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TECHNICAL NOTE

CRIMINALISTICS

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Cannabis Profiling Based on Its Elemental Composition—Is It Possible?

ABSTRACT: Elemental composition of 85 cannabis samples was established using GF AAS and ICP OES methods. The robustness of the method was determined by analyzing eight independently prepared replicates from a single cannabis plant. The accuracy of the method was established by analyzing four plant certified reference material samples. The ability of discriminant analysis using elemental compositions to distinguish between fiber cannabis samples collected from four different regions of Poland was evaluated. Then, a classification model was developed that correctly classified selected samples of known origin. Cannabis samples confiscated by law enforcement agencies have also been subjected to discriminant analysis. A classification model has been developed for four locations in Poland (Białystok, Kościerzyna, the environs of Skarżysko Kamienna, and Bydgoszcz), to help determine where samples of unknown origin could have been grown.

KEYWORDS: forensic science, cannabis profiling, elemental composition, inductively coupled plasma optical emission spectrometry, graphite furnace atomic absorption spectrometry, discriminant analysis

Drug addiction has been a major social concern in Poland and worldwide. There is a growing threat of drug crimes. The number of crimes associated with illegal production (or growing), distribution, and smuggling of narcotic drugs and crimes committed under the influence of narcotics has been continuously on the rise.

In Poland, we saw a 17-fold increase in the number of detected of drug crimes over the last several years! (1).

Statistical studies of the Polish police and other institutions shed some light on the real image of drug addiction and the consequences thereof. It appears that drugs are easily available: over 1/3 of middle and secondary school and university students have tried drugs at least once (2). In terms of doing drugs, marijuana is the most popular drug (2). It may be assumed that every 10th student regularly smokes marijuana (3).

It is therefore essential to undertake measures to limit access to drugs, and in particular, to marijuana and hashish.

Cannabis Profiling Based on Organic Compounds Contents

Cannabis may be profiled on the basis of its organic composition in order to trace the source of any sample.

Psychoactive cannabis and hemp grown for fiber may be identified using well-developed differentiation methods based on the content of cannabinoids: Δ^9-tetrahydrocannbinol (Δ^9-THC), cannabigerol (CBG), cannabidiol (CBD), cannabinol (CBN), tetrahydrocannabivarin (THV), cannabidivarin (CBDV), cannabichromen (CBC), and cannabigerol monoethylether (CBGM) (4,5), or based on THCA synthetase gene analysis (6). Despite detailed and well-documented studies on differentiating cannabis specimens, the differentiation process is limited to hemp grown for fiber and cannabis used for drug purposes.

ElSohly et al. (7) analyzed 157 samples from six different regions (Colombian, Jamaican, Mexican, Thai, Californian, and Hawaiian) by gas chromatography–mass spectroscopy (GC/MS). Chromatograms of 175 peaks were collected for each sample. Statistical analysis of 175 compounds, including K-nearest-neighbor classification, gave classification accuracies which ranged from 81% for Hawaiian samples to 100% for Jamaican samples. However, the similarity of the chemical compositions of Californian and Mexican samples led to some misclassifications. These authors also identified possibly chemical "markers," which were present in samples from one region, but are absent in samples from other regions. Despite their successful classifications, the authors noted that this method can only differentiate across relatively large regions, since the range of content of organic compounds is too small within a single country. Besides, analysis by GC/MS is time consuming (about 60 min per sample).

DNA fingerprinting methods have been also used in identifying (8) and profiling (9,10) cannabis samples. Genetic analytical methods include random amplified polymorphic DNA (RAPD) analysis using polymerase chain reaction (PCR). Fifty-one samples of cannabis plants have been analyzed using RAPD analysis. Samples originated from Australia, Papua New Guinea, and Tasmania. Significant genetic differences have been revealed in samples from Australia and Papua New Guinea.

Pinarkara et al. (11) analyzed by RAPD 290 psychoactive-type cannabis samples seized from 29 different locations of Turkey. The results were analyzed using cluster analysis and analysis of molecular variance (AMOVA). The results show separation of samples originating from western and eastern parts of Turkey.

