Isotopic and Molecular Characterization of Particulate Organic Matter in Coastal Waters

Luc Megens

Isotopic and Molecular Characterization of Particulate Organic Matter in Coastal Waters

The research presented in this thesis was conducted at the Centre for Isotope Research of the University of Groningen and at the Netherlands Institute for Sea Research, Texel.
Funding was provided by the Dutch Foundation for Scientific Research (NWO), The Hague, Netherlands.

STELLINGEN
behorende bij het proefschrift
van
Luc Megens

Isotopic and Molecular Characterization of Particulate Organic Matter in Coastal Waters

Bij het bepalen van de oorsprong van organisch materiaal in mariene of estuariene sedimenten en in de waterkolom is het noodzakelijk meer dan één parameter te gebruiken. De ^{14}C-activiteit is hiervoor een goede aanvulling op de veel gebruikte stabiele-isotopenverhouding van koolstof.
dit proefschrift

Particulair organisch materiaal in de Eems-Dollard bevat een aanzienlijke fractie afkomstig van mariene of estuariene algen met een relatief hoge leeftijd.
dit proefschrift

Wat betreft de aard van organisch materiaal in rivierwater, is de Amazone niet representatief voor de meeste rivieren elders in de wereld, getuige een vergelijking van de relatieve ^{14}C-concentratie daarin met recente ^{14}C-gegevens uit andere rivieren.

Het voor isotopenfractionering corrigeren van met AMS verkregen $^{14}C/^{12}C$-resultaten met behulp van met IRMS verkregen $^{13}C/^{12}C$-resultaten, is onjuist.

De toegankelijkheid van het min of meer klassieke verdampingsmodel van Craig & Gordon is ernstig belemmerd door het gebrekkige en inconsequente gebruik van de symbolen en door de gebrekkige toegankelijkheid van de betreffende proceedings.
(Craig, H. and Gordon, L.I., 1965. Deuterium and oxygen-18 variations in the ocean and the marine atmosphere. Spoleto Conference, Pisa: 1-22).

Het door hedendaagse architecten negeren van de richtlijn van de Romein Vitruvius (*De Architectura I,4,12)*, namelijk om geen nederzettingen te stichten nabij stilstaand water, door in nieuwbouwwijken veel waterpartijen te ontwerpen, en de huidige trend in het natuurbeheer om wetlands in het dichtbevolkte Nederland te creëren, kunnen op termijn een gevaar voor de volksgezondheid opleveren, met name indien ten gevolge van het broeikaseffect de gemiddelde temperatuur stijgt.

Dat iemand die wegens een misdrijf tot TBS is veroordeeld, maar onbehandelbaar blijkt, wordt vrijgelaten omdat hij zijn gevangenisstraf heeft uitgezeten, is een gebrek in het Nederlandse rechtssysteem.

Hoewel de massamedia het meestal hebben over wetenschappers die dieren klonen, is hier geen sprake van wetenschap maar van technologie.

RIJKSUNIVERSITEIT GRONINGEN

Isotopic and Molecular Characterization of Particulate Organic Matter in Coastal Waters

Proefschrift

ter verkrijging van het doctoraat in de
Wiskunde en Natuurwetenschappen
aan de Rijksuniversiteit Groningen
op gezag van de
Rector Magnificus, dr. D.F.J. Bosscher,
in het openbaar te verdedigen op
maandag 11 december 2000
om 16.00 uur

door

Luc Megens

geboren op 12 mei 1970
te Aalten

Promotores:	Prof. dr. W.G. Mook
	Prof. dr. J.W. de Leeuw
Co-promotor:	Dr. ir. J. van der Plicht
Beoordelingscommissie:	Prof. dr. R.W.P.M. Laane
	Prof. dr. W. Salomons
	Prof. dr. V. Ittekkot

CONTENTS

Introduction

Rivers transport organic matter produced by terrestrial organisms to the sea, partly as dissolved (DOM) and partly as particulate organic matter (POM). What happens to this terrestrial organic matter once it has arrived to the sea has intrigued many scientists, but after decades of research this question is still not fully answered (Hedges et al., 1997). However, insight in this matter may be essential for understanding the global carbon cycle and its anthropogenic perturbations (Siegentaler and Sarmiento, 1993).

The estimated amount of particulate organic carbon (POC) discharged by the rivers is approximately twice the total amount of organic carbon buried in all marine sediments (Berner, 1989; Hedges and Keil, 1995). Most of the organic matter in many rivers is highly degraded (Hedges et al., 1994) and appears to be derived from erosion of soil and peat (Mook and Tan, 1991; Hedges et al., 1986a,b; Meybeck, 1982). Because it survived complete degradation, the remnants are chemically and biochemically very resistant (Hedges et al., 1997). Between 17 and 35 % of POM in rivers is estimated to be labile organic matter (Ittekkot and Laane, 1991; Ittekkot, 1988). On a global scale terrestrial organic matter does not appear to be a major component of total organic matter content of marine sediment (Hedges and Keil, 1995): the major part appears to be derived from marine organisms. Unless the estimates of the global carbon budgets are very wrong, this would mean that much more than half of the terrestrial POC and more than just the 'labile' (Ittekkot and Laane, 1991) fraction discharged by the rivers is mineralized after it has entered the sea, despite its relative resistance to degradation.

To make quantitative estimates of the transport and mineralization of terrestrial POM in the marine environment, it is essential to have a reliable method for determining the relative contributions of terrestrial and marine OM to sediments and suspended matter. Several approaches have been developed. One is to quantify the amounts of biomarkers, selected organic compounds that are produced by specific organisms. The majority of these tracers for terrestrial organic matter are compounds specific to vascular plants, almost exclusively growing on land. Among these are long-chain n-alkanes, derived from plant waxes, hydroxylated carboxylic acids, derived from cutin, and methoxy-phenols, derived from lignin. If the concentration of the

biomarker in the terrestrial component of the organic matter mixture is known, the terrestrial component can be quantified. In the case of POM, the concentration of a certain biomarker is to be determined in fluviatile POM, which is the pure terrestrial POM source. However, use of this value might lead to biassed results, because part of the terrestrial POM is likely to be mineralized after it has entered the sea (Hedges et al., 1997). Since the different compounds in terrestrial POM probably degrade at different rates, this would lead to a change in the biomarker concentration (Hedges and Prahl, 1993).

Another way to discriminate between organic matter of marine and terrestrial origin is to measure the abundance ratios between the stable carbon isotopes in the organic matter. Due to small differences in the physical and chemical characteristics of isotopic molecules (i.e. molecules containing different isotopes of a specific atom), these ratios appear to be different in terrestrial and marine organic matter.

Carbon Isotopes and the Origin of Organic Matter

Carbon in nature consists of three isotopic species, ^{12}C, ^{13}C and ^{14}C. ^{12}C is the most common isotope, comprising ca. 99 % of all carbon. Approximately 1 % of all carbon is present as ^{13}C, while 1 out of every 10^{12} carbon atoms is ^{14}C. ^{12}C and ^{13}C are stable isotopes, ^{14}C is radioactive. Although these isotopes are chemically almost identical, small differences exist in diffusion velocities and reaction rates of compounds with different isotopes, due to mass dependent effects. Because at a given temperature the velocity of a molecule containing the heavy isotope is smaller than of the isotopically light molecule, the light molecule usually reacts faster than the heavier. Therefore, in incomplete irreversible reactions and in equilibrium reactions, the reaction product(s) will have a different isotopic composition than the initial reactant(s). This phenomenon is called isotopic fractionation. Isotopic fractionation resulting from irreversible physical or chemical processes is refered to as kinetic fractionation, while equilibrium fractionation is the isotope effect related to equilibrium reactions.

Because absolute isotope ratios are difficult to measure, the isotopic composition of a sample is measured relative to that of a reference material or standard, and is expressed as the δ value, which is defined (for ^{13}C) as:

$$\delta^{13}C = {}^{13}R_{sample}/{}^{13}R_{standard} - 1 \qquad (1)$$

where ^{13}R stands for the ratio $^{13}C/^{12}C$. The standard used for ^{13}C analysis is the internationally agreed V-PDB (Gonfiantini, 1984). Because natural $\delta^{13}C$ values are usually close to zero they are expressed in permil (0.001 = 1 ‰). A negative $\delta^{13}C$ value means that the $^{13}C/^{12}C$ ratio in the sample is lower than in the standard, or in other words is depleted in ^{13}C, or isotopically lighter. The range of $\delta^{13}C$ values in various carbon containing substances is shown in fig. 2.1.

Marine phytoplankton generally has a $\delta^{13}C$ value about 7 ‰ higher than land plants of the so called C-3 type, which are the most common in moderate climates (Fry and Sherr, 1984). The $\delta^{13}C$ values of both groups cover a wide range, respectively around –21 ‰ in marine phytoplankton in regions with a temperate climate and around –27 ‰ for terrestrial C-3 plants (e.g. Fry and Sherr, 1984). The difference in $\delta^{13}C$ between marine phytoplankton and terrestrial plants is widely used to determine the origin of organic matter in marine sediments or in suspended matter in coastal waters. From a measured $\delta^{13}C$ of OM in a sediment or suspended matter the fraction of terrestrial OM (x_T) can be calculated:

$$x_T = (\delta^{13}C_S - \delta^{13}C_M) / (\delta^{13}C_S - \delta^{13}C_T)$$

for which estimated average values for marine OM ($\delta^{13}C_M$) and terrestrial OM ($\delta^{13}C_T$) or measured values (e.g. when the source of the terrestrial component is one river in which the $\delta^{13}C$ of the suspended POM can be measured).

However, there are some complications that make the determination of the terrestrial contribution in marine POM less straightforward:

1) The $\delta^{13}C$ value of marine plankton varies with species, CO_2 abundance, growth rate, light intensity (e.g. Bidigare et al., 1999; Laws et al., 1995; Thompson and Calvert, 1995) and temperature (Fontugne and Duplessy, 1981).

2) Esspecially in semi-arid regions C-4 plants (e.g. maize, sugar cane, semi-arid grasses) are a major part of the total vegetation. These C-4 plants use a different mechanism for the assimilation of CO_2 than the C-3 plants, resulting in $\delta^{13}C$ values of around –11 ‰ (range –10 to –14 ‰; e.g. Deines, 1980), quite different from C-3 plants or marine algae. In the Gulf of

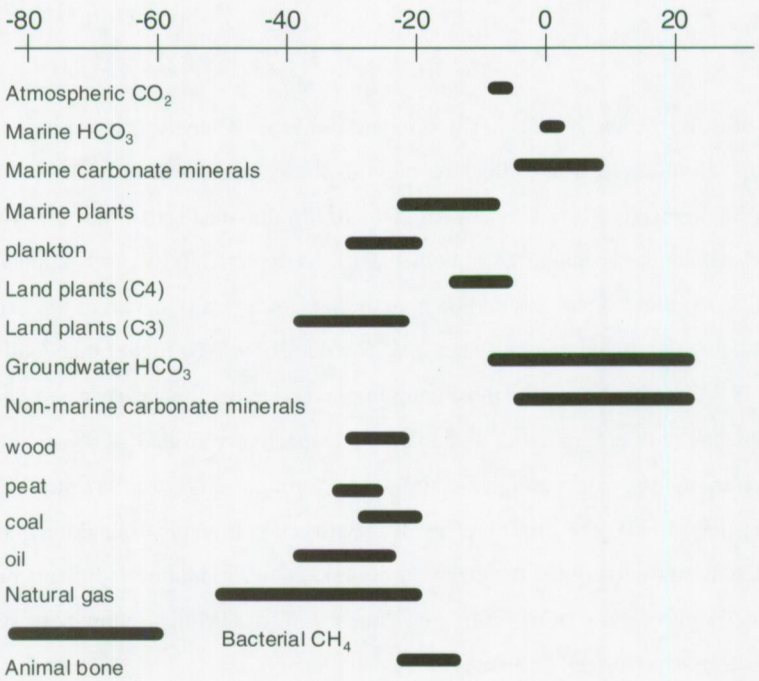

Figure 1 Overview of variations in $\delta^{13}C$ of natural compounds and organisms.

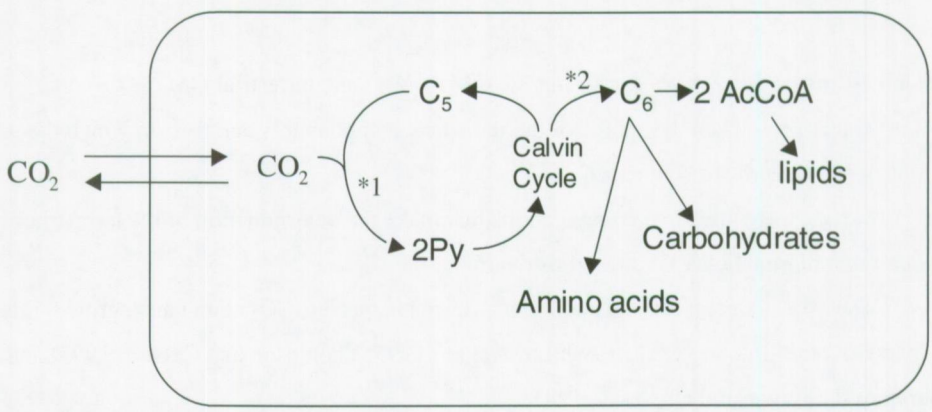

Fig. 2 Schematic view of the assimilation of inorganic carbon and the biosynthesis pathways to the various types of biochemicals. Two main reactions causing isotopic fractionation are *1: the Rubisco catalyzed reaction of CO_2 to pyruvate and *2: the pyruvate dehydrogenase catalyzed decarboxylation of pyruvate yielding acetyl-CoA, which is mainly responsible for the difference in $\delta^{13}C$ between lipids and carbohydrates/amino acids.

Mexico, e.g., Goñi et al. (1998) showed by means of molecular analysis that C-4 plant derived organic matter is an important component in sedimentary organic matter, and that ignoring this contribution leads to an underestimate of the terrestrial input to the sedimentary organic matter.

3) This thesis deals with another phenomenon that can affect the determination of the ratio of terrestrial to marine organic matter: within one particular organism chemical compounds of different chemical classes do not have the same isotopic composition. If during diagenesis some type of compound is more easily degraded than others (selective degradation) this might change the isotopic composition of the resulting mixture.

Isotopes in the different classes of chemical compounds

Photosynthesis converts inorganic carbon into glucose via a proces known as the Calvin-cycle. Subsequently, via a variety of reactions carbohydrates, amino acids and lipids are formed (fig. 2.2). Glucose is split into pyruvate, which is used in the synthesis of carbohydrates and proteins. It can be converted by pyruvate dehydrogenase into an acetyl group, the precursor of lipid molecules. The pathways to the various biomolecules have different isotopic fractionation effects (Hayes, 1993). Sugars and proteins (in autotrophic organisms) are ca. 5 ‰ higher in $\delta^{13}C$ than lipids (e.g. Deines,1980; Hayes, 1993). The main reaction responsible for this depletion of lipids is the formation of acetyl-coenzyme A from pyruvate by the enzyme pyruvate dehydrogenase (De Niro and Epstein, 1977), while sugars and amino acids are biosynthesized from pyruvate via other pathways. Even within compound classes considerable differences can exist between the individual compounds of one particular organism: $\delta^{13}C$ of individual amino acids from one organism may vary more than 5 ‰ (Macko, 1994), and also between different types of lipids the $\delta^{13}C$ values may vary up to 10 ‰ (Hayes, 1993; Schouten et al., 1998).

Sugars and proteins are more easily degraded (e.g. Harvey et al., 1995) than lipids or lipidic macromolecules (algaenans, found in cell walls of some algae; e.g. Gelin et al., 1999 or cutans, components of cuticles of plant leaves; e.g. De Leeuw et al., 1991) and lignin (Benner et al., 1987). Like lipids, lignin has a lower $\delta^{13}C$ value than carbohydrates in the same organism (Benner et al., 1987) which can be expected for the aliphatic macromolecules

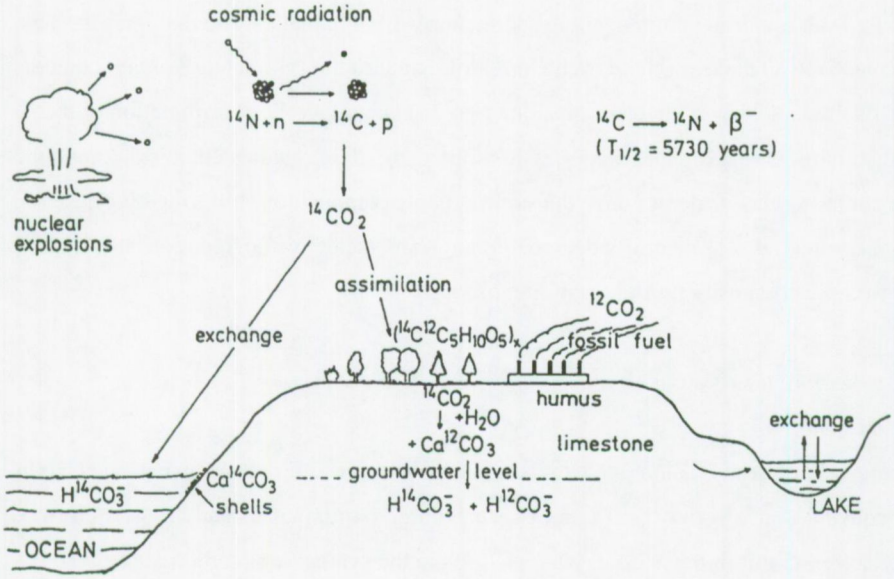

Fig. 3 Origin and distribution of ^{14}C in nature.

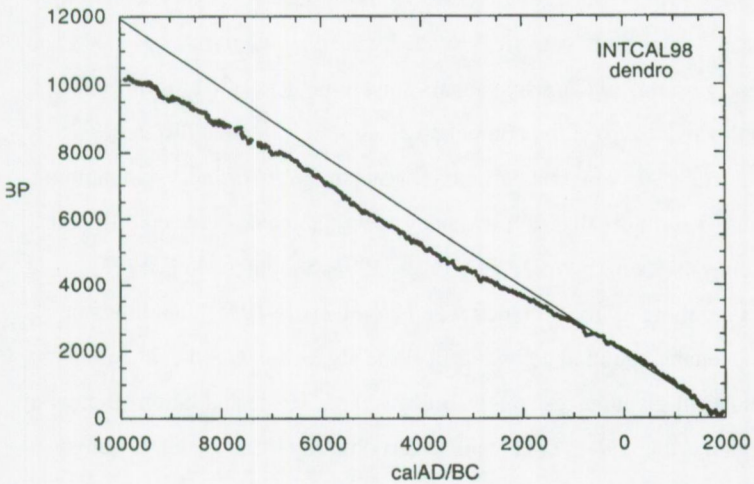

Fig. 4 ^{14}C calibration curve relating the ^{14}C age in years Before Present (y-axis) to calendar years (x-axis). Present in the ^{14}C timescale is defined as 1950 AD.

algaenan and cutan as well. Thus, partial degradation of organic matter can change the isotopic composition by specifically removing compounds with high $\delta^{13}C$ values. Laane et al. (1990) demonstrated that after incubation in the dark, the $\delta^{13}C$ of POM mainly consisting of (fresh) phytoplankton decreased by 4 ‰ from –19 ‰ to –23 ‰. Samples with lower initial $\delta^{13}C$ changed much less after incubation, probably because they contained a smaller amount of easily degradable compounds. A similar shift in $\delta^{13}C$ was observed during experimental diagenesis of vascular plant material, due to selective preservation of lignin (Benner et al., 1987). Therefore, a lower $\delta^{13}C$ value of organic matter in a sediment or suspended matter does not necessarily indicate a higher terrestrial contribution, but can also indicate selective degradation of compounds with a higher $\delta^{13}C$ value. This is a complicating factor for the use of bulk stable isotope measurements for the determination of the origin of the organic matter.

A solution could be the analysis of fractions of the organic matter that are homogeneous in isotopic composition or stability. Calculating the terrestrial component in each of this fractions thus eliminates the effect of selective degradation. The ideal situation would be to analyze all individual compounds, but the amount of different compounds in POM makes this a enormous task. The approach of this thesis is to separate the mixture of organic compounds in fractions roughly consisting of carbohydrates and other water soluble compounds, hydrolyzable amino acids, lipids, ester-bound lipids and base soluble compounds, and insoluble, non-hydrolyzable organic matter. A similar fractionation was performed by Wang et al. (1996, 1998), studying deep ocean POM and sediment.

In the projects described in this thesis stable carbon isotope analysis of isolated fractions is combined with the analysis of the ^{14}C age of or the relative ^{14}C abundance in the samples.

Radiocarbon

The radioactive carbon isotope ^{14}C is formed in the upper stratosphere from a nuclear reaction of ^{14}N with neutrons produced by cosmic radiation. It is subsequently oxidized to $^{14}CO_2$ which is mixed throughout the atmosphere. Due to exchange of CO_2 with the ocean ^{14}C also enters the oceanic carbon pool (fig 3).

^{14}C decays to ^{14}N emitting β^- radiation, with a half-life of 5730 years. When an autotrophic organism is taking up carbon from the atmosphere in the form of CO_2 or in case of

aquatic organisms from inorganic carbon dissolved in the water (dissolved CO_2 or bicarbonate), the ratio of ^{14}C to ^{12}C in the organism will be the same as in the carbon source, except for differences due to isotopic fractionation. However, this can be corrected for based on the stable carbon isotope ratio, so this complication no longer exists. When the organism dies, the $^{14}C/^{12}C$ ratio decreases because no more ^{14}C is taken up. ^{14}C in the organism is radioactively decaying, so that the $^{14}C/^{12}C$ ratio measured at a given time after the death of the organism compared to the original $^{14}C/^{12}C$ ratio of the living organism, is a measure of the time expired since the death of the organism.

It was assumed that the $^{14}C/^{12}C$ ratio in the atmosphere (and the ocean) was constant in (pre)historic times, and thus the ^{14}C activity in a sample could be compared to that in the modern atmosphere or modern materials, which was confirmed within the measurement precision by measurements of a number of samples with known age (ranging from 900 to 4900 years old; Libby et al., 1949). However, from the study of ^{14}C in tree rings by De Vries (1958) aiming at explaining discrepancies between ^{14}C ages and historical ages of objects from the Egyptian Old Kingdom and many subsequent studies (e.g. Olson, 1970), it became clear that the $^{14}C/^{12}C$ ratio in the atmosphere was not constant. Over the time for which treering chronologies exist, the atmospheric $^{14}C/^{12}C$ ratio decreased somewhat from 10 000 BC to 0 AD. Over the whole period there are also small variations (e.g. Radiocarbon calibration issue, 1998). With this treering data the ^{14}C measurements can be calibrated to calendar years (e.g. Van der Plicht, 1993; fig. 4).

The $^{14}C/^{12}C$ ratio (^{14}R or ^{14}A), or ^{14}C abundance of a sample is not determined absolutely, but relatively to the ^{14}C abundance of a reference material, measured under the same circumstances in the same equipment. This relative ^{14}C abundance (^{14}a) is thus defined as (Mook and Van der Plicht, 1999):

$$^{14}a = {}^{14}A_{sample} / {}^{14}A_{standard}$$

Like ^{13}C, ^{14}C is also subjected to isotopic fractionation in chemical processes. To be able to compare ^{14}C abundances in materials with different $\delta^{13}C$ values, a correction is made for this fractionation, thus giving a normalized relative ^{14}C abundance ($^{14}a_N$):

$$^{14}a_N = {}^{14}A_{sample} / {}^{14}A_{standard} * \{(1-25\,‰)/(1 + \delta^{13}C)\}^2$$

The age of the sample can be calculated from the $^{14}a_N$ value using the formula:

$$age = t_{0.5}/ \ln 2 * \ln (^{14}a_N) = -8033 * \ln (^{14}a_N)$$

This age is expressed as years BP (before present), were present is defined as AD 1950. ^{14}a values are relative to 0.95 times the activity or ^{14}C abundance of a batch of oxalic acid from 1950 ($^{14}A_{standard}$; Karlen et al., 1966). For archaeological applications a half-life of 5568 years (–8033 * ln 2) is used, which, during the first years of ^{14}C dating, was the best estimate of the ^{14}C half-life. Later determinations showed the half-life of ^{14}C was 5730 ± 40 years.

After 1950 test explosions of nuclear bombs in the atmosphere produced a considerable amount of ^{14}C, to a maximum of twice the concentration of ^{14}C in the atmosphere in 1963 (e.g. Meijer et al., 1994). After 1963 the ^{14}C concentration decreased rapidly due to exchange with DIC in the oceans, which is an approximately 50 times larger carbon pool than the atmosphere. Further dilution of atmospheric ^{14}C is caused by combustion of fossil fuels. Fossil fuels do not contain ^{14}C due to their high age, so that combustion adds ^{14}C free CO_2 to the atmosphere. Measurement of ^{14}C in tree rings showed that this effect already began during the Industrial Revolution in the late 19th century, when large amounts of fossil fuels started to be used (the Suess-effect). Due to this, the ^{14}C abundance of CO_2 in the atmosphere in 1950 was 2 % lower than before the Industrial Revolution. This explains the factor 0.95 between the standard abundance and the abundance of the oxalic acid standard, which was used to correct for the Suess-effect. The ^{14}C concentration in DIC in surface ocean water is in general lower than in atmospheric CO_2, because DIC in the upper water layers is mixed with old DIC by upwelling of water from deeper layers (the so-called reservoir effect; e.g. Stuiver et al., 1998).

Since the normalized relative ^{14}C abundance $^{14}a_N$ (further on in this thesis the symbol ^{14}a will be used) is corrected for isotopic fractionation, it is not affected by selective degradation of organic matter from a single source. It can be a useful parameter for the determination of the origin of POM in the aquatic environment. In the sea most autochthonous POM is assumed to be recent, i.e. from living or recently died organisms. Experiments have shown that only a very small fraction of phytoplankton, the main producer of organic matter, escapes from mineralisation within a short time after death (e.g. Harvey et al., 1995). Material escaping

mineralization sinks to the bottom in days or weeks (Eisma, 1991). Therefore, in deep seas autochthonous organic carbon in the upper water layers is of recent origin. In shallow waters older organic matter from the sediment might be resuspended into the water column. The terrestrial input of POM to the sea by rivers, is likely to consist mainly of relatively old, degraded material, eroded either from soils or peat deposits (e.g. Mook and Tan, 1991; Hedges et al., 1986a). However, Druffel et al. (1986) reported higher ^{14}a values in POM collected in the North-East Pacific than in sea surface DIC. This they attributed to the presence of young ^{14}C enriched terrestrial OM. The only published ^{14}C data on POM from a river are from the Amazon River, which show the presence of 'bomb' ^{14}C, i.e. ^{14}a values higher than 100 % (Hedges et al., 1986a). The material in this river is thus on average fresh, with a large component of less than 30 years old OM (the time between the first increase of atmospheric ^{14}C due to nuclear bomb tests and the measurements). However, this tropical river is probably not comparable to rivers in moderate climates, where terrestrial production rates and soil erosion are much less. ^{14}C data of surface sediments from the Rhine delta and the Dutch coast indicate that here the average ^{14}C age of river transported POM is rather high (Salomons and Mook, 1981).

The introduction of ^{14}C-Accelerator Mass Spectrometry (AMS) has made POM more accessible to ^{14}C analysis, because much smaller samples (equivalent to 20 to 1000 µg C) are needed than with radiometric ^{14}C analysis (typical sample size 1 g C), which is based on the detection radioactive decay of ^{14}C.

This thesis thus reports on the investigations of the stable and radioactive carbon isotope ratios in sedimentary and suspended particulate organic matter, both in bulk material and isolated fractions. Attempts are made to interpret the data obtained in relation to the origin of the organic matter. In chapter I the differences in carbon isotopic compositions between different fractions of a POM sample obtained by sequential extractions and hydrolyses, yielding different types of compounds, are reported. In chapter II size fractions of a surface sediment sample from the Ems-Dollard estuary are studied. In chapter III size fractions of recent sediments from the Washington coast (Eastern Pacific) obtained using SPLITT fractionation are studied with respect to the carbon isotope distributions. In chapter IV differences in carbon istopic and molecular compositions of POM along the salinity gradient in the Ems-Dollard during bloom and non-bloom periods are reported. In chapter V seasonal variations in the composition of POM in the middle part of the Ems-Dollard estuary are presented. In chapter VI POM from the North Sea

was studied in relation to POM from the main West-European rivers.

references

Benner, R., Fogel, M.L., Sprague, E.K., Hodson,R.E. (1987) Depletion of ^{13}C in lignin and its implications for stable carbon isotope studies. *Nature*, 329, 708-710.

Berner, R.A. (1989) Biogeochemical cycles of carbon and sulfurand their effect on atmospheric oxygen over Phanerozoic time. *Palaeogeography, Palaeoclimatology and Palaeoecology*, 73, 97-122.

Bidigare, R.R., Hanson, K.L., Buesseler, K.O., Wakeham, S.G., Freeman, K.H., Pancost, R.D., Millero, F.J., Steinberg, P., Popp, B.N., Latasa, M., Landry, M.R., Laws, E.A. (1999) Iron-stimulated changes in ^{13}C fractionation and export by equatorial Pacific phytoplankton: Toward a paleogrowth rate proxy. *Paleoceanography*, 14, 589-595.

De Niro, M.J. and Epstein, S. (1977) Mechanism of carbon isotope fractionation associated with lipid synthesis. *Science*, 197, 261-263.

Deines P. (1980) The Isotopic Composition of Reduced Organic Carbon. In: Fritz P. Fontes J.C. (eds.) *Handbook of Environmental Isotope Geochemistry.* Amsterdam. pp. 329-406.

Druffel, E.R.M., Homjo, S., Griffin, S. (1986) Radiocarbon in particulate matter from the eastern Sub-Arctic Pacific Ocean: evidence of a source of terrestrial carbon to the deep sea. *Radiocarbon*, 28, 397-407.

Eisma, D. (1991) *Suspended Matter in the aquatic environment.* Springer, Berlin.

Fontugne, M.R. and Duplessy, J.C. (1981) Organic Carbon Isotope Fractionation by Marine Plankton in the Temperature Range -1 to 31 °C. *Oceanol. Acta* 4, 85-90.

Fry, B. and Sherr, E.B. (1984) $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. *Contrib. Mar. Sc.* 27, 13-47.

Gelin, F.,Volkman, J.K. Largeau, C., Derenne, S., Sinninghe Damste, J.S., De Leeuw, J.W. (1999) Distribution of aliphatic, nonhydrolyzable biopolymer in marine microalgae. *Org Geochem.* 30, 147-160.

Gonfiantini, R. (1984) Advisory Group Meeting on Stable Isotope Reference Samples for Geochemical and Hydrological Investigations; *IAEA*: Vienna.

Goñi, M.A. Ruttenberg, K.C. and Eglington, T.I. (1998) A reassessment of the sources and importance of land-derived organic matter in surface sediments from the Gulf of Mexico. *Geochim. Cosmochim. Acta* 62, 3055-3075.

Harvey, H.R. Tuttle, J. H. and Bell, J.T. (1995) Kinetics of phytoplankton decay during simulated sedimentation: Changes in biochemical composition and microbial activity under oxic and anoxic conditions. *Geochim. Cosmochim. Acta* 59, 3367-3377.

Hayes, J. (1993) Factors controlling ^{13}C contents of sedimentary organic compounds: Principles and evidence. *Mar. Geol.* 113, 111-125.

Hedges, J.I., Ertel, J.R., Quay, P.D., Grootes, P.M., Richey, J.F., Devol, A.H., Farwell, G.W., Schmidt, F.W. and Salati, E. (1986a) Organic carbon-14 in the Amazon river system. *Science* 231, 1129-1131.

Hedges, J.I., Clark, W.A., Quay, P.D., Richey, J.F., Devol, A.H., Santos U.M. (1986b) Composition and fluxes of particulate organic material in the Amazon River. *Limnol. Oceanogr.* 31, 717-738.

Hedges, J.I., Ertel, J.R., Richey, J.E., Quay, P.D., Benner, R., Strom, M., Forsbberg, B. (1994) Origin and processing of organic matter in the Amazon river as indicated by carbohydrates and amino acids. *Limnol. Oceanogr.* 39, 743-761.

Hedges J.I. and Prahl, F.G. (1993) Early diagenesis, consequences for applications of molecular biomarkers. In *Organic Geochemistry*, (Engel, M.H. and Macko, S.A. eds.) Plenum New York. pp. 237-253.

Hedges, J.I. and Keil, R.G. (1995) Sedimentary organic matter preservation: an assessment and speculative synthesis. *Mar. Chem.* 49, 81-115.

Hedges, J.I., Keil, R.G., Benner, R. (1997) What happens to terrestrial organic matter in the ocean? *Org. Geochem.* **27,** 195-212.

Ittekkot, V. (1988) Global trends in the nature of organic matter in river suspension. *Nature*, 332, 436-438.

Ittekkot, V. and Laane, R.W.P.M. (1991) Fate of riverine Particulate Organic Matter. In *Biogeochemistry of Major World Rivers* (E.T. Degens, S. Kempe, J.E. Richie eds.) SCOPE vol. New York, 1991. pp.233-244.

Karlèn, I., Olsson, I.U., Kållberg, P., Killiççi, S. (1966) Absolute determination of the activity of two ^{14}C dating standards. *Arkiv f. Geofysik* 6, 465-471.

Laane, R., Turkstra, E. and Mook, W. (1990) Stable carbon isotope composition of pelagic and benthic organic matter in the North Sea and adjacent estuaries. In: *Facets of Modern Biogeochemistry* (Ittekot V., Kempe, S. Michaelis, W., and Spitzy, A., eds.), Berlin-Heidelberg, pp. 214-224.

Laws, E.A., Popp, B.N., Bidigare, R.R., Kennicutt, M.C., Macko, S.A. (1995)
Dependence of phytoplankton carbon isotopic compostition on growth rate and $[CO_2]aq$: theoretical considerations and experimental results. *Geochim. cosmochim. acta* 59, 1131-1138.

De Leeuw J.W., Van Bergen P.F., Van Aarssen B.G.K., Gatellier J.P.L.A., Sinninghe Damsté J.S. and Collinson M.E. (1991) Resistant biomacromolecules as major contributors to kerogen. *Phil. Trans. R. Soc. Lond. B*, 333, 329-337.

Libby, W.F., Anderson, E.C., Arnold, J.R. (1949) Age determination by radiocarbon content, world-wide assay of natural radiocarbon. *Science*, 109, 227-228.

Macko, S.A., Engel, M.H., Qian, Y. (1994) Early diagenesis and organic matter preservation - a molecular stable carbon isotope perspective. *Chem. Geol.* 114, 365-379.

Meijer, H.A.J., van der Plicht, J., Gislefoss, J.S. and Nydal, R., 1995. Comparing long-term atmospheric 14C and 3H records near Groningen,the Netherlands with Fruholmen, Norway and Izana, Canary Islands 14C stations. *Radiocarbon* 37, no.1, 39-50.

Meybeck, M. (1982) Carbon, nitrogen and phosphorus transport by world rivers. *Am. J. Sc.* 282, 401-450.

Mook W.G. and Tan F.C. (1991) Stable Carbon Isotopes in Rivers and Estuaries. In *Biogeochemistry of Major World Rivers* (E.T. Degens, S. Kempe, J.E. Richie eds.) SCOPE vol. New York, 1991. pp. 245-264.

Mook W.G. and Van der Plicht, J. (1999) Reporting ^{14}C activities and concentrations. Radiocarbon *Radiocarbon* **41,** 227-240.

Van der Plicht, J. (1993) The Groningen Radiocarbon Calibration Program. Radiocarbon 35, no. 1, 231-237. Calibration issue 1993, (M. Stuiver, A. Long and R.S. Kra, eds.)

Radiocarbon Calibration Issue. Stuiver, M. and Van der Plicht, J. (1998) *Radiocarbon*, 40, 1041-1164.

Salomons, W. and Mook, W.G. (1981) Field Observations of the Isotopic Composition of Particulate Organic Carbon in the Southern North Sea and Adjacent Estuaries. *Marine Geology*, 41, M11-M20.

Schouten, S., Breteler, W.C.M.K., Blokker, P., Schogt, N., Rijpstra, W.I.C., Grice, K., Baas, M., Sinnighe Damste, J.S. (1998) Biosynthetic effects on the stable carbon isotopic compositions of algal lipids: Implications for deciphering the carbon isotopic biomarker record. *Geochim. Cosmochim. Acta* 62, 1397-1406

Siegenthaler, U. and Sarmiento, J.L. (1993) Atmospheric carbon dioxide and the ocean. *Nature*, 365, 119-125.

Stuiver, M., Reimer, P.J., Braziunas, T.J. (1998) High-precision radiocarbon age calibration for terrestrial and marine samples. *Radiocarbon*, 40, 1127-1152.

Thompson, P.A. and Calvert, S.E. (1995) Carbon isotope fractionation by Emiliania huxleyi. *Limnol. Oceanogr.* 40, 673-679.

De Vries, Hl. (1958) Variation in concentration of radiocarbon with time and location on earth. Proc. Koninkl. Nederl. Akad. Wetenschappen B61, 1-9.

Wang, X.C. and Druffel, E.R.M. (1996) Radiocarbon in organic carbon classes in particulate organic matter and sediment in the deep northeast Pacific Ocean. *Geophys. Res. Lett.* 23, 3583-3586.

Wang, X.C. and Druffel, E.R.M., Griffin, S., Lee, C., Kashgarian, M. (1998) Radiocarbon studies of organic compound classes in plankton and sediment of the northeastern Pacific Ocean. *Geochim. Cosmochim. Acta* 62, 1365-1378.

I

Molecular, Radioactive and Stabile Carbon Isotope Characterization of Estuarine Particulate Organic Matter

Abstract

Organic Matter in sediments and suspended matter is a complex mixture of constituents with different sources, histories and stabilities. To study these components in a suspended matter sample from the Ems-Dollard Estuary we used combined molecular analysis with pyrolysis-gas chromatography-mass spectrometry and stabile and radioactive carbon isotope analyses of the bulk and separated chemical fractions. Carbohydrates and proteins, about 50 % of the total organic carbon, are much younger than the bulk sample and have a somewhat higher $\delta^{13}C$ value. Lipids and the final residue are considerably older and have lower $\delta^{13}C$ values. The final residue, ca. 17 % of the total carbon, consists mainly of aliphatic macromolecules that could be derived from algae or terrestrial plants. The $\delta^{13}C$ value points to a marine origin.

Introduction

Organic matter (OM) in sediments (SOM) and suspended matter (POM) is a complex mixture of constituents with different sources, histories and stabilities. Therefore these constituents can also differ in age so that ^{14}C-dating of a SOM and POM will give an average age of the contributing organic components instead of a date of sedimentation. Fractionation of the OM and successive complementary molecular and isotopic analyses of these fractions will however be useful to differentiate sources, histories and stabilities of these fractions and thus of SOM and POM as a whole.

POM in estuaries is derived from local primary production and transported material, both marine and terrestrial. Sources of the marine component can both be primary production or eroded sediment, and the terrestrial component eroded soil or peat. To discriminate the terrestrial from the marine organic component a linear mixing model based on the difference in the $^{13}C/^{12}C$ ratio between marine phytoplankton and terrestrial plants is used (e.g. Laane et al., 1990). Organic matter derived from marine phytoplankton - the main source of marine organic matter -

has $\delta^{13}C$ values of around –21 ‰, while terrestrial organic matter derived from C3 plants has $\delta^{13}C$ values of around –27 ‰ (Mook & Tan, 1990).

Sometimes this straightforward two end-member mixing model works nicely, but there are some complicating factors:

$\delta^{13}C$ values of marine phytoplankton can vary from –16 to –27 ‰ (Sackett et al., 1965; Fontugne & Duplessy, 1981), depending on environmental factors such as temperature, light intensity, salinity and nutrient availability.

