Biogeochemistry **45:** 303–327, 1999.

The quantitative variability and monosaccharide composition of sediment carbohydrates associated with intertidal diatom assemblages

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Accepted 19 October 1998

Key words: benthic diatoms, colloidal carbohydrates, mud flats, extracellular polymeric substances, GC-MS, EPS

Abstract. The extracellular secretions of epipelic diatoms (*Bacillariophyceae*) are an important source of carbohydrates on intertidal sediments. For analytical purposes, sediment carbohydrates have been operationally separated into colloidal and bulk fractions that are often assumed to be similar in their chemical properties. However, there has been little investigation into the nature of the two fractions. In this study, carbohydrate fractions were sampled *in situ*, isolated, purified and biochemically characterised using gas chromatography-mass spectrometry (GC-MS). Both carbohydrate fractions were found to contain similar sugars although in different proportions. Glucose represented more than 80% of the monosaccharides identified in the colloidal carbohydrate fraction while only 37% of monosaccharides present in the bulk carbohydrate fraction. Colloidal carbohydrate concentrations showed short-term variability and were correlated with diatom biomass (as chlorophyll *a*) suggesting the colloidal fraction is labile and may be of recent origin, perhaps representing diatom activity. Concentrations of the bulk carbohydrate fraction did not show significant short-term variation and was therefore more refractory. This combination of biochemical and field data suggested that the bulk and colloidal carbohydrate fractions were chemically and physically different. These findings have relevance to studies of estuarine carbon cycling.

Abbreviations: LTSEM – Low-temperature scanning electron microscopy; GC-MS – Gas chromatography-mass spectroscopy; EPS – Extracellular polymeric substances

Introduction

Intertidal sediment systems are dynamic and highly biologically productive environments. Diatoms (*Bacillariophyceae*) occur in most aquatic environments and are usually the main primary producers present on intertidal mud flats, although often showing considerable patchiness in biomass distribution.

Epipelic (mud-dwelling) diatoms are a major source of carbon on intertidal sediments through the secretion of simple sugars and extracellular polymeric substances (EPS) (Decho 1990). EPS is produced through the locomotive mechanism of epipelic cells (Edgar & Pickett-Heaps 1984) which undergo cyclic vertical migration in the upper millimetres of the sediment (Palmer & Round 1967; Hay et al. 1993). EPS is composed mainly of carbohydrates (80%, Myklestad 1974) and is important for bacterial metabolism, sediment stabilisation, sessile adhesion, resistance to metal toxicity and desiccation resistance (Peterson 1987; Decho 1990; Hoagland et al. 1993 and references therein, Sinsabaugh & Findlay 1995; Becker 1996; Sutherland 1996; Paterson 1997). However, Wetherbee et al. (1998) noted that the characterisation of the substances involved in diatom adhesion and locomotion remains an important challenge.

Carbohydrates have been isolated from diatom cultures, water and sediments using various extraction techniques such as water, EDTA and acid / alkali extractions, alcohol precipitation and filtration (Myklestad & Haug 1972; Handa & Mizimo 1973; Underwood et al. 1995; Sigleo 1996; McKnight et al. 1997). Carbohydrates that are easily extracted with water from sediments have been termed "colloidal carbohydrates" (Underwood et al. 1995) and include polymeric forms (EPS) and simple sugars. 20–25% of the carbohydrates in the colloidal fraction are polymeric and this EPS may be isolated directly by alcohol precipitation (Allan et al. 1972). The carbohydrates that remain in the sediment after colloidal carbohydrate extraction are termed "bulk carbohydrates" and this fraction contains more intracellular material than the colloidal fraction and material more closely absorbed to sediment particles.

Total sediment carbohydrates, bulk and colloidal phases have been measured in a number of studies (Rogers 1965; Grant & Gust 1987; Yallop et al. 1994; Underwood & Smith 1998) but the lack of biochemical information on the fractions has led to the assumption that they represent a purely operational separation. However, bulk and colloidal carbohydrates have recently been shown to differ in their vertical distribution with sediment depth (Taylor & Paterson 1998). Colloidal carbohydrates were concentrated in the surface 200 μm and decreased in concentration with sediment depth while bulk carbohydrates increased in concentration with sediment depth. These findings suggested that the colloidal and bulk carbohydrate fractions were chemically and ecologically different. Therefore, the objective of this study was to determine if the colloidal and bulk carbohydrate fractions could be differentiated by biochemical differences.

Of the few studies into sediment carbohydrate biochemistry (see Cowie & Hedges 1984), none deal with intertidal sediments or analyse the dissolved

phase within them. Several biochemical analyses have been performed on planktonic samples and diatom mono-cultures. In these studies, carbohydrates were isolated from the culture medium by lyophilisation and were generally found to contain galactose, fucose and rhamnose as major components while mannose, glucose, xylose and arabinose were present to a lesser degree (Myklestad & Haug 1972; Smestad et al. 1974). Sulphate was commonly found to comprise 7–9% of polymeric material (Allan et al. 1972). Aluwihare et al. (1997) identified the need for research into the link between carbohydrate accumulation and algal production. This is particularly important on intertidal sediments where microphytobenthic productivity may exceed that of phytoplankton, particularly where the water is turbid (Joint 1978).

Colloidal and bulk carbohydrate fractions from sediments inhabited by benthic diatoms were examined by GC-MS analysis. In addition, this information was complimented with data from field studies describing the natural spatial and temporal variability of sediment carbohydrates fractions.

Methods

Collection of sediment for biochemical analysis

Sediment was collected from a mid-intertidal station on the Eden Estuary, Fife, U.K. (56°22′ N 02°51′ E) on 9/4/96 at 13:30 hrs (2 h after low tide), the sediment temperature was 22°C and the salinity 25. The surface 10 mm of an area 50 × 30 mm was removed and transferred into a perspex tray. From this tray, 10 syringe cores of the upper 5 mm (1.8×10^{-3} m^3) were collected and pooled for monosaccharide quantification and identification.

In situ studies of intertidal mud flats

Investigations into temporal variation of surface carbohydrate concentrations were conducted on three intertidal mud flats (Table 1). Surface sediments were sampled on the Eden Estuary over the emersion period at two upper-intertidal sites (site 1 on 20 and site 2 on 22 April 1996). Sediment samples were collected by rapidly freezing the mud flat surface using liquid nitrogen vapour (LN_2) followed by LN_2 (liquid), using the cryolander method of Wiltshire et al. (1997). Cryolanded-samples were taken at 90 min intervals and one sample from each time interval was thin-sectioned to a depth of 1400 μm in a pre-determined series (0–200, 200–400, 400–600, 600–800, 1200–1400 μm). The upper 600 μm of the other 3 replicates were analysed for colloidal carbohydrates.

Table 1. Details of field sampling methodologies on the Eden and Humber Estuaries and at Baie de Marennes-Oléron (France).

Estuary	Sampling date/s	Sampling methods	Number of time intervals
Humber Estuary (53°38′ N 0°4′ E)	sites A and B 15/4/95	surface 10 mm (cores 30 × 130 mm)	8 (n = 4)
		core depth profiles with 0.2 mm resolution (0–10 mm)	3 (n = 1)
Eden Estuary (56°22′ N 02°51′E)	site 1 (20/4/96) site 2 (22/4/96)	depth profiles (0–1.4 mm) using cryolander sampler (Wiltshire et al. 1997)	6 (20/4/96) 5 (22/4/96) 0–0.6 mm, n = 4, 0.6–1.4 mm, n = 1
Baie de Marennes-Oléron (45°55′ N 1°10′ W)	mid-intertidal (10/4/97) upper-intertidal (11/4/97)	depth profiles (0–1.5 mm) using cryolander	3 (10/4/97) n = 5 6 (11/4/97) n = 5

On the Humber Estuary, surface carbohydrate concentrations were examined hourly at upper and mid-intertidal stations (100 m and 400 m from shore) over a spring tidal emersion period. Sediment core samples (20 mm diameter × 10 mm depth). Further sediment core samples (30 mm × 130 mm) were taken at the upper-intertidal site for high resolution depth profile analysis. These cores were frozen on site (–20 °C) and sectioned at eight depth intervals (0–200, 200–400, 400–600, 1000–1200, 2000–2200, 4000–4200, 6000–6200, 10000–10200 μm) for bulk and colloidal carbohydrate analysis. Finally, at Baie de Marennes-Oléron, samples were collected over a time series from a mid-intertidal site (approximately 2.5 km from shore) on 10/4/97 and an upper-intertidal site (approximately 500 m from shore), on 11/4/97. Three sediment cores (approx. 50 mm diameter × 20 mm depth) were collected using the cryolander method. Depth intervals analysed were 0–200, 200–400, 400–600, 1000–1200 and 1400–1600 μm. Colloidal carbohydrate and chlorophyll *a* were quantified in each depth section.

