Problem of fungal contamination in Precambrian palaeobiology : a cautionary note-I

C. Manoharachary, Manoj Shukla & Mukund Sharma

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The paper deals with the problem of fungal contamination in palaeopalynological preparations specially from the Precambrian sediments. The vegetative and reproductive structures of extant fungal groups show a broad similarity in morphology with the morphotypes described from the Precambrian. The recent fungi when subjected to chemical treatment similar to palynological preparations (maceration) do not show major physical and morphological changes. Nine common fungal genera were selected for this study. It has been observed that they withstand hydrochloric acid and hydrofluoric acid treatment without losing much of their morphocharacters. As these are common in soil profiles, one can easily be mislead when they occur amongst macerated residues. This data serves as a cautionary note to all palaeobiologists and specially dealing with Precambrian material, where every new evidence is important in adding to the meagre knowledge.

Key-words—Fungal contamination, Microfossils, Precambrian.

C. Manoharachari, Department of Botany, Osmania University, Hyderabad 500 007, India.
Manoj Shukla & Mukund Sharma, Birbal Sahni Institute of Palaeobotany, 53 University Road, Lucknow 226 007, India.

सारांश

कैम्ब्रिय-पूर्व पुराजैविक अध्ययन में कवकीय संदूषण की समस्याः सतर्कता हेतु उपलेख

सी० मनोहराचारी, मनोज शुक्ला एवं मुकुन्द शर्मा

प्रस्तुत शोध-पत्र में पुरापरागाणविक निर्मितियों में, विशेषतया कैम्ब्रिय-पूर्व अवसादों से, कवकीय संदूषण की समस्या पर विवेचना की गई है। वर्तमान कवकीय समूहों की शाकीय एवं जननांगी संरचनायें कैम्ब्रिय-पूर्व से वर्णित प्ररूपों से मोटे तौर पर आकारिकीय समानता प्रदर्शित करती हैं। वर्तमान कवकों पर जब परागाणविक मसृणन की तरह रासायनिक प्रक्रियायें की जाती हैं तो इनमें कोई विशेष आकारिकीय एवं भौतिक परिवर्तन व्यक्त नहीं होता। उक्त अध्ययन हेतु नौ सामान्य प्रजातियों को छाँटा गया है और यह प्रेक्षित किया गया है कि इनके आकारिकीय लक्षणों पर हाइड्रोक्लोरिक अम्ल एवं हाइड्रोफ्लोरिक अम्ल का कोई विशेष प्रभाव नहीं पड़ता। चूंकि ये मिट्टी की सतह में सामान्य रूप से मिलते हैं, अतः मसृणित-अवशेषों में विद्यमान होने के कारण कोई भी सरलता से भूल कर सकता है। प्रस्तुत शोध-पत्र सभी पुराजीव विज्ञानीयों जो कैम्ब्रिय-पूर्व नमूनों के अध्ययन में कार्यरत हैं, के लिए एक सतर्कतापूर्ण उपलेख है जहाँ कि प्रत्येक नया प्रमाण संकुचित ज्ञान में योगदान हेतु अत्यन्त महत्वपूर्ण है।

BIOGENIC activity during Precambrian is evidenced by the occurrence of forms with simple morphologies. Bacteria and cyanophytes, viz., coccoids (spheroidal-ellipsoidal), septate, unbranched filaments, tubular, unbranched microstructures, branched filaments and bizarre

PLATE 1 →

Bar in fig. 3B represents 50 μm for each photograph except 1B.

1A. *Aspergillus niger* van Tiegham: Conidiophores, vesicles and conidia. 1B. *Aspergillus niger* after treatment.

2A. *Alternaria alternata* Keissler, Hyphae, conidiophores and conidia; Fig. 1B, *A. alternata* after treatment showing hyphal fragment and conidia.

3A. *Cladosporium cladosporioides* de Veries. Hyphae and conidia: 3B, *C. cladosporioides* after treatment showing hyphal fragment and conidia.

