COURSE CODE: *MCB 405*

COURSE TITLE: *Environmental Microbiology*

NUMBER OF UNITS: *3 Units*

COURSE DURATION: *Five hours per week*

COURSE DETAILS:

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COURSE CONTENT:

The microbial environment; concepts in environmental microbiology; distribution, diversity and role of microorganisms in terrestrial; freshwater and marine environment. Environmental pollution: Air, soil and water. Eutrophication, sewages, effluents, indicator organisms. The aquatic environment-microbiology of water supplies, microbial contaminants and infectious agent in water, indicators of water quality, water treatment; microbiological test of water quality-multiple tube fermentation techniques, membrane filtration, water treatment and public health. Waste management: Hazardous and non hazardous waste; domestic and industrial waste; sewage and sewage disposal in the hot climates; Management approaches: sewage treatment, primary, secondary and tertiary. Environmentally transmitted pathogens. The principles of biodegradation of material; major microbial group involved in biodegradation and bioremediation; importance of bacteria in pesticide degradation; biocontrol. The role of microbes in different climates. Aero microbiology- sources, importance and control of air borne microorganisms. Environmental Agencies and Laws: Governmental/Non–governmental agencies: FEPA/SEPAS, NESREA, NOSRDA, & Ministry of environment, UNEPA, WWF, UNESCO, etc.

Practical: Techniques involved in water quality testing, soil analysis.

COURSE REQUIREMENTS:

This is a compulsory course for all students in the Department of Microbiology . In view of this, students are expected to participate in all the course activities and have a minimum of 75% attendance to be able to write the final examination.

READING LIST:

1. Madigan MT et al. BROCK Biology of Microorganisms. Pearson International edition. 2009.
2. Tortora GJ et al. Microbiology. Pearson International edition. 2007.
3. SCA. The Microbiology of drinking water. Part 1. Water quality and Public Health: Methods for the examination of waters and associated materials. Environment Agency. http:// www.environment-agency.gov.uk/common. 2010.

4. SCA. The Microbiology of Recreational and Environmental Waters.   Environment Agency. http:// www.environment-agency.gov.uk/common. 2000.
5. Cangelosi GA et al. Environmental Microorganism as human pathogens. A report from the American Academy of Microbiology. 2004

LECTURE NOTES

Definition and Scope

Environmental Microbiology is the study of microorganisms and the physical and chemical conditions influencing them. Environments are components of ecosystems. An ecosystem is a community of microorganisms and their physical and chemical environment that functions as an ecological unit.

The ecosphere or biosphere, constitute the totality of living organisms on Earth and the abiotic surroundings they inhabit. It can be divided into atmosphere, hydrosphere and litho-ecosphere to describe the portions of the global expense inhabited by living things in air, water and soil environments respectively. Microorganisms lives within the habitats of the ecospheres. The habitat is one component of a broader concept of the ecological niche, which include not only where an organism lives but also what it does there. The niche is the functional role of an organism within an ecosystem. Microorganisms may be autochthonous (or indigenous) or allochthonous or foreign.

ATMO-ECOSPHERE

The atmosphere consists of 78%, Nitrogen; 21%, Oxygen; 0.034%, carbon dioxide and trace amount of other gases. It is saturated with water vapour to varying degrees and it may contain droplets of liquid water, crystals of ice and particles of dust. The atmosphere is divided into regions delineated by temperature. The troposphere, the region nearest the Earth's surface. Above it is the stratosphere and above this lies the ionosphere.

The chemical and physical parameters of the atmosphere do not favour microbial growth and survival. Temperature decrease with increase in height in the troposphere. Temperature below the minimal growth temperate for microorganism. With increasing height pressure drops and the concentration of oxygen decreases to a point that precludes aerobics respiration. The low concentration of organic

carbon are insufficient to support heterotrophic growth, available water is also scarce. Hence limiting the possibility of autotrophic growth of microorganism in the atmosphere.

Even though the atmosphere is a hostile environment for microorganisms, there are substantial number of them in the lower troposphere, because of thermal gradients. There is rapid mixing.

Many microorganisms growing in the hydrosphere or lithosphere can become airborne, they are known as autochthonous atmospheric microorganisms. Methods of sampling organism in the atmosphere include viable plate count and direct count method. Cladosporium is a major fungal constituent of the atmosphere followed by *Puccinia graminis.*

HYDRO-ECOSPHERE

By its definition it contains water, which is necessary for microbial metabolism. It is more suitable for microbial growth than the atmosphere. Unlike the atmosphere, the hydrosphere contains autochthonous microbial population. Most of them are motile and able to grow at low nutrient concentrations.

The hydrosphere is divided into freshwater and marine habitats. Freshwater habitats include lakes, ponds, swamps, springs, streams and rivers. These habitats are collectively designated as Limnetic and their study is referred to as limnology. Marine habitats are the oceans and the estuarine habitats that occur at the interface between freshwater and marine ecosystems.

Freshwater Habitats

It could be stagnant (i.e. lentic) or moving (i.e. lotic), freshwater habitats include lakes, ponds, swamps, springs, streams and rivers.

The neuston is the uppermost layer of the hydrosphere, the surface microlayer, represents the interface between the hydrosphere and the atmosphere. It is characterized by high surface tension arising from the interfacing of water with gas. Under quiescent conditions, microorganisms from a surface film known as neuston. This layer is a favourable habitat for photoautotrophs because, 1^0 producers have unrestricted access to CO2 from the atmosphere and light radiation. Some mineral nutrients and metals

in water become enriched in the surface microlayer. 2^0 producers also proliferate here. Microbial number in the neuston are often ten-to a hundred fold higher than in the underlying water column. Devices including skimmers, touch-on screens and rotating drums have been developed for the qualitative and quantitative sampling of the neuston community.

