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THE CRUCIAL ROLE OF FORENSIC BOTANY IN THE SOLUTION OF JUDICIAL CASES

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ABSTRACT. A few years ago, two subjects disappeared and were found lifeless in the surrounding countryside. Understanding the causes of their death was particularly complex for the state of conservation of the human remains. A multi-disciplinary approach allowed experts in botany and geology to identify some algae traces on victims' belongings. Observations of microalgae, in association with other geological and botanical evidence, allowed investigators to geolocate the passage of one of the victims and to exclude that the victims' belongings were submerged under water. The present investigation proved how a traditional microscopic approach, based on the identification of associations of morpho-types of microalgae, could be decisive for linking the presence of one of the victims to specific sites of the territory where the body was found lifeless.

1. Introduction

The principle of Locard states that when two items or bodies interact, an inevitable contact responsible for a transfer of traces occurs. This principle, at the base of all forensic sciences, allows investigators to link a suspect to a victim or to a crime scene, because of the transfer of physical evidence. In outdoor crime scenes, in the countryside, geological and soil evidence may be represented by inorganic and organic traces. These latter may be animal and vegetal. Vegetal traces present in the soil evidence may be studied by forensic botanists in team with geologists, providing very useful information as demonstrated by Lombardi (1999) for the case of the kidnapping and homicide of Aldo Moro. Forensic botanists may investigate forensic traces of vascular and non-vascular plants. Among the non-vascular plants (*i.e.*, they do not have roots, stems or leaves), algae are unicellular or multicellular organisms widely distributed in all aquatic environments or simply in humid places, such as trees, walls, puddles, or moist soils (Sheath and Wehr 2015; Graham et al. 2019). Therefore, it is very likely that objects or people interacting in or near aquatic or wet environments encounter algae, which consequently become organisms potentially useful for linking a subject or an object to a site (D. W. Hall 2012; Hard and Wallace 2015; Levin et al. 2017; Magni et al. 2020). Aquatic ecologists have long recognized the critical importance of algae to the functioning of these ecosystems, as well as the role they play as determinants or indicators of water quality within them (Stevenson et al. 1996). It is this strong ecological foundation that led scientists to explore their role in forensic investigations (Keiper and Casamatta 2001; Cameron 2004; Scott et al. 2014, 2017; Thakar, Luthra, and Khattar 2018; Wanner et al. 2018; Spikmans 2019; Scott et al. 2021; M. K. Sharma, A. Sharma, and Bhatt 2021; I. Bogusz, M. Bogusz, and Elazna-Wieczorek 2022). Due to the prominent role and prevalence of algae in aquatic ecosystems, it is very likely that objects or people involved in an accident or crime that occurs in water (even partially as in a puddle) may preserve traces of algae transferred from the surrounding environments. Peculiar species of algae exist in certain terrestrial environments such as shaded tree bark (e.g., the north side in the northern hemisphere), shaded house facades or in the soil (Fitzpatrick and Donnelly 2021). Due to their prevalence and importance in nature, algae have a high probability of being a significative element in a forensic investigation. In cases of deaths in freshwaters, or in their proximity, the similarity of specific algae found as traces on clothing, vehicle or persons with the algal species growing at the scene of investigated events, or where physical evidence (such as a weapon), can provide valuable circumstantial evidence of correlation (Coyle et al. 2005). The comparison is possible between distinctive combinations of one or two species of indicator algae or, alternatively, between algal assemblages (*i.e.*, groups of species) that may not be typical of a defined location, but rather to a particular type of water body (e.g., freshwater rather than marine, streams rather than ponds, or waters near sewers or sources of thermal pollution, etc.) (Britton and Greeson 1989; Siver, Lord, and McCarthy 1994; Hard and Wallace 2015). Furthermore, the presence or lack of algae in body organs (such as lungs) can be used for positive or negative diagnosis of drowning death, and the relative abundances of diatoms (*i.e. microalgae* with a cell wall composed of silica) in such tissues can be used to infer the probable location of the drowning (Pollanen, Cheung, and Chiasson 1997; Pollanen 1998; Zimmerman and Wallace 2008; Diaz-Palma et al. 2009; Saini, Khanagwal, and Singh 2017; Liu et al. 2020; Zhou et al. 2020; Du et al. 2022). Differently for land plants, whose anatomy is rich in providing diagnostic characteristics (Ladd and H. C. Lee 2005; Aquila et al. 2018; Alotaibi et al. 2020), both vegetative and reproductive, algae in general, and especially microalgae, are tricky to identify at the species level (R. E. Lee 2018). Classic taxonomic identifications have focused on morphological characters observed at the light microscopy (Palmer 1959; Bellinger and Sigee 2015). However, modern research showed wide phenotypic plasticity and cryptic diversity and pointed on a rather different phylogenetic clustering (Verbruggen and Theriot 2008). A modern taxonomic approach generally does not fail of including ultrastructural (Scanning Electron Microscope - SEM and Transmission Electron Microscopy - TEM), phylogenetic, and phylogenomics data (Bhattacharya and Price 2020). However, in several professional contexts, such methods are not applicable and morphological identification at the light microscope is the standard for field samples (Cox and Cox 1996; Dillard 1999; Wehr, Sheath, and Kociolek 2015). The aim of the present paper was to verify whether a traditional morphological approach based on taxonomic identification of algae could be useful for exclusionary/comparative forensic purposes, bringing information for the comparison between samples of unknown origin with samples of known origin (Somma 2023c) related to a casework. An atlas of morphotypes (tentative *taxa*) recorded for each sample (both of known and unknown origin) was produced. A comparison was made of morphotypes identified in the samples of unknown origin to those of known origin and the peculiar associations of taxonomic entities in each sample

