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STUDIES ON THE OCCURRENCE AND ELEMENTAL COMPOSITION OF BACTERIA IN FRESHWATER PLANKTON

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Abstract

The occurrence and cation content of bacteria in a eutrophic freshwater lake (Rostherne Mere, Cheshire, UK) were investigated over a one year sampling period in relation to cation changes in the lake surface water and phytoplankton.

Scanning electron microscope examination of trawl-net and filtered samples demonstrated bacterial association with Anabaena, Aphanizomenon and diatoms. Direct counts of associated and unassociated bacteria showed that increases in bacterial population relate to population decline of major algal constituents.

Spectrophotometric determination of selected cation levels in the lake water demonstrated wide fluctuations throughout the sampling period, with elevated levels of transition metals before and at the end of Summer stratification. Zn and Pb also showed increased levels in relation to episodic events.

Mass fractions of spectrophotometrically-determined selected cations (Fe, Cu, Zn and Pb) in phytoplankton also varied considerably during the sampling period, with major increases apparently following peaks in water level.

X-ray microanalysis of whole, unassociated bacterial cells demonstrated high levels of soluble and bound cations, including K, Ca, Fe, Cu, Ni, Zn and Pb. Changes in the cation levels of bacteria did not follow a similar pattern to the general phytoplankton - probably due to differences in uptake or adsorption or to cycling of bacterial cells in the water column.

KEY WORDS: Bacteria, Plankton, Elemental composition, cations, X-ray microanalysis.

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INTRODUCTION

Bacteria form a major component of freshwater phytoplankton, occurring both as phytoplankton-associated and unassociated or free-living cells (Caldwell, 1977). The bacterial population, which frequently occurs at levels of 10^6 cells/ml, is important both in the primary productivity of the lake (Anderson and Dokulil, 1977) and in the cycling of inorganic nutrients.

The observations reported here were carried out at Rostherne Mere (Cheshire, UK) over a one year period (1985-86), and are part of a general research program on the cycling of freshwater micronutrients at this location. Previous studies by Reynolds (1978) have provided information on phytoplankton periodicity at Rostherne. This naturally-occurring eutrophic lake is supplemented by run-off from local motorways and agricultural land, and by periodic sewage overflows.

The object of this paper is to describe changes in the occurrence and elemental composition of the bacteria, and to relate these to both the biotic environment (particularly the phytoplankton) and the physico-chemical environment (particularly the levels of lead and transition metals in the surrounding aquatic medium).

Materials and Methods

Bacteria were examined in trawl-net samples and in bulk surface water samples, as outlined in Fig. 1. The surface water samples were double-filtered to separate macroplankton (size greater than 5μm) and picoplankton (size range 5-0.4 μm) from the aquatic medium. Samples were analysed in terms of fine structure (using scanning and transmission electron microscopy) and elemental composition (using atomic absorption spectrophotometry and X-ray microanalysis).

Filtration procedures

Macroplankton samples were obtained using a horizontally-trawled phytoplankton net. The sample was immediately passed through a zooplankton net, and divided into separate samples for elemental analysis and microscopy.

Bulk surface water samples were processed by passing 200 ml of surface water through a double

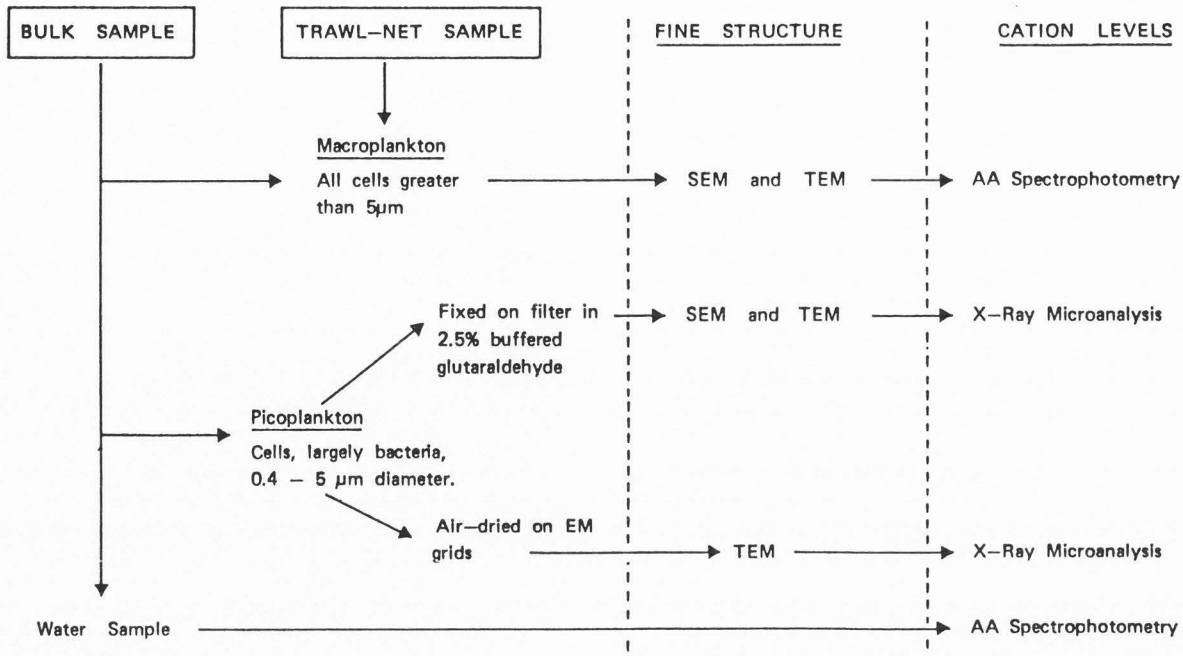


Fig. 1 Protocol followed for analysis of bulk and trawl-net samples.

filtration apparatus, containing a 5 µm followed by a 0.4 µm Nuclepore filter. The filtrate was used for water analysis.

