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X-RAY MICROANALYTICAL STUDIES OF FRESHWATER BIOTA: CHANGES IN THE ELEMENTAL COMPOSITION OF Anabaena spiroides DURING BLOOMS OF 1988 AND 1989

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Abstract

Samples of Anabaena spiroides were collected from a eutrophic freshwater lake (Rostherne Mere, Cheshire) during the bloom periods of 1988 and 1989, and analysed by SEM electron probe X-ray microanalysis. Vegetative cells, akinetes and heterocysts consistently had clear peaks of Mg, Si, P, S, Cl, K and Ca.

In comparison to vegetative cells, akinetes had generally higher elemental mass fractions (particularly K and P) while heterocysts had significantly lower levels of Mg, P, and Ca.

For both years, elemental mass fractions in vegetative cells showed significant changes during the course of the bloom, but these were not consistent from one year to the next. Mass fractions showed no correlation with water concentrations in 1988, but positive correlations - particularly with P and K occurred in 1989. In 1989, the availability of P in the lakewater was probably less than in 1988.

Significant correlations also occurred between certain elements within X-ray emission spectra, suggesting that specific associations occur in the cell.

Key Words: Phytoplankton, blue-green algae, algal blooms, X-ray microanalysis.

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INTRODUCTION

The elemental composition of freshwater algae has been extensively studied using electron probe X-ray microanalysis. The major part of this work has involved the transmission electron microscope (TEM) - including studies on the ultrastructural localisation of heavy metal ions (eg. Jensen et al.,1982; Silverberg, 1975) and composition of polyphosphate bodies (Sicko-Goad et al.,1975). Relatively few studies have been carried out involving analysis in the scanning mode, though Bistricki & Munawar (1982) have used this approach for detection of heavy metals under conditions of pollution, and Lehman (1985) used X-ray microanalysis in a study on nutrient levels in phytoplankton.

In contrast to TEM analysis, the use of the scanning electron microscope (SEM) typically involves relatively simple preparation techniques, with rapid freezing and cryodehydration of whole cell preparations rather than preparation of cryosections. As a result, the emphasis with SEM X-ray microanalysis is on whole cells rather than sub-cellular components, and this technique has particular potential for rapid analysis of individual algal cells, colonies and members of a particular taxonomic group within the mixed phytoplankton population.

The present study was carried out as part of a long term project on phytoplankton composition and elemental levels at a local eutrophic lake - Rostherne Mere, Cheshire, UK. This lake is one of a group of small, relatively shallow lakes in Cheshire, with an area of 48.7 hectares and a maximum depth of about 30 metres (Reynolds, 1978). The major objective of this work was to monitor changes in elemental composition of the resident Anabaena population (largely Anabaena spiroides) from early to late bloom, and to determine whether these changes relate to external factors (such as lakewater composition) or internal

factors (such as stage of growth cycle). Observations were carried out during two annual blooms (1988 & 1989) of this alga, which is a major constituent of the phytoplankton at Rostherne Mere. Previous studies at this site have been carried out on the determination of elemental levels in phytoplankton and bacteria using atomic absorption spectrophotometry and TEM X-ray microanalysis (Booth et al.,1987) and show major changes within the biomass during the annual cycle.

Materials and Methods

Samples of lakewater containing phytoplankton were collected from a central site at Rostherne Mere every three weeks during May-August,1988, and at weekly intervals from May-July, 1989. Samples were taken from the upper 5m of the lake using a polythene tube, and were processed immediately on the boat.

Chlorophyll determination

In 1989, total biomass within the sample was monitored by chlorophyll determination. 1 litre of lakewater was filtered onto a WHATMAN GF/C filter paper. Chlorophyll a extraction was carried out using the hot methanol procedure as detailed in HMSO (1980). Absorbance of the extract was recorded at 665nm (750nm background turbidity) using a PYE UNICAM SP8-100 ultraviolet spectrophotometer.

Cell culture

Cultured cells of Anabaena spiroides were used in the tests on beam penetration, and were of a similar appearance and size to those in the phytoplankton sample. Cells were obtained from the UK Culture Collection of Algae and Protozoa (ref: CCAP 1403/24 - originally isolated from Rostherne Mere) and were cultured in Jaworski's medium (Thompson et al.,1988).

Phytoplankton sample preparation

The polythene tube sample was used both for light microscoopy and scanning electron microscopy.

(a) Light microscopy. 200ml aliquots of sample were fixed in Lugol's iodine and examined by light microscopy to determine phytoplankton species composition. The number of filaments of Anabaena per ml of lake sample were counted using a Sedgewick-Rafter slide.

(b) Scanning electron microscopy. 50ml of sample were passed through a double filtration apparatus, containing 5um and 0.4um Nuclepore filters. The acidified filtrate was used for analysis of lakewater by atomic absorption spectrophotometry.

Macroplankton (including Anabaena spiroides) was deposited on the 5µm filter, which was subsequently divided and separately processed for SEM X-ray microanalysis and fine structural observation. Samples to be used for X-ray microanalysis were frozen in nitrogen slush, freeze-dried for 12h in an Edwards tissue-drier, mounted on SEM stubs and carbon coated. Samples for identification and fine structural observation were fixed for 2h in 2.5% glutaraldehyde in 0.1M sodium cacodylate (pH 7.2) at room temperature, then washed in buffer, dehydrated in an ethanol series, critical point dried and gold-coated according to standard procedures.

