Aquatic Sciences 55/1, 1993

1015–1621/93/010031–09 $1.50+0.20/0

Fossil carotenoids and paleolimnology of meromictic Mahoney Lake, British Columbia, Canada

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Key words: Paleolimnology, fossil pigments, carotenoids, okenone, photosynthetic bacteria, meromixis.

ABSTRACT

Vertical distribution of fossil carotenoids in a sediment core from meromictic Mahoney Lake was studied. Besides okenone and demethylated okenone, lutein and zeaxanthin and β-carotene isomers were identified. No carotenoids typical for purple nonsulfur or green sulfur bacteria were detected. The ratio of zeaxanthin to lutein (above 1:1 in all samples) indicates a dominance of cyanobacteria over green algae in the phytoplankton assemblages of the past. Okenone, which is found exclusively in Chromatiaceae, was the dominating carotenoid in all sediment zones.

The oldest sediment layers containing okenone were deposited 11 000 years ago. Between 9000 and 7000 and since 3000 years b.p., Chromatiaceae reached a considerable biomass in the lake. Vertical changes in okenone concentration were not related to changes of paleotemperatures. In contrast, okenone concentrations decreased during periods of volcanic ash input. During most of the lake history, however, mean okenone concentrations were positively correlated with sedimentation rates. This indicates that vertical changes of okenone concentration in the sediment reflect past changes of purple sulfur bacterial biomass in the lake.

According to these results, the past limnology of Mahoney Lake resembled that of the present with a sulfide-containing monimolimnion and a well-developed population of okenone-bearing purple sulfur bacteria.

Introduction

Recent studies of the phototrophic bacterial community in meromictic Mahoney Lake revealed an extraordinary high biomass accumulation of purple sulfur bacteria present in the permanent chemocline (Overmann et al. 1991). This worldwide outstanding cell density may be a relatively recent phenomenon or it could have persisted for a much longer period in the lake history.

In paleolimnological studies carotenoids of phototrophic bacteria may serve as a sensitive measure of past biomass changes (Brown et al. 1984, Züllig 1985). Due to

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the absence of light and oxygen, carotenoids are preserved well in the monimolimnion sediment of meromictic lakes (Leavitt and Carpenter 1990). The stable stratification of the bottom water reduces sediment resuspension. Due to the high hydrogen sulfide concentrations benthic invertebrates are absent thus preventing any bioturbation. Therefore the initial sequence of sediment layers is well conserved in meromictic lakes.

Mahoney Lake provides ideal conditions to elucidate the role of anoxygenic phototrophic bacteria in past lake metabolism by analysis of fossile carotenoids in sediment samples. In the present paper the vertical distribution and types of carotenoids were studied in sediments deposited over the past 13000 years.

Material and methods

Mahoney Lake is a small (19.8 ha, max. depth 15 m) meromictic and oligotrophic lake in the central South of British Columbia, Canada and is located 470 m asl. The lake originated after retreat of the Wisconsin ice sheet more than 13000 years ago and probably occupies a kettle basin. The high salt content of the lake water has been attributed to the presence of alkali-rich Tertiary rocks of the Marron Formation in the watershed (Northcote and Hall 1983).

A sediment core (length 6 m, diameter 5.5 cm) was obtained with a simple piston corer (McKean and Nordin, unpublished manuscript) from the deepest part in the center of Mahoney Lake in May, 1985. The core was extruded and split in half vertically with piano wire. Sections of one meter were stored at − 20 °C.

For analysis of carotenoids, one cm sections of the core were freeze dried and ground in a mortar. Pure cultures of the reference strains necessary for identification of carotenoids as well as *Amoebobacter purpureus* ML 1 isolated from Mahoney Lake (Overmann et al. 1991) were grown in batch culture in the basal medium described by Pfennig and Trüper (1989). Cells were harvested by centrifugation and the pellet freeze dried.

Pigment extractions were carried out in the dark. 50 mg of sediment or 10 mg of pure cell cultures were extracted for 20 min in 30 ml hot (60 °C) methanol/KOH (6% KOH v/v). All carotenoids detected in the Mahoney Lake sediment are stable under these conditions (Schmidt et al. 1965). The remaining pellet was extracted twice for 10 min with 10 ml acetone at room temperature. All extracts were combined in a separatory funnel and mixed with 30 ml diethyl ether/petroleum ether (1/10, v/v; boiling range of petroleum ether 35–80 °C) and 30 ml distilled water. After separation the methanol/water phase was discarded and the ether phases taken to dryness in a rotary evaporator at 35 °C. Dried pigments were dissolved in 3 ml acetone, transferred into glass vials, dried again in a stream of N_2 and stored in the dark at − 20 °C.

