Distribution, origin and transformation of amino sugars and bacterial contribution to estuarine particulate organic matter

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Abstract:

Amino sugars including bacterial biomarker muramic acid (Mur) were investigated in suspended particulate matter (SPM) to understand their distribution, origin, and biogeochemical cycling and the contribution of bacteria to particulate organic matter (POM) of the Mandovi estuary. SPM was collected from 9 sampling stations in the Mandovi estuary during the pre-monsoon (March) and monsoon (August). Total particulate amino sugar (TPAS) concentrations and yields varied spatially and were 2 to 5 times higher during the monsoon than the pre-monsoon. Negative correlation between salinity and TPAS-C yields [TPAS-C/particulate organic carbon (POC) × 100] indicates the influence of terrestrial organic matter on the transport of TPAS-carbon. Glucosamine (GlcN), galactosamine (GalN), and mannosamine (ManN) were abundant during the monsoon. Low GlcN/GalN ratios (<3) indicate bacteria as the major source of amino sugars. Higher amino sugar yields and lower GlcN/GalN ratios during the monsoon than the pre-monsoon indicate enhanced transformation and greater bacterial contribution to POM during the former season. Degradation trends observed with TPAS were well supported by those obtained with carbohydrates and amino acids. Based on Mur concentrations, bacteria accounted for 24 to 35% of the POC and 24 to 62% of the total particulate nitrogen (TPN). Intact bacterial cells, however accounted for a small proportion of POC (2.5 to 4%) and TPN (9 to 11%). Our study suggests that POM was subjected to extensive diagenetic transformation, and its composition was influenced by bacteria, especially during the monsoon.

Keywords: Amino sugars, Bacteria, Muramic acid, Mandovi estuary, Particulate organic carbon, Total particulate nitrogen.
1. Introduction

In rivers and estuaries, organic matter (OM) is derived from autochthonous and allochthonous sources. Rivers transport ~ 0.14 to 0.40 Gt-C year\(^{-1}\) of POC and 0.22 to 0.40 Gt-C year\(^{-1}\) of dissolve organic carbon (DOC) (Bauer and Bianchi, 2012). Transport of OM from rivers to coastal waters via estuaries is a very significant process in the global carbon cycle (Meybeck, 1982; Ludwig et al., 1996). During transport from rivers to coastal waters via estuaries, OM undergoes extensive degradation. Heterotrophic microorganisms are the primary agents of decomposition and transformation of OM. Therefore, estuaries are useful sites to study the distribution, sources and transformation of biochemical constituents including amino sugars of dissolved and particulate OM (Bianchi and Bauer, 2012; Bauer and Bianchi, 2012).

Amino sugars are constituents of the structural biopolymers chitin and peptidoglycan. Chitin, a homopolymer of N-acetyl-D-glucosamine, is produced by a large variety of marine organisms (Muzzarelli, 1977). Peptidoglycan is a structural polymer of bacterial cell walls and contains the amino sugars GlcN and Mur (Madigan et al., 2009). Additional sources of amino sugars include pseudopeptidoglycan, lipopolysaccharides, glycolipids, and glycoproteins (Benner and Kaiser, 2003; Madigan et al., 2009). Amino sugars accounted for 0.34 to 1.3% of organic carbon and 0.38 to 1.61% of organic nitrogen in marine POM (Benner and Kaiser, 2003). They are relatively more abundant in the dissolved phase (<0.1 µm), accounting for 0.6 to 2.5% of organic carbon and 2 to 7% of nitrogen of ultrafiltered dissolved organic matter (UDOM). This implies that amino sugars are important components of marine carbon and nitrogen cycles (Benner and Kaiser, 2003; Tremblay and Benner, 2009).

The concentrations and yields of amino sugars have been used as biogeochemical indicators to assess degradation state of OM (Benner and Kaiser, 2003; Lomstein et al., 2009; Carstens and Schubert, 2012). Moreover, the ratio of total particulate amino acid (TPAA)/TPAS is a useful tool to evaluate degradation state or freshness of OM, with higher ratios indicating relatively fresh OM (Jennerjahn and Ittekkot, 1999; Gupta and Kawahata, 2000; Lomstein et al., 2009; Carstens and Schubert, 2012).

Amino sugar composition changes during degradation of OM and the ratio of GlcN/GalN decreases with progressing degradation (Benner and Kaiser, 2003; Davis et al., 2009). The GlcN/GalN ratio has been used to differentiate OM inputs from chitin containing organisms and bacteria (Liebezeit, 1993; Benner and Kaiser, 2003; Fernandes et al., 2006; Niggemann and Schubert, 2006; Carstens et al., 2012). While degrading OM, bacteria leave their own imprint on the
degraded OM (Benner and Kaiser, 2003; Davis et al., 2009). Mur is exclusively found in bacteria (Schleifer and Kandler, 1972), and therefore often used as a biomarker for living bacteria and recent bacterial necromass (Moriarty, 1975; Moriarty, 1977; Benner and Kaiser, 2003; Niggemann and Schubert, 2006; Carstens et al., 2012). The rationale is based on the assumption that peptidoglycan is rapidly degraded after cell death. However, it appears that peptidoglycan is fairly resistant to degradation (Jørgensen et al., 2003; Nagata et al., 2003). Mur concentrations also have been used to quantify the contribution of bacterial carbon and nitrogen to total organic carbon and nitrogen of particulate matter and sediments (Tremblay and Benner, 2009; Lomstein et al., 2009; Bourgoin and Tremblay, 2010; Carstens et al., 2012).

