



Taxonomic study of freshwater microalgal diversity and its optimum culturing condition of District Karak, Pakistan

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Abstract

Algae are photosynthetic and predominantly aquatic organisms that produce up to half of the oxygen in Earth's atmosphere. In this study, the algal flora of district Karak has been isolated, identified, and explored for diversity based on its optimum in-vitro culturing and microscopic technique. Microalgal samples were collected from freshwater bodies of ecologically diverse sites of district Karak. The microalgae samples were collected from February to April (spring season) and August to September (summer season) in 2020-2022. In the aseptic environment, three different types of media (BBM, MBBM, and BG-11) were used to evaluate microalgal growth parameters. The fluctuation in temperature, pH, water density, and nutrient availability varies with species distribution; however, BBM media was shown to be more optimal and standard than others for algae cultivation. A total of 33 microalgae strains were investigated that belong to 4 classes, 10 orders, 12 families, and 17 genera. Among them 5 species were cyanobacteria, 16 species were green microalgae, and 12 species from Diatoms; in which Bacillariaceae was the dominant family with 6 species and their contribution was 19%. The 2nd most dominant families were Scenedesmaceae, Volvocaceae, and Desmidiaceae with each 4 species respectively and their contribution was 12%. The other families Oscillatoriaceae followed by Chlorococcaceae (9%) while some families represented only two species (6%) that were Fragilariaceae, Pinnulariaceae and Nostocaceae, and Gomphonemataceae, Naviculaceae, Chaetophoraceae were one (3%) species each. These species belonged to 17 genera and 12 families; three key categories of microalgae (Cyanobacteria, Green Algae, and Diatoms) were reported in this study area. This study's scope is to examine the scientific studies of microalgae diversity from various habitats of fresh water and investigate the optimal culture conditions for these algae growth which is essential for multiple applications. Hence, the present taxonomic findings demonstrate that District Karak is a rich source of microalgal biomes unexplored till now.

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Introduction

The word algae are derivative of the epithet "alga" in Latin & Greek, "phykos" means seaweed; which has a lack of defined body parts system like plant (Sahoo and Seckbach, 2015; Oyewumi and Olukunle, 2017; Selvaraj *et al.*, 2021). Microalgae are a planktonic vast and diverse group of microscopic unicellular may be prokaryotic or eukaryotic organisms (Masojídek *et al.*, 2013; Ghani *et al.*, 2020; Puchkova *et al.*, 2021). Blue-green algae also known as Cyanobacteria (Gram-negative bacteria) are among the most ancient photosynthetic microorganism due to the existence of a bluish pigment "Phycocyanin" in them (Singh *et al.*, 2016; Barinova *et al.*, 2018; Selvaraj *et al.*, 2021). They are oxygen-evolving microbes by photosynthesis process to get their energy; ranges from unicellular structures to colonial, branched, and un-branched filaments, non-motile and widely distributed organisms on Earth (Klm *et al.*, 2011; Shakir *et al.*, 2014; Tragin *et al.*, 2016;). Blue-green algae play a crucial role as primary producers in aquatic ecosystems with distinctive characteristics that provide lodging to varying environments (Raghuwanshi *et al.*, 2011; Halder, 2016; Narchonai *et al.*, 2019). They have massive phylogenetic diversity, as ancestors of plants from billion years of evolutionary history and often developed extremely habitats (Singh *et al.*, 2013; Barinova *et al.*, 2018; Arsad *et al.*, 2022). Microalgae are photosynthetic microbes that have generated rapid interest in applied research with multifunctional applications in the modern era (Abdelaziz *et al.*, 2013; Alam *et al.*, 2019; Ramos *et al.*, 2021).

Microalgae are assorted organisms with numerous prospective traits like cell organization, plastids, biochemical composition, morphological features, and habitat as well (Cheng, 2011; Wali *et al.*, 2017; Elisabeth *et al.*, 2021). They cover about 2, 00,000 – 8, 00,000 existing species, but currently, less than 5% of them are well-described (Guiry, 2012; Watanabe and Lewis, 2017; Puchkova *et al.*, 2021).

The wide variability of microalgae in terms of photosynthetic pigment compositions

"photosynthates" (storage polysaccharide), and the plastid structures are almost unexploited natural resources (Sahoo and Seckbach, 2015; Narchonai *et al.*, 2019; Selvaraj *et al.*, 2021). Based on plastid structures Algal world is divided into 10 groups, consisting of (Cyanobacteria, Glaucophyte, Rhodophyte (red algae), Chlorophyte (green algae), Haptophyte, Heterokontophyte, Dryptophyte, Dinophyte, Chlorarachnid, and Euglenid).

Microalgae are widely distributed in natural habitats almost in all ecosystems; they can grow in such habitats as sedimentary, deserts, soil, wall, stone, epiphytic, hot-spring water, salty lake, snow, freshwater, or seawater as well as in moist areas that have been adapted to extreme environments (Raghuwanshi *et al.*, 2011; Hopes and Mock, 2015; Alam *et al.*, 2019).

The physical and chemical parameters are essential for the standard medium and selections of suitable strains for the growth of microalgae; they can be grown in various bioreactors in the field, agar, liquid media, glycerol, cryoprotectant, and various organic wastes (Singh *et al.*, 2016; Rimsha *et al.*, 2020; Kamboj *et al.*, 2022). Temperature is a conditional factor for algae growth and development of green-algae grow up at 47°C, Diatoms develop up to 60°C, and Thermal blue-green algae at 74°C. There are several standard culture media are present for microalgae culturing; been reported that Light intensity, pH, and nutrient composition is capable in artificial habitats related to scientific experiments and environmental habitats (Chader *et al.*, 2011; Hokmollahi *et al.*, 2016; Jabeen *et al.*, 2021). Furthermore, microalgae can also maintain a unique relationship with other microorganisms in certain habitats observed naturally and artificially perform as symbiotic mutualism to support each other's life (Abdelaziz *et al.*, 2013; Watanabe and Lewis, 2017).

Freshwater algae were previously studied from various ecological zones of Pakistan including Naz and Hasan (2004) observed from the northern area, by Munir *et al.* (2012) from Kallar Kahar lake of salt

range, by Naveed *et al.* (2011) from Contra District Karak, by Khalid *et al.* (2014) from Taxila, by Ali *et al.* (2015), from Malakand, by Khan *et al.* (2017), from Tehsil Landi Kotal, by Suhaib *et al.* (2017) from Dir lower and later selected spots of district Peshawar were explored for algal communities by Imtiaz *et al.* (2018) as well as by Ullah *et al.* (2021) from District Mardan. In the past microalgae were classified based on morpho-anatomical characteristics by “Harvey” into four groups (Shakir *et al.*, 2014, Ten *et al.*, 2016), while presently according to “Lee” either prokaryote “cyanophyta” is one division or eukaryote based on chloroplast membrane (Suhaib *et al.*, 2017; Shah *et al.*, 2019; Tabassum *et al.*, 2021).

