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ELEMENTAL ANALYSIS OF NON-LIVING PARTICLES IN PICOPLANKTON FRACTIONS FROM OLIGOTROPHIC LAKE WATER

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Abstract—Electron microscopic (TEM) study of bacterial-sized planktonic fractions recovered from the water column samples of a small oligotrophic lake, consistently showed the presence of non-living organic and inorganic particles as prominent parts of the micro habitat. An all important objective of this work was to determine the composition and possible origins of the minute particles. They were of several morphological types, described by transmission electron microscopic (TEM) appearance as: spherical, oblong, cuboidal, plates and fibrillar. The TEM used in the scanning transmission mode (STEM) in conjunction with the Princeton Gamma Tech (PGT) System 4 energy dispersive X-ray spectrometer (EDX) allowed the determination of the elemental composition of individual particles.

The particles referred to as spherical were electron dense and were less than 1 μm in diameter. They were mainly composed of either Fe or Ti but not both. Cuboidal iron particles 37–75 nm in diameter were found in about equal abundance, but they were arranged in chains of individuals. Oblong particles were from 0.18 to 0.2 nm in width to 0.2 to 0.45 μm in length. Most were composed mainly of Ca, S and O but other elemental compositions were observed also. Plates with jagged edges composed of Al and Si were somewhat electron transparent. Both were less than 1 μm in largest dimension. Fibrillar particles were most abundant and were composed of Fe, Si, Cl, K, Na, O and C. They ranged from 90 Å to 37 nm in thickness and 1 μm to several μm in length. Particles of planktonic origin were observed with regularity including fragments of diatom cell walls and scales. These particles were composed of Si and O. Hexagonally shaped virus-like particles of several sizes were observed also. The relationship between elements in micro particles and their possible origins are discussed.

Key words—picoplanktonic fraction, non-living particles, oligotrophic lake water, elemental analysis

INTRODUCTION

Electron microscopic studies of bacteria-sized plankton recovered as a biomass from large water column samples (Corpe and Jensen, 1992) also revealed the presence of numerous, minute particles of various morphologies. These particles were described in a preliminary way (Jensen and Corpe, 1991) and likened to observations made earlier by Johnson and Seiburth (1982) of particulates in a marine picoplanktonic biomass.

Microbial metabolism and growth in natural waters may be stimulated by suspended particulate matter (ZoBell, 1943) perhaps because soluble nutrients are adsorbed and associated with cells growing at particulate interfaces (Heukelekian and Heller, 1940). Perret *et al.* (1990) suggested that trace elements could be transported by colloidal particles suspended in natural waters, thus controlling biogeochemical cycling. They also confirmed that pH, penetration of light and other physico-chemical properties of water could be affected by aquatic colloids as well (Perret *et al.*, 1991). The precise mechanisms by which exchanges of trace elements from colloids occur are largely unknown. The present study categorizes the representative free particles with regard to size, shape, composition and other details that allow the speculation about the origin and possible role of each in the ecology of a pristine aquatic system.

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MATERIALS AND METHODS

Three small oligotrophic lakes, Arthur, Sutherland and Alec Meadow, located in the Black Rock Forest near Cornwall, N.Y. were studied previously (Corpe and Jensen, 1992) for their picoplankton content. Water samples for this study were collected from Arthur's Lake once a month (May–November) in 1990. The other two were sampled twice in 1990 and 1991. The separation of picoplankton from other aquatic organisms was carried out with screens, filters and high speed centrifugation adapting the procedures developed by Johnson and Sieburth (1979). Surface water samples were by bucket. An open Niskin-type bottle was closed at a depth of 3.6 m, 0.3 m above the sediment, to provide deep samples. The temperature and pH values for each sample were recorded at the time of collection.

The glutaraldehyde (3%) fixed final concentrate (biomass) was stained with O_2O_4 and then embedded in Epon

(Luft, 1961). Thin sections, cut with a diamond knife, were post stained with uranyl acetate. Portions of the biomass, some glutaraldehyde fixed and some with no fixation, were air dried on Formvar-coated copper or nickel grids. Samples were examined in the TEM (Hitachi-7000) at 75 kV.