4A. *Chaetomium aureum* Chievers. Ascospores and hairs; 4B & C, *C. aureum* showing hairs and ascospores respectively after treatment.

5A. *Curvularia lunata* (Wakker) Boedijin. Hyphae, conidiophore and conidia; 5B, *C. lunata* conidia and hyphae after treatment.

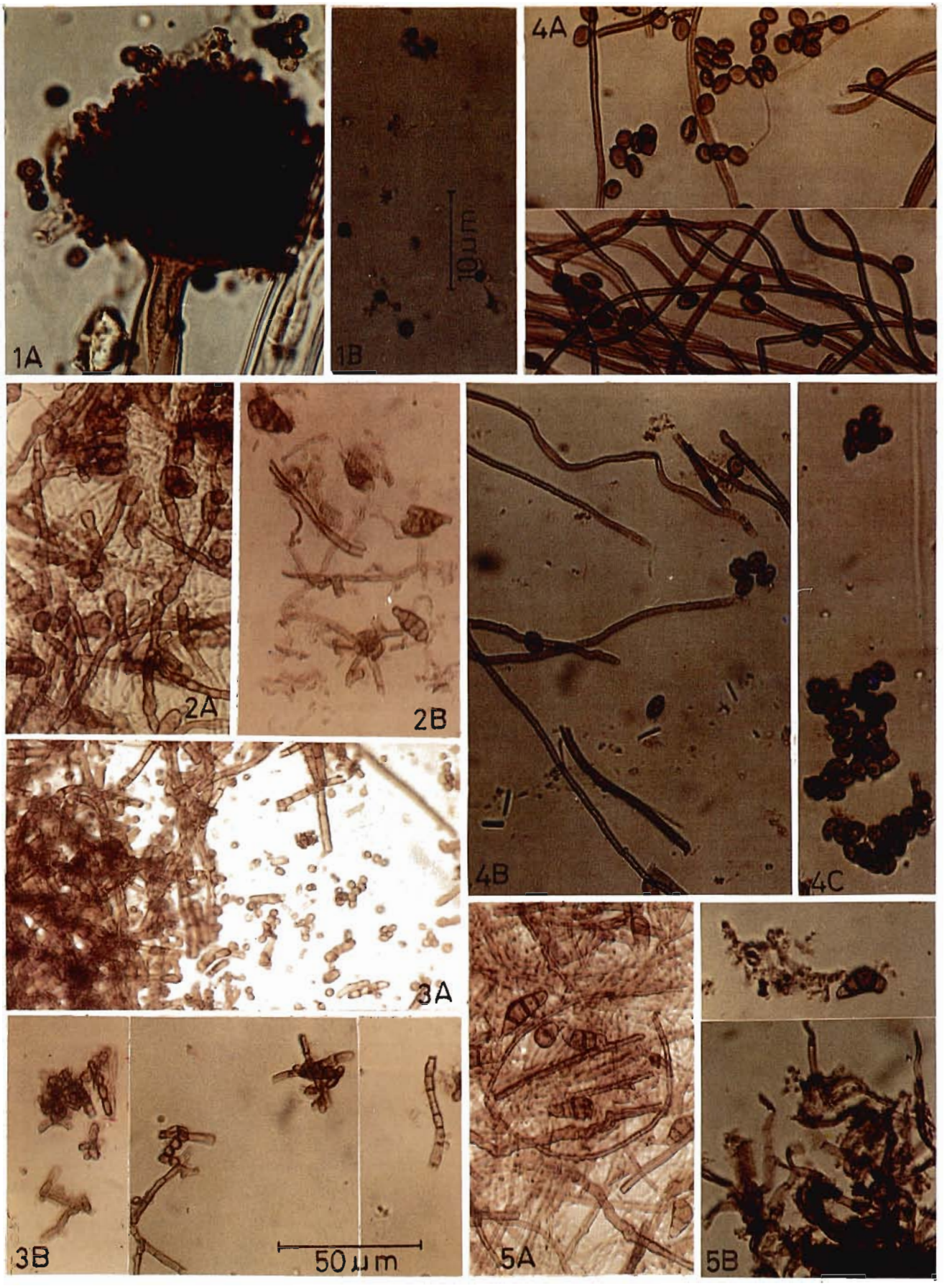

PLATE 1

forms, etc. are recorded (Hofmann & Schopf, 1983).

