الشركة القابضة
لمياه الشرب والصرف الصحى

برنامج المسار الوظيفي
للعاملين بقطاع مياه الشرب والصرف الصحي

دليل المتدرب

برنامج
(طحالب المياه العذبة)

Biological Analysis of Water

Freshwater Algae

كيميائي مياه- الدرجة الثالثة

تم إعداد المادة بواسطة الشركة القابضة لمياه الشرب والصرف الصحي
قطاع تنمية الموارد البشرية - الادارة العامة لتخطيط المسار الوظيفي
الإصدار الثانى - 2019.

مقدمة الإصدار الثانى

تهدف مجموعة البرامج التدريبية المعدة من إدارة المسار الوظيفى بالشركة القابضة لمياه الشرب والصرف الصحى إلى رفع كفاءة الكيميائين العاملين بالشركة القابضة لمياه الشرب والصرف الصحى والشركات التابعة لها وتنمية مهاراتهم ومعارفهم بالشكل الذى يضمن الوصول إلى كوب مياه نظيف وبيئة آمنة يرضى متطلبات وإحتياجات العملاء الكرام.

ويعتبر الإصدار الثانى من برامج المسار الوظيفى لوظيفة كيميائى مياه الشرب هو ثمرة جهود الكيميائيين العاملين بمعامل الشركات التابعة والمعمل المرجعى لمياه الشرب بالشركة القابضة بما تحمله من مزيج متجانس من الخبرات والكفاءات الذين لم يدخروا جهدا حتى يخرج هذا العمل بالطريقة اللائقة.

وجدير بالذكر أن هذا الإصدار يعتبر مكتبة مرجعية وافية وشاملة لجميع الجدارات المتضمنة المهارات والمعارف التي تجعل الكيميائى كفؤا لوظيفته.

ومما تجدر الإشارة إليه بأنه تم الاعتماد على منهجية للمسار التدريبى بحيث يكون المتدرب قد تلقى الدورات الحقلية بداية من التعرف على مراحل التنقية والمعالجة ثم الانتقال إلى الدورات المعملية داخل معمله طبقا للإطار الزمنى المحدد للمدد البينية لكل درجة وظيفية.

ولقد اعتمدنا على وضع معايير لكل مرحلة فى إعداد هذا الاصدار وكان من أهم هذه المعايير:

- المشاركة الفعالة للخبرات والكفاءات التدريبية بالشركات التابعة فى وضع المناهج بما يناسب عموم الكيميائيين على مستوى الجمهورية.
- عقد ورشة عمل متخصصة لكل مادة تدريبية يشارك بها جميع المدربين ذوى التخصص والخبرات سواء من المعمل المرجعى أو معامل الشركات فضلا عن أن يكون المدرب قد قام بتدريس هذه المادة مرات عديدة.
- استخدام وسيلة اتصال غير تزامنى بين جميع المدربين المعتمدين لكل مادة على حدة من خلال انشاء جروب على الفيس بوك لكل مادة على حده (مذكور فى دليل المدرب).
- وضع حقيبة تدريبية كاملة لكل برنامج معدة طبقا لأحدث النظم والمعايير العالمية تحتوى على (دليل المتدرب- شرائح العرض- ملحقات مقروءة ومرئية- دليل المدرب- بنك الأسئلة).
- بناء المحتوى لكل برنامج تدريبى طبقاً لأحدث المراجع العالمية ومن أهمها كتاب الطرق القياسية لتحليل مياه الشرب والصرف الصحى (الإصدار رقم 23) وبما يتوافق مع متطلبات آخر إصدارات الايزو(17025)، مع مراعاة التحديثات الخاصة بالتشريعات والقوانين ذات الصلة.

وجدير بالذكر أن الإصدار الثانى من البرامج التدريبية اعتمد فى تصميمه على عرض مبسط للمعلومات قدر الامكان طبقاً للأسس العلمية وطبقاً للجدارات المعتمدة على تحديد أهداف واضحة وصريحة لتدريب المتدربين، وتشـــتق تلك الجدارات من الفهم الواضـــح لدور المتدرب طبقا لبطاقة الوصـــف الوظيفى، وتتضـمن معارف ومهارات وسـلوك. مما يضـمن إكسـاب المتدرب مهارات سـلوكية بالإضـافة إلى المواد التخصصية.

كما تم تصـميم العديد من ورش العمل على أسـاس تسـهيل و تسـريع عمليتي التعلم و كسـب المهارات بما يسمح بتعظيم الفائدة من العملية التدريبية.

كذلك تم اســـتخدام أســـاليب التدريب الحديثة والاعتماد على التدريب التفاعلى والتركيز على الجوانب التطبيقية في استخدام الوسائل والأساليب المختلفة ، كما تم استخدام الطرق الحديثة للتعليم التفاعلي والغير تزامني كمصادر مساندة للتعلم من خلال انشاء جروب على الفيس بوك للمدربين المعتمدين (HCWW Trainers) .

وفى الختام نرجوا من الله أن يتقبل منا هذا العمل كما نأمل أن يكون هذا العمل علما نافعا للعاملين بقطاع المعامل بالشركة القابضة والشركات التابعة لما يشمله من معلومات فنية قيمة وأن يفيد العاملين الجدد بها ليصبحوا قادرين على تنفيذ مهامهم الوظيفية بالشكل الأمثل .

والله ولي التوفيق.

1. INTRODUCTION

1.1. Definitions

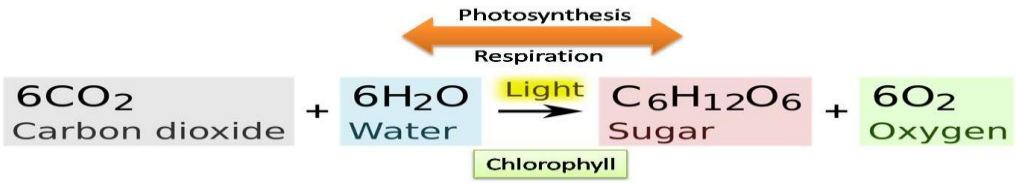
- **Planktonic algae (Phytoplankton):** algae occur as free-floating organisms (planktonic) and drift freely within the main body of water.
- **Benthic algae:** algae occur as substrate-associated (benthic) organisms.
- **Autotrophic Organisms:** (obtaining all their materials from inorganic sources) and photosynthetic– generating complex carbon compounds from carbon dioxide and light energy.
- **Biomarkers:** that give quick answers are able to act as an early warning system for the monitoring of environmental changes.
- **Bioindicators:** have a high ecological relevance and the ability to analyze environmental samples at any time after collection.

1.2. What are algae?

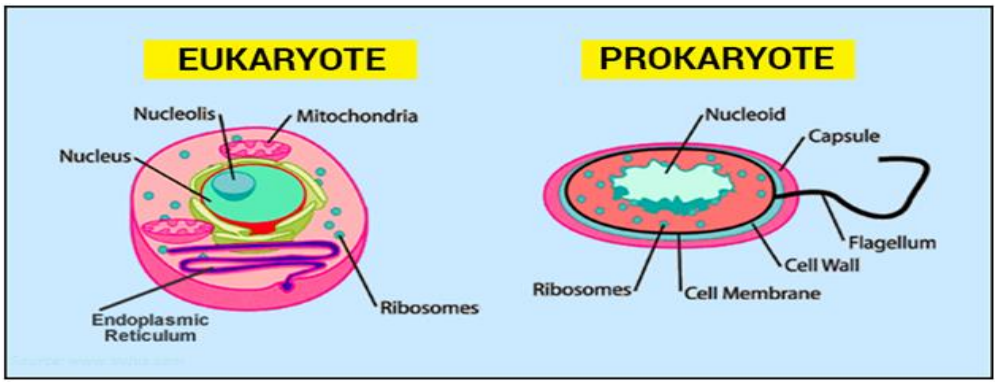
- The word ‘algae’ is applied to a broad assemblage of organisms that can be defined both in terms of morphology and general physiology.
- Algae were part of the first organisms in the world. They first emerged about 3.5 billion years ago.
- The term algae refers to both macroalgae (seaweeds) and a highly diversified group of microorganisms known as microalgae visible only with the aid of a light microscope.
- Algae can be aquatic or subaerial, when they are exposed to the atmosphere rather than being submerged in water.
- They are simple organisms, without differentiation into roots, stems and leaves and their sexual organs are not enclosed within protective coverings.
- They are typically present as: a- **Planktonic algae (Phytoplankton):** algae occur as free-floating organisms and drift freely within the lighted regions of the main body of water.

  b- **Benthic algae:** algae occur as substrate-associated (benthic) organisms.

- In terms of physiology, they are fundamentally autotrophic (obtaining all their materials from inorganic sources) and photosynthetic generating complex carbon compounds from carbon dioxide and light energy.