Elemental analysis was carried out using the electron microscope operating in the scanning transmission mode (STEM) at 75 kV in conjunction with a PGT System 4 EDX. An area of the grid was first examined in the TEM mode. To analyze a particle the instrument was then set in the STEM mode. The beam was then stopped from scanning (spot mode) and placed on the particle. The X-rays produced from elements in the spot were collected with EDX. Analysis was carried out at 100,000 × magnification. At this magnification the spot diameter was about 20 nm. This allowed for very good resolution because the X-rays generated will only come from the spot area (Baxter and Jensen, 1980). The condenser lens current was adjusted to give a count rate of approx. 1000 cps. A 100 s count time was used. A spectrum from the formvar or Epon was taken for each analysis and subtracted from the spectrum of the item of interest. Negatives were taken of particles examined either before or after spectroscopic analysis.

RESULTS

The picoplankton biomass was first viewed in thin sections in the TEM mode and negatives taken with the resulting excellent resolution of cells, but also non-cellular particles of several morphotypes which are shown in Fig. 1. The particles were put into groups on the basis of their morphology and form with the aid of both sectioned and air-dried material. A micrograph of the air-dried biomass which shows five of the seven small particle types found is shown in Fig. 2.

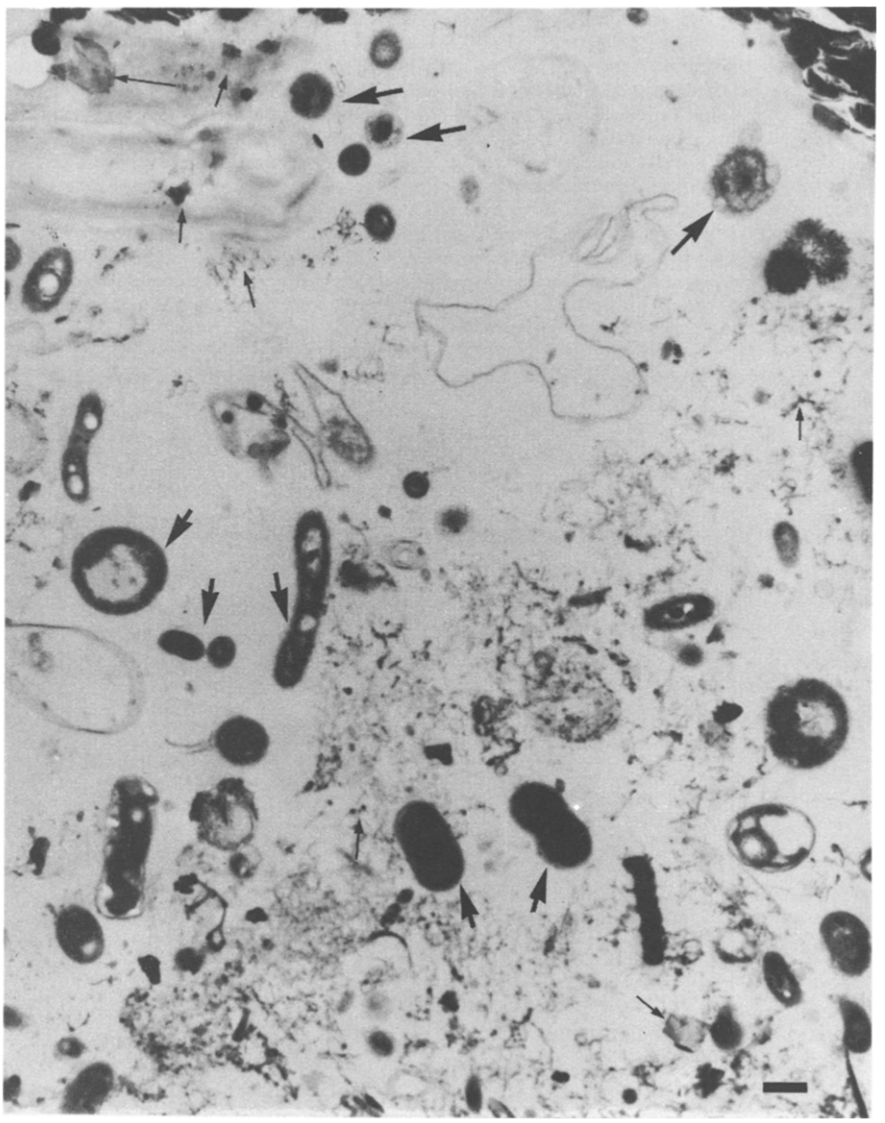


Fig. 1. Low magnification of an electron micrograph of a thin section of a concentrated biomass. Both particles (small arrows) and cells (large arrows) are shown. Bar = 0.2 μm.

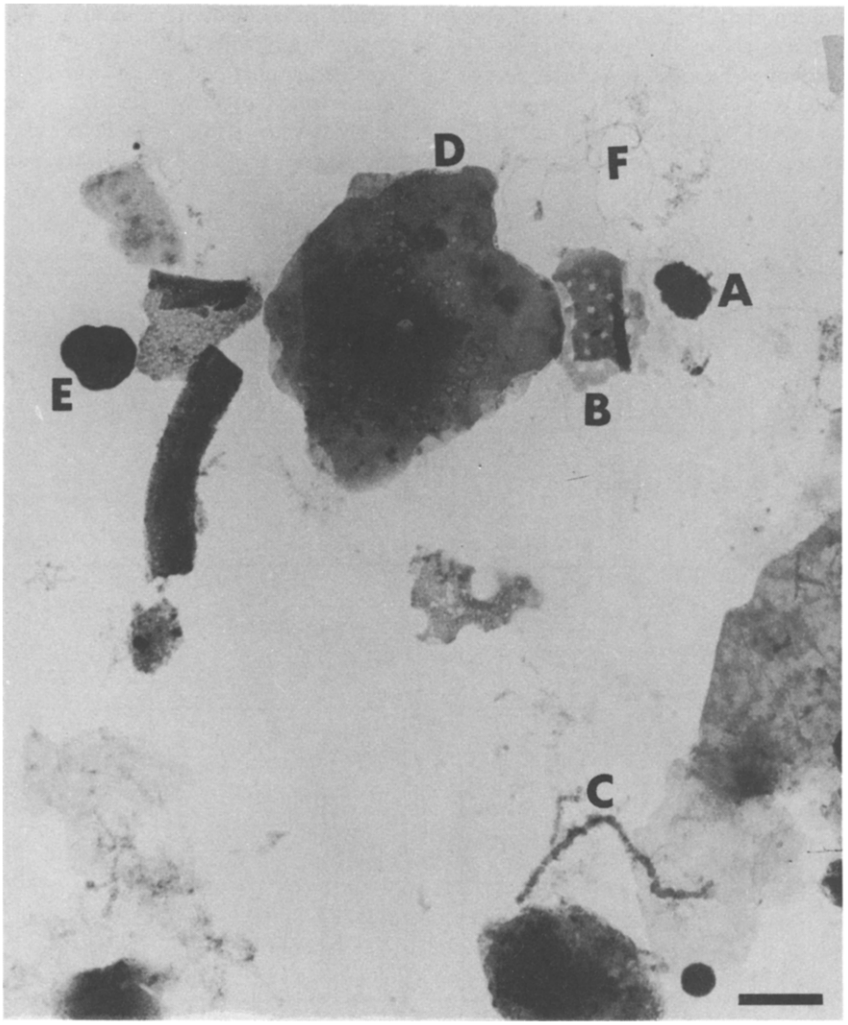


Fig. 2. Five types of particles are shown in this single air-dried preparation. The particle types are labeled and spectra are shown in Fig. 3. Bar = 0.2 μm.

