Ratios of biogenic elements for distinguishing recent from fossil microorganisms

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ABSTRACT

The ability to distinguish possible microfossils from recent biological contaminants is of great importance to Astrobiology. In this paper we discuss the application of the ratios of life critical biogenic elements (C/O; C/N; and C/S) as determined by Energy Dispersive X-ray Spectroscopy (EDS) to this problem. Biogenic element ratios will be provided for a wide variety of living cyanobacteria and other microbial extremophiles, preserved herbarium materials, and ancient biota from the Antarctic Ice Cores and Siberian and Alaskan Permafrost for comparison with megafossils and microfossil in ancient terrestrial rocks and carbonaceous meteorites.

KEYWORDS: Microfossils, Carbonaceous Meteorites, Orgueil, Murchison, Energy Dispersive X-Ray Spectroscopy; Biomarkers, Biogenic Elements, Elemental Ratios, Cyanobacteria, Extremophiles

1. INTRODUCTION

Astrobiologists have highlighted the importance of establishing the validity of chemical, mineralogical and morphological biomarkers in ancient rocks, meteorites and other Astromaterials. It is necessary to recognize that biomarkers exist in a variety of strength levels. Weak biomarkers only suggest biogenicity whereas strong, valid biomarkers provide clear evidence of biological activity. Valid biomarkers in ancient rocks, meteorites and other astromaterials must satisfy rigorous criteria. During the past decade, extensive Field Emission (FESEM) and Environmental (ESEM) Scanning Electron Microscopy investigations of the morphological characteristics and EDS analyses of the elemental compositions of minerals, terrestrial microfossils and known biological organisms have been investigated in the NASA/MSFC/NSSTC. Astrobiology Laboratory. These studies included a wide variety of minerals, rocks, and meteorites as well as living and fossil (Holocene to Archaean 2.8 Ga) eukaryotic and prokaryotic organisms (e.g., plants, hair, fungi, diatoms and other algae, cyanobacteria, sulfur and sulfate reducing bacteria. This research has resulted in the recognition that elemental ratios of certain life-critical biogenic elements can provide a powerful mechanism for distinguishing recent biological contaminants from ancient indigenous microfossils and recognizing valid morphological biomarkers in meteorites. This paper addresses the use of ratios of life critical biogenic elements (C, N, and S) for distinguishing recent biological contaminants from valid, indigenous microfossils in ancient rocks and meteorites. To be considered valid, the biomarkers must satisfy to rigorous criteria:

1.) **Valid biomarkers must be Unambiguously Biological**
2.) **Valid biomarkers must be Undeniably Indigenous**

Although many biominerals provide weak evidence of biogenicity, some can be considered strong biomarkers. These include conclusively recognizable biofilms; biogenic magnetites and magnetosomes in "chain of pearls" configurations. Other mineral biomarkers include silica biopolymers such as are found in the shell of diatoms, silicoflagellates, and radiolaria. More definitive evidence of biological activity is provided by valid biomarkers such as complex isoprenoid biomolecules, fatty acids, cholestane Pristane, Phytane and the diagenetic breakdown products

of other biological pigments and protein Amino Acids with unambiguous enantiomeric excess. Valid biomarkers also would include segments of RNA, DNA, genes, proteins, enzymes, or other complex biomolecules as well and unambiguously recognizable microfossils or consortia with distinctive chemical and cellular differentiation. It is crucial that the indigeneity of the biomarkers can be undeniably established. For this reason methodologies must be developed to distinguish recent biological contaminants from valid indigenous chemical and morphological biomarkers and microfossils in ancient rocks, meteorites and returned astromaterials.

Many important chemical biomarkers have been detected in carbonaceous meteorites during studies carried out by different investigators on a carbonaceous meteorite samples studied from the late-1800's to the present. **Table I** is a chronological summary for many chemical biomarkers detected in carbonaceous meteorites.

TABLE I. Chemical Biomarkers in Carbonaceous Meteorites

BIOMARKER	REFERENCE
Organic Matter ~ humus, peat & lignite coal[1-3]	Cloez, 1864a, 1864b; Pisani, 1864
Petroleum-like Hydrocarbons[4]	Berthelot, 1868
Amino Acids[5-7]	Nagy *et al.*, 1961; Kvenvolden *et al.*, 1970
Long-Chain Fatty Acids ~ Ancient Sediments[8]	Nagy and Bitz, 1963
Porphyrins[9,10]	Hodgson and Baker, 1964; 1969
Polymeric Matter ~ Kerogen[11]	Bitz and Nagy, 1966
Polycyclic Aromatic Hydrocarbons[12]	Commins and Harrington, 1966
Aliphatic Hydrocarbons w/Alkane Preference[13]	Nooner and Oró, 1967
Normal & Isoprenoid alkanes[14]	Gelpi & Oró, 1970
Nitrogen Heterocyclics & Nucleic Adid Bases Purines, Pyrimidines Triazines,Adenine/Uracil[15-19]	Hayatsu, *et al*. 1964, 1968 ; Folsomme *et al.* 1973; Hua *et al.*,1986; Stoks & Schwartz,198
Pristane, Phytane and NorPristane[13,20]	Nooner and Oró, 1967; Kissin, 2003
Protein Amino Acids with Enantiomeric Excess[21-29]	Oró *et al.*,1971; Engel *et al.*; 1980, 2003; Cronin *et al.* 1997; Ehrenfreund *et al.*, 2001

Some of these chemical biomarkers (e.g. PAH's, Petroleum-like Hydropcarbons, Amino Acids, etc.) can be produced by abiotic mechanisms (e.g., Miller-Urey or Fisher-Tropsch synthesis) and they consequently do not provide conclusive evidence of biological activity. However, many of the others are not produced by any known abiotic mechanisms and thus must be considered to represent valid chemical biomarkers in carbonaceous meteorites.

In 1967, Nooner and Oró[13] detected isoprenoids (Pristane, Phytane and Norpristane) in the Orgueil CI1 carbonaceous meteorite. Pristane (2,6,10,14-tetramethylpentadecane) is a naturally saturated terpenpoid alkane ($C_{19}H_{40}$) that is a biogeochemical derivative from phytol. Phytol is a well-known a decomposition product of Chlorophyll. This photosynthetic pigment, which is crucial for the complex process of photosynthesis, is common in phototrophic organisms on Earth. It plays a critical role in the flow of energy in the Biosphere. Phototrophs use trapped solar energy to convert carbon dioxide and water into sugars and more complex cellular components such as carbohydrates, polysaccharide sheaths, fatty acids, nucleic acids, DNA, RNA and a host of other biochemicals essential for life. The organic carbon that is fixed from carbon dioxide can then be used by other life forms. The stored solar energy is extracted by chemotrophic organisms via anaerobic fermentation processes that occur in the absence of oxygen, During aerobic respiration the organic carbon is completely oxidized and returned to the atmosphere as carbon dioxide, thereby completing the carbon cycle. Every carbon atom in every molecule in the organism is derived from carbon dioxide that is fixed into organic carbon by the photosynthetic process.

There are no known abiotic mechanisms that produce Chlorophyll. Pristane and Phytane are diagenetic derivative breakdown products of chlorophyll and are considered valid biomarkers. Kissin[20] has pointed out that the transformation of chlorophyll into its phytol chain and then to the isoprenoid alkanes pristine and phytane is a slow process. Phytane (3,7,11,15-tetramethylhexadecane) is only found on Earth in ancient oil shales and crude petroleum. The conversion of terrestrial biological matter into linear alkyl chains is a slow, multi-step process involving microbialy mediated enzymatic reactions. Kissin's experimental investigations established that the free saturated hydrocarbons in the Orgueil meteorite were not the result of contamination by oils used in the laboratory environment or recent microbiolocical contamination effects.

1. Biogenic Elements in Living Organisms and Meteorites

All organisms on Earth appear to possess the same fundamental requirements for liquid water, an energy source and requirement for a limited set of life-critical (biogenic) elements. By far the most critical elements in life on Earth are the six life critical elements (Carbon, Hydrogen, Oxygen, Nitrogen, Phosphorus, and Sulfur) are found in relatively large quantities in all organisms. The first four are by far the most abundant. Sulfur and Phosphorus are also critical for life, but they are found in much smaller quantities in living organisms. Minor biogenic elements present in organisms in lesser amounts include: Mg, Fe, Na, K, Ca, and Cl. Biogenic elements are needed for construction of cellular structural components, metabolism and respiration, and storage and transport of energy and information. A few other elements (e.g., Si, Mn, Al, I, Cu, Zn, As, Ni and F) are needed for enzymatic actions and specialized functions and they usually appear only in trace levels.

All of the major life-critical biogenic elements (along with Fe, Si, and Mg) are also the major components of both carbonaceous meteorites and comets. Comets may have played a major role in the dispersal of the biogenic elements throughout the Solar System.[30,31] All are found in the Orgueil, Murchison and other carbonaceous meteorites. The distributions of the major biogenic elements within the meteorite samples are very heterogeneous. Energy Dispersive X-Ray Spectroscopy (EDS) spot analysis and 2-D X-ray maps obtained during the present investigation clearly establish that the biogenic elements C, O, Mg, and S are heavily concentrated in the filaments found in the Orgueil CI1 carbonaceous meteorite.. Furthermore, there is a readily observable differentiation in abundances of the biogenic elements from observably different and distinguishable components of the filaments.

Nitrogen is very rarely found to be present in the filaments above the level of detectability of the EDS. Nitrogen is absolutely essential for life since it is present in all amino acids and in the purines (Adenine and Guanine) and Pyrimidines (Cytosine, Uracil, and Thymine) which are essential for the construction of life critical biomolecules such as ATP, RNA, DNA and proteins. All modern (and old but not fossilized) biological materials studied have been found to have detectable levels of nitrogen. However, nitrogen is absent in many ancient fossils as a result of the diagenetic losses that occur over many millions of years.