- Chlorophyll *a* is found in all photosynthetic algae, the other algal chlorophylls have a more limited distribution and function as accessory photosynthetic pigments.
- Chlorophyll *b* is found in the Euglenophyta and Chlorophyta. Chlorophyll *b* functions photosynthetically as a light-harvesting pigment transferring absorbed light energy to chlorophyll *a*. The ratio of chlorophyll *a* to chlorophyll *b* varies from 2:1 to 3:1.
- Chlorophyll *c* is found in the Dinophyta, Cryptophyta, and most of the Heterokontophyta.
- There are two basic types of cells in the algae, **prokaryotic** and **eukaryotic**. Prokaryotic cells lack membrane-bounded organelles (plastids, mitochondria, nuclei, Golgi bodies, and flagella) and occur in the cyanobacteria. The remainder of the algae are eukaryotic and have organelles.



1.3. Size and shape

1.3.1.Size range

- In the planktonic environment, algae range from small prokaryotic unicells (diameter <1 µm) to large globular colonies of blue-green algae such as *Microcystis* (diameter reaching 2000 µm).

- Planktonic algae are frequently characterized in relation to discrete size bands – picoplankton (<2 µm), nanoplankton (2–20 µm), microplankton (20–200 µm) and macroplankton (>200 µm).
- Each size band is characterized by particular groups of algae.

Size Range of Phytoplankton

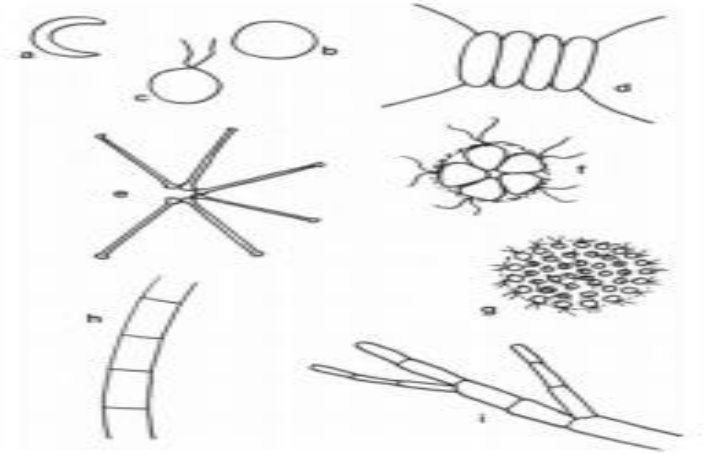
Category	Linear Size (Cell or Colony Diameter, µm)	Biovolume* (μm^3)	Unicellular Organisms	Colonial Organisms
Picoplankton	0.2–2	4.2×10^{-3}–4.2	Photosynthetic bacteria Blue-green algae *Synechococcus* *Synechocystis*	-
Nanoplankton	2–20	$4.2–4.2 \times 10^3$	Blue-green algae Cryptophytes *Cryptomonas* *Rhodomonas*	
Microplankton	20–200	4.2×10^3–4.2×10^6	Dinoflagellates *Ceratium* *Peridinium*	Diatoms *Asterionella*
Macroplankton	>200	$>4.2 \times 10^6$	-	Blue-green algae *Anabaena* *Microcystis*

Biovolume values are based on a sphere (volume = $^4/_3\pi r^3$).

1.3.2.Diversity of shape

- The shape of algal cells ranges from simple single non-motile spheres to complex multicellular structures.
- The simplest structure is a unicellular non-motile sphere, which may become elaborated by the acquisition of flagella, by a change of body shape or by the development of elongate spines and processes.
- Cells may come together in groups without defined number or shape or may form globular colonies that have a defined morphology (Fig. 1.2f,g).
- Cells may also join together to form linear colonies (filaments) which may be unbranched or branched (Fig. 1.2h, i).
- Although motility is normally associated with the possession of flagella, some algae (e.g. the diatom *Navicula* and the blue-green *Oscillatoria*) can move without the aid of flagellae by the secretion of surface mucilage.

- In many algae, the presence of surface mucilage is also important in increasing overall cell/colony size and influencing shape.
- Size and shape, along with other major phenotypic characteristics, are clearly important in the classification and identification of algal species.
- At a functional and ecological level, size and shape are also important in terms of solute and gas exchange, absorption of light, rates of growth and cell division, sedimentation in the water column, cell/colony motility and grazing by zooplankton.



General shapes of algae

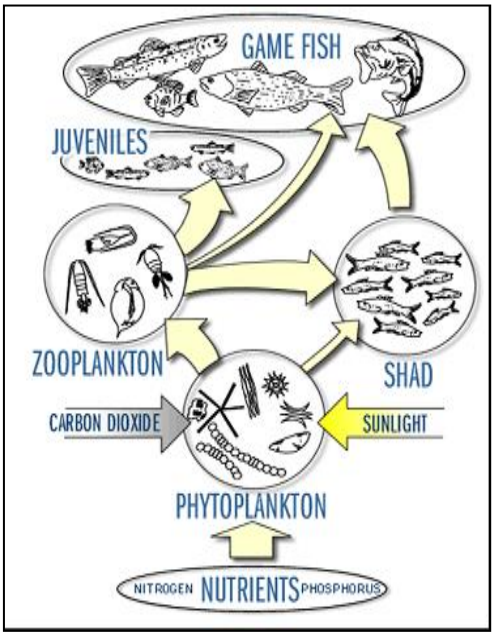
- Non-motile unicells: (a) *Selenastrum*; (b) *Chlorella*.
- Motile unicell: (c) *Chlamydomonas.*
- Non-motile colony: (d) *Scenedesmus* (e) *Asterionella*.
- Motile colony: (f) *Pandorina*; (g) *Volvox*.
- Unbranched filament: (h) *Spirogyra*.
- Branched filament (i) *Cladophora.*

2. Algae as primary producers:

- Algae as fixers of carbon and they are the pigmented autotrophs generators of biomass; initiating the food chain of any aquatic ecosystems and are the primary producers upon which the primary consumers, zooplankton depend.
- The level of primary production by algae in freshwater bodies can be measured as fixed carbon per unit area with time (mg Cm^{-3} h^{-1}), and varies greatly from one environment to another.
- Primary production varies with trophic status and with depth in the water column. Eutrophic lakes, containing high levels of available nitrogen and phosphorus, have very high levels of productivity in surface waters, decreasing rapidly with depth due to light absorption by algal biomass.

- In contrast, mesotrophic and oligotrophic lakes have lower overall productivity but this extends deep into the water column due to greater light penetration.

Aquatic Food Chain



Categories of Trophic Water Types

Trophic status	Phosphorus	Chlorophyll
Oligotrophic	**12 ppb**	**0-2.6 µg / L**
Mesotrophic	**12-24 ppb**	**2.7-20 µg / L**
Eutrophic	**25-96 ppb**	**20-56 µg / L**
Hypereutrophic	**> 96 ppb**	**> 56 µg / L**

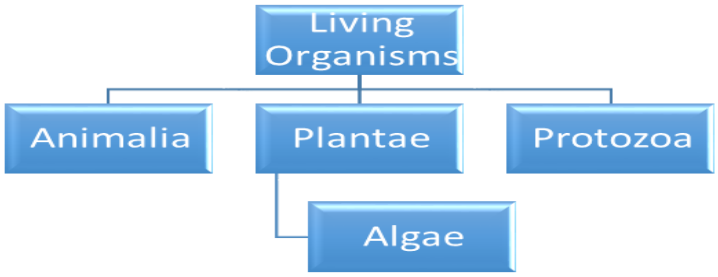
3. Taxonomy and Classification of Algae:

- They are distinguished from higher plants in terms of size and taxonomy, and from bacteria in terms of their photosynthetic characters and biochemical activity.
- Historically, the major groups of algae are classified into Divisions (the equivalent taxon in the zoological code was the Phylum) on the basis of pigmentation, chemical nature of photosynthetic storage product, photosynthetic membranes' (thylakoids) organization and other features of the chloroplasts, chemistry and structure of cell wall, number, arrangement, and ultrastructure of flagella (if any), occurrence of any other special features, and sexual cycles.

