CHAPTER I : INTRODUCTION

Diatoms are single celled algae that live in houses made of glass. They are the only organism on the planet with cell walls composed of transparent, opaline silica. Diatoms cell walls are ornamented by intricate and striking patterns of silica. Diatoms have light-absorbing molecules that collect energy from the sun and turn it into chemical energy through photosynthesis they occur either as solitary cells or in colonies, which can take the shape of ribbons, fans, zigzags, or stars. Individual cells range in size from 2 to200 micrometers. In the presence of adequate nutrients and sunlight ,an assemblage of living diatoms doubles approximately every 24 hours by asexual multiple fission; the maximum life span of individual cells is about six days. Diatoms have two distinct shapes: a few centric diatoms are radially symmetric, while most pennate diatoms are broadly bilaterally symmetric. Living diatoms makeup a significant portion of the Earth’s biomass.

The study of diatoms is a branch of phycology. Diatoms are classified as eukaryotes, organisms with a membrane bound cell nucleus, that seperates them from the prokaryotes archaea and bacteria. Diatoms are a type of plankton called phytoplankton, the most common of the plankton types. Diatoms also attached to benthic substrates, floating debris and on macrophytes. They comprise an integral component of the periphyton community. Another classification divides plankton into eight types based on size: in this scheme, diatoms are classed as microalgae. Several system for classifying the individual diatom species exists.

Diatoms are widely distributed and naturally abundant in a range of aquatic and terrestrial environments. Individual diatom species and population assemblages are diverse and environmentally specific due to their sensitivity to multiple variables including light, nutrient availability, pH and salinity. The hardened silica cell wall (SiO_2) is resistant to decay and retains diagnostic features enabling species identification and forensic comparison. Furthermore, the microscopic nature of diatoms increases their potential for use in a forensic capacity. It is highly unlikely that the transfer of diatom traces from a crime scene will be recognized by a perpetrator, enhancing the potential for diatoms to be recovered as evidence.

Diatoms have been established as reliable and naturally abundant environmental indicators in a broad range of applications including palaeoecological reconstruction water quality management and climate change research. The main application of diatoms in forensic science is currently pathological, assisting in the diagnosis of downing as a cause of death. Further research has been directed towards the use of algae and particularly diatoms in the estimation of the post mortem submersion interval (PMSI) of an item or cadaver recovered from water. Fossil diatom have also been recognized as important traces in soils and anthropogenic materials including paints, pesticides and safe ballasts.

Diatoms are used to monitor past and present environmental conditions and are commonly used in studies of water quality. Diatomaceous earth is a collection of diatom shells found in the earth's crust. They are soft, silica-containing sedimentary rocks which are easily crumbledintoa fine powder and typically have a particle size of 10 to 200m. Diatomaceos earth is used for a variety of purpose including for water filtration, as a mild abrasive, in cat litter, and as a dynamite stabilizer.[11]

The main goal of diatom analysis in forensics is to differentiate a death by submersion from a post-mortem immersion of a body in water. Laboratory tests may reveal the presence of diatoms in the body. Since the silica-based skeletons of diatoms do not readily decay, they can sometimes be detected even in heavily decomposed bodies. As they do not occur naturally in the body, if laboratory tests show diatoms in the corpse that are of the same species found in the water where the body was recovered, then it may be good evidence of drowning as the cause of death. The blend of diatom species found in a corpse may be the same or different from the surrounding water, indicating whether the victim drowned in the same site in which the body was found.

The centrifuge machine works on the principle of sedimentation principle, where the centrifugal acceleration causes denser substances and particles to move outward in the radial direction. At the same time, objects that are less dense are displaced and move to the centre. A centrifuge is a machine with a rapidly spinning container that uses centrifugal force to isolate fluids of different densities, like blood cells from plasma cells. The heat generated by the high-speed rotation of the centrifuge rotor could also degrade genomic DNA. It will also purifying chemical samples.

A compound microscope works on the principle that when a tiny object to be magnified is placed just beyond the focus of its objective lens, a virtual, inverted and highly magnified image of the object is formed at the least distance of distinct vision from the eye held close to the eyepiece. Compound microscope represent a significant step up in magnification, resolution and difficulty of use from the stereomicroscope. Polarized light microscopy provides both quantitative and qualitative information which is of value in observing, identifying and comparing microscopic particles, crystals and fibers.

