COLLECTION AND EXTRACTION OF DIATOMS IN AURANGABAD REGION IN FORENSIC DIATOMOLOGY

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Abstract: Diatoms are unicellular, eukaryotic and photosynthetic algae with unique cell wall made up of silica. They have selective living habitat which are variable according to climate, chemical factors and interference of other biological organisms. Diatoms are indicates past and present ecological questions and they are strongly related to the quality of aquatic system. Diatoms having different geometrical shapes with different taxonomic precision. Due to their unique characteristics they are used in nutrient supplements, antibiotic, anti-cancerous drug, nanotechnology and paleoecology to interpret historical condition from fossils. Present study will help forensic expert to compare with samples in drowning cases of Aurangabad region and to conclude pre and post- mortem drowning. It helps to link suspect and victim to crime and demonstrates the collection and extraction methods for diatoms with minimum loss.

Keywords: Diatoms, Frustules, H_2O_2extraction, Methyl red.

Introduction: Aquatic microscopic life termed as algae having large group known as diatom(Greek = "cut in half"). Diatoms are unicellular, eukaryotic and photosyntheticorganism and found almost in every aquatic and moist environment such as lake,river, ocean, sea, ditches and puddles. The cell size of diatoms ranges between5µm – 0.5 mm. Diatoms is including in class*Bacillariophyceae* having unique silica cell wall with distinct geometrical shapes.Diatoms are crucial in determining biological diversity with 200 genera including around 10-20 thousand species and primary productivity. Each diatoms species occurs according to its specific environmental tolerance and the species which are globally distributed are mostly pollution tolerant species. Diatoms are classified into two types on the basis of their habitat either Planktonic(free-floating) or Benthic (attached to a substratum).Diatoms are occur solitaryand sometimes forms colonies.

Diatoms are silica utilizing organisms with the most distinctive feature of siliceous cell wall known as frustule. As the silica present intransparent frustule like glass this organism is referred as 'algae in glass houses'. The frustule is dividing into two parts i.e., comprising of two valves. The valves overlap to form complete cell structure. A single frustule consists of an epivalve (larger valve), ahypovalve (smaller valve) and girdle bands that hold both valves together. Frustules have a distinct shape and intricate sculpturing with pores, spines, andelevations. Due to the differentiation of frustules into valve and girdleeach diatom cell can represent itself in two principal of orientation i.e.valver view and girdle view. Cells are must be seen in the valve view for identification because valves containing more features but in some genera such as *Rhizosolenia* girdle view is more useful for identification. Diatom taxonomy is based on morphological characters like size, Shape andpatterns of frustules. Taxonomists classify diatoms into two major groups on the basis of symmetry:

1) Centric – circular; radial symmetry,
2) Pennates – elongate; bilateral symmetry

The diatoms have specific pores known as areolae. These areolae allow the gaseous exchange and material between the cell and the environment. The central cytoplasmic bridge consist nucleus in it. It is surrounded by a large vacuole, which usually accounts for 70% of the total cell volume. Vacuole and deposit Photosynthetic deposit products into the vacuole attached to chloroplasts. Other organelles like silica deposition vesicles (SDVs) are closely associated with theGolgi complex and, in some species, mitochondria may be found in proximity to both. In diatoms Chlorophyll a3 and c4 carry out the process of photosynthesis. Golden-brown color of diatoms is due to the relative proportion of these and the accessory pigments, fucoxanthin and β-carotene. Diatoms store energy as unique polysaccharide chrysolaminarin or various lipid molecules. They are beingexplored as a source for biofuels. Some species are known to stay afloat by regulating intracellular lipids. Diatoms can be used in nutrient supplement, antibiotics, anticancerous drug and nanotechnology. Diatoms are indicates past and present ecological questions. Diatoms are used in paleoecology to interpret historical condition from fossils.

Material and method: Diatoms may be detected on substrata by feel (slimy or mucilaginous) or may beseen as a thin golden-brown film covering substrata. In some conditions or atcertain times of the year this film may become thicker and much more noticeable.The essential natural microhabitats are solid substrata, exposed damp sedimentsand the stems of rooted vegetation. Diatoms are also present in the suspendedcomponent of the phytoplankton. Man-made and other objects (paper or Plasticbags, pieces of wood, cloth) are also frequently colonized by diatoms. The water samples of lake water were collected by categorized habitat into foursampling categories. The four categories are given below:

1. Lake water sample
2. Benthic sample
3. Planktonic sample
4. Sample with artificial substratum

The samples were collected from the bank of lake, from where it was easier toreach at site of sample collection and nearer to the human locality. The sampleof Harsul Lake water referred as the **sample 'A'** and the sample ofSawangi Lake water referred as the **sample 'B'**.

