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E-SURVEY TO STUDY THE SITE SELECTION FOR DIATOMIC SAMPLE, CLEANING METHODS AND STAINING TECHNIQUES OF DIATOMS

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ABSTRACT:

Diatomic are unique unicellular algae, that represents a major taxonomic division of phytoplankton. The most distinguishing feature of this organism is extracellular coat which is composed of silica. Diatoms are native of all naturally occurring water bodies such as ocean, sea, rivers and puddles etc. They are major phytoplankton having importance in aquatic ecosystem to release oxygen and carbon fixation. Diatoms are shows diverse morphology and also having selective habitat. Due to distinct features they having application in water quality assessment, to design various Nano devices, as nutrient supplement, as medicine and having application in forensic science as well. This E- survey provides guidance on site selection in diatomic sample collection, cleaning methods of frustules and different staining techniques of diatoms.

Keywords: Diatoms, morphology, frustules, phytoplankton

Introduction:

Diatoms are major group of microscopic algae. They are unicellular and eukaryotic algae include into class (Bacillariophyceae). Usually occurring single but sometimes may be found in colonial or filamentous structure. Diatoms are pigmented and photosynthetic and some of them can structure heterotropically in the dark if get suitable source of organic carbon. The cell size of diatoms ranges from 5um-500um. They can found almost in each most and aquatic environment. Diatoms are a significant group of organism in terms of variation and freshwater system. They are uniquely accountable for production of 20-25 % of global oxygen. Also having role establishing a fundamental link in between primary and as essential component of marine (aquatic) ecosystem (Karthick Taylor, Mahesh and Ramchandra, 2010).

The diatoms are silica utilizing organisms with unique feature of siliceous cell wall names as frustule. As cell wall is made up of silica it is transparent look like glass due to this feature diatoms are also called as 'algae in glass houses'. The cell wall of diatoms is bipartite. The valves are frustule are overlapped on each other, which consists of epivalve and hypovalve. This two valve fits over as lid of a box. Frustules shows radial and bilateral symmetry with distinct shapes. Classification by taxonomist shows two major group of diatoms on based symmetry which include diatoms with elongated bilateral symmetry known as pennates and diatoms with circular radial symmetry known as centric. Their taxonomy is based on morphological feature, size and shape. They are one of extremely diverse group comprising of 10-12 thousand species and around 200 general as their morphological character they are diverse about their habitat and found marine, freshwater and some terrestrial habitat (Kale and Karthick, 2015).

Site Selection:

The site number and location should be select before Sampling so as to present Sampling in proper way. In case of water quality assessment changes in water should be marked. If sampling is intended to monitor the effect of all factor then upstream and downstream of water should be consider and sampling should be extend for an appropriate distance to know the pollution often on water.

Site selection can be considered at 3 distinct parameters or scale.

1. A very broad scale

2. An Intermediate scale

3. A fine scale

A very broad scale is concerned with site of sampling within a drainage area (catchment). An intermediate scale is concerned within a designated area sampling of site selection. A fine scale concerned within a reach from where sample should be collected. Diatoms are occur in wet places where there is a possibility of photosynthesis .on the basis of occurrence diatoms are classified into two types such as planktonic which are free floating diatom and benthic which are attached to the subtraction. (The Icfai university journal of soil and water Sciences (2010).

If benthic diatoms are being added to study, the samples site are likely to be situated near to the center or location of pre-exciting survey to aid the comparisons of diatoms found in the specific water body and other types of environmental information. Such process may include the choice of sample station to approximately 100 m area appropriate selected site will be determined by the choice of substratum. All data should be note down in the standard format which will help to aid the data interpretation. Local situation and need can determine the precise nature of the information that is required and the following should be considered.

