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A decarbonation and preservation method for the analysis of organic C and N contents and stable isotope ratios of low-carbonated suspended particulate material

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Abstract

The aim of this study was to determine a simple routine procedure to preserve, decarbonate and analyse low-carbonated filters of suspended particulate organic matter (POM) for particulate organic carbon and nitrogen content, $\delta^{13}C$ and $\delta^{15}N$. Our goal was to analyse these four parameters from a single and entire filter of POM without altering the organic material.

First, freezing (-20°C) versus oven-drying (60°C) were compared as the initial preservation step. Afterwards, non-acidified samples were compared to acid-treated samples using 0.12N HCl (diluted HCl rinsing at the end of the filtration) or 12N HCl (filters exposed to HCl fumes for four hours in a dessicator). Regarding the preservation methods, our results indicate that freezing increases the uncertainty of $\delta^{15}N$ measurements and, in combination with concentrated HCl treatment, leads to a loss of particulate nitrogen and an alteration of the $\delta^{15}N$ signature. Consequently, we recommend drying to preserve filter samples. Regarding acid treatments, we found that (i) diluted HCl would not be sufficient to fully remove the carbonate from our samples, (ii) in contrast, a four-hour exposure of the filters to the HCl fumes was enough to remove all the inorganic carbon, and (iii) the concentrated HCl treatment did not alter the nitrogen measurements (only when drying without freezing is used to preserve the filters). Consequently, we propose that low-carbonated POM filters are preserved by drying and carbonates are removed by exposing the filters to HCl fumes (four hours) for the analysis of particulate organic C and N content and isotope ratios.

1. Introduction

Over the last decades the techniques employed to determine organic carbon content, nitrogen content and stable isotope ratios ($\delta^{13}C$, $\delta^{15}N$) have been improved and are now widely used to determine the origin (marine vs. terrestrial) and fate of organic matter in the water column and modern sediments [1-3] as well as in ancient sediments for paleo-reconstruction [4-6]. In addition, carbon and nitrogen stable isotope values of organisms are used to determine food webs structure in numerous environments [7-9]. Most of these studies require seasonal sampling but, as the field-collected samples are not analysed immediately, storage under controlled conditions is necessary to prevent organic matter alteration. In addition, to avoid bias within the isotopic signal of particulate organic carbon (POC), carbonates must be removed. Indeed, particulate inorganic carbon (carbonate minerals, mainly calcite, aragonite and dolomite) and particulate organic carbon exhibit distinct isotopic signatures [10]. It is important that the procedure used to remove carbonate from total particulate material should not alter the organic matter.

Various storage and decarbonation treatments have been reported in the literature. The storage of suspended particulate matter (SPM) generally involves either drying [11] or freezing [12] the samples or applying both of these methods [13]. In the seventies and early eighties, the separation of inorganic and organic carbon, as well as the removal of carbonates from SPM or sediment involved either acidification with diluted H_3PO_4 [14-15] or HCl [16] or a loss on ignition [17-19]. Recently, acidification with diluted or

concentrated HCl has been the standard method for elemental and isotopic analysis [20-22]; however, in some cases, authors do not remove carbonates before analysis [23-24]. Storage and acidification may alter the organic matter and, consequently, the values of elemental and isotopic data. Some reports on elemental carbon measurements highlighted that acidification with diluted HCl was unsatisfactory as it could result in the loss of acid-soluble organic carbon during carbonate dissolution (between 5 and 45% of the organic carbon can be lost) [15,25]. In contrast, Hedges and Stern claimed that the vapour acidification method with concentrated HCl avoids loss of acid-soluble organic matter [26]. It is questionable how aggressive methods such as these affect the results when stable isotopes are not analysed. Similar inconsistencies also exist in the preparation and storage of marine organism samples for C and N analyses [27]. In particular, Bunn et al. have shown that weak-acid attack (HCl 10%) in soft tissues could lead to a 3‰ decrease in the mean $\delta^{15}N$ ratios, suggesting that those isotopic changes are due to loss of molecules during acid washing [28]. A common solution to avoid any deterioration of N results by acid exposure is to cut the filter into two portions: one is used for C analyses after decarbonation, and the other one for N analyses without decarbonation. This method is based on the assumption that the SPM is homogenously distributed on the filter, which for coastal water samples is unlikely. Cutting the filter also lengthens the protocol, and could introduce contamination.

