

Bio-monitoring a future tool for environmental studies

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Received : 11.10.2021; **Accepted** : 08.11.2021

ABSTRACT

The present paper is prepared to discuss the significance of utilizing bio-monitoring tool for environmental monitoring *vis-a-vis* conventional physico-chemical analysis. The innovative approach of bio-monitoring study is considered to be more effective by many environmental scientists as outcome of the study depicts more logical conclusion based on presence or absence of certain aquatic organisms which indicate the degree of pollution of an aquatic ecosystem.

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KEY WORDS : Bio-monitoring, Indicator organism, Pollution, Tools

Introduction

Testing for chemical pollution in our nation's streams has traditionally meant using analytical chemistry. In recent years, environmental agencies have endorsed biological monitoring to enhance or replace chemical monitoring. The theory behind biological monitoring (bio-monitoring) is to use the organisms living in the aquatic system as a measure of water quality. Aquatic organisms are subject to pollutants in the stream as it flows by, day and night. Consequently, the health of the organisms reflects the quality of the water they live in. If the pollution levels reach a critical concentration, certain organisms will migrate away; fail to reproduce, or die, eventually leading to the disappearance of those species at the polluted site. Normally, these organisms will return if conditions improve in the system.

For water quality assessment, measurements of aquatic biota summarize the preceding river conditions for weeks or months before their collection. For example, an episodic pollution event, such as a chemical spill, may go undetected by periodic water sampling regimes but damage to aquatic biota can be detected long after the cause of the impact has passed.

In bio-monitoring benthic macroinvertebrates are common inhabitants of lakes and streams where they are important in moving energy through food webs. The term "benthic" means "bottom-living", so these organisms usually inhabit bottom substrates for at least part of their life cycle; the prefix "macro" indicates that these

organisms are retained by mesh sizes of ~200-500 μ m.

A bio-monitoring study begins with an experimental design and site selection. The next step includes selecting the organisms appropriate for the stream and types of contamination of concern. After the organisms have been selected, the appropriate collection technique must be applied. Finally, once the samples have been collected, identified, and counted, the data must be interpreted. Therefore in addition to the physico-chemical analysis, monitoring of the river water quality with biological variables (bio-monitoring) is being considered now a days following the standard procedure.

Assumption

With increasing pollution, change will occur in the species present (*e.g.*, appearance of tolerant species), the number of species and change in abundance of species.

- Biomonitoring can not entirely replace standard physico-chemical water quality methods which provide information on water quality at a particular spatial unit during the time of sampling while bio monitoring provides some historic insights into the water quality.
- Standard physico-chemical water quality methods need to be carried out in conjunction with bio monitoring to comprehensively evaluate the health of rivers. This is particularly important when heavy metal or pesticide contamination is suspected. Fresh water benthic macro-invertebrates, or more simply "benthos", are larger than 1/2 millimetre and live on rocks, logs,

TABLE – 1 : Different parameters of water, sediments, heavy metals and pesticides

Water		Soil/Sediment		Heavy metals		Pesticides	
S. No	Parameters	S. No	Parameters	S. No	Parameters	S. No	Parameters
1.	pH	1.	Bulk Density	1	Zn	1.	Organo-chlorine
2.	Colour	2.	Moisture Content	2	Cr	2.	Organo-Phosphate
3.	Turbidity	3.	pH	3	Cd		
4.	Water Temperature	4.	Total Hardness	4	Fe		
5.	Conductivity	5.	Total Alkalinity	5	Mn		
6	Total Suspended Solids	6	Chloride				
7	Total Dissolved Solids	7	Nitrate				
8	Total Alkalinity	8	Phosphate				
9	Bicarbonate Alkalinity	9	Potassium				
10	Carbonate Alkalinity	10	Sodium				
11	Total Hardness						
12	Calcium Hardness						
13	Magnesium Hardness						
14	BOD ⁵						
15	COD						
16	Dissolved Oxygen						
17	Free CO ₂						
18	Inorganic Phosphate						
19	Organic Phosphorus						
20	Total Phosphorus						
21	Chloride						
22	Nitrate						

TABLE – 2 : Analysis of Phytoplankton Samples

Sampling Method	Spot sampling:
Sampler	Bottle, Ruttner sampler
Bottle	Polyethylene or glass, 100ml, in duplicate
Sampling	The bottle and cap are rinsed with the sample water and filled with the water collected from upstream. Collection of re suspended sediment is avoided. The bottles are kept clean from the sand and dust
Preservation	1 ml Lugol's solution is added to 100 ml sample (Lugol's solution: 60 gr KI+40 gr I2 crystals in 1 Lt. Aquadest)
Transport/storage	In the dark
Maximum time until analysis	6 months, although certain cells will distort or disrupt.

sediment and debris on river bed.

