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Identification and enlisting characteristics of algae collected from tehsil Kabal, district Swat, Pakistan

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ABSTRACT

In this modern era, the energy crisis is one of the major issues most countries including Pakistan are facing. Globally, scientists are shifting to find out sustainable and eco-friendly ways and methods to encounter this issue. A large number of benefits can be extracted from the algae generally found in every aquatic habitat. Algae are a heterogeneous group of first oxygen-evolving aquatic autotrophic thallophytes which lack a sterile jacket around their reproductive cells. Microalgae, being microbes undergo vegetative, asexually and sexually reproduction. Due to this, a higher growth rate and biomass are produced in short time duration. This biomass being rich in lipid and oil content can further be processed for biofuel production. Samples of algal species were collected from different regions of Tehsil Kabal, Swat, Pakistan followed by culturing in BBM (Bold Basal Media) and examined under a microscope. The results showed that Diatoms (Brown Algae) were present ina higher ratio compared to the Green Algae. Some of the prominent diatoms identified were *Synedra ulna, Amphora elliptica, Mastogloia elliptica, Cyclotella atomus, Cymatopleura solea, Nitzcschia frustulum, Nitzcschia radicula, Cymbella aspera, Navicula cuspidate, Coelestrum and Oocystis*. The Green Algae identified were *Spirgyra, Microspora indica, Microspora pachyderma, Scendesmus*. The Blue green alga identified in the sample was Anabaena. The present would be of further help for experimental work on algae as a renewable energy source in the region.

Keywords: Renewable Energy, Biofuels, Algae, Diatoms, Swat

1. INTRODUCTION

Algae according to modern phycologists are a heterogeneous group of first oxygen-evolving aquatic autotrophic thallophytes which lack a sterile jacket around their reproductive cells. They are supposed to be aquatic, oxygen-evolving photosynthetic autotrophs and can be unicellular, colonial, or constructed of filaments or composed of simple tissues [1]. An alga consists of Chlorophyll 'a' as its primary photosynthetic pigment. Algae belong to an important group of organisms that grow in a range of aquatic habitats which includes lakes, ponds, rivers, oceans, wastewater, barks of trees, etc. his group of plants has some unique properties making it distinct from other groups of organisms. An alga contains both Prokaryotic and Eukaryotic groups, huge varieties

of pigment systems, life cycles and a long evolutionary history [2]. With the discovery of Electron microscopes, the characteristics study of algae has shown a lot about the structure of algae. Due to high magnification power, electron microscopy has revealed a lot about the structure, flagella, flagella hair, flagellar swelling, eye spot, chloroplast, endoplasmic reticulum, thylakoid grouping, pit connections, vesicles, etc. A detailed scheme of classification was been proposed by the father of phycology Fritsch in 1935 in his book under the title "The Structure and Classification of Algae". Globally the Fritsch classification system is generally followed with some modification as suggested by the modern phycologists [3].

When it comes to encountering an energy crisis, sustainable approaches are been searched. Algae in this case are the best sustainable source that can be converted into energy (biofuels) which includes biodiesels, bioethanol, etc. It has shown a higher oil yield (non-edible) due to its fast growth. It is estimated that 50% of the algal weight is due to the oil content it carries and which can be converted into biofuels. Process for the production of biofuels from algae shows resemblance to those which are produced from food and non-food crops. Renewable sources of energy in comparison to fossil fuels are of great importance specifically for sustainable energy development and environmental protection [4]. Algae reproduce by three means i.e., Vegetative (Fragmentation, Hormogonia, Fission, Tubers, Budding, Amylum star, Bulbils, etc.), Asexually (Zoospores, Aplanospores, Hynospores, Akinetes, Endospores, Exospores, Nanospores, Palmella Stage, Autospores, Cysts, Daughter Colonies) and Sexually reproduction including Isogamy, Heterogamy, Conjugation, Autogamy, Hologamy [3].

