

31.3 per cent iron as free iron oxides (dithionite-soluble) after the coarse inorganic and organic particles had been removed by sieving. According to X-ray analysis and measurement of solubility in acidic ammonium oxalate (which gives a fairly good measure of amorphous iron oxides<sup>2</sup>) no crystalline oxides were present (sample 1). This material was aged under conditions extremely favourable for goethite formation—that is, boiling for some hours in strong alkaline solution (normal potassium hydroxide). Under these conditions, artificially prepared ferric hydroxide is readily transformed to well crystallized goethite in a few hours<sup>3</sup>. The natural material, however, did not crystallize (sample 3, Table 1), even after prolonged ageing (72 h). It was decided to treat it several times with 15 per cent hydrogen peroxide in order to destroy the organic matter; while the carbon content was reduced from 10.23 to 1.03 per cent carbon, the amorphous nature was unchanged (sample 2). After this material had been aged under the alkaline conditions already described, X-ray analysis showed broadened diffraction lines of goethite (sample 4).

In order to confirm this result, amorphous ferric hydroxide, which had been freshly prepared by precipitation of ferric nitrate with ammonia, was aged in the same way, except that on this occasion the hot normal potassium hydroxide extract of the natural material was used. This clear dark coloured extract contained 1.24 g of carbon/l. When the precipitate was treated with pure potassium hydroxide, goethite was formed in a few hours and this was accompanied by a considerable decrease in solubility (sample 7); however, when the potassium hydroxide extract of the natural material was used, no crystalline forms appeared and the solubility remained high (sample 8).

Table 1

Sample No.	Sample	Treatment	Analytical data	
			"Amorphous oxide" (percentage of total)	X-ray diffraction
1	Ditch precipitate	None	100.2	Amorphous
2	Same as 1	H <sub>2</sub> O <sub>2</sub>	96.3	Amorphous
3	Same as 1	4 h ageing in N KOH at 100° C	99.0	Amorphous
4	Same as 2	4 h ageing in N KOH at 100° C	61.7	Goethite (weak)
5	Same as 2	72 h ageing in N KOH at 100° C	52.3	Goethite (weak)
6	Freshly precipitated ferric hydroxide	None	100.0	Amorphous
7	Same as 6	4 h ageing in N KOH at 100° C	6.1	Goethite (strong)
8	Same as 6	4 h ageing in boiling N KOH extract of No. 1 at 100° C	81.3	Amorphous + goethite (very weak)

These results suggest that organic compounds in the alkaline extract may have an inhibitory effect on the crystallization of amorphous ferric hydroxide. Strong adsorption of organic anions by the amorphous ferric hydroxide may disturb or even prevent its rapid crystallization.

Adsorption of anions such as phosphate, molybdate or silicate, which are usually present in soils and sediments, by ferric hydroxide, may have the same effect. This has, in fact, been verified for both phosphates<sup>4,5</sup> and silicates<sup>6</sup>; thus it is possible that these anions played a part in the present experiments.

In the presence of organic matter, both natural and synthetic goethite, and amorphous ferric hydroxide, are not directly dehydrated to haematite on heating, but are first dehydrated to maghaemite<sup>7</sup>. The involvement of organic matter in these reactions may also explain why the sharp exothermic peak usually observed for artificial amorphous ferric hydroxide on dry thermal analysis does not occur with amorphous hydroxides in soils. Further more, the hardening of laterite, which is frequently observed after the vegetative cover has been removed, may also arise from a similar effect because the soil

organic matter would be decomposed on cultivation. Further experiments are being carried out.

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### A Microbiological Approach to the Problem of Soil Humic Acid Structures

CHEMICAL investigations of soil humic acids have indicated their possible components, but have not so far thrown any light on the structures involved in this complex group of compounds<sup>1</sup>. The use of biodegradation of humic acids as a means of analysing their constituents has attracted the attention of only a few workers<sup>2-4</sup>. Most studies concerned with the microbial utilization and degradation of humic acids have revealed the susceptibility of their nitrogenous portion to attack; however, the structures of these moieties are defined largely by the carbonaceous fraction.

