AL-FARABI KAZAKH NATIONAL UNIVERSITY

UDC 602.3: 579.8; 606:622.75 Presented as manuscript

HUMA BALOUCH

Study of biodiversity of microalgae of Almaty region and prospecting for biotechnological valuable strains

6D070100 – Biotechnology

Dissertation for Doctor of Philosophy (PhD) degree

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Almaty, 2021

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DEFINITIONS

In this dissertation, the following terms are used with the corresponding definitions:

1. **Saprobic index** is a ratio of the sum of products of abundance, saprobic value, and the indication weight of individual species, and the sum of products of their abundance and indication weight.
2. **Internal transcribed spacer (ITS)** refers to a piece of non-functional RNA situated between structural ribosomal RNAs (rRNA) on a common precursor transcript. ITS region includes the ITS1 and ITS2 regions, separated by the 5.8S gene, and is situated between the 18S (SSU) and 28S (LSU) genes in the nrDNA repeat unit.
3. **Bioindication** is the observation or experimental investigation of living organisms for providing qualitative or quantitative information on the state of the environment with special reference to trace metals.

NOTATIONS AND ABBREVIATIONS

DNA — Deoxyribonucleic Acid
PCR — Polymerase chain reaction
DW — Dry Weight
EDTA — Ethylenediaminetetraacetic Acid
MUSCLE — Multiple Sequence Comparison by Log-Expectation
NOx — Mono-nitrogen Oxides
PCR — Polymerase Chain Reaction
SSU — Small Subunit Ribosomal DNA
TEM — Transmission Electron Microscope
SEM — Scanning Electron Microscope
TAE — Tris-acetate-EDTA
mg — Milligram (s)
% — Percentage
°C — Centigrade
µg — Microgram (s)
mg — Milligram (s)
ml — Millilitre (s)
g — Gram (s)
kb — Kilobase (s)
M — Molar
MW — Molecular weight
min — Minute (s)
OD — Optical density
Bp — Base pair (s)
FAMEs — Fatty Acid Methyl Esters
GCMS — Gas chromatography–mass spectrometry
ITS — Internal Transcribed Spacer
SSU — Small subunit
rbcL — ribulose-bisphosphate carboxylase gene (rbcL)
PUFA — Polyunsaturated fatty acid

INTRODUCTION

General description of the research.

The overarching purpose of this Ph.D. dissertation is studying the diversity of microalgae from different freshwater habitats of Almaty region, selection, isolation and identification of the most promising strains of microalgae, which have good potential for use as source of biofuel feedstock, antibacterial compound and bio-indicators.

The relevance of the research.

Heavy reliance of developing countries on fossil and imported fuels to meet the growing energy needs has become a significant impediment to their socio-economic development. Combustion of the fossil fuel is by far the largest source of atmospheric pollution, and major factor responsible for climate change and global public health concern [1]. The need for transitioning away from fossil fuels has been on the national priority agenda of developing countries, beginning with the recognition of bioenergy as an alternate and sustainable method for producing carbon-neutral energy source. This is further viewed as a crisis because of the recognition that bioenergy, which includes both biomass and biofuel, will exert pressure come of exploiting agricultural lands for growing biofuel crops [2]. Research is being directed towards exploring ways and harnessing alternate, local or indigenous sources for biomass and biofuel without negatively impacting food security. With the current trend towards conservation and sustainable resource management, there is renewed interest in exploring microalgae for biofuel production, bioremediation, value-added industrial and biodegradable products.

Antimicrobial resistance (AMR) poses a severe, ongoing, and global risk to population and environment health. Recent estimate provided by World Health Organziation (WHO) suggest that more than a half a million deaths globally are attributable to the drug-resistant bacterial infections [3]. Extensive efforts are underway to find novel and unusual antibacterial compounds which expand beyond the known antibacterial motifs. Owing to their extraordinary physiological, ecological, molecular and regulatory mechanisms, microalgae can be found in number of environments and capable of surviving in extreme conditions. Thus, the novel natural products with diverse biological activities, notably antibacterial, are expected to be obtainable from microalgae, thriving in extreme conditions.

Ecological monitoring is fundamental to our understanding of how the above mentioned human interventions, particularly persistent metal pollution or contamination, impact key species and ecosystem functions. Understanding the specific changes to freshwater biota following exposure, bioaccumulation and toxicity of heavy metals pollutant is vital to assess the functional status of ecosystem. The identification of specific molecular, biochemical, physiological and behavioral changes in highly sensitive species can be used as tool to build the resilience of degrading ecosystem [4]. Recent decades have witnessed an increased appreciation of the role of microalgal diversity in ecosystem function and their significant value as source for bioindicators of water quality. However, thus far there has been limited success with restricted set of indicator species.

The comprehensive understanding of the afore mentioned ecological role and deeper exploration of biotechnological potential of microalgae in areas of basic, applied and industrial research requires the accurate identification of species. There have been few systematics investigations of microalgae species from unusual or extreme habitats thereby limiting our knowledge of their biodiversity, function and their potential as source of biotechnologically important bioactive compounds. The lack of basic information on microalgal species diversity at different taxonomic levels has significant implications for many aspects of ecosystem monitoring, conservation biology, and evolutionary biology [5]. Recent advancements in molecular biology, biomolecular engineering, informatics, and other related sciences has greatly facilitated scientists for tapping the maximum potential of microalgae with applications ranging from drug development, agriculture, industry, to bioenergy and sustainable environment.

The purpose of the research:

In the present study, investigations were focused on finding promising strain of microalgae from freshwater habitats of Almaty region, able to demonstrate biofuel potential, antimicrobial activity and bioindication.

The main tasks of the research:

1. To investigate the diversity of microalgae species in the Almaty region with the aim of providing baseline information needed for the assessment of changes in microalgae biological diversity in the Almaty region.
2. To develop approaches for accurate identification of isolated monoculture microalgae strains based on light and scanning microscopy, ITS amplicon sequencing, and rbcL-gene primer.
3. To isolate microalgae species with high biomass, high lipid content and suitable fatty acids, desired for producing biofuel.
4. To study the antimicrobial potential of microalgae isolates and to determine the zone of inhibition of methanol extracts against different pathogenic bacteria.
5. To assess the utilization of microalgae strain as bioindicators of heavy metal pollution in freshwater bodies.

The research objects and materials: Microalgae isolates including *Monoraphidium griffithii* ZBD-01, *Nephrochlamys subsolitaria* ZBD-02, *Ankistrodesmus falcatus* ZBD-03, *Parachlorella kessleri* ZBD-04, and *Desmodesmus pannonicus* ZBD-05, *Monoraphidium sp*. ZBD-06, and *Ankistrodesmus sp*. B-11.

Research methods.

Cultivation methods, lipid extraction, trans-esterification, Gas Chromatography Mass spectrometry - Fatty Acids Methyl Esters (FAMEs) analysis, agar disk-diffusion assay, minimum inhibitory concentration (MIC), DNA extraction, Polymerase Chain Reaction, DNA cloning, sanger sequencing, fluorescence assays.

The scientific novelty of the research. As a result of the research, 7 new isolates of microalgae were isolated and identified: *Monoraphidium griffithii* ZBD-01, *Nephrochlamys subsolitaria* ZBD-02, *Ankistrodesmus falcatus* ZBD-03, *Ankistrodesmus sp*. B-11, *Parachlorella kessleri*. ZBD-04, *Desmodesmus pannonicus*

ZBD-05, including a psychrophilic new strain *Monoraphidium sp.* ZBD-06, for which no information was available in GenBank.

The potential of isolated strains of green microalgae as valuable sources of fatty acids methyl esters for biodiesel fuel has been studied for the first time. Strains *P. kessleri* and *A. falcatus* were selected as species with high biomass and lipid productivity, while the study of fatty acid composition demonstrated the dominance of palmitic, stearic and oleic acids, which are known to be the most dominant components of biodiesel fuel. The presence of higher quantities of saturated fatty acids (C16 - C18) and high productivity of biomass confirms the prospects of their use in the bioenergy sector as a raw material.

The potential efficacy of a crude extract of isolated microalgae isolates as an antibacterial agent has been tested for the first time. Their ability to suppress the growth of some pathogenic gram-positive and gram-negative bacteria has been established. In the previous literature, very few studies have successfully reported the efficacy of antimicrobial activity of green microalgae against gram negative bacteria. However, in this study, it is noteworthy that the green microalgae isolates have good potential in screening bio-control agents and discovery of natural products with new structures.

The selected strain Ankistrodesmus sp. B-11, showed high sensitivity to cadmium ions, which can be used as a test object in assessing the ecological state of aquatic ecosystems contaminated with heavy metal ions. It was established for the first time that cadmium reduces the rate of electronic transport to the membrane of thylakoids, inhibits the transport of electrons and protons at the level of plastoquinone (PQ) during the transfer of electrons from PS II to PS I. It was found that the concentration of cadmium 0.01 mg/l causes a change in the ultrastructure, which primarily affect the photosynthetic apparatus, in particular, the modifications concerned the location of thylakoids in the stroma: an increase in interthylakoid spaces, resulting in a decrease in photosynthetic activity. A certain significant increase in the vacuolization of cells is due to the structural changes in the cytoplasmic membrane under the control of cadmium ions.

Theoretical and practical significance of the research: The main conclusions and provisions of the work expand the theoretical basis of this direction of research, deepen our knowledge associated with the basics of the influence of heavy metals on the photosynthetic activity and ultrastructure of microalgae cells. Also, the theoretical significance of this study lies in the fact that its results significantly supplement the currently available scarce data on the species biodiversity of the algal flora of aquatic ecosystems in the Republic of Kazakhstan, in particular, 5 lakes in the Almaty region. The results obtained expand the data on the properties of new strains of microalgae that are promising for use in various fields of biotechnology, including bioenergy. The study of the metabolic characteristics of lipid-producing strains of microalgae and their antibacterial properties is of fundamental nature, revealing individual features of the processes of lipid accumulation in microalgae cells. The data obtained suggest lines for further work on improving the accumulation of lipids by strains of microalgae using the synthetic and industrial biotechnology techniques. The data obtained on the antibacterial effect of the isolated isolates of microalgae and their fatty acid

composition significantly expand the understanding of the mechanisms of the antagonistic action of microalgae against pathogenic bacteria.

The practical significance of this research lies in obtaining pure microalgae cultures with great biotechnological potential and which, accordingly, can be used as raw materials in bioenergy, pharmaceuticals and medicine. In addition, the revealed changes in the induction curves of rapid and delayed fluorescence, being one of the first rapidly recorded parameters of microalgae cells after the action of cadmium, could be used to diagnose the condition of objects. The relative changes of chlorophyll fluorescence parameters indicated in this study can also be used for biotesting water quality in natural and artificial reservoirs.

The main provisions for the defence:

This study reveals that the majority of species, making up the phytoplankton community in freshwater bodies of Almaty region, were diverse and showed significant variations among different taxa.

In this study, six microalgal species based on parameters for fast growth were cultivated and their total lipid and FAME profiles indicated their high potential of two strains *Parachlorella kessleri* ZBD-04, and *Ankistrodesmus falcatus* ZBD-03 for use as feedstock for biofuel based on their calculated biodiesel properties (Cetane number 50 and 48, Iodine Value 103.6 and 83.4), more than the required limit (CN >47.0) in accordance with the EN14214 biodiesel standards.

The study showed high potential of microalgal isolated strains for biotechnological application in different aspects and their utilization as raw-material for the production of high-value products such as antibiotics.

The accumulation of heavy metals in cells of *Ankistrodemus sp*. and the observed ultrastructural changes indicated their sensitivity to the presence of potentially toxic heavy elements in the environment, thus indicative of water pollution.

Key research findings and conclusion:

These results obtained in this study have increased the scope of finding industrially important microalgae from the freshwater habitats of Almaty region and these isolates, which have been maintained as stock cultures, could be vital source for the discovery of industrially useful bioactive compounds. The microscopy results of the study gave an idea of the detailed morphological characteristics of the isolated strains namely, Monoraphidium griffithii, Nephrochlamys subsolitaria, Ankistrodesmus falcatus, Parachlorella kessleri, and Desmodesmus pannonicus, Monoraphidium sp., and Ankistrodesmus sp. B-11, which would also aid in the identification and characterization of the studied microalgal strains in future.

The ITS region and rbcL gene was examined in six species namely, *Monoraphidium griffithii* ZBD-01, *Nephrochlamys subsolitaria* ZBD-02, *Ankistrodesmus falcatus* ZBD-03, *Parachlorella kessleri* ZBD-04, and *Desmodesmus pannonicus* ZBD-05, *Monoraphidium sp.* ZBD-06, which has successfully provided additional sequence data in Genbank databases and has resolve better the debate on their taxonomic status. The morphological identification carried out for Ankistrodesmus sp. B-11 was sufficient at the genus level, with high morphological similrarity to another studied isolate Ankistrodesmus falcatus ZBD-03. Therefore the

marker based PCR analysis for identification was not performed for Ankistrodesmus sp. B-11.

The fatty acid compositions of four isolates were studied and the major fatty acids were palmitic acid, oleic acid and stearic acid comprising of 10-45%, 5- 34% and 5-30% of the total fatty acids. Collectively these fatty acids occupied up to 30-70% of the total fatty acids in two strains *Parachlorella kessleri* ZBD-04 and *Ankistrodesmus falcatus* ZBD-03 with Cetane number more than 47, which is an ideal component and number to be considered for biodiesel, hence, this study suggest that the studied isolates represent valuable resources for future research for microalgae-based biofuels.

The present study has shown the promising antimicrobial activities of methanol extracts of four strains against gram positive and gram negative bacteria. The preliminary (agar disk-diffusion) and secondary (minimum inhibitory concentration (MIC)) antimicrobial assays displayed significant antibacterial activity of methanol extract of *Parachlorella kessleri* against *Bacillus subtilis* (maximum zone of inhibition 0,8 mm), *Staphylococcus aureus* and *Klebsiella pneumoniae, Nephrochlamys subsolitaria* against *Bacillus subtilis* (maximum zone of inhibition 0,6 mm), *Pseudomonas aeruginosa and E. coli, Monoraphidium griffithii* against *Klebsiella pneumoniae* and *E. coli,* and *Ankistrodesmus falcatus* against *Klebsiella pneumoniae* and *E. coli.*

Study of the effect of low concentrations of cadmium ions on growth, photosynthetic activity and ultrastructure of cells of the microalga *Ankistrodesmus sp. B-11* revealed a high sensitivity of the strain to cadmium concentrations of 0.005-0.02 mg / l in the nutrient medium.

Relationship of the research with the scientific project.

The dissertation research work was undertaken under the framework of three projects including ‘AP08052402- Development of technology for obtaining bio-fertilizers based on nitrogen-fixing cyanobacteria’; AP08052481- Development of a technology for producing biodiesel based on active strains of microalgae’; ‘AP05131743- Development of scientific and methodological foundations for biomonitoring technology and forecasting the state of polluted aquatic ecosystems using phototrophic microorganisms.

Research approbation.

The main results and observations are presented and discussed at international conferences and symposiums:

- at the International Scientific Conference of Students and Young Scientists “Farabi alemi” (Almaty, Kazakhstan, April 2019);
- at the European Biotechnology Congress (Prague, Czech Republic September 2020);
- at the International scientific and practical conference on ‘Aspects and Innovations of Environmental Biotechnology and Bioenergy’ (Almaty, Kazakhstan, February, 2021);
- 5th Symposium on EuroAsian Biodiversity (SEAB-2021) – Online – (Muğla, Turkey, July 2021).

Publications.

The majority of this dissertation was published in 6 scientific works, including 1 research article with impact factor, indexed in Web of Science (WoS) and SCOPUS, 4 articles in scientific journals recommended by Education and Scientific monitoring Committee of Ministry of Education and Science of the Republic of Kazakhstan (CCESF MES RK), 4 abstract in the materials of international conferences. Two articles are under process of publication in reputable international peer reviewed scientific journals.

Dissertation structure: This dissertation is written in 104 pages, containing notations and abbreviations, and describes, in detail, introduction, literature review, materials and methods, results and discussion, conclusion, supported by 287 citations to technical literature (references), and contains 11 tables and 18 figures.

1. LITERATURE REVIEW

1.1 Microalgae

Microalgae are one of the potential biotechnologically valuable group of photosynthetic microorganisms and represent most promising resource for new products and applications. Cyanobacteria (Cyanophyceae) are prokaryotic microalgae whereas green algae (Chlorophyta) and diatoms (Bacillariophyta) are eukaryotic microalgae [6]. Microalgae, which represent a wide range of organisms in a range of environments, occur in all current earthly habitats, not only marine, but also terrestrial [7]. More than 50,000 species are estimated to be available, but only a small number of about 30,000 species are believed to have been have been studied and described [8]. The main requirements for growing algae are water, arable soil, sunlight and nutrients. Microalgae can fix CO_2 using solar energy in more sustainable, efficient and environmentally friendly way than terrestrial power plants with several additional technical advantages [9]. Microalgae make use of sunlight more effective than land plants, absorb harmful toxins, have limited resource requirements and are not competing for precious resources with food or agriculture [10].

Microalgae are one of the most promising resources for novel products and applications and one of the possible biotechnologically important clusters of photosynthetic micro-organisms. Over billions of years of growth, microalgae, with ancient origin, have adapted exclusively to extreme environments [11]. Between 22,000 and 26,000 species are known to exist, only a few of which were classified as valuable for commercial use [12]. The importance of micro-algae diversity as a source of important biological materials such as antibiotics, medicines, enzymes, herbicides, growth promoters and energy sources has been increasingly appreciated over the last few decades [13], The researchers use the full potential of microalgae to provide solutions to address the various global problems of the 21st century as a step towards a new and sustainable bio-based economy. A viable alternative source of energy to substitute fossil fuels [14-15], a precious additive for feed and food products [16], used as raw ingredients for amino acids, vitamins and production of valuable bioactive compounds [17-18], and so on, the unique characteristics of microalgae have expanded to include efficient nutrient recycling in modem farming and waste water processing systems [19].

1.1.1 Microalgal cultivation

The photosynthetic microorganisms obtain energy from sunlight or artificial light sources, within the photosynthetically active radiation (PAR), with wavelength of radiation between 400 and 700 nm [20]. Photosynthesis also requires additional inorganic carbon source, such as dissolved CO_2 and essential mineral major nutrients, like Nitrogen, Phosphorus, Sulphur, and micronutrients such as Magnesiumg, Calcium, Mangangese, Copper and others. Also important are medium conditions that are optimal for microalgae growth, including pH, temperature, and dissolved oxygen [21]. Thus quantitatively in microalgae cultivation, the main factors that affect or influence the building of microalgae biomass are light; the relative concentration of assorted

compounds in a liquid phase such as dissolved inorganic carbon, dissolved oxygen, and growth mineral nutrient; and physical conditions such as pH, temperature, and biological contamination [22].

The type of medium required for growth of microalgae strain vary from species to species based on their ability to tolerate salinity, acidity or alkalinity. Some of the species occurred over the whole pH range while for species from fresh water habitats, algal media is confined to low salinity to medium highly concentrated in salt ions [23]. Nitrogen, phosphorus, and carbon are the three major nutrients that influence microalgal growth, biomass, and biochemical composition. Lack of these nutrients has a significant impact on microalgae metabolism. [24]. There are two types of culture media used for microalgae cultivation, namely liquid culture media and solid media (agar) for isolation of microalgae as pure culture [25]. The most generally used approach for solid media is streaking, which can be accomplished by spreading microalgae across the surface of an agar plate in a petri dish or an agar slant in a test tube. Agar cultures have the advantage of being able to quickly separate pure culture from contaminants, but they also have drawbacks, such as difficulty scaling up. A batch or continuous culture can be done with a liquid culture, with batch culture being the most prevalent. Batch culture is carried out by inoculating microalgae inoculum into a culture vessel and adding a small amount of culture medium with axenic or co-culture treatments, which is then incubated in a favourable growth environment to increase biomass production [26].

Continuous flow-culture, on the other hand, is carried out in a photo-bioreactor, where microalgae development differs both qualitatively and quantitatively when the culture is harvested continually at the same flow rate with fresh culture material. The culture can stay in the exponential phase indefinitely using this strategy [27]. Microalgae culture can take place on a variety of scales, including plates, shake flasks, and photo-bioreactors. For preliminary research, plates and shaking flasks are chosen to determine the most advantageous growth conditions, such as nutrient medium composition. Culture media of various compositions can be used in plates or flasks. Generally, large scale cultivation of microalgae is achieved by using two methods, i.e. closed and open. Microalgae are cultivated in a closed pond by bubbling it with air or CO2 in a photo-bioreactor in a closed system. It produces dry microalgae with high specifications and is less likely to be contaminated during production since it provides highly controlled conditions. Meanwhile, the open method, which is typically used for commercial and mass production, will grow microalgae in a pond with free and continuous air contact. In comparison to closed systems, open systems are easier to operate [28].

Microalgae can fix carbon dioxide from three separate sources: atmospheric CO_2; heavy industry CO_2 discharge; and soluble carbonate CO_2. Microalgae assimilate CO_2 from the air under normal growth conditions. In most microalgae the amount of CO_2, typically up to 150,000 ppmv can be significantly high [29]. As a result, CO_2 from external sources such as power stations [30], as soluble CO_2 such as Na_2CO_3 and $NaHCO_3$ are supplied in the algae growth media in common production units [31]. Nitrogen, phosphorus and silicon are other inorganic nutrients essential for algae

development. Although some algae species can determine air-based nitrogen in the form of NO_x [32-33], most micro algae need urea to be the best source in soluble form [24]. Phosphorus, which is less essential during the algal growth cycle, is needed at smallest amounts but must be provided above basic requirements, for this reason not every additional P is bioavailable [35]. Phosphate ion binds to metal ions in very small amounts. Silicon is only essential to produce certain algae groups such as diatoms [36].

1.1.2 Occurrence and Biodiversity of Green Microalgae

Microalgae are extremely diverse, with approx 200,000-800,000 estimated species, of which only about 35,000 are described [37]. Traditionally, morphological findings and pigment profiles have discriminated against microalgal types. Methods of molecular discrimination are often very successful for their identification, especially for those pico-sized fractions with few morphological characters to identify [38]. The collection of appropriate genetic markers to identify, quantify, analyse diversity and isolate genomic DNA from environmental samples are factors which need to be addressed. Current approaches do not emphasise the quantity of microalgal cells, but cell enumeration plays a key role in field evaluation. On the other hand, routine molecular techniques and advanced technology such as next generation sequence technology (NGS) should concentrate on molecular quantification, typically a repetitive procedure, and also large-scale environmental microalgal detection [39].

The selection of microalgae can only be carried out for biotechnological applications in various ecosystems and environmental conditions. In ponds, rivers, tanks, other fresh and marine ecosystems or habitats, Microalgae live in abundance [40]. The microalgae's diversity profile shows the anthropogenic activity and level of contamination in water systems. Freshwater environments are commonly known as lentic ecosystems, and temple ponds are more susceptible to man's activities. Thus, large and diverse microalgae in temple ponds are more likely to be found or recorded.

1.1.3 Adaptation to extreme environment

With exceptional physiological, ecological, molecular and regulatory mechanisms, microalgae are located and can survive in extreme conditions in a variety of environments [41]. It is ubiquitous in the biosphere and has adapted to survival under a wide range of environmental strains, such as heat, cold, drought, saline, pHO, anaerobiosis, osmotic pressure and UV exposure [42] and may therefore develop under all available conditions from freshwater to extreme salination. This broad range of environments helps to synthesise the myriad of chemical compounds, thereby taking into account the specific ability of those involved in Blue Biotechnology [43].

The snow, seas (psychrophaloids), hot springs (thermophiles), and salt lakes (Halophiles) occupy a wide range of freshwater, aquatic and terrestrial ecosystems, including extreme conditions [44]. About 30 000 phytoplankton species are described, of which approximately 90% are eukaryotic. As regards organisms, the majority of microalgae (>50 percent) comprise diatoms [45], but the numbers are estimated to be much greater than those currently described. The secret to the success of microalgae is their evolution, allowing them to adapt to many different conditions. High and

fluctuating nutrient concentrations, turbulsion and low light conditions are characterized in coastal and estuarine regions. Turbulent conditions lead to sediment suspension and high concentrations of nutrients in the column of water [46]. Those countries are dominated by diatoms et prasinophytes [47] which are adapted to the acquisition of nitrate and phosphate [48] with high peak absorption and high growth rates [49].

The open ocean consists of water, low nutrients and high levels of radiation. It contains a number of micro algae including coccolithophores, prasinophytes, dinos and pelagophytes [50]. Affinity strategists like coccolithophores have low saturation constants that allow them to compete for low-concentration nutrients. A high affinity to phosphates but not nitrate is present in the major pelagic coccolithopher *Emiliania huxleyi* [51] which gives it benefits under phosphate-limiting conditions.

In aquatic polar systems, a number of extreme conditions are present in microalgae: cold temperatures, high summer radiation and complete winter obscuration. Freezing of surface water often contributes to high levels of salt and oxygen and small exchanges of gas for sea ice [52]. Polar oceans are dominated by pennate diatoms, as well as by haptophytes and dinoflagellates [53], indicating the adaptation of chromalveolates to polar marine ecosystem environmental conditions.

Through microalgae, which thrive under extremes, it is expected that new natural produce with various biological activities, especially antibacterial, will grow. To use their biochemical, ecological, evolutionary and industrial potential, it is crucial to understand the molecular and regulatory mechanisms of such microalgae. Via various cultural dependence and independents approaches to new biologically active molecules, new strains of microalgae surviving in extreme conditions have therefore been extensively explored over the past couple of decades [54].

Examples of Microalgae growing in extreme environments: Mastigocladus laminosus is a morphologically complex thermophilic cyanobacterium which grows at 55ºC and can survive at temperatures of 64ºC. It also has high freezing and drying resistance [55]. Among the most known thermophile eukaryotic algae known to live at temperatures between 45 and 60ºC are cyanidium caldarium and its families. It also grows in habitats of less than pH 5 [56]. A mixotrophic organism, Galdieria sulphuraria, found in extreme environments such as hot sulphur springs, with pH levels less than 4 and up to 56°C [57]. The high concentrations of heavy metals in many of these environments often needed adjustments to withstand these conditions. One of the known form of "snow algae," *Chlamydomonas nivalis* is which typically has a growth temperature of about 0ºC and causes pink snow. Spores that resist subzero temperatures in winter generally have high reserves of lipids, sugars and polyols. In cosmetic extracts, C. nivalis is already commercially used [58].

Phaeocystis maritima is well under Antarctic and Arctic ice and is krill's favourite feed. The existence of antifreeze molecules adapted to the cold means living at 0°C and occasionally below. The oils rich in polyunsaturated fatty acids such as ARA, EPA and DHA accumulate up to 40 percent of their weight [59]. *Aphanothece halophytica* is a colonial alkaliphylic halotolerant cyanobicide that is able to develop in a wide salinity

range from 14.5 to 170 g/l NaCl and with alkali pH conditions of a higher pH than pH 11. Betaine is also used to accumulate as an osmoprotective substance [60].

Dunaliella salina has an optimal level of salt at approximately 120 g/l and increases in NaCl concentration to 300 g/l. It has been documented that *D. viridis* is optimally grown at about 60 g/l of NaCl and tolerated up to 230 g/l (NaCl is 35 g/l) [61]. For beta-cartotene production D. salina is commercially used [62]. Nostoc communal develops extracellularly excreted and accumulated glycosylated mycosporin-like amino acids (MAAs) for protection from UV. Scytonemine, the rare alkaloid in the sheet around Nostoc cells, colours yellow-brown the cells and provides light absorption about 390 nm. An additional sunscreen pigment also found [63].

1.1.4 Assessing Diversity and its Importance

The term 'Microalgae' is being broadly defined for the long time as those algae where the individual organisms generally require a microscope to be recognized. However, given the enormous diversity of taxonomically unrelated microbial eukaryotes existing in unicellular, colonial and filamentous forms [64], possessing higher-level taxonomic placement in three kingdoms including Bacteria, Chromista, and protozoa [65], it requires significant rethinking to provide an absolute and acceptable definition which may differentiate microalgae from the macroalgae.

