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Study of microalgal diversity in two systems: open (Lake) and closed (Irrigation basin) in Ouargla- Algeria

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Devant le jury

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In front of the jury

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Dedication

I dedicate this modest work to:

*My beloved parents **Nacira** and **Salah** for their encouragement and sacrifices and for everything they have done for me, that's a debt I can never repay*

*My sister **Maria** and my brothers **Youcef** and **Aissa***

My whole family

*To the spirit of my uncles **Djamal** and **El Hadj**, and my aunt **El Zohra** may Allah bless them with his mercy, and may they rest in peace in their graves*

*All my friends without exception, **Oum Elkhir**, **Hania**, **Hind**, **Ahlem**, **Siham** ...*

All the people who helped me during my study

All, from the bottom of my heart I dedicate this work

Zahra

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Abreviation list

BBM	Bold's Basal Medium
BG-11	Blue Green -11
HBA APC	Hassi Ben Abdallah city hall
H-S-C # 10	Half Strength Chu #10
L	Liquid
pH	Hydrogen potential
S	Solid

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Introduction

Algae are a diverse group of simple photosynthetic organisms. Growing almost exclusively in aquatic environments ranging from freshwater, estuaries, ocean surface to coral reefs (AMANTE and EAKINS, 2009). Diversity of algae has been conservatively estimated at about 30,000 species, with a rough estimate of more than 1 million species (GUIRY, 2012). An alga is generally characterized on the basis of its size, color, shape, form or growth habit. Some algae are visible to the naked eye, whereas others can be seen with the help of a magnifying glass or a microscope. In most habitats they function as the primary producers in the food chain, producing organic material from sunlight, carbon dioxide, and water (LEE, 2008).

Microalgae constitute an extremely heterogeneous group gathered around a physiological coherence: oxygenic photosynthesis (ANDERSEN, 1992). They are a diverse group of microscopic plants with the wide range of physiological and biochemical characteristics (AVAGYAN, 2008). Patterns of species distribution and abundance are determined by a complex interaction of biotic and abiotic factors (SANTOS, 1993).

Microalgae have attracted great interest as an important biological resource with multiple applications. Algae and algae products are extensively applied in the pharmaceutical, cosmetic and food industries (SOUZA *et al.*, 2012).

Ouargla climate is of Saharan type, known by its aridity. This climate is characterized by strong insolation with low and irregular precipitations, also by high temperatures, which increases salinity rates in wetlands by evaporation.

Wetlands are the richest and the most productive ecosystems on the planet (BRANDER *et al.*, 2006). In Algeria, water bodies are very numerous especially in the south of the country. These environments are usually called Chotts and Sebkhas. They are often brackish, salty or hypersaline (GAUTHIER, 1928).

Humid biotopes in the large basin of Ouargla are composed of Chotts, lakes and Sebkhas. These areas are highly productive by the presence of food chains (BERREGUI, 2013) mainly microalgae.

Microalgal diversity is a very important subject to study, especially with the continuous discoveries of the benefits and the uses of microalgae and their products.

This subject is poorly studied with unpublished works in our region. From this interest, the question we are posing and trying to answer is whether all these favorable conditions of temperature, insolation, lakes, and salinity have an influence on the diversity of microalgal flora, also can these conditions make a difference in open and closed systems of freshwater bodies in our region?

Therefore, we have conducted this work as a contribution to study and to compare between microalgal diversity of an open system (Hassi Ben Abd Allah lake) and a closed system (Mehiriza irrigation basin) in arid and semi-arid zones of Ouargla.

Objectives of this work are:

- Census of microalgae that exist in Ouargla region's waters.
- Identification of microalgae from Ouargla region.
- Comparison between microalgae found in both systems.

This thesis is divided into three parts with four chapters. The first part is about, generalities about microalgae and classification of Microalgae, the second one contains study materials and methods that we have used during our work and the last part and the important one is about the results of our study followed by general conclusion.

Chapter I
Generalities about microalgae

I.1. Definition of microalgae

Algae are thallophytes; plants lacking roots, stems, and leaves (LEE, 2008). Microalgae are commonly defined as oxygen-producing photosynthetic microorganisms containing a plastid with chlorophyll *a*. They are mainly found as solitary cells, showing little or no cellular differentiation. Most species occur in aquatic habitats and can be isolated from fresh, brackish or saline waters, although some species can be found in the soil or rocks, in moist or even relatively dry environments (LEITE and HALLENBECK, 2012).

Microalgae form a wide and heterogeneous group with species spread among different phyla (LEITE and HALLENBECK, 2012). They are of prokaryotic (cyanobacteria) and eukaryotic nature. With respect to diversity they include 11 divisions and 28 classes (HOEK *et al.,* 1995). The numbers of approximately 30 000 species so far described are certain to increase substantially with new knowledge (NORTON *et al.*, 1996).

I.2. Morphology

The term "morphology" describes the shape, form or growth habit of an organism and its parts. Algae exhibit extremely diverse morphology, and can be categorized into several major groups (MAC, 2017).

I.2.1. Unicellular forms

The unicellular types are seen in all groups of algae with the exception of the class Phaeophyceae. Unicellular forms can be motile or non-motile, the motile forms are either rhizopodial or flagellates and non-motile forms are coccoid (SAHOO and SECKBACH, 2015).

Rhizopodial algae, lack flagella, the organs motion, but are able to perform amoeboid movement by means of cytoplasmic growth e.g, *Chrysamoeba* (Fig. 1.1) (ASTITVA, 2014).

Flagellates, the unicellular motile forms are the simplest type of thallus in algae. The flagellated unicelled structures are distinctive of certain classes e.g., *Euglenineae* (Fig. 1.2), *Cryptophyceae*, *Chrysophyceae* and *Dinophyceae*. Flagellated vegetative cells are absent in *Cyanophyceae*, *Phaeophyceae*, *Rhodophyceae* and *Bacillariophyceae*. The nature of flagellation, type and number of flagella and the attachment of flagella, is an important character in classification (SHARMA, 2015).

Fig. 1.1: *Chrysamoeba sp.* (http://penard.de/Stramenopiles/Chrysophyceae/)

Fig. 1.2: *Euglena gracilis* (https://classificationofthekingdoms.weebly.com/protista-examples.html)

Fig. 1: *Chrysamoeba sp.* (Left), *Euglena gracilis* (Right)

Coccoid forms, are simple spherical or elongated cells e.g., *Microcystis*, *Cylindrocystis*, *Pinnularia* (Fig. 2.1) (Bacillariophyceae); triangular as in *Tetragonidium* (Cryptophyceae) and *Trigonium* (Fig. 2.2) (Bacillariophyceae). The epiphytic or attached forms have a basal disc (SHARMA, 2015).

Fig. 2.1: *Pinnularia* sp. (http://protist.i.hosei.ac.jp/PDB/Images/Heterokontophyta/Raphidineae/Pinnularia/sp_06b3.html)

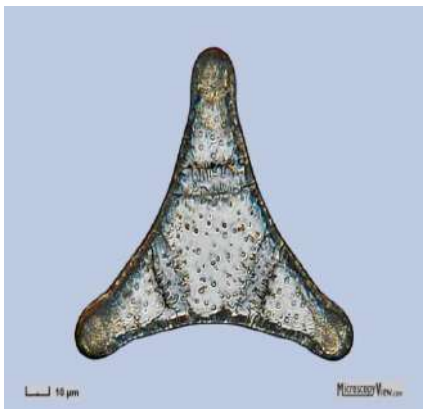


Fig. 2.2: *Trigonium glandiferum* (http://www.microscopyview.com/MENU/400-DIATOM/403-TRI/H403-6305.html)

Fig. 2: Coccoid microalgae. On the left: *Pinnularia* sp., on the right: *Trigonium glandiferum*

Spiral algae, unicellular, spiral filamentous. Example: *Spirulina* (Fig. 3.1 and Fig. 3.2) (SAHOO and SECKBACH, 2015).

Fig. 3.1: *Spirulina platensis* (https://utex.org/products/utex-lb-1926)

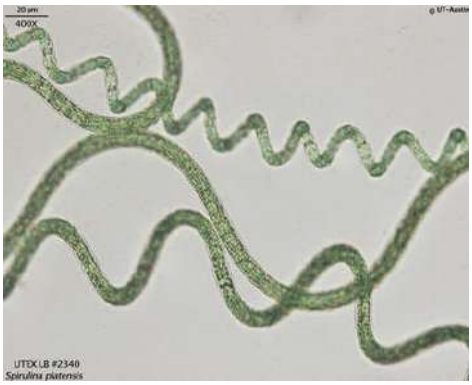


Fig. 3.2: *Spirulina platensis* (https://utex.org/products/utex-lb-2340)

Fig. 3: *Spirulina platensis*

I.2.2. Multicellular forms

Colonial aggregation, a colony is an assemblage of individual cells in which there may be either a variable number or predictable number of cells with an arrangement of cells that may remain constant throughout the life of the individual. Depending on the morphology the colonies may be coenobial, palmelloid, dendroid and rhizopodial (Fig. 4) (KHURANA, 2015).

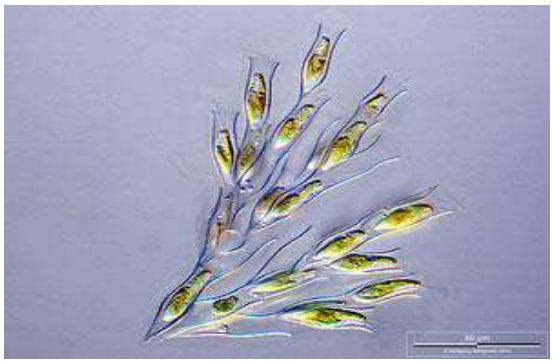


Fig. 4: *Dinobryon divergens* (http://www.diatom.org/lakes/taxa/chryso/Dinobryon/divergens/TB-Dinobryon_divergens.htm)

Filamentous forms, a common growth form among the algae is the filament, where daughter cells remain attached to each other following cell division forming a chain of cells joined end to end. Filaments may be unbranched or branched and may be uniseriate (a single series of cells) or multiseriate, where a few to many individual filaments fuse together to form a larger, more complex structure (Fig. 5) (KHURANA, 2015).

Fig. 5: *Cladophora glomerata*

(https://www.alamyimages.fr/photos-images/cladophora-glomerata-cladophora-glomerata.html)

Siphonaceous forms, they are a large multinucleate forms of various shapes without cross walls to separate the nuclei or other organelles. A cross wall does form during formation of the reproductive structures, which separates it from the parent filament. An example is the yellow-green alga *Vaucheria* (Fig. 6) (WEHR and SHEATH, 2015).

Fig. 6: *Vaucheria sp.*

(http://dbmuseblade.colorado.edu/DiatomTwo/sbsac_site/genus.php?g=Vaucheria)

I.3. Cellular organization

The eukaryotic microalgae possess a true membrane-bounded nucleus, which contains the major part of the genome distributed on a set of chromosomes, and the nucleolus. They have cytoplasm divided into compartments and membrane-bounded organelles (Golgi body, mitochondria, endoplasmic reticulum, vacuoles, centrioles and plastids) devoted to specific functions.

The DNA of prokaryotic Cyanobacteria and Prochlorophytes is not organized in chromosomes, lies free in the cytoplasm together with the photosynthetic membranes, and is not surrounded by a membrane. Moreover, the prokaryotes have no membrane-bounded organelles. Many microalgae are uninucleate, those with multinucleate cellular organization (coenocytic) usually have a peripheric cytoplasm containing nuclei and chloroplasts, which are the most important plastids (RICHMOND, 2004).

I.4. Distribution and Habitats

Algae most commonly occur in water, be it fresh water, marine, or brackish. However, they can also be found in almost every other environment on earth, from the algae growing in the snow of some American mountains to algae living in lichen associations on bare rocks, to unicellular algae in desert soils, to algae living in hot springs (Fig. 7) (LEE, 2008).

Fig. 7: Different habitats of microalgae

(https://www.pinterest.com/pin/35747390762324656/)

Tab. 1: Examples of some algae growing in diverse habitats (KHURANA, 2015)

Habitat	Genus
Aquatic	*Volvox, Hydrodictyon*
Terrestrial	*Vancheria terrestris*
Epiphytic (Grow on other aquatic plants)	*Oedogonium sp.*
Cryophilic (Grow in snow)	*Chlamydomanas nivalis*
Thermophilic (Grow in hot springs)	*Mastocladus laminosus*
Epizoic (Grow on the surface of other aquatic animals)	*Acrosiphonia* on limpets *Basicladia chelonum* on Blanding's turtles
Endophytic (Grow inside a plant)	*Coleochaete nitellarum*
Endozoic (Growing inside the body of vertebrates or aquatic animals)	*Prochloron* as extracellular symbionts of sea-squirts

I.5. Biology of Microalgae

I.5.1. Reproduction

Reproduction is one of the most important characteristic of all living beings. It is the production of one's own kind. It is necessary for the continuation of the species on earth and also to replace the dead members of the species. In this process, living organisms produce their offsprings for the continuity of the species by vegetative, asexual and sexual methods (TACLAN, 2016).

The vegetative reproduction is a type of reproduction where a part of thallus becomes specialized and gets detached from the parent to form a new offspring. The new individual thus formed in this way is genetically identical to parent and no variation is observed (Fig. 10.1) (SAHOO and SECKBACH, 2015). When vegetative reproduction takes place through specialised cells (other than sex cells), it is described as asexual reproduction (Fig. 10.2) (DUTTA, 1981).

Fig. 8.1: *Anabaena sp.* performs vegetative reproduction (http://www.infrastructura.info/2018/anabaena-sp.asp)

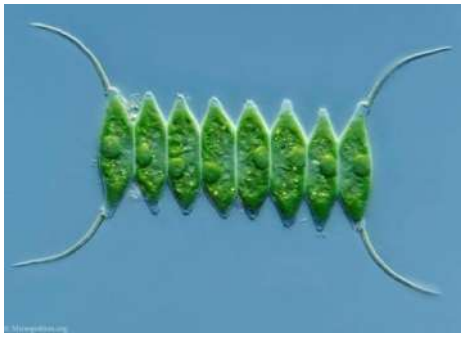


Fig. 8.2: *Scenedesmus sp.* performs asexual reproduction (http://www.photomacrography.net/foru m/viewtopic.php?p=32109&sid=232df 96b14c23 546ad3e88f151c5e77)

Fig. 8: Left: *Anabaena sp.* performs vegetative reproduction, right: *Scenedesmus sp.* performs asexual reproduction

Sexual reproduction has been reported from all members of algae except cyanophyceae. In sexual reproduction two opposite mating types (gametes) fuse to form a zygote (SAHOO and SECKBACH, 2015). Gametes are always haploid and may or may not be different in morphology. If both the sex cells look alike, they could be male called plus (+) or female called minus (-) mating types or strains. Gametes can fuse only when one is plus and the other is minus (Fig. 11) (DUTTA, 1981).

Fig. 9: *Chlamydomonas sp.* performs sexual reproduction (http://cfb.unh.edu/phycokey/Choices/Chlorophyceae/unicells/flagellated/CHLAMYDOMONAS/Chlamydomonas_Image_page.html)

I.5.2. Photosynthesis

All microalgae are photosynthetic. In fact much of what is known about photosynthesis was first discovered by studying the green alga *Chlorella* (ROGERS and EATON, 2017). Photosynthesis represents a unique process of sunlight energy conversion. In this process, inorganic compounds and light energy are converted to organic matter by photoautotrophs. Virtually, all forms of life on Earth depend directly or indirectly on photosynthesis as a source of energy for their metabolism and growth (Fig. 12) (RICHMOND, 2004).

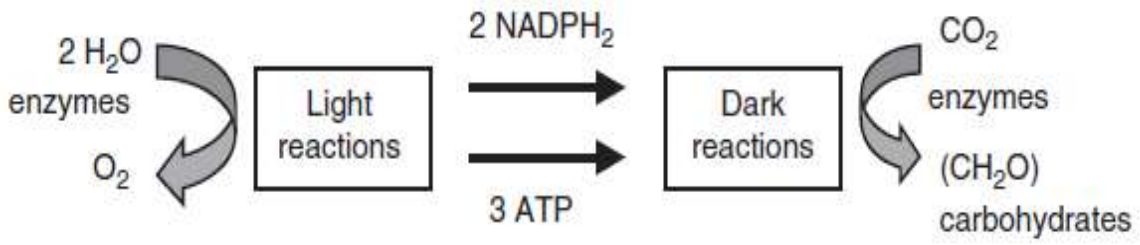


Fig. 10: Major products of the light and dark reactions of photosynthesis (RICHMOND, 2004)

The process of oxygenic photosynthesis is divided into two stages, the so-called light reactions and dark reactions. The light reactions include light absorption, transfer of excitons, electron and proton translocation resulting in the production of $NADPH_2$, ATP and O_2. The other phase, the dark reactions, which occur in the stroma, represent the reduction of carbon dioxide and the synthesis of carbohydrates using the $NADPH_2$ and ATP produced in the light reactions. All photosynthetic organisms contain organic pigments for harvesting light energy. There are three major classes of pigments: chlorophylls, carotenoids and phycobilins. The chlorophylls (green pigments) and carotenoids (yellow or orange pigments) are lipophilic, while phycobilins are hydrophilic (RICHMOND, 2004).

I.5.3. Microalgal growth modes: Autotrophic, Heterotrophic and Mixotrophic

Tab. 2: Microalgal growth modes (LEE, 2008)

Microalgal growth modes	Characteristics
Autotrophic	• They use inorganic compounds as a source of carbon • Photoautotrophic (photolithotrophic): using light as a source of energy • Chemoautotrophic (chemolithotrophic): oxidizing inorganic compounds for energy
Heterotrophic	• They use organic compounds for growth • Photoheterotrophs (photoorganotrophs): using light as a source of energy • Chemoheterotrophs (chemoorganotrophs): oxidizing organic compounds for energy • They usually require a vitamin They can also be: • Phagocytotic: absorbing food particles whole into food vesicles for digestion • Osmotrophic: absorbing nutrients in a soluble form through the plasma membrane • Saprophytic: live on dead material • Parasitic: live off a live host • Auxotrophic: requiring a small amount of an organic compound
Mixotrophic (facultatively heterotrophic)	• They use organic compounds supplied in the medium

I.5.4. Microalgae metabolism

Algal metabolism concerns the biochemical and transport processes by which algae take up nutrients and convert them into the materials needed for growth, reproduction and defence of the organisms.