1. A detailed description of the site on first visit and note subsequence visit on unusual occurrence must be taken.
2. Mapping of site should be done and map reference should be taken from the survey of India topographical sheets. This information should have co-ordinates like latitude longitude and altitude with the name of (village / hamlet)
3. A record form should be design for field to record the all the parameter at site which include the sampler name, measurement of width and depth, substratum composition extent of bank site, cover of algae and macrophyte etc.
4. Photographic record should be done for data interpretation which may help to note the limited major changes that have occur.

In case of stream bio monitoring site should be in 'riffle' where water flowing over stones and runs and glides are also suitable for collection with proper selection of substratum. Sampling should be done in moderate and high velocity of water which ensures the continuous exchange of the water with algae in surrounding and it prevent suit up of a local chemical environment like selection of cobbles from pools and ponded areas should be avoided for sampling. If the sampling changing aid of water quality assessment. If the river is deep then suitable sample should be collected from the bank of rivers, cobbles or other should be collected from the area which is near to the rivers bank from riffles with flowing water to where flow is > 20 cm/s. This based on the assumption that flowing water on edge having same physical and chemical quality at that in the main steam.

Diatom community in the stream can be determined by light regime and velocity of water because light regimes have effect on diatom community structure and physiological process influencing organism's response to pollutants. The sample has to be compared should collect from the similar light regimes. Heavily shaded area, area which is much closed to the bank

should be avoided because it may show varied water quality and increased sediment also have influence o diatom community.

Choice of substratum after site selection is one of the important factor because diatoms can be found on most submerged surfaces, however the community varies which is depend upon the chosen substratum. According to the round (1993) diatoms have distinct assembly that found associated closely with particular microhabitat. If the sampling is having an aid of particular community then the care should be taken that the sample should not get contaminate from other microhabitat diatoms are detected by observing the slimy or mucilaginous feel or with the thin golden – brow film covering substrata . They can colonies on artifacts, plastic bags, piece of wood etc. So sample can be collected from this substrata when alternative is absent. So the substrata can be Eplithon epiphyton, macrophyte, and artificial. (Taylor *et al.* ,2007)

Cleaning Technique:

Frustules of diatoms may be cleaned with either hydrogen peroxide acid. To resolve the structure of frustules in light microscopy which is used for identification cleaning technique are important. The organic components of cell must be removed to resolve structure and slide preparation should done with the complete removal of organic matter in the sample, foreign material should complete removed or absent in sample, The values of cover slip should not be clumped and spread over the entire area without edge effect, the mountant should be properly placed without air bubbles contamination a must be avoided in all the preparation of sample cleaning method should free from health hazard. The chemical used for preparation of samples may be carcinogenic and corrosive, adequate safety and health precautions should be taken in every step of sample preparation.

The common method of diatom cleaning for preparing diatom slide is acid oxidation. It effectively removes all organic of cell including membrane. Depending upon the preferred microscopic technique both acid and non-acid techniques hydrogen peroxide method is used because it is gentler than acid oxidation. Remaining untreated material or exception of calcium deficient water. it is necessary to dissolve the calcium with hydrochloric acid and then sample should rinsed. This is important if further processing of sulphuric acid is needed otherwise precipitate of calcium sulphate write form, which will difficult the process of identification. In absence of fume cabinet all methods with boiling acid must be avoided.

In all cleaning method is the original sample should allow to settle for 24 hrs. And supernatant water should cleared without losing diatoms material. The prepared sample should be proceed c bar slide preparation and microscopic analysis. After cleaning method final rising is need to rinse the remnant acid and also prevent the reaction in between mounting medium.

Hydrogen peroxide Method:

It is one of the best method used to clean the diatom sample that require little cleaning and where corrosion is very less. Hydrogen peroxide is much gentler method that the acid method and it is not corrosive. The choice of hydrogen peroxide method which may be either cold or hot depends upon the fume cabinet availability.

Hot H_2O_2 Method

- Diatom suspension should mixed and take 5-10 ml of the suspension in a beaker.
- Beaker should mark properly with sample number. (Preferably in several places).
- 20 ml of H_2O_2 should add to sample and the sample should heat on hot plate for 1 to 3 hr. at 90^0 c.
- Few drops of HCL should add and leave the sample to get cool.
- The samples should rinse as in HOT HCL and $KMnO_4$ method.