Carbohydrate fractionation

Sediment carbohydrates were operationally separated into colloidal and bulk carbohydrate fractions (Underwood et al. 1995). The colloidal carbohydrate fraction within the sediment was extracted using distilled H_2O. The water and sediment were mixed to form a slurry which was incubated at 20 °C for 15 min. The liquid phase containing colloidal carbohydrates was then separated by centrifugation (1500 g for 15 min). 200 μl of the colloidal fraction was used for carbohydrate quantification. The sediment pellet (formed through centrifugation) was frozen, lyophilised and the dry weight was noted. The pellet was then homogenised and 2 mg used to determine bulk carbohydrate content.

Carbohydrate quantification

The carbohydrate concentrations of both fractions were quantified using a spectrophotometric assay (Dubois et al. 1956). 200 μl of 5% w/v of phenol was added to 200 μl of the colloidal carbohydrate fraction and mixed. 1 ml of concentrated sulphuric acid (H_2SO_4) was added to the mixture using a pump dispenser. The mixture was incubated for 35 min and sugar concentration determined against glucose standards (Liu et al. 1973). To quantify the bulk fraction, 2 mg of the homogenised, lyophilised sediment was added to 200 μl distilled H_20 and the assay protocol followed as before. After incubation, the sediment reagent mixture was centrifuged and the absorbance of the supernatant read.

Tri-fluoroacetic acid hydrolysis of carbohydrate samples

Carbohydrate fractions were hydrolysed with 50 ml of 0.1 kmol m^{-3} trifluoroacetic acid (TFA) at 100 °C for 4 hours to purify the samples (Ferguson 1992). 10 g of the lyophilised and homogenised sediment (representing the bulk carbohydrate phase) and 30 ml of colloidal carbohydrate extract were used. The solutions were cooled and filtered using sintered glass filter funnel (Quickfit SF3 A33) containing cilite clay. The filtrates were placed in a rotatory evaporator (Büchi R-114) and evaporated to dryness. Ethanol was then added and evaporated off, to remove residual TFA and remaining distilled water. Samples for gas chromatography-mass spectrometry (GC-MS) were deionised using an ion exchange column (Dowex AG50, hydrogenated form). The de-ionised samples were re-dissolved in 100 μl of distilled water and carbohydrate concentrations of the bulk and colloidal samples were measured.

Monosaccharide analysis: Methanolysis and GC-MS

Methanolysis and GC-MS allows the identification of neutral sugars, *N*-acetyl hexoamines and sialic acid (Ferguson 1992). Trimemethylsilyl (TMS) derivatization of the deionised carbohydrate samples was undertaken before the GC-MS analysis. Glass capillary tubes (SMI size J, yellow band) were prepared by heat cleaning. One end of the tube was sealed and a carbohydrate sample which corresponded to a concentration of 5 μg ml^{-1} of glucose equivalents was added. A pre-prepared series of monosaccharide standards were added to the samples. These contained 0.5 nmol m^{-3} each of rhamnose, xylose, mannose, galactose, glucose, *N*-acetylgalactosamine and sialic acid. 1 nmol m^{-3} of *scyllo*-inositol was added to the samples. Further monosaccharide standards containing arabinose, fucose, xylose and *scyllo*-inositol were prepared and analysed after the samples. The microtubes were dried in a speed vacuum concentrator (Stratech Scientific). The contents were dissolved in 20 μl methanol and dried, then dissolved in 50 μl of 0.5 mol m^{-3} HCl in dry methanol. The capillaries were sealed and placed in a heating block at 80 °C for 4 h. Methanolysis causes the breakdown of glucosidic bonds in the monosaccharides, this results in the production of methylglycosides. After cooling, the capillaries were broken open and 10 μl of 2-methyl 2-propanol was added and then dried using the speed vac concentrator, followed by the addition of 20 μl of methanol, then 10 μl of pyridine to neutralise any remaining HCl. 10 μl of acetic anhydride was added to each capillary tube to *N*-acetylate any free amines and the samples were incubated for 25 min at 20 °C (Ferguson 1992). The contents were again dried and dissolved in 20 μl of methanol and dried once more to ensure thorough desiccation. Finally, 15 μl of trimethylsilyl (TMS) was added to the capillaries which were sealed with teflon tape and incubated at 20 °C for 10 min.

1 μl of the standards mixture was injected into the GC-MS (Hewlett Packard). The carrier gas was helium and the column was Econocap SE54 (30 m × 0.25 mm). Injections were made using a split/splitless injector. The programme was as follows: injection temperature 80 °C (1 min) starting temperature 140 °C (1 min) then increasing at 5 °C per min to 260 °C and finally 15 °C per min to 300 °C. The run time was approximately 45 min. There were sufficient carbohydrates in the samples to use 0.5 μl of the bulk and colloidal samples. The monosaccharide composition of the sediment samples was determined through the comparison of the sediment samples with the monosaccharide standards. The concentration of each monosaccharide in the samples (nmol l^{-1}) may be calculated after Ferguson (1992) and the accuracy of the technique is approximately ± 15%.

Low-temperature scanning electron microscopy (LTSEM)

Low-temperature scanning electron microscopy (LTSEM) was used to visualise sediment samples collected during the field study of the Eden Estuary (20–22/4/96). Samples were rapidly frozen *in situ* by liquid nitrogen (LN_2) vapour followed by LN_2, (liquid) using the method of Wiltshire et al. (1997). Samples were stored under LN_2 until examined (Paterson 1986). The samples were visualised using a SEM (Jeol JSM 35CF), modified for cryogenic examination (Oxford Instruments CT1500). LTSEM of samples prevents water loss and preserves the structure of hydrated material (Paterson 1995; Défarge 1997).

Results

Monosaccharide GC-MS analysis

The mean *in situ* concentration of colloidal carbohydrates (on surface sediments) used in the chemical monosaccharide analysis was 1.8 mg glucose equivalents g^{-1} dry sediment (n = 10). The importance of primary chemical purification by TFA hydrolysis was shown by a comparison of two colloidal carbohydrate samples (Figure 1). The untreated colloidal sample appeared brown in colour and the baseline of the Total Ion Chromatogram (TIC) was unstable (Figure 1a), suggesting contamination of the sample with other compounds, possibly lipids. Furthermore, monosaccharide amounts in the untreated colloidal sample were much lower than in the TFA treated sample, (Figure 1b), which may indicate that noncarbohydrate substances in the sample were masking the signal of the sugars. In the TFA treated samples, TICS show that colloidal and bulk sediment carbohydrate fractions were dominated by glucose and contained the same variety of pentoses and hexoses (Figure 2). However, the ratio of glucose to other monosaccharides in the colloidal and bulk carbohydrate fractions were markedly different (Figure 3, Table 2). The colloidal fraction had a greater proportion of glucose to other monosaccharides (82%) than the bulk fraction (37%).