Autochthonous neuston microbiota include: *Pseudomonas*, *Caulobacter*, *Neuskin*, *Hyphomicrobium*, *Achromobacter*, *Flavobacterium*, *Alcaligenes*, *Brevibacterium*, *Micrococcus*, and *Leptothrix*. Cyanobacteria: *Aphanizomenon*, *Anaebaena* and *Microcystis*. The filamentous fungi *Cladosporium* and various yeasts are frequently associated with the neuston. Algal genera viz: *Chromulina*, *Botrydiopsis*, *Codosiga*, *Navicula*, *Nautococcus*, *Proterospongia*, Protozoa found include: *Difflugia*, *Vorticella*, *Ariella*, *Acineta*, *Clathrulina*, *Stylonychia* and *Codonosigna*. Some of these same organisms are also found in the underlying water column.

Wetlands are shallow aquatic environemts dominated by emergent plants develops in poorly drained shallow basins, often as a result of gradual filling in of a lake by silt and vegetation. The water surface may be largely observed by emergent plant serve as the basis for wetland classification.

Lakes: They are divided into 3 zones based on the penetration of light. The combined littoral and limmetic zones are known as the euphotic zone. Profundal is the area of deeper water beyond the depth of effective light penetration. The littoral is an area of shallow water where light penetrate to the bottom. This area is dominated by submerged or partially submerged higher plants and attached filamentous and epiphytic algae.

The limnetic zone is an area of open water away from the shore. The dominant 1^0 producers here are planktonic algae. The limnetic zone descends to a level known as the compensation depth. The compensation depth is the lowest level having effective light penetration, where photosynthetic activity balances respiratory activity.

The bottom of the lake or benthos represents the interface between the hydrosphere and the lithosphere. Organisms in the profundal zone are largely secondary producers and are in most cases

dependent on the transport of organic compounds from the overlying zone. The benthic sediment habitat favours proliferation of microorganisms.

Lakes can be classified as either oligotrophic or eutrophic based on productivity and nutrients concentration.

Rivers are characterized by flowing water with zones of rapid water movement and pools with reduced currents. Zone of rapid water movementt tend to be shallower than pools. In pool zones, the decreased current velocity allow for the deposition of salt. There's a great deal of transfer of chemical into rivers waters through rain water runoff and erosion of river banks.

The upper course is usually characterized by swift flow, a high degree of oxygenation and low temperature. Shading by forests generally keeps 1^0 production in the upper course low and organic material input is derived mostly from the surrounding lithosphere. The middle course of a river is characterized by decreasing velocity, higher temperature and less shading, resulting in significant intrinsic primary production.

Microorganisms in rivers are attached to surfaces such as submerged rocks. Dissolved nutrients are rapidly absorbed by these attached organisms and are liberated upon death and decay only to be absorbed again a small distance downstream. Hence, nutrients do not move with the speed of the current but exhibits a slower movement. The path of a nutrient can be viewed as a spiral rather than a cycle, a phenomenon known as nutrient spiraling.

Rivers are often polluted with high amount of effluents from industries and municipalities. Municipal sewage discharge introduced light amount of organic compounds. Concentration are generally depleted thereby increasing the BOD of the water.

Industrial effluents may also introduce toxic chemicals such as heavy metals, which may adversely affect microbial activities.

Freshwater Microbial Communities

Autotrophic bacteria are autochthonous members of lakes and play an important role in nutrient cycling. Photoautotrophic bacteria normally found in lakes include Cyanobacteria and in anoxic zones, the purple and green anaerobic photosynthetic bacteria. Blooms of Cyanobacteria, often associated with mineral enrichment is common in freshwater. Genera: *Microcystis*, *Anabaena* and *Aphanizomenon* can be dominant.

Chemolithotrophic bacteria which include member of the genera: *Nitrosomonas NItrobacter* and *Thiobacilus* play important roles in Nitrogen, Sulphur and Iron cycling within lakes.

Microorganisms in sediments are usually different from the neustonic ones. Fungi are found on the debris that accumulates on the sediment surface. They include cellulolytic forms. Bacteria that are capable of anaerobic respirations are important members of sediment microbiota and include *Pseudomonas* species capable of denitriifcation activities. Within the sediment obligate anaerobic bacteria such as endospore forming ones - *Clostridium*, Methanogenic bacteria (produces – methane gas) *Desulfovibrio* species (produces H_2).

Many fungi in freshwater lakes, rivers and streams are considered to be allochthonous because they are associated with foreign organic matter that enters the water body. Many ascomycetes and other fungi are found on wood and decaying plant materials in water. When the plant material is degrade the fungi disappear. Yeasts are found in many freshwater bodies viz: fermentative members of *Torulopsis*, *Candida*, *Rhodotorula* and *Cryptococcus*.

Algae found in freshwater include members of *Euglenophycophyta*, *Chlorophycophyta*, *Chrysophycoplyta*, *Cryptophycophyta* and *Pyrrhophycophyta*. Protozoans present in freshwater are the phagotrophic flagellates which are important grazers of bacterial population, amoeboid, ciliated and flagellated protozoa. Among which include *Paramecium Didinium*, *Vorticella*, *Amoeba*.

Microorganisms play important role in freshwater (especially lake) productivity and the transformation of organic compounds.

DOMESTIC WASTES AND WASTE TREATMENT

Domestic waste water contain high levels of organic matter from human faeces, urine and graywater. Gray water results from washing, bathing and meal preparation. Water from various industries may also enter the system. People excrete 100 – 500 grams wet weight of faeces and 1 – 1.3 litres of urine per person per day. Three major tests are used to assess the amount of organic matter in waste water:

(i) Total Organic Carbon (TOC)

(ii) Chemical Oxygen Demand (COD)

(iii) Biochemical Oxygen Demand (BOD)

The major objective of domestic waste water treatment is to ensure that water released into the environment do not pose environmental and health hazards. This is done by the reduction of BOD, which may be either in form of solids (suspended matter) or soluble.

BOD is the amount of DO consumed by organisms during the biochemical oxidation of organic (Carbonaceous BOD) and inorganic matter. It was developed in the, 1930s. The 5-day BOD test (BODs) is a measure of the amount of O_2 consumed by a mixed population of heterotrophic bacteria in the dark at 20^0C over a period of 5 days. TOC is determined by oxidation of the organic matter with heat and oxygen, followed by measurement of the CO_2 liberated with an infrared analyzer.

COD is the amount of oxygen necessary to oxidize all of the organic carbon completely to CO_2 and H_2O. COD is measured by oxidation with potassium dichromate ($K_2Cr_2O_7$) in the presence of sulpheric acid and silver.

