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Technical note

Simple standard procedure for the routine determination of organic matter in marine sediment

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Abstract

For the routine determination of organic matter in marine sediment, a rough estimate is often obtained by measuring the loss of weight on ignition (Difference-On-Ignition method: D.O.I.). This D.O.I. is to be used in ecological studies where organic material in sediment is used as an environmental variable among others. A review of papers using this method showed a great variability within conditions used. These conditions are generally used without accurate justification. We propose a simple standard procedure based on grain-size fraction of sediment to determine optimal temperature and time of ignition, and the influence of the sample weight. The method proposed, using the loss of weight on ignition, will give the same accuracy in measurements of organic matter in marine sediments.

Introduction

The organic concentration of sediments is of primary importance in studies of benthic ecosystems. It is often considered as an important trophic source for the benthos (Cocito et al., 1990), and organic enrichment, both natural and anthropogenic, is one of the most common forms of disturbance in the benthos (Weston, 1990; Gee et al., 1985).

There are two commonly used methods for determination of organic matter in sediment (Buchanan, 1984):

1. For an accurate determination, the organic nitrogen and organic carbon are estimated.
2. For the routine determination, a rough estimate may be obtained by measuring the loss of weight on ignition.

While the determination by elemental analysis is more accurate, the loss of weight on ignition is still largely used in benthic ecology (Table 1) because this method gives a very quick and cheap estimation of organic matter contents in sediment.

The first method has been largely documented (Cauwet, 1975; Kristensen & Andersen, 1987; Telek & Marshall, 1974; Hedges & Stern, 1984; Hirota & Szyper, 1976; Froelich, 1980; Byers et al., 1978). The second one has been a standard procedure for a number of year in many marine ecological studies. However, few studies documented accurately this method (Mook & Hoskin, 1982). A review of papers using the loss on ignition method showed a great variability within the conditions used: sample weight, temperature of ignition, time of ignition (Table 1). The lack of standard method leads to difficult comparisons between results. Moreover, no rigorous reasons and results to support the choices made are found in literature.

The purpose of this paper is to propose a fast simple standard procedure based on loss of weight on ignition for a routine determination of organic matter in marine sediment. This method is only to be used in ecological studies where organic material content in the sediment is needed as an environmental variable among others. In many ecological studies of macrobenthic communities, only a rough estimation of organic mat-

