CHAPTER I

INTRODUCTION

Diatoms (dia-tom-os ‘cut in half’, from dia ‘through’ or ‘apart’; and the root of are a major group of Algae, specifically microalgae, found in the ocean, waterways and soils of the world. Living diatoms make a significant portion of the Earth’s biomass. The forensic significance of diatoms is it is used for the diagnosis of drowning deaths. Forensic Limnology is a sub-field of forensic botany, which examines the presence of diatoms in crime scene samples and victims. Different methods are used to collect this data but all identity the ratios of different diatom colonies present in samples and match those samples with locations at the crime scene. Fossil evidence suggests that diatoms originated during or before the early Jurassic period, which was about 150 to 200 million years ago.

Diatoms are almost ubiquitous. They live in aquatic environments, soils, ice, attached to trees or anywhere with humidity and their remains accumulates forming diatomite, a type of soft sedimentary rock. They are the dominant marine primary producers in the oceans and play a key role in the carbon cycle and in the removal of biogenic silica from surface waters. Diatoms are unicellular algae with golden-brown photosynthetic pigments. They have a siliceous skeleton known as frustules that comprise two valves, one overlaps the other like the two pieces of a petri dish. The frustule is ornamented with pores, processes, spines, hyaline areas and other features The size range is between 1 to 2000 μm in length.

They belong to the Division Chrysopthya, Class Bacillariophyceae and are divided in two Orders: The Centrales or Biddulphiales and the Pennales or Bacillariales. The Pennales or pennate diatoms have frustules that are elliptical or rectangular in valve view, with sculpture that is bilaterally symmetrical about a central line while the Centrales are characterized by frustules which are circular, triangular or quadrate in valve view and rectangular or ovate in girdle view. Diatoms could be single or could form colonies. The cell has two or more golden-brown

photosynthetic chloroplasts, a central vacuole, a large central diploid nucleus. Diatoms also store oils rather than starch and lacks of flagella or pseudopodia.

The first record of diatom frustules are centric forms from the Early Jurassic although very few remains are known before the Late Cretaceous, they were moderately affected by the massive extinction at the end of the Cretaceous. Living diatoms are very sensitive to parameters like salinity, oxygenation and other physical and chemical conditions, so they provide a valuable tool for studies of modern water quality and for the reconstruction of past environments, particularly for evidence of climatic cooling and changing sedimentation rates in the Arctic and Antarctic oceans. Also the evolutionary history of diatoms has been punctuated by several floristic turnovers which makes diatoms great tools for biostratigraphy correlations.

These specific qualities have meant that diatoms have been used in forensic science in a variety of ways, the most frequent being the diagnosis of death by drowning (Peabody & Cameron 2010). When a person drowns, water will enter the lungs and then the bloodstream through ruptures in the peripheral alveoli and be carried to the other organs, such as the liver and heart (Pollanen 1998). Naturally, the microscopic contents of the water, which will include diatoms, will pass into the blood. Detection of diatoms in various organs can contribute to the diagnosis of death by drowning, a process referred to as the ‘diatom test’. The use of diatoms (the ‘diatom test’) to diagnose a cause of death by drowning is often one of a number of independent techniques utilized by the Forensic Pathologist.

Fig.1.1: PENNATE DIATOM

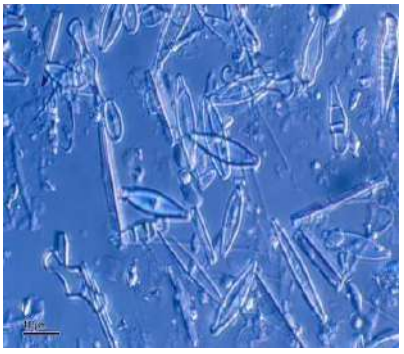

Fig.1.1: PENNATE DIATOM

CHAPTER II

LITERATURE REVIEW

2.1. G Sudhakar, B Jyothi, V Venkateswarlu (1994)

This study shows the role of diatoms as indicators of pollution gradients. In this water samples and algal samples were analysed at monthly intervals at three sites along the course of the river, along with raw effluents. The water before the confluence of effluents as well oxygenated with an alkaline pH. Diatoms constituted 61% by numerical abundance and were reduced to 25% of total algae at the entry of effluents. Mathematical equations were derived involving the physiochemical variables for better prediction of algal number.

2.2. Y Seeta, P Manikya Reddy (May 2015 to April 2017)

This study focus on the diversity of diatoms in the rivers of Telangana. Collection of algal samples from Krishna, Godavari and Manjira rivers covering a distance of 20 km has been carried out. Three sampling stations were selected along each river. Surface water and algal samples were collected seasonally. Samples were collected randomly from different spots. Diatoms maximum growth and development was observed during winter months and minimum during summer and rainy reasons. Among the diatoms the species of Cymbella showed rich diversity and variation.