Furthermore, even organic matter from one organism is still a mixture of different compounds covering a wide range of $\delta^{13}C$ values. Polysaccharides and proteins have on average higher $\delta^{13}C$ values than lipids (Deines, 1980). The $\delta^{13}C$ values of amino acids from one organism can cover a range of up to 10 ‰ (Macko et al., 1994) and also lipids from one organism show large differences in $\delta^{13}C$ (Schouten et al., 1998).

Third, in most cases POM only represents a fraction of the original biomass. Polysaccharides and proteins are much more labile than some lipids and other bio(macro)molecules. Because of the differences in $\delta^{13}C$ between these molecules this selective degradation will affect the total $\delta^{13}C$ value as well.
Combining the results of stabile carbon isotope analysis with ^{14}C-measurements of quantified well-characterized fractions of POM might be helpful in a validation of the processes and variables determining the molecular and isotopic composition of the POM.

Such an approach was followed by Wang et al. (1996) to trace the origin and history of POM and SOM in the deep ocean. Their results show large differences in ^{14}C activities and $\delta^{13}C$ in fractions of SOM and POM, suggesting preferential decomposition of OM and adsorption of old dissolved organic matter.

To establish the origin and history of estuarine POM we analyzed bulk and chemical fractions of POM from the Ems-Dollard Estuary, combining isotopic and molecular analyses to investigate if phenomena as found by Wang et al. (1996) in deep ocean POM and SOM can also be found in estuarine POM.

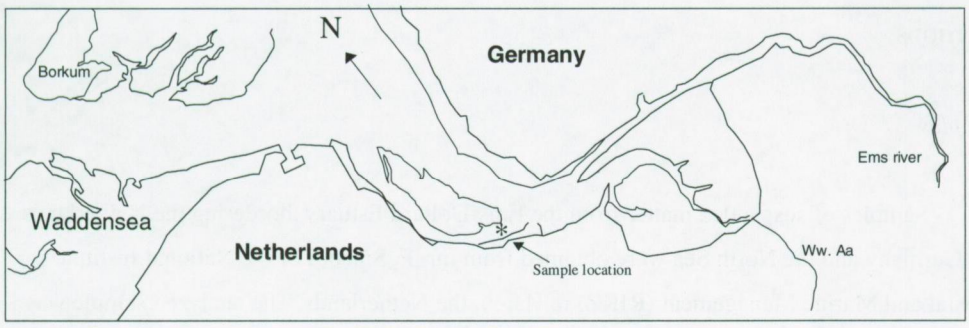

Fig. 1.1 The Ems-Dollard estuary. Sampling location indicated by the asrerisk.

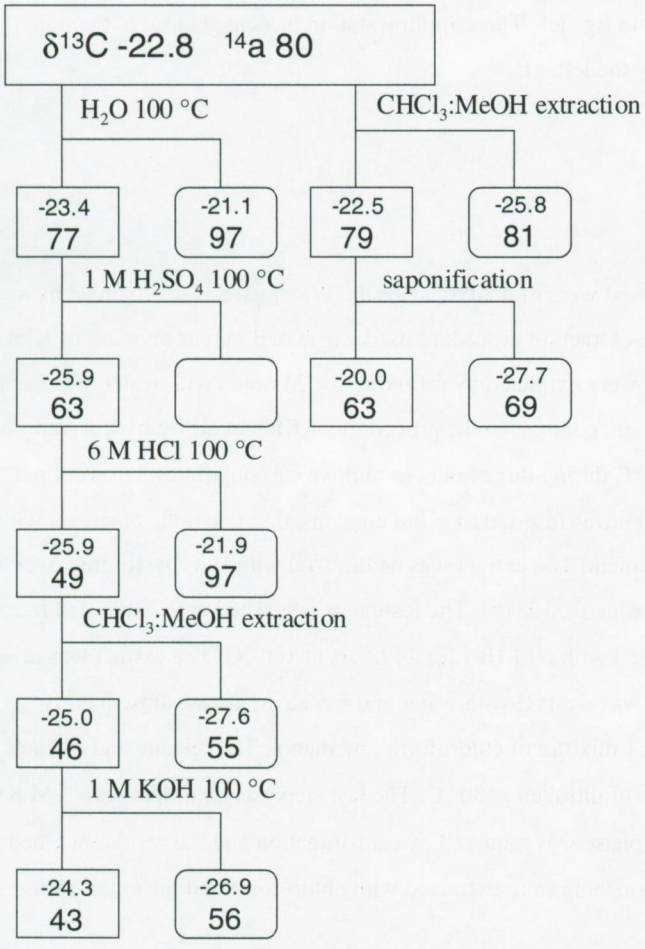

Fig. 1.2 Scheme of the extraction procedure. R=residue E=extract. See text for chemical identification.

METHODS

Samples

Samples of suspended matter from the Ems-Dollard Estuary, bordering the Netherlands and Germany and the North Sea were obtained from mr. F. Smedes of the National Institute for Coastal and Marine Management (RIKZ) in Haren, the Netherlands. The analyzed sample was taken on August 12, 1993 by continuous flow centrifugation during two hours. 1927 liters of water from 1.5 m depth were centrifuged. The resulting suspended matter sample was freeze dried. The sample area is shown in fig. 1.1. The sampling station is located close to the dutch town of Delfzijl and indicated by the letter E.

Extraction procedure

Solvents and chemicals used were of analytical grade. Water used was produced by a Millipore milli-Q apparatus. The extraction procedure used was based in part on work of Klok et al. (1981a, 1984). First, samples were extracted by refluxing for 24 hours with water. The residue and extract were freeze dried. Then, contrary to the procedure of Klok et al. we hydrolyzed the residue with 1 M H_2SO_4 at 100 °C during three hours to remove carbohydrates that were not extracted with hot water. This step was inserted to avoid condensation reactions of sugars with amino acids during the HCl treatment. The extract was neutralized with $BaCO_3$, filtered over a glass filter and freeze dried (Klok et al., 1981b). The residue was washed with water and freeze dried. This residue was hydrolyzed with 6 M HCl for 24 hours at 100 °C. The extract was dried in vacuo over KOH, the residue was washed with water and freeze dried and subsequently extracted ultrasonically with a 2:1 mixture of chloroform : methanol. The residue and extract were dried under a gentle stream of nitrogen at 30 °C. The last step was treatment with 1 M KOH at 100 °C for 1 hour. The liquid phase was removed by centrifugation and the residue washed repeatedly with water. The supernatants were extracted with chloroform and the extract was dried under nitrogen.

Alternatively, the sample was first 3 times ultrasonically extracted with chloroform : methanol (2:1). The extract and residue were dried under a stream of nitrogen. Then the residue was either treated with 1 M KOH at 100 °C for 1 hour or extracted with boiling water for 24 hours (*vide supra*).

From all the intermediate residues an aliquot was taken for analysis.

Analysis

Samples containing carbonates were treated with dilute hydrochloric acid and dried in vacuo over KOH. Samples were combusted for 6 hours at 600 °C in evacuated sealed quartz tubes with copper oxide as oxidator. The resulting gas was purified with a dry ice : ethanol cold trap to remove water and a silver oven at 400 °C to remove halogens and sulfur compounds. The purified carbon dioxide was analyzed for $\delta^{13}C$ with a VG SIRA 9 isotope ratio mass spectrometer. $\delta^{13}C$ values are reported relative to the V-PDB standard. The CO_2 was reduced to graphite with iron as a catalyst at 600 °C as described by Aerts-Bijma et al. (1997) and analyzed for ^{14}C concentration with the Groningen ^{14}C-AMS (Gottdang, et al., 1996). Results were reported as ^{14}a in percent of modern carbon (pMC).

Molecular analysis was performed using on-line pyrolysis gas chromatography - mass spectrometry as described by Van Heemst et al. (2000). The sample is pressed on a wire with a curie point of 610 °C and placed in a pyrolysis unit mounted on a gas chromatograph. The wire was heated under nitrogen by induction with a high frequency field. Pyrolysis products are led into the GC column. The GC is connected to a mass spectrometer that measures spectra of the separated compounds.

RESULTS AND DISCUSSION

The POM sample from the Ems-Dollard was fractionated according to the described procedure that is shown in fig. 1.2. The thus obtained fractions were analyzed molecularly and isotopically. Py-GC-MS analysis of fractions E1, E2 and R5 showed that the hot water extract (E1) consisted mainly of (poly)saccharides and some protein (cf. Klok et al, 1981a, 1984). The extract after hydrochloric acid treatment (E3) was derived from hydrolyzed protein. The pyrolysate of the final residue contained mainly homologous series of n-alkanes and n-alkenes (fig. 1.3). This material might be derived from algaenans or cutans, resistant aliphatic biopolymers from respectively plant cuticles or algal cell walls (De Leeuw et al., 1991). Sulfuric acid hydrolysis (E2) is a standard method to hydrolyze polysaccharides. E4 is the "free" lipid fraction and E5 probably contains saponified lipids and alkali soluble material.

A carbon balance was made based on the weights of the fractions and their organic carbon concentrations. About 50 % of the total organic carbon was extracted in the first three fractions as (poly)saccharides and protein. The final residue accounted for approximately 17 % of the total organic matter.

Isotopic analyses showed a considerable variation in the $\delta^{13}C$ values and ^{14}C activities (fig. 2) The polysaccharides and proteins have considerably higher ^{14}C activities than the bulk sample. These compounds can be degraded rapidly by microorganisms in the water column (Deines, 1980; Laane et al., 1987), so only relatively fresh material is present. The sulfuric acid extract could not be analyzed, probably because of the presence of to much sulfur oxide in the gas mixture.The residual organic material has a much lower ^{14}C activity. This indicates a highly resistant nature of this material (cf. De Leeuw et al., 1991).

The lipids extracted in this procedure have a much lower ^{14}a value (56 pMC) than lipids extracted directly from the untreated sample (^{14}a: 81 pMC). Also these fractions differ in $\delta^{13}C$ (–27.6 vs. –25.8 ‰). Maybe the youngest lipids were extracted in the previous treatments. When the hot water extraction and the HCl hydrolysis are performed after extraction of lipids, their $\delta^{13}C$ values are slightly higher (0.6 and 0.9 ‰ for the hot water and HCl extract respectively). This could indicate that they contain some lipids, since lipids have lower $\delta^{13}C$ values than carbohydrates and amino acids. However, lipids could not be extracted from the other extracts.

Mass balances calculated with ^{14}a, $\delta^{13}C$ and organic carbon yields are in good agreement with each other, which indicates that the extraction procedure gives valid results.

CONCLUSIONS

Chemical fractions of POM from the Ems-Dollard estuary show considerable differences in their ^{14}a and $\delta^{13}C$ values. Carbohydrates and amino acids have higher ^{14}a values and are lighter in ^{13}C than the bulk sample. The insoluble unhydrolyzable residue has a much lower ^{14}a value than the bulk. It has a highly aliphatic macromolecular nature. It may be derived from aliphatic biopolymers from the cell walls of algae or from terrestrial plant cuticles. $\delta^{13}C$ values of the fractions indicate a mainly marine algal origin.

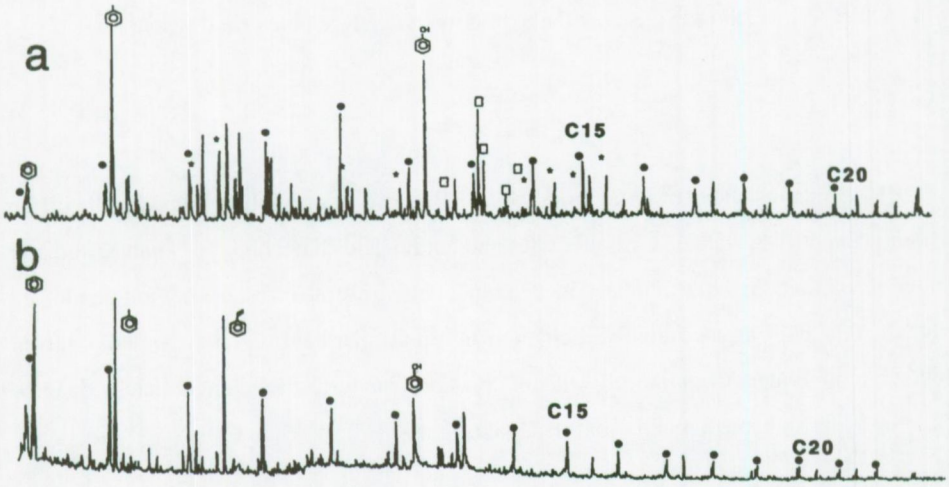

Fig. 1.3 a) TIC-trace of the pyrolysate of a bulk POM sample; b) TIC-trace of the insoluble non-hydrolyzable fraction. n-alkanes and n-alkenes are indicated by black circles, phenols by open squares and other aromatics by asterisks.

REFERENCES

Aerts-Bijma, A.Th., Meijer, H.A.J., Van der Plicht, J. (1997) AMS sample handling in Groningen. *Nucl. Instr. and Meth. in Phys. Res. B,* 123, 221-225.

Deines, P. (1980) The Isotopic Composition of Reduced Organic Carbon. In: Fritz, P. Fontes, J.C. (eds.) *Handbook of Environmental Isotope Geochemistry.* Amsterdam. pp. 329-406.

Fontugne, M.R., & Duplessy, J.C. (1981) Organic Carbon Isotope Fractionation by Marine Plankton in the Temperature Range -1 to 31 °C. *Oceanol. Acta* 4, 85-90.

Gottdang, A., Mous, D.J.W., Van der Plicht, J. (1995) The HVEE ^{14}C system at Groningen.*Radiocarbon* 37, 649-656.

Van Heemst, J. D. H. , Megens, L., De Leeuw, J. W., Hatcher, P. G. (2000) Characterisation of Estuarine Macromolecular Dissolved Organic Matter. *Org. Geochem.* **31,** 847-858.

Klok, J., Van der Knaap, J.M.M., De Leeuw, J.W., Cox, H.C., Schenck, P.A. (1981a) Qualitative and Quantitative Characterization of the Total Organic Matter in a Recent Marine Sediment. In: *Advances in Organic geochemistry 1981.* (Eds. Bjoroy, M. et al.) pp. 813-818.

Klok, J., Nieberg- van Velzen, E.H., De Leeuw, J.W., Schenk, P.A. (1981b) Cappillary Gas Chromatographic Separation of Monosaccharides as their alditol acetates. *J. Chromatogr.* 207, 273-275.

Klok, J., Baas, M., Cox, H.C., De Leeuw, J.W., Rijpstra, W.I.C., Schenck, P.A. (1984) Qualitative and Quantitative Characterization of the Total Organic Matter in a Recent Marine Sediment. *Org. Geochem.* 6, 265-278.

Laane, R., Turkstra, E. & Mook, W. (1990) Stable carbon isotope composition of pelagic and benthic organic matter in the North Sea and adjacent estuaries. In: *Facets of Modern Biogeochemistry* (Ittekot V., Kempe, S. Michaelis, W., and Spitzy, A., eds.), Berlin-Heidelberg, pp. 214-224.

Le Clercq,M., Van der Plicht, J., Meijer, H.A.J. and De Baar, H.J.W. (1997) Radiocarbon in marine dissolved organic carbon (DOC). *Nucl. Instr. and Meth. in Phys. Res. B,* 123, 443-446.

De Leeuw, J. W., Van Bergen, P. F., Van Aarssen, B. G. K., Gatellier, J. P. L. A., Sinninghe Damste, J. S., Collinson, M. E. (1991) Resistant biomacromolecules as major contributors to kerogen. *Philosophical Transactions, Royal Society of London, B.* 333, 329-337.

Mook, W.G. and Tan, F.C. (1991) Stable Carbon Isotopes in Rivers and Estuaries. In: *Biogeochemistry of Major World Rivers* (E.T. Degens, S. Kempe, J.E. Richie eds.) SCOPE vol. New York, 1991. pp. 245-264.

Sackett, W.M., Eckelman, W.R., Bender, M.L., Be, A.W.H. (1965) Temperature dependence of carbon isotope composition in marine plankton and sediments. *Science* 148, 235-237.

Schouten, S., Klein Breteler, W., Blokker, P., Schogt, N., Rijpstra, W.I.C., Grice, K., Baas, M., Sinnighe Damsté, J.S. (1998) Biosynthetic Effects on the Stabile Isotope composition of algal lipids: implications for deciphering the carbon-isotopic Biomarker Record. *Geochim. Cosmochim. Acta* 62, 1397-1406.

Wang, X.C., Druffel, E.R.M., Lee, C. (1996) Radiocarbon in Organic Compound Classes in Particulate Organic Matter and Sediment in the Deep Northeast Pacific Ocean. *Geophys. Res. Lett.* 23, 3583-3586.

II

Use of molecular, stable carbon and radiocarbon isotope composition for determination of origins of sedimentary organic matter in an estuary

ABSTRACT

Our study of the stable and radioactive carbon isotopic composition of particle size fractions of a surface sediment from the Ems-Dollard estuary shows, that carbon isotope ratios vary considerably with particle size. The analysis of the both $^{14}C/^{12}C$ and $^{13}C/^{12}C$ isotope ratios in various fractions shows a clear difference between fractions with particle sizes smaller and larger than 20 µm. The organic material in the fine fractions (<20 µm) has considerably higher ^{14}C values (^{14}a ~ 80 %) than that in the coarse fractions (52 %) and has higher $\delta^{13}C$ values (average of –23 ‰ and –25.6 ‰ respectively). This shows that OM in the fine and the coarse fractions has different sources. The organic carbon in the fractions with particle sizes < 20 µm is mainly imported from the North Sea. The contribution of material from the Ems river appears negligible. The carbon isotopic composition of the coarse fractions points to a terrestrial contribution. Discrete organic fragments are found of both terrestrial and marine/estuarine origin.

INTRODUCTION

An important part of the global organic carbon cycle is related to the flux of terrigenous organic carbon from the continents to the oceans (e.g. Hedges and Keil, 1995). Estuaries form an important interface between marine and fluviatile watermasses where fluviatile water is flowing

into the sea and complex processes affect the transported substances (e.g. Burton and Liss, 1976).

A major organic carbon pool in estuaries is Particulate Organic Matter (POM), present suspended in the water column and in sediments. POM in estuaries has several sources: marine, autochthonous and fluviatile primary production and terrestrial detritus discharged by rivers. It can cycle between the sediment and the watercolumn by settling and resuspension for considerable time (Eisma, 1993). To estimate the relative contributions of these sources, measurements of the stable carbon isotopic concentrations (expressed as $\delta^{13}C$ relative to a standard) in POM are frequently used based on the assumption that marine organic matter in general has a higher ^{13}C concentration than organic matter derived from terrestrial plants using the C3 carbon fixation pathway (e.g. Keil et al., 1997, Goñi et al., 1997, 1998; Laane et al., 1990; Fry and Sherr, 1984; Salomons & Mook, 1981).

Particulate matter is, however, not homogeneous in terms of particle size or chemical characteristics (Keil et al., 1994, Mayer, 1994, Bergamaschi et al., 1997). Furthermore, the organic component consists of a wide range of compounds with potentially different sources, characteristics and stability. Certain biochemicals such as polysaccharides and proteins are more labile than other compounds (Laane, 1982; Laane et al., 1987; Benner et al., 1987). Moreover, different classes of compounds from the same source have different $\delta^{13}C$ values. For example, polysaccharides and proteins have significantly higher $\delta^{13}C$ values than lipids (Deines, 1980; Hayes, 1993; Schouten et al., 1998). Also lignin, a polyphenolic macromolecule in vascular plants, has a lower $\delta^{13}C$ value than polysaccharides from the same plant (Benner et al., 1987).Thus, selective degradation of the more labile components (e.g. (poly)saccharides and

proteins) over time can change the ^{13}C concentration of POM drastically (Laane et al., 1990; Benner et al., 1987).

The normalized ^{14}C abundance (^{14}a; Mook and van der Plicht, 1999), however, is only dependent on the age of the material, because by definition a correction is made for isotopic fractionation, based on the generally known relation that fractionation of ^{14}C is twice that of ^{13}C (e.g. Mook and Streurman, 1983). In this way, using both ^{13}C and ^{14}C, a distinction can be made between fresh and old organic matter present in estuarine POM. Mook and Tan (1991) suggested that ^{14}C could be a useful tracer of the origin of POM in coastal waters because they assume that in general POM dischared by rivers originates from eroded peat or soil and therefore is relatively old. However, radiocarbon data show that POM in the Amazon river represents rather fresh material (Hedges et al., 1986). Trumbore (1993) showed that organic matter in soils in the drainage basin of the Amazon river has a very short residence time in the soil, but in soils of temperate regions the residence time is much longer.

Coarse and fine particles are usually not transported in the same way (Eisma, 1993). Coarse particles are usually transported via bottom currents (saltation) while finer particles can be reintroduced into the upper water column and transported as suspended matter. This difference in transport behaviour can be the cause of a difference in origin of the fine and coarse particles. Also the minerals differ, causing different absorption characteristics of organic compounds. For example amino acids have a high affinity for clay minerals (e.g. Wang and Lee, 1993). These differences between coarse and fine particles might be reflected in the composition and in the stable and radioactive carbon isotopic characteristics of the organic matter in different size fractions of POM (Bergamaschi et al., 1997; Keil et al., 1994).

Therefore, we have investigated the relations between particle size, molecular characteristics and stable and radioactive carbon isotope ratios in surface sediment from the Ems-Dollard Estuary, bordering the Netherlands and Germany.

MATERIALS AND METHODS

Sampling area

The Ems-Dollard estuary (Fig. 1) has a fresh water inflow from the river Ems (40 - 350 m^3/s) and a smaller inflow from the Westerwoldse Aa (ca. 30 m^3/s; Helder and Ruardij, 1982). The total length of the estuary is ca. 100 km with an area of 600 km^2. In the inner part (the Dollard) ca. 80 % of the area consists of tidal flats, in the outer part 40 to 50 %. The tide in these waters is semi-diurnal with a diurnal inequality and has an amplitude from 2.5 m in the outer estuary and 3 m in the Dollard. It is fully mixed over much of the estuary and partially mixed only at low salinities in the Ems and the Southern part of the Dollard. There is a net input of particulate matter from the North Sea as a result of an accumulation mechanism. Suspended matter transported inward by the flood current starts to sink when the current decreases. Since it takes time to settle the particles are still transported inward during sinking and reach a site where they can not be resuspended again by the ebb current (Postma, 1954, 1961).

The contribution of the North Sea to the particulate matter is estimated at $9 * 10^5$ tons per year, compared with a river input of ca. $1.4 * 10^5$ tons per year (Laane and Ruardij, 1988). The studied surface sediment was collected in the "Bocht van Watum", one of the two main tidal channels in the middle part of the estuary, close to the town of Delfzijl at a water depth of 4.5 m at low tide (fig. 2.1). ^{210}Pb profiles of sediments from the area do not allow calculation of sedimentation rates, indicating strong mixing of the sediment (unpublished results RIKZ). The average salinity at this location is 16 ‰.

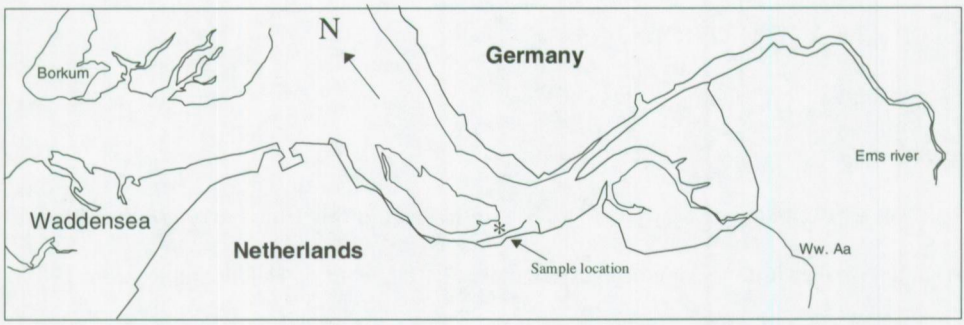

Fig. 2.1 Map of the Ems-Dollard estuary. The sampling station is indicated in the map by an asterisk. Thin lines are boundaries of tidal flats.

Samples

The surface sediment sample from the Ems-Dollard Estuary (about 40 kg) was collected in February 1994 by the National Institute for Coastal and Marine Management (RIKZ) with a grab sampler, sampling the upper 10-15 centimeters of the sediment. This sample was originally collected and size fractionated by F. Smedes from RIKZ to study different grain size correction procedures for the determination of organic micropollutants and heavy metals using various size fractionation methods (cf. Klamer et al., 1990). Later, this fractionated sample was used in this study of carbon isotopic and molecular composition. The sample was stored in a 50 l PE container at 4 °C until further processing. An aliquot of 6 kg of this sample, taken from the top of the container, was passed through 2 mm nylon netting to remove large fragments. This resulting sample (from now on named 'total sample'; fraction 1, see Fig. 2.2) was size fractionated by wet sieving and settling according to the scheme shown in Fig. 2.2, based on the work of Klamer et al. (1990). Sieves consisted of polycarbonate with nylon netting. The water used for sieving and decantation was pre-filtered sea water mixed with deionised water to a salinity of 16 ‰. The water was first equilibrated with a small portion of sediment in an ultrasonic bath for 20 minutes, and thereafter stored overnight at 4 °C to allow the particles to settle. The decantate was used for sieving. An aliquot of sample was put on a sieve placed over a collector dish on a vibrating table and precooled (4-10 °C) water was pumped onto the sieve. The water with the particles that passed through the sieve was led from the collector dish to a thermostated (10 °C) centrifuge with a flowthrough rotor, rotating at 22000 g. The outflowing water was used for sieving again.

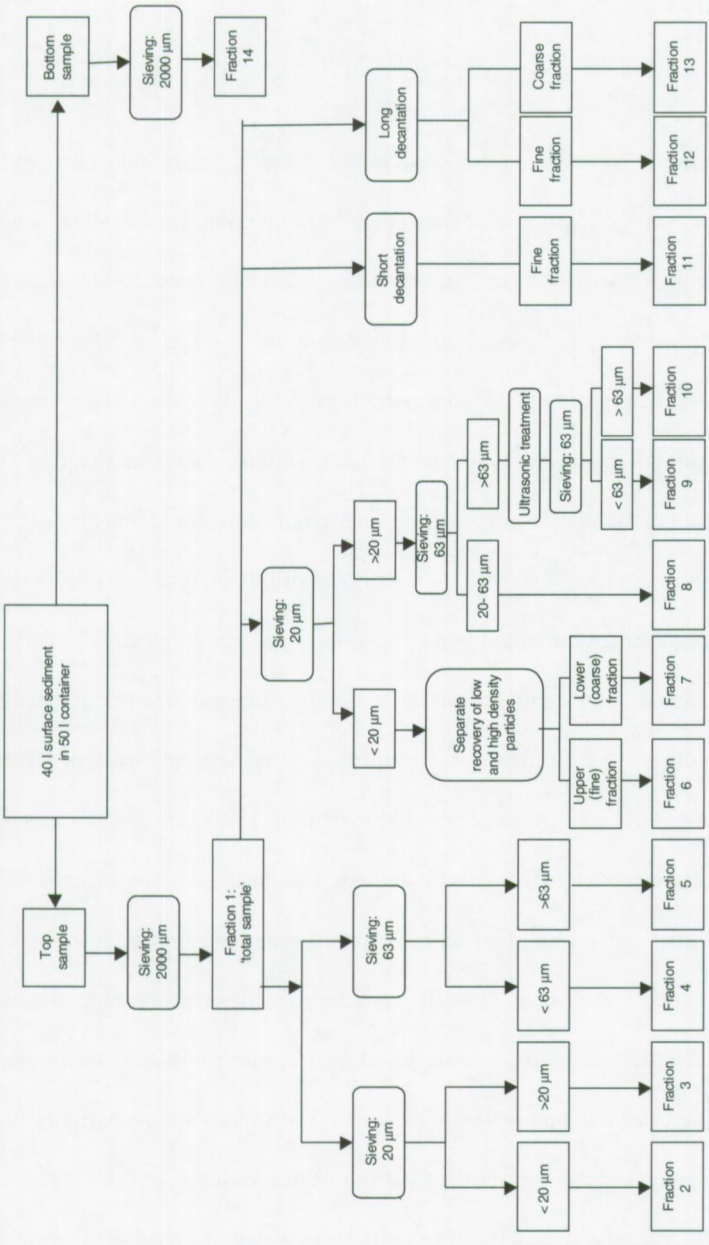

Fig. 2.2 Schematic representation of the sieving and settling procedure. Top sample refers to a subsample taken from the top of the sample storage container, bottom sample to a subsample taken from the bottom of the container.

Four subsamples were obtained by sieving over 20 µm and 63 µm netting respectively (fractions 2, 3 and 4, 5 respectively; see Fig. 2.2). Another aliquot of the total sample was sieved over 20 µm and the upper and lower layer of the particles accumulated in the rotor were collected separately, yielding a fine and a coarse fraction, both smaller than 20 µm (fractions 6 and 7). Rapidly settling particles were deposited close to the inlet of the rotor (the lower layer; fraction 7), while slower settling particles settled more close to the outlet of the rotor (the upper layer; fraction 6). The residue (> 20 µm) was sieved over 63 µm yielding the 20-63 µm fraction (8). The fraction larger than 63 µm was then sonicated for one hour at 200 W while cooled with ice. This was sieved again over 63 µm giving fractions 9 (< 63 µm) and 10 (> 63 µm).

In a seperate fractionation experiment, an aliquot of the total sample (1 kg) was suspended in a decantation beaker in 3 l water by gentle stirring. Water was introduced from the bottom of the beaker and the overflow led to a continuous flow centrifuge, collecting the lighter particles. Fraction 1 was the fine fraction collected by short decantation with slow stirring. Then, decantation proceeded with faster stirring and continued until approximately half the sample was decanted, yielding fractions 2 and 3.

Since larger particles tend to sink during storage, a subsample was taken from the bottom of the initial container, in order to check possible inhomogeneities in the initial sediment sample (no. 14).

All fractions were freeze dried in containers covered by a lid with a small hole to avoid contamination by diffusion. The freeze dried fractions were homogenized in a Retsch ball mill, with three 10 mm balls and one 30 mm ball at 50 % speed. Tests showed that these conditions did not affect particle sizes.

Particle size determination

Particle size fractions were determined according to the dutch norm NEN 5753 (NNI, Delft, The Netherlands). Briefly, organic matter is digested with boiling hydrogen peroxide, and carbonates are removed by addition of dilute hydrochloric acid. In the remaining mineral sample the fraction >63 µm is determined by wet sieving. In the sample passing the sieve the <2 µm and <16 µm fractions are determined by the so-called "pipet method". In a glass cylinder the sample is homogeneously suspended in water containing sodium pyrophosphate to prevent aggregation. At defined settling time and depth, calculated according to Stokes law, samples are taken from the waterphase by means of a volumetric pipet. The particle weight determined by evaporation and corrected for the amount of sodium pyrophosphate is a measure for the specific fractions <2 µm and <16 µm.

Microscopy

Some of the samples were studied using optical light microscopy. Aliquots in glycerol/water were used to prepare standard palynological slides. The material was examined using magnifications at 100x, 400x and 1000x to determine the identity of the various recognizable particles (Fragments < 10 µm can still be identified).

Organic Carbon determination

Organic Carbon concentrations were determined with a Carlo Erba elemental analyzer. Prior to analysis inorganic carbonates were removed by exposing the samples to hydrochloric acid vapour over night.

Carbon Isotope measurements

For carbon isotope analysis of the organic matter, aliquots of the fractions that contained ca. 1 mg organic carbon were acidified with dilute hydrochloric acid to remove inorganic carbonates, dried *in vacuo* over potassium hydroxide and combusted in an oven at 900 °C in a flow of oxygen. The combustion gases were led over silver in an oven at 400 °C to remove halogens and sulfur dioxide, an oven with copper oxide (800 °C) to oxidize carbon monoxide and through a cold trap (dry ice/ethanol) to remove water. The CO_2 was collected in a cold trap in liquid air. After the oxygen was pumped away the gas was led over an oven with copper (600 °C) to reduce nitrogen oxides. The CO_2 yields were determined by expanding the gas in a known volume and measuring the pressure. The ^{13}C concentrations of the purified carbon dioxide were measured with a VG SIRA isotope ratio mass spectrometer and expressed as $\delta^{13}C$ relative to the V-PDB standard. Subsequently, the carbon dioxide was reduced to graphite with an iron catalyst at 600 °C and a twofold excess of hydrogen (Aerts-Bijma et al., 1997). The ^{14}C activity (^{14}a) in the graphite was measured with the Groningen ^{14}C-dedicated Accelerator Mass Spectrometer. (AMS; Gottdang et al., 1997). The ^{14}a values are defined as the $^{14}C/^{12}C$ ratio (corrected for fractionation using the ^{13}C fractionation) in the sample relative to the same ratio in the oxalic acid standard and are reported in % (percent of modern carbon) (Mook and van der Plicht 1999).

Curie-point pyrolysis-gas chromatography (CuPy-GC)

Curie-point Pyrolysis-Gas Chromatography (CuPy-GC) analyses were performed using a Hewlett-Packard 5890 gas chromatograph, equipped with a cryogenic unit and a 25 m fused

silica capillary column coated with chemically bound CP Sil-5 (0.32 mm internal diameter, 0.45 μm film thickness). Helium was used as a carrier gas. A flame ionisation detector (FID) at 320 °C was used for detection. The temperature programme was as follows: initial temperature 0°C (5 min); heating rate 3°C/min; final temperature 300°C (10 min). In GC-MS analyses, the Hewlett-Packard 5890 gas chromatograph was connected to a VG Autospec Ultima mass spectrometer operated at 70 eV with a mass range of *m/z* 50-800 and a cycle time of 1 s.

A small amount of sample was pressed onto a flattened ferromagnetic wire with a Curie temperature of 610°C, and placed into a pyrolysis unit (FOM-4LX; Boon *et al.,* 1987). The pyrolysis unit was connected to a Fisher 9425 high frequency generator that heated the wires inductively in 0.15 s to the Curie temperature. This temperature was maintained for 10 s. From the coarse fractions carbonate was removed with dilute hydrochloric acid and ca. 500 mg was homogenized with an IKA universal mill and a pestle and mortar prior to Py-GC.

HPLC

Samples were analysed for the presence of tocopherols by HPLC based on a method of Barua et al. (1993). Samples were extracted three times ultrasonically with ethylacetate. The extracts were concentrated and analyzed on an Alltech econosphere C18 column by isocratic elution with a mixture of acetonitrile: dichloromethane:methanol:1-octanol (90:15:10:0.1).

RESULTS

To study the effect of grain size distribution on the stable and radioactive carbon isotopic and molecular composition and the applicability of this unique combination of analytical methods, fractions obtained by various size fractionation procedures from a surface sediment sample from a tidal channel in the Ems-Dollard estuary were analyzed. Results of the analyses are summarized in Table 2.1. The sample taken from the bottom of the initial container (no. 14) is somewhat enriched in coarse particles compared to the sample from the top of the container (the 'total sample', no. 1). The organic carbon content is lower, but there are no significant differences in the carbon isotopic composition. The total sample contains ca. 20 % material < 20 µm on a mass base. About 70 % of the total sample was separated as the > 63 µm fraction (no. 3). Particle size analysis of the fractions showed that there is a considerable amount of mineral particles larger than 63 µm in the fractions obtained by sieving over the 63 µm sieve. This might be the cause of the differences in the mass balance of the sieving over 20 µm (fractions 2 and 3) and 63 µm (fractions 4 and 5) on the one hand, and of the other series of sievings that yield the two fractions < 20µm (no. 6, 7), the fraction 20-63 µm (no. 8) and the two fractions obtained from the > 63 µm material (no. 9 and 10) on the other hand. In this second sieving procedure the > 63 µm fraction (no. 9 and 10) constituted only 58 % of the total by mass.

Organic carbon concentrations (%OC) are much higher in the fine fractions than in the coarse fractions. Most of the organic carbon is associated with fine grained material. However, the organic carbon mass balance calculated from the weight portions (Fm) and organic carbon concentrations of the fractions ($\%OC_f * Fm / \%OC_t$) yields lower estimates of the organic carbon

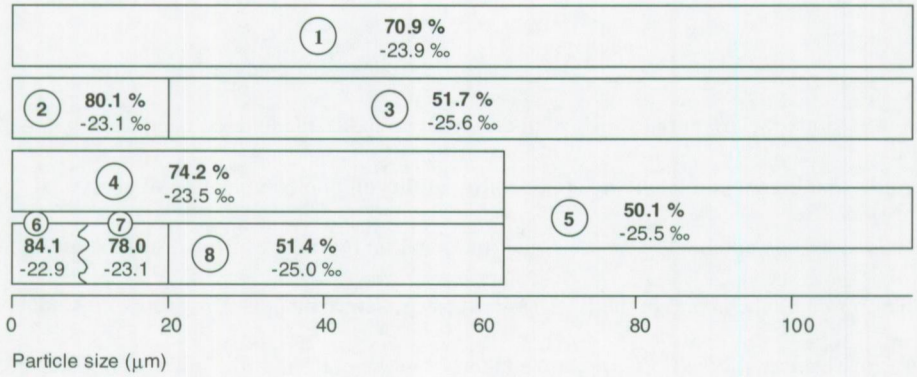

Fig. 2.3 Carbon isotopic compositions of OM in fractions obtained by sieving of the surface sediment. Numbers in circles are fraction numbers as in figure 2.2, the upper number in the rectangles is the normalized ^{14}C abundance (^{14}a in % relative to NBS oxalic acid), the lower number is the $\delta^{13}C$ (in ‰ relative to V-PDB).

concentration in the total sample than measured in fraction 1 that represents the total fraction (%OC_t; table 1). The organic carbon in the fractions obtained by sieving over 20 µm (no. 2 and 3) and over 63 µm (no. 4 and 5) is ca. 65 % of the organic carbon in the total sample (no. 1). In the second sieving experiment, 80 % of the organic carbon in the total sample (no. 1) is present in the obtained fractions (no. 6 to 10). The organic carbon in the fractions obtained by decantation (no. 11,12,13) match more closely that in the total sample (ca. 92 %).

We observe a remarkable difference in the ^{14}C activities of the fractions <20 µm and >20 µm (fig. 2.3). The coarser fractions have a ^{14}a value of around 50 % and the finer around 80 %. The $\delta^{13}C$ values of the fractions < 20 µm are ca. –23.0 ‰, the $\delta^{13}C$ of the coarse fractions ranges from –24.9 to –26.2 ‰. There is a clear correlation (r^2=0.80) between the ^{14}a and the $\delta^{13}C$ values of the fractions (fig. 2.4a). The correlation between the carbon isotopic composition and the organic carbon concentration is less significant (fig 4b).

