

NOTE

Simultaneous determination of particulate organic carbon, nitrogen and phosphorus collected on filters, using a semi-automatic wet-oxidation method

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ABSTRACT: A wet-oxidation method is presented for determining simultaneously particulate organic carbon (POC), particulate organic nitrogen (PON) and particulate organic phosphorus (POP) collected on filters. Conversion of POC, PON and POP to inorganic products, carbon dioxide, nitrate+nitrite and soluble reactive phosphate respectively, is performed by a persulfate wet-oxidation in slight alkaline condition. After oxidation, inorganic products are dissolved in the digestion mixture and concentrations are measured automatically by colorimetry using a 3 channel Technicon AutoAnalyzer[®]. Concurrent comparisons were made with dry combustion at 950°C for POC and PON (CHN analysis) and with former wet-oxidation procedure for POP. Results indicated high efficiency to oxidize natural suspended particulate matter from sea- and freshwater as well as phytoplankton cultures and sinking particles collected by sediment traps. This method also exhibits good reliability and precision in comparison with former procedures and is highly suitable for routine analysis and especially appropriate for shipboard work.

KEY WORDS: Wet-oxidation · Particulate matter · Carbon Nitrogen · Phosphorus

Particulate organic carbon (POC) and particulate organic nitrogen (PON) are generally determined simultaneously by high temperature dry combustion using a CHN analyzer. Particulate organic phosphorus (POP) is often analyzed by chemical wet-oxidation (CWO) using potassium peroxydopersulfate (Menzel & Corwin 1967). Recent work indicated that CWO is comparable to CHN analysis in precision and accuracy for PON collected on Whatman GF/F filters (Raimbault & Slawyk 1991). Pujo-Pay & Raimbault (1994) have pointed out the efficiency of CWO for simultaneous analysis of PON and POP. But up until now, there has been no procedure allowing the simultaneous determi-

nation of POC, PON and POP collected on the same filter. Based on the previous works cited above, we propose (1) a slight modification of CWO offering complete oxidation and dissolution of particulate matter and (2) a subsequent automated analysis of the oxidized C, N and P species by colorimetry. This procedure is applied here to describe the vertical distribution of POC, PON and POP at 1 station in the Mediterranean Sea and to follow the accumulation of POC, PON and POP in culture during phytoplankton growth.

Materials and methods. The procedure involves 5 analytical steps. (1) Collection of a particulate matter (PM) sample by filtration, (2) placement of the filter in digestion flasks containing deionized water, (3) elimination of inorganic carbon by acidification and bubbling, (4) digestion in an autoclave and (5) automated analysis of inorganic products using a Technicon AutoAnalyzer[®]. A volume of 250 to 500 ml was used to collect particulate matter from oligotrophic waters, while smaller volumes (10 to 100 ml) were used for particle-rich waters and phytoplankton cultures. PM was always filtered under low vacuum (100 mm Hg). Pre-combusted (450°C for 24 h) Whatman GF/F filters (25 mm in diameter) were routinely used for the filtration. Other filters, such as Gelman Teflon membranes (porosity = 0.2 µm, 25 mm in diameter) or Nuclepore polycarbonate membranes (porosity = 3 µm, 47 mm in diameter), were tested for blank values. These Nuclepore polycarbonate membranes were just rinsed with deionized Milli-Q water (DIW), but the hydrophobic Teflon membranes had to be wetted with a drop of 95% ethanol before DIW, rinsing and filtration as suggested by Libby & Wheeler (1994).

Reagent: All chemicals were of reagent grade quality and aqueous solutions were prepared using fresh

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deionized DIW. The oxidizing reagent was prepared as follows: 30 g of disodium tetraborate (Merck 6308) was dissolved in 250 ml DIW heated at 40 to 50°C. Then 15 g of potassium peroxodisulfate (Merck 5092) was added and rapidly dissolved by stirring. The oxidizing reagent was stored in a glass bottle protected from direct light. At ambient temperature, disodium tetraborate crystallizes in a few hours. To avoid this problem and to be sure of maximum oxidative efficiency, just the quantity needed for a batch of samples was prepared daily. The purification step recommended by Nydhal (1978) to reduce blank values is not required with these Merck products.

