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Analysis of Forensic Soil Samples Via High-Performance Liquid Chromatography and Ion Chromatography

ABSTRACT: Traditional forensic soil comparisons are performed via physical and/or chemical examinations of color, texture, and mineral content, leaving any organic- or water-soluble fractions unexamined. This study uses high-performance liquid chromatography (HPLC) and ion chromatography (IC) to assess the qualitative and quantitative variation in these fractions of soil. Soil samples $(n = 120)$ were collected over the course of 3 weeks from urban, suburban, and rural locations in and around Lansing, MI. Additional samples from six of these locations (two urban, two suburban, and two rural) were collected once a week for 10 weeks for temporal analysis. Nine additional samples, equally spaced over a 1 $m²$ grid, from these same six locations were collected for spatial analyses. Qualitative and quantitative analysis of the resultant chromatograms separated the 120 samples into 10 groups by HPLC and 23 groups by IC. This study shows that using HPLC and IC to analyze the organic- and water-soluble fractions of soil can successfully discriminate samples. Quantitative analysis of the results eliminates some false inclusions by providing further differentiation of samples. The results of this study indicate that adding HPLC and IC analyses to traditional forensic soil analysis schemes can improve overall sample differentiation. The methods used in this study were also able to detect both qualitative and quantitative variations in soil over a relatively small geographic area. This demonstration of soil heterogeneity underscores the importance of the collection of a representative known sample population when assessing a forensic soil comparison. Significant temporal variation was also demonstrated over the course of 10 weeks of sampling; however, samples were found to be consistent over shorter periods of time. Baseline levels of inorganic anions were determined via IC; these levels may be useful in assessing the significance of anions detected in soil from cases involving low explosives.

KEYWORDS: forensic science, forensic geology, soils, soil analysis, trace evidence, high-performance liquid chromatography, ion chromatography, low explosives

Traditional forensic soil comparisons often use a physical and microscopic examination of color, texture, density gradient, and mineralogical content, followed by a determination of particle size distribution and additional mineral identification using polarized light microscopy (PLM) (1–7). The inorganic mineralogical and/ or chemical composition is then confirmed by spectroscopy, including X-ray diffraction (XRD) and either energy or wavelengthdispersive spectroscopy (EDS or WDS), or by bulk chemical analysis via X-ray fluorescence (XRF) (7–13). Although these are useful methods for discrimination of soil samples, they primarily target the inorganic minerals in soil while organic- and water-soluble constituents are largely neglected. Chromatographic methods may provide additional discrimination; the compounds examined by these methods (humic materials and organic/inorganic contaminants) are independent variables not examined using traditional methods. If such discrimination is possible, analyses of this type could also be used as screening tools for soils, providing a relatively quick and simple method for eliminating soils from different sources while avoiding a lengthy mineralogical analysis that requires specialized experience.

One chromatographic method that has been used in the past to examine soil extracts is reverse-phase high-performance liquid chromatography (HPLC) (14–18). Research has shown that this technique is capable of separating various fractions of soil and differentiating samples, qualitatively and/or quantitatively,

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depending on the extraction method used. The incorporation of absorbance ratios into this technique has also been examined (14,17). Reuland and Trinler (15) analyzed acetonitrile extracts of soils from eight different locations and found that all samples could be distinguished either qualitatively (based on number and location of peaks) or quantitatively (based on relative peak intensities). This study also reported that samples taken 1 m apart were both qualitatively and quantitatively similar, while those taken 3 m apart were only qualitatively similar; samples taken from the same location over a 7-week period showed no qualitative or quantitative differences.

Ion chromatography (IC) has been extensively used in the analysis of inorganic anions in explosive residues and drinking water, but the application of this technique to forensic soil comparisons has not been reported.

The purpose of this study was to complete an extensive evaluation of the discriminatory power of HPLC and IC as applied to forensic soil comparisons. Methods for both techniques were developed and tested to provide optimal differentiation while using minimal sample. The results were analyzed both qualitatively and quantitatively to assess the evidentiary significance if these methods were to be used on case samples. The spatial and temporal variation at several locations was examined to assess the importance of representative sampling and/or time-of-collection in casework.

Methods

Soil samples were collected over the course of 3 weeks from 120 locations within a 10-mile radius of Lansing, MI, and were designated as urban ($n = 40$), suburban ($n = 40$), or rural ($n = 40$).

