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ORIGINAL ARTICLE

Forensic Study of Diatoms in Freshwater Sample Around Patna Region, Bihar, India.

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ABSTRACT

Diatoms are eukaryotic, unicellular algae that belong to a Bacillariophyceae family and it exists in water. Diatom is important in determining whether or not a person died as a result of drowning. When a corpse is retrieved from the water, it is generally believed that the death was caused by drowning, and that the body was drowned either before or after death. In such circumstances, the existence of diatoms in body tissues is a very useful evidence in determining the cause of death. Various types of diatoms found in dead body tissues are related to the types of diatoms present in bodies of water that are recovered from the body. The objective of this study was to find out which types of Diatom are present in different freshwater bodies around Patna district of Bihar, India. In this research, freshwater samples were collected by Random Sampling Technique from 17 different water habitats of Patna, during January to February, 2021. For diatom analysis, samples from ecological niches like river Ganga, ponds, and lakes were collected in clean water bottles with tight-fitting caps. Extraction of diatoms is done in two ways: The H_2O_2 method and the HNO3 method. The aim is to determine which extraction procedure — H_2O_2 or NHO_3 — is best for diatom extraction. After the extraction of diatoms, the examination and photography of diatoms species is performed by compound microscope (Leica DM750 with Leica ICC50 E camera). Different Diatoms species found in various water environments at different location were investigated in this research. Some of their names are Cymbella sp., Fragiliaria sp., Ehrenbergiulva granulosa, Coscinodiscus radiates, Cyclotella sp., Melosira sp., Actinoptychus sp., Triceratium sp., Nietzsche sp., Actinocyclus sp. The information from this experiment will help in forensic science laboratories to identify diatoms, criminal investigation and locating drowning sites or place of crime. In this analysis we found that nitric acid digestion method give better extraction as compare to hydrogen peroxide extraction method.

Keywords | Lie Detector, Forensic Evidence, Polygraph, Narco Test

INTRODUCTION

Diatoms are microscopic algae that are unicellular, photosynthetic organism with a worldwide distribution. They are the world's largest biomass producers, contributing 25% of the world's total oxygen production and being one of the most important contributor to the global carbon fixation.[1] Diatoms are highly effective species as assessed by their versatility, range, productivity and comparative antiquity. Diatoms have a siliceous cell wall made up of two intricately sculpted halves, which makes them exceptional for a wide variety of applications for understanding and analyzing varying degrees of species level complexity. The taxonomy of diatoms is

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based on morphological features such as frustule ornamentation shape, size, and patterns; the presence or absence of raphae; the number of striae, and so on. The diatoms are classified into two main orders based on the structure of the frustule: Centrales and Pennales. The Pennales have bilateral symmetry, while the Centrales are radially symmetric.[2]

Usually, similar types of diatoms occur in almost identical types of water bodies. It has been discovered that there is a significant shift in the diversity of diatoms with respect to seasonal changes. The quantitative and qualitative distribution of diatoms in water sources is strongly supported by climatic conditions.[3]

The significance of diatoms in drowning cases has always been recognized. When the cause of death cannot be determined by traditional postmortem examination, the presence of diatoms in the lungs and other body tissues can help determine whether or not the death was caused by drowning. Diatoms aren't found in the human body. When laboratory tests show diatoms in corpses that match those in the water where the corpses were discovered, drowning is indeed a strong possibility as a cause of death.[4]

Sample Collection and preservation

During the months of January and February 2021, water samples were collected from various locations in the Patna area in tidy and clean water bottles with tightly fitting caps to prevent contamination. 500ml of water samples were collected from each of the 17 water habitat from different location of Patna. The bottles were labeled properly with collection number, the location of sampling area along with date and month.

METHOD & MATERIALS

For sample preservation, we kept the samples in a refrigerator for a few days, at room temperature. When we needed to conduct the experiment, we took out the sample from the refrigerator and put it in a beaker with proper labeling. Then, into each beaker, we added 1% of Lugol's iodine solution and left it to rest overnight.[5] The next day we began the diatom extraction procedure.

In the study of diatoms, we used two extraction methods to see which one provided the better results: nitric acid digestion and hydrogen peroxide extraction.

