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on
Aquatic Environment Impact Assessment

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FOREWORD

Natural resources, for the past few decades, are being continuously being brutally exploited to provide for necessities, luxury and comfort to the growing populace. Aquatic resources are the worst sufferers in this regard, being the *ultimate sinks* of all environmental aberrations. The waste products generated on account of cultural eutrophication are directly discharged into the aquatic system, endangering the health of its denizens. This phenomenon has lead to accumulation of toxic substances, heavy metals and non-biodegradable chemicals, besides unabated organic loading of the system, affecting its biotic health. Siltation, increased water abstraction and river course modification have further compounded the problem. These unhealthy practices have lead to a significant shift in the biotic texture of the aquatic environment with gradual dominance of economically less preferred species over the desired ones.

The required increase in production and productivity on a sustainable basis in future requires a sense of caution at every level of production and development activities. We need set ecologically motivated priorities for the same to *anticipate and prevent* rather than *react and cure*. Environmental impact assessment studies are a tool to arrive at the required priority of anticipation and prevention.

Central Inland Capture Fisheries Research Institute, as a result of its work during the last decade, has been able to evolve technology of Environmental Impact Assessment of various types of changes being commonly encountered in aquatic environment in the country. A major impediment in the environmental impact assessment of the aquatic resources has been non-availability of the trained manpower. The present training program for scientists/ development workers/ administrators is a step towards overcoming the said impediment. This manual is a compilation of lectures delivered at the said training program. It is hoped that this would be of use to the participants as well as others concerned with development of aquatic resources.

M. Sinha
DIRECTOR

NEED FOR AQUATIC ENVIRONMENT IMPACT STUDIES

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Environmental perturbations are global phenomena affecting the biodiversity and production functions of all types of ecosystems. In recent years the alarm of environmental degradation has attracted the attention of experts and planners alike. The process of environmental degradation is mainly a function of various man-induced activities and is transboundary in nature. To be precise, it is a period of rapid change in population, economy and environment, increasing gap between rich and poor and increasing disequilibria between the growth and the capacity of the environment to sustain it. The world has entered an era of fast and rapid transformation of *population, production* and *consumption* of raw materials, energy and even culture. In such a scenario, the realisation that the current pace of economic growth would be impossible to sustain is emerging very fast. This is specially so in developed, or developing, countries as they have exploited, or are in the process of exploiting, the natural ecosystem too brutally to purchase extra luxury and comfort for their populace resulting in environmental mismanagement in general, which can lead to various health problems and productivity loss (Table 1).

The aquatic ecosystems, acting as the *ultimate sinks* of almost all environmental aberrations, can be considered as the mirrors of environmental degradations following various anthropogenic activities. The drainage basins as a whole, and the rivers in particular, are being devastated by intensive human activities. River Ganga, the lifeline of our cultural ethos, for instance, has brutally been assaulted to the extent that, barring the region around headwater, it has almost lost its originality in terms of ecological intricacies. The production functions, including fish and fisheries, the major produce; indicate a declining trend at a very rapid pace. A significant shift in the biotic texture, at various trophic levels, has taken place with gradual dominance of less preferred species over the desired ones. The civil construction in and around the rivers have contributed a lot in disrupting the normal grazing chains and breeding behaviour of fish species due to alarming alterations of their niche. Rivers in general have been converted into a receiving pots for all kind of waste, be it industrial or municipal, which in turn have vitiated the water quality and biota to the extent of irreparable state in certain stretches, and some streams have already been converted into sewage channels. The gradual accumulation of toxic substances, heavy metals and non-biodegradable chemicals in particular, besides unabated organic loading in the system, have assumed serious proportions as the danger of their entry in human body is lurking large. The elaborate base-line data on various aspects of environmental variables, generated by CIFRI through its sustained and regular monitoring of the river systems in the country, indicate that cellular and organ level damages in fish species is on the increase, threatening the fish as well as its consumers.

Natural enrichment of nutrients from allochthonous sources and aging of lakes are common phenomena, but in recent years the process has been accelerated on account of so many man induced interferences resulting in eutrophication of lakes, tanks and floodplain wetlands. The degree of eutrophication, however, varies depending on nature and quantum of waste influx in the systems. Eutrophication is also a type of pollution due to excessive loading of organic matter, which has all the potentialities to upset the ecological balance of an ecosystem, as observed in floodplain lakes in Ganga and Brahmaputra basins. It is not a welcome development from biodiversity and fisheries point of view.

The salient effect of environmental perturbations in aquatic environment can be summarised as under:

- The symptoms of biodiversity loss are emerging very strongly with appreciable shift in community size and structure towards lesser economically valued species.

- Reduced flow rate and water volume on account of dams/barrages, increased water abstraction and increased sedimentation have been found detrimental for all types of aquatic biota, including fish.

- Gradual transformation of benthic niche from mollusc dominance to chironomids and oligochaete abundance with the new recruitment of polychaetes in certain stretches is indicative of increased pollution load.

- Cellular and organ level deformities in fish species have become common, especially at stress points, due to accumulation of heavy metals/pesticides.

The expected increase in population of the country by the turn of the century would need considerable increase in productivity as well as production from all available resources on a sustainable basis. This is quite a contrasting situation in view of the above said present alarming state of environment, both terrestrial and aquatic. The required increase in production and productivity on a sustainable basis can only be possible with improvement in the state of terrestrial and aquatic environment. This would require a holistic effort towards utilisation of available resources. It would also require a sense of caution at every level of production and development activities for which we have to set ecologically motivated priorities, preferably ***to anticipate and prevent*** and not ***react and cure***.

Environmental impact studies are essential for this required priority of ***anticipation*** and ***prevention***. Until and unless such impact studies are done to collect the required information, it would not be possible to formulate policies for prevention or cure of the present or likely factors of environmental perturbations leading to habitat destruction. Since environmental factors have both synergistic and antagonistic effects, the impact study need also be taken up on similar lines.

TABLE : Principal Health and Productivity Consequences of Environmental Mismanagement

Environmental Problem	Effect on health	Effect on productivity
Air Pollution	Many acute and chronic health impacts: excessive levels of urban particulate matters responsible for 3 to 7 lakh premature deaths annually and for half of childhood chronic coughing;400 to 700 million people, mainly women and children in poor rural areas, are affected by smoky indoor air.	Restrictions on vehicles and industrial activity during critical episodes; effect of acid rain on forests, bodies of water and human artifacts.
Atmospheric changes	Possible shift in vector borne diseases; risks from climatic natural disasters; diseases attributable to ozone depletion (more3 lakh cases of skin cancers and 1.7 million cases of catract per year)	Sea rise damage to coastal investments; regional changes in agricultural productivity; disruption of aquatic food chain.
Solid and hazardous wastes	Diseases spread by rotting garbage and blocked drains. Risks from hazardous wastes are typically local, but often acute.	Pollution of groundwater resources
Deforestation causing siltation of rivers	Localized flooding, leading to death and disease.	Reduced fertility, loss of watershed stability and carbon storage by forests. Loss of nontimber forest products
Water pollution and water scarcity	More than 2 million deaths and billions of illnesses a year are attributable to water pollution; poor household hygiene and added health risks are caused by water scarcity.	Declining fisheries; rural household time (time spent in fetching water) and municipal costs of providing safe water; depletion of acquifers leading to irreversible compaction constraint on economic activities because water shortage.
Soil degradation	Reduced nutrition for poor farmers; greater susceptibility to drought.	Field productivity losses.
Loss of biodiversity	Potential loss of new drugs.	Reduction of ecosystem adaptability and loss genetic resources.

RIVER WATER QUALITY MONITORING IN CONTEXT OF INDIAN SCENARIO

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Introduction

Our understanding of the riverine system are far less adequate as compared to that of the lentic habitats. Our understganding of the Indian riverine systems are far from satisfactory, most of them confined to limited aspects. Most riverine and stream systems have had their flow regimes altgered by man. The foremost cause is due to forest clearance that alters the pattern of stream discharge. Such changes determine the flow pattern of rivers and streams and their beds are fundamental to most of the properties of biotic habitats in running waters. These aspects of river ecology are still largely neglected in biological studies. Apart from the increasing load of solids, through surface industrial effluents and land runoff the riverine systems also receive toxic and hazardous substances, such as pesticides, metals, polychlorinated biphenyl and other chemicals inimical to aquatic life. The problem of pollution in the inland open water is attaining alarming dimensions. During the last five decades the development of water resources for irrigation, generation of electricity, potable water supplies and industrial use has been the main thrust of our planning process. These ecological transformations have compounded their eco-degradation and threaten the productive cycles of the riverine systems. The deteriorating condition of the aquatic habitats is discernible from the declining yields from these resources. The fish yield have declined by 25-75% at various locations as compared to yields during 1960s and 1970s.

Riverine Resources

India has open water resources in the form of extensive Coastal lines, river systems, and an assortment of inland water bodies like, ponds, tanks, lakes, reservoirs, Oxbow lakes etc. The riverine resources have a total length of 45,000 Km.,. The Ganga river systems with its main tributaries like, Yamuna, Ganga, Ram Ganga, Ghagra, Gomtri, Kosi, Gandak, Chambal and Sone have combined length of 12,500 Km. The Brahmputtra in the east (4073 Km), Peninsular rivers like Mahanadi, Godavari, Krishna and Cauvery (6437 Km.) and Narmada & Tapti (3380 Km.), in the west and the other major river systems. Indian rivers carry a surface runoff of 167.23 mhm which is 5.6% ie., $1/8^{th}$ of the total runoff flowing in all the rivers of the world. The major rivers and their traverse through varied geo-climatic zones displaying a high diversity in their biotic and abiotic characteristics throughout their 28,000 Km., linear drift. Dotted with flood plains, Oxbow lakes, Quiescent backwaters, and interspersed deep pools, the rivers possess a mosaic of varying biotope raning from lotic to lentic habitats. A large number of man-made lakes have been created on different river basins for irrigation, hydropower, flood control, and industrial uses. Total water spread area under reservoir stand 3 million ha., and is expected to be doubled by 2000 AD. Recently 975 major reservoirs in the country (1000-10,000 ha) have been identified to cover an area of 1.7 million ha. All these resources offer immense scope and potential for developing the capture fisheries.

The river systems on the basis of source of water can be classified. The rivers which are fed by snow-melt tend to be high in spring. The glacier fed streams flow out at a high level in summer. The cold winter results in low discharges. Large rivers representing a summation of many mono or local effects show regularity than small ones. Because greater river require a large amount of water to alter their pattern of discharge. The pattern of discharge is dependent upon local climate, pattern of precipitation, evaporation and other related factors. Such differences in small and large systems can have important biological consequences. The temperature of rivers and streams vary much more rapidly than those in lakes but quite often over a much smaller range.

Status of Riverine Fisheries

The Ganga, Bramhaputtra, Godavari and Krishna the fish yield varied from 0.64 to 1.6 t per Km. Some disturbing trends are already discernible in riverine fisheries of the country., especially the Ganga. A number of man made changes in the riverine habitats due to large scale water abstraction for irrigation, construction of dams and barrages, siltation, soil erosion due to deforestation in the catchment area, and water pollution from industrial, agricultural and municipal wastes have all had devastating effects on the fish stocks of Indian rivers. Apart from a steady decline in total fish and spawn yield of the prized Indian major carps and the hilsa, there is an alarming swing in the population structure of the Gangetic Carps. The biologically and economically desirable species have started giving way to low value species. The anadromous hilsa, bountiful til 1972 at all centres above Farakka barrage in the lower Ganga has touched an all times low level due to obstruction on its up river migration created by the barrage. Large reservoirs increasingly becoming a characteristics component of riverine basins. A large number of man-made lakes are created in the country on different river basins for irrigation, power generation, flood control, and the industrial uses. These water sheets by virtue of their sheer magnitude, constitute an important Caputre Fisheries resources for the country. According to the one estimate total water spread under reservoirs which stood at 3 m ha., during the 70s is expected to swell to 6m. ha., by 2000 AD. There are 975 major reserovirs in the country in a size range of 1000-10,0000 ha., covering an area of 1.7 m. ha. Indian reservoirs on an average, produce a meager 14.5 kg/ha from the large class of reserovirs and above 100 kg/ha from the smaller ones.

Reserovirs alter river hydrology both up and down streams creating a new artificial aquatic environment. The quality of reserovirs water varies from watershed depending on soil, man's activities and climatic conditions. It also varies with the shape of the reserovirs basin, exposure to light and wind action and the amount of waste exchange. Due to these variables productivity of reserovirs can be estimated. Man's dependence on water streams from the premises of navigation, amenity, recreation, fishing, power generation and abstraction for domestic and industrial supplies. An accelerated pace of development in all these spheres over the decades have exerted pressure on the available water resources and the society has become more and more concerned about the need for conserving the pristine ambience of this vital resource. The water quality assumes a greater significance in the context of aquatic ecosystems where the harvestable commodities are products of the complex processes of community metabolism that depends directly or indirectly on water quality.

Water Quality and Environmental Constriants

The water quality is mainly deteriorated due to anthropogenic stresses. Mainly some of the man-induced stresses which adversely affect water quality vis-a-vis fishery activities are; over abstraction of water, siltation of river beds, and pollution due to urban, industrial and agricultural runoff. The stress exerted by these developmental processes culminate in mortality of fish and fish food organisms, destruction of breeding grounds and impediments in migration, instances of which are well, documented. Mostly following general classification are responsible for deterioration of aquatic environment;

1. Sewage wastes.
2. Industrial Wastes.
3. Land Erosion.
4. Agricultural Drainage.

Effects of some of the environmental constraints on water quality and the biotic communities are given in Trable 1.

Constraints	Source	Effects
Nutrient Loading	Fertilizer, Sewage	Algal blooms, marine life destruction
Chlroinated hydrocarbons	Agricultural runoff, Industrial wastes	Contaminated and diseased finfish, shellfish
Pesticides (DDT, PCBs etc) Petroleum hydrocarbon	Oil-spill, industrial discharge, urban runoff	Ecosystem destruction
Heavy Metals: As, Hg, Cd	Industrial wastes, mining	Diseased and Contaminated fishes
Cu, Pb, Zn Silt Load/Particulate matter	Soil erosion, poor basin management, flyash	Smoothers benthic communities, destroys Juveniles stages of finfish & Shellfish, affects recruitment, block light needed by aquatic flora.
Plastic litter	household wastes	Strangles and destorys natural habitats
Temperature	Thermal Plants	Fish kill, destruction of other biota, eutrophication
Reduced flow rates	Water abstraction	Acclerration of sedimentation rate, destruction of breeding grounds impeding migration irriational fishing

Relationship of Water Quality vs Fish & Environment

The industrial and municipal effluents are as divergent as they are obnoxious. There are number of harmful chemical toxicants emanating from different industrial units, their nature vary depending on products, production processes and materials used. Agricultural runoff carries heavy load of non-biodegradable pesticides. Domestic waste also contain a variety of chemicals, detergents, and organic laod. To protect the ecosystem from gradual degradation, we must have criteria that will protect the entire life cycle of the desirable species as well as the food chain on which these species depend. The validity of applying safe concerntration limits is rather limitted, as they are determined under controlled laboratory conditions. Some fish kills incidences from Indian rivers are depicted in Table 2.

Table 2. Some fish kill incidence in Indian rivers due to water quality deterioration

Place	Year	Pollutants
R. Gomti	1983,84,86	Distillery waste
R. Chaliyar	1974	Pesticide
R. Tungabhadra, Harihar	1984	Rayon Polyfibre
R. Ganga	1981	Fertilizer effluent
R. Ganga, Monghyr	1968	Oil Refinery
R. Adyar, Madras	1981,82	Tannery
R. Rend Rihand Reservoir	1970, 78, 80	Chemical, & thermal effluent

Impact of Indusrtrial Effluents

Various industries in India consume nearly 10 billion cubic meter of fresh water annually. The water requirements of different industries are depicted in Table 3. Mostly the same amount of water comes in the form of wastewater. The current practices adopted for disposal of industrial waste include discharge into public sewers, rivers, reserovirs or goes through creeks and estuaries with little or no treatment. This is evident from the fact that most of the regions with industrial activities have become the foci of pollution. There are reports of servere pollution in the river Hooghly. Ganga at Kanpur & Allahabad, Yamuna at Delhi, Kalu near Bombay. Pollution due to industrial effluents have also been observed in some reserovirs and lakes.

Table 3. Water requirement for some indusrtries in India

Industry	Volume of water used
Viscose rayon	16001/Kg fibre
Pulp & Paper	270-450 1/kg Paper
Tannery	40-50 l/Kg hide
Cotton Textile	20-70 l/Kg Cloth

Integrated Steel Mill	20-50 l/Kg Steel
Distillery	20 l/Alcohol
Sugar	15-40 l/kg Sugar
Dairy	6-10 l/Kg Milk
Urea	6-8 l/Kg Urea
Coke Oven	1.5-2.0 l/Kg Coal

Toxic and hazardous substances such as pesticide and metals are carried to the aquatic systems through sewage, industrial effluents and urban & agricultural runoff. The potential for accumulation of toxic substances within tissues increases the significance of certain pollutants which may be present in water at very low levels. Even the traces of these xenobiotic substances effect the growth and reproduction cycles of the majority of aquatic animals. Such a situation results in low fish output and facilitates transport of metals and pesticide residue to consume through the contaminated fish. The range of potential toxic substances include poisons, metals and pesticides have the greatest potential for bioaccumulation.

Metals

Metal pollution in fishes are in significant evidence in our country. Various workers have reported accumulation of metals in fishes in India. High Zinc bearing wastes from a rayon factory have been found to cause complete wiping out of molluscan population in the discharge area in river Tungabhadra. Biomagnification by 14.755 times in the kidney and 7.340 times in gonads of *Rita rita* and 4.300 times in molluscan and 1.4000 times in crabs have been reportred from the Hooghly widely differing toxicity depending upon its speciation. In combination metals such as zinc, copper and chromium have shown higher toxicity.

Heavy metals are stable and non-biodegradable. Therefore, unlike the other forms pollutants they not only linger in the ecosystem, but also get passed on to the living tissues in increased concentrations through biomagnification. The ecological implications of such residues are manifold. Apart from posing a public health hazard for the fish caters, heavy metal accumulation in the tissues of fishes and other organisms causes physiological disorders such as necrosis of liver, damage of nephrons in kidney, haemotological abberations, decline in growth rate, and fecundity, and enlargement of gall bladder. Fish food organisms such as Cyclops, and Daphnia are more sensitive to metals like, Zn. Presence of persistent pollutants in the water course not only creates unfavourable environment for fish, but also causes paucity of fish food organism. Zn, Cu and Cr in combination are many times more toxic to fish as compared to their inddividual toxicity. Retarded growth, anaemia and delayed reproduction of fish have been noticed at an exposure of 2 ppm of Zn, Cu, and Cr in combination for 120 days.

Pesticide

Many workers have reported presence of pesticides in alarming range in the fish and fish food organisms. Nearly 1,000 and 1,300 ppb and 1.300 ppb of BHC and methyl parathion in water in the river Cauvery near Srirangapatnam in Mysore and 20-200 ppb of BHC in drinking water in the Hassan district of Karnataka have been reported. BHC in Aranyar reservoir and

neary by ponds in Chitoor district of Andhra Pradesh have also shown presence of pesticide in significant amounts. A report on river yamuna indicates presence of DDT residues in water 90.602-3.416 ppb) and fish (0.059-7.575 ppm) near Delhi. Although significant residues of DDT have not been dected in water in the Hooghly estuary near Calcutta its presence has been dected in sediments (17-89 ppb), molluscs (65-953 ppb), fish (31-460 ppb) and plankton (15-130 ppb). DDT has been biomagnified by plankton, fish gastropods and biavalves by more than 2,550, 7,500, 3,660 and 15,800 times of its ambient level in water. It has also been reported that pesticides such as BHC, Endosulfan, Methyl Parathion in water and sediments of fish ponds in the Sunderban regions of West Bengal are also present in alarming concentrations.

The observations of many workers revealed that fish food organisms such as plankton and benthos are very sensitive to these chemicals as compared to fish. Thus their presence in aquatic ecosystems not only affects the fish directly, but also adversely affects the availability of fish food organisms. Most of the commonly used pesticides viz, DDT, BHC, Endosulfan, Ethyl Parathion,. Methy l Parathion, Dimethoate, Phosphomidon, Quinalphos, Carbryl have been screened to evaluate their toxicity to fish, pra;wn, and fish food organism. Enzyme activity (acid phosphatase) was markedly reduced in those fishes. There has been significant decline in growth and reduction of RBC count, HB and PCV in the fishes exposed to BHC. Organic mercury compounds particularly the alkyl mercury ones are extremely dangerous as they are more stable and remain accumulated in the fish tissues for longer time (biological half life: 86-435 days). They affect the nervous system and lead to crippling diseases in fish and ultimately in man. Crude oil and oil fractions may form coating over the gills leading to direct fish kills. Phenolic substances discharged from petro-chemical complexes and washings from coal mines may cause paralysis of nervous system and cardio-vascular congestion of fish. Chlorinated hydrocarbons which persist longer time in water gets accumulated progressively in different steps of food chain. Fishes like other animals, are capable of concentrating lipophillic compounds in their body tissues several hundred to several thousands times the ambient concentration in the water. DDT is one such pesticide which can accumulate in fish to levels more than 10,000 times the concentration present in the environment. Studies have often been reported indicating adverse effects at structural levels and at the metabolic level. Pesticides finding their way to aquatic habitats are found to interfere with the reporductive capacity of the fishes.

Effects of Sewage Wastes

The major adverse impacts on water quality of sewage wastes are; deoxygenation, high BOD load, rapid eutrophication, and accumulation of heavy metals, in the environment. Sharp fall in Dissolved Oxygen in water renders the biotic communities under severe stress. Apart from affecting the organisms at lowser levels, intensive rate of pollution from municipal sources often causes direct fish kill especially in smaller streams where the problem gets aggravated due to reduced water flow rate. The *Escherichia* and *Aserogenes* are the major coliform bacteria encountered in polluted waters. The presence of Escherichia will indicate definite faecal contamination and possibility of the spread of much virulent diseases as gastroenteritis, typhoid, cholera, etc. by a host of pathogenic bacteria. Increased use of synthetic detergents for domestic purpose, their incidences in the sewage effluents are escalating. Synthetic detergents impair the growth and reproductive capacity of fishes as they are absorbed into the body systems of fish. Detergents mixed with oil may be 60 times more toxic than the oil alone.