- The benthos include crustaceans such as crayfish, mollusks such as clams and snails, aquatic worms and the immature forms of aquatic insects such as stonefly and mayfly nymphs.
- Benthos do not move around much and with long life cycle allow detection of past pollution events such as pesticide spills and illegal dumping thereby help in determining decline in aquatic environmental quality.
- Few benthos are generally intolerant of pollution and their large presence in stream indicates good water quality. Large presence of pollution tolerant benthos shows that water is polluted. Benthos are visible to unaided eye, can be retained on a sieve with mesh size of 0.6 mm diameter
- Benthos in-habitat different substratum of rivers & streams.
- Water bodies with different quality support diverse benthos communities with well developed taxonomy.
- Benthos demonstrate an integrated effect of pollution and community response are sensitive to organic loading, thermal impacts, substrate alterations, toxic pollution *etc.*
- Bio-mapping include preparation of biological water quality colour map of a river basin. Different colours on a river basin map indicate various water quality classes in terms of clean, slight pollution, moderate pollution, heavy pollution and severe pollution of river.

Methodology

‘For bio-assessments, it is important to sample each site in the same season of each year to ensure

comparability of the data. Typically, sampling is done in the fall or late summer when the largest portion of the taxa in the stream are likely to be present in an aquatic life stage, flow conditions are optimal for safe sampling, and the stream that is to be sampling has been wet for most of the year.. The best time of year may vary depending on location. It should be noted that sampling must not be undertaken if streams are in flood.’

Sampling Technique

Study Designing and Site Selection

Before initiating sampling a study design should be developed on the basis of the objective using the required tools. Protocols for selecting a sampling location must be finalized depending on the type of site visited; reference or test site.

Reference sites

Along with the main sites, reference sites should be selected on the following basis.

Selecting a reference site

- A reference site should have no point sources of pollution and also have natural habitat features similar to those of exposure areas.
- Reference areas may not represent pristine conditions, but areas in which impacts are lowest or disturbance is minimal.
- Careful consideration is required, not only for the consistent application of the selection criteria for reference areas, but also in determining how many reference areas or stations will be examined for all study designs. The following specific points can be

TABLE – 3 : Analysis of Zoo plankton Samples

Sampling Method	Composite sample of 5x10L water, filtered over plankton net.
Sampler	Bucket and plankton gauze (55 µm)
Sampling depth	Surface water (0-0.6 m)
Bottle	100ml Polyethylene (LDPE), white mouth, in duplicate
Sampling	The conical plankton net is held in the water, with the upper ring about 10 cm above the water surface. This will minimize the damage to the organisms due to pressure on the filter. 50 liter of water sample is measured. Water, using a bucket is passed over the filter. The net is then pulled vertically out of the water, and a wash bottle is applied to wash down the organisms that are stuck to the inner side of the net and the water is allowed to flow away. The bottom holder is then removed and the sample is quantitatively transferred to the sample bottle.
Preservation	70% ethanol (alcohol)
Transport/storage	No special considerations
Maximum time until analysis	It can be holding even upto 6 months.

considered during the selection of reference sites for a benthic invertebrate assessment study.

- As reference areas are selected to match the habitat features of the exposure area(s), identification of the types of test sites must be assessed and their habitat features should precede the selection of reference sites.
- If non-point or other point source inputs occur upstream of the test sites, reference site(s) should be selected in the nearest comparable drainage basin with minimal development that occurs within the same eco-region.
- If additional sources of disturbance to the river valley are associated with the test sites, stressor effects may be confounded by the disturbance. Accordingly, additional physically matched reference areas should be selected.

