



Monosaccharide composition of suspended organic particulate matter in relation to its origin

Monosaccharide
HPLC
Particulate matter
Sea water
Brackish water

Sucres
HPLC
Matière particulaire
Eaux marines
Eaux saumâtres

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ABSTRACT

Samples of phytoplankton cultures and of suspended particulate matter from continental, brackish and marine waters were examined by HPLC for the monosaccharide content of their hydrolysable sugar fraction. Eight monosaccharides were currently assayed and their weight compared to HPLC-assayed chlorophyll *a* concentrations. With the exception of oligotrophic waters, these two variables are well correlated in the various prospected environments. Glucose was always the strongly dominant form, not only in fresh and brackish waters but also in marine samples with high phytoplanktonic biomass. If samples are compared on a salinity basis, only four monosaccharides showed significant differences in their relative concentrations, as a consequence of sampling location: ribose, galactose and mannose contents were higher in marine waters (salinity > 35) and arabinose in brackish or fresh waters (salinity < 25). On the other hand, no significant difference appears between the two respective average glucose relative concentrations. No direct relationship could be established between the origin of organic matter (marine or continental) and monosaccharide ratios or an order of monosaccharide predominance in hydrolysable sugar fraction. But the occurrence of phytoplanktonic bloom was frequently associated with an enhancement of glucose relative concentrations as compared to other monosaccharide forms. Moreover, ribose and galactose relative values can provide valuable information and appear to be linked to phytoplanktonic biomass in marine waters.

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RÉSUMÉ

Relation entre la composition en monosaccharides de la matière organique particulaire en suspension et son origine

Sur des échantillons de phytoplancton en culture et d'eaux continentales, saumâtres ou marines, a été étudiée, par HPLC, la composition en monosaccharides de la fraction glucidique hydrolysable. Huit formes ont été couramment mesurées, et leurs poids comparés aux concentrations en chlorophylle *a*. A l'exception d'eaux marines oligotrophes, ces deux grandeurs sont bien corrélées entre elles dans les différents types d'environnements. Dans tous les cas, le glucose constitue la forme dominante, non seulement dans la matière particulaire des eaux douces

ou saumâtres, mais aussi dans des eaux marines à biomasse phytoplanctonique élevée. Si les échantillons sont comparés en fonction de leur salinité, seules quatre formes présentent des teneurs relatives différentes selon l'origine des échantillons : les concentrations en ribose, galactose et mannose sont plus élevées dans les eaux marines (salinité > 35), et supérieures pour l'arabinose, dans les eaux dessalées (salinité < 25). Il n'existe pas par contre de différence significative, entre ces deux groupes, pour les pourcentages de glucose. On ne peut établir de relation directe entre l'origine de la matière organique (marine ou continentale), ni avec l'ordre de dominance relative des différentes formes de monosaccharides, ni avec l'évolution des rapports entre différentes formes. Cependant, l'existence d'une poussée phytoplanctonique est fréquemment associée à une augmentation des teneurs relatives en glucose par rapport aux autres formes de monosaccharides. De plus, dans de nombreux cas, il existe bien une association entre biomasse phytoplanctonique et évolution correspondante des teneurs relatives en galactose et ribose.

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INTRODUCTION

Sugars are universally represented in the biosphere and constitute an important fraction of the particulate organic matter in seawater. The percentage of glucidic carbon in total organic carbon weight (POC) may vary from 10 to 30 % (Handa and Yanagi, 1969; Artem'yev, 1973), and sometimes, exceed 50 % in eutrophic waters (Hama, 1983). In the phytoplanktonic biomass, the contribution of glucidic weight to the dry matter can be estimated from 15 to 40 % (Parsons *et al.*, 1961).

Organic matter in the ocean is of marine as well as terrestrial origin. The distinction between these two contributions to the oceanic carbon budget is of primary importance. In higher plants, the support tissues are mainly cellulose compounds, while the reserves are starch; both are glucose polymers. So glucose is a major constituent of the continental vegetal-matter sugar fraction. On the other hand, support tissues are absent in marine phytoplankton. It has often been suggested that the concentration ratios of glucose to other minor forms, such as ribose (Degens and Mopper, 1975), fucose (Delmas, 1983), galactose (Cowie and Hedges, 1984 *b*), or others (Ittekkot *et al.*, 1984), which are monosaccharides that are less involved in plant structures, might constitute a good index for inland organic matter inputs to the ocean. However, a glucose dominance was also reported from the extractable carbohydrate fraction of cultured (Handa, 1969; Myklestad, 1974) or natural phytoplankton (Handa and Yanagi, 1969; Liebezeit and Bölter, 1991). Most of the samples upon which the above-cited hypothesis is based were sediments (Degens and Mopper, 1975; Delmas, 1983) or particles collected by traps (Ittekkot *et al.*, 1984), which are generally richer in organic matter than suspended particles collected from sea water by filtration. In these kind of natural samples, analytical procedures are easier to conduct as a consequence of the higher organic matter levels by sample volume unit. Sugar analyses on suspended particulate matter are rarer. Some have been carried out on the monosaccharide composition of the hydrolysable sugar fraction on suspended par-

ticulate matter in sea water (as example: Cowie and Hedges, 1984 *b*; Ittekkot *et al.*, 1982; Liebezeit, 1984); others were conducted either on the whole particulate (Handa and Tominaga, 1969; Handa and Yanagi, 1969; Tanoue and Handa, 1987) or dissolved (Mopper *et al.*, 1980; Sakugawa and Handa, 1985) glucidic fraction and monosaccharide composition of water samples collected in surface or deep waters. Using gas chromatography, the latter authors have reported glucose levels ranging from 40 to 70 % or more of the total assayed monosaccharides recovered after hydrolysis. But these samples were collected in marine waters without special attention to the influence of continental water inflows.

