Int J Legal Med (2000) 114 : 6–14

ORIGINAL ARTICLE

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Diatom detection in the diagnosis of death by drowning

Received: 29 March 1999 / Received in revised form: 1 September 1999

Abstract A medicolegal/algological collaboration lasting several years aimed at developing methods for dealing with dead bodies found in water where the circumstances are not clear, has led to an interdisciplinary procedure. To enable algological analysis, sample preservation and preparation must be free from contamination or carry-over at the beginning of the autopsy, although it should be noted that the demands on the digestion method are very high. One or more water samples from the site of drowning (from surface and bed) should be taken. Microscopic-algological analysis should record quantitative (diatom density), qualitative (species) and morphological (description of diatom valves) details for every sample. Furthermore, the species index and dominance identity similarity indices are calculated during the analysis procedure. The algological conclusions are based primarily on the separation values of Kater as well as on pair-matching. The final report is the result of interdisciplinary collaboration.

Key words Diatoms · Drowning · Diagnosis · Methods

Introduction

The method referred to in research papers as diatom detection is understood as involving the detection and identification of diatoms to aid in the algological diagnosis of death by drowning. During the last few decades, a large number of studies have thrown light on the widest possible range of aspects of this subject [3, 15, 26, 29, 33]. However, apart from some individual cases, little attention has been paid to the detailed explanation of the methodology of sample preparation, microscopic detection and subsequent analysis of the diatoms. Some studies involved searching for the presence of diatoms only [41], whereas in other studies the number of diatom valves was estimated semi-quantitatively [10, 25] or determined quantitatively by counting [2, 8, 13, 15, 21, 29, 35, 37, 38]. The range of species occurring in individual samples is often determined in a similarly inconsistent way [10, 13, 16, 17, 22, 23, 25, 36, 43]. Information on the species level has been provided by a number of authors [e.g. 8, 15, 32, 35, 41]. Morphometric information on the diatom valves (e.g. maximum valve length) is usually not recorded in a systematic way, but determined only in connection with specific questions [12, 22, 29, 40]. Although it is known from the studies carried out by Tamaska [40] that only quite small diatom valves or valve fragments can penetrate as far as the bone marrow (femur) during drowning (according to Tamaska < 15 μm), thus making a magnification of between 630 × and 1000 × absolutely necessary for their detection, researchers tended to use much lower magnifications, even after the publication of Tamaska's work. Burger [6] used 200 × magnification, Staak [39] 500 ×, Schellmann & Sperl [36] 250 × – 400 ×, Antonenko & Feris [1] 100 × – 400 × and Kater [13] 80 × – 320 ×).

In the absence of a standardised method it is hardly surprising that diatom detection has often been the subject of severe criticism [24, 28, 36, 38]. A number of scientific discussions would not have been necessary if more importance had been attached to methodology. Papers on the subject published in recent years [2, 15, 25, 27, 29] at least allow the assumption that this view is justified.

We would therefore like to offer some basic thoughts from a diatomological point of view and describe some of our own methodological experience and knowledge. Because we have so far been mainly concerned with the methodological aspects and with dead bodies found in water where the circumstances were unclear, and did not deal with routine cases, we shall refrain from providing any statistical analysis concerning sensitivity or specificity of the type published by various other authors [2, 15, 29].

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Table 1 The amount of material to be removed, the amount to be used for the diatom analyses, and the normal amount used for each prepared microscope slide

Sample	Total weight of sample removed	Total weight of sample used	Weight of sample per microscope slide[a]
Liver	Approx. 250 g	Approx. 180 g	Approx. 20–25 g
Kidney	One capsule, 100–250 g	Approx. 100–180 g	Approx. 10–25 g
Bone marrow	Approx. 10–15 cm of femur	All, approx. 5 g	Approx. 0.5–0.7 g
Contents of duodenum	Total contents	As much as possible	
Contents of stomach	Approx. 100 g	Approx. 50–100 g	Approx. 5–10 g
Blood	Approx. 20 ml	Approx. 10 g	Approx. 2.5–5 g
Lungs	Approx. 20 g	Approx. 5 g	Approx. 0.5–0.7 g
Drowning medium, water	Approx. 1000 ml	Approx. 50–100 ml	Approx. 5–10 ml
Drowning medium, sediment	Approx. 200 ml	Approx. 50–100 ml	Approx. 5–10 ml

[a] After dissolving, the amount of sample to be used is added to 4 ml of diatom-free water and 0.5 ml of the resulting mixture is used for each microscope slide

Methodology

Sample collection

The prerequisite for meaningful diatom analysis is the collection of samples that are free from contamination or carry-over. All samples to be collected must therefore be removed in the prescribed sequence at the beginning of the autopsy (Table 1).

Instruments required

Forceps, knife, scissors, ladle, disposable syringe (at least 20 ml), saw (oscillating or handsaw), thread, artery forceps, wash-bottle containing diatom-free water, plastic bags (e.g. Minigrip) and plastic cups (100 ml and 50 ml).

Diatom-free water

Water that has been purified by osmotic filtration and ultrafiltration or water that has been filtered using a microfilter with a maximum pore size of 5 μm.

