BIOREMEDIATION OF OIL REFINERY WASTEWATER THROUGH INDIGENOUS ALGAL SPECIES

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BIOREMEDIATION OF OIL REFINERY WASTEWATER THROUGH INDIGENOUS ALGAL SPECIES

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بسم الله الرحمن الرحيم

DEDICATION

I dedicate my work to My Beloved Parents, Family members, friends

&

Respected Teachers

ACCEPTANCE BY THE VIVA VOCE COMMITTEE

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I ***Tariq Mehmood Khan*** (17-FBAS/PHDES/S16), student of Ph.D in Environmental Science (session 2015-22), hereby declare that the matter printed in the thesis titled "Bioremediation of Oil Refinery Wastewater Through Indigenous Algal Species" is my own work and has not been published or submitted as research work or thesis in any form in any other university or institute in Pakistan or abroad.

Date: 23-02-2023 **Signature of Deponent**

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FORWARDING SHEET BY RESEARCH SUPERVISOR

The thesis entitled “Bioremediation of Oil Refinery Wastewater Through Indigenous Algal Species” submitted by Tariq Mehmood Khan in partial fulfillment of Ph.D degree in Environmental Science has been completed under my guidance and supervision. I am satisfied with the quality of student’s research work and allow him to submit this thesis for further process to graduate with Ph.D Degree from Department of Environmental Science, as per IIUI rules & regulations.

Date: 23-02-2023

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Those who bear the throne (of Allah) and those near to Him glorify and praise their Lord. They believe in Him and implore His forgiveness for those who believe… (Quran, 40:7).

If you be thankful, I will increase surely you (in bounty)..... (Quran surah Ibrahim 14:7)

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ABSTRACT

Water quality remains one of the most significant environmental issues. Numerous local and large-scale programs aim to provide good ecological status of freshwater and marine ecosystems. Due to presence of contaminants, refinery wastewater cannot be considered suitable for irrigation purposes, human consumption and even enter into the natural water streams without treatment. To overcome this unfavorable scenario, there is dire need to treat refinery wastewater before entering into the environment. As non-biological treatment processes are not only quite expensive but are not eco-friendly, therefore alternative means including biological treatment processes have to be adopted. For this purpose, present study was accomplished to treat refinery wastewater through Phycoremediation. Phycoremediation/Bioremediation process employs microorganisms including microalgae for wastewater treatment.

Algae fall under a diverse group of unicellular, multicellular and photosynthetic eukaryotic microorganisms having over 500 genera and more than 8000 species reported till now globally. Algae are currently in use as Phycoremediation tool for treating industrial wastewater before entering into natural streams. Literature about algal diversity in habitats around refining industries is not available in abundance as compared to other industries or general habitats. Due to this problem, present study was designed and conducted to treat wastewater from streams of Attock Refinery Limited, Morgah, Rawalpindi (33°33′18.68″N 73°4′38.3412″E). For this purpose, collection and identification of indigenous algal species naturally occurring in the wastewater effluents of oil refinery was carried out and documented at initial stages of urbanization in refinery surroundings and can be used as baseline data for future studies. During this study, seven sampling locations were randomly selected. After sampling identification of species was

carried out on the basis of their taxonomical characters studied under the light microscope at 40x resolution. Further isolation and propagation of species was done by using BG-0 and BG-11 media under complete sterilized conditions. Taxonomic results showed that algal flora of wastewater channels at ARL is comprised of 18 species belonging to 4 phyla, 5 classes, 9 orders,

15 genera and 12 families. Majority of reported species fall under Phylum Ochrophyta (9) and Cyanobacteria (7). It was observed that *Lyngbya sp.*, *Tribonema sp.* and *Scenedesmus sp.* frequently found at every sampling site. Selected species were cultured after isolation process in the laboratory and allowed to interact with refinery wastewater. Various physio-chemical parameters like pH, Total Soluble Solids (TSS), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Oil and grease (O&G), Phenols, Sulphates (SO_4), Sulfides (S_2), Chlorides (Cl_2) and Total Dissolved Solid (TDS) were analyzed by using methods of American Public Health Association (APHA).

Laboratory trials showed quite promising results and predicted the potential of tested species to perform well under field conditions. It was observed that efficiency of each species was increased gradually up to completion of experiment (end of third week). Among all the three species *Scenedesmus sp.* performed best during third week i.e., pH (7.26), TSS (6.87), BOD (4.36), COD (9.71), O&G (0.37), Phenol (0.02), SO4 (162), S2 (0.01), Cl (155) and TDS (154) (values in ppm), (Although TDS showed lowest value in first Week of second season 106 ppm). Overall this study suggested that these three locally abundant species may be used in combination/proportions for improving wastewater quality before using them for irrigation or discharge into the natural environment. Results showed that refinery wastewater treatment through Phycoremediation on field scale could be cost effective, environment friendly and easy to operate solution for future.

CHAPTER 1

1. INTRODUCTION

Water is the foremost vital natural resource for life on earth and water quality is most prominent environmental issue. The accessibility and quality of water always had a significant influence for suitable habitat selection regarding humans and their quality of life. The freshwater shortages in particular areas are results of deterioration of freshwater sources due to increase in population and industrial development (Al-Hussieny *et al.,* 2020). Several countries utilize river water source to cope with the household and industrial consumption (Gani *et al.*, 2015). To mitigate the demands of expanded population for freshwater, techniques of wastewater treatment ought to be applied. Numerous local and large-scale programs aim at providing good ecological status of fresh water and marine ecosystems (Asiwal *et al.*, 2016).

Wastewater is a water that has been negatively influenced in quality by anthropogenic impact. Mainly it is of two types like Municipal and Industrial wastewater. Municipal wastewater comprises of effluents discharged by household dwellings, sewerage and storm drains. Industrial wastewater includes the certain quantity of pollutants. Wastewater effluents comprise of a wide range of potential contaminants and concentrations (Tsiknia *et al.*, 2014). Petrochemical industries including petroleum refineries generate a large quantity of pollutants and release waste materials into the wastewater channels and ultimately to the environment. A variety of wastes are produced in refineries either in gas, liquid and solid forms that must be treated and disposed of in an environmentally safe manner. Production, refining and conversion of crude oil into useful chemicals are associated with the direct or indirect release of waste materials and pollutants into the environment (Han *et al.*, 2021).

1.1 General Composition of wastewater

Wastewater is composed of effluents released by household residences, commercial properties, industrial areas and agriculture (Sanchis-Perucho *et al.*, 2018). The wastewater comprises 99% water and 0.1% of the other components including microorganisms, organic and inorganic compounds. The organic contents comprising protein, sugar content, oil and grease, coloring, solvents, phenols, cyanide, fat, vegetable content from food or detergents or pesticides and human excreta. Suspended solids are actually the organic matter that remains suspended and are insoluble particles. These materials are highly toxic, aesthetic disturbances (Xiong *et al.*, 2019). In order to remove most of the organic materials which are also considered as key pollutants, the wastewater needs treatment (Sun *et al.*, 2019).

The wastewater also comprises of biodegradable organic materials which leads to oxygen depletion in streams, lakes and rivers ultimately causing fish death and odors. Nutrients and heavy metals found in wastewaters such as nitrogen, potassium, ammonium, cadmium, copper, chromium, mercury, lead and nickel have a detrimental impact. Inorganic materials i.e. acids including hydrogen Sulphate and bases cause corrosion and altering the favorable conditions for flora and fauna as well (Xiong *et al.*, 2019).

Crude oil contains small number of water-soluble compounds but water and hydrocarbons are basically immiscible which makes wastewater treatment operations a challenge. The petroleum industry serves as an important pollution distributor discharging heavy metals in freshwater bodies. Petroleum refineries through refining processes convert raw crude oil and other petroleum hydrocarbon sources into different useful end products and intermediates. A large volume of wastewaters is generated through oil refining process. This wastewater contains a significant number of pollutants (Hodges *et al.*, 2017). The oil refineries are under immense pressure to comply with the rules and regulations related to environment protection and operation safety. Despite a regular generation of waste, there are inevitable discharge of pollutants in oil refineries owing to faulty plants, erosion, pumps leakage, corrosion and pipeline failure, etc. (Ajayan *et al.*, 2015).

The receiving bodies for wastewater releasing from petroleum refineries are rivers, lakes, irrigation systems and the marine environment. Major ecological constraints like eutrophication, foaming, dissolved oxygen depletion and fish kills are reported. Therefore, uncontrolled wastewater contributes to water resources degradation, reduces agricultural production and affects public health and biodiversity. On the contrary, the controlled use of wastewater, through treatment and planning, leads to water resources augmentation, particularly in the countries that suffer from water scarcity conditions, in addition to environmental protection. The use of wastewater in irrigation may also improve groundwater conditions, by recharging aquifers thereby lessening overdraft and preventing seawater intrusion in coastal areas.

1.2 Contribution of petroleum sludge

Petroleum sludge contains both organic components and heavy metals with typical range of concentrations (American Petroleum Institute (API), 1989). High concentration of metals in petroleum sludge was reported as Chromium (480 mg/kg), Copper (500 mg/kg), Iron (60,200 mg/kg), Nickel (480 mg/kg), Lead (565 mg/kg) and Zinc (1299 mg/kg) (Da Rocha *et al.*, 2010). The inappropriate dumping of petroleum sludge to the ecosystem, cause a serious risk as noteworthy changes in the chemical and physical properties of the soils of surrounding areas, leading to morphological changes (Murungi and Sulaimon, 2022). It also causes nutrient deficiency and reduce development of the plants cultivated in such soils (Al-Mutairi *et al.,* 2008). Sludge contains polycyclic aromatic hydrocarbons (PAHs) and petroleum hydrocarbons (PHCs) which are genotoxic to both human and other animals due to unproductive sludge treatment and inappropriate disposal of sludge into the ecosystem is a serious health issue (Murungi and Sulaimon, 2022). These components are recalcitrant due to their heavy molecular weight, strong bonding ability, water repelling ability and slightly soluble in water. The high consistency of the petroleum sludge fills the soil pores and reduce the aeration in the soil particles. Their presence also reduces the soil wettability, hygroscopic moisture and hydraulic conductivity. The high molecular weight of these elements develops a water repelling film resulting in availability of water-air movement in the soil (Tang *et al.*, 2012). These hydrocarbons are able to seep through the soil layers into the groundwater reservoirs, resulting in serious danger to the water bodies (Xu *et al.*, 2009) and reducing the diversity of microorganisms in soil (Suleimanov *et al.,* 2005).

1.3 Management of petroleum sludge

Appropriate management of petroleum sludge has attained considerable emphasis because of enhanced production and harmful nature. Numerous techniques have been applied to manage it for reducing the injurious contaminant concentration or allow them to settle down and then to reduce the effect on environmental and human health of this toxic matter. These techniques involve incineration, chemical handling, land farming / land filling, photo catalysis, pyrolysis, solidification or stabilization, solvent extraction, ultrasonic treatment and biodegradation (Xu *et al.*, 2009; Yan *et al.*, 2012). Among the aforesaid techniques only some of them are fruitful depending on the concentration of the dangerous waste material, cost of treatment and environmental policies. Few approaches of recycling hydrocarbons from waste are centrifugation, electromagnetic method, frost and melt treatment, forth floating, microwave radiation, sludge pyrolysis and surfactant enhanced oil recovery (EOR). Mostly treatment methods have three phases of waste management approaches are utilized (Al-Futaisi *et al.*, 2007), initially to reduce the petroleum sludge development at source, retrieving oil from the oily sludge and disposing off the un extractable petroleum outlets.

1.4 Significance of treatment of wastewaters

The ecological threats to natural ecosystems in general and water bodies in particular due to ever increasing industrial growth and resultant untreated wastewaters has becomes alarming. Very little intension has been made in the past on wastewater treatment facilities whereas treated water supply often received more priority than wastewater collection and treatment. However, due to global industrialization and continuous urban development, wastewater treatment has been given greater emphasis. Currently there is a growing awareness of the impact of wastewater contamination of water bodies including rivers and lakes; wastewater treatment is now receiving greater attention from the World Bank and government regulatory bodies (Rose, 1999).

Treatment of the wastewater effluents is executed through a series of on-site treatment technologies involving primary and secondary wastewater treatments. Wastewater has conventionally been treated by physical, chemical and biological approaches such as activated sludge (AS), gas floatation, hydro cyclones, electro and chemical coagulation, electrochemical oxidation; membrane filtration, evaporation ponds, waste stabilization ponds (WSP), trickling filters (Mohamed *et al.*, 2018), biological aerated filters and rotating biological contractor, etc. (Hodges *et al.*, 2017). A biological treatment is applied after these operations in a suspended growth procedure. However, these processes are typically associated with numerous operational problems, which include: poor ability of the sludge to settle due to low F/M (food to microorganism) ratio; production of extra-cellular polymers consisting of lipids, proteins and carbohydrates that adversely affect sludge settling; biological inhibition due to toxic compounds. Some shortcomings of these approaches are technical workers are compulsory, settled sludge in the sedimentation basin is highly expensive. Similarly wastewater approaches can eradicate the severe toxicity issues (Hodges et al., 2017).

The wastewater discharges contain high concentration of nitrate and phosphate into the freshwater bodies leads to eutrophication in lakes and rivers and results in other hazardous concerns regarding health and agriculture (Rajasulochana *et al.*, 2009). Due to addition of these domestic and industrial untreated sewage into fresh water bodies toxic algal blooms developed. This decreases water quality and causes hypoxia, anoxia, kill fishes and other trophic disturbances (Khan *et al.*, 2017).

Wastewater has high potential to reutilize in farming, particularly in arid and semiarid areas. Wastewater reuse increases food production by providing a stable supply of water and contributes to environmental security by reducing the pollution level of rivers and surface water (Zaidi, 2007). Treatment processes that couple carbon capture and wastewater treatment with low or no carbon emission (Mohamed *et al.*, 2018) can be considered as the most sustainable options. In order to resolve this issue, the treatment of refinery wastewater can be obtained through the use of algae and microalgae as Phyco or Bioremediation agents. Microalga use light, CO_2, nutrients and water to produce biomass through photosynthesis (Hsueh *et al.*, 2009).

1.5 Importance of algae in ecosystem

The term 'Algae' was first coined by Linnaeus in his renowned book Species Plantarum in 1753 (Linneaus, 1753) and it was de Jussieu, (1789) who classified the plants and delimited the algae from the rest of the plant world to its present status. They are either unicellular or large multicellular, eukaryotic organisms without any multicellular sex organs. Algae are heterogeneous group and photoautotrophic organisms playing a main role as primary producers in aquatic habitats. Algae assists the development of the food chain for other animals (Aliya *et* *al.,* 2009). Algae are of are highly diverse in size, form, structure, habit, color and habitat. The size ranges from microscopic to enormous forms reaching several meters (Ahmed *et al.*, 2016). Few of the algae species are motile having flagella and some of them are non-motile. Some species live independently, few form colonies or filamentous structure. The large multicellular algal species have specialized tissues, but true stems, roots and leaves absent as compared to complex, higher plants. They have a kind of complicated structure that can be differentiated into several parts carrying out specific tasks. Nonetheless, their cell wall is made of cellulose similar to plants. Whereas, the algae have pectin in their cell wall which made little slimy in appearance.

Even though most species of algae are phototrophic while other are mixotrophic, and some are heterotrophic. The species attain energy both from photosynthesis and organic carbon uptake are known as Mixotrophic pigment less and colorless called heterotrophic but still have essential genetic affinities with their phototrophic species (Pfandl *et al.*, 2009). The mode of propagation may differ from asexual to sexual reproduction.

1.5.1 Aquatic algae

Predominantly the algae are aquatic and are found in fresh water growing in ponds, pools, Lakes Rivers, tanks etc., in brackish water as well as in marine water. The commonly found fresh water algae are *Nostoc, Oedogonium, Chara, Cladophora*. Some freshwater algae have been reported from tropical waters e.g., as *Cyclotella sp., Cocconeis pediculus, Orthoseira roeseana; Microcystis sp*., *Nostoc sp., Oedogonium sp., Volvox sp*. etc. While some species have been collected from the temperate waters (*Pediastrum sp., Fragillaria sp., Nitzschia sp.*).

Based upon habit, freshwater algae can be classified as planktonic, benthic, neustonic algae. The planktonic algae float easily on the surface of water bodies and can be further categorized into groups. First group is Euplanktons (*Chlamydomonas, Cosmarium, Microcystis,* *Scenedesmus, Volvox,* etc.) and Tychoplanktons (*Cladophora, Oedogonium, Zygnema,* etc.). Second is Benthic algae are found in the bottom of water bodies e.g., shallow pools. Epizoic such as *Cladophora* grows on snail; Epilithic (*Batrachospermum monilisperme*, *Tribonema minus, Ulothrix tenuissima* etc.); Epipelic (*Clostarium sp., Cosmarium sp*., *Oedogonium sp.* etc.) Epiphytic (*Ulothrix sp., Vaucheria sp*.). The last group is Neustonic algae grow at air water interface for e.g. (*Botrydiopsis (Xanthophyceae), Chromatophyton (Chlorophyceae), Nautococcus (Chlorococcaceae)* (Sahoo and Baweja, 2015).

1.5.2 Supralittoral algae

These algae grow above the water surface as well as on the rocky shore e.g., *Prasiola stipitata* (a green seaweed), *Ulothrix flacca* etc. Sublittoral or infra littoral algae generally grow below the water level e.g*., Anabaena and Oscillatoria* etc. Littoral algae found grown in the areas with the periodic exposure of tides and the areas in-between shore and sea e.g *Dictyota sp., Gracilaria sp., Grateloupia sp., Polysiphonia sp., Rhodymenia sp., Chondrus crispus, Laminaria sp.* etc. Algal species recorded in intertidal zones are *Euglena sp., Fucus sp., Gigartina sp., Laminaria sp., Porphyra sp.,* etc. The aerophytes growing on the surface of walls, rocks, leaves, bark (*Chroococus, Pleurococcus, Scytonema, Trentepholia,*), Epiphloeophytes growing on the tree bark (*Cephaleuros Phycopeltis epiphyton, Somatochroon*), Epiphyllophytes found on the leaves surface (*Chlorococcales, Cyanoderma, Pleurococcus, Trentopholia, Trichophilus*). The cryophytic algae grow in Polar Regions of the world e.g., *Chlamydomonas nivalis, Gloeocapsa*, *Scotiella.* Endozoic algae grow inside the vertebrates or aquatic animals' bodies for e.g., *Anabaeniolum. Oscillatoria, Simonsiella, Zoochlorella, Zooxanthella.* Several algal species are found growing on the surface of other aquatic animals known as epizoic algae, e.g., *Bascicladia, Cladophora crispate, Dermatophyton, Oscillatoria, Protoderma* and *Stigeoclonium*. These grow on other aquatic flora are Epiphytic algae e.g *Chaetonema rivularia, Coleochaete nitellarum*, *Chaetophora cocconis.* Halophytic algae grow in highly saline waters e.g., *Chlamydomonas ehrenbergii, Dunaliella sp., Oscillatoria sp., Stephanoptera sp.,* and *Ulothrix sp.* Parasitic algal species dependent on other living organisms e.g., *Cephaleuros virescence, Ceratocolax sp., Polysiphonia fastigata and Rhodochytrium phyllosiphon.* Symbiotic algae such as *Anabaena azollae, Anabaena cycadaceae* and *Nostoc sp.* Algae growing on soils, logs, rocks etc. are called Terrestrial Algae e.g., *Botrydium, Fritschiella, Oedocladium* and *Vaucheria.* These algae grow in hot springs or hot waterbodies are therophyte algae (*Scytonema, Synechococcus, Synechocystis, Phormidium* etc.) (Madadi *et al.*, 2016).

1.6 Types and techniques of algal flora cultivation

Algal species can be grown anywhere with favorable conditions such as ample nutrients, suitable temperature and abundant sunlight. They also found in the marginal lands where cultivation of cereal crops is unable to grow e.g., rocks, hills, barren land, walls and rooftops (Wahlen *et al.,* 2011). Similar to the higher plants, carbon dioxide (CO_2) is essential for the growth and development of algal species. This feature is highly effective in CO_2 sequestration of the algal biomass production system. The CO_2 released from the industries, untreated raw biogas or crude natural gas from the gas well sites and also found in atmosphere. The conventional carbon dioxide removal from the gasses released with the help of chemicals like amine solvent can be successfully replaced by the cultivation of algal flora (Sarker *et al.,* 2016). Microalgae cultivation can be classified into two main categories, such as cultivation in Ponds and Photobioreactors (PBRs). The major difference between a pond system and a PBR is that the growing algal species in ponds generally depends on the natural resources, while PBRs have controlled environment. The PBR is very expensive and operating cost is higher than that of the pond system. Lately, about 90% of the total microalgae species are produced commercially in open pond system.

1.7 Growth modes

The growth characteristics and cell composition of microalgae are significantly dependent on the cultivation conditions. The cell composition and growing habits of microalgae are highly dependent on the culture conditions (Chojnacka and Marquez-Rocha, 2003). The nutrient and energy sources provided by each cultivation condition is different. The variation of lipid content and biomass production is observed in the microalgae grown in various cultivation conditions. However, oil production of the microalgae cannot be determined only by considering the lipid contents. Therefore, both lipid contents and biomass production of microalgae must be measured. The combined impact of oil contents and biomass productivity is more suitable index to determine the capacity of oil production of the microalgae (Chen *et al.*, 2011). Microalgae are highly adopted to attain nutrients or resources from their environment and enhance their efficacy to utilize these resources. They have the ability to cause metabolic shift depending on the environmental conditions (Mata *et al.,* 2010). The main cultivation conditions of microalgae are photoautotrophic, heterotrophic, mixotrophic and photo-heterotrophic cultivation. The cultivation method, types of nutrient sources, effects and other information of each condition are given below:

1.7.1 Photoautotrophic cultivation

Photoautotrophic cultivation is a condition where light sources are available for energy while inorganic carbon (i.e., carbon dioxide) as carbon source to form chemical energy through photosynthesis (Huang *et al.*, 2010). This is generally used cultivation condition for microalgae growth. By utilizing photoautotrophic cultivation condition, it is observed that the lipid content varies from 5-68% depending on microalgal species (Chen *et al.*, 2011). To enhance the lipid content of species during growth, a nutrient limiting or nitrogen limiting condition can be applied (Mata *et al.,* 2010). Nitrogen limited conditions inclines to increase the accumulation of lipids, the highest lipid productivity is attained when the condition persist amid increasing lipid content and harvesting time is crucial (Widjaja *et al.,* 2009). Photoautotrophic cultivation participates in Carbon dioxide sequestration from the atmosphere as the carbon source for the cell growth. Therefore, the microalgae should be encouraged to grow in the vicinity of the factories or power plants that are able to supply a large quantity of carbon sources for growth.

The combined cultivation of microalgae with yeast in autotrophic cultivation, the Carbon dioxide produced from the cultivation of yeast can be used as a carbon source for the growth of microalgae. This procedure strengthens the sustainable approach of microalgae cultivation from the sequestration of carbon released by yeast (Yen *et al.*, 2020). This combined microalgae and yeast culture cultivation has also increase the carbon dioxide bio-fixation rate and formation of lipid, where the biodiesel produced will have good oxidative stability (Zhu *et al.*, 2013). Furthermore, risk of contamination is less damaging in photo autotrophic cultivation condition than the other three cultivation conditions. Therefore, in-vivo cultivation system of algae is generally recommended to be done utilizing this cultivation condition in order to control the level of contamination. Since the biomass concentration of the microalgae enhanced under photoautotrophic condition resulting in the increase of harvesting cost of microalgae grown in this cultivation condition.

1.7.2 Heterotrophic cultivation

The heterotrophic cultivation is a condition in which micro-algae utilize organic compounds as carbon and also sources of energy (Chojnacka and Marquez-Rocha, 2003). Few micro-algal species can grow in both photoautotrophic and heterotrophic conditions depending on the availability of the light source. The issues related to the limited supply of light during photoautotrophic cultivation causing reduction in higher cell density in large scale photobioreactors can be avoided by utilizing heterotrophic cultivation (Huang *et al.*, 2010). Several researchers have reported that it is found that a higher biomass and lipid productivity of microalgae can be attained by utilizing heterotrophic cultivation condition. Some microalgae species result in enhancing lipid content utilizing heterotrophic cultivation, for instance *Chlorella protothecoides* has a 40% increase in lipid content after varying the cultivation condition from photoautotrophic to heterotrophic (Xu *et al.,* 2006). Numerous organic carbon sources can be consumed by microalgae during growth and development such as glycerol, fructose, galactose, glucose, lactose and sucrose (Liang *et al.,* 2009). Corn powder hydrolysate (CPH) is a cheaper organic carbon source and recently utilized in several studies. Though one of the major drawbacks of this condition is the contamination of the sugar when used as an organic carbon source (Chen *et al.*, 2011). Therefore, in-vivo system is not recommended for this cultivation condition and hence conventional microbial bioreactors are more appropriate cultivation medium for this mode of cultivation condition. Similarly, the utilization of the organic compounds enhances the substrate cost required for the microalgae growth and consequently increasing the total cost of production of the heterotrophic cultivation condition.

1.7.3 Mixotrophic cultivation

Some microalgae species perform photosynthesis as the main energy source and use both inorganic (carbon dioxide) and organic compounds as carbon sources for growth. The growth of microalgae species under such cultivation condition is called mixotrophic cultivation. These species are capable to grow under mixotrophic cultivation can live either photoautotrophic or heterotrophic condition, or both depending on the concentration of organic compounds and intensity of available light (Kong *et al.*, 2010). They assimilate organic and inorganic compounds as the carbon sources and the carbon dioxide produced during respiration will be trapped and reused under photoautotrophic cultivation condition at the availability of light (Mata *et al.,* 2010). The ability of growing under phototrophic cultivation, this mode can play a vital role in global carbon dioxide reduction as consumption of CO_2 contributes in providing carbon source for microalgal growth. Use of organic compounds could result in the several issues regarding contamination in this cultivation condition. Therefore, a closed photobioreactor is utilized for this cultivation where light sources can be provided and contamination can be reduced. In addition, microalgae that grow under mixotrophic cultivation condition have relatively high lipid and biomass productivities compared with heterotrophic cultivation condition (Shu and Tsai, 2016). Still, mixotrophic cultivation is less frequently utilized in growing microalgae for oil production than compared to photoautotrophic and heterotrophic cultivation conditions.

1.7.4 Photoheterotrophic cultivation

The cultivation condition where micro-algae require light while utilizing organic compounds as the carbon source is Photoheterotrophic cultivation, also known as photo-organotrophic, photo-assimilation or photo-metabolism (Mata *et al.,* 2010). As photoheterotrophic cultivation requires light while mixotrophic growth requires organic compounds to carry out this function (Chen *et al.*, 2011). Therefore, photoheterotrophic cultivation simultaneously requires both organic such as sugars and a light source (Chojnacka and Marquez-Rocha, 2003). Production of biodiesel using this approach is still infrequent, even though the production of some photo-regulated beneficial metabolites can be increased by utilizing photoheterotrophic cultivation (Ogbonna *et al.,* 1999). Like mixotrophic and heterotrophic cultivation conditions, contamination related issues arise in photoheterotrophic cultivation as organic compounds are used as carbon sources. Additionally, this mode requires a special design of photobioreactor as the medium of cultivation during production which increases the capital cost and operating cost of cultivation. Various carbon and energy sources are being used in different cultivation conditions. Therefore, species performance index (cell density) vary as the nutrient source differs. Several systems scale-up differently as various cultivation conditions require different operating conditions and environment.

1.8 Role of Petroleum Refineries

Petroleum industry has a prominent role in the industrial sector of Pakistan. Most efficient utilization and benefits from crude oil are attained by the production of several products such as fuel, lubricants, waxes and bitumen etc. On the basis of demand through processing in a refinery (Ojumu *et al.,* 2005). Though refineries and petrochemical industries are essential for the economy and improved quality of life but the injurious and environmentally inappropriate pollution impacts of the wastes produced from this industry have been reported on global level (Ruiz-Ordaz *et al.,* 2001). Petroleum hydrocarbons are most remarkable contaminants in the ecosystem, as they result in destruction of the adjacent ecosystems as well. The pollutants found in contaminated sites by refinery effluents contains organics, phenols, acid, heavy metals, polycyclic aromatic hydrocarbons, salts and trace elements such as cadmium, chromium, copper, lead, mercury, zinc etc. at varying concentrations (Pathak *et al.*, 2010).

The pollutants in refinery wastes are phenols and its derivatives. They are disseminated in several ecosystems as natural or artificial mono-aromatic compounds and cause a severe environmental problem owing to versatile use, toxicity and distribution in the ecosystems. Phenols used for domestic purpose released in the freshwater could cause a serious threat to human health. High density of the hydrocarbons in sediments have been traced to petroleum refinery effluents (Ojumu *et al.,* 2005)

The toxicity of the phenols and its compounds generally cause the reduction of wastewater bio treatment even at low concentrations (Abd-el-haleem *et al.*, 2003). While physico-chemical approaches have been utilized for reducing phenols and its compounds. Biological methods are considered more efficient as they are cost effective, easy to handle and environment friendly. It also helps in reducing the production of other useless toxic products as a result of physico- chemical methods requiring further processing (Thavasi and Jayalakshmi, 2003).