For the C and N elemental and stable isotope analyses, a few recent studies have dealt with the consequences of storage and decarbonation treatments on marine sediments [29], particulate material [30] and organisms [28,31]. However, none of these studies have focused on suspended particulate organic matter (POM). At present, the method for

decarbonation and preservation of particulate organic matter (POM) filters is still under debate and there is no definitive publication available for this procedure on coastal water samples.

This study compares the most common preservation and decarbonation treatments and investigates how they affect the C and N elemental and isotopic composition of suspended particulate matter (SPM) samples. For preservation, freezing (-20°C) and oven-drying (60°C) were compared, while for decarbonation, "no acid treatment" was compared with exposure to diluted liquid HCl and concentrated HCl vapour. Our aim is to determine a simple routine method that (1) removes carbonates, (2) preserves organic material and (3) allows the analysis of the four parameters (*i.e.* C and N organic content, $\delta^{13}C$ and $\delta^{15}N$) from one entire filter with minimal time and cost consumption.

2. Experimental

2.1 Material

All laboratory equipment was pre-washed with HCl 10%, rinsed with de-ionised water (DIW) and dried. In addition, the glassware (filter holders, filtration funnels, vials and filters) was pre-combusted (450°C, 4 h). Both treatments eliminated any trace of inorganic and organic carbon.

2.2 Sample origin

Seawater was collected in the Bay of Brest (France), a coastal macrotidal ecosystem. In spite of the narrow strait that connects the Bay with the Atlantic Ocean, it represents a real marine ecosystem [32-33] with strong tidal exchanges with the ocean

and little influence from the two rivers (the Aulne and the Elorn). The waters from the watershed are poorly carbonated, the surrounding rocks being mainly composed of illite, chlorite, kaolinite and micas [34], *i.e.* silicated minerals. Therefore, the influence of carbonates on particulate material, via river input, is minor.

2.3 Sampling protocol

On the 5th of June 2001, 30 L of seawater were collected for C and N analysis using a 5L-Niskin bottle. The seawater was pre-filtered (200 µm) to avoid heterogeneity in particle size, poured into two 15-L black carboys, and then placed in a cold dark room (6°C) before use. Before sampling these carboys were gently shaken in order to homogenize the seawater. Each 500-mL sample was filtered through a pre-combusted glass fibre filter (Whatman GF/F, 25 mm diameter). Prior to filtration, filters were rinsed with 10% HCl followed by DIW to eliminate any trace of inorganic carbon. The SPM filters were again rinsed with DIW immediately after filtering the samples, to eliminate Cl^- ions harmful to the performance of the elemental analyser (Schubert and Nielsen, 2000), and placed in glass vials.

After filtration, different treatments were tested on a total of sixty SPM filters. Thirty SPM filters were stored dried (D) and another thirty stored frozen (F). In each set (D and F), 10 samples were treated with concentrated HCl vapours (12 N) after storage (D-12 and F-12), 10 with diluted HCl (0.12 N) before storage (D-0.12 and F-0.12) and 10 were not treated with acid (D-0 and F-0). Three blanks were exposed to each procedure. The treatments and their identification code are summarised in Table 1. To avoid

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potential effects of sampling time on the data, the filters used for each treatment were chosen at random from the sampling series.

2.4 Preservation treatments

For the freezing treatment, the vials containing SPM filters were placed in a freezer (-20°C) immediately after filtration, and stored for one week. Before analysis, the filters were oven-dried for 12 hours at 60°C.

For the drying treatment, after filtration the filters were dried (12 hours at 60°C), enclosed within clean glass vials and stored in the dark for one week.