1.1 Carbon in carbonaceous meteorites and filamentous microfossils

Mason[32] reported in 1963 that carbonaceous meteorites can contain up to 4.8% (weight %) carbon. Otting and Zähringer[33] obtained a slightly lower value (3.19 wt %). Using the more sensitive Energy Loss Electron Spectrometer (EELS), the bulk Carbon content of the Orgueil meteorite was found to be 3.5% (by weight) and they found 5% of the carbon rich grains to also be enriched in Oxygen, Phosphorus and Sulfur (the COPS phase).[34] The Biosphere is critically dependent upon "carbon fixation" reactions. In this complex processes enzymes are used to catalytically convert atmospheric CO_2 into carbohydrates that can be used by living organisms. During the carbon fixation cycle, light energy is captured during photosynthesis by photoautotrophic microorganisms and stored in the chemical bonds of ATP and NADP. This stored energy can then be used to power the enzymatic reactions that convert molecules of carbon dioxide and water into the organic molecules. During the Calvin cycle in living organisms the fixation of CO_2 into carbohydrates is catalyzed by the (RuBisCo) enzyme.[35] The RuBisCo enzyme has both carboxylase and oxygenase activity and is also capable of fixing atmospheric oxygen during the process of photorespiration. RuBisCo comprises almost 50% of the protein of chloroplasts and is thought to be the most abundant protein on Earth. Many bacteria and archaea are chemoautotrophs or chemolithotrophs that are capable of deriving energy from inorganic energy sources and chemical reactions and synthesizing organic compounds from carbon dioxide or carbonate rocks. Biological fractionation occurs during the Calvin cycle since the light carbon stable isotope (C^{12}) is incorporated in preference to the heavier (C^{13}) isotope. Carbon isotope measurements for glycine in the Orgueil meteorite yielded a value of $\delta^{13}C=+22$ per mil (far above the terrestrial range of -20 to -35 per mil providing clear and convincing evidence that the Orgueil amino acids are extraterrestrial.[36] Biological fractionation of stable isotopes of carbon as seen in ancient long chain carbon biogeopolymers (kerogen) has been interpreted as geological evidence of biological activity preserved in the rock record for ~3.8 Ga. Stromatolites formed by filamentous prokaryotes and cyanobacteria extend back to at least 2.8 Ga[37-41]

1.2 Oxygen in the carbonaceous meteorites and filamentous microfossils

Oxygen is a life-critical biogenic component of virtually all organic molecules, RNA, DNA and proteins and it is crucial for metabolism. The Orgueil and Murchison meteorites are rich in oxygen. The mean oxygen content for

CI meteorites is 46%.[42] The EDS spot analysis reveals that some of the filaments have Oxygen levels above 60% (atomic) while others have values as low as 9% atomic. Some of the sheaths have C/O ratios similar to kerogen. For example C/O = 108 at spot X on the sheath of the graphitized filamentous microfossil in Fig. 2.a. This ratio is entirely unlike that obtained for living or recently dead biological matter. Elemental 2-D x-ray maps show many of the other filaments often have oxygen levels that significantly exceed the levels found in the adjacent or underlying rock matrix.

1.3 Nitrogen in the Biosphere

The Biosphere is critically dependent upon both "carbon fixation" and "nitrogen fixation" reactions. In these complex processes enzymes are used to catalytically convert atmospheric CO_2 and N_2 into forms that are useful to living organisms. Nitrogen is one of the most abundant elements in the Solar System and it critical to all living organisms. Nitrogen is an essential component of amino acids, nucleic acids, purines, pyrimidines, ATP and proteins. Nitrogen is required for synthesis of RNA and DNA and the regulation of many crucial biochemical pathways. Although nitrogen is abundant in the atmosphere (79%), atmospheric nitrogen cannot be used by living organisms until it has been converted by "nitrogen fixation" enzymatic processes. The N_2 gas molecule is unavailable for most organisms due to the triple bond that renders it virtually inert. Nitrogen is converted into a useable state by nitrogen fixing bacteria such as the heterocystous cyanobacteria. The process of nitrogen fixation involves the conversion of gaseous dinitrogen into ammonia NH_4^+, which can then be converted to nitrite or nitrate ions by nitrifying bacteria. Nitrogen fixation is accomplished by use of the enzyme complex nitrogenase which catalyzes the reaction:

$$N_2 + 8\,e^- + 8\,H^+ + 16\,MgATP \rightarrow 2\,NH_3 + H_2 + 16MgATP + 16\,P_i$$

Fixed nitrogen is precious and is scavenged after death by microorganisms and subsequently removed by a very slow diagenetic processes. Although the level of nitrogen can be fairly high (2 to 15% in living and dead modern biological materials, it is almost never encountered at levels above 2% in microfossils. Consequently, nitrogen levels and C/N ratios provide a useful tool for distinguishing recent biological contaminants from indigenous microfossils.

Cyanobacteria play a crucial role in nitrogen fixation on planet Earth. The nitrogen content of living cyanobacteria often amount to more than 10% by weight.[43] A nitrogen deficiency immediately affects the amount of phycobiliproteins and consequently their photosynthetic light harvesting efficiency. The nitrogenase enzymes are extremely sensitive to oxygen and therefore in order to fix nitrogen an anaerobic environment must be provided. Cyanobacteria are oxygenic photoautotrophic microorganisms that are primarily aerobic microorganisms and therefore they must provide specialized cells to facilitate this process. The diazotrophic cyanobacteria (capable of using N_2 as their sole source of nitrogen for growth) can be subdivided into three groups:

Group I: Heterocystous Cyanobacteria: Exclusively filamentous cyanobacteria that differentiate special cells (heterocysts) which have lost the capacity for oxygenic photosynthesis. Thick walled heterocysts form a diffusion barrier for gases and limit the entry of oxygen so that they can carry out diazotrophic growth under fully aerobic conditions. The heterocyst prevents oxygen from entering the cell where the nitrogen fixation is taking place. Heterocystous cyanobacteria include: *Calothrix, Anabaena, Nodularia,* and *Scytonema.* The Orgueil meteorite contains filaments interpreted as morphotypes of *Calothrix,* but the other Genera have yet been detected.

Group II: Anaerobic N_2-Fixing Non-Heterocystous Cyanobacteria: These are both filamentous and unicellular. They function by locating themselves in microbial mats in regimes that allow them to avoid oxygen and they may require sulfide to inhibit oxygenic photosynthesis. Examples include: *Oscillatoria limnetica, Plectonema boryanum,* and several species of *Lyngbya* and *Synechococcus.* Most of the filamentous forms found in Orgueil are consistent with morphotypes of species of *Oscillatoria* and *Plectonema.*

Group III: Aerobic N_2-Fixing Non-Heterocystous Cyanobacteria: These include common filamentous components of cyanobacterial mats such as: *Microcoleus chthonoplastes,* and various species of *Lyngbya, Oscillatoria, Trichodesmium,* and *Gloeothece.* The exact strategy is still not known but it may include temporal separation of the nitrogen fixation and oxygenic photosynthesis stages.

1.4 Nitrogen in carbonaceous meteorites and terrestrial fossils

Although Nitrogen is abundant in living organisms, it is severely depleted in meteorites.[44] During his initial investigations of Orgueil, Cloëz[1, 2] reported the detection of an ammonium compound at approximately 0.1% (weight %). He considered the ammonium to be present as a water soluble ammonium and chlorine salt that he called 'ammonium hydrochlorate'.[3] In 1963, Mason[45] concluded that the Orgueil ammonium salts were probably in the form

of NH_4Cl or $(NH_4)_2SO_4$. Moore[46] reported finding 2,400 ppm nitrogen (0.24% atomic) in Orgueil and 2,900 ppm in the Alais meteorite. Gibson *et al.*,[47] analyzed 27 carbonaceous meteorites and found that carbon-rich meteorites were also enriched in nitrogen. The broad variations in the nitrogen levels of individual samples show the heterogeneous distribution of nitrogen in Orgueil.

Nitrogen is also severely depleted in ancient fossils. It enters the geological cycle through the enzymatic fixation of atmospheric N_2 and it is transformed into ammonium. Gallien *et al.*,[48] used Nuclear Reaction Analysis (NRA) to investigate the nitrogen and carbon content of biogenic and abiogenic minerals in Paleozoic shales and found the following atomic C/N ratios:

Abiotic Devonian hydrothermal feldspars:	C/N = 0.13 - 0.26
Marine bacteria	C/N = 2.9 - 14.3
Biogenic minerals	C/N = 17 - 25
Proterozoic kerogens	C/N = 104 - 167
Archaean kerogens	C/N = 200 – 500

These published results are consistent with the data from the EDS investigations carried out at the NASA/MSFC Astrobiology Laboratory on a wide variety of recognizable microfossils in carbonaceous meteorites, as well as fossils of trilobites, fish and prokaryotic filaments in terrestrial rocks as well as the data obtained from EDS studies of living and ancient biological materials. Table 2 provides the C/N; C/O and C/S values for a number of representative examples. The Electron Energy-Loss Spectroscopy EELS or NRA systems[42] are much more sensitive to the low-Z elements than the Energy Dispersive X-ray Spectroscopy (EDS) systems employed in the present investigation. Even under ideal conditions the EDS rarely detects the low-Z element Nitrogen at levels as below 0.2% (2000 ppm). However, since these elements are detectable at levels above 5000 ppm (~0.5%) a value of 0.5% was used to estimate the lower limit for C/N and C/S ratios when Nitrogen or Sulfur values were returned as 0.00% by the Energy Dispersive X-ray Spectrometer software.

1.5 Sulfur, Phosphorus, and Magnesium in carbonaceous meteorites and terrestrial fossils

Sulfur is a major biogenic element that comprises a storage component for many bacteria. The strong covalent disulfide bond is crucial to the folding, structure, and function of proteins. Sulfur is a minor constituent in carbonaceous meteorites. The sulfur content is highest level (5.9%) in the CI carbonaceous meteorites and the carbonates and sulfates in CI and CM meteorites provides evidence for aqueous activity on the parent body.[43] Although sulfur is critical for life, it rarely exceeds a percent in living organisms. However, the sulfur content of the Orgueil filaments is extremely high (~10-50% in the filaments) but it is much lower in the Orgueil matrix. This point is dramatically illustrated in the 2D X-ray map of **Fig. 2b**, where the filaments glow brightly in Sulfur against the meteorite rock matrix background. The sulfur levels found in the filaments of the Orgueil meteorite are significantly higher than present in living cyanobacteria and other microbial extremophiles.

Phosphorus is a crucial component of the *RuBisCo* enzyme and it is essential for the nucleotide adenosine triphosphate (ATP) - the major energy currency of cells. However phosphorus is a minor component of the CI meteorites[44,45] and is present at ~0.08 weight% for the Orgueil meteorite. Phosphorous is rarely detected in the Orgueil filaments and it is typically at very low levels (< 0.5%) in living cyanobacteria, bacteria, archaea and diatoms.

Magnesium is a minor biogenic element in terms of the amount present in cells, but it is used in enzymes and plays a major role in photosynthesis as a component of chlorophyll. Magnesium is a major element in the CI meteorites, where it comprises ~9.6% by weight. Much of the magnesium is in water-soluble hydrated magnesium sulfate (in different stages of hydration) and as hydrated Mg layer-lattice silicates (serpentine or chlorite).[44]

2. RESULTS

2.1 Images and EDS elemental abundances of filaments in the Murchison CM2 Meteorite

During the past decade, the ElectroScan Environmental Scanning Electron Microscope (ESEM), the FEI Quanta 600 FEG and the Hitachi S-4100 Field Emission Scanning Electron Microscopes (FESEM) were used at the NASA Marshall Space Flight Center to produce high resolution images and Energy Dispersive X-ray Spectroscopy (EDS) analyses and 2-D X-ray maps of elemental compositions of embedded microfossils in ancient rocks and carbonaceous meteorites. Comparative studies with the same instrumentation was carried out on a wide variety of

megafossils (Trilobites, Fish, etc.) as well as known fossils of Proterozoic and Archaean cyanobacteria and associated filamentous prokaryotes and environmental samples and pure cultures of living cyanobacteria and axenic cultures of type strains of novel genera and species of bacteria and archaea. Freshly fractured interior surfaces of many of the CI and CM carbonaceous meteorites have been found to contain many complex and embedded filaments consistent in size, shape, and morphology with known species of cyanobacteria and associated filamentous trichomic prokaryotes.

