برنامج المسار الوظيفي للعاملين بقطاع مياه الشرب والصرف الصحي

دليل المتدرب

البرنامج التدريبي كيميائي مياه

FRESHWATER ALGAE- الدرجة الثالثة

تم اعداد المادة بواسطة الشركة القابضة لمياه الشرب والصرف الصحي
قطاع تنمية الموارد البشرية - الادارة العامة لتخطيط المسار الوظيفي V1 1-7-2015

Table of contents

FRESHWATER ALGAE

Introduction to Freshwater Algae
General introduction

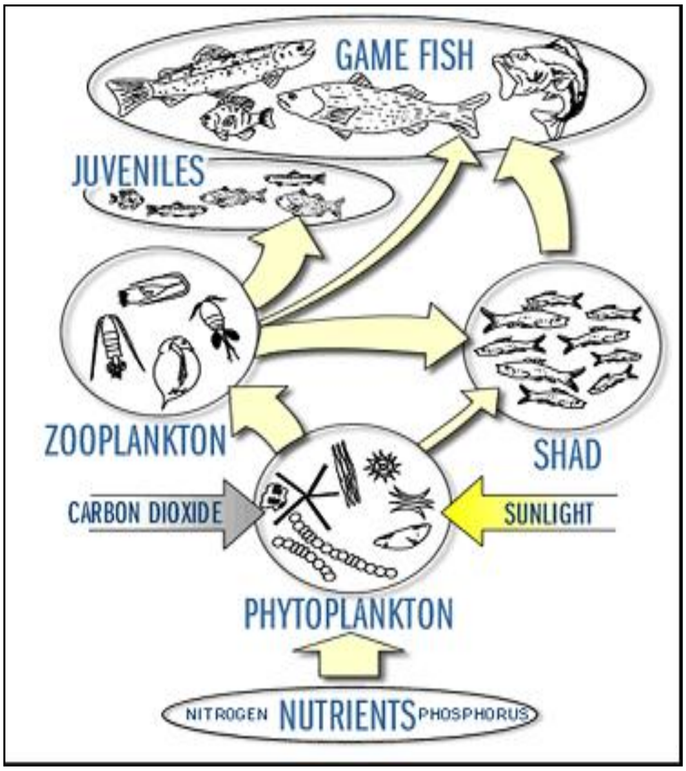
- Algae are widely present in freshwater environments, such as lakes and rivers.
- They are typically present as micro-organisms visible only with the aid of a light microscope.
- They have a major importance in the freshwater environment, both in terms of fundamental ecology and in relation to human use of natural resources.
- The word 'algae' originates from the Latin word for seaweed and is now applied to a broad assemblage of organisms that can be defined both in terms of morphology and general physiology.
- They are simple organisms, without differentiation into roots, stems and leaves and their sexual organs are not enclosed within protective coverings.
- 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.
- These organisms include both prokaryotes (cells lacking a membrane bound nucleus) and eukaryotes (cells with a nucleus plus typical membrane-bound organelles).

Algae as primary producers

- As fixers of carbon and generators of biomass, algae are one of three major groups of photosynthetic organism within the freshwater environment.
- They are distinguished from higher plants (macrophytes) in terms of size and taxonomy, and from photosynthetic bacteria in terms of their biochemistry.
- The level of primary production by algae in freshwater bodies can be measured as fixed carbon per unit area with time ($mgCm^{-3}\ h^{-1}$), 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. Algae are fundamentally autotrophic (photosynthetic).



Aquatic Food Chain

Categories of water types

Annual mean level of total phosphorus (mg/m^3)	Water Type	Annual mean concentration of chlorophyll "a" (mg/m^3)
	Ultra- Oligotrophic	
- 4.5		1.0
	Oligotrophic	
- 15		2.8
	Mesotrophic	
- 48		8
	Eutrophic	
- 150		22
	Hypereutrophic	

Planktonic and benthic algae

- Within freshwater ecosystems, algae occur either as free-floating (planktonic) or substrate associated (largely benthic) organisms.
- Planktonic algae drift freely within the main body of water with some species able to regulate their position within the water column.
- Substrate associated organisms are either fixed in position (attached) or have limited movement in relation to their substrate.
- These substrate associated algae are in dynamic equilibrium with planktonic organisms, with the balance depending on two main factors; the depth of water and the rate of water flow.
- Build-up of phytoplankton populations requires a low flow rate of water and adequate light levels.
- Benthic algae require adequate light (shallow waters) and can tolerate high rates of water flow, so predominate over phytoplankton in fast flow rivers and streams.
- Benthic algae also require adequate attachment sites – which include inorganic substrate, submerged water plants and emergent water plants at the edge of the water body.
- The distinction between planktonic and non-planktonic algae is ecologically important and is also relevant to algal sampling and enumeration procedures.

Size and shape

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	4.2–4.2×10^3	Blue-green algae Cryptophytes *Cryptomonas* *Rhodomonas*	
Microplankton	20–200	4.2×10^3–4.2×10^6	Dinoflagellates *Ceratium* *Peridinium*	Diatoms *Asterionella*
Macroplankton	>200	>4.2×10^6	–	Blue-green algae *Anabaena* *Microcystis*

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

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.

Taxonomic variation – the major groups of algae

Freshwater algae can be grouped into 10 major divisions (phyla) in relation to microscopical appearance and biochemical/cytological characteristics.

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.

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.

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

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.

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.

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. 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.

Major Divisions of Freshwater Algae: Microscopical Appearance

Algal Division (phylum)	Index of Biodiversity[a]	Typical Colour	Typical Morphology of Freshwater Species	Motility (Vegetative Cells/Colonies)	Typical Examples
1. Blue-green algae *Cyanophyta*	297	blue-green	Microscopic or visible – usually colonial	Buoyancy regulation Some can glide	*Synechocystis* *Microcystis*
2. Green algae *Chlorophyta*	992	grass-green	Microscopic or visible – unicellular or filamentous colonial	Some unicells and colonies with flagella	*Chlamydomonas* *Cladophora*
3. Euglenoids *Euglenophyta*	124	Various colours	Microscopic – unicellular	Mostly with flagella	*Euglena* *Colacium*
4. Yellow-green algae: *Xanthophyta*	73	yellow-green	Microscopic – unicellular or filamentous	Flagellate zoospores and gametes	*Ophiocytium* *Vaucheria*
5. Dinoflagellates *Dinophyta*	54	red-brown	Microscopic – unicellular	All with flagella	*Ceratium* *Peridinium*
6. Cryptomonads *Cryptophyta*	15	various colours	Microscopic – unicellular	Mostly with flagella	*Rhodomonas* *Cryptomonas*
7. Chrysophytes *Chrysophyta*	115	golden brown	Microscopic – unicellular or colonial	Some with flagella	*Mallomonas* *Dinobryon*
8. Diatoms *Bacillariophyta*	1652	golden brown	Microscopic – unicellular or filamentous colonies	Gliding movement on substrate	*Stephanodiscus* *Aulacoseira*
9. Red algae *Rhodophyta*	22	red	Microscopic or visible – unicellular or colonial	Non-motile	*Batrachospermum* *Bangia*
10. Brown algae *Phaeophyta*	2	brown	Visible – multicellular cushions and crustose thalli	Non-motile	*Pleurocladia* *Heribaudiella*

Major Divisions of Freshwater Algae: Biochemical and Cytological Characteristics

Algal Division (phylum)	Pigmentation[#] Chlorophylls	Pigmentation[#] Carotenes	Pigmentation[#] 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**	$\alpha, \beta, \varepsilon$		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

5. **Light:**

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

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.

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 as well as actinomycetes may be found in high population densities.

On the other hand, the **CHEMICAL** constituents of water affect plankton development and vise versa.

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.

2. **pH:**

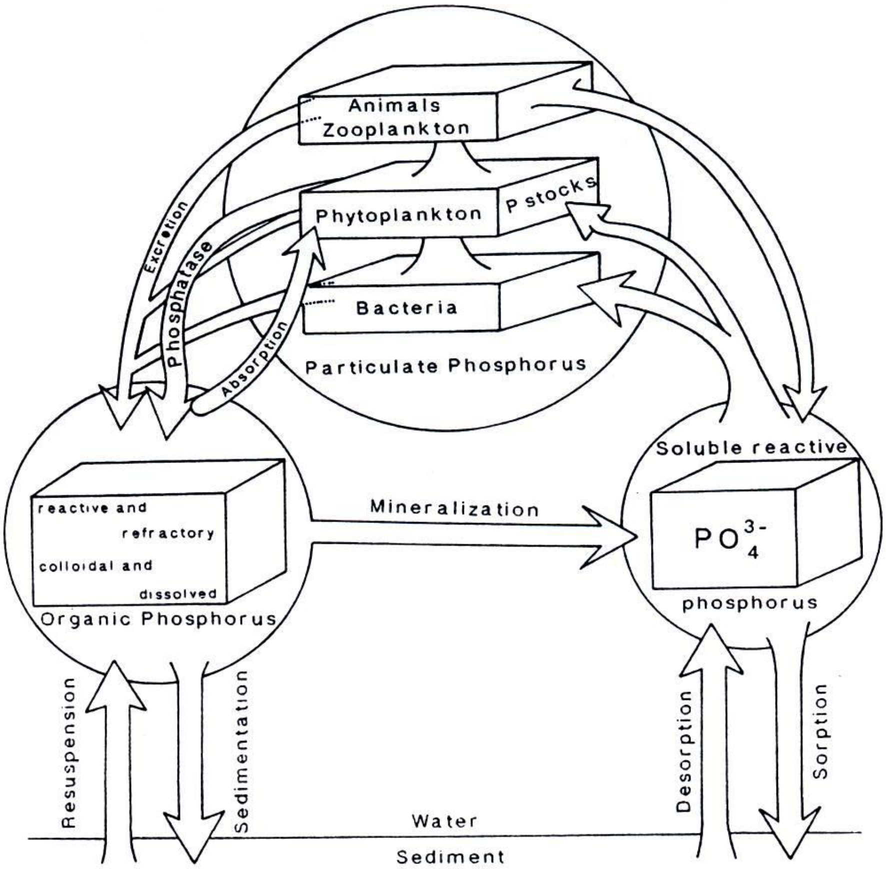
- The pH in natural water alters with its carbon dioxide content.
- Thus streams which possess some limestone in solution are well buffered and exhibit little variation on pH levels beyond the range of 6.8 – 8.8.
- Some species of algae are common at water with pH value of 1.8 e.g. Chlamydomonas sp., 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.

