

THE PRESENCE OF DIATOM FRUSTULES IN SELECTED RIVER AND SEA  
WATERS IN JOHOR

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To my beloved parents and sisters

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## ABSTRACT

Diatoms could be found in person whose death was caused by drowning whereby substantial amount of water was consumed into his body. In such instance, diatoms would usually be found in lungs as well as other internal organs. Presence of silica-cell wall makes diatoms resistant to enzymatic and acid digestion in human body, thereby of benefit to post-mortem analysis. Diatoms could be associated to dead body and to location of the drowning incident which assist in determining the cause of death. Hence, diatom is valuable in forensic research. The present study is a preliminary work to explore the morphologies of diatoms which is useful for forensic investigation. In this study, water samples from selected rivers and seas in the state of Johor, Malaysia were collected, preserved and kept under 4 °C. Acid digestion method was carried out to extract diatom cells and viewed under light microscope. The presence of diatom cells from both fresh and sea water were detected at the magnification of 400x and 1000x. Diatom frustules were observed and morphologies of these diatoms were examined. It was found that the diatoms of *Nitzschia* and *Navicula* were observed in most of the study regions. From their distinctive frustules appearances found in the water resources from Johor Bahru, Mersing and Pontian, the suggested diatom genera also included *Melosira*, *Skeletonema*, *Coscinodiscus*, *Thalassiosira*, *Gyrosigma*, *Cocconeis*. There were also several unidentified diatoms present that would need further studies. The present study has introduced a good exposure to diatom morphologies from selected water resources in supporting the use of diatoms in forensic aspect.

## ABSTRAK

Dalam kes di mana seseorang yang kematian disebabkan oleh lemas akan menelan jumlah air yang cukup banyak yang mengandung diatom. Oleh itu, diatom biasanya akan ditemui di dalam paru-paru dan organ-organ lain berkaitan. Disebabkan kehadiran dinding sel silika, diatom adalah tahan kepada pencernaan asid dan enzim di dalam badan manusia yang boleh dimanfaatkan untuk analisis post-mortem forensik. Kehadiran diatom boleh dikaitkan kepada mayat dan lokasi kejadian lemas berlaku. Ia juga akan menjadi bantuan dalam menentukan punca kematian. Penyelidikan ini merupakan satu kerja awal untuk mengkaji dan meneliti kegunaan diatom tersebut. Dalam kajian ini, sampel air dari sungai-sungai dan lautan terpilih di negeri Johor, Malaysia diambil dan dikawal pada suhu 4 °C. Pencernaan asid digunakan untuk mengekstrak sel-sel diatom sebelum dilihat di bawah mikroskop. Bentuk dan sifat sel-sel diatom serta genusnya dari kedua-dua air sungai dan air laut telah dicerap dengan pembesaran 400x dan 1000x. Dari bentuk frustules mereka, diatom genera *Nitzschia* dan *Navicula* dicerap di kebanyakan kawasan kajian. Dalam kajian sumber air dari Johor Bahru, Mersing dan Pontian, diatom genus telah dicadangkan, dengan genera yang dicerap adalah *Melosira*, *Skeletonema*, *Coscinodiscus*, *Thalassiosira*, *Gyrosigma*, *Coscinodiscus*. Terdapat juga beberapa diatom tidak khusus yang hadir yang memerlukan kajian lanjutan. Hasil kajian ini telah berjaya mengenalpasti genus dan genera diatom yang dicerap berdasarkan kawasan pensampelan terpilih dan telah menyediakan satu siri data awal diatom bagi kegunaan penyelidikan pada masa akan datang.

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## CHAPTER 1

### INTRODUCTION

#### 1.1 Background of Study

Diatoms are considered to have useful examining potential for forensic investigations, especially in drowning cases (Nopparut, 2011). With significant amount of diatoms present in the water, diatoms are able to first enter into lungs through inhalation. Along with the circulatory system in human body, diatoms will then be transferred into various parts of body (Kristic *et al.*, 2002).