Impact of Thermal Wastes

Fishes vary greatly in their ability to withstand heat, and the upper limits for different species vary from 22 ^{0}C to more than 42^0 C. Trouts diet at 25^0 C: their eggs will not hatch at temperature higher that 14 ^{0}C and they grow more rapidly at temperatures below 15.5^0 C than they do at higher temperature. On the other hand Carps can withstand temperatures of upto 35-38 ^{0}C, the lower figure being the limit for larger individuals and higher for small ones. The more ecological consequences of heated discharge into aquatic ecosystem are increase in water temperature, alteration in chemical parameters and change in metabolisms and life hisotry of aquatic communities. The heated discharge pushes up the temperature by 8-10 ^{0}C which may cause mortality of fish and fish food organisms. Temperature exerts a direct influence on toxicity. The studies revealed acidic nature, low alkalinity and high chloride contents in thermal wasrtes together with presence of metals in the ash. The studies indicated that fishes avoid the heated effluents discharge points by swimming away instinctively to safer places. The breeding grounds. got shifted at Rihand reservoir, Carps production is adversely affected due to deposition of fly-ash in the marginal areas of river/reservoir which constitute their breeding grounds. Fly ash will cover extensive areas in river bed, blanketing effect on the substratum resulting in retardment on the bottom over the years may seal the nutrients away from the water phase, thereby affecting productivity.

Effects of Water Abstraction

Many riverine fishes have a preference for a particular velocities and any man-made changes in the stream flow regimen can upset the physiological rhythm of fishes. Many fish populations are dependent upon annual flooding for food and spawning. Stream flow rate has a direct impact on the migratory habits of fishes. Discharge can cause migration (commence, create barriers at high or low flows, cause delays, disrupt normal routing and change the speed of travel. Some fish eggs require a flow of well-oxygenatged water through the gravels in which they are incubating. Discharge also influences the fish food species composition and total production as well as the availability of shelter. A number of workers have reported the relationships of ecological impacts of river modifications (Table 4).

Table 4. Ecological impacts of direct or indirect modifications of the river bed

Activity/Modifications	Effects
Construction of locks	Enhancement of eutrophication, Partial storage of fine sediments may result in anoxic interstitial waters.
Damming	Enhancement of eutrophication, bottom anoxia, high organic matter in surface waters etc., Complete storage of sediments resulting in potential fish kills during slucice gate operation (high ammonai, BOD, and TSS).

Dredging	Continuous high levels of TSS, and resultant silting of gravel spawning areas in downstream reaches. Regressive erosion upstream of dredging areas may prevent fish migration.
Felling of Riparian	Continous high levels of TSS, and resultant silting of gravel spawning areas in downstream reaches. Increased nitrate input from ground waters.
Floodplain reclamation and river bed	Loss of diversity, including specific spawning areas.
Channelisation	Loss of biological habitats, especially for fish.

Conclusion

Assessment of human activities impacts on fish population and ecosystems is urgently needed. Antghropogenic impacts on the hydrosphere are accelerating and many commercially important species have already been stressed. At present there are several technological limitations in adopting full proof and tight regulations in maintaining water quality of rivers. The chief ones among them are the limitations of existing waste treatment plants, non-point effluent flow to the river and un-controlled sub-ubran and agricultural drainages. Large rivers like the Ganga, meandering through several states, need a uniform quality code aiming at specific environmental objectibves. Such water quality management schemes should take into consideration the long-term goals based on well-defined mathematical models of water quality incorporating the variables (DO, BOD, DO Saturation, BOD decay coefficients, water flow volumes, downstream flow distance) etc.

There is need for catchment modification for the control of soil incursions and transport of fertilizers and pesticides into the river through agricultural runoff. The control of such pollutants can be effectively done by adopting best management practices (BMPs). In the agricultural fields and other land falling within the catchment area of the river. The BMPs involve managerial controls for adoption of suitable horticultural practices and afforestation, structural controls for making grassed water ways, detention ponds and terraces for soil erosion. There is urgent need for educating the farmers about the ecological implications of the indiscriminate use of agrochemicals. The water quality monitoring of Indian rivers can be successful only through peoples participation and awareness to combat pollution.

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ROLE OF PRIMARY PRODUCTIVITY STUDIES IN BIOMONITORING VIS A VIS SUSTAINABLE RESOURCE MANAGEMENT

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Introduction

Eco-regeneration, preservation of ecosystem and other ecological consideration mainly relate the attainment of the objective of sustainability of natural resources. There are two aspects to the concept of sustainability. One recognises that the economic growth is dependent on exploitation of natural resources, while the other asserts that the very existence and well being of human race depends on the long term maintenance of these natural resources. Vast stretches of river Ganga as well as other water bodies have been polluted by various activities connected with the economic progress of our country, which may lead to decline in natural productivity if proper care is not paid to abate pollution. Production of aquatic animals from an ecosystem is dependent on a very complex community matabolism in which the solar energy trapped by primary producers pass through different tropic level before a small fraction of it is converted to harvestable animals. So the environmental constrains that have no direct bearing on aquatic animals can also reduce aquatic productivity leading to poor growth and production of fish. Fortunately, in India, we have vast amount of inland water bodies. The rivers, lakes, reservoirs, tanks, ponds, estuaries and wetlands constitute the major resources having the potential to meet the 4.5 million ton of fish requirement by 2000 A.D. Scientific monitoring is essential for achieving high production from such water bodies.

Monitoring of polluted water bodies:

Aquatic pollution may be studied in two ways

a) Chemical analysis of water and sediment samples
b) Biological monitoring

a) Physico chemical study to identify environmental stress: The natural water bodies such as rivers, lakes, estuaries etc. frequently receive municipal and industrial effluents, which are as divergent as they are highly toxic and abnoxious. Industrial effluents generally contain harmful toxicants such as acids, alkali, heavy metals, organic chemicals etc. depending on product and production process. Agricultural run off carries different pesticides. Municipal effluents contain a variety of chemicals, detergents and organic matter. Industrial effluent may cause direct fish kill, destruction of habitat for benthos and plankton and toxicity to organisms and fish fry. Tannery, textiles, Jute and other organic wastes cause DO depletion and had high BOD load. The municipal effluent having high

BOD load cause D.O. depletion, rapid eutrophication and heavy metal contamination in the aquatic environment. Hot water discharge from thermal power plants into the water body may have adverse impact on its biota. For example, hot water discharge from NTPC power plant have adverse impact on Rihand reservoir.

Chemical analysis of water and sediment samples from the water bodies may clearly indicate the nature and extent of pollution in outfall region as well as the recovery zone. As expected, the environmental stress is maximum at the outfall region.

Biological monitoring of aquatic ecosystem

Environmental pollution is essentially a biological phenomenon. One of the most striking advantage of bio monitoring of water quality is that it can integrate many environmental factors over a long period of time. On many occasions, chemical monitoring may be ineffective either due to combined effects of pollutants or the concentrations being too low to be detected. In determining water quality in relation to fish, biomonitoring is very useful, since it provides a direct measure of the biological qualities conducive to fish production. Methods of biomonitoring are presented in Fig. 1.

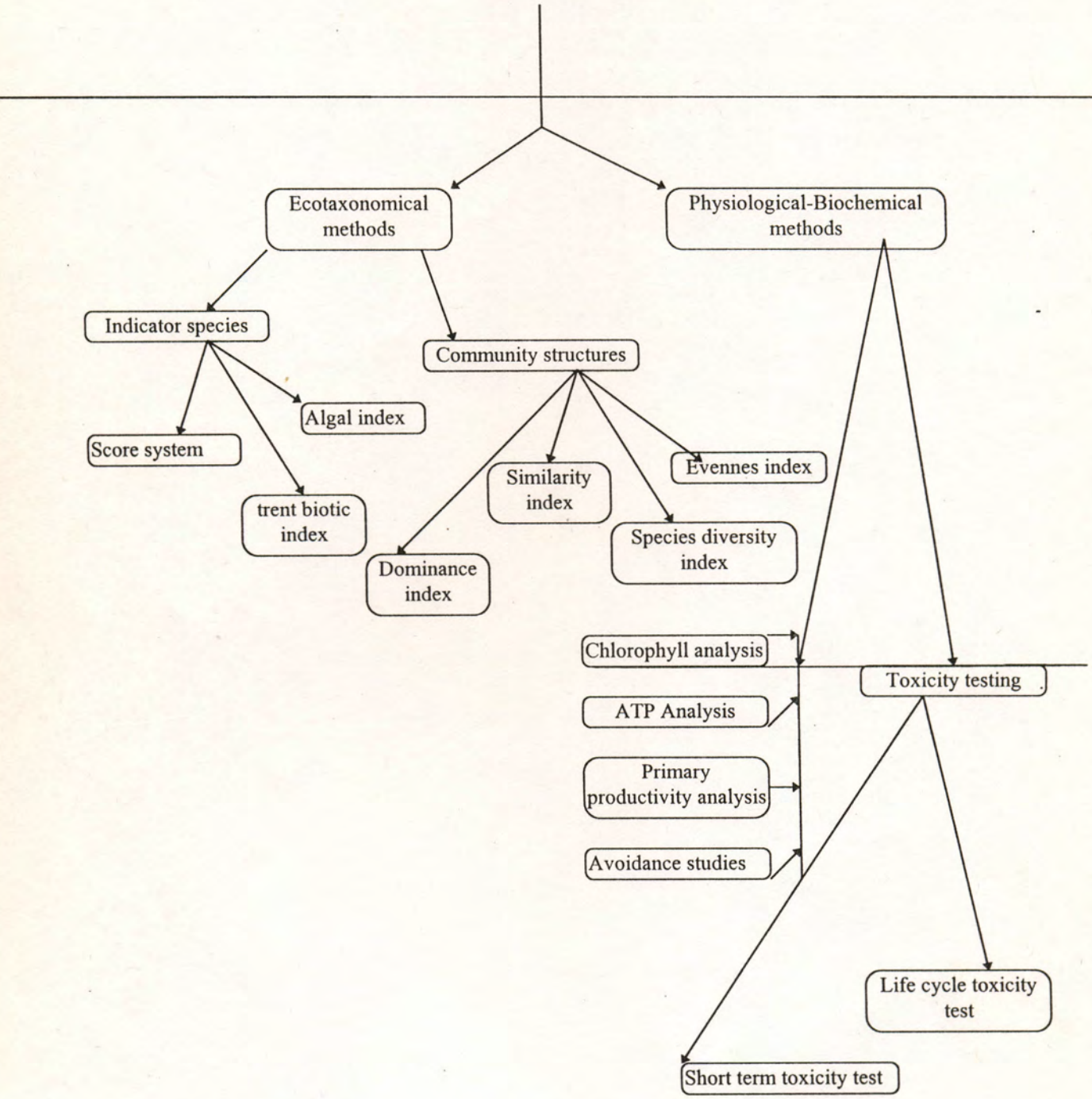
Trent biotic index, various score systems, species diversity indices are generally considered to be more useful in bio monitoring studies. But primary productivity study is also very useful for assessing the productivity status of a water body vis a vis to identify environmental stress on the ecosystem.

Primary production

Photosynthesis by green plant, phytoplankton or algae adds chemical energy and organaic matter to ecosystems, a fraction of which becomes available to consuming organisms. In most ecosystems organic matter is formed by primary producers (autotrophic organisms) and is degraded to carbon dioxide and nutrients by heterotrophs, mainly animals and bacteria. Formation and degradation pathways are called 'chains' or 'webs' according to their complexity. In any system the flow of energy coming from the sun's radiation is unidirectional and most of it is transformed to heat within the system. Nutrients such as carbon, nitrogen, phosphorus, silicon and many others, brought into the aquatic environment with inflowing water, rainfall, sewage etc. may cycle within the system before they are exported from it.

Primary production in a water body generally depends on sunlight, turbulance, turbidity, nutrient concentrations as well as on environmental stress. In Hooghly estuary Ghosh et al (1980b) conducted primary productivity study in relation to industrial pollution. He found lower primary production during summer and monsoon season around the outfall of pulp and paper mill (Soda process). It however, improved appreciably during winter. Ghosh *et al* (1977) have also observed that primary production was significantly lower at the outfall of sulphite pulp and paper mills. In case of tannery outfall, gross and net production were 71 and 81%, 87 and 74% and 43 and 41% respectively during summer, monsoon and winter seasons (Ghosh *et al*,1980a). Bagchi and Nath (1998) studied the primary production of Hooghly Matlah estuarine system with special reference to pollution in Hooghly estuary during the period 1982-1993. They recorded minimum primary production at Nawabganj centre near Barrackpore at Hooghly estuary. This

Fig. 1
Biomonitoring



zone is having many industrial units along the banks which discharged their untreated effluents into the estuary which badly affected the primary production of the system at this centre compared to other centres (Table 1.)

Table 1. Mean primary production (mgC/m3/hr) at different centres of Hooghly-Matlah estuary (1982-93)

P. P.	Nabadwip	Medgachi	Nawabganj	Uluberia	Kakdwip	Frazerganj
Gross	52.3	45.3	36.7	651.4	59.4	65.3
Net	29.5	32.5	20.1	32.5	33.3	34.4

The author studied the impact of hotwater discharge on primary productivity in Rihand reservoir. Estimation of carbon assimilation employing C-14 technique revealed wide fluctuation in primary productivity within various sectors of the reservoir. In Inlet Bay, the primary production was moderately good (Av. 21.01 mgC/m3/hr), which declined to 0.38-4.8 mgC/m3/hr in hotwater channel which recorded higher temperature showing an overage rise of 6.65 °C. The productivity improved in Waidhan Bay (2.21-91.58 mgC/m3/hr) where the temperature is lower than hotwater channel.

Primary productivity study may be employed as a useful tool for classifying the fish ponds. The primary productivity was trace (Nil-6 mgC/m3/hr) in a pond having acidic soil reaction (pH-3.5) which was found to be unsuitable for fish culture (Nath, 1986).

Primary production ranged between 200 and 432 mgC/m3/hr in Jalpaiguri Ponds having acidic soil reaction (pH 5.76-6.2), while at Malda having neutral soil reaction (pH 6.9-7.2) the primary production was significantly higher ranging between 526 and 762 mgC/m3/hr. (Nath *et al.* 1994). In fact Malda ponds were very productive compared to Jalpaiguri ponds.

In India, significant improvement in the water quality and primary productivity rate has been observed in Kanpur due to the positive impact of diversion and treatment of the effluents, prior to their release in the river since 1987 (Jhingran, 1991).

Thus, it may be concluded that primary productivity determination of natural water bodies may be a useful tool for bio monitoring studies. A sudden decrease of primary production in a system indicate industrial pollution or other stress in that water body. On the other hand, very high primary production also shows excess nutrient load, particularly phosphate and nitrogen, leading to algal bloom in the system. Excessive algal bloom is not conducive for natural ecosystem. The ultimate and best solution to the problem of eutrophication is removal of nutrients from the source water of any water body, but this may not be economically feasibile in many cases. So, efforts may be paid to develop ways in which lakes can assimilate higher quantity of nutrients without becoming obnoxiously degraded. Development of more efficient food chains so that the high productivity of eutrophic lakes is converted into desirable fish protein may be ideal. The addition to lakes of suitable fungi and viruses which attack blue green algae has been suggested as one means of keeping undesirable algal population in check.

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NEED FOR BIODIVERSITY CONSERVATION IN AQUATIC ECOSYSTEMS

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Introduction

Biodiversity or biological diversity may be defined as the variability & variety of flora, fauna and microbes in an ecosystem in given time. In recent years biodiversity has become a focal point of animated discussion at various fora throughout the globe. However, the level of debate has increased many folds ever since the Earth Summit in Rio de Janerio in 1992. The process of debate on biodiversity conservation also continues in India but with divergent views and varied perception regarding its management and conservation. The realisation of biodiversity conservation and its sustainable exploitation have no doubt made considerable dent in the planning processes yet nothing tangible could be achieved so far in absence of unanimity on priority areas of its management, specially in third world countries, owing too much pressure on resources in the face of ever increasing human population.

Biodiversity has very intimate relationship with the progress and development of human civilization. Most of the human needs are linked with biological resources, be it food, clothing, shelter, medicine or recreation. It is essential, therefore, that the biodiversity irrespective of its occurrence must be managed and conserved with utmost care and rationally so as to save the flourishing human civilization on the Earth. The level of information and the perception of biodiversity conservation is still very poor specially in developing countries including India. The understanding on aquatic biodiversity in particular has failed to touch the level it should have been. The aquatic resources covering 75% of the Earths' crust have been envisaged to play a very positive and significant role in food front of the world in general and the developing countries in particular. This assumption may appear to be at the conceptual stage for the time being, the per capita diminish of land & biological resources of terrestrial origin cannot be ignored with the rise in human population at a faster pace. In such a scenario when the land biodiversity is becoming critical the aquatic biodiversity may have to play very a crucial role in supplying food to the people.

The biodiversity in terms of gene-pool and genetic variability has been under a constant threat in recent years due to so many reasons like over exploitation or other man induced activities. The situation is grave and assuming serious proportion owing to the loss of plant and animal species beneficial to men. Many such species are becoming either rare or endangered at a very rapid pace besides many species have already become extinct. The conservation of biodiversity is thus important not only from an economic point of view but to preserve their aesthetic and social values also. An acceleration in the understanding of biodiversity conservation is the need of the hour for better management of biological resources and the euphoria generated at the Rhio conference must not be allowed to degenerate into a series of platitudes in which the words like biodiversity become only a catch words for convenience rather than words of meaning.

Definition of Biodiversity

In order to have better understanding of biodiversity a clear idea of the meaning of biodiversity besides its attributes and values is a must. The literary meaning of biodiversity is variability of plants and animals. At the Rio Conference it has been defined as :

> **"Biological diversity means the variability among living organisms from all sources including *inter alia*, terrestrial, marine and other aquatic systems and the ecological complexes of which they are part; this includes diversity within species, between species and of an ecosystem"**

The aforesaid definition suggests that biodiversity does not refer only to variability of species and conservation of threatened biota but it covers the whole spectrum of the natural environment, from microbes to landscape. Evidently, conservation of biodiversity must be viewed in its totality rather than restricting our observation to living ones only.

Biodiversity in India

It has been estimated that the Earth has a huge variability of plant and animal species, somewhere in between 5 and 50 millions, but only 1.7 million could have been identified till date. India, being the meeting ground of three divergent but major global biogeographic realms (Indo- Malayan, Eurasian and the Afro-tropical), is one among the worlds' top twelve mega-diversity nations. India has varied agro-climatic conditions with distinct ecological zones ranging from perpetual snow cover to equatorial & tropical conditions; from mangroves to humid tropics and from hot to cold deserts. The plant wealth of the country has an estimated number of 54,000 species which account for 12% of the total plants of the world. The flowering plants alone have been estimated to 15,000 species (16% of the global diversity). Nearly 33% of the plant wealth, available in India, is endemic and about 1000 species are endangered. The animal wealth on the other hand has been estimated to 68,371 which includes 60,000 insects, 1693 fish, and 372 mammals. Paradoxically, however, the marine life of India has not been estimated fully inspire of a long coastline of 7500 km with a continental shelf of more than 45 million ha and a an extended economic zone of 201 million ha.

Threats to aquatic biodiversity

The aquatic ecosystems have been subjected to various forms of environmental stresses, during the past few decades. Most of such environmental problems are not natural rather man induced. Increased human activities in the catchment areas of various natural aquatic systems have affected the natural processes of the systems adversely thereby threatening the normal growth of biotic communities. Some of such threats to aquatic biodiversity are as under:

Encroachment:

Changing land use patterns with the increase in human population has encroached upon the natural water bodies for various activities such as agriculture, urban expansion and so on.

Siltation:

Siltation of natural water bodies has been identified as one of the major problems affecting the biodiversity. Deforestation and other anthropogenic activities have accelerated the pace of soil erosion causing higher rates of siltation and the resultant shrinkage of physical resource and ultimately stress to biodiversity. The wetland ecosystems in India may be cited an example to this effect.

Weed infestation:

Presence of aquatic macrophytes provides stability to an ecosystem. However, excessive proliferation of macrophytes as weeds has been found detrimental to aquatic biodiversity due to locking of necessary nutrients in the hydrophytic chain. In weed infested ecosystems the phenomenon of *survival for the fittest* starts operating as a result many sensitive and fragile forms are either completely eliminated from the ecosystem or at least become threatened.

Pollution:

Water pollution has assumed a serious proportion in recent years affecting the aquatic resources adversely both in terms of physical as well as biological resources. Most of the natural water bodies have been subjected to an indiscriminate ingress of domestic sewage, factory effluents and solid wastes. Agricultural run off containing fertilizers and pesticides has made the problem still complex. The net out come of such developments in the aquatic systems is eutrophication or excessive nutrient enrichments leading to lopsided biotic growth like algal blooms and thus creation of an unfavourable aquatic regime not conducive to normal occurrence of various biota.

Over exploitation:

Over exploitation of aquatic systems for various economic activities viz. abstraction of excessive water for agriculture, industries & others; indiscriminate fishing & aquaculture etc. are some of the factors have been found detrimental in the maintenance of healthy biodiversity.

Symptoms of strain

The symptoms of strain due to the cumulative effects of aforesaid pressures may be reflected into:

- decrease in biological diversity specially of endemic and endangered species
- deterioration of water quality
- sedimentation and shrinkage in water area
- decrease in population of migratory birds, fish and other fauna
- prolific growth of unwanted & obnoxious aquatic weeds

In the event of such symptoms it may be prudent to believe that the biodiversity of that particular water body is under threat and needs necessary corrective measures to conserve the same.

Monitoring biodiversity

Conservation and preservation of biodiversity are very important to save this planet from disintegration. It is essential, therefore, to introduce a well designed monitoring protocol for its maintenance. The broad principles for biodiversity monitoring can be as under:

- *monitor to record integrity of sites (SIM) such as nature reserves, sanctuaries etc. in view of threat perceptions.*
- *monitor to ensure the quality of such sites (SQM)*
- *monitor to record the long term ecological effects of climatic changes*
- *monitor to detect the effects of over-grazing, over exploitation, irrigation, pollution or salinisation*
- monitor to record changes in the distribution of endangered or threatened endemic species
- monitor to keep a track of the richness or diversity of the biotopes that we value.

The study of biodiversity can be pursued adopting one or more than one mode of operation as the objective demands. These modes are **Survey** (recording at one time), **Surveillance** (repeated surveys), **Monitoring** (repeated recording with clear objectives and using a standardised approach)and **Census** (repeated recording of a single species including fluxes such as births and deaths).

Research options / priorities for conservation

In view of the importance of biodiversity conservation in the face of emerging threats in recent years concerted efforts are required not only to maintain the ecological balance, but also for sustaining human welfare through biological resources. It is necessary to have an integrated approach interlining various facets for the conservation and sustainable use of biological diversity (fig. I).