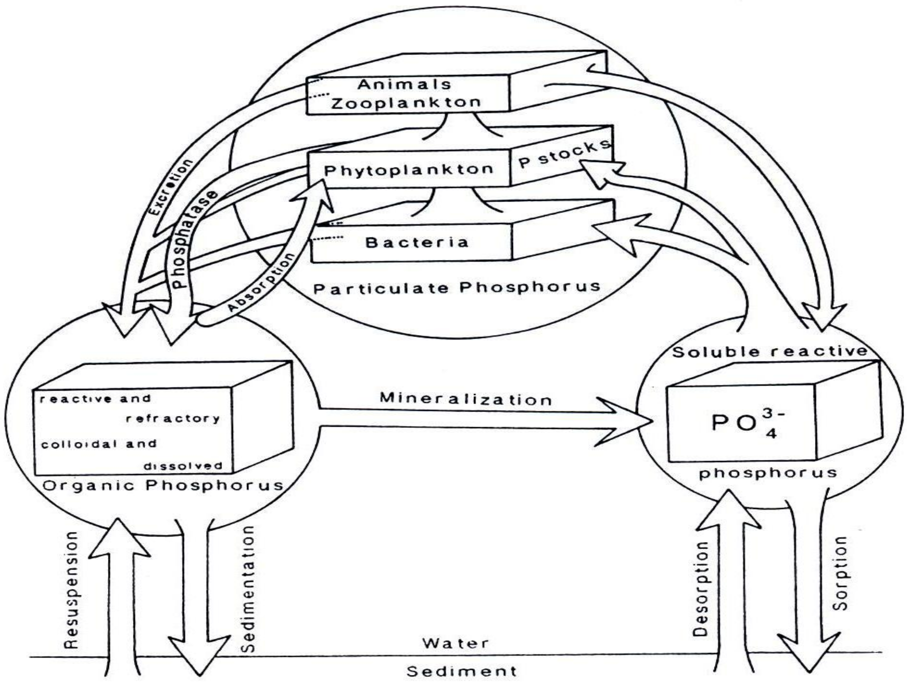
3. **Salinity:**

- Salinity in water is known to affect 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. **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.

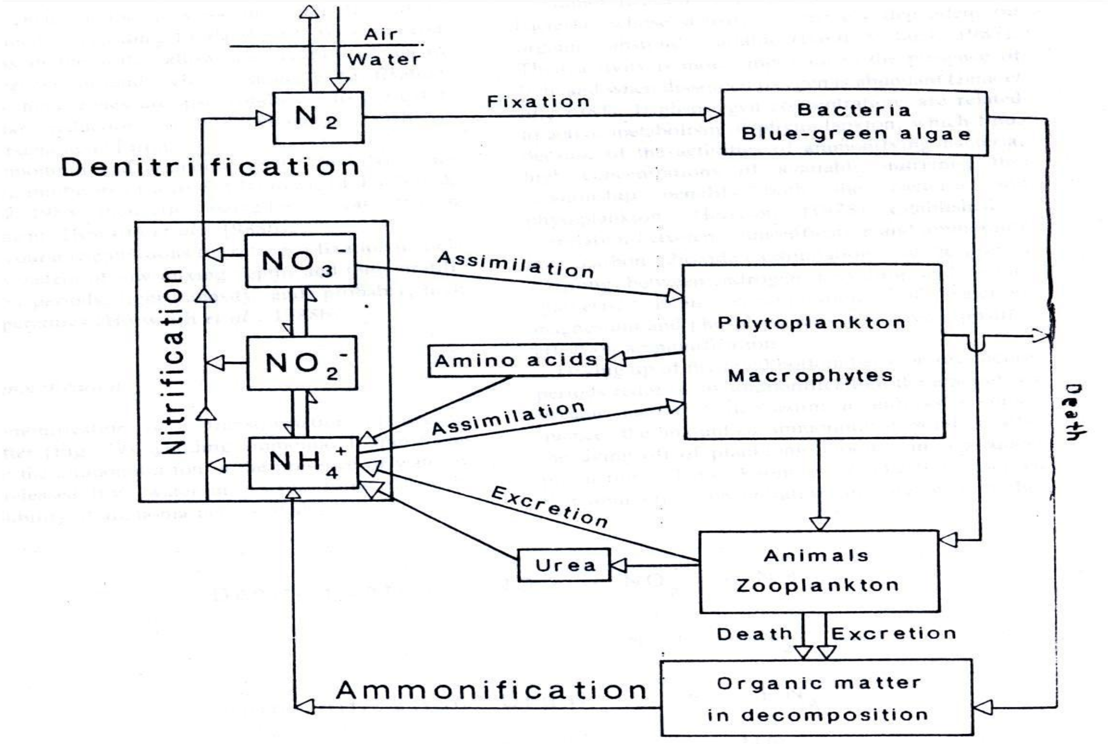




Phosphorus cycle in water

5. Nitrogen:

- Nitrates are carried out into streams by runoff water they tend to be more abundant at times of heavy rains.
- Its highest level was detected during winter months when planktonic growth is greatly reduced. The same tendency is seen for other nitrogen group.



Nitrogen cycle in water

Sampling, Biomass Estimation and Counts of Freshwater Algae

- Algal populations can be sampled in a number of different ways, depending on the nature of the habitat and the location of the algae within the water body.
- Algae within the water column (plankton) are in dynamic equilibrium with both attached and unattached forms associated with plant surfaces and sediments at the bottom of the water column.
- Techniques for sampling and enumeration can be conveniently separated in relation to these two main groups of algae – planktonic and non-planktonic (substrate-associated) organisms.

PLANKTONIC ALGAE

- Lake and river phytoplankton include all those photosynthetic free-floating organisms that are present within the main water body, and which generate oxygen by photosynthesis.
- This includes prokaryotic blue-green algae but excludes photosynthetic bacteria – which do not evolve oxygen and which do not normally make a major contribution to overall biomass formation.

Mode of collection

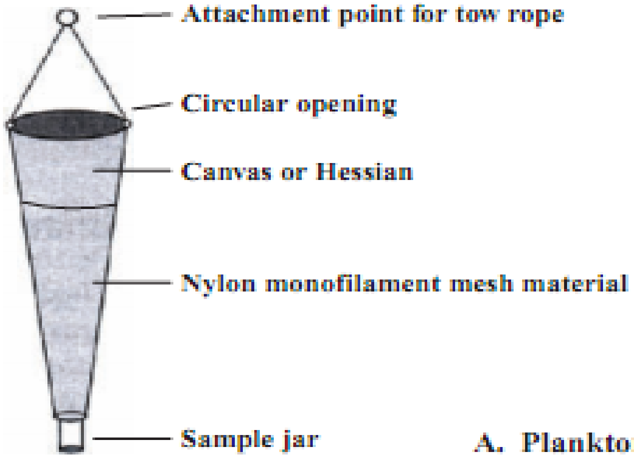
- Phytoplankton samples can be directly collected from the water body in three main ways – via a phytoplankton trawl net, volume sampler or as an integrated sample.
- In addition to this, samples of sedimenting phytoplankton can be passively collected over a period of time using sediment traps.
- In all cases, material for live examination should be brought back to the laboratory as quickly as possible and examined straightway.
- For chemically preserved material, the fixative should be added immediately (on the boat) after collection.

Phytoplankton Trawl net

- Plankton nets vary in design from basic tow-nets (conical or with truncated neck) to more complex devices fitted with flow meters and special opening/ closing mechanisms (Eaton et al., 2005).
- In their simplest form (Fig. 2.4), they consist of long cones with a circular opening at their mouth and some form of collecting chamber at the narrow end.
- Mesh size the type of netting and mesh size determine filtration efficiency (extent of removal of different particle sizes), clogging tendencies, velocity of water flow within the net and condition of sample after collection.
- Trawl nets of different mesh sizes can be classified in relation to the minimum size range of organisms collected and vary from largest zooplankton/ichthyoplankton nets (mesh aperture 1024

µm) to phytoplankton (53–76 µm) and nanoplankton (<50 µm) nets.

- A typical phytoplankton net mesh size of about 50 µm will collect the majority of algae in the microplankton and macroplankton range referred to collectively as 'net phytoplankton'.
- Small unicellular algae (nanoplankton) can be collected by smaller mesh sizes (commercially available nets go down to 10 µm), but problems with clogging and reduced water flow mean that these smaller organisms are best collected as bulk samples followed by sedimentation or centrifugation.
- In practice, routine collection of net zooplankton and phytoplankton involves using separate zooplankton and phytoplankton nets (mesh sizes about 250 µm and 50 µm respectively).
- Using the two nets in combination has the advantages that both samples are collected from the same volume of lake water, and that zooplankton is largely removed from the phytoplankton sample – reducing sample contamination and algal ingestion during transport to the laboratory.



A. Plankton net

B. Net mesh sizes and classification

Size of Aperture (µm)	Approximate Open Area %	CLASSIFICATION
1024	58	Largest zooplankton and ichthyoplankton
752	54	Larger zooplankton and ichthyoplankton
569	50	Large zooplankton and ichthyoplankton
366	46	Large microcrustacea
239	44	Zooplankton - Microcrustacea
158	45	Zooplankton - Microcrustacea and most rotifers
76	45	Net phytoplankton – Macroplankton and microplankton
64	33	
53	-	
10	-	Nanophytoplankton

Trawl net collection of phytoplankton.

(A) Simple conical phytoplankton net, with collection jar.

(B) General table of commercially-available net mesh sizes and their classification.

- The phytoplankton net would have mesh sizes in the range 53–76 µm. Larger mesh sizes would collect colonial blue-greens and other large algae but not the major part of the net phytoplankton.
- Each mesh size will collect the specified group of organisms, plus all larger size groups unless they are prevented from entry into the net by pre filtration.

Advantages and disadvantages

- The major advantage of trawl net collection is that a large sample of phytoplankton biomass can be obtained within a short period of time and that it is collected as a concentrated sample.
- This is particularly useful in oligotrophic waters and during winter in temperate mesotrophic/eutrophic lakes, where phytoplankton numbers may be very low.
- Trawl net samples can be used for bulk analysis of phytoplankton (e.g. nitrogen and phosphorus content, lipid content), microscopic analysis of the range of organisms present (e.g. light and electron microscopy, X-ray and infrared microspectroscopy) or simply where a rapid collection procedure is required to give an idea of the main large celled and colonial algae present in the lake.
- The major disadvantages of trawl net sampling are the following. Loss of small algae.
- Algae below the mesh size are largely excluded from the sample. Exclusion is not absolute, since the mesh becomes partly occluded by large-celled and colonial algae, so some smaller organisms are retained.
- Sample contamination: The phytoplankton sample tends to become contaminated by zooplankton, which selectively consume algae and contribute to overall biomass.
- Pre filtration using the zooplankton net removes larger zooplankton such as Daphnia, but smaller zooplankton such as rotifers and protozoa pass through.
- Small particulate inorganic and organic debris may also be retained with the phytoplankton, some of which (decomposing matter) may have an associated bacterial population.
- In stratified temperate lakes, debris contamination is particularly important during winter months, when the lake is vertically mixed and sediment at the bottom of the water column may become re-suspended.
- In streams, contamination of the net sample by non-algal particulate material is most likely to occur at night-time (sediment disturbance by the nocturnal benthic community) and during winter months (Richardson et al., 2009).
- Sample deterioration. Because of the concentrated nature of the sample, adverse changes in the water (e.g. oxygen

depletion) during transport may lead to death of some of the more delicate algae Problems in quantitation.

