Research Article

A Prospective Study on Morphological Identification and Characterization of Freshwater Green Algae Based on the Microscopic Technique in District Rawalpindi

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Abstract | The current study was carried out to isolate, identify and characterized fresh water green algae species from ecologically diverse habitats of Tehsil Gujar Khan, District Rawalpindi. A microscopic image data was used to identify algal species. A total of 30 species were recorded that belonged to 4 orders, 11 families, and 14 genera. *Clamydomonas reinardtii, Acutodesmus obliquus* and *Cosmarium isthmocondrum* are new record from Pakistan. Among identified taxa, *Scenedesmus* was the dominant genus with 8 species and their contribution was 26.6%. The 2nd most dominant genus was *Cosmarium* with 6 species and their contribution was 20%. The other genera *Chlorococcum*, *Ankistrodesmus, Coelastrum,* and *Closterium* were (6.6%). Some genera represented only one (3.3%) species that were *Chlamydomonas, Eudorina, Tetraspora, Chlorella, Westella, Pediastrum, Acutodesmus,* and *Stigeoclonium*. Hence, the current study reveals that Gujar Khan, District Rawalpindi is a rich source of green algae and an ideal place for their cultivation.

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Introduction

Green algae are one of the most diverse groups of algae, with at least 7000 species (Nelson and Garcia-Pichel, 2021). Green algae are characterized by several distinct features including filaments, colonies, branched, motile, non-motile, and blade-like thallus (Leliaert *et al.*, 2012). Chloroplast contained chlorophyll a, b and accessory pigments including carotenes and xanthophylls which are surrounded by double membrane with thylakoid arranged in lamellae. Pyrenoid are present and embedded in the chloroplast and surrounded by starch, the primary reserve carbohydrate (Forjan *et al.*, 2015). Green algae have variations in their morphology ranging from microscopic flagellated unicells to complex macroscopic thalli with varying degrees of morphological differentiation. They are a paraphyletic group from a taxonomic standpoint since they have a common ancestor with plants and have

the same pigments. They synthesize the same type of carbohydrates during photosynthesis as terrestrial plants (Nzoiwu *et al.*, 2017).

Algal taxonomy is a key discipline in phycology and is critical for algal genetics, physiology, ecology, applied phycology, and particularly bioassessment. Taxonomic identification is the most common analysis and hypothesis-testing endeavor in science. Errors of identification are often related to the inherent problem of small organisms with morphologies that are difficult to distinguish without research-grade microscopes and taxonomic expertise in phycology (Manoylov, 2014).

Microalgae are a diverse group of aquatic photosynthetic organisms having variations in cell morphologies, life cycle, and growth patterns, and favoring a variety of habitats (Alam *et al.*, 2019). Green algae may be found in land areas in large numbers, although certain species have specific ecological requirements. For example, flagellated chlorophytes are common in nutrient-rich standing waters. Filamentous conjugating green algae are present in the stagnant water of roadside ditches and ponds as well as the littoral zones of lakes where they can form free-floating mats or mix with other algae in attached or floating masses. Desmids are more prevalent in low-conductance ponds and streams with moderate nutrient levels and they frequently mix with macrophytes (Haworth, 2016). Some algal species have the ability to face stressful environments and some species are unable to adopt themselves a stress environment. Some algal species can face stressful environments and some species are unable to adapt themselves to a stressful environment (Mukhtar *et al.*, 2021). Many green algae may grow heterotrophically in the dark using an external source of organic carbon and this growth is faster than pure autotrophic conditions (Bell, 2013; Fan *et al.*, 2012). In mountains, biodiversity and distribution of green algae are fragmented and overall poor. Some environments, such as the snowpack at high elevations (Jacquemin *et al.*, 2019), have gained more attention (Hoham and Remias, 2020).

The diversity of green algae was reported in Pakistan i.e., (Shuaib *et al.*, 2017; Ali *et al.*, 2011; Khan *et al.*, 2017; Wali *et al.*, 2017; Ullah *et al.*, 2019, 2021; Zarina and Shameel, 2013; Imtiaz *et al.*, 2018; Mukhtar *et al.*, 2021; Jaffer *et al.*, 2019; Shah *et al.*, 2011; Asad *et al.*, 2011; Ghazala *et al.*, 2009). The present study, therefore, aims at exploring the diversity of green algae thriving naturally in the in Tehsil Gujar Khan, district Rawalpindi. The identified species were further assessed for their occurrence at global, regional, and local levels.

Materials and Methods

Physiological features

Rawalpindi city is a capital of Pakistan. It is situated in the northernmost part of Punjab and comprised an area of 5,286 km^2 with latitude 33.626057° N and its longitude 73.071442° E (Shabbir and Ahmad, 2015). Its elevation is 508 m. The distance between Rawalpindi to Gujar Khan is 44km and 861.9 meters. The average rainfall is 1,346.8 millimeters (53.02 inc), most of which falls in the monsoon season (Rehman *et al.*, 2021).

Study area

Green algae samples were collected from different stations of Potohar Gujar Khan Tehsil of district Rawalpindi. All samples were collected during the mid of February and the start of October 2019. Its latitude is 33.2616°N and longitude is 73.3058°E with 461 m elevation. Samples were collected from polluted (ditches, stagnant water, and dam) and non-polluted (Streams, running water, ponds and tube wells) sites. Five different sites of Gujar Khan selected for the collection of algal samples were Takia baba Rahim shah, village Sukho, Dehra Muslim, village Cheena, and Village Arrha.

