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DIATOMOLOGICAL MAPPING OF MAJOR WATER BODIES OF JHANSI REGION

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ABSTRACT

Diatoms are the microscopic algae which are known for their species diversity, reliability and abundance that can contribute to the forensic investigation of drowning cases. In the present study a total of six major water bodies situated around the district Jhansi, were selected for the study of diatoms. Different samples referring to different substrata such as epilithon, epiphyton and surface water were collected together from each water body and examined by acid digestion method for the presence of diatoms. Physical parameters of all the water bodies were also recorded in which the average temperature was found 31^0C, p^H ranging from 7 to 8.2 with maximum 8.2 for Pahuj reservoir and TDS varying from 126 ppm to 225 ppm. Pennate diatoms were the most frequent and dominating diatoms in all the water bodies. A total of 18 diatom species belonging to the 14 genera were identified in all the water bodies. *Nitzchia* and *Navicula* were found to be the most prevalent diatom genera identified in all the water bodies. Betwa river (11 genera) was found to be more differentiating in terms of number of diatom genera identified followed by Garhmau lake (8 genera), Pahuj river (6 genera), Antiya lake (5 genera), Pahuj reservoir (5 genera) and Parichha dam (3 genera).

Keywords: Forensic Science, diatoms, drowning, compound microscope, etc.

INTRODUCTION

Diatoms are the eukaryotic unicellular microorganisms belonging to the Kingdom Protista and Class Bacillariophyta[1]. Diatoms are related to the major aquatic vegetation known as algae, specifically microalgae present in both fresh and salt water, and belong to a large group of Protists called 'Heterokonts'. The color of heterokonts ranges from yellowish to brown due to the pigments such as carotenoid and Fucocoxanthin[2]. Most of the diatoms are non-motile but some move through flagellation. Diatoms morphology contains unique silica cell wall known as frustules with distinct geometrical shapes varying from box-shaped to cylindrical and symmetrical to asymmetrical. Another important characteristic feature of the diatoms is 'raphe' which is found mostly in pennate diatoms that divides the valve into two[3]. Diatoms cells have life expectancy of about six days and are confounded to live in a specific salinity, temperature, light intensity, pH and level of organic materials[4]. Diatoms study has been introduced to forensic since the late 18th century and since then more than 200 genera and one lakh species of diatoms have been discovered[5,6].

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In Indian subcontinent Biswas (1936), Gonzalves and Gandhi (1952), and Gandhi (1959) carried out extensive research on diatom taxonomy but their work mainly focused on the pinnate diatoms, with almost no or very little attention given to the centric forms[7,8,9]. In a major contribution to diatom studies, Desikachary (1987) illustrated and described marine and fossils centric diatoms from the Indian Ocean in his book "Atlas of the Diatoms"[10]. Guy and Taylor (1861) described that the aquatic particles found in the drowned bodies, can serve as a confirmatory particles for the drowning cases[6]. Hofmann first discovered the diatoms in lungs in 1896[11]. Different researchers have utilized different biological specimens to elicit the facts about diatoms. Kasparck (1937) investigated lung tissues after digestion in acidic medium to locate the diatoms[12]. Incze (1942) got successful in determining the presence of diatoms from blood and parenchymatous organ[13]. Later, Tamasaka (1949) successfully investigated drowned persons bone marrow to detect the presence of diatoms and their research was further modified by Timpermann (1972) who worked on the bone marrow specimens[14,15]. Timpermann (1972) emphasized that the one diatom in the bone marrow can help in establishing the cause of death as drowning but on the basis of later researches he opined that it cannot be a solid basis for such diagnosis[15]. Hendey et al., (1973) were the first who brought the specialized knowledge of diatoms study in the field of forensic[16]. Peabody (1980) evaluated the findings of different researchers and suggested the scrupulous examination of lung, liver, brain and bone marrow in both drowned and non-drowned subjects to better address the cases of drowning[17]. The presence of diatoms in non-drowned subjects have confused investigators and led them to believe that diatom analysis is not helpful in detecting drowning[18]. Therefore, it is important to note that the investigator must have sound knowledge of different diatoms because distinguishing the presence of diatoms from the background flora of the suspected drowning site is an essential part of the investigation. Furthermore, diatoms can also serve important role in locating the site of drowning where water currents may have moved a body or the body has been deliberately moved from its original position.

So far very limited researches on diatoms have been conducted in the district Jhansi, Uttar Pradesh. Jhansi is situated in the northern hemisphere and has a warm and temperate climate suitable for diatom growth. The region encompasses different types of water bodies that can provide habitats to diatoms. Moreover, Jhansi is a region that shares its boundary with the two states, i.e., Uttar Pradesh (2120 deaths) and Madhya Pradesh (5779 deaths) that were ranked 6th and 1st respectively in terms of the number of accidental deaths occurred due to drowning across the country (NCRB report, 2020).