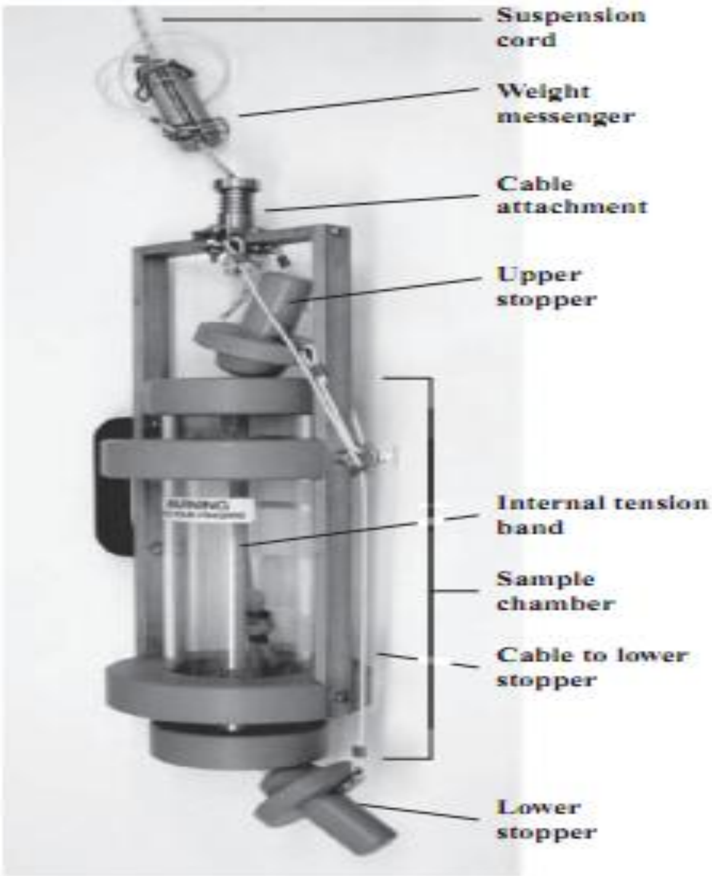
- For various reasons, net samples cannot be used to make an accurate quantitative assessment of phytoplankton populations (per unit volume of water) within the lake.
- During sample collection, the vertical position of the net varies within the surface waters, so the sample is not truly representative of the entire epilimnion.
- Even when trawling is carried with a flow meter, complexities of water circulation around and through the net (see above) make it difficult to know the actual volume of water that has been filtered.

Volume samplers

- Collecting phytoplankton samples within a volume of lake water has the advantages that biomass or species populations can readily be determined per unit volume of medium, and that all sizes of phytoplankton are obtained within the sample.
- A variety of collecting vessels are available including the Kemmerer, Van Dorn, Niskin and Nansen sampler.
- Phytoplankton samples at vertical points in the water column can be obtained by lowering a depth sampler such as the Van Dorn model to the required depth, then dropping a weight messenger to trigger closure.
- The volume of sample (usually 1–2 L) can be brought to the surface and analyzed.
- Care must be taken to ensure that depth samplers are completely watertight at the depth being sampled, and that surfaces in contact with the water sample are completely inert.
- Samples collected from volume samplers are typically divided into separate aliquots for determination of a range of parameters including inorganic nutrient concentrations, soluble organics, and phytoplankton and zooplankton biomass.
- These water samples can be very heterogeneous, however, and care must be taken that they are well-mixed (by pouring into a large container and stirring) before subdivision.

Van Dorn Volume Sampler

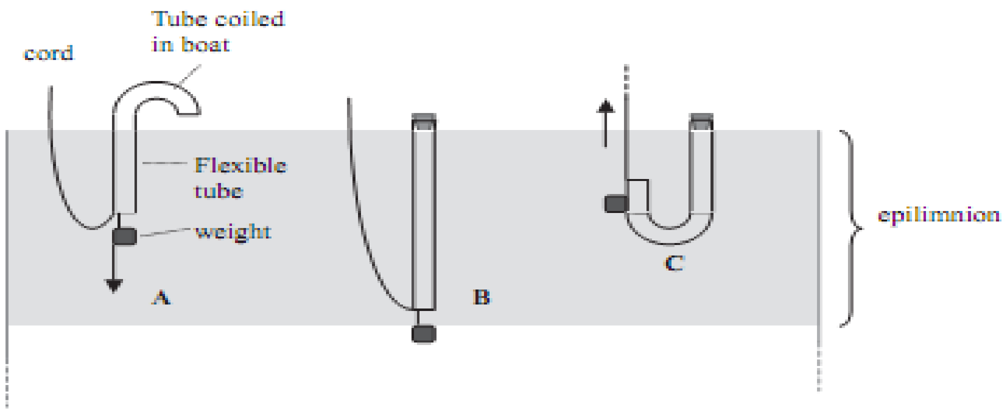
Shown in the open (sprung) position, the sampler is lowered to the required depth in the water column, then the weight messenger released down the suspension cord. This strikes the cable attachment, which releases the cables to the upper and low stoppers. These are then jerked into position at either end of the chamber by the internal tension band, creating a water-tight seal, which encloses the water sample. The sampler is subsequently pulled to the surface and the water sample collected.



Integrated sampling

- Analysis of the average community structure of the top part of the water column (epilimnion) of a stratified lake (Fig. 2.3(b)) is normally obtained from an integrated (composite) sample, using a collecting tube which is lowered from the boat.
- In the case of shallow unstratified lakes, the whole of the water column can be sampled.
- The sampler is typically a flexible plastic tube, internal diameter approximately 2.5 cm and about 1 meter longer than the depth through which the sample is to be collected, and is weighted at its end (Fig. 2.6).

- A long piece of cord is attached to the weighted end and the tube lowered slowly and vertically into the water.
- This process has to be carried out with care in order to collect a column of water in the tube as undisturbed as possible representing the water column of the lake.
- To ensure a vertical sample (tube not displaced by current), the tube should be sufficiently weighted and the boat should be stationary (attached to a buoy).
- The top of the tube is then closed by inserting a rubber bung. With the top still the weighted bottom end is raised out of the water so the tube forms a loop.
- The weighted end is placed in a suitable large receptacle and the rubber bung removed from the other end.
- The trapped column of water now flows into the container and totally mixes.
- The integrated sample of the lake surface (top 5 m) can then be separated into subsamples to analyse water chemistry, chlorophyll content and species composition.
- The use of integrated sampling techniques (e.g. Straskraba and Javornicky, 1973) provides information on the overall phytoplankton population over a part of the water column (stratified lake) – but loses detail such as phytoplankton gradients and strata of particular algal species.
- Comparison of vertical trawls from different sites on the lake is particularly useful where horizontal rather than vertical variation in the lake is being investigated.
- Once the lake water sample has been collected, the phytoplankton can then be analysed in various ways – including total biomass, species populations, biological interactions (e.g. fungal infection), physiological state, chemical composition and molecular characteristics.



Collection of integrated water samples at Lake Surface (not drawn to scale). (A) Weighted flexible tube is lowered vertically into water. (B) Top of tube closed by bung when fully extended in epilimnion. (C) Weighted end of tube is raised by attachment cord. The whole tube is then taken into the boat, the bung removed and the lake water poured as a mixed sample into a collecting vessel.

Preservation and storage

- The handling of samples after collection is a critical stage in most phytoplankton work.
- It is important to minimize quantitative and qualitative changes in the phytoplankton composition before further treatment of the samples takes place.
- This can be achieved either by proceeding rapidly to the next step, e.g. fixation of the sample, or by keeping the samples at a low activity rate until the processing of the sample can take place, e.g. when cultures are to be set up.

KEEPING SAMPLES ALIVE

- This procedure is limited to some special conditions such as inshore investigations (where the distance to the laboratory is reasonably short).
- Phytoplankton in water samples will keep their viability for some time provided they are not subjected to rise in temperature or light intensity.
- In net hauls or samples from blooms, however, the viable period may be rather short.
- In order to extend the period of viability it is recommended that the level of physiological activity of the species should be lowered.
- Water samples should be kept at water temperature if the latter is below 10° to 15°C> or cooled-(2° to 10° C) if temperature is above 15°C, and stored in total darkness.
- Water samples from bloom situations should be dealt with as net hauls.
- Net hauls should not be too dense, and they should be kept in a rather large amount of water (250 to 1,000 ml).

- Some light may be necessary to avoid oxygen depletion in the sample.
- Temperature should be low, 2° to 5° C, even in summer, because heterotrophic organisms, including bacteria, will multiply rapidly in such a dense sample.
- The release of dissolved organic matter and the, decay of many cells would provide a good substratum for such growth.

Fixation

- A great number of fixatives and preserving agents have been described, but they have all proved to be more or less selective in their fixing capacity.
- The most widely used fixatives for phytoplankton are formaldehyde and potassium iodide plus iodine (Lugol's solution).
- Both used on their own or with other compounds added.

The formaldehyde method (Formalin)

- Fixing and preserving agent. Use from 2 to 4 % buffered formalin (20 g sodium borate + 1 L 37 % Formaldehyde) to the sample. Shake the bottle immediately to facilitate an instantaneous fixation.
- For net hauls, add fixing/preserving agent to make up about one-third of the volume if the sample is dense.
- Mix well, It is important that fixation should take place immediately after the collection of each sample (the water sample should be added directly to the fixing agent) in order to prevent any adverse effects of e.g. light and temperature changes.
- This is particularly important for net hauls where the high cell density may otherwise cause rapid decay. Transport to laboratory or store.

Advantages of the method

- The neutral formaldehyde method (when properly carried out) renders coccolithophorids, diatoms and thecate dinoflagellates in identifiable condition, and a large number of naked flagellates can be recognized as such.
- In addition, cells devoid of flagella can be distinguished.
- The ingredients of the fixing/preserving agent are usually easy to obtain and the solution will keep well during storage.
- Samples preserved with formaldehyde may keep for years without further attention, when stored properly.

Disadvantages of the method

- Formaldehyde fixation is qualitatively selective especially in that it distorts cell shape of naked species and causes flagella to be thrown off in many flagellates.
- Cell content will bleach, making it very difficult to distinguish between pigmented and non-pigmented cells. Organelles, as e.g. flagella, may need phase-contrast microscopy in order to be revealed.

The iodine method (Lugol's fixation)

- The most commonly used alternative to the above method is Lugol's fixation, i.e. iodine dissolved in a potassium iodide solution. It is first proposed for freshwater.
- Fixing and preserving agent 100 g KI is dissolved in 1 liter of distilled water; then 50 g iodine (crystalline) is dissolved and 100 ml of glacial acetic acid is added.
- As the solution is near saturation, any possible precipitate should be removed by decanting the solution before use.
- For water samples add enough fixing agent to give the sample a weak brown color (About 0.3 % Lugol’s solution to sample), shake well.
- Use this fixative for water samples only: net hauls are usually better preserved with formaldehyde.
- Use clear glass bottles only, since colored bottles such as brown glass bottles would make it difficult to ascertain the amount of fixing agent needed.

- Avoid plastic bottles, as they will take up iodine from the solution.
- As for formaldehyde, it is important that fixation should take place immediately after sampling.
- It is important that an adequate amount of fixing agent be added, as too much iodine may give a heavy staining. Transport to laboratory or store.
- If samples are to be stored for some time, the concentration of iodine in solution should be checked regularly (check the color) and more preserving agent added if necessary.
- The loss of molecular iodine from the sample depends on the storage conditions and is increased by light.

Advantages of the method

- The main advantage is that a large number of flagellates will retain their flagella.
- Other organisms of the phytoplankton community will also fix reasonably well.
- The cells will stain brownish yellow and hence be easier to observe during the counting procedure, though many organic and inorganic particles will be stained as well.
- The staining will render phase-contrast microscopy less desirable than for the formaldehyde method.
- The ingredients are relatively easy to obtain, and the stock solution will keep well for many years.

Disadvantages of the method

- The acid Lugol's solution will dissolve; also, silica will dissolve with long storage time.
- Lugol's solution without any addition will turn slightly acid relatively soon with a resultant elimination of coccolithophorids.
- Samples preserved with iodine need attention during storage as iodine is oxidized with time.
- Many organisms are frequently over stained; a large amount of starch will often obscure the anatomy, and also make surface structures difficult to observe, e.g. thecal plates of dinoflagellates.

- However, cells stained by iodine may be cleared up by adding sodium thiosulphate, which reduces the iodine.