Sustaining natural biological structural and functional attributes of aquatic ecosystems is of great concern for the last few decades. Currently, the monitoring and assessment of pollutants of the aquatic environment are mainly based on the determination of some chemical parameters. However, due to high costs of complex chemical analyses, nature, sources, distribution and level of emissions of pollutants, chemical analysis is not the only feasible way to obtain information for effective environmental monitoring. More recently, much attention has been given to use of algal flora biodiversity as bioindicators to manage an aquatic ecosystem according to the habitat requirements [66-67].

By biological diversity, it is often understood as number of microalgal species in a particular habitat, and how relatively abundant each of the species is. It is believed that the more number of species (greater variety) present in a particular ecosystem, they will be more likely to be naturally resilient. Some species are much more vulnerable than others to the change in environment (for example, as a result of anthropogenic impact), that leads to the increased richness for the dominating species, occupying the newly created ecological niches [68]. Biodiversity thus not only affected by change in the number of species, but it also takes into the account all aspects relevant to species dominance and rarity. Moreover, the biodiversity illustrates the uniqueness of the community, thus can serve as a bio-indicator to assess ongoing ecological or environmental changes. Microalgae play the most vital role in the sustaining and formation of aquatic ecosystems, because they form the first level of aquatic trophic chains and foundation of interspecific relationships.

Despite their importance, much of the information on clear description, characterization of microalgal communities and strains designation is somewhat inaccessible. The number of species of algae is very large, estimates figures in excess

of over a million species [69], of which between 40,000 to 60,000 have been identified to date [70]. Since, according to some estimates, hundreds of thousands to millions of microalgal species are still unknown, thus the role of many species for processes and functions of ecosystems is still not understood [71]. A better knowledge of microlgal biodiversity and its interrelation with the environment is crucially important.

The lack of basic information on microalgal species diversity at different taxonomic levels has significant implications for many aspects of ecosystem monitoring, conservation biology, and evolutionary biology [72-74]. The multilevel analysis of microlagal biodiversity will provide a system to understand the mechanism contributing to generate diversity, assess the way diversity is organized, and confer the value it may have to the structure and function of entire community in a given area [75]. This is particularly important for microalgal species and strains of economic value or environmental concern. The isolation, identification of indigenous microalgal strains with promising properties is a key to improving the feasibility of bio-prospecting for microalgal-derived high value products [76-78].

1.2 Microalgae Bioprospecting

Recent decades have witnessed an increased appreciation of the role of microalgal diversity in ecosystem function and their significant value as source for important biological materials such as antibiotics, drugs, enzymes, herbicides, growth promoters and source of energy [79]. For transition toward a modern and sustainable bio-based economy, the researchers are harnessing the maximum potential of microalgae for providing solutions for addressing the diverse global challenges of the twenty-first century. Being instrumental for efficient nutrient recycling for modern agriculture and wastewater treatment systems [80], a viable alternative source of energy to replace the fossil fuels [81], valuable additive for food and animal feed products [82], serving as a raw material for amino acids, vitamins and productions of valuable bioactive compounds [83-84], the unique attributes of microalgae has widened the scope of their utilization in nearly every research field.

Despite the widely acknowledged value of microalgae virtually in all fields of biotechnology, the studies about their diversity, various ecological functions and properties from understudied environment are still scarce. Speculation that the numbers of still undiscovered microalgal specie exceed the known species has led researchers globally to search and collect new strains of microalgae, preserve, and explore their biotechnological potentials [85].

Owing to their extraordinary physiological, ecological, molecular and regulatory mechanisms, microalgae can be found in number of environments and capable of surviving in extreme conditions [86]. Thus, the novel natural products with diverse biological activities, notably antibacterial, are expected to be obtainable from microalgae, thriving in extreme conditions. It is important to understand the molecular and regulatory mechanism of such microalgae in order to use their biochemical, ecological, evolutionary and industrial potential. Therefore, over the past few decades, new strains of microalgae thriving in extreme conditions have been extensively studies

through various culture-dependent and independent approaches in pursuit of novel biologically active molecules [87-88].

1.3 Microalgae as a Source of Biodiesel

Depending on the microalgae strain, the content of the culture media, and experimental or physical circumstances such as temperature, pH, carbon dioxide, and photonic energy absorption, microalgal cells can accumulate lipids up to 80% in mass [89-90]. Finding a good strain that grows well and produces as much lipid as feasible across a wide range of temperature and pH throughout its culture is one of the primary obstacles in mass culturing microalgae for biodiesel. Another key challenge in algae growing is developing a low-cost photobioreactor that reduces contamination hazards while promoting rapid growth. Another issue is ensuring a steady supply of nutrients, as well as the high expense of extracting oil from microalgae.

Microalgae can be employed economically and environmentally friendly for the production of biofuels. These biofuels can be manufactured in combination with carbon dioxide reduction, wastewater treatment and high-value chemical processing. Efficiency is poor, but much can be improved. At least part of the climate change and energy crisis is viewed with the use of microalgae [91]. Many microalgae are extremely rich in the use of the current technologies to turn into biodiesel. Over 50% of their biomass is very popular as lipids, often up to 80%, and oils about 5-20% [92].

A normal pathway for evaluating the biofuel potential of microalgal starin, as illustrated in figure 1, involve: selection of microalgal strain based on growth rate and lipid content; selection of appropriate measuring and monitoring methods and instruments for observing microalgae growth; developing a technique for harvesting algae, cost-effective downstream process of obtaining dry algae biomass, oil extraction and biodiesel conversion, designing and constructing a laboratory scale PBR for algal growth, and developing a multi-factorial kinetic model of microalgae growth in batch reactor [93].

1.3.1 Advantages of Using Microalgae for Biodiesel Production

Many research publications [94-95] have discussed the advantages of employing microalgae for biodiesel synthesis over other existing feedstocks (first and second generation biofuels). Microalgae are simple to cultivate and can be grown on non-arable land, brackish water, and waste water with little or no attention. Furthermore, unlike conventional forestry or agricultural crops, microalgae may grow everywhere using water and sunshine to reproduce through photosynthesis, which is essentially the conversion of solar energy into chemical energy with a high growth rate. In comparison to crop plants, estimates of algae's maximal photosynthetic efficiency range from 5 to 10%. [96-97]. Some microalgae species can survive in adverse environments as high salinity and temperature fluctuations. Through carbon capture and usage in photosynthesis, microalgae can reduce greenhouse gas emissions from the atmosphere and industrial flue gases.

Heavy metals can be removed from the environment while also producing potentially valuable biomass using microalgae [98-99]. After oil extraction, the leftover

biomass fraction can be used as a high-protein feed for farm animals. It also includes sugars that can be utilised to make bioethanol.

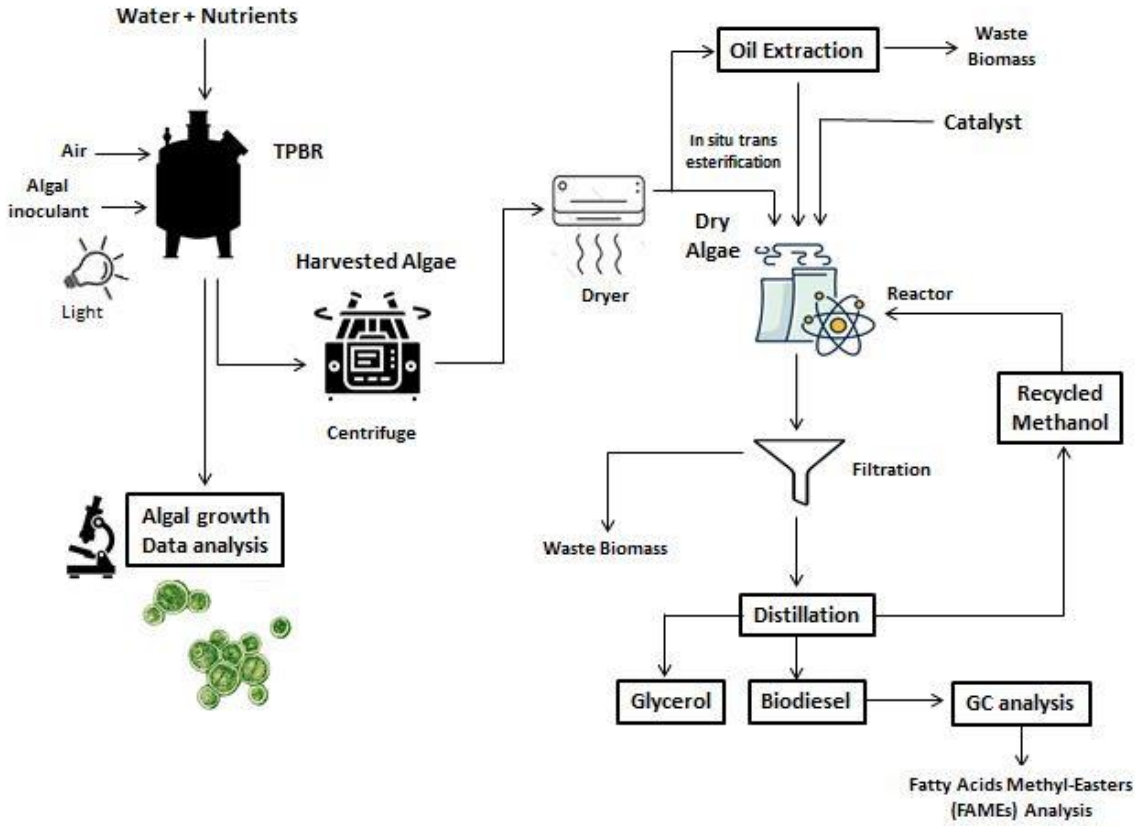


Figure 1: Process of making Biofuel from Microalgae [100]

1.3.2 Microalgal Lipids

The lipid, ubiquitous group of organic compounds, is often assumed to mean fats. However, fat is a group or subgroup of lipid molecules called triglycerols or triglycerides, long hydrocarbon chain terminated with carboxylic acid group. While virtually several fields of biotechnology are generally recognized as having a benefit of microalgae, the molecular, biochemical, and computational modeling analysis of microalgal microalgal from the understudied environment with potential diverse ecological functions and properties are still scarce. The researchers have been leading the world to explore and harvest new microalgae strains, to conserve and explore their biotechnological potential, given the fact that there is an even greater number of still undiscovered microalgae organisms.

There are global interests currently in search of latest, safe antioxidants, like plant materials, from natural sources for the prevention of oxidative food degradation and to limit oxidative damage to living cells [101]. Microalgae are photosynthetic micro-organisms that are able to quickly produce solar, CO_2 and nutrient biomass in water bodies. This biomass is made up of major metabolites like carbohydrates, oil and lipids for which high-quality goods, like animal and human nutritional supplements,

pharmaceuticals, industrial chemicals and transport fuels can be manufactured. A vast range of possible applications from aquaculture to health products, animal feed and human nutrition is available from Algal biomass and algae-derived products. Some algae are thought to be abundant in natural antioxidants [102]. In addition, through use of clean nutrient media to grow the microalgae it is possible to monitor the quality of microalgae cells, which do not contain any herbicides and pesticides, or any other harmful material [103].

Lipid metabolism and photosynthetic performance of microalgal cells can be strongly affected by culture conditions such as temperature, light and medium composition. Few studies [104-105] demonstrated that cells of high plants acquire the ability to accumulate relatively significant amount of fatty acids (FAs) during their growth cycle in low temperatures, allowing the microalgae to adapt extreme environmental temperature. It can be interpolated for photosynthetic microbes that efficiency of lipd metablosim would be increased at low temperatures. A study reported that algal lipid content depends only on algal strain, culture medium and light intensity [106]. In addition, another study [107] revealed the effects of changing culture medium concentrations and composition on algal lipid content and increase or decrease in specific fatty acid; for instance, efficacy of nitrogen source in culture media in modifying fatty acids (lipid) composition in Chlorella cells.

Microalgae contain essential fatty acids (EFAs) such as arachidonic and linoleic acids etc. that should be in nutrition for healthy development. These acids cannot be synthesised rapidly enough by body to satisfy needs [108]. The structural components of many lipids are faty acids, which differ considerably between algae in their types and quantities. In recent years, compositions of fatty acids have created considerable interest among researchers in large-scale microalgae production including marine algae. This primarily is due to the health effects in plants, including microalgae, of mono- and polyunsaturated fatty acids (MUFA and PUFA).

In addition, polyinsaturated Fatty Acids (PUFAs), including membrane fluid control, the transport of electron, oxygen and thermal adaptation, play key functions in cellular and tissue metabolism [109]. A sequence of reactions can be separated in two separate steps to create a biosynthesis of EPA. First, oleic acid synthesis from acetate, accompanied by linoleic acid conversion and α-linolenic acid. The following measures of slackening and elongation form a ínter-3 PUFA. Within the cell, EPAs are usually esterified to form complex lipid molecules (by cyclooxygenase and lipooxygenase) and play an important role in the developmental and regulatory physiology of higher animals and humans as the precursor in a series of eicosanoids, a hormonelike component like prostaglandins, thromboxans or leucotrienes [110].

Many health supplement stores are therefore currently selling the preparation, packaged with capsules or caplets, of microalgae, including spirulina and chlorella and also in food or beverages which have known treatment values for hypercholesterol, hyperlipidemia and atherosclerotics [111]. Microalgae's fatty acid content is determined by the climate and culture selected for growth [112]. Heterotrophic, photoautotrophic, nitrogen deficiency or relaxation are some of the environmental factors. While some microalgae are grown as sources of such fatty acids, transgenic

algae, including transgenic oil seed plants that are engineered to produce EPA may provide a sustainable alternative oil source for human consumption [113]. However, continued market antipathy to transgenic food products currently limits the prospect of deploying transgenic organisms nutritionally improved by EPA. The use of transgenic algae EPA as a high potential alimentary supplier in aquaculture will be an option. In this way, the major health benefits of these fatty acids could be incorporated into the human nutrition, without the need of direct consumption of food that is modified genetically.

1.4 Microalgae as Bioindicators

Bioindicators are of course used to evaluate the health of the population as well as being an effective instrument for the detection of positive or negative environmental changes and their resulting impact on human society. As said before, the key environmental factors affecting the efficacy of phtoplankton for bioindication including the transmission and intensity of light, water sediment, temperature and suspended solids. Bioindicators can be used to assess a region's natural state or the extent and severity of contamination [114].

Because microalgae contain chlorophyll and must survive and grow throughout the day, they are similar to terrestrial plants. The bulk of them are light and prefer to swim near the surface of the water. Development and photosynthesis are closely linked with each other, as light and food supplements are used. Algae are very susceptible to pollution, and this can be expressed in population or photosynthesis levels. In most cases, algae are as sensitive to contamination as other species, affects population development or photosynthesis. If the diversity of the plantoplankton species changes, it may indicate marine ecosystem pollution.

It is estimated that marine waters and freshwater account for more than 90% habitable space on earth. These ecosystems are regarded as the mean for vast array of bio-productive resources, and most of the primary productivity are result of tremendous microbial activity by the microalgae, in particular the pico-phytoplankton (0.2 – 2.0 μm), nano-phytoplankton (2.0 – 20 μm) and micro-phytoplankton (20 – 200 μm) [115]. The microalgal abundance and diversity in the aquatic systems can vary greatly from one aquatic ecosystem to another because of the variation in environmental variables. Therefore, the type and the increasing level of microalgae are used as indicators of the ecological conditions and water quality of their ecosystems.

Microalgae can be utilized as bioindicator organisms in studying the status of aquatic ecosystems, including those polluted by heavy metals. Microalgae represent a convenient material widely used in biomonitoring because they are more sensitive to pollution than multicellular organisms. The high specific surface area of microalgal cells promotes the rapid intracellular accumulation of toxicants [116]. The commonly used test functions of microalgae are based on integral characteristics, such as death rates, growth increments, and changes in ultrastructure and photosynthesis. The advantage of photosynthesis as a test function results from its sensitivity to numerous pollutants.

Fluorescence assays provide detailed information on the activity of photosynthetic apparatus under the action of toxic substances [117-118]. Such methods provide real-time information on physiological condition of microalgae. The emergent techniques for evaluating photosynthetic activity of higher plants and algal cultures comprise measurements of chlorophyll fluorescence induction curves with a high temporal resolution (starting from 10 μs) upon excitation with powerful flashes [119-120]. The high-resolution measurements of fluorescence induction curves take only a few seconds and are accomplished with devices like PAM (pulse-amplitude modulated fluorometers) and PEA (plant efficiency analyzers). Some recently developed devices allow concurrent measurements of chlorophyll fluorescence and the redox conversions of photosystem I (PSI) carriers; i.e., they allow simultaneous monitoring of individual reactions in PSII and PSI [121-122].

Although the action of heavy metals on plants was a matter of numerous studies, the influence of this metal salts on individual light reactions of photosynthesis and ultrastructure of algal cells remains insufficiently understood.

1.5 Antibacterial Potential of Microalgae

Among the major bioactive constituents of microalgae, proteins, polysaccharides, polyunsaturated fatty acids (PUFAs), especially EPA and DHA, amino acids, and antioxidants (polyphenols, flavonoids, and carotenoids) have been significantly coorelated with antimicrobial activity potential of microalgae [123-124]. Although the importance of microalgae as a source of unique bioactive compounds with antimicrobial activity is growing, the identification of compounds directly responsible for algae's antimicrobial potential remains an underrepresented field of research, owing to the discovery of new types of compounds in recent years.

Besides their estblished antioxidant activity, high content of polyphenols, carotenoids, and flavonoids in microalgae constitute a valuable source of natural and wide range antibacterial agents [125]. These antioxidants protect the human body from damage caused by reactive oxygen species (ROS), as well as providing a mechanism to counteract or prevent neurodegenerative disorders and inflammatory diseases with significant tissue injuries [126].

Antimicrobial activities of microalgae have been attributed to compounds belonging to several chemicals groups for example, the antimicrobial activity of supercritical extracts obtained from the microalgae *Chaetoceros muelleri* was linked to its lipid composition [127]. The antimicrobial activity is attributed to compounds belonging to several chemical classes [128]. However, not only various fatty acids, but also such combinations as — and -ionone, –cyclocitral, neophyltadiene and phytol can explain the antimicrobial activity detected in several *Dunaliella salina* pressurized extracts [129]. Efforts are ongoing, but are still relatively starting due to new groups of compounds that are specifically responsible for certain antibiotical functions. In attempts to substitute antimicrobial compounds of synthetic origin currently used — including subtherapeutic doses of antibiotics employed as prophilatory action in animal breeding, microalgal free-cell extracts are also being investigated as food and feed additives.

1.5.1 Antibacterial resistance

In view of the development of antibiotic resistance in bacteria, the researchers are experiencing an unprecedented push for the discovering natural and alternative sources for antibacterial bioactive compounds. The new line of secondary metabolites of antibacterial potential should also accomplish further objective to simplify the production process and significantly decrease the production costs. In this connection, freshwater and marine microalgae are emerging as a new generation of promising and novel ingredients at massive scale with major benefits in health industry and demonstrated antibacterial, antifungal, and antiviral activities [130-131].

Although significant research into algae's antimicrobial potential has only begun in the last two decades, the majority of studies to date have focused on the therapeutic and antibacterial/antiviral properties of algal chemicals, as well as their ability to suppress or kill clinical bacteria. [132-134].

The majority of studies have focused on the study of antibiotic resistance in bacteria for numerous reasons: I bacterial infections are responsible for the majority of nosocomial and community infections; ii) the wider and increasing number of classes of antibiotics are also a reason for prescence of a more diverse range of resistance mechanisms; and iii) further studies of the underlying molecular mechanisms are provided by the possibility to transfer the determinants of bacterial resistance to normal, characterized bacterial strains. [135].

1.5.2 Discovery of antibacterial compounds

A mixture of fatty acids, chlorellin, has been shown to have been responsible for cytotoxic activity in both the Gram+ and Gram- bacteria [136] isolated the first antibacterial compound from a microalgae, *Chlorella*. In the mean time there has been a boom in research into antibacterial active principles developed by the microalgae [137]. This has arisen, for example, from the risk of many MRSA strains, which have caused an increasing concern at worldwide health care institutions – because they are not susceptible to most traditional antibiotics.

It is therefore urgent that new antibacterial compounds can be discovered according to different biochemical action mechanisms. The complex, multi-layered cellular wall-structuring of gram-bacteria usually makes it less effective to prevent antibiotics from penetrating Gram-bacteria [138]. This is the reason why the supernatant (and methanolic extracts)' antibacterial activity is more effective than gram-bacteriums. The precise mechanism of action of fatty acids remains unclear: it may affect many cellular goals, even though cell membranes are the most probably, as the damage to the membrane is likely to result in cellular leakage and a decrease in the absorption of the nutrient, besides the inhibition of cell breath. Compounds synchronised with *Scenedesmus costatum* and partly purified from organic extract, exhibited activity against aquaculture bacteria, which appears to induce lysis of bacterial protoplasts as a result of their fatty acids longer than 10 carbon atoms [139]. Fatty acids have been well-known for some time to interfere with bacterial growth and survival, but recent structure-functional relation research suggests that this capacity

depends on both its chain length and its insaturation level. Cholesterol-like compounds can antagonise antimicrobial traits [140] to take account of both the composition and the free lipid concentration [141]. The antibacterial activity of polyunsaturated aldehydes deserves a special ention from microalgal-derived oxylipins. Diatoms such as *S. costatum* and *Thalassiosira rotula* synthesise these compounds. Moreover, it degrades the growth of various sea bacteria, such as (Gram-) *Aeromonas hydrophila* and (Gram +) *Planokoccus citreus* and *Micrococcus luteus* [142], such as *Alteromonas haloplankti*.

1.5.3 Extraction of Antimicrobials from microalgae

Novel extraction technologies and chemical extraction procedures are used to obtain a variety of extracts from microalgae-rich microalgae in order to obtain required functional/antimicrobial chemicals [143]. Various solid–liquid extraction techniques for producing extracts from seaweed have been developed since the late 1970s, primarily by mixing organic solvents, such as [chloroform: methanol] combinations or acetone. Some operations, such as ultrafiltration techniques, are also included to improve selectivity. Other methods employ ethanol to precipitate protein in the first phase, followed by extraction with hexane, butanol, or ethyl acetate in the second stage. Based on the high solubility of the compounds in the extractant mixture, recent studies show that ternary mixtures of solvents (ethanol–hexane–water [77:17:6]) can be used to form a homogeneous solution, with the advantage of significantly increasing the extraction yield and purity of the compounds extracted in a single stage [144]. The antibacterial potential of algal extracts is determined by the solvent's capacity to extract specific bioactive chemicals, as well as the bacteria's or fungi's sensitivity to these compounds [145]. Traditional extraction techniques including Soxhlet, solid–liquid extraction (SLE), and liquid–liquid extraction (LLE) are time-consuming procedures that need large amounts of solvent and produce low extraction yields.

Concerns about the use of clean technologies to extract bioactive compounds from algae have prompted the international scientific community and R & D engineers to invest in technologies such as supercritical fluid extraction, extraction using high intensity pulsed electric fields (PEFs), ultrasonically assisted extraction (USE) [146], microwave-assisted extraction (MAE), and accelerated solvent extraction (ASE), which uses pressure and temperature [147] to preserve as much as possible the quality and bioactivity of the extracted compounds . Degradation of cell walls by an enzymatic pathway has also been used in microalgal treatment (e.g. in *Chlorella vulgaris*), but it is still too expensive to be applied widely in industry [148].

One of the most advanced approaches for extracting bioactive chemicals from algae is supercritical fluid extraction with CO_2. Although it is a highly efficient and quick process for generating high-purity extracts that are rich in the desired functional chemicals, it is nonetheless quite expensive on an industrial scale [149].

Ultrasonically aided extraction (USE) is based on the mechanical effects of acoustic cavitation on algal cell walls. The efficacy of the extraction procedure at room temperature, and hence little loss of bioactive chemicals, is one of its key advantages.

Ultrasound is already being intensively employed in the field of microalgae, with promising results [150].

The employment of high-intensity electrical pulses (PEFs) is a technique in which high-voltage pulses break cellular material, allowing proteins, chlorophylls, and carotenoids, among other things, to be released. It has been successfully applied in bioactive extracts from Spirulina and Chlorella species [151-152]. The approach is based on the electroporation theory, which states that variations in cell membrane conductivity and permeability favour the production of microscopic pores that allow internal components to be released into the environment.

Microwave-Assisted Extreaction (MAE) has treated a variety of Dunaliella genus microalgal species to aid in the extraction of carotenoids. Microwave-assisted extraction aids extraction by providing ohmic heating and even temperature distribution. The application of this method to the species Dunaliella tertiolecta and Cylindrotheca closterium resulted in a process that included quick extraction, repeatability, and a high yield, as well as other antibacterial chemicals [153].

1.6 Molecular Identification

Recent advances in molecular biological techniques and bioinformatics have undoubtedly enabled the discovery and comprehensive assessment of thus-far-undiscovered forms of microbial life, including microalgae, in situ, without isolation into pure cultures. New species of microalgae are now being described and characterized combining morphological traits with molecular sequence data, utilizing either DNA sequence data and/or secondary structure of ribosomal DNA for phylogenetic applications. In particular, high-throughput amplicon sequencing of environmental DNA and/or RNA proved to be far more powerful and robust technique, when applied to characterize microbial diversity [154-156]. Gene-based biodiversity discovery has become an important application for biomonitoring diagnostic development and majority of the biodiversity studies have used this approach to not only improve the efficiency of biomonitoring, but also to expands its relevance for habitats and biota groups which have not been fully studied due to insufficient taxonomic knowledge or technical competency [157-159].

The DNA-based approach for the comprehensive assessment of microalgal communities at genus-, species- and strain-level utilizes various sequencing technologies to identify species provided as individual specimens or in environmental samples such as water, sediment or soil [160-1162].

1.6.1 Effectiveness of ITS-based markers

Multi Marker-based is increasingly being considered for revealing the entire gene repertoire of the community. This genetic analysis of genomes, by using high-throughput sequencing (HTS) of unpurified template DNA, has become the dominant source of publicly available sequence data. Marker gene based identification is viable method to determine a taxonomic distribution or fingerprint profile through PCR amplification and sequencing of evolutionarily conserved and variable regions in 18S rRNA or 18S rDNA [163-164].The DNA of individual specimens is typically analyzed

using the Sanger sequencing platform, whereas the amplicon-based metagenome from environmental samples is analyzed using high-throughput next-generation DNA sequencing platforms such as the Illumina Miseq sequencing and 454-Roche pyrosequencing platform. Another important initiative towards understanding the dynamics, ecology and environmental distribution of microalgae belonging to Chlorophyta in marine ecosystems is by [165]. The authors reviewed and summarized current knowledge on the phylogenetic, morphological and ecological diversity of unicellular marine and halo-tolerant Chlorophyta. Around 9,000 Chlorophyta 18S rRNA gene sequences from culture and environmental samples deposited in public databases were examined with the aim of assessing the extent of diversity and exploring their oceanic distribution based on a subset of 2,400 sequences for which geographical information is available. The study also evaluated the utility of using of the large subunit ribosomal rRNA (LSU) or ITS as potential suitable marker for explore microdiversity at the species level or below.

As exemplified throughout this review, the sequenced-based analysis of microalgae can be accomplished by one of the following methodological strategies: (i) High-throughput DNA sequencing of a clone library developed from PCR products of environmental DNA generated with a phylogenetic marker indicating the potential taxonomic origin (ii) sequenced based screening of random fragments to find a particular sequence or gene of interest, followed by sequencing of the adjacent regions to locate markers with improved taxonomic specificity. Currently, the distinction of microalgal individuals below the species level employ either shotgun metagenome sequencing or by focusing on intragenomic heterogeneity within phylogenetic (e.g., 18S rRNA or 18S rDNA) or functional gene targets.