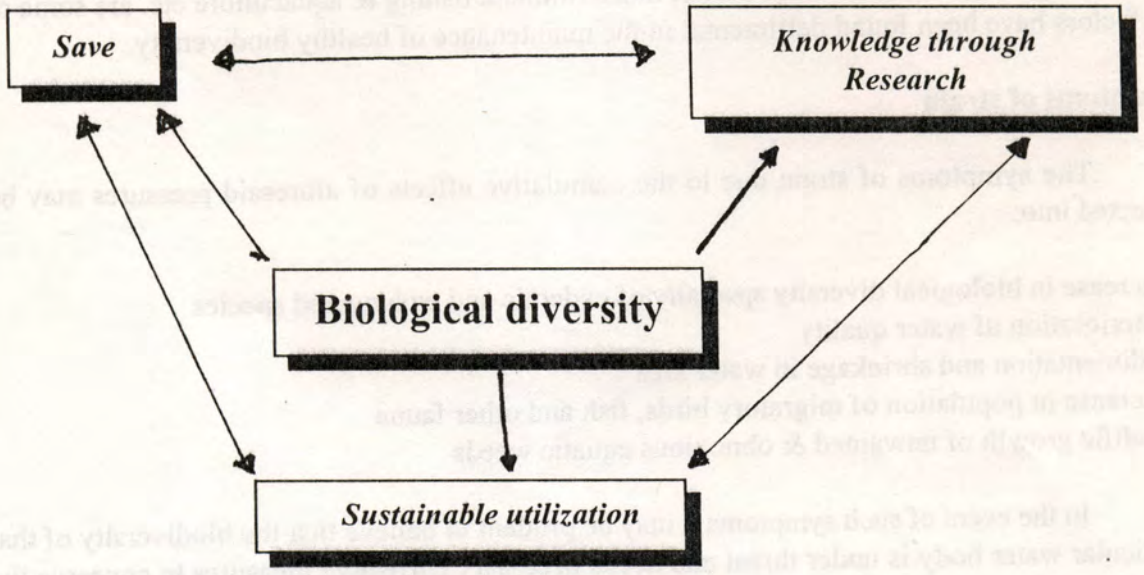

Fig.I: Inter-linkage between facets of biodiversity conservation

Effective conservation of aquatic biodiversity requires efforts from various quarters as many scientific disciplines may be involved for optimum results(Fig. II)

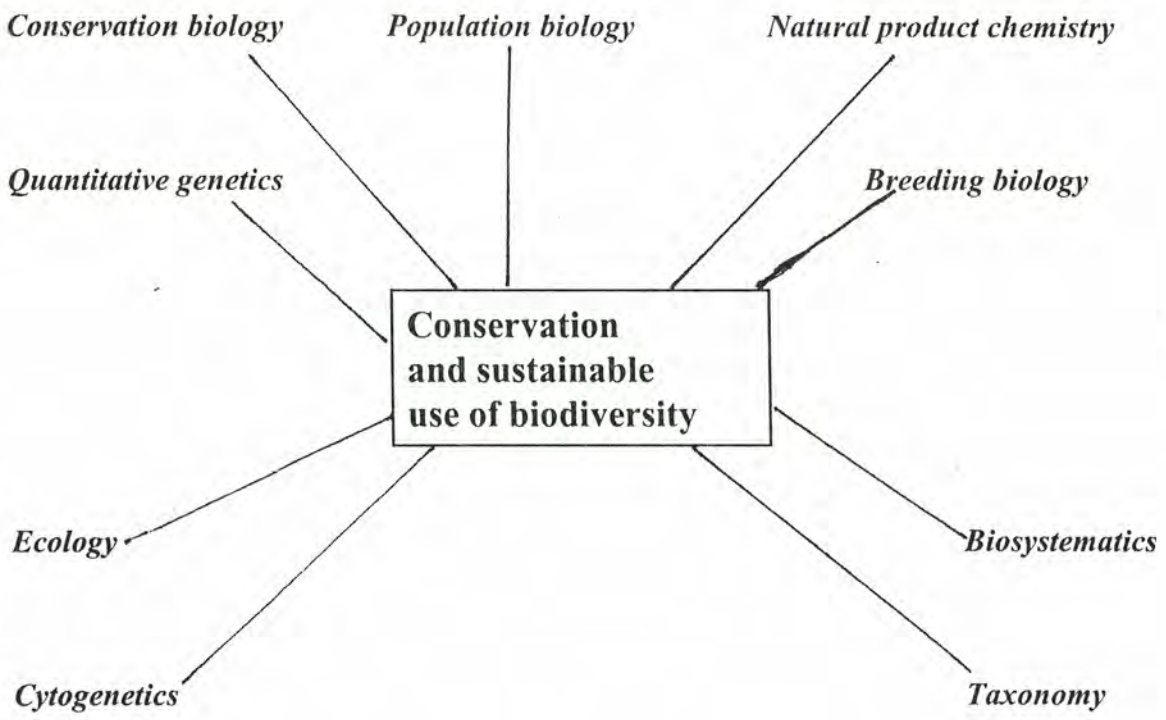

Fig. II- Different scientific disciplines in biodiversity conservation

Conserving biodiversity requires necessary strategies to conserve genes, species, habitats, and ecosystem *in situ.* This can be supplementing through restoration of lost species to their original habitats and by *ex situ* preservation of species in gene banks, sanctuaries etc. In the long term, however, both *in situ* & *ex situ* methods are necessary for which research needs have to be prioritised under the following headings:

1. Survey, 2. Composition, structure & function of ecosystems, 3. Monitoring, 4. Sustainable use, 5. Valuation, 6. Integration of traditional knowledge & skills, 7. Social, economic & cultural factors, 8. Development of restoring technologies, 9. Biotechnology application.

Importance of aquatic biodiversity conservation

Man induced activities have stressed the aquatic environment to such an extent that the aquatic biodiversity beneficial for human welfare is under a constant threat of total elimination. Available statistics indicate that almost half of the existing species may become extinct within next 100 to 300 years at the present rate of environmental perturbations (Wright *et al* 1993). The role of habitat loss is even a greater threat to aquatic biodiversity. In many countries such as in Philippines about 67% of the mangrove forests have been lost within a span of 60 years only

(Dugan, 1990). Similarly, majority of the wetlands of Asia in general and India in particular are reeling under a severe threat of resource loss (Scott & Poole, 1989 ; Sinha & Jha, 1997). On the other hand of the scale, at the molecular level, there is considerable reduction in the available genetic resources owing to extinction of plants and animals. In case of the total decline in genetic resources, the ability of taxa to adopt the changing conditions is bound to be affected and accordingly the population may not survive.

The moot point of debate is why is biodiversity so important? The answer is not simple as a range of arguments are there such as *precautionary, moral, indicative, aesthetic* and *economic.*

I. **Precautionary**: Our knowledge on biodiversity is still insufficient for making any judgement as too much loss of biodiversity an ecosystem can sustain without losing its ecological balance. In view of this and till our knowledge becomes sufficient. We must conserve the biodiversity as so as to use the resources on sustainable basis. The precautionary approach, thus is necessary to avoid the risk of losing valuable genes from the genetic pool.

II. **Moral**: The moral argument for biodiversity conservation is based on the fact that man being the most evolve animals on the Earth is morally bound to protect and improve the environment as a steward so that the same can be handed over to the next generation with pride. It implies, therefore, that the physical or biological resources need be exploited with utmost care so as to preserve these in reasonably good shape to hand onto the next generation.

III. **Indicative**: The indicative argument for biodiversity evaluation is based on the fact that it provides the necessary indication of the health of an environment. A change in the level or texture of biodiversity is often the first indicator of change in an ecosystem. The phenomenon of eutrophication in lakes, for instance, is a function of nutrient enrichment in the system wherein a considerable shift in community structure has been evident, specially at the level of plankton and other invertebrates.

IV. **Aesthetic**: Conservation of biodiversity also has cultural & emotional over tones such as the feelings that biodiversity land scape and natural ecosystems which support various species used to provide a sense of solace and a feeling of homeliness. However, in developing countries including India the aesthetic, moral and cultural values of biodiversity have been pushed in the rear as the priorities for biodiversity utilization are different in the face of high population growth and abject poverty often leading to over exploitation of biological resources.

V. **Economic**: The biological diversity can be considered a capital asset with enormous potential for yielding sustainable benefits. However, quantification and fixing of its realistic price are rather difficult. All the more the functional values of an ecosystem are much more difficult to price. The value of an aquatic ecosystem in relation to some of its attributes can be priced such as a value of its habitat as tourist attraction, fishing activities etc. There is a plethora of values for biodiversity which need not be viewed in a monetary term only. However, pricing of activities in an aquatic system would prevail for some time, specially in developing economy which have an inherent problem of bridging the gap between demand and supply of basic requirements. It can be expected, therefore, that the biological diversity of aquatic ecosystems may have to face the problem of over exploitation, a negative factor for healthy conservation of its biodiversity.

Broad action plan for biodiversity conservation

Broadly, the biodiversity of the aquatic systems can be conserved in two ways viz. *in situ conservation & ex situ conservation*. These two strategies are complimentary to each other and as such must be taken up simultaneously for an optimum result. Conservation of biodiversity is essential to promote sustainable economic and social development, specially in developing countries where biomass economy is predominant. In order to make the biodiversity conservation programme a success immediate attention on the following areas is needed.

- Prioritisation for conservation and sustainable use of biological diversity and agenda for Scientific and Technical research.
- Evaluation of potential economic implications of conservation of biodiversity and its sustainable use, and evaluation of biological and genetic resources.
- Technology transfer and financial issues
- Modalities of a protocol for transfer and handling of any living organisms resulting from biotechnology.

The above-mentioned strategies can be implemented through activities as under:

- *In situ* (in a site) conservation of target species or ecosystem
- *Ex situ* (off a site) conservation of target species
- Conservation through gene banks
- Through public awareness & training
- Through peoples' participation
- Motivating NGOs
- Developing a conservation net- work of various working groups
- identification of hot spots affecting biodiversity.etc.

Conclusion

Conservation of aquatic biodiversity is a must not only for ecological balance of aquatic environment but to save the entire civilization on this planet. The task is no doubt daunting but not impossibly provided the problem is being tackled in broad perspective rather than in isolation. It is imperative therefore, while conserving the biodivetrsity of aquatic system we must take into consideration the human use both the resources within the system and that external to it. Water quality, quantity and hydrology are known to be the essential component in catchment use and ecosystem performance. A quantitative assessment of these factors in the form of nutrient budget, sediment budget, hydrological profile etc. are must for effective biodiversity conservation of aquatic ecosystems. Various aquatic systems need to monitor in time scale so as to keep a track on the changing biodiversity in the face of increased human pressure. Effective laws are also the need of the hour to put a halt on the irrational exploitation of aquatic resources. Coordination and net-working between various developmental agencies is a must to make the task of biodiversity conservation smooth and relatively easier.

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MACROBENTHOS - A HANDY TOOL IN RAPID ENVIRONMENTAL IMPACT ASSESSMENT OF FRESHWATER ECOSYSTEMS

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Macroinvertebrates are the animals which inhabits the bottom substrates (sediments, debris, logs, macrophytes, filamentous algae, etc.) of freshwater habitats for at least part of their life cycle. Macroinvertebrates are those retained by mesh sizes $\geq$ 200 to 500 μm and are the main object of the study. Mostly, they belonged to group of insecta mollousca and annilida. Primarily macrobenthos are used in surveillance to know whether conservation measures are successful. The use of macro benthos to predict environmental impact prior to the start of a development is a specialized form of surveillance biomonitoring. The second major type of biomonitoring is done to ensure compliance-either to meet statutory requirement or to control long-term water quality. Benthic invertebrates can be used to test effluents and to ensure receiving water standards or they can be used to ensure that standards are mantained during and after construction of a project. Benthic macroinvertebrates are used to achieve the above objectives in a variety of way, including monitoring changes in genetic composition, bioaccumulation of toxicants, toxicological testing in the laboratory and field, and measurements of changes in population numbers, community compostion or ecosystem functionary.

Till a few decades ago, use of quantitative approaches, such as correlating the presence on approximate relative abundance of certain macro-invertebrates with pre-establish classifications of environmental quality was emphasised in water quality monitoring programme. This approach was based on saprobien system for assessing the pollution status of lotic habitats and by the lake typology concept of characterising the trophic status of lentic organisms present. However, a transition occurred in 1970's when the emphasis shifted towards quantitative approaches that typically included calculation of diversity indices, formal hypothesis testing that required replicate sampling units and detailed statistical analysis. In recent years, however, anothe transition has occurred, and a renewed interest in the use of qualitative techniques, primarily because of high cost of quantitative approaches. This shift resulted in the development of what are generally called "rapid assessment approaches". The purpose of applying rapid assessment approaches is to identify water quality. In recent years, a similar approach is being taken to assess status of fish communities.

Rapid assessment approaches are some what analogous to using thermometers in assessing human health; easily obtained values are compared to a threshhold that is considered to be "normal". The key questions in biomonitoring are: what population and community measures are biologically relevants, what are the threshhold against which they are being compared (normal body temp.). How much of a deviation from a threshold is a sign of "unhealth".

Advantage of using macroinvertebrates in biomonitoring

Benthic macroinvertebrates offer many advantage in biomonitoring, which explain their popularity. Some of these are intrinsic to the biology of the animals. First, they are ubiquitous, therefore, they can be affected by environmental perturbations in many different types of aquatic systems and in habitats within those waters. Second, the large number of species involved offers a spectrum of responses to environmental stresses. Third, their basically sedentary nature allows effective spatial analysis of pollutant or disturbance effects. Fourth, they have long life cycles compared to other groups, which allows elucidation of temporal changes caused by perturbations. As a result, benthic macroinvertebrates act as a continuous monitor of the water they inhabit, enabling long term analysis of both regular and intermittent discharges, variable concentrations of pollutants, single or multiple pollutants, and even synergistic or antagonistic effects.

Rapid Assessment Measures

The rapid assessment methods have been divided into five categories: **richness, enumeration, community diversity** and **similarity indices, biotic indices**, and **functional feeding groups measures.**

Richness method:- This method is based on the number of distinct, specified taxonomic units in a collection or at a site; richness is a components and estimate of community structure. Macroinvertebrate species richness, because it is based on specimens identified to the lowest taxonomic level, rather than on nominal species, which often requires rearing of specimens and taxonomic expertise for accurate identification. Often species are separated by perceived differences and are given designations (sp. A, sp. B, etc): these groups may, or may not correspond to distinct species. Separation of various stages of the same species into different taxa would result in over estimation of taxa richneas. More often, however, similar appearing species are not separated, which results in under estimation of taxa richness.

Enumerations method:- All collected organisms are counted to estimate relative abundances of different taxonomic groups (number of individuals in certain orders, families, or species or numerically dominant taxa in these groupings). Essentially no taxonomic effort is required for total number of individuals requires distinctions based on the group under consideration (e.g. numbers of individuals for a given order, family or species).

Community diversity and similarity indices methods

These measures of community structure usually require taxonomic distinctions at the species level (or at some higher taxonomic level of macroinvertebrate richness). Total

number of taxa provides a richness component in calculating the value of diversity indices; the number of individuals for taxon provides an eveness component. Few of the dozens of diversity indices that have been proposed, the regularly used is shannon's index. The index is based on information theory and may be defined as:

$$\bar{H} = - \sum_{i=I}^{S} \frac{ni}{n} \; Ln \; \frac{ni}{n}$$

Where S is the number of species in a sample, n the total number of individuals in the sample, and n, the number of individuals of each species i of sample from a population i.e. $n=\sum ni$.

The concept of species diversity is based on the theory that in aquatic biotic community living in a pollution free environment is characterized by the presence of a wide variety of species but only by a moderate number of each species. A change in the biotal community structure resulting in less species but greater abundance of select tolerant ones, reflects the advent of condition of environmental stress. Wilhm gave the different values of $\bar{H}$ to denote the aquatic pollution. Value of $\bar{H}$ between 3-5 indicates clean water, and 1.0-3.0 as moderately polluted and below 1.0 as substantially polluted. Staub *et.al* gave a slightly different value for $\bar{H}$ in terms of species diversity which is 3.0-4.5, 2-3, 1-2, 0-1, the degrees of pollution being slight, light moderate and heavy respectively, also indicating a negative correlation between $\bar{H}$ and pollution. Community similarity indices are used to compare community structure in space (*e.g.* among different sites) or overtime (year to year). Similar levels of taxonomic discernment among the communities being compared is implicit in their use. Some Community Similarity Indices stress richness (*e.g.* Jaccard index) or both richness and abundance (*e.g.* Pinkhan-Pearson Index).

Biotic Indices - It is an index of water pollution based on study of biota. Biotic Indices use prestablished water -quality tolerance values for taxa (families, genera, or species) that have been collected and identified. The relative abundance of taxa, weighted by tolerance values, sometimes may be included in the calculation of a biotic index. About 10 (ten) biotic indices are known out of these a few (Beak index, the Trent Index, Chandler's biotic score or CBS and Chutter index) are frequently used in pollution studies. For Ganga river system a scoring system has been developed. In this method all families or species present are listed, scores are prescribed to each of these families according to the values indicated in table 1, and score for all families are added to give the total cumulative site score. It will be appreciated that the better the biological quality at the choosen sampling site, the higher will be the biological score. In fact, values well in excess of 200 could be expected in Himalayan river reaches. Some common macrobenthic organisms found in open water are shown in Plate I and II.

Functional feeding groups methods

These measures are community measures that are based on the morphological structure and behaviors responsible for food acquisition by given species at a site.

Apparently, some discrepency exists to how functional group designations currently are made and how they were intended to be made. Functional groups, as currently used in the ecological data table, reflects trophic levels (*i.e., herbivores, detritivores, carnivores)* and are based on digestive tract analysis.

General comments

Sampling procedure:- Sampling may be done either by frame nets for shallow water bodies or by grabs in water bodies deeper than metre.

Taxonomic level:- In most measures generic level/species level identification is made, but other specify the family level as sufficient. While in some protocols, only taxa of Ephemeroptera, placoptera and Trichoptera are identified; in others, only taxa with an indicator value are identified or taxa are separated into species group but no identifications are actually done. The choice of taxonomic level represents a compromise between the desire for increased information count and the resulting usefulness of species level identification, and the cost of obtaining it.

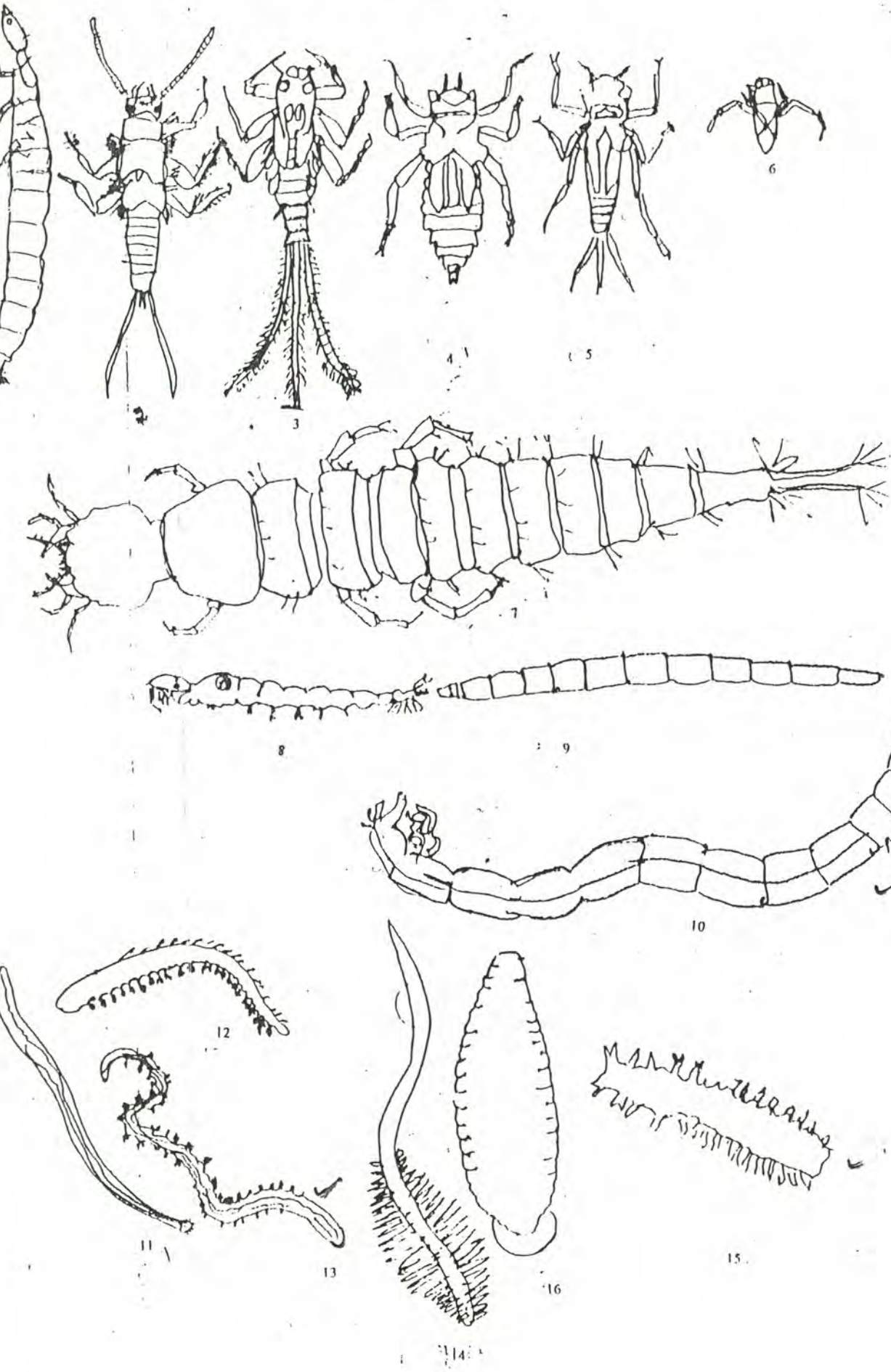
Measures used:- In most studies one or two methods are used, and that to based on established environmental tolerance of organisms, such as biotic index or numbers of tolerant species. According to a study in 67% of protocol taxa richness or some form of enumeration were used followed by diversity and similarity indices (26%) and functional feeding group measures (25%). It will be better if rapid assessment studies should be based by optioning two methods (Shannon H and Biotic Index) to reach at a conclusion of status of a water body.

