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THE IMPORTANCE OF BIOAEROSOL IN FORENSIC INVESTIGATIONS

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Abstract: The article discusses the importance of bioaerosol in terms of forensic microbiology and explains its usefulness in forensic investigations. Examples of investigative practice and crime investigations available in the literature are also presented, with special focus on the importance of biological aerosol analyses during evidence proceedings.

1. Introduction. 2. Biological aerosols. 3. Bioaerosols in forensic investigations. 4. Summary

ZNACZENIE BIOAEROZOLU W BADANIACH KRYMINALISTYCZNYCH

Streszczenie: W artykule omówiono znaczenie bioaerozolu pod kątem mikrobiologii sądowej oraz wyjaśniono jego przydatność w badaniach kryminalistycznych. Przedstawiono również dostępne w literaturze przykłady z praktyki śledczej i przeprowadzonych dochodzeń, ze szczególnym zwróceniem uwagi na przydatność analiz aerozoli biologicznych w postępowaniu dowodowym.

1. Wstęp. 2. Aerozole biologiczne. 3. Bioaerozole w badaniach kryminalistycznych. 4. Podsumowanie

Key words: air, bioaerosol, forensic science, fungi, pollen
Słowa kluczowe: powietrze, bioaerozol, nauki sądowe, grzyby, pyłki

1. Introduction

As defined, forensic science, or forensics, is the application of science to establish how historical events occurred and thereby provide impartial evidence that can be used in a court of law. This may be observed as the use of the scientific methods and processes in crime-solving, within the criminal justice system. Forensic sciences include medicine, biology, dermatoscopy, chemistry, traseology, and computer science. Forensic science can be used to identify decedents or suspects, establish a connection to a crime or crime scene, or prove elements of a crime [7, 22, 50]. The first mention of the use of medicine and biology to solve the crime appeared in the 13th century [46]. Using science to investigate crimes and identify criminals began in the mid to late 1800s. People who had a significant impact on the development of forensic sciences include Ambroise Paré, Fortunato Fidelis, Edmond Locard, Francis Galton, Carl Wilhelm Scheele, Henry Goddard, Hans Gross, Francis Galton, James Watson, Alphonse Bertillon, Sir William Herschel, Sir Alec Jeffreys, Frances Glessner Lee, and many others. They had an impact on the development of forensic medicine, toxicology, chemistry, ballistics, anthropometry, crime scene photography, biology, fingerprinting as well as DNA analyses Now, in the 21st century, forensic scientists have started using modern technologies in crime-solving (e.g. laser scanners, drones, photogrammetry). Thanks to the usage of new testing methods and modern techniques, many cases from many years ago have been solved [10, 19, 36, 41, 49, 50].

We still have to remember that forensic science is derived from biological, engineering, and social sciences, which often deal with issues distant from the world of crime but have been adapted for criminal investigations. There are many forensic sciences and each of them deals with researching a different segment of reality for the criminal trial.

Forensic science connects many sciences from different areas [18, 50]. One of the newer areas of forensic science is microbiology [9, 13, 30, 33, 48]. During an investigation, the main goal for scientists and investigators is to determine who committed the crime, so they have to focus on looking for evidence at the crime scene. Specialists of forensic microbiology may use biological proof to place people at crime scenes, investigate bioterrorism events, or determine the cause of and time of death [8, 11, 33]. Microorganisms isolated from the human body after death could be considered as etiological or a contributing factor to death. Also, the presence of isolated microbes at the crime scene may make it easier to determine the involvement of other people in committing a crime or transporting the

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corpses to another location after committing homicide [8, 41]. Microorganisms that are present in soil, water, or plants can be released into the air as bioaerosols and can be transported over long distances [37]. Their presence at a crime scene may be evidence or a source of important crime information.

In this article, the author describes the importance and role of bioaerosol in forensic investigations, based on the available information from the literature (including examples from case studies).

