Case Note

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Forensic comparative analysis of soils by the method of spore-pollen analysis using projection to latent structures discriminant analysis

For more than 50 years, spore-pollen analysis data have been used around the world to investigate crimes. Pollen and spores are most often studied while complex forensic examination of soils.

Extensive use of spore-pollen analysis is largely conditioned by such specific features of pollen and spores as their very-large-scale production in plants, the ability to preserve in soils, presence of characteristic morphological features enabling to distinguish and identify individual taxa, etc.

In Belarus, the method of spore-pollen analysis has also been implemented while forensic soil examination: it is increasingly applied to solve identification tasks in comparative analysis to establish the belonging of soil layers on physical evidence to the searched area. Its main advantage is that it is a multicomponent analysis allowing to evaluate both the composition of palynoflora and the percentage of several dozen components of spore-pollen spectra contained in soil samples. Therefore, to determine the belonging of soil layers on physical evidence to the searched area, most informative are data obtained while spore-pollen analysis, helping forensic experts on the basis of a specific taxonomic composition of pollen and spores and percentage of spectra components to draw the most valid conclusions.

Detection of pollen and spores in studied samples in a quantity sufficient for comparative analysis enables to statistically process data of samples microscopic examination. Statistical processing of results is one of the characteristic features of spore-pollen analysis as a method that distinguishes it from other methods implemented in multidisciplinary forensic examination of soil.

The authors declares no conflict of interest of relavance to this topic.

Keywords: *forensic palynology, soil, microscopy, taxonomic composition, percentage, projection to latent structures discriminant analysis.*

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Formulation of Research Problem. Enhancement of possibilities of forensic science depends mainly on the introduction of new research methods into forensic practice. The discovery of a huge number of features characterizing properties of studied objects is essential while group identification, because a more full study of compared objects enables to narrow the group of objects to which they belong, therefore to bring group identification to individual.

When conducting examinations of various physical evidence, experts usually extensively use research methods applied in biology, chemistry and physics. One of the methods which application can be important for forensic examination in the field of soil research is spore-pollen analysis (hereinafter referred to as SPA) aimed at identifying plants based on the study of their spores or pollen.

Analysis of Essential Researches and Publications. Specialists have been using spore-pollen analysis to study all types of soils for decades [1]. Foreign authors describe various methods and examples of using palynology to solve forensic tasks [2]. Research objects are pollen of gymnosperms and angiosperms and spores of cryptogram plants.

The main tasks of soil forensics are individual identification of a local area by layers on soil-contaminated objects or establishing the source of soil layers, that is determining the territorial belonging of soil layers if the event site is not established. Solution of the first issue is possible in the case of determining a unique set of identification features. At the same time, the more features are studied, the more successful solution for this task will be.

The need to solve tasks on establishing territorial belonging of soil layers on clothing and footwear, vehicles or other objects arises when the location of

[1] Horrocks M., Coulson S. A., Walsh K. A. J. Forensic palynology: variation in the pollen content of soil surface samples. *Journal of Forensic Science*. 1998. Vol. 43. Is. 2. P. 320—323. DOI: https://doi.org/10.1520/JFS16139J (date accessed: 20.02.2021) ; Marumo Y. Forensic examination of soil evidence. *Japanese Journal of science and technology for identification*. 2003. No 7. Is. 2. P. 95—111. DOI: https://doi.org/10.3408/jasti.7.95 (date accessed: 20.02.2021).