Still, high cost and low accessibility in forensic laboratories are the main disadvantages of this method.

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Choi et al. suggests a "metabolic" profiling to differentiate cannabis plantations (12). This method consists in identifying the chemical profile of metabolites in cannabis tissues. Twelve cannabis samples originating from various plantations have been subjected to ^{1}H NMR analysis. The following metabolites have been identified in sampled flower clusters: Δ^9-tetrahydrocannabinolic acid—Δ^9-THCA (the key component), Δ^9-THC, and CBN. Δ^9-THC and sterols have been identified in cannabis leaves. Other compounds detected are the following: sugars (α- and β-glucose), amino acids (asparagine, alanine, valine, glutamic acid). Principal components analysis (PCA) has been performed to differentiate sampled cannabis originating from various plantations.

Cannabis Profiling Based on Elemental Composition

There are some papers concerning differentiation of cannabis samples on the basis of their elemental composition.

Watling (13) used laser ablation inductively coupled plasma mass spectrometry technique (LA ICP MS) to determine elemental composition of cannabis samples. He analyzed 45 elements. A database for over 1400 cannabis samples was created. The elemental composition allowed for the comparison of the elemental composition of seized samples to those present in the database, and information on their provenance was henceforth obtained.

The author points out that LA ICP MS is a technique that is very difficult to calibrate for quantitative analysis because of several reasons: (i) availability of certified reference materials of similar matrix to analyzed samples is limited; (ii) pulse to pulse variations in laser coupling efficiency result in differing amounts of sample reaching the plasma.

Applying this technique in forensic laboratories requires availability of expensive instruments which do not provide quantitative results, and thus it is difficult to attain comparable results in different laboratories.

Shibuya et al. used isotope ratio mass spectrometry to analyze C and N isotopic compositions of cannabis samples originating from dry and humid regions of Brazil (14). They collected 90 cannabis samples from different Brazilian production regions, differing in both climate and geography. They found that although the isotopic profile of samples from dry regions was different from that of hot and humid and semi-humid tropical regions, it was difficult to discriminate samples seized in regions with similar climatic conditions solely on the basis of isotopic composition. Even when they extended their work with advanced statistical methods, they found that isotope analysis on its own was insufficient to unambiguously determine the origins of all their samples (15). They therefore included elemental composition data obtained with sector field ICP MS into their classification of cannabis samples from different Brazilian regions (16). Classification based on linear discriminant analysis gave good results, with almost 98% of samples being classified correctly. Although Shibuya et al. (16) were thus able to profile cannabis samples on the basis of their elemental and isotopic composition, they were applying it to samples from Brazil—a country with an area of over 8.5 million km^2, and with many different types of climate and geography.

The purpose of this study was to develop a fast, inexpensive, and effective profiling method of cannabis samples originating from various regions of Poland based on elemental composition of the plants, and to assign particular samples to specific cannabis growing regions.

Poland is a country with an area of over 312,000 km^2, and is situated in a moderate climate, so the analyzed cannabis plants were cultivated in one climatic region and in a relatively small area. First, we wanted to check if elemental composition of fiber cannabis plants cultivated without use of fertilizers exhibits differences according to their origin. Then samples seized by Polish Police were included in the study. All results were analyzed by discriminant analysis, which allows for classification of unknown samples on the basis of a defined model.

We used techniques available in many forensic laboratories (inductively coupled plasma optical emission spectrometry [ICP OES], graphite furnace atomic absorption spectrometry [GF AAS]). This is very important because creating a database of traceable results for cannabis samples requires availability of the equipment. What is more, ICP OES and GF AAS are cheaper techniques than RAPD analysis or ICP MS. Besides high cost of the instrument (ICP MS), special conditions must be fulfilled, such as accessibility of laminar flow box.

Materials Studied, Methods, and Techniques

Samples

Eighty-five cannabis samples have been included in the analysis (20 samples of fiber hemp; the remaining samples have been delivered from the Central Forensic Laboratory of the Polish Police in Warsaw).