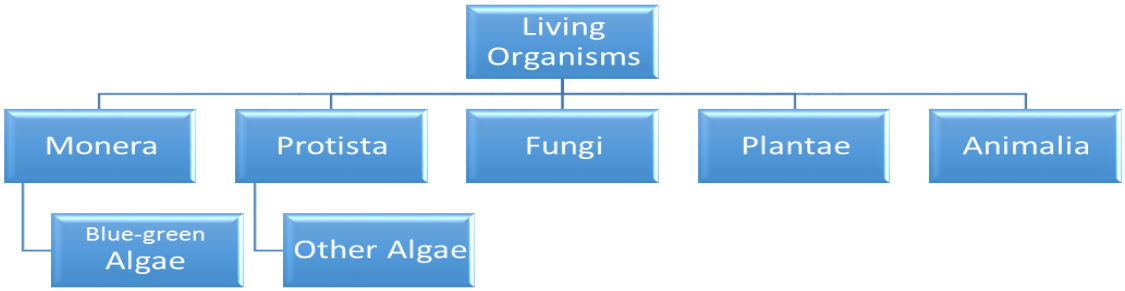
Algal Division (phylum)	Pigmentation# Chlorophylls	Carotenes	Diag.* Carotenoids	Starch-like Reserve	External Covering	Chloroplast Fine-Structure: Outer Membranes	Chloroplast Fine-Structure: Thylakoid Groups	Flagella (Vegetative Cells & Gametes)
1. Blue-green algae *Cyanophyta*	a	β	zea-	Cyano-phycean starch[α]	Peptidoglycan matrices or walls	0	0	0
2. Green algae *Chlorophyta*	a,b	α, β, γ	viola-	True starch[α]	Cellulose walls, scales	2	2–6	0–many. Similar (isokont)
3. Euglenoids *Euglenophyta*	a,b	β, γ		Paramylon[β]	Protein pellicle	3	3	1–2 emergent
4. Yellow-green algae: *Xanthophyta*	a,c_1,c_2	α, β		Chrysolaminarin[β]	Pectin or pectic acid wall	4	3	2 unequal (heterokont)
5. Dinoflagellates *Dinophyta*	a,c_2	β	peri-	True starch[α]	Cellulose theca (or naked)	3	3	2 unequal (heterokont)
6. Cryptomonads *Cryptophyta*	a,c_2	α, β	allo-	True starch[α]	Cellulose periplast	4	2	2 equal (isokont)
7. Chrysophytes *Chrysophyta*	a,c_1,c_2,c_3	α, β, ε		Chrysolaminarin[β]	Pectin, plus minerals and silica	4	3	2 unequal (heterokont)
8. Diatoms *Bacillario-phyta*	a,c_1,c_2,c_3	β, ε	fuco-	Chrysolaminarin[β]	Opaline silica frustule	4	4	1, reproductive cells only
9. Red algae *Rhodophyta*	a	α, β		Floridean starch[α]	Walls with galactose polymer matrix	2	0	0
10. Brown algae *Phaeophyta*	a,c_1,c_2,c_3	β, ε		Laminarin[β]	Walls with alginate matrix	4	3	2 unequal (heterokont) reproductive cells only

Classification of the Different Algal Groups According to Biochemical and Cytological Characteristics

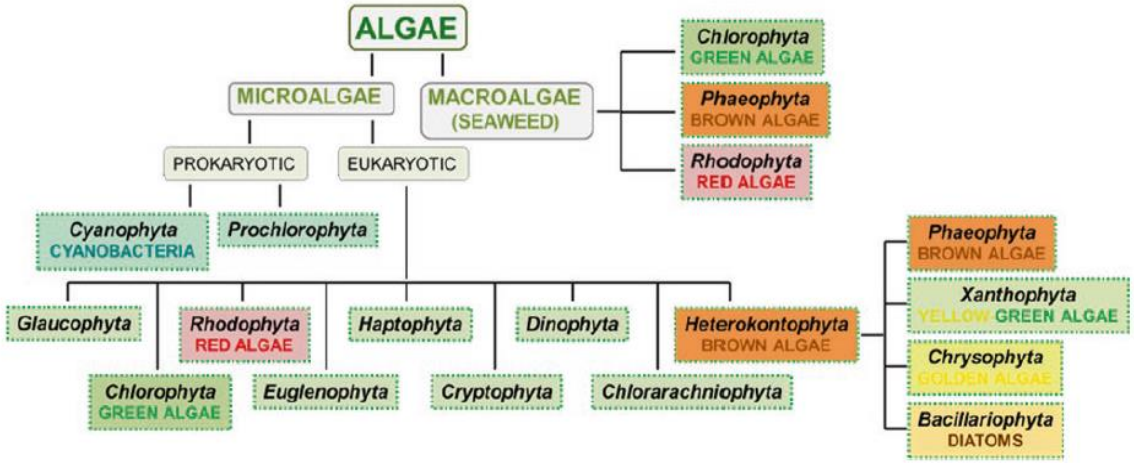
- **<u>In the past</u>**:



- As algae have cell walls and *chlorophyll a* for photosynthesis, they were classified under Plantae.
- **<u>Recently:</u>**



- Algae do not have vascular tissue, so; they no longer belong to the Plantae kingdom. Instead, they are now placed in two kingdoms: Monera & Protista.



- **Fig. -- Classification scheme of the different algal groups**

4. Environmental Factors Influencing Algal Growth and Algal Population

- Planktonic organisms are controlled by the physico-chemical properties of the water in which they dwell. Hence, different planktons mark different oceans, rivers and lakes.
- Plankton overgrowth may influence the physical and chemical composition of the water and changes its quality (e.g. colour, odour, turbidity, alkalinity, acidity, dissolved oxygen, surface tension and organic content).
- The average number of organisms present in a water body may be limited by the available amount of nutrients, temperature, light and turbidity.
- Accordingly the number of micro-organisms tends to vary from time to time and from one sector of stream to another.
- Phytoplankton structure and dynamic are influenced by physical (e.g., light availability, stratification and mixing process), chemical (nutrient, especially N and P), biological

(e.g., predation) and hydrological (e.g., water residence time) factors, which show temporal and spatial changes.

4.1. Physical factors:

which influence the types and numbers of phytoplankton in a river are mainly summed up as follows:

4.1.1.Size of Streams:

- As rivers become larger, certain changes in the plankton can be expected, specifically that small green and blue-green algae become relatively more important in the plankton than diatoms.

4.1.2.Current Rate:

- The vertical distribution of planktonic organisms in the river depends entirely on the current.
- The maximum speed of the water is usually attained near the surface and decreases sharply toward the bottom.
- It is clear that benthic forms are exposed much less current pressure than that of the surface water. The vertical distribution of plankton was found usually to be uniform when the current is swift.
- The velocity of the current is greater in the main river than near the littorals. Therefore, one may expect plankton organisms to be more abundant near the banks.

4.1.3.Water Level:

- Changes in chemical and biological characters of water may be accompanied by alternations in water level and hence forth, an unbalance in planktonic life may be expected.
- At time of low water, the volume of flow and current rate decline, nutrient depletion is increased and nutrient replacement is decreased.
- At flood time the water level increases and the average speed also greatly accelerated. Accordingly, flood waters bring great changes in planktonic distribution.

4.1.4.Depth:

- The water depth in a stream is very important to phytoplankton distribution.
- In case of deep rivers, the number of phytoplankton tends to decrease towards the bottom because of the high reduction in light penetration which interferes with algal photosynthesis. This makes it clear that algal growth is basically dependent on light.

4.1.5.Light:

- It is clear that algal photosynthesis and hence algal growth are depend on light as the same processes in higher green plants.

4.1.6.Turbidity:

- Turbidity in a water body can reduce light penetration to a point which completely prevents plant growth including that of phytoplankton.
- Silt, clay, planktonic over growth and other material (e.g. colloids) may accumulate in water to produce high turbidity levels.
- Under such conditions a decline in phytoplankton numbers due to the reduction in light penetration.

4.1.7.Temperature:

- Water temperature occupies an important role in the control of planktonic life.
- Temperature changes not only affect many of physiological processes of a cell but also influence the kind of life to be present in water.
- Water temperature is known to vary from one place to another and from one season to another.
- Warm climate is considered ideal for maximal growth for most algae.
- During the winter season, low temperature and increased rainfall may create an environment that is somewhat hazardous to the growth of many species of microorganisms. However, certain diatoms, flagellates and unicellular green algae may become quite numerous.
- In summer and early fall seasons, the heat intensity usually produced high evaporation rates which results in the concentrations of nutrients necessary for the growth of many types of microorganisms.
- In such instances, heterotrophic forms, filamentous green algae and several species of blue-green algae may be found in high population densities.

4.2. Chemical factors: chemical constituents of water affect plankton development and vice versa.

4.2.1.Dissolved Gases:

- Biological life in a stream influences the balance of gases dissolved in water such as oxygen and carbon dioxide.

- In highly polluted sections of a stream, the dissolved oxygen content of the water may be depleted, whereas the presence of algae in such areas add a new load of oxygen sometimes reaching a state of super-saturation as a result of photosynthetic activity during day time.

4.2.2.pH:

- The pH in natural water alters with its carbon dioxide content.
- Thus streams which possess some limestone in solution are well buffered and exhibit little variation on pH levels beyond the range of 6.8 – 8.8.
- Some species of algae are common at water with pH value of 1.8 e.g. *Chlamydomonas sp., Navicula sp., Desmidium sp.,* and *Euglena sp.* While, *Ulothrix sp., Stigeocolonium sp.,* and *Mougeotia sp.* were found at pH 4.0 or even lower.
- Neutral or slightly alkaline conditions are considered favorable for majority of river flora.

