Methodology for securing environmental traces in the form of diatomaceous material

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Abstract

The use of diatoms as environmental forensic traces is an ideal tool for linking a person to a specific place – environment, and may also be helpful in diagnosing drownings or determining the place of drowning. This is a standard tool used by law enforcement agencies in numerous European countries, as well as in Australia, China, India and the United States of America. The aim of this work is to indicate possible methods and principles that should be used to secure diatoms at the scene during procedural activities such as site examination and search, as well as to promote knowledge in the field of forensic diatomology in Poland. This methodology was developed based on recognized and widely used experience in obtaining and securing diatoms for the purposes of research based on their taxonomy and ecology as well as on the literature. We recommend a procedure for securing diatom material based on methods used and developed by scientists and adapted to the conditions and technical capabilities available to a Polish police officer – a foresnsic technician.

Keywords: forensic diatomology, diatoms, procedure for securing environmental samples

Diatoms, as single-celled algae, commonly occur in aquatic or humid environments. Apart from water, they require access to light to thrive. In addition to the widespread presence of diatoms, the species diversity is also noteworthy as it defines a specific environment both qualitatively and quantitatively and allows it to be distinguished from other aquatic environments. The widespread distribution and diversity of diatoms make them an excellent tool that can be used in preparatory proceedings as the so-called environmental traces (Levin et al., 2017; Scott et al., 2016; Bogusz et al., 2018; Scott et al., 2021; Scott et al., 2021; Bogusz et al., 2022). Diatomaceous environmental traces make it possible to link a person to a specific environment and can also be used to help in drowning diagnosis (Pollanen, 1998; Peabody, Cameron, 2010; Rana, Manhas, 2018; Bogusz et al. ., 2018). The application

of diatom analysis is used by law enforcement agencies in the detection process to help diagnose drownings and is an integral part of the procedure in numerous countries, including: France (Delabarde et al., 2013), India (Rana, Manhas, 2018), China (Zhao, 2019), Macedonia (Krstic, 2002) and Switzerland (Hürlimann et al. , 2000). This analysis is also used in Poland, albeit not on an extensive scale (Bogusz et al., 2018). In addition to diagnosing drownings, during the detection process, diatoms can be an important piece of evidence that allows for linking the perpetrator to a specific site. The popularity of this knowledge is constantly growing, being featured in scientific publications as well as during symposiums[1] and conferences[2]. Dissemination of knowledge in the field of forensic diatomology (Żelazna-Wieczorek, 2019) takes place on many levels, which causes an increase in awareness about the validity of securing diatoms as forensic environmental traces among forensic technicians. While knowledge about the possibility of using diatoms is effectively disseminated among police officers whose tasks include, inter alia, examinations of sites, persons and items, as part of which they reveal and secure traces and evidence of crime. There are no proper guidelines on how to secure the samples that will constitute material for diatom analysis. Apart from general instructions on how to secure samples with diatomaceous material (Makowska, Bogusz, 2020), there have been no publications so far containing rules and tips for properly securing diatomaceous material at the incident scene. The purpose of this study is to provide a list of the most effective methods of securing diatom material, which can be performed both during the site examination and during thesearch for the suspect, based on the current scientific literature, but also taking into account the technical possibilities available to the Polish police.

Diatom microhabitats in the aquatic environment

Recognition of diatom habitats is crucial for selecting the appropriate procedure for securing environmental traces for diatom analysis. Apart from floating in the water column, forming a part of the phytoplankton, diatoms also occur on substrates permanently immersed in water, collectively known as phytobenthos. The phytobenthos itself can be divided into: epilithon – algae growing on stones, epipelon – algae growing on soft sediments such as silt, epiphyton – algae growing on submerged vegetation, epipsammon – algae growing on sand, and epixylon – algae occurring on dead wood immersed in water (Kawecka and Eloranta, 1994).

