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APPLICATION OF MICROCHEMICAL TECHNIQUES: IDENTITY OF SOIL SAMPLES*

Lauren J. Goin and Paul L. Kirk

Paul L. Kirk, Professor of Biochemistry, University of California Medical School, writes of a newly developed laboratory procedure for differentiating between soil samples in criminal investigations. Based upon specific gravity studies similar to those used in glass problems (see *38*:416 and *38*:168) the method allows for rapid determination of the relative specific gravity of two specimens. Using this method laboratory workers will be able to differentiate between different soils as accurately but more rapidly than through microscopic analysis.

Lauren J. Goin who collaborated with Prof. Kirk on this study is a graduate of the University of California in Technical Criminology.—EDITOR.

Soil, usually in the form of mud, is of frequent occurrence among articles of physical evidence used in the solution of crime. It may be recovered often from shoes, and occasionally from other clothing, tools, tires, furniture, and the like. It is accordingly important to ascertain the extent to which different soils may be distinguished from each other, or expressed differently, to what extent identity of soils may be established, and what is the significance of a demonstrated identity.

Consideration will disclose that soils show wide differences in composition, not only from widely separated points but also from points quite close together. This is expected because soil represents not only original earthy constituents derived from the parent rock of the immediate locality, but rather, the accumulation of the ages, conditioned by the operation of natural forces and the activities of living organisms over millenia. The particles of alluvial soil may be derived from an almost infinite number of sources, and since the action of water and wind would in few cases be identical over long periods of time in different spots, great variations in composition would be expected. Colluvial soil, or soil in which some movement and intermingling of parts has occurred, would be expected to be less variable, and sedentary soil least variable of all. Most human habitations occupy alluvial regions, as in valleys, and thus the most variable soil is that which is also most often encountered in criminal evidence.

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Probably as important as the variations of surface soil is the fact that soil varies rapidly with depth. The admixture of soil from below the surface with surface soil is taking place constantly in excavating for pipes, paving, basements, and in agricultural operations. Thus, extreme surface variations may arise locally due to admixture with subsurface soil of the same region. Addition of fertilizers and soil conditioning materials and of human, animal, and plant wastes would cause further variations of a local nature.

Soil has been most studied by agriculturists, or from the standpoint of the relation of soil to agriculture. Its value in criminal investigation is mentioned in several standard references, but systematic methods for its identification appear to be lacking. This is at least partially due to the fact that the analytical methods of the agricultural analyst require large samples, and are usually concerned with elementary composition, particularly with regard to elements such as nitrogen or phosphorus which are of interest in determining fertility. Mechanical analysis for the distribution of particle sizes is also frequently employed and requires larger samples than are usually found in evidence. In view of its importance to the criminologist, some review of the origin and nature of the particles which make up common soils is given here. The information is compiled largely from more extensive treatments found in such references as that of Hilgard (1), Lyon and Buckman (2), and Robinson (3).

The basic components of soil originate primarily from mechanical and chemical decomposition of igneous, metamorphic, and sedimentary rocks. Since rocks are almost infinitely variable in composition, containing usually many different minerals, even sedentary soil formed from them will have a considerable area variation, but much less local variation. As the decomposed rock particles are moved by gravity forming colluvial soil, further changes occur. As the soil particles are washed, blown, or moved by gravity to the lowlands and become alluvial soil, still further mixing and alteration occur. The chief minerals which give rise to soil and may be found as soil constituents are listed below.

PRIMARY MINERALS

Included in this group are a wide variety of undecomposed rock fragments ranging from stones down through pebbles, sand, and silt. While almost any rock material may be included in this category, the most important constituents are probably:

1. *Quartz* (*Silica*). Quartz sand is an almost universal component of soil, being most prominent in the sandy soils. It is recognized by its hardness, translucency, and the presence of concholdal fractures. It originates primarily from igneous rock but much of the soil quartz is contributed directly by metamorphic and sedimentary rock (sandstone). Pure quartz is clear and transparent, but due to the presence of impurities it may be white or a variety of colors. Differences of hydration and crystalline form allow recognition of various quartz minerals, e.g. opal, tripolite, jasper, and quartz.

2. *Calcite* (*Limestone*). This mineral occurs widely but particularly in calcereous soils. It is white and reacts with acids with evolution of carbon dioxide gas.