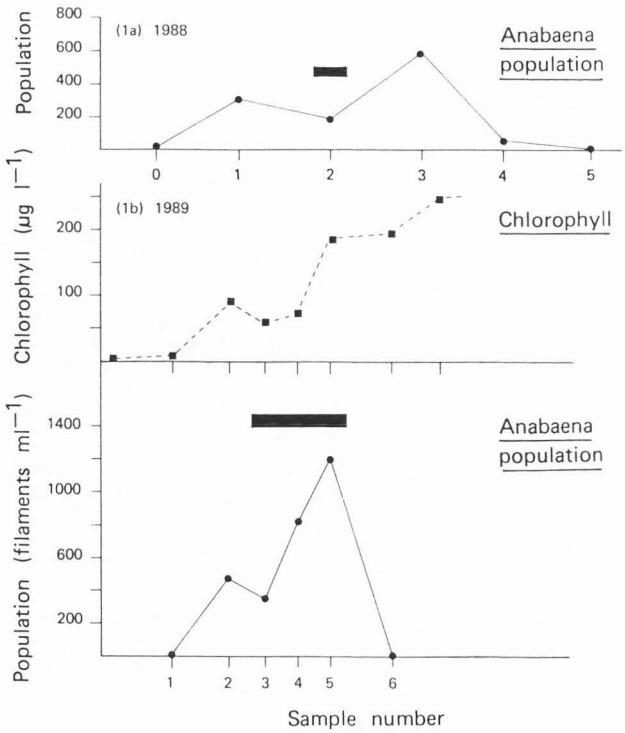
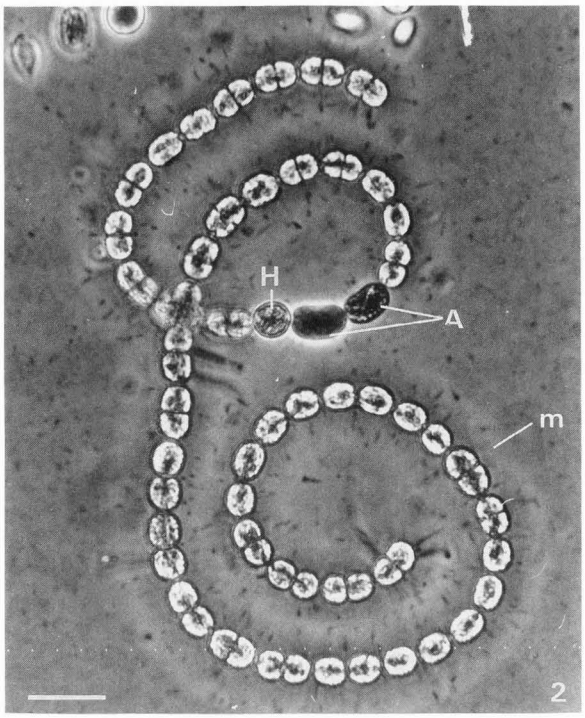


Fig.1 Changes in Anabaena population during 1988 (1a) and 1989 (1b). The temporal presence of akinetes is indicated by the black bar.
(Fig.1a) Sample dates: 0 (11th May), 1 (1st June), 2 (23rd June) 3 (13th July), 4 (3rd August), 5 (24th August).
(Fig.1b) Sample dates: 1 (18th May),2(31st May), 3 (7th June) 4 (14th June),5 (21st June), 6 (5th July).

X-ray microanalysis

Specimens were examined and analysed using a Cambridge 360 SEM with a LINK AN10,000 analyser (LINK Systems Ltd). X-ray emission spectra were obtained from individual vegetative cells, heterocysts and akinetes of Anabaena filaments, at an accelerating voltage of 15kV, 100s livetime, 10K magnification and with a reduced raster of constant size.

Quantitation was carried out at 15kV using inorganic standards, using cobalt as



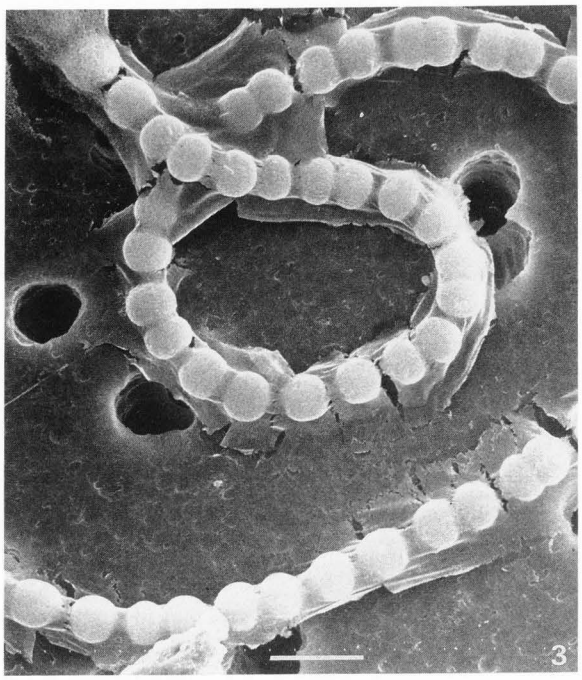


Fig.2 Light micrograph (phase-contrast) of filament of Anabaena spiroides. The close apposition of akinetes (A) and heterocysts (H) is typical of this species. The cells are surrounded by a thick layer of mucilage (m). Bar scale-10µm.

