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Electron microscopic characterisation of iron and manganese oxide/hydroxide precipitates from agricultural field drains. 1

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Abstract. Scanning and transmission electron microscopic examination of drain precipitates revealed the presence of a slime/organic layer and fungi, bacteria (including filamentous and Fe bacteria), and possibly actinomycetes. Most of the filamentous structures were encrusted with Fe and Mn compounds. Treating the samples with acidified $NH_2OH.HCl$ and leucoberbelin blue revealed some structures similar to *Hyphomicrobium* and *Pedomicrobium* spp., yeast cells, cocci, fungal spores, and relics of diatoms and amoebae. Both, scanning and transmission electron microscope-energy-dispersive analysis of X-rays showed a clear association of microbial structures with Fe and Mn oxides. It was suggested that Fe and Mn were being precipitated in the drains. However, the precipitates were not stable under natural conditions, and therefore we concluded that these precipitated oxides were also undergoing reductive dissolution. It thus appeared that precipitation of Fe and Mn, particularly Mn, had been mediated microbiologically in the drains.

Key words: Fe and Mn precipitates – Field drains – Scanning and transmission electron microscopy – EDAX analysis – Fe and Mn oxidising/precipitating microorganisms

Fe and Mn precipitation in agricultural drains has been observed in various parts of the World (Glathe and Ottow 1972; Streutker 1977; Grass et al. 1973a, b; Ford 1975; Ford and Tucker 1975; Wheatley 1988) and these compounds were mostly Fe oxides (Spencer et al. 1963). Precipitation of Fe and Mn compounds is considered to be a consequence of diverse chemical and microbiological processes (Meek et al. 1973, 1978). Oxidation and precipitation of Fe is mainly controlled by chemical processes, while that of Mn is considered to be controlled by biological processes. A large number of Mn-oxidising microorganisms have been shown to be associated with agricultural drain-line precipitates (Meek et al. 1973; Sojak and Ivarson 1977; Akhtar 1986). The objective of the present study was to obtain evidence that implicated microorganisms in the oxidation and precipitation of Fe and Mn, based on scanning and transmission electron microscopic characterisation and microchemical analysis by energy-dispersive analysis of X-rays of agricultural drain-line precipitates.

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Materials and methods

Study site and sample collection

Drain precipitate samples were collected from a farm, Talybont Ucha, at Bangor, Gwynedd, North Wales, UK (grid reference SH:605705). The farmland was under grass for sheep grazing. The main soil on the farm was the Denbigh series, a Brown Earth with a low base status, and a small area of the farm had soil of the Sannan series, a Brown Earth with gleying (Ball 1963). Clay-ware tile drains had been installed almost 150 years ago, and in some parts new tiles had been installed only 3 years previously. Precipitate samples of both the 150- and 3-year-old drains were obtained by carefully removing small portions of the drainpipes along with their coatings. These drain-tile fragments were rinsed gently in tap water.

Electron microscopy

The precipitate samples were dehydrated in graded acetone and distilled water solution and were critical-point dried using liquid CO_2 with or without glutaraldehyde fixation. Some of the samples were treated with $0.2\,M$ $NH_2OH \cdot HCl$ in 25% acetic acid or 0.4% (w:v) *N,N*-dimethyl-amino-*p,p'*-triphenylmethane-*O'*-sulfonic acid (leucoberbelin blue) in 2.5% acetic acid for 10 h, to reduce Fe and Mn oxides before proceeding to acetone dehydration and critical-point drying. The samples were either sputter-coated with gold, using a Polaron E5000 scanning electron microscope coating unit or C-coated in a vacuum evaporator and examined with a Hitachi S-520 scanning electron microscope. To determine the elemental composition of samples, a Cambridge stereoscan electron microscope (Mark 2A) equipped with energy-dispersive analysis of X-rays was used.

For transmission electron microscopy, the precipitate was removed from the 150-year-old drain tile, dehydrated, and critical-point dried as previously described. Ribbons of the precipitate samples, cut longitudinally with a stainless steel blade, were embedded in three resins: (1) Spurr, (2) Araldite, and (3) Tabb. The embedded specimens were cut into ultrathin sections of 1 and 1.3 μm with a microtome (LKB V), using a diamond knife. The sections were collected on copper grids with collodion support film and were stained (Reynolds 1963) and viewed with an AE Corinth 275 transmission electron microscope. Microchemical analysis was carried out with a Philips scanning/transmission electron microscope, EM301, equipped with energy-dispersive analysis of X-rays on 1.3 μm thin, unstained sections mounted on copper grids with Formvar support film.

