**CONTAMINATION OF NAKHLA BY TERRESTRIAL MICROORGANISMS.** J.K.W Toporski[1], A. Steele[2], D. Stapleton[3] and D.T. Goddard[4], [1] ***School of Earth, Environmental and Physical Sciences, University of Portsmouth, Burnaby Building, Portsmouth PO1 3QL, UK***, jan.toporski@port.ac.uk; [2] ***Mail code SN, NASA, Lyndon. B. Johnson Space Center, Houston, Texas, 77058, USA;*** [3]***BNFL, Springfields Site, Preston, UK;*** [4] ***Fujitsu Europe Ltd, Uxbridge, UK.***

**Introduction** Ever since meteorites have been known to come from an extraterrestrial origin people have speculated as to whether they could contain evidence of life. This was again exemplified by the claims of McKay et al. [1]. In each case the scientific community as a whole has regarded such claims of life with a certain amount of incredulity and upon the furnishment of evidence of terrestrial contamination, has dismissed the claims with alacrity. However, the actual sources of contamination within meteorites and the mechanisms of entry of such contamination still remain poorly understood. With the discovery of a probable terrestrial bacterial contaminant on ALH84001, the argument of contamination has taken another turn [2]. The findings of several researchers into the organic material within this meteorite concluded that contamination was present but then presented unfeasible arguments as to its origin from Antarctic ice [3, 4]. All the groups failed to detect an organism living on this meteorite. Not a good omen for future exploration of our solar system. With this in mind when the allocation of Nakhla became available it seemed sensible to screen this meteorite for contamination by terrestrial microorganisms.

**Materials and Methods** With the aim to detect possible terrestrial microbial contaminants, Scanning Electron Microscopy (SEM) investigations were carried out on samples from Nakhla. Our allocation consisted of chips taken from four sites across the meteorite, none of which was adjecent to macroscopic fractures. These samples were numbered I to IV. Sample I contained fusion crust, sample II was taken from just underneath the fusion crust, sample III originated from the interior of the meteorite, sample IV was from near the center of the meteorite. No visible fractures were observed in the meteorite.

Small chips were removed from each sample in sterile conditions under laminar flow. All instruments were presterilized by flaming or autoclaving. Chips were then visually characterised and the majority of each sample area allocation retained for culturing and organic surface analysis techniques. The small fractions of each sample chosen for SEM investigation were mounted onto aluminum stubs using carbon tape. To increase sample conductivity silver dag was sparingly used to connect the base of the samples with the stub. The samples were Au/Pd-coated for 45 seconds after allowing 30 hours drying time for the silver dag under contamination free conditions. All samples were imaged with a JEOL SM 6100 fitted with a light element Energy Dispersive X-Ray Analyser (EDX).

**Results** The results presented in this abstract are from extensive imaging studies of chips from all four sample areas. Culturing experiments have begun but the data are not available at the time of writing this abstract. Figure 1 shows the presence of a hyphae forming organism on sample I (fusion crust). The hyphae appear to be growing from a 2 μm diameter spherical structure, which strongly resembles a fungal spore. Figure 2 shows the terminal end of a single hyphae on the fusion crust. The hyphal structure appears to be disrupted and flattened. This is not surprising, as these samples received none of the pretreatment normally required for biological imaging. Small 100 nm circular objects are seen both associated and removed from the hyphal terminus.

Figures 3 and 4 are related to sampled IV. Figure 3 again shows the presence of hyphal filaments originating from an apparent spore-like structure. The surface appears to be covered with a thin film with the underlying mineral edges becoming indistinct. Figure 4 shows a hyphal structure appearing through the matrix of the meteorite and terminating after approximately 15 μm (terminus out of sight of the bottom of the image). Again, evidence can be seen of a coating, blurring the edges of the mineral phases, most notably in the bottom left-hand part of the image. EDX-analysis of the apparent coating showed consistently the presence of a carbon peak, which is not seen on the apparently uncoated mineral surfaces.

No evidence of any organisms was found on samples II and III although due to the heterogeneity of occurrence of the contaminant they may be present [5].

**Discussion** Figures 1 and 2 show the presence of probable fungal contaminants on the surface of the fusion crust. The 100 nm spheres shown in figure 2 have either formed mineralogically or from the dehydration of the hydrated polymeric substances normally secreted by microorganisms. Although these are the most plausible explanations, labeling these structures nanobacteria would require further collaborative evidence from culturing studies.

Figures 3 and 4 appear to show that this fungal contaminant has managed to infiltrate to the center of the meteorite. Even though no visible fractures could be observed, small cracks and fissures must exist to allowing the organism to infiltrate. These need be no larger than the diameter of the fungal hyphae (approximately 1 µm).