3. *Dolomite.* This material is similar to calcite except that a portion of the calcium is substituted by magnesium. Its occurrence and properties are similar to those of calcite.

4. *Mica.* At least three varieties of mica are found widely distributed through soils; (1) Muscovite, the common light colored mica; (2) Lepidolite; and (3) Biotite, also common, and usually a dark bottle green. Mica contributes little or nothing to the useful properties of soil, but its abundance and distinctive appearance (shiny scales) make it of great utility in identifying soil.

5. *Feldspars.* A great variety of feldspars are recognized, of which orthoclase, microcline, albite, oligoclase, labradorite, and anorthite are the best characterized. The feldspars include the aluminosilicates of sodium, potassium, and calcium. Their decomposition gives rise to clay along with more or less soluble salts of the metals named. For this reason feldspars are among the most important soil-forming minerals. The amounts of undecomposed feldspars found in a soil sample may be very small, however, due to the relative ease of decomposition of these minerals.

6. *Hornblende and Pyroxene* (*Augite*). These are two very similar crystalline minerals of wide distribution. They differ chiefly in their crystal form, both being complex materials containing calcium, sodium, magnesium, iron, aluminum, silicon, oxygen, and hydrogen. Black hornblende is the chief black component of rock and soil. Its color is due to its considerable iron content. Neither mineral has a definite composition but varies quite widely in the proportions of the various elementary constituents.

7. *Minor constituents.* Other primary minerals of rather

wide distribution are gypsum, talc, kaolinite, limonite, serpentine, and magnetite. In addition to these not uncommon materials some fifty other minerals may at times be found in soil. Some of these will be quite local in their distribution, such as cinnabar, the common ore of mercury, pyrites, etc.

CLAY MINERALS.

Clays are exclusively the products of decomposition of primary minerals such as feldspars. Pure clay is considered by mineralogists to be hydrated aluminum silicate. However, both the degree of hydration and the presence of many possible contaminants cause wide variations in clay, both in its physical behavior and in its appearance. Clays will be found in nearly all soils and is the major constituent of most heavy soils. It imparts to a soil cohesiveness and plasticity, and becomes hard and adherent on heating.

The color of clay soil varies from white to black through red, yellow, green, or blue, depending on the nature of the admixed impurities. Clay apparently arises primarily from decomposition of feldspars, hornblende, and other rocks, but the clay of soil may have originated from a wide variety of metmorphic and sedimentary rocks. The characteristics of the clay are largely a function of the moisture to which the rock was exposed during its disintegration. In most soils a number of the clay minerals will co-exist.

ORGANIC CONSTITUENTS

Organic matter, though not mineral in nature, is one of the most variable of all soil constituents and is of peculiar importance in the identification of soils. Primary soils will be almost or completely devoid of organic matter. This the soil will accumulate slowly as vegetation becomes established, and the soil micro-organisms, worms, and insects gradually invade the region. Agricultural land is likely to be particularly rich in organic constituents both from growth occurring on the land and from added materials such as manure, peat, and cover crops. Richest of all are the peat and muck soils which have been formed primarily from the constant decay of organic matter and contain only a small amount of residual minerals deposited mostly by flooding.

In the soil organic matter is degraded by natural processes into a mixture of compounds collectively known as humus, whose chemical ·omposition is largely unknown. This is recognized as a black partly decomposed residue which is an intimate constituent, not merely an admixture with soil minerals. In fact it is well known that humus is bound by some means to mineral particles, coating them tightly and being removed from them with considerable difficulty. Humus constitutes the most important black coloring matter of soil. It alters texture markedly, making clay soil less cohesive and sandy soil more so. From the standpoint of the present investigation, its most important effect is on the density of the soil, which diminishes in proportion to its organic content, since organic matter is lighter than any of the soil minerals.

It is apparent that the elucidation of the complete composition of a soil is a prohibitively difficult task which has rarely been accomplished even for research purposes. For identification it is never necessary that all constituents be identified as such or that they be separated. Any method which quantitatively distinguishes particles of characteristic appearance or properties will be successful in proving identity or nonidentity, depending on whether the distributions found in two soils are the same or different.