Organic carbon mass balances can be calculated from $\delta^{13}C$ and ^{14}a values, since these parameters in a bulk sample are the weighted average of the same parameters in its components. For example, in a mixture consisting of component A and B, the fraction A (x_A) in the mixture M can be calculated from the $\delta^{13}C$ values of the fractions and the mixture by solving the equation:

$$x_A \cdot \delta^{13}C_A + (1 - x_A) \cdot \delta^{13}C_B = \delta^{13}C_M$$

Comparison of organic carbon mass balances calculated from %OC and weight fraction, $\delta^{13}C$ and ^{14}a thus gives an indication of the quality of the analyses and separation procedure. The carbon mass balance calculations based on $\delta^{13}C$ and ^{14}a (with an absolute error of ca. 10 %) are

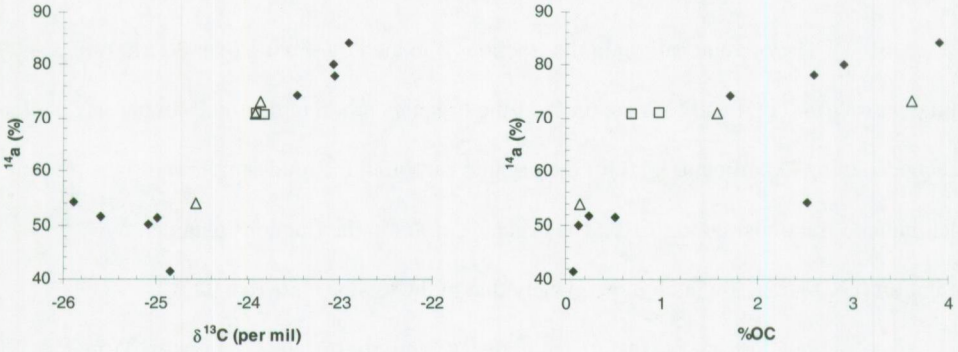

Fig. 2.4 Relation between d13C and 14a of OM in the size fractions of the surface sediment (left) and 14a vs. organic carbon concentration (%OC; right). Open squares indicate the total sample and the sample from the bottom of the initial sample container, closed diamonds the fractions obtained by sieving and open triangles the fractions obtained by decanting.

in reasonable agreement with the balance calculated from the weight portions and the organic carbon concentrations (Table 1). The calculations based on $\delta^{13}C$ and ^{14}a show that 70 % of the total organic carbon is associated with particles smaller than 20 µm and 90 % with particles smaller than 63 µm. 80 % of the organic carbon in the fraction larger than 63 µm is associated with particles smaller than 63 µm released after ultrasonic treatment (fraction 10).

Microscopy

Microscopic inspection of the coarse fraction >63 µm (fraction 5) shows that this fraction consists mainly of large mineral particles and large pieces of organic material which are identified as fragments of soft tissue of higher plants (parenchyma), copepod eggs, diatom fragments, *Pediastrum* (a fresh water alga) and cuticle fragments. The small fractions (<20 µm; fractions 2, 6 and 7) are quite homogeneous, consisting mainly of mineral particles with brown entities attached (most probably organic matter). Recognizable discrete organic fragments include spores, pollen grains and, possibly, small algae. Recognizable organic fragments in the fraction 20-63 µm (fraction 10) are bisaccates (pollen of gymnosperms), other pollen grains and spores, soft tissue of plants and *Pediastrum*. Fractions 12 and 13, which were ultrasonically treated, contain no visibly recognizable organic material.

Chemical Analysis

The chromatograms of the pyrolysates of a number of fractions are shown in Fig. 2.5. The pyrolysates of the finest fractions (<20 µm) contain a range of polar compounds - mainly furans,

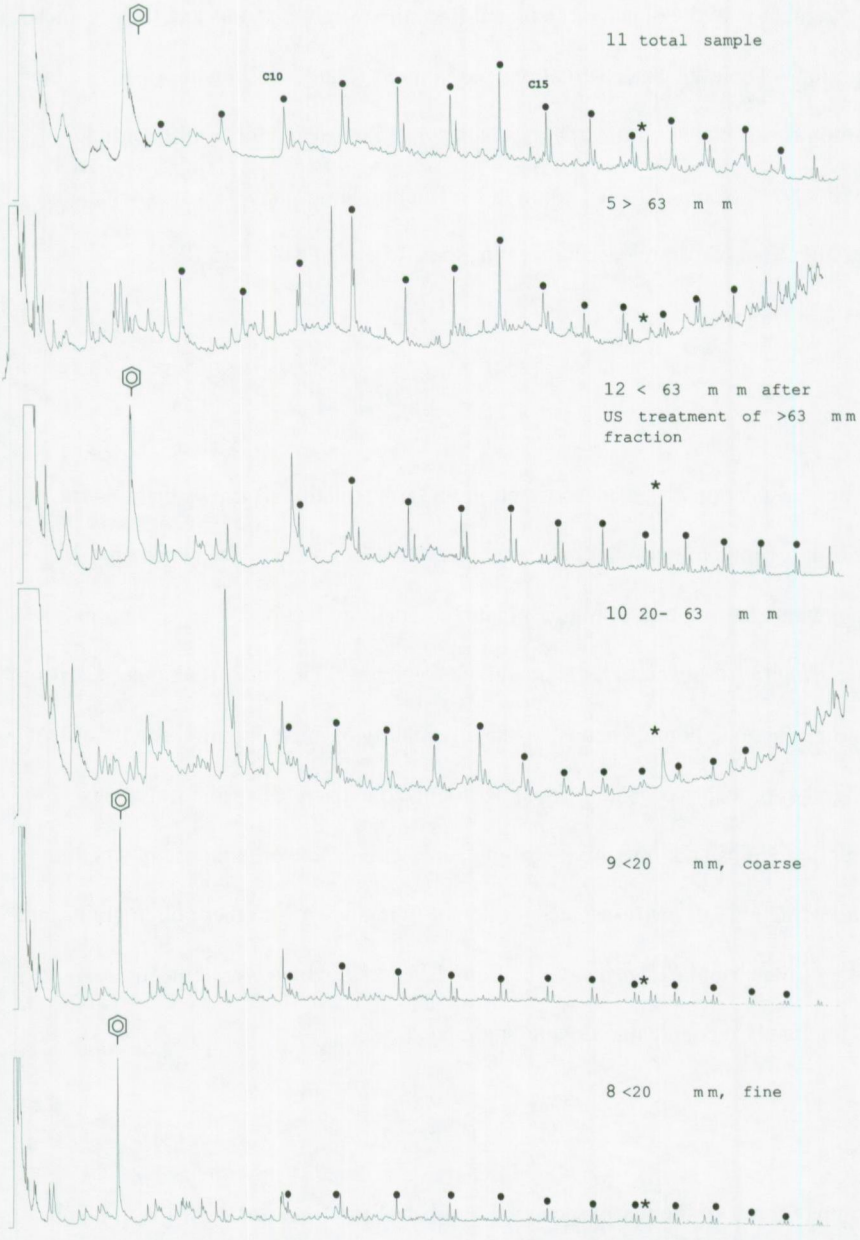

Fig. 2.5 Chromatograms (FID) of pyrolysates of several size fractions. Fraction numbers as in fig. 2.2. Dots indicate n-alkene/n-alkane pairs, asterisks prist-1-ene.

pyrroles, alkyl benzenes and phenols, typical pyrolysis products of polysaccharides and proteins - and a series of *n*-alkanes and *n*-alkenes. The coarser fractions appear to yield higher amounts of n-alkanes and n-alkenes, relative to the toluene peak. Furans and pyrroles were not detected. Mass chromatography showed that methoxyphenols, typical pyrolysis products of lignin (Saiz-Jimenez and De Leeuw, 1986), were not present in the pyrolysates of the fine nor the coarse fractions. Of the aliphatic compounds prist-1-ene is much higher compared with the *n*-alkanes and *n*-alkenes in fractions 10 and 12 than in the other fractions.

DISCUSSION AND CONCLUSIONS

The carbon isotope characteristics of the size fractions within this single sediment sample show considerable differences. The fractions with particle size larger than 20 µm, accounting for 30 % of the total organic carbon, have $\delta^{13}C$ values ranging from –25 to –27 ‰ and ^{14}a values around 52 %. The fractions finer than 20 µm have $\delta^{13}C$ values around –23 ‰ and have considerably higher ^{14}a values (78 to 84 %). These fine fractions are similar in their carbon isotopic composition when compared to suspended organic matter at this site that has average $\delta^{13}C$ values of –23.2 ± 0.4 ‰ and average ^{14}a values of 75 ± 3 % (Megens, 1999) indicating the close relation between the small particles in the sediment and in the watercolumn (e.g. Eisma, 1981). Although the seperation by sieving over 63 µm had some technical problems, this is of little influence to the observed difference between the fractions < 20 µm and >20 µm.

The finest fraction (fraction 6) has the highest ^{14}C activity (84.1 %), a high $\delta^{13}C$ value of –22.9 ‰ and the highest organic carbon content (3.90 %). The $\delta^{13}C$ value of organic carbon in the Ems river in surface sediments is about –27 ‰ (Salomons and Mook, 1981) and in suspended matter it ranges from –25 to –28 ‰ (Eisma et al., 1983; Eisma et al., 1991). Typical $\delta^{13}C$ values of organic carbon in sediments and suspended matter in Dutch coastal waters of the North Sea are around –23 ‰ (Laane et al., 1990). The ^{14}a values of suspended matter in the Dutch coastal waters range from 82 to 88 %. Therefore, the organic matter in this fraction appears to consist entirely of material imported from the North Sea.

The next fraction in size (the 'coarse' fraction < 20 µm, no. 7) has almost the same $\delta^{13}C$ (–23.1 ‰), but a significantly lower ^{14}a (78.0 %) indicating the presence of an 'older' component. Suspended POM from the Ems river has a similar ^{14}a value (77 %; Megens, 1999),

but a lower $\delta^{13}C$ value (–25.3 ‰, Megens, 1999; –25 to –28 ‰, Eisma et al., 1991). Therefore the OM in this fraction is not a simple mixture of OM from the Ems and the North Sea. Based on mineralogical investigations it was determined that no small particles from the Ems reach this point (Eisma, 1981; Favejee, 1960). Thus, the older component probably does not originate from fluviatile POM. Exchange with Dissolved Organic Matter (DOM) has been proposed as an explanation for the lower ^{14}a values in detrital aggregates at the deep ocean sea floor compared with sinking POM (Wang et al., 1996). Macromolecular DOM (> 1000 dalton) from the Ems-Dollard has ^{14}a values of 87 % (Van Heemst et al., subm.), and is therefore not the source of the old component. The ^{14}a of low molecular weight DOM in the Ems-Dollard is not known.

We conclude that the finest fraction (no. 6) consists of imported North Sea POM and that the next fraction in size (no. 7) is a mixture of North Sea POM and an older component. This is based on the reasonable assumption that the isotopic composition in North Sea POM is the same in all size fractions of suspended matter. Particle sizes in suspended matter in Dutch coastal waters range from 0.2 to 100 µm, and the distribution curve is bell-shaped with a peak around 10 µm (Eisma and Kalf, 1987). Carbon isotope distributions over different particle size fractions are not known for North Sea POM. Possibly, differences in isotopic composition in different size fractions in North Sea POM exist as well.

The lower $\delta^{13}C$ values of the coarser fractions indicate, using the simple two end member mixing model of marine and terrestrial organic matter, a higher contribution of terrestrial organic matter to the coarse fractions than to the fine fractions. However, the $\delta^{13}C$ values of phytoplankton in the estuary can cover a range from a marine value of –17 ‰ to values around –30 ‰, due to the gradient of $\delta^{13}C$ of dissolved inorganic carbonate (DIC) in the Ems-Dollard estuary, ranging from –11 ‰ in the river to +1 ‰ in the sea (Megens, 1999). Therefore, the

lower $\delta^{13}C$ values could be related to upstream produced phytoplankton derived OM as well. The considerably lower ^{14}a values (ca. 50 %, corresponding to a ^{14}C age of about 5500 yr BP) is an indication for a terrestrial source, because the Ems river flows through an area with large peat deposits that started to form ca. 7000 years ago (Dupont, 1986). The lower $\delta^{13}C$, however, can also indicate selective degradation of compounds with relatively high $\delta^{13}C$ values like carbohydrates and proteins. Chemical characterization by means of Py-GC shows that the organic matter in the coarser fractions is enriched in n-alkanes and n-alkenes series and that pyrroles and furans, pyrolysis products of (altered) carbohydrates and proteins (Pastorova et al., 1994; Van Heemst et al., 1997), are very low or absent. This indicates that the lower $\delta^{13}C$ values might be caused by selective degradation. The alkanes and alkenes are pyrolysis products of aliphatic macromolecules, which in nature are present in cell walls in some marine and lacustrine phytoplankton species (algaenans) and in cuticles of terrestrial plants (cutans; De Leeuw et al., 1991). These aliphatic macromolecules are highly refractory, therefore they can explain a high ^{14}C age. Typical pyrolysis products of lignin, which is often used as a marker of terrestrial OM (e.g. Hedges and Mann, 1979; Bergamaschi et al., 1997) are absent. This suggests that the lower $\delta^{13}C$ values need not be caused by a larger terrestrial component.

Microscopy, however, showed the presence of remains of higher plant in these fractions, but also fresh water algae and discrete organic particles associated with a marine or estuarine environment. We did not determine how much these discrete organic particles contribute to the total organic carbon in these fractions. In total sediment usually less than 10 % of the organic matter is present as discrete organic debris (Hedges and Keil, 1995), but in coarse fractions (> 38 μm) of sediment from the delta of the Amazon river more than 60 % was present as low density discrete organic particles (Keil et al., 1997).

In addition, we observed, that in the chromatograms of the pyrolysates of fractions 8 (20-63 μm) and 9 (the fine fraction obtained after ultrasonic treatment of > 63 μm material) the prist-1-ene peak is much higher than in the other fractions. Prist-1-ene has been shown to be a pyrolysis product of tocopherols (Goossens et al., 1984). However, tocopherols were not detected in ethylacetate extracts by means of HPLC. Pristene also has been thought to be derived from the phytyl side chain of chlorophyll (Bendoraitis et al., 1962). However, when pyrolysing chlorophyll and phaeophytin, a degradation product of chlorophyll, prist-1-ene is a minor component compared with a suite of other isoprenoid components (Larter et al., 1979). These are not present in our samples. Thus it is unlikely that the pristene is derived from intact chlorophyll or phaeophytin in the samples. Probably pristene in the pyrolysate is one of the pyrolysis products of the macromolecular component.

Our study of the stable and radioactive carbon isotopic composition and molecular characterization of one size fractionated surface sediment from the Ems-Dollard estuary shows that large differences exist in the stable and radioactive carbon isotopic composition of OM in fractions of this sediment with particle sizes <20 μm and >20 μm. The average ^{14}a (80 %) and $\delta^{13}C$ (–23 ‰) values of the fine fractions are much higher than those of the coarse fractions (52 % and –25.6 ‰). The main and possibly only source of OM in the fine fractions of this sediment sample is POM from the North Sea. The OM in the coarse fractions appears to be mixture of terrestrial and estuarine or marine material. The low ^{14}a and $\delta^{13}C$ values in combination with the presence of fragments of soft tissue of higher plants indicate a contribution of eroded peat. The lower $\delta^{13}C$ values can be explained partly by the selective degradation of carbohydrates and proteins, as is revealed by molecular characterization.

Nr.	Description	Fm	>63 µm	<63 µm	<16 µm	<2 µm	$CaCO_3$ mass%	%OC	$\delta^{13}C$	^{14}a (%)	OCt/OCf	CB13	CB14
1	< 2 µm upper part of sample	1.00	45.9	19.4	15.6	10.8	21.8	0.98	-23.91±0.07	70.9±1			
14	< 2 µm lower part of sample	1.00	52.0	14.3	11.2	7.6	24.3	0.7	-23.81±0.07	70.7±1			
2	< 20 µm	0.18	0.5	59.2	57.4	38.5	17.9	2.91	-23.08±0.07	80.1±1	49.0	69	68
3	> 20 µm	0.82	62.4	1.7	1.7	1.2	22.8	0.24	-25.60±0.07	51.7±1	15.7	31	32
4	< 63 µm	0.29	18.0	51.7	32.8	21.9	15.1	1.72	-23.47±0.07	74.2±1	54.0	74	86
5	> 63 µm	0.71	68.3	1.3	1.3	0.9	23.4	0.14	-25.08±0.07	50.1±1	10.6	26	14
6	< 20 µm upper frac	0.04	0.3	65.3	65.3	59.0	8.7	3.9	-22.91±0.07	84.1±1	15.7	*	23
7	< 20 µm lower frac	0.18	0.3	58.7	54.9	36.5	20.3	2.6	-23.06±0.07	78.0±1	48.3	*	44
8	> 20 µm; < 63 mm	0.19	37.2	35.0	4.2	3.0	12.4	0.52	-24.98±0.07	51.4±1	11.1	15	17
9	< 63 µm after us treatment	0.01	25.7	25.0	14.6	10.3	28.0	2.53	-25.89±0.07	54.2±1	2.5	4	7
10	> 63 µm after us treatment	0.57	64.5	0.6	0.6	0.5	28.7	0.08	-24.85±0.07	41.5±1	2.4	7	4
11	fine fraction decantation 1	0.07	0.9	58.5	53.3	36.6	17.5	3.62	-23.87±0.07	73.1±1			
12	fine fraction decantation 2	0.58	33.9	31.4	24.8	16.7	12.7	1.58	-23.92±0.07	71.0±1	99.94	*	*
13	coarse fraction decantation 2	0.42	65.6	2.6	2.6	1.7	25.3	0.15	-24.58±0.07	54.0±1	0.06	*	11

Table 2.1 Weight portions relative to the total sample (Fm) and results of mineral particle size analysis of the fractions after removal of OM and carbonates, Calcium carbonate content, organic carbon content (%OC), $\delta^{13}C$ values (vs. V-PDB) and ^{14}C normalized relative abundance (^{14}a) of the organic carbon. In the last three columns the distribution of organic carbon over the respective fractions is calculated from weight portions and organic carbon percentage (OC_f/OC_t; OC_f is %OC*Fm of the fraction and OC_t is the OC concentration of sample 1, the 'total' sample, $\delta^{13}C$ values (CB13) and ^{14}a values (CB14).

REFERENCES

Aerts-Bijma, A.Th., Meijer, H.A.J., Van der Plicht, J., 1997. AMS sample handling in Groningen. Nucl. Instr. and Meth. in Phys. Res. B, 123, 221-225.

Barua, A.B., Furr, H.C., Janick-Buckner, D., Olson, J.A., 1993. Simultaneous analysis analysis of individual carotenoids, retinol, retinyl esters and tocopherols in serum by isocratic non-aqueous reversed-phase HPLC. Food Chem. 46, 419-424.

Bendoraitis, J.G., Brown, B.L., Hepner, L.S., 1962. Isoprenoid hydrocarbons in petroleum. Isolation of 2,6,10,14-tetramethylheptadecane by high-temperature gas-liquid chromatography.Anal. Chem. 34, 49-53.

Benner, R., Fogel, M.L., Sprague, E.K., Hodson, R.E., 1987. Depletion of 13C in lignin and its implications for sable carbon isotope studies. Nature 329, 708-710.

Bergamaschi, B.A., Tsamakis, E., Keil, R.G., Eglington, T.I., Montluçon, D.B., Hedges, J.I., 1997. The effect of grain size and surface area on organic matter, lignin and carbohydrate concentration, and molecular compositions in Peru Margin sediments. Geochim. Cosmochim. Acta 61, 1247-1260.

Boon, J. J., Pouwels, A. D. and Eijkel, G. B., 1987. Pyrolysis high-resolution gas chromatography-mass spectrometry studies on beech wood: capillary high-resolution mass spectrometry of a beech lignin fraction. Biochem. Soc. Trans. 15, 170-174.

Burton, J.D., Liss, P.S., 1976. Estuarine Chemistry. Academic Press.

Deines, P., 1980. The isotopic composition of reduced carbon. In: Handbook of Environmental Isotope Geochemistry. Vol. 1, The terrestrial environment, A. (Fritz, P. and Fontes, J.C., eds.) Chap. 9, pp. 329-406.

Dupont, L.M., 1986. Temperature and rainfall variation in the Holocene based on comparative palaeoecology and isotope geology of a hummock and a hollow (Bourtangerveen, the Netherlands). Rev. Palaeobot. Palynol. 48 71-159.

Eisma, D., 1981. Supply and deposition of suspended matter in the North Sea. Spec. Publ. Int. Ass. Sediment. 5, 415-428.

Eisma, D., Boon, J., Groenewegen, J., Ittekot, V., Kalf, J., Mook, W.G., 1983. Observations on macro-aggregates, particle size and organic composition of suspended matter in the Ems estuary. Mitt. Geol.-Paläont. Inst. Univ. Hamburg 55, 295-314.

Eisma, D., Kalf, J., 1987. Distribution, Organic matter content and particle size of suspended matter in the North Sea. Neth. J. Sea Res. 21, 265-285.

Eisma, D., Bernard, P., Cadee, G.C., Ittekot, V., Kalf, J., Laane, R., Martin, J.M., Mook, W.G., Van Put, A., Schumacher, T., 1991. Suspended matter particle size in some west-European estuaries. Neth. J. Sea Res. 28, 193-214.

Eisma, D., 1993. Suspended Matter in the aquatic environment. Springer-Verlag.

Favejee, J.Ch.L., 1960. On the origin of the mud deposits in the Ems-estuary. In: Das Ems-Estuarium. (Ed. J.H. Voorthuizen) Verh. Kned. geol.-mijnb. Genoot. 19, 147-151.

Fry, B., Sherr, E.B., 1984. $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. Contrib. Mar. Sc. 27, 13-47.

Goñi, M.A. Ruttenberg, K.C., Eglington, T.I., 1997. Sources and contribution of terrigenous organic carbon to surface sediments in the Gulf of Mexico. Nature, 389, 275-278.

Goñi, M.A. Ruttenberg, K.C., Eglington, T.I., 1998. A reassessment of the sources and importance of land-derived organic matter in surface sediments from the Gulf of Mexico. Geochim. Cosmochim. Acta 62, 3055-3075.

Goossens, H., De Leeuw, J. W., Schenck, P. A., Brassell S. C., 1984. Tocopherols as likely precursors of pristane in ancient sediments and crude oils. Nature 312, 440-442.

Gottdang, A., Mous, D.J.W., Van der Plicht, J., 1995. The HVEE 14C system at Groningen.Radiocarbon 37, 649-656.

Hayes, J., 1993. Factors controlling ^{13}C contents of sedimentary organic compounds: Principles and evidence. Mar. Geol. 113, 111-125.

Hedges, J.I., Mann, D.C., 1979. The lignin geochemistry of marine sediments from the southern Washington coast. Geochim. Cosmochim. Acta 43, 1809-1818.

Hedges, J.I., Keil, R.G., 1995. Sedimentary organic matter preservation: an assessment and speculative synthesis. Mar. Chem. 49, 81-115.

Hedges, J.I., Ertel, J.R., Quay, P.D., Grootes, P.M., Richey, J.F., Devol, A.H., Farwell, G.W., Schmidt, F.W., Salati, E., 1986. Organic carbon-14 in the Amazon river system. Science 231, 1129-1131.

Van Heemst, J. D. H. , Megens, L., de Leeuw, J. W. and Hatcher P. G., submitted. Characterisation of estuarine macromolecular dissolved organic matter. submitted to Org. Geochem.

Helder, W., Ruardij, P., 1982. A one-dimensional mixing and flushing model of the Ems- Dollard estuary: calculation of time scales at different river discharges. Neth. J. Sea Res. 15, 293-312.

Keil, R.G., Tsamakis, E., Bor Fuh, C., Giddings, J.C. and Hedges, J.I., 1994. Mineralogical and textural controls on the organic composition of coastal marine sediments: Hydrodynamic separation using SPLITT fractionation. Geochim. Cosmochim. Acta 58, 879-893.

Keil, R.G., Mayer, L.M., Quay, P.D., Richey, J.E., Hedges, J.I., 1997. Loss of organic matter from riverine particles in deltas. Geochim. Cosmochim. Acta 61, 1507-1511.

Klamer, J.C. Hegeman, W.J.M., Smedes F., 1990. Comparison of grain size correction procedures for organic micropollutants and heavy metals in marine sediments. Hydrobiologia 208, 213-220.

Laane, R.W.P.M., 1982. Chemical Characteristics of the Organic Matter in the Waterphase of the Ems-Dollart Estuary. PhD dissertation, Univ. Groningen.

Laane, R.W.P.M., Ruardij, P., 1988. Modelling estuarine carbon fluxes. Mitt. Geol-Paläontol. Inst. Univ. Hamb. 66, 239-265.

Laane, R.W.P.M., Etcheber, H., Relexans, J.C., 1987. The nutritive value of particulate organic matter in estuaries and its ecological implications for macrobenthos. In Transport of Carbon and Minerals in World Rivers. (eds. Degens, E.T., Kempe, S. and Gan, W.-B.) Pt. 5 Mitt. Geol-Paläontol. Inst. Univ. Hamb. 64, 71-91.

Laane, R., Turkstra, E., Mook, W., 1990. Stable carbon isotope composition of pelagic and benthic organic matter in the North Sea and adjacent estuaries. In: Facets of Modern Biogeochemistry (Ittekot, V., Kempe, S. Michaelis, W. and Spitzy, A., eds.), pp. 214-224. Springer.

Larter, S.R., Solli, H., Douglas, A.G., De Lange, F., De Leeuw, J.W., 1979. Occurence and significance of prist-1-ene in kerogen pyrolysates. Nature 279, 405-408.

De Leeuw, J.W., Van Bergen, P.F., Van Aarssen, B.G.K., Gatellier, J.P.L.A., Sinninghe Damsté, J.S. and Collinson, M.E., 1991. Resistant biomacromolecules as major contributors to kerogen. Phil. Trans. R. Soc. Lond. B, 333, 329-337.

Mayer, L.M., 1994. Surface area control of organic carbon accumulation in continental shelf sediments. Geochim. Cosmochim. Acta 58, 1271-1284.

Megens, L., 1999. Stable and radioactive carbon isotopic and molecular characterization of POM in Dutch coastal waters. Ph. D. thesis Groningen.

Mook, W.G., Streurman, H.J., 1983. Physical and chemical aspects of radiocarbon dating. Proc. Groningen Symp. 14C and Archaeology (W.G. Mook and H.Tj. Waterbolk, eds.), PACT. 8, 31-55.

Mook W.G., Tan F.C., 1991. Stable carbon isotopes in rivers and estuaries. In Biogeochemistry of Major World Rivers (E.T. Degens, S. Kempe, J.E. Richie eds.), pp. 245-264. SCOPE.

Mook, W.G., Van der Plicht, J., 1999. Reporting 14C activities and concentrations. Radiocarbon (in press).

Pastorova, I., Botto, R.E., Arisz, P.W., Boon J.J., 1994. Cellulose char structure, a combined analytical Py-GC-MS, FTIR and NMR study. Carbohydrate Res. 262, 27-47.

Postma, H., 1954. Hydrography of the Dutch Wadden Sea. Arch. Neerl. Zool. 10, 405-411.

Postma, H., 1961. Transport and accumulation of suspended matter in the Dutch Wadden Sea. Neth. J. Sea Res. 1, 148-190.

Saiz-Jimenez, C., De Leeuw, J.W., 1986. Lignin pyrolysis products: Their structures and their significance as biomarkers. Org. Geochem. 10, 869-876.

Salomons, W., Mook, W.G., 1981. Field observations of the isotopic composition of particulate organic carbon in the southern North Sea and adjacent estuaries. Marine Geology, 41, M11-M20.

Schouten, S., Klein Breteler, W., Blokker, P., Schogt, N., Rijpstra, W.I.C., Grice, K., Baas, M., Sinninghe Damsté, J.S., 1998. Biosynthetic effects on the stable isotope composition of algal lipids: Implications for deciphering the carbon-isotopic biomarker record. Geochim. Cosmochim. Acta , 62, 1397-1406.

Trumbore, S., 1993. Comparison of carbon dynamics in tropical and temperate soils using radiocarbon measurements.Global Biogeochem. Cycles 7, 275-290.

Wang, X.C., Lee, C., 1993. Adsorption and desorption of aliphatic amines, amino acids and acetate by clay minerals and marine sediments. Mar. Chem. 44, 1-23.

Wang X.C., Druffel E.R.M., Lee C., 1996. Radiocarbon in organic compound classes in particulate organic matter and sediment in the deep northeast Pacific Ocean. Geophys. Res. Lett. 23, 3583-3586.

III

Stable carbon isotope, radiocarbon and molecular characterization of organic matter in size fractionated recent sediments from the Washington coast.

INTRODUCTION

The flow of organic carbon (OC) from land to sedimentary deposits in the sea is an important part of the global carbon cycle. It has been widely studied, but there are still many questions to be answered. Rivers discharge annually more particulate organic carbon (POC) to the ocean than the estimated total burial of POC in marine sediments (Berner, 1989; Hedges and Keil, 1995), which is the sum of marine and terrestrial POC. This could be explained by mineralization of a considerable part of the terrestrial organic matter (OM) after entering the marine environment. However, terrestrial OM delivered to the sea is thought to be highly degraded OM from soils or peat and therefore resistant to mineralization (Hedges et al., 1986ab; Mook and Tan, 1991). Also the region where terrestrial OM is deposited, is subject of debate.

Various methods have been developed to quantify the amount of terrestrial OC in marine sediments or suspended matter. One approach is to quantify molecules that are produced only by terrestrial organisms like lignin (Hedges and Mann, 1979) or long chain n-alkanes (e.g. Prahl et al., 1994). The disadvantage of these methods is that they not necessarily give accurate information about other types of molecules, that have different behaviour and stabilities. In recent sediments lipids, of which the long chain n-alkanes are a sub-fraction, are only a minor component of the total organic matter (OM). Lignin concentrations generally are even lower. Part of total OM consists of carbohydrates and proteins, having characteristics quite different than lipids or lignin. Therefore, their concentrations are not necessarily proportional to those of these compounds.

Carbon isotope ratios in OM carry information about the producer of the OM, both about its identity and about the circumstances in which it produced the OM. Differences in the stable carbon isotope ratios ($\delta^{13}C$) between marine and terrrestrial OM are widely used to estimate mixing ratios of marine and terrestrial OM, usually measuring the $\delta^{13}C$ as a bulk parameter.

However, $\delta^{13}C$ values of different compounds from one producer are not the same, due to differences in isotopic fractionation during the various biosynthetic pathways (Hayes, 1993). Lipids of plants and algae have lower $\delta^{13}C$ values than cabohydrates and proteins, on average ca. 5 ‰ (Deines, 1980) or even more (Schouten et al., 1998). Thus, the $\delta^{13}C$ of the total OM can change dramatically when the more labile components like carbohydrates and proteins are selectively degraded. This will seriously affect the possibility of determining the origins of the OM using $\delta^{13}C$.

Another way to distinguish particulate OM (POM) of different origins might be the use of ^{14}C analysis as proposed by Mook and Tan (1991). POM discharged by rivers to the sea is thought to exist mainly of POM from eroded soils or peat and therefore to have a higher age than marine POM. The age measured by ^{14}C dating, and the normalized relative ^{14}C abundance or ^{14}a, which is proportional to the age, is independent of isotopic fractionation.

Sediment grain-size might influence the concentration and distribution in marine sediments, as is suggested by various studies on bulk sediments (e.g. Premuzic et al., 1982; Prahl et al., 1992). A correlation is observed between the OC concentration and the particle surface area of coastal sediments (Mayer, 1994; Keil et al., 1994). Observations in sediments with different grain-size distributions led to the hypothesis that some types of organic compounds are associated with particles with specific grain-sizes (Prahl, 1985; Prahl et al., 1992). To study these effects in more detail fractions with well defined grain-sizes have to be selected. Several methods exist to separate sediment particles based on their size, like wet and dry sieving or flotation. Keil et al. (1994) were the first to apply the split flow thin cell lateral transport (SPLITT) fractionation technique in their study of sediments. SPLITT fractionation has the advantage that it can be used for separation in size classes below 20 μm, based on hydrodynamic sorting. It can be operated in continuous mode, allowing a high throughput of sample.
In the SPLITT fractions a close relationship between OC content and surface area was observed, in accordance with observations in bulk sediments. Particle size appeared to affect also the quality of the OM. The C:N ratio was higher in coarse than in fine fractions, correlating with the total clay mineral content, percentage of smectite and iron concentrations (Keil et al., 1994).

We applied a combination of stable and radioactive carbon isotope analysis to SPLITT size fractions of sediment samples from the slope and basin off the Washington coast to study the

effects of particle size on the carbon isotope concentrations. The OM was characterized by means of pyrolysis-GC-MS. Also bulk samples were analyzed using this combination of techniques.

Methods

Samples and sampling area

The continental margin along the Washington-Oregon coast is considered an important deposit of terrestrial OM. The main sediment source of the shelf is the Columbia river, with an approximate discharge of 10^7 tons of sediment per year (White, 1970). Using concentrations of various biomarkers (long chain n-alkanes and CuO oxidation products of lignin and cutin) , Prahl et al. (1994) estimated that 60 % of the OM in surface sediments on the shelf is of terrestrial origin. This value decreased to 30 % for slope sediments and to less than 15 % for sediments in the Cascadia Basin.

Sample locations are shown in fig. 3.1. Exact locations, water depth and sample depth below the sea floor are summarized in table 3.1. Samples were collected using a box corer during July/August 1994 aboard the RV Wecoma. The cores were stored frozen until subsampling. The subsamples were stored frozen until analysis. From core 202 and 217 the subsample immediately below the subsample used for bulk analysis was used for size fractionation.

SPLITT Fractionation

Samples were fractionated into hydrodynamically sorted size fractions using the Split flow, thin cell, lateral transport (SPLITT) method as described byKeil et al. (1994). The theory behind SPLITT fractionation has been described by Giddings (1985, 1988) and Springston et al. (1987). In a thin cell (381 µm thick, 20 cm long and 4 cm wide; fig. 3.2) a sediment suspension flow was continuously introduced through the top inlet. Simultaneously, sediment-free water was pumped through the bottom inlet. The two flows were initially separated by a stainless steel splitter, thus forming laminar flows towards the other end of the cell. The contact surface

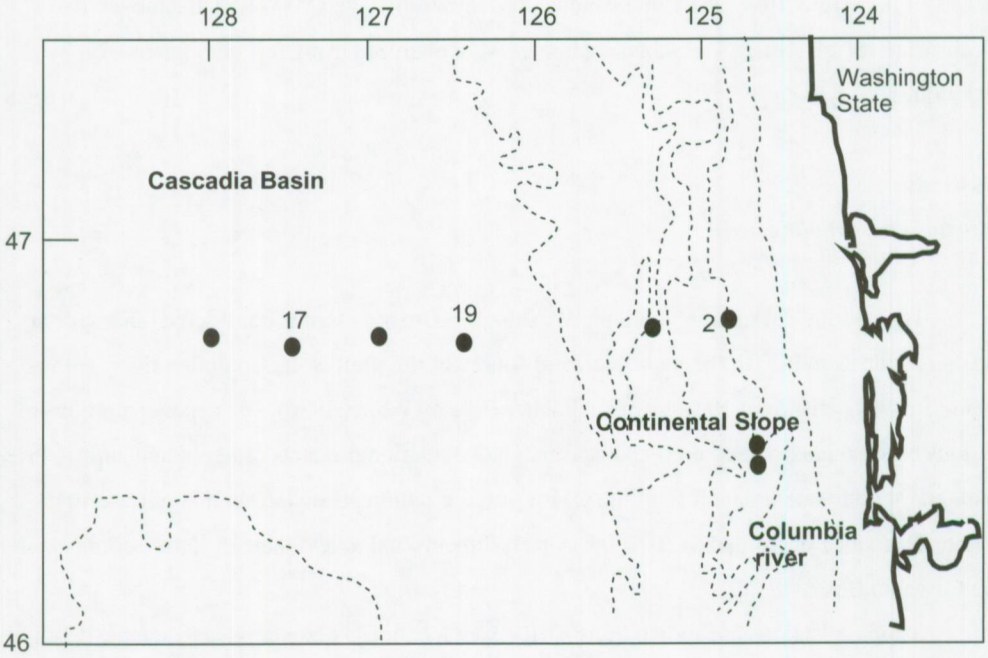

Fig. 3.1 Washington Margin study region. Sampling locations are indicated by numbered (sample number - 200) dots. The unnumbered dots are locations of cores dated by Hedges et al. (1999).

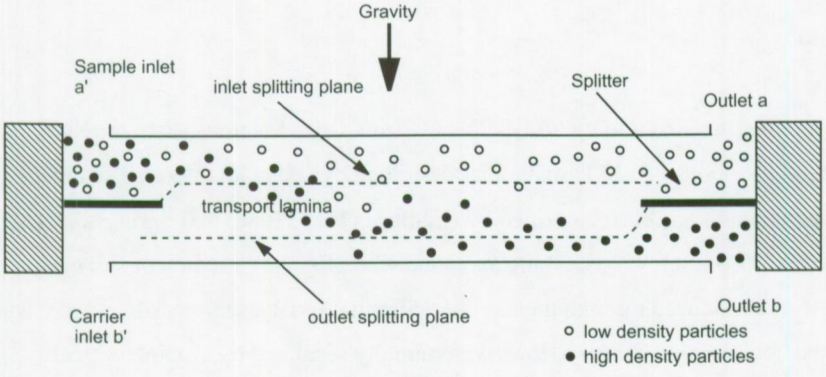

Fig. 3.2 Schematic view of the SPLITT cell. For description see text.

between the upper and lower stream is called the inlet splitting plane. By adjusting the two inlet flow rates (V(a') and V(b')), the position of the inlet splitting plane and thus the thickness of the streams can be tuned. During transport through the cell, particles sink out of the thin upper stream due to gravity. The speed with which they sink depends on their sedimentation coefficients, which are functions of the size, shape and density of the particles (Giddings, 1988). Further in the cell at the bottom a thin lamina is formed. The contact surface between this stream and the stream above it is called the outlet splitting plane. The position of this plane is controlled by the ratio of the two outlet flow rates (V(a) and V(b)). Only particles that had enough time to pass through the outlet splitting plane leave the cell through the lower outlet. Thus the particles are separated in particles with high and low sedimentation coefficients. In case of spherical particles with uniform density the sedimentation coefficient only depends on the particle size so that particles can be separated based on size. The cutoff diameter is controlled by adjusting the inlet and outlet flows and is calculated from these flows (Giddings, 1985, 1988).

Assuming spherical particles with a density of 2.4 g $cm^{-3,}$ flow rates were calculated for a given theoretical cutoff diameter (Keil et al, 1994; table 3.1). The actual maximum particle size in the fine fractions was determined by microscopy. For some separations, this actual cutoff deviated considerably from the theoretical value due to nonuniformity of the density and shape of the particles.

Samples were first sieved over 250, 65 and 37 µm mesh. The fine fraction was used for three subsequent SPLITT fractionations with cutoffs as shown in table 3.2. The fraction < 3 µm was separated in fractions coarser and finer than 1 µm by allowing a suspension to stand in a beaker for a time calculated by Stokes law.

sample	latitude (N)	longitude (W)	distance off shore (km)	water depth (m)
202	46°47'83"	124°54'41"	33.6	440
219	46°45'02"	126°29'93"	97.7	2153
217	46°45'49"	127°30'0"	137.8	2647

Table 3.1 Sampling locations, water depth at the sampling site and distance off shore of the three samples studied.

cut off (mm)		flow rates (ml min-1)			
theoretical	actual	a'	b'	a	b
8.6	17	3	30	30	3
7.2	8	4.2	30	23	11.2
4.3	3	1.8	10.5	8	4.3

Table 3.2 Parameters used in SPLITT cell to separate sediments into approximate size classes. The average density of the sediment particles was assumed to be 2.4 g cm^{-3}

Isotope Analysis

Samples for carbon isotope analysis were treated with dilute HCl to remove carbonates and dried in vacuo over KOH. The dried samples were sealed in vacuo in quartz tubes with an oxidized copper wire. The tubes were heated in an oven at 700°C for ca. 6 hours. The resulting gas was led through a dry ice-ethanol cold trap to remove water and over a silver oven at 400°C to remove halogens and sulphur compounds. The purified carbon dioxide was analyzed for $\delta^{13}C$ with a VG SIRA 9 isotope ratio mass spectrometer. $\delta^{13}C$ values are reported relative to the V-PDB standard. Repeated pretreatment and analysis of a POM sample showed a precision of 0.07 ‰.