The previously used sugar assay methods have many limitations when they are applied to measurements in seawater, either in the dissolved fraction or in particulate matter samples with low organic content such as oligotrophic or deep water samples. Most of the problems occur during the concentration (Dawson and Mopper, 1978), purification or isolation stages of carbohydrate fraction recovery and treatment (Cowie and Hedges, 1984 *b*).

By using HPLC methods for sugar separation and sugar-specific fluorochromes for UV detection, we have been able to compare, with a reduced treatment of the samples, the monomeric composition of the sugar fraction of the suspended particulate organic matter content of various freshwater and marine samples, including phytoplankton cultures.

MATERIALS AND METHODS

Sample collection

Samples were chosen in order to encompass a broad range of environmental situations for the study of monosaccharide composition in suspended particles. The monosaccharide content of phytoplanktonic cells (labeled PHY in the text) was studied by harvesting, at the end

of the exponential phase, laboratory cultures of twenty-one different species: three dinophyceae, nine diatoms, three cyanobacteria, two chrysophyceae, two chlorophyceae, one rhodophyceae and one prasinophyceae (for detailed list and culture conditions, *see* Morales-Loo and Goutx, 1990; Compiano, 1990). These samples were taken as a reference for the sugar content of phytoplanktonic biomass. Natural samples of particulate organic matter were collected from various situations (Fig. 1), including: - fresh water: four southern French rivers (Fig. 1B-RIV: Rhône, Durance, Arc, Touloubre); - brackish water: a coastal pond (Fig. 1B-POND), Étang de Berre (France), with the high primary production rate; - upwelling (Fig. 1C-UPW): euphotic layers (0-60 m) of the Somalia coast upwelling; - Indian Ocean: euphotic (0-60 m, Fig. 1C-INDSURF) or deep layers (100-3 000 m, Fig. 1C-INDEEP); - oligotrophic surface waters (Fig. 1A-TRANS) collected during an oceanographic cruise from Crete (Eastern Mediterranean) to the Indian Ocean through the Red Sea. Surface water sample collection in rivers (RIV) and in pond (POND) was carried out over a period of one year at monthly intervals (for details *see* Compiano, 1990). Samples labelled TRANS, UPW, INSURF and INDEEP were collected during *Oceat III* cruise (*see* Mihalopoulos *et al.*, in press).

Natural water samples were collected by a rosette system fitted with 10 l Niskin bottles and CTD sensors (for IND-SURF, INDEEP, UPW samples), by individual 10 l Niskin bottle (for POND) or by bucket for surface waters (RIV, TRANS).

All the samples were prepared in the same manner: the water was prefiltered over a 150 µm-nylon net and the suspended particulate matter was collected by filtration on precombusted (5 hours, 550°C) Whatman GF/F filters.

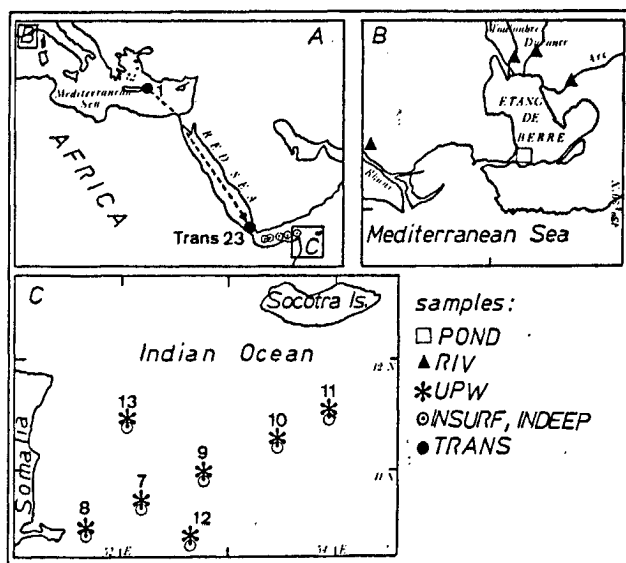


Figure 1

Maps of sampling locations. The positions of TRANS samples (Fig. 1A) from Crete to Djibouti determine their number (*see* text for the type of samples and abbreviations).

Carte des stations de prélèvements. La position des stations TRANS, de la Crète vers Djibouti correspond à leur numéro d'ordre (*voir* le texte pour le type de échantillons et leurs abréviations).