Formaldehyde versus iodine

- No single method can be expected to be suitable for the preservation of all types of phytoplankton.
- The fixing and preserving agent should be chosen with regard to the aim of the investigation.
- For instance, in a typical offshore area with thecate dinoflagellates and diatom domination, an acid formaldehyde fixation may be applied; in a tropical area with coccolithophorids predominating, neutralized formaldehyde is appropriate: in inshore areas with naked flagellates as an important part of the community, the acid iodine method may give the best result from a combined quantitative and qualitative view.

Concentrating phytoplankton samples

- The samples were shacked well and 500 ml sample were transferred to graduated cylinder of 500 ml capacity. The samples were left to settle for five days.
- The supernatants were then siphoned slowly with a small plastic tube ending with a fine net of 20-µm mesh diameter, until the sample was concentrated to about 50 ml.
- The reduced sub-sample were adjusted to 50 ml and kept in dark plastic vial for microscopic examination.

Counting techniques

1. The inverted- microscope method

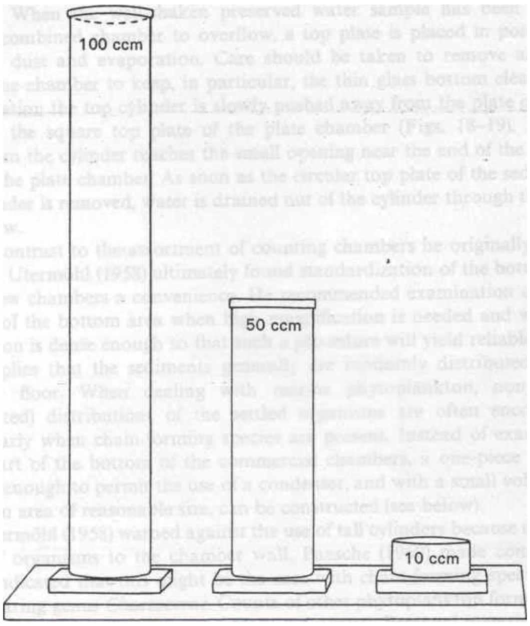
Utermöhl method (sedimentation method)

- Utermohl (1931) designed an assortment of counting (= sedimentation) chambers or cylinders in order to provide chambers for specific sample characteristics.
- A sample with a dense phytoplankton population or a lot of detritus is most easily examined in a chamber in which the bottom (floor) area is large relative to the volume.
- A sample with a sparse population and less detritus is best examined in a chamber in which the bottom area is small

relative to the volume, reducing the time wasted scanning the empty bottom.

- The principle is to remove the upper part of the chamber (the sedimentation cylinder) after sedimentation, leaving organisms in the bottom part, which has a height less than the working distance of the condenser.
- The model now in common use is a combined plate chamber consisting of a top cylinder (sedimentation cylinder) of 10, 50 or 100 ml capacity (5 and 25 ml may also be available) and a bottom-plate chamber. The parts of the plate chamber are a rectangular Perspex plate, a ring and a circular bottom (base) plate of cover slip thickness.
- The plate fits into the mechanical stage of the inverted microscope of the type for which it is constructed.
- It has a small opening close to one end, and a circular central opening of 26 mm diameter, which is slightly larger than the diameter of the sedimentation cylinders.
- The ring can be fastened to the underside of the rectangular plate by a key.
- The bottom plate is placed between the ring and the large opening of the Perspex plate and thus forms the floor of the plate chamber.
- The combined chamber is ready for use when the cylinder of the desired capacity is placed on top of the plate chamber.
- When the well-shaken preserved water sample has been poured into the combined chamber to overflow, a top plate is placed in position to eliminate dust and evaporation.
- Care should be taken to remove all water outside the chamber to keep, in particular, the thin glass bottom clean.
- After \ sedimentation, the top cylinder is slowly pushed away from the plate chamber by using the square top plate of the plate chamber.
- Pushing stops when the cylinder reaches the small opening near the end of the Perspex plate of the plate chamber.
- As soon as the circular top plate of the sedimentation cylinder is removed, water is drained out of the cylinder through the small hole below.
- Variations in preservation method and species composition probably account for the diversity of sedimentation time recommended in the literature.

- Utermohl (1931) assumed that all organisms would have settled by the day after preparation of the sample, while other scientists prefer approximate settling times necessary are as follow: 100 ml 100 hours 50 mL 50 hours 25 mL 25 hours 10 mL 10 hours 5 mL 5 hours 2 mL 2 hours.
- This method permits the examination of Nanoplankton cells and consequently it is more accurate than any other techniques.



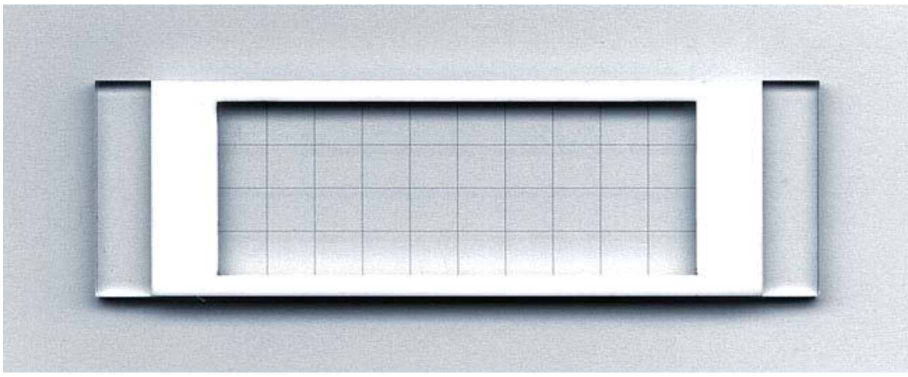
Sedimentation cylinders of Utermöhl method

Utermöhl chambers

2. The standard- microscope methods

Sedgwick Rafter cell

- The chamber is without rulings and is rectangular (50x20 mm), 1 mm deep, of area 1,000 mm^2 and volume 1.0 ml with No. 1.5 cover slips, most x20 N.A. 0.5 objectives can be used permitting magnification to x500.
- The largest phytoplankton's can be held in this slide and many species as small as 10 µm can be recognized in it.
- It is best suited to large and relatively scarce organisms, which can be detected in the concentrate just a few per ml.



Sedgwick Rafter cell

Palmer-Maloney counting cell

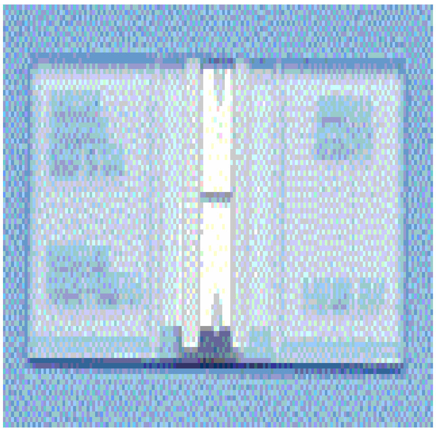
- The chamber is without rulings and circular, of diameter 17.9 mm, depth 400-µm area 250 mm^2, and volume 0.1 ml.
- It has a loading channel (slot) on each side. High- dry objectives can be used. Calls (of some species) as large as 150µm will enter and be reasonably well distributed in this chamber.
- Even the smallest phytoplanktons can be detected in it.
- Species in the concentrate at over 10/ml should at least detected.

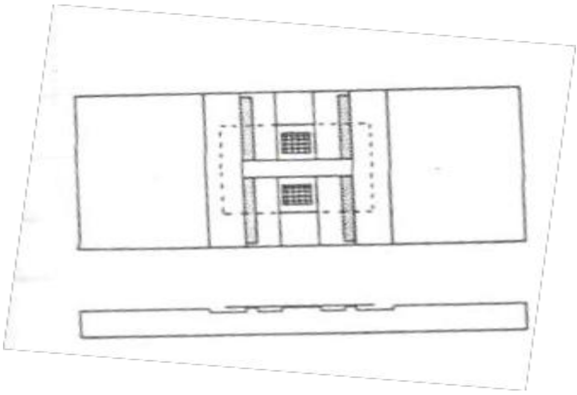
Palmer-Maloney counting cell

Haemacytomerter, 0.2mm deep

- Generally available with Fuchs- Rosenthal ruling which consists of sixteen squares each 1 mm on a side (and each further divided into sixteen squares 250 µm on a side).
- Slides with two such rulings (thirty-two 1-mm squares) thus hold a total of 0.0064 ml of plankton concentrate in the ruled areas.
- High- dry objectives can be used if the cover glass is thin; wide-field oculars are recommended.

- Species larger than c. 75 µm will seldom distribute them well, and long thin species, or those forming long colonies, will usually accumulate near the entry slit or at the chamber edges.
- Call densities as low as 103/ml in the concentrate is detectable; 5x103 cells /ml yield an average of one cell per 1-mm square.





Haemacytomerter cell

Specific technique for preparation of Diatoms sample (Cleaning Diatoms)

- One of the many methods in use for oxidation of organic material of diatom cells, used with success for planktonic diatoms, is as follows:
  1. Rinse the sample with distilled water by centrifugation, or passive settling.
  2. Add an equal amount of saturated $KMnO_4$. Agitate. Leave for 24 hours.
  3. Add an equal amount of concentrated HCl to the sample and $KMnO_4$ mixture. The solution turns dark brown. Heat gently over an alcohol lamp until it becomes transparent and colorless or light yellow- green.
  4. Rinse with distilled water until sample is acid- free.
  5. Cleaned diatom material should be stored in distilled water to which are added a few drops of the mixture of formaldehyde and acetic acid to hinder growth of bacteria and fungi and dissolution of silica.

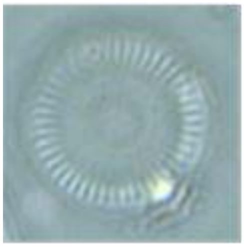
The main Phytoplankton Classes in River Nile

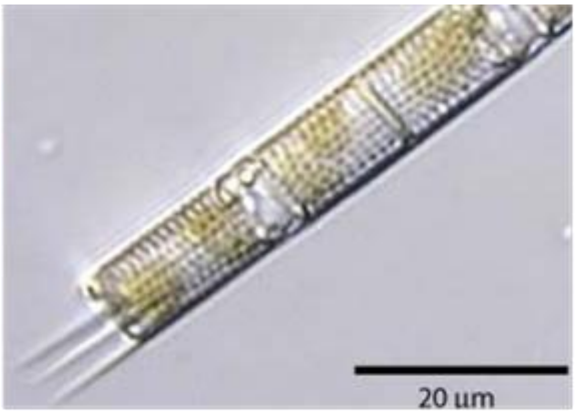
- Bacillariophyceae (Diatoms)
- Chlorophyceae (Green algae)
- Cyanophyceae (Blue-Green algae)
- Cryptophyceae (Cryptomonads)
- Chrysophyceae (Golden-Brown algae)
- Dinophyceae (Dinoflagellates)
- Euglenophyceae (Euglenoids)

Diatoms (Bacillariophyceae)

- They are a most important group of phytoplankton even though most species are sessile and associated with littoral substrata.
- Both unicellular and colonial forms are common.
- The group is commonly divided into the centric diatoms (Centrales), which have radial symmetry, and the pennate diatoms (Pennales), which exhibit essentially bilateral symmetry.