For ^{14}C analysis by means of AMS purified CO_2 samples were converted to graphite using iron powder as a catalyst at 600°C (Aerts-Bijma, 1997). The resulting graphite-iron mixture was pressed onto an target holder. The $^{14}C/^{12}C$ ratio in the graphite was measured with the Groningen ^{14}C -dedicated Accelerator Mass Spectrometer (Gottdang et al., 1996). The $^{14}C/^{12}C$ ratios are reported as relative activities (^{14}a) in percentages according to equation 1:

$$^{14}a = {}^{14}R_{Sample}/{}^{14}R_{RN} * \{(1 - 25\ ‰)/(1+\delta^{13}C_{Sample})\}^2 \qquad (1)$$

where $^{14}R_{RN}$ is the reference $^{14}C/^{12}C$ ratio which is defined as 0.95 times the $^{14}C:^{12}C$ ratio of the original NBS oxalic acid in 1950 (Mook and Van der Plicht, 1999).

			bulk			hot water extracts		
sample	water depth (m)	sample depth (cm)	^{14}a	$d^{13}C$	OC_x/OC_t	$^{14}a_x$	$d^{13}C_x$	age (yr BP)
202	440	22-23	70.6%	-22.1	0.10	76.0%	-21.5	2208
219	2153	19-21	47.9%	-22.6	0.09	62.5%	-19.4	3770
217	2647	23-27	38.0%	-22.9	0.10	49.4%	-19.9	5660

Table 3.3 Characteristics of the three bulk samples and of the hot water extracts of the bulk samples. The age of the extracts is not corrected for reservoir effects.

sample	water depth (m)	sample depth (cm)		size fraction (mm)							weigthed average
				<1	1-3	3-8	8-17	17-38	38-63	63-250	
202	440	23-30	fm	0.3%	0.6%	5.2%	13.5%	15.9%	13.5%	51.1%	
			%OC	1.90%	1.72%	1.19%	1.10%	0.66%	0.19%	0.27%	
			fOC	0.9%	2.0%	12.7%	30.1%	21.3%	5.4%	27.6%	
			$d^{13}C$	-23.6	-24.1	-23.1	-22.8	-23.0	-23.8	-23.3	-23.1
			^{14}a		52.1%	51.2%	50.7%	47.6%	42.8%	46.5%	48.0%
219	2153	23-27	fm	0.3%	6.4%	14.5%	49.5%	28.5%	0.7%	0.1%	
			%OC		1.60%	1.70%	1.50%	0.80%			
			fOC		7.8%	18.3%	55.9%	18.1%			
			$d^{13}C$		-22.6	-22.3	-22.4	-22.6			-22.4
			^{14}a		62.4%	61.2%	58.4%	59.6%			59%
217	2647	21-23	fm	0.0%	28.8%	18.3%	10.9%	41.5%	0.3%	0.2%	
			%OC		1.43%	1.29%	1.15%	0.97%	0.79%		
			fOC		35.1%	20.1%	10.7%	34.0%	0.2%		
			$d^{13}C$		-24.2	-23.6	-23.3	-23.0	-23.3		-23.6
			^{14}a		36.1%	40.9%	34.1%	34.7%	41.0%		36%

Table 3.4 Characteristics of the size fractions of the sediment samples. Fm is weight percent, fOC is the amount of OC in the fraction as a percentage of the OC in the total sample.

RESULTS AND DISCUSSION

Bulk Samples

Carbon isotopic compositions of three recent sediment samples from ca. 23 cm below the sediment-water interface off the Washington coast are shown in table 3.3. A depth profile of $\delta^{13}C$ and ^{14}a of core 219 was measured by Hedges et al. (1999). Our ^{14}a and $\delta^{13}C$ values fit well in their profile. Our isotope date from the other two samples agree well with data from other cores sampled near our sites. The $\delta^{13}C$ values of the samples range from –22.1 ‰ for the slope sample (202), to –22.9 ‰ for the sample from the Cascadia Basin located the farthest off shore (217). Not taking into consideration possible effects of degradation on $\delta^{13}C$, this would indicate that the relative contribution of terrestrial OC is highest in the farthest sample. However, Hedges et al. (1999), who studied a number of sediment cores from the Washington Margin, including the three cores reported here, show that OM in off-shore sediments is more degraded than in near-shore sediments. Therefore, partial degradation might be the cause of the lower $\delta^{13}C$ in the samples off-shore.

The $\delta^{13}C$ values of OC in surface sediments (0-2 cm sediment depth), sampled along a transect at the same latitude show the opposite trend: lowest on the shelf (–23.9 ‰ on average) and highest in the Cascadia Basin (–21.4 ‰; Prahl et al. 1994; Hedges and Mann, 1979). Isotope data from Hedges et al. (1999) are also lower in the upper centimeters of the near-shore slope sediment.

In this core described by Hedges et al. (1999) $\delta^{13}C$ increased from –24.2 ‰ at 4.5 cm to ca. –22.1 ‰ deeper in the sediment (22 - 32 cm), the same value as we measured at a similar depth. The ^{14}C content at 4.5 cm depth is lower than at 11.5 cm, which means that the sediment appears to be older at 4.5 cm than at 11.5 cm. There are a number of possible explanations for this: 1) At some time the sediment was covered with older sedimentary material resuspended from an other location. 2) Since depositing POM is probably a mixture of components with different ages (cf. Wang et al., 1996), the contribution of the older component was higher more recently than in the more distant past. If the older component were of terrestrial origin this would also explain the lower $\delta^{13}C$ at 4.5 cm, implying that the POM discharge from land was lower in the past or the in situ primary production higher. 3) If over time the ratio between the old and

fresh component was more or less constant, the older, terrestrial component would have been preferentially degraded. However, older OM is expected to be more resistant to degradation than fresh material, because the easily degradable compounds are probably already removed (e.g. Hedges et al., 1984).

In order to distinguish the effect of degradation on $\delta^{13}C$ of the OM from the effect of the terrestrial/marine mixing ratio, separated components have to be studied of which the $\delta^{13}C$ is not affected by degradation. For this the studied component has to consist of constituents with the same stability.

As an approximation of this we analysed hot water extractable OM from the samples. This extract consists mainly of (poly)saccharides and some protein (chap. 1). Around 10 % of the OM in the three samples is extractable by hot water. This is considerably less than can be extracted from fresh marine POM (Megens et al., 2000). The extracted fractions have higher $\delta^{13}C$ values than the bulk OM, –21.5 ‰ for the slope sample and –19.4 ‰ and –19.9 ‰ for the other samples (table 3.3). The latter two clearly indicate a marine algal origin of the OM in these fractions. The higher $\delta^{13}C$ for the slope sample (202) indicates that here some terrestrial OM contributes to the hot water extractable fraction. Taking the average $\delta^{13}C$ of the extracts of the Cascadia basin samples (–19.6 ‰) as the marine end member and a $\delta^{13}C$ of –25 ‰, estimated from data of Prahl et al. (1994) from the Columbia river (–25.5 ‰ on average for bulk OM) as the terrestrial end member, ca. 35 % of the OC in this fraction is of terrestrial origin. Prahl et al. (1994) calculated that ca. 60 % of the total POM in shelf and slope sediments was of terrestrial origin.

The ^{14}a values of the hot water extracts are higher than the bulk ^{14}a values (table 3.2), possibly due to post-depositional downward migration of the water-soluble component. However, it probably also reflects different sources of OM, since terrestrial OM discharged in the ocean – mainly eroded soil or peat OM – is probably older than the autochthonous OM produced by phytoplankton, reaching the sea floor in days or weeks (Eisma, 1991), a period of time negligible on the ^{14}C timescale.

Since the hot water extracted OM from the Cascadia Basin samples are of marine origin it could be assumed that their ^{14}C age can be used to date the sediment if this material remained in situ after deposition. The age of these fractions is 3770 and 5660 BP for station 219 and 217 respectively, or 3370 and 5260 BP (table 3.2), when corrected for a marine resevoir effect of 400

years (Stuiver et al., 1986). The age of 5260 BP at an average depth of 25 cm in the sediment at station 217 corresponds to a sediment accumulation rate of 4.8 cm/ka, which agrees well with rates calculated by Hedges et al. (1999) from bulk ^{14}C depth profiles in two other sediment cores from the Cascadia Basin (3.1 and 5.6 cm/ka respectively). For station 219 the age of the hot water extract corresponds to a accumulation rate of 7.4 cm/ka, in good agreement with the rate calculated by Hedges et al. (1999) for the same sediment core (7.9 ± 0.4 cm/ka).

The average age of the hot water extract of the slope sample is 2200 BP, i.e. 1800 BP if corrected for a resevoir effect of 400 years. Accumulation rates of slope sediments calculated by Hedges et al. (1999) range from 12 (off shore) to 15.6 (near shore) cm/ka. Since station 202 is located on the upper slope, the accumulation rate is probably similar to the high value of 15.6 cm/ka, which corresponds to an age of the sediment at the sample depth (22.5 cm) of 1500 years. The higher age of the extract is probably due to the presence of terrestrial OM, as is shown by the $\delta^{13}C$ value. The age of this terrestrial component is 2810 BP, using a terrestrial contribution of 35 % as calculated from the $\delta^{13}C$ values. The age of the terrestrial OM, soluble in hot water, at the time of deposition was therefore 1320 yr BP (^{14}a=84.9 %). Unfortunately, no ^{14}C data on POM from the Columbia River, the main source of terrestrial POM to the Washington Margin, are known to confirm this value. Bulk ^{14}a values of suspended POM from the Rhine river (Europe) range from 84 to 96 %, but hot water extractable OM have considerably higher ^{14}a values, up to 114 % (Megens et al., 1999). These values are clearly affected by the presence of 'bomb ^{14}C', and would have been lower in the pre-nuclear era.

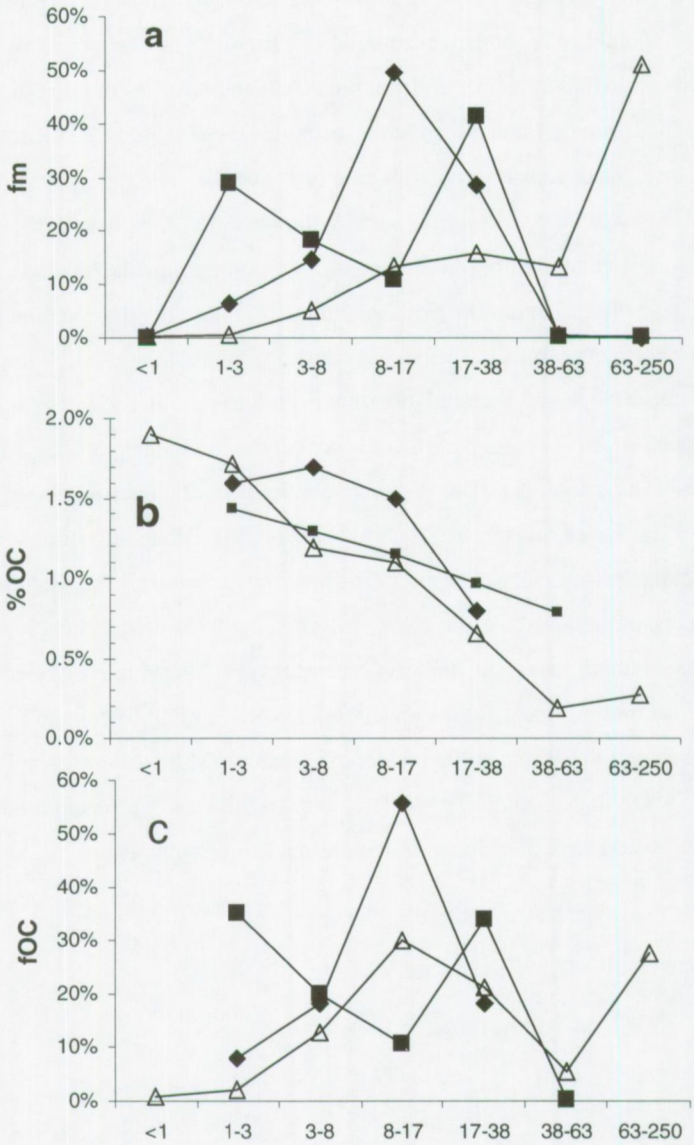

Fig. 3.3 a) Weight percent distribution of particles from the three samples. b) Organic carbon contents of the size fractions. c) Distribution of OC over the size fractions. sample 202: triangles, 217: squares, 219: diamonds.

Particle size fractions

Samples from the same sampling locations were size fractionated, using SPLITT fractionation. From site 202 a subsample was taken from the core immediately below the sample used for bulk analysis. Grain size distributions of the samples are shown in fig. 3.3a and table 3.4. The slope sample (202) contains the highest amount of coarse material (>63 μm), whereas the samples from the Cascadia Basin (219 and 217) are dominated by particles with sizes of 17 to 38 and 8 to 17 μm respectively. Organic carbon concentrations range from 0.2 % in the coarse fractions to 1.8 % in the fine fractions (fig. 3.3b). In 217 and 219 the amount of OC in the fractions >63 μm is negligible compared to the total amount of OC. In sample 219 most of the OC is present in the 8-17 μm fraction, whereas in sample 217 this fraction contains less OC than the 1-3 μm, 3-8 μm and 17-38 μm (fig. 3.3c). In sample 202 almost equal amounts of OC are found in the >63 μm and the 8-17 μm fractions.

The $\delta^{13}C$ and ^{14}a of OM in the size fractions of the three sediment samples are shown in fig. 3.4 (see also table 3.3). The weigthed average ^{14}a of the fractions of sample 202 is 22 % lower than that of the bulk sample that was taken from 4 cm higher in the core. Because of this a lower value is expected, but based on accumulation rates determined in nearby sediment cores (Hedges et al., 1999), it should be much closer to the ^{14}a value of the bulk sample. In these cores 4 cm corresponds to an age difference of about 300 years (ca. 2 % in ^{14}a for this age). During sample desalting prior to SPLITT separation it was clear that a certain amount of organic matter was lost. This might explain the lower ^{14}a value because water soluble material has a higher ^{14}a than the bulk as is shown by the analysis of the hot water extract of the bulk sample. The residue of the water extracted bulk sample has a ^{14}a of 50 %, 2 % higher than the sample from 4 cm deeper, in agreement with the accumulation rates determined on other cores. The average ^{14}a of the fractions of sample 217 is 2 % lower than that of the bulk sample from the same depth and slighty but not significantly lower than the residue of the bulk after hot water extraction.

The average ^{14}a value of the fractions of sample 219 is, however, more than 10 % higher than that of the bulk sample from the same depth, and corresponds to a depth of 11 cm based on the data of Hedges et al. (1999). Also from this sample OM was lost due to desalting, but since water soluble material is younger this should make the remaining sample older. Since the ^{14}a of

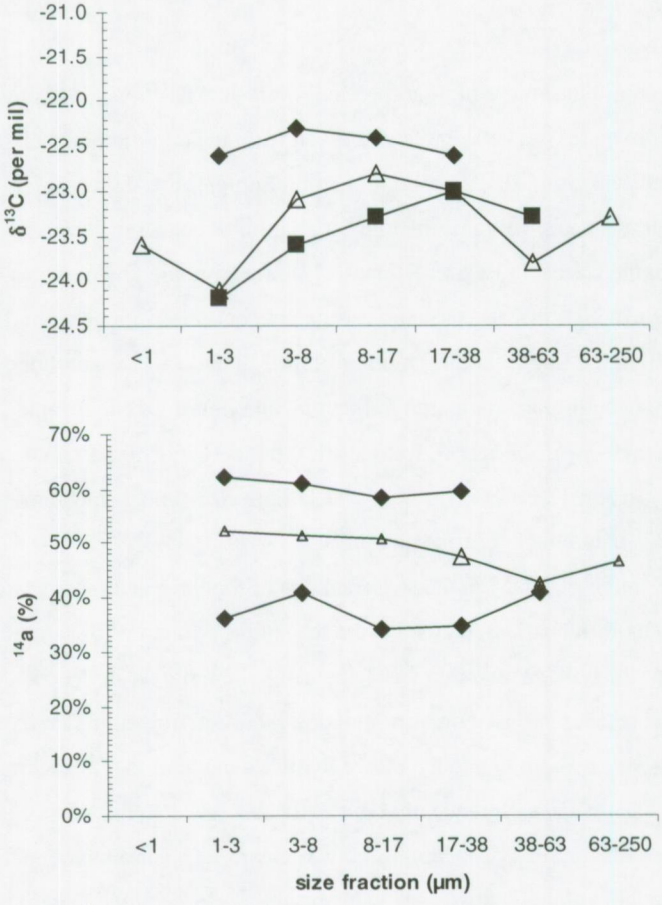

Fig. 3.4 d13C (‰ relative to V-PDB) and 14a (% relative to NBS oxalic acid) of the size fractions of the samples 202 (triangles), 217 (squares) and 219 (diamonds).

the bulk sample fits well in the data of Hedges et al., there is probably something wrong with the fractionated sample. It might be contaminated by fresh material (20 % of the total OC would be from contaminants in that case).

There is a clear variation in $\delta^{13}C$ of the size fractions of samples 202 and 217, but for sample 219 $\delta^{13}C$ values of the size fractions are very similar (around –22.5 %). The $\delta^{13}C$ value of sample 219 are 0.5 to 1.5 ‰ higher than those from the other two samples. The small $\delta^{13}C$ variation between the size fractions suggests that OM is qualitatively homogeneously distributed over the size fractions.

In sample 217, however, there is a clear increase in $\delta^{13}C$ with increasing particle size between 1 and 37 μm, suggesting a higher contribution of terrestrial OM in the smaller particles. There is no correlation between the $\delta^{13}C$ and ^{14}a values. This would be expected, because terrestrial POM is probably older than marine POM reaching the sediment, and thus a higher amount of terrestrial OM would cause a lower ^{14}a. Terrestrial POM, however, is most likely a mixture of components with different ages, as is the case for POM in rivers in North-West Europe (Megens et al., 1999). If some components end up specifically in one size class, this affects the ^{14}a of the fraction, disturbing the correlation.

For molecular characterization the fractions were analysed by means of pyrolysis-GC-MS. The pyrolysates are dominated by alkylbenzenes, probably derived from aromatic macromolecules. The main differences between the fractions exist of different distributions of these compounds. Furthermore, the pyrolysates contain benzonitril as a prominent component, (methyl)naphtalene and some aliphatic compounds. The m/z 55+57 trace of the pyrolysate of the coarse 17-38 μm fraction reveals the presence of a series of alkanes and alkenes, pyrolysis products of aliphatic macromolecules, but in the pyrolysate of the fine 1-3 μm fraction the alkenes are present in very low concentrations compared to the alkanes. Series of n-alkanes and n-alkenes in similar concentrations are produced upon pyrolysis of algaenans and cutans (De Leeuw et al., 1991). The low concentrations of alkenes in the fine fraction indicate that here a different type of aliphatic macromolecule occurs. A series of n-alkanes with n-alkenes in much lower concentrations was also observed in protosalvinia fossils (Mastalerz et al., 1998). Phenol, quite prominent in pyrolysates of suspended POM samples from the North Sea and an adjacent estuary (Megens et al., 1999ab), is present in similar concentrations as the C_3-alkylbenzenes, whereas in North Sea suspended POM the latter compounds are much less abundant. Phenol is a

pyrolysis product of polyphenolic macromolecules, e.g. lignin, or protein. Specific markers of lignin, methoxyphenols (Saiz-Jimenez and De Leeuw, 1987), were not detected in the pyrolysates. Other products of (altered) proteins, like pyrroles or indoles, nor markers for (altered) carbohydrates could be recognized.

In the slope sample (202) $\delta^{13}C$ varies over a range of 1.4 ‰, without a clear relation with particle size. Except for the 63-250 µm fraction ^{14}a decreases with increasing particle size from 52.1 % to 42.8 % (a difference of ca. 1600 years). This might point to a larger terrestrial component in the coarser particles, since the terrestrial POM discharged by rivers is expected to be older than the marine OM. However, $\delta^{13}C$ does not decrease with increasing particle size, which is expected if the coarser particles contain more terrestrial material. The differences in ^{14}a could be due to post-depositional migration of mobile compounds through the sediment, e.g. downward migration of water soluble compounds that preferentially adsorb to small particles rich in clay minerals.

The chromatograms of the pyrolysates of the fractions are similar to those of sample 217. The main difference between the pyrolysates of the fraction with the lowest $\delta^{13}C$ (–23.6 ‰, <1 µm) and that with the highest $\delta^{13}C$ (–22.8 ‰, 8-17 µm) is the high concentration of m- and p-dimethylbenzene in the <1 µm fraction. In both fractions n-alkenes occur in much lower concentrations than the corresponding n-alkanes, like in the fine fraction of sample 217. Also here markers of proteins and carbohydrates are almost absent.

Conclusions

Organic matter in sediments from the Washington Margin are mixtures of components with different ages and origins, causing the apparent age of the bulk OM to be higher than the age of the sediment. In sediments from the Cascadia Basin the age of the OM extracted by hot water gives a good approximation of the sediment age, if corrected for a marine reservoir effect of 400 years. As is shown by the $\delta^{13}C$ this material is derived from marine phytoplankton. The hot water extract from the slope sample contains around 35 % of terrestrial OM, that is approximately 900 years older than the marine component.

In the three samples, carbon isotopic compositions vary between particle size fractions obtained by SPLITT fractionation. Observed maximum differences in ^{14}a and $\delta^{13}C$ between

fractions are 10 % and 1.5 ‰, respectively. Relations between particle size and carbon isotopic composition vary between samples. In the slope sample a negative correlation between ^{14}a and particle size is observed, contrary to a positive correlation in one of the samples of the Cascadia Basin.

references

Aerts-Bijma, A.Th., Meijer, H.A.J. and Van der Plicht, J. (1997) AMS sample handling in Groningen. *Nucl. Instr. and Meth. in Phys. Res. B,* **123,** 221-225.

Berner, R.A. (1989) Biogeochemical cycles of carbon and sulfurand their effect on atmospheric oxygen over Phanerozoic time. *Palaeogeography, Palaeoclimatology and Palaeoecology*, 73, 97-122.

Deines P. (1980) The Isotopic Composition of Reduced Organic Carbon. In: Fritz P. Fontes J.C. (eds.) *Handbook of Environmental Isotope Geochemistry.* Amsterdam. pp. 329-406.

Eisma, D. (1991) *Suspended Matter in the aquatic environment.* Springer, Berlin.

Giddings, J.C. (1985) A system based on split-flow lateral transport thin (SPLITT) separation cells for rapid and continuous particle separation. *Sep. Sci. Technol.* 20, 749-748.

Giddings, J.C. (1988) Continuous separation in split-flow thin (SPLITT) cells: potential applications to biological materials. *Sep. Sci. Technol.* 23, 931-943.

Gottdang, A., Mous, D.J.W. and Van der Plicht, J. (1995) The HVEE ^{14}C system at Groningen.*Radiocarbon* **37,** 649-656.

Hayes, J. (1993) Factors controlling ^{13}C contents of sedimentary organic compounds: Principles and evidence. *Mar. Geol.* 113, 111-125.

Hedges, J.I. and Mann, D.C. (1979) The lignin geochemistry of marine sediments from the southern Washington coast. *Geochim. Cosmochim. Acta* 43, 1809-1818.

Hedges, J.I., Turin, H.J., Ertel, J.R. (1984) Sources and distributions of sedimentary organic matter in the Columbia river drainage basin, Washington and Oregon. Limnol. Oceanogr. 29,35-46.

Hedges, J.I., Ertel, J.R., Quay, P.D., Grootes, P.M., Richey, J.F., Devol, A.H., Farwell, G.W., Schmidt, F.W. and Salati, E. (1986a) Organic carbon-14 in the Amazon river. *Science*, 231, 1129-1131.

Hedges, J.I., Clarck, W.A., Quay, P.D., Richey, J.E., Devol, A.H., Santos, U.M., (1986b) Compositions and fluxes for particulate organic material in the Amazon river. *Limnol. Oceanogr.* 31, 717-738.

Hedges, J.I. and Keil, R.G. (1995) Sedimentary organic matter preservation: an assessment and speculative synthesis. *Mar. Chem.* 49, 81-115.

Hedges, J.I., Hu, F.S., Devol, A.H., Hartnett, H.E., Tsamakis, E., Keil, R.G. (1999) Sedimentary organic matter preservation: A test for selective degradation under oxic conditions. *Am. J. Sci.* 299, 529-555.

Keil, R.G., Tsamakis, E., Bor Fuh, C., Giddings, J.C., Hedges, J.I. (1994) Mineralogical and textural controls on the organic composition of coastal marine sediments: Hydrodynamic separation using SPLITT-fractionation. *Geochim. Cosmochim. Acta,* 58, 879-893.

De Leeuw J.W., Van Bergen P.F., Van Aarssen B.G.K., Gatellier J.P.L.A., Sinninghe Damsté J.S. and Collinson M.E. (1991) Resistant biomacromolecules as major contributors to kerogen. *Phil. Trans. R. Soc. Lond. B*, 333, 329-337.

Mastalerz, M., Hower, J.C., Carmo, A. (1998) In situ FTIR and flash pyrolysis-GC-MS characterization of Protosalvinia (Upper Deonian, Kentucky, USA): Implications for maceral classification. *Org. geochem.* 28, 57-66.

Mayer, L.M. (1994) Surface area control of organic carbon accumulation in continental shelf sediments. *Geochim. Cosmochim. Acta*, 58, 1271-1284.

Megens, L. (2000) Isotopic and molecular characterization of particulate organic matter in coastal waters. Ph.D. thesis Groningen University.

Mook W.G. and Tan F.C. (1991) Stable Carbon Isotopes in Rivers and Estuaries. In *Biogeochemistry of Major World Rivers* (E.T. Degens, S. Kempe, J.E. Richie eds.) SCOPE vol. New York, 1991. pp. 245-264.

Mook W.G. and Van der Plicht, J. (1999) Reporting ^{14}C activities and concentrations. Radiocarbon *in press.*

Prahl, F.G. (1985) Chemical evidence of differential particle dispersal in the southern Washington coastal environment.*Geochim. Cosmochim. Acta*, 49, 2533-2539.

Prahl, F.G., Hayes, J.M., Xie, T.M. (1992) Diploptene: an indicator of terrigenous organic carbon in Washington coastal sediments. *Limnol. Oceanogr.* 37, 1290-1299.

Prahl, F.G., Ertel, J.R., Goñi, M.A., Sparrow, M.A., Eversmeyer, B. (1994) An assessment of terrestrial organic carbon contributions to Washington coastal sediments. *Geochim. Cosmochim. Acta*, 58, 3035-3048.

Premuzic, E.T., Benkovitz, C.M., Gaffney, J.F., Walsh, J.J. (1982) The nature and distribution of organic matter in the surface sediments of world oceans and seas. *Org. Geochem.* 4, 63-77.

Saiz-Jimenez, C. and De Leeuw, J.W. (1987) Lignin pyrolysis products: Their structures and their significance as biomarkers. *Org. Geochem,* **10,** 869-876.

Schouten, S., Breteler, W.C.M.K., Blokker, P., Schogt, N., Rijpstra, W.I.C., Grice, K., Baas, M., Sinnighe Damste, J.S. (1998) Biosynthetic effects on the stable carbon isotopic compositions of algal lipids: Implications for deciphering the carbon isotopic biomarker record. *Geochim. Cosmochim. Acta* 62, 1397-1406

Springston, S.R., Myers, M.N., Giddings, J.C. (1987) Continuous particle fractionation based on gravitational sedimentation in split-flow thin cells. *Anal. Chem.* 59, 344-350.

Stuiver, M., Pearson, G.W., Braziunas, T. (1986) Radiocarbon age calibration of marine samples back to 9000 CAL YR BP. Radiocarbon **28,** 980-1021.

Wang, X.C. and Druffel, E.R.M. (1996) Radiocarbon in organic carbon classes in particulate organic matter and sediment in the deep northeast Pacific Ocean. *Geophys. Res. Lett.* 23, 3583-3586.

White, S. (1970) Mineralogy and geochemistry of continental shelf sediments off the Washington-Oregon coast. *J. Sed. Petrol.* 40, 38-54.

IV

Carbon isoptopic and molecular characterization of particulate organic matter along the salinity gradient in the Ems-Dollard estuary during bloom and non-bloom periods

ABSTRACT

Using a combination of stable carbon isotope and ^{14}C analysis and pyrolysis-gas chromatography-mass spectrometry, we characterized Particulate Organic Matter (POM) sampled along the salinity gradient in the Ems-Dollard estuary during spring and autumn. In spring the $\delta^{13}C$ and ^{14}a values of POM increase with increasing salinity (from –23.9 to –20.1 ‰ and 80.1 to 104 % respectively), albeit not linearly. The relative ^{14}C abundances in spring are higher than in autumn, when ^{14}a values in the inner and the outer part of the estuary range from 74 to 79 %, except for two stations in the middle part of the estuary. The higher ^{14}C abundances in spring are caused by the higher contribution of fresh phytoplankton during spring. Organic matter extracted by hot water has much higher ^{14}a values than the bulk POM, but comparison with $\delta^{13}C$ and ^{14}a values of Dissolved Inorganic Carbon (DIC) shows that most of the extracts contain, besides fresh phytoplankton derived organic matter, some old material. Combining the isotope ratios of the extracts with the relation found between the $\delta^{13}C$ and ^{14}a of DIC, enables the calculation of maximum contributions of fresh phytoplankton to the POM. The increase of the $\delta^{13}C$ and ^{14}a values in spring are mainly caused by higher primary production in the outer part of the estuary than in the inner part. Horizontal mixing of POM appears to be a minor cause of the increase of the isotope values.

The lower ^{14}a values of the autumn samples show that there is much less fresh organic matter present. This means that algal primary production is low and the organic matter produced during the spring blooms has been largely removed. Two POM samples from the middle part of the estuary have much lower ^{14}a and $\delta^{13}C$ values than the other autumn samples. In combination with molecular data obtained by pyrolysis-gas chromatography-mass spectrometry, this shows that the samples contain a high amount of old terrestrial material, possibly due to erosion of a peat layer that became exposed or due to dredging activities.

INTRODUCTION

Suspended Particulate Organic Matter (POM) in estuaries and coastal seas originates from various sources: primary production, resuspended sediment and terrestrial detritus discharged by rivers. To quantify the contribution of these sources the difference between the $\delta^{13}C$ values in marine phytoplankton and terrestrial plants can be used (e.g. Mook and Tan, 1991; Laane et al., 1990; Fry and Sherr, 1984; Salomons & Mook, 1981). Organic matter (OM) produced by marine phytoplankton has an average $\delta^{13}C$ of –21 ‰, while the average $\delta^{13}C$ of terrestrial C–3 plant derived OM is –27 ‰. However, there is a wide spread in these values. The $\delta^{13}C$ value of phytoplankton depends on light and temperature (Fontugne and Duplessy, 1981) and growth rate (Laws et al., 1995; 1997). In some areas of the world a considerable part of the terrestrial vegetation is formed by C–4 plants, which are enriched in ^{13}C by about 10 to 15 ‰ compared to C–3 plants. Goñi et al. (1997,1998) showed that the contribution of C–4 plant derived OM entering the Gulf of Mexico considerably affected the estimate of terrestrial input in sediments in the Gulf based on $\delta^{13}C$ values.

Furthermore, fractionation due to biosynthesis causes e.g. lipids to have lower $^{13}\delta$ values and carbohydrates and amino acids to have higher $\delta^{13}C$ values than the total biomass (Hayes, 1993; Deines, 1980; Eadie and Jeffrey, 1973). Generally, in plants and phytoplankton the average $\delta^{13}C$ values of lipids are ca. 5 ‰ lower than carbohydrates and amino acids (Deines, 1980). However, within compound classes considerable differences are observed, e.g. by Macko et al. (1990) for carbohydrates, Macko et al. (1994) for amino acids and Schouten et al. (1998) for lipids.

Carbohydrates and amino acids, isotopically enriched components, decompose more rapidly than other components (e.g. Harvey et al., 1995; Laane et al., 1990). Therefore, partial decomposition can change the $\delta^{13}C$ of bulk POM (e.g. Laane et al., 1990), and thus affects the quantification of the terrestrial contribution when using bulk $\delta^{13}C$ values.

Mook and Tan (1991) suggested that the relative activity of the radioactive isotope ^{14}C might discriminate between OM from different sources, in addition to $\delta^{13}C$ measurements. Terrestrial input of POM into coastal waters through rivers consists mainly of eroded material, and therefore can be expected to be older (lower ^{14}C content) than marine OM that consists mainly of fresh phytoplankton derived OM (Mook and Tan, 1991). Unlike the $\delta^{13}C$ value, the relative ^{14}C activity (^{14}a) is not affected by selective degradation, because it is the same for all

compounds derived from a single, short living, organism because it depends by definition only on the time elapsed since the death of the organism. Therefore, ^{14}C can be a useful additional tracer to estimate the contribution of the various sources. The development of Accelerator Mass Spectrometry (AMS) makes it now possible to analyze much smaller samples than was possible with conventional radioactivity measurement. This enables the analysis of both carbon isotopes of isolated organic fractions from POM samples, so that fractions with similar stabilities can be compared. In addition, qualitative chemical characterization will help to determine more adequately the sources of POM.

The Ems-Dollard estuary (fig. 4.1) is a well studied estuary between the Netherlands and Germany, and is part of the Wadden Sea. It has an area of ca. 600 km^2, half of which consists of tidal flats. The Dollard, the widened inland part of the estuary, consists for 84 % of tidal flats, while the area of the outer part consists for 45 % of tidal flats. Sediment accumulates in the estuary due to an accumulation mechanism described by Postma (1961). A turbidity maximum is located in the river Ems. The main fresh water tributary is the river Ems. Its catchment area consists for a large part of peatlands. POM in the Ems river appears to be almost entirely derived from erosion of peat deposits (Eisma et al., 1983; Mook and Tan, 1991).

The $\delta^{13}C$ of POM generally tends to increase with increasing salinity in the estuary, suggesting mixing of the river POM with marine POM (Eisma et al., 1983; 1991). However, the $\delta^{13}C$ of phytoplankton in an estuary can also vary, because $\delta^{13}C$ values of DIC in rivers is usually lower than the DIC in the sea (Mook and Tan, 1991), causing an increasing $\delta^{13}C$ of DIC and OM produced from it with increasing salinity.

Here we report on a combination of ^{13}C and ^{14}C data with molecular characterization of suspended POM sampled in the Ems-Dollard estuary along a alinity gradient from the river Ems towards the North Sea, in spring and autumn. To the best of our knowledge, this is the first time that suspended matter along a salinity gradient in an estuary is characterized with ^{14}C in combination with ^{13}C and chemical characterization by means of pyrolysis-GC-MS.

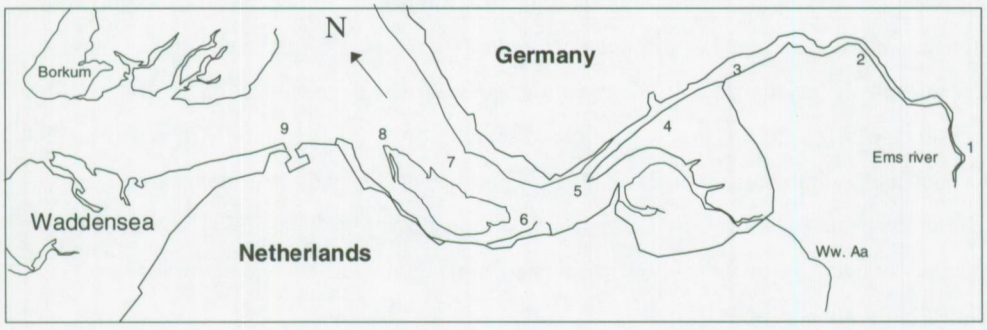

Fig. 4.1 Map of the Ems-Dollard estuary. Sampling stations are indicated in the map with numbers.

Methods

Samples

Samples of suspended matter were obtained on board the R.V. Navicula during cruises in September 1995 and April and September/October 1997. Approximately 100 l of water was pumped from 1–2 m depth below the water surface by a membrane pump into 20 l polycarbonate bottles. Suspended matter was immediately concentrated by cross-flow filtration with a CFP-2-E-6A microfiltration cartridge from AGT (Needham, USA). The suspension was pumped through the cartrigde by a centrifugal pump. The water sample was concentrated to a volume of ca. 2 l and stored frozen (–20 °C) until further processing. An aliquot of the filtrate was used for DIC analysis. In the laboratory the suspended matter concentrates were thawed and centrifuged at 10000 rpm in a MSE high speed centrifuge. The supernatant was discarded and the sample freeze dried for further analysis.

Carbon isotope analysis

For isotope analyses, POM samples were treated with dilute hydrochloric acid to remove carbonate and dried over potassium hydroxide in vacuo. Aliquots of samples were combusted during at least 6 hours at 700 °C in quartz tubes sealed under vacuum with copper oxide as oxygen donor. The combustion gases were led over silver at 400 °C to remove halides and sulfur dioxide. Organic carbon concentrations were determined by expanding the CO_2 in a known volume and measuring the pressure.

DIC was extracted as CO_2 from aliquots of the filtrate in a vacuum system by adding phosphoric acid according to established methods (Mook, 1970).

The $\delta^{13}C$ values of the purified CO_2 were measured with a VG SIRA 9 Isotope Ratio Mass Spectrometer (IRMS) in Groningen and are reported as $\delta^{13}C$ values in per mil relative to the standard V-PDB, according to:

$$\delta^{13}C = ({}^{13}R_{sample}/{}^{13}R_{standard}) - 1$$

where ^{13}R is the ratio of ^{13}C to ^{12}C in the sample.

The CO_2 was reduced to graphite with hydrogen and an iron catalyst at 600 °C (Aerts-Bijma et al., 1997). The $^{14}C/^{12}C$ ratio in the graphite was measured with the Groningen ^{14}C - dedicated Accelerator Mass Spectrometer (AMS; Gottdang et al., 1995). The $^{14}C/^{12}C$ ratios are reported as normalized relative activities (^{14}a) - relative to a standard activity corresponding to AD 1950 - in percentages (Mook and Van der Plicht, 1999):

$$^{14}a = {}^{14}R/{}^{14}R_{RN} * \{(1 - 25\,‰)/(1+\delta^{13}C)\}^2$$

where ^{14}R is the ratio ^{14}C to ^{12}C in the sample, $^{14}R_{RN}$ is the normalized standard activity and $\delta^{13}C$ is that of the sample. This includes correction for isotopic fractionation, which makes it possible to compare ^{14}a values of compounds with different $\delta^{13}C$ values. The ^{14}a value relates to the (average) age of the sample as:

$$age = -8033 * \ln(^{14}a)$$

which is an age in years before present (BP), which means before 1950.

Pyrolysis-Gas Chromatography-Mass Spectrometry

The chemical composition of POM samples was determined with Curie-point Pyrolysis-Gas Chromatography-Mass Spectrometry (Py-GC-MS). Analyses were performed using a Hewlett-Packard 5890 gas chromatograph, equipped with a cryogenic unit and a 25 m fused silica capillary column coated with chemically bound CP Sil-5 (0.32 mm internal diameter and a film thickness of 0.45 µm). Helium was used as a carrier gas. The Hewlett-Packard 5890 gas chromatograph was connected to a VG Autospec Ultima mass spectrometer operated at 70 eV with a mass range of *m/z* 50-800 and a cycle time of 1 s. The temperature programme was as follows: initial temperature 0°C (5 min); heating rate 3°C/min; final temperature 300°C (10 min). Samples were pressed onto flattened ferromagnetic wires with Curie temperatures of 610°C, and

placed into a pyrolysis unit (FOM-4LX; Boon *et al.,* 1987). The pyrolysis unit was connected to a Fisher 9425 high frequency generator that heated the wires inductively in 0.15 s to the Curie temperature. This temperature was maintained for 10 s.