HPLC sugar measurements

The particulate matter, collected on filters as above, was treated with 5 ml of H_2SO_4 -1.86N at 100°C for four hours (Ittekkot *et al.*, 1982; Mopper, 1977). As reported by Mopper *et al.* (1980), stability is good under these conditions. After centrifugation, the supernatant was brought to pH 4.0-4.5 and used for HPLC chromatography, using the method of Mopper and Johnson (1983), by precolumn fluoro-chrome-derivatization at 65°C for 20 minutes; the fluoro-chrome reagent was dansylhydrazine (DNS; Sigma, ref.D.7634; Avigad, 1977) and was used at 5 % (W/V). Depending on the carbohydrate concentrations of the samples, hydrolysed volumes in reaction vary from 20 to 200 µl, but the water/DNS ratio was kept constant by addition of a sufficient volume of bidistilled water in order to obtain a final reaction volume of 640 µl. Separation was carried out on a Beckman Ultrasphere C₁₈-ODS column (5 µm, 250 x 4.4 mm). Eluant A was 0.08M acetic acid (pH 4.0); eluant B was acetonitrile; flow rate was 1.2 ml.min⁻¹; and the gradient used was: 22 to 30 % B in 14 mn; 30 to 70 % B in 2 mn; isocratic at 70 % B for 3 mn; and 70 to 30 % B in 3 mn. The following monosaccharides were detected: galactose (gal); glucose (glc); mannose (man); xylose (xyl); arabinose (ara); ribose (rib); fucose (fuc); and rhamnose (rhm). Slight changes in elution gradient or in A-eluent pH were sometimes necessary in order to achieve optimum xylose/arabinose separation or when monosaccharide concentrations were low with regard to DNS reagent (*see* Compiano and Romano, 1988). Fructose, which reacts poorly with DNS (Avigad, 1977; Mopper and Johnson, 1983), often coelutes with arabinose; its response factor was normalized to 1/27 with arabinose; its concentrations were always low and its distribution highly variable (*see* Results) and must be considered here with caution. On a basis of one litre of filtered sea water, the detection limit varies from 5.5 nmol/l for rhamnose to 30 nmol/l for glucose, and reproducibility from 1 % for glucose to 6 % for arabinose. In order to minimize the masking influence of glucose dominance, mean values are calculated with reference to the total particulate sugar fraction for glucose percentages, but to the total minus the glucose part for the other forms, as recommended by Cowie and Hedges (1984 a).

The following HPLC apparatus was used: two Altex pumps (mod. 110A) monitored by an Altex gradient programmer (mod. 420); a Rheodyne injection valve fitted with a 100 µl loop; and a Schoeffel fluorescence detector (mod. 970). The fluorescent signal of the derivatized monosaccharides was read with an excitation wavelength of 370 nm and a 540 nm cut-off secondary filter for emission.

Other sea water analysis

HPLC measurements of chlorophyll *a* concentrations were carried out with the same equipment, on GF/F filters extracted by 5 ml of 90 % acetone in water, according to the method of Mantoura and Lewellyn (1983), as modified by de La Giraudière *et al.* (1989), using fluorescence detection with a wavelength of 430 nm for excitation and a 580 nm cut-off filter for emission.

Salinity, used here as an index of continental influence in seawater, was measured either by conductivity sensors (UPW, INDEEP, INSURF samples) or by a Beckman laboratory salinometer (RIV, POND, TRANS).

Statistical comparisons were made by non-parametric tests (Siegel, 1956), as called for by the dispersion of some sample groups.

RESULTS

Chlorophyll *a* (Chl. *a*), sum of sugars (Σ CHO) and sugar/chl. *a* ratios (Σ CHO/Ch) are presented in Table 1 in mean value form after clustering by types of sample, with the corresponding range of recorded salinity values.

If the chlorophyll *a* or sugar concentrations are considered on a per-unit of filtered seawater volume basis, the dispersion inside each group is strong as attested by standard deviation values. This is recorded, particularly when the group takes into account either seasonal (POND, RIV) or biological (PHY) variabilities, or vertical profiles (INDEEP). On the other hand, when more stable or more homogeneous environments are considered (INDSURF, TRANS), dispersions are weaker, inside the group or when two groups, exhibiting close characteristics, such as surface waters collected in eastern Mediterranean and Red Sea (TRANS) or Indian Ocean (INDSURF) are considered. Σ CHO/Ch ratios are lowest in PHY and UPW samples with high phytoplanktonic biomass, and highest either in oligotrophic waters (TRANS) or when the biomass is diluted in high particulate organic-matter loadings (fresh -RIV- or brackish waters -POND-). In deep water samples (INDEEP) the high recorded values of Σ CHO/Ch ratios are only the consequence of the fact that pigments were then measured at detection levels.

When all per-litre chl *a* concentrations in surface waters are considered (without regard to INDEEP samples) and plotted versus monosaccharide sum (Σ CHO), in a log scale (Fig 2), a good relationship exists: $[\text{chl } a] = 0.026 [\Sigma \text{ CHO}] + 1.47$ ($r = 0.724^{***}$; $n = 146$; $P < 0.001$). When natural

Table 1

Numbers (*n*) of samples, mean (*m*) and standard deviation values (SD) clustered by types of environmental conditions (see text for abbreviations). Concentrations of chlorophyll *a* (chl *a*) and weight of monosaccharide sum (Σ CHO) are expressed in $\mu\text{g/l}$, ratio as W/W.

Nombre d'échantillons (*n*), moyennes (*m*) et écart-types (SD) des différents types de prélèvements (pour les abréviations voir le texte). Les concentrations en chlorophylle *a* (chl *a*) et en poids total de monosaccharides (Σ CHO) sont exprimées en $\mu\text{g/l}$ et le rapport en P/P.

Types CHO of samples		n	sal.	chl. <i>a</i> ($\mu\text{g/l}$)	Σ CHO ($\mu\text{g/l}$)	$\frac{\Sigma \text{ CHO}}{\text{chl } a}$
PHY	m	23	-	269.6	7852	63
	SD			295.6	6377	82
POND	m	12	7-12	22.5	3133	122
	SD			16.3	4559	101
RIV	m	42	<2	6.6	376	112
	SD			7.9	853	100
UPW	m	24	35-36	0.8	41	44
	SD			1.2	102	22
INDSURF	m	14	35-36	0.2	11	72
	SD			0.2	6	46
INDEEP	m	26	35	<.001]	8	-
	SD			-	24	-
TRANS	m	20	35-38	0.1	18	270
	SD			0.1	10	217