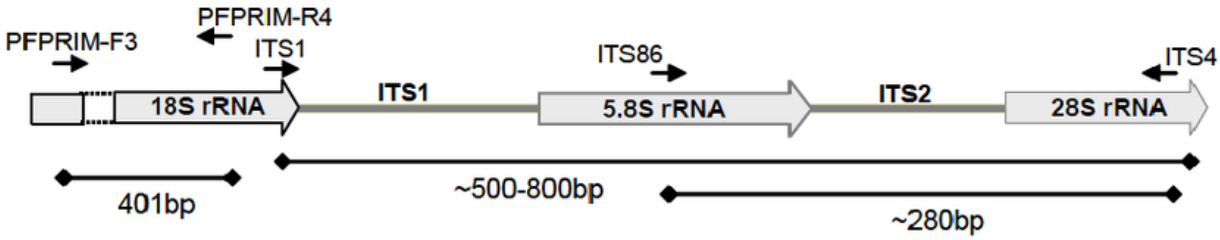


Figure 2 - Schematic representation of Microalgal ITS region [166]

The first approach refers to reconstructing large fragments or even entire genome without selecting any particular gene, alleviating biases from primer choice and enables the characterization of coding as well as non-coding components that can be used as phylogenetic markers [167-168]. In the later approach, specific internal conserved regions of DNA can be retrieved using taxonomical informative primer targets such as intergenic transcribed spacers (ITS) or the large ribosomal subunit (LSU) gene [169-170].

The research question enables the researchers to compare and determine the appropriate approach between assembly-based analyses and direct taxonomic classification of reads. The sequence conservation of regions of few genes has become

an unprecedented resource for taxonomy in addition to being a phylogenetic anchor that requires no prior knowledge of full gene sequence [171-172]. In some cases, the sequence homology of most genes of practical importance is often most difficult to be identified by PCR or hybridization due to their far too divergent nature. However, the nucleotide sequence for a few classes of genes is well-conserved to facilitate their identification by sequence instead of function.

Several recent studies have addressed the use of the 18S-ribosomal-DNA (rDNA), 18S-ribosomal-RNA (rRNA), 28S-ribosomal-DNA and its variable regions as taxonomic markers for the classification of Eukaryotes [173-175] and the validity and limitations of using them in the taxonomic profiling of metagenomes have already been discussed [176]. An extensive effort is being put to establish similar universal molecular markers for microalgal taxa [177].

Since the early 1990s, there was a progressive shift towards molecular taxonomic studies for microalgae [178]. Beside genes and spacer sequences, the past studies relied heavily on exploring ribosomal operon (e.g., actin, psba, rbcL, tufA, RUBISCO spacer, and other chloroplast genes [179-180]. However, while the SSU and LSU has proven efficient for delineation at high taxonomic levels, they are not considered as good for intraspecific differentiation. The suitability of marker based on ITS regions were increasingly recognized for microalgal phylogenetic and taxonomic studies due to their high degree of interspecific variability, conserved primer sites, and multicopy nature in the genome [181-182]. The former study proposed 5.8S + ITS-2 fragment as ideal candidate marker for microalgae owing to its broad taxonomic range (figure 2). The utilization of multiple markers based on four gene loci and their combined data was formalized by [183]. The study tested the efficiency of multiple markers based on four gene loci and their combined data (rbcL + tufA + ITS + 16S, rbcL + tufA and ITS + 16S), with three combined data having better resolution than single genes for higher intraspecific and interspecific divergence. Few studies reported tufA gene applicability most suitable for DNA barcoding and phylogenetic reconstruction based on its wide coverage and sequencing success [184-186]. A comparison of metataxonomic (marker gene-based) and metagenomics profiling is described in table 1.

1.6.2 Databases and Phylogenetic trees

Significant advances in next-generation sequencing technology have facilitated genome sequencing with high throughput at low costs. NGS technologies hold great potential to have profound impact in various areas of research, including several that, so far, have mainly used approaches based on de novo sequencing i.e., sequencing novel genomes where no reference sequence is available for alignment, and resequencing i.e., genomes sequencing from a species for which a reference genome is already available.

Although the technological advances in nucleotides sequencing has led to a substantial increase in the release rate of sequenced genomes at unprecedented scales and rates but it is computationally challenging. All these complex and comprehensive raw data are useless without utilization of correct tools for analysis, annotation, storage,

integration and translation. Resource integration and standardizing annotations are relevant for better understanding genetic diversity and deciphering complex mechanisms associated with microbial ecology, evolution, and diversity [187].

Since the publication of the first microalgal genome, red extremophile *Cyanidioschyzon merolae* in 2004 [188], over 100 microalgal genome projects have been launched and complete genome sequences of over 60 microalgal species been brought publicly available including green, and red microalgae as well as diatoms, dino-flagellates, nano-flagellates, and some uncommon species from underrepresented evolutionary branches (DOE Joint Genome Institute, http://genome.jgi-psf.org/; [189-191]; GOLD database, http://www.genomesonline.org/ [192]; Cyanobase, http://genome.kazusa.or.jp/cyanobase). During the last two decades, next-generation sequencing technology have greatly contributed to increasing number of sequenced microalgal genomes in public databases along with EST (expressed sequence tag) and transcriptome data sets and the breadth and depth of sequence assemblies and annotations are continuing to expand, with projects dedicated to filling in less characterized microalgal taxonomic groups [193-195].

The rapid expansion of genomic sequence data available and accessible in the afore-mentioned public repositories, and advances in databases analytic tools, makes it a daunting task for researchers to access, integrate, sort out and compare the best sequencing, specialized annotation and analysis strategies for microalgae. Fortunately, development of various customizable web-based genome browsers, model organism databases (MODs), molecule- or process-specific databases, and others has helped the researcher to find the needles in the haystack. A number of online databases are available for information on algal diversity and taxonomic studies, each with their own focus and limitations. There have been a number of studies published describing the microalgal genome sequences and this provides an opportunity to review what we have learned so far from sequencing the genomes of microalgae. At present, multiple data sets are available for ongoing more than 60 algae genome projects at the Department of Energy Joint Genome Institute (JGI), including status. Assembles, and annotations of sequenced genomes (http://www.algaeu.com/strains-of-algae-publications.html).

EST data from many microalgal species are available at the EST sequence databases of the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/genbank/dbEST/), a special division of Genbank, and the Taxonomically Broad EST Database (TBestDB), http://tbestdb.bcm.umontreal.ca/searches/welcome.php). Sequencing of mitochondrial and chloroplast genomes has been performed with even more microalgal species than in the EST or genome sequencing projects [196], which are available at the NCBI organelle database (http://www.ncbi.nlm.nih.gov/genome/ organelle/) and the Organelle Genome Database (GOBASE, http://www.bch.umontreal.ca/gobase/gobase.html).

Concerted efforts have been made to sequence complete organelle genomes on a large scale through the Organelle Genome Megasequencing Program (OGMP, http://gobase.bcm.umontreal.ca/). Furthermore, the genomic sequence information of various microalgal species has been updated in the Phytozome, a hub for genomic data

Table 1 - Comparison of Metataxonomic (marker gene-based) and Metagenomics profiling [197]

Technique	Method principle	Advantage and Challenges	Main applications
Meta-taxonomics	Using amplicon sequencing of 18S-ribosomal-DNA (rDNA); 18S-ribosomal-RNA (rRNA); 28S-ribosomal-DNA; or ITS or rbcL or tufA or 23S universal plastid amplicon (UPA)	+ Faster, cost-effective and more reliable identification to species level + accessible to non-specialists - inability to quantify taxon abundance - Amplification bias - more than one primer sets needed for maximizing diversity coverage and to offset primer biases - lack of comprehensively cured reference databases for assigning taxon to the OTUs	Biodiversity monitoring; Molecular phylogeny; Microbial ecology
Meta-genomics	Random shotgun sequencing of DNA or RNA (Sanger and 454/Roche sequencing) or long-read sequencing (Illumina/Solexa, SOLiD, PacBio SMRT System)	+ investigate uncultivable complete microbial communities in situ + No amplification bias + generated sequence reads do not require homology to known sequences (de novo profiling) - requires reference database of genes to classify sequence reads - requires high-quality DNA - requires more reads count for higher sensitivity	Structural and functional genomic screening contributing to discovery of novel genes; Phylogenetic profiling; Monitoring the biodiversity and the ecological status

from a few green microalgae (phytozome.jgi.doe.gov/pz/portal.html); the Greenhouse, largest eukaryotic algal genome collection available online

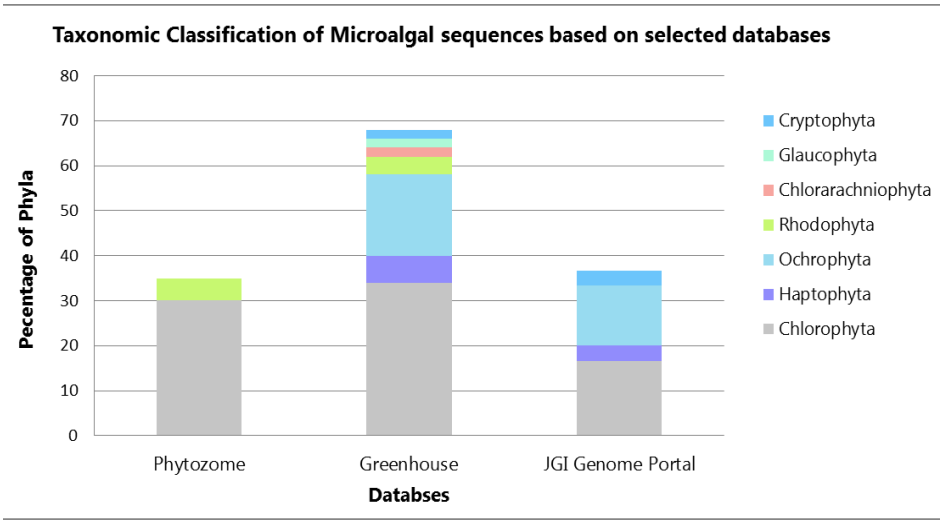


Figure 3 - Proportion of publicly available sequences of Microalgae

(https://greenhouse.lanl.gov/greenhouse/); realDB, a genome resource for red algae (http://realDB.algaegenome.org); pico-Plaza 2.0

(http://bioinformatics.psb.ugent.be/plaza/versions/pico-plaza/); CoGe database, which utilize the genomics comparison tools to analyze algal genomes of interest (https://genomevolution.org/coge/); Ensembl Plant database (http://plants.ensembl.org); EnergyAlgaeDB, functional genomics database for energy microalgae (http://www.bioenergychina.org:8989/); and EUKREF, reference database of 18S sequence barcodes that correctly represent algal lineage (http://eukref.org/).

Figure 3 shows the proportion of sequences from different phyla of microalgae in some of the above-mentioned databases. Chlorophyta (green microalgae) and Ochrophyta (heterokonts) are the most represented in all databases, and together they constitute more than 65% of the sequences publicly available. In the future, mass of unculturable genomes likely to be generated from metagenomic samples and next-generation sequencing in the next few years continue to make their way into the international databases, the distribution of the phyla and number of species will likely change.

A number of online databases are available for information on algal diversity and taxonomic studies, each with their own focus and limitations, such as Barcode of Life Data Systems (BOLD), a taxonomically curated database (http://www.barcodinglife.org); ITSoneDB (http://itsonedb.cloud.ba.infn.it/); and ITS2 Database (http://its2.bioapps.biozentrum.uni-wuerzburg.de/); and R-Syst::diatom (http://www.rsyst.inra.fr/en). The range of software tools being used in major steps of metagenomic data processing, Sequence Mapping; taxonomic profiling, sequence assembly and gene prediction has already been provided in detail [198-199].

A number of recent published studies has resulted in exponential rise in the microalgal genome datasets (table 2) and this provides an opportunity to review what we have learned so far from sequencing the genomes of microalgae. In the future, mass of unculturable genomes likely to be generated from metagenomic samples and next-generation sequencing in the next few years continue to expand the international databases interrelating genomic datasets to ecological data, the distribution of the phyla and number of microalgal species will likely change.

Table 2 - Characteristics and frequency of used sequencing platforms among genomes of microalgae published until 2018 [197, p. 12]

Platforms	Sequencing Principle	Read Length	Accuracy Reads %	Time run	Output data/run	No of genomes	% of total number of genomes
Sanger	Dideoxy sequencing	400~900 bp	99.999	20 mins~3 hrs	1.9~84 Kb	10	18.86%
454 GS FLX+/Roche	Pyrosequencing	600~800 bp	99.9%	24 hrs	0.7 Gb	4	7.54%
Solexa GAIIx/Illumina	Sequencing by synthesis	36~100 bp	98%	3~10 Days	600 Gb	18	33.96%
SOLiD4/Life Technologies	Sequencing by ligation	75 bp	99.94%	7 days	120 Gb	-	-
Ion Torrent (316 chip)/Life Technologies	synthesis	200~400 bp	98~99%	2 hrs	1 Gb	1	1.88%
PacBio/Pacific Biosciences	synthesis	Up to 60 kb	90%	10 hrs	1-10 Gb	3	5.66%
Sequencing platforms used in combination							
Combination of Sanger Sequencing and Roche/454	-	-	-	-	-	2	3.77%
Combination of Sanger Sequencing and Illumina/Solexa	-	-	-	-	-	2	3.77%
Combination of Sanger Sequencing and PacBio	-	-	-	-	-	1	1.88%
Combination of Roche/454 and Illumina/Solexa	-	-	-	-	-	8	15.09%
Combination of Illumina/ Solexa and PacBio	-	-	-	-	-	4	7.54%

To validate its use for bioassessment purposes, requires the researcher to place greater emphasis on data mining tools and statistical analysis and interpretation and illustrate the biologically significant patterns in the datasets. While it may be possible to align traditional morphological taxonomy- based approaches with DNA- based biomonitoring approaches, the metagenomic methods and reference genome libraries need further validation to be complementary source of information for biomonitoring programs on a large scale.

2 MATERIALS AND METHODS

2.1. Study Site Descriptions

The research covered five freshwater reservoirs of Almaty region: Issyk Lake (IL), Big Almaty Lake (BAL), Kolsay Lake (KL), Alakol Lake (AL) and Balkhash Lake (BL), the largest lake in the Central Asia (Figure 4, and Table 3). The Almaty region is located in southeastern part of Kazakhstan, commonly referred to as 'Seven Rivers': Zhetisu in Kazakh or Semirechie in Russian, because of the area's numerous lake, wetlands and streams coursing through the long range of mountains in the south and east part, and across the northern side of the region.

Alakol lake, a salt-water reservior, is semi-enclosed basin with surrounding cliffs of Terskey-Alatau range, strectched along the eastern border of Kazakhstan with Xinjiang Uyghur Autonomous Region (XUAR), China. Alakol lake is best-known for its clay minerals with therapeutic properties, and high water quality.

Kolsay lake is a system of three high lakes in the northern Tien Shan, in gorge Kolcaj. Algoflora of the Kolsay Lakes has not yet been practically thoroughly studied. With the appearance of an anthropogenic load on the lake during last few years, this area has become popular for tourism and camping.

Lake Issyk is an endorheic lake, which has its source glacial headwaters on the northern slope of the Trans-Ili Alatau mountain range, the Issyk river and its tributaries. The average maximum and minimum water temperature of Issyk Lake has been found to occur between 8°C in summers to below 4°C in winters, one of the limiting factor in the lack of lake's biodiversity.

Balkhash lake is one of the largest natural inland water reservoir in the south-eastern part of Kazakhstan. The eastern part of the lake is characterized by narrow, deep and high saline water, low and sandy shore, while the western side is high, rocky, wide with shallow freshwater. Four major rivers Aksu, Karatal, Lepsi and Ayaköz drain the eastern part of the lake while more than two-third of the total influx of Balkhash lake is received from snow and glacial fed Ile river, which originates from Tian Shan mountain range.

High-altitude (alpine) freshwater reservior originated as result of tectonic phenomena, Big Almaty Lake is located in the Trans-Ili Alatau mountains, on south of Almaty, a city in the southeastern region of Kazakhstan. The lake stands out for its distinctive turquoise color as streams draining the lake flow over the glaciofluvial deposits overlying the igneous and porphyritic rocks.

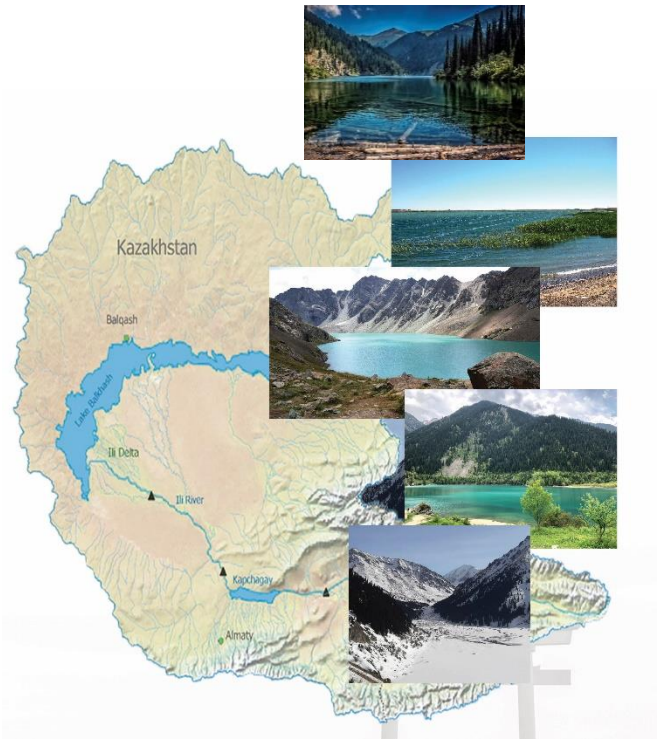


Figure 4 - Sampling sites – freshwater reservoirs of Almaty region.

Table 3 - Characteristics of sampling sites

Site code	Latitude	Longitude	Altitude (m)	pH	MAT (°C)	Lake area (km^2)	Sample type
IL	43°21'34.1496"	77°27'57.3084"	1760	6-6.7	12-14°C	0.70	Water
BAL	43°3'3.726"	76°59'7.4148"	2511	5.8-6	8-10°C		Water, Wet sediments,
AL	46°7'0.5376"	81°36'48.3624"	347	7-7.5	18-20°C	2650	Mats, water
BL	45°55'35.8824"	73°56'12.9876"	4221	8.33	18-20°C	16,996	Wet sediments, water
KL	42°56' 8.1564"	78°19'33.4164"	2254	7	15-18°C		Wet sediments, water

All samples were tested for temperature, pH, conductivity, total dissolved solids

(TDS) and salinity using a Thermo Scientific PCTestr 35 multiparameter. A volume of 1 L of seawater sample was taken using clean bottles at a depth of 0.5 m and then stored in cool boxes for transportation to the laboratory. The samples were taken from September 2018 to June 2019.

2.2 Microscopic Examination of Freshwater Samples

2.2.1.Light Microscopic Examination

The preliminary morphological identification of freshwater samples was done to assess the preliminary biodiversity of microalgae of Almaty region. The samples were observed under the light microscope, with a system of image capturing (MicroOptix OPTIX C600, Austria). Morphological identification was carried out based on some prominent morphological features for differentiation such as cell size (length to width ratio) and shape, solitary or colonial, presence or absence of mucilage etc., mentioned in the guidelines of description keys, taxonomic literature, manuals, manuscript and photo gallery available for microalgae species identification.

2.2.2. Sample preparation for Scanning Electron Microscopy

Following the identification of the isolates to the genus level, the Scanning Electron Microscope (SEM) technique was carried out at Electron Microscope Laboratory, Gazi University, Ankara, Turkey, as following: washing with phosphate buffered saline (PBS), centrifugation at 1000xg for 5 min, fixation with 5% glutaraldehyde, dehydration in ascending concentrations of ethanol, Critical Point Drying with CO_2 (Polaron CPD 7501), gold coating in a Polaron SC 502 sputer coater. The coated specimen was examined with JEOL JSM 6060 LV at accelerating voltage 10kV and SEM images were recorded.

2.3 Growth Medium

Fresh samples were maintained in liquid cultures of Bold's Basal Medium (BBM) and BG11. BBM and BG11 are generic medium designed to support the growth of a wide variety of green microalgae species. The composition of the medium and added microelements are described in Table 4. Liquid mediums were autoclaved and allowed to gas exchange for 24 hours before being used to cultivate microalgae. The liquid cultures containing the accumulative microalgae strains were incubated at 25°C under continuous illumination of 40 μmol photons m^{-2} s^{-1} for 2 weeks.

2.4 Isolation of Pure Microalgal Strains

To enumerate the growth of microalgae strains after successful direct isolation procedure using micropipette method and observable growth, the microalgae culture was transferred to separate 250-mL flask containing 100 mL of liquid BG-11 medium. The enrichment culture was kept at 22°C ±2 for about 20 days under fluorescent light with a photoperiod of 12 h, with shaker speed 100±10 rpm.

Table 4 - BBM and BG-11 Media Composition in g/L [200-201]

	BBM	BG-11		BBM	BG-11
Nutrients			Trace Metals		
KH_2PO_4	0.175		$ZnSO_4*7H_2O$	1.43	0.222
$CaCl_2*2H_2O$	0.025	0.036	$MnSO_4*4H_2O$	1.20	
$MgSO_4*7H_2O$	0.075	0.076	$CuSO_4*5H_2O$	1.24	0.08
$NaNO_3$	0.75	0.5	$(NH_4)6Mo_7O_{24}*4H_2O$	1.84	1
K_2HPO_4	0.075	0.04	$CoSO_4*7H_2O$	1.40	
$Na_2\ CO_3$,		0.02	$MnCl_2*4H_2O$		1.81
NaCl	0.025	-	MoO_3		0.015
citric acid,		0.006	$NaBO_3\ .4H_2\ O$		2.63
$FeSO_4$	0.005	0.006	$Co(NO_3\ )_2\ \bullet 2H_2\ O$,		0.02
EDTA	0.05	0.001			10
			$CaCl_3\ \bullet 2H_2\ O$, 1.2;		
			$FeSO_4\ .7H_2\ O$, 2.3;		
			H_3BO_3	3.09	2.86
			Deionized water	1.0 L	

2.4.1 Cleaning and Sterile Techniques

Later the microalgae were plated onto BG-11 agar medium (supplemented with antibiotics), which was placed at 22°C ±2 until single colonies of same size and similar appearance appeared. Upto one ml of each cell culture was centrifuged in 1.5ml eppendorf tube, and washed with sterile medium three times. Aliquot of cell suspensions were spread or sprayed sprayed aseptically onto BG-11 (Marine where appropriate) agar plates with antibiotic mix of azithromycin, cycloserin, kanamycin, and streptomycin at 100 μg ml-1 and incubated at 25°C under continuous light (40 μmol photons m-2 s-1) for 2 weeks. Absence of contamination was confirmed by keeping the treated microalgae cultures plates in the dark at ambient air temperature of 25°C for 1 week. The isolate was further purified by selecting a single colony and re-streaked, followed by microscopic examination, and individual pure colony was placed in autoclaved liquid medium to obtain axenic monoculture.

2.4.2 Cell Density Evaluation

Microalgae strains growth was measured throughout cultivation through measuring the absorbance (OD) at 630 nm using a EL800 microplate reader. The microplates were agitated for 25 min on a mini-orbital shaker prior to optical density readings. Growth of microalgae at varying cultural conditions and media composition was noted by cell counting using a hemocytometer. Growth parameters (growth rate, doubling time, and doublings per day) to monitor the growth microalgae were calculated based on the cell counts. Specific growth rates (μ, day^{-1}) at the exponential phase of growth were computed employing the equation $\mu = L_n\ (X_1 - X_0)\ /\ (t_1 - t_0)$, where X_0 and X_1 are the initial number of cells at the start (t_0) and number of cell after specific time or end (t_1) of the exponential phase. Doublings per day (k) and doubling time (T_2) were computed using the equations $k = \mu\ /\ Ln_{\ 2}$ and $T_2 = Ln_{\ 2}\ /\ \mu$ respectively.

Biomass productivity and lipid productivity was computed using the formula [202-203]:

$$\text{Biomass productivity (g L}^{-1}\text{day}^{-1}\text{)} = \text{biomass density (g L}^{-1}\text{)} \times \mu\ (\text{day}^{-1})$$

$$\text{Lipid productivity} = \text{biomass productivity} \times \text{lipid content (\%)}$$

2.5. Molecular Identification of Microalgal Species

2.5.1. DNA Extraction and Polymerase Chain Reaction

The microalgal cell suspension was collected (approximately 30mg) by centrifugation at 6,000 rpm for 10 min and genomic DNA of strain was extracted using Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research, D6005), as per the manufacturer's instructions. 50 – 100 mg (wet weight) microalgal cells that have been resuspended in up to 200 µl of water or isotonic buffer (e.g., PBS) to a ZR BashingBead™ Lysis Tube (0.1 mm & 0.5 mm). 750 µl BashingBead™ Buffer was added to the tube 2. The mixture secured in a bead beater fitted with a 2 ml tube holder assembly was processed at maximum speed for ≥ 10 minutes. The ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm) was centrifuged in a microcentrifuge at 10,000 x g for 1 minute. 400 µl of supernatant was transferred to a Zymo-Spin™ III-F Filter in a Collection Tube and centrifuged at 8,000 x g for 1 minute. 1,200 µl of Genomic Lysis Buffer was added to the filtrate in the Collection Tube from Step 4. 800 µl of the mixture was transferred from Step 5 to a Zymo-Spin™ IICR Column3 in a Collection Tube and centrifuged at 10,000 x g for 1 minute. The flow was discarded through from the Collection Tube and Step 6 was repreated. 200 µl DNA Pre-Wash Buffer was added to the Zymo-Spin™ IICR Column in a new Collection Tube and centrifuged at 10,000 x g for 1 minute. 500 µl g-DNA Wash Buffer was added to the Zymo-Spin™ IICR Column and centrifuged at 10,000 x g for 1 minute. The Zymo-Spin™ IICR Column was transferred to a clean 1.5 ml microcentrifuge tube and 100 µl (35 µl minimum) DNA Elution Buffer was added directly to the column matrix. The column mixture was centrifuged at 10,000 x g for 30 seconds to elute the DNA.

Following the DNA concentration measurements with the Spectrophotometer Thermo Scientific™ NanoDrop 2000, DNA extracts of each sample were stored at − 20 °C.

2.5.2 Standard Polymerase Chain Reaction (PCR) and Optimization

Nine different conditions were tested using a set of Four pairs of Universal primer, targeting the conserved regions of 18S, ITS1-5.8S-ITS2, 5.8S rDNAs and rbcL gene, since these are better suited for the identification of eukaryotes and green microalgae in particular (Table 5).

Table 5 - Primer Sequences used for amplification of microalgal *rbcL* and ribosomal ITS regions

Primer	Sequence (5′-3′)	Type	Source
ITS1	TCCGTAGGTGAACCTGCGG	Forward	[204]
ITS4	TCCTCCGCTTATTGATATGC	Reverse	
ITS1	AGGAGAAGTCGTAACAAGGT	Forward	[204]
ITS4	TCCTCCGCTTATTGATATGC	Reverse	
ITS5	GGAAGTAAAAGTCGTAACAAG	Forward	[204]
ITS4	TCCTCCGCTTATTGATATGC	Reverse	
rbcL	GCTGGWGTAAAAGATTAYCG	Forward	[205]
	TCACGCCAACGCATRAASGG	Reverse	

Each 50 µL PCR reaction mixture contained 27.75 µL of dH2O, 10 µL of GoTaq 5X PCR buffer, 6 µL MgCl2 25 mM, 1 µL dNTPs 10 mM, 0.25 µL of GoTaq DNA polymerase (5 U/µL) (Promega, USA), 1 µL of each primer (10 µM) and 3.0 µl of DNA template (30–50 ng/µL). The PCR programme was 95°C for 2 min, followed by 35 cycles of 95°C for 50 sec, primer-annealing temperature 49°C for 30 sec and 72°C for 1 min, with a final step of 10 min at 72°C.

2.5.3. Gel Electrophoresis

Amplification products were electrophorsed using 0.8 % agarose cast gels (Genepure LE agarose) in Tris-acetate-EDTA (TAE) buffer. The gel mix was stained with Ethidium bromide to a final concentration of 0.2 µg/ml, and samples were mixed with 6X loading buffer (0.25 % w/v bromophenol blue and 40 % w/v sucrose). Ten µl of 100 bpDNA ladder were loaded onto the agarose gel to evaluate band sizes and the electrophoresis was run at 80-100 watts for 30 to 45 min (BRL-Model 250, Life Technologie). Bands were visualized with a UV cabinet dark box and Fotodyne Foto/Prep UV Transilluminator.