MATERIALS AND METHODOLOGY

Site Selection

A total of six fresh water bodies situated nearby the district Jhansi representing rivers (Betwa and Pahuj), lakes (Antiya and Garhmau), dam (Parichha) and reservoir (Pahuj) were selected for sampling. All the sampling was performed during August to September, 2021 in which the climate remains wet throughout the region.

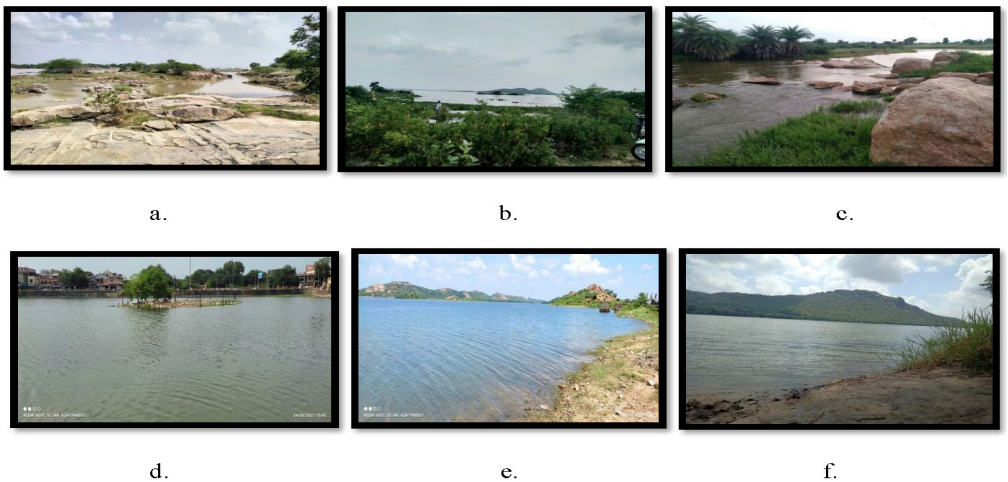


Figure 1: Different Water Bodies Selected for Sampling a. Parichha Dam, b. Pahuj Reservoir, c. Pahuj River, d. Antiya Tal, e. Garhmau Lake, f. Betwa River

Sampling Conditions

A rocky or shallow part of stream (riffle) was preferably chosen where the water is flowing over the stones ensuring the continuous exchange of water and preventing the local buildup of chemicals around the substrata. Sunlight and water velocity are the two major factors that can potentially affect the community of diatoms. So the samples were collected from the region where the velocity of water was moderate and completely exposed to the sunlight. Heavily shaded areas along with very high and low water velocity sites were avoided as they have serious implication on water quality, increased sedimentation, growth rate and relative abundance of diatom species. Samples were collected from both upstream and downstream discharge points. In case of rivers, samples were collected within 1 m of depth as the sun rays may not extend deep to the river and considerable difference may occur between the shallow and deep water. Epilithon with high filamentous green algae and boulders covered with layers of sediments (organic materials) were also avoided. Physical parameters of water bodies (pH, temperature and TDS) and sites (longitude and latitude) were recorded at the time of sampling.

Sample Collection

Samples referring to different attached substratum such as epilithon (boulders, stones, cobbles and pebbles), epiphyton (macrophyte plants) and free flowing water were collected from each water body and stored in a plastic container. **Epilithon** are considered to be the most preferred substratum providing habitat to the variety of diatom community across all the season. 5 to 6 epilithic substratums were collected from the water body and their attached organic material was removed by agitating the substratum briefly into the stream of water. The upper surfaces of the entire substratum were gently scrubbed in a plastic tray with the help of toothbrush to dislodge the diatom. After scrubbing, all the epilithic substratums were washed with river water and their rinsed suspension were poured into a labeled wide-mouth plastic bottle. **Epiphyton** substratums were collected from both emerged and submerged macrophyte plants, but the later was preferred over the previous one. Macrophytes were cut using the knife and taken into a plastic bag. The plastic bag was then shaken vigorously and squeezed so as to collect the brown suspension into the same wide-mouth labeled plastic bottle. 250 ml of water samples were also taken from each benthic, bank and surface



a. b. c.

Figure 2: Epilithon Substratums a. Stones and Pebbles, b. Boulders, c. Cobbles



d. e. f.

Figure 3: Macrophyte Plants d. Submerged, e. Emerged, and f. Surface Water Sample

region of the water body, and collected together in plastic bottle for the examination of diatoms.