2.3. Disha Rana, Deepika Bhandari (2016)

Diatoms plays an important role in Forensic Science and in the studies of water quality. Diatom acts as a supportive evidence for ascertaining the cause of death as well as the place of drowning. In present study 10 water bodies located in various part of Mumbai were sampled for study. Isolation of diatoms were carried out using acid digestion method. Total 35 species of diatoms were isolated and characterised. The information obtained helped to create a reference database on diatoms which can be utilized in future for ecological assessment and ready reference in drowning cases.

2.4 . Amarnath Dogiparti, Ravi Kumar Kurapati, Uday Ranjan T Joseph, Myla Chakravaravarthy (2013)

This study on distribution and diversity of phytoplankton in relation to physico-chemical parameters in Bhavanapadu Creek, Andhra Pradesh, India. A monthly sampling was carried out from December 2010 to November 2011 at 5 different stations. The four classes of phytoplankton comprise of 39 species. The phytoplankton population density and diversity depends upon the physicochemical parameters and showed significant correlation with the parameters like temperature, pH, ammonia, magnesium, phosphates and dissolved oxygen.

2.5 Jyothi Kaparapu, Mohan Narasimha Rao Geddadaa (2013)

The present study deals with seasonal variations, correlation coefficient and biodiversity indices of phytoplankton during April 2011 to March 2012 in the Riwada reservoir, Vishakhapatnam, Andhra Pradesh, India. Sampling was performed at five stations during pre-monsoon, monsoon and post monsoon. Maximum and minimum total phytoplankton population and percentages were recorded at station three in pre monsoon and at station two during monsoon. The diversity indices showed that the reservoir have a well-balanced phytoplankton community.

2.6 Andrew Bozarth, Uwe- G Maier, Stefan Zauner (2009)

This study focus on diatoms in biotechnology, modern tools and applications. Diatoms have played a decisive role in the ecosystem for millions of years as one of the foremost set of oxygen synthesizers on earth and as one of the most important sources of biomass in oceans. Efforts have been made to establish them as decisively useful in such commercial and industrial applications as carbon neutral synthesis of fuels, pharmaceuticals, health foods, bio molecules, bio remediates of contaminated water.

2.7 Kathleen M Ruhland, Andrew M Paterson, John P Smol (2015)

This study focus on lake diatom responses to warming, reviewing the evidence. Algae, the dominant primary producers in many aquatic ecosystems, are critical to

global biogeochemical cycling, and composition can cascade throughout aquatic food webs. Diatoms often dominate the algal communities in many fresh water systems. Study focus primarily on paleolimnological records. Using a weight of evidence approach, we conclude that recent climate change is main driver that has led to ecological tipping points resulting in the recent success of small planktonic diatoms that have been reported in many aquatic systems.

CHAPTER III

AIM AND OBJECTIVES

3.1 AIM

To identify the different diatoms, present in various water bodies of Kakinada region, Andhra Pradesh

3.2 Objectives

- To collect the water samples from various water bodies of Kakinada region.
- Isolation of Diatoms from collected samples and observation.
- To study the diversity of diatoms, present in various water bodies.

CHAPTER IV

MATERIALS AND METHODOLOGY

4.1 Apparatus:

Centrifuge, Beakers, Micropipette, Binocular Compound Microscope, etc.

4.2 Chemicals required

Nitric acid, Ethanol & Sulphuric acid.

4.3 Methodology

I have collected 40 water samples from four different water resources. From each water resource, I have collected 10 water samples. I took the water samples from VIVEKANADA PARK, BOAT CLUB, MADHAVAPATANAM & SARPAVARAM. I took the first sample from Vivekananda park, from that source I collected 10 water samples in different locations of ponds.

After sampling, 100 ml of water sample was taken for acid digestion. Concentrated Nitric acid was used for 48hrs acid digestion method. Then I took that water sample and transferred it into cuvettes by using micropipette and put for centrifugation. Repeated the centrifugation process for 3 times with filtrate to extract diatoms. After the process, I transfer the pellet of sample onto a slide by using loop, then made smear of it and air dried. After drying I examined it under Compound Microscope (40X). Dye like, Safranin, Malachite Green and Crystal Violet were used for colouring diatoms for visibility. Like the same procedure I have examined other samples also. By examining all those samples under compound microscope the different types of diatoms are visible.

Fig 4.1: WATER SAMPLES

I have taken various location screenshot of Vivekananda Park pond where I have collected water samples. Following are the location screenshots.

