DIATOMS AND FORENSIC SCIENCE

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Abstract—The application of diatom analysis in determining whether drowning was the cause of death has proved to be a valuable tool in forensic science. The basic principal of the “diatom test” in drowning is based on inference that diatoms are present in the medium where the possible drowning took place and that the inhalation of water causes penetration of diatoms into the alveolar system and blood stream, and thus, their deposition into the brain, kidneys, and other organs.

I provide an informal assessment of “reliability” of the “diatom test” through correlations between control samples and samples from organs and clothing in two case studies. In studies, all organ and clothing samples except one had matching analogues in the modern diatom dataset from the body recovery sites, reinforcing drowning as the cause of death. The analogue matching provides further information on the precise site of drowning, in particular differentiating between drowning in a bathtub versus a naturally occurring body of water.

INTRODUCTION

In 1904 Gregory Popp became the first scientist to present in court a case where the geological make up of soils was used to secure a criminal conviction (Ruffell and McKinley, 2005). Nevertheless, it was not until 1975 that the first textbook on forensic geology was published (Murray and Tedrow, 1975), and there is still only a very limited dedicated literature in the field (e.g. Saferstein, 2001; Murray, 2004; Pye and Croft, 2004; Ruffell and McKinley, 2005; Ruffell, 2006). Many general texts on crime scene investigations make little or no mention of forensic geology (Saferstein, 2001) and there is a lack of knowledge in the legal profession and police forces of its potential (Pye and Croft, 2004; White, 2004). However, one geologically-based forensic technique that has become established in forensic science is the “diatom test” for drowning (e.g. Peabody and Burgess, 1984; Pollanen, 1998; Cameron, 2004; Horton et al., 2006).

Drowning can be defined as death due to full or partial submersion in a fluid (Timperman, 1972; Krstic et al., 2002). The fundamental principal of the diatom test in investigation of drowning is based on the postulation that diatoms are present in the fluid where possible drowning took place and the inhalation of the fluid causes penetration of diatoms into the alveolar system and blood stream, and leading to their deposition into brain, kidneys, and other organs (Krstic et al., 2002). If the victim was dead before the body was submerged, the transport of diatom cells to various organs is prevented because of a lack of circulation.

Diatom analysis can be of further use in forensic science through identifying the provenance of individuals, clothing or materials from sites of investigation (Peabody, 1980, 1999; Siver et al., 1994; Pollanen et al. 1997; Krstic et al., 2002; Cameron 2004). Where materials have been submerged or there has been contact with littoral or riparian sediment or vegetation, diatom analysis of sediments or other diatomaceous traces present on clothing or footwear can be used to identify the type of habitat (Cameron, 2004). The comparison of external diatom assemblages is important for cases where a body has been transported a significant distance by currents or where a body may have been deliberately moved from the site of drown-

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ing (Cameron, 2004). There are strong environmental gradients in salinity, pH, and nutrient concentration in estuaries and rivers, thus diatom analysis will reflect this variation in water quality.

In this paper, we employ the “diatom test” in two case studies of drowning. We use a quantitative reconstruction technique, known as a transfer function to correlate diatoms from the study area and recovered from clothing and lung tissue/fluid to (a) reinforce drowning as a cause of death and (b) localize the site of drowning.

CASE STUDIES

In Case Study 1 the body of a woman was found floating face down in a river. Post mortem examination found the death to be suspicious. Death was attributed to the asphyxial effects of drowning due to homicide; however, a key aspect of the subsequent investigation was the precise site of drowning. Thus, we collected 12 samples for diatom analysis from five sites along a 50 km length of the river, including the body recovery site (Figure 1) to act as a control in the examination of diatom assemblages associated with lung fluid and