2.5 Acid treatments

Concentrated HCl treatment: after storage and just before analysis, the vials containing the dry SPM filters were exposed to concentrated HCl vapour in a glass dessicator four hours at room temperature. To drive off residual HCl and water, the filters were then placed inside a fume hood (3 hours) and then in an oven (overnight, 60°C).

In a preliminary study, three sets of three filters (replicates sampled in the Bay of Brest in February 1999) were exposed to HCl vapour for 4, 8 and 12 hours. C contents were 92.3 ± 5.7, 89.3 ± 4.0 and 95.0 ± 3.5 µg and $\delta^{13}C$ values -24.0 ± 0.1, -23.9 ± 0.1 and -24.1 ± 0.1‰ for an exposure of 4, 8 and 12 hours, respectively. Regardless of the decarbonation time, no significant differences were found between the three sets for both C content and $\delta^{13}C$ values. We therefore considered that a four-hour exposure was sufficient to eliminate traces of carbonate from this type of material containing relatively small quantities of carbonates.

Diluted HCl treatment: immediately after seawater filtration (before storage), 20 mL HCl 0.12 N were poured onto the SPM filter and left to stand for one minute without vacuum. The vacuum was started again to discard the HCl solution and the filters immediately rinsed with DIW before being transferred to vials for storage.

2.6 Elemental and isotopic analysis

After storage and acid treatment (no HCl, HCl 0.12 N or HCl 12 N) the filters were folded, placed into tin cups (9 mm height, 5 mm diameter) and kept in vials until analysis. SPM filters were analysed for C and N contents and isotope ratios using a Carlo Erba NA 2100 elemental analyser configured for C and N analysis and coupled to a Finnigan Delta S isotope ratio mass spectrometer.

Isotope ratios are reported in the classic δ notation:

$\delta^{13}C_{sample}$ or $\delta^{15}N_{sample} = (R_{sample} / R_{standard} - 1) * 1000$ where $R = {}^{13}C/{}^{12}C, {}^{15}N/{}^{14}N$. Pee Dee Belemnite and atmospheric nitrogen were used as standards for $\delta^{13}C$ and $\delta^{15}N$ respectively. Values are reported in parts per thousands (‰). EA-IRMS precision (standard deviation from ten replicate measurements, using glutamic acid as the working standard) is of $\pm 2\%$ ($\pm 1\sigma$) for C and N elemental analysis and of $\pm 0.1‰$ ($\pm 1\sigma$) for $\delta^{13}C$ and $\delta^{15}N$ analysis.

2.7 Data analysis

The effects of the different treatments on the four parameters (*i.e.* particulate carbon and nitrogen content (PC and PN), $\delta^{13}C$ and $\delta^{15}N$) were investigated by performing a one-way analysis of variance on all the data (ANOVA, Statgraphics Plus, 99%

confidence level). The equality of variances (sd) and normality had been previously tested with Bartlett's test and Kolmogorov's test, respectively, as prerequisites for any analysis of variance.

3. Results

Table 2 lists the measured values, means and standard deviations for particulate carbon and nitrogen content, $\delta^{13}C$ and $\delta^{15}N$. The data followed a normal distribution with 90% or higher confidence level (Kolmogorov's test). The equality of variances (s.d.) was also respected (Bartlett's test, $p > 0.05$) allowing the analysis of variance (ANOVA). Blanks were measurable for only particulate carbon (PC) analyses; for all other measurements, they were below the detection limit.

3.1 Particulate Carbon (PC)

The mean blank value for PC was 12.6 ± 5.0 µg for all of the treatments, corresponding to less than 7% of the mean gross PC value (184 µg). Net PC values were obtained by subtracting the blank mean value from the related gross PC for each treatment. The mean net PC value for all the filters was 172 (± 10.6) µg.

With the exception of F-0 samples, which differed from all the others ($p < 0.01$), no significant statistical difference was found between all the net PC mean values (Fig.1A and Table 2). So, for the same decarbonation treatment, there was no difference between freezing and drying (D-0.12 and F-0.12; D-12 and F-12); except for the non-acidified filters where frozen samples had significantly higher values (D-O differed significantly from F-0, $p<0.01$). When all the D values are pooled and compared to

pooled F values, there were no statistical differences between F and D data, indicating that the preservation method had no effect on particulate carbon values. No significant difference was observed between concentrated and diluted HCl treatments, whatever the preservation method used.