Collection of samples

All algal samples were in liquid form. Forceps, gloves, falcon tubes, notebook, EC meter, thermometer, barometer, polythene bags, permanent marker, and glass jars were used for collection purpose. The field data of collected samples are mentioned in Table 1. During the collection of samples, different parameters like the color of the substrate, temperature, humidity, vegetation, and habitat were recorded on the spot. The samples were brought to the laboratory and electrical conductivity (EC) and pH were measured. The collected samples were transferred to conical flasks containing 4% formalin and stored in a growth chamber at 24-25°C.

Microscopic observations

The collected samples were taxonomically investigated under a light microscope using different

Table 1: *Field data of collected green algae samples.*

Sample site	Samples code	Water colour	Temperature	Humidity	EC	pH	Vegetation	Habitat
Takia Baba Rahim Shah	LM1	Transparent	22.3°C	76%	570	6.92	Grasses	Stream
	LM2	Brown	23.6°C	69%	180	6.69	Grasses	Pond
	LM3	Black	26°C	69%	670	6.61	Grasses	Ditch
	LM4	Light green	25°C	76%	520	6.72	Grasses	Stream
	LM5	Muddy	26°C	70%	520	6.66	Grasses	Ditch
Village Sukho	LM6	Black	26.1°C	74%	580	5.82	Keekar, Cynodon	Stream
	LM7	Black	26.8°C	71%	430	6.49	Keekar	Pond
	LM8	Dirty green	28.6°C	66%	700	6.69	Keekar, Cynodon	Stream
Dehra Muslim	LM9	Muddy	28.6°C	64%	790	6.97	Grasses	Ditch
	LM10	Black	27.2°C	62%	650	6.27	Grasses	Stream
	LM11	Transparent	28.2°C	52%	400	7.11	Mulberry	Stream
Village Cheena	LM12	Transparent	28.2°C	46%	610	7.03	Mulbrry	Pond
	LM13	Muddy	28°C	62%	290	7.04	Grasses	Dam
Village Arrha	LM14	Muddy	30.7°C	50%	220	6.34	Grasses	Stream
	LM15	Green	31.7°C	41%	210	6.49	Grasses	Pond
	LM16	Brown	32°C	41%	410	6.61	Grasses	Pond
	LM17	Brown	37.1°C	32%	200	6.83	Grasses	Stream
	LM18	Transparent	31°C	45%	120	6.86	Grasses	Tube well

magnifications i.e., 10X, 40X, and 100X. To visualize and characterize green algae each specimen was mounted on a glass slide with the help of a dropper or micropipette. The glass slide was covered with a cover slip. A little drop of distilled water was added to the slide to remove dehydration Immersion oil was also used to observe the slide at 100X, and that oil drop was placed upon the cover slip. The dimensions of each species i.e., length and width were noted by using the calibrated eyepiece. All the morphological characters were recorded such as color, shape, pyrenoid, filamentous, branched, unbranched, unicellular, arrangement of chloroplast, and presence or absence of mucilage sheath. Each species was photographed using a camera and species were recognized by comparing them to legitimate literature. Identification was done by using authentic literature (Beherepatil and Deore, 2013; Rai, 2013b; Hegewald, 1997; Jena and Adhikary, 2007; Leghari, 2001b; Aquino *et al*., 2016; Vijayan *et al*., 2015; Ramos *et al*., 2015; Goldstein, 2015; Bhakta *et al*., 2011) and databases like algae base, UTEX, protist.com, and SAG.

Culturing procedure for algal growth

Different species of green algae require different nutrient media and different pH for optimum growth under laboratory conditions. Each purified sample were inoculated in BG11 (Blue-green algae media) (George *et al*., 2014), BBM (Bold Basal Media), and MBBM (Modified Bold Basal Media) (Bischoff, 1963). Algal samples were kept in the growth chamber for incubation at a temperature of about 25-28°C with continuous illumination at 2500-3000 lux. When algal cultures attained exponential growth, then they were transferred to 500 mL Erlenmeyer flasks with fresh media (Gerloff *et al*., 1950).

Results and Discussion

Summary of collected algal flora

In the present study thirty different species of green algae belonging to 4 orders, 11 families, and 14 genera, were identified from different habitats of Tehsil Gujar Khan, District Rawalpindi. Species were classified by following the Fritsch System of Classification (1944) as shown in Table 2. The most common genera were *Scenedesmus* (26.6%), *Cosmarium* (20%), *Chlorococcum, Ankistrodesmus, Coelastrum* and *Closterium* (6.6%). The other genera that represented only one (3.3%) specie were *Chlamydomonas, Eudorina, Tetraspora, Chlorella, Westella, Pediastrum, Acutodesmus,* and *Stigeoclonium.*

Growth response on different culturing media

Green algae species showed different growth on different media. Three media were used for the isolation and purification of algal species. The growth of green algae was maximum on BBM and MBBM media. While on BG-11 media, it showed minimum or no growth. The growth response of green algae on different culturing media is given in Table 3.

Table 2: *Classification of collected algal species.*

No.	Genus and species	Family	Order	Class
1	*Chlamydomonas reinardtii*	Chlamydomonadaceae	Volvocales (3)	Chlorophyceae
1	*Eudorina elegance*	Volvocaceae		
1	*Tetraspora gelatinosa*	Tetrasporaceae		
2	*Chlorococcum humicola, C. infusionum*	Chlorococcaceae	Chlorococcales (18)	
1	*Chlorella vulgaris*	Chlorellaceae		
1	*Westella botryoides*	Dictyosphaeriaceae		
1	*Pediastrum tetras*	Hydrodictyaceae		
2	*Ankistrodesmus falcatus, A. braunii*	Selenastraceae		
8	*Scenedesmus hystrix, S. denticulatus, S. bijuga, S. acutus, S. acuminatus, S. dimorphus, S. acunae, S. quadricauda*	Coelastraceae		
2	*Coelastrum pseudomicroporum, C. indicum*			
1	*Acutodesmus obliquus*			
1	*Stigeoclonium attenuatum*	Chaetophoraceae	Chaetophorales (1)	
6	*Cosmarium granatum, Isthmocondrum, C. lundellii, C. quadratulum, C. crenatum, C. angulosum*	Desmidioidiaceae	Conjugales (8)	
2	*Closterium subulatum, C. dianae*			
30	30	11	4	1

