

# Distribution of dissolved carbohydrates and uronic acids in a tropical estuary, India

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Carbohydrates including uronic acids are among the active components of dissolved organic carbon, and play an important role in biogeochemical cycling of organic carbon in marine environments. In order to understand their distribution, concentrations of total dissolved carbohydrate (TCHO), dissolved polysaccharide (PCHO), dissolved monosaccharide (MCHO), and dissolved uronic acid (URA) were measured in the Mandovi estuary, west coast of India during the monsoon and pre-monsoon seasons. The estuary experienced nearly fresh water condition during the monsoon season and marine condition during the pre-monsoon season. Concentrations of TCHO, MCHO and URA ranged from 17.7 to 67.3  $\mu\text{M C}$ , 4.1 to 15.5  $\mu\text{M C}$  and 2.3 to 10.8  $\mu\text{M C}$ , and their contribution to dissolved organic carbon (DOC) varied from  $\sim 11$  to 60%, 2.5 to 9.7%, and 1.8 to 5.3%, respectively. PCHO accounted for  $\sim 52$  to 92% of the TCHO. Generally, concentrations and yields of TCHO species were greater during the monsoon season. Phytoplankton abundance and bacterial cell numbers influenced the distribution of TCHO in the pre-monsoon season but not during the monsoon season. Transport of TCHO rich (11 to 60%) dissolved organic matter from the Mandovi estuary to the coastal waters during the monsoon season may affect ecosystem function by fueling biological activity of heterotrophic micro-organisms.

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## 1. Introduction

Carbohydrates are ubiquitous in marine environments, and are associated with biogeochemical processes taking place in marine environments (Bada and Lee 1977). Dissolved carbohydrates account for a large fraction ( $\sim 10$  to 85%) of dissolved organic carbon (DOC) in seawater (Romankevich 1984; Thurman 1985; Pakulski and Benner 1994; Hung *et al* 2001, 2005), pore waters (Burdige *et al* 2000) and ultrafiltered DOC (Amon and Benner 2003). Dissolved carbohydrates are derived from various processes including direct release by organisms (Biddanda and Benner 1997; Hama and Yanagi 2001), microbial degradation of organic matter (Hellebust 1965), viral or bacterial lysis of phytoplankton cells (Jumars *et al* 1989) and allochthonous DOC from rivers and soil (Gueguen

*et al* 2006). Dissolved carbohydrates are useful substrates for heterotrophic bacteria (Rich *et al* 1997; Kirchman *et al* 2001). Heterotrophic uptake and secondary production influence the abundance of dissolved carbohydrates in aquatic environments (Satoh *et al* 1986; Meon and Kirchman 2001). Due to their reactive nature, carbohydrates have been proposed as indicators of the bioreactivity and diagenetic state of natural dissolved organic matter (Skoog and Benner 1997; Amon and Benner 2003). Distribution of dissolved total carbohydrate (TCHO), dissolved monosaccharide (MCHO), and polysaccharide (PCHO) has been reported from different environments including rivers (Hung *et al* 2005; Gueguen *et al* 2006; Cai *et al* 2008), estuaries (Senior and Chevolut 1991), coastal and oceanic waters (Burney and Sieburth 1977; Pakulski and Benner 1994; Bhosle *et al* 1998; Hung *et al* 2001,

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2003; Wang *et al* 2006) and Antarctic sea ice (Herborg *et al* 2001).

In addition to neutral carbohydrates, many organisms produce acidic carbohydrates such as uronic acids (URA). URA can account for ~4 to 30% of TCHO, and 0.7 to 5% of DOC in river and estuarine waters (Hung *et al* 2001, 2005). Concentrations of URA generally decrease with water column depth suggesting their utilization by heterotrophic organisms (Khodse *et al* 2007). According to Hung *et al* (2003) URAs are less degradable than TCHO. Abundance of URA is of importance in humic acid formation (Yamaoka 1983), detoxification of toxic chemicals and removal of metal ions (Decho 1990; Santschi *et al* 1998; Quigley *et al* 2002), production of macroaggregates (Mopper *et al* 1995; Leppard 1997), microbial adhesion and biofilm development (Jain and Bhosle 2009).

Most of the above studies on dissolved TCHO species including URA have been carried out in temperate, Arctic and Antarctic waters. In contrast, numerous studies have been carried out to understand physical, chemical and biological processes in the tropical Mandovi estuary (Qasim and Sen Gupta 1981; De Sousa 1983; Devassy and Goes 1988; Ansari and Parulekar 1993; Shetye *et al* 1995, 2007; Ram *et al* 2003; Partihary *et al* 2009). However, little is known about biochemical characterization of dissolved organic matter in tropical waters, particularly in estuarine waters. Therefore, our aims were:

- to understand the distribution of TCHO, MCHO, PCHO and URA in the Mandovi estuary, and
- to evaluate the role of some factors in controlling the distribution of TCHO and URA in the Mandovi estuary.

## 2. Materials and methods

### 2.1 Study area

The Mandovi estuarine system is located in Goa, west coast of India between 15°15'N and 15°42'N and 73°45'E and 74°24'E. The Mandovi River originates in the Western Ghats of India and flows into the Arabian Sea. The total length of the estuary is about 75 km and the width at the mouth is about 3.2 km. The climate is humid, tropical with moderate temperature. The Mandovi River transports  $6 \times 10^{15}$  cm<sup>3</sup>/year of water to the Arabian Sea (Suprit and Shankar 2008). Ninety percent of this transport occurs during the monsoon season (June–September). The estuary is fed largely by the monsoon precipitation and receives a large

amount of land runoff from ore mining areas and forest land. In contrast, during the non-monsoon period, the input of fresh water is negligible and is regulated by the semidiurnal tides. The residence time of the water in the Mandovi estuary is 5–6 d during the monsoon season and about 50 d during the non-monsoon season (October–May) (Qasim and Sen Gupta 1981). Recently, physical, chemical and biological processes in this tropical estuary have been reviewed by Shetye *et al* (2007).

