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Research Article

Morpho-Taxonomic Identification and Seasonal Correlation between Algal Diversity and Water Physico-Chemical Parameters in District Bajaur Khyber Pakhtunkhwa

Nabi Ullah, Abdul Samad Mumtaz*, Lubna Anjum Minhas, Muhammad Kaleem, Rooma Waqar, Amber Jabeen and Ayesha Hanif

Department of Plant Sciences, Quaid-i-Azam University, Islamabad, 45320, Pakistan

Abstract | The current study was carried out to identify morpho-taxa and determine the seasonal link between algal diversity and water physico-chemical parameters in District Bajaur, Khyber Pakhtunkhwa. Total of 52 algal species belonging to 2 classes, 6 orders, 13 families, 22 genera, were reported from the four different sites (E1, E2, E3 and E4) of District Bajaur Khyber Pakhtunkhwa. Comprehensive investigation of four research stations revealed the phytoplankton diversity from Chlorophyceae to Cyanophyceae. All species were collected from the fresh water bodies during March spring to August rainy season in 2021. Collected Species were scrutinized taxonomically by using light microscope. In all studied sites genus *Scenedesmus* was dominant with fifteen species in both seasons, while all other micro-algal species were dominant at site E1, Salarzai Thali Dam in March spring session. During the study period, a total of 36 green micro-algal species and 16 blue-green micro-algal species were documented. Additionally, the sampling sites underwent thorough water physico-chemical analyses to explore any potential correlations with the diversity of phytoplankton. Conducted water physico-chemical analysis revealed that phytoplankton diversity greatly affects by various factors like Total soluble salts, Total Alkalinity, pH, TDS, EC, Temperature, Carbonate CO_3^{-2}, Bicarbonate HCO_3^{-1}, Chloride Cl^{-1}, Sulphate SO_4^{-2}, NO_3^{-1}, Ca^{+2}, Mg^{+2}, Na^{+1}, K^{+1}, BOD and COD, Turbidity. The supreme supportive water physico-chemical parameters for the algal development was recorded are the Temperature, pH, and the presence of nutrients like NO_3^{-1}, Na^{+1}, K^{+1}, Mg^{+2}, Ca^{+2}. Hence, the current study reveals that District Bajaur is a rich source of algae and an ideal place for their cultivation.

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***Correspondence** | Abdul Samad Mumtaz, Department of Plant Sciences, Quaid-i-Azam University, Islamabad, 45320, Pakistan; **Email:** as-mumtaz@qau.edu.pk
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Introduction

Algae are a broad category of photosynthetic organisms, that make up the majority of all living species' primary producers and they show a wide range of structures and sizes, from microalgae to enormous seaweeds (Gao *et al.*, 2021). They are the heterogeneous organisms can be found in a variety of habitats which can be aquatic, terrestrial, epizoic, endozoic, epiphytic, epileptic and may be symbionts (Minhas *et al*., 2023). Algae can exist in two different types of water which can be lotic fresh water bodies like Stream Rivers and lentic fresh water bodies like lakes and wet lands (Hook *et al*., 2020).

Algal taxonomy plays a vital role in the field of phycology and is crucial for various aspects such as algal genetics, physiology, ecology, applied phycology, and especially bioassessment. The process of taxonomic identification is the primary method used for analyzing and testing hypotheses in scientific research. However, identifying algae accurately can be challenging due to their small size and intricate morphologies, which often require specialized research-grade microscopes and expert knowledge in the field of phycology. As a result, errors in identification can occur, highlighting the importance of access to appropriate resources and taxonomic expertise in the study of algae (Manoylov, 2014).

A comprehensive study was conducted to investigate the algal diversity of District Peshawar, Sheikh Muhammadi during 2013. Total of 22 species were recorded belonging to 10 families among them Oedogoniaceae family appeared dominant with 5 species by (Ali *et al*., 2015). Several phycologists reported the biodiversity and seasonal variations of fresh water algae. Taxonomically thirty-five species belonging to fresh water were identified with water quality from river Panjkora at different sites during February 2015 to August 2016 by (Shuaib *et al*., 2017).

Later on fresh water algae were poorly studied, especially on the basis of taxonomy in district Marden during 2017-2018 to evaluate the distribution and seasonal variation of fresh water algae. The total 55 species belonging to 15 and other genera with most dominant genus *Spirogyra* were recorded from different localities of district Mardan, Pakistan by (Ullah *et al*., 2021). The diversity of fresh water algae of district Chitral was observed from various habitats with 52 species belonging to different 15 genera were recorded by (Ullah *et al*., 2019).

Among the different identified genera, the genus Mougeotia are dominant with 12 species. Different micro algal species from the selected regions of Khyber Pakhtunkhwa Peshawar valley Gulbahar, Ringroad and Hayatabad were investigated including altogether 30 species of algae belonged to 4 classes having 16 genera were collected from different water habitats (Salah-Ud-Din *et al*., 2017) in which Bascillariophyceae are the most prevalent and represented by 16 species. The diversity of algae depends upon the quality of water and temperature of water which play a major role in species richness distribution (Hussain *et al*., 2016). From all water bodies' algal species were reported, but for the growth of most species pound was found the most favorable among the stagnant water. Similarly, slow running water also favor for the algal growth (Ali *et al*., 2015). The purpose of the current study is to identify morpho-taxa and establish the seasonal relationship between algal diversity and water physico-chemical parameters in District Bajaur, Khyber Pakhtunkhwa.

Materials and Methods

Study area

District Bajaur is situated in Pakistan's Khyber Pakhtunkhwa province's Malakand Division, at the very tip of the Himalayan Range. In terms of latitude and longitude, it is located at 34.85'96" north and 71.42'99" east with an altitude of 870 meters (2854 feet).

To assess the algal diversity and their correlation with physicochemical parameters of water, four different sites of the District Bajaur were slected. The visited sites included Thali Dam Salarazai encoded samples E 1, Saddiq Abad Phattak encoded samples E2, Inayat Qilla Khwar encoded samples E3 and Khazani Chena encoded samples E4. Algal samples were taken from both polluted (such as ditches, stagnant water and dams) and unpolluted (such as streams, running water, ponds, and spring water) locations.All the samples were collected in two distinct seasons: The spring of 2021 (March) and the rainy summer of 2021 (August).

Collection of algal samples

50 ml Falcon tubes were used for the collection of water and algal samples and labeled according to dates, sample numbers and sampling sites. Water was added to tubes from collection sites and algal samples were shifted into falcon tubes with the help of forcep (Rehman *et al*., 2021). Each sample was collected in a separate falcon tube and then labelled with necessary informations. Field data of collected samples are mentioned in Table 1.