There are only a few studies on the distribution and biogeochemical cycling of amino sugars including ManN and Mur in aquatic environments (Benner and Kaiser, 2003; Tremblay and Benner, 2009; Bourgoin and Tremblay, 2010; Carstens et al., 2012). Estuaries, on the west coast of India, are strongly influenced by the southwest (SW) summer monsoon (Qasim and Sengupta, 1981; Shetye et al., 2007; Pratihary et al., 2009). However, little is known about the effect of the SW summer monsoon and pre-monsoon on the distribution, sources and biogeochemical transformation of OM in general, and amino sugars in particular in tropical monsoon driven estuaries (Khodse et al. 2010; Fernandes, 2011; Khodse and Bhosle, 2012). Therefore, we investigated the distribution and transformation of TPAS in a tropical monsoon driven estuary. In the present study, our aims were to: 1) examine the spatial distribution and seasonal variation of amino sugars, 2) determine the composition of amino sugars, 3) evaluate amino sugars as indicators of source and diagenetic changes, and 4) estimate bacterial contribution to POC and TPN.

2. Materials and methods
2.1. Study area

The Mandovi estuary is a tropical tidal river basin located in Goa between 15° 15’ N and 15° 42’ N and 73° 45’ E and 74° 24’ E along the west coast of India. The total length of the estuary is about 75 km, and width at the mouth is about 3.2 km. The temperature is moderate and the climate is humid and tropical. During the south west monsoon the average rainfall in Goa is 300 to 350 cm year\(^{-1}\) of which nearly 80% occurs in 3 months (June-August) (Unnikrishnan et al., 1997). During the south west monsoon river flow is high and westerly winds, wind induced waves, currents and tides are intense. Whereas, the dry northeast (NE) period between October to May is characterized with negligible rainfall, least terrigenous sediment discharge, and weak easterly winds. The current in the
estuary is tidally dominated, and saline waters occur several kilometers upstream from the river mouth during the dry season (Shetye et al. 1995). Thus, the channels of the estuary turn from mainly riverine during SW monsoon to highly saline for several kilometers from the mouth during the remaining 8 months. The residence time of the water in the Mandovi estuary is 5 to 6 d during the monsoon and about 50 d during the non-monsoon (October to May) period (Qasim and Sengupta, 1981). The banks of the Mandovi estuary are covered with mangrove trees.

During the monsoon season, nutrient concentrations are high (average nitrate 7 µM, phosphate 0.55 µM), primary production is low (7.5 mg C m⁻³ h⁻¹) due to light limited condition, and the estuary is dominated by heterotrophic organisms (Ram et al., 2003). In contrast, during the pre-monsoon with the improved light condition, the estuary is dominated by autotrophic organisms (Ram et al., 2003). Phytoplanktons are generally dominated by diatoms. Stable carbon and nitrogen isotope data suggest that POM is derived from terrestrial and marine sources during the monsoon and pre-monsoon, respectively (Fernandes, 2011; Khodse and Bhosle, 2012). POM is extensively degraded during the monsoon than the pre-monsoon (Fernandes, 2011; Khodse et al., 2010; Khodse and Bhosle, 2012). During the monsoon, carbohydrate rich dissolved and particulate OM is transported to the coastal waters (Khodse et al., 2010; Khodse and Bhosle, 2012). Clay mineral such as smectite and kaolinite are abundant near the mouth and the upstream stations of the estuary, respectively (Kessarkar et al., 2010).

2.2. Sample collection

Surface (~1 m) water samples (10 L) were collected using 5 L Niskin water samplers from 9 locations in the Mandovi estuary during the monsoon (August, 2005) and pre-monsoon (March, 2007) (Fig.1). Sampling was completed within 5.5 h. Immediately after collection, water was passed through 200 µm mesh nylon screen to remove large zooplankton, and then known aliquots (0.25 to 1.5 L) were filtered through pre-ashed (450 °C, 4h) and pre-weighted 47 mm GF/F filters (0.7 µm) for the measurements of SPM, POC, TPN, and TPAS. The filters were immediately transferred into clean screw capped glass vials and transported to the laboratory in an ice box. The samples were stored at -20°C until analyzed. To estimate total bacterial count (TBC), water sample (20 ml) was fixed with glutaraldehyde (final concentration 2%) and stored at 4 °C before analysis. In order to determine phytoplankton cell counts, 1L samples were preserved with a few drops of Lugol’s iodine.

2.3. Analysis of bulk parameters

The GF/F filter containing SPM was washed with distilled water to remove salts and the filter was dried at 40 °C for 24 h. The filter was cooled and weighed on a microbalance (AT-20, Mettler,
Precision = 0.002 mg) to obtain SPM. POC was analyzed spectrophotometrically by the wet oxidation method using glucose as standard (Parsons et al., 1984). TPN was estimated following the method of Raimbault and Gerd (1991) using EDTA as standard. Analytical variation for the methods used for POC and TPN analysis was 4.9% and 8.8%, respectively.

2.4. Phytoplankton cell numbers and bacterial cell counts

Phytoplankton cell numbers were determined by settling and siphoning procedure to obtain 20 to 25 ml of concentrated sample (Khodse et al., 2010). One milliliter of the sample was analyzed microscopically under inverted Olympus microscope (200X magnification) in Sedgewick-Rafter plankton counting chamber. In order to determine TBC, a known volume of seawater (2 to 5 ml) was stained with acridine orange (final concentrations 0.01%) for 5 min, filtered onto 0.22 µm black Nuclepore filter (25 mm). Bacterial cells were counted in at least 25 randomly selected fields using 100X objective, and an epifluorescence microscope (Nikon). Average cell number per field was calculated and used to estimate total bacterial cells following the procedure described by Parsons et al. (1984).