It includes mechanisms of light harvesting, carbon acquisition and aspects of nitrogen (N) and sulfur (S) assimilation as well as formation of unique secondary metabolites. It also shares many features in common with that of other living organisms but also differs in unique respects. Algal metabolism gives rise to a range of unique compounds, including secondary metabolites, some of which have toxicity to other organisms (BEARDAL and RAVEN, 2012).

I.5.5. Polymeric substances synthetized by Microalgae

The algae are phototrophs and need CO_2, H_2O, minerals nutrients, light, and suitable temperature. Via the photosynthetic process, these autotrophs produced simple sugars and more complex compounds while they release oxygen (SAHOO and SECKBACH, 2015).

Microalgae can biosynthesize, metabolize, accumulate and secrete a great diversity of primary and secondary metabolites including carotenoids, phenolic compounds, phycobilins, sulphated compounds and vitamins. Many of which are valuable substances with potential applications in the food, pharmaceutical and cosmetics industries (MUNIR *et al.*, 2013).

I.5.6. Needs of Microalgae - Factors influencing the growth of Microalgae

I.5.6.1. Physico-chemical parameters

I.5.6.1.1. Light

Light is the energy source that drives photosynthesis to convert nutrients into algal biomass (RICHMOND *et al.*, 1980). Light energy cannot be stored by microalgae, so the light should be supplied sustainably. Microalgae cannot use all the supplied light, because they cannot absorb all the photons. Besides that, too much light will cause light inhibition for the surface layer of the microalgae. The inner portion microalgae cannot reach the light and lack of photons (REN, 2014).

I.5.6.1.2. Temperature

Temperature is one of the most important environmental factors that influence algal growth rate, cell size, biochemical composition and nutrient requirements (RENAUD *et al.*, 2002). It determines the activity and reaction rates of intracellular enzyme, which will have an influence on algal photosynthesis, respiration intensity, affect the growth of microalgae and to limit its distribution (TAN *et al.*, 2009), it also influences the properties of cellular components such as lipids, proteins and carbohydrates (TAMBURIC *et al.*, 2014).

I.5.6.1.3. PH

The PH value affects the growth rate of microalgae. It is easier for microalgae to capture the atmospheric CO_2 when the growth condition is alkaline, leading to higher biomass production (ZANG *et al.*, 2011). When PH increases, the CO_2 present in water transfers into HCO_3, which is the mainly existing formation of carbon in weak alkaline. This is majorly used by microalgae (LIU *et al.*, 2005).

I.5.6.1.4. Salinity

Studies showed that microalgae have their own optimal growth salinity. When salinity gets higher or lower than the optimal value, it harms the algal growing rate (LIU *et al.*, 2006). Exposing algae to lower or higher salinity levels than their natural (or adapted) levels can change growth rate and alter composition (ZHILA *et al.*, 2011). Microalgae have their own system to adjust salinity range. Generally, seawater microalgae can tolerate higher salinity rather than fresh water microalgae (ZHU *et al.*, 2003).

I.5.6.1.5. Mixing

Microalgae grow well in lakes or streams because of the dynamics of the water. Water dynamic can promote every microalgae cell to obtain equal light source and nutrient (WANG, 2006).

Mixing is necessary to prevent sedimentation of the algae, to ensure that all cells of the population are equally exposed to the light and nutrients, also to improve gas exchange between the culture medium and the air (BARSANTI and GUALTIERI, 2006).

I.5.6.2. Nutritional needs

All organisms require basic nutrients for growth and multiplication, and most can meet all their cellular needs for their growth with a few key compounds; macronutrients, micronutrients (trace elements) and vitamins.

Nitrogen and phosphate are two important macronutrients for growth and metabolism of algal cells. Nitrogen is a fundamental element for the formation of proteins and nucleic acids. Being an integral part of essential molecules such as ATP, the energy carrier in cells, phosphate is another very important nutrient. Phosphate is also a part of the backbone of DNA and RNA, which are essential macromolecules for all living cells. Phosphorus is also a key component of phospholipids (HARRIS *et al.*, 1986). Carbon is one of the other major nutrients that must be supplied. It is essential for photosynthesis and hence algal growth and reproduction (BERMAN-FRANK and DUBINSKY, 1999).

In addition, silica (Si) is required for cell wall production by diatoms, and some chrysophytes and silicoflagellates. Although required in lesser amounts, sodium (Na), potassium (K), sulphur (S) and magnesium (Mg) are also considered macronutrients (ANDERSEN, 2005).

Micronutrients (trace metals) are metals present in algal cells in extremely small quantities but that are an essential component of phycophysiology. Iron (Fe), manganese (Mn), cobalt (Co), zinc (Zn), copper (Cu) and nickel (Ni) are the six most important trace metals required by algae for various metabolic functions (BRULAND *et al.*, 1991). Some microalgae also require vitamins such as B1 (thiamine), B12 (cyanocobalamin) and H (Biotin) for growth (ANDERSEN, 2005).

I.6. Biochemical composition of Microalgae

As with any higher plant, the chemical composition of algae is not an intrinsic constant factor but varies over a wide range. Environmental factors, such as temperature, illumination, PH-value, mineral contents, CO_2 supply, or population density, growth phase and algae physiology, can greatly modifie chemical composition (Tab. 3) (YAMAGUCHI, 1997).

Tab. 3: Distribution of the biochemical fractionation of a microalgae cell (SIALVE and STEYER, 2013)

Biochemical compartment	Fonction	Order of proportion (% by mass)
Proteins	Structure and metabolism	40-60
Lipids	Structure and energy reserve	5-60
Sugars	Structure and energy reserve	8-30
Nucleic acids	Support, vector and regulator of genetic information	5-10

I.6.1. Proteins

The high protein content of various microalgae species is one of the main reasons to consider them as an unconventional source of protein (SOLETTO *et al*., 2005). The amino acid pattern of almost all algae compares favorably with that of other food proteins (GUIL-GUERRERO *et al*., 2004). Amino acids composition, especially the free amino acids, varies greatly between species as well as with growth conditions and growth phase (BOROWITZKA, 1988).

I.6.2. Sugars

Carbohydrates (primarily starch) are another valuable component of the algal cell. Typical dry weight content of carbohydrates in algae range from 20% to 40% of total cell mass (HU, 2004). High starch strains including *Chlorella vulgaris* (with 37% dry weight starch), are being studied for potential use as high-yield feedstocks (HIRANO *et al.*, 1997).

I.6.3. Lipids

Microalgae have been known to be rich in lipids. It produces many different kinds of lipids, triglycerides and diglycerides, phospholipids and glycolipids, hydrocarbons and others. The relative composition of algal lipids depends greatly on the species used, the nutrient, environmental and developmental conditions in which the cells are cultured and harvested (HAN *et al.*, 2O11).

I.6.4. Pigments

One of the most obvious and arresting characteristic of the algae is their colour. In general, each phylum has its own particular combination of pigments and an individual

colour. Aside chlorophylls, as the primary photosynthetic pigment, microalgae also form various accessory or secondary pigments, such as phycobiliproteins and a wide range of carotenoids. These natural pigments are able to improve the efficiency of light energy utilization of the algae and protect them against solar radiation and related effects (VAN DEN BERG *et al*., 2000).

I.6.5. Vitamins and Minerals

Microalgae biomass represents a valuable source of nearly all essential vitamins (*e.g.* A, B1, B2, B6, B12, C, E, nicotinate, biotin folic acid and pantothenic acid) and a balanced mineral content (*e.g*. Na, K, Ca, Mg, Fe, Zn and trace minerals) (BECKER, 2004).

The vitamin content of an alga depends on the genotype, the stage in the growth cycle, the nutritional status of the alga and light intensity (photosynthetic rate). Vitamins cell content fluctuates with environmental factors, the harvesting treatment and the biomass drying methods (BROWN *et al*., 1999). The high levels of vitamin B12 and Iron in some microalgae, like *Spirulina*, makes them particularly suitable as nutritional supplements for vegetarian individuals (GOUVEIA, 2008).

I.7. Survival Strategies

The harsh conditions of extreme environments are at the edge of biological growth limits. Algae are growing in various severe extreme conditions. Prokaryotic phototrophs and eukaryotic algae can share some of the severe habitats or, can be separated, thriving in their individual harsh environment. Further away from the normal conditions algae and cyanobacteria can cope living in: temperature effects, elevated (thermophiles) or lower (psychrophiles) temperature levels, pH effects (acidophiles <4, or alkaliphiles >10), in hyper saline waters (halophiles), anaerobic conditions, living in toxic metals and desiccation (SAHOO and SECKBACH, 2015).

To combat this, algae has developed certain methods to carry over the period of severe conditions till the next growing season. However, fresh water and subaerial algae undergo perennation (which is defined as a temporary rest for algae, where all the metabolic activities are ceased till the onset of favorable season) by producing asexual thick walled spores, the marine algae avoids the tidal fluctuations by secreting a lot of mucilage which keeps them dehydrated during the low tides (VASHISHTHA *et al.*, 2007).

I.8. Culture modes

Batch, Continuous, and Semi-Continuous (Fed-batch) culture are the three basic types of phytoplankton culture (FAO FISHERIES TECHNICAL PAPER, 1996).

I.8.1. Batch culture

This is the most common method for cultivation of microalgal cells. In a simple batch culture system, a limited amount of complete culture medium and algal inoculum are placed in a culture vessel and incubated in a favorable environment for growth. Some form of agitation, such as shaking or impeller mixing, is necessary to ensure nutrient and gaseous exchange at the cell–water interface. The culture vessel can be a simple conical flask or an environment controlled fermentor (MULLER-FEUGA *et al.*, 1998).

Batch culture is widely used for commercial cultivation of algae for its ease of operation and simple culture system. Since the process is batch wise, there is low requirement for complete sterilization. For mass algal culture production, a portion of the culture could be retained as inoculum for the next culture batch (RICHMOND, 2004).

I.8.2. Continuous cultures

In continuous flow cultures, fresh culture medium is supplied to the homogeneously mixed culture and culture is removed continuously or intermittently. The approach is based on the observations that substrates are depleted and products accumulate during growth. Eventually, culture growth ceases due to depletion of the growth limiting substrate or accumulation of a growth-inhibiting product. To sustain cell growth, the growth-limiting substrate needs to be renewed and the growth inhibitory product needs to be removed or diluted by adding fresh culture medium (BUX and CHISTI, 2016).

I.8.3. Fed-batch (Semi-Continuous) culture

In a fed-batch culture, the medium is added continuously or intermittently whereas the culture is harvested periodically, thus the culture volume may not be constant and the rate of dilution varies with the culture volume. Fed-batch culture is the most widely used industrial continuous flow culture process, where concentrated culture medium is fed continuously or intermittently, and the culture is harvested at the end of the cultivation cycle (LEE, 1997).

Chapter II

Classification and application of Microalgae

Chapter II. Classification and application of Microalgae

II.1. History of classification

Classification is the systematic grouping of organisms into categories on the basis of relationships between them, where the relationship can be either evolutionary or structural (SHARMA, 2011). Taxonomic identification is the most common analysis and hypothesis-testing attempt in science (MANOYLOV, 2014).

The history of classification dates back to Carolous Linnaeous, who first classified plants into 25 classes based on "sexual system" considering the number of stamens and carpels in their flowers. Out of his 25 classes, in "Cryptogamia" which contains plants with "Concealed reproductive organs". Linnaeus proposed 14 algal genera of which only 4, *Conferva*, *Ulva*, *Fucus* and *Chara* are now considered as algae (DIXON, 1973).

Algal taxonomy is a key discipline in phycology and is critical for algal genetics, physiology, ecology and applied phycology (MANOYLOV, 2014). One of the first algologists is William Henry Harvey (1811–1866), who proposed the first descriptive algal classification (SAHOO and SECKBACH, 2015). Identification has been based on knowledge of morphological traits transmitted from generation to generation of planktologists in monographs or at the bench (Fig. 13) (MCMANUS and KATZ, 2009).

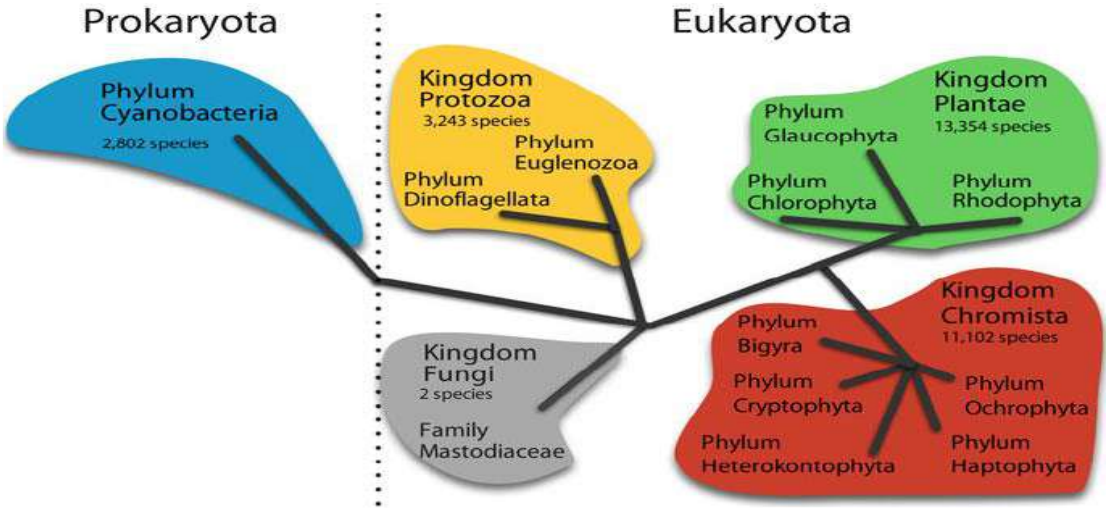


Fig. 11: Distribution of algae among groups in the Tree of Life as recognized by the Species 2000 in 2011 (http://www.itis.gov and http://www.catalogofl ife.org)

II.2. Different morphological classifications

Since W. H. Harvey, several classifications have been proposed based on a variety of characters including morphological, physiological, biochemical and more recently the molecular characters have also been considered.

The main characters which are being widely used for algal classification are:

a. Nature of photosynthetic pigments, chlorophylls, carotene, xanthophylls and phycobilins. The color of the thalli is best seen in the field, at least for algae visible to the naked eye.
b. Biochemical criteria, such as the nature of reserve substances.
c. Cellular criteria, such as the presence or absence of nucleus, cellular motility, plastid organization…etc.
d. Organization of the thallus, from unicellular microscopic forms to large complex algae.
e. Cycles and reproductive organs.
f. Diversity of environments and life modes (SAHOO and SECKBACH, 2015).

The major classification proposed by different algologists for algae are:

Classification proposed by William Henry Harvey (1836), Harvey described marine alage from many parts of the world including Britain, Australia, America, New Zealand and Antarctica, and divided them on the basis of their colour into the following three main groups (SHARMA, 1986):

1. Chlorospermae (Green algae)
2. Melanospermae (Brown algae)
3. Rhodospermae (Red algae)

Classification proposed by Felix Eugen Fritsch (1935), Felix Eugen Fritsch proposed the most acceptable and comprehensive algal classification. He published two volumes of "Structure and Reproduction of the Algae". His classification is based on different characteristics as pigmentation, chemical nature of reserve food material, flagellar arrangement (kind, number and point of insertion), presence or absence of organized nucleus in cell and mode of reproduction (SAHOO and SECKBACH, 2015).

Classification proposed by Gilbert Morgan Smith (1955), Smith believes that algae should be divided first into some divisions and then each division should contain some classes. His classification contains 7 divisions with 14 classes as following (SHARMA, 1986):

Tab. 4: Classification proposed by Gilbert Morgan Smith (1955)

Divisions	Classes
Chlorophyta	Chlorophyceae
	Charophyceae
Euglenophyta	Euglenophyceae
Pyrrohyta	Cryptophyceae
	Desmokontae
	Dinophyceae
Chrysophyta	Xanthophyceae
	Chrysophyceae
	Bacillariophyceae
Phaeophyta	Isogeneratae
	Heterogeneratae
	Cyclosporae
Cyanophyta	Myxophyceae
Rhodophyta	Rhodophyceae

Classification proposed by Robert Edward Lee (2008), Lee classified algae into two groups Prokaryota and Eukaryota which were further divided into divisions. Prokaryota has just one division Cyanophyta, whereas Eukaryota was further divided on the basis of nature of chloroplast membrane (Tab. 5) (SAHOO and SECKBACH, 2015).

Tab. 5: Classification proposed by Robert Edward Lee (2008)

	Groups	Divisions	Class
Prokaryota	I	Cyanophyta	Cyanophyceae
Eukaryota	II. Chloroplast surrounded by the two membranes of the chloroplast envelope	Glaucophyta	
		Rhodophyta	
		Chlorophyta	
	III. Chloroplast surrounded by one membrane of chloroplast endoplasmic reticulum	Euglenophyta (Euglenoids)	
		Dinophyta (Dinoflagellates)	
	IV. Chloroplast surrounded by two membranes of chloroplast endoplasmic reticulum envelope	Cryptophyta (Cryptophytes)	
		Prymnesiophyta (Haptophytes)	Prymnesiophyceae
		Heterokontophyta (Heterokonts)	Chrysophyceae
			Synurophyceae
			Dictyophyceae
			Pelagophyceae
			Bacillariophyceae
			Raphidophyceae
			Xanthophyceae
			Eustigmatophyceae
			Phaeophyceae

II.3. Classification proposed by Felix Eugen Fritsch (1935)

He classified algae into 11 classes as (FRITSCH, 1935):

Class I. *Chlorophyceae*

- Pigmentation: grass green chromatophores and contain the same four pigments (two green, two yellow), approximately in the same proportions as in higher plants.
- Chemical nature of reserve food material: starch, often accompanied by oil.

- Flagellar arrangement: It possesses a number of equal flagella (Commonly two or four) which arise from the front end of the swarmers and are all similarly orientated.
- Presence or absence of organized nucleus: present.
- Mode of reproduction: vegetative, asexual and sexual reproduction.
- Occurrence: The class is more widely represented in fresh than in salt water, and there is a marked terrestrial tendency.
- Cell wall: cellulosic.

