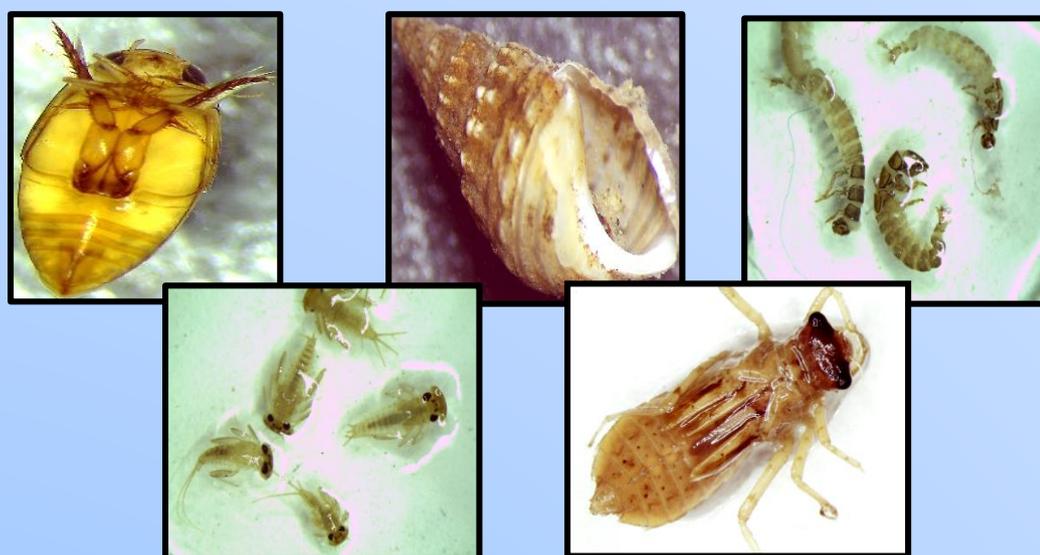


AN INTRODUCTION TO AQUATIC BIO-MONITORING USING MACRO-INVERTEBRATES



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PREFACE

Rivers are the lifelines of growth and culture for a country. Various human activities notably industrialization and urbanization have challenged the management of fresh water resources in India triggering the environmentalists all over the country to continuously monitor the equilibrium between pollution impact and sustainability of aquatic life. Traditional approach of water quality assessment through physico-chemical analysis alone does not provide complete information about the health of water body. Application of biological tools are also important to get information about the aquatic life. Both physico-chemical and biological characteristics are complementary to each other. Realizing the significance of bio-assessment, Central Pollution Control Board has developed bio-monitoring protocol using macro-invertebrates for surface water bodies. The protocol is being adopted for the biological water quality assessment of River Ganga and its tributaries under Water Quality Monitoring (WQM) project sponsored by National Mission for Clean Ganga (NMCG).

This report is a compilation of various facts related with bio-assessment of water bodies with the objective to make this approach more understandable and applicable. The efforts of Dr. Jaya Sharma, RA-III, Dr. Annu Goel, RA-III in report compilation & preparation, Sh. Navin Chandra Durgapal, (Ex-AD & DH, Bio-lab), Dr. P. K. Behera (Ad & DH, Bio-lab) as supervisors and encouragement for the work by Dr. Prashant Gargava, Member Secretary are appreciated.

Hope, it will give new insights to the environmentalists all over the country for better understanding of bio-monitoring as an effective tool to monitor health of surface water bodies and interpreting the biological water quality data into a non-ambiguous and precisely defined biological water quality criteria which is at par with internationally followed system.

Chairman

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1.0 Background

Significant development activities have been undertaken by the country in various sectors since independence. The work on various developmental activities is still going on. Natural resources of the country have been used on large scale causing negative impact on these sources and our aquatic resources are one of them. Aquatic resources are deteriorating due to significantly abstraction of water from various water bodies and on return unabated discharge of wastewater in these bodies. The deterioration of aquatic resources is assessed by various parameters or characteristics which may be categorized into two broader categories i.e. Cause parameters and Effect parameters. The Cause parameters are liable to cause damage to the environment and are mainly physico-chemical in nature. The outcome of cause parameters are generally reflected by the parameters known as Effect parameters and are generally biological in nature. Bio-monitoring or bio-assessment is one of such Effect parameter used to determine the impact of pollutants on aquatic life mainly of surface water bodies. Analysis of physico-chemical parameters has some limitations. Firstly, the water especially wastewaters are highly complex in nature and may contain thousands of chemicals, many of which may be present in such a low concentration that they may be beyond detection limit of existing analytical techniques and for many of them even the analytical techniques are inadequate. Moreover, the impact of these chemicals individually and in combination, on biological system varies significantly. Further, many of these chemicals and by-products are present as trace pollutants they may still be harmful even in low concentrations. To overcome these problems, application of summary parameters which are generally Effect parameters are increasing during the recent past to assess the status of aquatic water bodies. Bio-monitoring as a summary parameter sums all the effects of cause parameters in an easy to measure parameters.

Bio-monitoring, or bio-assessment is defined as ***“the systematic use of living organisms to determine the status of the environment.”***

Any change in the environment that may cause harm to the biological system is known as pollution. As per the definition, to assess the status of pollution in an aquatic environment, application of both group of parameters i.e. Cause and Effect parameters is required. Considering the significance of bio-monitoring, this tool is also incorporated recently by CPCB in the National Water Quality Monitoring Programme (NWQMP).

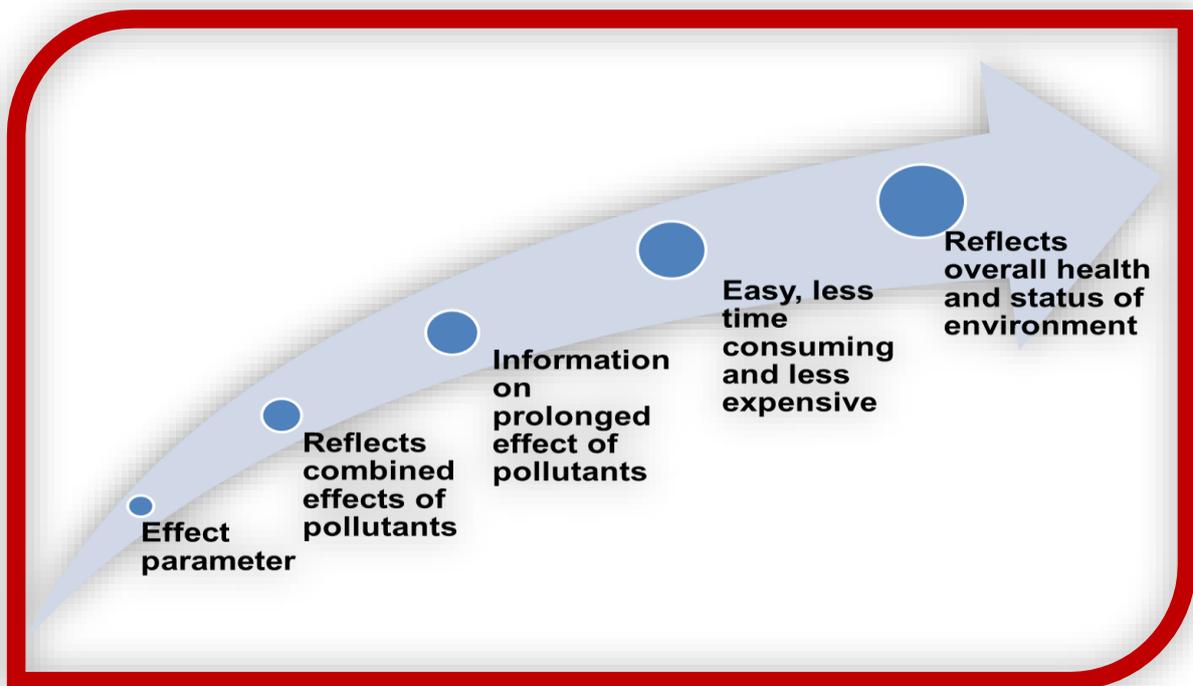


Fig.1: Advantages of Bio-monitoring

2.0 Types of biological assessment

Various methodologies used for bio-assessment are detailed below:

- **Ecosystem study** – This includes study of biotic community living in a prescribed area or physical habitat along with study of population of various group of organisms.
- **Measurement of primary production** – The most popular method used for measurement of primary production of a water body is the measurement of Oxygen production and its consumption through dark and light bottle experiment and chlorophyll estimation.

- **Observation of behavioral changes** - This includes changes in the behavior of aquatic organisms which includes feeding and predatory behavior, locomotor behavior, reproductive behavior etc.
- **Assessment of morphological and physiological changes** - These changes include physical appearance, deformities in various body parts and their abnormal functioning e.g. operculum movements, opening and closing of valves in Molluscs, growth inhibition etc.
- **Toxicity/Bioassay test** - To know acute or chronic effect of pollutants on biological system, this test can be used both in field as well as in laboratory by exposing specified number of test organisms directly in the water body or in test sample specified time period.
- **Bio-accumulation and bio-magnification studies** – In bio-accumulation certain chemicals taken up by the organisms through the entire body surface (as occurs in many annelids and simple plants) or through specific surfaces such as the gill membranes of fish. These chemicals/toxicants may tend to be retained by organisms in concentrations that exceed ambient levels. Bio-magnification is another type of bio-accumulation. Consumers at successive trophic levels in the food pyramid feed on populations much larger than their own. Therefore, any material that is retained in individuals at lower trophic levels may be further concentrated near the top of the food pyramid. The study of these two parameters is being used to have an idea accumulation of toxicants in food chain components at levels high enough to exert a toxic effect.

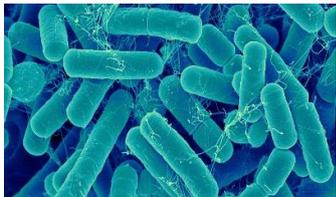
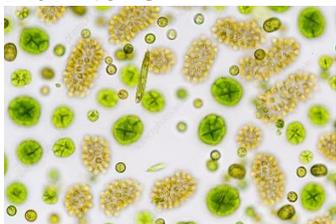
Among these methods, study of biotic community and population of different organisms are more widely used for bio-assessment because in an ecosystem all groups of organisms are interdependent on each other, any impact on one group of organisms affects the entire ecosystem. Similarly, population study of organisms provides information on density, natality (birth rate, mortality (death rate) age distribution, biotic potential dispersion etc. Population also possess genetic characteristics related to their ecology e.g. adaptability, fitness, persistence etc. The ecosystem study can also be used to detect slow changes in the ecosystem both structural and functional.

In this document the term bio-monitoring or bio-assessment is used for community/population studies.

3.0 Aquatic organisms used in Bio-monitoring

Several groups of organisms are being used for bio-monitoring (and belongs to various trophic levels of food chain. Decomposers include bacteria and protozoa, producers include phytoplankton and aquatic plants, herbivores consist zooplanktons, crustaceans etc., lower level carnivores comprise worms, insects, molluscs, small fishes etc. whereas, top level carnivores are large sized fishes, reptiles etc. The advantages and disadvantages of various groups of organisms in bio-assessment are summarised in Table 1.

Table.1: Advantage/Disadvantages of organisms used in Bio-monitoring

| Group of Organisms | Advantages | Disadvantages |
|---|--|--|
| Bacteria  | <ul style="list-style-type: none"> • Well-developed methodology for regular assessment. • Collection is easy. • Rapid response to changes, including pollution. • Good indicators of faecal contamination. | <ul style="list-style-type: none"> • Cells may not have originated from sampling point. • Populations recover rapidly from intermittent pollution. • Expertise and specific infrastructure is required for analysis. |
| Protozoa  | <ul style="list-style-type: none"> • Saprobic values well known. • Collection is easy. • Rapid responses to changes. | <ul style="list-style-type: none"> • Taxonomic expertise is required. • Cells may not have originated from sampling point. • Indicator species of impacts often present in normal environments also. |
| Planktons  | <ul style="list-style-type: none"> • Can tolerate pollution stress. • Good indicators of pollutants. • Good taxonomic keys are available for identification. | <ul style="list-style-type: none"> • Taxonomic expertise required. • Not very useful for severe organic pollution. • Sampling and enumeration problems with certain groups. • Not good for lotic environment |
| Macro-invertebrates | <ul style="list-style-type: none"> • Present in abundant numbers and belongs to diverse groups. • Many invertebrates are sedentary and are unable | <ul style="list-style-type: none"> • Sampling procedure is time consuming. • Quantitative sampling is difficult. • Occurrence is less in |

| | | |
|---|--|---|
|  | <p>to avoid the effects of pollutants due to limited mobility.</p> <ul style="list-style-type: none"> • Waterbodies that often do not support organisms of higher level of food chain but support macroinvertebrate communities. • Good indicators of pollution especially organic pollution. • Small size facilitates easy collection and identification. • Requires less sampling devices. • Good taxonomic keys are available for macroinvertebrates identification. • Reappears quickly when conditions become favourable. | <p>fast moving waters.</p> <ul style="list-style-type: none"> • Require taxonomic expertise for identification. |
| <p>Macrophytes</p>  | <ul style="list-style-type: none"> • Species usually attached, easy to observe and identify. • Good indicators of suspended solids and nutrient enrichment. | <ul style="list-style-type: none"> • Responses to pollution not well documented. • Often tolerant to intermittent pollution. • Mostly seasonal occurrence. |
| <p>Fishes</p>  | <ul style="list-style-type: none"> • Easy collection. • Effect of impact can easily be accessed through behavioural, physiological, morphological effects etc. • Can be used for measurement of long and short term effects. • Can indicate food chain effects. • Identification is easy. | <ul style="list-style-type: none"> • Able to migrate to avoid pollution. |

Among these groups of organisms, macro-invertebrates are found best suited for bio-monitoring and are used worldwide because of more advantages. Further, more ecological information available for their taxonomic groups and in bio-monitoring, taxonomic richness and composition characterization of macro-invertebrates are being used. Taxonomic identification of macro-invertebrates are done upto family level. One example of hierarchy of macroinvertebrates is shown in fig. 2 Moreover, majority of macro-invertebrates found in the water

bodies belong to the Phylum Arthropoda and Class Insecta, which is the highly diversified group among invertebrates and thus provides more indicators of water quality.