2.5. Analysis of particulate amino sugars

TPASs were determined following the method of Zhang and Amelung (1996) as described in Khodse and Bhosle (2010). The GF/F filter containing the SPM was transferred to a glass ampoule, and 10 ml 6M HCl followed by 50 µl of internal standard (myo-inositol) were added. The ampoule was flushed with nitrogen gas, sealed, and the sample was hydrolyzed for 5 h at 105 °C. The sample was cooled, neutralized with 0.4 M KOH, amino sugars were converted to aldononitrile derivatives (Guerrant and Moss, 1984), and analyzed by GC-Mass spectrometer (MS).

A GC-MS system (Shimadzu Model – GC-MS QP-2010) with electron impact ionization mode (70 ev) and a capillary column (RESTEK Rtx-5MS, 30 m, 0.32 mm id, 0.25 µm df) was used. The injector temperature was 250 °C, and the oven temperature was programmed from 120 °C to 250 °C at 10 °C/min, and held at 250 °C for 2.5 min and again increased to 270 °C at 20 °C/min, and maintained for 2 min. The interface and ion source temperatures of the MS were maintained at 280 °C and 250 °C, respectively. Sum of all the identified amino sugars has been defined as TPAS. Hydrolysis with 6M HCl usually gives slightly lower yields of GlcN and ManN and slightly higher yields for GalN compared with 3M HCl hydrolysis (Benner and Kaiser, 2003). Calculations of %C as total hydrolysable amino sugars in samples were based on the deacetylated form. Amino sugars measured in this study have six carbon atoms each and this value was used to calculate amino sugar carbon in samples (Benner and Kaiser, 2003). This results in underestimation of amino sugar carbon
by as much as 25% because acetylated forms of these amino sugars have eight carbon atoms each (Benner and Kaiser, 2003).

2.6. Quantification of bacterial contribution to particulate organic matter

C and N normalized yields of bacterial biomarker Mur measured in the samples were compared to yields measured in cultured bacteria (Kaiser and Benner, 2008; Bourgoin and Tremblay, 2010). The proportion of bacterial-C or N was calculated using the equation-

\[
\% \text{ Bacterial C or N} = 100 \times \left\{ \frac{(\text{biomarker}_{\text{sample}})}{(\text{biomarker}_{\text{bacteria}})} \right\} \quad (1)
\]

Where \((\text{biomarker})_{\text{sample}}\) represents the C-normalized or N-normalized yields of Mur in samples if % bacterial-C or % bacterial-N, respectively is estimated. \((\text{biomarker})_{\text{bacteria}}\) represents the C- or N-normalized yield of Mur in bacteria. Bacterial biomarker yields of 28.1 nmol mg C\(^{-1}\)Mur and 121.3 nmol mg N\(^{-1}\)Mur were taken from the literature (Kaiser and Benner, 2008). These values were observed for a mixture of 80% heterotrophic bacteria and 20% phototrophic bacteria (Kaiser and Benner, 2008).

Contribution of intact bacterial cells carbon and nitrogen to POM was estimated. Bacterial carbon was calculated by multiplying the bacterial cell counts with an average carbon content of bacterial cells obtained from literature. We used an average carbon content of 11 fg C cell\(^{-1}\) of estuarine bacteria to estimate bacterial carbon contribution to POC (Kawasaki et al., 2008; Bourgoin and Tremblay, 2010; Carstens et al., 2012). An average nitrogen content of 5.8 fg N cell\(^{-1}\) was used to estimate bacterial nitrogen contribution to TPN (Fukuda et al., 1998).

2.7. Statistical analysis

Correlations between the parameters were assessed using Excel software program. To evaluate spatial and seasonal variation paired t-test was carried out using log transformed TPAS, GlcN, GalN, ManN, and Mur data and STATISTICA software. Principal component analysis (PCA) was carried out using concentrations (µM-C) of TPAS, TPAA, total particulate neutral sugars (TPNS), deoxy sugars (rhamnose plus fucose), and glucose, and the ratios of C/N and hexoses (mannose plus galactose plus glucose)/pentoses (arabinose plus ribose plus xylose) to compare degradation trends using these parameters. PCA was performed on a personal computer using the statistical software package version 5.0 (Statsoft, Inc.). The data matrix used for PCA consisted of individual degradation indicators. The raw data matrix was normalized in order to nullify the influence of the components with higher values. Normalization was carried out by taking the logarithm of the parameter value plus 1, i.e. log \((x+1)\) where \(x\) was the value of each variable. Factors were extracted when the eigen values were more than 1.
3. Results

3.1. Bulk parameters, phytoplankton and bacterial cell counts

SPM, POC, and TPN showed significant \((p<0.001)\) spatial differences and increased from upstream station 8 to downstream station 1 during the monsoon, and generally, showed inverse distribution trends during the pre-monsoon (Table 1). The TBC varied from \(5.4 \times 10^8\) cells \(L^{-1}\) during the monsoon, and from \(17.1 \times 10^8\) cells \(L^{-1}\) during the pre-monsoon (Table 1). Phytoplankton cell counts at stations 4 to 8 were higher during the pre-monsoon (\(1.38 \times 10^4\) cells \(L^{-1}\)) than the monsoon (\(0.26 \times 3.84 \times 10^4\) cells \(L^{-1}\)) (Table 1).