Procedure: Digestion was carried out in 50 ml Pyrex bottles (Duran Schott) fitted with a screwcap equipped with a Teflon ring. New bottles were previously washed with 10% HCl and then submitted to a preliminary digestion to completely remove traces of organic matter. Forty milliliters of DIW was dispensed into cleaned digestion bottles, then filters retaining the particulate matter were placed in the bottles. Before oxidation the inorganic carbon was removed by adding 50 μ l of 5 N sulfuric acid, leading to pH around 2.5. After acidification, samples were bubbled for 5 min with a high purity oxygen/nitrogen gas stream passing through a charcoal/ascarite scrubber. Immediately after removal of inorganic carbon, 5 ml of oxidizing reagent was dispensed into each sample. After addition of the oxidizing reagent, bottles were tightly capped and autoclaved at 120°C (1 bar) for 30 min. Initial pH of the assay was higher than 9.0. After digestion the pH of the digestion mixture remained slightly alkaline (8.2 to 8.5) and trapped the CO₂ formed during carbon oxidation. After cooling at room temperature, the digestion mixture was analyzed for nitrate, phosphate and carbon dioxide. The digestion mixture was directly pumped from the digestion bottles for colori-

metric analysis of CO₂ using a specific manifold modified from Aminot & Kerouel (1990) where a buffered solution of phenolphthalein is discolored proportionate to the CO₂ concentration. The flow diagram is shown in Fig. 1. A 50 mm cell was used in the colorimeter and the wavelength of the filter was 520 nm. Water used for baseline was DIW acidified to pH 2.5 and sparged to remove CO₂. The sampling rate was 20 samples h⁻¹ (2 min sampling, 1 min washing). Nitrate and phosphate concentrations were measured using a conventional manifold (Tréguer & Le Corre 1975 in our case), but samples needed to be filtered when glass-fiber filters were used in order to eliminate filter fragments. A set of standards was prepared in the same digestion bottles as samples by dilution with acidified DIW of potassium biphthalate (200 mg l⁻¹), potassium nitrate and potassium phosphate. Filter blanks were made in replicate with each batch of samples. Filters used for blank determination were previously placed on the filtration system in order to reproduce the same experimental conditions as those for samples.

The procedure was applied to follow changes in POC, PON and POP phytoplankton culture during nutrient uptake. 5 μ mol l⁻¹ of nitrate and 1 μ mol l⁻¹ of phosphate were added to a nitrate-depleted culture of *Dunaliella tertiolecta*. Nutrient concentration was followed with time by chemical analysis on a Technicon AutoAnalyzer[®] according to Tréguer & Le Corre (1975) until nitrate was almost exhausted from the medium. A sub-sample of the culture was inoculated with ¹⁴C (20 μ Ci of NaH¹⁴CO₃) to measure carbon fixation (Steeman-Nielsen 1951). At each sampling time, parallel to nutrient and particulate matter analysis, a 50 ml sample was filtered onto Whatman GF/F filter at <100 mm Hg, and the filter was placed in a scintillation vial. To chase residual inorganic ¹⁴C, 250 μ l of 0.5 N HCl were added, and after 6 to 12 h the filters were

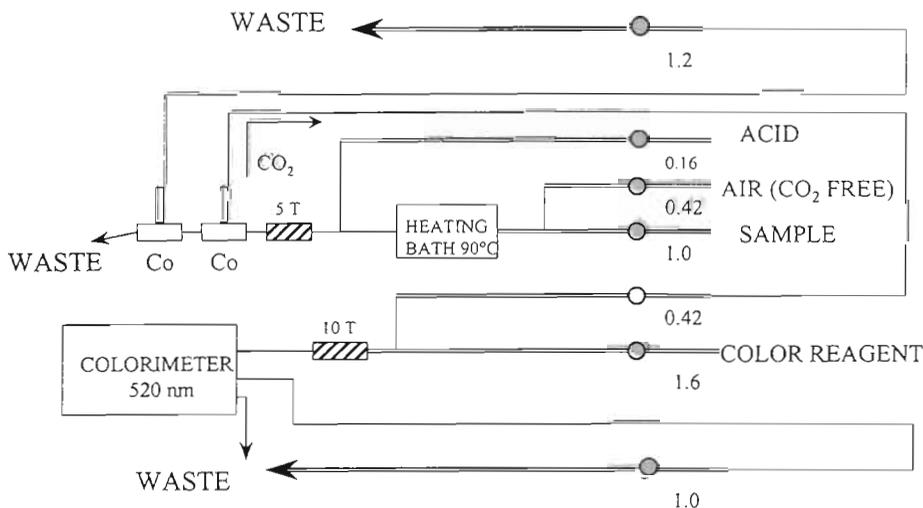


Fig. 1. Flow diagram of the automated system for the carbon dioxide analysis liberated after oxidation of particulate organic carbon. Flow rates of pumping tubes are given in ml min⁻¹. Color reagent is prepared as described in Aminot & Kerouel (1990). 0.4 ml of 1% phenolphthalein is diluted in 400 ml DIW; 5 ml of mixture (1/4 V/V) of 0.1 M Na₂CO₃ and 0.1 M NaHCO₃ were added. Acid was prepared by diluting 2.8 ml of 36 N sulfuric acid in 100 ml DIW; 7 g of hydroxylammonium chloride was added to prevent chloride formation

counted in 10 ml Aquasol on a Packard Tri-carb 2100TR scintillation counter. Carbon assimilation rates were calculated according to Platt & Sathyendranath (1993).