4.0 Macro-invertebrates sample collection

Macro-invertebrates colonize in different habitats and substratum of the water bodies like boulders, cobbles, pebbles, gravels, sand, silt, clay, macrophytic vegetation etc. Sample for bio-monitoring is collected in such a manner so that it represents all type of habitats exists at a location. The number of collected individual organisms in the sample shall represent the population of organisms in the community. Macro-invertebrates sample collection requires use of different sampling methods and devices depending on the type of substratum in which they exist.

4.1 Stony riverbed - boulders, cobbles, pebbles and gravels

- a) **Boulders and Cobbles:** The stones are lifted randomly and the organisms are picked up using soft forceps or brushed off into the white tray.



Fig.2.Collection of Macro-invertebrates in Substratum of Boulders & Cobbles

- b) **Pebbles and Gravels:** The hand net is placed firmly on the stream bed against the flow. The stream bed is kicked up by foot and the organisms are collected into the net. After this, the collected material is washed using sieve (recommended mesh size 0.6 mm as per ISO) and macro-invertebrates are collected into plastic bottles containing formalin (4%).



Fig.3. Collection of Macro-invertebrates in Substratum of pebbles & gravels

4.2 Substratum Consists of Sand or Silt

- a. **In deep-flowing and still water-** In such conditions, organisms are collected by drawing or pushing the hand-net through the surface layer of the substratum.



Fig.4. Collection of Macro-invertebrates in Substratum of Sand or Silt

- b. **In shallow stream with clay-** the grab samples are picked up using the shovel. Then, samples are washed using sieve to remove sediments and debris. Finally, the organisms are collected by hand or soft forceps as shown in fig. 5.



Fig.5. Collection of Macro-invertebrates in Substratum of Sand or Silt

4.3 Substratum consists of attached Macrophytes

If the river bed is covered with macrophytes then, BMIs are collected by uprooting the plants first and washing the roots with water into sieve and collected into white tray. From the tray organisms are picked up using forceps and preserved in 4% formalin for further study.



Fig.6. Collection of Macro-invertebrates in Substratum of attached Macrophytes

4.4 Non-availability of proper riverbed substratum

In case of following conditions, artificial substratum is installed. It should remain fixed for around 60 days for biological communities to establish.

- a. Non-availability of proper substratum
- b. Unstable substratum
- c. Constant alteration in substrate of water body
- d. Water bodies receiving shock load pollutants



Fig.7. Artificial Substratum for collection of Macro-invertebrates

Considering fluctuations in river hydrology, bio-monitoring study is conducted twice a year i.e. pre-monsoon/summer (April to June) and post-monsoon /winter season (November to February). Monsoon period (July to September) is not ideal for bio-monitoring studies especially for riverine system due to high flow and unstable substratum.

To obtain reliable and meaningful information number of organisms in the range of 50 to 250 are to be collected from each location. The collected material is washed using sieve (mesh size about 0.6 mm) to segregate macro-invertebrates. To avoid decomposition of organisms, the sample is preserved either with 4% formalin or with 70% ethanol.

5.0 Macro-invertebrates sample analysis

The health of aquatic ecosystems can be evaluated by studying composition of community (relative abundance) and richness (diversity) of macro-invertebrates. The generated data are used to assign a specific biological water quality class that reflects extent of alteration in aquatic ecosystem at a

specific location. To get high precision, macro-invertebrates are generally identified up to family level using stereo zoom microscope.



Fig.8: Sample analysis for Saprobic and Diversity score calculation

Measures of community composition provide information on the level of organic matter into a water body, increasing concentration of which causes replacement of pollution sensitive species with pollution intermediate and further with pollution tolerant ones.

Several bio-indices have been developed world wide to measure this species replacement quantitatively like Chandler biotic index, Trent biotic index, Biological Monitoring Working Party Score (BMWP), Average score per taxon (ASPT) etc., however, BMWP and Saprobic/ASPT scores, are the most preferred indices. BMWP has also been adopted as a standard method by an International Standard Organization (ISO) in 1979.

Bio-indices are represented as numerical expression that includes quantitative values of macro-invertebrates' diversity and qualitative information on sensitivity of each group towards organic pollution.

5.1 Biological Monitoring Working Party (BMWP) Score

The BMWP score was devised in the United Kingdom but was not specific to any single river catchment or geographical area. This scoring system which is based on study of macro-invertebrates' community is being used worldwide with modifications considering local conditions and type of invertebrates present/ absent in the aquatic system. The system uses sensitivity of invertebrates towards organic pollution (indicators of organic pollution) i.e. saprobic condition. All observed families have assigned a specific saprobic indicator value and are classified on the scale of 1 to 10. The families which are most sensitive to organic pollution are on the top of the list with weightage score of 10 while the tolerant families are at the bottom of the list with score value of 1. The other intermediately sensitive families are placed in between the scoring scale of 2 to 9.

BMWP Score is calculated by assigning all the observed families as per BMWP Score chart (Table 2). Total no. of families observed in one particular group is multiplied with its respective weightage value and then all multiplied values are added to generate BMWP Score. The original BMWP Score chart with some minor modifications, by including/ excluding families present/ absent in Indian conditions was adopted in India after thorough testing and discussion with experts. Table 2 summarises BMWP Scoring system adopted by CPCB.

$$\text{BMWP Score} = \sum \text{No. of families in one group} \times \text{Weightage score}$$

Table.2: BMWP score system adopted by CPCB

| Taxonomical families | Weightage Score |
|---|-----------------|
| Siphonuridae, Heptageniidae, Leptophlebiidae, Ephemerelidae, Potaminthidae, Ephemeridae, Prosopistomatidae, Neoephemeridae, Ameletidae, Taeniopterygidae, Leuctridae, Capniidae, Perlodidae, Perlidae, Aphelocheridae, Leptoceridae, Georidae, Lepidostomatidae, Brachycentridae, Sericostomatidae, Pseudoneuroclapsis, Glossosomatidae, Helicopsychidae, Leptohephidae | 10 |
| Chloroperlidae | 9 |
| Euphaidae, Protoneuridae, Plathycnemididae, Lestidae, Gomphidae, Cordulegastridae, Aeshnidae, Corduliidae, Libellulidae, Macromiidae, Psychomyiidae, Philopotamidae, Cheumatopsychidae, Chrysomelidae, Hydrenidae, Sciomyzidae, Limoniidae | 8 |
| Caenidae, Nemouridae, Rhyacophilidae, Polycalotropodidae, Limnephilidae, Stenopsychidae | 7 |
| Ancylidae, Hydrobiidae, Neritidae, Viviparidae, Thiaridae, Bithynidae, Unionidae, Pleuroceridae, Amblemidae, Septariidae, Assimnidae, Ampullaridae, Solecurtidae, Stenothyridae, Arcidae, Succinidae, Hydroptilidae, Palaemonidae, Atyidae, Genocentridae, Gammaridae, Potamidae, Parathelphusidae, Anthuridae, Niphargidae, Talitridae, Mysidae, Hymenosomatidae, Varunidae, Sesamidae, Gecarcinucidae, Nereidae, Nephthyidae, Nereididae, Sabellidae, Pisionidae, Histriobdellidae, Megascolecidae, Coenagrionidae, Agriidae | 6 |
| Mesovelidae, Hydrometridae, Gerridae, Nepidae, Naucaridae, Notonectidae, Pleidae, Corixidae, Vellidae, Hebridae, Belastomatidae, Haliplidae, Hygrobidae, Dytiscidae, Gyrinidae, Hydrophilidae, Noteridae, Dryopidae, Elminthidae, Psephenidae, Heteroceridae, Elmidae, Scritidae, Eulichadidae, Histeridae, Curculionidae, Hydropsychidae, Ecnomidae, Tipulidae, Culicidae, Blepharoceridae, Simuliidae, Nymphomyiidae, Sarcophagidae, Stratiomyiidae, Ceratopogonidae, Pyralidae, Planariidae, Dendrocoelidae, Carabidae, Hydrochidae, Staphylinidae | 5 |
| Baetidae, Sialidae, Corydalidae, Piscicolidae, Hirudinidae | 4 |
| Lymnaeidae, Planorbidae, Sphaeridae, Physidae, Corbiculidae, Onchididae, Glossophonidae, Hirudidae, Erpobdellidae, Haemadipsidae, Salifidae, Dugesidae, Aselidae, Cirolanidae, Aegidae, Stenasellidae, Cymothoidae, | 3 |

| | |
|---|---|
| Corallaniidae | |
| Chironomidae, Syrphidae, Ephydriidae, Muscidae, Psychodidae | 2 |
| Tubificidae, Naididae, Octochaetidae, Lumbricidae, Lumbricullidae | 1 |

5.2 Limitations of BMWP Score system

Though BMWP Score system classifies all the macro-invertebrates' families as per their sensitivity/tolerance towards pollution but have one limitation that it's value is greatly influenced by the number of individuals present in the collected sample.

5.3 Saprobic Score/ ASPT (Average Score per Taxon)

To overcome from the shortcoming of BMWP score system, saprobic score system has been used which is generated by dividing BMWP score with the total number of families observed. The score represents the average sensitivity of the families of macro-invertebrates as such is also known as the Average Score per Taxon (ASPT). High saprobic score reflects high DO and low biodegradable organic matter whereas, opposite condition is indicated by low score.

$$\text{Saprobic Score} = \frac{\text{BMWP score}}{\sum \text{Number of families encountered}}$$



a. Pollution Sensitive Taxa



b. Pollution Intermediate Taxa



c. Pollution Tolerant Taxa

Fig.9: Taxonomic families of macroinvertebrates

5.4 Advantages of using Saprobic score/ASPT

Saprobic Score/ ASPT is considered to be a better index in biological water quality evaluation due to facts as presented below:

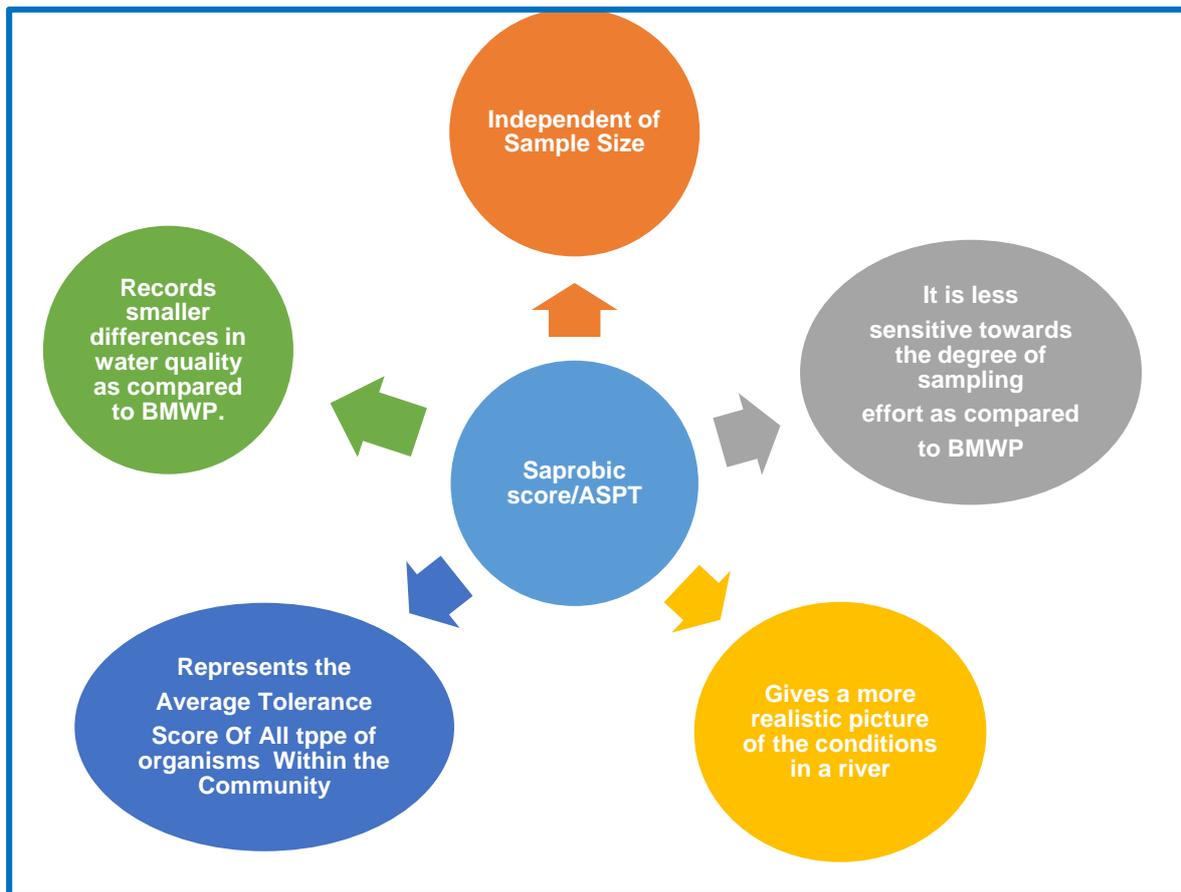


Fig.10: Advantages of Saprobic score/ASPT system

Saprobic score often gives anomalous information also due to sudden and significant change in water flow, change in composition of substratum, sudden release of toxic chemicals in the water etc.

6.0 Diversity score Index of macro-invertebrates

Diversity index is a statistical method and depends upon the different characters of a community such as number of existing taxa (**Richness**), distribution of individuals equally (**Evenness**) and total number of existing individuals. Different types of diversity indices are being used to determine the distribution of macro-invertebrates in aquatic habitat.