3.2. Total particulate amino sugar concentrations and composition

TPAS concentrations varied from 61.4 to 171.5 nM (average = 127 ±48 nM), and from 38.2 to 80.0 nM (average = 64 ±13) during the monsoon and pre-monsoon, respectively (Table 2). During the monsoon, the concentrations of TPAS were lower at upstream stations (5, 6, and 8) than at the downstream stations (stations 1, 2, 3, and 4), and generally, increased from the upstream station 8 to downstream station 1 with the exception of station 7 (Table 2). The concentrations of TPAS did not exhibit any particular distribution trend during the pre-monsoon, and were significantly lower \((p<0.001)\) than the monsoon (Table 2). TPAS showed significant spatial \((p<0.0001)\) and seasonal \((p<0.005)\) variations. Salinity did not show significant relationship with TPAS concentration during the monsoon \((r = 0.628, p<0.1)\) and pre-monsoon \((r = 0.025, p>0.1)\). However, TPAS-C yields showed fairly good negative correlation with salinity \((r = -0.698, p<0.05)\) during the monsoon. The ratios of TPAA/TPAS increased from upstream station 8 to downstream station 1 during the monsoon and did not produced any consistent pattern for the pre-monsoon (Table 2).

GlcN was the most abundant (average = 35 ± 13 mol\%) amino sugar followed by GalN (average = 31 ± 6 mol\%), ManN (average = 28 ± 16%), and Mur (average = 4.2 ± 1.9%) (Table 2). During the monsoon, the concentrations of GlcN and GalN generally decreased from the downstream station 1 to upstream station 8 while ManN did not exhibit any particular distribution trend (Fig. 2 a, b, c). As compared to other stations, concentrations of ManN were higher at stations 4 to 8 during the monsoon. Mur concentrations decreased from downstream station 1 to upstream station 5, and then increased again from station 5 to station 8 during the monsoon (Fig. 2d). GlcN, GalN, ManN, and Mur did not depict any distribution trend during the pre-monsoon (Fig. 2). The spatial differences in GlcN, GalN, ManN, and Mur were highly significant for the monsoon \((p<0.0004\) to 0.002), and pre-monsoon \((p<0.0001\) to 0.005). The concentrations of GlcN, GalN, and ManN were relatively higher \((p<0.0001\) to 0.002) during the monsoon than the pre-monsoon (Fig. 2). Mur concentrations did not
show significant differences during the monsoon and pre-monsoon ($p<0.83$). Measured Mur-N yields varied between 0.009 and 0.021 mgN per 100 mg C$^{-1}$ during the monsoon and from 0.004 to 0.016 mgN per 100 mg C$^{-1}$ during the pre-monsoon (data not shown). The Mur-N yields showed significant positive relationship with TPN, especially during the monsoon ($r = 0.705$, $p<0.05$). Average concentrations of GlcN, GalN, and ManN were higher during the monsoon than the pre-monsoon (Fig. 3).

TPAS-C yields varied from 0.95 to 3.29% (average = 2.04 ± 1.03) during the monsoon, and from 0.35 to 1.08% (average = 0.66 ± 0.25) during the pre-monsoon (Fig. 4a). TPAS-C contribution to POC decreased from upstream station 7 to downstream station 1 (Fig. 4a). Similarly, the TPAS-N yields (TPAS-N/TPN × 100) varied from 1.52 to 4.47% (average = 2.60 ± 0.97) and 0.26 to 0.89% (average = 0.53 ± 0.21) during the monsoon and pre-monsoon, respectively (Fig. 4b). TPAS-C yields showed significant spatial differences during the monsoon ($p<0.0008$) and pre-monsoon ($p<0.0001$). Similarly, TPAS-N yields also exhibit significant spatial differences during the monsoon ($p<0.0001$) and pre-monsoon ($p<0.0002$). Yields of both %TPAS-C ($p<0.002$) and %TPAS-N ($p<0.001$) showed significant seasonal variations. During the pre-monsoon, yields of %TPAS-C and %TPAS-N generally increased from upstream station 7 to downstream station 1 (Fig. 4a, b). GlcN/GalN ratio varied from 0.6 to 1.9 (average = 1.1 ± 0.4), and from 1.2 to 2.3 (average = 1.8 ± 0.5) during the monsoon and pre-monsoon, respectively (Fig. 4c).

Distribution of some of the carbohydrate and amino acid degradation indicators during the monsoon and pre-monsoon is shown in Fig. 5. Mol% glucose, TPAA concentrations and the ratios of hexoses (glucose plus mannose plus galactose) to pentoses (ribose plus arabinose plus xylose), TPAA/TPAS, and GlcN/GalN were higher and C/N ratios, mol% deoxy sugars, TPAS, %TPAS-C, and %TPAS-N lower during the pre-monsoon than the monsoon (Figs.4, 5, Table 2). PCA of the diagenetic indicators produced 2 factors, and accounted for 77% of the total variance. Factor 1 accounted for 51 % while factors 2 for 26% of the total variation. Factor 1 produced positive factor loadings for glucose, hexoses/pentoses ratio, TPNS and TPAA and formed a fairly close cluster (Fig.6). Factor 2 showed positive factor loadings for C/N ratio, deoxy sugars and TPAS (Fig. 6).