In a drowning case the victim may consume a substantial amount of water, allowing the entrance and deposition of diatoms in lungs as well as other organs viz. bone marrow, spleen, kidney, liver and intestine via circulatory movement of blood (Kristic *et al.*, 2002); a characteristic that is diagnostic for pathologist in ascertaining the cause of death. The fact that the assemblages of diatoms may be specific to certain habitats, associating the location of drowning especially in cases whereby the body was believed to be dead in a different location from the location of recovery may be possible (Pollanen, 1997). It has been reported that diatoms form about 25% of plant mass in the world (Round *et al.*, 1990) and they are resistant towards enzymatic and acid digestion due to the presence of silica-cell wall (Nadia, 2012), providing suitability for forensic analysis. Despite its possible use in forensic investigation, diatom test has not been commonly utilized due to the controversy over the reliability of the diatom test (Law and Jayaprakash, 2007). Issues pertaining to diatom testing included population based routine food related diatom

ingestion requires to be estimated and also the possibility of entry of diatom frustules into the systemic circulation via the digestive tract (Law and Jayaprakash, 2007). In this context, the present study which was aimed at investigating the distribution of diatoms across the different natural aquatic habitats (estuary and sea) at several locations in Johor, acquires forensic significance.

## **1.2 Statement of Problem**

In Malaysia, diatom test is not widely used as one of the parameters in investigating deaths, particularly drowning cases attributable to the lack of expertise in interpreting the results as well as insufficient database. Therefore, this present study which has been specifically designed to examine diatom presence in several aquatic habitats within Johor for the first time, acquires forensic significance.

## **1.3 Objective**

This study was designed:

- To identify the different genera of diatoms in selected river and sea waters in Johor.

## **1.4 Scope of Study**

In the present study, diatoms samples were collected from different sampling locations within three main regions in Johor, Malaysia namely Johor Bahru, Mersing and Pontian, both the estuaries and sea regions. Parameters included in this study were the diatom morphologies observed under microscope with the magnification of 400X and 1000X and the suggestive diatom genera identified. The result obtained

was to give a clear picture of diatom distribution in the selected water resources and therefore enable further researches in respect of forensic investigation and biological study.

### **1.5 Significance of Study**

The data gathered in this study may prove useful in enabling forensic pathologist to make better conclusion on the possible cause of death especially in drowning cases as well as in ascertaining the possibility of post-mortem relocation of bodies based on the genera of diatoms.

## REFERENCES

- Alberto A., Luisa O., Domenico A., and Marina M. (2005). "Life Cycle, Size Reduction Patterns And Ultrastructure Of The Pennate Planktonic Diatom *Pseudo-Nitzschia Delicatissima* (Bacillariophyceae)" *Journal of Phycology*, 41, 542–556.
- Alessia G., Cinzia D., Daniele G., Cristina C. (2011). "Diatom Extraction With HCL From Animal Tissues: A Technical Note" *Legal Medicine*, 13, 268–271.
- Andrew J. A. (2007). "Strong Purifying Selection In The Silicon Transporters Of Marine And Freshwater Diatoms" *American Society of Limnology and Oceanography*, 52, 1420–1429.
- Araujo C. F. C. & Souza-Santos L.P. (2013). "Use Of The Microalgae *Thalassiosira Weissflogii* To Assess Water Toxicity In The Suape Industrial-Port Complex Of Pernambuco, Brazil" *Ecotoxicology and Environmental Safety*, 89, 212–221.
- Auer A. & Mottonen M. (1998). "Diatoms And Drowning" *Journal of Legal Medicine*, 101, 87-98.
- Azparren J. E., Fernandez-Rodriguez A., Vallejo G. (2003). "Diagnosing Death By Drowning In Fresh Water Using Blood Strontium As An Indicator" *Forensic Science International*, 137, 55–59.
- Bahls L. (2012). *Stauroneis*. In *Diatoms Of The United States*. Retrieved November 26, 2013, from <http://westerndiatoms.colorado.edu/taxa/genus/Stauroneis>.
- Bold H. C. & Wynne M. J. (1978). "*Introduction to the Algae: Structure and Reproduction*" Prentice-Hall, Inc. Englewood Cliffs, New Jersey.
- Chen X. G., Zhang J., Huang Y., Hou Y. P. (2013). "Diatom Taxa Identification Based On Single-Cell Isolation And rDNA Sequencing" *Forensic Science International: Genetics Supplement Series*.

