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The Role of Microalgae in Solving Drowning Death Mysteries

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ABSTRACT

Forensic limnology is a branch of forensic science that focuses on the study of inland waters and their ecosystems. It plays a crucial role in various forensic investigations related to aquatic environments. Forensic limnology involves the analysis of the physical, chemical, and biological properties of lakes, rivers, ponds, and other water bodies, providing essential insights into cases such as drowning, disposal of evidence in water, connecting crime scenes, victims, and suspects, and estimating the time elapsed since submersion. A particularly noteworthy element within forensic limnology is the microalgae (phytoplankton), which are tiny, ubiquitous, and diverse organisms that thrive in the upper regions of water, where sunlight is abundant. Microalgae are invaluable tool in aquatic forensic investigations due to their microscopic size, not only they can easily enter the victim's body (ante-mortem drowning), but they can also easily transfer to the victim's body or belongings such as clothing and shoes. Forensic limnology is thus critical for examining the aquatic ecosystem and the evidence it contains, aiding in the resolution of various types of crimes. This review paper outlines the importance of forensic limnology in solving crimes in the aquatic environment. It emphasizes the role of microalgae (phytoplankton) in forensic science and demonstrates their applications in determining drowning cases, estimating postmortem submersion intervals, and establishing links between suspects, victims, and crime scenes. The chapter aims to raise awareness among forensic pathologists, forensic scientists, and forensic medico-legal experts about the potential utility of microalgae in solving the mysteries of cases involving aquatic environments.

Key words: microalgae, diatoms, drowning deaths, forensic limnology, immersion cases

Background

Forensic limnology is a branch of forensic science that makes an important contribution to a wide range of forensic investigations. The term limnology comes from the Greek, limne, meaning "lake", and logos, meaning 'knowledge". It is a subfield of environmental science or aquatic ecology concerned with the study of various properties (biological, chemical, physical, and geological) of inland waters. These may include flowing or standing, fresh or saline waters, either natural or man-made. This study encompasses lakes, ponds, rivers, springs, wetlands, and streams. Limnology also involves the study of aquatic organisms (e.g., plankton), linking it not only to environmental sciences but also to forensic sciences. Since many crimes are committed in or near water, analyzing the plankton present can help investigators link suspects and victims to a crime scene during a particular time of year. Planktons are microscopic organisms that live in freshwater and seawater. They are mainly divided into two types: microalgae and zooplankton. Microalgae can synthesize their own food through photosynthesis, while zooplankton feed on other organisms, such as microalgae, small crustaceans, and the eggs and larval stages of larger animals. Examples of zooplankton include cnidarians, amphipods, mysids, etc. Among plankton, microalgae are especially useful in forensic science for two reasons: Firstly, the cell walls of some microalgae species, such as dinoflagellates, diatoms, and green algae, are composed of a hard exoskeleton enabling them to withstand harsh environmental conditions (Díaz-Palma et al., 2009). Secondly, these organisms are highly diverse and ubiquitous in nature, making them valuable forensic evidence in a range of forensic investigations, including drowning cases, estimation of post-mortem submersion intervals, and linking suspects or victims to specific aquatic crime scenes.

Microalgae are tiny, ubiquitous, and often beautiful organisms that grow in the upper layers of water where sunlight is abundant (NOAA, 2024). They are found in both saline and freshwater ecosystems and represent one of the most important components of the natural food chain (Davis, 2015). Microalgae consist of a variety of microscopic unicellular and multicellular forms that occurs in different shapes, sizes, and colors that can be used to identify genera and species (Hall, 1997, Prescott, 1964; Reynolds, 1984; Wehr, Sheath and Kociolek, 2015). Their widespread occurrence in aquatic environments, along with their potential to distinguish between different aquatic habitats, makes them valuable in forensic investigations. Due to their microscopic size and invisibility to the naked eyes, microalgae can adhere to suspects, victims, their clothing, or crime weapons, thereby helping to link an individual to a specific crime scene (Siver et al., 1994). Many studies have reported that microalgae are a tool for investigating the environmental condition of a water body (Cheshmedjiev et al., 2010; Gao & Song, 2005; Padisák et al., 2006; Parmar et al., 2016; Pasztaleniec & Poniewozik, 2010), but their application in forensic science has been comparatively unexplored.

