An investigation of diatoms from abandoned water bodies in metropolitan cities

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Abstract- Diatoms are single-celled photosynthesising eukaryotic algae having great importance ecologically and forensically. They have a siliceous skeleton (frustule) and are found in almost every aquatic environment including fresh and marine water bodies. This review attempts to study the diatom ecology in the water bodies of Rajasthan. Diatoms are found in the body of the drowned individual and can be used as corroborative evidence in cases to answer the questions like the site of drowning, time of drowning, ante-mortem or post mortem drowning. It can also be served as reliable proof of site and time of drowning, even in the cases of contamination due to immersion of the body into the water for a longer time. This review study revealed that the most common diatoms reported in the waterbodies of metropolitan cities belong to species Navicula, Nitzschia, Cyclotella, Synedra, Fragilaria, Gomphonema.

Index Terms- Diatoms, forensically, siliceous skeleton and water bodies.

I. INTRODUCTION

INTRODUCTION:

Diatoms….The most beautiful microscopic organisms which are unicellular and photosynthetic in nature, these occur mostly as single celled but few species form colonies. They have certain unique features amongst the Kingdom Protista. The diatoms are divided into two groups that are common in freshwaters namely the centric diatom species which are spherical in shape and the pinnate diatoms which are of elongated shape. These organisms produce mucilage through different structures of the cell wall to enable the movement or attachment of the cells to the rock beds. This material is removed for the microscopic examination of the cell wall. After carrying out the practical steps for the slide preparation, at high magnifications only the silica structure is visible. This visible skeleton is usually stated as the Frustule. Chemical procedures dissolve all the organic material from both inside the cell wall as well as outside the cell wall. In drowning cases, post-mortem examination is one of the most difficult tasks in forensics. The water is more complicated than the land, which makes the recognition of cause of death more difficult. The diatom test has been suggested in the diagnosis of drowning.

Diatom analysis is more valuable and it is useful in diagnosis of drowning cases. The basic standard of diatoms test in drowning cases is based on the link between diatoms that are present in the medium where the probable drowning has taken place. These diatoms are deposited in the brain, kidney and other organs by inhalation of water. This fundamental fact is the key to the investigations as it gives scientific idea to know whether the person was dead or alive at the time of drowning (*Jc Taylor.et.al,2007*)

Most regular waters contain diatoms, a class of microscopic, unicellular algae, suspended in water. They have a siliceous cell wall which resists acid digestion, heat and putrefaction. Diatoms are traditionally divided into two orders: centric diatoms (Centrales), which are radially symmetric, and pennate diatoms, which are bilaterally symmetric (Pennales) but (*Pollanen.et.al,1997*), diatoms are catagorised into three classes: centric diatoms (Coscinodiscophyceae), pennate diatoms without a raphe (Fragilariophyceae), and pennate diatoms with a raphe (Bacillariophyceae). Most diatom exist singly, although some join to form colonies. They are usually yellowish or brownish, and are found in fresh and salt water, in moist soil, and also on the moist surface of plants.

Diatoms are aquatic unicellular plants that represent a major taxonomic division of the phytoplankton. The most distinctive feature of this unicellular organism is its extracellular coat or frustule, which is composed of silica. The frustule of the diatom is a crystalline structure that is characterized by unique patterns of symmetry and microstructure. On this basis, the vast structural diversity of the frustule leads to a remarkable number of morphologically-distinctive varieties of diatoms. This vast degree of morphological variation gives rise to a large number of species. Recent