Algae have an enormous importance and essential marker usually in the food chain, green energy, wastewater remediation, CO₂ cycling, toxic molecule assimilation, and biodegradability to life on the planet. Microalgae are photosynthetic autotrophic organisms with rapid growth that hold great promise as significant sources for new products and other roles (Ayubli and Valeem, 2019; Umen, 2020; Ramos *et al.*, 2021). Currently, biochemicals, pharmaceuticals, medicines, and biomass production for nutrients are the main commercial products produced by green microalgae. The souk for microalgae use is still developing, and new regions will be exploited.

The freshwater algae of district Karak have been studied poorly in the past and the baseline of algal flora is needed for the exploration of their potential. Hence, the present study scope & aims to examine the scientific studies on the diversity of microalgae found in freshwater environments and investigated the optimal conditions for growing these microalgae in culture, which is important for various applications. Furthermore, the present study was making a checklist of species diversity based on morphology and cytology.

Material and methods

Physiological features and sample collection

Topographically, Karak is a semi-arid region and shattered hills, located at 70.40° to 71.30° longitudes

& 32.48° to 33.23° latitudes, some 600–1400 meters above sea level. Karak is famous for deposits of natural oil, gas, and salt in the southern-west district of KP (Akhtar and Anees, 2019). Four different sites of District Karak (areas included Lachi village Kurram River, Bandar Khan village, and Shamoni Khattak) were visited for sample collection. The collection of samples was made from freshwater including rainwater, stream water, drinking water from tankers and ponds, etc. (Table 1). The samples were collected in 50ml falcon tubes with tag a unique sample code for every sample and noted important field data e.g., location, temperature, moisture, color, odor, and habitat of the algal sample (Ramos *et al.*, 2015; Goldstein, 2015). Global positioning coordinates of every sample was recorded through the Android mobile app (Galaxy A-32S).

Sample schedule and preservation

Random field visits were made a year in session 2020-2022, with 15 days of interval in seasonal variation (spring and rainy season). All the sample falcons in proper sample codes were kept in a growth room at 24- 25°C as preserved sample for further use (Beherepatil and Deore, 2013). Samples were preserved by the addition of 1–2ml of 4% formalin with the intervals of 1 month (formalin was prepared by the addition of 4ml of formalin in 96ml of distilled water). Samples were taken in the laboratory of Plant and algal genetics, Faculty of Biological Science, Quaid-i-Azam University Islamabad for taxonomic and cultural studies.

Microscopic analysis of crude culture

To ascertain the number of strains contained in the enriched crude culture, the sample was examined under a microscope. One drop of sample water was taken with a fine micropipette from each sample and observed at different lenses e.g., 10X, 40X, and at 100X under light (Leitz Wetzlar, Germany) microscope. In the observation under 100X, one drop of oil was placed upon the coverslip for the object amplification and lens safety. The characteristics like morphological features, color, shape, and size (by calibrated eyepiece) were noted during the observation (Goldstein, 2015; Minhas *et al.*, 2023).

Table 1. Field data of collected green algae samples

Sample Code	Location	Sample type	Substratum	Temperature	Humidity	Latitude	Longitude	Vegetation	PH	EC ($\mu\text{m}/\text{cm}$)
LIA	Lachi village	Rain water	Deep water	27.9°C	45%	33.3822	713381	Grasses,	6.75	820
LIB	Lachi village	Rain water	Rock surface	27.9°C	45%	33.3822	713381	Grasses,	6.79	880
S.P	Bandar khan village	pool water	Deepwater and scratched from wall	26.8°C	49%	32.873047	70.889083	Grasses	7.88	430
AG	Sarainaurang	Stagnant rainwater	Standing water	22°C	62%	32.827219	70.778039	Trees	7.9	830
K.R 1	Kurram River	Stagnant standing water	Standing water	22°C	50%	32.789183	70.826698	Phoenix, jacaranda	7.99	1530
K.R 2	Kurram River	Stagnant standing water	Standing water	22°C	50%	32.789183	70.826698	Grasses	7.24	1440
K.R 3	Kurram River	Moist soil along the water	Soil sample	22°C	50%	32.789183	70.826698	Phoenix Jacaranda	7.61	1330
K.R 4	Hamidan	Moist soil along the water	Soil sample	22°C	50%	32.789183	70.826698	Phoenix, jacaranda	7.41	1230
T.W1	Shamonikhat tak	Freshwater tubewell	Fresh deep water	27°C	69%	32.789183	70.826698	Triticum aestivum	6.46	1130
T.W 2	Shamonikhat tak	Freshwater tubewell	Scratched from deep walls	27°C	69%	32.789183	70.826698	Trees & Brassica campestris	6.7	820

Isolation of microalgae

Two distinct techniques, colony picking, and serial dilution methods, have been used to isolate microalgae strains from crude culture (Tragin *et al.*, 2016; Elshobary *et al.*, 2020). In the colony-picking technique, petri-plates were made by pouring BG-11 medium that had been agarized under a sterile circumstances. A small colony was once more picked up and transferred to newly prepared agarized BG-11 medium-containing Petri plates after the growth of microalgae culture in the Petri plate. The serial dilution procedure, in which liquid forms of the crude culture was transferred to BG-11 medium, was the second technique used to isolate microalgae strains from the crude culture. To acquire the single strains of microalgae, these procedures were repeatedly used (Akhtar and Anees, 2019; Selvaraj *et al.*, 2021).

Identification of isolated strains

Based on morphology and cytology, taxonomic studies of the diversity of algae were performed, and species identification was verified by comparison with related authentic literature and the algal database (Bhakta *et al.*, 2011; Beherepatil and Deore, 2013;

Vijayan *et al.*, 2015; Aquino *et al.*, 2016; Imtiaz *et al.*, 2018; Elshobary *et al.*, 2020; Minhas *et al.*, 2023).

If no novel species have been discovered after 10 microscopic fields, the identification of the microalgae species were done in multiples of 10; this concluded the diversity analysis. Species photos and observations of algal samples were obtained using a mobile camera. BG 11 medium and BBM were used to purify the identified species (Aquino *et al.*, 2016; Elshobary *et al.*, 2020; Lloyd *et al.*, 2021).

Culturing of pure identified algal strain

For culturing of green algal and blue-green algal strains, four different types of media were used in an aseptic environment. These different media are termed; BG-0, BG-11, BBM (Bold’s basal media), and SP (Spirulina media). Different species prefer different nutrient media and different pH for growth i.e. standard pH for BBM media was 6.8 to 7.2, for Spirulina media the standard pH was 9. For BG-11 and BG-0 media, the standard pH was 7.1 to 7.5 (Ramos *et al.*, 2015; Lloyd *et al.*, 2021).

Physio-chemical analysis of water

Chemical tests of sample water were conducted to determine the quality of the water (Estefan *et al.*, 2018). The pH, EC (electrical conductivity), and Total dissolved solids (TDS) of sample water were measured by using Atlas portable pH meter and smart EC meter respectively.