Each of these microhabitats will possess a diversity of diatoms. Therefore, it is important to secure samples not only of the water itself, because it will only contain diatoms found in the water column. Those that attach to substrates permanently immersed in water may be omitted. In addition, one should also take into account diatoms occurring in the subsurface layer of soil and constituting a group of organisms called edaphon (Antonelli et al., 2017).

When should material be collected for diatom analysis?

Diatoms may prove helpful in drowning diagnosis, but their mere presence in internal organs without their comparison with environmental samples taken from the site where the body was discovered will not provide information whether this place was also the drowning site. Only a thorough taxonomic analysis, carried out by an expert diatomologist, of diatoms present in internal organs (lung, kidney, bone marrow, stomach contents) and those found in environmental samples, may enable confirming or excluding the place where the body was found at the site of drowning or where the body was dumped into water after death. Therefore, it is important that in each case of discovery of a body, both in the aquatic environment and in its vicinity, comparative material is collected for qualitative and quantitative diatomological analysis.

Sampling should include all sites that may be inhabited by diatoms, hence the following sampling locations (photo: 1):

1. phytoplankton (from the water column);
2. phytobenthos (from the bottom and substrates permanently immersed in water):
   a) epipsammon (from the sand surface),
   b) epipelon (from soft sediments, e.g. silt),

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2 Conference in Gdynia in 2018 “Next generation of forensic biology laboratory” organized by the Central Forensic Laboratory of the Police

c) epilithon (from stones found in water),
d) epiphyton (from vegetation found in water):
   - from hydrophytes – plants submerged in water or floating on its surface,
   - from helophytes – plants rooted in water with stems above water surface,

3. epixylon (from a wooden substrate immersed in water);
4. edaphon (from the surface layer of soil).

Photograph 1. Available substrates with diatom material: 1 – water, 2 – mud, 3 – wood immersed in water, 4 – plants and bryophytes in contact with the aquatic environment, 5 – soil (photograph author: warr fc Kasper Molski)

Below, methods of securing diatom material from various types of substrates based on the available literature, are summarized in tables 1-6. In addition, a comment and a proposal is provided to adapt these methods for forensic technicians during procedural activities, such as scene examination or search.

Securing phytoplankton

In principle, a sample taken from the water column should be the primary sample secured during the examination of a corpse found in the aquatic environment. To ensure the optimum way of obtaining information on the diatom composition in this sample, strict rules should be followed.

Table 1. Instructions for collecting environmental samples from the water column in various types of ecosystems (Kawecka and Eloranta, 1994; Taylor et al., 2007; Żelazna-Wieczorek, 2019; Kolada, 2020)

No.	Reservoir type			Comment
	Standing water, deep (> 10 m)	Standing water, shallow (≤ 10 m)	Running waters	
	Description of activities			
1.	Rinse the plankton net in the reservoir/ watercourse.			• If no plankton net is available, collect water into a 10-litre bucket with a rope attached to the handle that enables submerging the bucket at greater depths. After collecting water into the bucket, set it aside for at least 1 hour (this can be done at the beginning of the scene examination). Setting the water aside will allow the diatoms present in the water column to sink to the bottom. After this time, gently pour out the upper layer of water, avoiding stirring, leaving only about 1 litre of water at the bottom of the bucket. • Pour the remaining water from the bottom of the bucket into a jar (it can be a jar used to secure osmological traces). • Remember that both the jar and the bucket must be previously rinsed in the same watercourse from which the sample is taken.
2.	Immerse the net to a depth of at least 5 m; pull it out in a vertical motion.	Immerse the net; drag several times under the water surface in a horizontal motion.	Immerse the net; hold still under the water surface for several minutes.	• In the case of deep standing waters, the occurrence of thermal stratification should be taken into account – immersing a net or bucket to a depth of at least 5 m will make it possible to collect diatoms from different layers of water. • In the case of shallow standing water bodies, where thermal stratification does not significantly affect the composition of the diatom community in the water column, there is no need to immerse the net to significant depths. • Samples from rivers should, if possible, be taken from the middle of the river course. • Samples from lakes and ponds should, if possible, be taken from their deepest points. • If samples are taken near a bridge, they should be taken from the side opposing the water flow, i.e. "facing the current". • If samples are taken in a dam reservoir, they should be taken at least several dozen meters from the shore.
3.	Pour the concentrated sample with a volume of up to 100 ml into a sterile sample container.			• Depending on the river, at the beginning of the vegetation season (March – April) and at its end (October – November), in order to collect a sample with a volume of close to 100 ml, it may be necessary to immerse the plankton net in the water column several times due to the low diatom counts. If no plankton net is available in the above periods, the sample should be collected into the bucket at least twice.