[2] Walter O. J., Adekanmbi O. H., Olowokudejo J. D. Palynological and Lithological Investigation of Forensic Materials at the University of Lagos, Nigeria: First Experimental Palynological Approach in Nigeria. *Journal of Forensic Science & Criminology*. 2019. Vol. 7. Is. 1. P. 104 ; Pereira J. S. R., Ribeiro H., Abreu I. Spatial and temporal environmental pollen analysis of footwear worn in the area of Barcelos, North-West Portugal, in a forensic context. *Aerobiologia*. 2019. Vol. 36. No 3. P. 1—6. DOI: https://doi.org/10.1007/s10453-019-09598-8 (date accessed: 20.02.2021) ; Reis C. I., Coimbra-Dores M. J., Rebelo M. T., Faria, M. S. Palynological analysis of soil in Portugal: potential for forensic science. *Palynology*. 2019. Vol. 43. Is. 4. P. 585—595. DOI: https://doi.org/10.1080/01916122.2018.1503199 (date accessed: 20.02.2021).

corpse or object does not correspond to a crime scene, which is the searched area; if it is necessary to establish the vehicle path, the place of material assets theft, etc. [1]

The advantage of SPA, compared to other methods applied in multidisciplinary soil forensics, is that it is a multicomponent analysis [2] enabling to assess both the composition of palynoflora and the percentage of several dozen components of spore-pollen spectra contained in soil samples. Therefore, when solving tasks of soil forensics, oftentimes it is data of spore-pollen analysis that allow forensic experts to draw the most valid conclusions based on a certain taxonomic composition of pollen and spores and the percentage of spectral components.

It should be stressed that in recent years, to assess results of a research on identified spore-pollen complexes, experts started to apply the methods of multivariate statistical analysis [3].

The **Article Purpose** is to consider practical approaches to determining the belonging of soil layers (from contaminated objects) to a specific local area with the help of SPA implementing elements of mathematical statistics.

Main Content Presentation. Apart from other issues, soil forensics should address the following issue: “Do soil layers obtained from 2 round-pointed shovels presented for research and a polymer bag with soil samples taken for a comparative study at 2 forest areas where the theft of the upper fertile soil layer was detected have a common genera (group) affiliation?” Along with other research methods, comparative research on the composition of spore-pollen complexes was performed.

To extract pollen and spores from the soil, the separation techniques developed by V. P. Hrychuk [4] were used. Zinc chloride dissolved in 2% hydrochloric acid with the specific weight of 1.9 was used as a heavy liquid.

[1] Wiltshire P. E. J. Protocols for forensic palynology. *Palynology*. 2016. Vol. 40, Is. 1. P. 4—24. DOI: https://doi.org/10.1080/01916122.2015.1091138 (date accessed: 20.02.2021).

[2] Хох А. Н., Рылова Т. Б. Доказательственное значение спорово-пыльцевого анализа при исследовании вещественных доказательств. *Вопросы криминологии, криминалистики и судебной экспертизы*. 2018. № 1 (43). P. 148—155.

[3] Ochando J. et al. Forensic palynology revisited: Case studies from semi-arid Spain. *Review of palaeobotany and palynology*. 2018. Vol. 259. P. 29—38. DOI: https://doi.org/10.1016/j.revpalbo.2018.09.015 (date accessed: 20.02.2021) ; Kumari M., Sankhla M. S., Nandan M., Sharma K., Kumar R. Role of forensic palynology in crime investigation. *IJournals: International Journal of Social Relevance & Concern*. 2017. Vol. 5. Is. 3. P. 1—13 ; Abdulrahaman A. A., Al Sahli A. A., Okoli J. U. The use of soil palynomorphs in forensics. *Journal of Applied Sciences and Environmental Management*. 2018. Vol. 22. No. 1. P. 85—89. DOI: https://doi.org/10.4314/jasem.v22i1.15 (date accessed: 20.02.2021) ; Riding J. B. A guide to preparation protocols in palynology. *Palynology*. 2021. Vol. 45. Is. 1. P. 1—110. DOI: https://doi.org/10.1080/01916122.2021.1878305 (date accessed: 20.02.2021).

[4] Ларина Т. В. Использование метода спорово-пыльцевого анализа при криминалистическом исследовании почв для локализации участков местности. *Проблемы экспертизы растительных объектов.* Москва, 1972. P. 104—106.