Method of sample collection

1) Lake water sample

Lake water samples were categorized into two type of habitat.

a. Flowing water sample

The flowing water sample was collected by using collection bag .The sample collection bag was dipped inside the lake deep as possible and sample was collected.

b. Stagnant water sample

The stagnant lake water was collected from the habitat where the water flow is not observed.

Fig1: Sawangi lake water sample with noted physical factors.

2. Benthic sample:

Benthic sample is the habitat where *Bacillariophyta* grows and attached to asubstratum as substratum is the rock where brown color biofilm is observed.The cobbles were preferred for benthic sample collection. If cobbles were notoccurred the boulder was taken. The biofilm was observed and it was scrabbedvigorously with toothbrush and the rock was washed with the lake water. Thebrown color water samples were collected into the collection bag. (Taylor J.C.,2007).

Fig 2: Benthic sample collection from rock substratum

3.Planktonic sample:

The Planktonic sample was collected from the habitat where the growth or mat ofmacrophyte was observed. The sample was kept into the polythene bag with lakewater and mixed thoroughly and the sample was collected in collection bag(Taylor, 2007).

Fig.3: Planktonic sample collection with macrophyte

4.Samples with artificial substratum

The sample was collected from the artificial substratum was the cloth sample .The clothsample was kept into a polythene bag with lake water .The polythene bagwas shaken vigorously and the cloth sample was removed .The brown coloredwater sample was collected in collection bag.

Separation Technique: If the sample was contained large sand grain or mud then the filter paper was usedto separate them. To resolve it more sample was filtered through the tissue paper.The porosity of filtering material was considered.

To avoid the sand particles, plant sediment or any artificial material samples werefiltered through net use for fishing.

Laboratory treatment:

Pre-examination

The sample was quickly examined to analyze the quality of water and quantity of diatoms. The maximum number of live cell showed the true quality of water. Ifthe majority of dead cells (frustules without chloroplast) observed then the samplewas discarded as it may not show the true reflection of water quality of samplingsite.

Cleaning techniques: Cleaning techniques are helps to clean frustules and also to remove organicmaterial from the sample. The sample can clean by two methods either aciddigestion or hydrogen peroxide. The conditions for cleaning process wereoptimized in present study for microscopic observations. Cleaning techniques aresignificant to clean the frustules structure and to identify diatoms.

For Sample ‘A’ and Sample ‘B’ Hydrogen peroxide method was used.Hydrogen peroxide is gentler than acid and it is a less corrosive method than aciddigestion method.

Hot H2O2Method: The sample was centrifuged after pre examination and the pelletof complete water sample was dissolved in to 10-15 ml distilled water. The pelletwas disturbed by using paintbrush or gentle shaking. Vigorous shaking or roughhandling may cause the damage to frustules or cell of diatom. Same amountofH2O2was added to the suspension and it was kept on water bath at 90° for 1-3hours. Leave sample to cool (Karthick B. and et al., 2010).

Cold H2O2 Method: The sample was centrifuged after pre examination and thepellet of complete water sample was dissolved in to10-15 ml distilled water. Thepellet was disturbed by using paintbrush or gentle shaking. Vigorous shaking orrough handling may cause the damage to frustules or cell of diatom. Same amountof H2O2 was added to the suspension and sample was leave for four days.(Karthick B. et al., 2010). After both procedure the sample was centrifuged at 2500rpm for 10 minutes at24°c and by same procedure 3-4 wash of distilled water were given to sample .By gently shaking the pellet was mixed in appropriate amount of wat

Preparation of diatom slides

(Franchini W., 2013)

1. Slides and cover-slips were cleaned thoroughly with detergent soap andbefore use it was
2. Cleaned with ethanol.
3. The sample was mixed thoroughly because the larger size ofdiatom tends to settle down; with the help of dropper thesample was placed on cover slip and kept sample dried or upto moist.
4. DPX used as the fixative and with help pointed needlethe fixative was mounted on the cover slip.
5. The cover slip was kept on to the microscopic slidewith the help of forceps.
6. The sample was leave to fix slide for sufficient time tillthe fixative get dried.
7. After fixation the sample was observed under 40 xs and 100 xsunder oil immersion.
8. The sample carefully labeled with the details.

Staining of diatom

Preparation of staining reagent:

The Methyl Red Indicator was prepared by dissolving o.o1gm of Methyl Red 30ml ethanol and make up it up to 50 ml with distilled water.

Staining of diatoms:

A drop of sample was taken on microscopic slide and methyl red indicator wasadded to it. Cover slip was placed on mixed sample with methyl red indicator andslide was kept at room temperature for 20 minutes. Slide was observed under oilimmersion**.** (Zetsche E.M. et al., 2012).