Table.3: Types of diversity indices

| Diversity index | Formula | Denotations |
|-----------------------------------|---|---|
| Shannon-Wiener Diversity Index | $H = \sum_{i=1}^s - (P_i * \ln P_i)$ | H = Shannon-Wiener diversity index; P _i = Relative abundance of each family; S = Number of Families; Σ = Sum from Family1 to Family S |
| Sequential comparison index (SCI) | $DS = \frac{n}{N}$ | DS = Diversity Score; n = Number of runs; N = Total number of individuals |
| Simpson Diversity Index | $1 - \Delta = \frac{\sum n_i (n_i - 1)}{N (N - 1)}$ | Δ = Simpson Diversity Index; n = Number of individuals belonging to i species N = Total number of individuals |
| Margalef Diversity Index | $d = \frac{(S-1)}{\ln N}$ | d = Margalef Diversity Index; S = Total number of species; N = Total number of individuals |
| McIntosh Diversity Index | $Mc = \frac{N - \sqrt{(\sum n_i^2)}}{[N - \sqrt{N}]}$ | Mc = McIntosh Diversity Index; n = Number of individuals belonging to i species N = Total number of individuals |

Among the various diversity indices, CPCB is using Sequential comparison index for its simplicity and Shannon-Wiener index for its reliability. These two indices are detailed ahead.

6.1 Sequential Comparison Index (SCI)

SCI is a simple method which is based upon distinguishing organisms by colour, size, shape and certain body parts. Evaluation of index requires no taxonomic expertise. SCI involves a pair wise comparison of sequentially encountered individuals in an observation tray. First observed animal is always different and scored as 1 run. If the next observed animal is different from the previous one, a new run starts and scored as ‘1’. If the organism found is same as previous it is counted as same run and scored as ‘0’. SCI/ Diversity Score values range between 0 and 1 where low value indicates low diversity of macro-invertebrates and high value indicates high diversity of collected macro-invertebrates.

Table.4: SCI method for analysis of Macroinvertebrate diversity

| No | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | Total Runs | Total Org. | Diversity Score |
|----|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|------------|------------|-----------------|
| 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 6 | 15 | 0.40 |
| 2 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 13 | 30 | 0.43 |
| 3 | | | | | | | | | | | | | | | | | 45 | |

6.2 Limitations of Sequential Comparison Index (SCI)/ Diversity Score:

This score does not provide information about type of organisms (sensitive or tolerant). In SCI method, chances of variation in index value of a sample exist and greatly depend upon the distribution pattern of organisms in observation tray.

For example, if a sample is collected with only two species A and B, both occurring in equal numbers and the sample is evenly distributed, there is a high probability that subsequent pile will alternatively yield the species A and B, resulting in a SCI of close to 1 which is obviously not reflecting the actual diversity.

Table.5: A & B- Distribution of organisms

Example A: Uneven distribution of organisms

| No | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | Total Runs | Total Org. | Diversity Score |
|----|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|------------|------------|-----------------|
| 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 4 | 15 | 0.26 |
| 2 | | | | | | | | | | | | | | | | | 30 | |

Example B: Even distribution of organisms

| No | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | Total Runs | Total Org. | Diversity Score |
|----|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|------------|------------|-----------------|
| 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 15 | 15 | 1 |
| 2 | | | | | | | | | | | | | | | | | 30 | |

6.3 Shannon-Wiener Diversity Index

The Shannon-Wiener index takes into account both taxon richness as well as abundance. Shannon-Wiener is most preferred index to assess macro-invertebrates diversity as its values are found more reliable compared to other diversity indices. Its values generally range from 0 to 5 for riverine system. However, typical values observed are in between 1.5 to 3.5. Values above 3.5 are rarely observed and below 1.5 indicate deterioration of ecosystem. Higher index value indicates greater diversity, as influenced by a greater number and/or a more equitable distribution of taxon, therefore reflects low impact of pollution sustainable ecosystem, whereas, low index values show high impact of pollution, less macro-invertebrates diversity and fragile ecosystem.

7.0 Biological Water Quality Classification based on Saprobic Score/ASPT

To classify the water bodies based on biological characteristics, saprobic score is used widely all over the world. Though, there are variations in the adopted criteria for biological water quality classification. CPCB has also developed criteria for such classification.

7.1 Earlier classification used by CPCB

CPCB had earlier classified water bodies into 5 classes of Biological Water Quality (BWQ) using both Saprobic and Diversity Score as detailed below:

Table.6: Initially adopted biological water quality criteria (BWQC)

| Range of Saprobic Score | Range of Diversity Score | Biological Water Quality |
|-------------------------|--------------------------|--------------------------|
| ≥7 | 0.2-1.0 | Clean |
| 6-7 | 0.5-1.0 | Slight Pollution |
| 3-6 | 0.3-0.9 | Moderate Pollution |
| 2-5 | 0.4 –Less | Heavy Pollution |
| 0-2 | 0-0.2 | Severe Pollution |

There are various drawbacks noticed while using this BWQC for biological water quality evaluation which are detailed below:

- No supporting literature is available for the use of 02 different scores in combination for biological water quality classification.
- The ranges of Saprobic Score and Diversity Score were overlapping in almost all the classes.
- Among five classes of BWQ, only uppermost class represents freshwater while all others indicate some degree of pollution in water.
- Anomalous combination of Saprobic and Diversity Score values have been observed which are unable to fit into a particular biological water quality class e.g. if, a sample has Saprobic score 4.78 and Diversity score 0.96, then no specific class can be assigned.

7.2 Modified biological water quality classification

Biological water quality criterion has been modified to overcome from the above mentioned shortcomings in earlier developed BWQC by CPCB. Now, it is based on Saprobic score/ ASPT only which has been divided into 5 classes. The

criteria of classification is mainly based on BWQC classification adopted by Ouse and Adur River Trust of United Kingdom, with slight modification in Saprobic Score range. Ranges of various classes have been fixed based on the field experience about the habitats and corresponding water quality which indicates various levels of Biodegradable Organic Matter/ Dissolved Oxygen level.

Table.7: Modified Biological Water Quality Classification

| |
|--|
| Biological water quality class I (Dark Blue): Unpolluted to very lightly polluted with Saprobic score 7 or above |
| River sections with this BWQ have very low Biodegradable organic material and are always saturated with Dissolved oxygen. Pollution sensitive macro-invertebrates families belonging to the taxa Ephemeroptera, Trichoptera, Plecoptera and Odonata dominate. |
| Biological Water quality class II (Light Blue): Lightly polluted with Saprobic Score 5.0 to 6.9 |
| River sections under this BWQ contain low biodegradable organic material without any appreciable dissolved oxygen depletion. Pollution Intermediate macroinvertebrates families belonging to the taxa Mollusca, Crustacea, Polychaeta, Coleoptera and Hemiptera dominate. |
| Biological Water quality class III (Green): Moderately polluted with Saprobic score 3.0 to 4.9 |
| River sections under this BWQ contain moderate organic pollution and enough amount of dissolved oxygen supply. Pollution Intermediate macroinvertebrates families belonging to the taxa Lepidoptera, Megaloptera, Hirudinea, Molluscs, small crustaceans (isopoda) and Planaria dominate. |
| Biological Water quality class IV (Orange): Heavily polluted with Saprobic score 1.1 to 2.9 |
| River under BWQC IV restricts living conditions due to high amount of biodegradable organic material and low or very low amount of dissolved oxygen. River sections having BWQC IV generally have turbidity due to suspended solids from wastewater. Macroinvertebrate families belong to taxonomic Order Diptera dominates which includes red midge larvae (Chironomids), larvae with siphon (syrphidae) etc. |
| Biological Water quality class V (Red): Severely Polluted with Saprobic score 1 |
| Water sections under this BWQC show the presence of excessive organic pollution as a result of oxygen-depleting organic waste waters. This section lacks in dissolved oxygen content due to the putrefaction process. Macro-invertebrate families namely Naididae, Lumbricidae, lumbriculidae, Tubificidae belonging to the subclass Oligochaeta dominates. |

8.0 Bio-monitoring Application and Web-portal

An android mobile based application named as “**Bio-monitoring App**” which can be downloaded & installed from Google play store and **Web portal** is available at url: <http://125.19.52.219/bio> have been developed by CPCB for

digitalization of bio-monitoring field and laboratory analysis data and generation of bio-assessment reports in various formats.

Bio-monitoring App assists in recording all the field observations in digital form. The app automatically takes details like date & time of sampling along with latitude & longitude of the sampling location. Other information like human & ecological influences, physico-chemical parameters like temperature, Dissolved oxygen etc. are added manually. The app can be used in off line mode also. The app data are required to be transferred to web-portal for further processing.

The web-portal automatically calculates the value for saprobic and diversity scores. For guidance, the portal also consists pictures of Macro-invertebrate families. After calculation of data, a brief report about Biological water quality status of the sampling location is generated. Bio-monitoring data is also depicted on web-portal for selected location/season/duration in the form of Geographic map, graph and table.