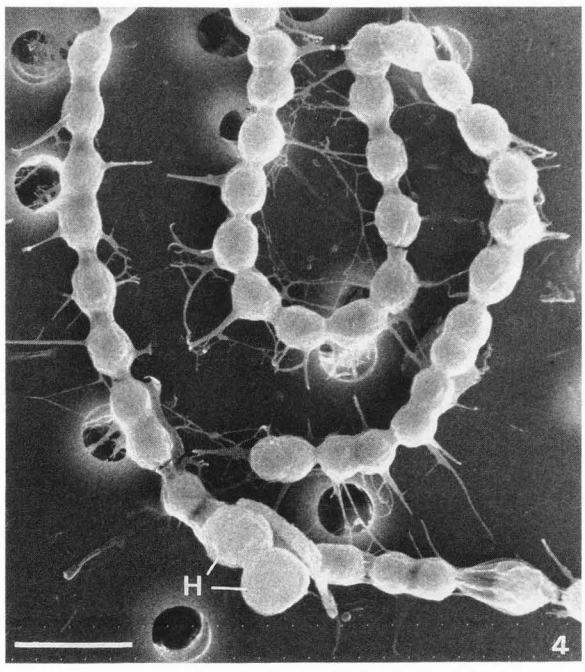


Fig.3 Scanning Electron Microscope (SEM) view of chemically fixed, dehydrated filament of Anabaena. The mucilage sheath appears as a continuous layer around the cells. Bar scale-5µm.

Fig.4 SEM micrograph of freeze-dried cells, with two heterocysts (H). The mucilage sheath is retained as strands of material attaching the algal cells to the underlying filter membrane. Bar scale-10µm.

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a reference for energy calibration. Elemental mass fractions (expressed as g/100g dry weight) were obtained using the LINK ZAF/PB program. This has elemental profiles stored on disk, and calculates peak/background ratios within the characteristic peak areas. Elemental peaks in the X-ray emission spectra were considered to be significant when -

$$P-B > 2\sqrt{(P + B)} \quad \ldots\ldots\ldots\ldots \quad (1)$$

where P= total peak integral
B= estimated continuum component of peak integral

For each sampling date, mean mass fractions were calculated from 20 separate vegetative cells, and (when available) 20 akinetes and heterocysts. Significant differences between mean levels of elements were statistically assessed using Duncan's multiple range test (95% significance level). Correlations between elements within spectra were determined over 20 analyses by calculation of Pearson correlation coefficients.

Results

Population counts

Changes in the population of Anabaena, expressed as number of filaments/ml, are shown in Fig.1 for the Summer blooms of 1988 and 1989. Measurements of chlorophyll[a] level (Fig.1b - 1989) demonstrate a marked rise in phytoplankton biomass during May (in parallel with the Anabaena population increase), continuing beyond the Anabaena decline.

Light microscopy

The typical appearance of filaments of Anabaena , as seen under phase contrast microscopy, is shown in Fig.2. Individual vegetative cells were frequently seen in a state of active division, and the presence of mucilage was observed as a layer of variable width around the filaments. In addition to vegetative cells, akinetes and heterocysts were also observed as large, highly refractive structures. Although akinetes only occurred in the mid-period of Anabaena dominance (Fig.1),heterocysts were present throughout the entire growth period.

The main species present was identified as Anabaena spiroides on the basis of cell size and filament morphology (Desikachary, 1959). Small amounts of Anabaena circinalis were also present, with slightly larger cells and a looser coiling of the filaments.

Electron microscopy

Scanning electron micrographs of Anabaena cells deposited on membranes are shown in Fig.3 (chemically-processed cells) and Fig.4 (freeze-dried cells). In fixed, ethanol-dehydrated cells, the mucilage typically appears as a continuous sheath, while in freeze-dried cells the mucilage was retained as strands of material connecting the algal cells to the filter membrane. The typical appearance of heterocysts is shown in Fig.4.

X-ray microanalysis of vegetative cells, akinetes and heterocysts

Beam penetration. With these preparations, where single cells are being analysed on a membrane surface, beam penetration is particularly important and is determined to a large extent by the accelerating voltage. At high voltages there is the danger that overpenetration of the beam through the cell into the underlying matrix will result in inaccurate determination of absolute mass fractions. In this situation, differences in cell size (eg. between samples) would also result in differences in the extent of matrix penetration, leading to errors in comparative values.

The possibility of overpenetration was tested by obtaining X-ray emission spectra from cultured Anabaena cells deposited on nylon mesh. Any contribution from the underlying matrix will be revealed by the presence of a titanium (Ti) peak, since this element is present at substantial levels in nylon (Fig.5c) but is not detectable in cells. Fig.5a shows a characteristic spectrum from an Anabaena cell at 15kV, and Fig.5b at 25kV. The presence of a clear Ti peak at 25kV but not 15kV is consistent with overpenetration at the higher but not the lower voltage, and all work was accordingly carried out at 15kV.

At 25kV, contribution of background from the underlying matrix resulted in smaller characteristic peaks compared to 15kV (Figs.5a,b) and decreased element detectability. Although clear peaks of Mg were routinely seen at 15kV, this element was close to the limits of detection at the higher accelerating voltage.

