Lipids in Soil

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Abstract

As much as 20% of soil humus occurs in the form of lipids. High values are characteristic of Podzol soils and highmoor peats. Lipids of the following types are known to be present: paraffin hydrocarbons, phospholipids, fats, waxes, fatty acids, and terpenoids. A long list of compounds have been reported; the identification of many of them require confirmation using modern analytical techniques. Some of the lipids known to occur in soil have phytotoxic properties; these may have a depressing effect on seed germination and on root and shoot growth. Waxes and similar materials may be responsible for the difficultly wettable condition of certain sands.

Introduction

SOIL ORGANIC MATTER, or "humus," can be separated into two groups of compounds: 1) humic substances, a series of brown to black, high-molecular-weight polymers formed by secondary synthesis reactions, and 2) nonhumic substances, consisting of compounds belonging to the well-known classes of organic chemistry, such as amino acids, carbohydrates, lipids, etc. (11). Past research has been concerned largely with the nature and origin of the humic substances, although, in recent years, increasing attention has been given to the amino acid and carbohydrate fractions. The lipid fraction of the soil has been largely ignored.

Lipids are distributed extensively throughout the soils of the world, ranging from the highly weathered laterites of the humid tropics to the weakly developed tundras of the arctic zone. In normal aerobic soil, the lipids probably exist largely as remnants of microbial tissue; low and variable quantities of these constituents may be associated with undecomposed plant residues and the bodies of living and dead microfaunal organisms.

Lipid Content of Soil Humus

The proportion of the humus in soil which occurs as lipids can best be estimated from data obtained using Waksman's (70,83–87) proximate method of soil analysis. In this method, a single sample of soil, peat, or compost is given a series of treatments designed to dissolve specific classes of organic compounds. Distribution of the different fractions in the humus of a series of brown and black prairie soils, as reported by Waksman and Stevens (87), is given in Table I. The data show that most of the organic matter occurred in the rather vague groups designated as "lignin-humus" and "organic nitrogen compounds"; only a small fraction existed as fats, waxes, and resins.

TABLE I

Approximate Composition of the Humus in Some Typical Prairie Soils[a]

Fraction	Treatment	%
1. Fats, waxes, resins	Ether plus alcohol extraction	1.2–6.3
2. Hemicellulose	Hydrolysis with 2% HCl	5.5–10.7
3. Cellulose	Hydrolysis with 80% H_2SO_4	2.8–5.2
4. "Lignin-humus"	Analysis of final residue for carbon	41.9–49.3
5. Organic nitrogen complexes	Analysis of final residue for nitrogen	30.4–37.4

[a] Adapted from Waksman and Stevens (87).

The range given in Table I for the lipid content of humus (1.2 to 6.3%) is probably characteristic of most agriculturally important soils of the world. However, higher values are by no means uncommon. Some typical values that have been reported for some mineral and organic soil types is illustrated in Figure 1. For the mineral soils, the highest values (up to 16% of the humus) occurred in the podzolic soils (Podzol great soil group). In the case of the organic soils, the highmoor peats contained larger amounts than the lowmoor and sedimentary peats.

The highest value reported for the lipid content of soil humus appears to be that of Piettre (43) who found that nearly one-half of the organic matter in some coffee plantation soils in Brazil was in the form of fatty or waxy material. Nearly one-third of the organic matter in some pollen peats examined by Minssen (36) was recovered as lipids.

The variations noted in the lipid content of humus (see Fig. 1) can be explained by differences in vegetation, pH, or a combination of these two factors. Podzol soils are developed on well-drained sites where mor is the characteristic type of humus on the forest floor—this humus typically occurs under coniferous forests growing on soils low in available calcium. On the other hand, Gray-brown Podzolic soils are formed on moderately well-drained sites where mull is the characteristic humus type on the forest floor—this humus typically occurs under deciduous forests growing on soils well supplied with calcium. Thus, Podzol soils are generally more acid than Gray-brown Podzolic soils, and, on the average, a higher proportion of the humus occurs in the form of lipids. Of

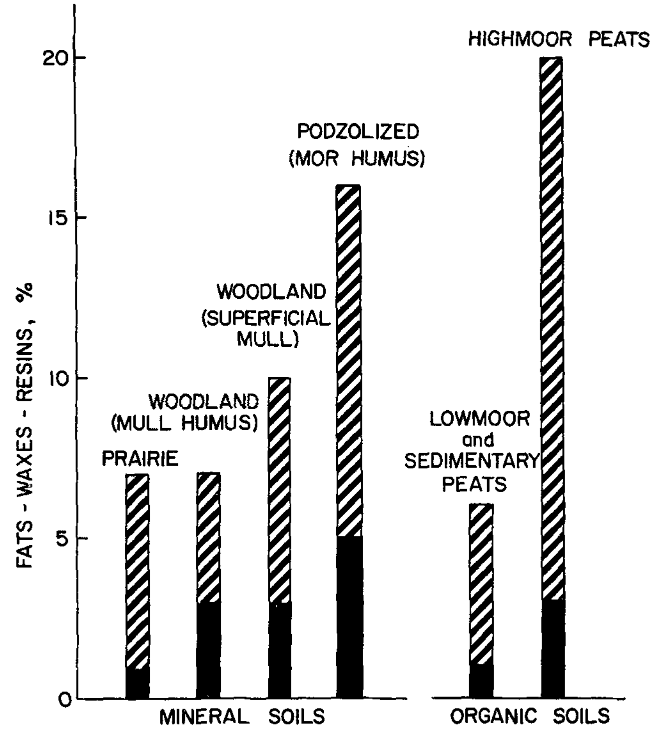

FIG. 1. Lipid content of the humus in several mineral and organic soils. The broken portions of the bars indicate the range of values reported. Data for the mineral soils were from Ashworth (1), Shewan (70), and Waksman and Stevens (87); for the organic soils, data were from Ashworth (1), and Waksman and Stevens (84–86).