estimates indicate that there are in excess of 10,000 different species of diatoms. Diatoms are most often encountered in naturally occurring bodies of water such as lakes, rivers, oceans, seas, ditches, and puddles. Some diatom species have preference for water of specific salinity, thus allowing general distinction between freshwater and marine diatom types. In addition, some diatom speciesare more frequently associated with soil and puddles than lakes. Fossilized diatoms are another major source of frustules in the biosphere, although, these frustules are derived from long dead diatoms. Such diatoms are mined for commercial purpose. The most Bodies in Water use and maybe as forensically important as live freshwater contemporary diatoms. Mined fossilized diatoms are used in several commercial products including the fine abrasives in polishing compounds. One seldom emphasized feature of diatoms are their complex population of body dynamics and ecology Diatom populations are constantly in flux and these fluxes are the result of complex and poorly understood nutrient water and aquatic cycles. The net result of these environmental variations is a monthly periodicity in the abundance of live aquatic diatoms with blooms of diatom populations in the spring and autumn (i.e. seasonal maxima). In addition, there are temporal and spatial variations in diatom concentration in any body of water in response to local factors including mineral content of water, temperature, water stratification, and acidity. A poorly understood aspect of diatom ecology is the variation in the species and it based on genus distribution over seasons. These ecological characteristics of diatom populations have great, and underutilized, forensic significance. Among 11 the most important forensically relevant feature of diatom populations is the monthly variation in water concentration of diatom frustules that should be the outcome of the diatom test for drowning during various times of the year. The widespread distribution of diatoms in water and their morphological specificity makes the diatom an important forensic marker of drowning. Analysis of diatoms present in the body tissues like lungs, liver, spleen, blood and bone marrow has been undertaken as supportive evidence in drowning cases. The theory behind the diatom test that when any person drowns, the diatoms present in that water will reach to the lungs and some of them because of their size penetrate into the alveoli. If the heart is still beating, the diatoms that have entered into the blood stream travel around the body and may lodge in distant organs such as the kidneys, brain and bone marrow before death (*Auer, 1991, Pollanen, 1997)*

The presence of diatoms in the internal organs most likely confirms the ante-mortem drowning if there any doubt about the drowning site, then water sample from the putative site of drowning can be collected and analysed to determine the similarity of different species of diatoms in the water and the body.

Most species occupy a size between 10 and 80 microns. They live free or unite to form colonies drifting either in plankton or attached to mud, sand, or any other solid substrate. Only a live body with a circulation can transport diatoms from the lungs to the brain or bone marrow from where they, may be detected microscopically after suitable treatment. The value of this test is disputed by various authorities.

The current technique for isolation of diatoms involves acid digestion of tissue-commonly bone marrow, lung, blood, or kidneys with subsequent centrifugation and washing. The final residue is examined by microscopy. Water taken from the presumed site of the fatality and suitable laboratory controls are also examined. (*Pollanen.et.al,1997*)

The diatom test is valid only if it can be shown that:

1 The deceased did not drink this water immediately before submersion.

2. The species recovered from the specimen are all present in the sample from the site of drowning.

3. The various species are present (if sufficient are found) in the same order of dominance for the admissible size range and in approximately the same proportions.

Since diatoms resist degeneration, diatom test may have some value in examination of decomposed bodies. The test is negative in dead bodies thrown in water and in dry drowning. Forensic limnology is a sub field of forensic botany, which examines the presence of diatoms in crime scene samples and victims. Drowning is a form of asphyxial death in which access of air to the lungs prevented by of the body in water or other fluid medium Complete submersion cover the nostrils and mouth being all that is required in order to determine, if drowning is the sole cause of death, a complete examination of all organs is essential to rule out any disease process which may have caused or contributed to death. As for example, incapacitating or fatal trauma to the head and neck may occur without external injury, Impact of the forehead on floor of the

pool may cause hyperextension of the neck loss of consciousness, and subsequent inhalation of some water (*S.Michael,1998*)

In such cases, the autopsy findings would consist of internal head injuries and haemorrhages in deep neck muscles with or without cervical vertebral fractures.

Autopsy findings: Diatom acts as a supportive evidence for ascertaining the cause of death as well as the place drowning Presence of diatoms in the distant vital organs is a method to distinguish between ante-mortem and post-mortem drowning. It is possible to pinpoint the potent site of drowning, if diatom species recovered from the tissues of the corpse are compared to that found in the water body from where the corpse is recovered. Asphyxial changes are seen in the body. Special attention should be devoted to:

1. Changes in the respiratory tract
2. Biochemical changes in blood
3. Presence and character of water in stomach and intestine.
4. Presence of diatoms tissues.

Diatoms are also used widely as biological indicators . They are used to determine and detect changes in the biological and chemical state of the water in streams, river, ponds because of their specific sensibility and tolerance to various ecological factors. Variety of ecological factors. Diatom preference and tolerance to factors such as pH, conductivity, salinity, humidity, organic matter, trophic state, oxygen requirements, nutrients, and current velocity in freshwater streams, rivers, lakes, wetlands, have been defined, and diatoms have also been used in study of sediments. Biotic indices is a scale for showing the quality of an environment by indicating the types and abundances of organisms present in a representativesample of the environment. It is often used to assess the quality of water in marine and freshwater to ecosystems using diatoms based on the relative abundance of the species (*David Penny, 1995).*

The role between abudance of diatoms and the environmental condition of the particular sourced can be quantified. Each diatom species possess different unique characteristic and accordingly, they need a certain physical and chemical characteristic in the habitat. The slight change in these parameters also results in the change of diatom community characteristic. Diatoms respond swiftly to any of the variations, and these variations can be in their biomass and structure (*Tauri Bere et al, 2013*).