Diatoms





Cyclotella **Melosira**



Navicula Nitzschia

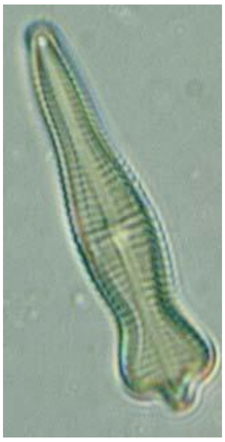

Synda

Diatoms



Amphora Caloneis Cocconeis


Cymatopleura Cymbella




Gomphonema Gyrosigma

Diatoms

Rhopalodia

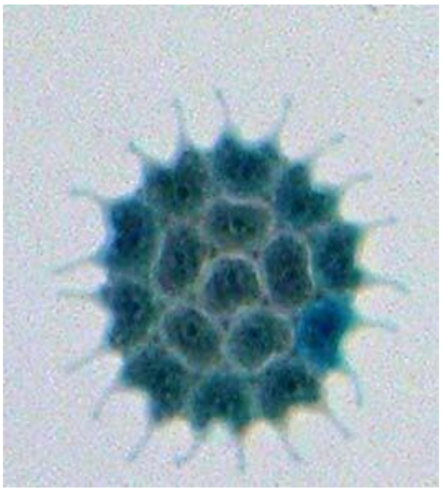
Stauroneis **Surirella**

Chlorophyceae (Green algae)

- These are an extremely large and morphologically diverse group of algae that is almost totally fresh water in distribution.
- A majority of the planktonic green algae belong to the orders Volvocales (e.g., Chlamydomonas, Sphaerocystis, Eudorina, Volvox) and Chlorococcales (e.g., Scenedesmus, Ankistrodesmus [2-3 µm], Selenastrum [6-7 µm], Pediastrum).
- Many members are flagellated (2 or 4, rarely more) at least in the gamete stages.
- Whereas the planktonic Volvocales and Chlorococcales are ubiquitous in distribution among waters of differing salinity within the normal limnological range, the distribution of most

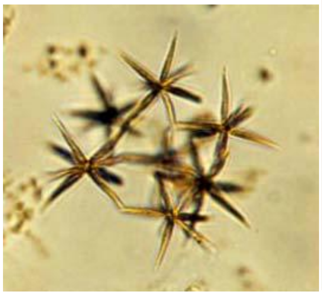
species of desmids of the Conjugales is limited to low concentrations of the divalent cations, calcium and magnesium.

- Although not totally restricted to waters of low salinity, the desmids are most common and the species diversity is greatest in soft waters draining landforms developed in granitic or other igneous rocks, and especially in waters with a high content of dissolved organic matter.
- Their abundance and diversity are often greatest in bog waters that drain through deposits of the moss Sphagnum.
- Many desmids are distributed widely, but as a whole, they are less cosmopolitan than most unicellular algae.

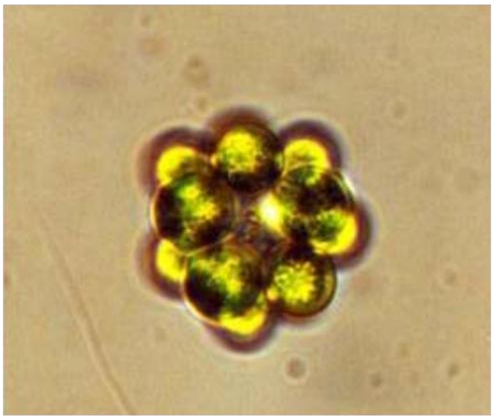


Chlorophyceae

Pediastrum

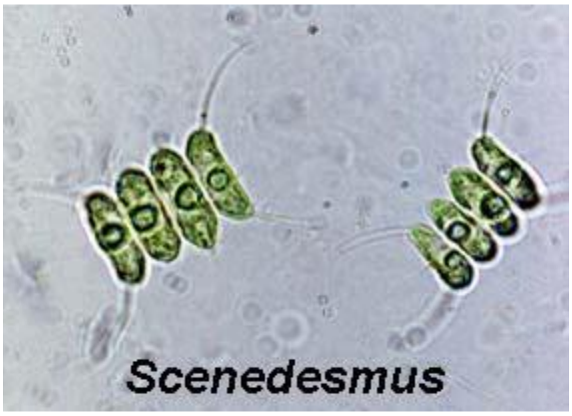


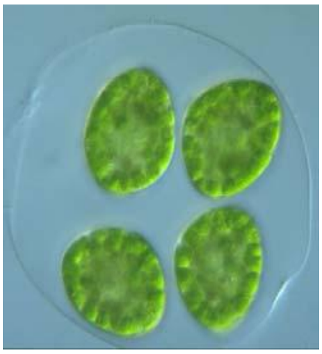
Actinastrum



Coelastrum

Dictyosphaerium

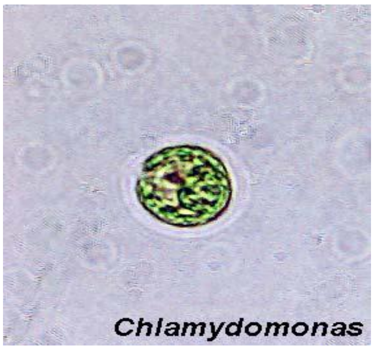

Scenedesmus



Planktonema Oocystis

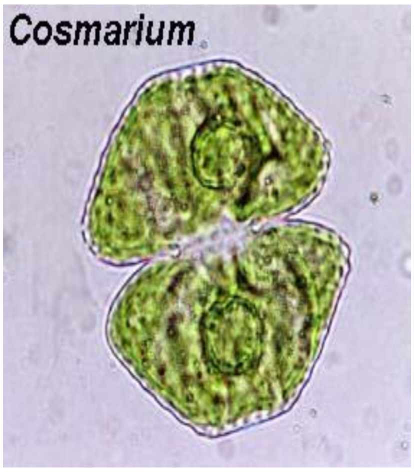
Chlorophyceae



Closterium

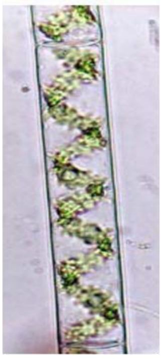

Chlamydomonas

Ankistrodesmus

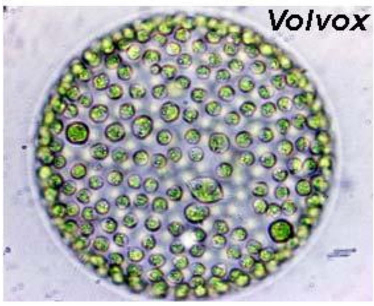

Cosmarium


Gonium

Mougeotia



Staurastrum


Volvox

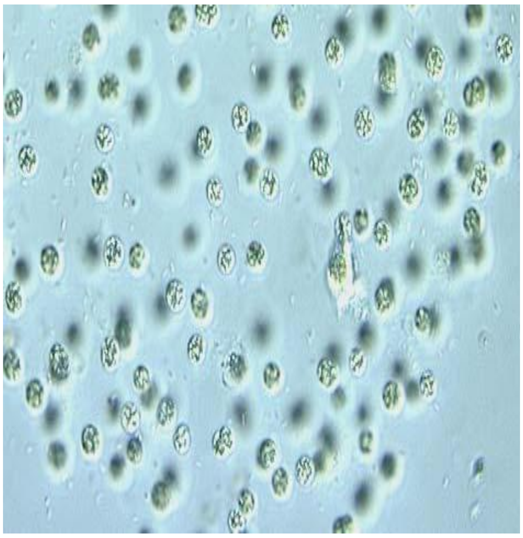
Spirogyra

Cyanophyceae (Blue-green algae)

- These are blue green bacteria, which were once called blue green algae. These are not even eukaryotes.
- The fact, however, that they are microscopic and photosynthetic has traditionally led to their receiving consideration in courses like this.
- Cyanobacteria were probably the first photosynthetic organisms and have been around for about 3.5 billion years.
- They exist as unicellular, colonies or filaments.
- They have a bacterial type cell wall, no nucleus and no flagella. In addition to bacterial chlorophyll, they have the pigments phycocyanin and phycoerythrin. Blue- greens are responsible for nitrogen fixation on land and in water.
- Like green algae, they can form lichen symbioses with fungi.
- A majority of the planktonic blue-greens consists of members of the coccoid family Chroococcaceae (e.g., Anacystis = Microcystis) and filamentous families Oscillatoriaceae, Nostocaceae, and Rivulariaceae (e.g., Oscillatoria, Lyngbya, Aphanizomenon [3-6 µm], Anabaena).

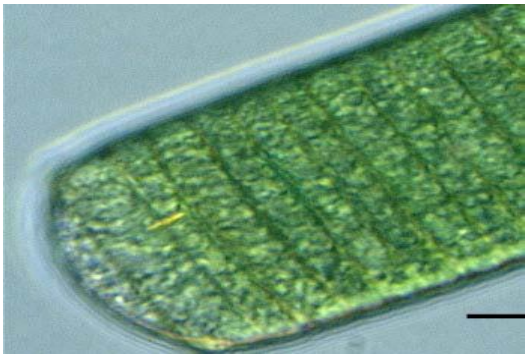
Cyanophyceae

Chroococcus

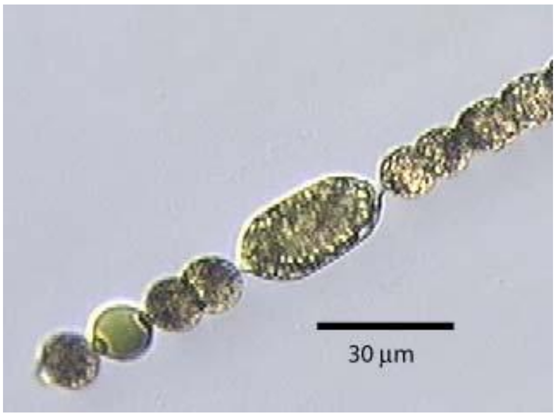


Microcystis

Lyngbya



Oscillatoria



Anabaena

Merismopedia

Cryptophycee

A small class of biflagellate unicellular algae (cryptomonads) in the chlorophyll a-c phyletic line (Chromophycota).

Cryptomonads are 4-80 micrometers long, ovoid or bean-shaped, and dorso- ventrally flattened. The cell is bounded by a moderately flexible periplast comprising the plasmalemma and underlying rectangular or polygonal proteinaceous plates. A tubular invagination (gullet, groove, or furrow) traverses the ventral cytoplasm and opens just below the apex of the cell. Pair of subequal flagella, which are covered with hairs and small scales, arise from the center or apical end of the gullet.

Chrysophycee

These have a dominance of carotene and specific xanthophyll carotenoids, in addition to chlorophyll a. Most of them are unicellular, a few are colonial; they are rarely filamentous. A number of them from important components of the phytoplankton. The unicellular species with a single flagellum (e.g., Chromulina, Chrysococcus, Mallomonas) are usually very small algal constituents of the nannoplankton (10-50 µm).Larger colonial forms such as Synura, Chrysosphaerella, Uroglena, and particularly Dinobryon are widely distributed. Certain species of Dinobryon and Uroglena develop in lakes of very low phosphorus concentrations, while other species of Dinobryon and Synura have high phosphorus requirements.

Dinophyceae

They **are** unicellular flagellated algae, many of which are motile. A few species of the order Gymnodiniales (e.g., Gymnodinium) are naked or without a cell wall; but most develop a conspicuous cell wall, the Peridiniales (e.g., Ceratium, Glenodinium, Peridinium).

