

# Amino sugars in suspended particulate matter from the Bay of Bengal during the summer monsoon of 2001

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Amino sugars (AS) are important constituents of organic matter. However, very little is known about their cycling in marine waters. In this research, we assessed the distribution and cycling of these compounds in waters of the Bay of Bengal. For this purpose, samples of suspended particulate matter (SPM) were collected from 8 depths (surface to 1000 m) at 6 locations during the 166th cruise of the ORV Sagar Kanya in the Bay of Bengal in July/August 2001. The SPM samples were analysed for particulate organic carbon (POC), particulate nitrogen (PN) and AS concentrations and composition. The AS varied between 0.4 and 17.5 nmol/l. Concentrations were high in the surface waters and generally decreased with increasing depth. AS concentration decreased from the south to north. AS accounted for 0.01 to 0.71% and 0.05 to 2.37% of POC and PN, respectively. Rapid decrease in AS-C% and AS-N% with depth indicates that these compounds were preferentially degraded relative to bulk POC and PN. The composition of AS suggests that glucosamine (GLU-N) and galactosamine (GAL-N) were present in the surface SPM samples, and their abundance decreased from surface downwards. Relatively, low values of GLU-N/GAL-N ratio indicate that the organic matter was mostly derived from the detritus of micro-organisms. Our data suggest that chitin, a polymer of the glucosamine produced by many marine organisms was not the major source of AS in the Bay. Rapid cycling of these compounds indicates their importance in the cycling of nitrogen in marine waters.

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## 1. Introduction

Amino sugars (AS) are unusual carbohydrates as they contain nitrogen. They are derived from many sources including microbial cell wall as peptidoglycan, lipopolysaccharides and pseudopeptidoglycan (Brock *et al* 1994). AS are also important constituents of many biopolymers such as polysaccharides, glycoproteins and glycolipids (Sharon 1965). Moreover, chitin, a polymer of amino sugar, N-acetyl glucosamine (Muzzarelli 1977; Benner and Kaiser 2003) is produced by many marine organisms (e.g., copepods and other crustaceans), and may thus form an important source of amino sugars in marine waters. Because of this, it is generally believed that, AS are widespread constituents of marine organic matter. Furthermore, most N

in marine and dissolved organic matter resides in amide functional groups, which are common to combined amino acids and acetylated amino sugars (McCarthy *et al* 1997; Benner and Kaiser 2003). Hydrolysable amino acids account for <15% of dissolved organic nitrogen in seawater, suggesting that AS could be important in the marine N cycle (McCarthy *et al* 1997). The distribution and cycling of AS in sedimenting particles and marine sediments have been well documented (Belluomini *et al* 1986; Haake *et al* 1992; Jennerjahn and Ittekkot 1997; Hashimoto *et al* 1998; Gupta and Kawahata 2000). Conversely, little is known about the distribution of AS in the SPM of marine waters (Muller *et al* 1986; Benner and Kaiser 2003; Unger *et al* 2005). For example, studies carried out in the Pacific and Atlantic Oceans suggest that AS of the

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SPM are rapidly utilized during the transport of the organic matter to greater depths (Benner and Kaiser 2003). Therefore, studies on AS will provide useful information on their distribution and cycling in marine waters.

There are numerous studies describing the physical, chemical and biological characteristics of the Bay of Bengal (Kumar *et al* 2002; Madhupratap *et al* 2003; Kumar *et al* 2004). In contrast, only a few studies have focused their attention on the characterization of organic matter with particular reference to the bulk parameters such as organic carbon, nitrogen, carbohydrates and proteins in the Bay (Nandakumar *et al* 1987; Bhosle and Dhople 1988; Bhosle *et al* 1992; Unger *et al* 2005). From this, it is evident that little is known about the molecular level characterization, distribution and cycling of organic matter in the Bay of Bengal. Therefore, the aims of the present study were to:

- assess the spatial and depth related distribution of amino sugars,
- identify the composition of amino sugars and
- evaluate the cycling of amino sugars in the Bay.

Such detailed studies will provide useful information about the dynamics, nutritive value, nature and degradation state of AS containing organic matter.