Class II. *Xanthophyceae (Heterokontae)*

- Pigmentation: yellow-green chromatophores with presence xanthophyll.
- Chemical nature of reserve food material: starch and oil.
- Flagellar arrangement: two very unequal flagella (or sometimes only one) arising from the front end.
- Presence or absence of organized nucleus: present.
- Mode of reproduction: vegetative, asexual and sexual reproduction.
- Occurrence: the class is more widely distributed in freshwater than in the sea.
- Cell wall: rich in pectic compounds.

Class III. *Chrysophyceae,*

- Pigmentation: brown or orange-coloured chromatophores containing one or more accessory pigments (phycochrysin).
- Chemical nature of reserve food material: fat and a compound leucosin.
- Flagellar arrangement: one or two (rarely three) unequal flagella attached at the front end.
- Presence or absence of organized nucleus: present.
- Mode of reproduction: vegetative and extremely rare Sexual reproduction.
- Occurrence: the class is widely distributed in colder freshwaters, but a few families are marine.
- Cell wall: silicified or calcified.

Class IV. *Bacillariophyceae* (Diatoms)

- Pigmentation: golden brown chromatophores with accessory brown pigments.
- Chemical nature of reserve food material: fat and volutin.
- Flagellar arrangement: present in Centrales.

- Presence or absence of organized nucleus: present.
- Mode of reproduction: vegetative and asexual.
- Occurrence: widely distributed in the sea and in all kinds of freshwaters, as well as in the soil and in other terrestrial habitats.
- Cell wall: composed partly of pectic substances and partly of silica.

Class V. *Cryptophyceae*

- Pigmentation: two large parietal chromatophores with very diverse pigmentation (commonly some shade of brown).
- Chemical nature of reserve food material: solid carbohydrates, in some cases starch, in others a compound similar to it.
- Flagellar arrangement: two slightly unequal flagella.
- Presence or absence of organized nucleus: present.
- Mode of reproduction: vegetative and sexual reproduction is only reported in one form.
- Occurrence: equally represented in the sea and in freshwaters.
- Cell wall: absent.

Class VI. *Dinophyceae* (*Peridinieae*)

- Pigmentation: discoid chromatophores which are dark yellow, brown, and contain a number of special pigments.
- Chemical nature of reserve food material: starch and oil (fat).
- Flagellar arrangement: two flagella.
- Presence or absence of organized nucleus: present.
- Mode of reproduction: sexual reproduction (rare).
- Occurrence: widely represented in the sea than in freshwaters.
- Cell wall: cellulosic.

Class VII. *Chloromonadineae*

- Pigmentation: discoid chromatophores having a bright green tint and with excess of xanthophyll.
- Chemical nature of reserve food material: Oil.
- Flagellar arrangement: two almost equal flagella.

- Presence or absence of organized nucleus: present.
- Mode of reproduction: vegetative.
- Occurrence: the class is only recorded from freshwaters.
- Cell wall: absent.

Class VIII. *Euglenineae*

- Pigmentation: several pure green chromatophores.
- Chemical nature of reserve food material: polysaccharide, paramylon.
- Flagellar arrangement: one or two flagella.
- Presence or absence of organized nucleus: present.
- Mode of reproduction: vegetative, sexual reproduction (rare).
- Occurrence: the majority of the members of this class probably inhabit freshwaters.
- Cell wall: Proteinaceous.

Class IX. *Phaeophyceae*

- Pigmentation: brown chromatophores with the usual pigments, the yellow tucoxanthin.
- Chemical nature of reserve food material: manitol, laminarin and fats.
- Flagellar arrangement: two laterally attached flagella.
- Presence or absence of organized nucleus: present.
- Mode of reproduction: sexual reproduction is of wide occurrence.
- Occurrence: mostly marine.
- Cell wall: cellulose, alginic acid and fucinic acid.

Class X. *Rhodophyceae*

- Pigmentation: red, blue chromatophores with the usual pigments and the red phycoerythrin and the blue phycocyanin.
- Chemical nature of reserve food material: solid polysaccharide similar to starch.
- Flagellar arrangement: absent.
- Presence or absence of organized nucleus: present.
- Mode of reproduction: sexual.
- Occurrence: mostly marine.
- Cell wall: outer pectic and inner cellulosic.

Class XI. *Myxophyceae (Cyanophyceae)*

- Pigmentation: chlorophyll, carotene, phycocyanin, and phycoberythrin.
- Chemical nature of reserve food material: sugars and glycogen.
- Flagellar arrangement: absent.
- Presence or absence of organized nucleus: absent.
- Mode of reproduction: vegetative and asexual.
- Occurrence: freshwaters and in terrestrial habitats.
- Cell wall: mucopeptides, amino acids, fatty acids and carbohydrates.

II.4. Molecular classification of algae

Molecular approaches for taxonomic identification of algae are being tested to augment or even replace morphological identification. Most molecular work has been related to characterizing biodiversity and systematic (DEANS *et al.*, 2012). Detecting species using nucleic, mitochondrial, and/or chloroplast DNA has become commonplace in recent years. DNA barcoding (HEBERT *et al.*, 2003) was proposed as a new system of species identification and discovery using a short section of DNA from a standardized region of the genome. This technique bypasses the need for laboratory cultivation and/or isolation of individual specimens. Molecular assessments of algal biodiversity have the potential for more automated, complete, and accurate characterization of taxa in natural communities (MANOYLOV, 2014).

Molecular approaches for taxonomic identification from environmental samples promise rapid, potentially inexpensive, and more thorough culture independent identification of all algal species present in a sample of interest (MANOYLOV, 2014). Analysis of DNA sequences technique involves (BELLINGER and SIGEE, 2010):

1. Collecting a sample of biomass from the entire microbial community.
2. Obtaining a DNA sample; this may involve extraction from a mixed environmental sample such as biofilm or soil.
3. Polymerase chain reaction (PCR) amplification of a specific nucleotide region, typically 16S or 18S rRNA genes.
4. Separation of the amplified strands by denaturing gradient gel electrophoresis (DGGE), or purification of the PCR products using a rapid purification kit.
5. Sequence analysis with comparison to a standard database; sequence identification is normally based on a match of at least 90%.

II.5. Microalgae applications

Microalgae are an enormous biological resource, representing one of the most promising sources for new products and applications (PULZ and GROSS, 2004). The current and forthcoming applications of microalgae are numerous and diverse, including food, feed, healthcare, industry and energy. Although the use of cyanobacteria in food dates back many hundreds of years, advances in this area were made in the 20th century (HABIB *et al.*, 2008).

In recent years microalgal studies have gained more attention. The use of *Haematococcus pluvialis* for commercial productions of astaxanthin (Fig. 8.1 and Fig. 8.2) (CHIA *et al.*, 2013), which is a carotenoid pigment and a potent radical scavenger and singlet oxygen quencher, with increasing amount of evidence suggesting that surpasses the antioxidant benefits of β-carotene, vitamin C and vitamin E (PAPADOPOULOS, 2008)**,** is already a reality, and encapsulated dried biomass of *Chlorella vulgaris* and *Spirulina platensis* as neutraceuticals are commercialized. All these applications can benefit from physiological investigations on microalgae, aiming at defined biochemical composition (CHIA *et al.*, 2013).

It is known that microalgae respond with physiological alterations to the environmental conditions where they grow (VALENZUELA-ESPINOZA *et al.*, 2002). This behavior can be viewed as a biotechnological attribute that can be manipulated in order to control the algae biochemical composition and growth, focusing on specific compounds and higher productivity (BAJWA *et al.*, 2017).

Fig. 12.1: Astaxanthin as powder and capsules (https://zovon.com/health-news/latest-health-news/astaxanthin-effective-improving-glucose-metabolism)

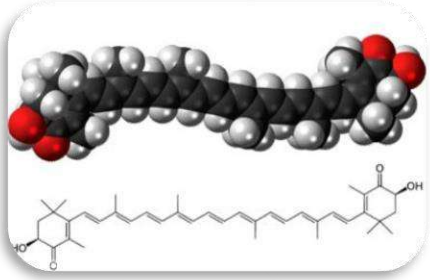


Fig. 12.2: Astaxanthin's molecular structure (https://www.superfoodly.com/natural-astaxanthin-foods-best-high-potency-food-sources/)

Fig. 12: Astaxanthin as powder and capsules (Left), and its molecular structure (Right)

II.5.1. Commercial application of Microalgae

II.5.1.1. Microalgae as human food

Microalgae are a rich source of carbohydrates, protein, enzymes and fiber. Many vitamins and minerals like vitamin A, C, B1, B2, B6, niacin, iodine, potassium, iron, magnesium and calcium are abundantly found in microalgae. Being such a rich source of essential nutrients, they are a major source of food, especially in Asian countries like China, Japan and Korea. Green micro-algae have been used as nutritional supplement or food source in Asiatic countries for hundreds of years (PRIYADARSHANI and RATH, 2012).

There pigments have commercial uses as a natural food coloring, such as Beta carotene. Microalgal Beta carotene is used as a food coloring, as a food additive to enhance the color of the flesh of fish and the yolk of eggs, and to improve the health and fertility of grain-fed cattle (BOROWITZKA and BOROWITZKA, 1987).

Some of the most biotechnologically relevant microalgae are the green algae (chlorophycea) *Chlorella vulgaris* (Biomass), *Haematococcus pluvialis* (Astaxanthin production), *Dunaliella salina* (β- carotene) and the Cyanobacteria *Spirulina maxima* (Biomass) which are widely commercialized and used, mainly as nutritional supplements for humans and as animal feed additives (GOUVEIA *et al.*, 2008).

II.5.1.2. Microalgae and cosmetics

Components of algae are frequently used in cosmetics as thickening agents, water-binding agents, and antioxidants. Some microalgal species are established in the skin care market (STOLZ and OBERMAYER, 2005), *Arthrospira* and *Chlorella*, are again those involved in the anti-aging (Porphyran, shinorine) and regenerative products (SPOLAORE *et al.*, 2006).

Some algal extracts are considered emollients, and are incorporated into anti-aging creams to prevent wrinkles and stimulate collagen synthesis; their ultraviolet (UV) protection properties are also being researched (SPOLAORE *et al.*, 2006).

II.5.1.3. Microalgae as potential source of High-value molecules

Microalgae can be a very interesting natural source of new compounds with biological activity that could be used as functional ingredients. In fact, some microalgae are organisms that live in complex habitats submitted to extreme conditions (for example, changes of salinity, temperature, nutrients, UV-Vis irradiation …etc.), therefore, they must adapt rapidly to the new environmental conditions to survive, producing a great variety of secondary (biologically active) metabolites such as lipids, protein and pigments, which cannot be found in other organisms (PRIYADARSHANI and RATH, 2012).

Bioactive lipids with a high proportion of polyunsaturated fatty acids (PUFA) found in marine microalgae (*Chlorella minutissima*, *Schizochytrium* sp., *Parietochlorisincise* …etc.), especially *n*-3 PUFA such as a-linolenic acid (ALA, C18:3*n*-3) (Fig. 9.2), eicosapentaenoic acid (EPA, C20:5*n*-3) (Fig. 9.1), docosapentaenoic acid (DPA, C22:5*n*-3) (Fig. 9.4), and docosahexaenoic acid (DHA, C22:6*n*-3) (Fig. 9.3), are one of these high-value molecules, which have been shown to be effective in preventing or treating several diseases including cardiovascular disorders, cancer, type 2 diabetes,…etc. (ABD El-BAKY *et al.*, 2002).

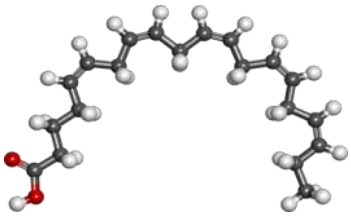


Fig. 13.1: Eicosapentaenoic acid (EPA)

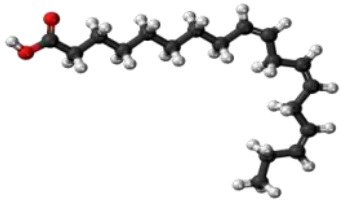


Fig. 13.2: A-linolenic acid (ALA)

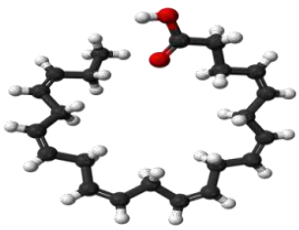


Fig. 13.3: Docosahexaenoic acid (DHA)

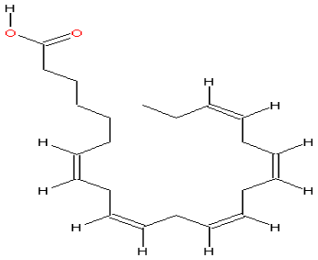


Fig. 13.4: Docosapentaenoic acid (DPA)

Fig. 13: Some of polyunsaturated fatty acids (PUFA) found in marine microalgae (https://lesystemenerveux.wordpress.com/author/luthopy/)

II.5.1.4. Microalgae as a source of biofuels

Microalgae have long been recognized as potentially good sources for biofuel production because of their high oil content and rapid biomass production. In recent years, use of microalgae as an alternative biodiesel feedstock has gained renewed interest from researchers, entrepreneurs, and the general public (AKSOY *et al.*, 2014).

It is proven that chemical compositions of microalgae fuels are similar to petrol and diesel since its crude oil has 80% of average energy content of petroleum, also can be used to replace fossil fuels for transportation (MILANO *et al.*, 2015). There are several ways to convert microalgal biomass to energy sources, which can be classified into biochemical conversion, chemical reaction, direct combustion, and thermochemical conversion. Thus, microalgae can provide feedstock for renewable liquid fuels such as biodiesel and bioethanol (DRAGONE *et al.*, 2010).

II.5.2. Industrial application of Microalgae

II.5.2.1. Uses of Microalgae as biofertilizer

Microalgae are employed in agriculture as biofertilizers and soil conditioners. The majority of cyanobacteria are capable of fixing atmospheric nitrogen and are effectively used as biofertilizers. Cyanobacteria play an important role in maintenance and build-up of soil fertility, consequently increasing rice growth and yield as a natural biofertilizer (SONG *et al*., 2005).

II.5.2.2. Uses of Microalgae in pharmaceuticals

Algal organisms are rich source of novel and biologically active primary and secondary metabolites. These metabolites may be potential bioactive compounds of interest in the pharmaceutical industry (RANIA and HALA, 2008). Microalgae contain numerous bioactive compounds that can be harnessed for commercial use. They have emerged as important sources of proteins and value added compounds with pharmaceutical and nutritional importance. The microalgae have a significant attraction as natural source of bioactive molecules, because they have the potential to produce bioactive compounds in culture, which are difficult to produce by chemical synthesis (BOROWITZKA and BOROWITZKA, 1992; KATIRCIOGLU *et al*., 2006).

Various strains of cyanobacteria are known to produce intracellular and extracellular metabolites with diverse biological activities such as antibacterial, antifungal and antiviral activity (MADHUMATHI *et al.*, 2011).

II.5.2.3. Microalgae as Aquaculture feed

Microalgae feeds are currently used mainly for the culture of larvae and juvenile shell and finfish, as well as for raising the zooplankton required for feeding of juvenile animals (CHEN, 2003). The most frequently used species in aquaculture are *Chlorella*, *Tetraselmis*, *Isochrysis*, *Pavlova*, *Phaeodactylum*, *Chaetoceros*, *Nannochloropsis*, *Skeletonema* and *Thalassiosira.* Mainly the microalgae *Spirulina* and, to some extent, *Chlorella* are used in this domain for many types of animals: cats, dogs, aquarium fish, ornamental birds, horses, poultry, cows and breeding bulls (SPOLAORE *et al*., 2006).

Chapter III

Materials and methods

III.1. Presentation of research area

III.1.1. Geographic localization

Ouargla region is located in the South East of Algeria. It is located at the bottom of a large basin of the Oued M'ya valley. The city of Ouargla, the administrative center of the state, is located at an altitude 157 m, its geographical coordinates are 31° 58' North latitude, 5° 20' East longitude, 800 Km south of Algiers (Fig. 14 and Fig. 15) (KORICHI, 2007).

Ouargla state covers an area of 163233 Km^2. It is limited:

- In North by Djelfa and El Oued states.
- To the South by Illizi and Tamanrasset states.
- In East by Tunisia.
- To the West by Ghardaia states.

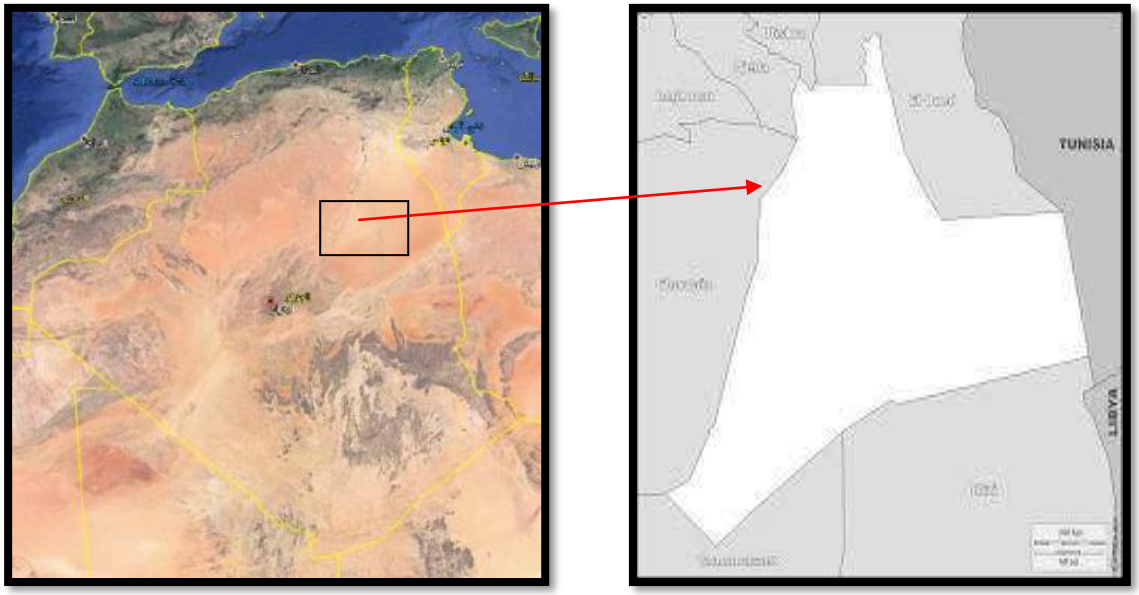


Fig. 14.1: Satellite image of Algeria (Google earth)

Fig. 14.2: Geographical map of ouargla (http://d-maps.com/carte.php?num_car=190720&lang=fr)

Fig. 14: Satellite image of Algeria (Left), Geographical map of ouargla (Right)

Fig. 15: Satellite image of Ouargla (Google earth)

III.1.2. Climatology

The climate of Ouargla is of Saharan type, characterized by a hot and dry summer, a rather mild winter, a weak rainfall and strong evaporation (KORICHI, 2007).

