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THE APPLICATION AND BENEFITS OF FORENSIC PALYNOLOGY IN ROBBERY EVENTS IN TURKEY

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ABSTRACT. Forensic science is required to elucidate crime, and it has been carried out through various induction methods. Forensic palynology, a branch of forensic science, is used to find offenders by investigating the connection between the crime scene, evidence, and offender, solving events, proving the reliability of the victim's narratives, building up the profile of the offender, reducing the number of suspects, assisting security forces in conducting accurate investigations and detecting smuggling of drugs and other materials. Palynological evidence is known to be extremely resistant to extremely high temperatures (over 400°C), strong acids (such as HCl-HF), and fungal and bacterial activity. One of the most important reasons for using palynological evidence as evidence in court is its high resistance. Property crimes account for most judicial cases in Turkey. Burglary accounts for a significant portion of property crime. We aimed to solve various burglary events in Turkey using palynological evidence in this study. We used various methods to analyze palynological particles found in forensic samples sent to our laboratories between March 2016 and May 2017. The findings were distributed to law enforcement officers and legal experts working in courtrooms (lawyers/attorneys/judges).

1. INTRODUCTION

"Forensic Palynology" deals with the spores, pollen, and palynomorphs found within biological evidence collected at the crime scene (such as soil, weapons, clothing, shoes, scarves, socks, etc.). Since the 1950s, palynological evidence has been utilized in numerous forensic cases and continues to be used up to the present day [1]. Studies in the field of palynology began with Grew's discovery of pollen in 1682 when he referred to these structures as "spermatic globules." [2]. Palynomorphs comprise microfossils such as acritarchs, dinoflagellate cysts, colonial algae, and chitinozoans with organic wall structures resistant to acids [3].

Concerning forensic palynology, Klaus Wilhelm conducted palynological examinations on mud samples from boots belonging to a suspect in a murder case in Austria in 1959. As a result of their research, they managed to determine the location where the murder had been committed [4].

Keywords. Forensic science, forensic palynology, robbery, palynomorphs, pollen, spore

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The same year, palynological tests were performed on mud samples taken from the victim's clothing in another murder case that happened in Sweden. As a result of these examinations, the location and time of the murder were determined. These two cases became the first documented examples in official court records [5].

During the 1960s and 1970s, Max Frei [6] utilized palynological data to solve numerous criminal cases in Switzerland, enabling the identification of crime scenes and suspects [1].

Forensic palynological studies in Turkey started in the 2000s. In 2004, the first article was published under the title "Biocriminal Palynology". In the article, a palynological study on sheep wool in Elmadağ district of Ankara province was carried out and the comparison of pollen and their percentages in the wool was given [7].

Dogan at al. (2006) contributed to the resolution of a case by using forensic palynology for the first time in Turkey with a study on a burglary case in Sakarya province [8]

Forensic palynological evidence can be collected from a wide range of sources. Some of these sources include various tools (residues on tools like guns, rifles, forks, knives, shovels, rakes, hoes, etc., suspected to have been used in the crime), pollen traces on packaging materials, mud and soil particles on the soles of shoes, sticky substances like honey, beeswax, pine resin, textiles such as blankets, carpets, rugs, paints, both painted and unpainted wood or similar materials, different parts of a corpse in homicide cases (respiratory passages, intestinal contents, skin, gaps between fingers and toenails), stains on various objects, dust, papers, and more.

In Türkiye, most criminal incidents involve crimes against property. Among these, theft cases constitute a significant proportion. This study aims to solve various theft cases in Türkiye using palynological evidence. Palynological particles discovered in forensic samples between March 2016 and May 2017 that were sent to the laboratory were analyzed using various techniques and the incidents tried to be solved.

2. MATERIALS AND METHODS

Obtaining Samples

Samples for which investigations were requested from various public prosecutors' offices or courts from various judicial events were sent and examined. The origins of the examined requests in this study are shown in Table 1.

TABLE 1. The origins of the examined requests.