In situ bulk and colloidal measurements over a tidal emersion period

The collection of surface sediments over a tidal emersion period revealed that concentrations of colloidal carbohydrates increased significantly at sites 1 and 2 on the Eden Estuary (Figure 4, 20/4/96, $F_{4,8} = 10.41$, $P = 0.003$; 22/4/95, $F_{4,10} = 7.17$, $P = 0.005$ one-way ANOVA), at both sites on the Humber Estuary (Figure 5, $F_{7,24} = 8.3$, $P = 0.000$, $F_{7,24} = 17.95$, $P = 0.000$), and at

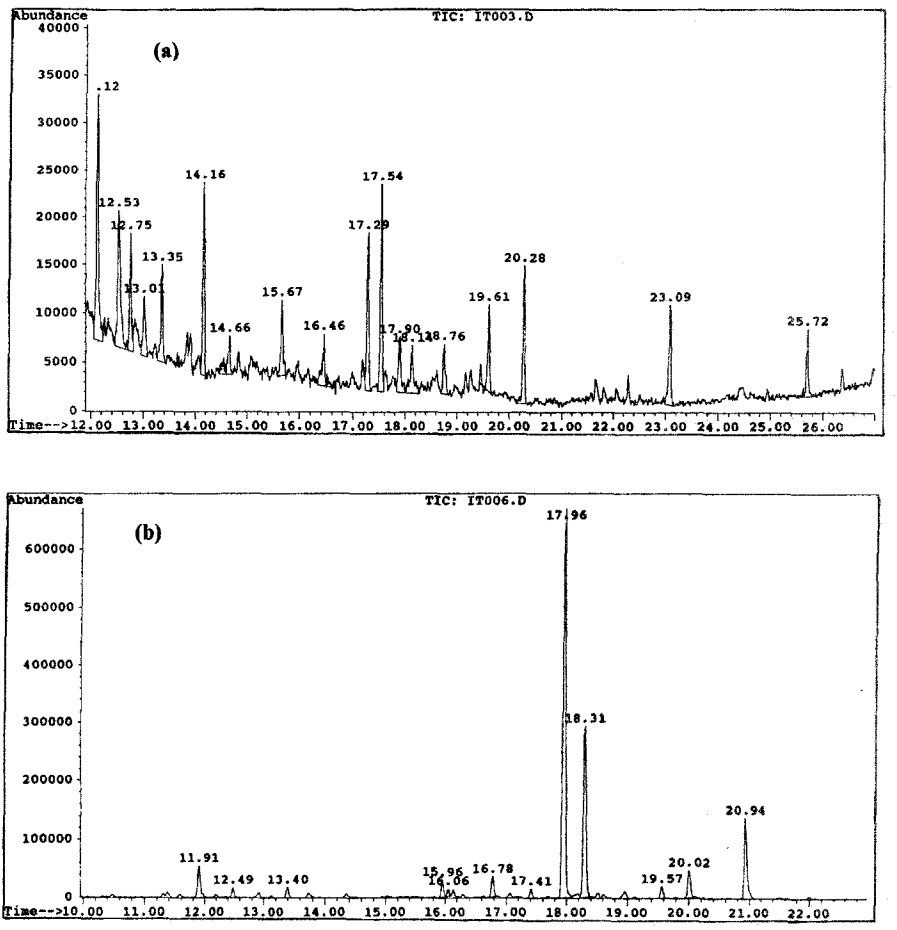


Figure 1. Two total ion chromatographs (TIC) illustrating the effect of TFA purification on sample integrity during GC-MS analysis. (a) Untreated sample. (b) TFA treated sample.

the upper-intertidal site at Baie de Marennes-Oléron (Figure 6a, $J^* = 10.9$, $P = 0.01$, $n = 32$, Jonckerre's test was chosen for this data set as the variances were heterogeneous, preventing the correct use of ANOVA, Siegel and Castellan 1988). However, the increases in colloidal carbohydrates over the emersion periods were not always consistent, for example, at site 1 on the Eden Estuary there was a decrease at the last measurement. In addition, on the Humber Estuary, colloidal carbohydrate concentrations increased sharply towards the end of the emersion period and the increase was steeper than those of the Eden Estuary. The concentrations of colloidal carbohydrate did not vary significantly over the emersion period at the mid-intertidal site at Baie de Marennes-Oléron (Figure 6b, $F_{2,57} = 1.86$, $P = 0.165$). The bulk

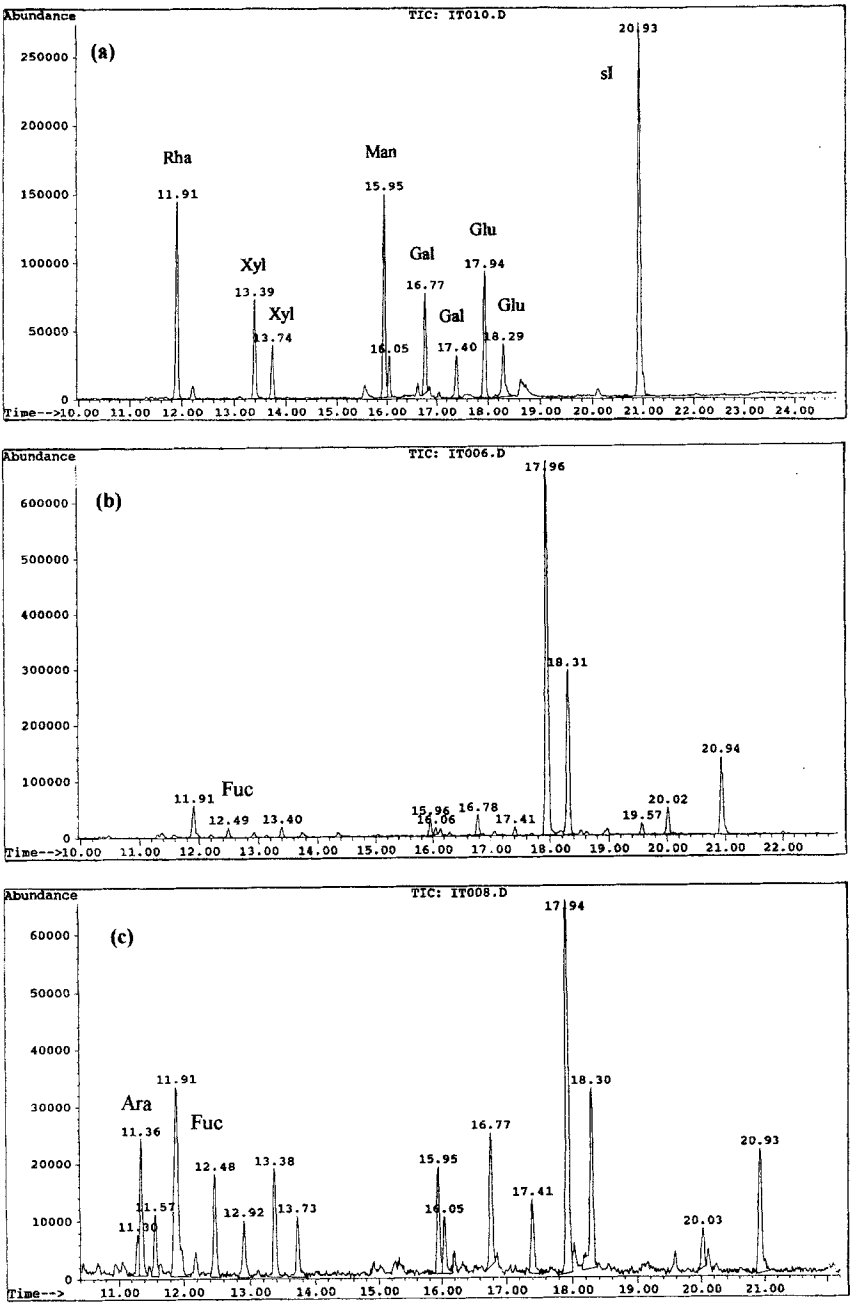


Figure 2. Total Ion Chromatograms (TIC) of the GC-MS carbohydrate analysis, (a) sugar standards (b) colloidal carbohydrate sample (c) bulk carbohydrate sample (Gal: galactose, Glu: glucose, Man: mannose, Xyl: xylose, Rha: rhamnose, sI: *scyllo*-inositol. Note; arabinose (Ara) and fucose (Fuc) standards, with *scyllo*-inositol, were run separately.

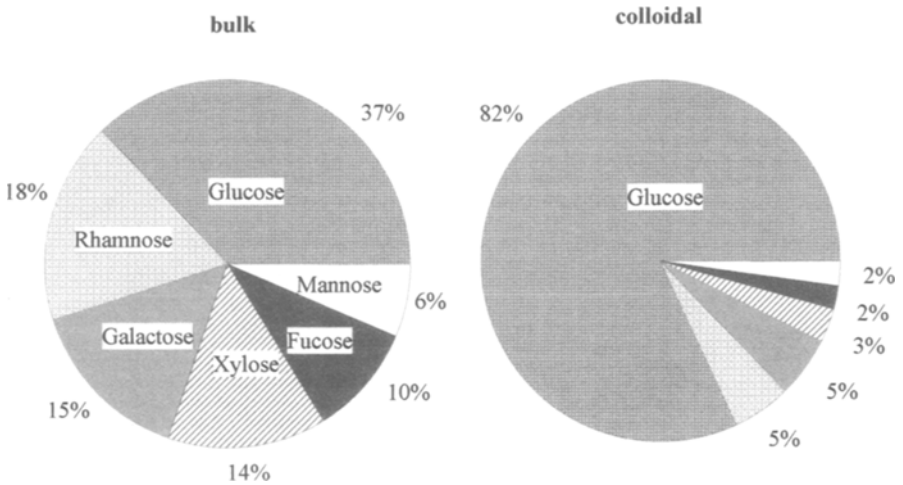


Figure 3. The variation in monosaccharide composition (%) of bulk and colloidal samples. Key for left diagram applies to both charts.