III.1.2.1. Temperature

This is a major factor conditioning the climate of the region. The temperature analysis will be made from data collected from the National Office of Meteorology Ouargla period (2008-2017). The average annual temperature is 23.34° C, with a maximum in July of 43.8° C (Average maxima 31.12° C), and a minimum in January of 5.22° C (Average minima 16.28° C).

III.1.2.2. Air humidity

It's the amount of water vapor that is in the air, it is often expressed as a percentage of saturation. The humidity of the air is medium. The annual average is 57.82 % (Appendix 1). It varies according to the seasons of the year.

During the summer, it drops to 34.7 % in the month of July, under the action of strong evaporation and hot winds; while in winter it rises and reaches a maximum average of 81.3 % in the month of December.

III.1.2.3. Wind

Wind is a characteristic element of the climate, characterized by its direction, speed and frequency (DUBIEF, 1963). The strongest winds of Ouargla blow from north-east to south (ROUVILLOIS-BRIGOL, 1975).

According to (Appendix 1), we notice that the winds are frequent throughout the year. The highest speeds are recorded during the period from February to September, with a maximum of 10.39 m / s during the month of May. The dominant direction of winds according to Dubief (1963) is the North-West.

III.1.2.4. Precipitation

Precipitation is very low and irregular, the annual average is 3.62 mm/ year (Appendix 1). They occur mainly in winter and autumn after a dry period usually between May and October. This lack of rainfall is accompanied by a marked irregularity in the rainfall pattern and considerable interannual variability, which accentuates the drought (OZENDA, 1983).

III.1.2.5. Evaporation

Evaporation is very important, controlled by temperature and accentuated by the evaporative power of dry winds. The annual average is 233.88 mm. The maximum is reached in July with an average of 433.33 mm. The minimum is recorded during the month of December with 83.42 mm.

III.1.2.6. Insolation

Because of the low cloudiness of the atmosphere, the amount of sunlight is relatively strong, which has a drying effect by increasing the temperature (OZENDA, 1983). The duration of insolation is obviously very important in the Sahara and varies quite significantly from one year to another and even according to the periods of the year considered (DUBIEF, 1963). The average duration of insolation is about 271.92 hours, with a maximum of 340.13 hours in August and a minimum of 230.97 hours in December.

III.1.3. Identification of sampling areas

Samples were taken from two different locations, Hassi Ben Abd Allah lake and Mehiriza irrigation basin. The sample taken from Hassi Ben Abd Allah Lake was collected in March, Mehiriza's sample was taken in April.

III.1.3.1. Hassi Ben Abd Allah

Hassi Ben Abd Allah municipality is located east of the city of Ouargla. This municipality resulting from the last administrative division (1984) is distant of 20 km of the chief town of the state and of 08 Km of the chief town of the administration department of Sidi Khouiled. It covers a total area of 1762 km^2 and an agricultural area of 1310 km^2.

It is limited: at North by the municipality of El-Hadjira, in the South by the municipality of Ain Beida, in the East by the municipality Hassi Messaoud, in the South-West: by the municipality of Sidi Khouiled (HBA APC). Geographical coordinates of Hassi Ben Abd Allah are: 32 ° 1 '33 "North latitude, 5 ° 28' 7" East longitude (Fig. 16).

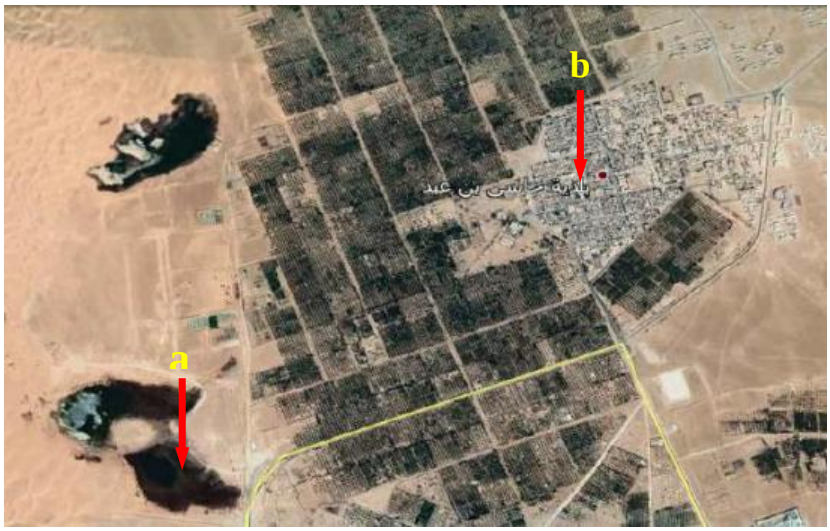


Fig. 16: Satellite image of Hassi Ben Abd Allah lake (a) and Hassi Ben Abd Allah city (b) (Google earth)

Hassi Ben Abd Allah lake, has an area of 10 hectares and a maximum depth of 4.7 m located at the bottom of the hollows to the west of the town (32 "01 'N. and 5" 44 'E) and bordered by sand dunes to the North (Ergs), South and East. By the N56 national road (Pic. 1) (HALFAOUI, 2008).

Pic. 1: Hassi Ben Abd Allah lake

III.1.3.2. Mehiriza

Mehiriza, also called Kahf El Soltan, is an agricultural zone designated to the cultivation of dates. It is located about 12 km away from Ouargla's municipality, by the N49 national road (Meniaa road). The municipality of Ouargla has distributed this land in the nineteen nineties. Its geographical coordinates: "44' 31°52 N. and "03'5°14 E (Fig. 17) (OUARGLA APC).

Fig. 17: Satellite image of Mehiriza (Google earth)

Pic. 2: Mehiriza irrigation basin

III.2. Measurement of physico-chemical parameters of sampling areas waters

Physico-chemical parameters of the environment, determine the growth, activities and the distribution of microalgae. For this, the hydrogen potential (pH) and electrical conductivity were measured using a HANNA portable pH-meter (Pic. 3) and Cond 315i/ SET portable conductivity meter (Pic. 4).

Pic. 3: HANNA portable pH-meter

Pic. 4: Portable conductivity meter

III.3. Sampling and conservation of samples during transportation

III.3.1. Sampling techniques

Sampling was done in the same period of the day between (10h-12h). Hassi Ben Abd Allah lake sample was collected in March Mehiriza's sample was taken in April. The samples taken contained both water and some of the soil and were collected from the surface and the bottom of the water bodies. They were taken directly by two methods:

- Glass containers (Pic. 5.1).
- Plastic bottle (Pic. 5.2).

Pic. 5.1: Sampling using glass container

Pic. 5.2: Sampling using plastic bottle

Pic. 5: Sampling using glass container (Up), sampling using plastic bottle (Down)

- The samples were kept in an insulated cooler and transported directly to the laboratory (Pic. 6).
- Some of Mehiriza irrigation basin sample was fixed at the laboratory using a Lugol solution (Pic. 7).

Pic. 6: An insulated cooler

Pic. 7: Fixed sample from Mehiriza irrigation basin

III.4. Culturing techniques (Isolation)

Four culture mediums were used to isolate as much as possible of the existing strains of microalgae. The culture mediums are: F/2 (for marine algae), Bold's Basal Medium (BBM) (for green algae), BG-11(for cyanobacteria) and Half Strength Chu #10 (for lake algae).

III.4.1. Culture mediums

Both solid and liquid mediums were prepared. Liquid mediums were prepared following the recipe of each medium, and they include adding macronutrients, trace metals solution and vitamins solution to distilled water or sea water in the case of F/2 Medium, to finally obtain 1 L of medium and then adjust their pH as mentioned in the recipe (Appendix 2, 3, 4, 5, 6, 7, 8 and 9). All ingredients should be prepared as stock solutions, because direct combinations of several compounds without any dilution in water may result undesirable precipitation (ANDERSEN, 2005).

To prepare solid medium, 15 g of agar powder is added to 1 L of heated medium solution. Both liquid and solid mediums are ready to be used after sterilization by autoclaving at 121°C for 20 min, they can be also stored for several months at 4°C (ANDERSEN, 2005). Petri dishes should contain 40 ml of solid medium.

N.B: We didn't use vitamins during preparation of the mediums, they were not available to us at that period.

Pic. 8: Culture mediums preparation liquid and solid, A: stock solutions of culture mediums, B: preparing liquid and solid medium on a hotplate

III.4.2. Enrichment, isolation and incubation parameters

a. Culture parameters of Hassi Ben Abd Allah lake sample

- **Enrichment on a liquid medium**

10 ml of sample water was transferred to an Erlenmeyer flask containing 100 ml of sterilized liquid medium, and then incubated at 25°C under continuous illumination using cool white lamp in Phytotron without agitation, for three weeks (LENGYEL *et al.*, 2012).

- **Isolation on solid medium by the dilution technique**

At the same time, dilutions were made reaching to 10^{-2}. Next, cultures were made by inoculating 0.1 ml of each dilution solution by spreading technique onto Petri dishes containing the mediums used in the enrichment solidified with 15g/L agar, incubated at the same conditions of the enrichment in Phytotron, for three weeks (LENGYEL *et al.*, 2012).


Pic. 9: Enrichment and isolation preparation of Hassi Ben Abd Allah lake sample

- **Isolation on solid medium after enrichment**

0.1 ml of enrichment solution was inoculated and spread into solid mediums. The mediums used are the same ones used in the enrichment, solidified with 15g/L agar at the same incubation conditions of the enrichment in Phytotron, for three weeks (ROGER and REYNAUD, 1977).

Pic. 10: Cultures in Phytotron (A), Phytotron (B)

b. Culture parameters of Mehiriza irrigation basin sample

- **Enrichment on a liquid medium**

1 ml of sample water was transferred to a test tube containing 10 ml of sterilized liquid mediums, and then incubated at 25°C under continuous illumination using cool white lamp in Phytotron without agitation, for three weeks (LENGYEL *et al.*, 2012).

- **Isolation on solid medium after enrichment**

0.1 ml of enrichment solution was inoculated and spread into solid mediums. The mediums used are the same ones used in the enrichment, solidified with 15g/L agar at the same incubation conditions of the enrichment in Phytotron, for three weeks (ROGER and REYNAUD, 1977).

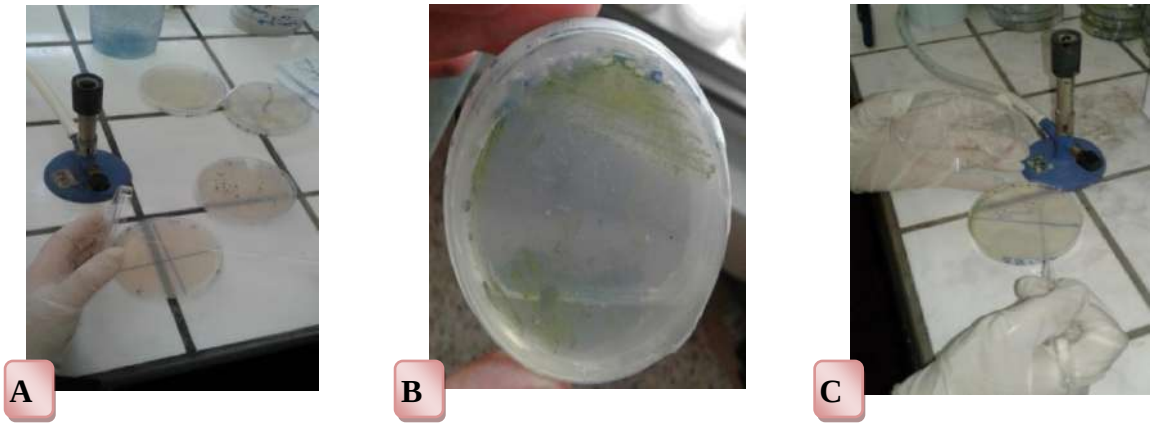
Pic. 11: Isolation on solid medium of Mehiriza irrigation basin sample after enrichment

III.4.3. Subculture (Purification)

In order to obtain pure unialgal cultures, numerous subcultures should be made. Subculture is made by taking a microalga colony at the tip of a Pasteur pipette, which is then inoculated into the new solid culture medium.

All the manipulations were carried out nearby Bunsen burner flame to avoid any risk of contamination and to maintain sterility of the working zone. Petri dishes that contained the strains were incubated at the same conditions of the culture (ROGER and REYNAUD, 1977).

A. For Hassi Ben Abd Allah lake sample, three successive subcultures were made at the same mediums where the colonies appeared (Pic. 13).



Pic. 12: Subculture of Hassi Ben Abd Allah lake sample, A: first subculture, B: second subculture, C: third subculture

B. For Mehiriza irrigation basin sample, one subculture was made at the four mediums where the colonies appeared (Pic. 14).

Pic. 13: Subculture of Mehiriza irrigation basin sample

III.5. Microscopic identification

After two weeks, we started the microscopic observations of the results that appeared in both liquid and solid mediums of the two samples using optical microscope with different magnifications.

During our microscopic observation, a fixative solution and a colorant were used. Lugol solution as a fixative and Brilliant cresyl blue as a colorant.

III.6. Preservation of strains

After purifying the strains obtained from the two samples by successive subculture, we proceeded to the final step which is the preservation of these strains.

This process is done by taking a colony from the last subculture and inoculates it in a test tube containing 10 ml of liquid medium and then place them in refregerater at 4 C°, the same medium used in the subculture. This method makes it possible to preserve the strains for a long time.

Pic. 14: Preservation steps

Chapter IV

Results and discussion

IV.1. Physico-chemical measurement results

The physico-chemical measurement results are shown in Table 6 and 7.

Tab. 6: Measurement results of Hassi Ben Abd Allah lake sample

Parameter	Value
pH	8.1
Temperature	23.1 °C
Electrical conductivity	29.1 $m.S^{-1}.cm^{-1}$

Tab. 7: Measurement results of Mehiriza irrigation basin

Parameter	Value
pH	8.3
Temperature	26 °C
Electrical conductivity	6.1 $m.S^{-1}.cm^{-1}$

Electrical conductivity mesurement is used to assess and monitor the overall mineralization of water (REJSEK, 2002). It follows the rate of salinity in all the locations studied, we have recorded 29.1 $m.S^{-1}.cm^{-1}$ electrical conductivity in Hassi Ben Abd Allah lake water and 6.1 $m.S^{-1}.cm^{-1}$ in Mehiriza irrigation basin water. Electrical conductivity is also influenced by the temperature and pH of the water (HADE, 2002).

The measurement results of physico-chemical parameters show a variation, in both samples. The recorded value of electrical conductivity of Hassi Ben Abd Allah lake water sample indicates that this water has a high concentration of minerals, which promotes and has a high impact on algal biodiversity (HADE, 2002) in this location with 35 species. On the contrary, the low value of electrical conductivity recorded in Mehriza irrigation basin water sample explains the low number of species identified (16 species).

Temperatures recorded in both locations are close to each other. pH mesurments results show that both of the water samples are slightly alkaline, which makes them a favorable environment for microalgal growth (GOLDMAN *et al.*, 1982).

IV.1. Culturing results

IV.1.1. Hassi Ben Abd Allah lake sample

Tab. 8: Enrichment results of Hassi Ben Abd Allah lake sample

	Color turn	Color	Color Intensity
BBM	+	Green	Intense
F/2	+	Green	Intense
BG-11	+	Green	Intense
Half Strength Chu #10	+	Brown	Weak

Positive results of Hassi Ben Abd Allah lake sample enrichment have appeared in all mediums (BBM, F/2, BG-11 and Half Strength Chu #10) used after three weeks with variation of color green and brown. Then, a high color intensity is noted in BBM, F/2 and BG-11. That indicates to the presence of microorganismes in Hassi Ben Abd Allah lake water (Tab. 8).

Results of isolation on solid medium by the dilution technique of Hassi Ben Abd Allah lake sample show that microalgal colonies appeared in all mediums after three weeks with the variation of number; 1 to 30 colonies; color green, white with black center, red, brownish green and black and size very small to big colonies (Tab. 9).

After three weeks, Hassi Ben Abd Allah lake sample results of isolation on solid medium after enrichment, show the appearance of a mat of microalgae on all of the solid mediums, which was followed later by a series of purification.

Tab.9: Isolation on solid medium by dilution technique results of Hassi ben Abd Allah lake sample

		Stock solution	Dilution 10^{-1}	Dilution 10^{-2}
BBM	Presence or absence	+	+	-
	Number	15	1	/
	Color	Green + Black	Black	/
	Size	2 medium + 13 very small	Small	/
F/2	Presence or absence	+	+	-
	Number	30	7	/
	Color	Green + White with black center	Red + Brownish green	/
	Size	Big + Medium + Small	Small	/
BG-11	Presence or absence	+	+	+
	Number	15	2	3
	Color	Green	Green	Green
	Size	Medium	Medium	Medium
Half Strength Chu #10	Presence or absence	+	+	+
	Number	5	2	3
	Color	Green	Green	Green
	Size	Small	Small	Small

IV.1.2. Sample from Mehiriza irrigation basin

Tab. 10: Enrichment results of Mehiriza irrigation basin sample

	Color turn	Color	Color Intensity
BBM	+	Brownish	Intense
F/2	+	Brown	Intense
BG-11	+	Green	Intense
Half Strength Chu #10	+	Pinkish	Medium

Enrichment of Mehiriza irrigation basin sample show positive results appeared in all mediums (BBM, F/2, BG-11 and Half Strength Chu #10) used after three weeks with variation of color green, brownish, brown and pinkish and its intensity. Then, a high color

intensity is noted in BBM, F/2 and BG-11. That indicates to the presence of microorganismes in Mehiriza irrigation basin water (Tab. 10).

Tab. 11: Isolation on solid medium results of Mehiriza irrigation basin sample

		Stock solution
BBM	Presence or absence	+
	Number	30
	Color	Green
	Size	Medium + Small
F/2	Presence or absence	+
	Number	40
	Color	Green
	Size	Medium + Small
BG-11	Presence or absence	+
	Number	55
	Color	Green
	Size	Medium + Small
Half Strength Chu #10	Presence or absence	+
	Number	50
	Color	Green + Brown
	Size	Medium + Small

Mehiriza irrigation basin sample results of isolation on solid medium after three weeks, show that microalgal colonies appeared in all mediums with the variation of number; 30 to 55 colonies, color; green and brown and with the same sizes; medium and small (Tab. 11).

