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A Preliminary Study on the Diatom Flora of Various Drowning Sites in Mizoram Rivers.

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ABSTRACT

In the present study, diatomological composition of main drowning sites of the three Rivers of Mizoram state in the Northeast India has been studied. The aim is to establish a database of diatoms to help in the diagnosis of suspected drowning cases occurring in this region. The observations of the present study model have evaluated the existing diatom flora and their distribution in the winter season in selected Rivers. The result shows significant differences of diatom distribution between the different water bodies (inter-water body distribution pattern), which can help in linking a drowned dead body to a specific water body source.

Keywords: Forensic diatomology, Drowning, Mizoram flora

Introduction

Drowning can be homicidal, suicidal or accidental. When a corpse is recovered from water, there is usually a suspicion whether the body was drowned before or after death. Sometimes, a criminal may throw a body in water after committing a murder or homicide somewhere else with an attempt to conceal his crime. In other cases, a body maybe recovered miles away from drowning sites. Forensic pathologist is often asked whether the drowning was ante-mortem or post-mortem along with whether the death really took place at the recovery site of body.

The diagnosis of drowning is one of the most difficult analyses in forensic investigations. Forensic diatomology has proven to be the most reliable method to diagnose death by drowning. If, both the quantitative and qualitative conditions of diatom distribution are agreeable then a positive conclusion with reference to the ante-mortem drowning can be offered. The concordance of the diatoms recovered in the body organs with diatoms of water samples collected from the suspected

drowning sites can also acts as a guide in establishing the most probable drowning site for the reconstruction of events.

It is estimated that there are 200 genera and approximately 1,00,000 species of living diatoms [1]. Various genera and species of diatoms establish themselves within different water bodies based on their specific nutrient and light requirements. This of course is fortunate for the forensic investigators engaged in drowning investigations to determine the drowning site. The diatoms are unicellular algae of class *Bacillariophyceae* under kingdom Protista. They are found in both fresh and marine water.. Normally similar types of diatoms exist in similar type of water bodies but unique ecosystem, some local environmental factors including mineral contents (especially silicon) of water and soil, temperature, water stratification, pH and pollution linkage can bring notable variations in quality and quantity of diatom population [2-8]. Emphasized on the detailed study of density (quantity), species (quality) and description of valve (morphology) of diatom for every sample before the diagnosis of death by drowning have been advocated because these parameters help knowing the entry of specific diatoms into the body organs from any putative drowning medium. Knowledge about the diatom flora in any water body and their correlation with the diatoms recovered from the drowned body could be a method of choice to resolve the questions related with the suspicions occurred on the drowning site [2-7, 10].

In the state of Mizoram in Northeast India drowning cases are very common. Literature survey could not provide any significant information about the studies made for the analysis of diatom flora of the Mizoram especially for forensic purpose.

Materials and Methods

In the present study, queries were made about the common drowning sites in Mizoram state. For this, the data of “Report on drowning cases in Mizoram during 2006, 2008-2011” (Fig. 1) was obtained from the Police Crime Branch, Aizawl District on 6^{th} and 9^{th} January 2012. Accordingly, three rivers in Mizoram namely Chite, Tlawng river, Tuirial river (Fig 2) were selected and water samples from common drowning sites were collected during January, 2012 during the winter season.

2.1. Sample collection

About 500ml. of water was collected in clean plastic bottles from both the bank and middle of each of the selected sites of all the three rivers and caps were sealed using brown tape to prevent any form of contamination. No amount of debris was observed in the collected samples. The samples were properly labeled so as to indicate its origin and prevent any form of error. The pH of the water samples were also checked during sample collection with the help of pH paper. All of them were found to be slightly alkaline. The samples when brought to laboratory were stored at room temperature.

2.2. Extraction and Identification of Diatom

Extraction of diatoms from the collected water samples was done using modified Acid Digestion Method [9]. 200 ml of water sample was transferred from the plastic bottle into a 500 ml clean glass beaker. 50 ml of concentrated nitric acid (HNO_3) and a pinch of Potassium Dichromate ($K_2Cr_2O_7$) were added into it. It was then left to stand undisturbed for digestion for about 2 hours. After digestion, the sample was transferred into a well- labeled centrifuge tube and centrifuged at 3000

rpm for 10 minutes. The aqueous supernatant was pipette out leaving behind the pellet at the bottom. The residual pellet was suspended in distilled water and was centrifuged again for 5 minutes at 3000 rpm. This step was done twice to ensure that all traces of acids were removed. Now to the final pellet, 2 drops of distilled water was added and mixed thoroughly. It was then marked properly and stored at room temperature.

10 microscopic slides were prepared for each of the selected sites (5 for the middle and another 5 slides for bank of the river) of different rivers. This was done in an attempt to narrow down the chance of diatoms being missed out.

For the preparation of permanent slides, one drop of residual mixture was pipette out and was spread evenly on the microscopic slide to make a smear. The slides were mounted permanently using a drop of DPX and then covered with a cover slip. The slides were examined under Nikon Eclipse 80i Light Microscope at 1000X magnification in oil immersion and Photo-micrographs were taken with the help of an attached digital camera. The diatoms were identified using available literature [10]. All the slides were analyzed and data was recorded in database record chart. The data so generated was subjected to morphological analysis, distribution pattern of identified diatom genera (for Inter and intra-river variations) and for the construction of diatomological maps.

RESULTS AND DISCUSSIONS

After the analysis of results, the profiles of diatom flora of selected water bodies were generated. The site wise record of diatoms was studied and compared thoroughly. Various commonly occurring and rare or site-specific diatoms species were noted.