## 2. Material and methods

### 2.1 Description of the study area

Bay of Bengal (BOB) is a semi-enclosed tropical basin in the northern Indian Ocean. It is bounded by the Indian Peninsula and Sri Lanka on the west and by the Andaman–Nicobar islands and Burma on the east. The uniqueness of the Bay is the reversing monsoons that cause seasonal changes in circulation and weather. Furthermore, the Bay receives large quantities of fresh water influx ( $1.6 \times 10^{12} \text{ m}^3 \text{ yr}^{-1}$ ) from the major rivers such as, Ganges, Brahmaputra, Mahanadi, Pennar and Cauvery (Subramanian 1993). The average annual discharges are very high for the Ganges–Brahmaputra and relatively low for the Pennar and Cauvery (Rao 1985). The fresh water inputs result in the stratification of the upper 50 m of the water column (Kumar *et al* 2002). Moreover, the frequently occurring cyclones are additional sources of phosphate and nitrates in the surface waters of the Bay (Vinayachandran and Mathew 2003). This nutrient supply results in the formation of intense localized blooms. Upwelling has been observed along the western boundary of the Bay

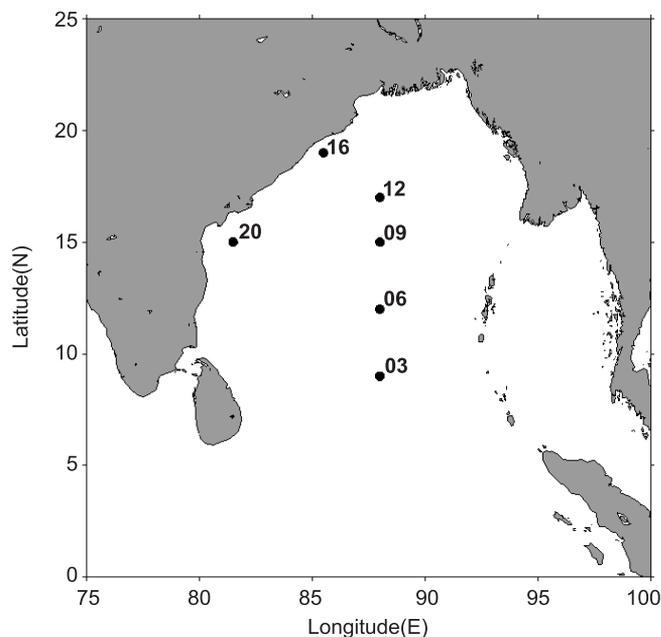


Figure 1. Map of the study site showing sampling stations in the Bay of Bengal.

(Shetye *et al* 1991). The Bay is slightly more oxygenated compared to the Arabian Sea.

### 2.2 Collection of samples

Suspended particulate matter (SPM) samples were collected from 8 discrete depths (2 to 1000 m) at six locations (figure 1) using Niskin water samplers (30l) attached to the CTD rosette system during the 166th cruise of ORV Sagar Kanya in the Bay of Bengal in the summer monsoon of 2001. Immediately after collection, the water samples (4 to 10l) were filtered through precombusted ( $450^\circ\text{C}$ , 4 h) 47 mm GF/F glass fibre filters ( $0.7 \mu\text{m}$  pore size) for the analysis of particulate organic carbon (POC), particulate nitrogen (PN) and amino sugars (AS) concentration and composition.

### 2.3 Analysis of samples

The SPM samples were de-carbonated overnight using fuming HCl in a desiccator (Ferrari *et al* 2003), dried at  $50^\circ\text{C}$  and then analyzed for particulate organic carbon (POC) and particulate nitrogen (PN) using a Perkin Elmer Elemental analyzer 2400 CHN analyzer. The precision of the methods was better than 7%.

Filters for amino sugar analysis were transferred to ampoules, and 2 ml of 6 N HCl were added to each ampoule. The ampoules were flushed with nitrogen gas and sealed. Samples were hydrolyzed for 22 h at  $110^\circ\text{C}$  (Bhosle *et al* 2005). The samples were cooled and neutralized using NaOH. An

aliquot was then reacted with *o*-phthaldialdehyde (OPA)-mercaptoethanol reagent and then analyzed by high performance liquid chromatograph (HPLC) as described previously (Lindroth and Mopper 1979; Bhosle *et al* 2005). This method has been routinely used to analyze amino sugars in sedimenting particles and sediments (Belluomini *et al* 1986; Haake *et al* 1992; Jennerjahn and Ittekkot 1997; Hashimoto *et al* 1998; Gupta and Kawahata 2000; Takano *et al* 2004).