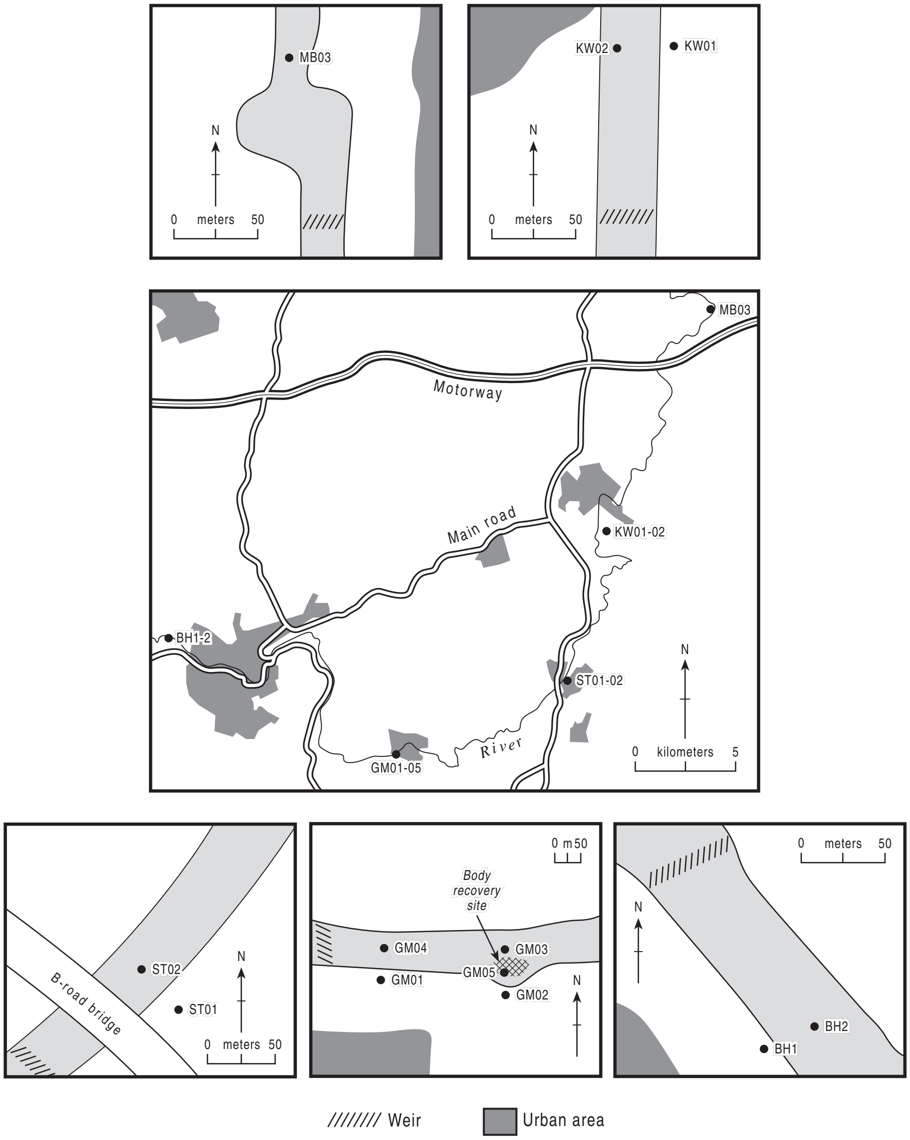

Figure 1—Location Map for Case Study 1 (modified from Horton et al., 2006).

clothing belonging to the accused (training shoe, sock, and T-shirt). At each sampling site, we collected diatom samples upstream of the local weir from a variety of river bed and river bank habitats to permit comparisons with the ecological conditions at the body recovery site (Horton et al., 2006).

In Case Study 2 the body of a boy was found face down floating in a pond. The post-mortem conducted at the time concluded that death was by vasovagal inhibition as a result of cold water immersion. The pathologist attributed death to fresh water drowning and concluded that death was not suspicious. However, the case was re-opened as it was thought the drowning may have been homicide by the child's mother (Horton et al., 2006). It was suggested that drowning took place in a domestic bath, and that the body was subsequently placed in the pond. Thus, we collected 14 samples for diatom analysis from four transects around the circumference of the artificial pond and two samples of sediment from the center of the pond to act as a control in the examination of diatom assemblages associated with three lung tissue samples (Figure 2).