Recently, the removal of pollutants produced by petroleum industry is becoming a severe problem. Although safe disposal, burial and incineration in protected landfills are highly expensive for the huge quantity of contaminants but considered accepted at current situations. Few approaches like gasification, incinerator, pyrolysis and secure landfills are still efficient but the production of contaminants during these methods have adverse effects on the ecosystems and public health (Taiwo and Otolorin, 2009). The other methods such as thermal and chemical generally used to remove hydrocarbons have less efficient and expensive. Therefore, bioremediation is recently most emerging approach for the treatment of refinery effluents and petroleum contaminated sites. Biological agents such as plants or microorganisms convert the complex organic contaminants into other simpler organic compounds due to their diverse metabolic abilities for the elimination and degradation of the various environmental pollutants (Medina-Bellver *et al.*, 2005). This process is applied by biological agents, generally bacteria, fungi, algae and plants are involved in the reduction or removal of toxic contaminants.

1.9 Impact of Bioremediation of wastewater

Bioremediation methods are being used by using naturally occurring microorganisms to degrade the environmental contaminants, especially organic wastes into less toxic concentrations (Kshirsagar, 2013). Bioremediation can be proved to be less expensive than other approaches which are previously used for the treatment of harmful waste (Vidali, 2001). It was first used during Roman's era (about 600 BC) to treat waste water. However, phytoremediation as a bioremediation process is not a new technique to remove heavy metals or organic compounds from wastewater or contaminated soil. About 300 years ago some plants like *Thlaspi caerulescens* and *Viola calaminaria* were reported to accumulate high concentrations of heavy metals. Recently, these efforts continued to add more plants that have the potential in removing toxic metals and organic compounds from soil and water. Bioremediation seemed a good alternative to conventional technologies in many countries across the world because it is cheap and easy to implement (Azubuike *et al.,* 2016). It was first concentrated on the degradation of the most toxic environmental pollutants into less toxic forms and to levels below the concentration limits established by local authorities. Therefore, phytoremediation techniques have been developed and seemed as good choice and have the potential as effective techniques to clean-up soils and water contaminated with toxic compounds. Some potential plants can be widely used to remove heavy metals or petroleum hydrocarbons from soil and water.

Phytoremediation has been considered as innovative bioremediation processes, non- destructive and clean in-situ technology that uses green plants and the associated microbes to remove, transfer, stabilize, metabolize, degrade, or volatilize contaminants in soil and ground water for environmental cleanup. Phytoremediation is a non-destructive and economic technology that uses plants to clean up soil and water from contaminants. Furthermore, plants may enhance the activities of some microbes to breakdown petroleum products in the rhizosphere. Also, aquatic plants, algae, fungi and lichens can be used to clean soil and wastewater from various types of pollutants and is widely used in many countries across the world (Shi *et al.*, 2021).

As a result of the great industrial and human developmental activities and urbanization in many parts of the world especially in the oil and gas producing countries, organic contaminants accumulate causing a lot of disturbances and damages to many sectors of life including economy, health, biodiversity and agricultures. In addition to the accumulation of heavy metals, plants are able to carry out degradation processes leading to complete or partial decomposition of organic contaminants. There are at least four methods which can be used by plants and the associated microorganisms to remediate soil and wastewater contaminated with petroleum hydrocarbons i.e. Phyto-degradation or Phyto-transformation, Phyto-extraction, Phyto-volatilization and rhizosphere biodegradation.

Industrial wastewater could have negative impact on various sectors of human life, not only because of organic pollutants but inorganic components mainly heavy metals which may cause great disruptive influence on the biological systems as well as disturbing the environment and ecosystem. Crude oil is rich of heavy metals like Ni, V, Cu, Cd, and Pb while some other heavy metals associated with crude gas like Hg, As and possibly other elements and compounds containing sulfur, halogen compounds (containing chlorine and fluorine), nitrogen compounds like amines, ammonia and nitrogen oxides (Henze *et al.*, 2002).

1.10 Role of algal species in remediation of wastewaters

Algal periphyton has great potential for removing metals from waste water (Han *et al.,* 2000; Olguín, 2003). Metal sorption comprises binding of metal on the cell surface and to intracellular ligands. The adsorbed metal is several times greater than intracellular metal. Concentration of metal and biomass in solution, pH, temperature, cations, anions and metabolic stage of the organism affect metal sorption. Algae can effectively remove metals from multi-metal solutions. Dead cells sorb more metal than live cells. Various pretreatments enhance metal sorption capacity of algae. $CaCl_2$ pretreatment is the most suitable and economic method for activation of algal biomass. Algal periphyton has great potential for removing metals from waste water (Han *et al.,* 2000; Olguín 2003). Therefore, the use of microalgae is the best way as it can absorb significant amount of nutrients because they need large amounts of nitrogen and phosphorus for proteins (45-60% microalgae dry weight) and metals as micronutrients for their growth. They have a potential to accumulate high concentrations of metals from the contaminated aquatic systems. Metal accumulation in algae comprises of two processes, like an initial fast (passive) uptake followed by a much slower (active) uptake.

The selection of algae strain to be used in wastewater treatment is determined by their effectiveness against wastewater. The major advantages of using microalgae over conventional methods as summarized by Noue *et al.,* (1992)are: (a) nutrients can be removed more efficiently, (b) no generation of toxic by-product such as sludge (c) biofuels can be produced from biomass harvested which are energy efficient (d) cost–effective.

Nutrient removal by algae involves various metabolic pathways of the algal cell. Carbon, nitrogen, phosphorous and sulfur are the main components responsible for algal growth. Metals such as potassium, sodium, calcium, iron and magnesium serve as micronutrients for the growth of algae. Microalgae has an ability to convert inorganic nitrogen only in the forms of ammonium, nitrite and nitrate to organic nitrogen through assimilation technique. Only eukaryotic algae can perform assimilation of inorganic nitrogen. During algae metabolism, phosphorus mainly in the forms of $H_2PO_4^-$ and HPO_4^{2-} is incorporated into organic compounds through a process called phosphorylation.

1.11 Efficacy of Phycoremediation of wastewaters

Physical, chemical and biological technologies have been developed to treat oily wastewater and restore environmental quality. However, their costs are high and most of them are difficult to use under field conditions, hence in such a condition there is an urgent need to study natural, simple, and cost-effective techniques to control pollution from industrial effluents and treating wastewater through Phytoremediation or Phycoremediation. Considering this, Phycoremediation was assumed to be very useful, as it is an innovative, eco-friendly and efficient technology in which natural properties of algae are used in engineered system to remediate hazardous wastes through physical, chemical and biological processes from wastewater (Ji *et al.*, 2017). Utilizing algae for biomonitoring eutrophication, organic and inorganic pollutants has been enhancing interest. It was possible to evaluate spectrophotometrically the total nitrogen content in water samples from aquatic systems to find eutrophication levels through using the chlorophyll formation of the algae (Abe *et al.*, 2004). Algae has considerable ability for accumulation of heavy metals due to tolerance mechanisms and several algal species synthesize phytochelatins and metallothioneins that can produce complexes with heavy metals and store them into vacuoles (Suresh and Ravishankar, 2004). Another benefit of utilizing algal species in Phycoremediation is the high biomass production by them resulting in high absorption and accumulation of heavy metals (Chekroun and Baghour, 2013).

Management of refinery wastewater through Phycoremediation has two major objectives. First is to protect the environment by reducing the pollution of fresh water resources and hence reducing the health as well as environmental risks and the other is to introduce an environment friendly, cost effective and sustainable refinery wastewater treatment facility for the petroleum industry.

1.12 Problem Statement

Wastewaters from oil refineries require certain level of treatment prior to discharge into natural water courses. Eutrophication and toxic algal blooms develop due to addition of untreated wastewater into fresh water bodies. This decreases water quality and causes hypoxia, anoxia, kill fishes and other trophic disturbances. Similarly, due to increasing scarcity of water in the world along with rapid population increase in urban areas gives reason for concern and the need for appropriate water management practices to compensate these problems.

Subsequently, the investigation of indigenous algal flora can be used for bio-remediation of metals from waste water and have a potential to reduce the number of risks from toxic metals and impact on environment of the area.

The phyco-remediation potential of indigenous algal species of Rawalpindi for the treatment of refinery wastewaters have not been discovered yet.

1.13 Objectives of Research

Following are major objectives of this research project;

- Identification and classification of the indigenous algal species of the wastewater effluents from ARL.
- To improve refinery's wastewater quality through Phycoremediation.
- Comparative study of Phytoremediation of wastewater by algal species.
- Introduction of a cost effective and environment friendly pilot project for wastewater treatment in petroleum industry.

1.14 Significance of Study

Phycoremediation is a cheap and efficient method of decontamination that has become increasingly popular now days to reduce environmental pollution. The effluent discharge from residences and industries constitutes a major source of water pollution. This study attempted to explore the algal flora found in the wastewater effluents of the Attock Refinery Limited (ARL). Moreover, it had essayed the usage of identified algal species for reclamation of refinery wastewater. This study may also help to report the efficacy of algal species regarding reclamation of refinery wastewater. Conferring to identify issues, suitable recommendations will be provided. Also, this study will provide management options for oil refineries, industries, commercial properties, municipal departments, government, NGOs and local level organizations and likely future scenario in light of current facts and figures predicted.

1.15 Hypothesis

Previous studies showed that indigenous algal species have an effective role in the Phyto/ Phycoremediation of oil refinery wastewater.

1.16 Limitations

Although, this study covers the proposed objectives comprehensively. The study lacks the laboratory analysis of heavy metals which may be cadmium, lead, copper, iron etc. as well as their sorption by the indigenous algal flora due to lack of time and financial resources. One of the major constraints faced during the study is the lack of access to some of research organizations due to COVID-19.7

CHAPTER 2

2. REVIEW OF LITERATURE

According to the World Bank, "The greatest challenge in the water and sanitation sector over the next two decades will be the implementation of low-cost wastewater treatment that will at the same time permit selective reuse of treated domestic as well as industrial effluents for agricultural and industrial purposes" (Looker, 1998). To prevent disease transmission, sanitation systems must have high levels of hygienic standards. The recovery of nutrients and water resources for recycling in agriculture and decreasing the overall user demand for water resources are some of the other goals of the treatment (Rose, 1999). The number of conflicts increases among agriculture and household utilization of scarce water resources. Therefore, treated wastewater is necessary for irrigation purposes. Wastewater is composed of over 99 percent water. In a developing urban society, wastewater generation is usually approximately 30 to 70 m^3 per person per year. The wastewater generated by a city of one million people would be adequate to irrigate around 1500 to 3500 hectares (Bdour and Asce, 2007).

2.1 Impact of wastewater on the ecosystem

In arid and semiarid areas, wastewater has a high capacity for reuse in agriculture. Consistent supply of treated wastewater enhances crop productivity and contributes to environmental security by decreasing the level of pollutants in the river and water surface. Major portion of river basin waters is conserved and dumping of municipal wastewater is a cost-effective and safe way. A very small percentage of wastewater is currently being used for irrigation in the world (Zaidi, 2007).

Wastewater reuse after treatment has an important role in water resources management, especially in areas having water scarcity like the Pothwar region. The treated wastewater is received by lakes, rivers, and irrigation systems. Dissolved oxygen depletion, eutrophication, foaming, and fish kills are major environmental issues. Uncontrolled wastewater from industries contributes to water resource degradation, reduces agricultural production, and affects public health. Wastewater reuse reduces the burden on freshwater resources and contributes to water conservation. Properly planned and managed wastewater treatment and reuse schemes have positive environmental and economic impacts. The use of treated industrial wastewater in irrigation improves groundwater conditions by recharging aquifers.

Before the reuse of wastewater for agriculture, it must be treated to a certain degree. The proper wastewater treatment system should provide cost-effective, slightly operational, and maintain requirements and release the effluent according to the recommended microbiological and chemical quality guidelines. The petrochemical industry generates a series of liquid effluents during the refining process which must be treated through depuration processes. The results of these processes are oil refinery sludge, potentially a waste product, that has a high content of petroleum-derived hydrocarbons (Marin *et al.,* 2005).

2.2 Significance of wastewater treatment

With the change from an agriculture-based society to an industrial one, wastewater treatment which is essential for aesthetic, health, ecological, and other viewpoints has become a serious problem (Güngör-Demirci and Demirer, 2004; Clarke and Baldwin, 2002). Both organic components and heavy metals are found in petroleum wastewater or sludge but a typical composition of petroleum sludge consists of the following heavy metals: Cr (480 mg/kg), Cu (500 mg/kg), Fe (60,200 mg/kg), Ni (480 mg/kg), Pb (565 mg/kg) and Zn (1,299 mg/kg) (Admon *et al.,* 2001);Da Rocha *et al.*, 2010). Until now, several options such as land application (Sommer and Hutchings, 2001), ground injection (Morken and Sakshaug, 1998) and constructed wetlands (Clarke and Baldwin, 2002)have been proposed for wastewater treatment/disposal. Though, the water bodies' plant-based treatment systems are cost-effective approaches that can be adopted by developing countries for the treatment of wastewater having a high concentration of heavy metals (Figueira and Ribeiro, 2005; Vandecasteele *et al.,* 2005; Jang *et al.,* 2005).

Few researchers also described a wetland system for wastewater treatment (Nzengy'a and Wishitemi, 2001). The research site continuously receives municipal sewage from two famous restaurants. The recycled treated wastewater is reused for several purposes in these restaurants. Physico-chemical features of the wastewater altered significantly as the wastewater flowed through the respective wetland. A contrast of wastewater influent contrasted with the effluent from the wetland showed the system's apparent success in water treatment, particularly in pH change, removal of suspended solids, organic load, and nutrients mean influent pH = 5.7 ± 0.5, mean effluent pH 7.7 ± 0.3; mean influent COD = 3749.8 ± 206.8 mg/l, mean effluent COD = 95.6 ± 7.2 mg/l; mean influent BOD_5 = 1603.0 ± 397.6 mg/l, mean effluent BOD_5 = 15.1 ± 2.5 mg/l; mean influent TSS = 195.4 ± 58.7 mg/l, mean effluent TSS = 4.7 ± 1.9 mg/l.

In a study carried out by (R. and A., 2004), treated wastewater by utilizing up-flow Anaerobic Sludge Blanket (UASB) followed by Rotating Biological Contactors (RBC). The efficacy of the UASB for the removal of BOD, COD, oil, grease, and TSS were 56%, 56%, 58%, and 85%, respectively. The effluent has been processed further using an RBC unit to improve the quality of treated effluents discharged for application in the irrigation of crops. Residual BOD, COD, TSS, and oil and grease, following RBC, were 40mg/l, 132mg/l, 44mg/l and 10mg/l respectively. Hence, the overall percentage removal of BOD, COD, TSS, and oil and grease were 94%, 91.5%, 96%, and 91%, respectively.

A study indicates that the association of phototrophic microorganisms and bacterium can result in better TPH removal efficiency as compared to systems employing only heterotrophic cultures in bioreactors. At N:P ratio of 19:1, 28.5:1, 38:1 and 47.4:1, the TPH removal efficiencies were found to be 98.6%, 99.4%, 99.4% and 99.3%, respectively. This association of *B. cepacia* and phototrophic microorganisms at a reactor scale could overcome several operational issues generally associated with hydrocarbon depletion. The major advantages of this system apart from high Total Petroleum hydrocarbon removal efficiency include good settle ability of sludge, low phosphorus requirement, no soluble carbon source requirement, and pH stability. This study presents a feasible technology for the treatment of wastewater generated from petrochemical industries and petroleum refineries using algal-bacterial biofilm in RBC (Chavan and Mukherji, 2008).

Wastewater treatment high-rate algae ponds (HRAPs) are presently the only economically viable way to produce algal biomass for conversion to biofuels with minimum environmental impact. Optimizing harvestable algal yield requires a better understanding of the influence of parameters such as CO_2 addition, species control, control of grazers and parasites, and natural bio-flocculation, as these parameters are currently poorly researched for wastewater HRAP systems. Further research on both fundamental and field-scale will assist optimization of harvestable algal yield and thus further improve the economic viability and the full-scale implementation of biofuel production from wastewater HRAP (Park and Craggs, 2010).

2.3 Biodiversity of Algal Species in Pakistan

The biodiversity of the algal species shows significant differences in species richness and variety of habitats in several regions. The anthropogenic activities lead to the deterioration of numerous ecosystems inducing changes in the biodiversity of the microphytes to very complex plants. Several studies have been conducted on freshwater blue-green algal species in different areas of Pakistan. Few studies have been carried out on the freshwater cyanophytes of Karachi (Farzana, 1979; Shameel and Butt, 1984) and other areas of Sindh province (Khalid and Shameel, 2011; Mahar *et al.*, 2009). The first systematic study of blue-green algae was made in the surrounding areas of Lahore and found 59 species, comprising nine new species and three new varieties (Ghose, 1924). Similarly, Randhawa, (1936) worked on *Chlorophyta* and then the addition of twenty-three species of blue-green algae in the identified species of this region. Twenty-five blue-green algal species were isolated from the rice fields of Kashmir (Khan, 1957) and ninety-five species were identified in some districts of Punjab province (Ali and Sandhu, 1972). Some freshwater green algae including Cyanophyta were isolated from the water bodies around Lahore (Masud-ul-Hasan and Yunus, 1978).

Blue-green algae are found in tropical and subtropical ecosystems and are capable of photosynthesis. They can be found in freshwater, marine, or polluted water bodies comprising more than 95% of the flora (Valeem and Shameel, 2005). To understand the diversity and distribution of algal species, few studies had been conducted in lower river swat Khyber Pakhtunkhwa (Sher and Hazrat, 2012). While Zarina *et al.,* (2010) found the diversity of blue-green algae (Cyanophyta) in northeastern Pakistan. Blue-green algal blooms have been found in the coastal areas of Balaji. The pools of the upper littoral region of sandy areas have first-ever shown excessive algal blooms. These algal biodiversity composite of members related to Oscillatoriales with two species of *Oscillatoria (O. Brevis* and *O. tenuis* species*)* and one species of *Lyngbya (L. contorta).* The limnological investigations revealed that *O. Brevis* is first time found in the coastal areas of Karachi (Munawar and Aisha, 2017).

In the freshwater ecosystem, algae are the major group of photosynthetic organisms. Sarim and Zaman, (2005) isolated the freshwater algae of several locations in the Charsadda district. They found 89 species of 31 genera of *Bacillariophyceae, Chlorophyceae, Cyanophyceae,* and *Xanthophyceae.* Furthermore, Ali *et al.*, (2005) identified the variations in biological and physiochemical characteristics of polluted water in a fish pond in the Muzaffargarh district. A survey conducted by Yaseen *et al.*, (2016) in Peshawar district, Khyber Pakhtunkhwa on freshwater algal species of Charophyta, Chlorophyta, Chrysophyta, Cyanophyta, and Xanthophyta. 13 genera and 51 species has been recorded in Peshawar district. A similar study has been done on a fraction of algal species of Kalpan stream and surrounding areas of the Mardan district and isolated 73 species belonging to 25 families and 34 genera of freshwater algae (Khan *et al.*, 2016). Recently, Ullah *et al.*, (2021) studied freshwater algae (Chlorophyta) on a taxonomic basis to elucidate the distribution and variation of algal species in different areas of the Mardan district. Almost 55 algal species belonging to 15 genera were reported from the local vicinity. The most prominent genus was Spirogyra with 17 algal species, *Tetraedron,* and *Cosmarium* with 5 species. Besides, *Cladophora, Mougeotia, Stigeoclonium, Oedogonium,* and *Zygnema* with 3 species. Whereas, *Chaetophora, Microspora,* and *Rhizoclonium* with 2 species in each genus, while *Closteriopsis, Uronema,* and *Ulothrix* were found with 1 species. The change in temperatures causes fluctuation in the distribution of Chlorophyta species, such as in summer the diversity of species increases, and in winter the diversity is drastically reduced.

To investigate the diversity and distribution of freshwater algal species, Aliya *et al.,* (2009) collected around 214 algae species belonging to 6 phyla, 86 genera of 33 families, 15 orders, and 10 classes were isolated and identified from various freshwater ecosystems of Karachi city. Among the species isolated, *Cyanophycota* was the dominant phyla with 82 species, followed by *Volvophycota* with 78 species, *Bacillarophycota* with 38 species, *Chlorophycota* with

10 species, *Euglenophycota, Chrysophycota* with 4 and 2 species respectively. Similarly, some other researchers (Ali *et al.,* (2010); Barkatullah *et al.,* (2013); Khuram *et al.*, (2019) had also conducted various studies on the green algal species found in diverse habitats of Pakistan. About 52 species belonging to 15 genera have been identified from several freshwater ecosystems of Chitral (Ullah *et al.*, 2019). Among these species, *Mougeeotia* were most prominent with 12 species, following *Spirogyra* comprising 8 species and *Zygnema* with 7 species respectively.

In a study conducted by (Naz and Masud-ul-hasan, 2004) forty-six algal species have been clustered belonging to class *Chroocophyceae* from freshwater habitats of several districts of Lahore, Attock, Khushab, Sargodha, Jhelum, Gujranwala, Sheikhupura, In Jhang and In Sialkot In of the In Punjab province, from Swat (Kalam) and Bahrain in the province of Khyber Pakhtunkhwa furthermore some samples were also collected from Muzaffarabad, Chenari and Neelum Valley in Azad Kashmir. These species of edaphic, edaphic, epipsammic, epioikotic, epilithic, and epiphytic blue-green algae were taxonomically inspected and found related to two orders, their families, and eleven genera. Among all identified samples only one was found related to the order *Chamaesiphonales* and the remaining belonged to *Chroococcales.* Only a single species was included in the family Entophysalidaceae, whereas the rest of the 44 species belong to *Chroococcaceae*. Among 11 identified genera, *Gloeocapsa* has the largest biodiversity with ten species, *Johannesbaptistia and Stichosiphon* (single species), *Aphanothece, Gleothece and Synechocystis* (two species), *Aphanocapsa* (four species), *Microcystis* (six species), *Chroococcus* and *Merismopedia* (eight species each). The plethora of species was first-ever identified and described from the freshwater or moist soil habitats of Pakistan.

To assess the diversity of freshwater algae species in Lahore city, (Masud-ul-Hasan and Yunus, 1978) isolated and identified the diversity of some fresh green algae belonging to *Cyanophyta* from several ponds and their vicinity. Research has been conducted reporting thirtyfive algal species related to fifteen genera, eleven families, five orders, five classes, and three phyla *Bacillariophycota, Chlorophycota,* and *Cyanophycota* (Jaffer *et al.,* 2018). The biodiversity found at various water bodies of Tehsil Kasur identified species belonging to five phyla, nine classes, twelve orders, and thirteen families. The dominant phylum was *Cyanophycota* with six genera. Four orders belong to the *Volvophycota. Bascillariophycota* has two genera in isolated species and *Charophycota* is represented by only one genus (Akhtar and Ghazala, 2011).

The first research on diatoms was conducted by (West and West, 1902). After that few investigations were reported on diatoms from the coastal areas of Pakistan (Salim and Iqbal, 1964; Ghazala and Arifa, 2009; Ghazala *et al.,* 2006; Saifullah and Moazzam, 1978). The diatoms recorded from the Saiful Malook, Kaghan valley, and Khyber Pakhtunkhwa comprise twenty-six species (Wazir, 2002). To assess the diversity of the diatoms in District Bannu, Khyber Pakhtunkhwa, the limnological study recorded about eighteen species related to eight genera from various water bodies i.e., Barran nallah, Barran dam, Doab, Kashu River, Kurram River, Khalboi alged, Kochkat River, Tangi alged, Tarkhobi alged and Tochi River. The prominent genus is *Cymbella* with five species, followed by *Gomphonema* with four species (Imtiaz, 2018). The blue-green algae have a high commercial and industrial valve. They are also regarded as bio-indicator of pollution (Moore, 1981). The blooms of these algal species were observed from the sandy portion of the upper littoral area showing an increased level of pollution due to anthropogenic activities. Leaf debris of mangrove forests provides favorable habitat for several blue-green algae species (Alvarenga *et al.*, 2015). The undescribed taxa were identified by studies conducted by a few researchers found in the mangrove areas (Saifullah and Taj, 1995; Saifullah *et al.,* 1997; Bano and Siddiqui, 2003). An investigation has reported that 9 algal species related to 5 genera, 3 families, 2 orders, one class, and one phylum were attained from the sample collected from Manora Karachi (Ayubi and Valeem, 2019). The species identified include members of *Nostocales* with three species of *Lyngbya* having two species of *Oscillatoria* and one species of *Gloeotrichia* genera. Followed by *Chroococcales* with two species of *Aphanothece* and one species of *Microcystis* genera.

Few taxonomic studies of algal flora based on the morphological and cytological characteristics of species were conducted. The distribution of algal species in the Kunhar river was reported for the first time (Leghari *et al.,* 2001). A similar ecological study was made on the diversity of algal flora of Wah garden District Attock (Leghari, 2003). The limnological study of algal species was conducted for the collection of algal flora from various freshwater bodies of Taxila and the surrounding areas. The identified twenty-five species of algal species including two species from the class *Ulvophyceae,* two species from *Zygnemophyceae,* seven *Chlorophyceae,* and the rest of fourteen species belonged to the class *Cyanophyceae* (Khalid *et al.*, 2014). The ecological study of algal species from several freshwater habitats of Khyber Pakhtunkhwa and Azad Kashmir (Imtiaz, 2018). The 115 species reported belong to two phyla, two classes, two orders, fifteen families, and thirty-one genera. The most abundant species (75 species) belonging to twenty-nine genera are related to *Bacillariophycota*. Euglena is the most prominent genus with thirty-four species.

To evaluate the diversity of algal flora of Rawal dam, Islamabad, and Wah garden Attock, a taxonomic study was conducted reporting 296 species, representing 114 genera of 11 phyla. It was also observed that the photosynthetic rate of phytoplankton was enhanced at a higher temperature. Similarly, Munir *et al.,* (2007) documented 102 species of algae from the freshwater stream in surrounding areas of Quaid-I-Azam University. Their taxonomic distribution is categorized into three groups. A limn logical study was conducted on Sawan River from four randomly selected sites to record algal species for three algal cycles (Ahmed *et al.*, 2016). The recorded 227 species representing 112 genera, 71 families, 38 orders, 15 classes, and 7 divisions. *Bacillariophyta* with 75 species, Chlorophyta with 65 species, Cyanophyta having 64 species and Charophyta having 48 species were the most prominent divisions. The most abundant genus was *Cosmarium* with 19 species, followed by *Phormidium* having 18 species.

To explore the algal floral diversity, a study was carried out on the four highly polluted locations of the Sawan River (Khan *et al.*, 2017). The taxonomical study recorded 36 algal species representing 5 phyla. Eight species were observed as rare, eleven of them as less common, eleven as common and six were abundant based on species mean annual importance value index. The disturbances in the diversity of algal flora led to the destruction of freshwater bodies' food web at Sawan River. Moreover, the reduction of phytoplanktons, hydrophytes, fish species, and regional or visitor birds are other rising outcomes. The increase in the formation of toxic algal blooms is also a key consequence. These studies were conducted on various sites of Sawan River found as wastewater and municipal waste disposal channels instead of a steady flowing natural freshwater source in Rawalpindi.

2.4 Role of algae in wastewater treatment

Several studies have been conducted to investigate the role of algae in wastewater treatment. Algae such as *Chlorella Vulgaris* and *Scenedesmus quadricauda* were used for the treatment. The role of microalgae in wastewater has been studied and found that different parameters like PH, phosphate, nitrate, biochemical oxygen demand, and chemical oxygen demand and showed that *C. Vulgaris* has the best removal capacity of nitrate and chemical oxygen demand while *S. quadricauda* has the capability of biochemical oxygen demand and phosphate reduction (Kshirsagar, 2013).

Green algae, particularly Cladophora species, are generally regarded as the best bio indicator of water bodies contaminated by both nutrients and heavy metals. An investigation reported that amount of toxic heavy metals (Ni, V, Cd, Pb, Cr) are found in algal biomass and to use bioaccumulation coefficients in correlated algae/water ecosystems in a sewer bay, which present in algal biomass and to establish the bioaccumulation coefficients in correlated algae/water environments in a sewage lagoon, which represents the tertiary stage of a refinery wastewater treatment plant. The accumulation of heavy metal aerosols from the surrounding industrial activities owing to the on graphic depression between the Eastern Alps and Western Carpathians and the characteristic circulation of air with the predominance of northwest wind direction is anticipated to contribute to the bioaccumulation capacities of algae for these metals. The refinery sewage lagoon has been examined for biochemical oxygen demand, ammonium, halogenated hydrocarbons, petroleum hydrocarbons, pH, phosphates, some poly-aromates, sulfates, and suspended matter. To return sufficient nutrients and proteins into the biosphere, the algal biomass is recommended based on the analyzed and acceptable toxic metals content for compost processing (Chmielewská and Medved', 2001).