Statistical analysis

All the statistical data analysis was performed using SPSS statistical software (version 17.0 for Windows). All of the data were subjected to a one-way analysis of variance (ANOVA), and the LSD (Least Significant Difference) test was used to determine the means with $p \geq 0.5$. Furthermore the diversity indexes like the "Simpson" index, the Margalef index, species richness, Equitability, and Dominant index.

Results and discussion*Algal diversity in district Karak*

In the current study thirty-three different species of microalgae belonging to three main categories into 10 orders, 12 families, and 17 genera, were identified from different habitats of district Karak, Khyber Pakhtunkhwa. Classification of collected species was based on the Fritsch System of Classification (1944) as shown in Table 2 (Akhtar and Anees, 2019; Junaid *et al.*, 2019). Among them 5 species were cyanobacteria, 16 species were green microalgae, and 12 species from Diatoms; in which Bacillariaceae was the dominant genus with 6 species and their contribution was 19%. The 2nd most dominant families were Scenedesmaceae, Volvocaceae, and Desmidiaceae with each 4 species respectively and their contribution was 12%. The other families Oscillatoriaceae followed by Chlorococcaceae (9%) while some families represented only two (6%) species that were Fragilariaceae, Pinnulariaceae and Nostocaceae, and Gomphonemataceae, Naviculaceae, Chaetophoraceae were one (3%) species each.

Algal diversity culturing response to different media

In an aseptic environment, three different types of media (MBBM, BG-11, and BBM) were used for the

isolation, purification, and evaluation of microalgal growth parameters (Halder, 2016; Arsad *et al.*, 2022). The fluctuation in temperature, pH, water density, and nutrient availability varies the species distribution and impacts algal growth. The maximum growth of algal biomes was reported on BBM and MBBM media; while BG11 media showed its minimum or negligible growth in vitro condition. The growth response of various algal species on three different culturing media is revealed in Table 3.

*A. Diversity of Cyanobacteria**i. Genus Oscillatoria*

Oscillatoria includes unbranched filamentous blue-green algae (cyanobacterium) commonly found in freshwater environments. It reproduces through fragmentation called hormogonia and survives through photosynthesis. This filamentous algal forename 'oscillatoria' derives from its rhythmic & slow oscillating motion found singly or in twisted mats form with around thin mucilaginous sheath. The genus *Oscillatoria* belongs to the family Oscillatoriaceae in the class Cyanophyceae of the phylum Cyanobacteria.

ii. Genus Phormidium

Phormidium is a genus of sheathed, filamentous usually expanded thallus cyanobacteria growing attached to the substrate. The filament texture varies in form without any branching; slightly waved to loosely, irregularly coil, and depending on environmental conditions, sheaths may occur from facultative to almost obligatory. Ecologically members of *Phormidium* are commonly found in a variety of habitats (found on wet rocks, damp soil, hot springs, desert soil, aquatic environment, and on other damp mats) worldwide. The genus *Phormidium* belongs to the family Oscillatoriaceae in the class Cyanophyceae of the phylum Cyanobacteria.

iii. Genus Anabaena

Anabaena is a genus of photosynthetic plankton forming filamentous cyanobacteria. These blue-green algae can be found in the form of colonies or either single cells or filamentous groups of cells. They are

known for their nitrogen-fixing abilities and symbiotic relationship with certain plants as natural fertilizers. *Anabaena* produces neurotoxins that are harmful to pets, animals, and local wildlife which contributes to its symbiotic interactions to protect the plant against grazing stress. The genus *Anabaena* belongs to the family Nostocaceae in class Cyanophyceae of phylum Cyanobacteria.

1. *Oscillatoria subrevis* (Mukhtar *et al.*, 2021)

Taxon Characters: Cells solitary or clumped together, thallus escalating, 0.8µm broad cells, 1-1.5µm long, Ends not reduced. Cell material is colorless, trichomes intertwined. Cells green in color without mucilage.

Remarks: Collected from the fresh water of the Kurram River and geographically distributed in Pakistan (Leghari, 2001) & Tajikistan (Barinova *et al.*, 2016).

2. *Oscillatoria tenuis* (Salah *et al.*, 2017)

Taxon Characters: Cells 4-5µm broad and 3µm long, Plant body blue-green, trichome straight, cells solitary, Mucilage absent.

Remarks: Collected from the fresh water of the Kurram River and geographically distributed in Pakistan (Leghari, 2001) & India (Singh *et al.*, 2013).

3. *Phormidium ambiguum* (Bellinger and Sigeo, 2010)

Taxon Characters: Trichomes tube-shaped, taper slightly towards the ends. Trichomes within the gelatinous mass, solitary, moving, Cells 7-12 µm wide and 8 µm long

Remarks: Collected from rainy water and geographically distributed in Pakistan (Leghari, 2001) & India (Singh *et al.*, 2013).

4. *Anabaena doliolum* (Karabi *et al.*, 2015)

Taxon Characters: Thallus mucilaginous, soft blue-green; trichome single, free swimming, straight, bent or slightly coiled, 3.6-4.2µ broad, apex slightly round with conical apical cells, cells cylindrical-shaped, heterocyst barrel or round, 6µm broad and 8µm long.

Remarks: Collected from the rock surface of the freshwater stream and geographically distributed in Pakistan (Leghari, 2001).

5. *Anabaena variabilis* (Karabi *et al.*, 2015)

Taxon Characters: Thallus gelatinous, dark green or yellowish-green trichomes rounded or intermingled, 4-8 µm broad, more often 3.2-5 µm broad, apex pointed, thickheaded; cells drum-shaped, heterocyst globular or oval, 7µm broad, up to 8µm long.

Remarks: Collected from the flowing stream of fresh water and geographically distributed in Malaysia (Ten *et al.*, 2016), Iran (Hokmollahi *et al.*, 2016) and India (Singh *et al.*, 2016).

B. Diversity of Green Algae

4. Genus *Scenedesmus*

Scenedesmus is one of the most common colonial freshwater green algae genus in plankton however, there are extremely diverse morphologies. They are spiny or feature bristles, nonmotile and colonial usually arranged in a row of 4, 8, 16, or 32 elliptical to spindle-shaped cells and reproduced by nonmotile spores called autospores. This genus is a good indicator of organic pollution and a rich source of protein content as well as a potential source of biodiesel. The genus *Scenedesmus* belongs to the family Scenedesmaceae in the class Chlorophyceae of phylum Chlorophyta.

5. Genus *Cosmarium*

Cosmarium genus includes fresh water and non-motile planktonic. *Cosmarium* is a comparatively large unicell characterized by an isthmus. A single haploid nucleus occupies the isthmus. Among the oldest, this large genus has a lot of variable cells but all are constricted in the middle leading to its unique recognized bi-lobed appearance called semi-cells. Members of this genus have a range of cell walls that maybe ornamented, granule, scrobiculations (pits), pores, spine, or smooth. The genus *Cosmarium* belongs to the family Desmidiaceae in the class Zygnematophyceae of phylum Streptophyta.