Pollen and spores identification was performed in a movable specimen using МИКМЕД 6 (ЛОМО) light microscope equipped with a digital camera while magnifying with a 50x to 1000x (with immersion). This was done so that each pollen grain or spore could be turned to both polar and equatorial positions, it is expedient to consider morphological features of the surface structure. In each specimen all pollen grains and spores were counted, encountered during sequential viewing of a specimen in parallel rows, starting from the upper left corner of the cover glass and ending with the lower right corner.

All samples contained a large quantity of pollen and spores (from 246 to 383). It helped to calculate the reliable percentage of spectral components. The composition of certain taxa, the number of pollen grains of each taxon and their percentage in each studied sample are presented in Table 1.

Table 1

Taxonomic composition, quantity and percentage of pollen and spores

Taxonomic composition of pollen and spores	Polymer bag		Shovel № 1		Shovel № 2		Forest area № 1		Forest area № 2	
	No.	%	No.	%	No.	%	No.	%	No.	%
Pollen of tree and shrub species	42,0	17,0	297,0	77,5	281,0	87,0	265,0	87,0	44,0	16,9
Pollen of herbaceous plants and prostrate shrubs	203,0	82,5	15,0	4,0	23,0	7,0	24,0	8,0	216,0	82,8
Spores	1,0	0,5	71,0	18,5	18,0	6,0	15,0	5,0	1,0	0,4
Picea abies (L.) H. Karst. (European spruce)	—	—	1,0	0,3	7,0	2,2	8,0	2,6	—	—
Pinus sylvestris L. (Scots pine)	40	16,3	164,0	42,8	243,0	75,5	218,0	71,5	42,0	16,1
Betula sp. (birch)	—	—	41,0	10,7	4,0	1,2	6,0	1,9	—	—
Alnus sp. (alder)	1,0	0,4	57,0	14,9	17,0	5,3	23,0	7,5	1,0	0,4

Taxonomic composition of pollen and spores	Polymer bag		Shovel № 1		Shovel № 2		Forest area № 1		Forest area № 2	
	No.	%	No.	%	No.	%	No.	%	No.	%
Quercus robur L. (common oak)	—	—	9,0	2,3	4,0	1,2	3,0	1,0	—	—
Ulmus sp. (elm)	—	—	1,0	0,3	—	—	—	—	—	—
Carpinus betulus L. (common hornbeam)	1,0	0,4	9,0	2,3	—	—	—	—	1,0	0,4
Corylus avellana L. (common hazel)	—	—	12,0	3,1	1,0	0,3	1,0	0,3	—	—
Salix sp. (willow)	—	—	3,0	0,8	5,0	1,6	7,0	2,3	—	—
Amaranthaceae subgen. Chenopodioideae (goosefoot family)	43,0	17,5	—	—	6,0	1,9	7,0	2,3	45,0	17,2
Polygonaceae (buckwheat family)	2,0	0,8	—	—	—	—	—	—	2,0	0,8
Apiaceae (celery)	—	—	1,0	0,3	2,0	0,6	1,0	0,3	—	—
Artemisia sp. (sagebrush)	2,0	0,8	2,0	0,5	1,0	0,3	—	—	2,0	0,8
Asteraceae (composites)	3,0	1,2	—	—	1,0	0,3	1,0	0,3	3,0	1,1
Asteraceae subgen. Cichorioideae (common chicory)	3,0	1,2	1,0	0,3	—	—	—	—	4,0	1,5
Poaceae (grasses))	15,0	61,0	11,0	2,9	6,0	1,9	6,0	2,0	16,0	61,3
Cyperaceae (sedges)	—	—	—	—	1,0	0,3	1,0	0,3	—	—

Taxonomic composition of pollen and spores	Polymer bag		Shovel № 1		Shovel № 2		Forest area № 1		Forest area № 2	
	No.	%	No.	%	No.	%	No.	%	No.	%
Typha angustifolia L. (lesser bulrush)	—	—	—	—	5,0	1,6	6,0	2,0	—	—
Myriophyllum spicatum L. (Eurasian watermilfoil)	—	—	—	—	1,0	0,3	2,0	0,7	—	—
Bryales (green mosses)	—	—	4,0	1,0	4,0	1,2	5,0	1,6	—	—
Sphagnum sp. (peat moss)	—	—	67,0	17,5	10	3,1	7,0	2,3	—	—
Lycopodium clavatum L. (club moss)	—	—	—	—	1,0	0,3	—	—	—	—
Polypodiaceae (polypody family)	1,0	0,4	—	—	3,0	0,9	2,0	0,7	1,0	0,4
Salvinia natans (L.) All. (floating fern)	—	—	—	—	—	—	1,0	0,3	—	—