was used to evaluate similarity among traces found on the victim' shoes and each collection site.

2. Forensic Botany

The methodologies and principles of botany can be applied in the investigation of criminal cases being one of the forensic sciences applied to criminal law (Picozzi and Intini 2009; D. W. Hall 2012; Saferstein 2017). Both objects or persons present in the scene of events can be linked to the scene itself thanks to the contact, and the consequent gained traces, with fragments, individuals or populations of land plants, algae or fungi (Coyle et al. 2005; Hard and Wallace 2015). Botanical elements useful in the forensic investigations are seeds, fruits, stems, leaves, roots, algal thalli, or fragments of any of them, both macro and microscopic, as well as in the latter dimensional scale, pollens, spores, and microalgae, which are mixed in the soil as its organic component (Ladd and H. C. Lee 2005) in continental environments. To either connect or dissociate victims to suspects or crimes scenes, the transfer of plant elements from the environment surrounding the victim or the suspect, especially from the ground, can be useful on any occasion, but inevitably in events that took place outdoors (Ladd and H. C. Lee 2005). In circumstances where the scene of events is known, it is advisable to produce a reference collection to be used for comparison with samples of unknown origin found on objects or persons (Ladd and H. C. Lee 2005). The questioned samples (unknown samples), when it is possible, are the first specimens to be examined for the taxonomic identification of the plant species present. After this phase, the same examination is usually carried out on the samples collected from the sites of the events of investigative interest (known samples). In addition, forensic botany also relies on anatomical, morphological, palynological, ecological, and vegetational observations (e.g., Horswell et al. 2002; Coyle et al. 2005; D. W. Hall 2012). More commonly, botanical traces provide potential class evidence, but occasionally, for example thanks to molecular analyses (DNA analyses), individual evidence can be obtained (sensu Saferstein 2017).

3. Case Study

A few years ago, two subjects (victims 1 and 2) disappeared and were found lifeless in the countryside. Understanding the causes of their death was particularly complex for the state of conservation of the human remains. The investigating authority tried not to neglect any element useful for the reconstruction of the events and consequently technical (Somma 2023b) investigations on the inorganic and organic traces found on the victims were also ordered (Somma *et al.* 2023c). Because events occurred outdoor, many vegetal traces were present on the victims' bodies and their belongings. These traces, showing potentially useful organisms for forensic investigation, were carefully analysed.

