

Microalgal Identification and Habitat Analysis of Selected Spots of Swat, Pakistan

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Abstract

This study investigates the taxonomic classification of algae, bacteriological analysis of water and physicochemical parameters across different areas. There are 25 algal genera and 64 species, reflecting high ecological diversity. The Zygnemaceae family is the most represented, with 12% of genera and 29.03% of species, followed by Desmidiaceae and Bacillariaceae, with each accounting for 12% of genera, and 11.29% and 4.84% of species, respectively. Bacteriological examination exhibited high microbial pollution. Haibat Gram water had most probable number 2.2 for every 100 milliliters of coliform bacteria, against World Health Organization standards (0 most probable number per 100 milliliters). Jalala water contained 140 colony-forming units per milliliter (World Health Organization standard: 50 colony-forming units per milliliter), with most probable number of coliforms at 3.6 per 100 milliliters and 2.2 most probable number per 100 milliliters of fecal coliforms. Gat Koto water contained 126 colony-forming units per milliliter and most probable number 2.8 per 100 milliliters, exceeding World Health Organization standards (0.2 most probable number per 100 milliliters). Physicochemical analysis revealed low turbidity in Haibat Gram water (total suspended solids: 5.33 milligrams per liter), with total dissolved solids: 141.67 milligrams per liter and biochemical oxygen demand: 3.20 milligrams per liter. Jalala water had the same trends (total suspended solids: 3.33 milligrams per liter, total dissolved solids: 144.33 milligrams per liter, biochemical oxygen demand: 6.20 milligrams per liter). Conductivity levels revealed freshwater suitability. These results reiterate the influence of microbial pollution on water quality, consistent with larger environmental stress physiology issues. They emphasize that more stringent water treatment measures should be implemented to suppress contamination threats.

Keywords: Taxonomic classification, Bacteriological Analysis, Physicochemical Parameters.

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Introduction

Algae constitute a highly variable assemblage of eukaryotic, photoautotrophic microorganisms comprising both unicellular and multicellular types and defined by thallus-like bodies. As autotrophic organisms, algae play a crucial role in ecosystems. They display phenomenal versatility with extreme conditions in their environments and have a capability of resisting high- and low-temperature environments. The algae are of varying sizes, from microscopic to large macroscopic species. Some species grow complex branching structures, while others grow in colonies. Algae are found everywhere, inhabiting a variety of habitats, such as soil, freshwater, and marine environments (Salah *et al.*, 2017). Among algae, unicellular organisms are of particular importance because they serve as a rich source of food and energy. Chlorella, for example, is a rich source of essential nutrients. Blue-green algae or cyanobacteria act as nitrogen-fixing organisms, making the soil fertile and playing an important role in increasing rice yields (Hussain and Shah, 2014). Algae are characterized by an impressive array of diversity, as around 500 green algae genera and 8,000 species of green algae have been found to date, and several others remain unknown (Kashif *et al.*, 2015). Being autotrophic, algae form a crucial component of ecosystems by promoting primary productivity and serving as the foundation of the food chain for a plethora of aquatic organisms (Hussain *et al.*, 2009).

Most of the seaweed and other algae are spread out in varied habitats, such as trees, soil and even animal surfaces, and they are able to grow at depths ranging up to 100 meters beneath the water level. These plants are highly adaptable, with certain species thriving in harsh environments like Arctic ice and hot areas. Algae can also grow on porous substrata like sandstone and limestone (Bellinger *et al.*, 2010). Algal size and morphology are highly diverse between habitats. Phytoplanktons, or microscopic algae occupy seas and lakes. (Sher *et al.*, 2012). The algal species contain a broad degree of morphological variation, as single cells aggregate to form colonies. The cells tend to assume filamentous formations in the forms of branched or unbranched chain-like structures (Nawaz and Sarim, 2004). One of the prime goals of the taxonomic exploration of unicellular green alga species is recording their habitat, which includes their geographical distribution, climatic and seasonal conditions, and the aquatic environment's pH. Furthermore, studies have also investigated the antimicrobial activity of methanolic extracts of algae against five bacterial isolates. The results supported the diversity of algal divisions and the antibacterial action of such extracts, with prospects for their utilization in biopharmaceuticals (Hussain *et al.*, 2011).