Distribution pattern of the identified diatoms

21 genera of diatoms were identified as shown in table 1 and Plates 1-4. Most of the identified diatoms belong to order “Pennales” with only a few “Centrales”. From the distribution pattern of identified diatom genera, it can be seen that variation is present in the distribution pattern of diatoms in the three selected rivers of common drowning sites in Mizoram. Nonetheless, some diatoms are present in all the selected drowning sites. The “inter-water body distribution pattern” of a diatom database can be helpful to reach a conclusion about the site of drowning. Accordingly diatomological map was constructed on the basis of commonly occurring diatoms, rarely occurring diatoms and water specific diatoms (Table 2).

From the selected three rivers, Tlawng River has maximum number of the identified diatoms genera i.e. 14 (Table 3), from Tuirial River 10 diatoms genera were identified (Table 4) and from Chite River 7 diatoms genera were identified (Table 5). Variation in pH, temperature, salinity, organic matter etc. may be responsible for the variation in their distribution pattern. From the study, it is seen that *Amphora, Bacillaria, Cocconeis, Cymbopleura, Diadesmus, Encyonama, Hitzschia, Surirella, Plagotropis, Psammothidium* were highly site specific while *Nitzschia, Cymbella, Gymphonema* were found in all the three selected water bodies. Tuirial River was found to be very rich in *Melosira* diatom. Different species were seen with large numbers. But apart from *Melosira*, not many varieties of diatoms were found.

In the study of intra-water body distribution of diatoms, it was found that diatoms were extremely less in the middle of the three rivers (Table 3, 4, 5). The slides from the middle of rivers had to be scanned under microscope meticulously. Many of the prepared slides were even found to be devoid of diatoms despite the careful and meticulous observation.

CONCLUSION

In the present study, three rivers of common drowning sites in Mizoram state were selected for the Diatomological study. Since all the samples were collected from running water bodies (rivers) during the winter season, the environment and the climatic conditions were not favorable for the growth of diatoms. 21 diatoms were identified up-to their genera level. Though all the samples had been collected in the same season (*i.e.* winter) with not much difference in the temperature and the pH (all the rivers were slightly alkaline), but still diversity was observed from river to river and from different sites of the same river (*i.e* middle and bank).

The conclusions derived from the present study are:

- In total, 21 diatoms genera were identified from the three selected water bodies. Most of them belong to the order Pennales with only a few Centrales.
- The result shows significant differences of diatom distribution between the different water bodies (inter-water body distribution pattern), which can help in linking a drowned dead body to a specific water body source.
- Intra-water body distribution pattern from the study shows considerable difference between the number of diatoms in two sites of a river (*i.e* the middle and the bank of a river). Diatoms were extremely less in the middle. This can help in cases where there is a question raised about the specific site of drowning within a single water body.
- Some of the diatoms like *Nitzschia, Cymbella* and *Gymphonema* were found present in all the selected water bodies.
- Diatoms like *Amphora, Bacillaria, Cocconeis, Diadesmus, Encyonama, Hitzschia* and *Surirella* were found to be site specific which is useful in determining the specific site of drowning.
- *Melosira* dominated the diatom population in Tuirial River while the other rivers have been equally populated by different diatom genera.

The present diatomological study is expected to be of help for forensic experts in certain medico-legal cases of suspected drowning in Mizoram state. But since the study was limited to a single season (winter season), there is a need for further research to generate diatom database for all the four seasons throughout a year. In future more water bodies of Mizoram can be covered to establish a bigger database which will be useful for forensic investigations and where diatoms can be identified up-to their species level.

Table 1: Identified diatom genera from the three selected rivers.

Diatom genus	Chite river	Tlawng river	Tuirial river
Amphora	-	+	-
Bacillaria	-	+	-
Cocconeis	-	+	-
Cymbella	+	+	+
Cymbopleura	-	+	-
Diadesmus	-	+	-
Encyonama	+	-	-
Fragilaria	-	+	+
Gomphonema	+	+	+
Gomphoneis	-	-	+
Hantzschia	-	-	+
Mastogloia	-	+	-
Melosira	+	-	+
Navicula	-	+	-
Nitzschia	+	+	+
Opephora	-	+	+
Plagiotropis	+	-	-
Psammothidium	-	-	+
Rhopaloida	-	-	+
Surirella	-	+	-
Synedra	+	+	-

[Note: Present = "+"; Absent = "-"]

Table 2: Distribution pattern of identified diatom genera in the three selected rivers.

River	Common	Rare	Specific
Chite	*Cymbella, Melosira*	*Synedra, Nitzschia, Gomphnema*	*Encyonama, Plagiotropis*
Tlawng river	*Fragillaria, Cymbella, Gomphonema, Navicula*	*Nitzschia, Opephora, Cymbopleura, Synedra, Diadesmus*	*Amphora, Bacillaria, Cocconeis, Mastogloia, Surirella*
Tuirial river	*Cymbella, Fragilaria, Melosira.*	*Gomphonema, Gomphoneis, Nitzschia., Opephora*	*Hantzschia, Rhopaloida, Psammothidium*

***Common**- Diatoms found present on average 5 or above per microscopic slide; **Rare**- Diatoms found present on average 1 to 4 per slide microscopic slide; **Specific**- Diatoms which are specific to a certain water body*

Table 3: intra-river variation in diatom distribution in Tlawng River

Diatom genus	Tm (middle of Tlawng)	Tb (bank of Tlawng)
Amphora	-	+
Bacillaria	-	+
Cocconeis	-	+
Cymbella	+	+
Cymbopleura	-	+
Diadesmus	+	-
Fragilaria	-	+
Gomphonema	+	+
Mastogloia	-	+
Navicula	-	+
Nitzschia	-	+
Opephora	-	+
Surirella	-	+
Synedra	-	+

