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Physico-Chemical and Microbial behavior of different water bodies with respect to algal growth and morpho-taxonomic identification of algae in district Nowshera, Khyber Pakhtunkhwa

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This study aims to find out the water quality of selected sites of Village Jalozai, District Nowshera and its relation to aquatic flora and microbes. Water samples from Mahajar camp canal, Anghor Gharai spring water and Jalozai Barani dam water were sent to PCSIR lab for physico-chemical analysis. The analyzed parameters such as water pH, water temperature, Magnesium, Chloride, Sulphates, Nitrites, Potassium, Dissolved oxygen and Total alkalinity were within the permissible limit and favors algal growth. Cadmium and lead (absent in Jalozai Barani dam) inhibits algal growth. Turbidity exceeded the prescribed limit which inhibits the algae growth and cause water pollution. Other parameters like Conductivity, Total dissolved solids, Total suspended solids, Total solids, Total hardness, Calcium, and Sodium also affect algal growth. Recorded mean water velocities of research spots (0.18m/s - 0.33m/s) favors algal distribution. Total plate count, coliform bacteria and Fecal coliform bacteria (absent in Jalozai Barani dam) confirmed bacteria- algae symbiotic relationship. A total of 32 species of algae were reported from the study area. The genus *Closterium* was prevalent and was represented by 6 species (19.35%). *Cladophora, Cosmarium, Fragilaria* and *Gomphonema* were represented by 2 species (6.45%). *Amphora ovalis*, *Chlorella vulgaris*, *Chroococcus turgidus, Chaetomorphaaerae*, *Cymbella affinis, Diatoms anceps, Euglena virdis*, *Hydrodictyon reticulatam, Microcystis aeruginosa, Mougeotia spp, Oedogonium spp, Spirogyra longata, Spirogyra mirabilis, Pediastrum boryanum, Pithophoraoedogonia*,*Melosira varians, Ulothrix zonata and Volvox* (3.2%) reported single species in this study. A further study is recommended to report seasonal changes in algal diversity and water quality of the research area.

Keywords: algal diversity, bacteria-algae symbiotic relationship, physico-chemical parameters, water quality, water pollution, water microbes

INTRODUCTION

Algae are larger group of plants having 7000 species. They range from unicellular microscopic structure to multicellular complex. They are having certain unique characteristics such as filamentous branched body, locomotory and non-locomotory features. They also form colonies. Their chloroplast contains photosynthetic pigments such as chlorophyll a, b, xanthophyll and carotenes. They also contain starch and pyrenoid (Minhas, 2023). Algae are found in stagnant, running and slow running water (Ullah, 2023). Algae are diverse groups of photosynthetic organisms ranges from microscopic to macroscopic (Gao, 2021). Algae exists in both lentic and lotic fresh water bodies (Hook 2020).

Distribution of algae in an area reflects the condition of water bodies of that area (Jang, 2014). Most of algal species form algal blooms and their diversity is affected due to temperature, light, presence and concentration of nutrients and other parameters (Wali 2017). Environmental factors affect the algal diversity in all habitats in different seasons such as pH, temperature, heavy metals, organic compounds and human activities caused pollution affecting base of water bodies (Dora 2010). Water quality is affecting by two key factors like water temperature and dissolved oxygen for water inhabiting organisms. Catabolism and anabolic processes, products of photosynthesis, water conduction property, density of water and dissolved gases especially oxygen in water is affecting due to water temperature (Pfannenstein, 2016).

Matter and constituents of aquatic plants were measured at different velocities in the field such as 0.03 m/s, 0.06 m/s, 0.10 m/s, 0.15 m/s, and 0.30 m/s. Results obtained from fields concluded that aquatic plants matter and their presence is badly affected by water flow (Li 2013). Water flow effects algae growth and provides important nutrients for algae growth. Removal of nutrients or pollutants or water flow which provides nutrients for algae growth can inhibit their growth (Azpura, 2013). All those water bodies which has low flow rate has less biomass which showed that flow rate effects the phytoplankton growth. At flow rate 0.06 m/s inhibits phytoplankton growth. When flow rate changes, it effects the competition of algae between blue-green algae and green algae for light. Blue-green algae blooms in still water while green algae in flowing water. The research findings indicate that critical flow rate can be used in reducing algal blooms occurrence by developing new methods (Zhang et al. 2015). Coliform are rod-shaped, gram-negative bacteria which do not forms spores. They are found in nature. Water quality analysis is based on the presence of these bacteria such as total coliform (TC), fecal coliform (FC), and Escherichia coli (Nabeela 2014).

The current study aims to identify morpho-taxa and distribution of algae in district Nowshera water bodies with respect to their physico-chemical nature and impact of native microorganism.