Results

Scanning electron microscopic examination of the precipitate surfaces from both the 150- and 3-year-old drains revealed the presence of an organic/slime layer covering the uppermost part of the precipitates (Fig. 1a, b). The precipitates contained Mn and Fe along with other mineral elements (Al, Si, P, S, Ca, and Ti), as observed with energy-dispersive analysis of X-rays (Fig. 1ia, ib). The organic/slime layer was more prominent on the older drain precipitate. When examined from the broken edges and crakes, the zone immediately below the top surface had a honeycomb-like morphology covered with fine strands (Fig. 1c). In contrast, the lowest zone, the zone adjacent

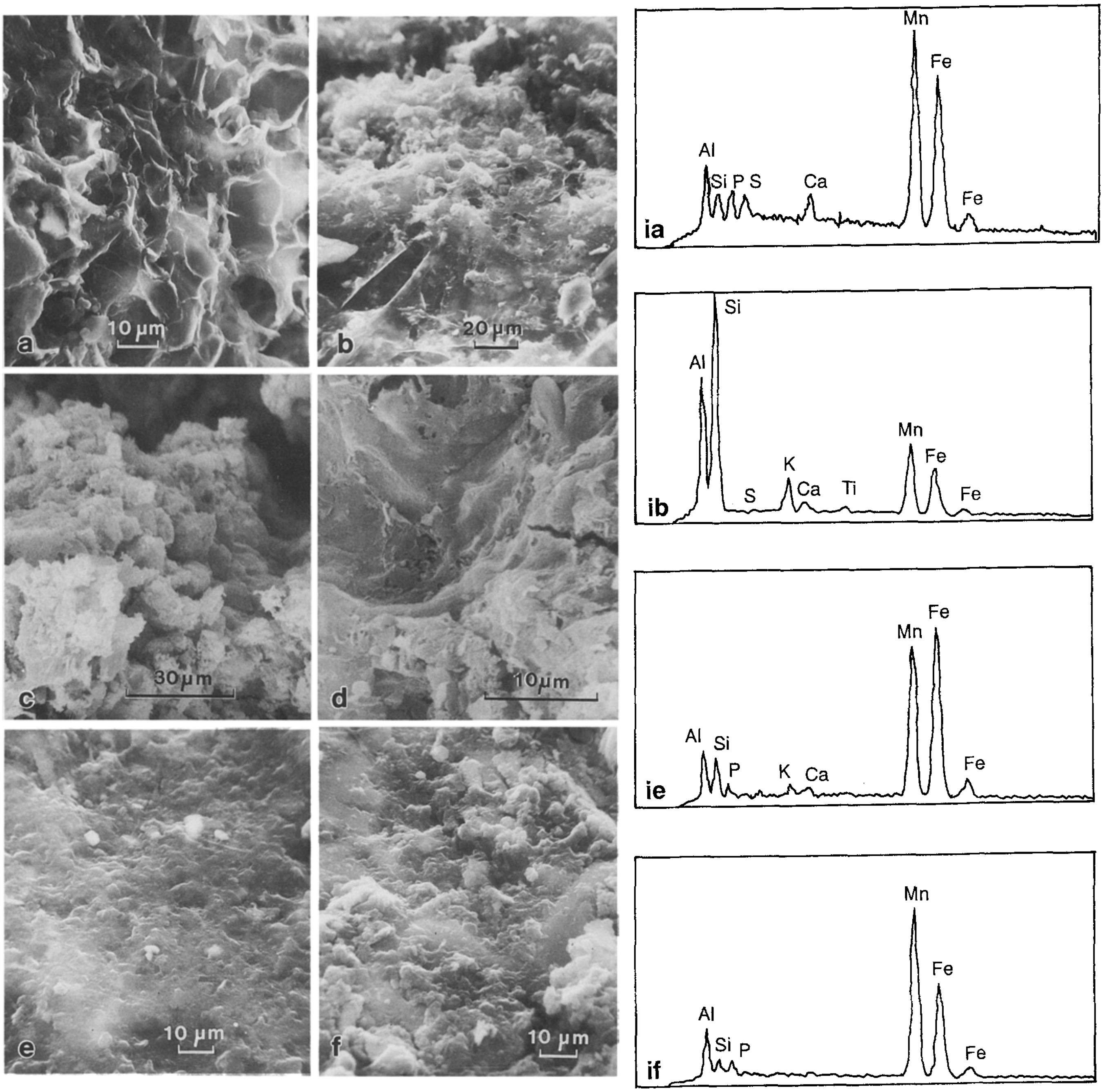


Fig. 1a–f, ia, ib, ie, if. Scanning electron micrographs showing: **a** a slime layer on a precipitate sample from a 150-year-old drain-line; **b** a slime layer on a precipitate sample from a 3-year-old drain-line; **c, d** top and bottom zones of a precipitate from the 150-year-old drain; **e, f** morphological features of Fe- (**e**) and Mn- (**f**)rich zone; **ia**, *ib*, **ie, if** energy-dispersive analysis of X-ray traces showing: **ia** chemical composition of the features presented in **a**; **ib** chemical composition of the features shown in **b**; **ie, if** chemical composition of the features shown in **e** and **f**, respectively

to the tile wall, was devoid of such fine structures (Fig. 1 d). A microchemical analysis of randomly selected points by energy-dispersive analysis of X-rays showed a variable and irregular distribution of Fe and Mn. Despite the variable composition, in general, higher Mn contents were found in the surface parts of the precipitate than in the deep zones near the tile wall. It was, however, difficult to morphologically distinguish either Mn- or Fe-rich zones (Fig. 1 e, f, ie, if).