Table 2. The MRRFm and concentrations of individual monosaccharides identified in each carbohydrate fraction using GC-MS analysis (μmol ml^{-1}, calculated from internal standards, Ferguson 1992).

monosaccharide	MRRFm*	bulk fraction	colloidal fraction	ratio (b : c)
arabinose	4.25	0.015	0.0015	1: 0.1
rhamnose	0.46	0.31	0.073	1: 0.2
fucose	0.31	0.17	0.029	1: 0.2
xylose	0.24	0.24	0.041	1: 0.2
mannose	0.51	0.11	0.030	1: 0.3
galactose	0.25	0.26	0.076	1: 0.3
glucose	0.32	0.64	1.134	1:1.8

*MRRFm = Molar Relative Response Factor of monosacharide (area of monosaccharide peak/area of internal standard peak).

sediment carbohydrate fraction was measured only for the Humber Estuary and did not vary significantly over the emersion period at the upper or mid-intertidal stations (Figure 7, $F_{7,24}$ = 1.38, P = 0.3; $F_{7,24}$ = 2.15, P = 0.07, respectively).

High resolution depth profiles of colloidal carbohydrate concentrations from the Eden, Humber and Baie de Marennes-Oléron mudflats showed that temporal variability was greatest in the upper 0.2 mm and least variable at the lowest depth intervals (Figures 8, 9 and 10). A positive correlation was shown between colloidal carbohydrate and chlorophyll *a* concentrations at

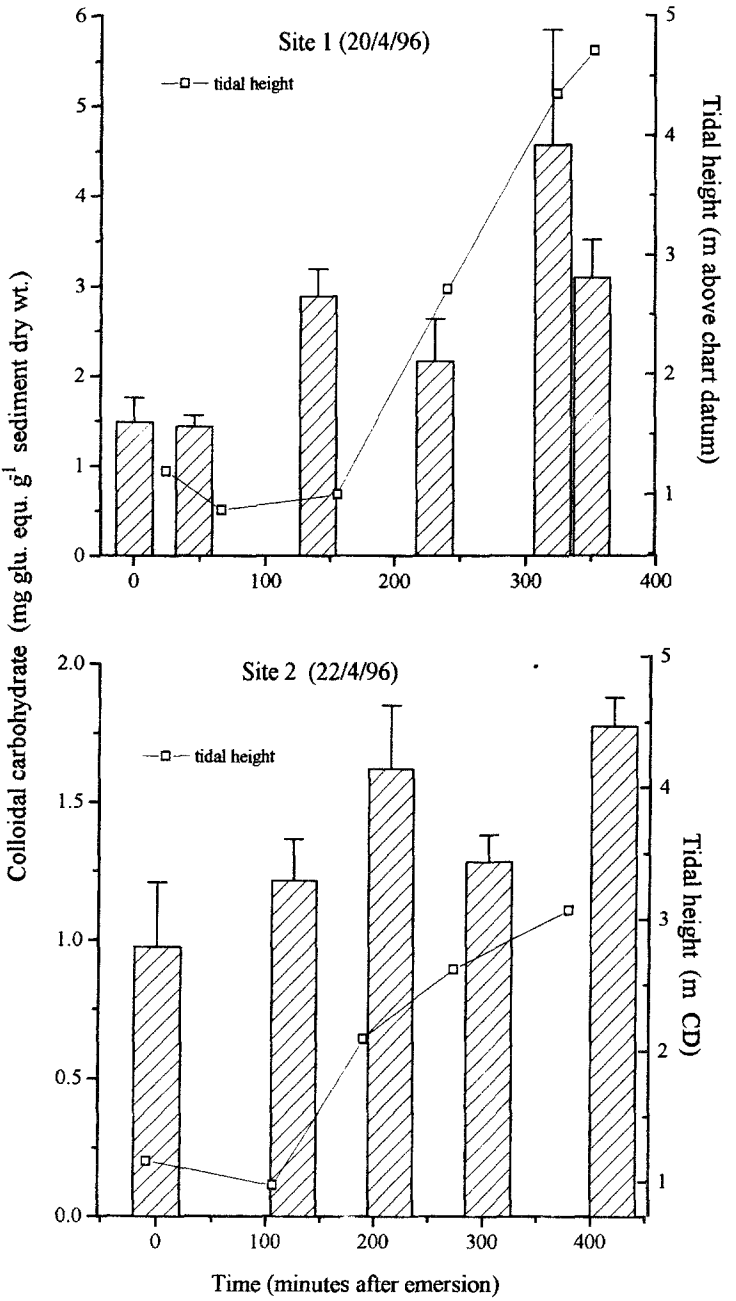


Figure 4. Increase in colloidal carbohydrate concentration (bars) over the emersion period at sites 1 and 2 on the Eden Estuary (n = 3, bars = 2 × standard error, SE). Background line indicates tidal curve for the site on the sample day.

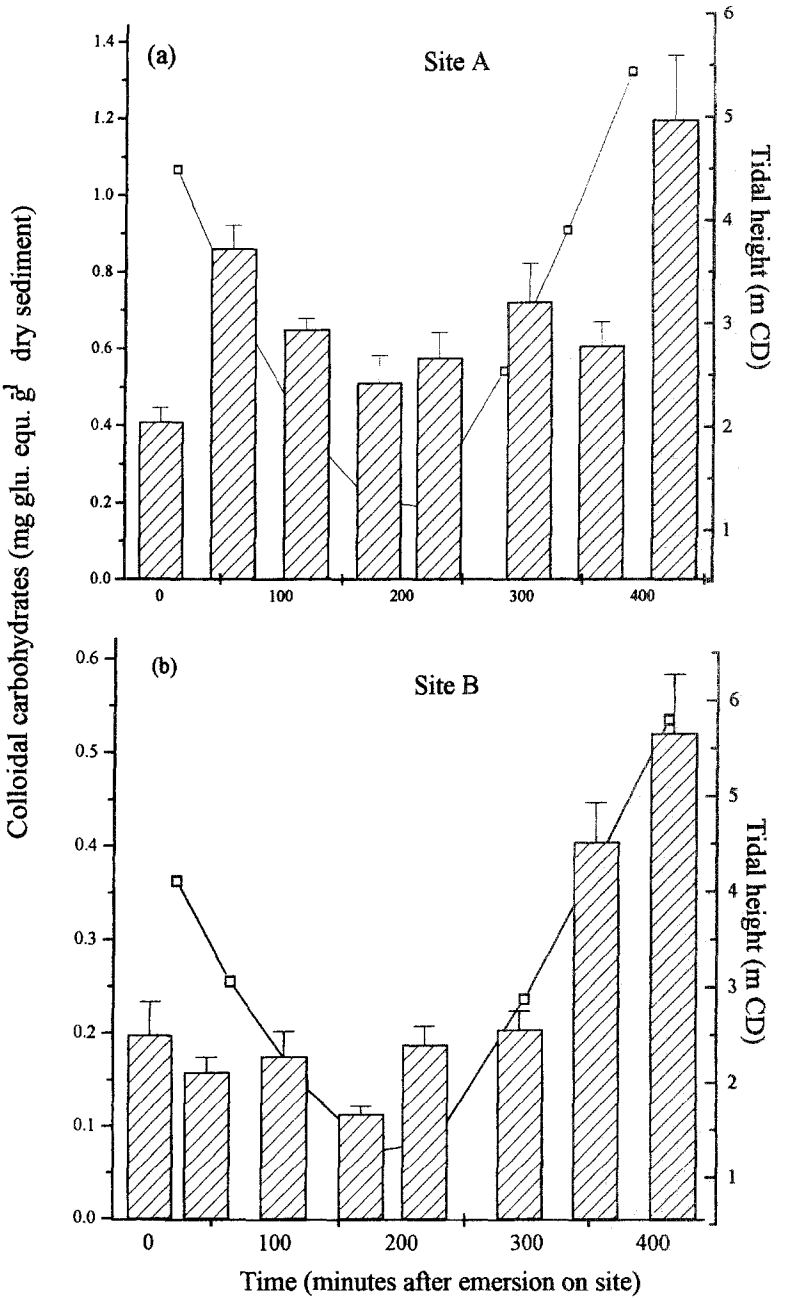


Figure 5. Increase in colloidal carbohydrate concentration (bars) over the emersion period at sites A and B on the Humber Estuary (n = 4, bars = 2 × SE). Background line indicates tidal curve for the site on the sample day.