Samples of hemp grown for fiber originated from four legal plantations in Bachórz (south-eastern Poland), Strzelin (north-eastern region), Nowy Korczyn (south), and Mleczewo (north). All sampled plants were grown from Benico seeds. Samples were collected approximately 2 weeks before harvesting. No fertilizers were applied, as declared by the respective growers. Samples were collected, immediately placed in paper envelopes, and dried at room temperature for a period of 2 months. Seeds were separated from flower clusters and flower clusters were collected for analysis.

Samples sent to the forensic laboratory originated from illegal plantations (36 samples) or were confiscated from drug dealers and drug users. The locations of illegal plantations were identified. As for confiscated samples, it has not been assumed that the place of confiscation pointed directly to the actual plant growing region.

Sample Preparation

Samples were homogenized with a Fritsh planetary ball mill. Next, 250 mg of the sample was weighted to a mineralization Teflon vessel, 3 mL of 65% nitric acid (Suprapur®; Merck, Darmstad, Germany) and 1 mL of 30% hydrogen peroxide (Suprapur®) was added. Next, samples have been placed in high-pressure *MULTIWAVE* sample preparation system made by Anton Paar (Perkin Elmer, Waltham, MA). Table 1 presents the sample mineralization program.

Next, samples were quantitatively transferred into 10 mL (class A, Brand®) flasks, and made up to the mark with deionized water (Milli Q, Billerica, MA).

Instrumentation

The instrumentation used for the determination of lead was a graphite furnace atomic absorption spectrometer (Avanta Ultra Z made by GBC). An inductively coupled plasma-optical emission spectrometer (Optima 3100XL made by Perkin Elmer) was used for the measurements of the following elements: B, Ba, Cu, Sr, Zn, Mn, Fe, Mg, and Ca. Tables 2 and 3 present operating parameters of the a/m spectrometers.

TABLE 1—*Mineralization programme of cannabis samples.*

Step	Power (W)	Time (min)
1	100–300	0–5
2	0	6–7
3	300–600	8–13
4	0	14–19
5	600–800	20–21
6	0	22–23
7	800–1000	24–29
8	0	30–31
9	1000	32–37

TABLE 2—*Operating parameters of ICP OES.*

Parameter	Value
Plasma gas flow (L/min)	15
Auxiliary gas flow (L/min)	0.5
Gas flow through atomizer (L/min)	0.5
Plasma power (W)	1450
Plasma observation height (mm)	15
Sample flow (mL/min)	0.65
Delay time (sec)	90

Calibration and Accuracy of Method

Calibration of spectrometers for each element was performed using aqueous calibration standards. Working standard solutions were prepared from 1000 mg/L stock solution (Merck) by dilution in 5% (v/v) HNO_3. For each element, five standard solutions of different concentrations were prepared. The working ranges of calibration curves are as follows: 0–10 mg/L for B, Ba, Cu, Sr, and Zn; 0–50 mg/L for Mn; 0–100 mg/L for Fe; 0–200 mg/L for Mg; and 0–500 mg/L for Ca. Pb was analyzed by standard addition method. The concentrations of lead in additions of a standard solution were as follows: 10, 20, 30 μg/L. The determination of elements by ICP OES was performed by internal standard method to compensate for the matrix effects caused by high concentration of nitric acid and easily ionizable elements. Beryllium and yttrium were chosen to be internal standards.

The quality control of the method was performed using the standard reference materials INCT TL1 (tea leaves), INCT MPH2 (mixed Polish herbs), CTA VTL2 (Virginia tobacco leaves), and CTA OTL1 (Oriental tobacco leaves). The measured values were within 95–105% of the certified values except for Zn in INCT TL1 (93%), Ba in CTA VTL2 (90%), and Sr in CTA VTL2 and CTA OTL1 (108% and 92%, respectively).

Determination results of elemental composition have been statistically analyzed in STATISTICA (version 6.0).