One of the authors (P. L. K.) has previously used soil examination as a means of proving commission of crime. In a case of child rape, a piece of dried mud was found on the child victim's bed. This was shown to agree in distribution of microscopically identifyable particles with mud removed from a suspect's shoe. The shoe contained also other mud which was shown to be completely different in the distribution of different types of soil particles. The method was used in various other cases of burglary and similar crimes in which mud samples were found on shoes. Determination of identity by this means was laborious because it involved extended counting of distinguishable particles under the microscope, in fields defined by an ocular grid micrometer. It suffered also from the difficulty of clean-cut distinction between certain similar but not identical appearing particles.

In connection with the investigation of a crime in which soil constituted important evidence, the method described here for proving identity or non-identity of soil samples was developed. It proved to be reasonably rapid, requiring only a few hours for completion, and to be capable of distinguishing between soils taken from contiguous regions, sometimes as little as a few inches apart. The method is indeed so sensitive to small changes in soil composition that the most serious problem in applying it is to obtain a standard soil from the exact spot of origin of the questioned sample. When this is not possible, it may still be practical by studying enough samples from the region, to localize the questioned soil within a few feet or inches of its origin. The proof of identity or non-identity of two soil samples is achieved by a careful study of the density distribution of the soil constituents when suspended in a series of liquids of different densities.

EXPERIMENTAL

The soil sample to be studied should weigh at least 25 mg. if possible, though smaller quantities down to about 5 mg. may be used. Larger samples offer no advantage except more uniform sampling. It is apparent that there is a relationship between the size of sample and the uniformity of sampling. In fact, a large enough sample properly obtained might be representative of the average composition of a rather large area. It is here desired, however, to obtain a sample representative only of the small region in question, and a small sample may very well be representative under these circumstances. For example, mud on a shoe certainly represents the surface mud of the footprint at which the sample was acquired.

The soil was prepared for study by disaggregating it as thoroughly as possible with a rubber stopper on a clean firm surface. It should not be ground in a mortar, since this crushes the small rock constituents which are normal to the soil in their original form. It was then sifted through a clean 100-mesh sieve and collected on a clean paper. The particles which would not pass the sieve were examined under low magnification, and if aggregates of small particles remained, these were broken and passed through the sieve. The sifted soil sample was weighed, and the same weight of standard samples prepared in identical manner were taken for comparison. A series of six or seven soils could be run simultaneously. This series might include two samples of the same original soil to prove the uniformity of sampling, the questioned soil, and other comparison soils.

Long glass test tubes were constructed, 15 to 18 inches in length and from 5 to 10 mm. outside diameter, uniform in a series. Suitable sizes of tubes for different sized samples are as follows:

50 - 80 mg.	samples	—	10 mm.	O.D.	tubing
30 - 50 mg.	"	—	8 mm.	"	"
10 - 30 mg.	"	—	6 mm.	"	"
(less than) 10 mg.	"	—	5 mm.	"	"

These were used as containers for the density distribution measurements.

A series of stock solutions of different known densities was prepared by mixing varying amounts of two or more organic liquids which were heavier than water. At various times, methylene iodide (d. — 3.325), bromoform (d. — 2.890), iodobenzene (d. — 1.332), bromobenzene (d. — 1.499), 2-chlornaphthalene (d. — 1.194), and nitrobenzene (d. — 1.158) were used. Due to the high cost of methylene iodide, and to the fact that only two liquids are actually necessary to prepare an entire tube, later runs were made with bromoform and bromobenzene mixtures only. It was convenient to recover liquids after use, but not to fractionate them into pure components. Many of the soil comparisons were accordingly made with filtered and distilled but impure liquids, and the actual density of the mixture in each case was found with a pycnometer (4). Such solutions did not have a predetermined simple density value but could be made variable in steps which were suitable for good comparison. A simple method of obtaining satisfactory stock solutions of reasonably well known density was to mix bromoform and bromobenzene in the proportions shown in Table I. The amount of each solution should be sufficient for at least all the tubes of a series. The amount of each solutions per tube is 0.6 to 1 ml. depending on the diameter of tube used. Thus, if seven soils are to be compared, a minimum of 4.2 to 7 ml. of each stock solution should be prepared in the ratios shown in Table I. Preparation of larger amounts of stock solutions is advisable if a considerable number of soils are to be compared, because it allows a completely valid comparison and the solutions are stable.