On both types of precipitate, a range (Fig. 2 a) of biological entities and mineral particles were observed. Some fungal hyphae (Fig. 2 b) and filamentous structures resembling sheathed bacteria (*Sphaerotilus-Leptothrix* spp.) encrusted with mineral matter (Fig. 2 c) were observed. On treating the precipitate of the 150-year-old drain with $NH_2OH.HCl$ in HOAc solution and/or acidified leucoberbelin blue solution, some filamentous structures of sheathed bacteria or actinomycetes (Fig. 3 a), *Hyphomicrobium* and *Pedomicrobium* spp. (Fig. 3 b, c), were observed. The other biological entities present were cocci or yeast cells (Fig. 3 d), fungal spores (Fig. 3 e), remains of diatoms (Fig. 3 f), and amoebae (Fig. 3 g, h).

The matrix of the older desposit was a network of very fine fibrillar material as seen with the transmission electron microscope in stained ultrathin sections. The fibrillars were probably composed of polysaccharide strands enmeshing diversified materials (Fig. 4 a). A few thin, rod-shaped structures completely covered with electron-dense material (Fig. 4 b, c) and helical structures similar to the morphology of *Gallionella* spp. (Fig. 4 d) were observed. Examination by transmission electron microscope with energy-dispersive analysis of X-rays of the similar to those presented in Fig. 4 b and c showed that they were composed mainly of Fe with traces of Al and P (Fig. 5 a, ia). Two patterns of Fe and Mn distribution were observed, (1) a uniform distribution of Fe and Mn contents around microbial sections and in the matrix (Fig. 5 b, ib), and (2) higher Fe and Mn contents around