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inated by sands, loamy sands, and sandy loams, with minor clay loam, silt loam, and muck soils. Criteria used to categorize each sample included the local human population, the amount of vehicle and pedestrian traffic, the distance from residential or commercial structures, and the general use of the land as commercial (urban), residential (suburban), or agricultural (rural). Additional samples from six of these locations (two urban, two suburban, and two rural) were collected once a week for 10 weeks for temporal analysis. Nine additional samples, equally spaced over a 1 m^2 grid, from these same six locations were collected for spatial analyses.

All samples were collected using a #9 soil plug to a depth of about 1 in. five times and stored in brown paper bags. Each sample was placed in a glass petri dish, dried in a 60[°]C oven for 2 h, and sieved through a $60/250$ mesh/ μ m Tyler-certified brass sieve. The fraction that passed through the sieve was stored in a vial for analysis.

The Dionex HPLC system (Dionex Corporation, Sunnyvale, CA) consisted of a P680 pump with an ASI-100 autosampler and a UVD340U diode array detector. The columns used were a Phenomenex Widepore C18 guard column (Phenomenex USA, Torrance, CA) (4 mm $L \times 2$ mm D) and an Alltech Widepore Econosphere C18 column (W. R. Brace & Co., Columbia, MD) (5 µm particle size, 250 mm \times 4.6 mm). Various combinations of extract concentrations, mobile phase compositions, and run times were tested to determine the optimal sample preparation method and system parameters to achieve sufficient peak resolution.

All samples were prepared for HPLC analysis using a 0.2 g/mL solution of sieved soil in acetonitrile. The solution was sonicated for 10 min and filtered consecutively through 0.45 and $0.2 \mu m$ syringe filters (Pall Corporation, East Hills, NY). The filtrate was evaporated to dryness, resolvated in HPLC-grade acetonitrile (J. T. Baker, Phillipsburg, NJ) to make a 1 g/mL solution, sonicated for an additional 10 min, and transferred to 2 mL HPLC vials containing $350 \mu L$ sample tubes. The mobile phase used was 65:35 HPLC-grade acetonitrile:reagent grade water (NERL, East Providence, RI). The sample run time was 100 min at a flow rate of 1 mL/min and a 10-µL injection volume. The guard column filter was changed after every 10–15 samples to maintain a functional system pressure.

A function verification standard containing either uracil (0.05 mg/mL), phenol (0.7 mg/mL), and N, N-diethyl-m-toluamide (6 μ L/mL) or uracil (0.015 mg/mL), phenol (0.7 mg/mL), N, Ndiethyl-m-toluamide (0.6 mg/mL), and toluene (4 mg/mL) was used daily to verify precision.

IC samples were run on a Dionex DX-120 ion chromatograph with a Dionex AS40 autosampler and an electrochemical detector. Samples were analyzed on two different columns and by two separate methods. The first method utilized an IonPac AS9-HC $(4 \text{ mm} \times 250 \text{ mm})$ column (Dionex) with a mobile phase of 9 mM Na₂CO₃ at a flow rate of 1.19 mL/min for 35 min and a 25-µL injection volume to detect and quantitate nitrite, bromide, chlorate, nitrate, phosphate, and sulfate. The second method utilized an AS16 (4 mm \times 250 mm) column with a mobile phase of 35 mM NaOH at a flow rate of 1.19 mL/min for 35 min and a 25-µL injection volume to detect and quantitate perchlorate, thiosulfate, and chlorate.

All samples were prepared for IC analysis using no less than 0.5 g of sieved soil in a 0.5 g/mL solution in reagent-grade water. This solution was sonicated for 10 min and filtered through a $0.45 \,\mu m$ syringe filter into two $0.5 \,\text{m}$ L IC autosampler vials. The daily standards for the two methods were one containing fluoride (1.7 mg/L), chloride (2.5 mg/L), nitrite (8.3 mg/L), bromide (8.3 mg/L), nitrate (8.3 mg/L), chlorate (8.3 mg/L), phosphate (12.5 mg/L), and sulfate (12.5 mg/L) prepared from Dionex Part No. 56933 and one containing chloride (8.3 mg/L), sulfate (5 mg/ L), thiosulfate (10 mg/L), iodide (20 mg/L), thiocyanate (20 mg/ L), and perchlorate (30 mg/L) prepared in the laboratory. The detection limits for the methods were experimentally determined to be 0.02 mg/L (nitrite), 0.05 mg/L (bromide), 0.02 mg/L (nitrate), 0.04 mg/L (chlorate), 0.31 mg/L (phosphate), 0.02 mg/L (sulfate), 0.07 mg/L (perchlorate), and 0.02 mg/L (thiosulfate).

