FREEZE-DRYING IN SOIL MICROSCOPIC PREPARATION : FURTHER EXAMPLES FOR THE PROTECTION OF ORGANIC STRUCTURES AND THE CONTROL OF DISLOCATIONS

by

S. Stephan (1)

Freeze-drying is a useful method for impeding any movements in soil samples during the preparation of thin sections. In our laboratory, it has been used since 1.968 in order to control the break-down of labile organic constituents (Stephan, 1.971). Electron microscopy requires a more sophisticated freeze-drying technique (see the papers of the 4th meeting). A simpler technique is still capable of yielding good results in light microscopy, especially in conjunction with autoradiography and chemical identification. This paper will give three examples.

Example 1. Study of organisms in an incipient soil

In a open-cut coal mining near Cologne, a flat area has been overlain by a thick layer of sand and gravel in 1.965. In December 1.970, the sparce pioneer vegetation consisted of mosses, algae and few modest phanerogams. In order to examine the effect of this vegetation, samples were taken in Kubiena boxes, frozen with liquid nitrogen, freeze-dried by a dry ice condenser, impregnated with Vestopal V 120 L (HULS) and styrene and thin sections (6 x 7 cm^2 slides) were prepared.

In deeper places, influenced by water runoff and covered with sandy material, a mat of green algae covers the soil and actually protects a certain part of the surface against erosion. The algae filaments are in a good contact with the mineral grains.

The more elevated parts are covered by patches of moss. Small soil animals (photo 1) are the consumers. The

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(1) Institut fuer Bodenkunde, BONN, Westgermany

tissue of moss (photo 9) is still quite visible in the animal's stomach (photo 10). It is interesting to note that the fine epidermal hairs of the animal are well preserved (photo 1). The animals produce droplets (photo 11) acting as initial soil plasma. Although small diatoms are capable of living autotrophically, they may well use organic substances of this plasma. These photographs represent the main food chain of the initial soil, with the exception of the microbes.

Example 2. Lake sediments with special reference to pyrite framboids

The freeze-drying method is also well suited for the study of submerged soils. The investigation of four cores taken from Laacher See sediment (Maria Laach, volcanic region of the Eifel mountains, W-Germany) gives an example.

The cores were taken by means of a box (2 m deep), dropped down from a rubber boat to the lake bottom at a depth of 10.3, 16.7, 9.8 and 20 m, respectively (Scharpenseel, pers. communication). The upper part was mostly too soft for this study. Before preparing, the core was allowed to settle down in the boxes. In this way, the pores seen in the thin section samples of the cores may be smaller than they would otherwise be. Samples were taken from the interior of the core, where no oxidation occured.

The fine details of the complicated microstructure were well preserved. The average participation of certain constituents was calculated by examining a number of fields (fig. 1). Possible mistakes may arise from overlapping and masking of smaller elements and overlooking of clear colloids situated between coloured particles. Nevertheless, the method gives relieable results when used for comparative studies and provides a good impression of the microstructure.

There are hardly any relationships between the different constituents which seem to vary more or less independently. Naturally, the pores were smaller in the sample from the core taken at the deepest level (core 4), and the carbonaceous fraction limits the amount of other constituents, especially of the clear colloids (deeper part of core 3). The stratigraphic relationships of the four cores are as yet unknown, as there were no clear marks or diagnostic features. ^{14}C dating was impossible as the water contained volcanic CO_2. There are so many and varied microscopic features which do not exist in terrestrial soils that we may consider only one sort of constituents.

The variability of pyrite framboids

Examination of these and other submerged soils shows a striking quantity of clear gelatinous globes (25-100μ m) containing white to yellowhish reflecting bodies of different size, largely of about 1 m. Love quoted by van Dam and Pons (1.972) named them pyrite framboids. They are found in sedimentary rocks of different age, ores, and acid sulphate soils (cat clay etc.) Their shape and pedochemical action have been studied (e.g. van Dam and Pons, 1.972; Miedema, Jongmans and Slager, 1.974). These studies however refer to framboids found in sediments which have undergone some diagenesis. They are stable enough to preserve their microstructure during preparation. Because the young framboids of uncompacted, wet sediments rich in organic matter undergo alteration during desiccation (photos 12, 13), it seems probable that there are types of framboids which have escaped detection.

In our samples of sedimens from the Laacher See, very weak forms of framboids have been left in a perfect state by means of freeze-drying. The participation of framboids is not related to the total amount of pyrite (fig. 1). Framboids seem to be bound to material rich inorganic substances which are absent from the pyrite-rich layers of core 1. Weak forms with a high content of colloids tended to accumulate in the upper part of sediment, when there were plenty organic remnants. Their grains may be arranged in a geometric order (photos 2.3). Without doubt, this forms of framboids represents gelatinous capsules of colonized sulphur bacteria, and the small bodies inside may well consist of sulphur as suggested by Stephan (1.973). But even in the upper part of the sediment, the grains of some framboids might increase and fill up the capsules (photo 14).

