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Original Research Article

Morpho-anatomical characters of few members belonging to Kingdom Protista from Safari Park, Lahore, Punjab, Pakistan

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Abstract

The present study was carried out on Safari Park, Lahore, Punjab, Pakistan. The sampling was carried out randomly in different seasons from different water bodies of the park especially from Lake. Thirty-seven species belonging to six genera i.e., *Pediastrum* (4 species & 11% abundance), *Trachelomonas* (4 species & 11% abundance), *Euglena* (11 species & 30% abundance), *Phacus* (12 species & 32% abundance), *Lepocinclis* (1 species & 3% abundance) and (5 species & 13% abundance). All genera belong to kingdom Protista, two phyla i.e., Volvocophycota and Euglenophycota, two classes i.e., Volvocophyceae & Euglenophyceae, two orders chlorocoocales and Euglenales and three families i.e., Hydrodictyaceae, Euglenaceae and Naviculaceae.

Keywords: Safari Park, Euglena, Phacus, Trachelomonas, Pediastrum, Lepocinclis, Navicula.

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INTRODUCTION

Lahore is the capital city of Punjab, Pakistan. Punjab is the largest province of Pakistan and is rich in a variety of water bodies such as rivers, canals, irrigation channels, rice fields, lakes and rain pools (Ali et al., 2010a). It is located at latitude 31°3259 N and longitude 74°2037 E. Its elevation is 217 m (712 ft). Climate of Lahore is hot semiarid. Ghose (1924) worked on the algal flora of Punjab province. He described number of blue green algae from Lahore (Ghose, 1924). Later on, Masud-ul-Hasan along with different workers have reported some algae including Cyanophyta from ponds of Lahore and adjoining areas. Nadeem-Ullah & Butt, 2017 on the basis of morphological and cytological characteristics, identified fifty-nine species belonging to Kingdom Monera. Mukhtar et al., 2021 reported 23 algal species belonging to 14 genera from different sites of district Lahore. Ecologically algae play a significant role in the nitrogen fixation, which takes part in increasing fertility of soil (Ali et al., 2010b). Algae are good indicator for pollution (Patrick & Reimer, 1966). They are one of the most important indicators of water quality because of their rapid reaction to environmental changes related to large animals and plants. Algae are the initiators of food chain (Ali et al., 2010b). So, by keeping in mind the necessity of the taxonomic studies of algal

species now a day, we collected algal samples from Safari Park Lahore, to studying morphological and cytological characters of different algal species (Nadeem-Ullah & Butt, 2017).

MATERIAL AND METHODS Collection site

Algal sample was collected to investigate/identified algal taxa up to species level from selected sites. The area includes Safari Park, Lahore which is located at latitude 31°22'50" N and longitude 74°13'05" E on Raiwind Road, Lahore.

Collection of material

Samples were collected with hands from different habitats of Safari Park. The samples were placed in plastic bottles and labelled with the help of permanent marker according to dates, sampling sites and sample numbers, for analysis of physiochemical variables. The sample bottles were filled as much necessary for investigation. Identification up to species level was done with the help of microscopic study.

Field Observation

Selected sites physical characters i.e., temperature, pH, GPS and EC were measured on spot to

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Preservation of the material

The samples were divided into two bottles, one of them preserved with 3-4% formalin (Aqueous solution of formaldehyde) by taking the intervals of 15 days while other was placed as such in the air for fresh algal sample. Then, transparent bottles containing preserved algal samples were placed at cool and dry place at room temperature.

Study of Material in the Laboratory

The research on algal taxonomy was carried out in the CBC laboratory at University of Central Punjab, Lahore. Collected samples were studied under microscopes for taxonomic identification. Samples were settled and upper layer is removed by siphoning process. With the help of forceps and needles, algal sample were taken from the preserved bottle and then placed onto the clean glass slide. The material was mounted and spread properly on the glass slide with the help of forceps. After that, coverslip was placed on the material and excess water was removed gently pressing the coverslip and was absorbed with the help of blotting paper. The material was observed and studied under the low and high magnification of the light microscope.