Selection of test sites

- The test sample site should be determined prior to going into the field according to the study parameters and desired outcomes.
- The test site should be selected based on a point of interest such as point source pollution and with consideration of other study objectives.
- The actual sample reach (site) should be determined

after physically visiting the site and performing some basic reconnaissance.

Creating site identifiers and unique codes

A site nomenclature should be established before conducting the field sampling. Sites are maintained in a database as unique entries therefore sites are assigned with a unique site code that identifies them in both space and time. Site codes should be assigned by the project leader and would reflect qualities of the site that help to define its location and other identifiers.

Site coding system

The structure of site nomenclature system will depend in part on the spatial and temporal frequency of sampling. Site nomenclature uses:

- 3 letters for a basin/sub-basin code;
- 2 numbers for a site number code;
- 2 numbers or letters for another level of sampling within a site (e.g. replication).

For example, NVD01 would be the site code for site #1 on the Narmada River. If there is a replication element, another identifier can be used with the site code. For example, the first sample could be designated with A (NVD01A), second sample with a B (NVD01B) and the third sample designated with C (NVD01C).

TABLE – 4 : Collection of samples and their preservation

Sampling Method	Collection by hand and hand net, grab sampler.
Sampler	Ekman Dredge Mud sampler
Sampling depth	Sediment from River bed
Polythene packs	Transparent ,Polyethylene wide mouth
Sampling	In this method, the conical plankton net is hold in the water, with the upper ring about 10 cm above the water surface. This will minimize the damage to the organisms due to pressure on the filter. 50 liter of water is then measured using a bucket and passed over the filter. The net is then pulled vertically out of the water, and a wash bottle is applied to wash down the organisms that are stuck to the inner side of the net and the water is allowed to flow away. The bottom holder is then removed and the sample is transferred to the sample bottle quantitatively.
Preservation	70% ethanol (alcohol)/ 5% Formaline
Transport/storage	In Ice pack transport container
Maximum timeuntil analysis	Within 15 days after collection

Site attributes determined on a map

There are a number of site attributes that would be determined prior to going into the field, using topographic maps. Some adjustments may be done as and when required.

Field Sampling Procedures

On arrival in the field, a Site Inspection Sheet should be filled out. The completion of this sheet will help in assessing the geographical and other related conditions by the field members.

Types of samples and measurements

1. Habitat Measurement
2. Samples for the physico chemical parameters
3. Samples for Benthic and Biological Parameters

Habitat Measurement

i. Channel Measurements

Characteristics of the channel in a stream reach often determine the abundance and distribution of benthic macro-invertebrates, so it is important to describe these

TABLE – 5 : Sampling techniques and the criteria for use

Sampling device	Good for use in the following situations
Grab or scoop samplers (Ekman dredge)	Devices that are designed to penetrate bottom sediment and collect a sample in standing or flowing water. Samples are sifted through a sieve to sort the organisms. Large items like twigs or stones are searched by hand .This technique are primarily used to collect macro-invertebrates and aquatic plants.
Nets	Used primarily to collect macro-invertebrate, fish, and zooplankton samples
Kick net	Useful in small streams with gravelly bottoms and good flow velocity. Nets are stretched across the stream and the bed upstream is disturbed by kicking; organisms flow into the net