4.4 COMPOUND MICROSCOPE

A compound microscope is a high power (high magnification) microscope that uses a compound lens system. A compound microscope has multiple lenses: the objective lens (typically 4x, 10x, 40x or 100x) is compounded (multiplied) by the eyepiece lens (typically 10x) to obtain a high magnification of 40x, 100x, 400x and 1000x. Higher magnification is achieved by using two lenses rather than just a single magnifying lens. While the eyepieces and the objective lenses create high magnification, a condenser beneath the stage focuses the light directly into the sample.

Compound microscopes are used to view small samples that cannot be identified with the naked eye. These samples are typically placed on a slide under the microscope. When using a stereo microscope, there is more room under the microscope for larger samples such as rocks or flowers and slides are not required.

Fig 4.2: BINOCULAR COMPOUND MICROSCOPE

4.5 CENTRIFUGE

A centrifuge is a laboratory device that is used for the separation of fluids, gas or liquid, based on density. Separation is achieved by spinning a vessel containing material at high speed; the centrifugal force pushes heavier. Centrifuges are used mostly in science. In this application, centrifugal force — the force from spinning that moves things away from the centre — separates liquids that have different weights. For example, a centrifuge is used to separate blood cells from plasma cells. The centrifuge works using the 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. There are two types of centrifugal techniques for separating particles: differential centrifugation and density gradient centrifugation. Density gradient centrifugation can further be divided into rate-zonal and isopycnic centrifugation.

CHAPTER V

RESULT AND CONCLUSION

From Vivekananda park - Pennate Diatom & Centric Diatom.

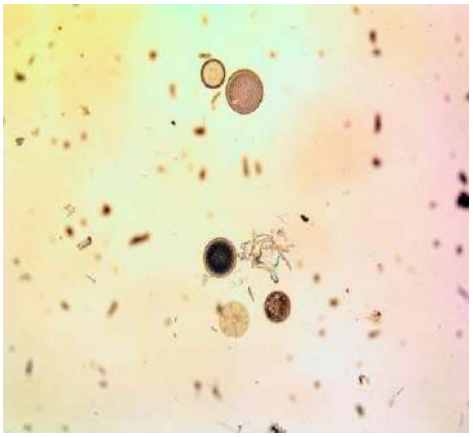

FIG 5.1: CENTRIC DIATOM

FIG 5.2: PENNATE DIATOM

From Boat club, The Pennate Diatom, Centric Diatom, Pennate & Centric Diatom.

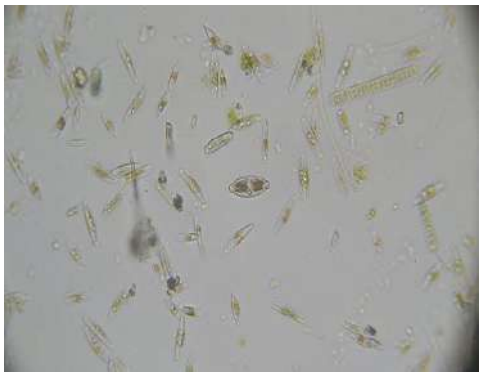

FIG 5.3: PENNATE DIATOM

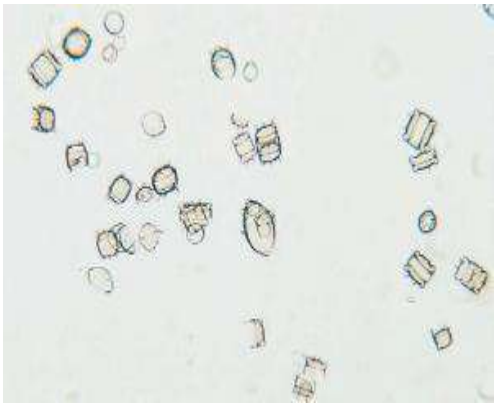

FIG 5.4: CENTRIC DIATOM

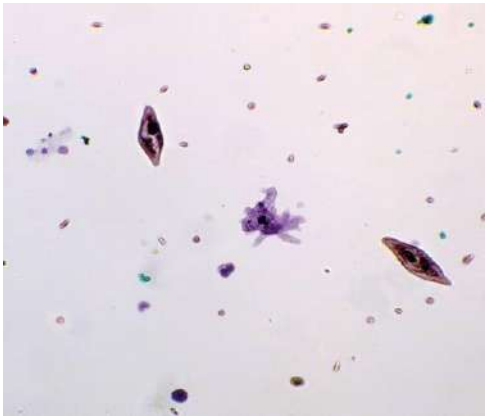

FIG 5.5: PENNATE AND CENTRIC DIATOM

From Madhavapatanam, - The Centric Diatom, Pinnate & Centric Diatom, Pinnate Diatom.

