EXAMINATION OF DIATOMS FROM THE TELUPID DISTRICT WATER SAMPLES BY DIGESTION TECHNIQUE USING CONCENTRATED NITRIC ACID (HNO_3)

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by

RICXLEY JOHN

**Dissertation submitted in partial fulfilment
of the requirements
for the degree of
Bachelor of Science (Honours) in Forensic Science**

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CERTIFICATE

This is to certify that the dissertation entitled EXAMINATION OF DIATOMS FROM THE TELUPID DISTRICT WATER SAMPLES BY DIGESTION TECHNIQUE USING CONCENTRATED NITRIC ACID (HNO_3) is bonafide record of research work done by Mr. RICXLEY JOHN during the period from October 2024 to February 2025 under my supervision. I have read this dissertation and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation to be submitted in partial fulfilment for the degree of Bachelor of Science (Honours) in Forensic Science.

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DECLARATION

I therefore declare that this dissertation is the result of my own research, unless otherwise mentioned and officially acknowledged. I further declare that it has not previously or concurrently been submitted in its entirety for any other degrees at Universiti Sains Malaysia or any other institution. I allow Universiti Sains Malaysia permission to utilize the dissertation for teaching, research, and promotion.

………… ………………

(RICXLEY JOHN)

Date:

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LIST OF ABBREVIATIONS

CT Computed tomography

DNA Deoxyribonucleic acid

mL Millilitre

mm Millimetre

μL Microlitre

μm Micrometre

HNO_3 Nitric acid

L/D Diatom content in lung tissue to in drowning medium ratio

MRI Magnetic resonance imaging

pH Potential of hydrogen

rpm Revolutions per minute

TN Total Nitrogen

TP Total Phosphorus

PEMERIKSAAN DIATOM DARI SAMPEL AIR DI DAERAH TELUPID MENGGUNAKAN TEKNIK PENCERNAAN ASID NITRIK PEKAT (HNO_3)

ABSTRAK

Diatom ialah sejenis alga yang diklasifikasikan dalam kumpulan mikroalga yang dapat menjalankan fotosintesis. Ia merupakan organisma eukariotik unisel yang mempunyai lapisan silika yang dikenali sebagai frustul. Diatom boleh ditemui hampir di setiap persekitaran akuatik, termasuk air tawar dan air masin, yang secara umumnya berada dalam persekitaran lembap. Dalam kajian ini, pemeriksaan terhadap diatom dijalankan ke atas tiga sampel air yang dikumpul dari lokasi berbeza di daerah Telupid, Sabah, seperti ladang kelapa sawit, kawasan rekreasi/taman, dan sumber air sungai yang tidak tercemar. Kaedah pencernaan asid menggunakan asid nitrik pekat digunakan untuk memerhati kehadiran diatom. Menariknya, dalam kajian ini, tiada diatom sentrik yang direkodkan dalam kesemua sampel air. Satu-satunya jenis diatom yang dapat ditemukan ialah diatom penat, yang mempunyai simetri bilateral. Secara keseluruhannya, data diatom yang dihasilkan dalam kajian ini boleh digunakan sebagai data pemeriksaan awal untuk kegunaan penyelidik lain dalam bidang yang sama.

EXAMINATION OF DIATOMS FROM THE TELUPID DISTRICT WATER SAMPLES BY DIGESTION TECHNIQUE USING CONCENTRATED NITRIC ACID (HNO_3)

ABSTRACT

Diatoms are a type of algae classified in a group of microalgae which undergo photosynthesis. They are unicellular eukaryotic organisms characterised by having a siliceous covering also known as frustule. Diatoms are found in almost every aquatic environment including fresh and saltwater, which is basically in moist environments. In this study, the examination of diatoms was carried out against three water samples collected from different locations in the Telupid District, Sabah to represent different location environments such as palm plantations, recreational area/park and undisturbed water sources or flow. An acid digestion method using concentrated nitric acid was used to observe the diatom. Interestingly, none of the centric diatoms were reported from all water samples. The only type of diatom was a pennate group diatom, which has a bilateral symmetry. In summary, the diatom data generated in this study can serve as a preliminary diatom database for other researchers in the same field.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Forensic science is becoming important nowadays as it helps forensic scientists to identify and solve many criminal cases. Despite many other forensic fields, forensic diatomology assists investigation by providing information to link the suspect to the crime site or victim. Diatom is a unicellular eukaryotic organism that is mostly found in a moist environment (Reid et al., 2021). Diatom also serves as a marker for ecological indicators because it keeps a history of the water environment in the past (Kutty et al., 2022). Meanwhile, in the forensic field, diatom has been used to determine ante mortem drowning (Li et al., 2019).