Table 1: *Field data of collected samples.*

S. No	Sample code	Location	Latitude	Longitude	Altitude	Substrate
01	E1	Salarzai Thali	34.81101	71.54671	892m	Dame water
02	E2	Saddiq Abad	34.72681	71.48661	911m	Sewage water
03	E3	Inayat Qilla Khwar	34.75728	71.46805	873m	Stream water
04	E4	khazany Chena	34.70684	71.46041	869m	Spring water

Water physico-chemical analysis

The physico-chemical parameters of the water samples were analyzed at the Chemical Testing Lab, specifically the Water Testing Section, located at Qarshi Research International (Pvt) Ltd in Haripur, Pakistan.

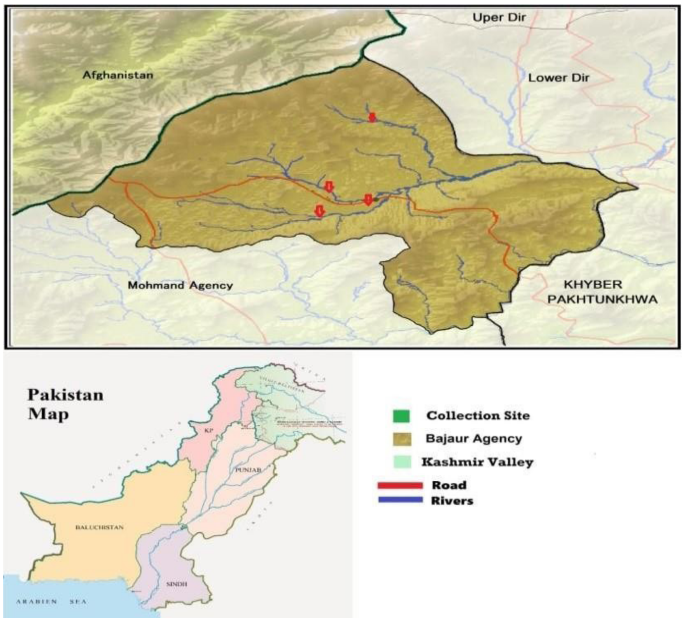

Figure 1: *Map of an Area; Distric Bajaur.*

Microscopic and experimental observations

Algal sample was taken from the falcon tubes with the help of forecep, and was placed on the clean slide. One drop of distell water was added and the material was spread with the help of toothpick on the slide. Coverslip was put on the material and lightly pressed with the help of thumb to remove extra water droplets from the material. After this, sample was observed under light micoscope using different magnification power e.g., 10X, 40X and 100X. Additionally, immersion oil was used to observe the slide at 100X, and a drop of that oil was applied to the cover slip. Using the calibrated eyepiece, the length and width of each species were measured. All morphological characteristics, including color, shape, pyrenoid, filamentous, branching, unbranched, unicellular, chloroplast arrangement, and presence or absence of mucilage sheath, were noted. Each species was captured on camera, and species were recognized by comparing the images to reliable sources of information (Minhas *et al*., 2023; Arguelles and Monsalud, 2017; Jacquemin *et al*., 2019).

Identification of the algal samples

Algal samples were observed and identified with the help of authentic literature (Hook *et al*., 2020; Beherepatil and Deore, 2013; Ramos *et al*., 2012; Chakraborty *et al*., 2020; Vijayan and Ray, 2015). The cell structure, shape, presence and absence of pyrenoid, chloroplast shape, quantity, and position were all analysed in depth.

Results and Discussion

From four different sites (E1, E2, E3 and E4) of District Bajaur, a total of 52 algal species belonging to 2 classes, 6 orders, 13 families, 22 genera were reported as shown in Table 2.

Spirogyra varians tipnee

The green filamentus algae without branching with a cylindrical cell body.The vegetative cells are 35 µm in width while as 38 µm long. One or various chloroplasts bands are spirally arranged with in the cell. Pyrenoids are present and septum with folded structure (Figure 2A).

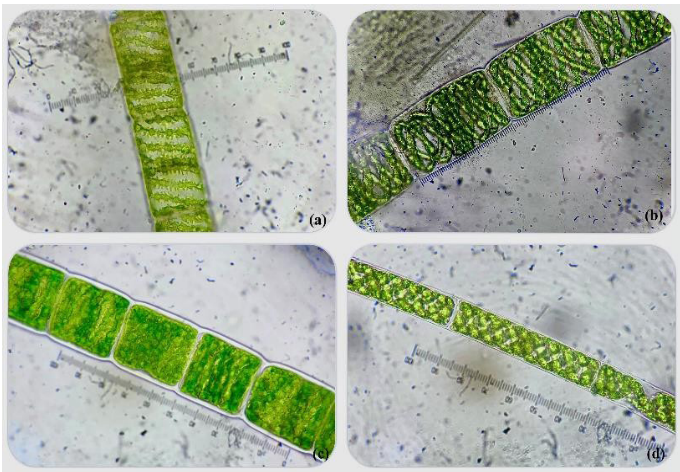

Figure 2: *a, b, c, d Spirogyra varians, Spirogyra maravillosa transeaue, Chaetomorpha aerea, Spirogyra corrugata.*

Table 2: *Classification of the micro-algal species reported from sites E1,E2,E3 and E4.*

Class	Order	Family	Genus and species
Chlorophyceae	Chlorococcales	Chlorococcaceae	*Chlorococcum humicola*
		Chlorellaceae	*Chlorella vulgaris*
		Dictyosphaeriaceae	*Westella botryoides, Dictyococcus varians, Dictyochloropsis splendid*
		Oocystaceae	*Oocystis pusilla*
		Coelastraceae	*Scenedesmus vacuolatus, S. ecornis, S. opoliensis, S. bijugatus, S. incrassatulus, S. dimorphus, S. platydiscus, Scenedesmus bijuga, S. quadricauda, S. armatus, S. quadricauda, S. bijugatus, S. abundans, S. bernardii, S. obliquus*
	Ulotrichales	Microsporaceae	*Microspora stagnorum, M. amoena*
		Ulotrichaceae	*Klebsormidium flaccidum*
	Conjugales	Desmidioidiaceae	*Cosmarium punctulatum, Desmodesmus abundans, D. baconii*
		Zygnemaceae	*Spirogyra varians, S. maravillosa, S. corrugate, Zygnema cruciatum, Macrochloris radiosa*
	Siphonales	Chaetosiphonaceae	*Chaetomorpha aerea, Monoraphidium contortum, M. circinal, M. nanum*
Cyanophyceae	Nostocales	Nostocaceae	*Nostoc linckia, N. spongiaeforme, N. punctiforme, Anabaena affinis, A. azollae,*
		Oscillatorieceae	*Phormidium chalybeum, Oscillatoria sancta, O. princeps, O. limnetica, O. tenuis, O. curviceps, O. limosa, Leptolyngbya vincentii,*
	Chroococcales	Chroococcaceae	*Gloeocapsopsis pleurocapsoides, Chroococcus disperses, Coelosphaerium pallidum Lemmermann*

Spirogyra maravillosa transeaue

The green falimentus aquatic algae, vegetative cells are approximately 28µm(20-30 µm) in width and 130 µm (100-250µm) in length along with plane end walls. The numbers of chloroplast 2-3 are spirally arranged and making 2-5 twists per cell (Figure 2B).