The instruments and gloves must be rinsed liberally with diatom-free water from the wash-bottle before commencing sample collection as follows:

1. Wash the corpse with tap water. After preparing the soft tissues of the thorax, create a pleural window to check the degree of distension of the lungs.
2. When opening the thoracic cavity (by removing the sternum) take care not to touch the lungs. In situ, use scissors to detach several strips of tissue (total weight 10–20 g, approx. 3–4 pieces) from the surface of each lung (Fig. 1) to a depth of not more than 5 mm. Place these in a plastic bag.
3. After opening the pericardium, lift up the heart at its apex using forceps or artery forceps (Fig. 2) to uncover the pulmonary veins. Use a disposable syringe to take 20 ml of blood from the left atrium and/or a pulmonary vein and transfer without any chemical additives to a plastic test-tube.
4. In situ, remove a piece of liver tissue weighting about 250 g from the left lobe of the liver, using scalpel and forceps and place it in a plastic bag (Fig. 3).
5. Mobilise the duodenum and use thread or a clamp to apply a postpyloric ligature at the pars horizontalis duodeni. Open the duodenum laterally and use a ladle to transfer the contents to a plastic cup (Fig. 4).
6. Open the stomach at the greater curvature by means of a firm stroke of the scissors. Empty the stomach using the ladle and save the stomach contents (Fig. 5). If the liquid-aqueous part of the stomach contents is already visible with the naked eye it should be saved separately from the solid part. Otherwise let the stomach contents stand for a short time and then separate the aqueous part for diatom analysis.
7. Remove the intestinal organs and save an entire kidney including the capsule (Fig. 6) in a plastic bag. This step can also take place during the autopsy.
8. Wash the thigh skin with a liberal amount of water. Use the saw to remove a 10–15 cm long piece of the shaft of the femur (Fig. 7) and place in a plastic bag.
9. Water samples at the suspected drowning site should be taken and consist of at least 1000 ml of water from the surface (i.e. surface water/plankton sample). If the suspected site of drowning is a stream, a river-fed lake or near the shore of a lake, a second sample should be taken from the lake bed (200 ml, periphyton, sediment). In the case of the second sample at least the most prevalent substrate should be sampled. Typically, this will be stone, sediment or an aquatic plant covering. Suitable techniques are scraping off the surface of stones with brushes, siphoning off the surface of the sediment and gathering up the aquatic plants. After adding filtered formalin (4% final concentration), store all water samples in the dark.

Choice of digestion method

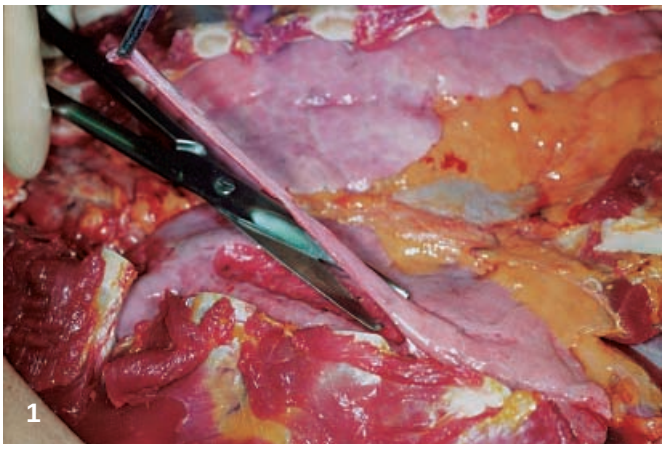
In principle we consider enzymatic digestion as described by Ludes et al. [16] to be very suitable. Our method is modelled largely on that described by Udermann & Schuhmann [42], Ranner et al. [32] and Kater [13] and is a quantitative digestion technique. We believe that the ideal digestion method should satisfy the following requirements:

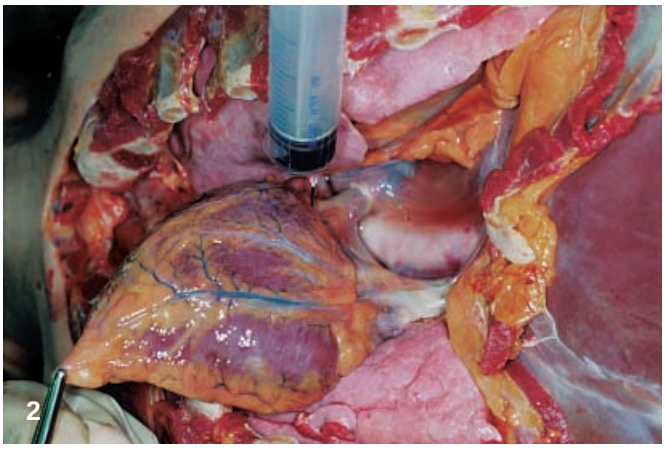
1. Only disposable equipment should be used for sample digestion to eliminate diatoms being carried over from one sample to the next.
2. For the lungs, blood and bone marrow the digestion method should be able to accept minimum sample amounts of between 5 g and 10 g, in the case of kidneys and liver the minimum amount should be 20 g. Smaller amounts considerably increase the time required for microscopy.
3. The quality of sample preparation must be high, i.e. the quantity of organic residues in the digested sample should be minimal.

