Environmental Pollution **49** 265–288

Downcore Sulphur Isotope Ratios and Diatom Inferred pH in an Artificially Acidified Canadian Shield Lake

M. Dickman

Biological Sciences Department, Brock University,
St. Catharines, Ontario, Canada L2S 3A1

H. G. Thode

Chemistry Department, McMaster University,
Hamilton, Ontario, Canada L8S 4K1

S. Rao

Microbiology Laboratories, National Water Research Institute,
Canada Centre for Inland Waters, Burlington, Ontario, Canada L7R 4A6

&

R. Anderson

Lamont-Doherty Geological Observatory of Columbia University,
Palisades, New York 10964, USA

(Received 5 March 1987; revised version received 14 May 1987;
accepted 31 July 1987)

ABSTRACT

Three gravity cores were removed from near the deepest point in Lake 223 on 9 June 1984, eight years after the Experimental Lakes Area (ELA) staff began the artificial acidification of the lake with sulphuric acid. The first of these cores was analysed for diatoms and pollen stratigraphy while the second and third were analysed for downcore sulphur isotope ratios (H. Thode) and downcore changes in sulphur reducing bacterial densities (S. Rao). Sediment core chronologies were based on lead-210 and cesium-137 data (R. Anderson) and the Ambrosia *pollen rise (M. Dickman).*

Analysis of the first core to the depth of the Ambrosia *pollen rise (9 cm) indicated that diatom inferred pH in Lake 223 at the time of the* Ambrosia

*rise (*circa *1890) was 6·8–7·0. At a sediment depth of 3 cm the diatom inferred pH was 6·7. Thereafter diatom inferred pH began a decline culminating in the present day (observed) pH range for 1984 (5·3–5·5). At a sediment depth of 1 cm, an increase in the abundance of two benthic alkalophilic diatoms occurred. The increase in the abundance of these diatoms was ascribed to an increase in hypolimnetic alkalinity following the artificial acidification of Lake 223. This is the first time that lake acidification has been linked to an increase in benthic alkalophilic diatoms associated with hypolimnetic alkalinity production following sulphate reduction.*

Sulphur in the anaerobic (black) sediment layers (0–1·5 cm) was isotopically light relative to the sulphur in the deeper layers. This was due to sulphur isotope fractionation resulting from the bacterial reduction of sulphate to hydrogen sulphide in the anaerobic portion of the water column. A jet black FeS-rich layer in the uppermost 1·5 cm of the lake's sediments was associated with an increase in the abundance of sulphate reducing bacteria (e.g. Desulfovibrio *spp.).*

INTRODUCTION

In the winter of 1976 personnel of the Freshwater Institute began acidifying Lake 223 each season with approximately 5500 litres of technical grade sulphuric acid (Schindler *et al.*, 1980*a*). A paleolimnological study of Lake 223 was carried out for three reasons: (1) to determine how diatom populations respond to artificial acidification, (2) to test the precision and accuracy of the diatom inferred pH technique in a lake where pH has been carefully monitored for the last fifteen years and (3) to determine the relationship between downcore changes in sulphur isotope ratios and the anaerobic bacterial mediated rate of sulphate reduction.

Ambient lakewater alkalinity is not a good predictor of the buffering capacity of lake sediments. The principal source of abiotic acid neutralisation is from the release of calcium ions (Schnoor & Stumm, 1985). Sources of sedimentary Ca^{2+} fluxes vary from 0–130 meq m^{-2} $year^{-1}$. These fluxes are the result of mineral weathering and advection of groundwaters. Porewater pH is confined to a narrow range close to the pK of carbonic acid (H_2CO_3). Porewater pH is indicative of a balance between excess CO_2 resulting from diagenetic reactions and the kinetics of silicate weathering. The primary factor determining redox status of surficial sediments is the availability of organic matter. Unproductive lakes exhibit oxidised surficial sediment potentials above 200 mV while those of more productive lakes are typically less than 200 mV (Stahl, 1986). Sulphate consumption in sediments also contributes to alkalinity production (Stahl, 1986). A significant portion of lake sediment porewater alkalinity is associated with Fe^{2+}, Mn^{2+} and

NH_4^+ (S. Schiff, pers. comm.). Sulphate consumption rates range from 5 to 160 meq m^{-2} $year^{-1}$. Permanent alkalinity from SO_4^{2-} requires sequestering of sulphur in sediments as organic sulphur or iron sulphides. Alkalinity production in epilimnetic lake sediments is an important component of the resistance of softwater lakes to anthropogenic acidification.

METHODS

On 9 June 1984, three short gravity cores were removed from the centre of Lake 223 (Fig. 1) with the assistance of Mr Richard Behrn and Mr Daniel Houle. The first was 28 cm long, the second was 26 cm long and the third was 44 cm long. The three cores were sectioned at 1 cm intervals with the exception of one core in which the uppermost 0–5 cm was sectioned at 0·5 cm intervals for diatom inferred pH analysis. Sediment core preparation techniques were similar to those described by Dickman & Thode (1985).

Pollen analysis methodology

The methodology for our pollen analysis has not been previously described. Five millilitres of 10% KOH were added to 0·25 g dry weight of lake sediment and the mixture was boiled for 5 min, stirring continuously. The mixture was then centrifuged, decanted and washed with distilled water. Following this, the sample mixture was dehydrated with 3 ml glacial acetic acid, centrifuged and decanted without washing the sediment. An acetolysis solution was prepared by adding one part concentrated sulphuric acid to

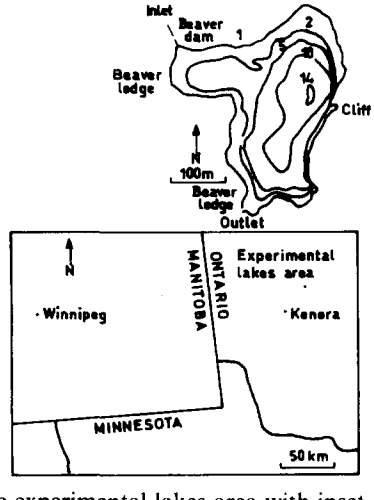


Fig. 1. The location of the experimental lakes area with inset of a morphometric map of Lake 223 (modified from Cook & Schindler, 1983).

nine parts acetic anhydride. Five millilitres of this solution was then added to a beaker containing the above sediment mixture and boiled for 5 min. After cooling, the mixture was centrifuged, decanted and washed with glacial acetic acid which was then also decanted. The final mixture was twice washed with distilled water and decanted each time. The resulting sediment was mounted on glass slides using corn syrup so the pollen grains could be rotated beneath the coverslip if required for identification purposes. Four hundred pollen grains were counted and the number of *Ambrosia* pollen grains counted were expressed as a percentage of this total. To avoid decimals in the presentation of our results, we expressed the *Ambrosia* counts as if 1000 pollen grains had been counted. Diatom counts were expressed in the same manner.

Diatom counts

Diatom enumeration was based on a minimum count of 800 diatom frustules per slide. Procedures for cleaning, mounting and counting the diatoms were described by Dickman *et al.* (1984). References used in the identification of the diatoms and the assignment of pH indicator status include Hustedt (1930–1937, 1938), Cleve-Euler (1951–1955), Patrick & Reimer (1966, 1975), Foged (1979), Beaver (1981) and Germain (1981).

The precision of the diatom inferred pH technique was estimated by making nine replicate slides of the sediment diatoms (3 from 2 cm, 3 from 3 cm and 3 from 7 cm). These slides were then coded to avoid unconscious bias and 800 frustules were counted per slide. The results of these coded slide replicate tests were used to calculate the standard deviation about the mean. The variance (two standard deviations about the mean diatom inferred pH) for any particular depth and core was represented by a vertical line atop the histogram plots from which replicate counts were made.

Water chemistry

Dissolved oxygen, pH, temperature, specific conductivity and light attenuation were measured in the field using a Hydrolab 4000 and a LiCor underwater light meter (Model 185B) quantum radiometer/photometer. Major ions had been previously analysed by Schindler *et al.* (1980*b*) and Cook & Schindler (1983).

