8.12	87.7	579	1727	289	0.28	4.11
NST	-	-	-	-	+	-	14.66	8	71.5	468	2137	234	0.23	3.52
NSA	-	-	+	-	+	-	14.38	8.02	87.4	501	1996	250	0.24	3.46
NSSCR	-	-	-	-	+	-	12.28	8.35	71.1	639	1565	320	0.31	3.75
BSP	-	-	+	+	+	-	10.86	9.58	65.6	203	4926	102	0.1	4.34
PSP	+	-	-	-	+	-	11.99	8.84	63.5	269	3717	135	0.13	4.19
BSMK	-	-	+	-	-	-	16.33	8.1	54.2	509	1965	255	0.25	3.58
WWP	-	-	-	+	+	-	18.86	8.92	28.5	513	1949	256	0.25	2.22
JGWM	-	-	-	-	+	-	16.62	9.28	74.9	257	3891	128	0.12	7.02

Note: presence (+) and absence (-)

Stream at the village of Bakhshali, in the Bada Stream at the village of Pabaini, and in the Warsak Wetland at the village of Warsak, in the Peshawar Valley (Figs 2, 3d). Langangen & Leghari (2001) also reported *Chara globularis* from the village of Nagar at the Karakoram Mountain range, the Indus River at the city of Hyderabad, and Lake Kinjhar and the Sonda pond in the Thatta District, which supports our results.

Chara vulgaris Linnaeus 1753

In the Peshawar Valley, *Chara vulgaris* was found in the Kabul River (Warsak village), the Kabul River (Haji Zai village), the Naguman River (Niyami village), the Naguman River (Naguman village), the Shah Alam River (Shah Alam village), the Swat River (Dildar Gharhi village), the Jindi River (Tangi Harichand Road), Jindi