Modern Wastewater Treatment

The essential goal of wastewater treatment is the removal and degradation of organic matter under controlled conditions. Sewage treatment is in 3 major steps.

- Primary treatment

- Secondary treatment

- Tertiary treatment

Primary treatment is the first step in municipal sewage treatment. It is a physical process that involve the removal of large solids, from the waste stream. The raw sewage passes through a metal grating that removes large particles such as twigs, plastics, stones etc. A moving screen then filters out smaller items after which a brief residence in a grit tank allows sand and gravel to settle out. The waste stream is then pumped into the primary settling tank/sedimentation tank or clarifier. Here the suspended organic solids settle to the bottom as sludge or biosolids. This sludge is referred to as primary sludge. Pathogens (especially microbes) are not removed effectively in the primary process.

Secondary treatment is a biological degradation process, in which the remaining suspended solids are decomposed and the number of pathogens is reduced.

Effluent from primary treatment may be pumped into a (1) trickling filter bed (2) an aeration tank or (3) a sewage lagoon.

Trickling filter bed is simply a bed of stoned or corrugated plastic sheets through which water drips. The effluent is pumped through a system ooverhead sprayer onto this bed, where bacteria and other microorganisms reside. These microorganisms intercept the organic material as it trickles past and decompose it aerobically. The media through which the waste trickles may be stones, ceramic materials, hard coal or plastic. Polyvinylchloride or polypropylene are now commonly used in modern high-rate trickling filters. Because of the lightweight of the plastic, they can be stacked in towers 6 to 10m high referred to as biotowers. The organic material passing through the filter is converted to microbial biomass which forms a biofilm on the filter medium surfaces. The biofilm that form on the

surface of the filter medium is called the **Zooleal film.** It is composed of bacteria, fungi, algae and protozoa. BOD removal by trickling filter is approximately 85% for low-rate filters. It is one of the earliest system for biological degradation of sewage.

Activated sludge

Also known as aeration-tank digestion. Effluent from primary treatment is pumped into a tank and a bacteria-rich slurry (activated sludge) is mixed with it. The mixture is aerated by pumping pure oxygen or air through it. This promotes bacteria growth and decomposition of the waste. It goes to a secondary settling tank where water is siphoned off the top of the tank and sludge removed from the bottom. Part of the sludge is re-used as inoculums for the incoming primary effluent (i.e. activated sludge).

One of the important characteristics of the activated sludge is the recycling of a large proportion of the bioman. This results in a large number of microorganisms that oxidize organic matter in a relatively short time. The detention time for sewage in the aeration basin varies from 4 to 8 hours. The content of the aeration tank is called the Mixed Liquor Suspended Solids (MLSS) while the organic part of MLSS is the Mixed Liquor Volatile Suspended Solids (MLVSS).

Tertiary treatment involves a series of additional steps after secondary treatment to further reduce organics, turbidity, nitrogen, phosphorus, metals and pathogens. Processes involve some type of physicochemical treatment such as Coagulation, filtration, activated carbon, adsorption of organics and additional disinfection. Constructed wet lands are now increasingly used in the treatment of liquid wastes and for bioremediation.

RECALCITRANTS AND XENOBIOTICS

Under favourable conditions all natural organic compounds degrade. For various purposes, the ingenious chemical industry now manufactures many compounds that greatly differed in their structure from natural organics. Some of these halo substituted, and nitro substituted organics such as propellants, regridgerants, solvents, PCBs, plastics, detergents, explosives and pesticides are now completely resistant (recalcitrant) to biodegradation. Some others degrade only slowly or give rise to

bound and polymeric residues of environmental concern. These compounds are referred to as xenobiotics. Reasons for a xenobiotic organic compound proving recalcitrant/include:

1) Unusual substitution – with halogens, mainly chlorine

2) Unusual bond or bond sequences – such as in tertiary and quaternary carbon atoms.

3) Highly condensed aromatic rings and excessive molecular size e.g. polyethylene and other plastics.

4) Failure of compound to induce the synthesis of degrading enzymes in microrganisms.

5) Failure of the compound to enter the microbial cell for lack of suitable permeases.

6) Unavailability of the compound due to insolubility or adsorption phenomena.

7) Excessive toxicity of the parent compound or its metabolic products. Examples of recalcitrant compounds are Propachlor, Aldrin, 2, 4, 5 – 6 trichlorophenoxy acetic acid (2, 4, 5 – T) and DDT.

MICROBIAL INDICATORS OF WATER QUALITY

Indicator organisms

Indicator microorganisms are used to predict the presence of and/or minimize the potential risk associated with pathogenic microbes. Indicator organisms are useful in that they circumvent the need to assay for every pathogen that may be present in water. Ideally, indicators are nonpathogenic, rapidly detected, easily enumerated, have survival characteristics that are similar to those of the pathogens of concern, and can be strongly associated with the presence of pathogenic microorganisms.

Rationale for the use of indicator organisms

The key criteria for ideal bacterial indicators of faecal pollution are:

- They should be universally present in large numbers in the faeces of human and other warm-blooded animals.
- They should also be present in sewage effluent, be readily detectable by simple methods.
- They should not grow in natural waters.

- They should also be of exclusive faecal origin and be present in greater numbers than faecally transmitted pathogens.

- No single indicator organism fulfils all these criteria, but the member of the coliform group that satisfies most of the criteria for the ideal indicator organism in temperate climates is *E. coli*.
- The presence of *E. coli* in a sample of drinking water may indicate the presence of intestinal pathogens.
- The absence of *E. coli* cannot be taken as an absolute indication that intestinal pathogens are also absent.

Other Indicator organisms

- Enterococci and
- Spores of sulphite-reducing clostridia, typified by *Clostridium perfringens*. Enterococci do not multiply in the environment and can occur normally in faeces. Numbers of enterococci in humans are greatly outnumbered by *E. coli* bacteria. When coliform bacteria are present in the absence of *E. coli*, but in the presence of enterococci, this can be indicative of the faecal origin of the coliform bacteria.

