Diatom analysis: Reviewing the strengths, weaknesses, and impacts of modern research

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Abstract The purpose of this paper is to review the theoretical and laboratory techniques which underpin diatom analysis and its ability to help determine death by drowning in a forensic context. Diatom analysis involves the recovery of diatoms (unicellular algae present in most natural bodies of water) which may be inhaled as part of a drowning medium (water) by an individual who has drowned prior to death. This literature review evaluates the strengths and weaknesses of the diatom analysis technique in the forensic context and evaluates recent research to discern whether it can be considered a reliable and valid forensic technique today. It is important to establish clarity as much of the original research conducted throughout the 1970's, 80's, and 90's have conflicting conclusions regarding the validity and reliability of diatom analysis. This literature review will show that modern research techniques used as part of the diatom analysis method have been able to reduce false positive results, increased the ability to distinguish between true and false positives, and found ways to mitigate many of the weaknesses noted in earlier research. Although some weaknesses such as diatom introduction into bodies prior to death and some details surrounding false positive results remain outstanding concerns, it can be seen in the literature that, when combined with existing strengths like seasonal variability and environmental specificity, diatom analysis is a valuable forensic tool, whose reliability has been strengthened by modern research, and can be relied upon to establish a definitive diagnosis of death by drowning.

INTRODUCTION

The forensic technique of diatom analysis is specific and uncommon but is a necessary and important diagnostic due to the complexities involved in determining death by drowning (Jian et al., 2019; Piette & De Letter, 2006). As Yukawa et al. (2013) note, diatom analysis is often considered to be "the gold standard" (p. 1) amongst the many laboratory tests to conclude death by drowning. Over 150,000 diatom species (unicellular algae) exist which can be found wherever sufficient light exists to support photosynthesis in most natural water bodies (Hendey, 1973). Diatoms preserve well, occur in high numbers, are detectible in almost all environments, and are environmentally specific (Horton et al., 2006; Pollanen et al., 1997). The main principle is that diatoms are present in the medium where drowning occurred and they will therefore also be present in body tissues (Horton et al., 2006; Krstic et al., 2002; Pollanen, 1998).

Diatom frustules, the subject of analysis, have hard box-like silica skeletons which are resistant to acid digestion and are almost indestructible, being soluble only in strong acid solutions (Fucci et al., 2015; Hendey, 1973; Seo et al., 2013), making post-mortem analysis possible (Pollanen et al., 1997). Diatom analysis allows forensic pathologists to determine the specific medium which the victim drowned in (e.g. fresh water, sea water, or lake water) because different diatom frustules can be found where environmental conditions differ due to narrow tolerances for temperature, light, salt content, environmental pollution, and pH levels (Levin et al., 2017; Tavassi et al., 2008). Thus, different species will be found in different bodies of water around the world (Auer, 1991; Coelho et al., 2016; Ludes, 2013; Peabody, 1977). Different species will also be present at different depths due to varying photosynthesis abilities (Auer, 1991).

Diatoms can be recovered from fresh, decomposed, and burnt tissues (Fucci et al., 2015)

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because prior to drowning, the victim would still have a beating heart which would allow diatoms to travel throughout the body via the circulatory system (Ago et al., 2011). Combining the inhalation of water and a beating heart allow diatoms to pass into the bloodstream as part of the oxygen exchange process (Rohn & Frade, 2006). As part of this process, diatoms then penetrate the lungs when water is inhaled, become lodged in alveoli, and can also be carried to internal organs such as the kidneys, heart, brain, and bone marrow (Ago et al., 2011; Antonenko & Ferris, 1987; Horton et al., 2006; Krstic et al., 2000; Pollanen et al., 1997). Thus, it is unlikely diatoms would be found in the internal organs or bone marrow of bodies disposed of in water post-mortem (Krstic et al., 2002). This makes diatom analysis helpful in determining whether a victim drowned or was disposed of in water post-mortem (Gruspier & Pollanen, 2000). In rare cases however individuals suffer rapid heart failure prior to drowning preventing circulation of diatoms and their entrance into internal organs (Antonenko & Ferris, 1987; Piette & De Letter, 2006).

Diatom analysis however remains controversial because diatoms can be found in tissues of people who died of causes other than drowning due to the widespread distribution of diatoms through the environment in soil, food, and air (Lucci et al., 2008). Diatoms can also implant passively into the lungs, stomach contents, and upper airways leading to unreliable analyses (Ago et al., 2011; Coelho et al., 2016). Diatom analysis remains in use however, because the diagnosis of drowning is highly complex (Ludes et al., 1999; Stephensen et al., 2019; Timperman, 1972), and other signs of drowning can in many cases can be insignificant or non-existent and may dissipate after death (Badu et al., 2015). Diatom analysis was recently used in a United Kingdom animal cruelty case which determined that an owner drowned their dog in a canal (BBC News, 2019; Johnson, 2019). As opinions on its usefulness remains controversial (Yukawa et al., 2013), it is important to evaluate the technique in greater detail.

