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Original Research Article

Forensic Characterization of Waterbodies In Malnad Region By Diatomological Mapping.

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Diatom test, Drowning, Diatomological mapping.

Abstract

Background: The 'Diatom test' is a reliable method which helps to determine the cause of death due to drowning based on quantitative and qualitative analysis of diatoms in human body and reference water sample. Positivity of this test is considered as an indicator of antemortem drowning. **Methods**: It was a prospective study where Samples were collected from ten different water bodies which included lakes, rivers, canals and reservoirs, for a period of one year and all the seasons were included by sampling once in two months and totally six samples of water were collected. The pH and temperature of water were recorded during sampling. From each of water samples, diatoms were extracted using concentrated nitric acid method. The sample was centrifuged, the resultant aspirate was poured over a clean microscopic glass slide, dried and analyzed with optical compound microscope fitted with light source at different magnifications. **Result:** The results show that, Total 22 genera of diatoms were identified. Most of them were pennales and few were centrales. Diatoms like Navicula, Cymbella and Synedra were present commonly in almost all water bodies, Melosira, Achnanthes and Brachysira were particularly seen in Lake water, Diatoma and Thalassiosira were commonly present in River water. Amphora and Ctenophora were commonly found in Lake water. Eunotia pectinalis and Tabellaria were particular to dam water. Thalassiosira, Surirella, Diatoma Cocconeis, Cyclotella were more commonly found in flowing water bodies. Melosira, Ctenophora, Brachysira, Achnanthes, Fragillaria were common to stagnant waters. **Conclusion:** Diatomological Mapping is a new tool in Forensic Biology as well as in Forensic Medicine, it must be constructed as organized research database.

1. Introduction

When a body is recovered from water, two critical questions require resolution: Was the victim alive or dead when he entered the water? Is the cause of death drowning? It is difficult to answer

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the both questions for the bodies recovered from water, especially in decomposed bodies in drowning or any other cause of death. It is also important to ascertain whether death of the person occurred at the place where the body was recovered. It imparts further necessity for the precise localization of site of drowning particularly when the body is found on land and no reference water body is available, or when the body is found away from the actual site of drowning which may be due to flow of water or any other reason.[1]

The diatom test where analysis of diatoms could provide a supplementary evidence, answers most of the above questions raised. The diatom test for the diagnosis of drowning is based on the assumption that diatoms, which are eukaryotic unicellular algae, reach the lung with inhalation of liquid and if effective cardio-circulatory activity exists, penetrate the pulmonary filter and disseminate to organs through the blood stream. Conversely, if a corpse is submerged Postmortem, the diatoms may penetrate passively into the airways, but, owing to the lack of cardiac activity, will not be transported to other organs.[2] Acid digested extracts of various internal organs demonstrate the presence of diatoms similar to those found in the drowning medium. Various genera and species of diatoms establish themselves within the specific water bodies based on their nutrient and light requirements and therefore they can differ from one water body to another both qualitatively and quantitatively with climatic or seasonal changes.[3] Some local factors like mineral content of water, temperature, water stratification, acidity, the distance from shore, the depth of sea and the tide, etc. do affect the diatom concentration in any water bodies.

Study of diatom flora over a period of time from different types of water bodies can be used as a suitable tool for generating Diatom profiles, which can be used not only as a standard for comparison with the diatom flora found in the tissues of drowned victims but also can be utilized to generate diatomological Maps which records the profiles of diatom flora of any water body with seasonal variation.[4] The foremost Global Burden of Disease (GBD) study by the World Health Organization (WHO) and the World Bank demonstrated that drowning is one of the most common causes of death throughout the world and reported 5,04,000 deaths due to drowning.[5] Hence here an attempt has done to collect and analyze water samples from different freshwater bodies likes rivers, lakes, dams, canals etc., from different parts of Malnad region for diatom distribution pattern and their seasonal variations. Thus, to create a profile of diatoms or diatomological map in at least ten water bodies of this area to help in positive diagnosis of drowning.

