Size fractionated CHLOROPHYLL <u>a</u> Serial filtration followed by fluorimetry after methanol extraction

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Chlorophyll <u>a</u> concentration was determined using the fluorimetric method proposed by Yenstch et Menzel (1963), adapted by Holm-Hansen et Rieman (1978) for pigments extraction by methanol, and described by Herbland *et al.*, 1985. Size fractionation was determined by filtration of 1.2-liter samples through polycarbonate filters (0.2, 0.6, and 2 μ m; 47 mm) using Sartorius systems (see photo below) and very low depression (drop by drop). The 0.2 and 0.6 μ m filters in the lower Sartorius system were separated by a nylon separator (NY8H04700, Millipore). Immediately after filtration, the filters (and the separator for the 0.2 μ m filter) were put on cryotubes with 5 mL of methanol for pigments extraction (30 mn at 4°C). Then, the fluorescence was measured with a Turner designs 10-AU-005-CE fluorimeter equipped with a chlorophyll <u>a</u> Kit (F4T45.B2 lamp) according to Welschmeyer (1994).

Fluorescence was converted in chlorophyll a using :

[*Chlorophylle a*] = Fo . Ko . v/V

 F_0 = fluorescence K_0 = calibration coefficient obtained with pure chlorophyll *a* (Sigma C5753) v = extraction volume (5 mL) V: filtrated volume (1200 mL)

References:

Herbland A., LeBouteiller A., Raimbault P., 1985. Size structure of phytoplankton in the Equatorial Atlantic Ocean. *Deep Sea Res.*, 32: 819-836.

Holm-Hansen O; et Rieman B., 1978. Chlorophyll a determination: improvements in methodology. *Oikos*, 30, 438-447.

Welschmeyer, N. A. 1994. Fluorimetric analysis of chlorophyll a in the presence of chlorophyll b and pheopigments. *Limnol. Oceanogr.*, 39 : 1985-1992.

Yentsch C.S. et Menzel D.W., 1963. A method for the determination of phytoplancton chlorophyll and phaeophytin by fluorescence. *Deep Sea Res.*, 10: 221-231.