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Continuous River Monitoring of the Diatoms in the Diagnosis of Drowning

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ABSTRACT: The diagnosis of drowning is one of the most difficult in forensic pathology. Diatom analysis has been proposed to provide supportive evidence of drowning but the reliability and applicability of quantitative and qualitative diatom analysis in the diagnosis of drowning is still disputed in the literature. In order to further examine such cases, the authors report the development of a water monitoring system based on algae performed each month at three aquatic locations where drowning victims are frequently found. Water samples and stones were taken from the surface and from the bed of the river. This protocol was performed during 1993 with analyses both on water samples and human tissue samples (30 bodies). The diatom profile of the drowning sites was compared with the tissue analysis. The extraction of diatoms from the tissues was performed with an enzymatic digestion method using Proteinase K.

Results indicate that the monitoring of river diatom populations is an accurate method of generating profiles of the river flora, which can then be compared with the diatom genera found in tissues.

KEYWORDS: forensic science, forensic pathology, death, drowning, diatoms

The diagnosis of drowning is one of the most difficult in forensic pathology (5,18). Diatom analysis has been proposed by several authors (8,9,14,15,16,17,21,22) as a means of providing supportive evidence for drowning. However, the relationship between diatoms and a diagnosis of death by drowning is very controversial because diatoms have been found at autopsy in non-drowned victims.

Several investigators, Gylseth and Mowé (7), Schellmann and Sperl (20) and Foged (6) are of the opinion that since diatoms can be found at autopsy in non-drowned subjects, they cannot be used as definitive evidence of drowning. On the other hand, Peabody (16,17) and Auer and Mottonen (1) believe that accurate diatom counts can, in most cases, discriminate between drowning and non-drowning cases. Such analyses are considered positive if 20 diatoms are identified per 100 μL of pellet obtained from a 10 g lung sample. For other organs, more than 5 complete diatoms, with exclusion of fragments, are required as an indication of water aspiration (12).

The purpose of this study is to develop a water-monitoring system based on diatoms recovered at sites where drowning victims are frequently found and to establish a database for comparison with diatoma recovered from victims' tissues.

When reference water samples taken at a site of submersion are not available, we propose using a system that continuously monitors the numbers and genera of diatoms present at a site of frequent drownings in order to establish a data base with which one can compare the diatoma present in the tissues. In fact, in the studies related to water quality, diatoms appear to present numerous advantages because of their global sensitivity to pollution and their resistance to organic pollution (4). These advantages lie in their cosmopolitan distribution and high diversity. Diatoms are also easy to collect and to store for reference purposes and constitute good indicators of environmental changes for both short and long periods.

Material and Method

Water Samples Analysis

Water samples were taken each month by the same person at three sites (river Ill, canal de la Marne au Rhin, canal du Rhône au Rhin) where drowning victims are frequently found. The diatom samples were taken by scraping the stones in the bed of the river or on the edges of the canals. The scraped surfaces were approximately 20 cm^2.

Diatom sampling was performed using standard techniques and ensured that glass containers and reagents were uncontaminated. A complete taxonomic analysis of the diatoms recovered from water samples and from the organs of the deceased was conducted (2,3).

This study was performed from April to November 1993. Samples were centrifuged at 2500 rpm for 15 min and diatoms were cleaned by incubation in hydrogen peroxide (130 vol%) at 80°C for 12 h. The fluid obtained was allowed to cool at room temperature and centrifuged at 2500 rpm for 15 min. The supernatant was decanted and replaced with diatom-free distilled water. The process was repeated three times until the fluid was transparent with a final spin at 3000 rpm to produce a pellet. After removing the supernatant, the sediment was air dried and mounted in Naphrax®. Examination of 300 diatoms was performed under light microscope (Zeiss®, Germany) equipped with an immersion objective (1000 ×) in order to determine the diatom taxa. The diatoms were identified using the flora of Krammer and Lange Bertalo (11). The determination of the dominant taxa was performed on the 300 examined frustules.

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Tissue Samples Analysis

Diatom analyses were conducted on material collected during 30 autopsies of bodies (18 males, 12 females) recovered by the Institut de Médecine Légale.

During the autopsy, 10 g of each of the following samples were taken for diatom analysis: a segment of peripheral lung tissue, kidney, liver, and brain. These samples were removed without contamination during the autopsies and minced with scissors. The tissues were then rinsed and mixed with 500 μL of 10 mg/mL Proteinase K and 100 mL of 0,01M Tris-Hcl buffer (pH: 7.5) containing 2% SDS (10,13).