3.1. Materials and Methods. Geological and botanical traces collected from the clothing and footwear finds of the victims (samples of unknown origin) were scanned both macroscopically and at the stereomicroscope to check the presence of possible algae. Aggregates of microalgae were also sampled from moist soil and fresh water from ponds collected on the sites under investigation (samples of known origin) (Figure 1) and fixed in formalin at the sampling site (final concentration at 4%). All samples were observed for comparative

purposes at the light microscopy (Laborlux 12, 40-1000 [o.i.] magnifications, LEITZ, Germany, equipped with a digital camera 12 MP, Apple Inc., USA). Microalgae were separated from the soil by dispersing them in sterile Petri dishes with distilled water and then small aliquots were mounted on microscope slides. Taxonomic identification of the microalgae was performed on a morphological basis¹ (Prescott, Croasdale, and Vinyard 1964; John *et al.* 2002; Bellinger and Sigee 2015; Wehr, Sheath, and Kociolek 2015). Where identification was not achievable at the species level, the smallest identifiable taxonomic level was recorded. Morphological characteristics (shape, size, color, taking into consideration the different state of conservation of the finds) and cellular characteristics (wall, unicellular, colonial, multicellular organization) were carefully observed. SEM images (microscope SEM QUANTA FEG 450, FEI Europe, Netherlands) were also taken and observed to scan for the presence of microalgae in the clothing of the victims.

4. Results

An aggregate mass mixed to geological evidence traces, a few millimeters in size, was found by the forensic geologist (R.S.) stuck in the sole of the shoe of victim 1 (unknown sample) (Figure 1A). It was characterized by a greenish color, but its plastic behaviour suggested that the material was not inorganic. This aggregate, observed by the botanist (M.M.), revealed to be made of a microalgal assemblage (Figure 1B). No algae were recorded on other part of the shoes or on victim 1's clothes.

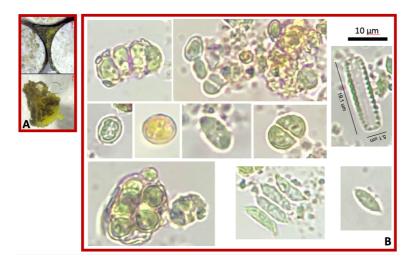


FIGURE 1. A) Close up of the sole of the shoe of victim 1 with the microalgal aggregate before sampling (upper) and after (lower). B) Algae isolated from the aggregate (unknown sample) at the light microscope.

¹M. D. Guiry and G. M. Guiry, "AlgaeBase. World-Wide Electronic Publication" National University of Ireland, Galway, 2023. URL: http://www.algaebase.org

Five distinct species of green algae (*Chlorophyta*), one morphotype of blue algae (*Cyanophyta*), determined at the level of order, and two diatoms (*Ochrophyta*), determined at the level of order, for a total of seven different taxonomic entities were identified on the unknown sample collected on the shoe of victim 1 (Table 1).

TABLE 1. Algae identified in the unknown sample of soil isolated from the sole of the shoe of victim 1.

Taxonomy

Deasonnia sp. (Chlamydomonadales, Chlorophyta) Deasonnia sp. young cells, or other (Chlamydomonadales, or Chlorococcales (Chlorophyta) Apatococcus sp. (Chlorellales, Chlorophyta) Apatococcus sp. (Chlorellales, Chlorophyta) Species of Chlorellales, or Chlamydomonadales (Chlorophyta) Scenedesmus sp. (Sphaeropleales, Chlorophyta) Desmodesmus sp. (Sphaeropleales, Chlorophyta) Chroococcales (Cyanophyta) in complex with Chlorophyta Diatoms (Pennales, Ochrophyta) (a more detailed identification is not possible as cells were in girdle view)

A single diatom (*Pennales, Ochrophyta*), determined at the level of order, was found in a SEM graph of the sole of a shoe of victim 2 (unknown sample) (Figure 2). No other types of algae were recorded on other part of the shoe or on victim 2's clothes.