Spherical bodies [Fig. 2(A) and (E)]

Numerous spherical bodies with a size range of 0.16–0.27 μm were observed. It was not possible, however, to predict differences in elemental composition on the basis of form or density. The spherical bodies had two main elemental compositions—Fe [Fig. 3(A)] and Ti [Fig. 3(E)]. The bodies with Fe [Fig. 3(A)] had, in addition, small amounts of O, C and very small amounts of S, Si, Al and Mg. The bodies which contained Ti had a small O peak and a trace of Al and S.

Oblong bodies [Fig. 4(A)]

These bodies varied in diameter from 0.18 to 0.2 μm and in length from 0.2 to 0.45 μm. They superficially resembled bacteria. Analysis of the bodies showed they contained mainly (a) Ca, S, O and small amounts of Mg or (b) Ca, P, Mn, S, Si, Mg, C and O or (c) were composed of mainly Ca, S, Si, Al, Fe, O and Mg or (d) were composed of mainly Ca and S with smaller amounts of O, Na, Mg, Si and K [Fig. 4(B)].

Cuboidal bodies [Fig. 4(C)]

Only one kind of body had a cuboidal morphology. These were composed of Fe with a small amount of O [Fig. 4(D)]. They are identical in morphology and elemental composition to the magnetite bodies found in magnetotactic bacteria which are a part of the picoplankton. Their dimensions range from 37 to 75 nm, and they seem to be arranged in chains.

Plates with jagged sides [Fig. 2(D)]

These bodies were found in great numbers in the samples studied. They varied in diameter from 0.6 to 0.9 μm. Elemental analysis showed that they were composed of Al and Si [Fig. 3(D)]. We suggest they are probably clay particles.

Fibrils [Fig. 2(C) and (F)]

Two different kinds of fibrils were observed with great frequency in the samples. Larger fibrils were about 37 nm in thickness and up to several

micrometers in length [Fig. 2(C)]. Elemental analysis of the larger fibrils shows they are composed of a substantial amount of Fe and smaller amounts of Si, S, Cl, K, Na, O and C [Fig. 3(C)].

The smaller fibrils were about 90 Å in thickness and often several μm in length [Fig. 2(F)]. Their small mass made it difficult to determine their composition with certainty. Analysis of the fibrils was attempted in areas where they had been compressed together. In these areas elemental analysis showed the *same* elemental composition as the larger fibrils. The fibrous particles tend to

