A MOLECULAR BASIS FOR, AND THE ALLEVIATION OF WATER-REPELLENCY IN SOILS

by

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SUMMARY

A literature survey of the extent and nature of water-repellency in soils showed that the phenomenon occurs world-wide and is a particular agricultural problem across southern Australia. A number of soils from areas notorious for non-wetting sands (South East of South Australia and coastal areas of South West of Western Australia) were obtained for examination of the water-repellent phenomenon.

In chronological order, this thesis covers the following aspects :

1. Effects of wetting, drying and salt treatments on water-repellent soils.

It was observed, that water-repellent soils were rendered wettable after freeze drying of moist soils, however, air and oven drying of the moistened soils only marginally reduced water-repellency. These effects have been attributed to a re-orientation of organic molecules. The orientation of hydrophobic and hydrophilic groups of organic matter is controlled by the presence of water-molecules. The hydrophilic groups tend to hydrogen bond to water molecules in wet situations and as the water is lost, polar groups hydrogen bond with each other causing the exposure of hydrophobic chains and a water-repellent surface. However, during the lower temperatures of the freeze drying process the water molecules are apparently lost without significant molecular rearrangements leaving a hydrophilic surface with exposed polar groups.

There were considerable changes observed in water-repellency as affected by treatments with some aqueous solutions and chloride salts. The type of cations present on water-repellent surfaces was noted to be important because cations also affect orientation of hydrophobic organic matter in soils.

Water-repellency in soils is known to be reduced by agitation due to the abrasive removal of organic matter from sand surfaces. However, floatable organic particles in soils are also hydrophobic. In soils such particles are intermixed with sand particles.

2. Extraction of causal agents from water-repellent soils.

Soxhlet extraction was chosen to minimize abrasion during the extraction of water-repellent materials from soils. The decrease in water-repellency of residual soil in the thimble was monitored as a function of time and provided clear data on the relative efficacy of various solvent mixtures.

Refluxing with an amphiphilic mixture of *iso*-propanol/15.7 M ammonia (7:3, by volume) completely extracted water-repellent materials from all soils examined. The residual soil in the thimble was readily wettable after 8 h extraction. By contrast, relatively non-polar lipid solvents, such as chloroform, ether and tetrachloroethylene, only marginally reduced water-repellency of soils. Thus the importance of polarity of solvent was established.

All of the extracted materials created water-repellency when they were coated upon acid washed sand. However, the degree of water-repellency induced at an arbitrary constant concentration of 400 mg kg^{-1}, as measured by the MED values, varied with the type of extractant. This variation may be ascribed to the presence of differing ratios of hydrophobic to hydrophilic components in the crude extracts.

3. Characterization of water-repellent materials.

Spectroscopic and chromatographic data established that water-repellent materials extracted by the mixture of *iso*-propanol/ammonia from sandy soils collected from Tintinara in the South East of South Australia were a mixture of long chain polymethylene waxes consisting of acids, alcohols and their compound esters. Major constituents of the extracted materials were long chain (16-32 carbon) fatty acids and long chain (14-28 carbon) alcohols. The most prominent acids were palmitic acid (C_{16}) and behenic acid (C_{22}), whereas up to 44 % of the alcohol content in the unsaponifiable fraction was cetyl alcohol (C_{16}).

Cetyl alcohol was observed to create highly water-repellent sands and has provided a useful model system for studies of water-repellency. It was necessary for the sand to be coated with an amount of cetyl alcohol equivalent to one uniform monolayer before it exhibited any measurable water-repellency; but to reach the plateau of maximum hydrophobicity (MED value 7), an amount of cetyl alcohol equivalent to 16 monolayers was required. A scanning electron micrograph of the sand coated with cetyl alcohol at a concentration of 400 mg kg^{-1} showed a papillate appearance of coalescent globules (diameter ~ 150-300 nm) covering all the sand grains.

4. Development of practical procedures for alleviation of water-repellency.

The degree of water-repellency produced by both natural and reference organic compounds on sand surfaces was found to be positively correlated with their concentration up until the maximum plateau level was reached and is also a function of the surface area of the sand. These data suggested, that the water-repellent problems of light textured soils might be nullified by simply increasing their surface area.

Laboratory experiments established that the application of fine particle materials such as oxides, lime, aerosil (a finely divided commercial silica) and clays to either Tintinara soils or acid washed sand coated with cetyl alcohol reduced the severity of their water-repellency. Incorporation of clays appears to be a useful approach to combat the problem, because clay would provide additional benefits, such as increased cation exchange capacity, to the soils. In laboratory studies the application of clay at a concentration of 10 g kg^{-1} (which is equivalent to 10 tonnes ha^{-1}) rendered a water-repellent Tintinara soil (MED value 3.5) wettable (MED value 0).

The dispersibility of clays, which determines their ability to mix with sands, was found to be an important feature in their effectiveness for minimizing water-repellency. This applies to both the natural sandy soils as well as the model cetyl alcohol-sand mixture. It is therefore important to consider factors influencing the dispersibility, such as sodicity and mineralogy, when selecting a particular clay to ameliorate the water-repellency of soils.

The initial field trial was commenced before the significance of dispersible clays was recognised. Nevertheless, the application of a flocculated clay to a sandy soil (MED value 3.1) near Keith in the South East of South Australia in a field experiment measurably reduced water-repellency of the soil. Clay spread on the surface at a rate of 20 tonnes ha^{-1} produced a continuous decrease in MED values from 3.1 to 0.8 over a period of 46 weeks. The incorporation of clay also improved the water regime of the sandy soil. However, there were no significant differences observed in lucerne establishment. With hindsight it is to be anticipated that better results will be obtained in the future with more dispersive clays.

DECLARATION

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any university. To the best of my knowledge and belief, no material described herein has been previously published or written by another person except when due reference is made in the text.

If accepted for the award of a Ph.D. degree, this thesis will be available for loan and photocopying.

Mansur Ma'shum

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to my children Rina, Anggi and Ami

CHAPTER 1

INTRODUCTION

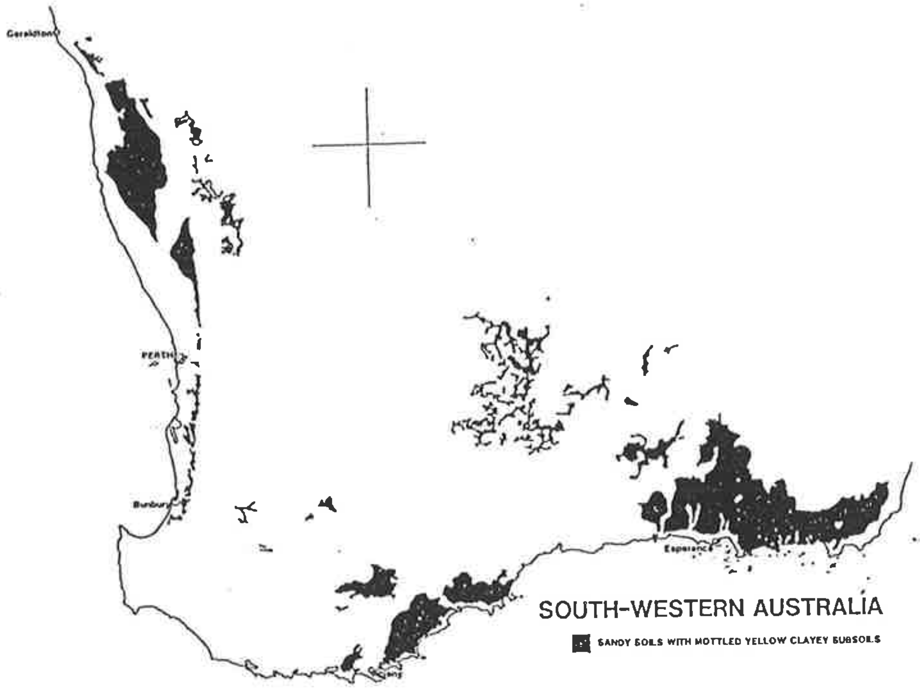
When water remains standing in the form of droplets instead of spreading on the surface of soil, the soil is said to be water-repellent. Two other terms, hydrophobic and non-wetting, have been used to refer to this phenomenon.

Water-repellency in soils was observed early in this century and has been subsequently reported to occur in a number of countries. Water-repellent soils are now widely considered as a serious conservation problem and a constraint on productivity across southern Australia. The phenomenon is dominantly associated with areas of siliceous sands with major occurrences in South Australia, western Victoria and Western Australia. Fig. 1.1. illustrates the extent of siliceous sands in the two states with an estimated area of about two million hectares. The map units are designated as sands or sandy soils with mottled clayey subsoils on the Atlas of Australian Soils.

Poor germination, patchy annual pastures, water and wind erosion are problems often associated with water-repellent soils. The total loss of production due to water-repellency in southern Australia is estimated to be over ten million dollars per annum. The loss by wind erosion of sands and all the nutrients concentrated in the top few centimetres is even more significant. Wetherby *et al* (1983) reported that water-repellency was a major contributing factor to sand drift events across the Eyre Peninsula in South Australia in the period 1975-1979.

Even though the discoveries on the origin of water-repellence are well documented, our present knowledge of the nature of materials responsible for water-repellency in soils is rudimentary and remains controversial. Organic materials have been implicated as the causal agent by most workers. However, there is no correlation between water-repellency and total organic matter contents. Water-repellency has also been associated with different vegetation such as Eucalyptus sp, chaparral, various shrubs, woody plants and grasses.

Fig. 1.1. : Areas of siliceous sands in south western and south eastern Australia.



SOUTH-WESTERN AUSTRALIA

■ SANDY SOILS WITH MOTTLED YELLOW CLAYEY SUBSOILS

SOUTH-EASTERN AUSTRALIA

■ SANDY SOILS WITH MOTTLED YELLOW CLAYEY SUBSOILS

Fungi have been implicated in inducing water-repellency. Many Basidiomycetes produce water-repellent hyphae and spores and there have been correlations between growth of such organisms with patches of water-repellency in fields and golf greens (Bond 1962, Bond and Harris 1964). In some cases, however, water-repellent sand grains have shown no evidence of the presence of hyphae or spores. McGhie and Posner (1980) reported that spore-forming fungi do not cause water-repellency in Western Australian soils and in some cases may reduce it. These conflicting reports of the biological origin of water-repellency suggested that it would be more profitable to examine the chemical nature of the causal agent(s) rather than its biological source.

A number of compounds including lipids, substituted phenols and less well defined substances such as humic acid fractions have been reported as the causal agents. From studies with phenolic compounds (Bozer *et al* 1969) it has been suggested that water-repellent molecules are amphiphilic with a hydrophilic group at one end for attachment to the surface and a hydrophobic tail, such as a hydrocarbon chain, as the accessible group on the surface. Because many naturally occurring lipids are also amphiphilic, they would be expected to be likely candidates for waterproofing agents in soils. However, the possibility that lipids could create water-repellency in soils was rejected until recently by some authors because soils which had been extracted with non-polar lipids solvents, such as chloroform and ether, remained water-repellent.

In Southern California the water-repellent phenomenon was found to be widespread in burnt brushland soils. Brushfires were subsequently shown to be a major cause of water-repellency and it is considered that during a fire, hydrophobic substances are vapourized and distil downwards along a decreasing temperature gradient where they condense on the cooler, lower layers (DeBano and Krammes 1966, *cit.* McGhie 1980). In Australia, however, association of water-repellency with fires has not been established.

To combat the problems caused by water-repellency three major approaches including cultivation techniques, the use of wetting agents and fine particle amendments have been suggested. However, the fact that water-repellent soils are still a major agronomic problem indicates limited success in dealing with water-repellency.

The objectives of this work have been to isolate and characterize as precisely as possible in chemical terms the causal agent for water-repellency in South Australian sandy soils and to develop a method to improve the wettability of such soils.

CHAPTER 2

LITERATURE REVIEW

2.1. Introduction

Water-repellency is a physical property of soil which has been recognized as having potential hydrological significance. Its occurrence has been reported from a number of countries in a variety of climatic zones.

Hydrophobic substances coating soil particles cause retarded and uneven entry of water into dry soils. It has been suggested that a range of materials are responsible for water-repellency, however, the substances have not been identified precisely. Fungal hyphae, humic acid and decomposing plant materials or litter have been implicated (Bond and Harris 1964, Roberts and Carbon 1972, McGhie 1980).

The occurrence of water-repellency in South Australian soils was first reported by Prescott and Piper (1932). They noted that water-repellency was often associated with a type of vegetation classified as "mallee" consisting of thick bush of Australian *Eucalyptus spp*. Further investigators reported that water-repellency in South Australian soils is important in soils with sandy surface horizons (Bond and Harris 1964, Bond 1968).

2.2. Discovery and distribution of water-repellency

The occurrence of water-repellency in soil was reported early this century. Probably the first scientists who reported this phenomenon were Schreiner and Shorey (1910) who suggested that the presence of special organic compounds induced water-repellency in Californian soils. In Australia, Greig-Smith (1911) had independently found water-repellency in some soils of New South Wales and believed the cause was fatty or waxy substances of apparently bacterial origin which were referred to as *agricere*. Shortly after Schantz and Piemeisel (1917) reported the occurrence of water-repellency in Colorado which was caused by basidiomycete fungi. In South Australian soils water-repellency was first described by Prescott and Piper (1932).

Water-repellency in citrus soils in Florida was reported by Jamison (1942). He observed that there was an interaction between citrus trees and the soil condition; the tree caused hydrophobicity to develop which in turn later affected the tree. It was suggested that water-repellency developed slowly with young trees and became intensified to a critical state in older groves of large trees. In further investigations Jamison (1946, 1947) suggested that water-repellency was a surface phenomenon and was a property of the organic matter coating the soil particles.

DeBano (1969a) in his review suggested that water-repellency occurred in ten western states of the United States and was mostly found on wild land areas. Water-repellency was also reported to be found in golf greens (Waddington 1969, Miller and Wilkinson 1977, Wilkinson and Miller 1978).

A series of studies in South Australia (Bond 1960, 1962 and 1964) described water-repellency in the south east of the state. The occurrence of water-repellent soils on the Eyre Peninsula was observed by Wetherby (1984). Generally, the phenomenon has been observed in sandy soils, however, McGhie (1980) reported water-repellency in fine textured soils in Western Australia. Water-repellency has also been reported in other states of Australia. Bond and Harris (1964) reported that some sandy soils in Victoria were water-repellent. Bond (1969a) described water-repellent soils in a small area in New South Wales and in the coastal plain near Perth.

Water-repellency is a wide-spread phenomenon in Australia and the United States and has been studied quite intensively in these two countries. Water-repellency has also been reported in other countries such as India (Adhikari and Chakrabarti 1976), Japan (Nakaya et al 1977a, 1977b), Italy (Giovannini and Lucchesi 1984) and North-East Scotland (Malik and Rahman 1985). As water-repellency can be intensified by fire (DeBano and Rice 1973) it can be predicted that this problem occurs in other countries in which the system of *shifting cultivation* is still practiced.

2.3. Formation of water-repellency

It has been recognized that there are two types of water-repellency, namely, natural and fire induced water-repellent soils (Savage 1975). The natural water-repellency appears in soils which have not been affected by unusual outside phenomenon, while the other form occurs in burnt-over soils. There is some evidence that natural water-repellent soil is induced by organic matter (DeBano 1981). The importance of organic matter in producing water-repellency was found by DeBano and Rice (1973), even in fire-induced water-repellent soil: organic matter must be present in the first stage of the formation of water-repellency. They concluded that the role of fire is to intensify an existing water-repellent condition. A corollary of the fire induced situation is that the causal molecules are at least partially volatile which was noted by Savage *et al* (1969b).

Bond (1960) suggested that fungi are a major cause of natural water-repellency due to the fact that fungi are consistently present in water-repellent soil. However, Roberts and Carbon (1971) contested this suggestion and proposed that fungi may be a partial cause of water-repellency.

Plant residues or litter have been implicated in the occurrence of water-repellent soils both in United States and Australia (Bond 1969b, Jamison 1942 and 1946, McGhie and Posner 1980).

2.3.1. *Organic coatings*

In the early stage of the investigation of water-repellent phenomenon Schreiner and Shorey (1910) found that some soils in California could not be properly wetted. They suggested that the peculiarity of the soil was induced by the presence of organic matter which when extracted had the properties of a varnish, repelling water to an extreme degree. Prescott and Piper (1932) reported the presence of an essential oil in South Australian water-repellent soils from "mallee" areas.

Jamison (1942) observed that water-repellency was caused by organic colloids. In subsequent studies Jamison (1945) tried to extract the material responsible for water-repellency with ether. As a result he found that the soil remained water-repellent after extractions. This finding led him to conclude that water-repellency seems to be a surface phenomenon and not due to the accumulation of oily or waxy water-resistant materials. Starting from Jamison's finding Wander (1949) employed different methods of extraction. A sample of water-repellent soil was extracted first with ether to remove any free fats or waxes, then with methyl alcohol. The treatment left the soil readily wettable. This result led him to conclude that the formation of metallic soap i.e. calcium and magnesium, on soil particles produced water-repellency.

Particles coated by hydrophobic substances were in some cases responsible for the observed water-repellent soil on the central plateau in the North Islands of New Zealand (Van't Woudt 1959). The coatings were so well adsorbed that the soil remained water-repellent after extracting with ether for 20 hours in a Soxhlet apparatus.

Emerson and Bond (1963) observed that water-repellency in South Australian soils was produced by an organic coating which could be removed by ignition at 500°C in a muffle furnace. However, according to Bond (1968) the organic coating was insoluble in cold organic solvents such as ether, methyl and ethyl alcohols, benzene, chloroform and acetone. Sodium hydroxide and sodium pyrophosphate were found to remove the coating. It was also found that hydrochloric acid was capable of reducing water-repellency of the samples examined. Similar results were reported by Roberts and Carbon (1972). Organic skins responsible for water-repellency on sandy soils of South Western Australia were resistant to removal by cold water, concentrated acid and organic solvents. However, they observed that prolonged treatment with hot diethyl ether, ethanol and benzene removed part of the coating. Treatment with dilute solutions of alkali removed the skin as suspended particles.

More recently, McGhie and Posner (1980) suggested that comminuted surface litter coating clay aggregates was the main cause of water-repellency in Western Australian soils. The effectiveness of the comminuted litter in causing water-repellency was found to depend on plant species and on the size of litter; the more finely ground material was found to produce the greatest degree of water-repellency.

2.3.2. *The role of microorganisms*

Microorganisms may cause water-repellency both directly and indirectly. Greig-Smith (1911) discovered that soil containing a fatty or waxy substance called "agricere" resulting from bacterial activities was water-repellent. Observations conducted by Bond (1960) have shown that the mycelia of some fungi are hydrophobic, with surfaces which repel water very strongly. He also suggested that some substances produced by the mycelia become dispersed in soils and imparted water-repellency to soils.

By examining sands collected from immediately below the fruiting bodies of *Basidiomycetes sp* Bond (1962) discovered that the presence of mycelia in those pockets of sands resulted in water-repellency. Further investigation conducted by Bond and Harris (1964) supported the previous findings. The metabolic products of microorganisms, especially fungi were responsible for water-repellency in soils in South Australia.

Savage *et al* (1969b) isolated eight species of fungi from water-repellent soils. The fungi were then incubated for various periods of time in silica sands. Two of them, *Aspergillus sydowi* and *Penicillium nigricans* caused limited water-repellency in these sands. It was observed that there was an inverse relationship of organic matter content with water-repellency of the sand culture. This suggested that the substances causing the water-repellency were either produced in the later stages of the fungal growth or were the lytic products of the fungi themselves.

Further evidence that microbial activity was linked with water-repellency was reported by Wilkinson and Miller (1978). They discovered that coatings on individual sand particles had the appearance of fungal mycelia even though they could not isolate the organisms. However, in Western Australian soils spore-forming fungi did not cause water-repellency and in some cases reduced it (McGhie and Posner 1980).

2.3.3. *Fire induced water-repellent soils*

The relationship between water-repellency and fire was firstly studied by Krammes and DeBano (1965). They found that the water-repellent phenomenon was widespread in burnt brushland soils of Southern California. There was an indication that the hydrophobic condition decreased when the soil was heated at 105°C for 48 hours. However, exposing soil samples to temperatures between 250 and 300°C for 18 minutes intensified water-repellency, while the condition was eliminated by temperatures of higher than 300°C for 30 minutes. In a subsequent study they also reported that water-repellency may be either intensified or destroyed by heating (DeBano and Rice 1973).

Two mechanisms by which subsurface water-repellency could be intensified were suggested. Firstly, heat intensifies the water-repellency *in situ* . Secondly, during a fire hydrophobic substances are vapourized and distilled along a temperature gradient to condense at a greater depth (DeBano and Krammes 1966, *cit.* McGhie 1980).

Cory and Morris (1969) reported that water-repellency of granitic soil can be completely removed by exposing the soil to 600°C or above for an hour. They suggested that firing soils under controlled temperature conditions has resulted in a downward movement of the organic layer so that the soil becomes water-repellent. These investigators also proposed the possible formation of an organo silicate complex between the translocated materials and the soil particle surfaces.

By conducting heating experiments on a fungal sand culture incubated for 53 days Savage *et al* (1969b) proposed a mechanism by which the sand culture became severely water-repellent after heating. The mechanism was purely physical wherein a nonpolar substance residing in localized areas of the sand was sublimed throughout the sample, creating a greater number of hydrophobic surfaces. An alternative mechanism was proposed by DeBano (1966, *cit.* Savage *et al* 1969b) that the organic vapours originated from pyrolysis rather than vapourization.

DeBano and Rice (1973) suggested that soil temperatures at different depths and large temperature gradients may intensify incipient water-repellency already existing at the various depths. Vapour and gases containing hydrophobic substances move downward in the soil profile and condense on cooler soil particles. After a fire, the soil has a water-repellent layer.

By conducting laboratory experiments Savage *et al* (1972) came to the conclusion that extreme water-repellency in burnt-over soils resulted from products derived from heating of the surface soil litter. The most effective substances in the products were aliphatic hydrocarbon-like materials.

Soil characteristics give clues to the potential severity of water-repellency. DeBano and Rice (1973) found that coarse-textured soil became more water-repellent than soils of finer texture. It was suggested that this feature is due to the fact that the surface area per unit mass was lower on coarse textured soil and they were more easily coated by the hydrophobic materials (Biswell 1975). Soil moisture content at the time of the burn has an important impact. The most severe water-repellent condition was produced by a 5 minute-burn over dry sand. In a wet sand water-repellency was concentrated nearer the surface and was very intense in the upper 0-1.5 cm of sand during the 225 minute burn (DeBano *et al* 1976).

2.3.4. *Chemistry of water-repellency*

It has been recognized that the substances responsible for causing water-repellency are definitely organic. However, the chemical characterization of the substances is complex. A series of organic compounds are able to produce water-repellency, hence a range of organic substances has been proposed to be the causal agent.

Bozer *et al* (1969) suggested that materials decreasing soil wettability include many organic compounds with amphiphilic characteristics. Substituted phenols were proposed as a prominent group of water proofing chemicals.

Other investigators believed that humic acids are the most likely substances responsible for water-repellency (Savage *et al* 1969a, Roberts and Carbon 1972, Adhikari and Chakrabarti 1976, Singer and Ugolini 1976, Tschapek *et al* 1973), however, not all humic acids produce water-repellency (Savage *et al* 1969a).

By treating a silica sand and soil with three microbial humic acids, a soil and peat humic acid Savage *et al* (1969a) observed that only the humic acid isolated from *Stachybotrys atra* culture induced hydrophobicity. Roberts and Carbon (1972) have shown that compounds within the stable humic acid complex were largely responsible for the hydrophobic properties of the organic skins on sand grains. A similar result was proposed by Singer and Ugolini (1976) who found that the humic/fulvic acid ratio (H/F) was highly correlated with water-repellency.

That humic acid of microbial origin is responsible for water-repellency (Savage *et al* 1969a) was supported by Adhikari and Chakrabarti (1976) who compared the effect of natural and microbial humic acid on the wettability of some Indian soils. These investigators observed that increased water-repellency was created in the presence of humic acid of microbial origin.

By measuring surface tensions of some solutions at various concentration levels of fulvic and humic acids Chen and Schnitzer (1978) found that the deficiency of fulvic acid in the soil solution resulted in water-repellency while an ample supply of fulvic acids increased soil wettability.

A result at variance with the conclusion of Chen and Schnitzer (1978) was a suggestion by Miller and Wilkinson (1977), that the infrared spectra of substances extracted from non wettable sands was characteristic of the spectra of fulvic acids.

Tschapek *et al* (1973) proposed that humic acids possess both hydrophobic and hydrophilic groupings, so that these acids fulfill the requirement to be the compounds responsible for water-repellency as indicated by Bozer *et al* (1969).

Other substances responsible for water-repellency were proposed by Savage *et al* (1969b). They treated silica sands with various organic compounds. The result was that amines were the only class of compounds which rendered the sand water-repellent without heating. The compounds capable of causing heat induced water-repellency, however, are quite numerous and varied as to type and include octadecylamine, octadecanol, palmitic acid and tristearin.

2.3.5. *Soil lipids and water-repellent properties*

Soil lipids represent a series of biological compounds which are insoluble in water but soluble in *fat* solvents such as ether, chloroform or benzene (Bloor 1925). The members of this group of substances range from relatively simple compounds such as fatty acids to more complex substances such as sterols, terpenes, fats, waxes and resins (Stevenson 1982). The term *bitumens* has also been used to refer to this group of compounds (Braids and Miller 1975, Jambu *et al.* 1978).

Lipids have been found to be widely distributed throughout soils of the world, however, these substances are probably the least studied components of soil organic matter possibly due to the fact that these compounds were thought to comprise but a small percentage of the total soil organic matter. Waksman and Stevens (1930) observed that only 1.23-6.24 % of the humus in mineral soils were present as lipids, while Jambu *et al.* (1978) reported that lipids in Podzolic soils represent close to 18 % of total carbon in such soils.

Soil lipids have both beneficial and detrimental effects to soils and hence to plants. An increase in structural stability is an example of the merits provided by soil lipids (Fehl and Lange 1965, Jambu *et al.* 1978 and Zobel 1946). However, there has been evidence that soil lipids are toxic in relatively low concentrations to plants growing in nutrient solutions (Schreiner and Shorey 1909, Schreiner and Skinner 1910 and Prescott and Piper

1932) and they also drastically reduced water availability to plants (Jambu *et al.* 1978).

That soil lipids have possible links to water-repellent properties was suggested along with discovery of the phenomenon by Schreiner and Shorey (1910) who identified the organic materials removed from hydrophobic soils as "varnish" materials which repelled water to an extreme degree. They observed that such soils, once freed of those substances, had a high water-holding capacity. In Australia, Greig-Smith (1911) found that the *agricere* i.e. fatty or waxy substances of apparently bacterial origin, induced water-repellency in some soils in New South Wales. Similar results were also reported by Prescott and Piper (1932) who considered that an essential oil induced hydrophobicity in South Australian soils. Removal of fats and waxes from a water-repellent soil using ether and then methanol left soil hydrophilic (Wander 1949). In addition, Wander (1949) noted that stearic acid created extreme water-repellency after treatment with calcium and magnesium hydroxide. Savage *et al* (1969b) found that palmitic acid induced hydrophobicity in sands after heating.

Despite this positive evidence, the possibility that lipids could induce water-repellency has been rejected by some authors (Jamison 1945, Van't Woudt 1959 and Bond 1968) because soils which had been extracted with ether and other lipid solvents remained water-repellent. The inability of less-polar solvents to remove lipids from soils has been well documented and it was suggested that some lipids may be linked with other compounds making them generally insoluble to less-polar solvents (Hance and Anderson 1963 and Johnson 1971). However, non-polar solvents would be expected to increase any hydrogen bonding between the surface and any acceptor or donor regions of hydrophobic materials.

2.4. Assessment of water-repellency

The physicochemical aspects of water-repellency of porous and non-porous surfaces have been discussed in detail by a number of workers (Letey et al 1962, Zettlemoyer 1969, Bond and Hammond 1970, Miyamoto and Letey 1971, McGhie 1980 and Nakaya 1982). A variety of methods have been used to assess the severity of water-repellency. Generally, the methods can be classified into four types : by measuring contact angle, the measurement of water drop penetration time, the use of critical surface tension of the infiltrating solutions and the use of infiltrometers.

2.4.1. *Measurement of contact angle*

A property which can be attributed to the wettability of a solid is the liquid-solid contact angle (Letey *et al* 1962). The contact angle can be measured geometrically for a plane surface soil. The measurement of contact angle can be conducted in various ways.

Letey *et al* (1962) described a method of calculating the contact angle by measuring downward water-infiltration or height of capillary rise. By assuming that soil is characterized by cylindrical tubes and using Poiseuille's approximation :

$$Q = \frac{\pi r^4 P}{8\ L\eta}$$

where Q is the rate of flow in volume per unit time; r is the radius of capillary; P is the pressure driving the water; L is the capillary length; and η is the viscosity of the solution; these investigators proposed the relationship between the rate of solution infiltrating into a soil column and the physical properties of solution, the effective pore radius and the liquid-solid contact angle.

The contact angle can then be calculated according to the following equation :

$$h = \frac{2\gamma \cos \Theta}{\rho\ g\ r}$$

where h is height of rise; γ is the surface tension; Θ is the angle between the water miniscus and the wall; ρ is the density of the liquid; g is the gravitational term and r is the radius of capillary.

The height of capillary rise was also developed to calculate contact angle (Bond 1962, Emerson and Bond 1963). The advancing contact angle was calculated from the ratio of the values of the height of rise obtained before and after ignition. This method was questioned by Yuan and Hammond (1968) who were concerned about the relation of the contact angle between ignited soil and water . They recommended the use of a soil-ethanol reference as a modification.

Pal and Varade (1971) measured the contact angle of water in soils by using the infiltration method while the contact angle of water on sands was measured by using the capillary rise method. They found that the Poiseuille's equation was only applicable to soils and not to pure sands.

The measurements of contact angle described above were not instantaneous contact angles but were influenced by the so called "reaction time" i.e. the period required for the water to react with soil particles sufficiently to obtain a stable measurement of the contact angle. This fact led Bahrani *et al* (1973) to introduce another method of measuring contact angle referred to as the infiltration procedure by using *n*-heptane as well as water.

2.4.2. *Water drop penetration time*

The most widely used method is the measurement of water drop penetration time. Krammes and DeBano (1965) applied this method to assess the wettability of soils in the field. A droplet of water was placed on the surface of soil and the length of time before the drop was absorbed was measured. They suggested that water droplets remaining longer than 5 seconds usually remained for several minutes and the soil could be classified as water-repellent.

Even though this technique is subject to several limitations, it is a very quick and easy procedure (Letey 1969). The water drop penetration time test is a method which tends to characterize the stability of water-repellency (DeBano and Letey 1969). Roberts and Carbon (1971) proposed an index of the severity of water-repellency based on the length of time the drop persists on the surface. A time of persistence of 1-10 s was arbitrarily considered as slightly water-repellent, 10-60 s as moderate and more than 60 s as severe water-repellent.

2.4.3. *Infiltration test*

The degree of water-repellency in soil can be assessed by using an infiltrometer (Bond 1962, King 1978 and 1981). A small ring infiltrometer consisting of plastic tube with diameter of 25 mm was applied to measure the severity of water-repellency in South Australian soils. The tube was embedded 5mm in flat soil. 5 ml of water was placed on the soil surface and the time required to soak away was measured. The severity of water-repellency was classified from very low to very severe with the samples having infiltration rates of 32 and 0.06 mm/minute respectively.

2.4.4. *Critical surface tension*

Letey (1969) suggested that soil water-repellency can also be assessed by the measurement of a critical surface tension. The critical surface tension is defined as the highest surface tension of a liquid which will wet the particular solid at a zero contact angle. This method was based on the close link between surface tension and contact angle as was proposed by Zisman (1964). A series of solutions having different surface tensions was applied in this method. A drop of solution of each surface tension was placed upon the soil and the rate of disappearance of the drop was observed. The surface tension of liquid applied was compared with the critical surface tension. If the surface tension of the liquid was lower, the liquid drop was absorbed instantaneously. Conversely, if it was higher, the penetration of the liquid was retarded.

2.4.5. *The MED values*

The background to the development of the aqueous ethanol penetration drop test has been clearly described by Letey *et al* (1962). In essence, pure ethanol is known to wet all surfaces. The key suggestion of Letey *et al* was to point out that the surface tension of an aqueous ethanol drop which just readily wetted a surface would provide a simple procedure for the ranking of water-repellent soils. Because the molarity of aqueous ethanol also determines its surface tension, the hydrophobicity of the surface can be simply expressed as the molarity of an ethanol droplet which will just penetrate (wet) the surface. Watson and Letey (1970) proposed an assessment method based on the correlation between the cosine of the contact angle -an index of water-repellency- and the surface tensions of liquids. They suggested that the lower the surface tension of aqueous ethanol solutions which remain standing on the soil surface for 5 s, the higher the initial water-repellency of the soils.

The molarity of an ethanol droplet which will penetrate the soil surface in 10 s is referred to as the MED value and has been used to measure the degree of water-repellency in South Australian soils (King 1981). Droplets of aqueous ethanol solutions are placed on the soil surface and the time required to be completely absorbed is recorded. According to this measurement the severity of water-repellent soils are classified into low (MED 0-1.0), moderate (1.0-2.4), severe (2.4-3.0) and very severe (>3.0). In fact this technique is a modification of the previously described method (Watson and Letey 1970).

Measuring MED values is a quick technique; as little as 1 g soil sample is required. The value recorded has been well correlated (King 1981) to the soil-water contact angle estimated by a capillary rise method of Emerson and Bond (1963). However, due to the temperature dependence of the surface tension of these solutions this method is temperature dependent. However, in a laboratory environment where temperature fluctuations can be controlled the results are remarkably reproducible, and have formed the basis for much of the progress achieved in this thesis.

2.5. Agricultural significance of water-repellent soils

Poor germination, loss in crop and livestock production, and both soil and water erosion are problems related to water-repellent soil. Water-repellency affects crops and pasture establishment in two possible ways : by reducing the amount of water available for germination and growth or inducing overland flow and erosion carrying the seeds off the site before they can germinate and become established.

In water-repellent soils the cultivated top 1 cm of soils is normally wet after recent rain, however, dry soil can be found at shallow depths. This uneven wettability results in patchy germination. Results from a field experiment near Cummins on the Eyre Peninsula region of South Australia (Bond 1972) indicated that the grain yield of barley sown in the top of ridges of a water-repellent sand was half of that sown in the furrows. The reduced yield was at least partly due to water-repellency. Ungerminated seed was recovered from the tops of the ridges three months after sowing during which time 100 mm of rain had fallen.

Based on experience on the Eyre Peninsula Wetherby (1984) reported that hydrophobic effects were more significant during a so called "Water-repellent Sand Year" which can be expected about one out of every three years. Economic assessment (Wetherby 1984) revealed that in an area of about 116 000 ha which exhibit low and moderate water-repellency the total value of lost of crop and livestock production was $ 3.5 million and $ 790 000 respectively.

Uneven establishment of both crops and pasture leaves such soils prone to wind and water erosion. Treating the water-repellent layer with a wetting agent reduces both run-off and erosion. Krammes and Osborn (1969) found that run-off from Californian water-repellent soils ranged from 40 to 100 mm during seasons when rainfall varied from 180 to 970 mm. By comparison, plots treated with wetting agents yielded only 20 to 70 mm run-off under the same rainfall. Water-repellency is considered to be the major factor contributing to the problem of sand drift in Eyre Peninsula (Wetherby *et al* 1983).

2.6. Alleviation of water-repellency

Three techniques have been employed to combat water-repellency.

2.6.1. Wetting agents

A series of wetting agents or surfactants have been used extensively in Australia and the USA. The compounds such as alkyl polyethylene glycol ether, alkyl polyoxyethylene ethanol and poly ethoxylate which are marketed under various trade names have been shown to be very effective in reducing hydrophobicity by lowering water-surface tension and allowing water to penetrate evenly and deeply. Earlier it was assumed that applying wetting agents is not a practical solution due to fact that they only increase wettability for a limited period and the high rate of application for a lasting effect making it an expensive approach (King 1974). However, more recently McGhie and Tipping (1984) found that such chemicals always improve soil wettability at rates of 50 litres ha^{-1}. These workers also reported that the effects of wetting agents examined have been found to persist for at least two or three years.

One problem which could arise is that such chemicals and their breakdown products can be phytotoxic (Valoras 1969 and Valoras *et al* 1974).

2.6.2. Cultivation techniques

Cultivation has been extensively used for lucerne establishment in the South East of

South Australia. Disc ploughing, cultivation and harrowing up to 10 cm depth in moist conditions increase wettability. In addition, deep ploughing 30-40 cm and further mixing surface and subsoil wets the soil quickly, however, such ploughed soils are prone to wind erosion (King, 1984).

2.6.3. Fine particle amendments

Application of high surface area materials such as clay and red mud appears to be one possible way to alleviate water-repellent problems. According to King (1974) Snyder of the University of Florida seems to have been the first to introduce this technique. Considerable decrease in water-repellency occurred on the plots treated with 3 and 5 % clay (King 1974) which is equal to approximately 30 and 50 tonnes ha^{-1} respectively. Bond (1978) found that addition of cores of two garden sandy loams with clay content of 12 and 16 % onto golf greens markedly increased the wettability of the golf greens. The rate of application was 12.5 kg per 1.5 m^2 which is equal to more than 83 tonnes ha^{-1}.

An alkaline fine residue from bauxite refining which had been neutralized by mixing with gypsum was effective in reducing water-repellency of a deep grey Gavin sand in Western Australia (Ward 1984). However, to achieve an agronomically tolerable water-repellency an enormous amount i.e. up to 2000 tonnes ha^{-1} of such residue had to be applied.

2.8. Concluding remarks

Water-repellency is a serious soil problem in Australia resulting in significant loss in crop and pasture production. In South Australia water-repellency is mainly observed on the surface of sandy soils in the South East of South Australia and in Eyre Peninsula.

It has been established that soils become water-repellent due to the presence of hydrophobic organic substances coating the sand particles. However, the specific chemical nature of these substances is still a mystery since a great diversity of organic compounds could be responsible for the hydrophobic problems. Humic acids have been regarded as the most likely candidates for the origin of water-repellency because strong alkali is well documented in its ability to convert water-repellent soils to hydrophilic soils. The possibility that lipids could produce water-repellency in soils has been rejected by some workers simply because soils which had been extracted with ether and other lipid solvents remained water-repellent.

There have been several approaches to the alleviation of water-repellent problems, however, none of the techniques suggested to date can be applied economically and universally.

Therefore, further study on the nature and behaviour of organic material causing water-repellency and on possible techniques to alleviate the problems appears justified.

CHAPTER 3

EFFECTS OF WETTING AND DRYING AND SALT TREATMENTS ON WATER-REPELLENCY

3.1. Introduction

Many difficulties have been encountered in assessing the effectiveness of extraction procedures aimed to isolate the compounds responsible for water-repellency in soils, since considerable changes in water-repellency can occur without removal of hydrophobic molecules. A very severe water-repellent clay-rich soil was always rendered wettable after extractions with either warm or hot water (50-100°C) for 1 hour. Conversely, water-repellency still persisted following extraction using various organic solvents such as benzene and ethanol (McGhie and Posner 1980). A more striking observation reported by these workers was that chloroform regenerated water-repellency of these soils which were hydrophilic following either water or 0.1 M NaOH extraction. This shows that the hydrophobic compounds had not been completely removed. In addition, King (1981) observed that water-repellency of sand is prone to abrasion, stirring or other agitation usual during extraction.

The effects of various aqueous extraction and drying procedures on the water-repellency of sandy surface soils and factors affecting the extraction have been studied. This work also aimed to determine whether the water-repellent organic matter is involved in salt like bonds with some metal ions.

3.2. Material and methods

3.2.1. Soil samples.

Soil samples were collected from two main regions in South Australia i.e. the South East and the Eyre Peninsula in which water-repellent soils have been reported to occur extensively (King 1981, Wetherby 1984). The A1 horizon (0-15 cm) of a solonized brown soil (Dy5.83) was sampled near Murray Bridge (sample MB). Samples were collected from the upper 15 cm of a siliceous sand (Uc2.21) at Tintinara (TA) and a Molineaux sand sample from Wharminda Eyre Peninsula (WH). These samples were air dried and sieved < 1 mm.

3.2.2. Assessment of water-repellency.

The ethanol drop test modified by King (1981) and the capillary rise technique of Emerson and Bond (1963) were employed to assess the severity of water-repellency.

3.2.3. Wetting techniques.

3.2.3.1. Evacuation procedure.

A soil sample (25 g) was wetted with distilled water. The mixture was evacuated for 5 minutes. When the vacuum was released the water was sucked into the sand. By this technique the soil was wetted thoroughly with minimum agitation. The mixture was then filtered under suction in a Buchner funnel.

3.2.3.2. Wetting with ethanol/water.

A soil sample (25 g) was placed in a Buchner funnel. 50 ml of 3.6M ethanol was poured onto the sample. The sample was easily wetted. The ethanol solution was then sucked out and the soil washed with 4x50 ml distilled water with suction applied continuously.

3.2.4. Drying techniques.

The wetted samples were air dried, oven dried or freeze dried.

3.2.5. Chloroform treatment.

Dried soils before and after aqueous extraction (25 g) were treated with 50 ml chloroform. The chloroform was allowed to evaporate under a fume hood. The samples were oven dried prior to measurement of water-repellencies.