2. Biological aerosols

Nowadays, one of the fast-evolving multidisciplinary science is aerobiology, related to the study of biological molecules in the air. It is an interdisciplinary science related to various fields of knowledge, including botany, palynology, mycology, phenology, meteorology, and allergology [1]. The air that we breathe consists not only of gases but also contains organic and inorganic particles. Particles of biological origin are present in the air in the form of bioaerosols. Their diameters range from 0.01 μm to 100 μm. The bioaerosol consists mainly of bacteria, fungal spores, viruses, pollen, products or fragments of fungi and bacterial cells (toxins), fragments of plants or insects (and other organic substances or biota) [15, 17, 28, 31]. Bioaerosols are present in enclosed spaces (e.g. inside buildings) and the external environment. Indoors, the presence of bioaerosol may be associated with the presence of humans, construction materials or room furnishings [4, 16]. The main sources of bioaerosol in the external environment are soil, natural and artificial water reservoirs, and living or dead organisms [17, 27, 40]. Also, the municipal waste facilities have a huge impact on the composition of the microbiome of the air (by emitting a specific biological aerosol which is typical for municipal landfills and sewage treatment plants) [5]. Bacteria or fungal spores are released into the atmosphere as a result of their removal from plant or soil surfaces, possibly through wind or thermal convection processes, and after spontaneous or rain forced emissions from natural water reservoirs [3, 37]. Microbes cannot grow during airborne transport but can survive in the air for some time. The survival time depends on the properties of the microorganisms and environmental conditions: access to nutrients and physical or chemical factors of environmental stress. The small components of biological aerosol retain their viability in the environment longer than larger microorganisms. Also, bacterial endospores can survive in the air for a long time. The most sensitive, vegetative forms die quickly. Due to the relatively small size of the cell, the most adapted forms of microorganisms are cocci and bacteria whose cells are covered with a layer of mucus or produce yellow and red carotenoid dyes that protect them from harmful UV radiation [25, 32, 39]. For example, in the air the survival time of *Aspergillus* and *Penicillium* is over 12 years; *Escherichia coli* and *Streptococcus faecalis* die within 30–60 minutes [16].

Sampling bioaerosols aims to efficiently capture (as much as possible) all biological particles from the air and then gather them to enable their subsequent detection, i.e. without changing and/or damaging their structure and maintaining their ability to grow on an appropriate microbiological medium. The most commonly used bioaerosol sampling techniques are impaction, filtration, and electrostatic precipitation [28]. The central topics of bioaerosols studies focus on health hazards (especially worker's health), effects on the atmosphere and climate, or bioterrorism [8, 24–26, 35, 38, 42, 44, 45, 47]. In addition to the fact that bioaerosols can be harmful to humans, they are also significant in other ways.

3. Bioaerosols in forensic investigations

The fungal spores can be isolated from soils, sediments, vegetation, and plant litter. Fungi can also grow on stones, bricks, wooden objects, leather, plastics, rubber, or textiles. Some of the fungi species that produce dry spores and grow on the aerial parts of living plants will be easier caught up in wind currents and can be transmitted more widely through the air (in low concentrations). The spores of certain species that are present on leaves (e.g. *Alternaria* or *Cladosporium*), can be observed in large concentrations in the air, especially during the autumn season [29]. Fungal spores may thus provide trace evidence. Even fragments of lichens or mold can become detached in items that are involved in criminal investigation [2, 6, 55].

Specialists in biology and forensic science point out the importance of microbiology, palynology, and mycology in investigations of committed crimes [1]. One of the factors that affect the possibility of resolving a crime are traces – amounts of material that might be valuable in a court as evidence. The cases described by Hawksworth and Wiltshire [22], show that microorganisms present in the air may be useful for this purpose. According to the authors, a young woman's body was found dumped in plants called 'stinging nettles' (*Urtica dioica*). Forensic microbiologists found two species of fungi (*Periconia* sp., *Torula herbarium*) in the suspect's car that were also found on the nettles (nettles support the growth of several species of fungi, especially about seventeen species are only found in association with these plants). It turned out that the suspect unconsciously picked up fungal spores on his clothing from the nettles. Some of these spores were transferred directly into the sus-

pect's car after being released into the air from his clothes. Based on this evidence, the police identified a suspect involved in the death of the woman. In the other case, the suspect (waiting in the forest for the victim to arrive) contaminated his clothes with a fungal plant pathogen from cypress trees at the crime scene. The murderer hid in a forested area and shot the victim upon arrival. Once again, the fungal spores were found in the murderer's car, placing him at the crime scene. There are also other cases (including accusations of rape) in which the biological material (in the form of fungal spores of specific species) constituted evidence during the investigation [20–22].