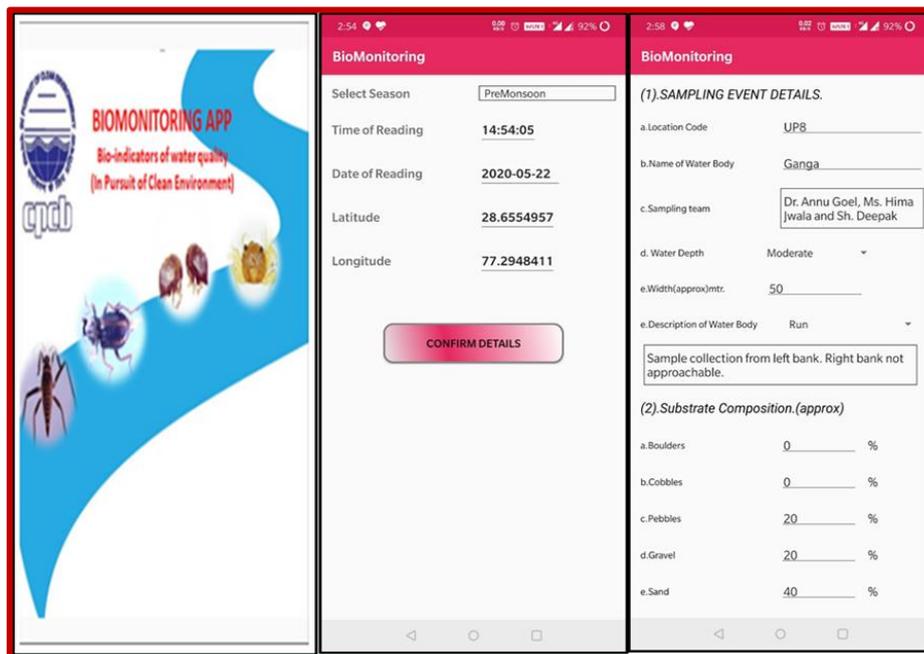


Fig.11: Bio-monitoring App showing Location and sampling event details

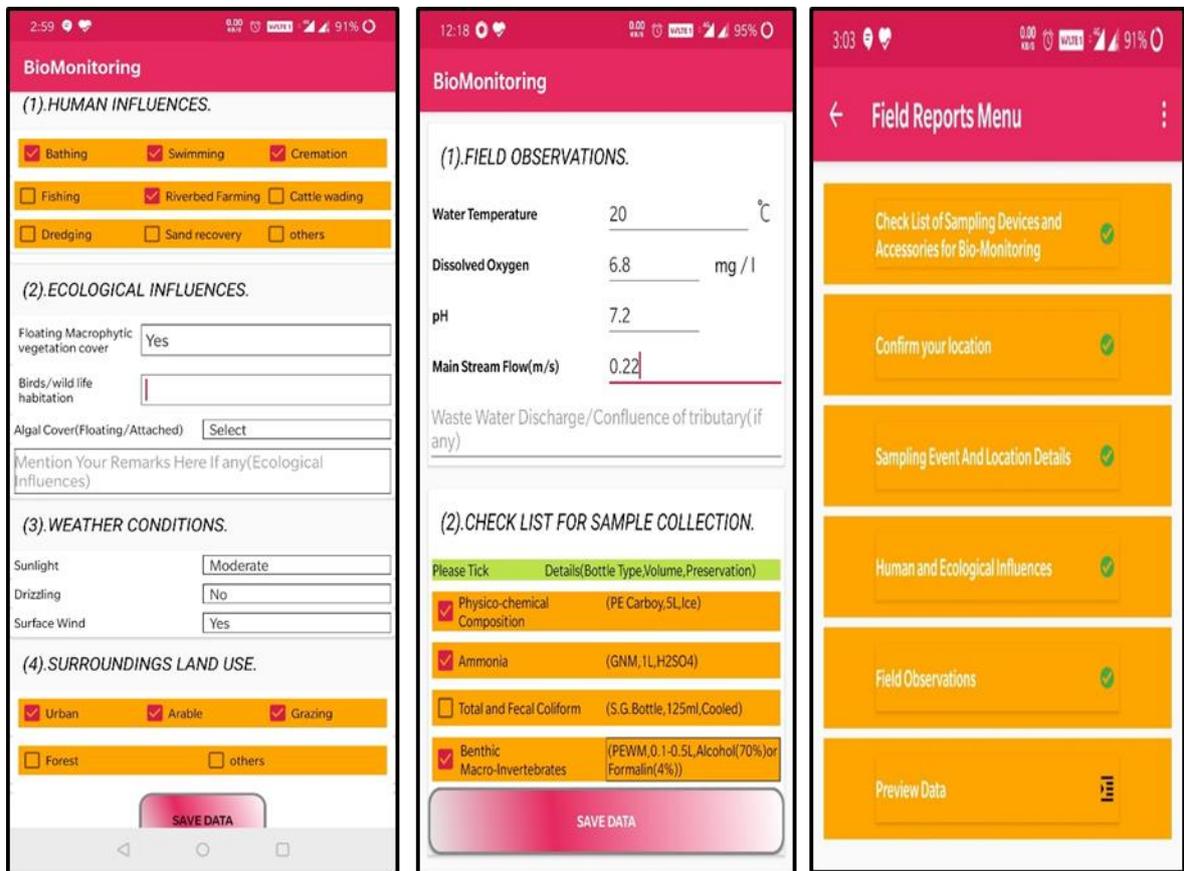
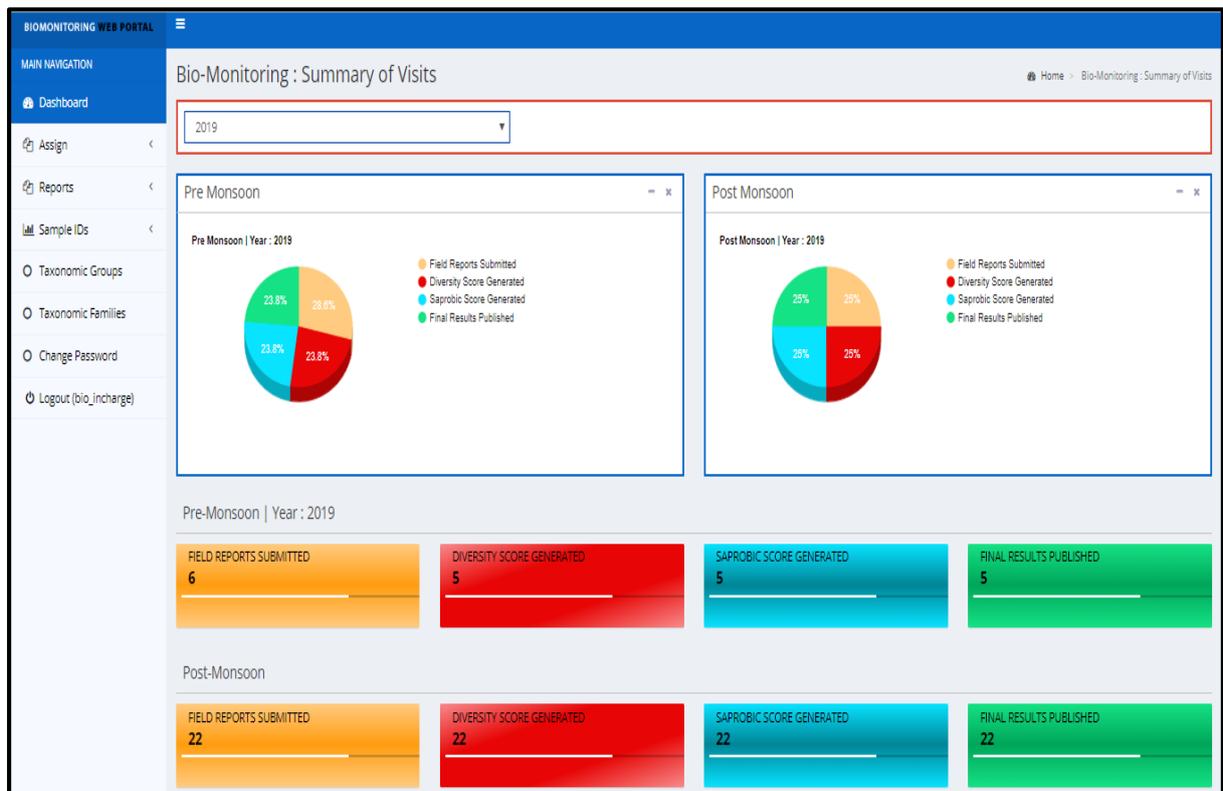
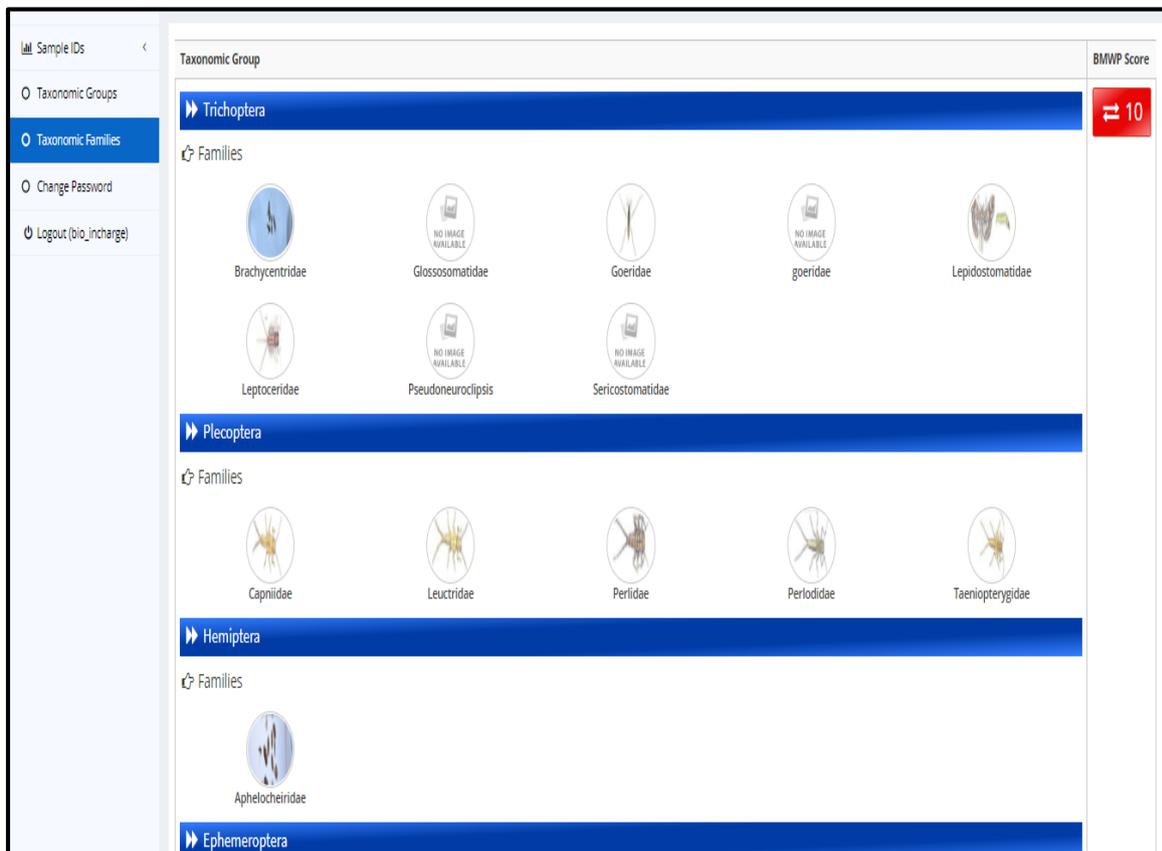


Fig.12: Bio-monitoring App showing required field observations and report menu



a. Dashboard of Biomonitoring Web-portal showing Summary of visits



b. Pictures of Macro-invertebrates on Web-portal

BIOMONITORING WEB PORTAL

MAIN NAVIGATION

- Dashboard
- Sample IDs
- Sample IDs List
- Taxonomic Groups
- Taxonomic Families
- Change Password
- Logout (annu_goe)

Diversity Score

Home > Diversity Score

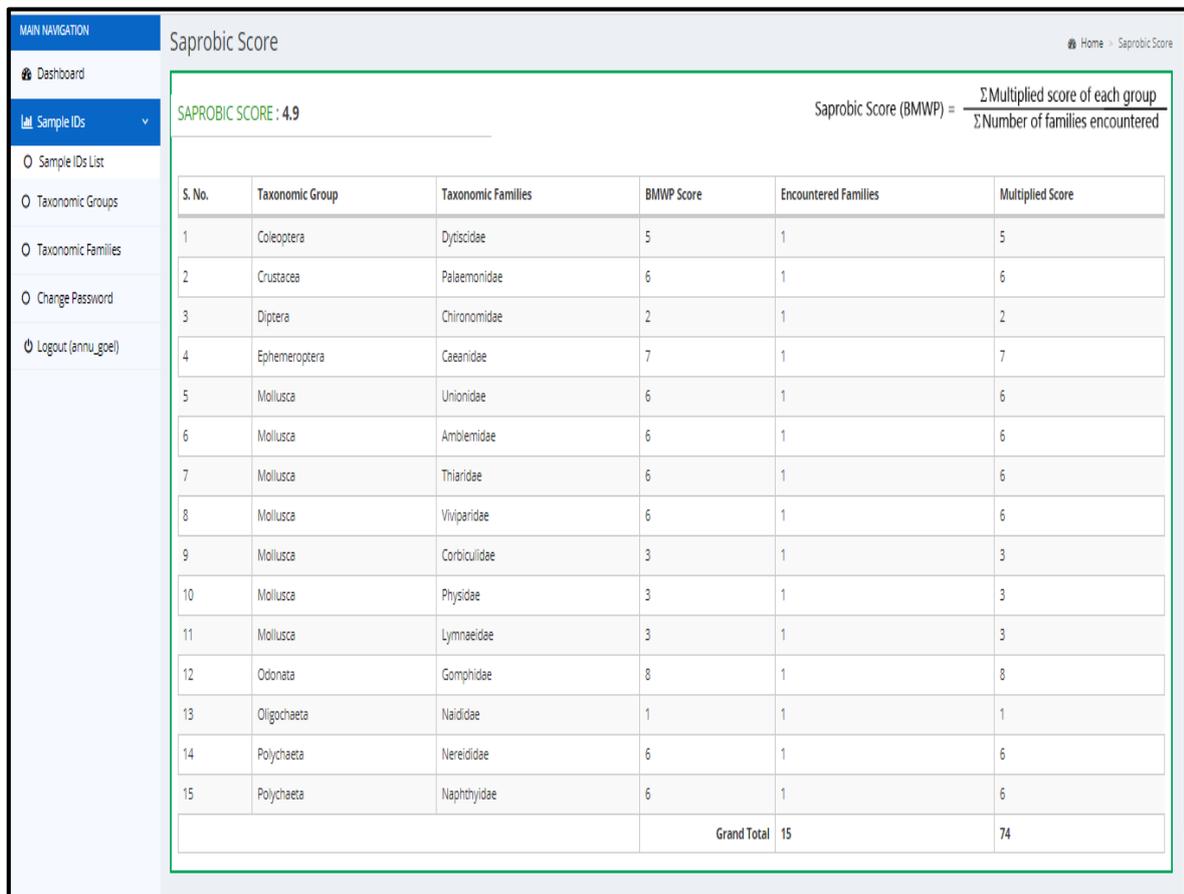
Diversity Score : 0.79

Σ Number of runs (f's) / Σ Number of organism counted

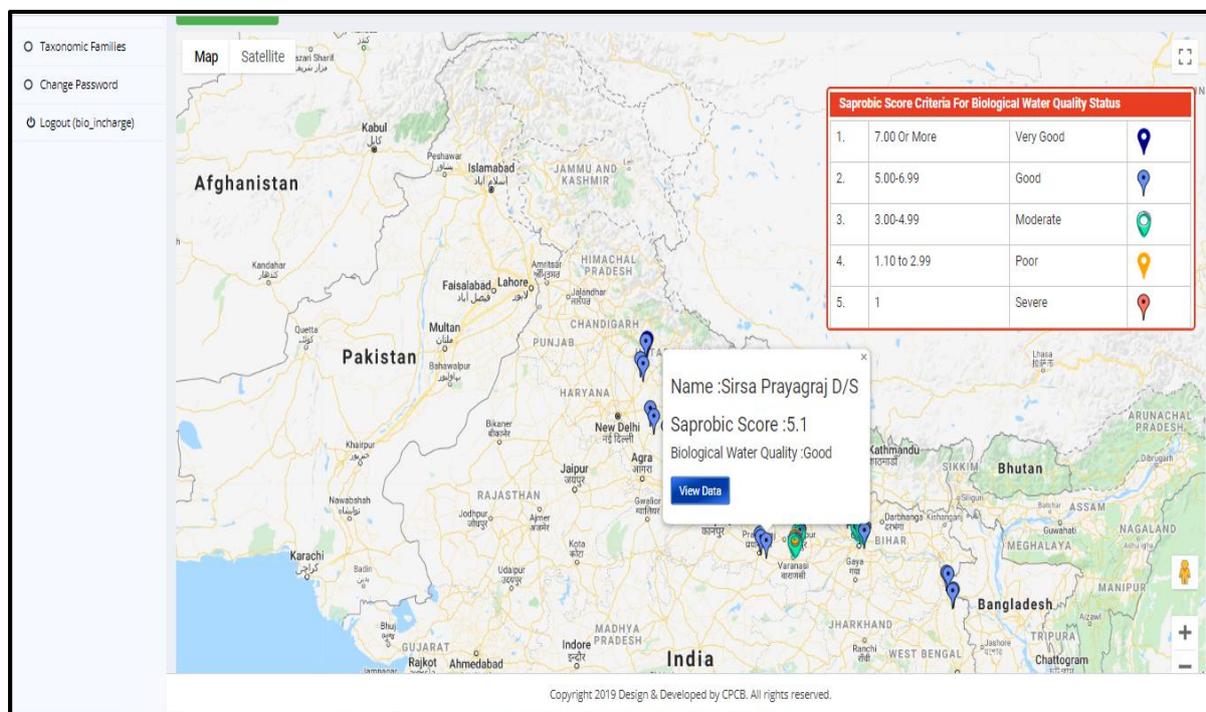
| No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | Total Runs | Total Org. | Diversity Score |
|-----|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|------------|------------|-----------------|
| 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 9 | 15 | 0.60 |
| 2 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 22 | 30 | 0.73 |
| 3 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 35 | 45 | 0.78 |
| 4 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 46 | 60 | 0.77 |
| 5 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 58 | 75 | 0.77 |
| 6 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 72 | 90 | 0.80 |
| 7 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 65 | 105 | 0.61 |
| 8 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | | | 93 | 118 | 0.79 |