At one site of the Gulf of Lions (northwestern Mediterranean Sea; 42° 25' N, 4° 04' E), 500 ml of sea-water were filtered on GF/F filters and on 0.2 µm Nuclepore membranes to determine vertical profiles of POC, PON and POP. Nitrate and phosphate were measured according to Tréguer & Le Corre (1975) and chlorophyll *a* concentrations were determined by fluorimetry using the methanol extraction procedure as described in Raimbault et al. (1988).

Results and discussion. DIW + reagent blank, total GF/F blank values (digestion reagent + DIW + filter) obtained in the 45 ml digestion mixture, and C, N, P contents by filter are given in Table 1. Whatman GF/F filters showed very low and very homogeneous C, N and P levels ranging from 0.53 to 0.76, 0.015 to 0.018 and 0.002 to 0.003 µmol filter⁻¹, respectively. These C and N values are equivalent to those of Hager & Harmon (1984) with a CHN analyzer. Nitrogen and phosphorus blanks are fairly similar to those obtained by Raimbault & Slawyk (1991), Libby & Wheeler (1994) and Pujo-Pay & Raimbault (1994) using similar CWO procedures. As shown in Table 1, variation in carbon blank measured in the 45 ml extract was mainly due to the variability of the DIW + reagent blank which ranged from 10 to 35 µmol l⁻¹ for the 3 sets of analyses made successively. Blank values were also determined for Teflon and polycarbonate membranes. Carbon blanks were significantly higher than for GF/F but were quite homogeneous (low standard deviation); nitrogen and phosphorus blanks were very low and often close to zero. Thus, in spite of their high C con-

tent these carbon-rich membranes were not significantly altered by the reagent and can be used with this method. POC and PON concentrations of particulate matter retained on Whatman GF/F filters were compared with those obtained with high temperature (950°C), performed with a CHN Leco 800 analyzer using glycine as standard. Comparison showed excellent agreement from suspended particulate matter (Fig. 2a,c). The recovery efficiency was close to 100% (at the 95% confidence level) with a very low standard deviation over a wide range of concentrations. POP values were compared with those obtained by the wet-oxidation procedure of Menzel & Corwin (1967) and comparison also showed a good agreement between the 2 methods (Fig. 2e). For sinking materials collected by sediment traps (Fig. 2b,d) the comparison also showed good efficiency of CWO for C and N, but with a greater scatter ($r^2 = 0.6$ to 0.8) than for suspended materials. A part of this latter heterogeneity could be due to the fractionation step required to analyze sediment trap samples. The thoroughness and precision of the method were checked by analyzing replicates of natural samples (Table 2). For C and N the relative standard deviation was generally low (<10%) in sea- and freshwater samples, except when particulate content was low (sample from below the euphotic zone for example) and for 1 sample of trap materials. The relative standard deviation was sometimes higher than 10% (up to 30%) for P analysis, but it should be noted that standard deviation was always very low (0.02 to 0.04 µmol l⁻¹); in this case the poor precision was mainly a consequence of the very low phosphorus content of the samples. These results indicate that the C/N/P ratio was close to the Redfield ratio (106/16/1) in

Table 1. Test of reproducibility for deionized Milli-Q water (DIW) + reagent blank and for filter blanks. GF/F = Whatman glass-fiber GF/F filter (25 mm diameter); Teflon = 0.2 µm Gelman Teflon membrane (25 mm diameter); PC = 3 µm Nuclepore polycarbonate membrane (47 mm diameter). Concentrations in the 45 ml extract are given in µmol l⁻¹ for DIW blank and for DIW + filter blank (filter blank). Quantities of C, N and P by filter are given in µmol. These latter values are derived from Filter blank × 45/1000

Sample	No. of samples	Carbon		Nitrogen		Phosphorus	
		in 45 ml (µmol l ⁻¹)	per filter (µmol)	in 45 ml (µmol l ⁻¹)	per filter (µmol)	in 45 ml (µmol l ⁻¹)	per filter (µmol)
1st set							
DIW blank	3	35.0±5.0		0.49±0.09		0.022±0.002	
GF/F blank	6	48.33±5.19	0.60±0.23	0.89±0.12	0.018±0.005	0.080±0.019	0.003±0.001
2nd set							
DIW blank	3	10.33±1.08		0.34±0.10		0.022±0.002	
GF/F blank	3	26.92±1.06	0.76±0.05	0.70±0.10	0.016±0.004	0.084±0.019	0.003±0.001
3rd set							
DIW blank	3	25±2.6		0.37±0.12		0.03±0.002	
GF/F blank	6	36.81±3.11	0.53±0.14	0.69±0.102	0.015±0.005	0.075±0.037	0.002±0.002
Teflon 0.2 µm	6	97.08±4.88	3.24±0.22	0.36±0.063	0.000±0.003	0.067±0.031	0.002±0.001
3 µm PC filter	5	130.5±4.27	4.75±0.19	0.55±0.021	0.008±0.001	0.006±0.002	0.0001±0.0001

