Indian Journal of Geo-Marine Sciences
Vol. 45(1), January 2016, pp. 16-28

Dissolved inorganic nutrients, organic matter and stable nitrogen isotopes as indicators of human impact in two contrasting estuaries in West Bengal, India, during winter monsoon

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Received 28 September 2012; revised 10 October 2012

The present study consists of the analyses of dissolved inorganic nutrients (NH_4^+, NO_3^-, NO_2^-, PO_4^{3-}, $Si(OH)_4$), dissolved organic nitrogen (DON), particulate nitrogen, particulate organic carbon, amino acids and $\delta^{15}N$ of particulate nitrogen and NO_3^- from Hooghly and Matla estuary. Hooghly estuary was sampled at 2 stations, at Kolkata and ca. 60 km downstream. Mangrove dominated Matla estuary was investigated along a N-S transect. Dissolved inorganic nitrogen, PO_4^{3-} and DON concentrations were low, especially for the Hooghly site at Kolkata. Human influence, however, was revealed by the more labile nature and elevated concentrations of amino acids in Kolkata. Release of nutrients from particulate matter during estuarine transport resulted in higher NO_3^- and PO_4^{3-} concentrations downstream Kolkata. In Matla estuary no human influence was detected and nitrogen fixation was observed.

[**Keywords**: Bay of Bengal, Sundarbans, dissolved inorganic nutrients, amino acids, $\delta^{15}N$, anthropogenic impact]

Introduction

Estuaries are sites of intense biogeochemical processes at the land-ocean interface[1-3]. Dissolved and particulate matter are subject to significant changes in relation to physical and chemical parameters as well as biological activity when entering the estuarine environments. Both the riverine delivery as well as the transformation processes within the estuary affect the export of dissolved and particulate components to the adjacent coastal and oceanic waters[4,5].

The input of fixed nitrogen to terrestrial ecosystems has more than doubled between 1860 and the early 1990s due to anthropogenic activities such as fertilizer production and atmospheric deposition of nitrogen released from e.g. fossil fuel combustion[6]. Considerable amounts of the anthropogenically fixed nitrogen enter the aquatic system and successively reaches the oceans via riverine and groundwater discharge[7]. In affected coastal areas nitrogen enrichment can cause eutrophication leading to oxygen deficits, toxic algae blooms or coral reef degradation[8,9]. In order to develop measures against eutrophication, information about the main sources of nitrogen has to be gathered. One approach for source identification is the use of $\delta^{15}N$ of particulate nitrogen and NO_3^- [e.g. 10,11,12].

Mangrove forests are highly productive ecosystems covering 60 – 75% of tropical coastlines[e.g.13].Due to their complex biogeochemistry they can act as source, sink or both for nutrients to adjacent coastal waters[14].

In this study, dissolved inorganic nutrients as well as the dissolved and particulate N load in the two contrasting Hooghly and Matla estuaries which are part of the large Ganges-Brahmaputra estuarine system were investigated. Our aim was to characterize the respective estuaries at the anthropogenically impacted Hooghly sites at Kolkata and Diamond Harbour as well as at the more pristine mangrove dominated Matla estuary, and to delineate the respective anthropogenic impact. The present study was carried out during the dry winter monsoon season in January 2011.

Material and Methods

The study area is located in the state of West Bengal, India, in the southwestern part of the Ganges-Brahmaputra Delta (Fig. 1). Sampling station Howrah Bridge (HB) is located alongside the city of Kolkata near Howrah Bridge, while the one named Diamond Harbour (DH) is located ca. 60 km downstream of HB. The Matla estuary (Matla) is located in the western part of the Sundarbans, which covers 9360 km^2 and represents the largest connected mangrove forest of the world[15,16]. The study area is strongly influenced by the subtropical monsoon climate. Annual precipitation is about 1600 to 1800mm[16].

Maximum precipitation occurs from June till September during southwest or summer monsoon. In December and January during northeast or winter monsoon the area receives very little precipitation. A corresponding precipitation pattern has been observed for the period August 2010-August 2011 (Fig. 2). Water discharge and sediment fluxes of the Ganges-Brahmaputra river system are strongly related to the seasonal variations of precipitation. Accordingly, highest discharge rates for Ganges and Brahmaputra are observed from June to October[17].

Sampling stations Hooghly estuary

DH and HB are located in the Hooghly estuary and in the Hooghly river, respectively. The Hooghly estuary represents the most western part of the Ganges-Brahmaputra delta extending approximately between 21°31‘-23°20’N and 87°45‘-88°45’E[18]. Water discharge rates of the Ganges and its side arms were significantly altered after the construction of the Farakka dam in the Ganges in the north of the state of West Bengal[19]. Farakka barrage was put on stream on 21st of April 1975 in order to guarantee an additional freshwater discharge for the Hooghly to avoid further silt accumulation at Kolkata port[18,20]. As a consequence, significant changes in salinity and ecology of the Hooghly estuary could be observed[18]. Today the estuary is considered to be salt free 60 km upstream of the river mouth at DH [21]. However, salinities of up to 1.5 PSU were detected in this study at DH, which can most probably be attributed to the reduced discharge during the dry season.

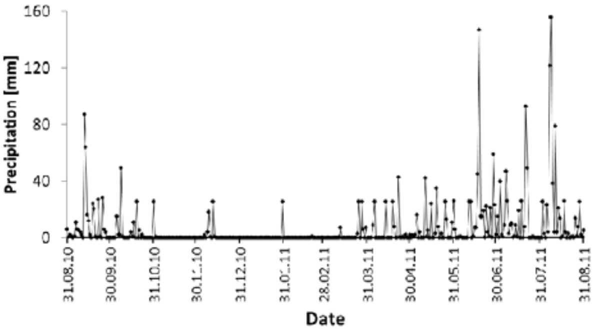

Fig. 2: Precipitation observed in Kolkata between August 2010 and August 2011. Sampling was carried out between 17th and 24th January 2011 during a persistent dry phase. Data available online: http://climate.usurf.usu.edu, accessed 15th January 2012.