the soil types given in Figure 1, the grassland (prairie) soils, represented by the Brunizens and Chernozems, have the highest pH values, and the humus in these soils is lowest in fats, waxes and resins. Fraps and Rather (16) recovered rather low amounts of lipid material from some Texas soils.

A pH relationship similar to that noted for the mineral soils appears to hold for the organic soils. Highmoor peats are formed in waters which are low in calcium (and other nutrients), whereas lowmoor and sedimentary peats are formed in places where the waters are relatively rich in calcium. Thus, highmoor peats are generally more acidic than lowmoor and sedimentary peats, and, on the average, they contain substantially higher amounts of lipid material (see Fig. 1). As much as 20% of the organic matter in individual layers of highmoor peats may exist in lipid constituents.

Information published by Fuestel and Byers (15) on the ether- and alcohol-extractable substances in a number of peat profiles having variable pH values show clearly that a relationship exists between pH and lipid content. The values they obtained, together with those reported for several other peat profiles (1,85,86) are summarized in Figure 2. The highest amounts (12 to 20%) occurred in the highly acidic Scottish sphagnum peat profile (pH 3.4 to 4.3); the lowest values (<1 to 2%) occurred in the Florida sedimentary peat profile (pH 7.1 to 7.5)

Cawley and King (7) concluded that the amount of wax which could be recovered from some Scottish peats was related to the nature of the vegetation from which the peat was formed; in general, lower yields were obtained from peats derived from Phragmite and Sphagnum than from peats derived from cottongrass, heather, and *Scirpus*. It is possible that the wax content of the samples could also have been related to pH.

It is not known whether the high lipid content of the humus in acidic soils results from inability of microorganisms to decompose completely the lipids occurring in plant remains, or if larger quantities of lipids are synthesized by microorganisms. It is instructive that fungi are often the predominant type of microorganism in acidic soils.

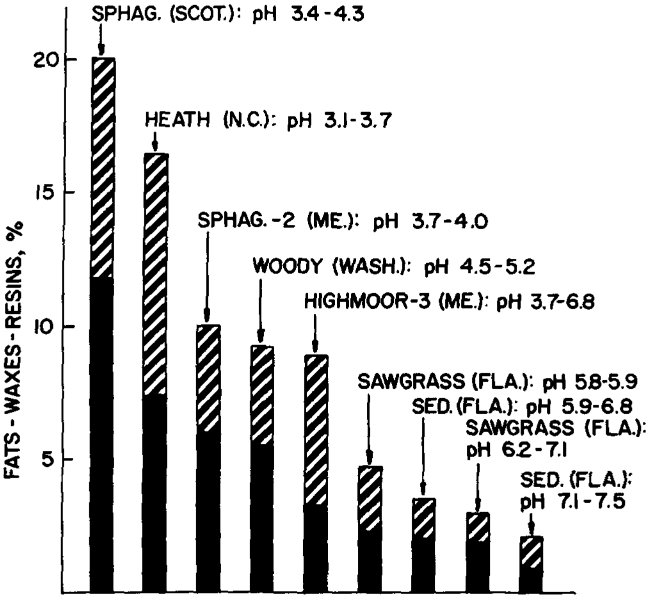

FIG. 2. Lipid content of various peat profiles in relation to pH. The broken portions of the bars represent the range of values found throughout the profile. The source of the data was: Sphagnum peat from Scotland, Ashworth (1); three highmoor peats from Maine, Waksman and Stevens (86); sedimentary peat from Florida, Waksman and Stevens (85); others from Fuestel and Byers (15).

Goldschmidt (17) estimated that there are 1.5 × 10^{12} metric tons of carbon in the pedosphere which occurs in terrestrial humus. Assuming that an average of 5% of the humus is composed of lipids, and that humus has an average carbon content of 58%, the quantity of lipid material contained in terrestrial soils of the earth is approximately 1 × 10^{11} metric tons.

Many of the estimates reported for lipids in soil may be low because of incomplete extraction. Feustel and Byers (15) found that when peat was treated with hot dilute HCl and reextracted with ether and alcohol, a further yield of waxy material was obtained. Shewan (70) concluded that a much longer extraction period was required for recovery of lipid material from soil than had been recommended by Waksman and Stevens (87). Hance and Anderson (21) found that a number of solvent mixtures gave higher recoveries of phospholipids from soil than ether and alcohol alone, although the difference could be eliminated by pretreating the dry soil with an acid mixture containing HCl and HF. Goring and Bartholomew (19) extracted bacterial tissues in the presence of added clay minerals and found that apparent lipid phosphorus decreased with increasing amounts of clay.