An ElectroScan ESEM image at 15 keV of a filament in the Murchison CM2 carbonaceous meteorite is shown in **Figure 1.a.** This image is interpreted as the mineralized remains of an emergent hormogonium from a morphotype of cyanobacteria (cf. *Nostoc* sp.). The EDS spectrum **(Fig. 1.b)** at spot X shows C 41.7%; O 16.8%; S 6.1% and N 0.0% (taken as N<0.5%) gives elemental abundance ratios: C/O=2.5; C/N>82; C/S=6.8. Detectable levels of Iron and Nickel are observed. The suite of elements is consistent with that observed in the meteorite matrix at point **Y** providing a clear indication that the filament belongs to the meteorite and it is not a recent biological contaminant.

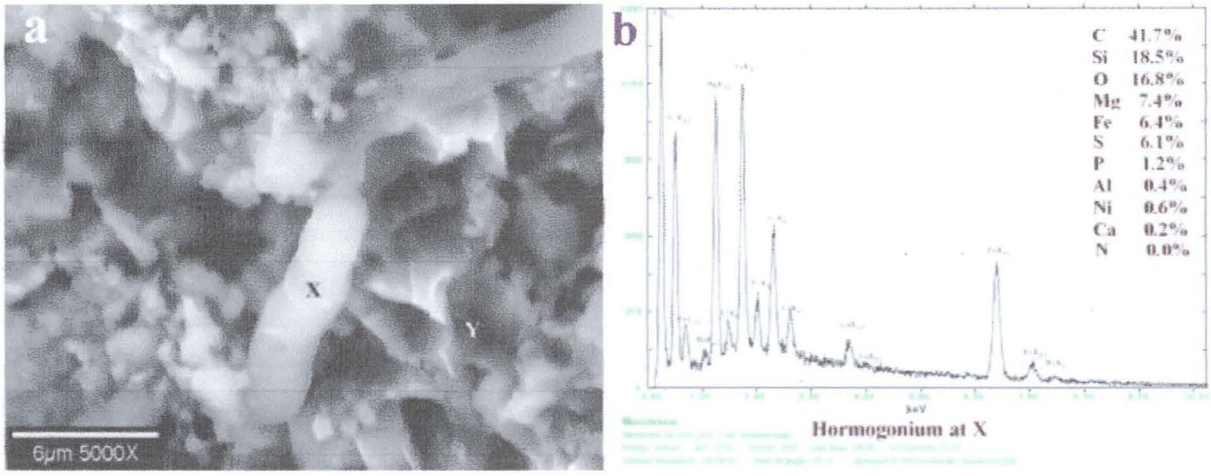


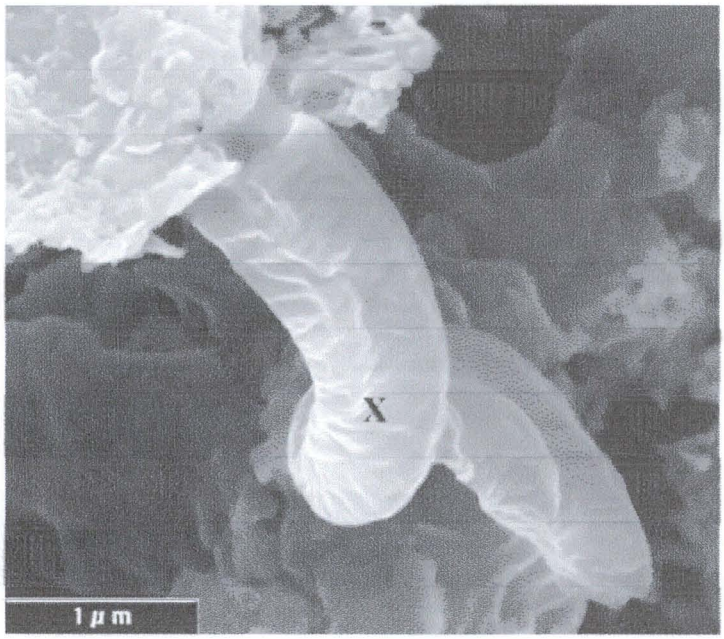
Figure1a. Filament with crosswall constrictions in the Murchison CM2 meteorite interpreted as emergent hormogonium of species of *Nostocacean* cyanobacteria. **b.** EDS spectrum at **X** shows elemental composition of filament similar to Murchison matrix but enriched in Carbon (C=42%; O=16.8%; S=6%; Ni=0.6%; N<0.5%; C/N=82; C/O=2.5;C/S=6.8).

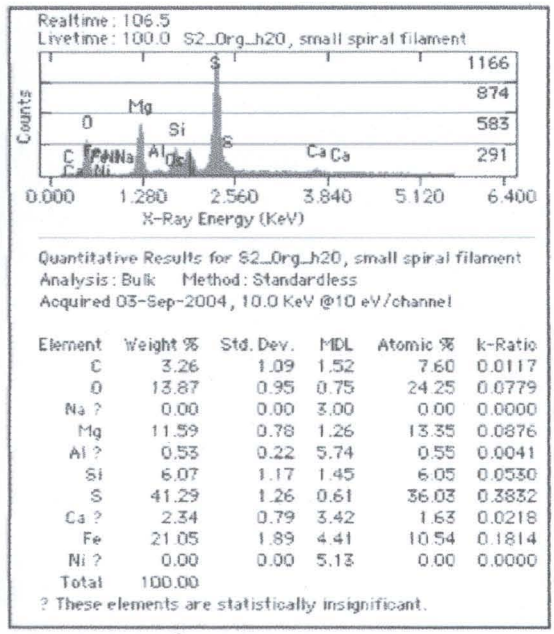
2.2 Images and EDS elemental abundances of filaments in the Orgueil CI1 Meteorite

The vast majority of the embedded filaments in the Orgueil CI1 carbonaceous meteorite have electron transparent carbon-rich sheaths enveloping a permineralized interior rich in magnesium and sulfur. This is interpreted as the result a fluid infused with a magnesium sulfate solution infilling the hollow carbonized sheath after death of the filamentous prokaryote. It has been known since shortly after the meteorite fell in 1864 that the Orgueil meteorite is a microregolith breccia that disintegrates immediately when it comes in contact with liquid water.[1-3] Consquently, it is suggested that the infilling of the interior of these carbon-rich envelopes must have occurred on the parent body prior to entry into the Earth's atmosphere. Some of the envelopes of the Orgueil filaments and sheath-like electron-transparent envelopes have over 80% (atomic) carbon. All of the filaments found have extremely high levels of magnesium and sulfur. This result is consistent with a magnesium sulfate rich fluis infilling hollow carbonaceous sheaths of cyanobacteria or filamentous sulfiur bacteria after death. This would require a flow of liquid water and since the Orgueil CI1 meteorite is destroyed by liquid water, this observation is interpreted as providing strong evidence that the permineralization of the filaments took place on the parent body prior to entry of the meteorite into the Earth's atmosphere. **Figure 2.a.** is a Hitachi Field Emission Scanning Electron Microscope (FESEM) image of a small spiral filament in Orgueil with size and morphology similar to known representatives of the modern helical cyanobacterium *Spirulina* sp. The EDS spectral data from spot X is shown in **Fig. 2.b.**

Figure 3a. is a a 1000X FESEM image of a very small (~120 µm) fragment of the Orgueil meteorite with **Fig. 3.b.** a 2D X-ray elemental map. This small region is densely populated with many different types of embedded filaments and electron transparent sheaths. Several of the filaments have complex morphological features that are well known in modern cyanobacteria and other trichomic prokaryotes. The major filaments and sheaths are clearly seen as bright features in the C, O, Mg and S maps and they appear as dark features in Si, Fe, and Ni maps due to the

relatively higher content of these elements in the underlying Orgueil meteorite rock matrix. Filament **1** can be clearly discerned in the Nitrogen map and the wrinkled, electron transparent, empty sheath **7** has a relatively high (47%) content of Carbon content. It is one of the only filaments ever found in the Orgueil meteorite with detectable levels of both Nitrogen (1%) and Phosphorus (0.8%). The irregular longitudinal striations of filaments **1** and **2** suggest these are multiseriate filaments in which multiple parallel oriented trichomes are enclosed within a common homogeneous sheath. Both of these filaments appear to be attached to and physically embedded in the rock or clay substratum of the Orgueil meteorite matrix and thus they may represent epilithic or epipelic forms.





a. **b.**

Figure 2.a. Morphotypes of *Spirulina sp.*in the Orgueil meteorite with **b.** EDS spectrum showing elemental abundances of Magnesium sulfate permineralized filament at spot X. (C 7.6%; N < 0.5%; O 24%; S 26%; C/N > 15; C/S = 0.3; C/O = 0.3)