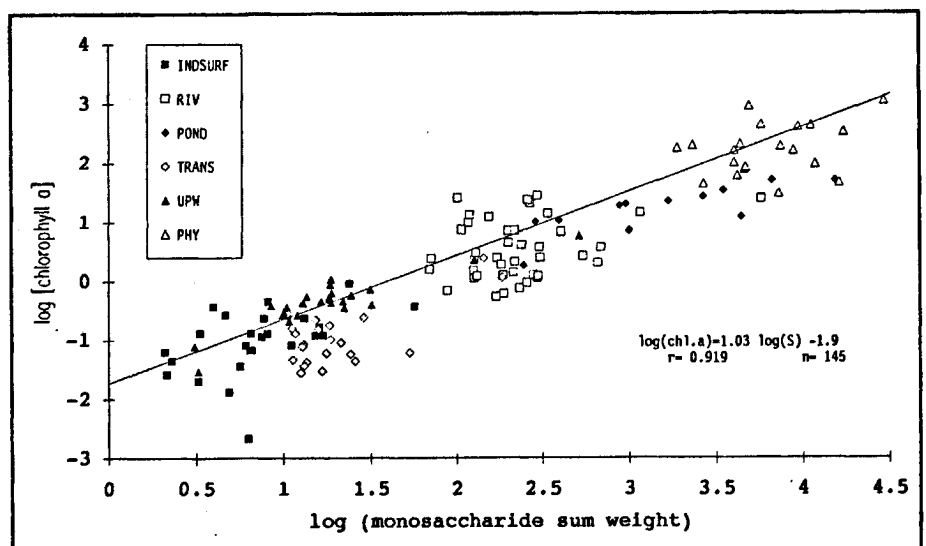
water samples alone are considered (PHY data excluded), this relationship remains strong $[(\text{chl.}a) = 0.043 (\Sigma \text{ CHO}) + 2.64$; $r = 0.755^{***}$; $n = 123$; $P < 0.001$].

Oligotrophic water samples (TRANS) exhibit the lowest respective per-litre concentrations of monosaccharide weight (Σ CHO) or chl *a* and phytoplanktonic cultures (PHY), the highest. Data from fresh and brackish water samples are located at the medium of the data cloud. In almost all the individual groups, the chl *a*-vs.-monosaccharide relationship is highly significant: UPW ($r = 0.981^{***}$; $n = 24$), POND ($r = 0.845^{***}$; $n = 12$), RIV ($r = 0.387^{***}$;

Figure 2

Relationship (log/log) between chlorophyll *a* concentrations ($\mu\text{g/l}$) and the weight of monosaccharide sum ($\mu\text{g/l}$) in the hydrolysable sugar fraction of the particulate matter (see text for the type of samples and abbreviations).

Relation (log/log) entre les teneurs en chlorophylle *a* ($\mu\text{g/l}$) et le poids de la somme des monosaccharides dosés dans la fraction glucidique hydrolysable. (voir le texte pour le type des échantillons et leurs abréviations).



$n = 41$). But, if in UPW and POND samples, chl *a* concentrations explain respectively more than 96 and 71 % of the monosaccharide distributions, for RIV samples, this value decreases below 15 %. Inside the TRANS group, no relationship exists between chl *a* and Σ CHO/ weight ($r = 0.382$; $n = 20$). For INDEEP data, where pigments are only measured at detection levels, the monosaccharide sum was measured at low but significant concentrations.

The mean percentage values and standard deviations of monosaccharide concentrations, referred to the whole glucidic hydrolysed fraction, are presented in Table 2. With the exception of fructose, the dispersions of monosaccharide percentage distributions (Tab. 2) were weaker, than the per-litre concentrations (Tab. 1), particularly in the case of samples collected in environmental conditions which can be considered as stable. Deep water samples (INDEEP) exhibit a more homogeneous monosaccharide composition than euphotic water samples. Moreover, when, in the same group of samples, important changes exist, as along the water column of the Somalian upwelling, important changes can be noted, not only in the per-litre amounts of particulate sugars, but also in the monosaccharide spectrum. The high standard deviation values recorded in UPW samples result from the great differences existing between productive and less-productive layers of the same water column.

In all cases, glucose is the strongly dominant form, as already widely reported. But glucose percentages reach their highest values in phytoplanktonic cells (PHY), whereas some of the lowest were recorded in continental waters (RIV). For the marine waters which can be considered as the least biologically-productive or as less influenced by inland inputs (as attested by sugar/chl *a* ratios higher than 200, Tab. 1: Indian Ocean deep layers - INDEEP- or Red Sea and Mediterranean oligotrophic waters - TRANS), the glucose percentages always exceed 45 % of the total sugar amounts.

Ribose percentages are higher (10 to 20 % of the sugar amount minus the glucose) in rivers or in some marine oligotrophic waters than in phytoplanktonic cells (PHY: 9.9 %) or in natural marine samples exhibiting high chlorophyll *a* content (UPW: 9.6 %). Highest mean relative fucose concentrations were not recorded in marine samples but in brackish (POND: 21.5 %) or continental waters (RIV: 24.1 %).

Phytoplanktonic cell cultures show relatively low concentrations of xylose and arabinose (0.8 and 1.9 % respectively) as compared to the other groups which exhibit significantly higher concentrations.

In natural samples, no clear relationship appears between the range of salinity (used to classify the samples as fresh or marine waters) and the relative concentrations of gluco-

Table 2

Numbers (*n*) of samples, mean (*m*) and standard deviation values (*SD*) of the data clustered by types of environmental conditions (see text for abbreviations) and range of salinity values (*sal.*). Percentages of glucose are referred (*) to the total amount of monosaccharide (W/W) and to the total minus the glucose for the other forms (**).