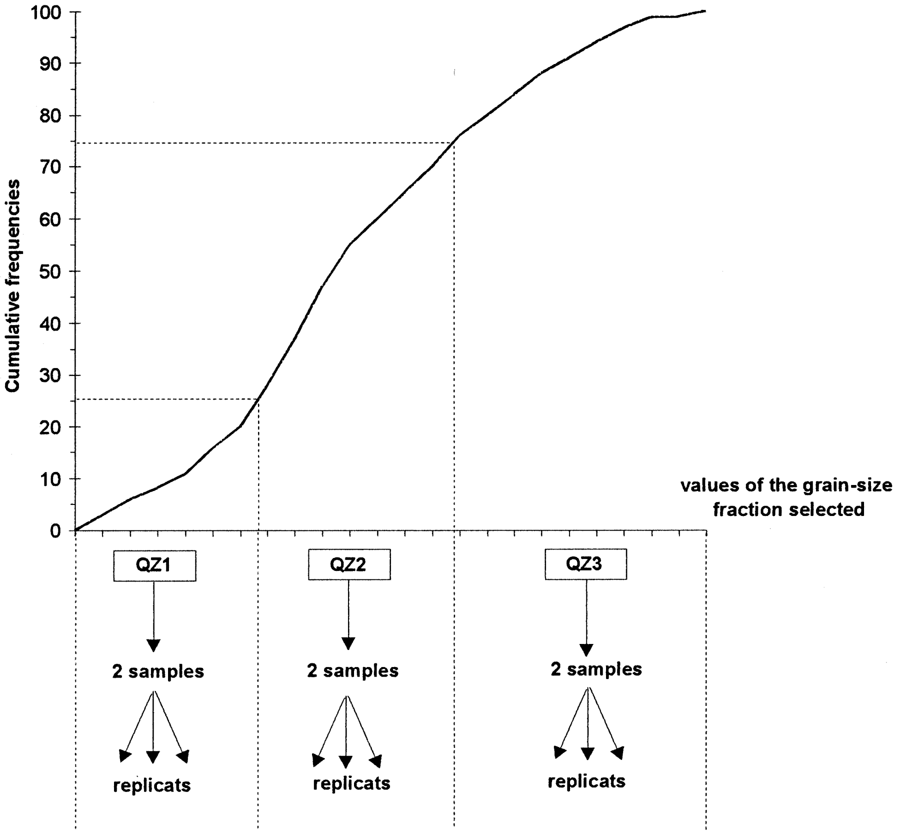

Figure 1. Scheme of the selection of samples.

Table 1. Review of parameters used by various authors in loss of weight on ignition method to determine organic matter content in sediment.

Weight (g)	Temperature (°C)	Time (hours)	Reference
2	480	4	Diaz Castaneda, 1989
–	480	5	Cocito et al., 1990
–	550	1	Buhl-Mortensen & Hoisaeter, 1993
–	100 to 800 (step 50)	16	Kristensen & Andersen, 1987
2	100 to 800 (step 100)	4	Mook & Hoskin, 1982
2	550	1 to 8 (step 1)	Mook & Hoskin, 1982
–	500	at least 4	Hirota & Szyper, 1975
–	200 to 1600 (step 100)	24?	Froelich, 1980
–	475–500	4–6	Byers et al., 1978
–	600	-	Buchanan, 1984
–	450	10	Hily, 1987

ter in sediment is needed. Elemental analysis would be in these cases too long and expensive. The aim of the method is to find rigorously the approximate limit between organic and inorganic combustion. For a more accurate study on the organic content, the first method based on elemental analysis (cf. *supra*) must be used.

Materials and methods

For this study sediments samples were used taken at several locations on the French coast in the Southern Bight of the North Sea, in the *Abra alba* muddy fine sand community. The different grain-size fractions were first determined for each sample.

The first step was to select samples corresponding to different range of values of the grain-size fraction that most discriminate the samples. This can be achieved, e.g., with Principal Component Analysis. In our case, silt content was the most discriminant grain-size fraction. Upper limit for the silt class is 0.05 mm following Larsonneur (1977). Cumulative frequencies distribution of the values of silt content was then plot with all samples, first and third quartiles were determined and 2 samples were randomly taken within each interval ('quartile zone') i.e. before quartile 1 (QZ1: <25%), between quartile 1 and 3 (QZ2: 25–75%), and over quartile 3 (QZ3: >75%) (Figure 1).

The second step was to check whether H_2O evaporation from clays between 100 and 150 °C is important or not. An important evaporation would make the method of D.O.I. impossible for sediments containing these clays (H. Golterman, pers. com.)

The third step was to determine optimal temperature, time and weight influence in the Difference-On-Ignition method (D.O.I.). 3 replicates were then taken in each of the samples selected.

1. *Determination of optimal temperature of ignition*

3 replicates were taken in each sample for each ignition. As we had no knowledge yet on weight influence and time of ignition, we used 2 g of sediment (Mook & Hoskin, 1982) and 4 hours of ignition (Kristensen & Andersen, 1987; Mook & Hoskin, 1982).

Sediment was first dried at 50 °C until constant weight was obtained. Sediment samples were then placed in crucibles, weighed and ignited at 300, 400, 450, 500, 550, 600, 650, 700 and 800 °C in a muffle furnace (Thermolyne model 62 700; precision ± 0.25%). This range of temperature was chosen because all results found in literature fall between 300 and 800 °C, and more accurately between 400 and 700 °C. We did not take into account the loss on ignition (L.O.I) peak found at 150–300 °C which is due to combustion of an easily combustible pool and evaporation of volatile low molecular compounds (Kristensen & Andersen, 1987).

