CHAPTER 16

MICROBIOLOGICAL ASSESSMENT AND MONITORING OF ENVIRONMENTAL POLLUTANTS

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Introduction

Individuals and governments have shown a great concern about the quality of the environment. Many governmental and non-governmental organisations are committed to activities that will reduce the effects of pollution of air, soil and water. Pollution does not only affect man, but also has grave effects on domestic livestock, wildlife, climate, forests and crops. Pollution management therefore needs to be prioritised by all governments.

In order to have a successful pollution management programme, some basic information about the pollutants is essential. The type(s) of pollutants present, their source, distribution and effects on the environment must be known. The cost of any remedial action must also be considered. It must be appreciated that the list of pollutants is extensive. For instance over 1,500 pollutants have been listed in the "black list" and the "grey list" depending on the levels of toxicity. (Mason 1991). It is also common to find more than one pollutant in specific environments and therefore effects on the environment could be complex.

Table 16.1: Categories of Pollutants found in Fresh Water
(***Source:*** Mason 1991)

Acids and Alkalis
Anions (eg sulphide, sulphite, cyanide)
Detergents
Domestic Sewage and Farm manures
Food Processing Wastes (including processes
taking place on the farm)
Gases (eg chlorine, ammonia)
Heavy metals (eg cadmium, zinc, lead)
Nutrients (especially phosphates and nitrates)
Oil and Oil Dispersants
Organic Toxic Wastes (eg formaldehydes, phenols)
Pathogens
Pesticides
Polychlorinated Biphenyls (PCBs)
Radionuclides

Table 16.1 shows the various categories of pollutants found in fresh water. These may either be *organic or inorganic* and can be *viable (in the case of microbes) or non-viable* and are caused by various factors.

General: Some Commonly Discussed Causes of Pollution

Some commonly discussed causes of pollution are heat, radionuclides, oil, heavy metals and organochlorines. Their effects on the environment and on living things are widespread.

Heat Pollution

A number of power utility industrial plants (e.g. electricity generating) generate heat which require large volumes of cooling water. This water is taken from the environment and later returned at a much higher temperature. This increase in temperature called thermal pollution changes the density and oxygen concentration of water. This will inevitably alter the biological species. For instance, Blue-green algae will dominate as they are tolerant of these elevated temperatures. Bacterial populations will also change with varying temperatures.

Radionuclide Sources of Pollution

There are various natural sources of radioactivity in nature. In nuclear power stations, water from the environment is used for cooling. This cooling water may acquire some radioactivity which is discharged into water bodies. Disposal of nuclear wastes in the environment also poses risks because disposal sites eventually leak and contaminate surface and ground water. The same may occur as a result of nuclear industry accidents. The occurrence of such radio nuclides may cause acute toxicity or chronic effects to man, fish, aquatic birds and shellfish.

Oil Pollution

The pollution of water bodies by oil affects fish, birds and plants. A number of micro-organisms are stimulated in the presence of oil. The ecology of water ecosystem changes in response to the generally deleterous effects of pollution.

Heavy Metals

Heavy metals are temed conservative pollutants since they are either not broken down or they are, over a long period of time. Examples are mercury, cadmium and lead, which are known to affect human life. Sources of heavy metal pollution are: weathering of rocks, bearing them, volcanic eruptions, fossils fuel, burning, paints, instruments, horticultural sprays. Fish take in heavy metals in their diet or through their gills. Mercury, for instance, accumulates in fish and while there is no apparent disadvantage to the fish, vertebrates (eg birds) that feed on the fish are at risk. Cadmium poisoning is associated with kidney damage in human and lead poisoning affects fish, water fowl and human.

Other Pollutants

Other important pollutants are **organochlorides** such as insecticides and polychlorinated biphenyls (PCBs) that are found in air and water. Toxicity to fish, various invertebrates and mammals has been demonstrated.

Effective Management of Pollution

For *effective management of pollution*, one needs to know the following:

- The substances entering the environment and their quantities, sources, distribution.
- Effects of these substances within the environment.
- Trends in concentrations and effects, and causes of these changes.
- How far these inputs, concentrations, effects and trends can be modified and by what means, at what costs.