Through evaluation of older and more recent literature, this paper aims to review the strengths and weaknesses of diatom analysis and determine whether it can still be considered a reliable technique in analysing possible cases of death by drowning. Much of the older literature on diatom analysis has been included as it is foundational to the evolution of the technique. Literature for this paper was collected through library resources at Simon Fraser University and additional online searches for related peer-reviewed literature. As reliability of analysis remains controversial amongst the scientific community (Carballeira et al., 2018), and testing historically laborious (Zhou et al., 2020), it is important to analyse the literature to determine whether the development of new techniques has increased the reliability. It will be seen in the literature that although weaknesses still exist, modern research and laboratory techniques have strengthened the reliability of diatom analysis showing that it should still be considered a strong forensic tool.

DISCUSSION

Strengths

Coelho et al. (2016), note the scientific principle of environmental specificity establishes the validity of diatom analysis. As specific diatom species are found in different water bodies, the presence of diatoms in the deep tissues or bone marrow of victims can lead to a definitive diagnosis of death by drowning (Auer, 1991; Pollanen, 1998). The diatoms present in bone marrow and other tissues can be compared to samples taken from the medium to determine an approximate drowning site to see if similar diatom species are present (Auer, 1991; Hürlimann et al., 2000; Pollanen, 1998). Environmental specificity makes diatom analysis a good indicator to use in suspicious death investigation when drowning is not suspected, because diatoms present in the bone marrow will match those found in the body of water where the victim was found (Pollanen et al., 1997).

When diatoms are present in high quantities, it is possible to pinpoint the exact location where drowning took place (Peabody, 1977), but only if the drowning area is localized such as in a pond or a ditch (Auer, 1991). It is possible however to approximate the location due to environmental specificity (Auer, 1991); diatoms would need to be sampled from surrounding areas and waterbodies if the exact location is unknown (Coelho et al., 2016).

Determining which diatom species are present in body tissues can also help establish the potential drowning location and the time of death due to seasonal variability. The prevalence of diatoms in waterbodies also varies seasonally (Hendey, 1973; Pollanen et al., 1997). Diatom blooms (when concentrations are highest) follow seasonal patterns with diatom concentrations spiking in April before decreasing throughout the summer and occurring in lower numbers through the winter months (Pollanen et al., 1997). Seasonal variability can be used to scientifically determine an approximate time of death as the diatom density in bone marrow would reflect water concentrations at the time of death (Hürlimann et al., 2000). As the success of diatom analysis is correlated with diatom concentrations in the environment, diatom analysis is most successful in summer months and least successful in winter months when concentrations are low (Pollanen at al., 1997).

The presence of even a few diatoms in organs other than the lungs can be a reliable indicator of death by drowning (Hendey, 1973). Peabody (1977) suggested that their discovery in bone marrow is an even better indicator. This is supported by Auer (1991), who specifies that analysis of diatoms found in bone marrow is the only definitive way to diagnose death by drowning in decomposed bodies. This is because diatoms can enter internal organs during the body decomposition process sometimes making analysis unreliable (Coelho et al., 2016; (Pollanen et al., 1997). Diatom analysis may be the only way to determine death by drowning after attempted resuscitation or post-mortem mutilation (Timperman, 1972). Lungs are excluded from analysis if the chest has been torn open and lungs have contacted water. Hendey (1973) and Pollanen et al. (1997), suggest that analysis of bone marrow from intact long bones like the femur are preferred. The absence of diatoms does not eliminate drowning as the cause of death, as it is possible that drowning may have occurred in water containing few or no diatoms (Pachar & Cameron, 1993; Peabody, 1977).

To solve this issue, minimum quantitative thresholds have been established and more research has been done regarding diatom density in different body tissues. Pachar and Cameron, (1993), established minimum thresholds for diatoms within a victim's organs and tissues to diagnose death by drowning, only making positive diagnoses when a significant difference existed between the number of diatoms in the lungs and those in closed organs such as bone marrows. In a study by Ago et al. (2011), drowning patterns were assessed based on diatom density in the drowning medium suggesting that diatom concentrations will be the highest in the lungs and will decrease as the diatoms circulate into deeper organs such as the bone marrow. The higher the diatom concentration is in the drowning medium, the greater the likelihood of positive results (Ago et al., 2011; Antonenko & Ferris ,1987).