Bacteriological procedures

Total and respiring bacteria in all water samples were estimated using the INT-formazan reduction technique (Zimmerman *et al.*, 1978). Immediately

on collection, a 10 ml portion of each water sample was poured into previously cleaned sterilised test tubes and 1 ml of 0·2% aqueous INT-dye (2-paraiodophenyl)-3-(*P*-nitrophenyl)-5-(phenyl) tetrazolium chloride, Sigma Chemicals) was added to each of the tubes. After mixing, the samples were kept in the dark for 20 min at *in situ* temperature. The reaction was stopped by adding 0·1 ml of 37% formaldehyde which also served as a preservative. The treated samples were then stored at 4°C. One millilitre of the INT-treated sample was filtered through 0·1 m pore size polycarbonate membrane (Nuclepore) which was presoaked for 24 h in a 0·0066% solution of Sudan Black (Merck) in 5% ethanol. The filter, while in the filter holder, was stained with 0·01% acridine orange for 3 min, dried and examined under oil immersion for total bacteria using epifluorescence microscopy under UV light. After epifluorescence counts, respiring bacteria were counted on the same slide using a combination of UV and transmitted bright light illumination (Zimmerman *et al.*, 1978). Because of anaerobic conditions and microbial layers on particles, sediment samples were not suitable for this procedure (Rao *et al.*, 1984*a*,*b*).

Sulphur cycle bacteria were estimated on all sediment samples using the 5 tube MPN procedure (Dutka, 1974). Densities of sulphur oxidising bacterial (*Thiobacillus* sp.) populations were measured using Postgate medium (Dutka, 1978). Sulphate reducing (*Desulfovibrio* sp.) bacterial populations were enumerated using Starky's medium with an anaerobic incubation. All MPN tubes were incubated for 21 days at 20°C.

Age-dating using lead-210 and/or cesium-137 analysis

Lead-210 per unit dry weight was measured at 1 cm intervals over the length of one of the three cores under the direction of Robert Anderson of Columbia University (pers. comm.). The 'total' lead-210 was measured by determining the granddaughter polonium-210 by means of isotope dilution (Nriagu *et al.*, 1982).

The cesium-137 activity was determined by gamma counting of an oven-dried subsample from each depth interval. A lithium-drifted germanium detector multichannel analyser was used. The basic technique was similar to that described by Pennington *et al.* (1973) and Edgington & Robbins (1976). Bioturbation factors such as those described by Fisher *et al.* (1980) were also considered.

Sulphur content and isotope ratio measurements

Standard methods were used to determine the sulphur content and sulphur isotope ratios of lake water sulphate. In general, the sulphur content was

determined gravimetrically either as $BaSO_4$ or as Ag_2S. The latter compound was converted to SO_2 gas for isotope analysis in the mass spectrometer.

In the case of the sediment samples, the total sulphur was extracted and determined using the 'Eschka' method. The samples of sediment, vacuum dried at 60°C and ground, are placed in a porcelain crucible well mixed with Eschka mixture (65% mgO, 35% Na_2CO_3). The conversion of all forms of sulphur to sulphate then takes place in a furnace at 800°C in 3 h. The sulphate produced was dissolved in water at 100°C, and precipitated as $BaSO_4$ from the filtered solution. Finally, the sulphate was converted in steps as indicated above to SO_2 gas for isotope analysis. Again, the sulphur content was determined gravimetrically either as $BaSO_4$ or as Ag_2S.

Isotopic analyses of SO_2 gas samples were performed using a high precision isotope ratio mass spectrometer described by Thode *et al.* (1951, 1961 and modified by Beaver (1973). Sulphur isotope ratios are expressed in terms of a notation where:

$$^{34}S\% = \frac{(^{34}S/^{32}S \text{ sample})}{(^{34}S/^{32}S \text{ standard})} - 1 \times 1000$$

The standard ratio is that of troilite sulphur in the Canyon Diablo meteorite. On this scale primary sulphur or magnetic sulphur have ^{34}S values of essentially zero.

RESULTS

Light attenuation (Table 1) is presented as the % of the surface light intensity (%SLI). Light was not detectable (ND) on the lake bottom (14 m). Secchi disc transparency (10·4 m) was measured on the sediment coring data (9 June 1984). Specific conductivity, dissolved oxygen (mg litre^{-1}), pH and temperature (degrees C) were measured at 1 m intervals near the centre of Lake 223 (Tables 1 and 2). For more extensive water chemistry data, see Schindler *et al.* (1980*a*) and Cook & Schindler (1983).

Lake 223 sediment core stratigraphy

The uppermost layer of sediments (0–14 mm) in all three sediment cores was jet black. Below this depth (14–33 mm) the sediments were blackish brown. Below this depth (33–120 mm) the sediments were brown. Microscopic analysis of these 33–120 mm deep sediments indicated that they were composed primarily of algal gyttja. From 120–200 mm a transition to less organic (lighter brown) materials occurred. The sediments at the base of the

TABLE 1
The Relationship Between Depth (m) and Percentage Surface Light Intensity (% SLI), Specific Conductivity micromhos cm^{-1}) and Dissolved Oxygen (mg $litre^{-1}$) for Lake 223 on 9 June 1984.

Depth (m)	*Light (% SLI)*	*Conductivity (micromhos cm^{-1})*	*Dissolved oxygen*
0	100	40	9·3
1	67	40	9·2
2	60	40	9·2
3	50	40	9·3
4	47	40	9·3
5	40	40	9·7
6	35	40	9·7
7	27	39	10·4
8	21	38	10·4
9	17	38	9·9
10	13	38	8·7
11	10	38	7·9
12	05	38	6·1
13	03	38	5·0
14	ND	39	0·2

ND = Not determined.

gravity core (200–340 mm) were brown and richer in clay than any of the overlaying sediments.

Chronologic information for Lake 223

During the last decade of the 1800s, ragweed (*Ambrosia*) became abundant in the Kenora area due to the disturbance of the natural vegetation by Europeans settling in that general region. This increase in *Ambrosia* pollen was detected in the sediments of Lake 223 by its increase above background levels (2–5 grains per thousand). The first significant increase in *Ambrosia* pollen (8 grains per thousand) occurred at a depth of 9 cm (Fig. 2).

The *Ambrosia* rise depth was used to calculate a mean sedimentation rate (0·8 mm year), for comparison with the cesium-137 (Fig. 3) and lead-210 (Fig. 4) generated sedimentation rates. The lead-210 generated sedimentation rate was 0·54 mm $year^{-1}$ while the cesium-137 generated sedimentation rate was 1·33 mm $year^{-1}$.

Downcore bacterial analyses

The surface sediments of Lake 223 contained 2300 sulphate reducing bacteria per millilitre. This is considerably higher than for any of the control

TABLE 2

The Relationship Between Depth (Eight Depths) and Dissolved Oxygen (mg litre ^{1}) on 13 June 1983, pH and Temperature (9 June 1984), Chlorophyll A and Alkalinity on 13 June 1983 and 7 October 1983. Data from 1983 were Provided by D. Schindler pers. comm.)

Depth	*DO 13/6/83*	*pH*	*Temperature (°C)*	*Chlor. A*[a] *6/83*	*Chlor. A*[a] *10/83*
0	9·1	5·3	15·3	0·5	2·4
1		5·3	15·3		
2		5·3	15·3		
3		5·3	15·3		
4	9·6	5·4	15·3	0·4	2·0
5		5·4	14·8		
6		5·4	13·8		
7		5·6	11·6		
8	11·9	5·7	9·9	2·1	1·9
9		5·7	9·0		
10	9·4	5·7	8·5	11·2	
11	6·6	5·7	8·2	14·5	23
12	4·9	5·7	7·9	15·1	27
13	3·1	5·7	7·6	17·3	29
14	2·3	5·7	7·7		

Depth (m)	*Alkalinity (microequiv litre^{-1})*	
	6/83	*10/83*[b]
1	−10	−2
2		
3		
4		
5	−9	−2
6		
7		
8	−3	+3
9		
10	11	48
11	24	40
12	27	674
13	24	857

[a] Chlorophyll was reported as micrograms litre^{-1}.
[b] The high alkalinity in October was associated with anaerobic hypolimnetic conditions.