A decision one has to make in monitoring the levels of pollution is whether to use biological or non-biological strategies. Biological species respond to intermittent pollution. In most environments, the activity of axenic cultures results in many different intermediates of pollutants. Biological samplers can easily pick these different substances. Chemical sampling programmes could miss these pollutants since they are programmed only for specific pollutants. It would be very costly to programme for all the pollutants available, let alone their intermediate breakdown products.

Normally, effluents contain a variety of pollutants and not single ones. Pollutants occurring together may act completely independently on the target, in that case one may find a dominant pollutant whose effects is most felt by the target; others may have an additive antagonistic or synergistic effects.

Biological Strategies

Once the decision to use Biological strategies to monitor pollution has been made, one has to find an organism that is suitable for bioassays. Such an organism must have the following characteristics:

- The organism must be *sensitive to the material or environmental factors* under consideration;
- It must be *widely distributed and readily available* in good numbers throughout the year;
- It should have *economic, recreational or ecological importance* both locally and nationally;
- it should be *easily cultured* in the laboratory;
- it should be *in good condition, free from parasites or disease;*
- it should be *suitable for bioassay testing.*

Small organisms with short generation times are preferred for bioassays. Fish are also used as they are important, in demonstrating long term effects of exposure to pollutants.

There are two types of bioassays: *biostimulation* and *toxicity tests:*

Biostimulation tests are used, for instance, to evaluate the nutrient status of a water body (eg. with algae), to distinguish between total and available nutrients to organisms and to determine effects of changing water quality on growth.

Toxicity tests on the other hand are used for screening of chemicals to determine the risks associated with various pollutants.

A number of organisms act as *bioaccumulators* (Mitchell, 1992). Pollutants are concentrated in the tissues of organisms. These pollutants can be measured on site. Bioaccumulators can be placed in the environment and rates of uptake can be measured over a period of time. Pollution levels at different sites and at different times of the year can be determined. There are various examples of bioaccumulators.

- **Mosses** are used to monitor heavy metals such as lead, copper cadmium;
- **Bivalves** have been used to monitor pesticide pollution by dieldrin.;
- **Eels** accumulate mercury and can be used for monitoring its levels.

Microorganism Interaction with Pollutants

Micro-organisms interact with pollutants in many ways (Yuerrero and Pedros-Alio 1992 and Mason, 1991). Toxic metal wastes in water are detrimental to health. Such metals tend to get concentrated in cells of various micro-organisms, such as fish and micro-organisms. There are also several microbe-metal interactions that enable us to use microorganisms in bioassays (Bitton, 1994):

- **Intracellular accumulation:** metals are found concentrated within microbial cells.
- **Cell Wall Associated Metals:** this is due to the presence of specific functional groups.
- **Metal Siderophore Interactions:** these are excreted by microbes and have strong affinity to iron. Some bind to copper and molybdenum.
- **Extracellular Mobilisation/Immobilisation** of metals by bacterial metabolites resulting in leaching of metals as a result of acid metabolite production by microbes or through metal oxidation.
- **Extracellular Polymer-metal Interactions:** extracellular polysaccharides produced by microbes may bind strongly to some metals.
- **Transformation and volatilisation of metals:** eg formation of elemental mercury.

Pollutants may be detoxified by physiochemical or microbiological processes. Microbes are involved in transformations that reduce the effects of pollutants in the environment by *bioleaching, biosorption and bioprecipitation.* Microbes do this for the following reasons:

- To satisfy their nutritional requirements;
- To satisfy energy requirements;
- to detoxify their immediate environment as part of metabolic processes; and
- Physical/chemical characteristics of microbial cell may offer no advantage to microbial population.

Examples of Physiochemical or Microbiological Activities

Examples of these activities inlude the following:

- *Cyanobacteria* - base metals;
- *Algae (chlorella)* - base metals, lead, precious metals;
- *Fungi* - radionuclides; and
- *Pseudomonas fluorescence* - chromate;
- *Alternaria (fungus)* - selenium;
- Non-living bacterial, fungal, lead, zinc, nickel, cobalt, algal biomass;
- *Thiobacillus thiooxidants*-iron chromium (leaching by H2SO4); and
- *Thiobacillus ferroxidants* - pyrite from coal (oxidation)

Biological assessment of water quality may be done in the field or in the laboratory.