Preparation for microscopy

Scanning electron microscopy was carried out on cells in suspension (trawl-net sample) or cells deposited on membranes (bulk surface water sample), fixed for 2h in 2.5% glutaraldehyde in 0.1M sodium cacodylate (pH 7.2) at room temperature. With both types of sample, fixative was added on the boat immediately after collection. Preparations were then washed in buffer, dehydrated in an ethanol series and critical point dried using standard procedures. Specimens were examined in a Cambridge S150 microscope.

For transmission electron microscopy, trawl-net samples were fixed in glutaraldehyde as above, washed in buffer, and post-fixed in 2% buffered osmium tetroxide. Ultrathin sections were mounted on carbon-formvar copper grids and stained with alkaline lead citrate.

X-ray microanalysis

Transmission X-ray microanalysis was carried out on free living (unattached) whole bacterial cells collected from the bulk water sample. Cells deposited on 0.4 µm membranes were resuspended in lake water, and droplets of bacterial suspensions placed on carbon-formvar nylon grids for two minutes - to allow sedimentation to occur. Grids were then drained, air dried at room temperature, and were then either analysed directly (determination of total cations) or after glutaraldehyde fixation and ethanol dehydration (determination of insoluble cations).

Microanalysis was carried out using a Corinth Analytical Microscope (CORA) fitted with Kevex detector and Link 860 analyser. Emission spectra were obtained using a 0.3 µm diameter probe, over a livetime of 200 s, beam current 60 nA. Elemental mass fractions were calculated using a computer program based on Hall (1971) quantitation for ultrathin specimens, using microcrystals as primary standards. The accuracy of the system was verified using selected metalloproteins as secondary standards (El-Masry and Sigee, 1986).

Atomic absorption spectrophotometry

Atomic absorption spectrophotometry was carried out on filtered lake water and trawl-net phytoplankton samples (Fig. 1). Filtered surface water samples were acidified, then analysed for their cation content using an Instrumentation Laboratory AA/AE Spectrophotometer 157. Phytoplankton samples were filtered on Whatman filter paper GF/C, air-dried to constant weight then digested in conc. Aristar nitric acid. The digest was dissolved in 10% HCl, made up to 10 ml with distilled water, then analysed by AA spectrophotometry.

Results

The occurrence and elemental composition of freshwater bacteria will be considered in three main sections - bacterial associations with phytoplankton, X-ray microanalysis of bacterial cells, and cation inter-relationships in the lake surface water system.

Bacterial associations with phytoplankton

Scanning electron microscopy of phytoplankton from the trawl-net sample consistently revealed direct associations of bacteria with the surface of blue-green algae (Fig. 2) and diatoms (Fig. 3). Transmission microscopy of ultrathin sections showed that the bacteria were typically embedded in surface mucilage (Fig. 4), where they frequently occurred as small groups of cells. The

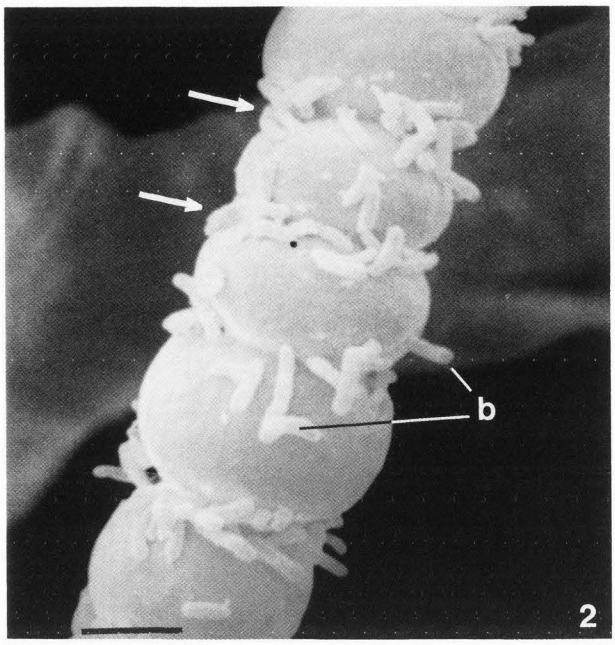
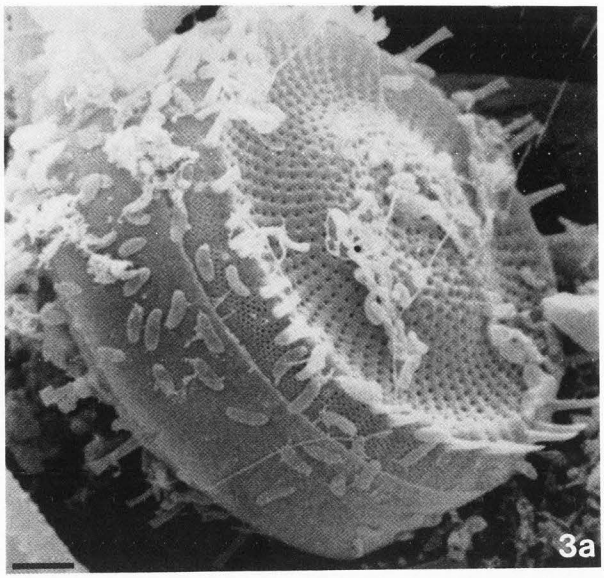