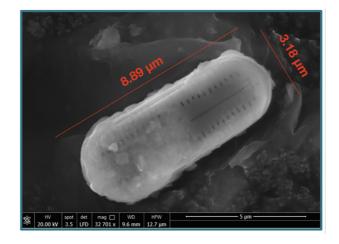


FIGURE 2. Diatom isolated from the sole of the shoe of victim 2 at the SEM (unknown sample).

Eight distinct species of green algae (five *Chlorophyta* and three *Streptophyta*), two morphotypes of blue algae (*Cyanophyta*), determined at the level of order, and several

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morphotypes of diatoms (*Ochrophyta*), determined at the level of order, for a total of eleven different taxonomic entities were identified in distinct associations in samples of known origin collected on the scene of events in freshwaters and ponds (Table 22; Figure **??**3).

TABLE 2. Algae identified in samples of moist soil and water from ponds collected on the investigated area (samples of known origin

Taxonomy

Deasonnia sp. (Chlamydomonadales, Chlorophyta) Deasonnia sp. young cells, or other (Chlamydomonadales, or Chlorococcales (Chlorophyta) Apatococcus sp. (Chlorellales, Chlorophyta) Chroococcales (Cyanophyta) in complex with (Chlorophyta) Clasterium sp. 1 (Desmidiales, Streptophyta) Clasterium sp. 2 (Sphaeropleales, Chlorophyta) Diatoms (Pennales, Ochrophyta) Filamentous Cyanophyta Scenedesmus sp. (Sphaeropleales, Chlorophyta) Species of Chlorellales o Chlamydomonadales (Chlorophyta) Spirogyra sp. (Zygnematales, Streptophyta)



FIGURE 3. A) The countryside surrounding the site of the discovery of the victims' bodies with the indication of the puddle of Figure 1 B (star); B) the mud of the puddle (known sample) where algae thrive, similar to those found in the shoes of victim 1 (unknown sample).

4.1. Description of *taxa* **identified in the unknown and known samples.** *Deasonia* sp. (*Chlamydomonadales, Chlorophyta*), *Deasonia* sp. young cells, or other *Chlamydomonadales* or *Chlorococcales* (*Chlorophyta*) (Figure 4 A-B).

Species of the genus *Deasonia* Ettl et Komárek have single cells (plurinucleate); young cells are elliptical, ovoid, while mature cells are always spherical with a thickened cell wall. They have parietal chloroplasts with bifurcations and indentations in mature cells, finally

reticulated, while cup-shaped perforated in young cells; in young cells pyrenoid is lateral in compact chloroplasts and moves to the center of the chloroplast in mature cells; vegetative stages are multinucleated, they can form biflagellate zoospores; the species are present in moist soil (Shubert and Gärtner 2015). The young cells have a more generic morphology which can be confused with other species of the same order (*Chlamydomonadales*) or of the order *Chlorococcales* (*Chlorophyta*).

Apatococcus sp. (Chlorellales, Chlorophyta) (Figure 4 C)

Species of the genus *Apatococcus* Brand *em.* Geitler have cells rounded to spherical or slightly flattened at the corners, more or less irregular cell packets formed by divisions in two or three consecutive perpendicular directions or multilayered pseudoparenchymatous cell aggregates, occasionally the beginning of the formation of short filaments can be observed; cell wall with smooth to rough surface, somewhat thickened in older cells; chloroplast in young parietal cells, in older cells partially constricted in the center, lobed, lacking pyrenoid (Shubert and Gärtner 2015). Rarely reported, but probably very common or cosmopolitan, the actual distribution is poorly known (Shubert and Gärtner 2015).