The extensive use of humic acids as the sole source of carbon during growth by micro-organisms has recently been reported<sup>5</sup>. Because enzymes are specific and their reaction conditions mild, the initial products of microbial enzyme cleavage should be more indicative of the constituents and structures of humic acids than is possible with chemical degradations, where artefacts can easily be produced. There are, however, some pitfalls in using a microbiological system which must be avoided. The by-products of microbial metabolism must not be mistaken for humic acid derivatives. Uncharacteristic portions of soil humus should not be used. Organisms which merely transform without degradation may provide little useful information. A number of organisms, in sequence or together, may be required to achieve a complete breakdown of humic acids.

Carbon dating techniques have indicated that the mean residence time of the carbon of "mobile" humic acids from a Black Chernozemic (Melfort) soil is about 800 years, thus indicating that the greatest proportion of this carbon has a very low turnover rate<sup>6</sup>. Certain fractions of the humic acids, such as the acid hydrolysable portion, however, were found to have a much higher turnover rate, indicating that micro-organisms in the soil are capable of attacking at least a portion of the humic acids<sup>7</sup>.

Micro-organisms capable of utilizing purified "mobile" humic acids from the Melfort Orthic Black soil as a source of carbon and/or nitrogen were isolated. Representatives of the genera *Penicillium*, *Aspergillus*, *Arthrobacter* and *Pseudomonas* were found to grow well with humic acids as a sole source of carbon. A *Penicillium* isolate was selected for further investigation. This fungus did not produce any pigment within a period of 8-9 weeks; it did not possess laccase, peroxidase, tyrosinase, proteinase, amylase or cellulase; it grew well on Czapeck-Dox sucrose broth and utilized humic acids as a sole source of carbon.

R spiration measurements indicated that the fungus utilized an inducible enzyme system, which required about 5 h for induction. It grew well at concentrations of 0.2 per cent humic acids or lower and, once adapted, continued to utilize humic acid carbon in the presence of glucose. Replacement cultures, shaken for 7 weeks, used up to 35 per cent of the humic acids (Table 1).

The extent of attack on the humic acids depended on the molecular size of these materials. The fractions having molecular weights of more than 50,000 were attacked to a

Table 1. UTILIZATION OF HUMIC ACIDS BY THE *Penicillium* ISOLATE

	Humic acid added (mg)	Humic acid recovered after 7 weeks (mg)	Amount used (per cent)
Control	60.0	60.0	—
Humate as carbon source at 0.020 per cent	60.0	40.8	32.0*
Humate as nitrogen source at 0.020 per cent	60.0	42.0	30.0
Humate as a source of both carbon and nitrogen at 0.020 per cent	60.0	39.0	35.0

\* Spore inoculation and incubation for a similar period resulted in 6 per cent utilization.

greater extent than those below 50,000 ('Sephadex' gel fractionation<sup>8</sup>). Elemental analyses of the surviving humates indicated that they were richer in nitrogen than the original. Functional group analyses indicated a considerable increase in the hydroxyl content, during degradation, with a smaller increase in the phenolic hydroxyl and the carboxyl groups available for titration.

Paper and gas chromatography used to study the intermediates produced by fungal cleavage, under conditions of restricted aeration, indicated that salicyl alcohol and salicylaldehyde were formed. Salicyl alcohol (also known as saligenin) is the aglycone of salicin—a glycoside found in plants belonging to the family of Salicaceae and a few others<sup>9</sup>. Willow and poplar, which are both members of the Salicaceae, formed an important portion of the flora covering this soil before the land was cleared for cultivation around 1900 (ref. 10).

It is interesting that one of the first known isolations of intermediates formed during the microbial cleavage of humic acids should yield two compounds which could not have been obtained by means of normal chemical degradation techniques. This implicates a plant glycoside in the synthesis of humic materials. Salicyl alcohol may also be produced by the reduction of salicylic acid, through salicylaldehyde, by several fungi which inhabit soil<sup>3</sup>. Salicylaldehyde has been implicated in the degradation of polyaromatic compounds such as naphthalene<sup>11</sup>. The *Penicillium* used in this investigation, however, did not reduce salicylic acid in either aerated cultures or low oxygen tension cultures, and this was so whether or not sucrose was present; moreover, it did not appear to utilize naphthalene.