Study area

Nowshera is a district of Khyber Pakhtunkhwa and Khyber Pakhtunkhwa is a province of Pakistan. It lies between $33^0$42 to 34^0 09 latitudes and 71^0 97 to 72^0 16 longitudes. It is bordered by Peshawar district to the west, District Mardan and Charsadda to the north, District Swabi is on the northeast, District Kohat is on the south and District Attock to the southeast. District Nowshera was a province of Afghanistan and was called as Nowkhaar. But later on, it became the part of British India through a Durand line agreement. It is divided into 47 union councils and covers an area of 181,610 acres (Ali 2018).Village Jalozai is attached with Attock-Cherat ranges and its water bodies are formed of alluvial depositions of sands, silt and gravel. Cherat ranges are rocky and receives water from river Indus and its five adjoining tributaries such as Jindai, Shah Alam, Naguman, Sardaryab and Khayale. Some important water resources are Gul Baba Chena, AnghorGharai spring, Mahajar camp canal and JalozaiBarani dam. Nowshera important rivers are bara river, kalpani river, Kabul River and many small dry water bodies or streams (Ali, 2016).

MATERIALS AND METHODS

Species sampling and preservation

The research was conducted at selected research sites and species were collected by picking and scrapping method. The samples were preserved in polythene bags and about 4% formalin solution was added to it. Bottles were washed 2 to 3 times to avoid contaminations. More than 25 samples were collected from different spots and were labelled with selected spots. Jang (2014) protocol was used for collection and preservation with slight modifications.

Species identifications

Samples were photographed with the help of camera Lucida and identification keys of Prescott (1964) and Tiffany and Britton (1971) were used for their identification.

RESULTS

Bacteriological analysis of water samples

Collected samples from Mahajar camp canal (sample 1), Anghor Gharai spring water (sample 2) and Jalozai Barani dam water (sample 3) were sent to PCSIR lab for various water quality tests.

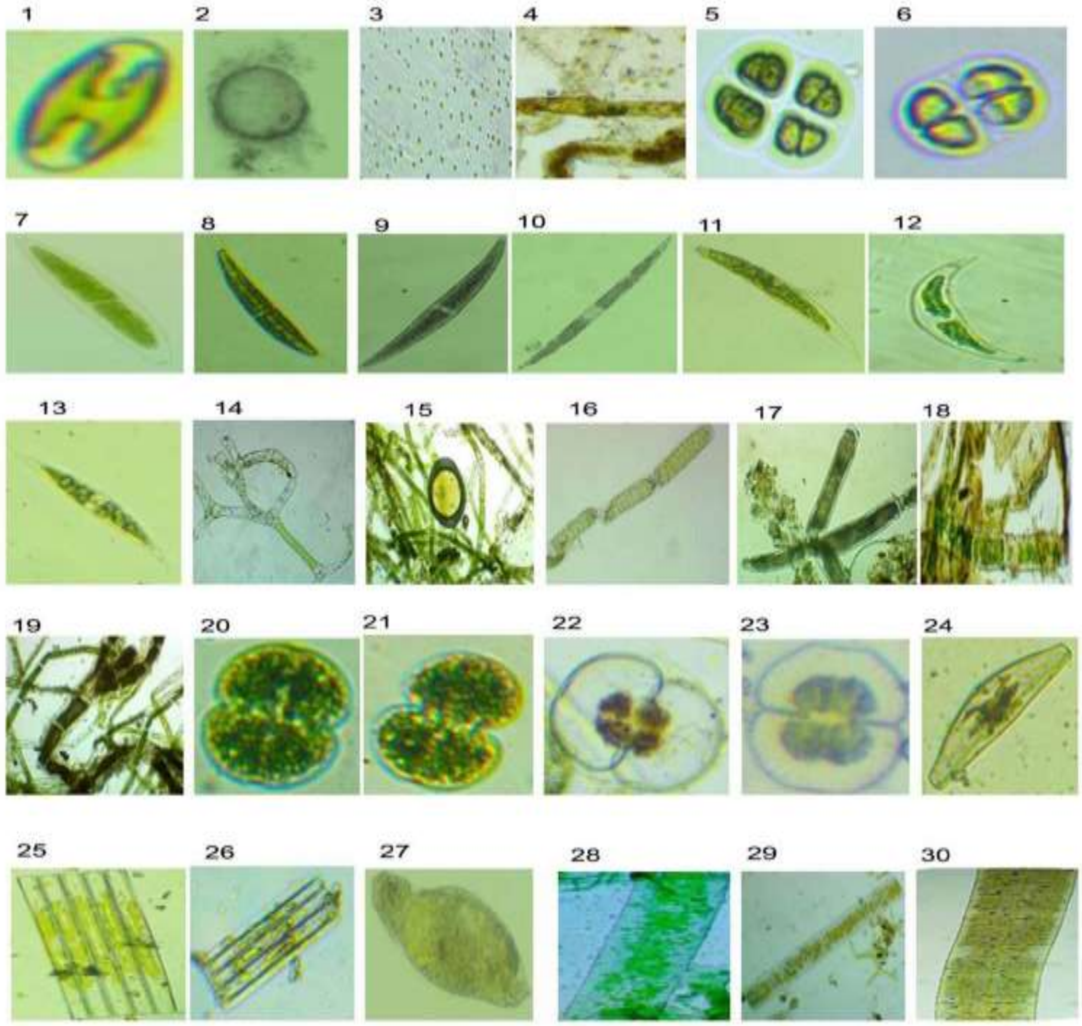
Table 1: **Bacteriological Analysis of Water**