The study demonstrated that microalgae biomass possesses the sorption capacity to remove heavy metal ions and other organic compounds. The findings show that *Oscillatoria quadriunctulata* and *Scenedes musbijuga* are growing vigorously, thus they can absorb a high quantity of heavy metals from aqueous solutions. The microalgal species grown in swage wastewater are more than those cultivated in the synthetic medium. Whereas the cultivation of *O. quadripunctulata* and *S. musbijuga* in sterilized petroleum industry effluents was relatively lower than that grown in the standard synthetic medium. The research also shows that the growth of microalgae in petrochemicals effluent has reduced the growth by 2.2% (*S. musbijuga*) and 8.8%

(*O. quadripunctulata*) compared to the standard medium. The bio sorption performance of *S. musbijuga* was high in accumulating Cu (57.5%), Co (29.6%), and Zn (42.7%) than *O. quadripunctulata*, whereas a low amount of Pb accumulation occurred by *S. musbijuga*. A good method for biological tertiary treatment of sewage and petrochemical wastewater is the micro-wastewater treatment systems (Ajayan *et al.*, 2015).

Another study reported that the green algae cultivated during the treatment of municipal and dairy farm wastewater were augmented with carbon dioxide for lipid productivity and nutrient removal. Dairy wastewater was treated outdoors in bench-scale batch cultures. The results showed that maximum lipid productivity also peaked at Day 6 of batch growth, with a volumetric productivity of 17 mg/day/L of the reactor and aerial productivity of 2.8 g/m^2/day, which would be equivalent to 11,000 L/ha/yr (1,200 gallons/acre/year) if sustained year-round. After 12 days, ammonium and orthophosphate removals were 96% and >99%, respectively. Hydraulic residence times (HRTs) system was used to treat municipal wastewater in semi-continuous indoor cultures for 2–4 days. Maximum lipid productivity for the municipal wastewater was 24 mg/day/L, observed in the 3-day HRT cultures. Over 99% of removals of ammonium and orthophosphate were achieved. The findings show that carbon dioxide-supplemented algal species from both types of wastewaters can simultaneously reduce dissolved nitrogen and phosphorus to low levels although producing a feedstock potentially beneficial for biofuel production (Woertz *et al.*, 2009).

The research was conducted on wastewater-born and settleable algal-bacterial culture, grown in a stirred tank photobioreactor under in-vitro conditions and it was utilized to reduce the carbon and nutrients in municipal wastewater and accumulate biomass simultaneously. It was reported that algal-bacterial culture exhibited good settling ability, as the total suspended solid (TSS) could be reduced to 0.016 g/l within 20 min sedimentation. The average removal efficiencies of chemical oxygen demand, total nitrogen, and phosphate were 98.2 %, 88.3%, and 64.8% within 8 days, respectively, while the average biomass productivity was 10.91g/m^{2}Sd. This study provides new insights into rapidly settle able algal-bacterial culture enrichment strategies and supplements the information on microbial ecology and diversity in algal-bacterial culture (Su, *et al.,* 2011).

Refinery wastewater recycling through Phycoremediation provides water for reuse which will help in water resource management through a pilot project. The quality of wastewater has been evaluated by analyzing BOD, COD, pH, EC, TDS, TSS, turbidity, and oil levels. The wastewater could be used for irrigation of lawns, golf courses, and college campuses (Okun, 1997), vehicle washing, fire protection, boiler feed water, concrete production (Santala *et al.,* 1998), and preservation of wetlands (Otterpohl *et al.,* 1999).

2.5 Phycoremediation of wastewater with different algal species

To eradicate the problem of wastewater pollution different kinds of Phyco-remediation techniques are used in previous studies with appropriate procedures and the most appropriate algal species. Some well-known algal species are suitable for the treatment of wastewater including *Caulerpa lentillifera, Chlorella Vulgaris,* and *Spirogyra sp.* which have been investigated for the successful removal of contaminants from wastewater (Khalaf, 2008; Logroño *et al.*, 2017). While *Botryococcus sp., Chlamydomonas sp., Chlorella sp., Scenedesmus sp.,* and *Spirulina sp.* have given successful results in treating municipal wastewater (Álvarez-Díaz *et al.*, 2017; García *et al.*, 2018). Following are the most significant results of previous studies done on wastewater treating algal species.

2.5.1 *Chlorella sp.*

An experiment was conducted by Subramaniyam *et al.*, (2016) on *Chlorella sp.* for the removal of nutrients from brewery wastewater, where *Chlorella sp.* effectively removed total nitrogen, phosphorus, and organic carbon along with its biomass production. Following are the other results of (*Chlorella sp.*) discussed here in detail;

2.5.1.1 *Chlorella vulgaris*

The capability of *Chlorella Vulgaris* for the removal and biodegradation of phenol from wastewater has also been tested where chlorella Vulgaris was tested on different concentrations of various chemicals for a total period of four days. For measuring phenol and cell growth spectrophotometric techniques were used for phenol measurement, and the Neubauer chamber and optical density were used for measuring cell growth. Chlorella *Vulgaris* removed pollutants at both concentrations in a short period but higher removal was achieved at a high phenol concentration of 100 ppm. The average removal rate of 75% was achieved at a solution of 50 ppm and 98% was achieved at a solution of 100 ppm. A reduction in phenol concentrations was also achieved proving that chlorella Vulgaris can treat wastewater and remove nutrients from contaminated wastewater (Baldiris-Navarro *et al.*, 2018).

Another research conducted by Travieso *et al.*, (2008) on *Chlorella vulgaris* reported the efficiency of the removal of waste from distillery effluent and reported that microalgae have the efficiency of reducing more than 98% COD and BOD releasing a safe effluent to the environment as well. Similarly, a study was conducted on *Chlorella vulgaris* efficiency for the removal of Chromium (Cr) and Selenium (Se) their combined effect on *Chlorella vulgaris* efficiency, where these species removed selenite-Se(iv) (49.5± 1.9%), selenate-Se (vi) (93.0± 0.5%) chromium nitrate- Cr (iii) (89.0± 3.2%) and dichromate- Cr (vi) (88.1± 1.3%) over 72 hours (Zou *et al.*, 2020).

An investigation on microalgae chlorella vulgaris was carried out in which a new and sustainable approach of microbial desalination cell (MDC) for saltwater (Jaroo *et al.,* 2019). In the cathode chamber, Microalgae *Chlorella vulgaris* were used to produce oxygen through the process of photosynthesis for the treatment of refinery wastewater and generation of bioelectricity. Within 24 days of the operation period, the optical density was measured in the biocathode chamber where the optical density showed an increase from 0.546 to 1.71. Oil refinery waste water's chemical oxygen demand (COD) of 97.33% was removed from the cathode and 79.22% was removed from the anode chamber. Volatile compounds which were causing odor from wastewater effluent were also effectively removed from wastewater by microalgae *C. vulgaris.* TDS 159.7 ppm/h was also removed with an initial 35000 ppm TDS in the desalination chamber.

Chlorella vulgaris a green algal species has also been used for the biodegradation of petroleum hydrocarbons. Two experimental durations were set of 7 and 14 days where microalgae chlorella vulgaris was treated with crude oil/water concentrations of 10 and 20 g/l. *Chlorella vulgaris* showed resistance to various pollutants and also removed heavy compounds from crude oil with a removal capacity of 94% of the light and 88% of heavy compounds in 14 days. With the increase in crude oil concentrations, *chlorella vulgaris* also showed an increase in its dry weight proving that algal growth is positively affected by crude oil concentrations (Kalhor *et al.*, 2017).

Recently, a study was conducted by Lu *et al.*, (2021) on the removal of heavy metal Cr from wastewater by culturing *Chlorella vulgaris* in a membrane photo bioreactor. A hollow fiber ultrafiltration module was used in a photo bioreactor with the in situ solid-liquid separations, hydraulic retention, and solid retention times controlled separately in the reactor. Significant results were achieved from SRT and HRT by removing toxic pollutants and producing high biomass. MPBR showed maximum results during operations by the reduction of chromium to
50% at a hydraulic retention time of 3 days and solid retention time of 40 days. 2 days of HRT and 40 days of STR removed chromium of 0.21 mg/L/d.

Another research study was conducted by Nagabalaji *et al.*, (2019), to remove nutrients from synthetic and industrial wastewater by microalgal species (*Chlorella vulgaris, Scenedesmus dimorphus, Chlorococcum sp.,* and *Chlamydomonas sp*.). Maximum nitrogen and phosphorus removal were achieved by *Chlorella vulgaris* in synthetic wastewater with 88.6% and 91.2% respectively compared to other microalgal species. The results obtained from secondary treated sewage treated with *Chlorella vulgaris* were in the following order, 68.6% removal for NH_4-N, 74% for NO_3-N, 71.5% for PO_4-P, and 90.2% for chemical oxygen demand (COD).

Removal of nitrogen and phosphorous from wastewater by two green microalgae (*Chlorella vulgaris* and *Scenedesmus rubescens*) was investigated by using a novel method of algal cell immobilization, the twin-layer system. In this system, microalgae are immobilized by self-adhesion on a wet, microporous, ultrathin substrate. Subtending the substrate layer, a second layer containing macroporous fibrous tissue provides the growth medium. Nitrate was removed efficiently from the wastewater by both species. Both algal species effectively removed ammonium, nitrate, and phosphate to less than 10% of their initial concentration utilizing secondary synthetic wastewater. It is conducted that immobilization of *C. vulgaris* and *S. rubescens* on twin layers is an effective means to reduce nitrogen and phosphorous levels in wastewater (Hernandez *et al.,* 2006).

2.5.1.2 *Chlorella pyrenoidosa*

A study was conducted using the microalgae species *Chlorella pyrenoidosa* for the treatment of wastewater and clean energy production (Kumari *et al.*, 2021). The growth of *C. pyrenoidosa* was achieved in real sewage treatment plant wastewater (STPW) and synthetic wastewater (SWW) where it reduced nitrate, phosphate, and chemical oxygen demand (COD) from SWW. While reduction occurred for nitrate, phosphate and chemical oxygen demand (COD) in STPW was 99.5%, 94.2%, and 87.1%, respectively. *C. pyrenoidosa* biomass production and biogas production by anaerobic digestion in the presence of anaerobic microorganisms were achieved. Total biogas produced was 479 ml and methane yielded 147 ml/volatile solid (g) from the untreated biomass of algae and the biomass of algae which was pretreated with Ultrasonic, thermal, hydrothermal and Fenton showed a 15.5%, 8.2%, 18.4% and 10.9% increase in methane, respectively.

2.5.1.3 *Chlorella sorokiniana*

The researchers Fernandes *et al.*, (2017) concluded in their study which was carried out for the removal of nutrients from concentrated toilet waste or black water, that *Chlorella sorokiniana* green microalgae successfully removed all inorganic N and P from wastewater where the ratio for N was 15 and that for P was 26. Initial removal and recovery ratios of N and P are independent of the initial ratios of N: P as in black water, the removal of N takes place when P is completely absorbed by microalgal cells. Overall removal of nutrients from black water was very effective with increased biomass production yielding 12 g dry weight/L. The nitrogen assimilation rate was 75% and Phosphorus was assimilated by green algae *C. sorokiniana* was 100% from toilet wastewater.

2.5.2 *Scenedesmus sp.*

A green microalgae *Scenedesmus sp.* was tested on fertilizer wastewater to remove nitrogen, phosphorus, chemical oxygen demand (COD), and biochemical oxygen demand (BOD_5). The results showed high growth of *Scenedesmus sp.* in wastewater having a growth rate of 0.3 to 0.38/day with dry biomass of 70.2 mg/L. Besides the growth and biomass algal species removed these inorganic toxins in the following pattern ammonium (NH^{+4}) 93%, nitrate (NO^{-3}) 84%, phosphate (PO_3^{-4}) 97%, total phosphorus (TP) 96%, COD 93%, and BOD5 84%. Removal of nutrients, COD, and BOD_5 also enhanced the formation of bio flocculation on wastewater effluent. After these results, it was concluded that fertilizer wastewater nutrients can be easily consumed by microalgae *Scenedesmus sp*. for its growth (Pham and Bui, 2020).

Another study conducted by Acevedo *et al.,* (2017) found that *Scenedesmus sp.* growth effectively occurred in a higher concentration of inorganic nutrients (nitrogen 150 mg/L and phosphorus 40 mg). The removal rate of phosphorus was 65% and that of nitrogen was 80% in real domestic wastewater. *Scenedesmus sp.* efficiently removed nutrients from moderate wastewater load with very few requirements for its maintenance. Suggesting *Scenedesmus sp.* for the treatment of DW, removal of nutrients, and production of biomass for getting biofuel.

2.5.2.1 *Scenedesmus obliquus*

Scenedesmus obliquus was reported to successfully remove almost all the pollutants present in the various wastewaters such as municipal poultry, swine, cattle breeding, brewery, and dairy industries (Ferreira *et al.*, 2017). A study examined the dual application of *Scenedesmus obliquus* in wastewater treatment and the accumulation of biochemical components in microalgal cells (Ansari *et al.*, 2019). Microalgae cultured in wastewater showed an adsorption and microelements removal rate in the following pattern $71.2 \pm 3.5\%$ COD, $81.9 \pm 3.8\%$ NH_4^+, 100.0% NO_3^-, and $94.1 \pm 4.7\%$ PO_4^{3-}. The growth rate of *S. obliquus* observed was 0.42 ± 0.02 $1 \cdot d^{-1}$ with a carrying capacity of 0.88 ± 0.04 g L^{-1}. The yield ($w \cdot w^{-1}$ of dry weight) obtained for lipid was $26.5 \pm 1.5\%$, for protein it was $28.5 \pm 1.5\%$ respectively and for carbohydrate, it was $27.5 \pm 1.6\%$. The biomass was then subjected to biochemical extraction which was already de-oiled, where protein yield obtained was $25.3 \pm 1.4\%$ and carbohydrate yield obtained was $21.4 \pm 1.2\%$, respectively. After the process of de-oiling biomass, biochemical extraction of biomass was done where protein and carbohydrate yields were obtained at 25.3 ± 1.4% and 21.4 ± 1.2%, respectively. Several functional groups (e.g., N H, CH_3, CH_2, C O, C N, P O, and Si O) were shown by Fourier transform infrared spectroscopy on the surface of biomass, confirming biochemical elements accumulated in microalgae. The cost-benefit analysis of the cultivation of microalgae revealed that this approach is economically feasible.

In a recent study, the different substitutions of lipid-free algal hydrolysate and/or waste glycerol were used in a growth medium to increase biodiesel production from microalgae *Scenedesmus obliquus* (Abomohra *et al.*, 2018). Maximum biomass productivity showed by a combination of different concentrations of WG with 15% LFAH, the productivity of biomass was 27.4, 30.5, and 28.9% over the control at a combined 5, 10, and 20 g L^{-1} WG, respectively. More accumulation of lipid occurred with different combinations of LFAH and 20 g/L WG. With the use of LFAH15-WG10 lipid, productivity gave a significant value of 59.66 mg L^{-1} in one day. FAMEs yield by 21.2% as compared to control was increased by LFAH15-WG10 while reducing the ratio of polyunsaturated fatty acids (PUFAs) reduced from 52.1% to 47.8% as of total FAMEs on the other hand mono saturated fatty acid ratio increased from 26.6% to 31.3% of total FAMEs.

Lutzu *et al.,* (2016)also conducted research on the purification of brewery wastewater by microalgae species (*Scenedesmus dimorphus*) where nitrogen and phosphorus were removed by more than 99% in one week: Nitrogen was reduced from 229 mg/L to less than 0.2 mg/L and Phosphorus reduced from initial concentration of 1.4-5.5 mg/L to less than 0.2 mg/L which was its final concentration.

2.5.2.2 *Scenedesmus abundant*

Experimental research was done on the mixotrophic growth of microalgal specie strain *S.*

abundance to purify domestic wastewater (Rajan *et al.*, 2019). The maximum biomass concentration of 3.46 mg^{-1} occurred over a cultivation period of 16 days. Rapid consumption of different pollutants occurred during the growth of microalgae *S. abundant*. Petroleum wastewater hydrocarbon accumulated by the biomass of microalgae. When the biomass of *S. abundance* of 4g was used for the bio sorption processes the COD value of simulated petroleum wastewater was around 8440mg/L and after bio sorption, it was reduced to 1239 mg/L. The biomass of *S. abundance* accumulated hydrocarbons from petroleum wastewater with an efficiency rate of 92.16%. Hydrothermal liquefaction of spent sorbent was done with a bio-oil yield of 43.4 wt. % at 300°C temperature. The reuse of HTL wastewater as a reaction medium increased bio yield by 47.91 wt. % in two cycles. The presence of higher molecular weight hydrocarbons C_8 and C_{21} were also revealed when further analysis of GC-MS liquid hydrocarbons was done. Heavy metals Pb (ii), Ni (II), and Cr (VI) were removed from wastewater using HTL bio-char as an adsorbent where 86.5% removal efficiency was recorded for ions of Ni (II).

2.5.2.3 *Scenedesmus sp.* ISTGA1

Research on microalgae strains *Scenedesmus sp.* ISTGA1 was carried out to check its ability in cleaning municipal wastewater and also to produce biodiesel from its biomass. Sterilized wastewater was taken in which microalgae strain was cultured for 14 days in different flasks with BG-11 medium. An increase in the PH of wastewater occurred from (6.3 ± 0.5 to 8.3 ± 0.2) and DO (2.7-fold). Heavy metals and organic contaminants were also removed when the wastewater was treated by *Scenedesmus sp.* strain ISTGA1, as analyzed by ICP-AES and GC-MS respectively. When ISTGA1 was grown in 100% wastewater the biomass production was 1.81 g/l and lipid production was 452 mg/l which were the maximum values. (C_{16} and C_{18}) saturated and unsaturated fatty acids when mixed in a balanced way produced biodiesel of better quality by microalgae confirming that microalgae *Scenedesmus sp.* ISTGA1 can be used for the

Phycoremediation of wastewater and biodiesel production from its biomass(Tripathi *et al.,* 2019).

2.5.2.4 *Scenedesmus vacuolatus* ACUF_053

Osorio *et al.*, (2019) conducted a study on the removal of nutrients from synthetic wastewater and the production of microalgal biomass in a new biofilm photobioreactor (PBR) configuration by using *Scenedesmus vacuolations* ACUF_053 and *Chlorella vulgaris* ACUF_809. Identification of *S. vacuolations* in five stages: attachment, biofilm formation, maturation I, adaptation, and maturation II done in biofilm development. High nutrient removal capacity was observed in biofilms of *S. vacuolatus* ACUF_053 and *C. vulgaris* ACUF_809. A higher amount of phosphate removal occurred in the attached growth of *S. vacuolatus* as compared to suspended cultures. During the formation and maturation phases of *S. vacuolatus* biofilm complete removal of nitrate and phosphate was recorded which were (60-240 mg l^{-1}) and (20-40 mg l^{-1}), respectively and 90% removal of nutrients removal occurred by *C. vulgaris* ACUF_809 in PBR semi-batch procedure.

2.5.2.5 *Scenedesmus sp*. HXY2

Another study was conducted on microalgae for the removal of nutrients from wastewater and the production of biomass (Ye *et al.*, 2020). For this purpose, eight species of microalgae were taken from the water and soil of the Hexi Corridor region, China, and an 18S rRNA gene sequence analysis was done to identify these species. The highest nutrient removal efficiency greater than 95% was recorded in *Scenedesmus sp*. HXY2 with the best growth was recorded under high total organic carbon and ammonia conditions. Biomass produced by *Scenedesmus sp.* HXY2 on its 12th day was 7.2 × 106 cells mL^{-1}. The lipid content and productivity recorded for this species were 15.56% and 5.67 mg L^{-1} day^{-1}, respectively. The lipids of *Scenedesmus sp.* HXY2 proved to be suitable for biodiesel production as the proportion of unsaturated fatty acids was

60.07%. *Scenedesmus sp.* HXY2 had great potential to produce lipids and purify wastewater from high ammonia and organic contents.

2.5.2.6 *Scenedesmus sp.* Z-4

Another microalgal mutant *Scenedesmus sp.* Z-4 was used to treat wastewater and produce lipids at a minimum temperature of 4, 10, and 15 °C (Ma *et al.*, 2017). Besides the optimal temperature of 25 °C, low temperatures of 4, 10, and 15 °C inhibited algal growth. The relevant biodiesel production at 10 °C was more for net energy output, proving that 10 °C suitable temperature for the recovery of energy and treatment of wastewater by using *Scenedesmus sp.* Z-4. When microalgae were cultivated in wastewater molasses with optimal COD concentration of 8000 mg l^{-1}, initial inoculation ratio of 15%, and C/N ratio of 15. COD, TN, and TP removal rates at 10 °C reached 87.2, 90.5, and 88.6%, respectively. However, the production of lipid content was 28.9% and lipid productivity was 94.4 mg/L/day.

2.5.3 *Spirulina sp.*

2.5.3.1. *Spirulina platensis*

A research study aimed to verify the elimination of penicillin as a pharmaceutical contaminant using the microalgae *Spirulina platensis* and *Chlorella vulgaris* from an aqueous solution (Yazdi *et al.,* 2018). The experiment was conducted on laboratory-scale synthetic samples and sewage samples from two hospitals in Birjand city, Iran. In this article, the influence of factors such as retention time, pH variables, and initial concentration on the disposal efficiency of the antibiotic penicillin was investigated by two types of microalgae. The results of this research study showed that when the number and biomass of microalgae were increased, the disposal efficiency of the antibiotic penicillin also increased, 7 pH or neutral was obtained as the optimal pH for the removal of penicillin for both algae. In addition, the contact time of 8 and 9 days was chosen as the optimal time for *C. Vulgaris* and *S. platensis*, respectively. The use of microalgae in major sewage water treatment of hospitals in Birjand effectively eliminated penicillin-based antibiotics. Therefore, this method can be effective in removing antibiotics of similar structure.

Recently, an olive-pomace oil bleaching experiment was carried out with the biomass of Spirulina platensis as a function of the contact time, the initial pH, and the biomass value and temperature in a batch system (Çelekli *et al.,* 2021). The surface of Spirulina platensis was characterized before and after bleaching olive-pomace oil at pH 2 by Fourier infrared spectroscopy (FTIR). The FTIR spectrum of S. platensis showed that olive-pomace oil bleaching depends on binding to amino and anionic groups due to electrostatic attraction. Rapid blanching was achieved with olive-pomace oil within the first 60 minutes of contact time. Then, the rate of increase of bleaching of olive pomace oil was reduced to a steady state. Bleaching of olive- pomace oil increases with an increasing amount of *Spirulina platensis*. These results showed that bleaching of olive-pomace oil was significantly achieved by microalgae *Spirulina platensis.* The bleaching behavior of olive-pomace oil is well described by the logistic model with a high correlation coefficient and low error values. The results indicated that *Spirulina platensis* has great potential for bleaching olive-pomace oil as an environmentally safe process.

Another experiment was carried out to clean secondarily treated wastewater with microalgae *Spirulina platensis,* experimental results showed that microalgae spirulina platensis was able to remove waste water nutrients and produce high-quality biomass for biodiesel production in an open and commercialized raceway pond (Chavan and Mutnuri, 2019). *Spirulina platensis* was able to remove chemical oxygen demand of 2.86 g/day, PO_4-P of 0.12 g/day, NH_4- N 0.82 g/day, NO_3-N 0.13 g/day, and total nitrogen 0.88 g/day. Bio methane was produced from the harvested biomass of algae at the capacities of 0.1-L and 5-L, Biomass consisted of lipids Dry weight of

26.65% with fatty acids ratios of C:16 and C18:1. Biogas of 165.0 ± 5.39 mL per g of volatile solids can be produced from the residual biomass of algae having 62.38 ± 2.12% of average methane contents. It was demonstrated in the study that microalgae *S. platensis* can treat water with low cost by removing nutrients from it for its consumption and growth and also produce high-valued biomass for biodiesel or biogas production.

The bio sorption experiment of toxic metals from industrial wastewater was carried out by (Rangsayatorn *et al.*, 2002) using algae strains *Spirulina platensis* and *Chlorella Vulgaris*, using the isothermal application, kinetics models, and process improvement to evaluate the effect of acid pretreatment on improving the percentage of toxic metal removal (%TMrem) from wastewater by algae strains (*Spirulina platensis* (SP) and *Chlorella vulgar* (CV) under different adsorbent doses (0.2–2.5 g), pH (4-8) and contact time (5-100 minutes). Acid treatment (Ac-T) changed the functional groups present on the surface of the algae which increased the electronegative groups and improved the percentage of TMrem of Al, Ni, and Cu. The treated SP was eliminated up to 95.0 %, 87.0% and 63.0% of Al, Ni, and Cu at optimum pH 5.5, 6.0 and 7.0 and absorption dose = 2.5 g/L respectively. %TMrem of 87.0%, 79.1%, and 80.0% were obtained with CV treated, respectively. Absorption of TMs in algae is endothermic, spontaneous, and follows Longmir and second-order kinetics. Zeta potential measurement shows that the absorption mechanism between the toxic metal (TM) and the algal strains is controlled by electrostatic interaction. Similarly, BioSource is a sustainable and effective technology for removing TM from wastewater.

2.5.3.2 *Spirulina maxima*

A study was conducted to treat soy sauce wastewater with *Spirulina* NCU-Sm and produce high-valued biomass from its growth. The results indicated that good growth of NCU- Sm occurred in 100% (undiluted) RSW (raw soy sauce wastewater). The biomass yield obtained from

NCU-Sm was 1.984g/L, and values of 93.86, 81.76, 84.08, 40.93, and 63.08%, were recorded in RSW for removal of NH_4^+-N, TN, COD, chromaticity, and salinity. When NCU-Sm was grown mixotrophically it recorded high removal of COD in 100% RSW (Han *et al.*, 2021). The growth of NCU-Sm in Soy Sauce Wastewater was affected by two factors the light spectrum and the other was pH. 11.11% biomass of, NCU-Sm increased when RSW pH was brought to 8, along with this protein contents, Chlorophyll-a and carotenoids were also increased to 8.69%, 37.14%, and 40.40%, and then reached 65.57%, 7.57 and 2.78 mg/g, respectively. Photosynthetic oxygen release efficiency of NCU-Sm was enhanced by red light and carotenoid contents of NCU-Sm were increased to 117.95%, and then reached 5.95 mg/g. growth and protein contents NCU-Sm were negatively affected with the use of blue light but chlorophyll-a content was increased significantly up to 10.99 mg/g. this study also suggested the dual role of spirulina when cultivated in RSW and best for high-valued biomass production which is rich in protein and natural pigments.

2.5.4 *Botryococcus sp.*

Botryococcus sp. was used to understand the biokinetic removal rate of Total Nitrogen (TN), Total Phosphorus (TP), and Total Organic Carbon (TOC) from artificial bathroom grey water (Mohamed *et al.*, 2018). The artificial bathroom greywater was made and the standard was checked using BOD, COD, SS, pH, and Turbidity. The whole process was carried out on a lab scale with 108 cell mL-1 of *Botryococcus sp*. it was noticed that there was a reduction of 51.5% of TN, 49.5% OF TP, and 42.6% of TOC. The measurement of removal of TN, TP, and TOC was determined between the gaps of 3, 5, and 7 days. The results showed a good reduction of TN, TP, and TOC from artificial grey water.

A study was conducted concerning the behavior and ability of two microalgae which includes *Tetradesmus obliquus* and *Botryococcus Brancusi*, a marine diatom and an *Arthrospira maximum* which is a photosynthetic cyanobacterium to grow on liquid digestates which are obtained from zoo technical, vegetable biomass and municipal solid waste (Massa *et al.*, 2017). Of all of these strains, MW LD is the bad medium for growth while *T.obliquus* and *B. braunii* grow on zoo technical (ZW LD). The study showed that *A. maxima* and T. obliquus have the highest removal values from 98.9-99.8 percent as compared to *P. tricornutum* and B. braunii whose values range from 79 and 88.5 percent. This shows that by applying batch cultivation, A. maxima and T. obliquus have a greater amount of carbohydrates, proteins, and ash.

The impact of Zn^{2+} on *Botryococcus braunii* as far as development and the photosynthesis-respiration and the capacity of this microalga to eliminate Zn present in squander water depicted was studied by Areco *et al.,*(2018). *B. braunii* decline as the photosynthetic and respiration rates are upset by the concentration of metal in the solution. The measure of the adsorption uptake concerning the time at constant pressure or concentration is followed by the pseudo-second-order model. The 1:1 proportion among H+ and Zn is reasonably acidic. Zn is eliminated inside 200 days with a worth of 3.4g g^{-1}. In the bioleaching process, the remediation of heavy metals and nitrates is gotten. The remediation of heavy metals in leachate is done by microalgae *B. braunii*. *Botryococcus. braunii* has the potential to increase the pH of acidic waters whereas photosynthesis and respiration are influenced by the increasing concentration of Zn. The zinc removal rate is increased with the pH and metal concentration.