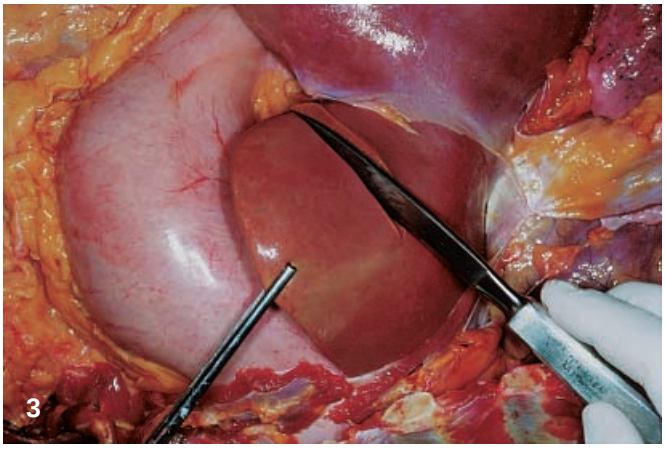
Microscopy

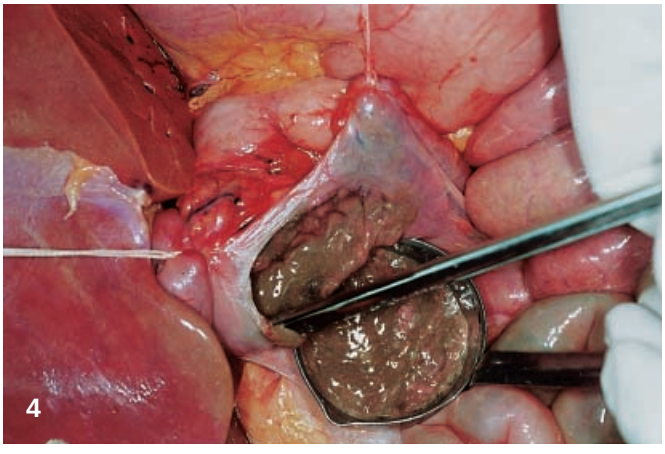
The diatoms were mounted in naphrax on a glass slide and examined using a magnification of 1000 × and oil immersion. Quantitative analysis and counting were carried out using phase contrast (100 × lens, oil immersion, 10 × eyepiece). The four standard works of Krammer & Lange-Bertalot [14] served as references for species identification.

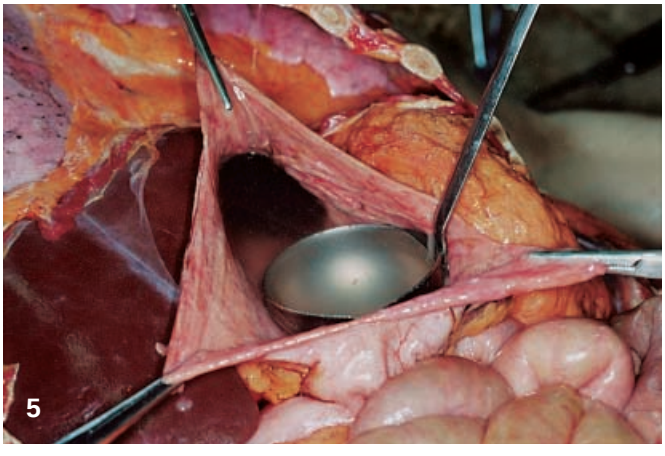
As far as possible, each diatom valve or valve fragment was identified and counted, and its maximum length measured. To simplify the length measurement, size classes with 5 μm increments (i.e. 0–5 μm, 6–10 μm, 11–15 μm, etc.) were employed. Counting was based on the valve or valve fragment. Cells comprising two valves or valve fragments were counted as two diatom units. Subsequently, the species and size category of each valve or valve fragment were listed.

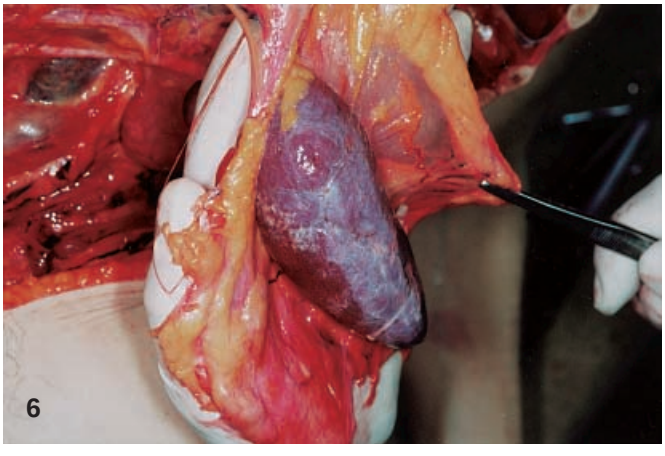












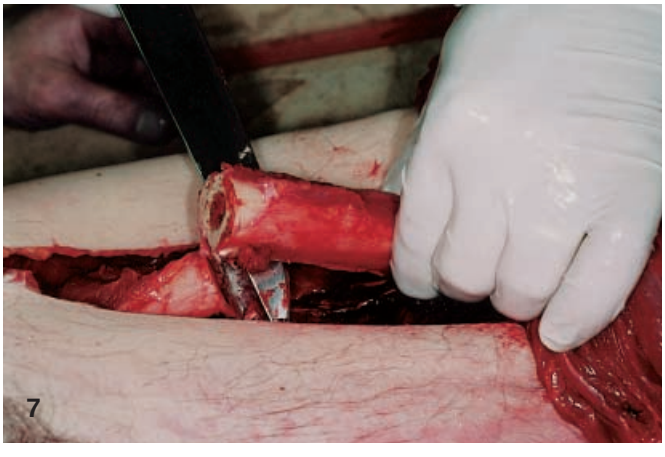


Fig. 1 Removing strips of peripheral lung tissue

Fig. 2 Blood puncture (from left atrium and/or pulmonary vein)