These fossil remains are meagre and often highly degraded. Due to this fact any new evidence is very important as it has a great bearing on the understanding of evolution of life and various other associated processes. Every new evidence of Precambrian microbiota requires close scrutiny.

Several of the 'fossils'—morphotypes described from the Precambrian sediments also compare morphologically with extant fungi. Instances of extant micro-organisms, artifacts and pseudofossils described as Precambrian microbiota have been put forward by Schopf (1975), Cloud (1976), Cloud and Morrison (1979), Schopf and Walter (1983), Fuxing and Qiling (1982), Horodyski (1981) and Karkhanis (1977). The frequency of misidentification depends upon the common soil organisms available in the sampled area and processing techniques. After the initiation of a multidisciplinary project on Indian Archaean palaeobiology a study on degradational aspect of extant micro-organisms was initiated with an idea to prepare a checklist of common modern contaminants. In this paper our results on the study of some common fungi are presented.

Fungi are known from a wide variety of habitats (Webster, 1980). They form a part of the air habitat (Gregory, 1971) and have also been reported up to the depth of 30 cm (Galiah, 1985; Manoharachary, 1986), in rock crevices and from surface samples such as laterite, sandstone, granite and alluvial soils (Manoharachary, 1986; Jarzen & Elsik, 1986). The vegetative hyphae and reproductive structures of these fungi which are often brown-black in colour have been confused with thermally altered fossil algal filaments and unicells. Such mistakes are more likely to occur when one is using maceration method (acid digestion of rock for concentration of organic matter) for fossil recovery. Since in the maceration method the organic remains are released from the rock matrix, it is not possible to make out the relationship of the detached organic matter whether, it is synsedimentary and syngenetic with the rock treated.

MATERIAL AND METHOD

Nine common fungi which commonly occur as soil biota, viz., *Aspergillus niger* van Tiegham, *Alternaria alternata* (Fr.) Keissler, *Cladosporium cladosporioides* (Fres) de Vries, *Chaetomium aureum* Chivers, *Curvularia lunata* (Wakker) Boedijin, *Drechselera rostrata* (Drechseler) Richardson & Fraser, *Phoma nebulosa* (Pers. ex S.F. Gray) Berk, *Rhizopus nigrecans* Ehrenberg and *Trichoderma viride* (Pers. E.M. Fries), were subjected to chemical degradation through inorganic acids, viz., HCl and HF which are commonly used for maceration of rocks. These fungi were taken from well-identified monosporic stock cultures available in the laboratory and grown on PDA medium (potato dextrose agar) under laboratory conditions. The fungi were treated initially with hydrochloric acid (40%) for two days and then after washing with distilled water were subsequently treated with hydrofluoric acid (commercial-40%) for 10 days. The residual fungal material was finally washed with distilled water and mounted in lectophenol. This chemical treatment is the same as used for maceration of rocks. Another set of slides was also prepared from untreated material of the same culture for reference and comparison.

All the photographs presented in Plate 1 and 2 have been taken on Leitz Orthoplan microscope in normal light using only natural density filter to enable the maximum exposures of natural colours of fungi without acid treatment and after treatment on Kodacolor-Kodak Gold film with the help of Vario orthomat 2 photographic attachment.

OBSERVATIONS

Following characters were observed in control and treated material.

