Different Types of Diatoms Seen in the River Narmada in India

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Abstract

Aim: To determine the different types of diatoms present in the waters of River Narmada flowing through 5 distinct cities of India and ending into the Arabian Sea. Different types of Diatoms were observed with change in the course of water. **Objective:** The study shall assist to determine the different types of diatoms present in the river Narmada. **Materials and Method:** The study does not require any special instruments and the technique used to extract the diatoms from the waters is also very simple to perform. In this study the acid digestion technique is not used but the technique is slightly modified in order to prevent the damage to the walls of the diatoms. **Result:** Various types of diatoms were seen and the diatoms present in the water collected from different cities of India are found to be different including the species unique to the area. Conclusion: The study describes the type of diatom present in the water of river Narmada collected from different places.

Keywords: *Diatoms, Drowning, Forensic, Narmada*

Introduction

Diatoms are unicellular, photosynthetic, eukaryotic organisms that are present in almost all the water bodies including springs, rivers, ponds, lakes ditches and also in freshwaters, brackish and marine waters (Smol and Stomer 2010), and also terrestrial habitats like wet rocks, mosses and soil (Falasco et al 2014;Tofilovska et al, 2014). Diatoms are large group consisting of 200 genera and approximately 13000 species, out of which 92 genera and about 569 species are reported in India. The diatoms are distinguished from other types of algae due to their structure and sculpturing of their walls.

The cell of the diatom consists of cell wall and protoplast. The cells are covered by a siliceous wall, called **frustule.** It consists of two overlapping halves called the theca. The upper one is **epitheca** and lower one is called **hypotheca**

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The theca consist of 2 portions

a. Valve: the upper flattened top

b. Connecting band or cingulum- incurved region

The common region of the connecting bands, where both the theca are fitted together, is called the **girdle.** Depending on symmetry, the cells are divided into 2 orders:

1. Pennales (bilaterally symmetrical)
2. Centrales (radially symmetrical)

In some pinnate diatoms, an elongated slit is present on their valves, called **raphe.** The raphe is interrupted at its midpoint by thickening of the walled called the **central nodule** as seen in figure 1.2.1 a and b**.** Similar thickening is also present at the ends called **polar nodules.** Some members of the order Pennales, do not have raphe, called pseudoraphe.

Based on electron microscopic studies, Hendey (1971) observed 4 basic secondary structures:

1. Punctae (small perforations on valve surface)

2. Canaliculi (tubelike narrow channels which run through the valve surface)

3. Areolae (large box like depressions)

4. Costae (riblike structures on the valve structures)

The cell wall of diatom is mainly made up of substances impregnated with silica. The content of silica varies from 1% to about 50% on the basis of dry weight of the cell.

Range of Diatoms

Different estimates of diatom diversity have been offered. Mann and Droop (1996) suggested there may be in excess of 200,000 species of diatoms (extent and fossil), but more recently Guiry (2012) gave the much reduced estimate of 20,000, with 12,000 already described (and accepted) 8000 species yet under discovery. Using modern diatom species concepts, and with the increased use of fine ultrastructural features and molecular analysis for species discrimination, a more realistic total number of species could be 30,000 to 100,000 (Mann and Vanormelingen 2013). It is probably agreed that around 15000 are currently accepted and known.

Drowning and Diatoms

Diatoms analysis is a valuable tool in forensic science and is useful in diagnosis of drowning cases. The basic principle with diatom test in drowning is based on correlation between diatoms that are present in medium where the possible drowning took place. As described in the morphology, the diatoms have silica-based skeletons that do not readily decay and can also be detected in heavily decomposed bodies. Diatoms do not occur naturally in the body hence if laboratory tests show their presence in the body, then it is a good evidence of drowning as the cause of death.