As a bioindicator diatom poses an important role for environmental health especially in relevance to environmental monitoring and water quality assessment. Due to their high sensitivity diatoms can detect even small changes in water quality such as the pH levels, nutrient load, and pollution (Smucker et al., 2020). The diatoms serve as an early warning system for environmental degradation. The capability of diatom in detecting environmental degradation has led to extensive use in water quality assessments, which is valuable for environmental management as well as conservation efforts (Evance et al., 2022). Different diatom species can indicate specific environmental conditions making them important as reliable markers for tracking changes in water bodies due to pollution or climate change.

On the other hand, diatoms can be very useful in forensic science, particularly in drowning case investigations where the diatom analysis could help to determine the causes of death by confirming the water inhalation as well as narrow down the investigation by linking with a specific location or the crime site. According to Kurshid et al. (2021) when a body recovers from the water identifying the cause of death becomes challenging because the body may decompose which creates difficulty in identification and the only way to determine is by diatom test from the victim organs such as lung, liver and bone marrow.

Examination of diatom used a concentrated acid either the nitric acid or sulfuric acid. This method is important especially to isolate and preserve the diatoms from complex samples by dissolving the organic matter in the water samples, leaving a clear sample for the examination of diatom.

1.2 Problem Statement

Diatom is one of the forensic biological evidence that can serve as a vital tool for both the forensic and ecological fields. According to a study conducted by Thakar and Singh (2010), diatoms act as gold indicators not only for determining the cause of death in drowning cases but also for determining the specific location of the drowning case. Their presence inside the water bodies allows for site-specific matching by comparing the diatoms found in a victim’s organs and those in the water. Besides that, diatoms are also recognised as highly sensitive bioindicators of water quality because of their response to specific changes, especially climate changes

However, based on the literature search there are limited studies conducted for diatom research, particularly in mapping the diatom across a variety of water bodies in different rivers, ponds or lakes from varying location sites in Malaysia. Therefore, the results obtained from diverse groups of research can be deposited in one diatom database to be used and accessed by other researchers in the same field. Other than that, the distribution of diatoms tabulated from the studied site can provide clues about the environment of the water sources.

1.3 Objectives

1.3.1 General Objectives

To identify the diatom communities from the water samples of Telupid District, Sabah.

1.3.2 Specific Objectives

1. To collect water samples from 3 different locations in the Telupid District, Sabah.
2. To examine the extracted diatom from the acid digestion through an optical microscope.
3. To classify diatom based on valve symmetry and frustule shapes.

1.4 Significance of Study

The relevance of the diatom study is underscored by the increasing number of water-related crimes such as drowning incidents, suicides, and water pollution in various water bodies. According to the Centers for Disease Control and Prevention (CDC), approximately 3,960 people die annually from drowning in pools, lakes, and other aquatic environments (30 Recent Drownings & Why They Happened, n.d.). Meanwhile, Satinder Ahuja (2021) has highlighted the incidence of decreasing water quality globally due to the release of various pollutants into the water bodies.

Hence, this study aims to examine the presence of diatoms in water samples collected from three water sources in the Telupid District, Sabah. The acid digestion method using concentrated acid is an established and standard method for the identification of diatoms. The information gained from the diatom communities in this area has a significant contribution to forensic and ecological conditions. Indirectly the diatom data gathered from this study can be used as a preliminary diatom database for future cases involving drowning or pollution in this area.

CHAPTER 2

LITERATURE REVIEW

2.1 Morphology of Diatom

Diatoms are a diverse group of unicellular eukaryotic organisms that are primarily found in aquatic environments, including freshwater and marine ecosystems (Reid et al., 2021). They play a significant role in the aquatic food web and are essential for various ecological processes. Their unique morphology, characterised by intricate silica cell walls known as frustules, distinguishes them from other phytoplankton. This study explains the morphology of diatoms, focusing on their structure, classification, and significance in both forensic and ecological fields.

Diatoms are photosynthetic organisms that utilise chlorophyll for photosynthesis, contributing significantly to global oxygen production (Kuczynska et al., 2015). They range in size from approximately 15 to 400 micrometres, with some species reaching lengths of up to 2 millimetres (Dipper, 2022). Diatoms can be classified into two major groups based on valve symmetry, which are centric (radial symmetry) and pennate (bilateral symmetry) diatoms.