Parameters	Sample1	Sample2	Sample3	Standard	Method No.
Total platet Count (cfu/ml)	415	470	200	<100	9215 A-B
Coliform Bacteria (MPN/100ml)	3.1	3.6	1.5	<1.1	9221 A-E
Fecal coliform bacteria	Present	Present	Absent	<1.1	9221 A-E

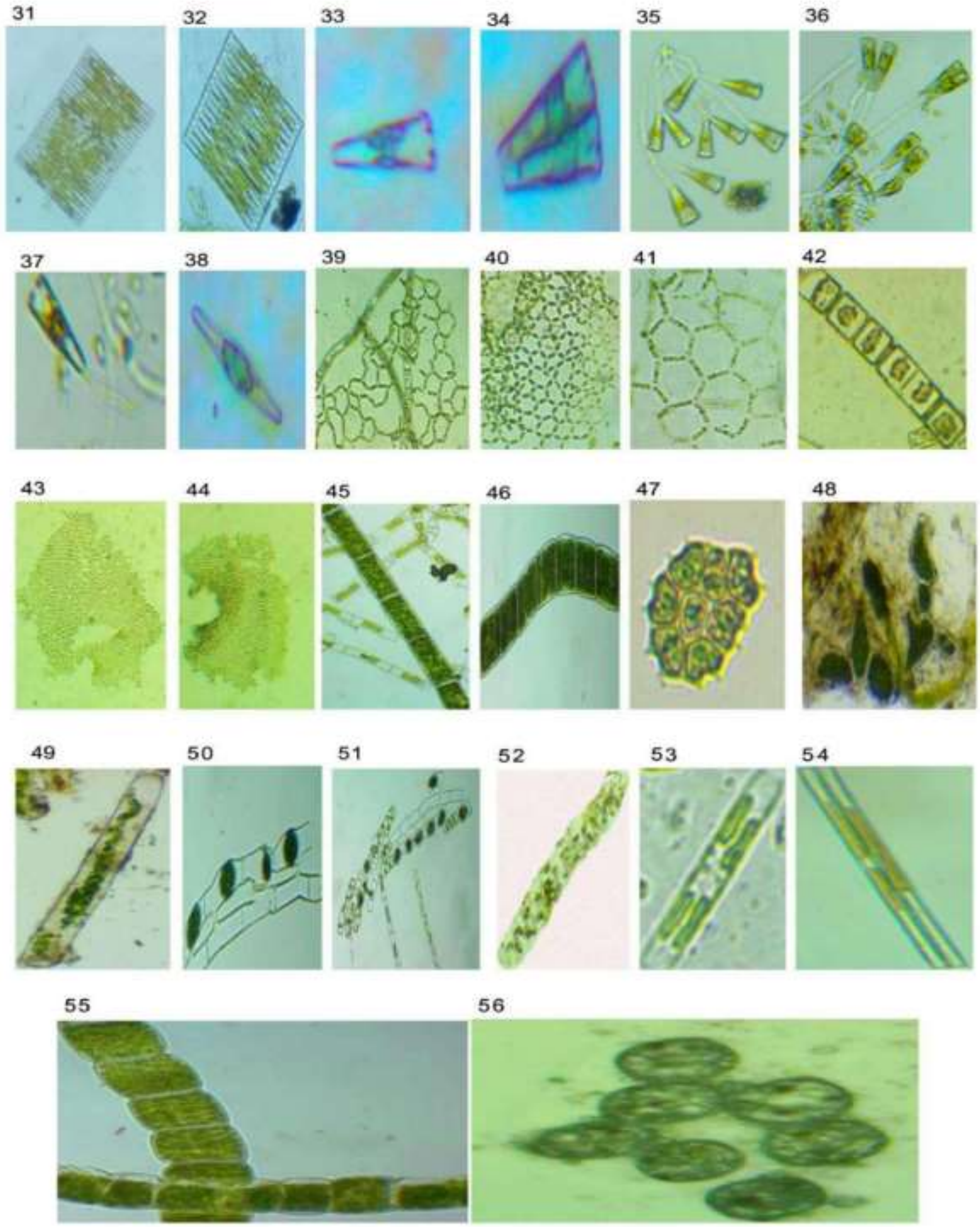
Table 2: Physico-Chemical analysis of water samples