The comparison of non-acidified and acidified filters led to different results depending on the preservation technique. Decarbonation resulted in a significantly reduced carbon content when the samples had been acidified and frozen ($p < 0.01$). On the other hand, no significant difference was noticed for drying preservation.

3.2 Particulate Nitrogen (PN)

The mean PN value for all filters was 33.1 (± 1.9) µg. The nitrogen content appeared homogeneous among the experiments except when freezing and concentrated acid treatment were combined (Fig.1B). Indeed, the F-12 mean value (30.5 ± 1.0 µg) significantly decreased compared to the other treatments ($p < 0.01$; Table 2 and Fig. 1B).

3.3 $\delta^{13}C$

Significant differences appeared among all the acid treatments (Table 2 and Fig.1C; $p < 0.01$). Acidified filters (D-0.12, D-12, F-0.12, F-12) were, indeed, significantly depleted in ^{13}C (- 21.3 vs. - 20.4‰, $p < 0.01$) when compared to the non-acidified ones (D-0, F-0). Concentrated HCl vapour treatments led to stable isotope values significantly lower than those with diluted HCl treatments ($p < 0.01$).

On the other hand, there was no significant difference between the preservation treatment regardless of the decarbonation method: D-0 did not differ from F-0, nor D-0.12 from F-0.12, nor D-12 from F-12.

3.4 $\delta^{15}N$

The overall mean $\delta^{15}N$ value was + 6.6 (± 0.4) ‰. F-0.12 differed significantly from the other treatments with a $\delta^{15}N$ value of 7.1‰ (p < 0.01, Fig.1E). When freezing preservation was used, there was an increase in the variability among mean $\delta^{15}N$ values (inter-value variability) together with an increase in standard deviation (intra-value variability, 0.38‰ vs. 0.26‰). When samples were dried, $\delta^{15}N$ mean values were more homogeneous, with only a slightly significant difference among D-12 and D-0.12 values (p < 0.01), but neither were significantly different from D-0 values.

4. Discussion

4.1 Characterization of the particulate organic matter

The stable isotope ratios reported here (Table 2) are within the range of those encountered in the literature for coastal suspended particulate organic matter in temperate ecosystems [2,35]. Furthermore, our data fall within the classical POM values measured in the Bay of Brest [36]. The POM was sampled the 5th of June 2001 at the SOMLIT station (Bay of Brest, France) during a phytoplankton bloom (see a time series at the address http://www.univ-brest.fr/IUEM/BIOFLUX/chloro.htm). During this period, the dominant species were diatoms (*Rhizosolenia stolterfothii* (100 000 cells l^{-1}) and

Leptocylindrus danicus (55 000 cells l^{-1}), Nézan, pers. com.), as is usually encountered during the first spring blooms in the Bay of Brest [33]. The chlorophyll concentration was approximately 3 µg.l^{-1}, a classical value for spring phytoplankton bloom in this area during the last decade [33]. The C:N and POC:Chl *a* ratios were 6.0 and 57.2, respectively, values characteristic of phytoplankton-dominated material [37-38]. In addition, due to the sampling method (200 µm pre-filtration; sea above), the contribution of large detritus and zooplankton on the POM was minimized. In summary, there is evidence that the sampled POM was dominated by phytoplankton.

From our results, the carbonate content of the samples (difference between decarbonated and non-decarbonated samples) is less than 5% of the total carbon content (Table 2). Consequently, one should be aware that our study deals only with material containing relatively small quantities of carbonates.

4.2 Drying versus freezing

For carbon results, freezing and oven drying without decarbonation (F-0 and D-0) did not affect the studied parameters (δ^{13}C and PC). However, the combination of freezing with concentrated HCl decarbonation (F-12) led to a significant loss of PN, and freezing with diluted HCl led to a significant increase of δ^{15}N (Fig. 1B, 1E, Table 2, p<0.01). Even if this combined effect cannot be separated, it clearly appears that freezing has an impact on particulate nitrogen and nitrogen isotopic values.