Draining grey water into the stream without treating it causes water pollution and aggravates mosquitos in rural areas. The aim of the study conducted by (Mohamed *et al.*, 2019) was to see the uptake of metal ions by *B. braunii* in artificial settings in the phytoremediation process. Calcium uptake is up to 99.7%, with 83% sodium, 96% magnesium, 64% for iron and

98.8% for zinc ions. Ca, Na, Mg, Fe, and Zn have the following uptake rate 0.93, 0.61, 0.92, 0.76, and 0.86 respectively. It is directly proportional to artificial greywater. Yield coefficients for Ca, Na, Mg, Fe, and Zn ions removal by the specific growth rate of *Botrycoccus sp*. were found to be 6.01, 10.35, 4.5, 0.57, and 0.54 respectively.

Excessive waste generation, global warming, climate change, and increased use of petrochemical fuels will cause an environmental crisis in the world due to increased population. Phytoremediation and sustainable hydrocarbon are being shown interest worldwide due to the upcoming problems. *Botyrococcus sp.* was isolated from the tropical rainforest with an optimum temperature of 23-33 °C and light intensity of 243 micromoles m-2s^{-1} (Gani, 2017). It is found that these species are more suited to the outdoor environment with integrated wastewater Phycoremediation. This was in terms of biomass productivity and bioremediation. Microalgae concentration was performed at 106 cells/mL for both wastewaters removal of nutrients occurred up to 100% and 9.8% in DW and FW, in outdoor conditions. Whereas it occurred at 95.4% and 76.4% in DW and FW in outdoor conditions. Heavy metals showed a significant reduction in both kinds of wastewater. Alum can be used to harvest from DW up to 99.3% and chitosan harvested about 94.9% from FW. Different kinds of cultures obtain different kinds of hydrocarbons. It can contribute to renewable energy sources by biofuel. The obtained hydrocarbons can be used as a chemical value in industries.

2.5.5 *Cyclotella glomerata*

Hammouda and Abdel-Hameed, (1994) studied different Algal species in the Nile, there were 8 different Bacillariophyta species in total, and they were all present during the purifying process. Diatoms reduced to 18.1% of the initial population as a result of treatment, which helped to gradually lower their overall density. The majority of all species were given by the

Bacillariophyta, with *Melosira granulatus* (20.2), *Nitzschia aciculatis* (19.5), *Cyclotella meneghiniana* (16.9), and *Bacillaria paradoxa* having the highest percentages of abundance (11.3). The percentage composition of these species was the greatest (7.4, 7.1, and 4.2 percent, respectively) among all the species at zero time, 14.5, 9.3, and 4.0 percent of the tested phytoplankton, and after a 16-day treatment.

2.5.6 *Fragilaria virescens*

Williams and Wetzel, (2020) have documented broadly, that Fragilaria virescens is a global species, and its existence is admitted in many regional floras. Since its illustration in 1843, the species has been categorized sometimes, yielding over 80+ names of varieties and forms that have been noted in various printed and online catalogs of jewels of the sea names. Some of the names in these catalogs are fully corresponding, or obvious synonyms, etc., but once repetition is eliminated, one must assume that the remaining infra-specific names were supplicated to represent some perceptible deviation from the recommended species – the minimum as acknowledged on the part of the investigator who made the title. What these names might be, and what they might show, regardless of the indistinct nature of the meaning beyond numerous infra-specific categories, is worth exploring. The resolution could be attained, to an exact degree at least, by examining any kind of specimens attached to every of these apparent infra-specific names, should the specimens still be obtainable. One result of these kinds of searches, along with finding new and non-documented taxa, is to throw up several nomenclatural issues. One is spotlighted herein.

2.5.7 *Oscillatoria terebriformis*

Richardson and Castenholz, (1987) regarded *Oscillatoria terebriformis* as a thermophilic cyanobacterium and achieved a diel vertical action pattern in Hunter's Hot Springs, Oreg. All-round most sunrise-to-sunset, community of *O. terebriformis* enveloped the surface of microbial mats in the hot spring spate under a hot spell limit of 54°C against gloom, trichomes make a move downhill by gliding mobility into the substrate to a depth of 0.5 to 1.0 mm, where the population remained until dawn. At dawn, the society swiftly comes back to the top of the mats. Field studies with microelectrodes express that the dense population of *O. terebriformis* make a move each night beyond an oxygen-sulfide interface, set foot in an anaerobic microenvironment as well as reducing, a dramatic contrast to the daytime domain at the mat surface in which oxygenic photosynthesis resulted in supersaturated O_2. Laboratory observation motility with apply of sulfide gradients manufactured in agar let out a negative reaction to sulfide at concentrations alike to those found within natural mats. The motility feedback may help spell out the presence of *O. terebriformis* beneath the mat surface at dark. The motion reverts to the surface at dawn pop up because of a mixture of phototaxis, photokinesis, and the onset of oxygenic photosynthesis which eat up sulfide.

2.5.8 *Oscillatoria subbrevis*

Discarding polythene among wastewater poses a major concern as they get mass in the environment. Submerged polythene in wastewater offers a classic substratum for algae to colonize. This paper highlights the biochemical composition of five cyanobacteria isolated from submerged polythene surfaces in domestic sewage water, Silchar town, Assam, (India). The carbohydrate, protein, lipid, vitamin C, and pigments (Chla, carotenoids, phycobiliproteins) fulfillment of five cyanobacterial species, *Phormidium lucidum*, *Oscillatoria subbrevis*, *Lyngbya diguetii*, *Nostoc corneum*, and *Cylindrospermum musicola* isolated from drowning polythene surface were examined (Sarmah and Rout, 2018). The extreme quantity of entire protein, carbohydrate, and lipid content establish in the *Oscillatoria subbrevis* and the least in *Cylindrospermum muscicola*. Vitamin C depends to be highest in *Oscillatoria subbrevis* and *Nostoc carneum* and least in *Cylindrospermum muscicola*. The total phycobiliproteins were maximum in the *Oscillatoria subbrevis* and minimum in *Cylindrospermum muscicola*. One-way analysis of variance (ANOVA) indicates significant differences amid the biochemical parameters of cyanobacteria unreachable from polyethylene surfaces.

2.5.9 *Pseudoanabaena lauterborn*

The genus *Pseudanabaena Lauterborn* described by Anagnostidis and Komárek (1988: 380) as a set of tiny cyanobacteria with *Pseudanabaena catenata Lauterborn* (1915: 437) (Chang, 1988) as the type species, *Pseudoanabaena* is famed for the alive of filaments with visible constrictions at cross-walls containing cylindrical cells. Hardly species that take both of them phycocyanin (PC) and phycoerythrin (PE) showed to be in tune with the chromatic modification that permits the adaptation of the PC/PE ratio to rise the absorption of accessible light (Kehoe and Gutu, 2006; Stomp *et al.*, 2004)

From that tip, Lauterborn realize Pseudoanabaena in 1915, and the taxonomy and systematics of this genus have been repeatedly updating. Initiatory, the genus grasp only two species, called, *P. catenate* and *P. constricta* (Szafer 1910: 164) Lauterborn (1915: 437), under Oscillatoriaceae (Geitler 1932). Fritsch, (1945) arranged Pseudoanabaena under the family Nostocaceae. This arrangement assaulted by Desikachary, (1959). Anagnostidis and Komárek (1988) gave a modern taxonomic system of cyanobacteria and allot the genus Pseudoanabaena under the subgenus Pseudanabaenoideae, family Pseudanabaenaceae, and command Oscillatoriales. As an outcome of the relentless switch within the cyanobacterial taxonomic system and the awareness of the latest species, Pseudoanabaena has nowadays made a large group with more than 33 species and narrated forms, of which some were anti-discrimination from the genera *Oscillatoria Vaucher ex Gomont* (1892: 198), *Phormidium Kützing ex Gomont* (1892: 156), *Lyngbya C. Agardh ex Gomont* (1892: 118), and *Spirulina Turpin ex Gomont* (1892: 96, 249) (Kling and Watson, 2003;Yu *et al.*, 2015).

2.5.10 *Lyngbya agardh ex gomont*

A new species of tropical marine cyanobacterium was uncoupled from the seashore of Singapore. Curren and Leong, (2018) recorded that *Lyngbya regalis* is a filamentous, non-heterocystous cyanobacterium that put up a brown-green macroscopic thallus of filaments that are linear to wavy. Filaments are enwrapped in a glassy, lamellated sheath with specific cross- wall tighten prominent and apical cells that are broadly rounded. The mixed phenotypic, phylogenetic (16S rRNA sequences) and ecological properties have been noticed, revealing that *Lyngbya regalis* is a novel species within the genus *Lyngbya C. Agardh ex Gomont*.

The filamentous cyanobacteria genus *Lyngbya C. Agardh ex Gomont* was primary outlined by Gomont (1892) in Monographie des Oscillariées and has undergo a maintained genus with morphologically cohesive taxonomic resources ever since. Species of Lyngbya have been explained beyond standard (Castenholz 2001) and newest categorization systems (Komárek 2014) to contain of bulky filaments having discoid cells enclosed in distinct sheaths. There are just about 69 different species in the genus *Lyngbya*, with some taxa be left unclear. It has been traditional that the systematic categorization of cyanobacteria is involved and unable-to rely on traditional morphological categorizations alone. The non-heterocystous genus *Lyngbya* was traditionally put down in Subsection III (Oscillatoriales) of cyanobacteria in Bergey's companion of Systematic Bacteriology (Castenholz *et al.*, 2001). Anyway, with the growth of molecular procedures, this genus was confirmed to be obviously polyphyletic and not monophyletic (Geng *et al.*, 2021; Engene *et al.*, 2013). Moreover, the genus *Lyngbya* has considered as a crypto genus, as subclades could not be changed based on morphological traits but could be understandably shown through phylogenetic analyses. To correctly characterize and identify species, the newest categorization now implies combining well-defined nomenclatural, heritable, morphological, and ecological properties in a polyphasic address (Komárek, 2018).

2.5.11 *Lyptolynbgya anagnotidis*

Wang *et al.*, (2014) making entire utilization of lipids and carbohydrates in microalgae for joint production of biodiesel and bioethanol may assemble a possible way to cut the expense of single biofuel manufacturing from microalgae. Liken with traditional unicellular oleaginous microalgae, filamentous microalgae *Tribonema* sp. is richer in lipid and carbohydrate pacify and lower protein content, thus, this study survey the suitability of *Tribonema* sp. as a substrate for joint production of biodiesel and bioethanol. Acid hydrolysis is the cost pep to saccharifying wall cells into fermentable sugar and releasing lipids. Microalgae biomass (50 g/L) was acid (3% H_2SO_4) hydrolyzed at 121 °C for 45 min to reach the maximum hydrolysis efficiency (81.48%). Subsequently, the lipid separated with hexane–ethanol from the hydrolysate was converted into microalgae biodiesel and the conversion rate was 98.47%. With yeast *Saccharomyces cerevisiae*, the maximum ethanol yield of 56.1% was reached from 14.5 g/L glucose in the hydrolysate.

The filamentous microalgae *Tribonema sp.* was cultured in the effluents of different stages (the primary clarifier, the anaerobic, and the anaerobic/oxic) of the traditional Anaerobic/Oxic (A/O) process for evaluating the recycling and treatment efficiency of the petrochemical wastewater in this paper. It was observed that *Tribonema sp.* grew in the anaerobic effluent and had the ideal growth states and wastewater treatment efficiency. The biomass concentration, the chemical oxygen demand (COD), and total nitrogen (TN) removal rates were 4.4 g/L, 98.4%, and 96.8%, respectively, for *Tribonema sp.* grown in the anaerobic effluent. Moreover, the total phosphorus (TP) and organic contaminants were almost completely removed and the oil content of the microalgae reached the highest (36.1%) in the anaerobic effluent. Wang *et al.*, (2014) recorded that the filamentous microalgae *Tribonema sp.* cultivation directly integrated with the treatment of anaerobic effluents is demonstrated as an alternative approach to the traditional toxic stage in the A/O process.

2.5.12 *Rhoicosphenia curvata*

Reproduction in *Rhoicosphenia curvata* (Kütz.) Grun. is isogamous. The two auxospores formed expand parallel to the apical axes of the gametangial cells. Expansion is bipolar and leads to the formation of a slightly curved, tapering cell, in which the initial valves are laid down. The perizonium consists of transverse and longitudinal bands. The transverse series, of 35 or so bands, is laid down centrifugally as the auxospore expands and can be classified into three groups based on band morphology. All except the central band are open hoops, orientated so that their ends lie in the midline of the less convex, ventral side of the auxospore. The bands have fimbriate margins on one or both sides and overlap one another from center to either pole. The longitudinal series includes 5 bands a wide central band, with two on either side: again, the bands overlap one another from the center outwards. The initial epivalve of the new generation forms beneath the dorsal side of the auxospore, on the opposite side from the longitudinal perizonial series. Mann, (1982) recorded that comparisons are made with other genera and the relevance of auxospore studies to an understanding of diatom morphogenesis is discussed.

During a study of the seasonal changes in the diatom flora of the River Wey, Surrey, it was found that valves of *Rhoicosphenia curvata* (Kütz.) Grunow was usually present in samples collected in the stretch of the river between Tilford and Guildford but not in any significant numbers either upstream or downstream of this region. A more detailed study showed that a tributary, the Farnham Wey, was the source of this particular diatom and that downstream of the confluence of these rivers the numbers steadily decreased. Valves of *Rhoicosphenia curvata* were most numerous in suspension in the river during the summer months, although the living organism is an epiphyte growing and dividing vigorously during the early spring and even in late winter. It is suggested by (Moss, 1977) that dead cells and detached valves are released into the river, from its tributary, during the summer, there being no evidence that this organism can actively divide or survive as a member of the river plankton. The valves form a convenient marker for the mixing of two distinct bodies of water.

2.5.13 *Stauroneis ehrenberg*

Two new aerophilic species of *Stauroneis ehrenberg* (Bacillariophyta) from the Eastern Himalayas. Wadmare *et al.*, (2022) documented two new diatom taxa associated with the genus *Stauroneis ehrenberg* were located in an aerophilic domain in Sikkim, India, a part of the Eastern Himalayas. The morphology of *Stauroneis sikkimensis sp. nov*. and *Stauroneis lepchae sp. nov*. are explained with light and scanning electron micrographs and compared with similar species in the genus. *S. sikkimensis* is characterized by lanceolate valves with a median constriction, bluntly rounded apices, radiate striae, and an H-shaped stauros. *S. lepchae* has broadly elliptical to lanceolate valves with obtusely rounded apices, radiate striae, and characteristic bow-tie-shaped stauros. These are the first two diatom species described from the Eastern Himalayas of India.

Stauroneis ehrenberg is usual and spread worldwide having some variation in frustule morphology. Lately, studies have investigated the molecular diversity of the *Stauroneis* from the temperate region, but there are no molecular data for tropical species. In the present study, four species of *Stauroneis* were investigated using morphological and molecular data. Morphological characters were analyzed using light microscopy (LM) and scanning electron microscopy (SEM), and the features of each taxon were compared with similar species within the genus. For the molecular analyses, *Stauroneis* strains were isolated, and cultured, DNA was extracted, and sequences from rbcL and 18S genes were analyzed to determine phylogenetic relationships. Our analysis describes three new species and records one previously known species. The new species are: (1) *Stauroneis* lateritic Wadmare, Kociolek & B.Karthick, characterized by small frustules with elliptical lanceolate valves and short substrate apices, broad central bow-tie shaped stauros, and few moderate striae centrally becoming strongly radiate towards the poles; (2) *Stauroneis* sholaii Wadmare, Kociolek & B.Karthick, which has broad lanceolate valves with short rectangular stauros and radiate striae; and (3) Stauroneis barytic Wadmare, Kociolek & B.Karthick, which has large, lanceolate valves with rounded apices, a stauros with 4-7 shortened striae and radiate striae. Ultrastructure and molecular data from India for the previously known, widespread species *Stauroneis gracilis Ehrenberg* are presented. The combined morphological and molecular approach supports the recognition of *S. lateritic*, *S. sholaii*, and *S. bartii* as new species.

2.5.14 *Pinnularia ehrenberg*

Study of França *et al.*, (2017) aimed to survey the genus *Pinnularia ehrenberg* (Bacillariophyta) in five preserved streams located in three conservation units (Brasília National Park, Chapada dos Veadeiros National Park and Terra Ronca State Park). Periphyton was collected in different substrata during the dry and rainy seasons (years 2012 to 2013), totalizing

25 sample units. Altogether, 23 taxa were identified, *P. subanglica, P. angustivalva*, and *P. butantanum* the most frequent. Pebble and sand were the richest substrates, with nine taxa each. Among the 23 taxa reported, 13 are cited for the first time for central Brazil: *P. angusta* var. *angusta, P. angustivalva, P. butantanum, P. divergens* var. *biconstricta, P. divergens* var. *mesoleptiformis, P. gibba* var. *subundulata, P. paulensis, P.*

persudetica var. *persudetica, P. subgibba* var. *angustarea, P. subgibba* var. *capitada, P. superpaulensis, P. viridiformis* var. *minor* and *P. undula* var. *undula*.

2.5.15 *Nitzschia sigmoidea*

Nitzschia sigmoidea is the type species of the diatom genus *Nitzschia.* Recent evidence implies that the genus is polyphyletic. As a reference for future studies by the (Knattrup *et al.*, 2007) of the genus, we here provide a thorough morphological examination of the silica frustule, including new details of copulae and poroids, as well as compiling previously published information on the cellular ultrastructure. Observed specimens of *Nitzschia sigmoidea* are linear in frustule view and sigmoid in girdle view with a length of 346–359 μm, and a width of 9–13 μm. Both nitzschioid and hantzschioid cells are found. The raphe is highly eccentric, elevated on a keel, without a central nodule. The raphe ends externally in a hook-shaped terminal fissure, internally in a helictoglossa forming an excrescence. Tubular fibulae are slim and irregularly distributed with a density of 6–9 in 10 μm. A conopeum runs the entire length of the cell. The striae are uniseriate with 4–5 periods in 1 μm, each with a hymen with a hexagonal pattern of perforations. Two types of copulae are found, both with two rows of periods, 3.2–3.4 periods in 1 μm. One type of copula is fimbriate with evenly distributed fimbriae, while the other type has a veil.

Initially, the growth of the diatom was restricted by the lack of carbon in the freshwater medium, after the addition of bicarbonate it grew well. HPLC analysis showed no domoic acid production by *N. sigmoidea.*

2.5.16 *Zygnema sp.*

In streptophytic green algae in the genus *Zygnema*, pre-akinete formation is considered a key survival strategy under extreme environmental conditions in alpine and Polar Regions. Arc et al., (2020) recorded that the transition from young, dividing cells to pre-akinetes is associated with morphological changes and the accumulation of storage products. Understanding the underlying metabolic changes could provide insights into survival strategies in polar habitats. Here, GC-MS-based metabolite profiling was used to study the metabolic signature associated with the pre-akinete formation in *Zygnema sp.* from Polar Regions under laboratory conditions, induced by water and nutrient depletion, or collected in the field. Light microscopy and TEM revealed drastic changes in chloroplast morphology and ultrastructure, degradation of starch grains, and accumulation of lipid bodies in pre-akinetes. Accordingly, the metabolite profiles upon pre-akinete formation reflected a gradual shift in metabolic activity. Compared with young cells, pre-akinetes showed an overall reduction in primary metabolites such as amino acids and intermediates of the tricarboxylic acid (TCA) cycle, consistent with a lower metabolic turnover, while they accumulated lipids and oligosaccharides. Overall, the transition to the pre-akinete stage involves the reallocation of photosynthetically fixed energy into storage instead of growth, supporting the survival of extreme environmental conditions.

2.5.17 Indigenous Microalgal Species in Bioremediation of Oil Refinery Wastewater

Recent research has emphasized the application of **native microalgae** isolated directly from petroleum refinery effluents, offering enhanced adaptability and remediation efficacy.

for instance, *Pseudochloris wilhelmii,* a pico-eukaryotic marine microalga, demonstrated exceptional removal of nitrogen (up to 0.823 mmol g^{-1} day^{-1}) and high lipid productivity (115 mg L^{-1} day^{-1}) when cultivated in oil refinery wastewater at ~24 °C and moderate light levels. Biomass lipid concentrations of 181–320 mg L^{-1} were recorded, showing its viability both for bioremediation and biofuel feedstock generation

A follow-up comparative study in July 2025 evaluated *Pseudochloris wilhelmii, Nannochloropsis gaditana,* and *Synechococcus sp.* MK568070, all indigenous or adapted to refinery wastewater. Here, *N. gaditana* exhibited the highest lipid content (37 % d.w.) and productivity (29.45 mg L^{-1} day^{-1}), while *P. wilhelmii* delivered the highest nitrogen uptake (0.895 mmol g^{-1} day^{-1}), volumetric productivity (93.9 mg L^{-1} day^{-1}), and refinery wastewater toxicity reduction (~76.5 %). These findings underline the interplay between nutrient removal and valuable biomass production Broader reviews (2024–2025) underscore the capability of microalgal species to remove refractory organic compounds, heavy metals, and pesticides, often through accumulation, adsorption, enzymatic degradation and metabolism. These processes contribute to sustainable and cost-effective bioremediation strategies while enabling downstream valorization of the algal biomass (e.g. biofuels). Moreover, consortium-based approaches pairing indigenous microalgae with hydrocarbon-degrading bacteria have shown synergistic effects. Co-cultures facilitate degradation of petroleum compounds via combined mechanisms: algae supplying oxygen and organic exudates, bacteria metabolizing hydrocarbons, and extracellular polymeric substances mediating pollutant sorption and detoxification

In an earlier settlement from 2023, oil refinery wastewater-adapted algal consortia were tested on kerosene-contaminated water. The system achieved simultaneous hydrocarbon removal and biodiesel precursor accumulation, showcasing dual benefits of remediation and bioproduct generation

Several studies also focus on indigenous bacteria from refinery-impacted sites. For example, *Acinetobacter venetianus* ICP1 and *Pseudomonas oleovorans* ICTN13, isolated from crude oil refinery wastewater, exhibited efficient alkane degradation via alkane hydroxylase gene expression and biofilm-supported hydrocarbon adhesion. Mixed strains outperformed individual cultures, suggesting that multi-strain microbial systems (bacteria + indigenous algae) can be optimized for refinery wastewater treatment

2.5.18 Integration with Previous Genera-Based Review

Together, these studies suggest a progression from genus-level taxonomic surveys (*Pinnularia*, *Nitzschia*, *Zygnema*) into applied bioremediation systems utilizing locally adapted species. While the earlier species descriptions emphasize taxonomy and survival strategies, the more recent work harnesses environmental resilience and functional properties of indigenous algae for treatment of complex refinery effluent streams.

By integrating these native species (or consortia of algae + bacteria) into Phycoremediation processes, your thesis can bridge the gap between descriptive taxonomy and applied biotechnology offering a robust framework linking ecological characterization to engineering applications in oil refinery wastewater remediation.

CHAPTER 3

3. RESEARCH METHODOLOGY

3.1 Study Area

The research work was conducted at Attock Refinery Limited (ARL), Morgah, Rawalpindi (33.5548° N, 73.0789° E). ARL is the pioneer of crude oil refining in the country with its operations dating back to 1922. Backed by a rich experience of more than 100 years of successful operations, refinery units have been gradually upgraded/replaced with state-of-the-art hardware to remain competitive and to meet modern challenges regarding equipment and economy. Several preliminary surveys of the Morgah, Rawalpindi including ARL were conducted to get familiarity and locate the sampling sites at ARL Morgah, Rawalpindi. The accessibility of these sites was also assessed for collection of water samples having micro-biota. A total of seven sampling sites were randomly chosen. During samples collection longitude and latitude by GPS (Global Position System), air and water temperature and pH of water and were determined.

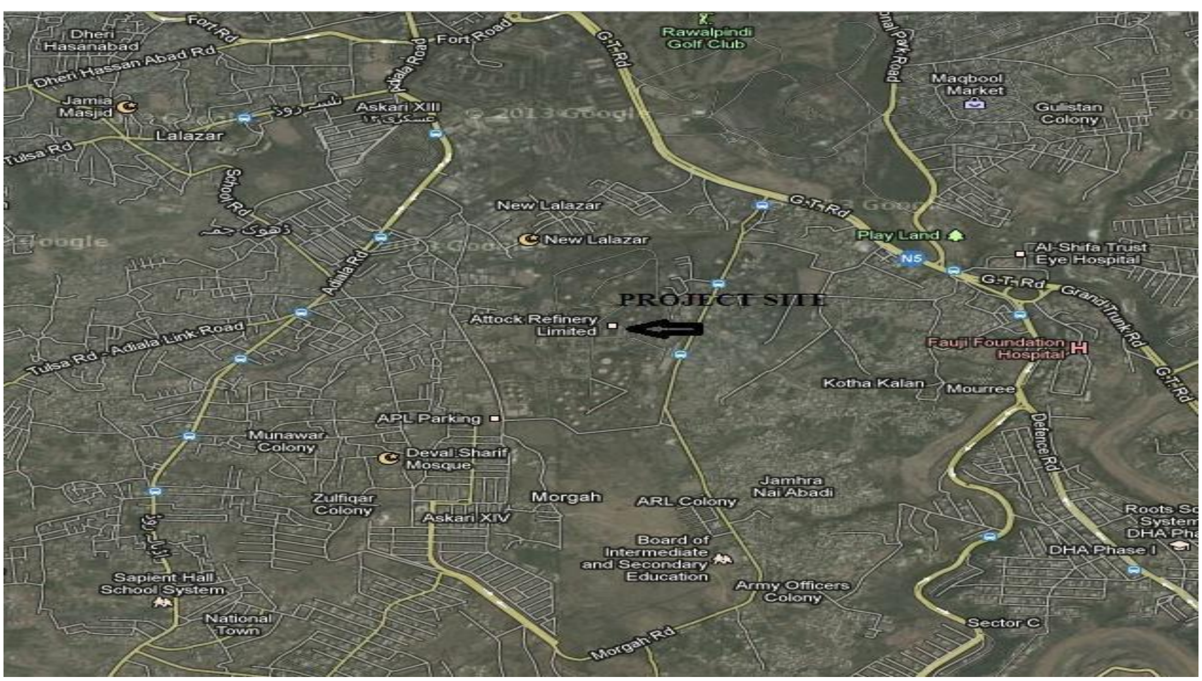

Figure 3.1 Map of the study area

3.2 Research Methods

3.2.1 Wastewater sampling and analysis

Wastewater samples were collected from various locations at refinery effluent drain by grab sampling techniques. These were mixed to form composite samples, filtered to remove coarse particles and divided into three replicates. Analysis for various physiochemical parameters like: PH, Total Soluble Solids (TSS), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Oil and grease (O&G), Phenols, Sulphates (SO4), Sulfur (S2), Chlorides (Cl) and Total Dissolved Solids (TDS) was carried out by using methods of American Public Health Association (APHA, 2005). Samples of untreated wastewater were stored at 4 degrees centigrade until used for Algae propagation.

3.2.2 Algal Sample collection and preparation

Periphyton, benthic and epiphytic species were collected with the help of pipette, knife, forceps and toothbrush using falcon tubes from seven different sites of wastewater streams located in vicinity of ARL. These samples were shifted to growth chambers to provide natural climatic conditions under controlled environment (Ahmad, Khan and Yasar, 2013; Taylor *et al.*, 2015; Neveux, Magnusson, Mata, Whelan, Nys, *et al.*, 2016).The algal species were isolated and multiplied BG-0 & BG-11 Agar media in growth chambers. Eighteen Algal species were identified whereas, on the basis of abundance at majority of sites three suitable species were selected for present study (Ahmad, Khan and Yasar, 2013).

3.2.3 Identification of species

On the basis of taxonomic characters identification of species was carried out under the light microscope at 40x resolution. A minute sample was placed on a microscope glass slides by using micro pipette and covered by the cover slips. Then these slides were placed under the light microscope to identify the species by observing their taxonomy and morphology. Various species were observed in the samples taken from different sites. After the identification process these species were preserved for isolation process.

3.2.4 Isolation of species

As different species were found naturally in the collected samples, it was very important to isolate these from each other to multiply for bulk preparation of each species in purified form. Isolation of algal species present in water samples in mixture form was done by using two types of media (BG-0 and BG-11) which was prepared according to the standard procedure. The whole process was carried out in well sterilized laboratory conditions. Agar was used in the media as solidifying agent and pH was maintained in circumneutral range. The prepared media was autoclaved at 121^{0}C for 15 minutes to eliminate the contamination. It took around 45 minutes to cool down the media after that the media was cooled down. Three sets of petri plates and test tubes were used for each sample. Pouring of media and shifting of algal species to petri plates and the test tubes were made under Laminar flow. UV light of laminar floor were turned on for

20 minutes for sterilization.