Nombre d'échantillons (*n*), moyennes (*m*) et écart-types (*SD*) des différents types de prélèvements (pour les abréviations voir le texte) et plage des valeurs de salinité. Les pourcentages de glucose (P/P) sont rapportées (*) à la somme des monosaccharides, et pour les autres formes, à la somme défalquée du glucose (**).

TYPES OF SAMPLES		sal.	glc (%) *	rib (%) **	fuc (%) **	gal (%) **	man (%) **	fru (%) **	ara (%) **	xyl (%) **	rhm (%) **
PHY	m	-	54.7	9.9	13.1	36.9	20.1	2.3	1.6	4.6	10.5
	SD		24.0	5.7	11.2	16.1	12.7	5.7	5.0	3.5	10.1
	n		21	21	21	21	21	21	21	21	21
POND	m	7-12	50.1	4.5	21.5	23.2	20.4	10.0	8.1	14.8	10.7
	SD		23.1	2.2	14.6	13.9	9.9	22.4	5.2	4.3	5.2
	n		12	12	12	12	12	5	8	8	12
RIV	m	<2	38.8	11.3	24.1	20.7	20.4	3.6	8.9	11.1	15.8
	SD		20.1	14.1	18.2	12.1	15.4	11.7	5.3	6.5	10.8
	n		43	43	37	43	43	15	19	19	36
UPW	m	35-36	37.3	9.6	10.9	27.6	21.8	6.3	7.8	12.4	11.7
	SD		20.7	5.0	7.2	7.7	8.9	13.5	6.0	3.3	6.0
	n		24	24	22	24	24	24	24	24	22
INDSURF	m	35-36	44.0	14.2	11.4	35.9	20.7	4.1	3.5	9.7	11.7
	SD		10.1	4.0	3.3	7.4	2.7	13.2	0.9	5.6	1.9
	n		14	14	10	14	14	14	14	14	7
INDEEP	m	35	50.9	10.8	18.2	28.8	36.0	9.4	2.9	12.5	7.0
	SD		11.5	5.4	17.3	12.9	12.6	23.9	2.1	8.0	8.5
	n		26	26	8	26	26	26	26	26	4
TRANS	m	35-38	50.9	8.0	11.4	39.1	20.1	nd	10.4	19.7	nd
	SD		9.0	2.9	4.4	6.4	3.9	-	3.7	3.7	-
	n		20	20	3	20	20	-	20	20	-

Table 3

Comparisons by a two-tailed Man-Whitney "U" test of the median values for relative percentages (W/W) of monosaccharide, to the sum (*) or to the sum minus glucose (**), between brackish (salinity < 25) and marine water samples (salinity > 35). PHo = probability of the risk for rejecting the null hypothesis (Ho = median identity); ns: no significance; ***: significance at P0.001 level; (m): median values; (SD): standard deviations; (n): number of samples.

Comparaison par un test "U" de Man et Whitney des pourcentages moyens de monosaccharides, par rapport à la somme (*), ou à la somme défalquée du glucose (**), entre les prélèvements d'eaux dessalées (sal < 25) et marines (sal > 35). PHo=probabilité du risque de rejet de l'hypothèse nulle (Ho postulant l'identité des médiane); ns: sans signification; ***: risque inférieur à 0.001; m= valeurs médianes; SD:écart-types; n: effectifs.

SALINITY		glc (%) *	rib (%) **	fuc (%) **	gal (%) **	man (%) **	fru (%) **	ara (%) **	xyl (%) **	rhm (%) **
< 25%	m:	41.3	9.8	20.5	21.7	20.4	5.2	8.7	12.2	14.5
	SD:	21.1	12.7	17.3	12.5	14.3	14.6	5.2	6.1	9.9
	n:	55	55	49	55	55	20	27	27	48
> 35%	m:	45.5	10.6	12.7	31.0	26.8	8.0	5.4	12.7	12.5
	SD:	18.9	5.0	10.0	10.9	11.8	20.4	4.7	6.9	8.1
	n:	134	134	71	134	134	112	134	151	55
U1		3076	2315	1386	2231	2388	1096	1124	1990	1112
PHo		0.5 ns	<.001 ***	0.18 ns	<.001 ***	<.001 ***	0.58 ns	.004 **	0.57 ns	0.47 ns

se and, on the other hand, ribose or fucose, two of the sugar forms most widely proposed as marine markers in the sugar fraction of the suspended particulate matter.

Two salinity ranges (above 25 and over 35) were selected, in order to compare marine and fresh (or fresh-influenced) waters (Tab. 3). On the weight assayed sugar forms, only four gave significant differences in percentage between the two salinity groups: ribose, galactose and mannose percentages were higher in marine waters and arabinose in continental samples. Despite the slight difference reported between the two median values for the ribose (9.8 and 10.6 %), and the high dispersion recorded (Tab. 2) within the first group (due to high variability in environmental features of brackish or fresh water samples), the marine particulate organic matter appears to be significantly more enriched in ribose as compared to continental samples. But in cultured phytoplankton cells (PHY) and in POND samples, the mean ribose percentages are among the lowest recorded (5.4 %), with a relatively low dispersion. In the two salinity groups, glucose percentages are close to each other (41 and 46 %) and no statistical difference is apparent. Standard deviations of glucose concentrations are low as compared to those of the minor forms. This confirms that glucose is well represented in all fresh water and marine environments.

In continental waters, the order of sugar relative amounts is glu/gal/fuc-man/rhm/xyl/rib/ara/fru and glu/gal/man/fuc-xyl/rhm/rib/fru/ara for marine waters. Although the percentage value for one specified form can differ in each salinity group, the order remains roughly similar. Behind glucose, the other forms appear at the same relative place in phytoplanktonic cell samples (glu/gal/man/fuc/rib-rhm/fru/xyl/ara), with the exception of ribose which comes higher in the order, and xylose which comes lower.