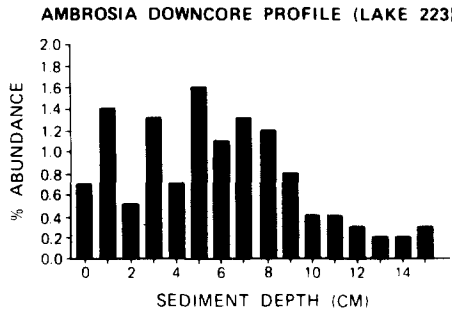


Fig. 2. Downcore (0–15 cm) *Ambrosia* pollen grain abundance expressed per thousand pollen grains. The *Ambrosia* rise (9 cm *circa* 1890) was statistically significant ($p < 0.05$).

lakes in the Wawa study area (Thode *et al.*, 1987). Heterotrophic bacteria (640 000 per millilitre) and sulphur oxidising bacteria (48 per millilitre) were also observed in the surface sediments (0–0·5 cm) of Lake 223 (core No. 3).

Sulphur contents and isotope ratios

The sulphur contents and isotope ratios were determined for Lake 223 sediment samples selected from 0 to 14 cm in depth. The sediment profiles of these values are plotted in Fig. 5 together with the Lake 223 water column data of Cook (1980) and Cook & Schindler (1983) for purposes of comparison. A single water column sample taken by us in June 1984 at a depth of 5 m gave a sulphate concentration of 10 ppm and a ^{34}S value of +13% (also included in Fig. 5).

Diatom inferred pH

The downcore pH for Lake 223 was inferred from the ratio of acid to alkaline diatoms in the sediments of this lake (Table 2). The log of this ratio

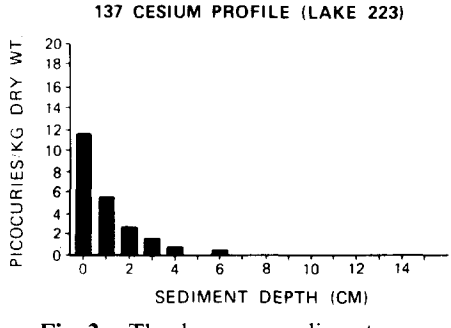


Fig. 3. The downcore sediment profile for cesium-137 in Lake 223. The cesium-137 generated sedimentation rate was 0·083 cm year^{-1}.

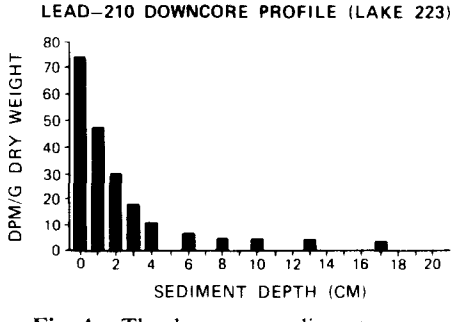


Fig. 4. The downcore sediment profile for lead-210 in Lake 223. The lead-210 estimated sedimentation rate was 0·054 cm year^{-1}.

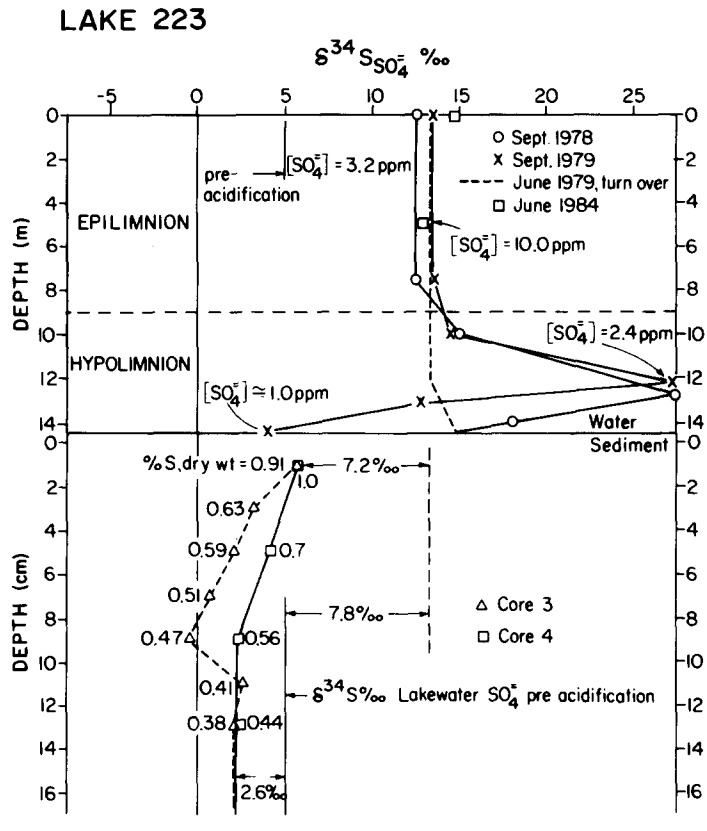


Fig. 5. Lake 223 water column and sediment core. Profiles of sulphur concentration and isotope ratios ($\delta^{34}S$). Water column data taken from Cook (1980). Sediment data and 1984 water column data—this work.

(log alpha, Merilainen, 1967, 1969) was modified by Renberg & Hellberg (1982) and Dickman *et al.* (1984) and referred to as Index B.

$$\text{Index B} = \frac{\begin{array}{r}(\%\ \text{Circumneutral diatoms}) + (5 \times \%\ \text{acidophilic diatoms}) \\ + (40 \times \%\ \text{acidobiontic diatoms})\end{array}}{\begin{array}{r}(\%\ \text{Circumneutral diatoms}) + (3{\cdot}5 \times \%\ \text{alkaliphilic diatoms}) \\ + (108 \times \%\ \text{alkalibiontic diatoms})\end{array}}$$

No alkalibiontic diatoms were observed in the sediments of Lake 223.

Although it is frequently difficult to distinguish between circumneutral and pH indifferent diatoms, the latter contribute little to the inference of lake pH and should be ignored as they increase the variance without increasing the precision of the diatom inferred pH estimates (Dickman *et al.*, 1984).

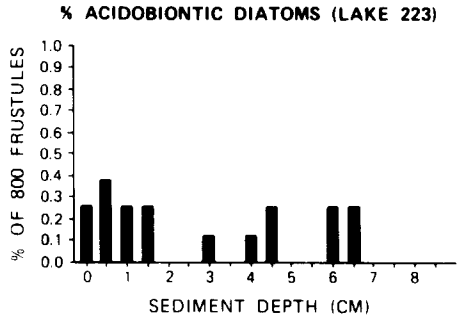


Fig. 6. Histogram of the per cent abundance of acidobiontic diatoms at 0·5 cm intervals downcore in the sediments of Lake 223.

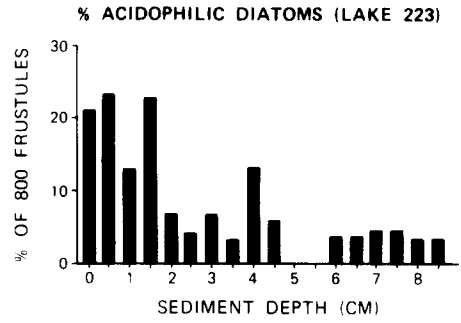


Fig. 7. Histogram of the per cent abundance of acidophilic diatoms at 0·5 cm intervals downcore in the sediments of Lake 223. To convert x axis to sediment depth divide by 2.

The log B diatom inferred pH was calculated from the equation: Diatom inferred pH = 6·4–0·85 Index B.