An HPLC system (Shimadzu, Model 1) consisting of quaternary pump, degasser, autoinjector, column oven, fluorescence detector, ODS guard column (4.6 mm ID, 4.5 cm length, 10  $\mu$ m particle size) and reversed phase Shim-Pack HRC-ODS analytical column (4.6 mm ID, 15 cm length, 5  $\mu$ m particle size) was used. A binary solvent system consisting of 50 mM sodium acetate buffer (pH 6.5) containing 3% of tetrahydrofuran as solvent A and methanol as solvent B was used to separate the AS. Using this solvent system the two AS, glucosamine (GLU-N) and galactosamine (GAL-N) could be separated from the standard mixture (figure 2A, B, C) and SPM samples (figure 2D). The sum of the concentrations of the two identified AS GLU-N and GAL-N is expressed as total hydrolysable amino sugars (AS). The precision of the method based on 5 replicates was  $\pm 4$  to 6%.

### 3. Results and discussion

POC and PN concentrations varied in the range of 2.43 to 14.25  $\mu$ mol/l and 0.11 to 2.84  $\mu$ mol/l, respectively (table 1). The surface values of POC and PN increased gradually from south (6.97  $\mu$ mol/l POC; 0.97  $\mu$ mol/l PN) to north (11.06  $\mu$ mol/l POC and 1.23  $\mu$ mol/l PN) for the deep oceanic stations (#03 to #12). A similar trend was recorded for the near shore stations (#16 and #20). The observed spatial variability in the POC and PN in surface samples may be due to differences in the input of autochthonous or allochthonous organic matter at these locations of the Bay. For example, it is possible that the northern stations may have received more inputs of terrestrial material due to their proximity to the area receiving river run-off that might have resulted in relatively higher abundance of organic matter at the northern stations. The POC concentrations reported here are relatively lower than those reported earlier for the Bay (Radhakrishna *et al* 1978; Bhattathiri *et al* 1980; Nandakumar *et al* 1987). This is due to different analytical methods used for the analysis of POC. Nevertheless, our POC concentrations are similar to those reported for other oceanic regions (Gordon *et al* 1979; Gordon and Cranford 1985).