The mixture was incubated at 50°C overnight; another 500 μL of Proteinase K (Boehringer Mannheim, Germany) was added and the solution was diluted with 100 mL of distilled water and centrifuged at 3000 rpm for 15 min, the upper layer was then removed. The sediment (100 μL/slide) was transferred onto a coverglass, mounted in Naphrax® and examined under the light microscope. Prior to the analysis, the SDS and Proteinase K buffers were checked for diatoms and filtered.

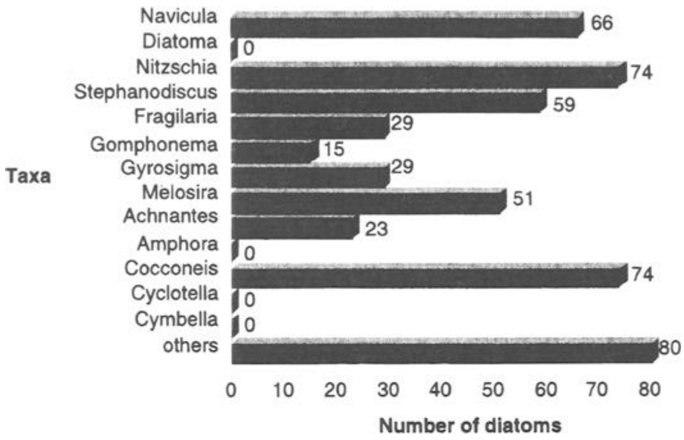

FIG. 3—*Dominant taxa in June (Site 3). Presentation of the dominant taxa from a water sample (% diatoms) taken at site 3 in June.* Nitzschia *and* Cocconeis *are the dominant taxa (%) followed by* Navicula *and* Stephanodiscus. Diatoma, Amphora, Cyclotella *and* Cymbella *were not found in the water samples.*

Results

The results of water monitoring conducted during the month of June are shown in Figs. (1,2,3). Only 13 taxa were considered during the study, the others were pooled in the group "other taxa" because of their low dominance.

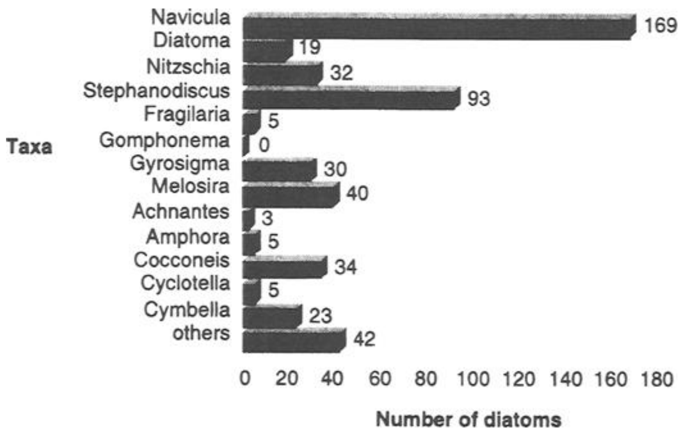

FIG. 1—*Dominant taxa in June (Site 1). Presentation of the dominant taxa from a water sample (% diatoms) taken at site 1 in June.* Navicula *is the dominant taxa followed by* Stephanodiscus *(%).*

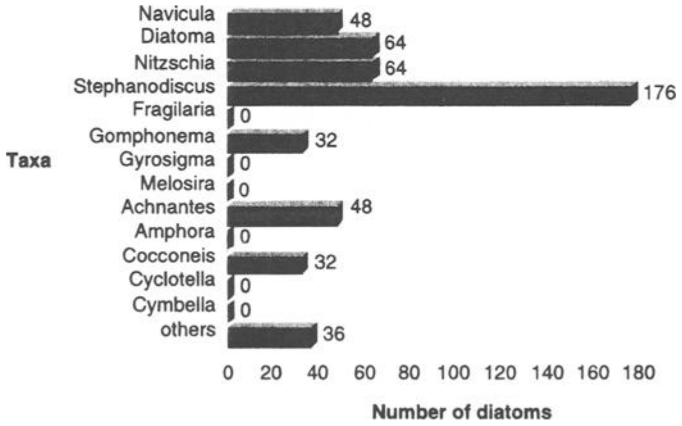

FIG. 2—*Dominant taxa in June (Site 2). Presentation of the dominant taxa from a water sample (% diatoms) taken at site 2 in June.* Stephanodiscus *is the dominant taxa followed by* diatoma *and* Nitzschia *(%). We found no* Fragilaria, Gyrosigma, Melosira, Amphora, Cyclotella *or* Cymbella.