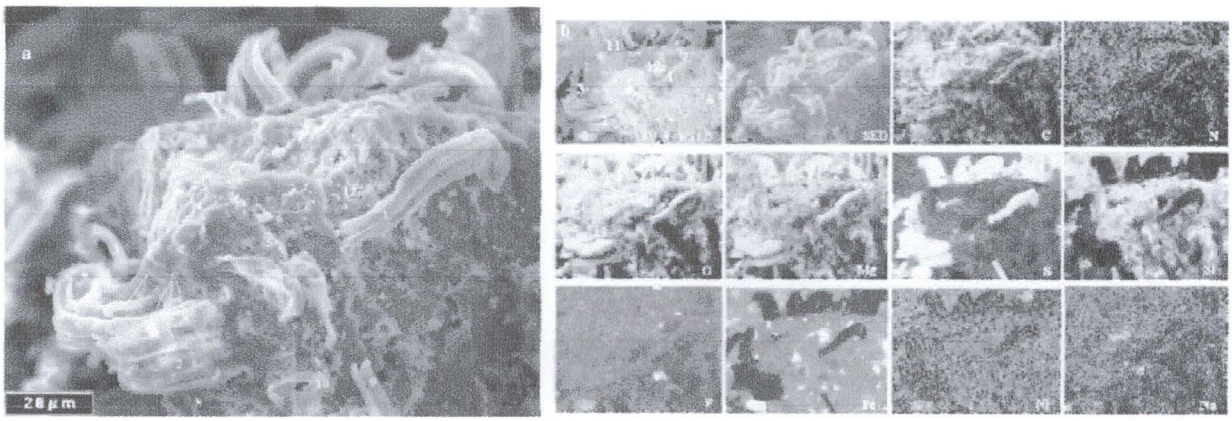
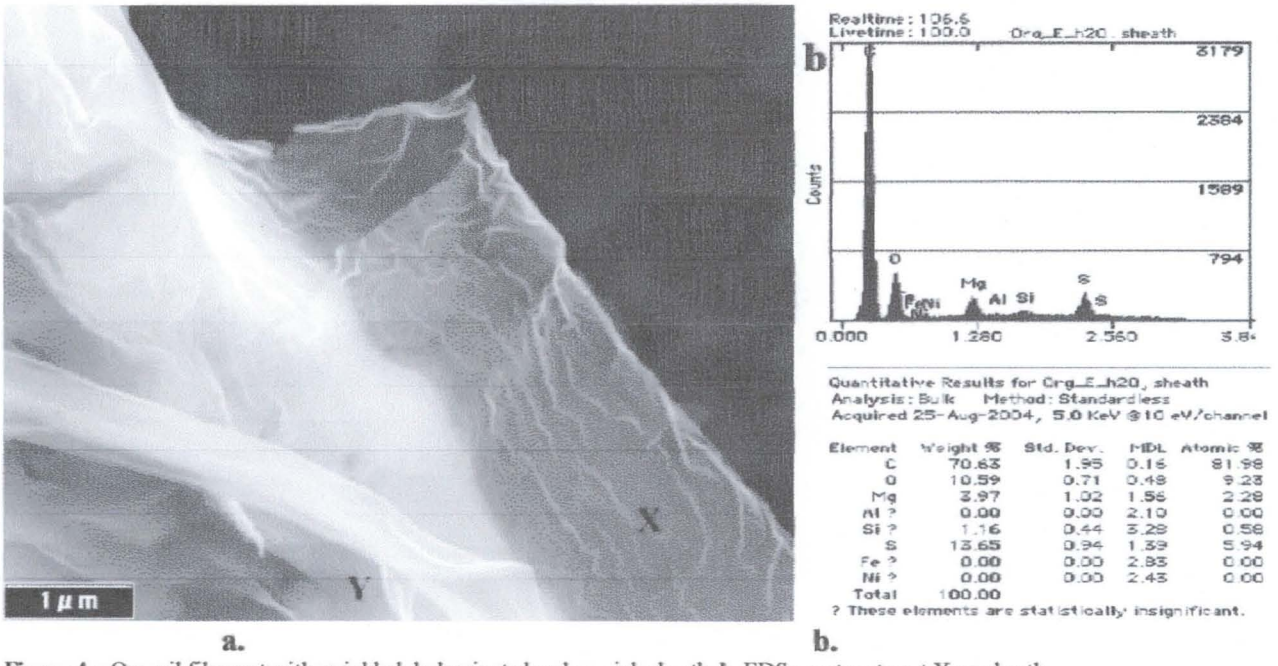


Figure 3. FESEM SED and BSED images and 2-D Elemental X-Ray Maps of Orgueil fragment with many different types of embedded filaments and empty carbonaceous sheaths. EDS spot data for numbered spots given in Table 2.

The end of Filament **1** widens slightly (~10 μm) where it joins the rock matrix and it appears to contain four trichomes with diameters ~2.5 μm /trichome. The larger filament **2** (~ 20 μm dia.) has longitudinal striations suggestive of ~5 trichomes with diameters ~4 μm/trichome. Faint cross wall constrictions are visible in Filament **2** suggesting the internal cells are ~ 4 μm in length and hence roughly isodiametric. The inferred configuration of filament **2** is that it consists of an ensheathed trichome bundle of parallel trichomes composed of isodiametric cells of

4 μm diameter as is well known in modern morphotypes of undifferentiated filamentous Oscilliatorialean cyanobacteria of the genus *Microcoleus* Desmazières ex Gomont) (See Castenholz, Rippka & Herdman[49]). Reproduction within this order occurs by trichome fragmentation and the production of undifferentiated short trichome segments (known as hormogonia) by binary fission of the cells in one plane at right angles to the long axis of the trichomes. The small multiseriate filament **1** is interpreted as representing a morphotype of the genus *Trichocoleus* Anagnostidis[50], which was separated from the genus *Microcoleus* on the basis of cell size and morphology. Trichomes of species of the genus *Trichocoleus* are typically of 0.5μm -2.5μm diameter. EDS spot spectral data on the meteorite rock matrix and several of the numbered filaments and sheaths are given in **TABLE 2.**



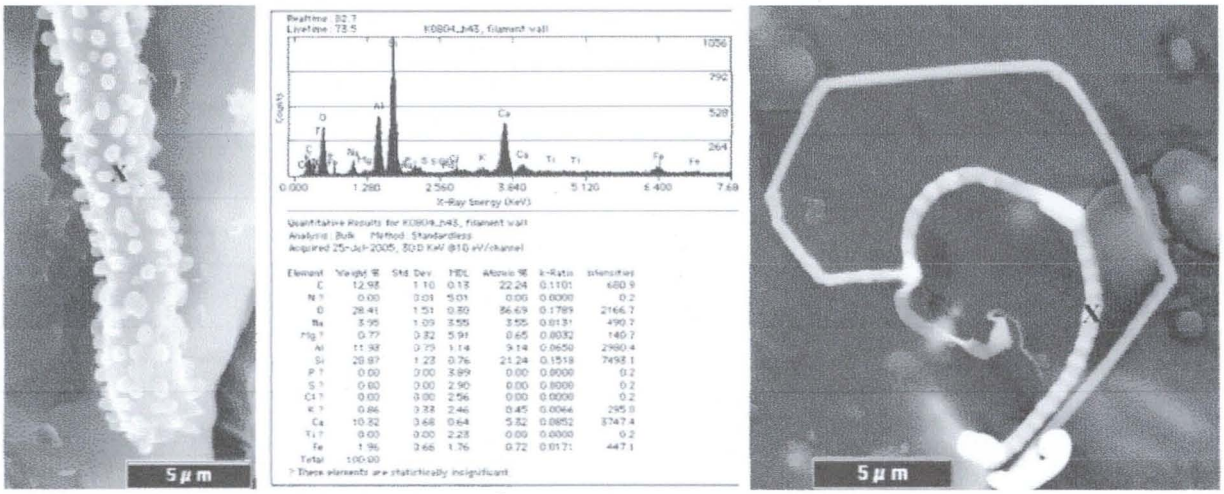
a. **b.**

Figure 4.a Orgueil filament with wrinkled de-laminated carbon-rich sheath. **b.** EDS spectra at spot X on sheath.

Figure 4.a. is a Hitachi FESEM image at 6000X of a complex, curved and polarized filament in Orgueil. The basal region of this branched filament is ~8 μm diameter and primary trichome tapers to 3 μm diameter at the apex. The terminal end of the filament is covered with an electron transparent mucilaginous sheath that encloses a 0.4 μm diameter terminal hair. The filament exhibits both "T" and "Y" branching configurations and the secondary trichomes are much narrower than the primary trichome. The secondary trichome at the lower center of the image forms a "Y" branch and then terminates in rounded empty sheaths. The Y branching and other features are suggestive of cyanobacteria of the Order Stigonematales, Geitler.[51] Some modern representatives of the genus *Fischerella* have branches that are much narrower than the main filament. This Order includes species that grow in thermal springs such as *Mastidocladopsis* Iyengar et Desikachary.[52] The Orgueil filament has a large nodule near the base that may represent lateral heterocysts, such as is sometimes seen in the genus *Mastidocladopsis*. This genus has not been extensively studied and only two tropical freshwater species (often found attached to stony substrates) have been described. The sheath of the Orgueil filament is wrinkled and laminated, which may be the result of the conditions in which fossilization took place. It should be also noted that the modern genus *Hapalosiphon* also has species with tapered and curved main filaments ~8 μm (e.g., *Hapalosiphon welwitschii* 5-7.5 μm). *Hapalosiphon hansgirgi* has 6-8 μm diameter main filaments narrowing to about 5 μm at the apex but none have been reported with such narrow branches. The EDS spectrum of the sheath **(4aX)** is exceptionally enriched in carbon (82 % C) and has a C/O ratio of 8.9. This is consistent with coal or kerogen but dramatically different from the samples of living, recently ancient (Pleistocene and Holocene) biological material. The EDS spectra for the filament interior **(4aY in Table 2)** shows it is permineralized with magnesium sulfate and has nitrogen content below the detection limit.

2.3 Images and EDS elemental abundances of Archaean filamentous prokaryotes

During this study we also evaluated the C/N, C/S and C/O ratios from fossil filamentous trichomic prokaryotes and cyanobacteria from the Upper Archean (lopian) rocks of Northern Karelia. Specimens from the Upper Archaean (lopian) deposits of Northern Karelia were collected by V.A. Matrenichev and N.A. Alfimova from the Institute of Geology and Geochronology of Precambrian of the Russian Academy of Sciences and provided by Prof. A. Yu. Rozanov, M. M. Astafieva, and Y.E. Malakhovskaya of Paleontological Institute (RAS). The samples were collected from the Northern part of Khisovaar structure (Parandovsk-Tikshosersk greenstone belt) which consists of thick complicated complex of volcanogenic-sedimentary rocks. The upper part of sedimentary complex was found to have isotopic age of 2706±7 million years and the lower portion 2803 ±35 million years. [53,54]



a. **b.** **c.**

Figure 5.a Mineralized filaments from carbonaceous shales of the Upper Archaean (lopian) rocks (~2.7 Ga) of Northern Karelia. **a.** Morphotype of oscillatorialean cyanobacterua with external nodules on isodiametric filament with **b.** EDS elemental composition . **c.** Unusual segmented filament of unknown affinity in Karelian rock. (EDS spectral data for spot X provided in Table 2 as 5cX.)