Chaetomorpha aerea (Dillwyn) Kützing

The color is bright grass green to yellow vivid green, generally 5-10 cm in height and the cells are morphologically cylindrical with 400-620 µm in diameter. Basal cells are elongated and 200-240 µm in diameter 5-10 times long and in contrast it similar to *Chaetomorpha linum*, attached to the bottom. It commonly seen in summer and leaves only for one year and generally found in streams and ponds where sufficient water exchanges (Figure 2C).

Spirogyra corrugata transeau

The aquatic green filamentus algae, generally 28-34 µm in width and 200-600 µm long with 2-3 chloroplasts are closely spiralley arranged making 3-5 turns per cell.The end walls are plane and septums are close to each other.The colour of chloroplast are dark and yellow green (Figure 2D).

Microspora amoena var. gracilis (Wille) de toni

Cell cylindrical 18-24 µm wide, 1-1.5 times longer then wide and wall thick about 2 µm. Slightly constriction at cross wall surface smooth and striated, junction between H sections clearly observable. Chloroplasts are in granular form and nearly filling cell (Figure 3A).

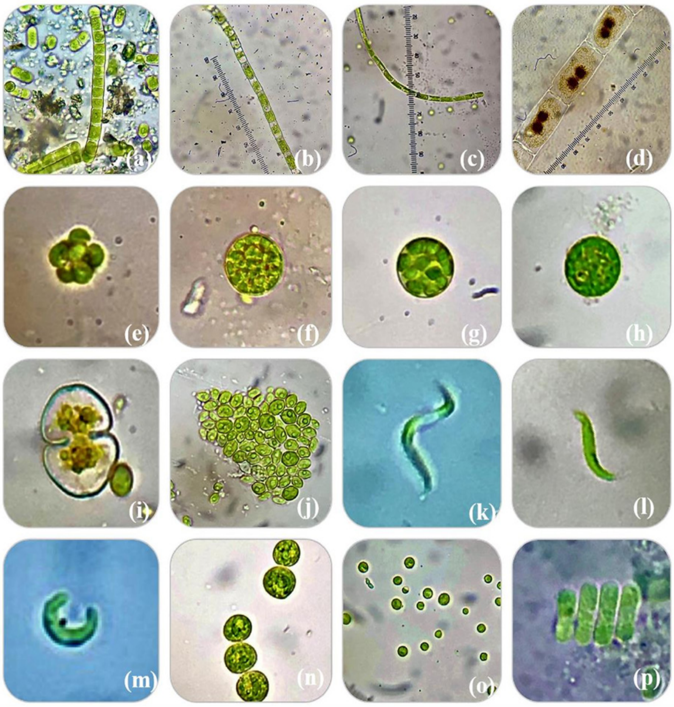

Figure 3: *a, b, c, d, e, f, g, h, I, j, k, l, m, n, o, p: Microspora amoena, Microspora stagnorum, Klebsormidium flaccidum, Zygnema cruciatum, Westella botryoides, Dictyococcus varians, Macrochloris radiosa, Dictyochloropsis splendid, Cosmarium punctulatum, Chlorococcum humicola, Monoraphidium contortum, Monoraphidium circinal, Monoraphidium nanum, Scenedesmus vacuolatus, Chlorella vulgaris, Scenedesmus ecornis.*

Microspora stagnorum (Kutzing) Lagerheim 1887
Filementus cylindirical cells, thin walled the two section not apperent. The cells as much as three times their diameter in length and slightly constracted at the cross wall. Chloroplast incomplete covering the wall in the form of granular sheet. Cells dimension; 8.7-9 µm wide,17-25 µm in length (Figure 3B).

Klebsormidium flaccidum (Kutz.)
Cylindrical cells, filimentus algae without branching. Filementus were very long, about more the thousands cells per filement. Cells were surrounded by mulicage and mature filament with blatant starch grains covering the pyrenoid. Dimension of cell, 6.4-7.3 µm in width while 10-14 µm long (Figure 3C).

Zygnema cruciatum (Vaucher) C. agardh
Cylindrical unbranched filamentus cells with two stellate chloroplasts per cell with a pyrenoid in each chloroplast. The width of cells are 32-36 µm while 45-50 µm in length (Figure 3D).

Westella botryoides (West) de wildeman
Ceonobia structured generally occure in 4-6 cells colonies formed with a 10-14 µm in diamater.Cells with a single pyrenoid and have parietal chloroplasts. Usally cells are spherical and smooth with a 4.0-7.0 µm in a diameter which creating irregular colonies (Figure 3E).

Dictyococcus varians gerneck
The cells are from 17-20 µm in diameter, cell wall is not sorrunded by mucilaginous sheath and were never surrounded by scales. The chloroplasts of mature cells of *Dictyococcus varians* are conjusted, because of close contact of plastids to each others (Figure 3F).

Macrochloris radiosa Ettl and Gärtner
The younger cells were orbicular to ellipsoidal and with a parietal chloroplast, single pyrenoid; one nucleus. The chloroplat was complately lobed and corrugated at the adult stage; vegetative cells had thick cell walls. But under the prosperous condition the protoplast division started with chloroplasts fragmentation. At this adult stage the vegetative cells diameter are between 45-50 µm (Figure 3G).

Dictyochloropsis splendid geitlervar
They are generally spherical with a diameter of 35-40 µm and with the exclusion of small cells the grille of chloroplast tightly fills the greatest part of lumina. The chloroplast structure could be very coarse or very fine (Figure 3H).

Cosmarium punctulatum breb
The cells are semitrunicate to sub semi circular; axile chloroplast are present with a small single pyrenoid. The length of the cell from 17.1-18 µm while 11-13.4 µm in width with a deep constraction, lateral margen scalloped with broadly trunacate apex (Figure 3I).

Chlorococcum humicola (Nageli) rabenhorst
Chlorococcum humicola is non-motile unicellular. Cells are solitary or seen in colonies with varied in size range from 22-29 µm. Cells have chloroplast completely filling the cell and with lateral notched single pyrenoid (Figure 3J).