Extraction and Test for the detection of diatoms from biological matter,

The different organs collected are Liver, Lung, Brain, Kidney. The other biological sample collected can be bone marrow from Femur, Sternum and also blood from the left side of the heart.

Extraction of Diatoms from Biological samples:

One gram of Kidney, Lung, Liver and Brain are taken in a glass beaker containing equal amount of concentrated Nitric acid and concentatred Sulphuric acid. It is diluted with distilled water and is kept on flame for digestion. After dissolvation of the organic matter, the glass beaker is kept overnight, covered with aluminium foil. The sediments are allowed to settle. After twelve hours, the sediments are transferred into a tarson tube using a dropper. The sample is then centrifuged at 2000 rpm for ten minutes, and the supernatant is discarded. This step is repeated three to four times. After, the sediments are taken and spread over a slide and then dried at 90 degree Celsius (*Nadia Fucci, 2012*).

Extraction and diatom from freshwater samples:

One litre of water should be collected from the targeted site, in a sterile container. Diatom test is done by adding Lugol's iodine to 200 ml of the sample and keeping it covered overnight. After 24 hours, the sample is centrifuged at 2000 rpm for five to ten minutes, atleast three times. The pellet obtained from centrifugation is placed in the microscope slide in form of a drop and the slide is kept to warm over 50 degree on a slide warmer. The dried pellet is finally dried and it is mounted with DPX and is dried again (*Vichar Mishra et al, 2011*) 7

II. METHODOLOGY:

Collection of water samples from different water bodies in the region of Bangalore city.

Sample collection: For the present study, six different fresh water samples from different regions of **metropolitan cities** were selected accordingly. The water samples were collected from various lakes namely: Lalbagh lake, Ulsoor lake, Yediyur lake, Doddai lake, Bairasandra lake, Sampangi lake.

Firstly, for the water sample collection, 2 zones are marked with constant distance between them and the water is collected from surface and also 6-12 cms below the surface of the water body.

Sample size : Appropriately sterilized plastic bottles of one litre capacity was labelled and was used for the sample collection. In case of the lake, sample was

collected in one litre capacity sterilized bottle, from the selected zone of water body.

PRESERVATION OF DIATOM SAMPLES:

Formalin solution prevents the growth of bacteria and algae in the samples collected.

1% Formalin preparation:

1ml formaldehyde + 99 ml distilled water. Addition of 1 ml of the prepared formalin to 500 ml of water. Shake well. After 2 hours, sediments are relaxed at the bottom and water becomes transparent.

PROCEDURE:

Lugol's iodine solution preparation:

Lugol's iodine = KI (potassium iodide) (10g) + Resublimed iodide crystals (5g) dissolved in 30 ml distilled water + diluted to 100 ml by adding 70 ml distilled water.

Sediments were left to settle at the bottom on the bottom. Half the amount of water from the top is discarded, rest of the water remaining is shaken well and 200 ml of water with sediments is transferred to a 250ml beaker. 3-4 drops of Lugol's iodine is added and is left undisturbed overnight. 4 slides for each sample and 1 permanent slide is prepared for each sample of water.

15 ml tarson centrifuge tube was taken and 15 ml sample was taken. Centrifuged at 1500 rpm for 10 minutes. Supernatant was discarded, leaving the pellet at the bottom. The water sample was again added and centrifuge was repeated for three more times to get proper pellet.

One drop of diluted pellets added on to the slide and cover with cover slip. Observed under microscope at both 10x and 40x.

Slide warmer (40-50° C). The slide was kept on the surface of the warmer and one drop of sample was added. The sample was left to dry out and after drying, DPX was added and covered with cover slip and further kept for drying in order to observe under oil immersion).

SLIDE PREPARATION:

For permanent slide preparation after drying process, one drop of DPX mountant was added and placed a square coverslip over it carefully without bubble formation. Again heat it on slide warmer for 30 minutes at 30-35 degrees. Now slide is ready for further examination. Place the slide under Compound microscope and examined it by 100x oil immersion magnification lens. Images of diatom are captured by the help of Digital Cam Recorder Unit which is attached with microscope and computer.

III. Observations and results

Doddai Lake: The Genera identified during the microscopic analysis were Cyclostephanos and Araphid.

Two species from Cyclostephanos Genera were identified after the observation process and four species of Araphid genera were identified.