Table 2. Taxonomic classification of collected microalgal taxon

Taxa	Genus	Family	Order	Class
1. <i>Oscillatoria subrevis</i>	<i>Oscillatoria</i>			
2. <i>Oscillatoria tenuis</i>		Oscillatoriaceae	Oscillatoriales	
3. <i>Phormidium ambigum</i>	<i>Phormidium</i>			Cyanophyceae
4. <i>Anabaena doliolum</i>	<i>Anabaena</i>	Nostocaceae	Nostocales	
5. <i>Anabaena variabilis</i>				
6. <i>Cosmarium pericymatium</i>				
7. <i>Cosmarium</i> sp.				
8. <i>Cosmarium tumidum</i>	<i>Cosmarium</i>	<i>Zygnematophyceae</i>	Conjugales	<i>Zygnematophyceae</i>
9. <i>Cosmarium rectangular</i>				
10. <i>Chlorococum infusionum</i>				
11. <i>Chlorococum minutum</i>	<i>Chlorococum</i>			
12. <i>Tetracystis chlorococcoides</i>	<i>Tetracystis</i>	Chlorococcaceae	Chlorococcales	
13. <i>Chlorella Rotunda</i>	<i>Chlorella</i>			
14. <i>Chlorella kessleri</i>				
15. <i>Volvox carteri</i>	<i>Volvox</i>	Volvocaceae		
16. <i>Eudorina unicocca</i>	<i>Eudorina</i>			Chlorophyceae
17. <i>Scenedesmus abundans</i>				
18. <i>Scenedesmus bijugatus</i>	<i>Scenedesmus</i>	Scenedesmaceae	Volvocales	
19. <i>Scenedesmus longus</i>				
20. <i>Scenedesmus opoliensis</i>				
21. <i>Fritschiella tuberosa</i>	<i>Fritschiella</i>	Chaetophoraceae	Chaetophorales	
22. <i>Nitzschia hungarica</i>	<i>Nitzschia</i>			
23. <i>Nitzschia navis</i>				
24. <i>Nitzschia oregona</i>		Bacillariaceae	Bacillariales	
25. <i>Cymbella hrengbergii</i>	<i>Cymbella</i>			
26. <i>Cymbella stuxbergii</i>				
27. <i>Cymbella turgid</i>				
28. <i>Diatoma anceps</i>	<i>Diatoma</i>	Fragilariaceae	Fragilariales	Bacillariophyceae
29. <i>Diatom</i> sp				
30. <i>Gomphonema parvulum</i>	<i>Gomphonema</i>	Gomphonemataceae	Cymbellales	
31. <i>Pinnularia viridis</i>	<i>Pinnularia</i>	Pinnulariaceae		
32. <i>Pinnularia major</i>			Naviculales	
33. <i>Navicula craticula</i>	<i>Navicula</i>	Naviculaceae		
Total :	33	17	12	10
				04

6. Genus *Chlorococum*

The *Chlorococum* genus is a type of coccoid, non-motile green algae found singly or in a layer on damp soil and rocks in fresh water. It is a polyphyletic genus of green algae that reproduces through spores and distributed worldwide. The single young cells are ellipsoidal or ovoid with essentially potlike chloroplast while in the mature form they are ellipsoidal—spherical to spherical with cup-shaped chloroplast. The cell wall is bound by hyaline, smooth, and may be thin or thick; sometimes with uni- or bipolar thickening. The genus *Chlorococum* belongs to the family Chlorococcaceae in the class Chlorophyceae of phylum Chlorophyta.

7. Genus *Chlorella*

Chlorella is a single-celled green algae genus. The Greek term *chlorella*, which means "green," is combined with the Latin "Ella" suffix, which means

"small." The cells are spherical in shape and present without flagella ranging from 2-10 µm in diameter. They contain chlorophyll as a green photosynthetic pigment in their chloroplast. Due to its high protein content and abundance of B-complex vitamins, *chlorella* is regarded as a superfood. The genus *Chlorella* belongs to the family Volvocaceae in the class Chlorophyceae.

8. Genus *Volvox*

The genus *Volvox* is a polyphyletic class of Chlorophyceae in the family Volvocaceae of the phylum Chlorophyta. They form the most developed structure of many flagellate cells along with single cup-shaped chloroplasts; to make circular colonies that are embedded in coenobium or hollow mucilaginous spheres. They live in an assortment of freshwater natural surroundings.

Table 3. Growth responds of microalgal species to different culturing media.

SL	Taxa	Family	BBM	MBBM	BG11
1.	<i>Oscillatoria</i> sp.	Oscillatoriaceae	+	+	+
2.	<i>Phormidium</i> sp.	Oscillatoriaceae	+	+	+
3.	<i>Anabaena</i> sp.	Nostocaceae	+	+	+
4.	<i>Cosmarium</i> sp.	Desmidiaceae	+	-	-
5.	<i>Chlorococcum</i> sp.	Chlorococcaceae	+	+	+
6.	<i>Tetracystis</i> sp.	Chlorococcaceae	+	+	-
7.	<i>Chlorella</i> sp.	Volvocaceae	+	+	+
8.	<i>Volvox</i> sp.	Volvocaceae	+	+	-
9.	<i>Eudorina</i> sp.	Volvocaceae	+	-	-
10.	<i>Scenedesmus</i> sp.	Scenedesmaceae	+	+	-
11.	<i>Fritschiella</i> sp.	Chaetophoraceae	+	+	-
12.	<i>Nitzschia</i> sp.	Bacillariaceae	+	-	+
13.	<i>Cymbella</i> sp.	Bacillariaceae	+	+	+
14.	<i>Diatom</i> sp.	Fragilariaceae	+	-	-
15.	<i>Gomphonema</i> sp.	Gomphonemataceae	+	+	-
16.	<i>Pinnularia</i> sp.	Pinnulariaceae	+	-	-
17.	<i>Navicula</i> sp.	Naviculaceae	+	+	-

9. Genus *Tetracystis*

There are many taxa of non-moving, spherical, unicellular algae that make up the *Tetracystis* algal flora, each with its own unique form and life-cycle phases. Tetrad is formed by solitary, 7-8 um in diameter, round to oval, greenish cells that contain mucilage and autospores. Solitary vegetative cells, groups of 2, 4, 8, or multiples of 2 or 4, or groups of more than 2 cells. The groupings' offspring cells first grouped before occasionally dissociating. Cells with a single pyrenoid, a hollow, numerous parietal chloroplast, and frequently with cracks running through them. The genus *Tetracystis* belongs to the family Chlorococcaceae in the class Chlorophyceae of phylum Chlorophyta.

10. Genus *Eudorina*

Eudorina is a paraphyletic genus in the Volvocine green algae clade and cosmopolitan distribution. It includes colonies of 16, 32 cells group and each colony possesses two mucilage sheath flagella which helps in locomotion. The colony has a hollow center, and the cells are organized in a ring around it. Among the most prevalent green algae is the species *Eudorina elegans*. The genus *Eudorina* belongs to the family Volvocaceae in the class Chlorophyceae of phylum Chlorophyta.