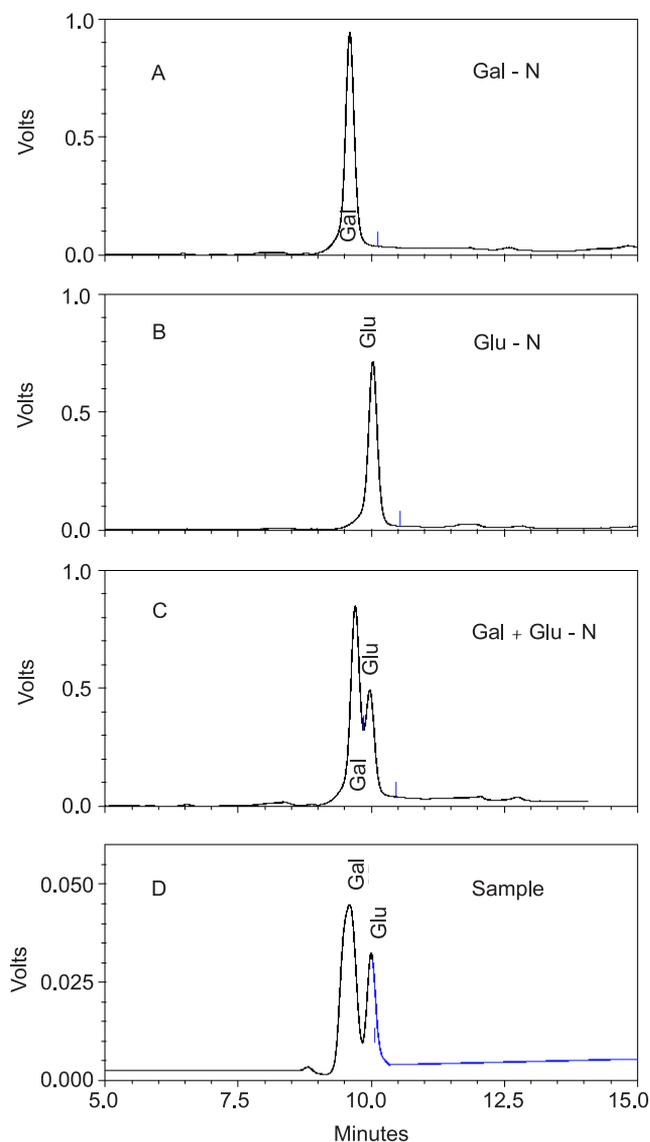


Figure 2. Chromatograms showing peaks of amino sugars separation of galactosamine (A), glucosamine (B), a mixture of galactosamine and glucosamine (C) and presence of these two amino sugars in a SPM sample (D) of the Bay.

Depth related variations were also observed at these stations. Both POC and PN were found to be higher in the surface waters and decreased with increasing depth but with some exceptions (table 1). This suggests utilization of these compounds by the mid water column organisms (Gordon and Cranford 1985). In contrast, at station 9, the distribution of both POC and PN was not influenced by the depth. This probably indicates a balance in the supply and utilization of these compounds. At station 12, concentrations of POC and PN were found to be relatively higher perhaps due to the inputs it receives from the rivers Ganges-Brahmaputra, Mahanadi, Pennar and Cauvery. At stations #03, 06 and 12 the concentrations of POC and PN decreased in the upper

Table 1. Distribution of POC, PN, C/N ratio, AS, AS-C%, AS-N% and GLU-N/GAL-N in the Bay.

Station	Position	Depth (m)	POC $\mu\text{mol/l}$	PN $\mu\text{mol/l}$	C/N ratio	AS nmol/l	AS-C%	AS-N%	GLU-N:GAL-N Molar
#03	9°N 88°E	2	6.97	0.97	7.1	10.4	0.57	0.64	0.35
		10	4.96	0.65	7.6	7.7	0.60	0.70	0.45
		30	5.86	0.78	7.5	7.2	0.48	0.55	0.36
		60	4.27	0.55	7.8	3.4	0.30	0.33	0.11
		100	4.08	0.52	7.8	4.2	0.39	0.71	0.29
		300	5.13	0.73	7.0	2.5	0.19	0.69	0.04
		500	4.49	0.60	7.5	2.0	0.17	0.50	0.06
		1000	7.92	1.17	6.8	3.3	0.16	0.65	–
#06	12°N 88°E	2	6.94	0.95	7.3	12.7	0.71	2.37	0.43
		10	5.63	0.86	6.5	9.1	0.62	1.04	0.43
		30	5.67	0.85	6.7	5.2	0.35	0.60	0.38
		60	4.79	0.89	5.4	3.9	0.31	0.33	0.34
		100	6.24	0.94	6.6	7.9	0.49	0.70	0.30
		300	6.49	0.89	7.4	3.6	0.21	0.35	0.07
		500	6.08	0.79	7.6	3.9	0.25	0.87	0.70
		1000	7.63	1.03	7.4	5.7	0.29	0.89	0.35
#09	15°N 88°E	2	6.53	0.96	6.7	7.6	0.45	1.16	0.15
		10	6.45	0.94	6.8	6.8	0.40	0.55	0.26
		30	6.60	1.14	5.8	3.2	0.19	0.29	0.73
		60	7.30	0.78	7.5	4.7	0.25	0.56	0.17
		100	6.77	0.71	9.6	2.0	0.11	0.26	0.38
		300	6.54	0.68	9.5	1.2	0.07	0.14	0.41
		500	6.49	0.81	8.0	1.1	0.07	0.20	0.30
#12	17°N 88°E	2	11.06	1.23	9.0	5.4	0.19	0.67	0.86
		10	13.44	1.41	9.5	10.3	0.30	0.73	1.35
		30	13.53	1.48	9.1	17.5	0.50	1.38	0.99
		60	11.53	1.27	9.1	4.1	0.14	0.45	0.71
		100	9.53	1.11	8.6	8.7	0.35	0.95	0.69
		300	14.25	1.63	8.8	5.4	0.14	0.54	0.76
		500	9.78	1.52	6.4	2.4	0.10	0.24	1.03
		1000	12.36	2.84	4.4	0.5	0.01	0.05	0.57
#16	19°N 85°E	2	6.58	0.78	8.4	0.8	0.05	0.06	0.72
		10	6.25	0.57	10.9	1.7	0.10	0.08	0.39
		30	4.17	0.50	8.3	1.2	0.11	0.08	0.57
		60	3.50	0.50	7.0	0.9	0.10	0.08	0.62
		100	4.0	0.43	9.3	1.2	0.12	0.12	0.47
		300	3.50	0.36	9.8	1.3	0.14	0.16	0.24
		500	3.08	0.36	8.6	0.4	0.05	0.08	0.34
#20	15°N 81°E	2	4.90	0.38	12.7	4.9	0.39	0.97	0.28
		10	5.34	0.42	12.8	2.3	0.17	0.31	0.21
		30	4.19	0.47	8.9	2.2	0.20	0.27	0.17
		60	4.27	0.28	14.8	1.8	0.16	0.23	0.24
		100	4.0	0.23	17.8	1.4	0.14	0.20	0.25
		300	3.81	0.11	33.9	0.6	0.06	0.11	0.32
		500	3.89	0.44	8.9	0.9	0.09	0.18	0.28
		1000	2.43	1.07	2.3	0.9	0.14	0.27	0.44