Fig. 3 Removing liver tissue from the left lobe of the liver

Fig. 4 Applying ligature to duodenum, opening the lumen

Fig. 5 Removing stomach contents, with aqueous part possibly already separated

Fig. 6 Removing kidney, collecting kidney with fibrous capsule

Fig. 7 Removing piece of the femur

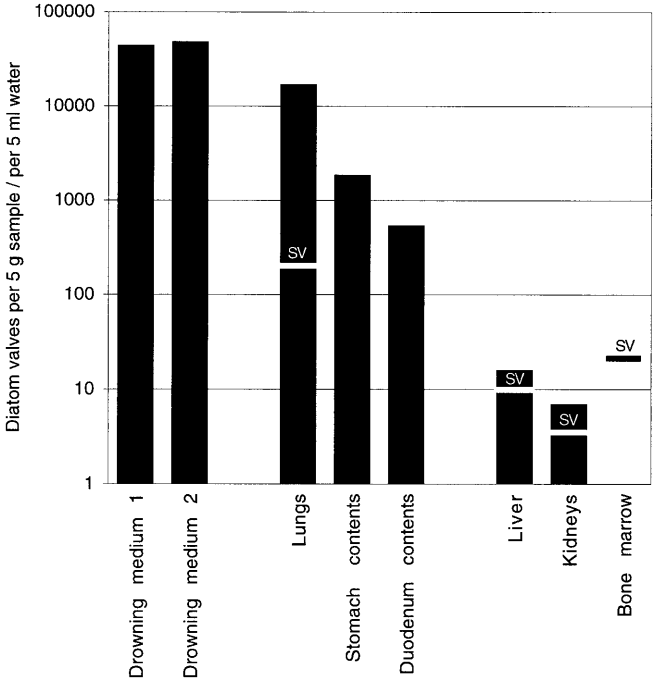


Fig. 8 Diatom density (per 5 ml water or per 5 g sample) found in drowning medium 1 (water at a depth of 1.5 m) and drowning medium 2 (surface water), in the lungs, in the contents of the stomach and duodenum, in the liver, in the kidneys and in the bone marrow of a case of drowning (case S 300–95) (In this particular case, no diatoms were found in the bone marrow. The horizontal white lines *SV* denote the so-called separation values of Kater [13] used to distinguish between death by drowning and death from other causes. Kater gives no separation values for the contents of the duodenum or stomach. In the case illustrated, the diatom densities found in the kidneys, liver and lungs exceed these separation values. This is typical for death by drowning)

Basic considerations concerning the recording of diatoms

Diatoms mounted permanently on a slide can be counted (diatom density), analysed (species determination) and measured (morphometry) under the microscope.

Determining diatom density, quantitative aspects

The diatom density is the number of diatom valves per area, per unit volume or per unit weight, respectively. The only matter of importance to be noted is that it is the number of diatom valves that must be counted. It is essential to include diatom fragments because the information they provide is just as important for algological forensics as that provided by intact valves (Fig. 8).

Assessment of the quantitative aspects (diatom density)

Empirical values published by various authors are available for the assessment of diatom density [e.g. 2, 13, 16, 30]. In the absence of our own empirical values and in view of the methodology used by us for sample preparation, we use the separation values listed by Kater [13] to distinguish between "water corpses" and "non-water corpses". Using a microscopic magnification of only 80 × – 320 ×, Kater established the following separation values for Germany (rounded figures): 200 diatoms per 5 g of lung (LU), 10 diatoms per 5 g of liver (LI), 4 diatoms per 5 g of kidney (KI) and 20 diatoms per 5 g of bone marrow (BM) but gave no figures for blood, stomach contents or duodenum contents. Kater found the following maximum values in non-water corpses (rounded figures): 54 diatoms per 5 g LU, 92 diatoms per 5 g LI, 22 diatoms per 5 g KI, and 30 diatoms per 5 g BM. Because basing the separation values on "diatoms" is inexact and because the microscopic magnification chosen by Kater was very low we converted the separation values (SV) and maximum (M) values into the following ranges (in diatom valves per 5 g of tissue): SV LU 200–400, M LU 54–108, SV LI 10–20, M LI 92–184, SV KI 4–8, M KI 22–44, SV BM 20–40 and M BM 30–60.

In order to be able to use the separation values of Kater [13] as modified by us, the diatom density per 5 g of the organ must be calculated for each sample. Because Kater gives only "diatoms" as the unit of density, and fails to specify whether he means valves or cells and whether fragments are included, we based this extrapolation on all diatom valves of which more than half the valve is present. The remaining diatom fragments are not used for calculating diatom density using the modified Kater values.