2.5.4. PCR Purification

DNA fragment was excized from the agarose gel slice with the help of a clean scalpel. All excess agarose was removed. The weight of the gel slice was measured and transferrred to a clean tube. For each 100 mg of agarose gel < 2 %, 200 µL Buffer NTI was added. The sample was incubated for 5–10 min at 50 °C. The sample was vortexed briefly every 2–3 min until the gel slice was completely dissolved. NucleoSpin® Gel and PCR Clean-up Column was placed into a Collection Tube (2 mL) and loaded up to 700 µL sample. Sample was centrifuge for 30 s at 11,000 x g. Flow-through was discarded and the column was placed back into the collection tube. 700 µL Buffer NT3 was added to the NucleoSpin® Gel and PCR Clean-up Column. Mixture was centrifuged for 30 s at 11,000 x g. Again flow-through was and place the column back into the collection tube. The collection tube was centrifuged for 1 min at 11,000 x g to remove Buffer NT3 completely. We made sure that the spin column does not come in contact with the flow-through while removing it from the centrifuge and the collection tube. The NucleoSpin® Gel and PCR Clean-up Column was placed into a new 1.5 mL

microcentrifuge tube. 15–30 µL Buffer NE was added and incubated at room temperature (18–25°C) for 1 min. Centrifuge for 1 min at 11,000x g.

2.5.5. Cloning of PCR Products

Each PCR product underwent ligation process, with composition of 50 µL PCR reaction mixture containing YµL of dH_2O, 1 µL of 10 ligation buffer, 1 µL PEG, 1 µL of vector (pGEM- T Easy Vector System (Promega) / pUCm-T Cloning Vector Kit (Bio Basic), XµL purified PCR product), 1 µL of T4 DNA ligase. The mixture was kept for 1 hour to overnight at 16-23°C. For transformation 100 µL of competent cells (Escherichia coli competent cells - JM101), were thawed on ice. 5 µL of ligation mixture was added and was kept on ice for 30 min. Then heat shock of 42°C s given on water bath for 1 min, followed by placing on ice for 2 min. 400 µL SOC culture was added and were place on shaker at 37 °C, 200-250 rpm for 1 hr. the mixture was centrifuged at 4000 rpm for 5 min. 400 µL of mixture was discarded from top using micropipette and cells were re-suspended with the culture. Bacteria were placed at Amp+plater which had been plated with 20 µL 100mM IPTG and 100 µL 20mg/ml X-gal. the suplus liquid was discarded after putting the late upward for 1 hr at 37°C. The plate was then placed downward for overnight. After 18 h of incubation, three white colonies were selected from each sample. Each selected colony was grown overnight in 3 ml of LB broth containing 50 ug/ml ampicilin at 37°C at 250 RPM.

2.5.6 Colony PCR

To verify successful cloning of desired DNA fragment, Colony PCR was performed using Gene Direx protocol. Each 25 µL PCR reaction mixture contained, 2.5 µL of PCR buffer, 2.5 µL $MgCl_2$ 25 mM, 0.5 µL dNTPs 10 mM, 0.25 µL of GoTaq DNA polymerase (5 U/µL) (Promega, USA), 0.5 µL of each primer (10 µM), single transformed colony and rest of dH2O to complete reaction mixture. The PCR programme was 94°C for 4 min, followed by 35 cycles of 94°C for 40 sec, primer-annealing temperature 49°C for 1 min and 72°C for 2 min, with a final step of 5 min at 72°C.

2.5.7 DNA purification (plasmid) and Sequencing

The plasmids were purified using Wizard® Plus SV Minipreps DNA Purification Systems kits (Promega). First step involved production of Cleared Lysate by pelletting 1–10ml of overnight culture for 5 minutes. Pellet was thoroughly resuspend with 250µl of Cell Resuspension Solution. 250µl of Cell Lysis Solution was added to each sample; inverted 4 times to mix. 10µl of Alkaline Protease Solution was add; inverted again 4 times to mix and then the mixture was incubated for 5 minutes at room temperature. 350µl of Neutralization Solution was added and mixed thoroughly by inverting the tube 4 times. The mixture was centrifuged at top speed for 10 minutes at room temperature. For binding of Plasmid DNA, the lysate was cleared into Spin Column by centrifugation at top speed for 1 minute at room temperature. Flow through was discarded, and Column was reinserted into Collection Tube. 750µl of Wash Solution (ethanol added) was added to the binding column and centrifuged again at maximum

speed for 1 minute. Flowthrough was discarded and column was reinserted into Collection Tube. This step was repeated with 250µl of Wash Solution and centrifuged again at top speed for 2 minutes at room temperature.

For elution of DNA for sequencing purpose, Spin Column was transferred to a sterile 1.5ml microcentrifuge tube, being careful not to transfer any of the Column Wash Solution with the Spin Column. 100µl of Nuclease-Free Water was added to the Spin Column and centrifuge at top speed for 1 minute at room temperature. The column was discarded, and DNA was stored at –20°C.

2.5.8. Sequencing

The products were sequenced in both directions by Sanger sequencing using the universal M13F/M13R primer pair. Plasmid DNA from each sample was sent out for sequencing to the Macrogen Europe, in Amsterdam, Netherland. The obtained sequences were analyzed against the database sequences in the GenBank nucleotide collection through the Basic Local Alignment Search Tool (BLAST) available on the National Center for Biotechnology Information (NCBI) website (www.ncbi.nlm.nih.gov/). Sequencing was done twice to minimize the probability of any Taq polymerase errors that could have occurred during the PCR process.

2.5.9 Phylogenetic analysis

The resulting ITS1/ITS4, ITS5/ITS4 and rbcL forward and reverse DNA sequences of microalgae 18S were aligned using sequence alignment editor Bioedit version 6.0 and search was carried out for similar sequences with the Basic Local Alignment Search Tool in the GenBank database of the National Center for Biotechnology Information (NCBI). Six sequences having the highest similarity to each query sequence were obtained and used for phylogenetic analyses with Isochrysis galbana strain (JX393298) as an outgroup. Multiple sequence alignment was carried out using the MUSCLE program with default parameters integrated in Molecular Genetics Analysis (MEGA) 6.06. The Phylogenetic tree was built by the Neighbor-Joining method (Kimura-2-Parameter algorithm) using bootstrap value (n = 1000 replicates) to determine the statistical reliability of the obtained topologies. The resulting sequences were deposited in the GenBank database for accession number. Living axenic cultures of all isolates were deposited in the Collection for Microalgae Cultures at the Biotechnology Lab, Al-Farabi Kazakh National University.

2.6. Lipid Extraction and Profiling

2.6.1 Lipid Extraction

For the conventional extraction method [206-207], 30-35 mg of wet biomass (*D. pannonicus, P. kessleri, M. griffithii, N. subsolitaria,* and *Ankistrodesmus falcatus*) was collected by centrifugation at 5,000 rpm for 5 min and blended with 6 mL solvent mixture of methanol and chloroform (2:1, v/v). The mixtures were shaken at 200 rpm on an orbital shaker for 3 h at 37°C. 2 mL of KCl (0.9%) and centrifuged at 3500 rpm for 5 min. the lower chlorophorm phase was transferred into a pre-weighed test tube and dried with air flow**.** After that, the extracted mixture was resuspended in 2.5 mL

Hexane. Acid-catalyzed trans-esterification of lipid content was done by adding 2 mL of methanolic H_2SO_4 (1% v/v). The reaction mixture was heated in a stoppered tube at 85°C for 2.5 h. After the sample had cooled down, 2 mL of KCl was added and subsequently the supernatant was transferred to 1.5 mL vial. The extraction solvent was evaporated with help of rotary evaporator in 15 min. Finally, centrifugation at 12000 for 5 min was performed and the supernatant containing FAMEs was collected in GC tube.

2.6.2 GC/MS analysis

GC-MS FAMEs analysis was performed on Agilent 7000A Triple quadrupole mass spectrometer, coupled to a gas chromatograph (Agilent 7890) equipped with an auto sampler. High purity of Helium gas was used as carrier gas. All standards and samples were injected in the split mode (split/column flow ratio 60:1). The injector temperature was 250°C; the oven temperature was 120°C). The mass spectrometer was operated in the electron impact (EI) mode at 70 eV in the scan range of 50–650 m/z [208]. The temperature of MS source and MS Quad was set to a value of 230 and 250°C, respectively. The injection sample volume was 1.8 μL. Mass Hunter software (Agilent) was used for data acquisition and processing. Peak identification of algal oil was performed by comparison with retention times of standards and the mass spectra obtained compared with those available in the Wiley and NIST libraries (Wiley Registry TM, 8th Edition Mass Spectral Library, and the NIST 08 Mass Spectral Library (NIST/EPA/NIH) 2008 version) with an acceptance criterion of a match above a critical factor of 80%.

2.6.3. Assessment of Fuel-derived properties on the basis of fatty acid profile

The fuel properties such as iodine value and cetane number (CN) were investigated to assess the fuel quality of microalgae strains, based on their fatty acid methyl esters (FAMEs) profile. Followig equations [209] were used to calculate saponification value, iodine value, and Cetane Number:

$SV = \Sigma\ (560 \times F)/MW$

$IV = \Sigma\ (254 \times F \times D)/MW$

$CN = (46.4 + 5458/SV) - (0.225 \times IV)$

Where SV is the Saponification Value, F = % of each fatty acid, MW= molecular weight of each fatty acid [210], IV is the iodine value, CN is the Cetane Number.

2.7 Antibacterial Assay

2.7.1. Preparation of crude extract

The cell density was calculated with a hemocytometer for quantifying the biomass. The Samples were centrifuged at 3500 rpm for 30 min to harvest the microalgal cells from 30 mL. The biomass (6 g wet weight) were suspended in 15 mL of Phosphate-buffered saline (PBS) cold buffer and disintegrated according to the previously reported procedure [211]. Sonication was performed (twenty cycles of 30 s each) with a Soniprep Ultrasonicator (Sartorius Labsonic), and the homogenates were re-centrifuged at 3500 rpm for 30 min to remove cell debris. The cultures' supernatant

was then discarded and 60% aqueous methanol was added to each pellet in 1:5 w/w ratio of the initial biomass concentration. The solutions were vortexed vigorously for 30 s, and then centrifuged at 3500 rpm for 20 min. The resulting supernatants were used to determine the antimicrobial activity of each strain.

2.7.2. Evaluation of antimicrobial activity

The Disk diffusion technique was employed to examine the antibacterial activity of Microalgae crude extracts against eleven bacterial strains, namely, Gram-positive (*Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228) and Gram-negative (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 13315, *Salmonella typhimurium* ATCC 14028, *Yersinia pseudotuberculosis* ATCC 911, and *Enterobacter cloacae* ATCC 13047). Pure colonies of test bacteria were obtained from the Microbiology Laboratory, Karadeniz Technical University. The Mueller-Hinton agar (pH = 7.2–7.4) plates were inoculated with broth cultures diluted to 0.5 turbidity ($\sim 1.5 \times 10^8$ cells·mL−1). Filter paper discs impregnated with 10 µL of methanol extract of the isolate, along with negative control disc of only methanol and antibiotic (rifampicin), were placed on the surface of an agar plate, pre-inoculated with bacterial suspension to be tested. Finally, the plates were incubated at 37°C for 16-24 hours and the diameter of zones of inhibition were measured in millimeter. All experiments were repreated three times for each strain.

2.7.3. Determination of Minimum Inhibitory Concentration (MIC)

The MIC was determined by adding crude extract in serial dilution (10 fold) to Mueller-Hinton Broth culture in sterile test tubes and bacterial strains were inoculated at the same concentration used for disk diffusion method. The tubes were incubated at 37°C for 24–48 h and were visually examined for bacterial growth. The MIC values were recorded as the dilution tube with lowest concentration of the extract that inhibited bacterial growth. The MIC is expressed as microalgal cells/mL at the onset of the extraction process as 6g wet weight of isolate was used for crude extraction [212].

2.8 Determination of the influence of Cadmium

2.8.1 Assay of cadmium influence on culture growth

Stock solutions of $CdSO_4 \cdot 8H_2O$ (OAO Reaktiv, Russia) were used for experiments. The stock solution was diluted with sterilized distilled water to a final concentration and added to the culture once per experiment. The examined cadmium concentrations were 0.002, 0.005, 0.02, 0.05, and 0.2 mg/L. Prior to experiments, the cells of an actively growing culture were sampled and inoculated into 500-mL flasks filled with 200 mL of medium without additions (control) or with the addition of cadmium. The cultures were grown for 2–10 days under round-the-clock illumination. The culture growth was assessed by counting cell numbers in a Goryaev hemocytometer.

2.8.2 Biophysical indicator assays

The induction curves of prompt and delayed chlorophyll fluorescence, as well as redox conversions of PSI components, primarily P700, were measured simultaneously using a Multi-function Plant Efficiency Analyzer (M-PEA-2, Hansatech Instruments, United Kingdom). Prompt and delayed fluorescence were recorded under alternating red light pulses (intensity 1500 μE/(m2s), 625 nm) and short dark intervals sufficient for recording delayed fluorescence. The dynamics of delayed fluorescence reflected changes in post-illumination light emission in the time range of 0.1–0.9 ms in the intervals between actinic light pulses. Changes in absorbance at a wavelength of 820 nm reflect the redox state of the PSI components, mainly P700, in the PSI reaction center. The intensity of modulated light at a wavelength of 820 ± 25 nm was 1000 μE/(m_2s). The data obtained were normalized to the value at t = 0.7 ms (MRO). Characteristics of M-PEA-2 and the measurement protocol were described in detail earlier. Since measurements of P700 redox state require the increased algal concentrations, the cells were concentrated on a membrane filter and kept in the dark for 10 min in a wet condition. Control measurements of S. quadricauda fluorescence using an Aqua-Pen CAP–C 100 fluorometer (Photon Systems Instruments, Czechia) and cell suspensions put into cuvettes showed that the procedure of cell enrichment on filters did not affect the physiological condition of analyzed cells. In short-term toxicological experiments using M-PEA-2, a diluted (10%) Prat medium was employed to prevent the binding of toxicants.

The primary photosynthetic processes were quantified by means of OJIP kinetic curves and the socalled JIP test. The JIP test exploits the following parameters of the fluorescence induction curve: fluorescence intensities at 20 μs (FO), 2 ms (FJ) 30 ms (FI), and 6 s (F6s) as well as FP (FM, maximum fluorescence intensity) and M0 (area above the OJIP kinetic curve and below the FM level). These measured values were used to calculate the following parameters:

(1) FV = FM – FO, the maximal variable fluorescence;
(2) FV/FM, the maximal quantum yield of the primary photochemical reaction in open reaction centers of PSII: FV/FM = ϕPo;
(3) VJ is the relative amplitude of the O-J phase (recorded at 2 ms of illumination). This parameter reflects the number of closed reaction centers with respect to the total number of reaction centers that can be closed; VJ = (FJ – FO)/(FM – FO);
(4) (VI is the relative amplitude of the O-I phase (determined at 30 ms of illumination). It reflects the ability of PSI and its acceptors to oxidize the plastoquinone pool; VI = (FI – FO)/(FM – FO);
(5) ϕEo is the quantum yield of electron transport beyond (at t = 0): ϕEo = [1 – (FO/FM)]*(1 – VJ), where VJ = (FJ – FO)/FV;
(6) DI0/RC = (ABS/RC) – M0·(1/VJ), the total amount of energy dissipated per reaction center (RC) as heat, fluorescence, and energy transfer to PSI at t = 0;
(7) ABS/RC, energy flux absorbed per active reaction center (RC); it characterizes the relative size of the antenna (ABS): ABS/RC = M0/VJ(1/ϕPo) = (M0/VJ)/[(FM – FO)/FM)];

(8) PIABS, performance index, an indicator of the functional activity of PSII, normalized to the absorbed energy: PIABS = [1 – (FO/FM)]/(M0/VJ)*[(FM – FO)/FO]*[(1 – VJ)/VJ];
(9) qE is the capacity of generating the pHinduced nonphotochemical fluorescence quenching: qE = (FM – F6s)/FV.

All measurements were made in at least five replicates. Data in figures represent mean values.

2.8.3. Electron Microscopy

The ultrastructure of microalgae was analyzed by means of transmission electron microscopy. Samples of *Ankistrodesmus sp*. B-11 were obtained by centrifugation at room temperature for 30 min at 3000 rpm on a Rotina 380R centrifuge (Hettich, Germany) and fixed with 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) for 2 h. The samples were triply washed in the same buffer and fixed at 4°C in 1% OsO4. After dehydration in ethanol and acetone, the samples were stained with 2% aqueous solution of uranyl acetate (pH 5.0). Finally, the samples were embedded in Spurr epoxy resin. Ultrathin sections (90 nm) were cut with a diamond knife using an Ultracut ultramicrotome (Leica, Germany). Cell structures were analyzed using a Jem 1200 EXII electron microscope (JEOL, Japan). This part of the work was performed in the laboratory of electron and confocal microscopy at the Adam Mickiewicz University in Poznan (Poland).

3. RESULTS AND DISCUSSION

3.1 Microalgae diversity profiling of environmental sample

Assessment of the species diversity of aquatic ecosystems is of growing importance for environmental monitoring. In view of the special attributes of phototrophic microorganisms, such as first level of aquatic trophic chain, short generation time, sensitivity to environmental change, microalgae serve as an important biomarker for assessing the quality of aquatic and marine ecosystem as well as an indicates the deterioation of water quality [213-214]. Besides this, many types of phototrophic microorganisms are highly resistant to various types of anthropogenic action and their increased biomass generation cause rapid eutrophication of water bodies [215]. By virtue of this they are particularly powerful bioindicator to assess the viability of anthropogenic measures (e.g. reclamation, remediation, and reintroduction).

Owing to their extraordinary physiological, ecological, molecular and regulatory mechanisms, microalgae can be found in number of environments and are capable of surviving in extreme conditions [216]. In the present study, the Algoflora of the Balkhash Lake comprised of 92 microalgae, represented by various varieties and forms related to 4 divisions, 9 classes, 9 orders, 17 families and 32 genera. The main biomass of microalgae identified in this lake was represented by green algae, with 35% share in total number of species. Then diatom species predominated in number, the number of which accounted for 29%. Cyanobacteria were found in third place and accounted for 27%, and about 9% of certain species of microalgae belonged to euglenophyta. The most frequently encountered species are: *Ankistrodesmus minutissimus*, *Chlorella vulgaris, Coelastrum microporum, Merismopedia minima*, *Gomphosphaeria lacustris, Phormidium foveolarum*. The saprobic index values indicated microalgae as the most dominant group of organisms, 50% of water capacity. The obtained data allowed us to relate the lake Balhash to "contaminated" by α-meso-probable type, and index value ranged from 3.51 to 2.5.

The second site studied was Lake Alakol. This is a salt free lake, located at a height of 347 m above the sea level in the Balkhash-Alakol lowland. Lake Alakol and small lakes located nearby, such as Sacykkol, Yyaly and others, form the Alakol Lake system [217]. Along the coast of the lake is located many capes, and bays, area 2652 km², and water mineralization ranges from 1.2 to 11.6 g / kg. As part of territorial recreational systems, these lakes are located on the coast near the villages Akshi, Koktyma, Kabanbai, Koktal, and are subjected to the great anthropogenic driving. Identified microalgae species in this lake were higher than in all investigated lakes. Main biomass was created by phytoplankton of the taxon Chlorophyta (39.32%). In Lake Alakol, there were 89 types of microalgae, which were related to the main five taxons (Bacilariophyta - 24, Cyanaprocaryota - 22, Chlorophyta - 35, Euglenophhyta - 7, Cryptophytfa - 1), belonging to 12 classes, 36 families and 55 kinds [218]. Cryptophytic algae were encountered less frequently, which shows their low adaptability to mineralization conditions. Presence of three divisions Chlorophryta, Bacilariophyta, and Cyanoprocaryota in all the study sites indicates the high ecological

relevance of species, and predetermines their ability to survive in salty conditions [219]. Bioindication of water quality was observed in β-mesosaprobic saprobic phase, thus Alakol lake was classified as weakly contaminated, with saprobic coefficient ranges within 1.5-2.5.

The algoflora composition of Kolsay Lake was found poor than Balkhash and Alakol. The composition of Algoflora in this lakes was furnished with 42 types, referring to 4 taxons: Bacillariophyta - 21 species, Cyanophyta - 7, Chlorophyta - 11, Dinophyta -3 (Table 6). The most abundant and varied in the form were diatom (Bacillariophyta), with 14 genera and 7 families. Diatoms including Cymbella, Cyclotella and Gomphonema were frequently found (figure 5).

The species from order Desmidiales, Chlorococcales were also encountered. The most frequently found genera included Cosmarium, Scenedesmus, Closterium Staurastrum, Pediastrum. Epiphytic microalgae species were also sampled along with filamentous algae Spirogira and Ulotrix. In the result of the analysis of the indicator-proof species of the lake microflora, we identified the presence of 20 species and variety of indicator species, of which oligo-xenos probes (x-o) - 8, oligos probes (o) - 6, xenos (x) - 4, meso probes (m) – 2 [220]. The assessment of the water condition of the lake by the indicator species of the phytoplankton showed it belonging to the category oligospecial. Saprobity index calculated by the method of Pantle-Bukka was equal to 1.5.

Another investigated lake named Issyk, in the Issyk gorge in Zailiyskiy Alatay, which is located at 40km east of Almaty, in Enbekshikazakh district of the Almaty region [221]. According to geologists, the lake formed about 8-10 thousand years ago as result of collapse of large mountain, which subsequently formed a water reservoir with a height of 300 meters [222]. The algological survey of Lake Issyk indicated 6 divisions and 29 genera. The largest number of types is represented by the taxa Bacillariophyta - 17, 50% of the total species composition. Representatives of Cyanoprokaryota included 9 species, the division Chlorophyta was represented by 5 types. Xanthophytes and Euglenophytes represented by 3 species. Main species diversity of lake algae Issyk are diatomaceous, blue-green and green algae, which account for 91% from the general species composition. Diatoms belonged to 14 genera in 7 families, out of which 3 families and 5 genera constitute the main part of the family and genus spectra. Cyanoprokaryotes were represented by 9 species, 7 genera, and 6 families, occupy the second position by the magnitude of bioformation and represent 26.5% of the total number of species.

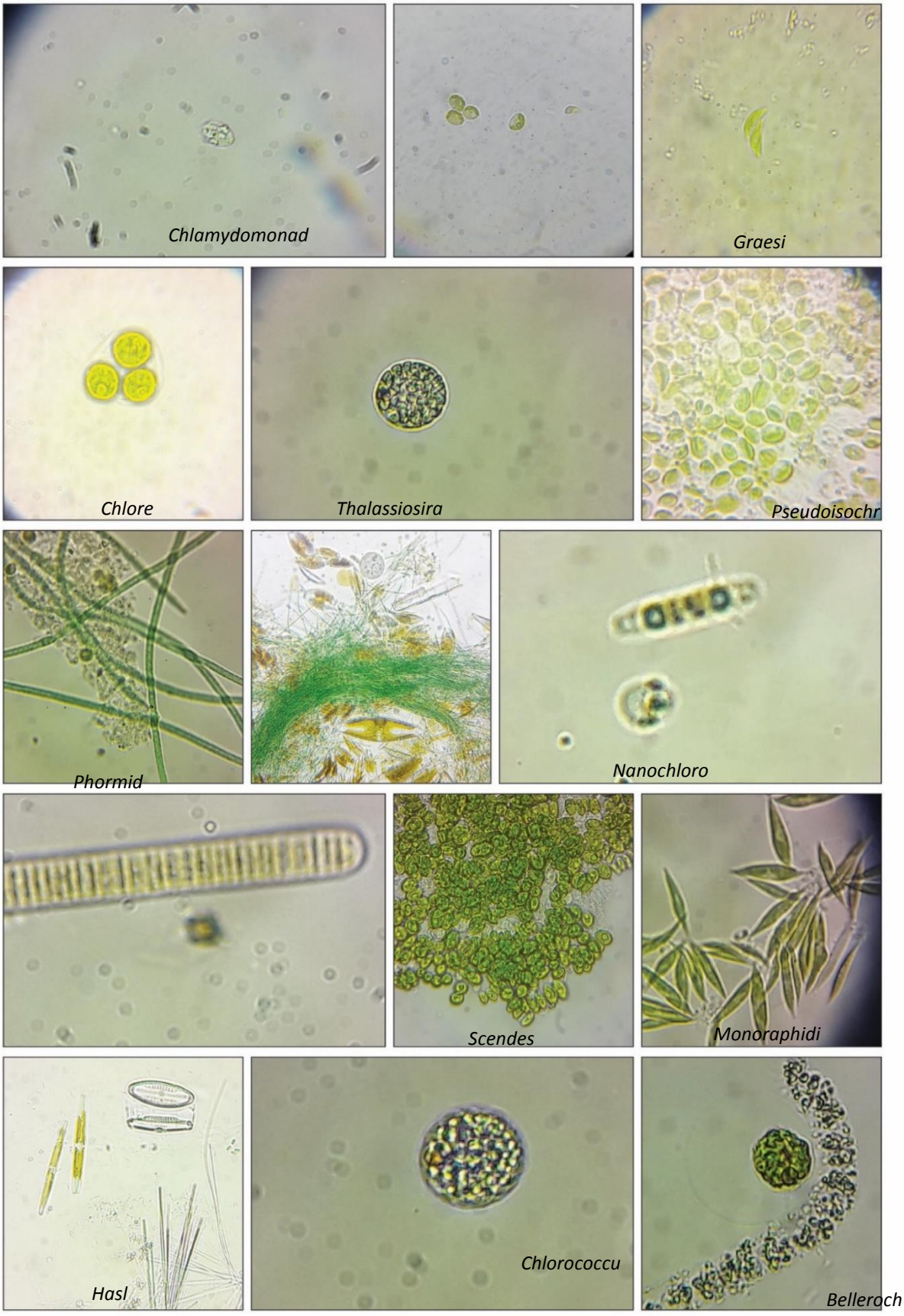


Figure 5 - Light Micrographs of different strains of microalgae from freshwater bodies of Almaty region

The lowest species diversity was recorded for green algae - 5 species belonging to 3 genera and 2 families. Also in Algoflore of Issyk there was an insignificant species variety of yellow-green algae (xanthophytes) - 1 species, gold and red algae - 1. It should be noted that these types of microalgae were not found in the rest of the explored water bodies. Analysis of the composition of indicative-probable microalgae showed the highest number of xenocaprobiotics, the most represented by the smallest radiation mesospecimens, and are sufficiently widespread types of intermediate level of contamination, which occupy an intermediate position between xenos-proof and oligos-proof zones. Evaluation of water state of Lake Issyk by indicator species of phytoplankton showed its necessity to the category oligocapable. Solid water index of Lake Issyk by the Pantle and Bukka method is equal to 1.25.

The lowest species diversity of microalgae was observed in the Big Almaty Lake (BAO). This high water reservoir is located in Zailiyskiy Alatay, at a distance of 15 km to the south the city of Almaty, at a height of 2510 m above the level sea [223]. The length of the cauldron of the lake, which has tectonic pass is 1.6 km, width - from 0.75 to 1 km. This is a small lake with a coastline length of 3 km, with a depth of about 40 m, while the volume of water is about 14 km^3 [224]. By comparison with others studied lakes, significantly less types of microflora are equipped in samples water discharged to the BAO.

When investigating BAO phytoplankton sample, we found 26 species microalgae belonging to seven taxons. Three species from the Cyanophyta division were identified, 2 species from Chrysophyta, 8 species from Bacillariophyta, 7 species of green algae of the Chlorophyta division, 4 representatives of Cryptophyta, and the smallest number are Dinophyta and Euglenophyta, 1 species each, respectively (Table 6). Diatom algae numerically predominated including: Navicula, Pinnularia, Gomphonema, Cymbella, Achnanthes (34.4%), which is characteristic a feature of the phytoplankton of cold lakes, so most of the representatives of this taxon microalgae are cold-tolerant species. The indicator types of microalgae were oligo-, xenocaprobes zone, of which xenos-proof microflora dominated, such as *Gloeocapsa sanguinea, Cyclotella comta, Pinnularia nobilis, Navicula graclis*. Capability index by the Pantle - Bukka method was equal to 0.5 (figure 6).