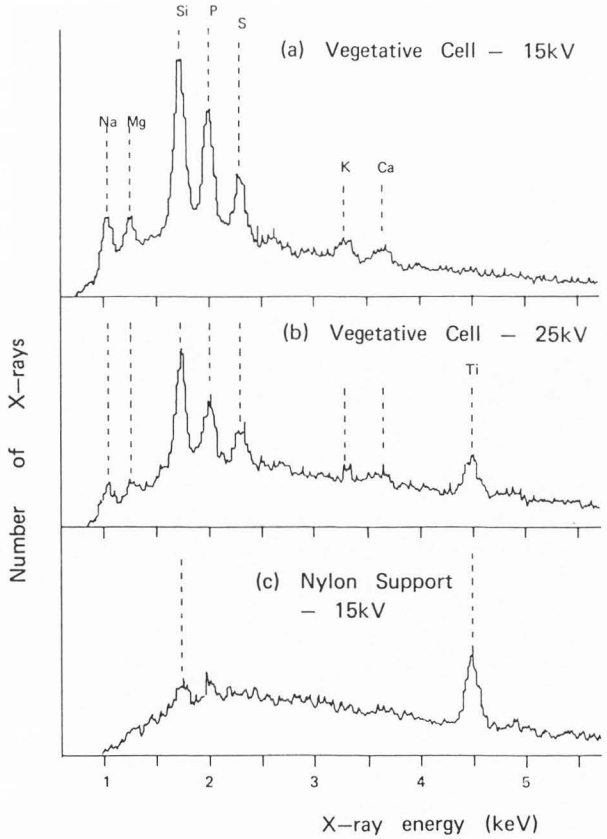

Fig.5 X-ray spectra and accelerating voltage.
(5a) X-ray emission spectrum from single freeze-dried cell of Anabaena, deposited on nylon mesh, taken at 15kV. Preparations of cultured cells differed from phytoplankton samples in having high levels of Na and Si and low levels of Cl. The absence of an extraneous Ti peak at 15kV suggests little beam penetration into the nylon support. (5b) Similar to 5a, taken at 25kV. The clear Ti peak indicates beam penetration beyond the cell at this kV. (5c) Spectrum from nylon support, showing substantial Ti peak.

Table 1. Elemental mass fractions* in vegetative cells, akinetes and heterocysts.

	Mg	P	S	Cl	K	Ca
Sample 3.1989						
Vegetative cells	0.27 (±0.03)	0.50 (±0.08)	0.28 (±0.04)	0.19 (±0.04)	0.46 (±0.08)	0.41 (±0.07)
Akinetes	0.33 (±0.04)	0.68 (±0.10)	0.32 (±0.04)	0.24 (±0.03)	0.59 (±0.14)	0.44 (±0.01)
Heterocysts	0.19 (±0.05)	0.30 (±0.07)	0.30 (±0.08)	0.13 (±0.04)	0.42 (±0.42)	0.22 (±0.06)
Sample 4.1989						
Vegetative cells	0.22 (±0.04)	0.36 (±0.09)	0.25 (±0.03)	0.55 (±0.12)	0.45 (±0.06)	0.31 (±0.07)
Akinetes	0.29 (±0.04)	0.60 (±0.14)	0.31 (±0.02)	0.65 (±0.16)	0.73 (±0.09)	0.32 (±0.08)

* Mass fractions are the mean of at least 15 spectra. Confidence limit are at the 95% probability level.

Sample 3 = 7th June,1989: Sample 4 = 14th June, 1989.

Analysis of different cell types. Although X-ray emission spectra from heterocysts and akinetes appeared generally similar to those of vegetative cells in terms of the range of detectable elements, quantitative differences did occur in elemental mass fractions (Table 1).

Spectra from akinetes typically had more pronounced characteristic peaks compared to vegetative cells, and mass fraction levels (particularly K and P) were generally higher. Heterocysts differed from vegetative cells in having significantly lower levels of Mg, P and Ca.

Seasonal variation in elemental occurrence

X-ray emission spectra from vegetative cells showed relatively little qualitative variation throughout the period of Anabaena dominance in 1988 and 1989, with the major peaks noted previously being consistently present. Considerable variation did occur, however, in the size of the peaks. An example of such quantitative variation is shown in Fig.6, where peaks of Mg, P and K were markedly higher in early bloom compared to late bloom (1989) material.

Changes in the mass fractions of detectable elements in vegetative cells during the periods of Anabaena dominance are shown in Figs.7-9 (1988) and Figs.10-12 (1989), together with elemental levels in the surrounding lakewater. Considering individual elements in order of atomic number -

Magnesium. The mass fraction of Mg varied from 0.2-0.4% in 1988, and 0.2-0.3% in 1989. In 1988, the level of Mg was considerably higher during the middle of the sample period (Fig.7) in 1989 there was a gradual decline throughout the bloom (Fig.10). The water level of Mg varied between 8-12 ppm in 1988, and fluctuated around 11 ppm in 1989.

Phosphorus. The mass fraction of P varied from approximately 0.4-0.8% over both sampling periods. This element followed a similar trend to Mg, with a mid-period peak in 1988 and a continuous decline from an initial high level in 1989. In both years, the level of P in lakewater showed a clear decline during the bloom period, falling to 0.08ppm in 1988 and to 0.02ppm in 1989.

Sulphur. This element was present in algal cells at mass fraction levels of about 0.2-0.4% (1988) and 0.2-0.3% (1989), reaching a maximum level during the middle of both sampling periods (Figs.8 & 11).

Chlorine. Changes in the mass fraction of Cl are shown in Figs.8 (1988) and 11 (1989), and are markedly different for the two seasons. Mass fractions reached higher levels (almost 0.6%) in 1989.