Identification of the collected material

The collected samples were identified by using T.V. Desikachary (1959) and Presscott (1962). The taxonomic description of collected species of algae was carried out after survey with the help of standard literature and monographs e.g., Cooke, (1882-1884); Randhawa, (1936); Kützing, (1895); Tiffany and Britton, (1952); Masud-ul-Hassan, (1978); Masud-ul-Hassan and Yunus, (1989), Nadeem-Ullah *et al.*, (2021) etc.

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Figure 1: Pediastrum tetras	Figure 2: P. boryanum	Figure 3: <i>P. duplex</i>	Figure 4: <i>P. granulatum</i>
Figure 5: Euglena promixa	Figure 6: Euglena minima	Figure 7: Euglena gracilis	Figure 8: E. amphigyrenica

Figure 9: Euglena velata	Figure 10: E. polymorpha	Figure 11: E. limnophilia	Figure 12:
The second	Tigate Will Poymorphic		
Figure 13: Euglena viridus	Figure 14: <i>E. cantabrica</i>	Figure 14: E. cantabrica Figure 15: E. sanguinea	
Figure 17: Trachelomonas volvocina	Figure 18: T. hispida	Figure 19: T. volzii	Figure 20: T. rugulosa
Figure 21: Lepocincilis texta	Figure 22: Phacus pyrum	Figure 23: P. caudatus	Figure 24: P. acuminatus

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Figure 25: P. longicaudata	Figure 26: P. alatus	Figure 27: P. ankylonoton	Figure 28: P. curvicauda
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Figure 29: P. hamatus	Figure 30: P. minutus	Figure 31: P. sesquitortus	Figure 32: P. acuminatum

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Figure 33: P. viguier	Figure 34: Navicula salinarum	Figure 35: N. menisculus	Figure 36: <i>N. gregaria</i>
Figure 37: N. tripunctata	Figure 38: N. pelliculos		

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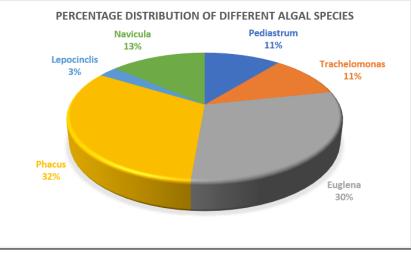


Figure 39: Percentage distribution of different algal species

RESULTS AND DISCUSSION

For the identification and taxonomic study of algae with their physico-chemical properties algal samples were collected from Safari Park, Lahore, Punjab. Identification and taxonomic study were based on morphological and cytological characteristics up to the species level. In this studies, 4 families, 6 genera and 39 species were identified.

Kingdom protista

Order Chlorococcales

Solitary or colonial; cells spherical, ovate, pyramidal or polygonal; reproduce by autospore formation.

Family Hydrodictyaceae

Coenocytic distinct members, various forms, mostly free floating some tychoplankton; cells cylindrical, triangular or cylindrical, form net or spherical coenobia, 2-64 cells or sometimes hundreds in a colony; chloroplast parietal with on to several pyrenoids; asexual reproduction is by division of parent colony in daughter colonies; sexual reproduction is by isogamy, gametes are biflagellate.

Pediastrum Meyen

Colony is flat, cushion-like, made up of multinucleate cells, arranged in a regular, geometrical fashion to form a circular plate; the cells being in multiple of two. The peripheral cells may be polyhedral and uni, bi, tri or tetra-lobed and somewhat different in shape from the internal cells. The colony may be continuous or perforate with interstices. There is a parietal, diffused chloroplast, with one pyrenoid and several nuclei. Besides multiplying by auto colonies, isogametes are produced in sexual reproduction.