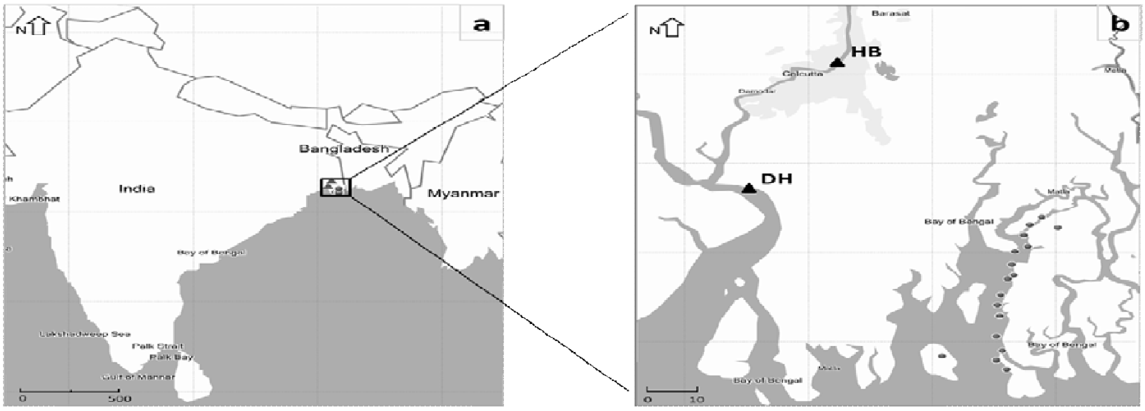

Fig. 1: Location of a) study area in India and b) sampling points at Hooghly estuary sites HB and DH (triangles) and along the Matla estuary (dots). Map created with BaseCamp, Garmin.

Average freshwater discharge through the Hooghly varies between 1000 and 3000 m^3/s during dry and rainy season, respectively[22]. The tide is predominantly semi-diurnal and the tidal range at the mouth of the Hooghly estuary varies between 1.8 and 5.2 m at neap and spring tide, respectively[21].

Tidal samples of HB were taken on 24th of January 2011 at the ferry transect from Baboo Ghat (Kolkata) to Rahmkrishnapur Ghat (Howrah) for HB and from Diamond Harbour to Kukrahati for DH (Figs. 1a, b). Tidal range at the sampling day was 4.5 m and 4.8. m for HB and DH, respectively. Samples were taken at tidal water heights of 1.0, 1.4, 1.7, 4.6 , 4.9 m and 1.1, 3.1, 5.2 m at HB and DH, respectively[23] as shown in Figs. 3 and 4. A total of 5 samples for HB and 3 samples for DH were taken during one tidal cycle at intervals of approximately 3 hours (Figs. 3, 4). At both sites, samples were taken in the middle of the river.

Sampling stations Matla estuary

In its southern, mangrove dominated sector, the Hooghly estuary is characterized by numerous distributaries and marshy islands. One of these distributaries is the Matla which together with the Hooghly occupies an area of ca. 0.8 million ha[18]. Matla estuary is located 65 km east of DH and stretches along ca. 70 km in a north-south direction (Fig. 1). The Matla does not receive constant freshwater supply from the hinterland. Therefore the estuary is under a strong marine influence. No significant freshwater inflow was detected during dry season between November 2008 and May 2009 at Bara Herobhanga Khal in the northern part of the estuary. Accordingly, salinities between 11 to 25 were measured in this period showing lowest values in November 2008 and highest values in May 2009[16]. Some inhabited islands, as well as agriculture and aquaculture in the northern part of the estuary are potential point and diffuse sources for anthropogenic nutrient inputs[16]. Apart from these, the Sundarban mangroves are generally considered to be a pristine ecosystem[24]. The tide is semi-diurnal as for the Hooghly estuary.

Matla samples were taken by boat at 17 nearly equidistant stations along a ca. 60 km N-S transect between 17th and 20th January 2011 (Fig. 1b).

Sampling and analytical procedures

Water samples were taken from water surface with a metallic bucket and stored on ice and in dark as 1 L aliquots in PP (polypropylene) bottles in Styrofoam boxes until filtration at NICED laboratory in Kolkata. Water physico-chemical parameters were measured in situ during sampling. Salinity was measured with Hach® HQ40d portable multi-parameter. The pH was measured with WTW® pH/Cond 340i multi-meter in combination with a WTW® SenTix 81 sensor.

Sample processing was completed within 10 days after the beginning of sampling. The water was filtered through pre-weighed and pre-combusted (12h, 450°C) GF/F filters (0.7 µm pore size) under constant pressure. After filtration, filters were dried at 40°C. Due to relative high salinities observed at Matla, salt residues of Matla samples were removed by rinsing the filters with ca. 15ml of ultrapure water. Total suspended matter (TSM) concentration was calculated by subtracting the weight of the empty filter from the weight of the dried filter after filtration and dividing the result by the respective volume of water filtered. For each station at least 4 filters were used to calculate the TSM content by arithmetic mean. 50 ml of the obtained filtrate were fixed with 150 µl $HgCl_2$ (concentration: 35g/l) for analyses of dissolved inorganic nutrients[25]. 15 ml of the filtrate were fixed with 30 µl phosphoric acid (υ (H_3PO_4) = 20 %) for analyses of total dissolved nitrogen (TDN). 900 ml of the filtrate were fixed with 900 µl hydrochloric acid (υ (HCl) = 35 %) for the analyses of $\delta^{15}N$ of nitrate**.** All filtrates were stored dark and frozen until analysis in the ZMT lab in Bremen.

Dissolved inorganic nutrients were analyzed by a continuous flow analyzer (Skalar San ++ system). Ammonium was quantified fluorometrically; silicate ($Si(OH)_4$), phosphate (PO_4^{3-}), nitrate and nitrite (NO_x^{--}) and nitrite (NO_2^-) were determined spectrophotometrically[26]. The concentration of nitrate (NO_3^-) was calculated as the difference between NO_x^- and NO_2^-. Detection limits were 0.324 µM, 0.029 µM, 0.041 µM, 0.094 µM and 0.776 µM for NOx^-, NO_2^-, NH_4^+, PO_4^{3-} and $Si(OH)_4$, respectively.