3.2.6. The effect of agitation.

Soil samples were wetted by the evacuation technique mentioned above. The mixtures were then shaken for 0.5, 1, 2, 4, 8, and 16 hours on an end-over-end shaker. One sample from each soil type was shaken gently i.e. by reversing the container 10 times and another sample was wetted without shaking. The mixtures were centrifuged. After decanting the supernatant the soil was stirred with a glass rod to mix in an organic rich fraction which tended to separate out from the sand. The soil was then oven dried.

3.2.7. Treatment with Na-EDTA and Na-acetate.

The most water-repellent sample i.e. Tintinara soil was wetted by the evacuation technique with the following solution : 0.05 M Na-EDTA with pH values of 3.75, 5.00, and 7.00; 0.2 M Na-acetate with the same pH values; and distilled water. After evacuation the samples were transfered into centrifuge tubes. The pH of each suspension was measured and they were shaken gently by end-over-end shaking twice over 4 hours at 1 hour intervals. pH values were measured before centrifugation. The pH values of the suspensions were adjusted to 8.00 and the colour of the extracts was measured at wavelengths of 400 nm. To remove either EDTA or acetate the soil was washed with 3 x 100 ml distilled water. After recording the final pH the soils were oven dried. Soluble cations in extracts were determined in an Atomic Absorption Spectrometer.

Both oven dried extracted samples and an original sample were wetted with distilled water by the technique of evacuation. The pH values of suspensions were measured and then adjusted to pH 3.00 by adding 1 M HCl. The suspensions were shaken gently, centrifuged, and washed with 10^{-3} M HCl. Excess chloride was removed by washing with distilled water. After measuring the final pH the soil samples were oven dried. A flow chart for these procedures is presented in Fig. 3.1.

3.2.8. Treatment with chloride salts.

The samples which had been treated with either Na-EDTA or Na-acetate were wetted with chloride salts namely 0.1 M $CaCl_2$, $MgCl_2$, , KCl, and NaCl. After measuring the pH the mixture was centrifuged and then washed with distilled water. The samples were oven dried before measurement of the MED values.

3.3. Results and discussion

3.3.1. Water-repellency and other properties of soil samples.

Water-repellency of soils used in this study (Table 3.1.) varied from moderate to very severe according to the classification proposed by King (1981). The order of severity of water-repellency based on the MED test agreed well with calculated contact angles using the formula proposed by Emerson and Bond (1963). The highest MED value corresponded to the largest contact angle and the least water-repellent sample possessed the smallest contact angle. Plate 3.1. illustrates the contact angle formed by a drop of water sitting on the surface of Tintinara soil. Such a drop remained unchanged for several hours on this type of soil. Agronomically serious situations in Australian water-repellent soils have been observed when MED values exceed 1.0 (King 1981). The soils at Wharminda have been less productive for both crops and pasture for the last 12 years (Mr. K.G. Wetherby, personal communication). Both Murray Bridge and Tintinara soils always exhibit poor germination, patchy annual pasture and marked wind erosion (Mr. P.M. King, personal communication). The results of mechanical analyses (Table 3.1.) support the general concept that water-repellency is characteristic of sandy soils (Bond and Harris 1964, Holzhey 1969 and Savage 1975).

Fig. 3.1. : Flow chart of Na-EDTA and Na-acetate treatment on water-repellent soils.

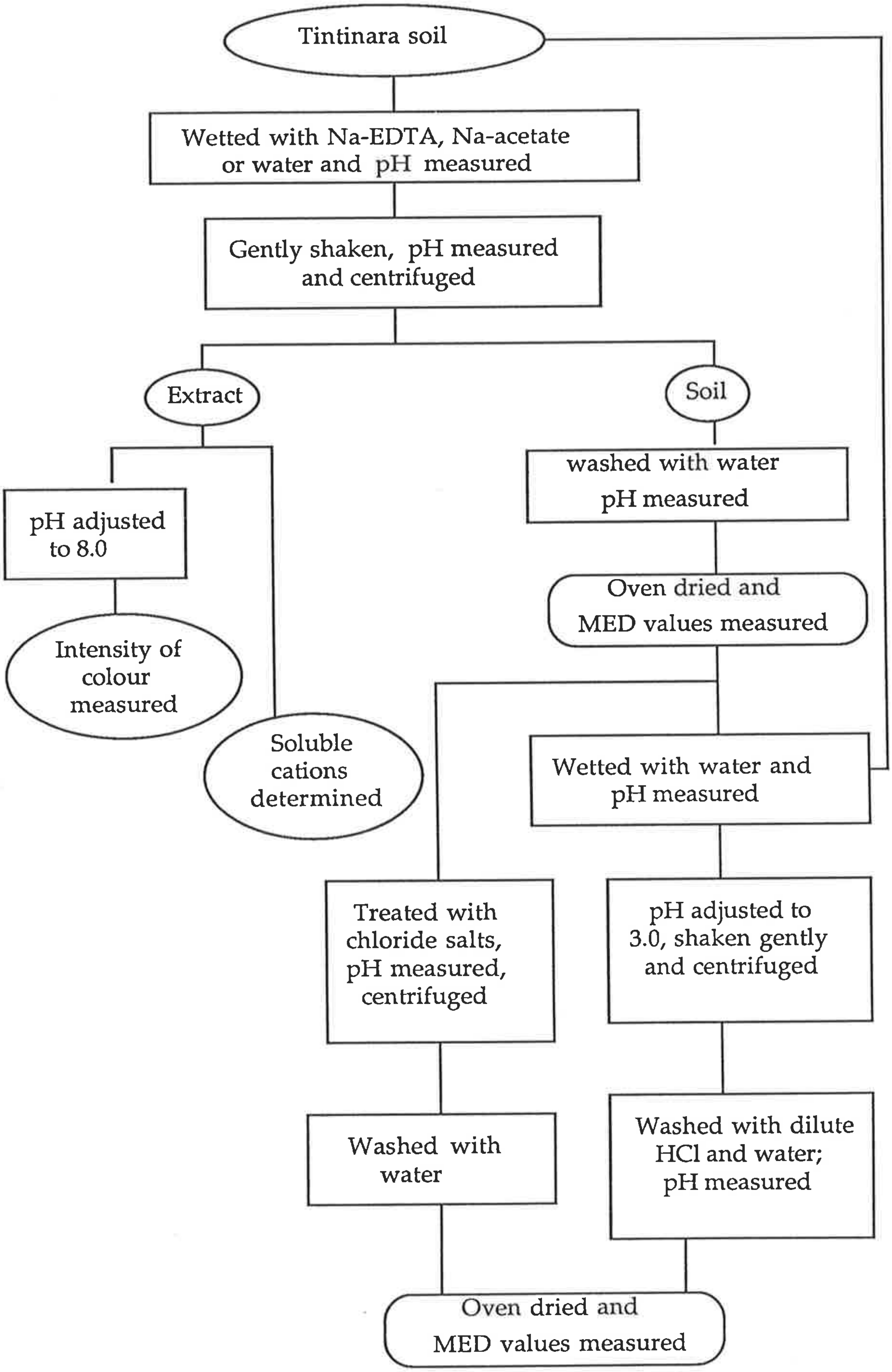

Tintinara soil
Wetted with Na-EDTA, Na-acetate or water and pH measured
Gently shaken, pH measured and centrifuged
Extract
Soil
pH adjusted to 8.0
Intensity of colour measured
Soluble cations determined
washed with water pH measured
Oven dried and MED values measured
Wetted with water and pH measured
Treated with chloride salts, pH measured, centrifuged
pH adjusted to 3.0, shaken gently and centrifuged
Washed with water
Washed with dilute HCl and water; pH measured
Oven dried and MED values measured

Plate 3.1. : A drop of water resides on the surface of Tintinara soil.

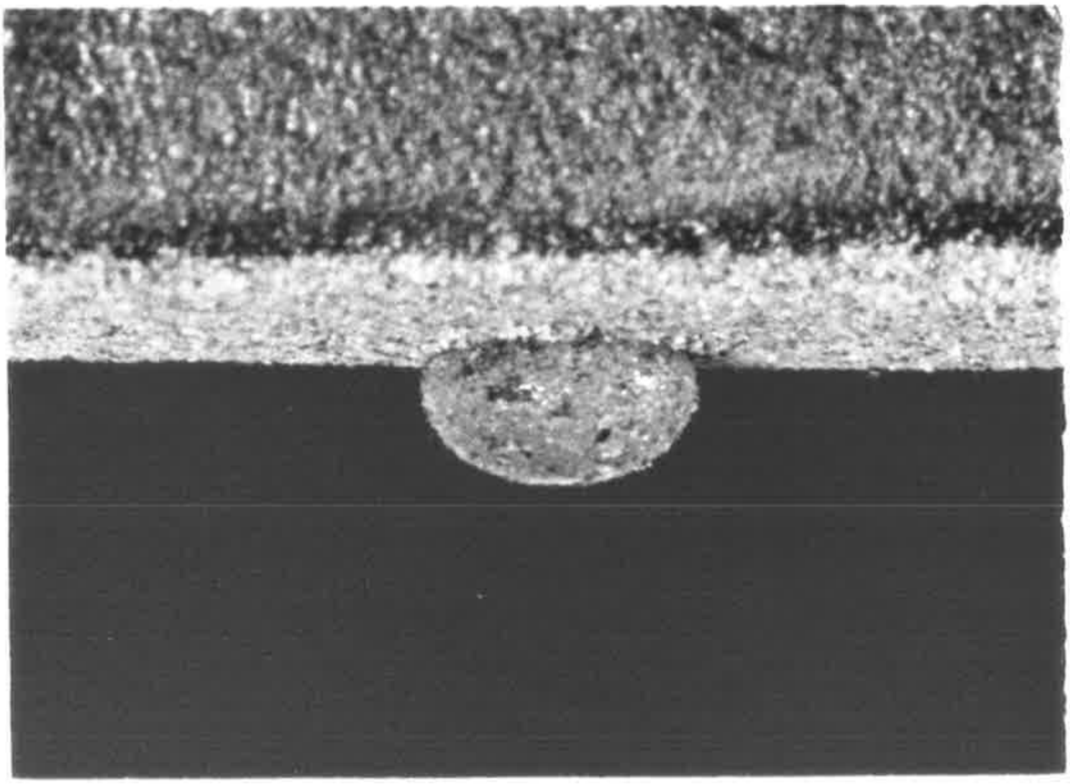


Table 3.1. Water-repellency and other properties of soil samples

Sample	Mechanical analysis			Total Carbon (%)	pH (1:5)		Water-repellency		
	clay (%)	silt (%)	sand (%)		H_2O	KCl	MED value	Height of capillary rise (cm)	Calculated contact angle (°)
MB	4.4	6.1	89.5	0.80	7.15	6.49	1.2	7.46	77.3
TA	6.9	8.3	84.8	1.25	6.72	6.15	3.5	-2.50	93.9
WH	4.2	4.9	90.9	0.53	7.45	6.95	1.9	6.41	80.5

3.3.2. Effects of wetting and drying techniques.

The water-repellencies of the soils examined were highly dependent on the drying procedure employed following water extraction (Table 3.2.). The effect of the two wetting techniques namely the evacuation technique and wetting with ethanol/water varied depending on the drying procedure. Wetting the soil with ethanol/water left the soil slightly more water-repellent than did the other techniques. This can be due to possible separation of an organic-rich fraction during extraction and handling during the evacuation procedure.

No drying procedure tested gave a product as water-repellent as the original soil, although samples oven dried from the moist condition still exhibited moderate water-repellency. Moist soils dried in air, even when subsequently oven dried were always less water-repellent than soils oven dried from the moist condition. For this reason oven drying of samples from the moist condition was selected in order to assess the effectiveness of aqueous extractants in removing water-repellency. As noted by McGhie and Posner (1980) chloroform treatment nearly always increased the water-repellency of the soils, however, considerable variation in the numerical extent of the increase in hydrophobicity was observed.

The most striking observation was that freeze drying removed water-repellency completely. The freeze dried soils were instantaneously wettable which was correlated with changes in capillary rise from -2.5, 7.46, and 6.41 to the values of 9.4, 9.9, and 10.1 for Tintinara, Murray Bridge and Wharminda soils respectively. Water-repellency was always recovered to some degree after rewetting and oven drying. It seems likely that this reversible phenomenon is due to changes in the molecular orientation of the organic matter which may be akin to a micellar inversion. Most forms of soil organic matter possess both hydrophilic and hydrophobic groupings. The hydrophilic groupings which include hydroxyl, carboxylic acid, carboxylate and amide will interact with water molecules when the soil is wet, but will tend to interact with each other in dry soils. In freeze dried soils, the molecular configuration of the wet organic matter will be preserved, so that the polar groups are exposed on pore surfaces in the organic matter, which is thus readily wetted.

Table 3.2. MED values following wetting and drying of the samples as indicated. Data along one line indicate successive treatments.

Sample	Untreated	Wetting techniques	Drying techniques			Rewetted and oven dried	$CHCl_3$ treatment
			Freeze	Air	Oven		
TA	3.5	Evacuation	0				0.7
				1.2	1.4		2.5
					2.2		2.6
			0		0	1.7	2.0
				1.2			2.2
		Ethanol/water	0				0.8
				2.3			2.6
					2.7		2.9
MB	1.2	Evacuation	0				0.3
				0.1	0.1		0.3
					0.2		0.5
			0		0	0.3	0.7
				0.1			0.5
		Ethanol/water	0				0.3
				0.8			1.0
					1.0		1.1
WH	1.9	Evacuation	0				0.5
				0.1	0.2		0.5
					0.3		0.7
			0		0	0.4	0.7
				0.1			0.5
		Ethanol/water	0				0.5
				1.0			1.4
					1.2		1.5

When the soil is oven dried, thermal reorientation of the polar groups to interact with each other can occur as water is lost, and the organic matter then presents largely non-polar groupings, for example methyl and methylene, on its surface. From this point of view, the marked hydrophobicity of the natural soils in the field could be interpreted as due to a particularly effective mutual interaction of organic polar groups. Again, the increase in water-repellency following chloroform treatment observed by McGhie and Posner (1980) may be due to a related molecular reorientation wherein the particulate hydrophobic organic matter in chloroform is redistributed by hydrogen bonding to hydrophilic particle surfaces on drying which would leave the hydrophobic ends exposed.

3.3.3. Effects of agitation.

After wetting, the soil tended to separate on sedimentation into a heavy, grey, sand-rich fraction; a black, finely particulate organic-rich fraction; and floating fraction consisting of larger plant remains. The composition of these fractions varied from one sample to another (Table 3.3.). The sand fraction was separated by decanting the organic-rich and floating fractions which remained in suspension after inversion of the soil-water mixture and allowing to settle for 10 s. This procedure was repeated 8-9 times until all the particulate organic-rich fraction was recovered.

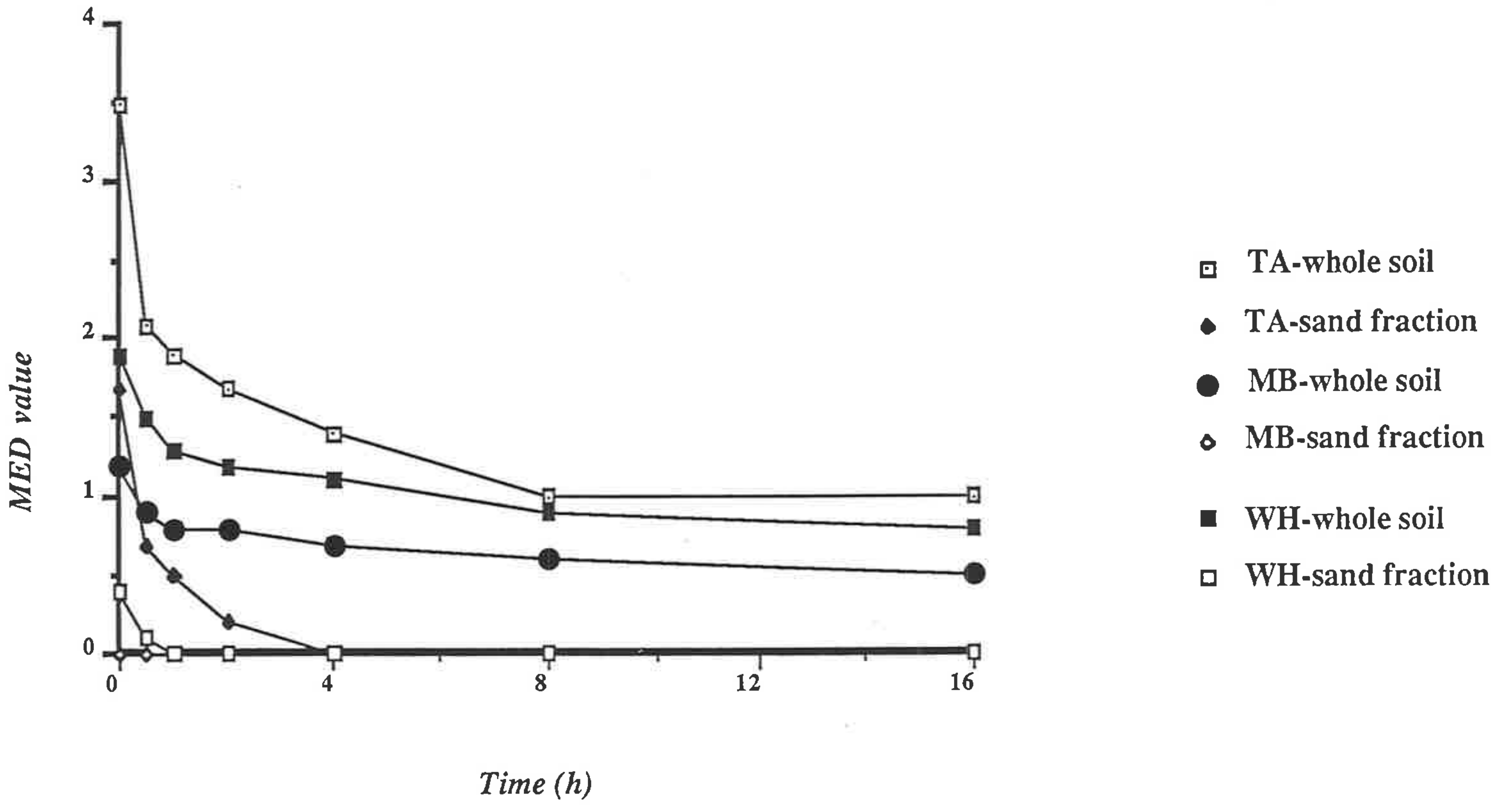
Table 3.3. Composition of organic and sand fractions and MED values of whole soils and sand fraction of Tintinara, Murray Bridge and Wharminda soils

Soils	Percentage of fractions			MED values	
	Floating material	Light fraction	Sand fraction	Whole soil	Sand fraction
Tintinara	0.47	5.89	93.64	3.5	1.7
Murray Bridge	0.07	6.18	93.75	1.2	0
Wharminda	0.19	3.55	96.26	1.9	0.4

It is likely that some water-repellent substances were present in both floating and light materials and in case of Murray Bridge soils, the bulk of the hydrophobic materials could be isolated by this simple technique. However, other techniques are necessary to remove completely these hydrophobic materials from other soils where they apparently coat the sand particles. Water-repellency of oven dried floating materials isolated from Tintinara soil was very severe with an MED value of 3.5. In addition, it is clear that water-repellency of the soils examined increased with an increase in the concentration of floating materials present in these soils. The importance of plant debris in inducing water-repellency was reported by Scholl (1971) who observed that wettability increased with depth below the litter zone and with decreasing organic matter. More recently, McGhie and Posner (1980) indicated that addition of 2 % of litter rendered a mallet soil more water-repellent. However, the effectiveness of comminuted plant litter in inducing water-repellency varied with plant species (McGhie 1980).

The effects of agitation were determined both on the whole soil and sand fraction. Water-repellency of soils wetted without shaking decreased slightly to MED values of 2.6, 1.0 and 1.6 for Tintinara, Murray Bridge and Wharminda soils respectively. The MED values of those samples which had been shaken gently were 2.4, 0.9 and 1.5. Fig 3.2. shows that sand fraction alone is much more susceptible to agitation becoming wettable in 4 and 1 h for Tintinara and Wharminda soils respectively. The sand fraction of Murray Bridge soils was originally wettable. These changes were probably due to the abrasive removal of organic matter from sand surfaces. A similar result was reported by Bond (1962) who considered that the decrease in water-repellency was due to removal of fungal hyphae coating the sand grains. Conversely, the whole soil retains significant hydrophobicity (MED values of 1.0, 0.5, and 0.8 for Tintinara, Murray Bridge and Wharminda soils respectively) even after 16 h shaking. Hence the residual water-repellency must be due to admixed discrete particulate organic matter. The hydrophobicity of the original soils therefore resides both on the sand particle coatings and the inter-mixing of partially decomposed plant remains with the soil.

Fig.3.2. : Effect of agitation on water-repellency of whole soils and sand fractions of three South Australian sandy soils


MED value
4
3
2
1
0
0
4
8
12
16
Time (h)
TA-whole soil
TA-sand fraction
MB-whole soil
MB-sand fraction
WH-whole soil
WH-sand fraction

These results indicate that, to estimate the effectiveness of an extraction technique, shaking should be restricted and care must be taken to redistribute discrete particulate organic matter through the residual soils.

3.3.4. Na-EDTA and Na-acetate treatments.

These treatments were only employed for the most water-repellent soils i.e. Tintinara soils and the results are presented in Table 3.4. below. Results indicated that generally Na-EDTA solutions reduced water-repellency of Tintinara soils more effectively than did Na-acetate solutions. One logical explanation for this observation is that Na-EDTA would have preferentially formed complexes with the elements Al, Fe, Ca, or Mg. For example, if the hydrophobic organic matter is represented as R-COOH one can formulate the reaction as :

$$(\text{R-COO})_2\text{Ca} + 2\,\text{Na-EDTA} \xrightarrow{} 2\,\text{R-COONa} + \text{Ca EDTA}$$

The salt R-COONa would not become soluble, however, the more wettable residual soil caused by the presence of the $[\text{Na}(\text{H}_2\text{O})_n]^+$ indicated that the R-COONa salt was formed. The importance of cations balancing the exchange capacity of organic matter in determining the water-repellency of organic matter was previously suggested. Davies (1957) reported that the COOH form of the carboxyl group was far less hydrophilic than the COONa form. Similarly Tschapek *et al* (1973) also found that humic acid was more hydrophobic than Na-humate. In addition, Wander (1949) indicated that occurrence of water-repellency in soils could be associated with the presence of calcium and magnesium forming non-wettable salts with fatty acids.

Table 3.4. The effects of Na-EDTA and Na-acetate treatments on water-repellency of Tintinara soils.

Treatment	pH of soil suspension	pH after shaking	Optical density at 400 nm	Final pH	MED values
Untreated		-	-	-	3.5
Water	6.23	6.26	0.10	-	2.2
Na-EDTA					
pH 3.75	3.86	3.96	0.79	5.76	2.0
pH 5.00	4.30	4.32	1.65	6.05	1.4
pH 7.00	6.74	6.75	4.60	7.72	0.6
Na-acetate					
pH 3.75	3.89	3.97	0.20	4.75	2.2
pH 5.00	4.95	4.98	0.21	5.87	1.9
pH 7.00	6.76	6.77	0.32	7.79	1.0

Data presented in Table 3.5. show that Na-EDTA extracted greater amounts of cations compared to those extracted by Na-acetate. As a chelating agent EDTA contains four donor groups which is sufficient to permit bonding with all coordination positions of cations. Hence, the complexes formed by this type of chelating agent are more stable than those formed by coordination with groups having monovalent ligands such as acetate (Welcher 1958 and Heald 1965). The removal of cations would have disrupted cation linkages within organic matter and between organic matter and sand particles which in turn resulted in an increase in the solubility of organic matter. More organic material was removed by Na-EDTA solution as revealed by the intensity of colour of the extracts. However, only a portion of alkaline soluble organic matter such as humic acids would have been isolated by this technique while aliphatic compounds would remain in the residual soil. Again the measurable water-repellency of residual soils may be ascribed to those unextractable compounds.

Table 3.5. Soluble cations in soil extracts

Extractants	Fe	Al	Ca	Mg	K
	(ppm)			(me 100 g^{-1})	
Water	2.0	0.8	0.80	0.40	0.04
Na-EDTA					
pH 3.75	101.6	95.2	4.32	0.31	0.16
pH 5.00	92.8	124.8	4.16	0.42	0.13
pH 7.00	104.0	144.8	4.72	0.54	0.14
Na-acetate					
pH 3.75	11.2	18.4	3.28	0.28	0.10
pH 5.00	14.4	41.6	2.24	0.24	0.08
pH 7.00	12.8	25.6	2.00	0.23	0.09

The effectiveness of both Na-EDTA and Na-acetate in removing organic material and hence in decreasing water-repellency is a function of the pH of the solutions. The more alkaline solutions rendered the soil more wettable. The importance of acidity or alkalinity of extractants was observed by Bond (1969b) who found that some alkaline reagents always yielded dark brown solutions and reduced the contact angle of wetting.

An attempt was made to return the soil to its original condition, without the Na^+ making it wettable. This was conducted by treating the extracted soils with HCl. Results (Table 3.6) show that there was a slight increase in water-repellency following removal of Na^+ from the most alkaline soils. This finding agrees well with the result found in our laboratory previously that hydrophobicity was always restored following acidification with formic acid (Mr. B.M. McKenzie, personal communication) . McGhie (1980) also found that water-repellency of comminuted mallet litter was reduced by increasing the pH to 10 with NaOH but the water-repellency was restored by acidifying to pH 3 with HCl.

Table 3.6. Effects on water-repellency of acidification of soil samples which had been treated with either Na-EDTA or Na-acetate

Samples	Original MED values	pH before adjusting	Final pH	Final MED values
Untreated	3.5	6.48	5.59	2.4
Treated with				
-water	2.2	6.22	5.37	2.1
-Na-EDTA				
pH 3.75	2.0	5.25	4.40	2.1
pH 5.00	1.4	6.11	4.90	1.4
pH 7.00	0.6	7.43	6.07	1.0
-Na-acetate				
pH 3.75	2.2	5.14	4.65	2.1
pH 5.00	1.9	6.09	4.70	2.1
pH 7.00	1.0	7.54	5.57	1.7

3.3.5. Treatment with chloride salts.

An increase in water-repellency was also detectable when the Na-EDTA and Na-acetate treated soils were further washed with calcium, magnesium or potassium chlorides (Table 3.7.), whereas sodium chloride washing further increased the wettability of the soils. This observation again emphasises the importance of cations present on water-repellent surfaces, yet the mechanisms of the role of cations needs further clarification. The presence of either calcium or magnesium would have affected orientation of hydrophobic organic matter which had not been removed by the aqueous solutions. The hydrophilic groups of such organic matter was possibly cation bridged to the sand surface and left hydrophobic tails exposed on the surface. Such an orientation would not be expected to occur in a sodium saturated system.

Table 3.7. Restoration of water-repellency as affected by cations

Sample	MED values				
	Before salt treatment	After salt treatment			
		NaCl	KCl	$CaCl_2$	$MgCl_2$
Na-EDTA treated					
-pH 3.75	2.1	1.7	1.4	2.8	2.1
-pH 5.00	1.4	1.2	1.0	2.4	1.9
-pH 7.00	0.7	0.3	1.2	2.1	1.7
Na-acetate treated					
-pH 3.75	2.3	1.9	1.4	2.1	2.4
-pH 5.00	1.9	1.7	1.2	1.9	1.9
-pH 7.00	1.0	1.0	1.0	1.6	1.7

3.4. Conclusions

The occurrence of water-repellency in South Australian soils is caused by organic materials present both in coatings on sand particles and the inter-mixing of partly decomposed plant remains into the soils. These hydrophobic substances may be partly extracted with aqueous solvents leaving the residual soils with some degree of water-repellency. The molecular configuration of hydrophobic organic molecules present, however, has an important role in determining whether or not a soil is water-repellent. Hence, other factors such as the type of cations occupying the exchange surfaces influencing the orientation of such molecules are also of importance.

To estimate the effectiveness of an extraction technique, shaking should be restricted and care must be taken to redistribute discrete particulate organic matter through the residual soils.

CHAPTER 4

EXTRACTION OF HYDROPHOBIC MATERIALS FROM SOILS

4.1. Introduction

The occurrence of water-repellency in soil is generally associated with coatings of organic materials on the sand particles. Hence, considerable efforts have been made to remove these materials from water-repellent soils. The usual "lipid solvents" such as ether and chloroform have removed some fats and waxes from soils but in nearly all cases treatments of water-repellent soils with non-polar solvents have left the soils hydrophobic. The only solvents found to be very effective in increasing wettability of water-repellent soils are mid to strong alkaline solutions such as sodium hydroxide, sodium carbonate and sodium pyrophosphate (Bond 1962, Roberts and Carbon 1972 and McGhie 1980). Roberts and Carbon (1972) observed that wettable ignited sand became water-repellent following treatment with the humic fraction from a sodium carbonate extract, and concluded that water-repellency in original soils was due to humic acids. However, Savage. *et al* (1969a) reported that only humic acid isolated from *Stachybotrys atra* induced water-repellency on sand and soil while after treatment with two other microbial humic acids, soil and peat humic acids sands remained hydrophilic.

It was clearly necessary to establish whether the failure by earlier workers to totally remove hydrophobic materials from the surface of soil particles was a consequence of covalent bonds to the surface which ruptured by alkali with consequent degradation of the molecules under these alkaline conditions or whether it was simply a matter of an inappropriate choice of organic solvents or condition. In order to answer this question the efficiency of various extraction procedures was examined and the water-repellency of both the extracted materials and residual soils were measured. Because the MED value is known to be reduced by agitation (King 1981), the Soxhlet extraction technique was chosen to minimize abrasion and hence exposure of hydrophilic surfaces during the extraction. The extracted hydrophobic components were then applied to wettable acid washed sand in order to determine to what extent the hydrophobicity of sands could be regenerated.

4.2. Materials and methods

4.2.1. Soil

Soil samples were obtained from Wharminda on the Eyre Peninsula South Australia, from Murray Bridge and Tintinara in the South East region of South Australia and from Western Australia.

4.2.2. Extaction with benzene/ethanol.

The soils were washed with 0.1 M HCl and with water to remove complexing cations that were combined with acidic organic matter before extraction with the mixture of benzene/ethanol (2:1, by volume) in a Soxhlet extraction apparatus for 8 hours. Residual soils and extract were dried in a fume hood. Water-repellency of dried residual soils was assessed by the MED test and the capillary rise technique.

4.2.3. Humic acid extraction.

Original soils and soils extracted with benzene/ethanol (25 g) were extracted with 50 ml of 0.5 M NaOH for 36 h. The soil-solution mixtures were inverted once every 8 h. The residual soil was recovered by centrifugation, acidified with HCl to pH 3.00, and washed with water three times by centrifugation. This residue was oven dried before determination of water-repellency. The dark coloured supernatant was centrifuged and the supernatant was filtered through glass wool to separate floating materials. The pH of the solution was adjusted to 1.00 by adding 5 M HCl to precipitate humic acid. The precipitate was allowed to settle overnight. The excess supernatant liquor was siphoned off and the remainder was centrifuged in a polythene bottle. Again the excess supernatant was siphoned off. The precipitate was purified by redissolving in 0.5 M NaOH, acidifying, washing with water until it was free from chloride and then freeze dried.

4.2.4. Fractionation of humic acid.

The humic acid obtained was fractionated by successive extractions with light petroleum ether and benzene/ethanol (2:1, by volume) in a Soxhlet apparatus. The extractions were terminated when the extract outside the thimble was colourless. The extracts were then evaporated to dryness in a fume hood.

4.2.5. Extraction with organic solvents.

The soils were extracted with various organic solvents (Table 4.2.) for 16 h or 48 h using a Soxhlet apparatus. The extracted soils were air dried in a fume hood and then oven dried at 105°C prior to assessment of their water-repellency. The extracts were evaporated to dryness in a fume hood.

4.2.6. Assessment of water-repellency of extracted materials and reference molecules.

The ability of humic acids and their fractions to induce water-repellency was investigated in the following experiments :

(1) Humic acids were dissolved in 7 M ammonia. Fractions were adjusted to 10 mg ml^{-1} of solvent. Two hundred µl of the solution was pipetted onto a 2 x 2 cm^2 glass paper and oven dried prior to the MED test.

(2) Dissolved materials were poured onto wettable acid washed sand (BDH Chemicals). After mixing the solvents were evaporated and the sand dried at 105°C for 2 h. The water-repellency of the coated sand was measured by the MED test.

The water-repellency of materials extracted with organic solvents from water-repellent soils and of reference molecules were assessed according to the procedure described above (2).

4.3. Results and discussion

4.3.1. Water-repellency of residual soils

All soils examined (Table 4.1.) were rendered wettable after successive extractions with benzene/ethanol, HCl , water and finally with sodium hydroxide. These soils were also rendered wettable after being extracted with the alkaline solution by itself even though agitation was minimised during extraction. The pale coloured residual soils remained hydrophilic after chloroform treatment. This suggested that the bulk of the water-repellent materials were in the dark coloured alkaline extract which could be removed from this type of soil. Such alkaline solutions have also been employed to extract hydrophobic materials from water-repellent soils of various origin (Bond 1962, Roberts and Carbon 1972, McGhie 1980). Calculated contact angles (Emerson and Bond 1963) of exhaustively extracted soils were 70.8°, 35.7° and 65.1° for Tintinara, Murray Bridge and Wharminda soils respectively compared to 93.9°, 77.3° and 80.5° for the original soils.

The relative effectiveness of various solvents for extracting water-repellent materials from Tintinara soil is shown in Fig. 4.1. From these data it is clear that there is a continual decrease in the residual water-repellency of extracted soils with time.

However, it is also apparent that removal of water-repellent materials is controlled by the polarity of the solvents. Non-hydroxylic solvents such as ether, tetrachloroethylene and chloroform had little effect in reducing water-repellency of soils. A similar result was reported by Jamison (1945) who concluded that any effect was due in part to the removal of small amounts of oils, waxes and other organic materials. Short term extraction of hydrophobic soils with ether (Van't Woudt 1959) or chloroform (McGhie and Posner 1980) showed either no significant effect or in the case of chloroform treatment actually increased water-repellency (McGhie and Posner 1980, Ma'shum and Farmer 1985). This increase has been ascribed to either molecular reorientation of water-repellent organic matter such as alkyl chains or redistribution of hydrophobic matter on exposed hydrophilic surfaces.

Morrison (1969) proposed that a mixture of benzene/ethanol (2:1, by volume) was the best solvent for extracting soil lipids. This amphiphilic solvent was certainly found to

Table 4.1. Water-repellency of extracted soil and concentration of materials removed

Sample	MED values (M)				Height of Capillary rise (cm)				Material removed with	
	Original	After extraction with			Original	After extraction with			Benzene/ ethanol	NaOH
		Benzene/ ethanol	HCl and water	NaOH		Benzene/ ethanol	HCl and water	NaOH	(mg kg^{-1})	
Tintinara	3.5	2.0	1.7	0	-2.50	4.38	4.05	12.22	687	5 600
	3.5			0	-2.50			13.52		5 700
Murray Bridge	1.2	1.1	1.1	0	7.46	2.47	5.89	27.66	899	3 200
	1.2			0	7.46			22.49		3 300
Wharminda	1.9	1.2	1.0	0	6.41	1.74	1.70	16.36	304	1 300
	1.9			0	6.41			17.89		1 300

Note : data in one line indicate successive treatments.

be more effective than the non polar solvents employed. However, Fig. 4.1. shows the extracted soil still retained a substantial degree of water-repellency. The most effective solvent in terms of its capability to render water-repellent soils wettable was the mixture of *iso*-propanol and 15.7 M ammonia (7:3, by volume). Under Soxhlet reflux conditions this amphiphilic solvent mixture completely removed the hydrophobic factor within 8 h and was equally effective on soils from Eyre Peninsula and Western Australia (Table 4.3) as well as on a non wetting compost. The extract were dark brown ($A^{1\%}_{273\ \mathrm{nm}}$ 450 nm = 5.63).

The inability of less polar solvents to remove lipids from soils has been well documented and it has been suggested that some organic compounds may be linked with other compounds making them generally insoluble to less polar solvents (Hance and Anderson 1963; Johnson 1971). However, the solubility and partition properties of the material extracted by the current Soxhlet procedure suggest that the major problem concerns the rupture of hydrogen bonds to or from the surface which are strengthened in aprotic solvents. Methanol or ethanol used as mixtures with chloroform or benzene have been employed to liberate protein-bound lipids, but by themselves they are poor solvents for most lipids. Kates and Eberhardt (1957) reported that they were able to remove total lipids from leaves using hot *iso*-propanol. Hara and Radin (1978) suggested that the mixture of hexane/*iso*-propanol has several desirable properties compared with conventional mixtures such as chloroform/methanol. The material extracted by hexane/*iso*-propanol was noted by these investigators to contain less non-lipid materials. Despite the superiority of *iso*-propanol in removing lipids, this solvent has not hitherto been applied to the problem of extracting hydrophobic materials from water-repellent soils.

Fig. 4.1. : Effect of solvents on water-repellency of Tintinara soil. The *iso*-propanol/ammonia data is coincident with the *iso*-propanol/water data at 24 h and beyond.

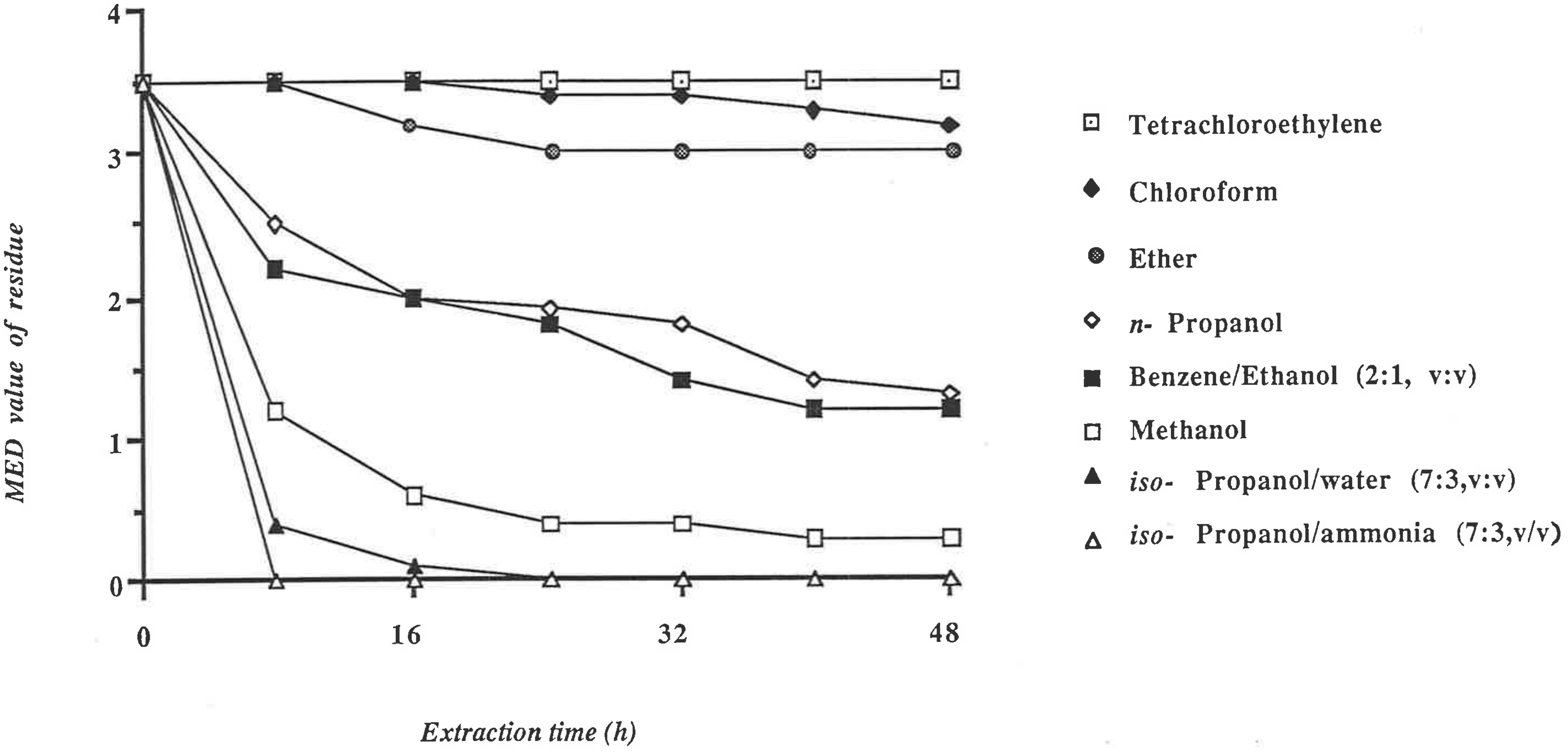

MED value of residue
4
3
2
1
0
0
16
32
48
Extraction time (h)
⊡ Tetrachloroethylene
◆ Chloroform
◉ Ether
◇ *n-* Propanol
■ Benzene/Ethanol (2:1, v:v)
□ Methanol
▲ *iso-* Propanol/water (7:3,v:v)
△ *iso-* Propanol/ammonia (7:3,v/v)

Table 4. 2 : Solvents used to extract water-repellent materials from a Tintinara soil (MED value 3.5)

Extractants	Amount of material extracted ($mg\ kg^{-1}$)	Hydrophobicity of residual soil (MED value)
Benzene/Ethanol (2:1,v:v)	1180	2.0
Chloroform	600	3.5
Ether	200	3.0
Tetrachloroethylene	650	3.5
Methanol	1230	0.6
n-Propanol	1330	2.2
iso-Propanol/water (7:3, v:v)	1690	0.1
iso-Propanol/15.7M ammonia (7:3, v:v)	1880	0

4.3.2. Water-repellency of extracted materials.

Humic acids extracted from Tintinara, Murray Bridge and Wharminda soils produced various degrees of water-repellency on glass paper and created MED values of 1.1, 0.3, and 0.5 respectively. However, acid washed sand coated with these humic acids at concentrations equal to their natural occurrence (Table 4.1.) remained wettable. A drop of water penetrated the coated sand immediately. The amount of humic acids coating the glass paper was 0.5 mg cm^{-2}. Acid washed sand used in this study had an estimated surface area of 10 $m^2\ kg^{-1}$. To obtain the same concentration as those on the glass paper 50 g humic acid was necessary to coat 1 kg of acid washed sand. Such a concentration (5 %) is unlikely to occur in most sandy soils. Application of humic acids to acid washed sand at concentrations up to 5 % did not influence the wettability. A chloroform treatment aimed to redistribute the hydrophobic materials did not alter the wettability of the sand.