In principle we consider the calculation of diatom density to be both admissible and necessary, even if relatively little is known about possible inhomogeneities in the organs concerned. Although inhomogeneity involves the danger of inadmissible extrapolation, the risk can be reduced by ashing as much of the organ sample as possible. We consider that specifying diatom density as the number of diatom units per microscopically observed quantity as well as standardising extrapolation to the number of diatom units per gram of tissue to be meaningful. In our view, all diatom units, i.e. also fragments, should be included when calculating diatom density or working out new separation values.

Species identification, qualitative aspects

Species identification should be carried out for every valve or valve fragment at the lowest possible level of identification (normally down to the species level, in some cases down to the variety level). It is recommended that each valve and each fragment found should also be visually documented (as a photograph, a video print or a digital image). In specimens with a high diatom density (e.g. drowning medium, lungs and stomach contents) the identification of 500 diatom valves or fragments is sufficient to characterise a diatom community. In specimens with a low diatom density (e.g. blood, liver, kidneys, bone marrow) all of the diatom valves present on the microscope slide should be identified (Table 2).

Assessment of the qualitative aspects (species composition)

As several authors [5, 15, 18, 19, 29, 30, 31, 35] have already mentioned, by simply comparing the species composition of the diatom communities present in the organ samples with those present in the drowning medium, it is possible to deduce the drowning site. Based on our own experience, we can confirm this statement. In order to compare the organ samples in relation to each other and with the drowning medium, we use two similarity indices (pair-matching):

1. SI: the species index, i.e. the species similarity between two samples according to Jaccard [11]
2. DI: the dominance identity, which is the relative occurrence similarity between two samples according to Renkonen [34]

$$SI_{1,2} = S_{1\cap 2}/S_{1+2^*}\ 100\ [\%] \quad (1)$$

$$DI_{1,2} = \sum_{i=1}^{S} q_i\,[\%] \quad (2)$$

where $S_{1\cap 2}$ is the number of species common to the two diatom communities in samples 1 and 2, S_{1+2} is the total number of species in the two diatom communities in samples 1 and 2, q_i is the smaller of the two relative occurrences of species i, and S all the species in samples 1 and 2.

Both indices are expressed as a percentage and range from 0% (no similarity) to 100% (complete similarity). Table 3 shows a simple example of calculating the two similarity indices. For other indices refer to Ludes & Coste [15].

Table 2 Abbreviated list of diatom species found in case S 300–95 and their relative frequencies of occurrence (rf) in each sample, illustrating the qualitative criteria mentioned in the text (all diatom species which attained an rf of 5% at least once per sample are listed. BM bone marrow, KI kidney, LI liver, DU contents of the duodenum, ST contents of the stomach, LU lung DM1, DM2 drowning medium 1 and 2. All values listed are percentages)

	Sample							
	BM	KI	LI	DU	ST	LU	DM1	DM2
Taxon								
Achnanthes biasolettiana		50			0.9	1.9	0.5	
Achnanthes minutissima var. *minutissima*			44	< 1	22	20	8.3	0.7
Asterionella formosa				17	2.6	2.9	1.6	1.5
Cocconeis placentula			11		5.2	4.8	0.5	
Cyclotella comensis				17	9.5	6.7	3.7	7.2
Cyclotella cyclopuncta					20	15	2.7	4.1
Fragilaria brevistriata			11					
Fragilaria ulna var. *acus*		< 1	11	11	6.9	13	64	79
Gomphonema minutum					4.3	7.7	2.4	0.4
Gomphonema pumilum				17		1.9		
Navicula lanceolata			11					
Navicula seminulum		50				1.0		
Navicula tripunctata			11				0.5	
Nitzschia pura				17				
Stephanodiscus parvus	100			< 1		2.9		0.2
Sum of remaining species with rf < 5%	0	0	0	22	28	22	16	7
Sum of all species	100	100	100	100	100	100	100	100
Number of diatom species found	1	3	6	7	30	27	33	19
Number of diatom valves or valve fragments counted	1	3	9	8	116	104	374	459

Table 3 Example of two diatom communities, illustrating the calculation of the two similarity indices DI (the dominance identity) and SI (the species index) (*LU* lung, *BM* bone marrow)

Diatom communities of samples			Similarity indices	
	LU	BM	$DI_{LU,BM}$	$SI_{LU,BM}$
Species A	30%	35%	30%	
Species B	20%	50%	20%	
Species C	20%	15%	15%	
Species D	15%	0%	0%	
Species E	10%	0%	0%	
Species F	5%	0%	0%	
No. of species	6	3		
$S_{LU \cap BM}$				3
S_{LU+BM}				6
Sum	100%	100%	DI = 65%	SI = 50%

When calculating the similarity indices the following should be taken into consideration:

1. The two diatom communities (samples 1 and 2) used for pair matching should contain approximately the same number of counted diatom units and should not differ by more than 20%. However, when comparing samples of bone marrow, blood, liver and kidneys with, for example, samples of the drowning medium, this requirement can seldom be fulfilled, because the drowning medium usually contains many more valves. In this case, one should check or calculate the percentage of the species present in the organ sample that are also present in the drowning medium.