It is notable that there are different forms of framboids probably representing different forms of microbes. On the other hand, there are different modes of development.

The growing grains, formed of pyrite, lost their geometric arrangement (photo 15). Framboids containing only single grains may lead to the formation of crystalline pyrite more rapidly (photo 4 b). Capsules with single grains may built chains during their propagation. In the deeper sediment layers, the concentration of pyrite increases and the formation of more or less extented pyrite layers takes place. The growth of pyrite grains is accompanied by a certain loss of water which makes the gelationous substance denser, as does with the adjoining material. For this reason, the formation of pyrite similar to that reported by van Dam and Pons (1.972) was an effect to both concentration and compaction. Furthermore, there are lobate forms of framboids (photo 4), either built directly by distinct organisms or as produced by stress. The random distribution of pyrite grains may be caused by stress and decomposition of multigrain framboids, or they may consist of framboids or chains of framboids with single grains; larger gelatinous aggregates may be preserved between such bulks of randomly distributed pyrite grains as shown in photo 5. Pyrite may also form within tube-like boides. We cannot exclude the possibility that in this case very weak framboids grow within algal tubes, although boundary lines were not visible, and normally, round framboids with small grains formed between algal tubes. Soot-like deposits of iron sulfide may arise from chemical precipitation without any biological action (photo 8).

The distance to the water- and sediment - surface is an important factor influencing the development of framboids (pressure, water content, age, diagenesis are connected with this factor). Although the connection with a certain content of organic matter has been noted above , there is no pyrite in the exceptionally good network of organic remnants partly filled with clear colloids, which normally contain plenty of pyrite framboids (photo 6). This may demonstrate the independence of features. Soot-like chemical deposits of iron sulfide have been found in layers rich in organic matter, but more so in clayey material (photo 8).

Some difficulties in studying the nature of framboids

As demonstrated by Babel (1.964) soil chemical compounds may be stained or bleached when situated at the surface of the section, e .g. organic structures by means of Giemsas stain (Stephan, 1.971). Staining by GIEMSA really gives good results with the organic remnants and detritus, but there is no certain effect with the framboids slime. Experiments with Iodine (in ethanol or as Lugols solution), with NaOCl , H_2O_2 and a corresponding use of HF had nearly no effect. This may indicate that there is a high content of water which is replaced by the resin and that the participation of slime is correspondingly small.

Investigating the nature of the grains, framboids with great, dark grains contain Fe^{++} which were clearly detectable as Turnbulls blue (method Kubiena, 1.938). However, framboids with finer grains failed to give this reaction. Nevertheless, it was also impossible to dissolve such grains by means of CS_2 in order to indicate their sulphuric nature. Because of the high refraction of the micro bodies it is not possible to distinghish between pyrite and sulphur using UV fluorescence. The weak framboid of photo 3, seen between crossed nicols, shows bright yellowish grains with the black cross as a sign of their spherical nature (photo 18). In view of the evidence, it seems probable that framboids with small grains do not contain pyrite but elemental sulphur, but this suggestion needs to be further investigated.

Example 2. Localization of ^{14}C labelled polychlorinated biphenyls (PCB)

There was another micromorphologic problem which called for the protection of soluble substances by freeze-drying. ^{14}C labelled PCB, applied to a column of undisturbed Hapludalf soil and percolated with 900 and 1.700 mm water, respectively, in the laboratory (Scharpenseel et al., 1.977), was to be localized. PCB is a poison, has long-term persistence in the environment, and may concentrate in the food-chains (Jensen, 1.972). Its fate in soils is still unknown. With the weak radiation of ^{14}C and the small concentration used, its detection required the use of low-level counting technique.

An important step in the preparation of samples for autoradiography is the application of an efficient freeze drying method. In 1.974, samples were cut out of the soil cores, rapidly frozen in liquid nitrogen, and freeze-dried to avoid dislocations and any change of the organic matter. One set of samples was infiltrated with rapid hardening Araldite, another set was impregnated with Vestopal which hardens in the course of several weeks. As there is no detectable PCB in the resin-filled pores and holes, we may suppose that the resins have no influence in the distribution of PCB. Sample preparation was difficult in the absence of a low-speed saw. Grinding was made by hand on grinding paper for avoid the formation of any radioactive dust particles. Each polished sample was protected by dipping it three times into a solution of 0.5% Pioloform in chloroform. This produced a very thin film. However, the film absorbes some activity, increased the distance between the sources of radiation and the stripping emulsion and so decreased the efficiency of radiation and increased scattering. All the same, the structures to which PCB was bound were great enough to give good results of both the orienting exposure to Definix medical film (KODAK) and the microscopic work done with KODAK AR 10 stripping film as described by Fischer & Werner (1.971).

Nearly all activity was found in the upper 8 cm of the soil column. It was not distributed uniformly over the soil plasma but was confined to definite structures. The structures must have fixed PCB very strongly as PCB is highly soluble in styrene and yet was not to be found in the pores. The stripping film stayed in contact with the sample allowing a perfect localisation, whereas the photographic recording is difficult.

