CHAPTER I: INTRODUCTION

Diatoms are almost ubiquitous photosynthesising algae, they have a siliceous skeleton (frustule) and are found in almost every aquatic environment including fresh and marine waters, soils, in fact almost anywhere moist.[12] Diatoms are microscopic,unicellular,and colonial algae found in marine,brackish,and fresh waters.They may be simple or branched,filamentous,and even enveloped in a gelatinous envelope or tube .[1] All diatoms are enclosed by a frustule that is made up of two valves fitted together by a connective zone called a girdle.They are non-motile, or capable of only limited movement along a substrate by secretion of mucilaginous material along a slit-like groove or channel called a raphe. Being autotrophic they are restricted to the photic zone (water depths down to about 200m depending on clarity). Both benthic and planktic forms exist.

Classification includes As algae,diatoms are protists.This means that they are eukaryotic organisms that are not specifically defined as plants,animals or fungus. Diatoms are formally classified as belonging to the Division Chrysophyta in Class Bacillariophyceae.This class of organism is distinguished by the presence of an organic cell wall that is composed of hydrated silica.Diatoms are also divided into two main orders.The Centrales or biddulphiales and the Pennales or Bacillariales.[9]

The pennales or pennate diatoms have frustules that are elliptical or rectangular in valve view,with sculpture that is bilaterally symmetrical about 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. The Chrysophyta are algae which form endoplasmic cysts, store oils rather than starch, possess a bipartite cell wall and secrete silica at some stage of their life cycle.The first record of diatom frustules are centric forms from the Early Jurassic although very few remains are known before Late Cretaceous ,they were moderately affected by the massive extinction at the end of cretaceous.[10]

Diatoms are commonly between 20-200 microns in diameter or length, although sometimes they can be up to 2 millimeters long. The cell may be solitary or colonial (attached by mucous filaments or by bands into long chains).Diatoms are domainant marine producers in the oceans and plays key role in carbon cycle and in removal of biogenic silica from surface waters.Living diatoms are very sensitive to the parameters like salinity,oxygenetion an other physical and chemical conditions,so they provide valuable tool for studies of modern water quality[2]

Diatoms do not occur naturally in the body. If laboratory tests show diatoms in the body that are of the same species found in the water where the body was recovered,then it may be a good evidence of drowning as the cause of death.Based on the study of drowning victims, where the diatoms are present in the medium, the penetration of diatoms into the alveolar system and blood stream has been caused by the breathing in of water by the drowning victims and then leads to the penetration of diatoms into other organs and parts of the body, such as bone marrow, the brain, kidneys and lungs . Hard bones (sternum and femur) and soft tissues (lungs and liver etc) of drowned bodies are usually sent to the Forensic Science Laboratories for the detection of diatom. While solving drowning cases, a correlation between the diatoms extracted from these tissue samples and the samples obtained from putative drowning medium has to be established for the successful determination of drowning site. The occurrence of diatoms in the bone marrow is a proof that the individual was alive when entered the water. This means that the cause of death was due to the drowning.[7]

When a person drowns in fresh water or marine water,diatoms are taken into lungs with water and distributed via blood vessels to internal organs.Their presence in lungs,stomach or even in bone marrow is used as evidence that the person was alive when they entered the water (antemortem immersion).The absence of internalized diatoms or failure to match internalized diatoms with those of recovery site would indicate that the victim possibly was dead to another cause prior to insertion into the water(post mortem immersiion) or was drowned elsewhere, respectively.It is also possible that a person did'nt internalize any diatoms during drowning.Diatoms may be extracted from Femur of a corpse and used to verify death by drowning.If victim also swallows water, diatoms may be found in stomach contents .Possible drowning have used the presence and identification of diatoms to confirm whether death occured by drowning or before the victim entered the water.It is also used to estimate how long a body has been in the water.Diatoms make for very interesting specimen under the microscope.They show complex patterns with very fine punctures on their surface.[3] The use of diatoms is to diagnose a cause of death by drowning often one of the number of independent techniques utilized by the forensic pathologist.