S. No	Parameters	MethodNo.	Units	Sample1 Result	Sample 2 Result	Sample 3 Result	WHO limit
1	Water pH	4500-H+.B	------	7.40	7.49	7.29	6.6-8.5
2	Water temperature (T)	2550. B	C0	13.30	14.00	13.00	NGVS
3	Turbidity	2130. B	NTS	27.60	19.88	120.88	<5
4	Conductivity	2510. B	µS/cm	2320.00	163.00	183.00	400
5	Total dissolved solids (TDS) (mg/L)	2540. C	mg/L	1447.00	1081.00	70.00	1000
6	Total suspended solids (TSS) (mg/L)	2540. D	mg/L	35.00	47.00	3.00	5
7	Total solids (TS) (mg/L)	2540. B	mg/L	1482.00	1128.00	73.00	1005
8	Total hardness as CaCO3 (mg/L)	2340.C	mg/L	563.16	470.29	86.19	500
9	Calcium as CaCO3 (mg/L)	3500-Ca. B	mg/L	331.97	207.48	112.03	200
10	Magnesium as CaCO3 (mg/L)	3500-Mg.B	mg/L	231.19	262.81	76.83	100
11	Chloride as Cl (mg/L)	4500-Cl. B	mg/L	188.27	77.07	35.13	500
12	Sulphates as SO4 (mg/L)	4500-SO4.B	mg/L	188.00	69.40	157.00	400
13	Nitrites as NO2 (mg/L)	4500-NO3.B	mg/L	30.44	24.69	25.00	50
14	Sodium as Na (mg/L)	3500-Na	mg/L	374.00	147.50	50.19	200
15	Potassium as K (mg/L)	3500-K	mg/L	11.60	7.90	5.00	30
16	Cadmium as Cd	Atomic absorption	mg/L	< 0.1	< 0.01	Nil	0.003
17	Lead as Pb	Atomic absorption	mg/L	<0.01	< 0.1	Nil	0.01
18	Dissolved oxygen	4500-O. G	mg/L	5.00	4.00	3.00	5
19	Total alkalinity as CaCO3 (mg/L)	2320.B	mg/L	590.64	505.04	14.04	30
20	Taste	2160.B	-----	Unacceptable	Unacceptable	Acceptable	Acceptable
21	Odor	2150.B	-----	Objectionable	Objectionable	Unobjectionable	Unobjectionable
22	Color	2120.B	-----	Greenish	Greenish brown	Bluish white	Acceptable

Identified genera:



1. ***Amphora ovalis*** **showing flat and rounded ends and crescent shaped valves**
2-4 ***Chlorella vulgaris*** **showing unicellular spherical and non-flagellated body; Colony of** ***Chlorella vulgaris*** **mixed with** ***Spirulina major*** **and** ***claophora spp***
5-6.*Chroococcus turgidus*, a fresh water blue-green algae showing clear sheath surrounding 2 or 4 0or 8 cells
7.*Closterium acerosum* **showing spindle shaped cells and having less thick and short apical cells**
8-9.*Closterium lunula* **cells showing more length than width and are with median girdle**
10.*Closterium gracile*s lender body showing central parallel part
11.*Closterium turgidum* **showing curved cells at apical side**
12.*Closterium leibleini* **showing inner margin have more deppression and is more curved**
13.*Closterium lanceolatum* **showing lens shaped body and chromatophore is present with 7-8 ridges; pyrenoids are present in center**
14-18*. Cladophora glomerta* **showing branched y-shaped clustered branches;** ***Cladophora specie*** **mixed with** ***chlorella*** **and** ***Diatoms species***
19.*Cladophora goensis* **showing branched thallus and apical cell with rounded tip**
20--21.*Cosmarium botrytis* **showing convex semi-cells joined with narrow isthmus**
20-23. ***Cosmarium quinarium*** **chloroplast showing two pyrenoids in each convex shaped semi-cell**
24. ***Cymbella affinis*** **showing lens-shaped body**
25-26. ***Diatoms anceps*** **showing chain form body**
27. ***Euglena virdis*** **showing anterior blunt end and middle wider and posterior pointed parts**
28-30. ***Fragilaria capucina*** **cells joined to form long filament**