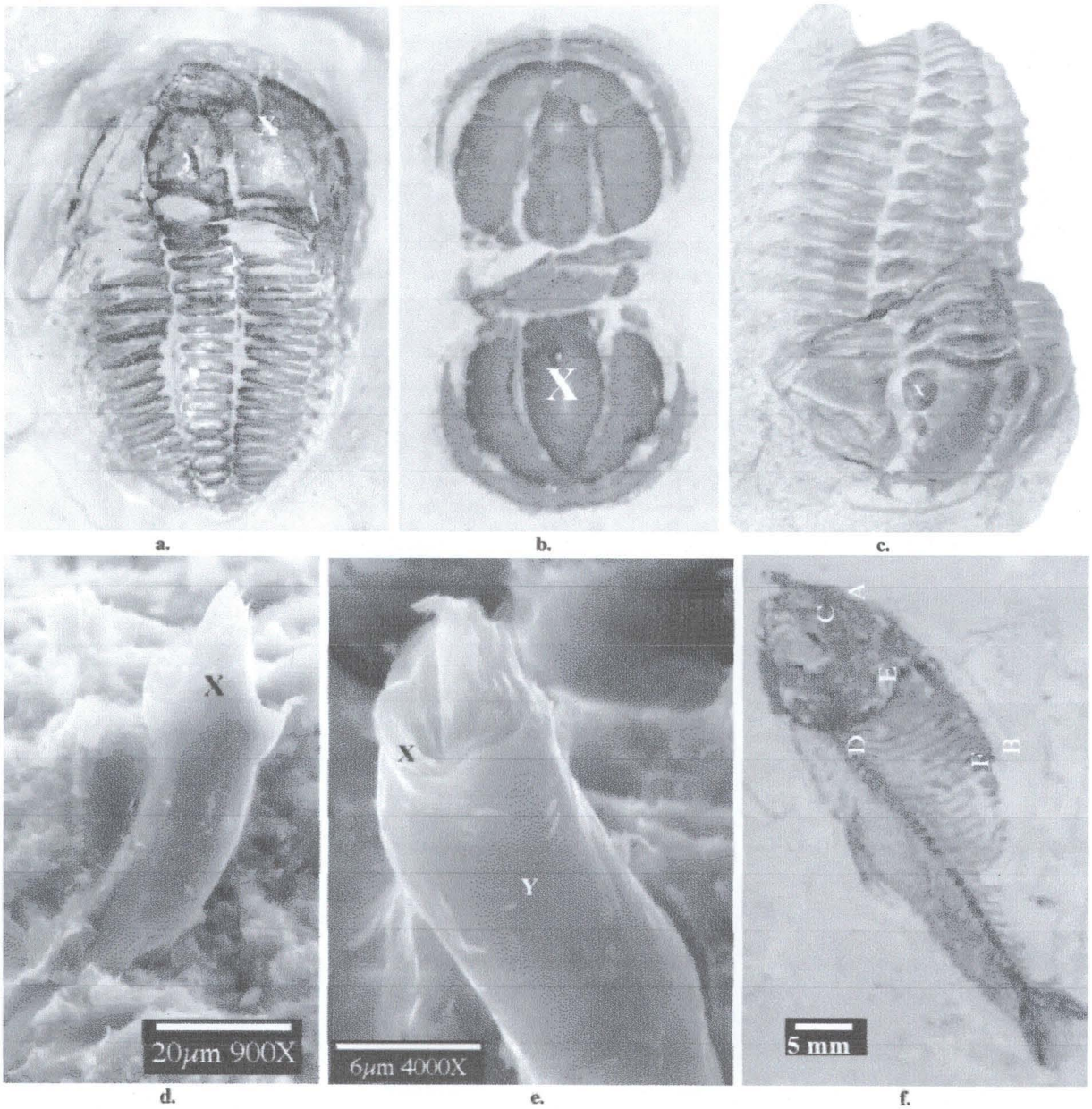
2.4 Images and EDS abundances of Cambrian and Ordovician trilobites, Devonian Mites, and Eocene fish

Energy Dispersive X-ray Spectroscopy analyses were also carried out to determine elemental compositions of well known terrestrial fossils such as Cambrian and Ordovician trilobites, Devonian mites and cyanobacterial filaments and Eocene fish. **Figure 6.a** is a visible light image of *Brachyaspidion microp.*[55] This is small well-preserved Middle-Cambrian (~500 Ma) trilobite of Order Ptychopariida was collected by the author in the Wheeler Springs Formation, House Range, Millard County, Utah. The Wheeler Shale mudstones are comprised exclusively of a fine-grained mixed carbonate mud and clay that accumulated below the influence of storm waves. The Wheeler Shale contains a very rich and diverse biota, including an abundance of benthic trilobites (e.g., *Asaphiscus wheeleri* and *Bollaspidella wellsvillensis* and many soft-bodied members of the Burgess Shale fauna. The Wheeler Formation accumulated in a deep, localized, fault bounded trough known as the House Range embayment on a broad sulfur-rich, carbonate platform.[57] The presence of Burgess fauna and Burgess Shale type preservation indicates an anoxic deposition in the absence of bioturbation. These are ideal conditions for extensive production of benthic, sulfur-oxidizing anaerobic chemolithoautotrophs, such as *Beggiotoa* and *Thioploca.*

These microbial communities could have provided a rich food source for Cambrian metazoans, such as the trilobites of the Wheeler Shale. **Figure 6.b** is a visible light image of the common Middle Cambrian Trilobite *Peronopsis interstricta* (Order Agnostida, Family Peronopsidea) from the Wheeler Formation, House Range, Utah. This small agnostid trilobite had no eyes and only two thoracic segments. **Figure 6.c** is a well-preserved Ordovician trilobite (~445 ma) *Reacalymene limba* from the Ashgill formation of North Wales. This inflated specimen is 27 mm long and has a semicircular cephalon and small, holochroal eyes.

A low magnification (900X) ESEM image of a fragment of an Orabatid mite with well preserved trichobotrias from Devonian graphite of the Botogol deposit of East Sayan (South Siberia)[58,59] is shown in **Fig. 6.d.** It is now accepted thate these raphites were formed by the conversion of highly carbonic sedimentary carbonate rocks

Fig. 6.e. is a 4000X ESEM image of a filament with size and morphology similar to known trichomic filamentous cyanobacteria of the Order Oscillatoriacæ. The spots at which the EDS data shown (Table 2) were obtained are marked on the filament. **Fig.6.f** is a visible light image of the small Eocene (~50 Mya) schooling fish (*Knightia sp.)* commonly found in the laminated sandstone of the Green River Formation of Kemmerer, Wyoming. The EDS spectral data for the marked spots on the trilobites and the spot C on the mineralized bone just beneath the eye socket of the Eocene fish are given in Table 2.



a. b. c.

d. e. f.

Figure 6. Middle Cambrian (~500 Ma) trilobites **a.** *Brachyaspidion microps* and **b.** *Peronopsis interstricta* from the Wheeler formation, near Swazey, Peak, House Range, Millard County, Utah **c.** Ordovician trilobite *Reacalymene limba* from Ashgill fm. (449-443 Ma) of North Wales. **d.** Image of Devonian Orabatid mite fragment and **e.** trichomic filament in the graphites from Botogol, South Siberia. **f.** Eocene (~50 Mya) schooling fish (*Knightia sp.)* with EDS spectra in Table 2 from Spot C of the bone just beneath the eye.

2.4 Images and EDS elemental abundances of Pleistocene filaments from Vostok Ice Cores

The Central Antarctic Glacier at Vostok is ~3.7 km thick ice sheet that overlays the 500 meter deep Lake Vostok. Sabit Abyzov *et al.*[60-62] have pioneered the study of viable microorganisms from the deep ice of the Central Antarctic ice sheet above Lake Vostok. They have shown that microorganisms can remain alive in a state of deep anabiosis for many thousands of years. Collaborative *in-situ* studies of Vostok ice and thawed ice cores precipitated on membrane filters were carried out at MSFC. These studies primarily used the Electroscan Environmental Scanning Electron Microscope (ESEM) to image ice fragments as they were allowed to melt in the instrument chamber. This made it possible to absolutely establish that the forms that were observed to emerge from the interior of the Vostok ice samples were indigenous and could not be interpreted as recent biological contaminants. Abundant microorganisms were found in all layers (102 M to 3611 M) of the ice sheet examined. Great variations in the composition, density and distribution of particulates and recognizable microorganisms were observed from one layer to another.

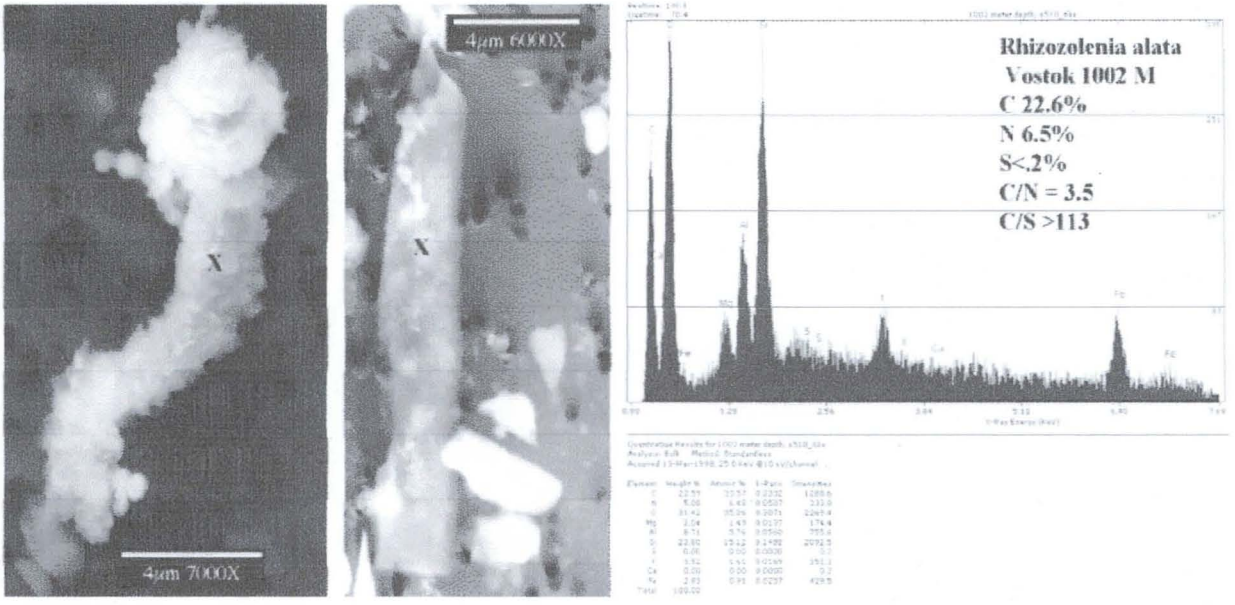


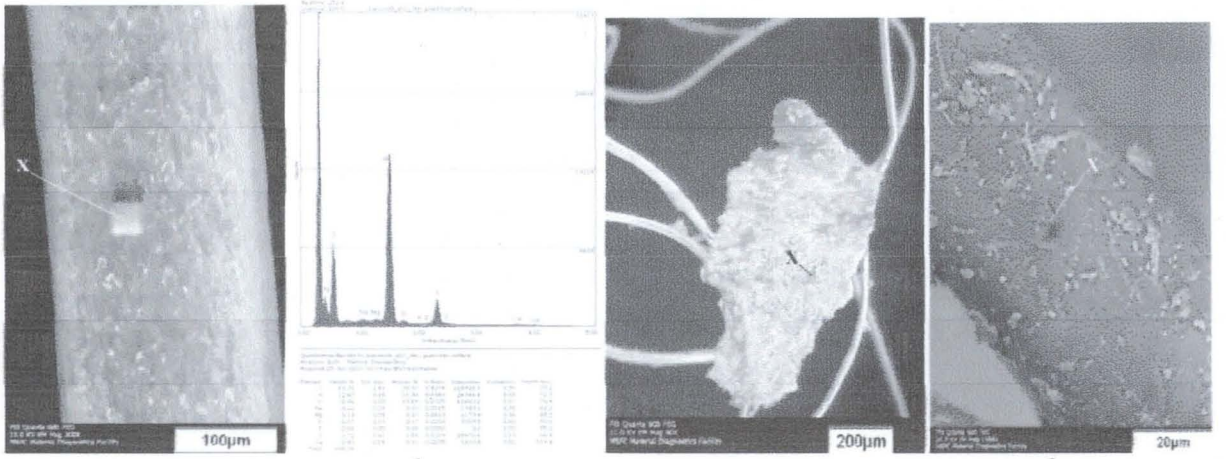
Figure 7. ESEM images of Pleistocene microbiota from Vostok ice cores: **a.** trichomic cyanobacterium from 1249M depth; **b.** diatom (cf. *Rhizosolenia alata* var. *gracillima*) from 1002 M deep ice layer with **c.** EDS spectrum at spot X.