TABLE-6 : The biological scoring system

Taxonomical	Class Taxonomical Families	BMWP Score
Ephemeroptera	Siphonuridae, Heptageniidae, Leptophlebiidae, Ephemerellidae, Pothamanthidae, Ephemeridae, Prosopistoatidae	10
Plecoptera	Taeniopterygidae, Leuctridae, Capniidae, Perlodidae, Perlidae	10
Hemiptera	Aphelocheiridae	10
Trichoptera	Leptoceridae, Goeridae, Lepidostomatidae, Brachycentridae, Sericostomatidae	10
Odonata	Lestidae, Gomphidae, Cordulegastridae, Aeshnidae, Corduliidae, Libellulidae, Euphaeidae, Plathycnemididae	8
Trichoptera	Psychomyiidae, Philopotamidae	8
Ephemeroptera	Caenidae	7
Plecoptera	Nemouridae	7
Trichoptera	Rhyacophilidae, Polycentropodiae, Limnephilidae	7
Mollusca	Neritidae, Viviparidae, Ancyliidae, Unionidae, Thiaridae, Hydrobidae, Bithynidae	6
Trichoptera	Hydroptilidae	6
Crustacea	Atyidae, Gammaridae, Plaeamonidae	6
Polychaeta	Nereidae, Nephthyidae	6
Odonata	Agriidae, Coenagriidae	6
Hemiptera	Mesovelidae, Hydrometridae, Gerridae, Nepidae, Naucoridae, Notonectidae, Pleidae, Corixidae, Hebridae, Veliidae, Belastomatidae	5
Coleoptera	Haliplidae, Hygrobiidae, Dytiscidae, Gyrinidae, Hydrophilidae, Noteridae, Psephenidae, Dryopidae, Elminthidae	5
Trichoptera	Hydropsychidae	5
Diptera	Tipulidae, Simuliidae, Culicidae, Blepharoceridae	5
Planaria	Planariidae, Dendrocoelidae	5
Ephemeroptera	Baetidae	4

Taxonomical	Class Taxonomical Families	BMWP Score
Megaloptera	Sialidae	4
Hirudinea	Piscicolidae	4
Mollusca	Lymnaeidae, Physidae, Planorbidae, Sphaeriidae	3
Hirudinea	Glossiphoniidae, Hirudidae, Erpobdellidae	3
Planaria	Dugesiiidae	3
Crustacea	Asellidae	3
Diptera	Chironomidae, Syrphidae, Ephydriidae	2
Oligochaeta	All families	1

attributes. The dimensions and shape of the channel and the substrate paving the bottom of the channel, a factor of critical importance to the benthos, are a result of the geology of the area and peak flows. Peak flows occur, on average, in two out of three years, are called “bank full discharge”. Erodible materials are carried through the stream reach, shape the dimensions of the channel (width, depth), and leave behind substrate material that the stream does not have enough energy to transport. The substrate material is crucial to the development of benthic macro invertebrate communities. It is possible to relate faunal distributions with a particular suite of hydrological variables by measuring channel characteristics (e.g. Cobb et al. 1992).

ii. Bank full Width, Wetted Stream Width, and Bank full-Wetted Depth

As flow decreases, water will cover less of the stream bottom, which will limit the available habitat for aquatic organisms. As flow increases, it erodes the banks of the stream and carries erodible materials down the stream and deposits them when the flow decreases again providing habitat for aquatic organisms. Two measures of stream width are made, bank full width to represent high flow conditions and wetted stream width to represent low flow conditions at the time of sampling. A third measurement, for bank full-wetted depth, is also made to determine the height from the water surface to bank full. This measurement, added to the wetted depths at each point in the transect, provides a user with the tools

necessary to calculate bank full discharge.

iii. Velocity and Depth of Stream:

Should be measured using velocity meter/Flow meter and sonometer

2. Collection and Analysis of Physico-chemical parameters

For analysis of physico – chemical parameters of the water body, water samples should be collected from identified stations as per criteria mentioned above with the standard methods of collection mentioned in APHA (1995). Following parameters can be analyzed for evaluating the impact of various physico-chemical parameters in sustainability of biotic community.

3. Collection and analysis of Benthic and biological samples

i. Phytoplankton

Sampling procedure – Phytoplankton

For collection of the plankton samples, plankton net (Nylon bolt No: 22) of mesh size 25 should be used. The collected should be preserved with 5% formaldehyde solution and iodine solution for analysis of zooplankton and phytoplankton sample respectively.

Analysis of the plankton samples should be carried out using Lacky’s drop count/Sedgwick Rafter Plankton counting methods under Tri-nocular microscope (Leica Image Analysis System). The results should be expressed in organism /liter.