In the overall composition of spectra of samples from round-pointed shovel No. 2 (3 samples) and taken from forest area No. 1 (10 samples), where theft of the upper fertile soil layer was detected, pollen of tree species constitutes 86—91 %, herbaceous plants and prostrate shrubs: 6—8 %, spores: 3—7 %.

Scots pine (*Pinus sylvestris L.*) prevails in the pollen of tree species: 71—76 %. Pollen of alder (*Alnus sp.*): 5—8 %, European spruce (*Picea abies (L.) H. Karst.*): 2—3 %, birch (*Betula sp.*): 0,3—1,6 %. There is also pollen of common oak (*Quercus robur L.*): 1—1,5 %, common hazel (*Corylus avellana L.*): 0,3—0,35 % and willow (*Salix sp.*): 1,1—2,5 %.

Among the pollen of herbaceous plants, pollen grains of goosefoots were mainly identified in specimens (*Amaranthaceae subgen. Chenopodioideae*):

1,8—2,6 % and grasses (*Poaceae*): 1,4—2,3 %. Constant presence in each sample of pollen of aquatic and semi-aquatic species is typical: lesser bulrush (*Typha angustifolia L.*): 1,5—2,2 %, Eurasian watermilfoil (*Meniantus spicatum L.*): 0,3—0,7 %, sedges (*Cyperaceae*): 0,3—0,35 %.

Cryptogram plants are represented by a small number of spores of sphagnum mosses (*Sphagnum sp.*): 2—3,3 %, green mosses (*Bryales*): 0,9—2 %, polypodies (*Polypodiaceae*): 0.4—1.1 %; there are single microsporangia of a floating fern (*Salvinia natans (L.) All.*) and club moss (*Lycopodium clavatum L.*): 0–0.3 %.

The composition of the pollen of tree species for samples from a polymer bag (3 samples) and taken at forest area No. 2 (10 samples) contains Scots pine: 16—16,5 %, as well as single pollen grains of alder: 0.4 % and common hornbeam (*Carpinus betulus L.*): 0,4—0,6 %.

In the composition of the pollen of herbaceous plants, in turn, representatives of the grasses were identified: 60,7—61,8 % and the goosefoots:17,0—17,8 %. Pollen grains of sagebrush (*Artemisia sp.*), buckwheat (*Polygonaceae*),composites (*Asteraceae*), common chicory (*Asteraceae subgen. Cichorioideae*) are found occasionally.

Cryptogam plants are represented by single spores of polypodies: 0—0,4 %. In samples from round-pointed shovel No. 1 (3 samples) in the total composition of the spectra, the pollen of tree species is 77.5 %, herbaceous plants 4 %, spores 18.5 %. Scots pine pollen prevails among tree species pollen 53 %. Alder pollen 15 %, birch 11.5 %, common oak 3 %, common hornbeam 3 %, common hazel 4 %, willow 1 %, elm (*Ulmus sp.*) 0.3 %. An insignificant amount of pollen of herbaceous plants was found in: grasses 3.5 %, single-celery (*Apiaceae*), sagebrush, common chicory.

Cryptogam plants are represented mainly by sphagnum moss spores: 18 %, occasionally spores of green mosses (Bryales) are found:1.5 %.

Later, obtained palynological data were analyzed using the principal component analysis (hereinafter referred to as PCA) [1]. For this purpose, *The Unscrambler X v.10.4.1 (CAMO, USA)* specialized program was used.