The frustule is the distinctive silica-based cell wall of diatoms, composed of two halves known as valves. The upper valve is called epitheca whereas the lower valve is called hypotheca (Figure 2.1). According to Rogato and De Tommasi, (2020), the diatom frustule is designed to be species-specific, allowing for identification based on its unique morphology. Each frustule is ornate and features numerous pores that useful in facilitating the exchange of gases, nutrients, and metabolic waste products.

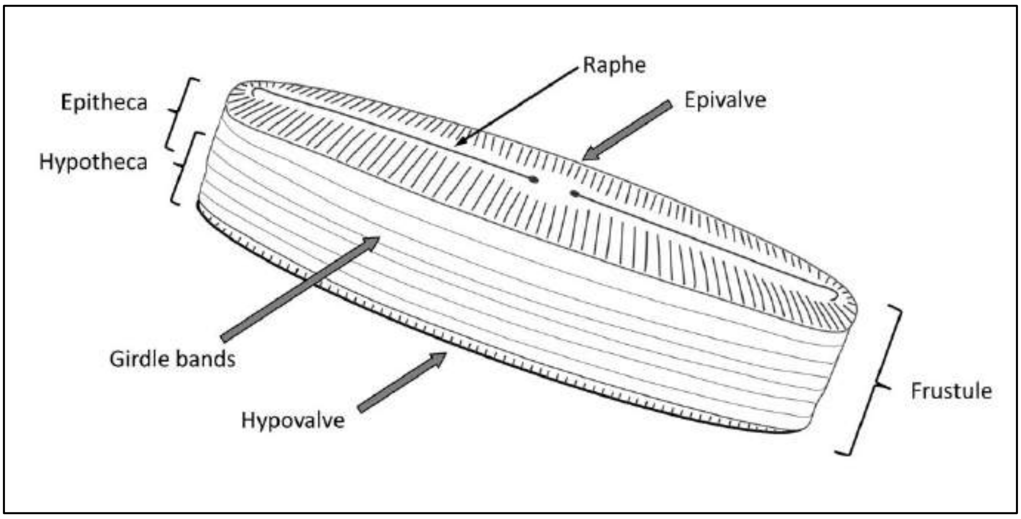

Figure 2.1: Structure of Diatom (Harbich, T., n.d.)

Diatoms extract silica from their environment to construct their frustules (Sun et al., 2023). The silica composition contributes to the durability of diatom frustules which mostly dissolve upon the death of the organism, some may be preserved under favourable conditions (Xu et al., 2021). Based on frustule shape, diatoms can be classified into two major categories namely the centric diatoms which is characterised by circular shapes and the pennate diatom has leaf-like forms and may vary in size from small to large. This classification highlights the morphological diversity within diatom species and their adaptation to different ecological niches.

It is important to understand the morphology of diatoms in order to discover their applications in both forensic and ecological fields. As primary producers in aquatic environments, they contribute significantly to global oxygen production and serve as indicators of environmental health. In forensic science, their presence in biological tissues aids in determining the circumstances surrounding the drowning incidents.

2.1.1 Centric Diatom

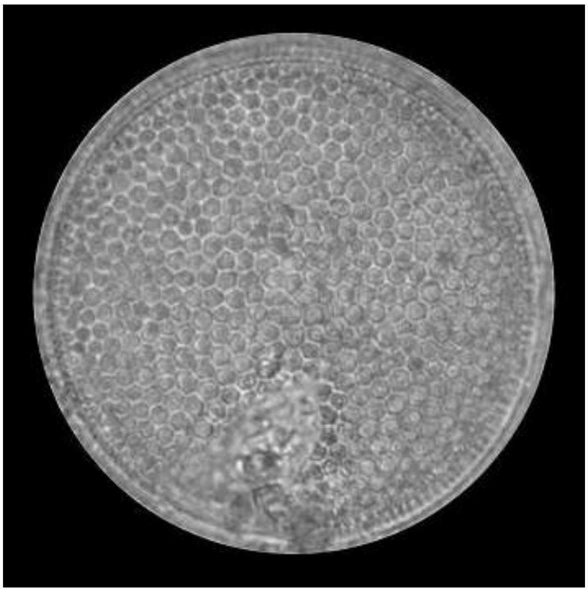

Figure 2.2: Image of Coscinodiscus, 1840 - A centric diatom (ucsd.edu., 2002)

Coscinodiscophyceae also known as centric diatoms are characterised by their radial symmetry, where they have multiple mirror images when divided along any diameter (Figure 2.2). This symmetry gives them a circular or disc-like appearance. To explain centric diatom, there are some features that must be considered such as structure, habitat, and reproduction.

