Benha University
Faculty of science
Botany Dept.
Time : 2hrs

4th year (Bot. & Chem.)
Standard analysis & Envir. Pollution
6, Jan. 2015 (1st semestre)
Course code : B453

All questions are to be attempted in sequence

Question I: Complete the following? 8mark

1. *Cultural methods for the quantitative study of aquatic microorganisms are* **the standard plate count & the Most Probable Number (MPN) method or the membrane filtration method**
2. *Niche or prime niche can be defined as* **the place where a microorganism is most successful**
3. *Environmental microbiology is* **the study of microbes in their natural habitats**
4. *Neutral buoyancy means* **organism keeping their body at the same density as water**
5. *Bioactive peptides are* **'peptides with hormone- or drug-like activity that eventually regulate physiological function through binding interactions to specific receptors on target cells leading to induction of physiological responses'**
6. *Benthic organisms (benthos) are* **organisms inhabiting the bottom sediment of aquatic environments**
7. *The genus of lactobacilli can be divided into three groups based on* **carbohydrate fermentation patterns**

Question II: Describe two only of the following? 20 mark

1. ***Standards Required of Water quality assay***

Water is used for many purposes, each of which requires that the water meets the standards, which in the main will ensure the health and safety of the users of the water. The standards required for water purity are.

1. Microbiological standards
2. Chemical standards

Water is required by the human body constantly and an average adult probably consumes up to one liter or more per day. Since water must normally be consumed every day, unlike other food constituents, which may be eaten now and again, standards must be carefully set with the aim of protecting human health. Several considerations enter into the selection of standards for drinking water. These include:

(a) The public health statistics relating to morbidity and mortality due to a pathogen or chemical

(b) The population exposed

(c) The physical and chemical state of the substance

(d) The toxicity of the substance to man or to suitable experimental animals.

(e) The amount of the substance likely to be found in other sources

Water meant for human consumption must be free from chemical substances and microorganisms in types and amounts, which can be hazardous to health. Not only must it be safe but it must also be aesthetically acceptable. It is for this reason that the governments of various countries around the world set standards to be met in drinking water. Ideally, the standards for drinking water should be uniform universally and used the world over. In practice, however, the standards depend on known and expected contaminants and the ability of the society or the government concerned to attain the standard; therefore, standards vary from country to country. The US standards, the European Union and the World Health Organization are recorded as standards for water quality.

In the United States, the US Environmental Protection Agency (EPA) sets the standards for drinking water. The Safe Drinking Water Act (SDWA), passed in 1974 and amended in 1986 and 1996, gives the EPA the authority to set drinking water standards. These standards are regulations that EPA sets to control the level of contaminants in the nation's drinking water.

Regarding, the Microbiological Standards, All the standards state that no sample should contain fecal coliforms. In addition, the US standards specify the absence of Cryptosporidium, Giardia lambia, and Legionella. It is recommended that, to be acceptable, drinking water should be free from any viruses which affect man. This objective may be achieved

(a) by the use of a water supply from a source which is free from wastewater and is protected from fecal contamination;

or (b) by adequate treatment of a water source that is subject to fecal pollution. Adequacy of treatment cannot be assessed in an absolute sense because neither the available monitoring techniques nor the epidemiological evaluation is sufficiently sensitive to ensure the absence of viruses.

2. *Bioremediation of heavy metals contaminated soil*

Heavy metals are the metals in the periodic table that have a molecular weight greater than 55. Heavy metal contamination, form both natural and anthropogenic sources, is recognized as a major environmental concern in marine ecosystems due to the pervasiveness and persistence of the contaminants. These include chromium (Cr), nickel (Ni), Copper (Cu), zinc (Zn), arsenic (As), selenium (Se), strontium (Sr), molybdenum (Mo), technicium (Tc), cadmium (Cd), mercury (Hg) and lead (Pb). However, some of these metals like Zn, Cu and Mo act as micronutrients in plant and animal growth as they function as cofactors in enzyme-catalyzed reactions.

When pollutants are applied at levels that kill most cells, the pollutants select for the few cells that have evolved resistance mechanisms and are able to persist. Exposure to heavy metals selects for resistance to heavy metals in the surviving microorganisms. There are several mechanisms of resistance to heavy metals:

extracellular detoxification,

altered permeability,

pollutant removal from the cell environment,

intracellular detoxification, and

abiotic binding or precipitation.

Microorganisms may persist in soil contaminated with extremely high heavy metal concentrations because those heavy metals may be extractable but not biologically available. The physiological state of the microorganism and the form of heavy metal are the two most important considerations when studying heavy metal effects on microbial populations.