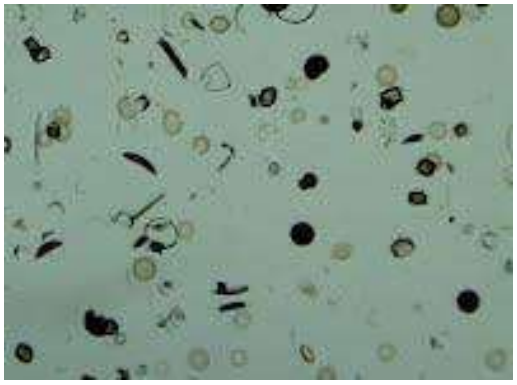

FIG 5.6: CENTRIC DIATOM

FIG 5.7: CENTRIC AND PENNATE DIATOM

FIG 5.8: PENNATE DIATOM

From Sarpavaram - the Centric Diatom, Centric and Pennate Diatom.

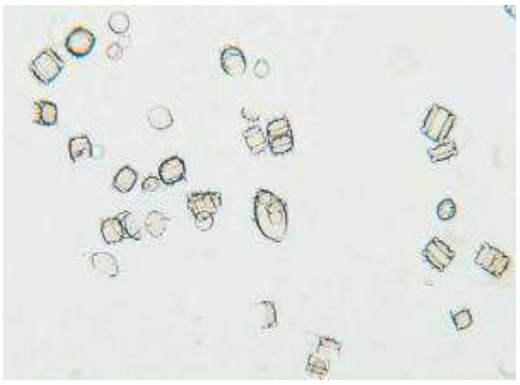

FIG 5.9: CENTRIC DIATOM

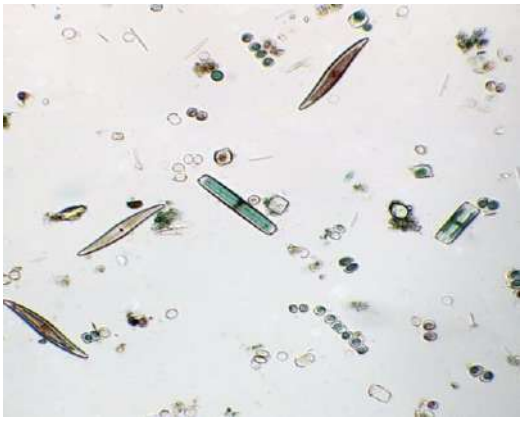

FIG 5.10: CENTRIC AND PENNATE DIATOM

CONCLUSION

In this study various diatoms are identified from 4 well known ponds of Kakinada. Diatoms can be used as evidence in drowning cases. Also this can be used as circumstantial evidences. Diatoms found examined in the project can play role standards of diatom species which are found in Vivekananda Park, Boat Club, Madhavapatanam & Sarpavaram. If any drowning cases are encountered in this particular water bodies, it will be easier for forensic investigation to identify drowning with the help of identify above mentioned diatom species.

Finally, this finding are considered as most effective and can be presented in court of law. Further studies can be conducted in many other water bodies. Forensic Databases should also include Diatoms for reference cross examination belonging to one particular area or segregating various species of diatoms according to their inborn locations.

REFERENCES

1. G Sudhakar, B Jyothi, V Venkateswarlu, Role of diatoms as indicators of pollution gradients, Environmental monitoring and assessment 33(2), 85-99, 1994.

2. Y Seeta, P Manikya Reddy, Diversity of diatoms in the rivers of Telangana, Diversity of diatoms in rivers has been investigated for a period of two years, May 2015 to April 2017.

3. Disha Rana, Deepika Bhandari, International journal of current Research and Review 8 (17), 13, 2016.

4. Amarnath Dogiparti, Ravi Kumar Kurapati, Uday Ranjan T Joseph, Myla S Chakravarty, International journal of Basic and Applied Science 2 (1), 1-10, 2013.

5. Jyothi Kaparapu, Mohan Narasimha Rao Geddada, Notulae Scientia Biologicae 5 (3), 290-295, 2013.

6. Andrew Bozarth, Uwe- G Maier, Stefan Zauner, Applied microbiology and biotechnology 82 (2), 195-201, 2009.

7. Kathleen M Ruhland, Andrew M Paterson, John P Smol, Journal of paleolimnology 54 (1), 1-35, 2015.

8. https://en.wikipedia.org/wiki/Category:Diatoms

9. https://en.wikipedia.org/wiki/Diatom

10. https://www.biocompare.com/Lab-Equipment/Laboratory-Centrifuges/

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