2. Materials and method

2.1 Materials used for analysis

1. **Plastic Water Sampling Jar of one litre capacity**
2. **Laboratory Thermometer** (from -5° C to +100°C with 0.5°C divisions)
3. **pH meter**- ESICO, Model 1012 microprocessor-based pH system
4. **Conc. Nitric Acid** - for extraction of diatoms by chemical digestion method
5. **Sterile conical measuring glass jar**- used to hold the water and acid mixture for acid digestion of particles in water
6. **Glass pipette**- to transfer test solutions
7. **Sterile plastic centrifuge tubes-** used for centrifugation in centrifuge machine
8. **Centrifuge Machine:** Remi, laboratory medical centrifuge.
9. **Glass microslides and coverslip-** to hold the water sample residue for analysis of diatoms
10. **Compound Microscope**

2.2 Methods

2.2.1 Collection of water sample:

This particular study is a prospective study for a period of one year, diatom samples were collected from different geographical localities of Malnad region. The water samples were collected from 10 different selected water bodies numbered S1- S10 (which include 3 – Lake, 2 – canals, 2 – rivers, 2 dams and 1 domestic source) once in 2 months covering all the seasons i.e., winter, spring, autumn and summer.[6] Six collections were made during a span of one year. The dates of sample collection were kept almost same. The water samples were collected just below the surface of water using sterilized plastic containers of one litre capacity. The pH and temperature of water were recorded during sampling. The pH was measured using a digital pH meter. Temperature was recorded using laboratory thermometer (**Table 1**).

2.2.2 Extraction and Analysis of Diatoms:

Extraction of diatoms from water was done using chemical digestion by concentrated nitric acid method. From each of the water sample 100ml of

water was taken and was transferred into an acid washed glass beaker. Samples were added with concentrated 25ml of nitric acid and then samples were allowed to stand undisturbed for 2 h. These samples were transferred to properly label plastic centrifuge tubes and centrifuged at 3000 rpm for 10 min. The supernatant was pipetted out leaving behind only a residual material at the bottom of tube. This residual material was suspended in distilled water and again centrifuged in the same way to ensure that even the traces of acid were removed. After final centrifugation except for 1ml the whole supernatant was discarded by pipetting out. The left-over aspirate is poured over a clean microscopic glass slide dried and mounted with DPX39 analyzed with optical compound microscope fitted with light source at different magnifications. Diatom species were identified on the basis of available literature.

Table-1: Description of Water Bodies

Station code	Water body	Source	Geographical area type
S-1	River	Tunga river	Plain
S-2	River	Badra river	Plain
S-3	Dam	Gajanur dam	Foot hills
S-4	Check dam	Purdal check dam	Foot hills
S-5	Lake	Mattur lake	Plain
S-6	Lake	Shimoga lake	Plain
S-7	Lake	Purale lake	Plain
S-8	Canal	Canal near jail road Shimoga	Plain
S-9	Canal	Canal near sharavathi dental college	Plain
S-10	Domestic source	Tap water	-

2.2.3 Diatom identification:

The diatom genera were identified by observing them at 100X magnification under compound microscope and the diatoms identified were compared with standard species chart available.