Sending Unit	Sending Year	Incident and Date
Turgutlu Chief Public Prosecutor's Office	Manisa	Bovine theft in Manisa province Ahmetli district, Dereköy Neighborhood Matdere Locality (Incident 1)-26.03.2017
Vezirköprü Chief Public Prosecutor's Office	Samsun	Attempt to steal animals from a house in Samsun province Vezirköprü district Aydınlı District (Incident 2)- 09.03.2017
Gordes Chief Public Prosecutor's Office	Manisa	A total of six cattle theft incidents from victim's barn in Salur Mahallesi Yaka Mevkii of Gördes district of Manisa province (Incident 3)- 04.01.2017
Posof Chief Public Prosecutor's Office	Ardahan	Attempted animal theft incident in Savaşır Village of Posof district of Ardahan province (Incident 4)-15.11.2016
Havza Public Prosecutor's Office	Samsun	Theft incident in the Celikhan District of Havza district of Samsun province (Incident 5)-16.04.2016
Çaycuma Chief Public Prosecutor's Office	Zonguldak	Four different cattle theft incidents occurred in Filyos Çaycuma district in Zonguldak province (Incident 6)-09.03.2016

Obtaining Palynomorphs, Spores, and Pollens from Samples

HF Acid Method

This method is used to obtain palynomorphs, spores, and pollen from soil samples. The process begins by weighing the sample to 10 g and initiating the drying step if the quantity is less than 10 g or drying the entire sample. The soil sample is placed in a plastic container and treated with HCl (hydrochloric acid), which is performed under a fume hood to protect from HCl vapor. This step aims to remove calcareous materials. After allowing the sample to settle for 24 hours, HCl is poured slowly onto the settled portion of the sample to continue the HCl reaction. It's added while monitoring the progress of the reaction. Completed samples are centrifuged, and washed, thus contamination is prevented [9].

Samples where the HCl reaction has finished are treated with HF (hydrofluoric acid) to remove mineral materials. Subsequently, the samples are allowed to settle with water, centrifuged, and subjected to acid treatment. Neutralized samples are treated with KOH (potassium hydroxide), washed, and filtered using fine mesh sieves.

After another centrifugation of these samples, distilled water and HNO_3 are added. The samples are mixed with sodium hexametaphosphate solution, and the

tubes are shaken after another centrifugation. The samples are centrifuged for ten minutes following this procedure. After that, KOH is applied to the samples once more. Following another washing of this mixture, the samples are liberated from surplus liquid. Following staining the samples, permanent preparations are made. By using this technique, soil samples are used to extract palynomorphs, spores, and pollen.

Wodehouse Method

This method is also applied in the preparation of pollen and spore slides for the analysis of samples such as clothing, textiles, soft furnishings, ropes, carpets, baskets, straws, etc.

Samples collected from the scene and sent to our laboratory have been washed with distilled water without contamination [10]. The distilled water containing pollen and spore samples is filtered through rice mesh screens with a diameter of 150-200 μm and transferred to centrifuge tubes. These tubes are centrifuged at 3500 rpm for 15 minutes. If the sediment level in the centrifuge tubes is high, the process is repeated 2-3 times; if the sediment level is low, it's repeated once more. After these steps, the distilled water in the samples is poured off. The tubes are inverted, placed on drying paper, and left for 1-2 minutes for the distilled water inside the tubes to drain. Following this stage, the samples undergo the staining process, and permanent slides are prepared.

Preparation of Permanent Slides

Preparation of Glycerin-Gelatine with Basic-Fuchsin

First, the gelatine is softened in distilled water. The softened gelatine is mixed with glycerin. To stain the pollen, basic fuchsin is added to prepare glycerin gelatine. Phenol is added to prevent mold. The mixture is heated, poured into petri dishes, and left to cool [11].

Pollen in tubes is transferred to slides using glycerin gelatine with basic fuchsin. The heated slides are covered with coverslips, labeled, and placed upside down on two parallel glass rods. Slides are awaited until freezing to diagnose spores and pollen [10].

Microscopic Examination of Slides

A binocular OLYMPUS CX41 light microscope was used to diagnose and count the pollen and spores. For counting, a 10x eyepiece and an x40 plan objective were utilized. The OLYMPUS E-330 camera (x100 plan oil-immersion lens) was used to take microphotographs of the palynological particles on the slides.

In the cases examined, two preparations were prepared for each sample sent by the prosecutor's office. Examples examined in the cases are given below in tables (Table 2-7). Diagnosis of pollen was made according to reference slides available

or prepared in the Hacettepe University Palynology Laboratory and publications on palynology [5, 11-15].

TABLE 2. Incident 1.