Result and Discussion:

Identification and systematic description of diatoms based on morphological characteristics in book 'An Illustrated Guide To Common Diatoms of PeninsularIndia by .Karthick B, Hamilton P.B &Kociolek J.P (2013), given as follows forsample 'A' (Harsul lake water sample, Aurangabad)

Asymmetrical biraphid

- Valves asymmetrical to apical axis/asymmetrical to the transapicalaxis, orboth
- Usually secrets mucilaginous stalks or mucilagous tubes
- Raphe system well developed

Symmetrical biraphid

- Valves with bilateral symmetry
- Valves symmetrical to both apical and transapical axes.
- Raphe system well developed and cells may be highly motile.

Nitzschoid

- Valves usually symmetrical to both apical and transapical axes
- Canal raphe system part of keel
- Keel positioned near or at the valve margin
- Keel can be flat or elevated from the valve surface.

Surirellaloid

- Frustules isopolar or heteropolar
- Raphe positioned along entire valve margin
- Raphe located on the canal ,which may be raised above valve surface

Monoraphid

- Valves with bilateral symmetry (symmetric about a line)
- Raphe system present on one (raphe valve)
- Raphe system absent on one valve (pseudoraphe valve)

Centric

- Valves with radial symmetry.
- Cells lack a raphe system and lack significant motility.
- Cells may possess fultoportulae and rimoportulae.
- Sexual reproduction is oogamous.

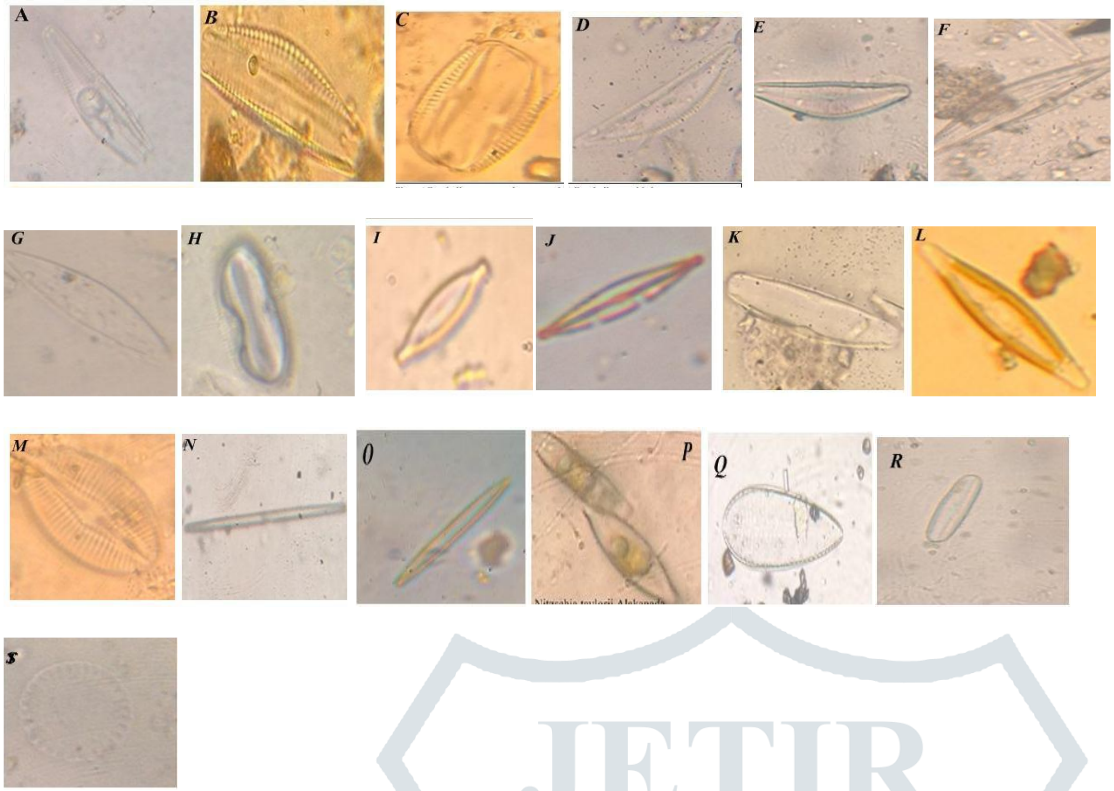
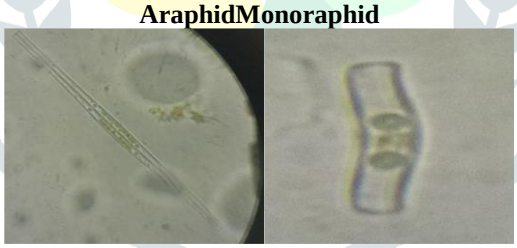