SUGGESTED BIOLOGICAL SCORING SYSTEM FOR THE RIVER GANGA

Siphlonuridae Heptageniidae Leptophelebiidae Ephemerellidae Potamanthidae	*10*
Ephemeridae ***(Ephemeroptera)***	*10*
Taeniopterygidae Leuctridae Capniidae Perlodidae Perlidae	*10*
Chloroperlidae ***(Plecoptera)***	*10*
Aphelocheiridae ***(Hemiptera)***	*10*
Phryganeidae Molannidae Beraeidae Odontoceridae Leptoceridae	*10*
Goeridae Lepidostomatidae Brachycentridae Sericostomatidae ***(Tricoptera)***	*10*
Lestidae Agriidae Gomphidae Cordulegasteridae Aeshnidae Corduliidae	*8*
Libellulidae ***(Odonata)***	*8*
Psychomyiidae Philopotanidae ***(Tricoptera)***	*8*
Caenidae ***(Ephemeroptera)***	*7*
Nemouridae ***(Placoptera)***	*7*
Rhyacophilidae Polycentropodidae Limnephilidae ***(Tricoptera)***	*7*
Neritidae viviparidae Ancylidae Unionidae ***(Mollusca)***	*6*
Hydroptilidae ***(Tricoptera)***	*6*
Corophiidae Gammaridae Paleamonidae ***(Crustacea)***	*6*
Nereidae Nephthyidae ***(Polychaeta)***	*6*
Platycnemididae Coenagriidae ***(Odonata)***	*6*

Mesovelidae Hydrometridae Gerridae Nepidae Naucoridae Notonecutidae	5
Pleidae corixidae **(Hemiptera)**	5
Haliplidae Hygrobiidae Dytiscidae Gyrinidae Hydrophilidae	5
Helodidae Dryopidae Elminthidae Chrysomelidae Curculionidae **(Coloptera)**	5
Hydropsychidae **(Tricoptera)**	5
Tipulidae Simuliidae **(Diptera)**	5
Planariidae Dendrocoelidae **(Platyhelminthes)**	5
Baetidae **(Ephemeroptera)**	4
Sialidae **(Megeloptera)**	4
Piscicolidae **(Hirudinea)**	4
Valvatidae, Hydrobiidae Lymnaeidae Physidae Planorbidae Sphaeriidae **(Mollusca)**	3
Glassiphoniidae Hirudidae Erpobdellidae **(Hirudinea)**	3
Asellidae **(Crustacea)**	3
Chrironomidae	2
Oligochaeta **(whole class)**	1

T 1. Phylopotamus 2. Stonefly nymph 3. May fly nymph 4. Dragonfly nymph 5. Damsel fly 6. Notonecta(adult) 7. Beetle larva 8. Chaoborus 9. Culicoides 10. Chironomid larva(1-10 insecta) 11. Dero 12. Nais 13. Tubifex 14. Branchiura sowerbyi 15. Nepthys oligobranchia 16. Oligobdella biannulata (11-16 annelids)



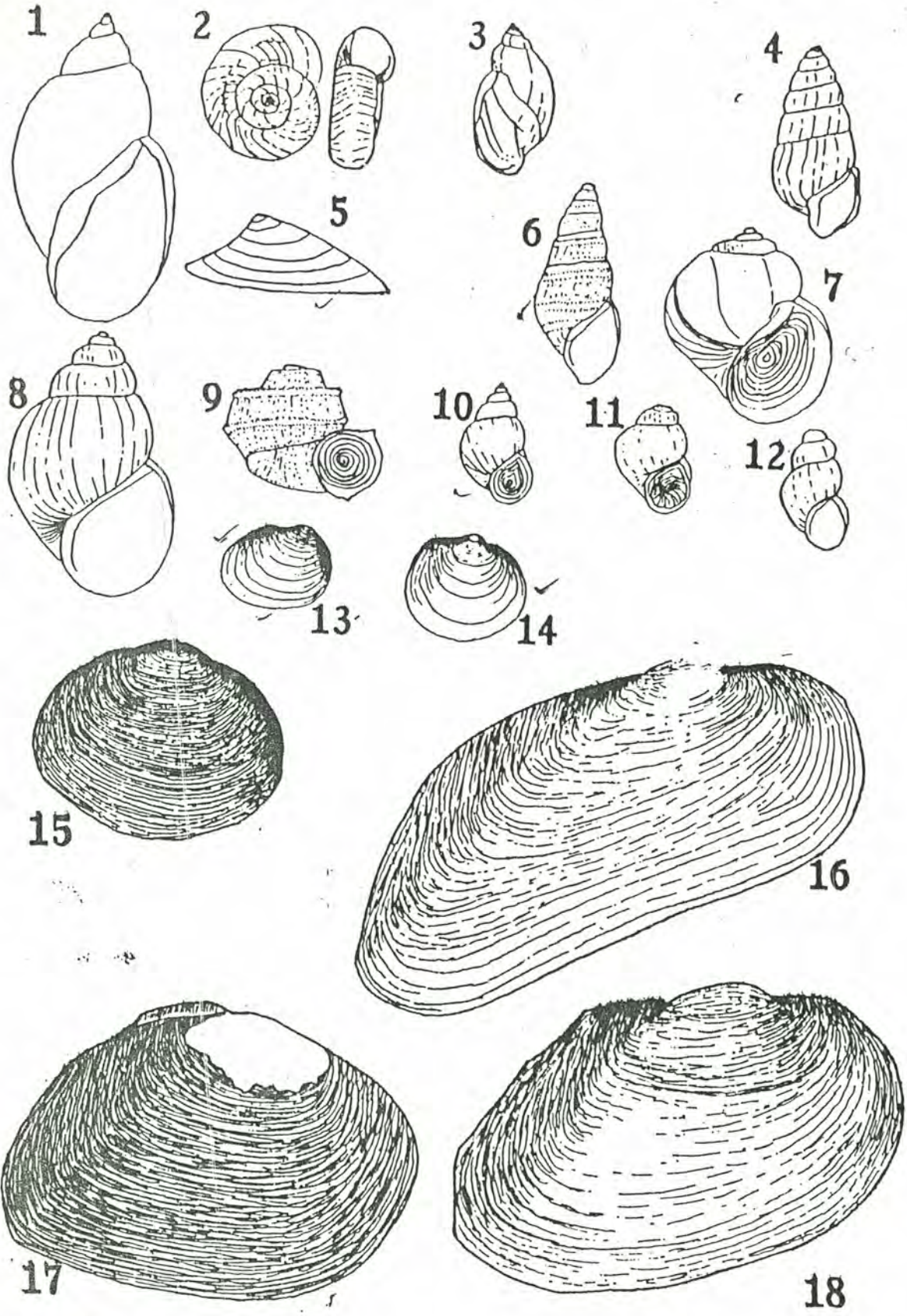


PLATE II

Figs.	Genera	Length	Dist.	Waters
11.	Amnicola	4	G	both
5.	Ancylus	5	E	both
18.	Anodonta	90	G	both
10.	Bythinia	10	NE	both
8.	Campeloma	25	N, S, E	both
6.	Goniobasis	22	G	both
1.	Lymnaea	10–50	G	both
16.	Margaritifera	30	G	both
14.	Musculium	9	G	both

Figs.	Genera	Length	Dist.	Waters
12.	Hydrobia	4	G	both
3.	Physa	16	G	both
13.	Pisidium	8	G	both
2.	Gyraulus	3–6	G	both
4.	Pleurocera	30	E, S, W	both
15.	Sphaerium	14	G	both
17.	Unio	100	E, S, C	both
9.	Valvata	5	G	both
7.	Viviparus	28		

MICROBIOLOGICAL STUDIES IN AQUATIC SYSTEMS- A NEW DIMENSION IN ENVIRONMENTAL IMPACT ASSESSMENT

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Introduction

Microbes are integral part of any ecosystem be it a forest, inland waters, marine waters, agricultural land etc. microbes include bacteria, fungi rickettsia and chalamydia. Among these bactria are the most common organisms found in any environment and play important role in an ecosystem. They break down many organic and inorganic substances and act as food for other organisms. Hence, they serve as important links in a food chain. The environmental parametrs like tempeature, humidity, pH, salinity and type of organic substances present dictarate the kind of bacteria that can thrive in an ecosystem. For example, in marine environment bacteria that can grow in high concentration are most abundant e.g. *vibrio* organisms. Any change in these parameter affects their population both in terms in quantity and quality. Hence, the study of micro-organisms in an ecosystem can be used as a tool for assessing the impact of any developmental/industrial activity.

Use of microbial communities for EIA

For the purpose of EIA sudy of bacteria can be used in following ways:

a) *Sewage Pollution:* Status of sewage pollution in water can be indicated by some representative species like *E. coli,* and *Klebsiella* collectively known as coliform bacteria. This group of bacteria normally inhabits human and animal intestines and are not normally found in open waters. Their number in a particular sample indicates the extent of sewage pollution.

b) *chemical Pollution:-* the discharge of toxic chemicals like ammonia usually results in decrease in number of microbes in general. However, some of the bacteria have specific afinity for metal ions. For example, presence of sulphur reducing bacteria (SRB) indicates presence of sulphur and indication of anaerobic conditions.

c) *Aquatic Productivity:-* The assessment of microbiota in an aquatic system can also be linked with aquatic productivity since bacteria are an important link in aquatic food chain. It can be done in two ways:

1) Measurement of productivity through estimation of bacterial load

2) Measurement of productivity from microbial consumption of phosphate-phosphorus

Microbial forms in aquatic system

Bacteria can be found in very diverse habitats and communities in aquatic environment. They are associated with all types of surfaces, including plants, rocks, animals sediments, manufactured objects, and plankton and they are found in environments that have extreme physical and chemical ranges: temperature - 4 to 50.0 ^{0}C, salinity 0 to 100 ppt, pressure 0 to 1000 atm., pH 5.5 to 8.5 an oxidation/reduction potential (Eh) + 400 to - 400 mv. The numbers, types and activities of these bacteria are basically dictated by their environmental setting. According to their habitat aquatic bacteria can be divided into following forms:

1) *Planktonic forms:* are free floating bacteria that are not attached to any specific substrate. But planktonic bacteria can be found attached to suspended particles that have a favourable effect on microbial growth because they are focal points for absorption and desorption process that provide nutrients for attached bacteria. Planktonic bacteria are mainly found in the neuston, the uppermost microlayer of water at the water-air interface, and the water colum.

2) *Epiphytic forms:* epiphytes may be defined as organisms growing on the surface of living plants. Epiphytic bacteria are usually found associated with algae. In this symbiotic relationship, the algae excrete organic materials for bacterial growth, and the bacterial metabolism supplies CO2 and inorganic nutrients for algal growth.

3) *Sediment bacteria:* are usually anaerobic decomposers which play important part in the formation of detritus in the sediment, hence, they play important role in the aquatic food chain.

Microbiology methods used in EIA

1) Direct microscopic count in water and sediment

Direct counting of total amount of bacteria is a most important method for quantitative study of the microflora, and it's reproduction, in a natural water body. Only by this method can all the bacterial cells present in a given volume of water or silt be enumerated with sufficient accuracy. The bacteria in water are counted on membrane filters or on preparations of suspensions of silt which are on slides.

A) *Counting of bacteria in water*

Water samples are taken in well-cleaned bottles washed several times with a part of the water to be examined. If transportation of the samples is necessary they are fixed with formaldehyde solution (1% at the final conc.).

Filtration: the pore size of the filters for direct microscopic count must not exceed 0.5 micron, and their working (upper dull) surface should not be contaminated. The volume of water to be filtered will depend upon the trophic level of the water body investigated. In an oligotrophic basin 10-15 ml of water pass through the working surface of the filter: in mesotrophic lakes the volume of water can be 5-10 ml, in eutrophic ones 2-5 ml, and in contaminated waters 0.5-1 ml.

Storage: After filtration bacteria on the filters are fixed in formaldehyde fumes, by placing the filters in a petri dish to which a few drops of concentrated formaldehyde are given inside the cover. The filters are then dried and stored.

Staining: For this purpose a sector of the filter is cut out, marked with a pencil and brought into a petri-dish at the bottom of which a round piece of 2-3 layers of netting, moistened with 3% erythrosin solution in 5% phenol, is placed. Staining takes one day. After this filter must be decolourised; hence, they are transferred to a beaker, filled with water, and left these until they are pale pink in colour. The filters are then dried and prepared for microscopic examination.

Examination: For this purpose the prepared filter sectors are placed on top of a drop of immersion oil on a microscope slide; another drop of oil is put on the surface of the filter and the whole is covered with a cover glass slide. These lides are ready for the examination when the filters have become transparent. Examination is usually made with an immersion objective X9Q and ocular X15 using a micrometer disc in one of the oculars ruled with 25 sections in an area of 25-35 mm^2.

The number of bacteria (N) in the initial sample can be calculated using the formula

$N= S.n.10^6/s.v$ cells/ml

Where S is equal to the area of the working surface of the filter (mm2), s is the area of the one cell of the micrometer disc (measured in micron sq. With a stage micrometer at the same magnification) n is the average number of bacteria per cell of the grid, and v is the volume (ml) of water which was filtered.

b) Direct microscopic count of bacteria in sediments

The most useful method of counting bacteria in sediments is by microscopic examination of preparations of sediment suspensions dried on slides and stained. The preparations are made in the following way. The sample of sediment (0.5 ml) is taken with marked glass tubes and transferred to a flask with 50-100 ml of 0.0005 N KOH. The flask is stoppered and shaken strongly for several minutes to separate the bacteria from detritus particles. The suspension is left for half a minute in order to allow large particles to sediment. A portion of the suspension is placed on the surface of the microscope slide which has been cleaned with alcohol and ether. To a drop of the suspension is added one drop of 0.05% agar which has been previously filtered through a membrane filter. The mixture is carefully and equally spread over the surface area of a slide (6 cm^2). The preparation is dried, fixed by flaming or by absolute alcohol and stained with erythrosin and dissolved fuchsin (1/300). Erythrosin is poured on the surface of the preparation and the latter is heated up until the erythrosin begins to vaporize. The dye is then is washed off, and dissolved fuchsin is poured on the preparation. After the same washing procedure the preparation is dried, a drop of immersion oil is placed on it's surface, and the slide is ready for examination.

2) Standard plate count

The standard plate count procedure provides a standard means of determining the density of aerobic and facultative anaerobic heterotrophic bacteria in water. The standard plate count is useful in judging the efficiency in operation of various water treatment processes and may have significant application as an in plant control test.

Procedure: Prepare two nutrient agar plates for each sample and confirm the sterility by keeping the plates at 35 ^{0}C. For each sample inoculate two culture plates, one with 0.1 ml and the other with 1 ml of the sample. Incubate the plates at 35 ^{0}C for 48 hours. Count the number of colonies in each plate and report the results as SPC in colony forming units/ml. Colonies should be counted promptly after completion of the incubation period. If counting must be delayed temporarily, store plates at 5 to 10 ^{0}C for a period of not more than 24 hours. For counting of colonies a counting aid like Quebec counter can be used.

The standard plate count is an empirical measurement because bacteria occur singly, in pairs, chains, clusters, or packets and no single growth medium or set of physical and chemical conditions can satisfy the physiological requirements of all bacteria in a water sample. Consequently, the number of colonies may be lower substantially than the actual number of viable bacteria present.

3) Total coliform and Faecal coliform count (Membrane filter technique)

The coliform group comprises all of the aerobic and facultative anaerobic, gram negative, non-spore forming, rod shaped bacteria that ferment lactose with gas formation within 48 hours at 35 ^{0}C. The standard test for the coliform group may be carried out either by the multiple-tube fermentation technique or by the membrane filter technique. The latter technique is simpler and less time taking. The results of the coliform test are being reported by the multiple-tube fermentation procedure as a Most Probable Number (MpN) index. It should be realised that this is merely an index of the number of coliform bacteria that, more probably than any other number, would give the results shown by the laboratory examination. It is not an actual enumeration of the coliform bacteria. By contrast, direct plaing methods such as membrane filter technique permit a direct count of coliform colonies. Here, the results are shown are as MF count per 100 ml.

Membrane Filter Count

a) *Selecting sample size:* Size of the sample will be governed by the expected bacterial density. An ideal quantity will result in the growth of about 50 coliform colonies and not more than 200 colonies of all types.

b) *Filtration of the sample:* Using sterile forceps, place a sterile filter (0.45 micron pore size) over the porous plate of the apparatus, grid side up. Carefully place the matched funnel unit over the receptacle and lock it in place. Filtration is then accomplished by passing the sample trhough the filter under partial vacuum. With the filter still in place, rinse the funnel by filtering three 20 to 30 ml portions of seterile dilution water between the samples. Unlock and remove the funnel, immediately remove the filter with sterile forceps, and place it on the sterile pad with MF endo medium with rolling motion to avoid the air. The petri dish with filter is then incubated at 35 ^{0}C for 24 hours.

c) *Counting:* the typical coliform colony has a pink to dark red colour with a metallic sheen on surface. The number of coliform colonies are counted with the aid of a low power 10 to 15 magnification) dissecting microscope or any other optical device.

d) *Calculation of coliform density:* Report the coliform density in terms of total coliforms per 100 ml. Compute the count, using membrane filters with 20 to 80 coliform colonies and not more than 200 colonies of all types per membrane by the following equation:

Total coliform colonies/100 ml= coliform colonies counted X 100/ml sample filtered

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HEAVY METALS AS AN INDICATOR OF STRESS CONDITION OF THE ECOSYSTEM

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Introduction

Biologically **stress** is a stimulus or succession of stimuli of such magnitude as to tend to disrupt the homeostasis of the organism. (Homeostasis - maintainance of internal constancy and an independence of the environment). The factors responsible for development of stress in the organisms of an ecosystem varies greatly with different magnitude of impact. Some of the stress factors may change the biochemical reactions of different enzyme and the organism can adopt to the changed situation, while in other case the organism may fail to adopt and may gradually disappear from the ecosystem which may even disrupt the existing food chain. The factors causing stress may be due to:

i) Physico-chemical alterations
eg: change in temperature, pH.

ii) Geomorphological alteration
eg: change in depth of an aquatic environment due to earthquake, siltation, weathering of rocks.

iii) Biological alteration
eg: change in population of an organism in a food chain.

iv) Anthropological interaction
eg: agricultural practice, industrial development, etc. leading to heavy metal accumulation; over-abstraction of water.

Many of the stress factors are interrelated in nature.

Heavy metals as a stress factor

The metals having specific gravity of approximately 5 or higher are called heavy metals eg: Cr, Mo, Mn, Fe, Co, Ni, Cu, Ag, Zn, Cd, Hg, etc. Some of these metals are essential nutrient elements of plants, animals and microorganisms (viz. Cr, Mo, Mn, Fe, Co, Cu, Zn) in trace amounts. Thus, their presence in desired concentration is very essential. Both very low and higher than the desired levels will create stress in different organisms. For nonessential elements, the lower concentration will not create any stress condition.

Source of heavy metals

The accumulation of heavy metals in an ecosystem may occur from any of the following sources-

i) weathering of rocks and minerals
ii) washings of opencast mines
iii) effluents of industrial and domestic origin
iv) agricultural practices like use of fertilizers, pesticides
v) movement of automobiles

Individual heavy metal

Survey of the literature indicates that mercury is most toxic among the heavy metals. A toxicity experiment of the metal chlorides on eels and cyprinodont fish *Orizias latipes* indicates the order of toxicity as

$$\begin{array}{ccccccc} Hg & \rangle & Cu & \rangle & Sn & \rangle & Fe^{2+} \rangle Mn \\ & & Zn & & Ni & & \\ & & Cd & & Fe^{3+} & & \end{array}$$

Mercury

It occurs at the levels of 10^{-6} ppm in sea water and is typically 0.001 - 0.007 ppm in fresh water, but concentrations may be much higher in waters receiving industrial effluents or the atmospheric deposition due to anthropogenic activities. In contaminated invertibrates it is at <0.1-10 ppm ww (wet weight) with 1-17% stored as methyl mercury. Fish store most of the methyl mercury in the muscle and thus is rapidly biomagnified through the aquatic food chain.

In general, the organomercurials are more toxic than the inorganic form. In an experiment of microbial growth with amended soil extract agar plate, $HgCl_2$ requirement was 20 times more than CH_3HgCl for similar growth. Mercury compounds can inhibit different enzyme activity and photosynthetic efficiency of microorganisms. Plants may absorb and concentrate Hg from soil; even to the extent that droplets of the metal have been found in the capsules of chick weed (*Holosteum umbellatum).* This build up of toxic metal may cause mitotic disturbances in the plant cells, which may prove to be lethal. Animals present at the top of a long food chain can accumulate very high amount of Hg in their body eg. in a food chain insectivorous bird-lacewings-aphid-plant grown on Hg treated soil, the birds can build up concentrations several thousand times than that of the soil concentration.

In case of fish with dietary exposure of mercury, the methylmercury form is absorbed about 5 times faster than the inorganic species with gut assimilation efficiency of 20%. Irrespective of the form of Hg and route of entry, significant portion of the body burden is accumulated in flesh. For many metals in contaminated lakes the body burden was found to increase linearly with size suggesting that there is an upper limit to the rate of excretion. For this reason highest concentrations of Hg have been found in large long lived specimen of tuna, soard fish, shark. When administered through food the toxic effect was expressed as regurgitation, extensive gastrointestinal and kidney damage. Mortality was notably absent even when the food was heavily contaminated @ 10 g/kg dw (dry weight) of $HgCl_2$. On the contrary, like other heavy

metals, the water borne component is very toxic to fish even at nanogram level. Acute LC_{50} 96 h on *Oreochromis mossambicus* was 0.0722 ppm

Accumulation of heavy metals in an ecosystem is always potentially harmful even if the form accumulated is safe to the organisms because the nontoxic form can undergo transformation by biological, chemical or physical forces to toxic form. One such incident occurred at Minamata, the industrial town of Kyushu island, Japan. Till date 400 people suffered, for more than 40 persons it proved fatal. Initial investigations considered the possibility of bacterial or viral infections and a range of heavy metals (Th, Pb, Mn) as being the causal agents of Minamata disease. The human deaths, in and around Minamata, coincided with large scale fish and shell fish mortalities. Latter it was discovered that methyl mercury was the causal agent. In the bay mud very high level (2100 ppm) of the toxicant was recorded. The cats, mice etc. were also affected. The question now appears where from the mercury came. Five years after the outbreak of the disease it was discovered that local chemical factory (Chisso) was using $HgSO_4$ as catalyst in acetaldehyde production and the relatively non toxic chemical was released with the effluents. That form of Hg was getting transformed to methylmercury. Now it is well known that under aquatic environment a number of fungus and bacteria under anaerobic or aerobic condition can produce methyl mercury. Fishing in Minamata Bay is highly restricted and it is estimated that about 400 tonnes of mercury are on the bottom of the bay close to the original source of effluent disposal area.

Copper

It is found in a variety of mineral deposits and therefore occurs naturally in sea water (0.0015-0.0582 ppm) and fresh water (<0.001 ppm). Pollution of the aquatic environment is mainly anthropogenic due to various industrial use and in the effluents the concentration may be as high as 90 ppm. The species of Cu present in the aquatic environment highly depends upon the pH of the medium. At pH <5, Cu^{2+} is the dominant form, at neutral pH with high alkalinity $CuCO_3$ predominates while at low alkalinity $Cu(OH)^+$ is formed. The organic matters readily complexes with copper and reduce both bioavailability and toxicity. For aquatic organism, the copper toxicity is due to Cu^{2+} and perhaps $Cu(OH)^+$ but not $CuCO_3$. The level of contamination in invertibrates is often 10-100 ppm dw but may be >1000 ppm dw in animals found near effluent discharge. Some of this contamination is passed on to fish through food chain but is not biomagnified since its accumulation can be well regulated, detected concentration <1-22 ppm dw. Copper is highly toxic to many microorganism, which are also used to control their growth. Gastropods are also affected greatly.