Table 3: *Growth response of different green algal species.*

S. No	Green algae	BBM	MBBM	BG11
01	*Chlamydomonas* sp	+	+	-
02	*Eudorina* sp	+	+	-
03	*Tetraspora* sp	+	+	-
04	*Chlorococcum* sp	+	+	+
05	*Chlorella* sp	+	+	+
06	*Westella* sp	+	+	-
07	*Pediastrum* sp	+	+	-
08	*Ankistrodesmus* sp	+	+	-
09	*Scenedesmus* sp	+	+	-
10	*Coelastrum* sp	+	+	-
11	*Acutodesmus* sp	+	+	-
12	*Stigeoclonium* sp	+	+	-
13	*Cosmarium* sp	+	+	-
14	*Closterium* sp	+	+	-

Diversity

1. *Clamydomonas reinardtii* P. A. Dangeard 1888: 130

Taxonomic characters: 4 cells present in mucilage, ellipsoidal in shape, visible pyrenoid, pyrenoid is present at the posterior end of the cell, width is 7.8 µm, green in colour, flagella at the anterior end, single chloroplast is present in cell, light green in colour Figure 1a.

Geographical distribution: India (Patil *et al.*, 2012), Malaysia (Ng *et al.*, 2011), Malaysia (Omar *et al.*, 2016).

Local distribution: New to Pakistan

Novelty: *Clamydomonas reinardtii* has not been isolated and describe previously from Pakistan and it is the new addition to algal flora of Pakistan.

2. *Eudorina elegance* Ehrenberg 1832: 78 (Goldstein, 2015).

Taxonomic characters: Colony of 14 cells present in mucilage**,** colony is elliptical in shape, cells are spherical in shape, cup-shaped chloroplast, pyrenoid is present, and colony is 52 µm in diameter Figure 1b.

Geographical distribution: India (Ja and Chandrab, 2012).

Local distribution: Swat (Ali *et al.*, 2010), Rawalpindi (Ahmed *et al.*, 2016).

3. *Tetraspora gelatinosa* (Vaucher) Desvaux 1818: 18

Taxonomic characters: 4 cells attached to each other through the gelatinous bag, cells are spherical in shape, look like a flower, colonial form, and chloroplast is present in cells, 10.4 µm in width, dark green in colour, cup-shaped chloroplast with pyrenoid, mucilaginous colony Figure 1c.