Species of Chlorellales or Chlamydomonadales (Chlorophyta) (Figure 4 D)

There are over 200 recognized genera among the non-motile and colonial coccoid green algae, with a range of morphologies and ecological habitats (Shubert and Gärtner 2015). *Scenedesmus sp. (Sphaeropleales, Chlorophyta)* (Figure 4 E)

Species of the genus *Scenedesmus* Meyen form colonies of 2, 4, 8 or 16 (rarely 32) cells, flattened, with long axes of parallel cells, laterally adjacent, arranged in a single linear or alternating series, ellipsoidal, ovoid or crescent or tapering towards each end, cell wall spineless, parietal chloroplast, usually with a pyrenoid (Shubert and Gärtner 2015). Reported worldwide and frequently abundant in nutrient-rich (especially high inorganic N) waters; it is commonly found in association with other coccoid algae genera (spiny-producing species, such as those of the genus *Desmodesmus*) (Shubert and Gärtner 2015).

Desmodesmus sp. (Sphaeropleales, Chlorophyta) (Figure 4 F)

Species of the genus *Desmodesmus* An, Friedl et Hegewald form colonies of 2, 4, 8 or 16 cells or unicellular forms, with long axes of parallel cells, laterally flanked, arranged in a single linear or alternating series; cells ellipsoidal, to ovoid, spines usually present on terminal cells and/or medial cells, occasionally absent, parietal chloroplasts, usually with a pyrenoid (Shubert and Gärtner 2015). An extremely common (and occasionally abundant) genus in continental water bodies (Shubert and Gärtner 2015). *Desmodesmus* species that typically produce colonial forms may occasionally produce single cells depending on environmental conditions, such as phosphorus concentration (Trainor 2014).

Closterium sp. (Desmidiales, Streptophyta) (Figure 4 G-H)

Species of the genus *Closterium* Nitzsch ex Ralfs have solitary cells, usually straight, arcuate, or lunate, many times longer than broad, in most species, tapering toward the apices, which may be pointed, broadly rounded, or truncated (J. D. Hall and McCourt 2015). Cells are unconstricted and may be inflated in the mid region in some species. Size varies by species from tens of micrometers in length to nearly a millimeter. In certain species, two semicells are visible due to intercalating wall material, with a central nucleus and two chloroplasts, axile and lobed with several pyrenoids. Sexual reproduction is known in several species, and zygospores may be irregularly globose or quadrate and are generally unornamented. Most species are benthic, loosely attached to sediments or on aquatic plants, but some are

planktonic. Some species of *Closterium* can be found in abundance on damp soils (Prescott *et al.* 1975).

Spirogyra sp. (Zygnematales, Streptophyta) (Figure 4 I)

Species of *Spirogyra* Link form long uniseriate filaments with cylindrical, unornamented, and unconstricted cells, usually attached to substrata by rhizoids, resting on sediments or floating on the surface (J. D. Hall and McCourt 2015). Cells have one to several parietal, spiraling chloroplasts per cell, a central nucleus and a large central vacuole. Species reproduce both sexually via zygospores and asexually by vegetative reproduction and aplanospores. All spores have multilayered walls that may be variously colored and ornamented. Zygospores are useful for species-level identification but are rarely encountered in the field. The genus *Spirogyra* is one of the most widespread and common of all freshwater algae. Species are tolerant of a wide variety of environmental and chemical conditions, including some that are remarkably tolerant of pollutants and a few that tolerate brackish conditions.

Filamentous Cyanophyta (Figure 4 K-M)

Cyanophyta, or Cyanobacteria, occur in a wide range of morphologies and ecological forms, which colonize almost all habitats and are ubiquitous in aquatic and terrestrial communities, including almost all types of moist soils (Komárek and Johansen 2015a,b). In recent decades, ecological features, ultrastructural features, and molecular evidence have substantially influenced the knowledge and understanding of this group. As a result, the classification of species is undergoing radical changes. The cellular morphological approach to classification, regardless its considerable limits, is still used mainly for the identification of natural material (Komárek and Johansen 2015a). Most soil cyanobacteria are filamentous species, but several coccoid forms are also common. Filamentous cyanobacteria comprise some of the most widely recognized and important freshwater algae in the world, and their classification is equally complex and intersecting with that of the orders of coccoid representatives (Komárek and Johansen 2015b). Many of the filamentous taxa are important members of lake and riverine phytoplankton, while others form thick and dense beds in a wide variety of benthic habitats (Komárek and Johansen 2015b). Others form conspicuous masses floating on the water surface of various water bodies, or grow attached to stones, or epiphytes on aquatic angiosperms and other algae, epipellic (*i.e.*, on muddy sediments) and epipsammic (i.e., on sand), and frequently colonize wet or damp lands (Komárek and Johansen 2015b).