For structure, centric diatom has a frustule (cell wall) that is composed of two valves which are the upper valve (epitheca) and the lower valve (hypotheca). These valves fit together snugly, allowing for protection and structural integrity. The intricate designs of these frustules often include various patterns and pores that facilitate nutrient exchange and gas diffusion (Reid et al., 2021).

As for habitat, centric diatoms are primarily planktonic, meaning that they float freely in the water column. They are commonly found in nutrient-rich environments, where they contribute significantly to primary production and serve as a food source for various aquatic organisms.

Last but not least, the reproduction of centric diatoms is mainly through vegetative division, where the cell divides into two daughter cells. This process leads to a decrease in size over successive generations, necessitating sexual reproduction to restore the original size (Jewson, 1992).

2.1.2 Pennate Diatom

Figure 2.3: Image of Naviculaceae, 2022 - A pennate diatom (Whitson, 2024)

In contrast to centric diatoms, fragilariophyceae, pennate diatoms exhibit bilateral symmetry, possessing a single plane of symmetry (Figure 2.3). This group can be further divided into two subcategories based on the presence or absence of a raphe, which is a slit-like structure that aids in movement:

1. Raphid Diatoms: Diatom with one or two lateral raphes along the sternum of the cell. The presence of a raphe allows these diatoms to move by gliding along surfaces, which is facilitated by the secretion of mucilage.

2. Araphid Diatoms: Lack raphes and do not exhibit movement. They typically rely on passive dispersal through water currents or sedimentation.

Similar to centric diatoms, pennate diatoms also possess frustules made of silica. However, their frustules are generally elongated or leaf-like in shape, adapting them to their benthic (bottom-dwelling) habitats (Phycokey, n.d.). Pennate diatoms can be found in both planktonic and benthic, often attached to substrates such as rocks or sediments. Their ability to inhabit various environments makes them versatile indicators of ecological health.

2.2 Forensic Diatomology

Forensic diatomology is a branch of the forensic field focuses mainly on examining and interpreting diatoms in various types of evidence including internal organs, lung tissues, and bone marrow, usually in water-related cases such as drowning or recovered body from aquatic environments. In these situations, the diatoms may get inhaled before or during water immersion by the person. The diatoms existence and distribution throughout the body tissues can provide a high value of information in order to determine the causes of the death as well as the location of immersion of the individual.

Diatoms were first observed in 1777, but their existence was formally recognised and described in 1783 by a French Naturalist, Jean Baptiste Lamarck. Subsequently, in 1896, a renowned professor of Forensic Medicine in Vienna, Hofman was the first person to detect and identify the diatoms in pulmonary fluid. He elaborated on the significance and relevance of the diatoms in asphyxia-related drowning deaths (Porawski, 1966). In the early nineteenth century, the initial research concerning about the identification and analysis of diatoms in forensic investigations was began (Timperman 1972, Karkola and Neittaanmäki 1981, Pollanen 1998, Andresen and Edlund 2001, Hu et al., 2013, & Marshall et al., 2023), In 1913, Rudolf von Hösslin, an Austrian scientist proposed the application of diatoms in the forensic field.

The methodology and technique for diatom analysis have been improved throughout the mid-twentieth century (Uitdehaag et al., 2010 & Scott et al., 2014). Usually, in post-mortem drowning investigations, if the person died after immersion, there will be present of diatoms inside the body. Hence, to extract and analyse the diatoms from different types of biological samples, such as lung tissues (Horton, 2007), bone marrow (Lunetta 2016 and Levkov et al., 2017), and from clothing (Zhou et al., 2020), scientists have developed few extraction and analysis techniques such as chemical digestion, filtration, and microscopic examination. Since the 1970s, the acknowledgement of forensic diatomology has been increased and become a valuable tool for criminal investigations, especially in drowning cases.

The evolution of standardised protocols and guidelines for diatom analysis helped in establishing a legitimate forensic discipline where this standardisation has become the chosen method for investigating cases such as suspicious drownings (Zgłobicka et al., 2021, Wagner et al., 2022, & Arumugham et al., 2023). Through this improvement, the recent advances in DNA technology had an impact towards forensic diatomology (Tantanasarit et al., 2013 & Rai et al., 2022). By extracting the diatom DNA from samples, researchers can improve the accuracy and reliability of diatom analysis. Hence, it can help to provide information about the origin and source of diatoms, thereby identifying the specific water body where a victim may have been submerged.

According to Fields et al. (2014), researchers are exploring the advantages and importance of advanced imaging techniques including scanning electron microscopy to improve the identification and classification of diatoms. In addition, there is research that has been carried out to improve the understanding of ecology and the distribution of diatoms which contribute to a more accurate and reliable forensic finding.