Sample No.	E.G. Content	Quantity
1	A sawdust sample from the incident.	1 Piece
2	A sawdust sample from the suspicious vehicle's body.	1 Piece

TABLE 3. Incident 2.

Sample No.	E.G. Content	Quantity
1	Wheat samples from the wheat Warehouse.	1 Piece
2	The black boots of the suspect.	1 Pair
3	Yellow-colored work gloves belonging to the suspect.	1 Pair
4	Brown-colored striped fabric pants belonging to the suspect.	1 Piece
5	The black coat belonging to the suspect.	1 Piece

TABLE 4. Incident 3.

Sample No.	E.G. Content	Quantity
1	Plant/soil samples from the area where the animals were loaded.	4 Package
2	Soil/straw/litter samples from the place where the animals were stolen.	3 Package
3	Samples from various parts of the suspect's vehicle.	5 Package
4	A soil sample from the left tool chest of the suspect's vehicle.	1 Package

TABLE 5. Incident 4.

Sample No.	E.G. Content	Quantity
1	Shoe samples of the suspects.	6 Pair
2	Sample from the floor mats of the suspects' Ford truck.	1 Package
3	Sample from the floor mats of the suspects' pickup truck.	1 Package
4	Sample from the victim's barn.	1 Package

TABLE 6. Incident 5.

Sample No.	E.G. Content	Quantity
1	Blue-colored jeans and a burgundy-colored hoodie worn by suspects.	1 Piece
2	Blue-colored jeans and cream-colored cardigan from the suspects.	1 Piece
3	Black jeans and a yellow coat from the suspects.	1 Piece
4	Wheat sample from wheat storage.	200 g

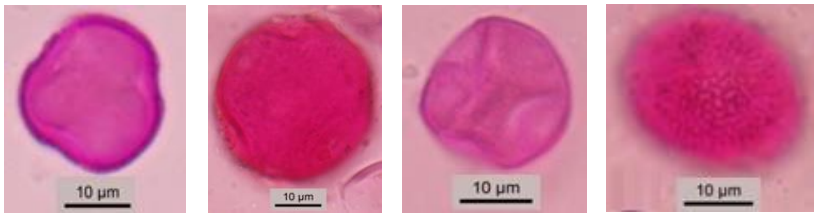
TABLE 7. Incident 6.

Sample No.	E.G. Content	Quantity
1	Sawdust samples found on the ground where the animals were loaded.	1 Package
2	Sawdust samples from the KIA truck body.	1 Package

3. RESULTS

In our study, forensic samples sent to our laboratory regarding the theft and attempted theft that occurred in various regions in Turkey between March 2016 and May 2017 were examined and evaluated from a palynological point of view.

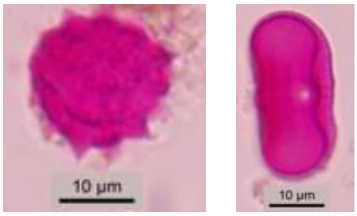
Various numbers of pollen belonging to Cupressaceae/Taxaceae, Pinaceae, *Plantago*, and Poaceae taxa were found in the examination made from the sawdust sample taken from the crime scene in incident 1. Brassicaceae, *Campanula*, *Cannabis*, and Oleaceae pollen found in the sawdust taken from the vehicle's body were not found in the sawdust sample taken from the crime scene (Figure 1). When these findings are evaluated together, the probability of the Fort Transit vehicle, which is suspected to have been used in the incident, to be found at the crime scene is very low.



a. b. c. d.

FIGURE 1. Microphotographs of pollen obtained from sawdust sample taken from the body of the suspect vehicle (a: Brassicaceae, b: *Campanula*, c: *Cannabis*, d: Oleaceae).

In the second incident, various numbers of pollen belonging to *Corylus*, Cupressaceae/Taxaceae, Pinaceae, and Poaceae taxa were found in the examinations made in the sample taken from the wheat warehouse. Pollens belonging to the insect-pollinated (zoogamous) *Bellis* and *Daucus* found in the samples taken from the wheat barn were not found in any of the suspect's belongings (Figure 2). When the results of our investigations are evaluated together, the probability of the suspect being present at the crime scene is seen as very low.



a. b.

FIGURE 2. Microphotographs of pollen obtained from a wheat sample taken from a wheat barn (a: *Bellis*, b: *Daucus*).