The resulting chromatograms were examined and differentiated on a qualitative basis, visually comparing the number and location of constituents in HPLC and the overall anion composition in IC. Samples within these groups were then examined on a quantitative basis, statistically comparing the relative ratios of components by HPLC and the anion concentrations by IC. If visual examination revealed obvious differences in ratios and/or concentrations, Analyse-It (Analyse-It Software Ltd., Leeds, U.K.) was used to determine whether the noted differences were statistically significant. This program generates box plots that graphically show the median, upper and lower quartiles, inter-quartile ranges (IQRs; middle half of the data), and overall spread for parametric and nonparametric statistics; the organic component ratios and inorganic anion concentrations in soil are nonparametric variables. Outlying observations are identified by the program as either "near" (between 1.5 and 3.0 IQRs from the upper or lower quartile) or "far" (more than 3.0 IQRs from the upper or lower quartile) outliers; soil samples identified as far outliers were considered to be statistically different. When variation in component ratios or amounts by visual inspection could be quantitatively confirmed as being statistically significant, samples were said to be differentiated.

Results and Discussion

HPLC Analysis

Preliminary qualitative examination of the HPLC chromatograms separated the sample population into two clearly distinct classes based on the number of components (Fig. 1). Further analysis of the number, location, and relative ratios of constituents separated the population into 10 distinguishable classes; the largest contained 82 samples and four classes contained one sample (Table 1). It was also noted that every sample contained five of the same major components in the same relative ratios to each other with retention times of 2.0, 11.4, 21.6, 40.9, and 78.4 min. Therefore, in this particular sample population, these components were not independently used as discriminating factors, only as reference points for the comparison of other, less common, components. Given the prevalence of these components in the sample population, the absence of one or more of them in a questioned or known sample would be significant.

Groups that appeared to be differentiated based on the relative ratio of two components were statistically analyzed to determine whether the variation was significant. For example, samples in Groups 1 and 2 were visually differentiated based on the ratio of the components at 2.0 and 5.4 min (Fig. 2). Statistical analysis verified this conclusion (Fig. 3). This plot shows the distribution of the value of this ratio in Groups 1 and 2. The solid-line boxes enclose the IQR (middle half) of the data, the top horizontal solid lines designate the upper quartiles, the bottom horizontal solid lines designate the lower quartiles, the dotted horizontal lines

FIG. 1—High-performance liquid chromatograms of two samples showing qualitative differences in the number and location of components.

represent the medians of the data, and the dotted vertical lines represent the spreads of the data from the lowest to the highest values within 1.5 IQRs of the upper and lower quartiles. This comparison clearly shows that the ratio of these two components is statistically different between Groups 1 and 2, i.e., there is no overlap in the distribution of the data.

The four samples separated into classes of one were qualitatively similar to nine samples in Group 4, but each appeared to have at least one significantly different component ratio (Fig. 4). In this example, the components eluting at 11.2 and 18.0 min appear to have different ratios and subsequent statistical analysis confirmed that the difference was significant. The other three individualized samples were distinguished in the same manner.

Examination of the HPLC results indicates that samples taken from urban locations have a more complex organic composition and exhibit more variability in the relative amounts of these components. Whereas samples taken from suburban and rural locations are both present in three of the 10 distinguishable groups,

TABLE 1—Number of samples from three location types (urban, suburban, rural) in each of 10 distinguishable classes as determined by high-performance liquid chromatography (HPLC) analysis.

Group	Number of Samples					
	Urban	Suburban	Rural	Total		
	14	37	31	82		
			n	13		
			റ	2		
				Q		
				າ		
h						
8						
10						
Total	40	40	40	120		

samples from urban locations are part of eight groups, including three of the four groups containing only a single sample. This is understandable, given the increased potential for contaminant transport to and from areas of this type.

IC Analysis

Qualitative examination of the overall anion composition including nitrite, bromide, nitrate, phosphate, sulfate, thiosulfate, and perchlorate separated the sample population into 13 distinguishable groups. Subsequent quantitative analysis further differentiated four of these groups, yielding a total of 23 groups, the largest containing 55 samples and 12 containing only a single sample (Table 2).