Aspergillus niger van Tieghem
Pl. 1, fig. 1A, B

Description—Hyphae pale, smooth

PLATE 2 →

(Bar in Fig. 1C represents 50 μm for each photograph)

1A, 1B. *Drechslera rostrata* (Drechsler) Richardson & Fraser. Hyphae, conidiophores and conidia; 1C, *D. rostrata* hyphae, conidiophores and conidia after treatment.

2A, 2B. *Phoma nebulosa* (Pera ex SF Gray) Berk. Pycnidia pseudoparenchymatous wall and pycnidia; 2C, *P. nebulosa* showing pseudoparenchymatous pycnidial wall after treatment.

3A. *Trichoderma viride* Pers ex. Fries. Hyphae and conidial mass; 3B, *T. viride* showing conidial mass after treatment.

4A, 4D. *Rhizopus nigricans* Ehrenberg. Columella, spores and hyphae; 4B & 4C, showing hyphae, columella and spore mass after treatment.

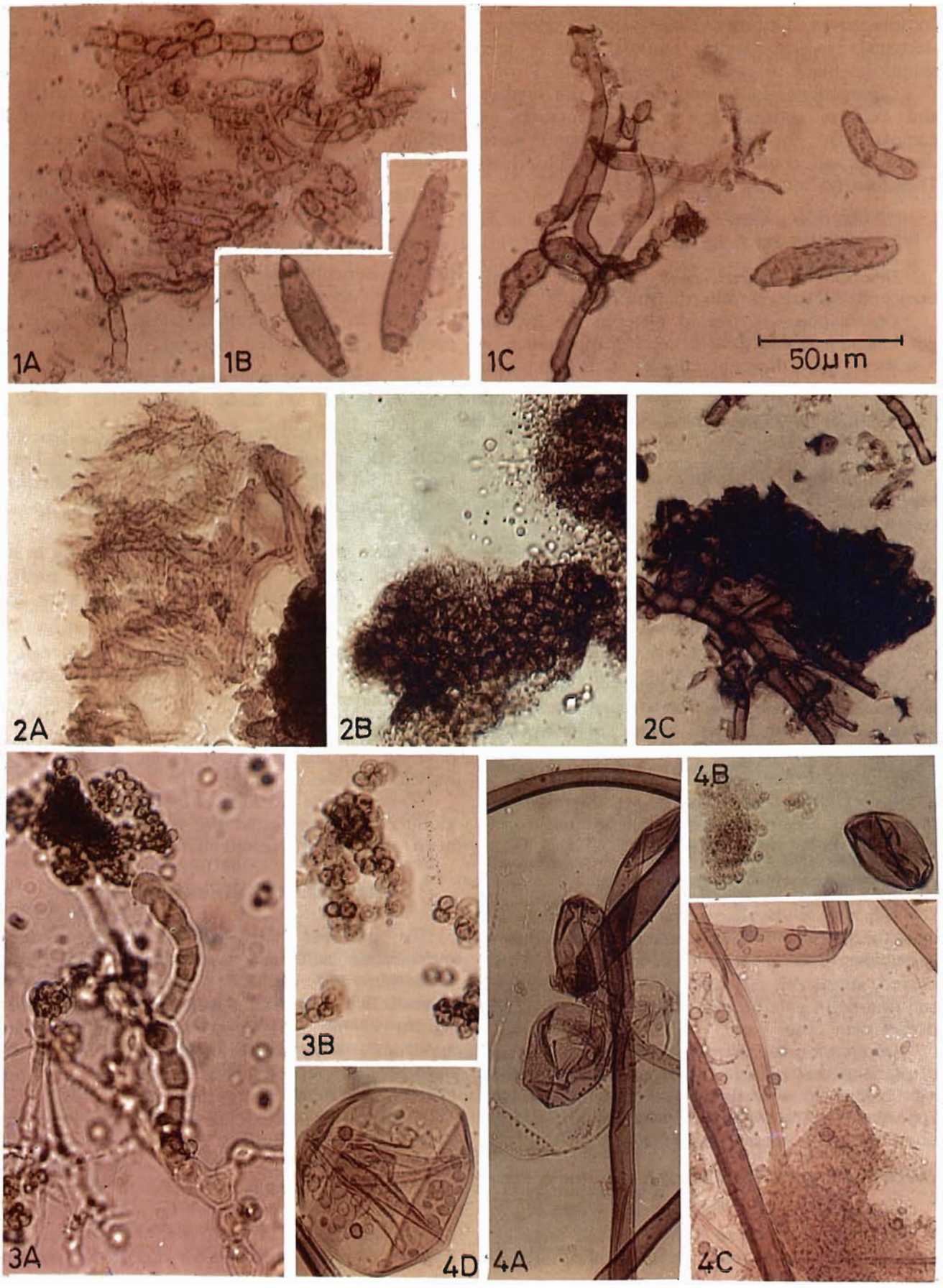

PLATE 2

conidiophores 1-2 mm long, vesicles globose, sterigmata in two series, conidia globose and spinulose, black in colour, spherical, 6.2-7.5 μm.

Observations—In treated material the hyphae and conidia showed loss of pigmentation as compared to the control. Conidiophores were dissolved and conidia became almost colourless. Therefore no remains were visible.

Alternaria alternata (Fr.) Keissler
Pl. 1, fig. 2A, B

Description—Hyphae pale brown to brown, branched, septate, conidia dictyosporous.

Observations—In treated material the hyphae got fragmented and gave deceptive appearance of fossilized algal filament, black or brown, with longitudinal transverse septa.

Cladosporium cladosporioides (Fres) de Vries
Pl. 1, fig. 3A, B

Description—Hyphae brown, septate, branched hyphal cells thick-walled; conidia unicellular or two-celled, found in chain, brown in colour.

Observations—Partial loss of pigmentation and reduction in the thickening of the septa were observed in treated material.

Chaetomium aureum Chievers
Pl. 1, fig. 4A, B, C