Fig**. A.** *Cymbella perparva krammer (kutzing)Grunow* ***B.*** *Cymbella turgidulgrunow* ***C.*** *Amphora pediculus* ***D.*** *HalamphoraVenetaKutzing***E** *Encyonemahustedtii krammer* ***F****.Cymbella bengalensis Grunow* ***G****.Navicula cryptocephala kutzing****H****.Mastogloiaelliptica (Agarth)cleve****I****.NavicularostellatakutzingJ. GhomphonemaAugustatum(kutzing)Rabenhorst****K.*** *Sellaphora Bacillium Var Jogensis Gandhi* ***l****. .Brachysiramicrocephala (Grunow)compere***M**. *Nitzschiacompressa(bailay)* Boyer **N**. *Nitzschia linearis (Agardh)smith* **O.** *Nitzschia umbonata(Ehrenberg)Lange-Bertalot* ***p****. Nitzschia tayloriiAlakananda , Hamilton &Karthick* **Q.** *Surirella lange-bertalotii kathik,Hamilton& kociol***R** *.Platessahustedtii (krasske)Lange –Bertalot***s.***Cyclotella meneghiniana Kutzing*

Identification and systematic description of diatoms have been done using book ‘An Illustrated Guide to Common Diatoms of Peninsular India by .Karthick B , Hamilton P. B &Kociolek J.P (2013) given as follows for sample ‘B’ (Sawangi Lake water sample, Aurangabad):

AraphidMonoraphid



UlnariaacusAchanthidiumminutissi(KutzingAboalss)(Kutzing)Czarnecki

Symmetric biraphid

Centric

MelosiraVariansAgardh

Assymetricbiraphid

Amphora copulata (Kutzing)Schoeman& Archibald

Discussion:

The study was designed and carried out to prepare database of diatoms by usingproper collection and extraction methods of diatom .The previously collectionmethod was done by using filter net on sample collection site. The present studyrepresents the modification in collection that sample was filtered aftercentrifugation process and dissolving pellet in 10-15 ml of water by usinglaboratory filter paper to remove the organic material from sample but the largesize of diatoms were not get filtered through it. The filtration by using fishing netwith small pore size was more significant than filter paper and it helps to removethe organic material or mud from sample. The rotation per minute ofcentrifugation was kept at low rotation in between 2500-3000 rpm which help toprevent damage to diatom cell.In cleaning procedure the use of equal amount of H_2O_2gave the good result and prevent the frustule damage and also breakage of diatom cell.Staining of diatomby using methyl red indicator helped to stain the cell wall of diatom withbrownish red color and it helped to visualize diatom cell also to differentiatediatoms.In sample 'A' the colonizationof *NitzschiataloriiAlakananda,Hamilton&Karthick occurs* prominently and in abundance. *NitzschiataloriiAlakananda,Hamilton&Karthick is* from taxa Nitzschioid having usually symmetrical valvesto both apical and transapical axes .also valves lanceolate to linear lanceolate withprotracted round to capitates apices. The raphe is continuous, with terminal apicesdeflected towards the valve face as a continuous loop across the apex mantle.Areolae on the valve are round to elongated depression. This taxa is mostlyoccurs in highly nutrient conditions(Karthicketal., 2013).In sample 'B', there are two species observed in Sawangi lake water samplewhich are more prominent and observed in colonies. The first species is

*Ulnariaacus (Kutzing)Aboalss*from taxa Araphid with bilateral symmetry of valveand lack of raphe and significant motility .valves are linear with sub-capitatespecies. Well defined hyaline area is present at the centre of valve, reaching onevalve margin only. This species occurs in the alkaline water with rich in oxygen. This species having valve length between 90-166µm.The valve lengths measured from prepared slides is 154.015 µm (Karthicket al., 2013).

The another prominent species in sample 'B'is*Achanthidiumminutissimum(Kutzing)Czarnecki*from taxa Monoraphid havingvalves with bilateral symmetry (symmetry about a line).Raphe system is presenton one valve known as raphe valve and valve with absent of raphe system knownas the pseudoraphe valve. Valves are linear – lanceolate with protracted, roundedapices.Raphe valves are narrow with broad centre area to form a transverseirregularly shaped central area. This species occurred in in well oxygenated, cleanwater and are slightly motile.(Karthick B. And et al., 2013).

Conclusion:

- Our study demonstrates the collection and extraction methods withMinimum loss of diatoms.
- The extraction procedure of diatoms with decrease in concentration ofH_2O_2 prevents the damage of frustules and also breakage of cell.
- Present study will help to forensic expert to compare with samples indrowning cases of Aurangabad region.
- The extraction procedure may help to recover diatoms from sample withminimum damage and help to conclude pre and post mortem drowning.

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