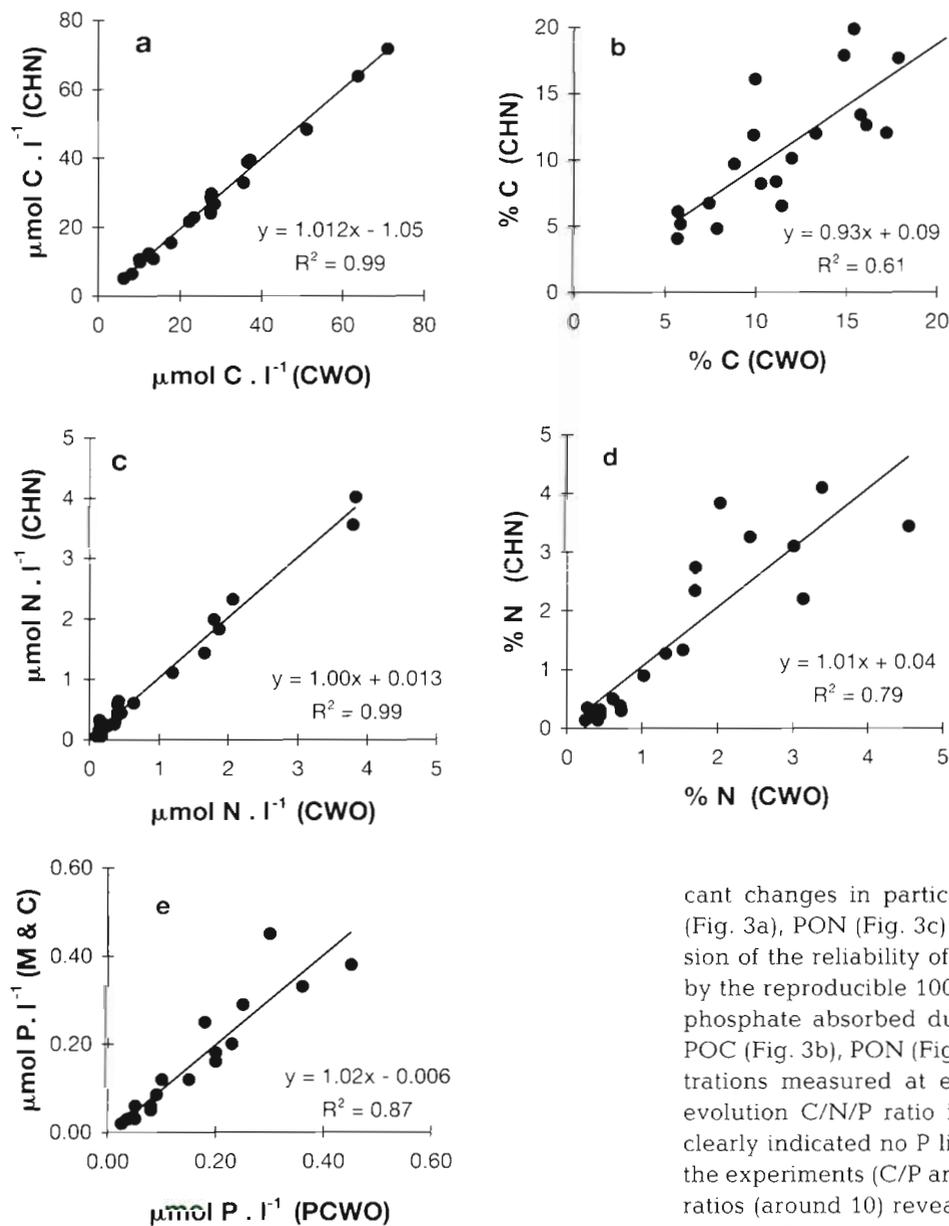


Fig. 2. Comparison between the chemical wet-oxidation (CWO) and the high temperature combustion (CHN) for (a) POC and (c) PON determination in suspended matter and for (b) POC and (d) PON in sinking materials collected by sediment traps. All particulate matter was retained on GF/F filters for analysis. For phosphorus determination (e) in suspended particulate materials collected on GF/F filter comparison is made between the present procedure (PCWO) and the Menzel & Corwin (1967) oxidation (M & C)

offshore samples, while a significant deviation occurred in the other samples. Samples from estuarine and lagoon waters as well as from coastal waters (influenced by the Rhône river) were relatively richer in carbon certainly because of the high content of detrital materials. One sample of sinking materials had a C/N/P ratio close to the Redfield value, indicating this trap collected fresh particles. The second sample corresponded to more degraded matter characterized by a significant P-deficiency relative to the Redfield ratio, since phosphorus is well known to be very labile and more rapidly recycled than nitrogen and carbon (Ammerman & Azam 1985).