Key to species:

- 1. Up to 16 cells per colony......2

 More than 16 cells per colony......3
- Diameter of colony up to 25 μm..... P. tetras Diameter of colony up to 85 μm.... P. granulatum
- 3. Cells walls sculpture*P.duplex* Cells walls granular.....*P. boryanum*

Name of species	Coenobia	No of cells	Diameter of cells	Marginal cells	Inner cells Diameter	Cell wall
P.tetras	Coenobia without holes	4-16	23.9 to 30.9 µm	7.4-8.7×9.8-12.4 μm	6.9-7.4 μm	Smooth
P. boryanum	Coenobia without holes	32	39.5-170 μm	6.2-12.3×10.0- 15.0 μm	7.4-34×4.9- 22.5 μm	Scarcely granular
P. duplex	Coenobia Circular	32-64	44.5-222.2 μm	7.4-19.8×10.0- 24.7 μm	10.0-12.5 μm	Fine Sculpture
P. granulatum	Coenobia circular	16	69.7-84.6 μm	18.9-23.5×17.9- 21.0 μm	12.3-18.2× 13.6-19.9 μ	Dense regular, granulation

Table 1: Morphoanatomical characters of different species of *Pediastrum*

Phylum euglenophycota

Pigmentation: Chlorophylls a & b; flagellation: two basal bodies, one or two, emergent, apical flagella, one with a spiraled row of fibrillar hairs; storage product: paramylon; cell wall constituents 80 % proteins, 11.6% lipid, 8.4% carbohydrates; proteinaceous pellicle in strips under the plasma membrane; mesokaryotic nucleus, characteristic type of cell-division, no sexual reproduction.

Class Euglenophyceae

Protozoan like protests; freshwater, sedentary or motile by 1-2 stout flagella; gullet and complex

vacuolar system present; chloroplast and pyrenoids present.

Order Euglenales

Possess paramylon a starch loke food reserve, reproduction by longitudinal division of the cell.

Family Euglenaceae

Chlorophyll localized in chloroplasts which may be disc shape, ribbon-like stellar; pyrenoids on chloroplasts; food reserve is paramylon; most members uniflagellate few are biflagellate.

Key to Genus:

- 3. Plastids without pyrenoids.....*Phacus* Plastids with pyrenoids....*Lepocincils*

Trachelomonas Ehrenberg

In this genus euglenoid cells are enclosed in a firm gelatinous shell which has an opening for the

flagellum. The shell or test has an almost endless variety of shapes and forms of decoration, and since these features are specific the taxonomy of the genus is based upon characteristics of the test rather than on those of the protoplast. The test is brown, often opaque, or tan to nearly colorless, according to the amount of iron compounds deposited in it. The test may be smooth or decorated with spines, warts, reticulations, punctations or combinations of these. The protoplast inside is highly metabolic and has the general features of the euglenoids. There is 1 flagellum, a red pigment-spot, and numerous ovoids, disc-like chloroplasts which may have pyrenoids. Reproduction is by cell division, which takes place within the test, one of the new cells escaping through the aperture and secreting its own shell.

Key to species:

1.	Lorica are round	2
	Lorica is long	3

- 2. Apical pore without collar.....*T. volvocina* Apical pore with annular thicking.....*T. rugulosa*
- 3. Length is 27.0-39.5.....*T. hispida* Length is 16.5-21.0....*T. volzii*

Name of species	Lorica shape	Apical pore	Ph	Season Of collection
T.volvocina	round to slightly oval	Apical pore without collar	7.80	Spring
T.hispida	Long	Apical pore without collar	7.57	Spring
T.volzii	long, elliptical	Apical pore with collar	7.80	Spring
T.rugulosa	Round	Apical pore with annular	7.35	Spring
		thickening		

Table 2: Morphoanatomical characters of different species of Trachelomonas

Euglena Ehrenberg

Cells elongated, fusiform or nearly cylindrical; round or somewhat flattened; chloroplast oval plates or rarely diffuse or ribbon like; paramylum in the form of one or two large or several small rods or sticks; tail piece present, formed by the gradual narrowing of the cell.