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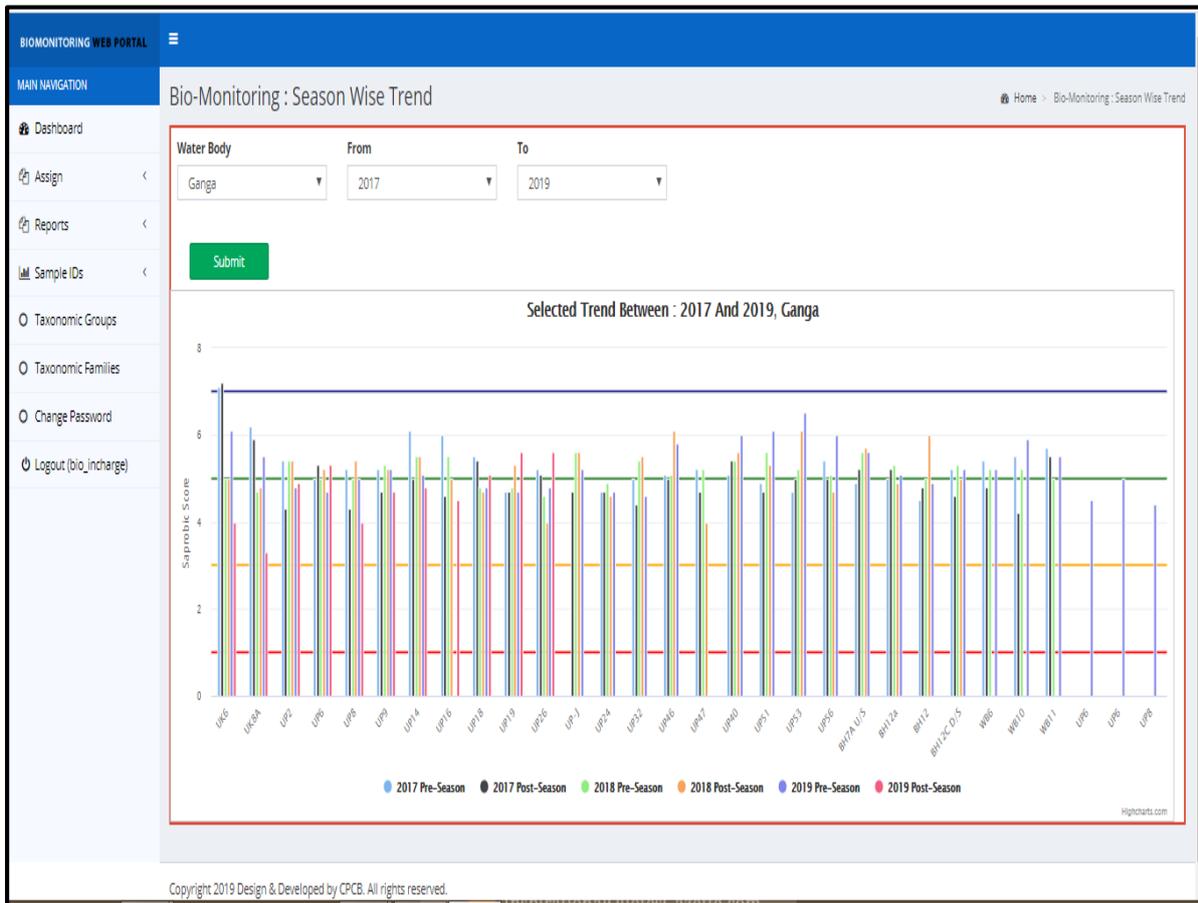
c. Diversity Score calculation on Web-portal



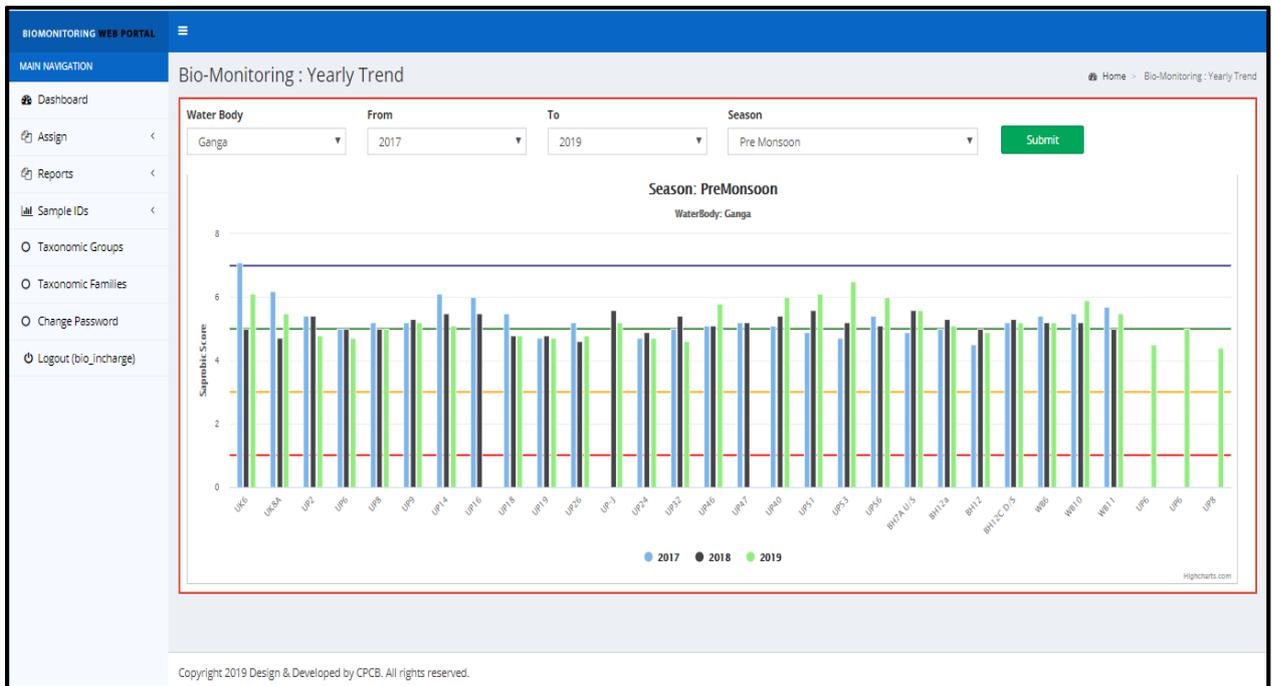
d. Saprobic Score calculation on Web-portal



e. Data of selected location on GIS map depicted on Web-portal



f. Bio-monitoring Season-wise trend over the years on Web-portal



g. Bio-monitoring Yearly trend on Web-portal

Fig.13 (a-g): Display of Bio-monitoring data on Web-portal

9.0 Biological assessment status of River Ganga (2017-20)

- ✓ CPCB had initiated Bio-assessment of River Ganga & its tributaries in the year 2014 at selected locations using aquatic macro-invertebrates. During the period 2014-16 survey cum bio-monitoring had been undertaken at 93 locations. Since 2017, the study was under taken more systemically on regular basis twice a year i.e. summer & winter at 41 locations at/near Real Time Water Quality Monitoring sites (RTWQMS). Out of 41 locations, 33 locations are situated on mainstream of Ganga River from Haridwar (Uttarakhand) to Diamond Harbour (West Bengal) and remaining 08 are located on the tributaries.

- ✓ Biological water quality of Ganga River at all the 04 studied locations during summer period of the year 2020-21 was found in Good Class with Saprobic Score range from 5.2 – 5.7. Winter study of 2019-2020 reflects Good biological water quality at 13 locations and Moderate at 08 locations. The comparison of biological water quality over the years (2017-2020) revealed that the quality remained stable at two locations i.e. Good at Sirsa, Prayagraj D/s and Moderate at Deori Ghat, Kanpur D/s. Biological water quality is declined slightly at Haridwar barrage from Very good (2017-2018) to Good class during rest of the studied period. At remaining locations, biological water quality fluctuates between Good and Moderate classes.

- ✓ Diversity score values ranged from 0.59 to 0.79 at all the 04 studied locations in 2020-21 summer monitoring. Diversity score values are in the range from 0.17 to 0.79 during post monsoon period of 2019-20, highest at Rajwari whereas, lowest at Ghatia Ghat. In comparison to over the years (2017-20), increasing trend in diversity score is observed at Srirampore D/s whereas, fluctuating trend is observed at remaining 31 locations of River Ganga.



Fig: 14. Sampling locations of River Ganga

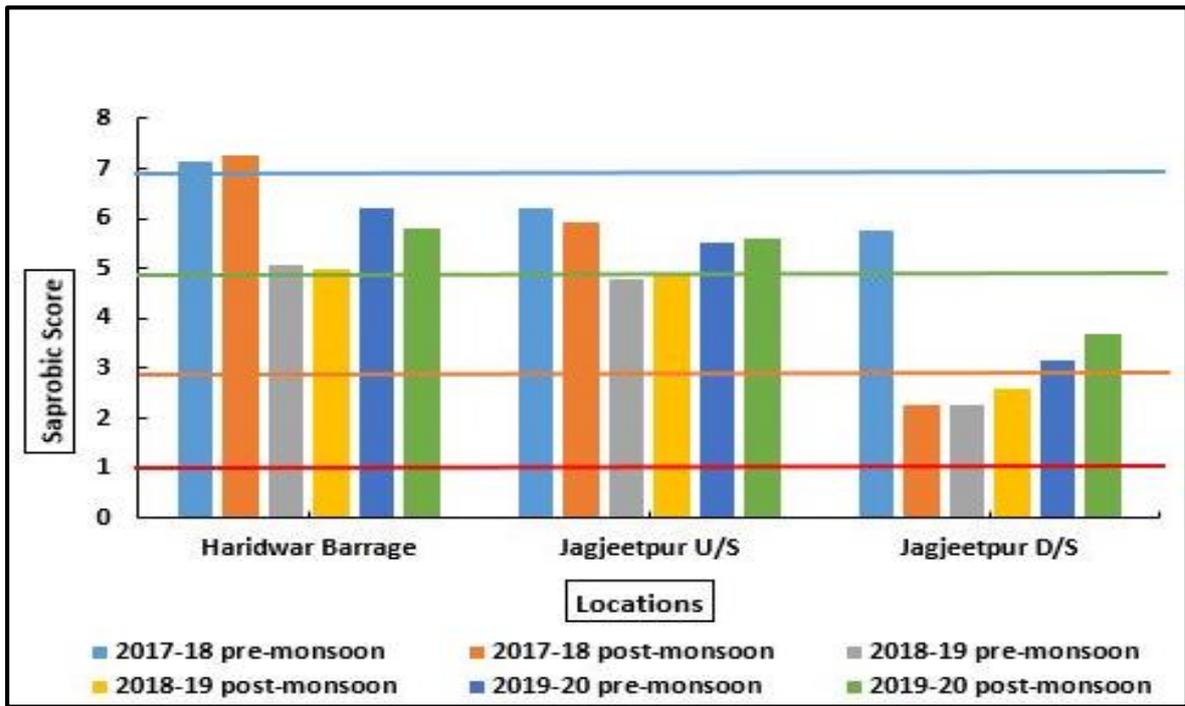
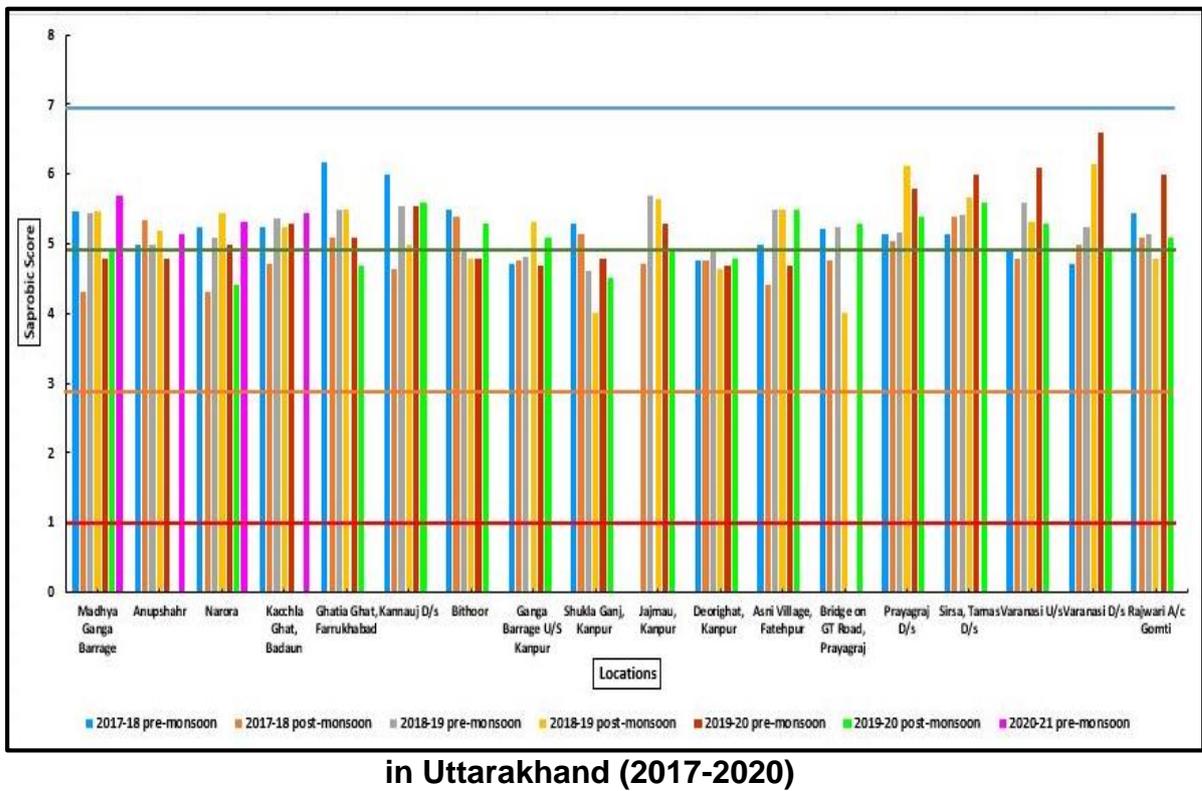