RESULTS AND DISCUSSION

Dissolved Inorganic Carbon

The $\delta^{13}C$ and ^{14}a values of DIC in the Ems-Dollard are plotted in figure 4.2. Due to storage problems only a limited number of samples were succesfully analyzed, but these samples show a strong correlation between $\delta^{13}C$, ^{14}a and salinity, due to mixing of fresh water and sea water with different $\delta^{13}C$ and ^{14}a values for DIC. The relation between the $\delta^{13}C$ and ^{14}a of DIC is linear:

$$^{14}a_{DIC} = 136.3 + 4.408 * \delta^{13}C_{DIC} \qquad r^2 = 0.999 \qquad (1)$$

The DIC of the Ems river has a $\delta^{13}C$ of –11.4 ‰ and a ^{14}a of 87 %. The $\delta^{13}C$ is low compared to that of ocean DIC (ca. +1 ‰), because the source of DIC in rivers are mainly carbonates and CO_2 in soils (Mook and Tan, 1991). Extrapolation of equation 1 to a marine $\delta^{13}C$ (0 to + 1 ‰), gives a ^{14}a of higher than 136 %. A ^{14}a value of DIC in water off the Dutch west coast of 120 % has been deduced from the ^{14}a of phytoplankton (Megens et al., 1999). The only known ^{14}a data of DIC from the North Sea were published by Le Clercq et al. (1997). They measured the same ^{14}a of 120 % of DIC and a $\delta^{13}C$ of –1.9 ‰ in the German Bight. These values are rather high compared to oceanic ^{14}C values (around 110 %, Nydal, 1998), and are probably caused by discharges of nuclear fuel reprocessing plants (Megens et al., 1999).

POM

The ^{14}a values of the POM samples from all locations, obtained in September 1995, April 1997 and early October 1997, are lower than the ^{14}a values of DIC at these locations (table 4.1). Since fresh phytoplankton has the same ^{14}a value (which is normalized for isotopic fractionation)

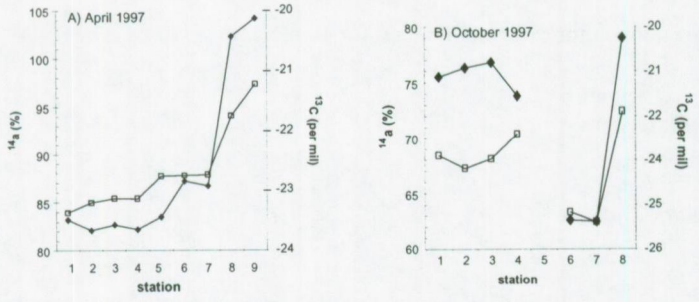

Fig. 4.2 $\delta^{13}C$ and ^{14}a of DIC as a function of salinity.

Fig. 4.2 $\delta^{13}C$ and ^{14}a of DIC as function of salinity.

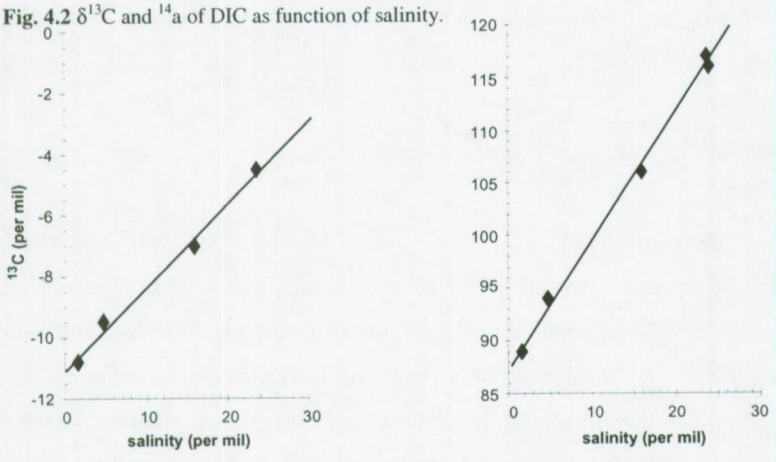

Fig. 4.3 $\delta^{13}C$ and ^{14}a values of POM at the sampling stations in spring 1997 (A) and Autumn 1997 (B).

as the DIC that it is made from, the POM is probably a mixture of relatively old material and fresh OM or does not contain any fresh phytoplankton biomass at all. In spring higher ^{14}a values were measured than in autumn, indicating a higher contribution of fresh OM in spring. In the Ems-Dollard estuary phytoplankton blooms occur generally in April-May (diatoms) and in May-July (phaeocystis; Colijn, 1983). The blooms occur mainly in the outer parts of the estuary. In the inner parts primary production is much lower, probably due to the higher turbidity (Colijn, 1983).

Spring POM

In spring a clear increase is observed in the $\delta^{13}C$ and ^{14}a values of POM with increasing salinity (fig. 4.3), but there is not a clear linear relation. The salinity increases sharply between stations 4 and 5, whereas the $\delta^{13}C$ and the ^{14}a of POM increase slowly until station 7 and are much higher at stations 8 and 9.

Stations 8 and 9 are located in the relatively deep outer part of the estuary. In spring phytoplankton blooms occur in the Ems-Dollard estuary, with high production rates mainly in the outer and middle part of the estuary. The high ^{14}a values of POM at these two stations (102 and 104 % respectively) indicate that a considerable part of the POM must consist of fresh OM. The ^{14}a values of DIC at these stations are 110 and 115 %, somewhat higher than those of POM. Therefore, the POM also contains some OM with a lower ^{14}a value. This can be older OM from resuspended sediment or from the river, but can also be due to a contribution of phytoplankton originating from more upstream parts of the estuary, where the ^{14}a of phytoplankton is lower due to the gradient in ^{14}a of DIC.

Stations 5 to 7 are located in the middle part of the estuary, where a considerable percentage of the area consists of tidal flats. Probably in this area resuspended surface sediment contributes more to the suspended matter than in the outer part. This would explain the relatively low ^{14}a and $\delta^{13}C$ values. In the inner estuary (stations 1 to 4) primary production is low (Colijn, 1983), but turbidity is much higher than downstream in the estuary (Eisma, 1983; 1991). Thus, the low ^{14}a and $\delta^{13}C$ values indicate that detrital material is the main source of POM. However, ^{14}a values are higher than in autumn, which shows that there is a significant contribution of fresh material.

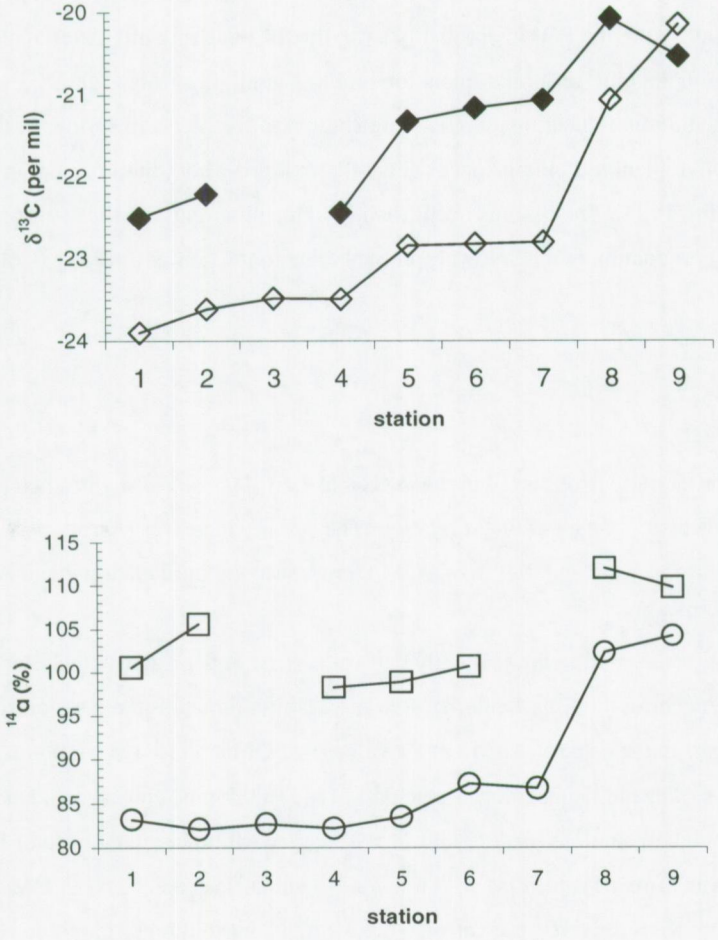

Fig. 4.4 d13C values of hot water extracts (closed diamonds) and of total POM (open diamonds) sampled during spring 1997 (top). 14a values of hot water extracts (squares) and of total POM (circles) during spring 1997 (bottom).

Because the $\delta^{13}C$ and ^{14}a of phytoplankton growing at different places in the estuary are not the same, due to the gradient of $\delta^{13}C$ and ^{14}a in DIC, it is not possible to determine the contribution of fresh phytoplankton directly. To solve this problem, a 'marker' of fresh phytoplankton has to be assigned. Since proteins and carbohydrates are easily degradable (Harvey et al., 1995) it is likely that carbohydrates and proteins in POM are representative for recently produced OM. As some protein and carbohydrates can be extracted by hot water we analyzed hot water extracts of the POM samples to see if they represent fresh phytoplankton with respect to ^{14}a (and $\delta^{13}C$).

The $\delta^{13}C$ values of the hot water extractable fractions of the POM samples increase more gradually than the bulk $\delta^{13}C$ values (fig. 4.4). The $\delta^{13}C$ values of the extracts are on average 15.2 ± 0.6 ‰ lower than of DIC for the samples from stations 5 to 9, where primary production is much higher than in the inner part of the estuary (Colijn, 1983). This rather constant difference suggests that the OM in these hot water extracts is indeed produced from the DIC at the same location. However, this difference is rather small compared with the difference between many published $\delta^{13}C$ values of marine phytoplankton (e.g. Fry and Sherr, 1984) and of marine DIC. The relatively small difference can probably be explained by the fact that these samples are from a bloom period. The $\delta^{13}C$ of marine phytoplankton has been shown to rise up to 7 ‰ after experimental iron fertilization of the ocean, causing a bloom like situation (Bidigare et al, 1999).

If the organic matter in the extracts is derived from fresh phytoplankton, there has to be the same relation between its $\delta^{13}C$ and ^{14}a values as for DIC, but corrected for the difference between hot water extractable phytoplankton organic matter and DIC. If we take for this 'fractionation factor' –14.7 ‰, the difference between the $\delta^{13}C$ of the hot water extracts of samples 6 and 8 and the DIC at these locations, the relation between the $\delta^{13}C$ of water extractable OM derived from phytoplankton and DIC is (eq. 2):

$$\delta^{13}C_X = \delta^{13}C_{DIC} - 14.7 \text{ ‰} \qquad (2)$$

combining equations 1 and 2 yields:

$$^{14}a_X = 201.0 + 4.408 * \delta^{13}C_X \qquad (3)$$

Only the ^{14}a and $\delta^{13}C$ values of the extracts of samples 8 and 9 are in agreement with equation 3, which indicates that they are derived from fresh phytoplankton (fig 4.5). The hot water extracts at the stations 5 and 6 have considerably lower ^{14}a values than calculated from their $\delta^{13}C$ values using equation 3. Therefore, these extracts contain a certain amount of relatively old OM. This shows that water extractable OM is not so labile as expected. Keil et al. (1994) showed that sediments of several hundreds of years old (dated by ^{210}Pb) contained a considerable amount of extractable OM, which degraded rapidly after extraction. They suggested that association of labile OM with mineral particles from the sediment makes it more resistant to degradation.

The $\delta^{13}C$ values of hot water extracts of the upstream samples (stations 1 to 4) are ca 12 ‰ lower than the DIC $\delta^{13}C$ values at the sampling locations, whereas the ^{14}a values are higher than those from DIC at the same locations. The relative high ^{14}a and $\delta^{13}C$ values indicate that part of the POM at these stations originates from fresh phytoplankton material from the outer parts of the estuary or from the sea. The ^{14}a and $\delta^{13}C$ values fit better into equation 3 than the values of samples 5 to 7 showing that the main source of the water extractable OM in these samples originates from fresh estuarine phytoplankton.

Because most of the extracts contain a certain, but not determinable amount of relatively old OM it is not possible to use the ^{14}a values of these fractions in the calculation of the contribution of fresh phytoplankton OM except for stations 8 and 9. Assuming that the POM in spring is a mixture of fresh phytoplankton and POM with the same $\delta^{13}C$ and ^{14}a as in autumn, when primary production is much lower, ca. 75 % and 85 % of the OM in sample 8 and 9 originates from fresh phytoplankton. When taking the ^{14}a of the hot water extracts as a minimum value for fresh phytoplankton OM in the other samples, maximum contributions of fresh OM in the other samples range from 20 to 45 % (table 4.1).

Autumn POM

The $\delta^{13}C$ values of POM in autumn at stations 1-4 and 8 (ranging from –24.2 to –22.9 ‰) point to a certain contribution of OM derived from phytoplankton from the outer part of the estuary or from the sea. It is even possible that the POM at these stations is entirely derived from phytoplankton, since the gradient in DIC shows that phytoplankton in the estuary can have these $\delta^{13}C$ values. Selective degradation of carbohydrates and proteins, that have higher $\delta^{13}C$ values than other compounds, can decrease the $\delta^{13}C$ of phytoplankton OM several per mil (Laane et al., 1991). Because of this, the measured $\delta^{13}C$ values can also indicate phytoplankton derived OM. Therefore, it is necessary to know more about the composition of the OM.

Molecular analysis by means of pyrolysis-GC-MS shows a wide variety of compounds present in the POM (fig. 4.6). Chromatograms of the pyrolysates show peaks corresponding to aromatic, aliphatic and (hetero)cyclic compounds. The dominant peaks in the chromatograms reflect toluene, phenol and *p*-methylphenol, pyrolysis products of aromatic amino acid moieties of proteins. Mass chromatography of m/z 195+209 also shows a pattern of peaks that correspond to pyrolysis products of adjacent aliphatic amino acids (Boon and De Leeuw, 1987). The (alkyl)pyrroles in the pyrolysate are probably products of moieties present in proteins as well (Van Heemst et al., 2000).

Besides *p*-methylphenol, possibly a pyrolysis product of tyrosine, also *o*- and *m*-methylphenol are present in the pyrolysate. These compounds do not exist as amino acid side chains. Their precursors are thought to reflect diagenetically transformed proteins (van Heemst et al., 1999).

Another potential source of alkylphenols is lignin, a polyphenolic macromolecule present in supporting tissue of vascular plants. Lignin is often used as a tracer of terrestrial OM in marine sediments (e.g. Goñi et al., 1998; Bergamaschi et al., 1997; Hedges and Mann, 1979). However, pyrolysis of intact lignin as well as altered lignin produces also methoxyphenols (Saiz-Jimenez and De Leeuw, 1986), compounds absent in the pyrolysates of the POM samples.

The pyrolysate further contains a broad variety of oxygen containing cyclic compounds, such as furans and cyclopentenones. These compounds are pyrolysis products of (partly altered) polysaccharides (Pastorova et al., 1994).

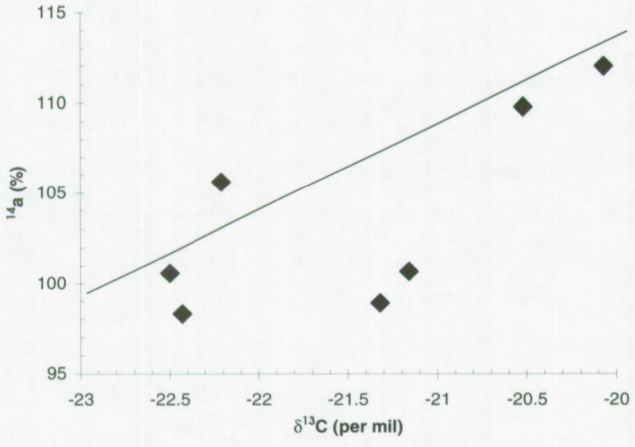

Fig. 4.5 14a vs. d13C of hot water extracts of samples collected during spring 1997. The solid line indicates the theoretical relation according to eq. 3.

Clearly present in the pyrolysates of these samples are series of n-alkanes and n-alkenes up to C25, reflecting aliphatic macromolecules, present in the cell walls of algae and in cuticles of terrestrial plants (De Leeuw et al., 1991).

Thus, Py-GC-MS indicates that the POM samples still contain partly altered polysaccharides and proteins. However, the pyrolysis data are not conclusive with respect to the source of the POM. The absence of detectable amounts of lignin might indicate that terrestrial OM is only a minor contribuant.

Like the spring samples some of the autumn POM samples were extracted with hot water. The extract of the sample from station 8, the outer part of the estuary, has a $\delta^{13}C$ of –21.1 ‰, typical for marine phytoplankton. Fresh phytoplankton-derived material with this $\delta^{13}C$ value has a ^{14}a of 108 %, according to equation 3. However, the ^{14}a of the extract (96.9 %) is significantly lower. Thus, the OM extracted by hot water is mainly derived from phytoplankton, but relatively old, or it is a mixture of fresh and relatively old OM. For organisms from the North Sea/Wadden Sea a ^{14}a value of 96.9 % corresponds to a date somewhere between 1956 (^{14}a 89 %; De Vries, 1958) and 1963 (^{14}a ca. 120 %; unpublished data).

The hot water extract from sample 1 has an almost identical ^{14}a value (96.3 %), which is higher than the ^{14}a value of DIC at this location. Therefore, at least part of the OM in this extract originates from a more downstream location, or of relatively fresh terrestrial OM (the ^{14}a of CO_2 in the atmosphere is higher than that of DIC at this location). The $\delta^{13}C$ value (–22.4 ‰) shows that a major part originates from the outer estuary. Because the ^{14}a value is lower than calculated from the $\delta^{13}C$ and equation 3 (96.3 vs. ca. 102 %), the OM in this extract also contains an older component, which could be derived from estuarine phytoplankton or could have a terrestrial origin.

The $\delta^{13}C$ and ^{14}a values of POM from stations 6 and 7 are much lower than those of the other samples. The lower $\delta^{13}C$ points to a larger terrestrial component or to degradation of isotopically heavy polysaccharides and protein. Py-GC-MS shows that furanaldehydes, pyrolysis products of polysaccharides, are present in much lower concentrations in the pyrolysate of sample 6 than in sample 3. Methylpyrroles, that are probably derived from (degraded) proteins (Van Heemst et al., 1999) are present in similar amounts in the pyrolysates of both samples, but

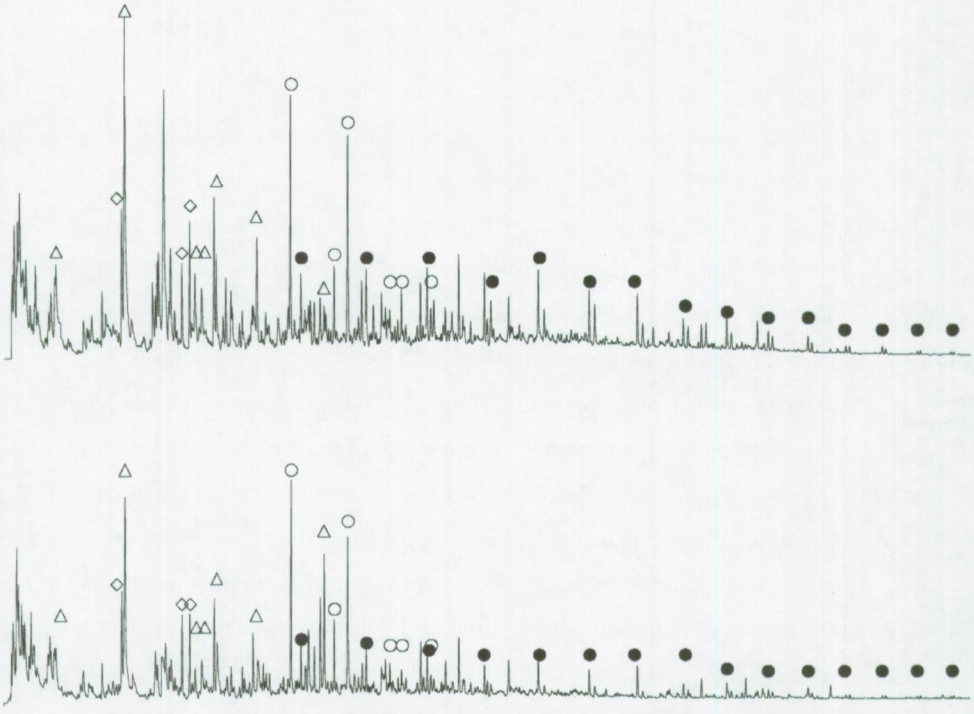

fig. 4.6 Chromatograms (TIC) of pyrolysates of autumn samples from (a) the inner and (b) the middle part of the estuary. Filled dots indicate alkane/alkene pairs, open circles indicate phenols, triangles (alkyl)benzenes and diamonds pyrroles.

the distribution of the two isomers differs. Peaks of acetaldehyde and acetophenone are high in the chromatogram of sample 6. Concentrations of long chain n-alkanes, markers of terrestrial OM, are higher in sample 6. Lignin is not present in either sample, as is shown by the absence of methoxyphenols. Thus, there is evidence for both selective degradation of polysaccharides and a higher terrestrial component in sample 6 as the cause of the lower $\delta^{13}C$ value.

CONCLUSIONS

Our analysis of POM in the Ems-Dollard estuary shows that the combination of ^{14}C-analysis with stable isotope measurements is useful studying the origins of POM, in particular if specific fractions of POM are analyzed and the data are combined with molecular analyses. We observed an increase in the $\delta^{13}C$ and ^{14}a values of POM with increasing salinity in spring, but not in autumn. Combining $\delta^{13}C$ and ^{14}a values of organic matter extracted by boiling water from the spring POM samples, with the relation found between the $\delta^{13}C$ and ^{14}a of DIC, made it possible to estimate a minimum value for the ^{14}C ratio of the POM-component derived from fresh phytoplankton. From this and the ^{14}a of the bulk POM a maximum contribution of organic matter originating from fresh phytoplankton to the total POM in each sample was calculated. This was not possible using only stable isotopes, because the $\delta^{13}C$ of phytoplankton is not the same in different places in the estuary, due to the gradient in $\delta^{13}C$ (and ^{14}a) of DIC. The observed increase in $\delta^{13}C$ and ^{14}a of POM with increasing salinity during spring is mainly due to higher primary production in the outer part of the estuary than in the inner part. Horizontal mixing of two POM pools is only a minor cause of the observed gradient.

In autumn all samples have lower ^{14}a values than the spring samples from the same locations, showing that algal primary production is lower and most fresh material produced during spring is removed from the water column. Two samples from the middle part of the estuary have much lower ^{14}a and $\delta^{13}C$ values, which, in combination with molecular evidence, shows that these samples contain a large terrestrial component.

	Autumn 1997			Spring 1997					
station	salinity	$\delta^{13}C$	^{14}a (%)	salinity	$\delta^{13}C$	^{14}a (%)	$\delta^{13}C$ X1	^{14}a X1	x_{new}
1	1.6	-23.89	75.5	0.9	-23.9	83.2	-22.5	100.6	29.1%
2	4.7	-24.19	76.3	3.1	-23.61	82.1	-22.21	105.6	20.6%
3	15.9	-23.97	76.8	5.7	-23.48	82.7	n.d.	n.d.	n.d.
4	23.9	-23.40	73.8	5.2	-23.49	82.2	-22.43	98.3	27.8%
5	25.2	n.d.	n.d.	17.8	-22.84	83.5	-21.32	98.9	32.7%
6	23.6	-25.17	62.6	16.3	-22.82	87.2	-21.16	100.7	45.2%
7	28.0	-25.39	62.5	21.2	-22.8	86.7	-21.06	n.d.	44.3%
8	n.d.	-22.89	79.0	20.2	-21.05	102.3	-20.07	112.0	73.0%
9				21.9	-20.14	104.2	-20.52	109.8	83.4%

Table 4.1 Carbon isotope data of suspended POM samples from stations along the salinity gradient in the Ems-Dollard estuary and the salinity (in per mil) at the stations at the time of sampling. Errors in $\delta^{13}C$ and ^{14}a are better than 0.1 ‰ and 1 % respectively. $\delta^{13}C$ X1and ^{14}a X1 are the respective values of the hot water extracts of the spring samples. x_{fresh} is the maximum fraction of OM derived from fresh phytoplankton, calculated as explained in the text.

References

Bergamaschi, B.A., Tsamakis, E., Keil, R.G., Eglington, T.I., Montluçon, D.B., Hedges, J.I. (1997) The effect of grain size and surface area on organic matter, lignin and carbohydrate concentration, and molecular compositions in Peru Margin sediments. *Geochim. Cosmochim. Acta* 61, 1247-1260.

Boon, J.J., De Leeuw, J.W. (1987) Amino acid sequence information in proteins and complex proteinaceous material revealed by pyrolysis-capillary gas chromatography-low and high resolution mass spectrometry. *J. Anal. Applied Pyrolysis* 11, 313-327.

Boon, J. J., Pouwels, A. D. and Eijkel, G. B. (1987) Pyrolysis high-resolution gas chromatography-mass spectrometry studies on beech wood: capillary high-resolution mass spectrometry of a beech lignin fraction. *Biochem. Soc. Trans.* 15, 170-174.

Le Clercq, M., Van der Plicht, J., Meijer, H.A.J. and De Baar, H.J.W. (1997) Radiocarbon in marine dissolved organic carbon (DOC). *Nucl. Instr. and Meth. in Phys. Res. B*, 123, 443-446.

Colijn, F. (1983) Primary production in the Ems-Dollard estuary. Ph.D. thesis University of Groningen.

Deines, P. (1980) The isotopic composition of reduced carbon. In: *Handbook of Environmental Isotope Geochemistry. Vol. 1, The terrestrial environment, A.* (Fritz, P. and Fontes, J.C., eds.) Chap. 9, pp. 329-406.

Eadie, B.J. and Jeffrey, L.M. (1973) $\delta^{13}C$ Analysis of Oceanic Particulate Organic Matter. *Mar. Chem.* 1, 199-209.

Eisma, D., Boon, J., Groenewegen, J., Ittekot, V., Kalf, J. and Mook, W.G. (1983) Observations on macro-aggregates, particle size and organic composition of suspended matter in the Ems estuary. *Mitt. Geol.-Paläont. Inst. Univ. Hamburg* 55, 295-314.

Eisma, D., Bernard, P., Cadee, G.C., Ittekot, V., Kalf, J., Laane, R., Martin, J.M., Mook, W.G., Van Put, A., Schumacher, T. (1991) Suspended matter particle size in some west-European estuaries. *Neth. J. Sea Res.* 28, 193-214.

Fontugne, M.R. and Duplessy, J.C. (1981) Organic Carbon Isotope Fractionation by Marine Plankton in the Temperature Range -1 to 31 °C. *Oceanol. Acta* 4, 85-90.

Fry, B. and Sherr, E.B. (1984) $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. *Contrib. Mar. Sc.* 27, 13-47.

Goñi, M.A. Ruttenberg, K.C. and Eglington, T.I. (1998) A reassessment of the sources and importance of land-derived organic matter in surface sediments from the Gulf of Mexico. *Geochim. Cosmochim. Acta* 62, 3055-3075.

Gottdang, A., Mous, D.J.W. and Van der Plicht, J. (1995) The HVEE ^{14}C system at Groningen.*Radiocarbon* 37, 649-656.

Harvey, H.R. Tuttle, J. H. and Bell, J.T. (1995) Kinetics of phytoplankton decay during simulated sedimentation: Changes in biochemical composition and microbial activity under oxic and anoxic conditions. *Geochim. Cosmochim. Acta* 59, 3367-3377.

Hayes, J. (1993) Factors controlling ^{13}C contents of sedimentary organic compounds: Principles and evidence. *Mar. Geol.* 113, 111-125.

Hedges, J.I. and Mann, D.C. (1979) The lignin geochemistry of marine sediments from the southern Washington coast. *Geochim. Cosmochim. Acta* 43, 1809-1818.

Van Heemst, J.D.H., Van Bergen, P.F., Stankiewicz, B.A., De Leeuw, J.W. (1999) Multiple sources of alkylphenols produced upon pyrolysis of DOM, POM and ancient sediments. *J. Anal. Appl. Pyrolysis* in press.

Van Heemst, J. D. H. , Megens, L., de Leeuw, J. W. and Hatcher P. G. (2000) Nature, Origin and average age of estuarine macromolecular dissolved organic matter as determined by molecular and carbon isotope characterisation. *Org. Geochem.* **31,** 847-858.

Keil, R.G., Montluçon, D.B., Prahl, F.G., Hedges, J.I. (1994) Sorptive preservation of labile organic matter in marine sediments. *Nature* 370, 549-552.

Laane, R., Turkstra, E. & Mook, W. (1990) Stable carbon isotope composition of pelagic and benthic organic matter in the North Sea and adjacent estuaries. In: *Facets of Modern Biogeochemistry* (Ittekot V., Kempe, S. Michaelis, W., and Spitzy, A., eds.), Berlin-Heidelberg, pp. 214-224.

Laws, E.A., Popp, B.N., Bidigare, R.R., Kennicutt, M.C., Macko, S.A. (1995)
Dependence of phytoplankton carbon isotopic compostition on growth rate and $[CO_2]$aq: theoretical considerations and experimental results. *Geochim. cosmochim. acta* 59, 1131-1138.

Laws, E.A., Bidigare, R.R., Popp, B.N. (1997) Effect of growth rate and CO_2 concentration on carbon isotopic ractionation by the marine diatom Phaeodactylum tricornutum. *Limnol. Oceanogr.* 42, 1552-1560.

De Leeuw J.W., Van Bergen P.F., Van Aarssen B.G.K., Gatellier J.P.L.A., Sinninghe Damsté J.S. and Collinson M.E. (1991) Resistant biomacromolecules as major contributors to kerogen. *Phil. Trans. R. Soc. Lond. B*, 333, 329-337.

Macko, S.A., Helleur, R., Hartley, G., Jackman, P. (1990) Diagenesis of organic matter - A study using stable isotopes of individual carbohydrates. *Org. Geochem.* 16, 1129-1137.

Macko, S.A., Engel, M.H., Qian, Y. (1994) Early diagenesis and organic matter preservation - a molecular stable carbon isotope perspective. *Chem. Geol.* 114, 365-379.

Megens, L., Van der Plicht, J., De Leeuw, J.W. (1999) Seasonal Variations in ^{13}C and ^{14}C Concentrations in Particulate Organic Matter from the Southern North Sea. *submitted to Geochim. Cosmochim. Acta.*

Mook, W.G. (1970) Stable carbon and oxygen isotopes of natural waters in the Netherlands. Proc. IAEA Conf. on Isotope Hydrology, Vienna. pp. 163-190.

Mook W.G. and Tan F.C. (1991) Stable Carbon Isotopes in Rivers and Estuaries. In *Biogeochemistry of Major World Rivers* (E.T. Degens, S. Kempe, J.E. Richie eds.) SCOPE vol. New York, 1991. pp. 245-264.

Mook W.G. and Van der Plicht, J. (1999) Reporting ^{14}C activities and concentrations. Radiocarbon *in press.*

Nydal, R. Brenkert, A. and Boden T. (1998) Carbon-14 measurements in surface water CO_2 from the Atlantic, Indian and Pacific oceans, 1965-1994. ORNL/CDIAC-104 NDP-057A

Pastorova, I., Botto, R.E., Arisz, P.W. and Boon J.J. (1994) Cellulose char structure, a combined analytical Py-GC-MS, FTIR and NMR study. *Carbohydrate Res.* 262, 27-47.

Postma, H. (1961) Transport and accumulation of suspended matter in the Dutch Wadden Sea. *Neth. J. Sea Res.* 1, 148-190.

Saiz-Jimenez, C. and De Leeuw, J.W. (1987) Lignin pyrolysis products: Their structures and their significance as biomarkers.*Org. Geochem,* 10**,** 869-876.

Salomons, W. and Mook, W.G. (1981) Field Observations of the Isotopic Composition of Particulate Organic Carbon in the Southern North Sea and Adjacent Estuaries. *Marine Geology*, 41, M11-M20.

Schouten, S., Breteler, W.C.M.K., Blokker, P., Schogt, N., Rijpstra, W.I.C., Grice, K., Baas, M., Sinnighe Damste, J.S. (1998) Biosynthetic effects on the stable carbon isotopic compositions of algal lipids: Implications for deciphering the carbon isotopic biomarker record. *Geochim. Cosmochim. Acta* 62, 1397-1406

De Vries, H. (1958) Atomic bomb effect: Variation of radiocarbon in plants, shells, and snails in the past 4 years. *Science* 128, 250-251.

V

Seasonal Variations in ^{13}C and ^{14}C Concentrations in Particulate Organic Matter from the Ems-Dollard

ABSTRACT

We analyzed a time series of Particulate Organic Matter (POM) from the Ems-Dollard estuary, spanning two years, for $^{13}C/^{12}C$ and $^{14}C/^{12}C$ isotope ratios in bulk POM and hot water extracts, lipid extracts, hydrochloric acid and base hydrolysates and insoluble, non-hydrolyzable OM from the POM samples. In addition we characterized samples on the molecular level by means of pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS). We observed a seasonal variation in the ^{14}a and $\delta^{13}C$ values of POM, with high ^{14}a values in spring and lower in the other seasons. This is due to phytoplankton blooms occurring during spring in the Ems-Dollard estuary. The seasonal variation was also observed in the isolated fractions of the POM samples. The average ^{14}a value of bulk POM in autumn and winter is 75 %, which indicates that the POM contains a relatively old component. The average ^{14}a values of the different components range from 97 % for the hot water extracts to 40 % for the residual fraction. Py-GC-MS shows that the hot water extracts consist mainly of (altered) carbohydrates and some protein. The ^{14}a values of the hydrochloric acid hydrolysates, mainly hydrolzed (degraded) proteins, are somewhat lower than those of the hot water extracts. The residual fraction and the base hydrolysate (^{14}a=60 %) consist mainly of relatively old material. The $\delta^{13}C$ values of all fractions are in the range expected for estuarine phytoplankton, but might indicate some terrestrial contribution as well.

The bulk $\delta^{13}C$ values of the spring samples are rather different. While the $\delta^{13}C$ of the spring '93 sample is much higher than the $\delta^{13}C$ in autumn and winter, the $\delta^{13}C$ of the spring '92 sample is a little lower than the average autumn and winter value. This is caused by considerably lower $\delta^{13}C$ values of the fresh components in spring '92 compared to those from spring '93.

INTRODUCTION

The origin and fate of Particulate Organic Matter (POM) in estuaries and coastal seas, though widely studied, are still not well known. The estimate of the amount of terrestrial organic carbon discharged by rivers to the sea exceeds the estimated amount of total organic carbon burial in the world's oceans (Hedges and Keil, 1995; Hedges et al., 1997). Terrestrial organic matter in the rivers is thought to be highly degraded and therefore rather resistant, but apparently a considerable amount of it is lost when entering the coastal seas (Keil et al., 1997).

POM in estuaries originates from several sources: in situ primary production, imported marine POM, resuspended sediment and terrestrial detritus discharged by rivers. To quantify the contributions of these sources the difference between the stable carbon isotope ratios in phytoplankton and terrestrial plants is used in a large number of studies. Organic matter (OM) produced by marine phytoplankton has an average $\delta^{13}C$ of –21 ‰, while the average $\delta^{13}C$ of terrestrial C-3 plant derived OM is –27 ‰ (e.g. Mook and Tan, 1991; Fry and Sherr, 1984). There is a wide spread in these values. The $\delta^{13}C$ of marine phytoplankton depends on light and temperature (Fontugne and Duplessy, 1981) and growth rate (Laws et al, 1995,1997;Bidigare et al., 1999). In some areas of the world a considerable part of the terrestrial vegetation is formed by C-4 plants, which are enriched in ^{13}C by about 10 to 15 ‰ compared to C-3 plants (e.g. Deines, 1980). Goñi et al. (1997) showed that it was necessary to take into account the contribution of C-4 plant derived OM to make an adequate estimate of terrestrial input in the Gulf of Mexico.

Furthermore, different chemical compounds in a single organism have different $\delta^{13}C$ values due to different isotopic fractionations caused by the different biosynthetic pathways followed (e.g. Hayes, 1993, Deines, 1980, Eadie and Jeffrey, 1973). Generally, in plants and phytoplankton lipids have $\delta^{13}C$ values that are ca. 5 ‰ lower than carbohydrates and amino acids (Deines, 1980). Even within these compound classes considerable differences can occur as reported, e.g. by Macko et al. (1990) for carbohydrates, Macko et al. (1994) for amino acids and Schouten et al. (1998) for lipids. Carbohydrates and amino acids, components enriched in ^{13}C, are decomposed more rapidly than other components (Harvey et al., 1995; Laane et al., 1990). Because of this, partial decomposition changes the $\delta^{13}C$ of bulk POM, and thus affects the quantification of the terrestrial contribution when using bulk $\delta^{13}C$ values.

Therefore, it is useful to study additional parameters in combination with the $\delta^{13}C$, like $\delta^{15}N$ and C:N ratio (e.g. Middelburg and Nieuwenhuize, 1998) or concentrations of biomarkers, chemical compounds that are specific to one kind of organism (e.g. Bergamashi et al. 1997). Mook and Tan (1991) suggested to use ^{14}C activities in organic matter to determine the origin of OM. Terrestrial input of POM into coastal waters through rivers consists mainly of eroded material, and therefore can be expected to have a higher age (lower ^{14}C content) than marine OM that consists mainly of fresh phytoplankton derived OM (Mook and Tan, 1991). The relative ^{14}C activity (^{14}a) is the same for all compounds derived from a single, short living organism because

it depends on radioactive decay and is by definition corrected for fractionation using $\delta^{13}C$ values (e.g. Mook and Van der Plicht, 1999). Therefore, ^{14}C is a useful additional tracer to estimate the contribution of the various sources. The development of Accelerator Mass Spectrometry (AMS) makes it now possible to analyze much smaller samples than was possible with conventional radiometry. It enables the analysis of both natural carbon isotopes of isolated organic fractions from POM samples, so that fractions with similar stabilities can be compared. In addition, qualitative chemical characterization will help to determine the sources of POM in a quantitative way.

The Ems-Dollard estuary (fig. 5.1) is a well studied estuary between the Netherlands and Germany, and is part of the Wadden Sea complex. It has an area of ca. 600 km^2, half of which consists of tidal flats. The Dollard, the widened inland part of the estuary, consists for 84 % of tidal flats, while the area of the outer part consists for 45 % of tidal flats. Sediment accumulates in the estuary due to an accumulation mechanism described by Postma (1961). A turbidity maximum is located in the river Ems. The main fresh water tributary is the river Ems, which flows through a large area of peatlands. A minor source of fresh water to the estuary is the Westerwoldse Aa. POM in the Ems river appears to be almost entirely derived from erosion of peat deposits (Mook and Tan, 1991).

The $\delta^{13}C$ of POM generally tends to increase with increasing salinity in the estuary, suggesting mixing of the river POM with marine POM (Eisma et al., 1983; 1991). This was most clearly seen between the upstream weir of Herbrum and the turbidity maximum.

Here we report on the results of the first investigations of seasonal variations in both ^{13}C and ^{14}C isotope ratios and chemical compositions of POM n a tidal flat estuary. The aim is to interpret the data with respect to the origins of POM in samples from the Ems-Dollard estuary.

METHODS

Samples of suspended matter from the Ems-Dollard Estuary (RIKZ sample station 155; indicated by the asterisk in fig. 5.1) were obtained from Dr. F. Smedes of the National Institute for Coastal and Marine Management (RIKZ), Haren, the Netherlands. Water depth at the time of sampling was between 2.7 and 4.9 m. Water from 1.5 m depth below the water surface was led

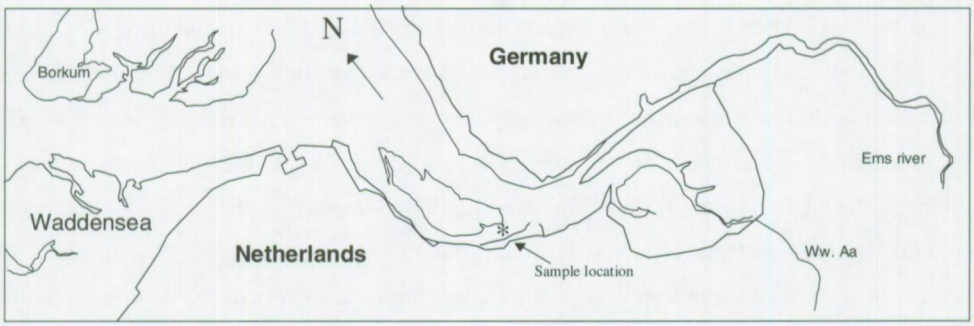

Fig. 5.1 Map of the Ems-Dollard estuary. The sampling location is indicated by the asterisk.