Based on the performed analysis, all studied samples were combined into 3 classes:

1) samples from a round-pointed shovel No. 1;
2) samples from a round-pointed shovel No. 2 and taken at forest area No. 1;
3) samples from a polymer bag and taken at forest area No. 2.

Fig. 1 shows that points reflecting the studied samples and corresponding to one class do not overlap and are located in different areas of the model regarding the 1st and 2nd principal components, indicating significant differences between them.

[1] Kherif F., Latypova A. Principal component analysis. *Machine Learning.* 2020. P. 209—225.

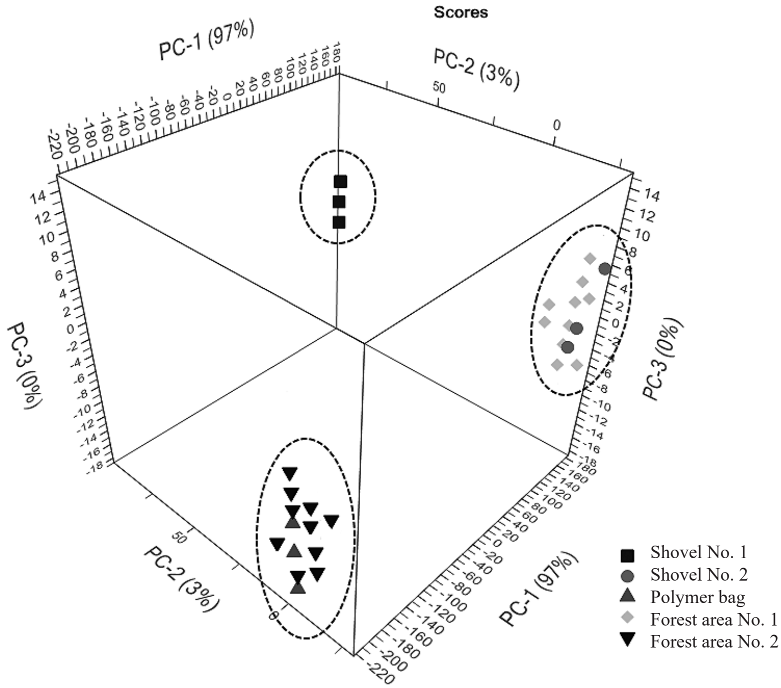

Fig. 1. Graph of PCA-calculations for 29 studied samples

After, projection to latent structures-discriminant analysis (hereinafter referred to as PLS-DA) [1] was conducted. We used the same data as for PCA, but at the same time samples from round-pointed shovels and a polymer package were assigned to the test dataset (test), and samples from two forest areas where theft of the upper fertile soil layer was detected were assigned to the training data set (training) and preliminary divided into 2 classes: site No. 1 Class *1*; site No. 2 Class *1*.

The accuracy of the designed PLS-DA-model assessed by the cross-validation method was 99.8 %.

Table 2 shows the significance coefficient of identified samples from round-pointed shovels and polymer bags. The mentioned coefficients have both positive and negative values depending on their contribution to the positive or negative areas of the model, their significance in this case does not depend on a sign and is determined only by deviation from zero.

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[1] Sampaio P. S. et al. Identification of rice flour types with near-infrared spectroscopy associated with PLS-DA and SVM methods. *European Food Research and Technology*. 2020. Vol. 246. Is. 3. P. 527—537. DOI: https://doi.org/10.1007/s00217-019-03419-5 (date accessed: 20.02.2021) ; Ruiz-Perez D. et al. So you think you can PLS-DA? *BMC bioinformatics*. 2020. Vol. 21. Is. 1. P. 1—10. DOI: https://doi.org/10.1186/s12859-019-3310-7 (date accessed: 20.02.2021).