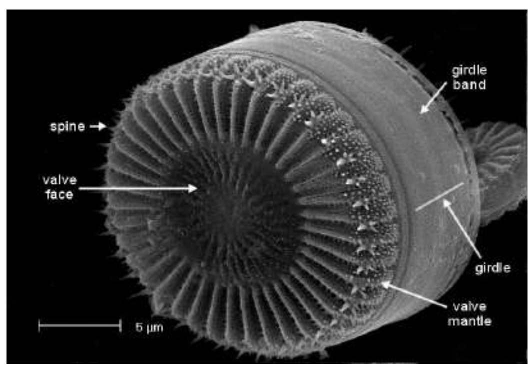

Figure 1: Centric Diatom

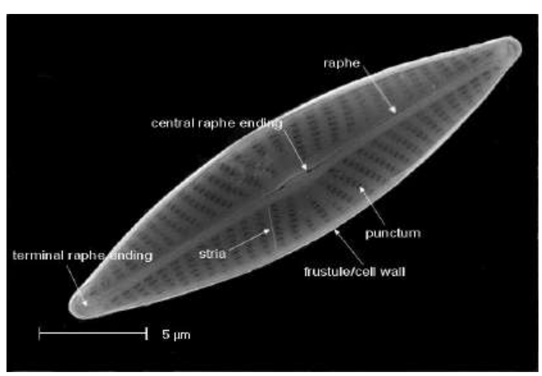

Figure 2: Pennate Diatom

CHAPTER II :LITERATURE REVIEW

Ajay Singh Rana et.al (2019) introduced a topic named A Systematic review on various Diatom species asssociated with drowning.When a person gets drowned into water containing diatoms,due to forceful aspirations they get enter into lungs and from lungs get logded into the body distant organs of body.Various tests have been developed for diagnosis of drowning deaths.The extraction methods of diatoms from tissue sample is based on digestive capability,reclaiming ratio of diatoms,destruction of diatom samples and time consumed for complete diatom digestion.This paper reviewed recent year' progress in diatom test,samples used and species of diatoms associated with drowning.

Yuanyuan Zhou et.al(2019) introduced a topic named Digital whole-slide image analysis for automated diatom test in forensic cases of drowning using a convolutional neural network algorithm,which states that diatom examinations have been widely used to perform drowning diagnosis in forensic practice.However,current methods for recognizing diatoms,which use light or electron microscopy,are time consuming and laborious and often result in false positive or negative decisions .In this study,demonstrated an artificial intelligence (AI)- based system to automatically identify diatoms in conjunction with classical chemical digestion approach.By employing transfer learning and data augmentation methods,trained convolutional neural network (CNN) models on thousands or tens of tiles from digital whole slide image of diatom smears.The results showed that trained model identified the regions containing diatoms in the tiles.This pilot study paves the way for future intelligent diatom examination,many efficient diatom extraction methods could be incorporated into automated system.

Lisha Gurung et.al (2013) made a research on Fresh water diversity in Deepor Beel-A Ramsar site conducted for an investigation of fresh water diatom diversity in deepor beel,ramsar site of assam,India.The samples were collected from six different sites on basis of habitat stratification.The results showed that there were 65 species of diatoms,belonging to 26 genera of which 53 were pennate and 12 were centric types.Abundance of diatom species provides evidence for their important role as primary producers in this wetland ecosystem of deepor beel of Assam.

Sakshi Manhas et.al (2018) made a review on Significance of diatoms in diagnosis of drowning deaths.Diatom test plays an important role for diagnosis and confirmation of drowning deaths.Diatoms species detection in both water and tissue sample gave a reliability of the diatom test in solving cases pertaining to drowning deaths by using Acid digestion method.The possible means and methods must be practiced for extration and identification of diatoms in near future.This paper reviews the recents year's progress on diatom test and its application in forensic science.

K.R Scott et.al (2018) introduced a topic named Freshwater diatom transfer to clothing:Spatial and temporal influences on trace evidence in forensic reconstructions.This study aimed to examine three of the spatial and temporal variables to inflluence the extent of an initial transfer of trace particulate, within context of freshwater diatoms to clothing.A series of experiments were designed to consider impact of recipient surface characteristics,source environmental conditions ,morphological variability,on total number and species richness of an evidential diatom sample recoverd from clothing.Diatoms were recovered using a H_2O_2 extraction technique and examined microscopically.The results demonstrated that diatom transfer to clothing varies with greater abundance and higher species richness treansferred to woven surfaces including acrylic,linen, and viscose.These findings highlight that clothing may offer valuable repository of fresh water datom trace evidence,the interpretation of evidential material shuold be approached within an exclusionary framework.