31-32 *Fragilaria crotonensis* showing ribbon-shaped body
33-37.*Gomphonema constrictum* showing swollen rounded valves and constriction at broad apical end
38. *Gomphonema gracile* showing lens-shaped body which is spherical in middle part
39-41. *Hydrodictyon reticulatam* showing water net-like shaped chloroplast.
42. Melosira varians showing square to rectangular cells in girdle view and has numerous discoidal golden-brown chloroplasts.
43-44. Colonies of *Microcystis aeruginosa*
45. Mougeotia specie mixed with *Oedogonium spp*, *Mougeotia* showing plate-like chloroplast

46. ***Oedogonium*** **specie showing unbranched filament**
47. ***Pediastrum boryanum*** **cells are of star shaped and forming circular plate-like arrangement**
48. ***Pithophora oedogonia*** **filament showing akinetes**
49 .***Spirogyra longata*** **showing spiral** ***Spirulina major*** **showing spiral filament.**
50-52. Lateral conjugation in ***Spirogyra mirabilis*** **and zygospore formation; the specie is showing single chromatophore which makes 4-7 turns in cell**
53-54. ***Synedra ulna*** **(Nitzsch) Ehrenberg showing narrow cells**
55. ***Ulothrix zonata*** **showing swollen or cylindrical cells and band-like chloroplast**
56.***Volvox spp*** **with daughter colonies**