Methodology

We collected a sample of surface sediment consisting of approximately 5 cm^3 volume (5 cm^2 surface sample by 1 cm thick) from control stations within the local/regional area. We were provided with diatoms slides from the lungs and clothing in both case studies by Crown Prosecution Service of England and Wales. In Case Study 1 the diatom slides were prepared from fluid in the cadaver's lungs and in Case Study 2 the diatom slides came from lung tissue taken at the original autopsy. The samples were digested in 70-100 ml of 20% H_2O_2 by heating gently in a water bath for up

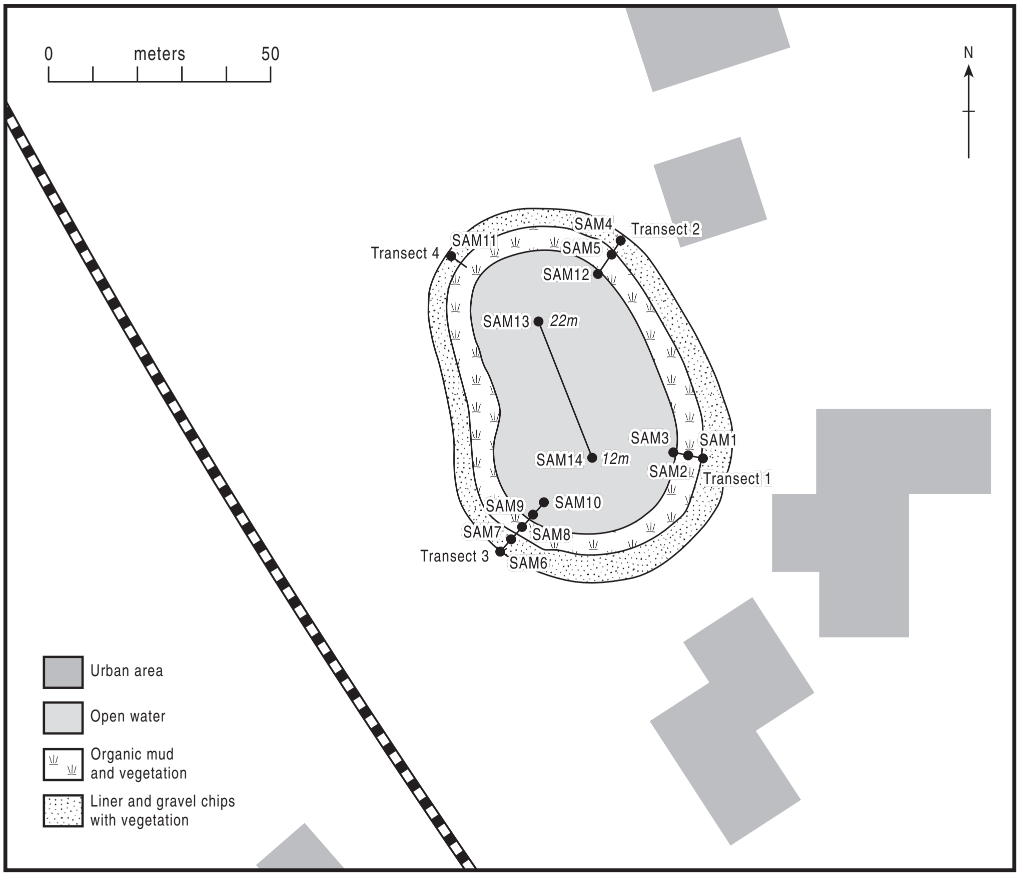

Figure 2— Location Map for Case Study 2 (modified from Horton et al., 2006).

to 24 hours, or until all organic matter was removed from the sample. Two and five drops of digested sample were pipetted onto two cover slips with 10 drops of distilled water and dried on a warm hotplate. Cover slips of differing concentration were then inverted and placed onto a glass slide using Zrax, which is a high refractive index medium mountant. After further gentle heating and cooling we counted a minimum of 250 diatoms at a magnification of 1000 times using the keys of Hartley (1996) and Van der Werff and Huls (1958-1974).