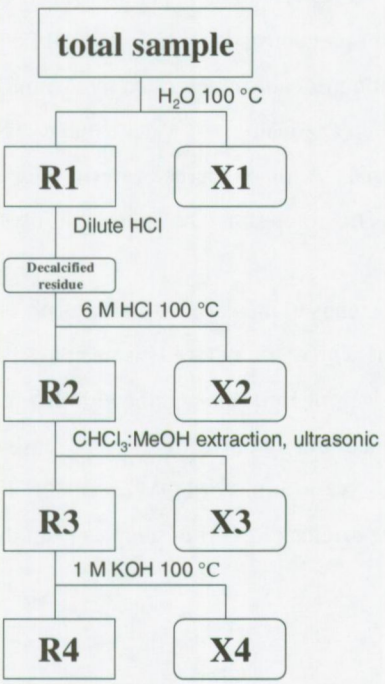

Fig. 5.2 Scheme of the extraction-hydrolysis procedure followed to obtain fractions of POM. All fractions except X3 were analyzed for $^{13}C/^{12}C$ and $^{14}C/^{12}C$.

through a flow-through centrifuge for two hours during ebb tide. Ca. 2000 l of water was centrifuged. The samples were freeze dried for storage.

The samples were fractionated by extraction and hydrolysis using a modification of the procedure reported previously (Megens et al., 1998). The procedure is shown schematically in figure 5.2. First the sample was extracted with water during 24 hours at 100 °C. After centrifugation (3000 rpm) the residue was washed repeatedly with water. Both the extract and an aliquot of the residue were analyzed. The residue was extracted with dilute hydrochloric acid (ca. 0.5 M) to remove carbonates. The residue was hydrolysed with 6 M hydrochloric acid for 24 hours at 100 °C. Extract and residue were separated by centrifugation and washing the residue with water. Then the residue was ultrasonically extracted with a mixture of dichloromethane and methanol (2:1). The extract was dried under a stream of nitrogen. The residue was dried in an oven at 100 °C for 1 hour. It was hydrolyzed with 1 M KOH (aq) for 1 hour at 100 °C. The residue was washed with water three times and freeze dried. The extract was neutralized with HCl and freeze dried.

$\delta^{13}C$ is defined as the relative deviation (expressed in per mil) of the $^{13}C/^{12}C$ ratio of the sample from the $^{13}C/^{12}C$ ratio of the international standard V-PDB according to the following equation:

$$\delta^{13}C = {}^{13}R_{sample} / {}^{13}R_{standard} - 1$$

The ^{14}C activity ratio ^{14}a is defined according to the following equation:

$$^{14}a = ({}^{14}R_{sample} / {}^{14}R_{standard})((1 - 25\,‰)/(1 + \delta^{13}C))^2$$

and is expressed in % (Mook and Van der Plicht, 1999). The standard used in ^{14}C analysis is natural oxalic acid with a ^{14}C activity defined for 1950. The ^{14}a is corrected for fractionation allowing the comparison of samples with different $\delta^{13}C$ values.

For isotope analysis samples were treated with dilute hydrochloric acid to remove inorganic carbonates and dried over potassium hydroxide in vacuo. They were combusted at a temperature of 700 °C in quartz tubes sealed under vacuum using copper oxide as oxygen donor.

The combustion gases were led over a silver oven to remove halogens and sulfur dioxide. $\delta^{13}C$ values of the purified CO_2 were measured with a VG SIRA 9 stable isotope ratio mass spectrometer.

For ^{14}C analysis, the carbon dioxide was reduced to graphite with hydrogen and an iron catalyst at 600 °C (Aerts-Bijma et al., 1997). The ^{14}C concentration in the graphite was measured with the Groningen Accelerator Mass Spectrometer (AMS; Gottdang et al, 1996).

The chemical composition of POM samples was determined with Curie-point pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS). Analyses were performed using a Hewlett-Packard 5890 gas chromatograph, equipped with a cryogenic unit and a 25 m fused silica capillary column coated with chemically bound CP Sil-5 (0.32 mm internal diameter and a film thickness of 0.45 μm). Helium was used as a carrier gas. A flame ionisation detector (FID) at 320 °C was used for detection. The temperature programme was as follows: initial temperature 0°C (5 min); heating rate 3°C/min; final temperature 300°C (10 min).
Samples were pressed onto flattened ferromagnetic wires with Curie temperatures of 358 and 610°C, and placed into a pyrolysis unit (FOM-4LX; Boon *et al.,* 1987). The pyrolysis unit was connected to a Fisher 9425 high frequency generator that heated the wires inductively in 0.15 s to the Curie temperature. This temperature was maintained for 10 s.

Results and Discussion

The $\delta^{13}C$ and ^{14}a values of suspended POM samples, sampled at three month intervals for two years, are shown in table 5.1. The ^{14}a values of organic carbon in suspended matter show a clear seasonal variability over the studied period with higher values in both spring seasons (May 1992 and 1993) and lower values in winter, autumn and also in summer (fig. 5.3c). In 1993 the $\delta^{13}C$ followed the ^{14}a with a high value in spring, but in 1992 the $\delta^{13}C$ of the spring sample is similar to the $\delta^{13}C$ values in the other seasons (fig 5.3b). The percentage of organic carbon in the suspended matter is higher in spring than in the other seasons, in 1993 higher than in 1992 (fig. 5.3a).

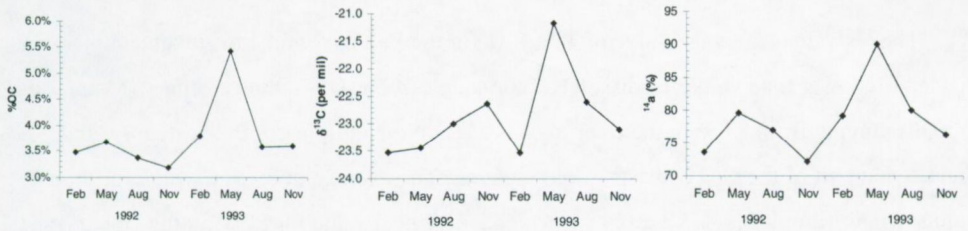

Fig. 5.3 Organic matter content in % (left), $\delta^{13}C$ of the organic carbon in ‰ relative to V-PDB (middle) and ^{14}a of the organic carbon in % (right) of suspended matter in the middle part of the Ems-Dollard estuary, sampled seasonally during the years 1992 and 1993.

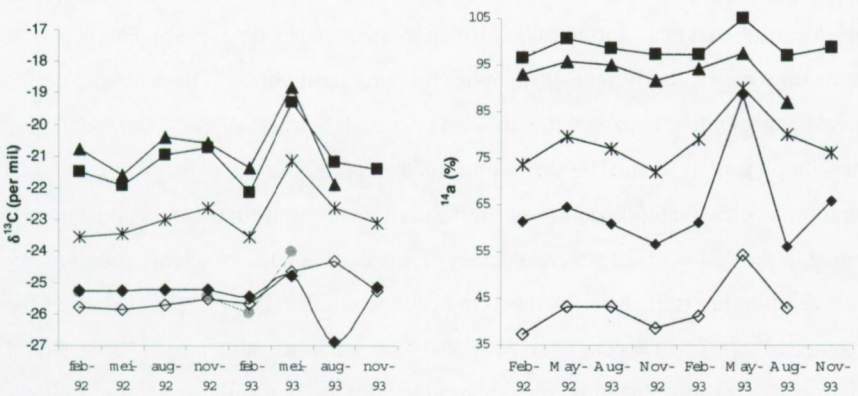

Fig. 5.4 $\delta^{13}C$ (left) and ^{14}a (right) of the bulk POM (crosses) and the fractions isolated as depicted in fig. 5.2: hot water extract: squares; HCl hydrolysate: triangles; KOH hydrolysate: closed diamonds; final residue: open diamonds.

Winter/Autumn/Summer

The ^{14}a values (average value of 75 ± 3 % for the February and November samples) are considerably lower than values of dissolved inorganic carbon (DIC) in this estuary (Megens et al., 2000) ranging from 87 % in the river to ca. 115 % in the outer part of the estuary. This indicates that part of the OM consists of old material. The average bulk δ^{13}C value for these autumn/ winter samples is –23.2 ± 0.4 ‰, which is a typical value for POM in the Dutch coastal waters (Laane et al., 1990). However, the ^{14}a of North Sea POM sampled in winter (Megens et al., 2000) is higher (87%) than the average of the autumn/winter samples studied here. Thus, POM in autumn and winter at this location in the estuary is not identical to POM from the North Sea, despite the similar δ^{13}C values. Possibly, the POM is a mixture of locally produced OM and material from the river. Bulk δ^{13}C values of POM in the Ems river show that OM in the river (δ^{13}C between –26 and –28 ‰) is not derived from algal primary production (δ^{13}C around –33 ‰), but from erosion of peat (Mook & Tan, 1991; Eisma et al., 1983; Eisma et al., 1991). This could explain the lower ^{14}a values of POM in the estuary, since the peat in the catchment area of the Ems is several thousands years old (Roeleveld, 1974).

Old material is mainly present in the insoluble non-hydrolyzable fraction (the final residue R4) and the base extract/hydrolysate (X4). The average ^{14}a value of the R4 fractions of the autumn and winter samples is 40 % (fig 5.4), equivalent to an age of 7360 years BP, which is approximately the time that peat started to be formed in the area (Dupont 1986). Gas chromatograms of pyrolysates of the R4 fractions (fig. 5.5) are dominated by n-alkane/n-alkene series, and peaks representing benzene, toluene and phenol. Branched aliphatic compounds and alkylbenzenes are present as well. The series of n-alkanes/n-alkenes are pyrolysis products of aliphatic macromolecules. Natural sources of this kind of macromolecules can be algaenans, which are found in the cell walls of several species of algae, or cutans, which are found in cuticles of leaves of higher (terrestrial) plants (De Leeuw et al., 1991). The δ^{13}C values of the R4 fractions (average of –25.7 ‰) appear to show that at least a considerable part of this residual OM has a marine algal origin. The aliphatic macromolecules are biosynthesized from lipids which have δ^{13}C values several permil lower than the whole organism (Deines, 1980). Therefore, if the source of the residual OM in the samples would be terrestrial plants, with bulk δ^{13}C values of around –28 ‰, a much lower δ^{13}C value is expected. Lipids from marine algae

have also lower $\delta^{13}C$ values than the alga as a whole.

The ^{14}a values of the residues of the autumn and winter samples are similar to these of winter residual POM in the North Sea (Megens et al., 2000). Also the chromatograms of the pyrolysates are quite similar. The $\delta^{13}C$ values however, are on average 0.7 ‰ lower.

The X4 fractions have higher ^{14}a values than the residues (average of 60 %) and therefore contain relatively more fresh material. The $\delta^{13}C$ values of the X4 fractions are very similar to the $\delta^{13}C$ values of the R4 fractions and of the free lipids (X3 fractions).They are probably a mixture of saponified lipids and base soluble (macro)molecules. Humic substances are defined as macromolecular organic matter soluble in base with a brown colour (e.g. Schnitzer, 1978). All the X4 solutions had a dark brown colour. Another base soluble group of macromolecules is hemicellulose. However, they are easily hydrolysed by hot cids (Sjöström, 1981) and therefore are already removed in the 6 N HCl hydrolysis step. Humic substances are thought to be random polymerisation products of the (degradation products of) main biochemicals: carbohydrates, proteins and lipids, where carbohydrates and proteins are far more abundant than lipids. In case of a marine algal origin a higher $\delta^{13}C$ value would be expected. Extraction with organic solvents does not change the $\delta^{13}C$ significantly. Therefore, it might be concluded that the X4 fractions contain a certain amount of terrestrial OM.

The $\delta^{13}C$ values of the free lipid fractions (X3) (average –25.6 ‰) show a mainly marine origin of these lipids. The ^{14}a values could not be determined accurately, but appear to be between the values of the X1 fractions and the R4 values, and thus are a mixture of old and recent organic matter.

The most recent material is found in the X1 fractions, which contain compounds extracted by hot water. The pyrolysates of these fractions consist of furanaldehydes and cyclopentenones and -pentanones, pyrolysis products of (altered) polysaccharides (Pastorova et al., 1994), but also of pyrroles, indoles and (alkyl)phenoles, derived from proteins or degraded proteins (Van Heemst, 1999). However, the average ^{14}a value (97.3 %) is lower than those of the corresponding fractions of the spring samples (101 and 105 % resp.). This suggests the present of some older material. On the other hand, it is possible that organic matter in the extracts of the winter and autumn samples was fresh, since the ^{14}a values are in the range of those of the DIC in the estuary (Megens et al., 2000). Degradation experiments of algae showed that carbohydrates

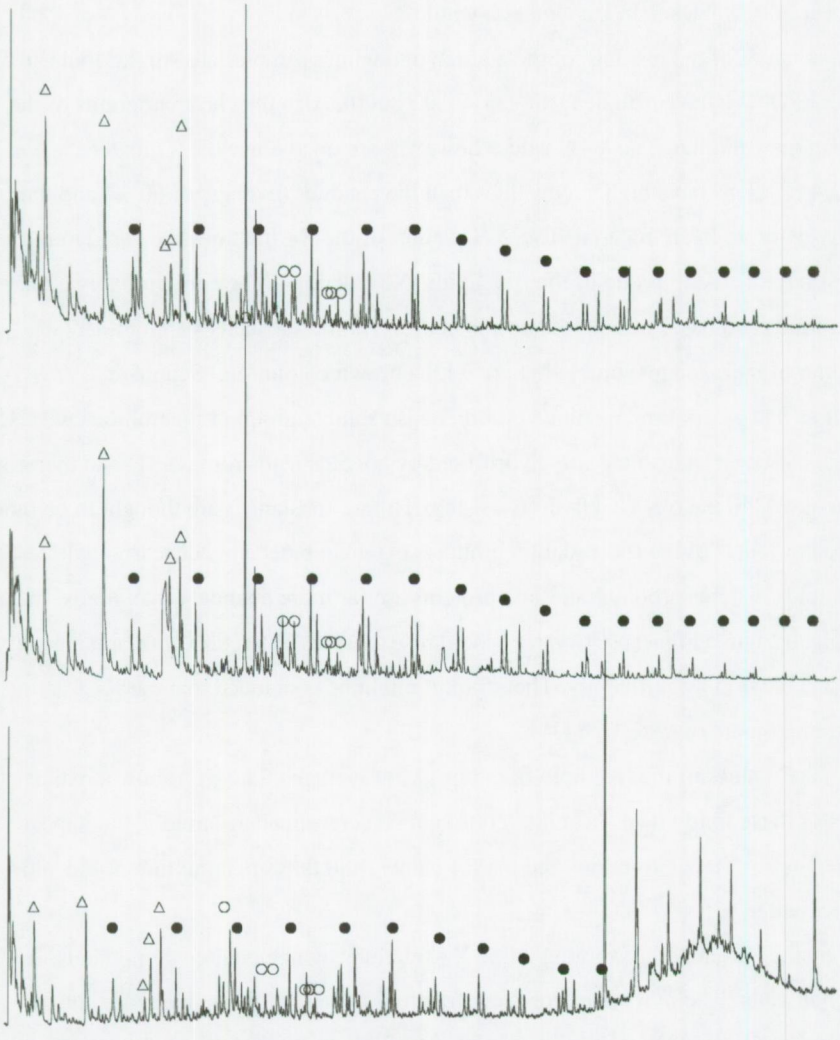

fig. 5.5 Chromatograms of the pyrolysates of the insoluble, non-hydrolyzable fraction of a winter (1992; top) and the two spring samples from 1992 (middle) and 1993 (bottom). Filled circles indicate n-alkene/n-alkane pairs, open circles phenols and triangles (alkyl-)benzenes. Both spring chromatograms contain peaks belonging to siloxanes. These and the contamination causing the big bump in the lower chromatogram appear to originate from contamination of the chromatographic equipment, as indicated by following tests. Unfortunately, not enough sample material was left to repeat the analysis.

and proteins are almost completely mineralized in one or two months (Harvey et al., 1995). However, it has been suggested that water soluble compounds are protected from degradation by adsorption to mineral particles (Hedges and Keil, 1995), which comprise the main portion of the suspended matter in autumn and winter. The $\delta^{13}C$ values are in agreement with an algal origin, considering the range of the $\delta^{13}C$ of DIC.

The X2 fractions, the soluble compounds after 6 N HCl hydrolysis have somewhat lower ^{14}a than the X1 fractions. The pyrolysates mainly consist of nitrogen containing compounds and some phenols, indicating that hydrolysed proteins are the main constituents. Proteins are easily degradable (Harvey et al., 1995), and therefore proteins in the suspended matter are probably mainly fresh. The lower ^{14}a values might be explained by the presence of some acid soluble humic substances, but also by protection of proteinaceous matter by adsorption to mineral particles.

Spring

The higher ^{14}a values in spring are probably caused by increased primary production by phytoplankton blooms in the spring season (cf. e.g. Laane, 1982; Colijn, 1983). In the Ems-Dollard area usually a diatom bloom occurs somewhere between the end of April and the end of May, and is followed by a Phaeocystis bloom peaking between the end of May and July. Measurements by Colijn (1983) in 1979 and 1980 showed peak values of daily primary production during the blooms of about 4000 mg C m^{-2}, whereas the primary production already had decreased with a factor 10 in August and was even lower in autumn and winter (Colijn, 1983).

In spring 1993 the bulk $\delta^{13}C$ value is relatively high (–21.2 ‰), but in spring 1992 the bulk $\delta^{13}C$ value is approximately the same as those in winter. All fractions of the spring 1993 sample have higher $\delta^{13}C$ values than the corresponding fractions of the other samples. However, the $\delta^{13}C$ values of the fresh fractions of the spring 1992 sample (the hot water extract and the HCl hydrolysate) are lower than the average autumn/winter values. Therefore the difference between the $\delta^{13}C$ values of the two spring samples is mainly caused by a difference between the $\delta^{13}C$ values of the fresh algal component. The $\delta^{13}C$ of algal organic matter produced in the Ems-Dollard estuary varies with the place where it is produced, since the $\delta^{13}C$ of the DIC in the Ems-

Dollard estuary ranges from around –11 ‰ in the river to around 1 ‰ in the North Sea (Megens et al., 2000). Due to isotopic fractionation between the DIC and the algal bulk organic matter, this corresponds to $\delta^{13}C$ values from –34 to –18 ‰ for estuarine algal organic matter. Thus, the higher $\delta^{13}C$ value of the spring 1993 sample is caused by a high contribution of algae from the outer part of the estuary to the POM.

The lower $\delta^{13}C$ of the algal component in spring 1992 could be caused by a lower $\delta^{13}C$ of the DIC. Because the $\delta^{13}C$ of DIC in the estuary correlates with the ^{14}a value, this implies a lower ^{14}a value of the algal organic matter as well, as is observed in the hot water extract and the HCl hydrolysate. Based on the linear relation between the $\delta^{13}C$ and ^{14}a of DIC (Megens et al., 2000), the difference between the $\delta^{13}C$ values of the hot water extracts of the two spring samples of 2.5 ‰, however, corresponds to a difference in ^{14}a of 11%, much more than the observed difference of 4 %. Therefore, the hot water extracts probably contain a fraction of older material, besides OM derived from fresh phytoplankton.

A difference in water temperature might also explain the difference in $\delta^{13}C$. Fontugne and Duplessey (1981) showed a relation between the $\delta^{13}C$ of algae and water temperature. The water temperature measured one week before suspended matter sampling in May 1992 was 2 °C lower than two weeks before sampling in May 1993 (monthly water temperature data from Rijkswaterstaat). According to the relation of Fontugne and Duplessy this corresponds to a 1.7 ‰ lower $\delta^{13}C$.

Another cause of the lower $\delta^{13}C$ of the algal organic matter in the spring 1992 sample might be a lower growth rate of the algae at the time of sampling (Laws et al., 1997). Based on the lower organic carbon contents, it appears that the bloom was not yet as strong as at the time of sampling in spring 1993. Bidigare et al. (1999) showed that a higher growth rate of phytoplankton in the tropical Pacific ocean, caused by experimental iron fertilization, raised the $\delta^{13}C$ of the phytoplankton by up to 7 ‰. Thus, a combination of lower water temperature and a carbon source with more riverine DIC in May 1992 might explain the low $\delta^{13}C$ value of the phytoplankton at that time.

The HCl hydrolysates (X2) have the same difference in $\delta^{13}C$ of 2.5 ‰ but the difference in ^{14}a is only 2 %, probably due to the lower ^{14}a of the fresh algal component in the hydrolysate of the spring 1992 sample. Assuming that the fractions are mixtures of fresh phytoplankton derived OM with the same ^{14}a value as the X1 fraction, and of older material with the same ^{14}a

value as the average of the winter and autumn X2 fractions, both the X2 fractions of the spring 1992 and the spring 1993 sample contain about 40 % fresh phytoplankton derived OM.

The base hydrolysates (X4) and the final residues (R4) consist, also in spring, mainly of relatively old material. The ^{14}a values, however, are higher than in autumn and winter indicating that some of the algal OM ends up in these fractions as well. Using again the ^{14}a values of the X1 fractions for the fresh contribution and the average ^{14}a value of the winter and autumn fractions for the old component, in spring 1992 the fresh component of both fractions is less than 10 % of the total organic carbon in the fractions, but in spring 1993 this is considerably more (table 5.1): ca. 60 % fresh material in the X4 fraction and ca. 20 % in the final residue. The fresh component in the residue might be algaenan, the non-hydrolyzable insoluble macromolecules found in some algae (De Leeuw et al., 1991), but it is also possible that in the extraction procedure some compounds like carbohydrates and proteins condensed.

The $\delta^{13}C$ values of the base hydrolysate (X4) and the final residue (R4) of the spring 1993 sample are higher than those of the corresponding fractions of the winter, summer and autumn fractions, but in spring 1992 these fractions have the same $\delta^{13}C$ values as in winter, summer and autumn. Using the contributions of fresh material in this fractions (calculated from the ^{14}a data), the $\delta^{13}C$ values of the fresh component in the X4 and R4 fractions of the spring 1993 sample are calculated to be –24.5 ‰ and –21.0 ‰ respectively. The value of –24.5 % shows that the fresh component in the X4 fraction consists of lipids. The difference with the $\delta^{13}C$ of the X1 fraction of 5.2 % is typical for the difference between carbohydrates and lipids in algae (Deines, 1980). The higher value of –21.0 ‰ of the fresh component in the final residue shows that it contains a considerable amount of isotopically heavy compounds, probably condensed carbohydrates and proteins.

CONCLUSIONS

The combination of stable carbon isotope analysis with radiocarbon analysis of both bulk material and isolated chemical fractions and with molecular characterization proves to be of great value for a better insight into the origins and history of POM in estuaries. Throughout the year, POM collected in the middle part of the Ems-Dollard estuary contains a considerable fraction with a relatively high age of several kyr. However, the composition is subject to seasonal variation. In spring phytoplankton blooms cause an increase in the ^{14}a of POM. The $\delta^{13}C$ signal of this bloom is highly variable and probably depends on the location of the bloom and variations in water temperature and growth rate. Combination of isotope analysis of isolated chemical fractions with chemical characterization shows that most of the old material has a marine or estuarine origin instead of eroded material supplied to the estuary by the river Ems. However, it also shows that POM in this part of the estuary is not the same as North Sea POM, despite the identical $\delta^{13}C$ values. Apparently, POM has a high residence time in the estuary, through repeated sedimentation and resuspension.

				$\delta^{13}C$			
sample date	%OC	bulk	X1	X2	X3	X4	R4
12-02-92	3.47%	-23.54	-21.50	-20.77	-25.30	-25.28	-25.78
20-05-92	3.67%	-23.45	-21.93	-21.61		-25.29	-25.85
27-08-92	3.37%	-23.00	-20.98	-20.43		-25.23	-25.72
03-11-92	3.19%	-22.63	-20.76	-20.60	-25.47	-25.24	-25.54
05-02-93	3.80%	-23.53	-22.16	-21.37	-25.97	-25.44	-25.66
19-05-93	5.42%	-21.17	-19.30	-18.83	-24.04	-24.80	-24.64
12-08-93	3.58%	-22.61	-21.20	-21.90		-26.90	-24.30
09-11-93	3.59%	-23.10	-21.42			-25.15	-25.26

	^{14}a					
sample date	bulk	X1	X2	X3	X4	R4
12-02-92	73.6	96.6	92.9		61.6	37.4
20-05-92	79.6	100.6	95.8		64.6	43.2
27-08-92	77	98.6	95		61.1	43.2
03-11-92	72.1	97.2	91.5		56.6	38.6
05-02-93	79.1	97.2	94.2		61.3	41.2
19-05-93	90	105	97.6		88.5	54.2
12-08-93	80	97	87		56	43
09-11-93	76.3	98.8			66	

Table 5.1 Organic carbon content and $\delta^{13}C$ (in ‰ relative to V-PDB; error of 0.07 ‰) and ^{14}a (in %; error of 1 %) of bulk samples and $\delta^{13}C$ and ^{14}a of the individual fractions. Fraction designations as in fig. 5.2.

References

Aerts-Bijma A.Th., Meijer H.A.J. and Van der Plicht J. (1997) AMS sample handling in Groningen. *Nucl. Instr. and Meth. in Phys. Res. B,* 123, 221-225.

Bergamaschi, B.A., Tsamakis, E., Keil, R.G., Eglington, T.I., Montluçon, D.B., Hedges, J.I. (1997) The effect of grain size and surface area on organic matter, lignin and carbohydrate concentration, and molecular compositions in Peru Margin sediments. *Geochim. Cosmochim. Acta* 61, 1247-1260.

Boon J. J., Pouwels A. D. and Eijkel G. B. (1987) Pyrolysis high-resolution gas chromatography-mass spectrometry studies on beech wood: capillary high-resolution mass spectrometry of a beech lignin fraction. *Biochem. Soc. Trans.* 15, 170-174.

Le Clercq,M., Van der Plicht, J., Meijer, H.A.J. and De Baar, H.J.W. (1997) Radiocarbon in marine dissolved organic carbon (DOC). *Nucl. Instr. and Meth. in Phys. Res. B,* 123, 443-446.

Colijn, F. (1983) Primary production in the Ems-Dollard estuary. Ph.D. thesis University of Groningen.

Degens E.T., Guillard R.R.L., Sackett W.M. and Helleburst J.A. (1968) Metabolic Fractionation of Carbon Isotopes in Marine Plankton - 1. Temperature and Respiration Experiments. *Deep-Sea Res.* 15, 1-9.

Deines P. (1980) The Isotopic Composition of Reduced Organic Carbon. In: Fritz P. Fontes J.C. (eds.) *Handbook of Environmental Isotope Geochemistry.* Amsterdam. pp. 329-406.

Dupont, L.M. (1986), Temperature and rainfall variation in the Holocene based on comparative palaeoecology and isotope geology of a hummock and a hollow (Bourtangerveen, the Netherlands). *Rev. Palaeobot. Palynol.* 48, 71-159.

Eadie, B.J. and Jeffrey, L.M. (1973) $\delta^{13}C$ Analysis of Oceanic Particulate Organic Matter. *Mar. Chem.* 1, 199-209.

Eisma, D., Boon, J., Groenewegen, J., Ittekot, V., Kalf, J. and Mook, W.G. (1983) Observations on macro-aggregates, particle size and organic composition of suspended matter in the Ems estuary. *Mitt. Geol.-Paläont. Inst. Univ. Hamburg* 55, 295-314.

Eisma, D., Bernard, P., Cadee, G.C., Ittekot, V., Kalf, J., Laane, R., Martin, J.M., Mook, W.G., Van Put, A., Schumacher, T. (1991) Suspended matter particle size in some west-European estuaries. *Neth. J. Sea Res.* 28, 193-214.

Fontugne, M.R. and Duplessy, J.C. (1981) Organic Carbon Isotope Fractionation by Marine Plankton in the Temperature Range -1 to 31 °C. *Oceanol. Acta* 4, 85-90.

Fry, B. and Sherr, E.B. (1984) $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. *Contrib. Mar. Sc.* 27, 13-47.

Goñi, M.A. Ruttenberg, K.C. and Eglington, T.I. (1998) A reassessment of the sources and importance of land-derived organic matter in surface sediments from the Gulf of Mexico. *Geochim. Cosmochim. Acta* 62, 3055-3075.

Gottdang, A., Mous, D.J.W. and Van der Plicht, J. (1995) The HVEE ^{14}C system at Groningen.*Radiocarbon* 37, 649-656.

Harvey, H.R. Tuttle, J. H. and Bell, J.T. (1995) Kinetics of phytoplankton decay during simulated sedimentation: Changes in biochemical composition and microbial activity under oxic and anoxic conditions. *Geochim. Cosmochim. Acta* 59, 3367-3377.

Hayes, J. (1993) Factors controlling ^{13}C contents of sedimentary organic compounds: Principles and evidence. *Mar. Geol.* 113, 111-125.

Hedges, J.I. and Keil, R.G. (1995) Sedimentary organic matter preservation: an assessment and speculative synthesis. *Mar. Chem.* 49, 81-115.

Hedges, J.I., Keil, R.G., Benner, R. (1997) What happens to terrestrial organic matter in the ocean? *Org. Geochem.* 27, 195-212.

Keil, R.G., Mayer, L.M., Quay, P.D., Richey, J.E., Hedges, J.I. (1997) Loss of organic matter from riverine particles in deltas. Geochim. Cosmochim. Acta 61, 1507-1511.

Laane, R., Turkstra, E. and Mook, W. (1990) Stable carbon isotope composition of pelagic and benthic organic matter in the North Sea and adjacent estuaries. In: Facets of Modern Biogeochemistry (Ittekot V., Kempe, S. Michaelis, W., and Spitzy, A., eds.), Berlin-Heidelberg, pp. 214-224.

De Leeuw J.W., Van Bergen P.F., Van Aarssen B.G.K., Gatellier J.P.L.A., Sinninghe Damsté J.S. and Collinson M.E. (1991) Resistant biomacromolecules as major contributors to kerogen. *Phil. Trans. R. Soc. Lond. B*, 333, 329-337.

Laws, E.A., Popp, B.N., Bidigare, R.R., Kennicutt, M.C., Macko, S.A. (1995)
Dependence of phytoplankton carbon isotopic compostition on growth rate and $[CO_2]$aq: theoretical considerations and experimental results. *Geochim. cosmochim. acta* 59, 1131-1138.

Laws, E.A., Bidigare, R.R., Popp, B.N. (1997) Effect of growth rate and CO_2 concentration on carbon isotopic fractionation by the marine diatom Phaeodactylum tricornutum. *Limnol. Oceanogr.* 42, 1552-1560.

Megens, L., Van de Plicht, J., De Leeuw, J.W. (1998) Molecular, radioactive and stable carbon isotope characterization of estuarine particulate organic matter. *Radiocarbon*, 40, 985-990.

Megens, L. (2000) Isotopic and molecular characterization of particulate organic matter in coastal waters. Ph.D. thesis University of Groningen.

Macko, S.A., Helleur, R., Hartley, G., Jackman, P. (1990) Diagenesis of organic matter - A study using stable isotopes of individual carbohydrates. *Org. Geochem.* 16, 1129-1137.

Macko, S.A., Engel, M.H., Qian, Y. (1994) Early diagenesis and organic matter preservation - a molecular stable carbon isotope perspective. *Chem. Geol.* 114, 365-379.

Megens, L., Van der Plicht, J., De Leeuw, J.W. (1999) Seasonal Variations in ^{13}C and ^{14}C Concentrations in Particulate Organic Matter from the Southern North Sea. *submitted to Geochim. Cosmochim. Acta.*

Mook W.G. and Tan F.C. (1991) Stable Carbon Isotopes in Rivers and Estuaries. In *Biogeochemistry of Major World Rivers* (E.T. Degens, S. Kempe, J.E. Richie eds.) SCOPE vol. New York, 1991. pp. 245-264.

Mook W.G. and Van der Plicht, J. (1999) Reporting ^{14}C activities and concentrations. Radiocarbon 41, *in press.*

Pastorova, I., Botto, R.E., Arisz, P.W. and Boon J.J. (1994) Cellulose char structure, a combined analytical Py-GC-MS, FTIR and NMR study. *Carbohydrate Res.* 262, 27-47.

Postma, H. (1961) Transport and accumulation of suspended matter in the Dutch Wadden Sea. *Neth. J. Sea Res.* 1, 148-190.

Roeleveld, W. (1974) The Groningen coastal area. Ph.D. Thesis University of Groningen.

Saiz-Jimenez, C. (1995) The origin of alkylbenzenes and thiophenes in pyrolysates of geochemical samples. *Org. Geochem.* 23, 81-85.

Schnitzer, M. (1978) Humic substances in the environment. In: Schnitzer, M. and Khan, S.U. (eds.) Soil organic matter. Elsevier. pp. 1-64.

Schouten, S., Breteler, W.C.M.K., Blokker, P., Schogt, N., Rijpstra, W.I.C., Grice, K., Baas, M., Sinnighe Damste, J.S. (1998) Biosynthetic effects on the stable carbon isotopic compositions of algal lipids: Implications for deciphering the carbon isotopic biomarker record. *Geochim. Cosmochim. Acta* 62, 1397-1406

Sjöström, E., (1981) Wood chemistry: fundamentals and applications. Academic Press, New York.

VI

Origins of Particulate Organic Matter from the Southern North Sea determined from ^{13}C and ^{14}C Concentrations

ABSTRACT

As a new approach for the characterization and determination of the origin of particulate organic matter (POM) in coastal waters, we measured the ^{14}C activity and $^{13}C/^{12}C$ isotope ratio and applied molecular analysis by means of AMS, IRMS and pyrolysis-GCMS. This combination of analytical techniques was applied for both bulk samples and isolated fractions of POM from the North Sea off the Dutch coast. This resulted in the first observation of a strong seasonal signal in both carbon isotopes. The isotope ratios $^{13}C/^{12}C$ and $^{14}C/^{12}C$ were high for spring and summer samples and significantly lower in autumn and winter. This can be explained by the high amount of marine phytoplankton in summer and spring, and the presence of detrital material in autumn and winter.

Phytoplankton as found in the spring and summer samples was ca. 20% enriched in ^{14}C with respect to natural values, very likely caused by ^{14}C contamination of the water from the English Channel by the nuclear fuel reprocessing plant at La Hague, Normandy, France.

The combination of $\delta^{13}C$ and ^{14}C activity shows that the winter sample was not a simple mixture of relatively recent marine phytoplankton and POM from the rivers Rhine and Meuse. The $\delta^{13}C$ values of isolated fractions of POM lead to a clearly different estimate of the terrestrial contribution to the winter POM than the $\delta^{13}C$ of bulk POM (40 vs. 60 % terrestrial material). This difference can be explained by selective degradation of labile, isotopically enriched components, i.e. carbohydrates, proteins.

INTRODUCTION

Suspended Particulate Organic Matter (POM) in estuaries and coastal seas can have several sources: in situ primary production, resuspended sediment and terrestrial detritus discharged by rivers. Estimation of the relative contribution of these sources has frequently been attempted by measuring the concentrations of the carbon isotope ^{13}C (e.g. Goñi et al., 1998; Laane et al., 1990; Fry and Sherr, 1984; Salomons and Mook, 1981). However, $\delta^{13}C$ values can vary substantially in organic matter, even from one source, thus limiting the use of bulk $\delta^{13}C$ values in a simple two-component mixing model. $\delta^{13}C$ values of phytoplankton depend on light, temperature (Fontugne and Duplessy, 1981) and growth rate (Laws et al., 1995; 1997; Bidigare et al., 1999). Furthermore, lipids generally have lower $\delta^{13}C$ values than total biomass, while the $\delta^{13}C$ of carbohydrates and amino acids are higher (Hayes, 1993; Deines, 1980; Eadie and Jeffrey, 1973). Decomposition then changes the $\delta^{13}C$ of bulk organic matter (e.g. Laane et al., 1990; Benner et al., 1987), because isotopically heavy components like carbohydrates and amino acids are decomposed more rapidly than other components (Harvey et al., 1995; Benner et al., 1987).

To estimate the terrestrial/marine ratio in organic matter more accurately, ^{14}C dating has been proposed (Mook & Tan, 1991). The ^{14}C age of biomass of short living organisms is, unlike $\delta^{13}C$, the same for all organic constituents, because the effect of isotopic fractionation is removed by the normalization procedure. Thus, complementary to $\delta^{13}C$, differences in ^{14}C concentrations in organic matter from various sources can be used to determine the contributions of these sources in a mixture. In POM the ratio can be determined between young organic matter and old carbon originating, for example, from eroded terrestrial organic matter. Applying Accelerator Mass Spectrometry (AMS) for ^{14}C analysis greatly extends the possibilities of ^{14}C analysis,

because for AMS much smaller samples are needed than for the conventional radiometric method. This specifically facilitates ^{14}C analysis of specific fractions of POM.

The southern North Sea (fig. 6.1) is a shallow shelf sea with an average depth of 40 m. Geomorphology, wind patterns and tidal motion cause an anticlockwise residual circulation. Water enters the North Sea from the Atlantic Ocean from the north and via the English Channel from the South. The residual circulation and the inflow from the English Channel result in a northward current along the Dutch coast.

Possible sources of particulate organic matter in the North Sea are: 1) autochthonous biomass, 2) POM from the ocean, 3) resuspended POM of bottom deposits and 4) fluviatile POM.

Annual phytoplankton primary production per m^2 is about a factor 2 higher in the eastern than in the western coastal waters or the central North Sea. In Dutch coastal waters the primary production is ca. 200 g C m^{-2} yr^{-1} (Joint and Pomroy, 1993). Phytoplankton primary production is especially high during spring and summer blooms.

