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Karl J. Reinhard

University of Nebraska at Lincoln, kreinhard1@mac.com

A. Barbosa

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Natural environment: protocol to recover pollen grains on human bodies

A.B. Gonçalves ^{a,*}, R.F. Alves ^b, S.A.M. Chaves ^c, A. Pott ^d, K.J. Reinhard ^e,

K.C.B.S Santos ^f, W.R. Oliveira ^g, H. Pistori ^h

^a Universidade Federal de Mato Grosso do Sul, Aquidauana (MS), Brasil

^b Secretaria de Estado para Educação, Esporte e Cultura, Aracaju (SE), Brasil

^c Fundação Oswaldo Cruz, Rio de Janeiro (RJ), Brasil

^d Universidade Federal de Mato Grosso do Sul, Campo Grande (MS), Brasil

^e University of Nebraska, Lincoln (NE), Estados Unidos

^f University of New England, Armidale, Australia

^g Coordenadoria Geral de Perícia do Estado de Mato Grosso do Sul; Universidade Católica Dom Bosco, Campo Grande (MS), Brasil

^h Universidade Católica Dom Bosco, Campo Grande (MS), Brasil

*Endereço de e-mail para correspondência: ariadne.gon@gmail.com. Tel.: +55-67-3027-3661

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Resumo

A perícia brasileira raramente utiliza a botânica devido à falta de especialistas nesta ciência para sua aplicação em casos reais. Apesar das vantagens, a palinologia forense ainda não é utilizada por especialistas brasileiros por não haver um protocolo padrão para ela. Nós relatamos um protocolo eficiente e rápido para amostragem de grãos de pólen coletados na pele de cadáveres. Oito cadáveres foram analisados. Com o uso de algodão e algumas gotas de glicerina, os corpos foram esfregados. Algumas precauções de segurança foram tomadas em todas as etapas para evitar qualquer contaminação da amostra de pólen. Foi possível recuperar um total de 134 grãos de pólen dos cadáveres e identificar 16 famílias de plantas. Além da aplicação dessa técnica em casos forenses, o protocolo pode ser usado em outras aplicações Palinológicas, uma vez que esfregar algodão em uma superfície, objeto ou animal pode ser uma maneira fácil de capturar grãos de pólen.

Palavras-Chave: Botânica Forense; Palinologia Forense; Ciência Forense; Investigação Palinológica; Cena de crime.

Abstract

Brazilian expertise almost never use botany due to the lack of specialists to deal with real cases. Brazilian experts do not apply forensic palynology because there is no standard protocol for application, despite its advantages. An efficient and rapid protocol for accepting pollen grains collected from the bodies skin is reported at this research. Eight bodies were studied, the pollen grain attached in the body was removed using cotton and a few drops of glycerin to rub the skin. To avoid any pollen contamination safety concerns were taken. It was possible to recover a total of 134 pollen grains from the bodies and identify 16 families of plants. In addition, the protocol can be used in other palynological applications, since rubbing cotton on a surface, object or animal can be an easy way to capture pollen grains.

Keywords: Forensic Botany; Forensic Palynology; Forensic science; Palynological investigation; Crime scene.

1. INTRODUCTION

Pollen is a relevant trace in forensic botany, it is very abundant, diversified, though rare in some places, it does not deteriorate easily such as leaves and wood, allowing us to create a unique palynological profile for different

locations [1]. Besides that, it can trap on objects and people's body for a long time, even after multiple wash cycles as reported in a recent experiment [2]. Furthermore, pollen documentation can provide additional information for the pollen identification, tracking

samples' origin and even the crime dynamics both temporally and spatially.

Brazil has six Biomes: Amazonia, Atlantic Forest, Caatinga, Cerrado, Pampa and Pantanal, being the most biodiverse country. The researchers believe that Brazil has one-six of the flora species in the world, besides many endemics [3]. Therefore, the unique microhabitats and many plants with different pollen grains are excellent to apply forensic palynology.

A famous Brazilian trial wherein forensic palynology could have been helpful during the investigation was the Eliza Samúdio case [4]. In 2010, the woman was missing, and the court condemned her ex-boyfriend for murder and body hiding. Nevertheless, the corpse was never found, a witness told it was fed to dogs, others said it was buried in an unknown place. If Brazilian police had collected pollen present on dogs or mud from tires of the vehicle used to carry the corpse, maybe they could detect some critical evidence of unusual pollen that could answer or give some clue on the body whereabouts.

Forensic palynology, since 1998, has been described as a science that few people know about. Regardless of a considerable increase in the number of cases that have been recently resolved through forensic palynology [5-7], not much has changed, and need more research on the area.

In Brazil, it is a new research line, and this paper is the first forensic palynology application in corpses reported. The use of botanical applications in forensic institutes in Brazil is limited [8] despite many specialists and research works developed in many other areas of Botany.