Monoraphidium contortum (Thuret) komarkova-legnerová
Cells are solitary maverick curved from sigmoidal to spiral forming 1.0-1.5 turns with gradually tapered apices and pointed ends. A single chloroplast is present with out pyrenoids. Cell dimensions from 7.5-12 µm in length while 1.5-2 µm in width (Figure 3K).

Monoraphidium circinale (Nygaard) nygaard
Cells are solitary, arched and semicircles in sideways viewed. They are in the contour spherical, slightly traped at the ends. A single parietal chloroplast without pyrenoids is present. The dimensions of cell from 1.5-2 µm in width; 4.5-10 µm in length (Figure 3L).

Monoraphidium nanum (Ettl) hindák
Cells are solitary; crescent shaped or reniform, to the end rounded. A single parietal chloroplast is present without pyrenoids. The dimensions of cell from 2.5-3.5 µm in width while 4-6 µm in length (Figure 3M).

Scenedesmus vacuolatus shihira and krauss
Oval or rounded in structure with yellowish-green colour, small vacuoles are present and the cells are 11-14.9 µm in diameter. Chloroplast is present with pyrenoid (Figure 3N).

Chlorella vulgaris beijerinck
The free living alga, cells are commonly lonely or in the form of colonies.The cells are globular with a thin cell membrane and usually 5-10 µm in diameter. The chloroplat lad shaped parietal with a pyrenoid which

is indistinct sometimes (Figure 3O).

Scenedesmus ecornis (Ehernberg) chodat

Colonies of 4 cells with diameter of 4.3-9.7 µm; 12-18 µm long arranged in a linear series.The end of the cells are rounded or speriodel without spines or projections like teeth (Figure 3P).

Scenedesmus opoliensis P.G. richter

Currently accepted name is *Desmodesmus opoliensis* (P.G. Richter) E. Hegewalad. Colonies of 2-4 cylindrical to sub fusiform cells arranged in a linear series. Adjacent cells along one of third in their length in contact, internal cells turgid in the median region and faded toward the ends. The terminal cells sub rectangular narrower and the poles of cells in colony ending in one or two spines. The spines at the terminal cells long more or less curved along 10-12.3 µm in length. Cells dimension, 4.2-4.5 µm broad and 13.3-15 µm long (Figure 4A).

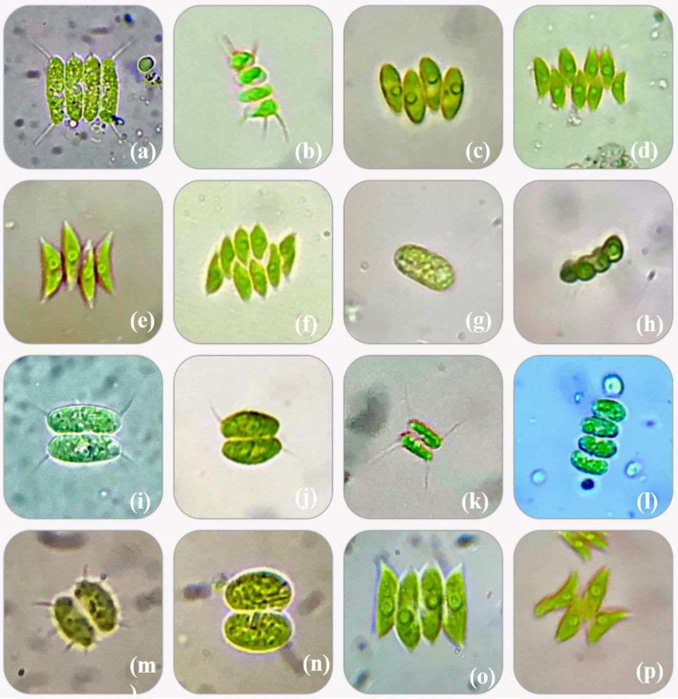

Figure 4: *a, b, c, d, e, f, g, h, I, j, k, l, m, n, o, p: Scenedesmus opoliensis, Desmodesmus abundans, Scenedesmus bijugatus Scenedesmus incrassatulus Bohlin, Scenedesmus dimorphus, Scenedesmus platydiscus, Oocystis pusilla Hansgirg var, Scenedesmus bijuga (Turp.), Scenedesmus quadricauda (Turpin) Breisson var, Scenedesmus armatus, Scenedesmus quadricauda (Trup.) Breb, Scenedesmus bijugatus Kuetzing, Desmodesmus baconii, Scenedesmus abundans (Kirchner) Chodat, Scenedesmus obliquus var.dimorphus, Scenedesmus bernardii G.M. Smith.*

Desmodesmus abundans (Kirchner) hegewald

Colonies of 2-4 celled in a linear arrangement. Cells are elipticas oblangas poles; at the terminal cell 2-3 spines are attached. Chloroplast is parietal; 1 pyrenoid. The dimension of the colony is 3-5.2 µm in width; 12.3-16.5 µm long (Figure 4B).

Scenedesmus bijugatus (Turp.) Kuetz. var. alternans (Reinsch) Hansg

Slightly curved flat colonies; generally, eight celled while sometime four celled colonies. Distinctly the cells arranged in alternating series with adjacent cells by short portion of their length. The four celled colony 18.4-25.7 µm in diameter. Cells are 5.3-8.9 µm broad and 10.2-15.8 µm long; spheroidal to ovid-spheroidal with rounded ends (Figure 4C).

Scenedesmus incrassatulus Bohlin, 1897

Colonies of 4-8 cells; spindle shaped arranged linear or sub alternating series. Cells fusiform curved with outer convex, slightly concave or straight inner side with stumpy ends and apical nodules. Colony of 8 cells 21-35 µm long, 16.3 µm wide. Single cell is 3.4 µm broad; 7-8.2 µm long (Figure 4D).

Scenedesmus dimorphus (Turpin) kuetzing

Colonies of four to eight celled. Cells are linear or sub linear arranged in a series, the eight celled colonies ever in sub alternatives series. The cells in the colony of *Scenedesmus dimorphus* differ from *Scenedesmus obliquns* in outer cells of the colony more or less crescent and attenuated at the apices. Cells are 3-7 µm broad and 15-23 µm long (Figure 4E).

Scenedesmus platydiscus (G.M. Smith) Chodat

Scenedesmus platydiscus has flat colonies of eight cells with a diameter of 25.3-33.7 µm. The cells are oblong and arranged in a double series; between the cells minute interstices or absent. The cells are 5.1-7.9 µm broad and 12.7-18.6 µm long (Figure 4F).

Oocystis pusilla Hansgirg var. maior Skuja

Elongated spheroidal cells; truncate to rounded ends with a slight median puffiness some time. Inside the old mother cell, cell wall solitary or 2-4 autospores; thin cell membrane without polar thickening and Chromatophores without pyrenoid. The width of the cell is from 7-11 µm; 17-23 µm in length (Figure 4G).