Fig. 2 Scanning electron micrograph of filament of Anabaena, collected by trawl-net during the July population peak. Numerous bacteria (b) are associated with the algal cells, particularly at the junctions between the cells (arrows). Bar = 2 μm



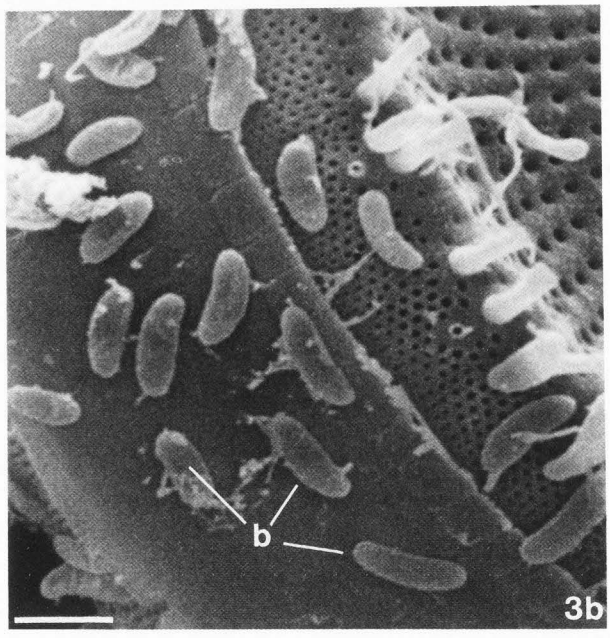


Fig. 3 (3a) Low power scanning electron micrograph of the diatom Stephanodiscus, with associated bacteria. Bar = 3 μm
(3b) Detail from 3a, showing high power view of attached bacteria. Bar = 2 μm

association of bacteria with particular constituents of the phytoplankton varied throughout the year, but was greatest at the time of population decline. This is shown in Fig. 5, where the percentage of algal cells with associated bacteria is shown for three major phytoplankton constituents - Anabaena, Aphanizomenon and diatoms. The time of maximal bacterial association occurs at different times of year, but in each case the peak relates to the end of the major algal growth phase.

Levels of free-living (unassociated) bacteria were determined from the 0.4 μm Nuclepore filter sample by making counts under the scanning electron microscope (Fig. 6). Population numbers changed considerably throughout the year (Fig. 7), varying from 0.5 - 2.5 x 10^6 cells/ml. The changing levels of free bacteria followed a complex pattern, but two major peaks in July (1985) and May (1986) appeared to relate to a peak in the populations of Anabaena and diatoms, respectively.

The results obtained at Rostherne are in broad agreement with those from other freshwater environments, where bacterial associations occur particularly with blue-green algae and diatoms (Caldwell and Caldwell, 1978) and peaks in the occurrence of associated bacteria follow peaks in algal population (Coveney et al., 1977).

The nature of the bacterial-phytoplankton association has been discussed by Cole (1982). Changes in the population of attached bacteria may relate to algal factors such as the release of photosynthetic compounds (Paerl, 1976), cell breakdown products (during senescence), and production of antimicrobial compounds (Gupta and Shrivastava, 1965).

X-ray Microanalysis of bacterial cells

In both air-dried and chemically-treated whole cell preparations, individual bacterial cells appeared highly electron-dense under the transmission electron microscope (Fig. 8). Emission spectra from both types of preparation (Fig. 9) showed a wide range of elemental peaks,

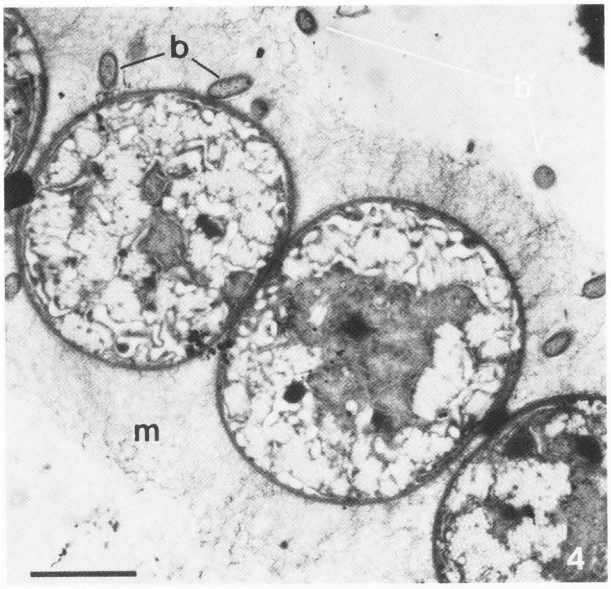


Fig. 4 Transmission electron micrograph of ultrathin section of Anabaena. The cells are enclosed in a layer of mucilage (m) which contains some embedded (b) and some surface (b') bacteria. Bar = 2 µm