Table I

STOCK SOLUTIONS OF KNOWN DENSITY

Density	Bromoform ml.	Bromobenzene ml.
2.890	1.00	0
2.800	1.00	0.069
2.700	1.00	0.158
2.600	1.00	0.263
2.500	1.00	0.390
2.300	1.00	0.736
2.000	1.00	1.506
1.499	0	1.00

The reason for the choice of the range of densities given is shown by an examination of Table II which shows the densities of the common soil minerals (5). It must be remembered that most of them will appear lighter than the values shown because of the presence of humus on the particles. The proportion of humus is particularly significant since it is one of the most

Table II

DENSITY AND COMPOSITION OF MINERAL CONSTITUENTS OF SOIL

Substance	Formula	Density
Quartz		
Opal	SiO_2nH_2O	1.9–2.3
Tripolite	$SiO_2 \cdot nH_2O$	2.1–2.3
Jasper	SiO_2	2.6
Quartz	SiO_2	2.6
Feldspars		
Orthoclase	$KAlSi_3O_8$	2.4–2.6
Microcline	$KAlSi_3O_8$	2.5
Albite	$NaAlSi_3O_8$	2.6
Oligoclase	$NaAlSi_3O_8 + CaAl_2Si_2O_8$	2.6
Labradorite	$CaAl_2Si_2O_8 + NaAlSi_3O_8$	2.7
Anorthite	$CaAl_2Si_2O_8$	2.7
Micas		
Muscovite	$H_2KAl_3(SiO_4)_3$	2.7–3.0
Lepidolite	$K_2Li_3Al_3(Si_3AlO_{10})_2(OH,F)_4$	2.9
Biotite	$K(Mg,Fe)_3Si_3AlO_{10}(OH)_2$	2.7–3.1
Hornblende	$Ca_2Na_{0-1}(Mg,Fe)_4(Al,Fe)(Si,Al)_8O_{22}(OH)_2$	2.9–3.4
Augite	$Ca(Mg,Fe,Al)(Si,Al)_2O_6$	3.3
Gypsum	$CaSO_4 \cdot 2H_2O$	2.3
Talc	$H_2Mg_2(SiO_3)_4$	2.8
Kaolinite (clay)	$H_4Al_2Si_2O_9$	2.6
Limonite	$Fe_2O_3 \cdot nH_2O$	3.6
Serpentine	$H_4Mg_3Si_2O_9$	2.6
Magnetite	Fe_3O_4	5.2
Calcite	$CaCO_3$	2.7
Dolomite	$CaMg(CO_3)_2$	2.9
Hematite	Fe_2O_3	5.0

variable of all the soil constituents, ranging from zero up to very high values. The density of this organic matter itself is so low that it would be expected to float on the lightest liquid used. Very little such floating material has been encountered. Apparently, nearly all the organic matter which passes a 100 mesh sieve is tightly bound to the soil minerals.

It will be noted that only a few of the common soil constituents are heavier than the heaviest fraction of liquid. In practice, it is uncommon to observe any appreciable fraction of the soil sink in the heaviest liquid, and any soil in which this occurred would be considered unusual. Many minerals are found in the earth's crust with heavier density, but these apparently occur in restricted regions only, or they are very minor constituents of ordinary soil.

Soil comparisons were carried out as follows: Into each tube of a series was placed a sample, all samples having been weighed and adjusted to the same value. The series of tubes were set up together and behind them was placed a cardboard with a series of parallel, equally spaced, horizontal ruled lines, 1 to 1½ inches apart. The heaviest stock solution was added to each tube up to the position of the lowest ruled line. All, or nearly all of the soil floated in every case. To each tube was then added the next heaviest liquid up to the level of the second ruled line. This addition was made slowly and carefully with a pipet so as to avoid mixing the solutions. These operations were repeated with all the liquids and each level, allowing a few minutes after each addition for the soil to distribute between the layers. After the addition of the last light layer, one hour was allowed to elapse for final equilibrium to be reached, after which a detailed examination of the tubes could be undertaken. At this time the series could be photographed by placing a fluorescent lamp and ground glass (or thin paper) behind the tubes and exposing against the lighted background. Soils taken from the same original sample invariably gave identical distributions at the solution interfaces.