The present study the aim is Extraction of diatoms from clothes. In past it has been found that there is only two study available related to Extraction of diatoms from clothes. In both the studies they use the cotton clothes for the extraction of diatom. But I done the extraction of diatoms in silk, jute, cotton and polyester clothes with the help of centrifuge machine and compound microscope.]12]

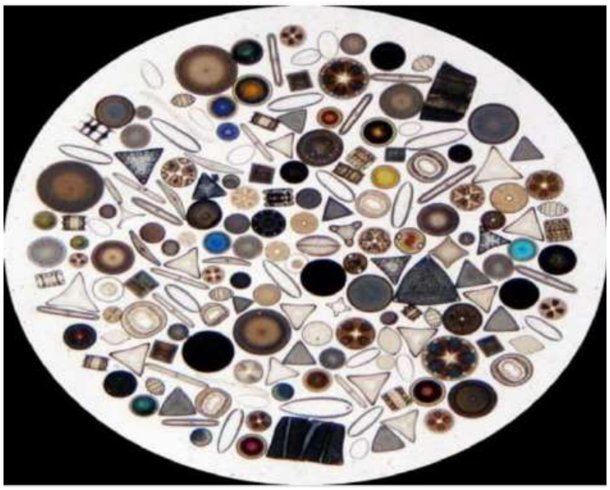

Figure 1: Diatoms

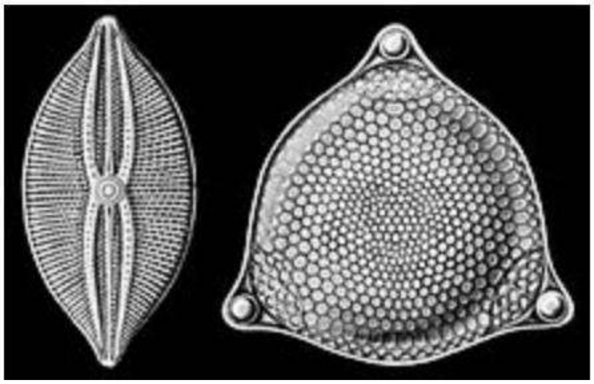

Figure 2: Types of diatoms

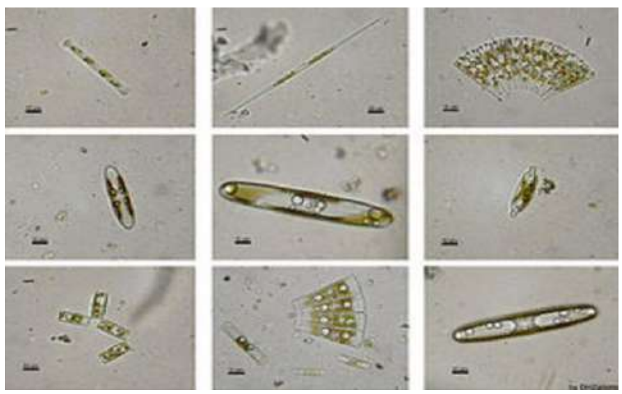

Figure 3: Light Microscope of several species of living freshwater Diatoms.

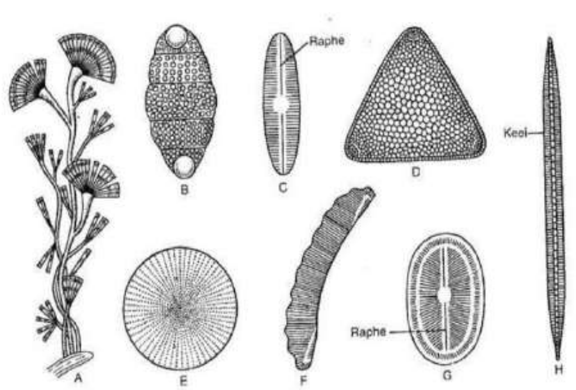

Figure 4: Different forms of Diatoms

CHAPTER II: LITERATURE REVIEW

Stefan Uitdehaag et.al (2010) studied about the extraction of diatom from clothing (cotton) for forensic comparison. Diatoms in clothing can be used to determine contact with surface water and contact with specific water source, which can help link suspects to crime scene. In this study they tested three methods for extracting diatoms from cotton clothing rinsing with water, rinsing with ethanol and dissolution of cotton with nitric acid and sulphuric acid. Therefore they present rinsing with ethanol as an effective extraction method for the quantitative and qualitative analysis of diatom in clothing.[1]