The amount of suspended matter from the ocean coming into the North Sea from the north is ca. 10 million tons annually. Approximately the same amount is entering through the English Channel (Eisma, 1981). Suspended matter from the north generally has a higher organic carbon content than suspended matter from the English Channel. About 0.4 million tons of Particulate Organic Carbon (POC) enters the North Sea in the north from the ocean per year and from the English Channel ca. 0.1-0.2 million tons per year (Eisma, 1981). Suspended matter entering the North Sea from the north is in part transported directly to the Norwegian Channel and Skagerrak where sedimentation occurs in these deep basins, and is in part transported by the

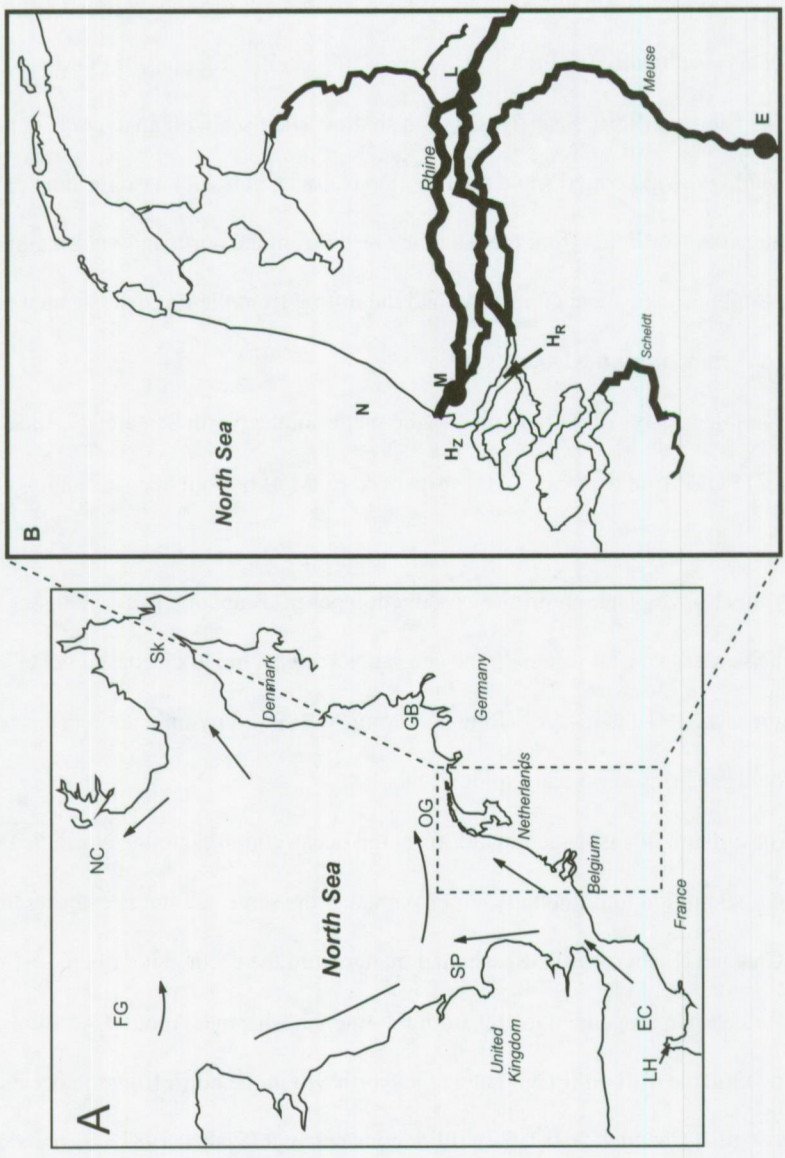

Fig. 6.1 Map of the North Sea (a) and the Dutch coast and rivers (b).EC: English Channel; FG: Fladen Grounds; GB: German Bight; LH: La Hague; NC: Norwegian Channel; OC: Oyster Grounds; Sk: Skagerrak; SP: Outer Silver Pit. N: Noordwijk; E: Eijsden; M: Maassluis; L: Lobith; H: Haringvliet (R: river; Z: North Sea near Haringvliet).

residual circulation southward through the western North Sea and then northward through the eastern North Sea after which it mainly ends up in the Norwegian Channel and Skagerrak. Repeated resuspension and sedimentation of POM in the North Sea results in an average residence time of ca. 2500 years (De Haas, 1997). In contrast, water transported along this route has a residence time of 2 to 3 years, whereas water masses entering the North Sea from the English Channel need ca. 4 months to reach the northern end of the North Sea. This water is transported in a narrow zone along the continental coast (Otto et al., 1990).

The sea floor of the North Sea mainly consists of coarse grained sandy sediments. However, in some areas the sediments are fine grained: the German Bight, the Oyster Grounds, the Fladen Grounds and the Outer Silver Pit (Eisma, 1981). These locations (indicated in fig. 6.1) are sources for suspended matter by means of resuspension.

The coastal waters of the Netherlands are influenced by three main rivers which end in the North Sea: the Rhine, Meuse and Scheldt. The rivers Rhine and Meuse together supply ca. 1.7 million tons of suspended matter per year to the sea (Eisma, 1981), which is equivalent to ca. 60,000 tons of organic carbon, assuming an organic carbon concentration in fluviatile suspended matter of 3 to 3.5 %.

Salomons and Mook (1981) measured the stable carbon isotope ratios in sediments and suspended matter in Dutch coastal waters in order to estimate the ratio of terrestrial to marine organic carbon. They measured $\delta^{13}C$ values in sediments lower than typical $\delta^{13}C$ values of marine phytoplankton and observed that low $\delta^{13}C$ values corresponded with a low ^{14}C activity. They concluded to the presence of relatively old and stable terrestrial OM in the coastal sediments. For suspended POM in the sea, no quantitative estimate was given for the terrestrial contribution. Based on stable carbon isotope concentrations in particulate **inorganic** carbonates

the authors deduced that the amount of suspended matter supplied by the Rhine and Meuse is negligible compared to total amount of suspended matter in the water column.

In this study we present variations of both stable and radioactive carbon isotope ratios combined with the chemical composition of a seasonal series of suspended particulate organic matter samples from the North Sea and samples from the rivers Rhine and Meuse. We analyzed both bulk POM and isolated fractions representing different chemical compound classes, in order to study possible effects of selective degradation. For the first time seasonal variations are observed in this combination of $^{14}C/^{12}C$ ratio, $^{13}C/^{12}C$ ratio and molecular composition of POM in a coastal sea. The data are compared with the first ^{14}C data of POM from rivers in a region of temperate climate.

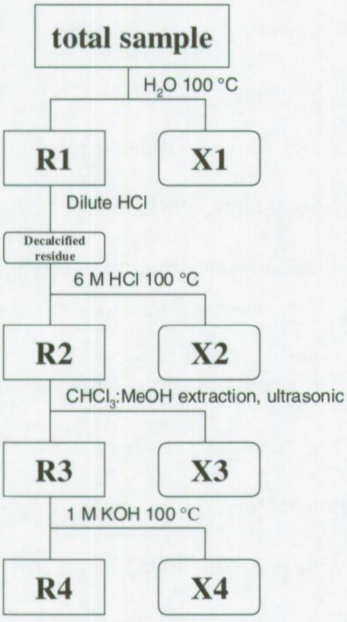

Fig. 6.2 Scheme of the extraction-hydrolysis procedure followed to obtain fractions of North Sea POM. All fractions (except X3) were analyzed for $^{13}C/^{12}C$ and $^{14}C/^{12}C$.

METHODS

Samples

Freeze-dried samples of suspended matter from the North Sea (RIKZ sample station Noordwijk 10) were obtained from the National Institute for Coastal and Marine Management (RIKZ), Haren, Netherlands. The sampling location is indicated in fig. 6.1 with the letter N. The water depth here is ca. 18 m. At this station suspended matter was sampled at three-month intervals. We analyzed samples from February, May, August and November 1994. The samples were obtained from three meters below the surface with a continuous flow centrifuge, concentrating suspended matter during 15 to 18 hours. Flow through the centrifuge was 650 l/h. The samples were stored frozen on board and freeze dried at the RIKZ.

Freeze dried samples of suspended matter from the rivers Rhine and Meuse were obtained from the National Institute for Freshwater Management (RIZA), Lelystad, Netherlands. Suspended matter at the locations of Lobith (Rhine, at the Dutch-German border; fig. 6.1, letter L), Maassluis (Nieuwe Waterweg; the estuary of the Rhine; fig. 6.1, letter M) and Eijsden (Meuse; at the Dutch-Belgian border; fig. 6.1, letter E) was sampled using a continuous flow centrifuge. Samples of suspended matter from the Haringvliet (H_R) and the adjacent North Sea (H_Z; fig. 6.1) were collected by the RIZA. The Haringvliet is a freshwater lake closed off from the North Sea by a dam. It recieves water from the Rhine and Meuse; water is discharged to the North Sea during low tide through sluices. However, during the week of sampling the sluices were left open during the whole tidal cycle.

Fractionation procedure

The Noordwijk samples were fractionated by extraction and hydrolysis using a procedure reported earlier (Megens et al., 1998), with some modifications. The procedure is shown schematically in figure 6.2. First the sample was extracted with water during 24 hours at 100 °C. After centrifugation (3000 rpm) the residue is washed repeatedly with water. Both the extract (X1) and an aliquot of the residue (R1) were analyzed. The residue was extracted with dilute hydrochloric acid (ca. 0.5 M) to remove carbonates. The resulting residue was hydrolysed with 6 M hydrochloric acid for 24 hours at 100 °C. Extract (X2) and residue (R2) were separated by centrifugation and the residue was washed with water. Then the residue was extracted ultrasonically with a mixture of dichloromethane and methanol (2:1). The residue (R3) was dried in an oven at 100 °C for 1 hour. It was hydrolyzed with 1 M KOH (aq) for 1 hour at 100 °C. The final residue (R4) was washed with water three times and freeze dried. The extract (X4) was neutralized with HCl and freeze dried. The stable and radiocarbon isotope concentrations were determined for all extract and residue fractions, except for the X3 fractions, which were to small for analysis.

table isotope analysis

For isotope analyses samples that still contained carbonates were treated with dilute hydrochloric acid to remove inorganic carbonates and dried over potassium hydroxide in vacuo. Aliquots of samples were combusted at 700 °C in quartz tubes sealed under vacuum with copper oxide as oxygen donor. The combustion gases were led over silver at 400 °C to remove halides and sulphur dioxide. Organic carbon concentrations were determined by expanding the CO_2 in a known volume and measuring the pressure. The $\delta^{13}C$ values of the purified CO_2 were measured with a VG SIRA 9 Isotope Ratio Mass Spectrometer (IRMS) in Groningen and are reported in ‰ relative to the V-PDB standard.

^{14}C analysis

The CO_2 was reduced to graphite with hydrogen and an iron catalyst at 600°C (Aerts-Bijma et al., 1997). The $^{14}C/^{12}C$ ratio of the graphite was measured with the Groningen ^{14}C - dedicated Accelerator Mass Spectrometer (AMS; Gottdang et al., 1995). The $^{14}C/^{12}C$ ratios are reported as relative activities, defined as $^{14}a = {}^{14}A/{}^{14}A_{RN}$. Here A is the activity; the subscripts R and N refer to reference and normalization, respectively. The reference corresponds to AD 1950; normalized means corrected for Isotopic fractionation to $^{13}\delta = -25‰$. The relative activity ^{14}a is given in % (Mook and van der Plicht, 1999).

Pyrolysis-Gas Chromatography-Mass Spectrometry

The chemical composition of POM samples was determined with Curie-point Flash Pyrolysis-Gas Chromatography-Mass Spectrometry (Py-GC-MS) at the Netherlands Institute of Sea Research. Analyses were performed using a Hewlett-Packard 5890 gas chromatograph, equipped with a cryogenic unit and a 25 m fused silica capillary column coated with chemically bound CP Sil-5 (0.32 mm internal diameter and a film thickness of 0.45 μm). Helium was used as a carrier gas. The Hewlett-Packard 5890 gas chromatograph was connected to a VG Autospec Ultima mass spectrometer operated at 70 eV with a mass range of *m/z* 50-800 and a cycle time of 1 s. The temperature programme was as follows: initial temperature 0°C (5 min); heating rate 3°C/min; final temperature 300°C (10 min).

Samples were pressed onto flattened ferromagnetic wires with Curie temperatures of 610°C, and placed into a pyrolysis unit (FOM-4LX; Boon *et al.,* 1987). The pyrolysis unit was connected to a Fisher 9425 high frequency generator that heated the wires inductively in 0.15 s to the Curie temperature. This temperature was maintained for 10 s.

RESULTS

Samples of suspended POM from the Noordwijk 10 sampling station (fig. 6.1), from the mouth of the Haringvliet and from the rivers Rhine (locations Lobith and Maassluis) and Meuse (location Eijsden) were collected. Organic carbon concentrations, $\delta^{13}C$ and ^{14}a values are summarized in Table 6.1.

North Sea: time series from Noordwijk 10

The organic carbon contents and carbon isotope ratios of bulk organic matter of POM from the Noordwijk 10 station in the North Sea (fig. 6.3) and carbon isotope ratios in the isolated organic matter fractions (fig. 6.4) clearly show seasonal variations: the ^{14}a values of the bulk organic matter are higher in spring and summer. During these seasons primary production is high (Joint and Pomroy, 1993), resulting in a relatively high amount of new organic carbon in the water column. The high values of ^{14}a in the May and August '94 samples correspond to high $\delta^{13}C$ values and high organic carbon contents. The organic carbon content of the August sample (35 % OC) indicates that this sample almost completely consists of organic matter. The $\delta^{13}C$ of –17.8 ‰ points to an algal origin. The freeze dried sample had a yellow-brown colour. By microscopic examination the particles in the sample appeared as a mixture of irregularly shaped green cells and elongated cell fragments, probably diatoms. The isolated fractions except the final residue R4 and the KOH hydrolysate (X4) have the same ^{14}a values as the bulk organic matter ($^{14}a \approx 120$ %), indicating the homogeneity of the organic matter *qua* source. The lower ^{14}a of the X4 and R4 indicate the presence of a small amount of older material. In theory this can also be caused by contaminants in solvents and reagents used, which are synthetic and therefore have a ^{14}C activity equal to zero. In that case 3 to 8 % of the organic carbon in these two fractions is contamination.

The chromatogram of the pyrolysate of the total sample is shown in fig 6.5c. The main constituents are aromatic and heterocyclic compounds and some short chain (branched) aliphatic

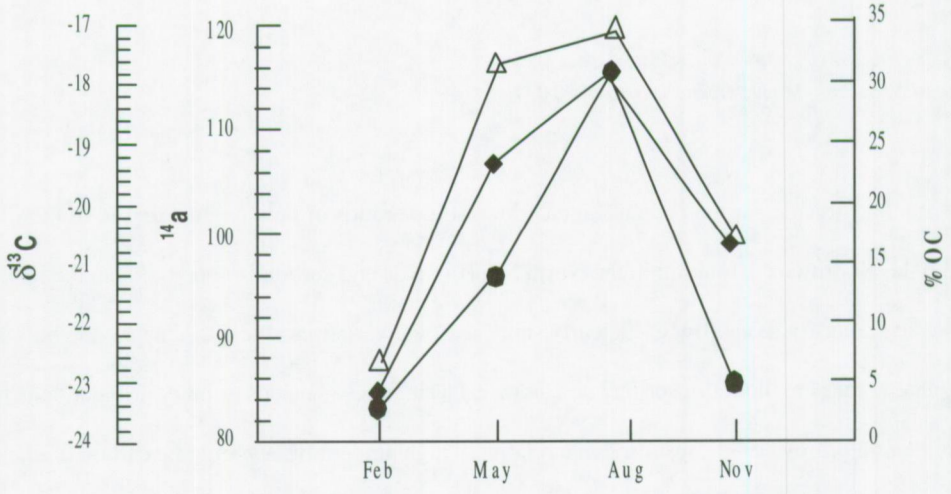

Fig. 6.3 Organic carbon concentrations of the suspended matter from the Noordwijk 10 sampling station in the North Sea (dots), $\delta^{13}C$ in ‰ vs. V-PDB (filled diamonds) and normalized ^{14}C relative abundance (^{14}a) in % (open triangles) of the organic component. This time series shows strong seasonal variations for both carbon isotopes and organic carbon content.

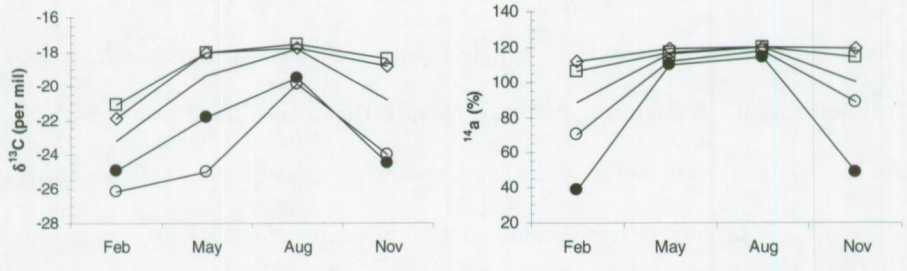

Fig. 6.4 $\delta^{13}C$ in ‰ (left) and ^{14}a in % (right) of isolated fractions of samples from the Noordwijk 10 sampling station in the North Sea. Diamonds represent hot water extracts (X1), squares the HCl hydrolysates (X2), open circles the KOH hydrolysates (X4) and closed circles the final residue (R4).

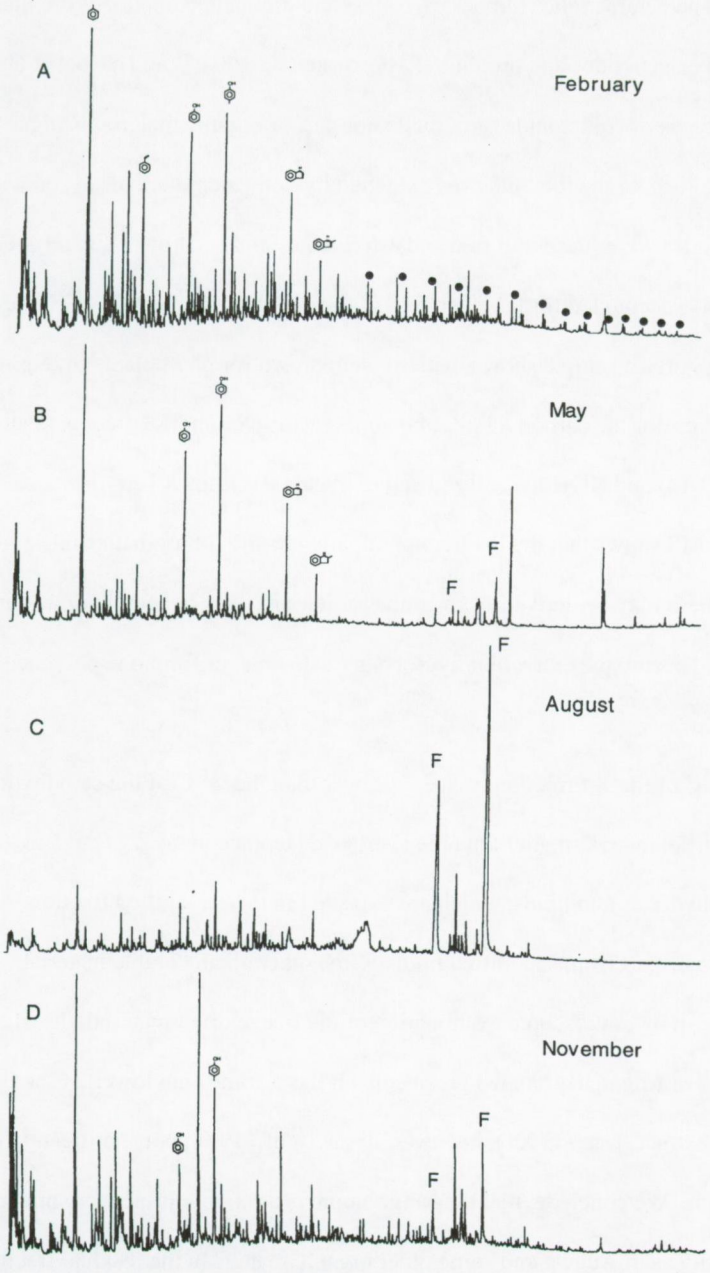

Fig. 6.5 Chromatograms of the pyrolysates of the four samples from the Noordwijk 10 sampling station in the North Sea. Dots indicate n-alkene/n-alkane series, F fatty acids.

compounds. The heterocyclic (furanes, pyrroles) and aromatic compounds are mainly pyrolysis products of polysaccharides and proteins (Pastorova et al., 1994; Van Heemst et al., 1999). These types of compounds form the major part of organic material in algae.

At least 70 % of the total OC was extracted by hot water and hydrolyzed with 6 N HCl. Much less organics were present in free and esterbound lipid fractions (X3 and X4), while the final residue (R4) accounted for less than 5 % of the OC. With respect to $\delta^{13}C$, the isolated fractions of the August sample show a pattern well known for phytoplankton (e.g. Deines, 1980), with higher values for the carbohydrates and amino acids (X1 and X2 fractions) and lower values for the residue (R4) and KOH hydrolyzed and extracted fraction (X4).

Py-GC-MS shows that the R4 fraction mainly consists of aromatic and some heterocyclic compounds. These may be derived from condensation products of carbohydrates or protein, possibly formed during the extraction/hydrolysis treatments, or from e.g. polyphenolic macromolecules.

The $\delta^{13}C$ of the X4 fraction is ca 2 ‰ lower than the $\delta^{13}C$ of the carbohydrates and proteins. The difference is smaller than the average difference in $\delta^{13}C$ of ca. 5 ‰ (Deines, 1980) between carbohydrates and lipids, which are expected in this saponified fraction. Apparently this fraction contains, apart from saponified lipids, some other alkali soluble material.

As shown by microscopic examination of the freeze dried material, the May sample mainly consists of irregularly shaped green cells. It has a somewhat lower ^{14}C activity of 116 %. The hot water extract (fraction X1), however, has a ^{14}a of 119 %, not significantly different from the August value. We conclude, that the May sample is a mixture of new organic matter with the same ^{14}C activity as in August and some older organic matter. In the residual fraction R4 the contribution of the old component is the highest. This fraction has the lowest ^{14}a value (110 %).

The X2 fraction (^{14}a 117 %) appears to contain a minor older component. As in the August sample most of the OC (ca. 60 %) is in the X1 and X2 fractions. The δ^{13}C values of the fractions show the same pattern (fig. 6.4).

The winter (February) POM sample has a much lower ^{14}C activity, indicating that it contains a considerable amount of older organic matter. The ^{14}a values of the fractions of the February sample show a wide variation, ranging from 112 % for the hot water extract to 39 % for the final residue, while δ^{13}C ranges from –21 ‰ to –26 ‰. The bulk δ^{13}C value of –23.2 ‰ is considerably lower than that of the spring and summer samples. The pyrolysate of the February sample contains aromatic and (hetero)cyclic compounds, pyrolysis products of polysaccharides and proteins, and a series of n-alkanes and n-alkenes (fig. 6.5).

The chromatogram of the pyrolysate of the final residue (R4) is dominated by peaks corresponding to C-0 to C-3 alkylbenzenes and a series of n-alkanes and n-alkenes. Typical pyrolysis products of polysaccharides and proteins are are only present in very low concentrations. The δ^{13}C value of this fraction is 3 ‰ lower than that of the hot water extract (X1) and 3.8 ‰ lower than the HCl hydrolyzate (X2).

The November (autumn) sample has a bulk ^{14}a value that is 12 % higher than that of the February sample showing that it contains more new organic material. The ^{14}a value of the hot water extract (X1 fraction) from November of 120 % and it's δ^{13}C value of –18.7 ‰ show that this fraction is (almost) entirely derived from fresh algal material. The other fractions have lower ^{14}a values pointing to a contribution of relatively old OM. The gas chromatogram of the pyrolysate shows both markers that indicate fresh material (relatively high peaks corresponding to C_{14} and C_{16} fatty acids) and old refractory material (n-alkane and n-alkene series; see fig 6.5).

Mouth of Haringvliet

POM sampled in March 1997 in the North Sea outside the Haringvliet, one of the arms of the Rhine-Meuse delta, is similar in bulk characteristics to the February 1994 sample from the Noordwijk 10 station. The average $\delta^{13}C$ and the average organic carbon concentration of 19 samples are -23.4 ± 0.4 ‰ and 3.0 ± 0.5 % respectively. Of three samples ^{14}C was measured, ^{14}a ranging from 82.6 to 86.7 %.

The Rivers Rhine and Meuse

Suspended matter was sampled in the Rhine and the Meuse where they enter the Netherlands (respectively at Lobith and Eijsden) and in two branches of the delta (near Maassluis in the Nieuwe Waterweg and in the Haringvliet; fig. 6.1). The Nieuwe Waterweg is open to the sea. The Haringvliet is normally closed off from the sea by a dam with sluices that open during low tide to discharge river water. However, suspended matter was sampled at day one and four in a period that the sluices were left open continously as part of a test to study the effects of turning the Haringvliet back into an estuarine system.

The $\delta^{13}C$ values of POM from the Rhine near Lobith (Dec. 1993) and the Meuse near Eijsden are the same as the average of measurements over several years reported more than a decade ago by Salomons and Mook (1981). The ^{14}a values, measured for the first time, are 92 and 96 % respectively. The $\delta^{13}C$ of POM sampled in the Rhine in March 1996 is 2.7 ‰ lower than in December 1993, while the ^{14}a (89.3 %) is lower as well. Py-GC-MS of the March sample

shows aromatics, heterocyclic compounds and series of n-alkanes and n-alkenes. The chromatogram is dominated by high peaks corresponding to C_{14} and C_{16} fatty acids and phytadienes, derivatives of the phytol side chain of chlorophyll, in the pyrolysate. This indicates a considerable contribution of new OM. Methoxyphenols, typical pyrolysis products of lignin (Saiz-Jimenez and De Leeuw, 1986), are clearly present. In the Nieuwe Waterweg (Maassluis) the $\delta^{13}C$ is slightly higher (–26.6 ‰) and ^{14}a is somewhat lower (90.7 %). In the Haringvliet (H_R) the $\delta^{13}C$ is similar (–27.2 ‰) to that of the upstream POM in the Rhine and Meuse, but the ^{14}a is significantly lower (ca. 85 %). The molecular composition is different from the Lobith samples. In the pyrolysate the series of n-alkenes and n-alkanes is absent.

DISCUSSION

Phytoplankton is one of the main sources of POM in North Sea water. Primary production is highest in spring and summer when phytoplankton blooms occur. Of the spring (May) and summer (August) sample, the latter consists almost exclusively of fresh phytoplankton. The ^{14}a value of 120 % is remarkably high compared to ^{14}a values of oceanic DIC and atmospheric CO_2 (ca. 110 % during 1994; Nydal, 1998). Comparably high ^{14}a values in DIC have been measured by Le Clercq et al. (1997) in the German Bight and west of Denmark, sampled in 1995. At the Frisian Front, (ca. 100 km north of the sampling station Noordwijk 10), the ^{14}a of DIC was 111 %. Macromolecular DOC sampled at the Frisian Front in May 1996, however, had a ^{14}a value of 121 % (Van Heemst et al., 1999) Such high ^{14}C concentrations, enriched with respect to natural concentrations, must originate from the nuclear industry. Le Clercq et al. (1997) suggested that the high ^{14}a observed in the German Bight was due to nuclear

power plants upstream the Elbe river. For the Noordwijk location, the likely source of the ^{14}C contamination is the nuclear fuel reprocessing plant at La Hague in Normandy, France. Approximately 95 % of the water flowing northward along the Dutch coast comes from the English Channel where this plant is located. Even higher ^{14}C concentrations, up to two times natural values, have been measured in both POC and DIC from the Irish Sea. These are attributed to discharges of the British Nuclear Fuels reprocessing plant at Sellafield (Cook et al. 1995).

The lower ^{14}a value of the May sample indicates the presence of some other OM with a lower ^{14}a value ('older OM'). A possible source for the old component is terrestrial POM discharged by the rivers, since the Noordwijk 10 station is in the zone (the plume) in which water discharged by the rivers Rhine and Meuse is transported northwards by the residual currents in the North Sea (De Ruijter et al., 1992). Another source can be resuspended sediment, which is the main source of suspended matter in the shallow North Sea (Bale and Morris, 1998). Due to transport by bottom currents, suspended matter is concentrated in the eastern North Sea (Eisma, 1981). Assuming that there is always a certain amount of qualitatively constant POM in the water column, diluted by fresh phytoplankton during spring and summer, the ^{14}a, $\delta^{13}C$ and %OC values of the February sample can be representative of the older component in the May sample. The ^{14}a value of the new phytoplankton component is known, as it is the same as in August and of the hot water extract. The $\delta^{13}C$ of the phytoplankton, however, is not necessarily the same, since it varies with temperature, light, species and/or growth rate. The $\delta^{13}C$ of the X1 fraction is quite similar (0.2 ‰ lower) to the X1 fraction of the August sample. Therefore, assuming the same difference in $\delta^{13}C$ between the X1 fraction and the bulk phytoplankton for both the phytoplankton species in May and in August, the $\delta^{13}C$ of the phytoplankton bulk OM in May is –18 ‰, somewhat lower than that of the August bulk OM.

Using the ^{14}a values in a carbon mass balance, we calculated that 90 ± 5% of the total OC in the sample is new organic carbon. This is consistent with the value obtained by calculating the mixing ratio using organic carbon concentrations. However, the $\delta^{13}C$ values point to a contribution of new phytoplankton OC of 73 ± 5 %. Therefore, the old component in the May sample does not have exactly the same characteristics as February POM, but has either a higher ^{14}a, a lower $\delta^{13}C$ or both. The suspended matter concentration in February was much higher than in May, most likely because of stronger resuspension due to seasonally varying weather conditions (Bale and Morris, 1998). Probably, in February more as well as different suspended matter occurs in the water column.

The February POM itself, as shown by its lower ^{14}a value, contains a high amount of relatively old material. The pyrolysate contains very small amounts of C_{14} and C_{16} fatty acids and phytadienes (fig. 6.5a). These compounds are rather dominant in the pyrolysate of the August sample and also quite clearly present in the pyrolysate of the May sample. Therefore, we consider them as markers for fresh phytoplankton derived OM. Their low concentration in the February sample also indicates a small contribution of fresh OM.

Another clear difference is the higher amount of styrene relative to toluene and phenol in the February sample compared to spring and summer. Styrene is even higher in samples from the river Rhine. Therefore, this appears to be a marker for terrestrial OM. Methoxyphenols, typical pyrolysis products of lignin (Saiz-Jimenez and De Leeuw, 1987), a macromolecule exclusively produced by vascular plants, were not detected. Lignin is often used as an indicator for the presence of terrestrial OM in marine sediments and suspended matter (e.g. Hedges and Mann, 1979; Bergamaschi, 1997). In the pyrolysate of POM from the Rhine, methoxyphenols could be detected as minor, but well detectable components. Thus, the absence of pyrolysis products of

lignin would indicate that the terrestrial component in this sample is negligible.

The winter bulk $\delta^{13}C$ of –23.2 ‰ seems to indicate a terrestrial contribution. On the other hand, a lower $\delta^{13}C$ can indicate degradation as well. A degradation experiment performed by Laane et al. (1990) shows a decrease of the $\delta^{13}C$ value of phytoplankton from the North Sea from –19 to –24 ‰ after 13 days incubation in the dark. This decrease can be explained by the selective degradation of labile components like carbohydrates and proteins which have relatively high $\delta^{13}C$ values (e.g. Deines, 1980). Thus, the lower winter $\delta^{13}C$ value can point to degraded phytoplankton organic matter as well. Extraction and hydrolysis of the sample show that it contains less carbohydrates and protein than phytoplankton, which could be a result of selective degradation.

However, all the isolated fractions have lower $\delta^{13}C$ values than the corresponding fractions of authochtonous phytoplankton (the summer sample). Assuming that these fractions are homogeneous with respect to stability, (labile compounds in the water extract and the HCl hydrolysate and more refractory compounds in the unhydrolyzable, insoluble residue, the KOH hydrolysate and the lipid fraction), they can not be affected by selective degradation. Therefore, in all fractions there appears to be a terrestrial component. Using the $\delta^{13}C$ values of the individual fractions of the sample and of phytoplankton in combination with estimated values for fractions of terrestrial OM, based on values of fractions from POM from the Haringvliet, terrestrial contributions to the individual fractions are calculated (table 6.2). The terrestrial contributions in all fractions are lower than the total terrestrial contribution calculated from bulk values. Thus, bulk values can not be used for an accurate estimate of terrestrial input. Apparently due to selective degradation, the isotopically lighter components were partly removed.

When comparing both $\delta^{13}C$ and ^{14}a values of bulk phytoplankton, POM from Rhine and

Meuse and the winter North Sea sample, it is clear that this sample is not a simple mixture of phytoplankton and POM from the rivers. Based on carbon and oxygen stable isotope analysis of inorganic carbonates, Salomons and Mook (1981) concluded, that suspended matter from the Rhine was at most a very small component in the suspended matter in the sea. Particulate carbonates from the Rhine could only be detected very close to the river mouth. Thus, the terrestrial OM component in this sample is not directly discharged by the rivers. According to Eisma (1981) the high concentration of suspended matter in the eastern part of the southern North Sea is due to landward transport by the residual currents in the North Sea of suspended matter in bottom water from deeper areas. Therefore, the terrestrial component probably originates from sea floor erosion. At several places in the North Sea floor terrestrial deposits such as peat are at the surface (Eisma et al., 1979), and are thus a likely source for terrestrial OM in the suspended matter.

The November sample contains considerably more new material than the February sample. The source of the new material could be phytoplankton growing during the period of the sampling or the phytoplankton blooms in spring and summer. Decay experiments performed by Harvey et al. (1995) have shown that carbohydrates from algal material disappear by bacterial mineralization in less than one month. Proteins are slightly more resistant, but also decay rapidly. Based on these experiments it might be assumed that the new organic matter in the sample is not a remnant from the summer bloom, but is mainly derived from biomass produced shortly before the sampling time.

The $\delta^{13}C$ value of the X1 fraction, entirely of algal origin as shown by its ^{14}a value, is 1.1 ‰ lower than that of the corresponding fraction of phytoplankton from August. If the difference in $\delta^{13}C$ between the X1 fraction and total OM of the producing algae is the same in August and

in November, this means that the $\delta^{13}C$ of algae growing in November is lower than that of algae growing in August. This can be caused by either a lower water temperature (Fontugne and Duplessy, 1981) or a different growth rate (Laws et al., 1995;1997; Bidigar et al., 1999). The water temperature in November was 9 °C lower than in August (12.1 and 21.3 °C resp.). According to the temperature-$\delta^{13}C$ relation found by Fontugne and Duplessy (1981) this causes the $\delta^{13}C$ of algae to be ca. 6 ‰ lower, much more than the observed difference. Also in May the temperature was ca. 9 °C lower than in August, but the $\delta^{13}C$ values were even closer together. From this it seems that the relation of Fontugne and Duplessy is not applicable in the North Sea. Another explanation can be depletion of DIC during a bloom as in spring and summer (Bakker, 1998). During a bloom the remaining DIC could become enriched in ^{13}C relative to DIC present during a period without a bloom, because algae preferably use isotopically light DIC. Thus, the organic matter synthesized in summer from the isotopically enriched DIC becomes enriched as well. This supports our hypothesis that the new component in the November POM is not produced during the summer bloom.

The ^{14}C data of POM from the Rhine and Meuse are the first published for rivers in temperate climate regions. To our knowledge ^{14}C measurements have only been reported for the Amazon river (Hedges et al., 1986). Amazon POM showed ^{14}a values higher than 100 % indicating the presence of 'bomb ^{14}C'. ^{14}C enrichment was mainly found in the coarse grained fraction, showing that a considerable part of POM in the Amazon river consisted of relatively new material. In the Rhine and Meuse, however, the ^{14}a values of POM are well below contemporary atmospheric values (cf. Meijer et al., 1994). This shows that the main constituent of POM in these rivers is relatively old.

Phytoplankton growing in the river Rhine has a relatively low ^{14}a value as well, because

the ^{14}a value of DIC is rather low (around 80%). This explains the lower ^{14}a value of POM in March 1996, which contains a considerable amount of new organic matter, as is shown by Py-GC-MS. The $\delta^{13}C$ value of this sample is lower, because DIC in the Rhine has a $\delta^{13}C$ value ranging from –11 to –9 ‰ (measured in the late 1960's; Mook and Tan, 1991), lower than the atmospheric $\delta^{13}C$.

CONCLUSIONS

In this paper we have presented the first observation of seasonal effects on the ^{14}C activity in POM in a coastal sea, and a detailed study of the isotopic composition of various components of POM. In addition, we have reported the first ^{14}C data on POM from rivers in a temperate climatic region.

We could show that, using a combination of both stable isotope and radiocarbon analysis of isolated fractions representing labile and refractive components of POM, and chemical characterization, important information about the origin of the POM is obtained that can not be obtained by measuring only stable carbon isotope ratios of the bulk OM. The use of radiocarbon in this type of study has been made possible by the advent of AMS for mg-sized samples. The use of bulk analyses leads to an overestimation of the terrestrial contribution, due to selective degradation of isotopically lighter compounds.

Particulate organic matter from the Dutch coastal waters of the North Sea in autumn and winter contains a considerable amount of relatively old organic matter. A direct input of the rivers Rhine and Meuse, the main fresh water suppliers to the area, is not detectable. The POM appears to be mainly resuspended surface sediment possibly mixed with material from the

English Channel. The oldest component has a ^{14}a value of less than 40 % and is found as insoluble non-hydrolyzable macromolecular material, mainly aliphatic in nature. These aliphatic macromolecules are found in both terrestrial plant leafs and in cell walls of algae. Based on its $\delta^{13}C$ value, this fraction appears to be for a considerable part of marine origin. This infers a high residence time of POM in the North Sea (cf. De Haas, 1997).

In spring and summer the suspended matter consists almost completely of algae, due to blooms during these periods. The algae are somewhat enriched with respect to natural values in ^{14}C (ca. 10 %), most likely due to discharge to the English Channel of radioactive inorganic carbon by the nuclear fuel reprocessing plant in La Hague, Normandy.

sampling date	SM (mg/l)	%OC	$\delta^{13}C$ (‰)	14a (%)	$\Delta^{14}C$ (‰)
North Sea					
North Sea Noordwijk 10					
3-2-94	13	2.7	-23.2	87.9	-121
6-5-94	4	13.7	-19.4	116.3	163
3-8-94	5	30.9	-17.8	119.5	195
2-11-94	2	4.5	-20.8	99.5	-5
North Sea Mouth of Haringvliet (Hz)					
11-3-97		2.3	-23.7	84.2	-158
14-03-1997A		2.2	-23.6	82.6	-174
14-03-1997B		3.3	-25.0	86.7	-133
average (n=19)		3.0±1.0	-23.4±1.4		
Rivers					
Rhine Lobith (L)					
29-12-93		3.1	-27.0	92.1	-79
13-3-96		5.9	-29.7	89.3	-107
Rhine Maassluis (M)					
10-11-93		5.0	-26.6	90.7	-93
Meuse Eijsden (E)					
12-1-93		4.4	-27.1	96.0	-40
Haringvliet (H_R; average values, n=5)					
11-3-97		3.3±0.3	-27.4±1.0	85.0±0.6	-150

Table 6.1 Suspended matter concentrations (SM), organic carbon concentrations of the suspended matter (%OC) and isotopic compositions of POM for the sampling locations shown in fig. 6.1. For the North Sea near the Haringvliet (H_Z) 19 samples were analyzed for $\delta^{13}C$ and a subset of 3 samples was analyzed for ^{14}C. Analytical errors in $\delta^{13}C$ were smaller than 0.1 ‰ and in ^{14}a around than 0.5 %.

		$\delta^{13}C$ plankton	terrestrial $\delta^{13}C$	$\delta^{13}C$ February	$x_{terrestrial}$
	bulk	-17.8	-27	-23.2	0.59
X1	H_2O extract	-17.6	-27	-21.9	0.45
X2	6N HCl hydrolysate	-17.5	-27	-21.0	0.37
X4	KOH hydrolysate	-19.9	-30	-26.1	0.61/0.5*
R4	final residue	-19.5	-31	-24.9	0.47

Table 6.2 Estimated terrestrial contribution ($x_{terrestrial}$) in the February sample, calculated from it's $\delta^{13}C$ values, using plankton $\delta^{13}C$ values measured in August as the marine end-member and estimated terrestrial $\delta^{13}C$ values.

References

Aerts-Bijma, A.Th., Meijer, H.A.J. and Van der Plicht, J. (1997) AMS sample handling in Groningen. *Nucl. Instr. and Meth. in Phys. Res. B,* **123,** 221-225.

Bakker, D.C.E. (1998) Process studie of the air-sea exchange of carbon dioxide in the Atlantic Ocean. Ph. D. Thesis Utrecht University.

Bale, A.J. and Morris, A.W. (1998) Organic carbon in suspended particulate material in the North Sea: Effect of mixing resuspended and background particles. *Continental Shelf Res.,* **18,** 1333-1345.

Bergamaschi, B.A., Tsamakis, E., Keil, R.G., Eglington, T.I., Montluçon, D.B., Hedges, J.I. (1997) The effect of grain size and surface area on organic matter, lignin and carbohydrate concentration, and molecular compositions in Peru Margin sediments. *Geochim. Cosmochim. Acta* **61,** 1247-1260.