[Note: Present = "+"; Absent = "-"]

Table 4: intra-river variation in diatom distribution in Chite River

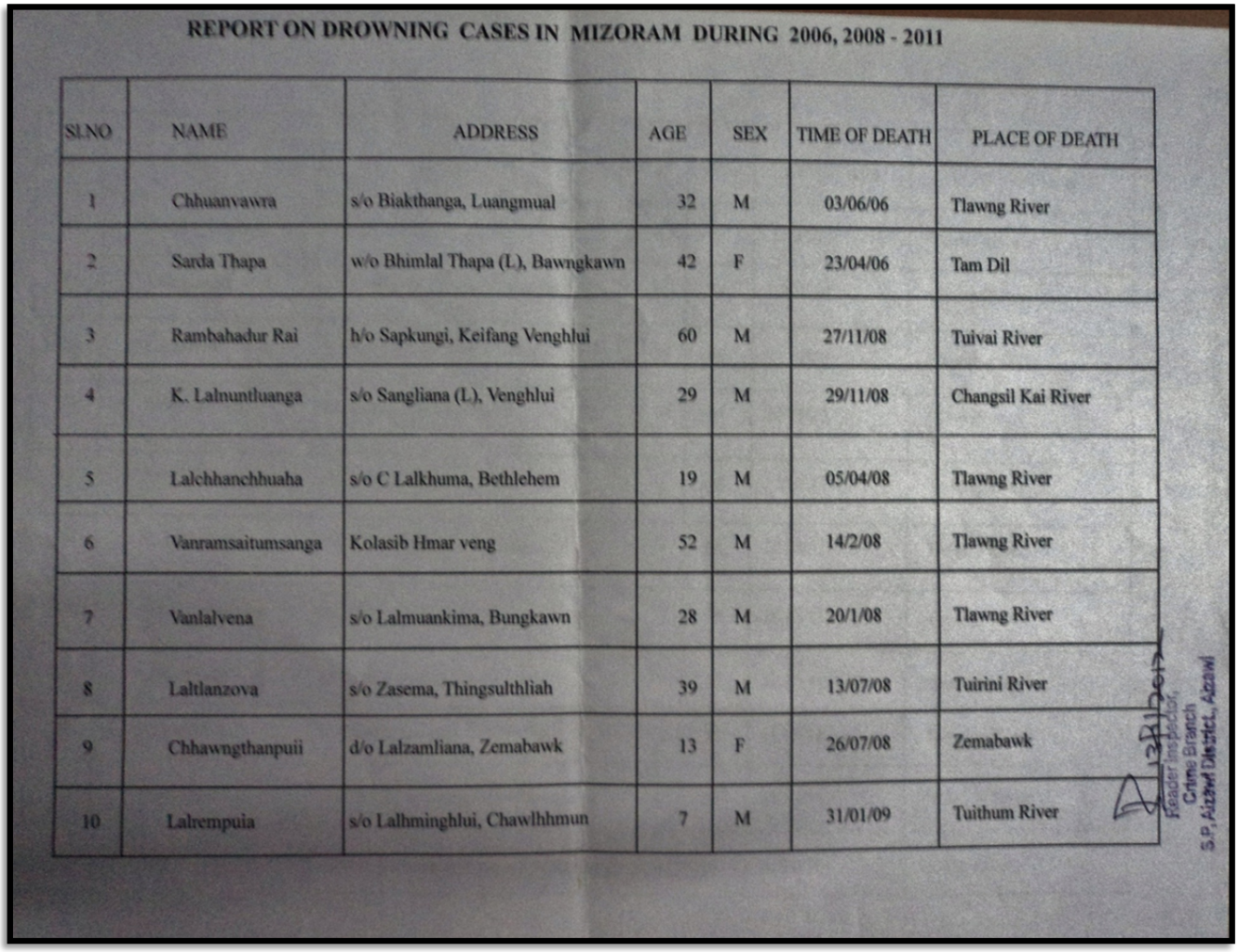
Diatom genus	Cm (middle of Chite)	Cb (bank of Chite)
Cymbella	+	+
Encyonama	-	+
Gomphonema	+	-
Melosira	+	+
Nitzschia	-	+
Plagiotropis	-	+
Synedra	-	+

[Note: Present = "+"; Absent = "-"]

Table 5: intra-river variation in diatom distribution in Tuirial River

Diatom genera	TRm (middle of Tuirial)	TRb (bank of Tuirial)
Cymbella	-	+
Fragilaria	+	-
Gomphoneis	-	+
Gomphonema	+	+
Hantzschia	-	+
Melosira	+	+
Nitzschia	-	+
Opephora	-	+
Psammothidium	-	+
Rhopaloida	-	+

[Note: Present = "+"; Absent = "-"]



REPORT ON DROWNING CASES IN MIZORAM DURING 2006, 2008 - 2011

Sl.NO	NAME	ADDRESS	AGE	SEX	TIME OF DEATH	PLACE OF DEATH
1	Chhuanvawra	s/o Biakthanga, Luangmual	32	M	03/06/06	Tlawng River
2	Sarda Thapa	w/o Bhimlal Thapa (L), Bawngkawn	42	F	23/04/06	Tam Dil
3	Rambahadur Rai	h/o Sapkungi, Keifang Venghlui	60	M	27/11/08	Tuivai River
4	K. Lalnuntluanga	s/o Sangliana (L), Venghlui	29	M	29/11/08	Changsil Kai River
5	Lalchhanchhuaha	s/o C Lalkhuma, Bethlehem	19	M	05/04/08	Tlawng River
6	Vanramsaitumsanga	Kolasib Hmar veng	52	M	14/2/08	Tlawng River
7	Vanlalvena	s/o Lalmuankima, Bungkawn	28	M	20/1/08	Tlawng River
8	Laltlanzova	s/o Zasema, Thingsulthliah	39	M	13/07/08	Tuirini River
9	Chhawngthanpuii	d/o Lalzamliana, Zemabawk	13	F	26/07/08	Zemabawk
10	Lalrempuia	s/o Lalhminghlui, Chawlhhmun	7	M	31/01/09	Tuithum River