In some samples, either total monosaccharide weight or glucose dominance can reflect the occurrence of phytoplankton blooms.

As a first example, Table 4, presents chlorophyll *a*, total sugar concentrations and percentages of glucose, ribose and fucose to the monosaccharide sum recorded at four stations in the Somalian coast upwelling. In the euphotic layers (0 to 50 m) of this upwelling (marine area located outside the direct influence of terrestrial inputs: no river outlet, presence of coastal deserts), when all samples are taken into account, the mean glucose contribution is one of the lowest recorded (37 %). This percentage appears to be directly linked to chlorophyll *a* concentrations and thus increases in phytoplankton biomass. Glucose percentages attain their highest mean values (45 %) in subsurface waters, in parallel with enhancement in chlorophyll *a* concentrations (1.2 µg/l); then, both regularly decrease to 100 m depth (24 % and 0.13 µg/l). Moreover, the highest recorded relative glucose percentage (90 %) is closely associated with highest algal biomass (chl *a* = 6 µg/l), mainly formed by diatoms (Mihalopoulos *et al.*, in press). On the other hand, as a consequence of strong glucose dominance, ribose and fucose percentages represent a minor part of the sugar fraction. Galactose percentage reaches its maximal values at station 8 (Tab. 4) with chl *a* concentrations, and remains high under the most productive layers. Mannose percentage exhibits an erratic distribution but a pronounced depletion in the upper-layer waters of station 8. Surprisingly, in one of these samples, (station 8-100 m), located under one of the most productive water layers, fructose, which is currently assayed at low levels, appears here to be the dominant monomeric form.

Another example of monomeric composition change and glucose enhancement with the increase of phytoplanktonic biomass was encountered during the collect of TRANS samples (Fig. 1). Figure 3 shows the evolution of chl *a* and sum of monosaccharide concentrations along the transect, in parallel with the trend of glucose/ribose ratio. From the Mediterranean (station 1)

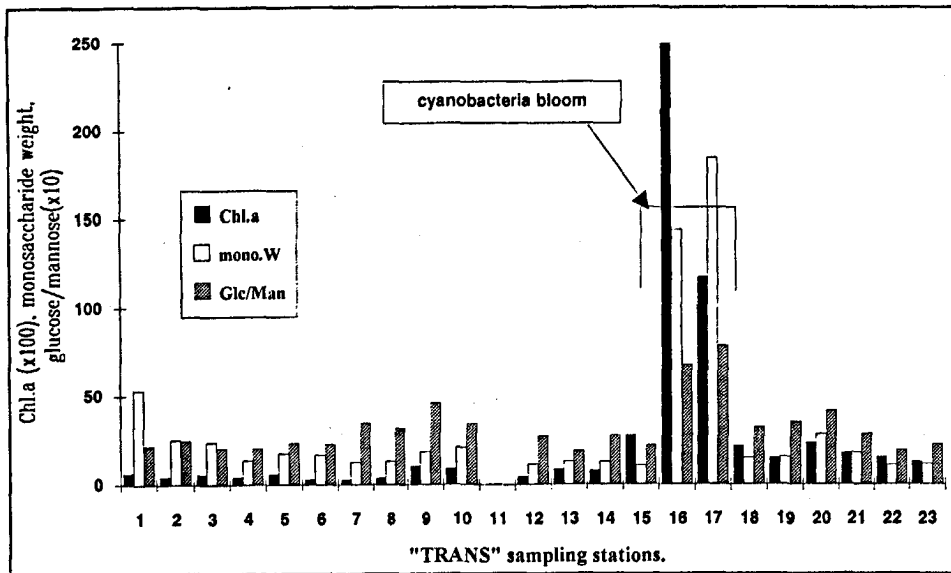


Figure 3

Evolution of chlorophyll a (in $\mu\text{g/l} \times 100$), the monosaccharide sum weight and glucoselmannose ratios ($\times 10$) in TRANS samples (see text) with a special mention of an *Oscillatoria* sp. bloom occurring at stations 16 and 17 (separated by about 80 km).

Évolutions des teneurs en chlorophylle a (en $\mu\text{g/l} \times 100$) et du poids de la somme des monosaccharides dans les échantillons de type TRANS, avec mention d'une poussée localisée d'*Oscillatoria* sp., constatée aux stations 16 et 17 (séparées par environ 80 km).

to the Indian Ocean, these oligotrophic waters exhibit, in general, relatively low values. In the Red Sea, the ship crossed (stations 16 and 17) a phytoplankton bloom, mainly formed by a cyanobacteria (*Oscillatoria* sp.). In these two samples (which are not considered above as a part of the TRANS sample group), we have measured a consecutive increase in chl a and monosaccharide concentrations. Glucose relative concentrations to the sum increased also, as attested by the recorded glc/rib ratio (or also by glc/xyl or glc/rib ratios, not presented here).

DISCUSSION

The results presented here show that high levels of glucose in the hydrolysable sugar fraction of particulate matter collected in natural water samples can be found in association not only with continental inputs, but also with marine phytoplanktonic biomass. This is roughly in accordance with data previously reported by Handa and Tominaga (1969), Handa and Yanagi (1969) or Tanoue and Handa (1987), for sea water samples collected in the North Pacific, a marine area which is located outside a significant continental water influence.