Water quality is central to deciding on public health and should not be underestimated (Herrero *et al.*, 2006; Rodri *et al.*, 2008). The population surge over the years has caused heightened water resource demands (Ghasemi *et al.*, 2004; Ghazala *et al.*, 2009). Yet, with more water use, water quality is progressively getting worse because of anthropogenic inputs like industrial growth and agriculture cover expansion (Jang *et al.*, 2014; Husain *et al.*, 2010). Different pollutants like chemical contaminants, microbial pathogens, industrial effluent, and toxicants cause unsafe drinking water (Zarina *et al.*, 2014). Polluted water is one of the principal reasons for waterborne diseases, resulting in serious health conditions like skin ailments, kidney damage, circulatory disease, gastrointestinal disorder, cancer, blue baby syndrome, nervous system disorder, and osteomalacia (Shuaib *et al.*, 2017). Alarming, waterborne diseases, most notably diarrhea, cause an estimated 53,000 child deaths each year (Joshi *et al.*, 2009). With the important role played by water quality in public health, the research on different algal species sought to offer holistic taxonomic information, as well as an evaluation of their habitat conditions, environmental factors, and pH fluctuations in aquatic environments.

Materials And Methods

The three main parts of research methods are algae identification, preservation, and collection. gathering water samples, from which algae are extracted, and analysing them chemically and microbiologically.

Samples Collection

From different spots in Swat Rani Zai, Haibatgram, Jalala, Gatkoto, and Barikot, fresh water algae are collected. Macroalgae are collected by hand, while algae on rocks are scraped using a knife and toothbrush. Forceps are employed for filamentous algae and desmoids flora are collected with a pipette. The diatoms are collected from stones with a toothbrush. Several types of freshwater habitats, such as rivers, ponds, streams, still water, shallow water, and aquatic plants, are employed for the collections. All of the samples of algae are collected and stored in glass vials with water added.

Preservation of Samples

After the sampling, all of the samples are preserved by the addition of a 4% formalin solution to avoid their deterioration. All of the algal samples are numbered and well labeled with their locations after fixation. All the samples are dispatched to Islamia College Peshawar's Botany Department.

Microscopy of Algae

Slides are prepared for algal microscopy. A single drop of the sample is put on a clean slide once it is taken. The slides are covered by a cover slip, and the bubbles are removed. The samples are observed under a light microscope with magnifications of 10X, 20X, and 40X once the bubbles had been removed. Diatom microscopy is done with 100X magnification.

Identification of Algae

Photos are captured following the observation of algae under a light microscope at different magnifications, and the species are identified from the literature available (Desikachary, 1959; Prescott, 1964; Shameel, 2001; Prescott, 2008).

Microbial Interaction

Water samples from the selected sites are obtained in Nestle bottles and labeled accordingly with the site name to ascertain the microbiological interaction. These water samples are transported to the PCSIR laboratory in Peshawar for the purpose.

Physicochemical Parameters of Water

The determination of physicochemical parameters, water samples are taken from the selected sites. Nestle bottles are employed for this purpose, and at each site, samples are taken and labeled with the site number and name appropriately. Mercury thermometer is employed for the measurement of the temperature of water at the site, while pH meter measured its pH. These samples are taken to the PCSIR laboratory in Peshawar for the purpose of identifying physicochemical parameters like conductivity, turbidity, chemical oxygen demand, biochemical oxygen demand, and total dissolved solids.