Figure 7.a.is an Electroscan ESEM images of a helical coiled cryopreserved filament from the 1249 M layer (~ 80,000 yrs) at Vostok. This filament has an emergent trichomic structure interpreted as a moprphotype of a filamentous cyanobacteria of the Order Oscillatoriacæ. **Figure 7.b.** is an ESEM image of a well-preserved diatom from 1002 meters depth (age ~70,000 yrs). This diatom has been identified as *Rhizosolenia alata* var. *gracillima* (Cleve) Gran, which is one of the smaller representatives of the family *Rhizosoleniacæ*. Some of the *Rhizosolenia* are gigantic. *Rhizosolenia. styliformis* has been reported with valve diameter up to 100 μm and lengths exceeding 1500 μm. The detection of this *R. alata* in the Vostok ice is interesting, since the family *Rhizosoleniaceae* is a marine planktonic diatom with no known freshwater forms.[63] The EDS spectrum in **Fig. 7.c.** shows that the nitrogen content of this ~80,000 year old diatom from the Vostok ice core is similar to that of modern living diatoms.

2.5 Images and EDS abundances of Hair/Tissue of Pleistocene Mammoth and Pre-dynastic Egyptian Mummy

The FEI Quanta FEG Scanning Electron Microscope was used to obtain high resolution images and conduct EDS Elemental analyses of ~15,000 year old samples of Wooly Mammoth guard hair, undercoat hair and tissue. of *Mammuthus primigenius,* Blumenbach, 1799. These samples were collected by the author in 1999 during the *International Beringia Expedition* to the Kolyma Lowlands of Northeastern Siberia. In 1977, an exceptionally well-preserved 40,000 year old frozen carcass of the baby Woolly Mammoth *Dima* was recovered near Magadan, Siberia from the permafrost near a tributary of the Kolyma river.[64] **Figure 8.a.** is an image of a ~200 μm diameter guard hair.

The square spot on this image is the result of beam damage to the hair by exposure to the 10 KeV electron beam as the EDS data for this area shown in **Fig. 8.b.** were obtained. **Figure 8.c.** is an image of a small fragment of Mammoth tissue with several undercoat hairs still attached. The **X** is linked to the dark square produced by beam damage when the EDS data was taken on the mammoth tissue sample and the 5000 year old hair **Fig. 8d.** from the pre-dynastic Egyptian mummy. All of these ancient hair and tissue samples show strong Nitrogen peaks with C/N and C/S ratios similar to living biological materials. Beam damage is the result of heating breaking down the proteins, polysaccharides and other organics. It is very frequently observed when studying modern, Holocene and Pleistocene biological materials. Beam damage is only rarely seen during investigations of the filamentous microfossils found in the carbonaceous meteorites or in the mineralized microfossils and macrofossils found in Archaean, Proterozoic, and Phanerozoic (except Pleistocene and Holocene) rocks on Earth.



a. **b.** **c.** **d.**

Figure 8.a ***Mamuthus primigenius*** guard hair with **b.** EDS spectra at spot X and **c.** Mammoth tissue and undercoat hairs and **d.** FEI Backscattered electron detector image of pre-dynastic Egyptian mummy hair (5,000 Yrs.) showing beam damage at spot X where EDS spectral data shows strong Nitrogen peak (C 64.3%; N 10.7%; O 19.6%; S 1.8%; P <.5%; C/N=6; C/S=52; C/O=3.3).

2.6 Images and EDS elemental abundances of modern diatoms, bacteria and cyanobacteria

The investigation also included determination of the ratio of biogenic elements in diatoms preserved for almost over 150 years on Herbarium sheets at the Henri Van Heurck Museum in Antwerp, Belgium as well as well as living cyanobacteria and bacteria (**Fig. 9**). The sample in **Fig. 9.a.** was collected by Hoffman Bang in 1816 and designated *Bangia quadripunctata* with an epiphytic filament attached to the diatoms. The EDS data for the filament at spots X and Y and the diatoms at spot Z are given in Table 2. The 500X FEI Quanta 600 FEG scanning electron microscope image of the type series of the diatom is shown in **Fig. 9.b.** These small naviculoid diatoms were collected by Lenormand in France in 1834 and mounted on an herbarium sheet and subsequently described by Kutzing[65] as *Schizonema lenormandi* Kutzing, 1849. These diatoms were observed by the author to emerge from broken ends of their gelatinous sheaths and begin swimming after sterile distilled water was added to a well slide containing a small fragment of the filaments. [66, 67] **Figure 9.c** and **d.** are Hitachi FESEM images of the living cyanobacteria *Lyngbya subtilis* and **d.** a collapsed filament of *Oscillatoria lud*,grown in pure culture at the NASA/NSSTC Astrobiology Laboratory. **Figure 9.e.** is a FESEM image of a living sample of axenic culture of the type strain *Spirochaeta Americana*, Hoover 2002 that was isolated from the sulfur rich black mud sediments of Mono Lake in California. The EDS spectral data **(Fig. 9.e)** at spot **X** on a clump of the tiny helical coiled filaments shows a clearly delineated Nitrogen peak (N = 10.7%, atomic) with C=62.3%; O = 20.8% and S = 0.4% (C/N=5.8; C/O=2.7; C/S= 156. **Figure 9.f.** is a FESEM image of a living sample of axenic culture of type strain of microbial extremophiles[68] *Spirochaeta Americana*, Hoover 2003 and the EDS spectra showing a clearly delineated Nitrogen peak is in **Fig. 9g.** The EDS data for the other samples are provided in Table 2

The EDS spectral data shown in Table 2 establish that the diatoms and filaments, which have been stored in dry condition since 1834, have Nitrogen levels and C/N ratios are consistent with living microorganisms. These measurements on mammoth and mummy hair and tissue and diatoms and their associated gelatinous envelopes preserved dry on herbarium sheets for over a century and a half provide solid data indicating that the loss of nitrogen from biological samples occurs over geological rather than historical time scales. Consequently the absence of

nitrogen in the filamentous microstructures found in freshly fractured interior surfaces of the carbonaceous meteorites indicates they should be interpreted are indigenous remains rather than recent microbial contaminants.

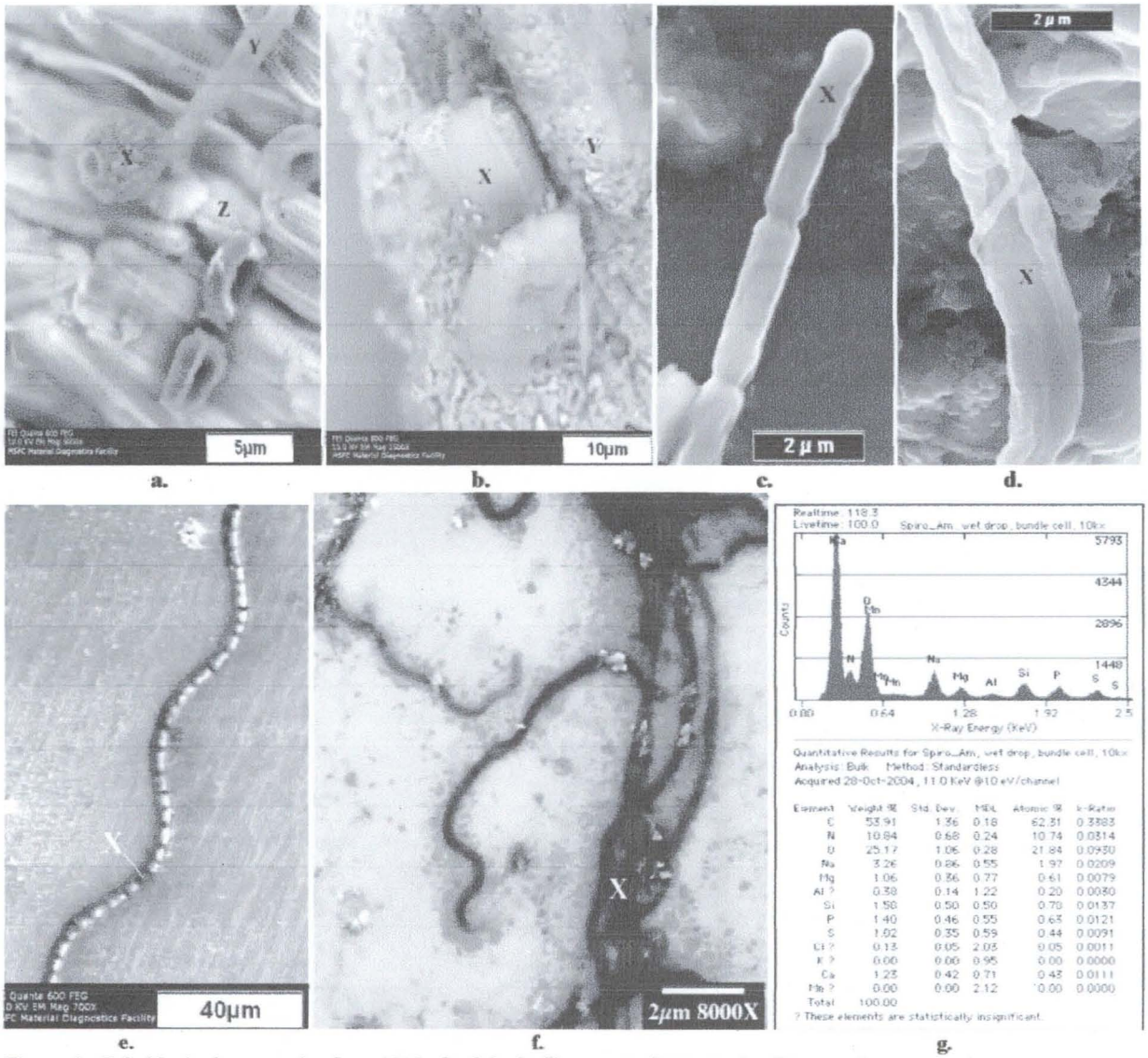


Figure 9. Dried herbarium samples from 1816 of epiphytic filamentous forms on the diatom **a.** *Bangia quadripunctata* and **b.** the diatom *Schizonema lenormandi* Kutzing, 1849 marked where EDS data were obtained. **c.** the living cyanobacterium *Lyngbya subtilis* and **d.** a collapsed filament from an axenic culture of *Oscillatoria lud*, **e.** living spiral filament of the cyanobacterium *Arthrosopirs platensis* showing beam damage and **f.** living sample of axenic culture of type strain of microbial extremophiles *Spirochaeta Americana*, Hoover 2003. and **f.** (EDS spectral data at spot **X** shows N = 10.7% (atomic) and S = 0.4% (atomic) and **g.** living EDS spectral data for other samples provided in Table 2.