- The rationle for enumerating heterotrophic plate counts has been to assess the general bacterial content of the water and to monitor trends or rapid changes in water quality.

Coliform bacteria

Coliform bacteria are defined as Gram-negative, non-spore-forming, rod shaped bacteria which are capable of aerobic and facultative anaerobic growth in the presence of bile-salts or other surface-active agents with similar growth-inhibiting properties. They usually ferment lactose at 37 °C within 48 hours, possess the enzyme β-galactosidase and are oxidase-negative. Coliform bacteria belong to the family Enterobacteriaceae and share similar cultural characteristics. Typical genera encountered in water supplies are *Citrobacter, Enterobacter,Escherichia, Hafnia, Klebsiella, Serratia* and *Yersinia*.

Faecal coliform bacteria

Faecal coliform bacteria possess the characteristics of coliform bacteria but are able to carry out lactose fermentation at 44 °C.   The term "faecal coliform" is not precise and has been used to describe coliform bacteria thought to be of faecal origin. The term "thermotolerant coliform" has been used to describe presumptive faecal coliform bacteria.

Escherichia coli

E. coli has long been used as an indicator of fecal pollution. *E. coli* is a coliform bacterium and has historically been regarded as the primary indicator of faecal contamination of both treated and untreated water. Most of the *E. coli* strains possess the enzyme β-glucuronidase, which can be detected using specific fluorogenic or chromogenic substrates.

Intestinal enterococci

Enterococci have been used successfully as indicators of fecal pollution. Intestinal enterococci are defined as Gram-positive cocci that tend to form in pairs and chains. They are non-spore-forming, oxidase-negative, catalase-negative, possess Lancefield's Group D antigen and hydrolyse aesculin. They can grow aerobically and anaerobically in the presence of bile salts, and in sodium azide solutions, concentrations of which are inhibitory to coliform bacteria and most Gram-negative bacteria. *Enterococcus faecalis* and some related species can reduce 2,3,5-triphenyltetrazolium chloride to the insoluble red dye, formazan.

Clostridium perfringens

 C. perfringens is an enteric, gram-positive, anaerobic, spore-forming, pathogenic bacterium found in human and animal feces. *Clostridium perfringens* is a member of the sulphite-reducing clostridia which is non-motile and is capable of fermenting lactose, reducing nitrate and liquefying gelatin. Most clostridia are strictly anaerobic, but a few species are capable of limited growth in the presence of low levels of oxygen.

Clostridium perfringens is the key species of the sulphite-reducing clostridia. *Clostridium perfringens* produces environmentally resistant spores that survive in water and in the environment for much longer periods than the vegetative cells of *E. coli* and other faecal indicators.

Colony count bacteria

Colony counts are enumerations of the general population of heterotrophic bacteria present in water supplies. The enumerations may represent bacteria whose natural habitat is the water environment or those that have originated from soil or vegetation. Historically, these bacteria have been enumerated on bacteriologically nutrient-rich media with incubation at 37 °C and 22 °C. It is well recognised, however, that only a small fraction of the viable bacterial population present in water is enumerated by the procedures normally employed. Monitoring of water supplies for colony count bacteria can be useful for monitoring trends in water quality or detecting sudden changes in quality.

Other potential indicators of faecal contamination

E. coli and related coliform bacteria, intestinal enterococci and *Clostridium perfringens* are currently recommended for use as indicator organisms of faecal contamination in water.

Other micro-organisms have been suggested for this purpose and these include the *Bacteroides fragilis* group, *Bifidobacterium* species or *Rhodococcus coprophilus*. Also bacteriophages that infect the coliform bacteria (coliphages) and the *Bacteroides fragilis* group have been used. Although some of these alternative indicator organisms have been applied with varying degrees of success to environmental waters, they are not considered suitable for the assessment of water treatment efficacy or treated water quality.

WATER-BORNE PATHOGENS

Bacteria

Campylobacter

Bacteria of the genus *Campylobacter* are members of the family Spirillaceae. Campylobacteriosis occurs most frequently in the summer months and the most commonly isolated species is *Campylobacter jejuni*. The organism can be carried asymptomatically by cattle, sheep, poultry and other birds,and is also isolated from natural waters.

Campylobacter species can survive in water for some days but are highly susceptible to chlorination or ultra violet disinfection at the doses typically used in water treatment and should, therefore, not be a risk in treated drinking water, unless it is subject to significant post treatment contamination.

E. coli O157

Some strains of *E. coli* can cause serious diarrhoeal disease. Several classes of diarrhoeagenic *E. coli* are now recognised, which are defined by the possession of distinct virulence factors. The most important of these are the Vero-cytotoxin-producing *E. coli* (VTEC), in particular VTEC of serogroup O157, but other *E. coli* serogroups may contain VTEC members. Typical symptoms of people infected with *E. coli* O157 range from mild diarrhoea, fever and vomiting to severe, bloody diarrhoea and painful abdominal cramps. In 10 - 15 % of cases, a conditionknown as haemolytic uraemic syndrone which can result in kidney failure. Individuals of all ages can be affected but children up to ten years old and the elderly are most at risk. The infectious dose for *E. coli* O157 is relatively low compared with other bacterial causes of gastro-enteritis, perhaps as low as 10 organisms.

Salmonella

Species of *Salmonella* are members of the family Enterobacteriaceae and are the causative agents of typhoid and paratyphoid fever, and milder forms of gastro-enteritis. The enteric fevers (typhoid, caused by *Salmonella typhi*, and paratyphoid, caused by *Salmonella paratyphi*) remain important contributors to water-borne disease world-wide, although nowadays very rarely in developed countries. Salmonellae can be subdivided into more than 2000 serotypes. S*almonella typhi* and *Salmonella paratyphi* are only associated with humans but the other salmonellae are found commonly in the faeces of animals and agricultural livestock, and have been found in poultry, eggs and meat products. Food-borne contamination is the major route of infection for these bacteria, but transmission can occur by water contaminated with faecal material. Survival in surface water is limited to hours or days, depending on the amount of contamination and the water temperature. Species of *Salmonella* are susceptible to normal methods of disinfection used in the water industry. *E. coli* is an adequate indicator for the presence and survival of *Salmonella* in water.