We observed a variation in the diatom flora by month and by site. This observation confirms earlier studies on diatoms (19). At a particular site, for a particular period, certain taxa (1 or 2) were dominant and quasi characteristic for that water site.

For example, the dominant taxa in May were *Diatoma, Cyclotella* and *Achnantes* at site 1, *Gomphomena* at site 2 and *Navicula* at site 3. In June, *Navicula* was dominant at site 1 (Fig. 1). *Navicula* and *Nitzschia* were observed in the same concentration at site 2 (Fig. 2) with *Stephanodiscus* being the dominant taxa. At site 3, *Nitzschia* and *Cocconeis* are dominant (Fig. 3).

The results of this monitoring (Fig. 4) showed that *Stephanodiscus* was dominant at site 2 from June to October, *Achnantes* at site 1 from July to October (Fig. 5). *Navicula* was dominant for the entire duration of the study at site 3 (data not shown).

In all the bodies examined, the chest cavity was closed and there was no direct contact between the immersion water and the lungs due to decomposition of the thorax. Diatoms were found in 27 lung samples, three kidney samples, five liver samples and one brain sample (Table 1).

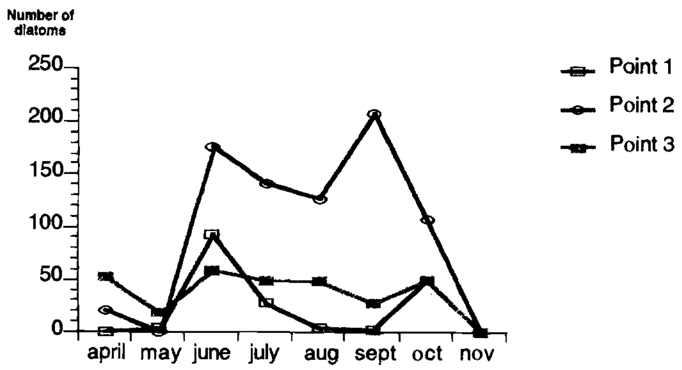

FIG. 4—*Seasonal variations of* Stephanodiscus *for the three points. The graphic presents the seasonal variations of* Stephanodiscus *for the three points. The concentration of* Stephanodiscus *is dominant from June to October in the samples taken from point 2. This taxa is absent at point 1 from August to September.*

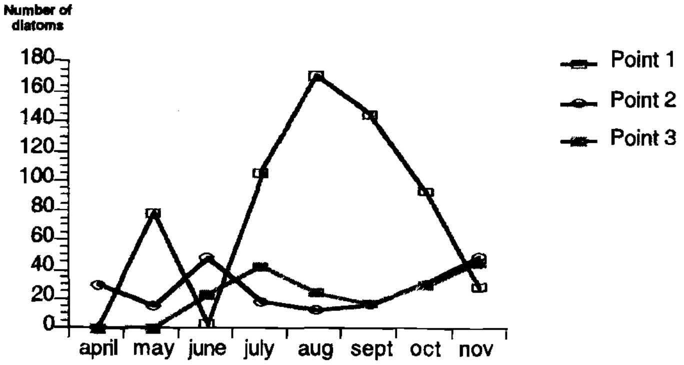

FIG. 5—*Seasonal variations of* Achnantes *for the three points. The graphic presents the seasonal variations of* Achnantes *for the three points. We observed a growth of this taxa during July and August.* Achnantes *is dominant at point 1 from July to October.*

In all cases where diatoms were recovered from the major circulatory organs, diatoms were also present in the lungs and vice versa. In 41% of the positive lung samples, more than 5 different taxa were identified. More than 60 diatoms/10 g of lung tissue were found in 66% of the samples. The maximum diatom content of samples from other organs was three different taxa and approximately 15 diatoms/10 g of tissue.

In nine cases, diatoms were found in water, lung and other organ samples. In 18 cases, diatoms were present in both water and lung samples but not in other organs. In three cases, diatoms were found in the water samples but not in the postmortem tissues. No diatoms were recovered from the reagents.

Similar morphological characteristics were found in the diatoms recovered from water, lung and other samples in the nine cases. They were considered positive cases, indicating death by aspiration of water into the lungs. The results of qualitative analysis in these cases showed good taxa correlation with the flora of the site of drowning when known (five cases).