Savage *et al* (1969a) did not observe any measurable water-repellency on sand and soil which had been coated with 0.05 and 0.5 % soil humic acids respectively. On the other hand, Roberts and Carbon (1972) reported that humic acids isolated from water-

Table 4.3. : Water-repellency of soils before reduction of their MED values to 0 by Soxhlet extraction with the mixture of *iso*-propanol/15.7M ammonia

Soil samples	Clay content (%)	Carbon content (%)	Water-repellency		Amount of hydrophobic materials extracted (mg kg^{-1})
			MED values	Height of capillary rise (cm)	
Tintinara 1	6.9	1.34	3.5	-2.5	1880
Tintinara 2	3.8	0.32	1.9	1.2	770
Murray Bridge	4.4	1.03	1.4	7.5	1810
Eyre Peninsula 1	4.2	0.60	1.9	5.6	890
Eyre Peninsula 2	2.1	0.47	2.4	3.8	1060
Eyre Peninsula 3	4.2	0.36	1.9	2.2	1700
Eyre Peninsula 4	2.1	0.67	3.9	-0.4	1750
Eyre Peninsula 5	4.2	0.24	0.5	6.3	450
Eyre Peninsula 6	4.2	0.33	2.2	2.2	720
Eyre Peninsula 7	2.1	0.49	3.1	0.8	1540
Tuart sand	2.1	1.18	2.0	1.7	2660
Trezise Gavin sand 1	2.1	3.41	1.4	2.2	4560
Trezise Gavin sand 2	4.2	2.22	1.4	0.4	2380
Soils under Brown Mallet Trees	14.6	7.14	3.0	-1.7	26290
Urrbrae	74.2	1.40	0	n.e. *	1830

* Not examined

repellent Gavin sands induced hydrophobicity on ignited sand. However, these workers did not specify the particular concentration of such humic acids deposited on the sand. Despite the differing effects of humic acids on wettability of sands, Tschapek *et al* (1973) suggested that humic acid itself is hydrophobic. They found that a drop of water placed on the surface of fine triturated humic acids tended to ball up making a contact angle of 101°. They also observed that a powder of humic acid floated on water. These workers indicated that humic acids possess both hydrophilic and hydrophobic groups, hence they meet the requirements necessary to be compounds which are able to create water-repellency on surfaces as suggested by Bozer *et al* (1969).

It is likely that the origin of humic acid is of particular importance in determining its ability to induce water-repellency. The findings of Savage *et al* (1969a) and Adhikari and Chakrabarti (1976) support this suggestion. These groups of workers reported the occurrence of water-repellency produced by humic acids of microbial origin. Therefore, apparently water-repellency induced by humic acids is a function of its concentration and its origin.

Humic acids extracted from these soils were further extracted with petroleum spirit (b.p. 60-80°C) and then with the mixture of benzene/ethanol (2:1, by volume) to isolate lipid materials. None of the humic materials were soluble in light petroleum. The amount of benzene/ethanol soluble materials and their water-repellency are presented in Table 4.4. Residual humic acids insoluble in both these solvents did not produce water-repellency upon acid washed sand at concentrations up to 50 g kg^{-1}. From Table 4.4. it can be seen that the hydrophobic components of these humic acid fractions represent no more than 15 % of the total humic acid content of the soils. And the restoration of this material to a hydrophilic sand at concentrations equivalent to their original loadings in the soil were insufficient to account for the original water-repellency as measured by the MED test. These results clearly demonstrated that organic materials responsible for water-repellency in humic acids were readily extracted by a benzene/ethanol solvent mixture. This suggested that with suitable solvent mixtures, the direct removal of such materials directly from water-repellent soils might render those soils measurably hydrophilic.

Despite the differing efficiencies of the solvents used in Table 4.2., all of them extracted some water-repellent materials from the soils and application of these materials to acid washed sand at an arbitrary constant concentration (400 mg kg^{-1}) resulted in significantly different degrees of water-repellency as measured by the MED values (Table 4.5.).

These data suggest that some components of the extracts were much more effective in inducing a water-repellent surface than others.

Table 4.4. Concentration and water-repellency of humic acid fraction soluble in benzene/ethanol.

Origin of humic acids	Soluble in benzene/ ethanol (%)	Amount (mg) deposited on 1 kg acid washed sand*	MED value	
			Humic acid	Original soil
Murray Bridge soil	15.0	476	1.1	1.2
Tintinara soil	11.9	608	2.1	3.5
Wharminda soil	15.0	192	1.5	1.9

* These amounts represent their calculated concentration in original soils.

The most striking observation was that the most effective solvent did not necessarily remove the most water-repellent materials. In addition, there was no correlation between the total amount of material removed and the water-repellency of original soils. This fact may be rationalized by assuming the presence of differing ratios of hydrophobic to hydrophilic components in the crude mixture. Consequently, in an overall explanation of the wettability of soils there is a need to examine the nature and concentration of the hydrophilic components as well as the nature and concentration of hydrophobic species. However, this thesis is primarily concerned with the latter.

Table 4.5. : Water-repellency of acid washed sand after treatments with materials extracted from Tintinara soils and several reference compounds.

Extracts and reference substances	MED values of acid-washed sands after treatments	
	Concentration 400 mg/kg	Concentration as naturally present
Benzene/Ethanol (2:1,v:v)	4.8	6.5 *(1180)
Chloroform	5.0	5.0 (600)
Ether	5.2	4.0 (200)
Tetrachloroethylene	5.0	5.2 (650)
Methanol	2.7	2.9 (1230)
n -Propanol	2.6	3.3 (1330)
iso -Propanol/water (7:3,v:v)	2.9	3.4 (1690)
iso -Propanol/15.7M ammonia (7:3,v:v)	2.9	3.4 (1880)
Palmitic acid	6.0	
Cetyl alcohol	7.2	
Tristearin	5.0	

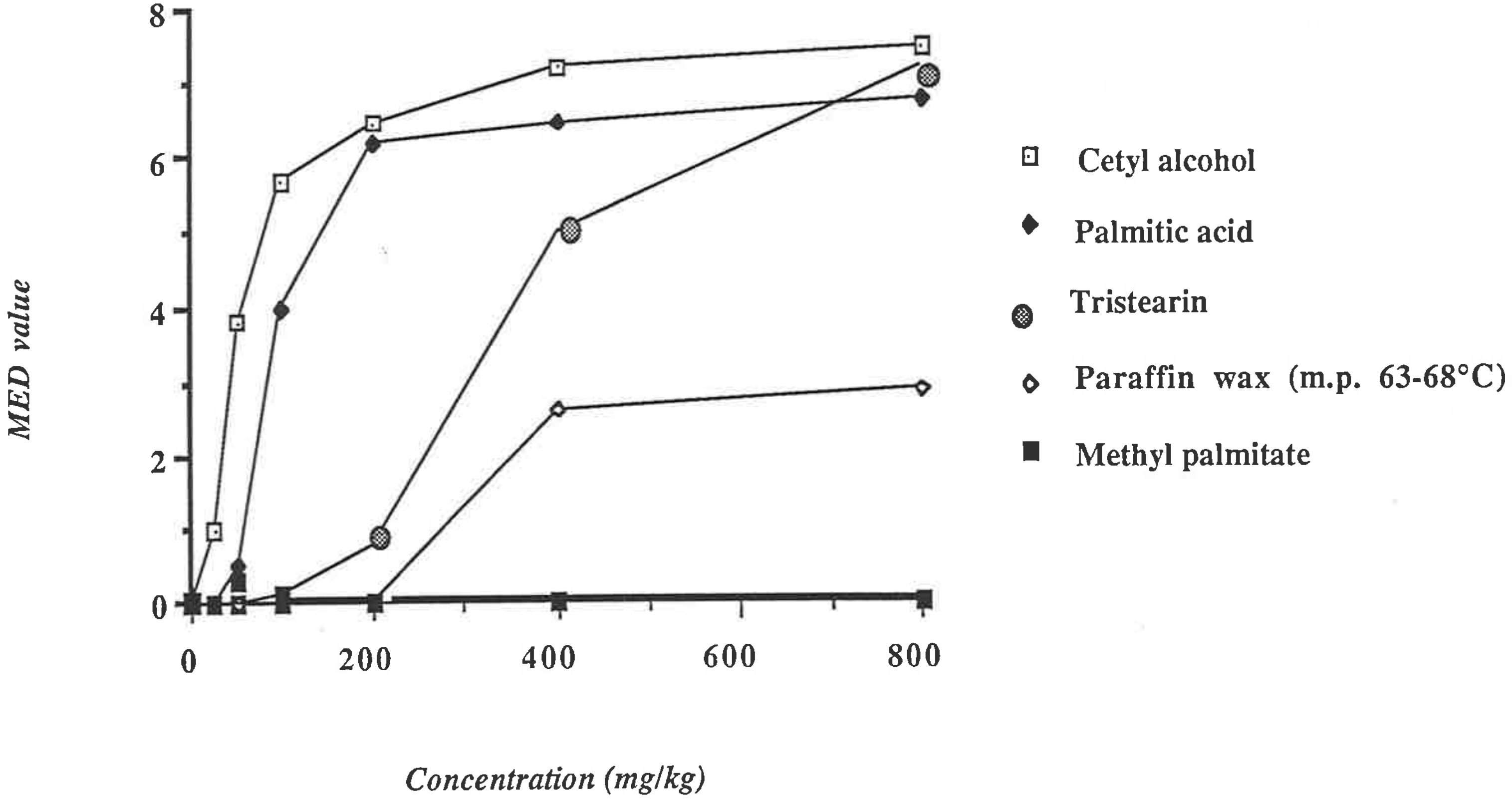
*From Table 4.2.

4.3.3. Concentration dependence of water-repellency.

The degrees of water-repellency of both extracted materials and reference molecules on acid washed sand were concentration dependent (Fig. 4.2.).

The close packed cross sectional area of a straight chain alcohol such as cetyl alcohol (16 carbon atoms) is 0.21 nm^2. This corresponds to 4.8 x 10^{18} molecules m^2 or 77.4 x 10^{-6} mol m^2 which equals to 77.4 μmol m^2 arranged end on to the surface. The commercial acid washed sand used in this study had an estimated surface area of 10 m^{2}kg^{-1} and would thus require approximately 77.4 μmol kg^{-1} for a uniformly distributed close packed monolayer. Hence in the case of cetyl alcohol (molecular weight of 242.43) only 18.7 mg kg^{-1} should suffice to provide such a layer. However, Fig. 4.2.

Fig. 4.2. : Water-repellency of acid washed sand after treatment with several reference compounds.


8
6
4
2
0
MED value
0
200
400
600
800
Concentration (mg/kg)
□ Cetyl alcohol
◆ Palmitic acid
● Tristearin
◇ Paraffin wax (m.p. 63-68°C)
■ Methyl palmitate

demonstrates that for cetyl alcohol at least 25 mg kg^{-1} or 103.3 µmol kg^{-1} were required to induce measurable hydrophobicity in the MED test and in order to reach the plateau region of maximum hydrophobicity at least 400 mg kg^{-1} or 1.653 µmol kg^{-1} were necessary. In other words, calculations show that measurable hydrophobicity only occured when the sand grains were uniformly coated by at least a close packed monolayer of cetyl alcohol and maximum water-repellency was attained only when there was a 16 fold excess. The thickness of the layer for maximal hydrophobicity with cetyl alcohol with a chain length of 2.24 nm would be nearly 36 nm thick. A scanning electron micrograph of the sand coated with cetyl alcohol (Plate 4.2.) shows a papillate appearance of coalescent globules (diameter ~ 150-300 nm) covering the sand grains. This suggests that the organic materials coat the sand grains non-uniformly. One explanation for such a vast excess of lipid material over that necessary for a monolayer formation in order to induce the water-repellency to the plateau region, could be that organic materials or in the case of cetyl alcohol rapidly coats the surface of the water droplet, such an explanation implies that droplet stabilisation by surface monolayer formation is an important factor in its hydrophobic response.

Fig 4.2. also indicated that the most effective organic compound to produce water-repellency was cetyl alcohol. In all cases, alcohols always produced greater hydrophobicity than the corresponding acids (Fig. 4.3.). Davies (1957) also reported that compounds with hydroxyl groups such as phenols and alcohols were less hydrophilic than carboxylic acids. Bozer *et al* (1969) suggested that hydroxylic phenolic compounds which are common constituents of natural resins and vegetative matter could be responsible in part for the water-repellence of "burned-over" watersheds.

4.4. Conclusions

The hydrophobic materials of the Australian soils examined in this study may be completely extracted in a Soxhlet apparatus with an amphiphilic solvent mixture of *iso*-

propanol/ammonia. All water-repellent soils studied were rendered wettable following extraction with these solvents. The extracted materials induced water-repellency on acid washed sand at levels comparable to the original soils.

Plate 4.1. A scanning electron micrograph of acid washed sand.

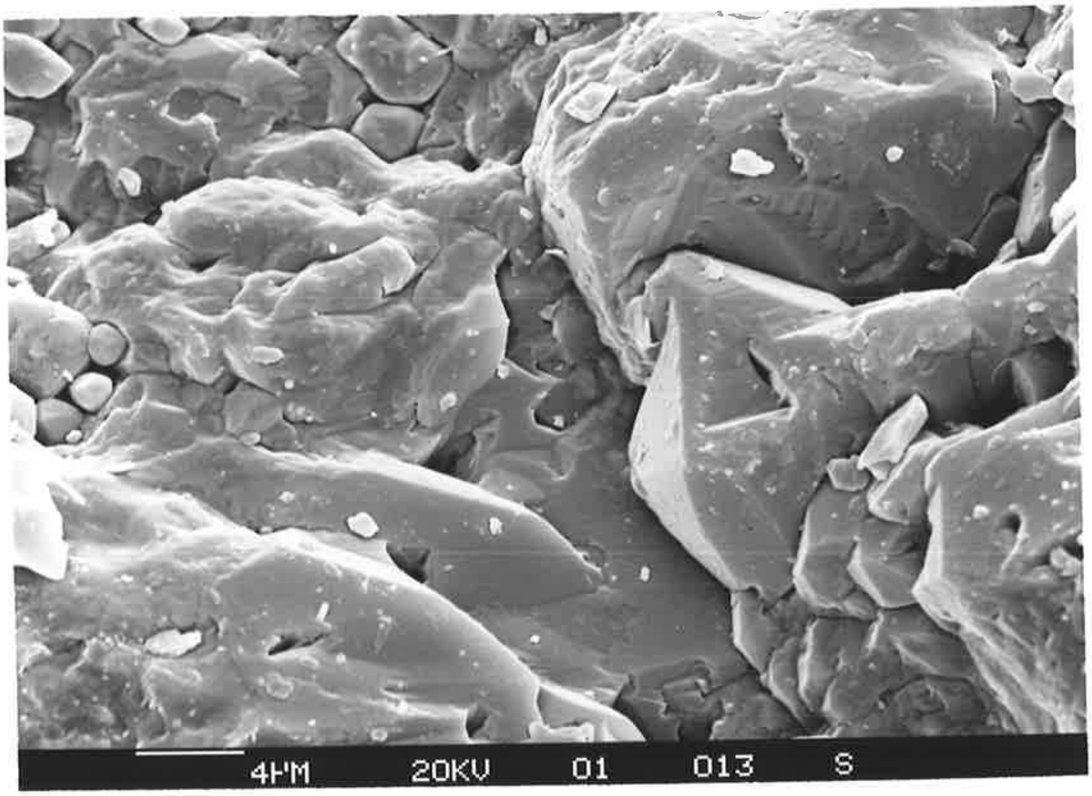

4µM
20KV
01
013
S

Plate 4.2. A scanning electron micrograph of cetyl alcohol-coated sand.

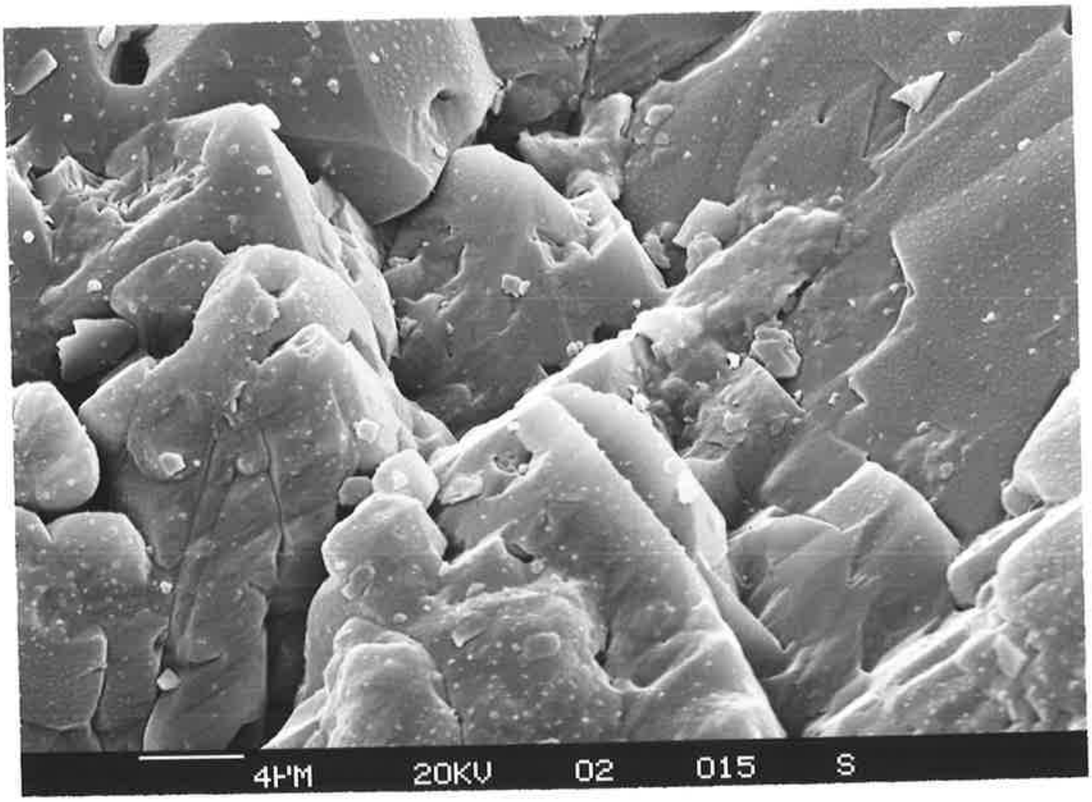
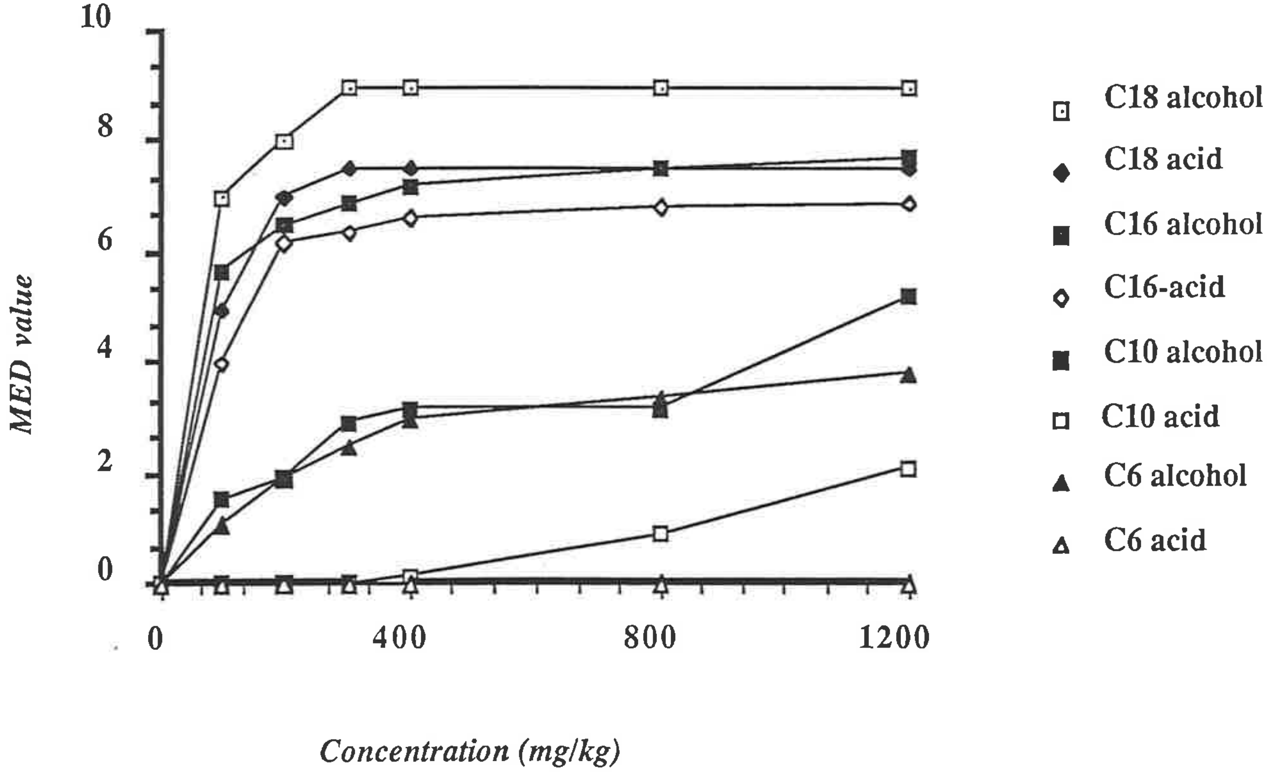

4μM
20KV
02
015
S

Fig. 4.3. Water-repellency of acid washed sand coated with reference fatty acids and alcohols.


10
8
6
4
2
0
MED value
0
400
800
1200
Concentration (mg/kg)
C18 alcohol
C18 acid
C16 alcohol
C16-acid
C10 alcohol
C10 acid
C6 alcohol
C6 acid

CHAPTER 5

CHARACTERIZATION OF HYDROPHOBIC MATERIALS

5.1. Introduction

As shown in Chapter 4 the materials responsible for water-repellency in Australian soils may be completely extracted with a mixture of *iso* -propanol/ammonia. The chemical characterization of the hydrophobic components of these extracts forms the subject of this chapter.

5.2. Materials and methods.

5.2.1 Soils.

Soils used in this study have been described in Chapter 3.

5.2.2. Extraction of cuticular waxes for comparative purposes.

Cuticular waxes from *Eucalyptus* leaves were isolated by washing with hexane (Horn *et al* 1964) and the solvent was evaporated in a fume hood.

5.2.3. Sublimation.

The crude benzene/ethanol extract was vacuum sublimed using the apparatus of Linstead *et al* (1955) with a slight modification at 240°C in a metal bath. This particular procedure is referred to as quasisublimation (Cheronis 1954) because the vapour was derived from the melt of materials. The melting points of the original extracted materials were 80-106°C.

5.2.4. Hydrolysis.

Crude lipid extract (100 mg) was refluxed for 16 h with 2 ml 1M ethanolic KOH. After cooling 5 ml of distilled H_2O was added. The mixture was extracted with 3 x 5 ml diethyl ether. The extract was collected after centrifuging and washed with 3 x 15 ml water in a separating funnel. Residual water in the ether solution was recovered by adding 2 g anhydrous sodium sulphate. This fraction is the unsaponifiable material containing alcohols, sterols, etc. The aqueous layer was mixed with the H_2O washing, and acidified to pH 1.5 with 1 ml 4 M HCl. This fraction was extracted with 3 x 5 ml diethyl ether. The ether soluble fraction was purified by reextracting with 3 x 15 ml H_2O and the residual water was absorbed by adding anhydrous sodium sulphate. This fraction contains the free fatty acids. Fig. 5.1. shows the flow chart for this procedure.

5.2.5. Oxidation.

Unsaponifiable materials were oxidized by the technique of Djerassi *et al* (1956). The oxidant was prepared by adding 23 ml of concentrated sulphuric acid to 26.72 g chromium trioxide and the mixture was diluted to a volume of 100 ml with distilled water. 150 mg of unsaponifiable materials was dissolved in 25 ml acetone. Oxidant (0.4 ml) was added and the mixture kept in an ice bath for 2 h before addition of 125 ml distilled H_2O. The precipitate was recovered by filtering in a Buchner funnel.

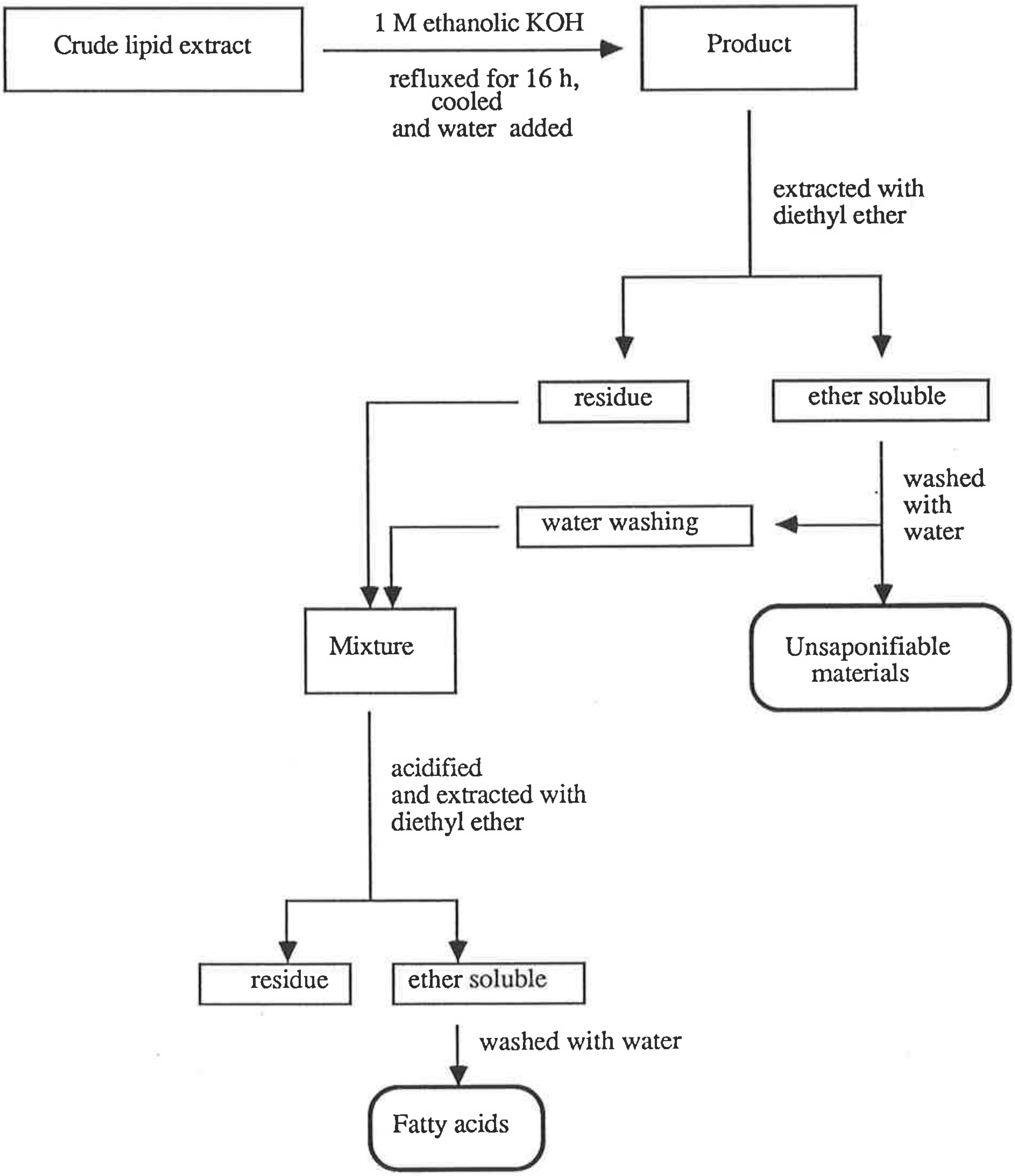
5.2.6. Hydroxamic acid test.

To 20 mg of crude materials 1 ml of 0.5 M ethanolic hydroxyl amine hydrochloride and 2 ml 6 M NaOH were added. The mixture was heated to boiling, cooled and 2 ml 1 M HCl and 1 drop 0.3 M $FeCl_3$ added. The appearance of colour was observed.

5.2.7. Elemental analyses.

The crude materials extracted by *iso*-propanol/ammonia from Tintinara soils and fractions soluble in chloroform, hexane and benzene/ethanol were sent to AMDEL Victoria for elemental analyses. The analyses included carbon, hydrogen and nitrogen

Fig. 5.1. : Diagram of the hydrolysis of water-repellent materials extracted by *iso* -propanol/ammonia from Tintinara soils.


Crude lipid extract
1 M ethanolic KOH
refluxed for 16 h,
cooled
and water added
Product
extracted with
diethyl ether
residue
ether soluble
washed
with
water
water washing
Mixture
Unsaponifiable
materials
acidified
and extracted with
diethyl ether
residue
ether soluble
washed with water
Fatty acids

(instrumental); oxygen (gravimetric) and ash.

5.2.8. Thin-layer chromatography.

The hydrophobic materials isolated from Tintinara soils and their fractions were dissolved in appropriate solvents and applied to starting spots on thin-layer chromatography (TLC) plates and then developed in chloroform. Spots were located by spraying with Rhodamin 6G (Holloway and Challen 1966) and copper acetate-phosphoric acid reagent (Fewster *et al* 1969).

5.2.9. Infrared spectra.

Infrared spectra were obtained using discs with a concentration of 1 mg material to be characterized in 180 mg potassium bromide or potassium chloride. Acid washed sand samples coated with soil lipids were located on a diffuse reflectance accessory for infrared spectroscopy. The spectra were recorded using a Perkin-Elmer 983 grating infrared spectrophotometer.

5.2.10. UV/VIS spectra.

For ultraviolet/visible spectra the crude materials extracted from Tintinara soils were dissolved in hexane and recorded with a Perkin-Elmer Lambda 5 spectrophotometer.

5.2.11. ^{13}C NMR (Nuclear Magnetic Resonance).

The chloroform soluble fraction of materials extracted from Tintinara soils were dissolved in $CDCl_3$ (Stohler, USA) to give concentrations of approximately 100 mg ml^{-1}. ^{13}C NMR spectra were recorded on a JEOL FX-90 Q Fourier Transform NMR spectrometer operating at a frequency of 22.45 MHz at an ambient probe temperature of 24° C. Between 25-50 K acquisitions were accumulated into 8 K memory addresses using a spectral width of 5000 Hz and 11 µs (45°) pulse with a recycle time of 2.8 s. Broad band proton decoupling was used and an exponential line broadening of 2 Hz was applied to the free induction decay prior to Fourier transformation. Peak positions were referenced against the centre peak of the $CDCl_3$ triplet which was taken as 77.0 ppm. Crude materials and fractions soluble in benzene/ethanol and hexane were dissolved in either deuteurated *iso*-propanol/ammonia, benzene/ethanol or hexane. Hence, the peak positions were referenced against the peaks of corresponding solvents.

^{13}C solid state NMR was conducted according to the method described by Oades *et al* (1987). The spectra were obtained with cross polarization (CP) from ^1H on a Bruker CXP 100 instrument operating at 22.6 MHz for ^{13}C. The pulse width was 5 µs. The samples were spun at the magic angle (MAS) in rotors constructed of boron nitride with a Kel-F cap. The spinning speed was 3-5 kHz. Chemical shift were measured relative to external hexamethylbenzene but are reported relative to tetramethylsilane. A 1 ms contact time was used and the recycle delay was 0.5 s. Up to 26330 scans were obtained. The IK FID acquired with proton spun temperature inversion and quadrative detection was zero filled to 4 K. A spectral width of 16 kHz was used employing a computer-controlled filter bandwidth and 12-bit digitizer resolution. Line broadening was 50 Hz.

5.2.13. Gas Chromatographic-Mass Spectrometer (GC-MS).

GC-MS was carried out on a Hewlett Packard 5992 GC-MS system equipped with a 12 m x 0.22 mm bonded phase vitreous silica column which was programmed from 180 to 280° C at 5° min^{-1}. The flow rate of the He carrier gas was 1 ml min^{-1}. The ionization voltage was 70 eV. The extracted materials were esterified with methanol/HCl prior to GC-MS which incorporated methyl pentadecanoate as an internal standard. For the methyl esters of oxidized alcohol analysis a 1m x 0.53 mm fused silica capillary column was employed.

5.2.14. High Performance Liquid Chromatography (HPLC) analysis.

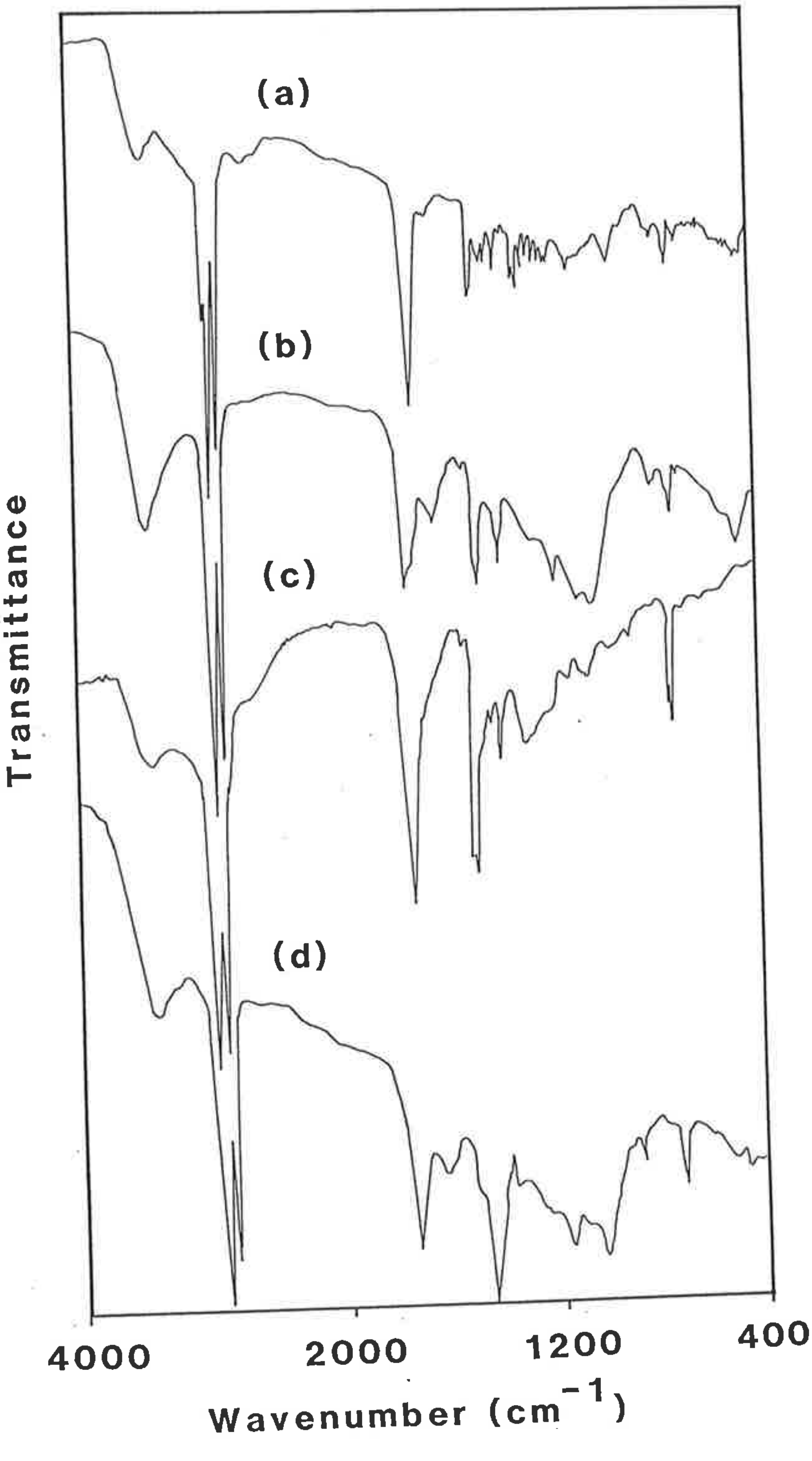
The alcohol content of extracted materials was analyzed using HPLC. The alcohols (50 mg) were derivatized as their dinitrobenzoyl esters by adding 2000 mg dinitrobenzoyl chloride in 20 ml of tetrahydrofuran along with 10 drops of pyridine and heated at 60° C for 4 h with stirring (Qureshi *et al* 1981). HPLC was performed with a system consisting of Waters Model 590 Programmable Solvent Delivery Module, Lambda-Max Model 481 LC Spectrophotometer. The spectra were recorded on Radiometer Servograph REC 51. The system was equipped with Waters µStyragel Gel Permeation Chromatography 30 cm column.

5.3. Results and Discussion

5.3.1. Benzene/ethanol extract.

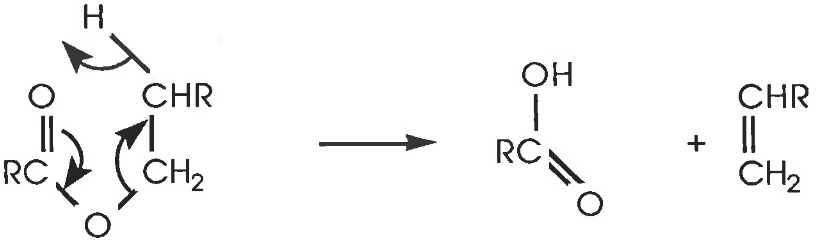
Infrared spectra of materials extracted with the mixture of benzene/ethanol from Tintinara water-repellent soils (Fig. 5.2.b.) suggested that the majority of the materials were long-chain polymethylene compounds as shown by strong C-H stretching at 2917 cm^{-1}, bending at 1460 cm^{-1} and rocking at 720 cm^{-1} frequencies. The major differences from the spectrum of palmitic acid (Fig. 5.2.a.) are that the spectra of the extracted materials showed a strong ester carbonyl peak at 1730 cm^{-1} and stronger O-H stretching and C-O stretching at 1000-1200 cm^{-1} absorbances suggesting that the crude extracts were apparently a mixture of esters, acids and hydroxylic compounds. The mixture was then fractionated by vacuum sublimation. The infrared spectrum of the vacuum sublimate (Fig. 5.2.c.) was characteristic of long chain fatty acids as revealed by a large peak of acid carbonyl at 1710 cm^{-1}. The most striking feature of the spectrum of the sublimate was the occurrence of doublet peaks of CH_2 rocking near 720 cm^{-1} which can be related to the length of polymethylene chains (Meiklejohn *et al* 1957) and to the alignment of chains in various crystal forms of fatty acids (Chapman 1965 *cit.* Freeman 1968). Meiklejohn *et al* (1957) presented infrared spectra of normal aliphatic acids from C_{10} to C_{36} showing that the acids with more than 16 carbon atoms tended to form doublet peaks near 720 cm^{-1}. The

Fig. 5.2. : Infrared spectra of (a) palmitic acid, (b) benzene/ethanol extract from Tintinara water-repellent soils, (c) vacuum sublimate of the extract and (d) residual sublimand.


(a)
(b)
(c)
(d)
Transmittance
4000
2000
1200
400
Wavenumber (cm^{-1})

residual materials which did not sublime appeared to be dominantly long chain esters indicated by an ester carbonyl peak at 1730 cm^{-1} (Fig. 5.2.d.).

Fuson (1962) suggested that certain esters may be pyrolysed into carboxylic acids and an olefin. Pyrolysis of such esters goes by way of a *quasi* six-membered ring.