Shigella

Species of *Shigella* are members of the family Enterobacteriaceae and cause bacillary dysentery (shigellosis) in humans. The *Shigella* group is divided into four main sub-groups differentiated by biochemical and serological tests. *Shigella dysenteriae, Shigella sonnei,Shigella flexneri* and *Shigella boydi* are the main organisms of concern. Person-to person contact, faecally contaminated food, and less frequently, water are the main sources of contamination. Survival in surface water is limited to hours or days, depending on the amount of contamination and the water temperature. Shigellae are susceptible to chlorination and ultra violet disinfection at the doses used in water treatment. *E. coli* is an adequate indicator for the presence and survival of *Shigella* in water.

Yersinia

Species of *Yersinia* are members of the family Enterobacteriaceae, of which some species cause diseases in humans and other mammals. Human plague, caused by *Yersinia pestis*, is not a water-borne disease. Other species, including *Yersinia enterocolitica*, *Yersinia intermedia*, *Yersinia kristensenii*, *Yersinia frederiksenii* and *Yersinia pseudotuberculosis*, may produce symptoms ranging from subclinical and mild diarrhoeal infections to rare severe infection including septicaemia. Some serotypes of *Yersinia enterocolitica* are more frequently associated with human disease than others. *Yersinia* species are susceptible to chlorination and ultra violet disinfection at doses normally used in water treatment. *E. coli* is an adequate indicator for the presence and survival of *Yersinia* in water.

Vibrio

Species of *Vibrio* are members of the Vibrionaceae. Some species, most notably, strains of *Vibrio cholerae*, cause gastro-enteritis in humans. *Vibrio* species occur naturally in brackish

and saline waters, and some can survive in fresh water systems. *Vibrio cholerae*, which causes cholera, can be divided into approximately 140 O-serovars. The strains that usually produce outbreaks of epidemic cholera are toxin-producing strains of the O1 serovar and a more recently reported serovar, O139. Some other serovars of *Vibrio cholerae* can also cause gastroenteritis. The primary route of transmission for cholera is contaminated water and outbreaks have also been reported following consumption of crops irrigated with sewage-contaminated water.

Vibrio parahaemolyticus also causes diarrhoea, often through the consumption of raw, contaminated seafood. *Vibrio fluvialis, Vibrio furnissii, Vibrio hollisae* and *Vibrio mimicus* are also recognised as causing diarrhoea. Other species of *Vibrio* are associated with wound infections or septicaemia following exposure to environmental waters. *Vibrio* species can grow in environmental waters, particularly when temperatures rise above 10 °C and may be associated with sediments, plankton and cyanobacterial blooms. *Vibrio* are susceptible to chlorination and ultra violet disinfection at doses normally used in water treatment.

Viruses

Norwalk-like viruses

Norwalk-like viruses (NLV) are classified within the *Caliciviridae* family. They are the most common cause of sporadic and epidemic viral gastro-enteritis in adults, but are also common causes of infections in children. Many strains of NLV have been recognised and they are currently divided into two major genogroups (I and II). The main route of transmission is via person to-person contact, but food-borne transmission may occur, especially involving raw or inadequately cooked shellfish. Water-borne outbreaks have occurred as a result of sewage contamination of drinking water supplies. The limited information currently available suggests that NLV are sensitive to chlorination. As these viruses may survive in the environment longer than bacteria, the absence of *E. coli* may not always equate with the absence of NLV.

Hepatitis A Virus

Hepatitis A virus is a member of the *Picornaviridae* family of viruses, and is the only member of the Hepatovirus genus in which there is only one serotype. No animal strains are known. The virus replicates in the liver and causes acute but self-limiting hepatitis. Transmission is by direct faecal-oral route and is most common in areas of poor hygiene and poor sanitation.   Water-borne outbreaks have been recognised after sewage contamination of drinking water. Hepatitis A viruses are sensitive to chlorination, but as these viruses may survive in the environment longer than bacteria, the absence of *E. coli* may not always equate with the absence of Hepatitis A viruses.

Other viruses

Enteroviruses (*Picornaviridae* family) are well-established indicators of human enteric viruses in the environment. This is due to the relative ease with which they can be concentrated from sewage-contaminated water and the availability of effective detection methods. Additionally, Enteroviruses replicate in the gastro-intestinal tract and are present in most populations throughout the year. The group includes poliovirus, Coxsackievirus B and echovirus. Enterovirus infections are commonly asymptomatic, but may cause flu-like symptoms, occasionally meningitis and, rarely, paralysis. Enterovirus infection does not result in gastro-enteritis unless as part of a more generalised illness. Vaccination campaigns utilising live poliovirus are undertaken world-wide resulting in widespread occurrence of the virus in the environment. Infections with other Enterovirus serotypes are common world-wide with different serotypes predominating from year to year. Water-borne transmission has not been confirmed, although as person-to-person transmission is the main route and results in many asymptomatic infections, it would be difficult to identify.

Rotaviruses (*Reoviridae* family) comprise six serogroups and are further divided into serotypes and genotypes. Serogroup A is the most common human rotavirus infection, although members of Group B and C can also infect humans. Infection first occurs below the age of one year and rotavirus is the most important pathogen causing gastro-enteritis of this age group. Subsequent infections throughout life are usually asymptomatic. A few waterborne outbreaks world-wide have been reported.

The Adenoviruses (*Adenoviridae* family), which include many different serotypes, replicate in the gastro-intestinal tract and are shed into sewage. Only serotypes 40 and 41 are known to cause gastro-enteritis in humans, mostly in babies. Infection involving drinking water has not been recognised. The Astroviruses (*Astroviridae* family) include at least eight serotypes that infect humans, causing gastro-enteritis, particularly in children. Water-borne infections have been reported.

The above viruses may survive in the environment for longer periods than bacteria, and the absence of *E. coli* may not be an adequate indicator for the environmental presence of these viruses in all circumstances. These viruses are sensitive to chlorination.

Classic calicivirus is the name given to a distinct group of Caliciviruses, and are recognised by clear cup-shaped markings on virions when examined by electron microscopy. This group is also known as Sapporo virus or Sapporo-like viruses. They are part of the family *Caliciviridae*, but are distinct from the NLV. No water-borne infections have been recognised.

