

# TO PREVENT ILLEGAL TRAFFICKING OF FISH SPECIES WITH THE HELP OF DIATOMS

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## **Abstract**

*Today illegal trafficking of aquatic organisms are very common in all over the world; especially Fish, crocodile, turtles, frogs etc. In this study we focused on fish, because fish are very common in India to import and export. We extracted diatoms from fish intestine with the help of aqua regia acid digestion method and compared it with the pond water samples, where a fish were taken. Two sites were selected for the collection of waters and fish samples, one from Kankaria Lake Ahmedabad and second from Shertha Village Lake Shertha (Gujarat). Species identification were done with the help of standard diatoms images from website sources. On the basis of diatoms, we can also differentiate one pond fish to another because each water body has its own diatoms species.*

**Keywords:** Diatoms, Drowning, Aquatic organisms, Acid digestion, Wildlife forensic.

## **INTRODUCTION**

Diatoms are photosynthesizing algae, which have siliceous skeleton frustules. It can be found in almost every environment including fresh and marine waters and soils.

The classifications are based on the shape of the diatoms. The Biddulphiales usually appear radially symmetrical and Pennales appears bilaterally symmetrical. Study reported that nine basic morphology categories of diatoms were found so far. The nine groups are centric, araphid, eunotioid, symmetrical biraphid, monoraphid, asymmetrical biraphid, epithemioid, nitzschoid and surirelloid. Diatoms species can be found on cobbles, rocks, damp sediments facing the water surface, stems of rooted vegetation and even on drowned victim's wet clothing. Some diatoms remain suspended within the aquatic habitat (i.e. planktonic) while other forms of diatoms are settled within the sediment (i.e. benthic) where they are a major food source for grazing protozoa and animals. Diatoms are mostly used in drowning cases for the establishment of anti-mortem or post-mortem drowning. Diatoms are useful in environment science to check the quality of water and pollutant inside the water body. Diatoms are also useful to establishment the time scene death. Diatoms are present in every water body like pond, river, wells, etc. In this study we extracted Diatoms from fish intestine with the help of aqua regia acid digestion method i.e. 3:1 HNO<sub>3</sub>: HCL, because its very powerful digestion method as compare to normal acid digestion like H<sub>2</sub>SO<sub>4</sub> method, benefit of this method is we can get the whole diatom structure, the breakdown of diatoms are very low as compare to other method. we selected two sites for water and fish sampling which were different in water diversity and also different in fish species, after that we extracted diatoms from water samples of one site i.e. Kankaria lake with normal extraction method without using acid and then we extracted diatoms from fish intestine with the help of aqua regia method and compared with water sample diatoms. Same processor we did in second site i.e. Shertha Village Lake. So on the basis of this study we might link a fish to water body.

## **MATERIALS AND METHODS**

### **A. Water Samples collection**

Water samples were collected from two different sources in Gujarat state, One from Kankaria lake Ahmedabad, and second from Shertha village Lake Shertha, Gandhinagar. Water samples were collected in 15ml falcon tube. 15 water samples were collected around the periphery of both the lake.

### **B. Fish samples collection**

The same sites were selected for the collection of Fish samples. At Kankaria lake we had kept only one catfish and from Shertha village lake we had kept 15 fish around the periphery of the lake same as we were collecting water samples. All the fish were kept at 4 degree so no degradation occurred.

### **C. Extraction of diatoms from water samples**

Total 10 ml water sample was taken in 15 ml centrifuge tube and centrifuged at 3000 g for 5min. The resulting supernatant was removed carefully while not disturbing the pellet. The pellet was suspended in 1.0 ml

sterilized distilled water. A drop of extracted sample was put on microscopic slide and analyzed under light microscope.