In the developed stripping film, the silver grains were largely formed over organic remnants (photos 16, 17) which comprised the whole range from brown, cellular tissues to coprogenous altered plant matter. The strongest attraction seemed to occur with organic particles changing into coal like substances. Those well shaped organic matter particles contain brown substances of different ori gin but similar chemical nature, listed by Babel (1.975)as leaf browning substances, phlobaphenes, lignin derivatives and fungal pigments. It is, however, difficult to establish that just these substances act as a sink for PCB. We could only say that PCB was strongly associated with the organic remnants of the soil.

Some consequences must be discussed : It has been thought that the adsorbing organic substances together with clay minerals form the soil plasma whereas the solid plant remnants forme an organic soil skeleton without special activity. It must be noted that Babel (1.975) doesn't regard these remnants as to be skeleton with respect to their short residence time. The example of PCB gives evidence for a more active role of the organic remnants and solid rotting products, as here adsorbing substances may be concentrated and, consequently, adsorbed compounds may accumulate.

In the case of PCB, its high concentration in only one compartiment of the soil, which is shortlived and may be consumed by the soil fauna, is clearly more dangerous than is a distribution in low concentration throughout the soil plasma. If there are similar situations for other substances, it may have ecological consequences, too. So the case of this PCB experiment may call for further investigations.

Legends

Fig. 1.-

Micromorphology of four cores from the Laacher See sediment. Each column represents a sample, divided into components. The presence of Surirellaceae (genus of big diatoms, number 1) and the notation of the framboids have been drawn outside the columns, the percentage of organic carbon in the dry matter is drawn from the left side (points, connected with lines; the length of the whole colum marks the 100 %). The following features have been calculeted: 2 = carbonate ; 3= skeleton, apart from 2 and 4; 4= opal, mostly diatoms; 5= light colloids; 6= rougher organic remnants, from which striking amounts of filiform algae may be separated as 7 ; 9= iron sulfide and perhaps some sulphur, in cases drawn as r= rarely; white field = = natural holes as detected with 25 x magnification by means of crossed polars and quartz red I; 8= difference to the full column, showing mainly the fine detritus and smaller pores. Right side: framboids, namely: – = no framboids present, r under 5 %, + 5-10 %, ++ 10-25 %, +++ 25-50 %, ++++ 50-85 %, +++++ more than 85 % of the sulfide and sulphure content.

Photo 1.- Section of a small soil animal which fed on moss plants. The small insert on the bottom shows a highly magnified part of the integument with fine hairs. Scale: 0.2mm for the main picture and 12μm for the insert.

Photo 2.- Very fine framboids from the upper layer of core 2. Scale : 20μm.

Photo 3.- Extremely weak object of the same section. Scale : 10μm.

Photo 4.- Lobate framboid, stained with GIEMSA. 4b : framboids with single grains of well crystallized pyrite . Core 1, 70 cm depth, scale 20μ m.

Photo 5.- Randomly distributed grains, core 2, 170 cm depth, scale 0.2 mm.

Photo 6.- Fibrous layer, 135 cm depth, core 2. Brown net of organic matter, partly filled with clear colloids. The peat-like fabric is well preserved. Scale 0.2 mm.

Photo 7.- Between clay colloids, there are framboids with connecting threads and well developed iron sulfide grains. Beneath the peaty layer shown in photo 6. Scale 0.2mm.

Photo 8.- Clay containing diffuse iron sulfide, core 4, 51 cm depth, scale 0.2 mm.

Photo 9.- Sporogon of the moss growing in and on the sandy to gravelly material. Phase contrast.

Photo 10 .- Stomach of the soil animal of photo 1 filled with moss tissue. Phase contrast.

Photo 11 .- Initial soil plasma consisting of droplets of the soil mesofauna. Note also the diatom.

Photo 12 .- Air-dried sample of lake sediment, disturbed throughout by shrinkage.

Photo 13 .- Freeze-dried sample of the same layer, microstructure well preserved, especially the framboids.

Photo 14 .- Round framboids with small and with thicker net-like grains, phase contrast.

Photo 15 .- Framboids with different infillings in a hole between organic remnants.

Photo 16 .- Silver grains (white reflecting) in the stripping film over an organic remnants.

Photo 17 .- Silver grains demostrating the activity of ^{14}C-PCB bound to an organic remnant.

Photo 18 .- The same framboid as seen in photo 3, between crossed plars.

SUMMARY

The use of freeze-drying for conserve weak organic structures, and impede dislocation effects is demonstrated in three examples.

1. The incipient plant cover of a recultivated, sandy area was consisting in mosses. Some photographs present the initial food chain. Leaf tissues have been found in the stomach of a small animal.