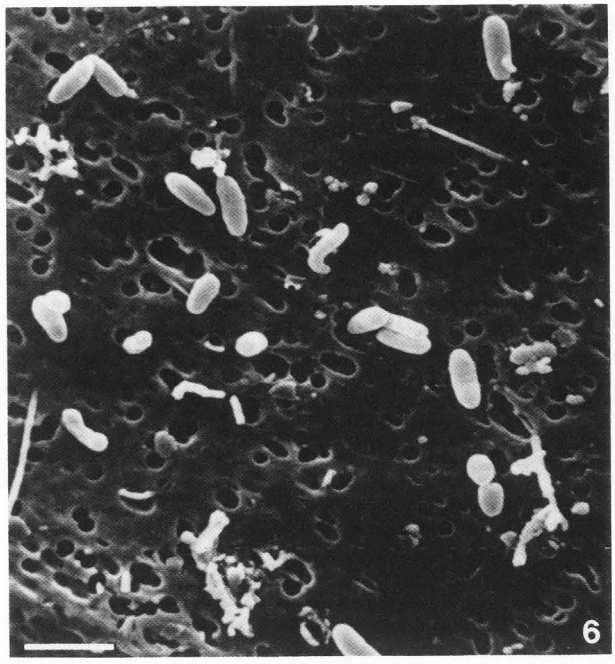
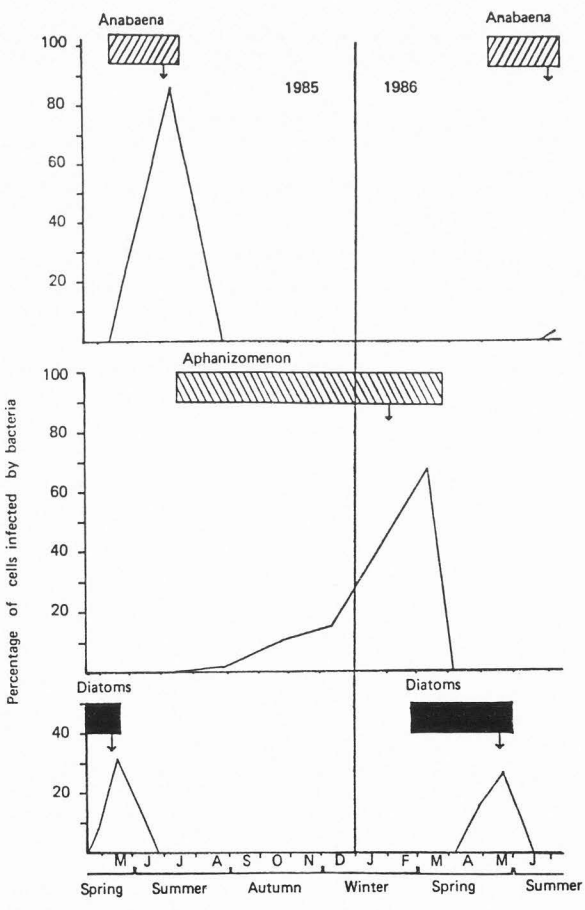


Fig. 6 Scanning electron micrograph of unassociated bacteria on Nuclepore filter. Bar = 2 µm



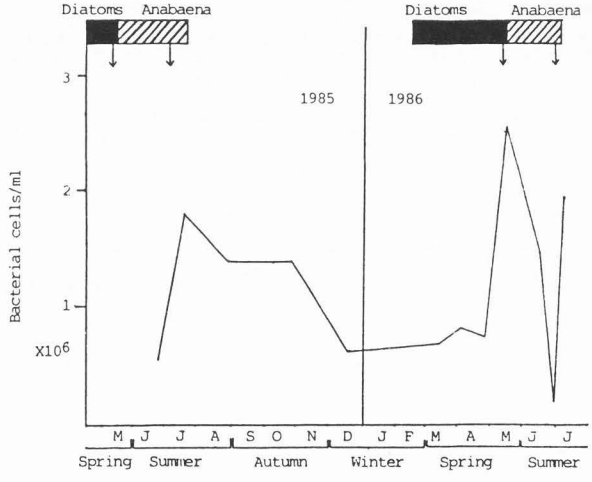


Fig. 7 Changes in the overall population of free-living (unattached) bacteria. High population levels and maxima in diatoms and Anabaena are indicated as in Fig. 5.

Fig. 5 Changes in the populations of algal-associated bacterial cells. For each graph, the bar insert shows the period of high algal population (over 20% of phytoplankton cell count) with population peaks (arrows).

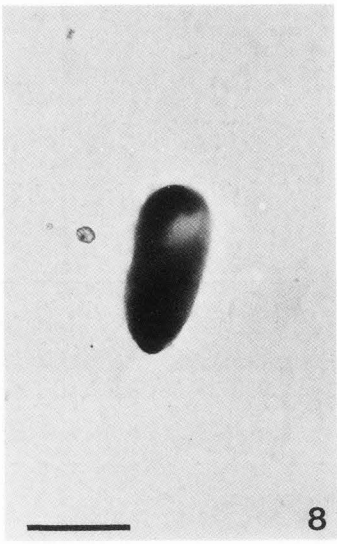


Fig. 8 Transmission electron micrograph of whole air-dried bacterial cell. Bar = 1 µm

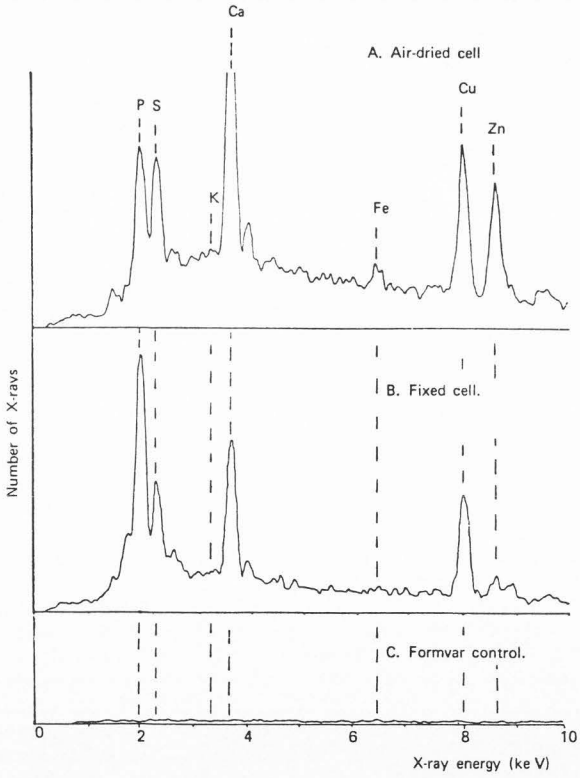


Fig. 9 X-ray emission spectra from air-dried and fixed whole bacterial cells.