Diatom stratigraphy

Major downcore changes in the relative abundance of acidobiontic, acidophilic, circumneutral and alkalophilic diatom taxa were primarily the result of changes in the percentage composition of 11 dominant taxa (Figs 6–21). The majority of the diatoms in the sediments of Lake 223 belonged to a single taxon, *Cyclotella stelligera* (Fig. 17).

Downcore changes in the relative abundance of the most common alkalophilic diatoms were of two types: (1) Benthic alkalophilic taxa which

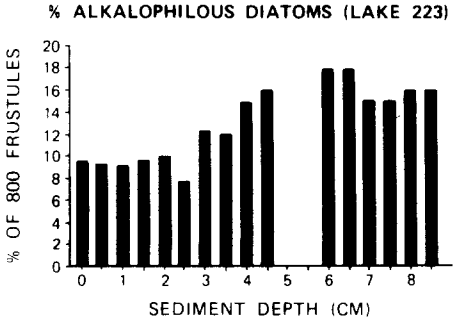


Fig. 8. Histogram of the per cent abundance of the alkalophilic diatoms in the surficial sediments of Lake 223.

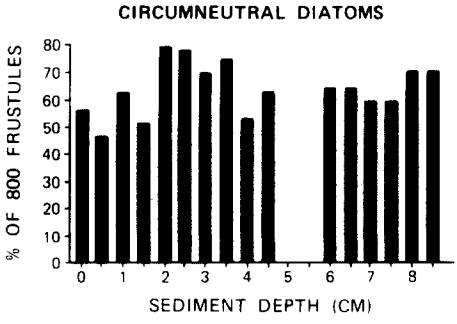


Fig. 9. Histogram of the per cent abundance of the circumneutral diatoms at 0·5 cm intervals downcore in the sediments of Lake 223.

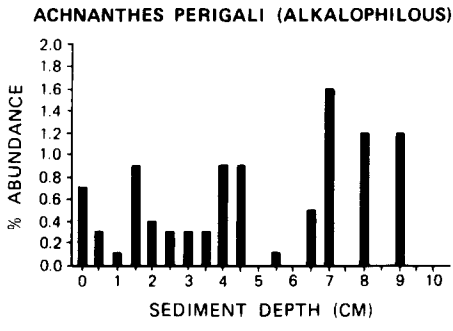


Fig. 10. The relative abundance of the benthic alkalophilic diatom *Achnanthes perigali* in the surficial sediments of Lake 223.

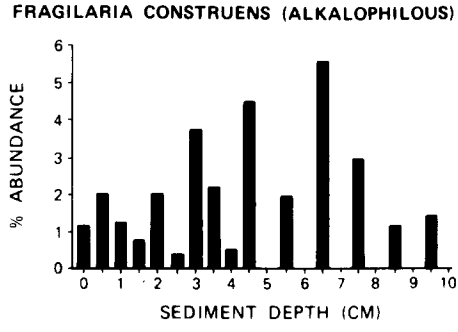


Fig. 11. The relative abundance of the planktonic alkaliphilic diatom *Fragilaria construens* in the surficial sediments of Lake 223.

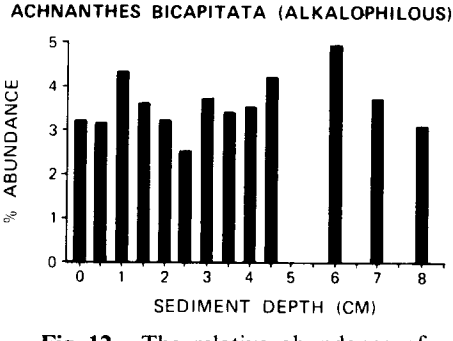


Fig. 12. The relative abundance of the benthic alkaliphilic diatom *Achnanthes bicapitata* in the surficial sediments of Lake 223.

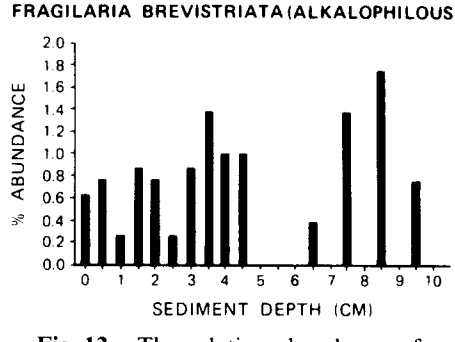


Fig. 13. The relative abundance of the alkaliphilic planktonic diatom *Fragilaria brevistriata* in the surficial sediments of Lake 223.

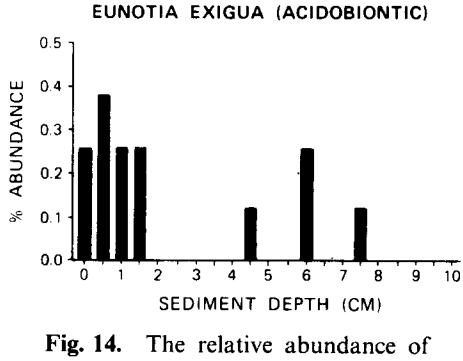


Fig. 14. The relative abundance of the acidobiontic diatom *Eunotia exigua* in the surficial sediments of Lake 223.

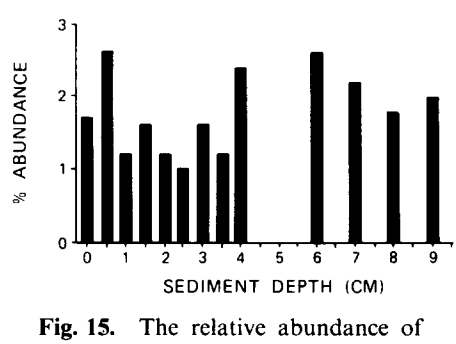


Fig. 15. The relative abundance of the acidophilic diatom *Tabellaria quadriseptata* in the surficial sediments of Lake 223.

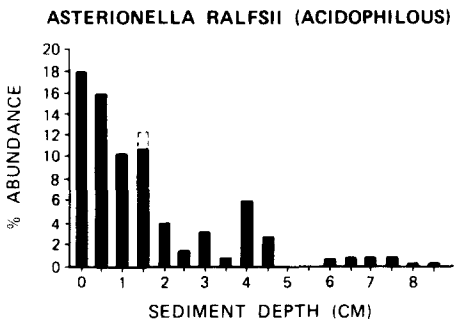


Fig. 16. The relative abundance of the acidophilic diatom *Asterionella falfsii var Americana* in the surficial sediments of Lake 223. Dashed line portions of the histogram (e.g. at 1·5 cm) in this and subsequent Figures represent the maximum range ($m = 3$).

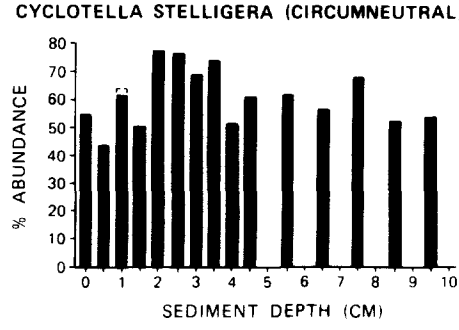


Fig. 17. The relative abundance of the circumneutral diatom *Cyclotella stelligera* in the surficial sediments of Lake 223.