Chroococcales (Cyanophyta) in complex aggregates with Chlorophyta (Figure 4 L)

Unicellular and colonial coccoid cyanobacteria were classified according to the traditional morphological system in the order *Chroococcales* (Komárek and Johansen 2015a). From modern investigations, mainly molecular and ultrastructural, it follows that coccoid cyanobacteria are a very heterogeneous group, developed in several distinct evolutionary lineages that led to a recent reclassification into phylogenetically distinct orders. Mucilaginous colonies of cyanobacteria can be spherical, oval, planar, or irregular (Komárek and Johansen 2015b). The structure of the sheaths and envelopes around the cells is different: mucilage can form a hyaline, amorphous mass, diffuse and marginal envelopes or structured and stratified sheaths (Komárek and Johansen 2015b). The gelatinous shells and sheaths (solid or structured outer layers) appear to perform a protective function in environments exposed to severe desiccation or solar radiation.

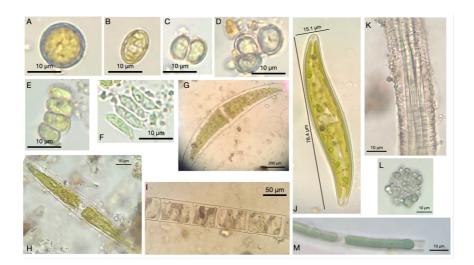


FIGURE 4. A) Deasonia sp. (Chlamydomonadales, Chlorophyta); B) Deasonia sp. young cells, or other Chlamydomonadales or Chlorococcales (Chlorophyta); C) Apatococcus sp. (Chlorellales, Chlorophyta); D) Chlorellales o Chlamydomonadales (Chlorophyta); E) Scenedesmus sp. (Sphaeropleales, Chlorophyta); F) Desmodesmus sp. (Sphaeropleales, Chlorophyta); G) Closterium sp. 1 (Desmidiales, Streptophyta); H) Closterium sp. 2 (Desmidiales, Streptophyta); I) Spirogyra sp. (Zygnematales, Streptophyta); J) diatoms (Pennales, Ochrophyta) in valve view; K, M) filamentous Cyanophyta; L) Chroococcales (Cyanophyta) in complex with Chlorophyta.

Diatoms (Pennales, Ochrophyta) (Figure 4 J)

Diatoms are unicellular algae present in almost all bodies of water, with a rigid cell wall composed of silica (SiO₂), known as a frustule, composed of two valves, a smaller hypovalve, which fits inside a larger epivalve, like two halves of a "box" (Kociolek, Spaulding, and Lowe 2015; Kociolek et al. 2015). Average cell size decreases in a population, because each valve produces a smaller complementary valve during cell division until it reaches a minimum size (usually one-third of maximum size) that induces sexual reproduction and restoration of maximum size (Edlund and Stoermer 1997). The siliceous walls of diatoms are often highly ornamented, and a large part of the taxonomy has always been based on their morphological characteristics, traditionally observed at the optical microscopy; currently, ultrastructural observations (SEM) of the frustule are considered essential for species identification, due to a strong morphological convergence between taxonomically distinct groups (Kociolek, Spaulding, and Lowe 2015). Among the diatoms, species with radial symmetry (Centrales) and others with bilateral symmetry (Pennales) are traditionally distinguished; among the latter, a subgroup has evolved a special structure involved in the movement of cells on surfaces, called raphe (Kociolek, Spaulding, and Lowe 2015). Modern phylogenetic systematics reorganizes these groups into several distinct lines. For simplicity, the Centrales and Pennales groupings had been used in this work, although we are aware that they are no longer taxonomically rigorous.