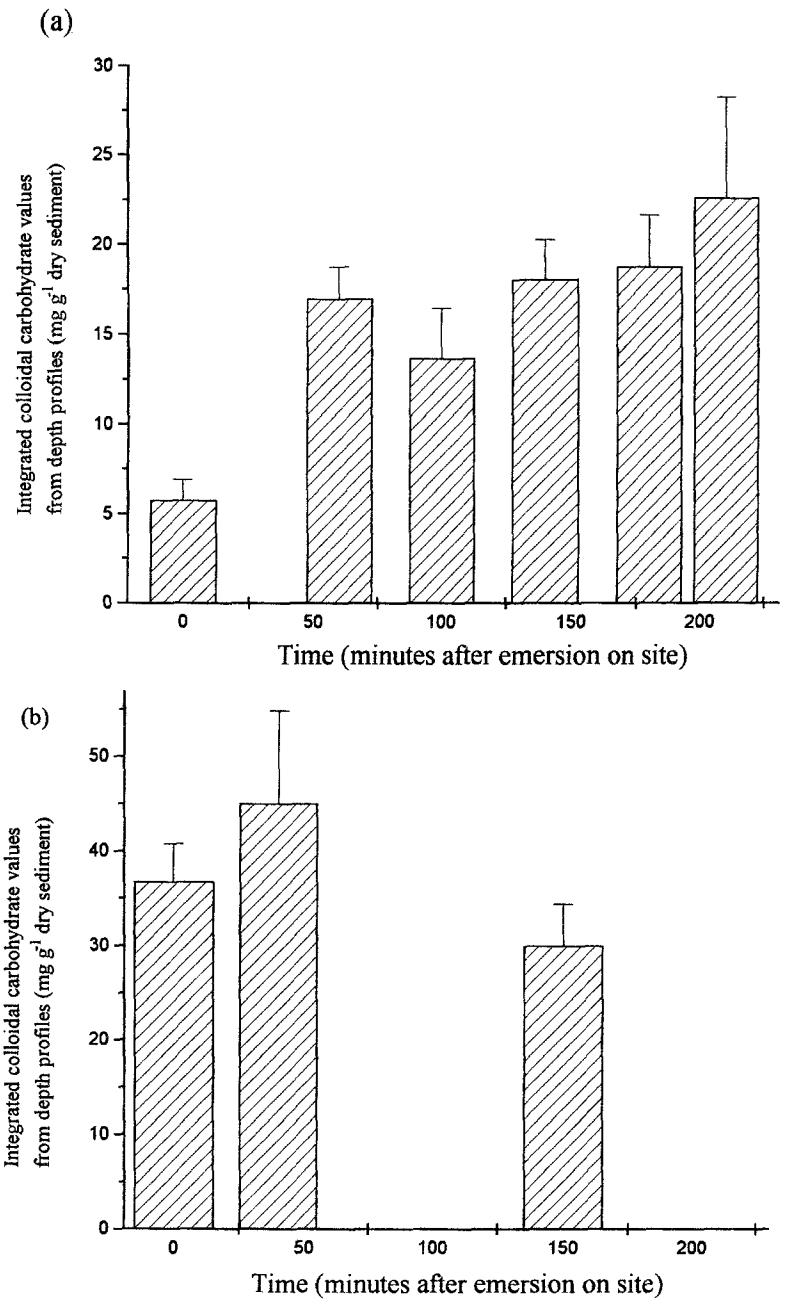


Figure 6. Colloidal carbohydrate concentrations over the emersion period at (a) the mid-intertidal and (b) upper-intertidal sites at Baie de Marennes-Oléron (n = 5, bars = 2 × SE).

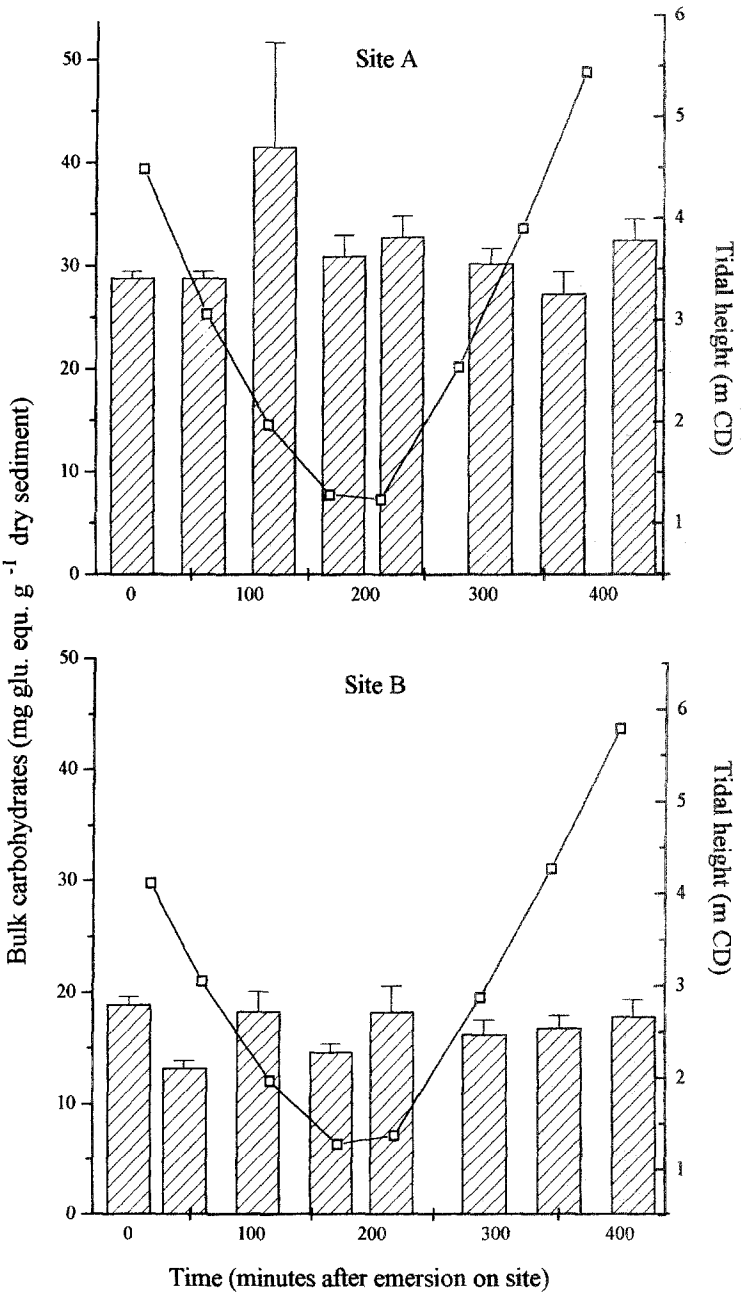


Figure 7. Concentrations of bulk sediment carbohydrates (bars) over the emersion period at (a) site A and (b) site B on the Humber Estuary (n = 3, bars = 2 × SE). Background line indicates tidal curve for the site on the sample day.