Samples that appeared to be differentiable based on the concentrations of various anions were statistically analyzed to determine whether the variations were significant. For example, within Group 2, sample U2 appeared to have a much greater concentration of nitrate than the rest of the samples with this particular anion composition (Fig. 5). Quantitative analysis of the actual concentrations of nitrate within Group 2 showed the difference to be significant (Fig. 6). Sample U2 is represented by the circle and, as in Fig. 5, this indicates that this particular value is a statistical far outlier to the population. Another sample represented by the " $+$ " symbol, U9, is a near outlier to the population but not considered to be differentiated from other samples in Group 2 according to the criteria of this study. Groups 1, 8, and 11 were quantitatively subdivided in the same manner based on significant differences in anion concentrations (i.e., nitrite, nitrate, and/or sulfate).

Generally, when examining the distribution of the anion concentrations, the samples taken from urban locations had the widest range of concentration values. This greater heterogeneity among urban samples is similar to the HPLC results. These locations were expected to have more variability in composition due to increased human activity.

FIG. 2—High-performance liquid chromatograms of samples from Groups 1 (top) and 2 (bottom) showing qualitative similarities with differences in the ratio of the components at 5.4 and 2.0 min.

The analysis of soil using a combination of both IC and HPLC provided greater discrimination than was possible using only one of the two methods. Whereas the 120 samples were separated into

FIG. 3—Boxplot comparison showing the distribution of the value of the ratio of the components at 5.4 and 2.0 min in Groups 1 and 2.

10 groups by HPLC and 23 groups by IC, the samples were separated into 40 groups when the results of both methods were taken into account. The largest of these groups contained 46 samples. Twenty-six of these groups contained only one sample, demonstrating the discriminating power of the techniques.

With regard to assessing baseline levels of anions that are often associated with the use of low explosives, IC analysis proved useful. Samples in this study exhibited nitrate concentrations up to 134 mg/L with an average of 15 mg/L and perchlorate concentrations up to 0.67 mg/L with an average of 0.34 mg/L. This indicates that the presence of these anions is not necessarily indicative of a low explosive having been used in the area as certain levels of these two anions are inherent to some soils (Table 3). However, as perchlorate was only detected in 18 of 120 samples and only at very low concentrations, the presence of this particular anion is more probative than that of nitrate (present in 113 of 120 samples at much higher concentrations). Also, chlorate does not seem to be an anion inherent to mid-Michigan topsoils as it was not detected in any samples in this study; its detection would indicate some form of foreign contamination, low explosive, or otherwise.

It is important to note that seasonal de-icing of walkways and roadways may spatially and temporally affect the occurrence and concentration of bromide and chloride anions. In such cases, it may be possible to discriminate soils from areas where de-icing agents were used. However, chloride was abundant in all samples and bromide was detected in only 12 of the 234 samples, with occurrences in urban, suburban, and rural locations. Therefore, differentiation of soils on this basis was not possible with this sample set.

Spatial Analysis

Five of the six locations sampled for spatial analysis showed qualitative consistency over 1 m^2 with regard to overall composition via both HPLC and IC. One location was consistent by HPLC results but showed the presence of perchlorate in one of the

FIG. 4—High-performance liquid chromatograms of samples from Groups 4 (top) and 9 (bottom) showing qualitative similarities with differences in the ratio of the components at 11.2 and 18.0 min.

nine samples in IC. Visual inspection of the relative ratios of components in HPLC indicated that spatial differentiation was possible at three of the six locations; further statistical analysis confirmed that these quantitative differences were significant at two of the locations. Quantitative analysis of the anion concentrations indicated that one of the nine samples at each of two locations could be separated from the remaining eight samples both visually and statistically based on nitrate or sulfate concentration.

TABLE 2—Number of samples from three location types (urban, suburban, rural) in each of 23 distinguishable classes as determined by ion chromatography (IC) analysis.

	Number of Samples			
Ion Composition			Group Urban Suburban Rural	
Nitrite, nitrate, phosphate, sulfate	1a	8	24	23
	1b	2	Ω	2
	1c	8	2	\overline{c}
	1d	1	1	$\mathbf{0}$
	1e	1	Ω	$\mathbf{0}$
	1f	1	2	$\boldsymbol{0}$
Nitrite, nitrate, phosphate, sulfate, perchlorate	2a	2	9	3
	2 _b	1	Ω	$\mathbf{0}$
Nitrate, phosphate, sulfate, perchlorate	$\mathbf{3}$	3	Ω	$\mathbf{0}$
Nitrite, nitrate, phosphate, sulfate, bromide	4	Ω	1	4
Nitrite, nitrate, bromide	5	Ω	Ω	1
Nitrate, sulfate, bromide	6	0	Ω	1
Nitrate, sulfate, thiosulfate	7	1	Ω	0
Nitrite, nitrate, sulfate	8а	1	Ω	2
	8b	1	Ω	θ
	8с	0	Ω	1
	8d	1	Ω	0
Nitrite, sulfate	9	1	Ω	0
Nitrate, sulfate	10	0	0	1
Nitrite, phosphate, sulfate	11a	4	Ω	0
	11 _b	0	1	0
Nitrate, phosphate, sulfate	12	3	Ω	0
Phosphate, sulfate	13	1	Ω	Ω