Key to species:

- 2. Cells are ovoid to suborbicular......3 Cells are not ovoid to subcylindric......5
- 4. Cell diameter 28-33 µm.....E.sanguinea

	Cell diameter 14.5-19 µmE.proxima
5.	Paramylon grains are not presentE. gracilis
	Paramylon grains are presentE.texta
6.	Cell are spindle7
	Cell are non-spindle9
7.	Flagella is withdrawnE. minima
	Flagella are present8
8.	Chromatophores are ribbon likeE. viridius
	Chromatophores are flat or stellateE. velata
9.	Cells are not cylindricalE.
	<i>limnophilia</i> Cells are cylindrical10
10.	Cell length 100-210µmE.
	amphigyrenica Cell length 36-84 mE.
	cantabrica

Table 3: Morr	ohoanatomical	characters of	of different s	pecies of Euglena
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Name of species	Shape of cell	Paramylon bodies	Length	pН	Pyrenoid
E. promixa	Metabolic	Numerous	50-95 μ	7.80	
E. minima	Sindle	Distinct	12-14 μm	7.35	Present
E. gracilis	Metabolic	Not observed	37-50 μ	7.35	Present
E. amphigyrenica	Cylindrical		100-210µm	7.80	
E. velata	Spindle	Varying number	98-100µm	7.80	Present
E. polymorpha	Metabolic	Small ovoid grains	80-90 μ	7.80	Present
E. limnophilia	Fusiform	Dimorphic	84-97 μm	7.80	Absent

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Name of species	Shape of cell	Paramylon bodies	Length	pН	Pyrenoid
E. viridius	Spindle to cylindrical	Numerous	78-80 μm	7.80	Present
E. cantabrica	Fusiform	Cluster	36-84 m	7.35	Present
E. sanguinea	Metabolic	Several	55-120 μ	7.35	
E. texta	Metabolic	Numerous	33-48 pm	7.35	Absent

Phacus Dujardin

Cells more or less flattened with a rigid pellicle, cup shaped or twisted. Periplast striations are always present and are quite distinct. Cells have a posterior spine or tail which is curved and pointed or may be straight and long. The plastids are in the form of small discs without pyrenoids. One long flagellum emerges out through the canal, other one small and emergent.

Key to species:

1.	Cells less than 50 µm long3
	Cells above than 50 µm long2

- 2. Width of celss above than 40 μm.....*P. caudatus* Width of cells upto38 μm....*P. longicauda*
- 3. Cells oval in shape.....4 Cells ovoid in shape.....5

4.	Cell more than 35 µm in width8	
	Cell less than 35 µm in width9	

- Tale piece tapering......P. pyrum Tale piece of anyother shape......6
 Cells upto 25 μm in width.......7 Cells more than 25 μm in width......P. minutus
 Tale piece short.....P. viguier
- Tale piece curved......P. alatus

 8. Chloroplast numerous per cell......10

 Chloroplast few per cell......11
- Cells upto 60 μm long......P. hamatus Cells above than 60 μm long.....P. sesquatortus
- 10. Cell ovali shape..... *P. acuminatus* Cell broadly ovoid to sub-orbicular...*P. curvicauda*
- Cell length upto 30 μm.....P. acuminatum Cell length upto 50 μm.....P. ankylocnoton

Table 4: Mor	ohoanatomical	characters of	different s	pecies of <i>Phacus</i>
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Name of Species	Cell Shape	Length	Periplast	pН
P. pyrum	Ovoid	28-30 µm	Spirally striated	7.57
P. caudatus	Oval	90-96 µm	Longitudinally striated	7.57
P. acuminatus	Oval	25-30 µm	Longitudinally striated	7.80
P. longicauda	Oval	70-85 µm	Longitudinally striated	7.80
P. alatus	Ovoid	19-24 µm	Longitudinally striated	7.80
P. ankylonoton	Oval	20-30 µm		7.80
P. curvicauda	Ovoid to suborbicular	25-30 µm	Longitudinally striated	7.80
P. hamatus	Ovoid to ellipsoidal	50-60 µm		7.80
P. minutus	Ovoid	20-28 µm	Longitudinally striated	7.80
P. sesquitortus		75-85 µm	Longitudinally or spirally striated	7.80
P. acuminatum	Oval	25-30 µm	Longitudinally striated	7.45
P. viguier	Ovoid	28-32 μ		