The Algological pure cultures of *Nephrochlamys sp*. and *Parachlorella sp*. were isolated from the lake Balkhash, *Ankistrodesmus falcatus* and *Monoraphidium griffthii* from Lake Issyk, *Ankistrodesmus sp. B-11* from Kolsay Lake and *Monoraphidium sp* from BAO. The smallest species diversity microflora was registered in Big Almaty Lake and the most rich Algoflora was observed in Lake Balkhash. According to the results of the work on extraction of pure cultures from samples taken from Lake Almati region, we obtained 7 Algological pure cultures (*Nephrochlamys subsolitaria, Parachlorella kessleri, Monoraphidium sp., Ankistrodesmus falcatus, Ankistrodesmus sp., Desmodesmus pannonicus,* and *Monoraphidium griffthii*) for further use in biotechnology.

Table 6 - Diversity of Microalgae in freshwater bodies of Almaty region

Species composition	Balkhash	Issyk	Alakol	Kolsay	BAO
	Number of species observed				
Bacillariophyta	27	17	24	21	8
Cyanophyta	25	-	-	7	3
Cyanoprocaryota	-	9	22	-	-
Chlorophyta	32	5	35	11	7
Euglenophyta	8	-	7	-	1
Cryptophyta	-	1	1	-	4
Chrysophyta	-	-	-	-	2
Dinophyta	-	-	-	3	1
Xantophyta	-	1	-	-	-
Rhodophyta	-	1	-	-	-
Total	92	34	89	42	26

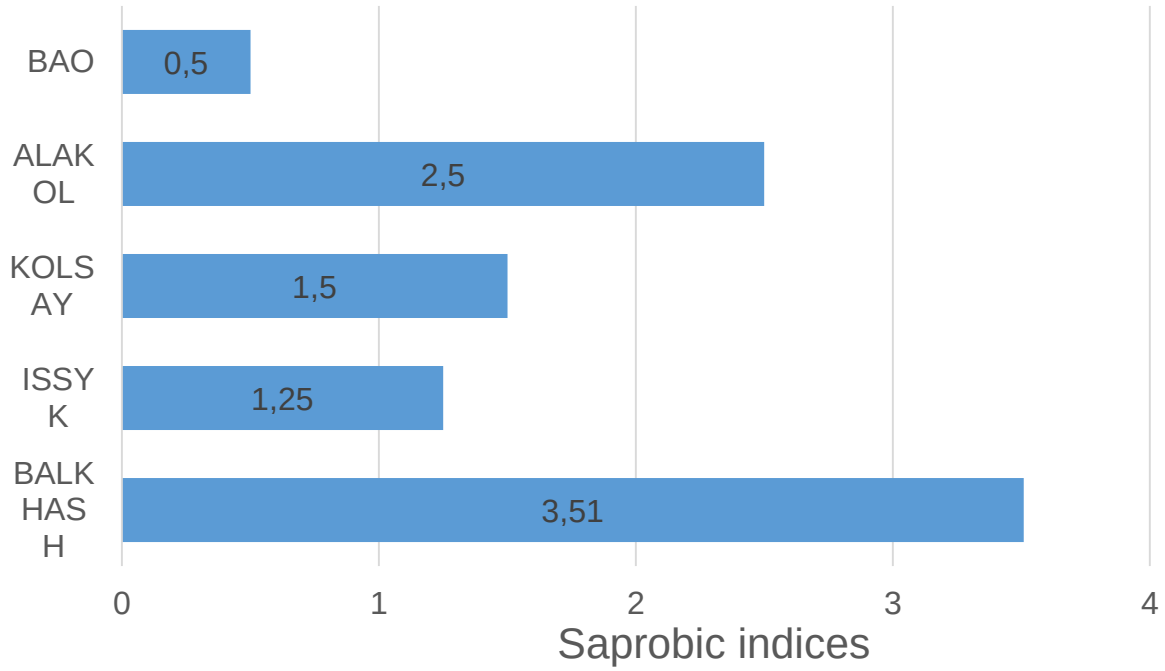


Figure 6 - Saprobic indices value of freshwater reservoir of Almaty region

Lakes Balkhash and Alakol had overall more balanced proportions and the highest diversity of microalgae, with more Chlorophyta and Bacilariophyta than other microalgae. The prescence of microalgae varied almost synchronously, which is indicative of taxonomic diversity and stable structure of phytoplankton community. Various environmental conditions significantly influence the richness and dimensional characteristics of phytoplankton. Therefore, the information on the average prescence of microalgal can be used in assessing the ecological state of a water body. Predominance of small-sized forms is likely to be sign of deterioration in water quality.

In the studied sites, the average composition of microalgae community found lower in BAO and Issyk Lake. This may partially explain the decrease in the contribution of small-sized species of microalgae and also of the improvement in their ecological state. This conclusion is supported by the decrease in saprobic index.

3.2 Morphological & Molecular Characterization of biotechnological valuable strain

3.2.1 Morphological identification of microalgal isolates

A total of 25 algal mixed cultures were collected from the five different lakes, of which seven green microalgal isolates (*Nephrochlamys sp., Parachlorella sp., Monoraphidium sp., Ankistrodesmus falcatus, Ankistrodesmus sp., Desmodesmus pannonicus, and Monoraphidium griffthii*) were selected based on the ease with isolation and cultivation of pure culture under our test conditions, species abundance, morphological homogeneity, and prescence of distinctive morphological features such as size, shape, colour, flagella, spines, mucilage presence etc. The cell morphology of seven microalgae isolates as observed under the light microscopy depicted in Figure 7 and 8. Although standardized morphological analysis is frequently used for taxonomic study of microalgae, however, it is not possible to accurately detail the identification at the species level, because the relationship between intra-specific variation of morphological characters and biological species boundaries are largely unknown in many micro-eukaryotes. The role of light microscopy was less obvious/efficient and more time consuming for precise identification at lower species level, however, microscopic analysis of the monocultures allowed preliminary identification of four isolates ZB-D01 & ZBD-06, ZBA-03 and B-11, ZBD-04, and ZBD-05 as genus *Monoraphidium, Ankistrodemus, Chlorella/Parachlorella, and Desmodesmu*s, while the strain ZBD-02 was ranked to the genus Raphidocelis or Nephrochlamys, respectively, using extensive monographs available for microalgae identification. These genera are green algae that belong to division Chlorophyta, class Chlorophyceae and orders Chlorellales and Sphaeropleales.

The light microscopy and scanning electron microscopy visualization of single cell of microalgal strain ZBD-06 revealed the cell size (Figure 8) varying from 4–6 μm in length and 0.78–1.23 μm in diameter. Solitary cells were predominantly observed having croissant shape to crescent shape with varying degree of curvature, rounded ends, occasionally appearing colonial in irregular arrangement. Solitary cells exhibited mucilage and wrinkled cell surface but no mucilaginous colonies were observed. Chloroplast occupied the majority of the cell volume. Such cell morphology shows that the ZBD-06 resembles the characterization of genus Monoraphidium and Ankistrodesmus.

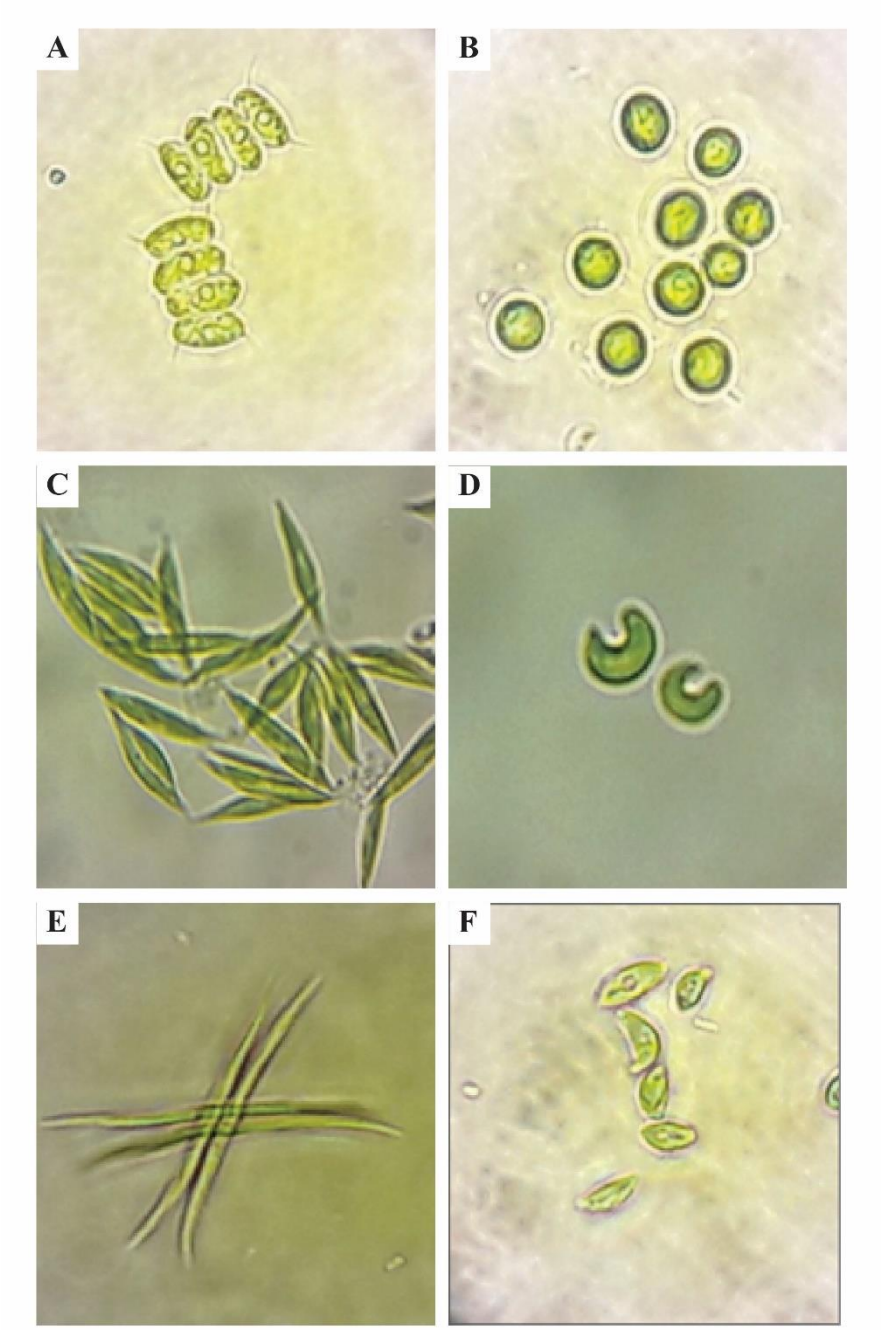
However, the strain was preliminarily identified as member of the genus Monoraphidium, for demonstrating cells dimensions and shape variations similar to *Monoraphidium subclavatum* as described in previous studies [225-226]. Both genus, classified under the family Selenastraceae, are similar in morphology, however the Ankistrodesmus differs by having larger cell dimensions, more or less straight cells, joined by mucilage to form colonies of various shapes, while cells of Monoraphidium

are distinguished from other genera in the family being strongly curved and lacking mucilage [227]. By assessment of all the morphological characteristic of the isolated microalgae, as illustrated in the Microphotograph of the specimen under 40x magnifications (Figure 7) the suspected species was *Monoraphidium subclavatum.*

The strain ZBD-05 was observed to be small green unicellular, non-motile, colonial and oblong-shaped microalgae. About 80-90% of colonies was composed of 4 cells, and sometimes was seen as chained colonies of 8-16 cells. The individual cells approximately ranged from 7 to 10 μm in length and 2.4 to 3.5 μm in width, with short spines on medial cell (400 to 600 nm) and longer spines at terminal cells as great or greater than cell's diameter (2 to 5.7 μm). The surface topology observation under SEM unveiled that the prescence of uninterrupted ribs, rosettes (circular structures) and warts on the outer cell wall layer confirming the strain to be *Desmodesmus* sp. as decribed previously in literature [228].

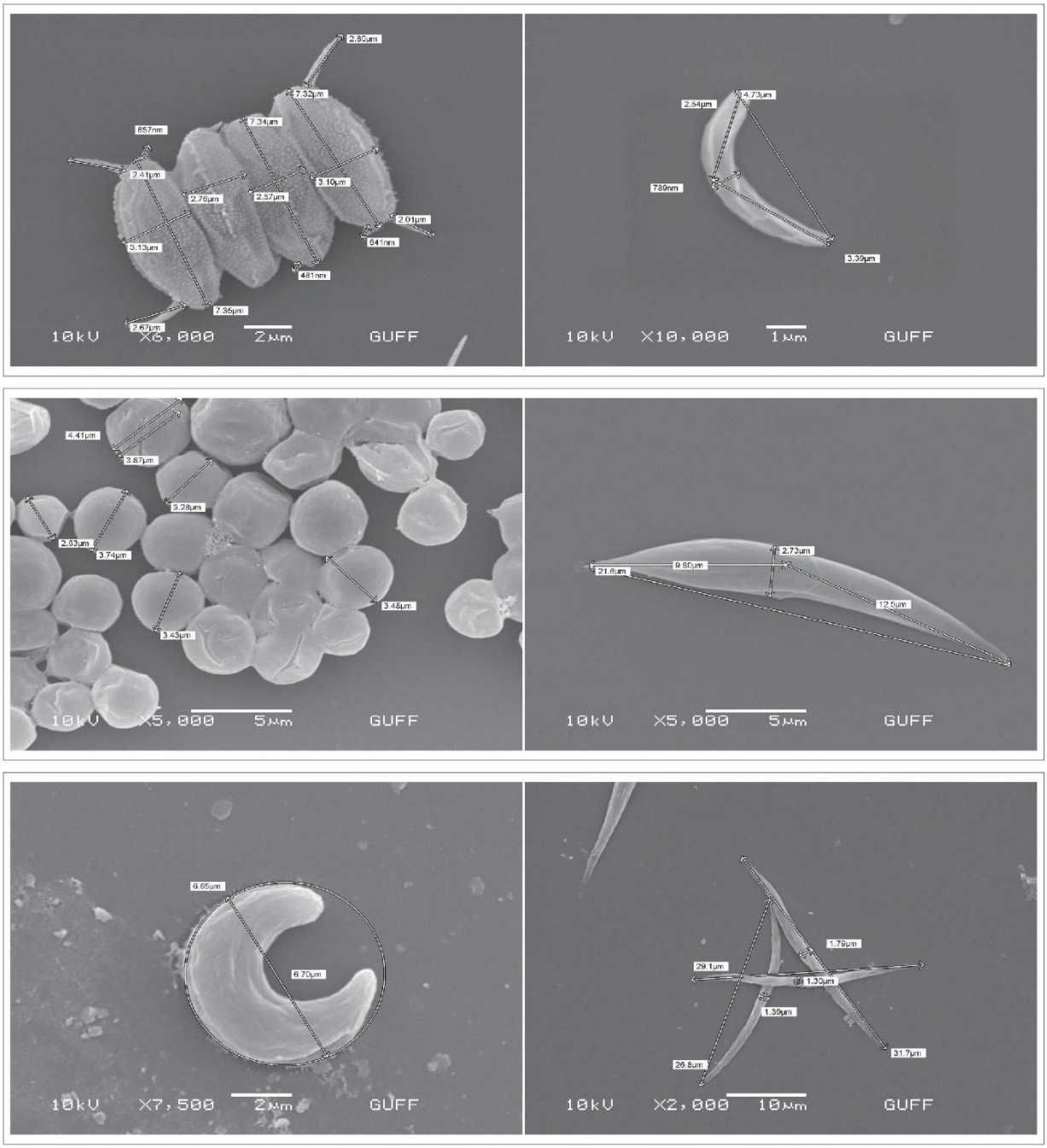
The strain ZBD-04 appeared to be coccoid green unicellular microalgae, 3-5 μm in diameter, spherical in shape, mantle-shaped chloroplast, with and without mucilage. From these morphological characteristics examined under the light and scanning electron microscopy, the isolate C1 was identified at genus-level as Chlorella/Parachlorella as depicted in previous literature [229].

In LM and SEM, the strain ZBD-01 tends to appear solitary falcate cells, about 18 to 24 μm in length, and less than 3 μm in width, with parietal chloroplast, distinctly sub-acute ends, usually straight over most of its length, only rarely the cells exhibited irregular shape, corresponding to the morphology of Monoraphidium genus [230]. The presence or absence of mucous lining appeared to be related to culture age and conditions.



(A) *Desmodesmus* sp. (B) *Parachlorella kessleri* (C) *Monoraphidium griffithi.* (D) *Nephrochlamys subsolitaria* (E) *Ankistrodesmus* sp. (F) *Monoraphidium sp*

Figure 7 (a) - Micrograph (40x) of isolated microalgae strains



(A) *Desmodesmus pannonicus* (B) *Monoraphidium sp.* (C) *Parachlorella kessleri* (D) *Monoraphidium griffithi* (E) *Nephrochlamys subsolitaria* (F) *Ankistrodesmus falcatus*

(B)

Figure 7 (b) – Scanning Electron micrograph of isolated microalgae strains

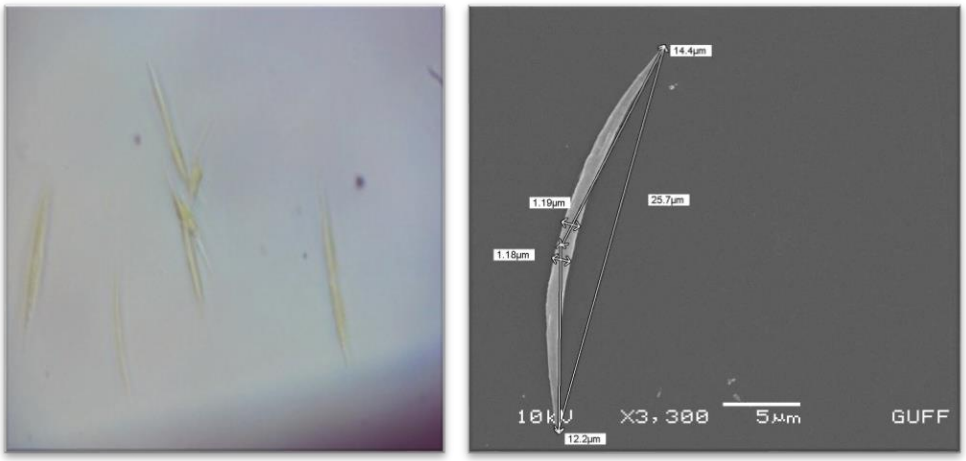


Figure 8 - Light (40x) and Scanning Electron Micrograph of *Ankistrodesmus sp.*

The strain ZBD-02 observed in this study was characterized by unicellular strongly curved (lunate) solitary cells, with ends rounded, attenuated and assymetric, cell diameter 5-7 µm, and slight mucilage secretion. These features appear morphologically similar to the genus Raphidocelis, Nephrochlamys, Selenastrum, Kirchneriella, and Tetranephris, however Selenastrum and Kirchneriella were excluded as cells rarely exists solitary in both genus [231].

The strain ZBD-03 exhibit elongated fusiform to needle-shaped cells, straight or slight curved, inclosed by mucilage, 1-3 x 20-40 µm in size, pointed ends, colonies of 2 or 4, rarely 8, radially arranged, shared features with the genus Ankistrodesmus [232]. The morphology of microalgae isolate B-11 was not differently observed from ZBD-03, except for abscence of colonial or radially arranged form, and cells slightly less wider as compared to ZBD-03.

Nevertheless, the morphological characteristics of the strains investigated in this study doesn't completely fit with the typical description at species level and, therefore, species-level identification could not be achieved based merely on morphological and ultrastructural characters. The identification results of six strains (*Monoraphidium griffithii* ZBD-01, *Nephrochlamys subsolitaria* ZBD-02, *Ankistrodesmus falcatus* ZBD-03, *Parachlorella kessleri* ZBD-04, and *Desmodesmus pannonicus* ZBD-05, *Monoraphidium sp.* ZBD-06) from the cell morphology were then verified by molecular identification through sequencing of the rbcL genes, ITS1-5.8S-ITS2 and 18S rDNA targeted region and alignment with sequences from the Genbank (NCBI). However, the morphological characteristics of isolate B-11 and ZBD-03 were similar enough to accurately identify both species to same genus, thus specific PCR analysis for sequencing was only performed for isolate ZBD-03.

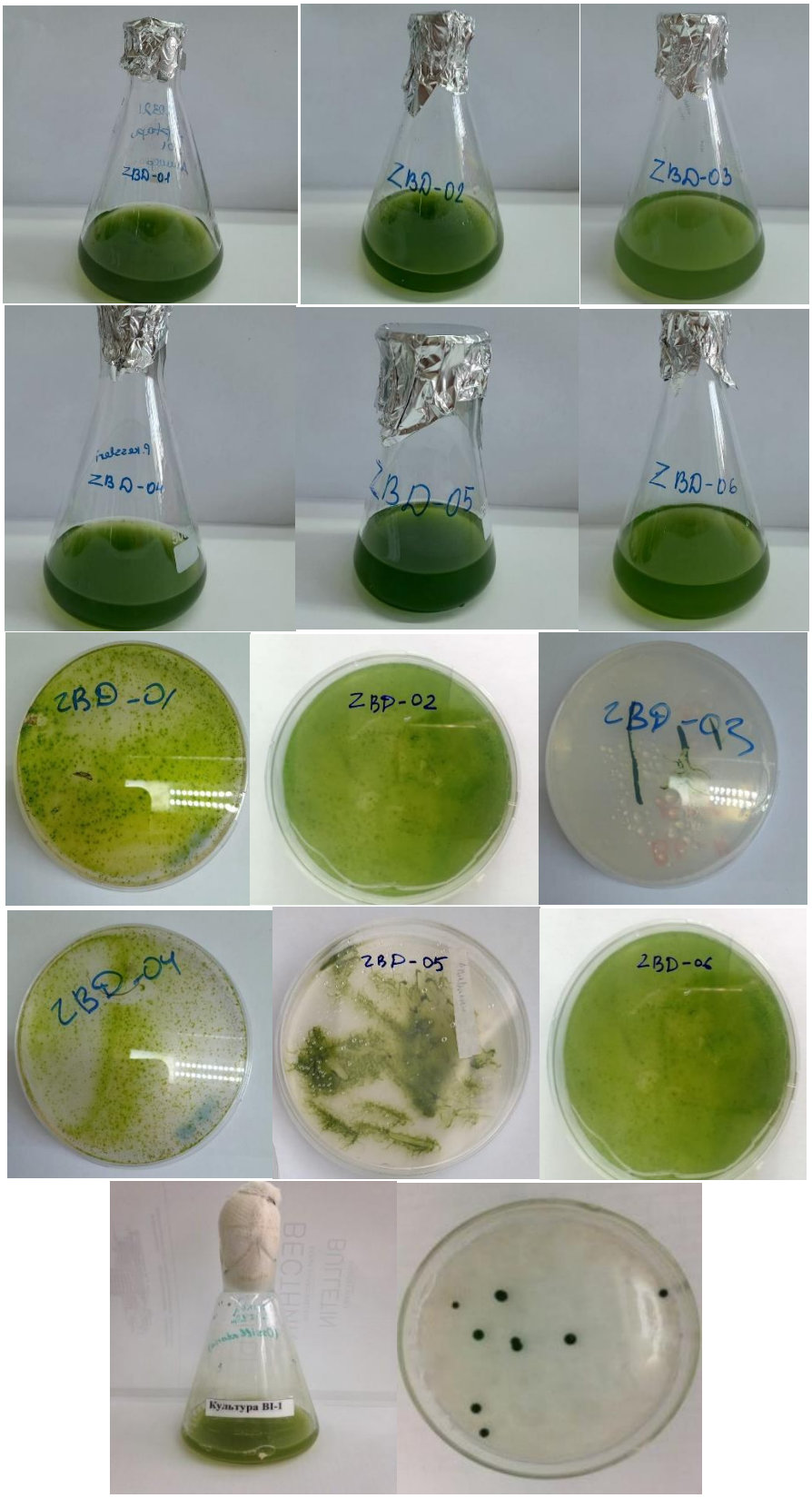


Figure 9 - Bacterial-free monoculture of seven strains of microalgae in liquid and agar medium

3.2.2 Molecular identification of microalgal isolates

The evolutionary, biogeographic, ecological, and conservation studies requires correct taxonomic identification of species and an expansion of knowledge on its true phylogenetic relationship. The phylogenetic inference of a species can also be utilizied for maximizing the probability of success in evaluating its closely related species for bioprsopsecting [233]. Among the various molecular and phylogenetic markers, which were identified, developed and optimized over the past few decades, Internal transcribed spacer (ITS) region of rDNA, particularly between 18S and 5.8 S and between 5.8 and 28 S rDNA subunits, is the most widely used region for accurately describing and restructuring phylogenies [234-235]. However, the species-level identification of few microalgae might not be possible using the ITS marker alone, therefore another reliable marker *rbcL* gene, based on the availability of large number of rbcL sequences for green microalgae at sequence databases such as the Barcode of Life Data (BOLD) System and National Center for Biotechnology Information (NCBI) System, was used in order to be discriminative enough for all tested isolates to reach species level.

In the present study, amplification of *rbcL* gene, 18S, ITS1-5.8S-ITS2, and 5.8S rDNAs partial sequence was carried out using universal primers. The PCR products of a unique single band varying in size from 550 bp to 800 bp were generated from all tested isolates with ITS and *rbcL* primers, with a exception where ITS5 primer failed to generate readable DNA sequence for strain ZBD-03 and rbcL primer failed to amplify ZBD-05 strain. Percentage similarity values obtained after pair-wise alignment of the *rbcL* gene and rDNA sequences of the isolates and qeuried against the NCBI database, with accession numbers indicated before the name of each strain, are listed in Table 7. The obtained sequences of four strains showed high degree of similarity (98-100%) with microalgae sequences deposited in GenBank database and the microalgal isolates ZBD-05, ZBD-04, ZBD-02, ZBD-03, and were identified up to species level as *Desmodesmus pannonicus, Parachlorella kessleri, Nephrochlamys subsolitaria, Ankistrodesmus falcatus*, and, based on 100%, 99%, 100%, and 98% sequence similarities, respectively, while sequence similarity between 89 - 93% was found for ZBD-01 with *Monoraphidium griffithi*. These sequence homology results strongly agree with the morphological findings in this study for these strains.

3.2.3 Phylogenetic Analysis

The ITS partial sequence-based phylogenetic analysis was performed for the five isolates of microalgae by comparing other published closest and related reference sequences (Figure 10). The identification of microalgae isolates ascertained by morphological and molecular analysis was supported by the phylogenetic results. The isolate ZBD-05 is strongly grouped in the same clade with *Desmodesmus pannonicus* with bootstrap value of 92% while ZBD-04 shared the same clade with *Parachloella sp*. and grouped with *Parachlorella kessleri* strains. The isolates ZBD-02 belonged to the clade of *Nephrochlamys subsolitaria* with percentage support value of 98%. The isolate ZBD-03 clustered with *Ankistrodesmus falcatus* with a bootstrap support value

of 100%. Th phylogram clearly confirmed the identification of isolates ZBD-05, ZBD-04, ZBD-02, and ZBD-03 as *Desmodesmus pannonicus, Parachlorella kessleri, Nephrochlamys subsolitaria, and Ankistrodesmus falcatus*. The observed phylogenetic clustering is in agreement with our initial morphological and molecular findings. However, the isolate ZBD-01 is located in the group of *Ankistrodesmus falcatus*, differing from the morphological identification result for this strain. The results of BLAST sequences homology search for this isolate showed low similarity to the known sequences, clustering distinctly from Monoraphidium genera.