Salicyl alcohol is known to undergo condensation to produce resin-like substances, and has a great affinity for amino compounds; it could therefore be involved in soil humus formation.

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### Age of the Youngest Hydrated Halloysite of Kyushu

Aomine and Miyauchi have concluded that the hydrated halloysite of a buried soil at Choyo in the Aso district of Kyushu was derived from volcanic ash that fell between 8,000 and 9,000 years ago<sup>1</sup>. The reasoning by which they arrived at this conclusion is open to question, and both

Table 1. (AFTER AOMINE AND MIYAUCHI)

Location	Horizon	Depth (cm)	Organic carbon (per cent)	In clay fraction	
				Allophane (per cent)	Hydrated halloysite (per cent)
Choyo	1	0-30	8.2	67	0
	2	30-80	2.4	75	0
	3	80-90	1.2	73	0
	4	90-140	1.7	68	0
	5	140-190	11.0	37	33
Kanoya	1	0-30	5.6	70	0
	2	30-70	12.1	72	0
	3	70-130	1.8	80	0
	4	130-175	7.1	74	0
	5	175-225	2.5	75	0
	6	225-275	4.0	80	35

the ash and the halloysite could be much older than 9,000 years.

They stated: "A buried surface soil containing hydrated halloysite would offer a suitable sample for dating the mineral by means of radiocarbon measurements, if the soil contains enough organic carbon and has been isolated from biotic activity since the soil was covered with ash. . . . So the age of volcanic ash in a previous surface layer can be estimated by the content of carbon-14 in the organic matter, provided that the period of time of carbon accumulation is negligibly short as compared with the ash-age."

They then gave details of a profile at Choyo (32° 53' N., 131° 0' E.) in which the upper four layers contained predominantly allophane in the clay fraction but no halloysite, and the fifth contained about equal amounts of allophane and hydrated halloysite (Table 1). This fifth layer was rich in organic matter and to arrive at the age of the ash of this layer they took the radiocarbon age of its organic carbon (8,650 ± 200 yr): "This value undoubtedly represents an average of organic carbon accumulated in the layer while it was on the surface". To this value they added an estimate of 330 yr ("or less") for the 11 per cent of carbon to accumulate, and concluded that "the hydrated halloysite of the fifth layer was derived from the ash that fell between 8,000 and 9,000 years ago. . . ."

While this is a possible interpretation, the following objections can be raised: (1) It implies that the ash of the fifth layer is only a few hundred years older than that of the fourth layer. It is improbable that a difference of a few hundred years in eight or nine thousand years would be sufficient to produce the sharp contrast in the clay mineralogy of the two layers. (2) In another profile, at Kanoya (31° 24' N., 130° 52' E.), Aomine and Miyauchi found that the organic matter of the uppermost layer containing hydrated halloysite, the sixth layer (Table 1), was beyond radiocarbon measurement, "that is, older than 30,000 years". In none of the layers above this buried soil however, is there any halloysite, although one or more of them is probably much older than 9,000 yr. (3) The age of the organic matter of a surface soil layer can be much younger than the time taken for the soil to form.

When soils form on newly deposited parent materials there is an initial period during which organic carbon rapidly accumulates, but there comes a time when a state of dynamic equilibrium is reached after which net accumulation ceases, or becomes very much slower, perhaps accompanying changes in the constitution of the clay minerals or changes in internal drainage. The level of this equilibrium differs according to climate and in some cases according to parent material, and usually this equilibrium will be reached in a few hundred years. Thereafter, although the concentrations of organic matter stay fairly constant, there is a continual breakdown of much of the existing organic matter and build-up of fresh organic matter<sup>2,3</sup>. Some very stable humus compounds are formed that do not enter into this process, but so far as I am aware their proportion has never been very great. Tamm collected humus from the B horizons of three podzol profiles on moraines at Garpenberg, Hartejarnsheden, and Haboskegen in Sweden, at sites that had been forested for as much as 9,000 yr, and, in commenting on the radio-