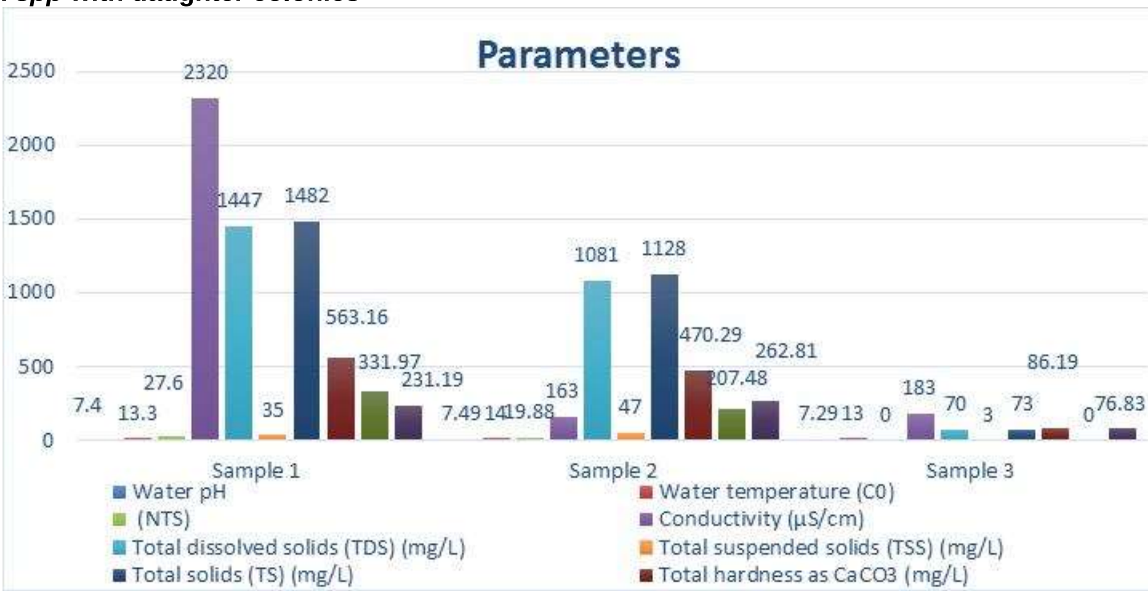


Figure 1: Physical and chemical parameters of sample 1, 2 and 3

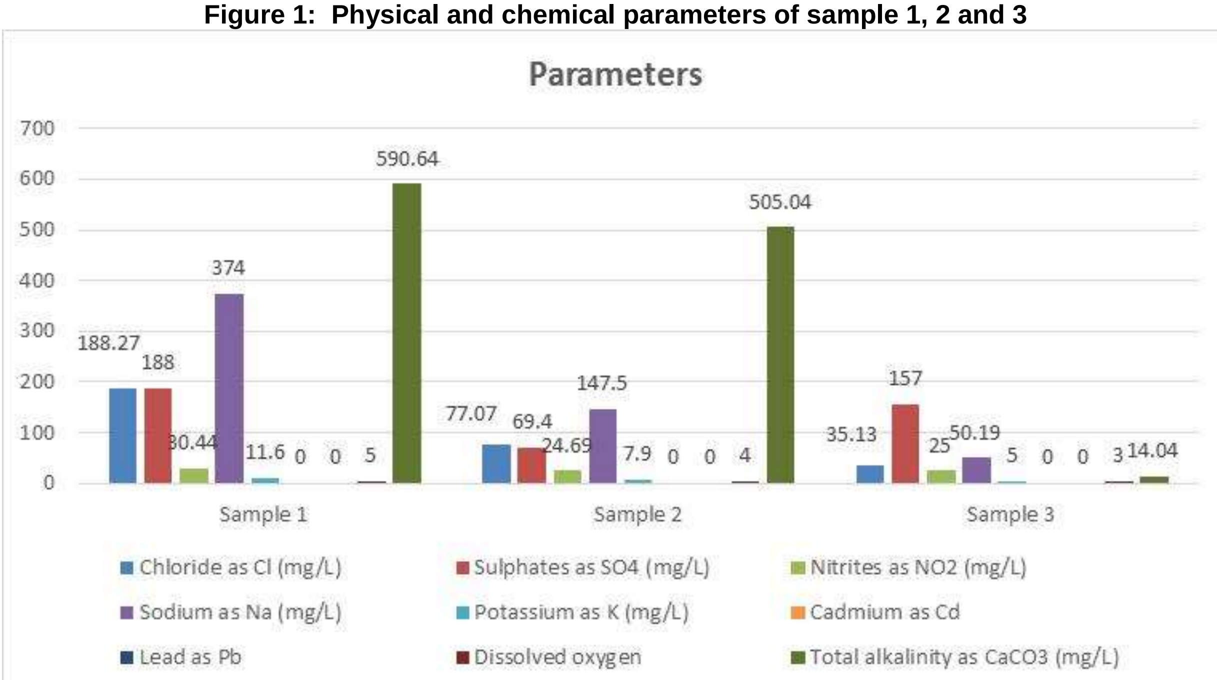


Figure 2: Physical and chemical parameters of sample 1, 2 and 3

DISCUSSION

A total of 32 species of algae were reported from the study area. The genus *Closterium* was prevalent and represented by 6 species (19.35%). *Cladophora, Cosmarium, Fragilaria* and *Gomphonema* were represented by 2 species (6.45%). *Amphora ovalis*, *Chlorella vulgaris*, *Chroococcus turgidus, Chaetomorphaaerae*, *Cymbella affinis, Diatoms anceps, Euglena virdis*, *Hydrodictyon reticulatam, Microcystis aeruginosa, Mougeotia spp, Oedogonium spp, Spirogyra longata, Spirogyra mirabilis, Pediastrum boryanum, Pithophora oedogonia*, *Melosira varians*, *Ulothrix zonata and Volvox (3.2%)* reported single species in this study. Further studies are recommended to report algal diversity and its relation to water microbes and quality in the research area.

Comparative analysis of samples parameters

All physical and chemical values of sample 1, 2 and 3 are dealt separately and analyzed.

Bacteriological analysis confirmed the presence of bacteria. The table 1 illustrates that total plate count and coliform bacteria for Jalozai Barani dam water was 200 and 1.5 respectively. Total plate count and coliform bacteria for Anghor Gharai spring water was 470 and 3.6 respectively. Total plate count and coliform bacteria for Mahajar camp canal was 415 and 3.1 respectively. Fecal coliform bacteria were present in Anghor Gharai spring water and Mahajar camp canal while absent in Jalozai Barani dam water. All these values were compared with standard values. These values show that bacteria are present in these research spots. Sen *et al* (2013) showed that microalgal form a mutual association with bacteria

Water pH from different spots of the said research area range between 7.29-7.4. Our findings of algal community suggest that it favors algae growth. pH effects algae growth. Leavitt (1999) reported that when pH was decreased from 6.6-5.0 it favors algae growth. As pH decrease favors algae growth so it can be concluded that low pH favors algae growth. Penderson (2003) reported that pH 9 cause algae growth inhibition (Carolyn et al. 2007).

Water temperature (T) from different spots of the said research area range between 13.30 C^0- 14 C^0. Our findings of algal community suggest that it is in optimum range which favors algal distribution. The increase and decrease in temperature effects algae growth. Singh and Singh (2015) evaluated that alga shows abundant growth at temperature ranges between 20 C^0- 30C$^{0.}$

Turbidity of different spots of the said research area range between 27.66 NTS-120.88 NTS. Our findings of algal community suggest that it has a negative effect on algal distribution. Algae and other aquatic life of streams and lakes is affected by turbidity. High turbidity limits aquatic life and algae because it reduces photosynthetic light (Denby et al, 1987). Water turbidity should be <5NTU in accordance with WHO guidelines. Usually, water bodies present above ground are turbid as compared to underground water. The Water other than fresh water turbidity ranges from <1 to 1000 NTU (Asadullah et al. 2013).

Conductivity of different spots of the said research area range between 163 µS/cm-2320 µS/cm. Our findings of algal community suggest that Jalozai Barani dam water conductivity is less than 400 µS/cm and has low total dissolved solids. It has negative effect on algal distribution. The other two research areas conductivity is more than 400 and has more total dissolved solids. They have positive effect on algal distribution. Conductivity (EC) value should not be greater than 400 µS/cm. this study showed that EC value was 179.3-20 µS/cm and its average value was 192.14 µS/cm. The above findings shows that research area having less dissolved solids and lower ions concentration (Meride and Ayenew, 2016).