### 2.2 Sample collection

Surface (~1 m) water samples were collected from nine locations in the Mandovi estuary during the monsoon (August 2005) and pre-monsoon (March 2007) seasons (figure 1). Water samples were collected using 5 litre Niskin samplers. Immediately after collection, water was passed through 200 µm mesh nylon screen to remove large zooplankton, and then filtered through mixed cellulose ester filter papers (0.22 µm, Millipore). This filtrate was defined as dissolved fraction. Filtered samples were preserved at –20°C for 15 days before analysis. Samples were also collected for chlorophyll *a* (Chl *a*) and phytoplankton cell counts. Chl *a* samples were filtered through 200 µm mesh nylon screen to remove large zooplankton, then through a Whatman GF/F filter. The filter was immediately transferred to a clean screw capped glass vial and transported to the laboratory in an ice box. For phytoplankton cell counts, one litre samples were preserved with a few drops of Lugol's iodine (1% w/v). In order to estimate total bacterial count (TBC), seawater sample (20 ml) was fixed with glutaraldehyde (final concentration 2%) and stored for 7 days at 4°C until analysis.

### 2.3 Ancillary measurements

Sea surface temperature and surface salinity were measured by the portable conductivity/temperature/depth (CTD) (Model SBE 19 plus, Sea-Bird Electronics, USA). Dissolved oxygen (DO) was fixed and analyzed following the Winkler's method (Parsons *et al* 1984). Dissolved nitrate, phosphate and silicates were measured using spectrophotometric methods (Parsons *et al* 1984). Chl *a* was extracted overnight in 90% acetone and measured following the method of Parsons *et al* (1984). Phytoplankton cells' count and composition were determined by settling and siphoning procedure to obtain 20 to 25 ml concentrated sample (Prabhu Matondkar *et al* 2007). One millilitre of the concentrated samples was analyzed microscopically under inverted Olympus microscope (200 × magnification) in Sedgewick–Rafter plankton counting chamber.

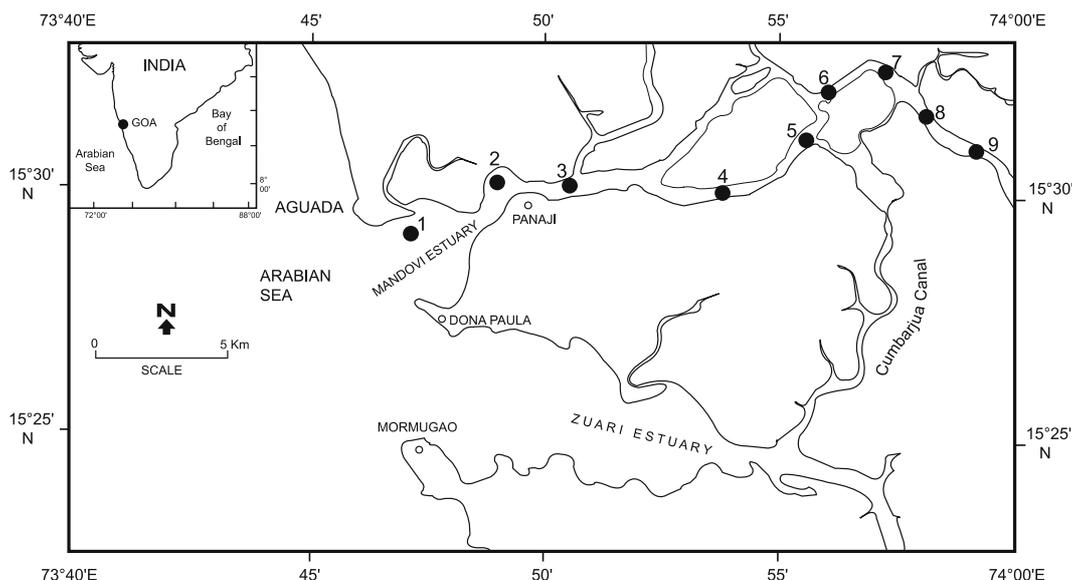


Figure 1. Sampling locations in the Mandovi estuary, west coast of India.

In order to estimate TBC, a known volume of seawater (2–5 ml) was stained with acridine orange (final concentration 0.01%) for 5 min, filtered onto 0.22  $\mu\text{m}$  black Nuclepore polycarbonate filter. Bacterial cells were counted in at least 25 randomly selected fields using 100 $\times$  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).

#### 2.4 Determination of DOC

DOC was analyzed by the high temperature (680°C) oxidation method using a Shimadzu TOC-5000 carbon analyzer. Sample values were corrected for the instrument blank (Benner and Strom 1993). Potassium hydrogen phthalate was used as a standard (50–400  $\mu\text{M C}$ ). Eight samples were analysed between injection of Milli-Q water and working standard solution. Analytical variation of this method was  $\pm 3.1\%$ .