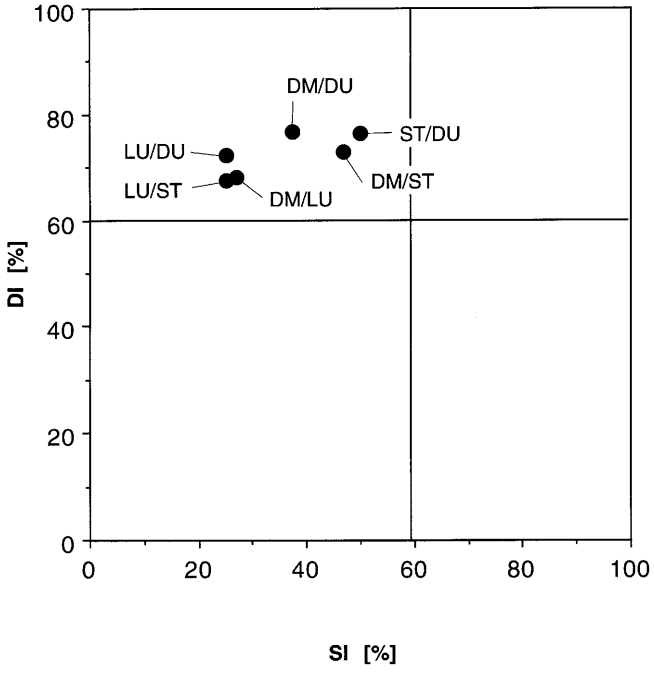


Fig. 9 Similarity of diatom communities in case S 315–95. Pairwise comparisons between drowning medium *(DM)*, lungs *(LU)*, contents of stomach *(ST)*, contents of duodenum *(DU)*, liver *(LI)* and kidneys *(KI)*. SI (species index) and DI (dominance identity) are similarity indices. Samples with similarity indices exceeding 60% are structurally very similar; experience has shown that such samples originate from the same diatom community