At high level of Cu exposure the fish gill structure is damaged with fusing of lamellae, lifting and swelling of lameller epithelium. Fresh water trout exposed to 0.311 ppm Cu at pH 7.9 caused 100% mortality within 24 h as a result of severe ionoregulatory and respiratory disturbances. Sea water trout under similar situation exhibited no significant change. This is due to relatively high concentration of Ca, more compensation of external binding sites, high carbonate alkalinity in sea water over fresh water. Dietary exposure of rainbow trout @ 3088 ppm for 8 weeks caused 28.8% mortality. Bioavailability of dietary Cu to rainbow trout is relatively less of 3.2%. At higher dose food regurgitation, epithelial lifting in the foregut were observed. Similar to mammals the muscle of fish does not become heavily contaminated. In channel cat fish *Ictalurus punctatus* at > 8 ppm dw Cu of food caused reduced growth and anemia, safe level was upto 4 ppm.

Zinc

Due to natural weathering of mineral deposits the freshwater Zn content is about 0.02 ppm and of seawater <0.0006 ppm but polluted effluents from anthropogenic source may contain upto 30 ppm of the metal. The invertibrates becomes polluted by Zn uptake from sediments and water and contain between 8-1290 ppm dw. Fish do not biomagnify the trophic contamination due to well regulation like Cu. For the same reason Zn level in fish is generally much lower than that of invertibrates from the same watercourse. Due to good regulatory system also Zn deficiency do not occur in fish.

The concentration of a metal in an organism do not itslef indicate that a stress condition is developed, sometimes the normal level in very high like hepatic Zn level of non-exposed squirrelfish is 2600 ppm.

The entry of Zn through diet caused no growth inhibition in the fingerlings of rainbow trout even at a concentration of 1700 ppm is food. Only Zn dissolved in water is of toxic concern. The major modifying physicochemical factor of Zn toxicity is hardness and pH of water. Typical 96 h LC_{50} concentrations are 1-10 ppm in soft water and 3-20 ppm in hardwater. The fishes of order Culpeiformes are found most sensitive. Zn causes hypoxia, caused by gross morphological damage to the gills - oedema, inflamation, cell sloughing and fusion- effects common for heavy metals in industrial concentrations. Low Zn concentration cause impairment of branchial Ca uptake lead to hypocalcemia. The most sensitive effect of Zn exposure is impairment of reproduction. In soft water 0.05 ppm Zn reduced the number of spawning and egg production. Oocyte production is dependent on vitellogenin synthesis with large amount of Ca requirement, absorption of which is hampered by Zn.

Cadmium

It is found at low levels (~ 0.0004 ppm) in natural waters. The invertibrates may bioconcentrate the metal, detected concentration is 0.13-56.6 ppm dw. These high levels of concentration are not biomagnified through the aquatic food chain to fish (0.02-0.09 ppm dw). The oral bioavailability of Cd is only 1-2% of the ingested dose in fish. Exposure of 10 g Cd/kg dw of food over 28 days in rainbow trout, equivalent to 68 mg/kg fish/day caused 42% mortality and should therefore be close to the LC_{50} dose. Sublethal dose caused hypocalcemia, great reduction of liver size, atrophy of proximal renal tubules etc. In mammals also Cd cause atrophy and distortion of renal tubules, leading to increased excretion of Ca. The intestinal Ca absorption is also impaired. All these lead to decalcification, skeletal deformation etc. popularly known as Itai-Itai (Ouch-Ouch) disease initially reported from northern Japan.

Lead

Surface layer of fresh water contain 0.0005 ppm of Pb while in open ocean it is <0.0001 ppm. Rivers in industrial regions often contain relatively large amount of Pb of 0.1 ppm. The natural dispersal of Pb is restricted due to its insolubility. The surface soil horizon of busy roads may contain Pb of > 1000 ppm. In microorganisms the metal binds with -SH, -NH_2, -NH functional groups of different enzymes and the bound Mn, Fe, Mg are lost. The water concentration of 0.05 ppm of the metal is toxic to the nervous system of fish. Black tails are the diagnostics of lead poisoning.

Chromium

The natural concentration in river water is 0.04 ppm and in sea water 0.00005 ppm. It is toxic in higher concentration to both plants and animals. Some algae show concentration factors of as high as 4000. Against *Oreochromis mossambicus* the 96 h LC_{50} was 30.83 ppm in one study.

Iron

Ferric (Fe^{3+}) form is much more toxic than ferrous (Fe^{2+}) form, at low pH the ferric hydroxide dominates and actually create toxicity. At 1.2-10.5 ppm level *Cyprinus carpio* was found to die due to precipitation of ferric hydroxide on gills, seen as brown deposits. Against *O. mossambicus* the 96 h LC_{50} was 83.20 ppm at pH 7 and 118 ppm at pH 8.5.

Nickel

Some clean streams are found to contain 50 ppm of Ni. Plants can accumulate Ni although it is not obligately required.

The recommended safe levels of heavy metals for fisheries purpose as given by the Environmental protection Agency (EPA), USA and for drinking water purpose as given by the Bureau of Indian Standards (BIS) are presented in Table 1.

Table 1. Permissible concentrations of heavy metals for different mode of use of the water resource.

Heavy Metal	Concentration in ppm or mgl^{-1}	
	Fish culture (EPA)	Drinking water (BIS)
Hg	0.0002	0.001
Cu	0.005 (10 ppm hardness) 0.02 (50 ppm hardness) 0.04 (100 ppm hardness)	0.05
Zn	0.01 (10 ppm hardness) 0.05 (>50 ppm hardness)	5.0
Cd	0.0004 (40 ppm hardness) 0.004 (400 ppm hardness)	0.01
Pb	0.005 (10 ppm hardness) 0.03 (> 10 ppm hardness)	0.1
Cr	0.05	0.05
Fe	1.0	0.3
Ni	0.01 (20 ppm hardness) 0.04 (320 ppm hardness)	-

Sample processing & Estimation

In general, the heavy metals are detected by Atomic Absorption or Plasma Emission Specrophotometric techniques. In AAS method the collected samples are first digested with strong oxidising acids like $HClO_4$, HNO_3, H_2SO_4 etc. to bring the biologically bound form of the metal to water soluble form. After preparation of sample by digestion, filtration, volume make up etc, the sample is ready for concentration measurement by AAS where metal specific cathode lamps are used as light source, the sample is atomised with the help of flame and excited by the use of cathode lamp (light source). The amount of light absorbed by the sample will depend upon the specific metal content. Standard curves are first prepared with the help of standard metal solutions and reading of unknown samples are matched to get its concentration. Proper care should be taken to avoid contaminations from the glass wares during sample processing and handling.

Heavy metal concentrations in the river Ganga and its tributaries

In the river Ganga, within the Rishikesh - Diamond Harbour stretch, the highest concentration of metals have been observed at Kanpur. In the tannery effluents in the area, the concentrations recorded during 1987 were Zn 0.285 ppm, Cu 0.179 ppm, Cr 0.200 ppm, Cd 0.014 ppm, Pb 0.026 ppm and Hg 0.001 ppm against the background levels of Zn 0.071 ppm, Cu 0.007 ppm and Cr, Cd, Pb, Hg below detectable limits at Hardwar. The heavy metal content of the Ganga river water at Kanpur indicates its unsuitability for fish culture. In the tidal stretch of the river, near Calcutta, very high levels of Zn (1.060 ppm) and Cr (0.588 ppm) in water were detected inspite of huge flushing action of tides in the region. In case of the river Yamuna also, the concentration of Zn and Cu were found to be higher than the safe levels.

The accumulation of heavy metals in different components of the ecosystems thus determine in many cases the healthy situation present in the system. The species composition may also be influenced greatly by the same factor due to their detrimental physical, physiological or genetic effects.

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BIOMONITORING OF PLANKTON IN CONTEXT OF POLLUTION ASSESSMENT

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The environmental pollution has become a serious problem in the country, because of rapid increase of population and concentration of factories around cities mostly near rivers. Lot of industrial waste water which is not being completely purified, is being discharged in open water systems of varied nature and causing various damages to biotic communities in aquatic ecosystem. Pollutants like organic, mercury and cadmium causing disease and oil contaminated fishes giving out abnormal smell while polluted mud problem is adversely affecting the biotops. On the other hand in agriculture sector the regular use of agricultural chemicals, fertilizers and water pollution by livestock excrement has become a serious problem for fisheries sector.

In aquatic systems such as rivers, coastal sea, inland sea, lakes and marshes where water exchange is insufficient, pollution has been increased by organic matters such as plankton, becuase the nutrient salts that contains a lot of nitrogen and phosphorus and the eutrophication has progressed because of addition of these elements from factories and household effluents. Accelarated eutrophication by phosphate and the foaming by Alkyl benzene sulfonate contained in synthetic detergent are also becoming serious problem for fishery. On the other hand, oil which run out from factory, waste oil and other industrial and life waste materials caused various damages to fishery. Thus various pollutants are adversely effecting the biodiversity of plankton in aquatic ecosystem.

Effect of water pollution on fishery

Pollution of water and river bed is producing serious effects directly or indirectly on fishery. In recent years causes of pollution are becoming more diverse and the effected area is spreading. Organic pollutant such as waste from cities and town bring about planktonic red blooms harmful for spawning and nursery area. Death of aquatic organisms and their escape from fishing ground result from decrease of micro-organisms.

Pollution of waterbodies and its beds by the accumulation of wastes and floating matters bring about the devastation of fishing grounds, makes sometimes the fishing impossible or decrease the effeciency and plankton productivity. Harmful matters *i.e.* heavy metals such as murcury and polychlorinated biphenyl from industrial wastes, besides giving physiological and ecological effect to aquatic biota, brings about the accumulation of these metals in fishes and shell fishes or appearance of deformities.

Discharge of warm waste water from thermal power plants also influences the aquatic biota.

Damage to aquatic biota from polluants

There are two ways in which the damage occurs to aquatic biota by polluants. As to the direct effect, the damage such as death of aquatic animals caused by inflow of deadly poisons or

of large amount of less poisonous substances. On the other hand, as to the indirect effect, damage appearing to aquatic biota as a result of the change of ecosystem and by the accumulation of poisons in bodies of aquatic animals.

Biomonitoring and prevention of damage caused by pollutants

For biomonitoring, the toxicity of agricultural chemicals against plankton was estimated in Japan by taking *Daphnia pulex* and *Moina macrocopa* as test animals and observing their median tolerance limit (TLM) after 3 hours. The chemicals were then divided into two groups according to their toxicity as shown in Table 1 below.

Table 1. Ranking standards for classification of Agricultural Chemicals by plankton toxicity

Rank	TLM 3 hrs. (Dephnids (ppm)
A	> 0.5
B	<0.5

A - No danger of toxicity is expected when used under the direction on the lebel.
B - Danger of toxicity is not usually expected but considerable care must be taken if used on a large scale.

For better biomonitoring results, as a matter of fact, it is important to carry out investigations and to choose safe agricultural chemicals according to the results obtained. Even among chemicals such as organophosphorus and carbomate insecticides which are not concentrated in living things but there are some which cause the appearance of deformities. So, we have to take good care in the use of agricultural chemicals. Kanazawa et al (1975) observed that carboryl and macbal of carbomate insecticides observed in soil particles become inactive against *Daphnia pulex*.

Eutrophication by nutrient salts such as nitrogen and phosphorus compounds in closed water region which together with other pollutants frequently brings about the appearance of red tide formed by plankton and does damage to fishery. As a result of investigations carried out in Japan on damage to fish by *Hemieutreptia anteque* HADA, the main plankton in red tide giving large damage, it is found that the main reason for the death of fish is that this plankton adheres to gills and cause the fish to die from suffocation. From experimentation on plankton effecting red tide, it has been observed that the minimum condition for the appearance of red tide is the concentration of nitrogen 0.1 ppm and that phosphorus of 0.015 ppm.

Chari and Kumar (1995) studied on doze mortality response of nuvan and bleaching powder on copepods. Field trials revealed that bleaching poweder @ 10 ppm and nuvan @ 50 μg per litre effectively killed copepods. However nuvan did not effect fish spawn while bleaching powder killed the spawn. Thus, use of insecticide like nuvan for agricultural crops is safe as its residual leaching in adjacent waterbodies will not effect plankton diversity. While contamination of bleaching powder in water bodies may have adverse effect on plankton diversity provided its concentration is considerably low.

Several studies have been made to show the trends of pesticide residue in various components of aquatic ecosystems. Thus, the residue levels (in ppm) in the four components of the aquatic ecosystems, namely, fish, plankton, water and mud were respectively 1.0, 0.1, 0.02 and 0.2 for lindane, heptachlor, aldrin and dieldrin; and 10.0, 2.0, 0.4 and 4.0 for endrin and methoxychlor (Hannon et al., 1970). In general, the pesticide risidue trend in aquatic ecosystems, appears to be as follows: water has the lowest average residue; bottom sediment, 18 times that of water; zooplankton and benthic algae, 37 times that of water; fish, 790 timesd that of water and aquatic insects, 7300 times that of water. Many studies indicate that the pesticides can be transferred from one organisms to the other through food chain. According to Rosato and Ferguson (1968) the fish exposed to 2 ppm of endrin for seven days, when fed to frogs, turtles water snakes, and birds, most of them died in 48 hrs. Obviously there is urgent need for judicious use of pesticides, so that the contamination of food chain in aquatic ecosystem can be avoided and better biodiversity of plankton could be sustained.

Environmental preservation

Biomonitoring of plankton in polluted openwater aquatic ecosystems is a difficult task but environment preservation, by maintaining natural surroundings in good condition and further promoting same through agriculture, forestry and fishery, seems to be the only answer to overcome pollutional problems. As these in integrated approach are meaningful for preservation of land and water, purification of water and air and protection of animals. It is, now, considered necessary to carry out the works on agriculature, forestry and fishery, taking necessary concordance with the reequired food productions, in such a way as to maintain the function of preservation of surroundings or even to promote it, as far as possible.

Prevention of eutrophication

Nutrient enrichment in water bodies accelerates densities of phytoplankton and inturn planktonic blooms are observed. This is indication of eutrophication which needs to be controlled through juducious biomonitoring of aquatic ecosystem. Fishing is a way to fix the incoming nutrient salt and to take it out of the water. The amount of fish cauhgt do not necessarily decrease the eutrophication in lakes and marshes, but some change occurs in the kind of fish suited for living and its growing rate: plankton-eating fish increases by eutrophication. Most of such valueless fishes is apt to bring nutrient back to water bottom soil and accelerates eutrophication in lakes and marshes.

If the average phosphorus content in the whole body of living fish is taken to be 0.4%, rate of collection of phosphorus by fishing is 1.4%-18% in the case of lakes in Japan. In shallow irrigation ponds, where circulation of water is good and moreover, there is no outflow of nutrient salts, but their recirculation, the ratio, at times reaches 20%-40%. By fishing, therefore, fairly large amount of phosphorus is collected. One way to increase the rate of collection further, may be to add fish-eating fish and to change the production system. At any rate it is necessary to bring up a group of fish, which has high flesh-increasing efficiency and is also suitable for fishing.

Thus, biomonitoring of plankton may serve a tool in assessing pollution in aquatic ecosystems. Also, it is necessary to establish appropriate techniques which will promote the preservation function of surroundings, at the sametime keeping harmony between production and environment.

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MACROPHYTE VEGETATION IN RIVER AS AN INDICATOR OF WATER QUALITY

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Introduction

With the growing industrialisation and chemo-mechanisation of agriculture all sorts of chemicals are continuously being introduced in the environment and these ultimately find their way into aquatic ecosystem. In the past attempts have been made to measure such qualitative changes in terms of purely chemicals, biochemicals and physical standards. However, after experiencing the difficulties in setting standards based on above methods only, the biological surveys and tests came into existence for better understanding and determining the quality of water.

Biological surveys deals with the interrelationship of living organisms-plants and animals with each other and with their environment. Plants are extremely imporant component of primary producers in aquatic ecosystem. They capture solar energy, produce oxygen, participate in nutrient cycle and provide habitat for a variety of aquatic life. In the last decade the importance of aquatic plants in the environment risk assessment process has been recognised. These are not only important as indicator of constraints of stresses but also serve as significant routes of chemical deposition, movement and bioavailability.

Plants as indicator of water quality

Use of macrophytes as indicator organisms of quality of river water is limitedly known and has been possible only in temperate climates where these aquatic vegetations have definite periodicity of appearance, and their diversity is not much in comparison to tropical climates. In India rivers in floodplains often contain wet or marshy areas where large strand of emergent vegetation or swamps develop (*eg. Typha, Phragmites, Cyperus, Scirpus, Monochoria* and grasses, etc.). In areas where there is not much tidal effect and conditions are suitably undisturbed, a typical gradation from emergent in shallow water to floating leaved (*eg. Ludwigia, Commelina, Paspalum, Hygrorhiza, Ipomoea, Alternanthera, Nymphaea, Nymphoides, Utricularia,* etc.) and finally submerged types (*eg. Ceratophyllum, Najas, Potamogeton, Vallisneria, Hydrilla,* etc.) in deeper water are found. Many of these river channels usually remain chocked with *Eichhornia crassipes*. This type of macrophyte colonisation is possible due to gradual siltation and accumulation of organic deposits in the river bed. There are however

rivers which have well defined channels with relatively small flood water, high current speeds and turbidity. These rivers tends to discourage dense growth of mariginal vegetation and support only stray patches of *Cyperus, Scirpus, Ipomoea cernea, Monochoria, Butomus* sp., etc.

Of the various factors responsible for distribution of a species in a particular site, the knowledge about its tolerance/intolerance to abiotic environment is most important and may help to use it as an indicator of a particular environment. A macrophyte species will be excluded from a site if it can not tolerate the abiotic condition to which it would be exposed through out its life cycle. On the other hand, the abundance of a species will be related to how nearer the conditions are optimal for its maximum growth. Such abiotic factors may be substratum, particle size, velocity of water, light, temperature, water chemistry and water level fluctuations, etc.

Macrophytes are however, not used as commonly as invertebrates species or microphytes as indicator of pollution in rivers. While reduction in the abundance of macrophytes that occasionally occur in the down stream of point of source of pollution can sometimes be ascribed to heavy metals or detergent toxicity, increased turbidity, and smothering of plants by silt or mining spoils. Macrophytes may also be shaded by dense population of sewage fungus, filamentous or epiphytic algae down stream of organic pollution. Heat pollution and changes in salinity may also alter species composition. Eutrophication is unlikely to increase macrophyte productivity in many low land rivers that already have sufficient phosphorus and nitrogen for optimum growth rates. On the other hand, it is usually associated with a loss of macrophyte diversity as highly competative species dominate.

For river basin survey it is thus imperative to identify the macrophyte infested areas and also to search out the reasons for their colonization. This besides helping in controlling organic contamination of the environment directly, would eliminate the problem of river bed chocking with macrophyte in due course of time.

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THE UTILITY OF BIOLOGICAL MARKERS IN ENVIRONMENTAL MONITORING PROGRAMME

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Aquatic environments are seen to be influenced by a number of ways. Sometimes the influences are natural events like the draught, flood, storm, geotectonic changes etc. in which the hydrological qualities of the environment are altered. Mostly, however, the influences are anthropogenic alterations like thermic, energetic, mechanical, acoustic, optical etc., caused by the processes of civilization, in which a number of substances are either added or discharged into the water. Many a times, toxic and harmful substances are released into the water bodies, like the discharge of a number of chemical and toxic compounds, by which process the water gets contaminated. These substances affect the physical and chemical qualities of water. The release of urban sewage to water also affects water bodies decreasing the dissolved oxygen.

Most of the living resources present in the water body remain sensitive to alterations in the environment and different biotic organisms are found to respond in different manners. The immediate responses may be the alterations in certain physiological pathways like the inhibition of some enzymes, reduction in growth and reproduction etc. The prolonged responses may lead to bioaccumulation of the harmful agents together with the possibilities of shifting to a more comfortable habitat. The severe responses, however, may lead to death of the organisms. Only when the responses of specific aquatic organisms to given changes have been ascertained, these organisms may then be employed to determine the quality of waterbody vis-a-vis the level of contaminating agents in the environment.

The biotic organisms, themselves, can be investigated in the aquatic environment which will exhibit the total effects of all impacts on the body of water. This study can be made over a prolonged period of time to observe changes with respect to seasons and the year. It can also be studied from place to place or at different locations in the aquatic environment and compared for relative changes in the quality of water or determining 'hot spots' in a stretch of environment. Additionally, laboratory studies following standard methods can be carried out with aquatic organisms using aliquots of water brought from the water body or effluents discharged into it. These tests, commonly known as 'biotests' can be used to obtain valuable information pertaining to the extent of adverse effects resulting from natural or man-made activities. This will also help in possible assessment of environmental impacts of compounds or effluents discharged into the system.

In some cases, either the biotests can be carried out alone or these tests may form a part of an integrated assessment approach in which data from biological tests are put together with data obtained from chemical tests, carried out on the water and sediments directly.

A further understanding of natural and man-made changes in the aquatic system can be better obtained by ways of combination of biological tests and ecological methods. These types of investigations are usually carried out for a prolonged period of time to obtain natural changes in biological variables as well as changes occurring by way of sudden activities or man-made slow interventions.

Uses of biological materials in environmental monitoring programme

The biological assessment of aquatic environment is gaining increasing importance in many countries and a large number of methodologies have been evolved that are being used, if not routinely, for the purpose. The presence of harmful substances of natural or artificial origin either causes a physical alteration in the habitat or alterations in aquatic environment for which a variety of changes are observed in biotic organisms. Broadly, these changes are : (i) alterations in species composition of aquatic communities or changes in the dominant group of a habitat. (ii) alterations in behaviour (avoidance, swimming mode), growth, reproduction, body physiological processes including histological changes and mophological deformations etc. (iii) alterations in survivality of whole community or their sensitive life stages like eggs, larvae etc. (iv) alterations in the natural microbial flora of the environment etc. Such changes are mainly caused by variations in the quality of aquatic environment. These changes, therefore, form the basis of biological method of assessment and monitoring of water qualities of wide and diverse nature. The changes allow accumulation of valuable information on the effects of anthropogenic activities on ecosystem including the presence of toxicants and deleterious effects on aquatic communities of the common pollutants like toxic metals, pesticides, organic compounds and other industrial and domestic discharges. The effects allow gathering information on the transformation of the pollutants in water, sediments or organisms or their long term effects like bioaccumulation, biomagnification etc. The effects permit determination of acute, chronic or genotoxicity etc. of the substances in the organisms. The observed changes finally permit the assessment of environmental protection measures. The biological methods of environmental monitoring programme can thus generate systematic information on the quality of water, define clean water by way of certain standards and permit management of aquatic bodies for raising valuable protein foods, like the fish and shell fish. An early warning system can also be devised from the observed changes.

Advantages of biological assessment

The advantages of biological assessment method are that they indicate changes in ecological system including the ecological damages. Since biotic communities integrate changes of environment throughout their life-cycle, the advantage lies in enabling the investigator to say about the past state of assessment as well as the present state. Protozoa, algae, bacteria etc. reflect water quality assessment for the past few weeks, whereas insects, worms, snails etc. reflect water qualities for periods much beyond that limit. Additionally, biological assessments are quicker and cheaper than chemical assessments which are costly and time consuming. The advantage of biological method does not, however, preclude the necessity for chemical analysis.

