Z Rechtsmed (1984) 93:71–77

Zeitschrift für
Rechtsmedizin

Evaluation of Microbial Soil Identity in Forensic Science

P. J. Van Dijck and H. van de Voorde

Catholic University of Leuven, Faculty of Medicine, School of Public Health, Department of Forensic Medicine, Vital Decosterstraat 102, B-3000 Leuven, Belgium

Summary. Microbiologic analysis of different soil samples was undertaken to assess the value of this method for forensic purposes. Three groups of micro-organisms were isolated on selective media: bacteria, fungi, and actinomycetes. Comparison of the morphology of the colonies led to the conclusion of non identity of the soil samples under examination. The method was applied to soil traces on articles of clothing, trousers, shoes, and stockings. It was found that the soil did not originate from the suspected scene of a crime. Examination of the microbiologic soil composition does not provide an absolute certainty of identity, but it is highly reliable.

Key words: Soil microbiology – Soil identity – Soil bacteria – Soil fungi – Soil actinomycetes

Zusammenfassung. Mikrobiologische Analysen verschiedener Bodenproben wurden durchgeführt mit dem Zweck, diese Methode für gerichtliche Untersuchungen zu überprüfen. Drei Gruppen Mikroorganismen des Bodens wurden auf selektiven Nährböden isoliert: Bakterien, Pilze und Aktinomyzeten. Durch den Vergleich der Morphologie der gewachsenen Kolonien konnte die Verschiedenheit der Bodenproben herausgefunden und bewiesen werden. Diese Methode wurde für sonstige Bodenspuren, die an Kleidungsstücken klebten, erfolgreich angewendet. Es konnte aus diesen Untersuchungen geschlossen werden, daß die Erde nicht von der Stelle, an der das Verbrechen vorausgesetzt wurde, herstammte. Die Untersuchungen hinsichtlich der mikrobiologischen Zusammensetzung von Bodenproben sind nicht absolut, aber sie sind bei kritischer Beurteilung sehr zuverlässig.

Schlüsselwörter: Bodenmikrobiologie – Bodenidentität – Bodenbakterien – Bodenpilze – Bodenaktinomyzeten

Since the macroflora of natural soil varies, it may be supposed that the microflora changes at the same time. It is known that a microbial soil ecology does exist [3, 13] and that also fungi are connected to given environments [2, 14].

Offprint requests to: P. J. Van Dijck (address see above)

Lambert and Chardez [12] applied the study of the soil fauna of *Thecamoebae* to a murder case and showed the applicability of their method in soil comparison in general.

Protozoan composition of the soil was already studied for ground identification. Some years ago Thornton et al. [15] have measured enzyme activity in soils and found that it varies greatly since the micro-organisms in soils show great variations.

Kissling [11] has recently described the value of *Azotobacter* isolation from soils to prove a suspect's presence at the site of a crime. Similar problems of soil identity have often been submitted to our forensic laboratory by investigating magistrates and could be solved by the examination of the microflora of the given soil. In this study, we tried to appraise this method of identification.

Materials and Methods

Soil Samples and Treatment

Soil samples were collected in sterile plastic specimen vials (Stayne Continental, Jumet, Belgium) from private gardens. The samples were taken at soil level with the spoon provided in the vial. The plating out for isolation was done the same day or after overnight preservation at room temperature. The soil samples obtained from the investigating magistrate were not collected under standard conditions, which might have introduced errors when a blank control of the container was not possible. Soil adhering to articles of clothing was scraped with a sterile scalpel, and a clean part of the clothing was used as a control.

Preparation of Suspension for Plating

Approximately 1 g soil was weighed and suspended in pyhsiologic saline until a concentration of 10 mg/ml was obtained. The suspension was mixed for 4 min in a desintegrator (MSE England) at the highest speed and diluted twice 1/10. A volume of 0.3 ml (30 µg sample) was then streaked on the surface of three different media in triplicate using a wire fold in a straight angle, while the plate was gently rotated (Denley, England). The surface was dried in a ventilated incubator.

Media Used

For isolation of bacteria, Tryptic Soy Agar (Oxoid CM 131) was used. The fungi were isolated on Potato Dextrose Agar (Difco Labs 0013-01), to which 20 µg chloramphenicol/ml was added. The recovery of the actinomycetes was performed on a semi-synthetic medium: 10 starch (from corn), 2 $(NH_4)_2SO_4$, 1 NaCl, 1 $MgSO_4$7aq, 3 $CaCO_3$, 1 K_2HPO_4, 15 agar, in g/l tap water. Of each medium, 15 ml was used on plates of 8.6 cm diameter. The plates were incubated overnight at 37°C for the isolation of bacteria (first result) and further at 25°C until most of the colonies were pigmented. For fungi and actinomyces, 25°C was chosen as the incubation temperature, and the colony morphology became visible after 6–7 days.