Fungal spores present in the air (in the form of a biological aerosol) can likewise affect the colonization of exposed human bones (under favorable conditions and long-time exposure). Also, the sporing cycle of fungi can help in determining the time of events. In one of the cases conducted in London, in 2004, it was possible to determine when the body of a young woman was placed in a shallow grave. It was possible due to the presence of black pustules on the underside of the leaves (present in the grave), while the leaf's tissue associated with them was red-purple. It was caused by one plant rust pathogen – *Phragmidium violaceum.* The spores of this fungus are produced and released (in conditions of sufficient humidity) during spring and autumn, after which they are transmitted in the air. Taking into account the color of the pustules, investigators made an estimate. The evidence indicated that the body had been deposited in the grave between late September and early November [52]. Many fungi are seasonal, but some of them (e.g. *Sarcoscypha* or *Flammulina*) have more limited occurrence and can provide important trace evidence during investigations [20]. The colonization of human corpses by some fungi commonly present in the atmospheric air (e.g. *Mucor*) may mean that the victim had been killed and stored for some time in another place and had lain at the deposition site for a short time [11, 14, 23, 33, 41, 43, 51].

For forensic purposes, it is more important to determine the degree of similarity of the samples taken than to identify 100% of all the pollen grains or spores revealed in the samples from the crime scene. Plant pollen is carried by vectors, mostly by wind (in the form of bioaerosol). So, pollen and spores are carried up and away from the parent plant. Pollen derived from wind-pollinated plants are common components in the airspora and are often over-represented. Crime scenes are often dominated by self-pollinated plants. In this case, the pollen falls to the ground around the plant and rarely gets into the air but it may be the most abundant on an item of footwear. In this situation, pollen from the air at the crime scene may form only a small proportion of the profile relevant to the criminal investigation. Pollen and spores can be relatively easily determined, collected, protected, and tested. The diversity of pollen and spores means that samples taken at a distance of several meters from each other may have different characteristics, and this can be a very valuable clue for establishing evidence [2, 6, 20, 34, 54, 56].

The importance of pollen in crime investigations can be illustrated by a case from South Wales, where a murder victim was buried on a hillside dominated by *Picea sitchensis.* Analysis of the surface soil samples around the grave showed that spruce pollen hardly registered in the samples because only a few trees had reached sexual maturity. However, in the soil samples, the presence of pine pollen was noted, and the source of that pollen was a small, but matured pine, growing about 100 meters from the grave. Therefore, pine pollen could have been associated with potential suspects. We have to remember that time is an important factor in forensic sampling, because pollen and spores falling at any one time will be mixed with pollen previously accumulated on the surfaces. Pollen and spore occurring rarely, associated with a specific area and spread over short distances are of major importance. Also, it would be advantageous if a forensic palynologist visited the crime scene [52, 53].

Plant pollen is most often revealed on clothing, footwear (with mud or soil), or other objects (paintings, furniture, firearms, documents). During breathing, sporomorphs (in the form of bioaerosol) are absorbed into the human respiratory tract and then penetrate the nasopharynx and lungs. Pollen was also isolated from the content from the coronary tract, and – in the form of a biological aerosol – can stick on hair (especially on the head) and may stay there longer than on the body surface (it does not wash out and rinse so quickly). It confirms that plant pollen (as bioaerosol component) present in the environment may constitute evidence in criminal casework [55, 56].