Cold H_2O_2 Method

- Diatom suspension should mixed and take 5-10 ml of the suspension in a beaker.
- Beaker should mark properly with sample number (Preferably in several places).
- 20 ml of H_2O_2 should add to sample and leave sample for four days.
- The samples should rinse as in HOT HCL and $KMnO_4$ method.

Bleach Method

- The samples should rinse to remove any preservative if present by centrifugation (3 runs at 2,500 rpm) with distilled water.
- Diatom suspension should mixed and take 5-10 ml of the suspension in a beaker.
- Beaker should mark properly with sample number (Preferably in several places).

- Commercially available bleach (sodium hypochlorite) should add to sample in quantity of 5-10 g and leave it for minimum one day (it depends on organic material in sample).
- The samples should rinse five times by distilled water.

Hot HCL and $KMnO_4$ Method

- The sample should shake well and pour 5-10 ml of thick suspension into beaker.
- Beaker should mark properly with sample number (Preferably in several places).
- 10 ml of saturated potassium permanganate solution should add in sample, mix it well and leave sample for 48 hrs.
- 10 ml of concentrated HCL should add to the sample and beaker should cover with watch glass.
- The samples should heat on hot plate at 90^0 c for 1 to 3 hrs. in fume cabinet until the sample changes to clear and yellow solution.
- Do not allow sample to boil and care should take so that sample not get cross contaminated.
- After oxidation 1 ml hydrogen peroxide should add drop by drop in sample. Absence of fumes will conclude the complete oxidation of organic material.
- After oxidation sample should allow to cool and transfer in centrifuge tube (10ml).
- Rinse the sample by centrifuging at 2,500 rpm for 10 min. with distilled water, followed by washing.
- During washing without losing any diatomic sample, supernatant should be poured off.
- Supernatant should decant and the centrifugation should repeat
- Sample should further wash for at least four time.
- After last washing, the diatoms again loosened by means of a jet of distilled water and sample should poured into glass storage labelled with necessary information.

Decalcification

Decalcification is necessary if sample later treated with Hot HNO_3 /H_2SO_4 method .In this method sample combine with acid and form insoluble precipitate .

- The sample should shake well and pour 5-10 ml of thick suspension into a heat – resistant beaker.
- Add few drops of dilute HCL in a fume cabinet and agitate gentlye; effervescence as the carbonate reduces to co_2 can observed.
- Continue addition of dilute HCL and agitate the beaker until effervescences stop.
- To remove the acid, pour the solution into centrifuge tube and add distilled water and centrifuge the sample.
- The sample should centrifuge and rinse with distilled water at 2,500 rpm for 10 min.
- The supernatant should decanted after centrifugation and washing is repeated for four times.

Hot HNO_3 / H_2SO_4 Method

- Sample should check for presence of calcify and decalcify the sample if necessary.
- The diatom sample should mix carefully and according to the density sample size should take ~ 10 ml.
- Beaker should mark properly with sample number (Preferably in several places).
- 5 ml Strong acid mixture should add (HNO_3 + H_2SO_4, 2:1) and sample should place on hot plate to heat in beaker covered with glass watch at 90^0c for 2-3 hrs. Cross contamination should avoid during boiling with acid.
- Sample should rinse and test for presence of organic material as in Hot HCL and $KMnO_4$ method. (Karthick B. Taylor J. C.Mahesh M. K. & Ramchandra,2010)

Staining Techniques of diatom:

Preparation of staining reagent:

The Methyl Red indicator was prepared by dissolving 0.01 g of Methyl Red 30 ml ethanol and make up it up to 50 ml with distilled water.

Procedure:

A drop of sample should take on microscopic slide and add methyl red Indicator to it. cover slip should place on mix sample with methyl red indicator and slide should kept at room temperature for 20 minutes, slide should observe under microscope.

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