Securing phytobenthos

Samples from the bottom and substrates permanently associated with the bottom, due to the different diatom species composition as compared with those occurring in the water column (phytoplankton), should be secured simultaneously with phytoplankton samples.

Securing epipsammon

Table 2. Instructions for collecting environmental samples from sandy substrates (Kawecka and Eloranta, 1994; Taylor et al., 2007)

No.	Description of activities	Comment
1.	Rinse the sediment dredger in the water body/watercourse.	• Instead of a sediment dredger, take a urine container with a capacity of 100 ml and use it to collect the surface layer of sand to a depth of 1 cm, or alternatively, use a jar used to secure osmological traces.
2.	Push the dredger with the truncated side into the sand and collect a layer of sediment several centimetres thick.	
3.	Transfer the collected sample to a sterile sample container.	

Securing epipelon

Table 3. Instructions for taking environmental samples from muddy substrates (Kawecka and Eloranta, 1994; Taylor et al., 2007)

No.	Description of activities	Comment
1.	Collect water into the pipette to rinse it.	• A plastic or glass pipette with a bulb should be cleaned between the attempts; additional rinsing with water from the place where the next sample is taken reduces the risk of contamination.
2.	Collect the surface silt layer with the pipette.	• Take care as not to stick the pipette into the bottom. This would cause it to become clogged with the silt taken in.
3.	Transfer the collected sample to a sterile sample container.	• Pour the contents of the pipette (the surface layer of mud) into a 100 ml plastic container (urine container).

Securing epilithon

Table 4. Instructions for taking environmental samples from natural stony substrates (Kawecka and Eloranta, 1994; EN 13946: 2003; Townsend & Gell, 2005; Taylor et al., 2007; Karthick et al., 2010; Zgrundo et al., 2018; Żelazna-Wieczorek, 2019; Kolada, 2020; Richards et al., 2020)

No.	Description of activities	Comment
1.	In order to collect epilithon (diatoms from stones), use a plastic tray and a toothbrush with hard bristles, which should be rinsed in the watercourse.	• Instead of the tray, a plastic bowl or other plastic container can be used. • Tools should be cleaned between each sample; additional rinsing with water from the site where the next sample is to be taken reduces the risk of contamination. • Alternatively, you can use a wooden spatula, a spoon, a knife or other sharp object to remove the diatomaceous layer from the stones.
2.	Pour up to 100 ml of distilled water into the tray.	• The volume of distilled water should be adjusted to the size of the tray or other container used.

3.	Select at least 5 small or medium-sized stones permanently immersed in water.	• The preferred stone size range is from 64 to 265 mm. • Select several stones, but no more than 10, so that the total area from which the diatom biofilm will be collected is approximately 10 cm^2. • Stones covered with filamentous algae and with a visible layer of bottom sediment should be avoided. • If more than 75% of the substrate in a given site is covered with filamentous algae, they should be removed from the surface of the stones and the following rules should be applied.
4.	Gently rinse each stone under the water surface of the water body/watercourse.	• This allows for the removal of contaminants loosely attached to the substrate.
5.	Place the stones in the tray, orienting them to their original position.	• This allows the substrate to be retained in its original position and the biofilm to be collected from its upper surface.
6.	Brush the upper surface of the stones in the tray vigorously, rinsing the brush in water from time to time.	• Rinsing the toothbrush bristles is intended to release diatom shells from among them. • The water should become cloudy and brownish in colour. • Remains of vegetation or stone fragments should be removed from the water. • If the amount of collected material is unsatisfactory, the sampling area should be increased.
7.	Transfer the collected sample to a sterile sample container.	• Due to the small volume of the sample, a plastic urine container with a volume of 100 ml will be optimal.