11. Genus *Fritschiella*

The green thallus of the *Fritschiella* genus has upright irregular branches of uniseriate filaments with a lack

of caps of end cells and colorless rhizoids that extend below the soil surface. Each cell growing above ground has a single parietal chloroplast with several pyrenoids. Specie were bluish-green whereas some cells were also yellowish-green in color. The shape of the cells varies in the filament some were round, barrel, rectangular shaped, etc. All cells were connected in a filament and different branches originated from the unbranched filaments of different lengths. These morphological features are an example of a parallel evolutionary adaptation to terrestrial life with land plants. The genus *Fritschiella* belongs to the family Chaetophoraceae in the class Chlorophyceae of phylum Chlorophyta.

6. *Scenedesmus abundans* (Strean, 1973)

Taxon Characters: Two-celled colony with small round-shaped pyrenoid in the center of the cell. Each cell had three pointed spines, one on the outer side of the cell and the third spine in the center of the cell facing outward. At 100x, the length of each cell was 18.2µm and the width of 7.8µm.

Remarks: Collected from the freshwater of the Kurram River and geographically distributed in Ireland (Guiry, 2012), Taiwan (Sahoo and Seckbach, 2015), and Karnataka, India (Singh *et al.*, 2016).

7. *Scenedesmus bijugatus* (Beherepatil *et al.*, 2013)

Taxon Characters: Colony 4-8 celled, present linearly, cells curved, oblong to elliptical in shape, ends

rounded. 4 cells colony is 16-22.5 µm long, single cell 7-10 µm wide.

Remarks: Collected from swimming pool fresh water and geographically distributed in Pakistan (Khalid *et al.*, 2014; Khalil *et al.*, 2021) and Iran (Hokmollahi *et al.*, 2016).

8. *Scenedesmus longus* (Beherepatil *et al.*, 2013)

Taxon Characters: Colony 4, 8 cells, 4 cells colony 8.9-15µm long, 7-9µm wide. Single cell around 4-5 µm broad. Cells oblong rounded at one end pointed at the other, present in linear or sub linear series.

Remarks: Collected from pool fresh water wall geographically distributed in India (Selvaraj *et al.*, 2021), and Taiwan (Sahoo and Seckbach, 2015).

9. *Scenedesmus opoliensis* (Beherepatil *et al.*, 2013)

Taxon Characters: Colonies of 2,4 cells, arranged linearly, cell rod-shaped, 17-26µm long, 3-7 µm broad, outer cells with large spiny plans 17-25µm long, inner cells some.

Remarks: Collected from fresh water of tubewell and geographically distributed in India (Singh *et al.*, 2013) and Taiwan (Sahoo and Seckbach, 2015).

10. *Cosmarium pericymatium* (Zarina *et al.*, 2012)

Taxon Characters: Cells solitary, overall cells outline elliptical, Semi cells rounded to elliptical at apices while basal angle not rounded, isthmus present at center and dark green or brownish green semi cells are 22.5- 27 µm broad, isthmus 17.7-20 µm broad.

Remarks: Collected from fresh water of tube well and geographically distributed in Pakistan (Zarina *et al.*, 2012) and Brazil (Ramos, 2015).

11. *Cosmarium* sp.

Taxon Characters: Solitary semi cells with tiny deep median isthmus, oval, round at apices. Cells 25-30 µm broad and 28µm long, dark green with mucilage sheath. Scattered chloroplast around isthmus and granules from cell wall pores.

Remarks: Collected from fresh water of the stream and geographically distributed in Pakistan (Leghari, 2001) and India Uttar Pradesh (Verma *et al.*, 2012).

12. *Cosmarium tumidum* (Zarina *et al.*, 2012)

Taxon Characters: Cells solitary, tiny to large deep median isthmus, Semi cells oblong-oval, round at apices, cells 32 to 35µm broad and 28 µm long, isthmus 5.2 to 6.0 µm broad and dark green. Mucilage sheath present and chloroplast scattered around the isthmus. The zigzag manner around the cell wall shows scattered granules and secreted mucilage from cell wall pores.

Remarks: Collected from freshwater tube well mixed with other free-floating algae and geographically distributed in Brazil (Ramos, 2015), Malaysia (Ten *et al.*, 2016) and India (Verma *et al.*, 2012; Singh *et al.*, 2016).

13. *Cosmarium rectangulare* (Kouassi, 2010)

Taxon Characters: Cells solitary, measuring 30-35µm broad and isthmus 6-8µm, hemi cells trapezoidal in shape and slightly flattened at apices, Chloroplast, and pyrenoid is present.

Remarks: Collected from fresh water of tube well mixed with other free-floating algae and geographically distributed in Pakistan (Naveed *et al.*, 2012; Wali *et al.*, 2017; Khalil *et al.*, 2021), India (Vijayan *et al.*, 2015; Singh *et al.*, 2016) and Island (Klm *et al.*, 2011).

14. *Chlorococum infusionum* (Watanab and Lewis, 2017)

Taxon Characters: Cells round to spherical or ellipsoidal, Yellowish green or dark green, 5- 6.5 µm in diameter, Pyrenoid visible, round to spherical, Chloroplast sponge-like covering the entire cell surface.

Remarks: Collected from pool freshwater and geographically distributed in Pakistan (Agha *et al.*, 2020) and Tajikistan (Barinova and Niyatbekov, 2018).

15. *Chlorococum minutum* (Elshobary *et al.*, 2020)

Taxon Characters: Cells solitary, oval, green in color, 4-5µm broad, chloroplast parietal with a prominent pyrenoid, Mucilage absent.

Remarks: Collected from fresh water of tube well and geographically distributed in Pakistan (Ghani *et al.*, 2020) and Russia (Bellinger and Sige, 2010).

16. *Chlorella Rotunda* (Bock *et al.*, 2011).

Taxon Characters: Cells solitary, spherical or oval shaped, 3–5 µm broad. Mucilage absent. Chloroplast single, with broadly spheroidal to spherical pyrenoid. Reproduce through autospores.

Remarks: Collected from the fresh water of rainfed stream and geographically distributed in India (Kamboj *et al.*, 2015) and Ireland (Guiry, 2012; Guiry, 2020).

17. *Chlorella kessleri* (Eldrin, 2019)

Taxon Characters: Cells solitary, spherical to spheroidal in shape, spherical pyrenoid is present. Cells 3.5-6.5 µm broad. Reproduce through 3-4 autospores.

Remarks: Collected from rainfed stream attached to substratum and geographically distributed in Pakistan (Jabeen *et al.*, 2021), India (Singh *et al.*, 2016) & (Chader *et al.*, 2011).

