

# Evaluation of the Effectiveness of an Alternate Diatom Extraction Procedure in Diatomological Study

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## Abstract

**Background:** The “Diatom Test” is based on the recovery of diatoms in high concentration in the organs of drowned persons because diatoms pass through the alveoli into the great circulation. Acid digested extracts of various internal organs and water collected from waterbodies demonstrate the presence of diatoms. If quantitatively and qualitatively these diatoms match to each other, that is the confirmatory diagnosis of death due to drowning in that drowning medium. This study was conducted to analyze the effectiveness of alternate method of diatom extraction using diluted Sulphuric acid solution and to compare its effectiveness with the traditional method of extraction of diatoms using concentrated nitric acid.

**Methods:** It was a prospective study. Samples were collected from ten different water bodies which included lakes, rivers, canals and reservoirs, for a period of one year in all the seasons. The pH and temperature of water were recorded during sampling. From each of water samples, diatoms were extracted using both classical and alternate method. And then compared by observing the diatoms at 100X magnification under compound microscope. Total 22 genera of diatoms were identified. Most of them were pennales and few were centrales. The time demanded for complete digestion of samples with strong acids like concentrated nitric acid was much lesser time than with diluted acids as it has stronger digestive capability in comparison to diluted sulphuric acid. The structures of different diatoms specifically Cyclotella, Cybella, Navicula were better found in almost intact state with clear background after digestion by diluted sulphuric acid.

**Conclusion:** Depending on different criterion, digestion by diluted sulphuric acid has procured better result than concentrated nitric acid digestion method. Therefore, for qualitative and quantitative diatom analysis alternate diatom extraction method is a better choice than traditional method.

**Key Words:** Diatom extraction, Drowning, Nitric acid, Diluted sulfuric acid

## Introduction

The diatom test for the diagnosis of drowning is based on the assumption that diatoms, which are eukaryotic unicellular algae, reach the lung with inhalation of liquid and if effective cardio-circulatory

activity exists, penetrate the pulmonary filter and disseminate to organs through the blood stream.<sup>1</sup> Conversely, if a corpse is submerged Postmortem, the diatoms may penetrate passively into the airways, but, owing to the lack of cardiac activity,

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will not be transported to other organs<sup>2</sup>. These diatom and algal communities can vary from one water body to another. Water bodies with similar chemical and physical compositions develop similar but not identical diatom community. Various genera and species of diatoms establish themselves within the specific water bodies based on their nutrient and light requirements and therefore they can differ from one water body to another both qualitatively and quantitatively with climatic or seasonal changes. Some local factors like mineral content of water, temperature, water stratification, acidity, the distance from shore, the depth of sea and the tide, etc. do affect the diatom concentration in any water bodies.<sup>3</sup>

Acid digested extracts of various internal organs and water collected from waterbodies demonstrate the presence of diatoms. If quantitatively and qualitatively these diatoms match to each other, then that is the confirmatory diagnosis of death due to drowning in that drowning medium.<sup>5</sup> In traditional method, this diatom test involves digestion of the tissues by strong acids: this treatment may destroy diatoms with the risk of a false negative test result.<sup>7</sup> On the other hand, the use of other methods such as enzymatic digestion with proteinase K, digestion by diluted acids are very uneconomical and not for the exhaustive extraction of some species of diatoms.<sup>8,9</sup>

As Malnad region of Karnataka has lot of freshwater resources which accounts to many deaths due to drowning, hence here an attempt was done to collect and analyze water samples for a period of one year from different freshwater bodies like rivers, lakes, dams, canals etc., from in and around Shimoga city for diatom distribution.<sup>4,6</sup> After that a comparative analysis was conducted between the traditional method and alternate method of diatom extraction to evaluate their effectiveness.

### Objective:

To compare the traditional method of diatom extraction using strong nitric acid at 90° C with alternate method of diatom extraction by addition of diluted 30% of H<sub>2</sub>SO<sub>4</sub> with a small amount of organic matter, maintaining overnight at room temperature.

### Material and Method

#### Type of the study:

It is a comparative analytical study

**Study period:** For a period of 1 years from March, 2014 to February 2015

#### Materials used for analysis:

1. **Water Sampling Jar of one litre capacity-** for collection of water sample from the water bodies.
2. **Laboratory Thermometer-** which has engraved graduations, with a range of from -5° C to +100°C with 0.5°C divisions.
3. **pH meter-** ESICO, Model 1012 microprocessor-based pH system
4. **Conc. Nitric Acid-** used for extraction of diatoms by chemical digestion method (classical method)
5. **30% Sulphuric acid-** used for extraction of diatoms by chemical digestion method (alternate procedure)
6. **Sterile conical measuring glass jar-** used to hold the water and acid mixture
7. **Glass pipette-** to transfer test solutions
8. **Sterile plastic centrifuge tubes-** used for centrifugation in centrifuge machine
9. **Centrifuge Machine:** Remi, laboratory medical centrifuge.
10. Glass microslides and coverslip
11. Compound Microscope