Description—Perithecia dark, olive brown with an ostiole and appendages or hairs, as a 8-spored, ascospores olive brown, ovate or elliptical, 12.7 × 5.9-6.8 μm in size.

Observations—Reduction in the size of ascospores and thickening of wall layers were observed in the treated material.

Curvularia lunata (Wakker) Boedijin
Pl. 1, fig. 5A, B

Description—Hyphae septate, brown, branched, conidiophores unbranched, erect, septate, brown, geniculate. Conidia with three transverse septae, curved, apical cell rounded, pale brown, basal cell sub-hyaline to pale brown, middle cell broader and darker than other parts.

Observations—The treated material showed loss of pigmentation both in hyphae and conidia which was higher in this fungus along with wrinkled appearance in the hypha wall, which was apparently due to loss of internal contents.

Drechslera rostrata (Drechsler) Richardson & Fraser
Pl. 2, fig. 1A, B, C

Description—Hyphae brown, septate, branched conidiophores flexuous, dark brown, septate, conidia clavate, elliptical, rostrate, 5-18 μm. Pseudoseptate, septa dark, end cells hyaline, middle cells brown.

Observations—Partial loss of pigmentation, internal contents and rigidity in the wall layers of hyphae as well as in conidia were observed in treated material.

Phoma nebulosa (Pers. ex SF. Gray) Berk
Pl. 2, fig. 2A, B, C

Description—Pycnidia brown, w. pseudoparenchymatous and brown, conidia hyaline.

Observations—Partial loss of pigmentation was observed in pseudoparenchymatous wall layers of pycnidia of the treated material.

Rhizopus nigrecans Ehrenberg
Pl. 2, fig. 4A-D

Description—Hyphae branched, brown, coenocytic, sporangiophores in clusters, up to 2 mm long, 10.5-17 μm wide, sporangia almost spherical, 85-195 μm in diameter, blackish brown, spores rounded, 5-6 × 3-4.6 μm in size.

Observations—Loss of contents resulting in an irregular shaped columella and also partial reduction of pigmentation in general were the effects in treated material.