In order to verify the very low concentrations of ammonium determined by the Skalar San++ System, ammonium concentration was also measured by the salicylate method based on Berthelot's reaction in a Shimadzu UV-1700 spectrophotometer[26]. Samples for the salicylate method were brought to the required pH of 13 with NaOH. Precipitates of calcium and magnesium hydroxides in the samples were removed via centrifugation with a Heraeus® Labofuge 400 (5 min, 4000 rpm) before measurement in the spectrophotometer. Total dissolved nitrogen (TDN) of the samples was measured using a Shimadzu® TOC-Vcpn total organic carbon analyzer based on the principle of high-temperature catalytic oxidation in connection with a unit measuring total nitrogen (Shimadzu® TNM-1). The dissolved organic nitrogen (DON) concentration was obtained as difference between TDN and dissolved inorganic nitrogen[26].

Particulate nitrogen (PN) and particulate organic carbon (POC) contents of suspended matter were analyzed by high-temperature combustion in an EuroVector EA3000 element analyzer[26]. For POC measurement, filters were treated with 1N HCl and subsequently dried at 40°C to remove carbonate before measurement. PN measurements were done on untreated filters. For measurement of $\delta^{15}N$ of NO_3^-, the adapted ammonia diffusion method for oceanic nitrate was applied[27]. This method is based on a reduction of NO_x^-- compounds to ammonia by an alloy of Cu, Al and Zn (Devarda's alloy) and a subsequent diffusion of ammonia out of aqueous phase onto an with phosphoric acid acidified glass fiber filter disk[27,28]. The nitrogen isotope composition ($\delta^{15}N$) of PN and NO_3^- was analyzed by high-temperature combustion in a Flash 1112 EA element analyzer connected to a Thermo Finnigan-Delta-plus gas isotope ratio mass spectrometer.

Total amounts of hydrolysable amino acids (AA) were analyzed with a Biochrom 30 amino acid analyzer after hydrolysis of a quarter to one filter with 6 N HCl at 110 °C for 22 hours. Hydrolyzed samples were evaporated, taken up in 2 ml of sodium citrate buffer and injected into the analyzer for chromatographic separation on a cation exchange column. Monomer detection was carried out fluorometrically after derivatisation with o-phtaldialdehyd and mercaptoethanol. For statistical analyses software JMP by SAS was used. Linear regression fits were executed to analyze correlations between salinity and parameters measured at Matla estuary. P-values <0.05 were considered to be significant.

Results

Arithmetic means of all parameters measured at the Hooghly and Matla sampling sites are presented in table 1. In the Hooghly estuary, highest water temperatures were measured at DH (22.5 ± 1.4 °C) and lowest at HB (18.4 ± 0.1 °C). Water temperatures measured at Matla showed an intermediate value of 20.8 ± 0.6 °C. Salinities revealed no marine influence at HB and only a minor one at DH (1.0 ± 0.6). Highest salinities were measured along the Matla estuary (22.6 ± 1.5). The pH values showed only minor variation between sites with an average value of 8.2 for all sampling stations. Concentration of $Si(OH)_4$ was much higher at Hooghly sampling stations (HB: 66.2 ± 6.7 µM, DH: 80.1 ± 4.0 µM) than at those in Matla (9.8 ± 3.1 µM). Ammonium was below detection limit at all sampling stations using the continuous flow analyses system. However, low concentrations of 1.3 µM could be detected using the salicylate method at HB and DH. At all Matla stations ammonium was below detection limit also when using the salicylate method. NO_3^- dominated the dissolved inorganic nitrogen (DIN) pool at all the sampling stations, with an average percentage contribution of 82, 83 and 97 % to DIN for HB, Matla and DH, respectively. Average concentrations of NO_3^- were 26.2 ± 1.1 µM at DH and considerably higher than low concentrations of 0.9 ± 1.0 µM and 3.4 ± 1.5 µM observed at HB and Matla, respectively. Concentrations of NO_2^- were 0.7 µM for HB and Matla and three times higher than at DH. Concentrations of PO_4^{3-} were also highest at DH (0.9 ± 0.3 µM) followed by Matla. At HB PO_4^{3-} was under detection limit. DON concentrations were with on average 11.9 ± 0.3 µM about twice higher at Hooghly than at Matla stations.

TSM, POC and PN concentrations were considerably higher at the Hooghly stations than at the Matla ones. Highest mean concentrations of TSM were observed at station DH (283 ± 156 mg/l), followed by HB (241 ± 85 mg/l) and Matla (18 ± 8 mg/l). POC and PN concentrations were highest at HB with arithmetic means of 342.4 ± 70.6 µM and 49.1 ± 7.2 µM, respectively. Relatively high values of POC (203.2 ± 86.3 µM) and PN (24.7 ± 10.4 µM) were also observed at DH. Concentrations of POC and PN at Matla were much lower than at the Hooghly stations with arithmetic means of 25.4 ± 8.7 µM and 3.3 ± 1.2, respectively. C/N ratio was higher for DH

Table 1: Physico-chemical parameters and concentrations (mean ± SD) of dissolved nutrients, particulate matter components, $\delta^{15}N$ of particulate matter and NO_3^-; b.d. = below detection limit, n.d. = no data, (n) = number of data points

