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Diatoms as a Forensic Evidence: Transfer of Marine Diatoms to Different Fabrics and Determination of Their Appropriate Extraction Method[A]

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Abstract: In forensic studies, diatoms are often used as trace evidence. In particular, diatoms attached to clothing are used for this purpose. However, studies show that the transfer of diatoms to clothing and the extraction method give different results on different fabrics. Therefore, this study was designed to determine the most effective marine diatom isolation method for the most commonly used fabrics. Fabric pieces were immersed in seawater for 4, 24 and 96 hours to ensure the transfer of diatoms. Diatoms were extracted from the fabrics using three different methods: rinsing with distilled water, rinsing with ethanol, and digestion with hydrogen peroxide. Results showed that the extraction method, fabric type, and immersion time affected the number of diatoms. 4 hours of immersion time was sufficient for the transfer of diatoms to viscose fabric. 4 hours of immersion time also seems to be sufficient for the transfer of diatoms for viscose+polyester fabric, but they could only be obtained from the fabric by the H_2O_2 method. However, it is understood that 24 hours of immersion time is required for diatom transfer to tencel+polyester fabric. Our results showed that the most effective method for all fabric types is H_2O_2 digestion. Therefore, we recommend the use of the H_2O_2 method, especially in cases where the residence time in seawater is unknown in forensic cases. If there is any signs that the evidence has been in seawater for a long time, other methods may be preferred.

Keywords: Diatom, Fabric, Forensic, Immersion-time, Marine, Trace-evidence

[A] The study does not require approval from an ethics committee. The article has been prepared according to research and publication ethics.

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Introduction

Diatoms are widespread in all aquatic and humid environments, such as lakes, oceans, and soil, and tend to colonize on artificial and natural surfaces. The colonization of diatoms can vary seasonally, and water quality also affects colonization (Totti et al., 2007). Unlike other microscopic algae, diatoms have siliceous outer walls (frustules). Since frustules are resistant to decomposition, forensic sciences use diatoms as trace evidence (Vijayan et al., 2020). Furthermore, because diatoms are highly sensitive to environmental variables, some species are habitat-specific and are used in science, including forensic science, as environmental indicators (Dahiya et al., 2024).

Forensic pathologists frequently use the diatom test in drowning cases. The diatom test can be used to determine whether the cause of death was drowning or post-mortem immersion in water (Zhou et al., 2020). In addition, diatoms collected from the body surface, bone marrow, and clothing can be used as trace forensic evidence (Marshall et al., 2023). This means that if indicator diatom species are found in the evidence, it helps to determine the actual crime scene. However, it is important that diatoms are properly isolated from evidence (Taylor et al., 2007). Recently, there have been studies investigating the immersion time required for the transfer of diatoms to clothing and the extraction methods of diatoms attached to clothing after immersion (Magni et al., 2020; Scott et al., 2018). Water washing, 70 % ethanol washing, ethanol washing with centrifugal force, acid digestion, and hydrogen peroxide digestion methods are widely used for the extraction of diatoms. These studies have generally been conducted in lakes and streams. Unfortunately, very limited research has been conducted in marine environments (Sidari et al., 1999).

Uitdehaag et al. (2010) stated that the highest number of diatoms in cotton fabric was obtained by the acid digestion method. They also showed that the water rinsing method was less effective on cotton fabrics. However, researchers suggested that the results obtained by the ethanol method were more suitable for forensic research since it provides a higher diversity of species. On the other hand, Scott et al. (2014) concluded that the most effective isolation method for cotton fabrics in contact with both freshwater and soil environments is digestion with H_2O_2. These studies show that the appropriate diatom isolation technique may be different for different fabric types. For this reason, this study was designed to determine the most effective diatom isolation method for the most commonly used fabric types in daily life and the answers to the following questions were investigated.

What is the minimum immersion time required to transfer diatoms to fabric?

What is the most effective extraction method to collect diatoms from fabric?