4.2. Comparative analyses. From the comparative analyses, a technical opinion of generic compatibility of the trace from the sole of the shoe of victim 1 (Table 1) with macroenvironments similar to the different freshwater points found in the area under investigation (Table 2) was expressed; in some site the peculiar association of microalgae were all present, in other ones only partially. Furthermore, a technical opinion of strong compatibility of the same sample of unknown origin with macro-environments similar to the bottom of a small artificial basin (known as "gebbia") and to an ephemeral pool in the ground, respectively fed by a pipeline drawing spring water and its loss, was expressed. It was not possible to express a technical opinion of compatibility of the diatom observed in the micrograph of the sole of the shoe of victim 2 with any diatom observed diatom, it was not possible to exclude that the failure to find it might be due to difficulties to identify minute organisms in complex matrices, but also might depend on the insufficient observation time enforced by the investigations.

5. Discussion and Conclusions

Forensic sciences imply the use of expertise, principles, scientific methods, and modern technologies related to a wide range of disciplines, in order to fully investigate crimes and physical evidence that might be presented in a court of law (Picozzi and Intini 2009; Saferstein 2017). By definition, a "holistic" multidisciplinary approach is crucial for not undervaluing data and information from all traces both from the scenes of events and the persons involved (Somma 2022, 2023a). Especially in crimes occurring outdoor, collected evidence and analyses involving an array of disciplines, such as botany (Caccianiga et al. 2021; Morabito, Mondello, and Somma 2023; Somma et al. 2023c), geology (Ruffell and McKinley 2009; Somma et al. 2023c,d; Spoto, Barone, and Somma 2023), or paleontology (Di Maggio et al. 2014; Somma and Maniscalco 2023), are crucial in investigation where soils and plants are widespread. In addition, forensic botanists with geologists, geographers, coroners, and environmental psychologists may also collaborate in multidisciplinary teams in cases regarding the geographic profiling (Canter and Youngs 2003; Canter 2005; Somma 2023b) or searches for clandestine graves (Somma and Costa 2022; Byrd and Sutton 2023; Marra 2023; Somma and Costa 2023; Somma, Sutton, and Byrd 2023; Tagliabue et al. 2023), where also the plants may play a significant rule in dating the age of the burial. Other illicit activities involving plants may concern fake fossils of plants, frauds, and detection of counterfeit goods (Marra, Di Silvestro, and Somma 2023; Spoto 2023). In this paper, such multidisciplinary approach allowed the botany and geology experts to ascertain the presence of the two subjects in the scene of event and to reconstruct the path followed by them in their last hours of their lives. In particular, observations of microalgae, in association with all other geological and botanical evidence (Morabito, Mondello, and Somma 2023; Somma et al. 2023c), allowed investigators to geolocate the passage of victim 1 in a specific puddle located close to the disappearance site. Nowadays, taxonomic identification of microalgae often uses phylogenetic and ultrastructural approaches, which needs the involvement of skilled experts and complex techniques. Consequently, the before mentioned approach in forensic investigations is neglected, with few notable exceptions (*i.e.*, diatoms in drowning deaths, Magrey and Raj 2014; Z. et al. 2016; Shen et al. 2019;

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Zhang *et al.* 2020; Khurshid *et al.* 2021). Conversely, the present investigation proved how a traditional light microscopic approach could be decisive to associate known samples with unknown traces of soil, basing on the identification of associations of morpho-types of microalgae. Furthermore, a multidisciplinary approach, as it can be accomplished by a diversified working team, should be desirable in forensic investigations and achievable only when the education system includes forensic competencies in the *curricula* of studies (Spoto, Somma, and Crea 2021; Somma 2022; Baldino *et al.* 2023; Somma *et al.* 2023a,b). The large amount of data that can be collected by means of a holistic approach may allow to test carefully alternative hypotheses and, when concordant, contributes to reconstruct past events and dynamics. In complex cases, such as the casework here presented, a such approach may assist judicial authority also in the understanding of the reasons of certain criminal behaviours.

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