TABLE- 7 : Biological Water Quality Criteria Developed by CPCB

Range of Saprobic Score (BMWP)	Range of Diversity	Water Quality	Water Quality Class	Indicator colour
7 and More	0.2-1	Clean	A	Blue
6-7	0.5-1	Slight Pollution	B	Light Blue
3-6	0.3-0.9	Moderate Pollution	C	Green
2-5	0.4 & less	Heavy Pollution	D	Orange
0-2	0-0.2	Severe Pollution	E	Red

ii. Zooplankton

Sampling procedure – Zooplankton

Zooplankton sample should also be analyzed following the method as adopted for phytoplankton.

iii. Macro-invertebrates:

Sampling procedure – Macro-invertebrates

Macro-invertebrate species are an important group for the study of effect of pollution stress on aquatic communities. There are many sedimentary forms, some of which are more susceptible to pollutant toxicity than others. Taxonomic knowledge is useful for identification of these species in the field.

Identification of Samples

Collected samples should be examined under a dissection or stereo zoom microscope (10X and above) and identified using standard taxonomic literature.

Samples should be assigned to a family or genus using taxonomic keys for that particular group.

The bio-monitoring scores should be obtained by summing the individual scores of all families present. Values for individual families reflect their pollution tolerance based on the current knowledge of distribution and abundance.

In general Pollution intolerant families have high BMWP scores, while pollution tolerant families have low scores (Sivaramakrishnan, 1992).

BMWP-ASPT: The Average Score per Taxon (ASPT) should be calculated by dividing the score by the total number of scoring taxa. A high ASPT usually characterizes clean sites with relatively large numbers of high scoring taxa. Disturbed sites generally have low ASPT values and do not support many high scoring taxa (Sivaramakrishnan, 1992).

TABLE- 8 : Calculation of Nygaard Index

Index	Calculation	Oligotrophic	Eutrophic
Myxophycean	Chlorophyceae/Desmidiaceae	0.00-0.04	0.1-3.0
Chlorophycean	Chloococcales/Desmidiaceae	0.00-0.7	-0.2-9.0
Diatom	Centric Diatom/Pennate Diatom	0.0-0.3	0.0-1.75
Euglenophycean	Euglenophyceae/Cyanophyceae+ Chloococcales	0.0-0.2	0.0-1.0
Compound	Cyanophyceae+ Chloococcales+ Centric Diatoms+ Euglenophyceae /Desmidiaceae	0.01-1.0	1.2-2.5