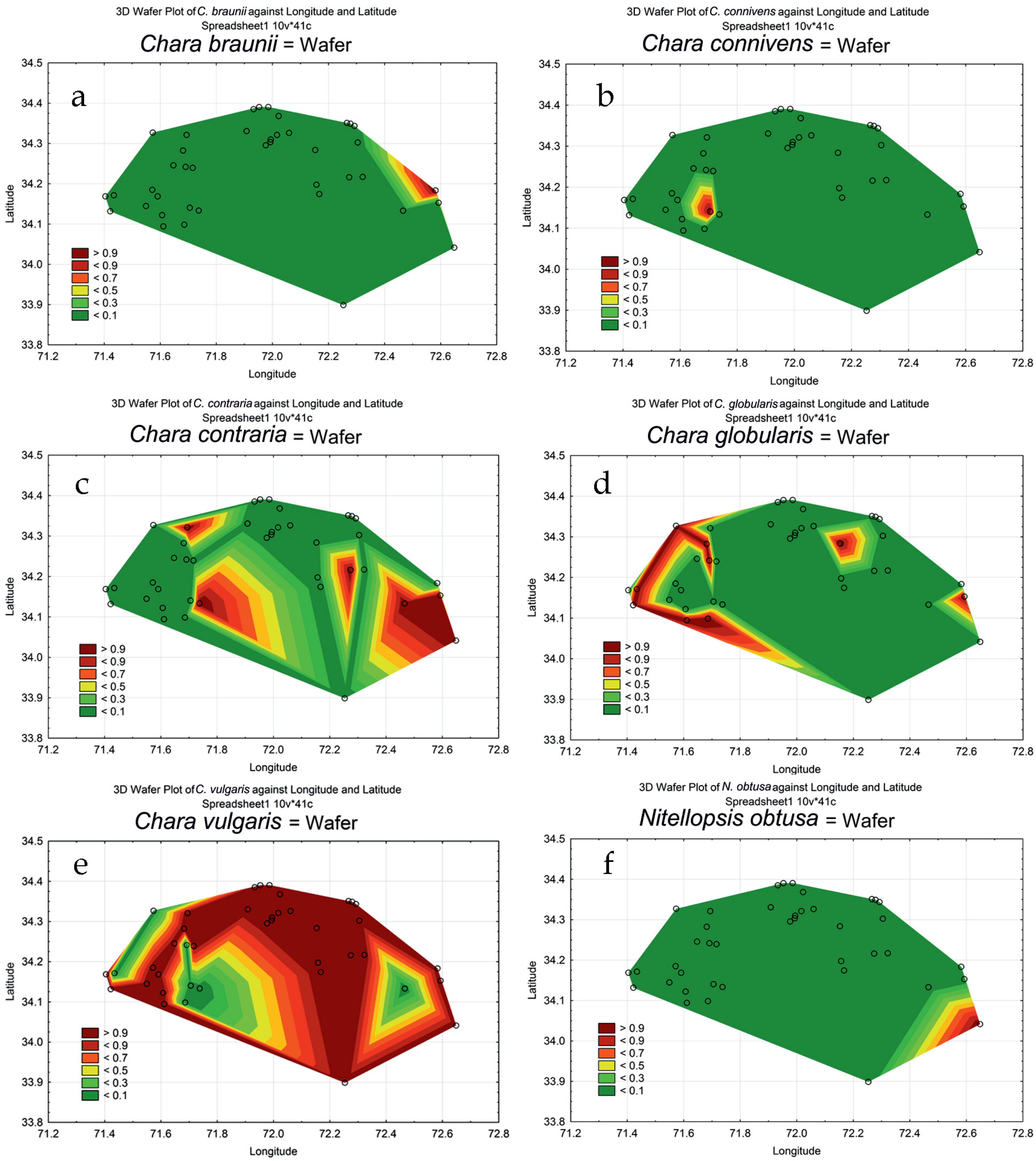

Figure 3

Spatial distribution of six Charophyta species at the sampling sites in the Peshawar Valley. *Chara braunii* (a); *C. connivens* (b); *C. contraria* (c); *C. globularis* (d); *C. vulgaris* (e); *Nitellopsis obtusa* (f)

River (Kanewar village), the Jindi River (Umarzai village), the Indus River (Galla village), the Indus River (Attock Khurd town), the Subhan Khwar Stream (Uchawala village), the Jalala Stream (Jalala village), the Jalala Stream (Mahabat Khan Koroona), the Uch Khwar Stream (Umar Abad village), the Bama Kandah Stream

(Hathian village), the Ghargo Kandah Stream (Spalano Dheri village), the Lund Khwar Stream (Lund Khwar village), the Shamsi Dan Stream (Shamsi Dan village), the Shamsi Dan Stream (Said Abad village), the Balar Stream (Hamzakot village), the Balar Stream (Bakhshali village), the Balar Stream (Gari Kapura village), the Pacha Tangi Stream (Cheena village), the Dagi Stream (Hera Wand village), the Machi Stream (Machi village), the Gadar Stream (Katlang Road), the Naranji Stream (Turlandi village), the Naranji Stream (Adina village), the Naranji Stream (Sim Canal Road), the Bada Stream (Pabaini village), the Panjman Stream (Panjman village), Warsak Wetland (Warsak village) and Jamal Gharhi Wetland (Jamal Gharhi village, Peshawar Valley; Figs 2, 3e). Langangen & Leghari (2001) also reported *Chara vulgaris* from Lake Bakar in the Sanghar District, Lake Chatar in the Mirpur District, Hanna Lake in the Queta District, Lake Manjosa in the Poonch District, Lake Rawla (Islamabad), the Tarbella dam in the Swabi District, and at the villages of Kochalk, Rawla Kot, Tando Adam and Tando Mitha Khan, and in the Haripur and Peshawar Districts, which supports our results.