E.A Levin et.al (2017) studied the transfer of diatom from freshwater to foot wear materials. An experimental study assessing transfer, persistence and extraction methods for forensic reconstruction. A series of experiments were studied out to transfer and persistence of diatoms upon common footwear materials. They use two extraction techniques that is hydrogen peroxide digestion, for a suite of five common footwear materials: canvas, leather, suede, rubber and polyurethrene. These finding confirm that footwear can represent useful repository of diatom in case work scenario for forensic reconstruction.[2]

K.R Scott et.al (2019) studied freshwater diatom transfer to clothing spatial and temporal influence on trace evidence in forensic reconstruction. Diatoms are recovered using H2O2 extraction technique and examined microscopically. The result demonstrated that diatom transfer to clothing various significantly fewer diatoms were transferred to clothes varies significantly with a greater abundance and a higher species richness transfer to coarse woven surface including acrylic ,linen and viscos.[3]

Kapil Verma (2013) studied role of diatoms in the world of forensic science. This article reviews the forensic aspects of diatoms analyses and acid digestion method for diatom extraction. A body recovered from water does not necessarily imply that death was due to drowning. In drowning related death case, a correlation between the diatoms extracted from bone marrow and samples obtained from drowning medium have to be established for the successful determination of drowning site in forensic laboratories. Diatom analysis should be consider positive when number of diatoms is about a minimal established limit ; 20 diatoms of pellet obtained from 10gm of lung samples and 50 diatoms from bone marrow and drowning site can strengthen this supportive evidence and positive conclusion can be drawn whether a person was living or not when drowned.[4]

Bertranel Ludes et.al (1996) studied continuous river monitoring of diatom in the diagnosis of drowning. Diatom analysis has been proposed to provide supportive evidence of drowning but the reliability and applicability of quantitative and qualitative diatom analysis in the diagnosis of drowning is still disputed in the literature. The diatom profile drowning site was compared with the tissue analysis. The extraction of diatom from the tissue was performed with an enzymatic digestion methods using proteinase K.[5]

Federica Bortolotti et.al (2011) tested the specificity of the diatom test: search for false positives. The diatom test is widely used by forensic pathologists as proof of drowning. The aim of the present study was to verify the claimed inaccuracy of the method caused by an excessive rate of false- positives related to inadvertent exposure to diatoms of the general population. The extraction of the diatoms was performed by incubation of the samples in nitric acid for 48 hours at 60 degree Celsius. The diatom entering living bodies through the respiratory or digestive tracts via air, water, or food, supporting the validity of the diatom test as proof of drowning.[6]

Jian Zhao et.al (2016) studied the quantitative comparison analysis of diatom in the lung tissues and the drowning medium as an indicator of drowning. The presence of diatom in the lung tissues, internal organs and bone marrow is considered as the supportive evidence in the diagnosis of death by drowning. They made a comparison analysis between medium using the ratio of diatom numbers in both samples (L/D ratio), utilizing Microwave Digestion – Vacuum Filtration-Automated Scanning Electron Microscopy method. This quantitative diatom analysis in the lung tissues, provides supportive evidence in determining if a body recovered in water was due to drowning or not.[7]

Vandana Vinayak et.al (2019) studied diatoms in forensics: A molecular approach to diatom testing in forensic science. Diatoms are eukaryotic, unicellular, golden brown microalgae with more than 20,000 species in number and are most often encountered in naturally occurring bodies of water. The diatoms however have come as a rescue tool to differentiate ante-mortem and post- mortem drowning. Presently the complete genome of only 5 diatoms has been done, that is Phaeodactylum tricornutum (pennate species), Thallasiosira pseudonanna (centric species), Fragilariopsis cylindrus (pennate species), Pseudonitzschia multiseries (pennate species) and Cyclotella cryptica (centric species). There is a limited homology between human diatom DNA it is easier to rule out a case of ante- mortem or post- mortem drowning by using molecular tools.[8]