Potassium. K, the major cation associated with Anabaena cells, showed considerable variation during the sampling periods - ranging from 0.4-1.0% in 1988 and 0.4-0.7% in 1989. The pattern of change in cell mass fraction was quite different in the two sampling periods (Figs.9 & 12). The

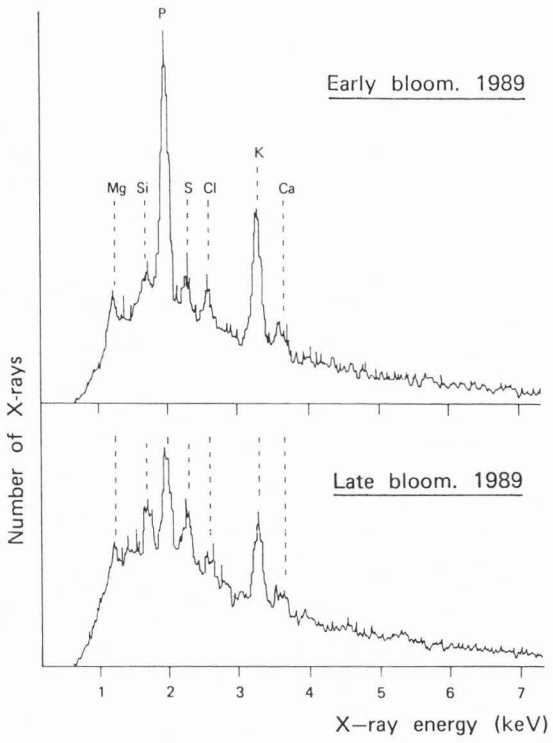

Fig.6 Spectra from early and late samples during the Anabaena bloom of 1989 (samples 1 & 5).

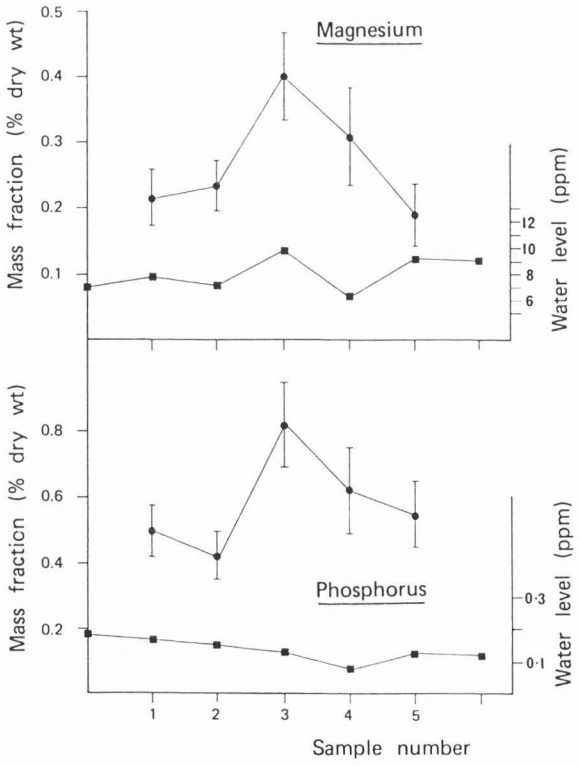

Fig.7 Changes in the level of magnesium and phosphorus during the Anabaena bloom of 1988. Duncan multiple range test. Pairs of samples significantly different at the 95% probability level were-
Magnesium: 3(1,2,4,5); 4(1,2,5)
Phosphorus: 3(1,2,4,5); 4(2)

water concentration decreased during the bloom period in both years, falling to under 4ppm.

Calcium. Ca was typically present at a mass fraction of around 0.3%, varying from a minimal value of 0.1% in 1988 to almost 0.5% in 1989. For both seasons, the water level remained within the range 30-40ppm.

Cell mass fraction correlations with water concentration. The extent to which elemental changes in cells relate to changes in water concentration can be determined by calculation of correlation coefficients, as shown in Table 2 for P and the major cations Mg, K and Ca.

Table 2. Correlation between Anabaena mass fractions and concentration of elements in water.

	Mg	P	K	Ca
1988	0.27	-0.41	-0 .04	0.27
1989	0.63	0.86	0 .92	-0.21

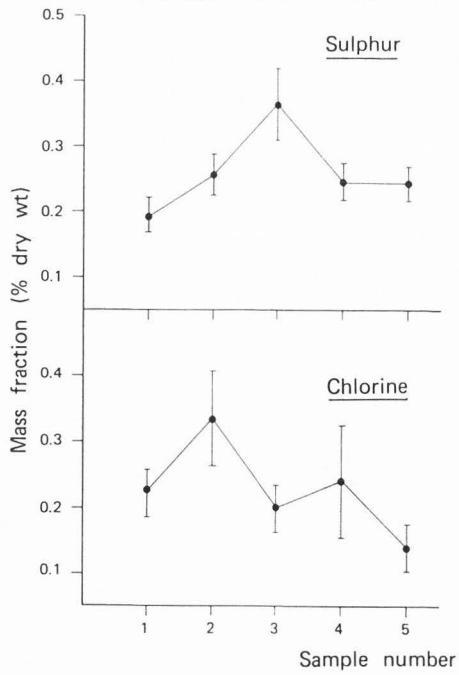

Fig.8 Changes in the mass fraction of sulphur and chlorine during the Anabaena bloom of 1988. Duncan multiple range test-
Sulphur: 1(2,5); 3(1,2,4,5).
Chlorine: 2(1,3,4,5); 5(1,4)