#### 2.5 Determination of carbohydrates

MCHOs were determined without hydrolysis using the 2,4,6 tripyridyl-s-triazine (TPTZ) spectrophotometric method of Myklestad *et al* (1997). Briefly, 1 ml of filtered (0.22  $\mu\text{m}$  pore size mixed cellulose ester filter paper, Millipore) water sample was mixed in a test tube with 1 ml of potassium ferricyanide (0.7 mM) and kept in boiling water bath for 10 min. Then 1 ml of ferric chloride (2 mM) followed by 1 ml of TPTZ (2.5 mM) were added immediately and mixed well on a vortex-mixer. After 30 min, absorbance was taken at 595 nm.

In order to measure TCHO, a known aliquot (10 ml) of the filtered (as above) estuarine water was evaporated to dryness at 40°C using a rotary vacuum evaporator, and treated with 1 ml of 12 M  $\text{H}_2\text{SO}_4$  for 2 h at room temperature ( $28 \pm 2^\circ\text{C}$ ) (Bhosle *et al* 1998). Subsequently, the sample was diluted to 1.2 M using Milli-Q water, transferred to a glass ampoule, flushed with nitrogen gas and sealed. The sealed sample was hydrolyzed at 100°C for 3 h (Bhosle *et al* 1998). After this, the sample was removed, cooled and neutralized with 2 N NaOH. A suitable aliquot was then used for the analysis of total dissolved carbohydrates by the TPTZ method as described above.

All the samples were analyzed in triplicate. Calibration curves were prepared using glucose (0.5 to 3  $\mu\text{g/ml}$ ) as a standard. The coefficient of variation of the method was  $< 4\%$ . The values of carbohydrates are expressed as glucose equivalent. The unit used is  $\mu\text{M}$  glucose assuming that all monomers were hexoses. The unit  $\mu\text{M C}$  is obtained by multiplying  $\mu\text{M}$  values by 6 assuming 6 mol of carbon per mol of hexoses. PCHO concentrations were calculated by subtracting MCHO concentrations from the concentrations of TCHO.

#### 2.6 Determination of URA

The concentrations of URA were analyzed by the method of Filisetti-Cozzi and Carpita (1991) as described by Hung and Santschi (2001). Briefly, 30 ml of estuarine water were passed through cation exchange resin column (Dowex 50W-8X, 50 to 200 mesh, hydrogen form, Sigma Company). The column was washed with three bed volumes of Milli-Q water to remove the adsorbed uronic acids

from the cation exchange column. This washing procedure gave 90 to 96% recovery of uronic acid. Hung and Santschi (2001) reported 84 to 89% recovery of uronic acids using the same procedure. All the washings (column passing solution) were combined, and reduced to a small volume using a rotary vacuum evaporator. To the 0.4 ml of the concentrated sample, 40  $\mu$ l of 2 M sulfamic acid was added and the solution was stirred on a vortex mixer. Subsequently, 2.4 ml of 75 mM sodium tetraborate in concentrated sulfuric acid was added to the vial, and the vial was heated at 100°C for 10 min in a boiling water bath. After cooling, 80  $\mu$ l of 0.15% m-hydroxydiphenyl was added and after 10 min, absorbance was measured at 525 nm. Absorbance of Milli-Q water blank was subtracted from sample absorbance. Glucuronic acid was used as a standard compound for calibration. Concentration was expressed as glucuronic acid equivalent. The unit used is  $\mu$ M of glucuronic acid. The unit  $\mu$ M C is obtained by multiplying  $\mu$ M values by 6 assuming 6 mol of carbon per mol of hexoses. The coefficient of variation of the method was <6%.

### 2.7 Statistical analysis

One-way ANOVA was carried out to understand spatial and seasonal variations in Chl *a*, DOC, TCHO, MCHO, PCHO and URA using STATISTICA program (5) loaded in the personal computer. Correlation between parameters was assessed using Excel software program (Sokal and Rohlf 1981).

## 3. Results and discussion

### 3.1 General hydrography and nutrient

Surface water temperature was low (27.4 to 28.3°C) in the monsoon, and high (30.7 to 31.8°C) during the pre-monsoon season (table 1). Although no particular trend was noticed, water temperature was higher at the upstream station #8 and lower at station #1 near the mouth of the estuary (table 1). During the monsoon season, nearly fresh water condition exists in the estuary because of heavy precipitation (table 1). In contrast, the estuary experience marine condition during the dry pre-monsoon season because of negligible freshwater discharge. DO concentrations varied from 2.9 to 5.8 mg/l (table 1), and concentrations were higher during the monsoon season (table 1). Concentrations of nitrate, phosphate and silicates showed strong seasonal and spatial variations. Concentrations of nitrate and silicates were higher at the fresh water end that generally decreased

towards the mouth of the estuary (table 1). During the monsoon season, salinity showed significant inverse relationships with nitrate ( $r = -0.814$ ,  $p < 0.01$ ), and silicates ( $r = -0.746$ ,  $p < 0.02$ ) suggesting that freshwater discharge was a major source for these nutrients. In contrast, phosphate concentrations increased with increase in salinity during the monsoon season ( $r = 0.856$ ,  $p < 0.01$ ), and the pre-monsoon season ( $r = 0.910$ ,  $p < 0.001$ ) implying that seawater was a source for phosphate in the estuary (table 1). In addition, inorganic nutrients are released in to estuarine waters from bottom sediments through vertical and horizontal mixing by winds, tides and bottom currents (Partihary et al 2009). This nutrient supply is mostly responsible for higher phytoplankton biomass and primary production in the estuary during the pre-monsoon season (Prabhu Matondkar 2007; Partihary et al 2009).