Protozoa

The enteric protozoa that cause human illness are usually transmitted by the consumption of food and drink, although environmental contamination and poor hygiene are also important transmission routes. Many cause particular problems in immuno-compromised patients, particularly in people infected with HIV and individuals with T-cell deficiencies. The protozoa that are of most concern are *Cryptosporidium*, *Giardia* and *Toxoplasma*, although **Cyclospora** has been identified in a number of food-borne outbreaks. Water-borne outbreaks of infection with protozoa have been reported.

Cryptosporidium

Cryptosporidium species are the cause of a diarrhoeal disease that can last for up to several days to a few weeks. A chronic life threatening infection with watery diarrhoea can occur in people with compromised immune systems. There have been several outbreaks of gastroenteritis linked to drinking water, contaminated swimming pool and recreational water use, and drinking water is an important identifiable source of human cryptosporidiosis. Other sources include contamination associated with farm visits and food-borne infection.

Cryptosporidium oocysts have a low infectious dose (from 10 to 1000 organisms) and individual strains have been found to differ in their infectivity. Natural water sources are commonly contaminated with oocysts from animal and human faeces. *Cryptosporidium* species includes a number of types that are infectious to humans.

The oocysts of *Cryptosporidium* are infectious when excreted in faeces, and pass into rivers and lakes via sewage works and agricultural run-off. The oocysts are resistant to environmental conditions and disinfectants such as chlorine, and can pass into drinking water when there are failures in filtration processes or contamination of source waters. There is increasing evidence that oocysts are susceptible to ultra violet disinfection. Conventional indicator bacteria are not good indicators of *Cryptosporidium* contamination, and water supplies that are at risk of contamination are subject to continuous monitoring.

Giardia

Giardia species are flagellated protozoans that parasitize the small intestines of mammals, birds, reptiles and amphibians, and giardiasis is a common cause of diarrhoea. The symptoms of giardiasis range from asymptomatic to a transient or persistent acute stage, with steatorrhoea, intermittent diarrhoea, and weight loss, or to a sub-acute or chronic stage that can mimic gallbladder or peptic ulcer disease. The cysts of *Giardia duodenalis* are relatively resistant to chlorine, although less resistant than *Cryptosporidium* oocysts. The cysts can remain viable in cold water for several months.

Cyclospora

Cyclospora is a coccidian parasite that causes protracted watery diarrhoea. It occurs worldwide, and is normally associated with travel to developing countries. Outbreaks linked to drinking water have been reported. Person-toperson contact is not thought to occur, because the oocysts need to mature (sporulate) under environmental conditions outside the host for one to two weeks before they become infectious. The oocysts have been reported to be relatively resistant to chlorine.

Microsporidia

Microsporidia are protozoa with characteristic morphology including a lack of mitochondria and possession of a distinctive coiled polar tube in the spores. Two species, *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*, are a common cause of chronic diarrhoea in immuno-compromised individuals, and they may infect a range of agricultural animals. As viable spores are passed by infected patients, person-to-person transmission and contamination of water with human waste are potential routes of transmission. The difficulty of isolating organisms by tissue culture means that reliable information on the sensitivity of spores to chlorine is not available for all species. The relatively recent emergence of species of Microsporidia as human pathogens and the difficulties of diagnosis mean that water-borne associations cannot yet been clearly demonstrated, although spores have been found in non potable water.

Toxoplasma gondii

Toxoplasma gondii is a parasite which forms oocysts in cats, and cysts within a secondary host's (other mammals or birds) tissues. The life cycle is completed when the carnivorous primary host consumes the secondary host. Humans are infected by consuming inadequately cooked meat from infected secondary host species such as agricultural animals, or from oocysts occurring in food or water. The sporulated oocysts of *Toxoplasma* are very resistant to environmental conditions and disinfectants. Water-borne infections arise through oocysts, from infected wild cats, getting into drinking water.

Entamoeba histolytica

Entamoeba histolytica causes amoebic dysentery and abscesses in the liver and other organs.
Water-borne infections mostly arise when consuming contaminated food or water in countries where it is endemic. Infection is common world-wide, particularly in poor countries with inadequate sanitation. Outbreaks of infection associated with drinking water are rare.

Microorganisms affecting taste, odour and appearance

Ideally, drinking water should be clear and acceptable to the palate. In practice, however, the

aesthetic properties of a drinking water will depend to a large extent on its source and any subsequent treatment or microbial activity. In most instances, when there is adverse comment regarding the appearance, taste or odour of a drinking water, the causes tend to be physical or chemical in nature rather than microbiological.

Nevertheless, musty, mouldy or earthy tastes and odours may result from the growth of fungi or actinomycetes in water pipes. These tastes and odours are primarily associated with the production of secondary metabolites (notably geosmin and 2-methylisoborneol) or the biomethylation of chlorinated phenols (for example, trichloroanisole from trichlorophenol).

Other compounds, which are produced via microbial decomposition, can impart fishy, swampy or septic odours to waters, whilst rotten-egg odours can be generated via the reduction of sulphate and sulphite to hydrogen sulphide by some bacteria (for example, *Desulfovibrio desulfuricans* and some species of *Clostridium*).

Micro-organisms growing in biofilms in pipes can result in the corrosion of iron pipes. A consequence of this is the discoloration of drinking water due to elevated levels of iron in the water, or to the accumulation of (brown) iron or (black) manganese deposits, or iron-stained material being dislodged from pipes or sediments.

Actinomycetes and certain algae can also cause taste problems by growth in the raw water, particularly storage reservoirs. This situation can often be controlled by the inclusion of appropriate use of granular or powdered activated carbon in treatment processes.

Cyanobacteria and animalcules
Cyanobacteria (blue-green algae)
Cyanobacteria occur naturally in many in-land, standing bodies of water and can often be seen forming a surface scum or bloom. These bacteria thrive in warm, shallow and nutrient rich lowland waters and examples include *Anabaena, Aphanizomenon, Microcystis* and *Oscillatoria*. Some species produce toxins that can be found in mucus material which is secreted by cells. There is no evidence, however, that these toxins pose a risk to public health via treated water supplies as they are destroyed by treatment. One of the main problem with Cyanobacteria is that raw water blooms can affect treatment efficiency by blocking filtration systems, and can cause adverse tastes and odours in the treated water, although these may be removed with appropriate treatment.