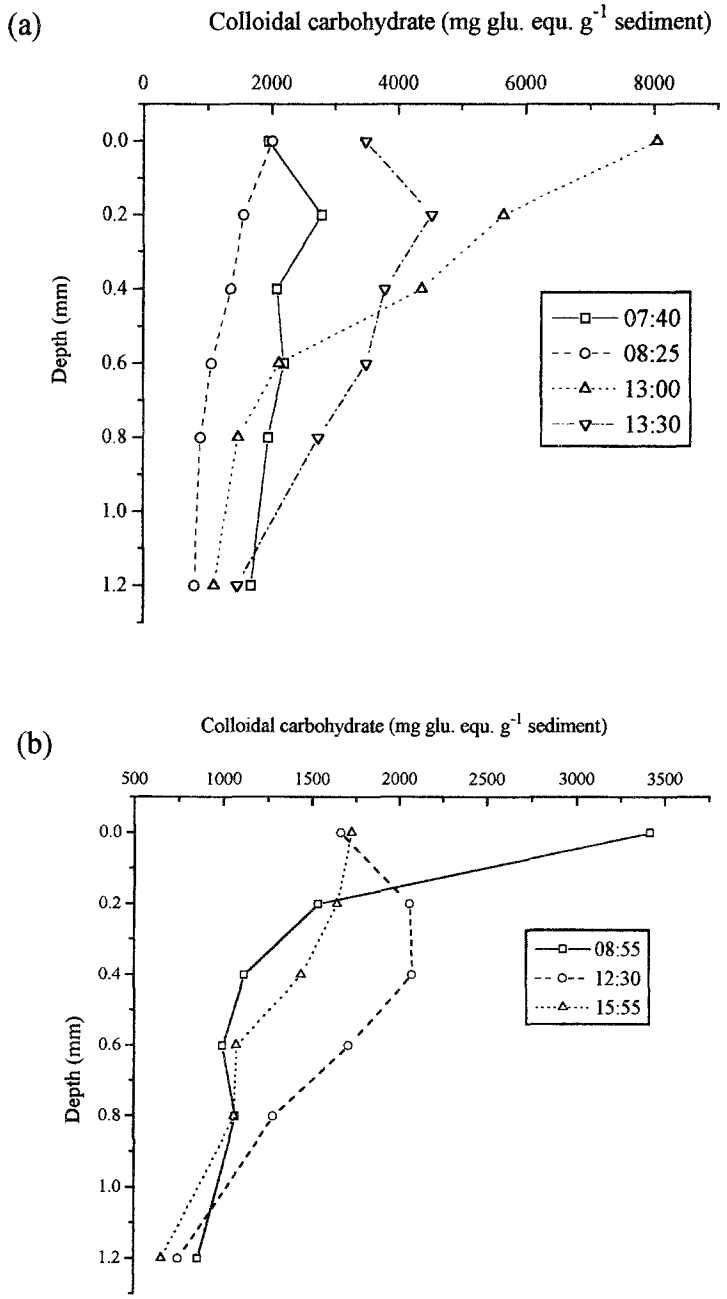


Figure 8. High resolution depth profiles of colloidal carbohydrates in the upper 1.4 mm over the emersion period from sites 1 and 2 on the Eden Estuary.

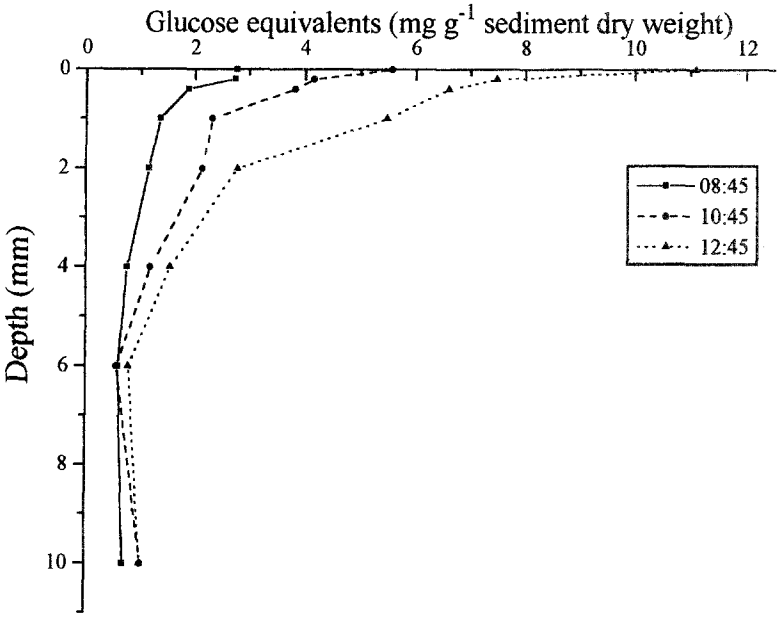


Figure 9. High resolution depth profiles of colloidal carbohydrates in the upper 10 mm over the emersion period from sites A on the Humber Estuary.

the mid-intertidal site (r^2 = 28%, $F_{1,56}$ = 21.82, P = 0.000) but not at the upper-intertidal site (r^2 = 2%, $F_{1,147}$ = 2.97, P = 0.09). In addition, the ratio of colloidal carbohydrate to chlorophyll *a* did not vary with sediment depth at either site (mean 54:1, $F_{4,52}$ = 0.2, P = 0.9 and 52:1, $F_{4,141}$ = 0.4, P = 0.8 at mid and upper-intertidal sites respectively, log 10 transformed data).

Low-temperature scanning electron microscopy (LTSEM)

LTSEM examinations of the surface sediments collected during the field studies on the Eden Estuary showed epipelic diatoms associated with clay minerals and the sediment matrix (Plate 1a, b). Cyanobacterial trichomes and several epipelic diatom species were observed on the sediment surface, including *Gyrosigma* spp. and *Navicula* spp. A polymeric matrix was clearly visible in several different forms including thin fibrils, thick extensive sheets surrounding the diatom cells and as thin sheets between sediment particles (Plate 1), probably formed as a result of the formation of ice crystal segregation zones (Jeffree & Read 1991). In addition, diatom cells were embedded in a globular matrix on the surface of intertidal sediments. The diatom assemblage on the mud flats of the Eden Estuary were dominated by *Diploneis crabro* (Droop pers. comm.) with *Navicula* spp., *Gyrosigma* spp., *Nitzschia* spp., *Surirella* sp. and *Rhoicosphenia curvata* also present.