4.2.3.Salinity:

- Salinity in water is known to effect on flora.
- Many species of freshwater algae are killed in saline water while few fresh water algae such as *Cladophora glomerata* may resist high salt content.

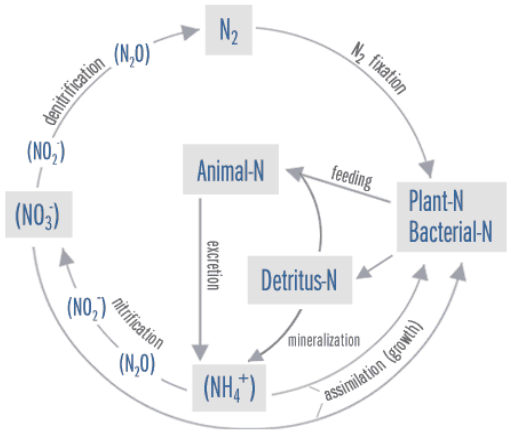
4.2.4.Phosphorus:

- Phosphates are vital to the presence and development of phytoplankton.
- Several investigation carried out on fresh waters show that phosphate content of a river reach its highest value during the winter months when algal growth is at its lowest.
- Concurrently, the same element is at their lowest during the spring and summer months when algal activity turns to be at its highest.
- If substantial amounts of agricultural drainage or sewage discharge into a stream, an increase in phosphorus is expected at and below this point. The favorable effect on algal growth is frequently striking in this region.

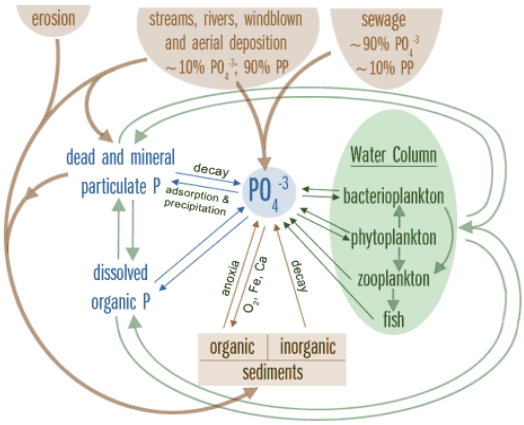
4.2.5.Nitrogen:

- Nitrogen occurs in the gas, liquid (dissolved in water), and solid phases. N can be associated with carbon (organic species) and with elements other than carbon (inorganic species).

- The cycling of nitrogen within ecosystems is complex due to the many transformations in form it goes through as a result of microbial processing.
- Important inorganic species include nitrate (NO^-_3), nitrite (NO_2 2), nitric acid (HNO_3), ammonium (NH^+_4), ammonia (NH_3), the gas N_2, nitrous oxide (N_2O), nitric oxide (NO), and nitrogen dioxide (NO_2).
- Prokaryotic algae (cyanobacteria) play a main active role only in nitrogen fixation and assimilation.
- For all eukaryotic algae, the only forms of inorganic nitrogen that are directly assimilable are nitrate (NO^-_3), nitrite (NO^-_2), and ammonium (NH^+_4).
- The more highly oxidized form, nitrate, is the most thermodynamically stable form in oxidized aquatic environments, and hence is the predominant form of fixed nitrogen in aquatic ecosystems, though not necessarily the most readily available form.
- Its highest level of nitrate was detected during winter months when planktonic growth is greatly reduced. The same tendency is seen for other nitrogen group.



Nitrogen Cycle



Phosphorus Cycle

5. Ecological and Economic Importance of Algae:

- Algae have ecological and economic importance in various spheres. It is well known that phytoplankton is a primary producer in many aquatic systems.
- Phytoplankton are at the base of aquatic food webs and of global importance for ecosystem functioning and services.
- Some species of algae are edible and can be consumed by humans.
- They also constitute an ingredient in animal feed and can be converted into organic fertilizers (including raw biomass, compost, dried meal, and extracts).

- Extraction of biologically active compounds from algae offers a new range of products, which can be used in the food, pharmaceutical, cosmetic, and agricultural industries.
- The phytoplankton has been used in the water quality assessment, mainly due to its short life cycle and prompt response to changes in the environmental conditions, which, eventually, may hinder the understanding of the interactions and associations of species with the chemical and physical variables.
- Algae are responsible for 50% of the photosynthetic processes that take place on Earth.
- Algae are efficient in removing nitrogen, phosphorus, and toxic metals from a wide variety of wastewaters (municipal, agricultural, and industrial).
- Some of algae are able to play the role of biomarkers / bioindicators of exposure to pollutants, i.e., quantitative measures of changes in the biological system that may be caused by exposure to the toxic effects of environmental chemicals.
- Phytoplankton also may be used to indicate the relative efficiencies of water treatment plants.
- Algae are known to be a rich source of biologically active compounds, such as oils, fats, polyunsaturated fatty acids, proteins, carbohydrates, minerals, antioxidants, and pigments.

Algae into High Value-Added Products

6. Problems caused by Algae:

- Growth of algae in water treatment plants can cause various problems within the process including: clogging of intake screens, clogging of sand filters, increased chlorine demand.
- Some species of plankton develop noxious blooms that can create offensive tastes and odors, color, turbidity and altering the pH of water in drinking water.
- Algal blooms may even create anoxic conditions or produce toxins that poison both aquatic and terrestrial organisms, resulting in animal or human illness or death. So algal blooms raise ecologic, economic and public health concerns.

7. Algae as Bioindicators

- Biological indicators (bioindicators) may be defined as particular species or communities, which, by their presence, provide information on the surrounding physical and/or chemical environment at a particular site.
- The basis of individual species as bioindicators lies in their preference for (or tolerance of) particular habitats, plus their ability to grow and out-compete other algae under particular conditions of water quality.
- The advantages of biological monitoring over separate physicochemical measurements to assess water quality are that it:
- Reflects overall water quality, integrating the effects of different stress factors over time; physicochemical measurements provide information on one point in time.

- Gives a direct measure of the ecological impact of environmental parameters on the aquatic organisms.
- Provides a rapid, reliable and relatively inexpensive way to record environmental conditions across a number of sites.

7.1. Characters of Bioindicators:

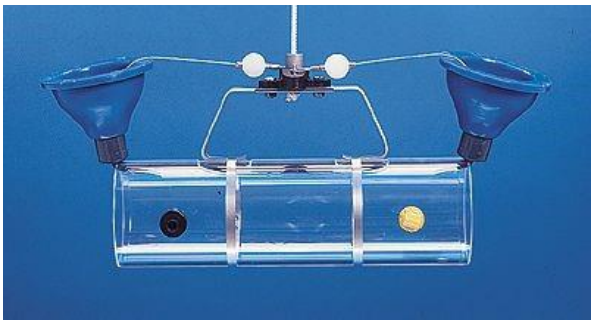
- **biologically relevant** i. e. easily related to the maintenance of ecological integrity;
- **socially relevant** i.e. of obvious value to those involved in the decision-making process, including the general public;
- **broadly applicable** to many stressors and sites;
- **sensitive to stressors**, preferably without an all or none response or excessive natural variability;
- **measurable,** in that it can be operationally defined and quantified using an accepted procedure with known precision and accuracy;
- **interpretable** i.e. capable of distinguishing acceptable from unacceptable conditions in a manner that is scientifically and legally defensible;
- **capable of** continuity of measurement through time and space;
- **of an appropriate** spatial and temporal scale for the assessment under study;
- **not redundant** with other measures included in the monitoring program;
- **integrative** by summarizing information from many other possible indicators that cannot be feasibly measured;
- **anticipatory** i.e. capable of providing a signal of ecosystem deterioration before serious harm has occurred;
- **timely** i.e. capable of providing information rapidly enough that management actions can be implemented before unacceptable damage occurs;
- **diagnostic** of the particular stressor causing the problem;
- **cost-effective** by providing the maximum amount of information per unit effort.

8. Identification and Enumeration of Algal groups:

8.1. Sampling:

- Use opaque sample containers because even brief light exposure during storage will alter *chlorophyll* values.

- Sample-storage bottles should be free of any acid residue and should be made of polyethylene or glass to avoid metallic ion contamination, which can lead to significant errors when making algal assays or productivity measurements.
- To avoid confusion or error, label each container with the sampling date, cruise number, sampling station, and type of sample.
- Use waterproof labels and waterproof ink.
- Avoid potentially overfilled sample bottles, inaccurate preservative additions, and contamination with potentially hazardous preservatives.
- Sample size depends on the type and number of determinations to be made If phytoplankton densities are expected to be low (e.g., in oligotrophic waters), collect a 1.0 L sample. In richer, eutrophic waters, collect a 0.1- to 1.0 L sample.
- In a field record book, note sample location, depth, type, time, meteorological conditions, turbidity, water temperature, salinity, other significant observations, and if possible, photo document and record sample coordinates using a hand-held global positioning system (GPS).
- Van Dorn usually is the preferred sampler for standing crop, primary productivity, and other quantitative determinations because it does not inhibit the free flow of water through the cylinder.