The recent research is a part of a struggle in which algal species identification along with the study of their characteristics has been made in order to identify the species of algae present in tehsil, Kabal, district Swat, Khyber Pakhtunkhwa, Pakistan.

2. MATERIALS AND METHODS

2.1. Sample collection

In order to collect the algal samples, proper survey was been carried in the village Garri, tehsil Kabal, district Swat, Pakistan. After the survey, suitable locations were marked and then sampling of Algae was carried out at different natural ponds, lakes and standing water. Some algae were collected from the downstream with the help of forceps. Rest of samples were collected by scoops and cans. The sampling was done in a transparent medium size plastic container. Distilled water was added to container in double the mass of the algae. These samples were brought to Biofuel Lab of Dept. of Agricultural Chemistry and Biochemistry with care for identification and further study.

2.2. Identification of Algae

Clean Slides were prepared for the identification of algae by following the standard procedure by Kalina [5]. On a clean slide, a single drop of sample was poured followed by fixing of coverslip and removal of bubbles. Microscopy was carried out for the identification in an efficient and effective way. Numerous species were identified in collected samples out of which 15 were selected for detailed study and culturing in different culture medium for present research. The comparison of the identified specie with manuals of Prescott, (1991) and different online source was done.

2.3. Preparation of Culture Media

2.3.1. Preparation of Bold Basal Media (BBM)

Bold Basal Media is generally used for almost all type of fresh-water algae and hence it was used during this entire experiment. 1 L of BBM media was prepared in which the entire sample were added. In order to prepare

Table 1: The composition of Bold basal medium

S. No.	Component	Amount	Stock Sol (Conc.)
1	$NaNO_3$	10 ml	10g/400mL
2	$CaCl_2.2H_2O$	10 ml	1g/400mL
3	$MgSO_4.7H_2O$	10 ml	3g/400mL
4	K_2HPO_4	10 ml	3g/400mL
5	KH_2PO_4	10 ml	7g/400mL
6	$NaCl$	10 ml	1g/400mL
7	$EDTA$	1 drop	--
8	$Iron\ Stock$	1 drop	--
9	$Boron\ Stock$	1 drop	--
10	$Bold\ Trace\ Stock$	1 drop	--

Table 2. Composition of Trace Stock Solution

S. No	Component	Stock Solution
1	H_2SO_4	10 mL
2	$ZnSO_4.7H_2O$	8.82g/L
3	$MgCl_2.4H_2O$	1.44g/L
4	MoO_3	0.71g/L
5	$CuSO_4.5H_2O$	1.57g/L
6	$Co(NO_3)_{2.}.6\ H_2O$	0.49g/L

the 1 L stock solution of BBM media, standard procedure was followed. The following solutions were prepared and finally diluted to 1 L volume with the help of distilled water (table 1).

2.3.2. EDTA Stock Solution

In order to prepare the EDTA stock solution, 50g of EDTA and 31g of KOH are added to 800mL of distilled water which is finally brought to 1 L and kept in dark.

2.3.3. Iron Stock Solution

For preparation of Iron stock solution, 10ml H_2SO_4 is added in 800 mL water after which 5 g of $FeSO_4.7H_2O$ is added. The solution is brought to the 1 L mark with the help of distilled water and kept in dark.

2.3.4. Boron Stock Solution

11g of H_3BO_3 is added to 900 mL of distilled water which is later brought to the final volume of 1 L.

2.3.5. Bold Trace Stock Solution

Trace elements are the necessary portion of the Bold Basal Medium and hence it can't be ignored. Following is the recipe of its preparation (table 2).

3. RESULTS AND DISCUSSION

It was observed that numerous algae were present in the sample. Out of which 15 were chosen as candidate algal species for the detail discussion. Enlist is the algae which were identified in the microscopy.