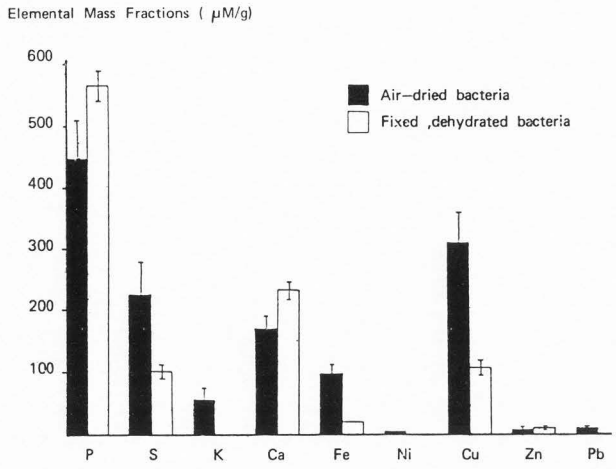


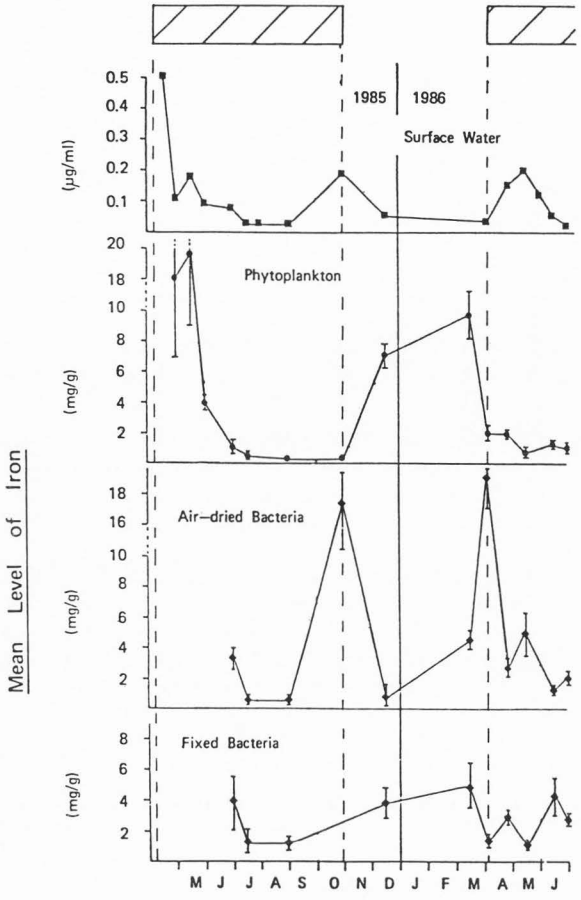
Fig. 10 Mean levels of detectable elements in free (unassociated) bacteria. Mean mass fractions of air-dried and fixed bacteria are given separately, and are each derived from a total of at least 20 analyses. The mass fractions of air-dried cells are total level of element/total dry weight, while the values for fixed cells are insoluble level of element/insoluble matrix dry weight. Confidence limits are at the 95% probability level.

with the clear presence of P, S, monovalent cation K, and divalent cations Ca, Fe, Cu and Zn. Ni and Pb were also frequently detectable.

Substantial variation in elemental composition normally occurred between individual bacterial cells within a single preparation - as shown by characteristically wide confident intervals for mean mass fraction values. Within a single sample, elemental mass fractions in the air-dried preparations typically showed considerable differences from the chemically-treated cells. This is shown in Fig. 10, where the fixed, dehydrated cells have considerably reduced levels of K and also reduced levels of major transition metals (in this case Fe and Cu).

The use of X-ray microanalysis in the determination of bacterial cation levels provides a novel approach to investigating elemental changes in this very important constituent of the freshwater biomass. The high spatial resolution of the technique permits separate determination of cation changes in picoplankton - as distinct from macroplankton, and the broad energy range of the emission spectra provides information on a wide range of elemental constituents at each analysis - including both monovalent and divalent cations.

The use of this technique with whole bacterial cells (rather than sectioned material) means that preparation of material is rapid and simple, with minimal alteration in cell composition. X-ray microanalysis of whole cells has been reported previously with laboratory-



Figs. 11-14 The duration of water stratification is indicated by the bar inserts.

Fig. 11 Mean levels of iron in surface water, phytoplankton and bacteria.

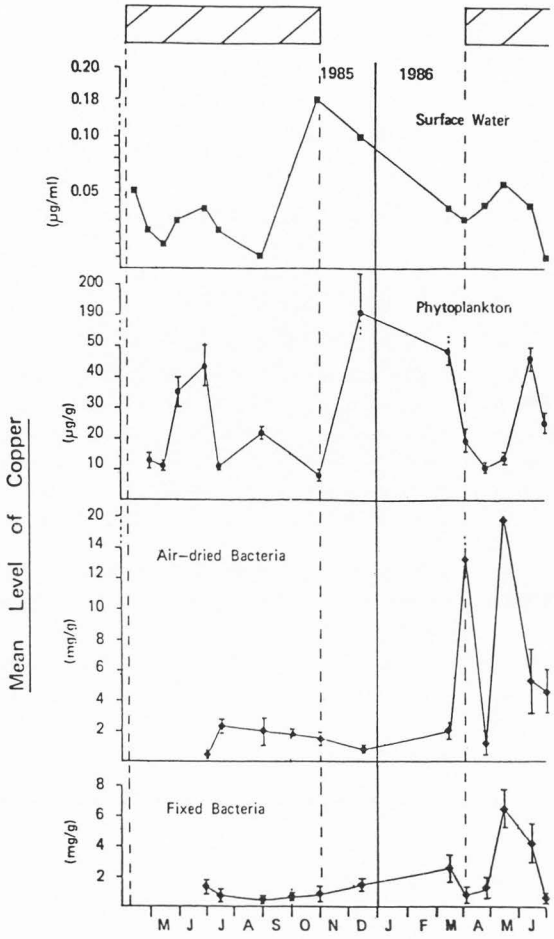


Fig. 12 Mean levels of copper in surface water, phytoplankton and bacteria.