While numerous studies have reported the use of microalgae been made to review the significance and application of microalgae, alongside with diatoms, in routine drowning diagnosis and related forensic analysis. This review does not propose the formal inclusion of microalgae in forensic protocols, but rather compiles real cases and existing research demonstrating their potential role in forensic science.

MICROALGAE AS EVIDENCE

Because of their unique characteristics, microalgae play an important role in the forensic investigation of drowning cases.

Size of Microalgae

The sizes of microalgae range from small unicellular forms (about 1 μm) to large colonies of blue-green algae (up to 200 μm) as specified in Table 1. Based on their characteristic size, microalgae can be classified into four types:

Table 1. Size Range of Different Types of Microalgae.

Category	Size range (μm)	Organisms
Picoplankton	0.2–2	*Aphanothece smithii* (Blue green algae)
Nanoplankton	2–20	*Achnanthes subhudsonis, adlafia multnomahii* (Diatom), *Tetraedron regulare, Chlorella vulgaris* (Green algae)
Microplankton	20–200	*Amphipleura pellucida* (Diatom), *Cosmarium corda* (Green algae), *Ceratium furca* (Dinoflagellates).
Macroplankton	>200	*Synedra ulna, Gyrosigma* (Diatom), *Closterium aciculare* (Green algae)

Shape of Microalgae

Microalgae exist in a variety of shapes and forms, ranging from unicellular (single-celled) organisms to more complex multicellular forms. Cells may aggregate into colonies, which can be spherical or irregular in shape. Some cells align in chains to form filaments, which may be branched or unbranched. In addition, certain microalgae possess flagella, spines, and other ornaments on their surface. The shapes of microalgae vary widely; they may be spherical, oval, ovoid, rod-shaped, conical, or compound (a combination of one or more shapes). Prescott (1964) proposed the following terminology for the various forms of microalgae:

- ✧ Acicular: Needle-like shape e.g., *Nitzchia acicularis* (Figure 1).
- ✧ Arcuate: Curved or bow-shaped e.g., *Scenedesmus arcuatus* (Figure 1).
- ✧ Cylindrical: Two round circles present at both ends are connected by two parallel lines, e.g., *Oscilltoria* sp. (Figure 1).
- ✧ Discoid: Flat circular shape, disk-like e.g., *Cyclotella* (Figure 1).
- ✧ Ellipsoid: Elongated with curved margins with sharply round margins e.g., *Oocystis* sp., (Figure 1)
- ✧ Fusiform: Spindly shaped, broadest in the mid-region and tapering at both ends, e.g., *Navicula cupsidata* (Figure 1).
- ✧ Lanceolate: Longer than broad, e.g., *Closteridium* sp. (Figure 1)

- ✧ Lenticular: Lens-shaped, e.g., *Diplopsalis lenticula* (Figure 1)
- ✧ Lunate: Cresent-shaped, e.g., *Closteridium* sp. (Figure 1).
- ✧ Oblate: Flattened spherical, nearly globular shape (Figure 1).
- ✧ Oblong: Curved and elongated with broadly rounded ends e.g., *Euglena caudate* (Figure 1)
- ✧ Oval: Elongated and convex margins and symmetrically curved ends, e.g., *Lepocinclis* sp. (Figure 1)
- ✧ Ovoid: Egg-shaped, e.g., *lepocinclis* sp. (Figure 1).
- ✧ Pyramidal: Pyramid-like, with a pointed tip, and broad base e.g., *Gonyaulax* sp. (Figure 1).
- ✧ Reniform: Kidney-shaped, e.g., *Cryptomonad* sp. (Figure 1).
- ✧ Trapezoid: Four-sided figure with two parallel sides e.g., *Crucigenia fenestrate* (Figure 1).
- ✧ Quadrate: Square or rectangular shape, e.g., *Crucigenia crucifera* (Figure 1)