These results indicate that the water- and organic-soluble components of soil can vary over a relatively small geographic area. Therefore, in forensic analyses it is important to collect and analyze a representative population whenever possible. Specifically, when collecting a known sample for comparison purposes, multiple samples from several locations at various distances from the suspected area of origin should be collected and analyzed to develop a compositional range to compare with a questioned sample. If the results from the questioned sample fall within the range of the results from the known population, the samples cannot be eliminated as having originated from the same location as the known one.

Temporal Analysis

Five of the six locations sampled for temporal analysis showed qualitative consistency over the 10-week period with regard to overall organic composition via HPLC. One location showed the progressive disappearance of a major component at 42 min. IC results showed that only one location had a qualitatively consistent anion composition over the 10 weeks of sampling; the other five locations had various anions (bromide, nitrite, phosphate, or perchlorate) that were only detected in samples from a few (one to four) of the 10 weeks. The concentrations of sulfate or nitrate differed both visually and statistically in samples from five of the six locations. HPLC analysis yielded three locations that had statistical differences in the relative ratios of components in one of the 10-weekly samples. The other three locations had small visual variations in ratios but these observations were not statistically supported.

The results of the temporal analysis indicate that, over a 10 week period, soil composition can vary significantly on both a qualitative and quantitative basis; one sample from at least half of the locations studied was differentiated from other weekly samples from the same location using either HPLC or IC. There was

FIG. 5—Ion chromatograms of samples from Groups 2a (top) and 2b (bottom) showing qualitative similarities with differences in the concentrations of nitrate.

FIG. 6—Boxplot showing the distribution of the nitrate concentration within Group 2.

temporal consistency over shorter periods of time; the organic composition at three locations was consistent over the full 10 weeks and for an average of 4 weeks at the other three locations. The inorganic composition at the six locations was also consistent for an average of 4 weeks at a time but there were many more individual samples that varied quantitatively, both in concentration and ratios of anions. The variability in these types of constituents is understandable given their water-soluble nature, possible changes in the moisture status of the soils, and microbial production and consumption of nitrate, nitrite, and sulfate anions (19). Because the soil was removed when sampled, all samples from the temporal study could not be removed from the exact same spot; therefore, some of the differences may be due to spatial heterogeneity.

The results of the temporal analysis at the six locations indicate that the time between the collection of a known sample and the suspected time of incidence is an important factor to consider when making conclusions of common origin; compositional differences could be temporally related and were not cause for ex-

TABLE 3—Concentrations of various anions as detected by ion chromatography (IC).

Ion	Number of Samples $(Pop. = 120)$	Concentration Range (mg/L)	Average Concentration (mg/L)
Nitrite	111	$0.01 - 21$	1.21
Bromide		$0.07 - 1.69$	0.61
Nitrate	113	$0.11 - 134$	15
Phosphate	110	$0.34 - 16$	4.07
Sulfate	120	1.70-1484	31
Thiosulfate		NA	7.87
Perchlorate	18	$0.14 - 67$	0.34

NA, not applicable.

clusion. As expected, the shorter the time between collection of samples, the less temporal variation existed.

Conclusions

This study shows that using HPLC and IC to analyze the often neglected organic- and water-soluble fractions of soil can successfully discriminate samples. Including quantitative analysis of the results eliminates some false inclusions by providing further differentiation of samples. To demonstrate that the variation observed via HPLC and IC analysis is an independent variable from the inorganic composition, 10 samples that were differentiated by these methods were examined via XRF. Some of the samples were broadly similar in elemental composition in a one-to-one comparison. Although this comparison was not performed with a population of known samples, the XRF data indicate that additional discrimination is possible when HPLC and IC analysis are added to traditional forensic soil analysis schemes.

The methods used in this study were able to detect both qualitative and quantitative variations in soil over a relatively small geographic area. This demonstration of soil heterogeneity underscores the importance of the collection of a representative known sample population when assessing a forensic soil comparison. Significant temporal variation was also demonstrated in this study; however, samples were found to be consistent over shorter periods of time.

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