Nitric Acid Digestion Method was used for the diatoms extraction from freshwater water samples. 10ml of water sample was taken and transferred into a separate 25ml acid washed test tube and the test tube were properly labeled. 2ml of concentrated nitric acid (HNO3) was added to the sample. Then the sample kept for hot water bath on hot plate at 40-45˚C for 30 minutes. After that the samples were kept undisturbed for 24 hours. Then the samples were moved to properly-labeled centrifuged tube and were centrifuged for 10 minutes at 2500rpm. The centrifugation was carried out repeatedly three times by taking 2ml of sample each time to increase the concentration. The supernatant was removed carefully with the help of a dropper. Again the pellets formed were suspended in the distilled and centrifuged at 2500rpm to remove the acid contents.[6]

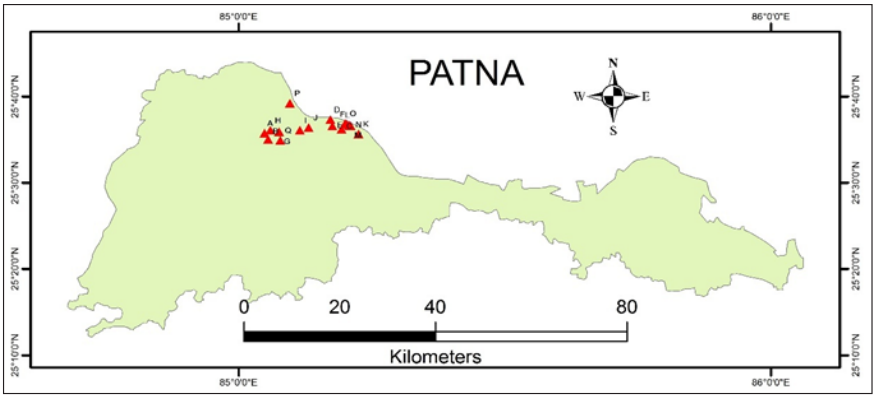
In the Hydrogen peroxide method, we took 10ml of water sample and transferred into a separate 25ml hydrogen peroxide washed test tube and the test tube were properly labeled. 10 ml of hydrogen peroxide (H2O2) were added to the sample. And then we follow the same procedure which we done in the case of Nitric Acid Digestion Method.[5]

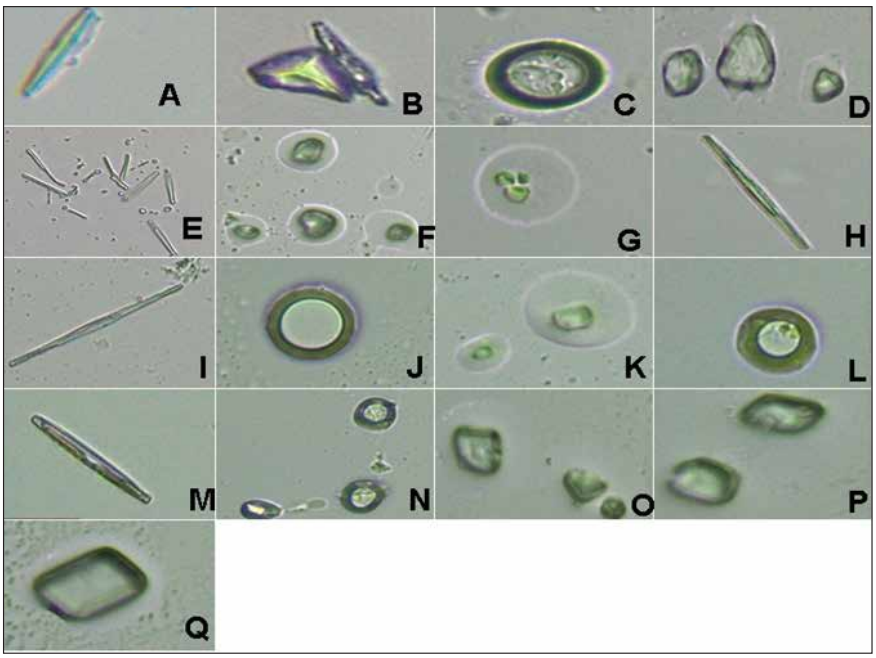
Mounting

After cleaning, the pellet is dropped onto the slide and covered with a cover slip. After that, the slides were dried on a heating plate at 30-40°C for 4-6 minutes. Then the slide was kept aside carefully for air dry, once the slide gets cool. It was examined under the compound microscope (Leica DM750) at magnification of 40X and digital images were taken with (Leica ICC50 E) camera connected to the compound microscope (Leica DM750). The diatom species were categorized based on the description provided by the various articles, site, book and reports.[7-11]