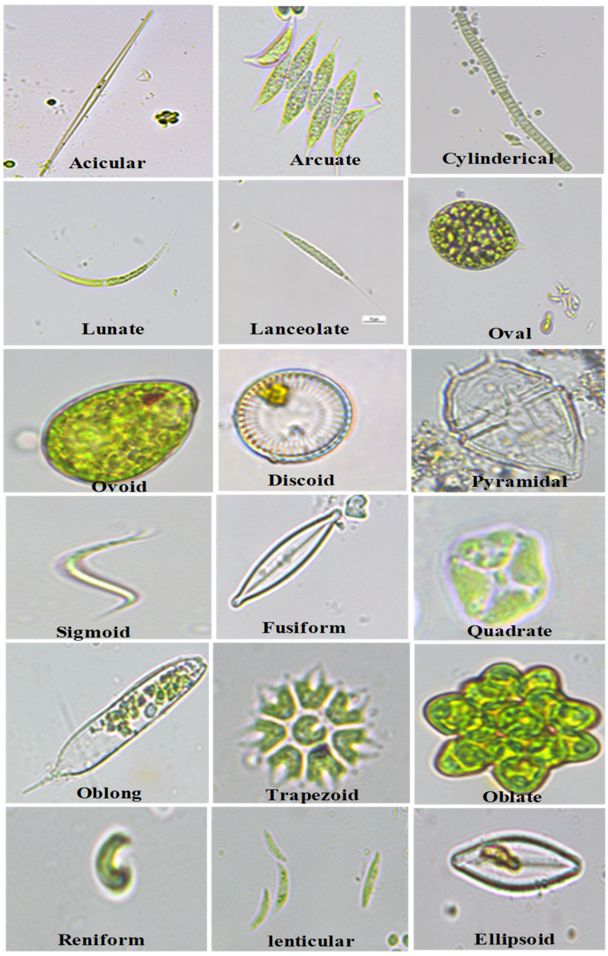

Fig. 1. Photomicrograph Showing Different Shapes of Microalgae

The Structure of Microalgae

Microalgae, or phytoplankton, include both eukaryotic and prokaryotic organisms that differ in their cellular organization. Among the various divisions of microalgae, cyanobacteria are the only division that includes prokaryotic organisms, while the others (algal divisions) contain eukaryotic organisms.

Microalgae differ greatly in the composition of their cell walls. In general, microalgae consist of two cell walls that enclose the cytoplasm in the middle (Figure 2). The outer cell wall is gelatinous and amorphous in nature, while the inner cell wall contains a fibrillar component that forms the structure of the cell wall. The outer cell wall has a different structure in the different genera. In the class Xanthophyceae, the cell walls contain pectin, while in the classes Chlorophyceae, Rhodophyceae, Phaeophyceae, and Dinophyceae, pectin is mixed with cellulose. In some classes of the microalgae division, coccoliths have calcareous scales, while in the Chrysophyceae, silica scales are present on the surface of the cell wall (Sharma, 1986). The representatives of the class Euglenophyceae lack the outer cell wall and are called "naked". They are surrounded by an inner cell wall called pellicle, which is rich in proteins. Various green algal taxa contain sporopollenin (also found in pollen grains) in their walls, which gives them strength and helps them withstand harsh environmental conditions (Burczyk & Dworzanski, 1988; De Vries et al., 1983), while the cell wall of prokaryotic cyanobacteria is made of peptidoglycan.

Within the cell wall, the cytoplasm forms a gel-like suspension that helps maintain the cell's osmotic balance. In eukaryotic microalgae, the cytoplasm contains various membrane-bound organelles such as mitochondria, the golgi apparatus, and the endoplasmic reticulum. At the center is the nucleus, enclosed by a double-layered nuclear membrane. Photosynthetic pigments such as chlorophyll a, chlorophyll b, and carotenoids, are embedded within the thylakoid (a membrane-bound structure inside the chloroplast). In contrast, prokaryotic cyanobacteria lack a well-defined nucleus that is not bounded by the nuclear membrane; instead, DNA fibrils hang freely in the cytoplasm. They also lack membrane-bound organelles such as mitochondria, the Golgi apparatus, the endoplasmic reticulum, etc. The thylakoid, which contains photosynthetic pigments such as chlorophyll a, carotenes, and xanthophylls, is also free in the cytoplasm. The generalized structures of eukaryotic and prokaryotic microalgae are shown in Figures 2 and 3. Table 2 specify the characteristics of microalgae and the class to which they are belonging.