3.2.5 Selection of suitable species

After identification and isolation of all the indigenous algal species, secondary data was consulted to select the suitable species for wastewater treatment. Species were selected on the basis of frequent availability in the indigenous environment and Phycoremediation potential of micro algal *sp*ecies.

3.2.6 In vitro-Experiment

The species showed better growth during isolation process and having potential to treat wastewater like *Lyngbya sp., Scenedesmus sp*. and *Tribonema sp.* were shifted in flask containing 500ml of wastewater collected from outlet of refinery effluent. Then these flasks were shifted to growth chamber where temperature was set to 26 ^{0}C ±2, humidity 80% and normal 8 hours of day light was provided. These species were allowed to grow in growth chamber for 2 weeks After the successful growth, samples from the flasks were taken and centrifuged at 13000 rpm for 15 minutes, as a result the sample differentiated into two layers the wastewater was settled down in Eppendorf tubes. Water samples were analyzed for above discussed parameters (3.3.1).

3.2.7 In Vivo-Experiment

The selected algal species were multiplied in the laboratory, transferred to refinery green/ laith houses for further multiplication. After acclimatization, algal species were transferred to the respective cells of experimental apparatus. Chambers were already contained fresh tap water and wastewater was allowed to gradually replace the fresh water. The fiber glass constructed chambers were used to avoid interference of chemicals from surrounding soil. The thickness of these chambers was 4 mm with 1% slope to facilitate the water circulation and flow. In order to ensure the smooth flow of wastewater, the PVC pipes were used from wastewater tanks to inlet of treatment chambers and for collection of the treated wastewater, polyethylene drain flex pipes were used at the exit. All the twelve chambers were be placed in three groups each having three chambers. After the completion of the acclimatization phases and complete replacement of tap water with wastewater in treatment chambers, the untreated wastewater was applied to cells by gravity flow via spherical valves and PVC pipes at constant Hydraulic Loading Rate (HLR) of 20 liters/day. Discharge rate of wastewater was readjusted based upon first pilot reading. Treated wastewater samples were collected and analyzed. Each sample having one liter volume was collected in sterilized jars. Collected samples were studied and analyzed in laboratory to check the performance of Phycoremediation process. Laboratory analysis works were carried out at Quality Control Laboratory (QCL) of ARL, Environmental laboratory of National Cleaner Production Center (NCPC) and in the laboratory of Department of environmental science, International Islamic University, Islamabad (IIUI). Chemical analyses were carried out as per the Standard Methods for concerned parameters (Bansal and Steel, 2018; Kiran *et al.*, 2018).

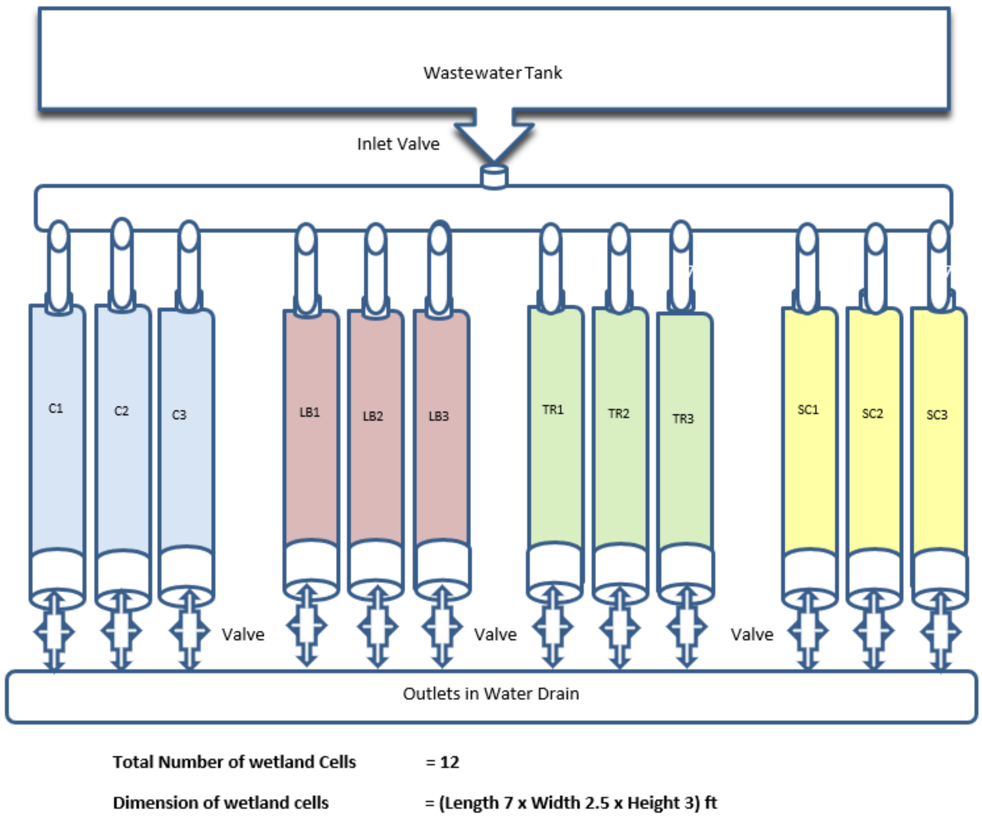

Figure 3.2 Experimental design

3.2.8 Statistical analysis

The experiment was laid in Randomized Complete Block Design (RCBD). This study had evaluated the means, standard deviation, variance (SDV) and standard deviation (SD), t-test and one way ANOVA using SPSS software.

CHAPTER 4

4. RESULTS AND DISCUSSION

4.1 Diversity of Algae in Wastewater Habitats around Refinery Area in District Rawalpindi, Pakistan

4.1.1 Introduction

Algae is diverse group of unicellular, multicellular and photosynthetic eukaryotic microorganisms with thallus bodies having variety of forms; more than 8,000 species of 500 genera belong to green algae have been discovered with many more possibilities of new species to be discovered (Ali *et al.,*2015). Various species have different chloroplast structures. Photosynthesis, which is carried out by the chloroplast, aids in the generation of food. Algae are one of the most essential species on the planet because they provide sustenance to aquatic life. Algae can survive in wide range of temperatures and range in size from a pinhead to a huge species. They also develop colonies, with many of them branching out. Algae can grow in rivers, seawater, soil and other damp places (Salah-Ud-Din *et al.,* 2017). Nitrogen-fixing organisms are also known as blue-green algae (Shah, 2014).

Algae play an important role in food chain formation for other species (Hussain *et al.,* 2008). Microscopic algae known as phytoplankton found in lakes and oceans having various size and habitats (Sher and Hazrat, 2012). Cyanobacteria are the earliest form of algae found on planet. Fossil record showed more than three billion years old algae from the rocks which lacking oxygen algae and other green plants produce oxygen through photosynthesis, they are the first plant to appear as photosynthetic about billion years ago (Hussain *et al.,* 2010). Except blue-green algae, they belong to Eukaryotic group of organisms. In Eukaryotic algae, there is an important organelle (chloroplast) which contains green pigments as chlorophyll that serves to catch light for photosynthesis process and few more yellow and brown pigments (carotenoids)

(Hussain *et al.,* 2008). Algae come in a variety of shapes and sizes, with single and independent cells combining and forming colonies. These cells generate filaments by attaching end to end in a branching configuration.

Periphyton algae has lot of potential for metals removal from wastewater (Han *et al.,*2000; Olguín, 2003). Metal sorption comprises intracellular ligands and metal's binding on the surface of cells. The intracellular metal is several times smaller than adsorbed metal. Various factors like pH, concentration of cations & anions, temperature, concentration of metal, biomass in solution and metabolic stage of the organism affect process of metal sorption. Algae can be used effectively for removal of metals from multi-metal solutions. Physical, chemical, and biological technologies have been developed to treat oily wastewater and restore environmental quality

Present study will act as baseline research around refining industry at the time when refinery is completing its hundred years of operations and urbanization is being launched by private housing colonies in the surrounding areas. It will also contribute to the documentation of algal flora of Rawalpindi which will ultimately help future studies regarding ecological distribution of algal flora in the area. The objective of this study is to determine the algal biodiversity of ARL wastewater channels during present era.

4.1.2 Materials and Methods

4.1.2.1 Study Area

The study was carried out at Attock Refinery Limited (ARL), Morgah, Rawalpindi, spreading over an area of about 600 acres and is pioneer crude oil refining industry of Pakistan operating since 1922. Several preliminary surveys of the Morgah, Rawalpindi including ARL Refinery were carried out to get knowledge of the area and to locate the sampling locations.

Seven sampling sites were randomly chosen (Fig. 4.1) and were also assessed for micro-biota collection.

Fig. 4.1 Satellite view of Study Area

Table 4.1. Sampling sites at Attock Refinery Limited (ARL)				
No.	**Sampling sites**	**Latitude (N)**	**Longitude (E)**	**Elevation (m)**
1	A	33.5581405	73.07859594	509
2	B	33.5550391	73.0783685	507
3	C	33.551696	73.0776096	501
4	D	33.5497999	73.0769714	510
5	E	33.5536012	73.0809313	500
6	F	33.5518149	73.0778448	501
7	G	33.54751542	73.07621023	504

4.1.2.2 Sample collection and preparation

Periphyton, benthic and epiphytic species were collected with the help of pipette, knife, forceps and toothbrush using falcon tubes from seven different sites of wastewater streams located in vicinity of ARL. These samples were shifted to growth chambers to provide natural climatic conditions under controlled environment (Taylor *et al.*, 2015; Ahmad *et al.,*2013; Neveux *et al.*, 2016).

4.1.2.3 Identification of species

Laboratory scale identification of species were carried out on the basis of their taxonomy under the camera mounted light microscope at 40x resolution. Minute sample was placed on a glass slide by using micro pipette and covered by the cover slips. Then these slides were placed under the light microscope to identify the species by observing their taxonomy and morphology. Various species were observed in the samples taken from different sites.

4.1.2.4 Isolation of species

Isolation process was done for purification and multiplication of different algal species present in the water samples in mixture form. Two types of media (BG-0 and BG-11) was prepared according to the standard procedure. All work was done in well sterilized laboratory conditions. pH of the media was maintained after adding agar was used in the solution as a solidifying agent and autoclaved at 121^{0}C for 15 minutes to eliminate the chances of contamination. It took around 45 minutes after that the media was cooled down. Three sets of petri plates and test tubes were used for each sample. Pouring of media and shifting of algal species to petri plates and test tubes were made under Laminar flow. UV light of laminar flow were turned on for 20 minutes for sterilization.

4.1.3 Results and Discussions

4.1.3.1 Isolation and identification of Algal Species

Samples were randomly collected from all sites in two seasons (August, 2020 (S1) and April, 2021 (S2)) from wastewater streams of ARL, Morgah Rawalpindi. Survey of these locations showed bloom formation was equally good in both seasons. Standard microbial techniques (several plating and microscopic observations) were used for isolation and purification of microalgal strains. Eighteen microalgal strains were isolated on the basis of morphological characteristics like cell shape and size which indicated that they belongs to the species (Table 3.1) as described by *Hegewald & Hanagata* (2000). Taxonomic identification was done using basic taxonomic guides (Desikachary, 1959), *Prescott GW*(1951), *Philipose MT*(1967)

4.1.3.2 Distribution and Morphology

Microscopic observations of morphological characteristics of algal species confirms the natural presence of eighteen different species (table 1) in the area under study from four phyla

Ochrophyta having 9 species (50%), Cyanobacteria 7 species (39%), Chlorophyta 1 specie (5.5%) and Charophyta 1 specie (5.5%).

Table 4.2 Diversity of algal flora of the wastewater effluents of ARL, Rawalpindi Pakistan

Phylum	Class	Order	Family	Genus /Species	Locality
Ochrophyta	Bacillariophyceae	Thalassiosirales	Stephanodiscaceae	*Cyclotella glomerata*	A, C, & D
		Bacillariales	Bacillariaceae	*Nitzschia hungarica Grunow*	C & G
				Nitzschia sigmoidea	B, C, E, & G
		Naviculales	Naviculaceae	*Navicula playstoma*	C, E, F & G
			Pinnulariaceae	*Pinnularia Ehrenberg*	C & E
			Stauroneidaceae	*Stauroneis Ehrenberg*	D & E
		Cymbellales	Rhoicospheniaceae	*Rhoicosphenia curvata*	A & F
		Fragilariales	Fragilariaceae	*Fragilaria virescens*	E & G
	Xanthophyceae	Tribonematales	Tribonemataceae	*Tribonema sp.*	A, B, C D , E , F & G

Cyanobacteria	Cyanophyceae	Nostocales	Oscillatoriaceae	*Oscillatoria sp.*	B, D & G
				Leptolyngbya anagnostidis	C & F
				Oscillatoria terebriformis	B, F & G
				Oscillatoria subbrevis	A, B, D, F
				Arthrospira sitzenberger	C, G
				Lyngbya agardh ex gomont	A, B, C, D, E, F & G
			Nostocaceae	*Pseudoanabaena Lauterborn*	C, E, F & G
Chlorophyta	Chlorophyceae	Sphaeropleales	Scenedesmaceae	*Scenedesmus sp.*	A, B, C, D, E, F & G
Charophyta	Conjugatophyceae	Zygnematales	Zygnemataceae	*Zygnema sp.*	D, E & F

Phylum: **Cyanobacteria**

Class: **Cyanophyceae**

Family: **Oscillatoriaceae**

Key to species: *Oscillatoria* (*simplicissima, terebriformis, subbrevis*)

Thallus

- Dark blue green colour. *O. simpilicissima*
- Dark blue colour *O. terebriformis*

Trichomes anatomy

1. Not constricted septa, having screw like slightly attenuated trichomes. *O. terebriformis*
2. Constructed at cross walls and not attenuated at apices. *O. simpilicissima* (Venter *et al.,* 2003).
3. Straight form, non-attenuated at the terminals/apices, non-granulated (Yaseen *et al*., 2016) at the end walls. O. subbrevis
4. Terminal cells are rounded in shape, calyptras not found. *O. subbrevis* (Ullah, N. 2019)
5. Round terminal cells, non-capitates and without calyptras. *O. terebriformis*
6. Hemi-spherical end cells with non-granulated septa (Venter *et al.,* 2003) *O. simpilicissima*
7. Free floating, planktonic in nature, having different in size either smaller or longer as single. *O.subbrevis*
8. Trichomes is yellowish blue green and straight. *O.simpilicissima* **(**Venter *et al*., 2019)
9. Facultative firm, thin hyaline sheets present.
10. Trichome is 4.5-10um wide and up to 77 um. *O. subbrevis*

Filaments

Loosely arranged (free floating, in flakes like clusters or mats). Straight and long

Measurements

- Cells 2.5-7.6um in diameter and Width: 3-8um. *O. terebriformis*
- 1.5-3.5um in length, 4.5-8.2um wide. *O. subbrevis*

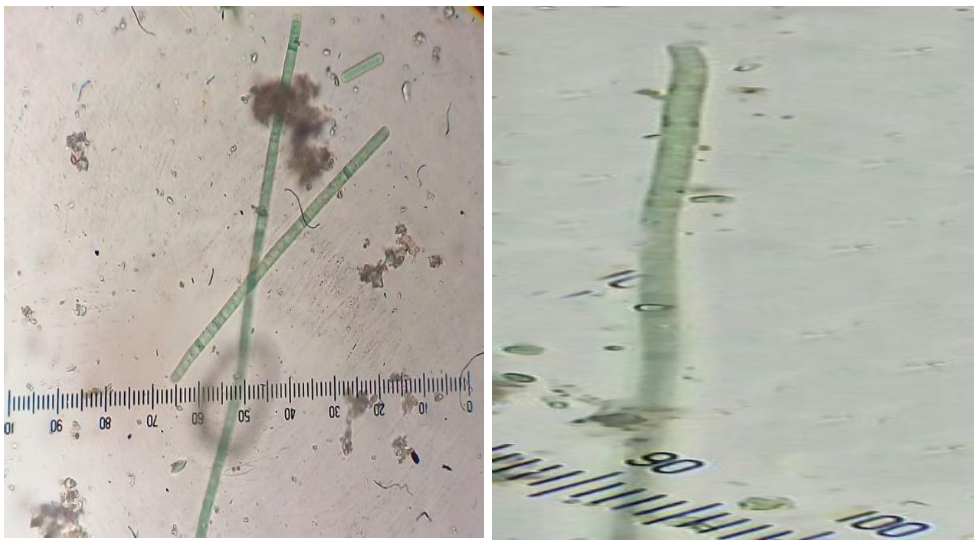

Figure 4.2 *Oscillatoria simplicissima,*

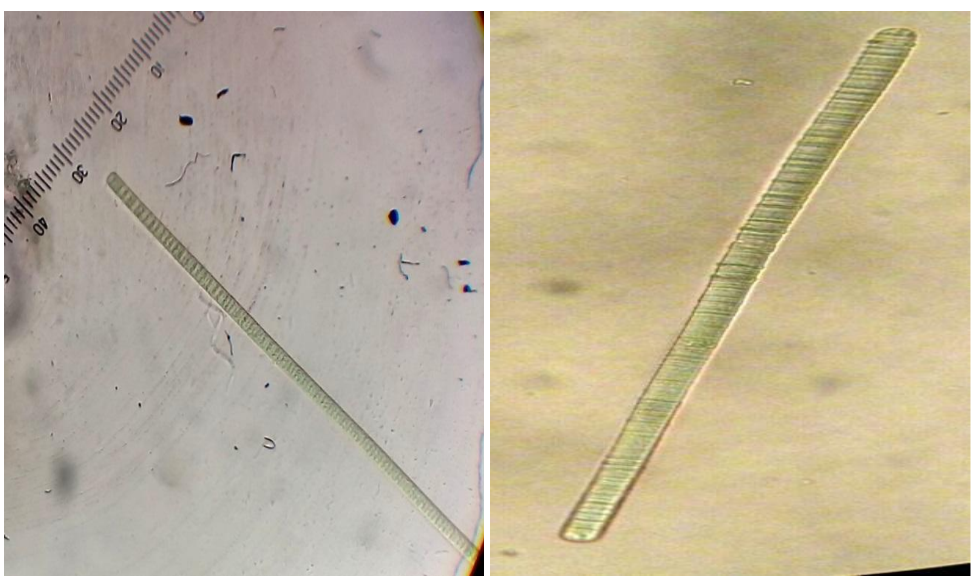

Figure 4.3 (a) *Oscillatoria terebriformis,* and Figure 4.3 (b) *Oscillatoria subbrevis*

Key to species: ***Leptolyngbya anagnostidis***

- Trichomes motile or non-motile with trembling.
- Ends are neither attenuated nor capitates. (Komárek, J. 2016).
- Trichomes are 0.5–3.5 um in width.
- The filaments of this species are mostly having un-attenuated and non-capitated terminal parts.
- Hyaline sheaths are found which are facultative, firm and thin in nature. (Komárek, J. 2016).
- Cells are cylindrical, lesser wide but mostly longer than widths. (Komárek, J. 2016).

Figure 4.4 *Leptolyngbya anagnostidis*

Key to species: ***Arthrospira sitzenberger***

- Thallus blue green in color.
- Trichomes multicellular, cylindrical, without sheath, loosely and regularly coiled, usually

of large diameter and large spirals, with comparatively short and fewer coils. (Ullah, N. 2019).

- Distinct cross walls, apices slightly or not at all tapering. (Ullah, N. 2019).
- Terminal cells rounded, calyptras absent.
- Cells nearly as long as broad 2-6 um.
- End cells broadly rounded.

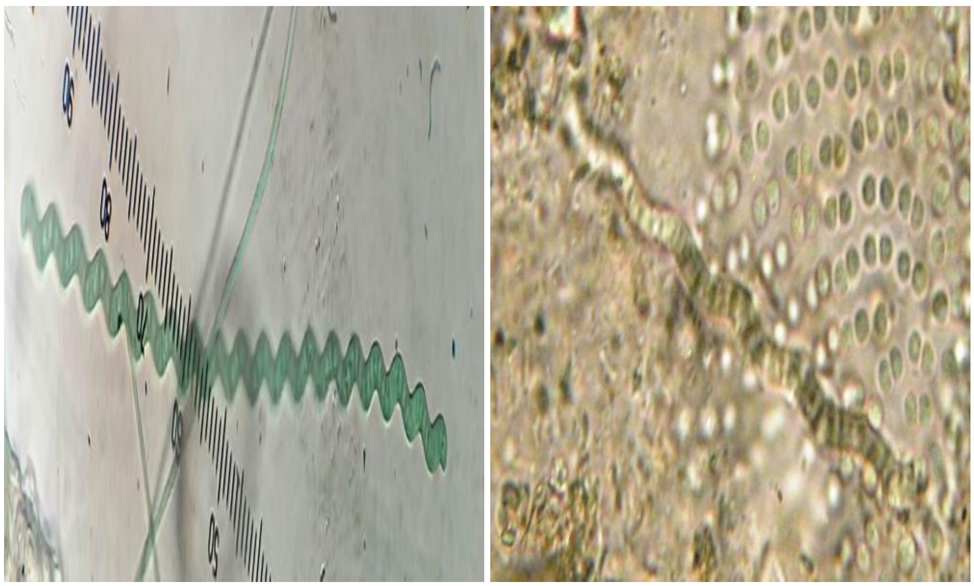

Figure 4.5 *Arthrospira stizenberger*

Key to species: ***Lyngbya agardh ex gomont***

- Trichomes single or free in a thin, massive thick sheath colorless, yellow to brown or red, blue to purple red (Komárek, J. 2016).
- Large filamentous mat-forming cyanobacterium usually unbranched. (Ullah, N. 2019).
- Lyngbya mats may be several inches thick and can cover large areas of the water surface or benthic (bottom) sediments. (Ullah, N. 2019).
- Free swimming or forming free thallus. (Ullah, N. 2019).

- Spirally coiled or attached at Entire filament or attached at the base or in the middle.

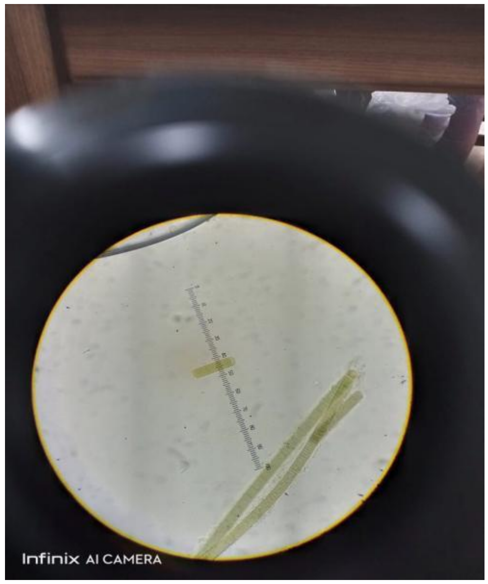

Figure 4.6 *Lyngbya agardh ex gomont*

Family: Nostocaceae

Key to species: *Pseudoanabaena lauterborn*

- Cells are cylindrical with rounded or oval ends.
- Dark blue-green filaments. (Khan, Z. *et al.,* 2017).
- Trichomes are straight, short and motile without oscillation and rotation, and distinctly constricted at cross-walls.
- Cells isodiametric or up to 2× longer than wide, 1.2–2.2 µm in width and 2–5 µm in length (Khan, Z. *et al.,* 2017).
- Apical cells round without polar aerotopes and calyptras (Khan, Z. *et al.,* 2017).
- It is benthic and can be found in muddy sediments of lakes and other water bodies. (Khan, Z. *et al.,* 2017).
- Cells length 2–5 µm, width 1.4–2.2 µm.(Khan, Z. *et al.,* 2017).

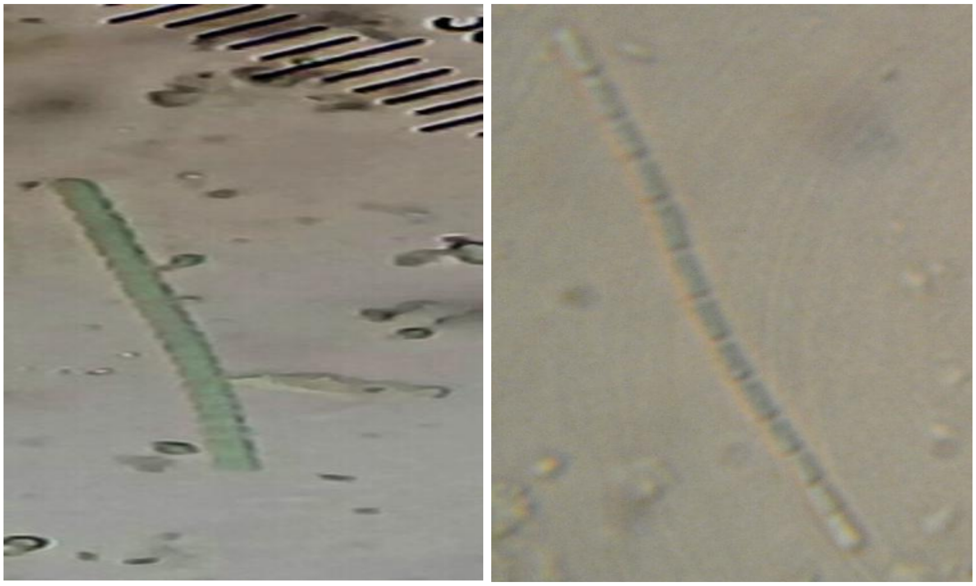

Figure 4.7 *Pseudoanabaena lauterborn*

Phylum: **Charophyta**

Class: **Charophyta**

Family: **Zygnemataceae**

Key to species: ***Zygnema sp.***

- Filaments simple with prominent two distinctive stellate chromatophore with central pyrenoids.
- Cells longer than broad.
- Cells had a diameter of ~25 µm with the nucleus in the center and two chloroplasts. (Ullah, N. 2019).
- The transparent cytoplasm in the periphery formed individual strands surrounding the large vacuoles.

- The central nucleus of young cells was surrounded by numerous endoplasmic reticulum (ER) cisternae and mitochondria.

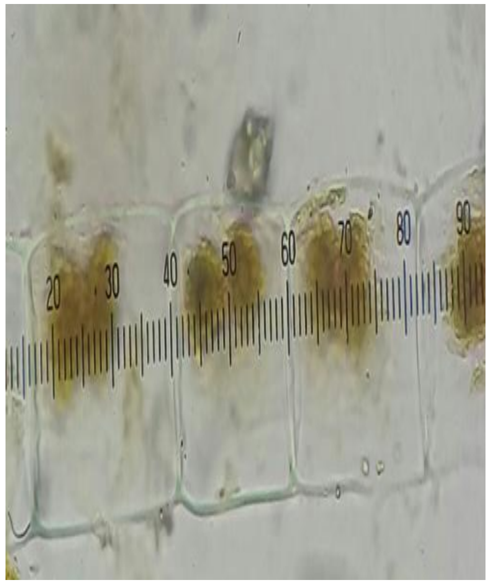

Figure 4.8 *Zygnema sp.*

Phylum: Ochrophyta

Class: Bacillariophyceae

Family: Stephanodiscaceae

Key to species: *Cyclotella glomerata*

- Cells formed colonies and intertwining chains. (Tuji, A. 2006).
- 4-10 u in diameter. (Tuji, A. 2006).
- Outer zone with radial strae 13-15 in 10u.(Tuji, A. 2006).
- Inner zone smooth or with ring of short striase in central part.(Tuji, A. 2006).

Figure 4.9 *Cyclotella glomerata*

Family: Bacillariaceae

Key to species: *Nitzschia* (*hungarica*, *sigmoidea*)

Cells

- Cells 6-9, 20-110µ *N.hungarica.*
- Cells 8-14 x 160-500u, curved and cuneate in gridle view, almost linear with parallel sides. *N.sigmoidea* (Ullah, N. 2019, Sadiq, B. 2013).

Valves

- Valves closely linear (parallel or little bitoncave sides and moderatey rostrate poles). *N. hungarica.*
- Valves naviculoid, with cuneate, acute, recurved poles. Ullah, N. (2019) *N. sigmoidea.*

Stritions

- Stritions 16-20 in 10u, obstructed by a equitably white fold, keel punctate 7-9 in 10u. *N.hungarica.*
- Keels slightly excentric with punctate 5-7 in 10; striations 23-26 in 10. *N. sigmoideau* **(**Ullah, N. 2019).

Figure 4.10 *Nitzchia hungraria grunow*, Figure 4.11 *Nitzchia sigmoidea*

Family: Naviculaceae

Key to specie: ***Navicula playstoma***

- Cells 15-20 × 37-50 µ (Wazir, S. M. 2002).
- valves elliptic-lanceolate with broadly and bluntly rostrate ends; (Wazir, S. M. 2002).