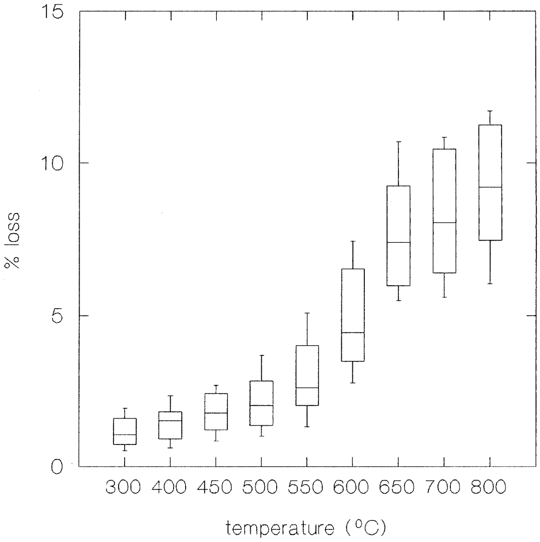

Figure 2. Box plot of the percentage loss of weight of all the sediment samples ignited at various temperatures ($n = 18$ samples at each temperature). The box represents the middle 50% of the series. The median is indicated by the horizontal line through the box. The ends of the box are the twenty-fifth and seventy-fifth percentiles. The error bars indicate the tenth and the ninetieth percentiles of data.

Percentage weight loss was calculated for each replicate. Due to variable temperature steps, the weight losses at each interval are given as % loss on ignition (%L.O.I. °C^{-1}) (Kristensen & Andersen, 1987).

2. *Determination of optimal time of ignition*

We used the same procedure as the determination of optimal temperature of combustion with the temperature of ignition previously determined. Sediment samples (3 replicates per sample) were also placed in crucibles and ignited for periods of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 hours.

3. *Influence of sample weight on D.O.I*

The influence of sample weight on D.O.I. method has never been studied yet. Table 1 indicates that very few studies precise the weight of sediment used. For this purpose 2, 4, 6, 8 and 10 g of sediment were placed in crucibles and ignited for the period and temperature previously determined. 1 replicate per sample was used for each ignition.

Percentage weight loss was calculated for each sample. The weight losses at each interval are given as % loss on ignition (%L.O.I.).

All weighing was performed using a Sartorius balance model R 160 P (precision ± 0.01 mg).