Materials and Methods

Fabric Types and Transfer of Diatoms

In this study, 87 % Viscose + 13 % Polyester (VIS+PES), 88 % Tencel + 12 % Polyester (TNCL+PES) and 100 % Viscose fabrics (VIS), which are widely used especially in women's clothing, were used. For the transfer of diatoms, an apparatus containing 9 cm^2 fabric samples cut from each type of fabric was prepared. This apparatus, schematized in Figure 1, contains a total of 12 pieces from each type of fabric. 9 of these were planned for 3 repeated analyses from 3 different extraction methods, and the rest were planned for SEM examinations and as backup against possible losses. Since the transfer of diatoms was examined in 3 different immersion times in the study, this apparatus was prepared in 3 duplicates. These apparatuses were immersed in the shores of Darıca / Kocaeli (Türkiye, Figure 2) and allowed for diatom attachment then removed from the sea after 4, 24 and 96 hours, respectively, and left to dry in the shade for later use in diatom extraction.

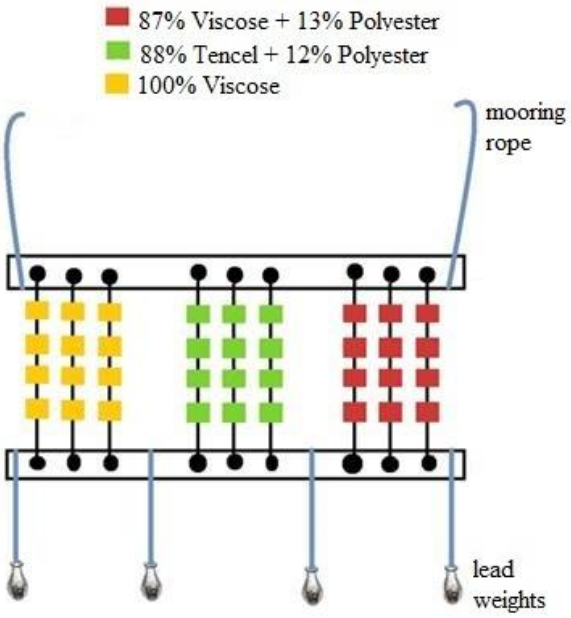

Figure 1. Schematic representation of the immersion apparatus for transferring diatoms to fabrics.

Diatom Extraction

Three extraction methods were used to obtain the diatoms attached to the fabric pieces. These are: rinsing with distilled water, rinsing with ethanol 70 % and digestion with hydrogen peroxide (H_2O_2).

Rinsing with Distilled Water (RW)

Fabric pieces were individually placed in a 100 ml glass bottle with 30 ml of distilled water and then shaken for 24 hours in a magnetic stirrer. After that, the fabric pieces were removed from the bottle. The contents remaining in the bottle were transferred to a new tube and centrifuged at 3500 rpm for 5 minutes. Pellets were diluted to 5 ml with distilled water and then the lugol solution was added drop by drop (Scott et al., 2014). All samples were stored at 4 °C. Rinsing with distilled water was performed with 3 repetitions for each fabric type.

Rinsing with Ethanol 70% (RE)

30 ml of 70 % ethyl alcohol was used instead of distilled water, and the same procedures were performed in the water washing method (Scott et al., 2014). Rinsing with ethanol was performed with 3 repetitions for each fabric type.

Digestion with Hydrogen Peroxide (H_2O_2)

Fabric pieces were placed into tubes containing 10 ml 30 % H_2O_2 and heated at 70 °C in a water bath for 3-hours. The fabric pieces were removed from the tubes, and the remaining content was centrifuged for 5 minutes at 3500 rpm. Pellets were washed three times with distilled water and then it was diluted to 5 ml as a final volume. Finally, the lugol solution was dripped and stored at 4 °C (Scott et al., 2014). Digestion with H_2O_2 was performed with 3 repetitions for each fabric type.

Figure 2. The place where the fabrics were kept in the sea for the transfer of diatoms to the fabric.