2. Bore-cores of lacustric soils may be prepared - using freeze-drying. It is true that the pores have changed their form; but principally the structure is well visi

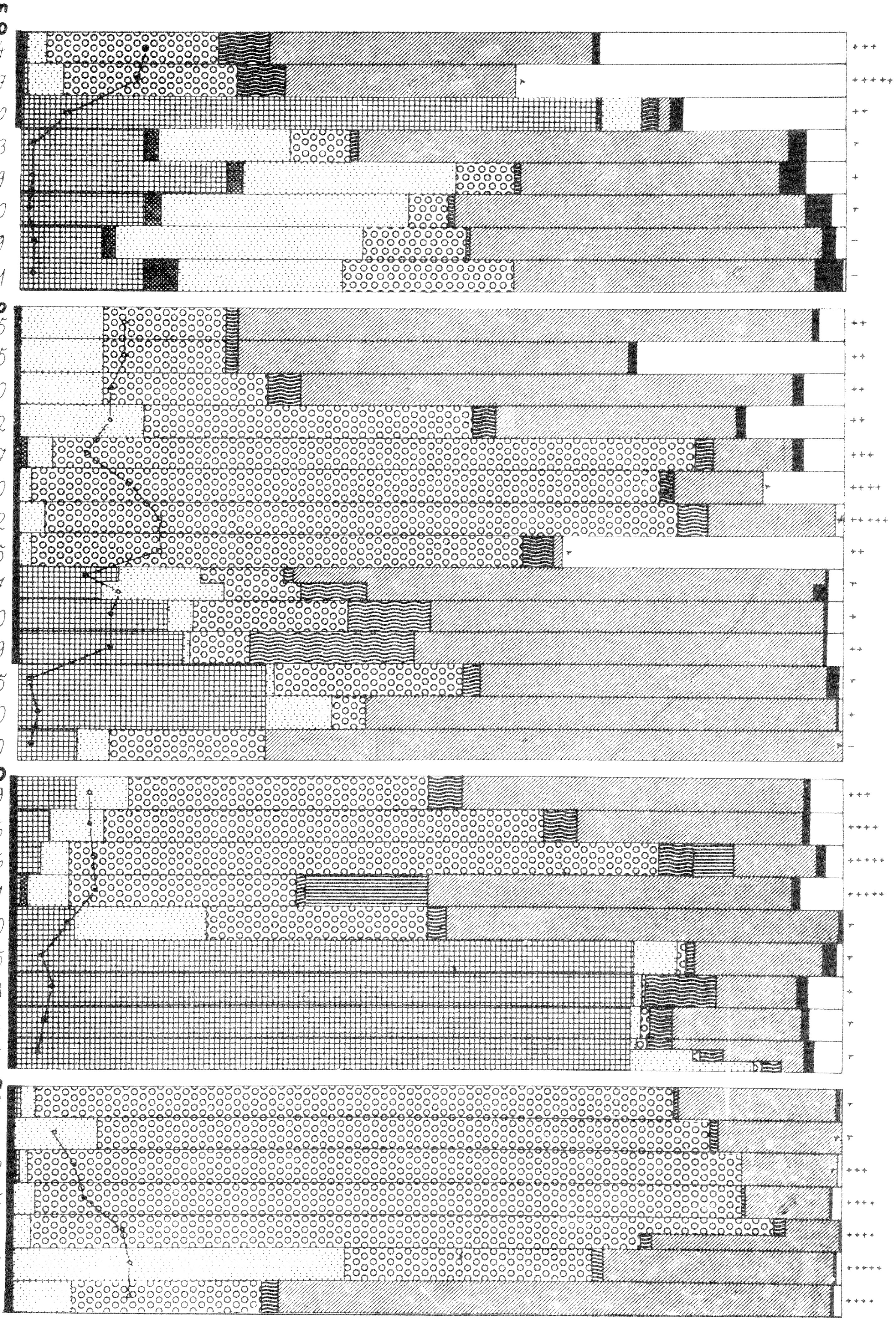
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Fig. 1