Nitellopsis obtusa **(Desvaux) J.Groves 1919**

In our study, *Nitellopsis obtusa* was found in the Indus River at the village of Galla, the Peshawar Valley (Figs 2, 3f). Langangen & Leghari (2001) reported *Nitellopsis obtusa* from Lake Bakar in the Sanghar District, Lake Kinjhar in the Thatta District, and the Breja spring in the Dadu District, which supports our results.

The water temperature ranges from 10.32°C to 19.37°C, pH from 5.63 to 9.58, ORP from −50.8 mV to 236.2 mV, EC from 203 µS cm^{-1} to 643 µS cm^{-1}, resistivity from 1555 Ω-cm to 4926 Ω-cm, TDS from 102 ppm to 326 ppm, salinity from 0.1 PSU to 0.32 PSU and DO from 2.22 mg l^{-1} to 8.09 mg l^{-1} (Table 2). The results showed that water quality parameters varied within a narrow range. They indicate that water at the surveyed sites was fresh, slightly saline, slightly alkaline and moderately saturated with oxygen. Water pH, EC, TDS, salinity and DO were within standard limits, while water temperature, ORP and resistivity showed deviations from good conditions (Wetzel 1983; Horne & Goldman 1994; Al-Badaii et al. 2013; Adhena et al. 2020; Alam et al. 2020). The sampling season, agricultural and nutrient runoff, domestic and industrial effluents were the major causes of fluctuations in water quality parameters, similar to previous studies conducted in habitats of the Upper Indus River (Ali et al. 2007; Barinova et al. 2013, 2016).

The distribution of the main environmental parameters is shown in Figure 4 in the form of statistically generated maps. Water temperatures in the studied water bodies were higher in small foothill rivers and streams (Fig. 4a). At only one site was the water pH below 7, reflecting the acidification impact (Fig. 4b). Salinity mapping showed that saline water was located in the valley plane (Fig. 4c). The highest dissolved water oxygen concentrations were found in several foothill habitats (Fig. 4d).

Canonical correspondence analysis (Fig. 5) showed that dissolved oxygen positively affected *Chara vulgaris* and negatively affected *C. contraria* and *Nitellopsis obtusa*. *Chara braunii, C. connivens* and *C. globularis* were positively affected by pH and resistivity, and negatively affected by total dissolved solids, electrical conductivity, and salinity. Temperature and oxidation reduction potential positively affected *C. contraria* and *Nitellopsis* and negatively affected *C. vulgaris*. Furthermore, CCA also revealed that total dissolved solids, electrical conductivity, and salinity have no effect on the spatial distribution of *Chara contraria*, *C. vulgaris* and *Nitellopsis obtusa*.

Statistical plots of Charophyta species in relation to water quality variables demonstrate habitat preferences of the identified Charophyta species (Figs 6, 7). *Chara vulgaris* demonstrated different preferences with respect to habitat parameters of the Peshawar Valley. Unlike other species, this species preferred more oxygenated water with elevated conductivity (Fig. 6), TDS, and salinity (Fig. 7). All six species preferred lower water temperature in the catchment of the Peshawar Valley (Fig. 7).

Comparative analysis of species abundance of microalgae communities associated with charophytes (Fig. 8) revealed high individuality of algae assemblages, as the percentage of similarity in Bray–Curtis analysis was below 25%. The most similar communities were those accompanying *Chara contraria* and *Nitellopsis obtusa*. The second similar pair were the communities of *Chara globularis* and *C. vulgaris*. The most distinct communities were those accompanying *Chara braunii* and *C. connivens*. Since microalgae communities show a clear response to the studied environment, we analyzed the distribution of Charophyta species in relation to environmental parameters (Figs 6, 7). Thus, in the pair of *C. contraria* and *N. obtusa*, which demonstrated similar preferences, the former species appears more indicative and can therefore be used as an indicator for this pair. Although, the communities of *C. globularis* and *C. vulgaris* were similar, the preference profiles were unique for each species (Figs 6, 7). At the same time, *C. braunii* and *C. connivens* have the same preferences for environmental parameters of waterbodies in the Peshawar Valley. Therefore, in this pair, both species can serve as indicator species.