IV.2. Species identification

Observation under an optical microscope, of morpho-anatomical characters of microalgae harvested, allowed us to identify 46 species, 35 species in Hassi Ben Abd Allah lake and 16 species in Mehiriza irrigation basin (Tab. 12, Tab. 13 and Tab. 14).

The species were identified using *Algaebase* database (http://www.algaebase.org) and with the help of Microorganisms Laboratory: Genome and Environment (France).

Characterization of algal species has shown the dominance of **Bacillariophyceae** class with 20 species followed by **Chlorophyceae** with 16 species, **Cyanophyceae** with 6 species then **Euglenophyceae** with two species and finally **Xanthophyceae** and **Chrysophyceae** with only one specie in each of these two classes. Most of the identified microalgae (35 species) were found in Hassi Ben Abdallah lake.

Results show a difference in microalgal flora between the two locations studied with some similarities. That may be due to environmental conditions and water composition (Tab. 13).

The mediums (BBM, F/2, BG-11 and Half Strength Chu #10) that we have used were all effective, positive results have appeared in all of them.

Most of the species identified were found in F/2 solid medium with 29 species, most of them are diatoms. Followed by BBM solid medium with 15 species, the majority were chlorophyts and 03 species of cyanobacteria (Tab. 14).

9 species: *Chlorella vulgaris*, *Ulnaria ulna*, *Caloneis elongata*, *Monoraphidium komarkovae*, *Neochloris pseudoalveolaris*, *Microcystis sp.*, *Prochloron sp.*, *Monodopsis unipapilla*, *Oscillatoria sp.* were found in several mediums in liquid and solid forms.

The fixed water sample from Mehiriza irrigation basin contained 08 species; *Navicula sp.*, *Ulnaria ulna*, *Caloneis elongata*, *Cosmarium sp.*, *Cosmarium laeve*, *Chlamydomonas sp.* (b), *Chroococcus turgidus*, *Oscillatoria sp.*, from three classes; Bacillariophyceae, Chlorophyceae, Cyanophyceae.

Tab. 12: Identified strains list according to class

Class	Specie
	Pinnularia sp. (a)
	Pinnularia sp. (b)
	Pinnularia sp. (c)
	Pinnularia sp. (d)
	Pinnularia sp. (e)
	Pinnularia sp. (f)
	Pinnularia sp. (g)

Bacillariophyceae	*Pinnularia sp.* (h)
	Diploneis subovalis
	Pinnularia sp. (i)
	Achnanthidium exiguum
	Navicula amphora
	Planothidium lanceolatum
	Fragilaria capucina
	Nitzschia hungarica
	Navicula sp.
	Planothidium lanceolatum
	Ulnaria ulna
	Navicula gregaria
	Caloneis elongata
Chlorophyceae	*Haematococcus pluvialis*
	Chlamydomonas monadina
	Chlamydomonas perpusillus
	Chlamydomonas sp.
	Chlamydomonas geitleri
	Gloeocystis ampla
	Trochiscia hystrix
	Neospongiococcum sphaericum
	Chlorella vulgaris
	Sphaerocystis schroeteri
	Cosmarium biretum
	Cosmarium laeve
	Cosmarium sp.
	Monoraphidium komarkovae
	Gloeocystis sp.
	Neochloris pseudoalveolaris
	Prochloron sp.
	Microcystis sp.
	Gomphosphaeria aponina

Cyanophyceae	*Chroococcus turgidus*
	Synechocystis sp.
	Oscillatoria sp.
Euglenophyceae	*Euglena sp.* (a)
	Euglena sp. (b)
Xanthophyceae	*Monodopsis unipapilla*
Chrysophyceae	*Mallomonas sp.*

Tab. 13: Identified strains list according to sampling areas

Sampling areas / Strain	Hassi Ben Abdallah lake	Mehiriza irrigation basin
Pinnularia sp. (a)	+	-
Pinnularia sp. (b)	+	-
Pinnularia sp. (c)	+	-
Pinnularia sp. (d)	+	-
Pinnularia sp. (e)	+	-
Pinnularia sp. (f)	+	-
Pinnularia sp. (g)	+	-
Pinnularia sp. (h)	+	-
Diploneis subovalis	+	-
Pinnularia sp. (i)	+	-
Achnanthidium exiguum	+	-
Navicula amphora	+	-
Planothidium lanceolatum	+	-
Fragilaria capucina	-	+
Nitzschia hungarica	-	+
Navicula sp.	-	+
Planothidium lanceolatum	-	+
Ulnaria ulna	-	+
Navicula gregaria	-	+

Caloneis elongata	+	+
Haematococcus pluvialis	+	-
Chlamydomonas monadina	+	-
Chlamydomonas perpusillus	+	-
Chlamydomonas sp. (a)	+	-
Chlamydomonas geitleri	+	-
Gloeocystis ampla	+	-
Trochiscia hystrix	+	-
Neospongiococcum sphaericum	+	-
Chlorella vulgaris	+	-
Sphaerocystis schroeteri	+	-
Cosmarium biretum	-	+
Cosmarium laeve	-	+
Cosmarium sp.	-	+
Monoraphidium komarkovae	+	-
Gloeocystis sp.	+	-
Neochloris pseudoalveolaris	+	-
Chlamydomonas sp. (b)	-	+
Prochloron sp.	+	+
Microcystis sp.	+	+
Gomphosphaeria aponina	+	-
Chroococcus turgidus	-	+
Synechocystis sp.	+	-
Oscillatoria sp.	+	+
Euglena sp. (a)	+	-
Euglena sp. (b)	+	-
Monodopsis unipapilla	+	-
Mallomonas sp.	-	+

Tab. 14: Identified strains list according to culture mediums

Strains \ Culture medium	BBM		F/2		BG-11		H-S-C # 10		Fixed water sample
	S	L	S	L	S	L	S	L	
Pinnularia sp. (a)	-	-	+	-	-	-	-	-	-
Pinnularia sp. (b)	-	-	+	-	-	-	-	-	-
Pinnularia sp. (c)	-	-	+	-	-	-	-	-	-
Pinnularia sp. (d)	-	-	+	-	-	-	-	-	-
Pinnularia sp. (e)	-	-	+	-	-	-	-	-	-
Pinnularia sp. (f)	-	-	+	-	-	-	-	-	-
Pinnularia sp. (g)	-	-	+	-	-	-	-	-	-
Pinnularia sp. (h)	-	-	+	-	-	-	-	-	-
Diploneis subovalis	-	-	+	-	-	-	-	-	-
Pinnularia sp. (i)	-	-	+	-	-	-	-	-	-
Achnanthidium exiguum	-	-	+	-	-	-	-	-	-
Navicula amphora	-	-	+	-	-	-	-	-	-
Planothidium lanceolatum	-	-	+	-	-	-	-	-	-
Fragilaria capucina	-	-	+	-	-	-	-	-	-
Nitzschia hungarica	-	-	+	-	-	-	-	-	-
Navicula sp.	-	-	-	-	-	-	-	+	+
Planothidium lanceolatum	-	-	+	-	-	-	-	-	-
Ulnaria ulna	-	+	+	-	-	+	-	+	+
Navicula gregaria	-	-	+	-	-	-	-	-	-
Caloneis elongata	-	+	-	+	-	+	-	+	+
Haematococcus pluvialis	+	-	-	-	-	-	-	-	-
Chlamydomonas monadina	+	-	-	-	-	-	-	-	-
Chlamydomonas perpusillus	+	-	-	-	-	-	-	-	-
Chlamydomonas sp. (a)	+	-	-	-	-	-	-	-	-
Chlamydomonas geitleri	+	-	-	-	-	-	-	-	-
Gloeocystis ampla	+	-	-	-	-	-	-	-	-
Trochiscia hystrix	+	-	-	-	-	-	-	-	-
Neospongiococcum sphaericum	+	-	-	-	-	-	-	-	-

Chlorella vulgaris	+	+	+	+	+	+	-	-	-
Sphaerocystis schroeteri	-	-	+	-	-	-	-	-	-
Cosmarium biretum	-	-	-	-	-	-	-	+	-
Cosmarium laeve	-	-	-	-	-	-	-	-	+
Cosmarium sp.	-	-	-	-	-	-	-	-	+
Monoraphidium komarkovae	+	-	+	-	-	-	+	-	-
Gloeocystis sp.	-	-	+	-	-	-	-	-	-
Neochloris pseudoalveolaris	+	-	+	-	-	-	-	-	-
Chlamydomonas sp. (b)	-	-	-	-	-	-	-	-	+
Prochloron sp.	+	-	+	+	+	-	+	-	-
Microcystis sp.	+	-	+	-	+	-	+	-	-
Gomphosphaeria aponina	+	-	-	-	-	-	-	-	-
Chroococcus turgidus	-	-	-	-	-	-	-	-	+
Synechocystis sp.	-	-	+	-	-	-	-	-	-
Oscillatoria sp.	-	+	+	+	+	+	-	+	+
Euglena sp. (a)	-	-	+	-	-	-	-	-	-
Euglena sp. (b)	+	-	-	-	-	-	-	-	-
Monodopsis unipapilla	-	-	+	-	-	+	-	-	-
Mallomonas sp.	-	-	-	+	-	-	-	-	-

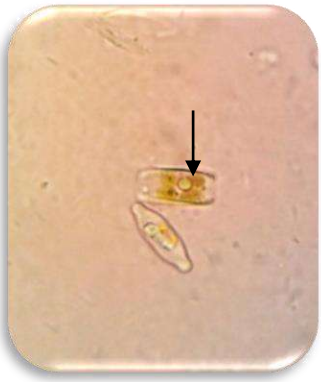
IV.3. Species Classification

Below are photos of microalgae taken at the time of observation, with different magnifications (Gx 60, Gx 100).

IV.3.1. Bacillariophyceae

Bacillariophyceae (Diatoms), with yellow or golden brown chromatophores containing, apart from the usual ones, accessory brown pigments of disputed nature. The products of photosynthesis are fat and volutin. They are unicellular or colonial. A cell-wall is always present and is composed partly of pectic substances and partly of silica. One set of forms (Centrales) is radially, the other (Pennales) bilaterally symmetrical. The Diatoms are a highly differentiated group. The members of this class are probably all diploid. Diatoms are

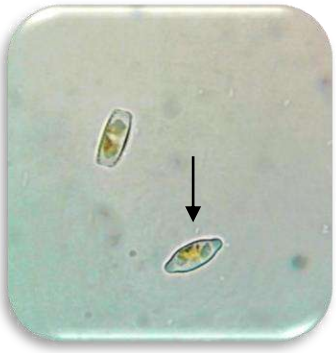
very widely distributed in the sea and in all kinds of freshwaters, as well as in the soil and in other terrestrial habitats (FRITSCH, 1935).



Pic. 15: *Pinnularia sp*. (a) (Gx100)

Pinnularia sp. (a) (Hustedt, 1935).

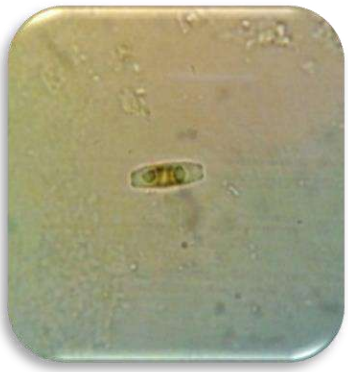
Kingdom Chromista
Phylum Bacillariophyta
Class Bacillariophyceae
Order Naviculales
Family Pinnulariaceae
Genus Pinnularia



Pic. 16: *Pinnularia sp*. (b) (Gx100)

Pinnularia sp. (b) (Hustedt, 1935).

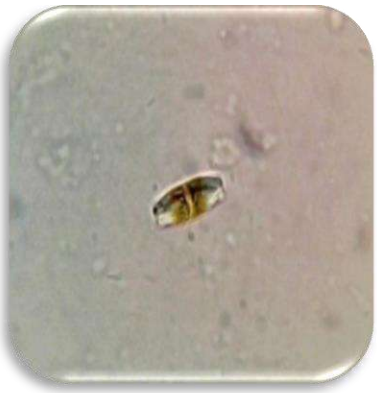
Kingdom Chromista
Phylum Bacillariophyta
Class Bacillariophyceae
Order Naviculales
Family Pinnulariaceae
Genus Pinnularia



Pic. 17: *Pinnularia sp.* (c) (Gx100)

Pinnularia sp. (c) (Hustedt, 1935).

Kingdom Chromista
Phylum Bacillariophyta
Class Bacillariophyceae
Order Naviculales
Family Pinnulariaceae
Genus Pinnularia



Pinnularia sp.(d) (Hustedt, 1935).

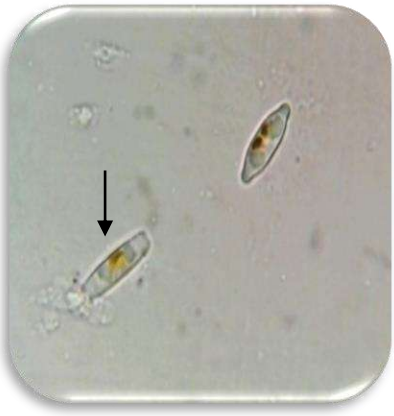
Kingdom Chromista
Phylum Bacillariophyta
Class Bacillariophyceae
Order Naviculales
Family Pinnulariaceae
Genus Pinnularia

Pic. 18: *Pinnularia sp.* (d) (Gx100)

Pinnularia sp. (e) (Hustedt, 1935).

Kingdom Chromista
Phylum Bacillariophyta
Class Bacillariophyceae
Order Naviculales
Family Pinnulariaceae
Genus Pinnularia

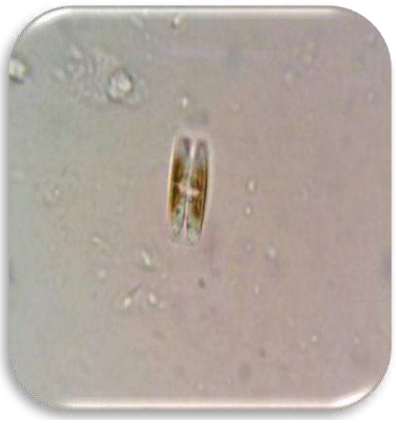
Pic. 19: *Pinnularia sp.* (e) (Gx100)



Pinnularia sp. (f) (Hustedt, 1935).

Kingdom Chromista
Phylum Bacillariophyta
Class Bacillariophyceae
Order Naviculales
Family Pinnulariaceae
Genus Pinnularia

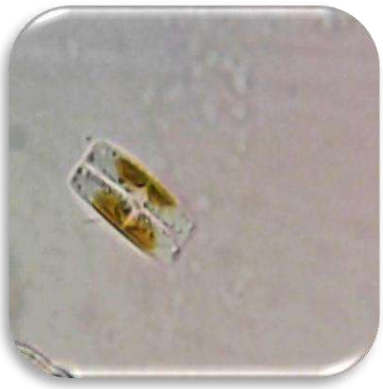
Pic. 20: *Pinnularia sp.* (f) (Gx100)



Pinnularia sp.(g) (Hustedt, 1935).

Kingdom Chromista
Phylum Bacillariophyta
Class Bacillariophyceae
Order Naviculales
Family Pinnulariaceae
Genus Pinnularia

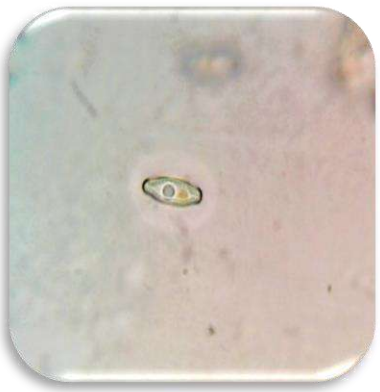
Pic. 21: *Pinnularia sp.* (g) (Gx100)



Pinnularia sp. (h) (Hustedt, 1935).

Kingdom Chromista
Phylum Bacillariophyta
Class Bacillariophyceae
Order Naviculales
Family Pinnulariaceae
Genus Pinnularia

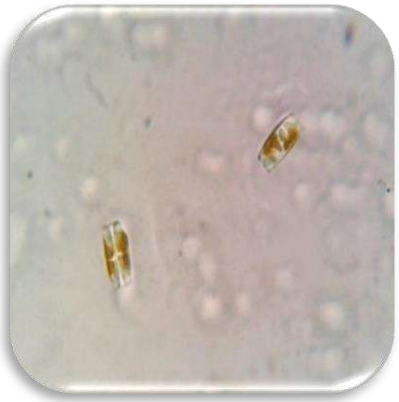
Pic. 22: *Pinnularia sp.* (h) (Gx100)



Diploneis subovalis (Cleve, 1894).

Kingdom Chromista
Phylum Bacillariophyta
Class Bacillariophyceae
Order Naviculales
Family Diploneidaceae
Genus Diploneis

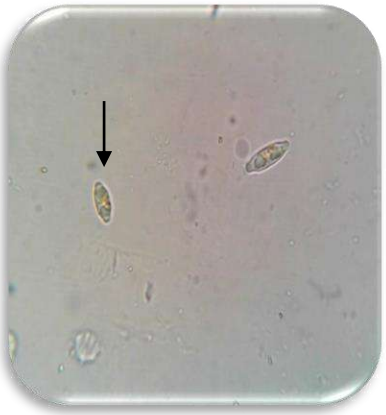
Pic. 23: *Diploneis subovalis* (Gx100)



Pinnularia sp.(i) (Hustedt, 1935).

Kingdom Chromista
Phylum Bacillariophyta
Class Bacillariophyceae
Order Naviculales
Family Pinnulariaceae
Genus Pinnularia

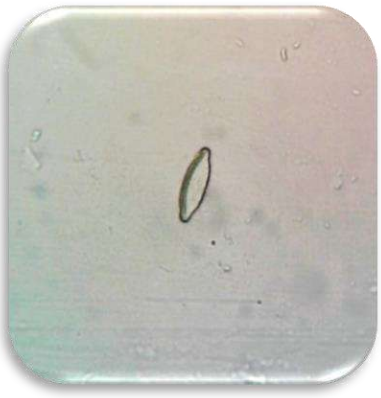
Pic. 24: *Pinnularia sp.* (i) (Gx100)



Achnanthidium exiguum (Czarnecki, 1994).