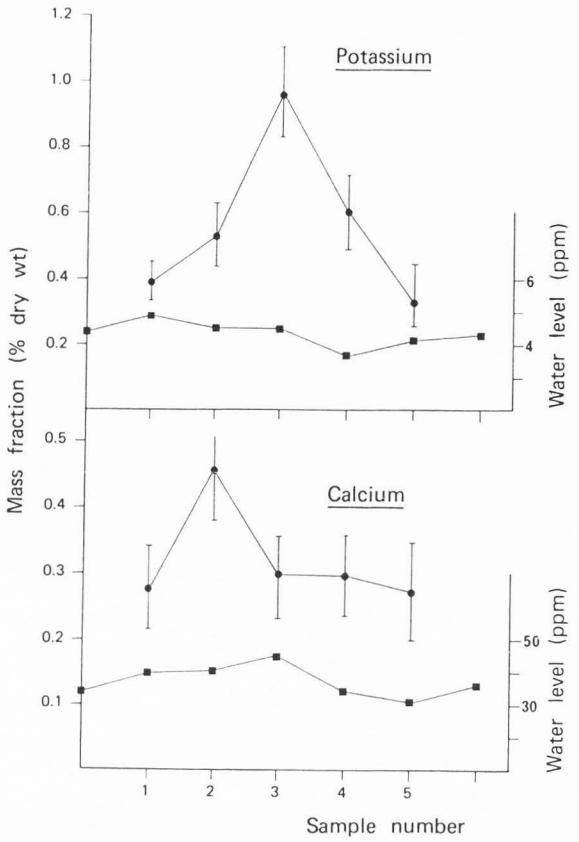

Fig.9 Changes in the level of potassium and calcium during the Anabaena bloom of 1988. Duncan multiple range test- Potassium: 2(1); 3(1,2,4,5); 4(1,5). Calcium: 2(1,3,4,5).

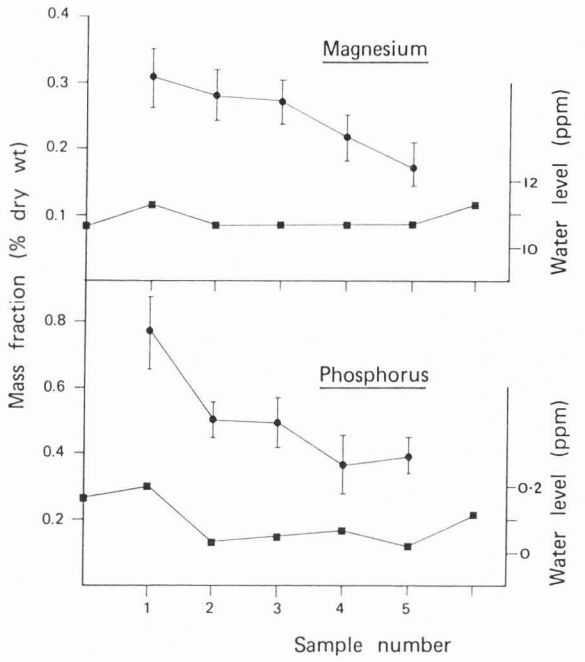

Fig.10 Changes in the level of magnesium and phosphorus during the Anabaena bloom of 1989 Duncan multiple range test- Magnesium: 1(4,5); 2(4,5); 3(4,5) Phosphorus: 1(2,3,4,5); 2(4); 3(4).

The results show that very little correlation occurred in 1988 between elemental levels in cells and in the surrounding medium, but in 1989 Mg, P and K all had high positive correlation coefficients.

Correlations between elements within cells

Correlations between elements within cells may provide useful information on elemental associations and compartmentation, and is shown separately for 1988 and 1989 in Table 3.

The results show that a large number of significant positive correlations occur between elemental levels within cells, involving correlations between positively charged molecules (cations- Mg,K, Ca) and negatively charged molecules (including P, S, Cl) in different combinations. The results also show that elemental correlations were not random but followed specific combinations (see discussion) and that relatively few correlations were negative.

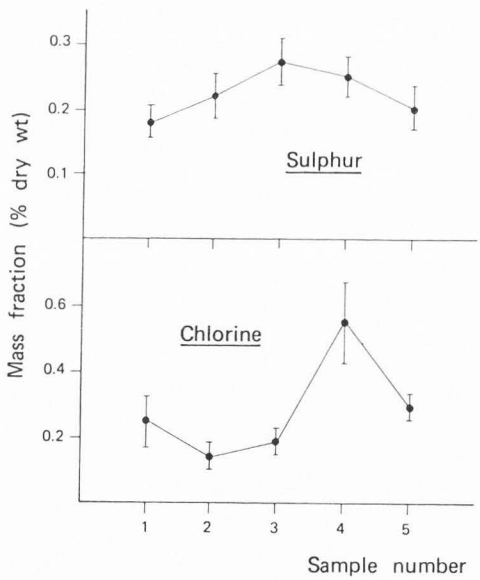

Fig.11 Changes in the mass fraction of sulphur and chlorine during the Anabaena bloom of 1989. Duncan multiple range test- Sulphur: 2(1); 3(1,2,5); 4(1,5). Chlorine: 1(2); 4(1,2,3,5); 5(2,3).

Table 3. Major Correlations between elements within cells

Sample No.	Mg-P	Mg-S	K-P	K-Cl	Ca-P	Ca-S	Ca-Cl	Mg-K	S-P
1988 Samples									
1	(+)	(+)	.	.	(+)	(+)	(+)	.	(+)
2	(+)	.	.	(+)	.	(+)	.	.	.
3	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
4	(+)	.	(+)	.	.	.	.	(+)	(+)
5	.	.	.	(+)	.	.	.	(+)	.
1989 Samples									
1	(+)	(+)	(+)	.	.	.	(+)	(+)	(+)
2	(+)	.	(+)	.	.	(+)	(+)	.	.
3	.	(+)	(+)	.	.	.	.	.	.
4	(+)	.	(+)	.	.	.	.	.	.
5	.	.	(+)	.	.	.	(+)	.	.