Kristie R Scott et.al (2017) studied the value of an empirical approach for the assessment of diatom as environmental trace evidence in Forensic Limnology. This paper outlines the current applications of limnology, particularly algae and diatom analysis, within forensic science and introduces new and ongoing research within the field. These studies demonstrate the analytical capability of the Scanning Electron Microscope (SEM) at various stages of investigation. The results highlight that the SEM provides a valuable tool during the initial stages of an investigation, determining the presence and abundance of a range of environmental indicators and directing further strategy for the more in depth collection and analysis of a forensic sample. [9]

Sunlin HU et.al (2013) studied the detection of diatoms in water and tissues by combination of microwave digestion, vacuum filtration and scanning electron microscopy. The detection of diatoms has been proposed to be useful in the diagnosis of drowning. In this presented paper, they describe a novel method for qualitative and quantitative analysis of diatoms in water and organs. The further scientific research in the field of automatic diatom identification using SEM images has to be done in order to automate the process of detection and identification of diatoms in water and tissues for the diagnosis of drowning. [10]

CHAPTER III : AIMS AND OBJECTIVES

AIM:

To identify the different diatoms present in various clothes like Jute, Silk, Cotton and Polyester**.**

OBJECTIVE:

- To study the various species of diatom in a particular water source.

CHAPTER IV: MATERIALS AND METHODOLOGY

Materials Required

Apparatus:

1. Conical flask
2. Beaker
3. Measuring jar
4. Gloves
5. Cotton
6. Micropipette
7. Centrifuge tube

Reagent:

1. Samples
2. Distilled water
3. Con Nitric acid

Instruments Required:

1. Smart R17 Plus Mhanil Centrifuge machine
2. Magnus MLX Plus Compound microscope

Figure 5: Centrifuge machine

Figure 6: Compound microscope

Method:

Four different cloth samples were collected and from 12 different location the water samples were collected from the Aditya college pond. For each cloth, three samples were taken. The clothes were dipped in the water sample. After sometime the water had been squeezed into the conical flask and add 50ml con nitric acid into it. After that put that samples for acid digestion about 48 hours. After 48 hours the water samples had been taken and with the help of micropipette it had been transfer into the centrifuge tube and put it for centrifugation about 15 minutes at 21^{o}C. After completing the centrifugation remove the supernatant from that sample and add same amount of distilled water into that sample and put it again for centrifugation. After that the samples had been transfer into the glass slide and observe under the microscope for diatoms. The photographs of the each diatoms was taken.

Figure 7: Cloth dipped in beaker

Figure 8: Sample is collected with the help of micropippete

Figure 9: Silk cloth

Figure 10: Jute cloth

Figure 11: Cotton cloth

Figure 12: Polyester cloth

Figure 13: Aditya college pond location

CHAPTER V : OBSERVATION

Silk Cloth samples

SL.NO	SAMPLES	TYPES OF DIATOMS
1	Silk sample 1	Pennate diatom
2	Silk sample 2	Centric & Pennate diatom
3	Silk sample 3	Centric diatom