Fig.15: Trend in Saprobiic Score at main stream locations of River Ganga



in Uttarakhand (2017-2020)

Fig.16: Trend in Saprobiic Score at main stream locations of River Ganga in Uttar Pradesh (2017-2020)

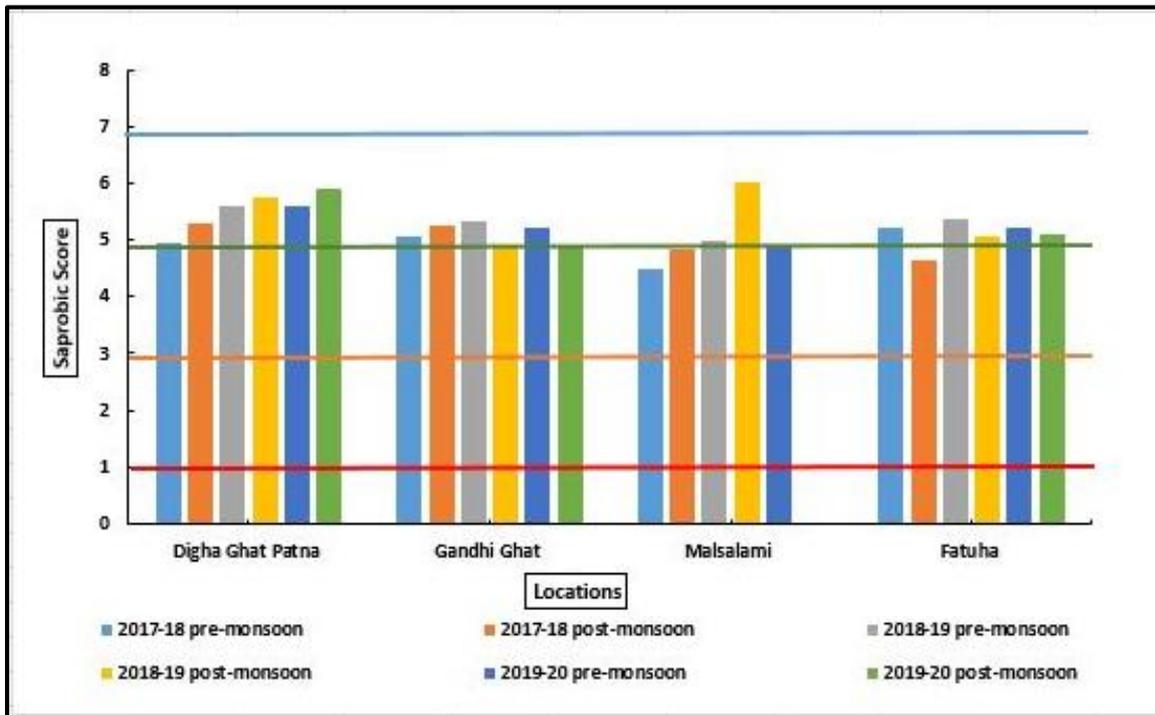


Fig.17: Trend in Saprobic Score at main stream locations of River Ganga in Bihar (2017-2020)

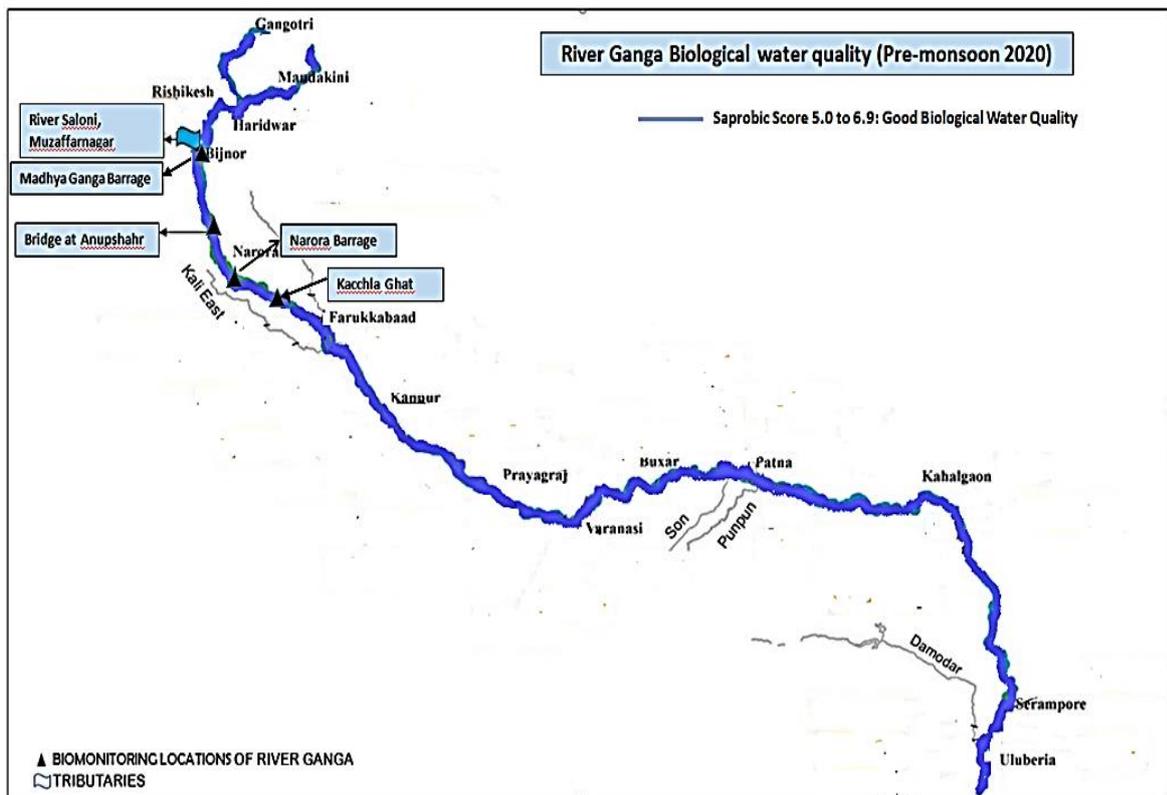


Fig.18: Biological water quality of River Ganga & its tributary (Summer, 2020)

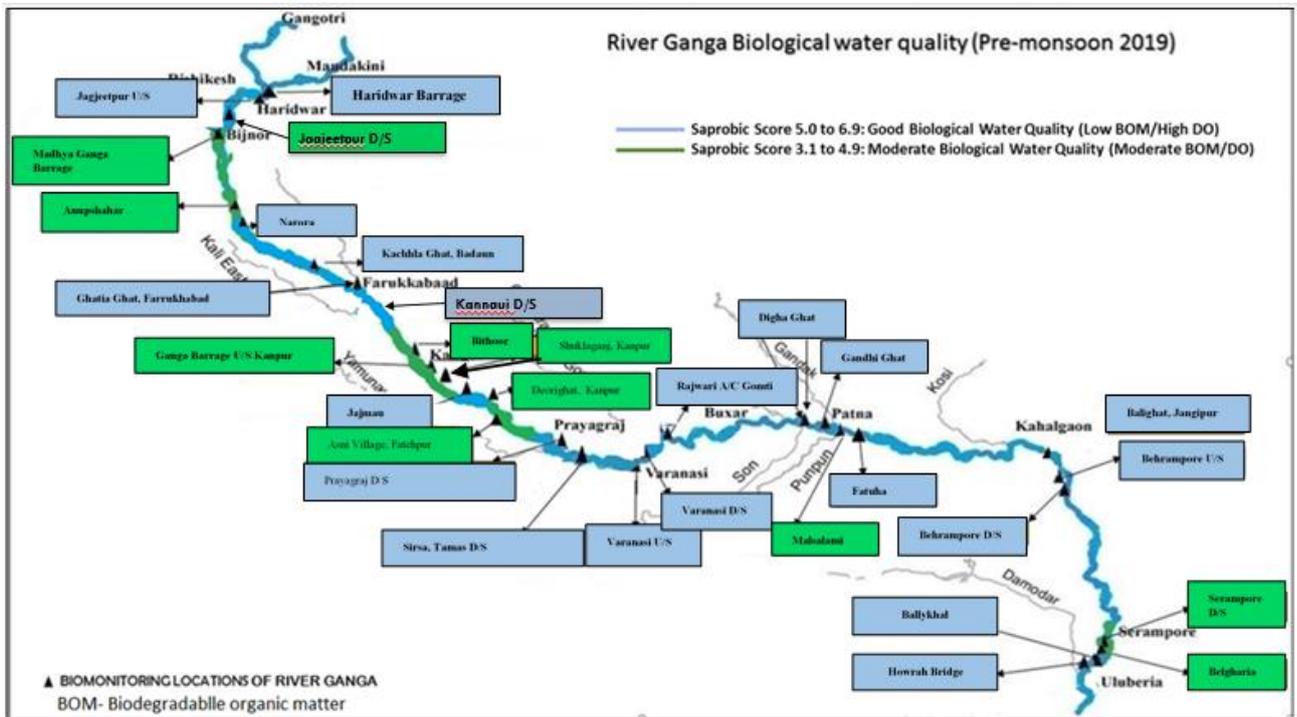


Fig. 19: Biological water quality of Main stream of River Ganga (Summer, 2019)

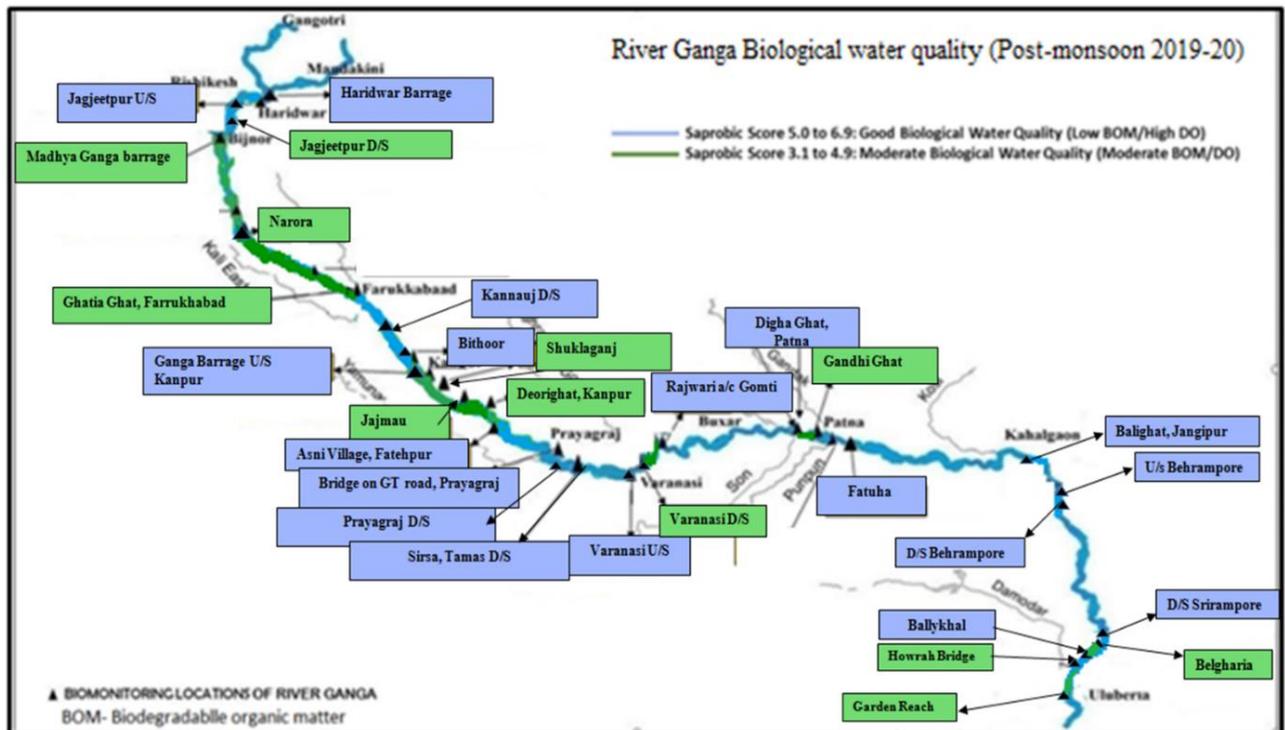


Fig. 20: Biological water quality of Main stream of River Ganga (Winter, 2019-20)