Fig. 3 clearly shows that phytoplankton growth (biomass increasing) can be easily followed by the signifi-

cant changes in particulate matter in terms of POC (Fig. 3a), PON (Fig. 3c) and POP (Fig. 3e). An impression of the reliability of the proposed method is given by the reproducible 100% recovery of ^{14}C , nitrate and phosphate absorbed during growth compared to the POC (Fig. 3b), PON (Fig. 3d) and POP (Fig. 3f) concentrations measured at each sampling time. The time evolution C/N/P ratio in particulate matter (Fig. 3g) clearly indicated no P limitation during the first 2 d of the experiments (C/P around 80). But the N/P and C/N ratios (around 10) revealed a slight N limitation (Fuhs et al. 1972) due to the initial nitrate/phosphate ratio (5/1). At the end of the experiment, when nitrate was depleted and phosphorus concentration was below $0.3 \mu\text{mol l}^{-1}$, N deficiency significantly increased (C/N = 20), while C/P ratio reached 110, a value close to the Redfield ratio.

In the field, profiles of POC, PON and POP collected on GF/F filters and $0.2 \mu\text{m}$ membranes were compared with the vertical distribution of chlorophyll and nutrients. The distribution of POC, PON and POP was well related to the chlorophyll biomass (Fig. 4). A maximum concentration of particulate matter in terms of C, N and P was observed in surfaces associated with a chlorophyll maximum (1 to $1.5 \mu\text{g l}^{-1}$). At depth, particulate matter fell to values ranging from 20 to 50% of the surface concentrations while chlorophyll levels