The greatest care was necessary to flow each solution on top of the last one, thus giving a stepwise density gradient with the soil particles grouped at the solution boundaries. In case the soil sample was too large for the size of tube chosen, the soil would pack at the interface and equilibrium was not reached in a reasonable time. When this happened, a fine glass thread was inserted and rotated in the soil layer to break it up before the next layer was added. This situation did not occur when the size of sample and the tube diameter were in the proper relationship.

After standing for several hours, the liquid layers gradually diffused into each other with establishment of a more or less uniform density gradient. The soil layers also redistributed themselves more or less continuously throughout the tube according to the density of the individual particles. At this stage, visual examination showed variations with more reliability than before the layers were diffused. For photographic reproduction, tubes in this state were less satisfactory because the soil was distributed more and did not show on the finished picture as well. In general, examination at 1 hour and at 7 to 24 hour periods should be made.

RESULTS

It was originally intended to use the stepwise density gradient tube for primary separation of soil fractions, each of which would later be examined microscopically or by other means. It became apparent early in the investigation, however, that the separation itself was as sensitive a method of soil comparison as was necessary, without further examination. The difficult problem became one of sampling comparison soils closely enough to the possible point of origin of a questioned soil to obtain agreement. The results of the investigation will be presented in logical, rather than chronological sequence.

Soils were obtained from the Soil Science Division of the University of California College of Agriculture and separated in stepwise density gradient tubes as follows:

1. Highly micaceous sand from Hermit Valley, Tajunga, Riverside, California.
2. Humus free, silty clay from Sonoma County, California.
3. Humus heavy, silty clay from the Stockton Delta region, California.
4. Red silty clay from 10 miles S. W. of Fresno, California.
5. Same as 4. Used for control.
6. Calcereous soil from Calexico, Imperial Valley, Mexico.
7. Colluvial Coast Range soil, from hills back of Berkeley, California.
8. Alluvial garden soil from Berkeley, California.

The appearance of these soils when separated is shown in Figure 1. The wide differences in composition are shown by the intensity of variations of different soils at the same density level. Tubes 4. and 5. show complete identity as would be expected. The height at which the majority of the soil separates is determined very largely by the organic content, modified by the density variations of the mineral constituents. However, variations between corresponding levels are always definite. It should be remembered that many of the layers showed significant amounts of soil which were still too small to register well in photographs. The appearance of the tubes after 11 hours standing is shown in Figure 2.

A test of the quantitative sensitivity of the method was performed by choosing two soils which were markedly dissimilar, viz. above samples 1 and 3 and preparing variable mixtures of the two in known amounts. The tubes, numbered as shown were arranged to contain in the order given:

14. Pure stock 1.
15. Stock 1 plus 1% by weight of stock 3.

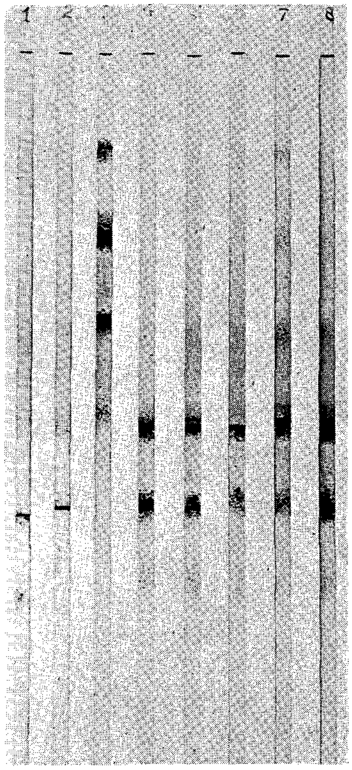

Figure 1.
Soils from
Widely Different Areas.
1 hour standing

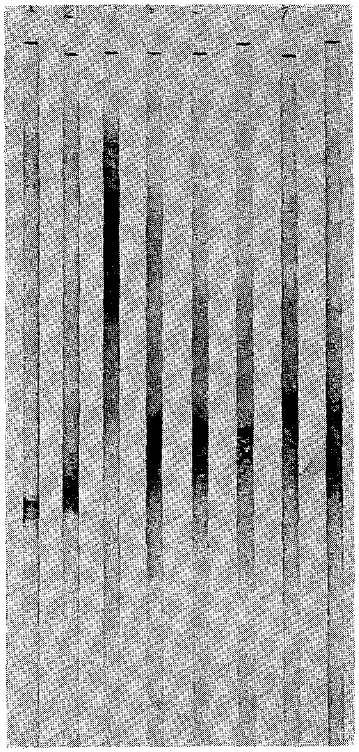

Figure 2.
Soils from
Widely Different Areas.
11 hours standing

16. Stock 1 plus 10% by weight of stock 3.
17. Stock 3 plus 10% by weight of stock 1.
18. Stock 3 plus 1% by weight of stock 1.
19. Pure stock 3.