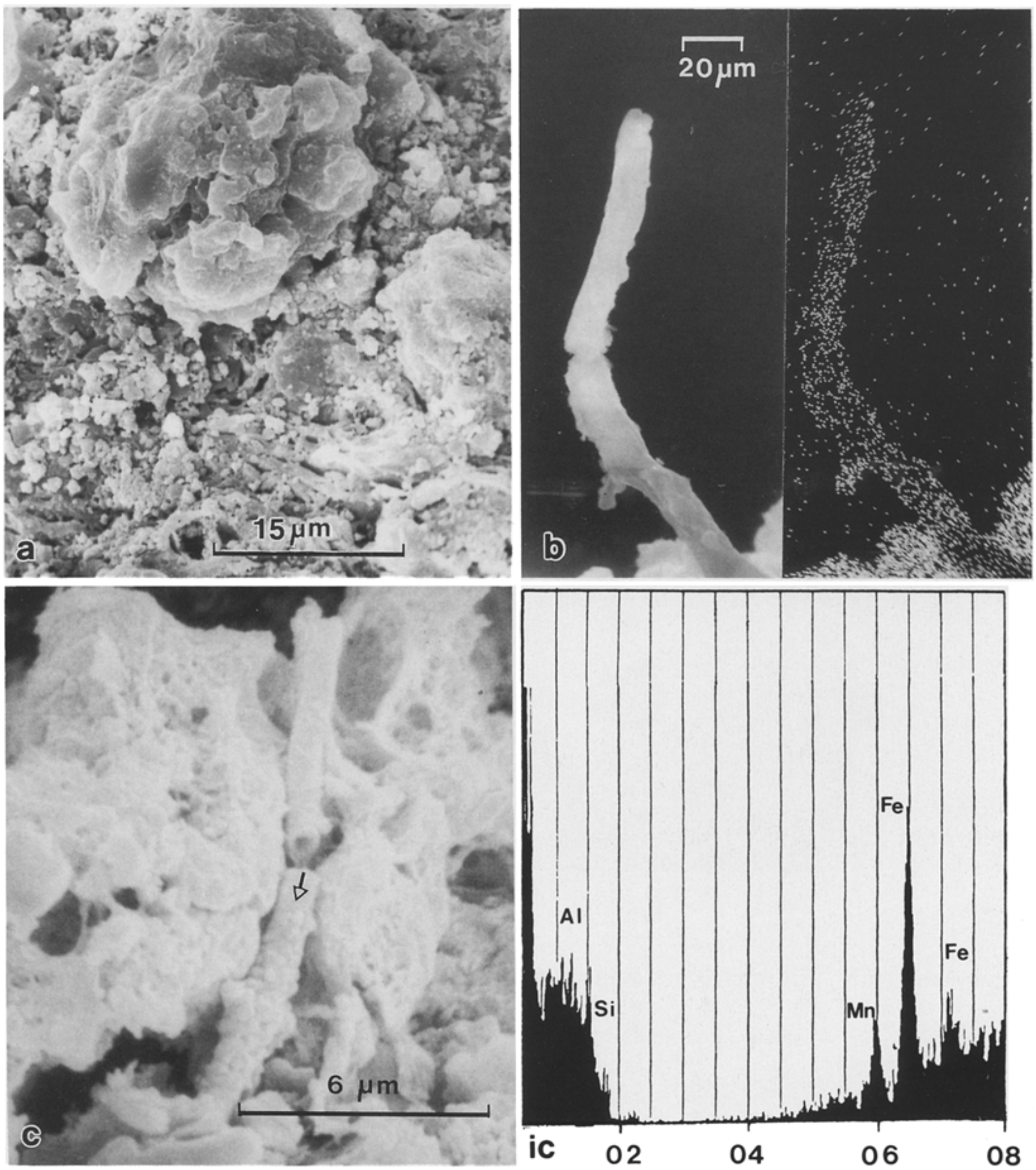


Fig. 2 a – c, ic. Scanning electron micrographs showing: **a** mineralogical and biological entities on a precipitate from a 150-year-old drainline; **b** a fungal hypha and its X-ray image of Mn distribution; **c** an encrusted filamentous structure resembling organisms of sheathed bacteria; **ic** chemical composition of the point marked in **c**