4. Discussion

4.1. Total particulate amino sugar concentrations and composition

TPAS concentrations observed for the Mandovi estuary are higher than those reported earlier for lakes, seas and oceanic waters. (Benner and Kaiser, 2003; Davis and Benner, 2005; Klauser,
Mandovi is a shallow estuary. Therefore, greater TPAS concentrations at downstream stations 1, 2, and 3 may be the result of re-suspension of bottom sediments due to strong westerly winds (5.1 to 5.6 m s\(^{-1}\)), wind induced waves (2 to 2.5 m wave height) and currents and stirring by tides (2 to 2.5 m) during the monsoon. In contrast, during the pre-monsoon, no particular distribution trend was evident for TPAS probably due to negligible river run-off, and absence of re-suspension because of weak easterly winds (1.8 to 2.6 m s\(^{-1}\)). Yields of TPAS-C (0.35 to 3.29%) and TPAS-N (0.26 to 4.47%) observed for the Mandovi estuary are in the range of values reported from other environments (Tremblay and Benner, 2009; Unger et al., 2012; Carstens et al., 2012).

GlcN and GalN were the most abundant amino sugars (Fig. 2a,b). All the individual amino sugars showed significant spatial and seasonal differences. These amino sugars are also abundant in pure cultures of microorganisms and natural assemblages of organisms collected from various environments. This suggests their widespread distribution and similar reactivity (Benner and Kaiser, 2003; Tremblay and Benner, 2009). GlcN and GalN showed similar distribution trends. Significant positive relationships between GlcN and GalN during the monsoon \((r = 0.812, p<0.01)\) and pre-monsoon \((r = 0.954, p<0.001)\) suggest common origin. During the monsoon, greater concentrations of TPAS, and GlcN at stations 1, 2, 3, and 7 were due to abundances of TBC at stations 1, 2, and 3, and of phytoplankton at station 7 (Fig. 2). Greater abundance of GalN at stations 1, 2, 3, and 5 during the monsoon was due to greater TBCs at these stations (Table 2). During the monsoon, TBC showed positive correlations with GlcN \((r = 0.599, p<0.1)\), and GalN \((r = 0.871, p<0.001)\) suggesting that these amino sugars were derived from bacteria.

ManN was relatively less abundant during both the monsoon and pre-monsoon. Distribution trend observed for ManN was different than those recorded for GlcN and GalN implying a different reactivity. ManN is present in phytoplankton, zooplankton, and bacteria (Benner and Kaiser, 2003). ManN is derived from mannose that is a structural monomer in algae. Field and laboratory studies suggest that mannose is comparatively inert (Cowie and Hedges, 1996). ManN may also be less degradable and therefore gets accumulated in POM. However, with data at hand it is not possible to explain the abundance of ManN during the monsoon.

Mur concentrations decreased from downstream station 1 to 5 and then increased again (Fig. 2d). This distribution trend was similar to that recorded for TBC. Moreover, Mur showed significant positive correlations with the TBC during the monsoon \((r = 0.799, p<0.01)\), and the pre-monsoon \((r = 0.666, p<0.05)\) suggesting that Mur was mostly associated with living bacteria or freshly derived bacterial necromass. During the monsoon, Mur also showed significant relationships with GlcN \((r =\)

0.708, \( p<0.05 \) and GalN \( (r = 0.771, p<0.01) \) suggesting common origin for these amino sugars. During the pre-monsoon, distribution of TPAS or individual amino sugar did not produce any particular trend, except that Mur concentrations were higher at stations 5, 6, and 9 due to greater TBCs at these stations.

4.2. Origin and diagenetic alteration of amino sugars

Amino sugar composition is a useful tool to identify the relative inputs of OM derived from zooplankton and bacteria in marine waters (Müller et al., 1986; Haake et al., 1993; Benner and Kaiser, 2003; Fernandes et al., 2006; Niggemann and Schubert, 2006). This approach is based on the fact that zooplankton is rich in GlcN. Due to high chitin concentrations, GlcN/GalN ratios are high (>20) for zooplankton rich POM (Müller et al., 1986; Gupta and Kawahata, 2000; Benner and Kaiser, 2003; Niggemann and Schubert, 2006). In contrast, soil and aquatic bacteria exhibit GlcN/GalN ratios that are lower (1 to 3) than those recorded for fungi, plants, and animals (Glaser et al., 2004; Klauser, 2007; Tremblay and Benner, 2006). For the Mandovi estuary, the GlcN/GalN ratio showed spatial and seasonal variations and varied between 1 to 3 (Fig. 4c). These GlcN/GalN ratios fall in the range of values reported for bacteria. This indicates that TPASs were mostly derived from bacteria. The GlcN/GalN ratios were significantly \( (p<0.001) \) lower during the monsoon (average = 1.1 ± 0.4) than the pre-monsoon (average = 1.8 ± 0.5) implying greater bacterial influence and diagenetic processing during the former season.

GlcN/GalN ratio decreases with progressing degradation of planktonic OM (Benner and Kaiser, 2003; Davis et al., 2009). Thus GlcN/GalN ratio also provides information on diagenetic transformation of OM. Davis et al. (2009) reported rapid biodegradation of purified or natural chitin in seawater by natural bacterial population during which GlcN/GalN ratios of zooplankton OM decreased from 8.1 to 1.1. The low GlcN/GalN ratio (~1.1) recorded is similar to that obtained for marine bacteria. This suggests that amino sugar signature of planktonic OM having high (>8) GlcN/GalN ratio was gradually replaced by that of bacteria with low (~ 1.1) GlcN/GalN ratio (Davis et al., 2009; Kawasaki and Benner, 2006). However, abiotic release of GlcN from particulate matter into dissolve phase may also account for low GlcN/GalN ratios in the Mandovi estuary (Davis et al., 2009).