Results

The dataset includes 25 genera representing 64 species across various algal families, indicating a diverse ecological composition. The most dominant families are zygnemaceae. This family has the highest representation, accounting for 12% of genera and 29.03% of species, highlighting its ecological significance. Desmidiaceae and Bacillariaceae both contribute 12% of genera, but with fewer species (11.29% and 4.84%, respectively). Genera and Species Ratio: Hydrodictyaceae and Scenedesmaceae each contribute 8% to both genera and species diversity, indicating balanced distribution. Underrepresented families such as Merismopediaceae and Volvocaceae each contain only one genus and

species (4% and 1.61%), suggesting they may play a limited role in this ecological community. Overall Contribution the top three families (Zygnemaceae, Desmidiaceae, and Oedogoniaceae) represent significant percentages of the total species count, emphasizing their importance in aquatic ecosystems (Table 1; Figures 1-3).

Table 1: List of family, genera, and species.

Family	Genera	Genera %	Species	Species %
Merismopediaceae	1	4%	1	1.61%
Volvocaceae	1	4%	1	1.61%
Hydrodictyceae	1	4%	5	8.06%
Scenedesmaceae	2	8%	5	8.06%
Closteriaceae	1	4%	1	1.61%
Desmidiaceae	3	12%	7	11.29%
Cymbellaceae	1	4%	2	3.23%
Fragilariaceae	1	4%	2	3.23%
Bacillariaceae	3	12%	3	4.84%
Gomphonemataceae	1	4%	3	4.84%
Naviculaceae	1	4%	2	3.23%
Pinnulariaceae	2	8%	3	4.84%
Zygnemaceae	3	12%	18	29.03%
Cladophoraceae	1	4%	1	1.61%
Chaetophoraceae	1	4%	1	1.61%
Ulotrichaleaceae	1	4%	2	3.23%
Oedogoniaceae	1	4%	7	11.29%
Total	25		64	

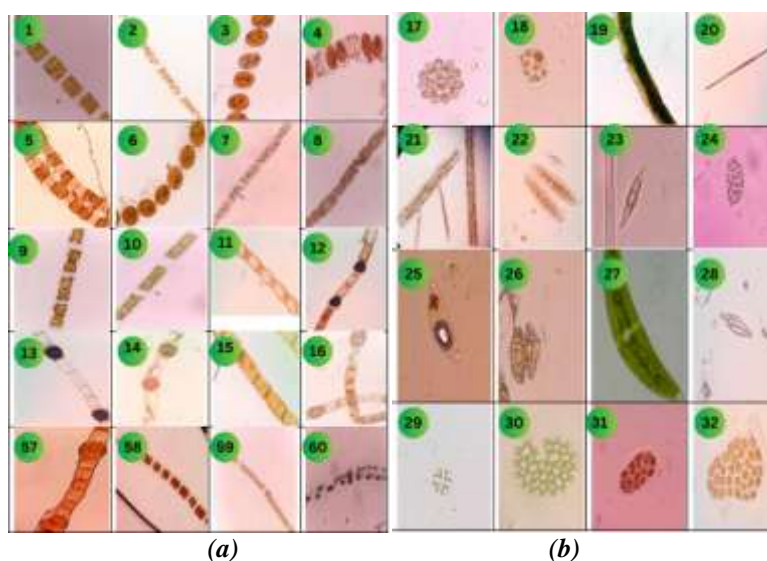


Figure 1: Microscopic image of different algae Spp. Including.

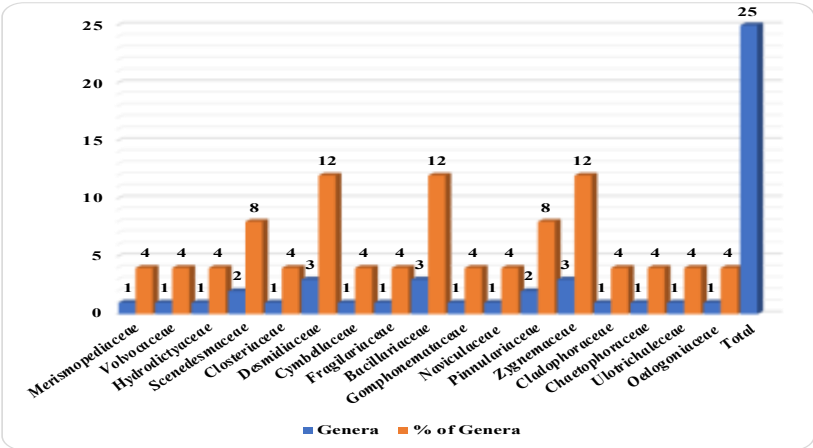


Figure 2: Percentage of the number of genera.