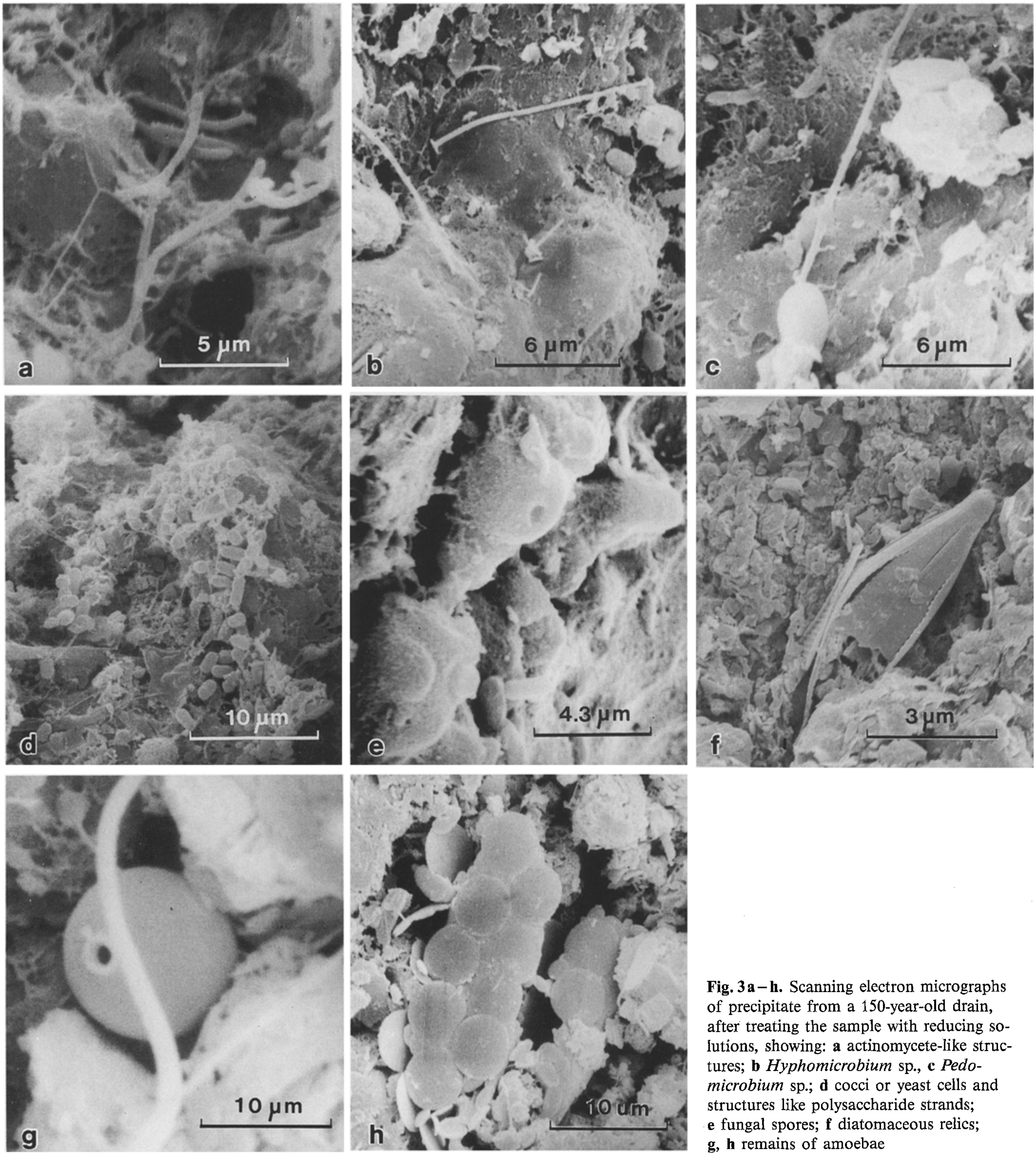


Fig. 3a–h. Scanning electron micrographs of precipitate from a 150-year-old drain, after treating the sample with reducing solutions, showing: **a** actinomycete-like structures; **b** *Hyphomicrobium* sp., **c** *Pedomicrobium* sp.; **d** cocci or yeast cells and structures like polysaccharide strands; **e** fungal spores; **f** diatomaceous relics; **g**, **h** remains of amoebae

microbial sections in comparison with the matrix (Fig. 5c, ic).

Discussion

The drain-line precipitates contained a range of microbiological and mineralogical components. Various processes contributed towards the formation and development of the heterogeneous precipitate. Silting of drains with fine sand and silt particles is common because these particles are easily eroded. Along with these mineral particles, biological entities may also have been washed into the drain. One bacterial and two fungal strains, capable of oxidising Mn, were isolated from the drain waters and deposit.