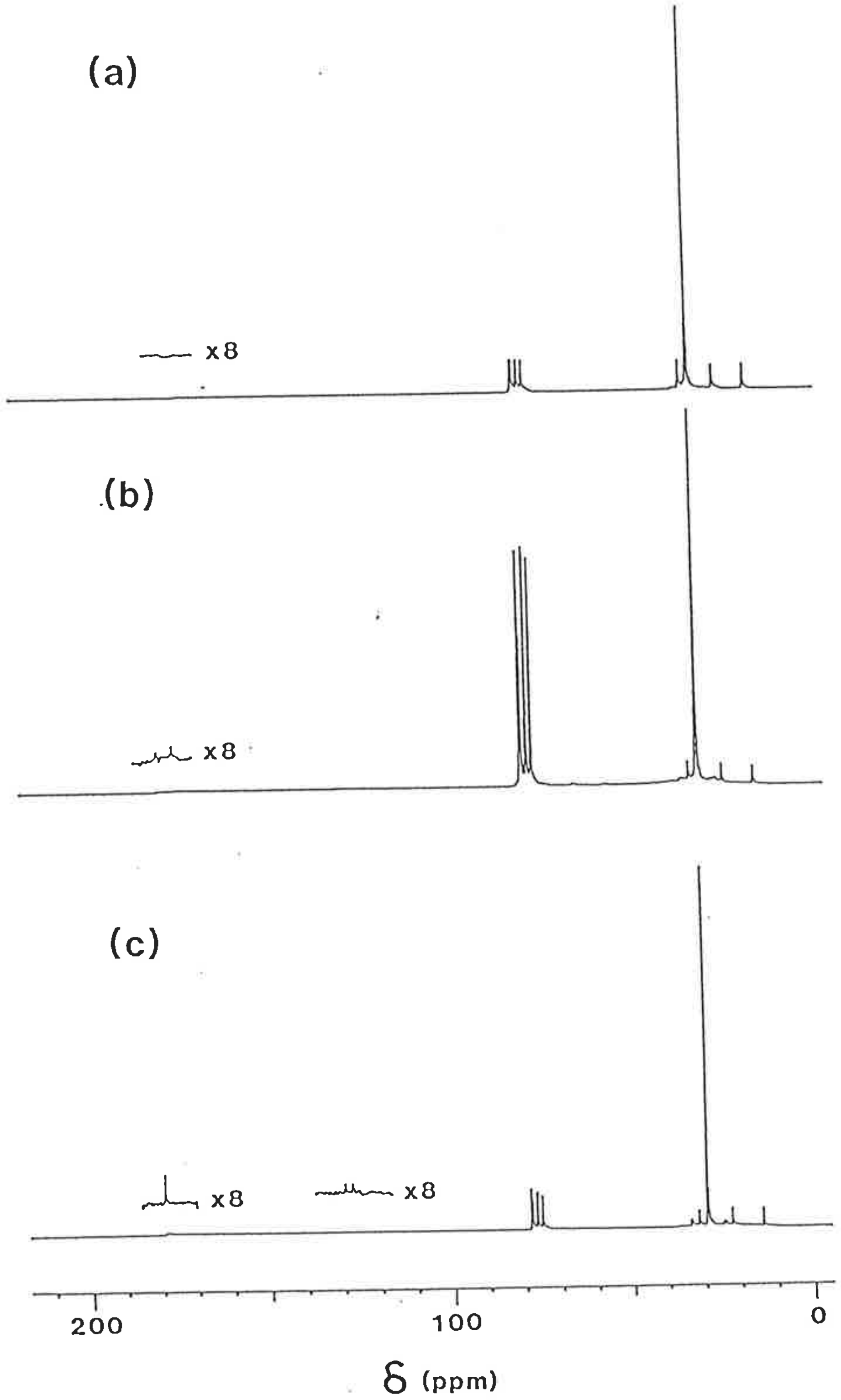


The presence of olefinic compounds in the vacuum sublimate was revealed by ^{13}C NMR spectra showing olefinic signals at 130.7 and 128.5 ppm (Fig. 5.3.c.). The large polymethylene resonance at 29.6 ppm together with $-CH_2-CH_2-CH_3$, $-CH_2-CH_3$ and $-CH_3$ resonances at 31.9, 22.6 and 14.1 ppm respectively (Levine *et al* 1972) confirm the infrared data that the materials extracted from Tintinara water-repellent soils with the mixture of benzene/ethanol (Fig. 5.3.b.) were made up of long-chain aliphatic compounds. The carbonyl resonances at 178.4 and 174.1 ppm which are barely detectable above background noise are the only features which differ from a spectrum of paraffin wax (m.p. 63-68°C) (Fig. 5.3.a.).

5.3.2. Humic acids.

The infrared spectrum of an alkaline extract of water-repellent Tintinara soil (Fig. 5.4.a.) is characteristic of the spectra of humic acids showing broad bands in the regions of 3400 cm^{-1} (primarily OH stretching), large band near 2920 cm^{-1} and small band at 2850 cm^{-1} (C-H stretching of aliphatic groups), small peaks at the region of 1730 cm^{-1} (C=O frequency COOH, C=O stretching of ketonic carbonyl), broad bands near 1650 cm^{-1}

Fig. 5.3. : ^{13}C NMR spectra of (a) paraffin wax (m.p. 63-68°C), (b) intact benzene/ethanol extract of Tintinara soils and (c) vacuum sublimate of the extract. The sharp triplet centred at 77.0 ppm is caused by the $CDCl_3$ solvent.


(a)
x8
(b)
x8
(c)
x8
x8
200
100
0
δ (ppm)

(aromatic C=C double bond conjugated with C=O and/or COO$^-$), 1420 cm^{-1} (symmetrical stretching of COO$^-$). Broad bands were also present near 1200 cm^{-1} (C-O stretching and/or OH deformation of COOH) and near 1030 cm^{-1} (C-O stretching of alcoholic and phenolic OH groups). The only feature distinguishable from other infrared spectra documented previously (Schnitzer and Skinner 1968, Khan and Schnitzer 1971, Kallianou *et al* 1987 and Ramunni *et al* 1987) is a prominent peak of long chain polymethylene at 2920 cm^{-1}. These humic acids are almost identical to humic acids extracted from Phragmites peat (Farmer and Morrison 1960) and from B horizon of a podzol and from Mud Lake, Florida (Stevenson and Goh 1971) as revealed by their infrared spectra.

The intensity of the transmittance at 2920 cm^{-1} drastically decreased while the peak at 2850 cm^{-1} disappeared (Fig. 5.4.b.) after extraction with benzene/ethanol mixture. An infrared spectrum of the humic acid fraction soluble in benzene/ethanol (Fig. 5.4.c.) is characteristic of long chain polymethylene compounds. The rocking bands at 720 cm^{-1} which together with C-H bending at 1460 cm^{-1} and C-H stretching at 2920 cm^{-1} indicated the presence of long chain aliphatic compounds and strong absorbance at 1710 cm^{-1} suggested the presence of terminal carboxylic groups.

Fig. 5.4.d. shows the infrared spectrum of materials isolated directly from Tintinara water-repellent soil with the mixture of benzene/ethanol which is similar to the spectrum of humic fraction soluble in benzene/ethanol (Fig. 5.4.c.) . However, it is likely that the materials isolated directly from soil by solvent extraction was dominated by esters (1730 cm^{-1}) rather than acids (1710 cm^{-1}) derived by saponification in the humic acid preparation.

These results indicate the presence of long chain polymethylene compounds in humic acids extracted from the water-repellent soil from Tintinara. These benzene/ethanol soluble polymethylene compounds which make up approximately 15 % of the humic acids account for the observed hydrophobic properties of the humic acid fraction. The residual humic acid (85 %) was quite hydrophilic.

Fig. 5.5. shows ^{13}C CPMAS (Cross Polarization-Magic Angle Spin) NMR

Fig. 5.4. : Infrared spectra of (a) humic acids extracted from Tintinara soils, (b) humic acid fraction insoluble in benzene/ethanol (2:1, by volume), (c) humic acid fraction soluble in benzene/etahnol, and (d) materials isolated directly from soils with benzene/ethanol.

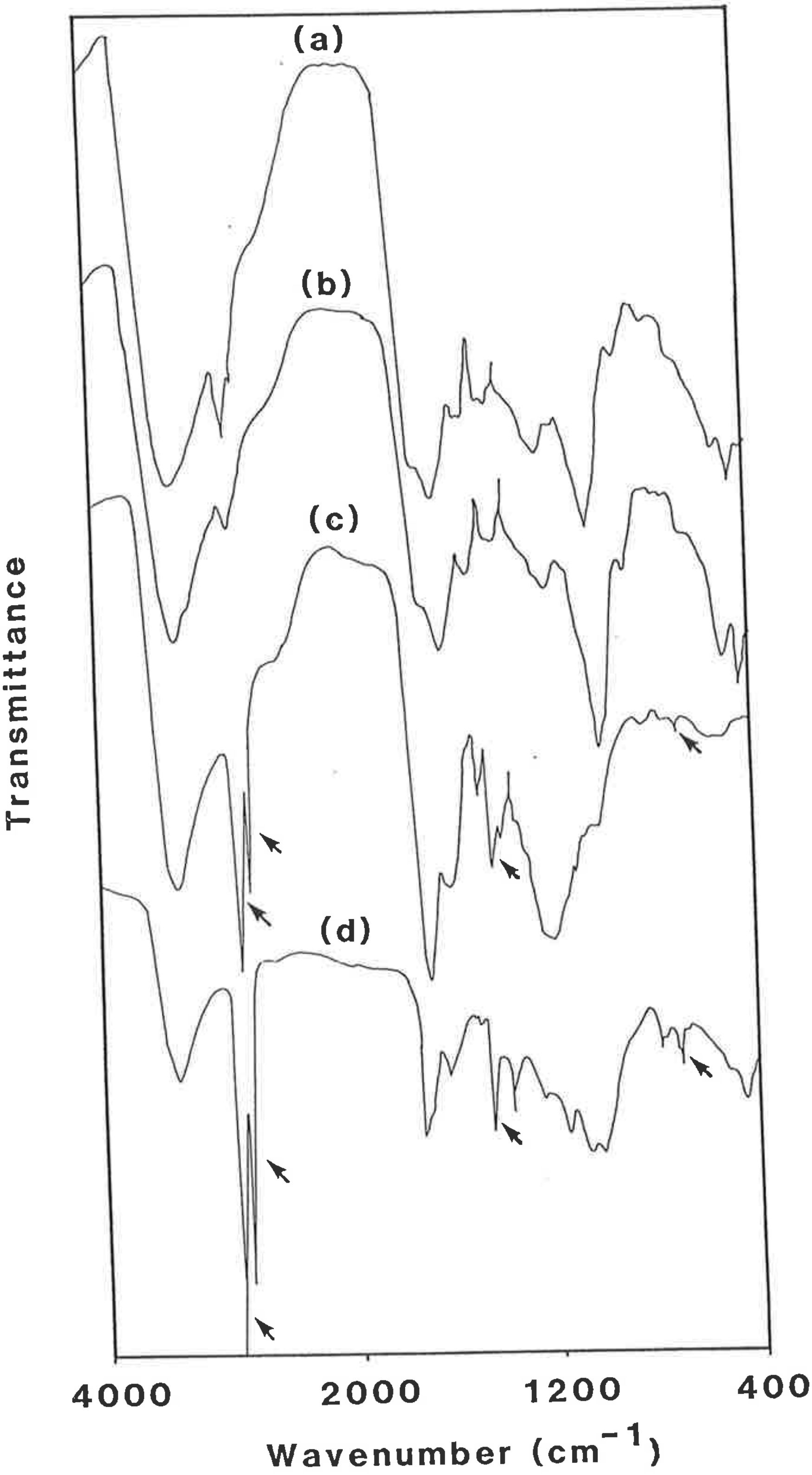
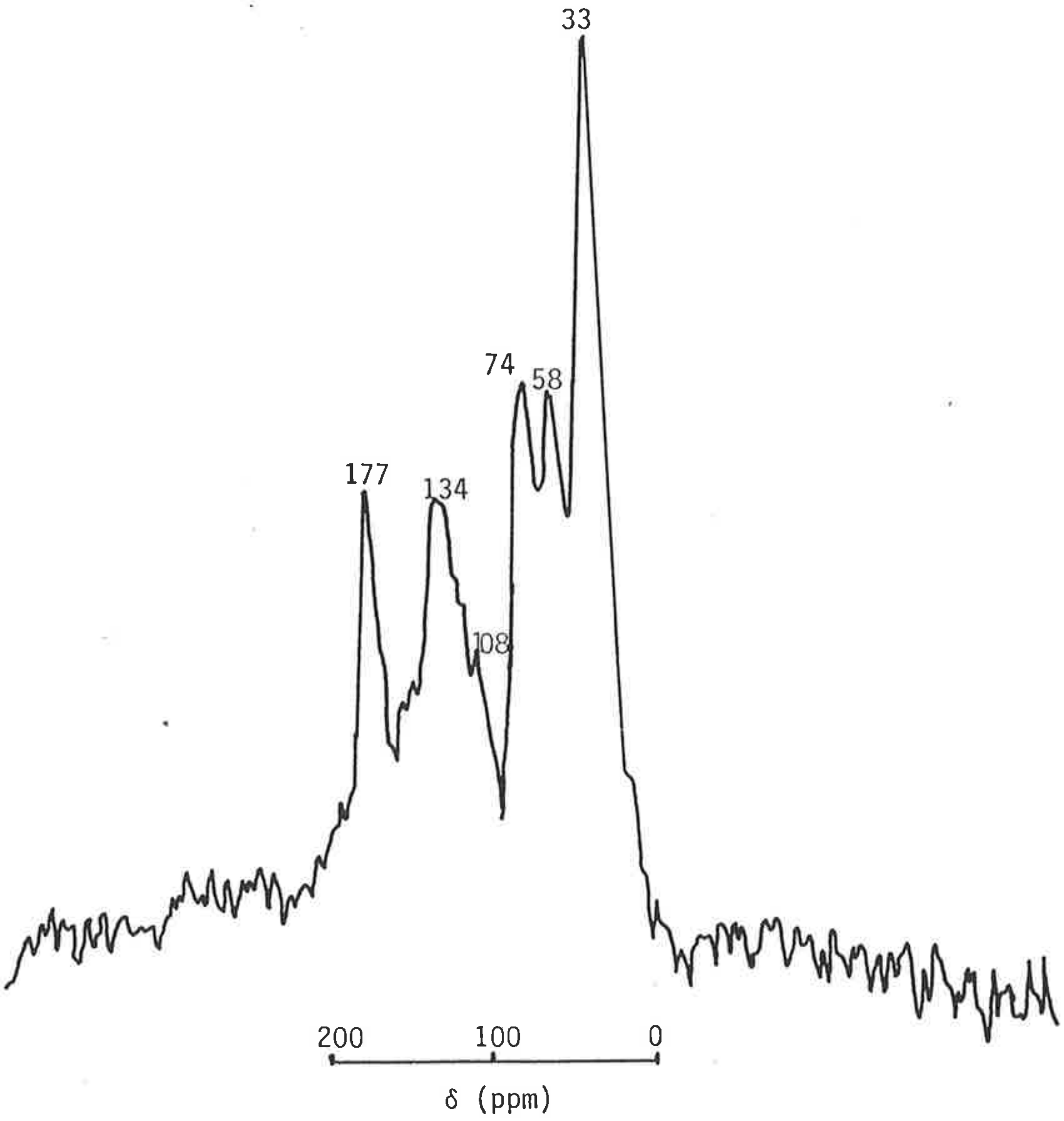

(a)
(b)
(c)
(d)
Transmittance
4000
2000
1200
400
Wavenumber (cm^{-1})

Fig. 5.5. : ^{13}C CPMAS NMR spectra of a humic acid fraction extracted from Tintinara soils.


33
74
58
177
134
108
200
100
0
δ (ppm)

spectra of humic acids extracted from Tintinara soil. The weak shoulder beyond 30 ppm can be assigned to the terminal methyl group of alkyl chains. The large and sharp peak at 33 ppm is referred to aliphatic carbon and indicates the domination of long chain polymethylene compounds. The prominent peak at 58 ppm suggests the presence of methoxyl groups, at 74 ppm oxygenated alkyl carbon of polysaccharides, at 134 ppm the presence of aromatic carbon and 177 ppm carboxyl carbon (Barron and Wilson 1981, Wilson *et al* 1981, Preston and Ripmeester 1982, and Preston and Schnitzer 1984). To summarize, this spectrum clearly indicates that the humic acids extracted from this type of soils is dominated by polymethylene compounds. The amount of carbon present as long chain aliphatic materials is close to 31 %. Oades *et al* (1987) reported that the intensity of the peak at this region was decreased slightly in a fraction 20-53 µm of Urrbrae soil after *iso*-propanol/ammonia extraction. These workers found that the extracted material was long chain polymethylene compound as revealed by infrared spectroscopy.

5.3.3. *Iso*-propanol/ammonia extract.

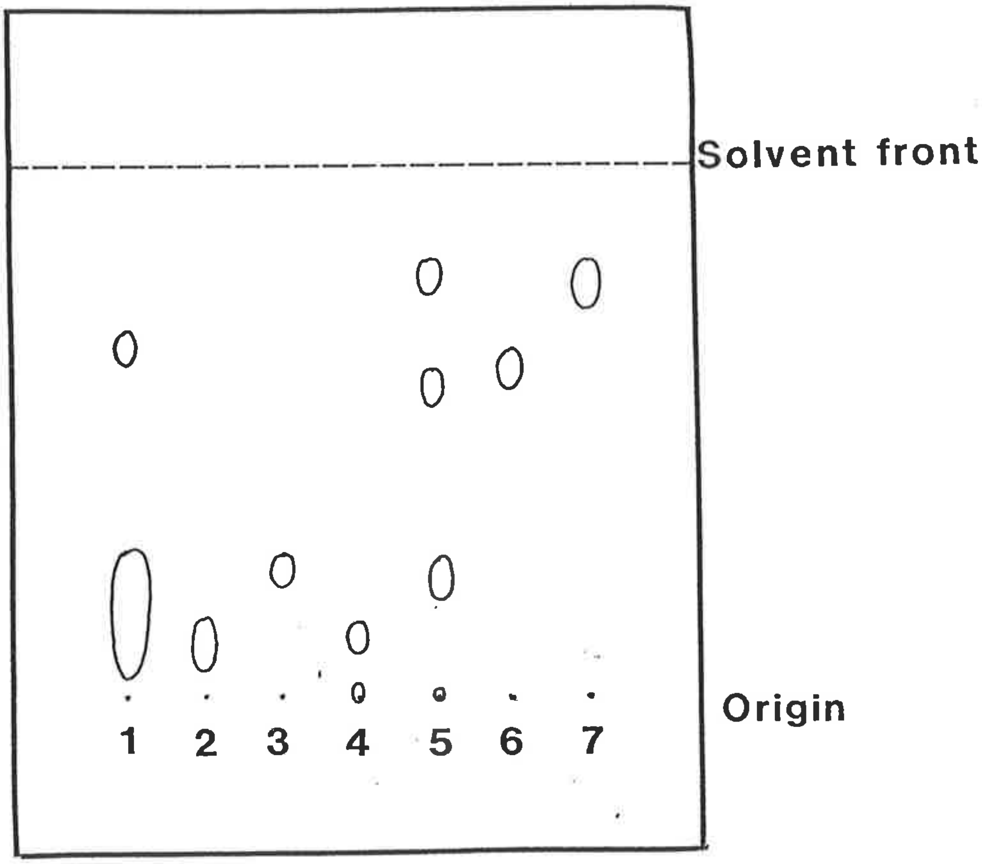
The materials extracted with the amphiphilic mixture of *iso*propanol/ammonia were characterized using combined spectroscopic and chromatographic techniques.

Thin layer chromatograms of the extracted materials (Fig. 5.6.) suggested that the major components of the hydrophobic extracts had Rf values, and hence partition coefficients which were similar to long chain acids, alcohols and wax esters. The detection of these relatively unreactive materials was successfully accomplished by the copper acetate-phosphoric acid charring procedure of Fewster *et al* (1969). Reference steroid spots showed a transitory colour change from red through deep blue to black as noted by Fewster *et al* (1969) but these colours were not observed with the hydrophobic soil extracts, indicating that common steroids were not major components of these materials.

An attempt to obtain appropriate UV/VIS spectra of the extracted materials was unsuccessful due to the poor solubility of the materials in the solvents recommended for ultraviolet

Fig. 5.6. : Thin layer chromatogram of soil lipids and some reference molecules. Adsorbent : Aluminium sheets Silica gel (without fluorescent indicator). Solvent : chloroform. Spots were located by copper acetate-phosphoric charring techniques.

1. Palmitic acid.
2. Cholesterol.
3. Cetyl alcohol.
4. Lipids isolated with *iso*-propanol/ammonia from Tintinara soils.
5. Lipids isolated with benzene/ethanol from Tintinara soils.
6. Methyl palmitate.
7. Paraffin wax (m.p. 63-68°C).


Solvent front
Origin
1
2
3
4
5
6
7

spectroscopy. However, a fraction of the extract which is soluble in hexane showed only absorption at 220 nm and lacked the peak at 273 nm . Horn *et al* (1964) indicated that peak at 273 nm is characteristic of β-diketones, a major component of cuticular waxes isolated from a number of *Eucalyptus spp.* . Hence, these data suggested that β-diketones were not major components of the extracted materials.

Fig 5.7. compares the infrared spectra of an *iso*-propanol/ammonia extract from Tintinara water-repellent soils, a hydrophobic compost mixture and a sample of the cuticular wax from the leaves of *Eucalyptus crucis*. In each case the dominant features include the C-H stretching at 2917 cm^{-1}, bending at 1460 cm^{-1} and rocking at 720 cm^{-1} frequencies characteristic of extended polymethylene chains. In general, the higher the MED value at an arbitrary level of 400 mg kg^{-1} the more prominent these C-H frequencies are. The relatively low intensity of signals for C=O stretching frequencies suggests that there is either a mixture comprising a small amount of compounds with carbonyl groups admixed with a large amount of paraffinic molecules devoid of carbonyls and/or both the carbonyl and polymethylene chains are present in the same molecule. In the latter case the unusual ratio of signals is brought about by the presence of a numerically large excess of CH_2 vibrations compared to the number of C=O stretching vibrations. The features of the infrared spectra in Fig 5.7. are also akin to previous published spectra for organic coatings extracted from sand grains of non wettable golf greens (Miller and Wilkinson 1977) , data from Western Australian hydrophobic plant litter (McGhie 1980) as well as material extracted from water-repellent Italian soils (Giovannini and Lucchessi 1984). Fig 5.8. shows the infrared diffuse reflectance spectra of acid washed sand which has been coated with increasing concentrations of the hydrophobic extracts from Tintinara soil. It is clear that C-H stretching region is readily measurable by this technique. The transmittance at 2917 cm^{-1} frequency is well correlated with the concentration of soil lipids added.

^{13}C solution NMR spectrum (Fig. 5.9.a.) of the hydrophobic *iso*-propanol/ammonia extract from Tintinara soils shows a large polymethylene resonance at 29.4 ppm together with $\underline{C}H_2\text{-}CH_2\text{-}CH_3$, $\underline{C}H_2\text{-}CH_3$ and $\underline{C}H_3$ resonances at 31.9, 22.6,

Fig. 5.7. : Infrared spectra of lipids isolated from : (a) Tintinara soils, (b) non-wetting compost mixture, and (c) *Eucalyptus crucis* leaves.

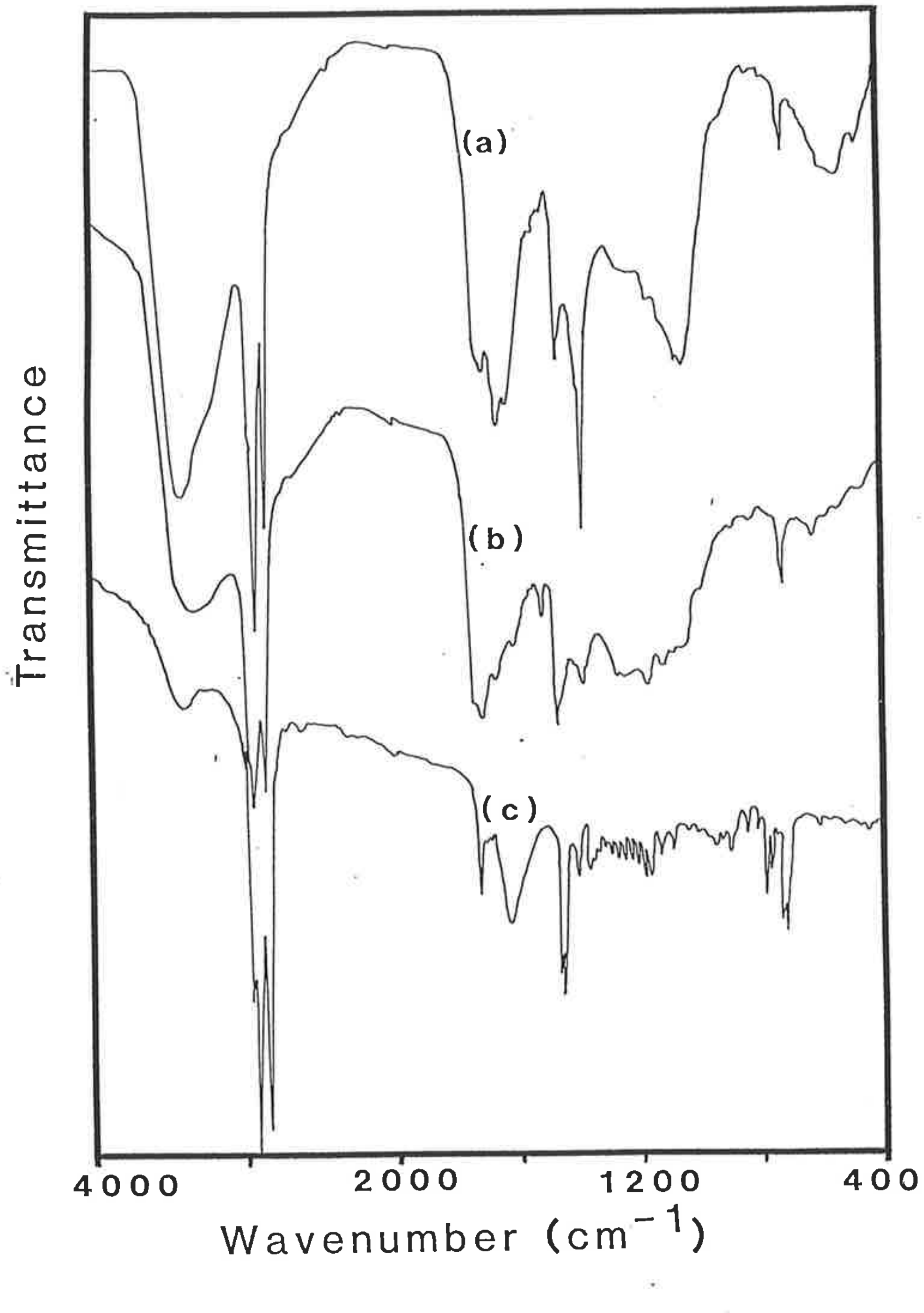

Transmittance
(a)
(b)
(c)
4000
2000
1200
400
Wavenumber (cm^{-1})

Fig. 5.8. : Diffuse reflectance infared spectra of acid washed sand treated with lipids isolated from Tintinara soils.

C = concentrations of lipids on acid washed sand (mg kg^{-1}).

M = MED values of the treated sands.

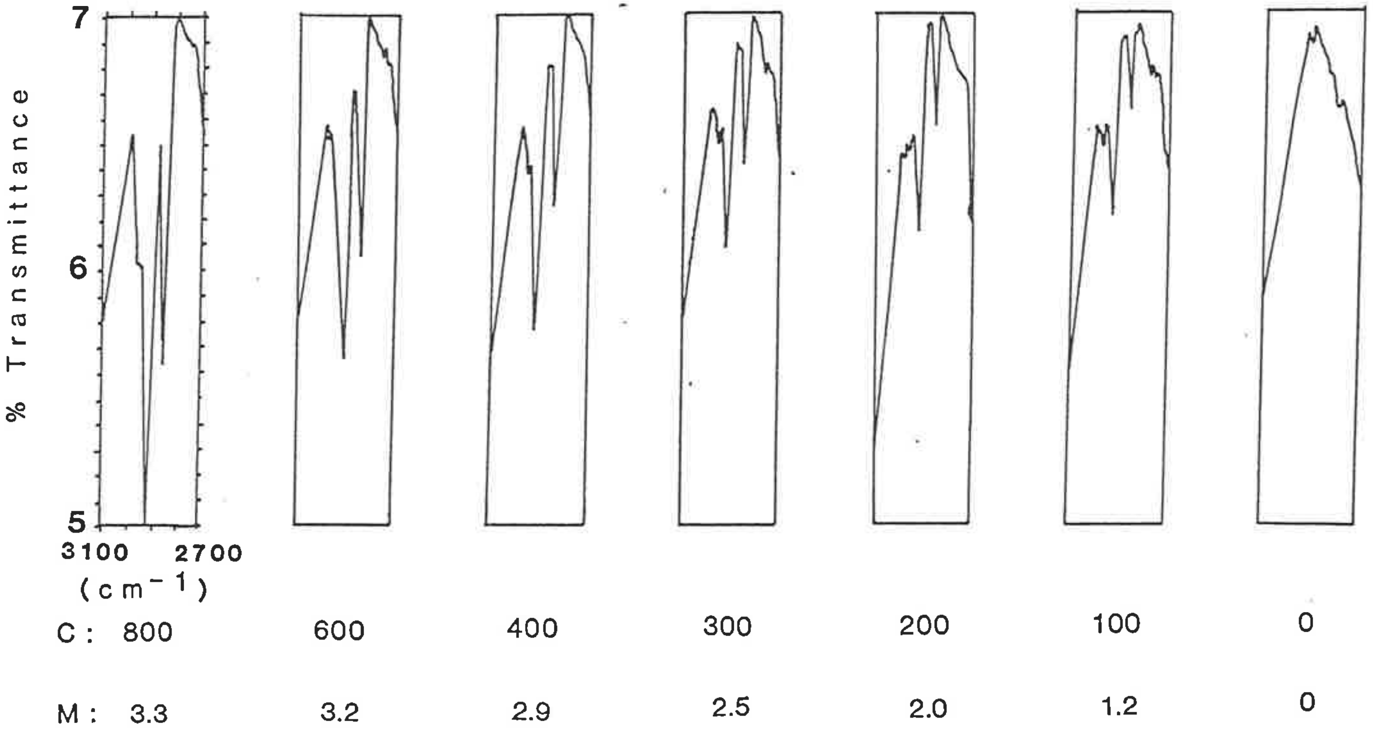
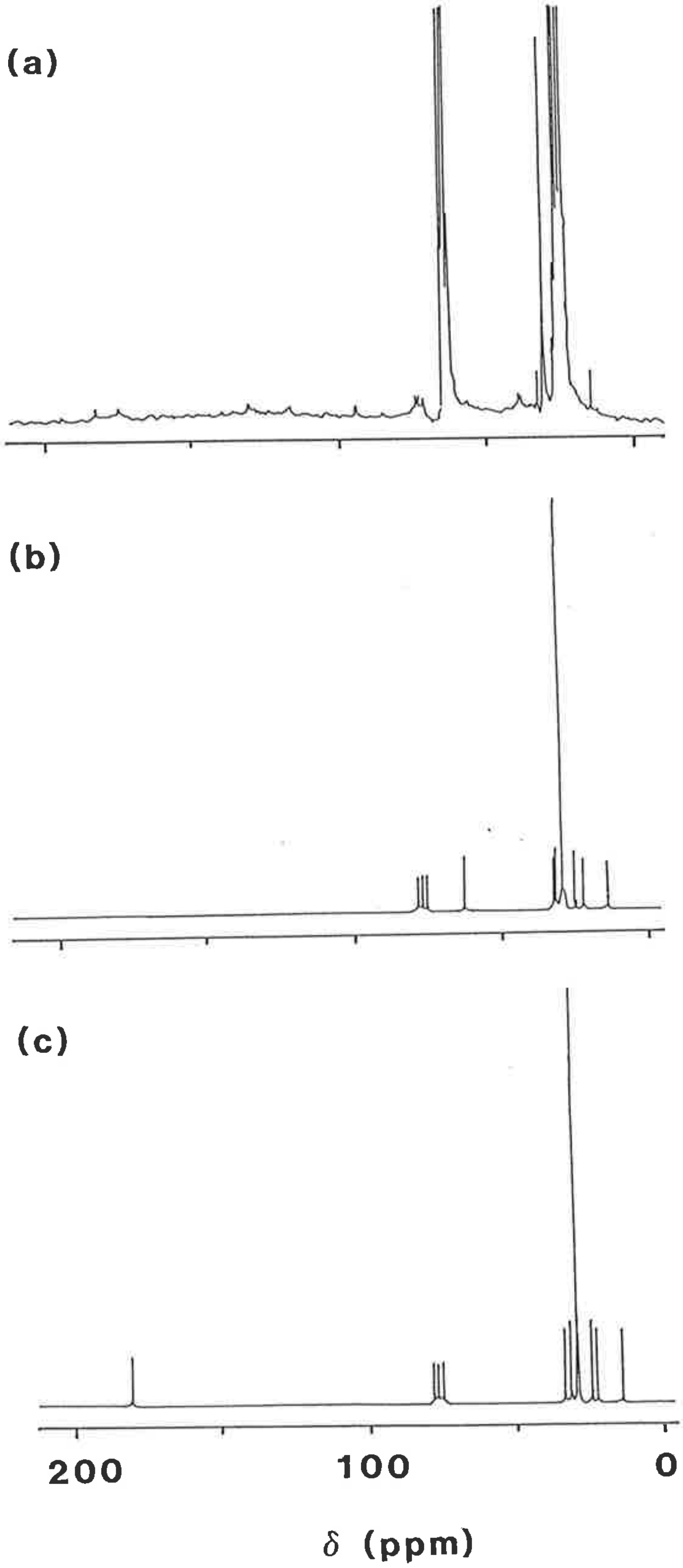

7
6
5
% Transmittance
3100
2700
(cm⁻¹)
C : 800
600
400
300
200
100
0
M : 3.3
3.2
2.9
2.5
2.0
1.2
0

Fig. 5.9. : ^{13}C solution NMR spectra of (a) intact lipids isolated from Tintinara soils, (b) cetyl alcohol, and (c) palmitic acids.


(a)
(b)
(c)
200
100
0
δ (ppm)

amd 14.1 ppm respectively (Levine *et al* 1972). Comparison of the spectra of both cetyl alcohol (Fig 5.9.b) and palmitic acid (Fig. 5.9.c.) with the soil extract (Fig.5.9.a) confirms the presence of carbons of similar type. However, although the polymethylene and carbonyl peaks are present, many of the signals are obscured by signals centred at 24 and 58 ppm which can be ascribed to residual *iso* -propanol in the sample.

GC-MS analysis indicated that the hydrophobic materials contained combined and uncombined long chain fatty acids containing 16-32 carbon atoms (Fig. 5.10.) with at least a bimodal distribution showing maxima at C_{16} and C_{22} when the 74 m/z ion was monitored. Such a distribution has also been reported for lipids from the sediment of an Andean lake (Simoneit *et al* 1980). 1-18 % of the carbon extracted by *iso*-propanol/ammonia was present as fatty acids.

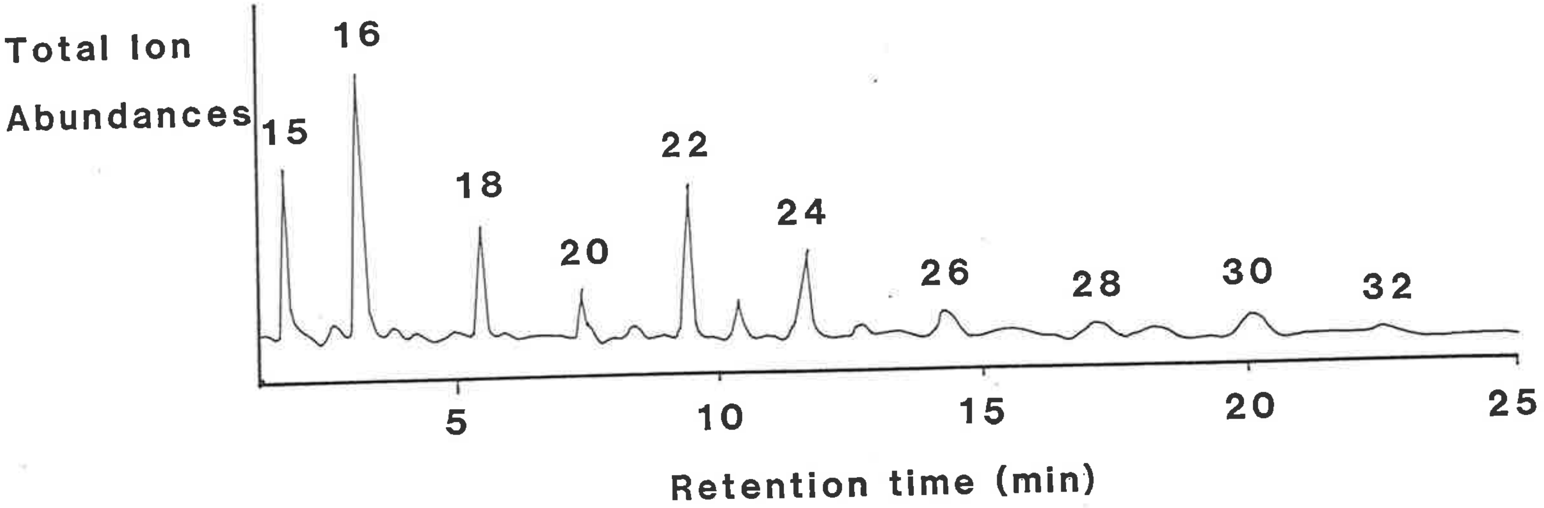
From these data it is clear that the crude hydrophobic extract consists mainly of long chain fatty acids. The involvement of such acids in water-repellent surfaces has been previously reported by Wander (1949) who noted that stearic acid created extreme water-repellency after treatment with calcium and magnesium hydroxide. Davies (1957) also reported that molecules with COOH and OH group such as alcohols and phenols produced hydrophobicity on surfaces. More recently Savage *et al* (1969b) found that palmitic acid induced hydrophobicity in sands after heating.

5.3.4. Hydrolysis.

On hydrolysis the crude materials extracted from Tintinara soils with the mixture of *iso*-propanol/ammonia was fractionated into 32.4 % free fatty acids and 9.7 % non-saponifiable fraction containing alcohols, sterols, etc. This indicates the presence of additional components in the crude extracts which were not lipid compounds. This conclusion was confirmed by the presence of up to 32 % of material in the crude extract which was water soluble.

Both the free fatty acids and non-saponifiable fractions induced very severe water-

Fig. 5.10. : Distribution of long chain fatty acids in soil lipids isolated with the mixture of *iso*-propanol/ammonia from Tintinara soils analyzed using GC/MS.


Total Ion
Abundances
15
16
18
20
22
24
26
28
30
32
5
10
15
20
25
Retention time (min)

repellency on acid washed sand. Application of an arbitrary concentration of 400 mg kg^{-1} produced MED values of 4.5 and 4.0 for the non-saponifiable fraction and free fatty acids respectively, compared to 4.3 induced by the intact materials.

Despite the failure to detect any positive steroid reaction in the intact lipids the presence of some steroid-like material in non-saponifiable materials was revealed by thin-layer chromatography(Fig. 5.11.). Deep purple spots at the first stage of charring as was observed by Fewster *et al* (1969) were detected in lanes 4, 5 and 6 tracers and are cross hatched. Infrared spectra (Fig. 5.12.) indicated that crude lipid extract (a) was mainly wax esters which can be fractionated into fatty acids (c) with strong carbonyl band at 1710 cm^{-1} frequency and molecules containing hydroxyl groups such as alcohols (b) with characteristic very strong OH bending at 1410 cm^{-1}. The presence of acids in the crude extract together with esters was suggested by prominent double peaks at 1710 cm^{-1} and 1730 cm^{-1} for acid carbonyl and ester carbonyl bands respectively. Esterification with methanol/HCl of fatty acids obtained from hydrolysis resulted in the movement of carbonyl peak from 1710 cm^{-1} to 1730 cm^{-1} (d). The result of the hydroxamic acid test supported the previously mentioned observation that the crude extract from water-repellent Tintinara soils chiefly consisted of wax esters. The intact extract produced a reddish brown colour, whereas methyl palmitate (reference), free fatty acids and methylated fatty acids from soil resulted in magenta, yellow and deep yellow colours respectively.

Alcohols obtained on hydrolysis were derivatized as the dinitrobenzoyl esters prior to HPLC analysis. Fig. 5.13. shows the HPLC separation of dinitrobenzoyl derivatives of soil lipids (a) and cetyl alcohol (b). Quantitative analysis of methyl esters of the oxidized alcohols using combined gas chromatography-mass spectrometer (GC-MS) indicated that up to 44 % of total alcohols present was cetyl alcohol. Distribution of alcohols in lipids extracted from water-repellent Tintinara soils as revealed by the methyl esters of oxidized alcohols (Fig. 5.14.) is similar to distribution of fatty acids in the same extract, however, the longest chain for alcohols observed was 28 carbon atoms compared with 32 carbon atoms for fatty acids.

Fig. 5.11. : Thin layer chromatogram of the hydrolysis product of soil lipids and some reference molecules. Adsorbent : Aluminium sheets Silica gel (without fluorescent indicator). Solvent : chloroform. Spots were located by copper acetate-phosphoric acid charring techniques.

1. Palmitic acid.
2. Saponified materials.
3. Saponified materials.
4. Cholesterol.
5. Nonsaponifiable material.
6. Nonsaponifiable material.
7. Cetyl alcohol.