Diatom Counting

1ml of solution containing diatom was counted with a light microscope (Nikon Eclipse E100) using haemocytometer (Neubauer). Each extract was counted 5 times and averaged. Thus, 3 average values for each extraction method were used in statistical analyses. The results were calculated from the formula below as the number of diatoms per ml, (LeGresley and McDermott, 2010) and then calculated for 5 ml (final volume of extract). Results were presented as number of diatoms per cm^2 of fabric (1 diatom = 2 valve).

$$The\ average\ number\ of\ diatoms\ per\ ml = Average\ count\ per\ large\ square\ \times 10000 \quad (1)$$

In addition, in order to determine which diatom species were widely attached to the fabrics, the extracts obtained from the fabric pieces were observed under light microscope and identified.

Scanning Electron Microscope (SEM)

Scanning Electron Microscope (SEM) was used to examine the fabric surface structure and to view the diatoms attached to the fabrics. Once the fabric samples were dried at room temperature, they were placed on aluminium disks with sticky carbon bands. The samples were then coated in gold-palladium at 40-50 nm thickness with the help of the BAL- TEC SCD 005 coating device. The coated samples were examined and photographed at 20-30 kV with the CARL ZEISS / EVO 40 electron microscope at Bursa Uludag University Art and Science Faculty Microscopy Laboratory.

Statistical Evaluations

Data are presented as the mean ± standard deviation (sd). Prior to all statistical analyses, data were tested for normality and homogeneity of variances using the Kolmogorov-Smirnov' test and Levene's test, respectively. Three-factor ANOVA was used to establish differences among fabric types, extraction methods, and immersion times. Additionally, each extraction method was evaluated within itself with two-way ANOVA to determine the differences between immersion time and fabric types. A posthoc test for multiple comparisons (Tukey's HSD) was performed when the data revealed significant differences at a level of $p < 0.05$. The analyses were performed using the commercial software program SPSS 23 (IBM Corporation).

Results

The surface texture of the fabric types used in the study is presented in Figure 3 using SEM and stereo microscope. The images show that the fabric surface structure and weave characteristics are different among the fabrics. The images show that VIS and VIS+PES fabrics have a rougher surface. The TNCL+PES fabrics appear to have a smoother surface compared to the other two fabric types. Microscopic examinations revealed that *Cocconeis* sp., *Tryblionella* sp., *Fallacia* sp., *Coscinodiscus* sp. and *Pleurosigma angulatum* were the predominant species attached to fabrics. Pleurosira laevis are less attached to fabrics compared to these species. Some of the species detected in light microscopy and SEM images are presented in Figure 4 and 5 respectively.