Figure 14: Silk sample slide 1

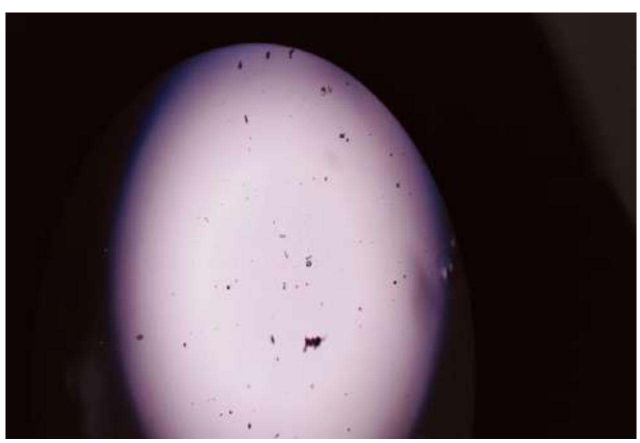

Figure 15: Pennate diatom

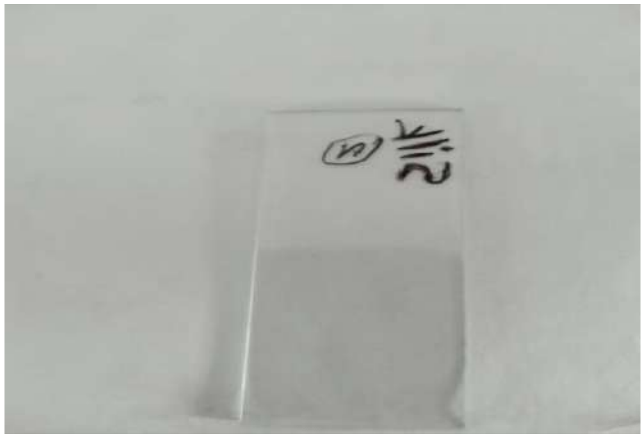

Figure 16: Silk sample slide 2

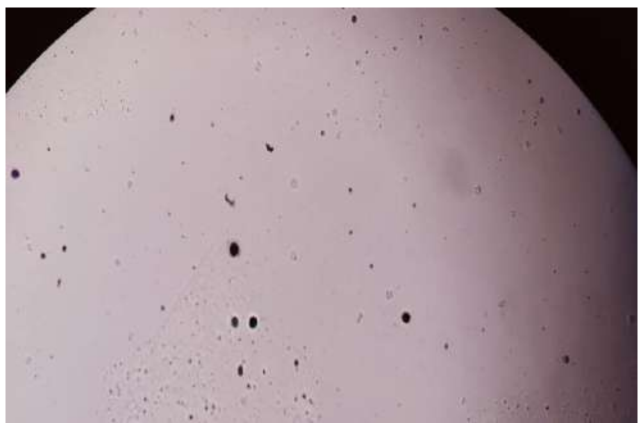

Figure 17: Centric and Pennate diatoms

Figure 18: Silk sample slide 3

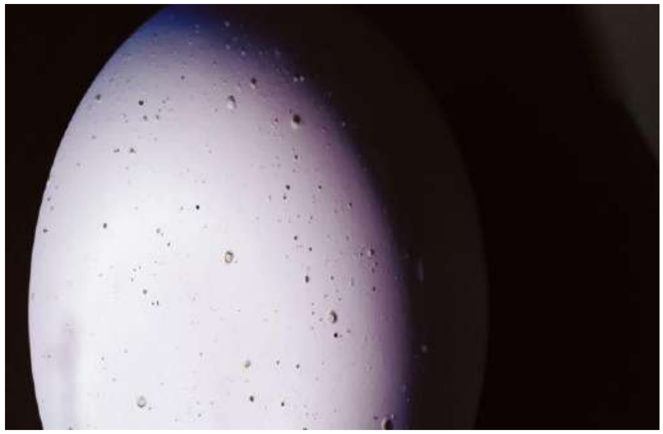

Figure 19: Centric diatoms

Jute Cloth Sample

SL.NO	SAMPLES	TYPES OF DIATOM
1.	Jute sample 1	Pennate and Centric Diatoms
2.	Jute sample 2	Pennate and Centric Diatoms
3.	Jute sample 3	Centric Diatoms