Table 2

Calculated significance coefficients

Sample No.	Significance coefficient	Deviation
Polymer bag No. 1	–0,9494005	0,085706
Polymer bag No. 2	–0,9619232	0,264547
Polymer bag No. 3	–0,9708145	0,049738
Shovel No. 1_1	3,621863	8,977525
Shovel No. 1_2	3,662567	9,069019
Shovel No. 1_3	3,703678	9,161437
Shovel No. 2_1	0,9100758	0,603048
Shovel No. 2_2	0,9250175	0,297527
Shovel No. 2_2	0,9394305	0,148326

According to the results of the performed research, studied samples from a polymer bag were assigned to Class *-1* of the corresponding forest area No. 2 (the average significance coefficient was –0.96), and the samples from the round-pointed shovel No. 2: to Class *1,* corresponding to the forest area No. 1 (the average significance coefficient was 0.92). In turn, the samples from the round-pointed shovel No. 2 were not assigned to any of the 2 classes, that is areas where the upper fertile soil layer was stolen (the average coefficient of significance was 3.7; Fig. 2).

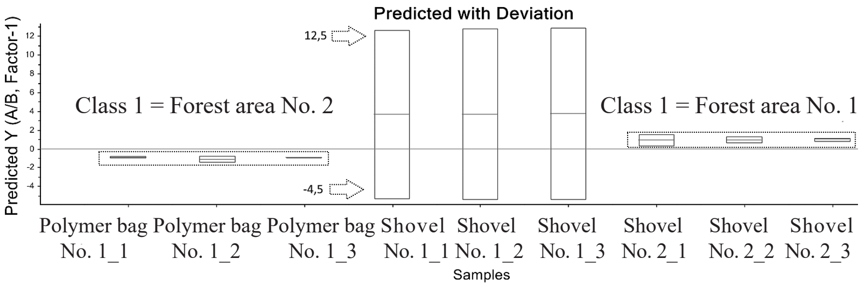

Fig. 2. Division of samples from test dataset

To assess the degree of variability influence of the taxonomic composition of pollen and spores on the distribution of objects from the test dataset (test) in principal component space and to determine the most informative parameters, an analysis of loads on the first and second principal components was performed. The results are presented in Fig. 3.

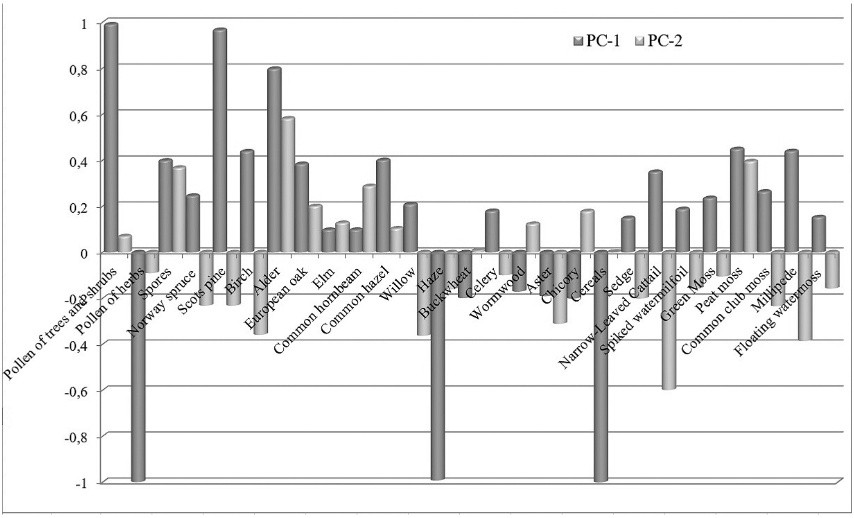

Fig. 3. Graphs of loads on the first and second principal components

As can be noted, the most informative (variable) parameters of the obtained spore-pollen spectra are the overall composition of the spore-pollen spectrum (the quantitative ratio of pollen of three main groups of plants (tree species, herbaceous plants and prostrate shrubs, as well as spores), along with the quantitative and percentage concentration of the following taxa: Scots pine, birch, alder, goosefoots and grasses; that is, it is these indicators that are increasingly responsible for dividing the studied samples into 3 different groups.