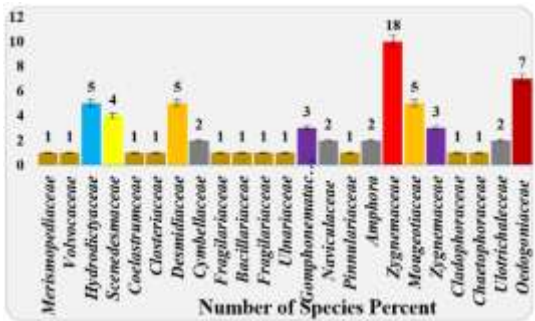


Figure 3: Percentage number of species.

Bacteriological Analysis of Water

Haibat Gram

The total Plate count of 86 cfu/ml exceeds the WHO standard of 50 cfu/ml. The results indicate a higher microbial contamination and poor water quality. Coliform Bacteria (MPN/100ml) result is 2.2 MPN/100ml, while the WHO standard is 0. This suggests the presence of coliform bacteria, which may indicate contamination from fecal matter or other sources. Fecal Coliform Bacteria (MPN/100ml): Similar to total coliform, the result is 2.2 MPN/100ml. The concerning as fecal coliforms are more specifically associated with fecal contamination, posing health risks (Table 2).

Table 2: Bacteriological analysis of water from Haibat gram.

Parameters	Results	WHO Standards
Total plate count (cfu/ml)	86	50
Coliform Bacteria (MPN/100ml)	2.2	0

Fecal coliform Bacteria (MPN/100ml)	2.2	0
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Jalala

The WHO threshold of 50 cfu/ml is exceeded by the result of 140 cfu/ml. This suggests possible contamination and insufficient water quality because it shows a high microbial contamination. The coliform bacteria (MPN/100 ml) are 3.6, which is more than the WHO recommendation of 0. The existence of coliform bacteria suggests that faecal or environmental contamination may be the cause. With 2.2 MPN/100 ml of faecal coliform bacteria, there is a direct risk to health since faecal coliforms are directly linked to faecal pollution.

Table 3. Bacteriological analysis of water from Haibat gram.

Parameters	Results	WHO Standards
Total plate count	140	50
Coliform Bacteria (MPN/100ml)	3.6	0
Fecal Coliform Bacteria (MPN/100ml)	2.2	0

Gat Koto

The total plate count is 126 cfu/ml. This suggests a significant degree of microbiological contamination, which is problematic for the quality of the water. Coliform Bacteria (MPN/100ml): 2.8 MPN/100ml is more than the WHO recommendation of 0. Coliform bacteria indicate contamination that could be harmful to one's health. Faecal Coliform (MPN/100 ml): This likewise surpasses the WHO threshold of 0.2 MPN/100 ml. Faecal coliforms are a sign of possible faecal contamination, which can cause major health problems (Table 3 and 4).

Table 4. Bacteriological analysis of water from Haibat gram.

Parameters	Results	WHO Standards
Total plate count	126	50
Coliform bacteria (MPN/100ml)	2.8	0
Fecal coliform (MPN/100ml)	2.2	0