In the field, this is done in three stages (Mason, 1991):

- **Surveys:** a programme of measurements that defines a pattern of variation of a parameter in space eg. measuring the level of a heavy metal 'X' present in effluent by determining its level in a sample gives information at a particular time.

- **Surveillance:** repeated measurements of a variable to establish a trend, eg. measuring for heavy metal 'X' in the effluent over a period of time.

- **Monitoring:** a policy for managing pollution might be put in place to lower the level of metal 'X' by some percentage. One must make observations of whether the reduction it adheres to standards.

Examples of Indicator Organisms

In the laboratory, one uses intrusive micro-organisms. For instance, in the monitoring of pathogens in water there is a heavy reliance on laboratory analysis. Several pathogenic microbes are associated with wastewater. The origin of these microbes are mainly faeces. These bacteria are grouped as follows:-

- Gram-negative facultatively anaerobic bacteria (eg *Vibrio, Escherichia, Klebsiella, Shigella*);
- Gram-negative aerobic bacteria (eg *Pseudomonas, Flavobacterium*);
- Gram-positive spore forming bacteria (eg *Bacillus*); and
- Non spore forming gram-positive bacteria (eg *Arthrobacter, Corynebacterium*).

A number of these micro-organisms cause diseases such as typhoid fever, cholera, *shigellosis, gastroenteritis*).

Proper sampling is important for accurate monitoring of water bodies:

- **Containers:** Samples are collected in bottles that have been cleansed and rinsed carefully. Final rinse should be with distilled water before sterilisation.

- **Dechlorination:** Add a reducing agent to containers intended for collection of water or wastewater effluents having residual chlorine or other halogens. Sodium thiosulphate neutralises residual halogens and prevents continued bactericidal action during sample transit.

- **Sampling:** Leave enough space in the bottle when sampling to enable adequate shaking of sample before examination. Samples should be representative (use sampling locations at critical sites). Sampling frequency is also critical. One can use manual sampling or sampling apparatus.

- **Size of sample:** The volume of sample should be adequate to carry out all tests. The normal size is 100 ml;

- **Sample identification**: Samples must be properly identified (location of sample, date and time of collection etc).

Proper laboratory analysis is also as important as sampling. One has to decide on which method of analysis to use: a heterotrophic plate count is done using either the pour plate or spread plate method. Sample should give 30-300 CFU/Plate (using a Quebec colony counter for manual counting or automatic plate counting instruments that use a television scanner coupled to a magnifying lens and an electronics package). One can use general, selective or differential media, to identify specific microorganisms.

- **Direct total microbial count** are obtained from counting stained cells under the microscope. Counts include viable and non-viable counts, and therefore tend to be exaggerated.

- **Membrane filter method** (MF): This method uses a microbiological filter. Large volumes of water are filtered through 47 mm, 45 m gridded MF, under partial vacuum. The filter is later placed on agar in a petri dish, incubated and the colonies that develop are counted.

- The most probable number method is used routinely in water analysis. Normally, this test is done in three steps, the presumptive, confirmed and completed test. Most Probable Numbers (MPN) tables are used to read the results of the three tube or five tube method. The MPN tends to give higher counts than other methods.

Table 2.2: Examples of Indicator Mechanisms

Indicators	Examples	Purpose	Character
Total Coliforms	*E.coli* Enterobacter Klebsiella	Determining Quality of potable water recreational waters	Aerobic & Facultative. Anaerobic gram negative, Rod-shaped bacteria, Ferment lactose with gas production within 48 Hrs @ 35°C
Faecal Coliforms	*E.coli* Klebsiella Pneumonia	Presence of faecal material from warm blooded organisms.	Ferment lactose at 44°C
Faecal Streptococci	Streptococcus Faccalis *S.bovis* *S.equinus* *S.Aviu*m	Faecal contamination in *water*	
Anaerobic Bacteria	Costridium Perfringens	Indicator of past pollution tracer to follow fate of pathogens eg faecal pollutions in marine environments	Gram-positive spore forming rod pores are resistant to many environmental stress
Bacterio-phages	Colihages	Water quality indicators in estuarie, estuaries, seawater, recreational water, potable water. Provides information of water treatment process such as coagulation, flocculation sand filtration absorption to activated carbon & disinfection.	