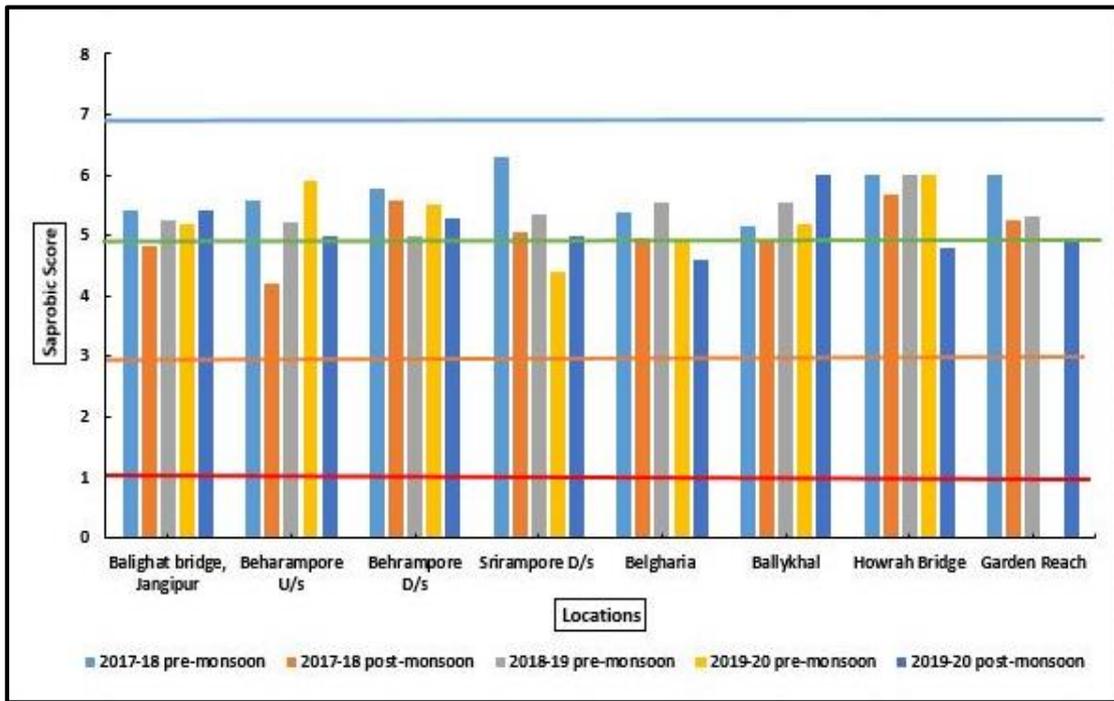


Fig. 21: Trend in Saprobiic Score at main stream locations of River Ganga in West Bengal (2017-2020)

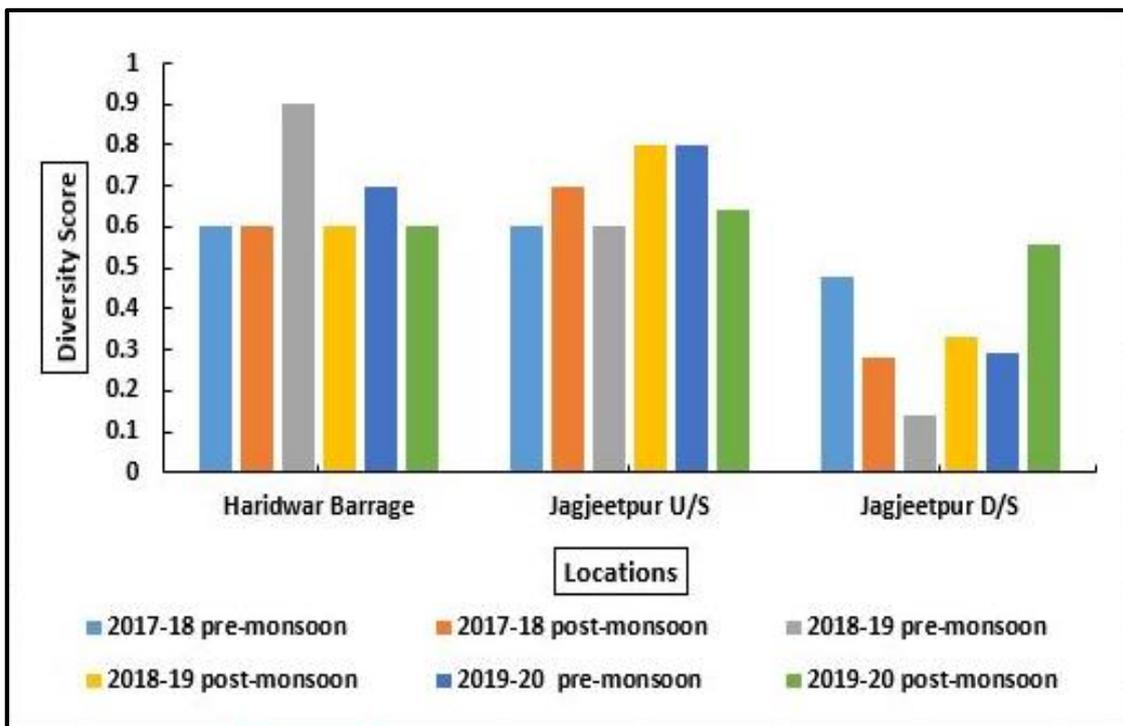


Fig. 22: Trend in Diversity Score at main stream locations of River Ganga in Uttarakhand (2017-20)

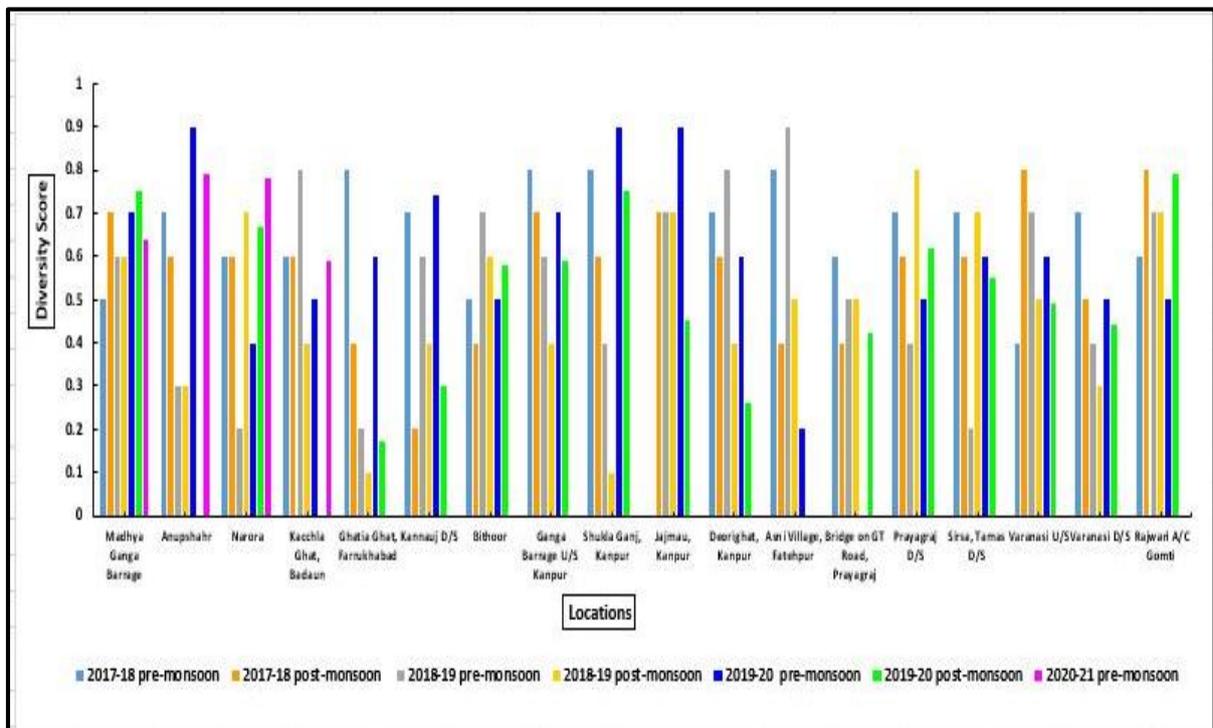


Fig. 23: Trend in Diversity Score at main stream locations of River Ganga in Uttar Pradesh (2017-20)

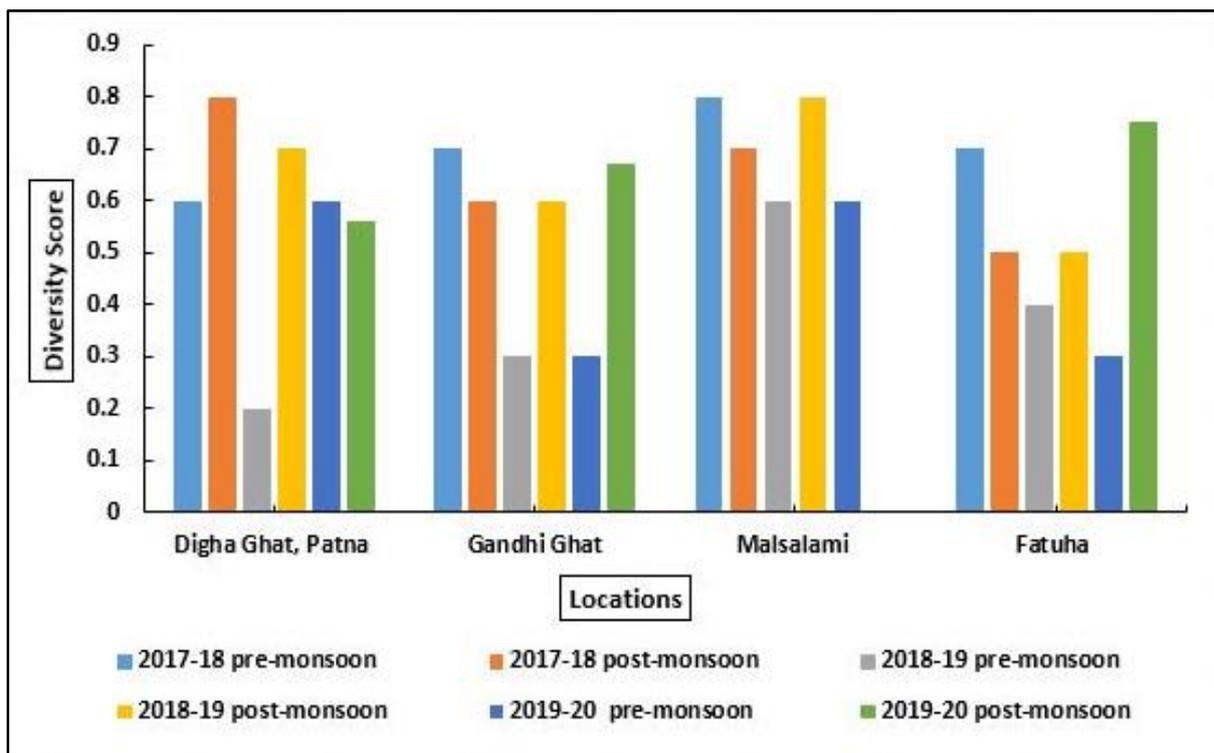


Fig. 24: Trend in Diversity Score at main stream locations of River Ganga in Bihar (2017-20)

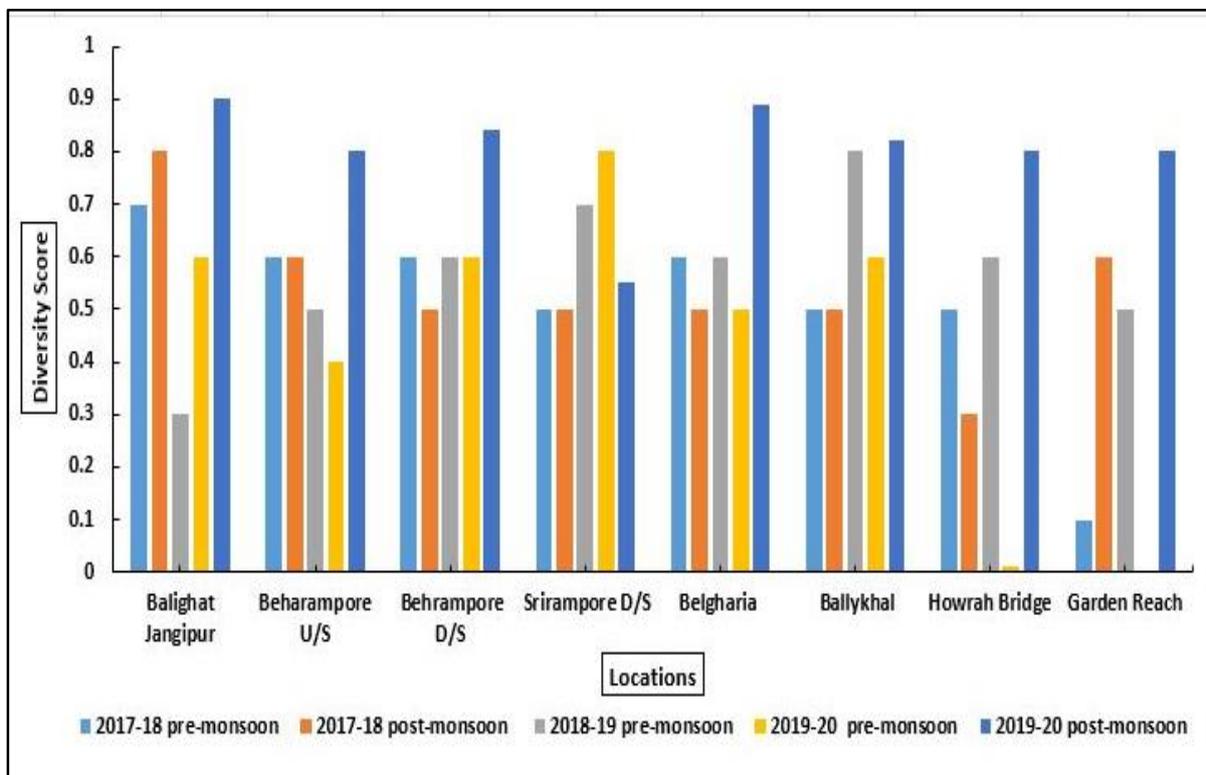


Fig. 25: Trend in Diversity Score at main stream locations of River Ganga in West Bengal (2017-20)

9.1. Distribution of Macroinvertebrates Families in river Ganga (2017-20)

The study of distribution and abundance of macroinvertebrates families during the bio-assessment of river Ganga (2017-2020) have shown that families which indicate good or moderate level of organic pollution with BMWP weightage score of 3 to 7, are abundant in entire stretch of river Ganga and as such the biological water quality of river stretch belongs to Good or Moderate class. Pollution sensitive families belonging to the orders Ephemeroptera, Plecoptera, Trichoptera and Odonata with BMWP score 8 to 10 are abundant at Haridwar barrage and Jagjeetpur U/s only and they are either absent or present in declined number in downstream locations. Similarly, pollution tolerant families belonging to the orders Oligochaete and Diptera are also found in very less numbers at all the locations of river Ganga which reflects that none of the locations of river Ganga is in Severe and Heavy BWQC.