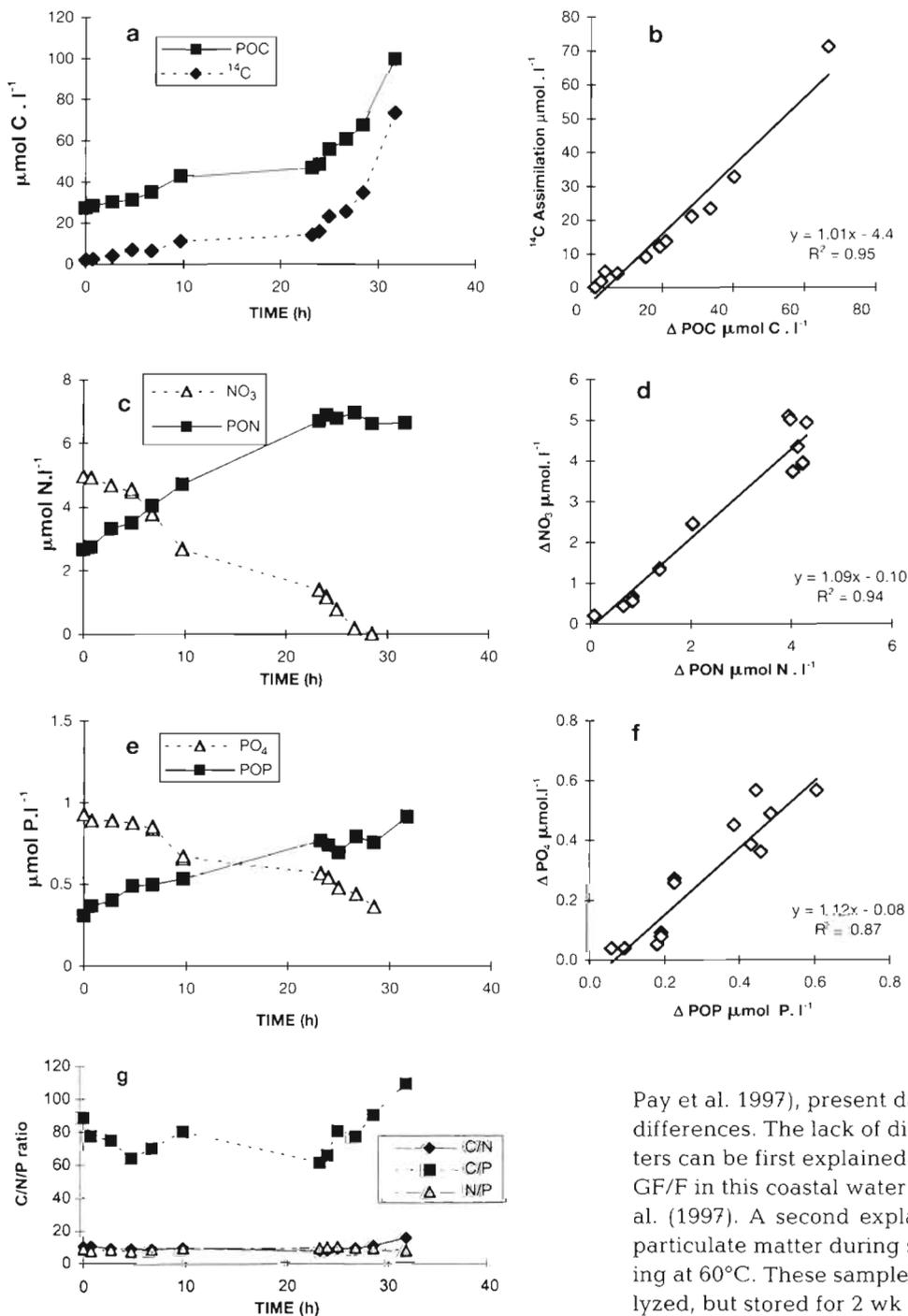


Fig. 3. Time course of the parameters studied during the algal growth of *Dunaliella tertiolecta*: (a) carbon assimilation (PO^{14}C) and POC; (c) concentration of nitrate (NO_3) and PON, (e) concentration of phosphate (PO_4) and POP. (b, d, f) Linear relationship obtained between inorganic nutrient assimilation and biomass increasing in terms of POC (ΔPOC), PON (ΔPON) and POP (ΔPOP), respectively. (g) Time-course of C/N/P ratio

became negligible ($<0.05 \mu\text{g l}^{-1}$). Vertical distributions of the $>\text{GF/F}$ and $>0.2 \mu\text{m}$ POC, PON and POP were similar, with maximum concentrations from 0 to 40 m and characteristically decreasing between 50 and 100 m. Although recent works have pointed out the better retention efficiency of the $0.2 \mu\text{m}$ membrane compared to the GF/F filter (Altabet 1990, Libby & Wheeler 1994, 1997, Slawyk & Raimbault 1994, Puj-

Pay et al. 1997), present data do not exhibit significant differences. The lack of difference between these 2 filters can be first explained by a low underestimation of GF/F in this coastal water as suggested by Pujoy-Pay et al. (1997). A second explanation could be the loss of particulate matter during storage of samples after drying at 60°C . These samples were not immediately analyzed, but stored for 2 wk at 60°C .

Libby & Wheeler (1994) have suggested that heat can cause low and variable PON determination with Teflon membranes. Then, immediate analysis following filtration is recommended or, if impossible, storage by freezing over a short period has to be preferred to drying, especially for Teflon filters. The C/N/P ratio noted in the 0 to 100 m water column (160/13/1) revealed a clear deviation from the Redfield ratio which could be due to: (1) phosphate deficiency (nitrate/phosphate ratio ranging from 40 to 120) as it is

Table 2. Test of reproducibility for POC, PON and POP analyses. Samples were collected in the northwestern Mediterranean Sea at 2 offshore sites (5 m depth) and at 1 coastal site (5 and 140 m depth), from lagoon (Berre lagoon) and estuarine waters (Rhône river) and from sinking materials (Sinking POM) collected during 4 d in a PPS5 sediment trap placed near the surface (140 m depth, collecting surface = 1 m²). All particulate matter were filtered on GF/F filters before oxidation. Values of blank filter have been subtracted. RSD%: relative standard deviation

Sample	No. of samples	Carbon		Nitrogen		Phosphorus		C/N/P
		$\mu\text{mol l}^{-1}$	RSD %	$\mu\text{mol l}^{-1}$	RSD %	$\mu\text{mol l}^{-1}$	RSD %	
Surface seawater (offshore)	5	5.74±0.31	5.4	0.73±0.052	7.1	0.050±0.002	4.0	114/15/1
Surface seawater (offshore)	5	5.30±0.17	3.2	0.66±0.23	3.8	0.043±0.008	18.6	123/15/1
Surface seawater (coastal)	4	5.58±0.39	6.9	0.48±0.041	8.5	0.026±0.004	15.4	215/18/1
Deep seawater (140 m)	4	2.28±0.17	7.5	0.19±0.061	32	0.011±0.002	18.2	223/18/1
Berre Lagoon	3	222.2±19.9	8.9	8.27±0.50	6.0	0.625±0.035	5.6	337/13/1
Rhône River	3	336.6±6.8	2.0	9.96±0.32	3.2	0.807±0.040	5.0	417/12/1
		$\mu\text{mol C}$		$\mu\text{mol N}$		$\mu\text{mol P}$		
Sinking POM	4	1723.3±28.6	1.7	180.0±8.6	4.8	12.9±0.77	6.0	134/14/1
Sinking POM	4	1167.8±328	28	105.7±20.0	18.9	4.00±1.33	33.3	311/29/1

