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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The present study consists of the analyses of dissolved inorganic nutrients (NH\textsubscript{4}\textsuperscript{+}, NO\textsubscript{3}\textsuperscript{−}, NO\textsubscript{2}\textsuperscript{−}, PO\textsubscript{4}\textsubscript{3}−, Si(OH)\textsubscript{4}), dissolved organic nitrogen (DON), particulate nitrogen, particulate organic carbon, amino acids and \(\delta^{15}\)N of particulate nitrogen and NO\textsubscript{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\textsubscript{4}\textsubscript{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\textsubscript{3}− and PO\textsubscript{4}\textsubscript{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\textsuperscript{1-5}. 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\textsuperscript{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\textsuperscript{6}. Considerable amounts of the anthropogenically fixed nitrogen enter the aquatic system and successively reaches the oceans via riverine and groundwater discharge\textsuperscript{7}. In affected coastal areas nitrogen enrichment can cause eutrophication leading to oxygen deficits, toxic algae blooms or coral reef degradation\textsuperscript{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\textsubscript{3}−\textsuperscript{e.g. 10,11,12}.

Mangrove forests are highly productive ecosystems covering 60 – 75% of tropical coastlines\textsuperscript{e.g.13}. Due to their complex
biogeochemistry they can act as source, sink or both for nutrients to adjacent coastal waters\textsuperscript{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\textsuperscript{2} and represents the largest connected mangrove forest of the world\textsuperscript{15,16}. The study area is strongly influenced by the subtropical monsoon climate. Annual precipitation is about 1600 to 1800 mm\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{19}. Farakka barrage was put on stream on 21\textsuperscript{st} of April 1975 in order to guarantee an additional freshwater discharge for the Hooghly to avoid further silt accumulation at Kolkata port\textsuperscript{18,20}. As a consequence, significant changes in salinity and ecology of the Hooghly estuary could be observed\textsuperscript{18}. Today the estuary is considered to be salt free 60 km upstream of the river mouth at DH\textsuperscript{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. 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.](image1)

![Fig. 2: Precipitation observed in Kolkata between August 2010 and August 2011. Sampling was carried out between 17\textsuperscript{th} and 24\textsuperscript{th} January 2011 during a persistent dry phase. Data available online: http://climate.usurf.usu.edu, accessed 15\textsuperscript{th} January 2012.](image2)
Average freshwater discharge through the Hooghly varies between 1000 and 3000 m³/s during dry and rainy season, respectively. 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.

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, 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. 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. 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. Apart from these, the Sundarban mangroves are generally considered to be a pristine ecosystem.

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.

Dissolved inorganic nutrients were analyzed by a continuous flow analyzer (Skalar San ++ system). Ammonium was quantified fluorometrically; silicate (Si(OH)₄), phosphate (PO₄³⁻), nitrate and nitrite (NO₃⁻) and nitrite (NO₂⁻) were determined spectrophotometrically. The concentration of nitrate (NO₃⁻) was calculated as the difference between NOₓ⁻ and NO₂⁻. Detection limits were 0.324 µM, 0.029 µM, 0.041 µM, 0.094 µM and 0.776 µM for NOₓ⁻, NO₂⁻, NH₄⁺, PO₄³⁻ 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. 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.

Particulate nitrogen (PN) and particulate organic carbon (POC) contents of suspended matter were analyzed by high-temperature combustion in an EuroVector EA3000 element analyzer. For POC measurement, filters were treated with 1 N HCl and subsequently dried at 40°C to remove carbonate before measurement. PN measurements were done on untreated filters. For measurement of δ¹⁵N of NO₃⁻, the adapted ammonia diffusion method for oceanic nitrate was applied. This method is based on a reduction of NO₃⁻ 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. The nitrogen isotope composition (δ¹⁵N) of PN and NO₃⁻ 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-phthaldialdehyde 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. NO₃⁻ 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 NO₃⁻ 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 NO₂⁻ were 0.7 µM for HB and Matla and three times higher than at DH. Concentrations of PO₄³⁻ were also highest at DH (0.9 ± 0.3 µM) followed by Matla. At HB PO₄³⁻ 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
(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 δ¹⁵N 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, δ¹⁵N values of NO₃⁻ could not be measured for HB, because of the low