Meinschein and Kenny (35) used a ball milling technique to extract lipids from some subsurface soils. The efficiency of extraction might be increased by using modern ultrasonic vibration techniques.

Significance of Lipids in Soil

Attempts have been made to relate the productivity of soils to the presence of lipids or their transformation products. Ashworth (1) emphasized that "better quality" soils are associated with a low average content of fats and waxes. Piettre (43) found that some exploited coffee plantation soils contained high amounts of lipids, "les toxins du sol." Greig-Smith (20) explained "soil exhaustion" on the accumulation of fats and waxes which he termed "agroire."

According to Schreiner et al. (58), terpenes are particularly toxic to plants. Low concentrations of borneol, $C_{10}H_{18}O$, and camphor, $C_{10}H_{16}O$, were found to be highly toxic. Turpentine, $C_{10}H_{16}$, also inhibited plant growth. Prescott and Piper (44) pointed out that many soils from the mallee area in South Australia contained substances which prevented seed germination. They concluded that oils from eucalyptus trees were responsible.

Schreiner and his associates (55,57–59,61) concluded that dihydroxystearic acid, $CH_3(CH_2)_7$-$CHOHCHOH(CH_2)_7COOH$, was toxic to plants and that it was one of the compounds responsible for the low fertility of worn-out soils. Out of 25 "good" soils, only 2, or 8%, contained the acid, whereas 18 out of 34 "poor soils," or 51%, contained this compound (55,57). Wheat seedlings showed abnormal growth when grown on soils containing small quantities of dihydroxystearic acid. The conditions under which dihydroxystearic acid accumulated were deficiency of lime, lack of good oxidation (poor aeration), and poor tilth.

Desai and Seshagiri (10), and Mitsui et al. (37), concluded that butyric acid inhibited root development of rice in paddy fields.

McCalla et al. (32), in a study of phytotoxic materials in the stubble-mulch system of farming, found that ethanol-soluble substances in wheat straw were among the compounds which had a depressing effect on germination and on root and shoot growth. An ether-soluble portion of the ethanol extract was separated into strong acids (soluble in 5% $NaHCO_3$), weak acids (soluble in 5% NaOH), neutral compounds, and bases (soluble in 5% HCl). Each fraction contained substances which inhibited plant growth.

Sandy soils are often made unproductive through cropping because the individual sand grains become coated with organic constituents; these coatings confer water-repelling properties to the sand (25,44). The compounds responsible for this phenomenon are unknown, but participation of lipids is suspected. As early as 1910, Greig-Smith (20) alluded to the waterproofing of soil particles by wax-like substances.

Burges (4) suggested that there was a relationship between the wettability of fungal and algal spores and the vertical distribution of fungi and algae in the soil. Presumably, organisms which produce spores with waxy nonwettable coats, such as various species of *Penicillium*, would be confined to the surface soil, whereas those with wettable coats, such as certain *Fungi imperfecti*, would occur in both the surface soil and the subsoil.

Persistence of Lipids in Soil

The primary source of soil humus is the remains of vegetative growth, the lipid content of which varies considerably. For example, as much as 25% of mature pine needles may occur as lipids, whereas only from 2 to 5% of rye tissue and corn stalks is in this form. In the decomposition of plant residues in soil, proteins, cellulose, hemicellulose and water-soluble substances are believed to be decomposed first, followed in order by certain ether- and alcohol-soluble substances and lignin.

Tenney and Waksman (76) reported a net loss of lipids when mature plant residues were incorporated into a mineral soil and allowed to undergo decomposition. For the soils examined, the lipid content of humus in the surface soil was lower than in the plant residues, but higher than in the humus of the subsoil. Ashworth (2) found that changes in fats, waxes and resins during decomposition in compost heaps were small and fluctuating.

Shewan (70) compared the ether- and alcohol-soluble content of loose forest litter with that of the decomposing layers, namely, the "F" layer (litter undergoing active decomposition) and the "H" layer (litter extensively decomposed, original plant structures lost). Figure 3 shows that there was, in general, a net loss of ether- and alcohol-soluble material during decomposition. On the other hand, the humus in the soil proper often showed an enrichment in ether- and alcohol-extractable substances. The higher values were invariably associated with podzolic (Podzol) soils (see Fig. 1).

Very little is known of the rates at which individual lipids decompose in soils. Those compounds most resistant to microbial decomposition, such as certain higher alcohols and paraffins, should persist for longer periods of time than those that are attacked readily by microorganisms, such as the true fats and phospholipids. Waxes protecting the surfaces of leaves, needles, trunks and fruit of many higher plants are particularly resistant to decomposition; these should be capable of surviving essentially unchanged over long geological periods (3). Plants belonging to the Coniferaceae and Myrtaceae produce rather large quantities of relatively stable terpenoid compounds.