cultured bacteria (Sigee et al., 1985a, 1985b, Sigee and Al-Rabaee, 1986).

In freshwater bacteria, consistent differences were apparent between air-dried and chemically treated preparations, allowing separate determination of total (mainly soluble) and insoluble elements. The distinction between the two types of preparation was much less than in previous studies on laboratory-cultured cells (Sigee et al., 1985b), however, where a range of transition metals was generally detected only in fixed, dehydrated cells - since these cations were present largely as bound (insoluble) elements.

Cation inter-relationships at the lake surface

The range of concentrations of selected cations occurring in surface water at Rostherne over the annual sampling period are shown in Table 1. Considerable variations occurred in the surface water concentrations of particular cations throughout the sampling period, with related changes in the cation levels of phytoplankton and bacteria. These three parameters are shown in Figs. 11-14, for three major transition metals (Fe, Cu, Zn) and Pb. In each case the annual cation changes are considered in relation to major alterations in the annual cycling of the water mass - without necessarily implying any direct causality between the two types of change. Alterations in the vertical movement of the water mass involve a period of non-cycling (stratification) over the Summer, followed by a period of intermixing of the surface and lower layers - which commences in October as the Autumn overturn.

Changes in the occurrence of the selected cations are as follows - a) Iron. The level of Fe in lake water (Fig. 11) shows a double annual

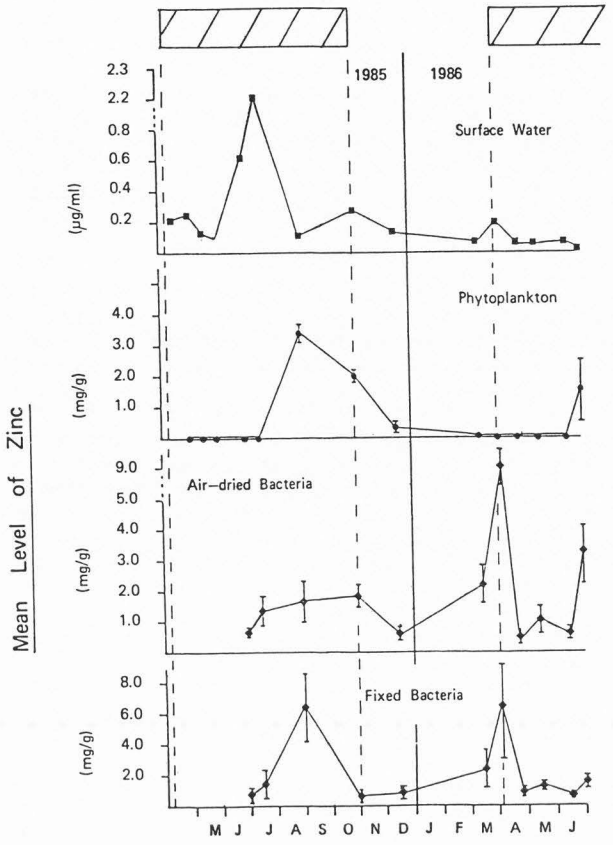


Fig. 13 Mean levels of zinc in surface water, phytoplankton and bacteria.

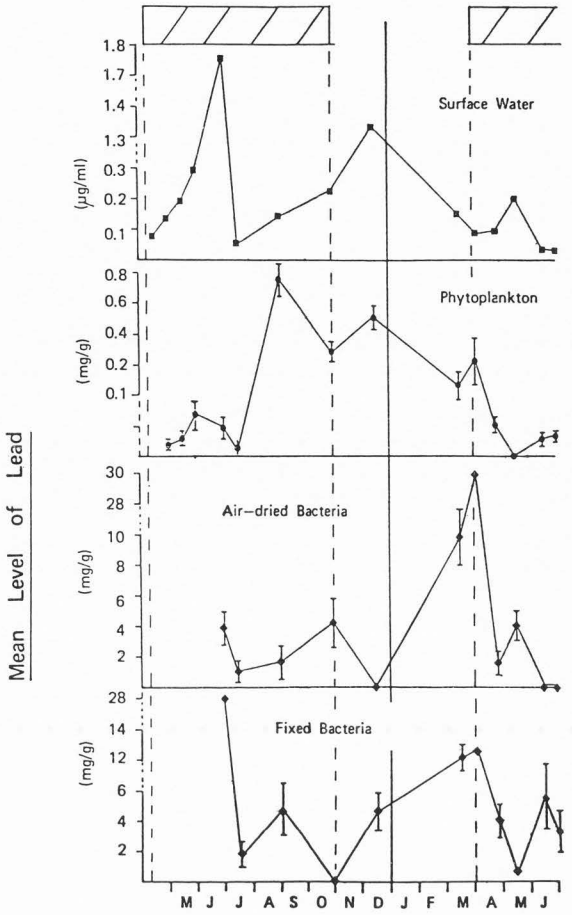


Fig. 14 Mean levels of lead in surface water, phytoplankton and bacteria.