3.1. Taxonomical Description of Identified Algae Specimen

Table 3. Systematic enumeration of algae identified

Phylum	Class	Genus	Specie
		Ulnaria	*Synedra Ulna*
		Amphora	*Amphora epliptica*
		Mastogloia	*Mastogloia elleptica*
		Cyclotella	*Cyclotella atomus*
		Cymatopleura	*Cymatopleura solea*
Ochrophyta	Bacillophyceae	Nitzschia	*Nitzschia radicula*
		Cymbella	*Cymbella aspera*
		Navicula	*Navicula cuspidate*
		Coelestrum	Specie Not Found
		Oocystis	Specie Not Found
Chlorophyta	Chlorophyceae	Spirogyra	Specie Not Found
		Microspora	*Microspora indica*
			Pachyderma
		Scendesmus	Specie Not Found
Cyanophyta	Cyanophyceae	Anabaena	Specie Not Found

The description to each of the genus and specie is based on the description available on AlgaeWeb, AlgaeBase and some other notable online sources suggested by Phycological literature (table 3).

3.1.1. Synedra ulna

The specie belongs to genus Ulnaria is a rod-shaped alga having bilateral symmetry. Silica is the main constituent which is present in the walls of algae's diatoms which is overlapping halves. This genus is having unicellular algae which grow in the spray-like clusters. The description of *Synedra ulna* in the literature states that the valves of *Synedra ulna* is linear with gradually reduced to beak-like projection or sliced-shaped end. The central area is no longer than broad while its axial end is narrow. The shape of the specie is found often square to rectangular. It is also observed that short striae on the margins are located in a parallel way. The length of valves in *Synedra ulna* ranges from 50 minimum to 100 µm usually while in some cases it has found up to 300 µm. The breadth is 15-9 µm while striae 11-14 in 10 µm. (figure 1 A). Study have shown that the fatty acid production of this specie is 6.40 (microgram/ml) and is suggested a good specie for biofuel production [6].

3.1.2. Spirogyra

Spirogyra sp. has a very prominent property of spiral chloroplast in its cells. A slimy filamentous mass of algae is been formed after the cell's nurtures. This genus undergoes both sexually and asexually. Smaller pieces can be formed from a filament when it is divided into fragments each one of which will be further capable of forming new cells. Two cells align with each other during sexual reproduction and form conjugation tubes. These are later connected between two cells which allow the exchange of genetic material (figure 1 B). Studies have shown that Spirogyra sp. are generally rich in polyunsaturated FA (PUFA), (Arachidic acid C20: 4, Erucic acid C22: 1 and Docosadienoic C: 22: 2) with a