### 3.2 Distribution of phytoplankton and TBC

Phytoplankton cell numbers and Chl *a* concentrations were higher in the pre-monsoon season (table 1). Highest phytoplankton cell count recorded at station #7 ( $3.84 \times 10^4$  cells/l) during the monsoon season was due to a dinoflagellates bloom (table 1). Nutrient concentrations were higher, whereas phytoplankton cell counts and Chl *a* concentration were lower during the monsoon season because of light limited condition and high suspended particulate matter (Prabhu Matondkar et al 2007). During the monsoon period, diatoms were more abundant (70 to 98%) at stations (#1 to #4) with relatively higher salinity, while dinoflagellates (64 to 98%) were abundant at stations (#5, #6, and #7) with lower salinity (table 1). During the pre-monsoon season diatoms and dinoflagellates accounted for 58 to 98%, and 2 to 42% to the total phytoplankton count, respectively (table 1). In surface waters, TBC varied from ( $5.4 \times 10^8$ ) to ( $42.1 \times 10^8$ ) cells/l (table 1). TBC was lower during the monsoon season and higher during the pre-monsoon season (table 1).

### 3.3 Dissolved organic carbon

In surface waters of the Mandovi estuary, DOC concentrations ranged between 80 and 195  $\mu$ M C (table 2). These DOC values are in the range of values earlier reported for the Mandovi estuary (Ram et al 2003), and also for some other estuarine and coastal environments (Lobbes et al 2000; Hung et al 2003, 2005; Kohler et al 2003). There was no correspondence between Chl *a* and DOC during both the monsoon and pre-monsoon seasons, indicating that phytoplankton biomass was

Table 1. Temperature, salinity, dissolved oxygen (DO), phosphate (PO<sub>4</sub>), nitrate (NO<sub>3</sub>), silicate (Si), chlorophyll *a* (Chl *a*), phytoplankton cell counts, diatoms and dinoflagellates percentage to total phytoplankton and total bacterial count (TBC) in the Mandovi estuary.

Station	Temperature (°C)	Salinity	DO (mg/l)	PO <sub>4</sub> (μM)	NO <sub>3</sub> (μM)	Si (μM)	Chl <i>a</i> (μg/l)	Phytoplankton (×10 <sup>4</sup> cells/l)	Diatoms <sup>a</sup> (%)	Dinoflagellates <sup>a</sup> (%)	TBC (×10 <sup>8</sup> cells/l)
Monsoon											
1	27.4	16.34	3.3	0.84	4.30	43.9	4.49	0.36	95.0	5.0	29.1
2	27.6	14.58	4.4	0.98	7.30	55.3	4.72	0.48	97.9	2.1	12.3
3	27.6	13.40	3.6	0.53	3.73	83.7	4.32	0.56	95.4	4.3	28.1
4	28.1	1.03	5.4	0.36	6.84	118.2	2.05	0.28	70.1	26.5	5.4
5	27.5	0.14	5.2	0.44	8.85	121.2	1.25	0.64	12.6	86.2	9.3
6	27.7	0.09	5.2	0.44	8.94	126.9	1.00	0.26	33.8	63.8	6.1
7	27.4	0.09	5.7	0.40	8.30	126.9	5.97	3.84	1.8	98.0	6.3
8	28.3	0.06	5.8	0.44	8.88	59.5	6.53	NS	NS	NS	6.0
Pre-monsoon											
1	30.8	34.58	2.9	0.75	1.65	7.0	9.17	0.56	97.7	2.3	20.3
2	30.9	34.06	3.3	0.67	3.79	5.5	6.30	0.27	88.2	11.8	24.2
3	30.7	33.86	3.3	0.66	4.00	6.0	5.85	0.35	95.3	4.7	17.1
4	30.8	32.65	3.4	0.79	4.08	5.7	14.66	1.63	86.9	13.1	24.3
5	31.2	30.19	3.3	0.54	1.94	2.5	14.02	1.45	84.6	15.4	33.6
6	31.3	29.13	3.4	0.55	1.85	3.2	13.94	1.38	76.3	23.7	42.1
7	31.5	24.44	3.9	0.42	1.53	1.2	11.02	2.87	58.1	41.9	22.8
8	31.5	21.33	4.1	0.43	2.45	2.3	17.24	3.21	86.4	13.6	19.8
9	31.8	17.46	4.3	0.37	1.18	1.8	9.33	0.41	98.1	1.9	41.5

<sup>a</sup>Percentage of the total phytoplankton cell count.

Table 2. Distribution of dissolved organic carbon (DOC), total dissolved carbohydrate (TCHO), free monosaccharide (MCHO), polysaccharide (PCHO), and dissolved uronic acid (URA) in the Mandovi estuary.