Photographs

A copy of the growth was obtained by a shade image. The plates were placed on hard photographic paper (Agfa 4 Brovira speed BH310PE), illuminated for 3 s. The paper was processed in the ordinary way.

Results

Soil samples were taken from four sites separated by a few meters. Three types of microflora were isolated from eighteen samples: bacteria, fungi, and actinomycetes. The picture shows the growth of bacteria and fungi obtained from two gardens, respectively (Soil 1 and Soil 2), and of two samples of each soil (A and B). In each case the vegetation was different, the left upper plate deriving from a sample near an ash tree *(Fraxinus excelsior)* and the right upper plate deriving from a sample taken from under an ornamental shrub *(Aucuba japonica)*. This garden had a sandy soil. The colonies of bacteria and fungi were different in morphology and color, even though the two samples of this garden were taken only 5m apart. In this case, samples taken a short distance apart may be recognized by the specific microflora. The natural view of the plates showed more clearly the difference than the photograph can do (Fig. 1). The plates for actinomycetes showed too little growth of typical colonies, so that conclusions with these isolations were not possible. The plates 2A and 2B show samples from another garden, 3km distant from the first one. A difference in the two types of microflora is also visible and is most striking for bacteria.

As above, the number of colonies of actinomycetes was too small to permit valid conclusions. On other occasions, actinomycetes isolation, however, has provided good results. Some soil samples taken 60km apart contained the same fungal flora, but the bacterial population differed as well as the actinomycetes. In this kind of research, it must be recommended that the three types of microflora should be examined.

Case Report

At the request of the investigating magistrate we checked the identity of soil adhering to articles of clothing and to shoes, of dirt under the nails, and of a soil sample taken from the suspected scene of a crime. On two occasions, the results were very striking: the appearance of the cultures were very different. It could not be made out if the suspect had been at the scene of the crime.

Important Details Applying to the Method

Perfect homogenization of the soil is very important. As a control, three samples were plated out in tenfold. The appearance of the plates was not always identical for the same sample. For fungi, the genus and, if possible, the species were determined: some genera appeared only on one or two plates. The phenomenon is illustrated in Table 1. With the non-homogeneous mixture, the fungal growth differed considerably in one sample, as did the bacterial or actinomycetes isolation, although to a lesser extent.

For fungi, overgrowth of *Mucor* or *Rhizopus sp.* may cause difficulties. Daily observation generally is necessary, and invasive growth can be prevented by cutting out and burning the disturbing colonies. The method is a semi-quantitative determination, so inoculation with a needle is not satisfactory,