Total dissolved solid (TDS) of different spots of the said research area range between 70mg/L-1447.mg/L. Jalozai Barani dam water has less dissolved substances and has negative effect on algal distribution. Our findings of algal community suggest that other two has a positive effect on algal distribution. Weber-Scannell and Duffy, (2007) suggested that TDS of natural water ranges from 30-600 mg/L while the water used for consumption should have <1000 ppm (Asadullah 2013). Kerekes and Nursall reported that algae growth inhibited at TDS concentration >1400mg/L. Evans and Prepas found that nitrogen fixation reduced in blue green algae when are exposed to about 2450mg/L TDS (Weber-Scannell and Duffy, 2007).

Total suspended solid (TSS) of different spots of the said research area range between 3 mg/L-47 mg/L. Jalozai Barani dam water has less suspended substances and has negative effect on algal distribution. Our findings of algal community suggest that other two has a positive effect on algal distribution. Bilotta and Brazier (2008) suggested that suspended solids cause water pollution, disturbs natural ecosystem, effects fish population, increases cost of water treatment and disturbs water bodies. Government has set water quality guidelines for suspended solids in freshwater bodies.

Total solid (TS) of different spots of the said research area range between 73 mg/L-1482.mg/L. Jalozai Barani dam water has fewer total solids and has negative effect on algal distribution. Our findings of algal community suggest that other two has a positive effect on algal distribution. Riaz (2017) reported that photosynthetic microalgae grow in a waste water container reduced the total suspended solids, total dissolved solids and oxygen concentration

Total hardness as $CaCO_3$ of different spots of the said research area range between 86.19 mg/L-563.16 mg/L. Jalozai Barani dam water and Anghor Gharai

Spring water value for total hardness of CaCO3 is less than WHO standard value and has positive effect on algal distribution. Our findings of algal community suggest that Mahajar camp canal water has negative effect on algal distribution. Asadullah (2013) found that Total hardness as $CaCO_3$ of streams and rivers ranges from 1-1000 ppm. Calcium and magnesium ions make water hard

Calcium as CaCO3 of different spots of the said research area range between 112.03 mg/L-331.97 mg/L. Jalozai Barani dam water has positive effect on algal distribution. Our findings of algal community suggest that Mahajar camp canal water has negative effect on algal distribution. Yarish and Edwards (2003) evaluated that calcium is required for algal both species growth at different salinity level. Its presence is affecting potassium concentration.

Magnesium as CaCO3 of different spots of the said research area range between 76.83 mg/L-262 mg/L. Our findings of algal community suggest that it has a positive effect on algal distribution. Microalgae requires magnesium for growth. It makes the chlorophyll structure and acts as cofactor for many enzymes. In a study, it was concluded that magnesium deficiency in Chlorella during photoautotrophic culture process effects several physiological processes, photosynthesis process and triacyl glyceride (Wang 2014).

Chloride as Cl of different spots of the said research area range between 35.13 mg/L-188.27 mg/L. Our findings of algal community suggest that it has a positive effect on algal distribution. Various concentration of NaCl were tested to check the effect the salinity. Organisms inhibiting marine environment were more halotolerant (8-10% NaCl than organisms inhibiting non-marine environment (2% NaCl) (Batterton and Baalen, 1971).

Sulphates as SO_4 of different spots of the said research area range between 69.4 mg/L-188 mg/L. Our findings of algal community suggest that it has a positive effect on algal distribution. Algae absorb Sulphur in the form of Sulphates like photolithotrophs. Phosphate concentration is high in oceans such a 29 mM. On the other hand, fresh water bodies have low concentration due to effect of seasonal and daily changes. The homeostasis process, photosynthesis reaction, metabolism and carbon and nitrogen uptake is affected by low concentration of Sulphates (Mario 2008).

Nitrites as NO_2 of different spots of the said research area range between 25 mg/L-30.544 mg/L. Our findings of algal community suggest that it has a positive effect on algal distribution. Results obtained in experiment supported the hypothesis that cyanobacteria show better growth in the large lakes having phytoplankton and low nitrogen and phosphate ratio (Seale and Warren, 1987).

Sodium as Na of the said research area range between 50.14 mg/L- 374 mg/L. Our findings of algal community suggest that Mahajar camp canal water has negative effect on algal distribution but other two has a positive effect on algal distribution. NaCl high concentration negatively effects growth by Na ions pressure during osmotic pressure. This inhibition cannot be overcome by changes other physical parameters such as light, temperature, pH or by changing medium chemical composition (Batterton and Baalen, 1971).