Table 5. Instructions for taking environmental samples from man-made rocky substrates, such as concrete bridge pillars and concrete bank reinforcements (EN 13946: 2003; Zgrundo et al., 2018; Kolada, 2020). The operation involves scraping the biofilm from the substrate below the water surface (Fig. 1). In order to avoid losing the scratched material with the current of a running watercourse, scrape the biofilm directly into a 0.9 L jar and leave it for at least 30 minutes.

No.	Description of activities	Comment
1.	Rinse the scraper in the water body/watercourse, simultaneously stirring up the water opposite the surface of the substrate from which the sample will be taken.	• Alternatively, a wooden spatula, a spoon, a knife or other sharp object can be used to collect the diatomaceous layer. • Disturbing the water removes contaminants loosely attached to the substrate.
2.	Scrape off the biofilm from a total area of approximately 10 cm^2. Repeat 2 times.	• Usually the obtained sample is very dense, but if the amount of collected material is unsatisfactory, the sampling area should be increased.
3.	Transfer the collected material to a sterile sample container.	• Due to the small volume of the sample, a plastic urine container with a volume of 100 ml will be optimal.

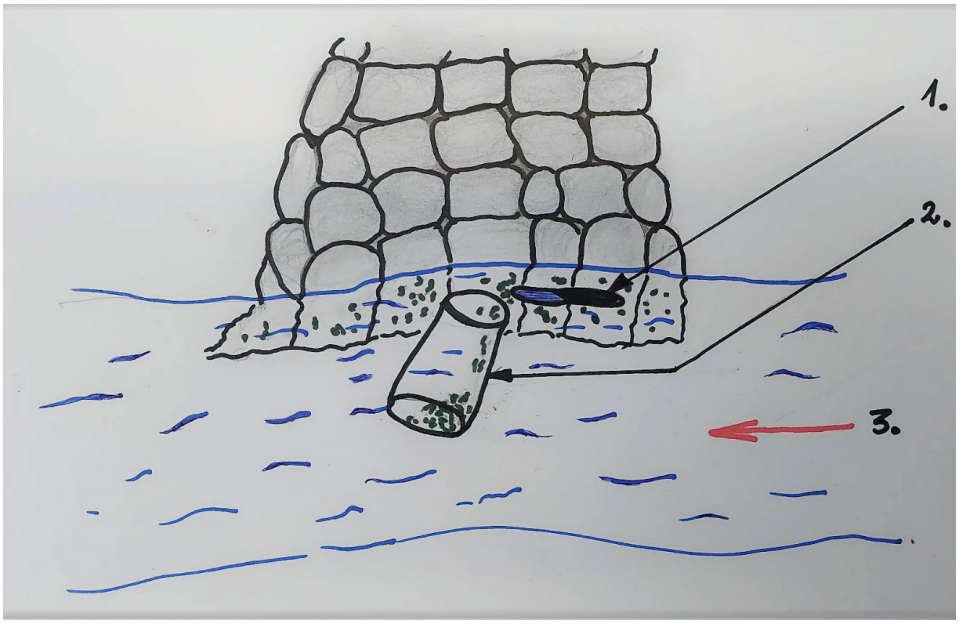

Fig. 1. Scheme of sampling from stony substrate in the stream of a watercourse: 1 – scraper for sampling, 2 – jar for sampling, 3 – direction of the current (author: Iwona Bogusz)