Only in some cases of great repercussion the Botany was used, e.g., algae [9] and diatomaceous [10] analyses were done in forensic samples. The use of pollen grains to aid in the elucidation of crimes is still an incipient science under study by Brazilian research groups.

Criminalists are also known as forensic scientists. These professionals examine important stuff and signals found in a crime scene aiming to recognize and interpret the evidence associated with crimes during the police inquiry. Brazilian police do not use to apply botanical evidence due to the lack of awareness on how important this science is in case resolution. In other countries, such piece of evidence is considered valuable to solve crimes, and courts accept it.

The main areas of forensic expertise used in Brazil are fingerprints, DNA, bloodstain, entomology and shotgun residue. Despite the advantages, Brazilians experts do not yet use forensic palynology because there is not a standard protocol for it. The purpose of this research is to describe a forensic palynology technique to recover pollen grains from dead bodies, which can be useful to Brazilian police.

2. DESCRIPTION OF THE PROTOCOL

Campo Grande is the capital city of Mato Grosso do Sul State in Brazil, the estimated population is 843,120 people [11], and its geographic position is between latitudes S 20° 37' and S 20° 58', and longitudes W 54° 51' and W 54° 71' (Figure 1). We sampled pollen grains from the skin of the whole body in corpses at Campo Grande Morgue (Institute of Legal Medicine and Dentistry - IMOL). This research was previously authorized by the Ethics and Research Committee of Don Bosco Catholic University (UCDB) at CAAE nº 53037716.8.0000.5162 approved by CONEP (Comissão Nacional de Ética em Pesquisa) in October 5th 2016. It had a partnership with the Department of Public Safety of Mato Grosso do Sul State (SEJUSP). Eight violent death cases were chosen randomly to be studied between January 2016 and December 2016 (Figure 1).

Falcon tube (50 mL), tube holder, cotton, glycerin, paper and pencil were the primary materials used for swabbing pollen from eight corpses in the morgue (Figure 2A). We used three units of cotton (size 5 cm x 5 cm) per corpse, 1 mL of glycerin was added to the cotton to aid trapping the pollen grains. Sequential identification and information such as place of death, climatic situation, and cause of death have been recorded for each body. We swabbed the head (hair, eyebrows, face, nostrils, ears), shoulders, arms, hands, legs and feet. Some belongings as string, shoes, clothes, helmet and cap were also swabbed (Figure 2B).

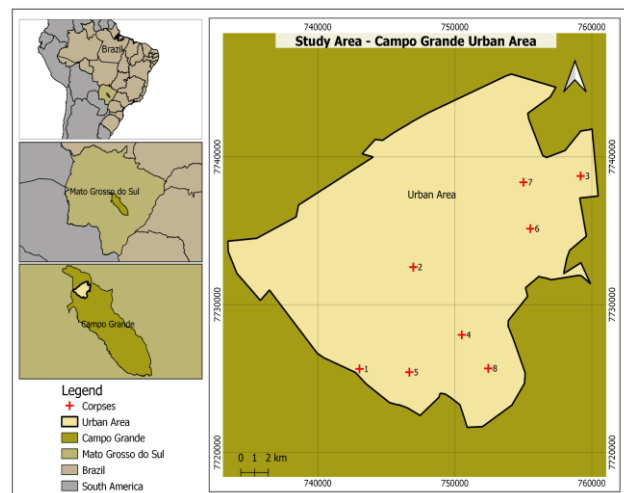


Figure 1: Map of the Campo Grande urban region showing the locations of the eight corpses taken to the morgue and from which we collected pollen samples.

To avoid any pollen sample contamination, some safety precautions were taken at all stages. 1- Clean hands; 2- Use of disposable gloves, spatulas and brushes; 3- New slides and coverslips; 4- Sterile tools; 5- Personal protective equipment (PPE) (Figure 2C); 6- The door and windows were kept closed, and eventual airborne pollen

was collected to detect any possible contaminant [12], also it is not recommending the air conditioner use.

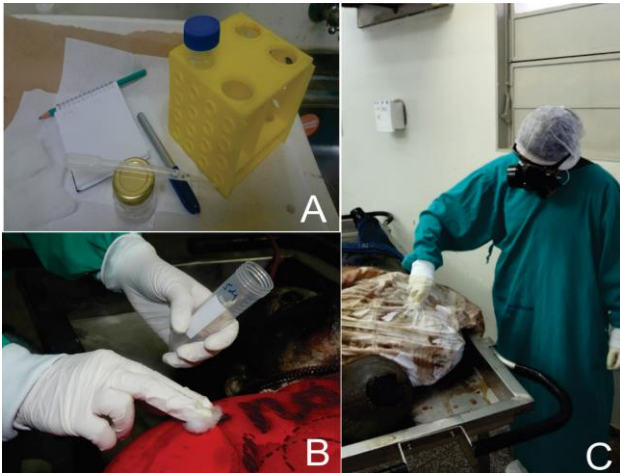


Figure 2: Forensic palynology application; A) Material used for pollen sampling; B) Recovering pollen from corpses in the morgue; C) Swabbing the corpse using the appropriated personal protective equipment.