Map of the Sampling Areas

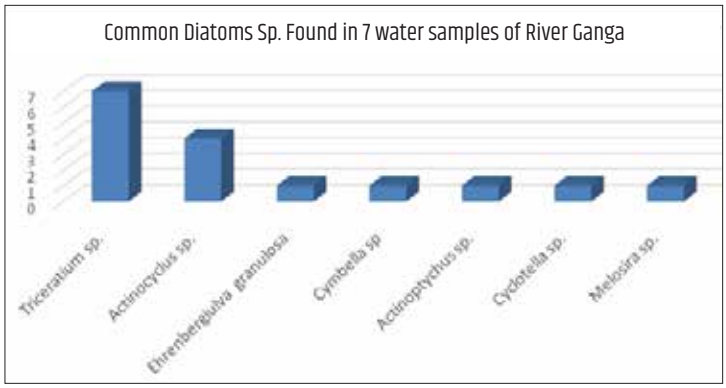
The following locations have been shown in the map. Namely, Eco Park lake, Adalat Ganj Talab, Mangal Talab, Kothwan pond, Digha Nahar, Adarshnagar Lake, B.M.P Mandir Pond, Bazar Samiti Talab, Danapur pond, Bajarang puri pond, Bhadra Ghat, Mahavir Ghat, Naujar Ghat, Hanuman Ghat, Gai Ghat, Gandhi Ghat, Digha–



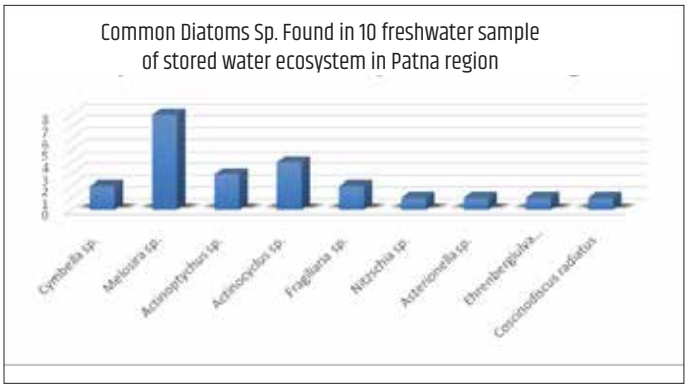


S.NO.	SAMPLING LOCATION	CO-ORDINATES	DIATOM SPECIES THAT FOUNDED
A	Kothwan pond	25.59588, 85.04866	Cymbella sp., Melosira sp., Actinoptychus sp.
B	Danapur pond	25.58417, 85.05563	Fragiliaria sp., Actinocyclus sp., Melosira sp.
C	Bajarang puri pond	25.60332, 85.19361	Actinoptychus sp., Actinocyclus sp.
D	Gandhi Ghat	25.62212, 85.17254	Triceratium sp., Actinocyclus sp.
E	Bazar Samiti Talab	25.60995, 85.17628	Nitzschia sp., Melosira sp.
F	Gai Ghat	25.61369, 85.20324	Triceratium sp., Actinocyclus sp.
G	B.M.P Mandir Pond	25.5986, 85.07604	Actinocyclus sp., Melosira sp.
H	Digha Nahar	25.60148, 85.05957	Cymbella sp., Actinocyclus sp., Melosira sp., Asterionella sp.
I	Eco Park lake	25.60179, 85.11492	fragilaria sp., Melosira sp.
J	Adalat Ganj Talab	25.60699, 85.13156	Ehrenbergiulva granulosa, Melosira sp.
K	Mangal Talab	25.59457, 85.22517	Coscinodiscus radiatus, Melosira sp.
L	Bhadra Ghat	25.61164, 85.20719	Ehrenbergiulva granulosa, Triceratium sp.
M	Mahavir Ghat	25.61074, 85.20891	Cymbella sp., Triceratium sp.,Actinoptychus sp.
N	Naujar Ghat	25.6099, 85.2108	Cyclotella sp., Triceratium sp., Melosira sp.
O	Hanuman ghat	25.61483, 85.20006	Triceratium sp., Actinocyclus sp.
P	Digha–Sonpur Rail Bridge	25.65375, 85.09635	Triceratium sp., Actinocyclus sp.
Q	Adarshnagar Lake	25.58215, 85.07821	Actinoptychus sp., Melosira sp.