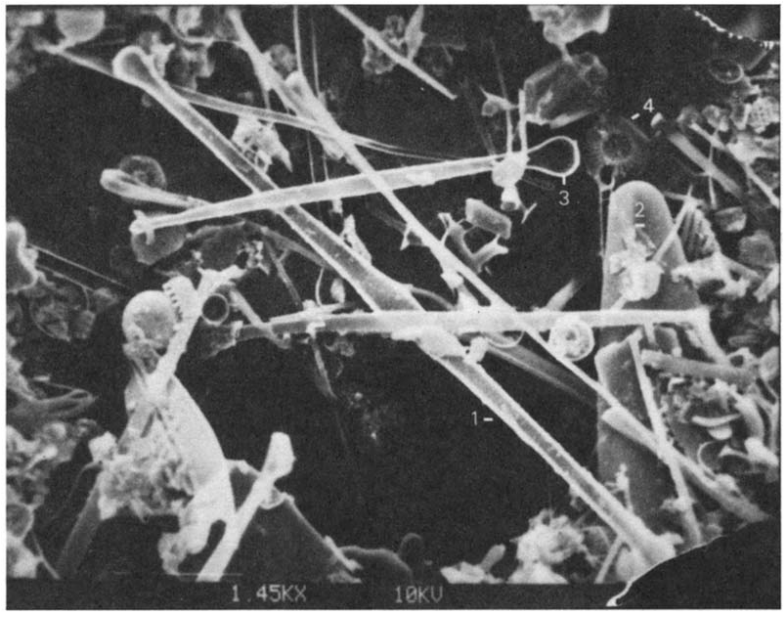
displayed a general upcore decrease with a statistically significant ($P < 0{\cdot}05$) increase at 1·5 cm (*Achnanthes perigali*, Fig. 10) and 1·0 cm (*Achnanthes bicapitata*, Fig. 12) following the artificial acidification of Lake 223 and (2) planktonic alkalophilic taxa which decreased in relative abundance after the artificial acidification of Lake 223 (Figs 10 and 13). The alkaliphilous, planktonic, diatom *Melosira italica* subspecies *subarctica* (Fig. 20) also decreased significantly ($P < 0{\cdot}05$) after the initiation of acidification as did shallow water taxa such as *Nitzschia gracilis* (Fig. 21).

The most abundant acidophilic diatom in the sediments of Lake 223 (*Asterionella ralfsii* var. *americana* Fig. 16) increased dramatically after the lake's acidification. This diatom increased most strikingly in the top 1·5 cm while the pH indifferent diatom *Asterionella formosa* increased at 1·0, 1·5, 3·0, 4·0 and 4·5 cm (Fig. 19). Acidophilic and acidobiontic taxa (e.g. *Asterionella ralfsii* var. *americana*, *Eunotia vanheurkii* (not figured), *Tabellaria quadriseptata* and *Eunotia exigua* Figs 14, 15 and 16) all increased in their relative abundance following the artificial acidification of Lake 223.

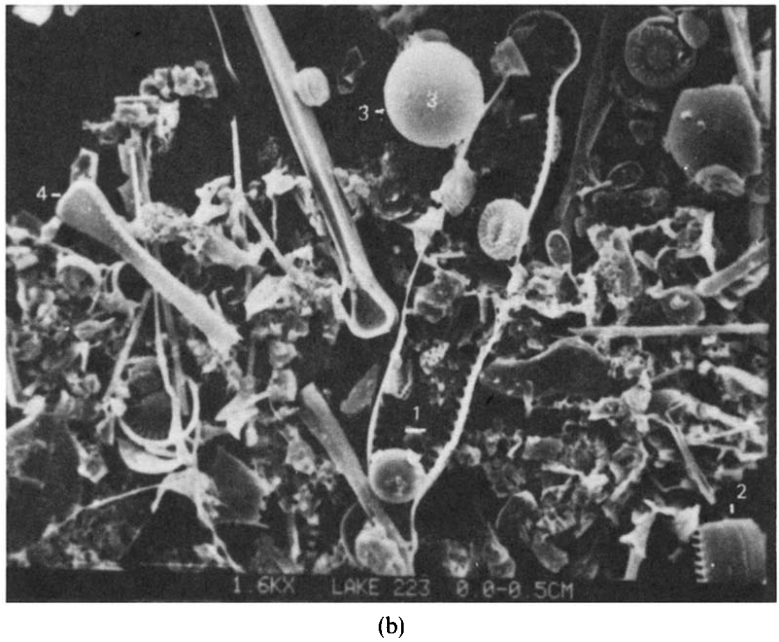
DISCUSSION

Sulphate reduction and the generation of alkalinity

Schindler *et al.* (1980*a*) reported that after the addition of sulphuric acid to Lake 223 in 1976 the freshly introduced sulphur was reduced by sulphate reducing bacteria. Based on our observations of the increase of



(a)



(b)

Fig. 18. Scanning electron micrographs (1,600 × magnification) of the common diatoms and chrysophyte cysts and scales found in the sediments of Lake 223. (1) *Pinnularia* sp., (2) *Cyclotella stelligeria*, (3) *Dinoryon* cyst and (4) *Tabellaria quadriseptata*.

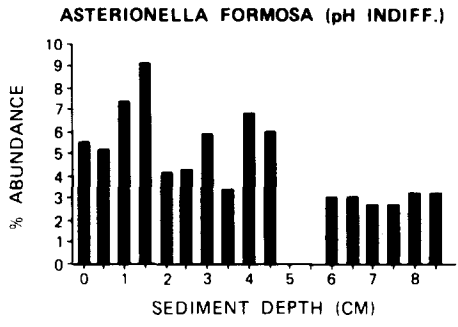


Fig. 19. The relative abundance of the pH indifferent diatom *Asterionella formossa* in the surficial sediments of Lake 223.

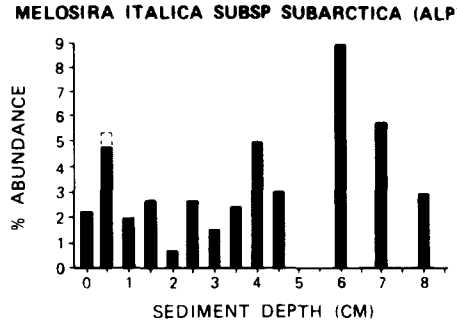


Fig. 20. The relative abundance of the alkaliphilic planktonic diatom *Melosira italica* subspecies *subarctica* in the surficial sediments of Lake 223.

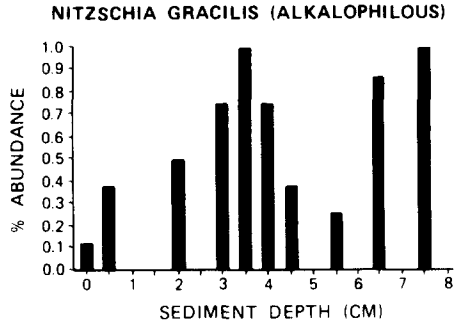


Fig. 21. The relative abundance of the alkaliphilic littoral zone benthic diatom *Nitzschia gracilis* in the surficial sediments of Lake 223.

Desulphovibrio spp. in Lake 223 sediment cores, we have concluded that some of this reduced sulphur quickly combined with ferrous ions in the anaerobic portion of the lake's hypolimnion to form a FeS-rich black sediment layer (0–14 mm).

In an iron-dominated lake such as Lake 223 where Fe^{2+} at 14 m was 1·6 mg litre^{-1} in July of 1983 (D. W. Schindler, pers. comm.), alkalinity production occurs as the result of H_2S build-up in the anoxic hypolimnion of the lake during late summer and autumn. It should be noted that pyrite formation will also generate alkalinity. Unfortunately, only qualitative (light microscope and SEM) information exists about the presence of pyrites in the surficial sediments of Lake 223. Redox reactions involving manganese and nitrogen also occur, but they are of secondary importance (Cook & Schindler 1983).

Estimates of sedimentation rate

We have hypothesised that the addition of 5500 litres of concentrated sulphuric acid (Schindler *et al.*, 1980*a*) to the epilimnion of Lake 223 in the winter of 1976 stimulated the formation of FeS in the lake's hypolimnion and shortly thereafter this material entered the lake's bottom sediments forming a black layer there. The sedimentation rate over the last eight years (1976–1984), as judged from the depths of the relatively uncompacted FeS-rich black layer at 14 mm, was 1·75 mm year^{-1}. This estimate of the sedimentation rate during the last decade was high due to the relative lack of compaction in the flocculant surface sediment layers. The average rate of sedimentation over the last century (*Ambrosia* rise *circa* 1890) was 0·54 mm year^{-1} (Fig. 2). During the last 50 years, the lead-210 estimated sedimentation rate (Fig. 3) was 0·85 mm year^{-1}. No attempt was made to correct these estimates for differential sediment compaction because attempts at making such corrections are usually based on the percentage water content of the dried sediments. These corrections were found not to be particularly reliable.