Euglenophyceae

Few species are truly planktonic. Almost all are unicellular, lack a distinct cell wall, and possess one, two, or three flagella. Their development in the phytoplankton occurs most often in seasons, strata, or lake systems in which concentrations of ammonia and especially dissolved organic matter are high. However, these algae are found most often in shallow water rich in organic matter such as farm ponds.

Identification and enumeration of Algal groups

1. Scope

- This method is applicable to the identification and enumeration of algae in drinking water, water during intermediate stages of treatment and raw water.

2. Principle

Method Summary

- Concentration of water sample using centrifuge to a definite volume and take 1ml of concentrated sample on Sedgwik Rafter cell (S-R cell) and examine it under the microscope to identify and count algal species.

3. Environmental conditions

- Carry out the test in air conditioned environment.

4.Equipment

- Measuring cylinders.
- Pipettes of volume: 1ml, 2ml.
- Centrifuge and Centrifuge tubes.
- Microscope.
- Sedgwik Rafter cell (S-R cell).
- Brown bottles.

5. Chemical and reagents

- Lugol's iodine solution.

6. Precaution

- Avoid the direct contact with Lugol's iodine solution.
- Keep the concentrated sample in dark bottles.

7. Procedure

- Receive the sample preserved with Lugol's iodine from the sampling section.
- Shake the sample well and gently to be homogenous.
- Take 1L from sample incase of treated water, 500ml incase of raw water.
- Concentrate the sample by centrifuge at 1000 g for 20 minutes.
- Discard supernatant gently and keep a small volume at the bottom of tube.
- Transfer this volume to a cylinder and measure the net final volume then transfer to a small brown bottle with complete information of the sample on label.
- Record all data of sample in concentration of algae sample form.
- Place the cover glass diagonally across the cell to prevent formation of air bubbles in the cell corners.
- Shake the sample well and gently to be homogenous.
- Take1ml from concentrated sample and put it in the S-R cell.

The cover slip will rotate slowly and cover the inner portion of S-R cell.

- Let the S-R cell stand for15 minutes to settle plankton.
- Examine the sample microscopically and then identify and count algal species in randomly selected fields horizontally and vertically across the S-R cell and record your results in log book for algal counting.
- Repeat the last 6 steps for each sample.
- Dilute portion of the sample if the sample is very condensed and the organisms overlapped.
- Record all data in algal sample log form

8. Calculation

- Calculate the algal count as (Org./ml) by using the following equation:

1. Strip counting (لعد مياه الطرد والخزانات والمرشحات والمروقات)

$$\mathrm{No./ml} = \frac{\mathrm{C} \times 1000/\mathrm{mm}^3}{\mathrm{L} \times \mathrm{D} \times \mathrm{W} \times \mathrm{S}}$$

٢. Field counting (لعد مياه النيل)

$$No/ml = \frac{\mathrm{C} \times 1000\ \mathrm{mm}^3}{\mathrm{A} \times \mathrm{D} \times \mathrm{F}}$$

- Take the mean value of the two duplicate counts.

9. Quality control

- Process the sample in duplicate.
- The difference between duplicate counts doesn’t exceed 20%.
- Fill the S-R cell with distilled water and investigate between each sample.

10. Method performance data

- Repeatability, Precision and Z score for enumeration of algae at different levels
- Reproducibility between analysts at different levels of algae.
- Bias.
- Homogeneity.
- Uncertainty of counting at different levels.

11. Reporting

- The final result expressed as count for (Chlorophyceae, Cyanophyceae, Bacillariophyceae, Other Group and Total) as Org. /ml.
- In case of absence of any group; it is recorded N.D. (Not detected).
- Concentration of algae sample form (F-B-03).
- Algae sample log form (F-B-05).

How to convert Centrifuge RPM to RCF or G-force?

Posted on Jul 15, 2012 by Kelly Gleason in Research

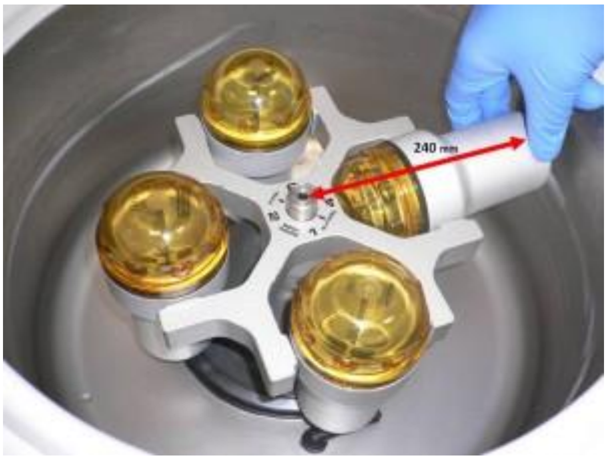
Translational research is finding its way into all phases of clinical trials which means more and more research nurses are finding themselves with the task of collecting and processing samples. These samples are very valuable resources and crucial to the overall success of research.

This requires many research nurses to learn and develop knowledge and skills in the lab not only to work safely in a laboratory setting but also to maintain integrity of the samples processed. It can feel challenging and overwhelming when first reading instructions and lab manuals to guide us in processing samples.When you are lucky, the lab handbook gives you the speed at which your samples must be spun in the same language spoken by your centrifuge, either RPMs or G-force. But what do you do when it doesn’t? The answer is easier than you think.

The force exerted on a particle in a centrifuge is a simple function of the rotation speed of the centrifuge and the radius of rotation. The actual equation is:

$$\text{RCF or G-force} = 1.12 \times R \times (RPM/1000)^2$$

R is the radius of rotation measured in millimeters. For example in the photograph below R is 240mm.



R can be measured at the top of the tube (R_{min}), the middle of the tube (R_{av}) or the bottom of the tube (R_{max}). If your protocol does not specify, you can use chose but if you are trying to pellet something out, you should probably use R_{max} as the pellet forms at the bottom of the tube.

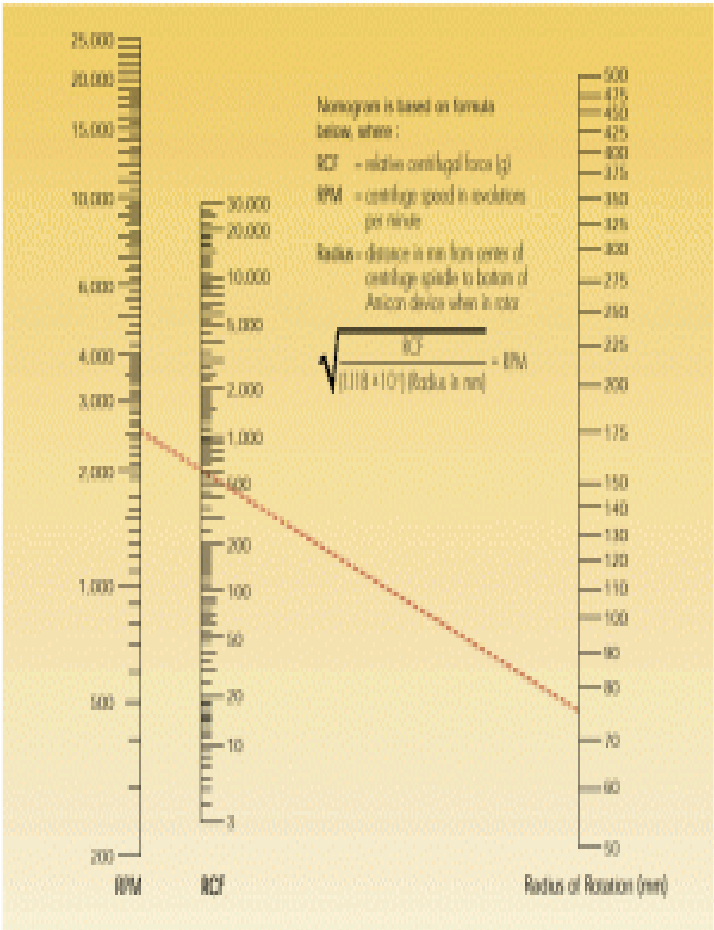
Sedimentation

Particles in a suspension will settle in the bottom of a vessel over time, this is called sedimentation. The particles fall to the bottom due to gravity, in the same way that herbs in a salad dressing settle at the bottom of the bottle. This force is expressed as G. Centrifugation increases the rate of sedimentation (i.e. accumulation of red and white bloods cells in the bottom of a blood tube) by spinning the blood samples and creating a centrifugal force that acts on the particles (in this case, red and white blood cells).

RPM stands for 'revolutions per minute'. This is how centrifuge manufacturers generally describe how fast the centrifuge is going (i.e. revolving). The rotor, regardless of its size, is revolving at that rate.The force applied to the contents, however, varies according to the size of the centrifuge as a larger centrifuge will have a longer radius and a smaller centrifuge will have a shorter radius.

For example, when revolving at 2000 RPM, a larger centrifuge with a longer radius length will spin samples at a higher g-force than a smaller centrifuge with a shorter radius length.

If you know at what g-force you need to spin your samples and you can measure the radius on your centrifuge, you can figure out the speed or RPM you need to set your centrifuge by using a Nomograph, as set out below.



You may also use this equation if you can't access a nomograph.:

$$\textbf{G-force} = 0.00001118 \times R \times RPM^2$$

When you know the G-force at which a sample should be spun, you can measure the radius of your centrifuge and determine the revolutions per minute at which to set your centrifuge.

The take home message is that centrifugation speeds quoted in RPM will only be constant for centrifuges with the same rotor radii. If you use an RPM setting from a protocol where someone used a centrifuge with a different radius from yours, you will get a different G-force. Often the difference will not be significant enough to affect the sample but it is always good practice to standardize as much as possible how samples are processed at various institutions using different equipment.

Sample collection and processing are very important aspects of clinical research and the research nurse's skills in the laboratory are crucial not only in maintaining a safe practice but also in ensuring sample integrity. Results of studies are dependent on the quality of the collection and processing of these samples and good lab skills help research nurses achieve this.

4. The formula is always good to have in case your internet is down or the above links cease to exist.

$$\textbf{G Force (RCF)} = \textbf{(rpm)}^{2} \times 1.118 \times 10^{-5} \times r$$

$$\textbf{RPM} = \sqrt{[RCF/(r \times 1.118)]} \times 1000$$

Where:

g = Relative Centrifuge Force

r = rotational radius (cm)

N = Revolutions per Minute (RPM)

$$RCF = \left(\frac{RPM}{1{,}000}\right)^2 \times r \times 1.118 \quad \Rightarrow \quad RPM = \sqrt{\frac{RCF}{r \times 1.118}} \times 1{,}000$$

12. References

- APHA (American public Health Association) (2005)

Standard Methods for the Examination of Water and Wastewater 21th A.P.H.A. AWWA. WPCF.American Public Health Association 1015 pp. 15th St. N.W. Washington D.C. 20005.

Determination of Microcystins — Method using solid phase extraction (SPE) and high performance liquid chromatography (HPLC) with ultraviolet (UV) detection

1. Scope:

- This method is applicable for the determination and quantification of microcystins (MCYST-RR, MCYST-YR, and MCYST-LR) in raw water (containing biomass) and different stages of drinking water treatment and finished tap water, using solid phase extraction (SPE) followed by high performance liquid chromatography (HPLC) with photo diode array (PDA) detector at the below indicated quantification and minimum detection limits (LOQs, RL and MDL)

2. Principle:

- Water samples containing cyanobacterial material (biomass) shall be filtered first.
- The biomass is extracted separately with a solvent (methanol/water).
- The extract is filtered, diluted and a solid phase extraction (SPE) is applied for sample clean-up. The filtrate is treated as a pure water sample.
- Pure water samples such as tap water are enriched using SPE. The microcystins are eluted from the SPE cartridges with acetonitrile containing 0.05 % by volume of trifluoroacetic acid (TFA).
- Microcystins are quantified by high performance liquid chromatography (HPLC) with diode array detector at 230-240 nm.