	Androphages	Index of Contamination.	Resists disinfection Present in high numbers.
Yeasts and Acid Fast Organisms	Mycobacterium Fortuitum M. phlei	Indicators of Disinfection, Efficiency.	Resistant to chlorine/ ozone than *E.coli.*
Heterotrophic plate count	Pseudomonas Aeromonas Klebsiella Flavobacterium Proteus Citrobacter	Sensitive for the removal & activators of microbial pathogens in wastewater.	Influenced by temperature chlorine residual assimilable organic matter.

When monitoring pathogens in water, indicator organisms are used. (Table 2 2). Micro-organisms may be used as indicators for the following reasons:

- ❑ Occurrence of faecal contamination;
- ❑ Efficiency of the treatment process;
- ❑ Efficiency of wastewater treatment plants; and
- ❑ Post process contamination of water during distribution.

For an organism to be used as an indicator in water analysis, it must satisfy the following criteria:

- ❑ It should be a *member of the intestinal microflora* or warm blooded animals.
- ❑ It should be *present when pathogens are present* and absent in uncontaminated samples.
- ❑ It should be *present in greater numbers than the pathogens.*
- ❑ It should be at least *equally resistant as the pathogen* to environmental insults and to disinfection in water and wastewater treatment plants.
- ❑ It should *not multiply in the environment.*
- ❑ it should *be detectable by means of easy rapid and inexpensive methods.*
- ❑ The indicator organisms *should be non pathogenic.*

Chemical Indicators of Water Quality

Some laboratories use chemical indicators to determine if a water body is polluted. Table 16.3 shows some chemicals that are used as indicators.

Table 16.3: Chemical Indicators of Water Quality

Indicators	Examples	Purpose
Faecal sterols	Coprostanol Coprosterol Cholesterol Coprostanone	Indicate faecal contamination

Total coliforms are detected by the most probable numbers (MPN) technique or by the membrane filtration technique. The MPN generally overestimates the coliform numbers. The recovery of coliforms depends on: type of medium, the diluent and membrane filter used, presence of non coliforms, turbidity, occurrence of injured bacteria in environmental samples (due to physical, chemical or biological factors).

Enzymatic Assays

The assays are specific, sensitive and rapid.

1) **Galactosidase Activity**

Galactosidase activity is deployed for total coliforms in water and wastewater. Based on the hydrolysis of o-nitrophenyl - B-D-galactopyranoside (ONPG) to yellow nitrophenol.

2) **Glucuronidase Activity**

Glucuronidase activity is deployed for detection of *E.coli.* The method is based on the hydrolysis of a fluorogenic substance. 4-methyllumbelliferone glucuronide (MUG) by glucuronidase, an enzyme found in *E.coli.* End products are fluorescent and are detected using UV light.

Monoclonal antibodies: *E.coli* can be detected by using **monoclonal antibodies** against outer membrane proteins or against phosphatase in the periplasmic space. This technique still needs to be developed further.

Polymerase Chain Reaction (PCR)/Gene Probe Detection: This is a very sensitive method. Specific genes of *E. coli* are amplified by the PCR and subsequently detected with a gene probe. (one can detect 1-5 cells/100ml water).

When one uses the MPN method, a change in the fermentation medium and temperature of incubation, can select for specific indicators. This method is simple and can be relied upon.

In many laboratories in developing countries, the methods that are used for monitoring faecal pollutants in water are: the HPC, MF and the MPN. These can be performed with basic equipment and when carefully done, they give reproducible results. They are therefore within the reach of most laboratories in the developing countries.

References

1. Bilton G. *Wastewater Microbiology.* Wiley-liss (1994).

2. Guerrero R. and Pedros-Aloi (Eds). *Trends in Microbial Ecology* (1992).

3. Mason C.F. *Biology of Freshwater Pollution* 2nd Ed. Longman Scientific and Technical (1991).

4. Mitchell R. *Environmental Microbiology*, Wiley-Liss (1992).

5. Stoner D.E. (Ed). *Biotechnology for the Treatment of Hazardous Waste* CRC Press (1993).