3. BIOGENIC ELEMENT RATIOS

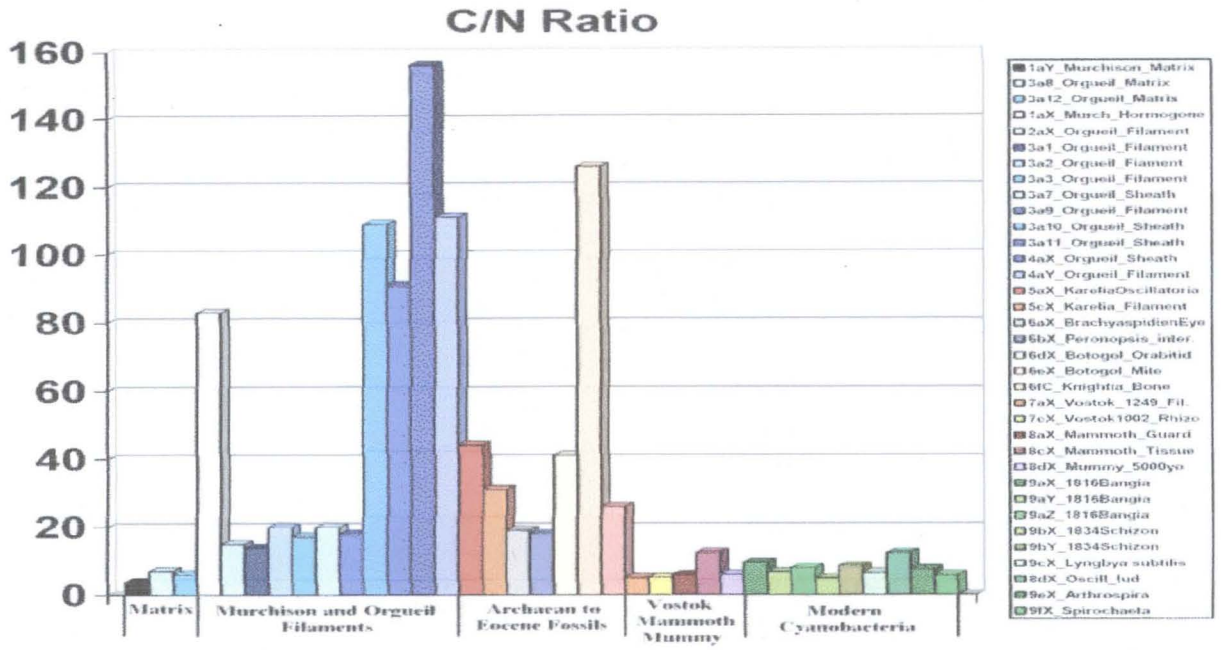
Table 2 provides the compilation of the elemental abundances measured for a number of the macrofossils and microfossils from carbonaceous meteorites, Archaean, Proterozoic and

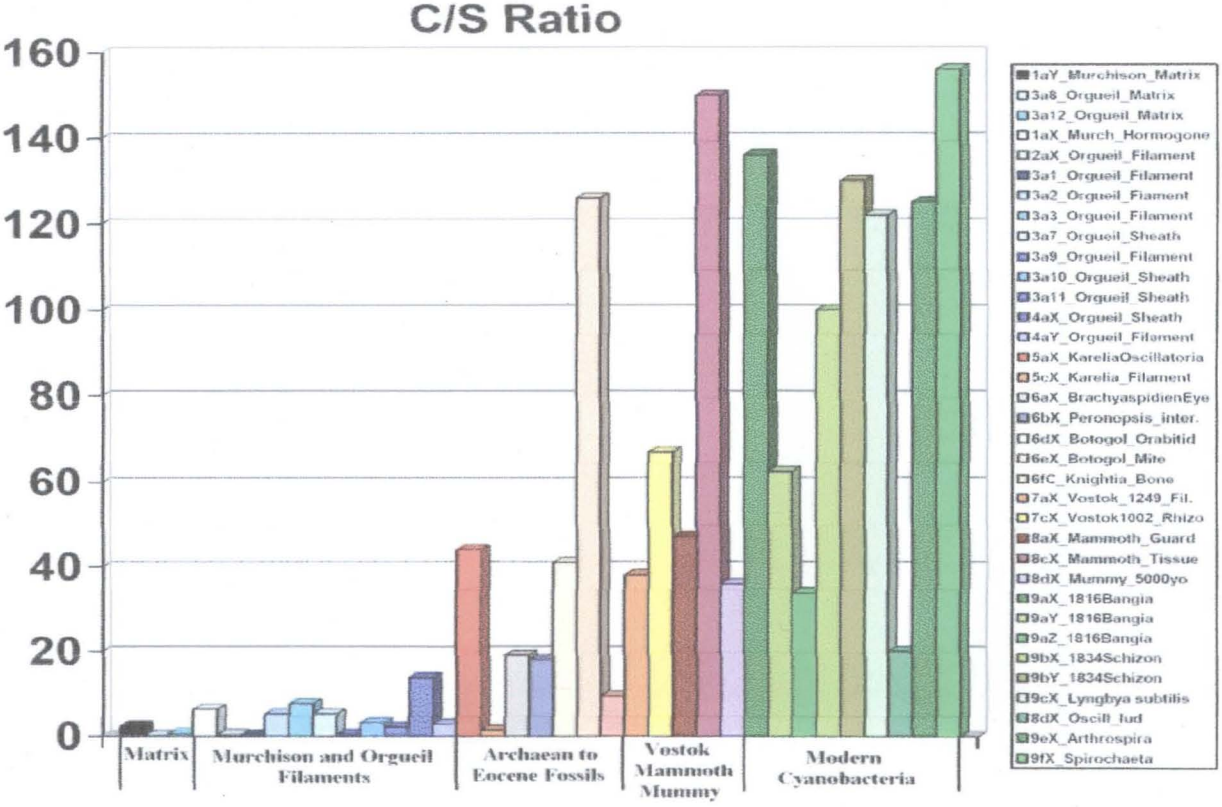
Phanerozoic rocks and Pleistocene, Holocene and living biological materials studied during this investigation.

TABLE 2. Biogenic Elements in Carbonaceous Meteorites, Archaean to Pleistocene Fossils, Holocene and Recent Diatoms and Cyanobacteria

FORM	C	O	N	P	Mg	S	Si	Al	Fe	C/O	C/N	C/S
Murchison_Matrix_1aY	6.2	50.1	1.6	0.3	7.5	2.6	10.8	0.9	6.1	0.12	3.8	2.4
Orgueil_Matrix_3a8	3.9	6.9	0.0	0.4	0.6	41.4	1.2	0.2	44.1	0.6	>7	0.1
Orgueil_Matrix_Balls_3a12	1.2	6.4	0.2	<.5	1.1	1.9	1.7	0.0	86.4	0.2	6.0	0.6
Murchison_Hormogon_1aX	41.7	16.8	<.5	1.2	7.4	6.1	18.5	0.4	6.4	2.5	>83	6.4
Orgueil_Filament_2aX	7.6	24.3	<.5	<.5	13.4	36.0	6.1	0.6	10.5	0.3	>15	0.3
Orgueil_Filament_3a1	7.2	39.8	0.8	0.2	13.9	34.8	1.3	0.2	1.1	0.18	>14	0.2
Orgueil_Filament_3a2	19.8	17.2	<.5	1.0	17.3	41.5	1.8	0.0	1.0	2.8	>20	0.5
Orgueil_Filament_3a3	22.4	35.4	1.3	0.2	10.9	23.4	2.9	0.4	1.7	0.6	17.2	7.7
Orgueil_Sheath_3a7	46.5	20.0	1.0	0.8	7.4	8.8	12.1	1.4	1.2	2.3	20	5.3
Orgueil_Filament_3a9	8.9	35.1	<.5	0.2	14.2	33.8	4.0	0.3	3.1	0.3	>18	0.3
Orgueil_Sheath_3a10	54.8	13.4	<.5	0.6	6.1	17.2	6.2	0.6	0.5	4.1	>109	3.2
Orgueil_Sheath_3a11	45.8	14.8	<.5	0.6	8.5	21.5	3.9	0.6	0.5	3.1	>91	2.1
Orgueil_Sheath_4aX	>78	9.2	<.5	<.5	2.3	5.9	0.6	0.0	0.0	8.9	>156	13.8
Orgueil_Filament_4aY	55.8	11.8	<.5	<.5	11.6	20	0.7	0.0	0.0	4.7	>111	2.8
Karelian_Oscillatoria_5aX	22.2	28.4	<.5	<.5	0.7	0.0	21.2	9.1	0.7	0.8	>44	>44
Karelian_Filament_5cX	15.6	6.8	<.5	1.3	0.0	11.2	1.6	52.3	0.0	2.3	>31	1.4
Brachyaspidien_Eye_6aX	9.6	44	<.5	<.5	2.0	0.0	24.5	5.6	2.2	4.4	>19	>19
Peronopsis_interstricta_6bX	9	40	<.5	<.5	1.6	0.0	4.5	2.5	4.9	0.2	>18	>18
Botogol_Orabitid_6dX	20.4	15.8	<.5	<.5	<.5	<.5	26.9	11.9	3.5	1.3	>41	>41
Botogol_Filament_6eX	62.9	3.7	<.5	<.5	0.0	0.3	15.4	8.8	2.3	17	>126	>126
Knightia_Bone_6fC	13.2	37.6	<.5	12.4	1.3	1.4	5.7	1.7	1.6	0.3	>26	9.4
Vostok_1249M_7aX	19.0	66.7	3.7	<.5	<.5	<.5	1.1	1.8	0.0	0.3	5.1	>38
Vostok_1002M_Rhizo_7cX	33.6	35.1	6.5	<.5	1.5	<.5	15.1	5.8	0.9	1.0	5.2	>67
Mammoth_guard_8aX	70.4	15.6	11.9	0.0	0.1	1.5	0.2	0.0	0.0	4.5	5.9	46.9
Mammoth_tissue_8cX	59.4	26.8	4.8	0.1	0.7	0.4	6.3	0.0	0.7	2.2	12.4	150
Mummy_Hair_8dX	64.4	19.6	10.7	0.0	0.1	1.8	0.2	0.0	0.0	3.3	6.0	36
1816_Bangia_9aX	67.9	21.9	7.1	0.2	0.1	0.5	1.7	0.0	0.0	3.1	9.6	136
1816_Bangia_9aY	62.4	22.4	9.3	0.7	0.0	1.0	3.1	0.2	0.0	2.8	6.7	62.4
1816_Bangia_9aZ	27.0	46	3.4	0.1	0.0	0.8	17.4	0.5	0.0	0.6	7.9	33.8
1834Schiz_lenormandi_9bX	50.0	30.4	10.2	0.0	0.0	<.5	8.5	0.9	0.0	1.6	4.9	100
1834Schiz_lenormandi_9bY	64.9	20.8	7.7	0.0	0.3	<.5	3.1	2.4	0.0	3.1	8.4	130
Lyngbya_subtilis_9cX	61.5	25.7	9.5	1.5	0.0	<.5	0.4	0.2	0.0	2.4	6.5	122
Oscillatoria_lud_9dX	51.9	20.9	4.2	0.1	5.4	2.6	0.4	0.0	0.0	2.5	12.3	20
Arthrospira_platensis_9eX	62.3	25.0	8.3	0.0	0.0	<.5	0.0	0.0	0.0	2.5	7.5	125
Spirochaeta_americana_9fX	62.3	21.8	10.7	0.8	0.2	0.4	0.8	0.2	0.0	2.7	5.8	156