To detect, describe and classify the distribution of diatoms at each local/regional study area we used two multivariate methods: (1) unconstrained cluster analysis, based on the unweighted Euclidean distance using incremental sum of squares with no transformation or standardization of the percentage data (Grimm, 2004), which classifies contemporary samples into more-or-less homogeneous zones (clusters); and (2) detrended correspondence analysis (DCA), an ordination technique, which represents samples as points in multi-dimensional space, where similar samples plot close together and dissimilar samples apart (ter Braak and Smilauer, 1997-2003). Thus, cluster analysis is effective in classifying the samples according to their diatom assemblage. The DCA gives further information about the pattern of variation within and between diatom zones, which is important because the precise boundaries between clusters can be arbitrary (Birks, 1986).

We also employed a transfer function, known as modern analogue technique (MAT), to compare the control samples with the lung and clothing samples (Juggins, 2004). The basic idea of the MAT is to compare numerically, using square chi-square distance dissimilarity coefficient, the diatom assemblage in a lung or clothing sample with the diatom assemblages in all available control samples. Having found the control sample(s) most similar to a lung or clothing sample, the local environment for the latter is inferred to be the locality of the analogous control sample(s) (Birks, 1995). Furthermore, the modern analogue technique is an important means of evaluating the likely reliability of reconstructions (Horton and Edwards, 2006). The 10th percentile of the dissimilarity range calculated between control samples is an approximate threshold value to indicate a "good analogue" (Birks, 1995; Horton and Edwards, 2006; Horton et al., 2006). Thus, the reconstructed location for lung and clothing samples was assumed to be reliable if a "good analogue" (dissimilarity coefficient ≤10th percentile) was indicated whilst the estimates associated with "no close analogue" (dissimilarity coefficient >10th percentile) samples should be treated with caution. For all statistical analyses we removed samples that had counts of less than 100 and all species groups that contributed less than 2% of any assemblage.

Case Study 1: The woman floating in a river

We identified 99 different diatom species from the twelve control samples from five sites along a 50 km length of the river. *Navicula radiosa*, *Cocconeis placentula* var. *euglypta* and *Achnanthes lanceolata* are the dominant diatom species. However, there are variations in the presence or absence of minor species and changes in the proportions of the dominant species; this is expected when one considers the complexity of in-channel microhabitats. Multivariate analyses divide the control samples into three zones (Figure 3). Zone A consists of samples GM/03, KW/02, BH/02 and GM/04, which are taken from in-channel habitats at a depth of 1.5 m or deeper with moderate flow. The zone is dominated by the benthic species *Navicula radiosa* and low percentages of the sessile/epontic species *Cocconeis placentula* var. *euglypta*. Samples GM/01, GM/02, ST/01, KW/01 and BH/01 comprise Zone B. These samples are from the river bank, above the water level. They are characterized by having the lowest percentages of *Navicula radiosa* and higher percentages of sessile/epontic species such as *Cocconeis placentula* var. *euglypta* and *Achnanthes lanceolata*. Zone C has three samples (GM/05, ST/02 and MB/03), which are from shallow water habitats (<1.5 m deep) and slow water flow. Zone C is composed of relatively high percentages of *Navicula radiosa*, and *Cocconeis placentula* var. *euglypta*.

Cocconeis placentula var. *euglypta*, *Melosira varians* and *Navicula radiosa* dominate the lung fluid (GCAF 16), and training shoe (KP31D) and T-shirt (KP87D) belonging to the accused. The MAT suggests that all lung and clothing samples have a "good