Physicochemical Parameters of Water of Haibat Gram

Temperature of freshwater environments is (18 °C) often maintain several kinds of aquatic species, and this water temperature falls within that range. 7.3 pH: For the majority of aquatic life, the pH level suggests neutral to slightly alkaline environments. Many freshwater species prefer a pH of 6.5 to 8.5. 5.33 mg/L of total suspended solids (TSS): Low turbidity and little particle matter that could block light penetration are indicated by this number, which is far below the NEQ limit of 150.00 mg/L. The total dissolved solids (TDS) concentration of 141.67 mg/L indicates high water quality with low quantities of dissolved ions, as it is

much lower than the NEQ of 3500.00 mg/L. Never Discovered Chemical Oxygen Demand (COD) for aquatic health, a COD value of "not observed" denotes extremely low levels of organic contaminants. The biological oxygen demand (BOD) level of 3.20 mg/L is below the NEQ of 80.00 mg/L, indicating that there is less organic matter breakdown taking place, which is good for the quality of the water. Turbidity (3.00 NTU): Clear water that permits sufficient light penetration for aquatic plants is indicated by a value below the maximum threshold of 10.00 NTU. The conductivity (214.00 $\mu\text{S}/\text{cm}$) measurement indicates the ion concentration in the water. Although there is no NEQ given, this level usually denotes low salinity, which supports freshwater environments (Table 5)

Table 5. Physicochemical Parameters of Water of Haibat Gram.

Parameters	Result	NEQs
Temperature	18°C	
pH	7.3	
TSS (Total Suspended Solids)	5.33 mg/L	150.00
TDS (Total Dissolved Solids)	141.67 mg/L	3500.00
COD (Chemical Oxygen Demand)	Not Detected	150.00
BOD (Biological Oxygen Demand)	3.20 mg/L	80.00
Turbidity	3.00 NTU	Max: 10.00
Conductivity	214.00 $\mu\text{S}/\text{cm}$	--

The pH is slightly above neutral, indicating good conditions for many aquatic species, as optimal ranges are typically between 6.5 and 8.5. A result of 3.33 mg/L for total suspended solids (TSS) is much lower than the NEQ of 150.00 mg/L, suggesting low turbidity and little particle matter, both of which are good for aquatic environments. The total dissolved solids (TDS) concentration of 144.33 mg/L indicates low levels of dissolved ions and high-water quality because it is significantly lower than the NEQ of 3500.00 mg/L. Chemical Oxygen Demand (COD) (Not Detected): The lack of COD suggests extremely low concentrations of organic contaminants, which is advantageous for preserving the quality of the water. Again, good for water quality, the biological oxygen demand (BOD) of 6.20 mg/L is below the NEQ of 80.00 mg/L, indicating low levels of organic material breakdown. Turbidity (3.00 NTU): This turbidity level is below the maximum threshold of 10.00 NTU, indicating clear water, which supports light penetration necessary for photosynthesis. Conductivity (221.00 $\mu\text{S}/\text{cm}$): This measure reflects the concentration of ions in the water; while no NEQ is specified, this level typically indicates low salinity, supporting freshwater organisms (Table 6).

Table 6. Physicochemical Parameters of Water of Jalala.

Parameters	Result	NEQs
Temperature	18°C	

pH	7.6	
TSS (Total Suspended Solids)	3.33 mg/L	150.00
TDS (Total Dissolved Solids)	144.33 mg/L	3500.00
COD (Chemical Oxygen Demand)	Not Detected	150.00
BOD (Biological Oxygen Demand)	6.20 mg/L	80.00
Turbidity	3.00 NTU	Max: 10.00
Conductivity	221.00 μ S/cm	--

Discussions

Taxonomic classification of Jalala and Haibat Gram

Algae are primary producers capable of converting atmospheric carbon dioxide into essential organic compounds. The current study is investigated of Jalala and Haibat Gram takht bhai. 64 species and 25 genera, are reported. Similar outcomes from Karachi in crisp water new water green growth environments uncovered (Manjare *et al.*, 2010) they ordered overview of new water green growth from various part of Pakistan (Muhammad *et al.*, 2016; Dohare *et al.*, 2014). A total of 68 blue-green algae species, belonging to 29 genera, are documented from various habitats, including lakes, streams, waterways, stale water, and wastewater, and are collected from the Malakand KPK, Pakistan, area by (Patil *et al.*, 2012). In wastewater bodies dominated in algal sprouts in the summer, algal differences are noted (Kumar *et al.*, 2010; Toma *et al.*, 2023). The same results are reported by Rehman *et al.* (2018) from Dir lower river Panjkora. Describe how the different types of algae in water bodies relate to the water's characteristics. The pH of distinct water bodies from distinct freshwater algal environments, as well as bodies of running and dormant water and wastewater, is clarified.