500 m of the water column and then increased again at 1000 m. It was interesting to note that the concentrations of POC and PN recorded at 1000 m were relatively higher than those recorded at the surface waters of these stations. At 1000 m organic matter had elevated levels of AS and low C/N ratio. This indicates that the organic matter present at this depth was derived from marine phytoplankton and bacteria (Karl and Knauer 1984). Concentrations of POC and PN showed significant positive

relationship ( $R = 0.89$ ,  $n = 44$ ) thereby suggesting their common origin.

C/N ratio is a useful tool to assess the nature of organic matter. The rationale behind this approach is that the fresh and degraded organic matter has low and high C/N ratio, respectively. For example, fresh phytoplankton and bacteria exhibit an average C/N ratio of 7 and 4, respectively (Lee and Furhman 1987; Nelson and Robertson 1993). The C/N ratio is found to increase with the microbial

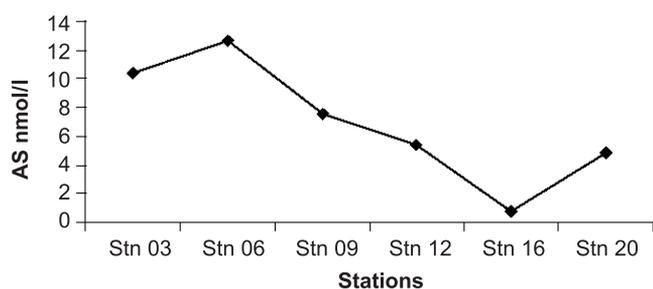


Figure 3. Spatial variation in the amino sugar concentration in the surface samples of Bay of Bengal.

decomposition of organic matter, since nitrogen is utilized faster than the carbon (Lehmann *et al* 2002). Similarly, the organic matter derived from terrestrial plants has high (>24) C/N ratio (Elser *et al* 2000). Most of the values of the C/N ratio of the SPM samples of the Bay are in the range of values recorded for phytoplankton and bacteria. High C/N ratio (>9) of the surface SPM at stations 12 and 20 indicate that the organic matter at these stations was relatively more degraded. Furthermore, a decrease in C/N ratio at 1000 m was also recorded at these two stations indicating the occurrence of relatively fresh organic matter at this depth (Nelson and Robertson 1993). The range of C/N values recorded for the stations sampled in the Bay suggests that organic matter was mostly derived from phytoplankton and bacteria.

Total concentrations of the AS ranged between 0.4 and 17.5 nmol/l (table 1). There were apparent variations in the AS concentrations with respect to location and the depth (figure 4). Distribution of AS in the Bay indicates that the concentrations of the AS increased from north to south (figure 3). These AS concentrations observed for the Bay are higher than those reported for other seas and oceans of the world. For example, Benner and Kaiser (2003) reported large variations (0.18 to 1.7 nmol/l) in total hydrolysable amino sugars of the SPM samples collected from various depths of the Pacific Ocean. The AS accounted for 0.01 to 0.71% of POC and 0.05 to 2.37% of PN in the Bay (table 1). These values are in the range of values (0.1 to 1.5% of organic carbon) reported for plankton and the suspended particulate matter collected from the Pacific Ocean (Benner and Kaiser 2003). The rapid decrease in the concentrations and the % AS-C/OC and AS-N/PN with increasing depth imply that these compounds were rapidly recycled in the upper ocean. AS concentrations showed fairly good correlation with POC ( $R^2 = 0.61$ ;  $n = 43$ ) and PN ( $R^2 = 0.64$ ;  $n = 43$ ) suggesting their common origin.