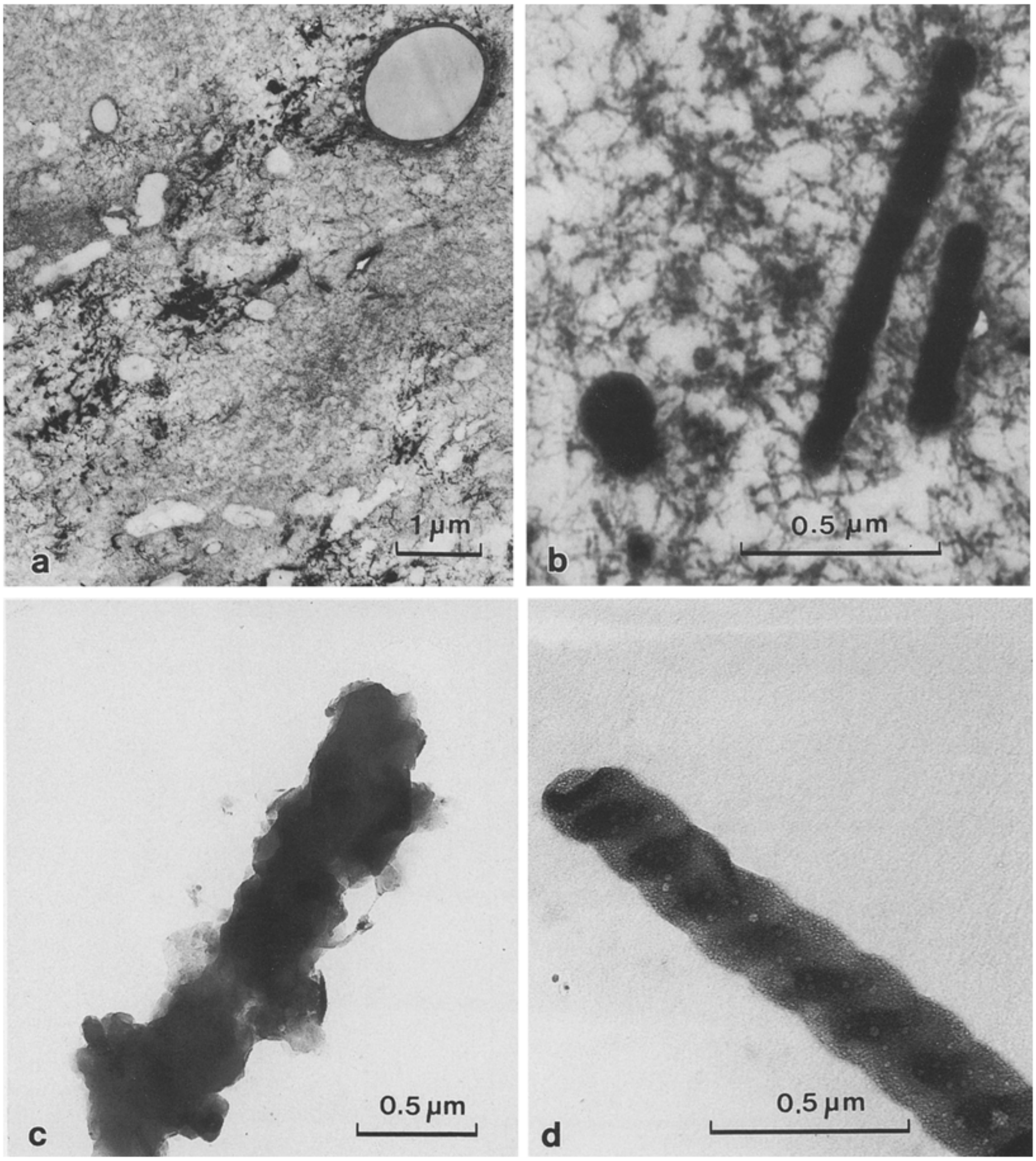


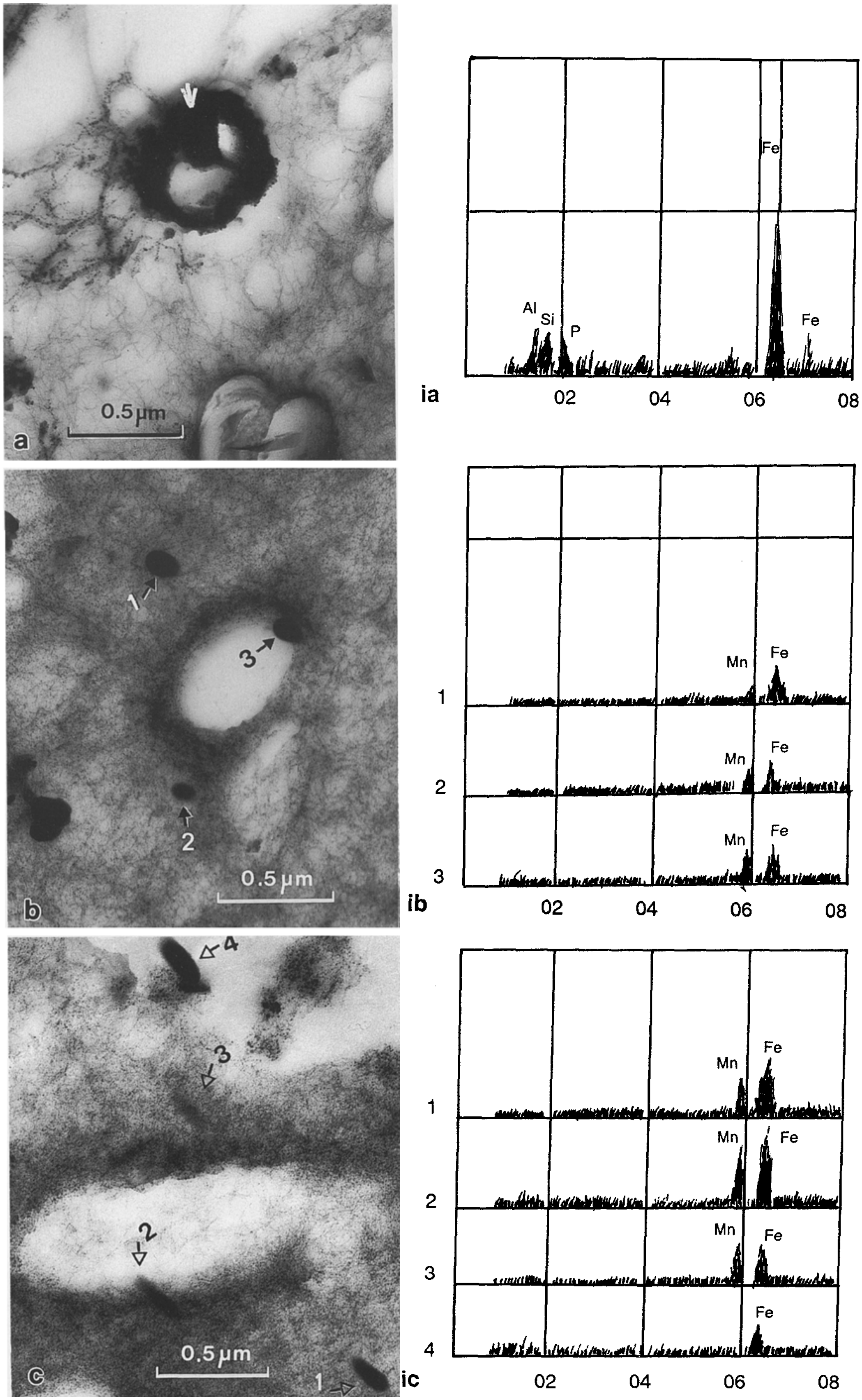
Fig. 4a–d. Transmission electron micrographs of a precipitate from a 150-year-old drain showing: **a** biological inclusions and strands of polysaccharide; **b, c** rod-shaped bacterial structures; **d** *Gallionella*-like structure

Also, a strong association between total heterotrophs and Mn-oxidising microorganism counts and rainfall was observed (Akhtar 1986), suggesting that a wide range of microorganisms had been washed in from the adjacent soils. Diverse Mn-oxidising microbial flora have previously been described in soil (Bromfield 1956; Ivarson and Heringa 1972; van Veen 1973), Mn concretions (Douka 1977), fresh water (Tyler and Marshall 1967; Gregory and Staley 1982; Gupta et al. 1987) sidements, ore and mineral samples (Greene and Madgwick 1989, 1991) and a river estuary (Vojak 1983).

The microbiological characterisation of precipitates showed a range of microorganisms, e.g., fungi, actinomycetes and/or filamentous bacteria, and relics of diatoms and amoebae. Some of the organisms may have established colonies at the interface of the drain-tile surface and flowing drainage water, as evident from the presence of an organic layer on the deposits and the attachment of bacterial microcolonies by slime on the surfaces of tile lines (Larock and Ehrlich 1975; Mack et al. 1975). It appeared that filamentous microorganisms were the most successful colonisers, as relatively large numbers of filaments were observed. Some other types of bacteria like *Hyphomicrobium* and *Pedomicrobium* spp. and cocci or yeast were also observed after treating the samples with reducing solutions to remove the masking Fe and Mn oxides.