### Methodology

#### Collection of water sample:

Water samples were collected from different geographical localities of Malnad region. The water samples were collected from 10 different water bodies numbered S1- S10 (which include threelakes, two canals, two rivers, two dams and one domestic source) once in 2 months covering all the seasons.<sup>6</sup> Six collections were made during a span of one year. The water samples were collected just below the surface of water using sterilized plastic containers of one litre capacity. The pH and temperature of water were recorded during sampling.<sup>4</sup>

#### Extraction and Analysis of Diatoms:

Extraction of diatoms from water was done using chemical digestion by two methods concentrated nitric acid method and 30% Sulphuric acid method. From each of the water sample 200ml of water was

taken and divided into two equal parts of 100ml each and was transferred into an acid washed glass beaker. Samples were added with concentrated 25ml of nitric acid in one and 25 ml of 30% Sulphuric acid into the other and then samples were allowed to stand undisturbed for 2 h. These samples were transferred to properly label plastic centrifuge tubes and centrifuged at 3000 rpm for 10 min. The supernatant was pipetted out leaving behind only a residual material. This residual material was suspended in distilled water and centrifuged again in the same way to ensure that even the traces of acid were removed. After final centrifugation except for 1ml the whole supernatant was discarded by pipetting out. The left-over aspirate poured over a clean microscopic glass slide is dried, mounted with DPX and analyzed with optical compound microscope fitted with light source at different magnifications. Diatom species were identified on the basis of available literature.<sup>3,4</sup>

#### Diatom identification:

Both the methods were compared by identifying the diatoms separately at 100X magnification under compound microscope.

### Results

After analysis of the water samples, diatoms were observed in all the samples. Totally 22 genera of diatoms were identified by these two techniques. Each of these diatoms showed distinguished pattern of morphological features. The cell wall or frustule of diatoms were of either of two body plans pennales and centrales, and most of them belong to pennales and few were centrales. The intact diatoms identified in different water bodies are summarized employing

the two different extraction methods (table 1&2). Two different extraction methods were compared based on these four criteria:

1. **Time-consumption for complete digestion:**  
Strong acids like concentrated nitric acid have taken much lesser time than diluted acids for complete digestion of equal quantity of tissue.
2. **Digestive capability:**  
The degree of digestion for different tissues in the limited time (2 h) was also compared by the weight of the precipitate after digestion. Concentrated nitric acid had the strongest digestive capability in comparison to diluted 30% sulphuric acid.
3. **Destruction to diatoms:**  
The structures of different diatoms specifically Cyclotella, Cybella, Navicula were found inmostly in intact state with clear background and little impurity after digestion by diluted 30% sulphuric acid. However, they were destroyed to greater extent by traditional method of using strong acid. Few intact Cyclotella, Cybella and their fragments could be found in the residue, but no intact Navicula could be discovered after using traditional method of tissue digestion.
4. **Reclaiming ratio of diatoms:**  
Cyclotella, Cybella, Navicula were better found and remained almost intact with clear background in alternative method of digestion, thereby giving a better reclaiming ratio of diatoms in comparison to the traditional method of tissue digestion.

**Table 1: Identification of intact Diatoms in various Water Bodies using classical method**

Diatoms	S-1	S-2	S-3	S-4	S-5	S-6	S-7	S-8	S-9	S-10
Achnanthes	-	-	-	-	+	-	+	-	-	-
Amphora	-	-	-	+	-	+	-	-	-	-
Astrerionella	+	-	-	+	-	+	-	-	-	+
Brachysira	-	-	-	-	+	-	+	-	-	-
Colonies	-	+	-	-	-	-	-	-	+	-
Cyclotella	-	-	+	-	-	-	-	-	-	-
Cymbella	+	-	+	-	-	-	+	-	+	-
Cocconeis	+	-	-	-	-	-	-	+	-	-
Ctenophora	-	-	-	+	-	+	-	-	-	-

Continue.....

Diatoma	-	+	-	-	-	-	-	-	+	-
Eunotia Pectinalis	-	-	+	+	-	-	-	-	-	-
Fragilaria	-	-	-	-	+	-	+	-	-	-
Gomphonema	-	-	-	-	+	-	+	-	+	-
Melosira	-	-	-	-	+	-	+	-	-	-
Navicula	-	-	-	-	-	-	-	-	-	-
Nitzschia	-	-	-	-	-	-	-	+	-	-
Pinnularia	-	+	-	+	-	-	-	-	-	-
Pleurosira	-	-	-	-	-	-	-	+	-	-
Surirella	+	-	-	+	-	-	-	-	+	-
Synedra	+	+	-	+	+	+	-	+	+	-
Tabellaria	-	-	+	+	-	-	-	-	-	-
Talassiosira	-	+	-	-	-	-	-	+	-	-