Scenedesmus bijuga (Turp.) Lagerheim

Colony of two to eight cells whiles 12-16 µm long and 4-8 µm in diameter (Figure 4H).

Scenedesmus quadricauda (Turpin) Breisson var. quadrispina (Chodat) G.M. Smith

Generally, 2-4 celled colonies; broadly ovoid and twice as long as broad. A short curved single spine presents at the poles of terminal cells. Cells are about 30 µm long; 25 µm in breadth (Figure 4I).

Scenedesmus armatus var. bicaudatus (Gugliemetti) chodat

Two to four celled colonies. Different from other types in having long spine at one pole of terminal cell, spines of the two terminal cells alternative to each other's. Cells are 2-4.3 µm broad; 8.4-11.3 µm long, usualy the longitudinal ribs seen in terminal cells (Figure 4J).

Scenedesmus quadricauda (Trup.) Breb var. quadrispina (Chodat) G.M. Smith

Colonies of 2-4 celled with 5.7-6.9 µm in diameter. The cells are broadly ovoid and as long as twice of its broad with smooth cell wall. At the poles of terminal cells have long spine with 7.2-9.8 µm long in length. The cells are 3-5 µm broad and long as 10-14 µm (Figure 4K).

Scenedesmus bijugatus kuetzing

Colony of two to four or eight cells arranged with in a linear series single categoric. Colony of 4 cells oblong to ovoid with broadly rounded ends; 13.5-17.1µm long; 13.4 µm wide. Single cell of colony is 9.6 µm to 12.1 µm long; 3.2-5 µ wide (Figure 4L).

Desmodesmus baconii M. Fawley, K. Fawley and E. Hegewald

Two celled colonies with multiple spines on the terminal cells. The long spine at the sub polar of the terminal cell was massive matched to the cells size and resemblance with large canine teeth of saber-toothed cats. Typically, the arcuate spines were some time straight nearly and at the poles of cells; one, two or three smaller spines are also seen. The cells colony is 6.5-7.9 µm in diameter. Single cell length 9.3-11.7 µm while 3.9-4.7 µm in width (Figure 4M).

Scenedesmus abundans (Kirchner) chodat

Colonies of 2 to 4 cells, cells are round oval shaped without spines shows intensive green colour. The cell wall is surrounded by mucilage; chloroplast without pyrenoid. Single cell of colony is 6.3 µm to 9.1 µm long and 4.2-6 µ wide (Figure 4N).

Scenedesmus obliquus var.dimorphus (Turpin) hansgirg

Colonies 4-8 celled; Cells shape ellipsoid fusiform or spherical with a well-defined apical crest. Cells arranged in a single series, shows intensive green colour. Each cell consists of single nucleus; cup shaped chloroplast surrounded by membrane and an eyespot. Dimension of the single cell, 3-4.2 µm broad and 8-9.7 µm long while colony, 10-12 µm long (Figure 4O).

Scenedesmus bernardii G.M. Smith

Four to eight celled colonies. Inner cells are crescent fusiform shaped and acute apices, arranged in alternate manner to adjacent cell by the apices in contact with apices or half portion of the other cell. Terminal cells crescent or fusiform, commonly attached to the apices of interior cell. Cells are 4-5 µm wide; 26.8-30 µm long (Figure 4P).

Anabaena azollae strasburger

Small or short filamentus blue-green algae with indistinct sheath and trichome constricted densely at cross wall, with apical rounded cell. The length of the cell is 3.2 µm while 2.3 µm broad (Figure 5A).

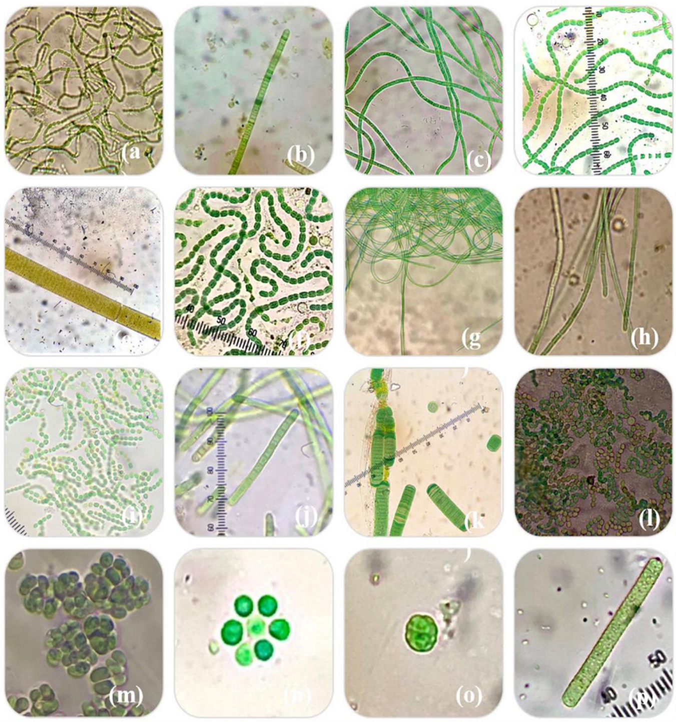

Figure 5: *a, b, c, d, e, f, g, h, I, j, k, l, m, n, o, p: Anabaena azollae, Oscillatoria sancta Kuetzing ex Gomont, Phormidium chalybeum, Nostoc linckia, Oscillatoria princeps Vaucher ex Gomont, Anabaena affinis, Leptolyngbya vincentii Komarek, Oscillatoria limnetica Lemmermann, Nostoc spongiaeforme, Oscillatoria tenuis Agardh ex Gomont, Oscillatoria curviceps Ag.ex Gomont, Nostoc punctiforme, Gloeocapsopsis pleurocapsoides, Coelosphaerium pallidum Lemmermann, Chroococcus dispersus, Oscillatoria limosa Agardh ex Gomont.*

Oscillatoria sancta Kuetzing ex Gomont 1892

Blue-green thallus; mucillagenous sheath present. Trichome straight or less and cross walls are mostly constricted. Length of the cell 8-12 µm whiles 4-5.5 µm in width. The end cells slightly capitate with a thickened membrane and hemispherical and attenuated (Figure 5B).

Phormidium chalybeum (Mertens ex Gomont) Anagnostidis and Komarek

Straight to curved long filaments, with thin sheath. Dark blue-green trichome constricted at cross wall. Attenuated slightly towards ends and hooked, finely granulated cell content. Apical cell obtuse, rounded widely. Cell dimension 4.5-5 µm in length while 1.3-2 µm in width (Figure 5C).