- Edlund M. B., Taylor, C. M., Schelske, C. L. and Stoermer, E. F. (2000). "Thalassiosira Baltica (Bacillariophyta), A New Exotic Species In The Great Lakes" *Canadian Journal of Fisheries and Aquatic Sciences*, 57, 610-615.
- Eileen J. (2006). "Achnanthes Sensu Stricto Belongs With Genera Of The Mastogloiales Rather Than With Other Monoraphid Diatoms (Bacillariophyta)" *European Journal of Phycology*, 41, 67-81.
- Esra Z. (2007). "Planktonic Diatom (Bacillariophyta) Composition Of Lake Kaz (Pazar, Tokat) Turk" *Journal of Biology*, 31, 203-224.
- Esther G., Adriana Z., Marina M., Beatriz R., Barrie D. (Eds.) (2001) "Life Histories Of Microalgal Species Causing Harmful Blooms". Majorca: European Commission.
- Fareha H., Leaw C. P., Lim P. T. (2010). "Fine Structure Of The Diatoms Thalassiosira And Coscinodiscus (Bacillariophyceae): Light And Election Microscopy Observation" *Annals of Microscopy*, 10, 28-35.
- Fareha H., Leaw C. P., Lim P. T. (2011). "Morphological Observation Of Common Pennate Diatoms (Bacillariophyceae) From Sarawak Estuarine Waters" *Annals of Microscopy*, 11, 12-23.
- Furnas, M. J. (1990). "In Situ Growth Rates Of Marine Phytoplankton: Approaches To Measurement, Community And Species Growth Rates" *Journal of Plankton Research*, 12, 1117-1151.
- Grethe T. & Eeik E. S. (1996). Marine Diatom, Chapter 2. In editor Carmelo R. T. Identifying Marine Diatoms and Dinoflagellates (Pages 5-385). *Elsevier London*.
- Hicks Y. A., Marshall D., Rosin P. L. (2006). "A Model Of Diatom Shape And Texture For Analysis, Synthesis And Identification" *Machine Vision and Applications*, 17, 297-307.



- Hu S., Liu C., Wen J. Dai W., Wang S., Su H. F., Zhao J. (2013). "Detection Of Diatoms In Water And Tissues By Combination Of Microwave Digestion, Vacuum filtration And Scanning Electron Microscopy" *Forensic Science International*, 226, 48–51.
- Joshua G. S. & Patrick J. K. (2013). "Several New Species Of *Amphora* And *Halamphora* From The Western USA" *Diatom Research*, 28, 61-76.
- Kociolek, P. (2011). *Fallacia Pygmaea*. In Diatoms Of The United States. Retrieved January 08, 2014, from [http://westerndiatoms.colorado.edu/taxa/species/fallacia\\_pygmaea](http://westerndiatoms.colorado.edu/taxa/species/fallacia_pygmaea).
- Kociolek, P. (2011). *Nitzschia Reversa*. In Diatoms Of The United States. Retrieved January 08, 2014, from [http://westerndiatoms.colorado.edu/taxa/species/nitzschia\\_reversa](http://westerndiatoms.colorado.edu/taxa/species/nitzschia_reversa).
- Krstic S., Duma A., Janevska B., Levkov Z., Nikolova K., Noveska M. (2002). "Diatoms In Forensic Expertise Of Drowning – A Macedonian Experience" *Forensic Science International*, 127, 198-203.
- Law Y. Y. & Jayaprakash P.T. (2007). "Prevalence Of Diatom Frustules In Non-Vegetarian Foodstuffs And Its Implications In Interpreting Identification Of Diatom Frustules In Drowning Cases" *Forensic Science International*, 170, 1-7.
- Ludes B. & Coste M. (1999). "Diatom Analysis In Victim's Tissues As An Indicator Of The Site Of Drowning" *International Journal of Legal Medicine*, 112, 163–166.
- Maya H. D., Nitsan G., Daniela E., Yehouda M., Ansgar G., Heiko W., Peter G. K., Aaron K. (2013). "The Role Of C4 Metabolism In The Marine Diatom *Phaeodactylum Tricornutum*" *New Phytologist*, 197, 177–185.
- Michel H. A. P. & Els A. L. (2006). "Drowning: Still A Difficult Autopsy Diagnosis" *Forensic Science International*, 163, 1–9.