This study introduces a new technique for extracting of diatoms from the water samples collected from different cities of India which include:

The 5 different places used for collection of samples include:

1. Amarkantak (0 km)- origin
2. Jabalpur (227 km)
3. Ahmedabad (1127 km)
4. Bharuch (1327 km)
5. Khambhat (1467 km)- sea (end)

Materials:

- Water samples from 5 different sites
- Micropipette- 1000 µl
- Micro-tips- 1000 µl
- Distilled water- diatom free
- Hot plate
- Centrifuge tubes 15 ml
- Centrifuge machine
- Microscopic slide
- Crystalline violet stain
- Light microscope
- Fluorescence microscope
- Immersion oil

Method:

Technique For Water Sampling:

Waters were collected from the following cities of India

1. Amarkantak plateau, Amarkantak
2. Gwari ghat, Jabalpur
3. Narmada Canal, Ahmedabad
4. Bhigurishi Temple, Bharuch
5. Sea Shore of Gulf of Khambhat

Extraction of diatoms from the water samples

The method used for extraction of diatoms from water samples requires no special treatment as well as no use of chemicals is done in the method mentioned below. The earlier used acid digestion technique uses nitric acid and hydrochloric acid which can damage the structure of diatom is not advised for the extraction of diatoms from the water sample.

Following steps were used to extract the diatoms

- Total 10 ml water sample was taken in 15 ml centrifuge tube and centrifuged at 4000 rpm for 5

minutes

· The resulting supernatant was removed carefully using a micropipette and by not disturbing the pellet

· The pellet was suspended in 1.0 ml sterilized distilled water

· From this prepared solution the slides were prepared for microscopic analysis

Microscopic Analysis:

· Slide preparation:

· A clean microscopic slide was taken and a drop of dissolved pallet was put on it. The slide was then heat fixed on the hotplate at 100^0C until the evaporation of water. After 1 to 2 minutes of heat fixing the slide it was stained with crystalline violet.

Diatom Staining Procedure

Simple staining: After heat fixing the slide either crystalline violet or safranin stain solution was used placed over the heat fixed slide using a dropper. The slide was then left for 1 minute and washed with distilled water. The slide was then observed under 100X oil immersion lens microscope.

Results

Various types of diatoms which were observed in the study are:

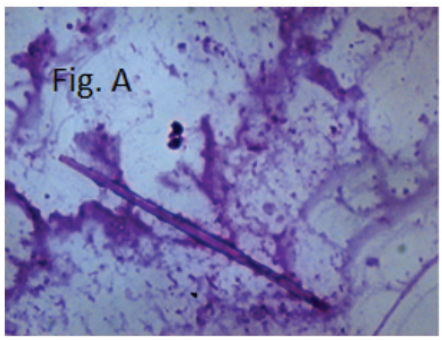
1. Nitzschia
2. Fragillaria
3. Navicula
4. Synedra ulna
5. Synedra Tabulate
6. Caloneis Bacillum
7. Fregilaria capunica
8. Fragellaria intermedia
9. Diatoma vulgaris
10. Nitzchia acidoclinata
11. Hyalosynedra lacvigata
12. Grammataphora subtilissima

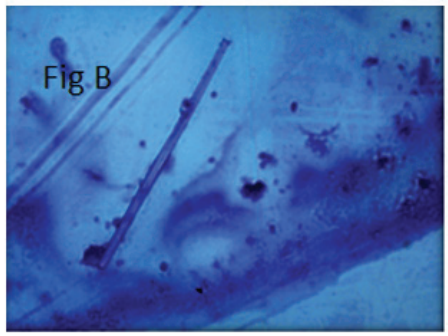
Microscopic Analysis

The analysis of samples showed different species of diatoms in different waters of river Narmada. The variation could not be observed in the form of species for all the diatoms among different cities. The photographs were taken with the help of a digital camera at 100X oil immersion magnification in light and fluorescence microscope.