Nostoc linckia Bornet and Flahault

Thallus blue green, densely entangled filaments. Diffluent gelatinous sheath present at the periphery. The vegetative cells are short and barrel shaped about 3.5-4 µm in width. The heterocystes have a spherical shape and are Akinetes subspherical, measuring 6-7 µm in width and 7-8 µm in length (Figure 5D).

Oscillatoria princeps Vaucher ex Gomont

Blue green thallus slightly curved and constriction at the cross walls absent. Individual colour of the filaments was blue green to olive green. The cells are very long in length 18-24 µm and 4-6 µm in length. End of the cell is celessightly capitate (Figure 5E).

Anabaena affinis lemmermann

Single trichome straight or bent and the cells mostly barrel shaped structure having 6 µm broad while 5 µm long and with round apical cells. Heterocyst's cells sub spherical and broad 5-7 µm. Sporogenous trichome making by long chain Akinetes (Figure 5F).

Leptolyngbya vincentii komarek

Cylindrical thin filaments; 0.6-1.8 µm broad; narrowed ends absent; pale greyish blue-green in colour. Very thin sheath longer then wide, cells isodiametric, cells end rounded (Figure 5G).

Oscillatoria limnetica lemmermann

Straight or slightly bent trichome, pale blue-green in colour and distinctly constricted at the cross walls. Cells are 1.5-2 µm broad; not capitate, not attenuated. The cells 2-4 times long as broad; about 35-45 µm long. The end cells are rounded, calyptra absent (Figure 5H).

Nostoc spongiaeforme C. Agardh ex Bornet and Flahault

Blue-green trichome, cells subcylindrical. Loosely elongated filaments, flexuous with diffluent sheath. Akinetes and heterocysts are subspherical. Cells are 3.6-7 µm in length while 3 -3.5 µm in width. Akinetes are 10-11 µm in length; 6.5-7 µm in width. Heterocysts are 7-8.5 µm in length; 7-8.3 µm in width (Figure 5I).

Oscillatoria tenuis Agardh ex Gomont

Blue green thallus; straight trichome and constriction not present at the cross walls. The cells are 5.5-6 µm broad; 4-4.5 µm long, uniformly granules present (Figure 5J).

Oscillatoria curviceps Ag. ex Gomont (after Gomont), Desikachary

Bright blue-green thallus, straight long trichome, hooked or loosely spirally coiled at the end. The apical cell rounded with thickened wall (Figure 5K).

Nostoc punctiforme hariot

Entangled densely filaments and flexuous with hyaline sheath. Blue-green trichome, cylindrical cells, very squeezed in dense sheaths with heterocysts exterior the sheath. Heterocysts are spherical; Akinetes are subspherical; Cell dimension 4-4.6 broad, 4.8-5.3 µm in length. Akinetes are 5.3-5.8 µm broad; 2-2.4 µm in length. Heterocysts diameter; 5-5.3 µm (Figure 5L).

Gloeocapsopsis pleurocapsoides (Novacek)

Cells are with irregular outline; Oval rounded shape blue green. Cell content finely granulated; Cell envelops lemellated, firm, and yellowish in colour. Cells colonies with irregular shape; Dimension of a single cell is 4-6.1 µm (Figure 5M).

Coelosphaerium pallidum lemmermann

Colony of small cells, an ovate cell crowded; Pale blue-green cell contents without pseudovacules. Cells are 2-3.5 µm long; 11-12.5 µm in diameter (Figure 5N).

Chroococcus disperses (Keissler) lemmermann

There colonies were small about 3-15 cells. Diameter of a single cell is 4-4.7 µm and surrounded with a mucous cover. The cells were spherical; pale blue green in colour (Figure 5O).

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Table 3: *Physico-chemical analysis of water samples of collection sites.*

S.	Chemical analysis (Parameters)	Sample E1 Salarzai Thali Dam (Rainy water)		Sample E2 Saddiq Abad (Sewage water)		Sample E3 Inayat Qilla Khwar (Stream water)		Sample E4 Khazani Chena (Spring water)	
		March	August	March	August	March	August	March	August
01	Total soluble salt in (ppm)	3.61	2.63	2.99	2.39	3.44	2.47	2.47	1.98
02	Alkalinity (ppm)	231	198	241	209	219	167	198	124
03	TDS (ppm)	229	202	226	197	221	186	201	148
04	Electrical conductivity (µS/cm)	809	754	813	783	801	723	767	639
05	pH	7.11	6.98	8.19	7.89	7.21	6.89	6.97	6.92
06	Sample temperature	26	38	31	37	25	33	22	28
07	Carbonate CO_3^{-2} (ppm)	3.31	3.02	3.21	2.87	3.04	2.09	3.04	2.37
08	Bicarbonate HCO_3^{-1} (ppm)	3.93	2.94	3.01	2.49	2.91	2.01	3.07	2.09
09	Chloride Cl^{-1} (ppm)	37.91	34.57	37.43	31.07	36.41	34.68	30.11	28.11
10	Sulphates SO_4^{-2} (ppm)	2.54	2.09	2.63	2.17	2.69	1.85	2.22	1.68
11	NO_3^{-1} (ppm)	3.92	3.39	3.01	3.02	2.87	2.63	2.09	2.03
12	Ca^{+2} (ppm)	9.45	7.88	8.81	7.92	8.95	7.02	7.07	6.14
13	Mg^{+2} (ppm)	4.22	3.61	4.19	3.93	4.13	3.09	3.01	2.30
14	Na^{+1} (ppm)	34.88	31.09	31.98	29.87	30.78	26.38	28.31	25.01
15	K^{+1} (ppm)	13.91	11.27	14.32	12.79	13.24	11.03	10.55	9.89
16	COD (mg/L)	27.41	24.98	25.19	23.96	26.24	23.09	21.45	18.93
17	BOD (mg/L)	8.09	7.04	7.99	6.79	8.02	7.03	6.07	5.70
18	Turbidity (NTU)	14.94	11.47	12.19	10.97	12.22	10.98	10.32	9.86

Oscillatoria limosa Agardh ex Gomont

Blue-green thallus; Straight trichome and doesn't constrict at cross walls. The cells are 4 µm long and 8-10 µm broad. Granules are uniformly distributed; the end cell thickened and rounded, no attenuated apex, frequently granulated cross walls (Figure 5P).

A comprehensive taxonomic investigation of algae from various habitats, including streams, rainy water, sewage, and spring water, revealed a total of 52 distinct species. These species exhibited a range of growth forms, including unicellular, colonial, filamentous, branched filamentous, and irregular forms. It is noteworthy that this study represents the initial exploration of algae in these diverse habitats.