Table 4

Stations in the upwelling off the Somalian coast (UPW, see Fig. 1): vertical profiles of chlorophyll a concentrations (in $\mu\text{g/l}$), sugar/chlorophyll a ratios ($\Sigma \text{CHO}/\text{Chl.}$ as W/W) and relative concentrations of glucose (%glc) to the monosaccharide sum and fucose (%fuc), ribose (%rib), galactose (%gal) and mannose (%man) to the sum minus the glucose concentrations.

Stations de l'upwelling des Somalies (UPW, voir Fig.1). Profils verticaux des concentrations en chlorophylle a (en $\mu\text{g/l}$), du rapport poids des monosaccharide $\Sigma \text{CHO}/\text{chl.}$ ($\Sigma \text{CHO}/\text{chl.}$ en P/P) et des concentrations en glucose (%glc) par rapport à la somme des monosaccharides, et de celles du fucose (%fuc), du ribose (%rib) et du mannose (%man) à la somme défalquée du glucose.

ST	Longitude Latitude	Z(m)	Chl. Σ	CHO/Chl.	%glc	%fuc	%rib	%gal	%man
7	52°08'E 10°24'N	0	0.73	43	44.8	3.6	14.0	14.1	46.9
		20	0.63	29	40.0	10.3	4.1	34.2	30.5
		50	0.54	25	27.0	7.4	4.5	29.2	34.1
		100	0.11	66	31.6	10.1	5.4	23.8	31.2
8	51°08'E 10°06'N	0	6.00	85	94.4	17.3	5.3	49.8	6.0
		20	2.32	55	93.3	11.4	9.7	40.6	9.0
		50	0.90	20	41.8	8.6	5.3	30.7	28.3
		100	0.37	155	(7.5)*	4.4	2.8	30.8	46.2
9	52°45'E 10°41'N	0	0.50	36	35.7	10.1	2.5	28.8	19.7
		20	0.45	36	32.5	6.8	2.7	32.5	18.3
		50	0.44	43	23.5	5.1	3.0	28.3	16.2
		100	0.13	51	22.8	5.2	4.4	22.4	22.0
10	53°20'E 11°00'N	0	0.58	41	27.3	8.4	5.7	22.1	21.1
		20	0.40	80	17.6	6.8	7.8	23.1	18.5
		50	0.21	52	23.1	8.3	6.3	20.6	22.9
		100	0.02	162	22.8	10.6	1.9	16.4	14.6

(* = fructose is the dominant form: > 80 %)

As attested by the strong relationship (Fig. 2) found between chl *a* concentrations and the sum of monosaccharide weights, sugars are closely related to biological fraction in suspended particulate matter. From oligotrophic marine waters (with low biomass levels) to phytoplankton cultures, all our examined environmental groups are regularly dispatched by the coupled variations of these two parameters. The chl *a* concentrations may be considered as an index of vegetal matter in waters. But chlorophyll levels do not have the same significance in marine as in brackish and fresh waters. In the former, they are closely associated to phytoplankton biomass; in the latter, chl *a* amounts result from a complex assemblage of freshwater phytoplankton cells and higher plant detritus, drained by continental inflows. This can explain why, in the different groups, the chl *a* distributions do not contribute at the same manner to the monosaccharide variability.

In our phytoplankton samples, which constitute an average monosaccharide composition from many various species, glucose is always the predominant form. It is followed by galactose and mannose and mean xylose percentage values account for less than 2 % of the total. In the highly productive waters of the Somalian coast upwelling, relative glucose levels are generally lower and galactose, arabinose and xylose higher than in cultures. But glucose remains the main monomeric form, as has been previously reported from the North Sea by Ittekkot *et al.* (1982) and Liebezeit (1984). Its dominance enhancement, as compared to other minor forms, are closely associated, in our samples, with the most productive waters. Since the monosaccharide composition of living cells can vary with species, age, trophic conditions and other factors, our laboratory culture data must be considered as no more than an averaged composition of phytoplanktonic material. In their analysis of the carbohydrate composition of natural phytoplankton samples collected from surface waters, Tanoue and Handa (1987) have also reported the same glucose dominance with percentages ranging from 36 to 58 % (M/M), and secondary dominance of mannose, galactose and xylose, but without clear trends for the minor forms. By using a triangular hexose plot to distinguish between terrestrial, marine and macroalgal carbohydrate contributions to the sediment, Liebezeit (1988) found marine samples to be dominated by almost equal concentrations of the hexoses when the effect of reserve polysaccharides could be excluded.

When the sugar composition of natural samples is studied, we must bear in mind that most of the monosaccharides are involved in polymers. Hydrolysis is a necessary step in order to determine the monosaccharide content of sugar fractions. In a chemical study of dissolved carbohydrates in water samples, Sakugawa and Handa (1985) have noted that the polysaccharide fraction (molecular weight > 4000) accounts for 50 to 70 % of the total dissolved polysaccharides and exhibits a significative enhancement in glucose percentages. On the other hand, Compiano and Romano (1988) have reported that the sugar fraction of coastal sediment and particles collected by traps released at different speeds their monosaccharide component, depending on their origin, when they are submitted to a mild acid hydrolysis. So, hydrolysis procedures are of great importance in