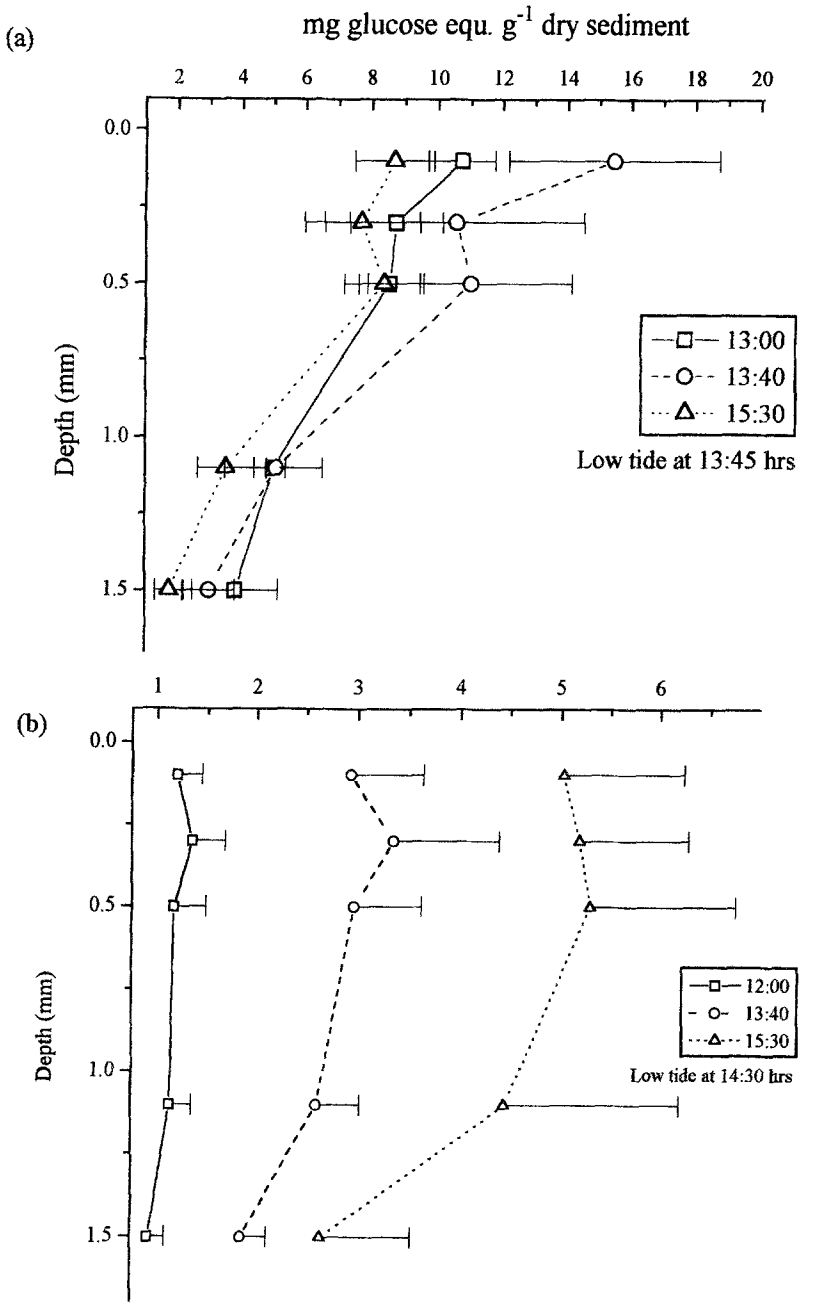
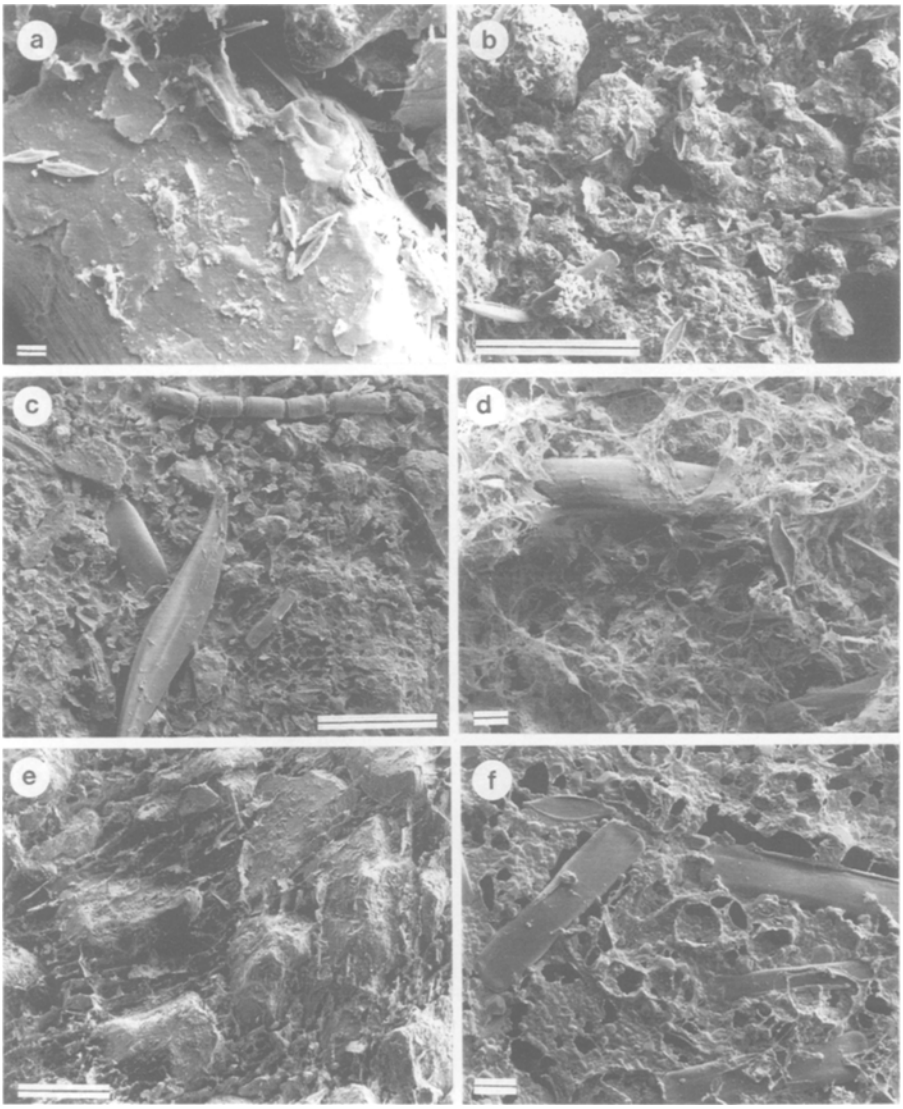


Figure 10. High resolution depth profiles of colloidal carbohydrate concentrations in the upper 1.5 mm at (a) the mid-intertidal and (b) upper-intertidal sites at Baie de Marennes-Oléron (n = 5, bars = 2 × SE).



Photographic Plate 1. Low-temperature scanning electron micrographs of surface sediments from the Eden Estuary (April 1996). (a) Epipelic diatoms on laminated clay minerals (scale bar 10 μm). (b) A mixed assemblage of epipelic diatoms present between sediment particles and organic debris (scale bar 100 μm). (c) *Gyrosigma sp.*, a chain of *Melosira sp.*, and other smaller epipelic forms connected to smaller particles by a thin fibrous matrix (scale bar 100 μm, negative Courtesy of Dr Karen Wiltshire). (d) A thick carbohydrate matrix, coating the sediment and diatom cells (scale bar 10 μm, negative courtesy of Dr Karen Wiltshire). (e) A fracture face view of the surface sediment showing sand grains surrounded by pore water (scale bar 100 μm). (f) Epipelic diatoms surrounded by a globular carbohydrate matrix (scale bar 10 μm).

Discussion

Biochemistry of the carbohydrate fractions

The traditional operational separation of the carbohydrate components into bulk and colloidal phases was examined (Underwood et al. 1995; Taylor & Paterson 1998). As with previous studies (Yallop & Paterson 1994; Underwood et al. 1995), the bulk carbohydrate phase was found in higher concentrations than colloidal material. This may reflect intracellular sugars from benthic organisms and sugars in complex structures, such as cellulose, occurring in the sediment. In this pilot study, a comparison of TFA purified and non-purified samples was conducted since protocols for the monosaccharide analysis of sediment carbohydrates were not available. The TFA purification was found to be necessary for the GC-MS analysis of sediment carbohydrates. The GC-MS analysis of TFA treated samples showed glucose to be the most abundant monosaccharide in both bulk and colloidal carbohydrate fractions. However, in the colloidal fraction glucose dominated the monosaccharide composition to a much greater extent than in the bulk fraction. The difference between the monosaccharide composition of the two fractions supports the suggestion that colloidal and bulk carbohydrate fractions are chemically different (Taylor & Paterson 1998). Similarly, a large-scale study of the Potomac River and Estuary showed no covariance between particle-associated carbohydrates and dissolved colloidal carbohydrates (Sigleo 1996). These studies suggest that biochemical and functional differences underlie the operational separation of carbohydrate fractions.

No information about the linkages between the monosaccharides could be derived. This is partly due to the very large number of possible polysaccharide structures. As stated by Alberts (1989): "*With current methods it takes longer to determine the structure of half a dozen linked sugars than to determine the nucleotide sequence of a DNA molecule containing many thousands of nucleotides.*" This is still the case. Also, carbohydrate extractions from the Eden Estuary contained a complex mixture of other organic compounds. Even after purification, traces of these substances could be seen as a hydrophobic sheen on the glassware, which makes linkage analysis difficult. Although GC-MS analysis does not provide a linkage analysis, the difference in the monosaccharide ratios suggests that the polymeric structure of the two fractions were different (Bertocchi et al. 1990). Further analysis is now required to support this preliminary study and elucidate the spatial and temporal variability of the monosaccharide composition of colloidal and bulk carbohydrate fractions. This will be complex since the abundance of sediment carbohydrates is influenced by many factors including dissolution, algal biomass, primary production and bacterial activity; factors which them-

selves exhibit temporal and spatial variability (Yallop & Paterson 1994; Smith et al. 1995). Investigations into these factors were not within the scope of this study.

Spatial and temporal changes of in situ *sediment carbohydrate fractions*

It has been established that 75–80% of the carbohydrates in the colloidal fraction exist as free sugars (Underwood et al. 1995). These provide a major and labile source of organic carbon within surface sediments (Smith et al. 1995; Ruddy et al. 1996). This suggests that much of the carbohydrate detected in the pore water (colloidal phase) would be of recent origin. This is supported by the field measurements that showed colloidal carbohydrate concentrations to vary significantly over a short period of time, in all but one mud flat site. The lack of variation in colloidal carbohydrates at the mid-intertidal site at Baie de Marennes-Oléron may be due to the low biomass and the shorter time-scale. The patterns of increases in colloidal carbohydrate concentrations were not predictable although there was a general increase with time at all but one site. Often, only the colloidal carbohydrate concentrations between the first and last time intervals were significantly different.