The current study was conducted in District Bajaur, Khyber Pakhtunkhwa, to identify morpho-taxa and ascertain the seasonal relationship (Table 4) between algal diversity and water physico-chemical parameters. Total of 52 algal species belonging to 2 classes, 6 orders, 13 families, 22 genera were recorded from different habitats including streams, ponds, springs, ditches and dam of District Bajaur KP. In all visited sites (E1, E2, E3 and E4) genus *Scenedesmus* was dominant with fifteen species and genus *Oscillatoria* was the second with six species. While other microalgae diversity was also rich at E1 Salarzai Thali Dam in March spring session as compared to other visited sites (Table 3).

Our findings demonstrated that District Bajaur, exhibited a high level of algal diversity. Similarly, in a previous study by Khan *et al.* (2011), a morpho-ordered characterization of 73 green algae species was conducted in the Kalpani stream and surrounding areas of Murdan. The study identified 34 genera, 25 families, 17 orders, and 9 classes, with the Chlorophyta family representing 65.75% of the total. Another study reported the presence of 138 species belonging to the Chlorophycean group, with 74 species (53.6%) classified under the Chlorococcales family. Both Cladophorales and Chaetophorales accounted for 3% of the total algal diversity (Ali *et al.*, 2010). Similarly, 30 species of green algae from District Gujar khan, Rawalpindi (Minhas *et al.*, 2023). Furthermore, Leghari (2001) conducted a study on green filamentous algae found in lakes and ponds of Sindh, identifying 31 species of Chlorophyta in freshwater and riverine lakes.

Table 4: *List of seasonal abundance of recorded planktonic species (green and blue-green algae).*

S. No	Name of taxa	Sample E1		Sample E2		Sample E3		Sample E4	
		March	August	March	August	March	August	March	August
1	Spirogyra varians	+++	-	-	-	++++	-	++++	-
2	*Spirogyra maravillosa* transeaue	+++	++	-	-	++++	-	++++	
3	Chaetomorpha aerea	-	-	-	-	+++	++	++	++
4	Spirogyra corrugata	++++	-	-	-	++++	-	++++	-
5	*Microspora amoena* var.gracilis (Wille) De Toni 1889	+++	-	-	-	++++	+	++++	+
6	*Microspora stagnorum* (Kutzing) Lagerheim 1887	-	-	-	-	++++	+	++++	+
7	*Klebsormidium flaccidum* (Kutz.)	-	-	-	-	++++	++	++++	++
8	Zygnema cruciatum	-	-	-	-	++++	++	++++	++
9	Westella botryoides	++++	-	-	-	-	-	-	-
10	*Dictyococcus varians* Gerneck	-	-	-	-	++	-	+++	++
11	Macrochloris radiosa	+++	-	++	-	++	-	++	-
12	*Dictyochloropsis splendid* Geitlervar	++++	-	++	-	++	-	++	-
13	*Cosmarium punctulatum* Breb	++++	++	-	-	++	-	++	-
14	Chlorococcum humicola	++++	++	-	-	++	-	++	-
15	Monoraphidium contortum	++++	-	-	-	-	-	-	-
16	Monoraphidium circinal	++++	-	-	-	-	-	-	-
17	Monoraphidium nanum	++++	-	-	-	-	-	-	-
18	Scenedesmus vacuolatus	++++	++	++	-	++	-	++	-
19	*Chlorella vulgaris* Beijerinck	++++	++	++++	-	++++	-	+++	-
20	*Scenedesmus ecornis* (Ehernberg) Chodat	++++	++	++	-	++	-	++	-
21	Scenedesmus opoliensis P.G. Richter	++++	++	++	-	-	-	-	-
22	*Desmodesmus abundans* (Kirchner) Hegewald	++++	++	++	-	-	-	-	-
23	*Scenedesmus bijugatus* (Turp.) Kuetz. var. alternans (Reinsch) Hansg.	++++	++	+++	-	-	-	++	-
24	*Scenedesmus incrassatulus* Bohlin, 1897	++++	++	+++	-	-	-	-	-
25	*Scenedesmus dimorphus* (Turpin) Kuetzing	++++	++	++	-	-	-	-	-
26	*Scenedesmus platydiscus* (G. M. Smith) Chodat.	++++	-	+++	-	-	-	-	-
27	Oocystis pusilla Hansgirg var. maior Skuja	-	-	++	-	-	-	++	-
28	*Scenedesmus bijuga* (Turp.) Lagerheim	++++	++	++	+	++	-	++	-
29	*Scenedesmus quadricauda* (Turpin) Breisson var. quadrispina (Chodat) G. M. Smith	++++	++	+++	++	+++	-	-	-
30	*Scenedesmus armatus* var. bicaudatus (Gugliemetti) Chodat	++++	++	++	++	-	-	-	-
31	*Scenedesmus quadricauda* (Trup.) Breb var. quadrispina (Chodat) G. M. Smith	++++	++++	+++	++	-	-	-	-
32	*Scenedesmus bijugatus* Kuetzing	++++	++	+++	-	-	-	-	-
33	Desmodesmus baconii	+++	++	++	-	++	-	-	-
34	*Scenedesmus abundans* (Kirchner) Chodat	++++	++	+++	++	-	-	-	-
35	*Scenedesmus obliquus* var.dimorphus (Turpin) Hansgirg	++++	+++	++	-	+++	-	-	-
36	*Scenedesmus bernardii* G.M. Smith	++++	++	+++	-	-	-	-	-
37	Anabaena Azollae	+++	-	++++	-	-	-	-	-
38	*Oscillatoria sancta* Kuetzing ex Gomont 1892	++++	++	++++	-	-	-	-	-
39	Phormidium chalybeum	++++	++	++++	++	++	-	-	-
40	Nostoc linckia	++	++	++++	++	-	-	-	-

Table continued on next page............

Links Researchers

S. No	Name of taxa	Sample E1		Sample E2		Sample E3		Sample E4	
		March	August	March	August	March	August	March	August
41	Oscillatoria princeps Vaucher ex Gomont 1982	++++	++	++++	++	++	-	-	-
42	Anabaena affinis	++	-	++++	++	-	-	-	-
43	*Leptolyngbya vincentii* Komarek	++	-	++++	++	-	-	-	-
44	*Oscillatoria limnetica* Lemmermann	++	-	++++	++++	-	-	-	-
45	Nostoc spongiaeforme	+++	++	++++	++++	-	-	-	-
46	*Oscillatoria tenuis* Agardh ex Gomont 1892	+++	-	++++	++++	-	-	-	-
47	*Oscillatoria curviceps* Ag.ex Gomont (after Gomont), Desikachary, 1959	+++	++	++++	++++	-	-	-	-
48	Nostoc punctiforme	+++	++	+++	++++	-	-	-	-
49	Gloeocapsopsis pleurocapsoides	-	-	++++	++	-	-	-	-
50	*Coelosphaerium pallidum* Lemmermann	++	-	++	++	-	-	-	-
51	Chroococcus dispersus	++	-	++	++	-	-	-	-
52	*Oscillatoria limosa* Agardh ex Gomont 1892	++	-	++++	++	-	-	-	-

-, absent; ++, less abundant; +++, abundant; ++++, Highly abundant. March spring season, August summer rainy, seasons.