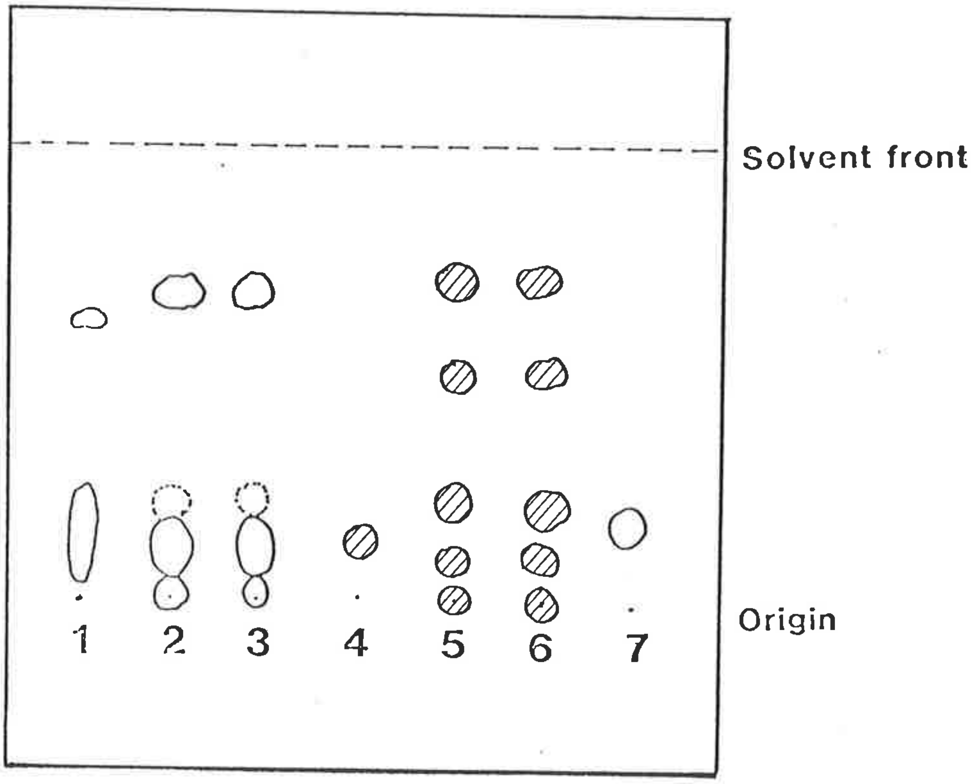

Solvent front
Origin
1
2
3
4
5
6
7

Fig. 5.12. : Infrared spectra of (a) intact lipids isolated from Tintinara soils, (b) non-saponifiable materials, (c) saponified product (free fatty acids) and (d) methylated fatty acids.

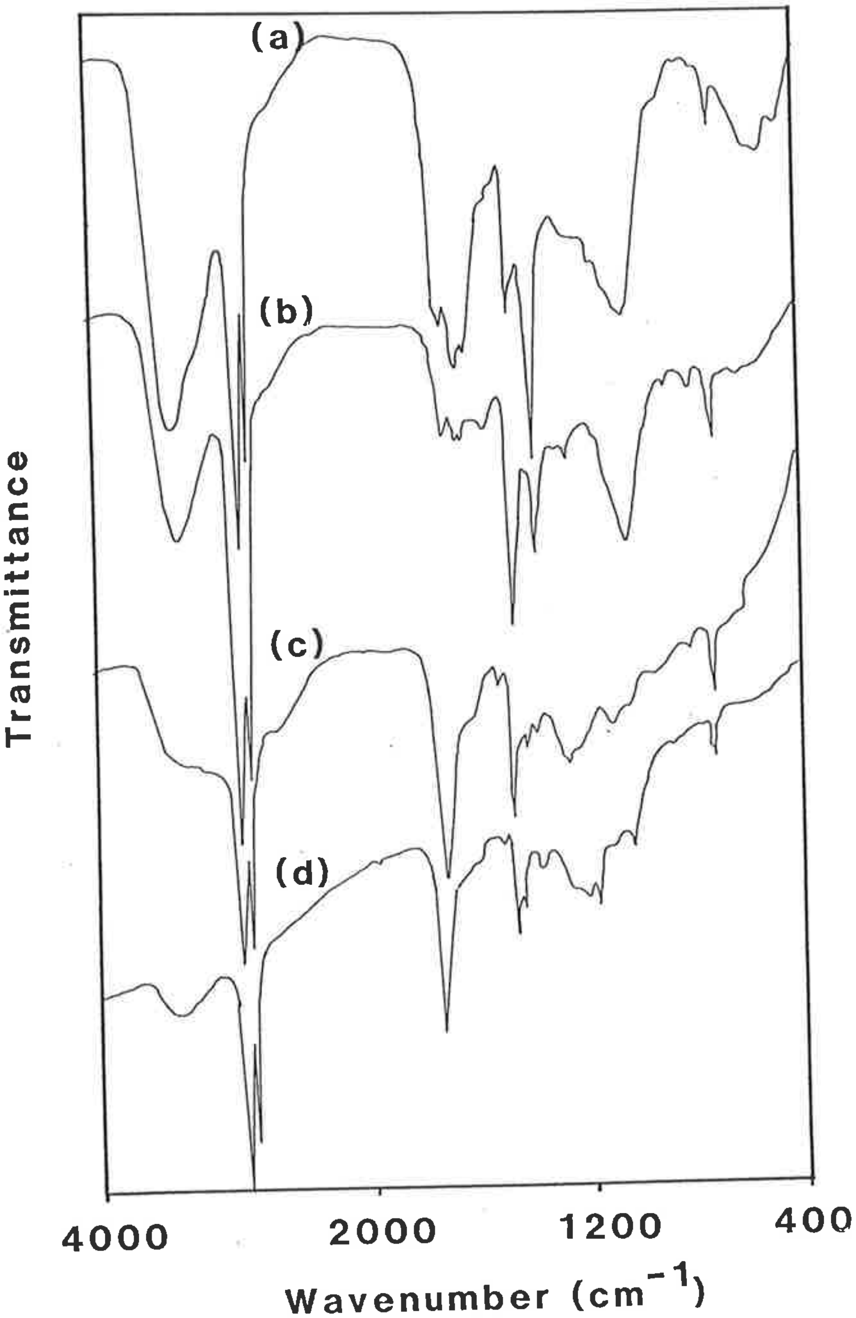

(a)
(b)
(c)
(d)
Transmittance
4000
2000
1200
400
Wavenumber (cm^{-1})

Fig. 5.13. : HPLC separation of dinitrobenzoyl derivatives of (a) soil lipids and (b) cetyl alcohol.

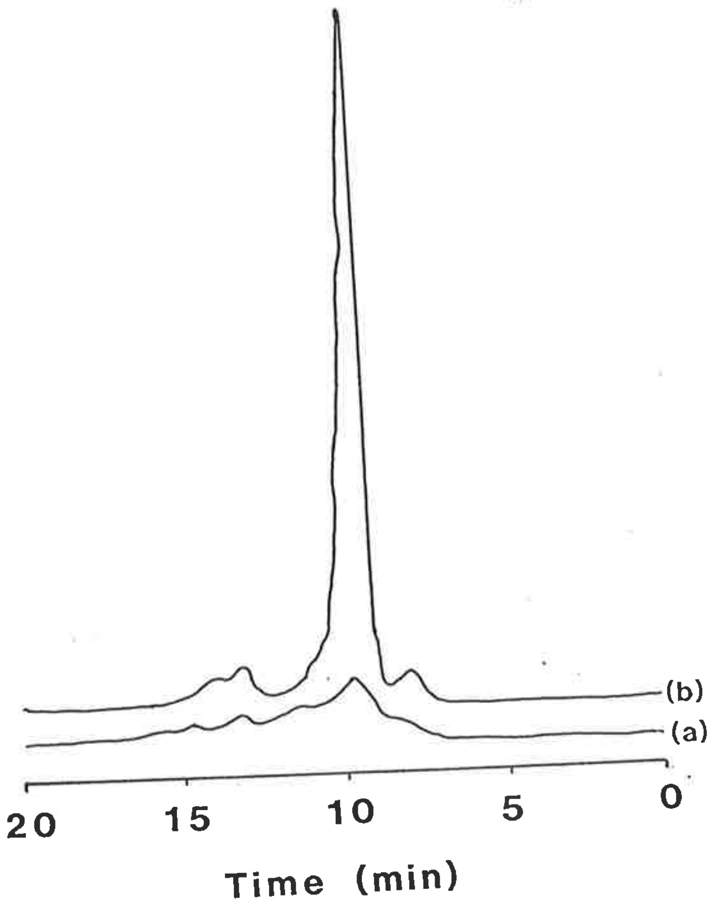
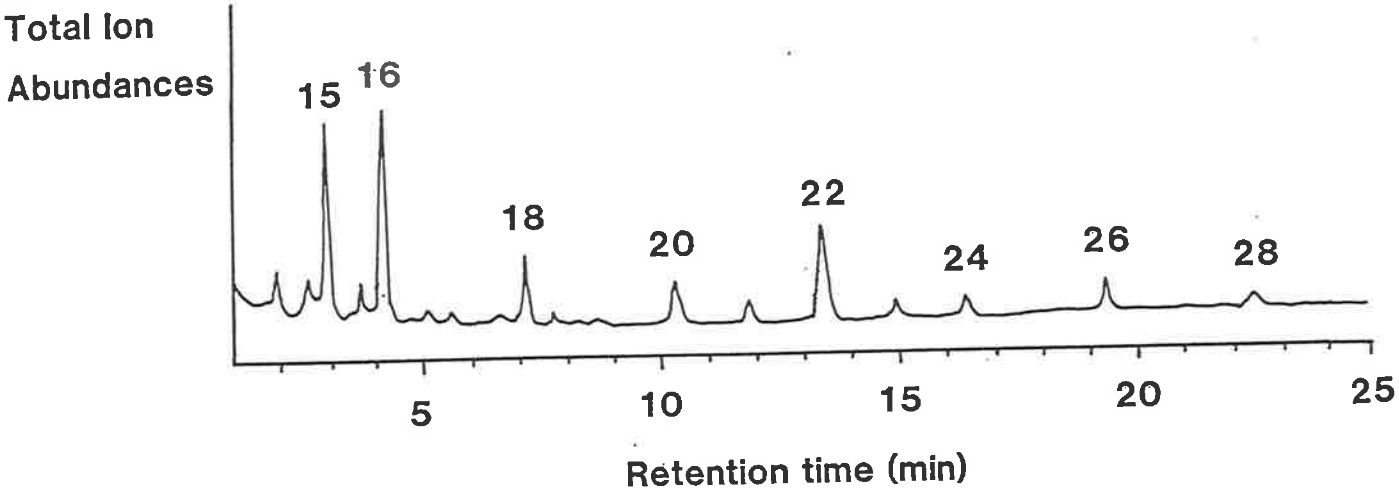

(b)
(a)
20
15
10
5
0
Time (min)

Fig. 5.14. : Distribution of long chain alcohols as revealed by GC/MS analysis of the methyl esters of oxidized non-saponifiable materials of lipids isolated from Tintinara soils.


Total Ion
Abundances
15
16
18
20
22
24
26
28
5
10
15
20
25
Retention time (min)

5.3.5. Fractionation of soil lipids.

Gross solvent fractionation of crude lipid materials was found to increase the specific hydrophobicity of the extracts. The lipid extract was fractionated based on its solubility in chloroform, benzene/ethanol, hexane and water (Table 5.1.). The most hydrophobic fraction was the hexane soluble fraction (Fig. 5.15.). However, some of water-repellent material was retained in the hexane insoluble fraction. By comparison, the mixture of benzene/ethanol (2:1 by volume) removed all hydrophobic components from the crude lipids, which again emphasises the importance of protic solvents in solubilizing the hydrophobic waxes.

Table 5.1. The solubility of the crude *iso* -propanol /ammonia extract from Tintinara soils in several solvents

Solvents	Solubility (% by weight)
Chloroform	70
Benzene/ethanol (2:1, by volume)	87
Hexane	37
Water	32

Infrared spectra (Fig. 5.16.) of those fractions shows that the presence of the ester carbonyl peak at 1730 cm^{-1} is correlated with the most water-repellent material. However, quantitative analysis using GC/MS indicated that compounds other than wax ester, such as acids and alcohols, are also important. Alcohols always induce more severe water-repellency on acid washed sand compared to their corresponding acids (Fig. 4.3.). In addition, to create a measurable water-repellency (MED 0.1) an acid must have at least 10 carbon atoms at a concentration of 400 mg kg^{-1}, whereas the 6 carbon alcohol (hexanol) created an MED value of 3.0 at this same level. These data clearly establish the importance of molecular size and structure to the water-repellent state.

Table 5.2. gives the elemental composition of soil lipids and various fractions.

Fig. 5.15. : Water-repellency of acid washed sand after treatment with soil lipid fractions.

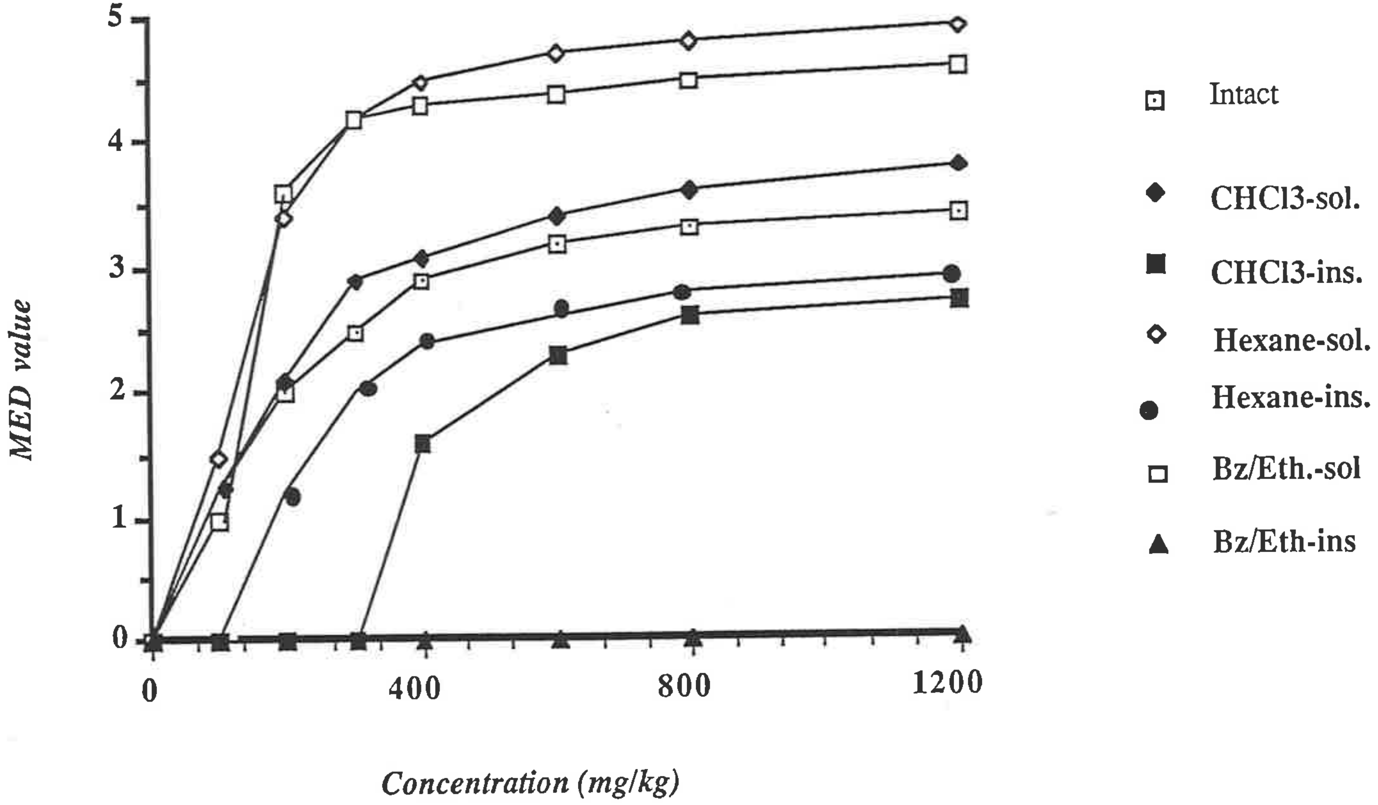
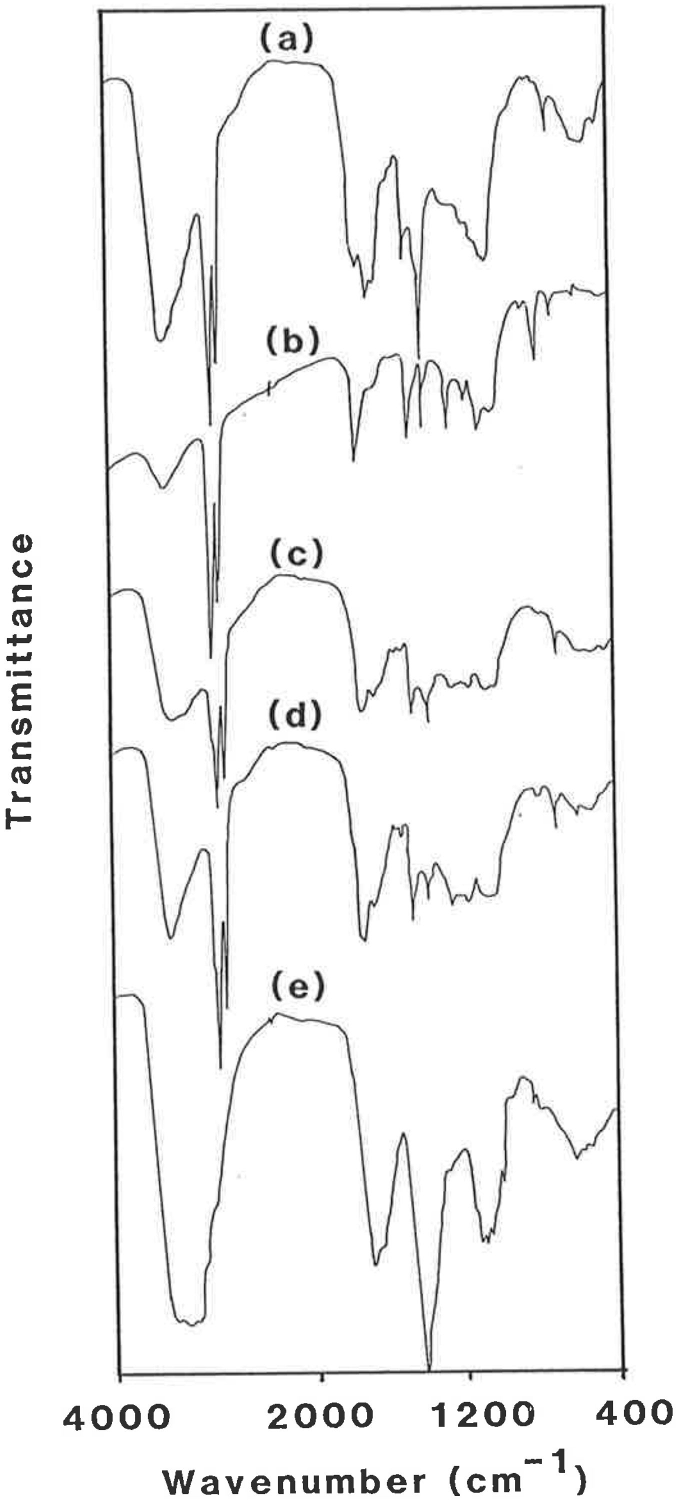

5
4
3
2
1
0
MED value
0
400
800
1200
Concentration (mg/kg)
⊡ Intact
◆ CHCl3-sol.
■ CHCl3-ins.
◇ Hexane-sol.
● Hexane-ins.
□ Bz/Eth.-sol
▲ Bz/Eth-ins

Fig. 5.16. : Infrared spectra of (a) intact soil lipids, (b) hexane soluble fraction, (c) benzene/ethanol soluble fraction, (d) chloroform soluble fraction and (e) water-soluble fraction.


(a)
(b)
(c)
(d)
(e)
Transmittance
4000
2000
1200
400
Wavenumber (cm^{-1})

These data agree well with previously described spectroscopic and chromatographic analyses indicating that the components of the materials were predominantly carbon, hydrogen and oxygen making up polymethylene compounds. The C:H ratios lie between 6.9 and 7.6 which are again consistent with the predominance of polymethylene compound signals in the infrared and nuclear magnetic resonance spectra as well as with the fatty acid composition as revealed by GC/MS analyses. The presence of nitrogen and the infrared absorbance near 1650 cm^{-1} of the intact lipid and benzene/ethanol soluble fraction suggests the presence of some long chain amides as well as acids and esters. However, as the extraction procedure involved the use of ammoniacal solutions, the nitrogen content may be an artifact of the extraction procedure.

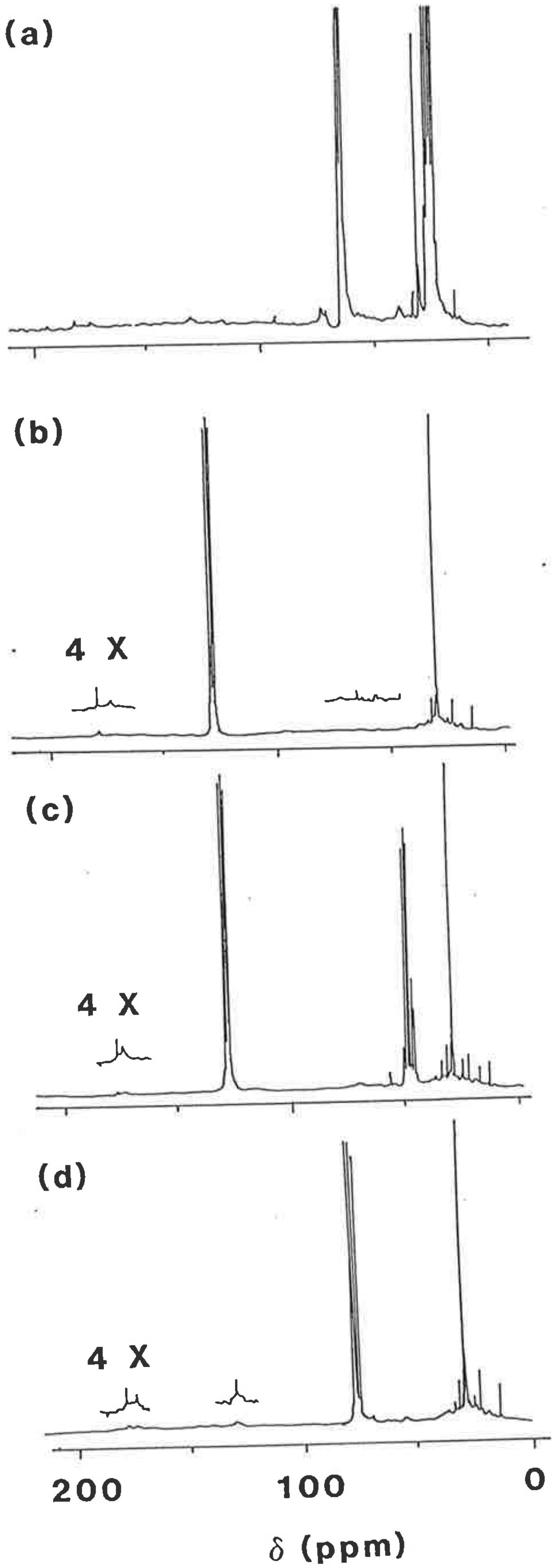
Table 5.2. Analytical data for soil lipids

Sample	MED value (400 mg kg^{-1})	Percent by mass C	H	N	O	Ash
Intact lipids	2.8	57.1	8.1	4.2	- *	6.2
Hexane soluble	4.5	72.0	10.5	2.3	12.8	1.4
Benzene/ethanol soluble	4.2	63.5	8.8	3.7	21.3	1.8
Chloroform soluble	3.0	69.8	9.2	2.3	17.1	1.3

Note : * Oxygen analysis was not carried out due to high ash content.

Fig. 5. 17. demonstrates the similarity of ^{13}C NMR of intact lipids (a) and hexane soluble materials (b), benzene/ethanol soluble materials (c) and chloroform soluble materials (d). All spectra show characteristic polymethylene resonance at 30.2 ppm. The only differing feature which can be detected on the spectrum of benzene/ethanol soluble fraction is another $\underline{C}H_3$ resonance at 17.9 ppm indicating that branched compounds are

Fig. 5.17. : ^{13}C solution NMR of (a) intact lipids, (b) hexane soluble fraction, (c) benzene/ethanol soluble fraction and (d) chloroform soluble fraction. Solvents used were *iso*-propanol/ammonia, benzene, benzene/chloroform and chloroform, respectively.


(a)
(b)
4 X
(c)
4 X
(d)
4 X
200
100
0
δ (ppm)

probably present in this fraction.

5.3.5. Origin of hydrophobic materials.

In most cases (Table 5.3.) soils under native vegetation were more water-repellent than nearby soils which had been cultivated. One possible explanation for this observation is that the hydrophobic organic matter which is probably derived from waxy *Eucalyptus* leaves is continually accumulated in undisturbed soils, whereas soil cultivation and cropping could have enhanced the distribution of such materials down the profile resulting in a decrease in their concentration in surface soils. Another possiblility is that by cultivating such soils one can induce a better environment for catabolic microorganisms which are able to degrade the hydrophobic compounds. McGhie (1980) postulated that water-repellence decreased with time from clearing due to sheet erosion of the water-repellent topsoils. However, he noted that severe water-repellency was detectable on sites which had been cleared for forty years. Data in Table 5.3. also indicated that water-repellency varies with type of vegetation. Similar observations were made by Bond (1969a) who found differences in wettability of soils beneath various plant cover.

Prescott and Piper (1932) reported that water-repellence is common in soils under mallee (*Eucalyptus sp.*) trees, however, the β-dicarbonyl ultraviolet spectrum at 273 nm and infrared absorptions at 1607 cm^{-1} which are characteristic of cuticular waxes from many *Eucalyptus* sp. (Horn *et al* 1964) are absent from the waxes extracted from Tintinara soils. This does not mean that they were not possible precursors, however, if they were, they have been oxidised to ester and acids.

Table 5.4. shows there was no obvious relationship between the total amount of material removed by solvent extraction and the water-repellency of original soils. There was no correlation between the total carbon content or the amount of carbon removed by *iso*-propanol/ammonia extraction with the total hydrophobic organic matter or with the severity of water-repellency of original soils (Table 5.4.). This observation may be

Table 5.3. Soil types, geology, vegetation and MED values of soil samples collected from Eyre Peninsula region.

No.	Location	Soil Types, Geology	Vegetation	MED values
1.	Kielpa	Mollineaux sand	Barley (*Hordeum vulgare* L.)	2.5
2.	idem	idem	Pasture	2.5
3.	idem	idem	Cereal Rye (*Secale cereale* L.)	1.5
4.	idem	idem	Narrow-leaved mallee (*Eucalyptus foecunda* Schauer)	2.0
5.	idem	idem	Broombush (*Melaleuca uncinata* R.Br.)	2.5
6.	idem	idem	Wheat (*Triticum aestivum* L.)	2.0
7.	Wharminda	idem	Veldtgrass (*Ehrharta* sp.)	2.5
8.	idem	idem	Common evening-primrose (*Oenothera stricta*)	3.0
9.	idem	idem	Barley (*Hordeum vulgare* L.)	2.5
10.	idem	idem	Broombush (*Melaleuca uncinata* R.Br.)	3.5
11.	idem	idem	Vetch	3.0
12.	Ceduna	Calcareous	Common iceplant (*Mesembryanthemum crystallinum* L.)	2.0
13.	idem	idem	Billybutton (*Craspedia* sp.)	0.5
14.	idem	idem	Barleygrass (*Hordeum* sp.)	0.5
15.	Streaky Bay	Red-brown sandy soil	Dryland tea tree (*Melaleuca lanceolata* Otto)	2.0
16.	idem	idem	Lincoln weed (*Diplotaxis tenuifolia*)	1.0
17.	Mangalo	Lowan sand	Lucerne (*Medicago sativa* L.)	0.5
18.	idem	idem	Barley (*Hordeum vulgare* L.)	1.0
19.	idem	idem	Ridge-fruited mallee (*Eucalyptus incrassata* Labill.)	3.5
20.	Eyre Highway	idem	Pasture	1.0
21.	idem	idem	Barley (*Hordeum vulgare* L.)	0.5
22.	idem	idem	Ridge-fruited mallee (*Eucalyptus incrassata* Labill.)	3.0
23.	Wanilla	idem	Pasture	3.0
24.	idem	idem	Barley (*Hordeum vulgare* L.)	3.5
25.	idem	idem	Ridge-fruited mallee (*Eucalyptus incrassata* Labill.)	4.5
26.	Maltalie	Coarse Lowan	Barley (*Hordeum vulgare* L.)	1.5
27.	idem	idem	Ridge-fruited mallee (*Eucalyptus incrassata* Labill.)	3.5

rationalized by the presence of differing ratios of hydrophobic to hydrophilic components in the crude mixture. Previous studies reported by Bond (1969a) suggested that even though the development of water-repellency is ascribed to the presence of plant residues, total organic matter content was believed to have no relation to the severity of water-repellency. He noted that some sands containing 5 % carbon were less water-repellent than others which have a contact angle of wetting above 90° but contained only 0.1 % carbon.

Table 5.4. MED values and carbon content of, and the amount of hydrophobic materials extracted with *iso*-propanol/ammonia from Eyre Peninsula samples.

No.	MED value (M)	Original C (%)	Extracted C (%)	Extracted materials (mg kg^{-1})
1.	2.5	0.375	0.027	846
2.	2.5	0.418	0.035	962
3.	1.5	0.396	0.005	928
4.	2.0	0.341	0.024	966
5.	2.5	0.474	0.129	1365
6.	2.0	0.391	0.047	1055
7.	2.5	0.543	0.051	915
8.	3.0	0.717	0.090	1163
9.	2.5	0.782	0.077	1503
10.	3.5	0.608	0.097	1783
11.	3.0	0.311	0.011	642
12.	2.0	3.290	0.050	3871
13.	0.5	2.980	0.270	1842
14.	0.5	4.300	0.080	2798
15.	2.0	11.750	0.050	8965
16.	1.0	11.450	0.100	1514
17.	0.5	0.333	0.033	616
18.	1.0	0.655	0.139	1472
19.	3.5	0.432	0.101	1562
20.	1.0	0.582	0.061	920
21.	0.5	0.879	0.127	1399
22.	3.0	0.479	0.081	905
23.	3.0	1.250	0.060	2133
24.	3.5	1.880	0.150	2764
25.	4.5	4.510	0.440	12260
26.	1.5	1.030	0.205	1467
27.	3.5	0.866	0.176	1830

5.4. Conclusions

These chemical studies have established the following points :

1. The hydrophobic factors for water-repellency in both the amphiphilic *iso* -propanol/ammonia and the humic acid extracts are consistent with the presence of polymethylene compounds as shown by infrared and NMR, elemental composition, thin layer chromatography and GC/MS of the components.

2. The presence of ester and carboxyl carbonyl bonds in the infrared spectra of the most hydrophobic fractions from *iso* -propanol/ammonia extracts and only carboxyl carbonyl in the humic acid fractions points to ester bond cleavage during the humic acid isolation procedure with an alkaline solution. Because both the long chain alcohols and acids from such a cleavage will also generate hydrophobic surfaces, simple hydrolytic procedures alone can be predicted to be ineffective for reducing water-repellency in soils.

CHAPTER 6

ALLEVIATION OF WATER-REPELLENCY IN SOILS

6.1. Introduction

The occurrence of water-repellent soils has been observed primarily on light textured soils with clay contents usually less than 10 % (Bond and Harris 1964, Bond 1968, Holzhey 1969 and Savage 1975). However, hydrophobic organic materials similar to those responsible for water-repellency in some South and Western Australian soils are present in other soils and have been extracted from the Urrbrae fine sandy loam (Chapter 4). The content of soil lipids in the hydrophilic Urrbrae soil was greater than the content in the series of hydrophobic soils examined. This finding suggests that there are factors other than the presence and concentration of hydrophobic materials which govern the creation of water-repellent surfaces.

There have been several studies which aimed to alleviate water-repellent problems in Australian soils. The use of wetting agents has been considered to offer the potential for increasing wettability of some Western Australian soils (McGhie and Tipping 1984). However, water-repellent soils at various locations in Eyre Peninsula retained some degree of water-repellency following treatment with wetting agents (Mr. K.G. Wetherby, pers. comm.). Cultivation techniques include rotary hoeing and deep ploughing have been extensively used for lucerne establishment in the south east of South Australia (King 1984). However, these procedures render the soils prone to both wind and water erosion.

Therefore, a study of the effects of fine particle materials on the water-repellency in soils was conducted.

6.2. Materials and methods.

6.2.1. Laboratory studies.

6.2.1.1. Soils and acid washed sand.

These materials have been described in previous chapters.

6.2.1.2. Water-repellency and surface area.

Acid washed sand was fractionated by dry sieving into 5 fractions *viz* 150, 250, 422, 500 and 1000 µm. Each fraction was coated with cetyl alcohol which was dissolved in chloroform. After evaporating the chloroform the sand was oven dried before measurement of hydrophobicity.

6.2.1.3. Admixture of fine particle materials.

Cetyl alcohol-coated sands (25 g) were mixed with fine particle materials including clay, lime (calcium hydroxide), aluminium oxide and aerosil (a finely divided commercial silica). The mixture was placed in a 50 ml plastic vial and shaken gently (end-over-end) for 2 minutes. As a control the water-repellent sands were shaken without clay. The water-repellency of the mixture was plotted against the concentration of clay applied.

Five clays with different surface areas, physical and mineralogical properties were fractionated by dry sieving into two fractions : < 53 µm and 53-90 µm. Fractions < 2 µm were obtained by a sedimentation procedure. The clay fractions were mixed with either Tintinara soil or acid washed sand coated with cetyl alcohol according to the procedure described above.

Two techniques of clay application were also examined.

1). A 1 g clod of oven dry clay was placed on the surface of 100 g of acid washed sand coated with cetyl alcohol packed in either a plastic or a glass column with a diameter of 2 cm. Water was supplied continuously using a peristaltic pump until 300 ml of leachate was collected. A 25 ml aliquot of leachate was oven dried in a pre weighed vial. The sand was taken out the column, oven dried and cooled before measurement of water-repellency.

2). Clay (200 mg) was suspended in 100 ml of water. The clay suspension was poured into columns containing either 100 g of acid washed sand (hydrophilic) or 100 g of acid washed sand coated with cetyl alcohol (hydrophobic). The column was washed with water until 200 ml of leachate was collected. The leachate (25 ml) was oven dried in a weighed container. The sand was oven dried, cooled and MED values measured.

6.2.1.4. Determination of dispersible clay.

Air dry soil (25 g) was placed in a 250 ml plastic bottle. To the soil 200 ml of water was added and the suspension shaken for 16 h on an end-over-end shaker. The mixture was washed quantitatively into a 500 ml settling cylinder, which was inverted several times and allowed to stand for 4 h 39 min. The top 50 ml of suspension was removed using a pipette and dried overnight at 105°C in a pre weighed beaker. Dispersible clay is expressed as a percent of oven dry soil.

6.2.1.5. Na- or Ca- saturated clay.

Clay fractions (< 2 µm) were obtained by dispersing 500 g of clay in 1000 ml of water, by shaking for 16 h in an end-over-end shaker. The suspension was allowed to settle for 15 h 29 min and the top 20 cm of suspension collected, concentrated in a rotary evaporator and freeze dried. 10 g of freeze dried clay fraction (< 2 µm) was suspended in 100 ml of either 1 M NaCl or 1 M $CaCl_2$ and liquid supernatant was decanted after centrifuging. The clay was washed twice with either 1 M NaCl or 1 M $CaCl_2$ and then with water until the clay began to disperse. The clay suspension was then dialyzed against reverse osmosis water (< 5 µS) until it was free from salt and finally freeze dried. These cation saturated clays were than mixed homogeneously with acid washed sand coated with cetyl alcohol according to the technique descibed previously.

6.2.1.6. Interaction of clay and hydrophobic sand.

To 100 g of either acid washed sand or cetyl alcohol sand 200 mg of clay was added. The mixture was packed in a 2 cm diameter plastic column. 100 ml of distilled water was then poured into the column. A part of the leachate (25 ml) was oven dried in a vial of known weight .

6.2.1.7. X-ray diffraction .

XRD was carried out with a Philips X-ray vertical goniometer using Co Kα radiation and a graphite post-diffraction monochromator. Oriented clay samples were prepared by drying suspensions onto glass slides prior to analysis.

6.2.2. Field experiment.

6.2.2.1. Location.

The field trial was situated in an area 16 km east of Keith towards Bordertown in the South East of South Australia. The soil showed a bleached A_2 horizon over a pedal subsoil. The factual key notation is Dy5.43 which is common for many non-wetting sands.

6.2.2.2. Experimental design.

Finely ground clay obtained from Millicent was incorporated into the water-repellent soils. The clay is based on palygorskite and smectite and is calcareous (Ug5.11) (Hodge *et al* 1984). The experiment was initiated on May 29, 1987.

There were five treatments :

1. Control
2. 2.5 tonnes/ha.
3. 5 tonnes/ha.
4. 10 tonnes/ha.
5. 20 tonnes/ha.

and two methods of application :

1. clay was spread on the soil surface.

2. clay was mixed with soil to a depth of 10 cm using a rotary hoe.

The design was a 5 x 2 factorial experiment. Treatments were placed according to a random technique and all treatments were replicated four times. The clay was treated with methyl bromide prior to application to kill any weed seeds. The plot size was 2 x 25 m.

Lupins (100 kg ha^{-1}) and a mixture of lucerne (5 kg ha^{-1}) and serradella (2 kg ha^{-1}) were sown. However, due to environmental effects resulting in poor germination of both lucerne and lupins in all plots these crops were destroyed. Seven weeks after clay application the plots were resown with barley and lucerne (Hunter Field). Three months later half of the barley plants in each plot were killed to provide a better growth environment for lucerne establishment.

6.2.2.3. Parameters.

Water-repellency tests were conducted five times : prior to clay application, and 6, 16, 28, and 46 weeks after treatment. Two composite soil samples *viz* 0-2.5 cm and 0-10 cm were collected from each plot. Establishment of barley and lucerne was monitored 9 and 39 weeks after sowing. Available water of the composite soil samples was determined 16 weeks after addition of clay.

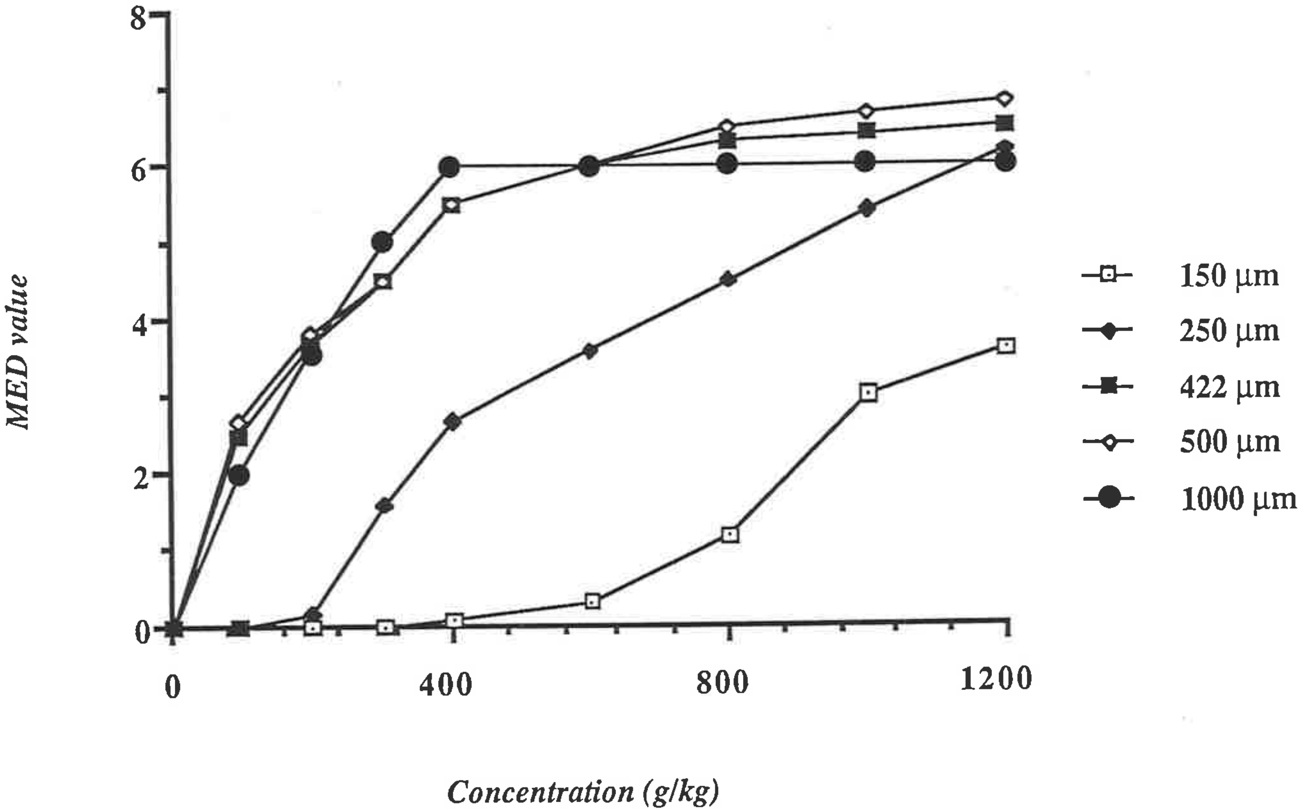
6.3. Results and Discussion.