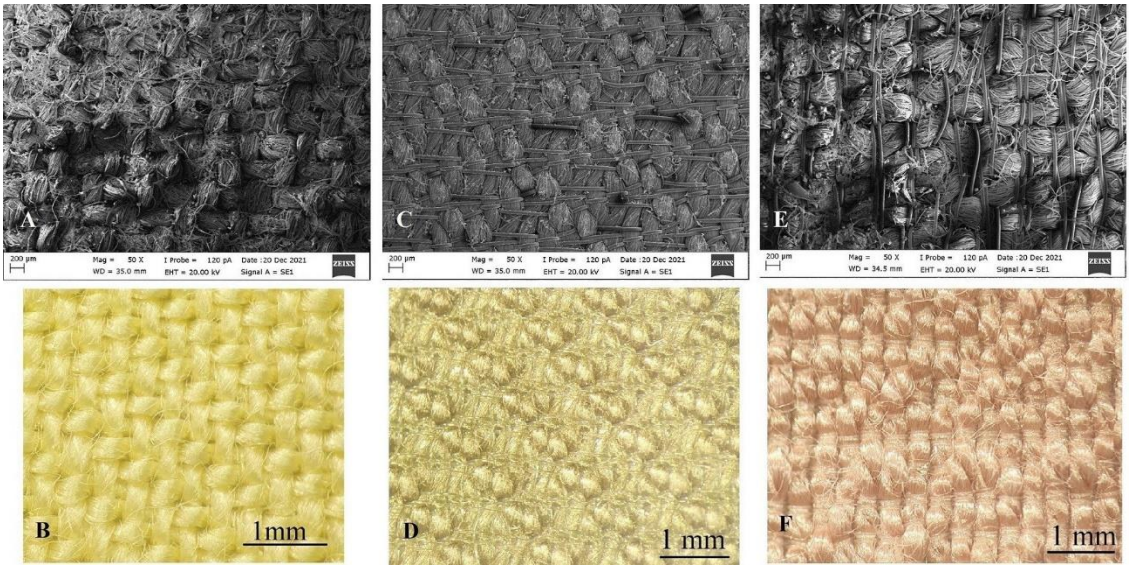

Figure 3. Surface images of different fabric types at the SEM and stereo microscope. (A-B 100 % Viscose; C-D 88 % Tencel + 12 % Polyester; E-F 87 % Viscose + 13 % Polyester)

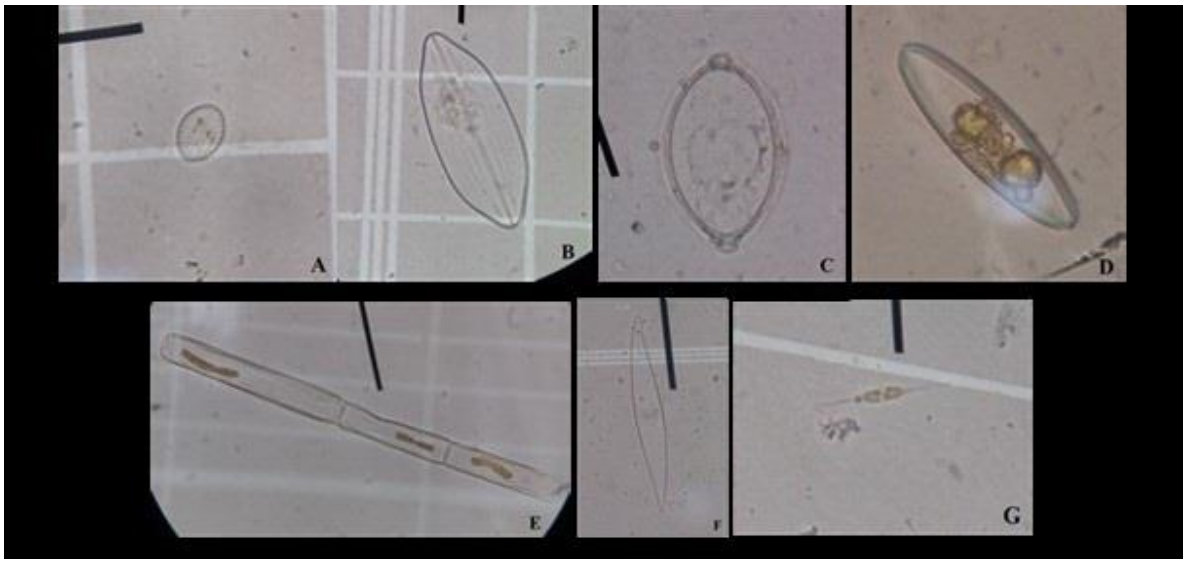

Figure 4. Light microscope images of diatoms on fabrics A) *Cocconeis* sp. (100x) B) *Fallacia* sp. (100x) C) *Pleurosira laevis* (100x) D) *Tryblionella* sp. (100x) E) Filamentous green algae (40x) F) *Pleurosigma* sp.(40x) G) *Nitschia acicularis* (100x)

Figure 6 presents the mean of the estimated diatom numbers by RW on different types of fabrics at three different immersion times. No diatoms were detected in fabric types except VIS after 4-hours of immersion. Diatoms were observed in VIS+PES and TNCL+PES fabrics after 24-hours and 96-hours of immersion. While the number of diatoms in the VIS fabric was almost similar after 4-hours (3009.26 ± 1181.23 diatom cm^{-2}) and 24-hours (3425.92 ± 1529.87 diatom cm^{-2}) of immersion, it increased radically after 96-hours (18 240.74 ± 7619.08 diatom cm^{-2}) of immersion. Statistical analysis shows that the number of diatoms estimated by RW differs both among fabric types ($F_{(2,18)}$=22.38; p=0.000) and between immersion times ($F_{(2,18)}$=13.17; p=0,000). The highest number of diatoms was found in VIS after 96 hours of immersion. Moreover, higher diatom count was obtained from VIS at all immersion times compared to other fabrics. Multiple comparison test also showed that VIS differs from other fabric types.