Water sampling 1: Amarkantak Waters

The diatoms species present were:



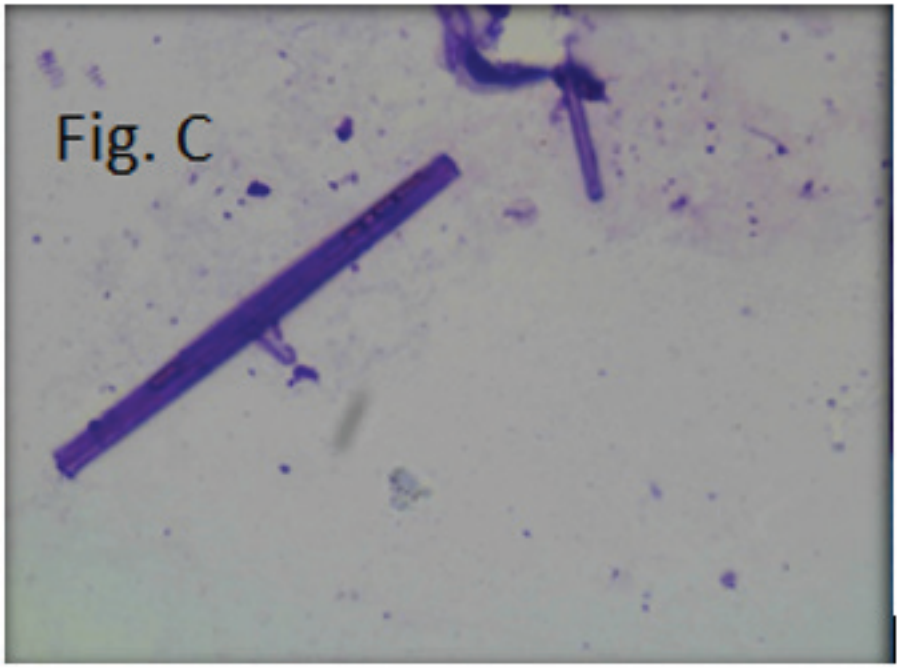


The above images show the following types of diatom

Figure A: Hyalsynedra Lacvigata

Figure B: Synedra Ulna

The different types of diatoms seen in Jabalpur waters of Narmada river are:



The different types of diatoms observed in the Ahmedabad water of Narmada river are:

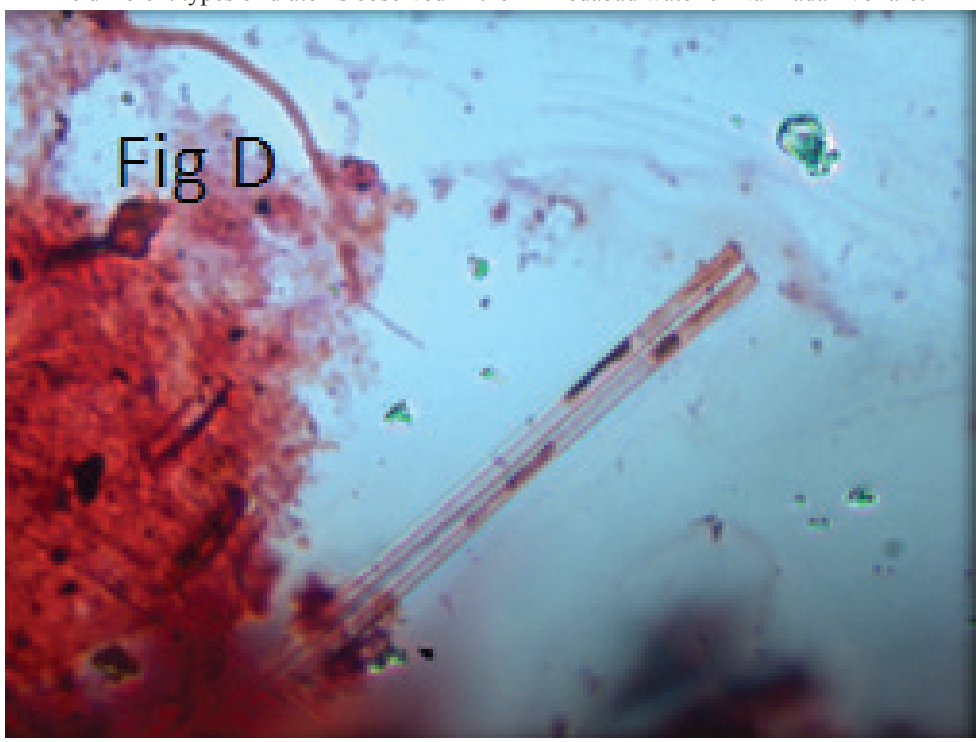


Figure B: Synedra Ulna

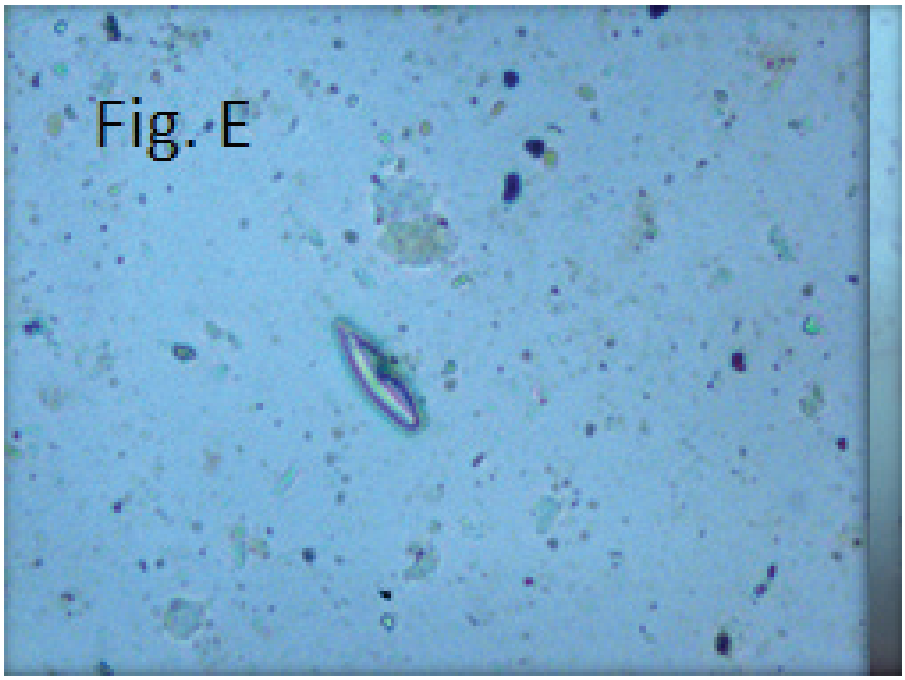
The different types of diatoms observed in the Ahmedabad water of Narmada river are:

The above images show the following types of diatom

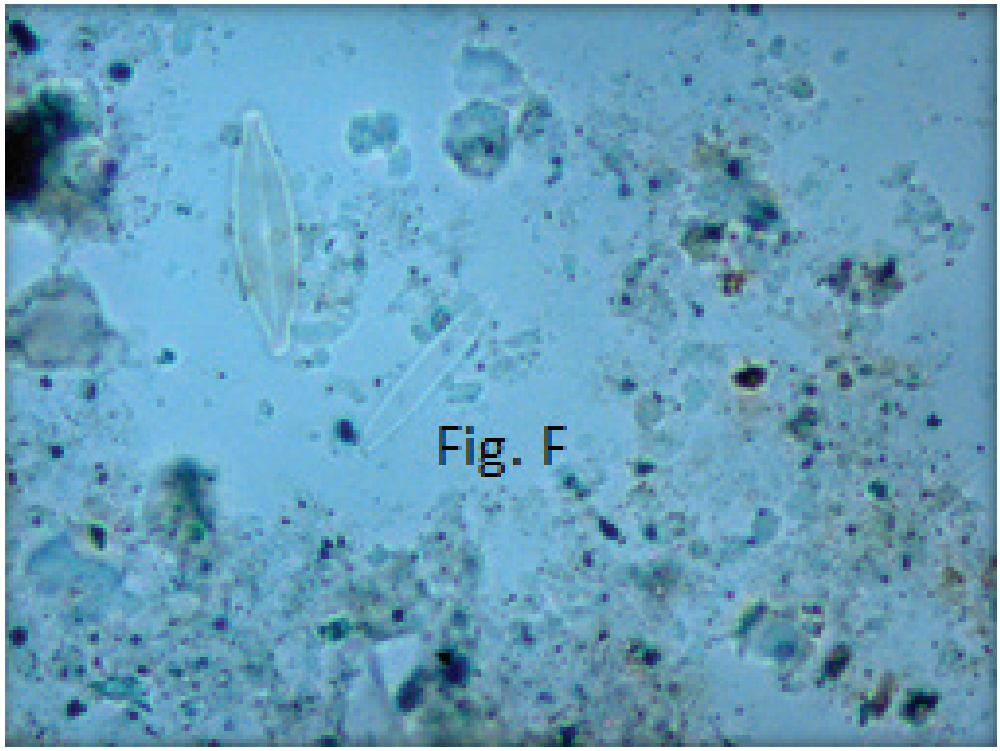
Figure C: Fregilaria apunica

Figure D:Fragillaria Intermedia

The different types of diatoms observed in the Bharuch water of Narmada river are:



The different types of diatoms observed in the Gulf of Khambhat sea where the Narmada river meets the sea are:



The above images show the following types of diatom

Figure E: Navicula

Figure F: Caloneis

Due to reduced distance between Bharuch and Gulf of Khambhat the navicular species of Diatoms were found to be dominant in the gulf of Khambhat, as similar environmental conditions persists.

Discussion

The results of the study mentioned in the above section were done using a limited number of teeth samples and thus the results obtained did not have an appropriate relevancy. The types of diatoms observed from the same river that is Narmada varied from city to city. Hence the environmental conditions play a very important role in the type of diatom found in the waters of the same river:

The variations seen in the type included:

1. Narmada river at origin (Amarkantak): Hyalosynedra lacvigata, Synedra Ulna, Synedra tabulata, Nitzschia,

2. Narmada river flowing through the city of Jabalpur: Navicula, Fregilaria capunica, Fragillaria intermedia, Grammatophora,

3. Narmada flowing in Ahmedabad: Nitzschia, Nitzchia Sicula, Nitzchia Acidonata, Fragillaria intermedia, Navicula

4. Narmada flowing in Bharuch: Navicula Hamiltoni, Navicula, Caloneis, Nitzchia

5. Narmada meeting the Arabian sea in the Gulf of Khambhat: Navicula, Caloneis, Nitzschia.

Diatom was first described in lung fluid by Hoffman in the year 1986[1]. However, Rovenstorff had successfully utilized it to solve a case of a drowning mystery in 1904. Incze successfully detected the diatoms in blood and parenchymal organs in 1942. Tamaska detected diatom in bone marrow in 1949. In the 1960s and 1970s, Timperman using large series of drowning cases, provided evidence for the validity of diatom test[2]. Detection of diatoms has been applied as an important sign of drowning in the beginning of 20th century and the use of diatoms for the diagnosis of drowning cases was debated soon after they were first debate. Over the past decades diatoms are screened for high lipid content. Geologists claim that much of crude oil comes from diatoms. Sanjay K.R, Nagendra Prasad M isolated diatom Navicula cryptocephala, from fresh water source was grown on suitable median for extracting and characterizing the oil for biodiesel production[3]. T.A. Nadia Fucci developed a simple and economical procedure for the destruction of human organic material for the diatom examination is presented. The author tested a minimal amount of H_2SO_4 diluted solution to detect diatoms in several tissues from human corpses under crime investigation, immersed in sea and river water. The method was compared with a traditional method that includes digestion with large amount of strong mixture of sulphuric and nitric acid (90%). The new procedure showed that all siliceous frustules of sea and river diatoms are more resistant to H_2SO_4 diluted treatment and are still recognizable after digestion, and observation under the microscope is better than the other procedure. Moreover, the microscopic observation of ameboid protozoa was also possible[4].

Conclusion

This study determines that the type and species of diatoms change, with the changes in the environmental conditions. The study serves to reduce the time to extract the diatoms and in order to come to a conclusion of cause of death. The extraction diatoms through this technique requires 3-4 hours to come to a conclusion which is very less when compared to other techniques used in forensic science. The technique used does not involve any damage to the evidence which is important as the diatoms are brushed from the surface of the diatom and no invasive techniques are used.

Ethical Clearance: N/A

Source of Funding: None

Conflict of Interest: None

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