Mercury is present in extremely low concentrations in most natural environments, averaging about 1 nanogram per litre. It is a widely used industrial product and is the active components of many pesticides. Because of its unusual tendency to concentrate in living tissues and its high toxicity, mercury is of considerable environmental significance. Around 3,000 metric tons of Hg are released into the atmosphere from the combustion of fossil fuels and Hg-containing compounds.

Mercury is toxic to microorganisms, and to survive in its presence, they transform Hg2+ into methylated compounds such as CH3Hg (methylmercury) and CH3HgCH3 (dimethyl mercury). These methyleted compounds forms of Hg are volatile and harmless to microorganisms since the Hg concentration decreases. However, methylated Hg compounds are readily adsorbed and retained by human tissue, where they cause neurological disorders. The molds and bacteria that have the ability to transform mercury belong to *Aspergillus, Neurospora, Bacillus, Mycobacterium, and Pseudomonas*. Along with methylation, these microorganisms release organically bound Hg during their heterotrophic activity.

$$RH\text{-}Hg\text{-}2H^+ \longrightarrow RH + Hg^{2+} + HCl$$

Demethylation and mercury precipitation from mercuric sulphides in anaerobic environments also occur:

$$CH_3Hg + 2H^+ \longrightarrow Hg^{2+} + CH_4 + H^+$$

$$Hg^0 \text{ or } Hg^{2+} + H_2S \longrightarrow HgS \text{ (ppt)}$$

Mercuric resistance is carried on plasmids that are exchanged between Gram-negative bacteria. The plasmid codes for a protein that initially binds Hg^{2+} to the cytoplasm where it is reduced by mercuric reductase to element mercury (Hg^0), which is lost from the cell by enhanced diffusion.

3. Diversity of organisms polluted water body

As a habitat for the existence of microorganisms, water has properties not found in other natural microbial habitats such as soil, plant and animal bodies; indigenous aquatic microorganisms are adapted to these conditions. Natural waters are generally low in nutrient content (i.e., they are oligotrophic); what nutrients there are, are homogeneously distributed in the water. The movement of water freely transports microorganisms; to counter this and offer themselves some protection; many aquatic organisms are either stalked or arranged in colonies immersed in gelatinous materials. To enable free movement in water, many aquatic microorganisms and/or their gametes have locomotory structures such as flagella.

Microorganisms are often adapted to, and occupy particular habitats in the water body; some occupy the air–water interphase (neuston), while others live in the sediment of water bodies (benthic). The conditions which affect aquatic microorganisms are temperature, nutrient, light, salinity, turbidity, water movement. The methods for the quantitative study of aquatic microorganisms are cultural methods (standard plate count and MPN), direct methods (microscopy and flow cytometry), and the determination of microbial mass. The microscopy methods are light (optical), epiflourescence, confocal laser scanning microscopy, transmission electron microscopy, and scanning electron microscopy. Microbial mass may be direct (weight after oven-drying) or indirect (turbidity, CO2 release, etc).

1. Bacteria

Early work (in the 1980s and earlier) on freshwater bacteria was based on bacteria which were culturable. Freshwater bacteria were merely grouped into:

(a) The flourescent group

(b) The chromogenic bacteria including violet, red
and yellow forms

(c) The coliform group

(d) The Proteus group

(e) Non-gas-forming, non-chromogenic, non-spore forming rods which do not produce Proteus-like colonies and may not acidify milk and liquefy gelatin

(f) Aerobic spore-forming rods

(g) White, yellow, and pink cocci

It was believed that there was no clear separation between soil bacteria and aquatic bacteria and those fresh water bacteria did not have a unique population of its own.

The advent of molecular techniques and especially the polymerase chain reaction (PCR) has made it possible to obtain information on microbial community composition directly, without cultivation. With new molecular techniques of the 1990s, it became possible to assess the microbial population of a natural environment through culture-independent techniques by isolating the nucleic acid in it, followed by the amplification and sequencing of bacterial 16S rRNA genes (Miskin et al. 1999).

There is a huge variation in the bacterial flora of a fresh water. The bulk of such bacteria are heterotrophic while a small proportion are chemoautotrophic.

The bacterial population consists of Gram-negative, non-spore- forming rods, especially Achromobacter and Flavobacterium. With increasing eutrophication, Pseudomonas, Proteus, Bacillus, and Enterobactetriaceae become more important. Other bacteria usually encountered include Vibrio and Actinomycetaes. When eutrophication is heavy, and due to organic material.

Most of the above-mentioned bacteria are Gram-negative; in general, Gram-negative bacteria are planktonic or tectonic. Among the "Aufwuchs" are actinomycetes, which are usually epiphytic. Most benthic bacteria are generally Gram-positive and include spore-formers, and Gram positive cocci, Clostridium, and pleomorphic forms (e.g., Arthrobacter, Nocardia). Chemo-autotrophic bacteria, e. g., Nitrosomonas and Nitrobacter are found in some lake waters while photosynthetic bacteria are found in some rivers.