Table 1. Range of cation levels in filtered surface water

Elment	Range of cation concentration
Calcium*	10 - 131 mg/l
Iron+	10 - 500 µg/l
Nickel"	0.2 - 1.2 µg/l
Copper+	10 - 180 µg/l
Zinc+	0 - 2210 µg/l
Cadmium"	4.8 - 33.8 µg/l
Lead+	30 - 1760 µg/l

All cation levels were determined from the 200 ml bulk water sample after removal of phytoplankton, zooplankton and bacteria by nuclepore membrane filtration. The atomic absorption spectrophotometric determinations were made -

+ using a flame method
" using a graphite furnace
* after addition of 10% Lanthanum chloride to the water sample

peak, one in the Spring (April/May) before Summer stratification and one following the Autumn overturn (October/November). The mass fraction of Fe in the phytoplankton rose during the Winter months (October to February) at a time when the water column was well-mixed and the total level of soluble Fe in surface water was relatively low. Major increases in the mass fraction of Fe in bacterial cells were detected in Autumn and Spring. These increases, seen particularly in the air-dried sample (ie. mainly soluble cations), coincided with increased levels of Fe in the surface water. The fixed bacterial sample showed considerably less variation in the level of (insoluble) cations, with a gradual increase over the Winter months. b) Copper. The level of Cu in the surface water (Fig. 12) undergoes a similar double increase to Fe, with a particularly high level detected in October. The mass fraction of Cu in phytoplankton showed elevated levels over the Winter months, rising to values of 190 µg/g in December. The level of Cu in the air-dried

bacterial samples showed a rise in Spring (May) in parallel to the surface water increase, but no corresponding increase in the bacterial Cu level occurred at the time of the Autumn overturn. Fixed bacterial cells showed a similar pattern of change in the level of insoluble Cu. c) Zinc. Zinc is the most commonly occurring transition metal cation in the surface water, rising to levels of over 2000 µg/l. The concentration of Zn in the surface water shows a small double peak (April/May and October - Fig. 13) - similar to Fe and Cu, plus a major peak in June/July. The mass fraction of zinc in the phytoplankton had risen sharply by August, remaining high until late October. A similar rise occurred in the level of insoluble Zn in fixed bacteria, with a second major peak in Spring. In air-dried bacteria, a low level Summer/Autumn increase was followed by a sharp rise to 0.9 mg/g in Spring. d) Lead. The occurrence of Pb in the surface water (Fig. 14) showed a different pattern from the transition metals, with major peaks in early Summer and late Autumn. The level in phytoplankton rose sharply during the period of lake stratification, and remained high until Spring. Both air-dried and fixed bacterial cells showed an increase in the level of Pb over Winter, reaching a peak in April, with a subsequent smaller rise in May/June in parallel with the increase in water level of Pb.

Discussion

The primary event that leads to a change in the level of a particular cation in phytoplankton and bacteria is a change in the level of that cation in the lake water.

Changes in aquatic levels of cations

The data presented in this report shows that major changes in aquatic concentration of lead and transition metals occur throughout the annual cycle, but the precise events leading to the changes await future elucidation. Factors which may be important include general weather changes, farming practices and vertical stratification or vertical mixing of the water during isothermal conditions. The results suggest that the latter may be of particular importance, since the transition metals investigated all showed a significant increase in aquatic level prior to stratification, and a second major increase at the autumn overturn. Episodic events are also probably important in producing cation increases, and the major peak of Zn in mid-Summer, as well as the peaks of Pb in early summer and late Autumn, may relate to periods of high rainfall - which had different effects at different times of year.

Cation uptake into phytoplankton and bacteria

The relationship between aquatic and primary biotic (phytoplankton and bacteria) metal levels is complex. It should be noted that the determination of cation levels by atomic absorption spectrophotometry and X-ray microanalysis does not distinguish between different species of a particular cation, and a rise in aquatic level of any cation does not necessarily imply an equivalent increase in availability to the biota. The results do indicate, however, that major rises in aquatic levels of cations can lead, after a lag phase, to elevated levels in the phytoplankton - suggesting a delayed biotic response to environmental change. Thus the increased levels of Fe and Cu in phytoplankton follow shortly after the aquatic Autumn peaks, while the levels of Zn and Pb in phytoplankton rise shortly after early Summer (episodic) aquatic peaks.

For each cation investigated, changes in the metal level of air-dried bacteria followed a markedly different pattern from the phytoplankton. This suggests either that different factors or rates may determine the accumulation of cations by these cells (eg. differences in rate and selectivity of uptake or surface adsorption) or that bacterial populations in surface water may be altered by cycling of bacteria from lower levels. Cation levels in fixed bacteria generally showed less change than in air-dried cells, suggesting that major fluctuations in total levels were mainly due to changes in soluble cations.

Bacteria as monitors of environmental cation levels

Both algal and bacterial cells are highly efficient at concentrating cations - either by taking the cations into the cell or by adsorption at the cell surface. The extent to which concentration of cations occurs can be judged from Figs. 11-14, where direct comparison can be made between aquatic concentrations (expressed as µg/ml) and biomass concentrations (expressed as mg/g). In the case of Fe, for example, the level in phytoplankton (per g dry weight) was approximately 0.2 - 20 x 10^4 times greater than in the surrounding water, and in the case of air-dried bacteria, from about 2 - 50 x 10^4 times greater - depending on the time of year.

