International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

A Study Investigating the Presence of Diatoms on Tooth Samples Using the Acid Digestion Method for the Estimation of Time Since Death in Drowning Cases

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Abstract

Teeth are a further provenance of diatoms in establishing the cause of death and time since death by analysis of the stage qualitative and quantitative evaluation of diatoms diversity. Trials also confirmed that diatoms have also been a useful tool in differentiating antemortem and postmortem drowning(Jian Zhao April 2014), This study mainly focuses on tooth immersed in a water sample from a known water body for 7 days to observe the variation and their stages of the life cycle. The method of extraction used is the acid digestion method using concentrated Sulphuric Acid. We could find the centric and pennate types of diatoms. Diatoms are also important as they indicate natural water quality(European Committee of Standardization 2004. According to the results, we cannot deny that teeth are a source for the extraction of diatoms and also an indicator of time since death when other methods fail.

Introduction

The definition of drowning given by the World Health Organization (WHO) is Drowning is the process of experiencing respiratory impairment from submersion/immersion in liquid. Drowning outcomes are classified as death, morbidity, and no morbidity (1). The diagnosis of drowning is recorded as the most challenging in Forensic Pathology (2-4). Over the years, several tests have been proposed for diagnosing drowning, but the diatom test is considered an important “biological marker” / “golden standard” for diagnosing drowning (5,6). Diatoms unicellular algae, eukaryotic microorganisms measuring from 5 microns -3 mm (Bate, 2004), belong to the class Bacillariophyceae which includes in excess of (15,000) species living in sea &fresh water. The skeleton of these algae is called a frustule having thecas which are the silica walls. Due to the hard silica skeleton, diatoms can be recovered from putrefied or injured tissues due to enzymatic or acid digestion (Ludes et all 1999) (7). The frustules comprise two halves called valves in which one half is smaller than the other. These fit perfectly together one inside the other. Depending on the shape and symmetry of the frustule, diatoms are classified as- Centrale (Biddulphiales) and Pennales (Bacillariales). Centrales are round and non-motile whereas Pennales are elongated which move with a gliding motion. (8)

Diatoms are considered as the biological marker for diagnosing drowning because when a victim is drowned, the diatoms which are already present in the waterbody enter into the human body further to the alveolar spaces in the lungs and penetrating the alveoli moving into the bloodstream and distributed to other organs and parts of the body such as bone marrow, brain, kidneys, and lungs. The presence of

diatoms in bone marrow is positive for antemortem drowning (Porawski 1996). The hard bones along with the soft tissues of the victim are sent for the detection of various species of diatoms. (8)

Objective

The objective of this study is to the estimate of time since death using tooth samples by the acid digestion method for the extraction of diatoms which is frequently used in the diatom tests for other bodily samples- sulphuric acid along the potassium dichromate (oxidizing agent).

Materials and methodology

Materials: 2 tooth samples, water samples, sulphuric acid, potassium dichromate, beaker, pipette, measuring cylinders, methylene blue, microscope.