Reader Inspector, Crime Branch S.P. Aizawl District, Aizawl

Fig 1: Report on drowning cases in Mizoram during 2006, 2008-2011 (Data obtained from Police Crime Branch, Aizawl, Mizoram)



SL.NO	NAME	ADDRESS	AGE	SEX	TIME OF DEATH	PLACE OF DEATH
11	Zohmingthanga	s/o Arason, Bungbangla	9	M	12/03/10	Tuirial River
12	Lalruatpuia	s/o Lalhluna, Ramhlun Venglai	27	M	19/03/10	Tlawng River
13	Laldawna	s/o Ramthanga, Armed Veng	48	M	04/06/10	Mirawng River
14	Lalduhawma	s/o Lalpara, Bethlehem	15	M	02/04/10	Chite River
15	Ricky Zosangzuala	s/o Laltani, Salem Veng	27	M	12/06/11	Tlawng River
16	R. Lalbiaknunga	s/o R. Lalbiakzaua, Sairang	23	M	28/08/11	Tlawng River
17	N.K.Vanlalringa	s/o Timo, Chite Veng	37	M	28/08/11	Tlawng River
18	Lalhriatpuia	s/o Lalnunmawia, Phuaibuang	21	M	14/10/11	Phunchawng
19	Lalhualhima	s/o Rochungnunga, Sakawrdai	33	M	03/10/11	Tuiruang River
20	Vanlaldina	s/o Hrangthanthianga, Sawkawrdai	42	M	03/10/11	Tuiruang River
21	Vanlalnghaka	s/o Saingura, Vanbawng	30	M	24/11/11	Tuivai River