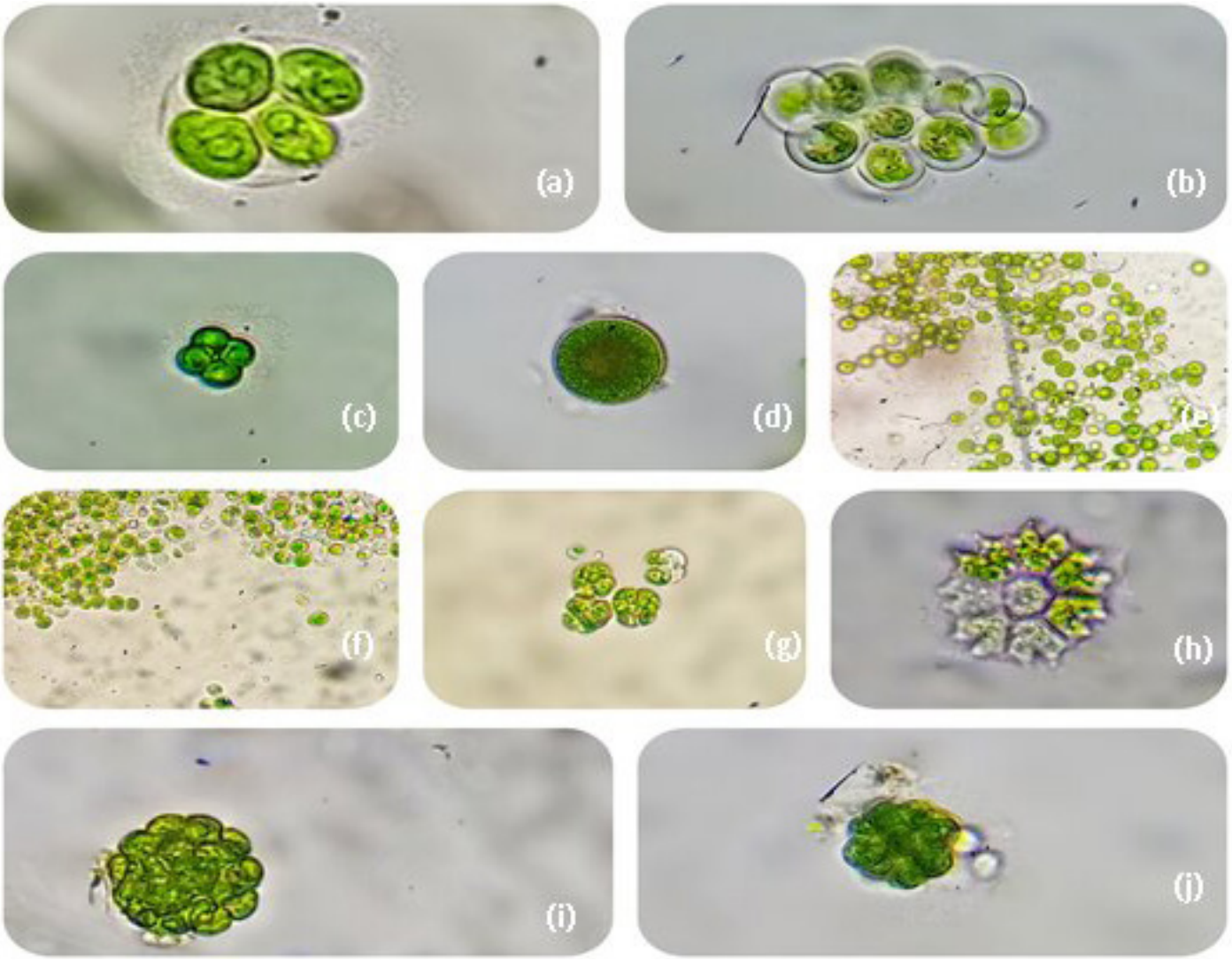

Figure 1: *Clamydomonas reinardtii P.A. Dangeard, Eudorina elegance Ehrenberg, Tetraspora gelatinosa (Vaucher) Desvaux, Chlorococcum humicola (Nägeli) Rabenhorst, Chlorococum infusionum (Schrank) Meneghini, Chlorella vulgaris Beijerinck (1980), Westella botryoides (West) De Wildeman, Pediastrum tetras var. tetraodon (Corda) Hansgirg, Coelastrum pseudomicroporum Korshikov, Coelastrum indicum W.B. Turner.*

Geographical distribution: Spitsbergen (Richter *et al.*, 2014), India (Hardikar *et al.*, 2019).

Local distribution: Sindh (Ghazala *et al.*, 2004).

4. *Chlorococcum humicola* (Nägeli) Rabenhorst 1868: 58 (Halder, 2016).

Taxonomic characters: Cell wall smooth, chloroplast scattered throughout cell, pyrenoid is present, single pyrenoid, and cells are spherical in shape, 12µm in diameter, without mucilage, multinucleate Figure 1d.

Geographical distribution: India (Bajpai *et al.*, 2019; Vijayan and Ray, 2015).

Local distribution: Karachi (Aliya *et al.*, 2009), Tiruchirappalli (Mubarak *et al.*, 2012), Swat (Ali *et al.*, 2010).

5. *Chlorococum* in fusionum (Schrank) Meneghini 1842: 27 (Vijayan *et al.*, 2015).

Taxonomic characters: Unicellular, width 13-5 µm, length 10.4 µm, without mucilage, present in colonies form, visible pyrenoid, without mucilage, spherical in shape, solitary, several cells combined to form cluster, light green in colour, internal cells are covered with chloroplast Figure 1e.

Geographical distribution: Philippines (Arguelles and Monsalud, 2017).

Local distribution: Kabul River (Barinova *et al.*, 2016).

6. *Chlorella vulgaris* Beijerinck (1980) (Ramaraj *et al*., 2016).

Taxonomic characters: Colonial form diameter is 23.4 µm, cup shaped chloroplast is present, single, or parietal pyrenoid is visible under microscope, circular in shape, elliptical or oval in shape, unicellular, green in colour, thin cell wall, young cells are spherical in shape Figure 1f.

Geographical distribution: Philippines (Arguelles and Monsalud, 2017).

Local distribution: Kabul River (Barinova *et al*., 2016), Sindh (Leghari *et al*., 2001), Tiruchirappalli (Mubarak *et al*., 2012), Islamabad (Valeem and Leghari, 2013), Swat (Ali *et al*., 2010).

7. *Westella botryoides* (West) De Wildeman 1897: 532, (Rai and Rai, 2018).

Colony of cells, single chloroplast is present, pyrenoid is not visible under microscope, cells are spherical in shape, cell wall is present, width is 31 µm, light green in colour, cells are arranged in groups Figure 1g.

Geographical distribution: Kerala (PS and Johan, 2020), Malaysia (Omar *et al*., 2016), India (Ja and Chandrab, 2012).

Local distribution: Islamabad (Valeem and Leghari, 2013), Swat (Ali *et al*., 2010).

8. *Pediastrum tetras* var. *tetraodon* (Corda) Hansgirg 1888: 112 (Rai and Rai, 2018)

Taxonomic characters: Colony of cells present in circular form, colony of 8 cells, intercellular spaces are not present in cell, diameter is 23 µm, 11 µm broad, single incision, double lobes present in cell, without mucilage, pyrenoid is not observed under microscope Figure 1h.

Geographical distribution: India (Patil *et al*., 2012).

Local distribution: Karachi (Aliya *et al*., 2009), Sindh (Leghari *et al*., 2001), Rawalpindi (Ahmed *et al*., 2016), Swat (Ali *et al*., 2010).

9. *Coelastrum pseudomicroporum* Korshikov 1953: 348 (Ramos *et al*., 2015).

Taxonomic characters: Each cell attached with protrusion of cell wall, plate-like structure, cells are spherical in shape, chloroplast is present, single pyrenoid, 28.6 µm width, without mucilage, colonies of cells range from 8 to 32 cells Figure 1i.

Geographical distribution: Kerala (PS and Johan, 2020).

Local distribution: New to Pakistan.

Novelty: *Coelastrum pseudomicroporum* has not been isolated and describe previously from Pakistan and it is the new addition to algal flora of Pakistan.

10. *Coelastrum indicum* W.B.Turner 1892: 161 (Ramos *et al*., 2015)**.**

Taxonomic characters: Cells attached with protrusion of cell wall, without mucilage, colonies of cell range from 8-32 cells, single pyrenoid, chloroplast is present, width 22 µm, without mucilage, spherical cells, free-living, colony have triangular holes Figure 1j.