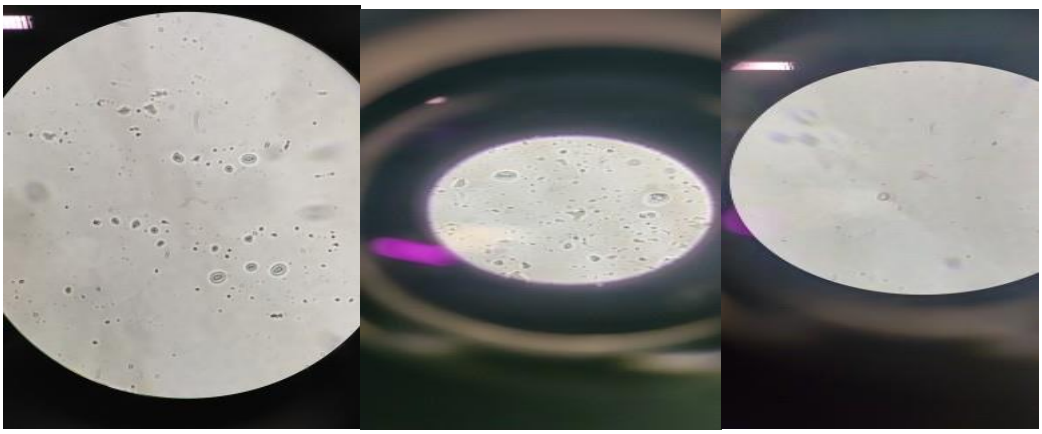
The water samples used for the project were collected from the Jumeirah Beach Residence, Dubai. Water samples were collected in washed and dried bottles to avoid contamination. In the laboratory, the diatom test was done to check for the presence of diatoms. The equipment used: beakers, pipettes, sulphuric acid solution, potassium dichromate, and measuring cylinders. 2ml of water sample was added to a beaker and concentrated sulphuric acid was added until the volume is twice that of the original sample followed by just enough potassium dichromate until it makes for a saturated solution. Let stand for 24 hours (10, 16-19). The samples are taken and slides are prepared. The sample is stained using methylene blue and observed for diatoms. One tooth sample is immersed in the water sample and placed in a beaker, concentrated sulphuric acid was added along with a smaller quantity of potassium dichromate and kept still for 24 hours. A drop of the sample along with methylene blue was placed on the glass slide and observed for the presence of diatoms.

The second water sample was kept for 7 days and the same preparation method was followed. The tooth sample was added and the preparation was repeated. A drop of the sample was placed on the glass slide along with methylene blue and checked for diatoms. Most of the pennate diatoms were skeletal structures as the lifespan of diatoms is 6 days. Multitudes of diatoms were seen in colonies