Furthermore, preservation by freezing led to an increase in δ^{15}N variability and standard deviation. Therefore, drying should be the preferred option for preserving samples until the analysis of the four parameters. Even though the storage duration was

not evaluated in this study — one week is not representative of usual storage duration, which may extend to weeks or months — our data reveal a direct effect of the preservation method: freezing to -20°C and defrosting samples affects the POM independently of storage duration.

4.3 Acid treatment

Decarbonation is necessary to remove all inorganic carbon from samples before measuring POC. Although our investigations showed no clear differences in particulate carbon between acidified and non-acidified samples (except in the freezing method, confirming the very low carbonate content of our samples), the $\delta^{13}C$ results pointed out the need of an acidification treatment. Indeed, with exposure to concentrated or diluted HCl, $\delta^{13}C$ mean value shifted from - 20.4‰ to - 21.3‰.

How can one explain a shift of 0.9‰ in $\delta^{13}C$ with such a small amount of removed carbonates? In temperate coastal ecosystems, the two potential particulate inorganic carbon (PIC) sources are drainage basin rocks and calcified benthic populations. The Bay of Brest drainage basin rocks are poorly carbonated, but 80% of the Bay surface is covered with calcified benthic populations: mainly maerl (calcareous algae), marine gastropods, pectinids and brittle stars [39-40]. Therefore, in this ecosystem, PIC is derived mainly from the benthic population (assuming that zooplankton was removed from filters by the 200-μm filtration). Typical values for carbonates (calcite and aragonite) in the literature range from - 5.5‰ to + 7‰ for organisms such as pteropods or molluscs [41-42], and normally between - 0.5 ‰ and + 1.5‰ for most of them. In Bay of Brest, $\delta^{13}C$ measurements on pectinid shells (*Pecten*

maximus) have revealed values within 0 and + 1‰ (Chauvaud, Lorrain and Dunbar, unpublished data).

To check the assumption of a carbonate effect in this 1‰ δ^{13}C difference between non-treated and acidified PC samples, we calculated an expected isotopic value for particulate organic carbon (POC). The following equation can be used to calculate δ^{13}C$_{POC}$:

$$\delta^{13}C_{POC} = \frac{[TPC]\delta^{13}C_{TPC} - [PIC]\delta^{13}C_{PIC}}{[POC]}$$

where the mean non decarbonated PC value, *i.e.* the mean total particulate carbon (TPC) value, was 177 µg, and the mean decarbonated PC value, *i.e.* the mean POC value, was 169 µg. The difference between TPC and POC corresponds to the particulate inorganic carbon (PIC) removed by the acid treatment. Using our values for [TPC], [PIC], [POC] and δ^{13}C$_{TPC}$ (Table 2), and assuming that the δ^{13}C$_{PIC}$ is + 0.5‰ (mean value from literature cited above), the calculated δ^{13}C$_{POC}$ value is - 21.4‰, which agrees with our results (mean value of all acidified samples = - 21.3 ± 0.2‰). This indicates that a very small quantity of inorganic carbon (less than 5% of the total carbon) can lead to a 1‰ increase in the δ^{13}C value. Bunn et al. reported a similar shift in the stable isotope ratio for seagrass nitrogen that was not accompanied by a detectable shift in elemental composition [28]. We believe that differences in PC content between the two carbonate removal procedures are not detected, since the PC analyses do not achieve the precision required to detect such small differences.

Because a small amount of carbonate significantly affects the δ^{13}C value — a 1‰ difference is indeed high enough to bias the future interpretation in food webs or organic

matter origin studies — acid treatment of the SPM samples is required for δ^{13}C studies, even in low carbonated waters.