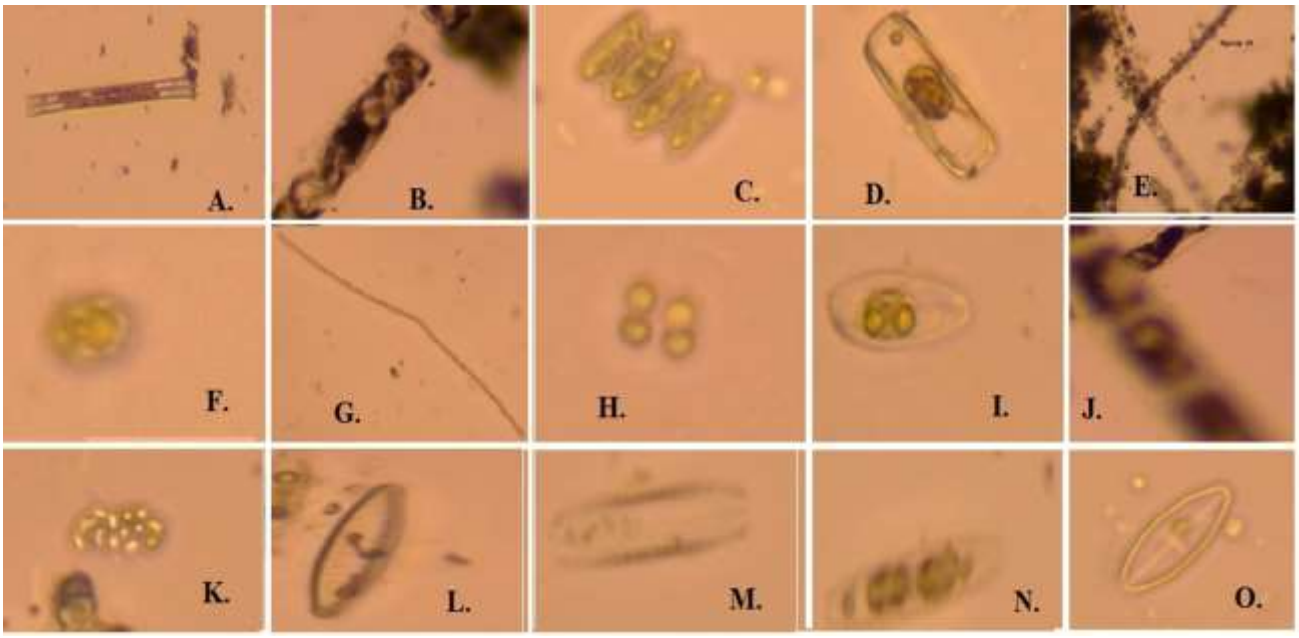

Figure 1. Showing the identifies algea: A. *Synedra ulna*; B. S*pirogyra*; C. *Scendesmus;* D. *Amphora elleptica;* E. *Microspora indica;* F. *Cyclotella;* G. *Anabaena;* H. *Coelestrum;* I. *Nitzschia radicula;* J. *Microspora pachyderma;* K. *Oocystis;* L. *Cymbella aspera;* M. *Cymatopleura solea;* N. *Mastogloia elleptica;* O. *Navicula cuspidata*

concentration of 89.970 µg/ml, 190.860 µg/ml and 101.280 µg/ml, respectively [7].

3.1.3. Scendesmus

The *Scendesmus* genus is mostly oblique single cells which are fused together in rows. The fused cells can range from two to thirty-two. These cells also have spine like appendages on their outermost cells of the colony. These are generally found globally expect from some zones i.e., Arctic and Antarctic. Mostly they are found in freshwater. This Genus plays a vital role in the biodiesel production in the recent years. These algae don't affect the environment when is cultivated in an abundance in a large quantity. A rapid increase in biomass and lipid content were observed in the algae of this genus under Nitrogen Concentration of 0.32 g/L. Hence, It makes it capable to create a biodiesel product. Study has shown that this specie has a potentially active growth rate. Hence, they are used for biodiesel production [8]. These algae are generally found in Freshwater, pond, streams etc. (figure 1 C).

3.1.4. Amphora elleptica

Cells belonging to this specie are solitary that can be motile. The cells appear oblique with flat shorten ends. The cell appears as a large slice of orange. The valves are of asymmetrical geometry. These are sometimes smaller or limited to each end of the cell. Both raphes lie on the same side of the valve. Generally, there are two or more plastids which occur in different positions throughout the cell. These plants are generally seen on stones, in mud and epiphytic on plants. Few species of this genus are freshwater (figure 1 D). Literature has shown under the optimized conditions the maximum lipid concentration were 43.3 ± 4.5 mg L−1 d−1. The fatty acids profile shows palmitoleic acid (39.8%), palmitic acid (31.9%), myristic acid (6.8%), oleic acid (4.7%), stearic acid (4.5%), arachidonic acid (3.9%), eicosapentaenoic acid (3.6%), linoleic acid (2.5%), tetracosanoic acid (1.7%), and linolenic acid (0.6%) [9].

3.1.5. Microspora indica

This specie is having an un-branched filament with cylindrical cell. The cell walls are contracted as they are lamellate. The genus undergoes via biflagellate isogametes. This genus is very useful as it removes the

heavy metals from the contaminated water. These algae are generally found in Freshwater, pond, streams etc. (figure 1 E)

3.1.6. Cyclotella

The cells of *Cyclotella* are short, drum-shaped, appearing as free-living or filamentous, chained or clustered united by mucilage. The number of plastids is high called as discoid. They are found mostly in freshwater and planktonic. Valves are found to be in circular, oval and triangular. Rows of areolae appears to be extended from valve center or absent from center which gives a ground like structure split into fascicles on the outer region of valve face. Areolae are poroidal, closed internally by cribra (figure 1 F). Literature has shown the lipid concentration of Cyclotella is 60.55 microgram, hence is best specie for concerned study [10].