Geographical distribution: South Korea (Kim, 2018), Bangladesh (Khondker *et al*., 2007).

Local distribution: Baluchistan (Aga *et al*., 2018).

11. *Scenedesmus hystrix* Lagerheim 1882: 62, (Beherepatil and Deore, 2013).

Taxonomic characters: Colony of 2 cells, arranged in a linear shape, cells oblong or cylindrical, cell wall covered with minute spines, 12-14µm ling, 4-5µm wide, rounded ends Figure 2a.

Geographical distribution: Eastern Nepal (Rai, 2013a), India (Patil *et al*., 2012), India (Patil and Saner, 2021).

Local distribution: Karachi (Aliya *et al*., 2009).

12. *Scenedesmus denticulatus* var. *linearis* Hansgirg 1888: 268

Taxonomic characters: Colony of 4 cells, cells are cylindrical and ovid, cells with rounded ends and arranged in linear shape, cells having straight spines, 4.5-5 µm broad, 8 µm long, cells arranged slightly in

zigzag pattern, dented projection at both ends of the cell Figure 2b.

Geographical distribution: India (Patil *et al*., 2012; Das and Keshri, 2015).

Local distribution: Karachi (Aliya *et al*., 2009), Sindh (Leghari *et al*., 2001).

13. *Scenedesmus bijuga* (Turpin) Lagerheim 1893: 158

Taxonomic characters: Colony of 2, 4, 8 cells, rounded apices, cells are ovoid and oblong, without spines, cells are 4.5 µm broad, 12 µm long, all cells in equal length, cells are arranged in a linear row, Internal cells are larger than external ones, internal cells narrower with rounded ends Figure 2c.

Geographical distribution: India (Reddy and Chaturvedi, 2015; Das and Keshri, 2015), India (Rishi *et al*., 2016).

Local distribution: Karachi (Aliya *et al*., 2009), Rawalpindi (Ahmed *et al*., 2016), Swat (Ali *et al*., 2010).