For both freezing and drying preservation, acid fumes led to further depleted δ^{13}C values compared to diluted acid (p<0.01). But even if this difference has a statistical meaning, a 0.3‰ difference is not truly meaningful in stable isotope studies. In any case, those results suggest that the weak acid treatment could not be sufficient to remove all carbonates from our samples. Furthermore, acid fumes, with respect to other measurements, do not seem to affect N values, when not combined with freezing (see §*4.2* above). Indeed, with the drying preservation method, PN and δ^{15}N values for both acid treatments were equivalent to the values of non-acidified samples (at a 99% confidence interval, one-way analyse of variance).

Since acid fumes treatment is needed for δ^{13}C analyses and since it does not alter the other parameters (especially PN and δ^{15}N) when drying preservation is used, we recommend removing carbonates from poorly carbonated samples by exposure to strong acid fumes for 4 hours.

5. Conclusion

Thorough examination of our results leads us to advise preserving particulate organic matter by oven-drying rather than freezing. This conclusion may be drawn in the absence of data showing the effect of storage time on dried samples, since even short-term freezing has a significant effect on POM analyses.

The carbon stable isotope analyses (*i.e.* the decrease of 1‰ in δ^{13}C values from non-decarbonated to decarbonated samples) indicates that carbonates must be removed in

order to avoid bias in the data, even for poorly carbonated samples. Since the diluted HCl treatment did not seem to be strong enough compared to the HCl fume treatment, and as the later did not alter the nitrogen measurements (PN and δ^{15}N), we recommend the use of strong HCl fumes to remove all carbonates from the samples.

To summarize, the protocol that we propose for the simultaneous determination of POC, PON, δ^{13}C and δ^{15}N from a unique SPM filter sample consists of (1) drying the filter after filtration for storage, and (2) exposing the filter to concentrated HCl fumes for 4 hours to remove carbonates. This protocol allows routine, accurate and precise analyses of the four parameters from a single filter, minimising the analytical time and costs.

Since this study focused on material containing low levels of carbonate, further investigations should be carried out to extend this procedure for the analysis of samples containing high levels of carbonate. It may be necessary to expose highly-carbonated filters to acid fumes for longer periods of time to remove all the carbonates. The effect of longer exposure times on organic material would also need to be investigated

References

[1] M.R. Fontugne, J. -M. Jouanneau, Estuar. Coast. Shelf Sci. 24 (1987) 377.

[2] J.N. Gearing, in: B.O. Jansson (Eds.), Coastal and estuaries studies, Springer-Verlag Berlin Heidelberg 22, 1988, 69.

[3] S.F. Thornton, J. McManus, Estuar. Cost. Shelf Sci. 38 (1994) 219.

[4] S.E. Calvert, B. Nielsen, M. Fontugne, Nature 359 (1992) 223.

[5] I. Bentaleb, C. Caratini, M. Fontugne, M-T. Morzadec-Kerfourn, J-P. Pascal, C. Tissot, NATO ASI series I 49 (1997) 475.

[6] M. Kienast, Paleoceanogr. 15 (2000) 244.

[7] N.J.P. Owens, in: J.H.S. Blaxter, A.J. Southward (Eds.), Advances in Marine Biology, 24, Academic Press, London, 1987, p. 389.

[8] B.J. Peterson, Acta Oecologica 20 (1999) 479.

[9] C. Rolff, R. Elmgren, Mar. Ecol. Prog. Ser. 197 (2000) 81.

[10] J. Hoefs, Stable isotopes geochemistry. 4th edition. Springer-Verlag, Berlin, 1997, 201 pp.

[11] G. H. Rau, J.-L. Teyssie, F. Rassoulzadegan, S.W. Fowler, Mar. Ecol. Prog. Ser. 59 (1990) 33.

[12] P.M. Williams, K.J. Robertson, A. Soutar, S.M. Griffin, E.R.M. Druffel, Prog. Oceanogr. 30 (1992) 253.

[13] P. Riera, P.A. Montagna, R.D. Kalke, P. Richard, Mar. Ecol. Prog. Ser. 199 (2000) 205.

[14] G. Cauwet, Chem. Geol. 16 (1975) 59.

[15] P.N. Froelich, Limnol. Oceanogr. 25 (1980) 564.