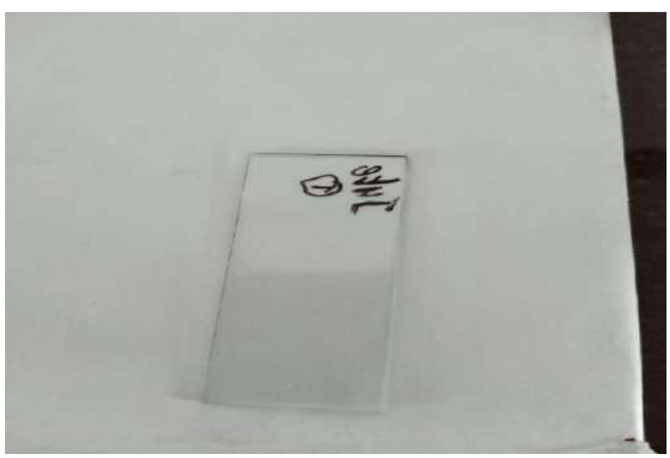

Figure20: Jute sample slide 1

Figure 21: Pennate and Centric diatoms

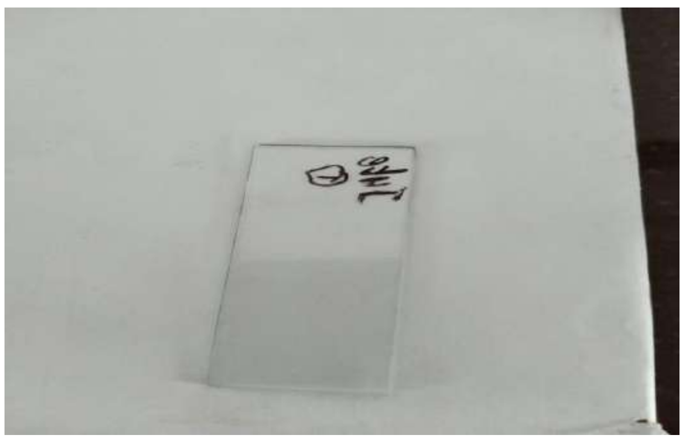

Figure 22: Jute sample slide 2

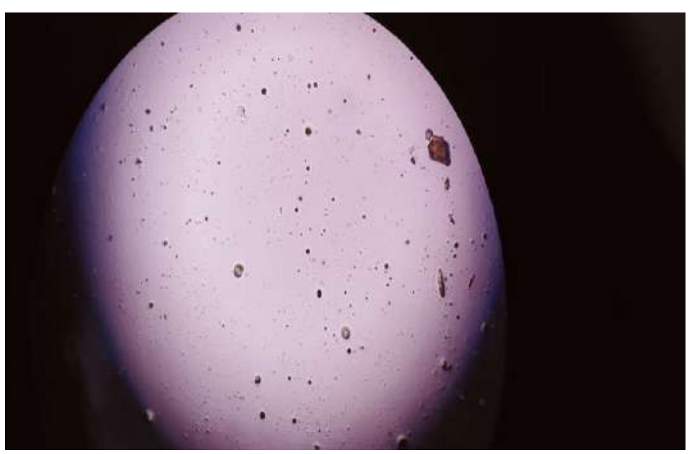

Figure 23: Pennate and Centric diatom

Figure 24: Jute sample slide 3

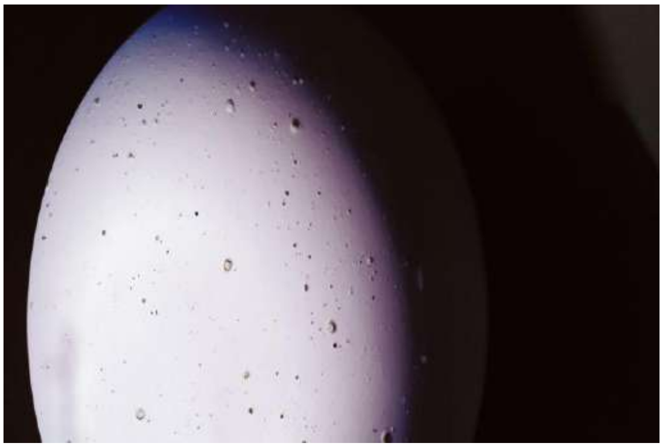

Figure 25: Centric diatom

Cotton Cloth Sample

SL.NO	SAMPLES	TYPES OF DIATOM
1.	Cotton Sample 1	Pennate and Centric Diatoms
2.	Cotton Sample 2	Pennate and Centric Diatoms
3.	Cotton Sample 3	Pennate and Centric Diatoms