WATER TREATMENT

The objective of water treatment is to produce wholesome water that meets the statutory requirements and is microbiologically and chemically safe for consumption, is not corrosive towards materials in contact with water and is aesthetically acceptable. The range of treatment processes includes clarification and sedimentation, filtration and disinfection. Depending on the source and nature of the water, one or more of these processes can be used. Whilst each of the treatment processes is able to reduce the numbers of particular micro-organisms, no process can ever ensure their complete removal. Disinfection (usually by chlorination) is the final safeguard against water-borne microbial contamination. When chlorine is used, the dose should be selected so that the chemical demand of the water is satisfied and that an adequate contact time is achieved before water is supplied to consumers.

Micro-organisms differ in their susceptibility to chlorine (in decreasing order of resistance: protozoan cysts, bacterial spores, enteric viruses and enteric bacteria). However, the combination of chlorine concentration and contact time necessary for inactivation of enteric viruses and pathogenic bacteria can be achieved by a well-managed water treatment works.

Treatment processes

Storage

The storage of water in reservoirs creates favourable conditions for the self-purification of the stored water, but may also cause undesirable changes in water quality. The benefits of storage include the provision of a continuous supply of water, reduction in turbidity, reduction in pathogens through the action of sunlight and sedimentation, dilution of undesirable substances that may accidentally enter the intake, and oxidation of impurities. It also provides a buffer should pollution occur in the river.

Presedimentation

Highly turbid surface water may require presedimentation before further treatment. Presedimentation basins are constructed in excavated ground or of steel or concrete. Such basins may be preceded by equipment for the addition of chemicals to provide partial coagulation during periods when the water is too turbid to clarify by sedimentation alone.

Prechlorination

Prechlorination to breakpoint has been widely used as an alternative to storage for water derived from lowland rivers and is also used when stored water contains much planktonic life. Its purpose is to reduce counts of faecal bacteria and pathogens, destroy animal life and algae, and oxidize ammoniacal nitrogen, iron, and manganese, thereby assisting in their removal. The combined and free chlorine which remains effectively discourages microbial activities, such as protozoal predation and

nitrification, as well as microbial growth during subsequent filtration. When used to disinfect raw water, the oxidative effect of chlorine and even more of ozone will result in the partial conversion of total organic carbon into biodegradable organic carbon which, if not removed by biological activity during treatment (e.g. during slow sand or granular activated carbon filtration), can result in the growth of nuisance organisms during distribution. It is important to balance the maintenance of the microbiological safety of drinking-water against possible hazards associated with the formation of such disinfection by-products.

Coagulation and flocculation

To remove particulate matter, a water-treatment plant will generally include equipment for coagulation and flocculation, followed by sedimentation and filtration. Coagulation involves the addition of chemicals (e.g. aluminium sulfate, ferric sulfate) to neutralize the charges on particles and facilitate their agglomeration during the slow mixing provided in the flocculation step. Floes thus formed co-precipitate, adsorb and entrap natural colour and mineral particles, and can bring about major reductions in counts of protozoa, faecal bacteria, pathogens, and viruses.

Sedimentation or flotation

The purpose of sedimentation is to permit settleable floe to be deposited and thus reduce the concentration of suspended solids that must be removed by filters. Flotation is an alternative to sedimentation, and has advantages when the amount of floe is small. The factors that influence sedimentation include the size, shape, and weight of the floe, viscosity and hence the temperature of the water, the detention time, the number, depth, and areas of the basins, the surface overflow rate, the velocity of flow, and the inlet and outlet design.

Rapid filtration

Typically, rapid sand filters consist of 0.4-1.2 m of sand, usually of an effective size of 0.5-1.0 mm, supported by gravel and underdrains. In recent years, single-medium filters have often been replaced by dual-medium or multimedia ones. During filtration, residual particles of floe not removed by sedimentation are trapped in the interstices of the bed, and may induce further flocculation of particles. A limited amount of biological activity may also occur, if it is not suppressed by prechlorination or by high flow rates. Both sand and mixed-media filters are normally cleaned by reversal of the flow though the bed (backwashing).

Slow sand filtration

Typically, slow sand filters consist of 0.5-1.5 m of silica sand with an effective size of 0.3-0.6 mm. The upper layer of fine sand is supported on gravel and a system of underdrains. Slow sand filtration is

simpler to operate than rapid filtration, as frequent backwashing is not required. It is therefore particularly suitable for developing countries and small rural systems, but is applicable only if sufficient land is available. On the other hand, the filters are readily clogged by algal blooms and do not remove heavy metals and many micropollutants efficiently. They effectively remove biodegradable organic carbon and oxidize ammonia.

Infiltration

Surface water can also be treated by infiltration of the raw or partly treated water into river banks or sand dunes, followed by underground passage; this is an effective means of removing undesirable microorganisms and viruses. Infiltration is applicable only in areas where suitable geological conditions exist. Pretreatment is required to prevent clogging of the infiltration area. In addition, water abstracted from the aquifer usually needs some additional treatment, such as aeration and filtration, to remove, e.g. iron and manganese present in anaerobic groundwater. The residence time underground should be as long as possible to obtain a water comparable in quality to groundwater.

Disinfection

The overall objective of disinfection is to ensure that the quality criteria are always met.

Terminal disinfection of piped drinking-water supplies is of paramount importance and is almost universal, since it is the final barrier to the transmission of waterborne bacterial and viral diseases. Although chlorine and hypochlorite are most often used, water may also be disinfected with chloramines, chlorine dioxide, ozone, and ultraviolet irradiation.

The efficacy of any disinfection process will depend on the degree of purity achieved by prior treatment, as disinfectants are highly active and will be neutralized to a greater or lesser extent by organic matter and readily oxidizable compounds in water. Microorganisms that are aggregated or adsorbed on to particulate matter will also be partly protected from disinfection. It is therefore recommended that the median turbidity of water before disinfection should not exceed 1 NTU; it should not exceed 5 NTU in any individual sample.