	HB		DH		Matla	
Salinity	0 ± 0	(5)	1.0 ± 0.6	(3)	22.6 ± 1.5	(17)
Water temperature [°C]	18.4 ± 0.1	(5)	22.5 ± 1.4	(3)	20.8 ± 0.6	(17)
pH	8.4 ± 0.2	(5)	8.1 ± 0.1	(3)	8.2 ± 0.1	(17)
$Si(OH)_4$ [µM]	66.2 ± 6.7	(5)	80.1 ± 4.0	(3)	9.8 ± 3.1	(17)
DIN [µM]	1.1 ± 1.2	(5)	26.9 ± 0.7	(3)	4.1 ± 1.7	(17)
NH_4^+ [µM] [a)]	b.d.	(5)	b.d.	(3)	b.d.	(17)
NH_4^+ [µM] [b)]	1.3 ± 1.2	(5)	1.3 ± 0.6	(3)	b.d.	(17)
NO_3^- [µM]	0.9 ± 1.0	(5)	26.2 ± 1.1	(3)	3.4 ± 1.5	(17)
NO_2^- [µM]	0.2 ± 0.2	(5)	0.7 ± 0.4	(3)	0.7 ± 0.2	(17)
DON [µM]	11.7 ± 3.8	(5)	12.1 ± 1.2	(3)	5.0 ± 1.4	(14)
PO_4^{3-} [µM]	b.d.	(5)	0.9 ± 0.3	(3)	0.2 ± 0.2	(17)
TSM [mg/l]	241 ± 85	(5)	283 ± 156	(3)	18 ± 8	(15)
POC [µM]	342.4 ± 70.6	(5)	203.2 ± 86.3	(3)	25.4 ± 8.7	(16)
PN [µM]	49.1 ± 7.2	(5)	24.7 ± 10.4	(3)	3.3 ± 1.2	(16)
C/N-ratio	6.9 ± 0.8	(5)	8.2 ± 0.3	(5)	7.8 ± 0.8	(5)
$\delta^{15}N$ (PN) [‰]	3.8 ± 0.3	(5)	3.6 ± 0.9	(3)	2.2 ± 0.9	(7)
$\delta^{15}N$ (NO_3^-) [‰]	n.d.	(0)	2.4 ± 0.7	(2)	3.8 ± 1.2	(8)
AA [mg/l]	2.4 ± 0.2	(5)	0.9 ± 0.3	(3)	0.1 ± 0.0	(6)
AA-N [%]	47.3 ± 8.1	(5)	31.2 ± 3.7	(3)	32.6 ± 11.7	(6)
Non-prot. AA [Mol-%]	1.0 ± 0.2	(5)	1.5 ± 0.2	(3)	1.1 ± 0.2	(6)

[a)] NH_4^+ detection via continuous flow analyzer system
[b)] NH_4^+ detection via salicylate method

(8.2 ± 0.3) than for HB (6.9 ± 0.8). Matla estuary showed an intermediate value of 7.8 ± 0.8.

Amino acids showed highest concentrations at HB (2.4 ± 0.2 mg/l). Concentrations at DH were about half those of HB. Matla stations showed lowest concentrations of AA (0.1 ± 0.0 mg/l). Contribution on AA-bound nitrogen to PN (AA-N%) was highest at HB (47.3 ± 8.1%). AA-N% was about the same at DH and at the Matla stations with arithmetic means of 31.2 ± 3.7 and 32.6 ± 11.7, respectively.

Highest average Mol% of the non proteinogenic amino acids (non-prot. AA) β-alanine (b-Ala) and γ-aminobutyric acid (g-Aba) was found at DH (1.5 ± 0.2 Mol%). The Mol% of non-prot. AA of HB and Matla stations were about the same with values of 1.0 ± 0.2 Mol% and 1.1 ± 0.2 Mol%, respectively.

Elevated average $\delta^{15}N$ values of PN were measured at HB and DH with arithmetic means of 3.8 ± 0.3 ‰ and 3.6 ± 0.9 ‰, respectively. Lowest average values of 2.2 ± 0.9 ‰ were observed at the Matla sites. $\delta^{15}N$ values of NO_3^- could not be measured for HB, because of the low nitrate concentrations . The values of Matla (3.8 ± 1.2 ‰) were higher than those of DH (2.4 ± 0.7 ‰).

Tidal variations of parameters at Hooghly stations

Impact of tidal variations on the concentrations of NO_3^-, NO_2^-, $Si(OH)_4$ and DON at HB are shown in Figs. 3a, b. These nutrients showed minimum values at low tide and maximum values at high tide. Observed concentration ranges were 0.0 – 2.6 µM, 0.0 – 0.5 µM, 56.8 – 75.1 µM and 8.7 – 17.8 µM for NO_3^-, NO_2^-, $Si(OH)_4$ and DON respectively. PO_4^{3-} concentration was below detection limit for all HB samples.

Lowest TSM concentration was observed at highest water level (101.1 mg/l). Highest TSM concentration was observed at the lowest water level (320.0 mg/l) (Fig. 3c).POC showed the same pattern as TSM, ranging between 230.4 µM and 424.1 µM (Fig. 3c). PN concentration showed highest value at 3:45 p.m. during high tide (56.4 µM) and lowest concentration 2 hours later also at highest water level during high tide (37.7 µM) (Fig. 3b). $\delta^{15}N$ of PN ranged between 3.5 and 4.3 ‰ (Fig. 3d).

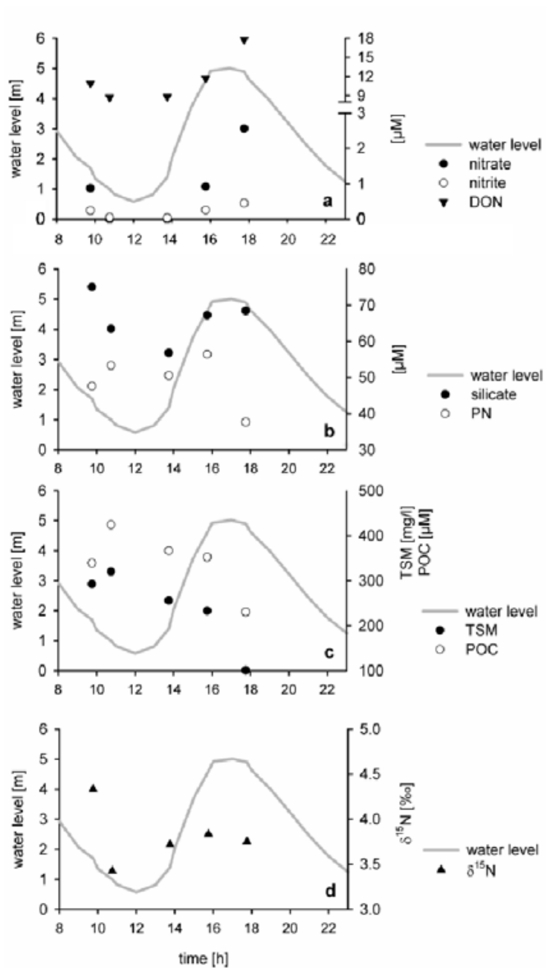

Fig. 3: Tidal variations at HB of concentration of a) nitrate, nitrite, DON (phosphate was under detection limit at all samplings) b) PN and silicate c) TSM and POC and d) $\delta^{15}N$ of PN.