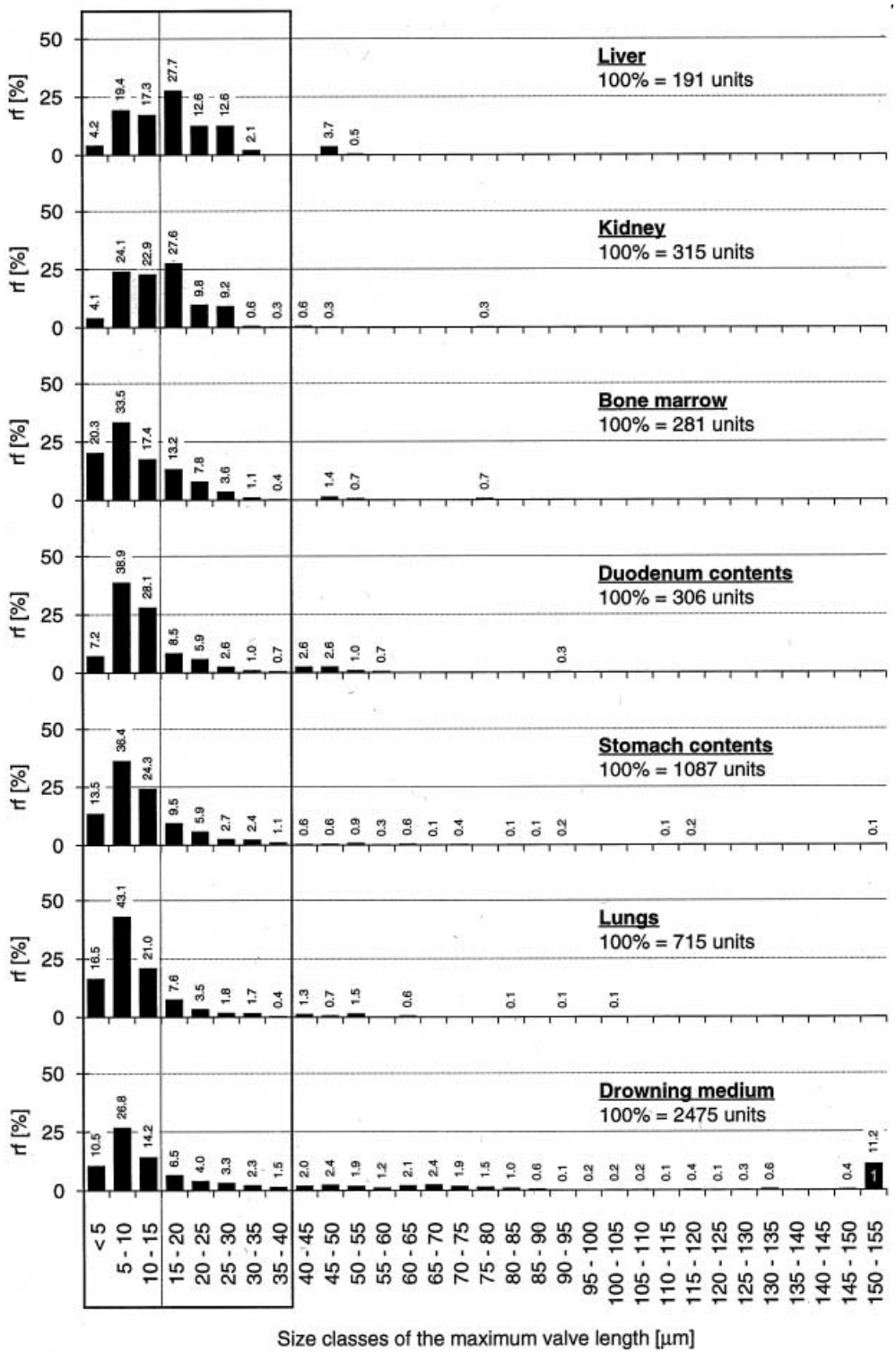


Fig. 10 Percentage relative frequency of occurrence (rf) of the valve lengths of all diatom units found in the liver, kidney, bone marrow, duodenum and stomach contents, lungs and in the drowning medium. Measurements from 12 cases. The two rectangles contain all diatom units < 15 µm and < 40 µm. *1* non-broken valves of *Fragilaria ulna* var. *acus*

2. Because only the small species can reach the blood, liver, kidneys and bone marrow during drowning, it is necessary to estimate which diatom valves and fragments contained in the drowning medium have the potential to get into these organs at all. An estimate of this potential, which is important, can be based on morphometric criteria (e.g. on maximum valve length). Pair matching should then also be based on this potential rather than on all the diatoms detected in the drowning medium. This concept of potential presence is likely to be taken sufficiently into account in most cases if the pair matching calculations are based only on valves having a maximum valve length of less than 40 µm.

The values of SI and DI above which two forensic diatom samples can be considered to be structurally very similar, are the subject of further investigation. Based on experiments with diatom communities and on field experience with streams [7, 9], it is not possible to distinguish between two diatom communities which have a

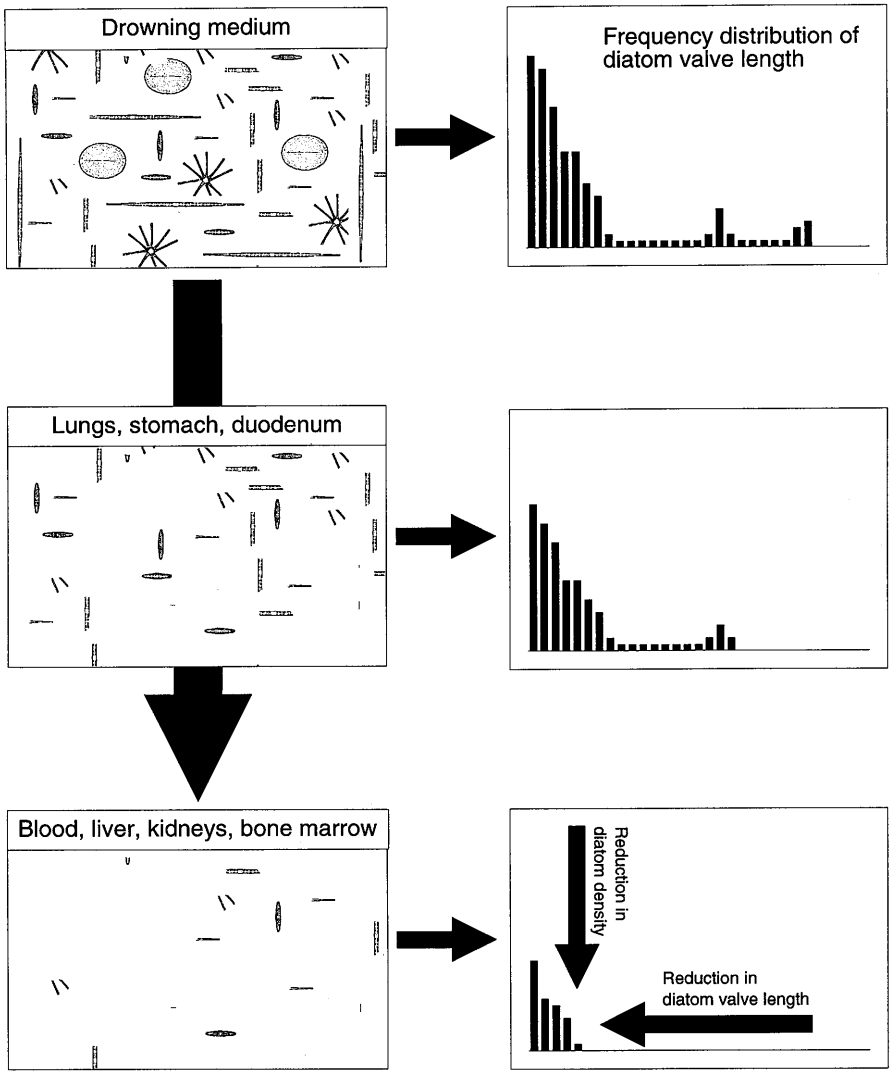


Fig. 11 Schematic representation of the filtration with respect to diatom density and size that occurs in the human body during death by drowning. Whereas diatom density and size in the lungs and in the contents of the duodenum and stomach will differ only slightly from the values found in the drowning medium, this difference is likely to be much greater in the case of those diatoms which pass into the circulatory system. In the blood, the liver, the kidneys and the bone marrow, only relatively few diatom fragments are found, with maximum lengths distinctly less than 40 μm

structural similarity exceeding 60%. However, this 60% level is too high for SI when comparing very different random samples.