The transmission electron microscopic examination revealed the presence of fibrous material and some structures similar to Fe-oxidising/precipitating bacteria such as *Leptothrix* and *Gallionella* spp. Whether these microorganisms proliferated in the drain or were washed in with soil material is yet to be ascertained. The occurrence of fibrous material in small amounts may imply that they were transported and were probably unable to develop colonies under these conditions.

The pattern of microorganism distribution suggested the presence of a zone with a relatively high level of bio-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logical activity on the surface of the precipitates. As the microbial cells assimilate organic and/or inorganic compounds from the drain water, these processes yield energy with which microbial cells reproduce, maintain internal structure, and form extracellular products. Thus, these processes are more likely to occur on the surface of the deposit. The thickness of an active zone is affected by concentrations of available substrates and by physicochemical conditions of the microenvironments (Hochn and Ray 1973; Characklis 1983). Obviously, when buried under thick deposits of minerals and bio-entities, the embedded microorganisms would suffer from a lack of nutrients (organic and/or inorganic) and of O_2, due to slow diffusion from the deposit surface. MacRae and Celo (1975) showed that washed suspensions of *Acinetobacter* sp., isolated from water supplies, caused colloidal ferric iron to precipitate at pH 6.0 and 7.6. The Fe-encrusted cells of the bacterium formed large aggregates, and endogenous respiration rates of the encrusted cells were 32–72% lower than that of unencrusted cells.

Fe and Mn oxides were preferentially precipitated around microbial structures in higher concentrations than in the matrix, as indicated by both scanning and transmission electron microscopy with energy-dispersive analysis of X-rays. Some microbiological structures were completely encrusted with Fe, some with both Fe and Mn compounds. However, the present energy-dispersive analysis of X-rays was unable to show whether the Fe and Mn were precipitated simultaneously, or whether the Mn oxide was deposited on top of a pre-existing Fe oxide. The irregular distribution of Fe and Mn in the matrix and crust also suggests that the interface was never stable under natural conditions. Most probably, the processes of oxidative precipitation and reductive dissolution occurred simultaneously with variable intensities and to varying extents at this interface. Mobilisation of Mn oxides was also observed with the addition of inocula obtained from the drain deposits (Akhtar 1986).

Mn, in particular, should not be considered stable, even after oxidative precipitation, as slight changes in redox conditions, i.e., low pH and Eh, can cause reductive dissolution (Hem 1964). In addition, Fe (II) is oxidised to Fe (III) at the expense of Mn (III)/Mn (IV) (Krauskopf 1979; Postma 1985). Mn oxides are also liable to dissolution, as reactions between these oxides and organic compounds can occur. For example, phenolic compounds are oxidatively polymerised to humic substances in the presence of Mn oxides, which are in turn reduced in these reactions (Shindo and Huang 1984; Lehmann et al. 1987).

Furthermore, Bromfield and David (1976) reported that *Arthrobacter* sp. reduced Mn oxides formed in earlier phases by the same bacterial culture simply on being transferred to a lower O_2 tension. The excretion of organic compounds by *Leptothrix cholodnii* able to reduce MnO_2 has also been reported (van Veen et al. 1978). MacRae and Edwards (1972), in a study of Fe precipitation in surface water supplies, showed that species of seven different bacterial genera became encrusted with Fe as they adsorbed Fe from solution. Under these circumstances, mixing of Fe and Mn and an uneven distribution would be expected at this dynamic interface. Seasonal variations in the weather would increase precipitate heterogeneity by changing the soil solution chemistry and altering the intensity of the washing-in processes.

In conclusion, it appears that microbial residue was the major component of the drain precipitates, and that precipitation of Fe and Mn, particularly Mn, was mediated microbiologically in these agricultural field drains.

Fig. 5a–c, ia–ic. Transmission electron micrographs and energy-dispersive analysis of X-ray traces of a precipitate from a 150-year-old drain showing: **a** a cross-section of a hollow rod-like structure and chemical composition (**ia**) of the marked point; **b** a cross-section of a microbial structure and chemical composition (**ib**) of the marked points, showing an even distribution of Fe and Mn; **c** a cross-section of a microbial structure and chemical composition of the marked points (**ic**), showing higher contents of Fe and Mn around the cross-section

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