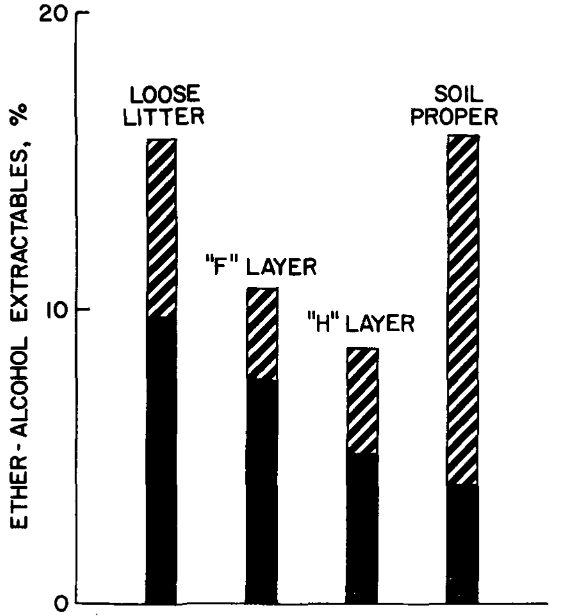

FIG. 3. Lipid content of forest litter and associated mineral soils. The broken portion of the bars represent the range of values reported. From Shewan (70).

Except for the paper by Piettre (43), no report has been published showing a significant accumulation of difficultly decomposable lipids in productive agricultural soil, even when large annual increments of residues and manures have been applied. Most cultivated soils apparently contain sufficient numbers of the proper kinds of microorganisms to destroy completely the lipid material contained in plant and animal residues. It should be noted in this respect that past studies on lipid transformations have been concerned with gross chemical changes accompanying decomposition, and that no attempt has been made to distinguish between lipids remaining as a remnant of the original material and those newly synthesized by microorganisms active in the decay process.

Oró et al. (38) concluded that high molecular weight hydrocarbons are degraded little if at all in the bovine digestive tract, and that they accumulated and outnumbered any hydrocarbons resulting from animal metabolism or synthesized by bacteria. The hydrocarbons in cattle manure were thus believed to be derived mainly from the plants which constituted the cattle's diet.

An interesting finding was recently reported by Chahal and Mortensen (8). These investigators found that a benzene-methanol extract of an Ohio peat (representing 1.9% of the total carbon in the peat) contained 40.3% of the C^{14} activity left behind after C^{14}-labeled rye tissue had been incorporated into the peat and allowed to decompose for 28 days. It was not known whether the material represented unaltered plant constituents or newly-synthesized microbial products.

Turfitt (79), in a laboratory incubation study, concluded that sterols were decomposed rapidly in soil, although in one experiment in which cholesterol was added to a mull soil, only 40% was decomposed the first six months and 60% within a year. Lack of aeration and high water content were factors which inhibited decomposition. In an early study, Rubner

(53) reported that he was unable to obtain complete destruction of fats added to soil. In one experiment, only 23% of butter added to a nonsterile soil had decomposed after one year, and only 38% was reported lost after 12 years.

Before the end of the eighteenth century the practice of burying butter in peat for purposes of preservation was widespread in Ireland; samples of "bog-butter" several hundred years old, and weighing as much as 100 pounds, have been recovered in recent years. The geochemical aspects of Irish "bog-butter" have been discussed by Bergmann (3).

Chemistry of Soil Lipids

In recent years, extensive use has been made of modern analytical methods (infrared, chromatography, etc.) for characterizing organic constituents in soil. The result has been a marked increase in our knowledge of the nature of the polysaccharide, nitrogen, and lignin-like fractions. Few examples can be cited where the newer methods have been applied to the study of soil lipids, and our knowledge of this material is highly fragmentary. Soils undoubtedly contain a wide variety of lipids, ranging from the rather stable paraffin hydrocarbons to ephemeral chlorophyll degradation products.

Waxes

Except for two studies (mentioned below), the research on soil waxes has been carried out using material extracted from peat. The vast majority of papers are dated 1940 or earlier (see reviews by Bergmann, 3; and Vallentyne, 80); a few papers have appeared between 1940 and 1950 (for example 6,7,48,75); and none seem to have been published since that time. It is apparent, therefore, that modern analytical techniques have not been adequately used to characterize peat waxes.

Many peat waxes have properties similar to fossil "plant" waxes isolated from lignites (26). These waxes, referred to as "montan waxes," are complex mixtures of free acids, esters and unknowns, which in a typical montan wax will occur in proportions of 17:53:30 (see Bergmann, 3). Other peat waxes have somewhat different chemical and physical properties (6,7,46–50,75,77). A wax from Irish peat examined by Reilly and his associates (47–49) had a melting point of 10° to 15° lower than montan wax, and it was softer in texture; this wax was named "mona wax." A wax separated from Rekinski peat by Rakowski and Edelstein (46) was especially rich in free acids (57%).

The most extensive studies on the nature of the wax material in mineral soils appear to be those of Meinschein and Kenny (34), and Butler et al. (5). In the study conducted by Meinschein and Kenny, a benzene-alcohol extract of soil was placed on a silica gel column prewet with n-heptane, and four fractions were recovered by successive elution with n-heptane, carbon tetrachloride, benzene, and methanol. The materials recovered with n-heptane and carbon tetrachloride consisted largely of saturated hydrocarbons; only the benzene eluate (wax fraction), representing about 5% of the extracted material, was examined in detail. The types of acids and alcohols in the wax esters were determined by converting the esters to saturated hydrocarbons by high pressure hydrogenation and analyzing them mass spectrometrically. Even carbon numbered (C-even) waxes were present in considerably higher amounts than their odd carbon number (C-odd) homologs (90% vs 10%). The waxes ranged from C_{36} to C_{52}, and the C-even waxes were formed from C-even normal aliphatic acids and normal primary aliphatic alcohols. The waxes in urea-nonadduct samples were chiefly esters of cyclic alcohols and normal aliphatic acids, a result that is in agreement with the observation that cyclic alcohols are more prevalent than cyclic acids in natural waxes.