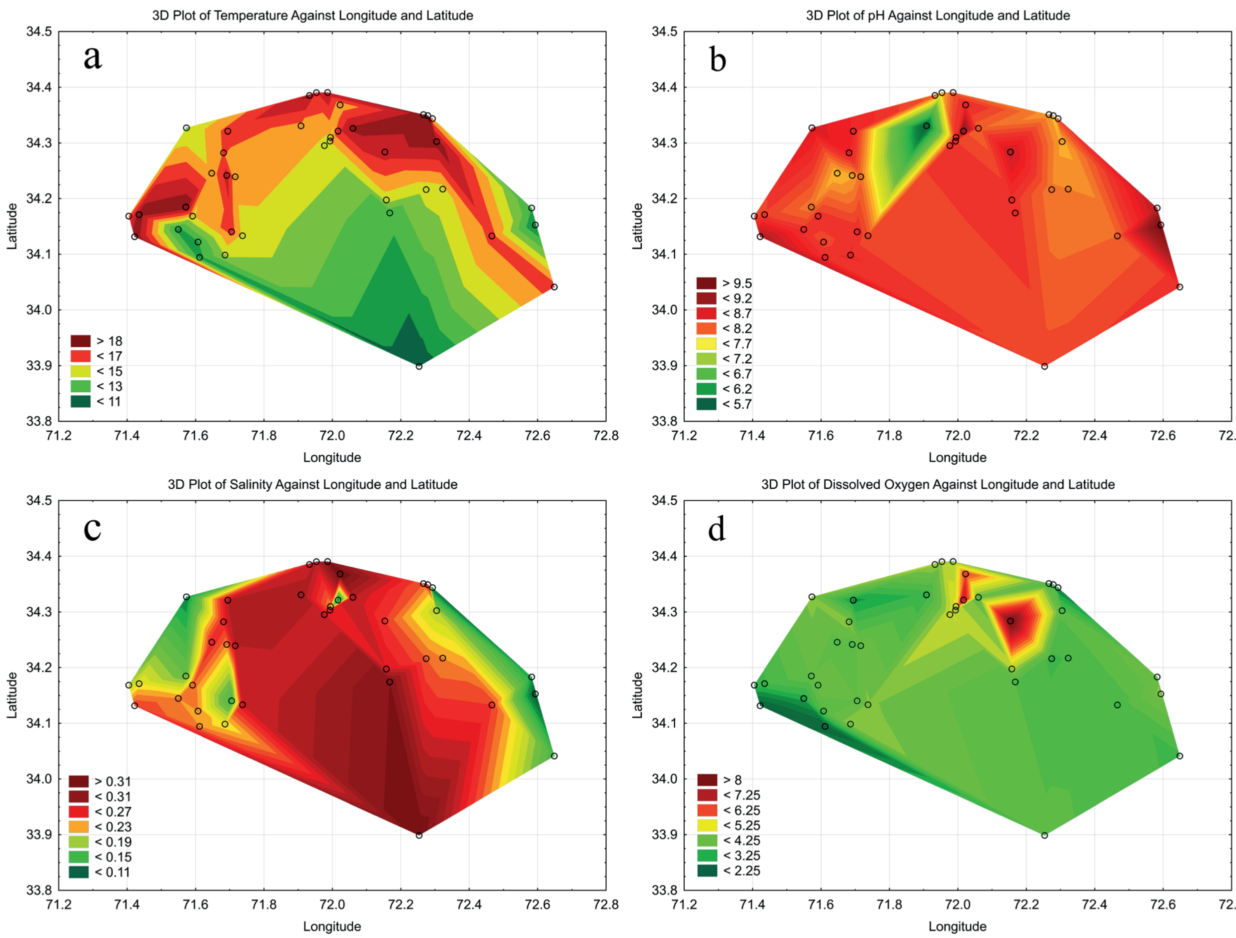

Figure 4

Distribution of water quality parameters in the water bodies of the Peshawar Valley. Water temperature (a); pH (b); Salinity (c); Dissolved Oxygen (d)