#### D. Extraction of diatoms from Fish samples

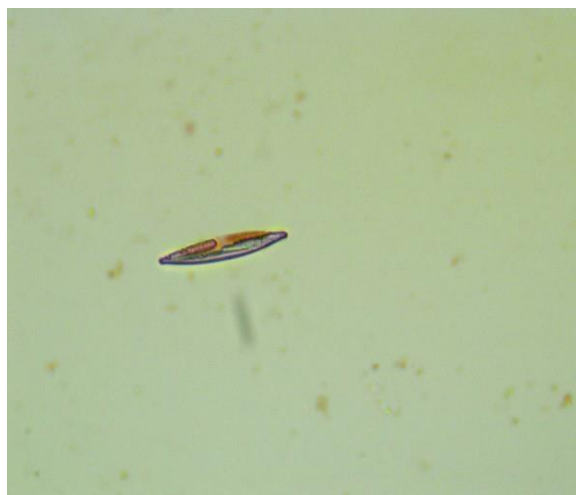
Approximate 5gm of Fish intestine was taken in a clean 100ml flask. In a fume cabinet, 100ml of lefort aqua regia solution was added i.e. 3:1Cons HNO<sub>3</sub>: HCL. Drops wise added H<sub>2</sub>O<sub>2</sub> until the bubbles is disappearing. A flask was put on hotplate at 96°C for 3hrs.After that the solution was poured into a centrifuge tube (10 ml). The samples were rinsed by centrifuging with distilled water at 2500 rpm for 10 min, the superannuate was decanted and the washing was repeated a further 4 times until the clean pallet was appear. Pallet was dissolved in 1ml of distilled water and seen under the light microscope.

#### E. Slide preparation

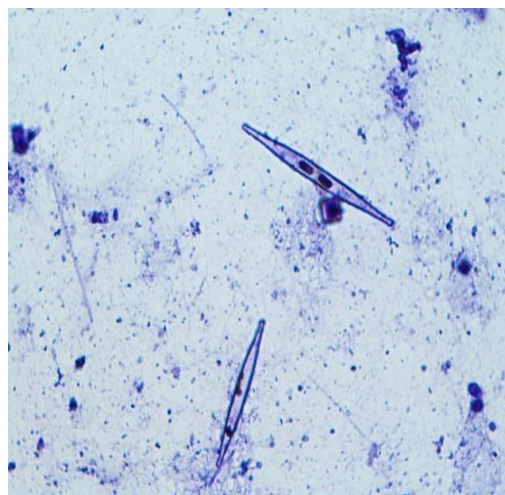
A clean slide was taken and put a drop of dissolved pallet and hit fix it on hotplate. After 1-2 minutes of heat fixing a slide was seen under Compound microscope (E200 Nikon) at 100x oil immersion lenses and images were taken.

### RESULTS

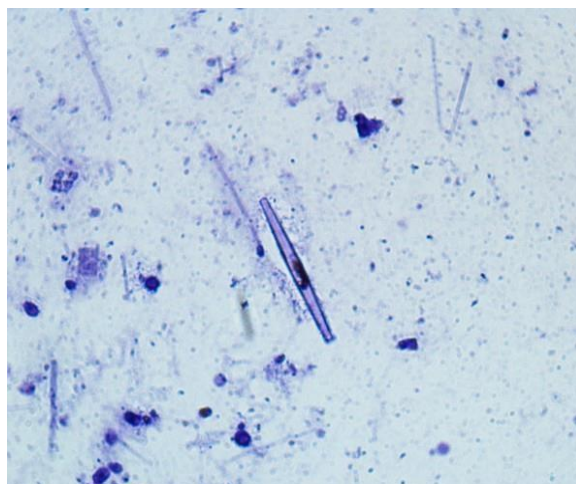
Analyzed samples were showed the following types of Diatoms collectively. The variation could not be observed in the form of species at different distances. The below are some of the images of Diatoms that were very frequently observed in water samples of Shertha lake.



