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A SIMPLE COST EFFECTIVE METHOD FOR DIGESTION OF DIATOM FROM FRESH WATER.



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Abstract

Diatom is one of important flora present in water in perspective of forensic science to determine cause of death due to drowning. Diatom helps not only in determination of cause of death, but also in establishment of a relation between site of drowning and site of recovery of corpus. In present study, House hold bleach Rin Fabric Whitener was used to digest diatom which was found effective, easy, less time consuming, less chemically hazardous and inexpensive digestion method of diatom from fresh water in comparison of acid digestion method and other proposed digestion methods. The physical morphology of diatom remained intact after digestion. The proposed method was found capable for digestion and diatom was visible under compound microscope at 400X magnification.

Introduction

All living lifes depend on water directly or indirectly and diatoms are one of those. Diatoms are eukaryotic, unicellular, autotrophic microscopic algae, found in fresh and marine water. They have a unique cell wall, made up of hydrated silicon dioxide (Sio2) and transparent like glass called frustule, has different patterns and arrangement of pores, ribs, minute spine which are utilized to define genus and specie of diatom. It consists of 200 genera and over 10,000 species, out of which 92 genera and about 569 species have been reported in India1. They belong to class Bacillariophyceae, order Centrales, and pennales.

There are several macroscopic findings of drowning such as froth on the mouth and nostrils, lung emphysema, odema aquosum etc9. These findings could not persist till long due to the putrification process, which makes identification difficult, in contrast, diatoms resist putrification and alive till million and billions of years due to their hard siliceous cell wall which keeps them safe from environmental changes and putrification. Besides this diatoms are having unique characteristics which help in identification, thus diatoms are used as Forensic markers in death occur due to drowning. From a medico-legal perspective, drowning can be classified in two broad ways, Antemortem drowning and Post mortem drowning. These medico-legal enigmas can be solved and the cause of death can be determined by testing of diatoms from the corpus recovered. Another fact can be established that whether the site of drowning and site of recovery are similar or not. Diatoms can be extracted from organs are collected during the postmortem

and water samples from the recovery site of the dead body. The diatom digestion test is based on the recovery of the silica wall of diatom through removing organic matter.

Large numbers of methods such as digestion with Conc HNO3, Conc H2SO4 & hot H2O2 have been introduced, collectively known as Acid digestion method, is time-consuming, costly and unsafe while performing. Other method is bleach method, but commonly commercial bleach is used to digest diatoms. In commercial bleach Sodium hypochlorite present in high percentage, more than 5%, available in pure form, no other chemicals are present in commercial bleach and not easily available because of the percentage of sodium hypochlorite depends on purpose of use2. In this study the bleach which was used, is Household bleach, is used to remove stains and clothes whitening, is cost-effective, easily available in any grocery store, less time consuming and safe in comparison of other proposed methods.

In this study digestion of diatoms was performed by household bleach "Rin Fabric Whitener" earlier known as "Rin Ala" with the composition of sodium hypochlorite 4%, Sodium hydroxide 1%, Amine oxide 1%.



Figure 1: Composition of liquid bleach Rin Fabric Whitener

Material and method

In this study, fresh water sample was collected from Narmada River. Water sample was collected from surface of the bank of river in clean plastic bottle and preserved in refrigerator. A 1.5 ml microfuge tube was taken and marked properly. Collected water sample was transferred to clean glass beaker and allowed to settle organic matter. Then 100 µl of water sample was taken up from bottom of the beaker to insure aspiration of organic material along with water. Then the Tube was kept undisturbed for some time and removed most of the water from tube. Then 200 µl of liquid house hold bleach Rin Fabric Whitener was added and mixed gently with the help of pipette and after every 20-30 min gently mixed the sample. The

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sample was kept for 3 hrs for digestion (digestion hours can be adjusted according to organic matter present in the sample). After the digestion 1 ml of distilled water was added in the sample for washing and centrifuged the sample at 3000 rpm for 3 min, repeat this process atleast 3 times or till complete removal of bleach. After centrifugation decant the supernatant and the pellet will remain at the bottom of the tube. Then diluted the pellet and made cloudy solution and viewed at 400X magnification under compound microscope.

Result

The new proposed method was found effective on fresh water diatoms. Time taken for digestion was only 3-4 hrs. Heating not required during procedure; only gentle mixing by pipette or dropper in between digestion. The morphological integrity of diatoms was found intact with fewer impurities in background. Diatoms found in fresh water samples were given below:-



Figure 2:- (a) Amphora, (b) Planotidium, © Nitzschia



Figure 3:- Cymbella

Discussion

Diatoms were successfully digested from fresh water by the proposed method without any destruction of morphology of diatom under microscope (at 400X) with minimum amount of impurities. During the procedure, it was found that, this method requires less time, less no. of major equipments and easy for performing. Carr et al. reported cleaning of diatom by commercially available bleach (5.25% Sodium Hypochlorite) and in present proposed method house hold bleach Rin Fabric Whitener was used to digest diatom, which is more cheap, easily available and equally capable for digestion of diatoms .The main advantage of this method over conventional acid digestion method (references) is very less hazardous, while performing. The method was performed on fresh water sample of Narmada River, other water samples from lack, pond etc. need to be performed by this method.

Conclusion

Diatom plays vital role not even in environment but also in diagnosis of drowning. Their resistance power against environment makes them valuable. Most common digestion method, which is used, is strong acid digestion method, which is capable for digestion from organ and water sample, but not safe while performing and morphology of diatom gets affect, whereas the current study findings conclude that method is efficient, safe, cost effective for digestion of diatom from fresh water sample and visibly good under compound microscope.

characteristics-occurrence-and-reproduction.

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