Kingdom Chromista
Phylum Bacillariophyta
Class Bacillariophyceae
Order Cocconeidales
Family Achnanthidiaceae
Genus Achnanthidium

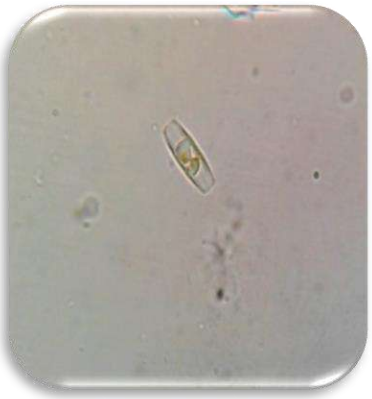
Pic. 25: *Achnanthidium exiguum* (Gx100)



Navicula amphora (Ehrenberg, 1832).

Kingdom Chromista
Phylum Bacillariophyta
Class Bacillariophyceae
Order Naviculales
Family Naviculaceae
Genus Navicula

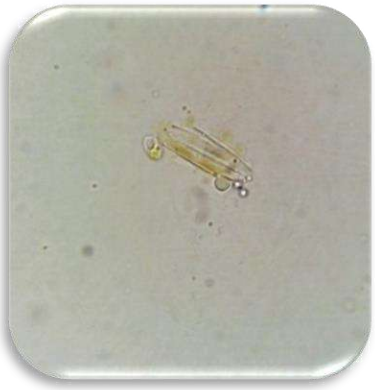
Pic. 26: *Navicula amphora* (Gx100)



Planothidium lanceolatum (Lange-Bertalot, 1999).

Kingdom Chromista
Phylum Bacillariophyta
Class Bacillariophyceae
Order Cocconeidales
Family Achnanthidiaceae
Genus Planothidium

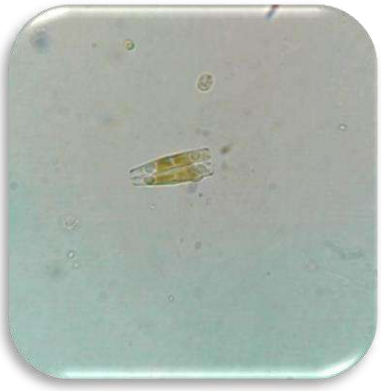
Pic. 27: *Planothidium lanceolatum* (Gx100)



Fragilaria capucina (Desmazières, 1830).

Kingdom Chromista
Phylum Bacillariophyta
Class Bacillariophyceae
Order Fragilariales
Family Fragilariaceae
Genus Fragilaria

Pic. 28: *Fragilaria capucina* (Gx100)



Nitzschia hungarica (Grunow, 1862).

Kingdom Chromista
Phylum Bacillariophyta
Class Bacillariophyceae
Order Bacillariales
Family Bacillariaceae
Genus Nitzschia

Pic. 29: *Nitzschia hungarica* (Gx100)

Navicula sp. (Bory de Saint-Vincent, 1822).

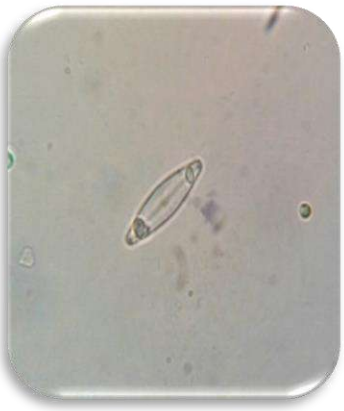
Kingdom Chromista
Phylum Bacillariophyta
Class Bacillariophyceae
Order Naviculales
Family Naviculaceae
Genus Navicula

Pic. 30: *Navicula sp*. (Gx100)

Planothidium lanceolatum (Lange-Bertalot, 1999).

Kingdom Chromista
Phylum Bacillariophyta
Class Bacillariophyceae
Order Cocconeidales
Family Achnanthidiaceae
Genus Planothidium

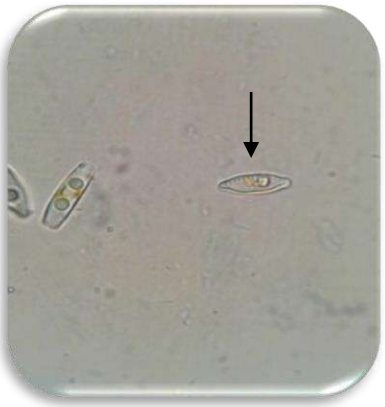
Pic. 31: *Planothidium lanceolatum* (Gx100)



Ulnaria ulna (Compère, 2001).

Kingdom Chromista
Phylum Bacillariophyta
Class Bacillariophyceae
Order Licmophorales
Family Ulnariaceae
Genus Ulnaria

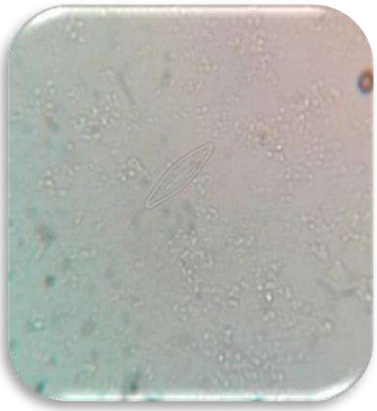
Pic. 32: *Ulnaria ulna* (Gx100)



Navicula gregaria (Donkin, 1861).

Kingdom Chromista
Phylum Bacillariophyta
Class Bacillariophyceae
Order Naviculales
Family Naviculaceae
Genus Navicula

Pic. 33: *Navicula gregaria* (Gx100)



Caloneis elongata (Boyer, 1927).

Kingdom Chromista
Phylum Bacillariophyta
Class Bacillariophyceae
Order Naviculales
Family Naviculaceae
Genus Caloneis

Pic. 34: *Caloneis elongata* (Gx100)

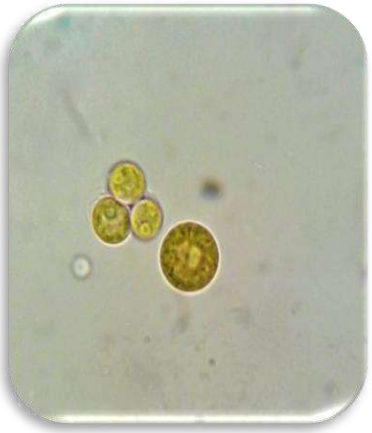
IV.3.2. Chlorophyceae

Chlorophyceae, with chromatophores which are grass green and contain the same four pigments (two green, two yellow) and approximately in the same proportions as in higher plants. Starch is the customary form of storage of the products of photosynthesis, often (especially in resting stages) accompanied by oil. The algal members have a cell-wall in which cellulose is often a prominent constituent. The majority of the members are algal and many exhibit sexuality (ranging from isogamy to advanced oogamy, usually with retention of the ovum). The class is more widely represented in fresh than in salt water, and there is a marked terrestrial tendency (FRITSCH, 1935).

Haematococcus pluvialis (Flotow, 1844).

Kingdom Plantae
Phylum Chlorophyta
Class Chlorophyceae
Order Chlamydomonadales
Family Haematococcaceae
Genus Haematococcus

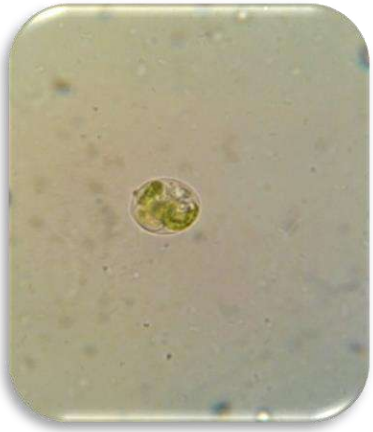
Pic. 35: *Haematococcus pluvialis* (Gx100)



Chlamydomonas monadina (Stein, 1878).

Kingdom Plantae
Phylum Chlorophyta
Class Chlorophyceae
Order Chlamydomonadales
Family Chlamydomonadaceae
Genus Chlamydomonas

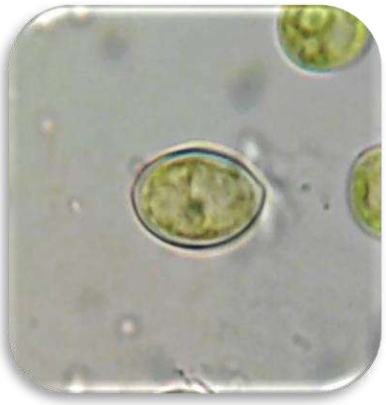
Pic. 36: *Chlamydomonas monadina* (Gx100)



Chlamydomonas perpusillus (Ehrenb, 1835).

Kingdom Plantae
Phylum Chlorophyta
Class Chlorophyceae
Order Chlamydomonadales
Family Chlamydomonadaceae
Genus Chlamydomonas

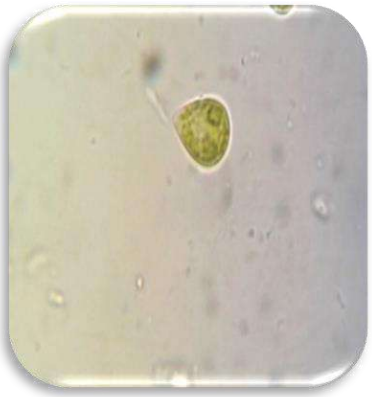
Pic. 37: *Chlamydomonas perpusillus* (Gx100)



Chlamydomonas sp. (Ehrenb, 1835).

Kingdom Plantae
Phylum Chlorophyta
Class Chlorophyceae
Order Chlamydomonadales
Family Chlamydomonadaceae
Genus Chlamydomonas

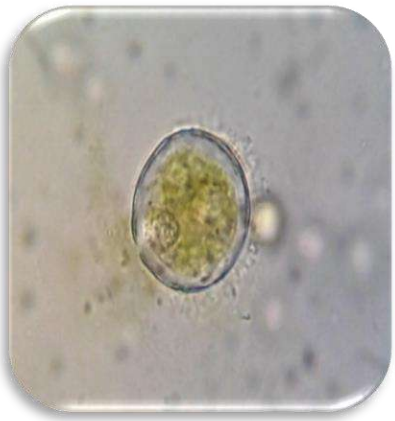
Pic. 38: *Chlamydomonas sp*. (a) (Gx100)



Chlamydomonas geitleri (Ettl, 1969).

Kingdom Plantae
Phylum Chlorophyta
Class Chlorophyceae
Order Chlamydomonadales
Family Chlamydomonadaceae
Genus Chlamydomonas

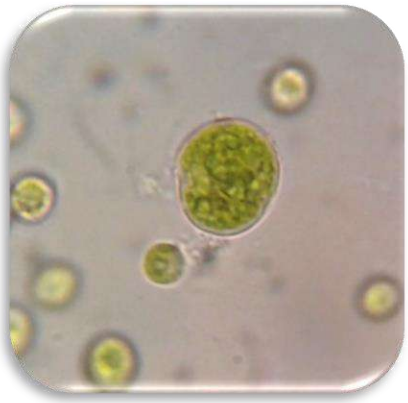
Pic. 39: *Chlamydomonas geitleri* (Gx100)



Gloeocystis ampla (Rabenhorst, 1863).

Kingdom Plantae
Phylum Chlorophyta
Class Chlorophyceae
Order Sphaeropleales
Family Radiococcaceae
Genus Gloeocystis

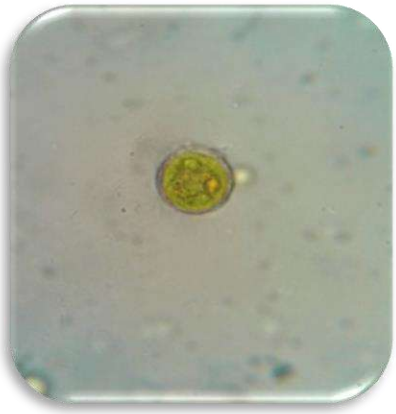
Pic. 40: *Gloeocystis ampla* (Gx100)



Trochiscia hystrix (Hansgirg, 1888).

Kingdom Plantae
Phylum Chlorophyta
Class Trebouxiophyceae
Order Chlorellales
Family Oocystaceae
Genus Trochiscia

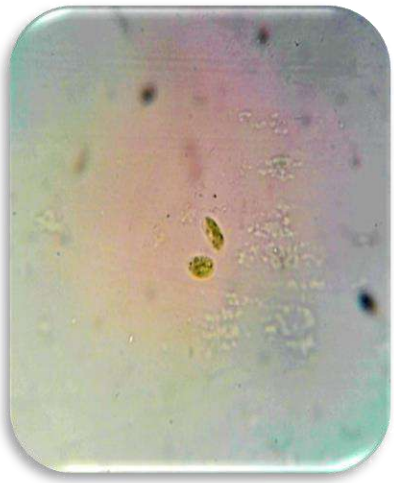
Pic. 41: *Trochiscia hystrix* (Gx100)



Neospongiococcum sphaericum (Deason, 1976).

Kingdom Plantae
Phylum Chlorophyta
Class Chlorophyceae
Order Chlamydomonadales
Family Chlorococcaceae
Genus Neospongiococcum

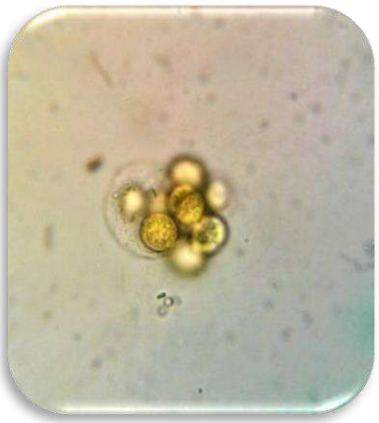
Pic. 42: *Neospongiococcum sphaericum* (Gx100)



Chlorella vulgaris (Beijerinck, 1890).

Kingdom Plantae
Phylum Chlorophyta
Class Trebouxiophyceae
Order Chlorellales
Family Chlorellaceae
Genus Chlorella

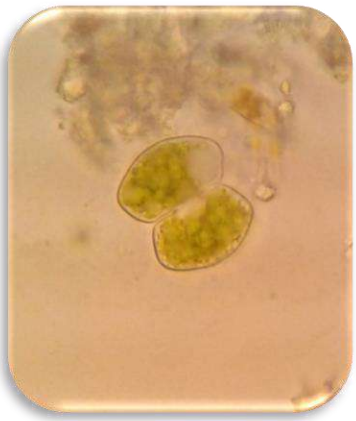
Pic. 43: *Chlorella vulgaris* (Gx100)



Sphaerocystis schroeteri (Chodat, 1897).

Kingdom Plantae
Phylum Chlorophyta
Class Chlorophyceae
Order Chlamydomonadales
Family Sphaerocystidaceae
Genus Sphaerocystis

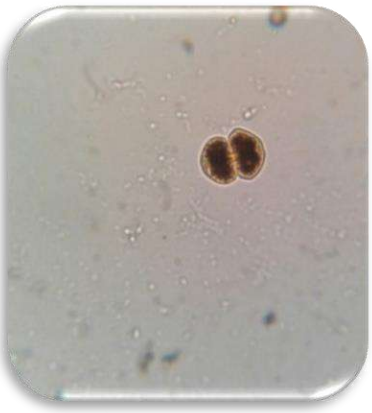
Pic. 44: *Sphaerocystis schroeteri* (Gx100)



Cosmarium biretum (Ralfs, 1848).

Kingdom Plantae
Phylum Charophyta
Class Conjugatophyceae
Order Desmidiales
Family Desmidiaceae
Genus Cosmarium

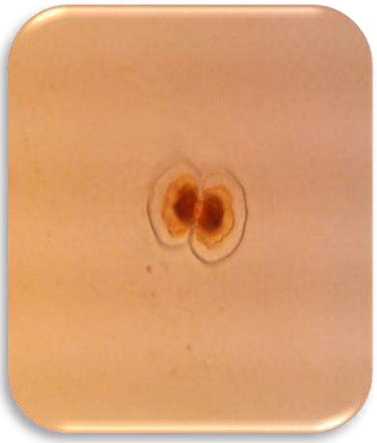
Pic. 45: *Cosmarium biretum* (Gx100)



Cosmarium leave (Rabenhorst, 1868).

Kingdom Plantae
Phylum Charophyta
Class Conjugatophyceae
Order Desmidiales
Family Desmidiaceae
Genus Cosmarium

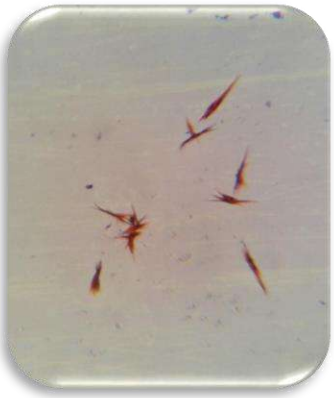
Pic. 46: *Cosmarium laeve* (Gx100)



Cosmarium sp. (Archer, 1861).

Kingdom Plantae
Phylum Charophyta
Class Conjugatophyceae
Order Desmidiales
Family Desmidiaceae
Genus Cosmarium

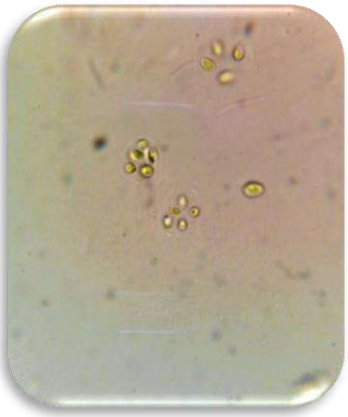
Pic. 47: *Cosmarium sp.* (Gx100)



Monoraphidium komarkovae (Nygaard, 1979).

Kingdom Plantae
Phylum Chlorophyta
Class Chlorophyceae
Order Sphaeropleales
Family Selenastraceae
Genus Monoraphidium

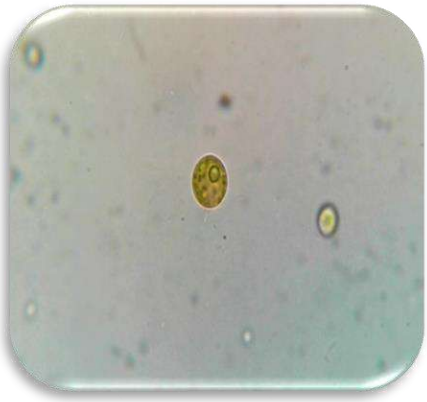
Pic. 48: *Monoraphidium komarkovae* (Gx100)



Gloeocystis sp. (Nägeli, 1849).