For separation and quantification the pigments were dissolved in 1 ml acetone and 20 µl samples were injected with a microliter syringe (Hamilton Bonaduz, Switzerland) in a HPLC system (Perkin-Elmer, Überlingen, Germany) equipped with a photodiode array detector (Waters 994, Waters, Eschborn, Germany). The chromatographic system used a pre-column packed with Nucleosil 50-5 and a 25 cm

separation column packed with Spherisorb ODS-1 (5 μm particle size). An isochratic mobile phase containing acetonitrile, methanol and isopropanol (85/10/5, v/v/v) was applied at a flow rate of 1 ml · min^{-1} (Ernst and Sandmann 1988).

For identification the absorption spectra and chromatographic mobility of pigments present in the sample were compared with those of pigments isolated from pure bacterial cultures and from *Lycopersicon esculentum*. All-*trans* isomers could be identified by comparison of the absorption spectra with respective all-*trans* standards and by the absence of the short wavelength absorption peaks characteristic for *cis*-isomers (e.g. 331 nm for lutein). According to Schmidt (1978) the following bacterial strains were chosen as sources for carotenoid standards: *Chromatium minus* strain 1211 (okenone), *Rhodospirillum rubrum* S1 (anhydro-rhodovibrin), *Thiocapsa roseopersicina* 6311 (spirilloxanthin), *Rhodospirillum rubrum* S1 (rhodovibrin), *Rhodomicrobium vannielii* (rhodopin) and *Thiothece* 2611 (demethylated okenone). Lycopene was obtained from *Lycopersicon esculentum*.

Carotenoid standards were separated and purified by thin layer chromatography on silica gel plates with a mobile phase consisting of 10% acetone in hexane (in the case of okenone, anhydro-rhodovibrin and spirilloxanthin), 20% acetone in hexane (rhodovibrin and rhodopene) or 30% toluene in hexane (lycopene). Demethylated okenone was separated on a grade II Al_2O_3 column with 20% acetone in hexane as the mobile phase and purified on silica gel G plates with 30% acetone in hexane. β-Carotene was purchased from Sigma (Deisenhofen, Germany) and purified on silica gel G plates with toluene (5%) in hexane.

The HPLC system was calibrated with chromatographically pure pigments. The concentration of each standard was determined spectrophotometrically employing the absorption coefficients given by Schmidt et al. (1965) and Davies (1976). The detection limit for carotenoids was 0.4 μg · (g Sediment)$^{-1}$.

Results and discussion

In most of the sediment samples carotenoids were detected. Separation by HPLC (Fig. 1) showed the peaks of six different carotenoids and their isomers. In addition to dominating all-*trans*- (No. 1) and *cis*-okenone (No. 2), demethylated okenone (small peak with a retention time of 6.2 min), all-*trans*-lutein (No. 3), all-*trans*-zeaxanthin (No. 4), α-carotene (No. 5) and β-carotene were identified. There was 56% of all-*trans*- (No. 6a), 27% of a nonspecified *cis*-isomer (No. 6b), and 18% of 15-*cis*-isomer (No. 6c) of β-carotene.

At present the population of anoxygenic phototrophic bacteria in Mahoney Lake is dominated by *Amoebobacter purpureus* (97.9% of the total number of phototrophic bacteria in the chemocline, Overmann et al. 1991). The chromatograms of carotenoid extracts of *Amoebobacter purpureus* ML1 isolated from the chemocline of the lake exhibited similar peaks of the three okenone isomers (Fig. 2).

Carotenoids were detected down to a depth of 4.9 m in the sediment core (Fig. 3, arrow). The vertical distribution of carotenoids in the Mahoney Lake sediment may be used as a measure of past changes of phototrophic bacterial biomass for the following reasons. Although vertical profiles of all carotenoids were similar, changes

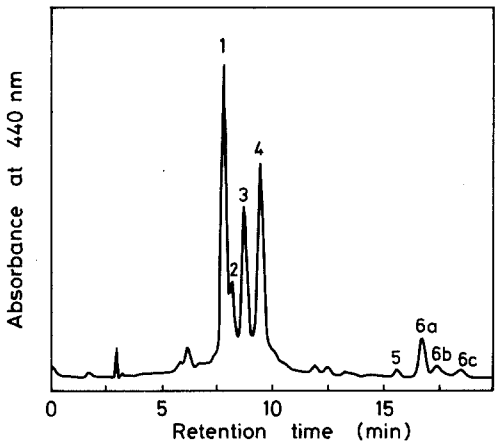

Figure 1. HPLC separation of carotenoids extracted from the sediment of Mahoney Lake. Sediment depth 61 cm. Small peak at retention time of 6.2 min = demethylated okenone. 1 = all-*trans* okenone, 2 = *cis*-okenone, 3 = all-*trans* lutein, 4 = all-*trans* zeaxanthin, 5 = α-carotene, 6a, b, c, = β-carotene isomers (56% all-*trans*, 27% of a non-specified *cis*-isomer, 18% 15-*cis*). In some of the other sediment depths, 15-*cis*-isomers of lutein and zeaxanthin were detected in small quantities