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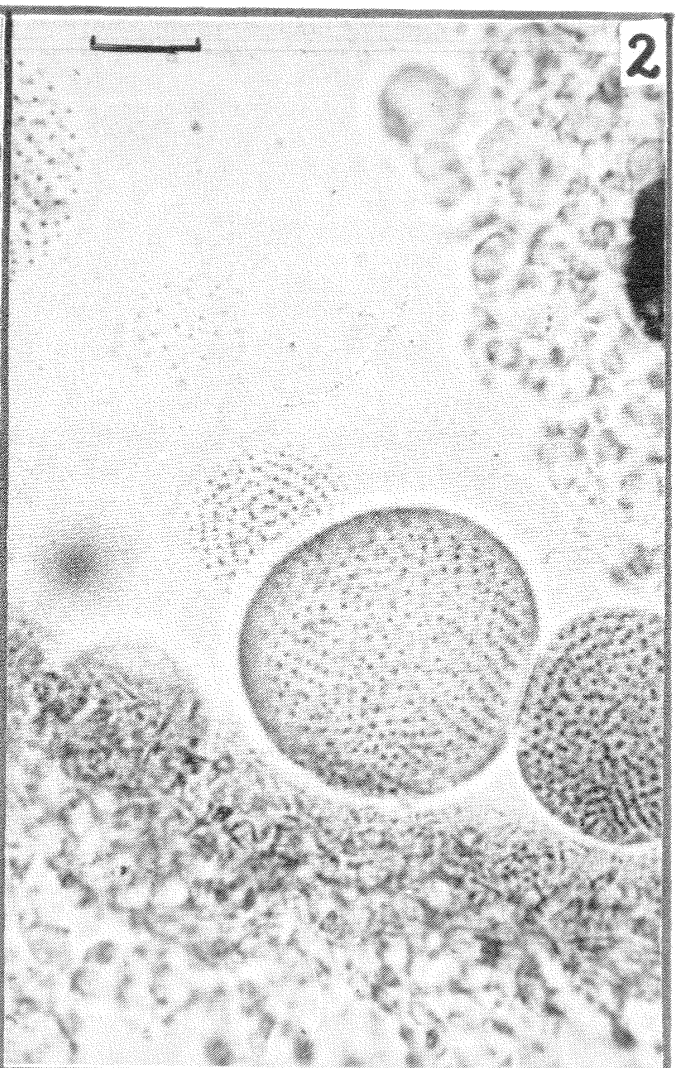
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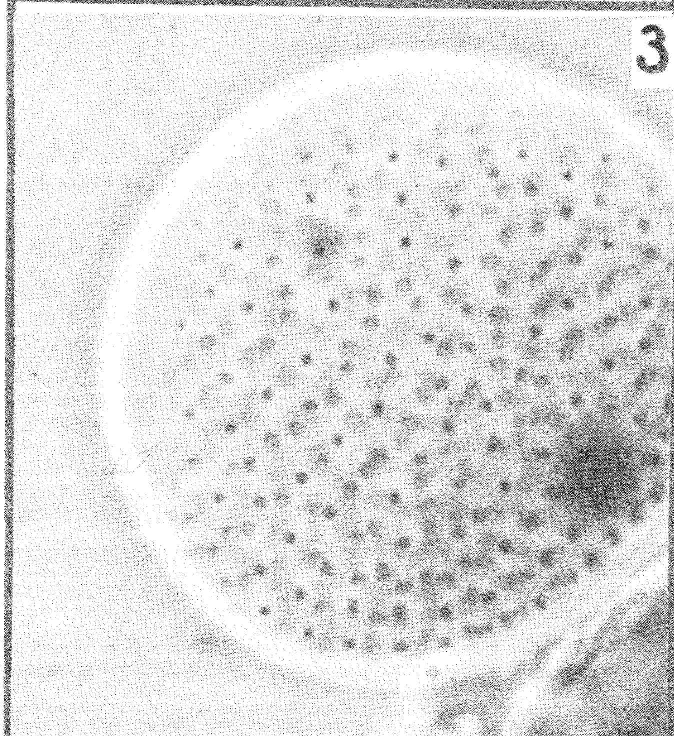
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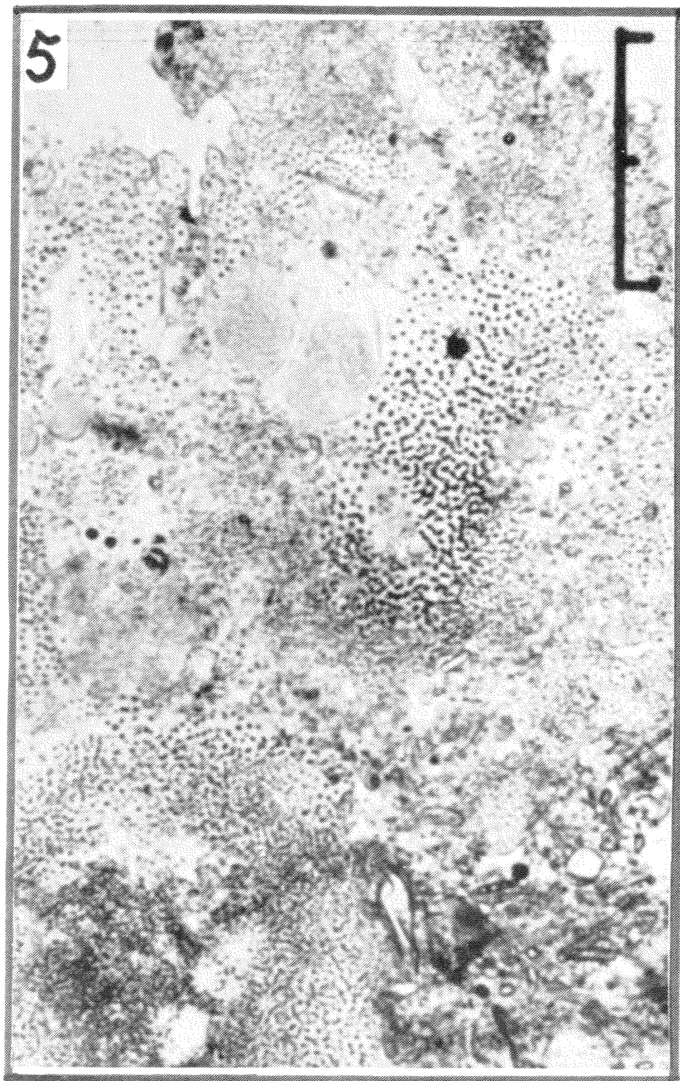