2.2.1 Roles of Diatom in Forensic Investigation

The most common application of diatom can be seen in cases such as drowning. According to Stephenson and Byard (2023), drowning is a state of respiratory impairment and asphyxiation caused by submersion or immersion in a liquid. This condition arises when the airway becomes filled with water or any other fluid, preventing the normal exchange of oxygen and carbon dioxide in the lungs (Auer and Möttönen 1988, Leth and Madsen 2017, & Levkov et al., 2017). This oxygen deprivation ultimately leads to asphyxia, respiratory failure, and potentially cardiac arrest. The seriousness of this situation is highlighted by the substantial worldwide consequences, leading to approximately 320,000 deaths every year making it the third most common cause of death, thus increasing the necessity of employing appropriate methodologies to conduct meticulous forensic investigations.

Diatoms, being ubiquitously present in almost all aquatic environments, play an important role in forensic diatomology. When an individual is submerged in a water body, the diatoms present in the surrounding environment can inadvertently be inhaled. These diatoms are then dispersed throughout various body tissues and fluids and even into the bone tissues through the bloodstream. In forensic investigations, detecting diatom frustules in bone marrow is considered one of the most reliable indicators of ante-mortem drowning. The identification of diatom species present in both the victim's tissues and water samples from potential drowning sites can corroborate evidence regarding whether drowning occurred at that location.

The drowning incidents are classified into primary drowning and secondary drowning. Primary drowning, also known as typical/wet drowning is further categorized into freshwater, where ventricular fibrillation is the mechanism of death and saltwater drowning, where pulmonary oedema leads to fatality (Taylor et al., 2007 & Levin et al., 2017). On the other hand, secondary drowning, also referred to as atypical drowning, refers to the death occurring within 30 min to several days after submersion and artificial respiration. In these cases, victims often succumb to conditions like pulmonary oedema, acidosis, or pneumonitis due to microbial infection. Secondary drowning is subdivided into dry drowning, where no water enters the gastrointestinal or respiratory tract, leading to mechanisms of death such as cardiac arrest, vagal reflex, and circulatory collapse.

Diatom analysis forms a keystone for distinguishing primary and secondary drowning cases. In primary drowning, diatoms are typically detected in significant quantities within the respiratory system due to water inhalation and the subsequent movement of water and diatoms into the lungs which can be identified through specialized microscopic techniques. Conversely, in secondary drowning, the lack of significant diatoms in the lungs aligns with the absence of actual water intake and also coincides with the cessation of blood circulation due to an individual's pre-existing deceased state.

Drowning holds significant medico-legal implications, particularly in homicide cases. When a person is found dead due to drowning, it's essential to carefully investigate the cause and manner of death. The forensic pathologist's primary task is to establish that drowning was indeed the cause of death while ruling out alternative causes.

In cases of suspected homicide, scene investigation is critical, as it can yield evidence such as signs of struggle, witness statements, and the absence of reasonable explanations for the victim's presence in the water. Autopsy findings play a pivotal role in determining whether trauma, injury, or pre-existing medical conditions may suggest foul play. Toxicology tests are conducted to detect the presence of drugs or alcohol that could have impaired the victim's judgment. Moreover, physical injuries indicative of a struggle or resistance may raise suspicion of a homicidal drowning.

Expert testimony, collaboration among forensic specialists, and meticulous evidence collection are key in determining the true nature of the drowning incident and its potential criminal aspect, aiding the justice system in holding individuals accountable when warranted. Careful examination of diatom concentrations and distribution patterns in various tissues and fluids can thus yield essential information to distinguish between these two categories of drowning incidents. While investigating drowning cases, many postmortem methods like CT scanning, MRI scanning, and biomarker analysis are useful for gathering evidence and make conclusions about the cause of death (Ludes et al., 1994, Yange et al., 1999, Singh et al., 2006, & Wagner et al., 2022).

By examining the diatom evidence present in different body tissues or fluids, forensic investigators can determine whether the person was alive or dead at the time of water immersion and potentially identify the location of immersion.

2.3 Ecological Diatomology

Diatoms play an important role in aquatic ecosystems as they serve as primary producers which form the base of the food chain for many microorganisms. The ability of diatom undergoes photosynthesis contributes significantly to oxygen level in the atmosphere. Thus, making the diatoms crucial for maintaining ecological balance. The differences in factors of aquatic environment such as light conditions and nutrient availability will distribute the diatom communities become vary in species composition and abundance. In addition, diatoms can also act as environmental indicators as their population numbers can reflect the shifting of water quality.