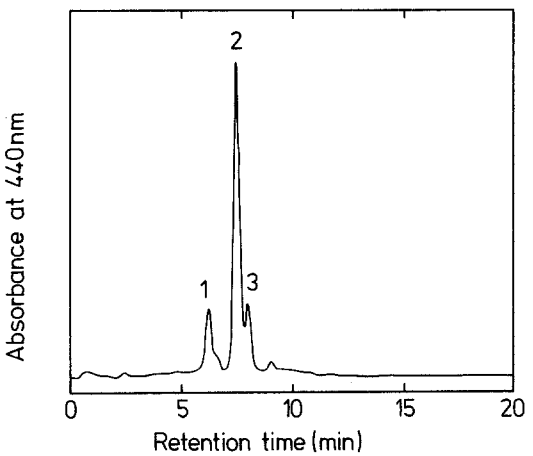

Figure 2. HPLC separation of carotenoids from *Amoebobacter purpureus* strain ML 1 isolated from the chemocline of Mahoney Lake. 1 = demethylated okenone (18.6% of total carotenoids), 2 = all-*trans* okenone (69.2%), 3 = *cis*-isomer of okenone (12.2%)

in absolute values did not occur in a proportional fashion (compare uppermost three data points for all carotenoids in Fig. 3). Allochthonous input of okenone seems improbable in Mahoney Lake because of its small drainage area (Northcote and Hall 1983). Furthermore, the chemical stability of carotenoids in sediments is very high; for example the reduction of double bonds in zeaxanthin was not found before 56000 years of burial in a marine sediment (Watts and Maxwell 1977). Okenone

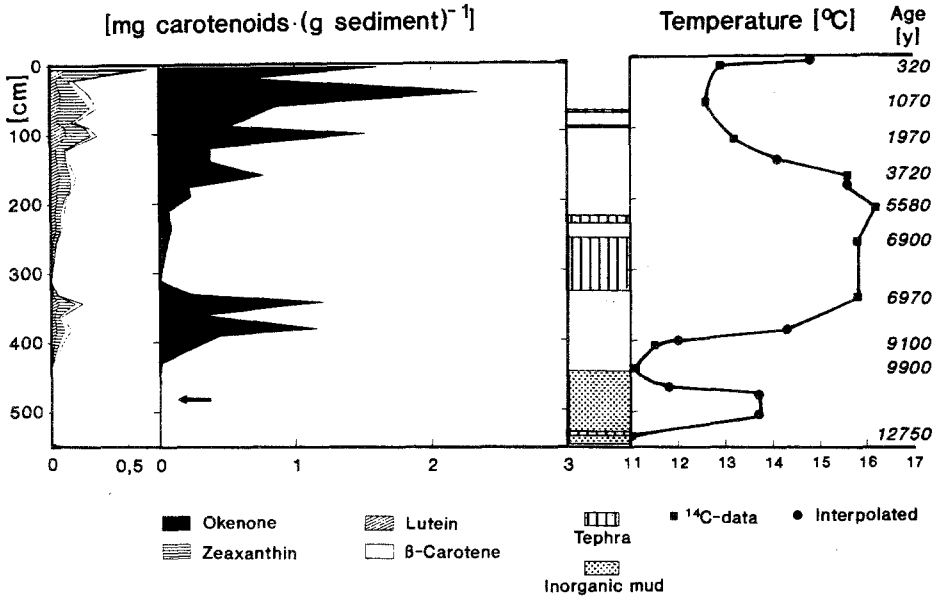

Figure 3. Vertical distribution of okenone, zeaxanthin, lutein and β-carotene (isomers as described in the text) in a sediment core from Mahoney Lake. Concentration given per dry mass of sediment. Arrow = oldest sediment layer containing detectable concentrations of okenone and β-carotene. Ash layers in the sediment were attributed to the following eruptions (from top of sediment core): 70 cm = Mount St. Helens; 95 cm = Mt. St. Helens; 230 cm = Mt. St. Helens; 260–330 cm = Mazama Mountain; 530 cm = Glacier Peak. The lowermost meter of sediment contains mainly inorganic mud. Paleotemperatures (mean of summer values) for Masset (British Columbia, Canada) and Humptulips (Washington, U.S.A.) after Lamb (1977). Rectangles = values for ^{14}C data, circles = temperature values determined after linear interpolation of age between adjacent ^{14}C data

concentrations in the sediment showed a positive correlation with sedimentation rates (see below). Thus, changes in the input rate of inorganic sediments cannot be the reason for changes in carotenoid concentration during most of the lake history.