- The triggering devices of these samplers are sensitive, so avoid rough handling. Always lower the sampler into the water; do not drop.
- Polyethylene or polyvinyl chloride sampling devices are preferred to metal samplers because the latter liberate metallic ions that may contaminate the sample.

8.2. Storing and Preservation:

- To examine live samples, partially fill containers and store them in a refrigerator or ice chest.
- If living material cannot be examined or if phytoplankton will be counted later, preserve the sample.
- There are multiple phytoplankton preservatives. Lugol's solution and Glutaraldehyde are the most commonly used; others include Formalin, Merthiolate, and "M3" fixative.

8.2.1.Lugol's solution:

- Lugol's solution, which can be used for most forms (e.g., naked flagellates), stains organisms that store starch (especially chlorophytes and cryptophytes) and tends to cause most cyanobacteria to settle.
- ***Preparation***: dissolve 20 g Potassium Iodide (KI) and 10 g Iodine crystals in 200 mL distilled water containing 20 mL Glacial Acetic acid.
- Add 0.3 mL Lugol's solution to 100 mL sample and store in the dark.
- For long-term storage, add 0.7 mL Lugol's solution per 100 mL sample, re-add Lugol's solution every 6-12 months.
- The sample should look like weak tea.

8.2.2.Formalin:

- Prepare Formalin by adding 20 g sodium borate ($Na_2B_2O_4$) to 1 L 37% formaldehyde
- To preserve sample with Formalin, add 40 mL buffered formalin to a 1L of sample immediately after collection.

8.3. Sample Concentration:

- The organisms in water samples often must be concentrated in the laboratory before analysis.
- The multiplication factor is a function of the concentrated sample's concentration and volume.

- Four techniques for concentrating phytoplankton are sedimentation, membrane filtration, centrifugation and sand filter / backwash.

8.3.1.Sedimentation/ Settling:

- Sedimentation is the preferred concentration method because it is nonselective (unlike filtration) and nondestructive (unlike filtration or centrifugation), although many Pico-plankton, smaller Nano-plankton, and actively swimming flagellates (in unpreserved samples) may not settle completely.
- This approach is too slow if results are needed quickly.
- For non-preserved sample allow 1 h settling per millimeter of column depth.
- For a sample preserved with Lugol's solution (2 to 4 ml/L), allow about 0.5 h settling/mm depth.
- Use cylindrical settling chambers with thin, clear glass bottoms. Apply a height-to-diameter ratio no larger than 5:1 to avoid excessive chamber wall influence and currents in the chamber.
- Fill settling chambers without forming a vortex, keep them vibration-free, and move them carefully to avoid non-random distribution of settled matter.
- When siphoning supernatant to obtain the desired concentrate (usually 2 to 3 mL; 5 mL for diatom mounts), do it slowly, do not agitate the water, and hold the end of the siphon or pipet directly below the water's surface.
- Store concentrated sample in a closed, labeled container (remember that samples preserved with Lugol's solution will need to be re-spiked every 6 to 12 months).

8.3.2.Membrane filtration:

- The filtration method permits the use of high magnification to enumerate small plankters (e.g., flagellates and cyanobacteria); it essentially concentrates the sample while providing a countable preparation.
- Filtration offers the opportunity to make permanent mounts, allows for fast sample preparation when rapid results are needed to support management decisions (as in water treatment), and enhances the use of auto-fluorescence (thin preparation).
- For samples with a low phytoplankton and silt content, this method increases the probability of observing less abundant forms.

- Delicate forms (e.g., “naked” flagellates) can be distorted by even gentle filtration. When populations are dense and the detritus content is high, the filter clogs quickly and silt may crush organisms or obscure them from view.
- Pour a measured volume of well-mixed sample into a funnel equipped with a membrane filter (25-mm filter diameter; 0.45-_m pore size). Apply a vacuum of less than 50 kPa (<25 mm Hg) to the filter until about 0.5 cm of sample remains. Break vacuum, then apply low vacuum (about 12 kPa, 2 to 3 mm Hg) to remove remaining water. Do not dry filter.
- Samples also may be concentrated on a filter, inverted onto a microscope slide, and quick-frozen so plankton can be transferred from the filter to the slide. Alternatively, oil can be added to make the filter slightly translucent.

8.3.3.Centrifugation:

- Plankton can be concentrated via batch or continuous centrifugation at 1000 g for 20 min.
- Discard supernatant gently and keep small volume at the bottom of the tube.
- Transfer the volume to a cylinder and measure the final volume then transfer to opaque bottle with complete information on it (Sample ID, date, initial and final volume).
- Although centrifugation accelerates sedimentation, it often damages fragile organisms, and is not preferable for quantitative analysis but still the most applicable.

8.4. Identification and counting:

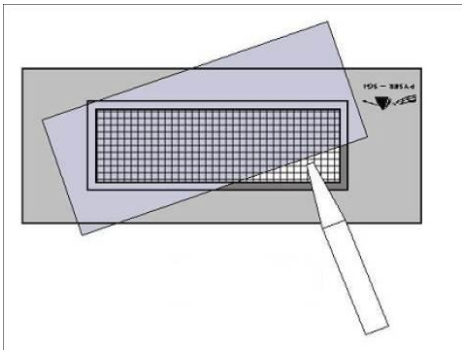
- Some phytoplankton are unicellular, while others are multicellular (colonial or filamentous). Listed below are suggestions for reporting concentration or density:

Enumeration Method	Counting Unit	Reporting Unit
Total cell count	One cell	Cells/mL
Natural unit count (clump count)	One organism (any unicellular organism, natural colony, or filament)	Natural Units/mL
Areal standard unit count	400 µm2	Units/mL

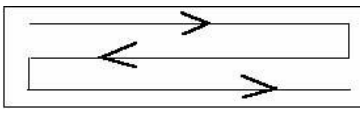
- A Sedgwick- Rafter (S-R) cell is commonly used for counting plankton because it is easily manipulated and provides reasonably reproducible data when used with a

calibrated microscope equipped with an eyepiece measuring device (e.g., the Whipple grid).

- The S-R cell is about 50 mm long _ 20 mm wide _ 1 mm deep. Its total bottom area is about 1000 mm2 and total volume is about 1000 mm3 (1 mL).
- The cell's greatest disadvantage is that high-magnification objectives cannot be used. As a result, the S-R cell is not appropriate for examining Nano-plankton.
- Place the cover glass diagonally across the cell to prevent formation of air bubbles in the cell corners.



- Shake the sample gently for homogeneity.
- Transfer 1 mL from the concentrated sample with a large pore pipette.
- The cover will rotate slowly and cover the inner portion of the S-R Cell during filling.
- Do not overfill because a sample depth greater than 1 mm would produce an invalid count.
- During lengthy examinations, do not permit large air spaces (caused by evaporation) to develop in the chamber. To prevent such air spaces from forming, occasionally place a small drop of distilled water on edge of cover glass.
- Before counting, let the S-R cell stand for at least 15 min to settle plankton. Count plankton on the bottom of the S-R cell.
- Identify and count different algal groups in random fields, the number of fields counted will depend on algal organism's density and statistical accuracy desired.