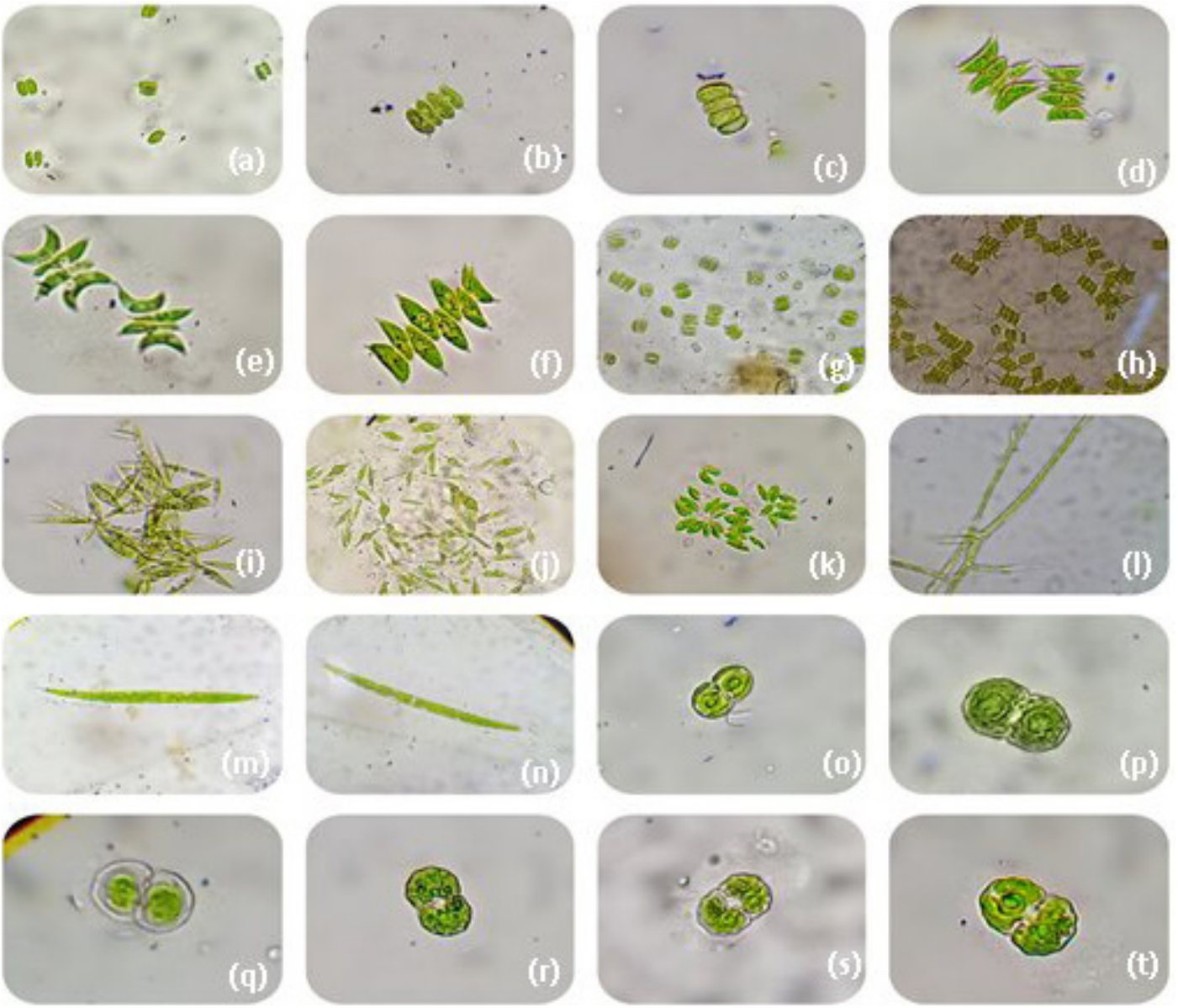

Figure 2: *Scenedesmus hystrix Lagerheim 1882: 62, Scenedesmus denticulatus var. linearis Hansgirg, Scenedesmus bijuga (Turpin) Lagerheim, Scenedesmus acutus var. dimorphus (Turpin) Rabenhorst, Scenedesmus acuminatus (Lagerheim) Chodat, Scenedesmus dimorphus (Turpin) Kützing, Scenedesmus bijugatus (Turp.) Kütz. var. graevenitzii (Bernard) Chodat, Scenedesmus quadricauda var. bicaudatus Hansgirg, Ankistrodesmus falcatus (Corda) Ralfs, Ankistrodesmus braunii (Nägeli) Lemmermann, Acutodesmus obliquus (Turpin) Hegewald and Hanagata, Stigeoclonium attenuatum (Hazen) Collins, Closterium subulatum (Kützing) Brébisson, Closterium dianae Ehrenberg ex Ralfs, Cosmarium granatum Brébisson ex Ralfs, Cosmarium isthmocondrum, Cosmarium lundellii var. ellipticum West and G.S.West, Cosmarium impressulum var. suborthogonum (Raciborski) Taft, Cosmarium crenatum Ralfs ex Ralfs, Cosmarium angulosum Breb.*

14. *Scenedesmus acutus* var. *dimorphus* (Turpin) Rabenhorst (Rai, 2013b)

Taxonomic characters: Colony of 4 cells, cells are in coenobium arranged in an irregular form, cells are curved in shape, internal cells straight, cell wall smooth, without spines, sharp pointed ends, cells are 10 µm long and 2µm broad, finger-like extended Figure 2d.

Geographical distribution: Eastern Nepal (Rai, 2013a), Turkey (Akgul *et al*., 2017).

Local distribution: Karachi (Aliya *et al*., 2009).

15. *Scenedesmus acuminatus* (Lagerheim) Chodat 1902:211, (Hegewald, 1997).

Taxonomic characters: Colony of 4 cells, curved colony, cells with sharp pointed ends, cell wall without spines, 13 µm long, 7.8 µm broad, cells are slightly curved in shape, cells are arranged in a linear or irregular form, single chloroplast, paranoid is visible Figure 2e.

Geographical distribution: Eastern Nepal (Rai, 2013a), India (Patil *et al*., 2012), India (Rishi *et al*., 2016), Turkey (Akgul *et al*., 2017), Malaysia (Omar *et al*., 2016).

Local distribution: Karachi (Aliya *et al*., 2009), Sindh (Leghari *et al*., 2001).

16. *Scenedesmus dimorphus* (Turpin) Kützing 1834: 608

Taxonomic characters: Colony of 8 cells, cells arranged in irregular series, internal cells less curved. cells apices sharp, without spines, external cells more curved in shape, visible paranoids, chloroplast present in the whole cell, cells are 15 µm long, 3.5µm broad Figure 2f.

Geographical distribution: Eastern Nepal (Rai, 2013a), India (Patil *et al*., 2012), India (Rishi *et al*., 2016), Malaysia (Ng *et al*., 2011), Malaysia (Omar *et al*., 2016).

Local distribution: Karachi (Aliya *et al*., 2009), Tiruchirappalli (Mubarak *et al*., 2012), Swat (Ali *et al*., 2010).

17. *Scenedesmus bijugatus* (Turp.) Kütz. var. graevenitzii (Bernard) Chodat (Rai, 2013)

Taxonomic character: Colony of 4 to 8 cells, cells are oblong, ellipsoid and ovoid in shape, without spines, cells arrange in alternate series, Cells width 3.7-5 µm, 10-12.5 µm length Figure 2g.

Geographical distribution: Nepal (Rai, 2013), India (Patil and Saner, 2021).

Local distribution: Tehsil Landikotal (Khan *et al*., 2017), Sindh (Leghari *et al*., 2001), Baluchistan (Aga *et al*., 2018).

18. *Scenedesmus quadricauda* var. *bicaudatus* Hansgirg 1890: 9 (Jena and Adhikary, 2007)

Taxonomic characters: Colony of 2, 4, 8 cells, cells arranged in a linear shape, cells are rigid at the middle, long spines, cells are 2.5µm broad, 13 µm long, and spines are 17 µm long, Spines are larger than cells, Cells are ovoid or cylindrical in shape, Internal cells have no spines, smooth cell wall Figure 2h.

Geographical distribution: Eastern Nepal (Rai, 2013), India (Patil *et al*., 2012), India (Reddy and Chaturvedi, 2015), India (Rishi *et al*., 2016), Turkey (Akgul *et al*., 2017), Malaysia (Ng *et al*., 2011), Malaysia (Omar *et al*., 2016).

Local distribution: Karachi (Aliya *et al*., 2009), Tiruchirappalli (Mubarak *et al*., 2012), Swat (Ali *et al*., 2010).

19. *Ankistrodesmus falcatus* (Corda) Ralfs 1848: 180, (Jena and Adhikary, 2007)

Taxonomic characters: Cells needle to spindle in shape, cells are in clusters, colony of 2-32 cells, without mucilage sheath, no visible pyrenoids, chloroplast is parietal, 32 µm long, 2 µm broad, straight, tapering ends, acute apices Figure 2i.

Geographical distribution: India (Das and Keshri, 2015), India (Ja and Chandrab, 2012).

Local distribution: District Dir lower (Shuaib *et al*., 2017), Sindh (Leghari *et al*., 2001), Rawalpindi (Ahmed *et al*., 2016), Mardan (Mursaleen *et al*., 2018), Islamabad (Valeem and Leghari, 2013), Swat

(Ali *et al*., 2010).