Station	DOC	TCHO	MCHO		PCHO		URA	
	a	a	a	b	a	b	a	b
Monsoon								
1	195	56.8 ± 5.2	6.3 ± 0.2	11.0	50.5 ± 5.5	89.0	6.9 ± 0.3	12.1
2	181	51.3 ± 5.7	5.4 ± 0.9	10.5	45.9 ± 2.8	89.5	5.7 ± 0.2	11.1
3	167	43.3 ± 2.6	4.1 ± 0.6	9.5	39.2 ± 3.5	90.5	4.5 ± 0.2	10.4
4	145	37.5 ± 3.6	5.6 ± 0.9	14.9	31.9 ± 4.7	85.1	4.7 ± 0.4	12.5
5	118	67.3 ± 8.0	5.4 ± 0.8	8.0	62.0 ± 7.6	92.0	6.7 ± 0.4	9.9
6	80	47.9 ± 4.3	4.8 ± 0.7	10.0	43.1 ± 5.1	90.0	3.4 ± 0.2	7.1
7	103	41.9 ± 3.6	5.3 ± 0.8	12.6	36.6 ± 3.0	87.4	2.3 ± 0.2	5.5
8	111	33.5 ± 3.1	8.1 ± 0.5	24.3	25.4 ± 2.8	75.7	5.0 ± 0.3	14.9
Mean SD	138 ± 41	47.4 ± 11.0	5.6 ± 1.2	12.6 ± 5.2	41.8 ± 11.4	87.4 ± 5.2	4.9 ± 1.6	10.5 ± 3.0
Pre-monsoon								
1	167	23.0 ± 4.0	8.0 ± 1.0	34.8	15.0 ± 4.0	65.2	7.7 ± 0.2	33.6
2	148	21.5 ± 3.3	8.5 ± 1.1	39.4	13.0 ± 2.5	60.6	3.7 ± 0.6	17.3
3	156	17.7 ± 0.8	8.5 ± 1.3	48.3	9.1 ± 2.3	51.7	3.2 ± 0.2	18.2
4	172	35.3 ± 3.5	7.5 ± 1.0	21.3	27.8 ± 7.4	78.7	8.5 ± 0.4	24.1
5	194	40.8 ± 2.3	10.5 ± 0.8	25.8	30.3 ± 2.8	74.2	9.2 ± 0.4	22.4
6	192	28.8 ± 3.8	9.6 ± 0.6	33.4	19.2 ± 4.2	66.6	7.0 ± 0.6	24.3
7	181	35.4 ± 3.0	8.5 ± 1.2	24.1	26.9 ± 4.8	75.9	10.8 ± 0.5	30.6
8	160	36.4 ± 1.9	15.5 ± 1.0	42.6	20.9 ± 2.1	57.4	8.8 ± 0.4	24.2
9	193	36.8 ± 2.0	9.0 ± 0.9	24.5	27.8 ± 3.1	75.5	9.4 ± 0.7	25.5
Mean SD	174 ± 18	30.6 ± 8.2	9.5 ± 2.4	32.7 ± 9.4	21.1 ± 7.5	67.3 ± 9.4	7.6 ± 2.6	24.5 ± 5.2

a:  $\mu\text{M C}$ ; b: percentage of TCHO.

not among the factors that controlled the abundance of DOC in the Mandovi estuary. DOC also serves as a source of carbon and energy for heterotrophic micro-organisms. Significant positive relationships between DOC and TBC during both the monsoon season ( $r = 0.756$ ,  $p < 0.01$ ), and the pre-monsoon season ( $r = 0.811$ ,  $p < 0.01$ ) indicate the influence of DOC on the abundance of bacteria in the estuary (Amon and Benner 2003).

### 3.4 TCHO species and yield

Concentrations of dissolved TCHO showed significant seasonal variations and ranged between 17.7 and  $67.3 \mu\text{M C}$  (tables 2 and 3). These TCHO values are in the range of values reported earlier for the estuarine and coastal environments (Witter and Luther 2002; Hung et al 2003, 2005; Wang et al 2006; Myklestad and Borsheim 2007). Distribution of TCHO is influenced by several factors including salinity, nutrient, phytoplankton biomass, and composition, and bacterial abundance (Handa 1969; Myklestad 1989; Meon and Kirchman 2001). During the monsoon season, the Mandovi estuary was characterized with low salinity, low phytoplankton biomass, low bacterial cell numbers and higher concentrations of nitrate, phosphate and silicates and TCHO (tables 1 and 2)

(Prabhu Matondkar et al 2007; Shetye et al 2007; Partihary et al 2009). TCHO did not show significant relationships with these parameters implying that they were not the major factors involved in controlling distribution of TCHO in the estuary during the monsoon season. In contrast, during the pre-monsoon condition, the estuary experienced nearly marine condition. TCHO showed fairly good relationships with salinity ( $r = -0.643$ ,  $p < 0.05$ ), Chl *a* ( $r = 0.748$ ,  $p < 0.01$ ), diatom cell numbers ( $r = 0.619$ ,  $p < 0.05$ ), and silicates ( $r = -0.747$ ,  $p < 0.01$ ) suggesting that they were among the factors that influenced TCHO distribution in the estuary during the pre-monsoon season.

MCHO concentration showed significant seasonal variations ( $4.1$  to  $15.5 \mu\text{M C}$ ) (tables 2 and 3), and concentrations were higher during the pre-monsoon season (average  $9.5 \mu\text{M C}$ ) as compared to the monsoon season (average  $5.6 \mu\text{M C}$ ) (table 2). Higher production by phytoplankton, especially diatoms, and/or *in situ* hydrolysis of polysaccharides or decomposition processes may be associated with higher concentrations of MCHO during the pre-monsoon season. The former seems possible as concentrations of MCHO showed fairly good relationships with concentrations of Chl *a* ( $r = 0.618$ ,  $p < 0.05$ ), phytoplankton cell count ( $r = 0.617$ ,  $p < 0.05$ ), and diatom cell count

Table 3. One-way ANOVA of the spatial (station) and seasonal variation in Chl *a*, DOC, TCHO, MCHO, PCHO and URA in the Mandovi estuary.