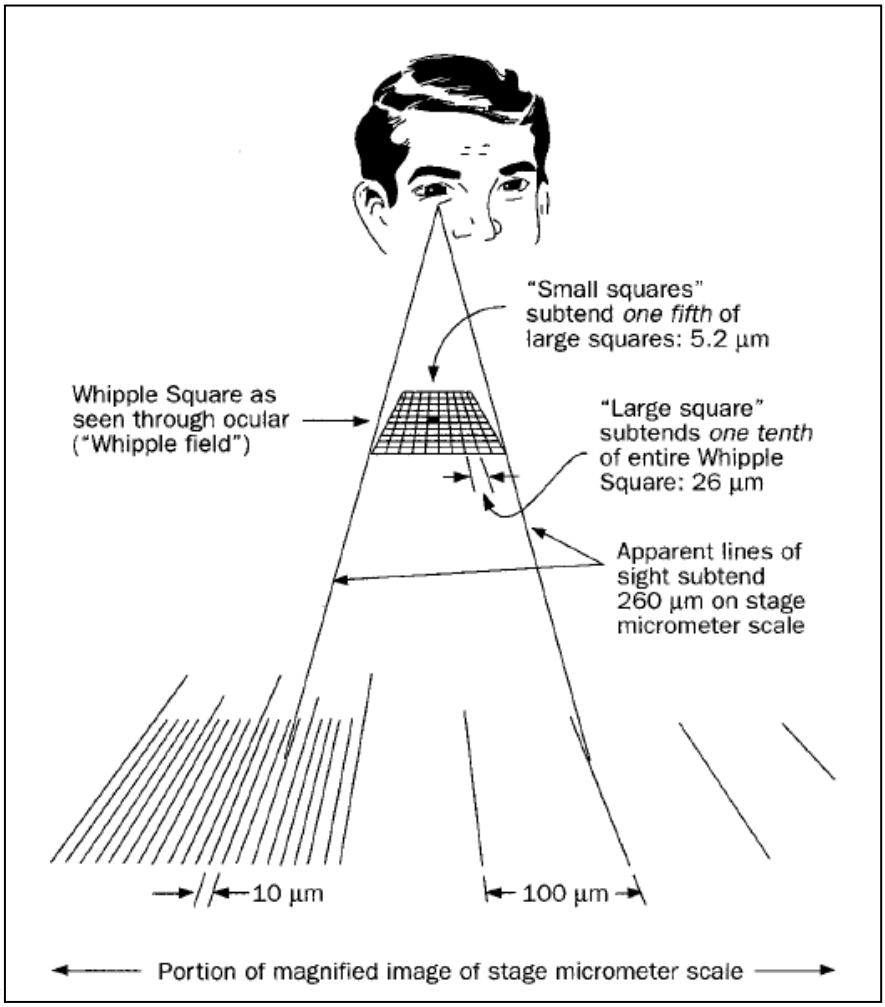
- Calculate the number of Organisms/mL for each algal group as follow:

$$\textbf{A}\text{lgal count (Org./ml)} = \left[\left(\frac{1000}{Counted\ Fields}\right) x \left(\frac{Net\ Vol.}{Sample\ Vol.}\right) x\ (No.\ of\ Counted\ Algae)\right]$$

- Calculate the total algal count in the sample as the sum of different algal groups count founded in the sample.

8.5. Microscope Calibration:

- Place the Whipple grid (Ocular micrometer) in an eyepiece of the microscope.
- Place the stage micrometer on the stage.
- Match the line at the left of the Whipple grid with the zero mark on the stage micrometer scale.
- Determine the width of the Whipple grid image to the nearest 0.01 mm from the stage micrometer scale.
- Divide the measure of the stage micrometer on the segments of Whipple grid.



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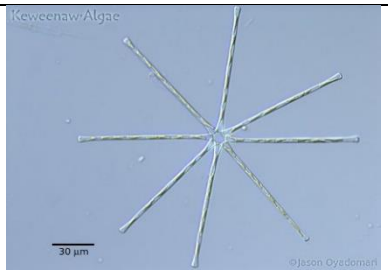
9. The main Algal Groups in the River Nile:

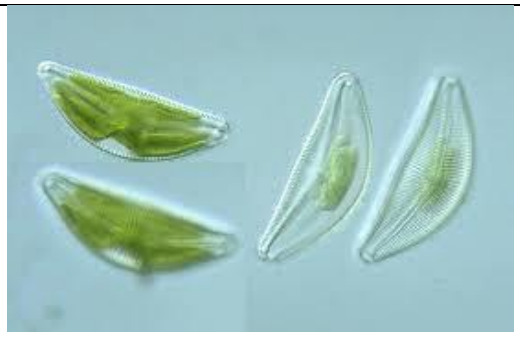
9.1. Bacillariophyta (Diatoms):

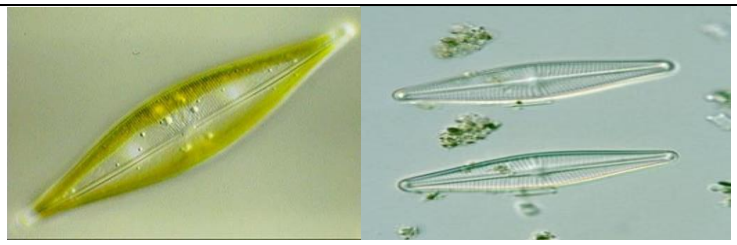
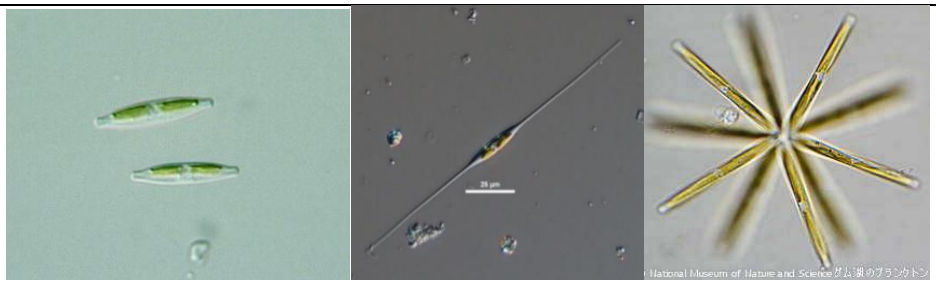
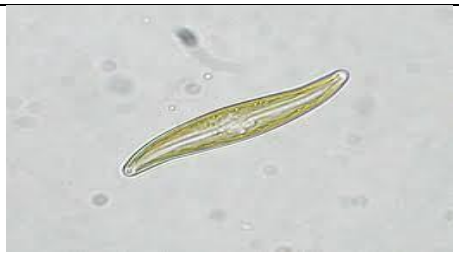
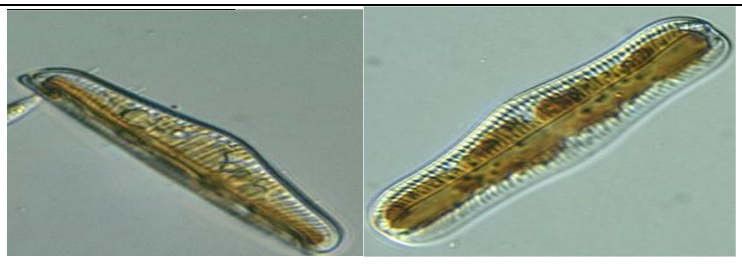
- They are a most important group of phytoplankton even though most species are sessile and associated with littoral substrata.

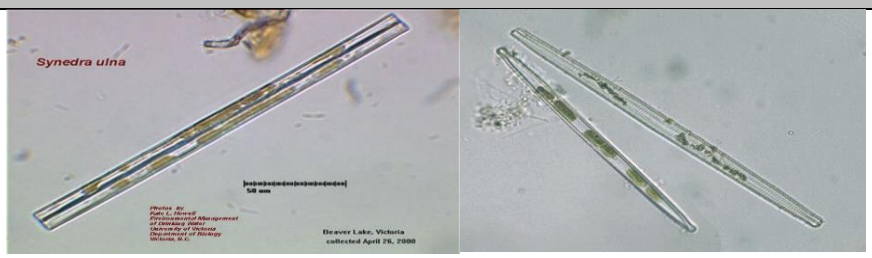
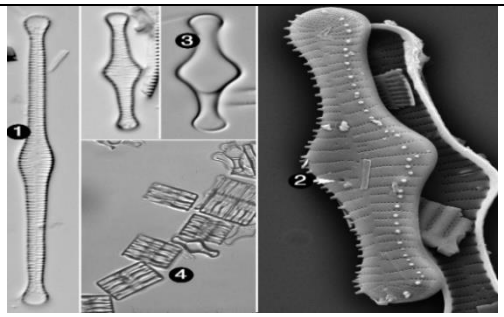
- Diatoms characterized with skeleton or frustule made of very pure silica coated with a layer of organic material.
- Present in both unicellular and colonial forms.
- The group is commonly divided into the centric diatoms (Centrals), which have radial symmetry, and the pinnate diatoms, which exhibit essentially bilateral symmetry. Many diatoms are slightly asymmetrical

The more founded Diatoms genera in the River Nile

Genus	Shape
Amphora sp.	
Asterionella sp.	
Cocconies sp.	
Cyclotella sp.	

Genus	Shape
Cymatopleura sp.	
Cymbella sp.	
Diatoma sp.	
Fragillaria sp.	
Gomphonema sp.	

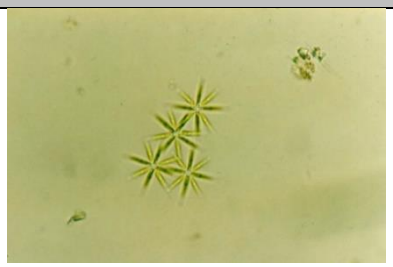
Genus	Shape
Gyrosigma sp.	
Melosira sp.	
Navicula sp.	
Nitzschia sp.	
Pleurosigma sp.	
Rhopalodia sp.	