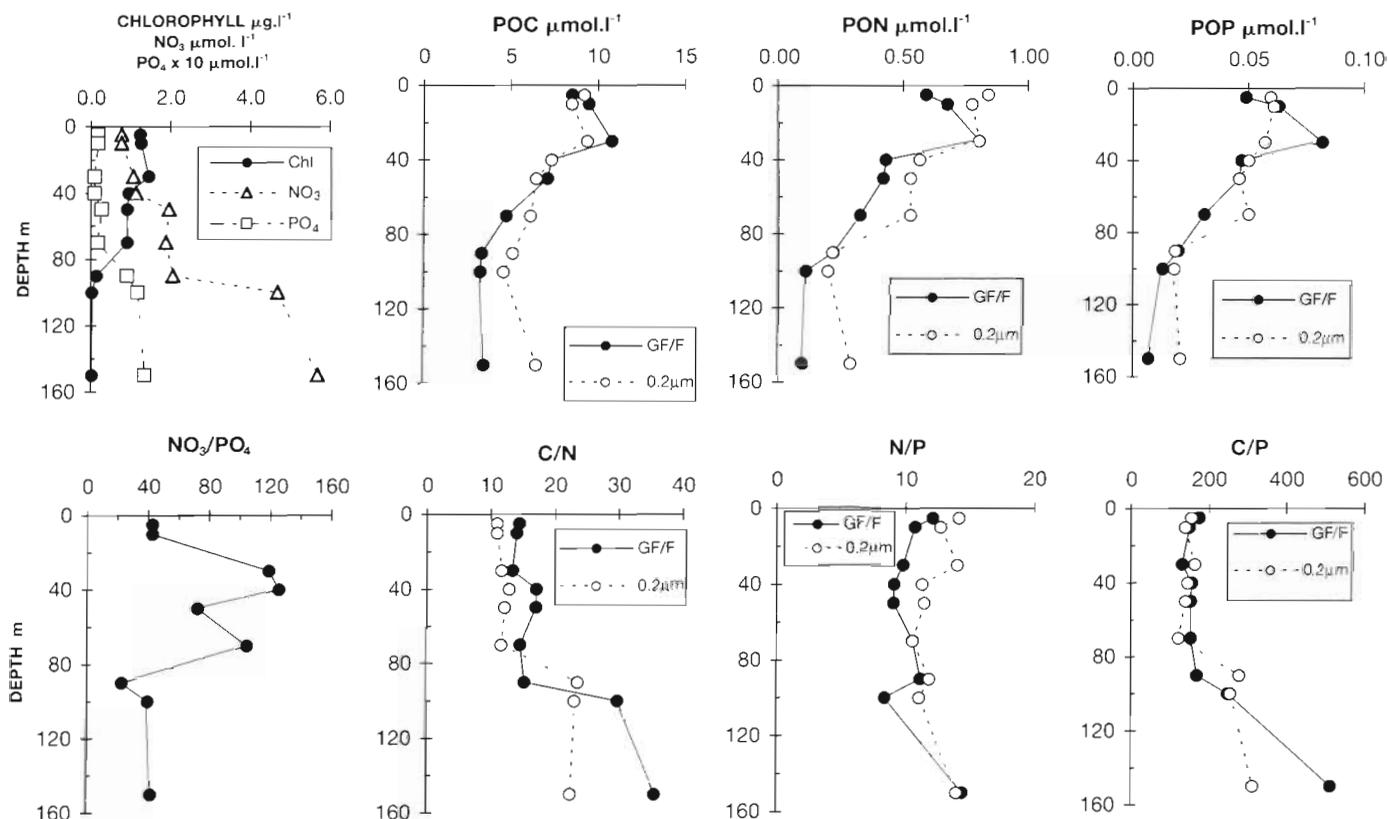


Fig. 4. Vertical distribution of POC, PON and POP fractions collected on GF/F filters and 0.2 μm Teflon membranes as well as vertical pattern of C/N/P ratio. Parallel profiles of nitrate (NO_3), phosphate (PO_4), nitrate/phosphate ratio (NO_3/PO_4), and chlorophyll retained on GF/F filters are given for comparison. The values were derived from a coastal station in the Gulf of Lions, northwestern Mediterranean Sea ($42^\circ 25' \text{N}$, $4^\circ 04' \text{E}$; 20 March 1998)

well known in the Mediterranean Sea (Berland et al. 1980), and (2) the influence of the Rhône river from which flowed carbon-rich materials (see Table 1).