The question of the relative degree of mixing and bioturbation of the recently deposited sediments of Lake 223 is critical to any stratigraphic analysis (Edgington & Robins, 1976; Fisher *et al.*, 1980). We hypothesised that the presence of pyrite framboids and FeS at a sediment depth of 14–33 mm indicated that some of the material deposited during the last eight years in the top 14 mm has been mixed (principally by chironomid bioturbation) to a depth of 33 mm. One of us (R. Anderson) plans to address the question of sediment mixing and bioturbation in his comparison of cesium and lead isotope downcore profiles in the sediments of E.L.A. Lakes.

Sediment sulphur

The sulphur content (% dry weight) was higher in the surface sediments (0–2 cm) of Lake 223 than in the deeper layers (12–14 cm) by a factor of 2·4 (Fig. 5). The big increase (*circa* 50%) which occurred between 2–4 and 0–2 cm, parallels in part a 2·5- to 3-fold increase in the lake's epilimnetic sulphate concentration following its artificial acidification with technical grade sulphuric acid. Cook (1980) reported an even higher sulphur content in the surficial samples (1 cm) for a Lake 223 core taken several years earlier.

The absence of isotopically light sulphur in the various external sources tested and the ^{34}S distribution patterns obtained for the lake sediments suggest an internal lake source for the isotopically light sulphur. We concluded that the shift in sediment ^{34}S values must be due to sulphur isotope fractionation resulting from the bacterial reduction of lake sulphate to hydrogen sulphide at or near the sediment–water interface. This biogenic

process was very likely stimulated by introduction into the lake of increasing amounts of anthropogenic sulphur, via acid (rain), Nriagu & Coker (1978, 1983). This has occurred since *Ambrosia* times in the case of the Algoma and Turkey lakes, and in addition via the direct addition of technical grade sulphuric acid since 1976 in the case of Lake 223 (Schindler *et al.*, 1980*b*).

This sulphur reduction to H_2S carried out anaerobically in the shallow sediments or in the lower hypolimnion for Lake 223 during summer stratification, in a partially open system, could account for the observed upcore shift in sulphur isotope ratios and sulphur content found for the lakes studied. The microbiological data obtained for the Algoma Lakes and Lake 223 are in accord with this conclusion. The Algoma Lakes' shallow sediments (0–2 cm) which showed the largest shift in ^{34}S displayed the highest concentrations of 'sulphate reducers' and the highest sulphur loading. On the other hand, the shallow sediments of lakes which showed only small shifts in ^{34}S had relatively low concentrations of sulphate reducers (Thode *et al.*, 1987).

It has been known for many years that sulphur isotopes are fractionated in the bacterial reduction of sulphate to hydrogen sulphide, the latter being depleted in isotopically light ^{34}S (Thode *et al.*, 1960). This process accounts for the isotopically light sulphur usually found in marine sediments where the requirements for this biogenic process, (1) the absence of oxygen, (2) the presence of sufficient sulphate and (3) the availability of an organic substrate with easily assimilated low molecular weight compounds, are easily met. In marine sediments, the bulk of the isotopically light hydrogen sulphide generated is unlikely to be immediately tied up by iron and other metals, but rather escapes to the oxygenated part of the environment where it undergoes rapid oxidation. Some of this isotopically light sulphur becomes incorporated in the various organic compounds in the sediments through the cycles of biologically mediated reactions (Thode *et al.*, 1960; Nriagu & Soon, 1985).

It has been generally assumed that the reduced sulphur in the sediments of soft water lakes of low sulphate concentrations is largely derived from the decomposition of organic matter, its isotopic ratio reflecting that of the lake water sulphate, since there is little or no sulphur isotope fractionation in plant (algae) metabolism of sulphate (Ishii, 1953; Kaplan *et al.*, 1963, Mekhtiyeva & Pankina, 1968). However, recent studies of Lakes 223 and 227 in the experimental lakes area (ELA) of northwestern Ontario by Cook & Schindler (1983), indicate that extensive bacterial sulphate reduction and isotopic fractionation occurs in anoxic zones of these lakes with sulphate concentrations as low as 30–40 moles litre^{-1} (3 to 4 ppm of SO_4^{2-}) contributing up to 70% of the total reduced sulphur in these sediments.

They suggest, however, that sulphate reduction may no longer be energetically favourable at sulphate concentrations below 20–30 μmol

litre^{-1} since these were the lowest concentrations of sulphate found in the bottom and pore waters of Lake 223 where bacterial reduction had occurred.

However, this seems unlikely since sulphate reduction by pure cultures of sulphate reducers *Desulphovibrio desulphuricans* has been reported at these low concentrations (Harrison & Thode, 1958). The minimum sulphate concentrations reported in these sediment pore waters could be due to oxidation of H_2S during sampling and in analytical procedures.

A more likely possibility is the competition between methanogens and 'sulphate reducers' for the organic nutrients present. A model for the distribution of sulphate reduction and methane genesis in fresh water lake sediments developed by Lovely & Klug (1986) indicates that at a sulphate concentration of $<30\,\mu$m litre^{-1} (*circa* 3 ppm) methanogens prevent 'sulphate reducers' from growing because of the dual limitations of low sulphate concentrations and organic nutrient consumption by methanogens. The small apparent sulphur isotope fractionation in the deeper sediments (10–12 cm) of the Algoma lakes and Lake 223 therefore suggests sulphate concentrations close to this threshold value, $20\,\mu$m litre^{-1} (2–3 ppm) some 60 to 80 years ago.

Downcore diatom inferred pH

When Lake 223 log B downcore diatom inferred pH was plotted against sediment depth, it became apparent that at a sediment depth of 1·5 cm there is a dramatic shift in diatom inferred pH. At this depth, both log B and log alpha suddenly shift from negative to positive values (Figs 22 and 23). This results in a statistically significant decrease in diatom inferred pH from above pH 6 to below pH 5·5 (Fig. 24). This was consistent with the reported

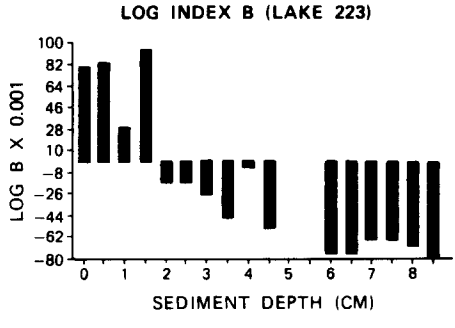


Fig. 22. Log (base 10) of index B as a function of sediment depth for the surficial sediments of Lake 223.

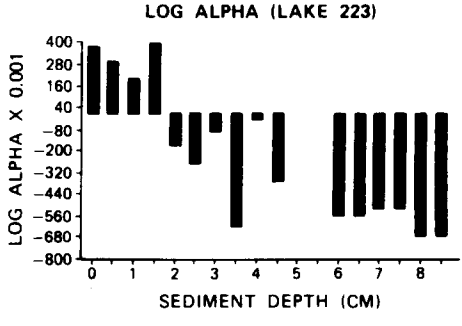


Fig. 23. The relationship between log alpha generated diatom inferred pH and sediment depth ($n = 3$).

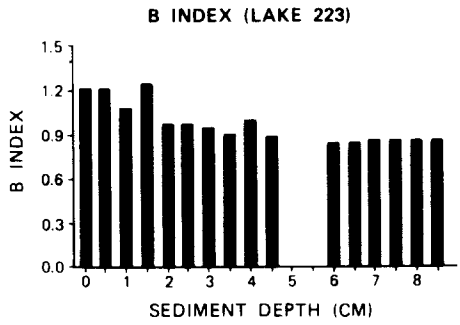


Fig. 24. Index B as a function of depth for the surficial sediments of Lake 223.

observations of pH in Lake 223 following its artificial acidification (Cook & Schindler, 1983). To assist the reader in interpreting Figs 22–24, the diatom inferred pH (log B Index) was plotted as a function of Lake 223 sediment depth (Fig. 25), and observed pH in 13 lakes was plotted as a function of log B (Fig. 26).