DH showed only minor tidal variation of salinity with values of 0.3, 1.2 and 1.5 at water levels of 1.1, 3.1 and 5.2 m, respectively. Tidal variation of concentrations of dissolved inorganic nutrients, DON and particulate matter are shown in Fig. 4a-d. Concentrations ranged between 24.9 – 26.9 µM, 0.4 – 1.1 µM, 0.6 – 1.2 µM, 75.8 – 83.6 µM and 10.7 – 14.4 µM for NO_3^-, NO_2^-,PO_4^{3-}, $Si(OH)_4$ and DON, respectively. NO_2^- and PO_4^{3-} showed minimum concentrations at maximum salinity, while NO_3^- showed highest concentration at maximum salinity. Highest silicate concentrations were observed at lowest salinity (Fig. 4b). Lowest TSM concentration (151.5 mg/l) was observed at mid water level and a salinity of 1.2. The highest TSM value (455.6 mg/l) was observed at lowest water level and minimum salinity (0.3) (Fig. 4c). POC and PN concentrations showed the same pattern as TSM and varied between 118.7 µM and 291.2 µM and 34.9 and 14.1 µM, respectively (Fig. 4b, c). $\delta^{15}N$ of PN ranged between 2.9 ‰ at low water and 4.6 ‰ at mid water level (Fig. 4d).

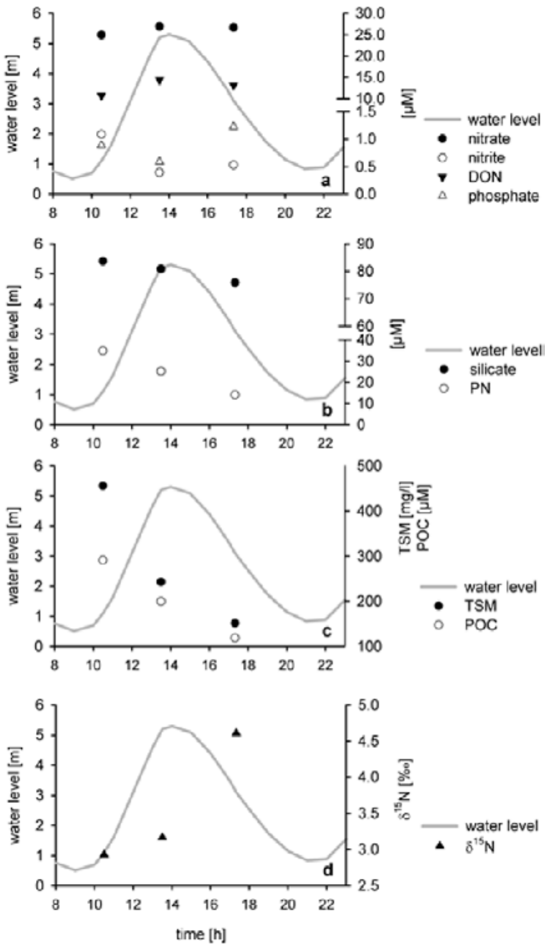

Fig. 4: Tidal variations at DH of concentrations of a) nitrate, nitrite, DON, phosphate b) PN and silicate c) TSM and POC and d) $\delta^{15}N$ of PN.

Spatial variation of parameters at Matla

Variations of individual parameters with salinity are shown in Figs. 5a-e. We observed a significant negative correlation of $Si(OH)_4$ (r=0.97, n=17, p<0.0001) and NO_3^- (r=0.93, n=17, p<0.0001) with salinity. Although NO_2^- showed lowest concentrations of 0.2 µM in the southern part of the transect, the negative correlation of NO_2^- with salinity was less significant (r=0.57, n=17, p=0.02). Phosphate concentrations were low in the Matla estuary; highest concentrations (0.4 µM) were unevenly distributed along the salinity gradient. PO_4^{3-} was undetectable in the middle and southern part of the estuary. The negative correlation of PO_4^{3-} with salinity was significant (r=0.61, n=17, p=0.01). Concentrations of DON, TSM, PN, POC as well as $\delta^{15}N$ of PN and of NO_3^- showed no significant correlations with salinity. Lowest $\delta^{15}N$ value of PN ($\delta^{15}N$ = 0.9‰ at Sal. = 22.6 PSU) was measured at a sampling station at the middle of the estuary.

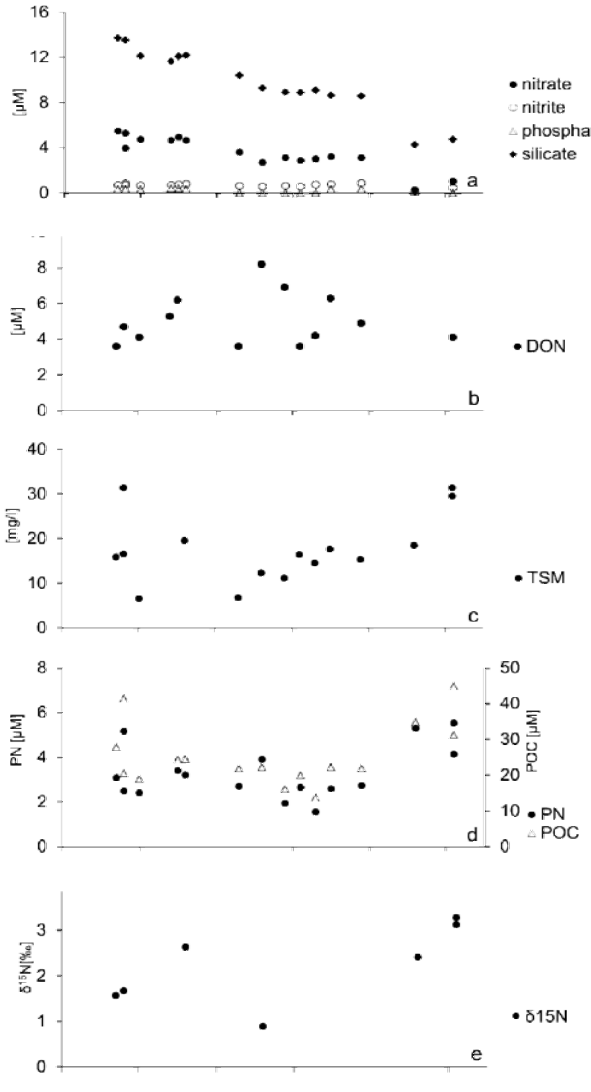

Fig. 5: Concentration of a) nitrate, nitrite, phosphate silicate b) DON c) TSM d) PN, POC and e) $\delta^{15}N$ of PN in the Matla plotted against salinity