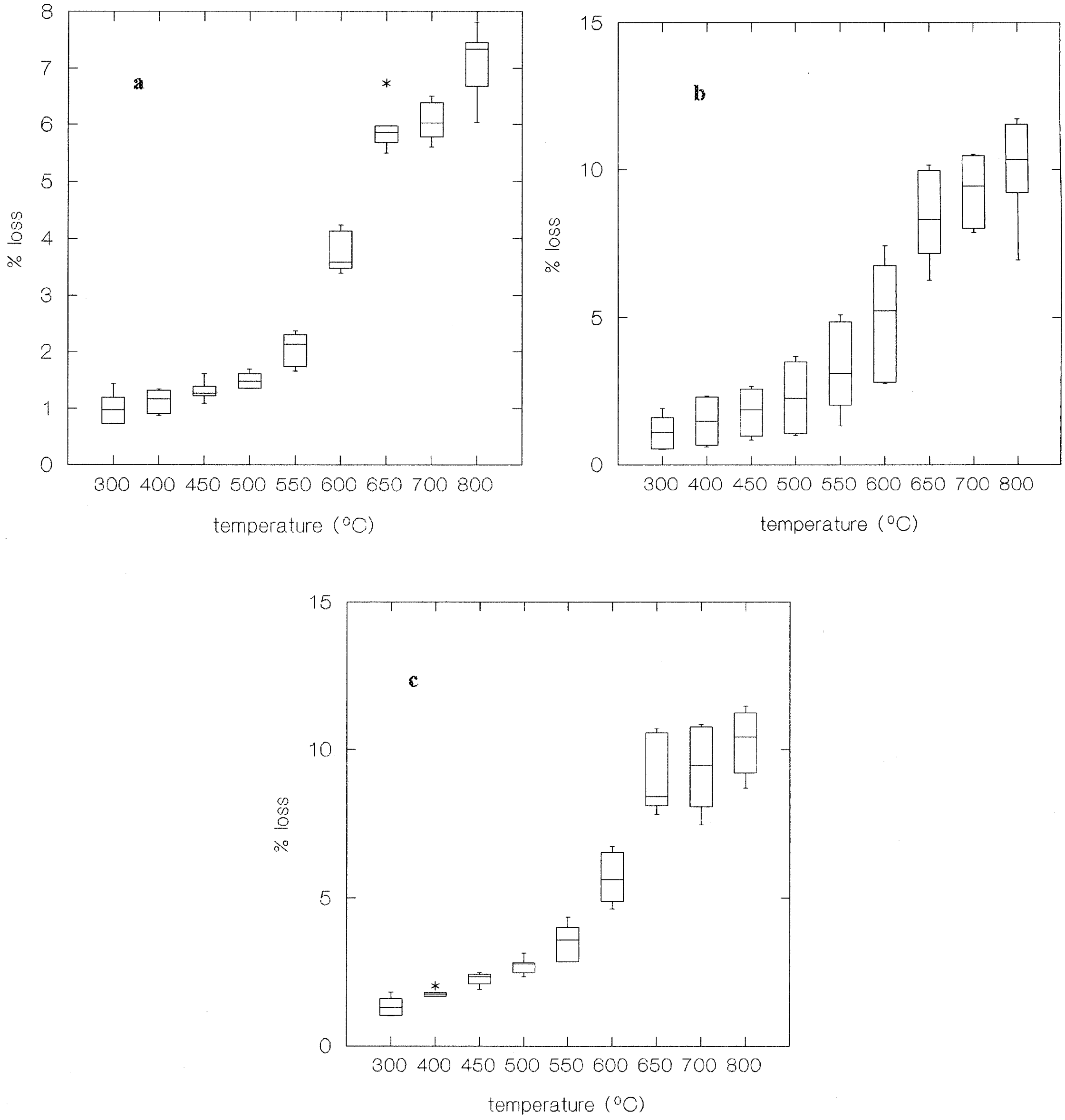

Figure 3. Box plots of the percentage loss of weight of sediment ignited at various temperatures in
(a) quartile zone 1 ($n = 6$ samples at each temperature);
(b) quartile zone 2 ($n = 6$ samples at each temperature);
(c) quartile zone 3 ($n = 6$ samples at each temperature).
The box represents the middle 50% of the series. The median is indicated by the horizontal line through the box. The ends of the box are the twenty-fifth and seventy-fifth percentiles. The error bars indicate the tenth and the ninetieth percentiles of data. Extreme values are shown as stars.

Statistical analysis

Because of inequality of variances among samples (heteroscedasticity), non-parametric methods were used. In the third step, Kruskal-Wallis one way analysis of variance was used to compare weight losses in all replicates and in each quartile zone.When the null hypothesis was rejected, we used a multiple comparison test: the Dunn's test (Dunn, 1964; Zar, 1984), to detect significant difference between samples.

Results

1. Determination of optimal temperature of ignition

The weight losses between temperature were significantly different when all replicates are pooled ($P<0.001$) (Figure 2) and in each quartile zone (QZ1, QZ2 & QZ3, $P<0.001$) (Figure 3). The Dunn's test showed that the weight losses increase was significant above 500–550 °C.