- Nadia F. (2012). "A New Procedure For Diatom Extraction In The Diagnosis Of Drowning" *Clinical and Experimental Pharmacology*, 2, 2161-1459.
- Nishitani Y., Fujii K., Okazaki S., Imabayashi K., Matsumoto H., (2005). "Weight Ratio Of The Lungs And Pleural Effusion To The Spleen In The Diagnosis Of Drowning" *Legal Medicine*, 8, 22–27.
- Nora G. M. (1998). "Use Of Epipelagic Diatoms For Evaluation Of Water Quality In The Matanza-Riachuelo (Argentina), A Pampean Plain River" *Water Research*, 32, 2029-2034.
- Nopparut S., Werawan R., Somsak V., Yuwadee P. (2011). "The Study Of Diatoms In Drowning Corpses" *Journal of the Microscopy Society of Thailand*, 4, 84-88.
- Philippe L., Arto M., Kristian S., Antti S. (2013). "False-Positive Diatom Test: A Real Challenge? A Post-Mortem Study Using Standardized Protocols" *Legal Medicine*, 15, 229-234.
- Pollanen M. S. (1998). *Forensic Diatomology And Drowning*, Elsevier Science, Amsterdam.
- Pollanen M. S. (1997). "The Diagnostic Value Of The Diatom Test For Drowning, II. Validity: Analysis Of Diatoms In Bone Marrow And Drowning Medium" *Journal Forensic Science*, 42, 286-290.
- Round F. E., Crawford R. M., Mann D. G. (1990). "The Diatoms: Biology & Morphology Of The Genera" *Cambridge University Press*, 27-28.
- Sarvesvaran R. D. M. J. (1992). "Review Drowning" *Malaysian Journal Pathology*, 14, 77 – 83.
- Spaulding, S., Edlund, M., Metzeltin, D. (2008). *Fallacia*. In *Diatoms Of The United States*. Retrieved November 26, 2013, from <http://westerndiatoms.colorado.edu/taxa/genus/Fallacia>.

- Spaulding, S., Edlund, M. (2008). *Cocconeis*. In *Diatoms Of The United States*. Retrieved November 26, 2013, from <http://westerndiatoms.colorado.edu/taxa/genus/Cocconeis>.
- Spaulding, S. (2011). *Gomphosphenia*. In *Diatoms Of The United States*. Retrieved November 27, 2013, from <http://westerndiatoms.colorado.edu/taxa/genus/gomphosphenia>.
- Spaulding, S., Edlund, M. (2009). *Gyrosigma*. In *Diatoms Of The United States*. Retrieved November 28, 2013, from <http://westerndiatoms.colorado.edu/taxa/genus/Gyrosigma>.
- Sterrenburg F.A.S., Tiffany M., Gordon, R., Nagy S. (2007). "Diatoms: Living In A Constructal Environment, In Seckbach J. (Ed.), *Algae And Cyanobacteria In Extreme Environments, Series: Cellular Origin, Life In Extreme Habitats And Astrobiology*", 11, 141-172.
- The Star. Be in the swim on drowning risks (April 28, 2013). Retrieve on 24 January, 2014 from <http://www.thestar.com.my/story.aspx/?file=%2f2013%2f4%2f28%2fnation%2f12847284>.
- Verma K. (2013). "Role of Diatoms in the World of Forensic Science" *Journal Forensic Research*, 4, 181.
- Wong S. L. and Hussain I. M. I. (Eds,) (2008). "A Study On Under Five Deaths In Malaysia In The Year 2006. Kuala Lumpur: *Clinical Research Centre*.
- World Health Organisation (2006). "*Guidelines For Safe Recreational Water Environments Volume 2: Swimming Pools And Similar Environments*". Switzerland: WHO.
- Wu J. T. (1999). "A Generic Index Of Diatom Assemblages As Bioindicator Of Pollution In The Keelung River Of Taiwan" *Hydrobiologia*, 397, 79–87.
- Xu G., Hu B., Shen R., Pan X., Zhou X. (2011). "Applications For Drowning Identification By Planktonic Diatom Test On Rats In Forensic Medicine" *Procedia Engineering*, 18, 417 – 421.