Reader Inspector,
Crime Branch
S.P, Aizawl District, Aizawl

Fig 2: Map of Mizoram showing the selected rivers and site of sample collection



Black dot= Tlawng river

Red dot= Tuirial rive

Green dot= Chite river

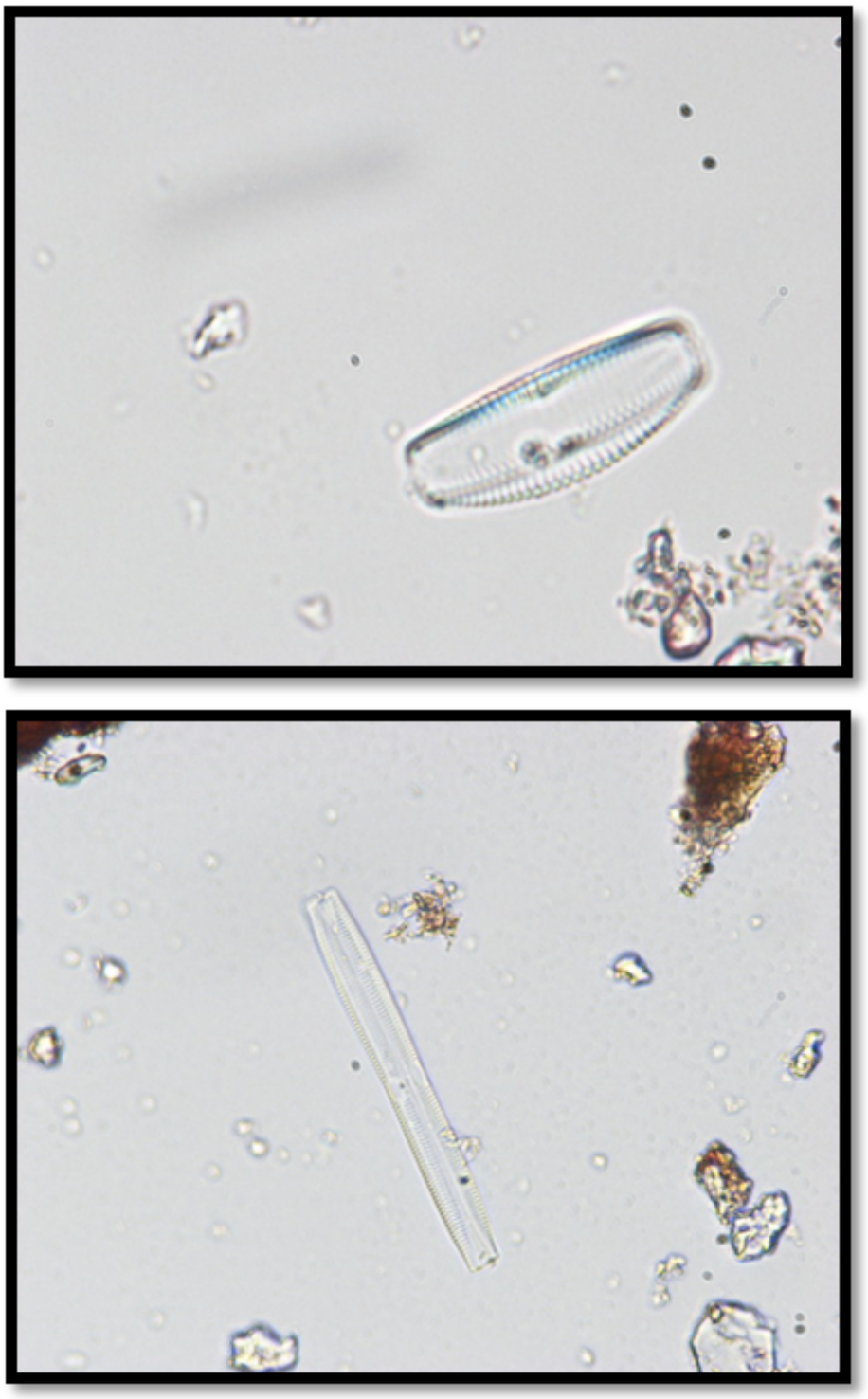
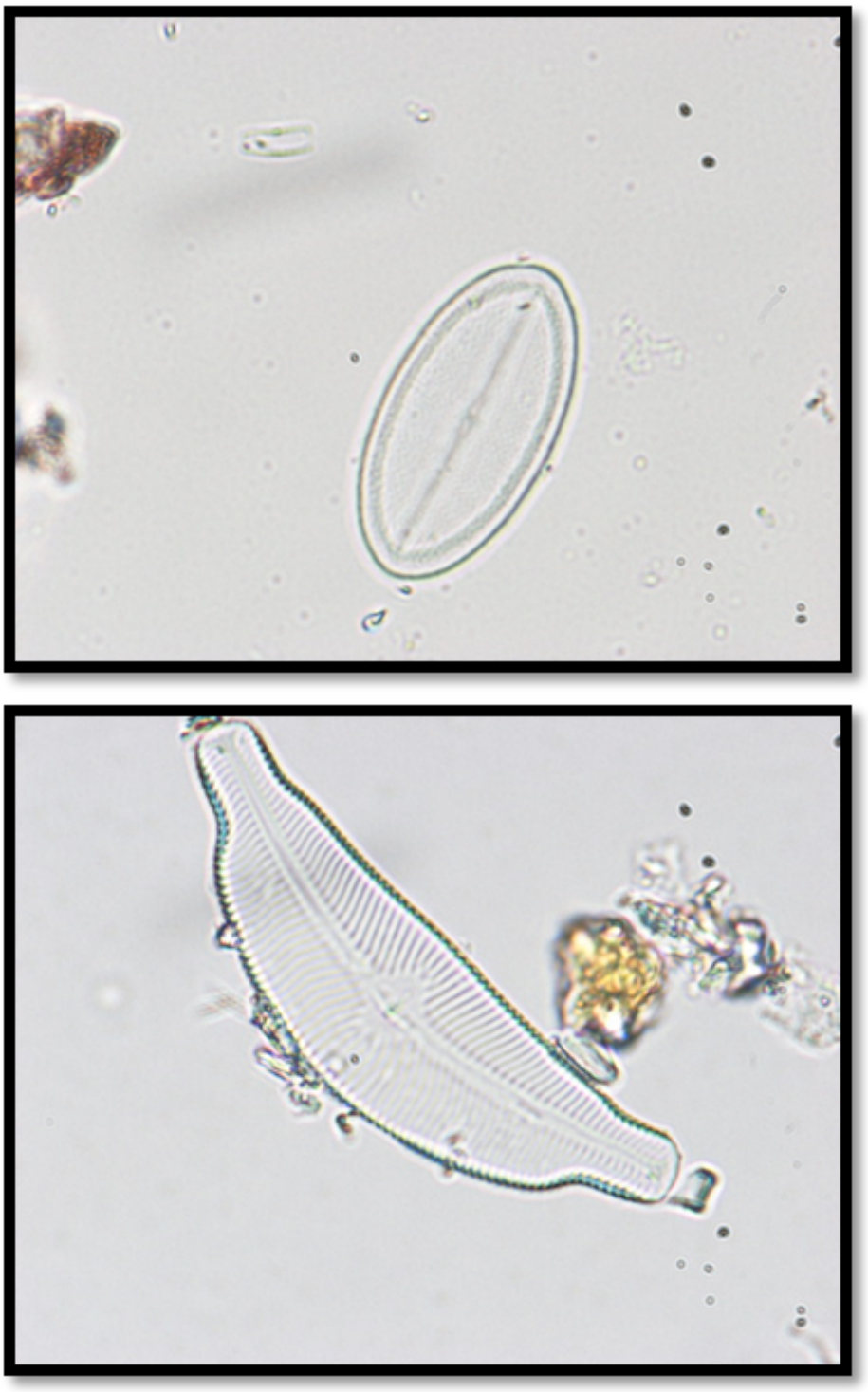
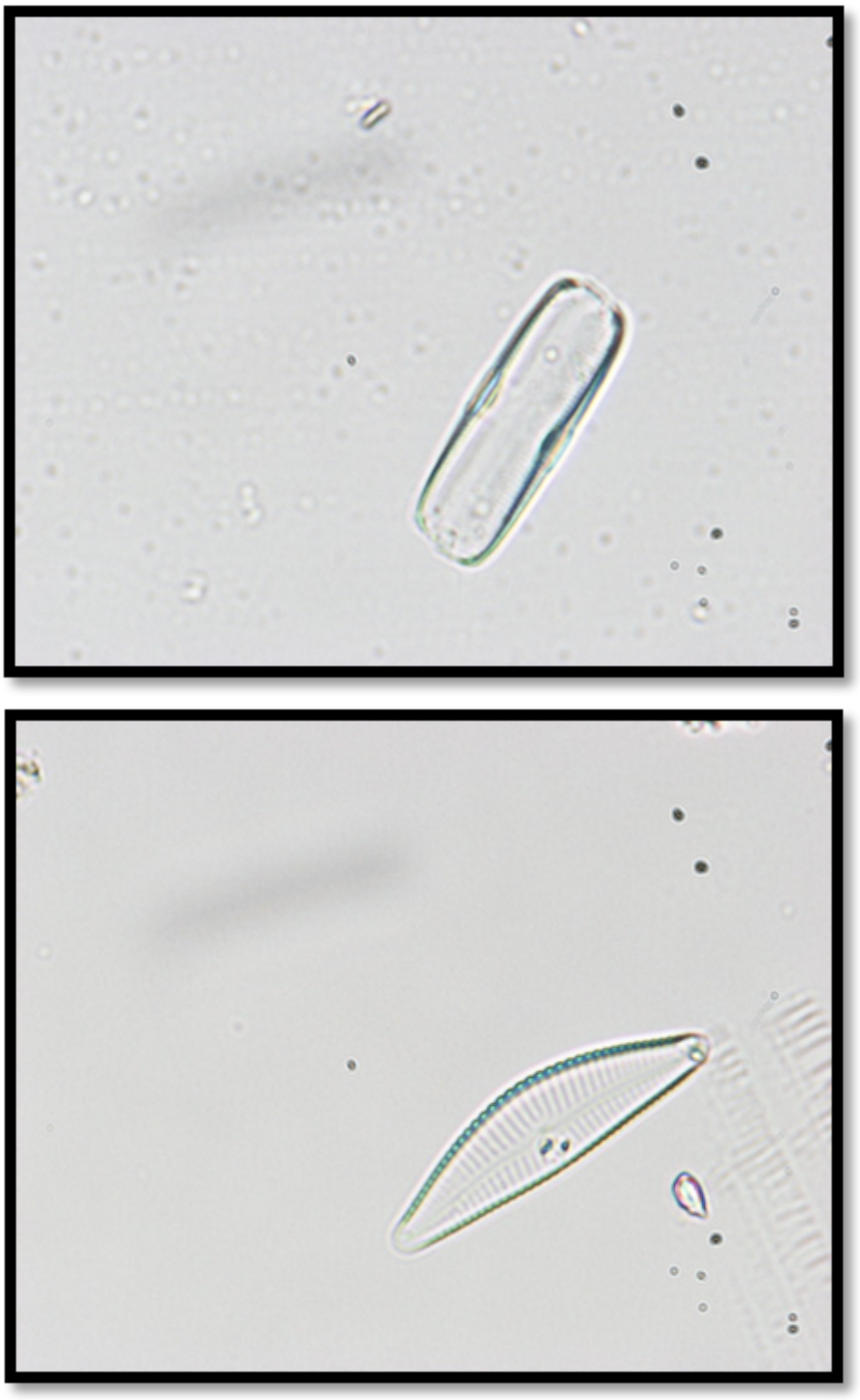