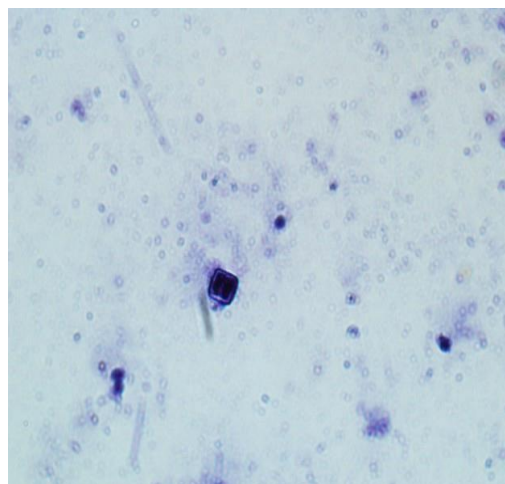
1. Navicula



4. Synedra tabulate



2. Synedra ulna



5. Melosirales



3. *Cyclotella meneghiniana*



6. *Nitzschia*

Figure 1. Different types of Diatoms were found from the 15 water samples of Shertha Village lake. Same as in Kankaria Lake around 15 water samples were analyzed and we found the following types of diatoms with the help of compound microscope.



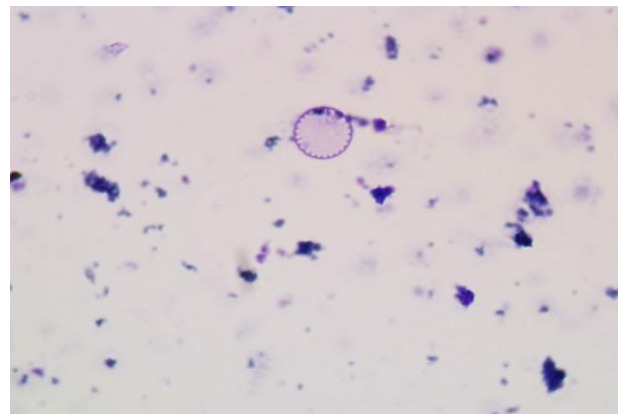
1. *Pinnularia viridis*



2. *Navicula*

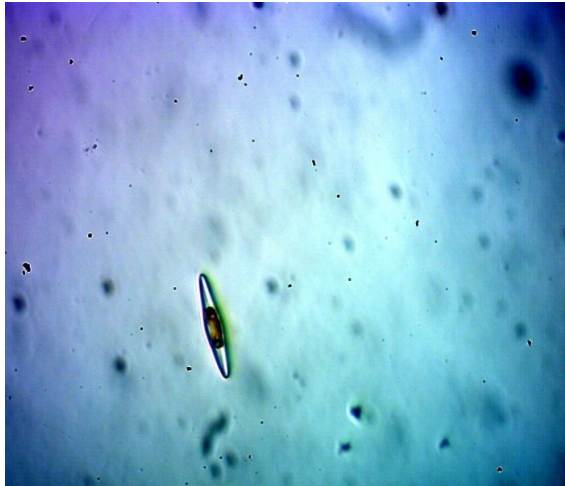