Weaknesses

Although there are many strengths with the diatom analysis technique, it is highly important to also discuss the various weaknesses. Diatoms can be introduced into the body through regular activities such as breathing, eating or drinking small diatom cells (Krstic et al., 2002). If exposure to materials containing diatoms occurs, the victim's organs cannot be used as evidence of drowning due to the pre-exposure (Taylor, 1994). Issues arise as marine diatoms can be well preserved in limestone, become aerosolized, and then inhaled, allowing them to enter the bloodstream (Moshkovitz et al., 1983). The lungs therefore are not good organs to analyse due to potential contamination (Horton et al., 2006; Pachar & Cameron, 1993). Inhalation however may be negligible (Antonenko & Ferris, 1987), as the body can naturally eliminate some foreign particles and prevent them from entering the organs (Pachar & Cameron, 1993). Thus further research is needed to analyse potential diatom build up in organs (Law & Jayaprakash, 2007).

Issues may arise however when victims are found in drowning mediums they were previously exposed to. Taylor (1994) notes a specific case where a victim was discovered in a body of water that they swam in every day for 15 years. Due to prior exposure, forensic scientists were unable to distinguish whether the victim last entered the water body alive or was environmentally predisposed to the same diatom species (Taylor, 1994). Further, little consensus exists regarding whether diatoms are accurate indicators of death by drowning, as diatoms can be found in swimmers or fishermen who were previously exposed to diatoms (Diaz-Palma et al., 2009). Thus, analysts must remain prudent in analysing victim records for previous diatom exposure events (Antonenko & Ferris, 1987).
Lab contamination can also impact findings in non-drowned victims as diatoms may be present in water used to wash lab instruments (Pollanen et al., 1997). Diatoms from instruments could mix with the bone marrow solution resulting in false positives (Pollanen et al., 1997). Lunetta et al. (2013) suggest that flasks and equipment be cleaned every 24 hours using sodium hydroxide and that old flasks with minor irregularities be replaced to prevent contamination, arguing that contamination is not an impediment once proper controls are implemented. Contamination however remains inevitable (Ago et al., 2011) although it can be mitigated through environmental specificity (Pollanen et al., 1997).

Diagnosing death by drowning is difficult in a body that has been severely dismembered or fragmented due to environmental exposure (Lunetta et al., 2013; Peabody, 1980). During decomposition, diatoms can passively enter soft tissues and organs (Hendey, 1973; Krstic et al., 2002), making the presence of diatoms in the lungs and stomach of potential cases of drowning inconclusive (Ago et al., 2011; Coelho et al., 2016). Large lacerations can also allow diatoms passive entry into the body, impacting the reliability of analysis (Hendey, 1973). As previously noted, diatoms can also passively enter airways through other means even when a victim did not drown (Bortolotti et al., 2011). Experts must therefore proceed with caution when interpreting diatom results from a decomposing body (Lunetta et al., 2013). Diatoms however are found in much higher concentrations in the bodies of drowning victims than of those disposed of in water post-mortem (Lunetta et al., 2013), and modern research techniques have been created to distinguish between true and false positives (Shen et al., 2019).

Rapid death can also prevent diatoms from circulating through the blood stream for a variety of reasons (Antonenko & Ferris, 1987; Piette & De Letter, 2006). Therefore, in some cases, other investigative methods must be used. Further, although finding high diatom concentrations may indicate voluntary water inhalation (Auer, 1991), lungs are not always a good organ for analysis as diatoms can passively enter them (Ago et al., 2011; Coelho et al., 2016; Pachar & Cameron, 1993; Timperman, 1972). In some cases, diatoms may only be found in the lungs and not other body tissues, meaning it would not be possible to definitively diagnose drowning as the cause of death (Bortolotti et al., 2011; Pollanen et al., 1997).

Horton et al. (2006) also suggest that it may be difficult to conclude if a death was a homicide or a natural drowning, as similar diatom species can be found in different water sources. For example, if a body was found in a lake that the city used for its water supply, it would be impossible to conclude whether the individual drowned in the lake or in a bathtub as the diatom species in either waterbody would not be distinct. Further, due to the low occurrence of positive tests, the likelihood of a false positive is relatively small as well, however this rate can be reduced through quantitative analysis (Ludes et al., 1999; Ludes, 2013; Lunetta et al., 2013; Pollanen et al, 1997). The establishment of a minimum threshold can help distinguish deaths by drowning due to much higher concentrations of diatoms often found in drowning victims (Shen et al., 2019). As Shen et al. (2019) found, significant statistical differences exist between false-positives and true cases of drowning lessening their impacts on analysis.