Another advantage lies in the use of acute toxicity tests in case of accidental contamination, which is of emergency nature. Since fish is an excellent tool for biological investigations; in case of accidental fish-kills, not only the samples of water is analysed chemically, toxicity tests using an aquatic organism from contaminated water are investigated simultaneously. This will enable the investigator to know whether toxic effects are present in one individual or it is a community kill. Although the responses of biotic organism will not indicate the type of contaminant or its concentration, the test will evidently allow the chemical examiner to pinpoint on the most toxic sample of water. Water quality managers and decision-makers can promptly take further necessary action. Remedial approaches can also be framed out of this.

Body response as an indicator

With respect to changes in the environment, the body responses of biotic organisms can be fruitfully utilized towards the assessment of water quality. Majority of the body determinations appear suitable for occasional assessments but fewer determinations appear suitable for routine assessments. Such body responses are developed with respect to particular water bodies, specific organisms etc. These are highly qualitative in analysis and are expensive. These tests are usually advanced ones, using say, determination of blood serum parameters like glucose or glycogen of specific tissues like the liver or muscle etc. They serve as indicators of stress in the environment. Sometimes, measurements of specific enzymes in tissues of aquatic organisms are done that can serve as indicators of oxygen depletion in the media or the presence of certain organic compounds.

Bioindicators and markers

While using the terms 'bioindicators' and 'biomarkers' in scientifically assessing the quality of an environment, the difference , if any, existing between the terms should be cleared. While the term 'bioindicator' emphasizes at the species level using the sensitivity of the species in demonstrating responses to a range of contaminants present in the environment; within the individuals of a bioindicator species, an in-depth study will reveal the ' markers' which will produce alteration in structure and functions of specific organs, particularly the physiological and morphological changes in cells and tissues as a consequence of exposure to contaminants. Thus, biomarkers are responses of living organisms (bioindicators) that may indicate exposure to contaminants including prediction of possible future effects.

A biomarker response states that a contaminant has been present in the environment that appeared available to the organism. It also ensures that the contaminant has reached the tissue or organ that has been affected by amounts over a period, considered adequate in exhibiting observed marker resposes. The harmful response depends on its progression level. The initial progression exhibits that a phenomenon called 'harm' has been started. Further, the adverse effect advances and that the deleterious effects started hindering growth, behaviour, reproduction etc. Finally the response leads to death of the organism that demonstrates significant population effects.

Although biomarkers are studied individually, the most practical application relates to a battery of markers to determine the total environmental impacts. A single biomarker test is usually carried out when only a single contaminant or a class of contaminants are present and where a highly sensitive biomarker test for their assessment is available. A number of markers are particularly useful in investigating various types or categories of contaminants.

Specific biomarkers for environmental monitoring programme

Metallothionein proteins

The Metallothioneins (MT) are low-molecular weight proteins (approx. 6000-7000 daltons) containing appreciably high amounts of sulphur-containing amino acid, the 'cysteine'(monoamino monocarboxylic amino acid) (approximately 30%). Cysteine of metallothionein contains - SH (thiol) group and this group is exceedingly capable of binding metal ions of Cu, Zn, Hg, Cd, Co, Bi, Ni etc. The protein is synthesized in the presence of the said metal ions and hence is an inducible protein, although some non-metal factors are also reported to induce MT. These proteins act as detoxyfying agents for metals. The metallothionein estimation is slow and costly. Therefore, in some cases, instead of the estimation of MT, the causative agents (metals) are directly estimated in the body to assess the quality of water with respect to contamination by metals. Yet, the estimation of the MT protein indicates the entry, pathway and time of exposure to the metal ions. Metal ions, on entering the cell, induce the synthesis of MT protein which then binds to the metal ions. More quicker methods of MT estimation are now-a-days coming up, that are linked to antibody-coupled reactions. Continuous upgradation of metallothionein assay method and its validation are necessary to apply it on a wider basis. The use of metallothionein estimation has been included as a biomarker for detecting metal contamination in the environment, as virtually all vertebrates and invertebrates appear to exhibit the presence of MT. Fish is reported to induce MT synthesis on exposure to metal ions and the amount of MT in fish tissues is considered as marker of the extent of contamination by metals.

Cholinesterase enzymes

The utility of the enzyme 'cholinesterase'as a biomarker has been indicated for various species including the fish. The cholinesterase-blocking pesticides like the organophosphates (malathion etc.) and carbamates (carbaryl etc.) demonstrate death in avian, mammalian and aquatic species by inhibition of this enzyme. Acetylcholine is involved in brain synaptic transmission. The enzyme hydrolyses the compound to choline and acetic acid, thus inhibiting brain responses and exhibiting fatal consequences. Although designed initially to kill insects and pests in agricultural fields, the ability of the organophosphates and carbamates to exhibit death in vertebrates provides an opportunity of assessing the effect of exposure of these compounds to fish. It thus appears possible to monitor fish and other aquatic organisms in different water bodies towards the possible contamination by organophosphate and carbamate pesticides. The cholinesterase activity is normally measured in brain tissues. However, blood measurements are also carried out. It is advantageous in the sense that, first of all, the enzyme is reported to be distributed in many tissues including the circulatory tissue (blood). Secondly, the estimation in blood does not necessitate the decapitation of the test species. Thirdly, the effects of continuous

exposure can be studied. More than half of inhibition of the brain enzyme activity together with presence of pesticide residue in the tissue are reported as the causes of death. Fish presents a valuable tool in demonstrating this, with reported brain enzyme inhibition varying between 40-80%. The carbamates do not exhibit as a consistent depressed activity as organophosphates exhibit towards the event of death.

Hepatic microsomal mixed function oxygenases (MFO)

The food substrates in the body are oxidized to carbon dioxide and water by the process called oxidation. In majority of the cases, these oxidation reactions remained coupled to energy productions that synthesize high energy phosphates (ATP). Such oxidations are usually carried out by the enzymes called dehydrogenases (carrying out oxidation) and oxidases (carrying out oxidation). However, there are altogether a different groups of oxidation reactions that are also being carried out in the body by the enzymes called 'oxygenases'. These oxygenases, instead of producing ATP, are involved in the breakdown of poisonous or harmful substanaces (drugs, pesticides or xenobiotics, toxic foreign chemicals etc.) that a living system encounters. Enzymes of this category catalyse the incorporation of oxygen into a foreign substrate (toxic compound, xenobiotic etc.). This takes place in two steps: i) oxygen binding to the enzyme at the active site and ii) the reaction in which the bound oxygen is transferred to the substrate. These oxygenases are divided into two categories: the dioxygenases (substrate + O_2 = substrate -O_2) and the monooxygenases (substrate - H + O_2 + ZH_2 = substrate - OH + H_2O + Z). While dioxygenases incorporate both atoms of molecular oxygen into the substrate, the monooxygenases incorporate only one atom of molecular oxygen to it. The other oxygen atom is reduced to water in the presence of an additional electron donor/acceptor (Z). Of particular interest is the monooxygenase enzyme system which is used as biomarker in assessing the presence of toxic compounds like the pesticides, drugs, polycyclic aromatic hydrocarbons (PAH), steroids etc. These systems are called microsomal MFOs because they are found in the microsome/endoplasmic reticulum of liver cells together with cytochrome - P450, which is a iron-containing (heme) protein. Both NAD/NADH and NADP/NADPH are involved in the transfer of reducing equivalents (electrons) for the back conversion of these cytochromes (reduction), which in turn, are again taken up by the substrates (foreign compounds) in a sequence of enzyme reactions, that can be viewed as hydroxylases also.

Drug/pesticide - H + O_2 + $2Fe^{++}$ + $2H^+$ —monoxygenase / hydroxylase— Drug/pesticide - OH + H_2O + $2Fe^{+++}$

(foreign compoound or xenobiotic) (Reduced cytochrome P450 protein) (metabolized xenobiotic) (Oxidized cytochrome P-450 protein)

This system is also called drug-metabolizing enzyme system and among the foreign compounds metabolized by this system are benzpyrine, aminopyrine, aniline, morphine, benzphetamine as well as drugs such as phenobarbital, several pesticides etc., that are capable of inducing the formation of hepatic microsomal MFOs and cytochrome- P450s. Proper conditions of estimation and specificity of response are required to be determined for each species with respect to the MFOs.

Most fishes are found to possess a very similar cytochrome P-450 dependent monooxygenase system that are induced by a number of PAHs, polychlorinated biphenyls (PCBs), dibenzo-p-dioxins and dibenzofurans.

There are about 50,000 commercially available chemicals, a large number of which are released into the biotic and abiotic world almost daily. Although, world chemical production exceeds 300,000,000 tons per year, it is still increasing and new range of products are constantly being manufactured. The damages posed by these amounts of chemicals to the life processes are easily imaginable. This strengthens the massive search for systems of elimination from, or detoxification processes by the body. One of the prominent examples of these systems is the monooxygenase system itself.

Cytochrome P-450 systems

A group of haemoproteins called cytochrome P-450s are there that are linked to detoxification processes in the body. These are present in almost all tissues, but found in exceedingly high amounts in liver. Being the central organ of metabolism, the liver receives special attention. These heamoproteins are found to be inducible and are induced by a variety of toxic, foreign and organic chemicals. In aquatic organisms, the activities of cytochrome P-450 dependent enzyme systems prove to be a meaningful bioindicator of pollution with respect to PAHs and PCBs. Chlorinated hydrocarbons like the organochlorine pesticides and insecticides, which are specific PAHs and PCBs and which are found in the aquatic system, induce cytochrome P-450 dependent enzymes in the hepatic microsomal fraction of fish. The enzyme is so named because it is blocked by the poisonous gas, CO and the CO-saturated complex shows absorption maxima at 450 nm of the spectrophotometric spectrum. Of particular interest is the environmental biomonitoring of aquatic bodies using fish, in which several environmental contaminants are able to induce the system. The cytochrome P-450 is reported to have two main types, the CyPIA and CyPIIB. The polyaromatic hydrocarbon (PAH) metabolizing enzymes like the Aryl hydrocarbon hydroxylase (AHH) and Ethoxyresorufin- O- dealkylase (EROD) are usually CyPIA-linked than CyPIIB linked; and in fish, CyPIA and not the CyPIIB is induced by the contaminants. Therefore, AHH and EROD are the cytochrome P-450 dependent monooxygenase estimations that are usually carried out in fish. Here, it differs a little from the other monooxygenases (CyPIIB). In mammalian and avian species, as there are CyPIIB family of cytochrome P-450s, those monooxygenases are normally assayed. The PAH type compounds are sometimes capable of elevating 100-fold inductions of certain monooxygenase activities. The fact that many of the inducing agents are well-known aquatic pollutants, they have greatly enhanced research with respect to fish cytochrome P-450 systems. Elevated levels of specific cytochrome P-450 activities in fish liver were reported near paper mills, bleaching craft mill effluents and oil-drill platforms.

Protein movements under electrophoretic fields

A more or less sensitive way of quantifying the pollutant induced cytochrome P-450 utilizes sodium dedecyl sulphate - polyacrylamide gel electrophoresis or SDS-PAGE. Coomassie brilliant blue R-250 may be used as a dye to stain the protein to demonstrate and quantify the presence of cytochrome P-450. Additionally, its binding with an antibody (polyclonal or monoclonal) specific to the cytochrome P-450 protein can be carried out

to develop Western Blot. The Western Blot methodology may permit assessment of cytochrome P-450 in samples that have not been stored for long time. In addition to mammalian and avian species tested so far, the technique finds application in aquatic species also. Whereas the constitutive cytochrome protein may have some activity in a particular monooxygenase assay, the Western Blot method may be specific for an individual or a family of induced cytochrome proteins.

Glutathione systems

An oxidative damage is sometimes seen in an organism when it is exposed to halogenated aromatic hydrocarbons, polyaromatic hydrocarbons, many other industrial organic solvents and some metals, like the selenium. Their metabolites are also seen to cause oxidative damages. When an oxidative stress or damage occurs, the protective systems which are usually the antioxidative systems exhibit adaptive responses. The cellular macromolecules may be modified. Finally, the tissue damages may occur. These alterations in the antioxidant systems or modification of macromolecules (proteins, nucleic acids, lipids) are used as biomarkers towards the exposure to contaminants. The protective systems may include oxidized glutathione (GSSG), reduced glutathione (GSH), glutathione reductase as well as some other enzymes like catalase, superoxide dismutase, peroxidase etc. and compounds like L-ascorbic acid (vitamin) and alpha- tocopherol (vitamin). Metallic selenium is reported to exhibit increased glutathione peroxidase and glutathione reductase activities, oxidized/reduced gluthathione ratio and lipid peroxidation. Polluted sites in aquatic environments exhibit elevated glutathione peroxidase activity, compared to unpolluted sites. Exposure to oils demonstrates varying levels of reduced glutathione. The utility of adaptive responses to oxidative stress in organisms, exposed to contaminants, is impressive. These markers can demonstrate effectiveness against a wide range of contaminants. However, it is better to use this marker in association with other markers, owing to slightly lesser specificities.

Thermoproteins

These are a group of proteins, called 'stress proteins', synthesized in the body in response to heat shock. Additionally their amounts are increased in response to a variety of chemicals and non-chemical stresses. These proteins can be used as biomarkers to some extent, against stress in general and thermoshock in particular. They are classed on the basis of molecular weights. Five classes of molecular weights have been reported so far that synchronizes with 90 K.dalton, 70 K.dalton, 60 K.dalton, 20 K.dalton and 7 K.dalton. The low-molecular weight heat stress proteins are usually not detected in non-stressed organisms. Heat stress is seen to induce proteins, the pattern of induction being specific to the types of stressors. The concept of heat stress proteins is modified increasingly.

Other biomarkers

Besides the above markers, a number of other bioindicators and biomarkers can be used in environmental monitoring programmes. They, however, require strict field and laboratory validations. Gall-bladder abnormalities and bile metabolites of contaminants in fish may reveal exposure to contamination. This observation is now-a-days extended to animals other than fish. Among other markers, the ATP synthesis and tissue energy

reserves, blood serum parameters of essential indices and DNA damages can be investigated. Aquatic plants, non-rooted and rooted vascular plants are sometimes used for indicating the presence of toxicant levels. Of particular interest may be the studies on *Eichhornia crassipes*. Microalgae (unicellular algae, diatoms, other phytoplanktons) and macroalgae (macrophytic algae) are sometimes studied. Their photosynthetic inhibition, in the presence of toxicants, is used as indicator. Zooplanktons, (rotifers etc.), periphytons, protozoa, etc. can also be conveniently used for the purpose. Mussels, sometimes, excellently indicate the extent, not only of metal pollution but also of several organic pollutions. Phase infection and replication on *E. coli* cells (coliphage) are seen to indicate extent of sewage pollution quite excellently.

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FISH AS A TOOL FOR BIO-MONITORING AQUATIC ENVIRONMENT

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Bio monitoring programmes have been very useful in identifying numerous local, regional and national ecotoxicological problems. Bio-indices used as tools in biomonitoring programme help in assessing bioavailability of contaminants, may serve as markers of specific classess of chemicals and serve as an early warning of population and community stress. Systematically, the bio-indices, more precisely biological responses of intrinsic or extrinsic factors can be obtained from an organism, a community or an ecosystem provided the exposure is adequate to influence biological functions or systems. Fish, at highest of the aquatic trophic level and hardy creatures with long life and wide adoptibility have the advantages to be considered most ideal and effective in biomonitoring programmes. Use of fish in this programme may be multipurpose, in chronic toxicity studies for assessing bio-accumulation, microlevel impact of toxicants on community components and also in evaluation of toxicity of various contaminants through acute bio-assay.

Fish toxicity bio-assay

Toxicity tests are principally testing of responses in living organisms exposed to toxic agents depending upon doses and time of exposure. Broadly, toxicity tests are categorised as acute toxicity which is usually designed to evaluate the concentration mortality relationship between the toxicant and test animals and the other chronic toxicity under taken for ascertaining long terms sublethal toxicity of toxicants on different life processes like growth, reproduction and survival of the exposed animals.

Acute toxicity

Acute toxicity most often measures effects of toxicants on survival over 24 to 96 hours period. In general, for fish as test animals, the small size, easy handling and widely available species are chosen for toxicological experiments. Fathead minnow (*Pimephales promales),* rainbow trout *(Oncorhynchus mykiss),* blue gill *(Leponus puntatus),* sheep head minnow *(Cyprintodon variegatus)* and gold fish (*Carassius auratus*) are widly used fish species in toxicity studies. Species like *Cyprinus carpio, Oreochromis mossambicus, Carassius auratus, Puntius ticto, P. sophore, Gambusia affinis, Clarius batrachus, Heteropneustis fossilis* and *Channa* spp. are often being used in such studies in India.

Mode of exposing test organisms in toxicants may be static or flow through depending on the nature of toxicant and also facilities available in hand. In general, flow through method of toxicity bio-assay is recommended for the toxicants which are highly degradable and changing original characteristics every now and then. Such substances are human wastes, city sewage, cattle shed wastes and so on. In static toxicity bio-assay, the media needs renewal every 24 hourly for the maintenance of consistancy in the doses of

exposed toxicants and also to avoid additive toxicity of the metabolise released by the exposed test animals. The fishes exposed in acute toxicity bio-assay are examined for behavioural changes, physiological stress like asphyxia, biological abnormalities leading capillary rupture and blood oozing and over sliming etc. Over and above the mortality percent at different times of intervals are recorded and the data obtained are used in statistical treatment for deriving the concentration mortality relationship for toxic substance and the tested animals.

Chronic toxicity

The term "Chronic"indicates any thing relating to long duration of time and for toxicity it is long term exposure for testing poisonous effects of toxicants to the exposed tests animals. When the concentration of any pollutant in environment is below acute toxicity range and unable to cause mortality of a exposed animals within a period of few hours or days is considered to be sublethal. The method of sublethal toxicity evaluation is quite different from acute toxicity bio-assay. Unlike mortality time and concentration of toxicants being the prime consideration in acute toxicity testing, sublethal toxicity evaluation is more dependent on the assessment of biological/physiological implication of the toxicants on the exposed animals. For chronic toxicity evaluation, the test animals are exposed in sublethal concentration of toxicants for the period so long the exposed animals are in a position to reflect adverse effect of toxicants on their biological activities like growth, respiration, digestion, excretion, reproduction and also hormonal, biochemical and haematological characteristics.

Fish by virtue of possessing well distinguised mechanisms of different physiological functions beside many of them being easy and captive breeders gained priority in chronic bio-assay experiments all over the world. In chronic toxicity bio-assay the early life stages of test organism are exposed to sublethal concentration of the toxicants and allowed to complete life cycle at least for the first generation. For fish, spawn and fry are most ideal life stages for conducting chronic bio-assay experiments.

Fish for contamination assessment

Most problematic issue in environmental pollution is with the handling of persistant non biodegradable substances like metals, pesticides, atomic wastes etc. which are very harmful to the biotic components even at lowerst concentration in the environment. Many of these toxic substances enter the aquatic system from non point sources and are difficult to be controlled/regulated from their introduction into the open water ecosystems. These contaminants contaminate the water and sediments in the first hand and gradually penetrate biotic communities inhabiting the ecosystem. The food chain components once in contact with accumulative toxicant start accumulating them in body tissue. Systematically the accumulated toxicants get magnified from lower to the higher trophic levels. Tissue wise assessment of contaminants and resultant impact evaluation become difficult task if the organisms are very small and delicate to handle. Moreover, the obtained results from any investigation using small test organisms may not be accurate and dependable. To overcome such difficulties fishes are in world wide use for monitoring the contaminating substances in the environment and evaluating their harmful effects on the biocommunities.

Fish as bio indicators

Use of bio indicators in pollutional studies is well established and being practised in particular for biological scoring in environmental monitoring programmes. The indicator organisms may be unicellular bacteria or nannoplankton to animals of higher trophic levels like fish and reptiles. The concept of using bio indicators in environmental monitoring is based on diversity in tolerance power of animals in adverse environmental conditons. Most tolerant, medium tolerant and least tolerant are the three general categories of animals in consideration of their tolerance power. However, apart from the use in saprobic system of zoning aquatic environment, the fish diversity has been accepted as an important index of community impact assessment.

Eutrification of lakes is very common, particularly those of medium and small size which are exposed to sewage drainage or runoff from the agricultural or diary activities. Though, as indicators of eutrification, small animals or plants of lower trophic levels are widely used fishes also may act as indicator organisms fulfilling similar objectives. The species with accessory respiratory mechanisms can withstand anoxic condition of an eutrophic ecosystem. *Channa* spp., *Clarius batrachus*, *Anabas testudeneous*, *Heteropteneustis fossilis* are most hardy species and their dominance in fish community indicates anoxic environment in the inhabited ecosystem. Similarly, *Ailia coilia*, a small catfish is very sensetive to environmental conditions and any change in the ecosystem quality affects their propagation and survival. Obviously presence of this species is an indication of conducive environment of the aquatic ecosystem.

Inclusion of fish in Environmental Impact Assessment is because of their direct or indicrect effects on ecosystem functioning. Fish predation is known to alter plankton community composition. In case of natural ecosystem evaluation, use of a single species may not be sufficient for natural fish communities for a number of reasons. First, inharent sensitivity of the individual species varies. Secondly, a variety of life history adaptation like feeding in differnet nitches by different species and also by different life stages of a same species, leads differential exposure to chemicals. However, only fishes are not adequate in biocommunity response assessment and must be supported by communities containing additional trophic levels for Environmental Impact Assessment. Karr and Colleagues developed an Index of Biotic Intregity (IBI) designed to reveal the integrative nature of fish communities as they respond to changes in water quality.

Metrics	**Ratiang of metric**		
	5	3	1
A. Species richness and camposition			
1. Total number of native species	Expected value for individual varies with stream order and region		
2. Number and identity of benthic species			
3. Number and identity of column species			

4. Number and identity of surface species			
5. Number and identity of tolerant species			
6. Percentage of individuals as to total species	<5	5-20	>20
B. Trophic composition			
7. Percentage of individuals as omnivores	<20	20-45	>45
8. Percentage of individuals as insectivores	>45	45-20	<20
9. Percentage of individuals as to carnivores	<5	5-1	<1
C. Fish abundance and condition			
10. Number of individual in sample	Expected value for number of individuals varies with stream order and physical habitat		
11. Percentage of individuals as hybrids or exotics	0	>0-1	>1
12. Percentage of individuals with disease, tumor skeletal abnormalities	0-2	>2-5	>5

Environmental stress and fish health

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The homoestatic control systems of fish are continualy impacted by the normal demands of the aquatic environment itself. Superimposed on this may be the effects of adverse environmental conditions including pollutants, land or water projects developments within the area and, in the case of fish in intensive culture, the effects of operating procedures such as handling, crowding, transporting and disease treatments.

Definition of stress

The term stress or stressor or stress factor is defined as the force or challenge in response to which there is a compensatory physiological change in fish. Thus, an environmental or biological stress is of significance if it requires a compensating response by a fish, population or ecosystem.