Physicochemical characteristics of water are directly related with the seasonal fluctuations and that is linked the phytoplankton distribution (Shuaib *et al*., 2017). Each phytoplankton taxon has variable resource requirements and responses to environmental physicochemical parameters, that results in fluctuation of microalgal diversity from month to month (Patritia and Martin, 2017; Shabbir and Ahmad, 2015). Such as the concentration of nutrients, the intensity of light and the temperature influence the primary production of microalgae (Kana *et al*., 1997). The water temperature at site E1 reached its highest point (38 °C) in August summer season while in March spring season showed best growth at 24 °C. Previously it was it was described that temperature for microalgae growth should oscillate between 20 °C and 30 °C (Pawlita-Posmyk *et al*., 2018). In March temperature at E1 site falls between the recommended temperatures that's why E1 site supported more algal diversity in March spring season.

Water pH is particularly essential for the growth of micro-algae because within narrow pH range there occur many biological reactions (Mohsenpour *et al*., 2021; Juneja *et al*., 2013). The pH ranges 7.11, 8.14, 7.21 and 6.97 was noted at the collection sites during March spring season while 6.98, 7.89, 6.89 and 6.92 was noted during August survey in summer season (Hajong and Ramanujam, 2018). Chemical analysis of water samples: the values of cations (Na^+) 34.88, 31.98, 30.78, 28.31 (K^+) 13.91, 14.32, 13.24, 10.55 ($Ca2^+$) 9.45, 8.81, 8.95, 7.07 and ($Mg2^+$) 4.22, 4.19, 4.13, 3.01 ppm, respectively) and anions (Cl^-) 37.91, 37.43, 36.41, 30.11 ($SO42^-$) 2.54,2.63,2.69,2.22 and (HCO3-) 3.93, 3.01, 2.91, 3.07, ppm, respectively) were the maximum in march in all sites. However, lower levels of the same ions, EC and TDS were detected in all locations in August. In addition, to comparatively higher concentrations of cations, anions EC and TDS were detected in sample E1 among all in March (Table 2). Algal development is influenced by a variety of environmental conditions, such as temperature, pH and presence of nutrients like NO_3^{-1}, Na^{+1}, K^{+1}, Mg^{+2}, Ca^{+2} (Pokhrel *et al*., 2021; Vazquez *et al*., 2011).

Seasonal variations of phytoplankton were also observed during study (Table 3). *Spirogyra varians, Spirogyra maravillosa* transeaue, *Spirogyra corrugata Klebsormidium flaccidum*, *Zygnema cruciatum* were high abundant in March spring season at site E3 and E4 from stream and spring moving water while absent or least abundant in August summer rainy season. *Monoraphidium contortum, Monoraphidium circinal* and *Monoraphidium nanum* were recorded at Site E1, from stagnant water of "Thali Dam" in March spring season while absent in the other site of collection (Roka *et al*., 2022). *Scenedesmus* species were recorded with higher number in March spring season from site E1, while absent or least abundant at other site of micro-algal collection. *Chroococcus dispersus, Coelosphaerium pallidum, Gloeocapsopsis pleurocapsoides, Leptolyngbya vincentii, Phormidium chalybeum, Oscillatoria, Nostoc* and *Anabaena* species were also more in numbers in March spring season at sites E1 Thali Dam while high abundantly recorded at site E2, from the stagnant sewage water of Markets and Homes and absent or least abundant in other sites of collection (Hajong and Ramanujam, 2018).

Within the District Bajaur, there are numerous unexplored aquatic sites that present opportunities for further investigation. Exploring these sites holds the potential to uncover new algal species that may be novel to the algal flora of Pakistan or even globally. It is crucial, therefore, to preserve the diversity of algae within local habitats and conduct systematic research on them. Such research can only be undertaken with a comprehensive understanding of the ecology and habitats of the distinct algal flora present. The discoveries resulting from these endeavors will serve as valuable resources for future research on freshwater algae in District Bajaur, located in the Khyber Pakhtunkhwa.

Conclusions and Recommendations

We collected 52 species of micro-algae from different four sites of District Bajaur Khyber Pakhtunkhwa, Pakistan. In total of 52 species 36 of green and 16 of blue-green micro-algae were recorded. Our study showed that most dominant genus in this area was *Scenedesmus* due to favorable ecological conditions such as Temperature, pH, and the presence of nutrients like NO_3^{-1}, Na^{+1}, K^{+1}, Mg^{+2}, Ca^{+2}. The stagnant rainy water at Thali Dam site E1, surrounded by agricultural area, supported the greatest number of micro-algal species, particularly *Scenedesmus* species. The highest number of micro-algal species was observed during the spring season, particularly in March, across various habitats. Notably, the Monoraphidium species were exclusively found at site E1, while a larger number of blue-green algae were recorded at site E2, specifically in the Saddiq Abad sewage water. During the March spring season, the species Spirogyra, Chaetomorpha, Microspora, Zygnema, and Klebsormidium were most abundant at sites E3 and E4. However, during the rainy season in August, these species were either less abundant or entirely absent, likely due to flood-related disturbances experienced during sample collection. The correlation between the algal diversity and physico-chemical parameters are the water temperature, water pH, and the presences of nutrients are all these factors that influence the algal development were recorded from the different site of collections. For the sake of future prospects, the District Bajaur is abundant in various species of algae. The species of *Scenedesmus* can be employed for the bioremediation of sewage, the generation of biodiesel, and the manufacture of nanoparticles.

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Novelty Statement

This has been the first ever report on true algal diversity with pictorial evidence from Bajaur, a hard to access area. Each group has been scrutinized at genus and species level revealing extended diversity in green (*Spirogyra* and *Scenedesmus*) and Blue Green (*Nostoc* and *Oscillatoria*) Algae and the study also revealed seasonal abundance of algal species during March.

Author's Contribution

Abdul Samad Mumtaz: Supervised the research project and provided laboratory facilities.
Nabi Ullah and Muhmmad Kaleem: original concept, study design, materials and field data collection from District Bajaur, performed microscopy drafting, editing and finalizing manuscript.
Lubna Anjum Minhas and Rooma Waqar: Physico-chemical analysis of water samples.
Amber Jabeen and Ayesha Hanif: Assisted in species identification, data collation and analysis.

Conflict of interest

The authors have declared no conflict of interests.

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