Ping peng et.al(2019) illustrated Spatial distribution of diatom assemblages in the surface sediments of Selin Co, central Tibetan Plateau, China, and the controlling factors. To illustrate spatial patterns of diatom communities and study the correlation with environmental variables, 143 surface sediments were sampled from the largest lake (Selin Co) on the central Tibetan Plateau. A total of 143 diatom species belonging to 30 genera were identified, most of which were halophilic and basophilic species**.** Through Canonical correspondence analysis, it was indicated that the spatial distribution of the diatom assemblages was mainly influenced by water depth, grain size and temperature, with water depth being the dominant factor. The results defined the spatial distributions and controlling factors of diatom assemblages from the different environments.

Min Chen et.al (2015) analysed diatoms collected from surface sediments in Chanthaburi and Welu Estuaries on northern coast of golf of Thailand.Total 144 species identified ,41 genera .Diatoms abundance range from 645 to 24,979 valves /g with average 7,215 vales/g .Using cluster and redundancy analysis ,four diatom assemblages representing different environmental conditions are identified. Main causes of differences in distribution of diatoms between Chanthaburi and Welu estuaries are Runoff,Estuary size,River width and Nutrient concentration.

T.Mangadze et.al (2018) assessed the water quality in small austral temperate river system in south Africa.Application of multivariate analysis and diatom indices.Canonical correspondence analysis indicated difference in diatom community assemblages explained by dissolved oxygen ,temperature, phosphate ,nitrate and conductivity. Several foreign indices like TDI(the trophic diatom index),SADI(south african diatom index) were used in the study.Therefore recommend wider use of SADI as Indicator of water quality conditions in South African lotic systems.

Omar Rodriguez-Al cala et.al(2019) analysed data set of epiphytic diatoms comprising 34 lakes from six European countries. It is large scale geographical and environmental factors and not geography.The results support validity of using diatom as ecological indicators across Europe.Also,gives support to use of epiphytic diatoms as biological indicators for shallow lakes .The results solved the pending questions in the spatial ecology of diatoms by proving that species turnover is stronger than nestedness at any spatial scale, and give support to the use of epiphytic diatoms as biological indicators for shallow lakes.

Janne Soininen et.al (2007) examined freshwater diatom community structure in relation to environmental and spatial gradients ,and to consider results in ecological context.Diatom literature stresses the importance of ion concentration and trophic status as major environmental drivers of diatom distribution in lakes and streams,while physical factors effect on community structure. Freshwater diatom communities seem strongly spatially structured.According to studies,from both lakes and streams ,pure spatial factors account for 20-30% of total community variation.Thereby suggest that diatoms lack strict ubiquitous dispersal.These results suggests that bioassessment programs utilising diatoms should consider spatial factors,for example,diatom communties are strongly spatially structured.

CHAPTER III: AIM AND OBJECTIVE

Aim:

To Identify and Extract the Diatoms from Estuary,Shore of Kakinada sea and Hope Island water resources

Objective:

- To extract the diatoms by Acid Digestion Method.

CHAPTER IV: MATERIALS AND METHODOLOGY

Materials:

Apparatus:

1. Conical flask
2. Samples
3. Beakers
4. Micro pipette
5. Vials
6. Glass slides

Reagents :

1. Nitric acid
2. Sulphuric acid
3. Hydrogen peroxide
4. Ethanol

Instruments :

1. Centrifuge
2. Compound Microscope

Figure 3: Centrifuge

Figure 4: Compound Microscope

Methodology:

In this study,the samples had collected from estuarine,shore and Hope Island of East Coast of Kakinada region.Three samples were collected from each location of each water sources.

In this study,the water samples which is of 100 ml each had been collected from estuarine,shore and hope island in east coast of kakinada.

The samples are taken in 1:1 ratio that is equal amount of samples and nitric acid solutions.These solutions are taken into a beaker and kept for digestion for 48 hours to let the diatoms settle down.

After 48 hours,0.5ml of the samples are pipette out with the micropipette into the vials and placed them in the centrifuge.The samples are centrifuged at 2500 rpm for 10 minutes.Then 0.25ml of supernatant is decanted and distilled water is added to the sample with the same amount.Again the samples are centrifuged with distilled water at 2500 rpm for 10 more minutes.it separated the supernatant from the solution.