6.3.1. Laboratory studies

6.3.1.1. Water-repellency and surface area.

Fig. 6.1. demonstrates the relationship between size of sand grains, and therefore specific surface area, and the amount of hydrophobic compound required to produce water-repellency. To form a close packed monolayer of cetyl alcohol 28.4 , 17.0, 10.1, 8.5 and 4.3 mg cetyl alcohol was required per kg acid washed sand with average particle size of 150, 250, 422, 500 and 1000μm respectively. These calculations indicate that to create a

Fig. 6.1. : Water-repellency of acid-washed sand is controlled by the concentration of cetyl alcohol applied and particle size (i.e. the surface area of the sand).


8
6
4
2
0
MED value
0
400
800
1200
Concentration (g/kg)
150 µm
250 µm
422 µm
500 µm
1000 µm

measurable water-repellency sand grains have to be coated with considerably more than a monolayer of cetyl alcohol. To produce water-repellency at the level comparable to naturally occurring Tintinara soils (MED = 3.5) as much as 1200 mg cetyl alcohol was necessary to coat 1 kg of sand with an average particle diameter of 150 µm. Such an amount would represent 42 monolayers. However, to create the same MED value on 1 kg of sand with grain diameters of 1000 µm required 200 mg of cetyl alcohol. This was sufficient to produce 46 monolayers. This implies that to produce water-repellency on very fine particles such as clay a very large amount of hydrophobic organic compound would be necessary. This explains why fine textured soils such as the Urrbrae fine sandy loam remain hydrophilic even though such soils contain substantial amounts of lipids similar to those materials responsible for water-repellency in sands. Bond and Harris (1964) reported that sandy soils are always more water-repellent than loams and other fine textured soils containing more than 5 % clay. DeBano and Rice (1973) found that coarse textured soil became more water-repellent than soils of finer texture. It was suggested that this feature was a consequence of the fact that the surface area per unit mass is lower on coarse textured soils and they are more easily coated by the hydrophobic materials (Biswell 1975).

A logical consequence of these observations, is that it should be feasible to decrease the water-repellency of light textured soils such as those near Tintinara by adding fine materials to increase their surface area. Two field experiments which involved the admixture of fine particle materials such as clay with the hydrophobic sands have been briefly reported. The application of this approach to combat water-repellency in Florida soils was noted in a report by King (1974). Bond (1978) incorporated cores of loam containing up to 16 % clay into non-wetting golf greens. This was undertaken as a special purpose solution to the problem of irregular turf growth. However, no detailed laboratory examination of the factors involved appear to have been reported in the available literature.

6.3.1.2. The admixture of fine particle materials with water-repellent sand.

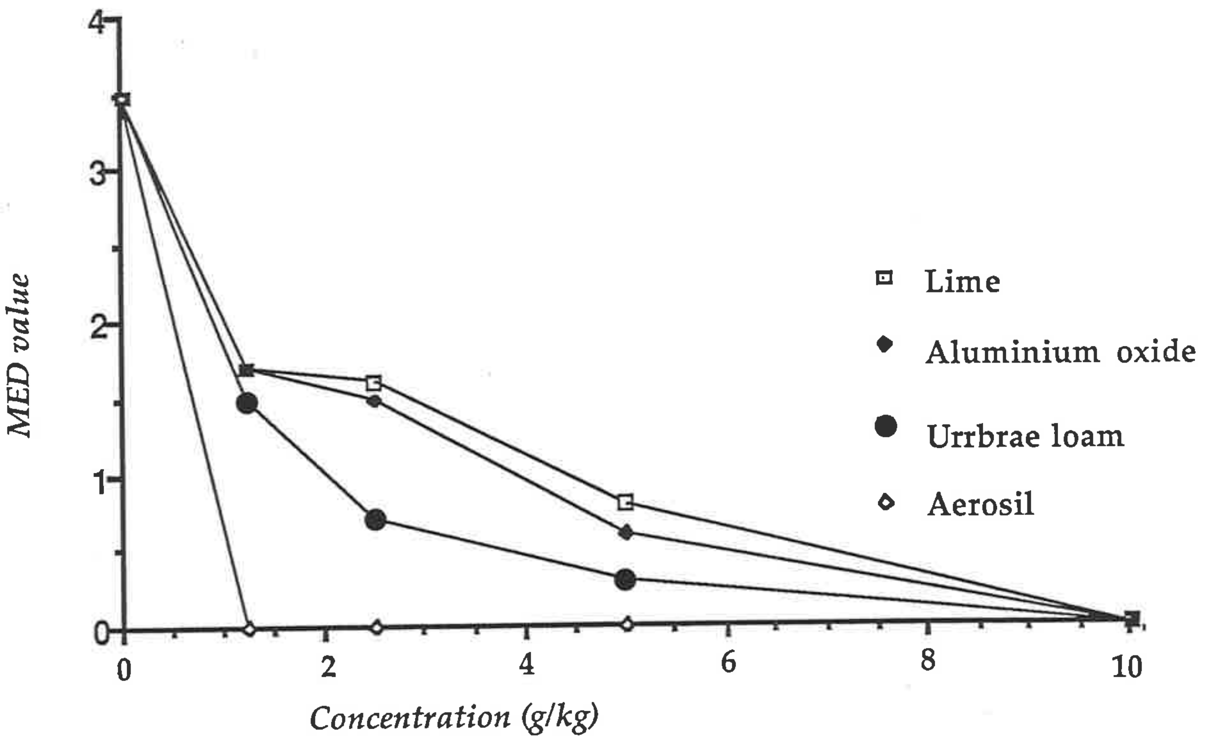
In laboratory studies, considerable reduction in water-repellency of Tintinara soil and acid washed sand coated with cetyl alcohol was noted following treatment with fine particle materials (Fig. 6.2. and 6.3.). It is obvious that aerosil (a finely divided commercial silica) with specific surface area of 380 m^2 g^{-1} was the most efficient ameliorator. However, the use of natural materials such as clay is more realistic, because clay would provide additional advantages, such as increased cation exchange capacity and water holding capacity, to the soils.

Table 6.1. Rates of application of various clays
to a water-repellent Tintinara soil

Clays	Rate of application (g kg^{-1})
Mundulla	5.0
Urrbrae	5.0
Millicent	7.5
Blanchetown	10.0

Application of clay at rates as low as 1.25 g kg^{-1} which is equal to 1.25 tonnes ha^{-1} (0-10 cm) markedly reduced the MED values of the Tintinara water-repellent soil (Fig.6.4.). The amount of clay required to render this soil wettable varied with the type of clay (Table 6.1.). The MED values of both Tintinara soils and acid washed sand coated with cetyl alcohol remained unchanged after being shaken by themselves suggesting that abrasive removal of the hydrophobic coatings had been avoided. It appears that the particle size of clay examined controlled to some extent its effectiveness in lowering MED values. The difference in effectiveness of various clay to alleviate water-repellency is also apparently caused by the condition of such clays. Generally ignited clays were less effective than the original clays in reducing MED values, however, with

Fig. 6.2. : Reduction in water-repellency of Tintinara soils as affected by admixture of fine particle materials.



MED value
4
3
2
1
0
0
2
4
6
8
10
Concentration (g/kg)
□ Lime
◆ Aluminium oxide
● Urrbrae loam
◇ Aerosil

Fig. 6.3. : Reduction in water-repellency of acid washed sand coated with cetyl alcohol as affected by admixture of fine particle materials.

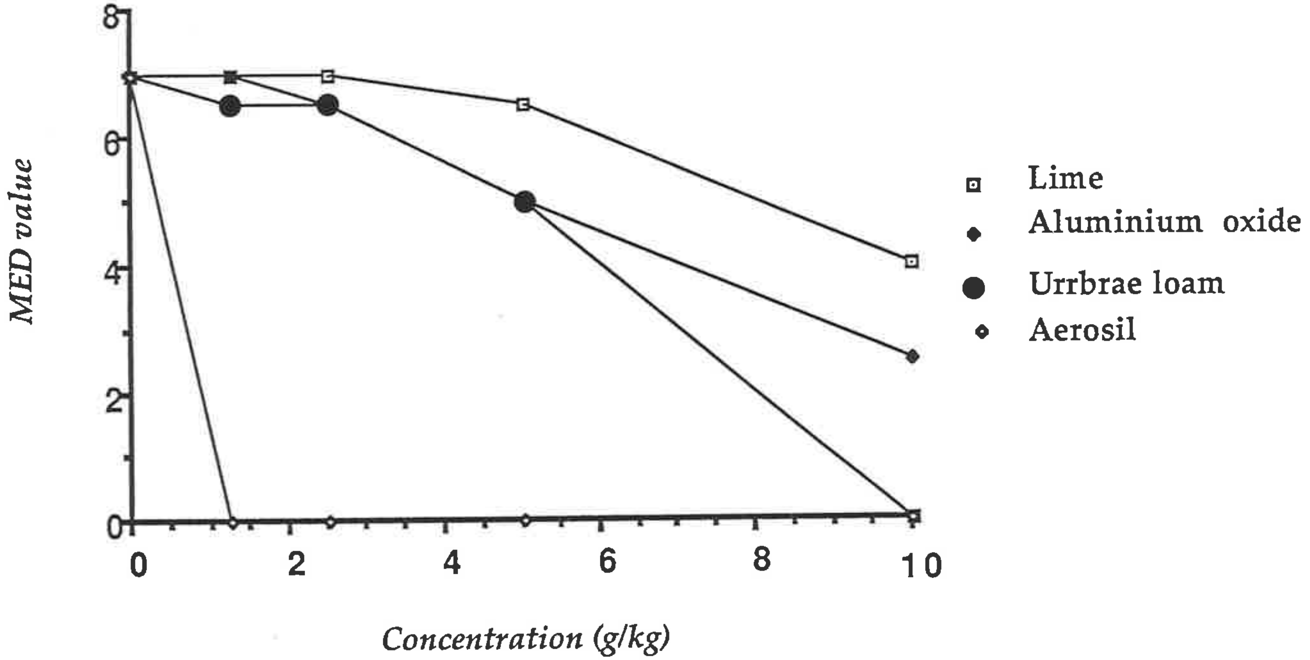
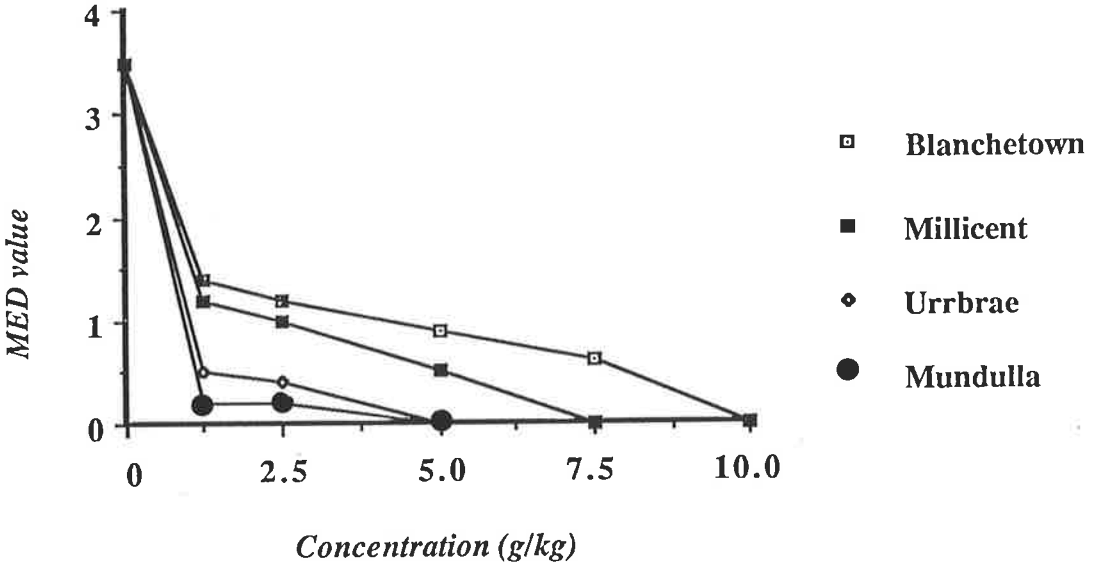

8
6
4
2
0
0
2
4
6
8
10
MED value
Concentration (g/kg)
Lime
Aluminium oxide
Urrbrae loam
Aerosil

Fig. 6.4. : Effects of clay admixture on water-repellency of Tintinara soils.

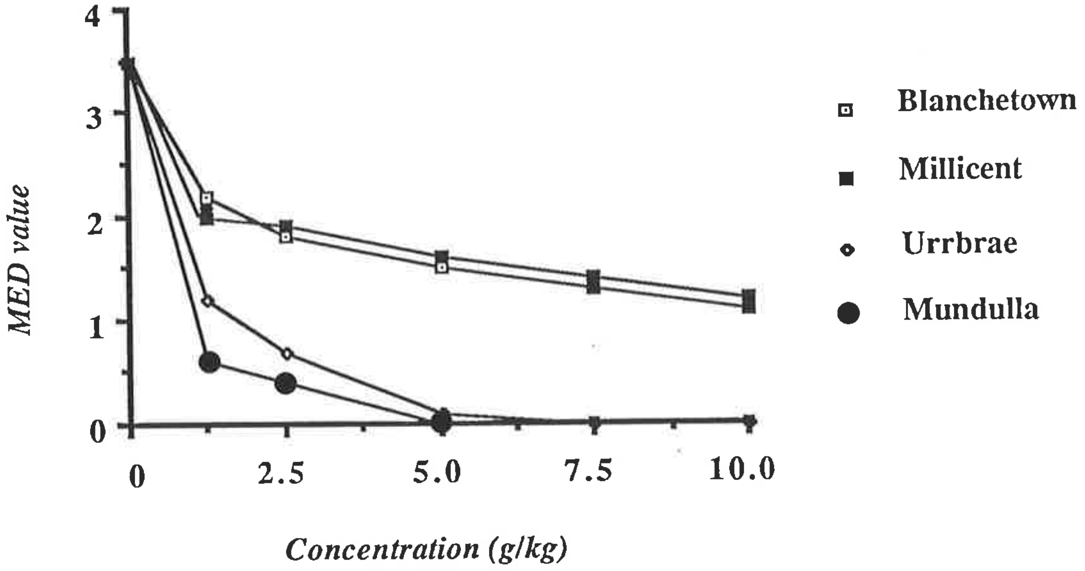
(a) < 53 μm and (b) 53-90 μm.

a).



MED value
4
3
2
1
0
0
2.5
5.0
7.5
10.0
Concentration (g/kg)
Blanchetown
Millicent
Urrbrae
Mundulla

b).



MED value
4
3
2
1
0
0
2.5
5.0
7.5
10.0
Concentration (g/kg)
Blanchetown
Millicent
Urrbrae
Mundulla

very effective clays such as Mundulla clay the difference was barely detectable suggesting that organic matter content is of lower significance than the physical nature of the clays. From the data shown in Table 6.2. it is apparent that the most effective clay (Mundulla) also contains the highest percentage of dispersible clay and the least effective contains the lowest percentage of dispersible clay.

Table 6.2. Dispersibility of clays

Clay	% dispersible clay
Mundulla	31.1
Urrbrae	14.1
Blanchetown	11.0
Millicent 1	9.7
Millicent 2	2.4

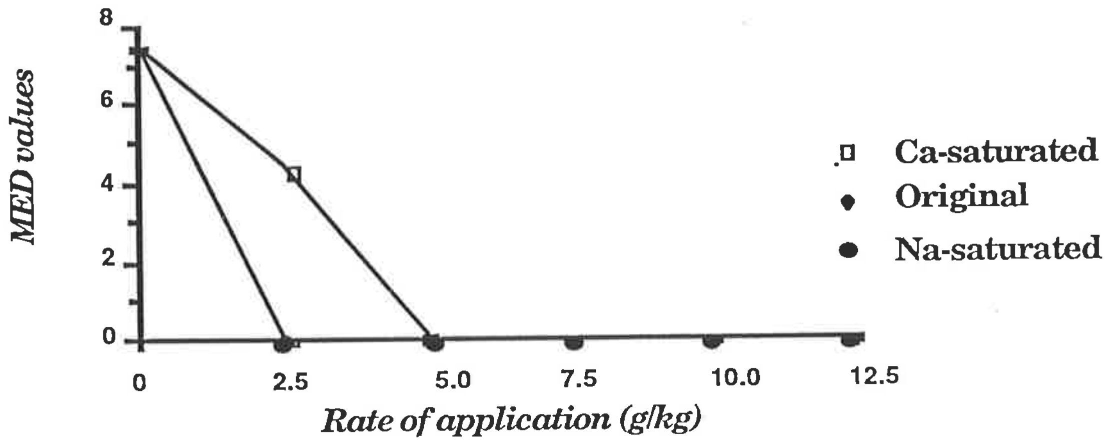
Sodium saturated clays are always more effective in reducing water-repellency than their original or calcium saturated states (Fig.6.6.a., b., and c.). This observation confirms the suggestion that dispersibility of clays is of particular importance in determining their effectiveness in reducing water-repellency in soils.

The mineralogy of clay applied is also of primary importance. Mineralogical examination revealed that the major component of Mundulla clay was kaolinite and this clay mineral was found to be as effective on a weight basis as illite. However, these clay minerals were much more effective than montmorillonite in reducing the hydrophobicity of the extremely hydrophobic model system consisting of acid washed sand coated with cetyl alcohol (Fig. 6.7.). The low effectiveness of Millicent clays on the other hand can be attributed to their mineralogy which is predominantly palygorskite and montmorillonite as well as to their less dispersive nature. Mineralogy controls to some extent the dispersibility

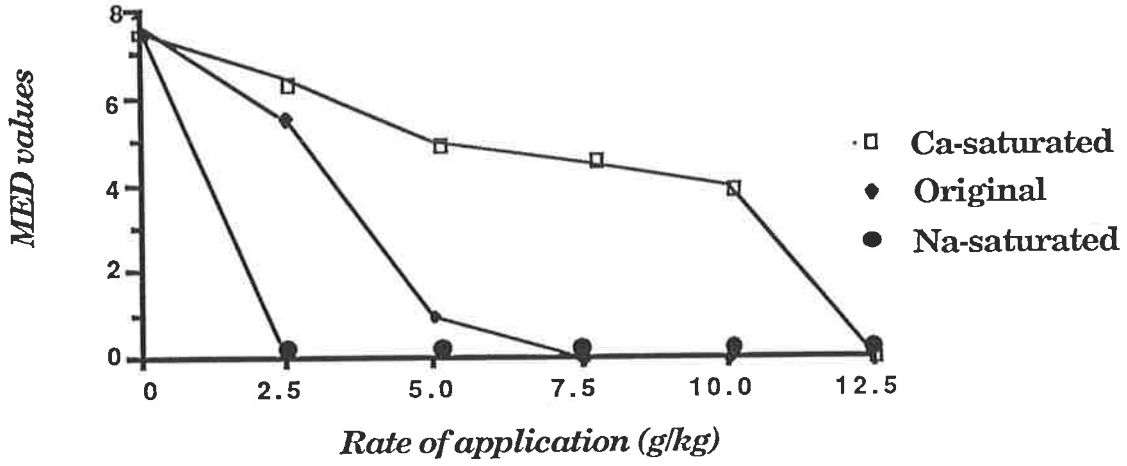
Fig. 6.6. : Comparison of cation saturation on the effectiveness of clays in reducing water-repellency of acid washed sand coated with cetyl alcohol.

(a). Willalooka illite, (b). Mundulla clay and (c). Red Hill montmorillonite.

a).


MED values
8
6
4
2
0
0
2.5
5.0
7.5
10.0
12.5
Rate of application (g/kg)
□ Ca-saturated
♦ Original
● Na-saturated

b).


MED values
8
6
4
2
0
0
2.5
5.0
7.5
10.0
12.5
Rate of application (g/kg)
□ Ca-saturated
♦ Original
● Na-saturated

c).

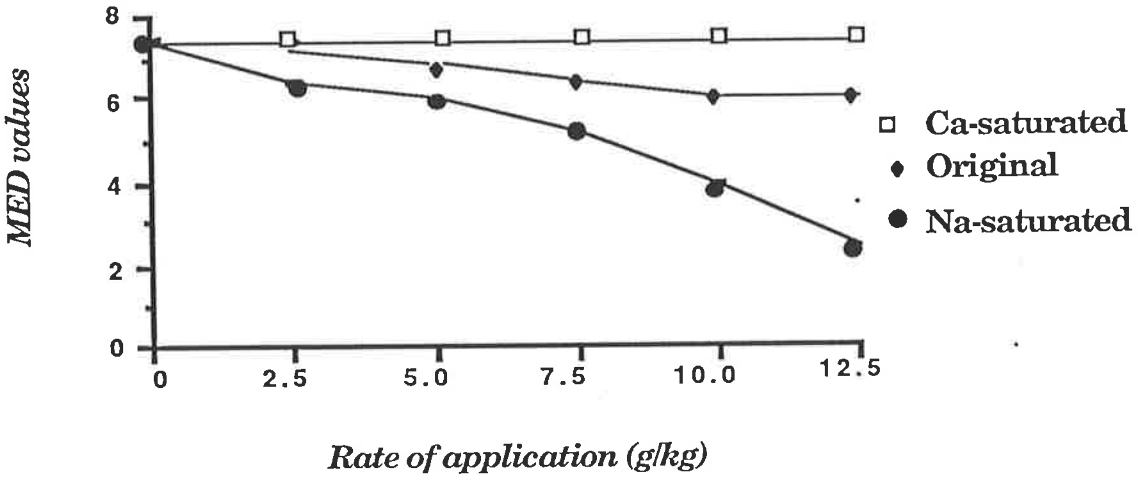
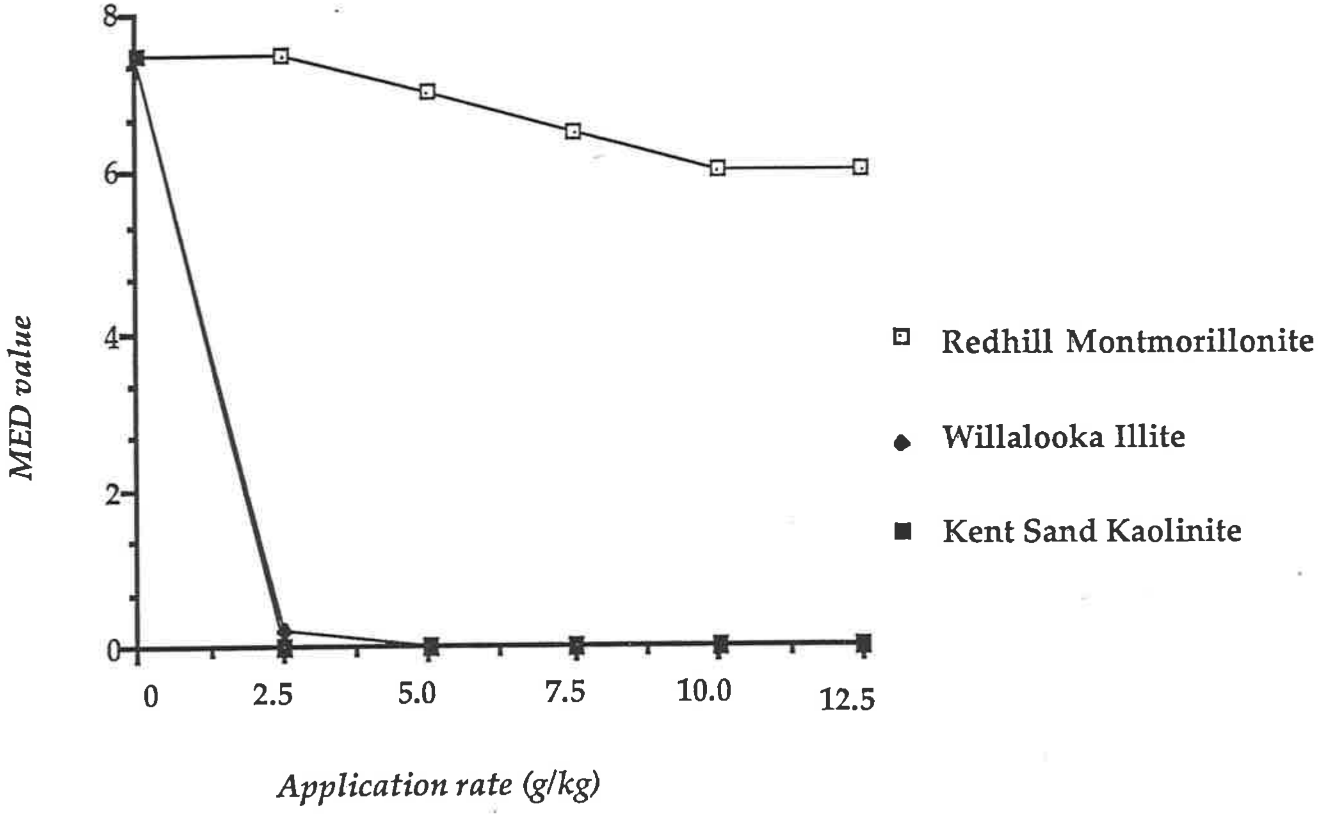

MED values
8
6
4
2
0
0
2.5
5.0
7.5
10.0
12.5
Rate of application (g/kg)
□ Ca-saturated
♦ Original
● Na-saturated

Fig 6.7. : Comparison of the effectiveness of clay minerals in decreasing water-repellency of acid washed sand coated with cetyl alcohol. The Willalooka Illite data is coincident with Kent Sand Kaolinite data.


8
6
4
2
0
MED value
0
2.5
5.0
7.5
10.0
12.5
Application rate (g/kg)
□ Redhill Montmorillonite
◆ Willalooka Illite
■ Kent Sand Kaolinite

of clays. Differences in clay mineralogy among the soils are believed to partly account for the differences in characteristics of clay dispersion (Arora and Coleman 1979, Velasco-Molina *et al* 1971 and Ali *et al* 1987). However, there were variations of the results reported by these workers which were attributed to the difference in salts used, electrolyte concentration and sodium adsorption ratio (SAR). The results reported by Arora and Coleman (1979) imply that soils with illitic clay are more dispersive than soils dominated by montmorillonite clay. Similar findings were suggested by Ali *et al* (1987) who believed that in a particular soil kaolinite and mica clays were apparently more dispersive than montmorillonite. The size and charge of exchangable cations and electrolyte concentration control repulsive forces between clay particles (Oades 1986). Spontaneous dispersion of Na-clays occurs when the electrolyte concentration falls below 0.1 M. However, Ca-clays do not disperse spontaneously, even in distilled water, but they can be dispersed mechanically.

The difference in the effectiveness of various clays in reducing water-repellence may also be attributed to the differences in size and shape of clay particles which are also largely determined by their mineralogy. The morphology of kaolinite is blocky-rigid with a width/thickness ratio of ~ 2, illite is platy-rigid with a width/thickness ratio of ~ 10 and montmorillonite is thin sheet-flexible a width/thickness ratio of ~ 100 (Oades 1986). Therefore, particles of kaolinite and illite can not be packed together as well as sheets of montmorillonite and aggregates of clay particles of kaolinite and illite are readily dispersed especially with Na on the exchange complex.

6.3.1.3. Methods of application.

Results presented in Fig. 6.4. were obtained by mixing clay with hydrophobic sand while they were air dry. Clay mixed by this technique was much more effective in alleviating water-repellency of sand than the two other methods examined. When a clod of clay was placed on the surface of a hydrophobic sand column and was washed down with water the MED value of the sand remained unchanged even though rates of application up to 10 mg kg^{-1} were used. Such rates applied dry rendered a very hydrophobic sand wettable. A similar result was observed when clay was applied in the form of a suspension. As much as 45 % of the dispersive Mundulla clay applied as clod was transported down the column when continuously wetted with water and 93 % of Na-montmorillonite moved down the column. However, less dispersive clays apparently did not move down and formed layers on the surface of the sand column. An important observation was that dry clay adhered more strongly to hydrophobic sands than to hydrophilic acid washed sand (Table 6.3.). This suggests that hydrophobic organic compounds such as cetyl alcohol act as a bridge between sand and clay through a mechanism which requires investigation.

Table 6.3. Transportation of clays through sand columns

Column	% of clay transport
Na-montmorillonite + hydrophilic sand	68.6
Na-montmorillonite + hydrophobic sand	10.6
Ca-montmorillonite + hydrophilic sand	4.9
Ca-montmorillonite + hydrophobic sand	2.5
Urrbrae loam + hydrophilic sand	5.6
Urrbrae loam + hydrophobic sand	3.1
Loxton clay + hydrophilic sand	22.8
Loxton clay + hydrophobic sand	17.7
Millicent clay + hydrophilic sand	4.1
Millicent clay + hydrophobic sand	2.4

6.3.2. Field experiment.

6.3.2.1. Water-repellency of original soils

MED values of the original soils are presented in Table 6.4. Statistical analysis indicated that there were no significant differences between plots. According to the classification of severity of water-repellency proposed by King (1981) the surface soil is categorized very severe (for MED >3.0) whereas the samples of 10 cm depth were severely water-repellent (for MED 2.0-3.0). These values are both higher than MED 1.0 which is regarded as critical for agronomic practices. The soil had exhibited severe water-repellency for several years resulting in very poor establishment of both pasture and crop (Mr. D. C. Lewis, pers. comm.). There is no record of previous attempts at alleviation of the problem on this property.

Table 6.4. Average MED values of original Keith soils

Treatment/technique	MED value	
	0-2.5 cm	0-10 cm
Control/spread	3.1 ± 0.1	2.9 ± 0.3
Control/mix	3.1 ± 0.0	3.0 ± 0.1
2.5 tonnes ha^{-1}/spread	3.1 ± 0.1	2.9 ± 0.3
2.5 tonnes ha^{-1}/mix	3.1 ± 0.1	2.9 ± 0.3
5 tonnes ha^{-1}/spread	3.0 ± 0.2	2.9 ± 0.3
5 tonnes ha^{-1}/mix	3.2 ± 0.1	3.0 ± 0.2
10 tonnes ha^{-1}/spread	3.2 ± 0.1	2.8 ± 0.2
10 tonnes ha^{-1}/mix	3.2 ± 0.1	3.0 ± 0.2
20 tonnes ha^{-1}/spread	3.2 ± 0.2	3.0 ± 0.2
20 tonnes ha^{-1}/mix	3.2 ± 0.1	3.1 ± 0.2
Mean	3.1 ± 0.1	2.9 ± 0.2

6.3.2.2. Changes in MED values.

Fig 6.8. shows that in this field trial, water-repellency of soil 6 weeks after clay treatment was still present and the treatments only marginally decreased the MED values. Further decrease in water-repellency was noticed after 16 weeks (Fig. 6.8.b.). However, statistical analysis of MED values at 6 and 16 weeks after treatment indicated that there were no significant differences between treatments or methods of application.

Fig. 6.8. : Effects of clay treatments on water-repellency of Keith soils.

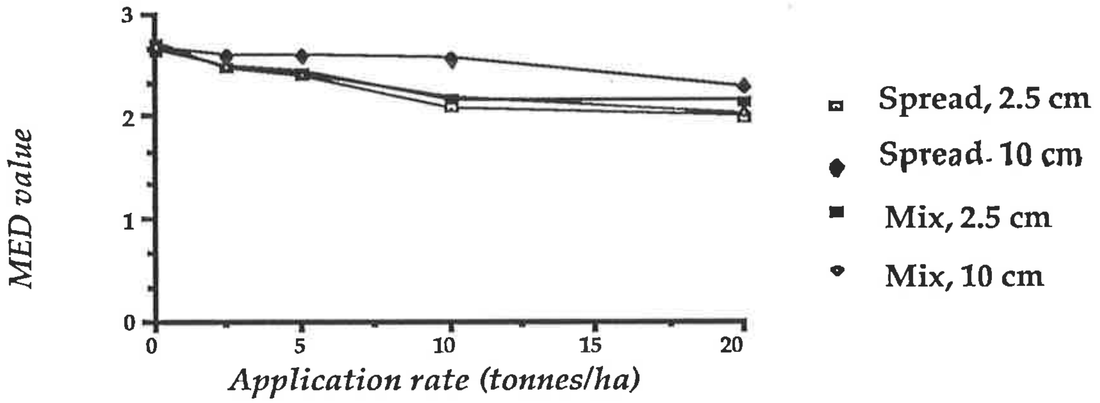
(a) 6 weeks after treatment.

(b) 16 weeks after treatment.

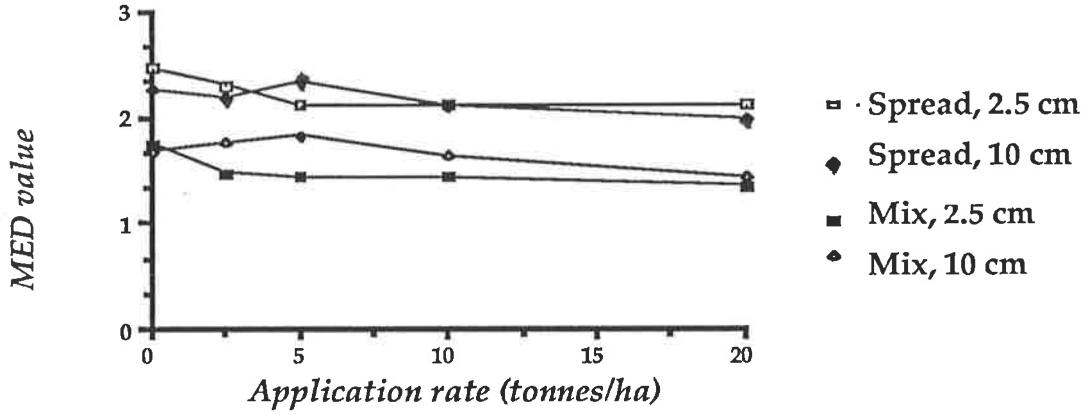
(c) 28 weeks after treatment.

(d) 46 weeks after treatment.

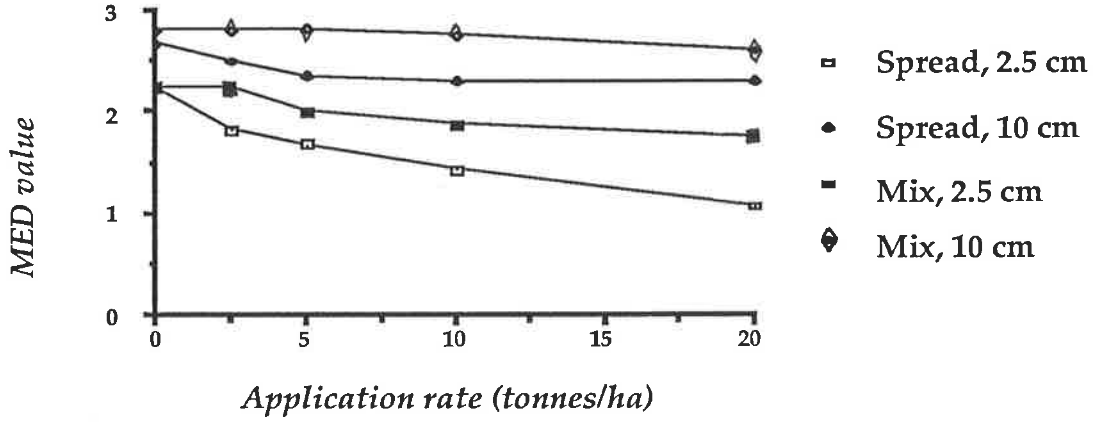
a).


MED value
0
1
2
3
0
5
10
15
20
Application rate (tonnes/ha)
Spread, 2.5 cm
Spread- 10 cm
Mix, 2.5 cm
Mix, 10 cm

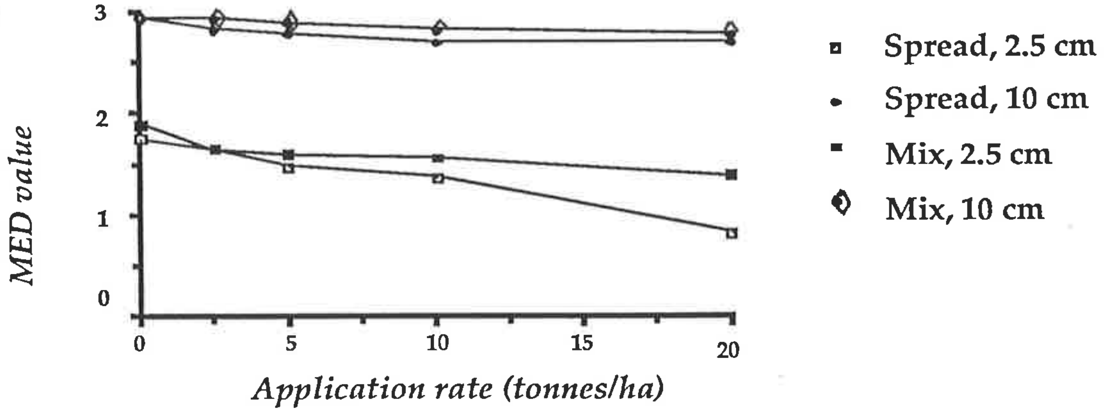
b).


MED value
0
1
2
3
0
5
10
15
20
Application rate (tonnes/ha)
Spread, 2.5 cm
Spread, 10 cm
Mix, 2.5 cm
Mix, 10 cm

c).


MED value
0
1
2
3
0
5
10
15
20
Application rate (tonnes/ha)
Spread, 2.5 cm
Spread, 10 cm
Mix, 2.5 cm
Mix, 10 cm

d).


MED value
0
1
2
3
0
5
10
15
20
Application rate (tonnes/ha)
Spread, 2.5 cm
Spread, 10 cm
Mix, 2.5 cm
Mix, 10 cm

Nevertheless, even at this stage, the MED data suggest that spreading clay on the soil surface was the more effective method of application and this treatment reduced MED values of the top 2.5 cm of soil. Surface application has the great virtue of simplicity and successfully alleviated water-repellency on a nearby property at Mundulla (Mr. C. Obst, pers. comm.). However, there are no records of the actual amounts of clay applied by this farmer, except that they were very large, probably approaching 100 tonnes ha^{-1} .

Soil samples collected 28 weeks after treatment were much less water-repellent, particularly those which had been treated with the highest rate of clay (Fig. 6.8.c.). Application of 20 tonnes ha^{-1} markedly reduced MED values of top 2.5 cm soil to 1.1 (spread) and 1.7 (mixed). This suggests that by spreading such clay at this rate the MED values of this soil nearly reached the agronomically acceptable MED value of 1.0 as proposed by King (1981). Statistical analysis of the MED values of the top 2.5 cm samples confirmed that there were significant differences at the 0.05 level between treatments and methods, however, there were no interactions between the two.

Further decreases in water-repellency of the soil were measurable at 46 weeks after the incorporation of clay (Fig. 6.8.d.). MED values of the top 2.5 cm soil which had been treated with the highest amount of clay (20 tonnes ha^{-1}) was reduced to 0.8 and 1.4 for spreading and mixing techniques respectively. Nevertheless, the statistical analysis indicated no significant differences among the treatments. The equivocal nature of the statistical analyses with time suggests that other factors apart from the clay itself are responsible for the measurable decrease in hydrophobicity. One such factor which has yet to be investigated in detail, concerns the microbial populations of the soils.

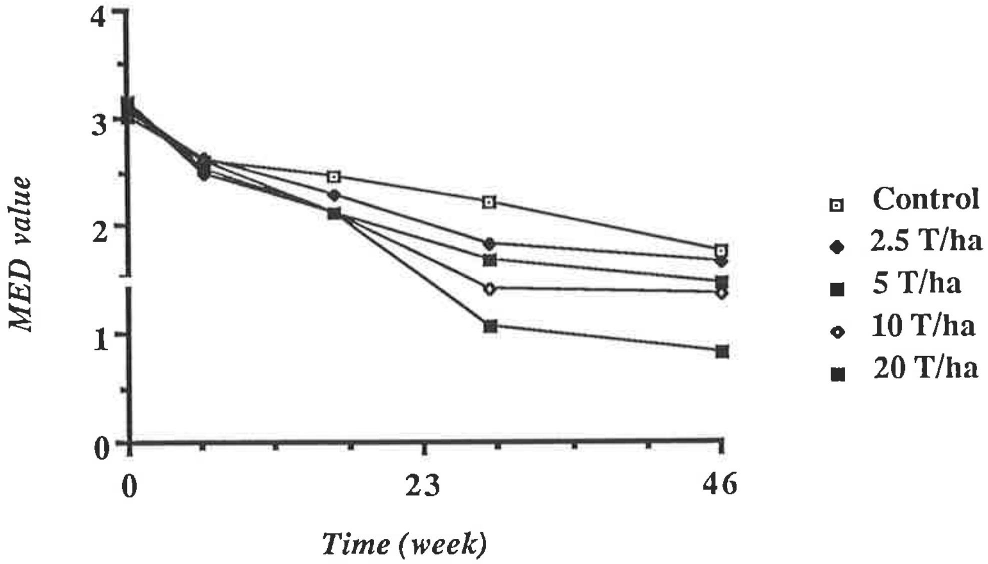
Fig. 6.9. a and b demonstrate that there was a continual decrease in water-repellency with time suggesting that the clay is gradually translocated and homogeneously mixed with sand particles. However, the fact that untreated soils became slightly less water-repellent than their original condition indicated that environmental factors have partially affected the stability of water-repellent status. Bond (1969a) confirmed that the intensity of water-repellency in the field varied with the season when the soil was tested

Fig. 6.9. : Changes in water-repellency of clay treated Keith sand with time.