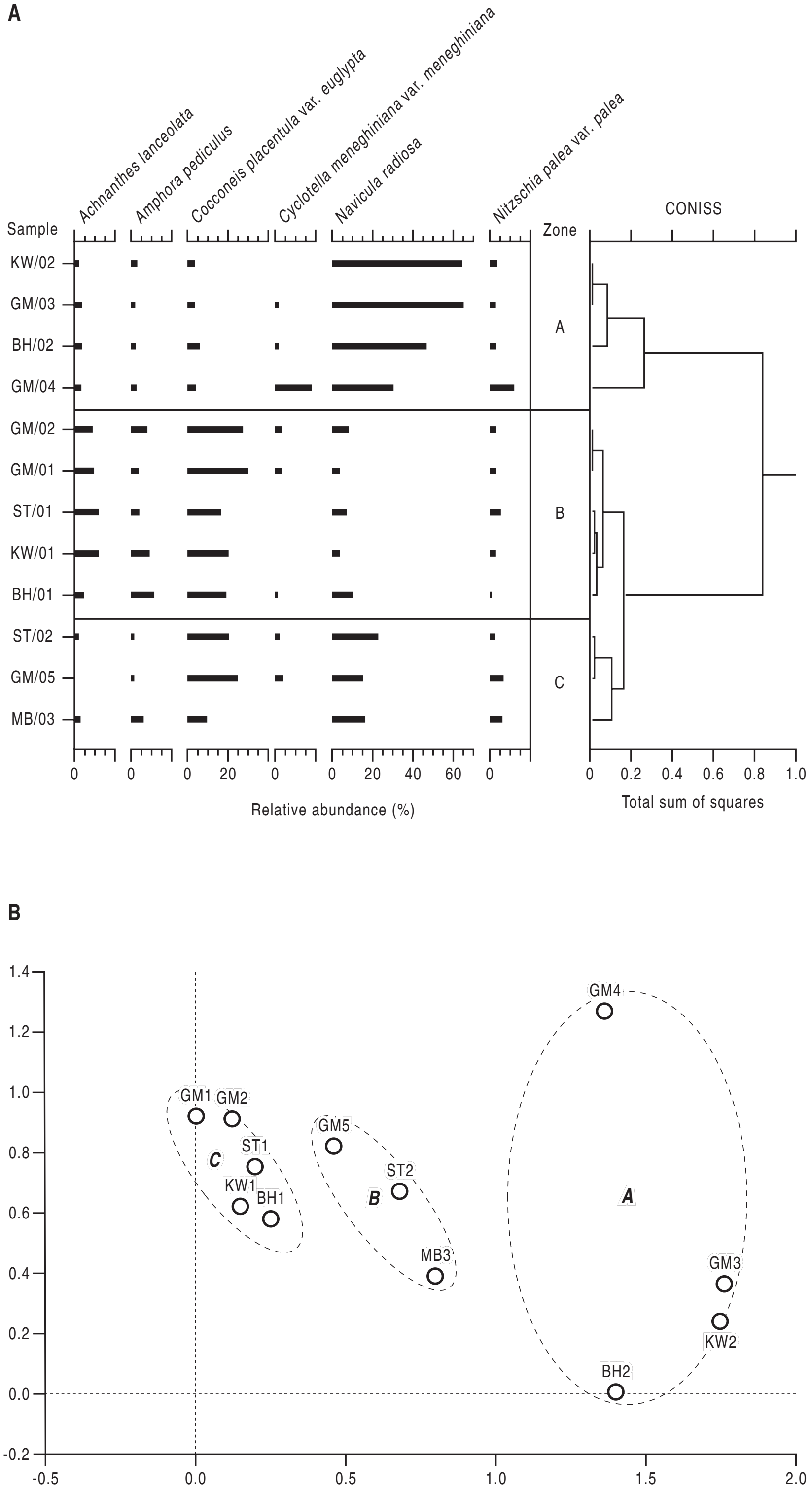

Figure 3—(a) Unconstrained cluster analysis based on unweighted Euclidean distances showing the diatom assemblages versus order of samples on dendrogram and (b) detrended correspondence analysis for Case Study 1. Only species with relative abundances greater than 10% in one sample are shown.

analogue" because 35 of the 37 diatom species from the lung and clothing samples are found in the control samples. The assemblages show similarities to all control samples from shallow water habitats with slow water flow (GM/05, ST/02 and MB/03). The MAT reconstructions suggest that the closest control analogy for the lung fluid, training shoe, and T-shirt is sample GM05 (Table 1). This sample is from the body recovery site, 100 m upstream of a weir, from the edge of an embayment, near the river bank with a water depth of 0.5 m. The sample from the sock is characterized by a relatively low percentage of *N. radiosa* and the higher percentages of *C. placentula* var. *euglypta*. The assemblage shows a strong similarity to control samples taken from the banks of the river, which all provide "good analogues" (GM/01, GM/02, ST/01, KW/01, BH/01). The MAT suggests the sock has the closest analogy with control sample GM02, which is upstream of weir at the bottom of the ramp/slipway adjacent to the body recovery site and 0.1 m above water level.