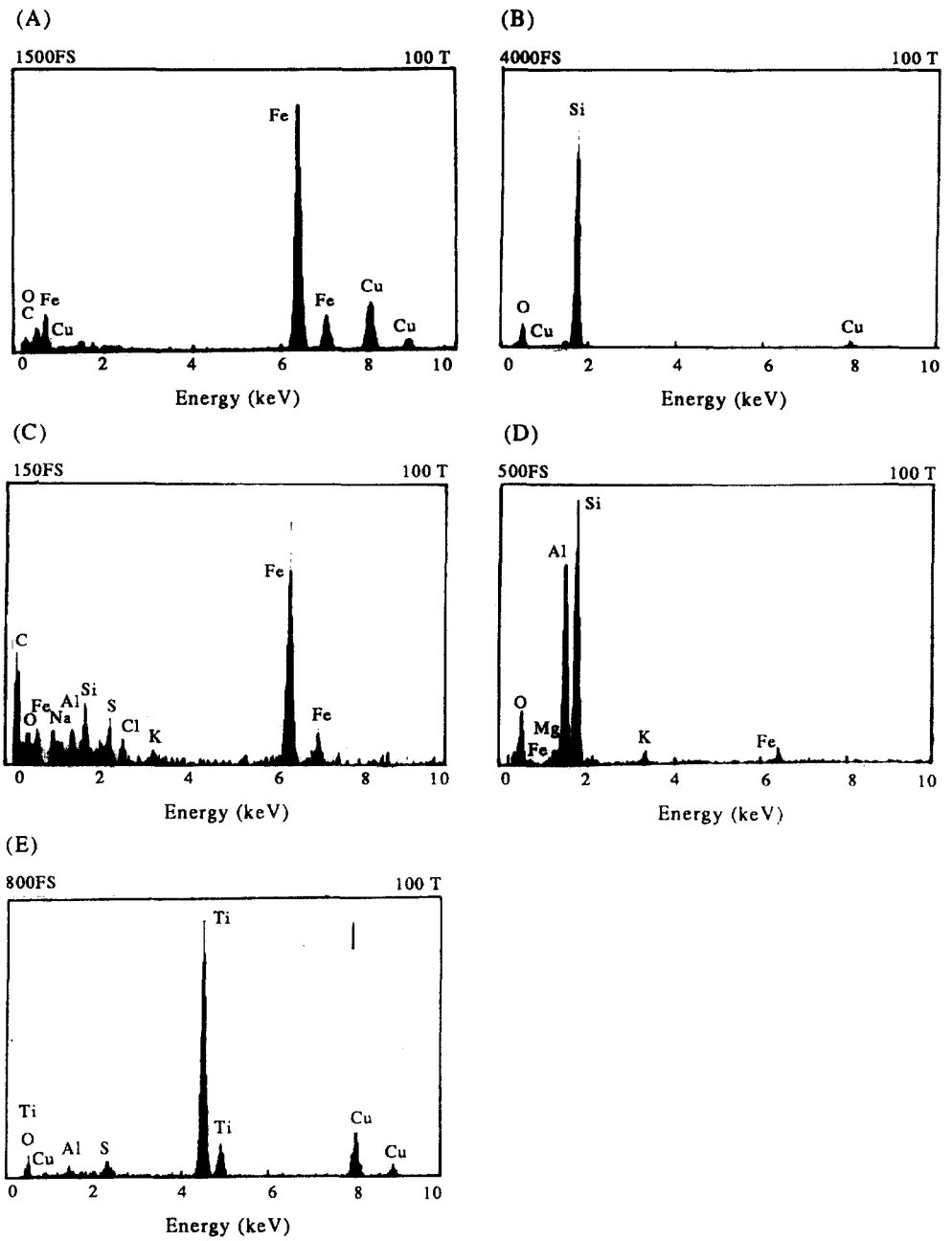


Fig. 3. (A) Elemental analysis of dense spherical particles such as are shown in Fig. 2 particle A. The major constituent is Fe with minor constituents of O and C. (B) Elemental analysis of particles such as are shown in Fig. 2 particle B. They are probably pieces of diatom cell walls or scales. The main element present is Si with small amounts of O. (C) Elemental analysis of thick filamentous particles such as are shown in Fig. 2 particle C. The main element present is Fe with smaller peaks generated by C, O, Na, Al, Si, S, Cl and K. (D) Elemental analysis of plate-like particles such as are shown in Fig. 2 particle D. The particle is composed mainly of Al and Si with smaller amounts of O, Mg, K and Fe. (E) Elemental analysis of dense particles such as are shown in Fig. 2 particle E. The composition of the spherical particles cannot be determined by their morphology. The major element present is Ti with small amounts of O, Al and S.