Using a pipette small amount of suspension is placed on a clean, dry glass slide.it should be smeared by using the forceps on the glass slide.After being placed on the slide, the diatom suspension is allowed to dry at room temperature in a dust free environment. After drying it got ready for examination.The samples are observed under the compound microscope.

Figure 5: Estuary

Figure 6:Shore

Figure 7:Shore

Figure 8: Hope Island

Figure 9:Hope Island

Fig 10: Estuary samples

Fig :11 Shore samples

Fig :12 Shore samples

Fig :13 Hope Island samples

Fig :14 Preparation of samples

CHAPTER V: OBSERVATION

Table 1:Location: ESTUARY (Korangi)

Samples	Diatom Species
Sample 1	Centric
Sample 2	Centric
Sample 3	Pennate

Table 2:Location:SHORE (Kakinada)

Samples	Diatom Species
Sample 1	Centric
Sample 2	Pennate
Sample 3	Pennate

Table 3:Location :SHORE (Uppada)

Samples	Diatom Species
Sample 1	Pennate
Sample 2	Pennate
Sample 3	Centric

Table 4: Location:HOPE ISLAND

Samples	**Diatoms Species**
Sample 1	Pennate
Sample 2	Pennate
Sample 3	Pennate

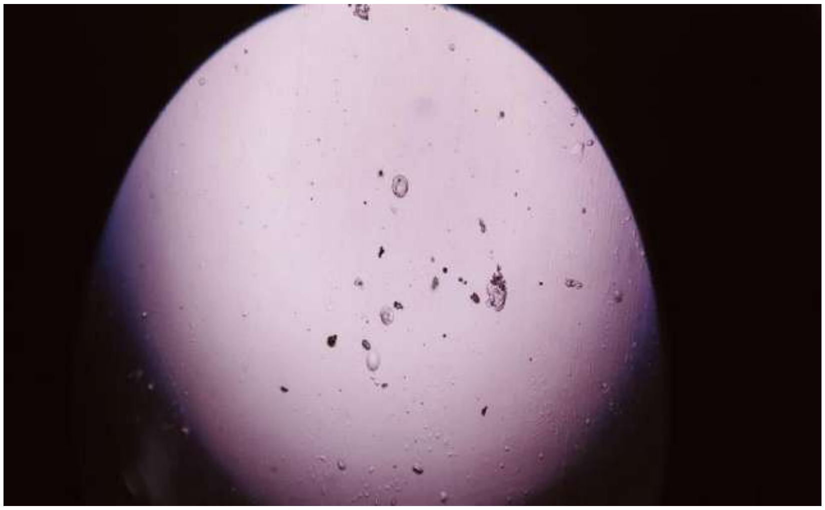

Fig15: Centric diatom