### Table 1: Physico-chemical parameters and concentrations (mean ± SD) of dissolved nutrients, particulate matter components, δ¹⁵N of particulate matter and NO₃⁻; b.d. = below detection limit, n.d. = no data, (n) = number of data points

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HB</th>
<th>DH</th>
<th>Matla</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salinity (‰)</td>
<td>0.0 ± 0</td>
<td>1.0 ± 0.6</td>
<td>22.6 ± 1.5</td>
</tr>
<tr>
<td>Water temperature [°C]</td>
<td>18.4 ± 0.1</td>
<td>22.5 ± 1.4</td>
<td>20.8 ± 0.6</td>
</tr>
<tr>
<td>pH</td>
<td>8.4 ± 0.2</td>
<td>8.1 ± 0.1</td>
<td>8.2 ± 0.1</td>
</tr>
<tr>
<td>Si(OH)₄ [µM]</td>
<td>66.2 ± 6.7</td>
<td>80.1 ± 4.0</td>
<td>9.8 ± 3.1</td>
</tr>
<tr>
<td>DIN [µM]</td>
<td>1.1 ± 1.2</td>
<td>26.9 ± 0.7</td>
<td>4.1 ± 1.7</td>
</tr>
<tr>
<td>NH₄⁺ [µM]</td>
<td>b.d.</td>
<td>b.d.</td>
<td>b.d.</td>
</tr>
<tr>
<td>NH₄⁺ [µM] b)</td>
<td>1.3 ± 1.2</td>
<td>1.3 ± 0.6</td>
<td>b.d.</td>
</tr>
<tr>
<td>NO₃⁻ [µM]</td>
<td>0.9 ± 1.0</td>
<td>26.2 ± 1.1</td>
<td>3.4 ± 1.5</td>
</tr>
<tr>
<td>NO₂⁻ [µM]</td>
<td>0.2 ± 0.2</td>
<td>0.7 ± 0.4</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>DON [µM]</td>
<td>11.7 ± 3.8</td>
<td>12.1 ± 1.2</td>
<td>5.0 ± 1.4</td>
</tr>
<tr>
<td>PO₄³⁻ [µM]</td>
<td>b.d.</td>
<td>0.9 ± 0.3</td>
<td>0.2 ± 0.2</td>
</tr>
<tr>
<td>TSM [mg/l]</td>
<td>241 ± 85</td>
<td>283 ± 156</td>
<td>18 ± 8</td>
</tr>
<tr>
<td>POC [µM]</td>
<td>342.4 ± 70.6</td>
<td>203.2 ± 86.3</td>
<td>25.4 ± 8.7</td>
</tr>
<tr>
<td>PN [µM]</td>
<td>49.1 ± 7.2</td>
<td>24.7 ± 10.4</td>
<td>3.3 ± 1.2</td>
</tr>
<tr>
<td>C/N-ratio</td>
<td>6.9 ± 0.8</td>
<td>8.2 ± 0.3</td>
<td>7.8 ± 0.8</td>
</tr>
<tr>
<td>δ¹⁵N (PN) [%]</td>
<td>3.8 ± 0.3</td>
<td>3.6 ± 0.9</td>
<td>2.2 ± 0.9</td>
</tr>
<tr>
<td>δ¹⁵N (NO₃⁻) [%]</td>
<td>n.d.</td>
<td>2.4 ± 0.7</td>
<td>3.8 ± 1.2</td>
</tr>
<tr>
<td>AA [mg/l]</td>
<td>2.4 ± 0.2</td>
<td>0.9 ± 0.3</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td>AA-N [%]</td>
<td>47.3 ± 8.1</td>
<td>31.2 ± 3.7</td>
<td>32.6 ± 11.7</td>
</tr>
<tr>
<td>Non-prot. AA [Mol-%]</td>
<td>1.0 ± 0.2</td>
<td>1.5 ± 0.2</td>
<td>1.1 ± 0.2</td>
</tr>
</tbody>
</table>

a) NH₄⁺ detection via continuous flow analyzer system
b) NH₄⁺ detection via salicylate method

Impact of tidal variations on the concentrations of NO₃⁻, NO₂⁻, 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 NO₃⁻, NO₂⁻, Si(OH)₄ and DON respectively. PO₄³⁻ 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).