The Charophyta species analyzed in this study were usually assessed not only as indicators of environmental quality but also as indicators of threats to algal communities. Charophytes make up the majority of algae biomass in most habitats, but if one species is endangered, the entire community is threatened. Since Pakistan does not yet have a Red List of Endangered Species, our results will serve as a basis for developing such a list for aquatic species. We compared the six species studied with the Red Lists of Balkans, Poland, Ukraine, the Czech Republic, and Germany in terms of the IUCN risk categories (Palamar-Mordvintseva & Tsarenko 2004; Blaženčić et al. 2006; Caisova & Gąbka 2009; IUCN 2012; Becker et al. 2016). We found that *Chara braunii, C. contraria, C. globularis* and *C. vulgaris* can be classified as being at low risk of extinction (LR). *C. connivens* is endangered in the Balkans and threatened with extinction in Poland (EN). *Nitellopsis obtusa* is a regionally very rare

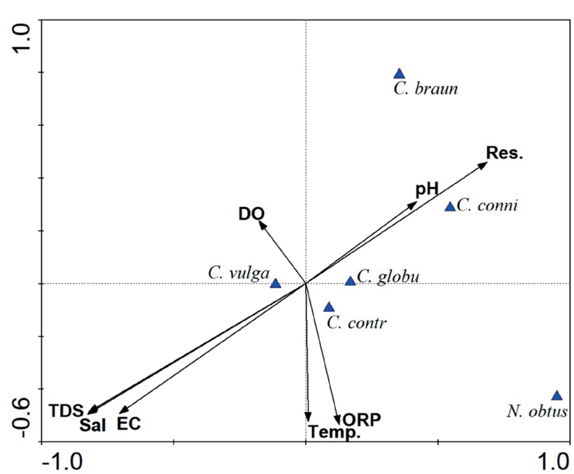

Figure 5

Canonical Correspondence Analysis (CCA) biplot of environmental variables in relation to species richness at the sampling sites in the Peshawar Valley