Results and Discussion

Elemental Composition of Cannabis Samples

In this study 85 cannabis samples were analyzed (20 fiber type and 65 drug type).

Table 4 presents analysis results for cannabis plants analyzed in this work together with the data obtained by Shibuya et al. and Landi.

For Zn, cannabis samples seized in Poland presented similar values, and for Ba, Cu, Fe, Mn, Pb, Polish samples presented values lower than samples seized in Brazil.

Levels of Ca, Cu, Fe, Mg, Mn, and Zn in Polish samples were higher than those reported by Landi. It is worth mentioning that samples from Brazil presented highly dispersed results compared to Polish and Italian samples. We can assume that the reason is that Brazilian samples were collected from a larger area, where soil composition differs substantially compared to Poland and Italy. The samples were collected from an area of about 312,000 and 150 km^2 in Poland and Italy, respectively.

It is very difficult to draw any conclusions on similarity of samples based on raw results. Advanced statistical analyses need to be applied, such as discriminant analysis (17,18).

TABLE 3—*Furnace temperature program used in lead content determination by means of GF AAS and palladium-magnesium modifier (0.1% Pd + 0.06% $Mg(NO_3)_2$).*

No.	Temperature	Growth Time (sec)	Maintenance Time (sec)	Internal Gas Flow (mL/min)
1	50	1	1	3
2	Sample introduction			
3	90	15	20	3
4	120	20	15	3
5	1100	10	10	3
6	1100	0	1	0
7	2300	0.7	0.8	0
8	2400	0.5	0.5	3

TABLE 4—*Elemental composition of cannabis samples.*

Element	Range of concentration (μg/g)		
	Results from This Work	Shibuya et al. (16)	Landi (19)
B	24–72		
Ba	1.1–99.2	5.4–671	
Ca	11495–110456		21,700–72,800
Cu	2.0–49.0	0.8–69	10–31
Fe	91–1589	110–7660	141–274
Mg	2532–8573		4400–6900
Mn	34–493	69–2066	24–93
Pb	0.20–3.40 (16.77)*	0.1–9.5	
Sr	20.1–346.6	17–662	
Zn	18.9–234.5	13–284	27.5–59

* Result obtained for only one sample.

Robustness of the Method

A plant is a very inhomogenous sample. Thus sample preparation must be evaluated very precisely to limit errors connected with inhomogenity. Repeatability of the method was established by analyzing eight samples collected from one plant of *Cannabis sativa*. Each of the samples was independently homogenized, digested according to procedure given in Table 1, and analyzed. From acquired analytical results, the coefficient of variation (CV) values were calculated, which are given in Table 5.

The CV values for Ba and Mn were 6.2% and 5.9%, respectively, while CV was lower than 5% for all other elements.

Discrimination and Classification of Samples of Fiber Hemp

Data on elemental composition of fiber hemp samples have been subjected to discriminant analysis. The analyzed results were not standardized prior to statistical analysis. The analysis results have revealed that the following elements are of paramount importance

TABLE 5—*The coefficient of variation (CV) values calculated from results acquired for eight independent samples collected from one* Cannabis sativa *plant.*

Element	Concentration	SD	CV (%)
B (μg/g)	77	3	3.4
Ba (μg/g)	27.3	1.7	6.2
Ca (%)	3.94	0.14	3.5
Cu (μg/g)	21.4	0.2	1.0
Fe (μg/g)	900	43	4.8
Mg (%)	0.584	0.012	2.1
Mn (μg/g)	128	8	5.9
Pb (μg/g)	3.09	0.16	5.1
Sr (μg/g)	87	4	4.3
Zn (μg/g)	70.8	2.2	3.1

TABLE 6—*Summary of discriminant analysis of concentrations of six elements.*

Element	Partial Wilks' Lambda	*p* Level
B	0.327	0.0051
Ba	0.768	0.3876
Cu	0.265	0.0017
Fe	0.550	0.0588
Pb	0.034	0.00000002
Zn	0.556	0.0812