[16] A.A. Roberts, J.G. Palacas, I.C. Frost, J. Sedim. Petrol. 43 (1973) 1157.

[17] W.E. Dean, J. Sedim. Petrol. 44 (1974) 242.

[18] G. Telek, N. Marshall, Mar. Biol. 24 (1974) 219.

[19] J. Hirota, J.P. Szyper, Limnol. Oceanogr. 20 (1975) 896.

[20] T. Nakatsuka, N. Handa, E. Wada, C.S. Wong, J. Mar. Res. 50 (1992) 267.

[21] S. M. Bernasconi, A. Barbieri, M. Simona, Limnol. Oceanogr. 42 (1997) 1755.

[22] J. Wu, S.E. Calvert, C.S. Wong, F.A. Whitney, Deep Sea Res. II 46 (1999) 2793.

[23] J. Faganeli, A. Malej, J. Pezdic, V. Malacic, Oceanol. Acta. 11 (1988) 377.

[24] J. Goering, V. Alexander, N. Haubenstock, Estuar. Cost. Shelf Sci. 30 (1990) 239.

[25] K. Weliky, E. Suess, C.A. Ungerer, P.J. Müller, K. Fischer, Limnol. Oceanogr. 28 (1983) 1252.

[26] J.I. Hedges, J.H. Stern, Limnol. Oceanogr. 29 (1984) 657.

[27] S. Kaehler, E.A. Pakhomov, Mar. Ecol. Prog. Ser. 219 (2001) 299.

[28] S.E. Bunn, N.R. Loneragan, M.A. Kempster, Limnol. Oceanogr. 40 (1995) 622.

[29] C.J. Schubert, B. Nielsen, Mar. Chem. 72 (2000) 55.

[30] J. Nieuwenhuize, Y.E.M. Maas, J. Middelburg, Mar. Chem. 45 (1994) 217.

[31] K.L. Bosley, S.C. Wainright, Can. J. Fish. Aquat. Sci. 56 (1999) 2181.

[32] J.C. Salomon, M. Breton, in : L. Cheung (Eds.) Environmental hydraulics, Balkema, Rotterdam, 1991, p. 459.

[33] L. Chauvaud, F. Jean, O. Ragueneau, G. Thouzeau, Mar. Ecol. Prog. Ser. 200 (2000) 35.

[34] P. Bassoulet, Etude de la dynamique des sédiments en suspension dans l'estuaire de l'Aulne (Rade de Brest), Thèse de doctorat de troisième cycle, Université de Bretagne Occidentale, Brest-France, 1979, 136 pp.

[35] E. Wada, A. Hatori, Nitrogen in the sea: forms, abundances and rate processes. CRC Press, Boca Ranton, Florida, 1991, 208 pp.

[36] N. Savoye, Origine et transfert de matière organique particulaire dans les écosystèmes littoraux macrotidaux, Thèse de doctorat, Université de Bretagne Occidentale, Brest, 2001, 324 pp.

[37] D.J.S. Montagnes, J.A. Berges, P.J. Harrison, F.J.R. Taylor, Limnol. Oceanogr. 39 (1994) 1044.

[38] L.A. Cifuentes, J.H. Sharp, M.L. Fogel, Limnol. Oceanogr. 35 (1988) 1102.

[39] J. Grall, L. Chauvaud, G. Thouzeau, S. Fifas, M. Glemarec, Y.M. Paulet, C. R. Acad. Sci. Paris 319 (1996) 931.

[40] L. Chauvaud, La coquille Saint-Jacques en rade de Brest : un modèle biologique d'étude des réponses de la faune benthique aux fluctuations de l'environnement, Thèse de doctorat, Université de Bretagne Occidentale, Brest-France, 1998, 266 pp.

[41] G. Wefer, W.H. Berger, Mar. Geol. 100 (1991) 207.

[42] E. Vander Putten, F. Dehairs, E. Keppens, W. Baeyens, Geochim. Cosmochim. Acta 64 (2000) 997.