The ecological diatomology is an important field that utilises diatoms to act as bioindicators to the ecosystem health such water quality and environmental changes. Through many researches about diatoms, scientist could use the advancements in methodology and technique to continually discover the importance of diatoms that can significantly tell the quality of fresh water and marine environment. By examining and analysing the diatoms communities in water environment, scientists can also assess the impacts of human activities towards the aquatic ecosystems. This will provide a crucial information about the quality level of the water which is can be useful for further conservational and management effort which aim to prevent and preserve the aquatic ecosystems.

2.3.1 Diatom as Water Quality and Toxicological Assessment.

According to a study conducted by Sophia Barinova and Karomat Mamanazarova (2021), diatoms can be very useful in ecological monitoring, especially in water quality assessment. The presence of diatom communities indicates that the water is less exposed to pollution. This is because when the water is less polluted, there is a high number of dissolved oxygen that is required by the diatoms to survive. Hence, their sensitivity to environmental changes makes them effective and reliable bioindicators for water quality assessment.

There are few indices have been developed to assess the water quality based on the composition and distribution of diatom communities. For example, the Specific Pollution Sensitivity Index (SPI) which serve as one of a metrics that assigns the scores to diatom taxa according to their sensitivity towards pollution (Blanco, 2024). This index has been successfully and widely applied in many studies to correspond the diatom communities with limnological variables such as phosphates and nitrites, strengthen its reliability as bioindicators. Besides, diatom-based indices also can provide insights into the health of aquatic ecosystems (Venkatachalapathy and Karthikeyan, 2015). The shifts in diatom communities indicate that there are environmental changes occurred such as the introduction of pollutants into water body (Shibabaw et al., 2021). By analyzing the diatom communities, it can help to conclude the ecological health of aquatic systems and identify potential toxicological risks.

Not only diatom is useful in water quality assessment, but it is also highly recognized as an indicator for toxicology especially in aquatic ecosystems. Due to the unique biological and morphological characteristic of diatoms, they have high value in detecting the presence of toxic substances in the water body. Therefore, the diatoms are crucial in toxicological assessments for biomonitoring since they have high sensitivity towards pollutants.

According to Saxena et al. (2020), diatoms respond rapidly to various pollutants, such as heavy metals, pesticides, and organic contaminants. This responsiveness of diatoms allows them for early detection of toxic conditions. Besides, the sensitivity of diatoms is vary based on different type of pollution which enables them to serve as early warning indicators for the presence of toxic substances in the water. Diatoms are more responsive to metal pollution than other organisms which they could explain the gradation in metal composition allowing an early identification for the presence of any heavy metal pollution in an aquatic ecosystem (Saxena et al., 2020).

The advancements in molecular techniques have enhanced the capacity to detect diatom species that potentially harmful. For example, according to (Dhar et al., 2015), researchers have developed species-specific oligonucleotide probe that can be used to identify diatom species known as *Amphora coffeaeformis* that associated with the production of domoic acid, a neurotoxin that could risks marine life and human health. This method eventually offers a reliable and sensitive approach that is not only will improve the detection capabilities but also facilitate the monitoring of different diatom species that may serve as indicator for water quality assessment.

In the nutshell, diatoms are valuable bioindicators that help in water quality and toxicological assessment in water ecosystems. The sensitivity of diatoms toward the changes in their surrounding with the help of various indices and molecular detection techniques making them invaluable indicators for environmental monitoring (Blanco, 2024). Due to introduction of new contaminants into aquatic environments by industrialization, deepen the knowledge in diatom will be important for understanding the health of aquatic ecosystem (Kim et al., 2024). Therefore, future study should be more focus on standardizing the methodologies for diatom analysis in water quality and toxicological assessments because it will improve the effectiveness of diatom in assessing aquatic ecosystems health.

2.4 Chemical Digestion for Diatom Detection

The most common chemical solution used in the acid digestion method is the strong acid. This method is used to extract the diatom from the water sample by eliminating organic matter in the water, preserving only the silica-based frustules (Caeiro, 2021). This to ensure that the diatom can be observed more clearly during microscopic examination. There are several steps in acid digestion including the digestion of organic materials, centrifugation, and followed by microscopic observation of diatoms.

Two common acids regularly used were known as nitric acid and sulfuric acid. Both acids function well in eliminating the other organic components in the water samples to ensure a clearer profile of diatom was observed under the light microscope. According to several studies, the acid digestion method has high reliability in isolating diatoms especially to solve cases involving drowning (Caeiro, 2021).