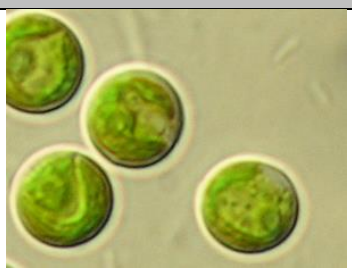
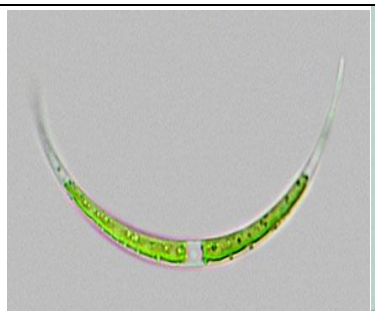
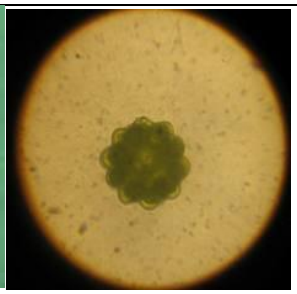
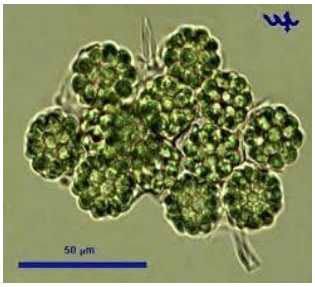
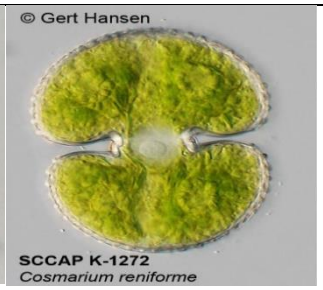
Genus	Shape
Synedra sp.	
Tabellaria sp.	

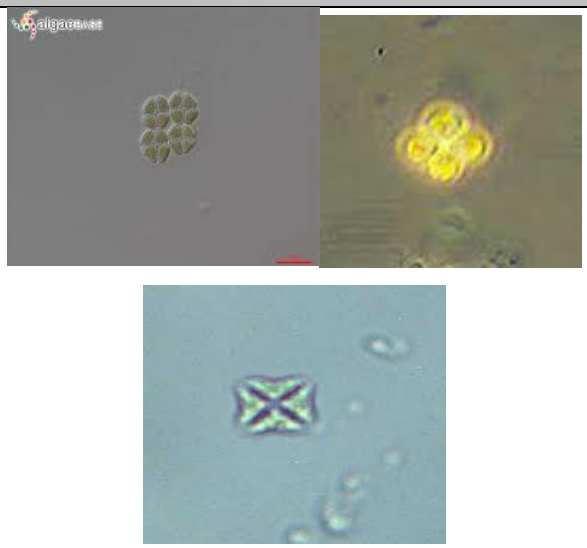
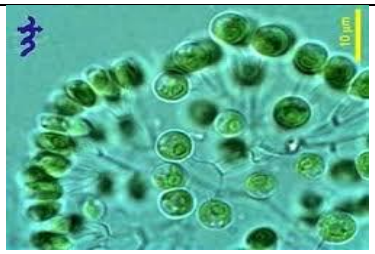
9.2. Chlorophyta (Green Algae):

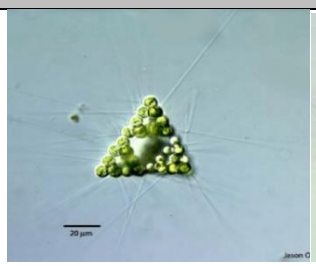
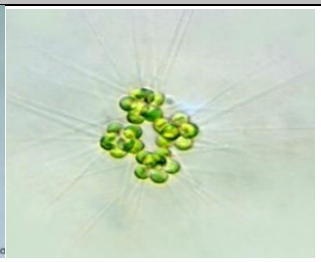
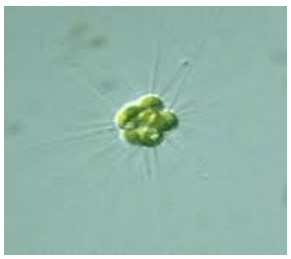
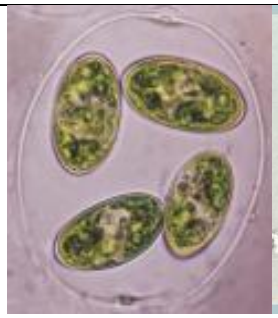
- These are an extremely large and morphologically diverse group of algae that is almost totally freshwater in distribution.
- Green algae usually appear in green because they contain the same ratio of chlorophyll a and b as that of higher plants and β-carotene.
- Green algae may appear to be filamentous, membranous, cylindrical, globular or coenocytic.
- Their photosynthetic products are starch and their cell walls are mainly composed of cellulose. Hence, it is generally believed that green algae are closely related to terrestrial higher plants in the theory of biological evolution.

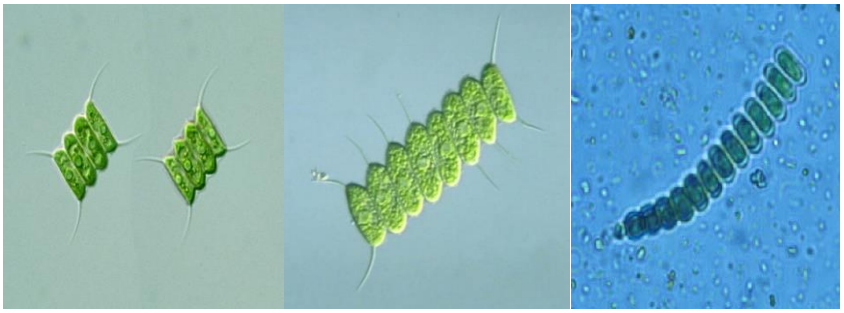
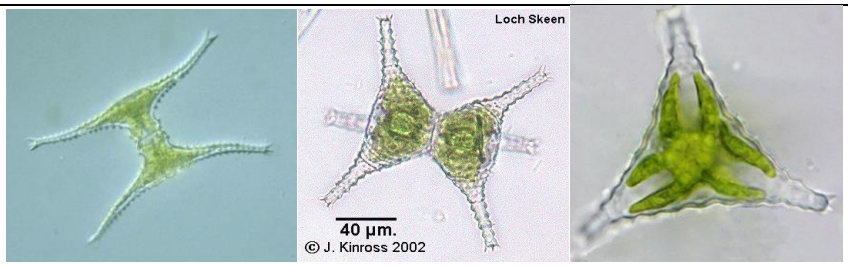
The more founded Green Algae genera in the River Nile

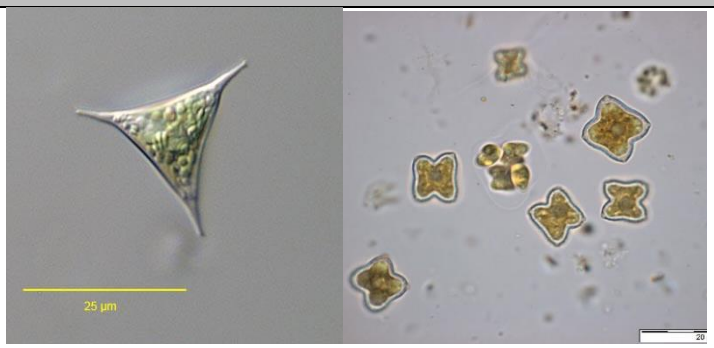
Genus	Shape
Actinastrum sp.	
Ankistrodesmus sp.	

Genus	Shape
Chlorella sp.	
Closterium sp.	 
Coelastrum sp.	  
Cosmarium sp.	 

Genus	Shape
Crucigenia sp.	
Dictyosphaerium sp.	
Golenkinia sp.	
kirchneriella sp.	

Genus	Shape
Micractinium sp.	  
Monoraphidium sp.	
Nephrocytium sp.	
Oocystis sp.	  

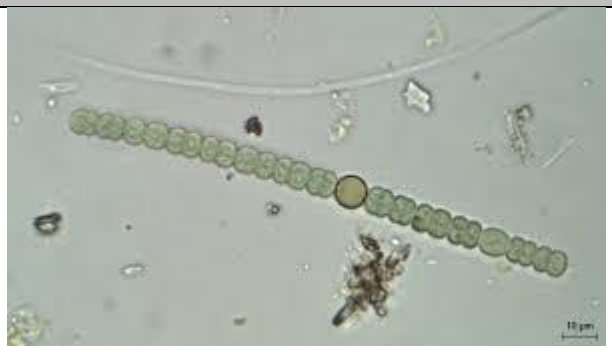
Genus	Shape
Pediastrum sp.	
Planktonema sp.	
Scenedesmus sp.	 
Selenastrum sp.	
Staurastrum sp.	