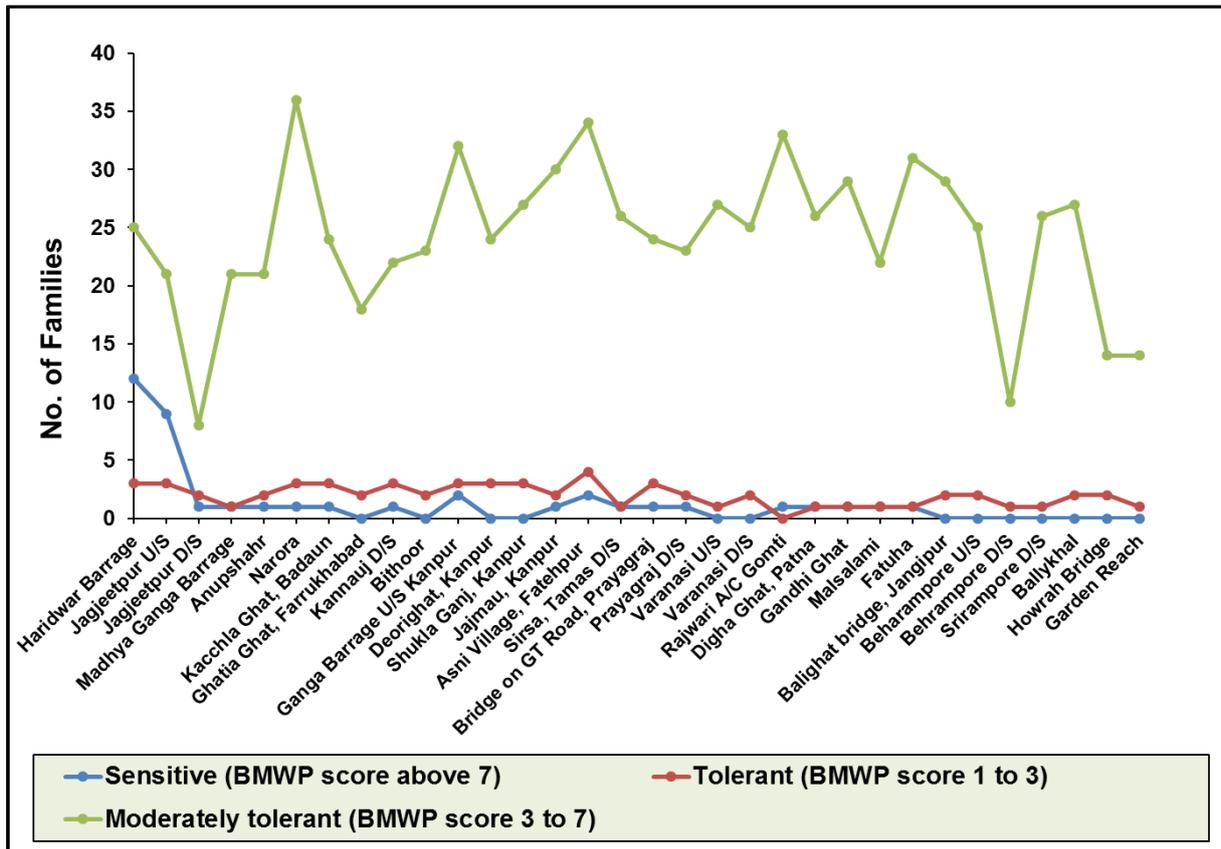


Fig. 26: Distribution of Macroinvertebrates Families (2017-20)

However, in June, 2020 at all four studied locations viz., Ganga barrage (Bijnore), Anupshahr, Narora barrage and Kacchla Ghat (Badaun), Pollution Sensitive May fly nymphs (Comb mouthed and flat headed) have been observed and that too in abundance. This might be due to various Covid-19 related restrictions imposed leading to reduction in pollution and less/no damage to the organism’s habitats which otherwise affected by instream human/cattle activities.



a. Comb mouthed



b. Flat headed

Fig. 27: Pollution Sensitive May fly nymphs

10.0 Biological assessment status of River Yamuna (2019-20)

The study has also been initiated for River Yamuna from Panipat upstream to Prayagraj (before confluence with river Ganga) in 2018 at 16 locations on annual basis. The bio-monitoring study of river Yamuna was carried out at 12 locations during winter season of 2019-2020 and at 05 locations in summer season of 2020-21.



Fig.28: Sampling Locations of River Yamuna

The study during 2019-20 winter season reflects moderate biological water quality at 07 locations and Good at remaining 05 locations while during summer season 2020-21, the biological water quality at 03 locations was in Good class while in remaining 02 locations, it was in Moderate class. In 2019-20 winter season, diversity score ranged from 0.27 at Palla in Delhi to 0.83 at Prayagraj in Uttar Pradesh and in summer season 2020-21, diversity score was in the range between minimum (0.20) at Nizamuddin bridge to maximum (0.70) at Palla and Palwal.

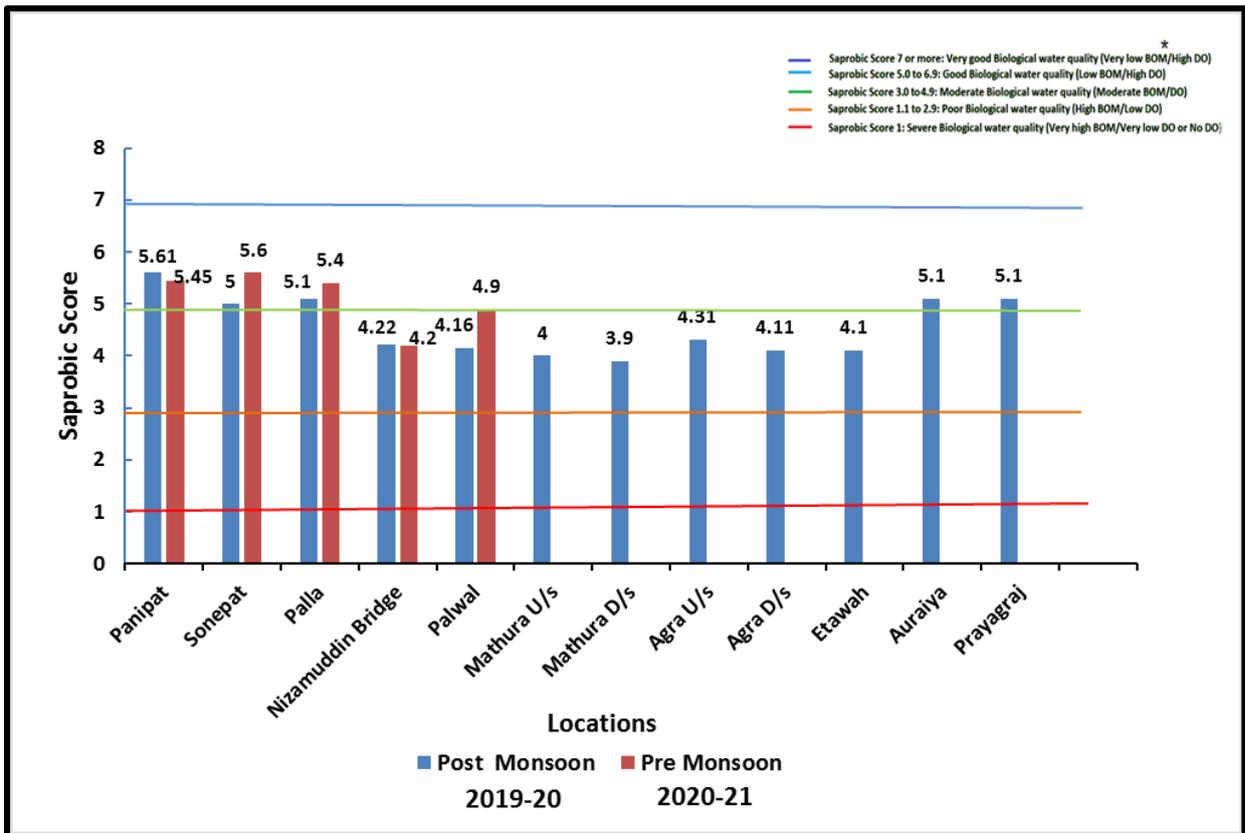


Fig.29: Saprobic Score at various locations of Yamuna River during 2019-20 (Winter) and 2020-21 (Summer)

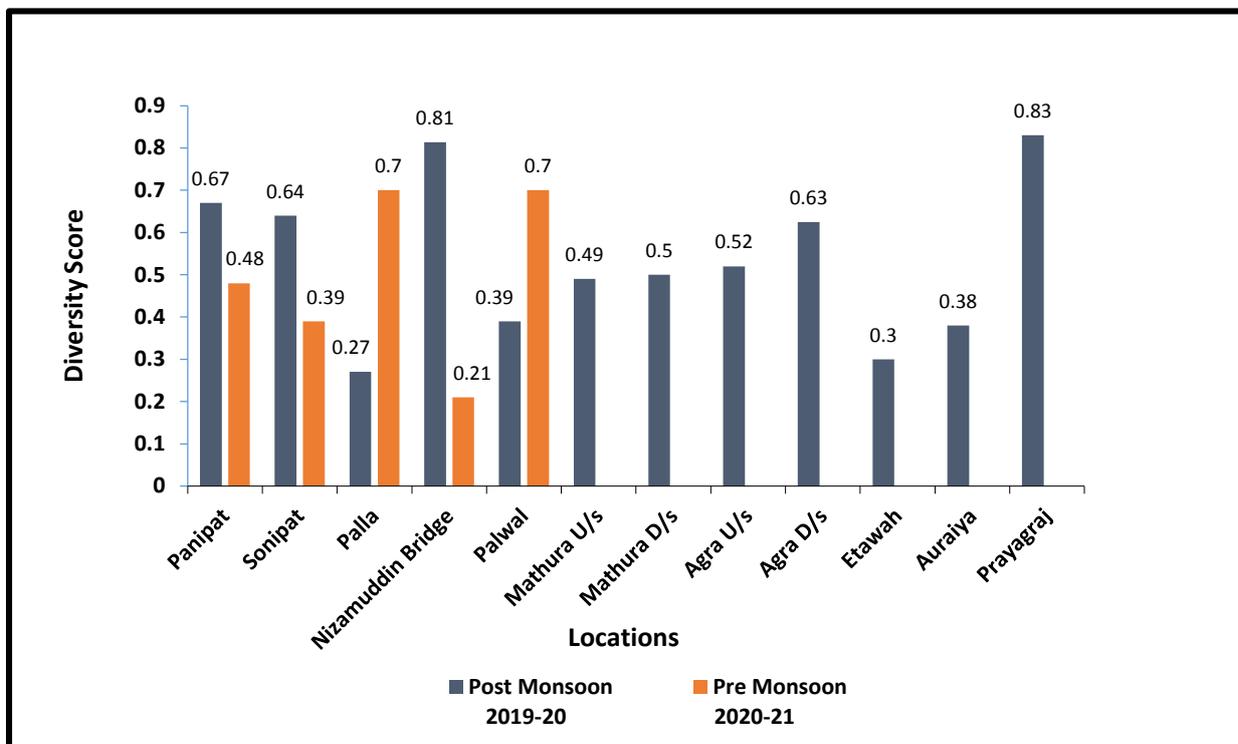


Fig. 30: Diversity Score of various locations of River Yamuna during 2019-20 (Winter) and 2020-21 (Summer)

11.0 New Macroinvertebrates' Families observed in River Ganga and Yamuna (2019-20):

11.1. Taeniopterygidae:

Pollution sensitive family Taeniopterygidae of order Plecoptera was observed in Yamuna river during summer season of Biomonitoring at Palwal, Haryana. This family has BMWP Score value 10 which indicates very less biodegradable organic matter and high dissolved oxygen in the water, thus, reflects the 'Very Good' biological water quality.

Characteristics:

- Larvae of Taeniopterygidae family are medium sized with distinct head patterns.
- They can be distinguished from other families by the sub equal length of tarsal segments as well as a prominent sub genital lobe or by single, finger-like gills at the base of the coxae.
- Some of the genus have very big wings, strong hair at femur and tarsus. Their abdomen may be dark brown with a light spot on tergite.



Fig.31: Order: Plecoptera; Family-Taeniopterigidae

11.2. Totricidae:

Family Totricidae of order Lepidoptera was found at Kachhla Ghat, Badaun, Uttar Pradesh and in river Yamuna at Panipat, Haryana. This family has BMWP score 5 which indicates moderate level of biodegradable organic matter in the water thus, reflects Moderate Biological water quality.

Characteristics:

- Anal fork or comb is present at the terminal segment of abdomen, or body is covered with tiny spicules (needle like spines); primary setae short; associated with nutgrass.
- The Tortricidae is a family of moths, commonly known as tortrix moths or leaf roller moths in the order Lepidoptera.



Fig.32: Order Lepidoptera; Family-Totricidae

12.0 CONCLUSION

Aquatic organisms act as useful indicators because they cope with chemical, physical, and biological influences in their habitat over the course of their entire aquatic lifecycle. Bio-monitoring of aquatic ecosystem using these organisms act as summary parameter that integrates the effect of all causative agents on aquatic life. Traditional physical and chemical measures of water quality are useful to help determine type of pollution, but unable to provide information on effects of pollutants on aquatic life through biological responses. Identification of indicator organisms can provide information about the type of pollution in the water bodies and health of aquatic ecosystem instead of analysing various physico-chemical parameters which require time, manpower and expertise. Bio-monitoring detects the cumulative impact of different stressors on aquatic organisms and thus, can reveal the overall health and status of environment. However, water quality assessment using physico-chemical parameters are also having its advantage to find out the pollutants that are affecting biological community. Therefore, both physico-chemical and biological assessment are complementary to each other and required to be incorporated in a monitoring plan to get complete information.

**“A river doesn’t just carry water it
carries life also”**

-Amit Kalantri