3. *Epithemia zebra*



4. *Cyclotella meneghiniana*





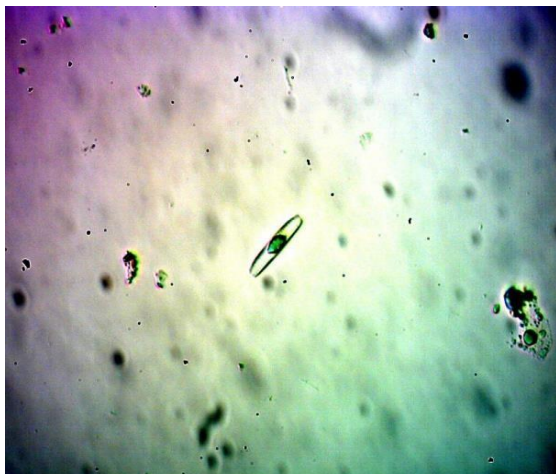
5. Caloneis



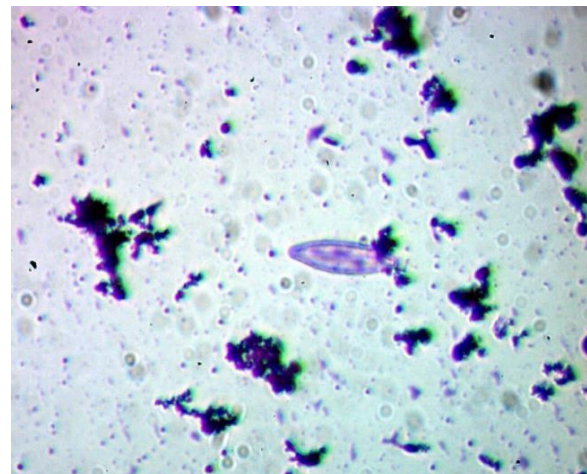
6. Nitzschia

Figure 2. Different types of Diatoms were found from 15 water samples of kankaria lake.

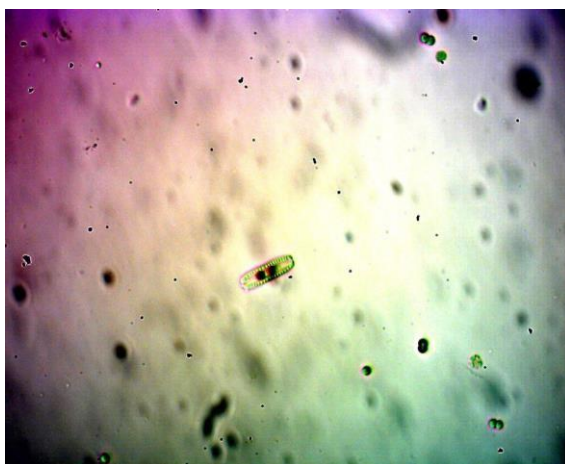
After the acid digestion of kankaria lake fish intestine we were found the same types of diatoms species which were present in kankaria water samples. Following are some types of diatoms which were found from kankaria fish sample