18. *Volvox carteri* (Umen, 2020)

Taxon Characters: Green in color, present in isolated gonidium form 40-48µm in each cell. The developed structure of many flagellate cells along with a single cup-shaped chloroplasts; make circular colonies (400-450µm) which are embedded in coenobium or hollow mucilaginous spheres.

Remarks: Collected from walls of the swimming pool and geographically distributed in Pakistan (Khalid *et al.*, 2014) and (Umen, 2020), Uttar Pradesh, India (Verma *et al.*, 2012).

19. *Tetracystis chlorococcoides* (Watanab and Lewis, 2017)

Taxon Characters: Cells solitary, circular to oval, greenish in color, 7-8µm in diameter, Mucilage present, autospores form a tetrad.

Remarks: Collected from swimming pool fresh water and geographically distributed in Malaysia (Ten *et al.*, 2016) and Ireland (Guiry, 2012).

20. *Eudorina unicocca* (Yamada *et al.*, 2008)

Taxon Characters: Cells surround whole colony, 8, 16, 32 celled colonies, pyrenoid not prominent and each the cell was 4-6µm in diameter.

Remarks: Collected from rain-fed stream rock and geographically distributed (Sahoo and Seckbach, 2015) and (Rimsha *et al.*, 2020).

21. *Fritschiella tuberosa* (M.O. P.lyengar, 1932)

Taxon Characters: *Fritschiella* species was bluish green in color, long filamentous body with various branches originating from the unbranched filaments. At 40x, the length of the small filament was 40 micrometer and the width was 4mm. The shape of the cells varies in the filament some were round, barrel, rectangular shaped etc.

Remarks: Collected from fresh water tube well and geographically distributed in Pakistan (Saleem *et al.*, 2011; Shah *et al.*, 2019; Salah *et al.*, 2020).

C. Diversity of Diatom

12. Genus *Nitzschia*

Nitzschia is a genus of common widely distributed marine diatom that may or may not be toxic. *Nitzschia* has green, photosynthetic cells with frustule-like silica cell walls that are bilaterally symmetric. The wide range of temperatures across *Nitzschia* species enables them to live in a variety of environments, from the open oceans to coastal waters. Increased nutrient levels, strong light exposure, and warm seawater temperatures are frequently linked to blooms. The genus *Nitzschia* belongs to the family Bacillariaceae in class Bacillariophyceae of phylum Gyrista.

13. Genus *Cymbella*

Cymbella species have benthonic and frequently bind to the substrate with a mucilaginous stalk. *Cymbella* members can be found in streams and other water bodies by forming colonial masses. Cymbellaceae members are asymmetrically about the apical axis. In the water bodies of old lakes, cymbelloid diatom diversity is exceptionally significant. Numerous species of *Cymbella* share similar valve architecture because of the genus' convoluted taxonomic history. The genus *Cymbella* belongs to the family Cymbellaceae in the class Bacillariophyceae of phylum Gyrista.

14. *Genus Diatoma*

Diatoma are well-known kinds of phytoplankton and are among the most significant group of microalgae. Diatoma are unicellular even though they can form colonies in the shape of fibers or strips, fans, crisscrosses, or stars. *Diatoms* are makers inside the food chain. Diatoma are photosynthetic algae, non-motile, have a siliceous skeleton (frustule), and have cosmopolitan distribution. Diatoma possess a bipartite cell wall and secrete silica at some stage of their life cycle. The genus *Diatoma* belongs to the family Fragilariaceae in class Bacillariophyceae of phylum Gyrista.

15. *Genus Gomphonema*

Gomphonema is a genus of diatoms planktons in fresh water. Unicellular diatoms in the genus mainly adhere to straight or branched gelatin stalks. The cells have a typical two-counter diatom shell. Identical valves, each with a raphe (the biraphid genus). In the girdle view, wedge-shaped, the characteristic dichotomy of diatoms to reproduce asexually and sexually reproduce through anisogamy. The genus *Gomphonema* belongs to the family Gomphonemataceae in the class Bacillariophyceae of phylum Gyrista.

16. *Genus Pinnularia*

Pinnularia is a genus of freshwater diatoms found in ponds and on moist soil. Its cell is oval and lengthy; it may have a simple or complicated raphe system. The key elements of the cell wall are pectic compounds, and a transverse cytoplasmic bridge suspends a single nucleus in the middle of the vacuole. Cytoplasm is arranged in the parietal layer and it moves by characteristic gliding movements. Two chloroplasts are present along the two sides of the cells. The genus *Pinnularia* belongs to the family Pinnulariaceae in the class Bacillariophyceae of phylum Gyrista.

17. *Genus Navicula*

Navicula genus includes boat shaped (incense-holder), motile and solitary diatom. *Navicula* cell shape varies from elliptic in girdle view to widely lanceolate in valve view. It is found important in global ecology as it produces a quarter of all the

oxygen within earth biosphere. A girdle of flowable mucilage threads surrounds the exterior of the *Navicula's* shell, serves as a tank track. The genus *Navicula* belongs to the family Naviculaceae in class Bacillariophyceae of phylum Gyrista.

22. *Nitzschia hungarica* (Salah *et al.*, 2017)

Taxon Characters: Rod shape, Yellow to dark green, apices bluntly rounded 37-95 μm in length, 6-9 μm in width, mucilage present.

Remarks: Collected from freshwater of tubewell and geographically distributed in Malaysia (Tan *et al.*, 2016) and (Salah *et al.*, 2017).

23. *Nitzschia navis* (Tan *et al.*, 2016)

Taxon Characters: Rod shape, solitary diatoms, brownish blue green, 60-80 μm in length, 6 μm in diameter, cells lanceolatae, apices rectangular, two brown chloroplasts are present.

Remarks: Collected from fresh water of tubewell stream and geographically distributed in Turkey (Blaginina *et al.*, 2021) and Asia (Romero *et al.*, 2011).

24. *Nitzschia oregona* (Sovereign, 1958)

Taxon Characters: Rod shape, yellowish green in color, 30-45 μm in diameter, 3 μm broad, linear lanceolatae valve, obtuse capitate ends.

Remarks: Collected from fresh water of tube well and geographically distributed in Malaysia (Tan *et al.*, 2016) and US (Du *et al.*, 2015).

25. *Cymbella hrengbergii* (Shameel, 2006)

Taxon Characters: Solitary rod shape, dark green in color, 50 μm in length, 16 μm in diameter, elliptic lanceolate valve, obtuse ends, raphe straight.

Remarks: Collected from fresh water of the stream and geographically distributed in India (Singh *et al.*, 2013), US (Du *et al.*, 2015) and (Shameel, 2006).

26. *Cymbella stuxbergii* (Shameel, 2006)

Taxon Characters: Solitary rod shape, yellowish brown in color, cell 60 μm in length, 20 μm in diameter, valve semi elliptic, obtuse and prolonged ends.

Remarks: Collected from the fresh water of the Kurram River and geographically distributed in Tajikistan (Barinova *et al.*, 2016) Russia (Pomazkina *et al.*, 2017).