L.O.I temperature scan from 300 to 800 °C showed a clear unimodal peak in every quartile zone (Figure 4). The separation between organic and inorganic matter occurs at 500–550 °C. This result confirmed the previous statistical tests. However, a weak peak was also visible between 400 and 500 °C. It represented the refractory organic matter pool following Kristensen & Andersen (1987).

We therefore choose 500 °C as ignition temperature.

The evaporation from clays between 100 and 150 °C was low: 0.53% (SD: 0.21).

2. Determination of optimal time of ignition

Because of a great range in weight losses between the different quartiles zones, the Kruskal-Wallis test on all replicates was not significant. The analysis in each quartile zone was however more informative. Weight losses at different times was statistically significant in QZ1 ($p<0.02$) (Figure 5a), in QZ2 ($p<0.01$) (Figure 5b) and in QZ3 ($p<0.05$) (Figure 5c). Weight losses significantly increased (Dunn's test, $p<0.05$) until 4 hours. The weight loss was then stable until 6 hours in all cases. At 7 hours and above, fluctuations are more important in each quartile zone. This is illustrated in L.O.I. time scan from 1 hour to 10 hours (Figure 6).

Fluctuations over 7 hours might be due to ignition of other factors beside those due to combustion of organic materials. Following these results, 6 hours of ignition seems to be a good compromise for complete combustion of organic matter without important interference with other factors (i.e. inorganic matter).

3. Influence of sample weight on D.O.I

There is no significant influence of initial sediment sample weight on weight loss by ignition. The L.O.I weight scan from 2 to 10 g (Figure 7) shows an immediate stabilisation of the weight losses in each quartile zone. We can then use 2 g of sediment sample in the D.O.I method, the same weight used by Mook & Hoskin (1982).

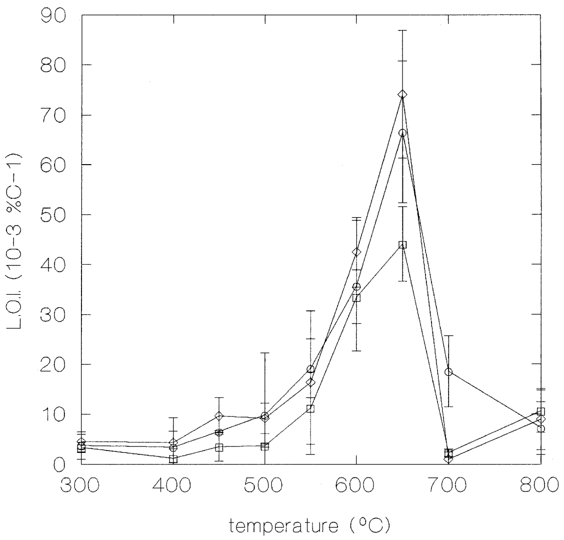

Figure 4. L.O.I. temperature scan from 300 to 800 °C on sediment samples ($n = 6$ samples per quartile zone at each temperature) in QZ1 (open squares), QZ2 (open circles) and QZ3 (open rhombs). Vertical bars represent ± 1SD. The values are given as % L.O.I °C^{-1} of initial dry weight.

Discussion

We found that an ignition of 2 g sediment sample at 500 °C during 6 hours is the best way to estimate organic matter in our samples by the D.O.I method. This result is in the range of those obtained in most studies published in term of temperature (Table 1). Published results using this D.O.I method were however very different in terms of time of combustion used (Table 1). The standard methodology proposed in this paper could lead to a more objective determination of time of ignition. Sediment sample weight did not influence the loss on ignition with our samples.

In order to check the results based on ignition, Org-C from subsamples were also estimated using a chemical method based on HCl and CHN analysis (e.g. Kristensen & Andersen, 1987). Relationship between Org-C estimated by HCl followed by CHN analysis and ignition method is high ($y = 1.05x + 0.025$; $N = 30$; $r^2 = 0.940$). The org-C concentration of the organic matter was about 43% (Org-C = 0.431. D.O.I. + 0.034; $N = 30$; $r^2 = 0.813$).