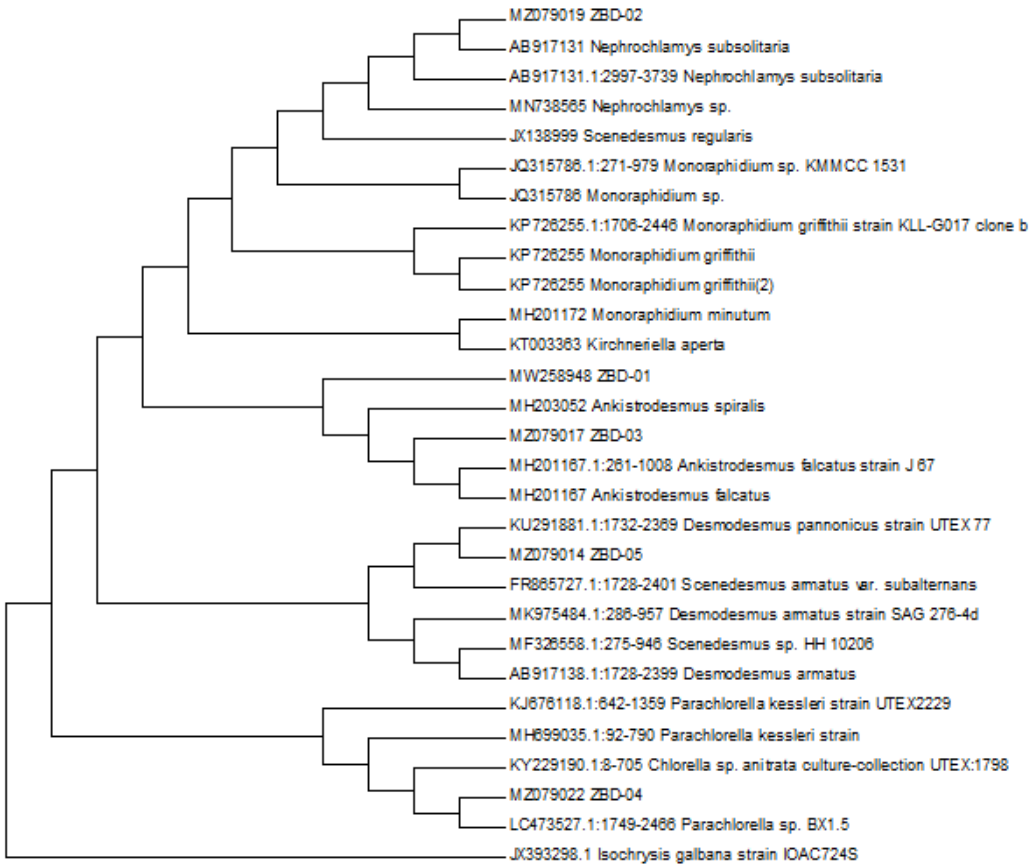


Figure 10 - Phenogram generated using the ITS1 region that includes five selected green microalgae isolates and homologous sequences with different similarity level.

Isolates	Alignments (ITS1)	Max/total score	Query	Identity	Alignments (ITS 1 (P))	Max/total score	Query	Identity	Alignments (ITS5)	Max/total score	Query	Identity	Alignments (rbcL)	Max/total score	Query	Identity
ZBD-05 MZ079014	*Scenedesmus armatus var. subalternans* FR865727	1240	73%	99.85%	*Scenedesmus armatus var. subalternans* FR865727	1201	91%	99.69%	*Scenedesmus armatus var. subalternans* FR865727	1225	91%	99.26%	No similarity found			
	Desmodesmus armatus MK975484	1194	73%	98.66%	*Desmodesmus armatus* MK975484	1155	91%	98.47%	*Desmodesmus armatus* MK975484	1179	91%	98.09%				
	Scenedesmus sp MF326558	1194	73%	98.66%	*Scenedesmus sp* MF326558	1155	91%	98.47%	*Scenedesmus sp.* MF326558	1179	91%	98.09%				
	Desmodesmus pannonicus KU291881	1179	69%	100.00%	*Desmodesmus pannonicus* KU291881	1140	86%	99.84%	*Desmodesmus pannonicus* KU291881	1164	86%	99.38%				
ZBD-04 MZ079022	*Parachlorella sp.* LC473527	1384	67%	99.60%	*Parachlorella sp.* LC473527	1345	54%	99.59%	*Parachlorella sp.* LC473527	1243	95%	95%	*Parachlorella kessleri* FJ968741	1092/2184	55%	99.50%
	Parachlorella kessleri KJ676116	1375	67%	99.34%	*Parachlorella kessleri* KJ676116	1338	54%	99.32%	*Parachlorella kessleri* KJ676116	1236	95%	94.36%	*Parachlorella kessleri* AB260912	1092	55%	99.50%
	Chlorella vulgaris AY948419	1375	67%	99.34%	*Chlorella vulgaris* AY948419	1338	54%	99.32%	*Chlorella vulgaris* AY948419	1236	95%	94.36%	*Chloroidium saccharophilum* MK295212	1048	55%	98.17%
ZBD-01 MW258948	*Monoraphidium griffithii* KP726255	1002	81%	91.03%	*Monoraphidium griffithii* KP726255	966	53%	90.80%	*Monoraphidium griffithii* KP726255	935	65%	89.23%	*Ankistrodesmus stipitatus* EF113406	894	60%	93.50%
	Nephrochlamys subsolitaria AB917131	965	81%	90.15%	*Nephrochlamys subsolitaria* AB917131	931	54%	89.90%	*Nephrochlamys subsolitaria* AB917131	902	65%	88.37%	*Ourococcus multisporus* KT369475	865	60%	92.67%
ZBD-02 MZ079019	*Nephrochlamys subsolitaria* AB917131	1373	82%	100.00%	*Monoraphidium griffithii* KP726255	702	56%	90.09%	*Nephrochlamys subsolitaria* AB917131	1358	68%	99.46%	*Ankistrodesmus stipitatus* EF113406	900	59%	93.67
	Nephrochlamys sp MN738565	1275	79%	93.57%	*Ankistrodesmus falcatus* MH201167	699	49%	93.40%	*Nephrochlamys* sp. MN738565	1267	65%	98.33	*Ourococcus multisporus* KT369475	870	59%	92.83
ZBD-03 MZ079017	*Ankistrodesmus falcatus* MH201167	1279	54%	98.48%	*Ankistrodesmus falcatus* MH201167	1291	51%	98.63%	No similarity found				*Ankistrodesmus stipitatus* EF113406	1005	63%	96.83%
	Monoraphidium griffithii KP726255	950	54%	90.52%	*Monoraphidium griffithii* KP726255	963	51%	90.70%	No similarity found				*Ankistrodesmus fusiformis* KT833576	994	57%	99.27%

Table 7 - The accession numbers and % similarities between amplified sequences, and the closest relative sequences for five isolated strains microalgae.

To perform the molecular identification of the isolate, the desired DNA fragments (~600 - bp) of isolate, amplified with universal primers of rbcL gene and ITS region were compared with the similar sequences in the database using BLAST online (http://www.NCBI.nim.nih.gov/). The similarity searches of the obtained amplicon sequences in the GenBank database were observed for the universal primer sets of ITS1/ITS4 and ITS5/ITS4, whereas the amplicon sequences generated from rbcL could not retrieved any blast match.

The blast result of only first set of primer ITS1/ITS4 was supportive and useful in species discrimination, while second set of primer ITS1/ITS4 and ITS5/ITS4 resulted in database mismatches with macro algae, uncultured microorganism and microalgae completely conflicting with morphological description. As the results with the second set of primers were not satisfactory to infer generic identity, therefore, the phylogenetic analysis was performed only with the first set of ITS/ITS4 primer. This reduced success in retrieving accurate sequence matches can be attributed to the fact that the number of well-described microalgae species are still not significantly represented in Genbank. The limitations in molecular identification of some microalgae at the species-level implied that further work is required to establish efficient DNA barcode markers for revealing sufficient heterogeneity for species identification, maximizing the gene database, as well as employing combination of molecular and morphological methods for accurate identification of species with extreme morphological plasticity.

The fifteen sequence entries with highest similarity to the obtained sequence of isolate ITS region were observed and multiple alignment of the sequences were generated with the MUSCLE program. The phylogenetic tree was constructed with MEGA software version 6 based on the evolutionary distances that were calculated by the Neighbor-Joining method [236] using Kimura-2-Parameter algorithm [237]. Statistical evaluation of the tree topologies was performed by bootstrap analysis with 1000 re-samplings [238]. *Isochrysis galbana* strain (JX393298) was used as an outgroup. Figure 11 represents the Neighbour-Joining showing phylogenetic position of strain and related taxa based on ITS1-5.8S-ITS2 region sequence comparisons.

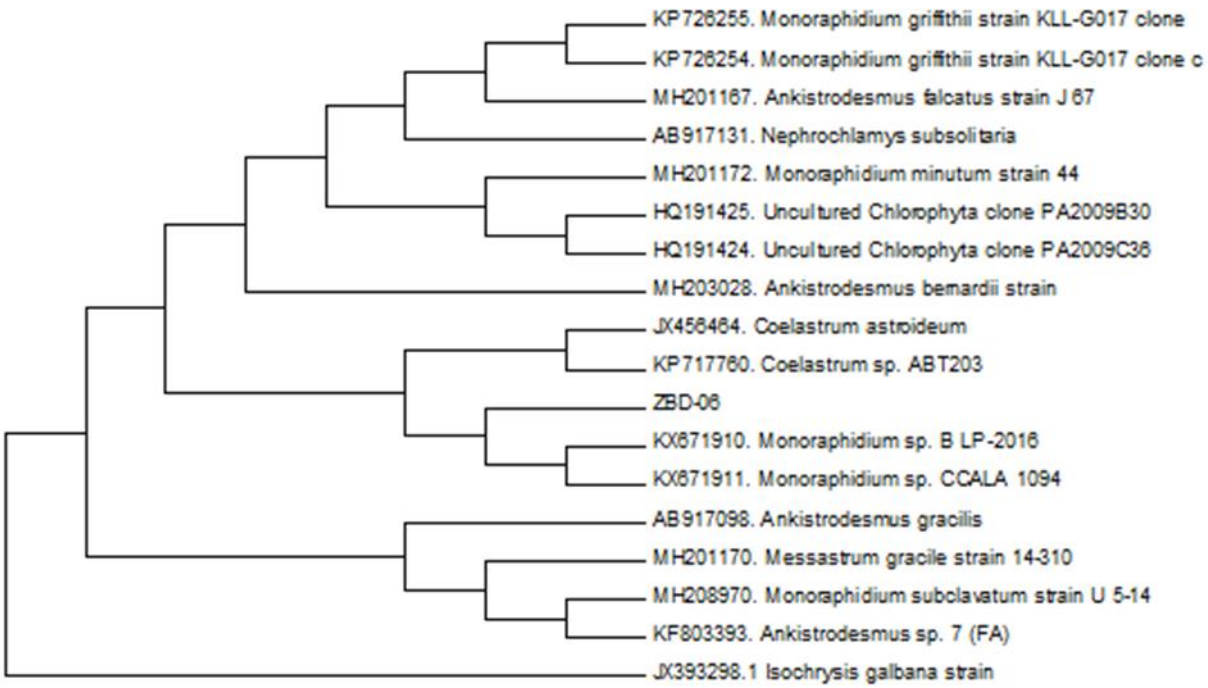


Figure 11 - Phylogenetic tree of the isolated microalgae and the closely related strains based on ITS1-5.8S-ITS2 region sequence comparisons

Phylogenetic tree consisted of basically three major clusters and one outgroup, each of which mostly contained different species of Genus Monoraphidium and Ankistrodesmus. The Phylogram (figure 11) shows that the ZBD-06 strain is closely related to *Monoraphidium sp*. B LP-2016 (score = 911; ident. = 93.33%), *Monoraphidium sp.* CCALA 1094 (score = 900, ident. = 93.28%) isolated from ice-covered lakes on James Ross Island (northeastern Antarctic Peninsula) [132, p. 19]. The clustering of Monoraohidium sp. from Almaty region with Monoaphidium sp. from Antarctic region determines the presence of closely related extermophilic species of microalgae in geographically far distinct location (continental level). The phylogram also depicts the relatedness of the newly isolated native strain to *Coelastrum astroideum* (JX456464), however, both strains are morphologically different [239]. Other closely related species were from the genus of Ankistrodesmus and Monoraphidium representing a polyphyletic group [240]. The overall morphological features and sequence-based phylogenetic analysis of ZBD-06 with one of the primers of ITS1 region presented a good correlation and served as important molecular target for identification and taxonomical position of the isolate to great extent based on the availability of sequence database. The obtained molecular result suggests the consideration of ZBD-06 strain as *Monoraphidium* sp. that was also based on light and SEM microscopic observation and the genus belongs to the family Selenastracaceae (Cholorophyceae). The ITS sequence of the isolate was submitted with accession number of MT178772 in NCBI database, respectively. The relative phylogenetic position based on the ITS1/ITS4 primer sequence might not determine species-level

resolution for this species, but when matched with the results of morphological studies, it provided more reliable identification of the isolate.

3.3 Evaluation of Biofuel potential

3.3.1 Growth evaluation of selected microalgal strains and Biomass productivity

Generally, growth rate, biomass yield, lipid content and fatty acid composition are important factors to consider in evaluation of microalgal strain as viable feedstock for biofuel production. An initial screening revealed lipid accumulation capacity of less than 20% of the cell biomass for all strains of this study, however *D. pannonicus, P. kessleri*, and *N. subsolitaria*, showed higher growth rate at 0.58 day^{-1}, 0.57 day^{-1}, and 0.42 day^{-1}, and biomass yield of 1.35 g, 1.42 g, and 1.21 g, respectively, per one Litre culture (Table 8). There was no significant difference between strains *M. griffithi* and *A. falcatus* (0.24 day^{-1} and 0.21 day^{-1}). All strains were grown as monocultures at the same laboratory condition in view of their robustness towards a wide range of environmental conditions, however it may not necessarily be the optimal culturing conditions for all strains of this study, and performance in growth, biomass and lipids productivity may vary if other nutritional or environmental requirements were accounted [241].

Table 8 - Comparison of growth coefficient, biomass, lipid content, and lipid productivity of five microalgal isolate

Isolates	Growth Coefficient	Average Biomass Concentration ($g\ L^{-1}$)	Total Lipid Content (% of dry weight)	Lipid Productivity ($mg\ L^{-1}\ day^{1}$)
Monoraphidium griffithi	0.24	1.12 ± 0.05	13.82 ± 0.1	19.6 ± 0.5
Nephrochlamys subsolitaria	0.42	1.21 ± 0.04	12.71 ± 0.4	22.4 ± 1.6
Ankistrodesmus falcatus	0.21	1.09 ± 0.14	13.41 ± 0.1	19.2 ± 0.7
Parachlorella kessleri	0.57	1.42 ± 0.08	16.43 ± 0.2	29 ± 1.2
Desmodesmus pannonicus	0.58	1.35 ± 0.04	14.51 ± 0	26.7 ± 0.7

3.3.2 Effect of temperature

The optimum temperatures for the studied six isolate were between 22-25 °C, while the minimal and maximal temperatures for the activity of six isolates were found to be 11 to 13 °C, and 33 to 36 °C, respectively. Results showed that isolates exhibited a similar trend of decrease in its growth rate/cell density by 20% at more than 30 °C. No growth of isolates was noted at/above temperature of 36 °C which can be considered to be the result of the interruption of microalgal growth or large number of dead cells. Despite of being collected from different lakes with different environmental and physical conditions, the maximum growth rate for all strains was obtained at nearly

same temperature. The studied strains had the best growth rate with BG-11 medium. The highest biomass yield was attained at 23±2 °C, after which the biomass of all isolates decreased or delayed with further increase in the temperature.

Temperature is one of the main factors affecting microalgae growth. Temperature greatly influences the biochemical composition, the uptake of essential micronutrients, rate of photosynthetic carbon fixation of nearly all species of microalgae [242]. It is known that phytoplankton potentially have optimal metabolic efficiency at specific temperature and growth rate will decrease drastically with increase or decrease in temperature.

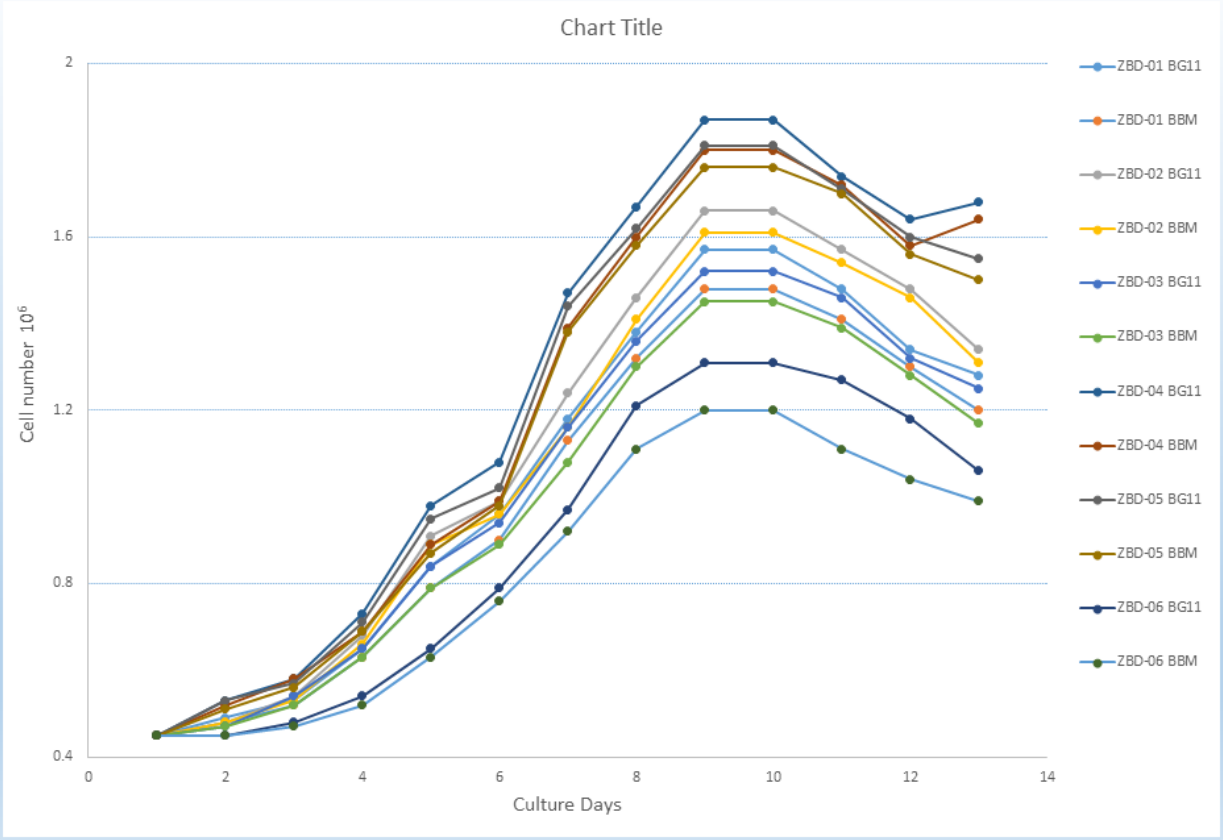


Figure 12 - Comparative Growth curve of Microalgae isolates in growth medium BG-11 and BBM

3.3.3 Effect of light

The effects of light quality (color) including white and yellow light and dark period was tested. The results indicated both light wavelengths are optimal for microalgae growth and no significant difference in results was noted. Our results also showed that optimal light wavelength along with the dark period are critical for microalgal growth. It was noted that growth of microalgae was negatively affected by prolong exposure to daylight without dark period.

There was relatively higher cell density (number of cells) when yellow and white light were alternatively used after 3 to 4 days of growth (light intensity 100 µmol/m^2/s). One previous study [243] suggested the red light as optimal for biomass productivity in green microalgae, as Chlorophytes have high concentration of chlorophyll and hence absorbs efficiently in the red wavelength area. However, another study [244] reported that red light was negatively associated with biomass growth of *Ch. vulgaris* biomass

and violet, green and orange light (250 μmol/m2 /s) were found more efficient for the same strain.

3.3.4 Effect of CO_2 concentration

The cell density of all studied isolates were positively correlated with elevated concentrations of CO_2 from 0.5 % upto 5%, however their tolerance range for further higher carbon concentration was limited. Studying the effect of different concentration on these strains was not feasible for longer period due to time and equipment constraints. However, the results obtained slightly coincided wuth previous study [245], where the maximum growth rate of green microalgae was found in the culture of 10 % CO_2. Another study [246] reported that the highest biomass yield of *Ch. vulgaris* was found when the microalgal culture were supplied with 8.5 % CO_2 and exposed to white light.

3.3.5 Effect of cultivation medium

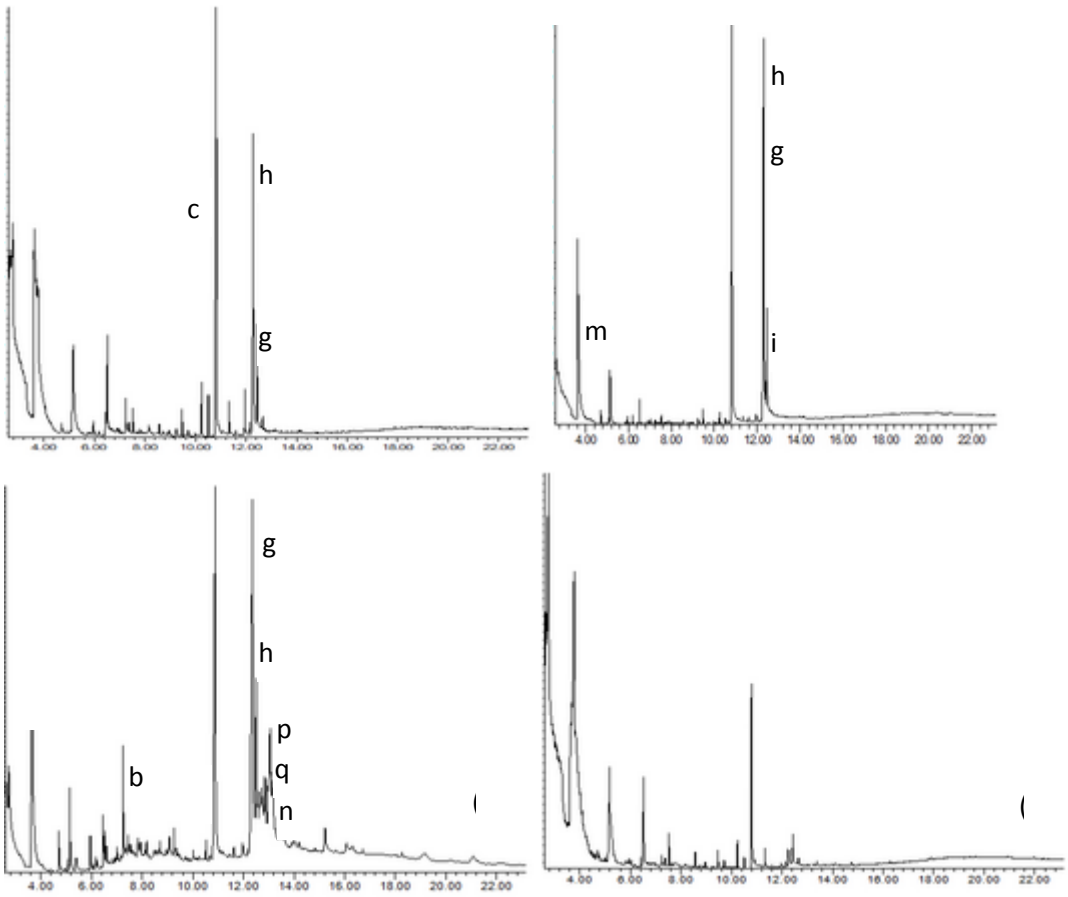
The growth curves for all isolates cultured in two different media types, including BG11 and BBM, are shown in Figure 12. The isolate *Parachlorella kessleri* with the BG-11 media showed the highest overall growth rate in both medium comparatively to all other isolates. *Desmodesmu*s and *Nephrochlamys* appeared to thrive in the BBM initially, but after 4 days of culture BG-11 enabled higher growth of these isolates. The growth coefficient and cell density were calculated to compare the growth of six species in the two mediums.

All isolates showed similar growth pattern in both mediums with provided same conditions of CO_2concentration, pH and temperature at specific time simultaneously. pH was noted to drop in first few days then gradually rise each day after that. The solubility of CO_2 in the liquid phase increases with increase in temperature, which lead to the change in pH and subsequently effect the growth of the microalgae.

3.3.6 Lipids profiling and Fatty Acid Analysis (FAMEs)

The derivatized fatty acid methyl esters (FAMEs) were analyzed for all strains, except for ZBD-02, using GC/MS. Consistently the Hexane extracts of four tested strains showed the prevalence of saturated fatty acids including palmitic acid, oleic acid and stearic acid as dominant fatty acids and low level of polyunsaturated fatty acids thus suggesting high potential of these strains for biodiesel feedstock consideration. Major fatty acid methyl esters observed in *P. kessleri* were grandiflorenic acid, palmitic acid, heptadecanoic acid, Oleic acid and stearic acid while the minor fatty acids were eicosapentaenoic acid (EPA), benzoic acid, lauric acid, tridecanoic acid, and 1-Phenanthrenecarboxylic acid. The largest peak area was noted for Octadecenoic acid (Stearic) at 16.86%, second largest peak area of 15.12% recorded due to the presence of n-Hexadecanoic acid (Palmitic) and the third peak was at 6.85% of Octamethyl cyclotetrasiloxane. Beside several saturated and unsaturated fatty acids ranging from C6 to C20, resin acids/diterpenoids such as abietic acid, palustric acid and callistric acid were also detected in P. kessleri, accounting for a percentage of 21.8% of the total fatty acids (Figure 13).

Monoraphidium griffithi and *Ankistrodesmus falcatus* also showed great potential as the sources of oleic acid, stearic acid and palmitic acid for renewable fuels such as biodiesel, as their prominent peaks were noted at 14.73%, 4.49% and 16.94%; and 33.27%, 7.26% and 30.08%, respectively. Fatty acids <C14 were found dominant in *Desmodesmus pannonicus* while n-Hexadecanoic acid (palmitic acid) and Hexadecanoic acid methyl ester are the only fatty acid present in considerable quantity with prominent peak of 20.03% and 11.59%. The GC/MS analysis (Table 9) revealed the fatty acids of four strains of this study which are known to be most common fatty acids for various biological activities such as antibacterial, antioxidant, medicinal applications and holds the potential as renewable chemical feedstocks in many industries such as biofuel, bio-plastic and bio-based products.



i) M. griffithii; ii) *A. falcatus*; iii) *P. kessleri*; iv) *D. panonnicus*. : a) 4-Phenyl-4H-1,2,4-triazole-3-thiol b) Tridecanoic acid C13:0, c) Pentadecanoic acid (C15:0), d) n-Hexadecanoic acid (C16:0), e) Hexadecanoic acid, methyl ester (C16:0), f) Heptadecanoic acid (C17), g) Octadecanoic acid (C18:0), h) 9-Octadecenoic acid (C18:1), i) 6-Octadecenoic acid, (Z)- (C18:1 cis-6), m) 3-Hydroxymandelic acid ethyl ester (C8), n) Abietic acid (C20), p) β-Pimaric Acid (C20), q) Callitrisic acid (C20).

Figure 13 - Gas chromatograms of fatty acid methyl esters from four studied species.

Cetane number (CN), Saponification value (SV), and Iodine value (IV), being an adequate criterion for estimating the properties of biodiesel, were calculated from the results of Fatty acid methyl ester (FAME) profiles (listed in Table 9). According to the ASTM D6751-02 and EN14214 standard for biodiesel, the required limit for CN is

>47.0 and >51.0, respectively, whereas the maximum value of 120 g I2/100 g fat is recommended for IV. The calculated CNs for isolates *P. Kessleri* and *A. falcatus* were found to be 48 and 50, and comparable with the etimated values reported for biodiesel obtained from other microalgae in the previous literature [247]. The calculated IVs and SVs of *P. Kessleri* and *A. falcatus* were 83.4 and 103.6 which are also in accordance with the EN14214 biodiesel standards and strongly suggest good oxidative stability of the isolates and their possible suitability as a feedstock for biofuel. The CNs of *M. griffithii* (34) and *D. pannonicus* (25.5) were less than those required for the biodiesel standard requirement, and their high iodine values178.3 and 194.9 suggests the isolates' low oxidative stability.