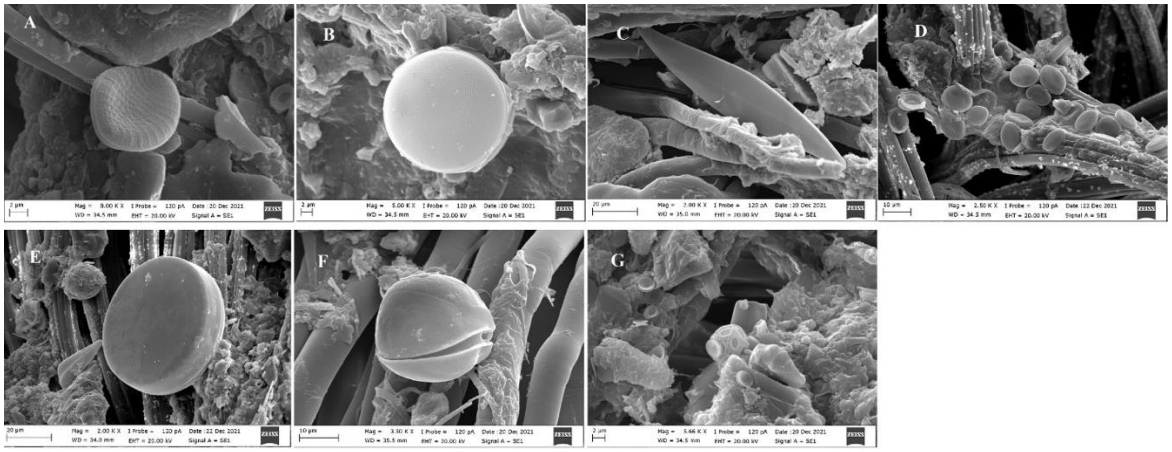

Figure 5. SEM micrographs of diatoms and other algae transferred to fabrics A) *Cocconeis* sp. B) *Coscinodiscus* sp. C) *Pleurosigma angulatum* D) *Cocconeis* sp. E) *Centric diatom* F) *Dinoflagellate* G) *Coccolithophore*

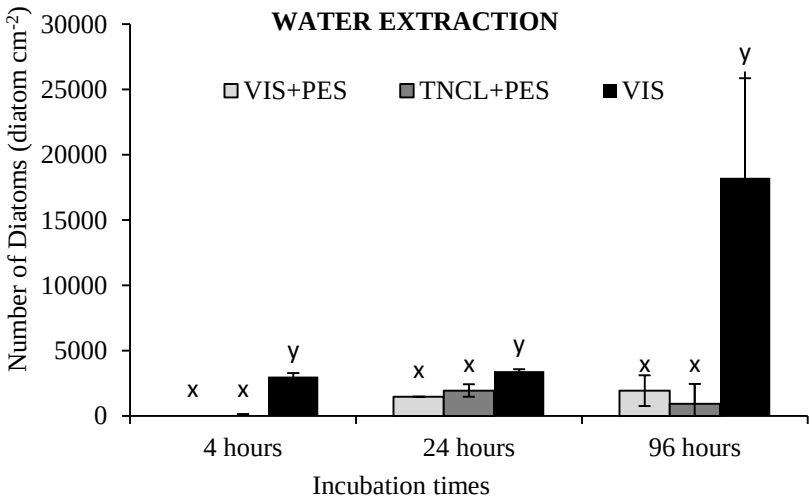

Figure 6. Diatom counts obtained by rinsed with distilled water in different fabric types (error bars indicate standard deviation). Different lowercase letters above bars indicate statistically significant differences between different fabrics.