27. *Cymbella turgid* (Guiry, 2012)

Taxon Characters: Solitary rod shape, green in color, 40–100µm in length, 25 in width, 7–9 striae in 10 µm, broader in middle than ends, central area straight.

Remarks: Collected from the fresh water of the Kurram River and geographically distributed in India (Verma *et al.*, 2012) and (Ding *et al.*, 2017).

28. *Diatoma anceps* (Salah *et al.*, 2017)

Taxon Characters: Solitary rod like, yellowish brown in color, 10-50µm in length, and 6-7µm in width, Valves apically and Trans apically symmetrical; linear with capitate ends.

Remarks: Collected from flowing water of rainfed stream and geographically distributed in Pakistan (Zaman *et al.*, 2011) and Russia (Pomazkina *et al.*, 2017).

29. *Diatom* sp

Taxon Characters: These consist of elongated narrow cells or rectangular. The cells are present in solitary. The valves are elliptical. At 40 x, the length was 10 µm and the width was 6µm.

Remarks: Collected from the freshwater of the rainfed stream and geographically distributed in Russia (Pomazkina *et al.*, 2017) and India (Singh *et al.*, 2016).

30. *Gomphonema parvulum* (Munir *et al.*, 2013).

Taxon Characters: Rod shape, yellowish green in color, 28µm in length, 8µm in diameter, clavate lanceolate valve, extends round ends.

Remarks: Collected from fresh water of rainwater and geographically distributed in Pakistan (Munir *et al.*, 2012) and India (Singh *et al.*, 2016).

31. *Pinnularia viridis* (Stanleycohn, 2001)

Taxon Characters: Rod shape, yellowish green in color, two slits in the cell wall, broad round apices, mucilage present, cell body 91-120µm long and 10µm broad.

Remarks: Collected from fresh water of rain and geographically distributed (Buaya *et al.*, 2015) and Tajikistan (Barinova *et al.*, 2018).

32. *Pinnularia major* (shamel, 2006)

Taxon Characters: Solitary rod shape, yellowish green in color, 100-120µm in length, 30µm in diameter, valve linear, broadly obtuse ends.

Remarks: Collected from the fresh water of rainfed stream and geographically distributed in the US (Du *et al.*, 2015) and (Shameel, 2006).

33. *Navicula craticula* (Bellinger *et al.*, 2010)

Taxon Characters: Rod shape, light green in color, 50 µm in length and 8µm in diameter, straight raphe, extended rounded tips, valves broadly lanceolate, two plate like chloroplasts are present.

Remarks: Collected from pool fresh water and geographically distributed in Pakistan (Junaid *et al.*, 2019; Khalil *et al.*, 2021) and India (Singh *et al.*, 2016).

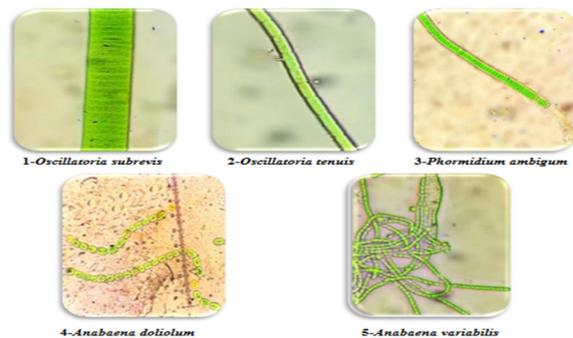


Fig. 1. 1-*Oscillatoria subrevis*, 2-*Oscillatoria tenuis*, 3-*Phormidium ambiguum* 4-*Anabaena doliolum* and 5-*Anabaena variabilis*.

A total of thirty-three different species of microalgae were collected from different habitats of which 20 were found to be unicellular, 7 colonial, 5 filamentous, and one in branched filamentous form (Figs 1, 2 & 3). The present study is the first taxonomic exploration of microalgae including cyanobacteria, green algae, and diatoms from different habitats and substratum including ponds, rainy water, streams and tube well. The current study revealed that district Karak has high algal diversity

and never been explored. Similarly, (Minhas *et al.*, 2023) studied 30 species that belonged to 4 orders, 11 families, and 14 genera from Tehsil Gujar Khan, District Rawalpindi.

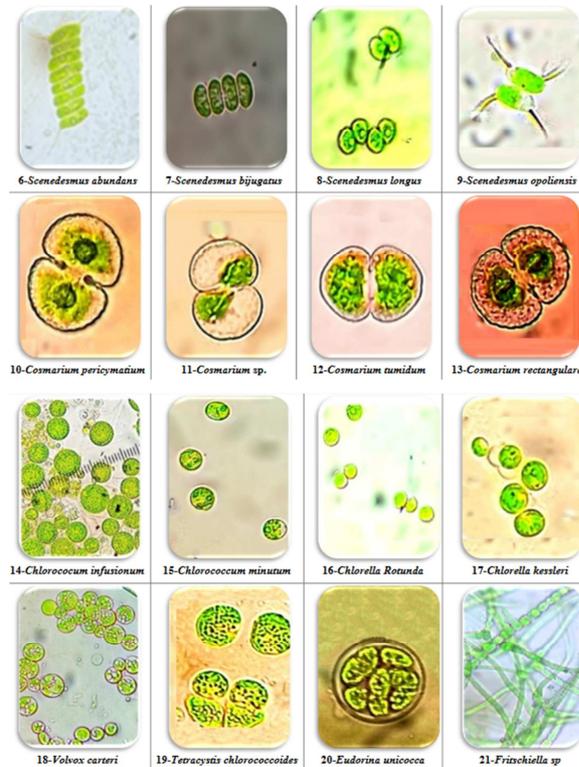


Fig. 2. 6-*Scenedesmus abundans*, 7-*Scenedesmus bijugatus*, 8-*Scenedesmus longus* 9-*Scenedesmus opoliensis*, 10-*Cosmarium pericymatium*, 11-*Cosmarium sp.*, 12-*Cosmarium tumidum* , 13-*Cosmarium rectangulare*, 14-*Chlorococum infusionum*, 15-*Chlorococum minutum*, 16-*Chlorella Rotunda*, 17-*Chlorella kessleri*, 18-*Volvox carteri*, 19-*Tetracystis chlorococcoides*, 20-*Eudorina unicocca* and 21-*Fritschiella tuberosa*.

In addition, morpho-ordered depictions of 73 freshwater green algae with 34 species, 25 families, 17 orders, and 9 classes were taken into consideration by (Khan *et al.*, 2011) in the Kalpani stream and its surrounding range in the Mardan region. There are 138 species of chlorophytes, according to another study, and 74 of them (or 53.6%) are members of the Chlorococcales family. According to (Ali *et al.*, 2010) the total diversity of the Cladophorales and Chaetophorales is 3%. In addition, (Leghari, 2001) reported the 31 different types of Chlorophyta found

in freshwater and Riverin lakes as green filamentous algae from Sindh's lakes and ponds. These findings are also related to previous work from Peshawar Valley (Saleem *et al.*, 2011; Salah *et al.*, 2017; Imtiaz *et al.*, 2018), Multan (Ghazala *et al.*, 2011), Mardan (Ullah *et al.*, 2021), and Maidan of district Dir (Tabassum *et al.*, 2021).