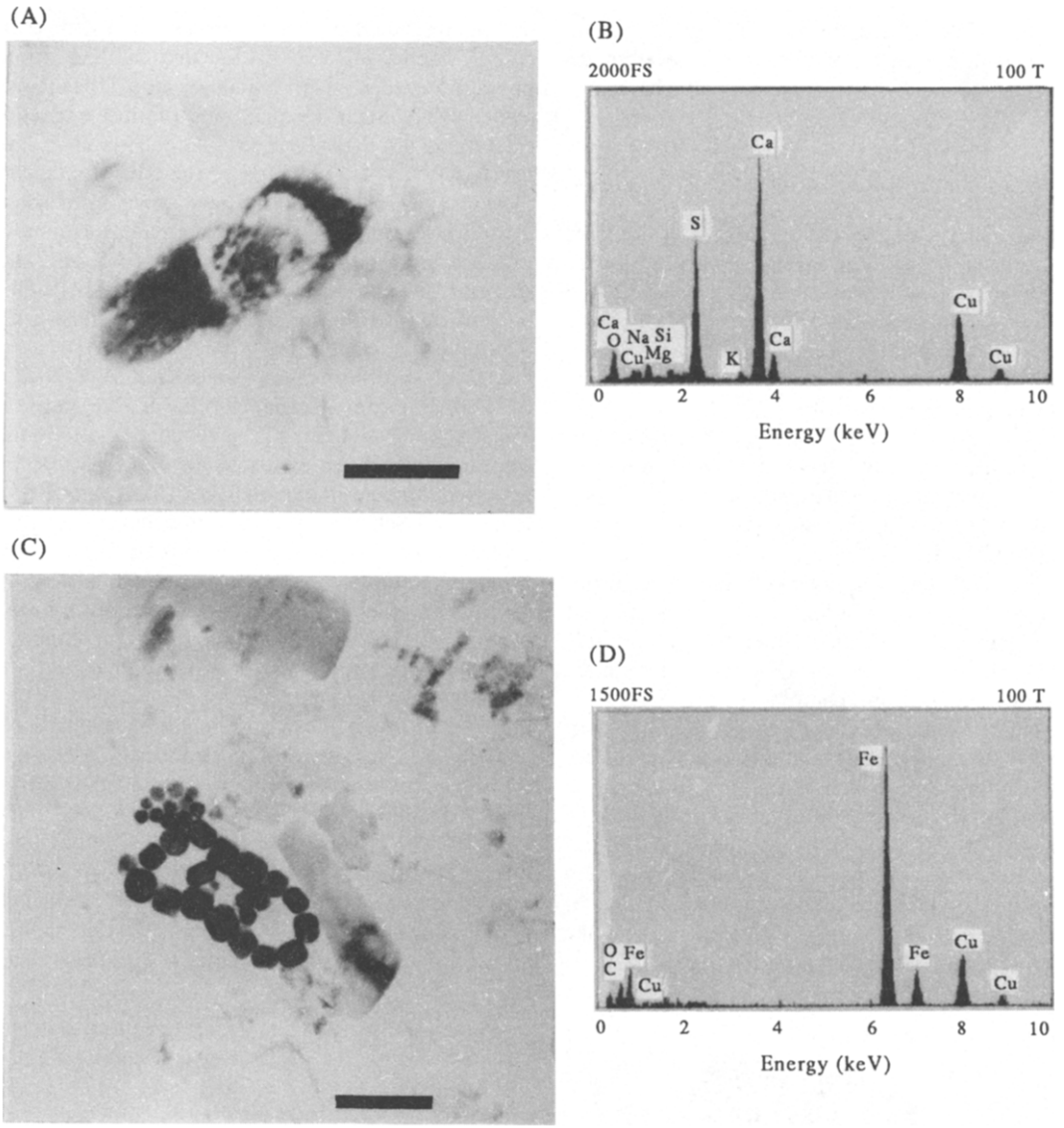


Fig. 4. (A) Oblong bodies which occur on a regular basis in the fraction. The central part of the body has been eroded by the beam during analysis. Bar = 0.2 μm. (B) Spectrum generated from one of the oblong bodies. The major elements present are Ca and S with smaller peaks generated by O, Na, Mg, Si and K. (C) Magnetite cuboidal bodies observed in a chain. Bar = 0.2 μm. (D) Spectrum generated from the cuboidal bodies. The main elements present are Fe and O.

clump together and are quite uneven in their distribution. Figure 1 depicts the biomass in a thin section. From the bottom of the micrograph diagonally upward to the right, is a mass of fibrous particles with various cells and other particles trapped within. The thin interwoven fibrils [Fig 2(F)] are clearly most numerous but impossible to count. This situation has caused the authors to describe certain particles as "more or less abundant" rather than as a percentage of the total.