The ratios of the biogenic elements C/N; C/S and C/O ratios for the carbonaceous meteorites and terrestrial fossils (Archaean to Eocene) and the Pleistocene and Holocene hair, tissue and filaments Woolly Mammoths, pre-dynastic Egyptian mummies and modern and living cyanobacteria, diatoms and other microbial extremophiles demonstrate. Although the C/O ratios are not very helpful, it is clear that C/N and C/S ratios may be effectively used to distinguish between indigenous fossils and recent microbiological contaminants. Plots of these biogenic ratios are shown in **Figure 10.**


C/N Ratio
160
140
120
100
80
60
40
20
0
Matrix
Murchison and Orgueil Filaments
Archaean to Eocene Fossils
Vostok Mammoth Mummy
Modern Cyanobacteria
1aY_Murchison_Matrix
3a8_Orgueil_Matrix
3a12_Orgueil_Matrix
1aX_Murch_Hormogone
2aX_Orgueil_Filament
3a1_Orgueil_Filament
3a2_Orgueil_Fiament
3a3_Orgueil_Filament
3a7_Orgueil_Sheath
3a9_Orgueil_Filament
3a10_Orgueil_Sheath
3a11_Orgueil_Sheath
4aX_Orgueil_Sheath
4aY_Orgueil_Filament
5aX_KareliaOscillatoria
5cX_Karelia_Filament
6aX_BrachyaspidienEye
6bX_Peronopsis_inter.
6dX_Botogol_Orabitid
6eX_Botogol_Mite
6fC_Knightia_Bone
7aX_Vostok_1249_Fil.
7cX_Vostok1002_Rhizo
8aX_Mammoth_Guard
8cX_Mammoth_Tissue
8dX_Mummy_5000yo
9aX_1816Bangia
9aY_1816Bangia
9aZ_1816Bangia
9bX_1834Schizon
9bY_1834Schizon
9cX_Lyngbya subtilis
8dX_Oscill_lud
9eX_Arthrospira
9fX_Spirochaeta


C/S Ratio
160
140
120
100
80
60
40
20
0
Matrix
Murchison and Orgueil Filaments
Archaean to Eocene Fossils
Vostok Mammoth Mummy
Modern Cyanobacteria
1aY_Murchison_Matrix
3a8_Orgueil_Matrix
3a12_Orgueil_Matrix
1aX_Murch_Hormogone
2aX_Orgueil_Filament
3a1_Orgueil_Filament
3a2_Orgueil_Fiament
3a3_Orgueil_Filament
3a7_Orgueil_Sheath
3a9_Orgueil_Filament
3a10_Orgueil_Sheath
3a11_Orgueil_Sheath
4aX_Orgueil_Sheath
4aY_Orgueil_Filament
5aX_KareliaOscillatoria
5cX_Karelia_Filament
6aX_BrachyaspidienEye
6bX_Peronopsis_inter.
6dX_Botogol_Orabitid
6eX_Botogol_Mite
6fC_Knightia_Bone
7aX_Vostok_1249_Fil.
7cX_Vostok1002_Rhizo
8aX_Mammoth_Guard
8cX_Mammoth_Tissue
8dX_Mummy_5000yo
9aX_1816Bangia
9aY_1816Bangia
9aZ_1816Bangia
9bX_1834Schizon
9bY_1834Schizon
9cX_Lyngbya subtilis
8dX_Oscill_lud
9eX_Arthrospira
9fX_Spirochaeta

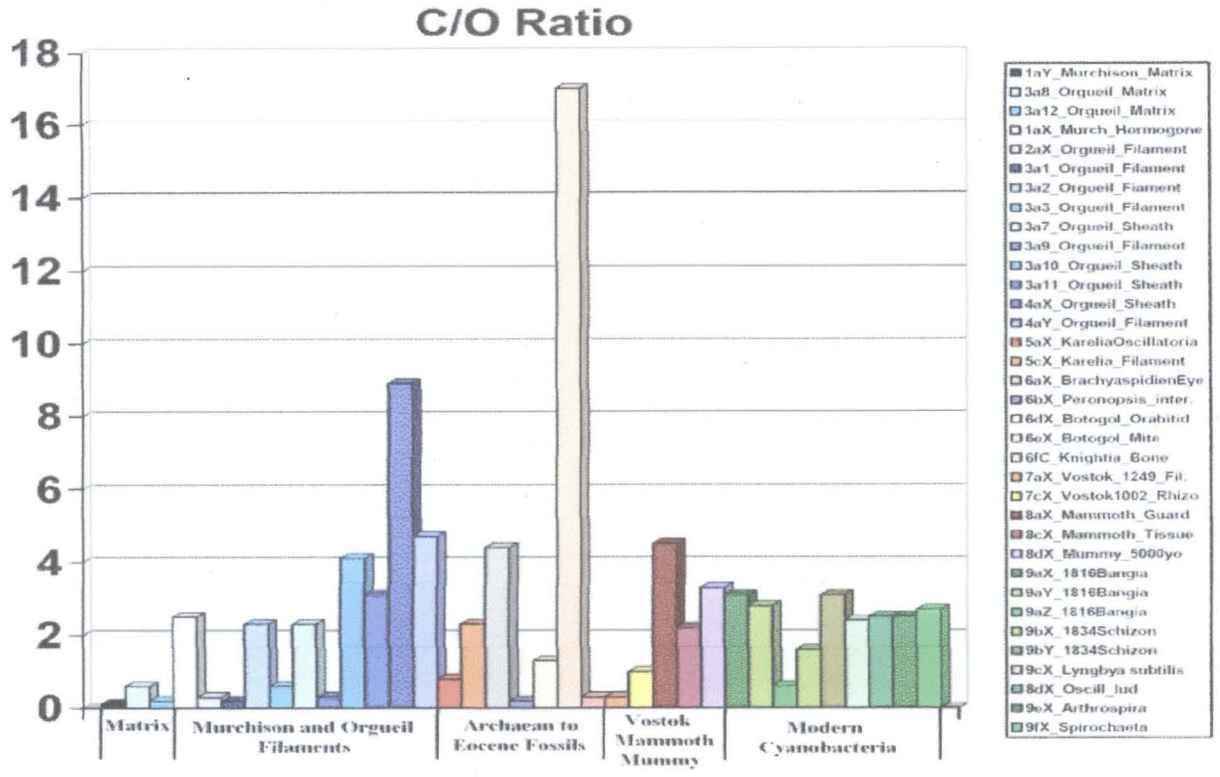


Figure 10. Plots of the C/N, C/S and C/O ratios for the fossils and living biological materials described.

A summary of the range of these biogenic element ratios is as follows:

	C/N	C/S	C/O
Orgueil Filaments:	**20 to > 156**	**0.2 to 13.8**	**0.3 to 8.9**
Archaean Filaments (Karelia)	**31 to > 34**	**1.4 to >44**	**2.3 to 4.4**
Cambrian and Ordovician Trilobites	**>18 to >41**	**>18 to >41**	**0.1 to 4.4**
Devonian Microfossils & Eocene Fish	**>41 to >126**	**>41 to >126**	**1.3 to 82**
Pleistocene/Holocene Hair	**5.1 to 12.4**	**20 to 156**	**0.3 to 2.7**

Although the FESEM EDS is not extremely sensitive to nitrogen, it is certainly capable of detecting nitrogen at the 0.5% level (5,000 ppm),. Under ideal conditions nitrogen can be detected as low as 0.2% (2,000 ppm) or less. Nitrogen levels as low as 0.2% were detected in the meteorite rock matrix and in a form interpreted as an akinete in the Orgueil meteorite. However, to be conservative the value 0.5% was used to avoid division by zero to determine minimum C/N levels of Table 2. EDS studies carried out with the same instrumentation have repeatedly demonstrated that the abundances of major biogenic elements found in the Orgueil filaments are distinctly different from that found in living organisms (bacteria, archaea, and cyanobacteria grown in axenic cultures, in enrichment assemblages, and in natural ecosystems), recently (~190 years or less) dead prokaryotic and eukaryotic microorganisms (cyanobacteria and diatoms of the Grunow Collection, Henri van Heurck Museum), or in ancient (~32,000 years) cryopreserved Pleistocene wood, moss, and bacteria from the Fox Tunnel of Alaska. The EDS analysis indicates that nitrogen is well above the level of detectability in all of the living and dead (herbarium material) cyanobacteria with abundances ranging from 4.6% to 12.6%. Nitrogen was also undetectable in the fossilized cyanobacteria found in the proterozoic phosphorites of Khubsughul, Mongolia and in the archaean rocks of Northern, Karelia in Siberia.[69-71]

4. CONCLUSIONS

The striking feature about the Archaean filaments and the Cambrian, Ordovician, Devonian and Eocene fossils, as well as the forms interpreted as microfossils of filamentous prokaryotes in the Orgueil and Murchison

meteorites is the almost universal absence of detectable nitrogen even though carbon is sometimes extremely abundant. However, the nitrogen levels detected in long dead biological materials (ancient filaments from Vostok ice cores, Woolly Mammoth hair, and a large number of hair and tissue samples from ancient mummies from Egypt and Chile) are not notably different from those found in living and recently dead cyanobacteria and other biological materials. The C/S ratios of many of the meteorite filaments, and known terrestrial fossils are also clearly distinguishable from modern and Pleistocene biological materials

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