The loss of $MgCO_3$ below 500 °C (Hirota & Szyper, 1985) together with the incomplete combustion of organic matter (mainly organic carbon) at this temperature may be responsible for the slight overlap observed at 500–550 °C in the L.O.I temperature scan (Kris-

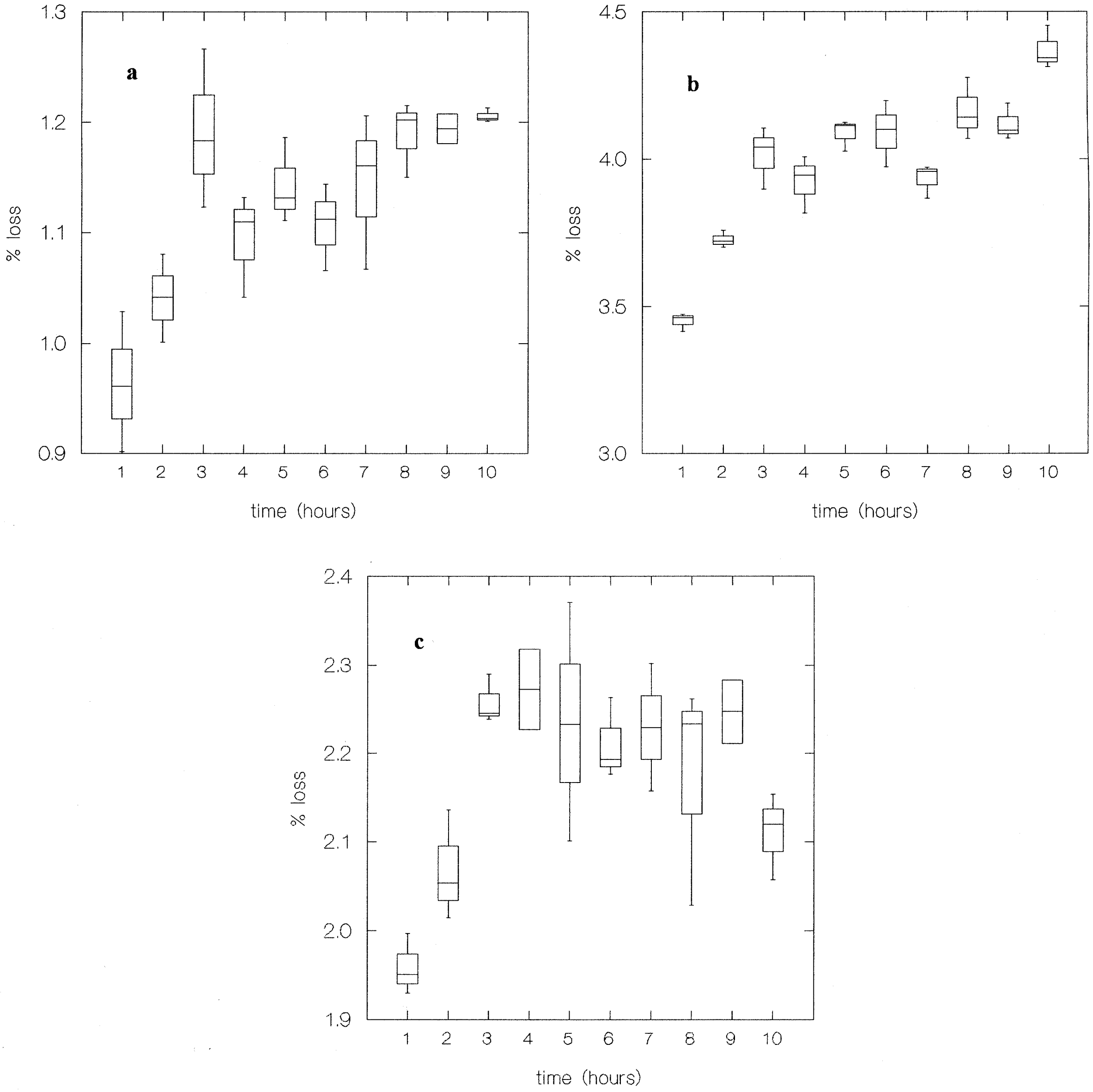

Figure 5. Box plot of the percentage loss of weight of sediment ignited at various times (at 500 °C):
(a) quartile zone 1 ($n = 6$ samples at each time);
(b) quartile zone 2 ($n = 6$ samples at each time);
(c) quartile zone 3 ($n = 6$ samples at each time).
The box represents the middle 50% of the series. The median is indicated by the horizontal line through the box. The ends of the box are the twenty-fifth and seventy-fifth percentiles. The error bars indicate the tenth and the ninetieth percentiles of data. Extreme values are shown as stars.