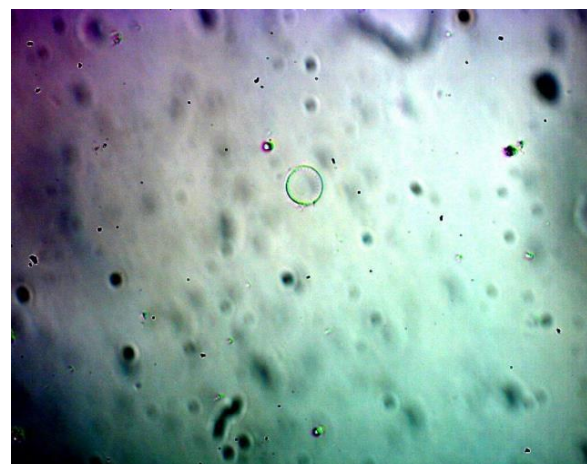
1. Pinnularia viridis



2. Navicula



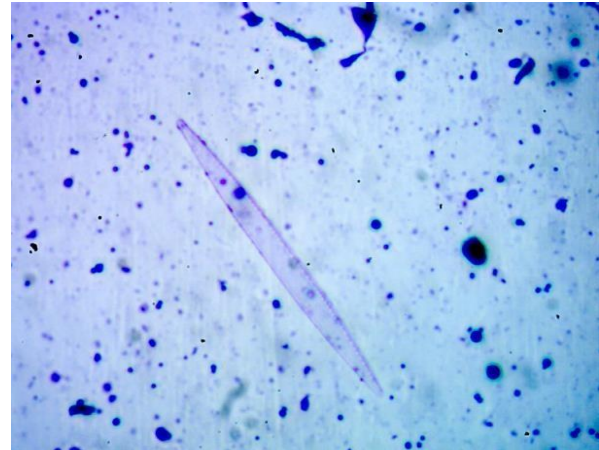
3. Epithemia zebra



4. Cyclotella meneghiniana



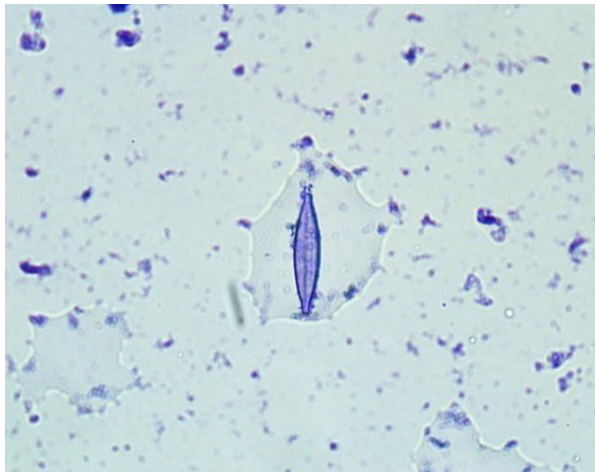
5. Caloneis



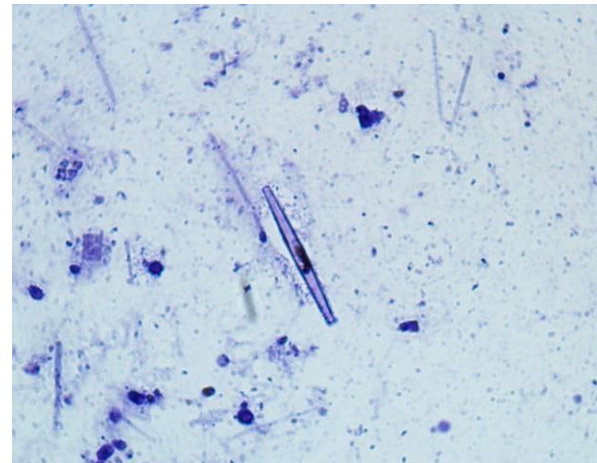
6. Nitzschia

Figure 3. Different types of Diatoms found around the 15 water samples of kankaria lake.

After the acid digestion of 15 fish samples which were kept at Shertha village lake, we were found the following types of Diatoms.