Potassium as K of different spots of the said research area range between 5 mg/L-11.5 4 mg/L. Our findings of algal community suggest that it has a positive effect on algal distribution. Potassium helps in switching on about 60 growth regulatory enzymes. It helps in altering enzymes forms and switching on the active sites of enzymes. It helps in neutralization of anions and some other compounds and also helps in stabilizing optimum pH between 7-8. The potassium concentration effects rate of enzyme reactions. It can be said that rate of a reaction depends on potassium ions concentration entering the cell (Iyer 2015).

Dissolved substances of different spots of the said research area range between 3 mg/L-5 mg/L. Our findings of algal community suggest that it has a positive effect on algal distribution. Oxygen solubility is affected by pH and temperature. In fresh water bodies, it is 14.6 mg/L at 0 C^0 and about 9.1, 8.3 and 7.0 mg/L at 20 C^0, 25 C^0 and 35 C^0 respectively. Pressure is 1atm during this experiment. The dissolved oxygen value is 9.0-7.0 mg/L at temperature 20 C^0 and 30 C^0. Low dissolved oxygen concentration affects aquatic life (Patel and Vashi, 2015).

Total alkalinity as CaCO3 of different spots of the said research area range between 13.30C^0-14C^0.Our findings of algal community suggest that it has a positive effect on algal distribution.

High pH favors in reducing algal population. Algae can withstand a specific ph. The photosynthesis rate limits in basic water. When alkalinity increases then it favors reduction of CO_2 for photosynthesis reaction (Carolyn et al. 2007).

Cadmium as Cd and Lead as Pb for Mahajar camp canal were <0.1 mg/L, and <0.01 mg/L. Cadmium as Cd and Lead as Pb for Anghor Gharai spring water were <0.01 mg/L and <0.1mg/L. Cadmium as Cd and Lead as Pb were absent in Jalozai Barani dam.

Studies in plants (Benavides et al. 2005 and Sharma and Dubey 2005) and algae (Szivak et al. 2009) have reported that both lead and cadmium are harmful for plants growth and it causes inhibitory effects and finally death of plant body (Carfagna et al. 2013).

Anghor Gharai spring water had unacceptable taste, objectionable odor and greenish brown color. Mahajar camp canal had unacceptable taste, objectionable odor and greenish color. Jalozai Barani dam water had acceptable taste, unobjectionable odor and bluish white color.

Algae produces odors and colors in water bodieS. Algae produces aromatic odor which resembles to flowers or vegetables. It also produces pungent, fishy and grassy odors (Sen et al. 2013).

Water velocity of Mahajar camp canal, Anghor Gharai spring and Jalozai Barani dam water are measured in March. Average velocity of Mahajar camp canal is 0.33 m/s, Anghor Gharai spring is 0.18 m/s and Jalozai Barani dam is 20 m/s.

Mean of water velocities from different spots of the said research area range between 0.18m/s- 0.33m/s. Standard deviation of mean of water velocities from different spots of the said research areas range between 0.01-7.8. Our findings of algal community suggest that it has a positive effect on algal distribution .All those water bodies which has low flow rate has less biomass which showed that flow rate effects the phytoplankton growth. At flow rate 0.06 m/s inhibits phytoplankton growth. When flow rate changes, it effects the competition of algae between blue-green algae and green algae for light. Blue-green algae blooms in still water while green algae in flowing water. The research findings indicate that critical flow rate can be used in reducing algal blooms occurrence by developing new methods (Zhang et al. 2015).

CONCLUSIONS

It has been concluded that water quality of the research site is affected due to presence of algae, aquatic microbes and various organic and inorganic compounds. The analyzed and identified algal species in research area were in symbiotic relationship with the present bacteria. Mahajar camp canal is more polluted than others and having least recorded water velocity value and algal species. The water velocity, bacteriological and physico-chemical parameters change from season to season and it needs to be studied in detail. Systematic research should be carried out to investigate other algal species and their effect on aquatic microbes and water quality.

Supplementary materials

Not applicable

Author contributions

Conceptualization, S.S. and W.Z.A.; methodology, R.R.; software, M.N.; validation, A.Q. and M.A.; formal analysis, S.S; investigation, A.Q and M.A.; resources, M.A.; data curation, M.A.; writing-original draft preparation, R.R.; writing-review and editing, S.S; visualization, W.Z.A.; supervision, M.A.; project administration, W.Z.A; funding acquisition, R.R. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

The study was approved by the Qurtuba University of Science and Technology, Peshawar, Pakistan

Informed Consent Statement

Not applicable.

Data Availability Statement

The data associated with these findings are available from the corresponding author upon request.

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Conflict of interest

The authors declared that present study was performed in absence of any conflict of interest.

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