Butler et al. (5) utilized gas chromatography to characterize the acids and alcohols produced by saponification of a neutral wax obtained from an Australian green soil. The acids (analyzed as the esters) ranged from C_{12} to C_{30}, with the even numbers predominating. The acids present in greatest amounts were C_{22} (13%), C_{24} (22%), and C_{26} (21%). The alcohols were separated into adducted (80%) and nonnadducted (20%) materials by treatment with urea. Conversion of the adducted alcohols to acids by oxidation with $CrO_3.H_2SO_4$, followed by chromatographic analysis of the esterfied material, gave a range of compounds similar to that listed above for the acids. The crude wax also contained a complex mixture of hydrocarbons (to be described in the next section). A pigment identified as a hexachloropolynuclear quinone of the dihydroxyperylenequinone or dihydroxydinapthylquinone type (chemical formula of $C_{20}H_4O_5Cl_6$) was isolated from the original extract containing the wax; this material was responsible for the green color of the soil.

The general observation that soil waxes are chiefly esters of the higher members of the homologous series of n-acids and n-alcohols, both having a preponderance of C-even carbon atoms, is in agreement with the obervation that animal and plant waxes are, for the most part, saturated straight-chain compounds containing primarily C-even acids and alcohols.

Organic Acids

More than a dozen free acids, including crotonic, $CH_3CHCHCOOH$, α-hydroxystearic, $CH_3(CH_2)_{15}CHOHCOOH$, dihydroxystearic, $CH_3(CH_2)_7CHOHCHOH(CH_2)_7COOH$, lignoceric, $C_{23}H_{47}COOH$, and 3 compounds identified as "agroceric," $C_{21}H_{42}O_3$, "humoceric," $C_{19}H_{34}O_2$, and "paraffinic," $C_{24}H_{48}O_2$, have been isolated from soil. These compounds were reported in the literature dated 1921 or earlier (51–61,63,67,71), and in reviews of Bergmann (3), Vallentyne (80) and Waksman (82). Confirmation for the occurrence of paraffinic and lignoceric acids in mineral soils was claimed by Tokuoka and Dyo (78). Stadnikov and Sabavin (72) reported the presence of caproic, $C_5H_{11}COOH$, heptoic, $C_6H_{13}COOH$, and caprylic, $C_7H_{15}COOH$, acids in peat.

Crude peat waxes have been found to contain variable quantities of free acids (see previous section). Rakowski and Edelstein (46) concluded that cerotic, $C_{25}H_{51}COOH$, heptacosanoic, $C_{26}H_{53}COOH$, and montanic, $C_{27}H_{55}COOH$, acids were present in the wax from Rekinski peat.

Low-molecular-weight organic acids, including formic, acetic, oxalic, and butyric, appear to be normal constituents of mineral soils (10,37,41,68). Some of these acids may be important agents in the mobilization and transport of metals, the weathering of rocks, and the solubilization of plant nutrients (especially phosphorus).

Organic acids of various types occur in small quantities in the rhizosphere of plant roots (52), and in raw podzol humus (54). Parker and Leo (42) detected fatty acids containing 12 to 20 carbon atoms in algal mat communities; underlying layers of black mud became progressively depleted in the unsaturated acids.

Mention was made previously of the observation of Schreiner and his associates (55,57–59,61) that soils low in fertility often contained high amounts of dihydroxystearic acid. Butyric acid, which is known to accumulate in paddy soils, may contribute to the "Aki-ochi" condition of rice (10,37).

Hydrocarbons

Normal paraffins in the C_{16} to C_{32} range have been found in the nonsaponifiable fraction of ether, alcohol and benzene-alcohol extracts of soil. The earliest reference to them was made by Schreiner and his associates (56,57,60,65,67), who isolated n-hentriacontane, $C_{31}H_{64}$, from several American peat and mineral soils. In later work, Titov (77) isolated n-pentatriacontane, $C_{35}H_{72}$, and n-tritriacontane, $C_{33}H_{68}$, from a Russian peat. In both of the abovementioned studies, the compound in question was identified using such criteria as melting point, carbon and hydrogen contents, negative addition of halogens, and general resistance to chemical reaction.

A study was made recently by Stevens et al. (73) of the distribution of normal paraffins in several soil types. Normal alkanes in the C_{23} to C_{31} range were observed, among which n-nonacosane, $C_{29}H_{60}$, and n-hentriacontane, $C_{31}H_{64}$, predominated. There was a preponderance of C-odd to C-even carbon atoms. This finding is of interest because normal paraffins in this range are widely distributed in plants, and they show a significant predominance of C-odd to C-even carbon atoms; n-nonocosane and n-hentriacontane are often major components.