Preservation of Suspension

Preservatives were required to stop cell division and decomposition of organic materials. No preservatives were used in diatom suspension, when the samples processed or analyzed within 24 hrs, in this condition samples were stored only in refrigerator at 4^0C. For short term storage (2 to 3 days) of diatom suspension Lugol's iodine (4 drops per 100 ml of suspension) was used as a preservative.

Sample Preparation

The diatom samples collected from different water bodies were centrifuged at 2500 rpm for about 10 min. After centrifugation, only sediment portion was used for decalcification and cleaning processes while the supernatant portion was discarded. If the sample did not processed on the same day, it was kept in the refrigerator at 4^0C and the next working day the procedure was followed. For decalcification, supernatant portion was treated with drop wise addition of dil. HCl till the effervescence stops. The sample was rinsed by centrifugation method at 2500 rpm for 10 min with distilled water. After centrifugation, the supernatant part was discarded every time and this washing procedure was followed 4 times. After washing, the final residual was subjected to cleaning procedure. For cleaning, a strong acid mixture containing Nitric acid and Sulfuric acid in the ratio 2:1 was used to remove organic materials present in the residual. The residual together with 8 ml of acid mixture and 10 ml of distilled water was taken into a beaker and covered with a glass plate. The beaker was then placed on to a hot plate at 90^0C for 2-3 hrs until the suspension was clear and transparent in appearance. Once the suspension was cleared it was allowed to cool at room temperature and rinsed by centrifugation method at 2500 rpm for 10 min with distilled water. This washing procedure was followed for 3 times and the final sediment was used for microscopic examination of diatoms.

Microscopic Examination

Few drops of diluted sediment were taken on a slide and a thin smear was made using the spreader. Without disturbing the slid, smear was dried on the hot plate and permanently mounted using DPX and cover slip. The slides were examined under a Compound Microscope at 100X, model no. JSI-656 manufactured by the Jyoti Photomaster at 100X. The images of the diatoms were captured using smart phone camera equipped with 60 mega pixels.

RESULTS AND DISCUSSION

The present study was carried out to study the diatom assemblage in the major water bodies situated around the district Jhansi. A total of six diversified water bodies including river, dam, lakes and reservoir were selected for the study of diatoms. The microscopic examination of the samples showed that the species of the diatoms did not differ much in both types and numbers. Genera of diatoms were recognized according to their cell shape and raphe. Pennate diatoms were found to be the most frequent and dominating diatoms across all the water bodies. The identification of the collected diatoms samples revealed the occurrence of 14 genera from all six different sites. The class Bacillariophyceae was found most frequent in Jhansi region. The most prevalent diatoms communities include biraphids (*Navicula, Nitzschia, Gomphonema*, *Cymbella*), followed by araphids (*Synedra, Fragilaria, Ulnaria*) and centric (*Cyclotella*). The number of diatoms was higher in rivers as compared to the lakes. A total number of 18 diatom species belonging to 14 genera were identified and listed in Table 1. *Nitzchia* and *Navicula* were found to be the most prevalent diatom genera identified in all the water bodies. *Frogilaria longiformis* was the most frequent diatom species found in Pahuj river and Pahuj reservoir. *Gomphonema*, *Synedra* and *Enconema* were the dominating diatom genera found in Pahuj river, Antiya lake and Garhmahu lake respectively.

The condition of the water body has significant impact on the growth and diversity of diatoms. Therefore, different physical parameters such as pH, TDS and temperature of each water body were also taken into account and reported in Table 2. The average pH of all the water bodies was 7.2 and there was no major difference observed in the pH of water bodies except Pahuj reservoir with pH 8.2. The average temperature of water bodies was about 31^0C. Of total TDS measured for water bodies, lake showed highest TDS (average 209 ppm) followed by the reservoir, river and dam. The lowest TDS was measured for Parichha dam (126 ppm) and the highest for Antiya lake (226 ppm).

Table 1: Diatom Species Identified in Different Water Bodies

Sl. No.	Water Bodies					
	River		Lake		Reservoir	Dam
Sites	Betwa , Baruasagar	Pahuj, Rampura	Antiya, Civil line	Garhmau, Garmau village	Pahuj, Grassland	Parichha, Parichha village
Identified General Species	Nitzschia sinuate, Navicula angilica, Cymbella, Cyclotella, Fragilaia, Tabularia, Eunotia, Staurosirella, Gomphonema, Fragilaria, Ulnaria	Fragilaria longifusiformis, Nitzschia sinuate, Navicula ingenua, Cymbella affinis, Gomphonema lagenula, Cyclotella	Amphora, Synedra famelica, Navicula ingénue, Encyonema langebertalotii, Nitzschia	Enconema gracilum, Gomphonema augur, Fragila biceps, Nitzshia palea, Nitzschia amphibia, Tabularia floculosa, Navicula, Fragilaria construens, Stauroneis,	Fragilaria longifusiformis, Nitzschia sinuate, Navicula, Cymbella, Cyclotella	Nitzschia filiformis, Fragilaria tenera, Navicula vilaplanii
Most Frequent Genera	Nitzchia, Navicula	Fragilaria, Gomphonema Navicula	Synedra, Navicula	Enconema, Nitzschia, Navicula	Fragilaria, Nitzschia, Navicula	Nitzschia Navicula