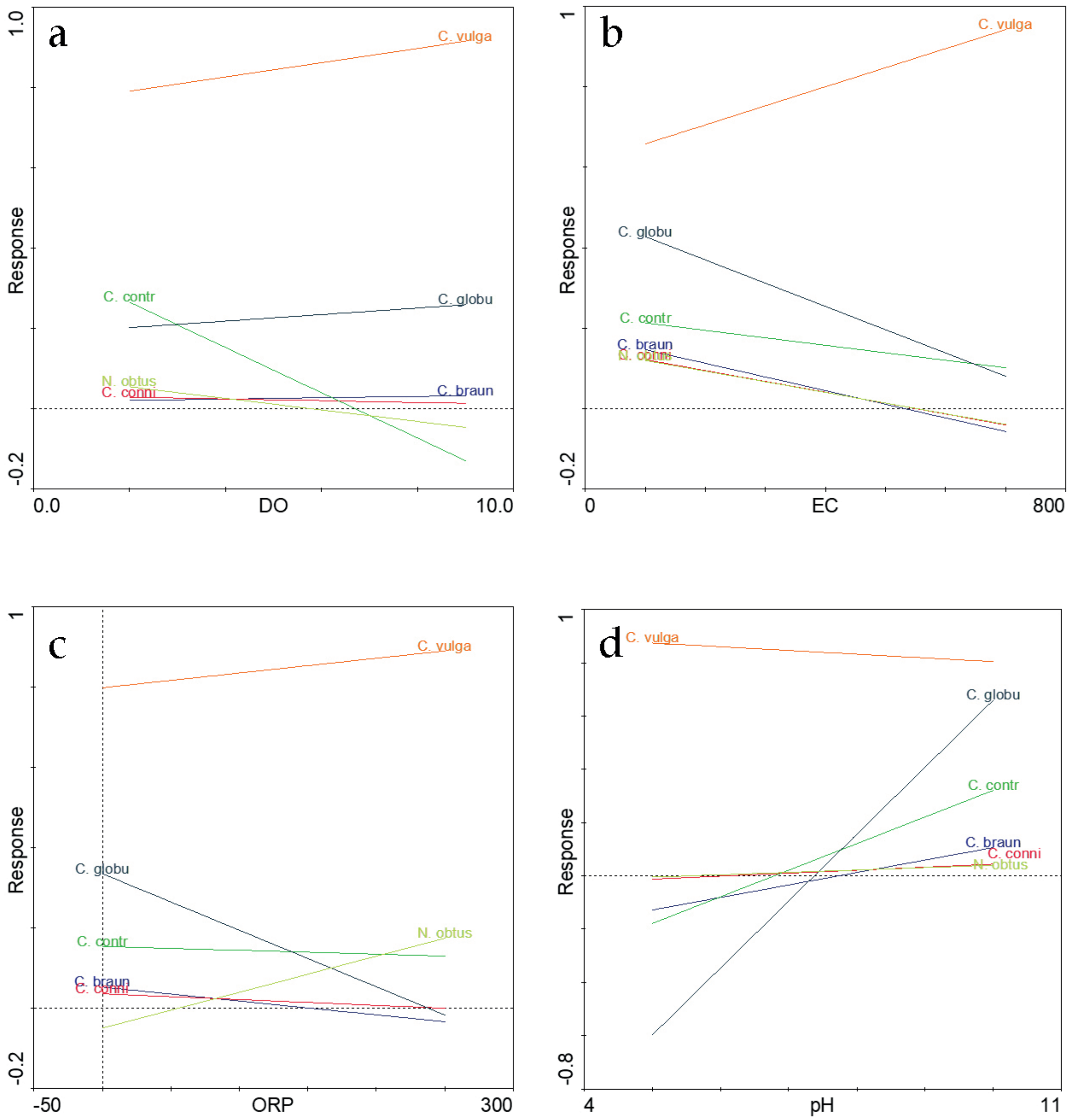

Figure 6

Statistical plots of Charophyta species in relation to water quality variables in the water bodies of the Peshawar Valley. Dissolved Oxygen (a); Electrical conductivity (b); ORP (c); pH (d)

species and has a disjunctive distribution worldwide. It occurs in water bodies in various countries of Europe and Asia. In Europe, it is classified as at high risk of extinction (VU). However, the determination of the VU category for this species in the flora of Pakistan requires additional argumentation and factual material on its vulnerability, which can be found in the Red List of Algae compiled for Pakistan.

It is known that pH, conductivity, salinity, total hardness, carbonate hardness, total phosphorus, total nitrogen, ammonia nitrogen are important for charophytes in European habitats (Becker et al. 2016). In Europe, *Nitellopsis obtusa* prefers slightly alkaline or neutral water, *C. globularis* and *C. contraria* thrive better in brackish water with high conductivity, *C. contraria, C. globularis, C. vulgaris*, and *Nitellopsis*