Very little is known of the relative distribution of hydrocarbons in microorganisms. Oró et al. (38) refer to unpublished research (part of which was their own) in which the hydrocarbons of bacteria, phyto- and zooplankton, and "coral" reef organisms were found to have a ratio of C-odd to C-even carbon atoms close to unity. The implication of these findings to the origin of the hydrocarbon material in soils is apparent. If the n-alkanes in soil represent microbially synthesized material, the ratio of C-odd to C-even hydrocarbons should be similar to that of the microbial mass of the soil.

A comparison of the relative amounts of n-alkanes in soil (C_{23} to C_{33} range) with that of leaves of pasture plants, cattle manure, crude oil, and some marine sediments is illustrated in Figure 4. Although variations occur in the relative distribution of C-odd to C-even hydrocarbons, only the n-alkanes in the crude oil fail to show a significant preponderance of C-odd to C-even hydrocarbons.

Mention was made previously to the study of Oró et al. (38) relative to the plant origin of the higher molecular weight alkanes in cattle manure. The results shown in Figure 4 for the hydrocarbons in the leaves of pasture plants, and in cattle manure, were based on their findings. The main difference between the hydrocarbon content of the cattle manure and the leaves of the pasture plant (spotted bur clover) which served as the bulk of the diet for the test animals, was that the cattle manure contained a relatively larger amount of the C_{33} hydrocarbon (n-tritriacontane); this was attributed to the presence of other plant species, which contained high concentrations of this hydrocarbon, in the cattle's diet.

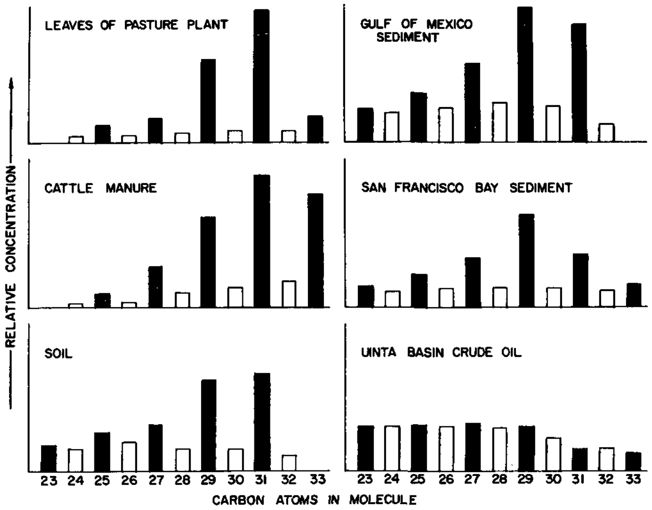

FIG. 4. Relative amounts of n-alkanes in pasture plants, manure, soils recent sediments, and crude oil. Source of the data was: pasture plants and manure, Oró et al. (38); soil and Gulf of Mexico sediment, Stevens et al. (73); San Francisco Bay sediment, Kvenvolden (30); Uinta Basin crude oil, Martin and Winters (31).

The hydrocarbon material in many ancient sedimentary rocks fail to show a predominance of C-odd to C-even carbon atoms. Some results published by Oró et al. (39) for the n-alkanes in chert from the Gunflint iron formation (1.9 × 10^9 years old) are of interest because the predominant n-alkanes are in the C_{16} to C_{24} range, and, as indicated above, there is no preference of C-odd to C-even carbon atoms. Earlier, Butler et al. (5), in connection with the isolation of a chlorinated pigment from an Australian green soil, had reported that hydrocarbons associated with the pigment consist primarily of compounds with odd and even numbers of carbon atoms in approximately equal amounts between C_{17} and C_{24}. A gas chromatographic pattern typical of their results was obtained (courtesy of J. H. A. Butler), and the relative concentration of n-alkanes was compared to that reported by Oró et al. (39) for the Gunflint chert. The remarkable similarity of the distribution of n-alkanes in the two samples is shown in Figure 5. To this writer's knowledge, the results obtained for the Australian soil represent the first instance where n-alkanes have been isolated from a recent sediment where C-odd hydrocarbons have not been present in considerably higher amounts than C-even hydrocarbons. It is of interest that the soil used by Butler et al. (5) was taken from olive-green patches associated with decomposing roots of eucalyptus trees, and that the hydrocarbons could have been synthesized by wood-rotting fungi.

Chrysene, a polycyclic aromatic hydrocarbon, was reported in soil by Kern (28). According to Cooper and Lindsey (9), this compound occurs in combustion gases and its presence in soils can be accounted for through the settling of dust particles.

Fats and Phospholipids

True fats have yet to be isolated from soils. Schreiner and Shorey (60,64) detected glycerol (acrolein test) in the product obtained by saponification of soil lipids.