Source of variation	df	Seq SS	Adj MS	F	<i>p</i> -value	F crit
<b>Chl <i>a</i></b>						
Station	7	0.005	0.001	1.002	0.4988	3.787
Season	1	0.034	0.034	46.050	0.0003	5.591
Error	7	0.005	0.001			
Total	15	0.044				
<b>DOC</b>						
Station	7	3441.729	491.676	0.336	0.9132	3.787
Season	1	4547.535	4547.535	3.109	0.1212	5.591
Error	7	10238.568	1462.653			
Total	15	18227.832				
<b>TCHO</b>						
Station	7	657.890	93.984	0.979	0.5107	3.787
Season	1	1233.551	1233.551	12.853	0.0089	5.591
Error	7	671.826	95.975			
Total	15	2563.266				
<b>MCHO</b>						
Station	7	43.958	6.280	3.607	0.0561	3.787
Season	1	63.141	63.141	36.264	0.0005	5.591
Error	7	12.188	1.741			
Total	15	119.287				
<b>PCHO</b>						
Station	7	687.985	98.284	1.107	0.4485	3.787
Season	1	1854.857	1854.857	20.886	0.0026	5.591
Error	7	621.662	88.809			
Total	15	3164.504				
<b>URA</b>						
Station	7	27.310	3.901	0.697	0.6769	3.787
Season	1	24.539	24.539	4.386	0.0745	5.591
Error	7	39.167	5.595			
Total	15	91.016				

Chl *a*, DOC, TCHO, MCHO, PCHO, URA are same as in tables 1 and 2.

( $r = 0.749$ ,  $p < 0.01$ ). In contrast, low concentrations of MCHO in the monsoon season were probably due to low rates of heterotrophic decomposition of PCHO and utilization of MCHO by heterotrophic populations. MCHO accounted for 8 to 48% of TCHO in the Mandovi estuary (table 2). Similar observations have been reported from coastal and oceanic waters (Bhosle *et al* 1998; Hung *et al* 2003). In contrast, MCHO accounted for 80% of TCHO in some temperate and Arctic rivers (Hung *et al* 2001, 2005; Witter and Luther 2002; Gueguen *et al* 2006; Wang *et al* 2006; Cai *et al* 2008).

In the Mandovi estuary, PCHO concentrations showed significant seasonal variation (9.1 to 62.0  $\mu\text{M}$  C, average  $30.8 \pm 14.1 \mu\text{M}$  C), and were 6 to 8 times higher than those recorded for MCHO (tables 2 and 3). PCHO accounted for

76 to 92% (average  $87.4 \pm 5.2\%$ ), and 52 to 79% (average  $67.3 \pm 9.4\%$ ) of the TCHO during the monsoon season and the pre-monsoon season, respectively (table 2). This indicates that PCHO was the major component of TCHO in the Mandovi estuary. Similarly, PCHO was also most abundant constituent of TCHO in marine waters (Pakulski and Benner 1994; Bhosle *et al* 1998; Hung *et al* 2003; Wang *et al* 2006). Contribution of PCHO to TCHO was higher (average  $87.4 \pm 5.2\%$ ) during the monsoon season (table 2). Mandovi estuary has mangroves vegetation along its banks. The monsoon season is characterized with greater precipitation and river run-off. River run-off will carry allochthonous organic matter derived from surface soils and polymers produced by micro-organisms (Wilkinson *et al* 1997). Structural polysaccharides such as cellulose, hemicellulose, and pectins are

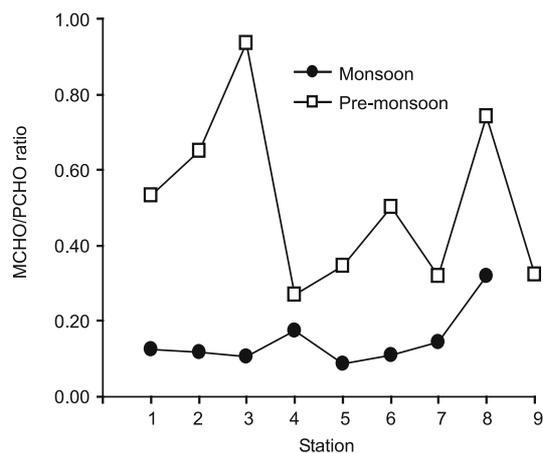


Figure 2. Variation of MCHO/PCHO ratio during the monsoon season and the pre-monsoon season in the Mandovi estuary.

abundant in vascular plant tissues (Aspinall 1970; Numan and Bhosle 2006). Moreover, decomposition of mangrove plant litter would also release structural polysaccharides such as cellulose, hemicellulose, and pectins, in estuarine waters thereby increasing PCHO contribution in TCHO during the monsoon season.

Differences in the relative distribution of MCHO and PCHO in the marine/estuarine waters and the temperate and Arctic rivers indicate different transformation pathways and mechanisms in TCHO pool (Wilkinson *et al* 1997; Hung *et al* 2005). This is probably because in marine waters TCHOs are mostly derived from *in situ* primary production by phytoplankton and bacterial metabolism with less decomposition and thus a higher PCHO fraction (Pakulski and Benner 1994; Cai *et al* 2008). In contrast, in Arctic rivers, most TCHO comes from allochthonous sources that undergoes intensive decomposition, and thus contain a lower PCHO fraction (table 2) (Hedges *et al* 1994; Hung *et al* 2001, 2005; Gueguen *et al* 2006; Cai *et al* 2008). Abundance of PCHO in the tropical Mandovi estuary strongly suggests higher inputs of PCHO from autochthonous and allochthonous sources and/or less degradation of PCHO.