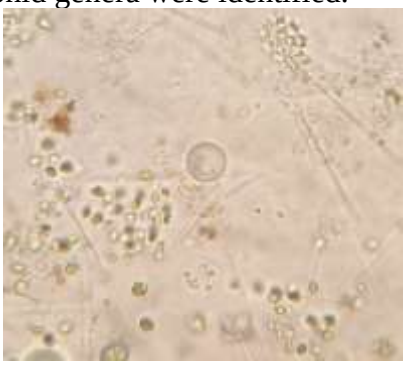


Figure 1. Cyclostephanos Diatoms

Class- Coscinodiscophyceae.

Order- Thalassiosirales.

These are characterized by having centric valves with distinct costae. In small specimens, the central areolae appear to be scattered randomly.

Figure 2. Araphid diatoms

Class- Fragilariophyseae

Order- Fragilariales

These are characterized by the valves with Bilateral symmetry. Frustules are elongated and linear in valve outline. The central part of valve has thickened area.

Byrasandra Lake: The Genera identified were Aulacoseira and Nitzschia. One species of Aulacoseira and Nine species from Nitzschia were found in the sample

Figure 3: Aulacoseira diatoms
Class- Coscinodiscophyceae.
Order- Aulacoseirales.
These have the characteristic features of deep mantle valve and are ornamented. Frustules form colonies joined by spines.

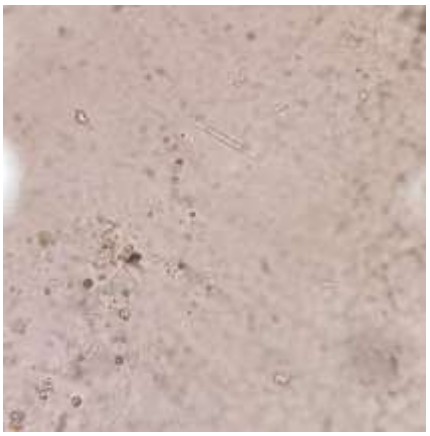
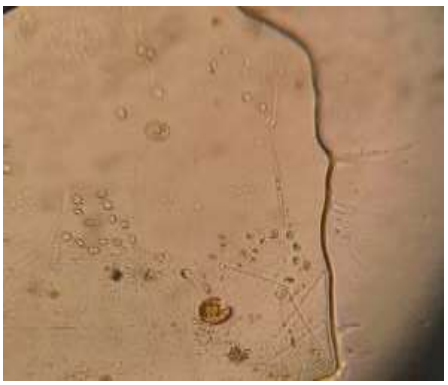


Figure 4 . Nitzschia diatoms
Class- Bacillariophyceae
Order- Bacillariales.
These are commonly pennate form of diatoms. Cells are elongate, fusiform or rectangular in girdle view.
Yediyur Lake: The Genera identified were Nitzschia and Araphid. Twelve species of Nitzschia and two species of Araphid were found in the sample.

Figure 5. Nitzschia diatoms
Class- Bacillariophyceae
Order- Bacillariales.
These are commonly pennate form of diatoms. Cells are elongate, fusiform or rectangular in girdle view.



2. Araphid.
Class- Fragilariophyseae
Order- Fragilariales
These are characterized by the valves with Bilateral symmetry. Frustules are elongated and linear in valve outline. The central part of valve has thickened area.
Sampangi Lake: The Genera identified were Cymbella and Nitzschia. Five species of Cymbella were observed and six species from Nitzschia were found.

Figure 6. Cymbella diatoms.
Class- Bacillariophyceae
Order- Cymbellales
These are the species with the vavles that are lanceolate and dorsiventral with bluntly rounded apices. The dorsal margins are moderately arched.

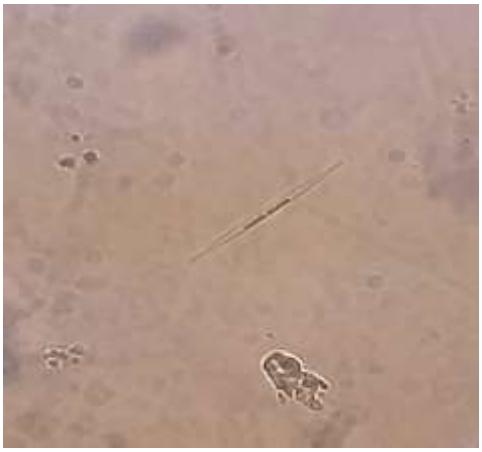


Figure 7. Nitzschia diatoms .
Class- Bacillariophyceae
Order- Bacillariales.
These are commonly pennate form of diatoms. Cells are elongate, fusiform or rectangular in girdle view.
Lalbagh Lake: The Genera identified were Synedra. The large number of synedra species were found in the sample.