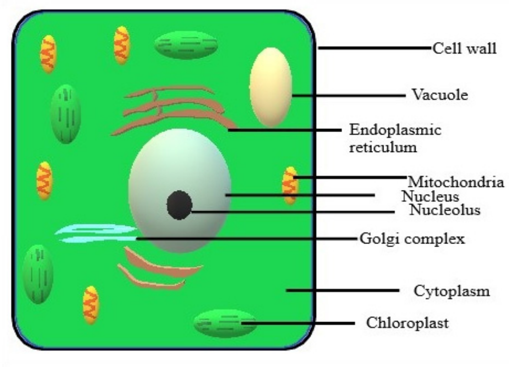

Fig. 2. Generalized Internal Structure of Eukaryotic Microalgae Cell.

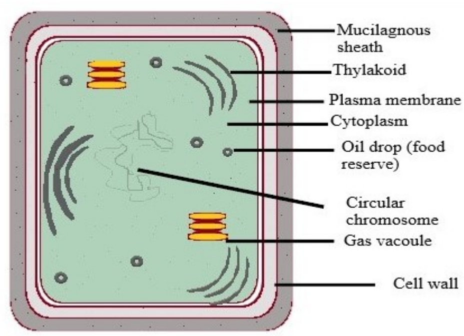

Fig. 3. Generalized Internal Structure of a Prokaryotic Cyanobacterial Cell.

Diversity and Classification of Microalgae

Aquatic ecosystems contain a wide variety of microalgae that differ in their morphological features, pigment types, presence or absence of flagella, and cell ornamentation (such as spines, bristles, etc.). These variations form the basis for classifying microalgae into seven algal divisions. Microalgae also exhibit significant diversity in their mode of reproduction, growth patterns, and community assemblages, which can vary depending on geographical location and environmental factors. This diversity in microalgae assemblage among different water bodies aids in the forensic characterization of aquatic environment based on microalgae assemblage. In the present study, algal divisions are classified according to Smith (1950). Among the seven algal divisions, Rhodophyta and Phaeophyta inhabit in oceans while other divisions have a cosmopolitan distribution, occurring in both terrestrial and the aquatic ecosystem (freshwater and marine).

Table 2. The Characteristics of Microalgae and the Class to which they Belong.

Divisions	Classes	Photosynthetic pigment	Cell wall composition
Chlorophyta	• Chlorophyceae • Charophyceae	Chlorophylls a and b. xanthophylls, carotenes	Polysaccharides or cellulose or cell wall absent
Chrysophyta	• Chrysophyceae • Bacillariophyceae • Xanthophyceae	Chlorophylls a and c and fucoxanthin	Cellulose or no cell wall Some with silica or calcium Carbonate
Euglenophyta (with 2 orders)	• Euglenophyceae	Chloropliylls a and b. carotenes in gencra with chloroplasts	No cell wall: protein –rich Pellicle
Dinophyta	• Cryptophyceae • Dinokonte • Desmokontae	Chlorophylls a and b and peridinin (a carotenoid)	Cellulosic plates known as Theca
Cyanophyta	• Cyanophycea	Chlorophylls a, phycoerythrin (red), Phycocyanin blue), allophycocyanin	Peptoglycan matrices or walls.

Role of Microalgae in Forensic Investigations

Microalgae can be identified and classified based on their characteristics such as shape, size, colour, cell wall composition, etc., making them valuable evidence in forensic investigations. To date, only a limited number of studies have reported the use of microalgae in forensics.