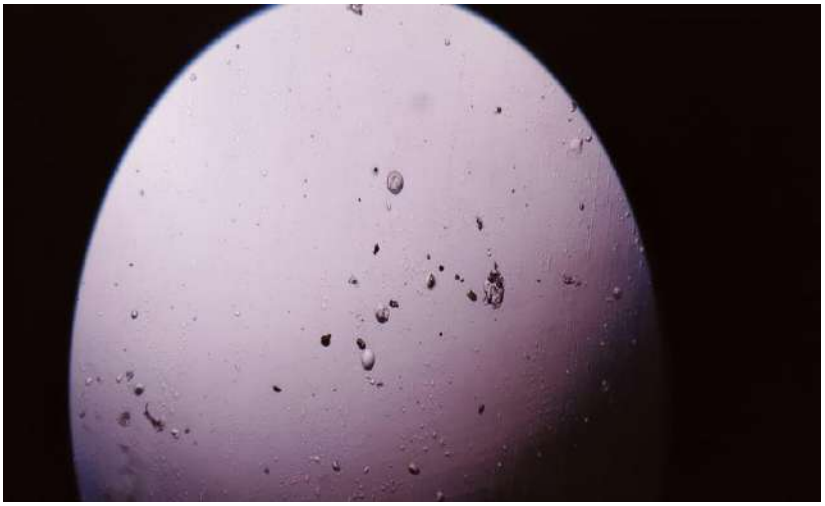

Figure 16: Centric diatom

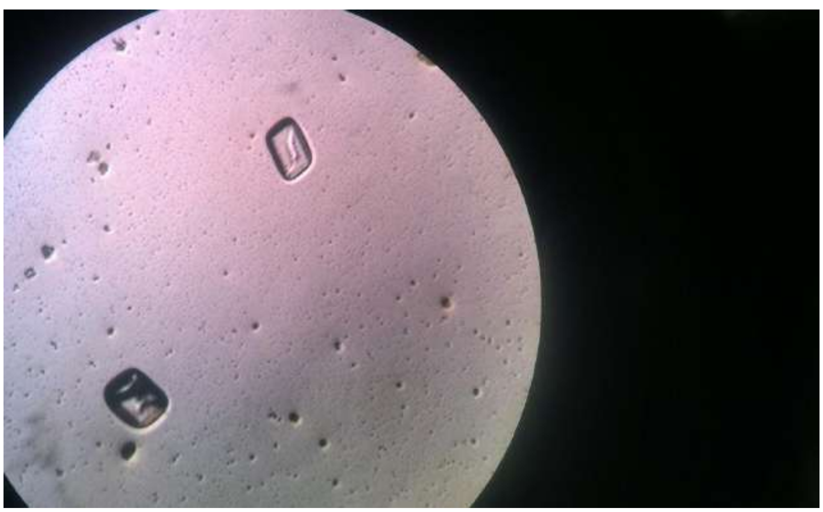

Figure 17:Pennate diatom

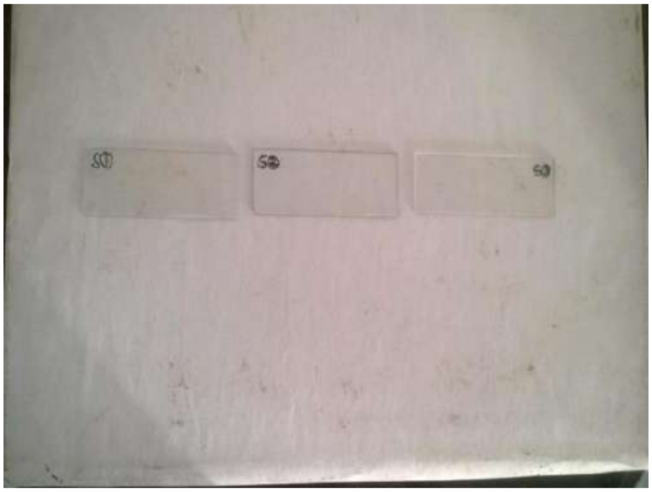

Figure 18:Glass slides containing samples

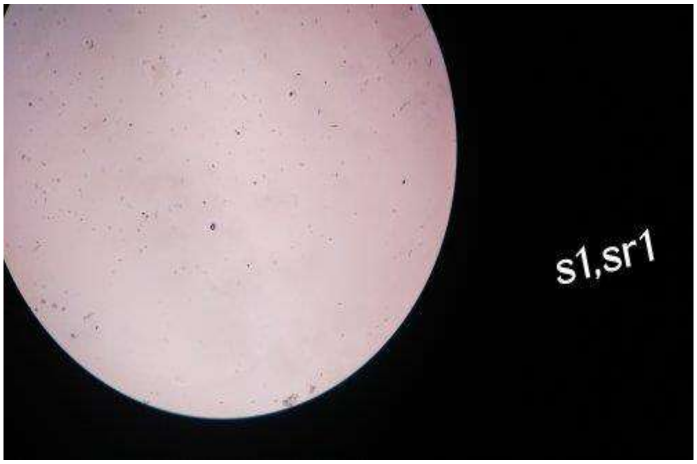

Figure 19:Centric diatom

Figure 20:Pennate diatom

Figure 21 :Pennate diatoms

Figure 22:Glass slides containing sample

Figure 23: Pennate diatom

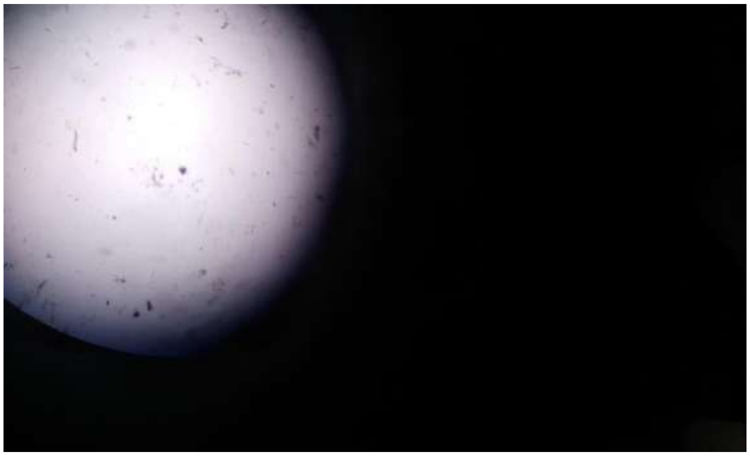

Figure 24:Pennate diatom

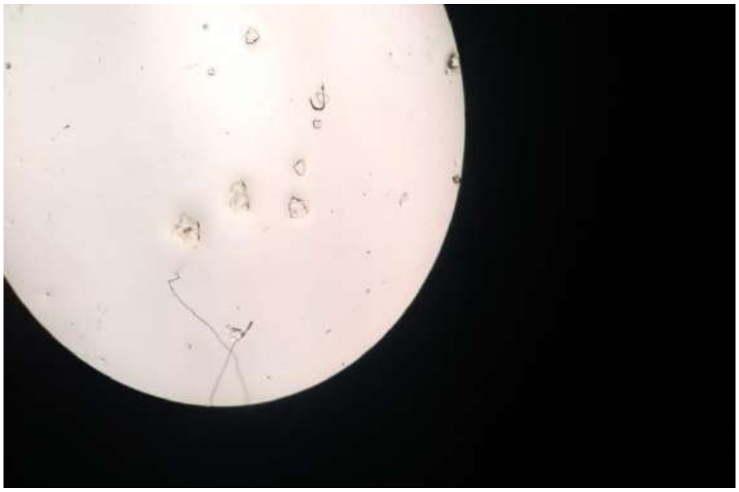

Fig 25:Centric diatom

Figure 26:Glass slides containing samples

Figure 27: Pennate diatom

Figure 28:Pennate diatom

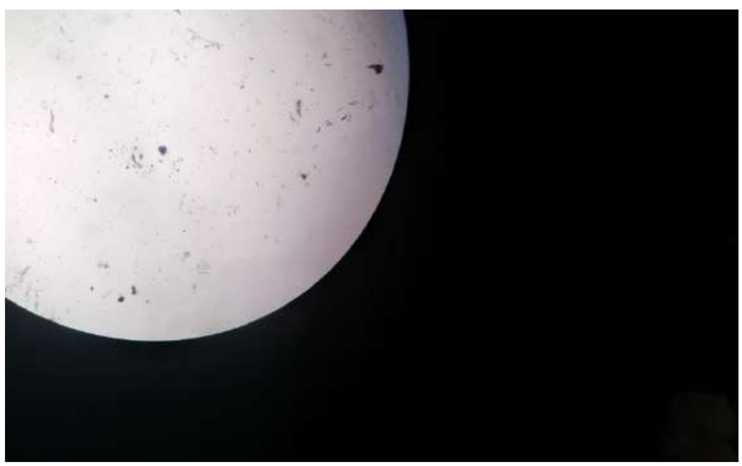

Figure 29:Pennate diatom

Figure 30:Glass slides containing samples

CHAPTER VI: RESULT AND CONCLUSION

Result:

The samples which were collected and extracted from various water resources are shown the results as Centric and Pennate species of diatoms under the microscopic examination as the following:

The samples which were collected from Estuary water bodies results in Sample 1,2 and 3 as Centric, Centric and Pennate diatom species respectively.Similarly,the samples which are collected from Shore water bodies of east coast of kakinada sea results in Samples 1,2 and 3 as Centric,Pennate and Pennate diatom species.From uppada shore water results in Samples 1,2 and 3 as Pennate,Pennate and Centric diatom species. Also,the samples which are collected from Hope Island water bodies results in Samples three of Pennate diatoms only.

Among all the samples, Centric diatoms are four in number and Pennate diatoms are eight in number.

Conclusion:

The diatoms were extracted and identified from various water sources of Estuary, Shore and Hope Island are found to be Pennate diatom species and some of Centric diatom species present in marine water.

Diatoms can be used as evidence in drowning cases, this method is very effective in such cases. Also it can be used as circumstantial evidences. Finally, these findings is considered as most effective,interpreted and present in court of law.In future, this study can help to identify various water resources of Kakinada and identification of various species of diatoms.

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