Boon, J. J., Pouwels, A. D. and Eijkel, G. B. (1987) Pyrolysis high-resolution gas chromatography-mass spectrometry studies on beech wood: capillary high-resolution mass spectrometry of a beech lignin fraction. *Biochem. Soc. Trans.* **15,** 170-174.

Le Clercq, M., Van der Plicht, J., Meijer, H.A.J. and De Baar, H.J.W. (1997) Radiocarbon in marine dissolved organic carbon (DOC). *Nucl. Instr. and Meth. in Phys. Res. B,* **123,** 443-446.

Cook, G.T., Begg, F.H., Naysmith, P., Scott, E.M. and McCartney, M.(1995) Anthropogenic ^{14}C marine geochemistry in the vicinity of a nuclear fuel reprocessing plant. *Radiocarbon*, **37**, 459-467.

Degens E.T., Guillard R.R.L., Sackett W.M. and Helleburst J.A. (1968) Metabolic Fractionation of Carbon Isotopes in Marine Plankton - 1. Temperature and Respiration Experiments. *Deep-Sea Res.* **15**, 1-9.

Deines, P. (1980) The isotopic composition of reduced carbon. In: *Handbook of Environmental Isotope Geochemistry. Vol. 1, The terrestrial environment, A.* (Fritz, P. and Fontes, J.C., eds.) Chap. 9, pp. 329-406.

Eadie, B.J. and Jeffrey, L.M. (1973) $\delta^{13}C$ Analysis of Oceanic Particulate Organic Matter. *Mar. Chem.* **1**, 199-209.

Eisma D., Jansen J.H.F. and Van Weering T.C.E. (1979) Sea floor morphology and recent sediment movement in the North Sea. In: The Quaternary history of the North Sea (Oele E., Shuettenheim, T.E. and Wiggers, A.J. eds.). Uppsala. pp. 217-231.

Eisma, D. (1981) Supply and deposition of suspended matter in the North Sea. *Spec. Publ. Int. Ass. Sediment.* **5,** 415-428.

Fontugne, M.R. and Duplessy, J.C. (1981) Organic Carbon Isotope Fractionation by Marine Plankton in the Temperature Range -1 to 31 °C. *Oceanol. Acta* **4**, 85-90.

Fry, B. and Sherr, E.B. (1984) $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. *Contrib. Mar. Sc.* **27,** 13-47.

Goñi, M.A. Ruttenberg, K.C. and Eglington, T.I. (1998) A reassessment of the sources and importance of land-derived organic matter in surface sediments from the Gulf of Mexico. *Geochim. Cosmochim. Acta* **62,** 3055-3075.

Gottdang, A., Mous, D.J.W. and Van der Plicht, J. (1995) The HVEE ^{14}C system at Groningen.*Radiocarbon* **37,** 649-656.

De Haas, H. (1997) Transport, preservation and accumulation of organic carbon in the North Sea. Ph.D. thesis

Groningen University.

Harvey, H.R. Tuttle, J. H. and Bell, J.T. (1995) Kinetics of phytoplankton decay during simulated sedimentation: Changes in biochemical composition and microbial activity under oxic and anoxic conditions. *Geochim. Cosmochim. Acta* **59,** 3367-3377.

Hayes, J. (1993) Factors controlling ^{13}C contents of sedimentary organic compounds: Principles and evidence. *Mar. Geol.* **113,** 111-125.

Hedges, J.I. and Mann, D.C. (1979) The lignin geochemistry of marine sediments from the southern Washington coast. *Geochim. Cosmochim. Acta* **43,** 1809-1818.

Hedges, J.I., Ertel, J.R., Quay, P.D., Grootes, P.M., Richey, J.F., Devol, A.H., Farwell, G.W., Schmidt, F.W. and Salati, E. (1986) Organic carbon-14 in the Amazon river system. *Science* **231,** 1129-1131.

Van Heemst, J. D. H. , Megens, L., de Leeuw, J. W. and Hatcher P. G. (1999) Characterisation of estuarine macromolecular dissolved organic matter. *submitted to Org. Geochem.*

Joint, I. and Pomroy, A. (1993) Phytoplankton biomass and production in the southern North Sea. *Mar. Ecol. Prog. Ser.,* **99,** 169-182.

Laane, R., Turkstra, E. & Mook, W. (1990) Stable carbon isotope composition of pelagic and benthic organic matter in the North Sea and adjacent estuaries. In: *Facets of Modern Biogeochemistry* (Ittekot V., Kempe, S. Michaelis, W., and Spitzy, A., eds.), Berlin-Heidelberg, pp. 214-224.

Laws, E.A., Popp, B.N., Bidigare, R.R., Kennicutt, M.C., Macko, S.A. (1995)
Dependence of phytoplankton carbon isotopic compostition on growth rate and $[CO_2]aq$: theoretical considerations and experimental results. *Geochim. cosmochim. acta* **59**, 1131-1138.

Laws, E.A., Bidigare, R.R., Popp, B.N. (1997) Effect of growth rate and CO_2 concentration on carbon isotopic ractionation by the marine diatom Phaeodactylum tricornutum. *Limnol. Oceanogr.* **42**, 1552-1560.

Megens, L., Van de Plicht, J., De Leeuw, J.W. (1998) Molecular, radioactive and stable carbon isotope characterization of estuarine particulate organic matter. *Radiocarbon*, **40,** 985-990.

Meijer, H.A.J., Van der Plicht, J., Gislefoss, J.S. and Nydal, R. (1994) Comparing long-term atmospheric ^{14}C and ^{3}H records near Groningen, the Netherlands with Fruholmen, Norway and Izaña, Canary Islands ^{14}C stations. *Radiocarbon* **37,** 39-50.

Mook W.G. and Tan F.C. (1991) Stable Carbon Isotopes in Rivers and Estuaries. In *Biogeochemistry of Major World Rivers* (E.T. Degens, S. Kempe, J.E. Richie eds.) SCOPE vol. New York, 1991. pp. 245-264.

Mook W.G. and Van der Plicht, J. (1999) Reporting ^{14}C activities and concentrations. *Radiocarbon* **41,** 227-240.

Nydal, R. Brenkert, A. and Boden T. (1998) Carbon-14 measurements in surface water CO_2 from the Atlantic, Indian and Pacific oceans, 1965-1994. ORNL/CDIAC-104 NDP-057A
Otto, L., Zimmerman, J.T.F., Furnes, G.K., Mork, M., Saetre, R. and Becker, G. (1990) Review of physical oceanography of the North Sea. *Neth. J. Sea Res.,* **26,** 161-238.

Postma, H. (1973) Transport and budget of organic matter in the North Sea. In: *North Sea Science (E.D. Goldberg ed.)* MIT Press. pp. 326-334.

Pastorova, I., Botto, R.E., Arisz, P.W. and Boon J.J. (1994) Cellulose char structure, a combined analytical Py-GC-MS, FTIR and NMR study. *Carbohydrate Res.* **262,** 27-47.

De Ruijter, W.P.M, Van der Giessen, A. and Groenendijk, F.C. (1992) Current and density structure in the Netherlands coastal zone. In: D. Prandle (ed.) Dynamics and exchanges in estuaries and the coastal zone. *Coastal and Estuarine Sciences,* **40,** 529-550.

Saiz-Jimenez, C. and De Leeuw, J.W. (1987) Lignin pyrolysis products: Their structures and their significance as biomarkers. *Org. Geochem,* **10,** 869-876.

Salomons, W. and Mook, W.G. (1981) Field Observations of the Isotopic Composition of Particulate Organic Carbon in the Southern North Sea and Adjacent Estuaries. *Marine Geology*, **41**, M11-M20.

Summary

This thesis reports on investigations of the stable and radioactive carbon isotope distributions in sedimentary and suspended particulate organic matter (POM) in coastal waters with the attempt to determine the origin of the POM. POM in coastal waters - estuaries or coastal seas - can have a (i) marine origin, i.e. the ultimate source of the POM is marine phytoplankton, or (ii) a terrestrial origin, as rivers and winds transport POM from the continents to the sea. The rivers alone transport annually more particulate organic carbon to the oceans than the total amount of organic carbon buried in all marine sediments. This means that terrestrial POM is largely mineralized once it enters the sea. In order to understand what is the fate of terrestrial POM in the sea it is necessary to be able to distinguish between terrestrial and marine POM. One method widely used is to analyze the abundance ratio between the two stable isotopes of carbon - ^{13}C and ^{12}C, conventionally expressed as the relative deviation from that of a standard ($\delta^{13}C$). In general there is a difference between this $\delta^{13}C$ value in (marine) phytoplankton ($\delta^{13}C$ around –21 ‰) and terrestrial plants ($\delta^{13}C$ around –27 ‰), due to a phenomenon called isotopic fractionation: the carbon isotopic composition of a POM sample is a measure of fractional contribution of terrestrial and marine organic matter. However, there are some factors that complicate the use of bulk $\delta^{13}C$ values: 1) $\delta^{13}C$ values of phytoplankton (and plants) varies, depending on a number of (environmental) factors; 2) different organic compounds in the same organism have different $\delta^{13}C$ values. Generally, carbohydrates and proteins have higher $\delta^{13}C$ values than lipids and other cell components. These compounds can degrade at different rates, causing the $\delta^{13}C$ of the total organic matter mixture to change in time depending on environmental conditions.

This thesis is the result of an extensive study of the ^{14}C abundance as an additional indicator of the origin of POM. The relative ^{14}C abundance (^{14}a), related to the age of organic matter, has been proposed as a possibly useful tracer, because in many rivers POM is mainly derived from erosion of old peat deposits and soils. Because conventionally ^{14}C abundances are corrected for isotopic fractionation (normalized ^{14}a values), the so corrected values are equal for all the components in short living organisms. Therefore, selective degradation, changing the $\delta^{13}C$, will not affect the ^{14}a of organic matter of a single source.

Apart from ^{14}C as a second indicator of the POM source of the bulk sample, the isotopic analyses were applied to different constituents of total POM, assuming that these fractions are more homogeneous with respect to stability and that, consequently, the $\delta^{13}C$ value would be much less affected by selective degradation.

To obtain additional information about the nature of the POM the samples were also subjected to pyrolysis-gas chromatography-mass spectrometry.

The first two chapters of this thesis are dealing with the variation in carbon isotopic distributions with particle size in recent sediments from the North-East Pacific off the coast of the state Washington (USA) and from the Ems-Dollard estuary in North-West Europe between the Netherlands and Germany. In the sediment samples from ca. 25 cm deep on the slope and in the so-called Cascadia Basin off the Washington coast, size fractionated by SPLITT-separation, small variations in carbon isotopic compositions are observed, up to 5 % for ^{14}a and 1.3 ‰ for $\delta^{13}C$. There are no clear trends common to the three samples studied. The bulk samples were also extracted with hot water. For the two deep samples from the Cascadia Basin the $\delta^{13}C$ values show that the hot water extracts are of marine origin. Their ^{14}C ages, corrected for a reservoir effect of 400 years, are in agreement with the sediment ages obtained by other authors. The bulk samples contain a considerable amount of older material. The hot water extract of the slope sample appeared to be a mixture of organic matter of marine and terrestrial origin, as shown by the $\delta^{13}C$ value. The terrestrial component is considerably older (ca. 1000 years) than the marine component. Py-GC-MS showed that the organic matter in the three samples is mainly aromatic. Specific markers of lignin were not detected. Specific pyrolysis products of proteins were not found either.

In the surface sediment from the Ems-Dollard estuary a large difference is observed between the ^{14}a of the size fractions finer and coarser than 20 µm. Organic matter in the coarser particles has an average ^{14}a of 52 %, contrary to 80 % in the finer particles. Also $\delta^{13}C$ differs considerably: –23.1 ‰ for the <20 µm fraction and –25.6 ‰ for the >20 µm fraction. Based on these results it appears that the coarse fraction contains a higher amount of organic matter from an old terrestrial source (peat), than the fine fraction. The pyrolysates of the fine fractions contained pyrolysis products of aliphatic macromolecules and compounds derived from proteins and carbohydrates. In the pyrolysates of the coarse fractions the concentrations of the latter compounds were hardly detected.

From the Ems-Dollard estuary also suspended POM was studied. In two sets of samples taken along the salinity gradient, from spring and autumn, the spring samples show an increase in $\delta^{13}C$ and ^{14}a with increasing salinity. As is shown by comparison with isotope data of the dissolved inorganic carbon (DIC) in the estuarine water and analysis of water extractable organic matter, this is caused mainly by the fact that in the outer estuary primary production is much higher than in the inner part of the estuary. However, some organic matter produced in water with a higher salinity is found in samples from lower salinity water. All samples contain an old fraction as well. In the autumn samples no clear increase in $\delta^{13}C$ and ^{14}a is observed. In the entire estuary, except at two stations in the middle part, the $\delta^{13}C$ values are quite similar to that of POM from the Dutch coastal waters of the North Sea (–24 to –23 ‰). The ^{14}a value, however, is considerably lower than that of North Sea POM, showing that it is not identical to North Sea POM. It might be material imported from the North Sea that already spent a relatively long time in the estuary during repeated sedimentation and resuspension. The two samples from the middle part of the estuary have considerably lower $\delta^{13}C$ and ^{14}a, indicating a large terrestrial component in the POM. The pyrolysates of these samples contain higher concentrations of long chain n-alkanes, indicators of terrestrial matter. Probably an old terrestrial deposit was eroding in the deep tidal channel near the sampling sites.

$\delta^{13}C$ and ^{14}a of suspended POM vary with season, due to estuarine algal blooms in spring. Not only were the bulk isotopic compositions of a time series of POM samples spanning the years 1992 and 1993 determined, but the samples were also fractionated into a hot water extract, hydrochloric acid hydrolysate, lipid extract, KOH hydrolysate and non-hydrolyzable, insoluble residue. The phytoplankton blooms occurring in spring cause the bulk ^{14}a to increase. The effect on the $\delta^{13}C$, however, is not always the same. In the two years studied, in one spring season the $\delta^{13}C$ of the POM increased, while in the other spring season it was the same as during non-bloom periods. As was shown by isotopic analysis of the isolated fractions, this was caused by a difference in the $\delta^{13}C$ values of the fresh phytoplankton component. The cause of this difference was probably a combination of: a difference in $\delta^{13}C$ of the carbon source and effects of water temperature, being lower at the time of the spring 1992 sampling, and growth rate, which was lower at the time of sampling in spring 1992, as indicated by the lower organic carbon content. The insoluble, non-hydrolyzable residue of all samples is relatively old material (^{14}a around 40 ‰), which, based on the $\delta^{13}C$ values, appears to originate for a considerable part from marine or

estuarine phytoplankton. The main component of POM in the Ems river is peat, that started to be formed in the area around 7000 years ago, which is approximately the same age as for the residues as well. Therefore, old marine organic matter is imported into the estuary or marine organic has a high residence time in the estuary.

Also in the North Sea a seasonal variation in $\delta^{13}C$ and ^{14}a is observed as well as in organic carbon content of a series of samples of suspended POM spanning the year 1994: high values in spring and summer and lower values in autumn and winter, as in the Ems-Dollard estuary, caused by phytoplankton blooms during these periods. All four samples were fractionated into a hot water extract, hydrochloric acid hydrolysate, lipid extract, KOH hydrolysate and non-hydrolyzable, insoluble residue. The summer sample appeared to be almost homogeneous in ^{14}a, showing that the sample consists almost entirely of fresh phytoplankton. The ^{14}a value of this phytoplankton is remarkably high (120 %), compared to DIC in the ocean (ca. 110 %), to DIC (around 80 %) and POM (85 to 95 %) in the main rivers discharging to this part of the North Sea, and to the atmospheric ^{14}a value (ca. 110 %). The high value might be caused by contamination of North Sea DIC by the nuclear fuel reprocessing plant at La Haye (France). Comparison of the isotope ratios of the winter sample with $\delta^{13}C$ and ^{14}a values of POM from the rivers Rhine and Meuse show that this sample contains a considerable old component of marine origin. The terrestrial contribution calculated from the $\delta^{13}C$ values of the individual fractions is lower than resulting from the bulk $\delta^{13}C$ (40 and 60 % respectively). This can be caused by the selective removal of carbohydrates and proteins, having higher $\delta^{13}C$ values than other components from the same organism. The winter sample contains, apart from a variety of polar compounds, aliphatic macromolecules, that may have a marine or terrestrial origin. In the summer sample, (almost) entirely consisting of phytoplankton, these macromolecules were not detected. However, the $\delta^{13}C$ of the insoluble, non-hydrolyzable residue, which contained these macromolecules, indicates that at least part of it is of marine origin.

SAMENVATTING

Van de mondiale koolstofkringloop, sterk in de wetenschappelijke belangstelling gekomen vanwege een mogelijke door de mens veroorzaakte klimaatverandering, zijn de atmosfeer, de landvegetatie en de oceanen de belangrijkste compartinenten. Van de ruwweg 5,5 Gigaton koolstof die de mens jaarlijks door verbranding van olie, gas en steenkool in de lucht brengt, vindt ongeveer 2 GtC zijn weg naar de zee, terwijl het mondiale plantendek een activiteit vertegenwoordigt van mogelijkerwijs 1 GtC. De resterende 3 GtC blijft in de atmosfeer achter en is daarmee verantwoordelijk voor de toename van het broeikaseffect.

De zojuist genoemde werking van het zeewater betreft het bestanddeel aan opgeloste anorganische koolstof, CO_2, bicarbonaat- en carbonaat-ionen, tezamen een concentratie van 24 mg koolstof per liter zeewater.

Zeewater bevat bovendien een bepaalde fractie organische koolstof, zowel in opgeloste toestand –de zogenaamde DOC, of wel dissolved organic carbon- als in vorm van particulair materiaal, POC, particulate organic carbon. De concentratie hiervan is zeer variabel. Sommige delen van de oceanen zijn door gebrek aan voedingsstoffen relatief arm aan plantaardige of dierlijke organismen, andere gebieden kunnen hoge gehaltes organische stof bevatten, althans tijdens bepaalde periodes van het jaar. Een voorbeeld hiervan zijn de zogenaamde planktonbloeien.

Het is te verwachten dat in gebieden met grote concentraties aan voedingsstoffen, zoals nitraten en fosfaten, maar ook bepaalde essentiële metalen, een grote biologische activiteit heerst. Met name kan dit het geval zijn in kustgebieden waar belangrijke hoeveelheden "meststoffen" in de zee terechtkomen, over het algemeen daarheen gebracht door rivieren. Om een kwantitatief beeld te krijgen van de rol die kustgebieden spelen binnen de mondiale koolstofkringloop is het verder van belang de herkomst van de daar voorkomende organische stof te kennen. Een deel zal afkomstig zijn van het land (van terrestrische oorsprong) en naar de zee worden gevoerd door de rivieren, een ander deel zal in het zeewater zijn gevormd (van mariene herkomst).

Om onderscheid te maken tussen organische stof van mariene of terrestrische oorsprong maken we gebruik van de mogelijkheden die de isotopensamenstelling van de koolstof bieden. We zullen hierbij kiezen zowel voor meting van de verhouding waarin de stabiele (niet-radioactieve)

isotopen van koolstof, ^{12}C en ^{13}C, of voor meting van de ^{14}C-radioactiviteit. Beide isotopen zullen blijken aanvullende informatie te leveren.

De concentratieverhouding tussen de twee stabiele isotopen ($^{13}C/^{12}C$ met een grootte van ongeveer 0,01) in organisch materiaal wordt voor een belangrijk deel bepaald door dezelfde $^{13}C/^{12}C$-verhouding in de anorganische koolstofverbinding die de bron is van de organische koolstof. Zo kunnen organisch materiaal afkomstig van mariene algen of landplanten in principe van elkaar worden onderscheiden.

Echter wordt de isotopensamenstelling ook beïnvloed door de aard van het chemische omzettingsmechanisme van de anorganische naar de organisch koolstof. De consequentie hiervan is dat materiaal van dezelfde herkomst (terrestrisch dan wel marien) een verschillend $^{13}C/^{12}C$ kan hebben doordat het bestaat uit verschillende hoeveelheden van verschillende types chemische verbindingen elk met een eigen $^{13}C/^{12}C$. Daardoor kan het zelfs voorkomen dat door gedeeltelijke afbraak van het materiaal de isotopenverhouding van organisch materiaal verandert: sommige verbindingen worden namelijk sneller afgebroken dan andere.

De verwachting dat de ^{14}C methode goed gebruikt kan worden om onderscheid te kunnen maken tussen terrestrisch en marien organisch materiaal in recente sedimenten en zwevend stof in kustwateren, is gebaseerd op de veronderstelling dat terrestrisch materiaal, door rivieren naar zee getransporteerd, hoofdzakelijk afkomstig is van oude, eroderende bodems of veenafzettingen, terwijl de mariene component afkomstig is van recent geproduceerd algenmateriaal. Hiervoor waren er uit het verleden enige aanwijzingen.

Naast toepassing van de genoemde isotopenmethodes is het materiaal ook moleculair gekarakteriseerd, dwz door gebruikmaking van karateristieke chemische samenstelling van de totale organische stof. Hierbij wordt gebruik gemaakt van pyrolyse-gas-chromatografie, waarbij grote organische moleculen in stukken worden gebroken door ze snel tot een hoge temperatuur te verhitten in een inerte atmosfeer. De brokstukken worden gescheiden met behulp van gas-chromatografie en kunnen vervolgens door middel van massaspectrometrische analyse worden geïdentificeerd (py-GC-MS).

Het organische materiaal dat in rivieren of estuaria of kustwateren voorkomt is aan een ingewikkeld samenspel van fysische processen onderworpen. Het wordt aangevoerd of gevormd in zwevende toestand en wordt zo aangeduid als gesuspendeerd materiaal. Afhankelijk van de stroming van het water kan het materiaal verder worden getransporteerd dan wel sedimenteren.

Bij veranderende stromingstoestand ter plaatse kan het opnieuw in gesuspendeerde toestand worden gebracht (re-suspenderen). De organische verbindingen zijn vaak gekoppeld aan anorganisch materiaal, zoals zand of slib. Dat bij de fysische processen de korrelgrootte en de soortelijke massa van de deeltjes een overheersende rol speelt, spreekt vanzelf. Daarom wordt bij het onderzoek daaraan veel aandacht besteed.
Bij onderzoek als het onderhavige is het van belang aan de verschillende compartimenten van het totale systeem, te weten de rivieren, de estuaria, de open zee en natuurlijk de kustwateren zelf aparte aandacht te besteden. Ieder hoofdstuk legt de nadruk op een bepaald aspect van het systeem.

Hoofdstuk 1 is een verslag van een verkennend onderzoek naar de variatie in $^{13}C/^{12}C$ en ^{14}C in fracties van particulair organisch materiaal (POM), afkomstig uit de Eems-Dollard en verkregen door middel van een relatief eenvoudige opeenvolging van extracties en hydrolyse-stappen (organische oplosmiddelen, kokend water en hydrolyse door zwavelzuur, zoutzuur en een kaliumhydroxideoplossing). Er blijkt een grote variatie te zijn, zowel in $^{13}C/^{12}C$ als ^{14}C: de wateroplosbare fractie en de oplosbare fractie na hydrolyse met zoutzuur, welke samen ongeveer 50% van het totale organisch koolstof in het monster omvatten, hebben een aanzienlijk hoger ^{14}C-gehalte (zijn jonger) en hebben hogere $^{13}C/^{12}C$-waarden dan de overige fracties. Het oudste is het onoplosbare, niet-hydrolyseerbare residu dat ongeveer 17% van de totale organisch koolstof bevat. Het verschil in ^{14}C tussen het residu en het heet-waterextract (resp. 43% en 97%) is meer dan 50%.
Moleculaire analyse met behulp van py-GC-MS toont aan, dat het waterextract en het zoutzuurhydrolysaat voornamelijk uit –respectievelijk– suikers en aminozuren bestaan. Het organisch materiaal in het residu blijkt voor een aanzienlijk deel alifatische macromoleculen te bevatten, zoals die aangetroffen worden in celwanden van algen (de zogenaamde algaenanen) en in huidmondjes van landplanten (cutan). $^{13}C/^{12}C$ van het residu is ongeveer 3‰ lager dan die van de suikers en aminozuren. Dit zou kunnen wijzen op een groter terrestrisch aandeel, maar wordt waarschijnlijk grotendeels veroorzaakt door het verschil in het type verbinding. Ook het lipidenextract en het KOH-hydrolysaat vertonen een aanzienlijk lagere $^{13}C/^{12}C$, nog lager dan die van het residue. De $^{13}C/^{12}C$-waarden van de fracties duiden op een aanzienlijk marien of estuarien aandeel, ook voor het oude materiaal.

Hoofdstuk 2 behandelt de variatie in $^{13}C/^{12}C$ en ^{14}C in fracties van estuarien oppervlaktesediment gescheiden op basis van deeltjesgrootte. Het monstermateriaal is afkomstig uit het Eems-Dollardestuarium. In fracties met deeltjes kleiner dan 20 µm zijn de ^{14}C-gehalten (gemiddeld 80%) en $^{13}C/^{12}C$ ($\delta^{13}C = -23‰$) aanzienlijk hoger dan in de grovere fracties (respectievelijk 52% en –25.6‰), erop duidend dat de herkomst van het organisch materiaal in de grove en fijne fracties een verschillende is. $^{13}C/^{12}C$ van de fijne fracties lijkt sterk op die van POM in de Noordzee, en ook de ^{14}C is vergelijkbaar (zie ook hoofdstuk 6). De lagere $^{13}C/^{12}C$ van de grove fracties wijst op een groter terrestrisch aandeel met een herkomst van, mede gezien de hoge leeftijd, geërodeerd veen. Er zijn echter ook aanwijzingen in de py-GC analyses: minder suikers en eiwitachtige materialen in de grove fracties dan in de fijne. Dit kan ook -althans gedeeltelijk- de lagere $^{13}C/^{12}C$ kan verklaren. Voor een bepaling van het relatieve balang van deze effecten is het nodig om het materiaal te scheiden in labiele en refractoire componenten en daarvan de isotopensamenstelling te bepalen, of kwantitatieve metingen van de verschillende soorten verbindingen te verrichten.

In **hoofdstuk 3** worden de variaties beschreven van $^{13}C/^{12}C$ en ^{14}C in groottefracties van recente bodemsedimenten van de oceaan voor de kust van de Amerikaanse staat Washington. De fracties zijn verkregen met behulp van SPLITT fractionering, en methode die berust op een hydrodynamische scheiding van zware en lichte deeltjes. Het voordeel van deze techniek is dat daarmee deeltjes op veel kleinere diameter kunnen worden gescheiden dan met behulp van zeven.

In de drie onderzochte monsters is de maximale variatie in $^{13}C/^{12}C$ en ^{14}C tussen de groottefracties respectievelijk 1‰ en 10%, zonder dat er sprake is van een duidelijke trend. In een van de monsters nam ^{14}C af met toenemende deeltjesgrootte. In alle drie monsters vertoont $^{13}C/^{12}C$ van de groottefracties een min of meer willekeurige spreiding. De heet-waterextracten blijken een hogere ^{14}C en $^{13}C/^{12}C$ te hebben dan de bulkmonsters. Uit de $^{13}C/^{12}C$ blijkt dat het extract van het monster afkomstig van een diepte van 440 m een mengsel is van marien en terrestrisch materiaal, terwijl de extracten van de twee andere monsters afkomstig van het diepe Cascadia Basin (2153 en 2647m diep) vrijwel volledig van mariene oorsprong zijn.

De extractleeftijden, gecorrigeerd voor een marien reservoireffect van 400 jaar –overeenkomstig de schijnbare ouderdom van het zeewater en dus ook van het daarin gevormde organische

materiaal– komen goed overeen met de leeftijd van het sediment op die diepte, zoals bepaald door andere onderzoekers met behulp van een diepteprofiel van bulk ^{14}C. Het extract van het minst diepe monster bevat naast een mariene component een terrestrische component met een hogere leeftijd, ca. 1300 jaar oud ten tijde van de depositie. Deze schatting is gebaseerd op een berekening van het aandeel van terrestrisch materiaal uit de $^{13}C/^{12}C$, ervan uitgaande dat de mariene component in het extract in leeftijd overeenkomt met het sediment, zoals in de andere twee monsters.

Deeltjesgrootte heeft hier een relatief klein effect op de isotopenverhoudingen, in vergelijking met de verschillen in tussen de groottefracties beschreven in hoofdstuk 2 en in vergelijking met de verschillen tussen typen verbindingen (hoofdstuk 1). De ^{14}C leeftijd van de waterextracten blijkt in de mariene sedimenten uit de diepe oceaan blijkt een goede benadering van de leeftijd van het sediment te zijn (d.w.z. hoelang geleden het sediment afgezet werd)..

Hoofdstuk 4 is een verslag van onderzoek naar het verloop van $^{13}C/^{12}C$ en ^{14}C van POM langs de saliniteitsgradient in het Eems-Dollard estuarium, zowel in de lente, een periode met sterke algengroei, als in de herfst met weinig algengroei. In de lente kon er een duidelijke toename in zowel de $^{13}C/^{12}C$ als ^{14}C worden waargenomen met toenemende saliniteit. Ook zijn de waarden hoger dan tijdens de herfst, te verklaren door de hogere primaire productie van fytoplankton tijdens het voorjaar. Ook van deze monsters is het heet-waterextract geanalyseerd. Vergelijking met de resultaten met $^{13}C/^{12}C$- en ^{14}C-waarden van opgelost anorganisch koolstof (DIC) toont aan, dat de extracten een oudere component moeten bevatten naast vers organisch materiaal afkomstig van fytoplankton. Ook moet op grond hiervan worden aangenomen dat de toename in $^{13}C/^{12}C$ en ^{14}C van POM met de saliniteit voornamelijk wordt veroorzaakt door de veel hogere primaire productie in het buitenste deel van het estuarium dan in het binnenste deel en in veel mindere mate door horizontale menging van POM. De lagere ^{14}C-waarden in de herfst tonen aan, dat het meeste tijdens de lente geproduceerde organische materiaal is verdwenen. In de herfst van 1997 worden in twee monsters uit het midden van het estuarium veel lagere ^{14}C en $^{13}C/^{12}C$ aangetroffen dan in monsters uit de rest van het estuarium. Waarschijnlijk is dit veroorzaakt door erosie van een oude veenafzetting in de buurt van de bemonsteringsplaats. De overige monsters vertonen weinig verloop, eveneens aantonend, dat de gradiënt in de isotopensamenstelling in de lente eerder een effect is van een gradiënt in de hoeveelheid primaire productie dan van menging van twee reservoirs van verschillende soorten OM.

Hoofdstuk 5 beschrijft een tijdreeks van POM uit het midden van het Eems-Dollard estuarium. Ook hier is duidelijk het effect van hogere primaire productie in de lente op ^{14}C te zien. $^{13}C/^{12}C$ is echter in maar één van de twee bestudeerde jaren hoger in de lente dan in de andere seizoenen. Analyse van geïsoleerde componenten (zie hoofdstuk 1) toont aan dat dit te wijten is aan de lage $^{13}C/^{12}C$ van de verse component, voornamelijk vertegenwoordigd door het heet-waterextract en het zoutzuurhydrolysaat. Gedeeltelijk is dit te begrijpen uit de lagere watertemperatuur en dus lagere groeisnelheid ten tijde van de monstername, daar deze factoren van invloed zijn op de isotopenfractionering door algen.

De gemiddelde $^{13}C/^{12}C$-waarden van de fracties van de zomer-, herfst- en wintermonsters zijn vergelijkbaar met de beschreven waarden in hoofdstuk 1, en lijken sterk op waarden van POM in de Noordzee. De ^{14}C activiteit is echter lager, hetgeen aantoont, dat POM in het estarium niert hetzelfde is als POM uit de Noordzee. De relatief lage ^{14}C van het bulkmateriaal, lager dan de laagste ^{14}C van DIC in het estuarium, toont aan, dat het POM uit deze seizoenen een oude component bevat. Het residue materiaal heeft een veel lagere gemiddelde ^{14}C van ca. 40% dan de heet-waterextracten en zoutzuurhydrolysaten (ca. 97%). Dit residu bestaat voor een groot deel uit alifatische macromoleculen, welke zowel afkomstig kunnen zijn van bepaalde types van algen als van terrestrische planten. Voor een terrestrische oorsprong ligt $^{13}C/^{12}C$ van het residu vrij hoog, maar wel binnen het bereik van de $^{13}C/^{12}C$ van lipiden uit mariene of estuariene algen. Ook van de overige fracties ligt de $^{13}C/^{12}C$ binnen de mogelijke waarden voor marien of estuarien fytoplankton. Gezien de $^{13}C/^{12}C$ waarde van POM uit de rivier is het niet waarschijnlijk, dat dit een belangrijke rol speelt bij de lagere ^{14}C. De data wijzen echter op een lange verblijftijd van POM in het estuarium.

In **hoofdstuk 6** worden de isotopenverhoudingen in POM afkomstig van de Noordzee vergeleken met die uit de Maas en de Rijn en uit de Maas-Rijn delta. In een tijdreeks van vier seizoenen gedurende 1994, bemonsterd voor de kust bij Noordwijk, is een duidelijke seizoensinvloed te zien. In de lente en zomer zijn $^{13}C/^{12}C$ en ^{14}C van POM veel hoger dan in winter en herfst, veroorzaakt door een sterke algengroei in de lente en zomer. Het zomermonster blijkt bijna volledig uit algenmateriaal te bestaan, op te maken uit de hoge organische koolstofconcentratie. ^{14}C in de geïsoleerde fracties was in de meeste gevallen vrijwel gelijk, hetgeen ook aantoont dat het organisch materiaal van één en dezelfde oorsprong is. ^{14}C (120%) is 10% hoger dan op grond van ^{14}C van DIC in de oceaan zou mogen worden verwacht voor

oceanisch fytoplankton. Deze hoge waarde is waarschijnlijk te verklaren uit radioactieve contaminatie van Noordzeewater door een nucleaire opwerkingsfabriek, bijvoorbeeld in La Hague in Normandië. Ook de ^{14}C van het lentemonster lag boven natuurlijke waarden maar was lager dan die van het zomermonster. Dit bevatte een relatief oude component, gezien de lagere organisch koolstofconcentratie van het monster waarschijnlijk afkomstig van gesuspendeerd sediment. Het wintermonster en een aantal monsters genomen voor het Haringvliet gedurende het einde van de winter van 1997 hebben een lagere ^{14}C (gemiddeld 85%), maar een hogere $^{13}C/^{12}C$ (gemiddeld –23,4‰) dan POM in de grote rivieren (^{14}C 90 tot 96% en $^{13}C/^{12}C$ ca. –27‰). Hieruit blijkt, dat POM in de winter geen mengsel van autochthoon marien materiaal en POM uit de rivieren is. De rivierinput is zelfs niet waarneembaar, in overeenstemming met eerdere studie van carbonaten, waarbij ook geen fluviatiele carbonaten in Noordzee POM werd aangetroffen. Dit toont aan, dat POM in de Noordzee een relatief oude component van mariene oorsprong bevat. Op grond van de bulk $\delta^{13}C$ kan zelfs niet worden uitgesloten, dat het materiaal volledig marien is. $^{13}C/^{12}C$ van de geïsoleerde fracties zijn echter alle lager dan van de corresponderende algenfracties uit de lente en zomer. De schatting van de grootte van de terrestrische component op grond van deze getallen is aanzienlijk lager dan op grond van de bulk $^{13}C/^{12}C$ (40 tegen 60%), mogelijk veroorzaakt doordat de verbindingen met hoge $^{13}C/^{12}C$ van het relatief verse algenmateriaal, zoals suikers en eiwitten, meer zijn afgebroken dan andere componenten van de algen.

List of publications

L. Megens, J. van der Plicht and J.W. de Leeuw, Molecular, radioactive and stable carbon isotope characterization of estuarine particulate organic matter. Radiocarbon, 40 (1998) 985-990.

L. Megens, J. van der Plicht and J.W. de Leeuw, Carbon isotopes in SOM, POM and DOM from Dutch coastal waters and estuaries. IAEA techdoc 1094 (1999) 237-242.

L. Megens, J. van der Plicht and J.W. de Leeuw, Temporal variations in ^{13}C and ^{14}C concentrations in Particulate organic matter from the Southern North Sea. Submitted to Geochim. Cosmochim. Acta.

J.D.H. van Heemst, L. Megens, P.G. Hatcher and J.W. de Leeuw, Nature, origin and average age of estuarine ultrafiltered macromolecular dissolved organc matter as determined by molecular and carbon isotope characterization. Org. Geochem. 31 (2000) 847-857.

H.A.A.M. Dirven, L. Megens, M.J. Oudshoorn, M.A.Dingemanse, B. van Ommen and P.J. van Bladeren, Glutathione conjugation of the cytostatic drug ifosfamide and the role of human glutathione S-transferases. Chem. Res. Toxicol. 8 (1995) 979-986.

Curiculum Vitae

Luc Megens werd geboren op 12 mei 1970 in Aalten.. In 1988 haalde hij het VWO diploma en ging Griekse en Latijnse Taal en Cultuur studeren aan de Kath. Universiteit Nijmegen. In 1989 begon hij daar aan de studie scheikunde. Bij GLTC volgde hij de hoofdrichting Klassieke Archeologie met als scriptieonderwerp de chemische samenstelling van pigmenten in Romeinse muurschilderingen. Bij scheikunde deed hij in het kader van het hoofdvak Chemische Toxicologie bij TNO-Voeding in Zeist onderzoek naar de reactie van alkylerende cytostatica met glutathion in verband met resistentie van tumoren tegen dit type medicijnen. Als bijvak werd ook stage gelopen op het Laboratorium voor Reumatische Ziekten van het St. Radboudziekenhuis in Nijmegen. Van beide studies legde hij in 1994 het doctoraal examen succesvol af.
Van 1995 tot 1999 werkte hij bij het Centrum voor Isotopenonderzoek van de Rijksuniversiteit Groningen en bij de afdeling Mariene Biogeochemie van het Nederlands Instituut voor Onderzoek der Zee te Texel aan een door NWO gefinancierd onderzoek naar koolstofisotopen in en chemische samenstelling van particulair organisch materiaal in kustwateren, waarvan de resultaten in dit proefschrift zijn neergelegd.
Sinds januari 2000 is hij werkzaam als systeemontwikkelaar bij Mn-Services te Rijswijk.

Nawoord

Nu dit proefschrift eindelijk voltooid is moeten hier zoals in elk proefschrift de mensen genoemd worden die het mogelijk gemaakt hebben mijn promotieonderzoek te doen. Allereerst de promotores en begeleiders: Wim Mook, van wie het idee voor dit onderzoek afkomstig was en die waardevolle input heeft gegeven bij het tot stand komen van dit boekje, en de dagelijkse begeleiders Hans van der Plicht en Jan de Leeuw. Een groot deel van dit onderzoek is verricht aan monsters verzameld door het Rijksinstituut voor Kust en Zee die mij ter beschikking zijn gesteld door Foppe Smedes, die ook verder zeer behulpzaam was. Bij de monstertochten die ik zelf heb ondernomen hebben de bemanning van de Navicula en op een aantal tochten Jasper van Heemst en Niels Brussee ondersteuning verleend. De analyse van de verzamelde monsters was niet mogelijk geweest zonder de analytische staf van het CIO en NIOZ. De $^{13}\delta$ analyses werden uitgevoerd door Henk Janssen of Bert Kers, en de door mij geproduceerde CO_2 werd door Anita Aerts, Dicky van Zonneveld en Fsaha Ghebru in grafiet veranderd voor de analyse op de AMS. Ook de gedachtenwisselingen met mijn medepromovendus gedurende de eerste twee jaar Martijn le Clercq hebben een bijdrage aan dit proefschrift geleverd. Verder ben ik dank verschuldigd aan Rick Keil die mij gastvrij heeft ontvangen op de University of Washington in Seattle, waar ik sedimentmonsters geSPLITT heb.