The ability of bacteria in particular to accumulate high levels of cations provides a potentially useful tool for environmental monitoring - since these cells can be rapidly extracted, analysed and a range of elemental mass fractions obtained. Previous studies with laboratory-cultured bacterial cells have shown that X-ray microanalysis can be very useful in relating internal cation changes to alterations in environmental level (Sigee and Al-Rabaee, 1986). The possibility of using freshwater bacteria for environmental monitoring is one of the major aspects of the Rostherne project, where the elemental composition of bacteria can be directly compared to that of lake water in the local (200 ml) environment. One apparent limitation in the use of bacteria for this purpose lies in the fact that they do not always respond to environmental change - possibly because the aquatic cation undergoing change may not be in a directly assimilable form. This is seen, for example, in the bacterial levels of Cu from August to December, where a decline in the bacterial level of cation contrasts with a conspicuous rise in the level of Cu in surrounding lake water.

Conclusions

Full interpretation of the data presented in this paper awaits further analysis, and also further sampling at Rostherne Mere. A number of tentative conclusions, however, can be drawn - (1) Increases in the populations of both attached and unattached bacteria relate to population peak and decline of particular algal species. (2) The level of cations in the lake water relates to major annual events (such as Spring stratification and Autumn overturn) as well as single (episodic) events. (3) Cation levels in the phytoplankton typically follow cation increases within the lake water as a delayed rise in the algal concentration. (4) Cation changes in the free-living (unattached) bacteria characteristically follow a different pattern from the phytoplankton, and frequently do not relate to surface lake water cation levels. (5) Algal and bacterial cells are able to concentrate cations to a high level (up to about 10^5 times). X-ray microanalysis of bacteria provides a rapid, but in some ways limited, system to monitor environmental cation levels.

Acknowledgements

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Discussion with Reviewers

J. Rowley: Were there recognizable peaks for Al or Mg from any of the atomic absorption spectrophotometric determinations or XRMA analyses? If not, was it due to the nature of the lake or the windows open during analysis?

Authors: Al and Mg were not included in the cations selected for analysis by atomic absorption spectrophotometry, and we have no data therefore on the occurrence of these elements in bulk biomass or water samples. Although recognizable peaks of Al and Mg were occasionally present in X-ray emission spectra, they were never substantial and were never present in 60% of analyses - the proportion arbitrarily set to allow calculation of mean mass fraction data. The lack of recorded levels of these elements from the X-ray emission data thus reflects their general occurrence at levels below the detectability of the technique rather than the absence of appropriate windows during analysis.

G.M. Roomans: While the differences between metal content of bacteria after air-drying and after fixation give an indication of the extent to which the particular metal is bound, surely it is too simplistic to refer to this difference as 'soluble' cations, since fixation also can lead to a loss of (weakly) bound cations.

Authors: Solubility of cell components is difficult to define and quantitate in absolute terms, and we accept that use of the terms 'soluble' and 'insoluble' in this context is to some extent simplistic. Constituents that are included in the soluble category include cations

that are weakly bound and cations that are bound to low MW proteins.

G.M. Roomans: It would be interesting to have data on the metal content per bacterium multiplied to the total bacterial mass. This would indicate the total amount of metal bound by the bacteria, and could be expressed relative to the total amount of metal present in the water.

Authors: We agree that it would be of considerable interest to convert the data obtained from single bacterial cells to an estimate for total biomass in the lake. This calculation could certainly be made for bacteria in surface water, though it is dangerous to extrapolate data obtained from a small number of cells in one part of the lake to a very much greater number of cells over a wide area.

G.M. Roomans: Are there any difficulties distinguishing between bacteria and inorganic precipitates in the material?
Authors: Filtration of lake water through the 0.4 µm membrane leads to the separation of bacterial cells plus debris (inorganic and organic) - both of which are transferred to the EM grid prior to microanalysis. Bacterial cells are quite distinct, however, under the transmission electron microscope in terms of size, shape and electron density, and are readily distinguished from other particulate matter.

G. Fahnenstiel: Does the use of 0.4 µm filters cause a severe underestimate of bacterial numbers?
Authors: We have no direct information on this from our own experiments, and it will certainly be worth putting a third filtration step in future experiments to test the possibility that cells are passing through the 0.4 µm filter. The bacterial counts that we routinely obtain of 10^6 cells/ml are, however, consistent with total levels obtained by other authors (eg. Cole, 1982), which would suggest that we are losing relatively few cells.

L. McMillan: What is the seasonal population flux for bacteria, and how is it differentiated from changes in algal growth due to heavy rainfall and sewage overflow?
Authors: Seasonal changes in overall bacterial population are shown in Fig. 5 (associated cells) and Fig. 7 (unattached cells). Both types of population appear to relate closely to algal growth - particularly diatoms and Anabaena.

G. Fahnenstiel: The authors have assumed that all organisms enumerated by SEM were heterotrophic bacteria. This may not be true, since small cyanobacteria may contribute substantially to numbers of total bacteria (Cronberg and Weibull 1981 Arch Hydrobiol. 60: 101-110; Drews et al. 1961 Arch fur Mikrobiologie 39: 101-115).
Authors: None of the cells that we have examined from 0.4 µm filters show any fluorescence for chlorophyll. We have concluded therefore that small cyanobacteria do not make any substantial contribution to our bacterial counts.

G. Fahnenstiel: More detailed information on phytoplankton composition would be helpful, particularly in relation to the association of attached bacteria with major phytoplankton.
Authors: Detailed studies on the cyclic changes in phytoplankton composition have been published by Reynolds (1978). The lake is dominated at different times of year by Asterionella formosa, Anabaena spiroides, Aphanizomenon flos-aquae and Microcystis aeruginosa - all of which are K-strategists and all of which have associated bacteria.