Table 6. Instructions for collecting environmental samples from epiphytes: plants submerged and floating in water – hydrophytes and plants submerged and rooted in water – helophytes (EN 13946: 2003; Taylor et al., 2007; Karthick et al., 2010; Zgrundo et al., 2018; Kolada, 2020)

No.	Description of activities	Comment
1.	Place 5 plants taken from the aquatic environment together with 50 ml of water from the body of water in a plastic zip lock bag.	• Instead of a bag, a 100 ml plastic container (for urine samples) or a jar for osmological traces can be used.
2.	Shake the contents vigorously to loosen the diatoms from the plant surface*.	• The water should become cloudy and brownish in colour. • If the amount of collected material is unsatisfactory, the sampling area should be increased. • *The shaking stage can be omitted and the plants can be secured entirely in a container or a jar with distilled water.
3.	In the case of helophytes select at least 5 stems permanently submerged in water, remove the stems from above the surface. Then cut off the stems above the substrate and transfer them to a plastic tray or other plastic container previously rinsed in the watercourse. Then pour 100 ml of water into the tray, or enough to cover the plant stems, and brush the stems vigorously** with a hard-bristled toothbrush.	• **The brushing step can be omitted and the plants can be secured entirely in a container or jar with distilled water.
4.	Pour the resulting rinsings (i.e. in point 2 and point 3) into a sterile test container.	• Due to the small volume of the sample, a 100 ml plastic container for urine samples or a jar for osmological traces will be optimal.

Securing epixylon – samples from a wooden substrate. In this case, taking environmental samples from wood fragments permanently immersed in water should be carried out in the same way as in the case of taking environmental samples from natural stony substrate (Table 4) (Żelazna-Wieczorek, 2019).

Securing edaphone – soil samples. In the case of bodies found in water, there is no need to secure soil samples. The described above secured phytoplankton samples and individual phytobenthos samples are sufficient to help diagnose drownings and to determine whether the site where the body was discovered was the same as the site of drowning.

However, it should be remembered that if an attempt is made to link the suspect to a specific place – the natural environment, it would be advisable to secure a soil sample from the place of the incident for the purpose of a comparative diatom analysis. This offers the opportunity to compare soil samples with evidence secured at a later stage in the form of muddy items, such as: tires, shoes, socks, trousers, shovels and spades with dried mud or soil. Such an analysis may confirm or exclude the presence of the perpetrator in a specific place.

Table 7. Instructions for collecting environmental samples from soil (Taylor et al., 2007; Antonelli et al., 2017; Wanner et al., 2018; Żelazna-Wieczorek, 2019)

No.	Description of activities	Comment
1.	To facilitate collecting the sample, clear the soil surface of any organic matter.	• Organic matter means all dead plants and animal remains.
2.	Using a soil corer, take a sample of 100 cm^2, to a depth of approx. 5 cm.	• A soil sample can be taken with a plastic urine container by inserting it into the soil to a depth of approximately 5 cm, and then collecting this layer of soil into the container. • Instead of a plastic container the jar used to secure scent marks can be used.
3.	Pour distilled water over the collected layer of soil, shake and pour through a sieve*.	• The obtained filtrate should be cloudy and brownish in colour. • *The soil sample can be secured as it is, in which case it should not be rinsed, but placed in a paper envelope and allowed to dry.
4.	Transfer the collected material to a sterile sample container.	• Due to the small volume of the sample, a plastic urine container with a volume of 100 ml will be optimal.

All the above samples should be fixed with 95% ethyl or methyl alcohol in a ratio of 1:10 (1 volume of alcohol to 10 volumes of water).

Handling of items that may contain diatomaceous material for use in comparative diatomaceous analysis:

Footwear

Muddy footwear can be secured in paper envelopes and then placed in a cardboard box and sent for diatomological examination.