3. Definitions:

- Laboratory Duplicates (D1 & D2): Two samples aliquots taken in the analytical laboratory and analyzed separately with identical procedures. Analyses of duplicates give a measure of the precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.
- Laboratory Reagent Blank (B): An aliquot of reagent water that is treated exactly as a sample including exposure to all

glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The blank is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

- Laboratory Fortified Blank (LFB): An aliquot of reagent water to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurement at the required method detection limit.

4. Environmental Conditions:

- This test method does not require specific environmental conditions but in general it is preferred to be applied in air conditional atmosphere.

5. Interference:

- Impurities contained in the extracting solvent usually account for the majority of the analytical problems. Solvent blanks are analyzed with each analytical batch of samples.
- An interference-free solvent is a solvent containing no peaks yielding data at greater than or equal to the LOQ and at the retention times of the analytes of interest.

6. Modification To Standard Test Method:

- N/A

7. Equipment:

- Glass microfiber filter paper, retention size 1 µm to 2 µm.
  - The maximum diameter of the filter should be 47 mm.
  - Filtration is needed only for the analysis of samples containing phytoplankton.
- SPE Manifold.
- Vacuum pump for SPE.
- Ultrasonic bath.
- Micro syringes of volumes; 50µl and 500µl.

- Laboratory centrifuge, 4000 min−1, relative centrifugal force (RCF) 10000 g.
- Adjustable horizontal shaker.
- Precision scale balance.
- Micropipete with disposable tips.
- pH meter.
- Vacuum filtration apparatus.
- Heating block with temperature control with the following characteristics: block-temperature 30 °C to 50 °C; and air free of oil delivery unit.
- Disposable filter unit, pore size < 0.45 µm.
  - Prior to use, verify that no microcystin losses occur during filtration (recovery testing).
- Sampling bottles, dark glassware, sterile and pre-cleaned.
- HPLC WATERS Chromatography system 2695, capable for inject till 1000 µl and suitable for volume flow rates between 0,3 ml/min and 1,0 ml/min with photo diode array detector, with auto- sampler, with column C-18 (250 x 4.6 mm, bonded fused silica column, 5 µm) and Em-power Chemistation system for acquisition and data analysis.

8. Chemicals and Reagents:

- Methanol, CH_3OH, HPLC grade.
- Acetonitrile, CH_3CN, HPLC grade.
- Trifluoroacetic acid, TFA, CF_3COOH.
- Standard dilution solution, SPE rinsing solvent, and re-dissolving solvent. Methanol/water [75/25 by volume].
- SPE elution solution: Methanol/water [90/10 by volume] containing 0.1 % by volume TFA.
- Sodium thiosulfate, solution: Dissolve 1 g of sodium thiosulfate $Na_2S_2O_3$ (anhydrous or with 5 H_2O) in 100 ml of water. The final concentration is ρ = 10 g/l (63 mmolar in case of anhydrous $Na_2S_2O_3$).
- Ammonium hydroxide solution: Commercially available ~ 1 mol/l of ammonium hydroxide solution, NH_4OH.
- Solid phase extraction cartridges (SPE) for microcystin enrichment.
- HPLC mobile phase solution (A): To a 1000 ml volumetric flask, add 800 ml of water and 800 µl of TFA and bring to volume with

water. Transfer this solution in a HPLC-eluent bottle. Degas the solution before use.

- HPLC mobile phase solution (B): To a 1000 ml volumetric flask, add 800 ml of acetonitrile and 450 µl of TFA and bring to volume with acetonitrile. Transfer this solution in a HPLC-eluent bottle.
- HPLC mobile phase gradient:

Table 1 – HPLC mobile phase gradient

Time (min.)	Eluent A% Water+ 0.0.5% TFA	Eluent A% CAN+ 0.05% TFA	Flow Rate (ml/min)
0.0	70	30	0.75
10.0	65	35	0.75
40.0	30	70	0.75
42.0	0	100	0.75
44.0	0	100	0.75
46.0	70	30	0.75
60.0	STOP		0.75

- Microcystins Standard.
- Microcystin stock solutions:
- Prepare a standard solution containing 2.5 µg/ml of MCYST-LR in the standard dilution solution. Store it below −16 °C. To avoid incorporation of water by condensation, do not open the vial until its contents have reached room temperature.
- If the solution is to be stored for a long period, use a hermetic vial. In case of doubt, weigh the vial and record any changes in mass during storage.
- Spiking solution for method control:
- Prepare a spiking solution by pipetting 200 µl of the microcystin stock solution into a 500 ml volumetric flask. Dilute it to the mark with water (tap-water or blank water from a natural lake),

and shake well.

- The concentration of this spiking solution is 1 µg/l of MCYST-LR.

9. Precautions:

- The method requires use of microcystin-containing solutions. Microcystins are highly hepatotoxic to humans. Laboratory wastes of microcystins shall be collected separately and disposed as highly toxic chemical waste. Long-term decontamination with concentrated sodium hypochlorite (NaClO) solution is also possible.
- Persons using this International Standard should be familiar with normal laboratory practice. This standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.
- It is absolutely essential that tests conducted according to this standard be carried out by suitably trained staff.
- Avoid the use of plastics whenever possible. This is necessary because the use of plastics (e.g. plastic pipettes, plastic tubing or plastic cartridges) may cause losses of microcystins through absorption on the surface walls.
- Use only reagents of recognized analytical grade and water complying with grade 3 as specified in ISO 3696:1987, unless otherwise specified.

10. Procedure:

10.1. Preparation of standard solutions of microcystin-LR for HPLC calibration

- Microcystins calibration standards are prepared from individual stock (neat/liq.) for the listed analytes at concentration of (0.025, 0.05, 0.1, 0.25 and 0.5 µg/L in methanol).

10.2. Sample preparation

10.2.1. Extraction of microcystin in biomass filtered on glass fiber filters or in freeze dried cyanobacterial biomass

- Take suitable sample volume:
  - 500 ml or samples with low algae density.

  - 250 ml for samples with visible algae density.
  - 100 ml or less for samples with high algae denity.
- Filter the taken volume on 47 mm glass fiber filter with medium velocity (a thin water jet should be visible).
- May be 1 filter is not enough for filtering all sample volume so replace the clogged one with another and continue filtration.
- Take the air dried filter sample in glass tube or glass vial.
- Don't take the tube if algae are too much on the filter.
- Freeze filter in tube or vial for 12 h till 1 day.
- After thawing freeze again for 12 h.
- Give 5-8 mg frozen dried algal in 5ml glass vial.
- Add 5 ml 75 % Methanol to vial with filter.
- Add1.2 ml of 75 % Methanol to vial with 5-8 mg dried algal.
- Shake vials on a shaker for 12 hours at least.
- Take with 3 ml Syringe 2.5 ml of sample through 25 mm syringe filter to clarify the sample.
- Evaporate to dryness in a water bath at 50 ºC under air free of oil.
- Reconstitute the residue in 200 µl 75 % Methanol and pay attention to residues on vial wall.
- Take the sample with 1 ml syringe and inject it in the auto sampler vial with glass insert through 13 mm or 4 mm syringe filter if necessary to clarify the sample.
- Analyze the sample on HPLC at 230 – 240 nm.
- Record all data in Extraction of Microcystin from Raw Water Sample.

10.2.2. Solid phase extraction of microcystin in water samples

- Take suitable sample volume:
  - 500 ml or samples with low algae density.
  - 250 ml for samples with visible algae density.
  - 100 ml or less for samples with high algae density.
- Filter the taken volume on 47 mm glass fiber filter with medium velocity (a thin water jet should be visible).
- May be 1 filter is not enough for filtering all sample volume so replace the clogged one with another and continue filtration.
- Take the filtrate and adjust the pH-value of samples with NH_4OH or TFA to pH = 5-8.
- For operation of pH meter refer to WI-MB-27.

- Transfer the sample after pH-adjustment in a 500 ml flask and add 5 ml Methanol.
- Conditioning the solid phase extraction cartridge as follow:
    - Add 2 x 5 ml Methanol 75%.
    - Then add 2 x 5 ml water HPLC grade.
- Do not dry the cartridge before using.
- Store the cartridge in water till use.
- Take the sample and filtrate it all through the SPE cartridge using SPE manifold.
- Adjust the sample at a flow rate not exceeding should form visible drops, has to be optimizes by tests.
- Wash the cartridge with 4 ml 20 % Methanol.
- Dry the cartridge by drawing air through it for 10-20 min to improve the elution step because of the less residual wetness.
- Elute microcystin in a 4 ml glass vial with 4 ml acetonitrile containing 0.05 % TFA (Wait after first 2 ml till “soak the sorbent” 3 min then add second 2 ml).
- Use a pipette ball or Nitrogen gas as residues remains in sorbent.
- Evaporate to dryness in a water bath at 50 °C under air free of oil (the evaporation of samples should be done in a fuming hood because of toxicity of the acetonitrile and TFA, don’t use too much air and don’t dry too fast).
- Reconstitute the residue in 500 µl of 75 % Methanol (wash the glass side several times using this 500 µl with a 2 ml syringe).
- Take the elute with this syringe and transfer to HPLC vial via a syringe filter if visual particle are present.
- Analyze the sample on HPLC at 230 – 240 nm.
- Record all data in Extraction of Microcystin from Drinking Water Sample

11. Calculation:

- Calculate the microcystin concentration according to the standard curve procedure.

12. Quality Control:

- Prepare the quality control sample from the microcystin standard as prepared in (10.2.).

- Calibration curves is checked by (0.1 µg/l) every analytical batch for microcystins over the concentration range 0.05-1.0 µg/l to confirm the verification of the linear response in these range of the System (correlation coefficient minimum 0.95).
- The lab analytical batch size is ≤ 20 samples.
- A reagent blank (B) is analyzed at the beginning of each analytical batch. The level of microcystins in the blank reagent has to be less than the LOQ.
- Laboratory duplicates (D1 & D2) are analyzed within each analytical batch. The absolute difference shall not exceed the assigned repeatability limit of each microcystin.
- Laboratory Fortified Blank (LFB) is analyzed within each analytical batch and the obtained recovery % shall be within 70-110 %.

13. Method Performance Data:

Compound	MDL	LOQ	S_r	S_R	Rec. %	MU	R^2
RR	0.05	0.1	0.04	0.05	70-110	30	0.999
YR	0.05	0.1	0.04	0.09	70-100	30	0.997
LR	0.05	0.1	0.04	0.04	70-110	30	0.998

14. Reporting:

- The unknown real sample result less than LOQ was reported <LOQ, the result more than LOQ was reported as 0.00 µg/l.

15. Related Documents and Records:

- Handling of Microcystin Standard Form
- Extraction of Microcystin from raw water sample form.
- Extraction of Microcystin from drinking water sample form.
- Working instruction of HPLC.
- Working instruction of Shaker.
- Working instruction of pH meter.
- Internal Report for Microcystin.