The estimated mean of total diatoms count by RE method are given in Figure 7. The data obtained from the RE method were found to be very similar to the data obtained from the RW method. While there were no diatoms in VIS+PES and TNCL+PES fabrics after 4-hours of immersion, diatoms were detected after 24 and 96-hours of immersion. Unlike other fabrics, diatom was detected in the VIS fabric after 4-hours of immersion. After 24 hours of immersion, diatoms were observed in all fabric types. The highest diatom count was determined in the VIS fabric after 96-hours of immersion, again similar to the RW method. Two-way ANOVA results also show that diatom counts vary depending on immersion time ($F_{(2,18)}$= 24.21; p=0.000). While there was no significant relationship between 4-hour and 24-hours of immersion (p>0.05), a significant relationship was found between 96-hours of immersion and other immersion times (p<0.05).

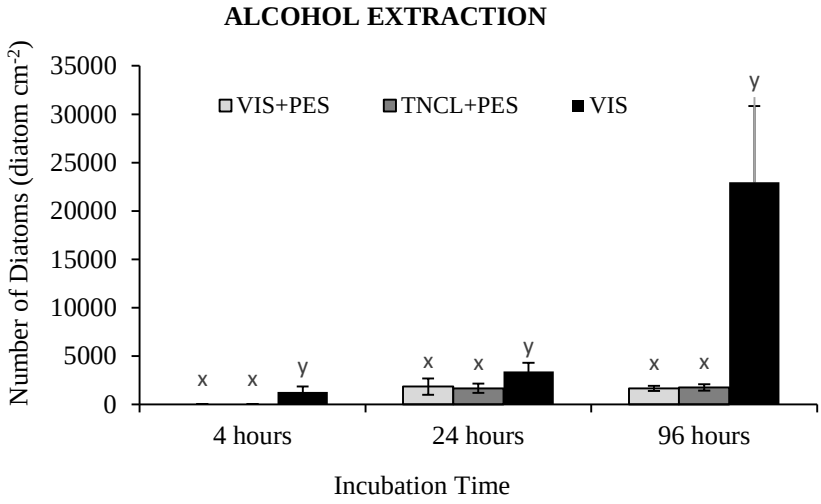

Figure 7. Diatom counts obtained by rinsing with ethanol in different fabric types (error bars shows standard deviation). Different lowercase letters above bars indicate statistically significant differences between different fabrics.

In contrast to RW and RE methods, diatoms were determined in VIS+PES fabric even after 4-hours of immersion by H_2O_2-digestion method (Figure 8). However, as in other methods, diatoms were not detected after 4-hours of immersion in TNCL+PES fabric. The highest total diatom count in all fabric types was found after 96-hours of immersion. Statistical analyses also showed that both fabric type ($F_{(2,18)}$= 96.23; p=0.000) and immersion time ($F_{(2,18)}$= 824.18; p=0.000) were effective in the transfer of diatoms to the fabric. The multiple comparison test showed that there was no significant difference between 4-hour and 24-hours of immersion, while 96-hours of immersion was different from the others. Among the fabric types, TNCL+PES was found to be statistically different from other fabric types.