tensen & Andersen, 1987) (Figure 4). A small amount of inorganic matter was lost and a small amount of organic matter was not combusted at 500 °C. Errors will then counteract each other when using the D.O.I method at 500 °C.

The D.O.I. method may suffer from the inability of precise separation of organic and inorganic matter (Froelich, 1980; Buchanan, 1984; Kristensen & Andersen, 1987). Even if this D.O.I method provide a rough estimate of the organic matter in sediment, this

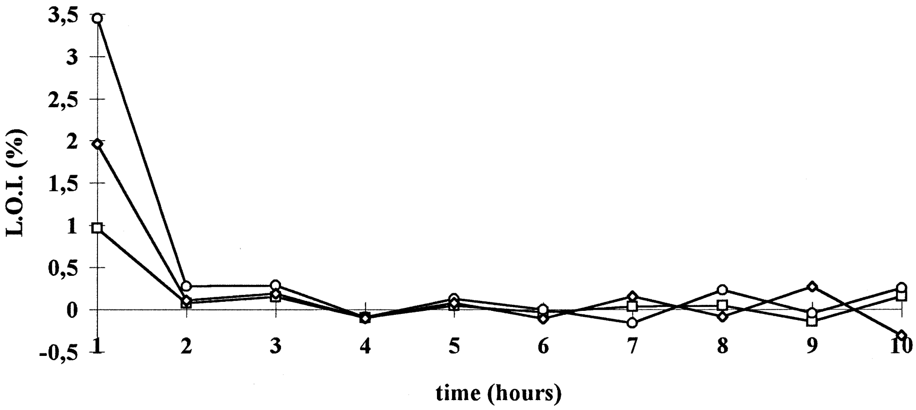

Figure 6. L.O.I. time scan from 1 to 10 hours on sediment samples ($n = 6$ samples per quatile zone at each time) in: QZ1 (open squares), QZ2 (open circles) and QZ3 (open rhombs). The values are given as % L.O.I. of initial dry weight.