Discussion

Tidal observations at the Hooghly estuary showed higher nutrient concentrations at the sea end (DH) than at the river end (HB) of the investigation area. TSM concentrations point at higher concentrations at the river end and a dilution with seawater at the sea end of the estuary. Even though salinity was 0 for all samples at HB the influence of flood and ebb tide could be detected in changing nutrient and TSM concentrations. The low salinities observed at DH further confirm the river dominance at both Hooghly sampling stations. In contrast to the river-dominated Hooghly stations, high salinity values at Matla sampling stations reflected the marine dominance of the estuary.

Concentrations of nutrients

The major part of dissolved silicate input into the oceans (ca. 80%) comes from rivers[29]. Higher dissolved silicate concentrations at HB and DH (Table 1) reflect the influence of the Hooghly river whereas comparatively low values of $Si(OH)_4$ at Matla stations are the result of dilution under the dominant marine influence at Matla estuary as evident from salinities. The difference in the concentrations of $Si(OH)_4$ between HB and DH could be a consequence of surface sealing due to urbanization and consequently lower rates of erosion, weathering and input of dissolved silicate[30] at HB on the one side, and the effect of outwelling from flood plains and the mangrove areas, as observed in Caeté estuary in Brazil[31], at DH on the other side. Brick industries at the riverside at DH could also contribute to elevated concentrations of $Si(OH)_4$ at DH. The lower mean values observed at DH during the dry season as compared to those measured during SW monsoon of 150.9 ± 21.8 µM[21] indicate the importance of precipitation and corresponding surface runoff and erosion generating higher inputs of silicate and resulting in higher $Si(OH)_4$ concentrations during rainy season[21,30]. The significant negative correlation of $Si(OH)_4$ with salinity at the Matla sampling stations points at conservative mixing of fluvial water masses with seawater. However, $Si(OH)_4$ values at Matla were much lower than concentrations (80 µM) measured in Caeté mangrove estuary in Brazil during dry period at similar salinities[13]. Silicate values for nearby coastal ocean surface water measured at a N-S transect at the Bay of Bengal during summer monsoon 2001 showed $Si(OH)_4$ values of about 2 µM which were much lower than those observed in our study[32] and point at a further uptake and dilution of $Si(OH)_4$ in offshore waters. Higher values in Matla and assumed conservative mixing behavior of $Si(OH)_4$ suggests a limited assimilation of silicate by diatoms probably due to low PO_4^{3-} values which limit primary production (see Table 1 and discussion below). Microscopy analyses of plankton of Matla estuary showed only small amounts of intact phytoplankton and relative high amounts of mineral detritus (own observation) confirming a low phytoplankton abundance at the investigated area.

Elevated NH_4^+ concentrations in river water are considered as an indicator for urban impact[33,34]. Therefore, we expected elevated NH_4^+ values in and close to the megacity Kolkata at the

Hooghly sampling stations. Surprisingly, the HB site was characterized by very low concentrations of NH_4^+ (1.3 ± 1.2 µM). Uptake of ammonium by phytoplankton can be neglected because of high TSM contents inducing light limitation. Another process which could lead to low NH_4^+ values is the consumption of ammonium by heterotrophic bacteria[e.g. 35]. However bacterial abundance was not very high at HB (total bacterial counts (TBC) = 2500 cfu/ml, A. Palit, personal communication) and DH (TBC = 1000 cfu/ml, A. Palit, personal communication) and therefore does not point to a significant consumption of NH_4^+ by heterotrophic bacteria at the Hooghly stations. Moreover, high TSM contents could allow for adsorption of ammonium to clay minerals[36] leading to low NH_4^+ concentration. This process is favored under freshwater conditions and low salinities as observed at HB and DH, respectively[37,38 and references therein]. Lower molar C/N values at HB (6.9 ± 0.8) than at DH (8.2 ± 0.3) and Matla (7.8 ± 0.8) hint at adsorbed NH_4^+ at the city of Kolkata. A plot of particulate organic carbon content against particulate nitrogen content can be used to verify this assumption[e.g. 39]. The intercept on the X axis of the HB plot (Fig. 6a) indicates a concentration of inorganic nitrogen of 8.5 µM adsorbed onto particles. However, the positive correlation of the few POC and PN values was not significant (r=0.85, n=5, p=0.06), and the widening of the confidence interval towards lower values shows that the zero intercept can vary within a wide range. At DH the plot of POC against PN (Fig. 6b) does not indicate adsorption of NH_4^+ to clay minerals. Even though correlation of POC with PN was significant (r=0.99, n=3, p= 0.048), the widening of the confidence interval towards lower values indicates that - same as for HB- more data points would be necessary to verify this assumption.

Even under consideration of the potential adsorption of NH_4^+, the concentration of NH_4^+ at HB is only 9.8 µM, which is much lower than expected for heavily anthropogenically impacted sites. NH_4^+ in the Scheldt estuary was reported to exceed 90 µM[40], and extreme high concentrations of up to 430 µM were observed under the influence of a large sewage plant on the Seine river downstream of Paris[34].