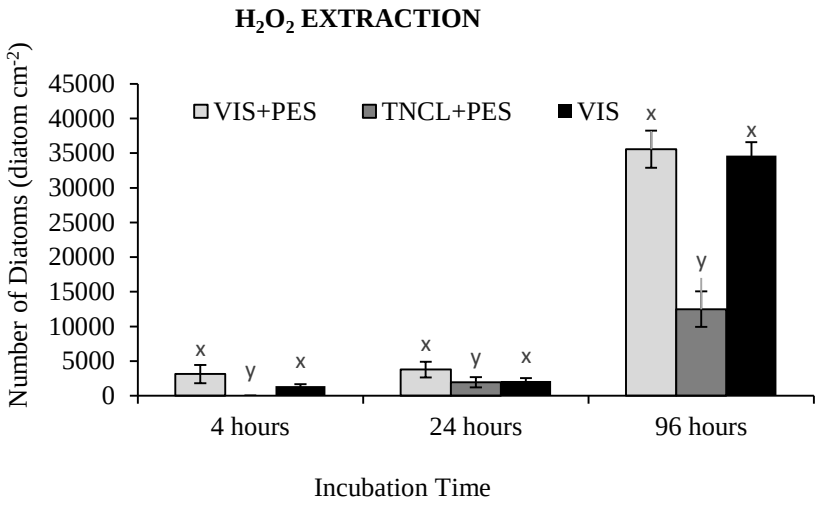

Figure 8. Diatom counts obtained by digestion with hydrogen peroxide in different fabric types (error bars shows standard deviation). Different lowercase letters above bars indicate statistically significant differences between different fabrics.

The three-factor ANOVA results showed that the diatom counts obtained from the fabric pieces differed both among the fabric types, immersion times and extraction methods (Table 1). Multiple comparison tests show that the H_2O_2-digestion method is different from the other methods. The highest diatom counts were obtained with the H_2O_2-digestion method throughout the study. All fabric types used in the study were also found to be different from each other. It was determined that there was no significant difference between the 4-hour and 24-hour periods for the transfer of diatoms to the fabrics, but the 96-hour immersion time was statistically different. In addition, the three factorial ANOVA results show that all factors in the study interact with each other. Accordingly, it was determined that there was an interaction between the extraction method and fabric types, between the extraction method and immersion time, and between the fabric types and immersion time.

Table 1. Three-way ANOVA results for diatom numbers determined in different fabrics (three levels: VIS+PES, TNCL+PES and VIS) extraction methods (three levels: RW, RE and H_2O_2-digerstion) and immersion times (three levels: 4, 24 and 96 hours). (df means degree of freedom, F means the value of F statistic, and Sig. means the p-value)

	df	**F**	**Sig.**
Extraction method	2	78.93	< 0.001
Fabric types	2	74.84	< 0.001
Immersion time	2	270.49	< 0.001
Extraction method * Fabric types	4	15.35	< 0.001
Extraction method * Immersion time	4	67.14	< 0.001
Fabric types * Immersion time	4	48.75	< 0.001
Extraction method * Fabric types * Immersion time	8	7.35	< 0.001

Discussion

The data obtained in this study showed that the method used, fabric type and the time of immersion affected the number of diatoms obtained from forensic evidence. Moreover, studies show that diatom morphology is also effective for adhering to the surfaces (Magni et al., 2020; Scott et al., 2021). The diatoms are morphologically divided into 2 groups: centrales (centric), which have a rounded appearance, and pennales (pennat), which have an elongated appearance (Round et al., 1990). It is known that centrales members are generally planktonic and pennales members are benthic, that is, they live by attaching to a substrate (Molino et al., 2016). In the study, among the commonly identified species on fabrics, *Cocconeis*, *Tryblionella, Fallacia, Pleurosigma,* and *Nitschia* have pennate morphology. *Coscinodiscus* and *Pleurosira laevis*, despite having centric morphology, have benthic habitat preferences.

In addition, it is known that the colonization success of diatoms on any surface is related to their raphe characterizations. The raphe is a slit in the silica cell wall of diatoms, and it is thought to be responsible for cell movement because the mucilaginous substance secreted from the cells is released from here (Siver and Velez, 2023). Stevenson (1986) categorized diatoms into three groups according to their colonization stages: pioneer colonist, late colonist and intermediates. Acs and Kiss (1993) stated that araphid and large-sized biraphid diatoms are pioneers, small-sized biraphid and monoraphid diatoms are intermediates, and medium-sized biraphid and monoraphid diatoms are late colonists. No araphid species were found in this study. The monoraphid or biraphid characters of the identified species suggest that they are late colonists or intermediates. Hoagland et al. (1986) also stated that raphid diatoms are the most abundant colonizers.