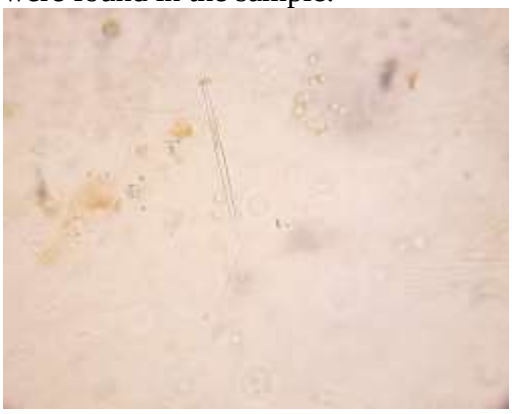


Figure 8. Synedra famelica diatoms**.**
Class- Fragilariophyceae.
Order- Fragilariales.
These are characterised by the cells clustered together at one point by a mucilage cushion that is secreted from the pore field on each cell.

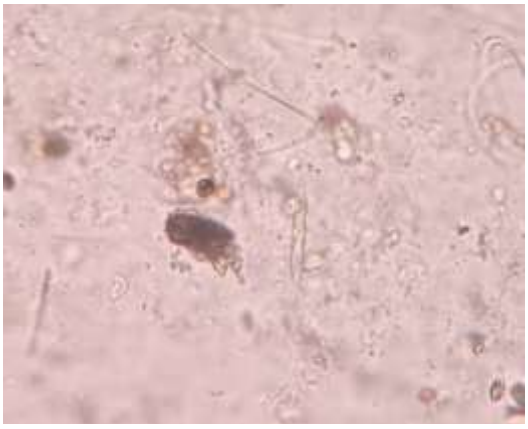


Figure 9. Synedra mazamaensis diatoms.
Class- Fragilariophyceae.
Order- Fragilariales.
These are characterised by the cells clustered together at one point by a mucilage cushion that is secreted from the pore field on each cell.
Ulsoor Lake: The Genera that were identified are Scoliopleuria and Nitzschia.
Four species of Scoliopleuria were found and seven species of Nitzschia were found in the sample.

Figure 10. Scoliopleuria diatoms.
Class- Bacillariophyceae
Order- Naviculales
These are characterised by the valves that are arched and elliptic to linear lanceolate with cuneate apices. Valves are slightly twisted. The longitudinal canal present on each side of the raphae.

Figure 11. Nitzschia diatoms.

Class- Bacillariophyceae

Order- Bacillariales.

These are commonly pennate form of diatoms. Cells are elongate, fusiform or rectangular in girdle view.

Table 1: Genera Observed

1.	Cyclostephanos
2.	Nitzschia
3.	Aulacoseira
4.	Cymbella
5.	Araphid
6.	Synedra
7.	Scolioplueria

LIMITATIONS:

The limitations of this work are, the water samples were collected only from only one or two zones of water body as there was no permission to enter the water body from other zones. The remaining water body may contain other varieties of Diatom species which may have got un-noticed. The environmental conditions may also have their effect on the reproduction process of the Diatom species. The observation through the microscope may hinder the clarity of the image due to different varying factors.

IV. DISCUSSION AND CONCLUSION:

Qualitative and Quantitative analysis of Diatoms can be done by the detection of Diatoms in the samples and also by identifying the species present in the samples. The Results of this study should also be interpreted in the context of post-mortem reports and police investigations. The Diatom test is significant even occasionally that diatom may also have been recovered from the internal organs of Non- drowning body. Since diatoms vary on basis of morphological and ecological characteristics, it is possible to find the location of Drowning. Further continuation of this study can be taken up and the Diatomological map of the entire region of Bangalore city can be prepared. Based on the study of drowning victims, where the diatoms are present in the drowning medium, the flow of diatoms into the alveolar system and also the blood stream has been caused by the inhalation of water by the drowning victim and further, which leads to the flow and accumulation 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 testing and detection of diatom. While solving drowning cases, a correlation between the diatoms extracted from these given tissue samples of an individual and the samples obtained from the targeted drowning medium has to be found and established for the successful determination and detection 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 (Rohn E J et al, 2006).