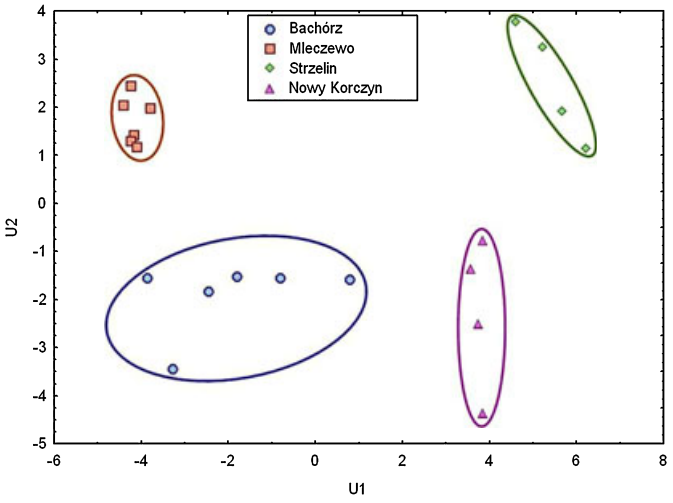

FIG. 1—*Scatter diagram of function 1 versus 2.*

in discriminating hemp: Pb, Cu, Zn, Fe, Ba, and B. However, only three elements may be considered significant in discriminant analysis of sampled fiber hemp: Pb, Cu, and B, which is confirmed in the value of partial Wilks' lambda and *p* level (Table 6).

Discriminant analysis has been applied in order to examine any possible significant differences between cannabis plants originating from various regions of Poland. The separation of the cannabis samples from different regions is clearly shown on a plot using the first two discriminant functions (Fig. 1).

Figure 1 presents no overlaps of groupings representing samples originating from various plantations. It may be concluded that hemp samples from plantations in various regions of Poland may be classified by means of elemental composition and discriminant analysis.

A classification model has been developed and revised for five samples of fiber hemp (Table 7). Those five samples were of known origin. They were marked as unknown to check correctness of classification by the developed model.

All samples have been correctly qualified by means of squared Mahalanobis distance. The result proved very promising, as fiber hemp samples may be correctly classified based on its elemental composition.

This applicability of classification model for fiber hemp has been also verified for other cannabis samples (grown for drug purposes). The analysis involved four cannabis samples from an illegal plantation in Busko Zdrój (Table 8).

All four samples have been classified to fiber hemp originating from Nowy Korczyn. The distance between these two localizations is relatively small (approximately 30 km), and the classification may be considered correct.

Discrimination and Classification of Samples of Cannabis

The last stage of analysis consisted of discriminant analysis and classification of analysis results of sampled cannabis collected at the forensic laboratory. These samples originated from illegal plantations and have been confiscated from drug dealers and drug users. Information concerning the locations of illegal plantations was available. As for confiscated specimen, it has not been assumed that the place of confiscation pointed directly to the actual plant growing region.

Discriminant analysis has been performed for 36 samples originating from four illegal plantations situated in four different locations in Poland (Biălystok, Kościerzyna, the environs of Skarżysko Kamienna, and Bydgoszcz).

It has been found that Mg, Sr, B, Zn, and Cu are elements that were found to be of the most statistical significance in this study (the value of *p* level for those elements is below 0.05) and were included in further discriminant analysis.

There were four groups of samples so three discriminant functions were achieved. Functions U_1 and U_2 contain almost 97% of the total discriminating power, so the third function (U_3) was excluded from further considerations.

Discriminant function U_1 drawn versus U_2 presented in Fig. 2 shows clearly separated three groups of samples.