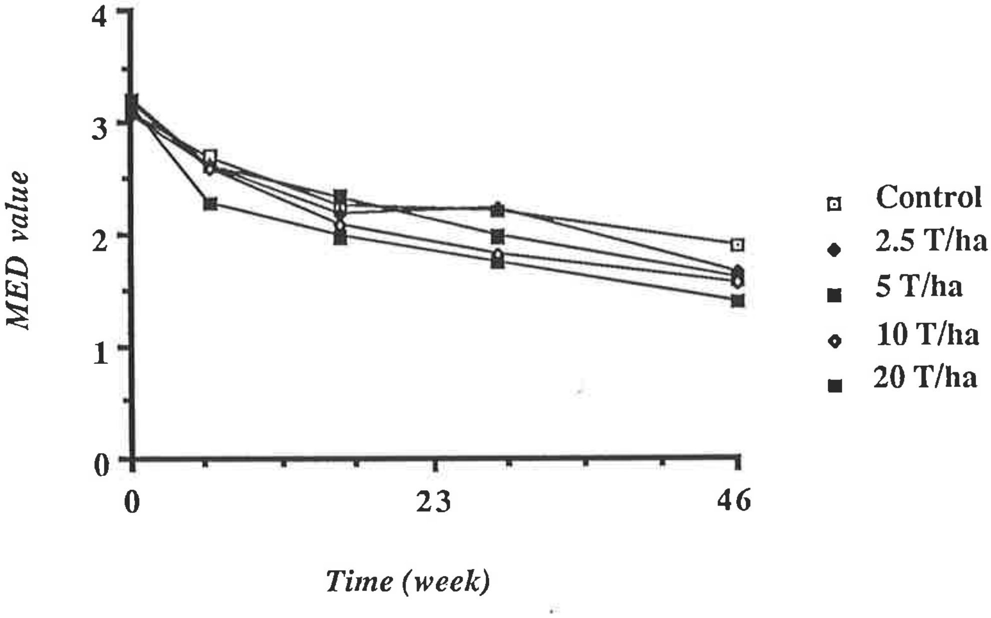
(a). 0-2.5 cm soil samples, clay was spread on the surface.

(b). 0-2.5 cm soil samples, clay was incorporated into the top 10 cm of soil.

a).


MED value
4
3
2
1
0
0
23
46
Time (week)
Control
2.5 T/ha
5 T/ha
10 T/ha
20 T/ha

b).


MED value
4
3
2
1
0
0
23
46
Time (week)
Control
2.5 T/ha
5 T/ha
10 T/ha
20 T/ha

and the nature of these environmental factors remains an important matter for future investigations.

6.3.2.3. Barley and lucerne establishment.

There was no statistically significant effect of clay treatment on the establishment of both barley and lucerne (Fig. 6.10.). However, it was apparent that lucerne establishment was marginally superior in plots with clay spread on the surface and is worthy of further study.

6.3.2.4. Available water.

Fig. 6.11. demonstrates that available water was elevated by clay treatment and this increase was statistically significant. Roberts (1966) also reported that incorporating fine particle materials namely clay, fly ash and silica into the top 10 cm of Gavin sand in Western Australia at the rate of ~ 34 tonnes ha^{-1} significantly improved water regimes in the top soil. He found that the percentage of water in soil samples from the 0-5 cm horizon taken 24 h after rainfall were 4.2%, 8.3%, 8.5% and 8.0% for control, silica treated, clay treated and fly ash treated respectively.

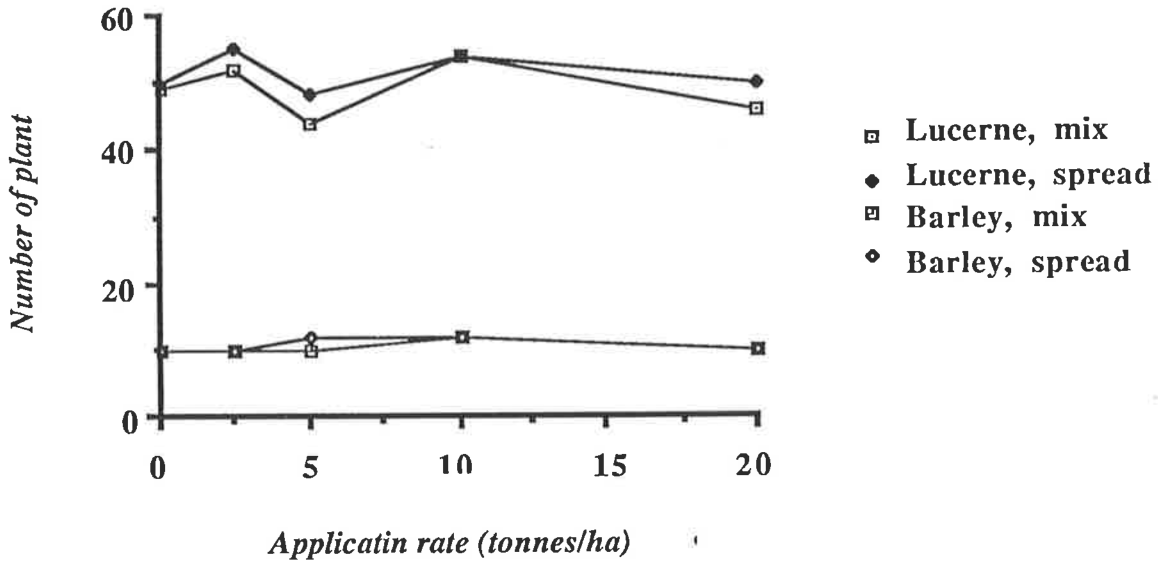
6.4. Conclusions

Water-repellency induced by organic compounds such as cetyl alcohol on acid washed sand is a function of both the concentration of the hydrophobic materials and the surface area of the sands. Sands with low surface area are easily rendered hydrophobic. Clays on the other hand require up to one hundred times more water-repellent materials to render them hydrophobic. The admixture of clay with water-repellent soils has been found to be particularly effective in alleviating water-repellency. However, the efficiency of clays in decreasing the severity of water-repellency of sands also depends on the ease with which

Fig. 6.10. : a. Barley and lucerne establishment 9 weeks after sowing

b. Lucerne establishment 39 weeks after sowing.

a).


60
40
20
0
Number of plant
0
5
10
15
20
Applicatin rate (tonnes/ha)
Lucerne, mix
Lucerne, spread
Barley, mix
Barley, spread

b).

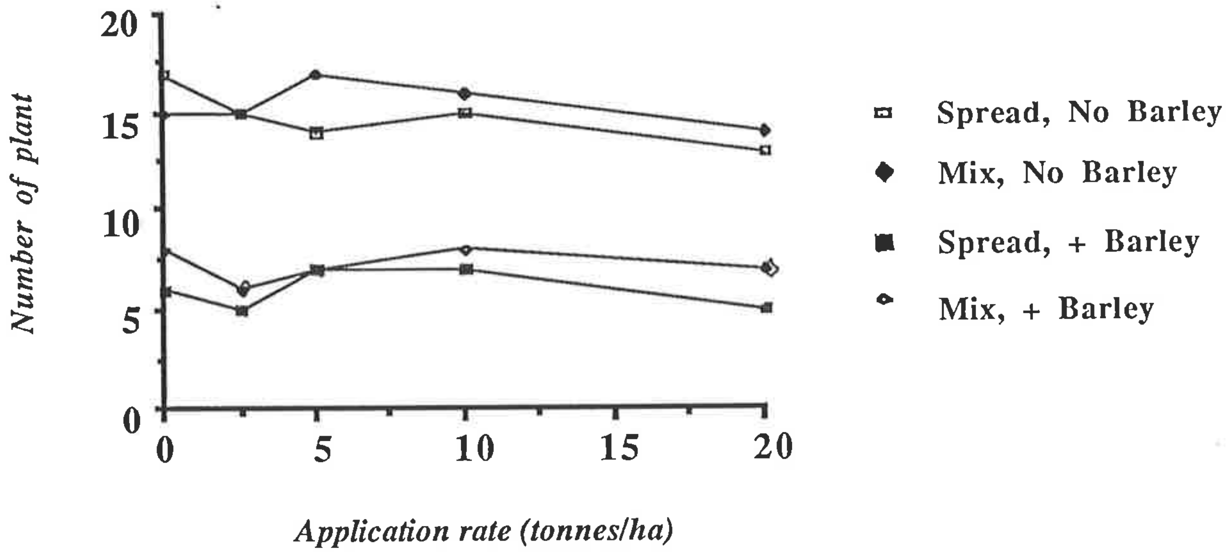
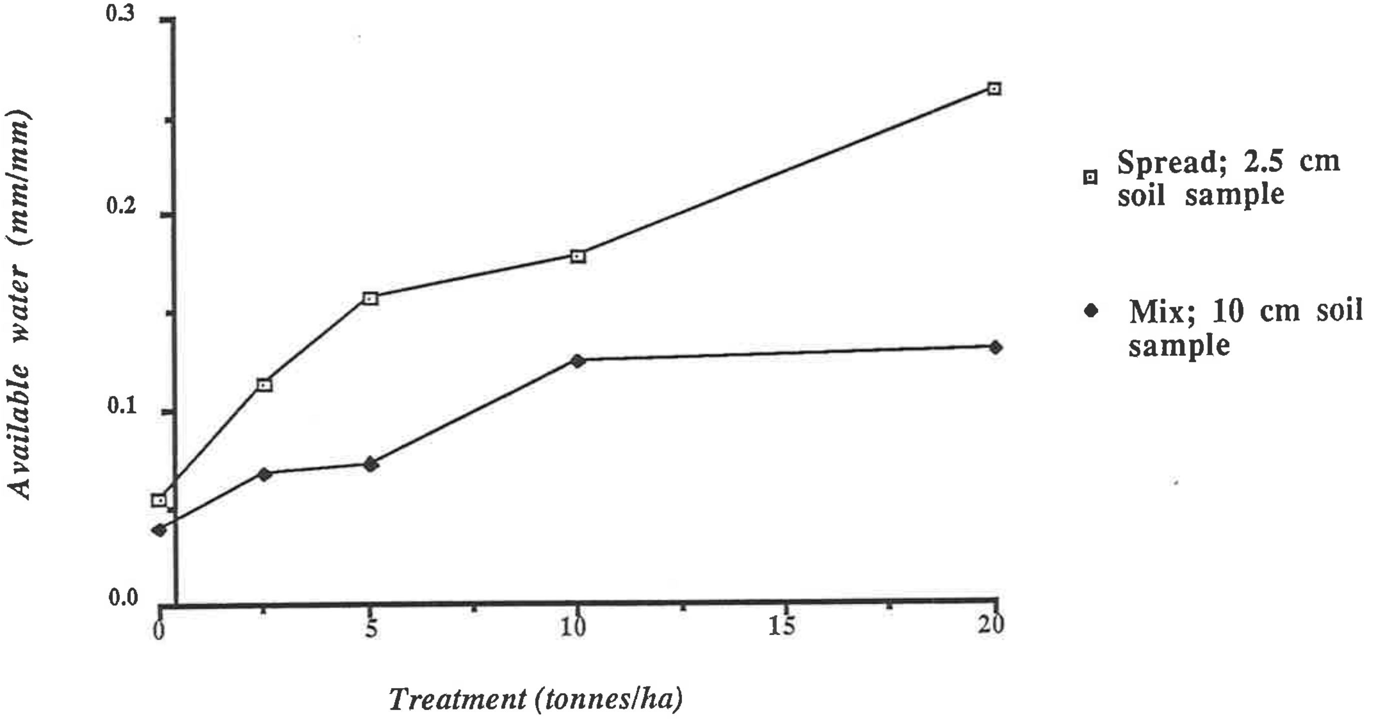

20
15
10
5
0
Number of plant
0
5
10
15
20
Application rate (tonnes/ha)
Spread, No Barley
Mix, No Barley
Spread, + Barley
Mix, + Barley

Fig. 6.11. : Effects of clay treatment on available water in Keith water-repellent sand 16 weeks after application.



0.3
0.2
0.1
0.0
Available water (mm/mm)
0
5
10
15
20
Treatment (tonnes/ha)
Spread; 2.5 cm soil sample
Mix; 10 cm soil sample

they can be dispersed. Dispersibility of clays is related to both their sodicity and particle shape which are largely determined by their mineralogy. Thus, because of the microstructure of clays kaolinite and illite have been shown to decrease hydrophobicity more efficiently than montmorillonite.

The first field trial was commenced before the significance of the dispersibility of clays became apparent. Nevertheless, despite its limited effectiveness, a non-sodic montmorillonite derived from Millicent was still able to reduce water-repellency of soils in a field experiment near Keith. However, the reduction to an agronomically acceptable MED value of 0.8 was observed only one year after treatment. An amount of 20 tonnes clay per ha was necessary to achieve such a reduction. I now anticipate much better results would be achieved using a sodium-kaolinite system. This system is often widespread in areas of water-repellent soils as an underlying clay layer. Field trials are now warranted as we have reliable simple methods for assessing the efficiency of various clays.

CHAPTER 7

BIOLOGICAL CATABOLISM OF WATER-REPELLENT COMPOUNDS IN SOILS

7.1. Introduction

With the identification of the causal agent for water-repellency in South Australian soils as a series of long chain polymethylene compounds, the question arose as to whether it is possible to alleviate the water-repellency by stimulating the microbiological catabolism of these waxes. The subject was brought to my attention by a report from Mr. K.G. Wetherby of South Australian Department of Agriculture at Cleve, who noticed that his share of a sample of a water-repellent soil decreased in MED value from 2.0 to 0 during storage, whilst a portion of the same soil stored at the Waite Institute remained hydrophobic, MED 0.7, after the same period of storage.

Long chain polymethylene compounds can be degraded by a number of microorganisms, such as *Corynebacterium* sp. (Ladd 1956), *Micrococcus cerificans* (Hankin and Kolattukudy 1968) and *Pseudomonas* sp. (Hankin and Kolattukudy 1969). There are a number of enzyme systems which catalyse the oxidation of fatty acids resulting in reduction of one or two carbon atoms in the molecules depending on the enzyme system which decompose the long chain fatty acids (Harwood and Russell 1984). Moucawi *et al* (1981) have studied the catabolism of polymethylene compounds in a range of soils and shown that this catabolism is inhibited in acid soils.

This chapter deals with exploratory laboratory studies concerning the possibility of utilizing naturally occurring microorganisms to alleviate water-repellentcy in soils.

7.2. Material and methods

7.2.1. Soils.

Soils used have been described in Chapters 3 and 5.

7.2.2. Treatment with sodium azide (NaN_3).

Soil samples (5 g) were placed in test tubes and wetted with 10 ml of 0.15 M NaN_3 or distilled water. Control solutions with no soil were established. On the top of solution 200 mg of acid washed sand coated with cetyl alcohol were sprinkled. The mixtureswere incubated at 28°C for 6 weeks. The behaviour of cetyl alcohol-coated sand was observed daily. At the end of the incubation period the soils were washed with 3 x 10 ml distilled water, oven dried, cooled and MED values measured.

7.2.3. Sterilization of soils.

Tintinara soil (100 g) was placed in an Erlenmeyer flask and autoclaved twice at 121°C and 225 kPa for 30 minutes. The soil was stored at room temperature between the two autoclavings.

7.2.4. Treatment with nitrogenous solutions and sodium chloride.

Either original or sterilized Tintinara soils (5 g) were placed in test tubes and wetted with 10 ml of one of the following solutions : distilled water, 0.001 M NaCl, 0.001 M NaN_3, 0.017 M NH_4NO_3 and 0.017 M $CO(NH_2)_2$ with pH values of 5.7, 7.1, 7.8, 6.6 and 6.9 respectively. The mixtures were incubated at 28°C for 1, 2, 3, 4, 5 and 6 weeks. At the end of each incubation, the soils were washed with 3 x 10 ml distilled water, oven dried, cooled and MED values measured.

7.2.5. Determination of carbon and nitrogen.

Total carbon content of soils was determined by dry combustion using a Leco CR-12 Carbon System with a furnace temperature of 1200°C.

Nitrogen determination was conducted according to a modified Kjeldahl method (Buresh *et al* 1982).

7.3. Results and discussion

Table 7.1. shows that non-wetting soils from Ceduna stored in a plastic bag in the laboratory of Department of Agriculture at Cleve Eyre Peninsula became wettable after 10 months, whereas the same soils stored in the same type of container for the same period at the Waite Institute still exhibited some degree of water-repellency. By comparison, there were no measurable changes in water-repellency of Tintinara soils after a much longer period of storage under the same conditions at the Waite Institute (Table 7.1.). Two possible explanations for this change in hydrophobicity of samples of the same origin stored at different locations may be considered. (1) If the decrease was simply due to the exposure of hydrophilic surface by physical forces such as vibrational abrasion or temperature fluctuations, then redistribution of the waxes on the exposed surfaces by means of a chloroform treatment should restore the level of hydrophobicity to levels similar to those originally observed (MED 2.0) or higher. (2) If the decrease was due to a net oxidative loss of hydrophobicity due to microbial action, then chloroform redistribution would never restore the hydrophobicity to the original level. Table 7.1. shows that although chloroform redistribution of the soils stored at the Waite Institute restored hydrophobicity to a degree (MED 2.3) in excess of the original (MED 2.0), the soils stored at Cleve exhibited a net loss of hydrophobicity (MED 1.2). These data clearly suggested that some form of microbial reduction in hydrophobicity may have occurred in the Cleve sample.

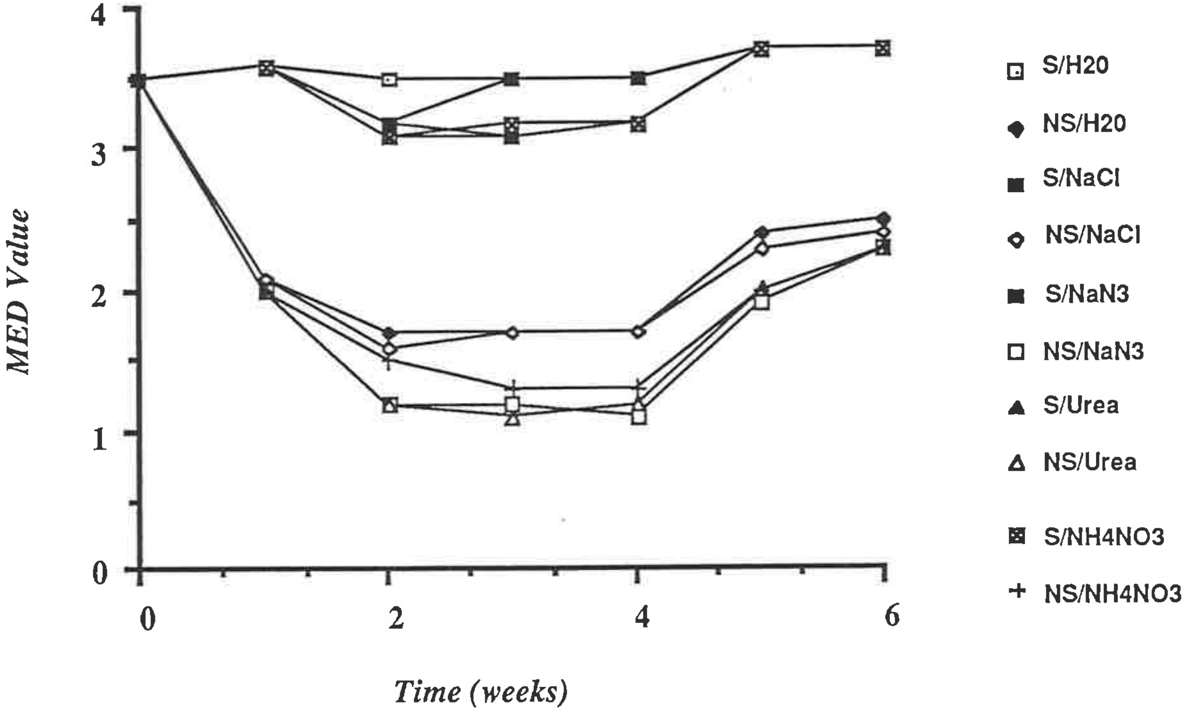
Table 7.1. Effect of NaN_3 treatment on water-repellency of soils from Ceduna and Tintinara

	Ceduna soils		Tintinara soils
	Stored at Cleve	Stored at Waite	stored at Waite
Original	2.0	2.0	3.5
After 10 months 3.5 years	0	0.7	3.5
$CHCl_3$ treatment	1.2	2.3	3.6
After 6 weeks :			
-wetted with H_2O	0	0	2.3
-wetted with 0.15 M NaN_3	0	0	0
$CHCl_3$ treatment of			
-H_2O wetted	0	0.5	3.0
-0.15 M NaN_3 wetted	0	0	0

An attempt to inhibit the microbial activity with a control incubation with 0.15 M sodium azide led to a complete loss of hydrophobicity in all incubations, even with the strongly water-repellent Tintinara samples Table(7.1.). Furthermore subsequent treatment with chloroform failed to restore hydrophobicity to any of the sodium azide treated samples. These data suggested, that either there was a pH effect (McGhie 1980) due to the alkaline (pH 9.8) sodium azide treatment, or that the azide was behaving as a nitrogen source for some microbial species whose proliferation resulted in a net loss of hydrophobicity.

In order to clarify which of these alternative proposals was more likely the experiment illustrated in Fig 7.1. was undertaken, whereby sterilisation was achieved through a double autoclaving sequence and the initial pH of the incubation mixture was controlled by using lower levels of sodium azide or other nitrogen sources. Fig. 7.1. clearly demonstrates the involvement of naturally occurring microorganisms in catabolizing

Fig. 7.1. Biological catabolisms of water-repellency in sterile (S) and non-sterile (NS) Tintinara soils.


4
3
2
1
0
MED Value
0
2
4
6
Time (weeks)
S/H20
NS/H20
S/NaCl
NS/NaCl
S/NaN3
NS/NaN3
S/Urea
NS/Urea
S/NH4NO3
NS/NH4NO3

the hydrophobic compounds as indicated by large differences between the MED values of heat sterilized and non-sterilized soils. It also shows that soils which had been incubated with nitrogenous solutions were in general less water-repellent than those incubated with water and sodium chloride.

Moucawi *et al* (1981) reported that the rates of decomposition of lipids appeared to be dependent on abundance and diversification of microorganisms, regardless of the chemical structure of the lipids. This emphasizes the need to embark upon a thorough study of the nutritional requirements (N, P, S and trace elements) of the soil microorganisms in the water-repellent sands of South Australia. Data presented in Table 7.2. show that some water-repellent soils in South Australia have very low nitrogen contents, varying from 0.05-0.17 %, suggesting that this nutrient would be one of the limiting factors for microbial activities. Although the importance of enriched media for optimal catabolic growth is well documented (Ladd 1956 and Jobson *et al* 1972), the observed rise in hydrophobicity after 4 weeks incubation suggests that some microbial species may actually be contributing to the net observed hydrophobicity measured by the MED test.

Table 7.2. Carbon and nitrogen contents of some water-repellent soils from South Australia

Sample	Total C (%)	Total N (%)	C/N ratio
Tintinara	1.34	0.13	10.3
Murray Bridge	1.03	0.08	12.9
Keith	1.25	0.10	12.5
Wharminda	0.60	0.05	12.0
Ceduna	3.29	0.17	19.4

7.3. Conclusions.

Unidentified microorganisms naturally present in Tintinara soils may be capable of utilizing hydrophobic materials as a substrate. The activities of such organisms may be limited by nutrient deficiencies. There is a good case for a future detailed study of the soil microbial population and the conditions under which it thrives. This can best be achieved through careful attention to nutritional requirements of the organisms in order to control their synthetic and catabolic activity towards the water-repellent polymethylene waxes.

CHAPTER 8

GENERAL DISCUSSION AND CONCLUSIONS

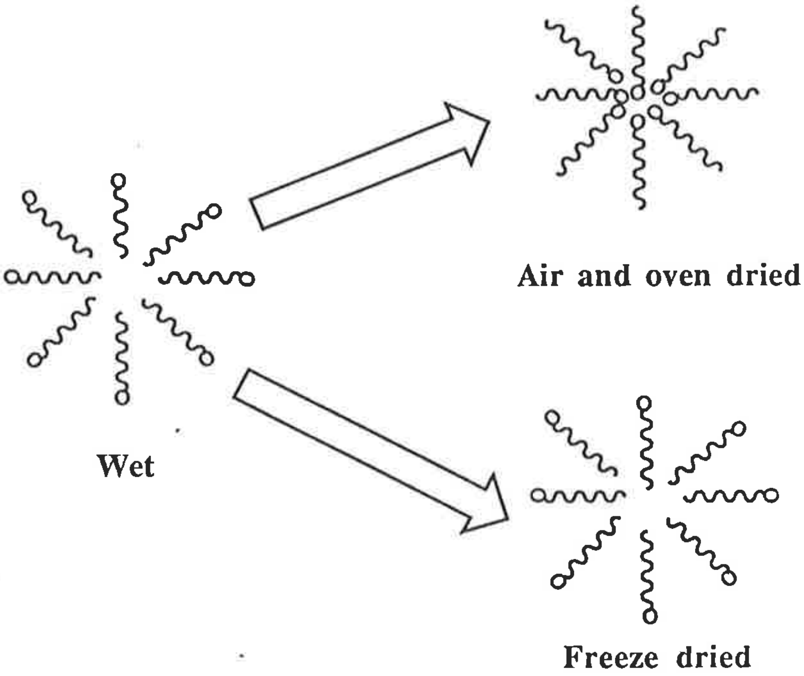
8.1. Organic matter and water-repellency

Results presented in Chapter 3 suggest that changes in water-repellency may occur under particular circumstances without extraction of the organic materials responsible. Configuration of organic molecules, for example, was found to be crucial. Wetting and drying which affect the molecular orientation of organic matter in soils had remarkable effects on water-repellency of some soils examined. Freeze drying of moistened water-repellent soils rendered these soils readily wettable and this effect was ascribed to a re-orientation of organic molecules (Ma'shum and Farmer 1985). The inversion of amphiphilic molecules shown in Fig. 8.1. illustrates a logical explanation of this phenomenon. Most forms of organic matter possess both hydrophobic and hydrophilic groupings. The orientation of these groups is largely controlled by the presence of water molecules. In wet situations polar groups such as carboxyl and hydroxyl tend to hydrogen bond to water molecules. As the water is lost, the polar groups interact with each other resulting in the exposure of hydrophobic chains and a water-repellent surface. However, it is apparent that during freeze drying the hydrophilic functional groups remained on the surface.

After wetting, the soil tended to separate on sedimentation into a heavy, grey, sand-rich fraction; a black, finely particulate organic rich fraction; and a floating fraction consisting of larger plant remains. It is likely that some water-repellent substances were present in the organic fractions.

Water-repellency in soils was also observed to be affected by agitation which may be ascribed to the abrasive removal of organic matter from sand surfaces. The effects of agitation were determined both on the whole soils and sand fraction. The fact that the sand fraction became more hydrophilic after agitation than the whole soils implies that the hydrophobicity of the original soils resides both in coatings on sand particles and the

Fig. 8.1. The configuration of organic molecules as affected by wetting and drying.


Air and oven dried
Wet
Freeze dried

presence of partially decomposed plant remains in the soils. These comparative observations on whole soil and the sand fraction lend support to the importance of plant litter in the development of water-repellency in soils which was noted by McGhie and Posner (1980).

8.2. Extraction of the causal agent

By monitoring the hydrophobicity of the residual soils in a Soxhlet extraction thimble as a function of time the importance of polarity of solvents was established. Non-hydroxylic solvents were found to only marginally reduce water-repellency of soils and confirmed earlier observations (Jamison 1945, Van't Woudt 1959 and McGhie and Posner 1980) indicating the relative ineffectiveness of the non-hydroxylic solvents, such as ether and chloroform, in reducing water-repellency of soils. It has been suggested that insolubility of some organic compounds such as lipids in less polar solvents is caused by the linkage of these compounds with other organic compounds (Hance and Anderson 1963 and Johnson 1971). However, the solubility and partition properties of the materials extracted by the current Soxhlet procedure suggest that the major problem, which was not sufficiently considered by earlier workers, concerns the rupture of hydrogen bonds which would be expected to be strengthened in aprotic solvents. The importance of amphiphilic solvents in removal of water-repellency was amplified by the fact that unlike chloroform or hexane a mixture of benzene/ethanol (2:1, by volume) completely dissolved the hydrophobic fraction from lipids and humic acids extracted from Tintinara water-repellent soils, leaving a residue of hydrophilic materials which had been coextracted during the Soxhlet extraction procedure.

Results presented in Chapter 4 shows that irrespective of the type of lipid solvent all of the extracted materials produced water-repellency on acid washed sand, however, the degree of water-repellency induced at an arbitrary constant concentration of 400 mg kg^{-1}, as measured by the MED values, varied with the type of extractant. These data indicate the presence of different ratios of hydrophobic to hydrophilic components in the crude mixture. Both components are of primary importance to the net water-repellency of the surface (Bozer *et al* 1969). The humic acid fraction from water-repellent Tintinara soils did not produce water-repellency on acid washed sand at concentrations up to 50 g kg^{-1} despite their ability to create hydrophobicity on glass paper. Savage *et al* (1969a) reported the occurrence of water-repellency on sand and soil which had been coated with microbial humic acids isolated from *Stachybotrys atra*, however, the sand and soil remained wettable after coating with humic acids from soil and peat. Adhikari and Chakrabarti (1976) confirmed the finding that some microbial humic acids were able to induce water-repellency. From the hydrophilic nature of humic acid fraction insoluble in benzene/ethanol, it is apparent that the ability of humic acid fractions to influence the water-repellency is determined by whether or not lipids are entangled in the fractions. Polymethylene compounds have been found to be important contributors to humic acids as revealed by ^{13}C NMR spectra (Schnitzer and Khan 1972, Wilson *et al* 1978, Schnitzer and Khan 1978 and Newman *et al* 1980). The origin and method of preparation of the humic acids apparently control the presence or absence of lipids in the extracted materials. The observed absence of ester carbonyls absorptions at 1730 cm^{-1} in the infrared spectrum of humic acid fraction soluble in benzene/ethanol indicates that extensive saponification has occurred during the humic acid extraction procedure. Conventional organic solvents, such as ether and ethanol, are often employed to remove the hydrophobic compounds prior to extracting the humic materials (Stevenson 1965 and Hayes *et al* 1975).

8.3. Characterization of water-repellent materials

In chapters 1 and 2 it was established that a commonly accepted view that lipids were not responsible for the water-repellency in soils is incorrect. The misapprehension has apparently been based on data involving the inability of conventional non-polar solvents, such as ether and chloroform, to extract completely the hydrophobic materials from soils.

The spectroscopic and chromatographic data in chapter 5 clearly establish that water-repellent materials extracted from Tintinara sandy soils with the mixture of *iso* -propanol/ ammonia may be characterized as a mixture of long chain polymethylene waxes consisting of acids, alcohols and their conjugate esters. as revealed by spectroscopic and chromatographic data (Chapter 5). The extracted materials contained long chain (16-32 carbon atoms) fatty acids (mainly C_{16} and C_{22}) and long chain (14-28 carbon atoms) alcohols. Up to 44 % of the unsaponifiable alcohols consisted of cetyl alcohol (16 carbon atoms).

The long chain polymethylene compounds in the water-repellent soils examined could have been derived from plant materials. The infrared spectrum of lipids extracted from Tintinara soils is similar to that of the leaves of Eucalyptus sp. Acids and primary alcohols are the main components of epicuticular waxes of most plants (Chibnall *et al* 1934, Tulloch and Hoffman 1973a and 1973b). Distribution of fatty acids and and alcohols in the lipids from the Tintinara soil also support the inference that the lipids are of plant origin. Tulloch (1976) confirmed that in plant waxes, the acids are predominantly straight chains, with even numbers of carbons and in some cases with a wide range of chain lengths (14-36 carbon atoms). Very long chain β-diketones (n-tritriacontan-16,18-dione) were reported to be the major components of waxes from many *Eucalyptus* species (Horn *et al* 1964). However, these compounds were not detectable in the lipids from the Tintinara soils (Chapter 5) suggesting that if these lipids are constituents of plant litter they are rapidly oxidized by microbial activity to smaller fragments. The guiding principle in the present investigation was to establish the general characteristics of the molecules responsible for water-repellency in natural soils rather than to assign them to any specific plant or microbial source.

Bacteria and fungi also produce waxes. In general, bacterial lipids contain straight chain isomers of fatty acids with C_{16} (palmitic acid) and C_{18} (stearic acid) being most often reported (Albro 1976) and C_{16} and C_{18} alcohols have been reported to be the major components of waxes isolated from *Bacillus cereus* (Kates *et al* 1962). ^{13}C NMR spectra of intact spores of this bacterium confirmed the presence of long chain polymethylene compounds (Lundin and Sacks 1988). Fungal fatty acids are found as homologous series of saturated and unsaturated aliphatic acids ranging from C_{10} to C_{26}. Again, the major components of fatty acids produced by fungi are C_{16} (palmitic acid) and C_{18} (oleic and linoleic acids) (Weete 1976). Fatty acids from mycelia of Basidiomycetes are mainly oleic and linolenic acids which comprised up to 63 % of the total fatty acids (Shaw 1966). Considering the fact that fatty acids in the hydrophobic organic materials extracted from Tintinara soils are dominated by palmitic acid, and that palmitic acid effectively waterproofed acid washed sand, it can be suggested that fungi may also have produced some of the water-repellent materials. Hence, the close link between fungi and water-repellent soils, as suggested by some previous workers (Bond 1962, Bond and Harris 1964), may be due to synthesis of hydrophobic long chain polymethylene compounds by these organisms as waxy materials coating their mycelia. However, as in the case of the crude humic acid fractions and lipids isolated from Tintinara soils, the observed net hydrophobicity will be dependent on the relative contributions of hydrophilic as well as hydrophobic components from these microorganisms.

8.4. Simulated water-repellency

Based on the fact that both long chain acids and alcohols are major components of water-repellent materials extracted from Tintinara soils, wettable acid washed sand was coated with a series of fatty acid and alcohol reference molecules (Chapter 4). Both cetyl alcohol and palmitic acid were found to effectively create water-repellency on sand surfaces. Cetyl alcohol in particular, because of its non-ionic nature and ready availability, has provided a valuable model for further understanding the nature of water-repellency in soils. The concentration dependent study established that the sand had to be uniformly coated with an amount of cetyl alcohol equivalent to one uniform monolayer before it exhibited any measurable water-repellency; but to reach the plateau of its maximum hydrophobicity (MED value 7.0), the equivalent of at least 16 monolayers of cetyl alcohol was necessary. A scanning electron micrograph of the sand coated with cetyl alcohol showed a papillate appearance of coalescent globules (diameter ~ 150-300 nm) covering the sand grains. Calculations indicated good agreement between the amount of cetyl alcohol in such globules with the amount necessary to give 16 monolayers. When poured into test tubes of water, this model system and the naturally occurring water-repellent Tintinara soils, both formed the characteristic irregular columns of highly water-repellent soil (Plate 8.1.) and in both cases when these columns are emptied into a Petri dish the inner contents were found to remain dry and friable.

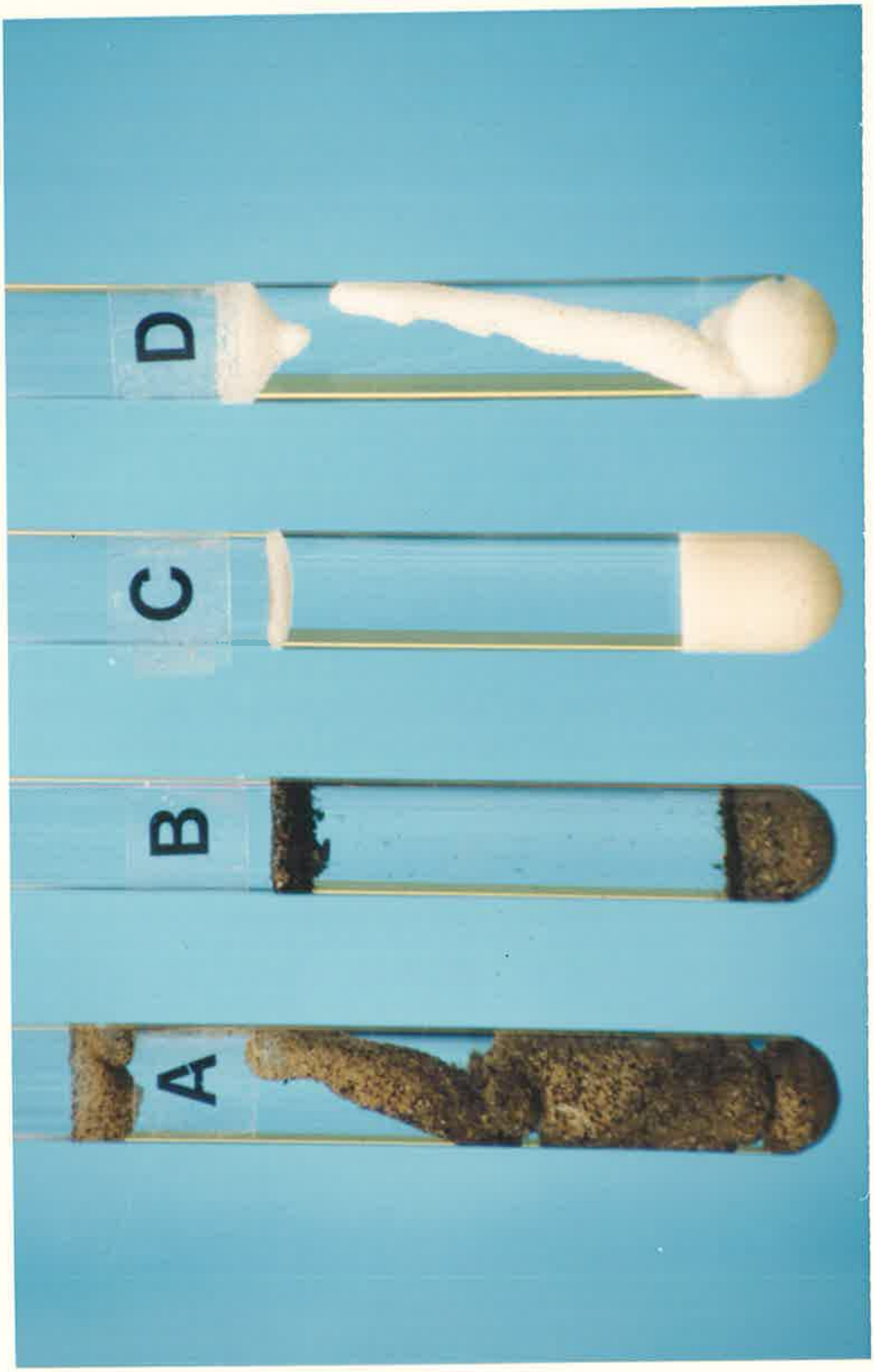
Re-application of either benzene/ethanol or *iso*-propanol/ammonia extracts to acid washed sand also produced water-repellent surfaces and the measurable MED values were also a function of the amount of extracts applied. The maximum MED value of 7 produced in the laboratory has not been observed in the field where values rarely exceed 4. It is possible that values greater than 4 may develop if further hydrophobic materials accumulate in the field.

8.5. Alleviation of water-repellency by clays

Several methods, such as the use of wetting agents and cultivation techniques, have been suggested to alleviate water-repellency in soils (Pelishek *et al* 1962, McGhie and Tipping 1984, King 1984 and Miyamoto 1985). However, the fact that water-repellent soils are still a major agronomic problem implies that there is a considerable room for improvement.

In the present study, it was observed that the degree of water-repellency induced by organic compounds on sand surfaces was positively correlated with the concentration of the hydrophobic organic compounds applied and with the calculated surface area of the sand (Chapter 6). Coarse particle materials with low surface area such as sand can be easily

Plate 8.1. Characteristic irregular columns of highly water-repellent Tintinara soils (a) and acid washed sand coated with cetyl alcohol (d). Such columns were not formed by Tintinara soils extracted with iso -propanol/ammonia (b) and acid washed sand (c).


D
C
B
A

coated with organic matter (Biswell 1975). However, to coat clay particles a much greater amount of organic material was necessary. Therefore, it is not surprising that water-repellency occurs mainly in light textured soils with clay contents usually less than 10 % (Bond and Harris 1964, Bond 1968, Holzhey 1968 and Savage 1975). These findings provide one possible approach to solving the water-repellent problems of light textured soils such as those in the South East of South Australia and in the Eyre Peninsula by simply adding materials of high surface area.