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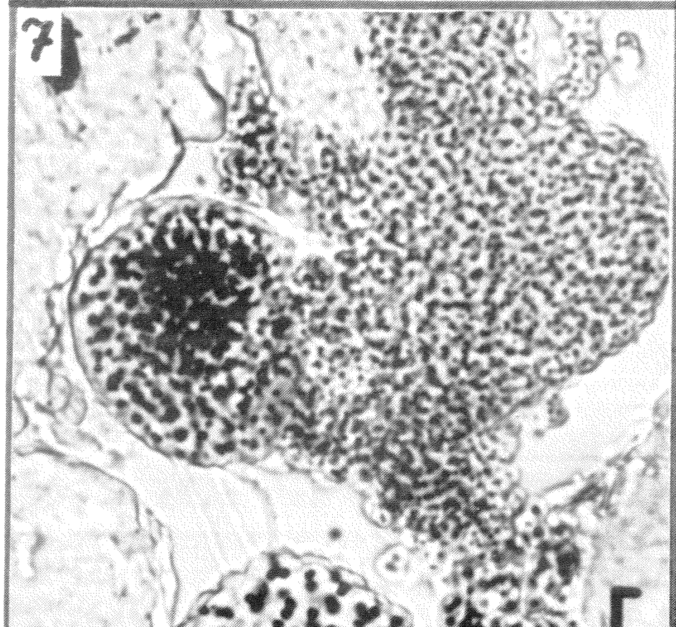

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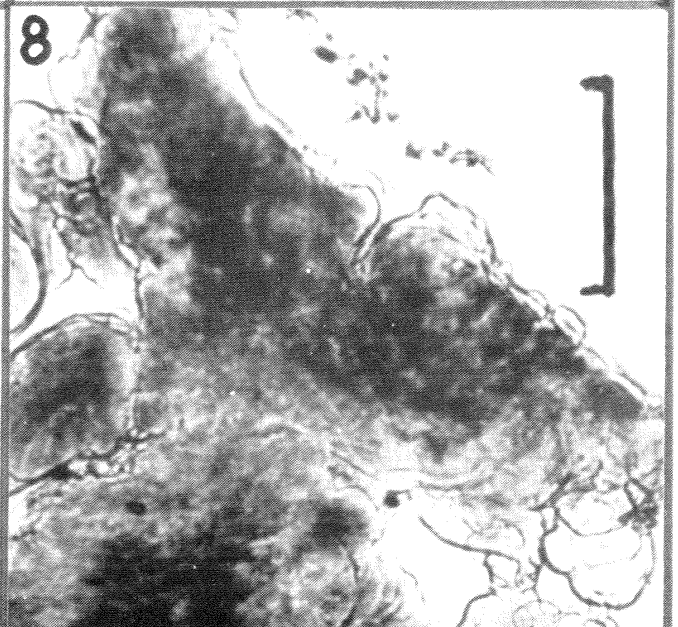

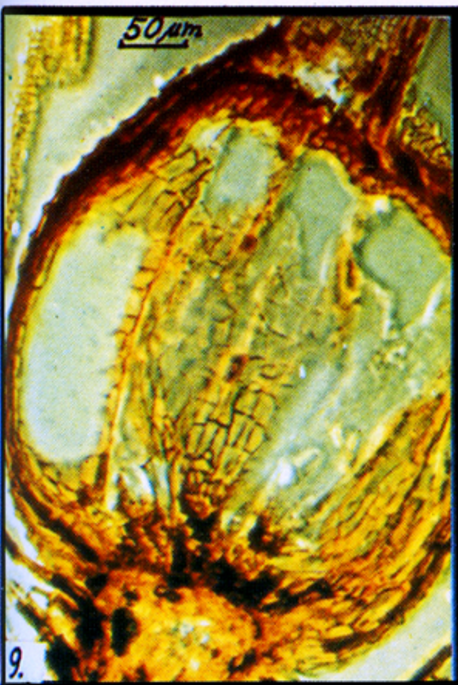

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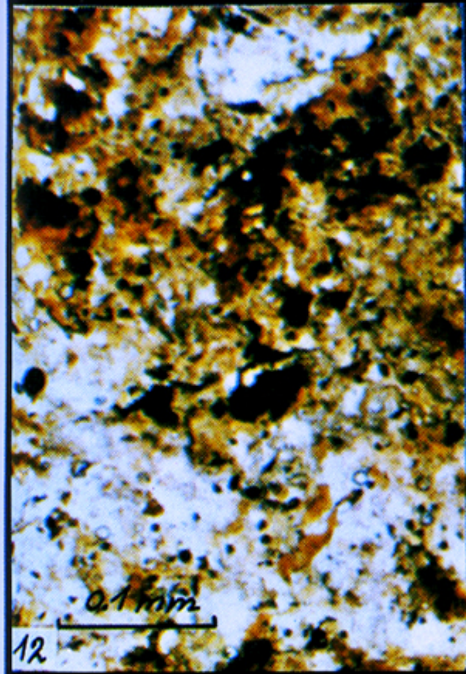