Aerosol particles also include cellular material and proteins emitted by humans and can be considered biological information – particles that carry biochemical information specific to living organisms. For example, living organisms, including humans, constantly emit a great deal of dead skin cells into the environment. Nowadays, with the development of analytical techniques, these particles could be isolated and characterized in terms of biochemistry. It may seem that a lot of biological structures and metabolic pathways are common to all people resulting in apparently common biochemical profiles, but these pathways and structures exhibit extensive polymorphisms and divergent post-translational modifications that reflect individual genetics. It can have a big impact and significance for development of biochemical forensics or medical profiling. Also, the concentration, degradation and type of aerosol

may depend on human presence at a specified place and time, and maybe this information can be used during crime investigation and court proceedings [12, 13].

4. Summary

Forensic microbiology is a science that is constantly evolving. Microbiological analysis of the evidence of crimes can influence the determination of potential offenders. Bioaerosols may have a huge importance in tracking down crime suspects. As the cases (described in the literature) show, biological material – in the form of bioaerosol – can be important evidence at crime scenes and bioaerosol analysis would appear to be an important tool that can be used during crime investigations.

References

1. Bednarz A.: The use of bioaerosol in forensic studies and their relationship with other fields of research. *Alergoprofil*, **12**, 135–139 (2016) (in Polish)
2. Boi M.: The Importance of Palynology in Forensic Investigations. *Forensic Sci. Add. Res.* **3**, 1–2 (2018)
3. Bowers R.M., McCubbin I.B., Hallar A.G., Fierer N.: Seasonal variability in airborne bacterial communities at a high-elevation site. *Atmos. Environ.* **50**, 41–49 (2012)
4. Brągoszewska E., Biedroń I., Kozielska B., Pastuszka J.S.: Microbiological indoor air quality in an office building in Gliwice, Poland: analysis of the case study. *Air Qual. Atmos. Health* **11**, 729–740 (2018)
5. Breza-Boruta B.: Bioaerosols of the municipal waste landfill site as a source of microbiological air pollution and health hazard. *Ecol. Chem. Eng. A.* 10.2428/ecea.2012.19(08)083 (2012) (in Polish)
6. Bryant V.M., Jones G.D.: Forensic palynology: Current status of a rarely used technique in the United States of America. *Forensic Sci. Int.* **163**, 183–197 (2006)
7. Budowle B.: Quality assurance guidelines for laboratories performing microbial forensic work. *Science Supporting Online Material*. doi:10.1126/science.1090270. (2003)
8. Budowle B., Schutzer S.E., Einseln A., Kelley L.C., Walsh A.C., Smith J.A.L., Marrone B.L., Robertson J., Campos J.: Building microbial forensics as a response to bioterrorism. *Science*, **301**, 1852–1853 (2003)
9. Budowle B., Schutzer S., Morse S.: Microbial Forensics. Academic Press (2019)
10. Burkhard M.: Handbook of Forensic Medicine. Sussex: Wiley Blackwell (2014)
11. Carter D.O., Yellowlees D., Tibbett M.: Cadaver decomposition in terrestrial ecosystems. *Naturwissenschaften*, **94,** 12–24 (2007)
12. Castillo J.A., Staton S.J.R., Taylor T.J., Herckes P., Hayes M.A.: Exploring the Feasibility of Bioaerosol Analysis as a Novel Fingerprinting Technology. *Anal. Bioanal. Chem.* **403**, 15–26 (2012)
13. Desikan P.: Our microbial signatures. *Indian J. Med. Microbiol.* **35**, 443–444 (2017)