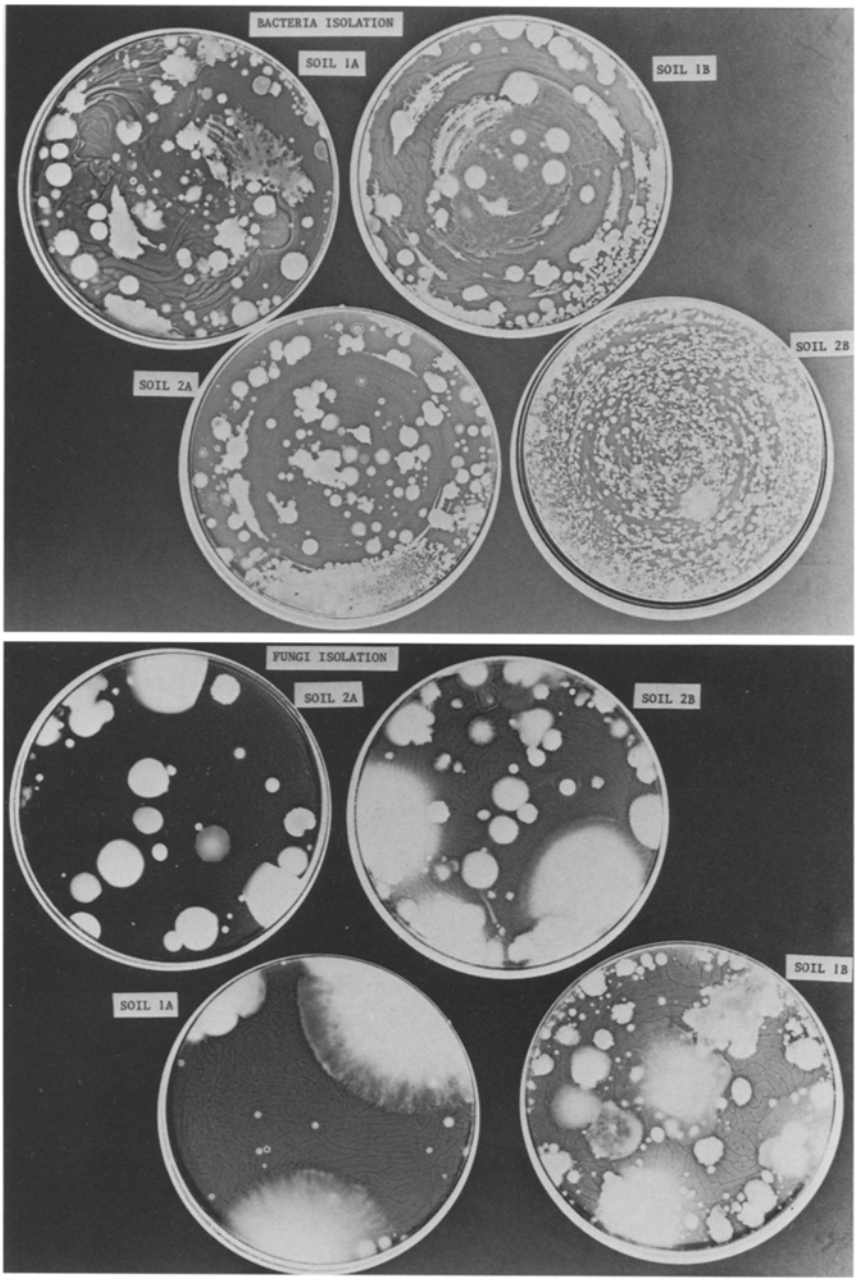

Fig. 1. Bacteria and fungi isolation of two different soil samples (gardens 1 and 2) on two sites of each soil (garden) (A and B). Growth on Tryptic Soy Agar for bacteria and Potato Dextrose Agar for fungi. Shadow picture of the colonies. The appearance of the bacterial or fungal composition is different in the eight plates (most striking for one plate of bacteria 2, B), and hence the origin of the soil samples is different

Table 1. Frequency of identified[a] fungi in one sample on ten plates

Plate no.	Soil 1				Soil 2			
	Sample A	Freq	Sample B	Freq	Sample A	Freq	Sample B	Freq
1	A fum	3	Pen	3	Pen	5	Pen	3
	Rhiz	1	Fus (1)	1	Muc (1)	1	Fus (3)	2
	Trich	1					Muc (2)	2
2	Pen	2	Fus (1)	1	Pen	3	Pen	2
	Trich	1	Fus (2)	1	Fus (2)	2	Clad	1
			Alt	2	A fum	1	Trich	1
			A fum	2				
3	A fum	4	A fum	2	Pen	5	Fus (3)	3
			Clad	2	A fum	2	Pen	4
					Fus (1)	1	Muc	1
							Ceph	2
4	Trich	1	Fus (1)	1	Pen	6	Pen	5
	Rhiz	1	Fus (2)	1	Muc (2)	1	Fus (3)	2
	Fus (2)	1	Clad	1	Clad	1	Muc (3)	2
5	A fum	3	Fus (1)	2	Fus (2)	2	Pen	5
	Trich	1	A fum	2	Pen	10	Fus (3)	3
					Clad	1		
					Trich	1		
6	A fum	1	Fus (2)	2	Muc (2)	1	Pen	2
	Muc (1)	1	Fus (1)	1	Pen	4	Fus (3)	2
			Pen	1	Clad	1	Ceph	6
					Fus	1		
7	A fum	3	Pen	5	Pen	8	Pen	4
	Rhiz	1	Fus (2)	1	Trich	1	Muc (3)	4
			A fum	1	Fus (2)	1	Horm	4
8	A fum	3	Pen	1	Pen	8	Pen	5
	Trich	1	Fus (1)	1	Muc (2)	1	Ceph	4
	Muc (1)	1	Fus (2)	1			Fus (2)	2
			Pen	1			Muc (3)	1
9	A fum	1	Pen	3	Pen	7	Pen	5
	Trich	1	Horm	1	Muc (2)	1	Ceph	3
							Muc (3)	1
							Fus (3)	1
10	Pen	4	A fum	2	Pen	8	Fus (3)	2
	Trich	1	Fus (1)	1	Fus (2)	1	A niger	1