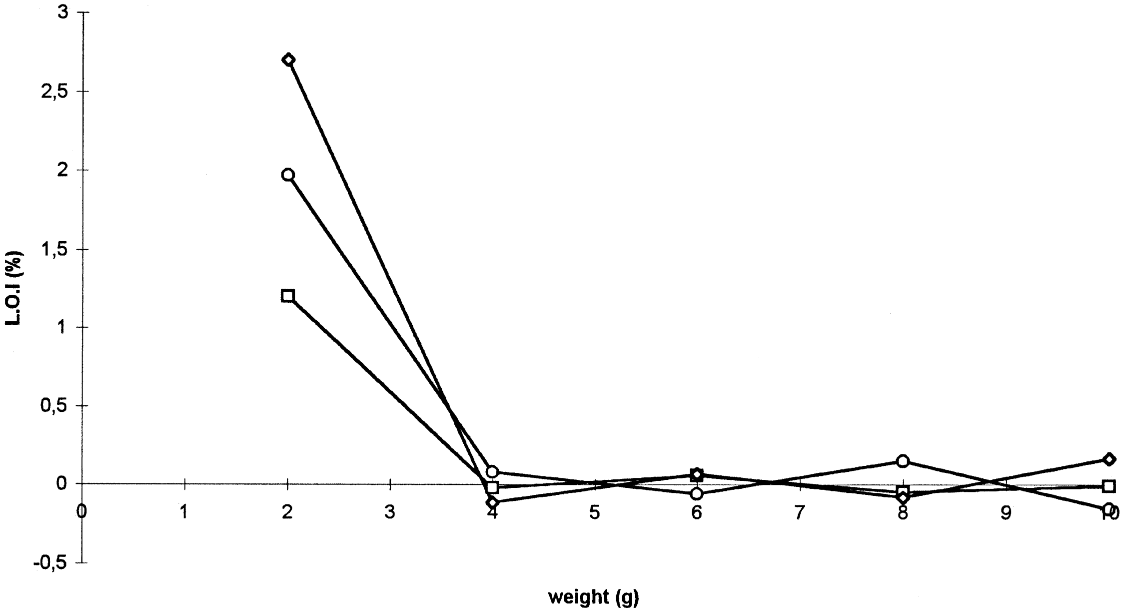

Figure 7. L.O.I. weight scan from 2 to 10 g. on sediment samples ($n = 1$ sample per quartile zone at each weight) in QZ1 (open squares), QZ2 (open circles) and QZ3 (open rhombs). The values are given as % L.O.I. of initial dry weight.

method can be mainly used to get a quick and cheap estimation of an ecological variable among others in ecological studies. However more accurate and reliable results could be obtained with the following cautions combined with our procedure.

Mook & Hoskin (1982) showed that the loss of structural water from clay minerals may become a significant source of error during combustion between 400 and 500 °C. They found 20% loss of weight due to structural water in clays with a mud sample containing 83% clay. In the present study, clay content was low (<1%). Bias due to this factor was therefore neglected. If clay content is important, it should be worthwhile to estimate the weight losses due to this factor following the procedure described in Mook & Hoskin (1982): before organic contents of sediments are determined by ignition, organics have to be digested from an aliquot of the sediment samples (at least one per quartile zone in our methodology) and the organic-free sediment be ignited at the temperature at which organic determinations are to be made.

Hirota & Szyper (1975) showed experimentally that the loss of carbonate may cause serious errors unless the temperature of combustion is kept at or below 500 °C. By keeping ignition temperature around this value up to 7 hours, there is a barely significant change in the $CaCO_3$ content of combusted samples. Above this temperature threshold of 500 °C, losses of carbonate may become important. When $CaCO_3$ is high in sediments, loss of weight on ignition should be used with great care and the method should be checked by determining the loss of weight after baking prepared samples of pure $CaCO_3$ (following Hirota & Szyper, 1975). To obtain carbonate-free sediment, samples are sometimes treated with HCl before ignition: i.e. samples are treated with 0.25 M HCl until no visible reaction occured and dried at 50 °C overnight (Buhl-Morensen & Hoisaeter, 1993) or sufficient 4N HCl is added to remove $CaCO_3$ (Schaff et al., 1992). Byers et al. (1978) found, however, that these methods involving acidification and washing to remove carbonates are highly suspect. They mentioned Roberts et al.(1973) who found that acidification removed 9–44% of the organic carbon in natural carbonate sediment.

The selection of grain-size fraction that most discriminate the samples before running the method should lead to a better identification of the potential inaccuracies present in the measurement due to problems of e.g. clay or carbonate (e.g. important shells contents...).

These recommendations are particularly important when the difference-on-ignition method is used to separate organic and inorganic carbon before carbon and nitrogen analysis with e.g. a CHN-analyzer.

Finally, using the standard procedure proposed, this methodology should give a similar accuracy in measurement of organic matter in marine sediments by D.O.I method and then comparisons between ecological studies using this method will become possible and more reliable.

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