Physicochemical Parameters

The current research compared eight physicochemical properties of water in Haibat Gram and Jalala, validating data by Sohail *et al.* (2022). The physicochemical properties of the Ganga River at Haridwar are also investigated, which affirmed observations as described by Pavlinac *et al.* (2018). Monthly fluctuations of major water quality parameters such as temperature, clarity, turbidity, total dissolved solids (TDS), pH, dissolved oxygen (DO), free carbon dioxide, total hardness, chloride, alkalinity, phosphate, and nitrates are measured in the Tamadalge Water Tank, Kolhapur, Maharashtra. These observations corroborated Mthembu *et al.* (2022) findings to highlight the importance of these variables in determining the quality of water for aquatic communities. An investigation of the River Swat, Batkhela, Pakistan, also found similar physicochemical characteristics; yet, the outcomes are not same as those observed by Mahfooz *et al.* (2019), whose water quality indices are based on 27

separate parameters, of which pH, color, TDS, and concentrations of a number of different metals are measured. The results of the current research are consistent with those of Lanjwani *et al.* (2021), who investigated various physicochemical parameters like color, temperature, acidity, pH, sulfate, chloride, DO, BOD, COD, and alkalinity. In addition, the findings of the study are corroborated by Sohail *et al.* (2021), who examined water temperature, clarity, turbidity, TDS, pH, DO, free carbon dioxide, total hardness, chloride, alkalinity, phosphate, and nitrate levels, which further established the importance of these parameters in water quality determination.

Bacteriological Analysis of Selected Water Bodies

Microbial contamination is still a major public health issue in rural and urban areas of Pakistan (Nsabimana *et al.*, 2021). The current study performed bacteriological examination of water samples from different sites in Swat Ranizai, Pakistan, consistent with the results of earlier studies (Talib *et al.*, 2019). The coliform group, being an indicator of bacterial contamination, is extensively employed to determine the potability of drinking water. The occurrence of coliform organisms is an indication of contamination by an unsafe water source, poor treatment, or post-treatment contamination (Wagh *et al.*, 2019). The World Health Organization (WHO, 1984) states that unpiped water supplies can have a maximum of 10 coliforms per 100 mL, but their regular occurrence indicates the necessity for better sanitary measures or a different water source. In the current investigation, 81% of the untreated and 38% of the treated samples are MPN positive for contaminated water samples that had high rates of contamination as well as risks to the general public health. Additionally, the presence of thermotolerant (fecal) coliforms is in 39% of positive samples, an organism that confirms proof of fecal contamination (Mark *et al.*, 1984). These results indicate declining contamination in the Kathmandu Valley; however, the findings are still alarming when compared against WHO drinking water standards (Alotaibi EL, 2009). Comparative investigations by southwestern Saudi Arabia (Rajendran *et al.*, 2006) and Tamil Nadu, India (Desikachary, 1959) indicated that all well water samples tested positive for coliform using the MPN method. Unlike in this research, which located a well devoid of total coliforms as a result of enhanced protective surrounding the well.

Conclusion

The study reveals a rich ecological diversity of algae, identifying 25 genera and 64 species, with the Zygnemaceae family being the most dominant. This highlights the critical roles these families play in aquatic

ecosystems. However, bacteriological analyses indicate significant water quality issues, with coliform and fecal coliform bacteria levels exceeding WHO standards, suggesting substantial contamination risks.

Recommendations

Regular monitoring of water quality in affected areas is essential to identify contamination sources and trends. Implement strategies to reduce pollution, especially from agricultural runoff and sewage discharge, to improve water quality. Educate local communities about the importance of maintaining water quality and the health risks associated with contamination. Conduct more in-depth studies on the relationships between algal diversity, water quality, and ecosystem health to develop better management practices

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