Pollens of taxa such as Cupressaceae/Taxaceae, Pinaceae, and Poaceae were found in the samples taken from the place where the animals were loaded and from the inside of the suspect vehicle, the front and rear plate, and various parts of the safe in the incident 3. In one of the samples taken from the place where the animals were stolen, starch grains that were found in uncountable concentrations were never found in the samples taken from various parts of the suspect vehicle. In addition, to consider the possibility of the suspect vehicle at the crime scene, it was expected that pollen belonging to taxa such as *Artemisia*, Boraginaceae, Caryophyllaceae, *Daucus*, and *Quercus* found in the samples taken from the crime scene would also be found in the samples taken from the vehicle. On the other hand, it is expected to encounter the shield hairs of the *Olea* (Olive) plant, which is abundant in the vegetation of the region (Figure 3). When these findings are evaluated together, the probability of the suspect being present at the crime scene is very low.

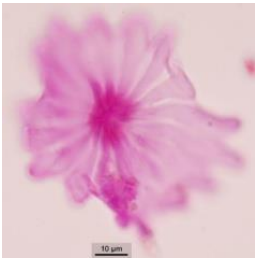


FIGURE 3. Microphotographs of the shield hairs of the Olea (Olive) plant.

Samples from the shoes and the mats of the Ford brand truck and pickup truck that belonged to the suspects in the fourth incident contained varying amounts of pollen from different taxa. Numerous *Chaetomium* spores and varying amounts of pollen from different taxa were found in the samples taken from the victim's barn (Figure 4). Pollen grains of Zoogamous taxa, Apiaceae, *Artemisia*, *Bellis*, and *Taraxacum*, were found in all samples examined. However, in this case, the absence of *Chaetomium* spores found in the sample taken from the victim's barn in any sample belonging to the suspects reduces the probability of the suspects being present at the crime scene. When these findings are evaluated together, the probability of the suspects being present at the crime scene is very low.

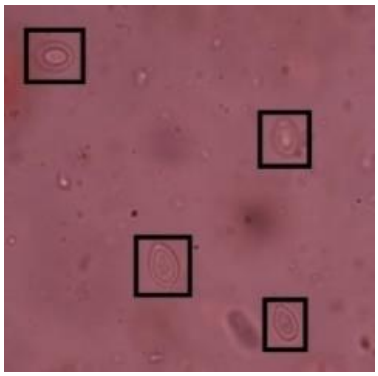
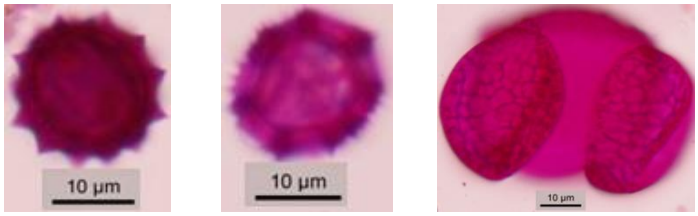


FIGURE 4. Microphotographs of the *Chaetomium* spores.

In the fifth incident, various pollen from multiple taxa was identified in the suspects' clothing. Pollen samples found in the examples were compared with the taxa they belong to, to determine whether the pollen from these taxa is similar or not. In this comparison, it was determined that the common taxa with pollen found on the suspects' clothing are *Bellis*, *Carex*, *Cistus*, Cupressaceae/Taxaceae, *Juglans*, Oleaceae, Pinaceae, Poaceae, *Quercus*, and Rosaceae. Another notable feature in this comparison is the high presence of pollen belonging to Pinaceae. Taxa from Pinaceae disperses their pollen by wind pollination and their pollen exhibits a wide distribution over large areas. The significant presence of Pinaceae pollen in the examined samples regarding this incident indicates that the suspects were in proximity to an environment where these taxa were present. The fact that Bellis, *Taraxacum*, and Pinaceae pollen were found in samples taken from the suspect individuals suggests that both these taxa and the individuals were present in the same environment (Figure 5). Due to the lack of detailed and sufficient photographs sent from the crime scene and its immediate surroundings, it has not been possible to establish a connection between the crime scene and the suspected individuals.



a. b. c.

FIGURE 5. Microphotographs of the suspects' clothes and pollen common to the area where the incident took place (a: *Bellis*, b: *Taraxacum* c: Pinaceae).