20. *Ankistrodesmus braunii* (Nägeli) Lemmermann 1908: 168

Taxonomic characters: Cells are solitary, no visible pyrenoids, pointed ends, chloroplast scattered at the center of the cell, cells are straight or in a curved shape, 2-5 µm long and 4.5 µm broad, cells are relatively broad, lateral view convex but irregularly Figure 2j.

Geographical distribution: Philippine (Arguelles and Monsalud, 2017), Ain Helwan (Shanab, 2006).

Local distribution: District Dir (Yaseen *et al*., 2016).

21. *Acutodesmus obliquus* (Turpin) (Hegewald and Hanagata, 2000).

Taxonomic Characters: Colony of 2-32 cells, pyrenoids are visible, pyrenoid is present at the center of the chloroplast, cells are in leaf-shaped, 26 µm long, 7.1 µm broad, coenobium is flower-shaped, pointed ends Figure 2k.

Geographical distribution: India (Reddy and Chaturvedi, 2015; Das and Keshri, 2015).

Local distribution: New to Pakistan.

Novelty: *Acutodesmus obliquus* has not been isolated and describe previously from Pakistan and it is the new addition to algal flora of Pakistan.

22. *Stigeoclonium attenuatum* (Hazen) Collins 1909: 301 (Bhakta *et al*., 2011).

Taxonomic characters: Filamentous algae, light green in colour, main filaments form branches, end of filaments have pointed ends, width of the main filament is 7.8 µm, width of branches is 5.2 µm, branches are alternate, sharp ends, cells are present in the main axis Figure 2l.

Geographical distribution: Tajikistan (Barinova and Niyatbekov, 2018).

Local distribution: Mardan (Mursaleen *et al*., 2018), District Dir (Yaseen *et al*., 2016).

23. *Closterium subulatum* (Kützing) Brébisson 1856: 154

Taxonomic characters: Curved shaped body, spindle-shaped ends, pyrenoid is present, cell wall smooth, chloroplast is present, ends are colourless, 13 µm width, 247 µm length, without mucilage, terminal vacuoles, several granules are present in vacuoles Figure 2m.

Geographical distribution: Lake Taabo (Groga *et al*., 2014), Bangladesh (Chakraborty *et al*., 2020), Bengal (Arpana, 2010), Oklahoma (Taft, 1931).

Local distribution: Peshawar (Sarim and Faridi, 1976).

24. *Closterium dianae* Ehrenberg ex Ralfs 1848: 168, (Rai and Rai, 2018).

Taxonomic characters: Body is slightly curved, rounded ends, pyrenoid is present, 273µm length, 13 µm width, chloroplast is present, empty spaces are present in chloroplast, attenuated toward apices, rounded apices, smooth cell wall, axial chloroplast is present Figure 2n.

Geographical distribution: India (Patil *et al*., 2012), Paranapanema river (Felisberto *et al*., 2014), Bangladesh (Chakraborty *et al*., 2020).

Local distribution: Rawalpindi (Ahmed *et al*., 2016), Swat (Ali *et al*., 2010).

25. *Cosmarium granatum* Brébisson ex Ralfs 1848: 96 (Leghari, 2001b).

Taxonomic characters: Pyrenoid is visible, one pyrenoid per semi-cell, 23µm long, and 18 µm broad, no mucilage is present, and 2 semi cells are connected from the base, deeply constricted in the middle, linear sinus, closed, lateral view elliptical in shape, cells are 1.5 times longer than broad Figure 2o.

Geographical distribution: India (Bansod and Patil, 2019), Brazil (Ramos *et al*., 2021).

Local distribution: Kabul River (Barinova *et al*., 2016), Rawalpindi (Ahmed *et al*., 2016), Mardan (Mursaleen *et al*., 2018), Tiruchirappalli (Mubarak *et al*., 2012), Swat (Ali *et al*., 2010).

26. *Cosmarium isthmocondrum* (Aquino *et al*., 2016)

Taxonomic characters: Visible pyrenoid, 15.5 µm long, 13 µm wide, semi-cells oblong, ornamented

with 2 granules, longer than wide, granules are more prominent, present at the center, chloroplast is granulated, semicircular semi cells Figure 2p.

Geographical distribution: Brazil (Aquino *et al.*, 2016).

Local distribution: New to Pakistan.

Novelty: *Cosmarium isthmocondrum* has not been isolated and describe previously from Pakistan and it is the new addition to algal flora of Pakistan.

27. *Cosmarium lundellii* var. *ellipticum* West and G.S. West 1894: 5

Taxonomic characters: Isthmus 6 µm long, pyrenoid is present at the center of the chloroplast, 37 µm long and 23 µm wide, constricted from the deep median, variable in shape, Semi-cells are hemispherical, spherical, rectangular, flattened Figure 2q.

Geographical distribution: India (Mhaske and Talwankar, 2018; Misra *et al.*, 2008), Rajasthan (Barupal, 2019), Arunachal Himalayas (Barupal, 2019).

Local distribution: Sindh (Leghari, 2001b).

28. *Cosmarium impressulum* var. suborthogonum (Raciborski) Taft 1945: 195

Taxonomic characters: Cells are 1.5 times longer than broad, deeply constricted in the middle, linear sinus, semi-cells rectangular in shape, cell wall thicker, apical view is elliptical in shape, lateral view is circular, one pyrenoid is present in the chloroplast, length is 29µm, width is 18 µm, Isthmus is 6 µm Figure 2r.

Geographical distribution: Brazil (Taniguchi et al., 2003).

Local distribution: Sindh (Leghari, 2001b).