order to determine monosaccharide composition of polymerized material. Some sugar polymers are highly resistant to acid hydrolysis, and the first point to be considered is the possible influence of hydrolysing procedures on the monosaccharide recovery. The acid hydrolysis method used here, as recommended by Mopper (1977) for suspended particulate matter, is not the strongest but the most widely used as a balance between monosaccharide recovery and further sugar degradation which may occur during stronger, or longer (Cowie and Hedges, 1984 *b*) hydrolysis. In brackish or freshwater samples, where highly refractory cellulosic polymers are present, this procedure cannot be considered as the most efficient, and should lead to underestimation of glucose contribution to the sugar fraction of continental matter samples. Through a comparison of wood, plankton and sediment trap material samples, Cowie and Hedges (1984 *b*) have shown that in wood samples, the glucose yields are increased by a factor 10.9 when a 72 wt % sulfuric acid pretreatment was applied to the samples, as compared to untreated samples. This yield decreases to a factor 2 to 3 in plankton or trap material. For the other monosaccharides, differences are weaker (about 10 % or lower), and differences are positive (best recovery) or negative (losses), depending on the monosaccharide and the origin of the material. The sugar fraction of sediment samples generally exhibits higher acid hydrolysis resistance than suspended particulate matter (Compiano and Romano, 1988). This is probably the consequence of previous degradation of labile compounds involved in cell metabolism or as reserve polymers, and mainly recovered as water-extractable carbohydrate fraction (Dawson and Liebezeit, 1982; Liebezeit, 1987).

But glucose percentages recorded in our samples of suspended particles, collected in continental (RIV) or brackish waters (POND), are comparable (40 to 60 %) to those found in marine water and sediment samples (Degens and Mopper, 1975; 1976) or in continental soils (Lowe, 1978). Moreover, if acid hydrolysis problems occur, they do not mainly concern the sugar fraction of phytoplankton cells, collected either from cultures (PHY) or productive waters (UPW). In these samples, the relative concentrations of glucose were found to be close to those recorded in natural samples influenced by continental water inputs (POND) and of the same order of magnitude as other literature data.

Degens and Mopper (1976) have considered that a value higher than 10 for the molar ratio (M/M) of glucose to ribose may be an index of the continental influence on marine sediments. But this conclusion cannot be extrapolated to suspended particulate matter. Examining the distribution of carbohydrates in marine environments, Liebezeit (1987) advances the conclusion that the glc/rib ratio can be useful for marine sediments where biomass is mostly absent, but that it cannot be applied to suspended particulate waters. Our results reach the same conclusion: when terrestrial-influenced and marine suspended particulate matter samples are examined, almost all our mean values (including phytoplankton samples), exceed this threshold, with the exception of surface waters of the Indian Ocean. The recorded range of variations for this ratio is wide and does not provide evidence for geochemical significance.

Nevertheless, one of the lowest mean glc/rib ratio values (Tab. 2) was recorded in the high production marine area of the Somalian coast upwelling and ribose, galactose or mannose show significant relative enhancement in the particulate matter of marine waters as compared to brackish or fresh water (Tab. 3). Cowie and Hedges (1984 *b*) have also used galactose, xylose and ribose in an attempt to distinguish between marine and continental influences in organic matter collected by traps, but for the particle fraction > 150 μm . Unfortunately, no monosaccharide is solely generated by one kind of environment. While galactose is one of the major components of diatom cell walls (Hecky *et al.*, 1973; Haug and Myklestad, 1976), it is also present in continental plant pectins (Aspinall, 1970). Xylose is a constituent not only of higher plant tissues but also of freshwater chlorophyceae cell walls (Percival, 1970). Moreover, it should be borne in mind that if glucose is involved in terrestrial plant polymers (cellulose, starch), it is also present in marine and freshwater phytoplankton cells. It plays a role, not only as a metabolic sugar, but also as a monomer of glucans which are, in part, reserve polysaccharides (Percival, 1968). This was attested by the high glucose percentages recorded in our algal cultures and in others (Handa, 1969; Myklestad, 1974).

In other words, our results show that when a broad spectrum of natural water samples is examined, no monosaccharide provides unequivocal information as to the origin of the particulate matter content. So we may conclude that information provided by monosaccharid form ratios, based on sediment studies, cannot be directly applied to the hydrolysable sugar fraction of suspended particulate matter, whether in sea water (Liebezeit, 1987) or in brackish or fresh water samples.

CONCLUSION

In continental and marine waters, suspended particulate organic matter is a mixture of algal cells and detrital mate-

rial. Each of these two sugar sources exhibits specific composition (as a consequence of its origin) and behavior during the course of the decomposition processes.

During the sinking of particles through the water column, the water-extractable carbohydrate fraction is greatly reduced as compared to the surface sediment (Dawson and Liebezeit, 1982; Compiano and Romano, 1988). In the sediment, degradation processes occurring in the top centimeters first consume metabolic sugars such as ribose or glucose, and then the more refractory cellulose compounds. So, in sediments from a marine area, the hydrolysable sugar fraction may be relatively lower in glucose, as compared to continental-water influenced samples, due to the fact that phytoplankton cell glucan could be previously partly degraded before deposition on the surface of the sediment (Handa and Yanagi, 1969; Liebezeit, 1987). The influence of degradative processes on the composition of sugar fraction probably does not have the same importance in the suspended particulate matter and in sediment samples.

In sediments, since some continental polysaccharides exhibit high refractory properties, the degradation of organic matter may bring about significant changes in the sugar fraction composition, and monosaccharide composition can be considered as a source of information about the origin of organic matter. But this leads to the conclusion that in the suspended particles of the waters, sugar ratios do not have the same geochemical significance as in sediments. In terrestrial-influenced environments, or in highly-loaded organic matter sea water samples (Compiano *et al.*, in press), the origin of the sugar fraction is complex, and monosaccharide ratios may lead to incorrect interpretations. On the other hand, in oligotrophic waters (the best represented type of water in the oceans, but also the poorest in suspended organic matter), the occurrence of phytoplankton blooms is closely associated with significant changes in the monosaccharide composition of hydrolysable sugar fraction. In this case, the information provided by the relationships between monosaccharides may constitute valuable data.

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