Furthermore, in all samples sent to our laboratory, wheat starches were found (Figure 6). These starch grains are most likely to have been transferred to the belongings of the suspect individuals from wheat storage. When these findings are considered together, the likelihood of the suspect individuals being present at the crime scene is quite high. To verify the accuracy of this possibility, we believe that our conclusion needs to be supported by additional evidence as well.

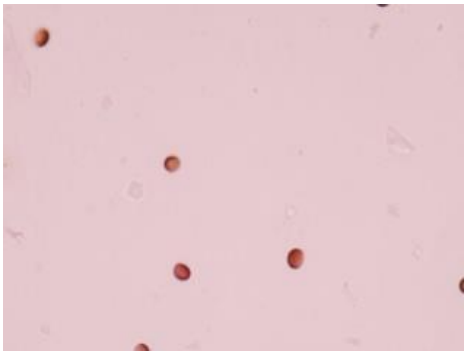


FIGURE 6. Microphotographs of wheat starches.

In the sixth incident, various pollen from different taxa were identified in the samples taken from the sawdust found at the location where the animals were loaded. Similarly, in the sawdust sample taken from the back of the suspect's truck, various quantities of pollen from different taxa were detected. In both examined samples, the common taxa with pollen were determined to be Cupressaceae/Taxaceae, *Lamium*, Pinaceae, and *Salix*. Cupressaceae/Taxaceae and Pinaceae are anemophilous plants, and their pollen can be found almost everywhere. The other common taxa were identified as *Salix* and *Lamium*. It can be stated that the pollen quantity of *Salix* is insufficient for comparison. While the pollen count for *Lamium* in the sample taken from the crime scene was 1229 grains, it was only 6 grains in the sample taken from the truck (Figure 7). These pollen quantities indicate a lack of consistency between the examined samples.

If the amount of *Lamium* pollen in the truck sample had been similar to the quantity in the sample from the crime scene, a clear connection could have been established between the truck and the crime scene. Additionally, taxa like *Alnus*, Asteraceae, *Fraxinus*, and *Populus*, whose pollen was found at the crime scene, were not observed in the truck sample. Although Oleaceae and Rosaceae pollen were found in the truck sample, they were not present in the sample taken from the crime scene.

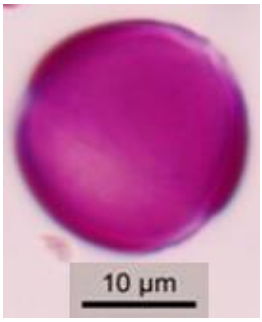


FIGURE 7. Microphotographs of *Lamium* pollen.

When these findings are considered together, the likelihood of the suspected vehicle being present at the crime scene appears to be a very weak possibility. To verify the accuracy of this possibility, we believe that our conclusion needs to be supported by additional evidence as well.

4. DISCUSSION

A burglary case from February 2006 was the first in Turkey to be solved using forensic palynology [8]. Since then, many cases have arrived at our laboratory, and research has been conducted on them. We have informed the legal system of our thoughts on these cases using the knowledge we have gained from these experiences. These cases' outcomes not only led to the capture of the offender but also saved the lives of innocent bystanders. In the studied cases, palynomorphs and spores were also used in addition to pollen. In case number five, starch grains were used. This demonstrates the need for those in this field to possess a strong grasp of both botanical science and palynology.

In addition, when we look at the examples of forensic palynology in the world [1, 16-20] we do not know how much our contribution is to the results of the cases, although it is known that it affects the results of the cases one-to-one.

Today, the United Kingdom is at the forefront of the use of forensic palynology, followed by New Zealand, Australia, Canada, a few Asian countries and the USA continue to use forensic palynology, albeit rarely [20]. In our country, the use of palynology in forensic cases is rarely utilized compared to these countries.

5. CONCLUSIONS

According to the study's data, pollen can be utilized as evidence in forensic investigations. It has been found that palynology can be utilized in forensic cases to establish a connection between the suspect's person or vehicle and the crime scene. Furthermore, our research will serve as a resource to enlighten scientists working on related problems.

Author Contribution Statements YH- specimen identification, data analysis, manuscript writing and editing, CD- supervising, specimen identification, data analysis

Declaration of Competing Interests The authors declare no conflict of interest.

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