Table 9 - Comparison of fatty Acids Composition of selected four Microalgae isolates

FAs Composition	*Fatty AcidsCompisition (% w/w)*			
	Monoraphidium griffithi	*Ankistrodesmus falcatus*	*Parachlorella kessleri*	*Desmodesmus pannonicus*
Hexadecanoic (palmitic, C16:0),	16.94%	30.08%,	15.12%	20.03%
Hexadecanoic acid, methyl ester	ND	ND	6.72%	11.59%
Octadecanoic acid (stearic, C18:0),	4.49%	7.26%	16.86%	ND
9-Octadecenoic acid (oleic, C18:1)	14.73%	33.27%	ND	ND
Diterpenoids such as abietic acid, palustric acid and callistric acid	ND	ND	21.08%	ND
Others (3-Hydroxymandelic acid, Dimethyl phthalate, Hexa and octamethylcyclotetrasiloxane, Lauric acid, Capric acid, Eicosapentaenoic acid (EPA), Tricosylic acid, 2,4-Di-tert butylphenol)	63%	30%	40%	68%
SV	195.4	199.5	260.8	236.4
IV	178.3	103.6	83.4	194.9
CN	34	50.3	48.4	25.5

Producing efficient and cost-effective microalgal biodiesel requires primarily optimizing genetic and regulatory processes in the potential strains of microalgae. Our research provided favorable microalgae candidates for biodiesel production, strain of *Parachlorella kessleri* and *Ankistrodesmus falcatus*, which showed content of suitable fatty acids and biodiesel properties under normal growing conditions of BG-11 medium. As the current study was conducted on microalgae strains collected from cold conditions, together with other limiting factors (e.g nutreints availability, and high solar irradiation) further research should be conducted to investigate tolerance to extreme environments by differentiating the growth medium and environmental conditions, particularly the differential effect of elements such as nitrogen, phosphorus and iron on biomass production and lipid yield of different microalgal strains.

3.3.7 Biofuel feedstock opportunity

Microalgal lipid content and compositions have direct effect on biodiesel properties. The fatty acid content of the lipid (rate of unsaturated FAs to saturated FAs) affects the properties of the resulting FAMEs. The biochemical composition of the biomass of microalgae towards higher lipids content and desirable fatty acids can be regulated by varying the cultivation conditions. The obtained peaks of fatty acid methyl esters and their relative percentages in GC/Ms results of this study indicates that lipid content of *P. kessleri* and *A. falcatus* of saturated and monounsaturated fatty acids. The saturation level of fatty acids considerably effects the storability and stability of biofuel. The lipid profile of *M. griffithi* also showed the presence of Fatty acids with chain lengths from C16 to C18 to C21-24, however polyunsaturated methyl esters were recovered from this isolate in relatively higher amount (63%) comparatively to combine percent relative weight of saturated and monounsaturated fatty acids (34%), which resulted in low cetane number and high Iodine value.

As per the EN14214 biodiesel standards, biodiesel with iodine value within the maximum limit of 120 g l_2 100 g^{-1} is a better quality one. The iodine value of *P. kessleri* and *A. falcatus* of < 120 g l_2 100 g^{-1} indicates a good quality of potential fuel engine with high heat combustion. The combustion of biofule is also determined by the abovementioned parameters. Fatty acids with higher ratio of saturated and monounsaturated fatty acids are expected to have higher heat of combustion as lower degree of unsaturation is directly related to ratio of hydrogen to carbon. The presence of unsaturated bonds in the FAMEs of *M. griffithi* lowered the cetane number and increased the iodine value considerably that reflects its proness to be oxidizied when exposed to air. Hence despite the prescence of unique and desirable fatty acids methyl esters in microalgae, the presence of unsaturated FAMEs lowers the quality of the biodiesel.

3.4 Examination of antimicrobial activity

Our results revealed the existence of valuable constituents, such as saturated fatty acids and terpenoids in microalgae strains, that also provide additional value other than obtaining biofuel and contribute to interesting biological activities, including antibacterial activity. Green microalgae have been reported to produce several pharmacologically important bioactive compounds such as anti-inflamatory n-3 fatty acids (Eicosapentaenoic acid (EPA), cytotoxic fucosterol, anticancer borophycin, antiviral cyanovirin, and nutraceutical β-carotene [248].

To comprehensively asses the antibacterial potential of green microalgae, the methanolic extracts of all microalgae isolates were tested for their antibacterial activity against eleven bacterial strains, namely, Gram-positive (*Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228) and Gram-negative (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 13315, *Salmonella typhimurium* ATCC 14028, *Yersinia pseudotuberculosis* ATCC 911, and *Enterobacter cloacae* ATCC 13047) by

disk diffusion assay. As shown in figure 14, four strains of microalgae exhibited diverse inhibitory effect on two gram positive bacteria (*B. subtilis, S. Aureus*) and three gram-negative bacteria (*E, coli, K. pneumonia, and P. aeruginosa*). *Parachlorella kessleri* and *Nephrochlamys subsolitaria* showed highest antibacterial activity against *B. subtilis*, as appeared from the inhibition zone of 0,6 mm in diameter, respectively. The antagonistic effect of *Parachlorella kessleri* was also noted against *S. aureus* and *K. pneumonia*. Isolated strains of *Monoraphidium griffithi* and *Ankistrodesmus falcatus* showed moderate antimicrobial activity against only gram negative bacteria (*E. coli* and *K. pneumonia*). *Nephrochlamys subsolitaria* also displayed active inhibitory effect against *E. coli* followed by lesser inhibitory action against *P. aeruginosa*.

Table 10 - Antimicrobial activity of Microalgal isolates against selected bacterial pathogens by disk diffusion method

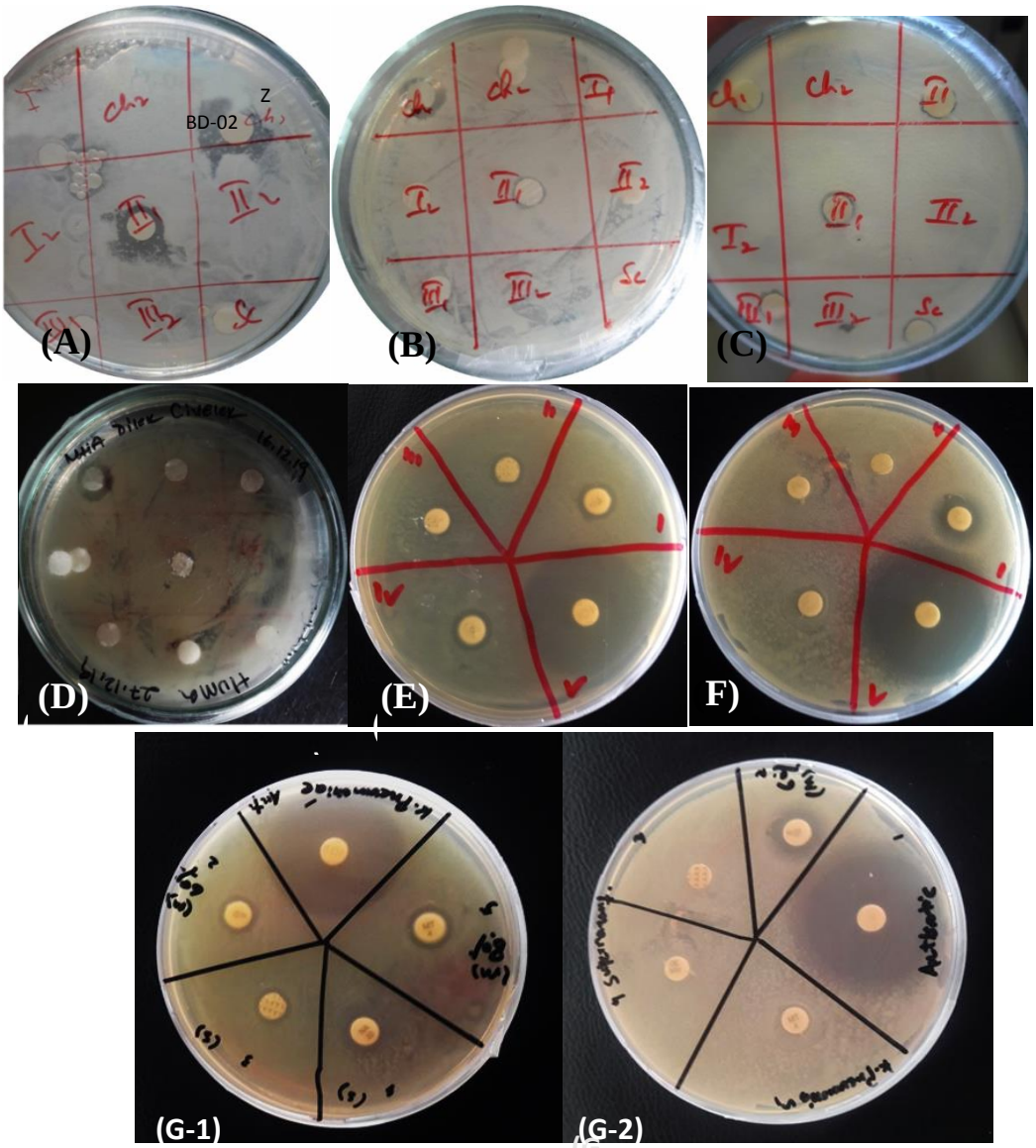
Isolates	**Species**	***B. subtilis***	***S. aureus***	***E. coli***	***P. aeruginosa***	***K. pneumonia***	**MIC values (microalgal cells/mL)**
ZBD-05	*D. pannonicus*	−	−	−	−	−	0.75×10^8 cells/mL
ZBD-04	*P. kessleri*	+++	++	−	−	+	1.2×10^8 cells/mL
ZBD-01	*M. griffithi*	−	−	+	−	+	0.90×10^8 cells/mL
ZBD-02	*N. subsolitaria*	+++	−	++	+	−	0.70×10^8 cells/mL
ZBD-03	*A. falcatus*	−	−	+	−	+	0.45×10^8 cells/mL

−: no antibacterial activity.
+: diameter of inhibition zone < 0,2 mm
++: diameter of zone of inhibition ≥ 0,4 mm.
+++: diameter of zone of inhibition ≥ 0,6 mm.

The methanol extracts of the same four strains, ZBD-01, ZBD-02, ZBD-03, and ZBD-04 were able to inhibit the growth of same tested bacterial strains in minimum inhibitory concentration (MIC) assay. The highest antibacterial activity was recorded of *Parachlorella kessleri* against *B. subtilis, S. aureas* and lower activity was recorded for *K. pneumonie*. *Nephrochlamys subsolitaria* minimum inhibitory concentration against *B. subtilis*, *E. coli*, and *P. aeruginosa* was observed in second dilution tube, respectively. Whereas, the lowest activity was showed by *Ankistrodesmus falcatus* against *E. Coli* and *K. pneumoniae* with MIC observed in first dilution tube. The MIC value of *Monoraphidium griffithi* was also observed against *E. coli* and *k. pneumoniae* in first dilution tube. Table 10 illustrates the minimum inhibitory concentration of methanol extract of four microalgae strains observed in dilution tubes.

A range of microalgal species are known for its antibacterial potential, owing to

to their metabolic potential to produce a large variety of bioactive molecules such as proteins, polysaccharides, polyunsaturated fatty acids (PUFAs), vitamins, sterols and antioxidants (polyphenols, alkaloids, phycobilins, flavonoids, terpens, tannins, saponins and carotenoids). Until now, considerable number of studies on bioactive compounds from freshwater and marine microalgae exhibiting antimicrobial activity have been published in past three decades. Despite these findings, the data about specific antibiotic properties of these bioactive constituents and their mechanism of inhibitory action are still scarce.



(A) against *B. subtilis*; (B) against *S. aureaus*; (C&D) against *E. Coli*; (E) against *K. pneumonia*; (F) against *P. aeruginosa*; (G-1) 10 µL of extract against *K. pneumonia*; (G-2) 30 µL of extract against *K. pneumonia*.

Figure 14 - The antibacterial activity of methanol extracts of microalgae strains (agar disc-diffusion method):

Few studies demonstrated that microalgae respond to the presence of bacteria by production and secretion of membrane- derived Free Fatty Acids (FFAs) as defense strategy [249]. The bacteriostatic (growth-inhibiting) or bactericidal (killing) potency of poly-and mono-unstaurated fatty acids, predominantly palmitic acid, oleic acid, linoleic acid, myristic acid, arachidonic acid, docosahexaenoic acid (DHA) and eicosapentaenoic acids (EPA) of microalgae, frequently produced under environemtal stress conditions, has been investigated extensively against a wide range of bacteria [250]. To the best of our knowledge, there have not been many reports regarding the possible antibacterial potential of microlagae strains of the present study. Thus, the aim of the present study was to investigate the ability of crude methanol extract of these microalgae strains isolated from Almaty region to inhibit the growth of various pathogenic bacteria.

The strongest antibacterial activity with maximum zone of inhibition was observed with methanolic extract of *Parachlorella kessleri*. Few studies have been conducted on the fatty acid composition and bioactive compounds of *P. kessleri* and reported that it consists predominantly of C13:0, C14:0, C16:0, C16:1, C18:0, C18:1 [251]. Considering that these unsaturated fatty acids have been found to have antimicrobial activity in previous literature, the activity of the P. kessleri can be supposed to be due to these compounds. This study suggests *N. subsolitaria* as a rich source of chemically diverse bioactive compounds having broad spectrum antibacterial potential. The genus Nephrochlamys is generally enriched with monounsaturated and saturated fatty acids and α-linolenic, oleic, palmitic, and hexadecatetraenic have been identified as characteristic fatty acids [252]. The possible prescence of these fatty acids in our strain *Nephrochlamys subsolitaria*, can be made accountable for the remarkable antimicrobial properties demonstrated against *B. subtilis, E. coli* and *P. aeruginosa*.

Ankistrodesmus falcatus and *Monoraphidium griffithii* are reported to contain saturated and unsaturated FFAs and PUFAs with omega groups, notably myristic acid (C14:0), palmitic acid (C16.0), oleic acid (C18:1), linoleic (C18:2), and linolenic acid (C18:3) [253-254], and most of these well-known metabolites for enormous potential of inhibiting several bacterias have been profiled in these strains in this study. The antibacterial and MIC assay of the present study suggests that *A. falcatus* and *M. griffithii* are of great importance as potential antibacterial source for production of bioactive compound specifically effective against gram negative bacteria (*E. coli* and *K. pneumonia*). The difference in sensitivity of gram positive and gram negative bacteria to antibacterial effect of bioactive compound or secondary metabolite is generally attributed to differences in cell wall structure of Gram-positive and Gram-negative bacteria [255]. Few studies allow to state that flavonoids show more potent antibacterial activity against gram negative bacteria as comparaed to gram positive bacteria [256]. One study also demonstrated the specific inhibitory effect of lectin extracts from the red alga *Solieria filiformis* against several Gram-negative bacteria including *K. pneumoniae* with no inhibition against Gram-positive *Staphylococcus aureus* and *Bacillus subtilis*, possibly because of binding of lectin from the extract with mannan (linera polymer of mannose sugar) on the cell surface of Gram-negative

bacteria, stimulating an immune response [257]. In this context, extensive screening studies are needed in order to identify the responsible bioactive compound in these two strains and to elucidate the mechanism of its antibacterial action.

The most susceptible microbes to specimen methanol extract of ZBD-06 were Gram-positive bacteria than Gram-negative bacteria. However, surprisingly the methanol extracts of *Monoraphidium sp*. ZBD-06 exhibited the notable antibacterial activity against *Klebsiella pneumoniae* ATCC 13883. The strain showed a positive response in an inhibition zone assay (0,4±2 mm with 10ul and 0,6±2 mm in diameter with 30uL) against Gram-negative bacteria *Klebsiella pneumoniae* ATCC 13883, Fig.14. (G 1-2). In order to confirm the antimicrobial action of strain, the Minimum Inhibitory Concentration (MIC) of the methanol extract was performed against the same bacterial isolate. The MIC value of the extract of ZBD-06 was observed at the first well (2-1).

The FAMEs results showed that these isolates contain several metabolites in lower concentration such as Phenols, alkaloids, terpenes, and flavonoids which could be potentially responsible for antibacterial activity of the extract against Gram negative bacteria. One previous study on assessment of antimicrobial potential of marine green microalgae *Desmococcus olivaceus (D. olivaceu*s) and *Chlorella vulgaris (C. vulgaris*), revealed that the solvent choice influences the physical and chemical properties of the extract, such as high phenol content was only found in methanol extract, and amount of falvonoids, terpenes, carbohydrates and alkaloids differed when extracted with different solvents such as ethanol, methanol and chloroform and diethyl ether extract [258].

The multilayered and complex cell envelope of the Gram-negative bacteria is one of the major obstacles, which restrict the entrance of antibacterial agents [259]. However, certain biomolecules can damage the bacterial membrane component, such as terpenoid and phenolic compounds [260]. Antibacterial action of *Monoraphidium sp*. ZBD-06 against *Klebsiella pneumoniae* is possibly related with presence of high content of key molecule, which was specific in action for growth inhibition of the bacterium. Taking into account the isolation of the strain from the freezing lake and continued growth at 24°C retaining optimal functionality of their macromolecules suggests that adaptation to extreme environment (low temperature) in this strain confers a particular ability to produce unique antifreeze proteins, intracellular molecule or membrane structure to protect the cell from freezing damage, and bioactive compounds of potential antibacterial activity [261]. Another possible explanation of the antimicrobial activity of the studied microalgal extract can be attributed to its potential contents of fatty acids, as the psychrophilic microalgae maintain their membrane fluidity at low temperature by incorporating a higher level of polyunsaturated fatty acids (PUFAs) in membrane lipids [262], thereby making them a potential source of polyunsaturated fatty acids for antibacterial product.

The obtained result invokes the necessity of further exploring this great potential and specific action of the *Monoraphidium sp.* strain ZBD-06 by screening biotechnologically appealing bioactive molecules, investigating its source in the cell (chloroplast, cytoplasm, mitochondria, etc.) and deciphering the main biosynthetic

pathways of the bioactive molecules. Because antimicrobial activity against *Klebsiella pneumoniae* have, to our knowledge, not previously been reported for Monoraphidium genus, the identification of novel bioactive metabolites may be possible in this strain, which probably either independently or synergistically act together against the bacterium.

Five strains of the investigated microalgae appeared as highly valuable species for possessing borad spectrum antibacterial activity while *Desmodesmus panonicus* ZBD-01 did not exhibit any antibacterial activity. The nature of solvent used for extraction and cultural conditions (temperature, pH, light, and medium constituants) influence the antibacterial activity of microalgae. Recent years have witnessed an increased interest in exploring various properties of microalgae for antibiotic production. However, in view of the limited amount of knowledge on the antibacterial and biotechnological potential of the strains used in this study, further studies need to be performed for screening bioactive compounds, evaluating their efficacy and mechanism of action against pahogenic bacterias.

3.5 Effect of heavy metals on the growth, biophysical parameters and ultrastructure of microalgae cells

Microalgae can be used as test organisms for studying the state of aquatic ecosystems, including those contaminated with various heavy metals. They are a convenient material widely used in biomonitoring because they are more sensitive to contamination than multicellular organisms. The high specific surface area of microalgae cells contributes to the rapid intracellular accumulation of toxic substances and, accordingly, gives a quick response of the living system to pollution. Commonly used test functions of microalgae are based on integral characteristics such as mortality rates, increments and changes in ultrastructure and photosynthesis. The advantage of photosynthesis as a test function is due to its sensitivity to numerous pollutants. New methods for assessing the photosynthetic activity of higher plants and algal cultures include measuring the fluorescence induction curves of chlorophyll with a high temporal resolution (starting from 10 μs) upon excitation by powerful flashes. The purpose of this section was to study changes in the growth, photosynthesis and ultrastructure of microalgae under the influence of different concentrations of metals in the medium and to select the most sensitive parameters of chlorophyll fluorescence for their prospective use in biotesting.

3.5.1 Selection of a microalgae strain characterized by high sensitivity to heavy metals

Viability of of most suitable strain among three isolates *Monoraphidium griffithii* ZBD-01, *Parachlorella kessleri* ZBD-04, and *Ankistrodesmus sp.* B-11, in waters polluted with Cadmium, and Chromium ions were assessed by studying the influence of various concentrations of these metal on growth (cell number/cell density) of microalgae in model experiments. The strain demonstrating sensitivity to these metals at small lethal concentration was further investigated based on parameters

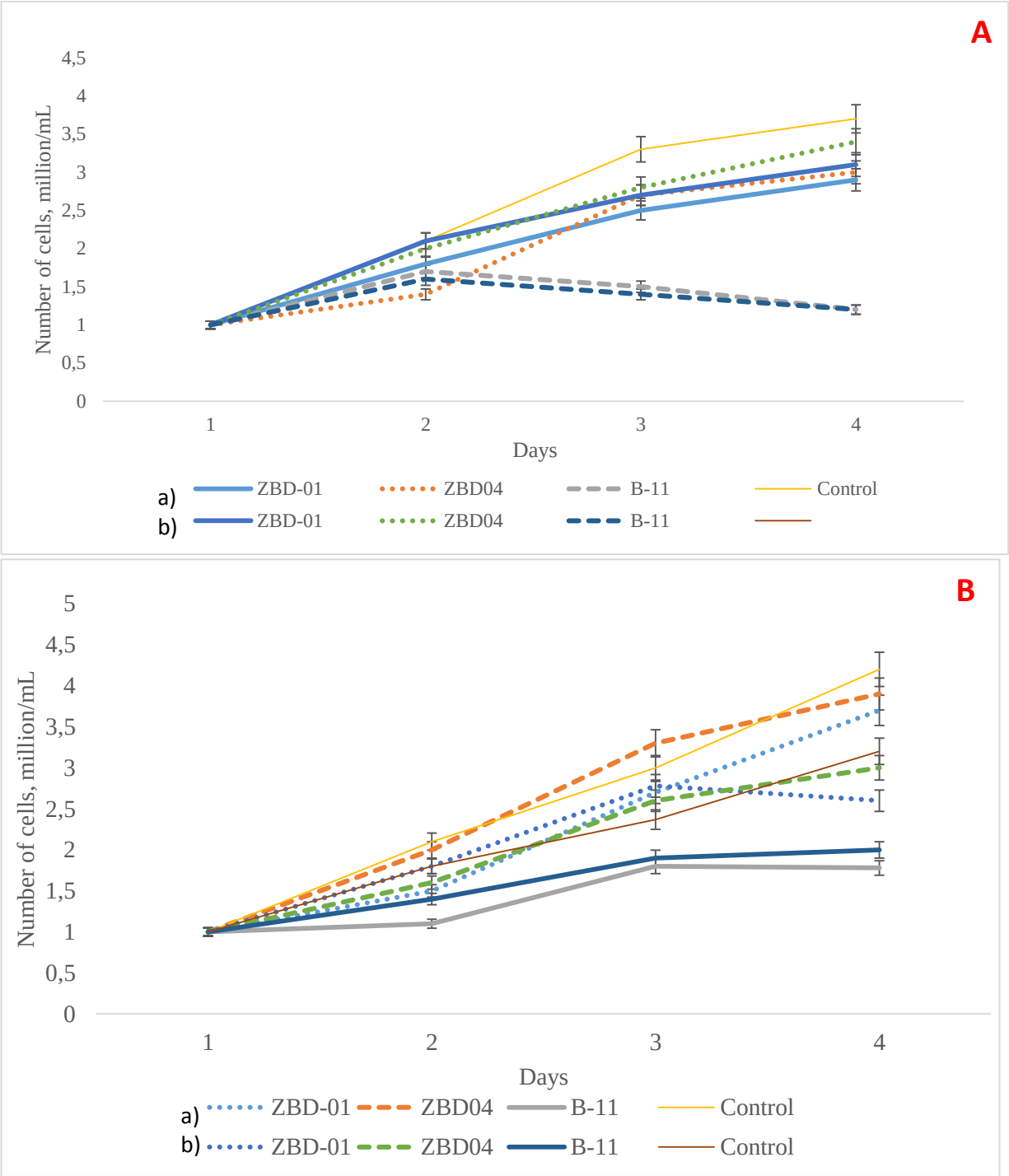
characterizing strain growth dynamics, cell ultrastructure, and changes in photosynthetic parameters of cell cultures).

Overall, the prescence of Cd, and Cr limited the growth rate of all studied isolates, however no striking promoting or declining effect was noted in growth rate of *Monoraphidium griffithii* ZBD-01 and *Parachlorella kessleri* ZBD-04, for initial 12-14 days. Later, the observed metal tolerance in these strains reduced gradually. However, Ankistrodesmus sp. B-11 was found overly sensitive to small concentration of Cd in initial days of cultural growth. This was further supported by examining the changes in photosynthetic activity and cell ultrastructure of *Ankistrodemus sp.* B-11. Therefore, Ankistrodemsu sp. B-11 was selected for further extensive studies to analyze the influence of heavy metal Cadmium on this isolate (figure 15).

3.5.2 Effect of Cadmium on the Dynamics of Microalgae Growth

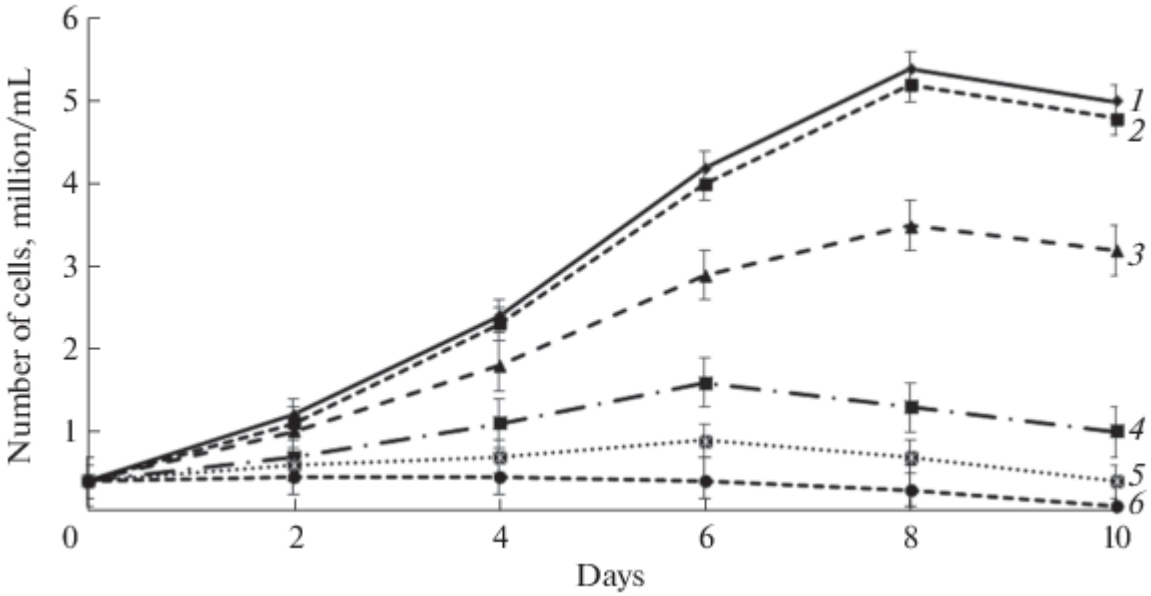
Figure 16 shows growth curves of *Ankistrodesmus* sp. B-11 in the presence of various concentrations of cadmium in the medium. The number of cells at the beginning of the experiment was 400 000 cells/mL. The examined culture of *Ankistrodesmus* sp. B-11 was able to grow in media containing 0.002–0.005 mg/L cadmium. The lowest concentration of 0.002 mg/L for this metal was close to the maximal permissible concentration (MPC) for water bodies. At this given concentration, the total number of algal cells was kept at the control level throughout the experiment. At a cadmium concentration in the medium of 0.005 mg/L, the number of cells was 36% lower than in untreated culture; nevertheless, the cadmium-treated culture exhibited sustained growth throughout the entire experiment, i.e., over 8 days of cultivation, similarly to the untreated (control) culture. Later on, the growth rates decreased, like in the control experiments.

An increase in cadmium concentration up to 0.02 mg/L caused a sharp decrease in the relative number of cells compared to the control values, already after the first day of cultivation. This finding indicates a high sensitivity of the *Ankistrodesmus* sp. B-11 to the given concentration of cadmium. On the sixth day of cultivation at this cadmium level, the total number of cells (maximum record) was only 38% of the control value.