LINKING SUSPECT AND VICTIM TO A CRIME SCENE

Establishing a connection between a suspect, and victim, and a crime scene is essential for case reconstructing. In some instances, the body is discovered far from the actual crime scene. To conceal the crime perpetrator may dispose of the body and the murder weapon in a water body, making the murder appear to be an accident or suicide. In such cases, microalgae present in the water can be compared with those found on a suspect's clothing, footwear or belongings. For instance, in a case study from suburban Connecticut, USA, two boys were fishing at a pond when they were attacked by several teenage assailants. The victims were beaten, bound with duct tape, and thrown into the pond to drown. One of the boys managed to escape and seek help from local residents. In this case, both the suspect and victim were linked to the crime scene through the analysis of their footwear. Various species of diatoms and scaled Chrysophytes (planktonic algae) were recovered from the shoes and matched with reference samples from pond sediment (Siver et al., 1994).

INVESTIGATION OF DROWNING-RELATED CRIME SCENE

Investigating the cause of death in bodies recovered from water is one of the most difficult and demanding tasks for forensic investigators. In such cases, the coroner must not only determine the cause of death but also opine the equally important question of where the drowning actually occurred. Traditionally, coroners rely on the detection of diatoms (microalgae) in tissue samples from drowned bodies. However, under certain environmental conditions or at a specific time of the year, diatom concentration may be very low. Researchers have found that other type of microalgae (microalgae) can also provide crucial circumstantial evidence, helping not only to answer these questions but also to establish links between suspects, victims, and the crime scene (Hall, 1997; Keiper & Casamatta, 2001; Siver et al., 1994). The significance of microalgae in solving drowning cases became evident when Chardez and Lambert (1985) reported the presence of aquatic organisms such as chlorophyta, dinoflagellates, invertebrates, protozoan ciliates, and bacteria in the blood of drowned individual. Later, Qu and Wang (1992) introduced a new approach by examining chlorophyll (a) of plankton in the lung samples of two drowned persons using a spectrofluorophotometer (SFPM). This method allowed accurate diagnosis of drowning, leading them to conclude that Chl. detection in lung tissues could serve as a reliable diagnostic method in forensic practice. Further advancement came with the development of Soluene-350 method, developed by Yoshimura et al. (1995) for extracting microalgae from the tissues of drowned victims. This method was applied to bodies recovered from the Yodo River in Osaka, Japan. Analysis revealed many types of green algae and diatoms, which were still detected in the river water samples, even a month later. In case study 1, diatoms such as Melosira and Staurastrum, were identified in lung samples, while a fragment of Navicula was found in the liver. In case 2, species such as Navicula, Cymbella, Staurastrum and a species of Zygnemataceae were found in the lungs and liver of a drowned individual. Díaz-Palma et al. (2009) extended and standardized the microalgae detection procedure as an enhancement of the traditional diatom test. They concluded that dinoflagellates and certain chlorophytes possess strong cell walls compared to diatoms, allowing their recovery from drowned tissues. Kim (2011) analyzed samples from three water bodies in the Gwanju area of South Korea, where drowning incidents are frequently found. He detected 16 diatom species, 20 green algae species, 6 cyanobacteria species, and 6 other algae species.