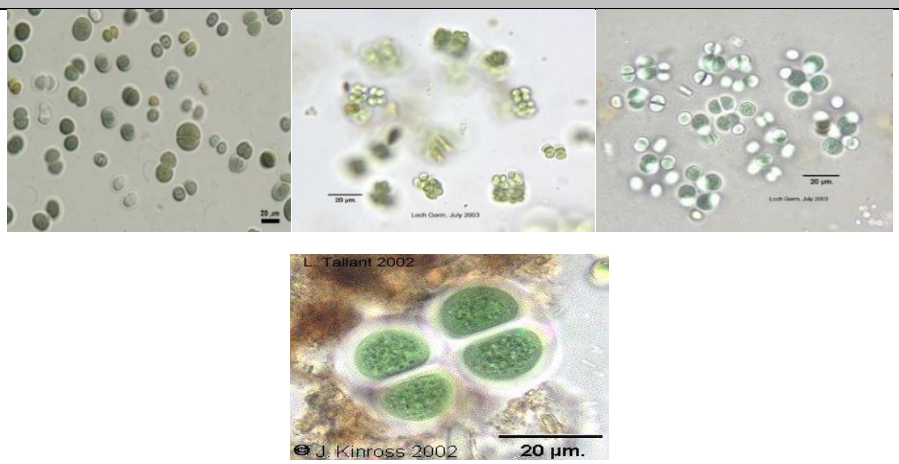
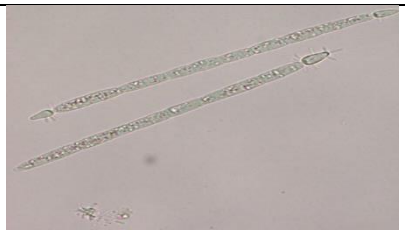
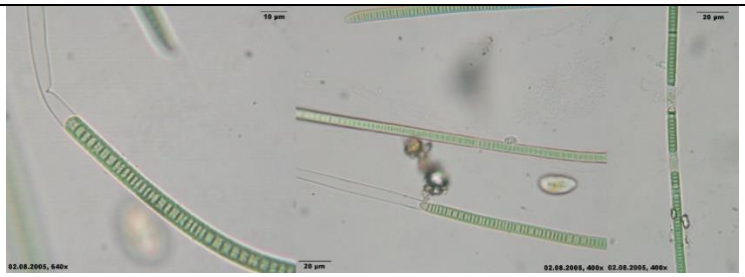
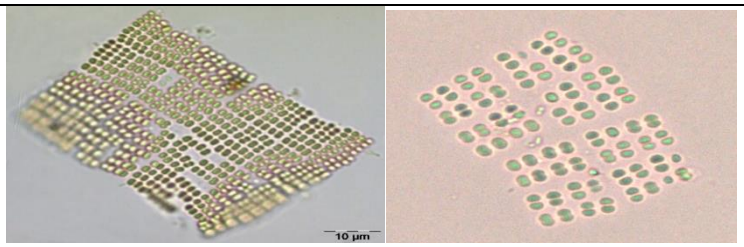
Genus	Shape
Tetraedron sp.	

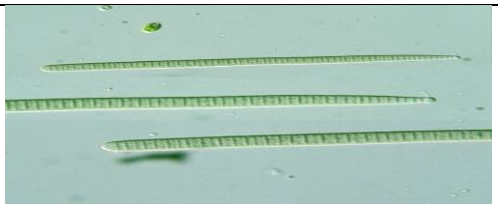
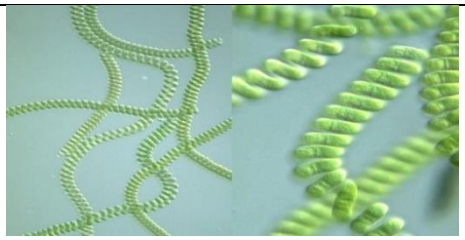
9.3. Cyanophyta (Blue-green Algae):

- Cyanobacteria or blue-green algae is a large heterogeneous group of prokaryotic, principally photosynthetic organisms.
- Cyanobacteria resemble the eukaryotic algae in many ways, including morphological characteristics and ecological niches, and were at one time treated as algae, hence the common name of blue-green algae.
- Cyanobacteria contain only one form of chlorophyll, chlorophyll *a*, a green pigment. In addition, they contain various yellowish carotenoids, the blue pigment phycobilin, and, in some species, the red pigment phycoerythrin. The combination of phycobilin and chlorophyll produces the characteristic blue-green color from which these organisms derive their popular name.
- Cyanobacteria may be unicellular or filamentous. Many have hyaline sheaths to bind other cells or filaments into colonies.
- Most cyanobacteria do not grow in the absence of light (*i.e.*, they are obligate phototrophs); however, some can grow in the dark if there is a sufficient supply of glucose to act as a carbon and energy source.

The more founded blue-green genera in the River Nile:

Genus	Shape
Anabaena sp.	

Genus	Shape
Chroococcus sp.	
Cylindrospermopsis sp.	
Lyngbya sp.	
Merismopedia sp.	
Microcystis sp.	

Genus	Shape
Oscillatoria sp.	
Phormidium sp.	
Spirulina sp.	

للاقتراحات والشكاوى قم بمسح الصورة (QR)

قام بإعداد الإصدار الأول من هذا البرنامج:

د/ محمد بكر محمد	المعمل المرجعى– الشركة القابضة
د/طارق رشدى	المعمل المرجعى– الشركة القابضة
د/ عاصم عبدالرحمن	المعمل المرجعى– الشركة القابضة
د/محمد أحمد السيد	المعمل المرجعى– الشركة القابضة
د/إبراهيم شوقى	المعمل المرجعى– الشركة القابضة
د/ صبرى زغلول وهبة حنا	المعمل المرجعى - الشركة القابضة
د/تامر إمام	المعمل المرجعى– الشركة القابضة
د/ سناء أحمد الإله	شركة مياه الشرب والصرف الصحى بالفيوم
د/ شعبان محمد على	شركة مياه الشرب والصرف الصحى بالفيوم
د/ حمدى عطيه مشالى	شركة مياه الشرب والصرف الصحى بالغربية
د/ سعيد أحمد عباس	شركة مياه الشرب والصرف الصحى بالغربية
د/ عبدالحفيظ السحيمى	شركة مياه الشرب بالقاهرة الكبرى
د/ مى صادق	شركة مياه الشرب بالقاهرة الكبرى

قام بإعداد الإصدار الثانى من هذا البرنامج:

د/ صبرى زغلول وهبة حنا	الشركة القابضة لمياه الشرب والصرف الصحى
د/ عماد حمدى محمود صبحى	الشركة القابضة لمياه الشرب والصرف الصحى

قام بالمشاركة وابداء الرأى لهذا البرنامج :

كيميائية/ إبتهال محمد عبده	شركة مياه القليوبية
كيميائى/ ابراهيم حسن سميسم	شركة مياه دمياط
كيميائية/ إيمان عوض الله اسكندر	شركة مياه البحيرة
كيميائية/ زينب رجاء حسن	شركة مياه القاهرة
كيميائية/ سارة حسين احمد ابو طالب	شركة مياه القليوبية
كيميائية/ سعاد سعد منصور	شركة مياه كفر الشيخ
كيميائى/ شريف سمير الشافعى	شركة مياه الدقهلية
كيميائى/ صفاء مصطفى طه	شركة مياه الاسكندرية
كيميائى/ عبد الحميد محمد الكباش	شركة مياه البحيرة
كيميائية/ عطاء عصمت احمد	شركة مياه قنا
كيميائى/ على خليفة على	شركة مياه القاهرة
كيميائية/ فايزة على بهيج	شركة مياه قنا
كيميائى/ محمد رضا عبد اللطيف البرعى	شركة مياه دمياط
كيميائى/ محمد رفعت محمود	شركة مياه الجيزة
كيميائى/ محمد محمود أبو عامر	شركة مياه المنوفية
كيميائى/ مصطفى حمدى	الشركة القابضة لمياه الشرب والصرف الصحى
كيميائى/ مصطفى محمود مصطفى	شركة مياه الجيزة
كيميائية/ مي مصطفى اسماعيل	شركة مياه الفيوم
كيميائية/ نورا محمود ربيع	شركة مياه الفيوم
كيميائية/ ولاء موسي احمد الشيمى	شركة مياه القليوبية
كيميائى/ وليد صالحين عبد الباسط	شركة مياه مطروح
كيميائى/ يحي اسماعيل صالح	شركة مياه الجيزة
كيميائى/ يوسف محمد عبد المقصود	شركة مياه كفر الشيخ

قام بالتنسيق الفني والإخراج لهذا البرنامج:

كيميائى/ محمود جمعه	الشركة القابضة لمياه الشرب والصرف الصحى

كيميائى/ الحسن الصادق الشركة القابضة لمياه الشرب والصرف الصحى