Treatment of groundwater

Groundwater extracted from well protected aquifers is usually free from pathogenic microorganisms, and the distribution of such groundwater without treatment is common practice in many countries. However, the catchment area must be protected by effective regulatory measures and the distribution system adequately protected against secondary contamination of the drinking-water. If the water, in its passage from source to consumer, cannot be protected at all times, disinfection and the maintenance of adequate chlorine residuals are imperative.

Treatment of surface water

The extent to which faecal bacteria, viruses, and parasites are removed by properly designed and operated equipment for flocculation, coagulation, sedimentation, and rapid filtration is equivalent to that achieved by slow sand filtration. Additional treatment, such as ozonation, will have a considerable disinfecting action besides converting part of the total organic carbon into a biodegradable form. If it is followed by activated carbon treatment or other biological filtration stage, some of the biodegradable organic carbon will be removed by microbial activity, thus reducing the potential for aftergrowth of nuisance bacteria in distribution networks.

ENVIRONMENTAL PATHOGENS

1. **Overview**

❖ Environmental pathogens are defined as micro-organisms that normally spend a substantial part of their lifecycle outside human host, but when introduced to humans cause diseases with measurable frequency.

❖ They are borne in water, soil, air, food and other elements of our surroundings and they affect almost every individual on the planet.

❖ They have the ability to survive and thrive outside the host which is not affected.

❖ They move from the environment into the host (i.e. from outside to inside) and cause diseases.

❖ They cause diseases in normal or immuno-compromised individual when acquired from the environment.

❖ They have adverse effects on human health and productivity.

❖ They can infect scores of individual from a single source.

❖ They do not necessarily rely on infection y person-to-person contact or engineered dispersal as is the case with other pathogen.

❖ The key difference between environmental pathogens and other human pathogens is their ability to survive and thrive outside the host.

2. Range of environment in which environmental pathogens can be found

S/n	Environment	Organisms
1.	**Aquatic** Drinking water supplies, recreational water (including fresh water, seawater, and even chlorinated system), wastewater, air conditioning cooling towers, recirculating hot water systems, and other water system.	*Legionella pneumophila, Mycobacterium avium complex* (MAC)*, Vibrio cholerae*
2.	**Food** Food harvesting, processing, handling and livestock feeding practices can introduce new routes of infection and new pathogen into food supplies.	*Listeria monocytogenes, E. coli O157:H7, Campylobacter jejuni*
3.	**Soil**	*Histoplasma, Coccidioides, Aspergillus fumigatus*
4.	**Air** Pathogens in soil and other environment may become airborne and can be found in indoor or outdoor air.	
5.	**Vectors** Organisms that play an active role in transmitting a pathogen e.g. biting insects (including mosquitoes, fleas, and ticks). Arthropod vectors e.g. mites, sand flies, and mosquitoes.	West Nile Virus, Plague, Lyme disease (*Borellia burgdorferi*)
6.	**Living reservoir** Organisms in which a pathogen lives and multiplies without damaging the host. Reservoir may include vectors or may include organisms that do not actively participate in transmission of the disease to humans. E.g. Beaver	*Giardia lamblia*
7.	Products of human activity including dwellings and fomites (objects on which pathogens are deposited and transmitted to humans).	

3. Examples Of Disease and Major Clinical Signs of Infection Caused By Environmental Pathogen

	Pathogen	Disease	Clinical Signs
Bacteria	*Vibrio cholerae*	Cholera	Diarrhea
	Vibrio parahaemolyticus	Gastroenteritis; wound infection; septicaemia	Diarrhea, abdominal pain, nausea, vomiting, headache, fever
	Campylobacter species	Gastroenteritis	Diarrhea
	Mycobacterium avium complex (MAC)	Pulmonary, localized, or disseminated MAC	Lymphadenitis and tuberculosis-like disease
	Coxsiella burnetti	Q-fever	Pneumonia
	Legionella speices	Legionnaires' disease	Pneumonia
	Rickettsia typhi	Endemic typhus	Fever
	Bartonella henslei	Cat scratch disease	Swollen lymph nodes, fever
	Borrelia burgorferi	Lyme diseases	Fever, rash, arthritis
	Listeria monocytogenes	Listeriosis	Gastroenteritis
	Bacillus anthracis	Anthrax	Fever, respiratory illness
	Burkholderia cepacia		Pneumonia
Viruses	SARS Virus	Severe Acute Respiratory Syndrome	Pneumonia
	Influenza viruses	Flu	Fever, cough, headache
	West Nile Viruses	West Nile fever, encephalitis	Fever, headache, altered mental status
	Hantavirus	Hantavirus pulmonary syndrome or haemorrhagic fever	Cardiopulmonary or renal malfunction
Eukaryotes	*Histoplasma capsulatum*	Histoplasmosis	Pneumonia
	Coccidioides immitis/posadasli	Valley fever	Pneumonia, chest pain, fever, cough, malaise
	Cryptosporidium parvum	Cryptosporidiosis	Diarrhea
	Cryptococccus neoformans	Cryptococcosis	Meningitis, fever, headache

4.	**Incidence and Epidemiology of Environmental Diseases**

❖	Surveillance is the key to understanding the incidence and epidemiology of environmental diseases. From a combined approach of active and passive surveillance and point prevalent studies.

❖	Epidemiological surveillance (Systematic collection of the number of cases of human of human or animal diseases).

❖	Microbiological surveillance (Determining the number of a given organism in the environment). Surveillance should be targeted to specific environments at specific times.

❖	Virulence factors determination (types and levels). This distinguishes between pathogenic and non pathogenic strains, degree of virulence/Virulence Factor Activity Relationships (VFAR). Virulence can be studied by combining phenotyping and genotyping.

5.	**Climatic change and environmental Diseases**

•	Linking weather events to changes in incidence (correlations).

•	Climatological data (precipitation, airborne particulates, surface water temperature, water pH and dissolved oxygen) and microbiological data (disease incidence and microbiological counts in environmental samples).

•	Remote sensing and Geographical information System (GIS).