The main disadvantage of the diatom test however, is its sensitivity (Pollanen, 1998). The study by Pollanen et al. (1997) only obtained a positive result in 30% of freshwater drownings, meaning that diatom frustules will not be detectible in the majority of cases. As can be seen by the cited literature however, much of the original foundational analysis was completed in the 1970's, 80's, and 90's. It is therefore important to analyse the impact that modern techniques have had on diatom analysis as a technique to determine cause of death. Liu et al. (2020) note that the substantial technological changes in recent decades have led to the development of new techniques that have increased the accuracy and reliability of diatom analysis. These methods, as Carballeira et al. (2018) note, have helped further validate it in the forensic sciences.

Impact of modern research advances

Although the weaknesses of diatom analysis are significant, it can be seen that modern research from 2013 to present shows that the reliability of diatom analyses can improve with new techniques and that controversies surrounding it have been reduced.

Many digestion techniques have been developed to separate the diatoms from organs.

Analysis can be completed with a much higher sensitivity using modern microwave digestion techniques, vacuum filtration, and high-resolution electronic microscopy (Hu et al., 2013). Fucci et al. (2015) also found that using hydrogen peroxide led to the discovery of nine new diatom species compared to the traditional hydrochloric acid method. Zhao et al. (2017) developed a new microwave digestion technique using scanning electron microscopy which led to a 97% positive test rate when evaluating closed organs (liver and kidneys). This was a was an approximate 70% increase from the 27% positive test rate from Pollanen et al. (1997), showing that reliability of diatom analysis has increased substantially with the development of new techniques which produce more accurate and reliable results.

Researchers have also found ways to reduce issues surrounding false positive results which is the greatest threat to diatom analysis reliability (Shen et al., 2019). Shen et al. (2019) found that statistical differences in diatom concentrations in bodily tissues existed between cases of true drowning and false positive results. Although diatoms can be found in victims who did not drown due to passive entry, the number of diatoms found in all organs are significantly greater in cases where victims were known to have drowned (Shen et al., 2019). This new method developed by Shen et al. (2019) shows that parameters can be set to allow forensic pathologists to distinguish between true and false positive cases and to better determine when false positive results emerge. Research by Li et al. (2019) further highlights the use of the ratio of diatom concentration in the lung tissues to the diatom concentration of the drowning medium to increase reliability.

To reduce the impact of contamination, Seo et al. (2013), used DNA-binding properties to isolate the diatoms from the heart blood sample after chemical digestion, which allowed for the separation of diatoms from other impurities, easing detection of diatoms. Seo et al. (2014) also developed a technique using silica-coated magnetic beads to separate diatoms from contaminants which showed significant improvements over previous methods. Zhou et al. (2019) also discovered that artificial intelligence (AI) could be used to automatically identify diatoms on a slide which increases the efficiency of analysis. As diatom analysis remains "time consuming and burdensome" (Zhou et al., 2019, p. 1), the discovery of the reliable use of AI as a rapid and objective method is encouraging to the forensic sciences. Zhou et al. (2019) note that additional research is still necessary to further explore the use of AI. Kakizaki et al. (2019) also worked to develop a new diatom digestion method using papain – a vegetable enzyme from papaya – instead of the more laborious and hazardous traditional acid-digestion method. They noted that this method was promising and that additional development should be undertaken. These studies show that additional research can further refine diatom analysis methods so external factors no longer present grave impediments.

CONCLUSIONS

Although the reliability of diatom analysis has been questioned due to the prevalence of false positive results and past issues with sensitivity in low diatom concentrations, it can be seen that modern research and laboratory techniques have addressed many of the existing concerns (Shen et al., 2019). Modern research has helped strengthen diatom analysis and it remains an important and necessary forensic tool, as diagnosing death by drowning can be highly complex. Along with its existing strengths, which include the presence of diatoms in almost every drowning medium, environmental specificity, seasonal variability, and significant quantitative differences between drowned and non-drowned victims, the reliability of diatom analysis continues to increase due to the use of modern research techniques and additional research. Since the foundational research of the 1970's and 80's, many significant developments have improved the reliability of diatom analysis and reduced many of its grave weaknesses including the ability to better distinguish between true positive and false positive results. As diatom analysis remains one of the only definitive ways to determine death by drowning due to the lack of alternative methods, the literature shows that additional refinement and research can continue to improve the technique, making analysis easier, less hazardous, and less time consuming, so that forensic pathologists continue to have this tool at their disposal and that its important role in forensic sciences can continue.

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