The appearance of the tubes is shown in Figure 3. No difficulty was encountered in distinguishing all tubes from each other with the exception of 18 and 19. One percent of the heavy soil added to 99% of light soil changed the volume relationships of these soils inappreciably, and since it is the visible volume that is distinguished, no definite distinction could be seen. However, 1% of light soil added to 99% of heavy, made a very definite difference which was seen quite easily.

One additional general test was applied to determine the practical sensitivity of the method. Five soil samples were taken, one from under each of five windows of the same Berkeley residence. All were garden soil of similar appearance and texture. They were separated in stepwise gradient tubes as described,

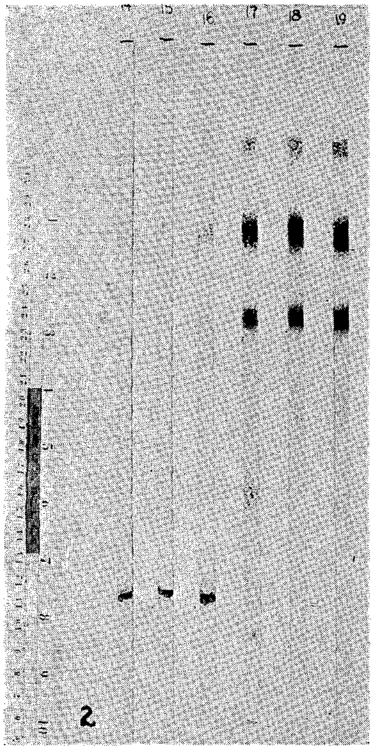

Figure 3.
Quantitative Comparison
of Mixed Soils.

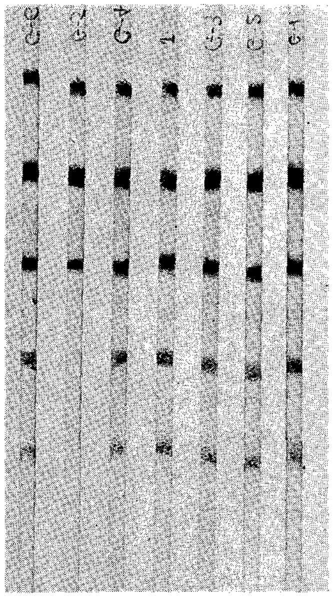

Figure 4.
Comparison of Soils
from Grave.

and each could be differentiated from all of the others. Those taken from flower beds were more similar than two which were not under so intensive cultivation. However, the ability to distinguish soils with this sensitivity was considered very significant.

The technique described was used in two criminal cases, and the evidence obtained was presented in court, accepted, and led to convictions in each instance. The first case concerned the burial of a murdered child's body. A shallow grave was dug in cultivated soil, presumably with a tool found in the possession of one of the suspects. The soil on the tool weighed 42 mg. It was separated by layers in the density tube in comparison with six samples of soil taken symmetrically from the grave, in a region about 2 by 4 ft. The surprising result was that each of the six samples was distinguishable from all of the others, and from the questioned soil. With one exception, all these samples were quite similar to each other and to the questioned soil. The latter varied less from the mean of the 5 similar samples than