Environmental alterations commonly encountered creating fish stress

Aquatic environment due to anthropogenic activities are subjected to various physico-chemical stresses either of short or prolonged duration. These effect the health of resident fish populations. Some of the very interesting examples of alteration in the aquatic environment investigated resulting in stress to fish populations are given below.

A. A sewage fed freshwater wetland

This wetland receives municipal sewage from Calcutta and fish. Indian major carps and *Tilapia* are cultured. The environmental parameters discussed below obviously act as predisposing stress factor for fish. The environmental parameters of importance creating stress to fish are given in Table 1.

Here the dissolved oxygen ranges between 0 and 18 mgl^{-1} during 24 hrs. The rate of change of DO per hour in the midday and during night is around 3 mg l^{-1} indicating high rate of photosynthesis in day time and high rate of respiration after dusk. Microbial consumption of DO per hour is 2.4 mgl^{-1}, indicating that DO is exhausted at night in the absence of light at a very fast rate. Transparency range is between 12 and 14 cm mainly because of planktonic bloom. The range of unionised ammonia present during the investigation is high.

It is thus evident from the observation that the high organic load, low transparency and high phosphate level is indicative of entrophic condition in the ecosystem. The high microbial consumption of DO per hour (2-4 mg l^{-1}) indicates exhaustion of DO for a few hours at night creating a stressed condition to fish. Moreover, the high fluctuation in pH, and consistently high levels of unionised ammonia create chronic stress to fish.

B. Sewage fed saline wetlands

Observations on the water quality in some of these wetlands where prawn and fish culture is done are given in Table 2.

These wetlands had organic matter in the decomposing phaze with consequent higher concentration of unionised ammonia. As a result in all these wetland except Agamaru the fishes are stressed and fish and prawn diseases are prevalent.

C. Water quality of some EUS affected water bodies

The water quality of some of these water bodies where fishes are affected by EUS is given in Table 3. In the outbreak of EUS low alkalinity and hardness associated with the low calcium acidic soils acts as a pre disposing stress factor for outbreak of EUS. This is very much evident in some of the water bodies in Assam, Tripura, Meghalaya, Bihar, Kerala where intensity of the disease outbreak is severe.

Methods for stress diagnosis

Several biochemical and physiological procedures have been developed to assess the severity of the physiological effects resulting from stress. The physiological parameters of importance for assessing stress in fish at the primary, secondary and tertiary levels are discussed below.

General Adaptation Syndrome (GAS)

The various physiological changes that occur as a fish respond to stressful stimulus are compensatory or in other words it is adaptive in nature and are required for acclimation. Collectively these phenomenon has been termed General Adaption Syndrome.

Conceptual Frame work of Stress response

The conceptual frame work is to consider the stress response in terms of primary secondary and tertiary changes.

i) *Primary response :* Following perception of a stressful stimulus by the central nervous system the stress hormones *viz.*, cortisol and epinephrine are synthesized and released into the blood stream.

ii) *Secondary response :* Changes in the blood and tissue chemistry and in the haematology occur, such as elevated blood sugar levels and reduced clotting time. Diuresis begins followed by blood electrolyte losses and osmoregulatory dysfunction. Tissue changes, include depletion of liver glycogen and interrenal Vit. C, hypertrophy of interrenal body.

iii) *Tertiary response :* Manifest in reduction of growth, resistance to diseases, reproductive success and survival. These may decrease recruitment to succeeding life stages as a result population decline occur.

A. Use of the physiological response as indicators of stress

Several of the many changes that occur in response to stress can be used as measurable indices of the severity of stress on fish. These changes are a direct or indirect result of the physiological response to environmental changes and can be quantified and used as predictive indices.

Primary stress response

Plasma cortisol : A relatively direct assessment of the severity and duration of the primary stress response can be obtained by monitoring the rise and fall of plasma cortisol or catecholamines (epinephrine and nor epinephrine) concentrations.

Secondary stress response

The secondary changes that occur mainly in the blood chemistry also characterize the severity of stress in fishes *viz.,* blood glucose, chloride, lactic acid. They are frequently used for assessing stress response. Hyperglycemia for blood glucose and hypochloremia for blood chloride is the physiological effect of concern during stress response. Accumulation of lactic acid in muscle or blood hyperlacticemia is also an indicator of stress due to bright or severe exertion.

The haematological parameters also provide useful information about an animals tolerance to stress.

Haemoglobin/Haematocrit : Its increase or decrease following acute stress can indicate whether haemodilution or haemoconcentration has occurred.

Leucocyte decrease (leucopenia) commonly occur during the physiological response to acute stressors. The blood clotting time and changes in the leucocyte count are among the most sensitive parameters indicating stress response.

Histopathology : Since many of the biochemical changes that occur in response to stress are the end result of cellular pathology histological examinations can frequently provide information on the effect of stress factors on fish. For example interrenal hypertrophy, atrophy of the gastric mucosa and cellular changes in gills are indicative of stress response.

The physiological tests of importance and their interpretations are given in Table 4.

Tertiary stress response

Experience have shown that several tertiary stress responses including changes in the metabolic rate, health, behaviour, growth, survival and reproductive success can indicate that unfavourable environmental conditions have exceeded acclimation tolerance limits of fish.

Metabolic rate : It is a fundamental aspect of animals performance and is affected by stress.

Reproduction : Detrimental effects on reproduction as manifested by oocyte atresia, spawning inhibition and decreased fecundity and hatching success are taken into consideration for assessing stress response.

Disease : Incidence of fish disease is an important indicator of environmental stress. Fish disease is actually the outcome of the interaction between the fish, their pathogens and the environment. If the environment deteriorates stressed fish is unable to resist the pathogens that they normally can resist. Certain diseases are proving to be useful indicators that tolerances of adverse environmental conditions have been exceeded.

B. Biological indicators of stress

In all of these water areas mentioned above the very common initial symptom of stress exhibited by fishes is excessive secretion of mucus from gills and body surface. In fact this physiological aspect of fishes can be fruitfully utilized for fish stress detection or detection of suboptimal water quality.

There are certain trichodinid parasites (*Trichodina* sp., *Tripartiella* sp.) ubiquitously present in fish gills especially of Indian major carps which can serve as good indicator of stress in fish. Excessive mucus secretion serve as substrate for these trichodinids which increase in number. A methodology has been developed where the presence of these trichodinids above 20 numbers in 0.05 ml of gill mucus in indicative of stress. (Table 5).

Conclusion

Thus it is apparent that knowledge of the tolerance limits for acclimation to the single or cumulative effects of various biotic and abiotic stress factors is an important part of the data base for species habitat relationship needed for effective fishery management. Such information will solve many problems ranging from prediction of the tolerance fish will have for proposed habitat alterations to evaluation of the effects on fish health exerted by modern intensive fish culture.

TABLE - 1

TIME IN HOURS	pH	DO mg^{-1}	TRANSPARENCY cm.	GROSS PRIMARY PRODUCTION $mgCm^{-3}hr^{-1}$.	PHOSPHATE-P mg^{-1}	AMMONIUM-N mg^{-1}	FREE AMMONIA mg^{-1}	NITRATE—N mg^{-1}	CARBONDIOXIDE mg^{-1}	BICARBONATE mgl^{-1}	SALINITY ppt.	TEMP. °C	CARBONATE mg^{-1}
03.00	7.9	0.5	13.0	1800.0	0.80	1.40	0.084	0.10	16.0	188.0	0.35	29.0	nil
09.00	8.0	3.5	-	-	2.00	1.20	0.108	0.25	6.0	210.0	0.36	33.5	nil
15.00	9.1	18.0	-	-	0.08	1.10	0.605	0.28	nil	180.0	0.35	36.0	nil
21.00	8.0	nil	-	-	1.20	1.00	0.090	0.38	6.0	200.0	0.35	32.5	nil

TABLE - 2

Name of	pH	Alkalinity ppm	Hardness ppm	Unionised Ammonia PPm	Salinity
Agamura (Unaffected)	8.3	127	18000	0.1	9.02
Beel Samily	8.5	126	3000	0.5	10.25
Kathore	8.6	127	2800	0.3	6.4
Tripley	9.0	147	3200	0.2	7.0
Maligada	8.6	125	3000	1.1	9.0

TABLE - 3

State	pH	Alkalinity (mgl^{-1})	Hardness (mgl^{-1})	Chloride (mgl^{-1})	Free CO_2 (mgl^{-1})	Ammonia (mgl^{-1})	Salinity ppt
Assam	7.1-7.5	13-74	11-38	4-23	4-10	N-0.4	
Tripura	6.7-7.6	7-49	9-45	3.5-18	2-8	N-O.6	
Meghalaya	6.5-7.5	7-14	10-15	2-12	4-6		
West Bengal	6.7-7.5	10-170	6-180	2.9-13	2-7	N-0.6	N-1.0
Bihar	6.1-6.8	25-30	13-20	4.7-7	4.0	1.8-2	
Orissa	6.8-7.4	44-138	55-180				1.5
Uttar Pradesh	7.5-8.0	40-217	42-234	0-5.8			
Tamil Nadu	7.8-8.3	103-139	105-158				
Rajasthan	8.0-8.2	140-150	80-90				
Maharashrta	7.5-9.5	30-115	48-140		2.5-3.0		
Kerala	6.3-7.0	0-11	8-17	0.34			1.0

Table 5 : Recommended physiological tests to assess the tolerance limits of fish for abiotic and biotic stress factors (compiled from Passino 1984; Buckley *et al.*, 1985). The interpretations of responses listed are general but not necessarily universal; investigators should be aware that there may be some stressful situations that do not evoke a change in one or more of these physiological conditions.

Physiological test	Interpretation if results are	
	Low	High
Blood cell counts	**Blood variables**	
Erythrocytes	Anemias, hemodilution due to impaired osmoregulation	Stress polycythemia, dehydration, hemoconcentration due to gill damage
Leucocytes	Leucopenia due to acute stress	Leucocytosis due to bacterial infection
Thrombocytes	Abnormal blood-clotting time	Thromobcytosis due to acute or chronic stress
Chloride, plasma	Gill chloride cell damage, compromised osmoregulation	Hemoconcentration, compromised osmoregulation
Cholesterol, plasma	Impaired lipid metabolism	Chronic stress, dietary lipid imbalance
Clotting time, blood	Acute stress, thrombocytopenia	Sulfonamides or antibiotic disease treatments affecting the intestinal microflora
Cortisol, plasma	Normal conditions	Chronic or acute stress
Glucose, plasma	Inanition	Acute or chronic stress
Hematocrit, blood	Anemias , hemodilution	Hemoconcentration due to gill damage, dehydration, stress polycythemia
Hemoglobin, blood	Anemias, hemodilution, nutritional disease	Hemoconcentration due to gill damage, dehydration, stress polycythemia
Hemoglobin, mucus	Normal conditions	Acute stress
Lactic acid, blood	Normal conditions	Acute or chronic stress, swimming fatigue
Leucocrit	Acute stress	Leucocytosis, subclinical infections
Blood osmolality, plasma	External parasite infestation, contaminant exposure, hemodilution	Dehydration, salinity increases in excess of osmoregulatory capacity, diuresis, acidosis
Blood total protein, plasma	Infectious disease, kidney damage, nutritional imbalance, inanition	Hemoconcentration, impaired water balance
Tissue variables		
Adenylate energy charge, muscle and liver	Bioenergetic demands of chronic stress	No recognized significance
Gastric atrophy	Normal conditions	Chronic stress
Glycogen, liver and muscle	Chronic stress, inanition	Liver damage due to excessive vacuolation, diet dtoo high in carbohydrates
Interrenal hypertrophy, cell size and nuclear diameter	No recognized significance	Chronic stress
RNA : DNA ratios, muscle	Impaired growth, chronic stress	Good growth

BIOMONITORING IN THE RIVER GANGA - A CASE STUDY

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Introduction

During recent years the water quality and quantity of the mighty river Ganga has gone down considerably due to increased deforestation in its catchment areas, rapid development of industries on the river banks, development of irrigation projects, many fold increase in the discharge of domestic, industrial, agricultural wastes into the system and river modifications along with populations explosion. These have affected adversely the river biota including the fish community. With a view to assess soil and water quality, community organisation of plankton, periphyton, macrozoobenthos and fish fauna, an exploratory survey was carried out by the Central Inland Capture Fisheries Research Institute during 1995 to 1996 at 43 selected centres of the river Ganga, Bhagirathi and Hooghly estuarine system from Tehri to Gangetic delta (Sunderbans).

Study area

The Ganga is 2,525 km long and its basin drains about one fourth of the countries area. At present the surface water availability in the Ganga basin is about 446 million acre feet (MAP). The annual flow of freshwater in the Ganges is estimated at 142.6 billion m^3 resulting from the melting of snow in Himalayas during the spring and hot months and monsoon rains during June to September. The river Ganga has an annual runoff of 493 km^3 and carries 616 x 10^6 tonnes of suspended solids to the Hooghly estuary. The Ganga, considered as the second major river of the world in terms of suspended load is the main contributor of sediment to the Bengal Fan which is the largest deep sea fan in the world. Many major and minor tributaries join the river Ganga in its entire course. The Ganga river basin is well developed becausxe of communication, industrialisation and urbanisation. Accordingly, both the banks of the entire river course are thickly populated covering Uttar Pradesh, Bihar and West Bengal states. Along the course from Gangotri to Sagar, there are 29 major cities, more than 70 towns and thousand of villages along with 132 large industrial units (86 in U.P., 3 in Bihar and 43 in West Bengal). Each of the cities, Haridwar, Farukhabad, Mirzapur and Bhagalpur expell 16 MLD of sewage effluent which Allahabad and Varanasi discharge 100 MLD of sewage into the river Ganga. At Patna the river receives 154 MLD sewage and at Kanpur 275 MLD. The total volume of waste water discharge over the 92 km length of river Hooghly works out to be 1,135.6 MLD.

The exploratory survey was carried out at 43 selected centres. Of these, nine centres (Tehri, Deoprayag, Rishikesh, Haridwar, Bijnor, Garhmukteswar, Anupsahar, Farukhabad and Kannauj) are situated in the upper stretch; eight centres (Kanpur, Dalmau, Allahabad, Mirzapur, Varanasi, Ghazipur, Buxar and Patna) are in the middle stretch; eight (Sultanpur, Barauni, Munger, Bhagalpur, Kahalgaon, Manickchak, Farakka and Dhulian) are in the lower stretch; two centres (Berhampur and Katwah) are on Bhagirathi river; while rest sixteen centres are situated in the Hooghly-Matlah estuarine system. Among these centres, Nabadwip, Krishnanagar, Tribeni,

Titagarh, Dakshineshwar, Uluberia, Roychowk, Kakdwip and Frazerganj are on the main Hooghly channel. Haldia, Bhagabatpur and Moipeeth centres are located in the Haldi, Saptamukhi and Thakuran estuaries respectively, Canning and Jharkhali centres are on Matlah estuary, while Bagna and Hasnabad centres are on Roymangal and Ichamati estuaries respectively.

Soil and physico-chemical characteristics

The study inferes that the entire river bed from Haridwar to Patna has been transformed into sandy soil with 79 to 99.7% sand and nil to 12% clay. The stretch upto Farakka is already under threat where the sand percentage is 48 to 54%. In the stretch between Sultanpur and Uluberia (upper delta region) the bed soil is more or less loamy in texture. The bed soil contains 31 to 79% sand, 12 to 60% silt and 5 to 30% clay. Lower zone of Hooghly estuary and adjacent estuaries contain high percengate of silt and clay particles forming 25-58% and 11 to 36% of the bed soil respectively. The study revealed that the stretch between Tehri and Patna suffers severely from textural deformity and the entire stretch is blanketted by sand drifted through a number of tributaries *viz.*, Ramganga, Yamuna, Gomti, Ghagra, Sone and Gandak. The denuded catchment washings are also responsible for the deformation of the river bed. The sandy bed of the upper and middle stretches of river Ganga can naturally contribute very little to the aquatic productivity.

The entire river bed soil has been found to have slightly alkaline to alkaline pH. Moderately alkaline (pH 7.9 to 8.9) soil has been observed in the estuarine stretch between Roychowk and Hasnabad. The allochthonous materials carried by Ganga is generally deposited in this zone making it alkaline. Soil with alkaline reaction are comparatively less responsive than neutral soil, both for agriculture and aquaculture production. Organic carbon, total nitrogen and available phosphate contents in bed soil was low in the freshwater stretches of the river Ganga as compared to estuarine stretch, indicating that the estuarine region is more productive than freshwater region.

The water pH observed was generally true reflection of the soil of the area. The present value of water pH of upper, middle and lower stretches of the Ganga ranged from 7.3 to 8.6; 7.0 to 8.8 and 7.3 to 8.8 respectively. These values were almost similar to observed values of pH in 1960 and 1984. This is so because the Ganga water has a high buffering capacity. Almost similar trend was observed in case of Hooghly estuary. Appreciable improvement in dissolved oxygen content of water was noticed in the middle (3.4 to 11.9 mg/l) and lower (4.8 to 9.6 mg/l) stretches of the river system as compared to 1985-90 period. Considerable increased value of dissolved oxygen (6.0 to 8.2 mg/l) was also observed in the estuarine system. As regards phosphate content, the entire upper stretch from Tehri to Kannauj had very low values (trace to 0.31 mg/l). In the middle stretch, besides Kanpur, Allahabad and Varanasi, the value of phosphate was found to be trace to 0.4 mg/l during summer months. Higher values were recorded at Kanpur (2.5 mg/l), Allahabad (0.8 mg/l) and Varanasi (1.05 mg/l) in the present study as compared to values in 1960 and 1985-90 periods. In the lower stretch, phosphate value was observed between 0.045 and 0.130 mg/l.

In the estuarine system the fluctuation of phosphate value was 0.020 to 0.160 mg/l. While during 1953-55 period, the phosphate content was very low. After commissioning of Farakka barrage the phosphate content has shown some improvement. The present values of nitrate (trace to 0.86 mg/l) in the river water as compared to earlier values indicate the improved

condition of water quality as well as lower degree of pollution. Increased level of nitrate (0.05 to 0.54 mg/l) was also recorded from the estuarine stretch barring Tribeni centre in the present study as compared to very low values ranging from 0.03 to 0.12 mg recorded during 1985-90. Silicate content was moderately high (4.5 to 9.4 mg/l) in the riverine stretch between Tehri and Katwah. In the freshwater zone of Hooghly estuary, it was also moderately high (5.4 to 12.4 mg/l). However, it was poor (0.5 to 5.9 mg/l) in the marine zone of the estuary. A critical analysis of the earlier works during pre Farakka barrage period and post barrage period relevant to hydrology revealed that additional discharges of freshwater through Farakka barrage had changed the ecology of the system significantly by reducing salinity and converting the earlier gradient zone into almost freshwate one. The present study indicates that the salinity incursion of the Hooghly estuary was observed upto Diamond Harbour (near Roychowk) situated 60 km from the mouth of estuary.

Plankton

The present study on plankton indicates that the density has considerably decreased in the middle and lower freshwater stretches of the Ganga as compared to 1960 but the composition of plankton has not changed much. The maximum density of plankton was 80,291; 45,613; 25,125; 7,685; 765; 1,444 and 2,390 units/l at Kanpur, Allahabad, Varanasi, Buxar, Patna, Bhagalpur and Rajmahal respectively, while it was decreased to 3,649; 2,400; 434; 765; 365; 675 and 936 units/l in the respective centres during 1995-96. But, the abundance of pollution indicator species such as, *Ankistrodesmus, Coelastrum, Pediastrum, Scenedesmus, Actinastrum (under* Chlorophyceae*), Cymbella, Cyclotella, Fragillaria* (under Bacillariophyceae) and *Anabaena, Lyngbya, Merismopodia, Spirulina* (under Cyanophyceae) was less in the lotic waters of Ganga during the present study which indicates better water quality. On the contrary, there is in general an increase in plankton density in the estuarine stretch in the present study as compared to pre-Farakka barrage period. This is positive effect of increased flushing of freshwater into the estuary after commission of Farakka barrage.

Periphyton

The periphyton flora in the riverine as well as estuarine stretches depicted almost similar trend like that of phytoplankton in respect to qualitative and quantitative abundance. A dominance of bacillariophyceae was observed followed by chlorophyceae and cyanophyceae in the entire Ganga, Bhagirathi and Hooghly stretches. In the upper most stretch, the periphyton concentration varied from 512 to 2,338 u/cm^2, while in the middle and lower stretches it varied from 224 to 6,080 u/cm2 and 900 to 2,000 u/cm^2 respectively.

Macrozoobenthos

Considerable decline in the macrozoobenthic density was also observed in the middle and lower stretches of the river Ganga. The present study indicates that maximum denisty of macrozoobenthos was 1,432; 418; 2,584; 1,709; 950; 889 and 319 unit/m^2 as compared to 21,143; 3,436; 2,214; 264; 8,415; 473 and 1,859 unit/m^2 at Kanpur, Allahabad, Varanasi, Ballia (Buxar), Patna, Bhagalpur and Rajmahal (Manikchak). While many fold increase in macrozoobenthic density was recorded in the Hooghly estuarine stretches. In the upper stretch between Tehri and Kannauj, the macro-zoobenthic fauna varied from 18 to 4,598 u/m^2 and the

population at Tehri was minimum in all the seasons which varied between 18 and 111 u/m^2. It was also observed that occurrence of pollution indicator groups such as ologochaeta, members of Ephemeroptera and Trichoptera was very negligible in the present study which also indirectly reflects the improved water quality of the river system.

Fishery

The fisheries scenario shows that the contribution of Indian major carps, has gone down miserably. *Catla catla* was almost absent in the middle Ganga. The absence of certain commercially important species such as *Notopterus chitala, Labio fimbriatus, Ompok pabo, O.bimaculatus, Pangasius pangasius* and *Mystus vittatus* were very less in the middle and lower stretches of the river Ganga. Considerable reduction in spawning grounds as well as lower degree of recruitment of IMC have also been observed in the middle and lower Ganga due to changes in river morphology, hydrography in terms of flow and flow rate, water abstraction for canal projects etc. and irrational fishing. Drastic decline in hilsa fisheries in middle and lower stretches of the Ganga after commissioning of Farakka barrage is also one of the main reasons for depletion of overall fisheries in the area. On the contrary, manifold increase in fish yield has been observed in the estuarine zone during post Farakka barrage period. The average annual prawn and fish yield from the estuary increased from 9,481.5 tons during pre barrage period (1966-67 to 1974-75) to 33,341 tons during post barrage period (1984-85 to 1994-95) and further to 42,703.2 tons durisng 1995-97. With the commission of Farakka barrage and higher flow of freshwater in the estuary, the general habitat for in the estuary has improved for its migration, breeding and growth, resulting in its increased landing from 1,457.1 tons in 1975 to 5,045.8 tons in 1995-97.