HPLC method is routinely employed for the analysis of AS in sedimenting particles and sediments (Belluomini *et al* 1986; Haake *et al* 1992;

Jennerjahn and Ittekkot 1997; Hashimoto *et al* 1998; Gupta and Kawahata 2000; Takano *et al* 2004). Using this method glucosamine (GLU-N) and galactosamine (GAL-N) were detected in the SPM samples collected from different stations sampled in the Bay of Bengal (figure 2). Concentrations of the GLU-N and GAL-N in the SPM samples varied from 0.1 to 8.7 nmol/l and 0.29 to 8.9 nmol/l, respectively (figure 4). Both the AS were also abundant in the SPM of other seas and oceans, the pure cultures of micro-organisms and the natural assemblages of organisms collected from various environments (Benner and Kaiser 2003). This suggests their widespread distribution and similar reactivities. It was interesting to note that the concentrations of both GLU-N and GAL-N decreased rapidly with the increasing depth suggesting their utilization. This AS distribution pattern contradicts the distribution pattern of these compounds observed in the sedimenting particles (Belluomini *et al* 1986; Haake *et al* 1992; Hashimoto *et al* 1998; Gupta and Kawahata 2000). In sedimenting particles the concentrations of amino sugars generally increased with water depth. The apparent difference may be due to the fact that the sedimenting particles are generally derived from large particles such as faecal pellets and faecal matter produced by chitinous zooplankton and/or contamination due to the chitinous swimmers that get caught in the traps.

The GLU-N/GAL-N ratio is a useful parameter to differentiate the relative inputs of the organic matter derived from zooplankton and micro-organisms in marine waters (Muller *et al* 1986; Haake *et al* 1993; Benner and Kaiser 2003). This approach is based on the fact that GLU-N content is very high in zooplankton, whereas, it is relatively low in micro-organisms. Our samples were relatively rich in GAL-N compared to GLU-N (figure 2). As a result, our GLU-N/GAL-N ratios are lower (0.04 to 1.4) than those (1 to 2.5) reported by Benner and Kaiser (2003) for the SPM samples collected from the Pacific Ocean. Similarly, our GLU-N/GAL-N ratios are also lower than those (6.3 to 8.69) recorded for the SPM of the Kara Sea (Unger *et al* 2005). The GLU-N/GAL-N ratio for the SPM of the Bay of Bengal samples is <2 thereby suggesting that the organic matter was mostly derived from heterotrophic micro-organisms. Absence of high (>4) GLU-N/GAL-N ratio also indicated that the contribution of chitin, a polymer of the N-acetyl glucosamine, produced by many marine organisms, to the marine organic matter was relatively very poor. Conversely, Muller *et al* (1986) reported a very high abundance of GLU-N and could not detect GAL-N in the suspended particles of the south Atlantic due to the abundance of zooplankton in the study area.