There was no significant change in bulk carbohydrate concentrations over the same period. The bulk material is associated with the sediment particles rather than with pore water and appears more refractory. Indeed, the absorption of organic matter to sediment particles can slow remineralisation by up to 5 orders of magnitude (Keil et al. 1994). When colloidal carbohydrates are available, they will be preferentially broken down by bacteria (and algal heterotrophy) leaving more complex materials including bulk carbohydrates untouched. Colloidal carbohydrates may represent a more dynamic fraction and a strong indicator of epipelic diatom metabolism. In this study, colloidal carbohydrates at the mid-intertidal site at Baie de Marennes-Oléron were found to be correlated to algal biomass (as chlorophyll *a*), agreeing with the study by Underwood and Smith (1998). No correlation was found between these two parameters at the upper-intertidal site, which may reflect the lower algal biomass at this site (0.08 ± 0.06 mg Chl. *a* g^{-1} sediment in the upper 0.2 mm compared to 0.29 ± 0.18 mg Chl. *a* g^{-1} sediment at the mid-intertidal site).

These *in situ* studies show that the colloidal and bulk carbohydrate fractions exhibit different temporal variability patterns. By taking into account the information obtained through GC-MS analysis and the differences found in the spatial distribution of the sediment carbohydrate fractions (Taylor & Paterson 1998), there is strong evidence to suggest that bulk and colloidal sediment carbohydrate fractions are derived from different sources and have variable turnover rates.

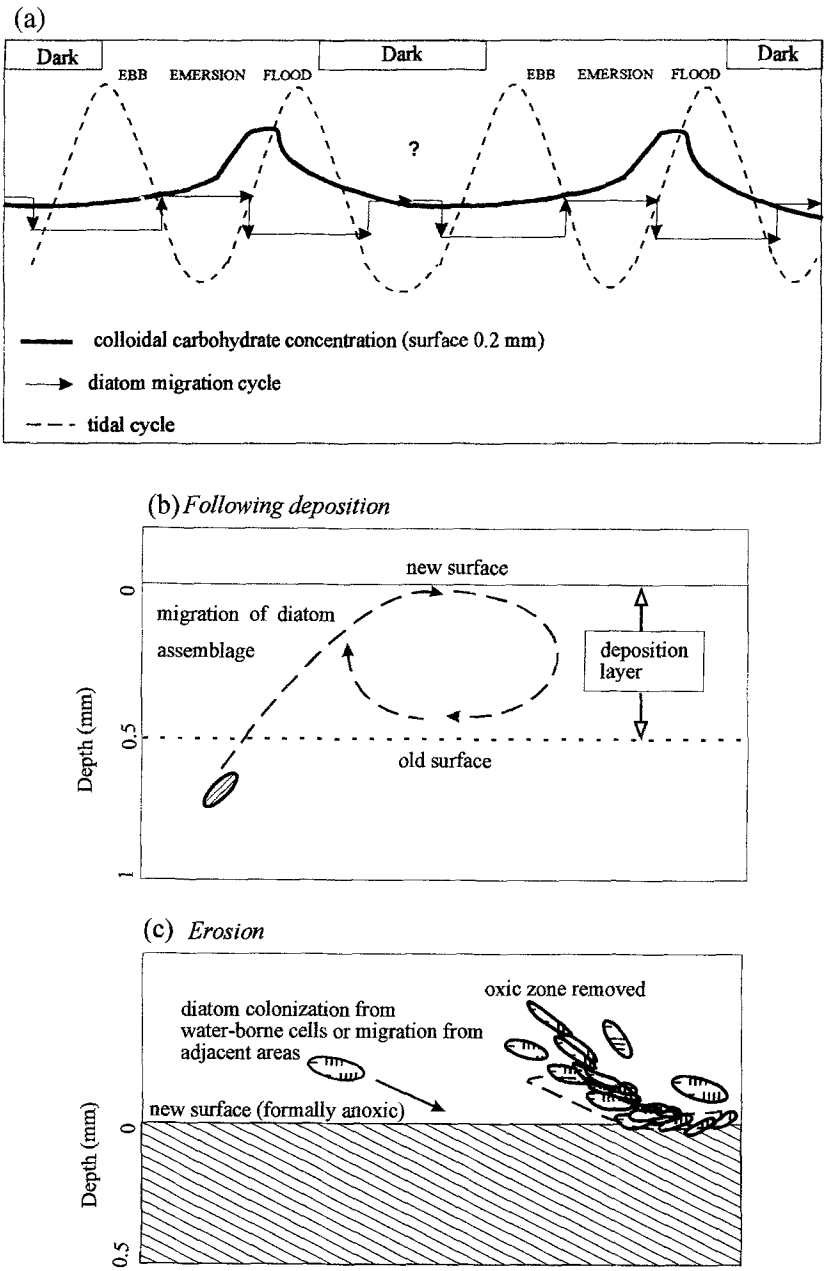


Figure 11. (a) Conceptual diagram showing the upward migration of diatoms to the sediment surface and the observed increase in colloidal carbohydrate concentrations during the emersion period. Hypothesised decreases in the carbohydrate concentrations occurs upon the flood tide, after diatoms have migrated from the surface. (b) Colloidal carbohydrates are found in freshly deposited sediments following the upward migration of epipelic diatoms. (c) Sediment erosion will lead to lower colloidal carbohydrate concentrations on surface sediment but production of carbohydrates will slowly increase and contribute to sediment stability.

A conceptual model of carbohydrate dynamics on surface sediments

Field measurements have shown surface carbohydrate values to be generally lowest at the beginning of the emersion period, when diatom cells have yet to surface. At this point, we may speculate that colloidal carbohydrate concentrations and algal biomass peak below the sediment surface (Figure 11a). Once cells migrate to the surface, primary production occurs at a maximum rate coinciding with maximum cell numbers (Brown et al. 1972). In addition, primary production is influenced by the length of the tidal exposure period (Guarini et al. 1998). Colloidal carbohydrate concentrations fluctuate and commonly increase until the cells migrate from the surface at the end of the emersion period. As the incoming tide floods the substratum, it is very likely that some colloidal carbohydrates, associated with algal biomass, will also be resuspended (Paterson 1986) and a froth is often visible on the tidal front. These carbohydrates will be broken down by bacteria in the water column or may adsorb suspended particulate matter and subsequently settle out (Karner & Rassoulzadegan 1995; Smith et al. 1995). During immersion, bacteria will also act on the sediment carbohydrates and reduce the level, without replenishment from diatoms, until the beginning of the next emersion period.

Following deposition

Where the hydrodynamic energy is low, sediment flocs will settle onto the bed, creating a new surface layer. This will effect the concentration of surface carbohydrates since the newly deposited layer may have low carbohydrate concentrations, due to the activity of bacteria in the water column. Depending on the thickness of the deposition layer, it is likely that diatom cells will migrate through the fresh deposits to the surface in one or more tidal cycles (Figure 11b).

Following erosion

Erosion has a dramatic effect on surficial carbohydrate production on intertidal mud flats, since it removes the upper sediment layers containing diatom cells and carbohydrates (Baillie & Welsh 1980). A new surface, formally anoxic, is then exposed (Figure 11c). In this situation, where colloidal carbohydrates are not available, bacteria may utilise the less energetic bulk material. Diatom cells are deposited on the new surface from the water column and from surrounding sediments (Underwood & Paterson 1993).

Conclusion

This study has shown biochemical and ecological distinctions between the commonly extracted colloidal and bulk carbohydrate fractions. This suggests that the two carbohydrate fractions are derived from a range of organic material (from glucose-rich exudates to recalcitrant detritus) and are affected by different environmental processes. An awareness of the extraction procedure used and the carbohydrate fraction measured is advised when relating *in situ* carbohydrate concentrations with metabolic processes or sediment characteristics such as erosion resistance. The conceptual model of surface carbohydrate dynamics is speculative but facilitates theoretical considerations and the development of further hypotheses. Such an approach is useful for understanding complex systems such as estuarine sediments.

Acknowledgements

The authors would like to acknowledge the laboratory assistance and advice provided by Shona Smith and Rob Field. Also, thanks to Mike Ferguson, Irvine Davidson, Julie Kelly, Claire Honeywill, Karen Wiltshire and Stephen Droop. This research was funded by NERC award GST/02/787 and received additional logistic and staff support from NERC GST/02/759 and EU Contract MAS3-CT95-002. This support is gratefully acknowledged. The authors would also like to thank the anonymous reviewers for their helpful comments.

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