The species now spawns in the entire freshwater zone of the estuary whose area has also increased with increased freshwater discharge. Certain freshwater fishes and prawn species *viz., Eutrophiichthys vacha, Clupisoma garua, Rita rita, Wallago attu, Aorichthys seenghala, A.aor, C.catla, Labeo bata and Macrobrachium rosenbergii* have made their appearance in the entire upper estuarine zone up to Uluberia and these species were not reported prior to pre-Farakka barrage period up to this extent. The freshwater zone of the Hooghly estuary is a potential source of hilsa and prawn (particularly *M.rosenbergii*) seed. Like freshwater zone of the estuary, lower marine zone is also considered as very potential for estuarine prawn and fish seed resources. The seed of giant freshwater *M.rosenbergii* was also available in certain stretches of Sunderbans and gradient zone of Hooghly estuary where salinity range varied from 5 to 12 ppt during May to July. The present study also reveals that the gradual decline in catch per unit of effort in winter fishery of lower estuary which contributes over 70% of the total estuarine catch indicated over exploitation and is thus alarming.

The present study has conclusively proved a general improvement in the water quality of river Ganga than what it was during 1985-90. The average annual fish production of the stretch above Farakka barrage has decline due to siltation, increased water abstractions, river course modifications and irrational fishing.

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SAMPLING & ANALYSIS
(ABIOTIC & BIOTIC PARAMETERS)

METHODS FOR ESTIMATION OF WATER AND SOIL QUALITIES OF AQUATIC ECOSYSTEMS

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Maintenance of a healthy aquatic environment and production of sufficient fish food organisms in a water body are two very important factors for fish production. To keep the water body conducive for fish growth, physical and chemical parameters like temperature, transparency, pH, dissolved oxygen, total alkalinity, free CO_2 and nutrient elements like nitrogen and phosphorus may be monitored regularly. Where the physico chemical factors are in normal range, the water body is usually productive, but when they are present in quantities above or below the optimum range the fishes and other aquatic organisms may be under stress which may lead to fish disease or fish mortality in due course.

1. **Temperature :**

The temperature is noted with the help of a centigrade thermometer or by temperature selective electrode.
Optimum range for carp growth : 23 - 30°C.

2. **Transparency :**

Transparency of a water body is recorded with a Secchi disc. Dip the Secchi disc in water until it is invisible.
Note the depth of the disc from water surface in cm.
Optimum range : 20 - 50 cm.

3. **pH :**

The pH of water sample may be determined accurately by using a pH meter which has been standardised against two buffer solutions of known pH.
Optimum range : 7.4 - 8.2

4. **Dissolved oxygen :**

Winkler's method :
Reagents :

i. Alkaline iodide : Dissolve 500 gm NaOH and 150 gm Potassium iodide in one litre distilled water. Keep the reagent in polyethylene container.

ii. Manganous sulphate : Dissolve about 480 gms of Manganous sulphate in one litre distilled water.

iii. N/40 Sodium thiosulphate : Dissolve 6.205 gms of pure Sodium thiosulphate in one litre of distilled water. Add 1-2 beads of NaOH as stabiliser. Keep in a brown glass bottle. This thiosulphate solution may be standardised against N/40 $K_2Cr_2O_7$ solution.

iv. N/40 $K_2Cr_2O_7$ Solution : Weigh 1.226 gms of pure $K_2Cr_2O_7$ and dissolve it in one litre distilled water.

Place 25 ml of dichromate solution in a conical flask, add 1 ml alkaline Iodide, acidify with 2 ml conc. H_2SO_4 and keep in dark for 10 minutes. Dilute with distilled water and titrate the iodine with the (N/40) thiosulphate using starch as indicator. Adjust the strength of thiosulphate to exactly N/40.

v. Starch : Take 1 gm soluble starch in 100ml water, boil for one minute. Add a few drops of acetic acid as stabilizer.

Procedure : Collect water sample in 125 ml D.O. bottle, add 1 ml of Manganous sulphate solution and then 1 ml of alkaline Iodide solution. Replace the stopper and keep the bottle in dark for 10 minutes. Then add 1 ml of conc. H_2SO_4 and shake to dissolve the precipitate. Transfer 50 ml of the solution to a conical flask, add 1-2 drops of starch solution and titrate the solution with N/40 thiosulphate to a colourless end point.

Calculation:

No. of ml of thiosulphate required x 4 = ppm of O_2 .

Optimum range : 5 - 10 ppm.

Ion selective electrode method:

Electrode is first calibrated and then reading is taken accordingly.

5. Free CO_2 :

Reagents : i. N/44 NaOH

Prepare 0.1 N NaOH by dissolving 4 gm of AR NaOH per litre and standardise it against 0.1N H_2SO_4 using phenolphthalein as indicator. Dilute 100 cc of this 0.1 N NaOH to 440 ml with distilled water. This is N/44 NaOH. Store it in a polyethylene bottle.

ii. Phenolphthalein indicator :

Dissolve 0.5 gm phenolphthalein in 100 ml 50% alcohol.

Procedure :

Take 50 ml of water sample in a conical flask, add 2 drops of phenolphthalein indicator. Add N/44 NaOH dropwise till the solution turns slight pink.

Calculation:

No. of ml of N/44 NaOH required x 20 = ppm of free CO_2.

Optimum range for carp culture ponds : 5 - 10 ppm.

6. **Total alkalinity:**

Reagents : I. N/50 H_2SO_4

ii. Methyl orange indicator solution.

Procedure :
Take 50 ml of water sample in a conical flask and add 1-2 drops of methyl orange indicator. Titrate with N/50 H_2SO_4 until the solution turns pink.

Calculation:
ml of N/50 H_2SO_4 consumed x 20 = ppm of total alkalinity.

Optimum range : 80 - 150 ppm.

7. Total hardness:

Estimation : Total hardness is determined by titration with standard ethylene diamine tetra acetic acid (EDTA) disodium salt using Eriochrome black-T as indicator. The end point is from reddish brown to blue (APHA, 1980).

Optimum range : 20 ppm and above

8. Dissolved Inorganic Phosphate :

Reagents :

i. 50% $H_2 SO_4$
ii. Ammonium Molybdate (10%)
iii. Acid ammonium Molybdate
Add 15 ml of 50% $H_2 SO_4$ to 5 ml of 10% ammonium molybdate.
iv. Stannous chloride solution
Dissolve 1 gm stannous chloride AR in 100 ml of glycerine.

v. Standard phosphate solution.
Dissolve 4.388 gm KH_2PO_4 in 1 litre distilled water. This stock solution is 1000 ppm phosphate.
Dilute 10 ml of this stock solution to 1 litre with distilled water. This is 10 ppm phosphate.

Procedure :

Place 50 ml of water sample in a Nessler tube, add 2 ml of acid ammonium Molybdate and 2 drops of stannous chloride. Mix and wait for 10 minutes. Measure the blue colour in a spectrophotometer at 690 nm. Similarly take four standard phosphate solutions in Nessler tubes and develop the blue colour by adding ammonium molybdate and stannous chloride. Measure the colours of the standard solutions by spectrophotometer. Determine the phosphate content of sample from the calibration curve drawn from standard phosphate solutions.

Optimum range for carp culture ponds: 0.2 -0.6 ppm.

9. Nitrate nitrogen :

Reagent :

i) Phenoldisulphonic acid
ii) 12 N NaOH
iii) Standard Nitrate solution (10 ppm)
Dissolve 0.722 gm of KNO_3 in distilled water and make upto 1 litre. Dilute 10 ml of this stock solution to 100 ml containing 0.01 mg N/ml = 10 ppm N.
iv) Aluminium sulphate solution (10%).

Procedure :

Evaporate to dryness 50 ml sample in a white porcelein basin on water bath. Cool and add 2 ml of phenoldisulphonic acid and rub it with a glass rod. Wait for 5 minutes and add 2 ml of Aluminium sulphate solution. Now add 12 N NaOH solution slowly until it is alkaline. Add 20 ml distilled water and filter the solution. Take filtrate, make up the volume to 50 ml. Measure the yellow colour of the solution by spectrophotometer at 410 nm . Prepare four standard solutions of nitrate from the standard nitrate solution (10 ppm). Evaporate the solutions to dryness, add phenoldisulphonic acid, mix by glass rod and then add 12 N NaOH to make the solutions alkaline. Dilute with distilled water and make up the volume (to say 50 ml). Measure the colour of these four solutions by spectrophotometer at 410 nm. Prepare a standard curve from the standard solutions. Determine the concentration of unknown solution from the standard curve.

Optimum total nitrogen content in carp culture ponds : 1.0 - 2.6 ppm.

10. Nitrite

Nitrite is estimated by measuring the colour of the reddish purple azo dye produced at pH 2.0 to 2.5 by coupling diazotized sulphanilic acid with N(1 naphyl) ethylenediamine dihydrochloride. The colour is measured by a spectrophotometer at 543 nm.

11. Free ammonia

Ammonia content in water sample may be estimated colorimetrically by Nesslarization. The yellow to brown colour produced by Nessler reagent on complexation with ammonia is measured by a spectrophotometer at 400-425 nm.

12. Specific conductivity:

Specific conductivity of water sample may be estimated easily by using a conductivity meter.

Optimum range for carp culture ponds : 250 - 1000 μmho/cm.

13. Biochemical Oxygen Demand (BOD)

Biochemical Oxygen Demand (BOD) is the measure of the amount of oxygen required by the microorganisms in stabilizing the biologically degradable organic matter under aerobic conditions.

BOD of water sample may be estimated by determining its oxygen consumption during 5 days incubation at 20 ^{0}C or 3 days incubation at 27 ^{0}C.

Method

Take water sample in two of 250 ml BOD bottle. Estimate the dissolved oxygen content of one bottle by Winkler method or by oxygen probe. Keep the other bottle in a BOD incubator at 27 ^{0}C for 3 days. After the incubation period, estimate the oxygen content of the bottle.

Then, BOD = Initial oxygen content - Final oxygen content .

If the BOD of the sample be high, then dilute the sample with distilled water or low BOD tap water. Record the dilution factor. Say 100 cc of the sample is made 1 liter with distilled water. Then the dilution factor is 1000/100 = 10

Now determine the initial oxygen content of this diluted sample and oxygen content of diluted sample after 3 days & incubation at 27 ^{0}C.

Then BOD of this sample = $(D_1-D_2) \times t$
where D_1 = initial oxygen content of the diluted sample.
D_2 = final oxygen content after 3 days & incubation.

t = dilution factor

14. Chemical Oxygen Demand (COD)

Chemical Oxygen Demand (COD) is the measure of oxygen consumed during the oxidation of the oxidizable organic matter by a strong oxidizing agent like $K_2Cr_2O_7$ with conc. H_2SO_4 in the presence of mercuric sulphate (to neutralize the effect of chlorides) and silver sulphate (catalyst).

Method

Take 20 ml of sample water in a 250 ml round bottom flask, then add 1.0 ml of 0.025 N $K_2Cr_2O_7$ solution(if COD more than 50 ml, extreme care should be taken for low COD).

Then add a pinch of Ag_2SO_4 and $HgSO_4$. Add 30 ml. conc. H_2SO_4 gently. Then reflux the flask on COD set for 2 hrs.

After reflux, add distilled water to make final volume to about 140 ml. Then titrate with 0.1 (N) ferrous ammonium sulphate with ferroin indicator. Run the same for a blank.

Calculation

COD (mg/l) = (b-a) x 10, where a = ml of titrant with sample
b = ml of titrant with balnk

SOIL ANALYSIS:

Collection : Collect soil samples from several locations of the water body by Ekman dredge. Mix the samples. Dry the samples in air. Powder it with a wooden hammer, strain through a 2 mm and then a 80 mesh seive and again air dry. Analysis may be done with the air dried sample but result should be expressed on the oven dry basis.

1. Soil pH :

Electrometric method :
Procedure : Take 10 gm soil in 50 c.c. beaker and add 25 ml of distilled water. Shake for half an hour. Dip the electrode of pH meter in the suspension and take the pH reading.
Optimum range : near neutral (6.5 - 7.5).

2. Organic carbon :

Reagents :

i) N $K_2Cr_2O_7$
Weigh exactly 49.04 gm of AR $K_2Cr_2O_7$ and dissolve it in 1 litre of distilled water.

ii) N Ferrous solution
Dissolve 278 gm Ferrous sulphate or 392.13 gm Mohr salt in distilled water, add 15 ml conc. H_2SO_4 and make up the volume to 1 litre. This soluiton should be standardised against N $K_2Cr_2O_7$ so that 1 ml Ferrous solution = 1 ml of N dichromate.

iii) Diphenyl amine indicator.
Dissolve 1 gm Diphenylamine in 200 ml of conc. H_2SO_4 and 40 ml of water.

iv) Phosphoric acid (85%)

v) Conc. H_2SO_4 .

Procedure :

Take 1 gm soil sample in a 500 ml conical flask. Add 10 ml of N $K_2Cr_2O_7$ and 20 ml of conc. H_2SO_4 . Allow the mixture to stand for 30 minutes. Dilute with water to 200 ml and add 10 ml of phosphoric acid. The excess of dichromate is titrated with N $FeSO_4$ using 1 cc of diphenylamine as indicator. The end point is green from a bluish colour.

Calculation :

(10 - No.of ml of $FeSO_4$ solution required) x 0.3 = Organic carbon (%)
Optimum content in carp culture ponds : 1.0 - 2.5%

3. Available phosphorus :

Trough's method :
Reagents :

i) 0.002 N H_2SO_4 .
Dilute 100 ml of standard 0.02 N H_2SO_4 to 1 litre.
Adjust the pH to 3.0 with ammonium sulphate.
ii) 50% H_2SO_4
iii) 10% Ammonium Molybdate
iv) Acid ammonium Molybdate reagent
v) Stannous chloride solution.
vi) Standard phosphate solution (1 ml = 0.01 mg P.)
The methods for preparing reagents are the same as given for determination of phosphate in water.

Procedure :

Place one gm air dried soil sample in a 250 ml bottle. Add 200 ml of 0.002 N H_2SO_4 (pH-3), shake the mixture for 30 minutes in a mechanical shaker. Keep it for 10 minutes and filter. Take 50 ml of filtrate in a Nessler tube and determine its phsophate as for water.

Calculation :

ppm of phosphate in solution x 20 = mg P/100 gm soil.

Optimum content in carp culture ponds: 9-19 mg/100 gm soil.

4. Calcium carbonate :

Rapid Titration method :

Reagents : i. N HCl : Dilute 175 ml of conc. HCl to 2 litres.
ii. N NaOH : Take 80 gm of NaOH in 2 litre of water.
iii. Bromothymol Blue indicator.

Procedure : Take 5 gm soil sample in a 250 ml bottle. Add 100 ml of 1 N HCl and shake for one hour. Allow to settle the suspension and pipette out 20 ml of the clear liquid in a conical flask. Titrate it with N NaOH using Bromothymol Blue indicator till it is just blue. Note the reading and carry out a blank taking 20 ml of 1 N HCl in a flask and titrating it in the same way.

Calculation :

(Titre for blank - Titre for soil solution) x 5 = % $CaCO_3$
Optimum content in carp culture ponds : 1.2 - 2.5%.

5. Available Nitrogen:

Reagents :

i. 0.02 N H_2SO_4
Dilute 100 ml of 0.1 N H_2SO_4 to 500 ml with distilled water.

ii. 0.02 N NaOH
Dilute 100 ml of 0.1 N NaOH to 500 ml with distilled water.

iii. Methyl red indicator
Dissolve 0.1 gm methyl red in 25 ml of ethyl alcohol and make up the volume to 50 ml with water.

iv. 0.32% $KMnO_4$
Dissolve 3.2 gm of $KMnO_4$ in 1 litre distilled water.

v. 2.5% NaOH
Disssolve 25 gm NaOH in 1 litre distilled water.

Procedure :

Place 10 gm soil sample in a 500 ml Kjeldahl flask. Add 100 ml of 0.32% $KMnO_4$ solution, 100 ml of 2.5% NaOH, 2 ml of liquid paraffin and some glass beads. Distill the mixture and collect the distillate in a conical flask containing 20 ml of 0.02 N H_2SO_4 and a few drops of methyl red indicator. Collect about 75-80 ml of distillate. Titrate the excess of 0.02 N H_2SO_4 with 0.02 N NaOH to a colourless end point.

Calculation:

(20 - No of ml of 0.02 N NaOH) x 2.8 = Available nitrogen (mg/100 g soil).
Optimum content in carp culture ponds: 50-65 mg/100 g.

6. Total Nitrogen

Regeants:

1. Conc. H_2SO_4
2. Salicylic acid
3. Sodium thiosulphate
4. 12 N NaOH soln.
Dissolve 480 grams of NaOH in 1 litre distilled water. Keep it in plastic or polyethylene bottle.

5. 0.1 N NaOH
   Dissolve 4 g NaOH in 1 litre distilled water and standardise against 0.1 N H_2SO_4.

6. 0.1 N H_2SO_4
   Dilute 100 ml of N H_2SO_4 to one litre. Standardise against 0.1 N Na_2CO_3.

7. Potassium sulphate
8. Copper sulphate
9. Methyl red indicator

Procedure

Take 10 g soil sample (or one g feed sample) in a Kjeldahl flask. Add 20 ml conc H_2SO_4 and 0.5 g salicylic acid and keep for half an hour. Then add 2 g sodium thiosulphate and 1 g copper sulphate and 5 g potassium sulphate and digest the mixture until a white or bluish colour liquid is formed, cool, dilute wtih water. Make it alkaline with 80 ml 12 N NaOH, add a few glass beads and distill. Collect the distillate in a conical flask containing 20 ml 0.1 N H_2SO_4 and a few drops of methyl red indicator. Collect about 120-150 ml of distillate. Titrate the excess of 0.1 N H_2SO_4 with 0.1 N NaOH till the solution turn.

Calculation:
For soil sample (20 - ml of NaOH required) x 0.014 = Total nitrogen (%).

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COLLECTION, PRESERVATION AND ANALYSIS OF BIOTIC SAMPLES IN BIOMONITORING STUDIES

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1. PLANKTON:

The collection and analysis of plankton sample depend on the nature of water bodies and the objectives one wants to achieve. In general, however, collection of plankton samples are done using a plankton net made-up of nylobolt clothings with 173 mesh per linear inch. The plankton nets are generally of cone- shaped so as to facilitate smooth filtration.

Mode of sampling

Sampling of plankton assemblage in an ecosystem can be done in two ways viz. I) surface sampling & ii) vertical hauling.

A) Surface sampling : In case of shallow water bodies it is presumed that the entire water column has homogenous distribution of plankton population owing to extended euphotic zone from surface to bottom. A known volume of water is being filtered through a hand plankton net wherein the plankton concentrate used to accumulate in the specimen tube fitted at the tail end of the net. The quantity or volume of water to be sieved depends on the availability of plankton population in an aquatic system. More the concentration of plankton population less the volume of water to be filtered and vice-versa. To be precise where the plankton population is sparsely distributed at least 100 L of water must be filtered, whereas in case of densely populated water bodies 10 to 25 L of water may be sufficient. The moot point is that one must collect a sample which is representative for that particular system with minimum of errors. B) The sampling centre may be sub-divided into five or more collection points and from each point a uniform quantity of water may be dawn for making a composite mother sample. A minimum of two litres of water drawn from the mother sample may be preserved and brought to the laboratory. A 72 hours sedimentation time may be allowed before the analysis work is being started. It is advisable to add few drops of Lugols' solution for accelerating the process of settlement of the plankton assemblage.

B) Vertical hauling: In deeper water bodies like lakes and reservoirs active & passive sinking of plankton population is a common phenomenon due to stratification of physico-chemical properties. Collection of plankton through surface sampling, therefore, may be deceptive as many organisms prefer to live below the sub-surface water may most likely be omitted. In view of this vertical or columnar hauling of plankton, using a cone shaped plankton net, need be exercised for getting a representative sampling. The plankton net is allowed to drift- down to a known depth of the water column and pulled-up gently. The plankton population of the column thus is being accumulated in the specimen tube fitted at the tail end of the net which is transferred to another container and preserved.

Preservation :

The plankton samples are preserved with the help of 2 -5% formal-dehyde or 90% ethyl-alcohol.

Analysis:

A 1. **Quantitative(surface)**- The quantitative evaluation of plankton samples are done as per Welch, 1935 by using the formula

$$N = \frac{a \times c}{l}$$

Where,

N = Number of plankton per litre of water
a = Number of plankton counted per ml of water in a counting chamber or by drop methods

A 2. **Quantitative (Vertical hauling)**- The calculation of plankton assemblage in this case also is being done by following the above mentioned formula. However, to estimate the volume water filtered from the column in litre is ascertained by using the following formula;

$$V = 1/3 \times \frac{\Pi r^2 \text{ (area of the net mouth) } \times h}{H - h}$$

Where,

V = Volume of water filtered in litre
h = length of the plankton net
H = height of the water column

B1. **Qualitative (phytoplankton)**: Qualitative analysis of phytoplankton be done following Lakeys' drop count method and a minimum of three drops must be observed.

B 2. **Qualitative (zooplankton)**: Qualitative analysis of the community must be done by using a plankton counting chamber such as Sedgwick- Rafter counting cell.

In addition to the aforesaid, separate and specialised methods are required for the collection of *nanno* and *pico* plankton. Good quality millepore filters of 0.5 μ pore size are generally used in a filtration unit to trap *nanno* & *pico* plankters.

2. PERIPHYTON

Mode of sampling:

Periphytic organisms are collected either by exposing artificial objects like microscopic slides in the water column or through scrapping/rinsing of natural submerged substratums like stones, plants etc.

Preservation:
(as in case of plankton)

Analysis:

Numerical abundance of periphyton samples is generally evaluated based on simple arithmetic calculation, as the counting of periphytic organisms is being done from a known area of the substratum either natural or artificial and is expressed in terms of number per unit of area such as **Number / cm^2**. The **qualitative texture** of the stratified organisms, however, done as per standard keys available for different group of organisms.

3. MACROPHYTES

Mode of sampling:

Sampling of macrophytes is done with the help of random quadrat method both from littoral and pelagic zones.

Analysis:

Qualitative analysis of the macrophytic stands is being done as per the standard methods for identifying phanerogams, generally based on morphological features. The **quantitative** analysis on the other hand is generally expressed in terms of biomass either wet weight (kg / m^2) or dry wet (gm / m^2).

4. BENTHOS

Mode of sampling:

Collection of benthic invertebrates is done with the help of a Grab sampler randomly on a transect basis so as to make the sampling a representative one. The bottom muds thus collected are sieved through sieves of various pores. And the organisms are picked with the aids of a forceps and kept in a jar for further analysis.

Preservation:

Benthic samples are preserved in 5% formal-dehyde.

Analysis:

The **quantitative** analysis of benthic invertebrates is being done based on simple arthematic abundance and is expressed in terms of number per unit of area, preferably as number / m^2 . The **qualitative** analysis is generally done based on grouping of the community into various taxonomic groups.

Suppose,

Length of the Grab = 0.22 m
Width of the Grab = 0.22 m

Therefore,

Area = L X W = 0.22 X 0.22 = 0.0484 m^2

Let ,

number per Grab = 50

Therefore,

NUMBER OF BENTHOS = 1034 m^2

The biomass of the benthic organisms can also be expressed in terms of fresh weight or dry weight per unit of area such as gm / m^2

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