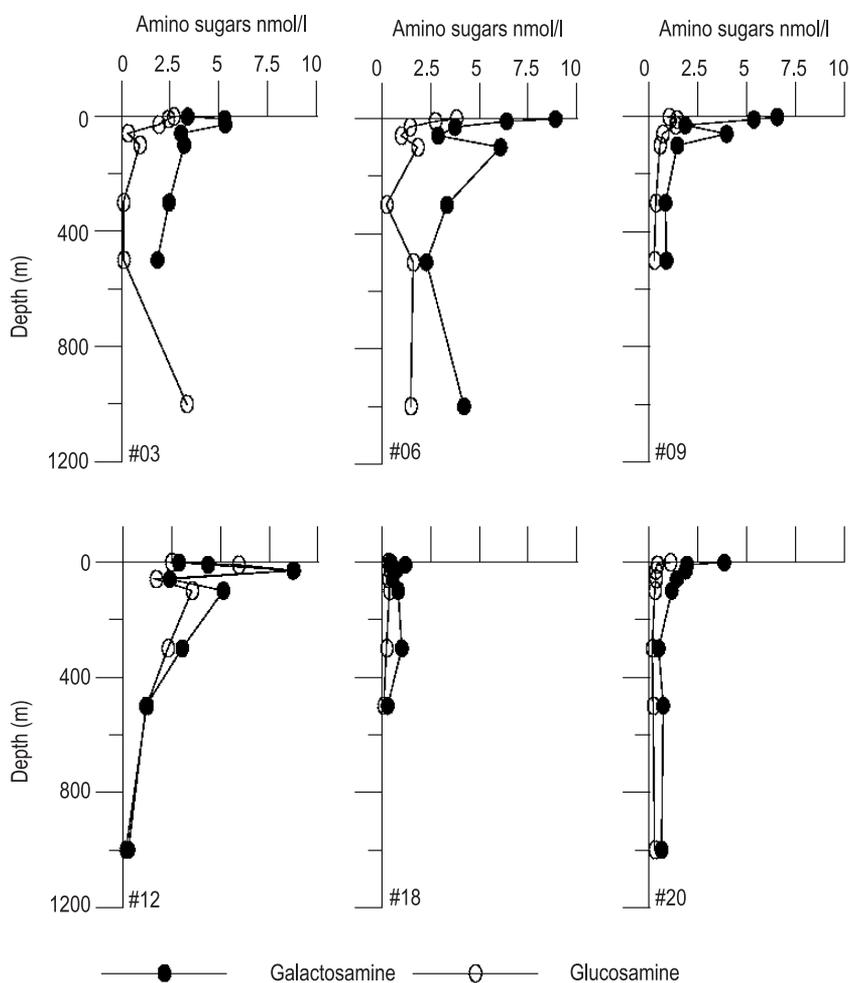


Figure 4. Depth profiles of galactosamine and glucosamine concentrations of the Bay of Bengal.

The concentrations of both GLU-N and GAL-N decreased with depth, however the decrease in the concentrations of GLU-N was more rapid. This results in decreasing GLU-N/GAL-N ratio with the increase in water depth. This is probably due to different sources of amino sugars and/or preferential degradation of GLU-N. It is argued that the decreasing GLU-N/GAL-N ratio is more indicative of preferential degradation of GLU-N than of different sources (Unger *et al* 2005). They reported that low GLU-N/GAL-N ratios were associated with reduced values of reactive index. In order to support this it was further argued that glucose as a major building block of GLU-N is an important storage compound whereas GAL-N is derived from galactose that is a structural monomer in algae. It is well documented that the storage polymers are preferentially degraded compared to structural polymers (Ittekkot *et al* 1982; Hernes *et al* 1996; Unger *et al* 2005). In view of this, it is hypothesized that the preferential utilization of GLU-N rather than different sources may be responsible for the observed low GLU-N/GAL-N ratio in our samples. This conclusion is also supported by a fairly good

correlation ( $R = 0.76$ ;  $n = 44$ ) between these two amino sugars suggesting a common origin.

Amino sugars are unique among the carbohydrates due to the presence of nitrogen atoms in their molecules. They are important components of the marine N-cycle. For example, total hydrolysable amino acids (THAA-N) account for a much greater percentage of marine organic nitrogen (42–72%) than amino sugars but amino sugar concentrations decrease more rapidly with depth than amino acid concentrations. This suggests that amino sugars are more efficiently recycled in the upper water column (Benner 2002; Benner and Kaiser 2003). Similarly, the decrease in amino sugar concentrations was greater than that recorded for the POC. This indicates that amino sugars are considerably more reactive than POC in the upper ocean waters.

#### 4. Summary

Amino sugars, which are the unique sugars with a nitrogen molecule, were found to be the important

constituents of the organic matter in the Bay of Bengal. Both GLU-N and GAL-N were present at all the stations. Rapid decrease in amino sugar concentration, AS-C% and AS-N% clearly suggested that the compounds are rapidly mineralized in the water column. Low GLU-N/GAL-N ratio (<2) indicates microbial detritus and not chitin as the major source of amino sugars in the Bay.

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