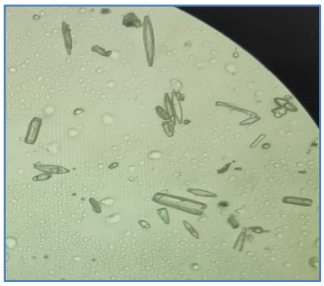
3. Observation & result

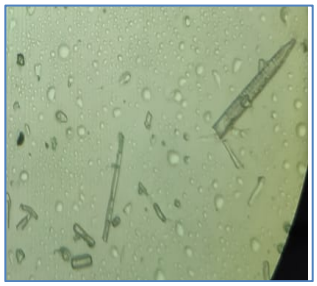
After analysis of water samples from 10 different water bodies (S-1 to S-10) during different seasons for a period of one year, diatoms were observed in all samples. Totally 22 different genera of diatoms were identified. The cell wall or frustule of diatoms were of either of two body plans pennales and centrales, and most of them belong to pennales and few were centrales. The observed diatoms were –Achnathesis, Amphora, Astrerionella, Brachysira, Calonies, Cyclotella, Cymbella, Cocconeis, Ctenophora, Diatoma, Eunotia Pectinalis, Fragilaria, Gomphonema, Melosira, Navicula, Nitzschia, Pinnularia, Pleurosira, Surirella, Synedra, Tabellaria, and Talassiosira. Out of which melosira, cyclotella, talassiosira belonged to centrales (**Figure 1-3**).

In this study the diatom of slightly larger size is more observed in lakes than in river suggesting that larger ones are more common in stagnant waters than moving waters.

Figure no. 1 : Microscopic View of Diatoms

Figure no. 2 & 3: Microscopic View of Diatoms





Noticeable divergences of diatoms are noted depending on pH level of the water body. Distribution pattern of diatoms has shown characteristic variation among the selected sites. Navicula genus was seen in all water bodies including the domestic source tap water. And they were present in all samples in all seasons but in varying quantity. Synedra genus was present in all the fresh water bodies except in the domestic source tap water. Cymbella was seen in most of the water bodies except in two lakes. Navicula, Cymbella and Synedra were common to

most of the water bodies studied. Melosira, Achnanthes and Brachysira were particularly seen in Mattur Lake and Purle Lake. Diatoma and Thalassiosira were commonly present in Badra River. Amphora and Ctenophora were commonly found in Purdal check dam and Shimoga Lake. Eunotia pectinalis and Tabellaria were particular to Purdal check dam and Gajanur dam. Thalassiosira, Surirella, Diatoma Cocconeis, Cyclotella were more commonly found in flowing water bodies Melosira, ctenophora, Brachysira, Achnanthes, Fragillaria were common to stagnant waters (**Table no-2**).

Table-2: List of Diatoms and their distribution in various Water Bodies

Diatoms	S-1	S-2	S-3	S-4	S-5	S-6	S-7	S-8	S-9	S-10
Achnanthes	-	-	-	-	+	-	+	-	-	-
Amphora	-	-	-	+	-	+	-	-	-	-
Astrerionella	+	-	+	+	-	+	-	-	+	+
Brachysira	-	-	-	-	+	-	+	-	-	-
Calonies	-	+	-	-	-	-	-	-	+	-
Cyclotella	+	+	+	-	-	-	-	-	-	-
Cymbella	+	+	+	+	-	-	+	+	+	+
Cocconeis	+	-	-	-	-	-	-	+	-	-
Ctenophora	-	-	-	+	-	+	-	-	-	-
Diatoma	-	+	-	-	-	-	-	-	+	-
Eunotia Pectinalis	-	-	+	+	-	-	-	-	-	-
Fragilaria	-	-	-	-	+	+	+	-	-	-
Gomphonema	-	-	-	-	+	-	+	-	+	-
Melosira	-	-	-	-	+	-	+	-	-	-
Navicula	+	+	+	+	+	+	+	+	+	+
Nitzschia	-	-	-	-	-	-	-	+	-	-
Pinnularia	-	+	-	+	-	-	-	-	-	-
Pleurosira	-	-	-	-	-	-	-	+	-	-
Surirella	+	-	-	+	-	-	-	-	+	-
Synedra	+	+	+	+	+	+	+	+	+	-
Tabellaria	-	-	+	+	-	-	-	-	-	-
Talassiosira	-	+	-	-	-	-	-	+	-	-

Noticeable divergences in the seasonal distributional of diatom species were recorded in the selected water bodies. On the whole Diatoms were present in large numbers during the early post monsoon season and in a considerable amount during the summer season. Their population steeply decreased during winter season and monsoon.