A slight modification of the conventional chemical wet-oxidation method with potassium persulfate has produced a reliable method with sufficient sensitivity and precision to simultaneously analyze POC, PON and POP in seawater. The automated colorimetry analysis of oxidized products offers rapid treatment of a large number of samples (20 h^{-1}). The present procedure shows consistent agreement with the combustion method, exhibits good reliability and precision (less than 10%) in comparison with former procedures but presents 3 distinct advantages. First, its ease of sample handling and analytical procedure is based on a simple apparatus, making it very suitable for shipboard determination. Second, it offers C, N and P determination on the same filter. Third, polycarbonate and Teflon membranes can be used in contrast to high combustion technique offering the possibility to study C, N, P content in different particulate size fractions. This procedure is very sensitive, simple and cheap and very convenient for shipboard analysis. It would be a valuable tool in future aquatic nutrient studies to evaluate the C/N/P relationship in different oceanic and freshwater ecosystems, as well as in phytoplankton cultures.

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LITERATURE CITED

- Altabet MA (1990) Organic C, N, and stable isotopic composition of particulate matter collected on glass-fiber and aluminum oxide filters. *Limnol Oceanogr* 35:902–909
- Aminot A, Kerouel R (1990) Improvement of a commercial continuous flow method for the determination of dissolved organic carbon in fresh water and seawater. *Analysis* 18: 289–295
- Ammerman JW, Azam F (1985) Bacterial 5'-nucleotidase in aquatic ecosystems: a novel mechanism of phosphorus regeneration. *Science* 227:1338–1340
- Berland B, Bonin DJ, Maestrini SY (1980) Azote ou phosphore? Considérations sur le paradoxe nutritionnel de la mer Méditerranée. *Oceanol Acta* 3:135–142
- Fuhs GW, Demmerle DL, Canelli E, Chen M (1972) Characterization of phosphorus-limited algae (with reflections on the limiting nutrient concept). In: Likens GE (ed) *Nutrients and eutrophication*. Am Soc Limnol Oceanogr, Spec Symp 1, p 113–133
- Hager SW, Harmon DD (1984) Chemical determination of particulate nitrogen in San Francisco Bay. A comparison of two estimates. *Estuar Coast Shelf Sci* 19:181–191
- Libby S, Wheeler PA (1994) A wet-oxidation method for determination of particulate organic nitrogen on glass fiber and $0.2 \mu\text{m}$ membrane filters. *Mar Chem* 48:31–41
- Libby S, Wheeler PA (1997) Particulate and dissolved organic nitrogen in the central and eastern equatorial Pacific. *Deep-Sea Res* 44:345–361
- Menzel DW, Corwin N (1967) The measurement of total phosphorus in sea water based on the liberation of organically bound fractions by persulfate oxidation. *Limnol Oceanogr* 10:280–282
- Nydhal F (1978) On the peroxodisulfate oxidation of the total nitrogen in water to nitrate. *Water Res* 12:1123–1130
- Platt T, Sathyendranath S (1993) Fundamental issues in measurement of primary production. *ICES Mar Sci Symp* 197: 3–8
- Pujo-Pay M, Raimbault P (1994) Improvement of the wet-oxidation procedure for simultaneous determination of particulate organic nitrogen and phosphorus collected on filters. *Mar Ecol Prog Ser* 105:203–207
- Pujo-Pay M, Conan P, Raimbault P (1997) Underestimation of particulate nitrogen concentrations in open ocean by the use of GF/F filters. *CR Acad Sci* 324:401–407
- Raimbault P, Slawyk G (1991) A semiautomatic, wet-oxidation method for the determination of particulate organic nitrogen collected on filters. *Limnol Oceanogr* 36:405–408
- Raimbault P, Rodier M, Taupier-Letage I (1988) Size fraction of phytoplankton in the Ligurian Sea and the Algerian Basin (Mediterranean Sea): size distribution versus total concentration. *Mar Microb Food Webs* 3:1–7
- Slawyk G, Raimbault P (1994) Simple procedure for simultaneous recovery of dissolved inorganic and organic nitrogen in ^{15}N -tracer experiments and improving the isotopic mass balance. *Mar Ecol Prog Ser* 124:289–299
- Steeman-Nielsen E (1951) Measurement of the production of the organic matter in the sea by mean of carbon 14. *Nature* 167:684–685
- Tréguer P, Le Corre P (1975) Manuel d'analyse des sels nutritifs dans l'eau de mer. Utilisation de l'AutoAnalyzer 2 Technicon, 2nd edn. Univ Bretagne occidentale, Brest

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