Considerable attention has been given from time to time to the occurrence of phospholipids in soil,

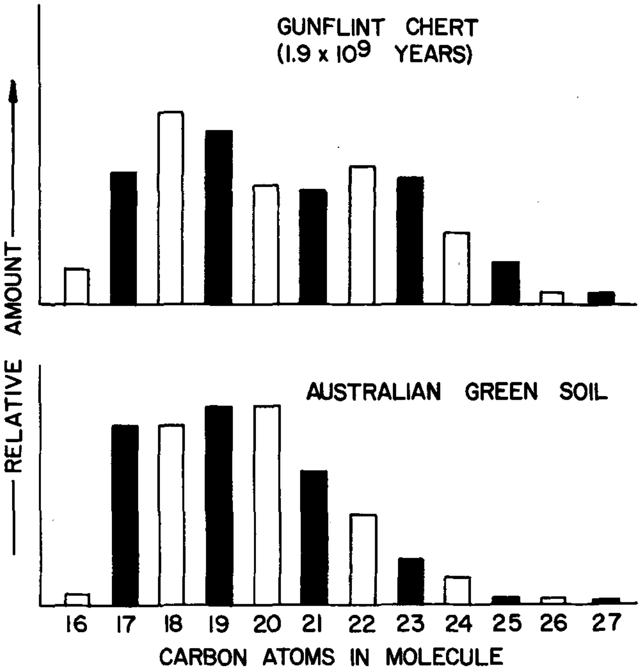

FIG. 5. Relative amounts of n-alkanes in Gunflint chert and an Australian green soil. Source of the data was: Gunflint chert, Oró et al. (39); Australian soil, Butler et al. (5) and private communication with J. H. A. Butler.

because these compounds are potential sources of phosphorus for plant growth. The earliest indication of their presence was from the work of Shorey (71), who detected choline in a soil extract. Small amounts of phosphate are extracted from soil with "fat solvents," and it has frequently been assumed, without justification, that this phosphate occurs in the form of glycerophosphatides. The highest value reported for "lipid phosphate" in soil appears to be that of Stoklasa (74), who recorded a value of 3.4 mg P per 100 g soil. The values Hance and Anderson (21) obtained for five soils ranged from 0.31 to 0.70 mg P per 100 g soil, equivalent to from only 0.6–0.9% of the total organic phosphate.

Direct evidence for the presence of phospholipids in soil was obtained recently by Hance and Anderson (22). These investigators used chromatographic techniques to analyze the products formed by alkaline hydrolysis of a phosphate-rich lipid fraction of soil. Glycerophosphate, choline, and ethanolamine were recovered from the hydrolysis mixture. The molar ratio of glycerophospate to choline plus ethanolamine was approximately unity (see Table II), and phosphatidyl choline was the predominant ester in the fraction. The latter finding is in agreement with the fact that the major glycerophosphitide in many plants and microorganisms contains choline. Little, if any, of the soil phospholipid material appears to be in the form of inositides (22).

TABLE II

Molar Ratios of Glycerophosphate, Choline, and Ethanolamine in a Soil Lipid Fraction[a]

Soil number	Molar ratios		
	Glycerophosphate	Choline	Ethonolamine
1	1	0.9	$\sim$0.2
2	1	0.7	$\sim$0.3
3	1	1.0	$\sim$0.3
4	1	0.7	$\sim$0.1

[a] From Hance and Anderson (22).

Porphyrins

The occurrence of porphyrin pigments in fresh-water and marine sediments, and in petroleum, has been recognized for many years (see reviews by Dunning, 12; and Vallentyne, 80). The major porphyrins are regarded as being derived from chlorophyll; they have been referred to as "chlorophyll derivatives" and "sedimentary chlorophyll degradation products."

Changes produced in chlorophyll during attack by microorganisms may involve loss of the phytol group (formation of a *phyllin*), loss of the magnesium atom (formation of a *pheophytin*), or losses of both (formation of a *pheophorbide*). Other alterations may result in the formation of structures of the types illustrated in Figure 6. *A priori,* one would expect to find porphyrin structures in soil characteristic of the types shown under "sediments" and "intermediate."

Gorham (18) identified chlorophyll derivatives in a number of surface woodland soils. Absorption spectra of the crude preparations were similar to pheophytin A (see Fig. 6). Chlorophyll content was related to acidity, namely, the amount recovered from some acidic mor humus layers was about twice that recovered from some neutral or faintly acidic mull humus layers.

Data obtained by Gorham (18) showed that, per unit of carbon, the chlorophyll content of woodland soils was an order of magnitude lower than that observed for lake muds. This result can be explained on the basis that deficiency of oxygen in the latter inhibits chlorophyll destruction. Poorly drained (humic gley) soils may contain porphyrins in amounts approximating that detected in fresh-water sediments.

Johnson and Thiessen (26) observed that a green substance accompanied the waxy material isolated from a wooded peat; this was subsequently separated from the wax and identified as chlorophyll. Kamoshita (27) observed reddish fluorescence in acetone extracts of soil and concluded that chlorophylls were present.

Pristane (2,6,10,14-tetramethylpentadecane) and phytane (2,6,10,14-tetramethlhexadecane) have been observed in ancient sedimentary rocks, including the one-billion-year-old Precambrian Nonesuch formation (13,34) and the two-billion-year-old Precambrian chert from the Gunflint iron formation (39). According to Oró et al. (39), these compounds are probably derived from phytol, the alcohol of chlorophyll. As yet, no attempt has been made to determine if hydrocarbons of chlorophyll origin occur in terrestrial soil.