The acid digestion method has high efficiency in extracting diatoms from water samples which is very helpful for diatom preservation for more accurate identification and observation. Besides, this method also is applicable to many sample types such as biological tissues and environmental matrices. Hence, it is frequently used in forensic investigations. However, there are still issues and limitation regarding of this method. For instance, even the diatom has a silica-based frustules, but it can be destroyed due to prolonged acid exposure or excessive heat. This will lead to false-negative results during diatom analysis. Besides, the leftover chemical could seriously pollute the ecosystem if not discharge appropriately (Marezza et al., 2021). In addition, the repeated high-rpm centrifugations could lead to contamination and loss of diatoms in the samples (Bhardwaj et al., 2021).

2.5 Water Bodies

Water body can be referred as to any accumulation of water on the Earth's surface which encompasses a wide range of water forms including oceans, seas, lakes, and lakes. Water body is mostly natural, but some are created by human activities and can be vary in sizes and characteristics. Some water bodies are navigable and are referred to as waterways. For examples are rivers and canals. In addition, water bodies could provide various vital functions towards human activities such as fishing, commerce and transportation, and provide water for hydropower, irrigation, and recreational purposes (Bindu and Mohamed, 2016).

2.5.1 Lake: Water Reservoir and Recreational Lake

A lake is defined as a relatively large body of water that is surrounded by land and occupies an inland basin. Unlike rivers, which are flowing bodies of water, lakes are characterized by still or slow-moving water, classifying them as lentic ecosystems (Saros & Anderson, 2015). Lakes can be natural or anthropogenic (human-made) and vary significantly in size, depth, and nutrient content (DeNicola et al., 2004).

The distribution of diatoms is influenced by several factors, including water quality, depth, and nutrient levels (Laird et al., 2010). Understanding these relationships provides valuable insights into the ecological status of lakes, such as the lake water reservoir in palm oil plantations and recreational lakes (Saros & Anderson, 2015).

Water quality is one of the most critical factors affecting diatom communities. Diatoms are sensitive to changes in their environment, making them effective bioindicators (DeNicola et al., 2004). Variations in nutrient levels can significantly impact diatom assemblages. Eutrophic conditions, characterized by high nutrient availability, often lead to a dominance of species that thrive in such environments. This phenomenon highlights how nutrient enrichment can alter the community structure of diatoms within a lake (Reid et al., 2021; Yu et al., 2021).

Depth also plays a vital role in determining diatom distribution. Research indicates that benthic diatom communities vary with depth due to differences in light availability and substrate characteristics (Laird et al., 2010). Shallow littoral zones typically host different diatom species compared to deeper areas of a lake. This variation indicates the importance of physical characteristics in shaping diatom communities (Saros & Anderson, 2015).

In artificial reservoirs like those found in palm oil plantations, diatom communities can reflect the impacts of agricultural runoff (DeNicola et al., 2004). These environments may exhibit elevated nutrient levels, leading to shifts in species composition towards those tolerant of eutrophic conditions (Yu et al., 2021). In contrast, recreational lakes often experience fluctuating nutrient inputs due to human activities such as fishing. Diatom analysis in these lakes can reveal temporal changes in water quality, aiding in the management of their ecological health (Reid et al., 2021).

By studying diatom communities in the water reservoir and recreational lakes, it can give valuable insights into the ecological status and health of these aquatic systems. This understanding is important for effective management and conservation efforts that help to maintain the biodiversity and ecosystem functionality in freshwater habitats (Saxena et al., 2023; Yu et al., 2021).

2.5.2 River: Unpolluted Upstream River

A river is defined as a natural freshwater stream that flows in a channel towards another body of water, such as an ocean, lake, or another river. Rivers are essential components of the Earth's hydrological cycle and play a significant role in shaping landscapes and ecosystems (Maharjan et al., 2020; Reid et al., 2021).

In water, the distribution of diatoms is influenced by various factors such as physical and chemical properties of water (Bhardwaj et al., 2021). Understanding how water quality impacts diatom communities can provide further detail about the health of ecosystems, particularly in unpolluted environments such as upstream rivers. For instance, variations in nutrient levels, such as total nitrogen (TN) and total phosphorus (TP), can lead to shifts in diatom assemblages (Jüttner et al., 2020). In clean, unpolluted upstream rivers, diatom species that prefer low nutrient levels tend to dominate, reflecting the overall health of the ecosystem (Lobo et al., 2016).