Particles of plankton origin [Fig. 2(B)]

Irregular-shaped particles with dimensions of from 0.1 to 2.0 μm were observed frequently. Elemental analysis showed they were composed of Si and O [Fig. 3(B)]. These were therefore pieces of cell walls of algae such as diatoms and/or pieces of scales such as are shown in Fig. 5(A). Intact scales or frustules were not observed in the picoplankton fraction but were observed in fractions passed through only a 20 μm filter.

Virus particles (hexagonal shaped) were observed in some abundance but these did not possess enough mass to generate elemental analysis in our EDX system. Virus-lysed prokaryotic and eukaryotic cells were seen previously in water column samples (Corpe and Jensen, unpublished observations) so the presence of free hexagonally-shaped viruses were anticipated.

Surprisingly no particles were observed which had only C and O as their elemental make up such as one would expect from plant cell wall

fragments. They were presumably degraded or solubilized in the anaerobic sediments, never to reappear as colloidal-sized fragments in the water column.

DISCUSSION

The fact that only particles of very small size were found was of course determined by the method of recovery we employed. Macroorganic debris in the water would be expected to become part of the sediment and presumably be degraded anaerobically; but recalcitrant residues, such as humic substances and other colloids, seem to be the only organic residues to find their way into the water column. Other minute particulate components and some soluble substances may be eluted from soil and decaying vegetation by rainfall and washing into the water mass (Wetzel, 1983).

Organic residues from lysed cells and other products from plant and animal residues may be difficult to recognize by electron microscopy or the TEM–EDX system unless the residues have a unique elemental composition. Diatom frustule fragments are prominent examples [Figs 2 and 3(B)], when both the TEM photomicrographs and the EDX spectrum provide convincing evidence that the particle is a fragment of the eukaryotic organism found in the sediment.

A few small, cuboidal-shaped, dense particles were similar in size and conformation to magnetite bodies observed in certain minute cells found in the Lake Arthur samples (Corpe and Jensen, 1992). Confirmation that such particles were similar to those in magnetotactic bacteria is indicated by their morphology and their elemental composition (Blakemore *et al.*, 1989). Such arrangements of free magnetite crystals, a good deal larger than observed in the bacteria, were also observed in the lake biomass so they may in all probability have come from some other source. Certainly results presented here show iron-bearing particles have a range of sizes and shapes. They may be particles of iron oxide or other inorganic derivatives of iron or organic particles with the ability to chelate iron.

The yellowish-brown lake water samples, yielded a dark brown picoplanktonic biomass. Such coloration of lake water suggested a high content of humic substances which represents a substantial part of the dissolved organic matter in lakes (Malcolm, 1985). Ghosh and Schnitzer (1982) found that humic substances prepared for electron microscopy were fibers whose thickness and configuration were influenced by neutral salts. They were similar in appearance to the fibrous particles from the Lake Arthur biomass shown in Figs 1 and 2. Most of these fibers did not show any metal content but about 10% showed the presence of iron (Ghosh and Schnitzer, 1982).

There are a variety of fibrous capsular and surface materials produced by prokaryotes, as cited by Corpe (1980), Sutherland (1990), and by freshwater algae (Schnepf *et al.*, 1980; Strycek *et al.*, 1992; Longhurst *et al.*, 1992). These materials pass through 0.4 μm filters and often blur distinctions between particulate and dissolved organic matter in seawater (Koike *et al.*, 1990). Even 0.2 μm pore size filtration and