Molecular biology has also been employed to complement traditional morphological methods. Kane et al. (1996) designed two sets of specific primers for picoplankton Synechococcus (Cyanobacteria) abundant in Lake Biwa, Japan. Using PCR amplification of 16S rDNA segments, they successfully detected picoplankton DNA in lung tissues fixed in formalin. He et al. (2008) developed a sensitive PCR-DGGE (Denaturing Gradient Gel Electrophoresis) method for analyzing the 16S rDNA of plankton in drowned and non-drowned rabbits, as well as in two human drowning cases. They used primers, forward and reverse primers used for the amplifying the region of 16S rDNA were CYA-F 5′-GGGGAATYTTCCGCAATGGG-3′, CYA-R (a) 5′ GACTACTGGGGTA TCTAATCC CATT-3′ and CYA-R (b)5′ GACTACAGGGGT ATCTAATCCCTTT -3′. Planktonic DNA was extracted from the lungs, liver, kidneys, blood, and brain of drowned humans and rabbits, whereas planktonic DNA was found only in the lungs of non-drowned rabbits, which may be due to passive water ingress. Other researchers have also applied PCR-based technique. Abe et al. (2003) and Suto et al. (2003) used primers targeting chlorophyll-related genes (Euglena gracilis EG1 and EG2) and fucoxanthin-chlorophyll a/c harvesting proteins (Skeletonema coastatum, SK1 and SK2). Chen et al. (2013) designed primers D512 (18S) and D978 (18S) for the V4 region of 18S rDNA, enabling genus-level diatom identification, though species-level diagnosis was not possible. They suggested that this molecular approach could complement conventional diatom test. Since accurate identification depends heavily on the complete reference sequence in databases. Rácz et al. (2016) applied the DNA-PCR technique in four drowning cases. DNA was extracted from microalgae such as diatoms, green algae, and blue-green algae from both postmortem tissue and water samples. However, while diatom DNA was not always detectable, DNA from other algal groups was successfully extracted, reinforcing the importance of expanding forensic focus beyond diatoms to include other microalgae samples.

MICROALGAE AS INDICATORS OF POSTMORTEM-SUBMERSION INTERVAL (PMSI)

Microalgae have also been investigated for their potential in estimating postmortem intervals. While studying insect colonization on submerged corpses, Merritt and Wallace (2009) observed algal colonization and proposed that algae could serve as indicators of postmortem submersion due to their diversity and abundance in the aquatic environment. They speculated that algae could be used to determine postmortem inundation interval due to their diversity and abundance in aquatic environments.

Casamatta and Verb (2000) tested this hypothesis by submerging rodent carcasses in a mid-order river. The experiment involved immersing rats in both a low-flow pool and a high-flow run for 31 days. Carcasses were retrieved after 3, 8, 12, 17, 21, 25, and 31 days to study the algal succession. Result showed extensive algal flora colonization as decomposition progressed. After 17 days, algal colonization increased at both sites, and after 21 weeks, the colonization process slowed down and stabilized. Initial colonization was dominated by diatoms, followed by green algae (desmids) and additionally diatom species after 17 days. The green algae, such as desmids, colonized the submerged rats quickly and proved to be a good indicator of the post-submergence period. Haefner et al. (2004) further investigated the relationship between immersion time and algal colonization by submerging six pigs and six ceramic tiles in two water bodies (a low-flow pool and a high-flow run). Samples were collected every four days, and colonization was assessed using chlorophyll-a concentration. Stronger algal colonization was consistently observed on pig substrate compared to ceramic tile, and a clear correlation was found between immersion time and chlorophyll concentration. Zimmermann et al. (2008) conducted similar semi-quantitative studies, immersing piglets and ceramic tiles in two ponds of similar salinity. They found that diatom diversity was greater on piglet carcasses than on ceramic tiles substrate. This suggested that with animal model provided more accurate representation of algal colonization in drowned body and that non-biological substrates such as tiles or stones, are unsuitable substitutes for studying PMSI in aquatic environments.

CONCLUSIONS

From the present study, it can be concluded that microalgae provide valuable evidence in forensic investigations, which not only assist in solving drowning-related cases but also can establish links between the suspect, the victim and the crime scene. In addition, their role in determining the postmortem submersion interval (PMSI) cannot be overlooked. Despite their potential, relatively few studies have focused on the forensic application of microalgae. Since the development of the diatom test in the 18th century, the methodology has remained largely unchanged. Forensic scientists continue to rely on the principle of detecting the presence or absence of diatoms in the tissue of drowned victims. However, when diatom concentrations are very low, they may not be detected, and their absence does not necessarily rule out drowning. Although microalgae testing is not yet a routine procedure, its importance has been demonstrated, such as in the juvenile case mentioned earlier, where microalgae evidence proved valuable in court. However, since the test procedure has not been fully validated across all cases, further research is essential. It is therefore necessary to consider an additional group of microalgae beyond diatoms, when interpreting forensic findings to ensure more reliable and comprehensive results.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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