The presence of large areas of a carbon rich film (fig. 3 and 4) is puzzling as the hyphal structures do not appear to be part of the film, rather they seem to sit on top of it. The appearance of the coating would neither be expected if, as it appears, it was a product of microbial activity, as any bacterial coating would dehydrate to strand-like appearance [6]. Therefore, it could represent deposits of a type of insoluble organic material indigenous to the meteorite and maybe providing a nutrient source for the fungal colonies inhabiting this meteorite.

From this study we conclude that all organic analysis conducted on this new Nakhla allocation should be undertaken with reservations. It is our contention that adequate screening methods be developed to ensure the accurate analysis of any indigenous organic material. However, as these organisms are living on extraterrestrial material they probably provide one of the best tools to study how life may exist elsewhere in the solar system.

**References** [1] McKay D.S. et al. (1996) *Science*, *273*, 924. [2] Steele A. et al. (1999): Imaging an unknown organism on ALH84001. *Abstracts to the 30$^{th}$ Lunar and Planetary Science Conference 1999*. [3] Bada J.L. et al. (1998) *Science*, 279, 362-365. [4] Jull A.J.T. et al. (1998) *Science*, 279, 365-369. [5] Steele A. et al. (1998) *Meteoritics & Planetary Science*, **33**, 4. [6] Steele A. (1996) PhD Thesis, University of Portsmouth, Portsmouth, UK.

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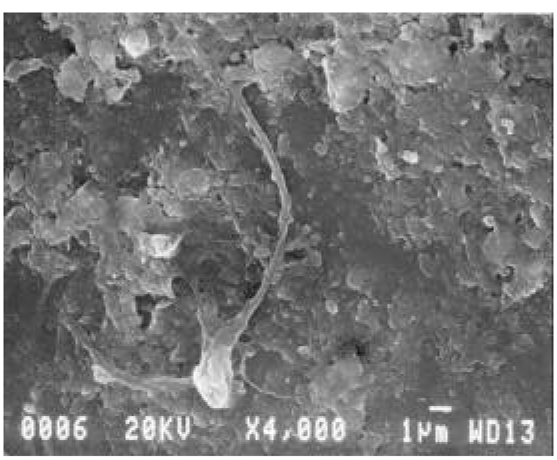


**Fig. 1.** SEM image of a hyphae forming organism on the fusion crust of sample I.

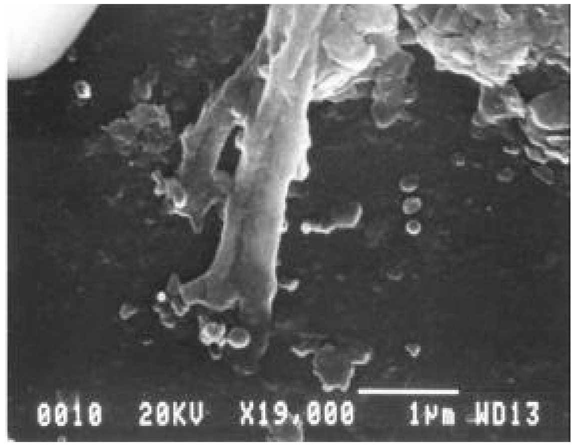


**Fig. 2.** SEM image showing the terminal end of a single hyphae with small spheres on the fusion crust.

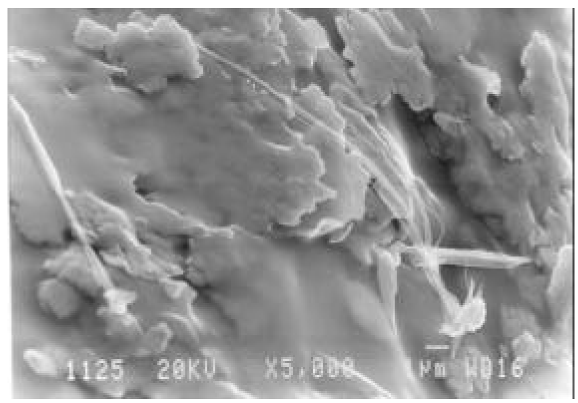


**Fig. 3.** Hyphal filaments originating from an apparent spore-like structure and coating film on sample IV.

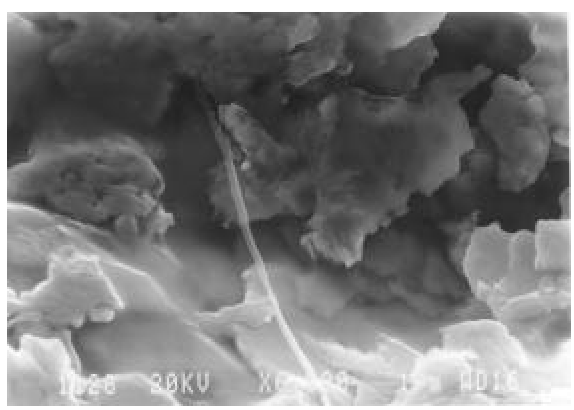

**Fig. 4.** Single filamentous hyphae structure on sample IV; again, the mineral surfaces appear to be coated.