Kingdom Plantae
Phylum Chlorophyta
Class Chlorophyceae
Order Sphaeropleales
Family Radiococcaceae
Genus Gloeocystis

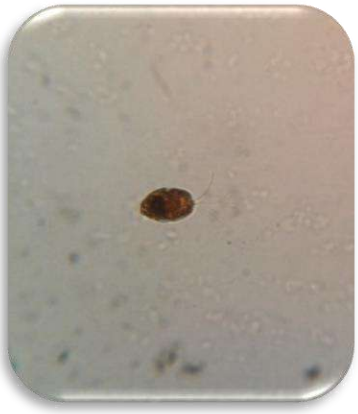
Pic. 49: *Gloeocystis sp*. (Gx100)



Neochloris pseudoalveolaris (Deason and Bold, 1960).

Kingdom Plantae
Phylum Chlorophyta
Class Chlorophyceae
Order Sphaeropleales
Family Neochloridaceae
Genus Neochloris

Pic. 50: *Neochloris pseudoalveolaris* (Gx100)



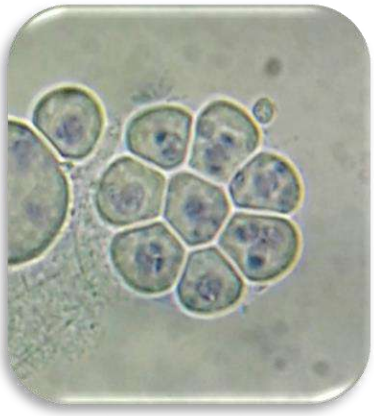
Chlamydomonas sp. (Ehrenb, 1835).

Kingdom Plantae
Phylum Chlorophyta
Class Chlorophyceae
Order Chlamydomonadales
Family Chlamydomonadaceae
Genus Chlamydomonas

Pic. 51: *Chlamydomonas sp.* (b) (Gx100)

IV.3.3. Cyanophyceae

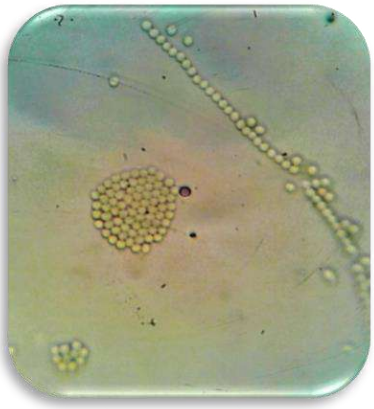
Cyanophyceae, with a simple type of cell, containing at the best only a very rudimentary nucleus (central body) and without a proper chromatophore, the photosynthetic pigments being diffused through the peripheral cytoplasm. The pigments present are chlorophyll, carotene, phycocyanin, and phycberythrin, the last two in varying proportions, the colour of the cells being very commonly blue-green. The products of photosynthesis are sugars and glycogen. No motile stages are known and all the members have a membrane around the cell. There is no sexual reproduction. The members of this class are of simple organization and many propagate entirely by simple division or by vegetative means. They occur very abundantly in freshwaters and in terrestrial habitats, and are not uncommon in the sea (FRITSCH, 1935).



Prochloron sp. (Lewin, 1977).

Kingdom Eubacteria
Phylum Cyanobacteria
Class Cyanophyceae
Order Synechococcales
Family Prochloraceae

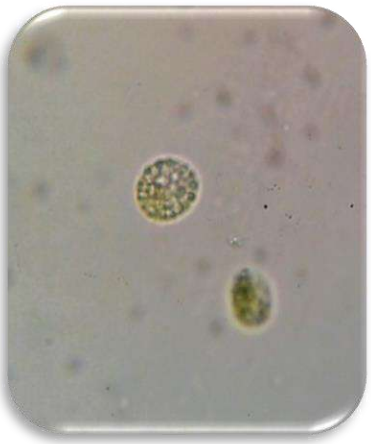
Pic. 52: *Prochloron sp.* (Gx100)



Microcystis sp. (Kützing, 1833).

Kingdom Eubacteria
Phylum Cyanobacteria
Class Cyanophyceae
Order Chroococcales
Family Microcystaceae
Genus Microcystis

Pic. 53: *Microcystis sp.* (Gx100)



Gomphosphaeria aponina (Kützing, 1836).

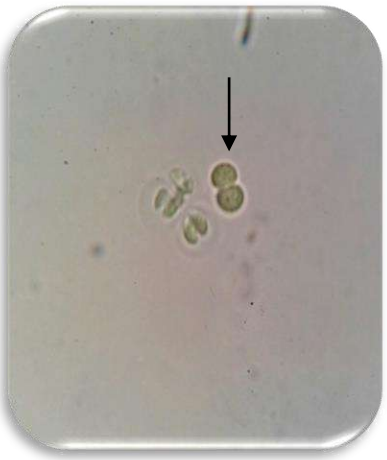
Kingdom Eubacteria
Phylum Cyanobacteria
Class Cyanophyceae
Order Chroococcales
Family Gomphosphaeriaceae
Genus Gomphosphaeria

Pic. 54: *Gomphosphaeria aponina* (Gx100)

Chroococcus turgidus (Nägeli, 1849).

Kingdom Eubacteria
Phylum Cyanobacteria
Class Cyanophyceae
Order Chroococcales
Family Chroococcaceae
Genus Chroococcus

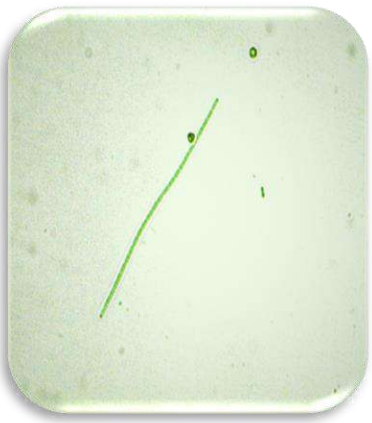
Pic. 55: *Chroococcus turgidus* (Gx100)



Synechocystis sp. (Sauvageau, 1892).

Kingdom Eubacteria
Phylum Cyanobacteria
Class Cyanophyceae
Order Chroococcales
Family Nitrospiraceae
Genus Synechocystis

Pic. 56: *Synechocystis sp.* (Gx100)



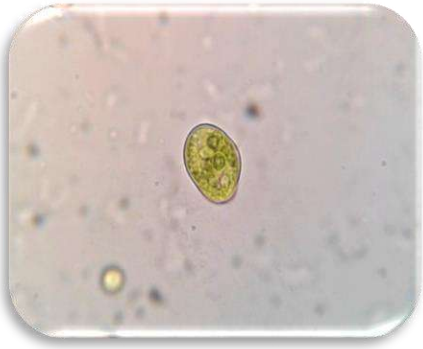
Oscillatoria sp. (Vaucher ex Gomont, 1892).

Kingdom Eubacteria
Phylum Cyanobacteria
Class Cyanophyceae
Order Oscillatoriales
Family Oscillatoriaceae
Genus Oscillatoria

Pic. 57: *Oscillatoria sp.* (Gx 60)

IV.3.4. Euglenophyceae

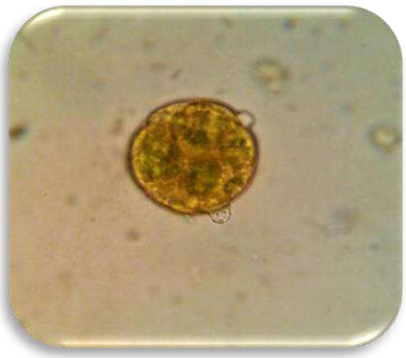
Euglenineae, with pure green chromatophores, each cell usually with several. The product of photosynthesis is a polysaccharide, paramylon, which occurs in the form of solid grains of diverse and often very distinctive shape. Only flagellate members are known and the majority are motile with the help of one or two -flagella which arise from the base of a canal-like invagination at the front end. There is a complex vacuolar system and a large and prominent nucleus. Only few cases of sexuality are known and these are not quite fully substantiated. The bulk of the members of this class probably inhabit freshwaters. The class is highly specialized and there are no really simple forms (FRITSCH, 1935).



Euglena sp. (Ehrenberg, 1830).

Kingdom Protozoa
Phylum Euglenozoa
Class Euglenophyceae
Order Euglenales
Family Euglenaceae
Genus Euglena

Pic. 58: *Euglena sp.* (a) (Gx100)



Euglena sp. (Ehrenberg, 1830).

Kingdom Protozoa
Phylum Euglenozoa
Class Euglenophyceae
Order Euglenales
Family Euglenaceae
Genus Euglena

Pic. 59: *Euglena sp.* (b) (Gx100)

IV.3.5. Xanthophyceae

Xanthophyceae, with chromatophores which are yellow-green owing to the presence of an excess of the yellow xanthophyll. Starch is absent, oil being the customary storage-product. The algal members have a cell-wall which is often rich in pectic compounds and which is frequently composed of two equal or unequal pieces overlapping at their edges. The motile cells posses's two very unequal flagella (or sometimes only one) arising from the front end. As a general rule the cells contain a number of discoid chromatophores. The majority of the members are algal, but sexual reproduction is rare and always isogamous. The most advanced forms have a simple filamentous habit. All are probably haploid. The class is more widely distributed in freshwater than in the sea (FRITSCH, 1935).

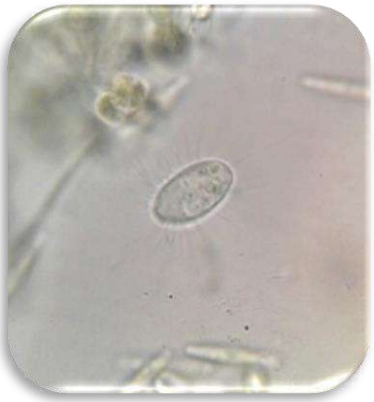
Monodopsis unipapilla (Reisigl, 1964).

Kingdom Chromista
Phylum Ochrophyta
Class Xanthophyceae
Order Mischococcales
Family Pleurochloridaceae
Genus Monodus

Pic. 60: *Monodopsis unipapilla* (Gx100)

IV.3.6. Chrysophyceae

Chrysophyceae, with brown or orange-coloured chromatophores containing one or more accessory pigments (phycochrysin). Starch is absent, fat and a compound leucosin occurring in the form of rounded whitish opaque lumps, are the customary forms of food-storage. A large proportion of the members are flagellate and devoid of a special cell-membrane. The motile cells possess one or two (rarely three) flagella attached at the front end. The cells typically contain one or two parietal chromatophore. The most advanced habit is that of a branched filament. Sexual reproduction is extremely rare and not yet quite clearly established in any case. The class is widely distributed in colder freshwaters, but a few families are marine (FRITSCH, 1935).



Mallomonas sp. (Perty, 1852).

Kingdom Chromista
Phylum Ochrophyta
Class Chrysophyceae
Order Synurales
Family Mallomonadaceae
Genus Mallomonas

Pic. 61: *Mallomonas sp.* (Gx100)

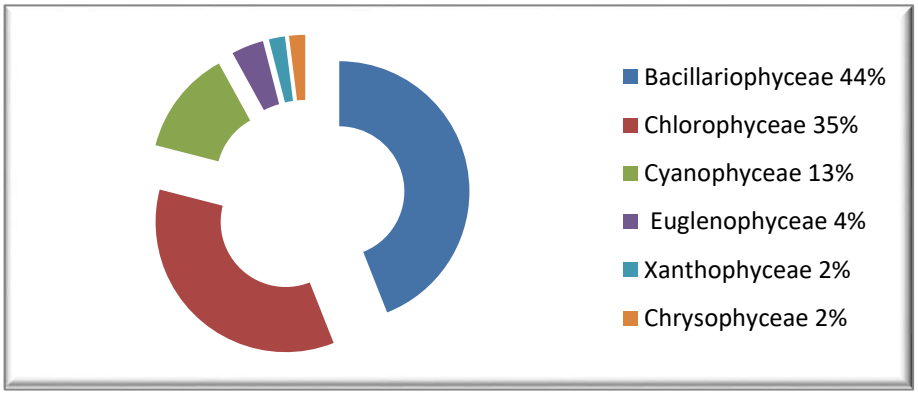


Fig. 18: Global graphic representation of microalgae found in both locations

The microalgal flora found is dominated by Bacillariophyceae which represent 44% of the global algae classes identified. Moreover, Chlorophyceae represent the second predominant class with 35% followed by Cyanophyceae with about 13% (Fig. 18).

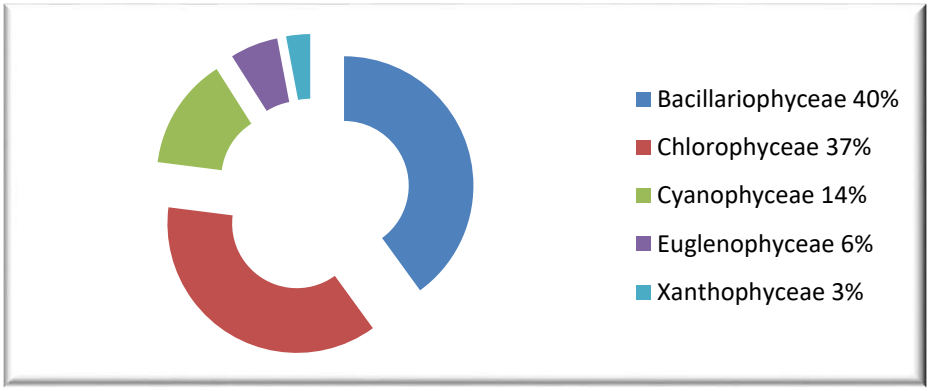


Fig. 19: Graphical representation of microalgae found in Hassi Ben Abd Allah lake

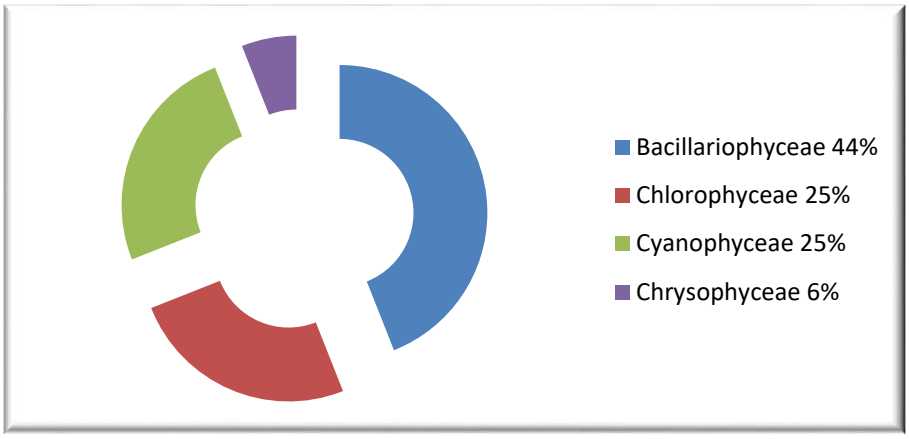


Fig. 20: Graphical representation of microalgae found in Mehiriza irrigation basin

The results (Fig. 19 and Fig. 20) show that Bacillariophyceae class is dominant in both locations, followed by Chlorophyceae with close values. Classes of microalgae appeared only in one location, like Euglenophyceae and Xanthophyceae in Hassi Ben Abd Allah lake and Chrysophyceae in Mehiriza irrigation basin.

The abundance and diversity of microalgal species in the lake compared to the irrigation basine has several reasons, which are:

- The lake is an older and more stable water body than the irrigation basin.
- Basin water is regularly renewed for irrigation.
- Lake water is highly eutrophic by the presence of urban waste.

Algae are ideally suited for water quality assessment because they have rapid reproduction rates and very short life cycles, making them valuable indicators (WAN MAZNAH WAN, 2010). Biological indicators (bioindicators) are defined as particular species or communities, which, by their presence, provide information on the surrounding physical and/or chemical environment at a particular site (BELLINGER and SIGEE, 2010).

Diatoms have been used extensively in water quality assessment and in monitoring human impacts on freshwater systems (BELLINGER and SIGEE, 2010). They exist in a wide range of ecological conditions, colonizing almost all suitable habitats; they can thus provide multiple indicators of environmental change (WAN MAZNAH WAN, 2010). The presence of diatoms indicates that the water is highly eutrophic (DESCY, 1993).

Their dominance in both locations indicates that these waters contain nutrient elements that are suitable for their growth in varying proportions, where Hassi Ben Abd Allah lake has the higher concentration with the bigger percentage (40%).

By comparing our work with other studies previously done on the microalgal diversity in Ouargla region, it's shown that there is a difference between the species identified. BENAKLI and MEGHCHOUCHE (2016) with 17 species divided into: 06 species of Diatoms with 35%, 02 species of Cyanobacteria representing 12%, 05 species of Chlorophyceae with 30% and Mediophyceae with 04 species representing 23%, NAOUIHA and NAOUIHA (2014) with 11 species divided into: Diatoms with 07 species representing 63.63% and 04 species of Dinoflagellates with 36.36% and BABAOUSMAIL (2014) with 19 species divided into: 05 species of Diatoms with 22%, Cyanobacteria with 06 species representing 33% and 08 species of Chlorophyceae with 45%.

Among the identified microalgae, we have found two species that are known by their industrial and nutritional uses, *Chlorella vulgaris* and *Haematococcus pluvialis*.

Chlorella vulgaris is used as a food source that contains a unique and diverse composition of functional macro- and micro-nutrients including proteins, omega-3 polyunsaturated fatty acids, polysaccharides, vitamins and minerals (PANAHI *et al.*, 2016). In stressful conditions *Haematococcus pluvialis* produces astaxanthin which is a very potent antioxidant used as a feed additive and as a human nutraceutical (OLAIZOLA, 2008).

Conclusion

Morphological analysis of the obtained strains from the two locations (Hassi Ben Abd Allah lake and Mehiriza irrigation basin) after performing several methods of isolation; Enrichment, isolation on solid medium after enrichment and isolation on solid medium by the dilution technique, has allowed us to identify 46 species, which are divided into 6 classes with 28 genuses :

Bacillariophyceae, with genuses as follows: *Pinnularia*, *Diploneis*, *Achnanthidium*, *Navicula*, *Planothidium*, *Fragilaria*, *Nitzschia*, *Ulnaria* and *Caloneis* representing 44%.