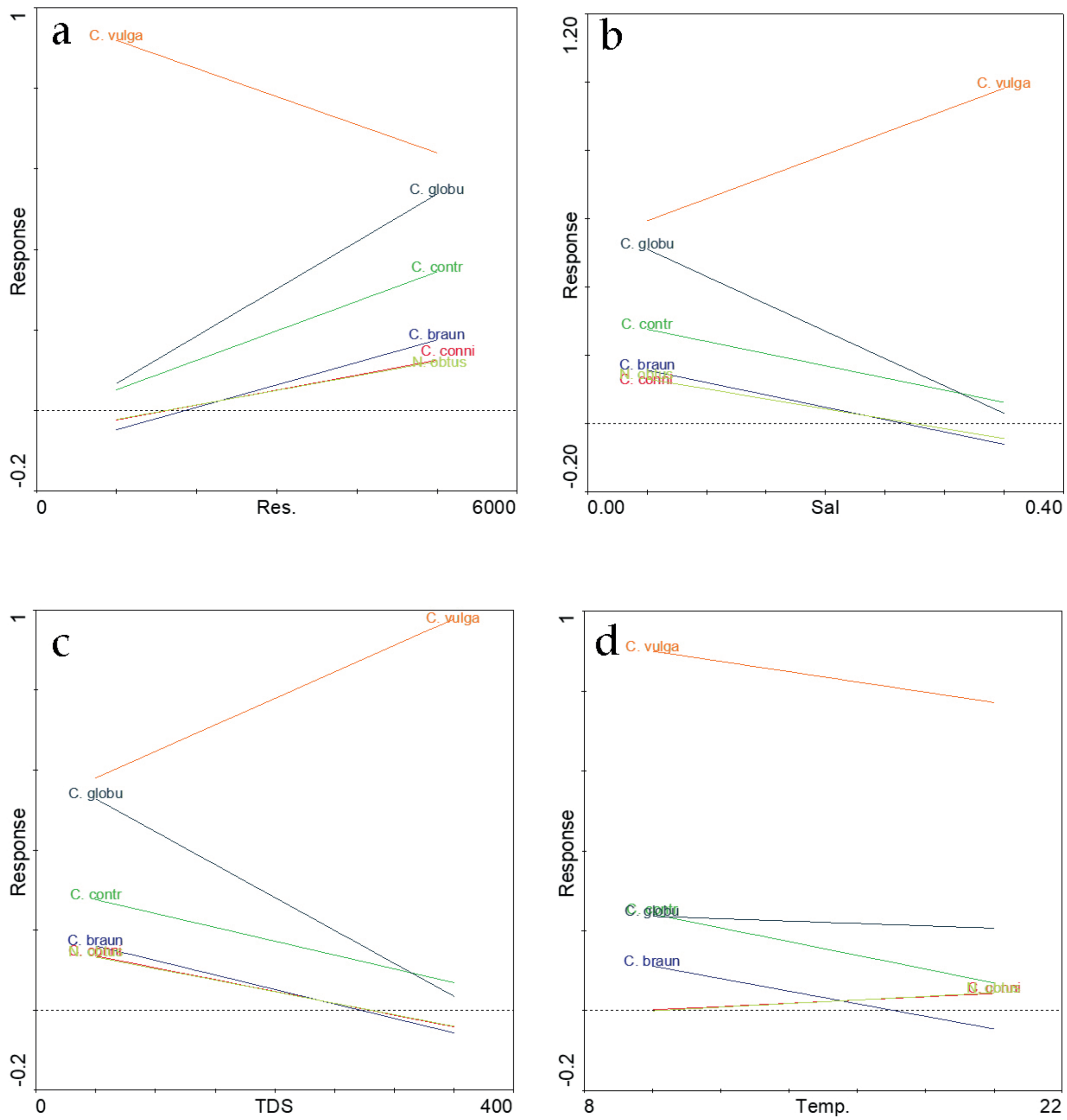

Figure 7

Statistical plots of Charophyta species in relation to water quality variables in the water bodies of the Peshawar Valley. Resistivity (a); Salinity (b); TDS (c); Water temperature (d)

obtusa tolerate moderate organic pollution. We confirmed that rare *Nitellopsis obtusa* in the ecosystems of the Peshawar Valley prefers low alkaline waters with low temperature, low salinity and low TDS, which extends the ecology of this species. Since *Nitellopsis* was recorded at only one site (Fig. 3f), the species was classified as VU. *Chara contraria* communities were paired with *Nitellopsis* classified as VU, because they avoid polluted waters at the inlet of the Swat and Kabul rivers into the Peshawar Valley (Fig. 3c,f), which was also reported from Poland (Blaženčić et al. 2006). *C. vulgaris* and *C. globularis*, on the other hand, were more common in the Peshawar Valley (Fig. 3d,e). They prefer slightly contaminated water bodies. Therefore, neither of the species in this pair could be included in the IUCN conservation categories in our study area. The last pair of species with similar community responses to the environment are *C. braunii* and *C. connivens*, both of which are rare in the Peshawar Valley. Both species prefer low saline and low alkaline