3.1.7. Anabaena

Anabaena is trichomes with spherical cells having intercalary heterocysts with available dissolved nitrogen mostly in the form of nitrate, nitrite or ammonium ions. Staining is usually applied in order to see the thin sheaths when presents. The cells are cylindrical or bend but overall, it gives a look like a string of a bead. The color variant can be blue green to yellow green cells. Species can have colored or uncolored mucilage (figure 1 G). Study on Anabaena has shown the presence of 40 % oil content in Anabaena and hence is a good source of renewable biofuels including biodiesels and bioethanol. [11]

3.1.8. Coelestrum

These are thalli forming entities consists of 4, 8, 16, 32 or 64 (-128) celled coenobia. They organize as spherical, pyramidal or cuboid, free-floating colonies. Cells are more-or-less densely aligned but usually with empty spaces between wall gaps. Cell walls appear to be smooth, but may contain wrinkled surface structurally. Initially the cells are uni-nucleate but later becomes multi-nucleate prior to reproduction in which mitotis involves centrioles cytokinesis which are linked with phycoplast. A single Chloroplast is present and parietal with single pyrenoid. The formation of daughter cells colonies lead to asexual reproduction known as coenobium which is present inside parental cells. All stages of mitosis are occurred prior to cytokinesis and coenobial formation during asexual reproduction. Species differentiation is happened based on the cell sizes; number of cells per coenobia, shapes the species is distinguished and patterns of cell wall enhancement as well nature of wall processes. Several species groups have been distinguished based on cell morphology in lateral view cells with single, snout-like or well-developed free projections that are more or less triangular or 5-6 angled and cells with several processes that are localized in apical portion of cell or distributed around the whole cell surface. (figure 1 H)

3.1.9. Nitzschia radicula

The cells of *Nitzschia* Genus are often stretched. Its width can vary widely in the valve view. The valves can be straight to sigmoid. It can lie in either valve or girdle view. The center in valve view can be slightly swollen with rounded edges at each pole. The capsized margin on one valve faces the margins of the other cell with no capsizes and capsize appears to cross the cell diagonally. Striate occur along the valve face of the cell. The formation of cells can be unicellular or form chain like colonies, sometimes forming within mucilage tubes. Girdle view of this species results in the detection of Frustules present in rectangular shape. Valves are flat ending with a wedge like shape. The valves are lanceolate. Apices of these species are beak-like rounded. The raphe is positioned at the valve margin. Striae are visible in large specimens. Fibulae are long (1-2 µm) and irregularly spaced, 6-8 in 10 µm (figure 1 I). Research done on the diatom *Nitzschia* shows that species

belonging to this genus can be cultured well in outdoor vertical-bed photobioreactors to produce biodiesel [12].

3.1.10. Microspora pachyderma

This specie is having an unbranched filament with cylindrical cell. The cell walls are contracted as they are lamellate. The genus undergoes via biflagellate isogametes. This genus is very useful as it removes the heavy metals from the contaminated water. These algae are generally found in Freshwater, pond, streams etc (figure 1 J). Study done on the growth of microspore has showed good growth and utilization for biodiesel. Studies have shown that the growth of mixed algae culture was promising and the biodiesel produced was in accordance with the ASTM standards [10].

3.1.11. Oocystis

Cells are present in solitary or celled colonies within a thin, hyaline mucilaginous envelope or within an expanded mother cell wall. The shape of colony is oval to ellipsoid. Chloroplasts are present ranging from one to many, disc like shaped without a pyrenoid. The mode of reproduction is asexual reproduction by 2-4-8-16 autospores releasing by rupturing the mother cell wall (figure 1 K). Literature has shown lipid content (13.9 wt %) for Oocystis sp. Fatty acid profile has shown 60% of polyunsaturated fatty acids (PUFAs) compared to saturated which 29.4% and monounsaturated fatty acids (9.9%) [13].