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$_2^-$ 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).

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 δ15N of PN and of NO3– showed no significant correlations with salinity. Lowest δ15N value of PN (δ15N = 0.9‰ at Sal. = 22.6 PSU) was measured at a sampling station at the middle of the estuary.

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 rivers29. 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 silicate20 at HB on the one side, and the effect of outwelling from flood plains and the mangrove areas, as observed in Caeté estuary in Brazil31, 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 µM21 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 season21,30. 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 salinities13. 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 study32 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 PO43− 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 NH4+ concentrations in river water are considered as an indicator for urban impact33,34. Therefore, we expected elevated NH4+ 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\footnote{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\footnote{36,37,38}, leading to low NH$_4^+$ concentration. This process is favored under freshwater conditions and low salinities as observed at HB and DH, respectively\footnote{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\footnote{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\footnote{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\footnote{31}.

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 NH$_4^+$ to clay minerals (Fig. 6c) at Matla sampling stations. Non-detection of NH$_4^+$ 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\footnote{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\footnote{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) 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.

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 and concentration of Caeté estuary (13.5 µM) during dry season at similar salinities. 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. Therefore, harmful impacts on the coastal marine ecosystem like algal blooms due to elevated DON concentrations and citations therein should not be expected in our study area, at least not during dry season.

PO₄³⁻ concentrations can be influenced by population density, use of fertilizers and change of land use like deforestation and references therein. 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. The PO₄³⁻ concentrations observed in our study were as low as those from some turbid Chinese rivers (<1.5 µM), resulting from the adsorption of PO₄³⁻ onto particulates. This mechanism could also explain PO₄³⁻ concentrations below detection limit at HB (Table 1). The increase of concentration of PO₄³⁻ between DH and HB can be explained by remobilization of PO₄³⁻ 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. Another possible explanation for general low phosphate values at all the sites is the use of phosphate by heterotrophic bacteria and references therein. As for the uptake of NH₄⁺ (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 PO₄³⁻ by heterotrophic bacteria. Dissolved reactive phosphate concentrations measured during monsoon at DH showed higher values of 2.6 ± 0.4 µM in comparison to PO₄³⁻ values measured in our study, indicating a higher input of phosphate during rainy season.

Concentrations of PO₄³⁻ (0.75 µM) measured in the Caeté estuary during dry season at similar salinities were higher than the very low values measured along the entire Matla estuary. The low PO₄³⁻ concentration and the significant negative correlation of Si(OH)₄ and NO₃ with salinity as well as the degraded nature of the POM evident from AA indicates PO₄³⁻ as the limiting nutrient in the Matla estuary leading to the low concentration of fresh organic matter.

Nitrogen isotopy of NO₃ and PN

Severe influence of sewage is indicated by δ¹⁵N values of PN > 9 ‰. 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 δ¹⁵N value (δ¹⁵N = 0.9 ‰) detected at the middle of the Matla estuary indicates nitrogen fixation by cyanobacteria like Trichodesmium, which is common in mangrove habitats and references therein. At the same site, we identified the cyanobacterium Anabaena gerdii via epifluorescence microscopy, further supporting the presence of nitrogen fixation in this section of the Matla estuary.

The low δ¹⁵N of NO₃ observed in this study (Table 1) makes us exclude sewage or manure as a major source for nitrate for DH and Matla, as typical δ¹⁵N values for these contributions typically vary between 7 and 20 ‰ and references therein. δ¹⁵N values of NO₃ coincided with signature of nitrogen observed in most soils (δ¹⁵N = 2 to 5 ‰) for both stations and references therein. Stable nitrogen isotope values of NO₃ could also indicate for nitrate from atmospheric deposition (δ¹⁵N = -7 to 9 ‰) and references therein, however atmospheric deposition is mainly related to rain which can be neglected for dry season (Fig. 2). Nitrogen from synthetic fertilizer (δ¹⁵N = 0 ± 3 ‰) and references therein as possible source for NO₃ is unlikely to occur in mangrove area at DH. Low concentrations of NO₃ at Matla estuary do not point at significant fertilizer input into the estuary. In fact, δ¹⁵N values of NO₃ at Matla were similar to values observed at river Barro Branco in the Amazonas for which δ¹⁵N values of 4.5 ± 0.8 ‰ 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.

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