Certain aquatic bacteria are considered as nuisance bacteria in drinking water. These include iron, sulfur, and sulfate-reducing bacteria. These nuisance bacteria may cause odor, taste or turbidity in water as well as destroy water pipes. "Iron" bacteria withdraw iron which is present in the environment and deposit it in the form of hydrated ferric hydroxide in mucilaginous secretions· and this imparts a reddish tinge to water and may stain clothes. Some well-known iron bacteria genera include Sphaerotilus, Leptothrix, Toxothrix, Crenothrix, Callionella, Siderobacter, and Ferrobacillus.

The sulfur bacteria include the green photosynthetic and nonphotosynthetic members. Many of the photosynthetic bacteria produce H2S which may impart odor to drinking water. Among some well-known colorless (non-photosynthetic) sulfur bacteria are Thiobacterium (short rods which deposit sulfur within or outside the cells), Macromonas (large slow-moving organisms which contain $CaCO_3$ as, well as sulfur), and Thiovulum (spherical cells up to 20 m). A well-known colorless sulpur bacterium is Thiobacillus, an autotrophic organism, the best known of which is T. thioxidans. It oxidizes thiosulphate first to sulfur and then to sulphuric acid. The H_2SO_4 may then corrode pipes and concrete sewers. All the above usually occur singly. Beggiatoa and Thiothrix are usually filamentous. Beggiatoa move by gliding and contain sulfur granules. Some species may be up to 50 m in diameter. Thiothrix is non-motile.

2. Fungi

Fungi are primarily terrestrial, but some are aquatic. Most of the water-dwelling fungi are Phycomycetes, although representatives of the other groups (Ascomycetes, Basidiomycetes, and especially Fungi imperfecti (Hyphomycetes)) contain some aquatic counterparts. Aquatic Hyphomycetes have been well described.

It is perhaps not surprising that it is only among the Phycomycetes that fungi with motile zoospores are to be found. Among the Phycomycetes, the following orders are aquatic: Chytriditlles, Blastocladiales, Monoblepharidales, Hyphochytriales, Leptomitales and Lagenidiales. These fungi are saprophytes or parasites on various plants and animals or their parts in water. In other words, they are mostly, “Aufwuchs,” that is attached and may be found on any aquatic plant or animal, algae, fish or even other fungi.

Among the more common genera of fungi encountered in water are: Allomyces, Achyla, Sapromyces, and various chytrids. Fusarium (not a phycomycete but in the Fungi Imperfecti) is also common in water. Aquatic yeasts have been discovered in large numbers in recent times particularly in waters with high organic matter contents and many Hyphomycetes (Fungi Imperfecti) have also been recorded. There are more than 600 species of freshwater fungi with a greater number known from temperate, as compared to tropical, regions.

The Fungi Imperfecti (called mitosporic fungi by some authors) are classified into two main classes, namely hyphomycetes, and coelomycetes. The hyphomycetes produce conidia directly from vegetative structures (hyphae) or on distinct conidiophores (a specialized hypha that bears conidiogenous cells and conidia whereas, the coelomycetes produce conidia within asexual fruit bodies called pycnidia.

The freshwater Fungi Imperfecti (mitosporic fungi) is classified into three groups: The Ingoldian hyphomycetes (also called the aquatic hyphomycetes), the aeroaquatic hyphomycetes, and the dematiaceous (dark colored) and hyaline (light colored) hyphomycetes and coelomycetes.

(a) The Ingoldian hyphomycetes

The aquatic or fresh water hyphomycetes are also known as the Ingoldian hyphomycetes, after Ingold who first described them in 1942. They are Fungi Imperfecti (i.e., Ascomycetes whose perfect or sexual stages have not been described) as well as some Basidiomycetes. Ingoldian hyphomycetes produce conidia that are mostly unpigmented and branched or long and narrow, and are adapted for life in running water. The Ingoldian hyphomycetes most commonly occur on dead leaves, and wood immersed in water.

(b) Aeroaquatic hyphomycetes

The aeroaquatic hyphomycetes produce purely vegetative mycelium in substrates under water, but produce conidia with special flotation devices, only when the substrates on which the fungus is growing are exposed to a moist aerial environment. They are found in stagnant ponds, ditches, or slow flowing freshwater. Their vegetative hyphae grow on submerged leaves and woody substrates under semi-anaerobic freshwater conditions and are found around the world.