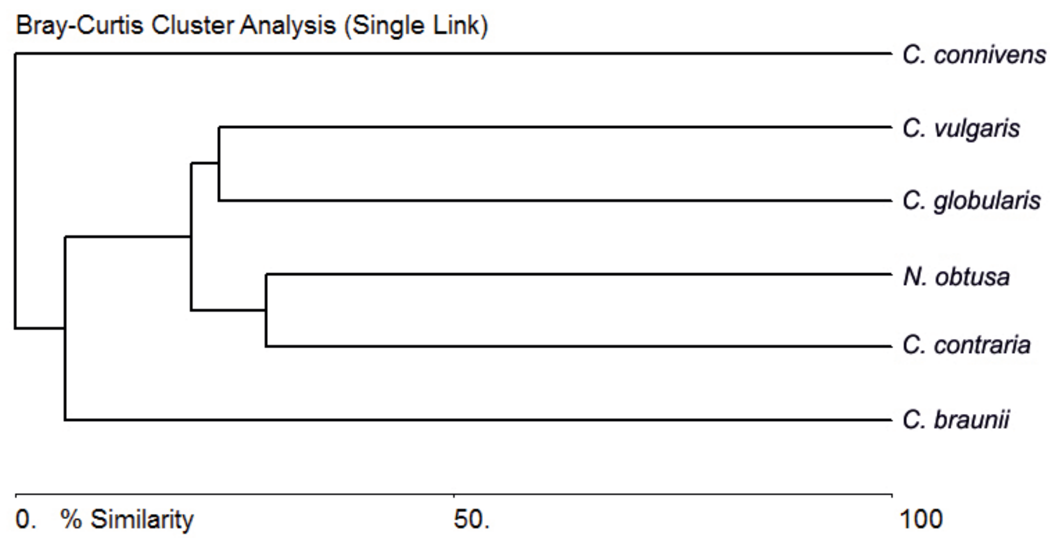

Figure 8

Bray–Curtis similarity tree of Charophyta communities at the sampling sites in the Peshawar Valley

waters and avoid polluted areas as *Nitellopsis*. This allows us to assign them to categories from LR to EN, as in Europe.

4. Conclusion

Charophytes in the Peshawar Valley were represented by six species from the genera *Chara* and *Nitellopsis*, which were identified through the analysis of 126 samples collected from 41 sampling sites in 2018–2019. Ecological preferences of each species were revealed using different statistical programs. The similarity of accompanying algal species in terms of their abundance (Khuram et al. 2017, 2019) was also calculated to confirm the ecology of Charophyta at the study sites. Three pairs of species with similar responses to environmental parameters of water quality were identified. *Chara braunii* and *C. connivens* prefer low saline and low alkaline water and avoid polluted water, which allowed us to assign them to IUCN conservation status from LR to EN, as in Europe. *Chara contraria* and *Nitellopsis obtusa* avoid polluted waters and prefer low saline and low alkaline water with low temperature. The extinction risk for both species in the Peshawar Valley can be defined as VU and their habitats could be protected. The last pair, *Chara vulgaris* and *C. globularis*, tolerates pollution and prefers high salinity, high alkaline and well oxygenated water. Both species were found in heterogenous habitats of the Peshawar Valley and did not avoid polluted sites where the Swat and Kabul rivers enter the valley, so they do not need protection.

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