Underlined names means that genera were only found in one sample

[a] Identification was in most instances only to the genus (one name)

Freq, frequency of the fungus on one plate; (1), means a given species; A fum, *Aspergillus fumigatus;* Alt, *Alternaria sp.;* Horm, *Hormodendrum sp.;* Fus, *Fusarium sp.;* Muc, *Mucor sp.;* Rhiz, *Rhizopus sp.;* Trich, *Trichoderma sp.*

since the quantity of liquid inoculated with a standard loop of 3 mm diameter differs from 0.005 to 0.01 ml. The measure error of a 0.3 ml inoculum is much smaller than that obtained with a loop.

Discussion

Routine comparison of soils includes the study of the particle size distribution using a Coulter counter [6, 8], mineralogic examination [9], color matching [4], and density gradient distribution [7]. The analysis of inorganic [10] and organic compounds in soil, such as saccharides [5] and polycyclic aromatic hydrocarbons [1] represents alternative evidence in certain cases.

Modern methods are aimed at traces, such as bacterial flora, but only a few applications have been described until now [11].

The proposed three groups of micro-organisms, viz., bacteria, fungi, and actinomycetes, make it possible to determine the identity or the difference of soil. Characterization of an ecologic site is also possible by the study of members of the protozoan class, especially the *Testacea* as mentioned [12]. This method of identification supposes a good knowledge of the protozoan morphology, which is not so common as for microbiology. Moreover, large ecologic areas, e.g., water, forests, or cultivated soil, may present a similar protozoan composition, but show a different microbiologic structure.

The proposed microbiologic method is not conclusive, and different soils may show the same microflora. None of three groups was identical in different samples.

A disadvantage of the microbiologic method is the dessication of soil adhering to articles, since in dried soil, fungi and actinomycetes are more valuable than bacteria. In this case, isolation of sporulated *Clostridia* may give a result as may other species, e.g., *Azotobacter* as mentioned by Kissing [11].

One may accept that besides soil other environments or objects may also be identified microbiologically in forensic testing, such as the specific atmosphere of a room, used tools, or food.

Acknowledgment. The authors wish to thank Dr. A. Wauters, Station for Soil Analysis, Heverlee, Belgium, for his valuable advice concerning chemical examination of soils.

References

1. Andrasko J (1978) An analysis of polycyclic aromatic hydrocarbons in soils and its application to forensic science. Int Microfilm J Leg Med 13:135–141
2. Corbaz R (1979) Les champignons opportunistes de l'atmosphère. Dermatologia [Suppl 1] 159:56–59
3. Dellucca AJ, Simonson JG, Larson AD (1981) *Bacillus thuringiensis* distribution in soils of the United States. Can J Microbiol 27:865–870
4. Dudley RJ (1975) The use of colour in the discrimination between soils. J Forensic Sci Soc 15:209–218
5. Dudley RJ (1976) A colorimetric method for the determination of soil saccharide content and its application in forensic science. Med Sci Law 16:219–229

6. Dudley RJ (1976) The particle size analysis of soils and its use in forensic science—the determination of particle size distribution within the silt and sand fractions. J Forensic Sci Soc 16:219–229
7. Dudley RJ (1979) The use of density gradient columns in the forensic comparison of soils. Med Sci Law 19:39–48
8. Dudley RJ, Smalldon KW (1978) The objective comparison of the particle size distribution in soils with particular reference to the sand fraction. Med Sci Law 18:278–282
9. Graves WJ (1979) A mineralogical soil classification technique for the forensic scientist. J Forensic Sci 24:323–338
10. Hoffman CM, Brunelle RL, Snow KH (1969) Forensic comparison of soils by neutron activation and atomic absorption analysis. J Crim Law Criminol Police Sci 60:395–401
11. Kissling E (1980) The detection of *Azotobacter* and its significance in criminal technical soil examination. Arch Kriminol 165:27–34
12. Lambert J, Chardez D (1978) Intérêt criminalistique de la microfaune terrestre. Rev Int Criminol 33:158–170
13. Marshall KC (1982) Advances in microbial ecology, vol 6. Plenum Press, New York
14. Segretain G, Segretain G (1979) Ecologie des champignons opportunistes. Dermatologia [Suppl 1] 159:56–59
15. Thornton JI, Crim D, Maclaren AD (1975) Enzymatic characterization of soil evidence. J Forensic Sci 20:674–692

Received February 29, 1984