Figure 1: Suspended particulate material results for the six preservation-decarbonation treatments (notation explained in Table 1): (A) Particulate Carbon- content (PC, µg), (B) Particulate Nitrogen (PN, µg), (C) $\delta^{13}C$ (‰), (D) $\delta^{15}N$ (‰). Means ± 1 standard deviation, n = 10.

Tables

Table 1: The six different treatments applied to the POM filter samples with their identification code.

Name	Acid treatment			Preservation	
	HCl 12N	HCl 0.12N	None	Dried	Frozen
D-12	X			X	
D-0.12		X		X	
D-0			X	X	
F-12	X				X
F-0.12		X			X
F-0			X		X

Table 2: Carbon and Nitrogen contents, $\delta^{13}C$ and $\delta^{15}N$ values from the preservation (D = Dried, F = Frozen) and HCl treatments investigated (n.d. means not determined).

Elemental parameters	PC (µg)						PN (µg)					
Storage	D			F			D			F		
Acid treatment (HCl N)	0	0.12	12	0	0.12	12	0	0.12	12	0	0.12	12
	170	154	161	184	156	162	32.5	32.7	32.9	34.2	32.2	30.5
	159	162	172	187	169	171	30.9	32.8	34.3	33.6	35.2	30.6
	180	175	175	182	155	176	35.4	35.5	33.9	35.3	30.2	31.7
	169	169	178	187	172	177	33.0	32.6	34.7	35.7	34.3	31.3
	182	172	165	199	185	158	35.4	33.5	33.2	36.0	34.6	29.2
	177	166	165	201	166	163	35.2	33.5	32.4	35.7	33.1	30.5
	172	180	175	168	160	171	32.6	32.5	33.1	31.8	31.7	29.5
	169	170	184	170	169	176	34.7	34.9	35.0	29.7	34.4	32.0
	173	157	178	178	160	159	34.0	32.9	34.3	32.8	32.3	30.1
	164	162	190	n.d	187	160	32.4	33.4	37.6	n.d.	34.5	29.5
Mean	171	166	174	184	168	167	33.6	33.4	34.1	33.9	33.3	30.5
Standard deviation	7.0	8.1	9.0	11.3	11.1	7.7	1.6	1.0	1.5	2.1	1.6	1.0

Isotopic parameters	$\delta^{13}C$ (‰)						$\delta^{15}N$ (‰)					
Storage	D			F			D			F		
Acid treatment	0	0.12	12	0	0.12	12	0	0.12	12	0	0.12	12
	-20.1	-21.1	-21.3	-20.3	-20.9	-21.3	6.7	6.4	6.3	7.0	7.1	6.1
	-20.5	-21.1	n.d	-20.4	-21.1	-21.3	6.3	6.5	n.d	6.9	7.3	6.1
	-20.4	-21.3	-21.4	-20.4	-21.0	-21.3	6.8	6.4	6.4	6.5	7.3	6.4
	-20.3	-21.2	-21.5	-20.4	-21.3	-21.7	6.5	6.7	6.8	7.3	7.4	6.3
	-20.4	-21.3	-21.5	-20.3	n.d	-21.3	6.9	7.0	5.9	6.9	n.d	6.5
	-20.4	-21.1	-21.4	-20.4	-20.9	-21.3	6.5	6.7	6.3	6.6	7.1	6.5
	-20.3	-21.8	-21.3	-20.8	-21.0	-21.6	6.3	6.3	6.6	6.1	7.2	5.8
	-21.1	-21.2	-21.8	-20.0	-21.0	-21.2	6.4	7.0	6.2	6.3	7.3	7.3
	-20.2	-21.1	-21.7	-20.6	-21.0	-21.3	6.6	6.8	6.8	6.4	6.4	6.6
	-20.1	-21.1	-21.6	n.d.	-21.4	-21.4	6.7	6.7	6.1	n.d.	6.7	5.8
Mean	-20.4	-21.2	-21.5	-20.4	-21.1	-21.4	6.6	6.7	6.4	6.7	7.1	6.3
Standard deviation	0.29	0.22	0.17	0.22	0.17	0.16	0.21	0.25	0.31	0.38	0.33	0.44