- Central area widened, rhombo-elliptic; transverse striations radial, 16-18 in 10 at the center, about 25 in 10 µ at the ends. (Wazir, S. M. 2002).

Figure 4.12 *Navicula playstoma*

Family: **Pinnulariaceae**

Key to specie: ***Pinnularia ehrenberg***

- Cells solitary and free-floating. (Ullah, N. 2019, Sadiq, B. 2013).
- Filament rarely short.
- Symmetric, rectangular in girdle view, girdles smooth, intercalary bands absent. (Ullah, N. 2019, Sadiq, B. 2013).
- Valves usually with straight sides, sometimes medially inflated or undulate, generally with broadly rounded poles. (Ullah, N. 2019, Sadiq, B. 2013).
- Axial field usually broad, expanded both polarly and medially, with complicated straight or sigmoid raphe. (Ullah, N. 2019, Sadiq, B. 2013).

Costae with internal openings, smooth, radial or transverse (convergent at the poles) with two longitudinal lines visible in the costate part of the valve.

- Chromatophores two, laminate, usually with pyrenoids. (Ullah, N. 2019, Sadiq, B. 2013).

Figure 4.13 *Pinnularia ehrenberg*

Family: Stauroneidaceae

Key to specie: *Stauroneis ehrenberg*

- Cells 6-8 x 70-100, solitary, without polar septum (Wadmare, N. *et al.,* 2022).
- Valves lanceolate, with obtuse ends (Wadmare, N. *et al.,* 2022).
- Axial area narrow, distinct (Wadmare, N. *et al.,* 2022).
- Stauros not extending to the margin (Wadmare, N. *et al.,* 2022).
- Transverse striations about 27 in 10 μ. (Wadmare, N. *et al.,* 2022).

Figure 4.14 *Stauroneis ehrenberg*

Family: Rhoicospheniaceae

Key to species: *Rhoicosphenia curvata*

- Cells 4-8 X 12-75 µ **(**Thomas, E. W. 2016).
- Curved and cuneate in girdle view (Thomas, E. W. 2016, Yaseen, *et al.,* 2017).
- Valves clavate, transversely striate with 12-15 striae in 10 µ (Yaseen, *et al.,* 2017).
- Hypovalve concave, with filamentous raphe and small central area epivalve convex with linear pseudoraphe frequently on filamentous algae.

Figure 4.15 *Rhoicosphenia curvata*

Family: **Fragilariaceae**

Key to species: ***Fragilaria virescens***

- Cells 5-10 X 12-120 µ, united into long chains (B. Ghazala, & Arifa Habib, 2011).
- Valves linear with straight to slightly convex sides. (Ullah, N. 2019).
- Rounded poles and very narrow pseudoraphe (Ullah, N. 2019, B. Ghazala, & Arifa Habib, 2011).
- Transverse striations moderately fine, parallel, 12-19 in 10 µm.

Figure 4.16 *Fragilaria virescens*

Class: **Xanthophyceae**

Family: **Tribonemataceae**

Key to species:

Tribonema sp.

- Filaments simple, un-branched (Ullah, N. 2019).

- Cell cylindrical, cell wall thick and lamellose. (Ullah, N. 2019).
- Chromatophores numerous, yellow green, disc shaped with oil droplets. (Ullah, N. 2019).
- Filaments are long, not easily fragmented. (Ullah, N. 2019).
- Cells are twice as long as broad.
- Cells 13-15 µ m wide; 25-52 µm long.

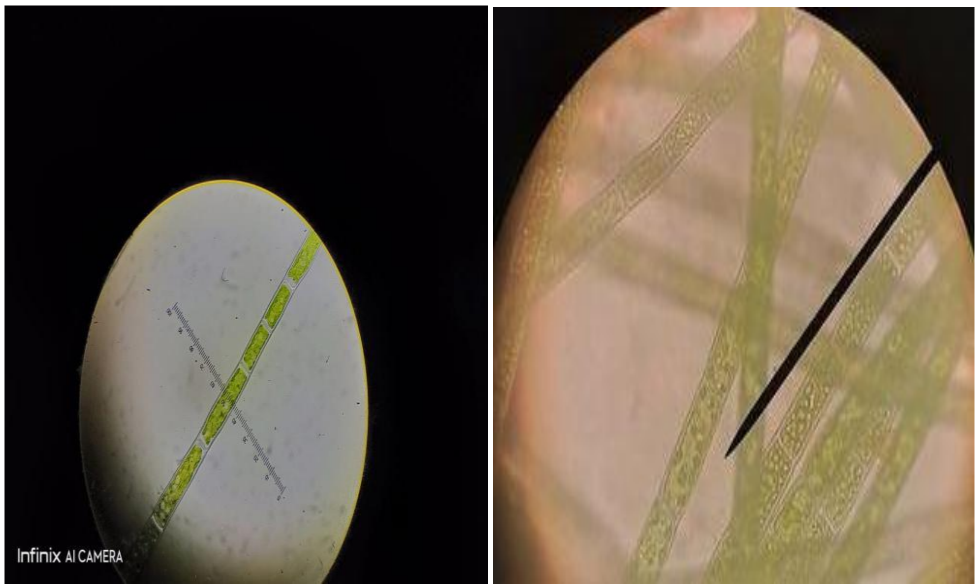

Figure 4.17 *Tribonema sp.*

Phylum: Chlorophyta

Class: Chlorophyceae

Family: Scenedesmaceae

Key to species: *Scenedesmus sp.*

- Four celled colonies, slightly alternating with cells adnate to each other (Ullah, N. 2019), (SADIQ, B. 2013).
- Cells having rounded ends with ovoid- ellipsoid shape (Ullah, N. 2019, Sadiq, B. 2013).

- Slightly longer than broad (Ullah, N. 2019, Sadiq, B. 2013).
- Ranges 3-4 µm in width while 6-9 µm long.

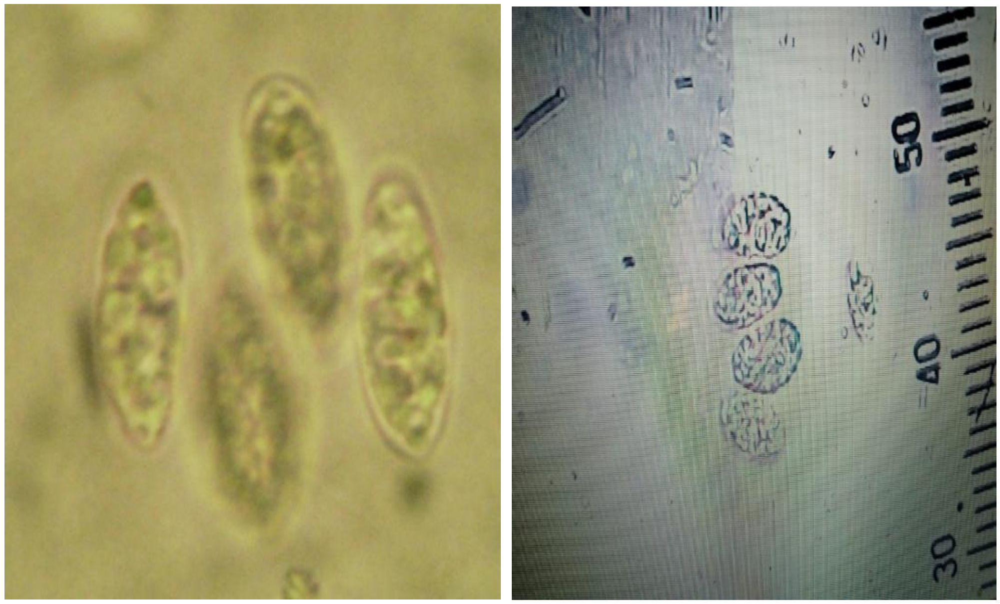

Figure 4.18 *Scenedesmus sp.*

4.1.3.3 Discussions

Present study identified eighteen different species belonging to fifteen genera, twelve families, nine orders, five classes and four phyla of algae in wastewater stream of ARL (Table 1).

Out of these following species *Oscillatoria subbrevis* (Yaseen, et., al., 2016, Ullah, N. 2019) *Arthrospira sitzenberger* (Ullah, N. 2019) *Lyngbya agardh* (Ullah, N. 2019) *Pseudoanabaena lauterborn* (Khan, Z. *et al.,* 2017) *Zygnema sp.* (Ullah, N. 2019) *Nitzschia hungarica* (Ullah, N. 2019, Sadiq, B. 2013) *Nitzschia sigmoidea* (Ullah, N. 2019, Sadiq, B. 2013) *Navicula playstoma* (Wazir, S. M. 2002) *Pinnularia ehrenberg* (Ullah, N. 2019, SADIQ, B. 2013) *Rhoicosphenia curvata* (Yaseen, *et al.,* 2017) *Fragilaria virescens* (B. Ghazala, & Arifa Habib, 2011, Ullah, N. *2019) Tribonema sp.* (Ullah, N. 2019) *Scenedesmus sp.* (Ullah, N. 2019, SADIQ, B. 2013) has been already identified and reported from different parts of Pakistan. Presented study is reporting them in Refinery waste water for the first time. Which not only shows their tendency to grow in different ecological conditions but also indicate that these can also be used effectively to control refinery wastewater pollution.

Moreover isolated species of *Oscillatoria (Simplicissima, Terebriformis, Subbrevis*) showed all of these have thallus bodies, trichomes and filaments. Beside these further microscopic observations showed dissimilarities from each other like; different color, cell size, shape etc. All of above discussed features also reported by Venter *et al.,* 2003, Yaseen, *et al.,* 2016, Ullah, N. 2019, Venter *et al.,* 2019.

Furthermore, *Leptolyngbya anagnostidis* also belongs to above mentioned Class and all the observed features/characteristics identified also reported by Komárek, J. (2016).

Next member of this class is *Arthrospira sitzenberger* which has been identified in North East Punjab, Pakistan (Ullah, N., 2019). Second last identified member of this Class was *Lyngbya agardh ex gomont* it is also reported by Komárek, J. (2016) and Ullah, N. (2019). Furthermore it has already identified in North East Punjab, Pakistan Ullah, N. (2019). Last identified member of this class was *Pseudoanabaena Lauterborn* which belongs to Family: Nostocaceae. Similar characters and features has also been reported by Khan, Z., *et al.,* 2017- and first-time present study has reported it in refinery waste water of ARL, Rawalpindi, Pakistan.

Only one species *Zygnema spp.* of Phylum Charophyta, Class Charophyta, and Family Zygnemataceae has been identified in refinery waste water. This specie has been also reported in Pakistan by Ullah, N. (2019).

Cyclotella glomerata from Phylum Ochrophyta, Class Bacillariophyceae, and Family Stephanodiscaceae first time has been identified in Pakistan. All the observed characteristics and features of this species has been reported by Tuji, A (2006).

Further few more species from same class but different families has been identified in refinery waste water. Like *Nitzschia Hungarica Grunow* and *Nitzschia sigmoidea* from Family Bacillariaceae, later one has also been reported by (Ullah, N., 2019, Saqib, B. 2013). Moreover *Navicula playstoma (*Family Naviculaceae) also reported by Wazir, S. M. (2002) in Pakistan. Subsequent identified member *Pinnularia Ehrenberg* (Family Pinnulariaceae) has been reported by Ullah, N. 2019 and Saqib, B. 2013 in Pakistan. All the observed characteristics and features of *Stauroneis ehrenberg* (Family Stauroneidaceae) are exactly same as reported by Wadmare, N *et al.,* 2022. *Rhoicosphenia curvata* (Family Rhoicospheniaceae) has been reported by Thomas, E. W. (2016). *Fragilaria virescens* (Family Fragilariaceae) has been reported by Ullah, N. (2019) in Pakistan. In spite few of these species found in Pakistan but present study identified them first time in refinery waste water stream.

Within above discussed phylum only one species *Tribonema sp.* from Class Xanthophyceae and Family Tribonemataceae has been identified and all the identified characteristics and features are also reported by Ullah, N. (2019) in Pakistan but this one is also reported first time in refinery waste water.

Last identified species is *Scenedesmus sp.* from Phylum Chlorophyta, Class Chlorophyceae, and Family Scenedesmaceae. It has also been reported at multiple locations in Pakistan by Ullah, N. 2019 and Saqib, B. 2013. But first time reported in refinery waste water stream by the present study.

Above mentioned all identified species presence in refinery waste water showed/indicate that these can effectively play role in cleaning pollutant in this water stream. Moreover *Lyngbya*, *Scenedesmus* & *Tribonema* species are present at all sampling points (Table 1). Their occurrence showed that, these can use as potential species to reduce refinery waste water pollution. It is suggested that these above-mentioned species can be used to control pollution.

4.1.4 Conclusion

Presented study identified the indigenously available algal species in drainage water course of Attock Refinery Limited, Morgah, Rawalpindi. This study helps to explore the existing status of algal flora and build up its baseline data for future studies during the initial stages of urbanization in surrounding areas of ARL. As there was huge gap regarding documentation of algal flora in the vicinity of any oil refinery in the region. During the study, 18 species belonging to 15 genera, 12 families, 9 orders, 5 classes, and 4 phyla were identified (Table 1) which reflects the presence of rich diversity of indigenous algal species. It also showed *Lyngbya sp.*, *Tribonema sp.* and *Scenedesmus sp.* (Table 1) were present at every sampling site. This study further suggested to extend such work around all oil refineries in Pakistan for development of database and germplasm collection which is currently not available. Moreover, developing regional GIS map for such studies will also helpful for future studies.

4.2 In-vitro Performance Evaluation of Various Indigenous Algal Species for Refinery Wastewater Treatment

4.2.1 Introduction

Water quality remains one of the most significant environmental issues. Numerous local and global programs aim at providing good ecological status of fresh water and marine ecosystems (Asiwal et al., 2016). Wastewaters from residences and industries require certain level of treatment prior to discharge into natural watercourses. Wastewater has traditionally been treated using waste stabilization ponds (WSP), activated sludge (AS), trickling filters, etc. (Mohamed et al., 2018). Eutrophication and toxic algal blooms developed due to addition of domestic and industrial untreated sewage into fresh water bodies. This decreases water quality and causes hypoxia, anoxia, kill fishes and other trophic disturbances (Khan et al., 2017).

Wastewater has a high potential for reuse in agriculture, especially in arid and semi-arid areas. Wastewater reuse increases food production by providing a stable supply of water and contributes to environmental security by reducing the pollution level of rivers and surface water (Zaidi, 2007). Treatment processes that couple carbon capture and wastewater treatment with low or no carbon emission (Mohamed et al., 2018) can be considered as the most sustainable options. Microalgae use light, CO2, nutrients and water to produce biomass through photosynthesis (Hsueh et al., 2009).

Algae being an autotrophic considered very important in the ecosystem. Algae help the formation of the food chain for other animals (Aliya et al., 2009). Algae are of different types, more than 500 genera and 8000 species of green algae have been discovered and a large number is yet to be discovered (Ali et al., 2015; Kamyab et al., 2015). Algae can effectively remove metals from multi-metal solutions. Dead cells sorb more metal than live cells. Various pretreatments enhance metal sorption capacity of algae. CaCl2 pretreatment is the most suitable and economic method for activation of algal biomass. Algal periphyton has great potential for removing metals from wastewater (Han et al., 2021; Olguín, 2003).

Attock Refinery Limited (ARL), Morgah, Rawalpindi is the pioneer of crude oil refining in the country with its operations dating back to 1922. Backed by a rich experience of more than 100 years of successful operations, refinery units have been gradually upgraded and replaced with state-of-the-art hardware to remain competitive and meet new challenges regarding environment and economy. To overcome the wastewater treatment challenge, ARL established effluent treatment plant in 1996.

The main objective of the study is to identify and check the capacity of algal species to reduce refinery wastewater pollution.

Thus this study would serve as a first ever baseline findings from the area which may help in future related projects like phyco-chemistry, ethno-phycology, phytoremediation, restoration strategies/impacts and management of aquatic ecosystems.

4.2.2 Materials and Methods

4.2.2.1 Study Area

The research work was conducted at Attock Refinery Limited (ARL), Morgah, Rawalpindi (Fig.4.19). Several preliminary surveys of Morgah, Rawalpindi including ARL Refinery were conducted to get familiarity and locate the sampling sites of the area. The accessibility of these sites was also assessed for collection of micro-biota and water samples. A total of seven sampling sites were randomly chosen (Table 1).

Table 1. Sampling sites of different areas of Attock Refinery Limited (ARL)

No.	Sampling sites	Latitude (N)	Longitude (E)	Elevation (m)
1	A	33º33′29″	73º4′43″	509
2	B	33º33′18″	73º4′42″	507
3	C	33º33′6″	73º4′39″	501
4	D	33º32′59″	73º4′37″	510
5	E	33º33′12″	73º4′51″	500
6	F	33º33′7″	73º4′40″	501
7	G	33º32′51″	73º4′34″	504

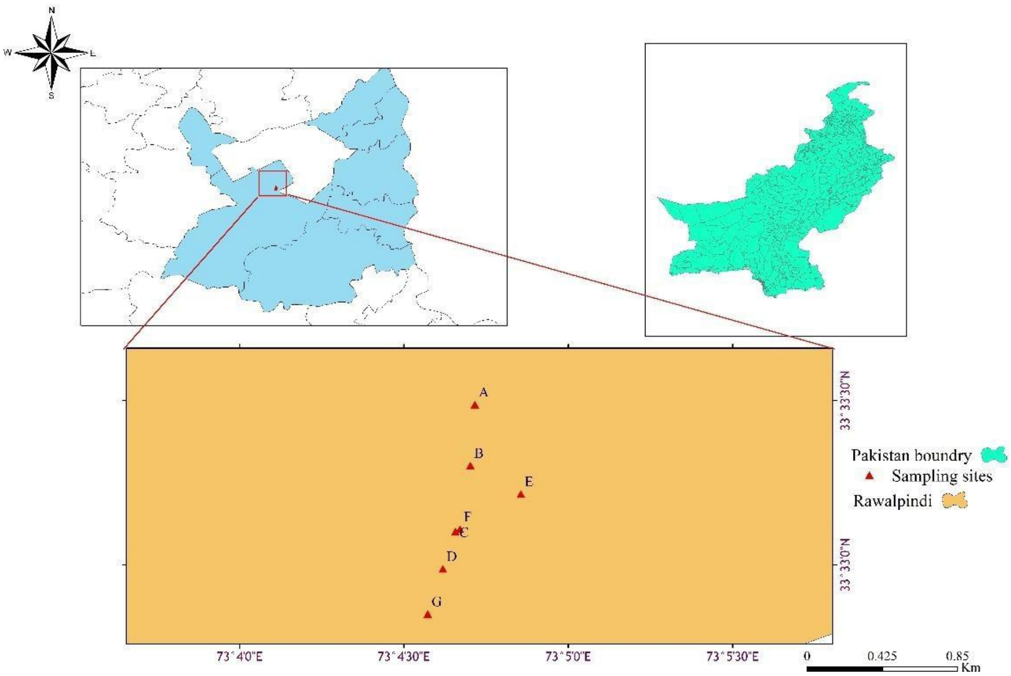

Fig. 4.19 Map of Study Area

4.2.2.2 Wastewater sampling and analysis

Wastewater samples were collected from various points at refinery effluent drains by grab sampling techniques. These were mixed to form composite samples, filtered to remove coarse particles and divided into three replicates. Analysis for various physiochemical parameters like: pH, Total Soluble Solids (TSS), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Oil and grease (O&G), Phenol, Sulphate (SO4), Sulfur (S2), Chlorides (Cl2) and Total Dissolved Solid (TDS) were carried out by using methods of American Public Health Association (APHA, 2005). Sample of untreated wastewater was stored at 4 degrees centigrade until used for Algae cultivation.

4.2.2.3 Algal Sample collection and preparation

Periphyton, benthic and epiphytic species were collected with the help of pipette, knife, forceps and toothbrush using falcon tubes from seven different sites of wastewater channels located in downstream of ARL. These samples were shifted to growth chambers to provide natural climatic conditions under controlled environment (Taylor *et al.*, 2015; Ahmad *et al.,* 2013 and Neveux *et al.*, 2016). The algal species were isolated and multiplied by using BG-0 & BG-11 Agar media in growth chambers. Eighteen Algal species were identified whereas, on the basis of abundance at majority of sites, three species were selected for present study (Ahmad *et al.,* 2013).

4.2.2.4 In-vitro Experiment

The species that performed better to grow during the isolation process like *Lyngbya sp.*, *Scenedesmus sp.* and *Tribonema sp.* were shifted in flasks containing 500 ml of wastewater collected from different selected sites. Then these species were allowed to grow in the wastewater and shifted to growth chamber where temperature was set to 26 $^{o}C \pm 2$, humidity was kept eighty percent (80 %) and normal light (10000 lux) was provided. These species were allowed to grow in growth chamber for 2 weeks, after that samples from the flasks were taken and centrifuged at 13000 rpm for 15 minutes, as a result the sample differentiated into two layers the wastewater was settled down in Eppendorf tubes. Water samples were analyzed for above mentioned parameters.

4.2.3 Results and Discussion

All three selected species were tested in the laboratory for three consecutive weeks. The statistical analysis of variance and Independent T-test at $p \leq 0.05$ (significance level) used to analyze the whole data set of all parameters. Fig. 1, 2 & 3 shows the percentage reduction using algal species.

4.2.3.1 Effect of algal species on pH

Laboratory analysis of wastewater treated with algal species showed that all species significantly reduce the pH of wastewater as compared to control in all three weeks. Maximum reduction in mean pH of wastewater was observed in third week *Lyngbya sp.* (7.03 ppm) exhibited maximum reduction in pH followed by *Scenedesmus sp.* (7.07 ppm) and *Tribonema sp.* (7.20 ppm). Minimum reduction in pH value was observed after first week. *Scenedesmus sp.* (7.40 ppm) performed very well in first week compared to *Lyngbya sp.* (7.53 ppm) and *Tribonema sp.* (7.63 ppm).

Furthermore, species comparison endorsed that all three species showed similar trend in decreasing pH value from first to third week. Time wise and species wise performance concluded that *Scenedesmus sp.* performed well as compared to *Lyngbya sp.* and *Tribonema sp.*

4.2.3.2 Effect of algal species on Total Soluble Solids (TSS) concentration

Total Soluble Solids (TSS) data showed that all species significantly reduced the TSS values as compared to control during all three weeks. Maximum reduction in mean TSS of wastewater was observed in third week *Scenedesmus sp.* (7.33 ppm) exhibited in TSS followed by *Lyngbya sp.* (8.67 ppm) and *Tribonema sp.* (10.67 ppm). Minimum reduction in TSS value was observed after first week. *Scenedesmus sp.* (11.67 ppm) performed very well in first week compared to *Lyngbya sp.* (14.0 ppm) and *Tribonema sp.* (15.33 ppm).

Furthermore, species wise comparison endorsed that all three species presented similar trend in term of decreasing TSS values from first to third week. Overall, it was concluded that *Scenedesmus sp.* performed well as compared to *Lyngbya sp.* and *Tribonema sp.*

4.2.3.3 Effects of algal species on Biological Oxygen Demand (BOD)

Data on Biological Oxygen Demand (BOD) showed that all three species significantly reduced BOD during each week except in case of *Tribonema sp.* which showed no significant difference in the values of second and third week. Moreover, all species showed gradual decrease in BOD as compared to the control treatment. *Scenedesmus sp.* and *Lyngbya sp.* showed lowest mean value i.e (7.67 ppm) followed by *Tribonema sp.* (9.33 ppm) as compared to control treatment (13.33 ppm) during third week. Minimum reduction in BOD value was observed after first week. *Scenedesmus sp.* (10.67 ppm) performed very well in first week compared to *Lyngbya sp.* (11.67 ppm) and *Tribonema sp.* (12.33 ppm). Overall, It was concluded that comparatively, *Scenedesmus sp.* performed well in all three weeks.

4.3.3.4 Effect of algal species on Chemical Oxygen Demand (COD) values

Data regarding Chemical Oxygen Demand (COD) revealed that all species significantly decrease COD during each week as compared to the control treatment. Wastewater treated with *Scenedesmus sp.* showed lowest mean value of (21.00 ppm) followed by *Lyngbya sp.* (22.67 ppm), *Tribonema sp.* (25.0 ppm) as compared to control treatment (33.67 ppm) during third week. Minimum reduction in COD value was observed after first week. *Scenedesmus sp.* (28.33 ppm) performed very well in first week compared to *Lyngbya sp.* (30.33 ppm) and *Tribonema sp.* (31.67 ppm). Overall, *Scenedesmus sp.* performed well as compared to other two species.

4.2.3.5 Effect of algal species on Oil and Grease (O&G) concentrations

Data regarding Oil and Grease (O&G) concentrations data revealed that no significant difference was shown by *Lyngbya sp.* during all the three weeks. *Scenedesmus sp.* showed no significant difference during second and third week. Similarly, *Tribonema sp.* showed no significant difference in all three weeks. Wastewater treated with *Scenedesmus sp.* showed lowest mean value (0.37 ppm) followed by *Lyngbya sp.* (0.38 ppm) and *Tribonema sp.* (0.44 ppm) as compared to control treatment (0.60 ppm) during third week. Minimum reduction in O&G value was observed after first week. *Scenedesmus sp.* (0.41 ppm) performed very well in first week compared to *Lyngbya sp.* (0.43 ppm) and *Tribonema sp.* (0.46 ppm). While comparing with other two species *Scenedesmus sp.* performed well during all three weeks.

4.2.3.6 Effect of algal species on Phenol concentration in refinery wastewater

Data regarding Phenol concentration revealed that all three species showed no significant difference among all three weeks. Wastewater treated with *Lyngbya sp.* and *Scenedesmus sp.* showed lowest mean value of (0.01 ppm) during third week followed by *Tribonema sp.* (0.02 ppm) as compared to control treatment (0.04 ppm). Furthermore, all species performed well during third week as compared to second and first weeks. Minimum reduction in Phenol value was observed during first week. *Scenedesmus sp.* (0.02 ppm) performed well during first week compared to *Lyngbya sp.* and *Tribonema sp.* (0.03 ppm). Overall *Scenedesmus sp.* performed very well while comparing with other two species.

4.2.3.7 Effect of algal species on Sulphates (SO_4)

Data about Sulphates (SO_4) showed that all three species significantly reduced the Sulphate concentration in refinery wastewater samples during all three weeks. After third week wastewater treated with *Scenedesmus sp.* showed lowest mean value (256.00 ppm) followed by *Lyngbya sp.* and *Tribonema sp.* (268.00 ppm) as compared to control treatment (323.33 ppm). Minimum reduction in Sulphate value was observed during first week. *Scenedesmus sp.* (299.33 ppm) performed very well in first week compared to *Lyngbya sp.* and *Tribonema sp.* (306.67 ppm). Overall, *Scenedesmus sp.* proved as the best performer species during all three weeks.

4.2.3.8 Effect of algal species on Sulfides (S_2) concentration

Sulfides (S_2) data revealed that all species showed significant difference among their mean values during third weeks. Wastewater treated with *Tribonema sp.*, *Lyngbya sp.* and *Scenedesmus sp.* showed lowest mean value (0.01 ppm) as compared to control treatment (0.03 ppm) in third week. Minimum reduction in sulfides concentration was observed during first week. All species showed lowest mean (0.03 ppm) in during week of the treatment. Overall, Lyngbya *sp., Tribonema sp.* & *Scenedesmus sp.* performed well during all three weeks.

4.2.3.9 Effect of algal species on Chlorides (Cl_2)

Data of Chlorides (Cl_2) revealed that all species showed significant difference during all three weeks values except *Tribonma sp.*, which showed no significant difference. Wastewater treated with *Lyngbya sp. & Scenedesmus sp.* showed lowest mean value (139.33 ppm) followed by *Tribonema sp.* (218.33 ppm) as compared to control treatment (282.67 ppm) during third week. Minimum reduction in concentration of chlorides was observed with *Lyngbya sp. & Scenedesmus sp.* showed mean value (239.33 ppm) followed by *Tribonema sp.* (256.00 ppm) after first week of treatment. Overall, *Tribonema sp.* showed good results to remove chlorides.

4.2.3.10 Effect of algal species on Total Dissolved Solids (TDS)

Date regarding Total Dissolved Solids (TDS) revealed that all species showed significant difference except third week of *Lyngbya sp.* and *Tribonema sp.* which showed no significant difference. Wastewater treated with *Scenedesmus sp.* showed lowest mean value (879.33 ppm) followed by *Lyngbya sp.* (900.67 ppm), and *Tribonema sp.* (1096.67 ppm) as compared to control (1179.67 ppm) during third week. Minimum reduction in TDS value was observed during first week. *Scenedesmus sp.* (1114.33 ppm) performed very well during first week compared to *Lyngbya sp.* (1124.00 ppm) and *Tribonema sp.* (1159.67 ppm). Overall *Scenedesmus sp.* performed better and showed low values as compared to other two species.