Case Study 2: The boy floating in a pond

We identified 37 different diatom species from the control samples of the pond. *Achnanthes lanceolata*, *Achnanthes hungarica*, and *Navicula cryptocephala* are the dominant diatom species. The results of the multivariate analyses show that the samples are divided into two reliable zones (Figure 4): Zone A consists of 33 species of diatom and is dominated by *A. hungarica*, *A. lanceolata*, *Cocconeis placentula*, and *N. cryptocephala*. The *Achnanthes* species are epontic diatoms that have an optimum in eutrophic water with pH values of about 7, but with widest distribution at a

Case Study	Sample	Dominant diatom species	Location
1	Lung fluid	*Cocconeis placentula* var. *euglypta* *Melosira varians* *Navicula radiosa*	GM05
	Training shoe	*Cocconeis placentula* var. *euglypta* *Melosira varians* *Navicula radiosa*	GM05
	T-shirt	*Cocconeis placentula* var. *euglypta* *Melosira varians* *Navicula radiosa*	GM05
	Sock	*Cocconeis placentula* var. *euglypta* *Melosira varians*	GM02
2	Lung tissue A	*Hantzschia amphioxys* *Nitzschia palea*	SAM06
	Lung tissue B	Indeterimate pennate species	SAM07
	Lung tissue C	*Navicula accomoda*	SAM06

Table 1—The inferred location of the control sample generated by the modern analogue transfer function as the closest analogue (modified from Horton et al., 2006).

pH >7, and are adapted to periodic/wet subaerial environments. *Cocconeis placentula* is an epiphytic species and its presence is most likely to be associated with the peripheral and emergent vegetation within the pond, whereas *N. cryptocephala* is a benthic species. The samples within Zone A come from the bottom and edge of the pond. Zone B is characterized by low percentages of *A. hungarica* and one sample with a high percentage of *Hantzschia amphioxys* (SAM06). Zone B has a lower proportion of alkalibiontic to alkaliphilous, mesotrophic-eutrophic, periodically wet subaerial and epontic diatoms than other zones. The samples within this zone are from ephemeral habitats at the edge or landward of the pond that are prone to frequent desiccation.

The diatom flora of the control samples from the pond today are much more species diverse than the dia-