Plate 1: Photomicrographs of the identified diatom genera

Amphora Bacillaria



Cocconeis Cymbella



Diadesmus Encyonama

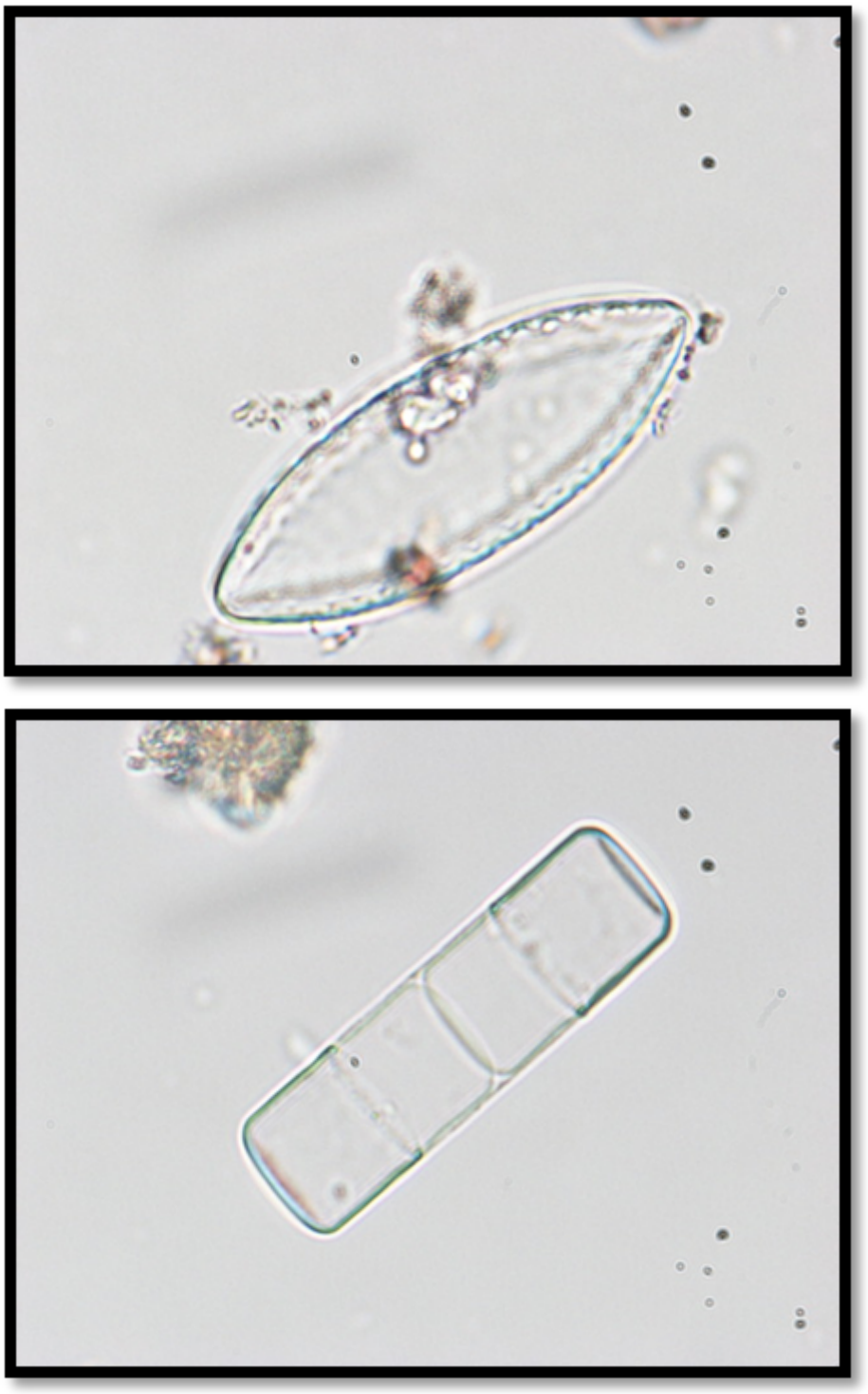