Substitute samples for the drowning medium

In Fig. 9, the DI and SI similarity indices of the samples from the drowning medium, the lungs, and the contents of the stomach and duodenum are shown alongside each other. With a value of more than 60%, the relative occurrence similarity DI was very high. On the other hand, the SI value showed a similarity of at most 50%. The poorer similarity of the species index (SI) is typical, and can be due to the fact that under normal circumstances there is a lower diatom density in the lungs, the stomach and duodenum contents than in the drowning medium, depending on the volumes swallowed and aspired.

As shown in Fig. 9 not only the diatom distribution in the lungs, but also that in the contents of the stomach and duodenum exhibit great similarity to that found in the drowning medium. This fact makes it possible to use the latter as a substitute for the drowning medium itself. This is mainly of importance when the site of drowning is unknown and postmortem effects are unlikely.

Diatom morphology

The morphology of a valve, for example the maximum size of the valve or valve fragment, the valve thickness and its shape (round, oblong, oval, etc.) may to a large extent be decisive for the penetration of a valve into the body [20, 29]. It is highly likely that large valves only reach the lungs, stomach and duodenum during drowning. On the other hand, small valves can get into the blood, liver, kidneys and even into the bone marrow without difficulty. If an accumulation of very large valves or valve fragments with a valve length in excess of 40 μm is found, for instance, in the bone marrow, this can indicate the intake of diatoms via the gastro-intestinal wall (while alive), or postmortem (e.g. methodological) contamination. In Fig. 10, all the diatom valves and fragments found in 12 cases are illustrated and the relative proportions of the maximum valve length classes are shown for each sample. From this it can be seen that with the exception of the drowning medium, at least two out of three diatom valves have a maximum length of less than 15 μm, and well over 90% have a maximum valve length of under 40 μm. In microscopic work, therefore, a magnification of at least 630 × –1000 × is essential, and one should avoid working with less.

We consider that taking all sizes into account, i.e. also those < 5 μm, is justified, although such fragments can also enter the body while alive. However, it is our experience that in every case many of these small units originate from the species pool of the drowning medium and thus do not enter the body before drowning.

This is especially easy to understand in the case of drowning media in which small valves dominate (e.g. *Navicula minima*, small forms of *Cyclotella* or *Stephanodiscus*) or contain long, narrow valves which are prone to disintegrate into small fragments (such as *Fragilaria ulna* var. *acus* or *Asterionella formosa*). Almost without exception, such small diatom units are identifiable and represent an important information content. Beutler [4] arrived at similar results and conclusions during his analysis of blood.

Processes relevant during drowning

Several factors are probably significant in the quantitative and qualitative distribution of diatoms in the body. These are the diatom density in the drowning medium and the filtration with respect to size that occurs as the diatoms pass from the lungs into the blood (Fig. 11).

According to the example shown in Fig. 8, the diatom density decreases by a factor of between 10 and 100 when passing from the drowning medium to the lungs and/or to the stomach and duodenum. The further decrease in diatom density from the lungs to the blood, kidneys, liver and bone marrow corresponds to a factor on the order of 100–1000. Even though this density gradient is not the same in every case and is only intended to indicate the approximate order of magnitude involved, it can be assumed that under normal circumstances the density of diatoms in the drowning medium is decisive for whether diatoms are found in the body of a drowned person. Studies based solely on diatom density will therefore only show a positive result when the actual drowning medium has a relatively high diatom density.

The filtration of the diatoms with respect to size can be traced based on the percentage distribution of the maximum valve length, as shown in Fig. 10. In the example illustrated, long, narrow valves of *Fragilaria ulna* var. *acus* (valve length approx. 150 µm) did not reach the lungs. Surprisingly, they were also not observed in the contents of the stomach or duodenum. However, we do not have a plausible explanation for this, other than that the species is possibly too brittle. In the transition from the lungs to the blood, as well as to the organs and to the bone marrow, there is a further reduction in maximum valve length. Most of the diatom valves and fragments in these samples have a maximum length of less than 20 µm.

Based on these facts, it can be assumed that the diatom potential in the blood, the liver, the kidneys and the bone marrow can be deduced from the drowning medium parameters (e.g. diatom density, species, dimensions).

Conclusion

To sum up, our joint algological/medicolegal experience has led to the following procedure in cases of drowning where the circumstances are not clear:

1. The first step involves the preparation and algological analysis of samples of the drowning medium, the lungs, the blood and the bone marrow. The remaining samples (stomach, duodenum contents, kidney and liver) are kept as substitute samples.
2. Microscopic-algological analysis records quantitative, qualitative and morphological details for each sample. In addition, the diatom densities and the similarity indices SI and DI are calculated for each sample.
3. The algological conclusions are based primarily on checking the separation values according to Kater [13] as well as on pair matching.

However, the use of separation values according to Kater should be replaced by more precisely defined values that are internationally recognized. We see this procedure as the first step towards standardization. Provided that the emerging methodological progress in the fields of enzymatic digestion [16] and image analysis can be realized, the foundation would be laid for future proficiency testing on an international basis.

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