

**European Mountain lake Ecosystems: Regionalisation, diagnostics &
socio-economic Evaluation**

EMERGE

19

SEDIMENTARY PIGMENT ANALYSIS

C, N, S ELEMENTAL ANALYSIS

A. Lami
T. Buchaca

C.N.R. Istituto Italiano di Idrobiologia, Pallanza
University of Barcelona

EMERGE - PROJECT

Sedimentary pigment analysis

C, N, S elemental analysis

Field and shipping instruction:

For the details on how to handle the cores in the field refer to EMERGE PROTOCOLS: SEDIMENT CORES.

During the fieldwork, the sediment should be protected against direct sun light and excessive warming. After slicing the core, the sediment samples should be deep frozen (-20°C) as soon as possible and shipped frozen to Pallanza.

As regards pigment stability, the frozen samples are stable for ca. 6 months. EXPRESS COURIER in a “ice-cream” like box with dry ice could send sediment samples (not mandatory). Warning: Be sure that the ink or label on the bags is resistant to very low temperature such as with dry ice.

We need some basic data performed by Site operator on each sample: Water content and Loss On Ignition at 550°C.

The amount of sediment needed to perform pigment and CNS determination is ca. 3-5 g of fresh sediment.

Laboratory Methods

Two laboratories will carry out pigment analysis simultaneously. Although the laboratory methods are different, we expect comparable results. We will draw up a protocol to perform a calibration between laboratories and we will organise a session for intercomparison.

Method used by CNR Istituto Italiano di Idrobiologia.

Algal and bacterial pigments will be extracted (overnight, at 10 °C, in the dark and under nitrogen) from ca. 1-2 g wet sediments using 90% acetone and then centrifuged at 3000 rpm for 10 min in 15 ml glass centrifuge tubes. Duplicates samples will be extracted. **The same sediment samples, after extraction, will be used to perform zooplankton analysis.**

Chlorophyll derivatives (CD) and total carotenoids (TC) will be extracted with 90% acetone and expressed as in Guilizzoni et al. (1983) and Züllig (1982), respectively.

Specific pigments will be determined by ion pairing, reverse-phase HPLC (modified from Mauntoura & Llewellyn, 1983, and Hurley, 1988) and expressed as nanomoles per gram of organic matter. The ion pairing (tetrabutyl ammonium phosphate 10^{-3} M) allows for greater resolution of the dephytolated acidic chloropigments (Chl c, chlorophyllide a, and pheophorbide a). The equipment employed consisted of a gradient pumping system and dual channel variable wavelength UV-VIS detector (set at 460 nm and 656 nm for carotenoids and chloropigments, respectively) controlled by a computer (Beckman System Gold). An auto-sampler for sample injection was connected through a precolumn to a reverse-phase C18 ODS column (5 µm particle size; 250 mm x 4.6 mm i.d.). After sample injection (300 µl of acetone extract not dried in a rotary evaporator), a gradient program that ramped from 85% mobile-phase A (80:20, by vol. methanol: aqueous solution of 0.001 M ion-

pairing and 0.001 M propionic acid) to 100% mobile-phase B (60:40, acetone: methanol) in 30 min with a hold for 20 min provided sufficient resolution of all pigments of interest. Flow rates from 1 ml min⁻¹ to 2 ml min⁻¹. The column was re-equilibrated between samples by linear ramping to 85% mobile-phase A for 5 min and maintenance for 10 min before sample injection. With this procedure, we are able to separate zeaxanthin from lutein and β -carotene from phaeophytin *a*. Analysis of replicates sediment samples yielded a C.V. of 4.5%- 11.5%, depending on pigments.

Method used by University of Barcelona.

Frozen sediment samples will be freeze-dried. Extraction will be carried out from 150 mg dry sediment samples sonicated in 2 ml 90% acetone for 40 seconds and centrifuged at 3000 rpm. Then the extract will be separated from the sediment, and we will repeat the same procedure without centrifugation. Finally, the 4 ml will be mixed again with the sediment, sonicated for 40 seconds and centrifuged. This final extract will be filtered with Whatman ANODISC 25 filter (0.1 μ m pore diameter) and analysed immediately. Before freeze drying, a sub-sample will be used to perform zooplankton analysis.