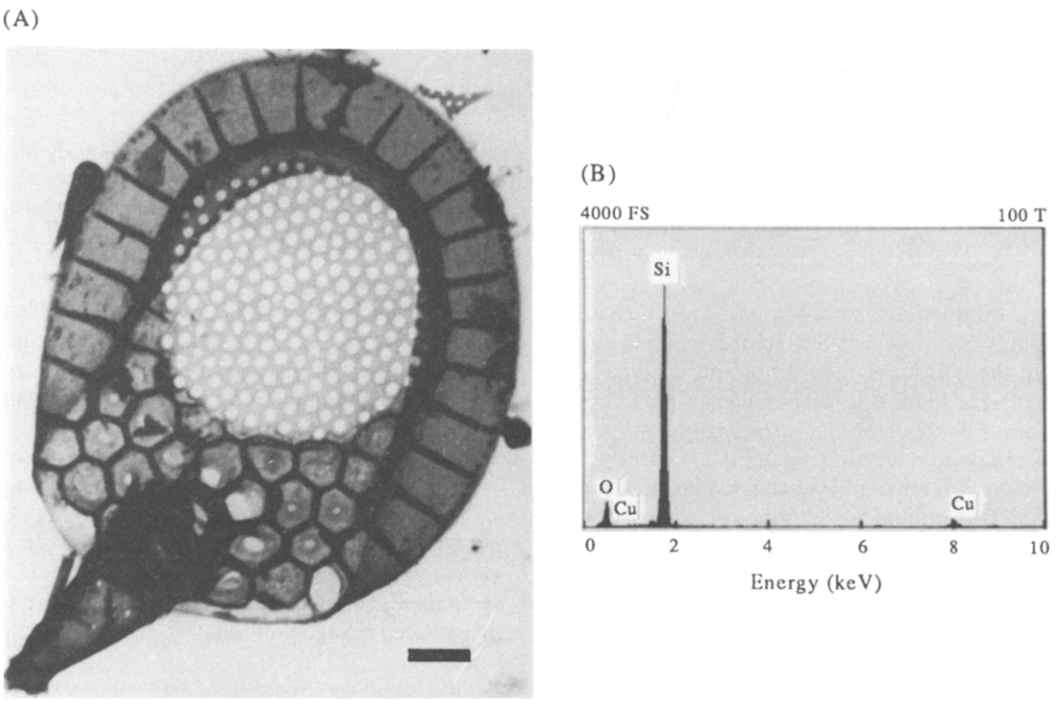


Fig. 5. (A) Scale of a eukaryotic algae. Bar = 0.5 μm. (B) Spectrum generated from scales. They produce emissions from the elements Si and O.

centrifugation do not make particle class distinctions (Munster and Chrost, 1990). Using resistive-pulse particle counters, Longhurst *et al.* (1992), however, were able to quantify the particulates in sea water and study contributions from several sources.

Beckett (1990) states that "A widely accepted concept suggests that surface layers of all particles can become coated with a common matrix of hydrated metal oxides (Fe, Al, Si) and natural organic matter." This present study cannot confirm that view. At least the minute particulate materials described do not contain these metals although many particles are actually composed of large amounts of iron [Fig. 3(A)], Al silicate [Fig. 3(D)] or pure silicic acid. Particles composed mainly of mineral elements do not show the presence of unique organic residues with EDX analysis.

The average size of the minute particles observed were of the size range commonly recognized as dissolved material (Wotten, 1990). Suspended colloids of this dimension, including the picoplankton, probably have little tendency to sink (Fogg, 1986). The microhabitat of the picoplankton is probably viscous rather than turbulent so that molecular diffision is all important in determining transport of materials to and from the cell (Fogg, 1986). The small size of bacteria and the particles described here indicate that turbulent mixing will not bring them into contact. Shear may have an effect but not predominantly. The main means of contact of the bacteria and the particles would be by Brownian motion. Our samples of biomass were removed by high speed centrifugation from a screened and filtered water sample so it is difficult to accurately visualize spacially the microenvironment.

The method used in this study will allow for the determination of the elemental composition of very small particles in other kinds of environments. This information, coupled with other ecological data, will help provide a more complete understanding of the aquatic environment.

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