Laboratory experiments established that application of fine particle materials such as oxides, lime, aerosil (a finely divided commercial silica) and clays reduced water-repellency of both Tintinara soils and the model waterproofed sands. The incorporation of clays appears to be a good approach to the problem in most instances, because the clay would provide some other benefits, such as increased cation exchange capacity, to the soils. This technique has been applied in Florida (King 1974) and Australia (Roberts 1966, Bond 1978 and Ward 1984). The establishment of crops and pasture on a farm, was markedly improved by spreading layers of clay on the surface of water-repellent sand near Mundulla in the South East of South Australia (Mr. C. Obst, pers. comm.), however, no attempt was made to measure the amount of clay used. In laboratory studies application of clay at a concentration of 10 g kg^{-1} which is equivalent to 10 tonnes ha^{-1} rendered the water-repellent Tintinara soil wettable. Again, it was noted that the particle size of clay used was of particular importance in determining the efficiency of clays in reducing water-repellency of sands. More importantly, the dispersibility of clays which determines their ability to mix with sands controlled to some extent their effectiveness in minimizing water-repellency of sandy soils and cetyl alcohol-coated sand. It is therefore necessary to consider factors affecting the dispersibility such as sodicity and mineralogy when selecting a particular clay to ameliorate water-repellency in soils. The use of illitic and kaolinitic clays which are known to be more dispersive than montmorillonite (Arora and Coleman 1979 and Ali *et al* 1987) would be appropriate. These types of clays are often widespread as an underlaying clay layer in areas of water-repellent soils in South Australia. Unfortunately, this information was not available at the time the first field experiment on clay addition was inititated early in 1987.

Nevertheless, results obtained from the field trial (Chapter 6) indicated that a flocculated clay from Millicent marginally reduced water-repellency at 16 weeks after application and a steady decrease in MED values was observed to occur over the next 30 weeks. By spreading 20 tonnes clay per ha on the surface of sandy soils near Keith, the MED value declined from 3.1 to 0.8 at the end of 46 weeks; a value which is agronomically acceptable according to King (1981). The incorporation of clay also measurably improved the water regime of the sandy soils and the increase in available water was a function of the rate of application. However, in this first trial no significant differences in lucerne establishment were observed. Better results would be anticipated using more dispersive clays such as sodic illite and kaolinite.

These data suggest that addition of clay can help to reduce water-repellency and could be used by farmers as part of their management of sandy soils to improve the productivity of these soils. The clay treatment will also provide some other beneficial effects for soil and water conservation. The amount of water penetrating non-wetting sands treated with clay should be increased and at the same time run-off should be minimised. Hence erodibility of these soils should be reduced. Incorporation of clays should improve aggregation of such sandy soils and hence their resistance to sand drift. Based on the laboratory observations, the application rate tested in the field trial could be reduced to ~ 5 tonnes ha^{-1} by using the more easily dispersed clays. The question arises as to whether these clays would be washed down the soil profile and also whether frequent applications would be required. Laboratory investigation using the model cetyl alcohol-coated sand (Chapter 6) revealed that water-repellent sands hold clays more firmly than that of hydrophilic sands suggesting that transportation of clays down the profile would not be a problem. However, further study is necessary to establish the mechanism of interactions between water-repellent sand, hydrophobic organic matter and clay particles.

8.6. Biological catabolism of water-repellency

The ability of several microorganisms to utilize and degrade some waxes *in vitro* is well established (Albro 1976). The oxidation of fatty acids can be catalyzed by a number of enzyme systems (Harwood and Russel 1984). There are three mechanisms of oxidation of fatty acids : α-oxidation is the oxidation of the carbon adjacent to the COOH and results in the loss of a single carbon, β-oxidation refers to the removal of two carbon fragments from the carboxyl end and ω–oxidation is the oxidation of the methyl end of the alkyl chain to yield dicarboxylic acids. To break long chain polymethylene compounds present in water-repellent materials several stages of oxidation will be necessary.

Short chain alcohols (less than 6 carbons) and fatty acids (less than 10 carbon atoms) did not induce water-repellency on sand surfaces (Chapter 5). Therefore, the breakdown of long chain polymethylene compunds into shorter chains would be another approach to alleviate water-repellency. Initial laboratory experiments (Chapter 7) revealed that certain as yet unidentified microorganisms, which are naturally present in Tintinara soils, play an important role in the catabolism of the waxes causing water-repellency. However, the prevailing nutrient and water conditions of the Tintinara soils is not optimal for the catabolic activity of these microorganims. Westlake *et al* (1977) suggested that degradation of polymethylene compounds can be accelerated by employing microorganisms if the environment is modified to support microbial growth and activity. Incorporation of nitrogen and phosphorus fertilizers were found to stimulate the bacterial population which was followed by an increased rate of utilization of long chain *n* -alkanes (Jobson *et al* 1974 and Westlake *et al* 1977).

The current soil conditions in non-wetting sand areas apparently inhibit the catabolic activities of microorganisms and their ability to degrade polymethylene waxes. Fertilizers applied to sandy soils in the South East of South Australia are leached down the soil profile because there are no mechanisms for retention. Lewis *et al* (1987a) reported that nearly 10

% of phosphorus applied to the soils in the Hundreds of Coles, Willalooka and Senior were accumulated in the 10-30 cm soil layer. Such an accumulation was also observed in the lower layer. These workers also noticed increases of sulfur in those layers (Lewis *et al* 1987b). It is probable that a similar fate would apply to other nutrients. These data suggest that a limited supply of nutrients for soil microorganisms, has restricted their catabolic activities. Clay treatments to reduce water-repellency, can also be expected to improve the retention of nutrients in top soils. Incorporation of clay may be a necessary pretreatment if we are to employ microorganisms to catabolize water-repellent materials in soils.

8.7. Conclusions

At this point a number of conclusions can be made :

1. Water-repellency in soils is a function of the orientation of amphiphilic molecules. The molecular orientation is to some extent affected by the presence of water molecules. Oven drying of moistened water-repellent soils yields hydrophobic surfaces whereas lyophilization of the same soils generates a hydrophilic surface.

2. The causal agents for the water-repellent condition of South Australian soils are a series of amphiphilic polymethylene waxes in which long chain fatty acids, alcohols and their esters predominate.

3. The water-repellent condition of natural soils can be modelled by coating acid washed sand with long chain polymethylene compounds such as cetyl alcohol. This model system has established the dependence of water-repellency on both the concentration of hydrophobic organic matter and the surface area of the sand.

4. Water-repellent South Australian soils can be made hydrophilic by the addition of relatively small amounts (10-20 t ha^{-1}) of clays. A relatively non dispersive Millicent clay reduced the water-repellency of soils near Keith to an agronomically acceptable level with an improved water regime after one year.

5. Readily dispersive sodic clays are always more effective on a weight base than their flocculated calcium counterparts.

6. Microbial activity capable of catabolizing the hydrophobic factors has been detected in non sterile soils from areas of non-wetting sands near Tintinara.

8.8. Future work

The present studies have focussed attention on two important aspects of the water-repellent soils worthy of further investigation.

Firstly, the crucial part played by surface area of the soils and the nature of the bonding between the amphiphilic lipid molecules and the hydrophilic soil surfaces. Further study on the use of more dispersive clays to ameliorate water-repellent soils and on the techniques of application are justified.

Secondly, the effect of autoclaving and nutrient solutions on the water-repellency of naturally occurring Tintinara soils provides the basis for a new approach to the control of the water-repellent soil problems. It is likely that a combination of clays with appropriate nutrients to stimulate catabolic activities of microorganisms may provide a long term solution to the problem.

APPENDICES

1. Origin and assessment of water-repellency of a sandy South Australian soils.
Mansur Ma'shum and V.C. Farmer
Australian Journal Soil Research
Vol. 23 (1985) 623-626.

2. Extraction and characterization of water-repellent materials from Australian soils.
M. Ma'shum, M.E. Tate, G.P.Jones and J.M.Oades
Journal of Soil Science
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Ma'Shum, M. & Farmer, V. C. (1985). Origin and assessment of water repellency of a sandy South Australian soil. *Australian Journal of Soil Research*, *23*(4), 623-626.

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Extraction and characterization of water-repellent materials from Australian soils

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SUMMARY

Organic materials responsible for water-repellency in some Australian soils were extracted with an amphiphilic mixture of *iso*-propanol/15.7 M ammonia (7 : 3, v : v) in a Soxhlet apparatus, after which the water-repellent soils were rendered wettable. The successful extraction by an organic solvent system indicates that the bulk of hydrophobicity in these soils is not covalently linked to the surface of the sand. The extracted materials restored hydrophobicity on acid washed sands or ignited sands at levels comparable to the original soils.

Spectroscopic and chromatographic examination of the extracted materials indicated that both free and esterified long-chain, 16–32 carbon atom, fatty acids were present with a bimodal distribution showing maxima at C_{16} and C_{22}. The ^{13}C-NMR and infrared spectra of the most hydrophobic extract suggest that hydrophobicity is caused by molecules with extensive polymethylene chains. Calculations with model compounds indicate that at least a close packed monolayer is required before measurable hydrophobicity can be detected with the molarity of ethanol droplet penetration test.

INTRODUCTION

It has been recognized for many years that water-repellent soil is a function of organic matter (Schreiner & Shorey, 1910; Prescott & Piper, 1932; DeBano & Rice, 1973). Although ignition or strong alkali removes hydrophobicity, no satisfactory organic solvent system has been found to extract completely the water-repellent material from such soils. The possibility that lipids could induce water-repellency has been rejected by some authors (Jamison, 1945; Van't Woudt, 1959; Bond, 1968) because soils which had been extracted with ether and other lipid solvents remained water-repellent. However, non-polar solvents would be expected to increase any hydrogen bonding between the surface and any acceptor or donor regions of hydrophobic molecules. Because strong alkali is well documented in its ability to remove hydrophobic materials, humic acids have been regarded as the most likely candidates for the hydrophobic factors (Savage *et al.*, 1969a; Roberts & Carbon, 1972; Adhikari & Chakrabarti, 1976; Singer & Ugolini, 1976; Tschapek *et al.*, 1973). However, not all humic acids produce water-repellency (Savage *et al.*, 1969a) and the possible cleavage of covalent linkages under those conditions makes it difficult to interpret what is happening in the intact situation. Ma'shum & Farmer (1985) have provided evidence to indicate that molecular orientation of organic matter determines whether or not a soil is water-repellent.

We have now investigated the use of amphiphilic solvent mixtures in a Soxhlet extraction procedure to remove hydrophobic materials from water-repellent soils. The ability of the extracted materials to induce water-repellency was tested by addition of the materials to acid washed sand. The extracted materials have been characterized by chromatographic and spectroscopic techniques and shown to contain extensive polymethylene chains including both long chain fatty acids and esters.

MATERIALS AND METHODS

Soils

Water repellent soils were collected from South and Western Australia. Some of them were studied earlier (King, 1981; McGhie & Posner, 1980; Ma'shum & Farmer, 1985).

Assessment of water-repellency

The degree of water-repellency was assessed by measuring MED values, *viz* the molarity of aqueous ethanol droplets which were absorbed by the soil in 10 s (King, 1981), an extension of a technique first suggested by Watson & Letey (1970). The capillary rise technique proposed by Emerson & Bond (1963) was also employed to measure water-repellency of some soils.

Extraction of water-repellent material

The soils were extracted by various organic solvents (Table 1) for 16 h or 48 h using a Soxhlet apparatus. The extracted soils were air dried in a fume hood and then oven dried at 105°C prior to assessment of their water-repellency. The extracts were evaporated to dryness in a fume hood for further analysis.

Assessment of water-repellency of extracted materials

The materials isolated from the soils were redissolved in chloroform (300 μl of chloroform per 1 g sand) and then poured onto wettable acid washed sand (BDH Chemicals, screen sizes between 40 to 100 mesh; this corresponds to surface areas in the range 0.005–0.015 m^2g^{-1} and is comparable to the Tintinara soils). After mixing, the solvent was evaporated and the sand dried at 105°C for 2 h. Finally, the water-repellency of the sand was assessed by measuring MED values.

Identification of water-repellent materials

The materials were applied to thin-layer chromatography (TLC) plates and then developed in chloroform. The most satisfactory technique for visualization of soil lipid extracts was the copper acetate-phosphoric acid charring technique of Fewster *et al.* (1969).

Infrared spectra were obtained using discs with a concentration of 1 mg material in 180 mg KBr. Acid washed sand samples coated with soil lipids were located on a diffuse reflectance accessory for infrared spectroscopy. The spectra were recorded using a Perkin-Elmer 983 grating infrared spectrophotometer. For UV/VIS spectra, samples were dissolved in dichloromethylene and recorded with a Perkin-Elmer Lambda 5 spectrophotometer.

For ^{13}C-NMR analysis samples were dissolved in $CDCl_3$ (Stohler, USA) to give concentrations of ~100 mg ml^{-1}. The ^{13}C-NMR spectra were recorded on a JEOL FX-90Q Fourier Transform NMR spectrometer operating at a frequency of 22.45 MHz at an ambient probe temperature of

Table 1. Solvents used to extract water repellent materials from a Tintinara soil (MED value 3.5 M) with hydrophobicity of residues

Extractants	Amount of material extracted (mg kg^{-1})	MED values (M)
Benzene/ethanol (2 : 1, v : v)	1180	2.0
Chloroform	600	3.5
Ether	200	3.0
Tetrachloroethylene	650	3.5
Methanol	1230	0.6
n-Propanol	1330	2.2
iso-Propanol/water (7 : 3, v : v)	1690	0.1
iso-Propanol/15.7 M ammonia (7 : 3, v : v)	1880	0

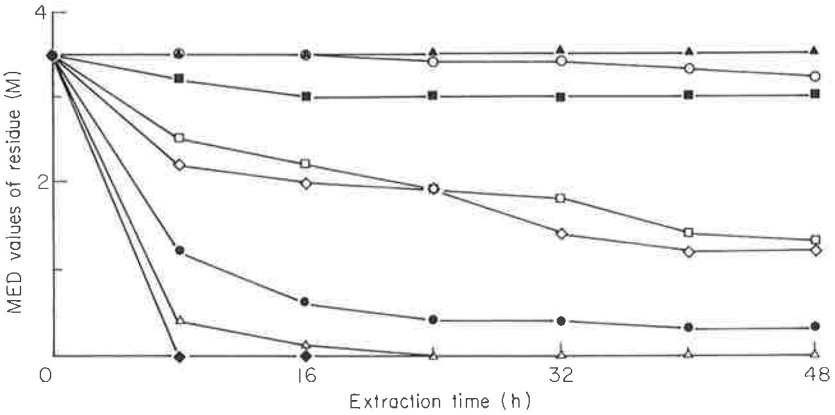

Fig. 1. Effect of solvents and duration of extraction on water-repellency of a Tintinara soil. ▲ Tetrachloroethylene, ○ Chloroform, ■ Ether, □ *n*-Propanol, ◇ Benzene/ethanol (2 : 1, v/v), ● Methanol, △ *iso*-Propanol/water (7 : 3, v/v), ◆ *iso*-Propanol/15.7 M ammonia (7 : 3, v/v). The *iso*-propanol/ammonia data are coincident with the *iso*-propanol/water data at 24 h and beyond.

24°C. Between 25–50 K acquisitions were accumulated into 8 K memory addresses using a spectral width of 5000 Hz and 11 μs (45°) pulse with a recycle time of 2.8 s. Broad band proton decoupling was used and an exponential line broadening of 2 Hz was applied to the free induction decay prior to Fourier transformation. Peak positions were referenced against the centre peak of the $CDCl_3$ triplet which was taken as 77.0 ppm.

Gas chromatography-mass spectrometric analysis were carried out on a Hewlett Packard 5992 GC/MS system equipped with a 12 m × 0.22 mm bonded phase vitreous silica column which was programmed from 180 to 280°C at 5° min^{-1}. The flow rate of the He carrier gas was 1 ml min^{-1}. The ionization voltage was 70 eV. The extracted materials were esterified with methanol/HCl prior to GC/MS analysis which incorporated methylpentadecanoate as an internal standard.

RESULTS AND DISCUSSION

Extraction of water-repellent materials

Fig. 1 shows the relative effectiveness of various solvents for extracting water-repellent materials from the Tintinara soil; this exhibits the most severe water-repellency of those soils which have been examined to date. From these data it is clear that there is a continual decrease in the residual water repellency of soils with time of extraction. However, it is also apparent that removal of water-repellent materials is controlled by the polarity of the solvents. Non-hydroxylic solvents such as ether, tetrachloroethylene and chloroform had little effect in reducing water-repellency of soils. A similar result was reported by Jamison (1946) who concluded that any effect was due in part to the removal of small amounts of oils, waxes and other organic materials. Short term extraction of hydrophobic soils by ether (Van't Woudt, 1959) or chloroform (McGhie & Posner, 1980) showed either no significant effect or, in the case of chloroform treatment, actually increased water-repellency (McGhie & Posner, 1980; Ma'shum & Farmer, 1985). This increase has been ascribed to either molecular reorientation of water-repellent organic matter, e.g. alkyl chains, or a redistribution of hydrophobic matter on exposed hydrophilic surfaces.

Morrison (1969) proposed that a mixture of benzene/ethanol (2 : 1) was the best solvent for extracting soil lipids. This amphiphilic solvent was certainly found to be more effective than non-polar solvents (Table 1). However, Fig. 1 shows the extracted soils still retained a substantial degree of water repellency. The most effective solvent in terms of its capability to render water-repellent soils wettable was mixture of *iso*-propanol and 15.7 M ammonia (7 : 3, v : v). Under Soxhlet reflux conditions this amphiphilic solvent mixture completely removed the hydrophobic factors within 8 h

Table 2. Clay and carbon contents, and two measures of water-repellency of 14 soils, together with amounts of hydrophobic materials extracted with *iso*-propanol/ammonia

Soils	Clay (%)	Carbon (%)	Water-repellency MED values (M)	Water-repellency Capillary rise (cm)	Hydrophobic materials extracted (mg kg^{-1})
Tintinara 1	6.9	1.34	3.5	−2.5	1880
Tintinara 2	3.8	0.32	1.9	1.2	770
Murray Bridge	4.4	1.03	1.4	7.5	1810
Eyre Peninsula 1	4.2	0.60	1.9	5.6	890
Eyre Peninsula 2	2.1	0.47	2.4	3.8	1060
Eyre Peninsula 3	4.2	0.36	1.9	2.2	1700
Eyre Peninsula 4	2.1	0.67	3.9	−0.4	1750
Eyre Peninsula 5	4.2	0.24	0.5	6.3	450
Eyre Peninsula 6	4.2	0.33	2.2	2.2	720
Eyre Peninsula 7	2.1	0.49	3.1	0.8	1540
Tuart sand	2.1	1.18	2.0	1.7	2660
Trezise Gavin sand 1	2.1	3.41	1.4	2.2	4560
Trezise Gavin sand 2	4.2	2.22	1.4	0.4	2380
Soil under Brown Mallet Trees	14.6	7.14	3.0	−1.7	26290

Table 3. Water-repellency (MED values) of acid-washed sand after treatment with materials extracted from Tintinara soil at two concentrations, or with three reference compounds

Hydrophobic material	MED values at two concentrations of hydrophobic materials: Standard (400 mg kg^{-1} sand) (M)	MED values at two concentrations of hydrophobic materials: Concentration as natural soil (see Table 1) (M)
Tintinara soil extractant		
Benzene/ethanol (2 : 1, v/v)	4.8	6.5
Chloroform	5.0	5.0
Ether	5.2	4.0
Tetrachloroethylene	5.0	5.2
Methanol	2.7	2.9
n-Propanol	2.6	3.3
iso-Propanol/water (7 : 3, v/v)	2.9	3.4
iso-Propanol/15.7 M ammonia (7 : 3, v/v)	2.9	3.4
Reference compound		
Palmitic acid	6.0	—
Cetyl alcohol	7.2	—
Tristearin	5.0	—

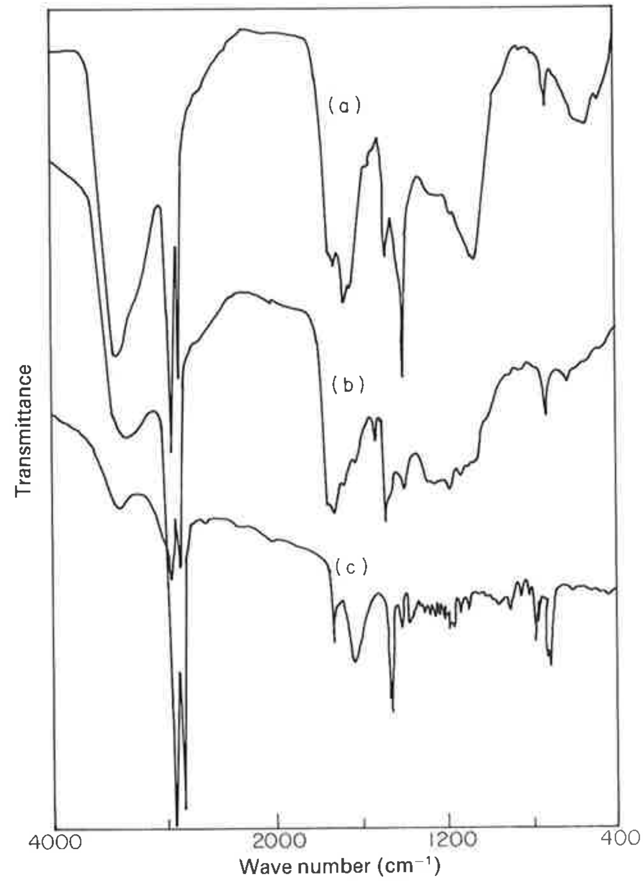

Fig. 2. Infrared spectra of lipids isolated from: (a) Tintinara soils, (b) non-wetting compost mixture, and (c) *Eucalyptus crucis* leaves.

and was equally effective on soils from Eyre Peninsula and Western Australia (Table 2) as well as a non-wetting compost mixture. The extracts were dark brown ($A^{1\%}_{1\,\mathrm{cm}}$ 450 nm = 5.63) and after drying were 70% soluble in chloroform; the $CHCl_3$-insoluble residue remained hydrophobic. However, alcohol/benzene extracted all hydrophobic materials from the dried extract leaving a hydrophilic residue (13% by weight). Control experiments demonstrated no detectable hydrolytic cleavage of methyl palmitate by this mildly alkaline extraction procedure.

Water repellency of extracted materials

Despite the differing extraction efficiencies of the solvents used, all of them extracted some water-repellent materials from the soils and application of these materials to acid washed sand at an arbitrary constant concentration (400 mg kg^{-1}) resulted in significantly different degrees of water repellency as measured by MED values (Table 3). These data suggest that some components of the extracts were much more effective in inducing a water-repellent surface than others. Thus there is a need to examine the nature and concentration of the hydrophobic components as well as the nature and concentration of hydrophilic species. All of these factors can be expected to contribute to the overall severity of the observed water-repellency, but only the hydrophobic factors are examined in this study.

Characterization of water-repellent materials

Fig. 2 compares the infrared spectra of ammoniacal-*iso*-propanol Soxhlet extracts from the

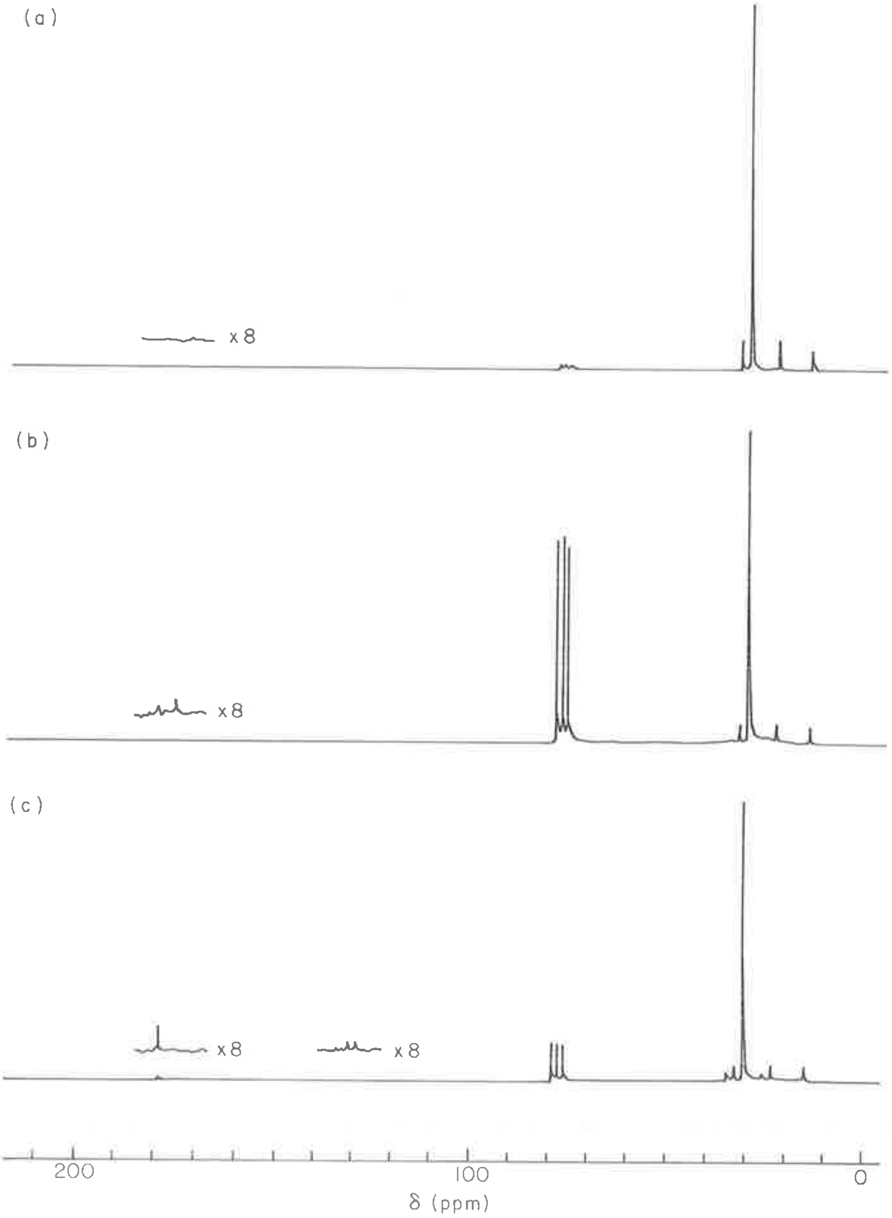

Fig. 3. ^{13}C NMR spectra of (a) paraffin wax (m.p. 63–68°C), (b) intact lipids isolated from Tintinara soils, and (c) sublimate of the soil lipids. The sharp triplet centred at 77.0 ppm is caused by the $CDCl_3$ solvent.

Tintinara water-repellent soils, a hydrophobic compost mixture and a sample of the cuticular wax from *Eucalyptus crucis* leaves prepared by the method of Horn *et al.* (1964). In each case the dominant features include the C–H stretching at 2917 cm^{-1}, bending at 1460 cm^{-1} and rocking at 720 cm^{-1} frequencies characteristic of extended paraffinic chains. In general, the higher the MED value at an arbitrary level of 400 mg kg^{-1} the more prominent these C–H frequencies are. The relatively low intensity of signals for C=O stretching frequencies, suggests that there is either a mixture, comprising a small amount of compounds with carbonyl groups admixed with a large amount of paraffinic molecules devoid of carbonyls and/or both the carbonyl and paraffin chains are present in the same molecule. In the latter case the unusual ratio of the signals is brought about by the presence of a numerically large excess of CH_2 vibrations compared to the number of $C=O$ stretching vibrations.

The ^{13}C NMR spectrum (Fig. 3b) of the extremely hydrophobic benzene/ethanol extract from Tintinara soils (Table 1) shows a large polymethylene resonance at 29.6 ppm together with $-CH_2\text{-}CH_2\text{-}CH_3$, $-CH_2\text{-}CH_3$ and $-CH_3$ resonances at 31.9, 22.6 and 14.1 ppm respectively (Levine *et al.*, 1972). The carbonyl resonances at 178.4 and 174.1 ppm, which are barely detectable above background noise, are the only features which differ from a spectrum of paraffin wax (mp.

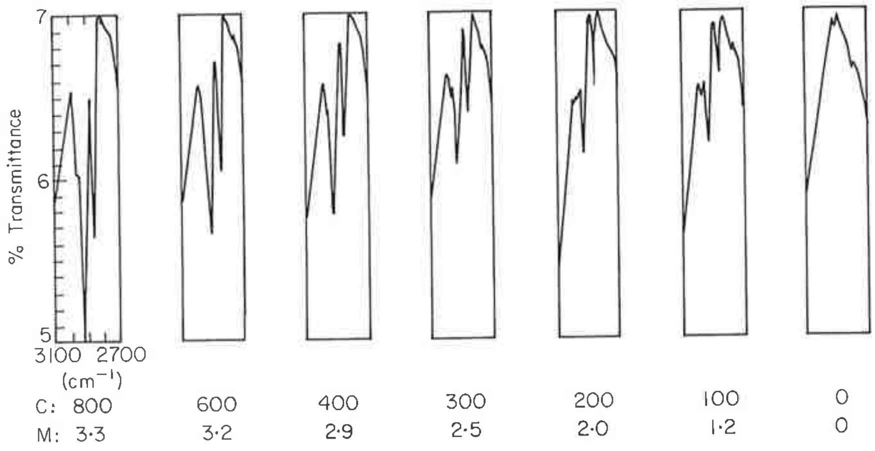

Fig. 4. Diffuse reflectance infrared spectra of acid washed sand which has been treated with lipids isolated from Tintinara soils. C = concentrations of soil lipids on acid washed sands (mg kg^{-1}), M = MED values of the treated sands (M).

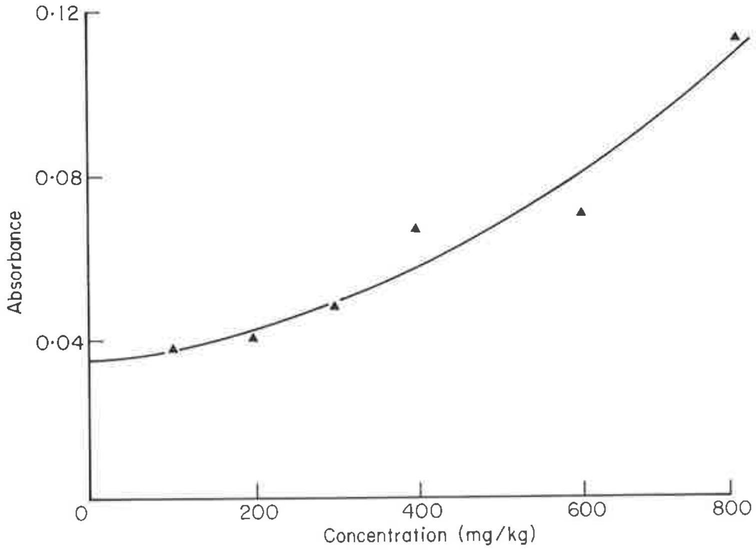

Fig. 5. Relationship between the absorbance (A) at 2917 cm^{-1} of diffuse reflectance infrared spectra of acid washed sand coated with soil lipids from Tintinara soils and the concentration (C) of those lipids. The regression line ($r^2 = 0.95$) is given by

$$A = 0.0356 + 1.38 \times 10^{-5} C + 9.61 \times 10^{-8} C^2$$

63–68°C) (Fig. 3a). The vacuum sublimate of this Tintinara soils extract, however, shows a clear ^{13}C carboxyl resonance (Fig. 3c) at 178.9 ppm together with olefinic signals at 130.7 and 128.5 ppm. Also observed in this spectrum are the $CO\text{-}CH_2$ and $CO\text{-}CH_2\text{-}CH_2-$ resonances at 34.4 and 24.7 ppm, respectively.

The features of the infrared spectra in Fig. 2 are also akin to previous published spectra for organic coatings extracted from sand grains of non-wettable golf greens (Miller & Wilkinson, 1977) as well as data from Western Australian hydrophobic plant litter (McGhie, 1980) and material extracted from water-repellent Italian soils (Giovannini & Lucchesi, 1984).

Fig. 4 shows the infrared diffuse reflectance spectra of acid washed sand which has been coated with increasing concentrations of the hydrophobic extract from the Tintinara soil. It is clear that C–H stretching region is readily measurable by this technique. The absorbance at 2917 cm^{-1} frequency is well correlated with the concentration of soil lipids added (Fig. 5).

Partition properties

Thin-layer chromatograms of the extracted materials also suggested that the major components of the hydrophobic extracts had Rf values, and hence partition coefficients, which were similar to long chain acids, alcohols and wax esters. The detection of these relatively unreactive materials was successfully accomplished by the copper acetate-phosphoric acid charring procedure of Fewster *et al.* (1969). For nondestructive isolation white hydrophobic spots on a translucent background were also detected after spraying with water (Tate & Bishop, 1962).

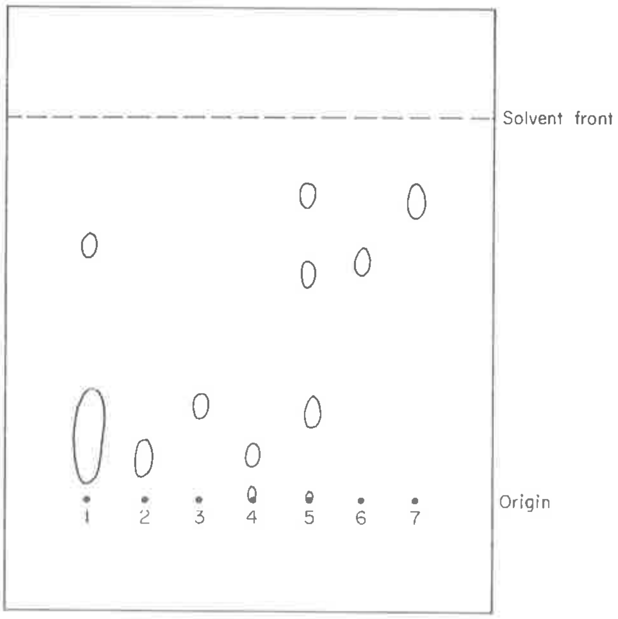

Fig. 6. Thin layer chromatogram of soil lipids and some reference molecules. Adsorbent: aluminium sheets silica gel (without fluorescent indicator). Solvent: chloroform. Spots were located by copper acetate-phoshoric acid charring techniques.
(1) Palmitic acid.
(2) Cholesterol.
(3) Cetyl alcohol.
(4) Lipids isolated with the amphiphilic mixture of *iso*-propanol/ammonia from Tintinara soils.
(5) Lipids isolated with benzene/ethanol from Tintinara soils.
(6) Methyl palmitate.
(7) Paraffin wax (m.p. 63–68°C).

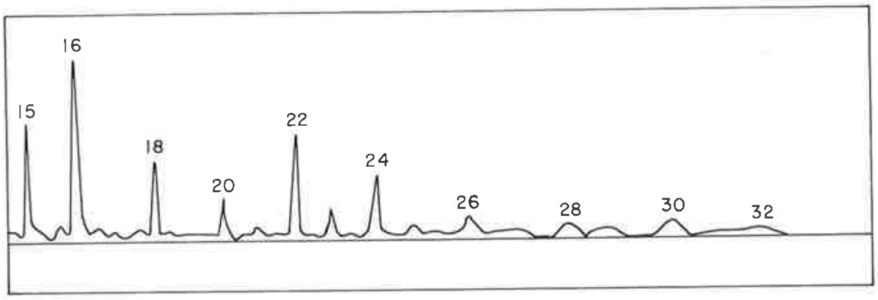

Fig. 7. Relative abundance of long chain fatty acids in soil lipids isolated with the mixture of *iso*-propanol/ammonia from Tintinara soils using GC/MS.

Fig. 6 shows the thin layer chromatographic behaviour of extracted hydrophobic materials compared with some reference compounds. Reference steroid spots showed a transitory colour change from red through deep blue to black as noted by Fewster *et al.* (1969), but these colours were not observed with the hydrophobic soil extracts, indicating that common steroids are not major components of these materials.

Fatty acid components

GC/MS analysis indicated that the hydrophobic materials contained combined and uncombined long-chain fatty acids with 16–32 carbon atoms (Fig. 7) and at least a bimodal distribution showing maxima at C_{16} and C_{22} when the $74\,m/z$ ion was monitored. Such a distribution has also been reported for lipids from the sediment of an Andean lake (Simoneit *et al.*, 1980). 1–18% of the carbon extracted by *iso*-propanol/ammonia was present as fatty acids. The involvement of long-chain fatty acids in water-repellent surfaces has been previously reported by Wander (1949) who noted that stearic acid created extreme water-repellency after treatment with calcium and magnesium hydroxide and also by Savage *et al.* (1969b) who found that palmitic acid induced hydrophobicity in sands after heating. Neither of these studies examined the concentration dependence of the phenomenon.

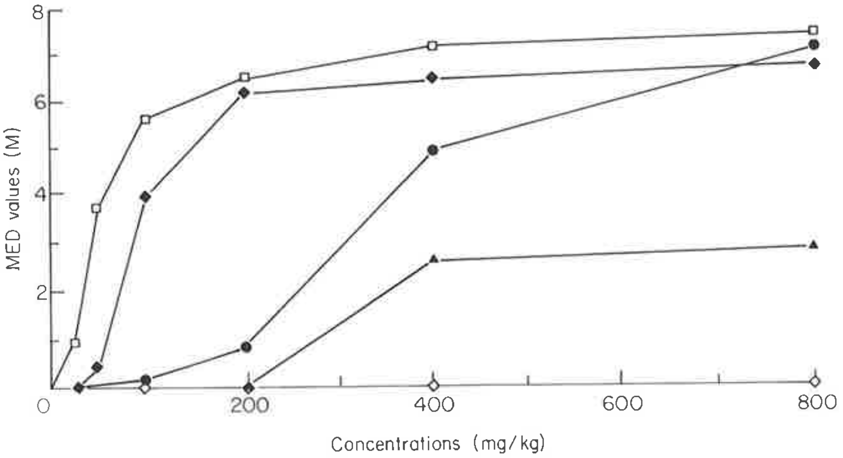

Fig. 8. Water-repellency of acid washed sand after treatment with several reference compounds. ■ Cetyl alcohol, ◆ Palmitic acid, ● Tristearin, ▲ Paraffin wax (m.p. 63–68°C), ◇ Methyl palmitate.

Concentration dependent hydrophobicity

The close packed cross sectional area of a straight chain acid or alcohol, such as the $C_{16:0}$ cetyl alcohol, is 0.21 nm^2. This corresponds to 4.8×10^{18} molecules m^{-2}, or 7.74 μmol m^{-2}, arranged end-on to the surface. The commercial acid washed sand used for Fig. 8 in this study had an estimated surface area of 10 m^2 kg^{-1} and would thus require approximately 77.4 μmol kg^{-1} for a uniformly distributed close packed monolayer. Hence, in the case of cetyl alcohol (MW = 242) only 18.7 mg kg^{-1} should suffice to provide such a layer. However, Fig. 7 demonstrates that for cetyl alcohol at least 25 mg kg^{-1}, or 103.3 μmol kg^{-1} are required to induce measurable hydrophobicity in the MED test and to reach the plateau region of maximal hydrophobicity at least 400 mg kg^{-1}, or 1.653 mmol kg^{-1}, are necessary. In other words, the calculations show that measurable hydrophobicity only occurs when the sand grains are uniformly coated by at least a close packed monolayer of cetyl alcohol and maximal hydrophobicity is attained only when there is a 16-fold excess. Two questions arise. Firstly, is the material uniformly distributed? In this case the thickness of the layer for maximal hydrophobicity with cetyl alcohol with a chain length of 2.24 nm would be nearly 43 nm thick (19.2 × 2.24 nm). If it were not uniformly distributed, then discrete particles of even larger diameter should be detectable. The second question concerns the necessity for such a vast excess of lipid material over that necessary for a monolayer in order to induce this hydrophobic state, and why very polar lipids such as cetyl alcohol and palmitic acid are much more effective than paraffin wax hydrocarbons or methyl palmitate. One conceivable explanation is that this excess is required not only to coat the surface of the sand, but also to coat rapidly the surface of the water droplet; such an explanation implies that droplet stabilization by surface monolayer formation is an important factor in its hydrophobic response.

CONCLUSION

It is clear that *iso*-propanol/ammonia removed the majority of lipids from water-repellent soils. The inability of less-polar solvents to remove lipids from soils has been well documented and it was suggested that some lipids may be linked with other compounds making them generally insoluble to less-polar solvents (Hance & Anderson, 1963; Johnson, 1971), but the solubility and partition properties of the material extracted by the current Soxhlet procedure suggest that the major problem concerns the rupture of hydrogen bonding links which are strengthened in aprotic solvents. Methanol or ethanol used as mixtures with chloroform or benzene have been employed to liberate protein-bound lipids, but by themselves they are poor solvents for most lipids. As long ago as 1957 Kates & Eberhardt reported that they were able to remove total lipids from leaves using hot *iso*-propanol and it is surprising that these early studies have not been applied to the problem of extracting hydrophobic materials from non-wetting sands. Recently, *n*-alkanes and alkanoic acids were extracted from soils and humic materials by supercritical gas extraction procedure with *n*-pentane (Schnitzer *et al.*, 1986).

The hydrophobic materials of the Australian soils examined in this work may be completely extracted in a Soxhlet apparatus with an amphiphilic solvent mixture of *iso*-propanol/ammonia which does not detectably cleave long chain ester bonds. The extracted materials restore hydrophobicity on acid washed sand or ignited sand at levels comparable to the original soil and their spectroscopic properties indicated they contain esters as well as long chain acids. The successful extraction by an organic solvent system indicates that it is almost certain that these compounds are not covalently linked to the surface of the sand. The chain length distribution showed by GC/MS analysis was predominantly composed of even numbered straight chain acids from 16 to 32 carbon atoms in length and which represent 1–18% of carbon extracted.

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