Pigments will be separated on a Waters 600E Multisolvant Delivery System with a Waters 717 Plus Autosampler with a refrigeration unit (4°C) and a C-18 column (Spherisorb ODS-1, 250 x 4.6 mm, 5 μ m particle size). Detection will be performed with a Waters 996 Photodiode Array Detector, set at 440 nm for peak integration, and carrying spectra in the range 300 to 800 nm. Analytical separation is achieved by linear gradient (1.2 ml/min, constant flow) using a modification of the system described by Wright *et al.* (1991). After sample injection (40 μ l of extract), pigments are eluted by linear gradient from 100% eluent **A** (MeOH / ACN / MilliQ (51:36:13) + 23.124 g AA); to 75% eluent **A** in 5 min followed by an isocratic hold for 5 min at 75% **A** and to 100% eluent **B** (EtilAcet / ACN (70:30)) in 20 min. The solvent composition is returned to initial conditions with a 5 min gradient, followed by 5 min of system equilibration before injecting the next sample.

For both methods, identification of all pigments was confirmed by comparison of spectral characteristics and chromatographic mobility of pigments isolated from sediments with those obtained from: TLC analysis (Züllig, 1982; Guilizzoni *et al.*, 1986), commercial standards (Sigma Chemical Co.), standards kindly donated by Hoffmann-La Roche of Basle, water samples of known phytoplankton composition, and published values (Foppen, 1971; Davies, 1976; Züllig, 1982; Mantoura & Llewellyn, 1983). Standard of okenone was obtained from culture of *Chromatium okenii* kindly provided by Dr. H. Züllig. Spectra will be obtained with a Perkin-Elmer Lambda 6 spectrophotometer.

Concentrations of pigments will be determined, following Mantoura & Llewellyn (1983), on the basis of molar extinction coefficients at the detection wavelengths. The molar extinction coefficient E_{1%460} and E_{1%656} is derived from the E_{1%} max reported in Davis (1976) and Mantoura & Llewellyn (1983).

Total carbon and nitrogen will be determined on dry sediment using a CNS analyser (Carlo Erba). The inorganic C will be measured with the CNS analyser on a sub-sample previously ignited at 550 °C. Total nitrogen is essentially organic N, since inorganic N is usually negligible (<2% dry weight).

References:

- Davies, B. H., 1976. Carotenoids. In: T.W. Goodwin (Ed.), Chemistry and Biochemistry of Plant Pigments, Academic Press London, New York. San Francisco, Vol. 2: 38-165.
- Guilizzoni, P., G. Bonomi, G. Galanti & D. Ruggiu, 1983. Relationship between sedimentary pigments and primary production: evidence from core analyses of twelve Italian lakes. *Hydrobiologia* 103: 103-106.
- Guilizzoni, P., A. Lami, D. Ruggiu & G. Bonomi, 1986. Stratigraphy of specific algal and bacterial carotenoids in the sediments of Lake Varese (N. Italy). *Hydrobiologia* 143: 321-325.
- Foppen, F.K., 1971. Tables for the identification of carotenoid pigments. *Chromatogr. Rev.* 14: 133-298.
- Hurley, J.P., 1988. Analysis of aquatic pigments by high performance liquid chromatography. *J. Anal. Purif.* 3: 12-16.
- Mantoura, R.F.C. & C.A. Llewellyn, 1983. The rapid determination of algal chlorophyll and carotenoid pigments and their breakdown products in natural waters by reverse-phase high-performance liquid chromatography. *Analytica Chimica Acta* 151: 297-314.
- Rowan K.S., 1989. *Photosynthetic Pigments of Algae*. Cambridge Univ. Press.
- Wright S.W., S.W. Jeffrey, R.F.C. Mantoura, C.A. Llewellyn, T. Bjørnland, D.Repeta, N.Welschmeyer, 1991. Improved HPLC method for the analysis of chlorophylls and carotenoids from marine phytoplankton.
- Züllig, H., 1982. Untersuchungen über die Stratigraphie von Carotinoiden im geschichteten Sediment von 10 Schweizer Seen zur Erkundung früherer Phytoplankton-Entfaltungen. *Schweiz. Z. Hydrol.* 44: 1-98.