MCHO/PCHO ratio varied from 0.08 to 0.93 in the Mandovi estuary (figure 2). During the monsoon, the ratio was low (0.08 to 0.3; average 0.14) while it was several times higher (0.27 to 0.93; average 0.51) during the pre-monsoon season (figure 2). The higher ratios indicate higher stability and biomass during pre-monsoon season. The higher MCHO/PCHO ratio may result from excretion from healthy cells (Myklestad 1989), and/or cellular and *in situ* degradation (Ittekkot *et al* 1981; Myklestad *et al* 1982; Smith *et al* 1992; Meon and Kirchman 2001). Ittekkot *et al*

(1981) reported increasing amounts of MCHO during the development of phytoplankton bloom, and the MCHO/PCHO ratio reached  $\sim 1$  at the stationary stage of the bloom. Furthermore, increase in water temperature during the pre-monsoon, microbial activity and photochemical reactions could be responsible for the observed seasonal variations in the MCHO/PCHO ratio.

The contribution of TCHO-C to DOC is defined as carbohydrate yield. Carbohydrate yield is a useful tool to assess degradation state of organic matter. Yield is high for the freshly derived organic matter, and low for the degraded organic matter. TCHO accounted for  $\sim 26$  to 60%, and  $\sim 11$  to 23% of DOC during the monsoon and pre-monsoon season, respectively (figure 3a). These yields are in the range of values recorded for freshly derived organic matter (15 to 30%) from phytoplankton (Pakulski and Benner 1994) and terrestrial sources ( $\sim 65\%$ ) (Opsahland Benner 1999). MCHO-C contribution to DOC varied from 2.5 to 7.3%, and 4.4 to 9.7% during the monsoon season and the pre-monsoon season, respectively (figure 3b). PCHO-C accounted for 22 to 54% and 6 to 16% of DOC during the monsoon season and the pre-monsoon season (figure 3c).

The Mandovi estuary experiences a shift in ecosystem production from net autotrophy during the pre-monsoon season to net heterotrophy during the monsoon season. This results in enhanced heterotrophic respiration, and a very low primary production:respiration (P:R) ratio of 0.28 (Ram *et al* 2003). Higher concentrations of TCHO, especially PCHO (22 to 54%) were recorded during the monsoon season. TCHO may serve as a source of carbon and energy for the heterotrophic activities during the monsoon season. In the Mandovi estuary, bacterial cell numbers showed a significant negative correlation with TCHO/DOC ratio suggesting preferential microbial degradation of carbohydrates (figure 4a). Bacterial abundance was influenced by water temperature (table 1). Microbial activity increased with increasing water temperature, preferentially utilizing specific and more labile DOC compounds (Apple *et al* 2006). This was also supported by the significant negative correlation between the TCHO/DOC ratio and the *in situ* water temperature (figure 4b). Hung *et al* (2005) reported that microbial utilization of carbohydrates was strongly influenced by *in situ* water temperature. During the monsoon season,  $\sim 98\%$  of terrestrial organic carbon was used for heterotrophic activity in tropical estuaries along the coast of India (Balakrishna and Probst 2005; Sarma *et al* 2009). Transport of TCHO rich dissolved organic matter from the Mandovi estuary to coastal waters will have strong impact on the functioning of microbial community.

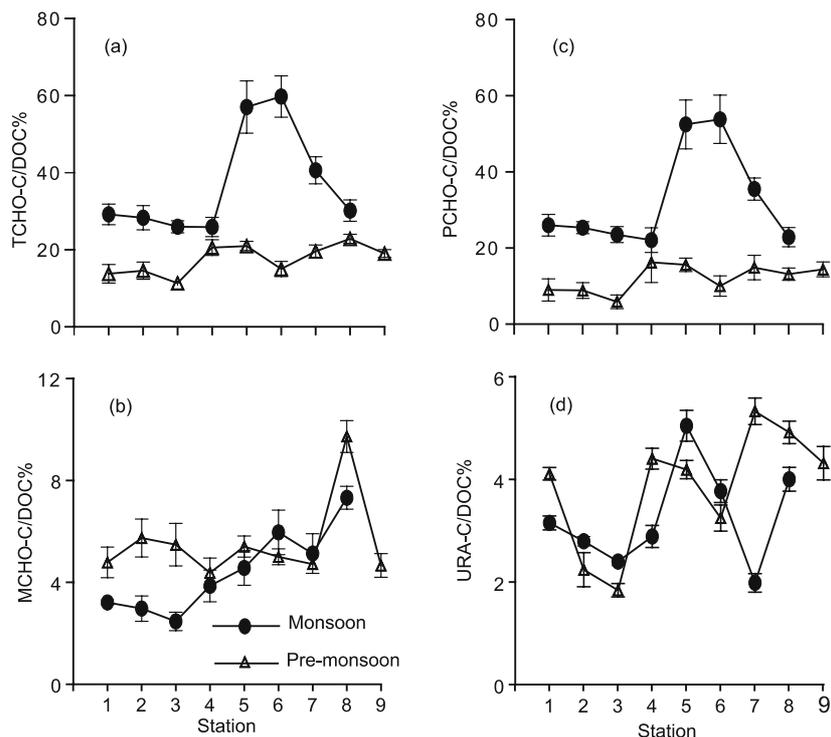


Figure 3. Contribution of TCHO-C (a), MCHO-C (b), PCHO-C (c), and URA-C (d) to DOC in the monsoon and the pre-monsoon seasons in the Mandovi estuary. Error bars represent the  $\pm$  SD.