**Table 2: Identification of intact Diatoms in various Water Bodies using alternate method**

Diatoms	S-1	S-2	S-3	S-4	S-5	S-6	S-7	S-8	S-9	S-10
Achnanthes	+	+	-	-	+	-	+	-	-	-
Amphora	-	-	-	+	-	+	-	-	-	-
Astrerionella	+	-	+	+	-	+	-	-	+	+
Brachysira	-	-	-	-	+	-	+	-	-	-
Colonies	-	+	-	-	-	-	-	-	+	-
Cyclotella	+	+	+	+	+	+	-	-	+	-
Cymbella	+	+	+	+	-	-	+	+	+	+
Cocconeis	+	-	-	-	-	-	-	+	-	-
Ctenophora	-	-	-	+	-	+	-	-	-	-
Diatoma	-	+	-	-	-	-	-	-	+	-
Eunotia Pectinalis	-	-	+	+	-	-	-	-	-	-
Fragilaria	-	-	-	-	+	+	+	-	-	-
Gomphonema	-	-	-	-	+	-	+	-	+	-
Melosira	-	-	-	-	+	-	+	-	-	-
Navicula	+	+	+	+	+	-	+	+	+	+
Nitzschia	-	-	-	-	-	-	-	+	-	-
Pinnularia	-	+	-	+	-	-	-	-	-	-
Pleurosira	-	-	-	-	-	-	-	+	-	-
Surirella	+	-	-	+	-	-	-	-	+	-
Synedra	+	+	+	+	+	+	+	+	+	-
Tabellaria	-	-	+	+	-	-	-	-	-	-
Talassiosira	-	+	-	-	-	-	-	+	-	-

### Discussion

For qualitative and quantitative diatom analysis as far as the applicability concerned, an ideal diatom test should have-

(1) Simple, safe and time saving digestive process

(2) Cheaper instruments and reagents required

(3) The damage due digestive reagents to diatoms should be mild with higher reclaiming ratio of intact diatoms.

(4) The organic residue should be minimal and hardly interfere with microscopic observation.

Therefore, Proper digestive method should be chosen to increase the sensitivity of the diatom test to its maximum value.<sup>10</sup>

Our study compared the effectiveness of traditional and alternative digestive technique for diatom extraction in respect to the time demanded by the methods, digestive capacity, damage to the tissues and reclaiming ratio of the diatoms. Morphological examination of Diatoms revealed 22 genera of diatoms in both digestion methods. Most of these identified diatoms were belonged to "Pennales" and few were of "Centrale" diatoms.

The identification of diatom shells in lung and other internal organs requires the complete destruction of the organ tissues to be examined except for the diatom frustules. The most common extraction technique consists of chemical digestion by nitric or sulfuric acid, solubilizers (e.g., soluene 350), or enzymes (e.g., proteinase K). Incineration has been used for fatty-rich samples; the resulting ashes being treated by oxidizing acid.<sup>7</sup>

Several procedures have been developed to replace the original acid digestion method for extracting frustules from human tissues. Most of the methods rely on alternative chemical or physiochemical means to solubilize the tissue. Although many methods have been suggested as alternatives to acid digestion, there is no evidence for improved yield of frustules as in acid digestion.<sup>9</sup>

During acid digestion the tissue sample is digested using strong acids where the tissues get completely digested leaving behind the diatom frustules which are made up of silica shells and resist acid digestion.<sup>10</sup>

The extraction of diatoms is usually done by classical method. The addition of diluted 30% Sulphuric acid showed better results than the classical method in respect to the reclaiming ratio of diatoms. The advantage over this technique proposed in the literature is that it is also possible to use a smaller amount of organ and smaller volume of already diluted acid without increasing sediment and avoiding the consequent difficulties observed in microscopic analysis.<sup>11</sup>

In a study conducted in Italy on ten dead bodies where the investigator tested minimal amount of

diluted Sulphuric acid solution to detect diatoms in tissues and the method was compared with traditional method of digestion with large amount of strong mixture of Sulphuric acid and nitric acid where diluted Sulphuric acid showed better results than the classical method with Sulphuric acid and nitric acid at high concentration.<sup>12</sup> Similarly in our study on water bodies also better results were observed using dilute Sulphuric acid rather than using concentrated nitric acid methods.

Regarding digestive capacity and the time required to digest same amount of tissue, traditional method was found to be more effective rather than the alternate method because of its aggressive nature. But the proposed alternate procedure is less chemically hazardous for the operator and the laboratory. It is also cheaper and yields more reliable results than those for the enzyme and the traditional methods.<sup>13</sup>

## Conclusion

The purpose of an extraction system is that it should not be too aggressive and should not destroy the siliceous material, but it will ensure the complete extraction of these algae from the human organs. Our study demonstrated that different diatoms have different resistance to different digestive reagents. Therefore, in respect to the reliability and applicability of the diatom test concerned, digestion by diluted sulphuric acid is of the better choice than concentrated nitric acid. The classic extraction causes aggressive digestion with lesser amount of intact demonstrable diatoms and higher presence of destroyed fragments. So, this alternate method can be the substitute of the traditional extraction method.

**Ethical Clearance:** Yes. (Ref. no: SIMS/IEC/88/13-14- By IEC, Shimoga Institute of Medical Sciences, Karnataka)

**Conflict of Interest:** None

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