The cotton balls were placed in falcon tubes (50 mL), identified according to each corpse number and taken to the laboratory for sample processing. In the laboratory, the falcon tubes were filled up with 20 mL of alcohol 95% and 2 mL of safranin 50%. The tubes were centrifuged at $3000 \times g$ during 10 minutes. After centrifugation, the pollen was decanted at the bottom of the tube, and the supernatant was discarded [13]. This process was performed three times, after that the cotton was discarded with the discard of the last supernatant. The sterile glycerin jelly (1 x1 cm) was swabbed in the tube base for pollen grain trapping. For microscope analyses the jelly contain the pollen grains was placed on a slide and covered with a coverslip.

3. RESULTS AND DISCUSSION

Suicide, homicide, and traffic crimes were the death's causes studied. The proposed protocol allowed recovering pollen grains from all eight corpses in the morgue for palynological analysis. The protocol allowed recovering 134 pollen grains classified in 16 families. These were Acanthaceae, Anacardiaceae, Arecaceae, Asteraceae, Cannabaceae, Caryocaraceae, Chenopodiaceae, Fabaceae, Malpighiaceae, Malvaceae, Myrtaceae, Poaceae, Podocarpaceae, Rubiaceae, Sapindaceae and Urticaceae. Myrtaceae had the highest number of pollen grains, found 55% more than other families. Pollen grains that could not be identified were labeled as not determinable (ND) and represented 4% of the total. The pollen grains found in each corpse is reported by Gonçalves and collaborators [14]. Figure 3 shows the percentage of pollen grains classified per family.

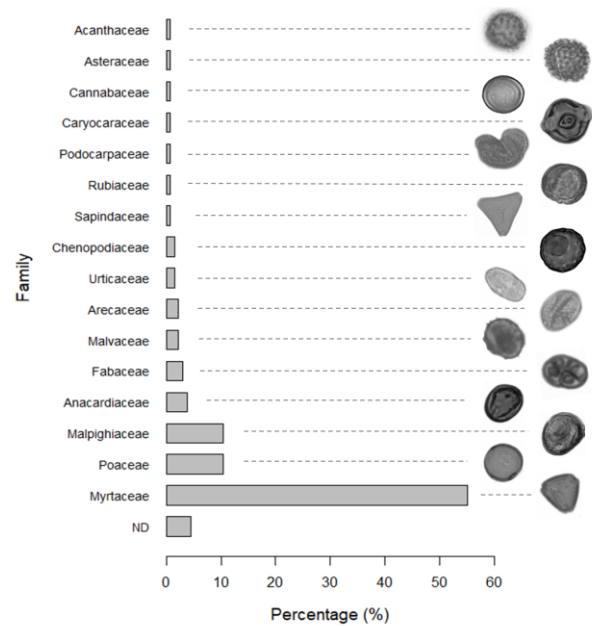


Figure 3: Percentage of pollen grains per family recovered from corpses and some of the pollen grains found respective to each family. ND: Not determinable.

There are some ways to sample pollen grains from forensic objects and corpses [12,15-17]. Vacuum is an excellent way to be used on objects like clothes, laptops, vehicles and a few others [15].

However, it is not an effective technique to use on corpses since the contact with the skin can block the air passage; moreover, blood can be aspirated and infect the equipment tube. The use of the method reported in our research can be used in both corpses and objects to sample pollen grains from the surface. The vacuum can be recommended to obtain samples needing deep suction, as vehicle seats, pillows, mattress and others. In addition to not being efficient in sampling pollen grains from the skin, vacuum equipment is expensive and not readily available in countries where it is not manufactured, e.g., Brazil.

Montali and collaborators [12] sampled pollen grains from corpses in the morgue in Italy. They utilized spatulates to sample nostrils, but they did not say what they used for face, eyebrow and hair sampling. Those authors likewise in our research avoided chemical treatment of the pollen grains, using dye cell as haematoxylin-eosin and fuchsine instead.

Hair washing to sample pollen grains from hair surface is another technique to recover pollen grain from corpses [15]. Using cotton with some drops of glycerin allows trapping pollen grains in the cotton swabbing the whole hair carefully. This simple method could avoid spending much time in the morgue. In addition, this technique is cheaper than washing the hair that needs some water, detergent or shampoo and takes an extended processing time in the laboratory with many centrifugations.

4. CONCLUSION

We report a simple and efficient method for sampling forensic pollen grains from corpses and their accessories. The technique can be used not only for forensic cases, but in other palynology applications, since to swab a cotton ball on a surface, object or animal could be an easy way to trap pollen grains.

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