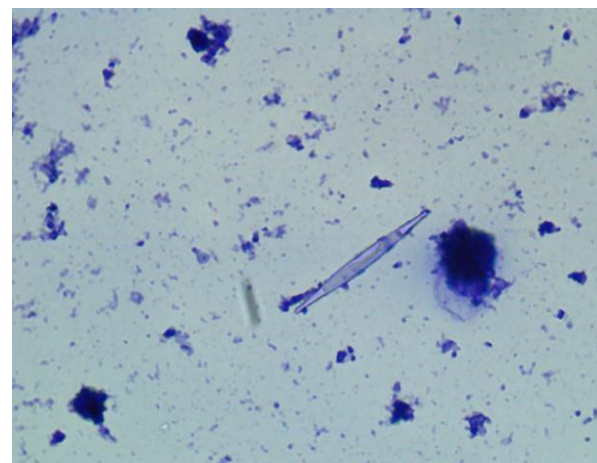
1. Navicula



2. Synedra ulna

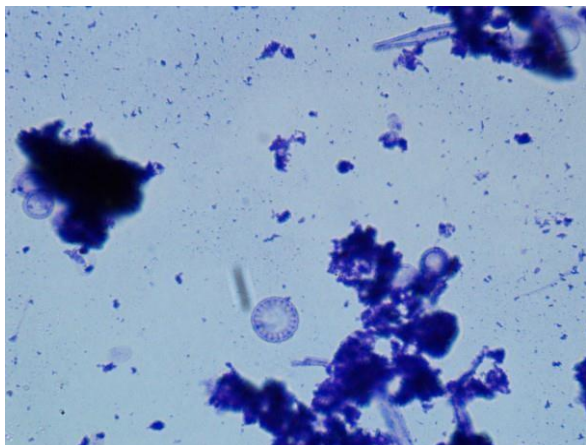


3. Nitzschia

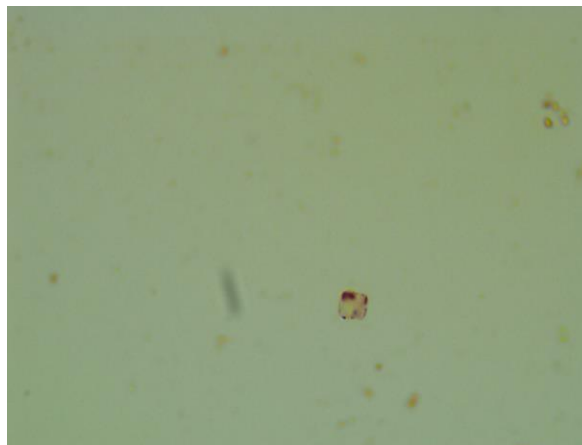


4. Synedra tabulate





5. *Cyclotella meneghiniana*



6. Melosirales

Figure 4. Different types of Diatoms found around the 15 Fish samples of Shertha lake.

## DISCUSSION

The results given in the previous page gives an idea about the species and types of Diatoms were found in both the water samples i.e. Shertha village lake water samples and Kankaria lake water samples and also give an idea about the Diatoms found in Fishes sample.

In Shertha lake water samples *Navicula*, *Nitzschia*, and *Melosirales* frequency were very high in all the water samples. Same as in Shertha lake Fish samples *Navicula*, *Nitzschia*, and *Melosirales* frequency were very high in all the fish samples. In Kankaria lake water samples *Caloneis*, *Navicula* and *Nitzschia* frequency were very high in all the water samples. Same as in Kankaria lake Fish sample *Navicula*, *Nitzschia* and *Caloneis* frequency were very high in Kankaria lake fish sample.

After comparison of both samples i.e. water and Fish with the stander Diatoms we were found that some species of diatoms were common in both the samples.i.e. *Cyclotella meneghiniana*, *Navicula*, *Nitzschia*, *Synedra ulna*, *Melosirales*, *Synedra tabulata*.

In Kankaria lake samples the following species of diatoms were same in both the samples. *Cyclotella meneghiniana*, *Navicula*, *Nitzschia*, *Epithemia zebra*, *Pinnularia viridis*, *Caloneis*. So from above observation we were found that the 3 species of Diatoms were same in both the sites water samples and Fish samples i.e. *Cyclotella meneghiniana*, *Navicula*, *Nitzschia*, but 3 species of Diatom were different in both the sites water samples and Fish samples i.e. *Synedra ulna*, *Melosirales*, *Synedra tabulata*, were found only in Shertha village lake and *Epithemia zebra*, *Pinnularia viridis*, *Caloneis*, were found only in Kankaria lake.

All of the above observation we were found that the Shertha lake had its unique Diatoms species which was different from the Kankaria lake, so on the bases of that different Diatoms species, we differentiated two water body systems and also differentiated two Fish. Because both the Fish had different Diatoms species.

## CONCLUSION

In above discussion we found that both the sites i.e. Kankaria Lake and Shertha Village Lake had a different Diatoms species. Fish which were caught from Kankaria lake had a same species of Diatoms which were found in Kankaria lake water samples. Same as Fish which were caught from Shertha Village Lake had a same species of Diatoms which were found in Shertha village Fish samples. Some species of Diatoms were unique to a particulate site.i.e.3 species of Diatoms which were not found in Shertha lake and 3 species of Diatoms which were not found in Kankaria lake. So on the basis of that different species we could different both the water body and differentiate their aquatic organism.i.e.Fish,So we can proved a hypothesis that a particulate water body has their unique Diatoms diversity which are different to other water body. On the basis of this hypothesis we can link a Fish to its water body and we may stop some illegal trafficking of aquatic organism like Fish, Crocodile, turtle etc. Which are dependent on water, so it's very important in specially Wildlife Forensic to stop these aquatic organism illegal trafficking.

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