TABLE 7—*Classification of fiber hemp samples.*

		Squared Mahalanobis Distance			
Case No.	Observed Classification	Bachórz	Mleczewo	Strzelin	Nowy Korczyn
B6	Bachórz	3.49203	14.43605	60.66659	30.31122
M5	Mleczewo	24.56944	1.45249	73.16001	72.57020
M6	Mleczewo	16.34434	0.09662	66.56302	63.30293
S4	Strzelin	52.13493	81.84285	4.15267	12.96573
NK4	Nowy Korczyn	25.23187	67.61858	27.03053	0.10526

TABLE 8—*Classification of cannabis samples grown for drug purposes using the model developed for fiber hemp.*

Case No.	Observed Classification	Squared Mahalanobis Distance			
		Bachórz	Mleczewo	Strzelin	Nowy Korczyn
BZ1	Nowy Korczyn	163.6678	260.9881	101.7135	68.66891
BZ2	Nowy Korczyn	23.8428	79.4503	76.3803	16.47945
BZ3	Nowy Korczyn	50.2603	120.3064	68.8271	14.03384
BZ4	Nowy Korczyn	63.7096	139.5697	64.2550	22.06333

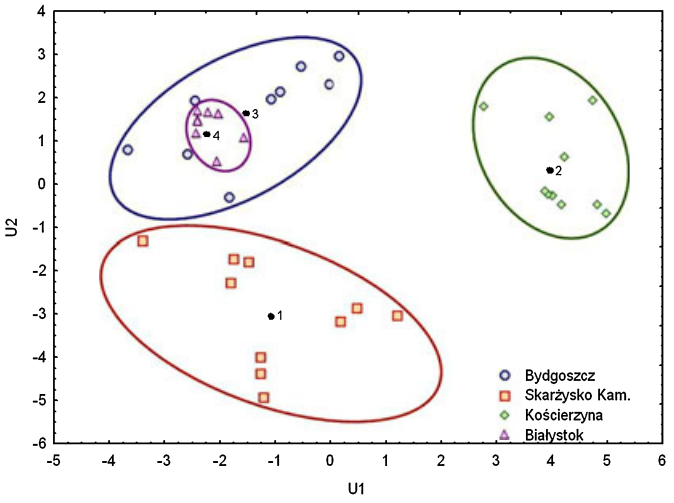

FIG. 2—*Scatter diagram of discriminant function 1 versus 2 (pt. 3 is a centroid point for samples from Bydgoszcz, and pt. 4-z—for samples from Biălystok).*

Note that samples from the environs of Skarżysko Kamienna and Kościerzyna constitute two separate groups. On the other hand, points representing samples from Biălystok and Bydgoszcz are mixed. This lack of clear distinction between the samples from Bydgoszcz and Biălystok is disadvantageous for sample classification, which may be mistaken.

Despite the overlapping of groupings from Biălystok and Bydgoszcz attempts to classify samples have been made. Five classification models were created. In each model a different combination of samples included in the learning set was used. The learning sets of the models were based on the results obtained for six samples from Bydgoszcz, seven samples from Kościerzyna, five samples from Biălystok, and seven samples from the environs of Skarżysko Kamienna. The remaining samples coming from the illegal plantations situated in those locations (which were not included in the learning set) were used to check the correctness of classification.

Irrespective of samples selected for the development of classification model, samples from Kościerzyna and Biălystok have been identified correctly. It might have been expected that samples from Kościerzyna would be classified correctly, as these samples used in the development of classification model constituted a separate group.

Surprising can be the fact that samples from Biălystok were classified correctly even though in Fig. 2 an overlap of groupings can be seen for those samples. However, the points representing samples from Biălystok form a very compact group of samples, which can explain correctness of classification of those samples.

The classification of the samples from Bydgoszcz was mistaken in all the models. For those samples 15 classifications were performed overall (five models, three samples). In 9 cases out of 15 the classification was incorrect. Classification of samples from Skarżysko Kamienna was mistaken in only one model and for only one sample.

Correctness of classification models varied from 8% to 25% of misclassifications. Considering the fact that a small number of samples have been used in discriminant analysis and that two intermixed groups existed, the correctness of classification models appears satisfactory.

The classification model which gave the best results of classification (the number of misclassified samples was the lowest) was applied to cannabis samples confiscated from drug dealers or drug users. The classification model of these samples has been created for four cannabis growing locations in Poland. It is therefore accurate for the classification of samples originating from the respective regions only. Samples confiscated from dealers may have been grown in other regions, therefore the classification of samples of unknown origin is provided as a reference only (Table 9).