Trichoderma viride Pers. ex. Fries
Pl. 2, fig. 3A, B

Description—Hyphae pale or light green, branched, septate, conidiophores not distinct, phialidic, conidia in groups, green, smooth, thick-walled, globose or ovoid.

Observations—Reduction in the compaction of globular cell masses and also in pigmentation have been observed in treated material.

DISCUSSION AND CONCLUSIONS

In general, the material treated with hydrofluoric acid and hydrochloric acid shows reduction and loss in pigmentation both in reproductive structures as well as in hyphae. Loss of rigidity in the wall layers resulting in wrinkled appearance of hyphae is also common. Size of the hyphae and reproductive structures were not affected in general. Compaction of the cells and the parenchyma formed by the hyphae were affected. The fragmented hyphae which lost septa or coenocytic (Pl. 1, figs 4B, 5B) do show comparison with the algal sheaths described in Precambrian literature and may be mistaken for *Eomycetopsis* and *Animikiea*. The septate hyphae which get fragmented can be mistaken for algal trichomes. The

easiest method to differentiate them from true fossil algal trichomes is to watch for bulbous or knuckel-shaped structure conspicuous in fungal hyphae. The globular conidial masses as found in *Trichoderma viride* (Pl. 2, fig. 3A, B) can be mistaken for *Myxococcoides* and *Aphanocapsa*-like taxa described from Precambrian sediments. The fragmented conidial spores (Pl. 1, figs 4C, 3B) compare closely with unicellular taxa such as *Eontophysalis*, *Huronispora*, etc. The sporangia with broken columella (Pl. 2, fig. 4C) can mislead Precambrian palaeobiologists into identifying them as *Kildenospharea* and various other acritarch taxa that are commonly recorded amidst shale biotas. The chances of contamination are more in shale biotas.

Doubts were expressed by Schopf (1970, 1975), Cloud (1976), Venkatachala (1986, 1987) and Schopf and Walter (1983) earlier about the possibility of fungal contaminants being introduced during material preparation and described as Precambrian micro-organisms. The extant fungal remains which are normally available in soil rock crevices and in humid areas such as river banks and outcrops in the valley section, mines and weathered-outcrops, where post depositional concentration of ores have taken place, can withstand treatment by inorganic acids. They take morphological shapes which are broadly comparable with the morphotypes generally described from the Precambrian. The present work establishes beyond doubt that fungal contamination can pose a serious problem. The Precambrian biologists should take note of this major source of contamination and acquaint themselves with the extant algal and fungal flora. The problem of contamination is not only with the macerations but also with the preparation of stubs for SEM studies particularly when the replica method is used. The process provides more exposures to the atmosphere which can introduce the contaminants (Oberlis & Prashnowsky, 1968; Schopf, 1975, p. 235; Cloud, 1976, p. 357; Cloud & Morrison, 1979, p. 89; Schopf *et al.*, 1965; Hofmann & Schopf, 1983, p. 329). To quote "It is either due to over enthusiasm or due to the thrill associated with such findings, such reports are increasing and the net gain in the advancement of Archaean and Precambrian palaeobiology remains static. The mistakes of earlier workers was due to lack of available literature and experience but it is not justifiable to commit such mistake in this age of advanced knowledge and instrumentation. If only we could interact with botanists engaged in the study of extant algae and fungi such erroneous identification can be avoided" (Venkatachala, 1987).

In view of above it is recommended that the results are checked for the possibility of extant taxa when we work on surface samples using the maceration method. Some of the precautions needed are: Preparation of a checklist of fungal taxa present in the fossil localities and their aerospora, the checking of fungal growth and avoidance of growth promoting conditions during curating.

These safety measures can minimise misleading reports. Some of the biotic remains recovered from maceration are closely comparable to the fungal hyphae and spores which withstand acid treatment In this situation, this paper we hope, will help avoid unintentional reporting of extant contaminations as Precambrian microbiota.

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