Among the fabrics, the highest diatom count was generally recorded in VIS fabric at all immersion times. Our results showed that VIS fabric is suitable for the transfer of diatoms. Compared to other fabrics, even 4 hours of immersion time was sufficient for the transfer of diatoms to VIS fabric. In fact, 4 hours of immersion time also seems to be sufficient for the transfer of diatoms for VIS+PES fabric, but they could only be obtained from the fabric by the H_2O_2 method. It appears that, 24 hours of immersion time was required for the transfer of diatoms to TNCL+PES fabric. It is thought that this difference between the fabrics for the transfer and subsequent colonization of diatoms is due to their surface structures. PES, a petroleum-based material, is one of the most produced synthetic fibers and has a hydrophobic character (Zhang et al., 2021). On the other hand, VIS has a semi-synthetic and hydrophilic character and is produced from natural cellulose. Unlike polyester, VIS fabric, which has nanopores, appears to have a rougher surface structure. For this reason, we believe that diatoms can more easily attach to VIS fabric. Although Tencel fabric, which is semi-synthetic and produced from wood cellulose, is similar to VIS fabric in terms of fiber, our results have shown that diatom attachment does not occur in a short time. Whilst to VIS fabric, when polyester is added to the fabric structure, it is understood that diatom attachment becomes more difficult.

The immersion time statistically affected the number of diatoms extracted from the fabrics. Although it was detected in VIS and VIS+PES fabrics with various extraction methods, diatoms were not found in TNCL+PES fabric after 4 hours of immersion. Even with the H_2O_2 digestion method, which is recommended as the most successful method (Scott et al., 2014), the presence of diatoms could not be detected. Therefore, it is clear that 4 hours of immersion will not be sufficient for diatom binding in all studied fabric types. On the contrary, Scott et al. (2014) recorded the highest number of diatoms in cotton fabric with 3 hours of immersion in a pond in Greenland, while they recorded the highest number of diatoms in 30 minutes in a stream. This difference may be

thought to be due to the fact that lake waters are stagnant, and rivers have more water movement compared to sea water, rather than the fabric type. In addition, the density of diatoms in the aquatic environment in which the fabrics were immersed may also affect the transfer time of diatoms to the fabric. In another study with different footwear materials (Canvas, suede, leather, rubber and polyurethane), the presence of diatoms was determined at 3 minutes of immersion time in freshwater (Levin et al., 2017). The researchers stated that diatoms transfer was observed even in 30 seconds with canvas, but the number of diatoms increased as the time increased. It is understood that 24 hours of immersion time is sufficient for diatom attachment and initiation of colonization with all studied fabrics and all examined methods.

In this study, 3 different methods were tried to obtain diatoms from fabrics. Among these, the highest diatom numbers were counted in the H_2O_2 digestion method with all studied fabrics and immersion times. Similar results were reported by Scott et al. (2014) for cotton fabric, and by Magni et al. (2020) for cotton, denim acrylic, and blend fabric. Levin et al. (2017) reported that diatoms were disintegrated by the H_2O_2 method. He also stated that they obtained more diatoms by the heating and shaking method compared to the H_2O_2 method. In another study, he reported that the most effective method for diatom isolation is the H_2O_2 method, but this method damages the fabric structure (Tekçeer, 2017).

Conclusion

As a result, our data show that the number of diatoms to be obtained from forensic materials varies according to the immersion time, fabric type and method used. While diatoms binding occurs in a very short time in some fabrics, it may take longer in other fabrics. Even if diatom binding occurs in a short time, diatom may not be obtained with the method used. For this reason, in forensic cases where the diatom test will be used, especially in cases where the duration of seawater residence is unknown, it may be recommended to use the H_2O_2 method, which is the most reliable method. However, the possibility that this method may damage the evidence should also be taken into consideration. If the evidence obtained is small and will be required for further procedures, it would be more advantageous to use the rinsed with alcohol method instead of the H_2O_2 method. Similarly, if the evidence is considered to have been in seawater for a long time, other methods may be preferred over the H_2O_2 method.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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