Classification results for Kościerzyna (GD11–14) need to be examined closely. These samples have been confiscated at the

TABLE 9—*Classification of samples of unknown origin.*

Sample	Region of Confiscation	Observed Classification	Sample	Region of Confiscation	Observed Classification
GD11	Kościerzyna	Kościerzyna	W1	Warszawa	Kościerzyna
GD12	Kościerzyna	Kościerzyna	W2	Warszawa	Kościerzyna
GD13	Kościerzyna	Kościerzyna	W3	Warszawa	Bydgoszcz
GD14	Kościerzyna	Kościerzyna	W4	Warszawa	Bydgoszcz
G1	Grzywna	Skarżysko Kam.	W5	Warszawa	Kościerzyna
G2	Grzywna	Skarżysko Kam.	W6	Wŏlomin	Kościerzyna
G3	Grzywna	Skarżysko Kam.	W7	Wŏlomin	Kościerzyna
D1	Dęblin	Skarżysko Kam.	SP1	Sokŏlów Podlaski	Biălystok
ND1	Niedźwiada Duża	Biălystok	SE1	Serock	Kościerzyna
BY1	Bychawa	Biălystok	SE2	Serock	Kościerzyna
BY2	Bychawa	Biălystok	SE3	Serock	Kościerzyna
BY3	Bychawa	Biălystok	SE4	Serock	Kościerzyna

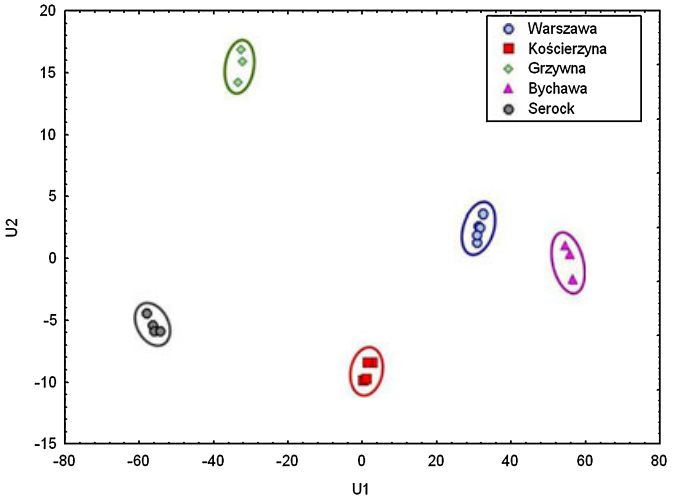

FIG. 3—*Scatter diagram of cannabis samples confiscated under various investigations.*

home of a person suspected of growing cannabis and have been assigned to the group from Kościerzyna. However, samples used in developing classification model (GD1–10) originated from an illegal plantation owned by the suspect. Nevertheless, it is likely that the person illegally growing cannabis stored samples from his own plantation. It may be therefore assumed that the samples confiscated at the home have been grown at a plantation located in this particular region. This information is of paramount importance for the law enforcement authorities.

Apart from verification of sample classification, all samples at the forensic laboratory have been examined for any similarities. For this purpose, discriminant analysis has been performed only for samples collected under one investigation. A scatter diagram of discriminant functions (Fig. 3) indicates a clear separation of samples confiscated in various regions, which may point to significant differences between the samples. Moreover, points within particular groups are not significantly scattered, which is a proof of sample similarity within particular groups.

Conclusion

In conclusion, the studies have shown that cannabis samples may be correctly classified in terms of their origin based on the result of elemental composition and discriminant analysis.

However, to make the classification model even more accurate, routine analyses of cannabis samples would have to be introduced to forensic laboratories, determining elemental composition of each plant sample. The results of such analyses and detailed sample characteristics would have to be collected and processed to compile a suitable database for correct classification model covering all regions of Poland.

Furthermore, ICP MS would be preferably used to gather more information on elemental composition of cannabis samples.

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