Steroids and Triterpenoids

There can be little doubt but that steroids and triterpenoids of various types occur in soil. Schreiner and his co-workers (56,60,66) reported the presence of "phytosterol" in the saponified material from a soil preparation. The sterol appeared to be present as an ester with a higher fatty acid. Preparations from other soils (56,57,59,62) contained a crystalline material which had chemical properties typical of the cholesterol group, but which had a melting point dissimilar to any known substance in this group—the compound was subsequently referred to as "agrosterol." According to Bergmann (3) this compound was probably related to betulin.

Stigmasterol and β-sitosterol, in association with the triterpenoid friedelan-3β-ol, were isolated from a humified peat by McLean et al. (33). In an earlier study, Ives and O'Neill (23,24) reported the presence,

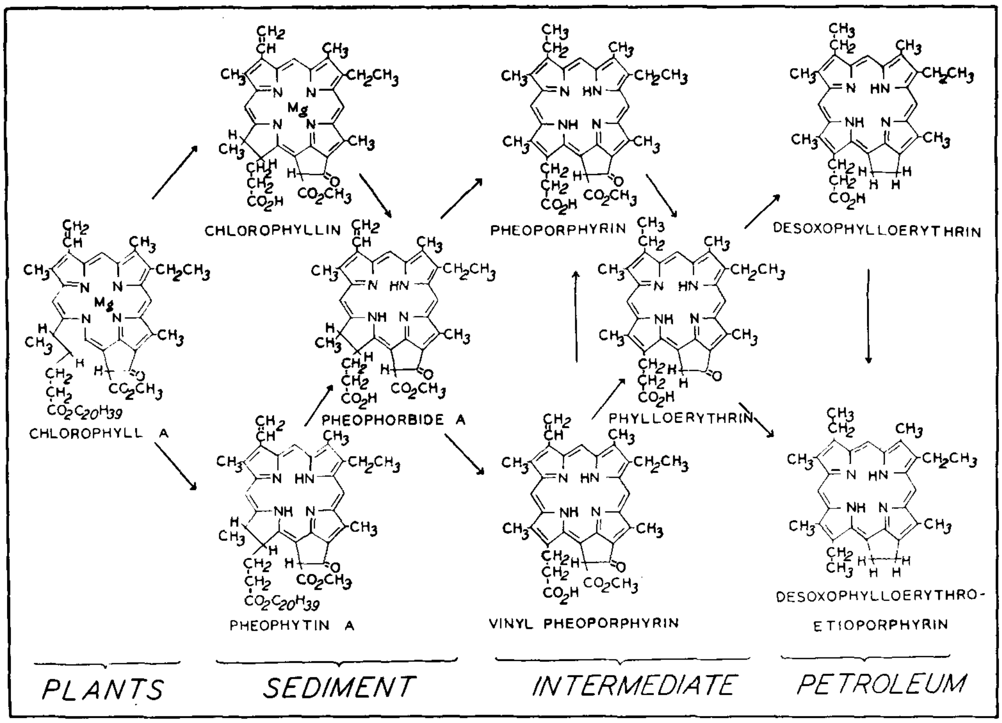

FIG. 6. Chemical changes involved in the formation of porphyrins. From Orr et al. (40).

in sphagnum peat moss, of the sterols mentioned above plus the triterpenoids α-amyrin, taraxerone, and taraxerol. Meinschein and Kenny (35) detected sterols and penta- and hexacyclic compounds (tentatively identified as triterpenoids) in the benzene extracts of several mineral subsoils. Bergmann (3) reported the presence of β-sitosterylacetate in a garden soil.

Turfitt (79) analyzed a number of English soils for their sterol content (colorimetric analysis) and found that the highest values were characteristic of poorly aerated and acidic soils, such as bog and fen peats. Schwendinger and Erdman (69) concluded that sterols were widely distributed throughout recent marine sediments.

Carotenoids

Carotenoids are, in general, sensitive to heat and light, and they are readily oxidized in the presence of air; thus, the conditions in most soils are suitable for their rapid decomposition. No report seems to have been published on the occurrence of carotenoids in agricultural soils, although they are undoubtedly present in trace amounts, particularly in poorly drained soils. About 20 different carotenoids have been found in wet sediments; only α-carotene, β-carotene, and rhodoviolascin, however, can be considered to have been identified positively (80). Detailed information concerning the carotenoids in peat, swamps, and marine- and fresh-water sediments has been given in reviews by Dunning (12) and Vallentyne (80).

Research Prospectus

Because of the diverse nature of soil and the complexity of the organic constituents contained therein, characterization of lipid components will require the skill and patience of scientists working in several disciplines of science. It is hoped that this review will stimulate additional research on this subject. It is apparent that many of the new separation and analytical techniques developed by lipid chemists can be applied directly. Gas-liquid chromatography has been extended to a wide variety of lipids, including the triglycerides (29), and, with conventional apparatus and the hydrogen flame detector, the technique is suitable for the analysis of nanogram amounts of fatty acid (14), steroids (81), and hydrocarbons (Peake and Hodgson, this symposium). The elegant new techniques of thin-layer chromatography are useful not only for separations but for quantitative determinations; these methods have been reviewed by Privett et al. (45). Of further interest and general applicability are the liquid-column chromatographic techniques described in a recent review by Rouser et al. (51). The rapidly accumulating body of reliable knowledge on the lipid composition of plants, animals, and microorganisms will undoubtedly be of great assistance to those working in soil chemistry.

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