- If the footwear is also to be the subject of traceological and biological tests, first of all, swabs should be taken for the purposes of biological tests: from the inside of the footwear and from any brown stains that could be of blood origin (to verify if this is blood, strip tests can be used, e.g. Peroxtesmo or Hemophan). Next, place the shoes in a zip-lock plastic bag and pour 1 litre of distilled water over them. After closing the string bag, shake it vigorously to wash out the diatomaceous sediment.
- Then, after rinsing, pour the water into a jar and fix the sample with 95% alcohol in a ratio of 1:10 (1 volume of alcohol per 10 volumes of water). The footwear should be dried in natural conditions and after drying it may be subjected to traceological testing.

Tires

Muddy tires should be rinsed with 1 litre of distilled water over a bowl or tray appropriate to the size of the tire. In order to obtain accurate diatomaceous material, the tires should be additionally scrubbed with a brush with hard bristles, but larger than a toothbrush, e.g. a hand brush.

- After rinsing, pour the water into a jar and fix the sample with 95% alcohol in a ratio of 1:10 with water.

Tools: shovels, rakes, spades and other objects covered in mud (Photograph 2a-2h)

If these items are to be subjected to biological testing or they exhibit traces resembling dried blood, before securing the diatom samples, swabs should first be taken from appropriate sites (strip tests can be performed to confirm presence of blood).

- Pour 0.5–1 litre of distilled water into a zip-lock bag (the amount depends on the size of the tool).

Photograph 2a-2h. Obtaining a sample from a tool; the order of performed activities is marked in photographs from A to H (photograph authors: warr fc Kasper Molski, warr Michał Zakrzewski)

- Immerse the working part of the tool together with the part of the handle (in the areas where soil is present) in a bag with water. After zipping or tightening the bag over the working part, shake the bag vigorously to rinse out the soil.
- After rinsing, pour the water into a jar and fix the sample with 95% alcohol in a ratio of 1:10 with water.

Muddy clothing, e.g. trousers, socks, gloves, sweatshirts, etc. (Photograph 3a-3g)
Secure everything by packing it into paper packages, which should be placed in paper envelopes.

- If the above-mentioned clothing items are the subject of other biological testing – genetic tests or analysis of the mechanism of bloodstain formation – they can be sent for diatom analysis after these tests have been performed.
- An alternative method is to crumble the mud that dried on clothing or brush it with a hard-bristled toothbrush into paper packets, which should then be placed in paper envelopes.

Photograph 3a-3h. Securing a sample of clothing using an example of trousers covered in mud; the order of activities performed is marked in the photographs from A to G (photograph authors: warr fc Kasper Molski, warr Michał Zakrzewski)

Necessary equipment for securing diatom samples:

- 10 L bucket with string;
- 0.9 L or 1 L jars;
- plastic containers with a capacity of 100 ml (for urine samples);
- a plastic tray, bowl or other container adapted to the size of the sample to be secured;
- glass pipette with a bulb;
- plastic zip lock bags adapted in size to the samples to be secured;
- hard bristle toothbrush;
- hand washing brush;
- distilled water;

- a strainer for pouring water from soil samples;
- ethyl or methyl alcohol 95%;
- paper envelopes;
- grey paper for packets;
- cardboard boxes.

An alternative method to storing wet samples for a long time is to secure them in the following manner: place filter paper on a clean tray, shake and pour out the entire wet sample, indluding the sediment, and leave to dry (Fig. 2). Then fold the tissue paper into a package with the dried sediment inside and pack it in a paper envelope.