In the present study, during the monsoon, POM had very low GlcN/GalN ratios (average = 1.1). This indicates bacterial signature and progressing diagenesis. Such low GlcN/GalN ratios have also been reported for degraded POM and sediment OM samples from river (Tremblay and Benner, 2009), lakes (Carstens et al., 2012), estuaries (Unger et al., 2012), sea and oceanic waters (Benner
and Kaiser, 2003; Davis and Benner, 2005) and marine sediments (Klauser, 2007; Lomstein et al., 2009). This implies that irrespective of the environmental setting, degradation of OM results in the production of OM with low (~ 1.1) GlcN/GalN ratio.

In peptidoglycan, GlcN and Mur occurs in 1:1 ratio (Schleifer and Kandler, 1972). Using this ratio, GlcN contribution by bacterial peptidoglycan to particulate GlcN was calculated (Table 2). In the Mandovi estuary, bacterial cell walls accounted for on an average 14 ± 11.4% and 16 ± 7% of the particulate GlcN during the monsoon and pre-monsoon, respectively (Table 2). This can also be the result of less phytoplankton production during the monsoon than the pre-monsoon. These values compare well with those reported for the POM from other environment (6% and 15%) (Carstens et al., 2012).

During the monsoon, with some exceptions, TPAS-C and TPAS-N yields generally decreased, from upstream station 8 to downstream station 1 due to mixing of water masses (Fig. 4a, b). It also was evident from the significant inverse relationship (r = - 0.698, p<0.05) between salinity and %TPAS-C. This implies that transport of amino sugar rich degraded terrestrial material from land surface to estuarine waters as well as the mixing of water masses played important role in influencing the distribution of amino sugars in the Mandovi estuary.

Amino sugar yield is a useful tool to understand degradation state of OM (Benner and Kaiser, 2003; Carstens, 2012). Generally, distribution trend recorded for TPAA/TPAS ratios was in contrast with that observed for TPAS yields (Fig. 4a, b, Table 2). During the monsoon, TPAS yields were higher and TPAA/TPAS ratios lower at the upstream stations (4 to 7) than those recorded for the downstream stations (1, 2, and 3) (Fig. 4,Table 2). Lower TPAA/TPAS ratios at upstream stations 4 to 7 reflect preferential utilization of TPAA resulting in the accumulation of amino sugars and/or transport of amino sugar rich degraded OM at these stations. This is in agreement with the observation that yields of amino sugar carbon and nitrogen are relatively higher in degraded than fresh OM (Dauwe and Middelburg, 1998; Benner and Kaiser, 2003; Kawasaki and Benner, 2006). Carstens and Schubert (2012) reported decrease in amino acids/amino sugar ratios with increasing water column and accumulation of amino sugars in degraded OM. Moreover, increase in amino sugar yields at upstream stations 4 to 7 may also be due to production of amino sugars by bacteria during degradation of OM. Whereas, high TPAA/TPAS ratios at stations 1, 2, and 3 reflect removal of amino sugars or input of protein rich OM at these stations. Distribution trends of TPAS yields and TPAA/TPAS ratios suggest that during the monsoon upstream stations (4 to 7) experienced extensive diagenetic alteration of OM than the downstream stations (1, 2, and 3). In contrast, during the pre-monsoon, yields of TPAS-C and TPAS-N generally decreased from downstream station 1 to
upstream station 7 implying that OM was diagenetically less altered at the upstream stations than the downstream stations (Fig. 4a, b). Interestingly, yields of TPAS-C and TPAS-N were higher during the monsoon than the pre-monsoon suggesting that OM was subjected to extensive diagenetic alteration during the monsoon than the pre-monsoon. This conclusion was also supported by TPAA/TPAS ratios which were lower during the monsoon than the pre-monsoon. Significant negative correlations of TBC with % TPAS-C (r = -0.742, n = 16, p < 0.001) and % TPAS-N (r = -0.562, n = 16, p < 0.02) reflect the influence of bacteria on biochemical cycling of amino sugars in the Mandovi estuary.

4.3. Comparison of amino sugars with other degradation indices

Carbohydrate and amino acid concentration and composition have often been used to understand biogeochemical processes occurring in aquatic environments (Cowie and Hedges, 1984; Ittekkot and Arain, 1986; Opsahl and Benner, 1999). Glucose content, TPAA concentrations, TPAA/TPAS ratio, and hexoses to pentoses ratio are relatively higher and C/N ratio, TPAS concentration, TPAS yields, and deoxy sugars are lower in freshly produced OM than in degraded OM derived from both marine and terrestrial sources (Cowie and Hedges, 1994; Tremblay and Benner, 2009; Carstens et al., 2012; Unger et al., 2012). Higher glucose content, TPAA concentrations, hexoses to pentoses ratios, and lower C/N ratio, TPAS concentrations, and deoxy sugars during the pre-monsoon than the monsoon suggests that OM was relatively fresher during the pre-monsoon than the monsoon (Fig. 5) (Fernandes, 2011; Khodse and Bhosle, 2012). This observation was also supported by relatively lower abundances of GlcN and GalN, %TPAS-C and %TPAS-N and higher TPAA/TPAS ratios during the pre-monsoon than the monsoon. Degradation trends obtained with TPAS were similar to those obtained using carbohydrate and amino acid concentration and composition suggesting that amino sugars are useful indicators to assess diagenetic changes in OM.