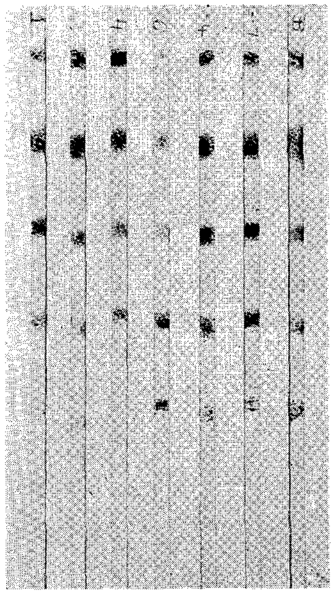

Figure 5.
Comparison of Soils
from a Locality.
1 hour standing

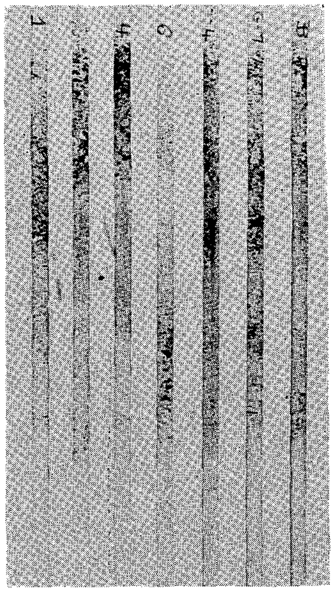

Figure 6.
Comparison of Soils
from a Locality.
41 hours standing

they did from each other. Figure 4 shows the photographic record of this comparison. The sample labelled "J" is the questioned soil, those labelled G1, G2, G3, G4, G5 and G6 being the comparison soils from the grave. In order to establish the variability of these soils as compared with other soil from the district, eight samples were taken in the neighborhood, some close to the grave and some as far as 10 miles in various directions. A portion of these were eliminated on the basis of their appearance and texture alone, and the remainder which appeared similar to samples from the grave were compared in density tubes with two such samples and with one ("B") which was removed from the bed spread in which the body was wrapped. The comparison of these soils is shown photographically in Figures 5 and 6. Although all soils were taken in a broad agricultural valley and in a region considered by soil scientists to have the same type of soil, it is apparent that the differences are very considerable between every sample except 1 and the grave samples, and between all of the comparison soils.

One additional criminal case involving an attack and rape was solved largely on the basis of soil comparison by this method. Fresh soil found on the shoe of the suspect was shown to match almost exactly fresh soil on the stocking of the victim. Both were very similar but not absolutely identical with soil from the scene of the crime, and different from several other comparison soils taken at distances from a few feet up to about 100 feet from the scene of the crime. In this case, the validity of sampling of the comparison soils was again the critical point, since it was impossible to determine within a few feet the spot from which the questioned soils could have been acquired during the crime. The sensitiveness of the procedure is such that a variation of a few feet may well give rise to soil differences sufficient to prevent the establishment of identity. In this instance, the fact of identity between the soil on the clothing of both victim and suspect carried much more weight than the near identity with soil from the scene of the crime.

Of the 41 samples of soil which have been examined by this procedure, in one case only was there an absolute identity of soils coming from two different samples. In this instance, both soils came from a park, some 60 ft. apart. It is probable that in gardening operations some transfer of top soil was made from one point to another, or that soil brought in from elsewhere was placed in both areas. However, no proof of this could be obtained. If such was not the case, it would indicate one possible exception in 41 cases. It is to be noted that with the exception of the tubes shown in Figures 1, 2, and 3, no comparisons of widely different soils as understood by agricultural experts have been made. In all other instances the soils which were compared were very similar as commonly understood. Thus, the method received a more critical test than would be true otherwise.

It should be clearly understood that refinements of this technique are easily possible, and extensions of the technique may be readily added. For example, the number of layers into which the soil is divided is entirely arbitrary. Instead of using 8 layers as reported here, the investigator could employ 10 or 20 if desired to break the soil into more fractions, and the density differences between successive layers can be made as small as desired. We believe that 8 to 10 layers are ample, since any finer differentiation merely makes the sampling problem more acute. By means of a pipet and suction line, it is possible to remove each layer separately after distributing the layers, and to expose the soil to any further study desired, either physical, chemical, or microscopic. This also is believed unnecessary in most instances, but a case might arise in which such extension of the investigation would be highly desirable. The technique is well adapted to any such procedure, since it does not destroy or alter the soil, but merely segregates its components on the basis of density differences.

SUMMARY

A simple and sensitive method is described for the comparison of density distribution of small soil samples as a means of determining identity or non-identity.

It is shown that even very similar soils can be differentiated and that the differences may be demonstrated in soils taken in very close proximity.

The successful use of the method in two criminal prosecutions is described.

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