Plate 2: Photomicrographs of the identified diatom genera

Fragilaria Gomphoneis



Gomphonema Hantzschia



Mastogloia Melosira

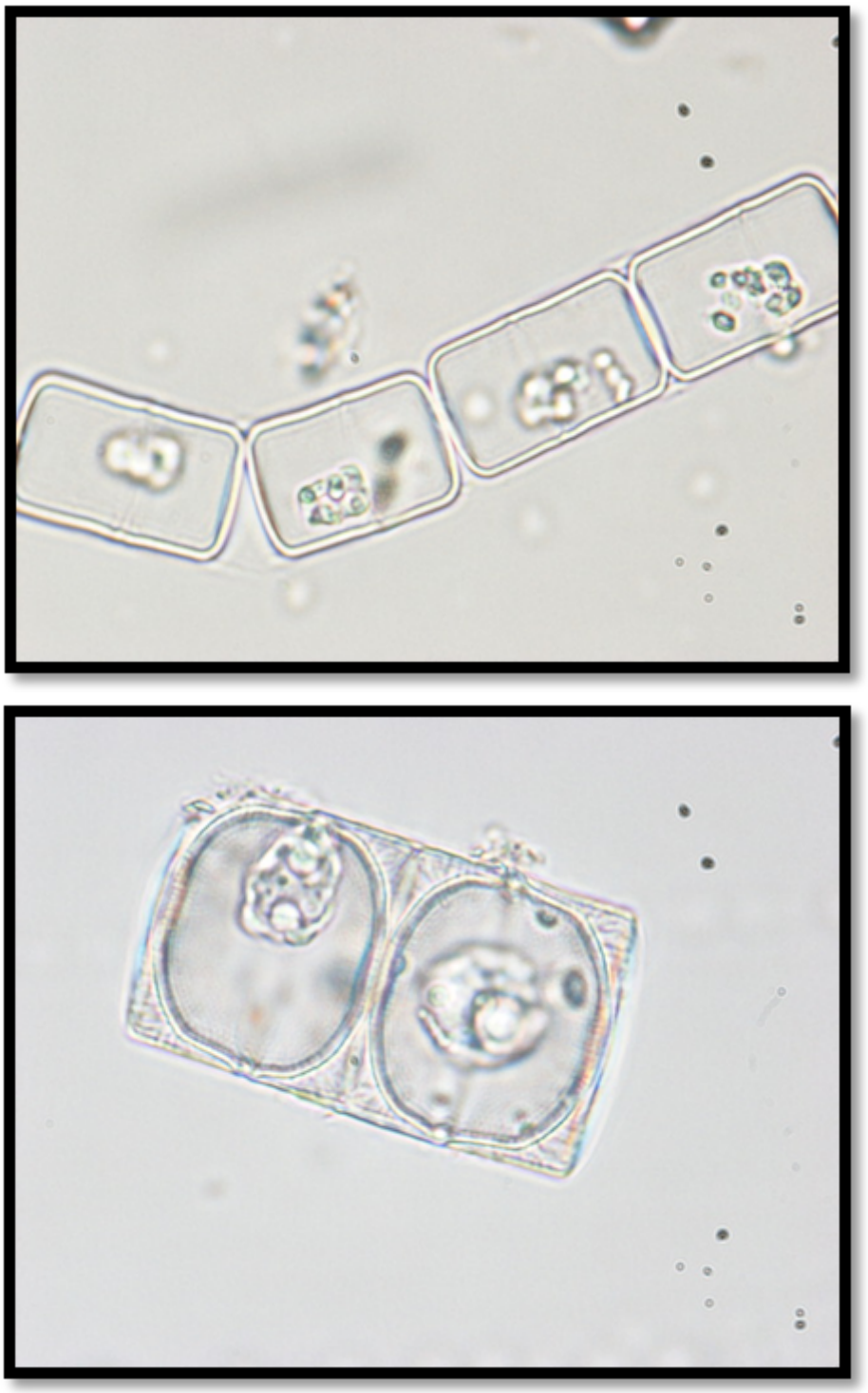
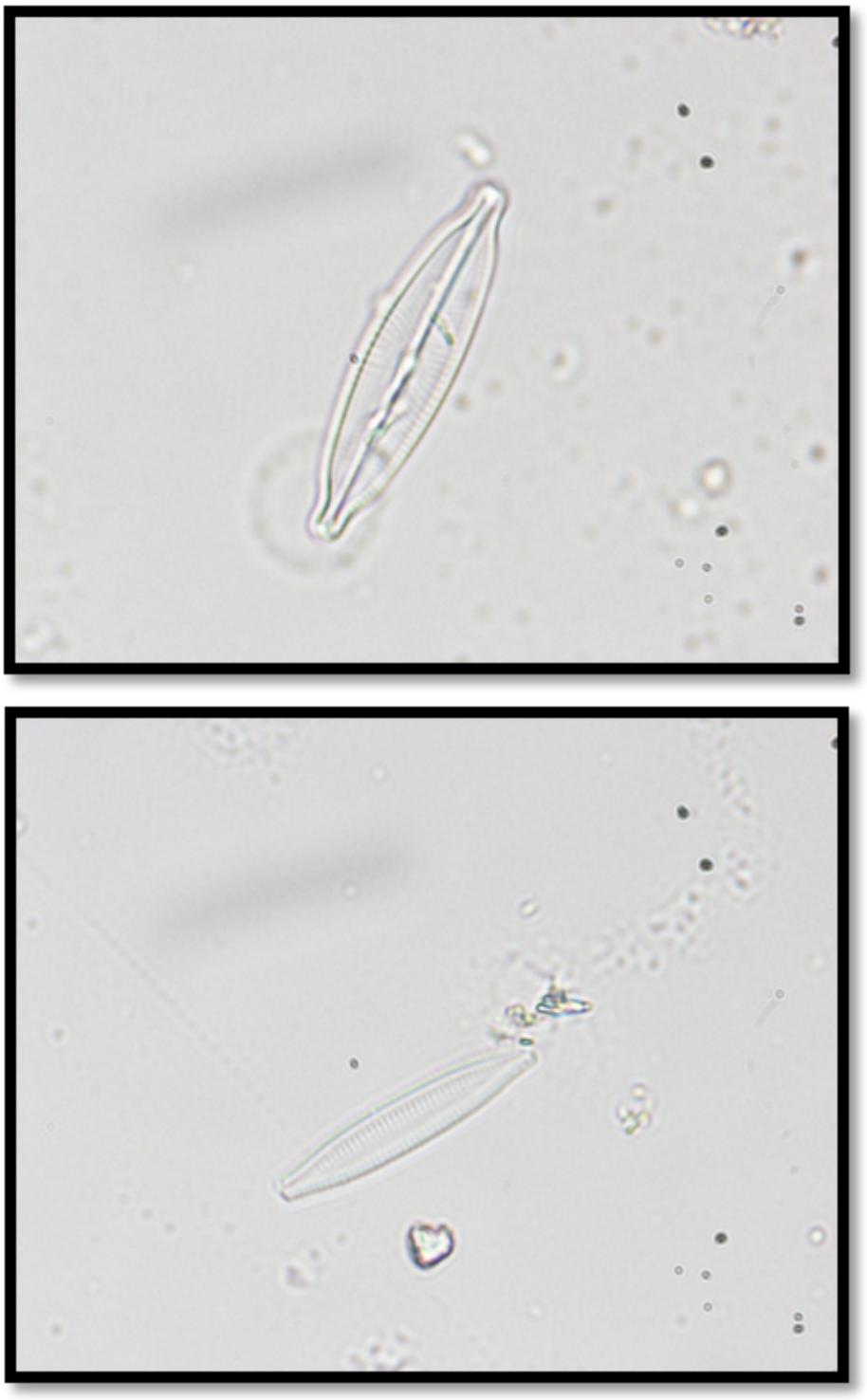