Diagenetic trends obtained using amino sugars were further compared with well established diagenetic indicators of amino acids and carbohydrates using PCA (Fig. 6). High positive factor loadings for glucose, TPNS, TPAA and hexoses/pentoses ratio suggest that the factor 1 was mostly controlled by freshly derived OM. In contrast, high positive factor loadings for deoxy sugars, TPAS and C/N ratio indicate that the second factor was influenced by degraded OM (Fig. 6).

4.4. Estimation of bacterial contributions to particulate organic matter

The detection of Mur indicates the presence of bacterial peptidoglycan or its remnants in the POM of the Mandovi estuary. We used Mur C and N yields (pl see material and method) to calculate
bacterial contribution to bulk C and N of POM using average bacterial yield taken from published literature on bacterial assemblages typically found in soils, fresh and estuarine waters (Tremblay and Benner, 2009; Bourgoin and Tremblay, 2010). Range of values depends on the proportion of Gram-positive bacteria assumed in natural assemblages (5 to 15%, Tremblay and Benner, 2009). Bacterial-C accounted on an average 35 ± 10%, and 24 ± 9% of POC during the monsoon and pre-monsoon, respectively (Table 1). The average contribution of bacterial-N to TPN was 62 ± 25% and 24 ± 8% during the monsoon and pre-monsoon, respectively. These bacterial C and N contributions to bulk POM compare well with those reported from other environments using Mur concentrations (Kaiser and Benner, 2008; Bourgoin and Tremblay, 2010).

Our estimates of bacterial contributions to bulk POM from Mur compare well with those earlier reported by Fernandes (2011) for the Mandovi estuary using D-alanine plus D-glutamic acid. Conversely, for the Amazon River system, Mur based bacterial contributions to the bulk POM were lower than those estimated from D-alanine plus D-glutamic acid (Tremblay and Benner, 2009). Mur is a biomarker for living bacteria (Moriarty, 1977) or recent bacterial necromass (Benner and Kaiser, 2003; Niggemann and Schubert, 2006). As compared to bacterial molecules D-alanine and D-glutamic acid or bulk POM, Mur is relatively more reactive (Nagata et al., 2003; Tremblay and Benner, 2006). This indicates that POM of the Mandovi estuary was relatively fresher than the Amazon River system.

Intact bacterial cells accounted for 2.5% of POC and 11% of TPN during the monsoon, and 4% of POC and 9% of TPN during the pre-monsoon. This suggests that most of the bacterial contribution estimated here comes from cellular debris. This conclusion is also supported by GlcN/Mur ratios which varied between 2.4 and 20.1, and from 3.5 to 19 (data not shown) during the monsoon and pre-monsoon, respectively. These GlcN/Mur ratios are much higher than that of bacterial peptidoglycan which has the ratio close to one. This is consistent with earlier observations that bacterial contribution mostly comes from cellular debris and not from intact bacterial cells (Kaiser and Benner, 2008; Tremblay and Benner, 2009).

The relationship between carbon normalized Mur-N and TPN in natural samples is another indicator to understand the influence of bacteria on POM (Tremblay and Benner, 2009). Carbon normalized concentrations of bacterial biomarker Mur-N showed significant positive relationship with TPN ($r = 0.705; p<0.05$), particularly during the monsoon. This relationship indicates that changes in bacterial-N content generally reflected changes in TPN of the Mandovi estuary. The importance of bacteria as a source of N in decomposing plant material transported by the Mandovi estuary is very much evident from this relationship.
4.5. Effect of monsoon on amino sugar yield and composition

In the Mandovi estuary, primary production is reduced due to light limited condition prevailing during the monsoon. The estuary becomes predominantly heterotrophic with primary production/respiration ratio of 0.28 (Ram et al., 2003; Shetye et al., 2007). OM is mostly derived from terrestrial sources (Fernandes, 2011; Khodse and Bhosle, 2012). POM of terrestrial origin has undergone extensive diagenetic alterations was well reflected in higher concentrations and yields of amino sugars during the monsoon. GlcN, GalN and ManN were relatively more abundant during the monsoon than during the pre-monsoon. These amino sugars tend to accumulate during degradation probably because they occur in structural biopolymer matrices (Tremblay and Benner, 2006; Kawasaki and Benner 2006). Moreover, extensive diagenetic alteration of OM probably due to extensive runoff of degraded terrestrial OM during the monsoon was also evident from lower TPAA/TPAS, and GlcN/GalN ratios during the monsoon than the pre-monsoon (Table 2). GlcN/GalN ratios during the monsoon indicate that terrestrial OM has strong imprint of bacterial OM. Thus during the high discharge monsoon period degraded terrestrial POM with bacterial imprint will be transported to the coastal waters. Conversely, increase in water temperature and salinity, improved light conditions, availability of nutrients, and negligible river run-off favour phytoplankton growth during the pre-monsoon. This was evident from higher phytoplankton cell numbers during the pre-monsoon than the monsoon. Freshly produced OM has low concentrations and yield of TPAS as well as relatively higher TPAA/TPAS and GlcN/GalN ratios.

5. Conclusions

Analyses of TPASs were suitable tools to understand dynamics, sources, nature and transformation of POM in the tropical monsoon driven estuary. The data presented above showed systematic changes in the nature of OM during the monsoon and pre-monsoon. In the Mandovi estuary, POC associated with the high discharge monsoon period contains much greater concentrations of amino sugars and lower concentrations of labile constituents such as sugars and amino acids and has already undergone considerable degradation (Fernandes, 2011; Khodse and Bhosle, 2012). In contrast fresh material was produced during the pre-monsoon.