Okenone is found exclusively in 10 Chromatiaceae species (*Chromatium okenii*, *C. weissei*, *C. minus*, *C. purpuratum*, *Thiocystis gelatinosa*, *Lamprobacter modestohalophilus*, *Amoebobacter purpureus*, *Thiopedia rosea*, *Thiocapsa halophila* and – besides spirilloxanthin – in some strains of *Thiocapsa roseopersicina*) (Caumette et al. 1985, Eichler and Pfennig 1988, Pfennig and Trüper 1989, Caumette et al. 1991).

The oldest sediment layers containing okenone were deposited 11 000 years ago during the Alleröd. In a period between 9000 and 7000 y.b.p. Chromatiaceae obviously reached a considerable biomass in the lake. High okenone concentrations in the sediments deposited during the late preboreal (9000–8500 years b.p.) and early boreal (8500–6900 y.b.p.) indicate a conspicuous bloom shortly after glacial lake genesis.

In contrast to the high okenone concentrations, no carotenoids of purple nonsulfur bacteria or green sulfur bacteria were detected. Similar to the present situation (Overmann et al. 1991), okenone-containing Chromatiaceae species

obviously dominated the phototrophic bacterial population in the past. In the presence of sulfide as an electron-donating substrate, these bacteria have a selective advantage over purple nonsulfur bacteria. If constant physiological properties of Chromatiaceae are assumed for the last 11 000 years, the results of the present study indicate the presence of anoxic, sulfide-containing bottom water during most of the history of Mahoney Lake.

The ratio of zeaxanthin to lutein was higher than 1 in all samples. Zeaxanthin, lutein and β-carotene are present in green algae (Chlorophyceae) and higher plants. In green algae the proportion zeaxanthin: lutein is below 1 (e.g. 0.4 for *Dunaliella*, *Ankistrodesmus*) and even lower (≈ 0.1) in terrestrials plants (Goodwin 1980). In contrast, cyanobacteria and Chrysophyceae do not contain lutein. As the degradation rates of zeaxanthin and lutein in oxic lake water are similar (Leavitt and Carpenter 1990), a change in the relative proportion of both pigments prior to deposition in the sediment is unlikely. Therefore the results of carotenoid determination indicate that cyanobacteria or Chrysophyceae may have dominated the phytoplankton population in the past. Yet, echinone which is a typical carotenoid of many cyanobacterial species (van den Hoek 1978, Goodwin 1980, Züllig 1985) was not detected. The same holds true for fucoxanthin which is found in Chrysophyceae and Bacillariophyceae. However, in the cyanobacterium *Synechococcus nidulans* (Chroococcaceae) the dominating carotenoid is zeaxanthin. Today the cyanobacterium *Chroococcus* sp. (closely related to *Synechococcus*) is the dominant phytoplankton species in Mahoney Lake (Northcote and Hall 1983). Within the last century higher amounts of zeaxanthin were deposited (Fig. 3). Possibly this indicates an increase of cyanobacterial biomass in the phytoplankton assemblage during that period.

In order to elucidate the causes for the changes in okenone concentrations during the past 11 000 years, paleo-temperatures during the deposition of different sediment layers were determined after Lamb (1977) (Fig. 3, right panel). The vertical profiles of temperature and okenone concentration do not parallel each other indicating that long-term climatic changes did not influence the amount of carotenoids deposited in the past. For example, the temporary temperature maximum in the Alleröd period (12 000–11 000 y.b.p.) did not result in a bloom of Chromatiaceae. Carotenoid concentrations declined sharply during the late Boreal and during the climatic optimum of the Atlantic (6500–4500 y.b.p.). In contrast, highest okenone concentrations were found in times of low average temperature (Sub-Atlantic, since 3000 y.b.p.).

Comparison with the depth and vertical extent of volcanic ash layers (Tephras) in the core (Fig. 3) indicates one possible explanation for the vertical variation of carotenoid concentration. Within the ash layers only low carotenoid concentrations were detected. A conspicuous decrease occurred during the input of volcanic ash after the eruption of Mazama Mountain (Oregon, U.S.A., 6900 years ago). Within 70 years an 80 cm thick ash layer was deposited in the lake. This layer was also detected in many soils of southern British Columbia.