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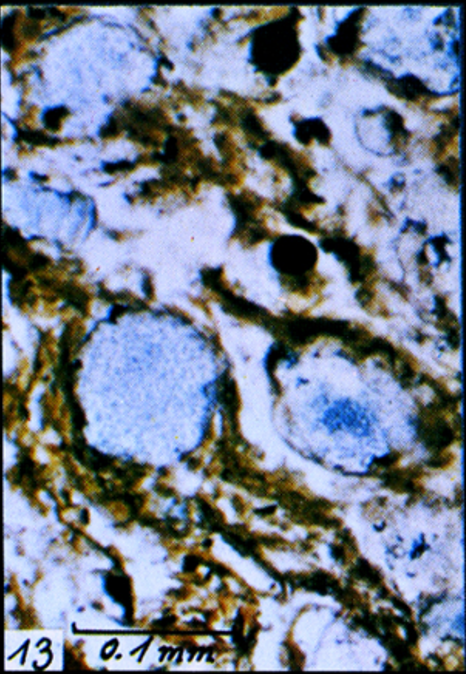

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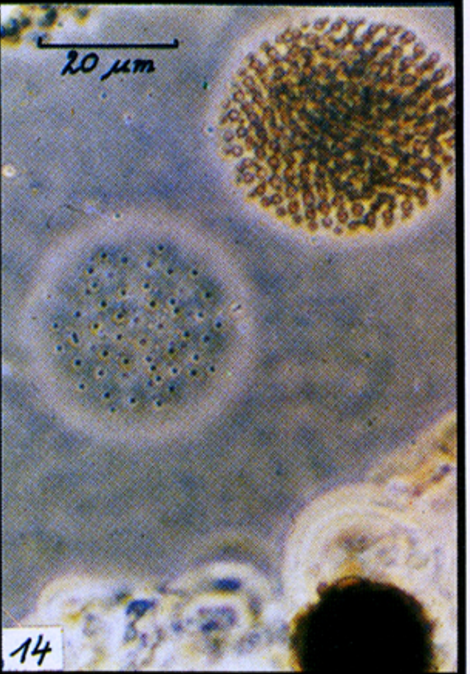

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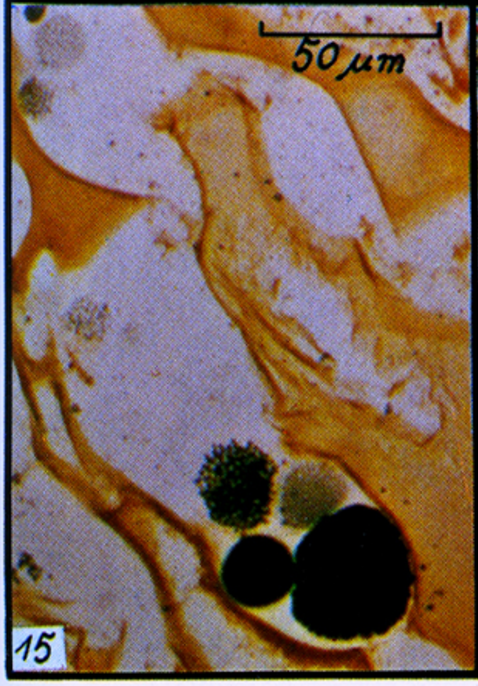

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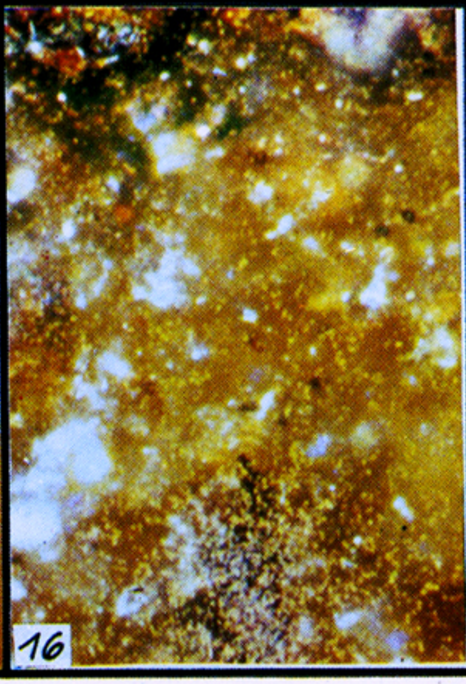