Figure26: Cotton sample slide 1

Figure 27: Pennate and Centric diatoms

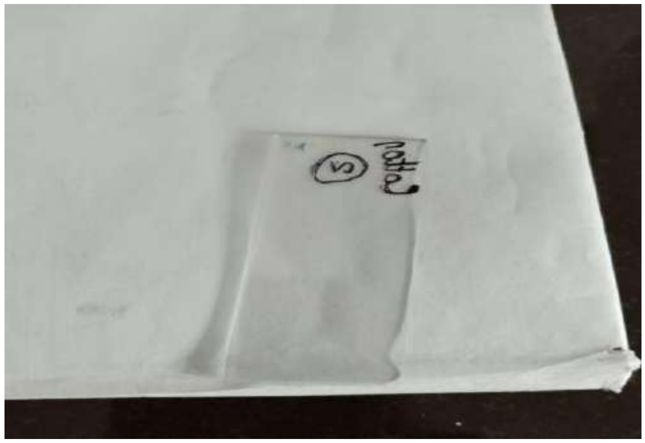

Figure 28: Cotton sample slide 2

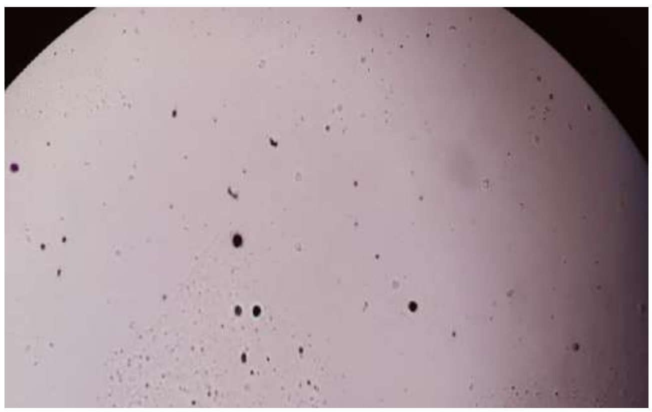

Figure 29: Pennate and Centric diatom

Figure 30: Cotton sample slide 3

Figure 31: Centric and Pennate diatom

Polyester Cloth Sample

SL.NO	SAMPLES	TYPES OF DIATOM
1.	Polyester sample 1	Absent
2.	Polyester sample 2	Absent
3.	Polyester sample 3	Absent

Figure 32: Polyester sample slide 1

Figure 33: Polyester sample slide 2

Figure 34: Polyester sample slide 3

CHAPTER VI: RESULT AND CONCLUSION

RESULT:

From the three Silk cloth samples both Pennate & Centric Diatoms are found. From the three Jute Cloth samples both Centric and Pennate Diatoms are found. From the three Cotton samples both Pennate and Centric Diatoms are found. From the three samples of polyester samples no Diatoms are found.

CONCLUSION:

The findings in this study indicate that the diatoms can be extracted from the clothes in which the clothes absorb the water content. As the cotton cloth will be having more water absorbances on that water sample the diatom is more followed by jute and silk samples. In the polyester cloth the water will not be absorb so by the analysis from that water sample no Diatoms are found.

This study provides an insight into the most effective methods for the collection of diatom particulates on a variety of substrates within criminal investigation.

In this study, the Diatoms are present in different cloth materials are found as Pennate and Centric. In future, study can be done in order to extract the species of Diatoms from other types of cloth materials such rayon, nylon, cotton silk, woolen ,etc..

CHAPTER VII: REFERENCES

1) Stefan Uitdebug et.al,Extraction of diatoms from (cotton)clothing forforensic comparisons,Forensic science international (2010) 112-116

2) A.J .Peabody, Forensic science and diatoms, in :E.F. Stoermer,J.P.Smol(Eds), the diatoms: applications for the environmental and earth sciences, Cambridge university press, Cambridge(1999) 413-418

3) K.R Scott et.al , Freshwater diatom transfer to clothing : spatial and temporal influences on trace evidencein forensic reconstructions , Science & Justice (2019) 292-305.

4) Kapil Verma, Role of diatoms in the world of Forensic science, Forensic Res (2013) 18-118.

5) Bertranel Ludes et.al Continuous river monitoring of diatom in the diagnosis of drowning,Forensic science (1996)425-428.

6) Federica Bortolotti et.al , The specificity of the diatom test:search for false positives,Medicine, Science and Law (2011) 7-10.

7) Jian Zhao et.al , The quantitative comparison analysis of diatoms in the lung tissues and the drowning medium asan indicator of drowning, Forensic and legal medicine (2016) 75-78.

8) Vandana Vinayak et.al Diatoms in forensics: A molecular approach to diatom testing in forensic science ,Diatoms: Fundamentals and applications (2019) 435-470.

9) Kristie R Scott et.al, The Value of an empirical approach for the assessment of diatoms as environmental trace evidence in forensic limnology, Archaeological and Environmental Forensic science (2017) 49-78.

10) Sunlin Hu et.al , Detection of diatoms in water and tissues by combination of microwave digestion , vacuum filtration and scanning electron microscopy , Forensic science international (2013) e48-e51.

11) https://en.m.wikipedia.org/wiki/Diatom

12) https://diatoms.org

13) https://study.com/academy /lesson/what-is -centrifugation-definition- process-uses.html

14) https://en.m.wikipedia.org/wiki/centrifuge

15) https://www.microscopeinternational.com