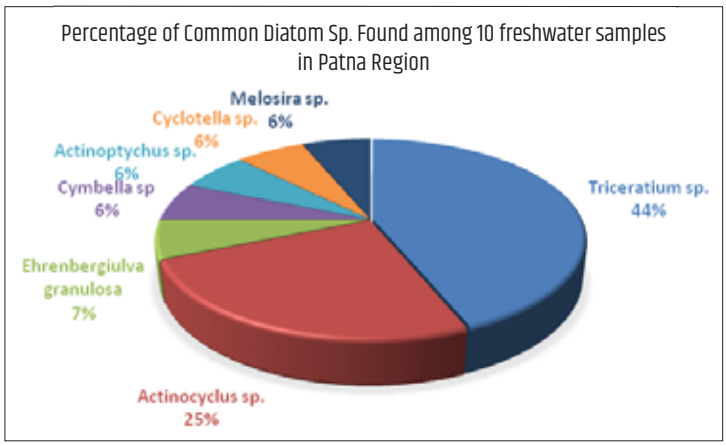
Table 1: Sample collection size 18-20km (during January to February, 2021)

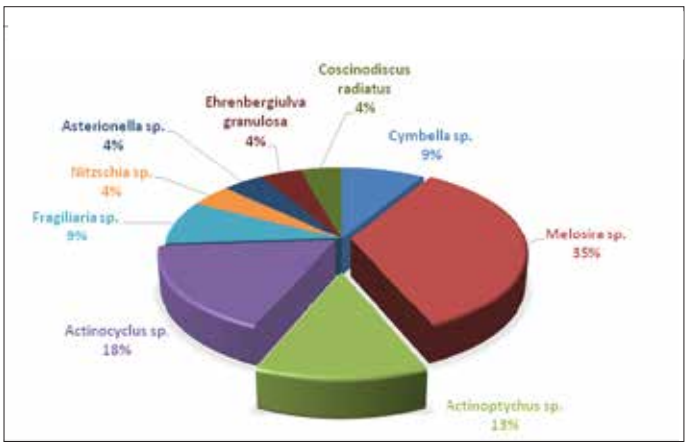


Graph 1: Some common diatoms found in the Patna water bodies



Graph 2: Some common diatoms found in the Patna water bodies





Sonpur Rail-Road Bridge (River Ganga).

Sample collected from River Ganga in different location are Bhadra Ghat, Hanuman Ghat, Gandhi Ghat, Mahavir Ghat, Naujar Ghat, Gai Ghat and Digha–Sonpur Rail-Road Bridge over Ganga River.

RESULT & DISCUSSION

In the drowning cases, diatoms play an important part. It is very helpful in deciding not just the cause of death, but also the site of drowning. The presence of a sufficient amount of diatoms in vital body organs (i.e. lungs and in between long bone) will confirm ante-mortem drowning to a certain degree. The scattering of diatoms in any body of water, and their interrelation with the diatom species retrieved from the drowned body, may be the method of choice for addressing the drowning site question. The presence of diatoms in water and biological samples can reveal a lot of information about the cause of mortality, season of death and the location.

In certain drowning cases, the outcome may be false positive, such as when a person drinks water from a pond, lake, or river on a frequent basis. As a consequence, when investigating drowning cases, authorities must understand this perspective.

Using the diatom test, we'll figure out whether the death was caused by drowning or not. Criminals frequently throw bodies into the river after committing a murder to it seem like the victim drowned.[12] The plume of froth on the

nose and mouth, respiratory emphysema, odema aquosum, froth in the trachea, Paltaufs spots, increased lung weights, and peripheral edema are the result in the change finding of autopsy in drowning deaths.[13] since diatoms can tolerate putrefaction, they are most useful in situations where decomposition has progressed and post-mortem drowning signs have faded. According to drowning death research, only the diatom test can detect ante mortem drowning in decomposed corpses and bodies' during late stages of decay. The diatom test identifies diatoms in organ samples and compares them to a control water sample.[14,15] From a scientific viewpoint, the study of drowning deaths allowed for a sensitive, accurate, and easily applicable examination. The diatoms test has emerged as the most effective test for detecting drowning deaths in forensic laboratories.