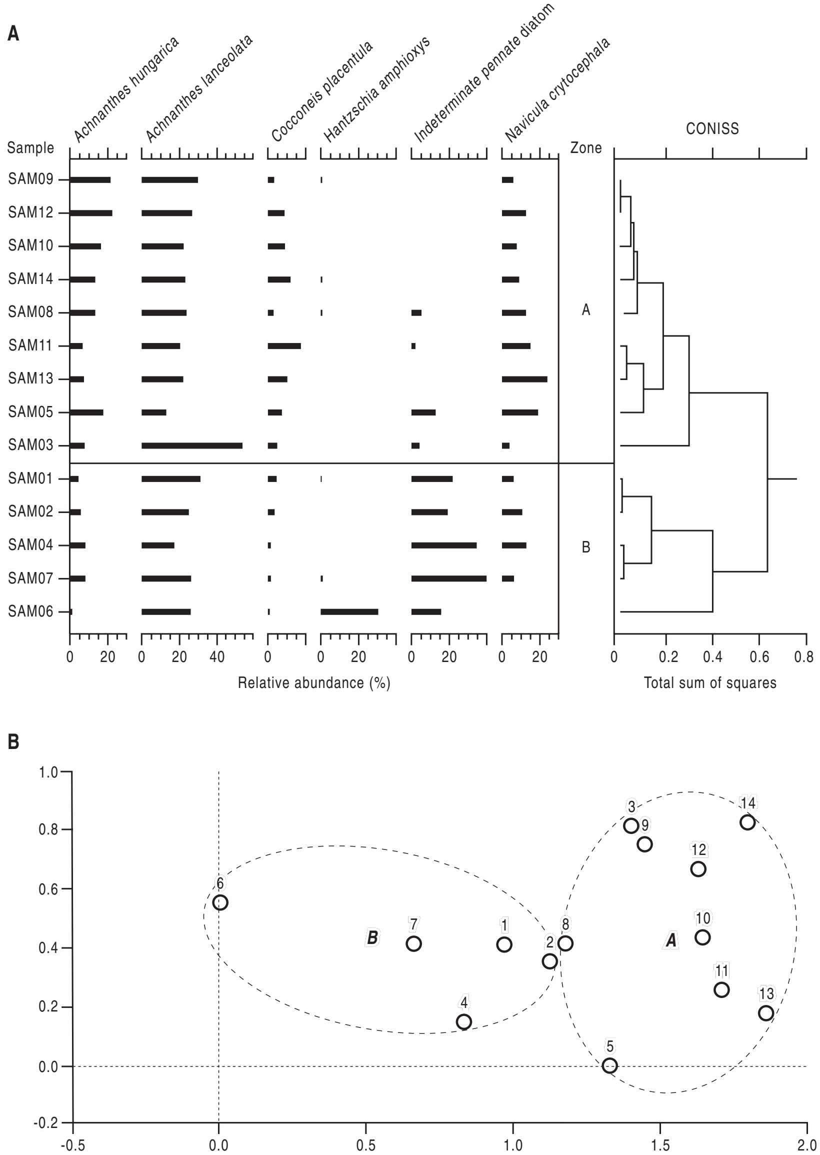

Figure 4— (a) Unconstrained cluster analysis based on unweighted Euclidean distances showing the diatom assemblages versus order of samples on dendrogram and (b) detrended correspondence analysis for Case Study 2. Only species with relative abundances greater than 10% in one sample are shown.

tom flora in the lungs, which were collected more than a decade ago. Nevertheless, the diatom assemblages in the lungs are comprised of many species that are also found in the diatom flora of the pond. The habitat preferences of the diatom species found in the lungs are likely to be a body of water with a pH of around 7 or slightly higher where the water is eutrophic or mesotrophic-eutrophic, that is prone to frequent desiccation around the periphery and/or changes in water depth. The aquatic habitat would also have a variety of substrates providing an array of microhabitats/ecological niches for benthic and epontic species. The MAT supports this inference; two of the three lung tissue samples have a “good analogue” in the control samples. The closest analogues for all three lung samples are SAM06 and SAM07 (Table 1). Samples SAM06 and SAM07 are from the landward edge of the longest transect consisting of five samples (SAM06 - 10). Sample SAM06 is taken from an area of mossy vegetation at the base of the bank and SAM07 is from the sediment overlying the pond liner and gravel (Figure 2). These samples are inundated periodically in response to local climate and water table variations. Lung tissue C has a moderate abundance of *Navicula accomoda*, which does not occur in the control samples and thus a “no close analogue” situation occurs.