(+) denotes a positive correlation significant to at least 90% probability level.
Other significant correlations noted were:
1988 Samples: 1 S-Cl; 2 Si-Cl; 3 K-S, Si-Cl; 4 Si-Cl;
5 Mg-Si(-),K-Si(-). Si-Cl(-)
1989 Samples: 1 K-S; 2 Si-P, 3 Mg-Cl, S-Cl

Discussion

Blue-green algae are typical of many mesotrophic and eutrophic freshwater sites, where they may exhibit extensive growth to form blooms. The factors responsible for bloom formation are not completely understood, but increased availability of nutrients - particularly nitrates and phosphates , are generally regarded as being important (Stewart et al., 1978). In this study, the intracellular levels of P and other elements relating to nutrient status are examined in detail during the bloom period over two successive years.

The major elemental mass fractions calculated in this study by SEM X-ray microanalysis (P, K, Ca) are closely similar to those obtained previously by TEM microanalysis of ultra-thin cryosections (Booth, 1988). The P mass fraction values obtained by SEM X-ray microanalysis are also in line with those obtained by other workers using spectrophotometric techniques (Healey, 1982). This general correspondence to other published data on Anabaena elemental composition suggests that the SEM quantitation system is valid and provides further evidence that potential problems arising out of beam overpenetration do not arise.

In general terms, changes in cell elemental concentrations may be determined by either intrinsic factors (eg. algal growth phases) or extrinsic factors (eg. external water concentrations.

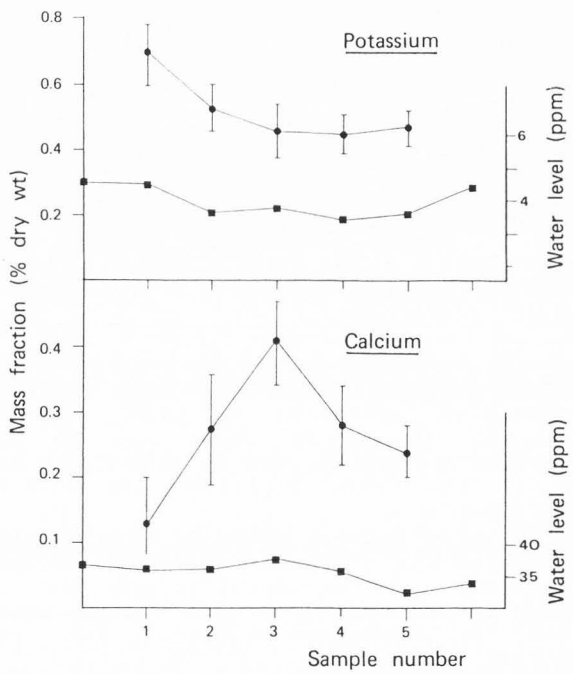

Fig.12 Changes in the level of potassium and calcium during the Anabaena bloom of 1989. Duncan multiple range test-
Potassium: 1(2,3,4,5)
Calcium: 2(1); 3(1,2,4,5); 4(1); 5(1)

Intrinsic factors and elemental mass fractions.

Elemental analysis of algae collected in lakewater samples at a particular site in the lake show significant changes in the mass fraction of major elements such

as Mg, P, S, Cl, K and Ca - indicating that substantial changes in elemental concentration occur during the course of a major bloom. The elemental changes observed in consecutive years, however, were quite different - suggesting that there is no consistent transition in the composition of algal cells during the different growth phases of the Anabaena bloom.

Internal composition of cells, particularly the occurrence of macromolecules and compartmentisation, may also be important in the overall presence of particular elements. The X-ray emission spectra that are taken from whole Anabaena cells give no direct information on the ultrastructural location of particular elements, which may be present in an intracellular compartment, within the cell wall, or in surface mucilage. Some indication of the chemical associations of particular elements is provided, however, by the correlation coefficients. A positive correlation would be expected to occur where two elements occupy a similar site in a non-competitive manner, or are chemically associated. A negative correlation will occur where two elements occupy a similar site competitively. The results suggest that certain associations are particularly prevalent -

(1) Mg and K (but not Ca) are particularly correlated with the presence of P. This element is largely present in nucleic acids and polyphosphate bodies within the cell, suggesting that Mg and K are directly associated with these cell components. The strong correlation between Mg and K is also consistent with these elements occupying a similar intracellular site non-competitively.

Studies by other workers (see, for example, Kagawa & Togashi, 1989) have also demonstrated a close correlation between cation levels and phosphorus content in planktonic algae.

(2) Mg and Ca (but not K) show strong correlations with S, possibly indicating an association with S-containing proteins.

(3) Ca is the most frequent cation correlated with Cl, possibly relating to a simple ionic association within a zone of hydration.

If particular cations are preferentially associated with particular macromolecules, then synthesis of these components may alter internal elemental mass fractions irrespective of external concentrations (where these are not rate limiting).

Extrinsic factors and elemental mass fractions

The possibility that elemental changes in algal cells might relate directly to water composition was examined by comparing these parameters within a tube sample - which represents a mean sample within the euphotic zone. Because of the small size of the sample within the lake as a whole, cell composition is being related to the immediate aquatic microenvironment. The results obtained suggest very little correlation between internal (algal) and external (environmental) element levels in 1988, but some correlation (particularly P and K) in 1989. X-ray microanalytical studies on phytoplankton by other workers have generally failed to demonstrate any clear correlation between cell mass fractions and water concentrations (Lindahl et al.,1983; Booth et al.,1987).