In this analytical study of diatoms we collect, preserves, extract and examine the diatom using a controlled water sample which is collected from various location of a Patna, Bihar region. Below are images of a few different diatom species. Few diatoms could be classified up to species level due to poor photographs. Since the samples were taken during the winter, the climatic conditions were not favourable to diatom formation, so there were few diatoms to be found. The growth of diatoms slows down because the climatic factors are not conducive for diatom growth in the winter, but only a few water samples had a stable diatom population.

We identified 11 diatom species in various water ecosystems in the Patna district region during this research, and their names are Cymbella sp., Fragiliaria sp., Ehrenbergiulva granulosa, Coscinodiscus radiatus, Ehrenbergiulva granulosa, Cyclotella sp., Melosira sp., Actinoptychus sp., Triceratium sp., Nitzschia sp., Actinocyclus sp.

In most of Patna ponds, lakes and nahr, the diatoms species we identified are Cymbella sp., Melosira sp., Actinoptychus sp., Fragiliaria sp., Nitzschia sp. and Actinocyclus sp. In Ganga river of Patna, diatoms species we found are: Triceratium sp., Actinocyclus sp., Ehrenbergiulva granulosa, Cymbella sp., Actinocyclus sp., and Cyclotella sp.

Common diatoms Species were discovered in seven water samples taken from the River Ganga in Patna, Bihar are Actinocyclus sp., Triceratium sp., Cymbella sp., Actinocyclus sp., Ehrenbergiulva granulosa, Cyclotella sp. and Melosira sp.

Among the seven water samples of Ganga River 44% Triceratium sp. Presented, 25% Actinocyclus sp. Presented, 7%Ehrenbergiulva granulosa presented, and 6% each Cymbella sp., Cyclotella sp. and Melosira sp. Presented.

Common Diatoms sp. was discovered in ten freshwater samples from the Patna region's store water ecosystem are Cymbella sp., Fragiliaria sp., Actinoptychus sp., Nitzschia sp., Actinocyclus sp., Ehrenbergiulva granulosa, Coscinodiscus radiatus, Cyclotella sp., Melosira sp.

Among the ten water sample of store water ecosystem, 35% Melosira sp. Presented, 18% Actinocyclus sp. Presented, 13%Actinoptychus sp. presented, 9% Cymbella sp., Fragiliaria sp. and 4% each Nitzschia sp., Ehrenbergiulva granulosa, Coscinodiscus radiatus, Cyclotella sp., Melosira sp. Presented.

CONCLUSION

Diatoms can be studied qualitatively and quantitatively by detecting and counting the diatoms species within the samples. Diatom analysis on a large scale and by area can aid the forensic science laboratories in identifying and locating drowning sites.

In this analysis, we found 11 diatom species in various water habitats in the Patna district area, and their names are: Cymbella sp., Fragiliaria sp., Actinoptychus sp., Triceratium sp., Nitzschia sp., Actinocyclus sp., Ehrenbergiulva granulosa, Coscinodiscus radiatus, Ehrenbergiulva granulosa, Cyclotella sp., and Melosira sp.

In most of the water ecosystem in Patna region, we identified the similar types of diatoms such as Cymbella sp., Melosira sp., Fragiliaria sp., Nitzschia sp., Actinocyclus sp., Ehrenbergiulva granulosa and Actinoptychus sp. In the waters of River Ganga, we found and identified mostly 7 different types of diatoms: Triceratium sp., Cymbella sp., Actinocyclus sp., Actinocyclus sp., Ehrenbergiulva granulosa and Cyclotella sp.

In this analysis of each 17 different slides of Diatoms which were extracted by nitric acid digestion method and hydrogen peroxide, we found that nitric acid digestion method gave

better extraction as compared to hydrogen peroxide extraction method. Using 'Hydrogen peroxide' to oxidise organic content is not adviced since washing off peroxide traces from samples is difficult due to the solution's bubbling, while nitric acid can be washed off quickly using the acid digestion process. As a practice for diatoms extractions, instead of hydrogen peroxide method, the use of nitric acid digestion method is advisable. IJFMP

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Conflict of Interest:

The author declares that there exists no commercial or financial relationships that could, in any way, lead to a potential conflict of interest.

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