DISCUSSION

The value of the diatom test in diagnosing drowning requires that certain conditions are fulfilled: similar diatom assemblages are detected contemporaneously in drowning fluid samples and in the lungs and peripheral organs (liver, kidneys, and/or bone marrow) of drowned subject(s); the number of diatoms found in the lungs and/or other organs are significant (e.g., >20 diatoms for each 100 µl of pellet obtained from 10 g of lung); and any possible contamination is avoided during sampling and preparation (Sidari et al., 1999). Pollanen (1997) supported the validity and utility of the diatom tests for drowning through the analyses of 771 cases of drowning. In 90% of cases in which the sample of drowning medium was available, diatom in bone marrow matched assemblages in the drowning medium. However, there are potential problems associated with the diatoms from tissues and organs. Diatoms have been found in victims who were not drowned (Schneider, 1980) and this raised a whole series of questions relating to how diatoms can come to be present in body tissues. For example, diatoms may be lifted up into the air and transported great distances, therefore, providing a source of diatoms that can be inhaled (Dayan et al., 1978). Furthermore, Schneider (1980) suggested that diatoms frustules in human tissues can be introduced from food and drinking water. Diatoms may also enter into tissues due to their degredation during prolonged submersion of the body in water (Krstic et al., 2002). In contrast, diatoms may be absent or in very low numbers in tissues from victims who did drown. Krstic (2002) suggested that rapid death could prevent the penetration of diatoms into the bloodstream and their subsequent deposition in the organs. Diatom assemblages are also further changed as the result of diagenetic processes in human tissues and lungs. These include selective preservation of diatoms, which depends on frustule composition and structure, and transportation of frustules away from (loss) and into the assemblage (mixing) (Zong and Horton, 1998, 1999; Cameron, 2004; Horton and Edwards, 2006). Problems can further arise when analyzing the diatoms present on clothing. When individuals run or walk through a garden pond or a stream, or indeed any body of water, particulate material in the water (including diatoms) can remain on clothing afterwards (Peabody, 1999). Thus, there is a potential problem of population superposition (i.e. the analytical effects of exposing an item of clothing to several successive environments prior to recovery).

The transfer function approach offers a quantitative method to provide an informal assessment of reliabilityof correlations between control samples and samples from organs and clothing (Horton et al., 2006). However, it is important that the analogue matching reconstructions are not considered in isolation, but are evaluated by comparison with other supporting forensic data. For our two case studies, all organ and clothing samples except one (Case Study 2, Lung tissue C) have matching analogues in the modern training set, reinforcing drowning as the cause of death. The analogue matching provides further information on the site of drowning. Results from Case Study 2 suggested that two of the three available lung samples show a statistically significant relationship to samples from the pond, indicating that the pond was the location of drowning. This was an essential piece of evidence in the acquittal of the accused woman of drowning the boy in a bathtub. In Case Study 1 the diatom-based reconstruction technique suggests strong similarities between lung and clothing samples, and control samples from shallow water habitats. Furthermore, analogue matching implies that the site of drowning was at the body recovery site. These conclusions were pieces of evidence that assisted in the conviction of a man for murder.

CONCLUSION

The "diatom test" for drowning is one of the most often applied and studied application of diatom analysis in forensic investigations and has become an established forensic technique because diatoms have many attributes that are applicable to forensic science. They are found in most aquatic environments and the systematic and taxonomic investigations of modern and fossil diatoms have been supported by numerous studies of distributional ecology. Furthermore, diatoms can provide a record of environmental conditions because their relationship to water quality and aquatic habitat is known and the diatom cell wall, which is silicified to form a frustule, is well-preserved, easily detectable and occurs in high numbers in sediment and water.

The basic principal of the "diatom test" in drowning is based on inference that diatoms are present in the medium where the possible drowning took place and that the inhalation of water causes penetration of diatoms into the alveolar system and blood stream, and thus, their deposition into the brain, kidneys, and other organs. The transfer function approach presented in this paper offers a quantitative method to provide an informal assessment of "reliability" of the "diatom test" through correlations between control samples and samples from organs and clothing. For our case studies, all organ and clothing samples except one (Case Study 2, Lung Exuviate C) had matching analogues in the modern diatom dataset, reinforcing drowning as the cause of death. The analogue matching provides further information on the precise site of drowning. Several circumstances arise that make localization of the precise site of drowning an important medicolegal issue. One situation is the differentiation of drowning in a bathtub versus a naturally occurring body of water when a body is recovered from the latter. In cases such as this, the modern analogue technique can compare the diatoms present in the two sites with those occurring in organs and clothing samples. Results from Case Study 2 suggested that two of three lung samples show a statistically significant relationship to samples from the pond, indicating that the pond was the location of drowning.

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