The differences in environmental correlations at Rostherne between 1988 and 1989 may reflect the decreased nutrient status of the lake from one year to the next, with water concentrations in 1989 being growth limiting. The reduced availability of phosphorus in 1989 is shown by the measured water concentrations (which fell from 0.2mg/l at the beginning of bloom to 0.02mg/l at the end), and also by the low cell mass fractions - which at 0.4% (or 4mg/g) resemble the cellular level of P04-deficient Anabaena determined by Healey (1973).

The low levels of P encountered in 1989 partly arose due to environmental factors, with a generally reduced rainfall leading to less inflow from streams and less run-off from surrounding agricultural land. There is also a long term reduction in the nutrient status of this lake over the last 10 years, as shown by changes in elemental water concentrations.

In general conclusion, SEM X-ray microanalysis can be used to provide detailed information on changes in the internal concentration of a range of important elements during the period of a bloom of Anabaena. The results obtained show that these elemental changes vary considerably from one year to the next and may relate to a number of internal and external factors.

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Discussion with Reviewers

J.A.Nott: Did the spot size match the size of the specimen? If so, the beam intensity would be greater at the centre than at the margins, giving uneven irradiation over the specimen.
Authors: Spectra were obtained using a standard-size raster, which typically scanned an area lying just within the periphery of the cell. This approach ensured equal irradiation over a constant area of specimen.

J.A.Nott: Have the authors tried similar analyses on an SEM cold stage ? This might reduce some loss of chlorine when the specimen is irradiated by the beam.
Authors: We have not yet carried out analyses on a cold stage. Chlorine loss does not appear to be a major problem at room temperature, however, since the mass fraction of chlorine shows no substantial variation with livetime.

G.M.Roomans:: Could the authors comment on beam penetration in these specimens, since normally with analysis of bulk freeze-dried biological material (density 0.3-0.5g cm-3) at 15 kV, the expected beam penetration would be 8-10um.
Authors: Although beam penetration through the algal cells at 15 kV might be expected, the absence of a Ti peak from the nylon support at this accelerating voltage indicates that this does not occur. This conclusion is further supported by close similarities in the elemental mass fraction data obtained by SEM and TEM X-ray analysis (see text), since overpenetration does not represent a problem in ultrathin specimen preparations. The apparent lack of beam penetration beyond the specimen at 15kV may arise due to an unusually high density in these prokaryote cells. Transmission electron micrographs of Anabaena show that the protoplasm is closely packed with membranes and ribosomal matrix.

B.Bergman: Does the elemental quantitation include externally absorbed elements? If so, would it be possible to wash the cells with EDTA to get rid of external elements?
Authors: Bulk analysis of these preparations includes all those elements that are present at the cell surface and in the cell wall in addition to internal elemental composition. In looking at the relationship between elemental composition of the biomass and the environment it is important to consider total elements associated with the cells and not just internal composition. Although it would be possible to specifically eliminate external elements by treatment with EDTA or removal of surface mucilage, there is the danger that this would also affect cell wall and internal cellular levels.

J.Morgan: Would you please summarise the advantages, from preparative and quantitation points of view, of conducting your analytical study with a scanning as opposed to a transmission electron microscope?
Authors: Scanning electron microscopy has a number of advantages over transmission electron microscopy in looking at the elemental composition of phytoplankton -

(1) The specimen can be rapidly prepared as a monolayer of cells for analysis, without the need for cryomicrotomy, (2) Different species of phytoplankton can be more readily identified (and analysed) in whole cell compared to section preparations.

Ideally, SEM and TEM X-ray microanalysis are carried out in parallel, with transmission microscopy providing useful information on elemental localisation within cells.

J.Morgan: You do not explain the functional significance of the elemental changes within algal cells during the different stages of their developmental cycle. How do you account for the compositional differences between vegetative cells, akinetes and heterocysts ?
Authors: The reasons for differences in elemental composition between the three cell types is a matter for speculation. Fine structural studies do suggest, however, that polyphosphate bodies are decreasingly present in akinetes / vegetative cells /heterocysts, which may explain why the level of phosphorus shows a similar transition. Other elemental differences may relate to this.

J.Morgan: I assume that K is a physiologically-active ion in the algal vegetative cells. How, therefore do you explain the very considerable changes in the [K] in these cells during different bloom phases, assuming that the [K] in the lake is not limiting ?
Authors: The role of K in the algal cells is highly complex since this cation occurs both in bound form (probably associated with nucleic acids) and in the cytosol (which may relate to the correlation with Cl). Changes in [K] during the bloom period may thus reflect a variety of changes in the insoluble or soluble parts of the cell and may be influenced by the uptake of quite different cations or anions.

J.Morgan: How do you justify the use of non-discriminate correlation analysis, when some of the observed relationships may in fact be entirely spurious ?
Authors: The application of correlation analysis to the whole range of detectable elements represents a broad approach intended to uncover as many statistically significant correlations as possible. Although these correlations are potentially useful in indicating different types of elemental association that may occur, they do not constitute proof.

C.Reynolds: I understand Rostherne Mere to be very rich in nutrients. If you can measure 20ppm of phosphorus in solution it is not reasonable to talk about cells being P-limited. Can you comment on this ?
Authors: It is difficult to know the precise conditions under which P-limitation occurs, and the levels of P in the cells may relate more to internal associations than to an external lack of availability.