Essential Equipments for Sampling Aquatic Insects

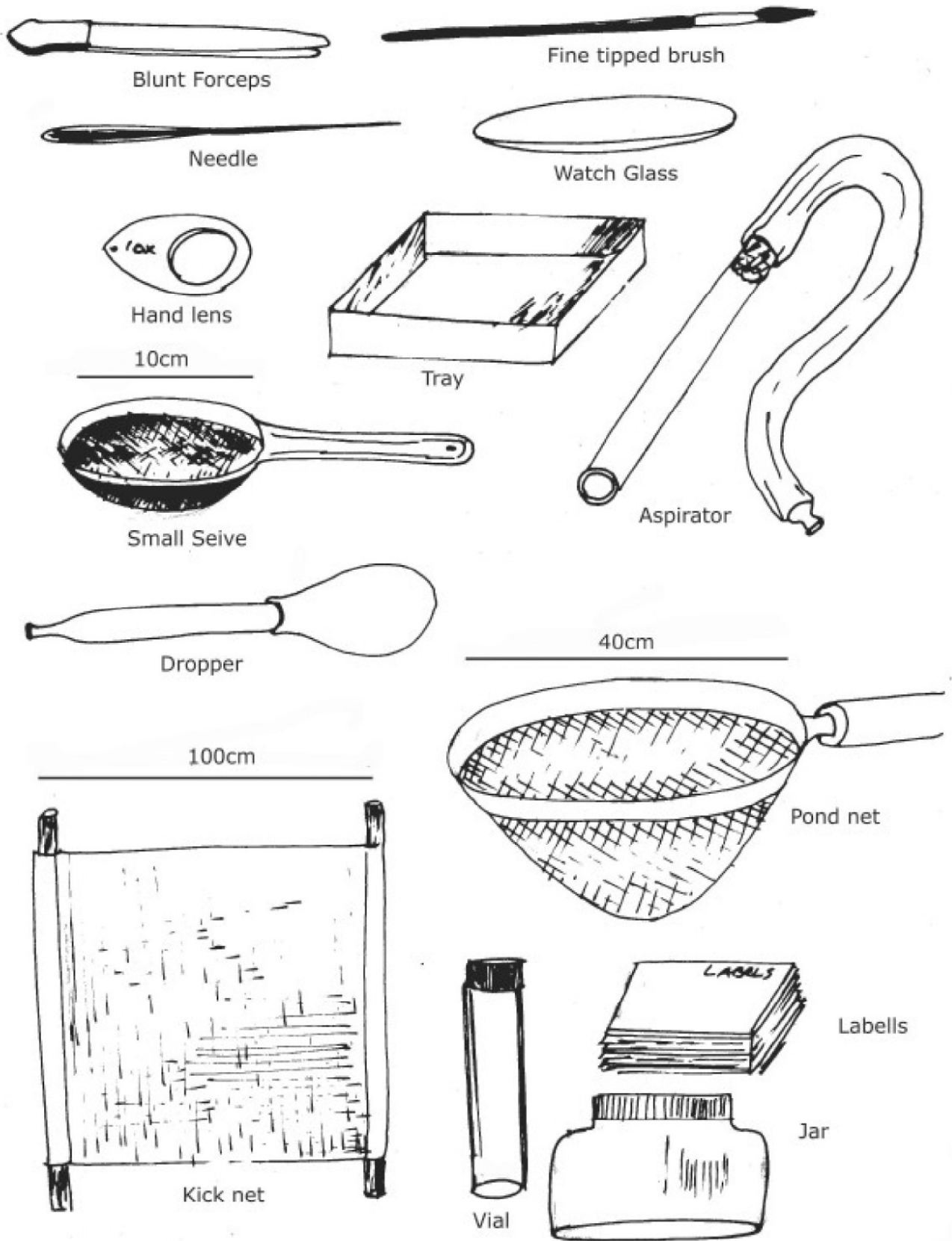


Fig. 1 : Tools used for collection of Macro-invertebrates

Benthic Saprobic Score Method (BMWP)

The benthic saprobity should be evaluated according to the method described by **Biological Monitoring Working Party (BMWP score system)**. This score system has a scoring of Zero to Ten of different benthic communities. The site score so derived is averaged which should be in the range of Zero to Ten. This score is then transformed into the benthic saprobity index by multiplying by a factor of 10 to produce a score from Zero to Hundred.

Calculation

1. The Saprobic Index

$$S = \sum (s.h) / \sum h$$

Where S = Saprobic Index, s = saprobic value for each indicator species, h = frequency of occurrence of each species. The value of S normally ranges from 1 to 4 for ambient waters.

2. The Diversity Index

$$H' = \sum Ni / N \log_2 Ni / N$$

Where H' = index value, N = total number of individuals of all species collected, and Ni = number of individuals belonging to the ith species.

3. Calculation of Shannon index

Formula

$$H' = \sum_{i=1}^R p_i \log_2 p_i$$

Where P is the proportion of characters belonging to the ith type of letter in the string of interest

Quality Assurance and Control in Sampling

Quality Assurance and Control (QA/QC) is an ongoing process which has the goal of prevention, early detection, and correction of field and analytical data

collection errors. For this-

1. Trained and experienced staff will be used for collection of samples.
2. The collection of sample would be authenticated by electronic gadget / certification by the field supervisors
3. Data sheets filled up by the field staff will be crossed checked and monitored regularly
4. The sample collection procedure shall be co-ordinated and managed as per the standard method mentioned in Canadian Bio-monitoring Network Manual (CABIN)
5. A coordinator/project manager would be deputed who will ensure that field staff are trained and who will also coordinate the management of the data.
6. Only trained participants will receive a username and password to enter data into the database.
7. All members of the field staff shall ensure all data sheets are filled in correctly and completely before leaving the site.
8. All members of the field staff shall determine if the data are reasonable before they leave the field, and if not, the measurements should be repeated before leaving.

Interpreting and Presenting Results

Results of impacted site should be compared with reference site to know how the benthic community has responded to habitat change along with the trophic status to be determined with the help of Shannon and Nygaard Index. The results thus obtained should be presented as simple tables and charts. Key results should be highlighted and presented in simple language. Graphical representation of results through maps and charts should be presented using GIS technology (colour bands) to reporting agency. The corrective measures for management action plan should be suggested based on the analysis report.

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