(A), Chromium (B) on growth dynamics of microalgae *Monoraphidium griffithi* (ZBD-01), *Parachlorella kessleri* (ZBD-05), and *Ankistrodesmus sp*. B-11: (a) 0.002 mg/L; (b) 0.005 mg/L

Figure 15 (A-B) - Influence of various concentrations of Cadmium on studied three microalgae strains



(1) control; (2) 0.002 mg/L Cd2+; (3) 0.005 mg/L Cd2+; (4) 0.02 mg/L Cd2+; (5) 0.05 mg/L Cd2+; (6) 0.2 mg/L Cd2+

Figure 16 - Influence of various concentrations of cadmium on growth dynamics of microalgae Ankistrodesmus sp. B-11:

At cadmium concentrations of ≥0.05 mg/L, the number of cells increased even slower or did not increase at all (Figure 16). It should be noted that, unlike the continuous increase in cell number over 8 days in the control culture, the number of cells in the culture grown at the initial cadmium concentration of 0.2 mg/L remained almost unchanged for the first 3 days, after which the bleaching of microalgal cell culture began. The gradual retardation of cell culture growth after 8 days of cultivation in the control experiments and in the treatments with cadmium concentrations of 0.002–0.005 mg/L was probably caused by the nutrient depletion and by the accumulation of metabolites inhibiting growth of microalgae [263].

3.5.3 Effect of Cadmium on Light Reactions of Photosynthesis in Microalgae

The maximum quantum yield of primary photochemical reactions FV/FM (φPo) in control *Ankistrodesmus sp*. B-11 cells was 0.6 (Table 11). In the presence of cadmium salt at concentrations of 0.05 and 0.2 mg/L, this value decreased already on the day following incubation. It should be noted that the absorption spectra of algal suspensions remained almost unchanged during short-term (24 h) incubation of algae with cadmium (data not shown), which indicates that cadmium ions had no influence on the pigment apparatus in this period.

Changes in photosynthetic activity of Ankistrodesmus sp. B-11 were characterized in detail by simultaneous measurements of the induction parameters of prompt and delayed fluorescence as well as the redox state of PSI electron carriers (mainly P700). Figure 17a shows the kinetic curves of fluorescence induction (OJIP) normalized to the FO level. In the control culture, the kinetics of fluorescence induction in response to high-intensity light comprised several stages known as O-J-I-P transients. The initial O-level corresponds to the intensity of chlorophyll fluorescence

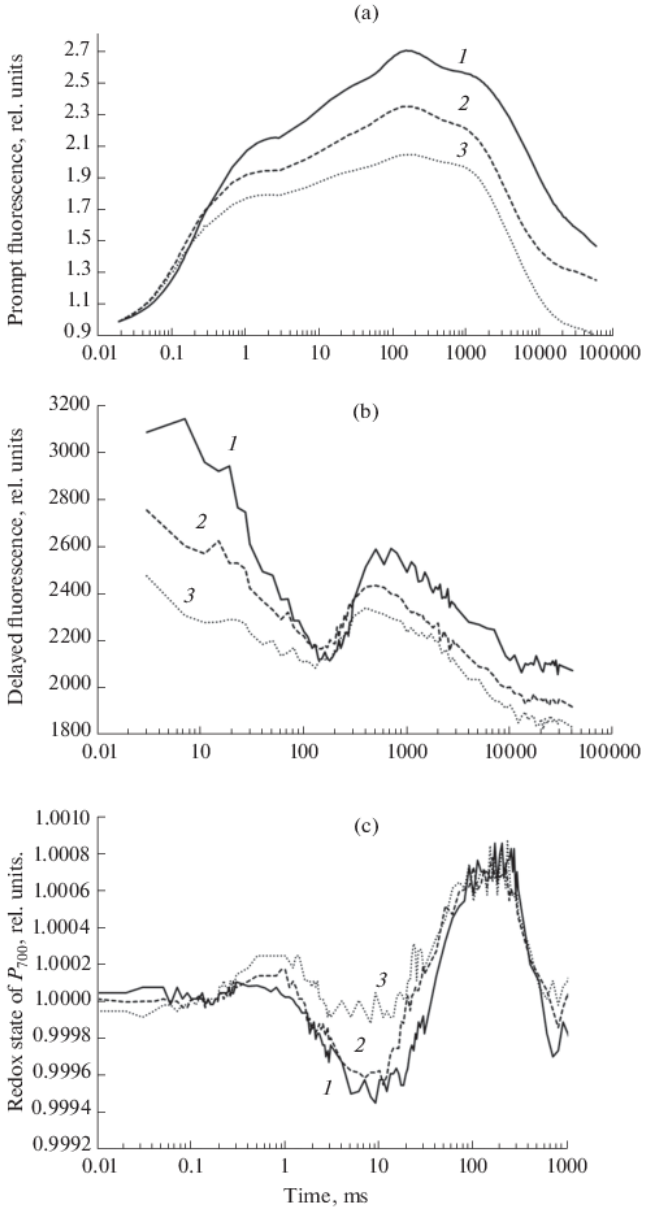
under “open” reaction centers of PSII (FO), when all QA acceptors are oxidized. The O-J phase reflects the light-induced reduction of QA, whereas the subsequent phases reflect mainly further accumulation of reduced QA due to the decelerated reoxidation of QA along with the reduction of QB acceptors and the plastoquinone pool (PQ).

Table 11 - Mean values of OJIP test parameters determined from the kinetics of fluorescence induction in the microalga Ank-istrodesmus sp. B-11 after 24-h incubation at various concentrations of cadmium

Parameters	**Control**	**0.05 mg/L**	**0.2 mg/L**
F_V/F_M	0.6 ± 0.02	0.52 ± 0.03*	0.46 ± 0.02*
F_V/F_O	1.73 ± 0.05	1.44 ± 0.06*	1.24 ± 0.04*
V_J	0.65 ± 0.03	0.67 ± 0.02	0.73 ± 0.03*
V_I	0.86 ± 0.03	0.86 ± 0.04	0.89 ± 0.04
φ_{Eo}	0.21 ± 0.01	0.17 ± 0.02*	0.12 ± 0.02*
PI_{ABS}	0.24 ± 0.02	0.12 ± 0.02 *	0.06 ± 0.03 *
ABS/RC	3.2 ± 0.4	4.48 ± 0.03 *	5.1 ± 0.2 *
DI_0/RC	1.29 ± 0.04	2.14 ± 0.05 *	2.75 ± 0.05*
q_E	1.63 ± 0.04	1.86 ± 0.03 *	1.89 ± 0.04*

* Asterisks mark significant differences between the treatment and the control culture at $P < 0.05$.

Characteristics of primary photosynthetic processes were quantified by means of the JIP test based on the parameters of the O-J-I-P kinetic curve of chlorophyll fluorescence (Table 11). In the presence of cadmium salt in the medium, the shape of the O-J-I-P curve was altered and the extent of the J-I-P photochemical phase was decreased, which indicated the disturbance of electron flux from PSII to the quinone pool [264]. The quantum yield of PSII-driven electron transport (φEo) in algal cells was diminished in the presence of cadmium ions. Hence, one of the main targets of cadmium impact was the acceptor side of PSII. An increase in the OJ phase corresponding to elevation in parameter Vj indicated the increased fraction of QB-nonreducing reaction centers in PSII. The JI phase (3–30 ms) corresponds to the reduction of the PQ pool, and the parameter VI is a suitable indicator of the PQ pool redox state in the dark [265]. The presence of cadmium ions had no influence on this parameter, indicating that the extent of oxidized plastoquinone molecules at the QB site in algae was unaffected by this heavy metal.



(a), delayed fluorescence (b), changes in reflection at a wavelength of 820 nm (c) in Ankistrodesmus sp. B-11 microalgae under the action of various concentrations of cadmium: (1) untreated culture, (2, 3) after 24-h incubation of cells in the presence of cadmium at concentrations of 0.05 and 0.2 mg/L, respectively. Prompt fluorescence was normalized to the FO level. The intensity of actinic red light was 1500 μE/(m2 s). Simultaneous measurements of all three signals were performed with an M-PEA-2 analyzer.

Figure 17 - Induction curves of prompt fluorescence

The parameter ABS/RC reflecting the size of light-harvesting antenna per reaction center in algal cells was higher in the presence of cadmium than in untreated samples; these changes resulted from the decreased proportion of active reaction centers due to disorders in protein D1 biosynthesis [266].

The performance index PIABS is a generalized indicator of PSII functional activity [267]. It was higher in the control cells than in the cadmium-treated cells. Low PIABS values for cadmium-treated algae indicated a low functional activity of PSII, mainly due to the decreased content of active reaction centers and the enhanced

quenching of excitations in the antenna. The decline in efficiency of excitation energy transfer from the light-harvesting complex to the reaction center should be accompanied by the increased dissipation of unutilized light energy. Indeed, the dissipated energy per reaction center (DI0/RC) in cadmium-treated cells was at a high level. These changes correlated with an increase in ΔpH-dependent non-photochemical quenching qE, as calculated from the fluorescence decline after the peak (qE = (FM – F6s)/FV). The increase in non-photochemical quenching was also noted by the PAM fluorometry method [268].

The millisecond component of delayed fluorescence arises as a result of secondary recombination of charges in PSII reaction centers; its intensity depends on the magnitude of the proton electrochemical gradient across the thylakoid membrane that lowers the activation energy for the reverse reaction (recombination) [269]. Therefore, the delayed fluorescence parameters provide the means to monitor the proton gradient changes across the thylakoid membrane in whole cells. The experimentally observed peak of the delayed fluorescence in the millisecond time range coincided with the J-I rise on the induction curve of prompt fluorescence (Fig. 17b). This peak originates from the accumulation of radiative redox states responsible for the backward recombination of charges and the emission of delayed fluorescence (emitting states); it also reflects the enhancement of delayed fluorescence due to the electric potential Δψ formed at the thylakoid membrane. The peak of the delayed fluorescence in the time range of seconds is supposedly related to photo-induced formation of the transmembrane proton gradient ΔpH that also reduces the activation energy for radiative transitions (emission of delayed fluorescence) in PSII reaction centers [270]. In cells exposed to cadmium ions, the intensity of delayed fluorescence on the induction curve was significantly reduced at 10–50 ms and 1 s (Fig. 17), which was probably determined by the suppression of noncyclic electron transport and the consequent deenergization of thylakoid membranes.

Measurements of modulated reflection at 820 nm provide the means to monitor redox transitions of plastocyanin and, particularly, P700 in PSI reaction centers. Switching on the light caused the initial oxidation of P700 followed by its subsequent reduction (Fig. 17 c). The fluorescence signal reflecting QA reduction and the reduction of P700 attained the plateau levels almost synchronously. The parallel accumulation of reduced P700 and QA reflected the reduction of electron carriers in the entire intersystem segment of the electron-transport chain; this reduction resulted from the absence of electron efflux on the acceptor side of PSI under conditions when ferredoxin–NADP reductase (FNR) was inactivated by dark incubation. Under prolonged illumination (~1–10 s), a second wave of P700 oxidation was observed, which can be explained by the outflow of electrons from PSI upon the activation of FNR and Calvin cycle enzymes [271].

The algal cells treated with low cadmium concentrations retained the ability of P700 photo-oxidation (Fig. 17c). However, the rate of P700 reduction by electrons arriving from PSII was decelerated in these cells. This is consistent with the analysis of the induction curves of prompt fluorescence. High concentrations of cadmium were found to suppress P700 oxidation reactions in PSI.

3.5.4 Influence of Cadmium on the Ultrastructure of Microalgae

The ultrastructure of microalgal cells was studied using transmission electron microscopy after 4-day cultivation, when the cells were in the phase of active growth. The concentration of cadmium ions in the medium was 0.05 mg/L. *Ankistrodesmus sp*. B-11 cells grown under optimal conditions were collected at the phase of rapid growth and used as control samples.

Inspection of ultrathin sections of untreated *Ankistrodesmus sp*. B-11 cells revealed the organelles, structures, and inclusions characteristic of this species (Fig. 18 a). The cell wall was smooth, thin, and colorless; its structure was typical of green microalgae and comprised the outer cellulose–pectin layer adjacent to the plasmalemma. The cytoplasm was perforated by the unevenly distributed system of tubules. On cell micrographs, the nucleus region and a large electron-dense nucleolus were distinctly visible. The intracellular inclusions comprised starch grains. Each cell had one chloroplast adjoining the cell wall. The chloroplast occupied almost the entire cell periphery and had no pyrenoid. The thylakoids occupied the entire stroma and were aligned typically of Ankistrodesmus: from the periphery to the cell center. In the plane of the cross section, the thylakoid contours lay parallel to each other and were curved. The thylakoid membranes were tightly pressed against each other.

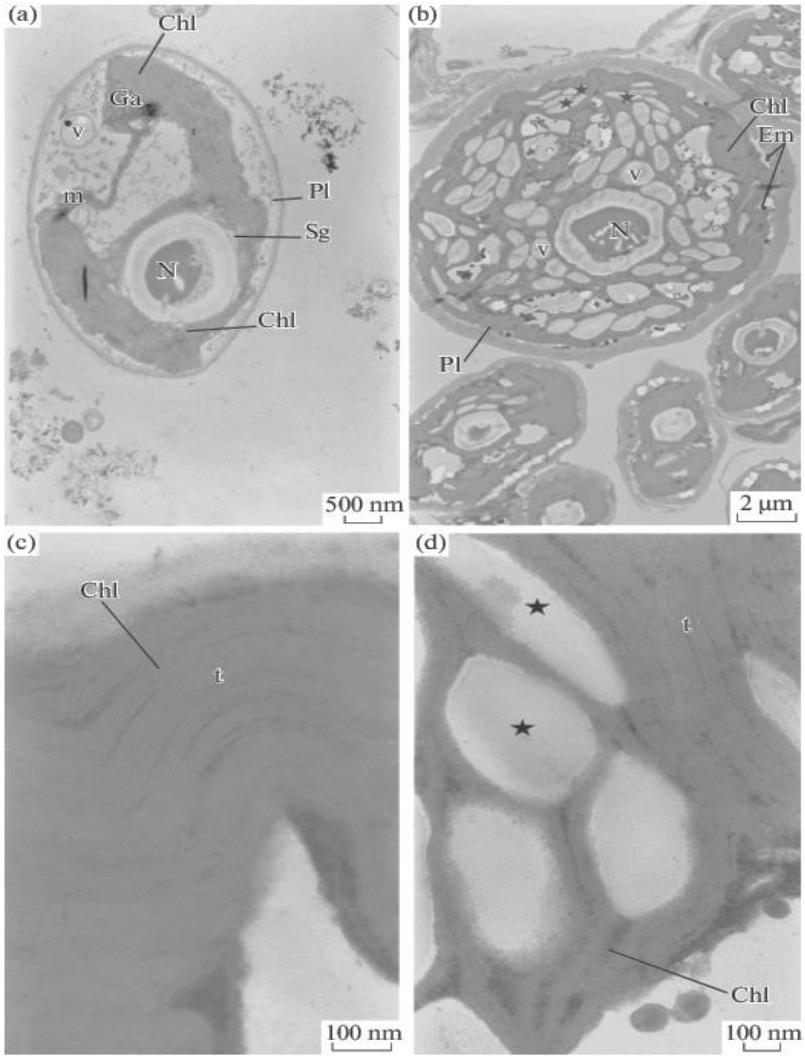
Electron microscopic examination of *Ankistrodesmus* sp. B-11 cells exposed for 96 h to 0.05 mg/L cadmium revealed ultrastructural changes in the photosynthetic apparatus (Fig 18 b). Specifically, the arrangement of thylakoids within the stroma was altered. We noted the increased distances between thylakoids, the disintegration of thylakoids, and swelling of the intrathylakoid spaces (Figs. 18 c, 18 d). In addition, electron-dense inclusions of an unidentified nature noted in some cells.

The increased activity of the Golgi apparatus in cadmium-treated cells should be noted: the number of excretory vacuoles was substantially higher in almost all the treated cells than in the control algae. In many cells, we noticed swelling of chloroplasts, the loss of tight connections between the membranes, vacuolization, and the enlargement of the interthylakoid spaces (Fig. 18 b). Furthermore, slight detachments of the cell wall from the protoplast were observed as well as the deformations of the original cell shape. These changes were apparently caused by the altered permeability of the cytoplasmic membrane (Fig. 18 b).

According to the published data, microalgae are very sensitive to the presence of metals in the medium [272]. The responses of algae to additions of lethal and sublethal concentrations of heavy metals may differ significantly. Different sensitivities of microalgae from various taxonomic groups are attributed to diverse mechanisms of heavy metal toxicity and to variations in metabolic features and morphology of algal cells. Our study revealed that the presence of cadmium in the nutrient medium at concentrations of $\geq$0.005 mg/L significantly decreased the number of *Ankistrodesmus* sp. B-11 cells with respect to the control values.

Current concepts of electron flow along the photosynthetic electron transport chain imply the sequential participation of two photosystems (PSII and PSI), at which the electron carriers reduced by PSII serve as electron donors for PSI. The

interconnection of PSII and PSI is manifested in chlorophyll fluorescence whose level depends on the redox state of quinone acceptor Q_A. The photoreaction of PSII reduces Q_A, thus increasing the fluorescence level, whereas the PSI activity oxidizes Q_A and leads to the fluorescence decrease]. The kinetics of light induction of variable fluorescence in the millisecond time range reflects changes in electron transport within the PSII complex and between PSII and PSI [273].



(a) ultrastructure of control cells, (b) changes in cell ultrastructure in the presence of 0.5 mg/L cadmium salt, (c) chloroplast of the control cell, (d) swollen chloroplasts with enlarged interthylakoid spaces. Designations: v—vacuole; Chl—chloroplast; t—thylakoids; Pl—plasmalemma; N—nucleolus; m—mitochondrion; Ga—Golgi apparatus; Sg—starch grain; Em—electron-dense material. Asterisks indicate the area with an expanded interthylakoid space. Scale: (a) ×10 000, (b) ×4000, (c) ×75 000, (d) ×50 000.

Figure 18 - Ultrastructure of Ankistrodesmus sp. B-11 cells

Evaluation of the PSII functional activity using conventional indicators, such as F_V/F_M (maximum quantum yield of PSII reaction) and PI_{ABS} (performance index),

proved that cadmium inactivates PSII, which is consistent with the toxic effects of cadmium on PSII observed earlier with other photosynthetic materials.

Simultaneous recordings of the induction curves for prompt and delayed fluorescence, as well as kinetics of P700 redox state using an M-PEA-2 device, revealed the primary targets of cadmium impact on photosynthetic reactions in the alga *Ankistrodesmus* sp. B-11. Analysis of the induction curves showed that one of the primary targets of cadmium action is located on the acceptor side of PSII, between Q_A and Q_B. The decrease in the quantum yield of electron transport in PSII led to a drop in the performance index (PI_{ABS}). This parameter, compared to F_V/F_M, was found to be more sensitive to cadmium toxicity and can be recommended in biotesting the water quality of natural and artificial reservoirs. The decrease in the number of active reaction centers and changes in electron transport promoted the enhanced nonphotochemical losses, which was evident from the elevation in quantum efficiency of energy dissipation (DI_0/RC) and in ΔpH-dependent nonphotochemical quenching (q_E). Experiments with higher plants showed that cadmium salts can affect the oxygen-evolving complex of PSII [274]. In our work, the F_V/F_O parameter related to changes at the donor side of PSII was found to decrease at cadmium concentration of 0.05 mg/L; these observations corroborate the possibility that cadmium compounds also affect the donor side of PSII. The decrease in the F_V/F_O parameter under the action of cadmium was previously noted on the green microalga *Scenedesmus obliquus* [275].

Unlike the reactions of PSII, photo-oxidation of P_{700} in the alga *Ankistrodesmus* sp. B-11 was less sensitive to cadmium. The inhibition of photosynthetic electron transport by heavy metals may possibly result from changes in ultrastructure of chloroplasts, the damage to thylakoids in particular.

The metals are known to have a strong influence on the ultrastructure of microalgae. The action of metals may lead to the formation of atypical cell shapes, nuclear inclusions, and changes in the composition of storage products. In addition, heavy metals cause disorders of chloroplasts' ultrastructure, which might be an important factor for the decrease in pigment content and the net rate of photosynthesis [276].

Our results suggest that cadmium induces ultrastructural changes that primarily occur in the photosynthetic apparatus. Specifically, the arrangement of thylakoids within the stroma was impaired, the interthylakoid distances enlarged, the thylakoids underwent disintegration, and the interthylakoid spaces were expanded. These complex alterations inhibited the photosynthetic activity. In addition, the extent of cellular vacuolization increased significantly due to structural changes of the cytoplasmic membrane. The cadmium-induced changes in cell structure observed in our study are consistent with the results of other authors [277]. For example, the cultivation of cyanobacteria in the presence of heavy metal salts was accompanied by changes in the ultrastructure of cyanobacterial cells. Some authors reported that heavy metal ions promoted cell wall thickening, plasmolysis (detachment of the cell wall from the protoplast), and destruction of thylakoid membranes. The nucleoplasm became enriched with polyphosphate granules that apparently play a key role in binding the metal ions and their detoxification. It was noted that the cytoplasm lost its

characteristic granularity, and extensive light areas appeared on cell cross sections [278].

Similar changes in cell ultrastructure in the cyanobacterium *A. flos-aquae* were observed in the presence of 0.05 mM copper: the authors noted a significant expansion of interthylakoid spaces and the accumulation of polyphosphate granules [279]. The treatment of *Synechocystis* sp. PCC 6803 with cadmium and mercury impaired the cell ultrastructure, which was evident from the disturbed integrity of chloroplasts, disordering of thylakoids, and the increased number and size of polyphosphate granules [280]. In particular, the exposure to cadmium affected the structure of the chloroplast envelope and thylakoid membranes. The curving and stretching of these membranes might result from the altered proportions of saturated and unsaturated fatty acids in membrane phospholipids as well as from hydrolysis of lipids and the release of fatty acids [281]. In addition, the number of grana was reduced and their shape was distorted in the presence of heavy metals. The grana lost their regular shape and became composed of fewer thylakoids [282]. The treatment of green microalgae *Chlamydomonas reinhardtii* and *Dunaliella salina* with selenium and catamine was accompanied by intense formation of destructive vacuoles: the degradation of these algal cells was caused by the release of the vacuolar contents into the cytoplasm and nucleus [283]. Nishikawa et al. [284] observed similar ultrastructural changes in *Chlamydomonas acidoph-ila* cells exposed to cadmium, copper, and mercury. The authors noted that the largest ultrastructural changes in microalgal cells were caused by cadmium ions. They stated the abundance of excretory vacuoles, enlargement of starch granules, and the decreased dimensions of pyrenoids and mitochondria [285].

Similar changes in the murein layer were demonstrated in various strains incubated with a variety of heavy metals, e.g., upon the exposure of *A. flos-aquae* to Cd^{2+}, after treating *A. variabilis* and *Synechococcus* with Zn^{2+}, and after adding Ca^{2+} and Sr^{2+} to *Bacillus stearothermophilus*. The cell wall is known to represent the first barrier to heavy metals [286]. At the primary stage, the metals are adsorbed on the cell surface and then enter the cell by means of passive or active transport. When the metals reach the cell membrane and bind to various ligands and functional groups occurring at the plasma membrane, such as –COOH, –SH, $–NH_2$, and $–P_3O_4$, they perturb the plasma-membrane permeability, thus causing the release of intracellular Na^+ and K^+. Such a leakage of Na^+ and K^+ ions as an indicator of membrane permeability changes was observed in *Nostoc muscorum* cells after their exposure to Ni^{2+}, Cr^{2+}, Pb^{2+}, and Ag^{2+} [287].

Generally, our experimental data demonstrate the effect of 0.05 mg/L cadmium on growth rates and the ultrastructure of *Ankistrodesmus* sp. B-11. The observed disorders in the structure of chloroplasts inactivate the oxygen-evolving centers of PSII and, consequently, decelerate electron transport. These changes suppress the photosynthetic activity of algae, which can be readily diagnosed by measuring the induction curves of chlorophyll fluorescence. The results of this work can be used for assessing the ecological status of aquatic ecosystems contaminated with heavy metal ions.

CONCLUSION

1. The diversity of microalgae in Lake Issyk, Lake Balkhsash, Lake Kolsai, Lake Alakol and Lake Bolshoye Almatinskoe, located in the Almaty region, was preliminarily studied using light microscopy and various microalgae descriptors and identified at the genus level. The result showed that representatives of the divisions Bacillariophytes, Cyanophytes, and Chlorophytes dominated almost all freshwater bodies, and their maximum diversity was observed in lakes Balkhash and Alakol.

2. Seven new isolates of microalgae were isolated from the lakes of the Almaty region. The morphology visualized by light microscopy (LM) and scanning electron microscopy (SEM) has led to the identification of seven strains of green microalgae, *Monoraphidium griffithii, Nephrochlamys subsolitaria, Ankistrodesmus falcatus, Parachlorella kessleri, Desmodesmus pannonicus*. *Ankistrodesmus sp.* B-11 (morphologically), which were later confirmed by PCR-based sequence analysis. Sequence analysis of the internal transcribed ribosomal DNA spacer part (18S, ITS1-5.8S-ITS2, 5.8S) and the ribulose bisphosphate carboxylase (rbcL) gene made it possible to identify seven microalgae up to the species level, *Monoraphidlamium griffithii* ZBD-01 (accession number MW258948), *Nephrochlamys subsolitaria* ZBD-02 (accession number MZ079019), *Ankistrodesmus falcatus* ZBD-03 (accession number MZ079017), *Parachlorella kessleri ZBD-04 (accession no. MZ079022), Desmodesmus pannonicus ZBD-05 (*accession no. *MZ079014). Monoraphidium sp*. (accession number MT178772).

3. It was found that of the isolated strains *P. kessleri* has the highest biomass production (1.42 ± 0.08, g / l), lipid productivity (29 ± 1.2 g / l) and the content of fatty acids C16 - C18 (90%), followed by *A. falcatus* and *M. griffithi*.

4. Gas chromatography / mass spectrometric analysis showed that the dominant fatty acids in *P. kessleri, A. falcatus* and *M. griffithi*. were palmitic acid (C16: 0), stearic acid (C18: 0) and oleic acid (C18: 1), with saturated fatty acids being at a higher concentration than unsaturated fatty acids. acid. Our results indicate that *P. kessleri* and *A. falcatus* are promising strains for biodiesel production due to their high lipid productivity and relatively high oleic acid content.

5. The results of studying the antibacterial activity of the biomass of the isolated strains showed a significant antibacterial activity of the methanol extract of *Parachlorella kessleri* against *B. subtilis* (highly active), *S. aureus* and *K. pneumonia; Nephrochlamys subsolitaria* against *B. subtilis* (highly active), *P. aeruginosa* and *E. coli; Monorafidium sp.* against *K. pneumonia; Monoraphidium griffithii* and *Ankistrodesmus falcatus* against *K. pneumoniae* and *E. coli*.

6. The potential of the isolated microalgae in the biomonitoring of the aquatic environment was assessed by studying the effect of low concentrations of heavy metals Cd, Zn and Cu on cell survival in *Monoraphidium griffithii* ZBD-01, *Parachlorella kessleri* ZBD-04, and *Ankistrodesmus sp*. B-11. The high sensitivity to the data of TM *Ankistrodesmus sp*. B-11.

7. The influence of cadmium ions on the growth, photosynthesis and ultrastructure of cells of the microalga *Ankistrodesmus sp*. B-11. The addition of cadmium to the nutrient medium at a concentration of 0.005–0.02 mg / l led to a significant decrease in the abundance of *Ankistrodesmus sp*. B-11. The addition of cadmium at a concentration of> 0.05 mg / L completely stopped cell growth.

8. It was found that cadmium ions caused ultrastructural changes in the location of thylakoids inside the stroma, detachment of thylakoid membranes with the formation of void intertilakoid spaces and a significant increase in vacuolization of microalgae cells. Simultaneous measurements of fluorescence induction curves and redox transformations of the components of photosystem I on a microsecond time scale using an M - PEA-2 fluorometer showed that cadmium ions inhibit electron transfer in photosystem II (PSII). It was found that the quantum yield of electron transport in PSII (φEo) and the efficiency index (PIABS) decrease; photoreduction of P700 pigment was slowed down, while energy dissipation (DI0 / RC) and ΔpH-dependent non-photochemical quenching (qE) were significantly increased by cadmium.

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