29. *Cosmarium crenatum* Ralfs ex Ralfs 1848: 96 (Aquino *et al.*, 2016)

Taxonomic characters: Semi cells pyramidal, pyrenoid is not visible, chloroplast present in partial, isthmus 6 µm, 25 µm long and 16 µm broad, deep median constriction, cells are in medium size, cells are longer than wide Figure 2s.

Geographical distribution: Brazil (Aquino *et al.*, 2016).

Local distribution: Kabul River (Barinova *et al.*, 2016), Swat (Ali *et al.*, 2010).

30. *Cosmarium angulosum* Breb (Vijayan *et al.*, 2015)

Taxonomic characters: 36 µm long, 19 µm broad, 5 Isthmus, no mucilage sheath, pyrenoid is present at the center of the chloroplast, 1 pyrenoid, deeply constricted in the middle, linear sinus, lateral margins are smooth, straight, basal angle are rectangular, lateral view oblong Figure 2t.

Geographical distribution: India (Misra *et al.*, 2008).

Local distribution: Karachi (Aliya *et al.*, 2009), Kabul River (Barinova *et al.*, 2016).

A total of thirty different species of green algae were found to be unicellular, colonial, filamentous, branched filamentous and irregular forms. All of these were collected from different habitats. This is the first taxonomic investigation of green algae from different habitats including streams, ponds, ditches, and tube well.

The current study revealed that Gujar Khan has high algal diversity. Similarly, (Khan *et al.*, 2011) considered morpho-ordered portrayal of 73 crisp water green algae in Kalpani stream and connected range of area Murdan, with 34 genera, 25 families, 17 orders, and 9 classes. Out of it, 65.75% belong to the Chlorophyta family. Another study revealed 138 Chlorophycean species. A total of 74 species (53.6%) belong to the Chlorococcales family. Cladophorales and Chaetophorales both a have 3% total diversity (Ali *et al.*, 2010). Moreover, (Leghari, 2001a) described green filamentous algae from Sindh's lakes and ponds revealing 31 kinds of Chlorophyta from new water and Riverin lakes.

The genus *Scenedesmus* was dominant in all ponds and ditches. The presence of *Scenedesmus* sp. indicates that ponds and ditches have a high level of organic compounds and nutrient content because species of this genus prefer these conditions for their growth. These species are thought to be indicators of highly

organic contaminated water. In some studies, similar results were explained about the *Scenedesmus* sp. diversity in highly contaminated organic water (Verma *et al*., 2012) and (Singh *et al*., 2013).

Results of our study showed that all 30 species were found in polluted and non-polluted water. From these species *Chlamydomonas reinardtii, Acutodesmus obliquus* and *Cosmarium isthmocondrum* found in our collection, are being reported here for the first time from Pakistan. These sites have some common species and have unique adaptive features which make them able to survive in both types of conditions. Similar studies were conducted by Barinova *et al*. (2006a) and explored 145 algal species from Alexander River (Central Israel), and Barinova *et al*. (2006b) reported 126 algal species from Hadera River, Israel depicting that the algae are the indicators of environmental conditions.

It is primarily of ecological, biotechnological, and commercial importance to isolate, cultivate and identify green algae from different habitats. The present work also investigated the response of green algae on three different media namely, BG-11 BBM and MBBM. Results of media response showed that BBM and MBBM media support better growth as compared to BG11 media. In this study, green algae were isolated using BBM, a standard chemically defined medium. In this medium, we were able to isolate thirty different species of green algae. We recognize that some genera are difficult to cultivate in this medium, thus we claim that our study is representative of Gujar Khan's overall green algal biodiversity (Lloyd *et al*., 2021). The study revealed that sampling areas with varied ecological variables play important role in influencing the distribution and diversity of subaerial algal communities. Hence, most of the readily responding types are potential candidates for biomass production and research for high value compounds deemed industrially important.

Conclusions and Recommendations

The present study depicts that Tehsil Gujar Khan, District Rawalpindi is a rich source of freshwater green algae. The most dominant genus in this region is *Scenedesmus* and the 2nd most dominant genus is *Cosmarium*. Species of other genera like *Chlorococcum*, *Ankistrodesmus, Coelastrum, Closterium*, *Chlamydomonas, Eudorina, Tetraspora, Chlorella, Westella, Pediastrum, Acutodesmus,* and *Stigeoclonium* also prevail in this area. Three species *Chlamydomonas reinardtii, Acutodesmus obliquus* and *Cosmarium isthmocondrum* are new record to the algal flora of Pakistan. From this research it is also inferred that green algae prefer culturing on BBM and MBBM. In the district of Rawalpindi many other aquatic sites are that are yet to be explored. Exploration of remaining sites would provide a chance to record new species that might be new to alga flora of Pakistan or new to algal flora of the world. As a result, it is important to maintain algal diversity in local habitats and to conduct more systematic research on them which can only be done after the understanding of ecology and habitats of distinct algal flora. These discoveries will be useful in future research to explore freshwater green algae in Gujar khan, District Rawalpindi.

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Novelty Statement

Chlamydomonas reinardtii, *Acutodesmus obliquus,* and *Cosmarium isthmocondrum* has not been isolated and described previously from Pakistan and it is a new addition to the algal flora of Pakistan.

Author's Contribution

Abdul Samad Mumtaz: Supervised the research project and provided laboratory facilities.
Lubna Anjum Minhas: Original concept, study design, performed microscopy drafting, editing and finalizing manuscript.
M. Kaleem: Isolation and culturing experiments.
J. Annum: Material and field data collection from Rawalpindi and suburbs.
R. Waqar: Assisted in species identification, data collation and analysis.

Conflict of interests
The authors have declared no conflict of interest.

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