Plate 3: Photomicrographs of the identified diatom genera

Melosira Melosira



Navicula Nitzschia

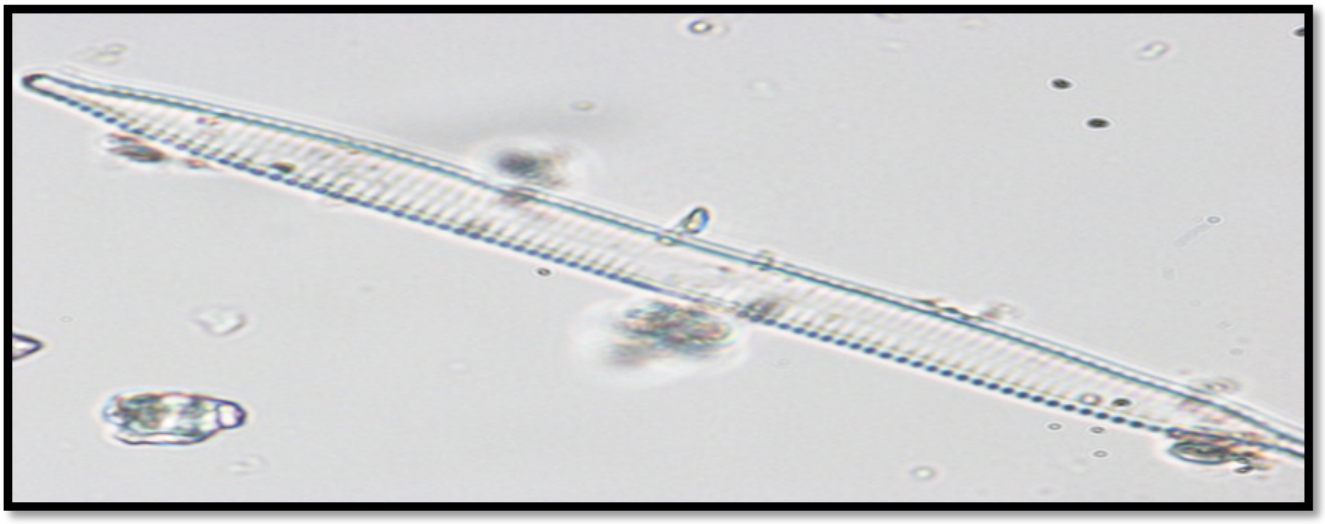


Opephora Plagiotropis



Plate 4: Photomicrographs of the identified diatom genera

Psammothidium Rhopaloida



Synedra



Surirella

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