Chlorophyceae, represented by: *Haematococcus*, *Chlamydomonas*, *Gloeocystis*, *Trochiscia*, *Neospongiococcum*, *Chlorella*, *Sphaerocystis*, *Cosmarium*, *Monoraphidium* and *Neochloris* genuses representing 35%.

Cyanophyceae, with genuses as follows: *Oscillatoria*, *Synechocystis*, *Chroococcus*, *Gomphosphaeria*, *Microcystis* and *Prochloraceae* representing 13%.

Euglenophyceae, represented by: *Euglena* genus representing 4%. Xanthophyceae with the genus *Monodus* representing 2%. Chrysophyceae with the genus *Mallomonas* representing 2%.

The lake (open system) water makes more favorable environment for microalgal development than the irrigation basin (closed system) water due to the high value of electrical conductivity recorded from the lake water, which indicates the richness of the water with nutrients that are necessary for the development and growth of microalgae.

Our work remains preliminary and needs to be pursued by further researches on microalgal diversity. This will allow us to better value the natural and the biological resources of our region.

Our recommendations for further researches are:

- Use other culture mediums and other sampling techniques.
- Provide all the incubation parameters for the microalgal cultures.
- Test the strains obtained for bioactive compounds.
- Isolation of strains for industrial uses.
- Make a molecular identification of the isolated strains.

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Appendix

Appendix

Appendix. 1: Climate data of Ouargla region (2008-2017).

Parameters / Month	T°			H. (%)	W. (m/s)	P. (mm)	E. (mm)	I. (hour)
	MinT °C	MaxT °C	MedT °C					
January	5.22	19.28	35.4	78.6	7.96	8.51	93.54	248.47
February	6.89	21.34	28.3	68.7	8.81	3.94	124.6	241.46
March	10.57	25.63	23.9	63.4	9.32	5.76	182.3	268.45
April	15.29	30.89	19.5	53.7	10.09	1.48	234.38	289.16
May	20.07	35.44	16.6	45	10.39	1.975	307.45	309.28
June	24.75	40.35	15.2	40.8	9.92	0.81	366.46	234.35
July	27.99	43.8	13.3	34.7	8.83	0.35	433.33	319.78
August	27.38	42.71	14.8	39.1	8.61	0.38	384.46	340.13
September	23.54	38.14	20.2	52.5	9.18	5.73	271.48	264.23
October	17.35	31.82	25	61.9	7.89	7.75	203.66	266.92
November	10.39	24.39	31.2	74.1	7.24	2.98	121.51	249.86
December	5.91	19.64	36.7	81.3	7.06	3.8	83.42	230.97
Annual average	16.28	31.12	23.34	57.82	8.77	3.62	233.88	271.92
Standard deviation	8.46	9.00	8.11	15.95	1.08	2.81	119.60	35.40

MedT: Medium temperature in °C

MinT: Minimum temperature in ° C

MaxT: Maximum temperature in ° C

T°: Temperature in °C

H: Relative humidity (%)

P: Precipitation (mm)

E: Evaporation (mm)

W: Wind (m/s)

I: Insolation (hour)

Appendix

Appendix 2: BG-11 Medium, Modified (Allen 1968, Allen and Stanier 1968, Rippka et al. 1979)

Into 900 mL of dH_2O, add 1 mL of the Fe citrate solution, and then add the remaining components. Autoclave. Final pH should be 7.4.

Component	Stock Solution ($g \cdot L^{-1}$ dH_2O)	Quantity Used
Fe Citrate solution		1 mL
Citric acid	6	1 mL
Ferric ammonium	6	1 mL
citrate	-	1.5 g
$NaNO_3$	40	1 mL
$K_2HPO_4 \cdot 3H_2O$	75	1 mL
$MgSO_4 \cdot 7H_2O$	36	1 mL
$CaCl_2 \cdot 2H_2O$	20	1 mL
Na_2CO_3	1.0	1 mL
$MgNa_2EDTA \cdot H_2O$	(See following recipe)	1 mL
Trace metals solution		

Appendix. 3: Trace Metals Solution

Component	Stock Solution ($g \cdot L^{-1}$ dH_2O)	Quantity Used
H_3BO_3	-	2.860 g
$MnCl_2 \cdot 4H_2O$	-	1.810 g
$ZnSO_4 \cdot 7H_2O$	-	0.220 g
$CuSO_4 \cdot 5H_2O$	79.0	1 mL
$Na_2MoO_4 \cdot 2H_2O$	-	0.391 g
$Co(NO_3)_2 \cdot 6H_2O$	49.4	1 mL

Appendix

Appendix. 4: Half-Strength Chu #10 Medium (Nalewajko and O'Mahony 1989)

Into 950 mL of dH2O, individually dissolve each component. Bring the final volume to 1 liter and autoclave.

Component	Stock Solution ($g \cdot L^{-1}$ dH_2O)	Quantity Used
$Ca(NO_3)_2$	20.0	1 mL
K_2HPO_4	2.5	1 mL
$MgSO_4 \cdot 7H_2O$	12.5	1 mL
Na_2CO_3	10.0	1 mL
Na_2SiO_3	12.5	1 mL
$FeCl_3$	0.4	1 mL
Trace metals solution	(See following recipe)	1 mL
Vitamins solution	(See following recipe)	1 mL

Appendix. 5: Trace Metals Solution

Component	Stock Solution ($g \cdot L^{-1}$ dH_2O)	Quantity Used
H_3BO_3	2.48	1 mL
$MnSO_4 \cdot H_2O$	1.47	1 mL
$ZnSO_4 \cdot 7H_2O$	0.23	1 mL
$CuSO_4 \cdot 5H_2O$	0.10	1 mL
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.07	1 mL
$Co(NO_3)_2 \cdot 6H_2O$	0.14	1 mL

Appendix. 6: Vitamins Solution

Component	Stock Solution ($g \cdot L^{-1}$ dH_2O)	Quantity Used
Thiamine · HCl (vitamin B1)	-	50 mg
Biotin (vitamin H)	2.5	1 mL
Cyanocobalamin (vitamin B12)	2.5	1 mL

Appendix

Appendix. 7: Bold's Basal Medium (BBM) (Bold 1949, Bischoff and Bold 1963)

Into 936 mL of dH2O, add 10 mL of the first six stock solutions. Add 1 mL each of the alkaline EDTA, acidified iron, boron, and trace metals solutions. Autoclave. The final pH should be 6.6.

Component	Stock Solution ($g \cdot L^{-1}$ dH_2O)	Quantity Used
Macronutrients		
$NaNO_3$	25.00	10 mL
$CaCl_2 \cdot 2H_2O$	2.50	10 mL
$MgSO_4 \cdot 7H_2O$	7.50	10 mL
K_2HPO_4	7.50	10 mL
KH_2PO_4	17.50	10 mL
NaCl	2.50	10 mL
Alkaline EDTA Solution		1 mL
EDTA	50.00	
KOH	31.00	
Acidified Iron Solution		1 mL
$FeSO_4 \cdot 7H_2O$	4.98	
H_2SO_4	-	
Boron Solution		1 mL
H_3BO_3	11.42	
Trace Metals Solution		1 mL
$ZnSO_4 \cdot 7H_2O$	8.82	
$MnCl_2 \cdot 4H_2O$	1.44	
MoO_3	0.71	
$CuSO_4 \cdot 5H_2O$	1.57	
$Co(NO_3)_2 \cdot 6H_2O$	0.49	

Appendix

Appendix 8: f/2 Medium (Guillard and Ryther 1962, Guillard 1975)

Into 950 mL of filtered natural seawater, add the following components. Bring the final volume to 1 liter with filtered natural seawater. Autoclave.

Component	Stock Solution ($g \cdot L^{-1}$ dH_2O)	Quantity Used
$NaNO_3$	75	1 mL
$NaH_2PO_4 \cdot H_2O$	5	1 mL
$Na_2SiO_3 \cdot 9H_2O$	30	1 mL
Trace metals solution	(See following recipe)	1 mL
Vitamins solution	(See following recipe)	0.5 mL

Appendix. 9: f/2 Trace Metals Solution

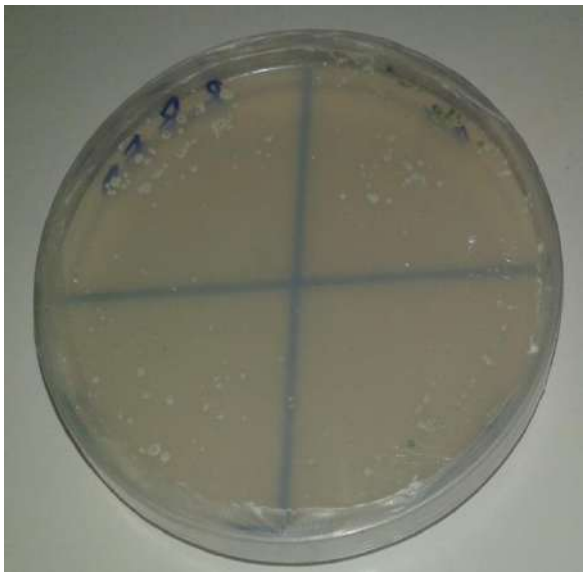
Component	Stock Solution ($g \cdot L^{-1}$ dH_2O)	Quantity Used
$FeCl_3 \cdot 6H_2O$	-	3.15 g
$Na_2EDTA \cdot 2H_2O$	-	4.36 g
$MnCl_2 \cdot 4H_2O$	180.0	1 mL
$ZnSO_4 \cdot 7H_2O$	22.0	1 mL
$CoCl_2 \cdot 6H_2O$	10.0	1 mL
$CuSO_4 \cdot 5H_2O$	9.8	1 mL
$Na_2MoO_4 \cdot 2H_2O$	6.3	1 mL

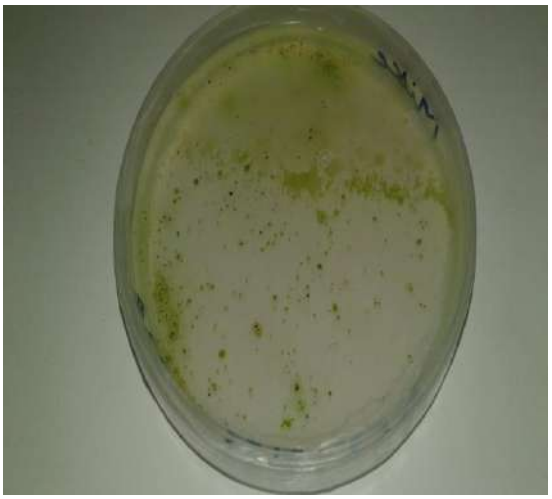
Appendix. 10: Laboratory equipment

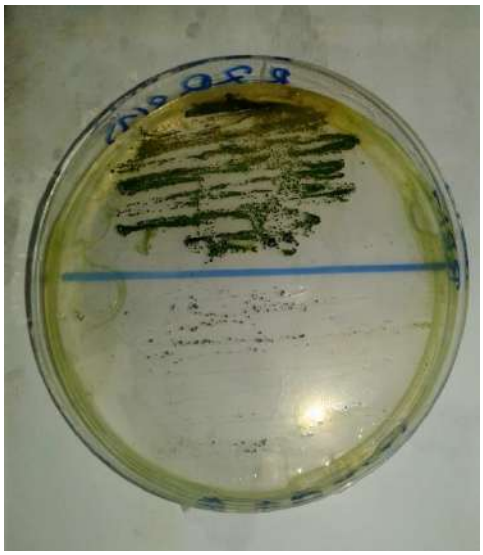
Laboratory wares	Reagents
Erlenmeyer flasks	Lugol solution
Glass bottles	Ethanol
Petri dishes	Brilliant cresyl blue
Stirring rods	Hydrogen chloride (HCL)
Test tubes	Sodium hydroxide (NaOH)
Beakers	Bleach
Pipettes	
Pasteur pipette	
Cylinder	
Analytical balance	
Hotplate	
Magnetic stirrer	
Bunsen burner	
Tube holder	
Micropipette	

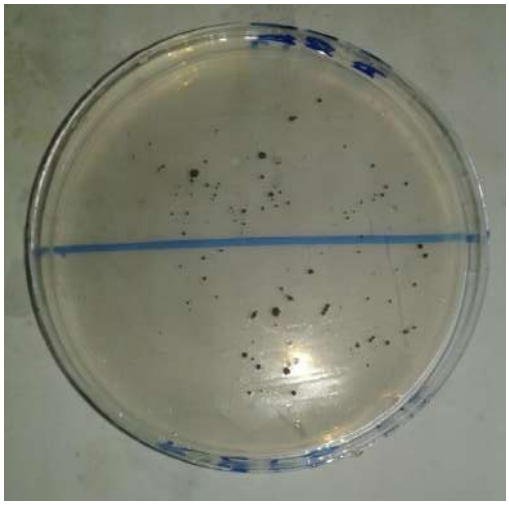
Appendix

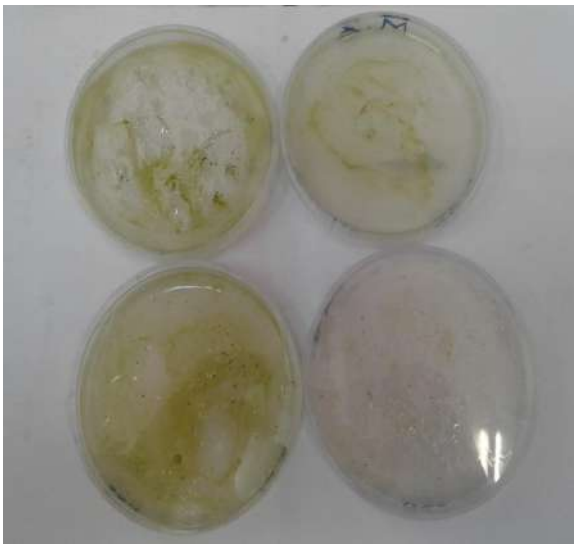
Appendix. 11: Some photos of isolation results











الملخص

دراسة تنوع الطحالب المجهرية في نظامين: مفتوح (بحيرة) ومغلق (حوض الري) في ورقلة - الجزائر

الطحالب المجهرية هي مجموعة غير متجانسة من الكائنات الحية الدقيقة التي تقوم بعملية التركيب الضوئي تنتمي إلى عدة مملكات. تعتبر هذه الكائنات مكمن رئيسي من الموارد البيولوجية مع استخدامات متعددة لها. يهدف عملنا إلى إجراء إحصاء وتحديد الأنواع الموجودة في منطق ورقلة، و إجراء مقارنة بين نظامين، النظام المفتوح (بحيرة حاسي بن عبد الله) والنظام المغلق (حوض الري محيريزة. تمت هذه الدراسة بأخذ عينات من المكانين و العمل عليها في المخبر باستعمال عدت طرق. و من هذا تحصلنا على النتائج التالية: تمكننا من تحديد 46 نوع من الطحالب المجهرية المقسمة بالنسب التالية: 44% دياتوم, 35 % الطحالب الخضراء,13 % البكتريا الزرقاء,4 % الأوغلينا,2 % الطحالب الصفراء المخضرة, 2 % الطحالب الذهبية. حيث احتوى النظام المفتوح (البحيرة) على أغلبية الأنواع (35 نوع) و احتوى النظام المغلق (حوض الري) على 16 نوع, و هذا يدل على أن النظام المفتوح يشكل بيئة أفضل لتكاثر الطحالب المجهرية لغناه بالمواد الأساسية التي تحتاجها. هذه الدراسة تحتاج إلى متابعة بمزيد من البحوث لنتمكن من فهم و تقييم أفضل لمواردنا الطبيعية و البيولوجية.

الكلمات المفتاحية: الطحالب المجهرية، إحصاء، تحديد الأنواع، مقارنة، نظام، ورقلة

Abstract

Study of microalgal diversity in two systems: open (Lake) and closed (Irrigation basin) in Ouargla- Algeria

Microalgae are a heterogeneous group of photosynthetic microorganisms that belongs to several kingdoms. They constitute a major reservoir of bioresources with multiple applications. Our work aims to make a census and to identify species that exist in our region Ouargla, then make a comparison between the two selected systems, the open system (Hassi Ben Abd Allah Lake) and the closed system (Mehiriza irrigation basin). This study was conducted by taking samples from both locations and working on them in the laboratory using different methods of isolation. From this we obtained the following results: We were able to identify 46 types of microalgae divided at different proportions: 44% Diatoms, 35% Chlorophyceae, 13% Cyanobacteria, 4% Euglena, 2% Xanthophyceae, 2% Chrysophyceae. The open system (lake) contained the majority of species (35 species) and the closed system (irrigation basin) 16 species, indicating that the open system is a better environment for the proliferation of microalgae due to its richness in essential nutrients. This study must be followed by further researches to better understand and evaluate our natural and biological resources.

Keywords: Microalgae, Census, Species identification, Comparison, System, Ouargla

Résumé

Etude de la diversité des microalgues dans deux systèmes: ouvert (lac) et fermé (bassin d'irrigation) à Ouargla-Algérie

Les micro-algues sont un groupe hétérogène de micro-organismes photosynthétiques appartenant au plusieurs royaumes. Ils constituent un réservoir majeur de bioressources à des applications multiples. Notre travail vise à recenser et identifier les espèces qui existent dans la région d'Ouargla, et faire une comparaison entre les deux systèmes choisis, le système ouvert (Lac de Hassi Ben Abd Allah) et le système clos (Bassin d'irrigation de Mehiriza). Cette étude a été réalisée en prélevant des échantillons des deux endroits et en travaillant au laboratoire en utilisant différent méthodes d'isolement. De ceci nous avons obtenu les résultats suivants: Nous avons pu identifier 46 types de microalgues divisés par les proportions suivants: 44% de Diatomées, 35% Chlorophyceae, 13% de cyanobactéries, 4% d'Euglena, 2% Xanthophyceae, 2% de Chrysophyceae. Le système ouvert (lac) contenait la majorité des espèces (35 espèces) et le système fermé (bassin d'irrigation) 16 espèces, indiquant que le système ouvert est un meilleur environnement pour la prolifération des microalgues due à son richesse en nutriments essentielles. Cette étude doit être suivie par d'autres recherches pour mieux comprendre et évaluer nos ressources naturelles et biologiques.

Mots clés: Micro-algues, Recensement, Identification, Comparaison, Système, Ouargla