Table 4.3 Comparison between Species over three week's retention time

Parameter	Species	N	1	2	3
pH (ppm)	*Scenedesmus*	12	7.22		
	Lyngbya	12	7.28	7.28	
	Tribonema	12		7.42	
	Control	12			7.70
	Sig.		0.87	0.20	
TSS (ppm)	*Tribonema*	12	9.44		
	Scenedesmus	12	11.11	11.11	
	Control	12		13.11	
	Lyngbya	12			18
	Sig.		0.142	0.055	
BOD (ppm)	*Tribonema*	12	9.11		
	Scenedesmus	12	9.67	9.67	
	Control	12		10.89	
	Lyngbya	12			13.33
	Sig.		0.81	0.212	1
COD (ppm)	*Tribonema*	12	24.89		
	Scenedesmus	12	26.78		
	Control	12	28.44		
	Lyngbya	12		33.67	
	Sig.		0.071	1.000	
O&G(ppm)	*Tribonema*	12	0.39		
	Scenedesmus	12	0.40		
	Control	12		0.45	
	Lyngbya	12			0.50
	Sig.		0.191		1
Phenol (ppm)	*Tribonema*	12	0.02		
	Scenedesmus	12	0.02	0.02	
	Control	12		0.027	
	Lyngbya	12			0.03667
	Sig.		0.55	0.052	
SO4 (ppm)	*Tribonema*	12	277.56		
	Scenedesmus	12	287.11		
	Control	12	287 .11		
	Lyngbya	12		323.33	
	Sig.		0.45	1	
S2 (ppm)	*Scenedesmus*	12	0.02		
	Control	12	0.02		
	Tribonema	12	0.02		
	Lyngbya	12		0.033	
	Sig.		0.98		
Cl (ppm)	*Scenedesmus*	12	193.78		
	Tribonema	12	193.78		
	Control	12		234.89	
	Lyngbya	12			282.67
	Sig.				
TDS (ppm)	*Tribonema*	12	1003.56		
	Scenedesmus	12	1020		
	Control	12		1133.11	
	Lyngbya	12		1179.67	
	Sig.		0.958	0.49	

Table 4.4. Week wise comparison of Algal species

Parameter			Control	*Lyngbya*				*Scenedesmus*				*Tribonema*			
	Week	N	1	Week	1	2	3	Week	1	2	3	Week	1	2	3
pH	1	4	7.70	3	7.0			3	7.07			3	7.20		
	2	4	7.70	2		7.27		2	7.20			2		7.43	
	3	4	7.70	1			7.53	1		7.40		1			7.63
	Sig.		1.00	Sig.	1.0	1.00	1.00	Sig.	0.07	1.00		Sig.	1.00	1.00	1.00
TSS (ppm)	1	4	18.00	3	8.7			3	7.33			3	10.7		
	2	4	18.00	2		10.67		2		9.33		2		13.33	
	3	4	18.00	1			14.00	1			11.67	1			15.33
	Sig.		1.00	Sig.	1.00	1.00	1.00	Sig.	1.00	1.00	1.00	Sig.	1.00	1.00	1.00
BOD (ppm)	1	4	13.33	3	7.7			3	7.67			3	9.33		
	2	4	13.33	2		9.67		2		9.00		2	11.0	11.00	
	3	4	13.33	1			11.67	1			10.67	1		12.33	
	Sig.		1.00	Sig.	1.00	1.00	1.00	Sig.	1.00	1.00	1.00	Sig.	0.07	0.15	
COD (ppm)	1	4	33.67	3	22.7			3	21.00			3	25.0		
	2	4	33.67	2	27.3	27.33		2		25.33		2	28.7	28.67	
	3	4	33.67	1		30.33		1		28.33		1		31.67	
	Sig.		1.00	Sig.	0.08	0.29		Sig.	1.00	0.16		Sig.	0.13	0.23	
O&G (ppm)	1	4	0.60	3	0.38			3	0.37			3	0.44		
	2	4	0.60	2	0.41	0.41		2		0.39		2	0.45		
	3	4	0.60	1		0.43		1		0.41		1	0.46		
	Sig.		1.00	Sig.	0.08	0.12		Sig.	1.00	0.07		Sig.	0.13		
Phenol (ppm)	1	4	0.04	3	0.01			3	0.01			3	0.02		
	2	4	0.04	2	0.02	0.02		2	0.02	0.02		2	0.03	0.03	
	3	4	0.04	1		0.03		1		0.02		1		0.03	
	Sig.		1.00	Sig.	0.09	0.09		Sig.	0.09	0.09		Sig.	0.09	0.09	
S04 (ppm)	1	4	323.33	3	268			3	256.00			3	268		
	2	4	323.33	2		286.67		2		277.33		2		286.67	
	3	4	323.33	1			306.67	1			299.33	1			306.7
	Sig.		1.00	Sig.	1.00	1.00	1.00	Sig.	1.00	1.00	1.00	Sig.	1.00	1.00	1.00
S2 (ppm)	1	4	0.03	3	0.01			3	0.01			3	0.01		
	2	4	0.03	2		0.02		2		0.02		2		0.02	
	3	4	0.03	1			0.03	1			0.03	1			0.03
	Sig.		1.00	Sig.	1.00	1.00	1.00	Sig.	1.00	1.00	1.00	Sig.	1.00	1.00	1.00
Cl_2 (ppm)	1	4	282.67	3	139			3	139.33			3	218.3		
	2	4	282.67	2		202.67		2		202.67		2	230	230.33	
	3	4	282.67	1			239.33	1			239.33	1		256.0	
	Sig.		1.00	Sig.	1.00	1.00	1.00	Sig.	1.00	1.00	1.00	Sig.	0.45	0.06	
TDS (ppm)	1	4	1179.7	3	901			3	879.33			3	1097		
	2	4	1179.7	2		1035.3		2		1017.0		2	1143	1143.0	
	3	4	1179.7			1124					1114.33			1159.7	
	Sig.		1.00	Sig.	1.00	0.11		Sig.	1.00	1.00	1.00	Sig.	0.10	0.68	

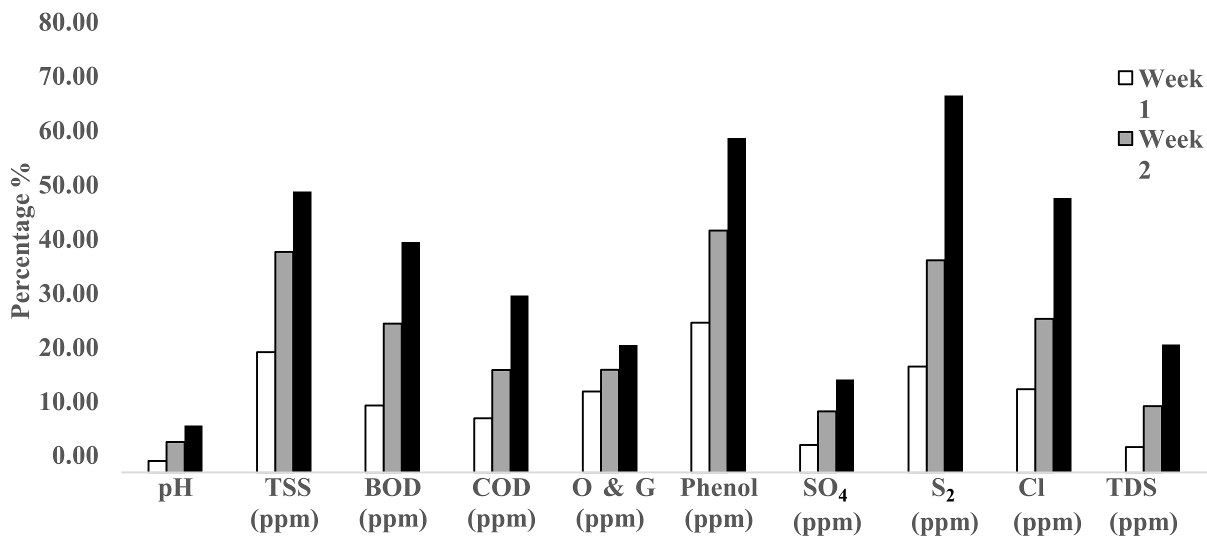

Fig. 4.20 Percentage Reduction in Concentration of all Parameters using *Lyngbya Sp.*

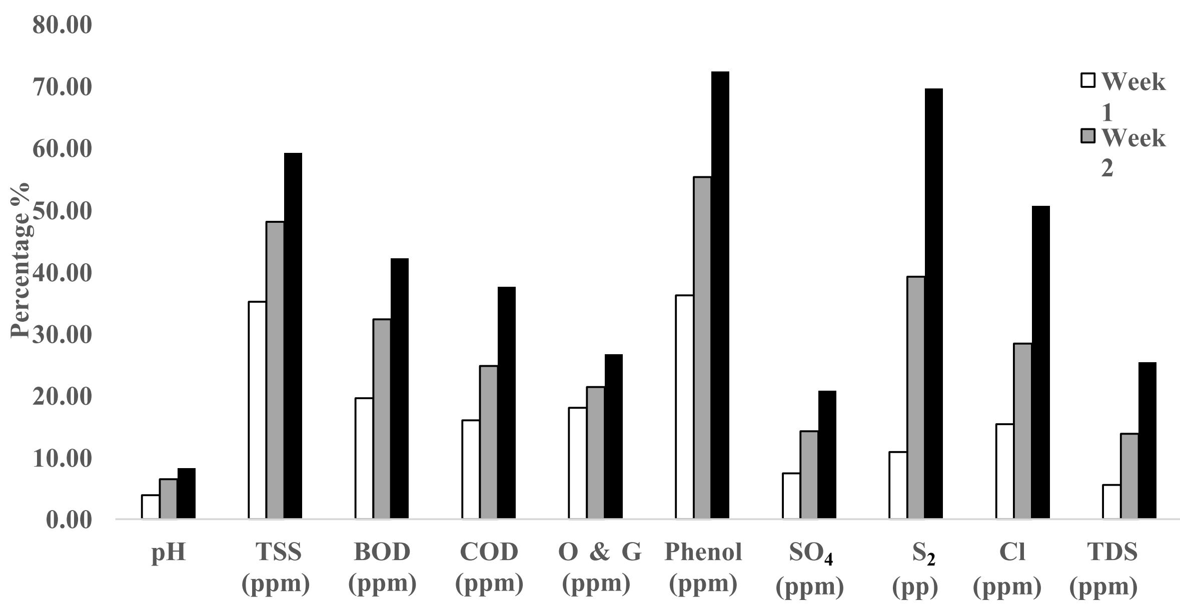

Fig. 4.21 Percentage Reduction in Concentration of all Parameters using *Scenedesmus sp.*

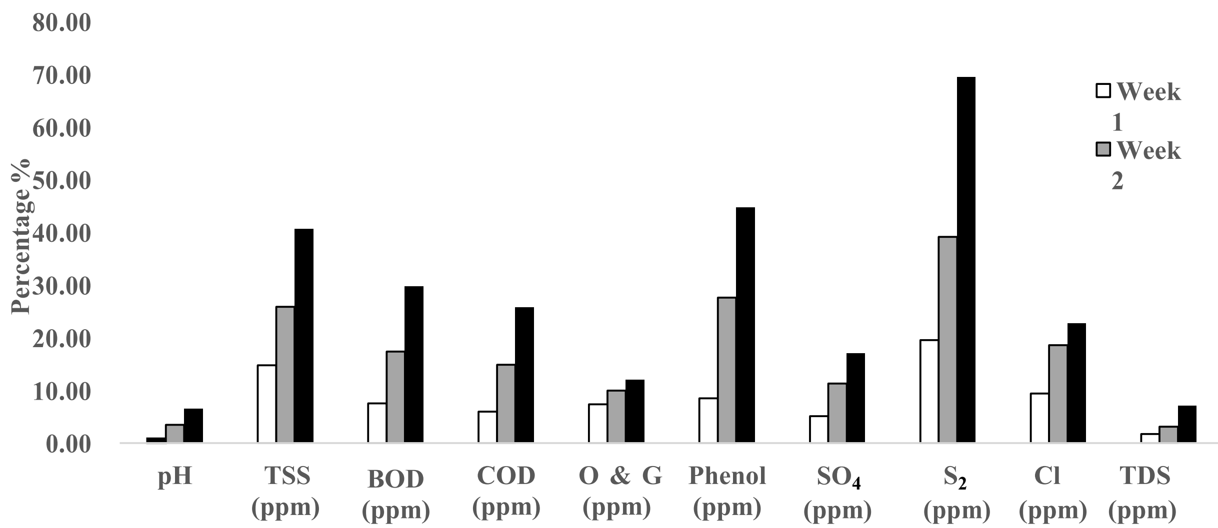

Fig. 4.22 Percentage Reduction in Concentration of all Parameters using *Tribonema Sp*

4.2.3.11 Discussion

Previous studies strongly supported that algal species can be used as alternative, ecofriendly, economical and efficient method for wastewater treatment (Chinnasamy *et al.*, 2010). But few studies also raise the question on using non- native proven and tested algal species to use for Phycoremediation as may native algal species start competing with them. Furthermore, growth and action of non- native species may be affected by local climatic and environmental condition and unable to give desired results. That's why it is being emphasized the need of exploration and testing of native and abundantly available algal species.

Laboratory trial showed quite promising results and predicted the potential of tested species to perform well on field scale. Algal species proved successful for reduction in pH, COD, BOD, TDS, TSS, Phenol, oil and grease, SO_4, S_2 and Cl_2 after first week of treatment which was further become effective with the passage of time up to the third week of every treatment. Which exhibit that long term treatment of algae is useful in term of Phycoremediation. Furthermore, with the increase in retention period algal biomass also increased, which enhance the efficiency of Phycoremediation process and ultimately play important role in pollution control. Mahapatra, *et al.,* (2013) also reported similar results and showed to decrease in pollution level from 50 % to 60 % at field level.

After treatment, pH value did not decrease below 7 over the period of time. pH value above and equal to 7 suggested that these species are using more carbon dioxide from water and decreasing its level in water due to increase in photosynthesis rate. So it means algal biomass is increasing with the passage of time, which is good indicator and proved to be helpful to control water pollution by increasing retention period (Oberholster *et al.,* 2021).

However, decrease in COD also correlate with increase in biomass level as it increased the chemical demand of dissolved oxygen level. Further decrease in BOD also correlate with increase in algal biomass in waste water (Oberholster *et al.*, 2019; Sharma *et al.*, 2013)

Initially, algal species absorb and convert many harmful pollutants into non- harmful forms in present capacity but later on with the increase in growth of algal species absorption surface area also increased, which enhances the absorption of TSS and TDS. Moreover reduce in the value of TSS confirm the active phytoremediation activity due to algal species. Other studies also supported that it reduced the turbidity (Sharma *et al.*, 2013) in wastewater. Decrease in TDS value is due to two factors one is because of evaporation and second is due to absorption of TDS by plants and algal species due to rapid growth. Furthermore, it also remediate the other pollutants in wastewater.

Decrease in Phenols, Oil & grease, SO_4, S_2 and Cl_2 also indicate the rapid algal growth and increased algal biomass (Ugya *et al.,* 2019; Amare *et al.,* 2018 and Liu *et al.*, 2010) which not only increase absorption of above mention pollutants but regulate all nutrient cycles like nitrogen and carbon cycles and also helps to decrease above discussed pollutants in refinery wastewater. Moreover, reduction in SO_4 also acknowledged the fact that it contribute to regulate many physiological characteristics of macro and micro-phytes in water which indirectly regulate and decrease many pollutants in water (Zhang *et al.*, 2008; Li *et al.*, 2012; Li *et al.*, 2012 and Ugya A. Y., 2017).

All three species performed very well in laboratory condition in term of Phycoremediation. As these showed promising results and proved to be good ecofriendly alternative for refinery wastewater treatment. But overall *Scenedesmus sp.* performed better compared to *Lyngbya sp.* and *Tribonema sp.* Overall above discussed results are promising which encourage the field scale experiment and provided base line study for refinery wastewater researchers.

4.2.4 Conclusion

Overall results confirmed that all the three species are good to treat refinery wastewater before discharging into natural streams. It was also concluded that all species have potential to improve the wastewater quality if exposed to the wastewater for longer times. Resultantly, the treated wastewater can be used for agriculture or recycled for refinery processes. The results also encourage to test all of these species on field scale as cost effective, environment friendly and chemical free solution to regulate water nutrient cycles.

4.3 Comparative Study of Phycoremediation through Micro Alga for Refinery Wastewater Treatment

4.3.1 Introduction

Wastewater treatment through traditional means, i.e. Bioremediation and Phycoremediation is the need of day in context of heavily urbanized/industrialized future. Phycoremediation (Mustafa and Hayder, 2021) Bioremediation process employs microorganisms such as microalgae for wastewater treatment. Phycoremediation has been known from last 40 years (Ahmad et al., 2013; Oswald et al., 1957) was the first to apply this discovery. Phycoremediation has several applications like: (i) nutrient removal from municipal wastewater (ii) organic matter-rich effluents removal (iii) nutrient and xenobiotic compounds removal by using algae-based bio sorbents (iv) wastewater and acidic treatment (v) CO_2 sequestration (vi) Xenobiotic transformation and degradation (vii) detection of toxic compounds using algae-based biosensors. On the basis of nutrient removal, a clear distinction may be drawn between microalgae and other conventional technologies (Danouche et al., 2021; Lavoie & De la Noüe, 1985).

Due to significant energy consumption, current wastewater treatment technologies are costly so that most of the industries in developing countries released it untreated into the fresh water bodies which cause serious health issues in humans and animals due to the presence of pathogens. Long-term exposure through irrigation and drinking may results in a variety of illnesses including bowel disease, cancer and birth defects in children (Singh & Mishra, 1987). Previous research work could not mainly be focused on refinery wastewater, most of the literature is based on industries like fabric, textile, paper mills automotive and tanneries etc. Due to ever increasing global energy demands, new refineries are being added therefore, it was utmost important to work on refinery wastewater. Aim of this study was to analyze and compare the effectiveness of different micro-Algal *sp*ecies to improve the refinery wastewater quality through Phycoremediation.

4.3.2 Materials and Methods

4.3.2.1 Study Area

The research work was conducted at Attock Refinery Limited (ARL), Morgah, Rawalpindi. ARL is the pioneer of crude oil refining in Pakistan with its operations dating back to 1922. Several preliminary surveys of Morgah, Rawalpindi including ARL Refinery were conducted to get familiarity and locate the sampling sites of the area. The accessibility of these sites was also assessed for collection of micro-biota and water samples. A total of seven sampling sites were randomly chosen (Fig. 4.1).

4.3.2.2 Wastewater sampling and analysis

Wastewater samples were collected from various points at refinery effluent drain by grab sampling techniques. These were mixed to form composite samples, filtered to remove coarse particles and divided into three replicates. Analysis for various physiochemical parameters like: PH, Total Soluble Solids (TSS), Biological Oxygen Demand (BOD), Chemical Oxygen Demand

(COD), Oil and grease (O&G), Phenol, Sulphate (SO4), Sulfur (S2), Chlorides (Cl) and Total Dissolved Solid (TDS) was carried out by using methods of American Public Health Association (APHA, 2005). Sample of untreated wastewater was stored at 4 degrees centigrade until used for Algae cultivation.

4.3.2.3 Algal Sample collection and preparation

Periphyton, benthic and epiphytic species were collected with the help of pipette, knife, forceps and toothbrush using falcon tubes from seven different sites of wastewater streams

located in vicinity of ARL. These samples were shifted to growth chambers to provide natural climatic conditions under controlled environment(Ahmad, Khan and Yasar, 2013; Taylor et al., 2015; Neveux, Magnusson, Mata, Whelan, Nys, *et al.*, 2016).The algal *sp*ecies were isolated and multiplied BG-0 & BG-11 Agar media in growth chambers. Eighteen Algal *sp*ecies were identified whereas, on the basis of abundance at majority of sites three suitable *sp*ecies were selected for present study(Ahmad, Khan and Yasar, 2013).

4.3.2.4 Method for Phycoremediation

The selected three *sp*ecies were multiplied in the laboratory, transferred to refinery green/ laith houses for further multiplication. After acclimatization, algal *sp*ecies were transferred to the re*sp*ective cells of experimental apparatus. Chambers were already contained fresh tap water and wastewater was allowed to gradually replace the fresh water. The fiber glass constructed chambers were used to avoid interference of chemicals from surrounding soil. The thickness of the chamber was 4 mm with 1% slope to facilitate the water circulation and flow. In order to ensure the smooth flow of wastewater, the PVC pipes were used from wastewater tanks to inlet of treatment chambers and for collection of the treated wastewater polyethylene drain flex pipes were used at the exit. After the completion of the acclimatization phase and complete replacement of tap water with wastewater in treatment chambers, the untreated wastewater was applied to cells by gravity flow via *sp*herical valves and PVC pipes at constant Hydraulic Loading Rate (HLR) of 20 liters/day. Discharge rate of wastewater was readjusted based upon first pilot reading. Treated wastewater samples were collected and analyzed. Each sample having one liter volume was collected in sterilized jars. Collected samples were studied and analyzed in laboratory to check the performance of Phycoremediation process. Laboratory analyses were carried out at Quality Control Laboratory (QCL) of ARL, Environmental lab of National Cleaner

Production Center (NCPC) and in the laboratory of Department of environmental science, International Islamic University, Islamabad (IIUI). Chemical analyses were carried out as per the Standard Methods for concerned parameters (Bansal and Steel, 2018; Kiran *et al.*, 2018).

4.3.3 Results and Discussion

All three selected *sp*ecies were tested in the field for three consecutive weeks in two different growing seasons (August, 2020 (S1) and April, 2021 (S2)). The statistical analysis of variance and Independent T-test at $p \leq 0.05$ (significance level) used to analyze the whole data set of all parameters.

4.3.3.1 Effect of algal species on pH of refinery wastewater

pH data (Fig. 1) revealed that all *sp*ecies significantly reduce the pH as compared to control in both seasons. During S1 maximum reduction in mean pH of wastewater was observed in third week through *Tribonema sp*., *Scenedesmus sp*. and *Lyngbya sp*. (7.24, 7.26 and 7.33 ppm re*sp*ectively). On the other hand, minimum reduction in pH value was observed after first week by *Tribonema sp*. (7.72 in S1 and 7.96 in S2) followed by *Scenedesmus sp*. (7.59 in S1 and 7.76 in S2) and *Lyngbya sp*. (7.53 in S1 and 7.48 in S2).

The comparison of results revealed that all *sp*ecies showed significant difference in the mean values of pH after all the three weeks of both seasons except *Lyngbya sp*. in second week (7.39 in S1 and 7.41 in S2). The effluent treated with *Tribonema sp*. performed best (7.24) in third week of S1 followed by *Scenedesmus sp*. (7.26) and *Lyngbya sp*. (7.33). Overall it was observed that *Lyngbya sp*. performed well as compared to *Scenedesmus sp*. and *Tribonema sp*.

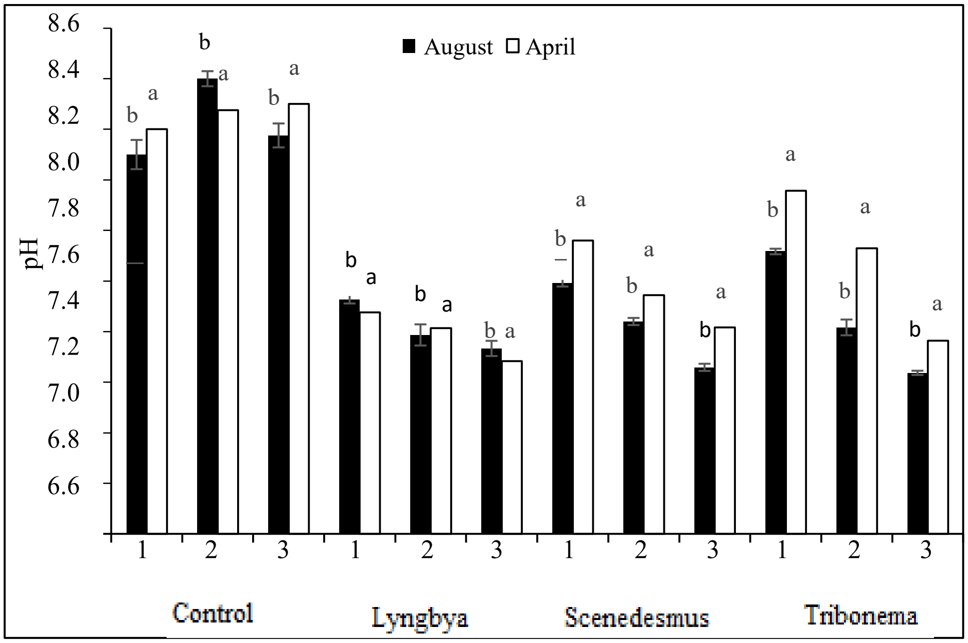

Fig. 4.23 Comparison of pH reduction by Algal species

4.3.3.2 Effect of algal species on Total Soluble Solids (TSS) of refinery wastewater

Total Soluble Solids (TSS) data (Fig. 2) revealed that all *sp*ecies significantly reduced TSS during S1 and S2. *Lyngbya sp*. showed lowest mean value (5.00 ppm) followed by *Tribonema sp*. (6.83 ppm), *Scenedesmus sp*. (6.87 ppm) as compared to control (28.4 ppm) in third week of S1. However, in S2 lowest mean value was observed in wastewater treated with *Scenedesmus sp*. (8.33 ppm) followed by *Tribonema sp*. (8.67 ppm) and *Lyngbya sp*. (17.0 ppm) as compared to control treatment (32.7 ppm) in third week. Overall in comparison with other two *sp*ecies, *Scenedesmus sp*. performed well in both seasons.

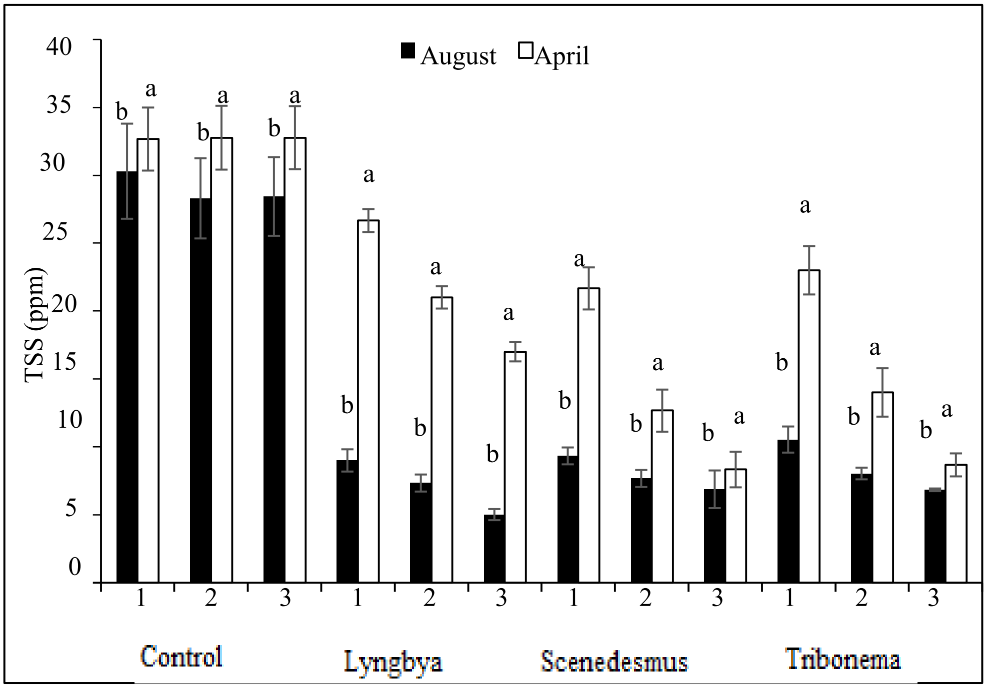

Fig. 4.24 Comparison of TSS reduction by Algal species

4.3.3.3 Effect of algal species on Biological Oxygen Demand (BOD) of refinery wastewater

Data on Biological Oxygen Demand (BOD) showed (Fig. 3) that all three *sp*ecies significantly reduced BOD during each week of both seasons except *Tribonema sp*. which showed no significant difference in the values of week three of both seasons. Moreover, all *sp*ecies showed gradual decrease in BOD as compared to the control treatment except *Lyngbya sp*. which showed highest value in first week of S1 (18.67 ppm), it may be due to human error at any stage during analysis. Furthermore, during S1 *Scenedesmus sp*. showed lowest mean value i.e (4.36 ppm) followed by *Tribonema sp*. (7.27 ppm) and *Lyngbya sp*. (12.67 ppm) as compared to control treatment (15.44 ppm) in third week. During S2 *Tribonema sp*. had lowest mean value (7.43 ppm) followed by *Scenedesmus sp*. (8.67 ppm) and *Lyngbya sp*. (10.0 ppm) as compared to control treatment which shows (17.43 ppm) in third week. It was observed that comparatively, *Scenedesmus sp*. performed well in both seasons.