4. Discussion

Among 22 different genera of diatoms identified, Navicula, Synedra were common to most of the water bodies these were the most frequently observed diatoms in all water bodies in all seasons, thus they were found to be widespread or common to this region. This was similar to the results obtained in a study conducted by Sane R, Verma P in Indore (MP) where they found Navicula in almost every water body with variation in their quantity.[7] Mattur Lake and Purle Lake where both the lakes are habitat for various aquatic plants. Melosira, Achnanthes and Brachysira were particularly observed from these two water bodies making them more specific for water bodies with aquatic vegetation and also these two water bodies were more alkaline in nature compared to the others. Amphora and Ctenophora were commonly found in Purdal check dam and Shimoga Lake. Both the water bodies have clear water and minimal pollution.

Eunotia pectinalis and Tabellaria were particular to Purdal check dam and Gajanur dam. Both of which reservoirs and are present at the foot hills. Thalassiosira, Surirella, Diatoma, Cocconeis, Cyclotella were more commonly found in flowing water bodies. Melosira, ctenophora, Brachysira, Achnanthes, Fragillaria were common to stagnant

waters. Nitzschia, Pleurosira were commonly found in the canal near Lakshmi theatre. This canal is highly polluted by domestic activities and lot of waste disposal. Nitzschia and Pleurosira were more common for the polluted waters. The size of the diatoms also varied with the conditions, water bodies with stagnant water like lakes had comparatively larger size diatoms, while small size diatoms were more frequently present in flowing waters. This was in contrast to the results obtained in a study conducted by Thakar MK, Singh R [8] in Punjab where they found larger size diatoms were more frequently observed in flowing waters.

Selected water bodies showed significant differences in the cyclical distributional of diatom species. Diatoms were present in large numbers during the early post monsoon season and in a considerable amount during the summer season. Their population steeply decreased during winter season and monsoon. Water samples collected from five water sites (Lake, canal, well and pond) on a seasonal basis, winter (December), spring (March), summer (June) and autumn (September) from the different geographical area of Haryana, India (Himalaya foothill, plain area and Aravali hills also revealed the significant difference in diatom distribution as the environmental condition varies in these selected sites.[9] Similar observations were also made by Pollanen M S[10], the diatom test was most likely to be positive in April (40%), and November (30%) and least likely to be positive in the Winter months. In a study undertaken by Tyagi GD, Dogra TD[11] wherein an analysis of the water samples of various ponds and lakes of Delhi showed two population maxima one in spring and another in autumn and 14 species of diatoms were observed.

Winter season has short days, and temperature declines to a very low level. Intensity of natural light remains very low and conditions are mostly dry. The temperature in summer were reaching maximum up to 32.2.0°C and that in winter up to 19.5°C. pH was in range greater than 6.6 and less than 8.6. pH was more towards acidic during summer and towards alkaline during rainy and winter.

Distribution of diatoms in water bodies of different geographical localities were affected by some physical and environmental parameters. The locality with low temperature, less salinity and less polluted water had less quantity of diatoms.[9] Generally, winter had a 'dilution effect' on diatom diversity because climatic conditions are not favorable for the growth of diatoms therefore very low population of diatoms existed in winter. But a substantial diatom bloom occurs in autumn season when range of temperature increases and a huge amount of natural light helps in photosynthesis and ultimately in growth of a variety of diatoms. A significant change in the qualitative and quantitative distribution of diatoms takes place in warm summer season. Due to the higher temperature growth conditions of diatoms follow a very slow and tidy pace. Time to time monitoring of water bodies is necessary for the updating of diatomological Mapping.[12]

5. Conclusion

The objective of the study was to understand the distributive pattern of diatoms in different water bodies of Shimoga. Recognition and study of regional distribution of diatoms can be used in postmortem diagnosis of drowning and to locate the particular area of drowning. Results have shown that there is diatom diversity at different water bodies and also slight changes during the different seasons within the same water body. It might have happened due to the difference in the geographical conditions that can affect the nutrient content of the water for the growth of diatoms. Change in the diatom diversities in different water bodies can be very interesting and useful in Forensic point of view in postmortem diagnosis of drowning.

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