The correlation between sedimentation rates and mean okenone concentration in the 9 time intervals between ^{14}C-dating was examined. No correlation ($r = -0.11$, $n = 9$) was found if all intervals were considered, but a weak positive correlation was

detected ($r = 0.75^*$, $n = 8$) if the period of high input of Mazama ash (6970–6900 y.b.p.) was left out of the analysis. This parallel development of pigment concentrations and sedimentation rates indicates that a considerable part of the variation in sedimentation rates was due to biomass variations in the lake.

Variations in the amount of sedimenting inorganic materials were important only during short time intervals. Obviously the increased sedimentation rate during the input of volcanic ash (especially Mazama ash) into the lake decreased pigment concentrations in the sediment. A similar effect due to increased input of clastic materials originating in lake watersheds has been described by Vallentyne (1956) and Züllig (1985). Additionally the decline of carotenoid concentrations during this period may be due to increased shading of phototrophic microorganisms by the ash suspension in the water column. After the input of Mazama ash there appears to be a trend of increasing okenone concentrations with obvious spikes. It is evident from the core that calcium carbonate precipitation in warmer water diluted the carotenoid pigments in the core during some of the warmer periods between 3500 and 6000 years ago. This may also help to explain some of the lower concentrations that occurred in the last 3000 years.

The highest okenone concentration reported in the literature was 1.08 mg · (g dry sediment)$^{-1}$ (Lago di Cadagno, Züllig 1985). The concentration in Mahoney Lake was 2.3 times higher. However, the sedimentation rate in Lago di Cadagno (180 cm per 1000 years) was 2.6 times higher than that of Mahoney Lake (68 cm in the last 1000 years). Thus the amount of okenone deposited during this time interval is comparable in both lakes. The present biomass of purple sulfur bacteria in Mahoney Lake is extraordinarily high (20880 µg bacteriochlorophyll a per liter; Overmann et al. 1991) compared to the low biomass in Lago di Cadagno (80 µg bacteriochlorophyll a per liter; S. Lehmann, pers. comm.). Possibly the historical sedimentation of Chromatiaceae in Mahoney Lake was decreased by a steep density gradient as nowadays (Overmann and Pfennig 1992).

In the sediments of other lakes studied so far, okenone comprised only a smaller fraction of total carotenoids (average 2–30%, calculated from Züllig 1985). In contrast, the average okenone concentration in the Mahoney Lake sediment was higher (53.4% of total) than that of phytoplankton carotenoids. In the six layers of maximum carotenoid concentration (Fig. 3), okenone even comprised 70.0 (S.D. $\pm$ 10.1)% of total carotenoids. This indicates oligotrophy of the lake also in the past. However, as the shading of phototrophic bacteria is decreased under oligotrophic conditions, carotenoids of green sulfur bacteria (β-isorenieratene) are usually found in the respective sediment layers, whereas low concentrations of okenone and the absence of β-isorenieratene have characterized eutrophic periods in small meromictic lakes (Brown et al. 1984, Züllig et al. 1985). Our results do not agree with this general pattern. The absence of carotenoids of green sulfur bacteria and a low portion of phytoplankton carotenoids at the same time may be explained by oligotrophic conditions and a strong competition for light in the chemocline. Once more this indicates an extraordinarily dense accumulation of Chromatiaceae in the chemocline in the past.

Okenone has also been found in other lake sediments more than 10000 years old (Züllig 1984, 1985). In contrast to other lakes (e.g. Lago di Cadagno, Lobsigensee,

Rotsee; Züllig 1985), there is no evidence for eutrophication and concomitant increase of phototrophic bacterial populations in Mahoney Lake. High concentrations of okenone, low concentrations of phytoplankton carotenoids and the absence of carotenoids of green sulfur bacteria are similar to the present limnological situation (Overmann et al. 1991). This indicates that the environmental conditions for growth of phototrophic sulfur bacteria in the past resembled those of present day. Possibly the meromixis of Mahoney Lake has existed already for several thousand years.

ACKNOWLEDGEMENT

We are indebted to Prof. N. Pfennig, University of Konstanz, Germany, for the supply of phototrophic bacterial cultures and for helpful discussions during the present work.

C. J. P. McKean and R. N. Nordin provided coring equipment and helped to take the sediment core. ^{14}C dating was organized by J. Green, University of Waikato, Hamilton, New Zealand. We thank J. T. Beatty for sampling assistance.

This study was supported by the Deutsche Forschungsgemeinschaft through its SFB 248.

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Received 2 March 1992;
revised manuscript accepted 8 September 1992.