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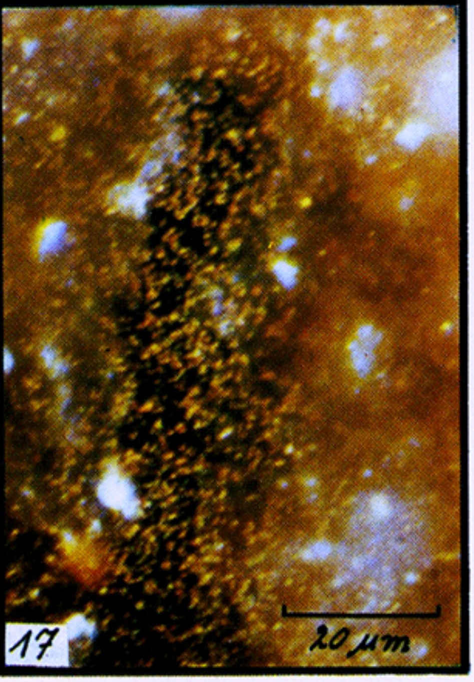

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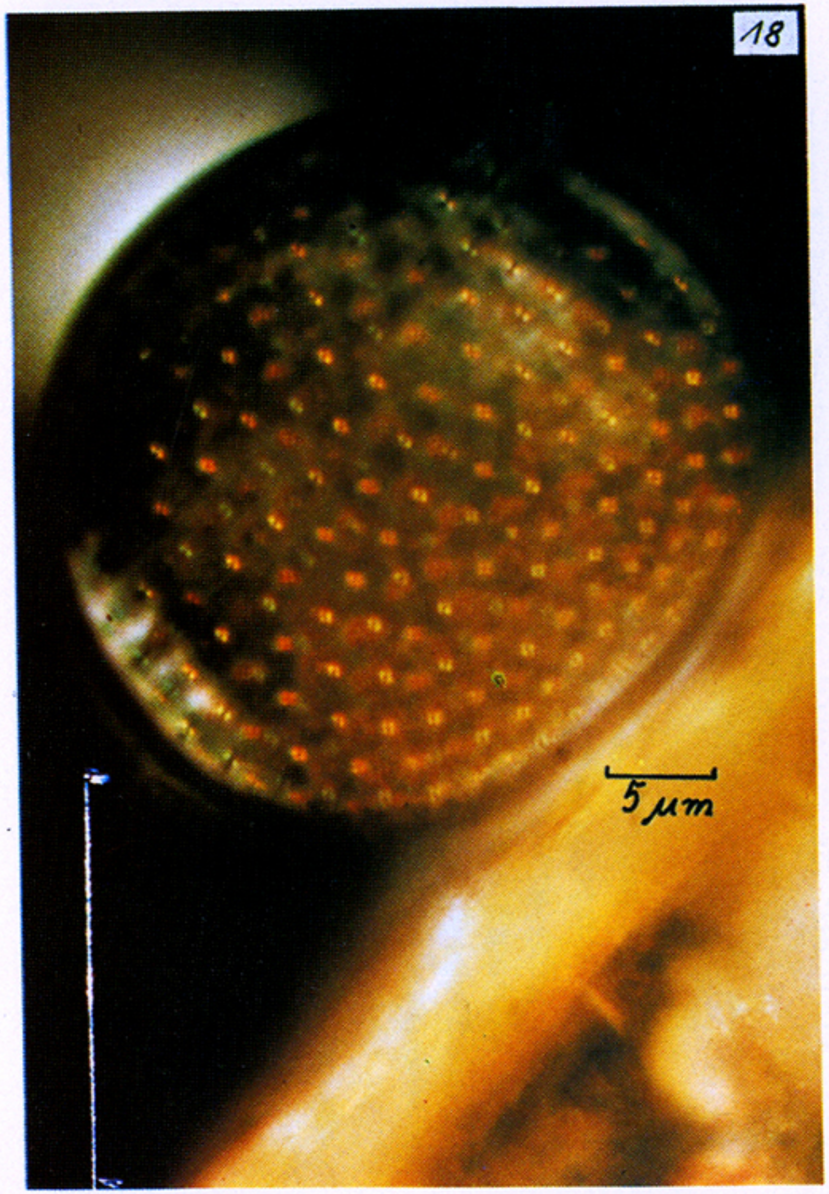
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ble. Most attention is called to the different forms of framboids. After freeze-drying, even colloid globes with a very small content of sulfide or sulphur are conserved.

3. The distribution of ^{14}C-labelled PCB in a soil column is studied by autoradiographic technics. During desiccation, dislocation of the soil solution is prevented by means of freeze-drying. Strong concentration of PCB in organic remnants suggests that rot products of microscopic size may be more important than diffused colloids, at least in the used Hapludalf soil.

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