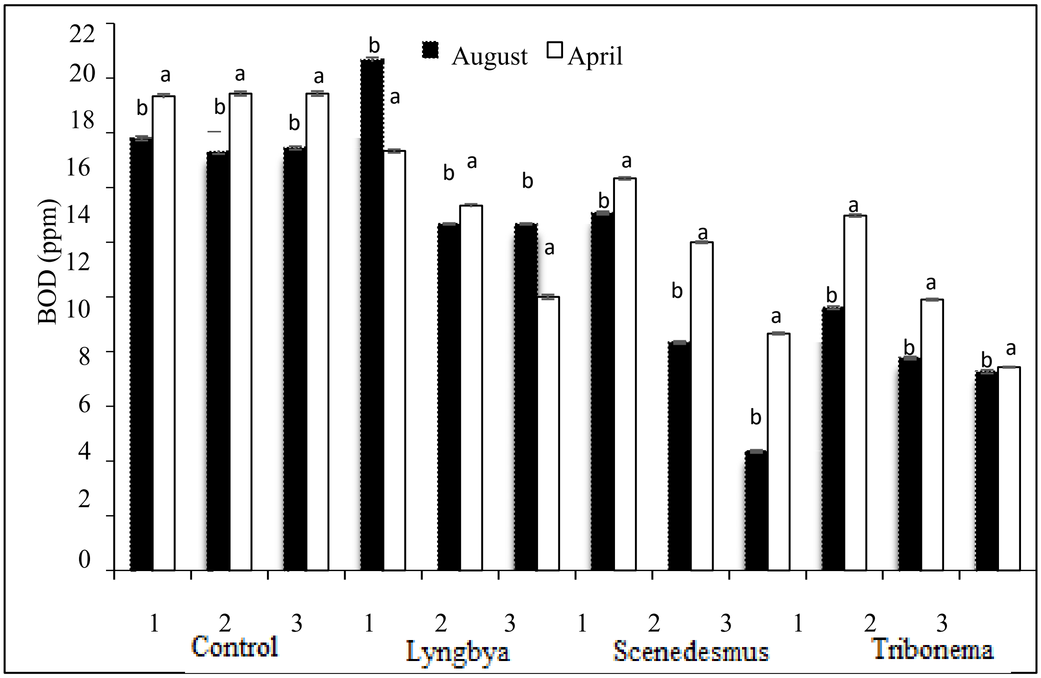

Fig. 4.25 Comparison of BOD reduction by Algal species

4.3.3.4 Effect of algal species on Chemical Oxygen Demand (COD) of refinery wastewater

Values of Chemical Oxygen Demand (COD) (Fig. 4) revealed that all *sp*ecies significantly decrease COD during S1 and S2 as compared to the control treatment which showed no significant difference in values of both seasons. Wastewater treated with *Scenedesmus sp*. showed lowest mean value of (9.71 ppm) followed by *Tribonema sp*. (11.6 ppm), *Lyngbya sp*. (14.0 ppm) as compared to control treatment (37.7 ppm) in third week of S1. However, in S2 lowest mean value was observed in wastewater treated with *Tribonema sp*. (13.6 ppm) followed by *Scenedesmus sp*. (16.7 ppm) and *Lyngbya sp*. (18.6 ppm) as compared to control treatment (37.1 ppm) in third week. In both seasons *Scenedesmus sp*. performed well as compared to other two *sp*ecies.

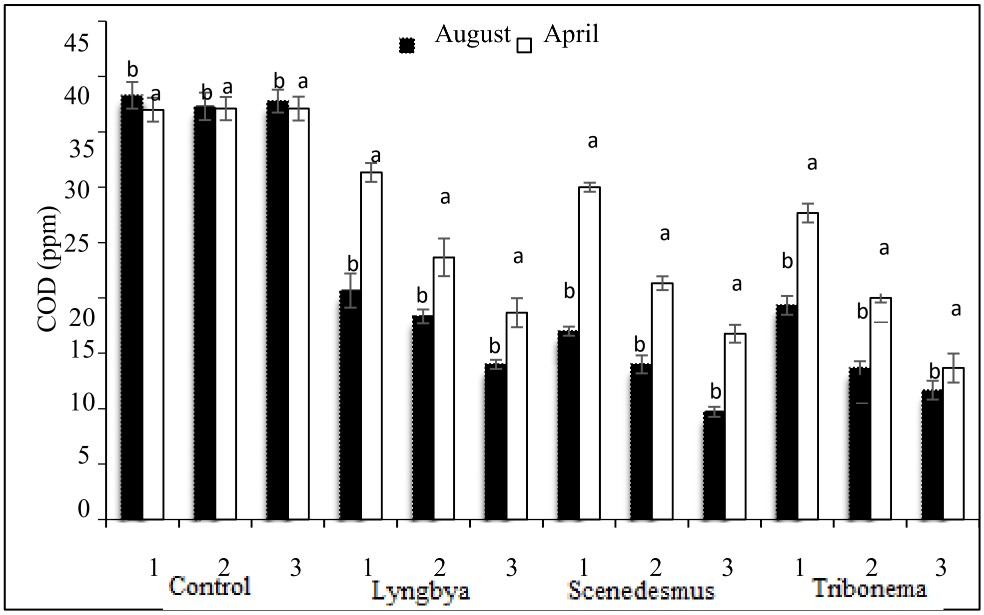

Fig. 4.26 Comparison of COD reduction by Algal species

4.3.3.5 Effect of algal species on Oil and Grease (O&G) of refinery wastewater

Oil and Grease (O&G) data (Fig. 5) revealed that no significant difference was shown by *Lyngbya sp.* during all the three weeks of S1 & S2. *Scenedesmus sp.* showed no significant difference in first two weeks while showed significantly difference value during third week of both seasons. Similarly, *Tribonema sp*. showed significant difference of its values in all weeks of both seasons (0.37 ppm in S1 & 0.42 in S2). Wastewater treated with *Tribonema sp.* showed lowest mean value (0.24 ppm) followed by *Lyngbya sp.* (0.36 ppm) and *Scenedesmus sp.* (0.37 ppm) as compared to control treatment (0.61 ppm) in third week of S1. However, during S2 lowest mean value was observed in wastewater treated with *Lyngbya sp.* (0.37 ppm) followed by *Scenedesmus sp.* (0.42 ppm) and *Tribonema sp*. (0.43 ppm) as compared to control treatment (0.60 ppm) in third week. In comparison to the other two *sp*ecies, *Lyngbya sp.* performed well in both seasons.

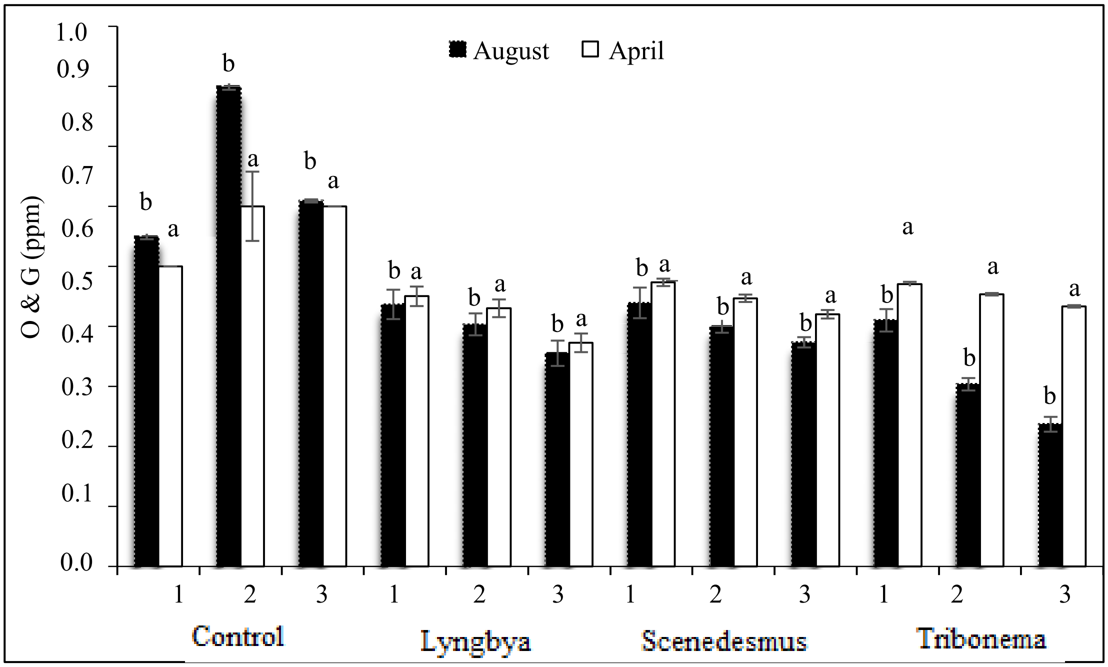

Fig. 4.27. Comparison of O&G reduction by Algal species

4.3.3.6 Effect of algal species on Phenol of refinery wastewater

Data regarding Phenol (Fig. 6) revealed that *Lyngbya sp.* & *Tribonema sp.* showed no significant difference during S1 and S2 while *Scenedesmus sp.* showed no significant difference in values of both seasons. During S1 Wastewater treated with *Tribonema sp.* showed lowest mean value of (0.007 ppm) in third week followed by *Lyngbya sp.* (0.010 ppm) and *Scenedesmus sp.* (0.023 ppm) as compared to control treatment (0.162 ppm) in third week in S1. Furthermore, in all treatments third week performed well as compared to second and first week. However, during S2 lowest mean value (0.017 ppm) was observed in waste water treated with *Tribonema sp.* followed by *Lyngbya sp. and Scenedesmus sp.* each having value (0.027 ppm) as compared to control treatment (0.173 ppm) in third week. Overall *Lyngbya sp.* performed well in comparison of other two *sp*ecies during both seasons.

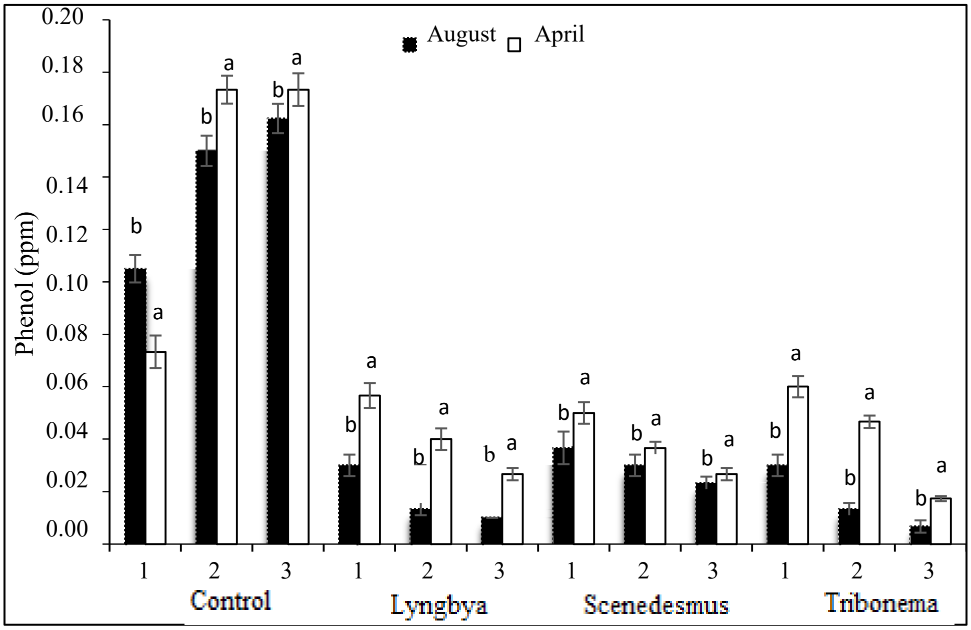

Fig. 4.28 Comparison of Phenol reduction by Algal species

4.3.3.7 Effect of algal species on Sulphate (SO_4) of refinery wastewater

Data about Sulphate (SO4) (Fig. 7) revealed that all three *sp*ecies significantly reduced the sulphate concentration in wastewater samples during both seasons except *Scenedesmus sp.* which showed no significant difference during second and third week. After third week of S1 wastewater treated with *Tribonema sp.* showed lowest mean value (128 ppm) followed by *Lyngbya sp.* (141 ppm) and *Scenedesmus sp*. (162 ppm) as compared to control treatment (406 ppm) in third week. Similarly, after third week of S2 wastewater treated with *Scenedesmus sp.* showed lowest value (165 ppm) followed by *Tribonema sp.* (166 ppm) and *Lyngbya sp.* (223 ppm) as compared to control treatment (396 ppm). Overall all species performed better during S1 as compared to S2. Furthermore, *Tribonema sp.* proved as the best performer *sp*ecie in both seasons.

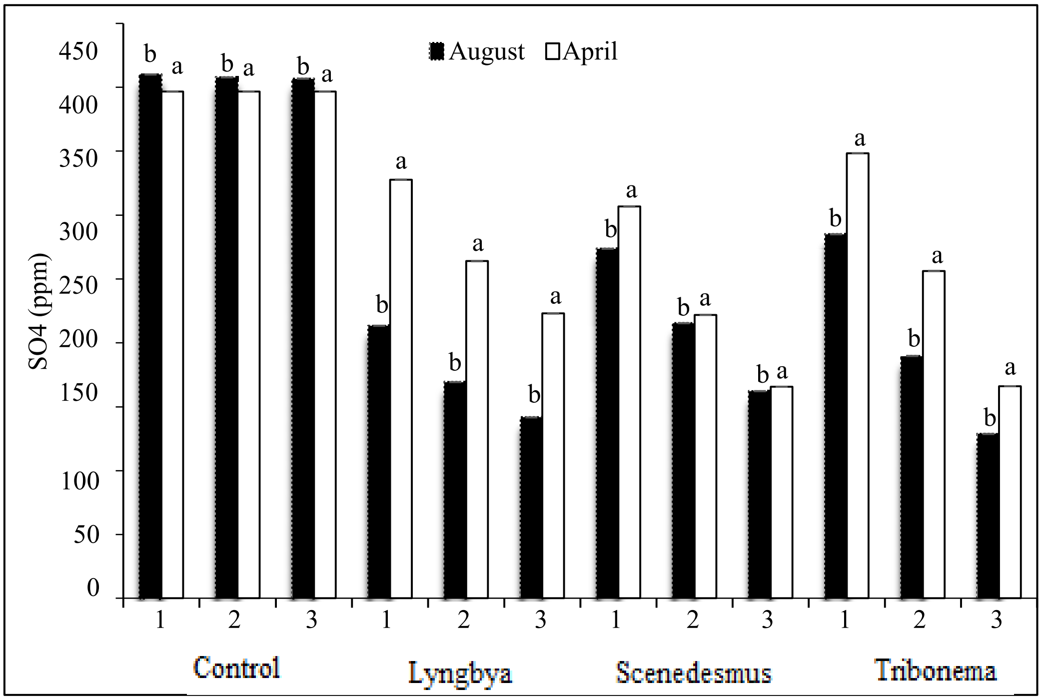

Fig. 4.29 Comparison of Sulphates reduction by Algal species

4.3.3.8 Effect of algal species on Sulphur (S_2) concentration in refinery wastewater

Sulphur (S_2) data **(**Fig. 8) revealed that all *sp*ecies showed significant difference among their mean values during both seasons (S1 & S2). However, *Lyngbya sp.* & *Scenedesmus sp* showed no significant difference in third week of both seasons (S1 & S2). In S1 wastewater treated with *Tribonema sp.*, *Lyngbya sp*. and *Scenedesmus sp*. showed lowest mean value (0.01 ppm) as compared to control treatment (0.16 ppm) in third week. In S2 wastewater treated with *Lyngbya sp. and Scenedesmus sp.* showed lowest value 0.01 ppm followed by *Tribonema sp.* (0.02 ppm) as compared to control treatment (0.15 ppm) in third week. Overall, S1 showed maximum decrease in Sulphur concentration in all treatments during all three weeks as compared to S2. Although *Lyngbya sp.* & *Scenedesmus sp.* perform well in both seasons.

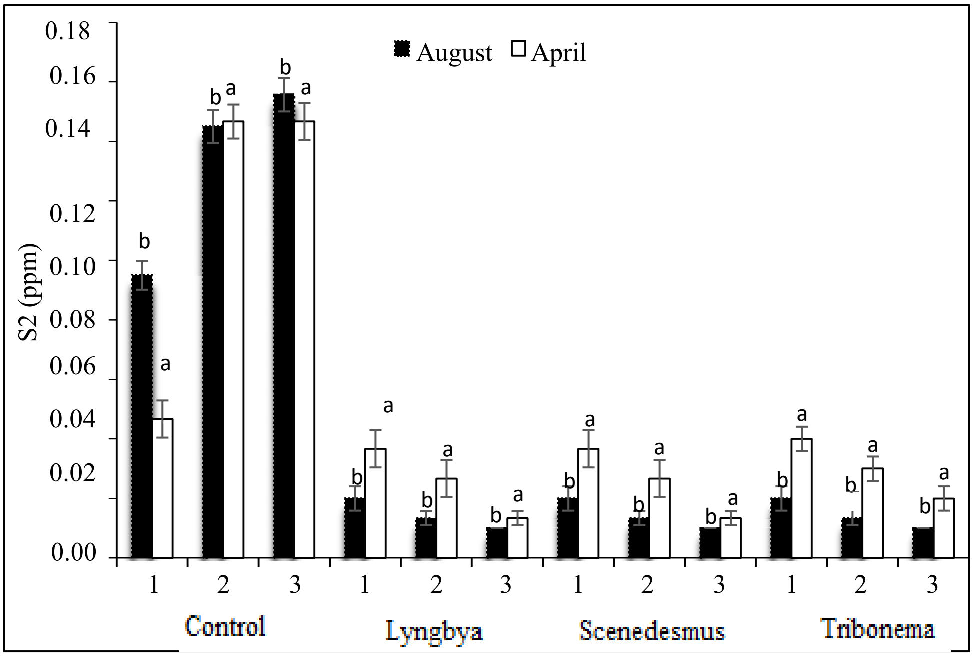

Fig. 4.30 Comparison of Sulphur reduction by Algal species

4.3.3.9 Effect of algal species on Chlorides (Cl_2) in refinery wastewater

Data of Chlorides (Cl_2) (Fig. 9) revealed that all *sp*ecies showed significant difference during S1 and S2 values except *Lyngbya sp.* which showed no significant difference in third week and *Scenedesmus sp.* in second week only. During S1 wastewater treated with *Tribonema sp.* showed lowest mean value (99 ppm) followed by *Lyngbya sp*. (114 ppm) and *Scenedesmus sp*. (258 ppm) as compared to control treatment (365 ppm) in third week. During S2 wastewater treated with *Lyngbya sp.* and *Tribonema sp.* each showed lowest value (133 ppm) followed by *Scenedesmus sp.* (155 ppm) as compared to control (353 ppm) in third week. Overall *Tribonema sp.* and *Lyngbya sp.* showed better performance in S1 as compared to S2. Overall, *Tribonema sp.* proved good results to remove chlorides in both seasons.

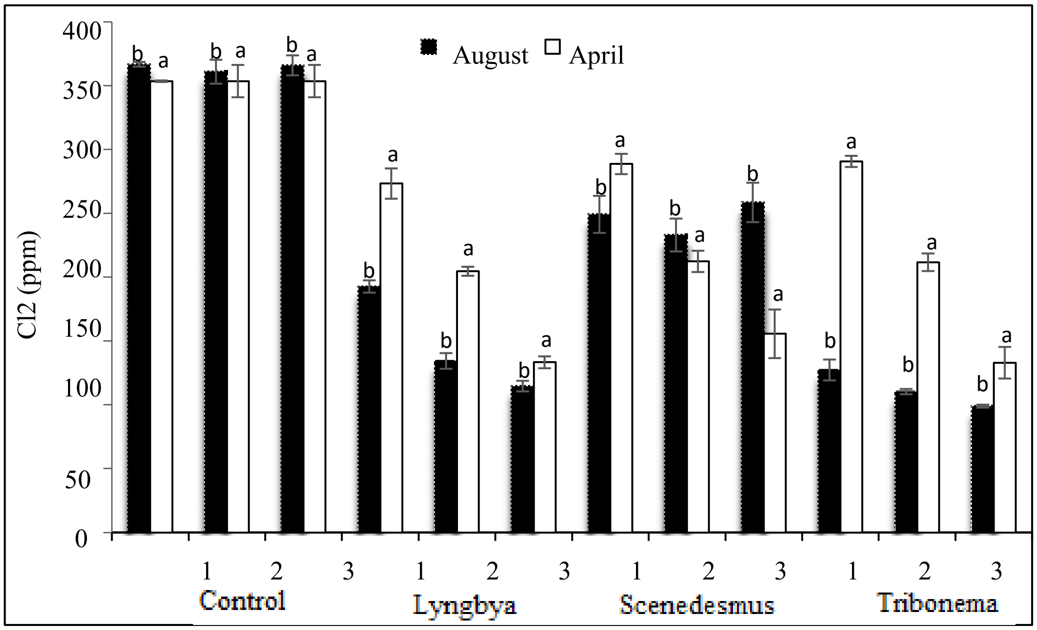

Fig. 4.31 Comparison of Chlorides reduction by Algal species

4.3.3.10 Effect of algal species on Total Dissolved Solid (TDS) of refinery wastewater

Total Dissolved Solid (TDS) data (Fig. 10) revealed that all *sp*ecies showed significant difference in S1 and S2 except third week of both seasons in case of *Lyngbya sp.* and *Tribonema sp.* which showed no significant difference. During S1, wastewater treated with *Tribonema sp*. showed lowest mean value (568 ppm) followed by *Lyngbya sp*. (1238 ppm), and *Scenedesmus sp*. (1542 ppm) as compared to control (1617 ppm) in third week. In S2, wastewater treated with *Tribonema sp.* showed lowest value (557 ppm) followed by *Scenedesmus sp.* (596 ppm) and *Lyngbya sp.* (1196 ppm) as compared to control (1669 ppm) in third week. Overall *Tribonema sp.* performed better and showed low values as compared to other two *sp*ecies.

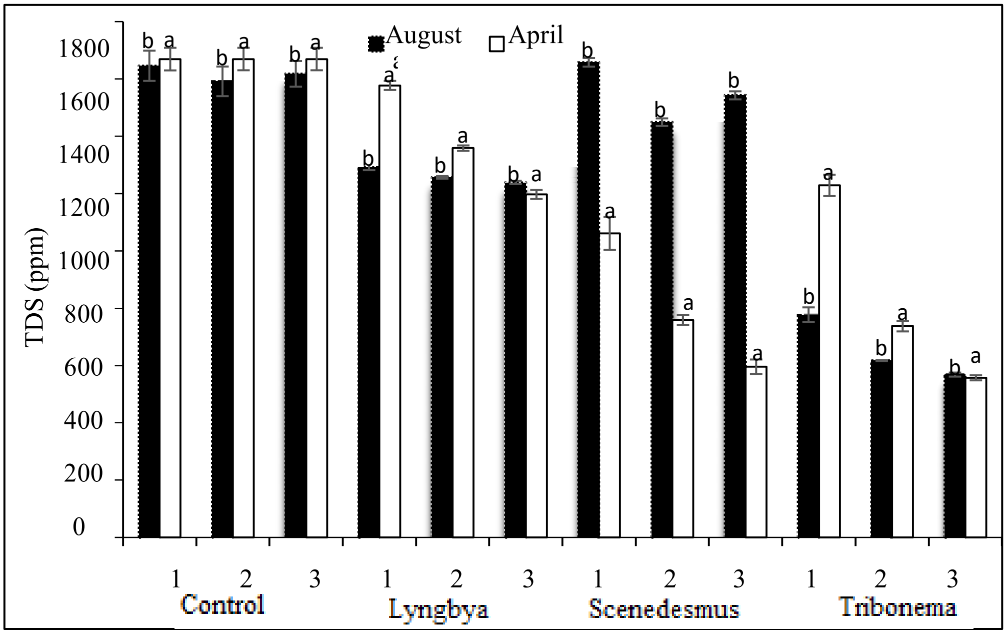

Fig. 4.32 Comparison of TDS reduction by Algal species

4.3.3.11 Discussion

Biological contamination control in wastewater is getting more popular due to its sustainability and environment friendly nature. In this regard Phycoremediation is one of the best natural treatment processes to deal with the problem. (Hodges *et al.*, 2017; Al-Hussieny, Imran and Jabur, 2020) reported the use of algal *sp*ecies to treat effluents from the refineries. The result of the above study is in line with present study in which *Lyngbya sp.* Significantly reduced the oil and grease (0.36 ppm) and Phenol (0.01 ppm) content of the refinery wastewater. The findings of present study showed the reduction of COD, BOD, TDS and TSS consecutively highlighted the beneficial use of photosynthetic microorganisms in the wastewater treatment which are similar to results of current study (Ashwaniy, Perumalsamy and Pandian, 2020).

Scenedesmus obliques showed BOD and COD level were reduced (4.36 ppm and 9.71 ppm re*sp*ectively) similar to the results reported by (Rajasulochana et al., 2009). Reduction of BOD and COD levels might be happened due to removal of the soluble organic compound and derivatives some extent from the wastewater during treatment process. Treatment of refinery effluents with *S. obliques* is an effective technology in the degradation of pollutants. Furthermore, *Scenedesmus obliques*. Successfully reduced the Oil and Grease (0.37ppm) and Phenol (0.02 ppm), also remain active in contaminated refinery wastewater and showed its abilities that are essential for Phycoremediation. Present results are also endorsed by (Cao, Xiong and Lund, 2013).

4.3.4 Conclusion

This study revealed that Alga are clearly effective organisms for reducing biological and chemical contaminants in refinery wastewater. Algae may easily thrive in wastewater medium due to the availability of necessary nutrients, as evidenced by their growth rate. As the measured parameters were within the limits of National Environment Quality Standards (2000) (NEQS, 2000) with all three species of algae treated wastewater and it can be used for irrigation purposes. The results of this study revealed that Phycoremediation process is effective in the reduction of pollutants during both seasons and was quite effective to keep concentrations within acceptable limits. As a result, it was envisaged that algal-based treatment could be a long-term, low cost and natural solution for refinery wastewater treatment. Overall, all the three selected species performed very well in general but *Scenedesmus sp.* showed the best results and proved good at treating organic as well as inorganic materials from refinery effluents.

CHAPTER 5

5. CONCLUSION AND RECOMMENDATIONS

The examination and identification of 18 indigenous local algal species collected from the wastewater effluents of Attock Refinery Limited reported the presence of blue green algae (*Lyngbya sp.*) green algae (*Scenedesmus sp.*, *Tribonema sp.*, *Zygnema sp.*) Cyanobacteria (*Oscillatoria sp., Arthrospira sp.*) and diatoms (*Navicula sp.*, *Pinnularia sp.*). The study is pioneer regarding estimation of algal biodiversity in refinery wastewater.

The study also evaluates the potential of Phycoremediation of the wastewater of petroleum refining industry. The results revealed that the algal species have significantly reduce the level of Total Soluble Solids, Total Dissolved Solids, Phenols, Sulphates, Sulfur, Chlorides, Biological Oxygen Demand, Oil & Grease and Chemical Oxygen Demand. They also improve the pH of the water to enhance its ability to be reuse in agriculture and other domestic chores.

The comparative investigation conducted on the differences among the potential of algal species to recommend the most effective algal species for Phycoremediation approaches. The study revealed that the *Scenedesmus sp.* are highly effective in the sorption of organic and inorganic matter from the refinery wastewater. However, blend of all three species can be more effective to treat wastewater. The bioremediation of the pollution load of the oil refinery effluent with indigenous algal species can be used as an effective technology in the reduction of pollutants like inorganic and organic compounds. Furthermore, the better performance in field conditions gave positive indication of their usefulness in the treatment of refinery effluent. Phycoremediation is a simple, natural, and cost-effective approach. The system, when standardized would not only be economical but also eco-friendly and sustainable. The information generated would help to scale up the process and moreover, to assess the economic feasibility of the technology.

Overall, the selected species have the ability to perform Phycoremediation approaches more effectively than other laborious practices. Based on the results, it is strongly recommended to:

- Explore indigenous biodiversity of the algal flora in the wastewater bodies for the identification of useful species in terms of wastewater treatment. This also helps in predicting the level of pollutant in the wastewaters, algal blooms, eutrophication and other threats to the environmental security.
- Effective utilization of the species for the reclamation of the wastewaters as well as environmental sustainability by avoiding the toxic effects of chemicals and cost-effective approach.
- Multiplication of remarkable species for industrial application, interactions of algal flora with biodiversity conservation and adaptability with climate change for long-term environmental sustainability in cost effective and environment friendly way.

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