The genera *Scenedesmus* and *Cosmarium* species were dominant in all ponds and rainy freshwater which indicates the high level of organic compounds and nutrient content in such habitat spots for both genera. These species are thought to be indicators of highly organic water because members of this genus prefer these conditions for their good growth. The similar previous results were also conducted from Peshawar (Zaman *et al.*, 2011), River panjkora of district Dir (Suhlab *et al.*, 2017), Karachi (Shahnaz *et al.*, 2018), Naran and Lahore (Junaid *et al.*, 2019), Kashmir (Khalil *et al.*, 2021) and Lahore (Mukhtar *et al.*, 2021).

In some studies, similar results were explained about the *Scenedesmus sp.* diversity in highly contaminated organic water (Verma *et al.*, 2012), (Singh *et al.*, 2013) and (Minhas *et al.*, 2023). Our current study showed that all 33 species were found in polluted and non-polluted water fresh water and are being reported here algal diversity for the first time in the southern area of Pakistan. Some common species of algae from these site areas have unique adaptive features which make them able to survive in both summer and spring types of conditions. Another the component that was seen during the study was the profundity of water, as more species were gathered from a stream, tubewell, and swimming pool contrasted with kurma and water around tree trunks because the depth of the stream, swimming pool, and tubewell was more than kurma and standing water around a tree trunk, where more algal communities.

Similar studies were carried out by (Barinova *et al.*, 2016), who investigated 145 algal species from the Alexander River in Central Israel, and by (Barinova *et al.*, 2018), who reported 126 algal species from the

Hadera River in Israel, demonstrating that the algae are the markers of environmental conditions. The isolation, cultivation, and identification of varied algae from various habitats are primarily of ecological, biotechnological, and commercial relevance. The current experiment additionally examined how three distinct culturing media, BG-11, BBM, and MBBM, affected the reaction of the diversity of algae. According to the results of the media reaction, BBM and MBBM media encourage growth more effectively than BG11 media. Our finding relived with previous data by (Singh *et al.*, 2013), Balochitan (Agha *et al.*, 2020), Charasada (Shah *et al.*, 2019), Chitral (Ullah *et al.*, 2019), Cholistan (Alam *et al.*, 2019) and (Minhas *et al.*, 2023).

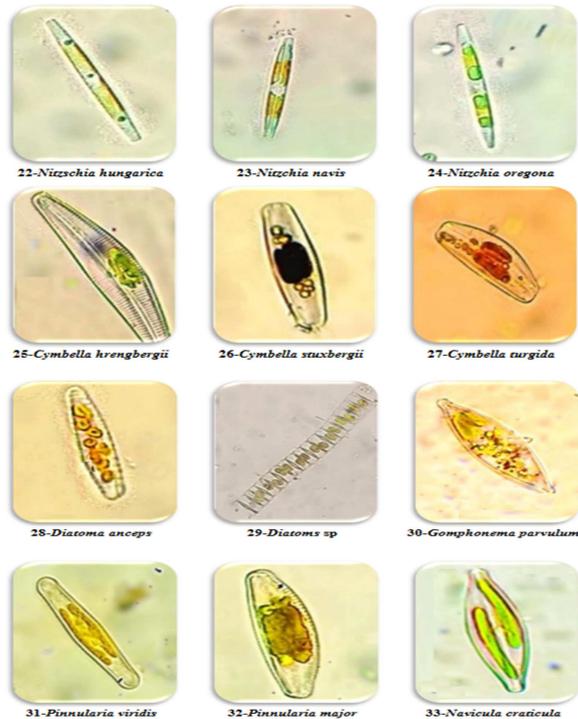


Fig. 3. 22-*Nitzschia hungarica*, 23-*Nitzschia navis*, 24-*Nitzschia oregona*, 25-*Cymbella hrengbergii*, 26-*Cymbella stuxbergii*, 27-*Cymbella turgida*, 28-*Diatoma anceps*, 29-*Diatoms sp.*, 30-*Gomphonema parvulum*, 31-*Pinnularia viridis*, 32-*Pinnularia major* and 33-*Navicula craticula*.

Algae were isolated in this study employing BBM in a regular, chemically specified medium. We were able to isolate 33 different species of algae diversity in this medium. We assert that our study is indicative of the

overall green algal biodiversity in the Karak district even if we are aware that some taxa are challenging to grow in this environment (Khan *et al.*, 2011; Lloyd *et al.*, 2021). The study found that the distribution and diversity of subaerial algal communities are significantly influenced by sampling locations with a variety of ecological factors. Previously this data was reported from Karachi (Ayubli and Valeem, 2019), Karachi (Jabeen *et al.*, 2020), Fiaslabad (Rimsha *et al.*, 2020), Kallar Kahar lake from Chakwal (Ghani *et al.*, 2020) and India (Kamboj *et al.*, 2022).

The distinction of species in different regions and their dissemination could be due to the quality of the water. Therefore, the majority of the easily responsive varieties are likely candidates for biomass production and research into highly valuable compounds thought to be crucial for the industrial sector.

Conclusion

This study appears to be a novel investigation as it suggests a taxonomic study of microalgae in freshwater bodies in the district of Karak, Pakistan, along with an assessment of the optimal conditions for their cultivation. It was concluded that a total of 33 species of algal diversity belonging to 17 genera were collected from different sites area of the District Karak. The study provides a comprehensive and novel analysis of the algal diversity in District Karak, southern west, Pakistan, highlighting the dominance of *Scenedesmus* and *Cosmarium* genera, and the potential for further exploration and discovery of new algal species in the region. Species of other genera i.e *Oscillatoria*, *Phormidium*, *Anabaena*, *Chlorococcum*, *Tetracystis*, *Chlorella*, *Volvox*, *Eudorina*, *Fritschella*, *Nitzschia*, *Cymbella*, *Diatoma*, *Gomphonema*, *Pinnularia* and *Navicula* also prevailed in this area. It is the first algal diversity from various habitats in district Karak, southern area; recorded to the algal flora of Pakistan. It is also inferred from the current study that microalgal diversity prefers culturing on BBM and MBBM as compared to BG11 which showed minimum culturing. Furthermore, in the district Karak, many other interesting aquatic spots are, that are yet to be

explored. Exploration of the remaining sites would offer the potential to record novel species that may be unique to Pakistan's or the entire world's algal flora. As a result, it is crucial to preserve algal diversity in local habitats and to carry out more systematic research on them, both of which can only be done until the ecology and habitats of various algal flora have been fully understood. This research could provide valuable information on the diversity and potential uses of microalgae in the region, as well as offer insights into the environmental conditions that facilitate their growth. Additionally, the study could contribute to the development of strategies for the sustainable management and utilization of freshwater resources in the area.

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