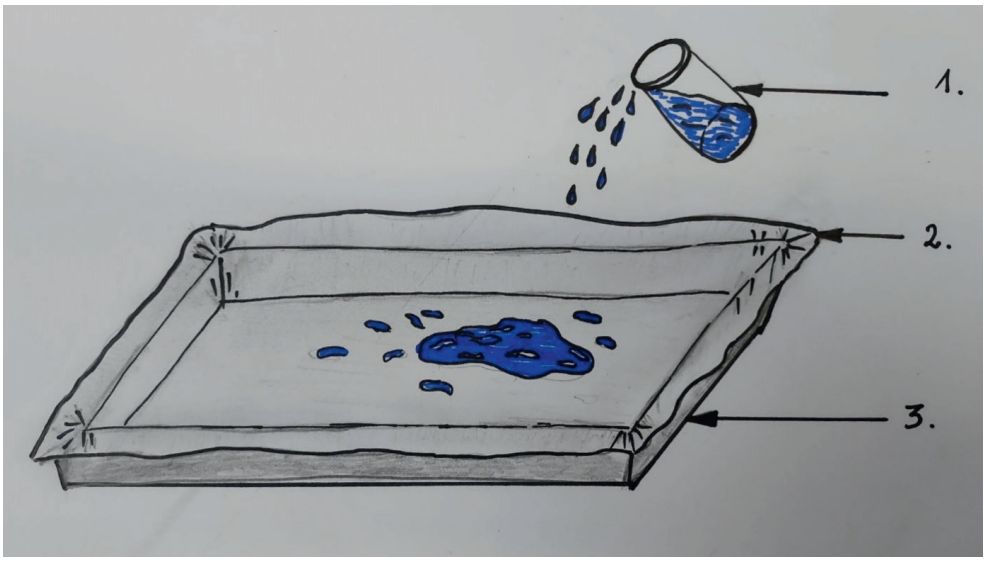

Fig. 2. Method of laying out the filter paper and pouring the sample in order to fix it by drying: 1 – jar with sample, 2 – tissue paper, 3 – cuvette (author: Iwona Bogusz)

Please remember that all secured samples, whether in glass jars or plastic containers, paper envelopes, or cardboard boxes, should be permanently attached to trace labels. Depending on the process activity, the secured sample will be called an object, a trace, or a comparative material. This decision belongs to the person conducting the proceedings and the forensic technician performing a given activity.

Final conclusions

Published sources indicate the use in the detection process of environmental traces in the form of plant pollen (Horrocks, Walsh, 1999; Wiltshire, 2006; Mildenhall, 2006; Mildenhall et al., 2006), fungi (Hawksworth, Wiltshire, 2010; Wiltshire, 2016), insects (Benecke, 1998; Pai et al., 2007; Cervantès et al., 2018; Matuszewski, Mądra-Bielewicz, 2019; Matuszewski, 2021), soil (Ruffell, McKinley, 2005, Morgan, Bull, 2005; Ruffell, Mc Kinley, 2006, Bull et al., 2006), including sand grains (Bull, Morgan, 2006; Morgan et al., 2008; Konopinski et al., 2012) and diatoms (Pollanen, 1998; Horton et al., 2006; Peabody, Cameron, 2010; Scott et al., 2016; Levin et al. , 2017; Scott et al., 2021; Bogusz et al., 2022). Taking this into account, securing the material in the form of samples from the environment should not be omitted for later use in the detection process. Environmental tests are often used by law enforcement agencies in Western countries, they play a huge role not only in creating forensic versions, but can also constitute an excellent evidence material. Moreover, it should not be forgotten that forensics is a constantly developing field and what was impossible to investigate 20 years ago is not a problem for forensic experts today. However, in order for experts to be able to perform proper analyses and draw appropriate conclusions in the future, they must be provided with properly secured study material. This work recommends guidelines for securing diatomaceous material, which, even if not applicable for ongoing proceedings, may also be used in the future. Taking into account the seasonal variability of the structure of diatom communities and their variability due to various environmental factors: water pH, salinity, conductivity and trophy (Kawecka, Kwandrans, 2000; Hall, Smol, 2001; Battarbee, 2001; Potapova, 2001; Witkowski

et al., 2001, Silva et al., 2022), omitting the securing of diatomological samples during the scene examination may be a significant error that cannot be corrected. The alternative equipment necessary to secure diatomaceous samples listed in this publication is relatively inexpensive, and the activities that need to be performed in connection with their securing are not complicated.

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