Conclusions. In the case considered in this research paper, the abundance of pollen and spores in the samples under study and the possibility of determining these plant remains helped to statistically process the data of samples microscopic examination. Comparison of spore-pollen spectra for samples from round-pointed shovel No. 1 with the rest of the studied samples showed the existing sharp differences, as confirmed by the results of projection to latent structures-discriminant analysis. At the same time, the spore-pollen spectra (1) for samples from the round-pointed shovel No. 2 and taken at forest area No. 1, and also (2) for samples from a polymer bag and taken at forest area No. 2 had a similar percentage of pollen and spores in the main groups of plants in the overall composition of the spectra, in addition, they are characterized by coincidence of the composition of taxa of tree species (regional components), herbaceous and spore plants (local components) and their quantitative content. According to the results of the classification by the PLS-DA method, it was concluded that the samples from the round-pointed shovel No. 2 and taken from forest area No. 1 have a common group affiliation; samples from a polymer bag and forest area No. 2 also had a common group affiliation. At the same time, it should be emphasized that (1) and (2) have a different generic affiliation. The classification accuracy turned out to be high and amounted to 99.8%. Consequently, pollen and plant spores are an integral part of any type of soil, and therefore (along with a complex of soil morphological characteristics) they can be efficiently used in solving tasks of soil forensics.

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Судебно-экспертные сравнительные исследования почв методом спорово-пыльцевого анализа с использованием дискриминантного анализа проекций на латентные структуры

В статье рассмотрены практические подходы к использованию спорово-пыльцевого анализа в установлении принадлежности почвенных наслоений на загрязнённых предметах конкретному локальному участку местности. Показана возможность интерпретации палинологических данных с применением дискриминантного анализа проекций на латентные структуры. По результатам проведённого исследования сделан вывод, что пыльца и споры растений наряду с комплексом морфологических признаков почвы можно использовать как информативный показатель при решении задач судебной почвоведческой экспертизы.

Ключевые слова: *судебная палинология, почва, микроскопия, таксономический состав, процентное содержание, дискриминантный анализ проекций на латентные структуры.*

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Судово-експертні порівняльні дослідження ґрунтів методом спорово-пилкового аналізу з використанням дискримінантного аналізу проєкцій на латентні структури

Уже понад 50 років у всьому світі під час розслідування злочинів використовують дані спорово-пилкового аналізу. Найчастіше пилок і спори досліджують під час виконання комплексної криміналістичної експертизи ґрунтів.

Широке застосування спорово-пилкового аналізу зумовлено значною мірою такими специфічними особливостями пилку та спор, як величезна кількість їх продукування рослинами, здатність зберігатися в ґрунтах, наявність характерних морфологічних особливостей, що дають змогу розрізняти й визначати окремі таксони і т. ін.

У Білорусі метод спорово-пилкового аналізу також знайшов застосування під час виконання судової ґрунтознавчої експертизи: його частіше *застосовують для вирішення ідентифікаційних завдань під час порівняльних досліджень для встановлення належності ґрунтових нашарувань на речових доказах ділянці місцевості. Його головна перевага полягає в тому, що це багатокомпонентний аналіз, який дає змогу оцінювати як склад палінофлори, так і відсотковий вміст одразу декількох десятків компонентів спорово-пилкових спектрів, що містяться в ґрунтових зразках. Тому для визначення належності ґрунтових нашарувань певній локальній ділянці місцевості найбільш інформативними є саме дані спорово-пилкового аналізу, що дають змогу експертам на підставі певного таксономічного складу пилку і спор та процентного вмісту компонентів спектрів дійти найбільш обґрунтованих висновків.*

Виявлення пилку і спор у досліджуваних пробах у кількості, достатній для порівняльного дослідження, дає змогу статистично обробити дані мікроскопічного вивчення зразків. Статистичне оброблення результатів — одна з характерних ознак спорово-пилкового аналізу як методу, що відрізняє його від решти методів, які застосовують у комплексній експертизі ґрунтів.

Ключові слова: *судова палінологія, ґрунт, мікроскопія, таксономічний склад, відсотковий вміст, дискримінантний аналіз проєкцій на латентні структури.*

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