14. Fernández-Rodríguez A., Cohen M.C., Lucena J., Van de Voorde W., Angelini A., Ziyade M., Saegeman V.: How to optimise the yield of forensic and clinical post-mortem microbiology with an adequate sampling: a proposal for standardisation. *Eur. J. Clin. Microbiol. Infect. Dis.* **34**, 1045–1057 (2015)
15. Gołofit-Szymczak M., Górny R.L.: Bioaerosols in office buildings. *Kosmos*, **66**, 491–502 (2017) (in Polish)
16. Górny R.L.: Particles of fungi and bacteria as components of rooms aerosol: properties, emission mechanisms, detection. Institute of Occupational Medicine and Environmental Health, Sosnowiec (2004) (in Polish)
17. Górny R.L. Microbial Aerosols: Sources, Properties, Health Effects, Exposure Assessment – A Review. *KONA Powder and Particle Journal*. doi:10.14356/kona.2020005 (2019)
18. Halicki K.: Polska i zagraniczna literatura oraz czasopisma z kryminalistyki, kryminologii, medycyny sądowej oraz opracowania dotyczące metod walki z przestępczością w okresie dwudziestolecia międzywojennego. *Problemy Kryminalistyki*, **278**, 69–83 (2012)
19. Hawkins S.: Using a Drone and Photogrammetry Software to Create Orthomosaic Images and 3D Models of Aircraft Accident Sites. *UK AAIB* (2016)
20. Hawksworth D.L.: Report on Identification of Fungal Spores in Palynological Preparations from Comparator Samples and Footwear. Report to Lincolnshire Police (2009)
21. Hawksworth D.L.: Report on Identification of Fungal Spores. Report to Wiltshire Constabulary (2009)
22. Hawksworth D.L., Wiltshire P.E.J.: Forensic mycology: the use of fungi in criminal investigations. *Forensic Sci. Int.* **206**, 1–11 (2011).
23. Hitosugi M., Ishii K., Yaguchi T., Chigusa Y., Kurosu A., Kido M., Nahai T., Tokudome S.: Fungi can be a useful forensic tool. *Legal Med.* **8**, 240–242 (2006)
24. Jiayu C., Qiaoqiao R., Feilong C., Chen L., Jiguo W., Zhendong W., Lingyun C., Liu R., Guoxia Z.: Microbiology Community Structure in Bioaerosols and the Respiratory Diseases. *J. Environ. Sci. Publ. Health* **3**, 347–357 (2019)
25. Kaiser K., Wolski A.: Control of microbiological air purity. *Technika chłodnicza i klimatyzacyjna* **4**, 158–162 (2007) (in Polish)
26. Kim K.H., Kabir E., Jahan S.A.: Airborne bioaerosols and their impact on human health. *J. Environ. Sci.* **67**, 23–35 (2018)
27. Kozdrój J., Frączek K., Ropek D.: Assessment of bioaerosols in indoor air of glasshouses located in a botanical garden. *Build. Environ.* **166,** 1–9 (2019)
28. Kulkarni P., Baron P.A., Willeke K.: Aerosol measurement: principles, techniques, and applications. John Wiley and Sons, Inc., New York (2011)
29. Lacey J.: The aerobiology of conidial fungi. In: G.T. Cole, B. Kendrick (Eds.), The Biology of Conidial Fungi, Academic Press, New York, 373–416 (1981)
30. Lehman D.C.: Forensic Microbiology. *Clin. Microbiol. News.* **36**, 49–54 (2014)
31. Ławniczek-Wałczyk A., Górny R.L.: Endotoxins and β-glucans as markers of microbiological contamination – characteristics, detection and environmental exposure. *Ann. Agric. Environ. Med.* **17**, 193–208 (2010)
32. Menetrez M.Y., Foarde K.K., Dean T.R., Betancourt D.A.: The effectiveness of UV irradiation on vegetative bacteria and fungi surface contamination. *Chem. Engin. J.* **157**, 443–450 (2010)
33. Micozzi M.S.: Postmortem Changes in Human and Animal Remains: A Systematic Approach. Springfield (1991)
34. Mildenhall D.C.: Civil and criminal investigations. The use of spores and pollen. SIAK J. 4, 35–52 (2008)
35. Paśmionka I.: Evaluation of microbiological quality of atmospheric air in a selected sewage treatment plant in Lesser Poland. *Aerobiol.* doi:10.1007/s10453-020-09627-x (2020)
36. Parker R.J.: Forensic Analysis and DNA in Criminal Investigations: Including Cold Cases Solved. RJ Parker Publishing (2015)

