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 NH2OH.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.

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 gleys (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 CO2 with or without glutaraldehyde fixation. Some of the samples were treated with 0.2 M NH2OH.HCl in 25% acetic acid or 0.4% (w:v) N,N-dimethylamino-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 colo-
dion 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 cracks, 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

![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.](image)