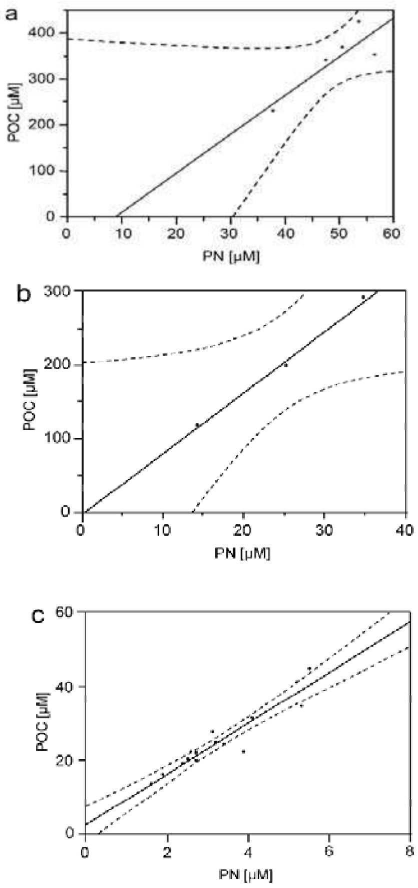

Fig. 6: Plot of POC against PN for a) HB b) DH c) Matla. Straight lines represent the linear regressions. Dotted lines represent confidence intervals. Plots created with JMP, SAS (graphically modified).

Another process which could be responsible for low concentrations of NH_4^+ at the Hooghly sites is nitrification. However, high values of NO_3^- would be the consequence which were not observed at HB. The elevated NO_3^- concentrations at DH are probably the result of remineralization processes (see discussion below).

The plot of POC against PN (r=0.94, n=16, p<0.0001) does not indicate adsorption of NH4+ to clay minerals (Fig. 6c) at Matla sampling stations. Non-detection of NH4+ at all Matla stations is consistent with results from near-pristine tropical estuaries in Australia where concentrations of 0.2 µM were measured at similar salinities during dry season[41]. This is in contrast to a one year study from the Caeté estuary in Brazil, where NH_4^+ outwelling from the mangrove sediments was the dominant species of the DIN pool and reached an average value of about 7 µM during dry season[31].

In contrast to the Caeté estuary our results do not indicate significant NH_4^+ input from porewater. As bacterial abundance was relatively high at Matla (TBC = 2.800.000 cfu/ml, A.Palit, personal communication) the uptake of NH_4^+ by heterotrophic bacteria[e.g. 35] could contribute to low NH_4^+ concentrations. This process has been particularly observed at elevated salinities and under oligotrophic nutrient conditions[35,42-44] and could therefore account for the nutrient poor, marine dominated Matla estuary.

NO_2^- concentrations were in general low in comparison to anthropogenic influenced estuaries like Scheldt estuary where concentrations up to 20 µM[e.g. 33,40] were measured. Higher NO_2^- concentrations at DH than at HB could be an indicator for transformation processes within the nitrogen pool at DH as NO_2^- is an intermediate product arising during nitrification and denitrification[e.g. 45]. Because of high NO_3^- concentrations at DH in comparison to HB and changes observed in the composition of particulate amino acids (see discussion below), nitrification processes are most probably taking place between HB and DH with elevated NO_2^- concentrations as a consequence.

NO_3^- concentrations are another indicator for human impact, reflecting the input of nitrate from e.g. fertilizers or aquaculture facilities[33,46,47]. Concentrations at HB were very low in contrast to a study in Kerala where NO_3^- concentrations of urban areas of up to 172 µM were measured[48]. As NH_4^+ and NO_2^- also showed only low concentrations, a contamination by DIN at HB is not evident.

DIN concentrations measured at DH resembled concentrations of 27.2 ± 2.1 µM measured in an earlier study during postmonsoon at DH[21]. The noticeable high concentrations at DH in comparison to HB might be a result of remineralization of particulate nitrogen. Transformation processes of the particulate phase were indicated by a reduction of POC concentration by 139.2 µM between HB and DH (Table 1). Similarly, we observed a decline of PN between the two sites by 24.4 µM, which corresponds approximately to the increase of DIN concentration of 25.8 µM indicating a transfer of nitrogen from particulate organic phase to dissolved inorganic phase.

Composition of particulate amino acids can be used to identify the degradation state of particulate organic material (POM)[e.g. 49,50,51]. The Mol% of non-prot. AA increases with the degree of degradation of POM and indicates bacterial decomposition of POM[49]. The composition of amino acids in the particulate phase showed considerable changes between HB and DH. Higher AA concentration and contribution of AA-N% at HB together with a lower Mol% of non-prot. AA as compared to DH points at a larger input of fresher proteins at HB (Fig. 7), probably due to anthropogenic sewage[51] as contribution from in situ production is probably negligible due to high turbidity. The differences in concentration of PN, DIN and in composition of AA between HB and DH confirm the release of DIN associated with particulate matter during estuarine transport, which was determined by a biogeochemical model of a study between Diamond Harbour and Sagar Island[21]. At the Matla estuary AA concentrations were low, showed relative low AA-N% values and high Mol% of non-prot. AA which points at the absence of fresh POM from planktonic production.

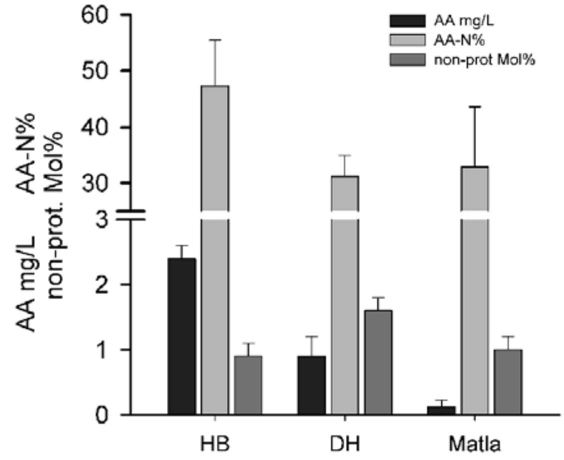

Fig. 7: Average AA concentration, AA-N% as well as non-prot. Mol% at HB, DH and Matla.

In contrast to NH_4^+, concentrations of NO_3^- in the Matla were higher than average concentrations in the Caeté estuary (0.8 µM) during dry season[31]. However, NO_3^- concentrations were still low and do not indicate any major anthropogenic impact due to agriculture or aquaculture in the hinterland. The small DIN concentrations measured in our study verified the pristine conditions during dry season. The significant negative correlation of NO_3^- with salinity at Matla sampling stations points at estuarine dilution as observed for $Si(OH)_4$.