37. Polymenakou P.N.: Atmosphere: A Source of Pathogenic or Beneficial Microbes? *Atmosphere* **3**, 87–102 (2012)
38. Prażak Z., Kowalska M.: Biological factors in occupational environment of nurses and possibilities of reducing exposure. *Hyg. Publ. Hlth.* **52**, 111–118 (2017) (in Polish)
39. Puspita I.D., Kamagata Y., Tanaka M., Asano K., Nakatsu C.H.: Are uncultivated bacteria really uncultivable? *Microbes Environ*. **27**, 356–366 (2012)
40. Redford A.J., Fierer N.: Bacterial succession on the leaf surface: a novel system for studying successional dynamics. *Microb. Ecol.* **58**, 189–198 (2009)
41. Riedel S.: The value of postmortem microbiology cultures. *J. Clin. Microbiol*. **52**, 1028–1033 (2014)
42. Robertson S., Douglas P., Jarvis D., Marczylo E.: Bioaerosol exposure from composting facilities and health outcomes in workers and in the community: A systematic review update. *Inter. J. Hyg. Environ. Hlth.* **222**, 364–386 (2019)
43. Sidrim J.J.C, Moreira Filho R.E., Cordeiro R.A., Rocha M.F.G., Caetano E.P., Monteiro A.J., Brilhante R.S.N.: Fungal microbiota dynamics as a postmortem investigation tool: focus on *Aspergillus*, *Penicillium* and *Candida* species. *J. Appl. Microbiol.* **108,** 1751–1756 (2010)
44. Simon X., Duquenne P.: Assessment of workers' exposure to bioaerosols in a French cheese factory. *Ann. Occup. Hyg.* **58**, 677–692 (2014)
45. Skórska Cz., Golec M., Mackiewicz B., Góra A., Dutkiewicz J.: Health effects of exposure to herb dust in valerian growing farmers. *Ann. Agric. Environ. Med.* **12**, 247–252 (2005)
46. Song Ci., McKnight BE.: The washing away of wrongs: forensic medicine in thirteenth-century China. University of Michigan (1981)
47. Soroka P.M., Cyprowski M., Szadkowska-Stańczyk I.: Occupational exposure to mycotoxins in various branches of industry. *Med. Pr.* **59**, 333–345 (2008) (in Polish)
48. Spagnolo E.V., Stassi C., Mondello C., Zerbo S., Milone L., Argo A.: Forensic microbiology applications: A systematic review. *Legal Med.* **36**, 73–80 (2019)
49. Thompson K.M.: Crime Films: Investigating the Scene. Wallflower Press, London (2007)
50. Ubelaker D.H.: The Global Practice of Forensic Science. Wiley Blackwell (2014)
51. van de Voorde H., van Dijck P.J.: Determination of the time of death by fungal growth. *Zeitschrift fur Rechtsmedizin*, **89**, 75–80 (1982)
52. Wiltshire P.E.J.: Estimate of Deposition Time for Skeletonised Remains Found in a Shallow Grave. Report to Thames Valley Police (2004)
53. Wiltshire P.E.J.: Report on Palynological Analyses of Exhibits and Control Samples for a Case of Cruelty Against Badgers. Report to the Royal Society for the Protection of Cruelty to Animals, Shrewsbury (2004)
54. Wiltshire P.E.J.: Palynological and Botanical Comparisons of the Crime Scene, Other Pertinent Places, and Exhibits. Report to the Metropolitan Police Service (2008).
55. Wiltshire P.E.J.: Forensic ecology, botany, and palynology: some aspects of their role in criminal investigation, in: K. Ritz, L. Dawson, D. Miller (Eds.), Criminal and Environmental Soil Forensics, Springer Science and Business Media, Dordrecht, 129–149 (2009)
56. Zachuta A.: Wybrane zagadnienia z kryminalistyki: ślady roślinnych pyłków i zarodników. *Kultura Bezpieczeństwa. Nauka-Praktyka-Refleksje*, **12**, 28–50 (2012)