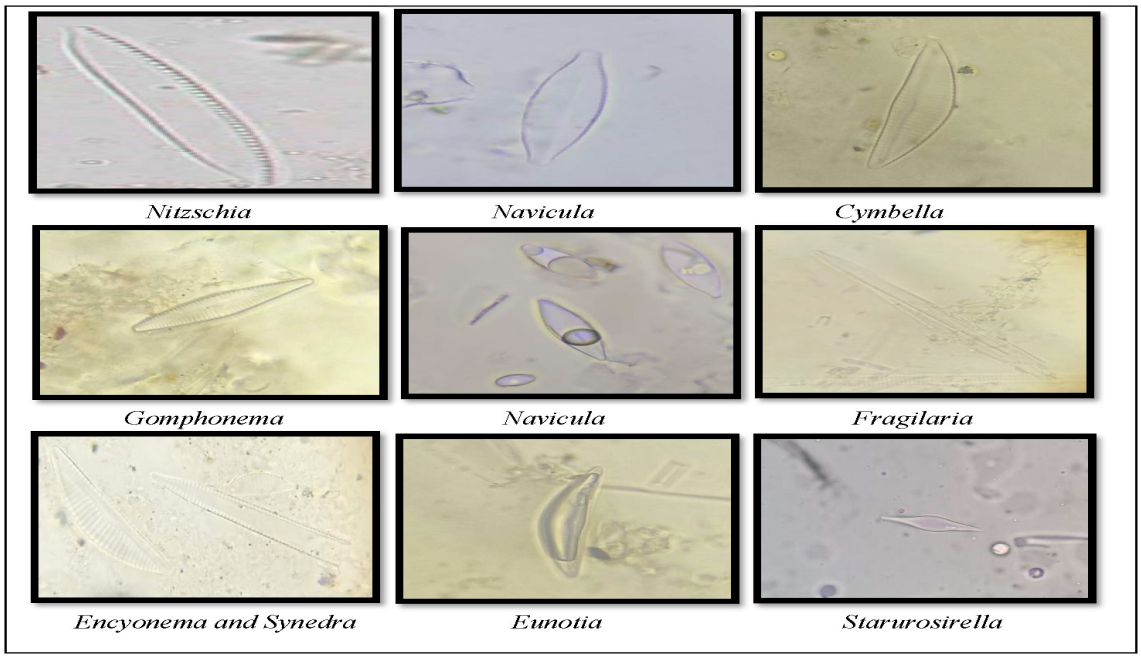


Figure 4: Photomicrographs of Some of Diatom Genera Identified in the Water Bodies

Table 2: Different Physical Parameters Recorded for Different Water Bodies

Sl. No.	Water Bodies	Sites	Physical Parameters				
			pH	Temp. (°C)	TDS (ppm)	Longitude	Latitude
1	River	Betwa	7.2	32.4	132	25.408 E	78.681 N
		Pahuj	7.3	30.3	195	25.449 E	78.518 N
2	Lake	Antiya,	7.4	30.9	226	25.456 E	78.567 N
		Garhmau	7.1	33.4	192	25.511 E	78.668 N
3	Reservoir	Pahuj	8.2	30.1	196	25.507 E	78.526 N
4	Dam	Parichha	7.0	29.6	126	25.519 E	78.778 N

The present study was conducted in the district Jhansi of Uttar Pradesh (2120 deaths) that stood sixth position and touching the boundary of Madhya Pradesh (5779 deaths) state that ranked first in terms of number of accidental deaths occurred due to drowning across the country. Drowning as a cause of accidental death had a fair share of about 10.1% (37238 deaths) of the total accidental deaths (366992 deaths) reported across the country in which males were found to be more prone to accidental drowning with a total of 29178 deaths as compared to the females accounting for 8059 deaths during 2020[19].

According to the NCRB suicidal deaths report 2020, Uttar Pradesh the most populous state has accounted for 3.1% of the total suicides (153052) reported in India. A total of 7977 deaths were reported due to drowning in Uttar Pradesh which accounted for 5.2% of the total suicidal deaths during 2020 in India. A total share of drowning as mode of suicide in males (5306) was higher as compared to the females (2671). Drowning (7977 deaths) was found to be the third major mode adopted for committing suicide after hanging (88460 deaths) and consuming poison (38336 deaths)[20].

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