V. REFERENCES:

1. Rana, A., Manhas, S. (2018, July 12). Significance of diatoms in diagnosis of drowning deaths: A review. https://doi.org/10.32474/PRJFGS.2018.01.000121

2. Harding,W.R., Archibald,C., Taylor,J.C. (2007, January). A Methods Manual for the Collection, Preparation and Analysis of Diatom Samples. (PDF) A Methods Manual for the Collection, Preparation and Analysis of Diatom Samples (researchgate.net)

3.Scott,K.R.,Jones,V.J., Cameron, N.G. ,Young , J.N.,Morgan, R.M. (2021,July 3). Freshwater diatom persistence on clothing I: A quantitative assessment of trace evidence dynamics over time. https :// doi. Org / 10. 1016/j.forsciint.2021.110898

4.Alakananda, B, Mahesh, K.M. Ramachandra T.V. (2013,December). Role of environmental variables in diatom distribution in urban wetlands of Peninsular

India. (PDF) Role of environmental variables in diatom distribution in urban wetlands of Peninsular India (researchgate.net)

5. Cameron, N.G. (2004,January 1). The use of diatom analysis in forensic geoscience. https://doi.org/10.1144/GSL.SP.2004.232.01.25

6. Gautam, Shristy. (2019). Diatoms in Forensics: A Molecular Approach to Diatom Testing in Forensic Science. 10.1002/9781119370741.ch18.

7 Levkov, Zlatko & Williams, David & Nikolovska, Dragica &Tofilovska, Slavica & Cakar, Zdravko. (2017). The use of diatoms inForensic science: advantages and limitations of the diatom test in cases of drowning. TheArchaeological and Forensic Applications of A Deeper Understanding of Human History.

8. Diatoms and Forensic Science. Published online by Cambridge University Press: 21 July 2017Benjamin P. Horton

9. Krstic, S., Duma, A., Janevska, B., Levkov, Z.,Nikolova, K., & Noveska, M. (2002). Diatoms in forensicexpertise of drowning—a Macedonian experience. Forensicscience international, 127(3), 198–203.https://doi.org/10.1016/s0379-0738(02)00125-1

10. A review on diatoms of freshwater bodies of Rajasthan. European Journal of Molecular & ClinicalMedicine, (2020). 7(7), 4657-4664198–203.

11. Sudhakar, G., Jyothi, B., & Venkateswarlu, V. (1994). Role of diatoms as indicators of pollution gradients. Environmental monitoring and assessment, 33(2), 85–99. https://doi.org/10.1007/BF00548591 24

12. Shaikh, Junaid & Ghuge, Arun & Sangle, Sandeep & Suryawanshi, Sagar. (2021). Diatomological mapping of Aurangabad Region for Unrevealing drowning Mystery. 10.21203/rs.3.rs-235655/v1.

13. Morin, S., Duong, T. T., Dabrin, A., Coynel, A., Herlory, O., Baudrimont, M., Delmas, F., Durrieu, G., Schäfer, J., Winterton, P., Blanc, G., & Coste, M. (2008). Long-term survey of heavy-metal pollution, biofilm contamination and diatom community structure in the Riou Mort watershed, South-West France. Environmental pollution (Barking, Essex : 1987), 151(3), 532–542. https:// doi.org/ 10.1016/ j. envpol .2007.04.023

14. Lowe, Rex. (1974). Lowe, R. L. 1974. Environmental requirements and pollution tolerance of freshwater diatoms. EPA-670/4-74-007, 340Pp..

15. Ivorra, Núria & Barranguet, Christiane & Jonker, Martijs & Kraak,Michiel & Admiraal, Wim. (2002). Metal-induced tolerance in the freshwater microbenthic diatom Gomphonema parvulum.Environmental pollution (Barking, Essex : 1987). 116. 147-57.10.1016/S0269-7491(01)00152-X. olerance

16. Bere, T., Mangadze, T., & Mwedzi, T. (2014). The application and testing of diatom-based indices of stream water quality in Chinhoyi Town, Zimbabwe. Water SA, 40(3), 503-512.

17. Fucci, N., Pascali, V. L., Puccinelli, C., Marcheggiani, S., Mancini, L., & Marchetti, D. (2015). Evaluation of two methods for the use of diatoms in drowning cases. Forensic science, medicine, and pathology, 11(4), 601-605.

18. Vinayak, V., Goyal, M. K., Mehta, R., Mishra, V., Rai, A., & Kumar, A. (2011). The role of diatom test in aquatic dismemberment and drowning. J Forensic Med Toxicol, *28(2)*

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