(c) Dematiaceous and hyaline fungi imperfecti

The dematiaceous and hyaline hyphomycetes and coelomycetes are distinct from Ingoldian hyphomycetes, because the conidia are not specifically adapted for aquatic existence. The fungi occur mainly on decaying herbaceous plant material and woody debris in aquatic and semi aquatic habitats worldwide. They are classified into two main groups: indwellers and immigrants. Indwellers have been reported only from freshwater habitats, whilst immigrants have been reported from terrestrial as well as freshwater habitats.

Freshwater fungi are thought to have evolved from terrestrial ancestors. Many species are clearly adapted to life in freshwater as their propagules have specialized aquatic dispersal abilities. Freshwater fungi are involved in the decay of wood and leafy material and also cause diseases of plants and animals.

3. Algae

Algae are primarily aquatic organisms and hence are to be found in large numbers in water, including freshwater. Some algae commonly encountered in drinking water include the blue-green algae: Microcystis aeroginesa (which yields a material toxic to man and animals), Aphanozomenon flos-aquae, and Anabaena circinalis, all of which hamper filteration processes in water purification.

The following diatoms also cause filteration problems: Asterionella formosa, Nitzschia acicularis, Stephanodisus astrea, Melosira spp. as well as the Xanthophyte, Tribonema bombycinum.

4. Protozoa

The Protozoa also show a pattern of succession in their use of bacteria as food. The earliest occurring Protozoa during the eutrophication of a fresh body of water are Sarcodina and Mastigophora which are found in large numbers only in freshly contaminated waters.

<u>Question III:</u> ***Clarify indicator organisms as water-fecal pollution monitors? 10 mark***

The greatest hazard associated with drinking water is that it may recently have been contaminated with sewage or by human (or even animal) excrement. Water recently contaminated by feces from patients or carriers of waterborne pathogens, for example, cholera, Salmonella, and Shigella may carry the live pathogens and thus be a source of fresh outbreaks. It is, however, not practicable to isolate and identify these pathogenic organisms as a routine practice. When pathogens are present in sewage or feces, they are, however, usually out-numbered by bacteria normally present in the alimentary canal and hence in the feces. These normal inhabitants are easier to detect. If they are not found in water it can be inferred in general that the water is free of pathogens, but it should be borne in mind that viruses may well be present.

An ideal indicator should

1. Be present whenever the pathogens are present

2. Occur in much greater numbers than the pathogens

3. Be more resistant to disinfection and to the aqueous environment than the pathogen.

5. Grow readily on simple media

6. Yield characteristic and strong reactions enabling as far as possible an unambiguous identification of the group

7. Be randomly distributed in the sample to be examined, or it should be possible to obtain a uniform distribution by simple homogenization procedures,

8. Grow widely independent of other organisms present when inoculated into artificial media (i.e. indicator bacteria should not be seriously inhibited in their growth by the presence of other bacteria)

The organisms which are used as indicators of fecal contamination are the following: *E. coli, Streptococcus fecalis, Clostridium welchii (C. perfringens), Bifidobacteria*.

Among these the most commonly used indicators are E. coli and the coliform group as a whole. E. coli is of fecal origin and E. coli Type I (Eijkmann positive), which is of human (and warm-blooded animal) origin grows at 44.5°C. Hence, the presence of fecal E. coli is a definite indication of fecal pollution. All coliforms in general are not necessarily of fecal origin; nevertheless, since they are not indigenous to water their presence in drinking water should cast suspicion on the water and should indicate pollution in the widest sense.

Question IV: ***A biosensor is a two-component analytical device. In the light of this statement show the following: 10 mark***

a- Composition of biosensor

* A biosensor consists of 3 parts

1. the sensitive biological element (biological material, a biologically derived material or biomimic),

2. the transducer and

3. the detector element.

b- Characters of biosensor

1. The biosensor must be highly specific for the purpose of the analyses
2. Show good stability over a large number of assays (i.e. much greater than 100).
3. The reaction should be independent of such physical parameters as stirring, pH, and temperature as is manageable.
4. The response should be accurate, precise, reproducible and linear over the useful analytical range, without dilution or concentration.
5. The complete biosensor should be cheap, small, portable and capable of being used by semiskilled operators.

c- Biosensor application as environmental monitors

- Glucose monitoring in diabetes patients
- Biomedical monitoring
- Determining levels of toxic substances before and after bioremediation
- Drug discovery and evaluation of biological activity of new compounds.
- Environmental monitoring, a part from being done at industrial level for monitoring their effluents is in itself a very large field and biosensor can promisingly monitor the environment at all levels (pollution and purity) making the environment healthier to live. The biosensor monitor pollution levels in air, water up to very low levels of detection, measure BOD, detect soil and groundwater contamination, in water monitoring, check purity of drinking water, measure pH of soil and determine its acidity or alkalinity.

Best wishes

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