### 3.5 Dissolved URA

Distribution, fate and cycling of URA in estuarine and marine waters are not well known (Mopper 1977; Bergamaschi *et al* 1999; Hung *et al* 2003, 2005, 2009; Khodse *et al* 2008). In the Mandovi estuary, URA showed seasonal variations (table 3), and varied from 2.3 to 10.8  $\mu\text{M C}$  (table 2). Average URA concentrations were higher during the pre-monsoon season ( $7.6 \pm 2.6 \mu\text{M C}$ ) than those recorded for the monsoon season ( $4.9 \pm 1.6 \mu\text{M C}$ ) (table 2). These URA concentrations are in the range of values earlier reported by Hung *et al* (2001, 2005) for the Galveston Bay (1.0 to 8.3  $\mu\text{M C}$ ) and the Trinity River (5.2 to 25.7  $\mu\text{M C}$ ). The contribution of URA to TCHO was lower during the monsoon season ( $\sim 5.5$  to 14.9%), and higher in the pre-monsoon season ( $\sim 17.3$  to 33.6%) (table 2). Hung *et al* (2001, 2005) reported that URA accounted for  $\sim 4.0$  to 17% and 10 to 30% of TCHO in Galveston Bay and in Trinity River, respectively. The contribution of URA to TCHO varied from  $\sim 5.0$  to 23% in the suspended particles of the northern Indian Ocean (Khodse *et al* 2007). URA accounted for 9.89% of TCHO of the sedimenting particles collected from the Dabob Bay (Bergamaschi *et al* 1999). The contribution of URA-C to DOC varied from 2.0 to 5.0%, and 2.2 to 5.3% during the monsoon season and the pre-monsoon season, respectively

(figure 3d). URA yields for the suspended particulate matter of northern Indian Ocean varied from 0.2 to 6.3% (Khodse *et al* 2007). The average URA yield for the suspended particles collected from the Gulf of Mexico was 0.64% and 0.70% in 2000 and 2001, respectively (Hung *et al* 2003). For the sedimenting particles of the Sagami Bay, Japan, URA yield was 0.7% (Hamanaka *et al* 2002). For the Trinity River, URA yields varied from 1 to 5% (Hung *et al* 2005). URA showed fairly good positive relationships with DOC in the monsoon season ( $r = 0.614$ ,  $p < 0.1$ ) and the pre-monsoon season ( $r = 0.664$ ,  $p < 0.05$ ). Similarly, URA also showed positive relationships with TCHO during the monsoon ( $r = 0.614$ ,  $p < 0.1$ ) and the pre-monsoon ( $r = 0.869$ ,  $p < 0.001$ ). These correlations indicate a common origin for these compounds.

During the pre-monsoon season, URA concentrations varied with changes in salinity ( $r = -0.626$ ,  $p < 0.05$ ), Chl *a* ( $r = 0.636$ ,  $p < 0.05$ ), nitrate ( $r = -0.689$ ,  $p < 0.02$ ), and silicates ( $r = 0.670$ ,  $p < 0.05$ ) implying their importance in influencing URA distribution in the Mandovi estuary. In contrast, concentrations of URA were not influenced by the changes in the above parameters during the monsoon season. URAs also are produced by vascular plants, and many other organisms (Bergamaschi *et al* 1999; Hung *et al* 2003), therefore additional inputs and selective preservation may account for variations in the

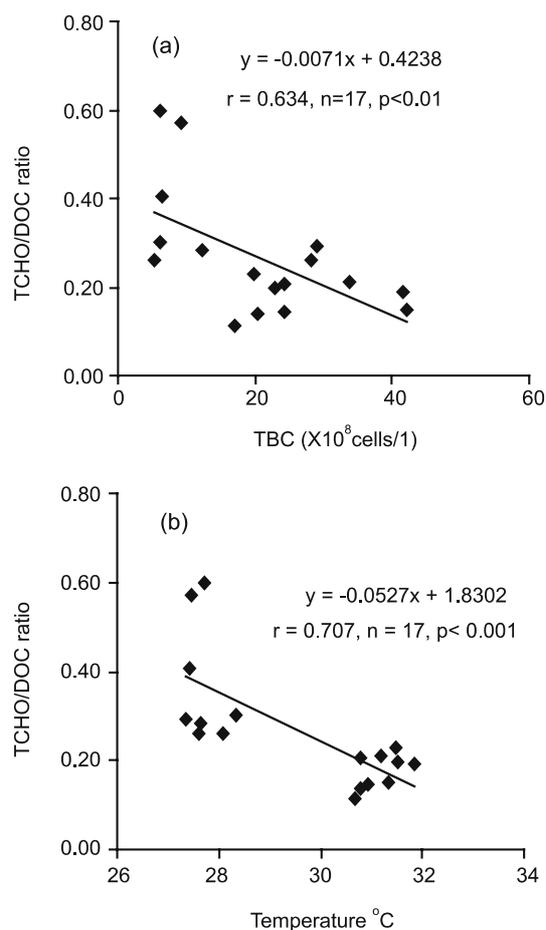


Figure 4. Relationship between TBC and TCHO/DOC ratio (a) and temperature and TCHO/DOC ratio (b) in surface water of the Mandovi estuary.

distribution of URA. Moreover, concentrations of toxic metal ions and adsorption by suspended particles are some other factors that may influence URA distribution in the Mandovi estuary (Hung et al 2001, 2005).

#### 4. Summary

TCHO, MCHO, PCHO and uronic acids were monitored at nine stations in the Mandovi estuary during the monsoon and pre-monsoon seasons. TCHO concentrations and yields were higher during the monsoon season. PCHO was the most abundant constituent of TCHO. Concentrations of both TCHO and URA were influenced by changes in salinity, Chl *a*, bacterial cell numbers and nutrients during the pre-monsoon season. In contrast, during the monsoon season, these factors had little impact on the distribution of carbohydrate species in the estuary. During the monsoon season, TCHO can serve as source of carbon to fuel heterotrophic activities in estuarine and coastal waters.

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