3.1.12. Cymbella aspera

Algae of this genus comprises of valves which contains rounded apices dorsiventral within itself. The dorsal margin is arched with a humped in middle. The ventral border is concave. The specie is wider in center compared to axial area which is narrow. The central area is asymmetric while its dorsal area is rounded. The shape is flat on its ventral side which is surrounded by areolae in an irregularly spaced manner, the specie lack stigma. At the proximal ends, the raphe is narrow and hence is deflected slightly toward the ventral margin. The split ends are expanded. The raphe fissures are sickle-shaped and deflected dorsally. Striae are radiate throughout. Areolae are present in 9-11 number and 10 µm in size (figure 1 L). The lipid productivity reported for the specie is 37 mg L−1 day−1 when cultured. Studies have shown that there are 13 different fatty acids showing variation in their percentage of dry cell weight. Hence, showing the efficiency of the mentioned diatom for renewable biodiesel production [14].

3.1.13. Cymatopleura solea

The cells are comprised of valves which are elliptic with short apiculate apices which 2 in general. Undulations are usually observed across the valve. Fibulae ranges between 8-9 µm in size and are regularly spaced. The raphe is there along the margin of the valve. Striae are composed of areolae, irregular in shape and unsystematic usually. The size of areolae is 10-15 µm (figure 1 M). Studied conducted has showed that microalgae are rich in various lipids, carotenoids, sterols and isoprenoids. They also states that several groups of diatom are of great interest due to their potential practical application as energy sources (biofuel) [15].

3.1.14. Mastogloia elleptica

The Valves of the cells are elliptical to linear-elliptical with convex to nearly parallel sides, densely rounded apices. The axial area is thin, wider than the raphe, and the central area is circular. Raphe branches are crosswise and twisting, with weakly expanded proximal ends. Pretecta of same size are numerous all over. The Striae consist of single rows of areolae. The Striae are radiated throughout. Areolae are coarse and number 16-20 (figure 1 N).

3.1.15. Navicula cuspidata

Navicula is a genus having boat-shaped algae shape. It is primarily marine, eukaryotic, photosynthetic organisms. The genus *Navicula* range in size from a single cell. *Navicula* is a diatom and it plays a vital role in global ecology. A valve in this specie consists of lance like head with prolonged rostrate ends. The width size ranges from 7.0-7.7 µm while the length ranges from 30-37 µm. The raphe is filamentous. The axial area is fine and linear. The central area is oval to irregular. These are often present with alternately long and short striae. Near the valve center, the Striae are radiate and curved, or irregularly curved. At the valve apices, striae become convergent. Striae are marked with little lines within range of 14-15 µm (figure 1 O). Study has shown best performance for genus *Navicula* in context of oil quality for the production of biodiesel. The high content is obtained via acid catalysis technique. It contains low sulfur and an improved cetane number [16].

4. CONCLUSION

It was evident from the experiment that the ratio of diatoms was observed in a high amount compared to freshwater. Modern research emphasizes biofuel production through microalgae culturing. Diatoms, commonly called brown algae produce higher biomass within shorter time duration. Apart from the high biomass production, increased lipid concentrations for the given diatoms have been reported in the literature. Hence, Diatoms are the best source to cultivate for biofuel production. Green Algae which are present in the sample are also the best source of biodiesel production. These algae also have a high growth rate and can double their size in a short time period. Being high in lipids content, the high yield and high biomass make it ideal as a source for biodiesel production.

5. RECOMMENDATION

- Each of the diatoms identified in the sample should separately be grown in a photo-bioreactor tank with controlled conditions except CO_2.
- Culture media should be changed for both Diatoms and Green Algae in order to obtain the optimum algal growth and biomass production.
- For efficient biofuels production utilizing microalgae, metabolic engineering techniques should be induced.

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NA

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NA

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