Discussion

Diatoms are abundant and diverse in aquatic habitats. This uniqueness makes them of forensic value in cases of suspected drowning. The results of diatom analysis, however, must be interpreted in light of the possibility of contamination and the possible presence of diatoms in the lungs of non-drowned individuals. Diatoms can passively enter the lungs and upper air ways of persons exposed to submergence who have not drowned. Likewise diatoms can come into contact with internal organs when submerged bodies are exposed to trauma or decomposition. In these cases diatom analyses may prove helpful in associating victims with particular aquatic sites, but not in the determination of drowning.

In the case of putrefied bodies with open chest or abdominal cavities, diatom analyses can be performed only on bone marrow, which is a less contaminated tissue (21).

TABLE 1—*Victim analyses results.*

Cases	Water	Lungs	Brain	Kidney	Liver
18	+	+	−	−	−
3	+	+	−	+	−
1	+	+	+	−	−
5	+	+	−	−	+
3	+	−	−	−	−

The presence of diatoms in the organs of non-drowned persons has been used as one of the arguments against this method. In fact, few diatoms in the organs of non-drowned individuals have been reported. Pachar and Cameron (15) showed that control samples contain from 5 to 25 diatoms/100 g of lung tissue and a maximum of 10 diatoms/100 g in the closed organs. In our study, no diatoms could be recovered from the lungs and organs of non-drowned individuals (15 corpses).

The diagnosis of drowning by diatom analyses should be considered positive when the number of diatoms is above a minimal established limit in the lungs and in closed organ samples. This limit, according to our previous study (12), is 20 diatoms per 100 μL of pellet (obtained from 10 g of lung samples) and five complete valves for other organs.

Diatoms recovered from a victim's organs can be taken as an indicator of aspiration of water if the same taxa are also identified in the water samples at the site of discovery. This particular drowning group represented 30% of our series, which is between the 20% reported by Neidhart and Greedyke (14) and the 57.9% given by Auer and Mottonen (1).

A difficult interpretation problem was raised by the presence of diatoms in both water and lung samples and their absence in other organs. This group represents 60% in our series and is higher than the 30.8% reported by Auer and Mottonen (1). According to these authors, if the number of diatoms in the lungs is above the established limit, death by drowning can be assumed but no definitive conclusion can be reached because diatoms could have entered the lungs passively.

Conversely, only a small quantity of water may have been inhalated by such victims and therefore did not migrate in the closed organs. Timpermann (21) suggested that finding diatoms in the lungs alone is strongly indicative of rapid death after a short period of agony. When the comparison between the taxa found in water and in the tissues is not possible because of a lack of water samples, the results of the river monitoring and the profile of the dominant taxa may be of help. The comparison of dominant taxa may be done for the lungs but not for the other organs due to the shape and diameter of the great frustules which may not penetrate in the organs.

Finnish authors (1,3) have associated the diatom profile in the tissues of the victims with a specific aquatic site of drowning.

In this study, we found the same dominant taxa in the lung samples as in monthly diatom data base when a corpse was found at one of the three investigated rivers or canals. The diatom profile from the data base also matched the water samples taken at the site where the corpses were found.

The systematic sampling of locations where submerged remains are frequently encountered allows for the creation of a predictive diatom database. Such a database is suitable for comparison with recovered tissues. Comparison between the diatoms found in the tissues and the algae of the water site also allowed us to exclude the possibility of air inhalated diatoms before death.

The network established for water monitoring of diatoms must be extended to other rivers and streams to complete the network of the French water agencies in order to provide more extensive reference data bases for use in cases of drowning.

Conclusion

Quantitative and qualitative diatom analysis in victims found in the water can give strong evidence of death due to aspiration of water. Our experience showed that a positive diagnosis of drowning could only be made if significant numbers of diatoms of similar taxa were found in both water and lung samples as well as in other circulatory organs. The correct interpretation of the results involves a complete taxonomic analysis of the diatoms recovered from water samples and from the organs of the deceased. In the case of an absence of water samples, the continuous water monitoring of the diatom taxa is an appropriate tool for the knowledge of the river flora which can be compared to the diatom genera found in the body tissues especially the lung tissue.

If the diatom profiles of the tissues match those of the water, diatom analyses will be very useful for the forensic pathologist to state about the cause of death, such as drowning. The determination of the dominant taxa may also indicate the site of drowning.

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