Besides the DIN, DON is a potential nitrogen source for phytoplankton in rivers and estuaries[52]. However, only a part of DON is bioavailable. Depending on its source, 0 to 73% of DON was bioavailable for estuarine plankton in a study in New Jersey, USA, where DON from different sources such as forests, agriculture areas and urban stormwater runoff was investigated[53]. The relative contribution of DON to total dissolved nitrogen (TDN) was 91%, 31 % and 55 % for HB, DH and Matla, respectively, and thus represents a significant portion of dissolved nitrogen. Nevertheless, DON concentrations at all stations were lower than annual mean concentration (18.6 µM) of different world rivers[33] and concentration of Caeté estuary (13.5 µM) during dry season at similar salinities[31]. Elevated DON concentrations as observed in the Delaware river (46.5 µM) or in anthropogenically impacted rivers in China (>50 µM) were not observed in our study[52,54]. Therefore, harmful impacts on the coastal marine ecosystem like algal blooms due to elevated DON concentrations[54 and citations therein] should not be expected in our study area, at least not during dry season.

PO_4^{3-} concentrations can be influenced by population density, use of fertilizers and change of land use like deforestation[e.g. 47 and references therein,55]. Extremely high concentrations have been measured for example at urban sites of Kerala (86 µM) or in the anthropogenically altered Scheldt estuary (50 µM) in the 1970ies[40,48]. The PO_4^{3-} concentrations observed in our study were as low as those from some turbid Chinese rivers (<1.5 µM), resulting from the adsorption of PO_4^{3-} onto particulates[54,56]. This mechanism could also explain PO_4^{3-} concentrations below detection limit at HB (Table 1). The increase of concentration of PO_4^{3-} between DH and HB can be explained by remobilization of PO_4^{3-} sorbed to mineral particles due to lower pH values and changing physical-chemical parameters (ΔpH: 0.3; ΔTemperature: 4.1 °C; ΔSalinity: 1.0 PSU; ΔTurbidity: 113.7 NTU) in the mixing zone of the estuary[57]. Another possible explanation for general low phosphate values at all the sites is the use of phosphate by heterotrophic bacteria[e.g. 35]. As for the uptake of NH_4^+ (see discussion above) this process could more likely occur at Matla as bacterial abundance was relatively high at Matla stations. At Hooghly stations the bacterial abundance was probably too low for significant uptake of PO_4^{3-} by heterotrophic bacteria. Dissolved reactive phosphate concentrations measured during monsoon at DH showed higher values of 2.6 ± 0.4 µM in comparison to PO_4^{3-} values measured in our study[21], indicating a higher input of phosphate during rainy season.

Concentrations of PO_4^{3-} (0.75 µM) measured in the Caeté estuary during dry season at similar salinities[13] were higher than the very low values measured along the entire Matla estuary. The low PO_4^{3-} concentration and the significant negative correlation of $Si(OH)_4$ and NO_3^- with salinity as well as the degraded nature of the POM evident from AA indicates PO_4^{3-} as the limiting nutrient in the Matla estuary leading to the low concentration of fresh organic matter.

Nitrogen isotopy of NO_3^- and PN

Severe influence of sewage is indicated by $\delta^{15}N$ values of PN > 9 ‰[12]. Accordingly, the values observed in the study area do not point at any major anthropogenic impact. This appears consistent with the low nutrient concentrations observed at all sampling stations and the reduced AA concentration at DH and the Matla estuary. The low $\delta^{15}N$ value ($\delta^{15}N$ = 0.9 ‰) detected at the middle of the Matla estuary indicates nitrogen fixation by cyanobacteria like *Trichodesmium*[58], which is common in mangrove habitats[31 and references therein]. At the same site, we identified the cyanobacterium *Anabaena gerdii* via epifluorescence microscopy[59], further supporting the presence of nitrogen fixation in this section of the Matla estuary.

The low $\delta^{15}N$ of NO_3^- observed in this study (Table 1) makes us exclude sewage or manure as a major source for nitrate for DH and Matla, as typical $\delta^{15}N$ values for these contributions typically vary between 7 and 20 ‰[60 and references therein]. $\delta^{15}N$ values of NO_3^- coincided with signature of nitrogen observed in most soils ($\delta^{15}N$ = 2 to 5 ‰) for both stations[61 and references therein]. Stable nitrogen isotope values of NO_3^- could also indicate for nitrate from atmospheric deposition ($\delta^{15}N$ = -7 to 9 ‰)[61 and references therein], however atmospheric deposition is mainly related to rain which can be neglected for dry season (Fig. 2). Nitrogen from synthetic fertilizer ($\delta^{15}N$ = 0 ± 3 ‰)[61 and references therein] as possible source for NO_3^- is unlikely to occur in mangrove area at DH. Low concentrations of NO_3^- measured in the Matla estuary do not point at significant fertilizer input into the estuary. In fact, $\delta^{15}N$ values of NO_3^- at Matla were similar to values observed at river Barro Branco in the Amazonas for which $\delta^{15}N$ values of 4.5 ± 0.8 ‰[62] were reported.

In the Barro Branco, NO_3^- was assumed to originate predominantly from the remineralization of organic matter within the stream, which could also be a possible source of NO_3^- in the Matla estuary.

Conclusion

The parameters analyzed in this study indicated a small anthropogenic impact on the investigated estuaries. As a significant phytoplanktonic production is unlikely due to high turbidity, obtained AA data suggested the input of OM from external sources (e.g. sewage inputs) in Kolkata. Remineralization of this material resulted in higher downstream NO_3^- concentrations. Observed dissolved nutrient concentrations were still much lower than expected from this highly populated region, which in part might be explained by particle adsorption. The Matla estuary showed much lower concentration of suspended matter reflecting the reduced riverine influence during dry season. Low dissolved nutrient concentrations limited primary production and suggested pristine conditions in this region. The present study complements earlier biogeochemical studies from this rarely investigated site of major riverine input to the world ocean.

Acknowledgements

Financial travel support to D. Unger and P. Fischer by the International Bureau of the BMBF (Grant No. IND10/A20) as well as project funding by ZMT (Project NitroBob), Deutsche Forschungsgemeinschaft (BIOVIBEN, LA 868/12-1) and the Department of Science and Technology (India) are greatly acknowledged.

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