16. References:

- ISO 20179:2005

Protozoa

- Protozoans are found in all moist habitats, but we know little about their specific geographic distribution.
- They are unicellular organisms that range in size from a few microns to several hundred microns.
- Polluted waters often have a rich and characteristic protozoan fauna. The relative abundance and diversity of protozoa are used as indicators of organic and toxic pollution.
- Protozoa play a role both as herbivores and as consumers in the decomposer link of the food chain.
- Protozoa are members of the Kingdom Protista. There are about 20,000 known species of protozoa that live in water and soil.
- Some feed on bacteria while others are parasites and feed off their hosts.
- Most protozoa are asexual and reproduce in one of three ways: fission, budding, and multiple fission.
- Some protists are sexual and exchange genetic material from one cell to another through conjugation, which is the physical contact between cells.
- A protist can survive in an adverse environment by encapsulating itself with a protective coating called a **cyst.**
- The cyst defends the protist in extreme temperatures against toxic chemicals and even when there is a lack of oxygen, moisture, and food.

Factors Affecting Growth and Distribution

Most free-living protozoa reproduce by cell division (exchange of genetic material is a separate process and is not involved in reproduction in protozoa). The relative importance for population growth of biotic versuc chemical-physical components of the environment is difficult to ascertain from the existing survey data. Protozoa are found living actively in nutrient-poor to organically rich waters and in fresh water varying between 0°C (32°F) and 50°C (122°F). Nonetheless, it appears that rates of population growth increase when food is not constrained and temperature is increased.

Nutrition

Protists receive nutrients by breaking down organic matter and can grow in both aerobic and anaerobic environments, such as protists that live in the intestine of animals. Some receive nutrients from organic matter and photosynthesis because they contain chlorophyll. These protists are considered both algae and protozoa. Protists obtain food in one of three ways: absorption, ingestion, and engulfing. Food is digested in the vacuole after the food enters the cell. The vacuole is a membrane-bound organelle. Waste products are excreted using a process called **exocytosis**.

Protozoa in the Wastewater Treatment Process

Most protozoa are free-living in soil and water and enter the activated sludge process through inflow and infiltration. The number of protozoa within the activated sludge process or mixed liquor varies greatly by process and operational conditions, especially hydraulic and organic loadings. The relative abundance of protozoa may be less than 100 per milliliter or greater than 100,000 per milliliter.

Protozoa, especially ciliated protozoa, perform several beneficial roles in wastewater treatment. These roles include cropping action, coating action, and recycling of mineral nutrients.

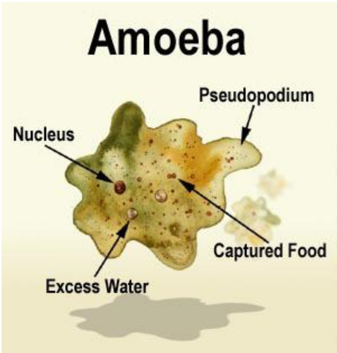
Bacteria are the primary food source for protozoa, and the consumption of suspended or dispersed bacteria by protozoa is referred to as "cropping" action. Cropping action removes many dispersed bacteria from the bulk solution.

Dispersed growth as well as colloids and particulate materials, collectively known as "fine" solids, also are removed from the bulk solution by the "coating" action of ciliated protozoa. This group releases sticky secretions that cover the surface of fine solids. Through coating action, the surface charge of fine solids is made compactible for adsorption to floc particles in the activated sludge process. The adsorption reduces the quantity of fine solids in the final effluent.

Protozoa also release excretions to the bulk solution. These excretions contain many mineral nutrients, including nitrogen and phosphorus, and help to recycle mineral nutrients in the activated sludge process. These nutrients then are available for bacterial activity in degrading wastes, the biochemical oxygen demand (BOD).

Protozoa in the activated sludge process commonly are placed in one of five groups: amoebae, flagellates, free-swimming ciliates, crawling ciliates and stalked ciliates. Amoebae and flagellates are considered "lower" life forms, while crawling ciliates and stalk ciliates are considered "higher" life forms. Free-swimming ciliates are considered "intermediate" life forms. Some treatment plant operators often perform routine microscopic examinations of the protozoa in the activated sludge to determine the health of the activated sludge process.

Typically, operators base the health of the process on the protozoan groups that are dominant as revealed by microscopic examination (Table below). For example, if lower life forms are dominant, the activated sludge is considered unhealthy and unacceptable. An unhealthy activated sludge produces an aeration tank effluent having a BOD greater than 30 mg/L. If the higher life forms are dominant, the activated sludge is considered healthy and acceptable. A healthy activated sludge produces an aeration tank effluent having a BOD less than 30 mg/L.

Protozoan Groups and Bioindicator Values	
Amoebae 	Rarely predominant except for start-up conditions and conditions that mimic start-up such as over-wasting, recovery from toxicity, washout, and organic overloading
Flagellates, plant-like 	Dominant under high organic loading, dispersion of floc particles, such as through chlorination, and start-up conditions or conditions that mimic start-up. Also may dominate in the presence of excess soluble phosphorus.
Flagellates, animal-like 	Except for the presence of excess soluble phosphorus, these are dominant for operational conditions listed for plant-like flagellates and usually follow plant-like flagellates as the dominant group.

Free-swimming ciliates 	Transition group that dominates between healthy and unhealthy conditions and proliferates when large numbers of free-swimming bacteria are present.
Crawling ciliates 	Dominant in the presence of mature floc particles and low BOD in the bulk solution. Alternate with stalked ciliates as the dominant group.
Stalked ciliates 	Dominant in the presence of mature floc particles and low BOD in the bulk solution. Alternate with crawling ciliates as the dominant group.

PHYLUM NEMATODA

- "roundworms"
- Most are free living, but every organism on earth allegedly has a nematode parasite. Therefore nematodes outnumber all other organisms?

Nematodes are aquatic animals present in fresh, brackish waters, salt waters, and soil worldwide. Freshwater nematodes can be present in sand filters and aerobic treatment plants. They are present in large numbers in secondary wastewater effluent, bio-filters or biological contactors. A freshwater nematode inhabits fresh water below the water table; a species utilizing oxygen dissolved in fresh water. Nematodes feed on bacteria, fungi, small protozoa, and sometimes other nematodes.

Nematodes can be distinguished from other aquatic worms by their cylindrical body and by the typical S-shaped, wriggling movement which they use to propel themselves through the water. The phrase "tube-within-a-tube" is a convenient way to think of nematode body structure. Most are microscopic, but a few can grow much larger, up to several feet in length. Nematodes may live in water, in soil, or as parasites. There are several species of parasitic nematodes which attack humans, but these species are believed to be associated with contaminated food rather than with contaminated water.

The free-living aquatic nematodes eat bacteria, rotifers, and other worms and can be very important in wastewater treatment. Nematodes crawl upon floc particles and move in whip-like fashion when in the free-swimming mode. Nematodes also secrete a sticky substance to be able to anchor to a substrate so that anchored nematodes can feed without interference by currents or turbulence. Nematodes are abundant in sludge and are especially common in percolating filters. They help stabilize sludge as well as controlling film accumulation in percolating filters. A lack of nematode activity can be one of the bio-indicators of a toxic condition that may be developing in the treatment process.

Rotifera

Detection of Parasites, fresh water Nematode and Rotifera

1. **Scope**

The direct method is suitable for the detection of Parasites, fresh water Nematode and Rotifera in drinking, raw or ground water samples. The staining method is suitable for the detection of Microsporidia.

2. **Equipment**

- 47 mm stainless steel filtration manifold.
- Centrifuge
- 47 mm filter membranes, no greater than 0.8µm pore size.
- Vacuum pump
- Petri dishes 47 mm
- Centrifuge tubes.
- Microscope
- Glass slide

3. **Chemical and reagents**

- Distilled water.
- Autoclaved distilled water
- Methanol
- Ethyl alcohol 95%

- Glacial acetic acid
- Ethyl alcohol 100%
- Xylene
- Chromotrope 2R
- Fast green
- phosphotungstic acid

4. Precaution

The biohazard associated with, and the risk of infection from, oocysts and cysts is high in this method because live organisms are handled. Samples and some reagents may contain high concentrations of biohazards and toxic compounds, and must be handled with gloves. Quality control sample must also be handled with gloves and laboratory staff must never place gloves in or near the face after exposure to solutions known or suspected to contain oocysts and cysts. Laboratory personnel must change gloves after handling filters.

5. Procedure

- Water samples take in container (1 L at least)
- Samples should be transport and stored at (5 ± 3) °C unless they are to be analyzed immediately.
- Samples should be analyzed within 24 h of collection.
- Place the membrane filter into the housing and clamp on the upper part.
- Pump the water sample through the filter.
- Rinse the container with 1 L of filtered distilled water and pump the washings through the filter after sample filtration to confirm there were no parasites on the surface of the water tank of the filtration unit.
- Remove the filter from the filter housing and place into a suitable clean Petri dish.
- Add suitable amount of distilled water in the Petri dish and gently scrap the surface of the filter for 1 min.
- Decant the washings into a 10 ml centrifuge tube refer to Working instruction for centrifuge.
- Repeat the wash procedure with further distilled water and add this to the centrifuge tube.

- Centrifuge the tube at 2000 rpm for 15 min.
- Genteelly and carefully aspirate off the supernatant leaving the pellet. If no pellet is visible, extra care shall be taken to avoid aspirating oocysts and cysts during this step.
- Spread the obtained pellet on the glass slide and examine by light microscope to detect parasites, fresh water Nematode and Rotifera.
- The slide after direct examination on microscope is left for drying then stained with modified trichrome stain for the detection of microsporidia as follow:
- The sample on the slide to air dry, Fix the specimen by methanol for 5 min, Flood the slide with Chromotrope-based stain and leave for 90 min.
- Rinse under a running tap for 1 min to remove excess stain
- Rinse in acid alcohol (0.45% glacial acetic acid in ethyl alcohol) for 10 sec
- Rinse briefly in 95% alcohol, Place the slide in 95% alcohol for 5 min
- Place the slide in 100% alcohol for 10 min
- Place the slide in Hemo-De (a xylon substitute) for 10 min
- Air dry and examine using a high power (1000x) objective to WI of research microscope.

6. Quality control

Blank sample contain autoclaved distilled water for negative control and positive sample from natural contaminated source as a quality control samples should be examined before the sample batch examination.

6.1. General:

6.1.1. Direct examination:

Positive: A proven positive natural water samples have freshwater Nematode, Rotifera or parasites.

Negative: Autoclaved distilled water contains no living organism.

6.2. Equipment cleaning

Wash all equipment that is reused thoroughly in water containing detergent and then rinse in distilled water to remove any oocysts and cysts that may be attached to the equipment.

Wash equipment used for positive control procedures separately (if possible in a separate area) from equipment used for the analysis of samples.

7. Method performance data

- Measurement of specificity =100
- Measurement of sensitivity = 91.6667
- Measurement of trueness=95.83333
- Measurement of Def Kappa=0916667
- Measurement of repeatability = 0.958333

8. Reporting

Presence or absence of live or dead Nematode, Rotifera, parasites and microsporidia in 1 L water.

9. References

- ISO / FDIS 15553: Water quality — Isolation and identification of Cryptosporidium oocysts and Giardia cysts from water.
- SMWW Standard Methods for the Examination of Water and Wastewater (2005).
- Health Canada (2004) Guidelines for Canadian Drinking Water Quality: Protozoa, Giardia and Cryptosporidium.
- Health Protection Agency (2007). Staining Procedures. National Standard Method. Issue 1.

المراجع

- **تم الإعداد بمشاركة المشروع الألماني GIZ**
- **و مشاركة السادة :-**

➢ 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