After the acidification of Lake 223, there was a differential response in the planktonic and benthic diatom communities. The planktonic alkalophilous and circumneutral species such as *Nitzschia graciles* (Fig. 21), *Rhizosolenia eriensis* and *Synedra acus* (not figured) were replaced by acidophilous taxa, primarily *Tabellaria quadriseptata* and *Asterionella ralfsii* (Figs 15 and 16, respectively). Similar observations were made by Davidson (1986). Benthic diatoms, on the other hand, responded quite differently. Those deep dwelling taxa which were exposed to anoxic conditions from time to time were dominated by alkaliphilic taxa rather than the anticipated acidophilic ones. The reasons for this are provided in the following section.

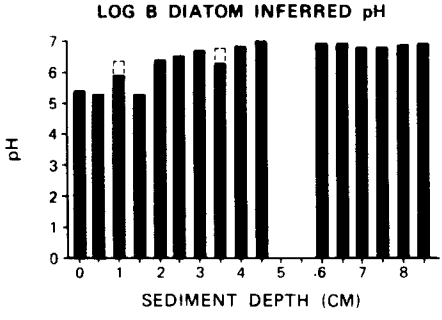


Fig. 25. Log B generated diatom inferred pH versus sediment depth for the surficial sediments of Lake 223. Dashed line portion of the 1·0 and 3·5 cm histograms represents maximum range on replicate counts.

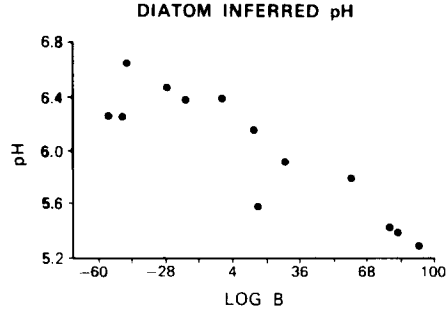


Fig. 26. The relationship between observed lake pH and surficial sediment (0–0·5 cm) diatom inferred log B for 13 lakes located north of Lake Superior (correlation coefficient = 0·87, $p < 0·01$).

Hypolimnetic alkalinity and diatom species composition

A number of benthic alkaliphilic taxa such as *Achnanthes perigali* and *A. bicapitata* initially decreased in relative abundance after the artificial acidification of Lake 223 and then increased at 0 and 1·5 cm (*A. perigali*, Fig. 10) and at 1 cm (*A. bicapitata*, Fig. 12). These statistically significant increases ($p < 0{\cdot}05$) were interpreted as resulting from short-term (e.g. Aug.–Nov.) anoxia and accompanying enhanced hypolimnetic alkalinity.

In addition, the meroplanktonic, alkalophilic taxon *Melosira italica* subsp. *subarctica* increased suddenly at 0·5 cm (Fig. 20). This too, was ascribed to the observed increase in the lake's hypolimnetic alkalinity following the onset of hypolimnetic anoxia in late summer.

The following hypothesis is advanced in an attempt to explain the observed short-term increase in some alkaliphilous diatoms following the acidification of Lake 223. According to Cook & Schindler (1983) the addition of sulphuric acid to Lake 223 increased the penetration of light by precipitating humic matter. In addition, the abundance of planktonic algal taxa was reduced. Both of these factors increased the depth of light penetration in Lake 223 providing benthic diatoms with an opportunity to colonise sediments at greater depths due to increased light penetration. Acidification also resulted in an increase in sulphate reduction rates from 3 to 10×10^{-4} mol m^{-2} day^{-1} (Cook & Schindler, 1983). This, in turn, caused the bottom tenth of the lake's hypolimnion to become anoxic and more alkaline.

It is our belief that some of the benthic, meroplanktonic or tycho-planktonic, alkalophilic diatoms in Lake 223 were responding to this increase in hypolimnetic light and alkalinity. To the best of our knowledge, this is the first report of an observed response of benthic alkaliphilic diatoms to an increase in hypolimnetic alkalinity resulting from the addition of sulphuric acid to a natural lake.

CONCLUSIONS

Visual inspection of the sediments removed from the three gravity cores which were taken from Lake 223 in June of 1984 indicated that the top 14 mm were jet black. This was attributed to FeS production following sulphuric acid introduction to Lake 223. Bioturbation was implicated in the downcore distribution of the FeS-rich material to a depth of 3 cm.

The log B index diatom inferred pH for Lake 223 from 1890 to 1967 ranged from pH 6·0 to pH 7·0. In 1976 when Lake 223 was acidified, diatom inferred pH suddenly began to decline. The pH of Lake 223 at the time the sediment cores were taken in June 1984 was 5·1–5·3 at the surface and 5·7

near the mud–water interface. The diatom inferred pH from the uppermost sediment layers (0–0·5 cm) was 5·3.

The artificial acidification of Lake 223 resulted in an increase in the relative abundance of acid indicator planktonic diatoms. Alkaline indicator benthic diatoms were rare relative to the planktonic taxa. Nevertheless, it was concluded that the generation of alkalinity by sulphate reduction in the anaerobic portions of Lake 223 and the increase in lake transparency following its artificial acidification stimulated the growth and reproduction of some benthic, meroplanktonic and tychoplanktonic alkaline indicator diatoms during periods of hypolimnetic oxygen depletion (Aug.–Nov.).

The upcore shifts in sulphur content and sulphur isotope ratios which chronicle past changes in lake sulphate loading were confirmed by this study. Our observations of upcore increases in the abundance of *Desulphovibrio* spp. were correlated with 2- to 3-fold upcore increases in the percentage dry weight of sulphur in the uppermost (0–2 cm) sediment layers. In addition, the sulphur in these uppermost sediment layers was isotopically light relative to the deeper layers. We have interpreted this to support the hypothesis that during periods of hypolimnetic hypoxia the *Desulphovibrio* reduced sulphate to sulphide and, by so doing, generated isotopically light sulphides. Previous studies (Dickman *et al.*, 1985; Thode *et al.*, 1987) have reported this phenomenon in lake sediments, but this is the first report of its occurrence in the water column of a lake.

ACKNOWLEDGEMENTS

The authors are grateful to P. Hayes of Brock University who assisted in the diatom enumeration, Mark Taylor of Waterloo University and Roger Sweets of Indiana University who assisted in the diatom identification, and Ben Baliat of McMaster University who assisted in the sulphur isotope ratio analyses. We are also grateful to the S.E.M. staff at East Carolina University in Greenville, North Carolina, for permission to use their S.E.M. facility and to NSERC for funding this research in the form of an operating grant to M. Dickman.

REFERENCES

Anderson, R. F. (1984). Personal communication. Lamont-Doherty Geological Observatory of Columbia University, Palisades, New York 10964 USA.

Beaver, E. M. (1973). An automatic radio readout system for a double collection mass spectrometer. *Mass Spectr.*, **21**, 37–44.

Beaver, J. (1981). Apparent ecological characteristics of some common freshwater diatoms. Ontario Ministry of Environment Report and the Technical Report Section, Central Region, 150 Ferrard Drive, Don Mills, Ontario M3C 3C3, Canada.

Cleve-Euler, A. (1951–1955). Die Diatomeen von Schweden und Finnland, Kunglia Svenska Velenskapsacademien Avhandlingar. Naturskyddsarenden Almqvist and Wiksell, Stockholm. **4**(1), 1–158, **4**(5), 1–255.

Cook, R. B. (1980). *The biogeochemistry of sulphur in two small lakes.* PhD Thesis, Columbia University, New York.