The physical characteristics of water also play an important role in determining diatom distribution. Factors such as temperature, pH, and dissolved oxygen levels can influence diatom growth and community composition (Maharjan et al., 2020; Yu et al., 2021). Higher temperatures typically favor diatom growth during summer months, while cooler temperatures in winter can lead to decreased densities (Wu et al., 2020). Additionally, sufficient silica is essential for diatom development, and rivers with adequate silicate concentrations will support robust diatom populations (Reid et al., 2021).

In unpolluted upstream rivers, stable water quality parameters support diverse diatom communities. Studies have demonstrated that diatoms can serve as early indicators of ecological changes due to their rapid response to alterations in water quality (Jüttner et al., 2020). This responsiveness makes them particularly useful for monitoring environmental health over time (Lobo et al., 2016; Caeiro, 2021).

In summary, the distribution of diatoms is closely linked to water quality parameters such as nutrient levels, temperature, and silica availability (Bhardwaj et al., 2021; Yu et al., 2021). Unpolluted upstream rivers provide an ideal environment for sensitive diatom species that indicate healthy aquatic ecosystems. Monitoring these communities can offer valuable insights into the ecological status of freshwater habitats and help guide conservation efforts aimed at maintaining biodiversity and ecosystem functionality (Caeiro, 2021; Saxena et al., 2023). Understanding how diatoms respond to environmental changes not only enhances ecological knowledge but also supports sustainable practices in managing aquatic resources (Bhardwaj et al., 2021; Yu et al., 2021).

CHAPTER 3

METHODOLOGY

3.1 Apparatus and Materials

All chemicals, consumables and apparatus employed in this study are listed in Table 3.1.

Table 3.1: List of items used in this study.

Apparatus	Materials
Pipette	Water samples
Microcentrifuge tube (1.5 mL)	Distilled water
Light microscope	Concentrated Nitric acid, HNO_3
Falcon tube (15 mL)	Coverslips
Beaker (30 mL)	Microscope slide
Hot plate	
Smartphone camera	

3.2 Research Method

Details of the experimental steps that followed in this study are shown in Figure 3.1.

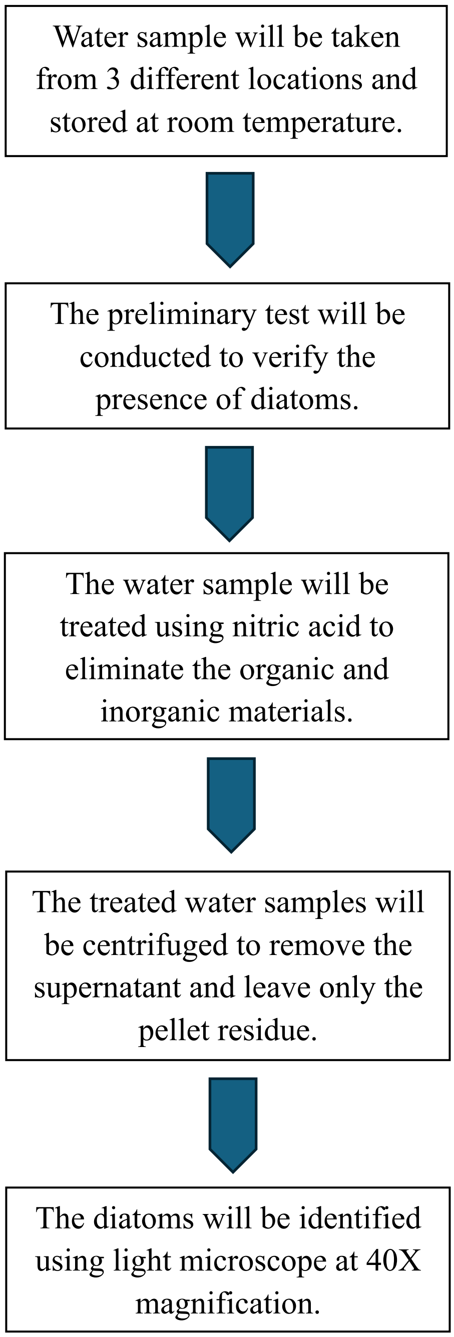

Figure 3.1: The flowchart of the study.

3.3 Sample Collection

A sampling of water samples was done in three different water sources located in the Telupid District, Sabah. All samples were stored in a mineral water bottle and tightly fitted to prevent leaking. An approximately 230 mL of water sample from each location was taken and named Water Source A (Figure 3.2), Water Source B (Figure 3.3), and Water Source C (Figure 3.4). Samples were kept at room temperature before being brought to USM Health Campus for further analysis

Figure 3.2: Map showing the location of water sampling for Sample A (**5.468354, 116.987468**).