Lepocinclis Perty

Cells ovoid, ovate, elliptical or fusiform, sometimes nearly spherical, with a firm and usually spirally striated periplast, round in cross section; posteriorly extended into an abruptly pointed tail-piece (rarely gradually tapering); a gullet in the anterior end where there arises a single flagellum that is once or twice the cell in length; chloroplasts numerous parietal discs; pigment-spot laterally placed in the anterior region; reserve food in the form of 2 large, lateral paramylon rings, the 2 together sometimes nearly encircling the cell.

L. texta (Dujardin) Lemmermann emend. Conrad Reference: Salla, (2018).

General Character

Obovoid to ovoid cells are 43-65 x 32, 2-40,7 μ m in size. The apex is asymmetrical, with lateral depression, while the posterior end is enlarged, with or without a caudal appendage. The taxon is cosmopolitan. In Africa, it has been identified in the following

countries: Burkina Faso and Burundi. It is sampled at the Abou station.

Locality: Safari (site 2)

Remarks: The algal species were collected from safari stagnant water with temperature 33.2°C and pH 7.35 was observed.

Family Naviculaceae

Frustules of values are symmetrical at both axes. Valve may be elliptical, lanceolate or boat shaped in outline. The sagittal axis is usually linear; it may be sigmoid. Each valve has a raphe with distinct central and polar nodules. Valves have transverse or striae. There were two laminate chromatophores in each specimen.

Navicula Børy de Saint-Vincent

Frustules free-floating, solitary or in colonies; girdle straight, rectangular, valve linear, lanceolate or fusiform; ends capitate, rounded or rostrate, raphe straight with central and polar nodules; striae or costae transverse, parallel or radiate, axial area narrow, linear; chromatophores two, laminate, lie on both sides of the valve with a bridge like connection in the middle, sometimes they are split up into numerous small, rounded granules.

Key to species:

- 1. Striae are lineate.....N.salinarum

 Striae are radiate......2
- 2. Raphe system on both valves.....*N. pelliculos* Raphe is straight or lateral......3
- 3. Central area is about one half.....*N.meniculos* Central area is elliptical......4
- 4. Areolae is 25-32 in 10 μm.....*N.gregaria* Areolae in 30-32 in 10 μm....*N.tripunctata*

Name of species	Shape of Valve	length	Width	Striae in 10 µm	pН
N.salinarum	Elliptical to lanceolate	23-35µm	8-9 µm	13-17	7.57
N. menisculus	Lanceolate	11-25 µm	6-7.5 μm	10.5–15	7.57
N. gregaria	Lanceolate	16-35 µm	4.1-7.0 μm	14–18	7.35
N.tripunctata	Linear-lanceolate	32-60 µm	6–10 µm	9–12	7.35
N. pelliculos	Elliptical	9-12µm	4-6µm	70-80	7.35

DISCUSSION

From the above study it was concluded that most of the species of *Euglena* and *Phacus* remain dispersed through out the year in different environmental and climatic conditions with 30 and 32% abundance respectively. Summer and rainy seasons are the most favorable for the growth of these genera. Similar kinds of results were discussed by Nadeem-ullah *et al.*, 2021. Nadeem-ullah *et al.*, 2021 postulated that most of the members of family Euglenaceae are the indication of polluted water and was water quality tests prove that lake water of Safari Park, Lahore is slightly Eutrophic water. proved that *Lepocinclis* was found with least growth having only one species with just 3% abundance and it was observed in slightly alkaline pH and moderate temperature.

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