Our results revealed that during the monsoon, distribution and abundance of TPAS, GlcN, GalN and Mur in the Mandovi estuary were influenced by bacterial abundance. However, high and variable GlcN/Mur ratios argue against major inputs from peptidoglycan. Similarly, low GlcN/GalN ratios (<3), particularly during the monsoon suggest that chitin was not the major source of amino
sugars. The importance of bacterially derived OM was also supported by the greater contribution of D- amino acids during the monsoon (Fernandes, 2011). ManN was more abundant (11 to 55 mol%) during the monsoon than the pre-monsoon. However, with data at hand, it was not possible to identify the factors responsible for the abundance of ManN. POM enriched in bacterial OM will be transported to coastal waters during the high discharge period.

Further studies on amino sugar distribution from various aquatic environments including estuaries are required to explain various processes influencing their dynamics and transformation. There also is a need to determine amino sugar concentration and composition in bacteria, fungi, micro-algae and terrestrial plants to develop biomarkers to identify sources of amino sugars. Studies using compound specific carbon and nitrogen isotope analysis might help to better understand amino sugars biogeochemistry in estuarine environment.

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Figure captions:

Fig. 1. Map showing the location of sampling stations in the Mandovi estuary, the west coast of India.

Fig. 2. Spatial and seasonal variation of glucosamine (a), galactosamine (b), mannosamine (c), and muramic acid (d) in the suspended particulate matter during the monsoon and pre-monsoon in the Mandovi estuary, the west coast of India.

Fig. 3. Variation in the average concentration of glucosamine (GlcN), galactosamine (GalN), mannosamine (ManN), muramic acid (Mur) of the suspended particulate matter during the monsoon and pre-monsoon in the Mandovi estuary, the west coast of India.

Fig. 4. Spatial variation in the contribution of total particulate amino sugar-carbon (TPAS-C) to particulate organic carbon (POC) (a), total particulate amino sugar-nitrogen (TPAS-N) to total particulate nitrogen (TPN) (b), and glucosamine:galactosamine (GlcN/GalN) ratios (c), during the monsoon and pre-monsoon in the Mandovi estuary, the west coast of India.

Fig. 5. Spatial and seasonal variation of total particulate neutral sugars (TPNS) (a), glucose (b), deoxy sugars (rhamnose plus fucose) (c), and hexoses/pentoses ratio (d), in the suspended particulate matter during the monsoon and pre-monsoon in the Mandovi estuary, the west coast of India. Data of TPNS, glucose, deoxy sugars and hexoses/pentoses ratio were taken from Khodse and Bhosle (2012).

Fig. 6. Plot of the principal component analysis (PCA) loading factors of TPAS, TPNS, glucose, deoxy sugars, hexoses/pentoses ratio, TPAA, and C/N ratio, for suspended particulate matter during the monsoon and pre-monsoon in the Mandovi estuary, the west coast of India. The variance accounted for by each principal component is shown on X and Y axis. Abbreviations used are given in Fig. 5, and Table 2. TPNS, glucose, deoxy sugars and hexoses/pentoses ratio data were taken from Khodse and Bhosle (2012), and TPAA data from Fernandes (2011).
Fig. 1.
Fig. 2.

Fig. 3.
Fig. 4.
Fig. 5.

Fig. 6.
Table 1.
Spatial and seasonal variability of salinity, suspended particulate matter (SPM), particulate organic carbon (POC), total particulate nitrogen (TPN), C/N ratio, phytoplankton cell counts, total bacterial cell counts (TBC), and percentage (%) contribution of bacterial cell carbon and nitrogen to POC and TPN of the Mandovi estuary during monsoon and pre-monsoon.

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<th>POC μM N</th>
<th>TPN μM N</th>
<th>C/N ratio (x10⁴ cells L⁻¹)</th>
<th>Phytoplankton (x10⁸ cells L⁻¹)</th>
<th>TBC (x10⁸ cells L⁻¹)</th>
<th>Bacterial contribution (%) Bac-C/POC</th>
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- = no data; * = data from Khodse et al, 2010; Bacterial-C or N (%) = {Biomarker sample/Biomarker bacterial} × 100. Bacterial biomarker yields in bacterial cells used for these calculations were 28.1 nmol mg C⁻¹ Mur and 121.3 nmol mg N⁻¹ Mur (Kaiser and Benner, 2008); SD = standard deviation; Bac-C = bacterial carbon, Bac-C was calculated using the average carbon content of 11 fg C cell⁻¹ (Kawasaki et al., 2008). Bac-N = bacterial nitrogen, Bac-N was calculated using an average nitrogen content of 5.8 fg N cell⁻¹ (Fukuda et al., 1998).
Table 2.
Spatial and seasonal variability of total particulate amino sugars (TPAS), total particulate amino acids (TPAA), TPAA/TPAS ratio, amino sugar compositions and GlcN<sub>peptidoglycan</sub> contribution (%) to the particulate GlcN of the Mandovi estuary during monsoon and pre-monsoon.

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</tr>
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</table>

- = no data; *= data from Fernandes, 2011; ** = GlcN<sub>peptidoglycan</sub> (%) = 100 – {(GlcN-Mur)/GlcN} × 100, where GlcN and Mur indicates molar concentrations.