Cook, R. B. & Schindler, D. W. (1983). The biogeochemistry of sulfur in an experimentally acidified lake. *Ecol. Bull.* (Stockholm), **35**, 115–27.

Davidson, G. A. (1986). *Palaeolimnological reconstruction of the acidification history of an experimentally acidified lake.* MSc Thesis, University of Manitoba, Winnipeg.

Dickman, M. & Thode, H. G. (1985). The rate of lake acidification in four lakes north of Lake Superior and its relationship to downcore sulphur isotope ratios. *Water, Air and Soil Pollution*, **26**, 233–53.

Dickman, M., Dixit, S., Fortescue, J., Terasmae, J. & Barlow, R. (1984). Diatoms as indicators of the rate of lake acidification. *Water, Air and Soil Pollution*, **21**, 375–86.

Dickman, M. D., Rao, S. S. & Thode, H. G. (1985). Effects of lake acidification on sediment bacteria, sediment sulfur isotope downcore profiles. *Environment Canada National Water Research Institute. NWRI Report Series*, 85–87.

Dutka, B. J. (1978). Methods for microbiological analysis of water, wastewaters and sediments. Inland Waters Directorate, Scientific Operations Division, CCIW, Burlington, Ontario.

Dutka, B. J., Bell, J. B. & Liu, D. L. S. (1974). Microbiological examination of offshore Lake Erie sediments. *Fish. Res. Board Can.*, **31**, 299–308.

Edgington, D. N. & Robbins, J. A. (1976). Patterns of deposition of natural and fallout radionuclides in the sediments of Lake Michigan and their relation to limnological processes. In: *Environmental Biogeochemistry, Vol. 2. Metal transfer and ecological mass balance*, ed. by Nriagu, J. O. 705–29, Michigan, Ann Arbor Science.

Fisher, J. B., Lick, W. J., McCall, P. L. & Robbins, J. A. (1980). Vertical mixing of lake sediments by tubificid oligochaetes, *J. Geophys. Res.*, **85**, 3977–4006.

Foged, N. (1979). *Diatoms in New Zealand, the North Island.* J. Cramer Struss and Cramer Publ. Hirschberg, Germany.

Germain, H. (1981). *Flore des Diatomees.* Paris, Solciete Nouvelle Des Editions Boubee.

Harrison, A. G. & Thode, H. G. (1958). Mechanism of the bacterial reduction of sulphate from isotope fractionation studies. *Faraday Soc. Trans.*, **54**, 84–92.

Hustedt, F. (1930–37). Die Kieselalgen von Deutschland, Osterreich under der Diatomeen: Flora von Java, Bali and Sumatra. *Archiv. Hydrobiologie Suppl.*, **16**, 1–155 & 274–394.

Hustedt, F. (1930–37). Die Kieselalgen von Deutschland, Osterreich under der Schweiz. *Rabenhorst's Kryptogamen-Flora*, **7**(1), 1–920, **7**(2), 1–736.

Ishii, M. (1953). *Fractionation of the sulphur isotopes in plant metabolism of sulphur.* MSc Thesis, McMaster University, Hamilton, Canada.

Kaplan, I. R., Emery, K. O. & Rittenberg, S. C. (1963). The distribution and isotopic abundance of sulphur in recent marine sediments off Southern California. *Geochim. Cosmochim. Acta*, **27**, 297–331.

Lovely, D. R. & Klug, M. J. (1986). Model for the distribution of sulphate reduction and methanogenesis in freshwater sediments. *Geochim. Cosmochim. Acta*, **50**, 11–18.

Mekhtiyeva, V. L. & Pankina, R. G. (1968). Isotopic composition of sulphur in aquatic plants and dissolved sulphates. *Geochem. Intern.*, **5**, 624.

Merilainen, J. (1967). The diatom flora and the hydrogen ion concentration of the water. *Ann. Bot. Fen.*, **4**, 51–8.

Merilainen, J. (1969). Distribution of diatom frustules in recent sediments of some meromictic lakes. *Mitt. Internat. Verein. Limnol.*, **17**, 186–92.

Nriagu, J. O. & Coker, R. D. (1978). Isotopic composition of sulphur in atmospheric precipitation around Sudbury, Ontario. *Nature*, **274**, 883–5.

Nriagu, J. O. & Coker, R. D. (1983). Sulphur in sediments chronicles past changes in lake acidification. *Nature*, **303**, 692–4.

Nriagu, J. O. & Soon, Y. K. (1985). Distribution and isotopic composition of sulfur in lake sediments of Northern Ontario. *Geochim. Cosmochim. Acta*, **49**, 823–34.

Nriagu, J. O., Wong, H. K. T. & Coker, R. D. (1982). Deposition and chemistry of pollutant metals in lakes around the smelters at Sudbury, Ontario, *Environ. Sci. Technol.*, **16**, 551–60.

Patrick, R. & Reimer, C. W. (1966). *The diatoms of the United States. Vol. 1.* The Academy of Natural Sciences of Philadelphia, **13**, 688.

Patrick, R. & Reimer, C. W. (1975). *The diatoms of the United States. Vol. 2.* The Academy of Natural Sciences of Philadelphia, **13**, 213.

Pennington, W., Cambray, R. S. & Fisher, E. H. (1973). Observations on lake sediments using fallout Cesium-137 as a tracer. *Nature*, **242**, 324–6.

Rao, S. S., Jurkovic, A. A. & Nriagu, J. O. (1984*a*). *Environmental Pollution Series A*, **36**, 195–205.

Rao, S. S., Paolini, D. & Leppard, G. C. (1984*b*). Effects of low pH stress on the morphology and activity of bacteria from lakes receiving acid precipitation. *Hydrobiologia*, **114**, 115–21.

Renberg, I. & Hellberg, T. (1982). The pH history of lakes in south-western Sweden, as calculated from the subfossil diatom flora of the sediments. *Ambio.*, **11**, 30–33.

Schiff, S. (1986). Personal communication. Institute of Groundwater Research, University of Waterloo, Waterloo, Ontario.

Schindler, D. W., Hesslein, H., Wageman, R. & Broecker, W. S. (1980*a*). Effects of acidification on mobilization of heavy metals and radionuclides from the sediments of a fresh water lake. *Can. J. Fish Aquat. Sci.*, **37**, 373–7.

Schindler, D. W., Wageman, R., Cook, R. B., Rusczynski, R. & Prokopowich, J. (1980*b*). Experimental acidification of Lake 223 Exp. Lakes Area: Background data and the first three years of acidification. *Can. J. Fish. Aquat. Sci.*, **37**, 342–54.

Schnoor, J. L. & Stumm, W. (1985). Acidification of aquatic and terrestrial systems, In: *Chemical processes in lakes*, ed. by W. Stumm. 318–38, New York, John Wiley and Sons.

Stahl, H. (1986). *Influence of sediment redox status on lake acidification in northeastern Ontario.* MSc Thesis, Laurentian University, Sudbury, Ontario.

Thode, H. G., Kleerekoper, H. & McElcheran, D. E. (1951). Isotope fractionation in the bacterial reduction of sulphate. *Research*, **4**, 581–2.

Thode, H. G., Harrison, A. G. & Monster, J. (1960). Sulphur isotope fractionation in early diagenesis of recent sediments of North East Venezuela. *A.A.P.G. Bull.*, **44**, 1809–17.

Thode, H. G., Monster, J. & Dunford, H. B. (1961). Sulphur isotope geochemistry. *Geochim. Cosmochim. Acta*, **25**, 159–74.

Thode, H. G., Dickman, M. & Rao, S. S. (1987). Effects of acid precipitation on sediment downcore profiles of diatoms, bacterial densities and sulphur isotope ratios in lakes north of Lake Superior. *Arch. Hydrobiol.*, **74**, 397–422.

Zimmerman, R., Iturriaga, R. & Brick, J. B. (1978). Simultaneous determination of the total number of aquatic bacteria and the number thereof involved in respiration. *Environ. Microbiol.*, **36**, 926–35.