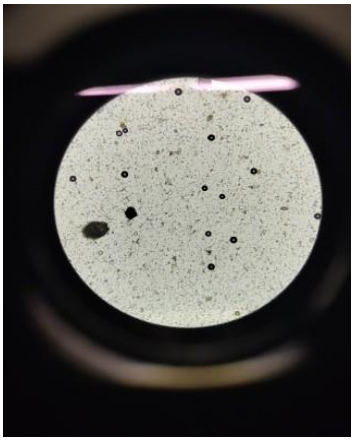
WATER SAMPLES

TEETH SAMPLES

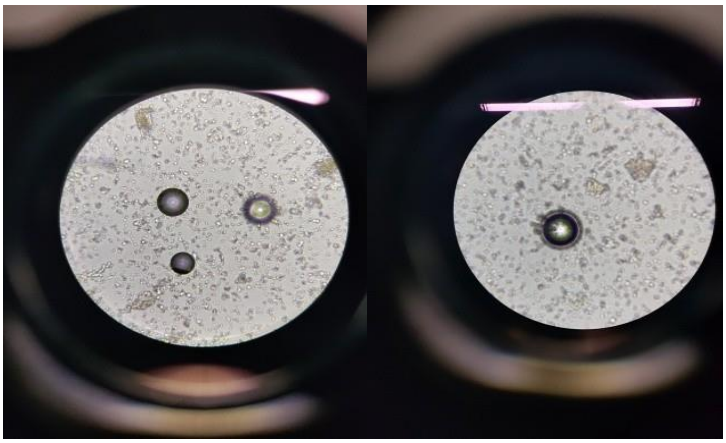
Result



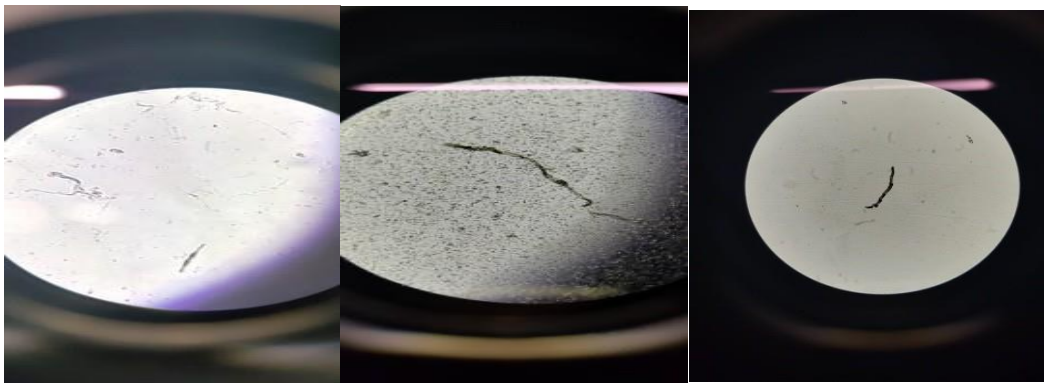
WATER SAMPLES OBSERVATION UNDER THE MICROSCOPE 40X



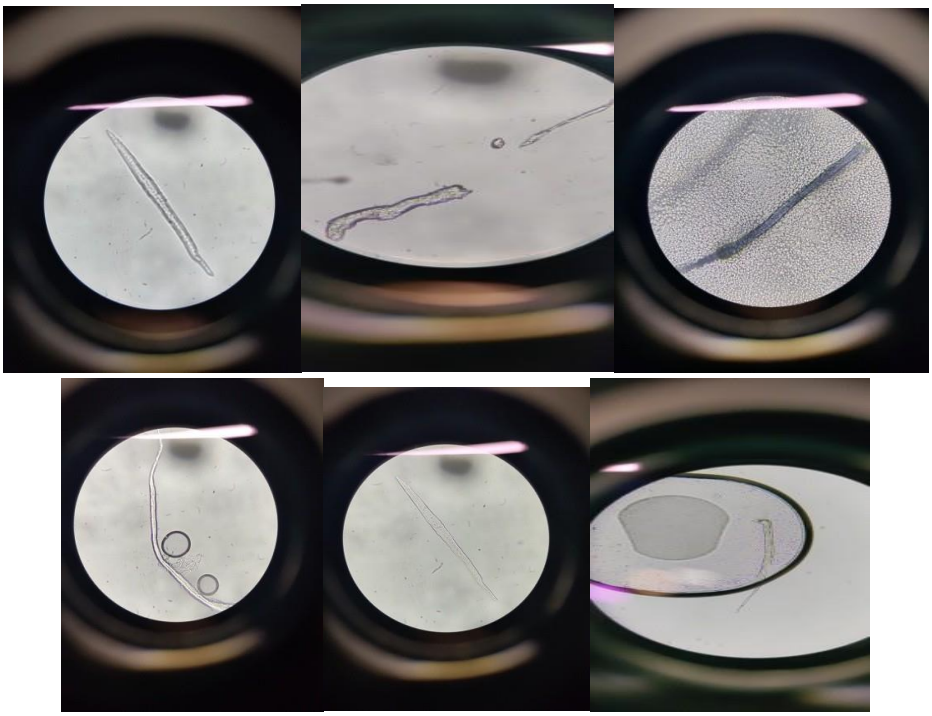
Before immersion of tooth sample (24hrs) 40X



After immersion of tooth sample (24 hours)- 100X



Before immersion of tooth sample (7 days)



Pennale diatoms are observed with just the silica skeletal remains after 7 days immersion in water

Conclusion

The morphological identification of diatoms is done from both of the tooth samples `and the water sample collected in the 1 and 7 days period showed similar diatoms. Water samples contained both centric and elongated types of diatoms.

In the 24-hour sample, we could observe the majority of the diatoms were the centric type of diatoms

In the 7 days sample, we observe that both the central and pennate diatoms are present. Only the skeletal structure remained. This is because the life span of diatoms is 6 days.

This study concludes with a positive result for tooth samples as that of previous studies regarding other bodily samples

Tooth samples can be used in the estimation of the cause along with the time estimation since the death in drowning cases.

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